CONNECTIVITY THROUGH ALLOCHTHONY: RECIPROCAL LINKS BETWEEN ADJACENT AQUATIC AND TERRESTRIAL ECOSYSTEMS IN SOUTH AFRICA

Report to the

WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

Background

An important aspect of the dynamics of nutrients and pollutants in natural systems is captured in the concept of allochthony, founded on the observation that nutrients and energy in a variety of forms are transferred between adjacent habitats, communities and ecosystems that are not routinely considered as connected. Different forms of nutrients and energy move across the conceptual boundaries of habitats via organisms' activities or physical processes such as wind or water currents, and these transfers can represent important food subsidies. Such cross-partition ecological subsidies can augment the nutritional condition, biomass and biodiversity of communities, particularly where local production (or autochthony) alone may be inadequate to support local food webs. Furthermore, organic subsidies can influence population dynamics, community interactions and ecosystem processes, and can represent dominant flux inputs in ecosystem budgets. Our intention was to explore organic nutrient fluxes in relation to a primarily lotic (i.e. flowing) aquatic system at the scale of a hydrological catchment.

Aim

The over-arching aim of this study was to identify and quantify invertebrate- and vertebrate-mediated reciprocal transfers of organic nutrients among aquatic (freshwater, estuarine, near-shore marine) and terrestrial habitats (including the buffer zone between aquatic and terrestrial habitats) in a South African hydrological catchment.

Methods

We assessed the flux of organic nutrients in a freshwater/estuary/terrestrial region represented by a hydrological catchment primarily using stable isotope ratios, augmented with stomach contents and/or fatty acid analysis in certain cases. Wherever possible, the isotope and lipid tracer techniques have been used concurrently to cross-validate diet composition of consumers in the habitats of interest (river, riparian zone, land, estuary).

Three estuarine and six freshwater sites were selected in the Kowie River, Eastern Cape. Sampling at each site was aimed at assessing as many elements of the food web as possible. Wherever possible, collections included algae, submerged and emergent macrophytes, dominant riparian trees, suspended particulate matter, zooplankton, in-falling terrestrial invertebrates, emerging freshwater invertebrates, aquatic invertebrates, migratory fish, amphibians, wading birds and select aerial predators.

Results and discussion

Basal resources

Carbon and nitrogen stable isotope values in basal sources (benthic algae, aquatic macrophytes and terrestrial leaves) and the mixed organic groups (suspended particulate matter and detritus) indicated that there were shifts in the contributions of the sources to the mixed pools across time and space. Terrestrial leaf contributions to bulk detritus generally increased in the downstream direction, whereas aquatic macrophyte contributions decreased from the upper to the lower reaches. Contributions of allochthonous materials were more substantial in bulk detritus, whereas autochthonous materials were generally more substantial in the particulates. However, the detritus was dominated by aquatic macrophytes and benthic algae in the upper reaches in all sampling periods, with detritus in the lower reaches dominated by terrestrial sources.

We hypothesised that the organic matter fuelling the suspended mixtures varies along the continuum, with the middle reaches of the system (i.e. the lower river) dominated by autochthonous and the upper reaches by allochthonous material. The influence of benthic algae in suspended particulate matter followed our predictions, but terrestrial plants did not dominate the particulates (or the bulk detritus) in the upper river. A second hypothesis (that the relative contributions of different sources change over time) was supported by the isotope models, although these changes were complex owing to the dynamic nature of the river over time.

Invertebrates

The diets of freshwater invertebrates did not always match the availability of sources identified in the mixed organic pools. The isotope models identified autochthonous (mainly aquatic macrophytes and attached algae) matter as the main food fuelling consumers in most freshwater parts of the river, although allochthonous organic matter was important at the headwaters and at the mouth of the estuary. Among all the functional feeding groups analysed in the river, there was a gradual shift from the assimilation of allochthonous to autochthonous material from headwaters to the downstream freshwater regions. The benthic food webs were measurably different along the length of the estuary, and trophic interactions within each site varied with season. Consumers assimilated carbon sources produced locally, as these consumers had similar $\delta^{13}C$ values to those of the local resources where they resided (such as suspended particulates, microphytobenthos or salt marsh grass). The models demonstrated that suspended particulate material was highly utilised by consumers in the upper estuarine reaches, whereas large contributions of marsh grass detritus were detected in the diets of consumers in the downstream region. These results were further supported by fatty acid and stable isotope data of sediment organic matter in the system, which indicated that the marsh grass *Spartina maritima* contributed substantially to the sediments in the lower reaches of the estuary.

We evaluated the contributions of aquatic and terrestrial prey to terrestrial spiders at varying distances from three locations in the Kowie River across several seasons. The proportions of aquatic food sources in spider diets corresponded to proximity to the river and the biomass of aquatic insects, but the strength of the relationship varied across seasons (more pronounced relationship during summer). Regardless of type of site, the extent of aquatic contributions to spider diets was taxon-specific. We found a larger influence of aquatic subsidies onto land at the sites with larger stream widths (downstream sites), as the influence was only from 0 to 2 m into the land at the upstream site. The stream width affected insect emergence, with larger benthic areas allowing for increased emergence.

Contributions of aquatic food to spider diets were highly variable among the spider taxa, therefore it was difficult to detect seasonal changes in diets. We expected seasonal differences, as prey emergence was much greater in summer than winter, for example. However, the seasonal aspects in the spiders' diets were complex and aquatic subsidies to their diets were not always greatest in summer. Additional factors (e.g. habitat complexity, microhabitats, etc.) are certainly influencing spiders' diets besides distance from the river and insect emergence.

Vertebrates

The tracer and gut content results illustrated that several species and life-stages of amphibians had well-differentiated diets, with some relying mainly on aquatic-derived food sources, others relying on terrestrial-derived sources, and yet others feeding on a mixture of the two. Clear spatial differences in the diet characteristics of the anurans were detected, with limited dietary ranges in the upstream community, and much greater variety in species occurring downstream.

The indigenous Cape stumpnose *Rhabdosargus holubi* fed on different food sources in linked aquatic habitats of the Kowie system during its life cycle. We confirmed the migration of fish among the marine, estuarine and lower riverine areas from the differing fish size classes occurring in these habitats. Consumption of food resources originating from the different regions was clear from the shifts in stomach contents, stable isotope ratios and fatty acid profiles. Our results implied that *R. holubi* shows site fidelity and narrow philopatry at differences stages of its life cycle within the Kowie system. All three datasets indicated large variability in the diet of *R. holubi* as it developed and traveled along the salinity gradient, with invertebrate prey increasing with fish size from the river to the sea. By shifting its diet and migrating among adjacent habitats during its life cycle, *R. holubi* contributes to connectivity within the Kowie system, mainly through transport of biomass and energy among the marine, estuarine and riverine regions. In turn, juveniles are consumed by piscivorous fish and some birds in estuaries, and this biomass is further transported within and outside the system. Immigration of postlarvae from the ocean into the estuary and, later in the life cycle, the downstream migration of juveniles back to the ocean all contribute to longitudinal energy transfers. These are all important conduits for connecting neighboring partitions.

Indigenous fish formed an important part of the diet of the largemouth bass *Micropterus salmoides* in the lower Kowie River. The prevalence of indigenous fish in the bass diet is cause for concern, as several authors have illustrated potential threats posed by the presence of *M. salmoides* in other regions of the world.

The isotope models for the estuarine birds suggested that the Cape Shoveller, Cape Teal and Yellow-bill Duck populations were composed of generalist feeders, while Ruff and Little Egret were specialist feeders. Little Egret display an “opportunistic” specialist feeding regime, whereby the indigenous fish *Mugil cephalus* was the preferred food source. The degree of overlap of the isotopic niches of Cape Shoveller, Cape Teal and Yellow-bill Duck was high, suggesting that these species are in direct competition with one another for dietary resources during some times of the year. Micro-invertebrates were a prominent food source amongst these consumers, while the interspecific competition for micro-invertebrate resources increased with the arrival of Ruff. These dietary data provided insights into the removal of aquatic resources from the estuary by terrestrial vertebrates.

Bloukrans tributary

In the Bloukrans River (a large tributary feeding into the Kowie system), environmentally stressful conditions were identified by shifts in macroinvertebrate communities, with low diversity in the upstream areas due to water pollution inputs and more diverse communities at sites located downstream. The macroinvertebrate communities in the Bloukrans River were negatively impacted by organic nutrient pollutants from urbanization and sewage effluents. The mean environmental scores for the whole tributary were relatively low compared to the standard reference scores that indicate an undisturbed stream in South Africa. These findings indicated major deterioration in water quality of the stream and a need for management and conservation interventions to restore its intrinsic natural value.

Flux model

We have developed a multi-phase model, whereby complexity is added in a stepwise manner to produce a spatially explicit model of the Kowie River system with terrestrial/aquatic interactions, multiple trophic levels, and the integration of disparate food webs. This ambitious platform can serve as an invaluable management tool, and a theory-building initiative, whereby the fate of biologically conserved molecules can be traced through multiple food webs and locations. It is clear from the flux model results that further model refinements are necessary so that the empirical dietary data of some consumers more closely match what the model is calculating at each site. Small adjustments to the model parameters can have large influences on the general outcome.

Conclusions

- Trophic connectivity between freshwater and terrestrial systems, or freshwater and estuarine systems, is ecosystem- and area-specific, and a variety of environmental factors contribute to the variability among locations and through time.
- The size of the riparian buffer zone along a river varies depending on the organisms considered, the morphology of the river, and a variety of environmental factors.
- Spatial and temporal patterns that occur in the organic composition of the basal food sources do not necessarily transmit to the consumers in a system, so it is important to examine each trophic level.
- Primary consumers in the Kowie River system more closely matched the variability in organic matter composition (i.e. allochthonous vs. autochtonous contributions) than did higher consumers, particularly owing to the greater mobility and selectivity among higher consumers.
- Pollutants entering a freshwater system can readily influence the community composition and hence the food web structure, leading to shifts in connectivity between adjacent communities.
- The application of ecosystem modelling to questions of connectivity and organic matter inputs may help managers to view a river basin in a holistic way that may in turn enhance the preservation of natural and ecologically important connections among habitats.

ACKNOWLEDGEMENTS

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The project team acknowledge and appreciate the participation of the Reference Group members.

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LIST OF ABBREVIATIONS

ANOSIM	analysis of similarity
ANOVA	analysis of variance
ASPT	average score per taxon
BFA	bacterial fatty acid
C	carbon
Chl *a*	chorophyll a
DEA	Department of Environmental Affairs
DO	dissolved oxygen
E	estuarine
EFA	essential fatty acid
Epipelon	organisms living on the surface of sediments
Epiphyton	plants that grow upon other plants (not parasitic)
EPT	Ephemeroptera, Plecoptera and Tricoptera richness index
ESC	ecosystem synthesis concept
EwE	Ecopath with EcoSim
F	freshwater
FAME	fatty acid methyl ester
FL	fork length
FPC	flood pulse concept
FPOM	fine particulate organic matter
GC	gas chromatograph
GF/F	glass fibre filter
HPFA	higher plant fatty acid
HUFA	highly unsaturated fatty acid
IHI	index of habitat integrity
IRI	index of relative importance
N	nitrogen
nMDS	non-parametric multidimensional scaling
MS	mass spectrometry
MUFA	monounsaturated fatty acid
P/B	production/biomass
PCA	principal component analysis
Periphyton	complex mixture of algae, cyanobacteria, heterotrophic microbes and detritus growing on submerged surfaces
PERMANOVA	permutational analysis of variance

POM	particulate organic matter
PUFA	polyunsaturated fatty acid
Q/B	consumption/biomass
RCC	river continuum concept
RDA	redundancy analysis
RES	river ecosystem synthesis
RPM	river productivity model
SASS	South African scoring system
SD	standard deviation
SEA	standard ellipse area
SFA	saturated fatty acid
SIAR	stable isotope analysis in R
SIBER	stable isotope Bayesian ellipses in R
SIMPER	similarity percentages
SL	standard length
SOM	sediment organic matter
SPM	suspended particulate matter
TDS	total dissolved solids
TL	total length
TP	trophic position
YOY	young-of-the-year

1 INTRODUCTION

1.1 Personnel and projects

This report documents the work contained within several student theses and postdoctoral research, and the overall project represents a complex and collaborative network of students, postdoctoral researchers and supervisors.

Leandro Bergamino (PhD): Spatial variation in the trophic connectivity within an estuarine environment: benthic-pelagic and terrestrial-aquatic linkages via invertebrates
Tatenda Dalu (PhD): Plankton dynamics in a riverine/estuarine continuum
Sydney Moyo (PhD candidate): Trophic linkages in aquatic food webs: the role of insects
Lenin Chari (PhD candidate): Nutrient fluxes between water and aerial habitats: the roles of aerial predatory invertebrates in trophic cross-subsidisation
Jeff Hean (PhD candidate): Reciprocal aquatic/terrestrial trophic subsidies through aquatic birds in a South African hydrological catchment
Likho Sikutshwa (MSc): Trophic linkages in aquatic food webs: the role of amphibians
Mandla Magoro (MSc): Predation by alien largemouth bass, *Micropterus salmoides* Lacepéde 1802 (Centrarchidae, Perciformes), on indigenous marine fish species in the Kowie River
Simphiwe Gininda (MSc candidate): Effect of pollution on community assemblages of a small riverine system: A case of Bloukrans River, South Africa
Laure Carassou (Postdoctoral Researcher): Identification and quantification of the contributions of migratory fish to reciprocal transfers of organic nutrients between adjacent aquatic habitats (river/estuary/ocean) associated with a South African hydrological catchment
Supervisors: Nicole Richoux, Martin Villet, Alan Whitfield, Dan Parker, William Froneman, Adrian Craig
International collaborators: George Arhonditsis, Gurbir Perhar, Felicity Ni

2 PERMITS AND ETHICS

Collecting permits were obtained for the different organisms and sites targeted:

Marine collection permits RES2012/05, RES2013/51, RES2014/12
Insect collection permits CRO 14/12CR and CRO 15/12CR, CRO 16/13CR and CRO 17/13CR, CRO 12/14CR and CRO 13/14CR
Kowie River collection permit CRO 116/12CR

Freshwater fish and invertebrates collection permit CRO 3/12CR and CRO4/12CR
ECPTA permit for Waters Meeting collections from Sept 2012 to December 2014
Waterbird collection permits CRO 52/13CR and CRO 53/13CR

Ethics clearances were obtained from Rhodes University and/or SAIAB for the vertebrate research:

Waterbird ethics clearance ZOOL-09-2012
Amphibian research ethics clearance ZOOL-15-2012
Fish research ethics clearance ZOOL-02-2012 (Rhodes University) and #2012/04 (SAIAB)

3 SUMMARY OF OUTPUTS TO DATE

3.1 Journal articles

1. Richoux NB, Bergamino L, Dalu T, Moyo S (*in preparation*) Fatty acid profiles indicate changes in the nutritional quality of suspended and benthic basal resources along a temperate river-estuary continuum. *Hydrobiologia*
2. Carassou L, Whitfield AK, Bergamino L, Moyo S, Richoux NB (*submitted*) Trophic dynamics of the Cape stumpnose (*Rhabdosargus holubi*, Sparidae) across three different aquatic environments: insights from stomach content and stable isotope analyses. *Marine and Freshwater Research*
3. Dalu T, Richoux NB, Froneman PW (*submitted*) Assessing habitat connectivity using stable isotope ratios: contributions of allochthonous and autochthonous materials to suspended particulate matter and detritus along a river-estuarine continuum. *Hydrobiologia*
4. Dalu T, Galloway AWE, Richoux NB, Froneman PW (*submitted*) Effect of substrate type on phytobenthos essential fatty acid availability in an austral temperate river system. *Hydrobiologia*
5. Magoro ML, Whitfield AK, Carassou L (**2015**) Predation by introduced largemouth bass *Micropterus salmoides* on indigenous marine fish in the lower Kowie River, South Africa. *African Journal of Marine Science*, DOI: 10.2989/16085914.2014.983889
6. Bergamino L, Richoux NB (**2015**) Food preferences of the estuarine crab *Sesarma catenata* estimated through laboratory experiments. *Marine and Freshwater Research,* http://dx.doi.org/10.1071/MF14122

7. Bergamino L, Richoux NB (**2015**) Spatial and temporal changes in estuarine food web structure: differential contributions of marsh grass detritus. *Estuaries and Coasts* 38(1):367-382
8. Dalu T, Taylor JC, Richoux NB, Froneman PW (**2015**) A re-examination of the type material of *Entomoneis paludosa* (W. Smith) Reimer and its morphology and distribution in African waters. *Fottea* 15(1):11-25
9. Bergamino L, Dalu T, Richoux NB (**2014**) Spatial and temporal changes in sediment organic matter composition within an estuarine environment: stable isotope signatures and fatty acid profiles. *Hydrobiologia* 732:133-145
10. Bergamino L, Dalu T, Whitfield AK, Carassou L, Richoux NB (**2014**) Stable isotope evidence of habitat coupling by a predatory fish (*Argyrosomus japonicus*: Sciaenidae: Teleostei) in the Kowie Estuary, South Africa. *African Journal of Marine Science* 36(2):207-213
11. Dalu T, Richoux NB, Froneman PW (**2014**) Using multivariate analysis and stable isotopes to assess the effects of substrate type on phytobenthos communities. *Inland Waters* 4:397-412
12. Dalu T, Froneman PW, Richoux NB (**2014**) Phytoplankton diversity along a river-estuary continuum. *Transactions of the Royal Society of South Africa* 69(2):107-116
13. Dalu T, Froneman PW, Chari LD, Richoux NB (**2014**) Colonisation and community structure of benthic diatoms on artificial substrates following a major flood event: a case of the Kowie River (Eastern Cape, South Africa). *Water SA* 40(3):471-480

3.2 Conference presentations

1. Richoux NB, Bergamino L, Moyo S, Dalu T, Chari L, Carassou L, Villet MH (22-27 February **2015**) Connectivity through allochthony: reciprocal links between adjacent aquatic and terrestrial ecosystems in South Africa. Association for the Sciences of Limnology and Oceanography Aquatic Sciences Meeting, Granada, Spain
2. Carassou L, Whitfield, AK, Bergamino L, Moyo S, Richoux NB (24-26 February **2015**) Trophic dynamics of the Cape stumpnose (*Rhabdosargus holubi*: Sparidae) across a gradient of aquatic habitats: insights from stomach contents and stable isotopes. 2nd International Conference on Fisheries, Aquaculture and Environment in the Indian Ocean. Sultan Qaboos University, Muscat, Oman
3. Hean JW, Richoux NB, Craig AJFK (18-23 May **2014**) The role of wading birds in linking terrestrial and estuarine habitats. Joint Aquatic Sciences Meeting, Portland, Oregon

4. Hean JW, Richoux NB, Craig AJFK (13-16 March **2014**) The role of waterbirds in energy transfer between estuarine and terrestrial habitats. Birdlife South Africa, Learn About Birds Conference. Mont-aux-sources Hotel, Royal Natal Nature Reserve, KwaZulu-Natal, South Africa
5. Dalu T, Froneman PW, Moyo S, Richoux NB (2-6 December **2013**) Succession and community structure of benthic diatom assemblages on artificial substrates: A stable isotope approach. Biodiversity Southern Africa Conference. University of Cape Town, Cape Town, South Africa.
6. Gininda S, Dalu T, Richoux NB (2-6 December **2013**) Does benthic macroinvertebrate community structure and diversity reflect changes in water quality along a freshwater stream? Biodiversity Southern Africa Conference. University of Cape Town, Cape Town, South Africa.
7. Moyo S, Dalu T, Villet MH, Richoux NB (2-6 December **2013**) What does stable isotope analysis reveal about food webs and trophic relationships in temperate streams of the Eastern Cape, South Africa? Biodiversity Southern Africa Conference. University of Cape Town, Cape Town, South Africa.
8. Carassou L, Whitfield AK, Richoux NB (18-23 August **2013**) Contribution of the Cape stumpnose (*Rhabdosargus holubi*, Sparidae) to trophic connectivity between adjacent aquatic habitats in a South African estuarine system. 48th Annual European Marine Biology Symposium, Galway, Ireland.
9. Hean JW, Richoux NB, Craig AJFK (30 June – 4 July **2013**) Reciprocal aquatic/terrestrial trophic subsidies through aquatic birds in a South African hydrological catchment: project outline and preliminary findings. Southern African Society of Aquatic Scientists 3013 Conference. Arniston, Western Cape.
10. Sikutshwa L, Richoux NB, Parker DM (30 June – 4 July **2013**) The role of amphibians in linking terrestrial and aquatic habitats in the Kowie catchment. Southern African Society of Aquatic Scientists 3013 Conference. Arniston, Western Cape.

3.3 Completed student theses

Magoro ML (**2014**) Predation by alien largemouth bass, *Micropterus salmoides* Lacepéde 1802 (Centrarchidae: Perciformes), on indigenous marine fish species in the Kowie system, South Africa. MSc thesis, Rhodes University, Grahamstown.

Bergamino L (**2014**) Spatial and temporal variations in trophic connectivity within an estuarine environment: benthic-pelagic and terrestrial-aquatic linkages via invertebrates and fishes. PhD thesis, Rhodes University, Grahamstown

Dalu T (**2015**) Spatio-temporal variation in the phytobenthos and phytoplankton community structure and composition of particulate matter along a river-estuary continuum assessed using microscopic and stable isotope analyses. PhD thesis, Rhodes University, Grahamstown

Sikutshwa L (**2015**) The diets of co-occurring anurans in a small South African river: assessments using stomach contents, stable isotope ratios and fatty acid profiles. MSc thesis, Rhodes University, Grahamstown

4 INTRODUCTION AND OBJECTIVES

4.1 Connectivity of landscapes through food webs

Central to the issues of quality and availability of water is the question of whether organisms (including humans) are under threat due to pollution, food limitation, or over-harvesting in both fresh and salt water systems. An important aspect of the dynamics of nutrients and pollutants in such systems is captured in the concept of allochthony, founded on the observation that nutrients and energy in a variety of forms are transferred between adjacent habitats, communities and ecosystems that are not routinely considered as connected. The reality is that no natural habitat is truly closed and uninfluenced by adjacent (or even distant) regions. Even local communities that appear discrete are open and connected in a myriad of ways to outside influences, with connectivity in natural systems varying enormously from nearly isolated to strongly linked (Polis et al., 1997). Different forms of nutrients and energy move across the conceptual boundaries of habitats via organisms' activities or physical processes such as wind or water currents, and these transfers can represent important food subsidies (Polis et al., 1997; Baxter et al., 2005). Such cross-partition ecological subsidies can augment the nutritional condition, biomass and biodiversity of communities (Sanzone et al., 2003; Burdon and Harding, 2008), particularly where local production (or autochthony) alone may be inadequate to support local food webs. Furthermore, organic subsidies can influence population dynamics, community interactions and ecosystem processes (Lindeman, 1942; Carpenter et al., 2005; Marcarelli et al., 2011), and can represent dominant flux inputs in ecosystem budgets (Marcarelli et al., 2011).

Cross-ecosystem subsidies are complex, difficult to assess and hence generally poorly understood, even in clear ecotone (boundary) regions. However, the importance and implications of allochthony have been recognised through research into a variety of ecosystems and circumstances, and there is growing evidence of the significance of cross-boundary subsidies between aquatic (stream, river and lacustrine) and terrestrial (island or continental) environments (Carpenter et al., 2005; Shurin et al., 2006; Bartels et al., 2012).

For example, oceans are linked to inland waters through processes such as eel, mullet and salmon migrations and to islands and continental margins through predator activities and flotsam deposition, and freshwater systems are linked to land through a variety of passive and active transfers of plant, animal and human matter (Polis et al., 1997). Acknowledging the movement of nutrients and consumers within and among adjacent habitats is critical to our general understanding of food web dynamics, as the health and sustainability of aquatic food webs can be strongly influenced by these movements of nutrients, detritus and organisms across contiguous habitats (Polis et al., 1997; Nakano et al., 1999; Guest et al., 2004; Heck et al., 2008). Our intention was to explore organic nutrient fluxes in relation to a lotic (i.e. flowing) aquatic system, but at the scale of a hydrological catchment.

Connectivity in relation to lotic systems can occur in a variety of ways: longitudinally (e.g. headwater to estuary), laterally (e.g. between aquatic and riparian/terrestrial areas), and even vertically (e.g. through pelagic and benthic links; Wiens, 2002). Lateral connectivity can be mediated through organisms such as aquatic insects that emerge from rivers and subsidise terrestrial consumers (Ward, 1989), while longitudinal connectivity links upstream and downstream areas and typically is accomplished by the active migrations of organisms and the passive delivery of organic and inorganic materials downstream (Ward, 1989, 1998; Wiens 2002). Vertical connections can occur through shifts in organisms' habitat use during their life cycles, and through settlement/resuspension processes (Wassmann, 1990; Kemp and Boynton, 1994). In this project, we have addressed these different aspects of connectivity concurrently within one hydrological catchment. The overall project objectives were to identify and quantify the transfers of organic nutrients that connect different habitats within a model catchment region.

4.2 Freshwater and terrestrial invertebrates

The freshwater macroinvertebrate community is represented by a variety of organisms including planktonic crustaceans, benthic molluscs (e.g. mussels, snails) and aquatic insects (e.g. caddisflies, mayflies, dragonflies). Some of these taxa can play critical roles in providing ecosystem services. For example, detritivores can accelerate leaf litter breakdown through their feeding activities (Dudgeon and Gao, 2011), and some species can process up to 73% of the leaf litter that falls into the headwaters of a river system (Covich et al., 1999). The detritivores that feed exclusively on decaying leaf litter represent the functional feeding group *shredders* (e.g. crabs). Shredders are important producers of fine particulate organic matter (POM), a food source that is important for the *collectors* or *filter-collectors* (e.g. blackfly and caddisfly larvae; Ciborowski et al., 1997). Additional functional feeding groups of invertebrates include the *grazers* (e.g. snails, midges) and the *gatherer-*

collectors (e.g. shrimps). *Predators* include dragonfly and damselfly nymphs and giant water bugs, and these feed on other invertebrates, and the top predators in the aquatic community are usually represented by fish (Vannote et al., 1980; Allan, 1995). Food sources of the herbivorous invertebrates are either allochthonous – produced outside the river (e.g. terrestrial plants), or autochthonous – produced locally (e.g. phytoplankton and phytobenthos). Similarly, infalling terrestrial invertebrate material represents an allochthonous food source for predators, whereas aquatic invertebrates are autochthonous food. Food web studies in freshwater systems can be approached from a variety of scientific perspectives, and we approached our research questions by assessing the relevance of published conceptual models for the catchment system of interest.

Conceptual models for lotic systems

Conceptual models proposed to help explain the shifting biological and physical processes that occur along a river system include the River Continuum Concept (RCC; Vannote et al., 1980), the Flood Pulse Concept (FPC; Junk, 1984), the River Productivity Model (RPM; Thorp and Delong, 1994; 2002) and the Ecosystem Synthesis Concept (ECS; Thorp et al., 2006). The RCC assumes that a river is an open system characterized by important interactions along its banks related to the changes in the physics and chemistry of the river along its length (Vannote et al., 1980). As such, the nature and composition of food sources change along this longitudinal continuum, whereby headwater sections of the river are narrow, shaded and relatively cold, and because production of aquatic primary producers is assumed to be limited here the relative proportions of allochthonous material inputs into the food web are high. Further downstream, rivers widen and energy inputs change as sunlight reaches the benthos and supports significant autochthonous production (Vannote et al., 1980; Nilsson et al., 1999). The biological processing of coarse particulate organic matter (POM) arising from the headwater regions results in downstream transport of large amounts of fine POM (Vannote et al., 1980; Townsend et al., 1987; Nilsson et al., 1999). The mid regions of rivers are important transition areas, and the RCC predicts the greatest variety of organic matter inputs here where light is relatively high and the water is warmer, hydrologically calmer, and having finer POM compared with upstream regions (Vannote et al., 1980; Statzner and Higler, 1985). Many studies on temperate lotic food webs have provided general support for the predictions of the RCC (Rounick et al., 1982; Hicks, 1997; Greathouse and Pringle, 2006; Jiang et al., 2011). For example, King et al. (1988) found a dominance of fine POM in the stomachs of invertebrates inhabiting the downstream regions of a South African stream. Similarly, Chang et al. (2012) showed that the quantity and composition of POM in the Lanyang River were generally consistent with the predictions of the RCC in that the contributions of leaves to coarse POM decreased from the headwaters to mouth, whereas those of locally-produced periphyton and macrophytes increased (Chang et al., 2012).

The variable inputs of different sources of organic material along a lotic continuum can influence the food available to aquatic consumers. For example, Cole et al. (2011) found that the diet of zooplankton in a freshwater system was comprised of up to 40% terrestrial material. However, field and laboratory experiments have also provided evidence that contradicts the notion that terrestrial inputs into consumers can be relatively large, and that autochthonous food sources are more important owing to their greater nutritional and less refractory composition (Mantel et al., 2004; Brett et al., 2009; Lau et al., 2009). Most published studies suggest that the RCC overemphasises the role played by allochthonous food sources and may not adequately reflect the relative importance of autochthonous resources, especially periphyton and green algae, which have proved important to consumers in temperate streams (Bunn et al., 2003; Torres-Ruiz et al., 2007).

Food web studies in temperate streams often support the generalizations made by the RCC (Rounick et al., 1982, Hicks, 1997, Hall et al., 2000). The FPC, an alternate view to food web structure, promulgates the importance of lateral connectivity between main channel and floodplain habitats and predicts that inundation of the floodplain by a flood pulse is the major driver for mobilising terrestrial organic matter sources. The FPC suggests that the movement of this terrestrial organic matter fuels food webs in floodplain rivers (Junk et al., 1989). The RPM, a popular alternative to the RCC, emphasises the importance of both autochthonous production (phytoplankton, benthic algae and aquatic plants) and carbon inputs from riparian zones into food webs, particularly during flood pulses. However, the main prediction of the RPM is that secondary production in rivers having a constricted channel and firm substrates is fuelled primarily by phytoplankton and phytobenthos (Thorp and Delong, 1994). Some production originates in the main river channels, but the major sites of carbon fixation occur in shallower areas having slower currents, where materials are better retained (Thorp and Delong, 1994; 2002). In-stream primary production is predictably more important because the material is relatively labile and thus more readily assimilated by heterotrophs. Evidence is accumulating in the literature regarding the dominant role of autochthony in freshwater systems overall (e.g. Minshall, 1978; Minshall et al., 1983; McCutchan et al., 2003; McNeely et al., 2007). For example, non-shredding primary consumers derived up to 71% of their biomass from autochthonous food sources in a Hong Kong stream (Li and Dudgeon, 2008). Similarly, Minshall et al. (1992) found that benthic algae represented a substantial food source for organisms in large rivers with narrow channels (Salmon and Mackenzie Rivers). Lewis et al. (2001), Hein et al. (2003) and Delong and Thorp (2006) all demonstrated that autochthonous organic matter represented the major energy source supporting secondary production in different rivers. Autochthonous sources are evidently important to aquatic consumers in a variety of tropical streams (March and Pringle, 2003) and in subtropical (Bunn et al., 2003) and temperate lotic systems (Torres-Ruiz et al., 2007).

Although aquatic and terrestrial macrophytes represent potential food for primary consumers, they tend not to be grazed directly due to their low digestibility and high levels of cellulose and lignin (Allan and Castillo, 2007). As such, macrophytes are relatively unimportant trophically until they decay and enter the detrital pool (Allan, 1995; Hedges et al., 2000; Lewis et al., 2001). Essentially, this means that even if terrestrially-derived carbon constitutes the greatest portion of transported POM, autochthonous production can remain the dominant food source for consumers (Thorp and Delong, 1994). Despite some evidence of direct consumption of macrophytes (Lodge, 1991; Newman, 1991), studies utilising stable isotope techniques have also provided evidence that contributions from macrophytes, through either direct herbivory or detrital pathways, to higher trophic levels are limited (Hamilton et al., 1992; France, 1996; Lewis et al., 2001; Delong and Thorp, 2006). The debate is on-going regarding the origins and contributions of organic matter to aquatic food webs, so additional studies are needed to further explore the nature and variability of these processes in different systems.

Compared with the herbivorous trophic pathways, less research has been focussed on stream predators and the contributions of local versus allochthonous carbon to their diets. Inputs of terrestrial invertebrates to vertebrate stream consumers via fortuitous infall has been recognized (Cloe and Garman, 1996; Wipfli, 1997; Kawaguchi et al., 2003), and in temperate regions this infall varies with season (with peaks occurring in summer and troughs in winter; Kawaguchi and Nakano, 2001). When terrestrial invertebrate infall to a Japanese stream was reduced experimentally, fish biomass also declined, thus suggesting that the population dynamics of the fish were influenced by allochthonous inputs (Nakano et al., 1999b). Reduced terrestrial subsidies have also induced trophic cascades, forcing fish to shift from terrestrially-derived food to local aquatic prey, which can ultimately decrease aquatic insect emergence and cause detrimental effects on riparian predators such as spiders (Baxter et al., 2004). Thus, seasonal variations in terrestrial invertebrate inputs to streams may be an important driver of ecosystem processes, at least in temperate regions. Terrestrial insects constituted up to 84% of the diet of bleak (*Alburnus alburnus*) in a German lowland lake (Mehner et al., 2005), and 50% of the diet of rainbow trout (*Oncorhynchus mykiss*) in a Californian stream (Rundio and Lindley, 2008). Terrestrial insect inputs into lotic systems can reach 8.7 g m^{-2} year^{-1} in forested streams (Kawaguchi and Nakano, 2001; Rundio and Lindley, 2008) and 5.1 g m^{-2} year^{-1} in grassland reaches of streams (Kawaguchi and Nakano, 2001). Apart from these studies, there are relatively few quantitative accounts of invertebrate subsidies to and from rivers, and there is little understanding of how such fluxes differ regionally around the world. There is particularly limited information on the predation by aquatic invertebrates such as dragonflies and giant water bugs on terrestrial invertebrates (Baxter et al., 2005; Ohba and Nakasuji, 2006).

We aimed to assess whether the riverine concepts [namely the RCC, FPC and RPM] developed for large temperate rivers would hold true for a small temperate river in the southern hemisphere. Based on the predominance of published literature showing the dependence of aquatic consumers on autochthonous carbon, we hypothesised that the carbon fuelling consumers is primarily derived from autochthonous food sources. Furthermore, we hypothesised that the contributions of autochthonous basal resources decreases in importance as the stream widens (from headwaters to downstream reaches), in line with the decreasing contributions of autochthonous materials to SPM in the Kowie River (see **Section 7.2**). To address these questions we (i) used stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to differentiate organic matter sources, (ii) determined the spatial and temporal differences in stable isotope values of basal resources and consumers along the longitudinal gradient, and (iii) estimated the contributions of organic matter sources to the primary and secondary consumers over space and time.

Emergent insects

Emerging insects can represent an important transfer of organic matter from water to land, and they can constitute an important dietary component for a wide variety of terrestrial consumers (Baxter et al., 2005). Aquatic insects have complex life histories, and they typically spend their immature stages submerged in freshwater. The insects undergo metamorphosis and emerge to terrestrial habitats as winged adults (Bland, 1978). The emergence of insects represents the final component of insect production, and a potential net export of material from an aquatic system (Davies, 1984). Several studies have documented the timing and rate of emergence of adult aquatic insects (e.g. Poepperl, 2000), and the dry mass flux has been estimated at ~4.3 g m^{-2} $year^{-1}$ on a global basis (Baxter et al., 2005; Gladyshev et al., 2009). Insects can thus act as a conduit of energy flow between aquatic and terrestrial habitats (Richardson et al., 2010). On emergence they may influence food webs as prey or consumers, since emergent dragonflies feed on insects (Knight et al., 2005) and are in turn consumed by birds (Nakano and Murakami, 2001). More information is available regarding emergent insects as prey subsidies than as predators.

Adult aquatic insects constitute an important dietary item for a host of consumer types such as birds (Gray, 1993; Nakano and Murakami, 2001; Yard et al., 2004), bats (Collier, 1991; Shiel et al., 1998), lizards (Sabo and Power, 2002a, 2002b), insects (Hering and Plachter, 1997) and spiders (Henschel et al., 2001; Collier et al., 2002; Kato et al., 2003; Akamatsu et al., 2005, 2007; Paetzold and Tockner, 2005; Burdon and Harding, 2008).

Paetzold (2004) reported that emerging aquatic insects along rivers in Switzerland and Italy constituted ~80% of a rove beetle's diet, and ~50% of a lycosid spider's diet. In a study of 26 riparian deciduous forest plots in northern Japan, Iwata et al. (2003) showed that an increase in the number of emerging insects coincided with increased abundance of insectivorous birds in the riparian zones, with aquatic insects constituting up to 82% of the diets of many of the birds. A study on spider communities along a Japanese stream showed a positive correlation between aquatic insect emergence and the density of tetragnathid spiders (Iwata, 2007). The evidence from these studies indicated that insects are important in coupling aquatic and terrestrial systems. The flux of energy (through insect emergence) to the terrestrial environment is likely significant, as only small percentages (e.g. ~1 to 3%) of emergent insects return to the water in the form of carcasses and either sink and decompose, or become consumed (Jackson and Fisher, 1986; Gray, 1989). Adults that fail to return to the water represent a net loss of organic nutrients from the aquatic system and potential food for consumers on land. Gratton et al. (2008) quantified midge infall into terrestrial habitats adjacent to Icelandic lakes at 1200 to 1500 kg midges ha^{-1} year^{-1}. Rates of midge infall to land varied among lakes, declined with distance from the lakeshore, and in some lakes were large enough to have a potential fertilizing effect on terrestrial plants. An important aspect of our project was to determine the role of emergent insects in connecting aquatic and terrestrial habitats.

Riparian zones and aerial invertebrates

Riparian habitats represent the buffer zones between rivers and land and can be defined as the areas of direct interaction between aquatic and terrestrial habitats. Riparian habitats may carry high densities of consumers, as the habitat complexity and resource exchange between aquatic and terrestrial zones can provide ideal growth conditions for animals (Naiman et al., 1993, Polis and Hurd, 1995, Naiman and Decamps, 1997, Fagan et al., 1999, Nakano and Murakami, 2001, Power, 2001, Sabo and Power, 2002b). Defining the extent of riparian zones is therefore important for both ecological and managerial reasons.

The role of the riparian zone as a buffer for nutrient transfer can be evaluated in a number of ways, the first by recognising that insect emergence and dispersal may vary predictably with increasing stream size and geomorphology. Gratton and Vander Zanden (2009) proposed that insect flux across the land-water ecotone increases with stream size, and that lateral fluxes of aquatic insects into terrestrial systems follows a negative exponential decline with distance from the water's edge. As such, emerging insect subsidies are likely spatially constrained and vary with distance from the aquatic systems (Petersen et al., 1999; Lynch et al., 2002; Gratton et al., 2008). Iwata et al. (2003) showed that birds concentrated their foraging close to exposed sections of meandering streams to take advantage of the open habitat for foraging on emerging insects. Researchers studying a temperate stream in the United Kingdom reported that most aquatic insects remained within 20m of the banks, as revealed through examination of lycosid spider diets (Briers et al., 2005). Spiders close to the stream derived ~40% of their diets from aquatic insects, whereas those living 20m from the stream derived less than 1% of their food from aquatic resources (Briers et al., 2005). In contrast, Power and Rainey (2000) reported that individuals of a sheet-weaving spider (Linyphiidae) derived at least 50% of their carbon from emergent insects, even when located hundreds of meters from a northern California river. The lateral dispersal of adult aquatic insects from streams certainly affects the spatial extent (i.e. the size of the buffer zone) of water-to-land energy subsidies. To assess this aspect of aquatic-terrestrial connectivity, we have evaluated the diets of web-building spiders along a spatial transect away from the freshwater source.

It is generally difficult to determine with much certainty the exact prey consumed by aerial predators such as spiders. For web-building spiders, the term “putative prey” is often used in reference to all flying insects. However, web-building spiders select and consume only a subset of the insects trapped in their webs (Castillo and Eberhard, 1983), and both web type and foraging strategy affect prey selection (Craig, 1989). As a result, many researchers studying predator-prey interactions have assumed that predators are generalists that prey on most insect species. Web-building spiders offer an attractive model for monitoring organic nutrient fluxes from aquatic to terrestrial habitats, as the webs serve as effective aerial sampling devices (although not unbiased) that have direct biological relevance. Most spiders are biased generalist predators that are abundant in terrestrial ecosystems (Nentwig, 1986; Nyffeler, 1999; Snyder and Wise, 2001), and spiders are important controlling agents for insect communities through their predatory behaviour (Young and Edwards, 1990). The importance of emerging insects to the diets of some web-building spiders has been evaluated in a few areas, but studies have mainly been confined to temperate regions (e.g. Kato et al., 2003, 2004; Iwata, 2007) and recently in areas with Mediterranean climates (e.g. Paetzold et al., 2005; Rundio and Lindley, 2012). We have tested whether spiders located nearest to the river rely more heavily on emergent aquatic insects as a food source compared with spiders distant from the river.

4.3 Invertebrates in estuarine systems

Estuaries represent important transitional zones between marine and terrestrial habitats, where food webs are influenced by a variety of organic matter sources (Deegan and Garritt, 1997). Spatial gradients in salinity and associated environmental variables (e.g. nutrients, particulates) structure the local estuarine communities. As contributions of different organic matter sources shift longitudinally, the trophic structure can also change along these environmental gradients. Phytoplankton, marsh plants, and benthic microalgae are primary producers potentially supporting most of the secondary production in estuaries (Kwak and Zedler, 1997; Stribling and Cornwell, 1997; Connolly et al., 2005; Martinetto et al., 2006; Richoux and Froneman, 2007). The predominant evidence indicates that consumers use food sources produced in the same region of the estuary in which they reside, potentially within a few tens of meters or less (Guest et al., 2004; Guest and Connolly, 2004, 2006), whereas the assimilation of terrestrially derived material can be minimal (Deegan and Garritt, 1997). However, most of this evidence arises from studies of microtidal environments with minimal freshwater input, so the transport of detritus was likely small in these cases. Relatively little attention has been focused on trophic connectivity between estuary and river regions, or estuary and land (but see Guest et al., 2004; Guest and Connolly, 2006). The diverse nature of the organic matter sources available to estuarine organisms make it difficult to assess the connectivity between estuaries and adjacent habitats, so the estuarine portion of our project was aimed at obtaining insight into the trophic functioning of a South African estuary, and clarifying the roles of the estuarine invertebrates in habitat connectivity.

Studies in South African estuaries have indicated that benthic invertebrates, zooplankton and fish can assimilate a variety of potential carbon sources including microphytobenthos, the epibionts associated with seagrasses, bacteria associated with the sediments, and salt marsh or terrestrial plants (Whitfield, 1989; Froneman, 2001a; Paterson and Whitfield, 1997; Richoux and Froneman, 2007). Mud prawns, shrimps, nereid polychaetes and brachyuran crabs utilise detritus derived from a combination of seagrasses and cordgrasses (Richoux and Froneman, 2007). Paterson and Whitfield (1997) studied a freshwater-deprived estuary, and their data suggested that marsh grass, seagrass, and epiphytes supported the littoral community of crustaceans, gobies, mullet and sparids, whereas the channel community of zooplankton and fish assimilated a mixture of phytoplankton, terrestrial plant material and C_4 macrophyte detritus. In some South African estuaries, phytoplankton production is minor (particularly the large cells that invertebrates can feed on) compared to that of the macrophytes, and therefore phytoplankton does not represent an important food source for most of the consumers (Whitfield, 1989; Perissinotto et al., 2003; Richoux and Froneman, 2007). Salt marsh vegetation is rarely considered a major food source, although it may represent locally important carbon sources for some invertebrates (Froneman, 2001a; Richoux and Froneman, 2007). Our project was focussed on determining the differences in the diets of benthic and pelagic invertebrates along a spatial estuarine gradient.

4.4 Amphibians as habitat connectors

Most anurans are generalist predators that exhibit opportunistic foraging behaviours (Santos et al., 2004). Adults are predators of invertebrates such as annelids, centipedes, millipedes, arachnids and a variety of insects (Toft, 1981; Duellman, 1994; Santos et al., 2004), while juveniles are detritivores and/or herbivores (Altig et al., 2007). Some of the larger adults can feed on small vertebrates including fish, birds, rodents, and amphibians (Duellman, 1994). Despite their label of 'generalist predators', some anuran species can show degrees of diet specialization (Simon and Toft, 1991). For example, some dendrobatids and bufonids are myrmecophagous (specialized for eating ants), and they consume ants in higher proportions than their availability (Toft, 1980, 1981).

The complex life cycles and habitat diversity of amphibians make them unique among the tetrapods. For example, anurans are significant consumers in both terrestrial and aquatic habitats (Najera-Hillman et al., 2009), and their life history cycles include dramatic ontogenetic changes in morphology, physiology and behaviour (Enriquez-Urzelai et al., 2013). Because they shift habitats and foraging behaviours through their development, amphibians can represent important avenues for connecting terrestrial and aquatic systems (Regester et al., 2005; Whiles et al., 2006). Energy from the terrestrial habitats are transferred to the water via eggs and embryos, whereas late stage tadpoles transfer aquatic energy to terrestrial systems when they metamorphose into adults (Trakimas et al., 2011). A valuation of the trophic relationships and ecological roles of this group is necessary to enhance our understanding of the transfer of energy between habitats (Houlahan et al., 2000; Trakimas et al., 2011).

Of the diverse consumers inhabiting freshwater systems, anurans are one of the least understood groups in terms of their trophic ecology (Petranka and Kennedy, 1999; Altig et al., 2007). Anuran diets are usually confirmed by direct observations or through stomach content and faecal analyses (Dalerum and Angerbjörn, 2005). França et al. (2004) examined trophic and spatial niches of two large species of *Leptodactylus* in Southeastern Brazil and identified a diverse range of prey consumed by these two species including aquatic and terrestrial prey.

In the last decade we have seen an increasing number of molecular-based studies on trophic ecology of various amphibian (Kupfer et al., 2006; Verburg et al., 2007; Araújo et al., 2009; Barnum et al., 2013). For example, Verburg et al. (2007) used nitrogen and carbon stable isotopic signatures to identify tadpoles and adult amphibians as intermediate links in the aquatic and terrestrial food webs, respectively. In a study using both carbon and nitrogen isotopes, one of New Zealand's most widespread native frogs (*Leiopelma hochstetteri*) was placed at an intermediate trophic level amongst predators (including *Anguilla australis*, *Rattus rattus* and *Galaxias fasciatus*), as the diet of adult *L. hochstetteri* was heavily influenced by terrestrial invertebrates (Najera-Hillman et al., 2009). Furthermore, a stable isotope study by Trakimas et al. (2011) revealed that terrestrial carbon was present in the eggs and embryos of amphibians, thereby indicating a terrestrial subsidy to an aquatic system, and they also discovered that algal carbon was probably responsible for tadpole growth preceding metamorphosis to adults. Such results point out the dynamic nature of resource transfers at the terrestrial-aquatic boundary and the general significance of fluxes across ecosystem boundaries as facilitated by amphibians (Trakimas et al., 2011).

In consideration of the biodiversity crisis involving amphibians (Connelly et al., 2008), the necessity for quantitative information on trophic interactions and the ecological roles of these consumers is growing. The large losses in biodiversity that continue to occur may have negative effects on important processes in freshwater habitats, such as reducing energy transfers between and within habitats (e.g. Whiles et al., 2006; Verburg et al., 2007). The primary aim of this aspect of the study was to determine the role of amphibians in connecting aquatic and terrestrial habitats. To achieve this, we examined the spatial and temporal variability in the diets of different life stages (adults and tadpoles) of anurans.

4.5 Fish as vectors of connectivity

Fish are a prevalent component of all aquatic habitats, including freshwaters, estuaries and coastal marine areas. Fish represent the largest group of vertebrates on Earth, and as such they contain species representing a myriad of reproductive strategies and ecological roles. Most fish species have complex life cycles, during which shifting life stages occur within different habitats (Whitfield, 1998; Miller and Kendall, 2009). For example, diadromous fish species migrate between freshwater and marine habitats for reproductive purposes (Potts and Wootton, 1984). Anadromous fish born in freshwater spend most of their life at sea and return to freshwater to spawn (e.g., salmon, smelt, shad, striped bass and sturgeon). Catadromous fish do the opposite; they live in freshwater and enter salt waters to spawn (e.g., eels). Many marine species also utilize estuaries as nurseries, with different species having variable levels of dependency on this habitat type (estuarine-dependent and estuarine-opportunistic species; Able, 2005). Other fish species reside in a single habitat throughout their entire life cycles (i.e., freshwater, estuarine or marine residents). Resident freshwater species can, however, occur in estuaries during periods of high river flow, and inversely, some resident estuarine species are found in both estuaries and adjacent rivers during high tides and/or periods of low river flow (Whitfield, 1998; Elsdon and Gillanders, 2005). Fish therefore can represent a major vector of connectivity between adjacent aquatic habitats, either by transferring biomass from one habitat to the other through their direct movements, or by consuming food resources from one habitat before migrating, temporarily or permanently, to another (Deegan, 1993; Gillanders et al., 2003; Peterson, 2003; Ray, 2005; Secor and Rooker, 2005; Platell and Freewater, 2009).

Environmental conditions (i.e., climate, river flow, tides, habitat alteration) have a strong influence on the connections described above (Bunn and Arthington, 2002; Able, 2005; Hodgson et al., 2006; Sheaves and Johnston, 2008; Vinagre et al., 2011). For example, periods of low river flow may hinder fish migrations by causing the emergence of sand bars and terrestrial vegetation (Berggren and Filardo, 1993; Erkinaro et al., 1999; Hodgson et al., 2006; Sheppard et al., 2012). Similarly, the construction of dams and weirs, or the introduction of alien predatory species, both resulting from human-related activities, can interrupt fish migration routes and cause the isolation of habitats or populations that are normally connected (Bentley and Raymond, 1976; Raymond, 1979; Griffiths et al., 1992; Drinkwater and Frank, 1994; Gehrke et al., 1995; Nyström et al., 2001; Latini and Petrere, 2004; Propst et al., 2008). We have explored the connectivity of adjacent aquatic habitats in one hydrological catchment by assessing the diets of several fish species.

Approaches to investigate connectivity via fish

A variety of methods are available to address the extent to which fish contribute towards linking adjacent habitats. Analyses of changes in fish abundance and size across multiple habitats has provided some understanding of how fish are spatially distributed throughout their life cycles, and how environmental factors or human activities interfere with these spatial patterns (Deegan, 1990; Gillanders, 1997; Henriques and Almada, 1998). However, such observational studies generally require long-term monitoring of fish populations in a particular system, and as such are costly and time-consuming. Another common method of estimating how fish connect habitats is to directly track their movements using either external or internal artificial tags (Ennevor and Beames, 1993; Morton et al., 1993; Dewey and Zigler, 1996; Jones et al., 1999; Courtney et al., 2000), or acoustic telemetry (Cowley and Whitfield, 2001a; Kerwath and Götz, 2005; Chateau and Wantiez, 2008; Bishop et al., 2010; Reynolds et al., 2010). Certainly these studies have increased our knowledge of animal migrations, although the expenses involved in utilising these technologies can be prohibitive, and the consequences of fish migrations to the food webs of the source and sink habitats typically remain unknown.

Examination of stomach contents can be a useful approach in beginning to explore fish contributions to habitat connectivity, as identifiable contents provide information about food items that have recently been consumed (potentially in a source habitat prior to migration; Luz-Agosthino et al., 2008; Clark et al., 2009; Pitt et al., 2009; Buchheister and Latour 2010). Identification and enumeration of parasites has also aided researchers in identifying recent habitats of some fish species (Olson and Pratt, 1973; Williams et al., 1992; MacKenzie and Abaunza, 1998). Additional indirect methods of estimating connectivity through fish movements involve the use of natural compounds derived from the environment that are incorporated into growing fish tissues through their feeding and metabolic processes. The presence and relative quantities of certain natural compounds in fish can thus reflect their recent environment, and can be used to trace fish habitat use through time. Natural tags used in fish studies have included trace elements in calcified tissues such as otoliths (Gillanders and Kinsgford, 1996; Campana, 1999; Yamashita et al., 2000), stable isotope ratios in soft tissues (Fry et al., 1999; Kennedy et al., 2000; Weber et al., 2002; Veefkind, 2003; Herzka, 2005; McMahon et al., 2011), and fatty acid composition of soft tissues (Suthers et al., 1992; DeSilva et al., 1998; Iverson et al., 2002; Veefkind, 2003; Gillanders, 2005; Turner and Rooker, 2005a and 2005b; Alfaro et al., 2006; Rooker and Turner, 2006; Sakdullah and Tsuchiya, 2009; Elsdon, 2010; Costalago et al., 2011). Each method has inherent advantages and disadvantages, and researchers adopting a combination approach have been successful in creating a more comprehensive understanding of the processes underlying, and consequences of, fish movements as avenues of connectivity (Rieley et al., 1999; Cocheret de la Morinière et al., 2003; Gillanders et al., 2003, Johnson and Hesslein, 2004; Alfaro et al., 2006; Rooker and Turner, 2006; Bertrand et al., 2011; Buchheister and Latour, 2011).

Our study incorporates a combination of complementary methods of stomach contents and stable isotope ratio analyses to address how fish movements contribute to the transfer of organic nutrients across a continuum of freshwater, estuarine and coastal marine habitats. An additional aspect was explored involving the effects of a potential barrier to fish migration (largemount bass – an alien predatory species) on this connectivity.

Largemouth bass as a potential barrier to connectivity

The largemouth bass *Micropterus salmoides* Lacepéde 1802 (Centrarchidae, Perciformes) was introduced into South Africa in 1928 for the purpose of recreational angling (DeMoor and Bruton, 1988). It is a facultative piscivore (Weyl and Hecht, 1999) having a preference for clear lentic or calm lotic regions with floating and submerged vegetation (De Moor and Bruton, 1988; Skelton, 2001). The largemouth bass is indigenous to North America, but authorised and unauthorised introductions have expanded its distribution to such an extent that it now inhabits freshwater bodies in parts of Europe, southern Africa, South America, Asia and numerous islands (Jackson, 2002; Froese and Pauly, 2012).

Largemouth bass can vary its diet depending on the availability of prey, and it exhibits cannibalism when levels of inter- and intra-specific competition are high (Weyl and Hecht, 1999). The largemouth bass shifts its diet through development, with small juveniles typically feeding on small crustaceans, larger juveniles on insects, and adults on fish (Weyl and Hecht, 1999; Schumann et al., 2001; Post, 2003; Wasserman et al., 2011). Once largemouth bass is introduced into a new environment, individuals can consume a variety of indigenous fish (Weyl and Hecht, 1999). Largemouth introductions can therefore pose a serious threat to biodiversity, particularly in areas having threatened endemic species, and possibly leading to severe impacts on ecosystem functioning (Gozlan et al., 2010). In some locations, *M. salmoides* has established so well it became the most abundant predator, and subsequently the focal point for subsistence fisheries (Weyl and Hecht, 1999). Since the dietary habits of the bass can vary among aquatic systems (Anderson, 1984; Weyl and Hecht, 1999; Weyl and Lewis, 2006; Wasserman et al., 2011), the nature of the impacts of this species on indigenous fish is important to investigate.

The main avenue of exploration that is particularly relevant to our larger project is the question of whether *M. salmoides*, through its feeding on indigenous migratory fish, represents a significant ecological barrier to connectivity in our model hydrological catchment. More precisely, the main objective of this particular component is to determine whether *M. salmoides* preys on indigenous juvenile fish migrating into the freshwater section of a South African river. To investigate this question, we have determined the diet composition of the bass, and hence the net transfer of organic nutrients to this alien species.

4.6 Birds as connectors of aquatic/terrestrial habitats

Conventional models of aquatic food webs assume that fish represent the most abundant organisms at the apex trophic level (Vannote et al., 1980; Fry, 1991; Wellborn et al., 1996). However, terrestrial predators such as birds feed in aquatic systems, and therefore can represent important additional components of upper trophic levels, and hence significant drivers of food web dynamics (Steinmetz et al., 2003; Žydelis and Kontautas, 2008). South African rivers and estuaries provide refuge to several hundred bird species that fill a myriad of ecological niches (Day, 1981). Avifauna feed on a large variety of food items including aquatic plants, algae, molluscs, crustaceans, polychaetes, annelids, amphibians and fish. Water birds play pivotal roles in nutrient cycling, maintenance of prey populations, and even the dispersal of seeds (Day, 1981). Fresh- and saltwater-based avifauna are terrestrial-based consumers that utilise food sources from adjacent habitats. As marine prey consumption by seabirds rivals that of global fisheries landings (Brooke, 2004), most research has been directed towards seabird trophic ecology (Barrett et al., 2007). However, we know much less about feeding relationships of avifauna in estuaries and rivers.

Predation by avifauna can result in substantial export of benthic macrofaunal biomass from aquatic systems (Baird et al., 1985; Moreira, 1997; Baird, 2011). The net energy consumption of invertebrates by shorebirds in the Riet River (including the estuary) in South Africa was estimated at 52% of the total energy consumed within the system (Kalejta-Summers et al., 2001). Similarly, wading birds consumed more than 26% of the net annual invertebrate production in the Berg River estuary, South Africa (Kalejta, 1992). Several researchers have demonstrated significant connections between insect emergence and avifaunal dynamics (Gray, 1993; Murakami and Nakano, 2002; Iwata et al., 2003; Uesugi and Murakami, 2007; Macdade et al., 2011), but efforts are needed to further examine questions about trophic connectivity. Our project has focussed on the feeding dynamics of wading birds in a South African estuary, with an aim towards defining the temporal changes in the diets of local and migratory birds.

4.7 Bloukrans tributary

Freshwater macroinvertebrates include organisms such as insect larvae, annelids (leeches), oligochaetes (worms), crustaceans (crayfish and shrimp), molluscs (clams and mussels), and gastropods (snails). Some of these organisms (i.e. insect larvae) spend their larval or nymph stages in water attached to submersed rocks, vegetation or logs, and then move to terrestrial environments as adults (Cummins, 1974; Reynoldson et al., 1997), and others remain part of the benthic community throughout their life cycles. Benthic macroinvertebrates are used in the biomonitoring of rivers because they integrate the effects of, and respond relatively quickly to, environmental changes and they are a diverse group that reacts strongly and predictably to human influences (Quinn and Hickey, 1990). Water quality of lakes and rivers has been studied extensively in certain parts of the world (e.g. Mesa, 2010; Roque et al., 2010), and studies in Southern Africa are relatively rare. This aspect of the greater project was designed to provide some insight into the distribution and abundances of macroinvertebrate communities in a polluted stream that enters the main river channel of interest for the greater project. We hypothesised that the invertebrate community structure changes along the longitudinal gradient of the stream, with the cleaner regions having greater diversity and overall health.

4.8 General approach

We have assessed the flux of organic nutrients in a freshwater/estuary/terrestrial region represented by a hydrological catchment primarily using stable isotope ratios, and augmented with stomach contents and/or fatty acid analysis in certain cases. Wherever possible, the tracer techniques have been used concurrently to cross-validate diet composition of consumers in the habitats of interest (river, riparian zone, land, estuary).

Traditional stomach content analysis represents a reasonable first step in determining consumer diets, as identifiable contents provide information about food selected by consumers (Pitt et al., 2009), although this is an invasive and destructive technique for studying most consumers. Analyses of pellets or faeces are not destructive, but they suffer from inherent biases involving differential digestion rates among prey items (Votier et al., 2003; Barrett et al., 2007), and all conventional methods provide dietary information about recent feeding only. Alternatively, stable isotope ratios and fatty acid profiles both provide details on the sources of nutrition assimilated (rather than merely ingested) over longer periods (Michener and Schell, 1994; Dalsgaard et al., 2003), although they are affected by biases related to metabolic processes in consumers and natural variation in or similarities among dietary sources. Field observations can provide additional information about the diets of larger consumers, although visual methods are typically time-consuming, logistically difficult, and biased for a variety of logistical reasons (Barrett et al., 2007). Fortunately, the limitations of one technique can be ameliorated through the incorporation of a combination of independent techniques.

Fatty acids as tracers

Fatty acids are components of some lipids, and they occupy important roles in membrane structure and energy storage (Budge and Parrish, 1998). The potential for using lipids in trophic ecology lies in their heterogeneous nature, which allows information to be garnered by tracing the paths of individual and groups of fatty acids through food webs (Budge and Parrish, 1998; Parrish et al., 2000; Hudson et al., 2001). Detailed lipid analysis can be particularly useful for studying the feeding ecology of organisms that are omnivorous and/or logistically difficult to study.

Much of the trophic research on fatty acid composition of invertebrates has been focused on marine systems (Parrish et al., 2000). Freshwater and estuarine systems are not as well studied, although organisms in these habitats contain appreciable quantities of essential fatty acids (EFAs) having a similar composition to marine organisms (Ackman, 1999). However, over the past two decades, lipid research in freshwater and estuarine systems has seen increasing attention. Aquatic systems generally yield greater amounts of the EFAs that tend to be limiting in the terrestrial environment (Gladyshev et al., 2009). EFAs are manufactured in variable proportions by different primary producers but very few consumers, so heterotrophs must obtain these largely from their food (Brett and Müller-Navarra, 1997). As a result, the fatty acid compositions of most consumers reflect those of their food sources, therefore allowing the paths of organic food to be traced through food webs. Because the most physiologically important fatty acids for animals are relatively scarce in terrestrial ecosystems, aquatic systems may serve a critical role in supplying them to terrestrial systems (Gladyshev et al., 2009). Fatty acid analysis therefore represents an ecologically-relevant method that can be used to investigate the subsidies via organic materials and consumers to and from the various habitats of interest in our model hydrological catchment.

Marine, estuarine and freshwater phytoplankton and phytobenthos are generally rich in polyunsaturated fatty acids (PUFAs), some of which are the EFAs that are exceedingly important for the structure, function and development of all animals (Arts et al., 2001). The fatty acid compositions, also known as profiles or signatures, of phytoplankton and phytobenthos are complex and more variable than those of vascular plants (Goecke et al., 2010). The typical fatty acid profiles of different producers can be characteristic of a particular class, family, genus or (rarely) species, although the use of many biomarkers is complicated by the variability in profiles occurring even within genera (Hofmann and Eichenberger, 1997; Khotimchenko et al., 2002; Kelly and Scheibling, 2012; Piepho et al., 2012). Macroalgae, vascular plants and bacteria are also potential food sources for consumers, and these have characteristic fatty acid compositions that can be used to identify their contributions to higher trophic levels (Kelly and Scheibling, 2012).

Hanson et al. (1985) compared the lipid contents in aquatic insects with those of terrestrial invertebrates in various streams of the United States. They found that aquatic insects had greater levels of PUFAs (particularly arachidonic and eicosapentaenoic acids) than terrestrial insects. Other studies on lipids in aquatic systems generally support the idea that aquatic insects are rich in PUFAs compared with terrestrial insects (Hanson et al., 1985; Bell et al., 1994; Ghioni et al., 1996; Torres-Ruiz et al., 2007; Torres-Ruiz et al., 2010). Aquatic insects lay their eggs in rivers, where the larvae then develop and accumulate PUFAs (Gladyshev et al., 2009), and because of their complex life cycles, aquatic insects effect transfers of PUFAs to the terrestrial system when they emerge and fall prey to terrestrial predators (Bland, 1978; Burdon and Harding, 2008). Preliminary estimates of PUFA export to the terrestrial landscape via insect emergence have a large range from 0.1 kg km^{-2} year^{-1} to 672 kg km^{-2} year^{-1} (Gladyshev et al., 2011), although these estimates were based on coarse averages of data obtained from a large diversity of ecosystems. Moreover, PUFA concentrations in aquatic insects have been measured in larvae, whereas data on adults are virtually absent (but see Gladyshev et al., 2011). Insects may thus play a major role in the translocation of PUFAs from aquatic to terrestrial systems. Larger consumers such as water birds have been identified as key pathways of export of highly unsaturated fatty acids (HUFA), and initial global export rates of 19 to 167 kg HUFA km^{-1} year^{-1} via birds have recently been estimated (Gladyshev et al., 2009).

Fatty acid profiles within organic materials are dynamic in nature. In a Swedish lake, Goedkoop et al. (2000) used fatty acids to determine the food sources of chironomids and midges, and how their diets varied through time. Other authors have similarly utilised the fatty acid trophic marker approach to infer the seasonal shifts in importance of autochthonous and allochthonous food sources to macroinvertebrates. In their study of fatty acids in a stream in New York, Torres-Ruiz et al. (2007) determined the primary diets of mayfly and caddisfly larvae, and that these diets shifted seasonally. As such, temporally-constrained studies may provide limited information about stream food webs that are dynamic in space and time (Torres-Ruiz et al., 2007). Tropical and sub-tropical systems may fluctuate seasonally based on water availability rather than temperature changes, and we expected that any seasonality in our model catchment would reflect these types of environmental changes.

Diet studies of organisms at higher trophic levels (e.g. fish and birds) have additional challenges to deal with. Fatty acids have been used in a variety of studies to assess spatial and temporal shifts in the diets of avifauna (Käkelä et al., 2007; Wang et al., 2009), or to assess dietary differences among or within species (Richoux et al., 2010). Several physiological processes affect the utility of fatty acid analyses for assessing bird diets, including assimilation, deposition and metabolism (Williams and Buck, 2010). It is most beneficial if researchers can determine the influences of these different processes on the changes in fatty acid composition from food to consumer through captive feeding trials (Käkelä et al., 2009), but these are not often logistically possible. Furthermore, different tissues such as adipose tissue, blood, or egg, contain dietary information derived by birds over different time scales ranging from days to months (Barrett et al., 2007), and knowledge about these variations remains sparse for most species.

Overall, fatty acids can be effectively used to resolve feeding relationships and nutritional condition of organisms, although the data obtained must be interpreted with caution. No single fatty acid can be assigned uniquely to any one food source, and most fatty acids change with environmental variations (e.g. light, temperature, nutrients) and metabolic condition or reproductive status of organisms (De lange and Van den Brink, 2006). As such, knowledge of fatty acids in food sources and consumers is important both for obtaining basic dietary information on consumers within one habitat, and for assessing the greater nutritional implications of connectivity between habitats. Incorporation of additional methods helps to augment the data derived from any fatty acid study, and stable isotope tracers are increasingly used in this corroborational manner.

Stable isotope tracers

Stable isotope analysis is used by ecologists to make determinations on the food sources of organisms, the length of food chains, and the transfer of contaminants through food webs (Peterson and Fry, 1987; Yoshii et al., 1999; Michener and Kaufman, 2007; Marty and Planas, 2008). Consumers assimilate carbon and nitrogen from their diets, and they preferentially respire the lighter carbon isotopes (^{12}C) and excrete the lighter nitrogen isotopes (^{14}N; Marty and Planas, 2008). Consumers are generally enriched with the heavier carbon isotope (^{13}C) in relation to their food by a fractionation factor of ~1‰, making carbon isotope ratios (δ^{13}C) suitable for tracing food sources (Peterson and Fry, 1987), whereas nitrogen isotope ratios (δ^{15}N) change by 3 to 4‰ from food to consumer, thereby allowing estimation of the trophic position of a consumer (Vander Zanden and Rasmussen, 1999). Provided that potential food sources differ in their isotopic signatures, the contributions of these different sources to a consumer's diet can be estimated using models (Phillips et al., 2005; Rasmussen, 2010). Stable isotope tracing offers two potential advantages over traditional diet methods such as gut contents analysis; δ^{13}C and δ^{15}N ratios of animal tissue represent the integration of carbon and nitrogen over prolonged periods rather than once in time, and they reflect the assimilation of food items rather than simple ingestion (Finlay, 2004). We have utilised stable isotope ratios as a molecular-level technique (along with fatty acids in some cases) to study the connectivity in our model hydrological catchment.

As in the fatty acid technique, one important requirement for successful use of stable isotope ratios is distinct isotopic signatures among potential food sources. Rounick et al. (1982) showed that allochthonous (-27‰) and autochthonous (-35‰) plant materials were isotopically distinct in small New Zealand streams, thereby providing a basis for identifying food resource utilization by aquatic animals. Bunn et al. (1989) studied a tundra river and showed that terrestrial detritus signatures (-28‰) were different from algal signatures (-23‰) in small tributary streams and rapids of the mainstem Koroc River, and that terrestrial detritus was the most likely source of energy fuelling the communities. In contrast, Gu et al. (2006) showed that the average δ^{13}C signature of particulates in Lake Wauberg, Florida, was -19‰, consistent with an autochthonous origin from phytoplankton production, and the signatures of the particulate matter shifted seasonally with water temperature, pH, CO_2 concentration and phytoplankton biomass.

Stable isotope data have been used to test the predictions of the RCC in different systems (March and Pringle, 2003). In their study of alpine streams, Füreder et al. (2005) identified the main food sources for aquatic insects (predators and filter feeders) using δ^{13}C signatures. Similarly, Mihuc and Toetz (1994) used carbon isotopes to demonstrate the importance of periphyton to aquatic insects, and Zah et al. (2001) used δ^{13}C and δ^{15}N ratios to assess the spatial changes in the contributions of autochthonous and allochthonous food sources to consumers in a Swiss glacial stream. They reported that filamentous algae and epilithic diatoms (autochthonous material) were consistently more important than terrestrial materials (allochthonous), a result contrary to the predictions of the RCC. Stable nitrogen isotopes identified the trophic positions of predatory and herbivorous consumers, including dragonflies, mayflies, stone-flies and caddisflies, in the Saint Lawrence River of North America (Anderson and Cabana, 2007). Stable isotopes revealed that horizontal web-building spiders derived up to 92% of their carbon from aquatic insects within riparian areas of a Japanese stream (Akamatsu et al., 2005). Similarly, aquatic prey constituted at least half of the carbon consumption by linyphiid spiders living hundreds of meters away from the South Fork Eel River in north-western California (Power and Rainey, 2000).

As with the fatty acid profiles, with an increase in trophic level, evaluation of consumer diets using stable isotope ratios becomes increasingly challenging for a variety of reasons. Initially used (e.g. Hobson and Wassenaar, 1997) and further developed as tools to study avian migrations (Norris et al., 2005; González-Solís et al., 2011), the stable isotope technique has resulted in important advances in the field of avian trophic ecology. Not only can the method provide information on basal diets, it can also inform us about foraging niches (Herrera et al., 2003; Fort et al., 2010; Ceia et al., 2012), resource partitioning (Herrera et al., 2003), and seasonal shifts in the diets of birds (Karnovsky et al., 2008; Davies et al., 2009) and other higher consumers. One important factor that can be particularly useful in this research is that stable isotope ratios analysed on different tissues can provide information on consumer diets at very different time scales (Hobson and Clark, 1992; Pearson et al., 2003). For example, isotope ratios of feathers provide a record of a bird's diet during the growth period of the feather (which can vary depending on development and moulting rates). In contrast, whole blood or blood plasma represent shorter-term records of bird feeding history (Quillfeldt et al., 2008). When used inclusively, isotopic data from different tissues can provide a composite picture of avian feeding habits. For example, Bearhop et al., (2006) demonstrated significant differences in summer and winter diets of seabirds in the Southern Ocean using isotopic signatures in blood and feathers.

Although stable isotope analysis is useful in identifying trophic relationships in many habitats, ambiguities arise whenever isotopic signatures of food sources overlap (e.g. terrestrial detritus and epilithic algae, or seagrasses and epibionts; Finlay, 2001; Richoux and Froneman, 2007). As a result, the importance of different food items to a consumer may be difficult or impossible to estimate. Moreover, the use of stable isotopes is complicated by the poor understanding of isotopic fractionation from food to consumer, as these values can vary with a variety of factors that can be species- or habitat-specific, including growth rates, body size, diet and physical conditions (Martínez del Rio et al., 2009). These inherent limitations provide ample motivation and need for the use of additional and independent analytical techniques to investigate food webs whenever possible.

Combined approach

A combination of two or more methods is beneficial for resolving food web questions (e.g. Kharlamenko et al., 2001; Kiyashko et al., 2004; Richoux and Froneman, 2007, 2008; Lebreton et al., 2011). For instance, although stable isotope ratios could distinguish seagrass and microalgae on the west coast of Australia, red and brown algal species were isotopically similar, but distinct in their fatty acid signatures (Hanson et al., 2010). Similarly, on the French Atlantic coast, fatty acids enabled the discrimination of seagrasses and microphytobenthos, which was not possible using stable isotopes alone (Lebreton et al., 2011). Furthermore, the combination of fatty acid, isotopic, stomach contents and/or other analyses is important in clarifying trophic relationships in food webs that might otherwise not be apparent from using one method alone. For example, in a study of an Australian coastal area, mixing models for stable isotopes yielded ambiguous results and indicated that brown algae and seagrasses were both feasible food sources for the amphipod *Allorchestes compressa* (Crawley et al., 2009). However, the presence of the fatty acids 20:4ω6 and 18:4ω3 in both the brown algae and the amphipods, viewed in combination with the isotopic data, together suggested that the amphipod was primarly consuming brown algae (Crawley et al., 2009). Results such as these provide sound evidence that a combination approach is more ideal for resolving complex food web dynamics.

Flux model

Our aim was to develop a steady state mass balance model that simulates an ecosystem at a single point in time and space. As such, we describe the relative contributions of allochthonous and autochthonous inputs and exports to the system, and thereby describe the links between the adjacent aquatic and terrestrial habitats (and aquatic and aquatic habitats in some cases). Using the mass-balance analysis software, *Ecopath with Ecosim* (EwE), we have developed a conceptual model that integrates the biotic and organic components of the catchment system. Few studies have attempted to describe the trophic interactions from a large ecosystem perspective, and our study is unique in that we have empirical data for multiple sources and pathways of transfer between habitats. This promises to be a novel modelling effort with regard to scale and resolution, and holds the potential to deepen our understanding of underlying trophic linkages and mass flows.

4.9 Motivation and aims

There is a general need for ecologists to transcend from a population (i.e. autecological) to a multi-community (i.e. metasynecological) perspective of trophic connectivity, especially when developing strategies for resource management or conservation (Wootton et al., 1996). Our study encapsulates this need by approaching the general question of connectivity through trophic linkages and subsidies via organic materials at the holistic level of a catchment system in South Africa. Most studies on subsidies between freshwater and terrestrial habitats are geographically biased, and much of the published knowledge is confined to the northern hemisphere (reviewed by Bartels et al., 2012). There is a need for holistic investigations in additional geographic zones including semiarid and arid regions where vegetation is more sparse (Bartels et al., 2012). Our study will contribute novel large- and multi-scale information on trophic subsidies in a poorly studied region of the world.

Overall aim:

The over-arching aim of this study was to identify and quantify invertebrate- and vertebrate-mediated reciprocal transfers of organic nutrients among aquatic (freshwater, estuarine, near-shore marine) and terrestrial habitats (including the buffer zone between aquatic and terrestrial habitats) in a South African hydrological catchment.

The specific aims were to:

1. quantify the contributions of autochthonous and allochthonous material to the suspended particulate matter (SPM) from different locations and during different seasons in the river/estuary continuum, and relate any variations to the local physical conditions and the composition of the primary producers,

2. quantify the contributions of aquatic versus terrestrial food sources to any abundant aquatic herbivores and predators by assessing their diets in relation to the food sources available (through space and time),

3. quantify the biomass and quality of insect emergence from freshwater to terrestrial habitats at different locations and during different seasons,

4. determine the variations in terrestrial invertebrate biomass infall to the lotic system through space and time,

5. define the size of the trophic buffer zone along the river by assessing the diets of aerial predators at increasing distances from the water,

6. quantify the nutritional contributions of aquatic prey to the terrestrial environment over time, through estimating the relative proportions of aquatic prey that spiders consume in comparison to terrestrial prey,

7. determine the spatial differences in the diets of benthic and pelagic invertebrates along the estuarine gradient to assess the contributions of autochthonous and allochthonous food sources to the food webs,

8. determine the spatial and temporal differences in the type and quality of diets in wading bird species in the estuarine environment,

9. quantify aquatic to terrestrial subsidies through bird activities by combining new field observations and previously published information on bird feeding rates and nutritional values of different prey items for the dominant wading bird species,

10. identify the main dietary items and nutritional condition of migratory fish and the sources of their food,

11. determine the migratory pathways of the indigenous fish through their isotopic signatures,

12. determine whether the largemouth bass represents a significant barrier to the migrations of indigenous fish,

13. determine the role of amphibians in connecting aquatic and terrestrial habitats,

14. determine the effect of pollution on a small riverine food web (in a tributary of the main river studied),

15. integrate the different components into an ecosystem flux model.

5 STUDY REGION

The region selected for this project was the Kowie River in the Eastern Cape Province of South Africa. The Kowie River source is in the hills of Grahamstown Heights, and the river drains a relatively small catchment area of ~800 km^2 (Heydorn and Grindley, 1982; Whitfield et al., 1994). The system flows in a south-east direction, draining the major part of the Bathurst region, and is artificially open permanently to the ocean (**Figure 1**). Its major tributaries are the Bloukrans, Brakrivier and Lushington (or Torrens) Rivers. Multiple streams feed into the river upstream of the sharp estuarine-to-freshwater transition zone at Waters Meeting Nature Reserve in Bathurst. The total length of the Kowie River is ~90 km, and the river has a meandering course cut deeply into the Bokkeveld shales which make up most of its catchment, with the upper reaches of the tidal water lying in a narrow valley with steep, high and densely wooded slopes. Farms for pineapples, citrus, chicory, fodder crops, beef cattle and goats utilise part of the upstream waters of the river. Marine water extends up to 30 km upstream from the river mouth (Heydorn and Grindley, 1982). Minimum and maximum air temperatures of the Kowie River region are 1.5 °C and 39.8 °C, respectively, with an average daily temperature of 10.6 °C (Bok, 1983).

The Kowie Estuary is about 21 km in length and varies in width and depth between 30 to 150 m and 2 to 8 m, respectively (Heydorn and Grindley, 1982; Whitfield et al., 1994). The upper reaches have steep banks, often vegetated down to the water's edge, the bottom is comprised mainly of fine sand and silt, and the intertidal zone is narrow (<10 m wide). The middle reaches broaden to ~100 m in width and 3 m in depth. Intertidal salt marshes and mud banks >50 m wide occur in some areas. The lower reaches consist of an artificial channel ~80 m wide that is linked to the Port Alfred Marina, which covers an area of 45 ha. Water depths in the estuary mouth channel range between 2.7 m and 6.0 m at spring high tide (Heydorn and Grindley, 1982). The channel and marina canals have walls of granite which drop vertically to a sandy bottom 24 m deep.

Salt marshes and a large intertidal mud flat >100 m wide occur in the lower estuary. The spring tidal range is ~ 1.1 to 1.7 m in different sections of the estuary, and strong tidal current speeds between 0.12 to 0.20 ms^{-1} have been recorded in sections of the estuary (Whitfield et al., 1994). Strong flows (>2 ms^{-1}) occurred in the lower reaches of the estuary when river floods coincided with an outgoing spring tide (Heydorn and Grindley, 1982). The Kowie River catchment falls within a temperate climate zone, with rainfall occurring mainly during spring and autumn (60%). Mean annual rainfall is 650 mm (Heydorn and Grindley, 1982) and estimated mean annual river discharge, excluding major flood events, is 20 x 10^6 m^3 (Whitfield et al., 1994). Episodic river pulses greater than l000 m^3s^{-1} have been recorded at the monitoring station on the river, but increased rainfall in the catchment does not always result in an increased river flow (Heydorn and Grindley, 1982; Bok, 1983; Whitfield et al., 1994).

The Kowie River has a high pH (mean 8.2) and alkalinity (139-185 ppm $CaCO_3$). The water is usually clear with a mean Secchi disc depth ranging from 0.7 to 1.03 m and maximum values up to 2.50 m, but light penetration is limited when there are high concentrations of dissolved organic materials (Bok, 1983; Whitfield et al., 1994). River water entering the estuary during low flow conditions is brackish ranging in salinity from 2 to 6 (Heydorn and Grindley, 1982). Salinity in the estuary is typically >30 and may increase to 40 in dry years. During prolonged river floods, the surface water of the entire estuary is almost fresh, although seawater may be present in the mouth region. Salinity stratification following flood events is often strongly developed in the lower and middle reaches of the estuary. Seasonal water temperatures recorded in the mouth region range from 14 to 22 °C, and in the upper reaches from 11 to 27 °C (Heydorn and Grindley, 1982).

6 METHODS

6.1 Sites

Three estuarine (E) and six main freshwater (F) sites were selected according to their accessibility and their locations from the headwaters, river branches, and ocean (**Table 1; Figure 1**). The main river sites run from F1 at the headwaters to F5 at the river/estuary boundary, plus F6 in the Bloukrans tributary. Site E1 is near the head of the estuary and is influenced by the river, Site E2 is near the middle reaches of the estuary, and Site E3 is located near the river mouth where extensive marine influence occurs. Sampling at each site was aimed at collecting as many elements of the food web as possible. Collections included, wherever possible, algae, submerged and emergent macrophytes, dominant riparian trees, suspended particulate matter (SPM), zooplankton, in-falling terrestrial invertebrates, emerging freshwater invertebrates, aquatic invertebrates, migratory fish, amphibians, wading birds and select aerial predators. Sampling sites for the fish and birds differed from those of the invertebrates and amphibians, and site information for these collections is available in the methods sections on fish and birds.

Table 1. Location of the main study sites along the Kowie system. E = estuarine site, F = freshwater site.

Site name	Coordinates	
	South	East
F1 Featherstone (Southwell road)	33°20'59.2"	026°33'37.6"
F2 Coleridge Farm	33°21'57.7"	026°37'38.0"
F3 Hollingrove Game Reserve	33°27'23.4"	026°41'22.0"
F4 Small Bridge (Bathurst)	33°30'16.0"	026°44'40.9"
F5 Waters Meeting Nature Reserve	33°32'45.6"	026°47'04.2"
F6 Bloukrans tributary	33°23'28.0"	026°42'25.3"
E1 Upper estuary	33°32'23.6"	026°48'13.0"
E2 Middle estuary	33°32'51.3"	026°49'02.3"
E3 Lower estuary	33°34'55.8"	026°51'41.8"

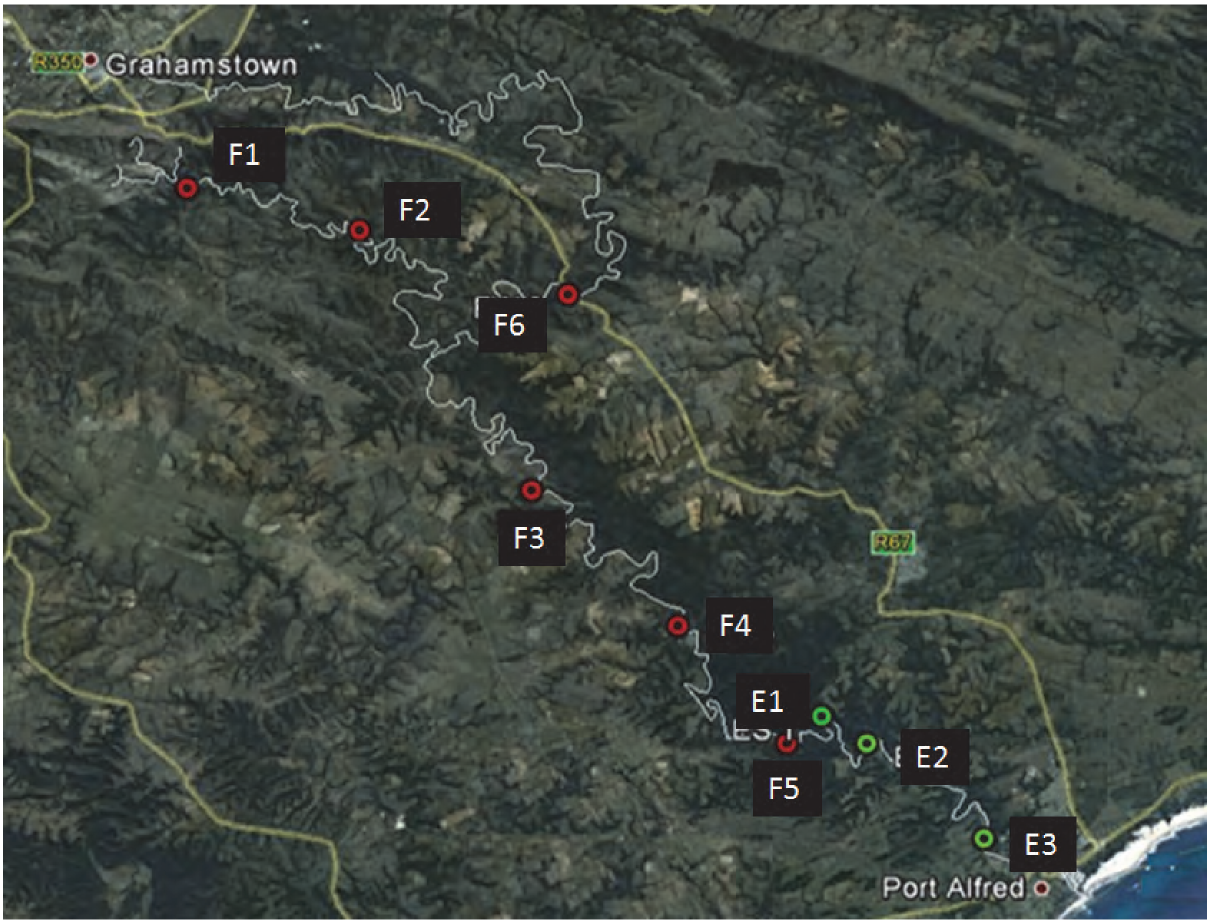

Figure 1. Locations of the nine collection sites along the Kowie River (F1 to F6) and Estuary (E1 to E3). Source: Google Earth

6.2 Primary producers/basal resources

6.2.1 Sampling and lab procedures

To address **Aims 1 and 2**, basal resources were collected during September and December 2012, and February and June 2013. Samples of plants (aquatic macrophytes and terrestrial plants) were collected during September and November 2012, and February, May and October 2013. Samples were collected from all sites when available (**Figure 1**). Our intention was to determine the seasonal and spatial variability in the contributions of allochthonous and autochthonous organic matter to SPM and larger detritus along a lotic continuum. Drawing from the river continuum concept (Vannote et al., 1980), we hypothesized that (i) the organic matter fuelling these suspended mixtures varies along the continuum, with the middle reaches of the system (i.e. the lower river) dominated by autochthonous benthic algae material and the upper reaches by allochthonous plant material, and (ii) these relative contributions change over time based on shifting environmental parameters such as freshwater inputs. To address these hypotheses, we measured $\delta^{13}C$ and $\delta^{15}N$ isotope signatures and fatty acid compositions of SPM, bulk detritus, and potential allochthonous and autochthonous organic matter sources and incorporated them into models to determine the source contributions to SPM and detritus at different locations of the riverine system over time.

<u>Chemical and physical parameters</u>

Chl-*a* and nutrient concentrations, air and water temperature, pH, dissolved oxygen, electrical conductivity, current velocity, salinity, suspended particulates and water transparency were measured. The physical parameters were measured using various probes (CyberScan Series 600, Eutech Instruments, Singapore; Flo-mate portable flowmeter Model 2000, Marsh McBirney, Maryland). The water column was sampled in triplicate (1L aliquots of surface water) at each site to permit chlorophyll-*a* (chl-*a*) measurements to serve as proxies for phytoplankton biomass and nutrient concentrations. In the laboratory, 250 mL of each water sample were filtered onto 0.7 µm Whatman glass fibre filters (GF/F). Each filter was extracted in 90% acetone at -20°C for 24 hours in the dark. Chl-*a* concentration was determined fluorometrically using a Turner 10AU. Aliquots of 500 mL were processed to determine phosphate, nitrate and ammonia concentrations. Ammonia was analysed using a Nessler method, nitrate with a cadmium reduction method, and phosphate with an amino acid method, all using an HI 83203 multiparameter bench photometer (Hanna Instruments Inc., Rhode Island).

Biological parameters

Detailed sample types included suspended particulate matter (SPM), periphyton (river only), epipelon, epiphyton, aquatic plants, terrestrial plants, bulk detritus, and bulk sediments (estuary only; n ≥ 3 for each sample type at each site and time). Twenty litre surface water samples were collected in buckets to obtain SPM. Periphyton in the river was brushed from pebbles and rocks using a toothbrush, and epipelon was collected in the top 1 cm of sediments from the littoral (river) and intertidal (estuary) areas. In the laboratory, the surficial sediments were placed in several 35 x 22 cm trays and layered with a 64 µm mesh and 5 mm of sterile sand (pre-washed with 1 M hydrochloric acid and ashed at 500 ^{o}C for 5 hours). The trays were incubated under moist conditions and artificial light for 15 hours to promote the migration of microalgae into the sand layer, after which the algae was extracted from the sand using pressure washing and concentrated in a 63 µm mesh sieve (Antonio et al., 2010). Aquatic plants (*Cyperus eragrostis*, *Potamogeton pectinatus*, *Phragmites australis*, *Schoenoplectus brachycerus* in the freshwater regions and *Spartina maritima* and/or *Chenolea diffusa* in the estuary) were collected by hand whenever they were present, and the epiphyton was removed from the leaves and/or stems using a toothbrush. Leaves were collected from live terrestrial plants (*Eucalyptus globulus*, *Olea* sp. and *Searsia* sp.) and bulk detritus was collected in a 50 cm diameter net (100 µm mesh) left in the water for 30 min. Bulk sediments (estuary only) were gathered from the top 1 cm within the lower intertidal zone. Bulk detritus, all samples containing phytobenthos (periphyton, epipelon and epiphyton) and water samples were concentrated onto Whatman GF/F glass fibre filters (pore size 0.7 µm) for further processing, and any visible non-algal material was removed with forceps. After collection, all samples were immediately stored on ice in the field and transferred to -20ºC in the laboratory until processing occurred within a day or two of collection.

6.2.2 Stable isotope protocol

Three samples of each type from each site and time were processed to obtain stable isotope data. Samples were freeze-dried at -60ºC (VirTis Benchtop 2K), and any large materials (e.g. aquatic macrophytes, terrestrial matter and detritus) ground to fine homogeneous powders using a mortar and pestle. Subsamples of ~1 mg were added to tin capsules. For the filtered samples, about 1 mg of dried material was scraped off each filter and placed into the tin capsules. Prior to encapsulation, algae samples concentrated on filters were vortexed for two minutes in 2 M hydrochloric acid (to remove calcium-based components), centrifuged for five minutes at 3600 rpm, washed in deionised water, centrifuged again, dried at 50ºC and homogenised in a Retsch Mixer Mill.

Stable isotope analyses were conducted using a Europa Scientific 20-20 Isotope Ratio Mass Spectrometer linked to an ANCA SL Prep Unit. Carbon and nitrogen isotopic signatures were expressed as the relative differences between isotopic ratios in the sample and conventional standards [internal (beet sugar and ammonium sulphate) standards and certified Casein protein standard], using the equation $\delta^{15}N$ or $\delta^{13}C$ (‰) = $[(R_{sample}/R_{standard})-1] * 1000$, where R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$.

6.2.3 Fatty acid protocol

All samples were placed into separate foil envelopes and lyophilized using a VirTis Benchtop 2K (SP Industries, USA) for 24 to 36 hours. Dried materials were scraped off filters (where necessary), ground finely with a mortar and pestle and weighed into glass test tubes (10-300 mg, depending on the sample type). Lipids were extracted and the fatty acids derivatized into fatty acid methyl esters (FAMEs) using a one-step method (Richoux and Ndhlovu, 2014). Gas chromatographic (GC) analyses of FAMEs suspended in hexane were completed using an Agilent 7890A GC equipped with a Zebron-Waxplus 320 column and helium as the carrier gas. Aliquots (1 µL) of the sample were analysed (see Richoux and Ndhlovu, 2014 for oven protocols) and the peaks integrated using Chemstation chromatography software, and identified using an Agilent QQQ mass spectrometer (MS) and MassHunter software. Each fatty acid was measured as a proportion of the total fatty acids (% TFA) and reported in the shorthand x:aωb, where x is the number of carbon atoms in the acyl chain, a is the number of double bonds, and b is the location of the first double bond counted from the methyl end of the acyl chain. The proportions of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), bacterial fatty acids (BFA; sum of odd-number and branched chain fatty acids), and higher plant fatty acids (HPFA; sum of 18:2ω6 and 18:3ω3) were summed, and several indices calculated in the SPM and phytobenthos pools [PUFA:SFA, a freshness index ref, and two ratios that indicate the relative dominance of diatoms: 16:1ω7 : 16:0 = diatom index 1, and (16:1ω7 + 20:5ω3) / (18:1ω9 + 18:4ω3 + 22:6ω3) = diatom index 2; Antonio and Richoux, 2014].

6.2.4 Data analysis

Permutational analysis of variance (PERMANOVA; McArdle and Anderson, 2001) was conducted on non-transformed isotopic data using PRIMER v6 with the add-on package PERMANOVA+ (Anderson et al. 2008). Site and period were the main factors in Euclidean-based PERMANOVA to determine any differences in the stable isotope signatures ($\delta^{13}C$ and

$\delta^{15}N$) aquatic macrophytes, benthic algae, terrestrial leaves, SPM and detritus among the study sites and times. Significant outputs were further investigated using PERMANOVA pair-wise comparisons (Anderson et al., 2008).

Bayesian stable isotope analysis in R (SIAR, Parnell et al., 2010) was used to assess the relative contributions of benthic algae (epipelon + epiphyton + periphyton), aquatic macrophytes and terrestrial leaves to SPM and detritus (the pooled organic material in the SPM and detritus categories were treated as the 'consumers' in these stable isotope models; Bergamino et al., 2014a). Differences ($p > 0.05$) were not detected in isotopic signatures of the benthic algae types (epipelon and epiphyton) in the estuary, so we combined these into one source group for simplicity. For the river section we combined periphyton and epiphyton within each season, excepting sites F2 and F3, where we combined epipelon, epiphyton and periphyton, as they were statistically identical ($p > 0.05$). Several SIAR models were run, each using data arising only from the relevant site and time. Fractionation factors of 0.5 were applied to the carbon and nitrogen data based on decomposition experiments in rivers (Keough et al., 1988, Schweizer et al., 1999; Dehairs et al., 2000).

We further examined the spatial and temporal variations in the trophic structure (i.e. relative nutritional composition) of the suspended and benthic basal resources in the Kowie system by performing a series of non-parametric multidimensional scaling analyses (n-MDS) with the fatty acid profiles of all the basal resources collected at each site and time. Differences in fatty acid profiles among the basal resources were tested using analysis of similarity (ANOSIM), followed by similarity percentage analyses (SIMPER) to identify the most influential fatty acids. To test the hypothesis that benthic algal pools are the most nutritionally-rich resources regardless of season or location, we ran PERMANOVA and pairwise comparisons using PUFA, EFA, PUFA/SFA, diatom index 1 and diatom index 2 as dependent variables and sample type, season and location as the independent variables. To determine the spatial and temporal variations in the contributions of allochthonous versus autochthonous matter to the SPM and benthic algal pools in the Kowie system, we performed a series of Pearson's rank correlations between PUFA levels and markers for the main potential contributors (HPFA, BFA, diatom index 1, diatom index 2) at each sampling time in addition to the location along the continuum (distance from F1, km). These fatty acid groups were graphed according to the distance of each site (km) from the most upstream site (F1). Site F6 was included at the confluence of the main and tributary channels, although it was located several km upstream within the tributary (**Figure 1**).

6.3 Freshwater invertebrates

6.3.1 Sampling and lab procedures

Aquatic communities

To further address **Aim 2**, aquatic macroinvertebrates were collected at each freshwater site over four seasons. Macroinvertebrates were collected by kick sampling (Lau et al., 2011) at each freshwater site using a SASS net (Dickens and Graham, 2002). The live animals were decanted into a white sorting tray, picked out with tweezers, and separated visually by order or family using Gerber and Gabriel (2002). Refer to **Sections 6.2.2** and **6.2.3** for laboratory procedures used to obtain stable isotope ratio and fatty acid data.

Emergence and infall

Emergence and pan trap data (to address **Aims 3 and 4**) were collected during each season. Pan traps (40 cm x 55 cm x 13 cm) were used to quantify the infall of terrestrial invertebrates to the river sites. Each pan was filled with water (2cm depth) mixed with several drops of a surfactant (detergent) to reduce the surface tension of water (Nakano and Murakami, 2001). Pan trap contents were sieved through a 500 µm mesh net and stored in 70% ethanol. Floating pyramidal emergence traps (0.37 m^2 base, PVC pipe construction covered with a 0.08 mm mesh) were used to measure the emergence rates of aquatic insects (Davies, 1984). The base of the traps was anchored into the stream substrate with bricks to prevent insect drift from entering the traps.

Invertebrates were identified and counted under a stereomicroscope. Adult aquatic insects were identified at least to family level, while most of the terrestrial invertebrates were identified to order, suborder (some Diptera), or family. Body lengths of invertebrates were measured using Vernier callipers (for specimens greater than 10 mm) or an ocular micrometre fitted to a dissecting binocular microscope (for specimens less than 10 mm). Measurements were made to the nearest 0.05 mm from the anterior part of the head to the anus. Appendages extending beyond these points (wings, ovipositors, caudal cerci, styles, etc.) were disregarded. Aquatic insects in the pan traps were excluded from the analysis. We estimated dry mass of invertebrates from length-mass regressions (methods as per Sample et al., 1993; Towers et al., 1994; Ganihar, 1997; Benke et al., 1999; Johnston and Cunjak, 1999; Sabo et al., 2002; Gruner, 2003; Höfer and Ott, 2009).

6.3.2 Data analysis

PERMANOVA (version 1.03, PRIMER v 6; Clarke and Gorley, 2006) with Monte Carlo p values were used to test for differences in isotope signatures among food sources (dependent variables were $\delta^{13}C$ and $\delta^{15}N$ values, and co-factors were season, time, and season * time). SIAR models were run to determine the proportional contributions of each food source to consumers (Parnell et al., 2013). The trophic fractionation factors used were 2.30‰ (± 0.18 SD; nitrogen) and 0.50‰ (± 0.13 SD; carbon; McCutchan et al., 2003), values based on the average fractionation derived from studies on streams and rivers (Anderson and Cabana, 2007; Cremona et al., 2010).

Species were grouped according to published information on their feeding behaviour (Merritt and Cummins, 1996) and their isotopic values. To determine which food sources should be included in each mixing model, we initally graphed isotope values of all sources and consumers and those very distant from each other were deemed unrelated (Phillips et al., 2014). This method allowed for each model to be reduced to include only a few main food sources. This simplifying protocol was important for ensuring that the models were not underdetermined and that unique solutions were possible in the majority of cases (Phillips et al., 2005; Newsome et al., 2007). We excluded SPM from the mixing models of the primary consumers for several reasons. First, SPM is a complex matrix of algae and decomposing terrestrial and aquatic vascular plants (Hamilton et al., 2005), so it was logical to include only the ultimate source components in the mixing models. Second, there is evidence that macroinvertebrates can feed selectively on particular components of SPM (Thorp et al., 1998; Raikow and Hamilton, 2001; Clapcott and Bunn, 2003). Third, since the objective of our study was to identify the importance of allochthonous *versus* autochthonous materials (*in situ*) to consumers in the riverine food web, it was logical to include only the primary autochthonous and allochthonous components. A similar approach was successfully utilized in several studies (*sensu* Hadwen and Bunn, 2004; Hadwen et al., 2007; Hadwen and Arthington, 2007).

To determine the contributions of organic matter sources to predators, we ran SIAR models using the primary consumers (filter-collectors, gatherer-collectors and grazers) as the potential prey. As the primary consumers, in turn, have diets based on differential inputs of basal organic materials, we then adjusted (using proportions) the contributions of primary consumers to predators to reflect the autochthonous and allochthonous sources that originally fueled the primary consumers.

We calculated daily aquatic invertebrate biomass (mg m^{-2} day^{-1}) using three emergence trap replicates, and daily terrestrial invertebrate biomass from three pan traps (mg m^{-2} day^{-1}). Data were square root transformed to balance the effects of dominant and less dominant taxa, and a similarity matrix (type III sums of squares, 9999 permutations) was created including all samples, followed by a two factor (site vs season) PERMANOVA to determine whether emergence or terrestrial infall differed with season or location.

6.4 Aerial invertebrates

6.4.1 Sampling and lab procedures

Two of the project aims were to 1) define the size of the trophic buffer zone along the river by assessing the diets of aerial predators at increasing distances from the water (**Aim 5**) and 2) quantify the nutritional contributions of aquatic prey to the terrestrial environmental over time (**Aim 6**). Different sites with varying stream widths were chosen to establish the relationship between width and the size of the buffer zone. Web-building spiders were collected along a distance gradient away from the river's edge up to 64 m into the terrestrial habitat at F1, F4 and F6. Collections were done during four periods: November 2012 (summer), March 2013 (autumn), June 2013 (winter) and October 2013 (spring). The main reason for the inclusion of F6 to address **Aim 5** was its high abundances of large spiders, particularly bark spiders (*Caerostris* spp.) and golden orb-web spiders (*Nephila* spp.), that were rare in the other two sites. Large spiders have the advantage that they consume larger flying insects that most spiders may fail to capture. Dietary tracers (stable isotopes) were used applied to determine the distances at which web-building spider communities become independent from aquatic insect subsidies. Flying insects of aquatic- and terrestrial-origin were collected and their trace signatures have been used to determine what spiders are eating across the distance gradient. Refer to **Sections 6.2.2** for laboratory procedures used to obtain stable isotope ratio data.

6.4.2 Data analysis

SIAR mixing models were run to determine the percentage contributions of aquatic and terrestrial food sources to the diets of spiders living at increasing distances from the river, and at different times of the year. Variability in δ^{15}N and δ^{13}C isotopic values of invertebrates (i.e. spider prey) was extremely large, so we used the isotopic values for the producers that the prey fed on to infer diet contributions of aquatic- and terrestrial-derived food sources (see Vander Zanden and Rasmussen, 2001; McCutchan et al., 2003). Terrestrial food sources were represented by C3 and C4 plants, whilst aquatic sources were represented by macrophytes and different types of algae (epiphyton, epipelon and periphyton). Producers with similar isotopic composition were grouped to minimize the number of sources so that we could simplify the range of possible solutions (Phillips et al., 2005; 2014).

Trophic fractionations of 2.3‰ (± 0.24 SD; $\delta^{15}N$) and 0.5‰ (± 0.19 SD; $\delta^{13}C$) were utilized between invertebrates and producers (McNabb et al., 2001; Vander Zanden and Rasmussen, 2001; Oelbermann and Scheu, 2002; Paetzold et al., 2005), and between invertebrate prey and spiders (McCutchan et al., 2003). We assumed that spiders preyed predominantly on herbivorous insects and less so on invertebrate predators, therefore spiders were assigned to a trophic level between 3 and 4 (generally 3.5). In some cases a trophic level had to be adjusted slightly so that the corrected isotopic values would fall within the convex hull bordered by the available sources (Phillips et al., 2014). Fractionation from the producers to the spiders was therefore adjusted to 5.75‰ (± 1 SD; $\delta^{15}N$) and 1.25‰ (± 0.19 SD; $\delta^{13}C$) after factoring in a trophic level of 3.5. The diet contributions of different producers were combined and grouped into two categories: terrestrial and aquatic sources. Aquatic and terrestrial source proportions were then related to distance from the river at each site and season. Regression analysis was done to investigate the relationship between distance from the river and the proportion of aquatic food sources occuring in spider diets, and to ultimately make predictions of how far inland the aquatic subsidies extend.

6.5 Estuarine invertebrates

6.5.1 Sampling and lab procedures

To address **Aim 7**, invertebrates (and some fish) were collected over four periods between September 2012 and June 2013 at E1, E2 and E3 (**Figure 1**). Benthic invertebrates were collected using a dip net (mouth area 0.3 m x 0.3 m, mesh size 1.0 mm) pushed perpendicular to the shore over a 2 m distance in replicates of three at each site and during each period. Invertebrates were washed of debris and kept alive in estuarine water for several hours to allow for their guts to clear. Three to five similar-sized individuals of the smallest species were pooled to obtain adequate biomasses. Zooplankton samples were obtained at night by towing a 200-µm mesh net for two minutes. All samples were stored at -80^{o}C until they were processed in the laboratory.

Specimens were identified to species using Day (1969) or Whitfield (1998). For the zooplankton samples, individuals of the main copepod species (*Pseudodiaptomus hessei* and *Acartia longipatella)*, crab zoea and mysids were isolated under a dissecting microscope and transferred to filtered estuarine water to allow for gut clearance overnight. Copepods were pooled (~50 individuals per species) to ensure that each sample had adequate material. Samples intended for tracer analyses were rinsed with distilled water to remove salts, lyophilized, and ground to a powder using a mortar and pestle. Refer to **Sections 6.2.2** and **6.2.3** for laboratory procedures used to obtain stable isotope ratio and fatty acid data.

6.5.2 Data analysis

To examine the trophic structure in the different estuarine regions and seasons, the trophic positions (TP) of the consumers were estimated using nitrogen isotope data (Post, 2002):

$$TP_{consumer} = 2 + (\delta^{15}N_{comsumer} - \delta^{15}N_{baseline})/F$$

where $TP_{consumer}$ is the trophic position of a consumer, $\delta^{15}N_{consumer}$ is the nitrogen isotope signature of the consumer, $\delta^{15}N_{baseline}$ represents the nitrogen isotopic ratio value of a baseline (amphipods in this case, assigned TP 2 in the equation) and F is the fractionation factor of nitrogen per trophic level (2.9‰; McCutchan et al., 2003). Amphipods were selected as the baseline since they were herbivores with limited mobility, and so they best reflected the base of the food web in each area of the estuary.

Spatial and temporal differences in the isotopic signatures of species occurring at all three sites were assessed using two-way ANOVA (main fixed factors were 'site' with 3 levels, and 'season' with 4 levels) followed with a Tukey's test. This analysis was performed on pooled data from benthic specimens collected at each site and season: *Hymenosoma* sp., *Exosphaeroma hylecoetes, Grandidierella lignorum*, *Palaemon peringueyi*, *Glossogobius callidus*, *Solea bleekeri*, *Sesarma catenata*, *Diogenes brevirostris* and *Upogebia africana*. This pooled approach enabled us to make community-based comparisons, as each species was not represented at every site and during every period.

SIAR models were run using each of the benthic consumers collected from each site to assess intra-specific spatial variability in the diets. Potential food sources for each consumer included in the models were based on literature reports of gut content data. The potential prey for the epibenthic crab *Hymenosoma* sp. included plant detritus, amphipods, isopods and copepods (Lucas, 1980; Whitfield, 1989). For the gobiid *Glossogobius callidus*, copepods, amphipods and SPM were selected as potential food sources (Wasserman, 2012), and for the soleidae *Solea bleekeri* we considered benthic crustaceans as potential prey (Cyrus, 1988). For the brachyuran crabs, SPM, plant detritus and small crustaceans were included as potential prey (Vorsatz, 2009), and for the mugilids *Liza dumerilii*, *Liza richardsonii* and *Mugil cephalus* we considered benthic algae, SPM and plant material as the potential food sources (Whitfield, 1988). Diet sources for the amphipods and isopods included detrital aggregates represented by SPM, sediment organic material (SOM) and marsh grass detritus (Whitfield, 1989). We used the fractionation factors of 2.9 ± 0.3‰ for

$\delta^{15}N$ and 1.3 ± 0.3‰ for $\delta^{13}C$ as these values were derived from muscle tissues of similar consumers (McCutchan et al., 2003). Time-averaged isotopic signatures of each potential food resource were used since isotopic values change with habitat parameters and organism growth and hence factors such as body size, protein turnover and temperature (McCutchan et al., 2003).

Stable isotope Bayesian ellipses in R (SIBER) models were used to examine the isotopic niche width (a proxy for trophic niche size) of selected species (e.g. those present during all or most sampling occasions) including the gobiid *Glossogobius callidus*, the epibenthic crab *Hymenosoma* sp., the caridean shrimp *Palaemon peringueyi,* the soleidae *Solea bleekeri* and the salt marsh crab *Sesarma catenata* at each site. Ordination using nMDS was done to examine spatial and temporal similaries in the non-transformed fatty acid profiles of the consumers common to all three sites. Differences in profiles among sites and seasons were tested using analysis of similarity (ANOSIM), and similarity percentages (SIMPER) identified the most influential fatty acids. Significant differences in the proportions of specific fatty acids in benthic consumers among sites were detected using one-way ANOVA, followed with a Tukey's test. Statistical analyses were completed using Statistica 7 (StatSoft, Tulsa, OK) and PAST 3.01 (Hammer et al., 2001).

6.6 Amphibians

6.6.1 Sampling and lab procedures

To address **Aim 13**, collections of adult frogs and tadpoles were done primarily during the breeding season between February and April 2013, which was the optimal period to conduct frog surveys as most amphibians were visible during this time. Additional collections were completed outside the breeding season (November 2012, May and June 2013). Tadpoles and adult *Amietia angolensis, Strongylopus grayii, Amietophrynus rangeri* and *Xenopus laevis* were collected from two sites (F1 and F4). A minimum of three individuals per species per site was collected for processing. The study sites were actively searched for frogs starting two hours after sunset (as most frog species are nocturnal) with the aid of headlamps and sweep nets. Specimens were searched for in pools, along pools and stream edges, underneath decaying logs, and under leaf litter on the river banks (i.e. all potential frog microhabitats in and around the river). Tadpoles were collected during the day by dip-netting for 15 minutes per site (Conradie et al., 2011). Three-array design drift-fences made of green shade cloth and two open-ended funnel traps were left at the sites for 72 hours to aid in the collection of adult frogs, specifically *Xenopus laevis*. The length of the fence was 3 m and it was attached to 0.5 m high metal stacks with cable ties. All specimens were transported alive to the laboratory for processing.

Recordings of frog calls were done during the night betwee 19H00 and 22H00 using a multi-function stereo recorder (Olympus WS-811). The recordings were noise-filtered using Raven Lite 1.0 software, and the calls identified using an audio CD (Du Preez and Carruthers, 2009). Recordings were used to confirm the presence of different frog species in each study area.

Four potential basal food sources were considered for tadpoles (epiphyton, benthic algae, periphyton and SPM; see **Section 6.2**). The main items in the adult diet typically consist of invertebrates including annelids, arachnids, molluscs and arthropods (Santos et al., 2004; Najera-Hillman et al., 2009), so these potential prey items were collected (**Sections 6.3 and 6.4**).

Anuran specimens were euthanized for 10-15 minutes in an aqueous solution of tricaine methanesulfonate (MS-222) buffered with sodium bicarbonate (Heyer et al., 1994). Measurements of the snout-vent length were recorded for each adult frog, and the average total length of a sub-sample of tadpoles. Adult frogs were identified to species level and tadpoles to family level using a field key (Du Preez and Carruthers, 2009). Gender of the adults was determined from examination of the gonads, and thigh and liver tissues were excised. Tadpoles were cut lengthwise, the head and gut removed, and the remainder of the body processed whole for obtaining tracer data. Tissues were stored at -80 ^{o}C, lyophilized at -60 (VirTis BenchTop 2K) for 48 hours and homogenized to a fine powder with a mortar and pestle. Refer to **Sections 6.2.2** and **6.2.3** for laboratory procedures used to obtain stable isotope ratio and fatty acid data.

Recent ingestion by adult frogs was assessed through stomach contents analysis. Stomachs were removed through a longitudinal abdominal incision, preserved in 10% formalin and later transferred to 70% ethanol. Prey items were counted and identified to the finest taxonomic level possible (order and family where possible, although some items like Hymenoptera were identified to family; ants were identified to Formicidae, hence all subsequent mention of Hymenoptera as anuran prey excludes ants) using a dissecting microscope and a key (Picker et al., 2004).

6.6.2 Data analysis

Stomach content data were recorded as the frequency of occurrence, or the number of stomachs containing a specific prey item as a percentage of all sampled stomachs, and prey items were categorized into terrestrial, aquatic or both terrestrial and aquatic. For example, larval aquatic insects were classified as aquatic, while flying adults were considered terrestrial. To estimate the trophic niche breadth, we used the Levin's measure (*B* and B_{st}= *B* standard; França et al., 2004), calculated as:

$$B = 1/(\sum_{i}^{n} p_i^2)$$

where *p* = the proportion of the total food items in food category *i* and *n* = the total number of food categories. Niche overlap was estimated using Pianka's index (Pianka, 1974):

$$Q = \frac{\sum p_{ik} p_{ij}}{\sum p_{ij}^2 p_{ik}^2}$$

where *pij* and *pik* represent the abundance of prey category *i* in the stomachs of consumers *j* and *k*. The value of Q varies between 0 (no common resource) and 1 (perfect overlap).

Isotopic signatures of potential terrestrial prey were pooled as 'terrestrial sources', and signatures of potential aquatic prey (including larval stages of some terrestrial insects) as 'aquatic sources'. The proportional contributions of aquatic and terrestrial sources to the diets of adult frogs and tadpoles at each site were estimated using SIAR models (Parnell et al., 2010). Variations in stable isotope values of both consumers and prey, and variation in isotopic fractionation factors, were incorporated into the models (Parnell et al., 2010). Isotopic fractionation values incorporated were 2.3 ± 0.24‰ (δ^{15}N) and 0.5 ± 0.19‰ (δ^{13}C; error terms represent standard deviations; Gillespie, 2013; Schriever and Williams, 2013). The results estimating the proportional contribution of each source to consumer diets were represented as boxplots with 25, 75 and 95% Bayesian credibility intervals (Parnell et al., 2010). The trophic positions (TP) of the various anurans were estimated as outlined in **Section 6.5.2**, except that the baseline used here was periphyton, assigned TP 1, and the fractionation factor of nitrogen per trophic level was 2.3‰ (Schriever and Williams, 2013).

Ordination (n-MDS), ANOSIM and SIMPER were performed using the qualitative fatty acid data to determine any differences within and among species and identify those fatty acids responsible for notable patterns. The data were also processed using principal components analysis (PCA) to assist in visual representation of the influential fatty acids in the n-MDS plots (factor loadings with > 0.2% contributions to variability were considered influential and superimposed in the n-MDS graphics). ANOSIM outputs include R values (1 indicates complete dissimilarity and zero complete similarity), mean ranks of variability within and between groups, and a p-value for each factor. Statistical analyses were performed using PAST 3.0 (Hammer et al., 2001).

6.7 Indigenous fish

The spatial and temporal distributions of fish populations, and the utilization of freshwater, estuarine and coastal marine habitats by different life stages, have been relatively well described in the Kowie River system (Blaber, 1973a; Bok, 1983; Whitfield, 1994; Whitfield, 1998; Whitfield, 1999; Vorwerk et al., 2003; Kruger and Strydom, 2010; Wasserman, 2010). Baseline information about the species occurring in the river, the estuary and/or the coastal ocean, as well as their life cycles, migration patterns, and general feeding strategies, is thus available. Our study focused on the role of migratory fish in connecting adjacent aquatic habitats. Anadromous fish (marine species spawning in freshwaters) are absent from South African waters (Whitfield, 1998); consequently, one catadromous and one marine estuarine-dependent species were selected based on their high abundances. The species targeted was the Cape stumpnose *Rhabdosargus holubi* Steindachner 1881 (Sparidae, Perciformes).

The Cape stumpnose is a marine estuarine-dependent species. Adults are found exclusively in nearshore marine waters, where they spawn in spring and summer. Larvae and juveniles use the estuary as a nursery before returning to the sea to spawn (Blaber, 1973a; Whitfield, 1998). Larval *Rhabdosargus holubi* feed on zooplankton, whereas juveniles have tricuspid dentition specialized for the consumption of filamentous algae, epiphytic invertebrates and aquatic macrophytes (Blaber, 1973a). However, we hypothesised that juveniles digest only the epiphytic diatoms covering leaves of plants, as they lack a means of breaking down higher plant tissues and digesting cellulose (Blaber, 1973a). At the end of the juvenile stage, the dentition of *R. holubi* develops into the molariform teeth characteristic of adults, which consume echinoderms, molluscs, crustaceans and polychaetes at sea (Blaber, 1973a; Paterson and Whitfield, 1997). The rationale behind this drastic dietary shift with development remains unresolved. For example, we do not know whether food consumed by adults in the marine environment is nutritionally superior compared with that of juveniles in the estuary, nor do we know whether this dietary shift can be explained by some other ecological requirements of the different life stages.

6.7.1 Sampling and lab procedures

Indigenous fish were collected to address **Aims 10 and 11** [hypothesis: indigenous migratory fish contribute in transferring organic matter among the freshwater, estuarine and marine sections of the Kowie system through ontogenetic variations in diet and feeding habits]. Seven sites were visited quarterly over one year (**Figure 2; Table 2**): one coastal marine site (MAR), three estuarine sites located in the lower (E3), middle (E2) and upper (E1) sections of the estuary, and three riverine stations (FW1, FW2 and FW3) in the lower section of the river (above the ebb and flow) that differed from sites visited in the other parts of the larger project. The lower section of the river was specifically targeted because of the rare occurrence of migratory fish in the upper portions of the Kowie River. We attempted to collect indigenous fish upstream at F4 (**Figure 2**) with little success. FW2 and FW3 were positioned upstream and downstream of a 5 x 50 m concrete weir and fishway located in the

Waters Meeting Nature Reserve, 21 km from the mouth of the Kowie River (**Figure 2**). FW1 was above the ebb and flow area. Each site was sampled in March/April 2012 (autumn), August 2012 (winter), November 2012 (spring), and February 2013 (summer). Additionally, eight specimens of *R. holubi* at larval/early juvenile stages (10-12 mm standard length) were collected in November 2013 at the mouth of the Kowie Estuary when larvae immigrated into the estuary from the marine environment. These additional specimens were used as representatives of the initial part of the species' life cycle; later juvenile stages residing in the estuary and adults from marine waters were collected previously.

Indigenous fish were collected using a combination of cast-nets, seine-nets, dip-nets, gill-nets and spearfishing (**Table 2**). For each species, a total of ± 10 fish was targeted at each site and period, and this value varyied depending on seasonal abundance and catchability. All captured fish were killed by gentle immersion in a cooler filled with ice (see **Section 2** for permits and ethics approval) and returned to the laboratory on ice. Fish were measured (standard, total and fork lengths, cm, hereafter SL, TL, and FL, respectively) and weighed (g). The stomachs and intestines were dissected via an incision to the mid-ventral line, from the anterior of the anal opening through the pelvic and pectoral girdles, to marginally behind the branchiostegal membrane (Willers, 1991). Fish stomachs and intestines were preserved in 4% formaldehyde for 48 hours before transferral to 70% ethanol. Two to three chunks of flesh of ~1 cm^3 were excised from the dorsal muscle of each fish, lyophilized for up to 36 hours, and stored at -80 °C.

Figure 2. Sampling sites for indigenous fish collections in the Kowie River (yellow insert and red circles), estuary (green circles), and marine area (yellow star). See **Table 2** for latitudes and longitudes.

Table 2. Sampling design used for fish collections in the Kowie system.

Site	Latitude	Longitude	Cast-net	Gill-net	Seine	Spear-fishing	Fishing rod (*M.salmoides*)
F4	S33.50434	E26.74462	X				
FW1	S33.54417	E26.78125	X	X			
FW2	S33.54593	E26.78641	X				X
FW3	S33.54557	E26.79046	X				
ES1	S33.53988	E26.80362	X		X		
ES2	S33.54759	E26.81731	X		X		
ES3	S33.58300	E26.86160	X		X		
MAR	S34.01222	E28.12833				X	

Table 3. Number of fish collected at each site. Sampling periods are autumn: March-April 2012, winter: July-August 2012, spring: November 2012, summer: February 2013. Study sites are depicted in **Figure 2**.

	Rhabdosargus holubi (Cape stumpnose)			
Site	Fall	Winter	Spring	Summer
F4	0	0	0	0
FW1	0	0	0	0
FW2	10	0	6	12
FW3	10	1	10	10
E1	29	0	10	17
E2	9	0	10	10
E3	11	21	11	11
Marine	13	2	3	15
Total	82	23	50	75
	230			

Gut contents analysis was conducted on both stomach and intestinal contents of all specimens of *R. holubi*. The preserved stomachs and intestines were washed with water to remove the ethanol, the stomach was separated from the intestine and, when possible, stomach fullness was visually quantified (%). Stomachs and intestines were cut open and their contents emptied into separate Petri dishes. The prey items found in each organ were classified into broad taxonomic groups, and their respective proportions visually estimated (% of total stomach and intestine contents, respectively) under a dissecting microscope.

Refer to **Section 6.2.2** for laboratory procedures performed to obtain stable isotope ratio data. For each fatty acid sample, 10-90 mg dry mass were used, depending on the amount of available material. Fatty acid analyses followed protocols modified by NB Richoux from Folch et al. (1957) or Indarti et al. (2005). Most samples were analysed using a one-step procedure following Indarti et al. (2005), as outlined in **Section 6.2.3**. A second, longer procedure was used for some samples (i.e. fish muscles). This protocol involved total lipid extraction followed by column chromatography for isolation of the neutral lipids in the fish tissues. Phospholipids are not as readily affected by dietary changes in secondary or higher consumers (these are the structural lipids, whereas neutral lipids represent stored lipids mainly obtained from the diet; Budge et al., 2006). Neutral lipids were used to derive fatty acid information from the fish samples. Fatty acid methyl esters (FAMEs) were created by adding sulphuric acid and methanol to each sample, and heating the mixture to 100°C for one hour.

6.7.2 Data analysis

The analytical approach for the fish component relies on a comprehensive and synthetic statistical procedure that allows for comparisons of fish trophic dynamics using complementary methods. The first stage in the data analysis consisted of summarizing habitat occupancy by different stages of *R. holubi*. This allowed us to confirm that this species actually migrates between adjacent aquatic habitats throughout its life cycle, therefore contributing in biomass and energy transfers. The average size (standard length, cm) of fish collected in different habitats and seasons was compared using ANOVA and student's t-tests. Seasonal variations in the *R. holubi* diet were assessed using sampling periods as the comparison factor (four levels: autumn, winter, spring, summer). Spatial variations were analysed following several approaches, where habitats were either based on ecosystems defined *a priori* (three levels: river, i.e., FW2 and FW3, estuary, i.e., E1-3 vs. MAR) or salinity levels, defined based on average salinities measured at each site throughout the study (three levels: low, i.e., mean salinity ≤ 15‰, medium, i.e., 15 < mean salinity ≤ 30‰, or high, i.e., marine site, with assumed mean salinity > 30‰). Fish sizes were divided into four qualitative classes based on the overall size distribution of the fish collected: very small (SL ≤ 5 cm SL), small (5 < SL ≤ 7 cm SL), medium (7 < SL ≤ 15 cm SL) or large (SL > 15 cm SL).

Ordination (nMDS) and ANOSIM were used to test for differences among seasons, habitats and fish sizes using 1) prey relative proportions (%) in stomachs, 2) $\delta^{13}C$ and $\delta^{15}N$ composition of dorsal muscles, and 3) neutral fatty acid composition in dorsal muscles. The proportional fatty acid data were used for analysis (% of total fatty acids), as relative data generally provide better statistical performance in multivariate methods. For each data set (stomach contents, stable isotopes and fatty acids), several data transformations were tested: log(x+1), presence/absence, square root and fourth root and those producing the best nMDS statistical performances (lowest stress values) were retained. As such, stomach content and fatty acid data were square-root transformed, while stable isotope data were untransformed.

R-statistic values provided by ANOSIM were used to compare the strength of spatial, seasonal or size-related variations in fish diet detected among methods. SIMPER were used to identify prey types and fatty acids responsible for differences among seasons, habitats and fish size classes. Patterns characterizing those prey and fatty acid relative proportions, and $\delta^{13}C$ and $\delta^{15}N$ compositions, were plotted as a function of season, habitat and fish size class. These statistics were performed using JMP 5.0.1 and PRIMER.v6 software.

6.8 Alien fish

6.8.1 Sampling and lab procedures

Samples to contribute towards **Aim 12** were collected from the lower Kowie River (**Figure 2**). FW1, FW2 and FW3 are consistent with the sites for collecting indigenous fish, and F4 was used in the larger study and is located further upstream, adjacent to a road causeway. *Micropterus salmoides* were collected quarterly (once every three months) with a combination of seine nets, gill-nets, dip-nets, cast-nets and conventional rod-fishing. The field sampling took place in April 2012, August 2012, November 2012 and February 2013. Difficulty was experienced in obtaining specimens from all sites, and it was possible to obtain specimens from all four sites only during the fourth sampling season. Fish were euthanized immediately after capture by immersion in a container filled with ice and then transported back to the laboratory. Due to a failure to obtain sufficient samples using gill nets during the second sampling time (30 July to 3 August 2013), a fishing competition was organised and fishermen collected the required number of samples using fishing rods. This event took place on 26 August 2012 and involved students from the Ichthyology Department at Rhodes University.

In the laboratory, fish were measured to standard (SL, mm) and total length (TL, mm), weighed (g) on a Mettler Toledo XP205 balance, and then individually labelled and frozen at -20°C. All fish dissections occurred within a few weeks of collection. Stomachs of *M. salmoides* were extracted and fixed in 10% formalin for at least seven days before transferral to 70% ethanol. The preserved stomachs were washed with distilled water to remove the ethanol. Stomach fullness was visually rated as empty, 25%, 50%, 75% or 100% full (Wasserman et al., 2011). Stomachs were cut open and the contents emptied into a Petri dish. The prey items were counted and identified under a dissecting microscope to family, genus or species level, depending upon the level of digestion (Hyslop, 1980). Invertebrates were identified using Gerber and Gabriel (2002) and Day (1981).

Grouped prey items of the largemouth bass were analysed for their volumetric contributions to all fish sampled during one period. A volumetric cylinder was partially filled with water and the volume noted in millilitres. Thereafter, one category of prey item was added and the final volume noted. The displacement volume of each prey category was obtained by subtracting the initial volume from the final volume (Hyslop, 1980). Prey and water were filtered through a fine mesh sieve, and prey were returned to their vials in preparation for gravimetric analysis. The pre-weighed vials were placed in a drying oven for 12 hours at 50 ˚C, and the dry masses of the grouped prey items recorded (mg). Results from both methods were analysed as a function of fish size for each sampling period, i.e., fish were grouped as “small”, “medium” and “large”, and prey proportions combined accordingly for each group.

For tracer analysis, three pieces of dorsal muscle were removed from each fish. The tissue type was selected based on the prevalent literature, which usually relies on analyses of fish dorsal musculature (e.g. Perga and Gerdeaux, 2005; Murdoch et al., 2013). Stable isotope samples were stored in foil envelopes, frozen at -80°C, and lyophilized with a VirTis BenchTop 2K at -60 °C for 30 hours. Dried samples were homogenized by grinding with an ethanol-cleaned mortar and pestle in preparation for stable isotope analyses. Individual homogenised samples were weighed and processed to obtain isotopic data as per **Section 6.2.2**. To determine if there was any significant effect of lipid presence on isotope samples of *M. salmoides*, lipid extraction was performed on randomly selected samples. This was done by adding a 2:1 chloroform and methanol solution into a test tube containing the isotope sample. The test tubes were capped and placed in a fume cabinet for two hours, and then the solution discarded. The procedure was repeated twice to ensure complete lipid removal. Samples were then dried at 50˚C for 24 hours, re-homogenized, and portions weighed into tin capsules using a Mettler Toledo XP205 balance. For samples intended for lipid analysis, 50 mg dry mass of muscle tissue extracted from the dorsal musculature were utilised. Fatty acid procedures were documented in **Section 6.2.3** and **Section 6.7.1**.

6.8.2 Data analysis

Stomach contents of *M. salmoides* were analysed using the index of relative importance (IRI; Hyslop, 1980), calculated as follows:

$$IRI = (\%N + \%V) \times \% F,$$

where %V is the volume of each food category expressed as a percentage of the total volume of all food items, %N is the number of individuals as a proportion of all prey items, and %F is the number of stomachs containing a certain food item expressed as a percentage of all stomachs in the sample (relative frequency of occurrence). For each prey category, the %IRI was determined as proportion of IRI values of all prey categories (e.g, $\%IRI= (IRI / IRI_{tot}) \times 100$; Wasserman et al., 2011).

ANOVA were used to test for variations in isotope signatures of *M. salmoides* among sites, seasons and size classes after validating conditions of data normality. Linear regressions were used to investigate the relationships between $\delta^{13}C$, $\delta^{15}N$ and C:N and fish size. The effect of lipid content on isotope signatures was tested using a one-way ANOVA. Due to significant variation in the $\delta^{15}N$ and $\delta^{13}C$ values of *M. salmoides*, individual biplots representing food webs were constructed for each site. SIAR models were used to estimate the contributions of prey items to the diet of *M. salmoides*. SIAR models were completed for fish in different size classes, and from different seasons and sites. The data entered into each model were the $\delta^{15}N$ and $\delta^{13}C$ of consumers, and the mean $\delta^{15}N$ and $\delta^{13}C$ values and standard deviations of potential prey. Following McCutchan et al. (2003), the trophic enrichment factors used were 2.8±0.40‰ for $\delta^{15}N$ and 1.1±0.35‰ for $\delta^{13}C$. Prey incorporated into the models (fish and invertebrates) were derived from the other sections of this document.

For the lipid analysis, the main focus was on the proportional fatty acid data. PCA of proportional fatty acid data were used to explore the within-species similarities and variance of *M. salmoides* samples across the three size classes and among the four sites. All proportional fatty acid data were arcsine square root transformed to minimise the effects of the dominant fatty acids. The PCA for predator fatty acids was performed separately from the food sources, and a third PCA was used to investigate the relationships between the consumer *M. salmoides* and the potential food sources together. In all cases, only fatty acids which contributed more than 1%TFA were included.

6.9 Birds

6.9.1 Sampling and lab procedures

To contribute towards **Aims 8 and 9**, waterbirds were captured in the lower estuary (**Figure 3**; collection locations were stipulated by Mr Fanie Fouche, Regional manager of Department of Environmental Affairs, Port Alfred). Collections of waterbird tissues included sacrificing some individuals of each focal species using approved methods (see **Section 2** for collecting permits and ethical clearances) so that sufficient material could be collected while leaving as small an imprint as possible on the community.

Individuals of Cape Shoveler (*Anas smithii*), Yellow-bill Duck (*Anas undulata*), Cape Teal (*Anas capensis*), Little Egret (*Egretta garzetta*) and Ruff (*Philomachus pugnax*) were collected from the mudflats of lower reaches of the Kowie Estuary. Using DEA approved methods (rifle shot to cause instantaneous death), up to five individuals of each species were collected in winter (June-August 2013), spring (September-November 2013), summer (December 2013-February 2014) and autumn (March-May 2014). Ruffs were captured only during spring/summer, as they are seasonal migrants from Siberia and eastern Europe. Blood samples were collected immediately, placed into lithium-heparin lined blood collection tubes (LASECTM mini-collect VGRV450478) and kept on ice. Individual birds were placed into sealed plastic bags and placed on ice until laboratory analysis.

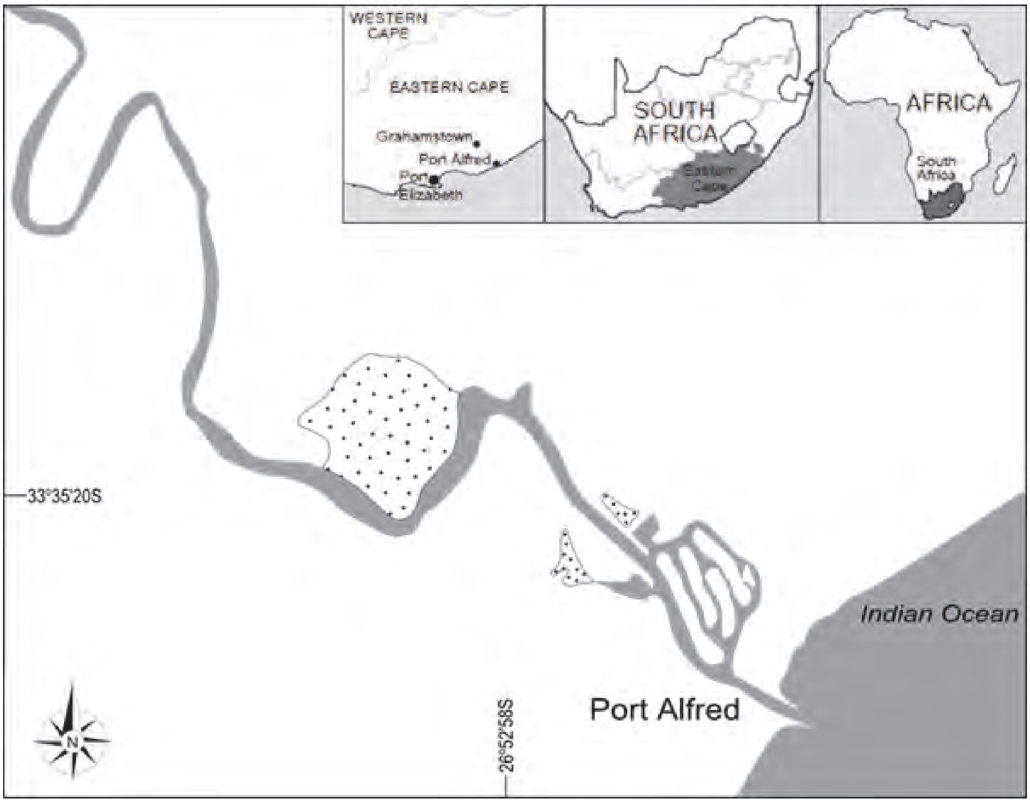

Figure 3. Waterbird collection and observation sites (dotted areas) within the reaches of the Kowie River estuary, Port Alfred.

Morphological measurements (wing length, bill length, total mass and moulting stage) of each bird were recorded, and the birds were dissected. The entire digestion system from stomach to rectum were removed and placed into 50ml plastic jars with 30ml of formalin until stomach content analysis could be conducted. Stomach samples were analysed under a dissecting microscope, and all identifiable diet items recorded. Each diet item was identified to species (where possible) and the total numbers of each species and percentage of total stomach contents calculated.

Samples of breast muscle, liver and adipose tissue were collected and placed into foil envelopes. These samples were immediately placed at -80°C for 72 hours and then lyophilized. Blood samples were separated into their constituent components by centrifuging at 2000 g (6000 rpm; C1008-G LASECTM mini centrifuge) for 15 min. Sub-samples of whole blood, blood plasma and red blood were placed onto 1.2 µm 47 mm glass fibre discs (MunktellTM; grade MGC) for stable isotope processing. Freeze dried bulk materials homogenised using a mortar and pestle and weighed into tin capsules for isotope analysis. Primary flight feathers, non-flight chest feathers, down feathers from the chest, and claw samples were collected and placed into 15 ml glass vials. These samples were cleaned of lipids using 1 ml chloroform:methanol (2:1) mixed with 1ml of MilliQ water, dried in a 50 °C oven, homogenized and weighed into tin capsules for stable isotope analysis.

6.9.2 Data analysis

Analysis of variance (ANOVA) was used to determine whether $\delta^{13}C$ and $\delta^{15}N$ stable isotope values were significantly different among seasons in each species. Mean $\delta^{13}C$ and $\delta^{15}N$ values for each species per season were used in SIAR models to determine the proportional contributions of selected food items from the estuarine environment into the bird diets. The trophic level of each waterbird species was calculated per season, and for the whole study year, using the equation outlined in **Section 6.5.2.,** with the trophic fractionation of 2.9‰ used between adjacent trophic levels (DeNiro and Epstein, 1981).

SIAR models were run for each species of waterbird using mean $\delta^{13}C$ and $\delta^{15}N$ values of all individuals sampled for each season, and potential prey were selected from the estuarine invertebrates (**Section 7.5;** Bergamino et al., 2014b). Preliminary biplots using $\delta^{13}C$ and $\delta^{15}N$ data from each waterbird species and potential prey were used to determine which prey would be best suited for the models. Because of non-significant differences in the $\delta^{13}C$ and $\delta^{15}N$ of certain potential prey items, $\delta^{13}C$ and $\delta^{15}N$ data were pooled to create one unique prey item source value, i.e. *Hymensoma obiculare* + *Sesarma catanata* ="crabs", and Amphipoda + Copepoda + Mysidacea + crab zoea = microinvertebrates. The following potential prey items were used in the mixing models: 1) Cape Shoveller and Cape Teal: *Palaemon peringuyii*, *Spartina maritima*, microinvertebrates and crabs, 2) Yellow-bill Duck: *P. peringuyii*, Polychaeta, *S. maritima* and microinvertebrates, 4) Little Egret: *P. peringuyii*, crabs, *Mugil cephalus*, *Myxus capensis* and *Sole blekerii*, 5) Ruff: Mysidacea, Amphipoda, Copepoda, Isopoda and crab zoea.

Convex hulls and Bayesian ellipses were created using $\delta^{13}C$ and $\delta^{15}N$ stable isotope values for each species using SIBER (stable isotope Bayesian ellipses in R; Jackson et al. 2011). The SIBER program is sensitive to small sample sizes, and results may be skewed or misrepresented when samples sizes < 6 are used. Therefore, winter and spring $\delta^{13}C$ and $\delta^{15}N$ stable isotope values were pooled to represent the "winter" isotopic niche of each species. Similarly, summer and autumn samples were pooled to create the "summer" niche. In addition, all $\delta^{13}C$ and $\delta^{15}N$ stable isotope values of each species across all seasons were pooled to create the "yearly" isotopic niche. Bayesian ellipse size and overlap of each species were calculated using SIBER and statistically compared using ANOVA.

Radial diagrams were produced to show the direction and magnitude of change in the mean $\delta^{13}C$ and $\delta^{15}N$ values of each species seasonally. For example, the mean $\delta^{13}C$ and $\delta^{15}N$ values of individuals from each species collected in winter was subtracted from the mean $\delta^{13}C$ and $\delta^{15}N$ values of individuals of the same species collected in spring. These new x ($\delta^{13}C$) and y ($\delta^{15}N$) co-ordinates were then used to calculate "r" (the hypotenuse of a right angled triangle). Theta was then calculated using trigonometry. The resulting "r" and "theta" values were plotted onto a radial plot which describes the magnitude (i.e. difference in ‰) and direction of change in the mean $\delta^{13}C$ and $\delta^{15}N$ stable isotope values as one season shifts to the next.

6.10 Bloukrans tributary

6.10.1 Sampling and lab procedures

The Bloukrans River is a tributary of the Kowie River that drains from the residential area of Grahamstown. The river is subject to various anthropogenic sources of pollution such as domestic garbage, overflowing sewage, agricultural inputs and livestock. As the river leaves Grahamstown, there is a wastewater treatment plant which discharges volumes of partially treated organic effluent which eventually increases water velocity. The lower sections of the river are largely impacted by agriculture through irrigation. Further downstream is the Bloukrans Nature Reserve with a pool that is regularly used for religous and traditional practice. The water quality within the Bloukrans River affects downstream river and estuary sections of the Kowie system, and therefore the Bloukrans River is of both ecological and social importance.

The main objective of this aspect of the project (**Aim 14**) was to establish how benthic macroinvertebrate assemblages in the Bloukrans River system vary across a human activity gradient. The primary assumption was that the main source of variation in community composition across the activity gradient may reflect a shift from intolerant to tolerant taxa as the extent of human activity increases. More specifically, this aspect of the larger study addresses the following three questions: (1) do the community assemblages differ in relation to changes in environmental stressors, (2) do the feeding relationships among members of the community differ in relation to these environmental stressors, (3) how different are the Bloukrans communities relative to the main pristine Kowie River channel.

The collection locations were based on the pollution gradient, with the pollution levels increasing upstream (i.e. from B4 to B1; **Table 4, Figure 4**). Samples were collected every three months over two day periods (two sites per sampling day) in April, July, and October 2013 and January 2014.

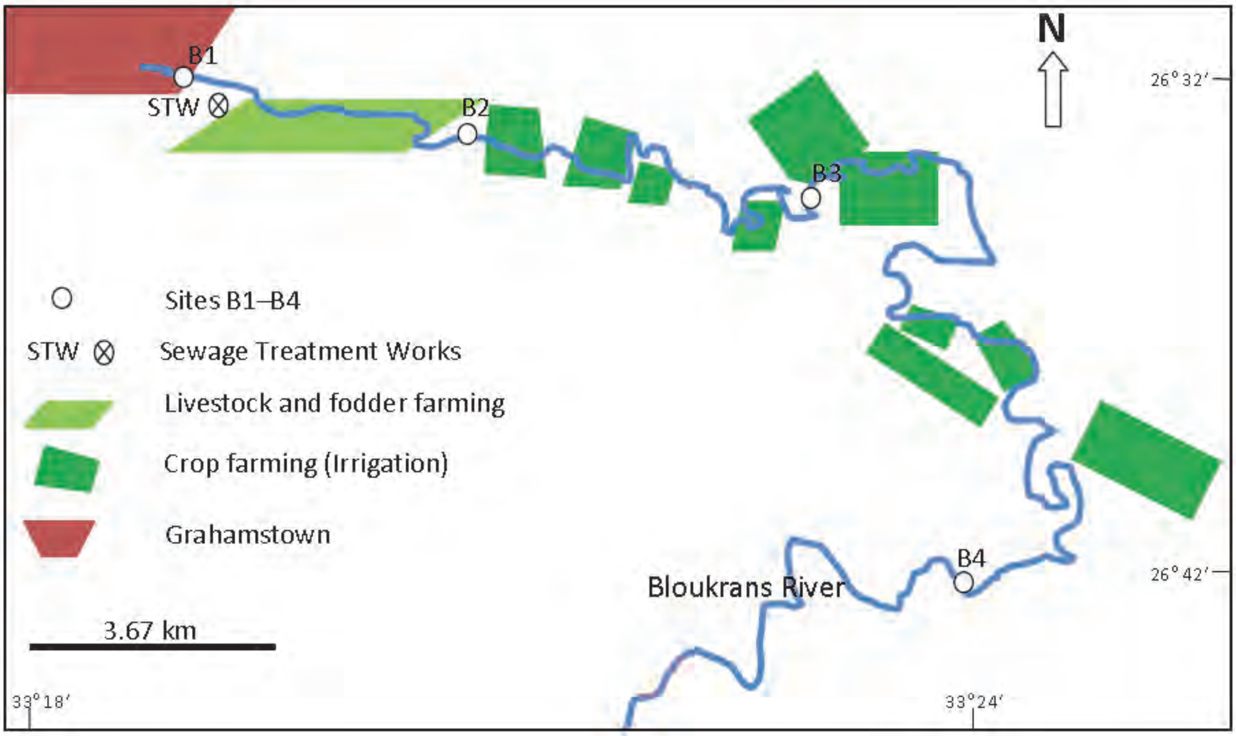

Figure 4. Locations of four study sites along the Bloukrans River (B1 to B4). Site B4 is equivalent to Site F6 in the larger study.

Table 4. Coordinates of sampling sites in the Bloukrans River.

Site Name	**Coordinates**	
	South	**East**
B1	33°18'51.9"	26°33'07.01"
B2	33°19'25.1"	26°36'01.81"
B3	33°19'47.08"	26°38'47.41"
B4	33°23'29.04"	26°42'32.60"

To establish the environmental quality at different regions of the river, we measured the following variables: flow rate, temperature, pH, conductivity, total dissolved solids (TDS), salinity (chlorides), resistivity, oxygen reduction potential, dissolved oxygen, suspended solids, N-ammonium, N-nitrites, N-nitrates, and P-phosphates. A water proof portable multi-probe meter (Cyber Scan Series 600, Singerpore) was used to measure the temperature, pH, conductivity, sodium chloride, resistivity and total dissolved solutes. Stream flow was measured using a portable flow meter (Marsh McBirney 2000, USA), and dissolved oxygen was measured using a portable device (YSI- incorporated 550A, USA). Channel depth and width were measured using a tape measure.

Water samples for nutrient and chlorophyll analysis were collected in to 500 mL polyethylene bottles and stored on ice. For the nutrients, a 250mL of the sample was analyzed using chemical reagent test kits and a multi-parameter Bench photometer (HI

83203, Hanna Instruments Inc., USA). The remaining 250 mL was filtered through 20µm nylon net mesh filters, 2µm isopore membrane filters and 0.7µm glass microfiber filters using vacuum <5 cm Hg and analyzed to determine chlorophyll *a* concentrations. These filters were soaked in 90% acetone for 24 hours in the dark and then analyzed using a Turner 10AU fluorometer.

Macroinvertebrates were collected using a 35 cm x 35 cm kick sampler SASS net with 0.08 mm mesh, and the catch placed in a tray for further sorting. The net was vigorously pulled in the upstream direction while moving back and forth through the same area with the net kept below the water surface. Hand picking was done where animals were seen attached on rock substrates. The animals were sorted in white rectangular trays and placed into 250ml plastic sample jars with 70% ethanol, and eventually counted and identified to family level using the Gerber and Gabriel (2002) field guide and placed in foil envelopes.

6.10.2 Data analysis

Macroinvertebrate community structure was evaluated using biotic indices. Macroinvertebrates were categorized into functional feeding groups according to Cummins et al. (2005) to investigate their responses to water quality fluctuations. For example, the ratios of filtering collectors and scrapers to the total abundance were calculated.The communities were analysed for total abundance, taxon richness, Shannon diversity index, percentage of Ephemeroptera, Plecoptera and Trichoptera (EPT) richness index, and the ratio of EPT to Chironomidae. The South African Scoring System (SASS) for invertebrates was used to assess the water quality at each site. Developed by Chutter (1998), SASS has been adopted as the standard method for water quality assessments of South African and Southern African rivers (Dallas, 1997). The SASS method of analysis allocates a score from 1-15 based on the degree to which a macroinvertebrate taxon (family level) is sensitive or tolerant to pollution. The most sensitive family receives the highest score (up to15), and the most tolerant a low score (down to 1). Generally, the higher the scores, the more sensitive the animals are to pollution. The Average Score per Taxon (ASPT) indicates the average tolerance score of all identified taxa within the community, calculated by dividing the SASS scores by the number of families present in the sample per site. The reference scoring criteria for SASS scores and ASPT are shown in **Table 5**.

Table 5. The standard scoring criteria for SASS Scores and ASPT used as water quality measures for the Bloukrans River. Information extracted from Chutter (1998).

SASS Scores	ASPT	State of water quality
> 100	> 6	Natural water quality with high habitat diversity
< 100	> 6	Natural water quality with reduced habitat diversity
> 100	< 6	Water quality may be natural but starts deteriorating
50-100	< 6	deterioration in water quality
< 50	ASPT variable	Major deterioration in water quality

To analyse the SASS scores, information about the in-stream and riparian habitat characteristics were analysed following a method by Dallas (2005). An index of habitat integrity (IHI) for riparian zone and in-stream was then determined using the formula:

$$\mathrm{IHI} = 100 - [\left(\frac{\Sigma\left(\frac{\mathrm{Mg}}{\mathrm{MV}}\right) \times \mathrm{Wt}}{\mathrm{MV}}\right) \times 100]$$

where: IHI= index of habitat integrity, expressed as a percentage, Mg = criterion rating values, Wt = criterion weight, MV = the total maximum scaled value per criterion (based on the weights of identified criterion). Substratum composition was characterized by giving scores for different subtrates present within the river bed using the standard method derived from Dallas (2005).

Normality tests on the data set were performed using Shapiro-Wilks and statistical effects of environmental variables were tested using Kruskal-Wallis tests, and differences among macroinvertebrate taxa using one way ANOVA in STATISTICA 12 (StatSoft. Inc. 1984-2013). Redundancy analysis (RDA) was conducted using PC-ORD software (version 5.10, 2006) for determining the redundancy of 43 macroinvertebrates taxa with 18 environmental parameters.

6.11 Flux model

6.11.1 Model design

We have developed a multi-phase model, whereby complexity is added in a stepwise manner to achieve **Aim 15**: a spatially explicit model of the Kowie River system with terrestrial/aquatic interactions, multiple trophic levels, and the integration of disparate food webs. This ambitious platform can serve as an invaluable management tool, and a theory-building initiative, whereby the fate of biologically conserved molecules can be traced through multiple food webs and locations.

The first phase involved the mapping out of lower food web interactions and mass flows at one site. Additional complexity has been incorporated in the forms of higher trophic levels (i.e., fish and amphibians), and including the effects of emergence and infall, linking terrestrial and aquatic ecosystems. This model building process can be repeated for each study site, after which they can be stitched together. An added complexity factor will be the migratory characteristics of species from upstream to downstream sites. We have chosen to use a steady-state modelling approach for this first phase for two reasons. First, data availability is inconsistent across sites and trophic levels. As such, the risk of propagating framework error in a dynamic setting is high. Second, uncovering the interactive dynamics between the various trophic levels and food webs in the Kowie River system is an on-going task. Using a steady-state approach afforded us the ability to *learn the system*, while estimating parameters and fractions not available in our observed data sets. Using parameter estimates and lessons learned from food web building and ecosystem-stitching, our modelling efforts shifted to phase two. We intend to incorporate a molecular layer on top of the developed ecosystem models to elucidate the flow of biologically conserved molecules across/between ecosystem components. Using this layered approach, the transport of molecules across boundaries (aquatic/terrestrial, saline/freshwater, upstream/downstream) can be studied, uncovering potential *keystone vectors*.

6.11.2 Software

Ecopath with Ecosim (*EwE*) is a modelling framework based on energetic budgets developed by Jeff Polovina (Polovina, 1984). *EwE* is comprised of three individual software suites: a static, mass balance analysis tool (*Ecopath*); a time dynamic simulation platform (*Ecosim*); and a dynamic spatial and temporal simulation platform (*Ecospace*) (Christensen and Walters, 2004). *Ecopath* utilizes a linear governing equation for balancing food web energetics and production. Eq. (1) expresses the steady-state model for each ecosystem component:

$$(1)\ B_i\left(\frac{P_i}{B_i}\right)EE_i = Y_i + \sum_{j=1}^{n} B_j \left(\frac{Q}{B}\right)_j DC_{ji}$$

where B_i is the biomass of group *i* during the study period, the ratio $(P/B)_i$ is the production/biomass of functional group *i* and is equal to total mortality under the equilibrium assumption, EE_i is the ecotrophic efficiency, i.e., the fraction of production consumed, Y_i is group *i*'s yield, B_j is the biomass of the consumer *j*, while the fraction $(Q/B)_j$ is the consumption/biomass ratio of consumer *j* and DC_{ji} is the fraction of group *i* in the diet of group *j*.

EwE can be used to compute parameters and indices corresponding to food web characteristics. In addition, it includes a suite of network analysis tools that can be leveraged for analysis of non-linear dynamics in complex food webs. Examples include system response to species' invasion (see Bondavalli et al., 2000; Feroz Khan and Panikkar, 2009; Villanueva et al., 2008) and general external perturbations (see Christensen and Pauly, 1992; Walters et al., 1997; Walters et al., 1999). *EwE* is currently in its sixth generation, and it has been used to model various ecosystems around the world, yielding insight into system dynamics and evolution (Christensen and Pauly, 1992; Walters et al., 1997; Walters et al., 1999). *EwE* is freely available from www.ecopath.org, and for a detailed overview see Christensen et al. (2005).

6.11.3 Parameterisation

The model was parameterised to represent the average state of F4's ecosystem spanning the months of September to January. Based on ecological significance, the organisms inhabiting F4 were grouped into nine functional groups. **Figure 5** illustrates the approximate trophic hierarchical breakdown of these functional groups. The apex group (predators) includes *Aeshnidae, Pseudagrion* spp*., Belostoma* spp*., Tabanidae, Hydaticus bivittatus, Lestes* spp*., Aulonogyrus* spp*., Appasus* spp*.,* and *Nepidae*. The mid-trophic level groups include the shredders (*Potamonautes* spp*.* and *Leptocerus* spp*.*), grazers (*Baetidae, Leptophlebiidae, Caenis* spp.), and filter-collectors (*Simulium* spp*., Hydropsyche* spp*.*). The generic primary producers (i.e., periphyton, phytoplankton, epiphyton, epipelon, and POM) were labelled "aquatic vegetation". We separated largemouth bass (*Micropterus salmoides*) into two categories to reflect dietary differences: adults and young-of-the-year (YOY). Adults were classified as fish with a length greater than 30 cm, while YOY fish were those less than 30 cm (Hossain et al., 2013). Anuran species (*Amietia angolensis*, *Amietophrynus rangeri*, *Strongylopus grayii*, *and Xenopus laevis*) were combined into an important group that linked the aquatic and terrestrial habitats. Spiders were divided into three categories to reflect dietary differences: horizontal, vertical, and tangle web builders. Previous research has suggested that differences in diets can arise from differences in web orientation and architecture (e.g. Akamatsu et al., 2004).

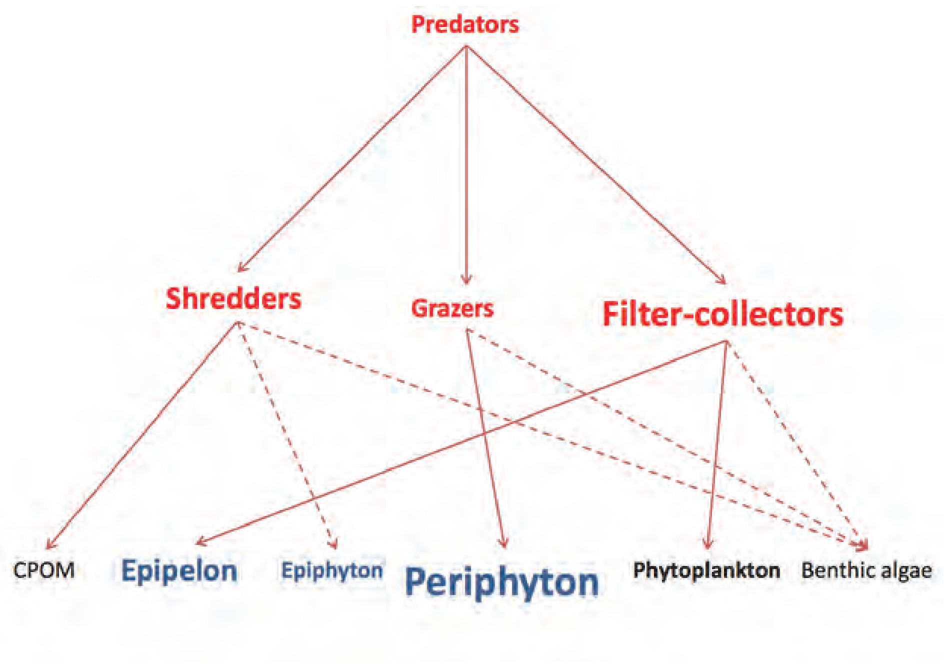

Figure 5. Functional groups and hierarchy for F4, the food web in the lower freshwater reaches. Consumers are depicted in red, and basal sources in black or blue. Larger fonts indicate a larger relative biomass.

6.11.4 Biomass estimates

Biomass estimates were derived from population estimates (**Tables 6** and **7**). To account for the sexual dimorphism in frogs and toads that lead to a size differentiation in males and females, biomass estimates were averaged for males and females and the biomass estimate for a given species was based on an average of the two sexes. This procedure assumes an equal number of males and females in the population and accounts for any sampling biases with regard to gender. Estimations for POM were obtained from drift measurements. Drift from the river was collected and weighed as dry weights. These dry weights were converted to spatial densities using stream flow rate, sampling time, and the net length. For groups containing multiple species, the biomass was estimated as a function of species abundance, sampling area, total area, and mass or spatial density for a given species.

Table 6. Predator, shredder, grazer, and filter-collector biomass data for the initial model.

Sampling Date	Functional Group	Individuals/m^2	Dry Mass (mg/m^2)	SD
Thursday, September 13, 12	filter-collector	93	62.28	8.75
Tuesday, December 4, 12	filter-collector	90	63.82	16.66
Wednesday, February 20, 13	filter-collector	158	148.42	45.23
Wednesday, December 4, 13	predator	13	2605.89	222.51
Friday, September 13, 13	predator	82	6616.44	558.29
Wednesday, February 20, 13	predator	67	6971.78	568.83
Tuesday, December 4, 12	shredder	44	8637.07	131.15
Friday, September 13, 13	shredder	117	8644.70	148.78
Wednesday, February 20, 13	shredder	34	36442.61	584.43
Friday, September 13, 13	grazer	63	52.37	5.10
Wednesday, December 4, 13	grazer	53	23.77	2.51
Wednesday, February 20, 13	grazer	48	30.04	3.02

Table 7. Primary production and aquatic vegetation biomass data for the preliminary model.

Functional Group	Sep (t km^{-2})	Dec (t km^{-2})	Feb (t km^{-2})
periphyton	5.821	1.873	9.857
phytoplankton	38.8	50.8	68.667
epiphyton	1.356	0.390	2.254
POM	46.1	87.4	66
epipelon	1.677	1.371	1.711

6.11.5 Diet composition

For fish species found in the Kowie River system, their gut contents were used to develop a diet matrix of the percentage of each consumed species. For largemouth bass, the main group was split into two to allow for cannibalism to be reflected in the model; adult fish preyed on YOY fish. Gut analyses from frog and toad species were averaged to compose a diet breakdown for the overall frog and toad group. Diet matrices for the remaining species, where gut content analysis was unavailable, were obtained from literature.

6.11.6 Production and composition rate estimation

Production per biomass estimates for fish were calculated using formulae derived by Randall and Minns (2000), depending on the category of the fish (adult vs. juvenile). Weight-at-maturity (W_{mat}) estimates for mature largemouth bass were calculated as the average weight of fish over 30 cm using an allometric relationship with maximum weights:

(2) $\log W_{mat} = -0.762 + 0.931 \log W_{max}$

Production per biomass estimates for these individuals were calculated as follows:

(3) $\frac{P}{B_W} = 1.32 \times W_{mat}^{-0.35}$

We applied the following formula for fish under 30 cm to calculate production per biomass estimates:

(4) $\frac{P}{B} = \frac{K(L_\infty - \bar{L})}{\bar{L} - L_c}$

where L_c is the minimum body length, $\bar{L}$ is the average body length, and K and L_∞ are parameters from von Bertanlaffy's growth equation estimated from length measurements:

(5) $L_t = L_\infty(1 - e^{-K(t-t_0)}$

For fish species with insufficient data, weight-at-maturity estimates were obtained from Fishbase.

Consumption rates were estimated through empirical relationships based on the von Bertanlaffy equation, water temperature, and caudal fin measurements (Palomares and Pauly, 1988):

(6) $\log\left(\frac{Q}{B}\right) = 7.964 - 0.204 \log W_\infty - 1.965T' + 0.083A + 0.532h + 0.398d$

where W_∞ is a parameter from the von Bertanlaffy weight growth equation, T is the mean annual water temperature whereas T' expressed a function of T in Kelvin (Kelvin = ˚C + 237.15),

(7) $T' = \frac{1000}{Kelvin}$

A is the aspect ratio of the caudal fin, and h and d reflect to what extent the group is herbivorous or detritivorous. For herbivorous groups, h = 1 and for detritivorous groups, d = 1; otherwise, these numbers were set to equal 0, and:

(8) $W_{\infty} = aL_{\infty}^{b}$

The aspect ratio was calculated as a function of the height of the caudal fin (l) and the surface area of the fin (s):

(9) $A = \frac{l^2}{s}$

Caudal fin measurements were sampled from Fishbase.

7 RESULTS AND DISCUSSION

7.1 Environmental, physical and chemical data

Monthly average discharges (m^3/s) of the Kowie River were 0.29 in September 2012, 0.54 in December 2012, 0.55 in February 2013 and 1.10 in June 2013 (Department of Water Affairs and Forestry database, http://www.dwaf.gov.za/hydrology; **Figure 6**). In October 2012, the Kowie catchment experienced flooding conditions, hence the highest water discharges with a monthly average of 94.1 m^3s^{-1}, followed by a decrease in November 2012 with an average of 2.2 m^3s^{-1} (**Figure 6**).

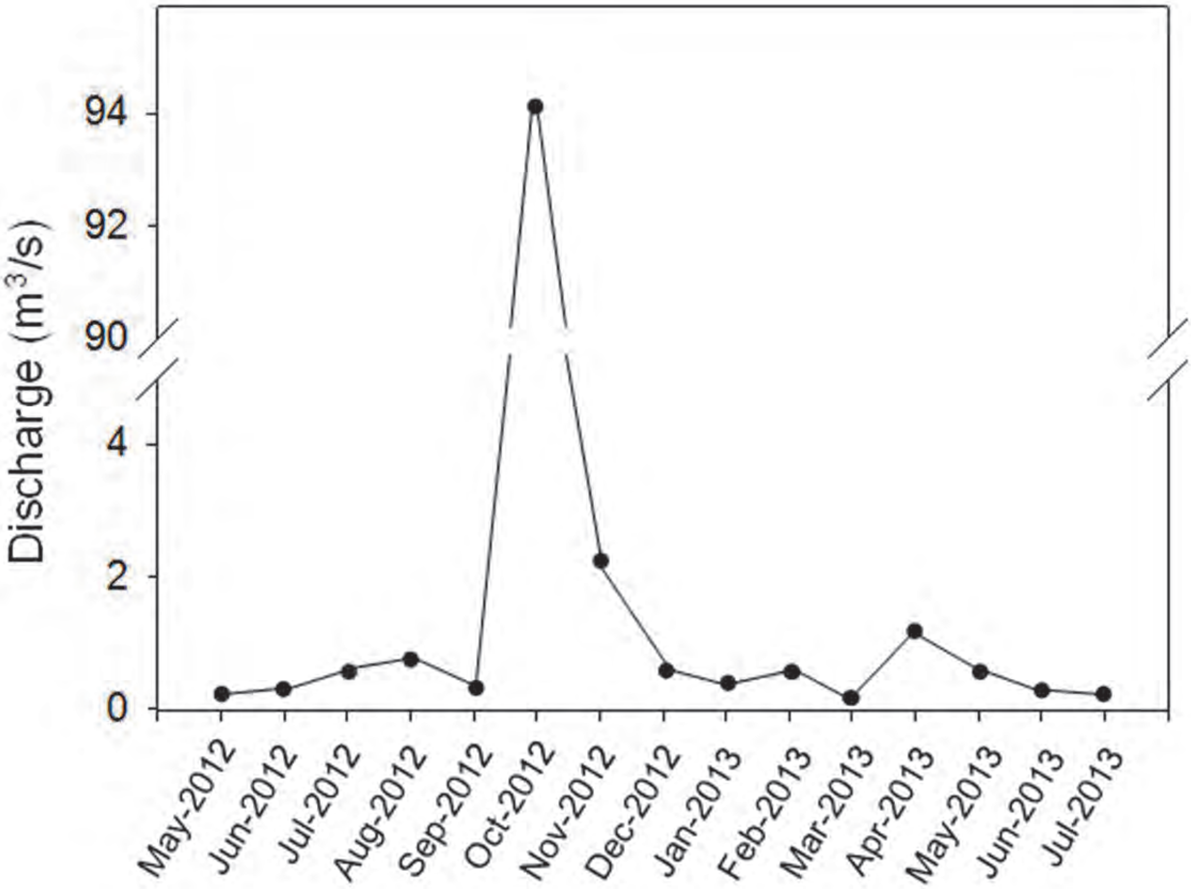

Figure 6. Monthly averages of discharge (m^3/s) in the Kowie system from May 2012 to July 2013. Data were sourced from the Department of Water Affairs and Forestry database, South Africa, http://www.dwaf.gov.za/hydrology

<u>Chlorophyll *a*</u>

Chl-*a* concentrations generally increased from the headwaters towards the downstream direction, with a peak in midstream, after which concentrations decreased again (**Figure 7**). The freshwater sites F1 to F5 had higher chl-*a* values (up to 15.9 mg m^{-3}) compared to the estuarine sites (up to 5.3 mg m^{-3}). Lower nutrient levels and differences in physico-chemical factors that affect phytoplankton communities may have influenced the decreased chlorophyll contents in the estuarine waters. Particularly high chl-*a* concentrations were observed after flooding occurred during October 2012 (**Figure 7**). In May/June, the chl-*a* concentrations were >1 mg m^{-3} at sites F1 to F4, and increased to a maximum at F5 (3.1 mg m^{-3}). Two-way ANOVA showed significant differences in chl-*a* concentrations over time (F = 3.68, p = 0.025) and among sites (F = 9.97, p = 0.004).

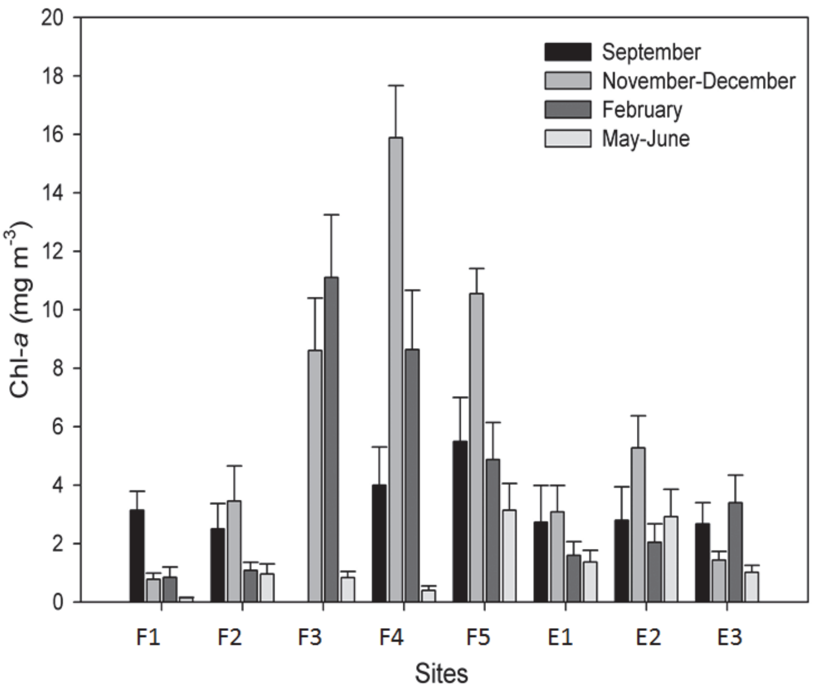

Figure 7. Chlorophyll-*a* concentrations (± standard error) along the Kowie system continuum (September 2012 to May/June 2013). No data available for Sept at F3.

The chl-*a* biomass in the Kowie system conformed to the general principles of the RCC (Vannote et al., 1980) in that the greatest autochthonous production (in suspended form in this case) occurred in the midstream regions owing to the decreased canopy cover. Further downstream the photosynthetic rates may have been hampered by the higher turbidity, mixing and shifts in other factors such as salinity.

Some of the other physico-chemical factors measured varied over space and/or time (**Table 8**), with values of most of the factors increasing in a downstream direction. Electrical conductivity ranged from 0.4 to 3.4 mS cm^{-1} in the river and from 4.1 to 36.8 mS cm^{-1} in the estuary. Salinity ranged from 0.1 to 1.8 in the river and from 2.0 to 27.4 in the estuary, while total dissolved solids were between 0.2 and 7.7 ppt in the river and between 2.8 and 29.1 ppt in the estuary. Stream depth ranged from 0.4 to 6.6 m and stream width ranged from 0.8 to 16.4 m. Ammonia concentration was higher in the estuarine sites (up to 1.04 mg L^{-1}) than in the riverine sites (up to 0.5 mg L^{-1}). Nitrate concentration ranged from 0 to 37.4 mg l^{-1} in the river and 0 to 24.4 mg L^{-1} in the estuary. Phosphate concentration ranged from 0 to 9.3 mg L^{-1} in the river and 0 to 6.2 mg L^{-1} in the estuary). Three factors (dissolved oxygen, temperature and water flow) showed significant differences among the four periods, whereas conductivity, total dissolved solids, salinity, water depth and ammonia differed significantly among sites (**Table 8**).

Table 8. Mean (± standard error) and Kruskal-Wallis ANOVA summary of physico-chemical factors and diversity indices at different sites and season along the riverine-estuarine continuum (Kowie system). Bolded Kruskal-Wallis statistics indicate $p < 0.05$
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parameters	**Sept 2012**		**Nov 2012**		**Feb 2013**		**June 2013**		**Kruskal-Wallis output**	
	estuary	river	estuary	river	estuary	river	estuary	river	period (df = 3)	site (df = 1)
dissolved oxygen (mg L^{-1})	6.64±0.42	6.55±0.41	4.76±0.12	6.23±0.31	5.83±0.23	5.56±0.49	7.33±0.12	7.36±0.46	**7.08**	0.13
conductivity (mS cm^{-1})	13.16±7.24	1.69±0.45	14.73±10.4	2.43±0.54	16.62±9.79	1.83±0.42	19.72±8.79	1.90±0.47	0.12	**20.80**
total dissolved solids (ppt)	8.68±4.88	2.63±1.49	9.86±6.94	2.07±0.15	11.11±6.51	1.23±0.28	14.50±7.42	1.24±0.31	0.08	**15.94**
salinity	7.24±4.39	0.85±0.23	8.51±6.34	1.50±0.11	9.55±6	0.91±0.2	13.05±7.27	0.88±0.23	0.20	**18.73**
resistivity (Ω)	75.63±32.9	1928±1488.7	88.39±36.5	446.66±259	62.21±27.5	556.1±303	42.16±18.7	539.2±279	0.62	0.01
pH	8.04±0.01	7.48±0.38	6.96±0.28	7.81±0.35	7.51±0.13	7.1±0.35	8.06±0.01	7.78±0.32	1.42	2.43
temperature (ºC)	19±0.42	15.7±0.61	25.1±0.40	21.76±0.71	25.17±0.96	22.28±1.39	13.23±0.47	13.96±0.45	**39.15**	1.42
oxygen reduction potential (mV)	-68.6±0.92	-29.83±19.89	22.27±29	-45.4±21.3	-85.07±8.43	-61.02±22.3	-79.87±0.7	-64.4±17.4	2.61	2.50
depth (m)	3.56±0.78	0.68±0.10	4.66±0.98	0.92±0.19	3.56±0.77	0.77±0.18	3.37±0.82	0.76±0.2	0.48	**45.47**
water flow (m s^{-1})	0.13±0.06	0.15±0.01	0.21±0.06	0.52±0.1	0.24±0.04	0.36±0.09	0.26±0.09	0.16±0.09	**3.72**	4.18
ammonia (mg L^{-1})	0.43±0.31	0.10±0.05	0.29±0.05	0.15±0.05	0.55±0.09	0.26±0.06	0.63±0.12	0.10±0.05	0.99	**13.25**
phosphate (mg L^{-1})	2.13±2.03	0.95±0.54	0.23±0.07	2.12±1.8	0.13±0.09	0.68±0.31	1.33±0.79	0.68±0.2	0.39	0.01
nitrate (mg L^{-1})	0.00	0.5±0.31	3.67±1.86	10.58±7.3	13.33±5.54	3.18±0.91	2.97±0.97	5.2±3.21	1.25	0.14
chl–*a* (mg m^{-3})	2.73±0.04	3.78±0.65	3.26±1.11	7.85±2.66	2.34±0.54	5.30±2.03	1.76±0.58	1.09±0.53	**3.68**	**9.97**

7.2 Basal resources

7.2.1 Stable isotope ratios

Spatial and temporal variability in basal signatures

Significant variations were detected in stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) signatures ($p < 0.05$) in benthic algae, SPM, terrestrial leaves and $\delta^{15}N$ for detritus, while spatial variation was not detected in detritus $\delta^{13}C$ ($p > 0.05$; **Table 9**). Pairwise comparisons of detritus $\delta^{13}C$ values indicated significant differences between sites F2 and F4 [t = 2.28, p (Monte-Carlo (MC)) = 0.0279] and between November and February [t = 5.54, p (MC) = 0.0114]. The $\delta^{13}C$ values of benthic algae were relatively high (-10 to -25‰) overall, while SPM had low $\delta^{13}C$ values (-24 to -32‰) compared to other organic matter sources. The estuarine region (sites E1 to E3) had low $\delta^{13}C$ values in SPM (-22 to -26‰), and the SPM in middle regions of the system (freshwater areas) were even lower (-28 to -32‰). The $\delta^{13}C$ values of most organic matter sources decreased from upper to mid reaches of the entire system before increasing at the most downstream sites (**Figure 8**). In benthic algae and detritus, the $\delta^{13}C$ values increased in the downstream direction. The $\delta^{15}N$ values in all sample types increased in the downstream direction before decreasing slightly in the lowest reaches during all study periods (**Figure 9**).

The SPM had a wide range in $\delta^{13}C$ values (-22.5 to -32.1‰) throughout the study, and detritus a more narrow range (-25.5 to -31.1‰), but a greater range in $\delta^{15}N$ values (**Figures 8 and 9**). The $\delta^{13}C$ values of terrestrial leaves ranged from -25.2 to -30.4‰ throughout the study, but values in the benthic algae varied significantly over time (range -12.9 to -28‰; $p < 0.05$; **Figure 8**). The benthic algae (range 4.1-14.7‰) and terrestrial leaves (range 1.4 to 13.5‰) had low $\delta^{15}N$ values relative to other basal sources. High C:N ratios (8.9 to 19.2) were measured in benthic algae and low ratios (7 to 9.3) in SPM in Sept. In November, the C:N ratios of SPM were low before increasing in February. C:N ratios of SPM ranged between 7.5 and 8.6 in Sept, from 7.8 to 8.4 in Nov/Dec, from 8.1 to 9.7 in Feb, and from 7.4 to 10.6 in May/June.

Table 9. Permutational ANOVA results of the effects of site and season on the stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of organic material in the Kowie system. df – degrees of freedom, * significant at $p < 0.05$, ** significant at $p < 0.01$

		df	**F**
Benthic algae			
$\delta^{13}C$	Site	7	49.47**
	Season	3	24.29**
	Site×season	20	22.76**
	Residual	250	
$\delta^{15}N$	Site	7	36.82**
	Season	3	20.29*
	Site×season	20	21.71*
	Residual	250	
Detritus			
$\delta^{13}C$	Site	7	1.12
	Season	3	0.95
	Site×season	16	1.67
	Residual	78	
$\delta^{15}N$	Site	7	107.81**
	Season	3	27.83**
	Site×season	16	29.81**
	Residual	78	
SPM			
$\delta^{13}C$	Site	7	116.06**
	Season	3	13.76**
	Site×season	20	18.49**
	Residual	56	
$\delta^{15}N$	Site	7	226.58**
	Season	3	47.39**
	Site×season	20	11.14**
	Residual	56	
Terrestrial leaves			
$\delta^{13}C$	Site	7	2.39*
	Season	3	4.08*
	Site×season	16	1.52
	Residual	109	
$\delta^{15}N$	Site	7	107.81**
	Season	3	27.83**
	Site×season	16	29.81**
	Residual	109	
Macrophytes			
$\delta^{13}C$	Site	7	17.21**
	Season	3	24.59
	Site×season	16	16.51
	Residual	109	
$\delta^{15}N$	Site	7	16.3**
	Season	3	10.32**
	Site×season	16	21.46**
	Residual	109	

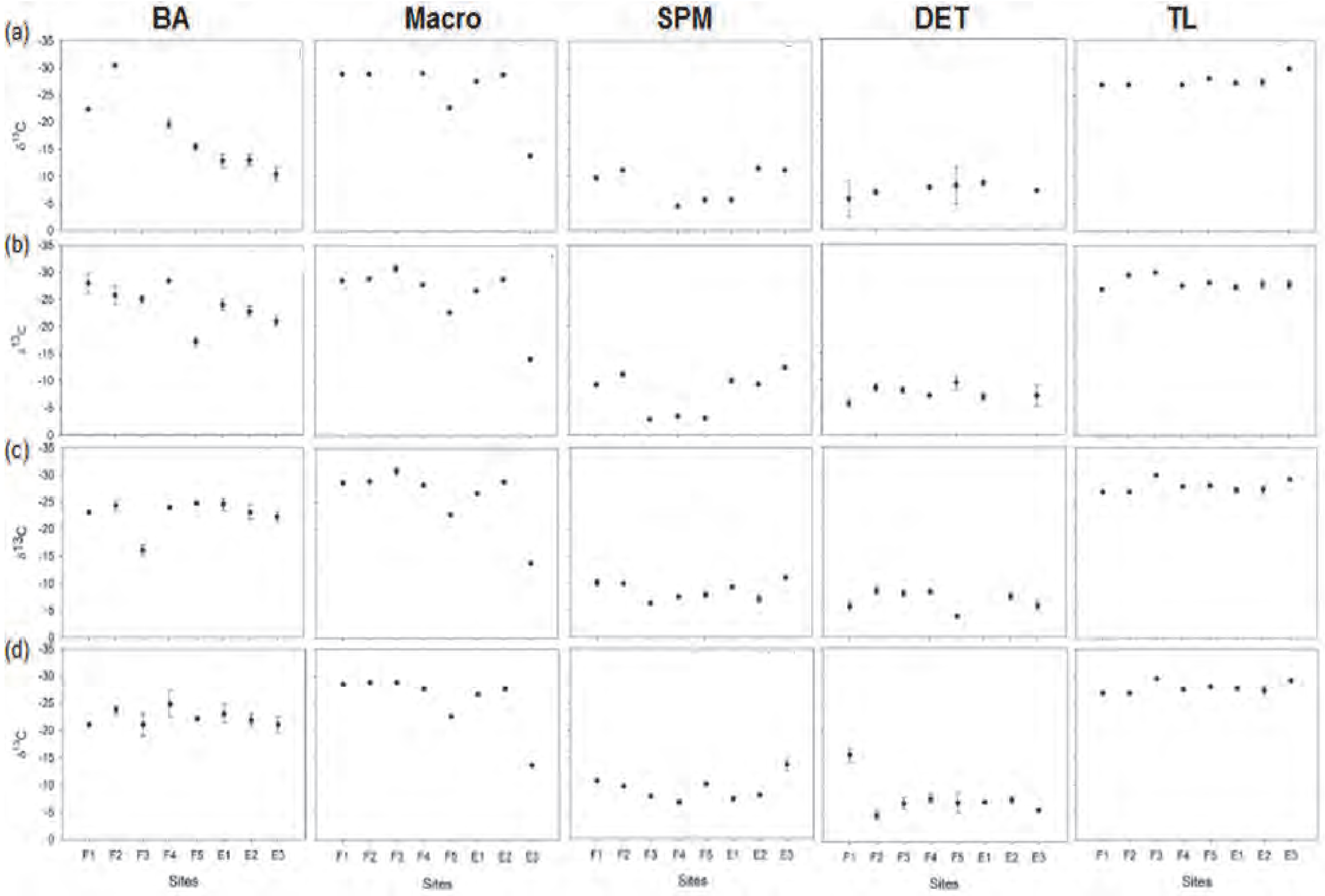

Figure 8. Mean $\delta^{13}C$ (‰, ± standard deviation) for organic matter sources in the Kowie system from September 2012 (early spring) to May/June 2013 (winter) : (a) Sept, (b) Nov/Dec, (c) Feb and (d) May/June. BA benthic algae, Det detritus, Macro macrophytes, SPM suspended particulate matter, TL terrestrial leaves.

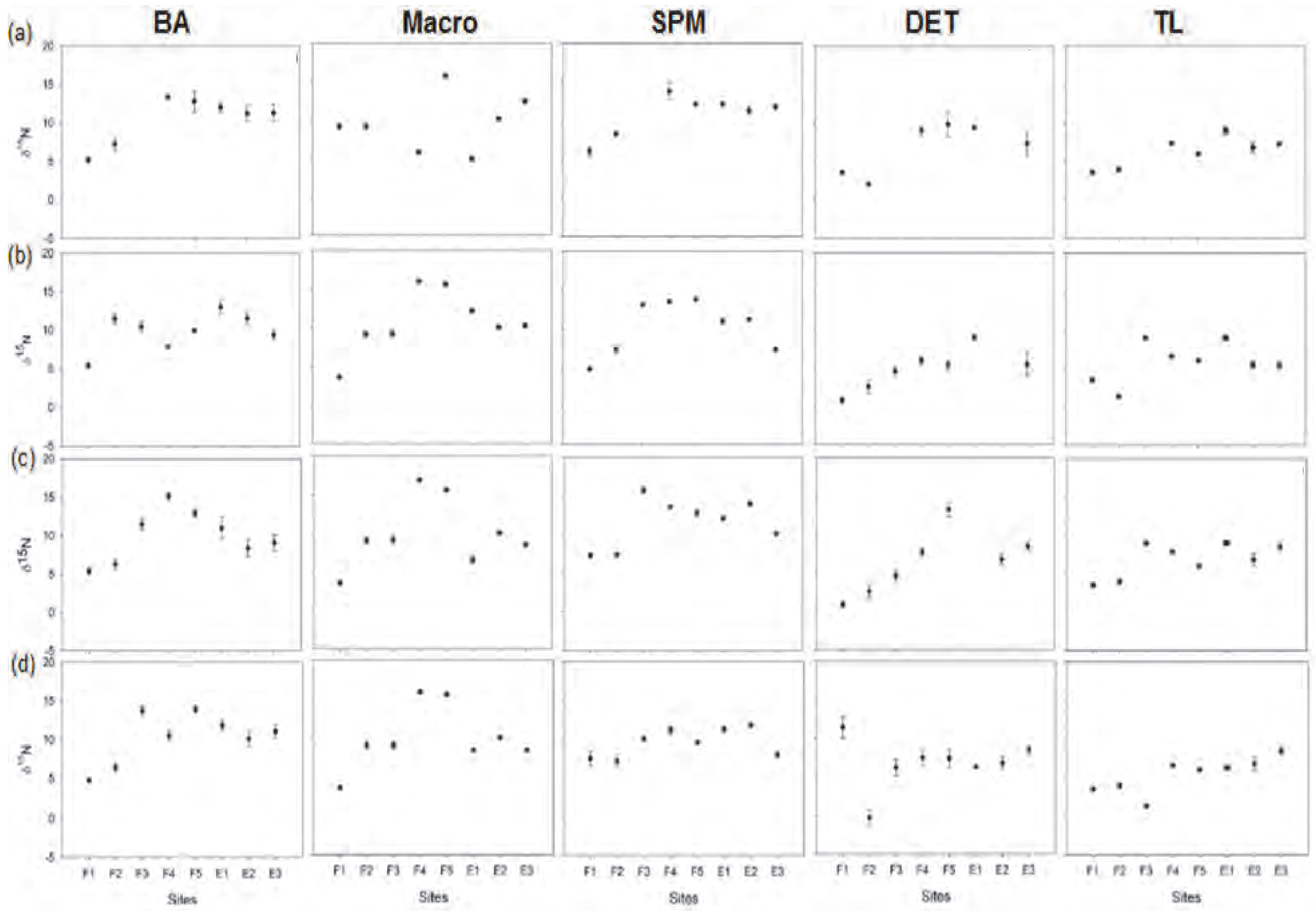

Figure 9. Mean δ^{15}N values (‰, ± SD) of organic material in the Kowie system from September 2012 (early spring) to May/June 2013 (winter) : (a) Sept, (b) Nov/Dec, (c) Feb and (d) May/June. BA benthic algae, Det detritus, Macro macrophytes, SPM suspended particulate matter, TL terrestrial leaves.

Relative contributions of basal organic sources to SPM and detritus

The SIAR models for detritus indicated spatial variations in the contributions from terrestrial leaves, with maxima occurring in the estuary (>70%; **Figure 10**). The contributions of aquatic macrophytes and benthic algae to detritus were variable, but generally these autochthonous sources were more influential in the upper freshwater regions than the lower reaches. Overall, the contributions of aquatic macrophytes and benthic algae to detritus decreased, while terrestrial leaves input increased, from upstream to downstream sites (**Figure 10**). The SIAR models estimated that both autochthonous sources were the main contributors to SPM in the mid to lower reaches, and aquatic macrophytes had a greater influence in the upper freshwater sites. Small contributions of terrestrial leaves to the SPM occurred throughout the study.

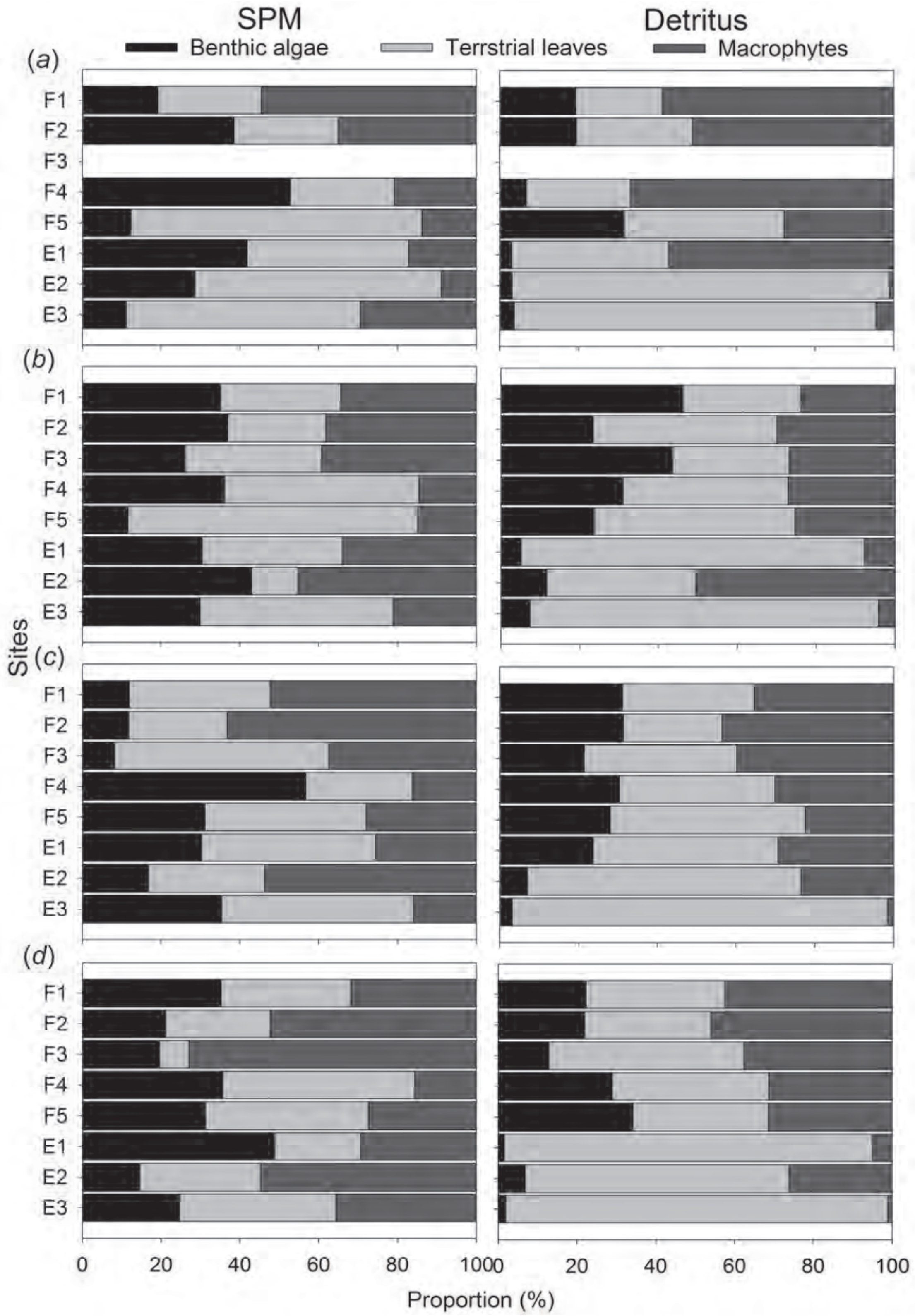

Figure 10. Relative mean percentage contributions, as estimated by SIAR, of benthic algae and terrestrial and aquatic plants to suspended particulate matter (SPM) and detritus in (a) Sept, (b) Nov/Dec, (c) Feb, and (d) May/June

Interpretations

Carbon and nitrogen stable isotope values in basal sources (benthic algae, aquatic macrophytes and terrestrial leaves) and the mixed organic groups (SPM and detritus) indicated that there were shifts in the contributions of the sources to the mixed pools across time and space. Terrestrial leaf contributions to bulk detritus generally increased in the downstream direction, whereas aquatic macrophyte contributions decreased from the upper to the lower reaches (**Figure 10**). Contributions of allochthonous materials were more substantial in bulk detritus, whereas autochthonous materials were generally more substantial in the particulates. However, the detritus was dominated by aquatic macrophytes and benthic algae in the upper reaches in all sampling periods, with detritus in the lower reaches dominated by terrestrial sources.

The relative contributions of allochthonous and autochthonous matter into the organic pools of the river-estuary continuum were spatially and temporally variable because the environmental conditions in the system changed through space and time. Hydrogeomorphological factors such as the types of riparian vegetation present, water velocity and channel width influence the balance between inputs from the terrestrial *versus* aquatic habitats (Bouillon et al., 2000; 2004; Abrantes and Sheaves, 2008). In the Kowie system, the channel width and relative biomass of aquatic macrophytes increase in the downstream direction, whereas the riparian vegetation cover decreases (Dalu et al., 2014b). However, a correlation analysis indicated no relationship ($p > 0.5$) between seasonal rainfall and terrestrial leaf composition in detritus and SPM, thereby the importance of rainfall in linking the land and water regarding the mixed organic pools remains complex. The absence of measurable relationships between rainfall and the organic pools may reflect the resilience of the system, in that effects of rainfall are temporally limited. We suspect that there was a lag between the sampling and flooding of October 2012, so by the time Nov/Dec samples were taken, the system had reset to some degree (at least as far as stable isotope ratios could detect).

The trends observed in the Kowie system contrast with the RCC, which predicts that terrestrial inputs are highest in headwaters and autochthonous production (and hence autochthonous inputs into the mixed organic pools) is increasingly important in the downstream direction where the river is wider and the canopy cover is lower. As such, the RCC predictions are not applicable to bulk detritus in the small temperate system that we have studied. The decreased influence of autochthonous sources in the bulk detritus in the downstream direction indicated that the growth of aquatic macrophytes and benthic algae colonizing the detritus was not necessarily curtailed by greater canopy cover in the headwaters (particularly in summer and autumn when ambient light levels tend to be relatively high). We expect that as the stream width widened in the downstream direction, the inputs of aquatic macrophytes and benthic algae were increasingly diluted by terrestrial leaves entering the entire length of the system. In turn, differential decomposition rates of different organic sources (with terrestrial leaves likely having the lowest decomposition rates) affected the relative proportions of different sources in the bulk detritus. Furthermore, fast-growing bacteria could potentially be outcompeting slow-growing benthic algae colonizing the detritus as the material moved downstream and experienced drastic changes in the environment (particularly salinity once the material reached E1).

SIAR models specifically indicated that the contributions of aquatic macrophytes to the SPM were greatest in the upper and lower reaches, and somewhat smaller in the mid reaches (particularly site F4), where benthic algae showed its greatest influence (**Figure 10**). The contributions of terrestrial plants to the SPM increased in the downstream direction during Sept and Nov, but were relatively equivalent among sites during Feb and May. Unlike the bulk detritus, the enhanced contributions of benthic algae to the SPM in the middle reaches supported the RCC (Vannote et al., 1980). The midstream of the Kowie system showed the greatest phytoplankton biodiversity and highest chl-*a* concentrations (Dalu et al*.,* 2014b), thereby supporting the RCC, but we were unable to include a pure phytoplankton signature in our models, so the influence of this suspended source on particulate pools remains to be investigated. Benthic algal communities in the middle reaches of the Kowie make up large proportions of the phytoplankton community due to re-suspension processes (Dalu et al., 2014a, b), so phytoplankton contributions to the SPM may mirror the benthic algal contributions.

The contributions of terrestrial plants to the SPM were quite different from those to the bulk detritus, and they showed different longitudinal trends (**Figure 10**). The large influence of terrestrial leaves in the bulk detritus, but not the SPM, in the downstream direction likely reflected the limitations of micro-organisms in breaking down the bulk material into smaller particles. Habitat connectivity (between water and land) is particularly interesting to consider in light of the contrasting contributions of terrestrial plants to the detritus and SPM. If one considers the bulk detritus alone, the two habitats can be considered highly coupled owing to the large proportions of terrestrial material into the mixed pool (particularly in the lower reaches). However, if one considers the SPM alone, the terrestrial contributions were much smaller, hence reflecting a decreased level of connectivity between the habitats. The next step in examining degree of connectivity in a system is to evaluate the assimilation of different components by the consumers in the river.

Our hypothesis that the organic matter fuelling the suspended mixtures varies along the continuum, with the middle reaches of the system (i.e. the lower river) dominated by autochthonous and the upper reaches by allochthonous material, was partially supported. The influence of benthic algae in SPM followed our predictions, but terrestrial plants did not dominate the SPM (or the bulk detritus) in the upper river. The second hypothesis (that the relative contributions of different sources change over time) was supported by the isotope models, although these changes were complex owing to the dynamic nature of the river over time. Organic matter pools can vary greatly from one site to the next within an aquatic system, and through time, and further research will help to illuminate the factors that affect these changes and, in turn, how these changes affect higher consumers. Our study provides a basis for investigating potential food web variability at a regional level based on shifts in the contributions of basal sources in any given time or place.

7.2.2 Fatty acids

Figures 11 to 14 represent the relative dissimilarities among the basal resources according to their fatty acid nutritional profiles in different sections of the Kowie system over the four periods. A relatively repetitive and orderly pattern was apparent at most of the freshwater and estuarine sites (particularly during Feb and some sites during May/June; **Figures 13 and 14**), with the terrestrial and aquatic plants consistently distinct based on enhanced levels of higher plant fatty acids (HPFAs; especially 18:3ω3 and sometimes 18:2ω6), the SPM most frequently dominated by the SFAs 16:0 and 18:0, and the phytobenthos pools (epipelon, periphyton and epiphyton) most frequently influenced by highly nutritional fatty acids typically associated with diatoms (20:5ω3 and 16:1ω7). In the estuary (E1-E3), these orderly relationships shifted and the patterns noted in the freshwater sections were altered in all periods.

Increased within-sample type variability was apparent at most sites during early and late spring (Sept and Nov; **Figures 11 and 12**), with SPM profiles the most variable in November (after extensive flooding occurred in the region). The benthic pools also showed increased variability during the spring months, but less so than the suspended pool.

The levels of polyunsaturated fatty acids (PUFA) in the SPM were highly influenced by inputs of HPFA, particularly during Sept and Nov, and by the diatom indices in Sept, Feb and May (**Figure 15; Table 10**). In Nov following intense flooding, there were no correlations between the PUFA and the diatom indices within SPM. Bacterial fatty acids (BFA) in SPM correlated negatively with PUFA during Nov, Feb and May. PUFA levels peaked at different locations in the continuum depending on sampling period, with peaks closer to the headwaters during Sept, and closest to the river mouth during Feb and May (leading to significant correlations between PUFA and distance; **Figure 15; Table 10**). Highest PUFA levels overall occurred during Nov (**Figure 15**).

Table 10. Pearson rank correlations with PUFA. * p < 0.05, ** p < 0.01, *** p < 0.001

	early spring		late spring		summer		winter	
SPM	R	p	R	p	R	p	R	p
BFA	-0.23	0.11	**-0.34**	*****	**-0.50**	*******	**-0.45**	******
HPFA	**0.65**	*******	**0.61**	*******	**0.34**	*****	**0.58**	*******
Diatom 1	**0.29**	*****	0.04	0.81	**0.66**	*******	**0.71**	*******
Diatom 2	**0.69**	*******	0.20	0.23	**0.69**	*******	**0.88**	*******
distance	0.26	0.06	-0.00	0.98	**0.42**	******	**0.52**	*******
epiphyton								
BFA	0.07	0.69	**-0.52**	******	0.24	0.20	-0.15	0.40
HPFA	**0.61**	*******	**0.50**	******	0.36	0.05	-0.01	0.95
Diatom 1	-0.22	0.22	**0.49**	******	-0.07	0.70	**0.53**	******
Diatom 2	-0.25	0.16	-0.29	0.11	-0.03	0.87	**0.87**	*******
distance	-0.05	0.77	**0.43**	*****	**0.67**	*******	**0.40**	*****
periphyton								
BFA	-0.47	0.08	**-0.71**	******	-0.18	0.48	-0.30	0.24
HPFA	0.44	0.10	0.46	0.09	-0.10	0.72	-0.27	0.30
Diatom 1	0.12	0.67	**0.65**	******	0.30	0.23	**0.72**	******
Diatom 2	-0.19	0.50	0.50	0.06	**0.54**	*****	**0.83**	*******
distance	**0.55**	*****	-0.36	0.19	0.04	0.88	0.28	0.28
epipelon								
BFA	-0.38	0.07	0.03	0.89	-0.21	0.38	-0.27	0.26
HPFA	**0.58**	******	0.05	0.84	0.25	0.28	**-0.59**	******
Diatom 1	-0.35	0.10	-0.07	0.78	-0.06	0.79	**0.56**	*****
Diatom 2	-0.20	0.34	-0.07	0.76	0.12	0.62	**0.82**	*******
distance	-0.01	0.96	**0.66**	******	0.17	0.48	-0.02	0.94

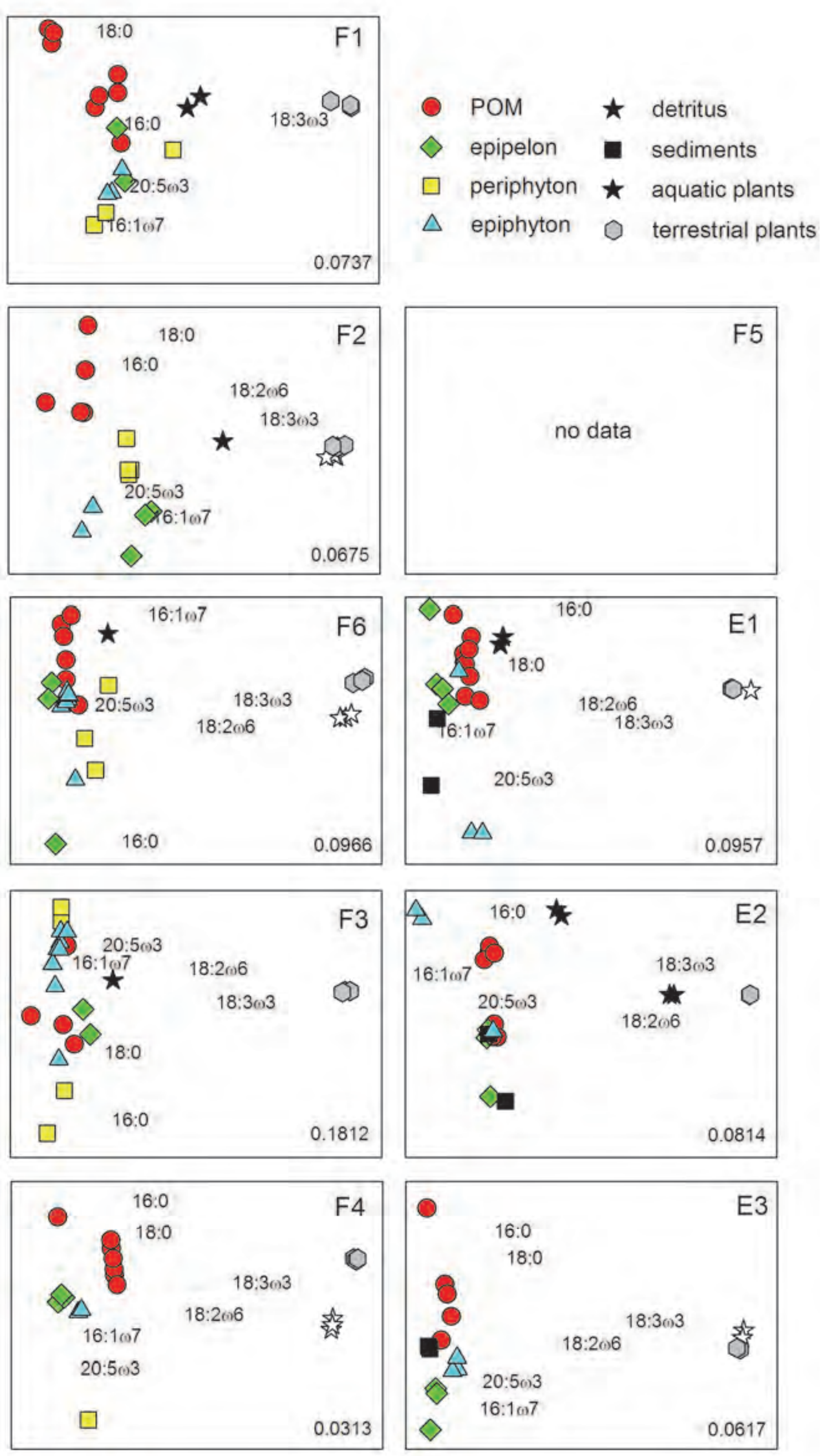

Figure 11. nMDS ordination of basal sources in the Kowie system during Sept 2012 (early spring). Influential fatty acids are superimposed, and values in lower left corners depict stress.

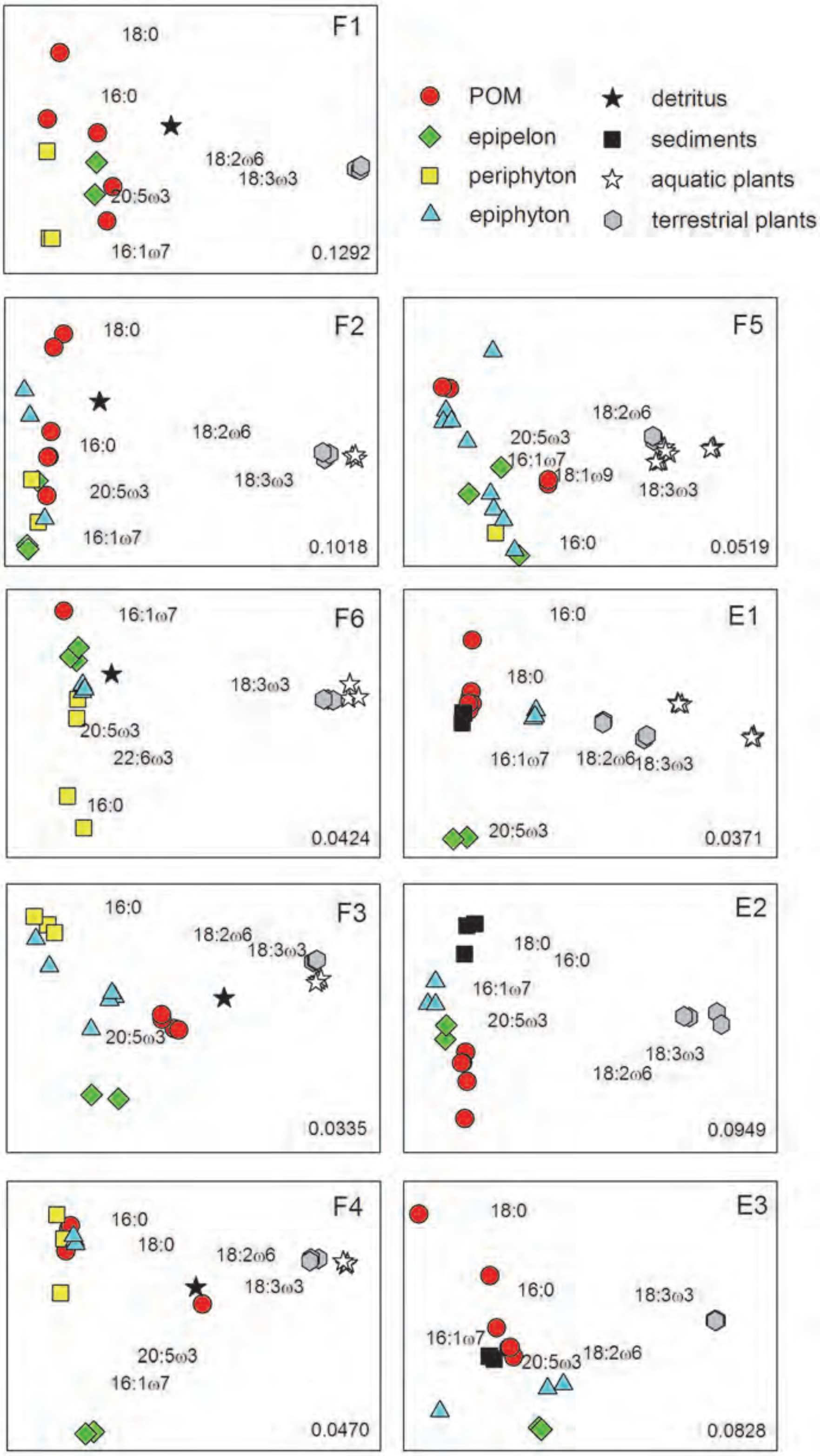

Figure 12. nMDS ordination of basal sources in the Kowie system during Nov 2012 (late spring). Influential fatty acids are superimposed, and values in lower left corners depict stress.

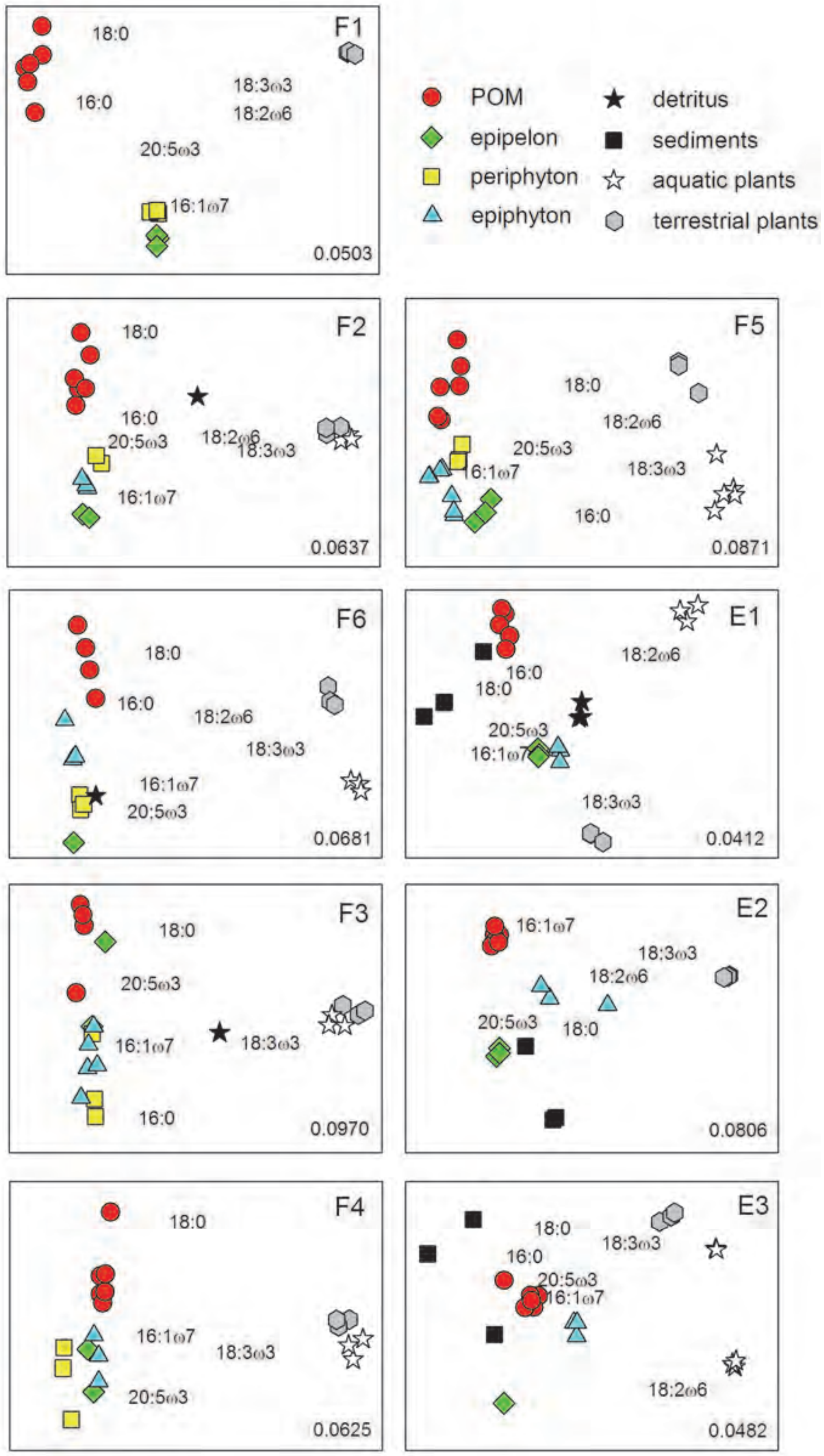

Figure 13. nMDS ordination of basal sources in the Kowie system during Feb 2013 (summer). Influential fatty acids are superimposed, and values in lower left corners depict stress.

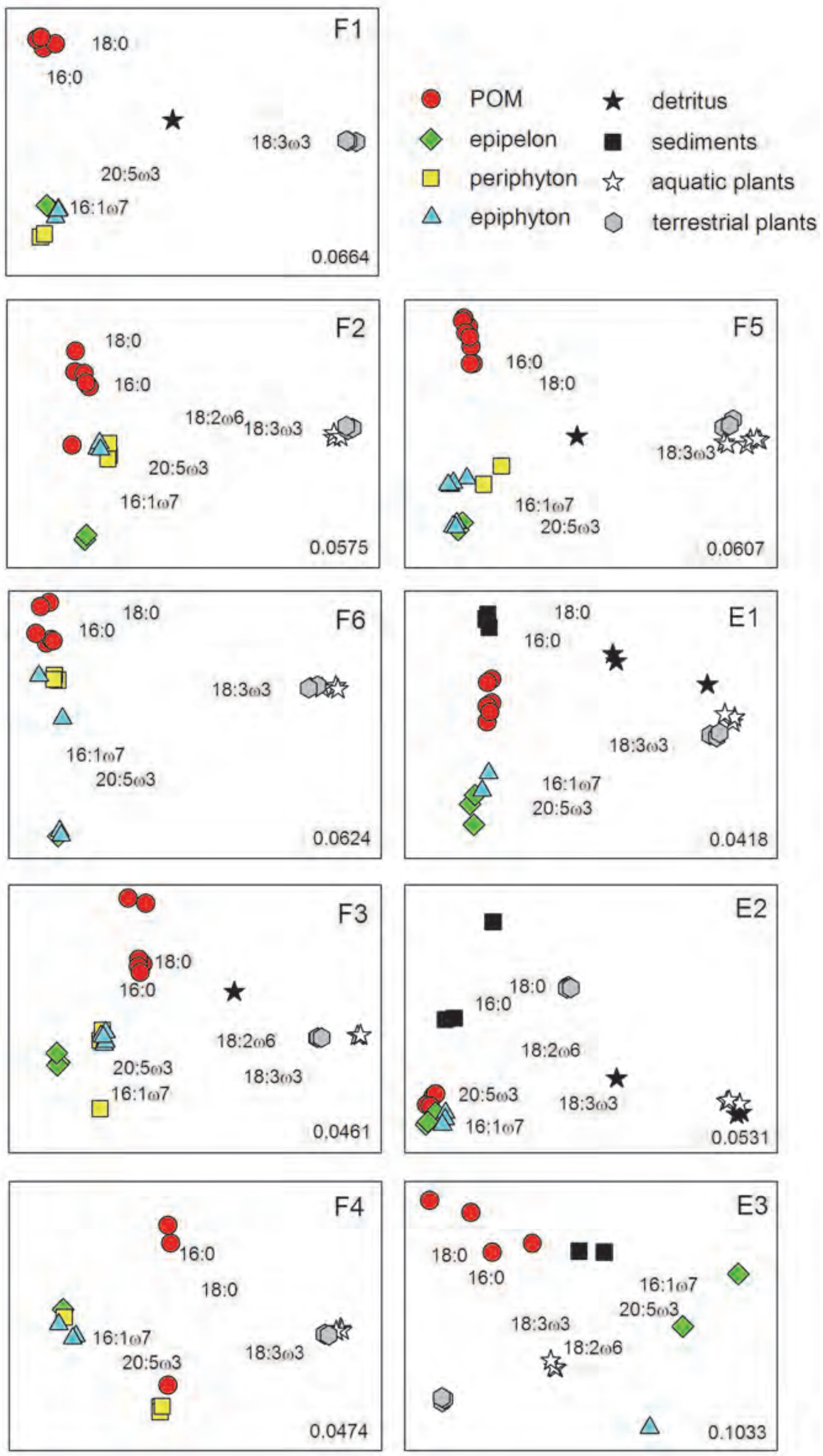

Figure 14. nMDS ordination of basal sources in the Kowie system during May/June 2013 (winter). Influential fatty acids are superimposed, and values in lower left corners depict stress.

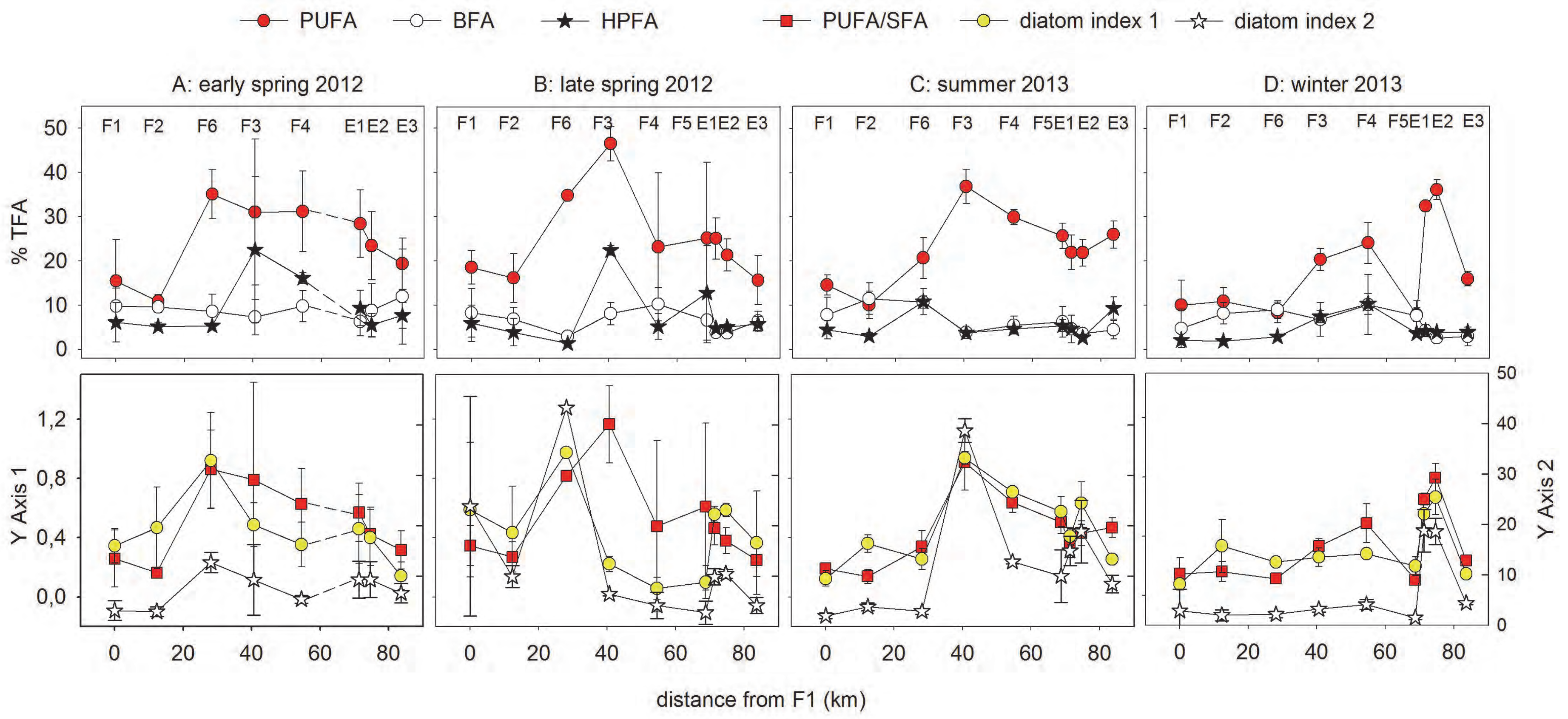

Figure 15. SPM dynamics in the Kowie system. Diatom index 1 (16:1ω7:16:0) and PUFA/SFA correspond to Y Axis 1, Diatom index 2 (16:1ω7+20:5ω3)/(18:1ω9+18:4ω3+22:6ω3)] corresponds to Y Axis 2.

7.3 Freshwater consumers

Available food sources for invertebrates varied along the freshwater sections of the Kowie system (**Table 11**) and were factored into the analyses. Black fly larvae (*Simulium* spp.) and caddisfly larvae (*Hydropsyche* spp. and *Cheumatopsyche* spp.) represented the filter-collectors; freshwater crabs (*Potamonautes* spp.) represented the shredders; snails (*Physella* spp.), midges (Chironomidae), some groups of mayflies (Baetidae) and cased caddisflies (*Leptocerus* spp.) represented the grazers; small square-gill mayflies (*Caenis* spp.) and the freshwater shrimp (*Caridina nilotica*) represented gatherer-collectors; and damselfly nymphs (Coengronidae and Chlorolestidae), Gomphidae (*Paragomphus* spp.) dragonfly nymphs (Libellulidae and Aeshnidae), horsefly larvae (*Tabanus* spp.) and giant water bugs (*Belostoma* spp. and *Apassus* spp.) represented the predatory invertebrates (**Table 12**).

Table 11. Basal autochthonous and allochthonous resources included as potential organic matter sources for freshwater invertebrates. x data available, – source absent from site

Organic matter source	code	F1	F2	F3	F4	F5	F6
Allochthonous sources							
coarse particulate organic matter	CPOM	x	x	x	x	x	x
Searsia spp.	LEAVES	-	x	-	-	-	-
Olea africana	LEAVES	-	x	x	x	-	x
Eucalyptus spp.	LEAVES	-	-	-	x	-	x
Eucalyptus globulus	LEAVES	x	-	-	-	-	-
Autochthonous sources							
benthic algae	BENTH	x	x	x	x	x	x
epiphyton	EPI	x	x	x	x	x	x
periphyton	PERI	x	x	x	x	x	x
filamentous algae	FA	-	-	x	x	x	-
Cyperus eragrotis	MACRO	x	x	x	x	x	x
Potamogeton pectinatus	MACRO3	-	-	-	-	x	-
Juncus effusus	MACRO1	-	-	-	-	x	-

Table 12. Functional feeding groups of invertebrates at each study site between Nov 2012 and Sept 2013

Functional feeding group	Taxon	F1	F2	F3	F4	F5	F6
Grazer							
Baetidae		x	x	x	x	x	x
Chironomidae		-	-	-	-	x	-
Leptoceridae	*Leptocerus* spp.	-	-	-	-	x	-
Amphipoda	*Amphipoda* spp.	-	-	-	-	x	-
Physidae	*Physella* spp.	-	-	-	-	x	-
Leptophlebiidae	*Castanophlebia calida*	-	-	-	x	x	-
	Adenophlebia auriculata	-	x	x	-	-	-
Filter-collector							
Simuliidae	*Simulium* spp.	x	x	x	x	x	x
Hydropsychidae	*Hydropsyche* spp.	-	-	-	-	x	-
	Cheumatopsyche thomasetti	-	-	-	x	-	-
	Cheumatopsyche afra	x	x	x	-	-	x
Gatherer-collector							
Atyidae	*Caridina nilotica*	-	-	-	-	x	-
Caenidae	*Caenis* spp.	x	x	x	x	-	x
Shredders							
Potamonautidae	*Potomonautes* spp.	x	x	x	-	-	x
	Potomonautes sydneyi	-	-	-	x	x	-
Predators							
Tabanidae	*Tabanus* spp.	x	x	x	x	x	x
Coenagrionidae	*Pseudagrion* spp.	x	x	x	x	x	x
Aeshnidae		x	x	x	x	x	x
Libellulidae		-	x	-	x	x	-
Belostomatidae	*Apassus* spp.	-	-	-	-	x	-
	Belostoma spp.	x	x	x	x	-	x
Chlorolestidae		-	x	x	x	x	-
Gomphidae	*Paragomphus* spp.	x	x	-	-	-	x
Platycnemidae		-	x	x	x	x	-

7.3.1 Isotopic values of consumers

At F5 (downstream), δ^{13}C values of most invertebrate taxa ranged from -29.7 to -22.1‰. The freshwater crab (*Potamonautes sydneyi*) showed the most enriched value (mean -25.9‰) during 2013, while the filtering black fly (*Simulium* sp.) had the most depleted δ^{13}C values (mean -30.6‰). The δ^{15}N of consumers at F5 ranged widely (13.0 to 20.3‰). Some species such as amphipod sp1 and the freshwater shrimp (*Caridina nilotica*) had highly differentiated stable isotope values over time (F = 38.8 for δ^{15}N, F = 8.7 for δ^{13}C; $p < 0.01$; **Figures 16 and 17**).

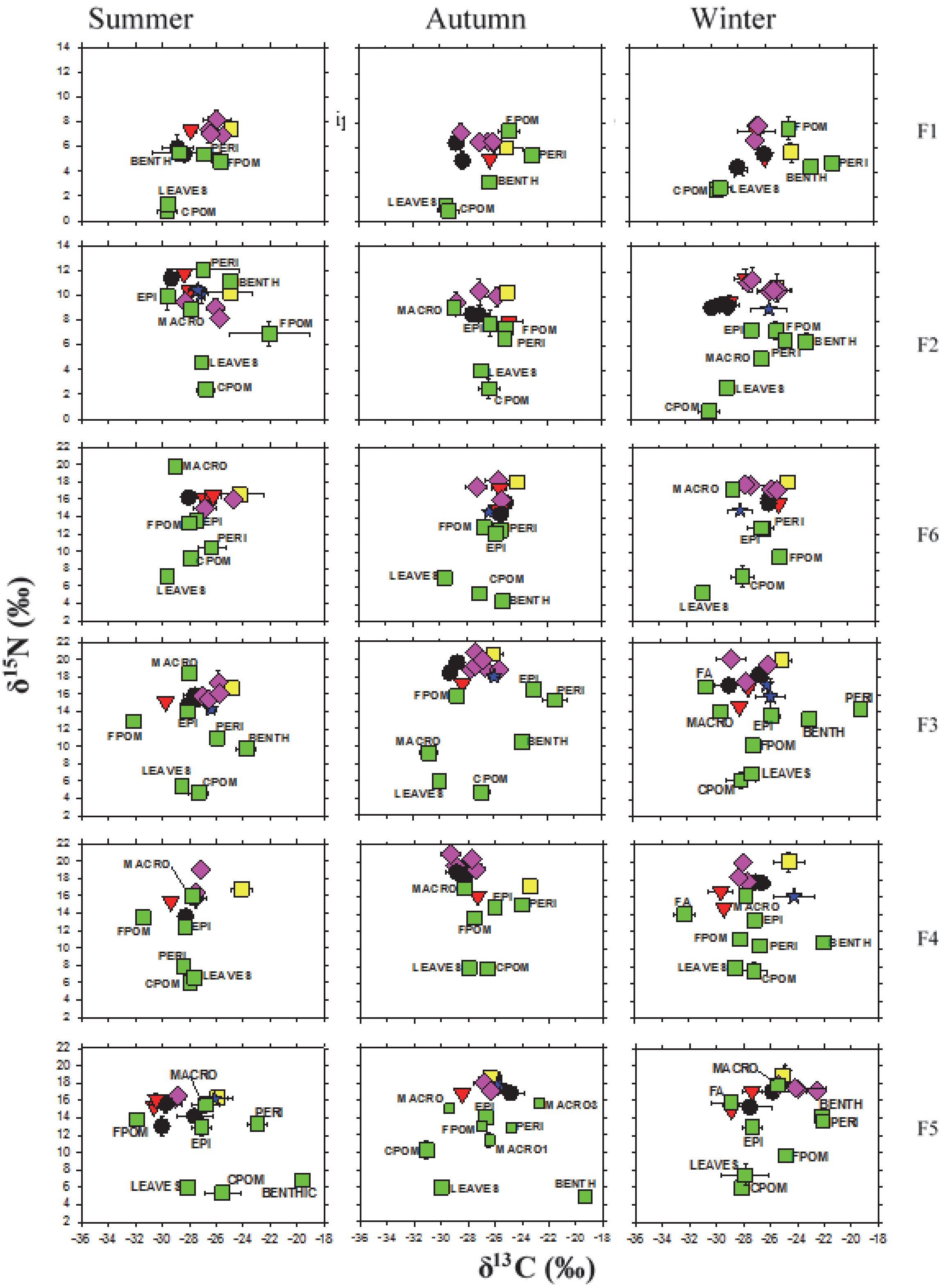

Figure 16. Mean stable isotopic ($\delta^{13}C$ and $\delta^{15}N$, ‰ ± SD) values of potential food sources (green squares) and consumers from the Kowie River from November 2012 to December 2013. Consumers include filterer-collectors (red triangles), gatherer-collectors (blue stars), grazers (black circles), shredders (yellow squares), predators (pink diamonds). F2 and F1 are at a different scale on the $\delta^{15}N$ axis, and F6 is between F2 and F3 owing to its position in the field (**Figure 1**)

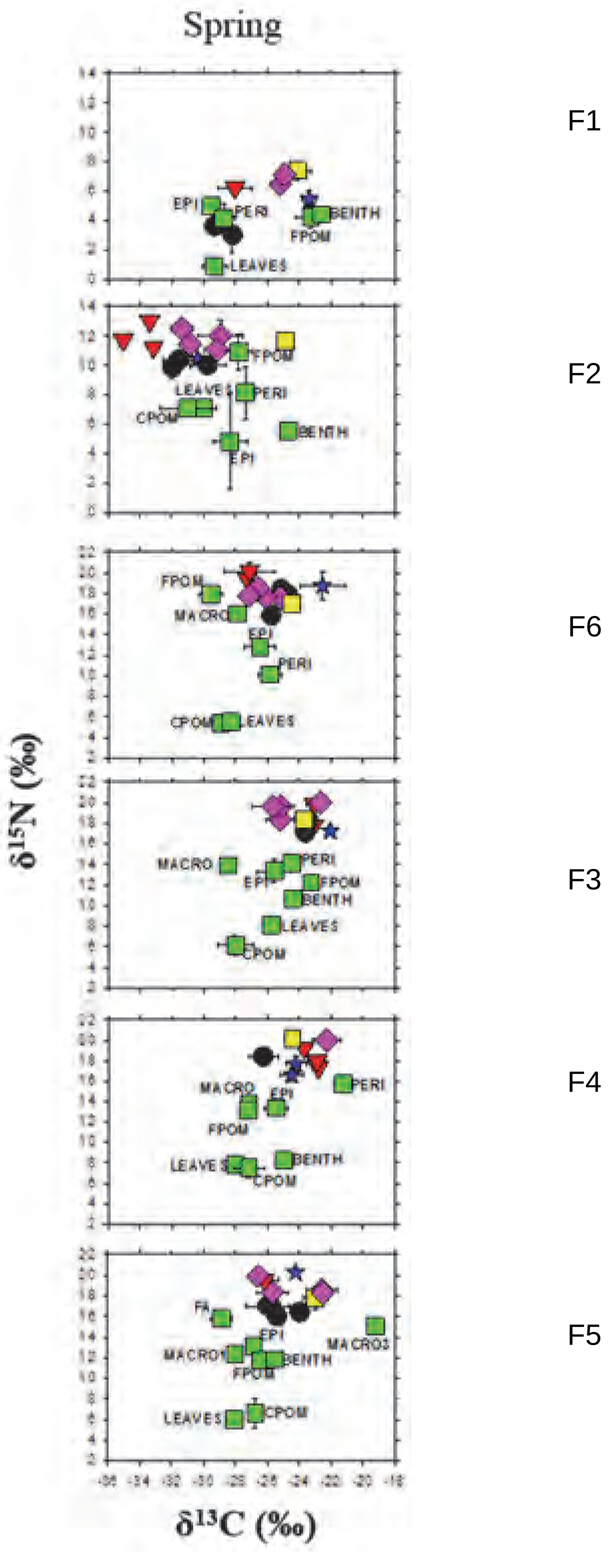

Figure 17. Mean stable isotopic ($\delta^{13}C$ and $\delta^{15}N$, ‰ ± SD) values of potential food sources and consumers from the Kowie River during winter (September) 2013. Consumers include filterer-collectors (red triangles), gatherer-collectors (blue stars), grazers (black circles), shredders (yellow squares), predators (pink diamonds). F4 and F5 are at different scales on the $\delta^{15}N$ axis

At F4, the $\delta^{13}C$ values of the consumer species ranged from -29.6 to -21.3‰, although these were similar in most organisms across sampling periods. The $\delta^{15}N$ of consumers ranged from 13.6 to 20.9‰. The predators in the group Tabanidae (*Tabanus* sp.) showed the highest $\delta^{15}N$ values, while the grazing mayfly (*Castanophlebia calida*) had low $\delta^{15}N$ values (13.6‰). At F3, $\delta^{13}C$ ranged from -30.1 to -22.1‰ in all invertebrate consumers, and $\delta^{15}N$ ranged from 14.8 to 20.8‰. The horsefly (*Tabanus* sp.) exhibited a broad range in $\delta^{15}N$, whereas the filtering blackfly (*Simulium* sp.) had a narrow range of $\delta^{15}N$ values across all periods (**Figures 16 and 17)**.

At F2 and F1 (the headwater reaches), consumers had low values of $\delta^{13}C$ (-35.05 to -23.38‰) and $\delta^{15}N$ (3.04 to 12.54‰) relative to those collected downstream. Most consumers except for the caddisfly larvae (*Cheumatopsyche afra*) exhibited considerable variations in both $\delta^{13}C$ and $\delta^{15}N$. While signatures of most of the consumers at the upper reaches fell within the range of the food sources, filter-feeders from F2 during Sept (range -33.14 to -35.05‰) fell outside the carbon range of the sources (**Figures 16 and 17)**.

At F6 (the main tributary that drains enters the Kowie River between F2 and F3), consumer $\delta^{13}C$ values ranged from -30.3 to -22.6‰, while $\delta^{15}N$ values ranged from 14.4 to 20.2‰. The values of $\delta^{13}C$ and $\delta^{15}N$ varied broadly across periods. For example, the caddisfly (*Cheumatopsyche afra*) was more depleted in ^{13}C and ^{15}N during summer and winter, and more enriched in spring (**Figures 16 and 17)**.

7.3.2 Diets of primary consumers

Model outputs indicated important contributions (up to 80%) of autochthonous carbon sources (epiphyton and aquatic macrophytes) to the grazer diets (**Table 13**), particularly in the downstream region of the river. Epiphyton contributed up to 42% towards grazer diets at F5 during Nov 2012*.* The greatest contributions of epiphyton to grazers (57%) occurred during Feb. Contributions of aquatic macrophytes (*Cyperus eragrotis*) to grazers at F4 increased to 88% in the May and Sept.

Periphyton and benthic algae contributed to the diets of most of the grazers at the headwater sites (range 2 to 40%), but not the downstream sites. While terrestrial carbon was not important to consumers in the lower reaches, terrestrial contributions were significant during autumn at F3, F2 and F1. In the headwater, characterised by high canopy cover (72%), leaves contributed substantially to the consumer diets (≥ 50%). Filamentous algae occurred in the system only during the May and Sept 2013 and contributed to the diets of grazers at F5 (ranged from 44% in May to 53% in Sept) and F3 (53% in May).

Autochthonous (epiphyton and aquatic macrophytes) and allochthonous sources (terrestrial leaves) both contributed to filter-collector diets (caddisfly and blackfly larvae; **Table 14**). SIAR results suggested that the autochthonous material made major contributions to filter-collectors at downstream sites (F5 and F4), mid reaches (F3) and in the tributary (F6) across all periods. Autochthonous material contributions to filterer diets were particularly substantial (> 70%) in the downstream and middle reaches. Increased riparian organic matter was assimilated by filterers (16 to 43%) at F2 and F1 (headwaters). Additionally, filamentous algae was assimilated by filterers in the downstream region (53% of the diet in May and 38% in Sept).

Terrestrial organic matter made relatively small contributions to gatherer-collector diets in the downstream (4-17%) and mid reaches (F3; 14-22%; **Table 15**). Terrestrial organic matter was more influential in consumers from the headwaters (F2 in particular), with the highest contributions occuring in the Sept. The only gatherer-collectors at F1 were spring moths, for which benthic algae was the major energy source (69% of the diet). Autochthonous material was the main energy source fuelling gatherer-collectors in the tributary (F6), where autochthonous carbon contributed more than 74% towards the consumer diets across all the periods. Autochthony was generally important at all sites, though there was a shift from some dependence of terrestrial organic matter in the headwater region to autochthony becoming more important downstream.

Shredders derived most of their energy from autochthonous material at all sites (**Table 16**). Terrestrial carbon was important to consumers in the headwaters (10-32% contributions), with the highest contributions recorded at F2 (Nov) and F1 (Feb and May). While solutions could not be generated for some of the sites, autochthony was generally important to consumers and terrestrial organic matter played a major role only in the headwaters.

Table 13. Mean contributions (%, 95% credibility intervals in parentheses) of sources to grazers in the Kowie River estimated from SIAR. Major basal resources with lower limits greater than 1% are bolded

		terrestrial	**aquatic macrophytes**	**epiphyton**	**benthic algae**	**periphyton**	**filamentous algae**
F1							
	Summer 2012	**59 (54-65)**			**23 (2-40)**	18 (0-37)	
	Autumn 2013	**77 (43-96)**			15 (0-41)	8 (0-22)	
	Winter 2013	**96 (90-100)**			2 (0-6)	2 (0-7)	
	Spring 2013	**35 (1-65)**			25 (0-47)	**40 (1-76)**	
F2							
	Summer 2012	10 (0-25)	37 (0-73)	**52 (18-92)**			
	Autumn 2013	**51 (41-59)**	**30 (11-46)**	19 (0-43)			
	Winter 2013	5 (0-11)	8 (0-18)	**87 (78-95)**			
	Spring 2013	**88 (72-100)**	7 (0-19)	5 (0-16)			
F3							
	Summer 2012	10 (0-20)		**79 (66-91)**		12 (0-24)	
	Autumn 2013	**38 (1-69)**	**48 (18-48)**	**14 (8-21)**			
	Winter 2013	15 (0-32)	32 (0-59)				**53 (31-77)**
	Spring 2013	17 (0-46)	35 (0-69)	**48 (8-92)***			
F4							
	Summer 2012	21 (0-38)	**31 (2-54)**	**48 (11-91)**			
	Autumn 2013	5 (0-11)	**88 (74-99)**	7 (0-21)			
	Winter 2013	4 (0-10)	**76 (62-91)**	**20 (1-36)**			
	Spring 2013	20 (0-48)	**45 (4-86)**	**35 (1-62)**			
F5							
	Summer 2012	10 (0-20)	**47 (21-77)**	**42 (4-74)**			
	Autumn 2013	11 (0-34)	32 (0-68)	**57 (17-98)**			
	Winter 2013	12 (0-28)		**43 (3-77)**			**44 (15-74)**
	Spring 2013	4 (0-10)	13 (0-32)	**30 (1-54)**			**53 (34-74)**
F6							
	Summer 2012	18 (0-49)		**73 (36-100)**		9 (0-29)	
	Autumn 2013	16 (0-43)		**67 (22-100)**	17 (0-47)		
	Winter 2013	5 (0-14)	**24 (4-64)**	**71 (41-94)**			
	Spring 2013	4 (0-11)	**70 (49-90)**	**26 (1-48)**			

Table 14. Mean contributions (%, 95% credibility intervals in parentheses) of sources to filterer-collectors in the Kowie River estimated from SIAR. Major basal resources with lower limits greater than 1% are bolded. “No solution” indicates that no solution was possible as the consumers fell outside the convex hull formed by the sources.

		terrestrial	aquatic macrophytes	epiphyton	benthic algae	periphyton	filamentous algae
F1							
	Summer 2012	**43 (13-70)**			33 (0-63)	24 (0-48)	
	Autumn 2013	**39(21-56)**			31(0-66)	**30(11-47)**	
	Winter 2013	**NO SOLUTION**					
	Spring 2013	**24 (1-47)**			27(0-56)	**49 (15-87)**	
F2							
	Summer 2012	**16(1-30)**	**32(3-56)**	**52 (33-71)**			
	Autumn 2013	**44 (23-64)**	9 (0-23)	16 (0-37)		**32 (1-56)**	
	Winter 2013	**33 (1-56)**	23 (0-49)	**43 (2-83)**			
	Spring 2013	**NO SOLUTION**					
F3							
	Summer 2012	17 (0-37)		**62 (38-83)**		21 (0-44)	
	Autumn 2013	**NO SOLUTION**					
	Winter 2013	10 (0-23)	**60 (45-74)**	**30 (16-43)**			
	Spring 2013	9 (0-30)	28 (0-62)	**63 (26-99)**			
F4							
	Summer 2012	13 (0-26)	**42 (20-66)**	**45 (8-79)**			
	Autumn 2013	**30 (20-38)**	**54 (29-71)**	16 (0-45)			
	Winter 2013	16 (0-30)	20 (0-39)	18 (0-39)			**46 (30-60)**
	Spring 2013	6 (0-19)		**47 (27-63)***		**47 (36-58)**	
F5							
	Summer 2012	10 (0-20)	**47 (21-76)**	**42 (5-74)**			
	Autumn 2013	**24 (2-44)**	**43(10-76)**	**33 (15-50)**			
	Winter 2013	17(0-33)		30(0-56)			**53(31-76)**
	Spring 2013	19 (0-44)	14 (0-29)	28 (0-54)			**38 (8-70)**
F6							
	Summer 2012	11(0-36)		**73(32-100)**		16(0-44)	
	Autumn 2013	19 (0-52)		**73 (33-100)**	8 (0-28)		
	Winter 2013	4 (0-14)	24 (0-47)	**71 (41-98)**			
	Spring 2013	11 (0-36)	**66 (29-99)**	23 (0-51)			

Table 15. Mean contributions (%, 95% credibility intervals in parentheses) of sources to gatherer-collectors in the Kowie River as estimated from SIAR. Major basal resources with lower limits greater than 1% are bolded. “No sample” indicates that there were no taxa that fell into the functional feeding group. “No solution” indicates that no solution was possible as the consumers fell outside the convex hull of the sources.

		terrestrial	aquatic macrophytes	epiphyton	benthic algae	periphyton
F1						
	Summer 2012	**NO SAMPLE**				
	Autumn 2013	**NO SAMPLE**				
	Winter 2013	**NO SOLUTION**				
	Spring 2013	14 (0-46)			**69(22-100)**	17 (0-50)
F2						
	Summer 2012	**30 (15-44)**	**49 (20-80)**	21 (0-41)		
	Autumn 2013	**NO SAMPLE**				
	Winter 2013	10(0-31)	26(0-56)	**64(27-93)**		
	Spring 2013	**65 (19-99)**	15 (0-48)	20 (0-52)		
F3						
	Summer 2012	10 (0-27)		**44 (23-65)**		**46 (21-68)**
	Autumn 2013	22 (0-47)	22 (0-44)	**56 (38-73)**		
	Winter 2013	14 (0-40)	23 (0-46)	**64 (35-89)**		
	Spring 2013	17(0-45)	32(0-65)			**52(13-95)***
F4						
	Summer 2012	**NO SAMPLE**				
	Autumn 2013	**NO SAMPLE**				
	Winter 2013	17(0-32)	**43 (15-71)**	**40 (1-76)**		
	Spring 2013	11 (0-35)	**37 (0-74)**	**52 (13-96)**		
F5						
	Summer 2012	13 (0-35)	**40 (8-70)**	**47 (11-83)**		
	Autumn 2013	4 (0-12)	33 (0-72)	**63 (24-100)**		
	Winter 2013	14 (0-43)	**64 (23-94)**	22 (0-51)		
	Spring 2013					
F6						
	Summer 2012	**NO SAMPLE**				
	Autumn 2013	**22 (5-45)**	**18 (4-34)**	**60 (23-85)**		
	Winter 2013	**26(14-40)**	**38(18-59)**	**36(5-63)**		
	Spring 2013	8(0-26)	**57(23-90)**	34(0-64)		

Table 16. Mean contributions (%, 95% credibility intervals in parentheses) of sources to shredders in the Kowie River estimated from SIAR. Major basal resources with lower limits greater than 1% are bolded. "No solution" indicates that no solution was possible as the consumers fell outside the convex hull of the sources.

		terrestrial	aquatic macrophytes	epiphyton	benthic algae	periphyton	filamentous algae
F1							
	Summer 2012	10 (0-21)			**39 (1-72)**	**51 (15-93)**	
	Autumn 2013	**23 (2-41)**			**37 (2-70)**	**40 (22-59)**	
	Winter 2013	**28 (18-41)**			**41 (12-71)**	31 (0-60)	
	Spring 2013	**NO SOLUTION**					
F2							
	Summer 2012	**32 (10-52)**	**36 (1-68)**	**33 (1-58)**			
	Autumn 2013	16 (0-39)	16 (0-41)	28 (0-54)		**40 (3-77)**	
	Winter 2013	**NO SOLUTION**					
	Spring 2013	24 (0-55)	**50 (10-96)**	26 (0-57)			
F3							
	Summer 2012	13 (0-32)	**38 (23-53)**	25 (0-48)		**24 (1-45)**	
	Autumn 2013	25 (0-49)	24 (0-47)	**50 (30-69)**			
	Winter 2013	**NO SOLUTION**					
	Spring 2013	11 (0-38)	20 (0-54)			**69 (27-100)***	
F4							
	Summer 2012	9(0-21)	**64(40-86)**	27(0-50)			
	Autumn 2013	3 (0-9)	9 (0-27)	26 (0-58)		**61 (27-97)**	
	Winter 2013	**NO SOLUTION**					
	Spring 2013	**NO SOLUTION**					
F5							
	Summer 2012	13 (0-37)	**43 (11-73)**	**44 (7-76)**			
	Autumn 2013	19 (0-47)	32 (0-66)	**49 (8-93)**			
	Winter 2013	14 (0-39)	**44 (7-83)**	19 (0-44)			22 (0-47)
	Spring 2013	19 (0-45)	**44 (26-63)**	**37 (6-61)**			
F6							
	Summer 2012	28 (0-58)		**43 (2-83)**		29 (0-60)	
	Autumn			**NO SOLUTION**			
	Winter 2013	26 (0-52)	**35 (1-63)**			**39 (1-75)**	
	Spring 2013	8 (0-26)	**57 (23-90)**	34 (0-64)			

7.3.3 Diets of secondary consumers

Autochthonous carbon (adjusted to represent the basal resources contributing to the primary consumers that were prey to the predators) was important for all secondary consumers in the Kowie River. Terrestrial carbon was important only for predators in the headwaters, while autochthony increased in importance in the downstream direction. Most predators consumed grazers, which were clearly dependent on autochthonous carbon sources (**Table 17**). The contribution of autochthonous carbon was notable throughout all periods. There was a gradual increase in assimilation of autochthonous carbon from headwaters (57%) to the downstream sites (88%). Predators assimilated only small proportions of terrestrially derived organic matter overall.

Table 17. Mean percentage contributions (95% credibility intervals in parentheses) of prey to the diets of predacious insects in the Kowie River from SIAR. The major basal resources with a lower confidence limit greater than 1% are bolded.

		Filterers	**Grazers**	**Gatherers**
F1				
	Summer 2012	**86 (62-100)**	14 (0-38)	
	Autumn 2013	**65 (46-84)**	**35 (16-54)**	
	Winter 2013	16 (0-33)	**45 (18-72)**	**39 (19-59)**
	Spring 2013	**41 (21-61)**	**59 (39-79)**	
F2				
	Summer 2012	27 (0-58)	16 (0-43)	**57 (20-97)**
	Autumn 2013	**42 (8-71)**	**58 (29-92)**	
	Winter 2013	12 (0-30)	21 (0-49)	**67(33-98)**
	Spring 2013	**4 (0-13)**	**59 (21-98)**	37 (0-73)
F3				
	Summer 2012	13 (0-31)	28 (0-56)	**59 (30-89)**
	Autumn 2013	**51 (23-81)**	**49 (19-77)**	
	Winter 2013	18 (0-41)	**52(23-83)**	30 (0-56)
	Spring 2013	**47 (7-85)**	**53 (15-83)**	
F4				
	Summer 2012	34 (0-67)	**66 (33-100)**	
	Autumn 2013	**44 (23-63)**	**56 (37-77)**	
	Winter 2013	**65 (51-80)**	**28 (7-47)**	7 (0-17)
	Spring 2013	**24 (1-47)**	**40 (14-64)**	**36 (18-53)**
F5				
	Summer 2012	31 (0-59)	**69 (41-100)**	
	Autumn 2013	**54 (36-72)**	**46 (28-64)**	
	Winter 2013	37 (0-78)	**63 (22-100)**	
	Spring 2013	7 (0-22)	**93 (78-100)**	
F6				
	Summer 2012	**59 (16-100)**	41 (0-84)	
	Autumn 2013	**52 (28-77)**	**48 (23-72)**	
	Winter 2013	20 (0-43)	24 (0-48)	**56 (37-76)**
	Spring 2013	12 (0-35)	**88 (65-100)**	

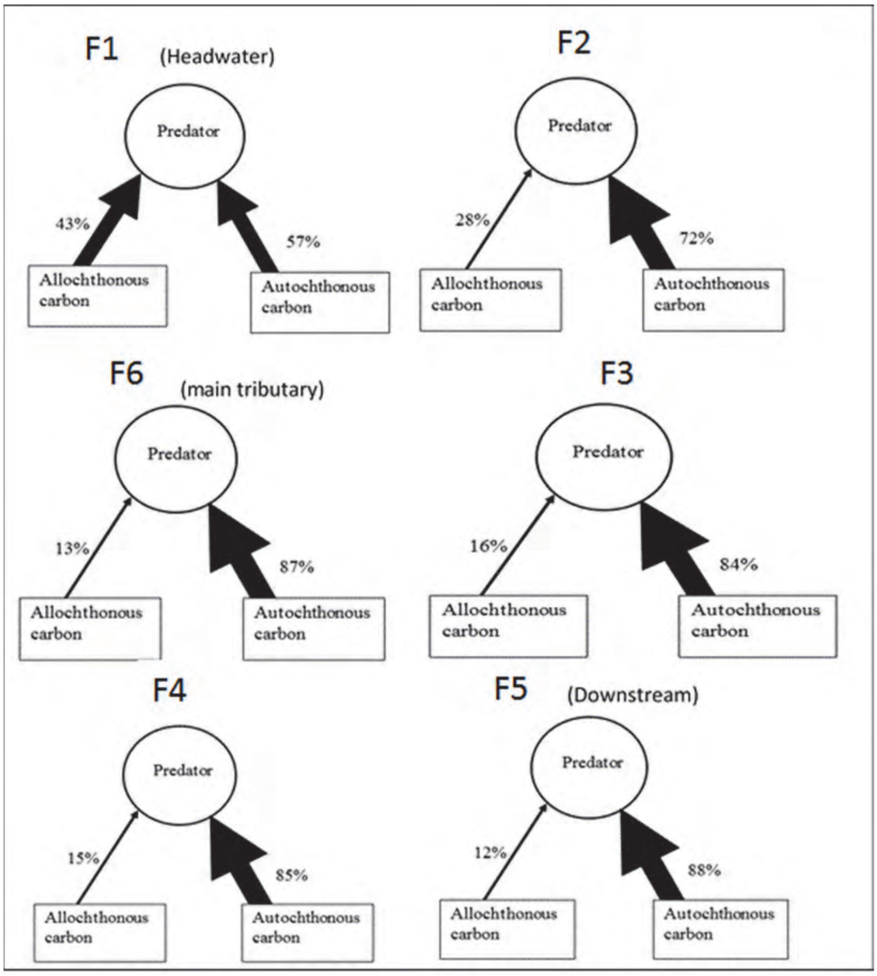

Figure 18. Schematic illustration of organic matter sources supporting secondary consumers in the Kowie River between November 2012 and September 2013. Contributions represent the primary consumers as prey, recalculated from their diet composed of basal resources

7.3.4 Interpretations

While both $\delta^{13}C$ and $\delta^{15}N$ values of basal resources varied widely among sites and periods, it was possible to discriminate between allochthonous and autochthonous food sources using a site-oriented approach. The $\delta^{13}C$ signatures of invertebrates were usually within the range of potential food sources from their respective sites. Although the SIAR models identified autochthonous (mainly aquatic macrophytes and attached algae) matter as the main food fuelling consumers, allochthonous organic matter was important at the headwaters. Among all the functional feeding groups analysed, there was a gradual shift from the assimilation of allochthonous to autochthonous material from headwaters to the downstream freshwater regions.

Our findings contrasted with those of several studies that indicated that terrestrial organic matter was the main driver of aquatic food webs (e.g. Huryn et al., 2001; Zeug and

Winemiller, 2008; Reid et al., 2008). We had hypothesized that carbon derived from local production would make up the bulk of the organic matter supporting riverine consumers (*sensu* Thorp and Delong, 1994). Our results are consistent with some other studies that have demonstrated that autochthonous carbon (aquatic macrophytes and algae) was highly influential in the food webs of lakes (Batt et al., 2012), tropical streams (March and Pringle, 2003) and large temperate rivers (Delong and Thorp, 2006). For example, Jepsen and Winemiller (2007) studied the food webs in Venezuelan rivers and found little evidence of terrestrial organic matter sources being important to consumers, whereas a combination of algae and macrophytes provided the major food source for the consumers. In the Kowie River, connections between secondary consumers and basal resources also showed that autochthonous organic matter provided the bulk of the organic matter that moves into the food web. Aquatic macrophytes made notable contributions to the basal diets of the higher consumers, with the greatest contributions occurring at the downstream sites. This finding supports conclusions made in other studies where aquatic macrophytes are potentially important in sections of large rivers (Hoeinghaus et al., 2007; Jepsen and Winemiller, 2007). The Flood Pulse Concept (FPC) suggests that river food webs are dependent on terrestrial sources and are highly dependent on flooding events (Junk et al., 1989). Our results support the importance of terrestrial organic matter to some consumers, mainly at downstream sites (**Figure 18**). However, there was limited support for the FPC from the Kowie system data, as the consumers derived more of their energy from autochthonously produced material. Floods in the Kowie River are rare and transient (typically lasting a few days) compared to the rivers used in formulating the original FPC, and they may not provide the time needed for decomposition of flood plain material which can then enter the metazoan food web. Research in some flood plains has also indicated that autochthonous derived carbon, rather than terrestrial carbon, supports consumers during long-term flooding periods (Delong et al., 2001). Clearly the link between freshwater inputs and autochthony *versus* autochthony is a complicated issue that warrants further attention.

The River Productivity Model (RPM) differs from the FPC in that it emphasizes the importance of algal grazer pathways in fuelling consumers in large rivers. The RPM emphasises that a combination of *in situ* production (including riparian zone producers) during periods not limited to flood pulses are the major sources of carbon (Thorp and Delong, 1994). In the Kowie River, the macrophytes and attached algae contributed significantly to the diets of most consumers. The assimilation of macrophytes by consumers is a particularly interesting outcome. Aquatic macrophytes have been described as potential food sources for primary consumers, but these sources are rarely attacked by aquatic herbivores due to their low digestibility and high levels of cellulose and lignin (Allan, 1995; Allan and Castillo, 2007). However, some researchers have argued that direct consumption is more common and more important to ecosystem functioning than previously thought (Lodge, 1991; Newman, 1991). Whilst there are no field studies showing direct consumption of macrophytes by herbivores, an experimental study on aquatic insects revealed that macrophytes contributed over 50% to the diet of *Atalophlebia albiterminata* (Ephemeroptera) and the case-building Trichopterans (*Lectrides varians*, *Notalina bifaria*, *Triplectides similis*; Watson and Barmuta, 2011). The RPM predicts that though direct herbivory on macrophytes is unlikely, the consumption of macrophytes can occur once the macrophytes have entered the detrital pool. In fact, the labile nature of autochthonous organic matter in rivers makes it a more preferred food choice compared with less labile allochthonous components (Thorp and Delong, 2002).

The River Continuum Concept (RCC) proposed that the main source of carbon driving consumers is fine particulate organic matter derived from upstream regions. Essentially, coarse terrestrial material in upstream regions is broken down into fine particles with increasing distance from the headwaters. In the Kowie River, SPM had a variable composition along the longitudinal gradient (**Section 7.2**), so the organic material transported downriver was certainly not of purely terrestrial origin. In fact, the SPM in the river was dominated by autochthonous material. Other researchers have noted the dominance of autochthonous sources in temperate streams (Thorp et al., 1998; Delong and Thorp, 2006), rivers in south eastern Brazil (Martinelli et al., 1999), rivers in Columbia (Kendall et al., 2001) and the upper Mississippi River (Delong and Thorp, 2006). Overall, the RCC is not adequate to describe/predict the organic matter sources fueling consumers in all systems. For example, in the Kowie system, the dominant shredding crab (*Potamonautes* spp.) was dependent mainly on algal resources, whereas according to the RCC they should consume terrestrial leaves. Synthesizing our results into a conceptual diagram (**Figure 19**), we suggest that the RPM is the most appropriate model for the Kowie River. However, the different models can be viewed together and may complement each other in explaining energy flow in a small temperate stream.

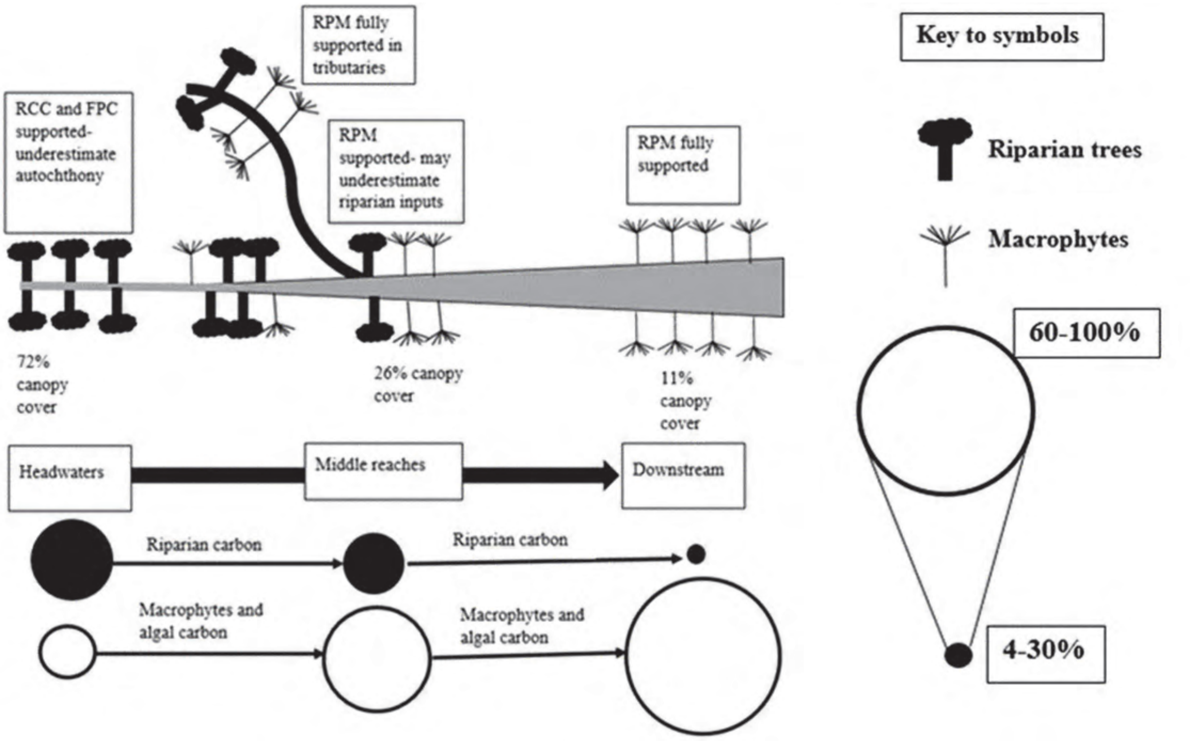

Figure 19. Conceptual diagram illustrating the contributions of organic matter sources supporting consumers in different reaches of the Kowie River. The relevant energy flow concepts (RPM River Productivity Model, RCC River Continuum Concept, and FPC Flood/Pulse Concept) are highlighted. Only carbon sources that contributed to consumer diets are included.

7.3.5 Temporal dynamics

Food web studies using stable isotopes are often limited to one season and one habitat (e.g. Delong et al., 2001; Brito et al., 2006). While these studies have provided invaluable information on the flow of carbon in river food webs, they are missing the seasonal changes in contributions of different organic matter sources. Food webs are dynamic over both temporal and spatial scales (Polis et al., 1996), and by sampling multiple food sources across multiple seasons we were able to elucidate the complexity of energy sources in the Kowie River across sites and seasons. For example, filamentous algae was found only during certain seasons (and only at certain sites), but it constituted part of the diet of consumers during those periods. This potential food source would have been missed if sampling had been confined to a single time. The change in food sources spatially and temporally justifies sampling of food sources at several locations and on multiple occasions. Our findings indicated that while there were major changes in isotopic values of sources and consumers over space and time, autochthonous carbon remained the primary source supporting consumers. Delong et al. (2001) noted that phytoplankton-derived carbon dominated the transported organic matter in a river over a year. Likewise, other temperate rivers showed temporal changes in $\delta^{13}C$ and $\delta^{15}N$ that suggested increases in the amounts of terrestrial organic matter input during certain periods, although autochthonous sources remained the primary constituent of consumer diets (Pingram et al., 2014). In contrast, chironomids can rely on carbon sources originating from detritus and periphyton in the September, but riparian plants and seston in the November (Fisher et al., 2001). Such contrasts further justify the need to adequately sample food sources and consumer diets across periods.

At some sites (and during some seasons), discernment of trophic levels was relatively distinct and variation in $\delta^{15}N$ within trophic levels was small. The high variation and overlapping isotopic values at certain sites suggested that some of the animals are omnivorous, capitalising on available food resources. Such plasticity in feeding modes has been reported (Palmer et al., 1993; Miyasaka and Genkai-Kato, 2009), where insects such as stone flies (predators) shifted their diet and assimilated up to 55% algae (Lancaster et al., 2005).

Our results from the Kowie River did not entirely support the predictions of the RCC and FPC, and rather the data suggested a strong reliance of consumers on autochthonous rather than on allochthonous matter. The RPM predicts that a mixture of local autochthonous production (by phytoplankton, benthic algae or macrophytes) and direct inputs from the trees in riparian zones are the major sources of carbon assimilated by consumers in large rivers (Thorp and Delong, 1994). As our data from the Kowie River more closely match the predictions of the RPM, this model may not be limited to large temperate rivers and may also be appropriately applied to small temperate rivers. One promising model that may help to further refine riverine ecology is the riverine ecosystem synthesis (RES). The RES (an heuristic model) suggests that the carbon sources supporting consumers are primarily driven by features such as geomorphological complexity and lateral connectivity (*sensu* Thorp et al., 2006). Additional research will be needed to evaluate the roles of hydrogeomorphology to the carbon sources fuelling consumers in temperate rivers.

7.3.6 Insect emergence and infall

Emergence and pan trap data from 3 sites (**Figure 20**; to address **Aims 3 and 4**) are presented here. Terrestrial inputs and emergence changed seasonally, and flux patterns were similar among sites in different periods. The highest emergence (by biomass) was recorded in Feb at all three sites (range 707 to 1870 mg m^{-2} d^{-1}; **Figure 21**). During the May/June, emergence dropped to 14 to 80 mg m^{-2} d^{-1}. Aquatic emergence was significantly influenced by site and season (**Table 18**). Terrestrial invertebrate infall was influenced by season, but not by location (**Table 18**, $p < 0.05$). There was an interaction between site and season regarding aquatic emergence ($F = 21.1$, $p < 0.01$), whereas terrestrial invertebrate inputs did not have a significant interaction between site and season ($F = 2.2$, $p < 0.01$).

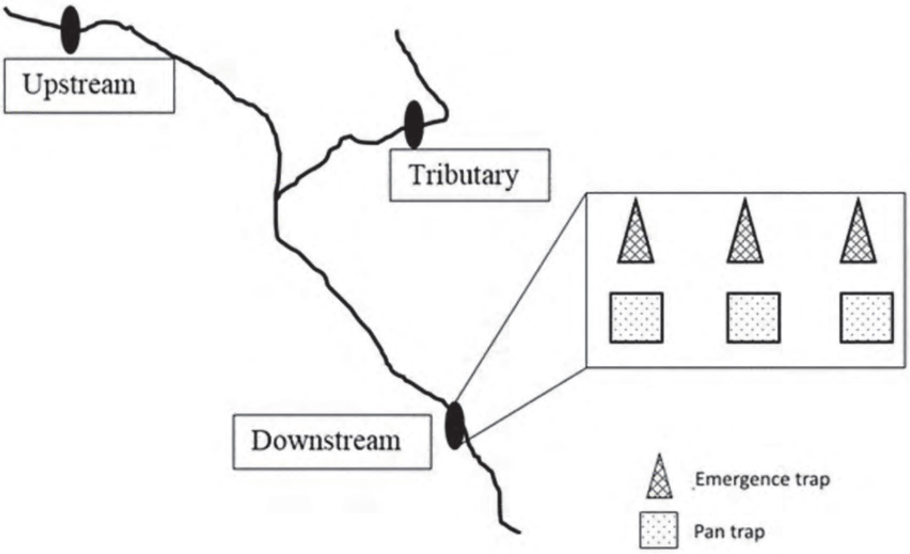

Figure 20. Location of the emergence and infall study sites (upstream corresponds to F2, tributary corresponds to F6, and downstream corresponds to F4) in the Kowie River, South Africa. Triangles and squares show the arrangement of traps at each study site.

The assemblage of emerged aquatic insects was composed of Diptera, Ephemeroptera, Coleoptera, and Trichoptera and included at least 18 families. Overall, composition of the emerging community was relatively similar among sites and was dominated numerically by Diptera (primarily Simuliidae). Terrestrial invertebrate infall comprised 21 orders over the two seasons and included small-bodied taxa such as Diptera, Hemiptera, and Thysanoptera (which were most numerous). The larger-bodied taxa such as Arachnida, Hymenoptera, Lepidoptera, and Orthoptera contributed the most biomass.

Table 18. Two-way PERMANOVA output for dry mass of aquatic invertebrates (mg m^{-2} d^{-1}), and dry mass of terrestrial invertebrates (mg m^{-2} d^{-1}) inputs in the Kowie River.

Factor	df	sums of squares	mean square	F	p
		Aquatic invertebrates (mg m^{-2} d^{-1})			
Season	1	23132.0	23132.0	227.0	<0.01
Site	2	2501.3	1250.6	12.3	<0.01
Season*time	2	4299.2	2149.6	21.1	<0.01
Error	12	1222.6	101.9		
		Terrestrial invertebrate inputs (mg m^{-2}day^{-1})			
Season	1	16058	16058	74.8	<0.01
Site	2	990.8	495.4	2.3	0.12
Season*time	2	964.4	482.2	2.2	0.13
Error	11	2362.2	214.8		

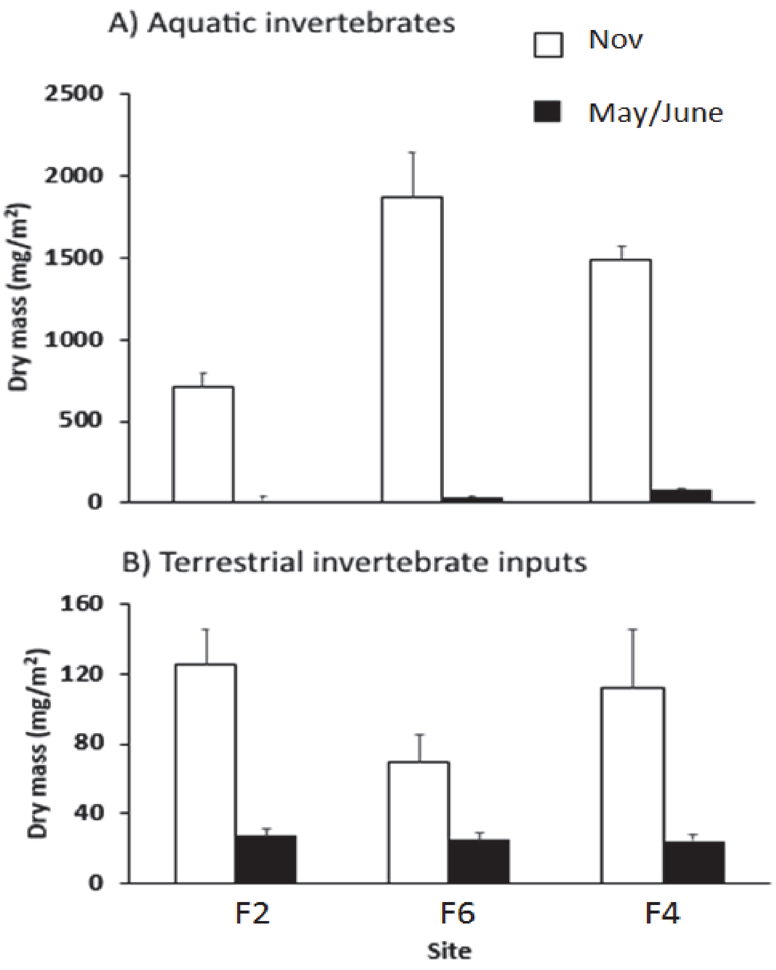

Figure 21. Mean (+ SD) seasonal dry mass of (A) aquatic invertebrates (mg m^{-2}; 3 replicate samples from emergence traps) and (B) terrestrial invertebrates inputs (mg m^{-2} day^{-1}); 3 replicate pan traps per site) at three sites along the Kowie River from November 2012 (late spring) to June 2013 (winter)

Our results corroborate previous findings that reciprocal subsidies between terrestrial systems and aquatic systems fluctuate periodically and that subsidy exchanges are dynamic (Power and Rainey, 2000; Nakano and Murakami, 2001). However, we did observe some differences in the seasonal patterns of insect emergence and infall compared with those of Nakano and Murakami (2001). The annual terrestrial infall in the Kowie River was much smaller than emergence across the two seasons, while in Japan, terrestrial inputs substantially exceeded emergence (Nakano and Murakami, 2001). The taxonomic composition of the subsidies may be important to understanding the patterns we observed among sites and between periods. Terrestrial inputs were composed of large and small taxa which varied considerably over time. Taxonomic diversity at the order level was lower for the emerging community, but contributions from the four different orders of aquatic insects also varied substantially over time.

Our findings emphasise the importance of incorporating both space and time into questions about linkages between habitats. Clearly there is much greater transfer occuring between habitats during November compared with May/June, and emergence was far more influential compared with infall. For example, an increase in riparian leaf input (which usually occurs in autumn in areas dominated by deciduous trees) can result in an increase in the terrestrial input to aquatic systems (Edwards and Huryn, 1996). The changes in inputs from terrestrial insects are less predictable, but other studies have indicated greater contributions from aquatic systems to terrestrial habitats than *vice versa* (e.g. Richardson et al., 2010).

7.4 Aerial invertebrates

The models revealed a general decline in the dietary contributions of aquatic food sources to spider diets with increasing distance from the water at the tributary (F6) (**Figure 22**) and F4 (downstream site) in the Kowie River (**Figure 23**) during most seasons. No patterns could be detected across the distance gradient at the upstream site F2 (**Figure 24**). Regression analysis (**Table 19**) revealed a strong and significant ($p < 0.05$) relationship between the distance from the river and the dietary proportion of aquatic sources at the downstream ($R^2 = 0.71$-0.98) and tributary ($R^2 = 0.85$-0.97) sites in all seasons except autumn (March) in the polluted ($R^2 = 0.58$, $p > 0.05$) site. Distance was not significantly correlated (**Table 19**, $p > 0.05$) to the proportion of aquatic food sources in spider diets at the upstream site during all seasons. However, the proportion of aquatic food sources generally varied across seasons, peaked in autumn (**Figure 24b**) and dropping to its lowest values in winter (June) (**Figure 24c**). In contrast, aquatic source contributions at the downstream site were considerably high in winter (**Figure 23c**) and very low in spring (Oct) (**Figure 23d**).

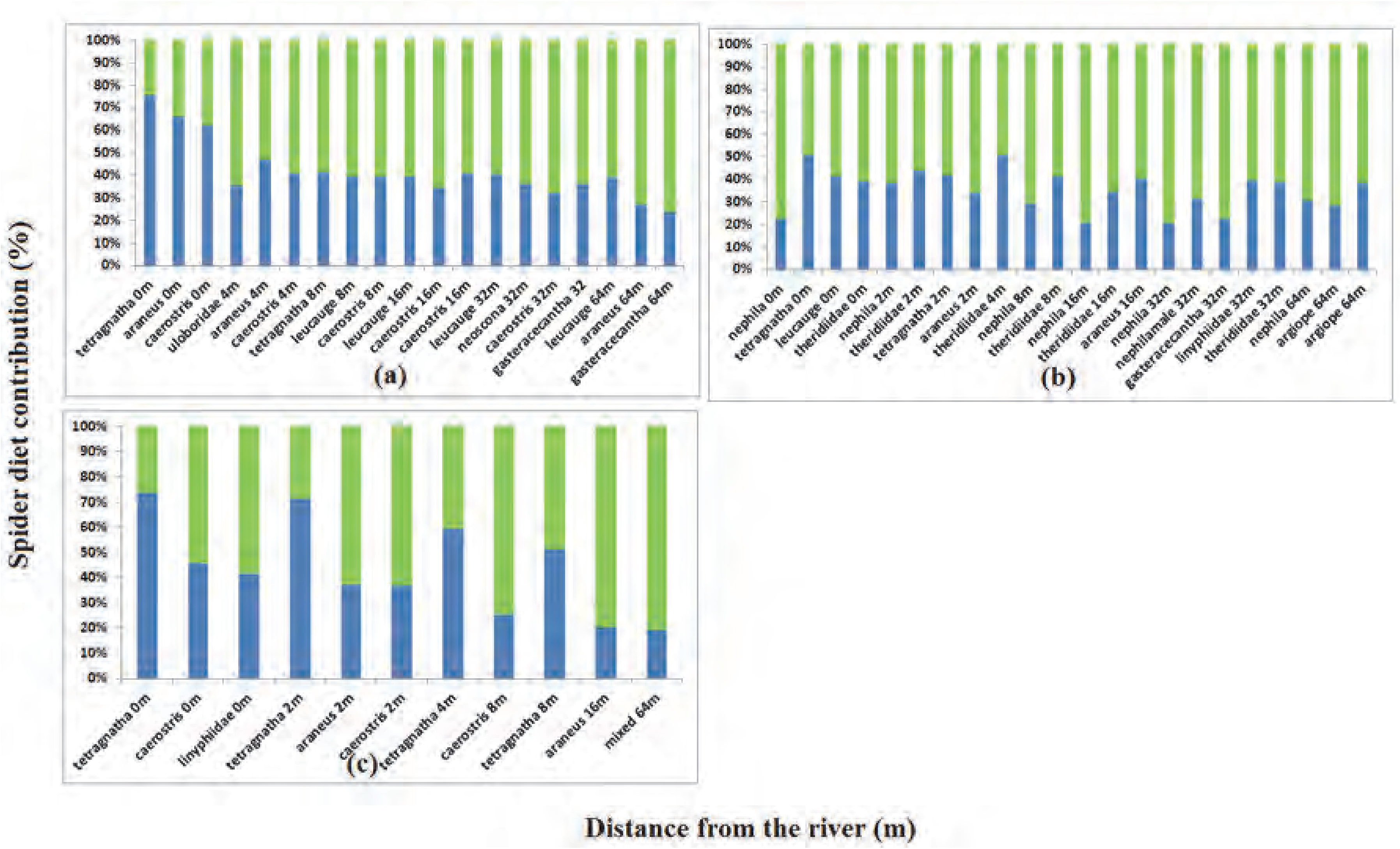

Figure 22. SIAR generated aquatic (blue) and terrestrially (green) derived contributions (%) to spider diets at varying distances (m) from the river channel at F6. The letters a, b and c represent three seasons; summer, autumn and winter, respectively

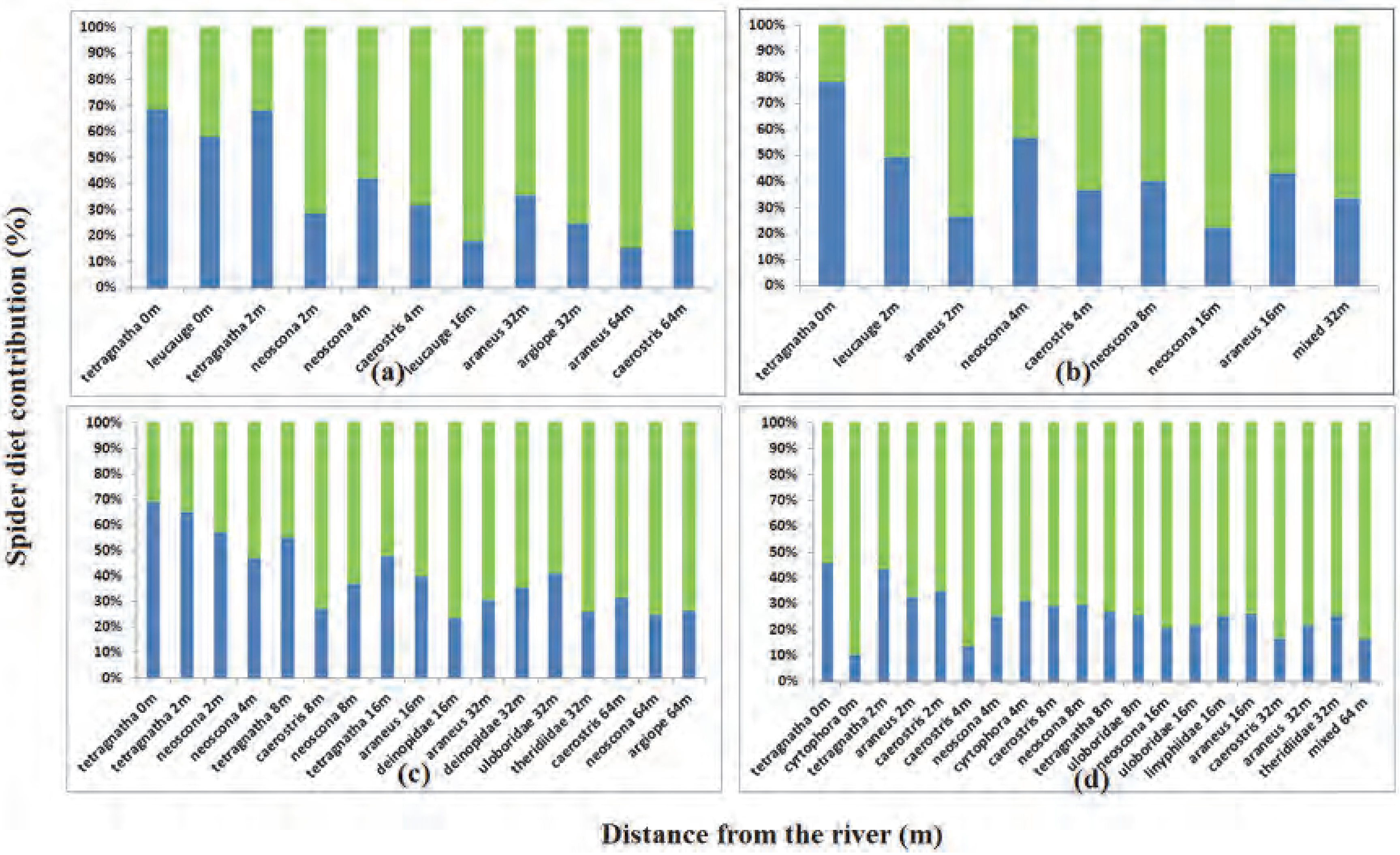

Figure 23. SIAR generated aquatic (blue) and terrestrially (green) derived contributions (%) to spider diets at varying distances (m) from the river channel at the downstream site (F4). The letters a, b, c and d represent four seasons; summer, autumn, winter and spring

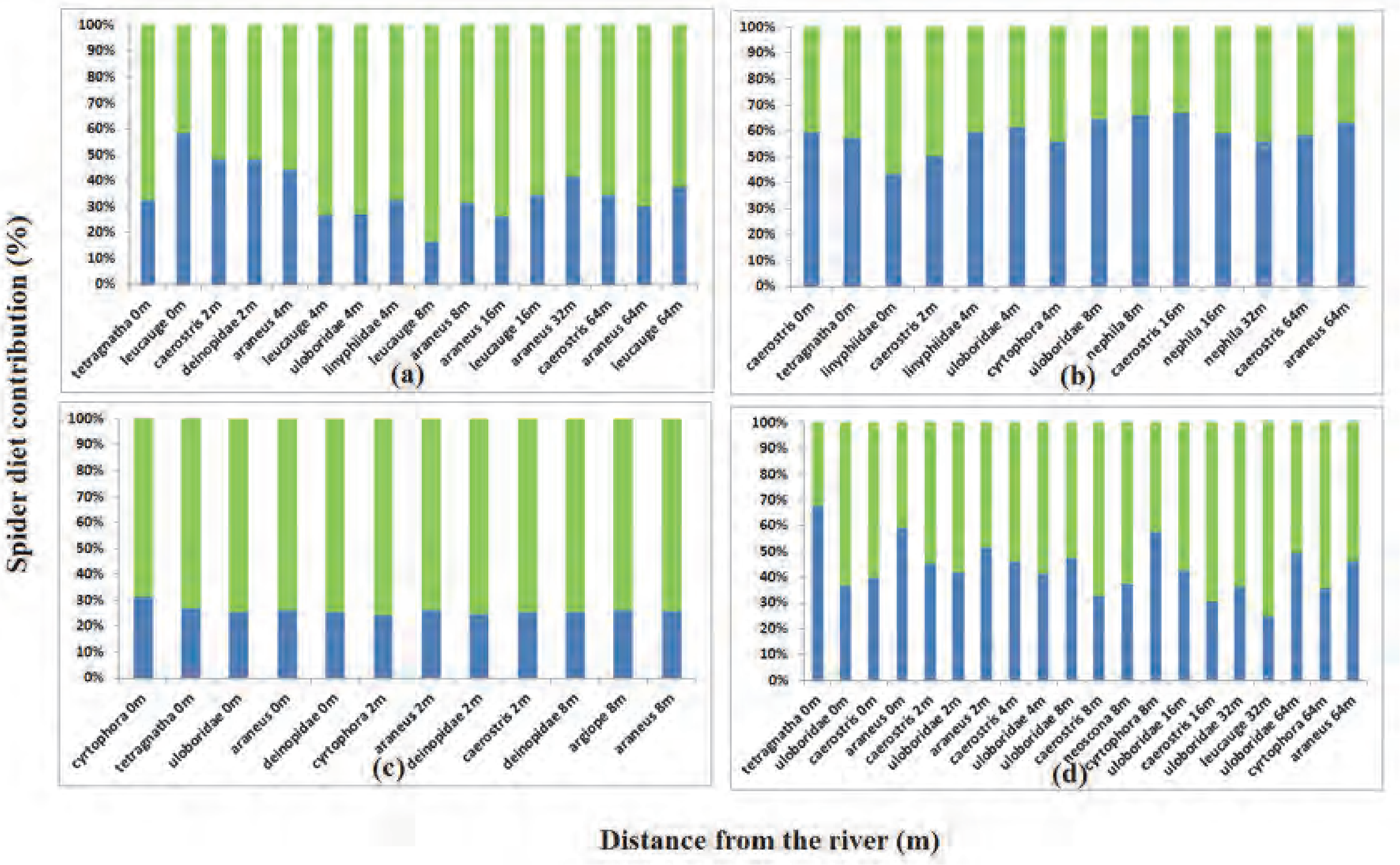

Figure 24. SIAR generated aquatic (blue) and terrestrially (green) derived contributions (%) to spider diets at varying distances (m) from the river channel at the upstream site F2. The letters a, b, c and d represent four seasons; summer, autumn, winter and spring

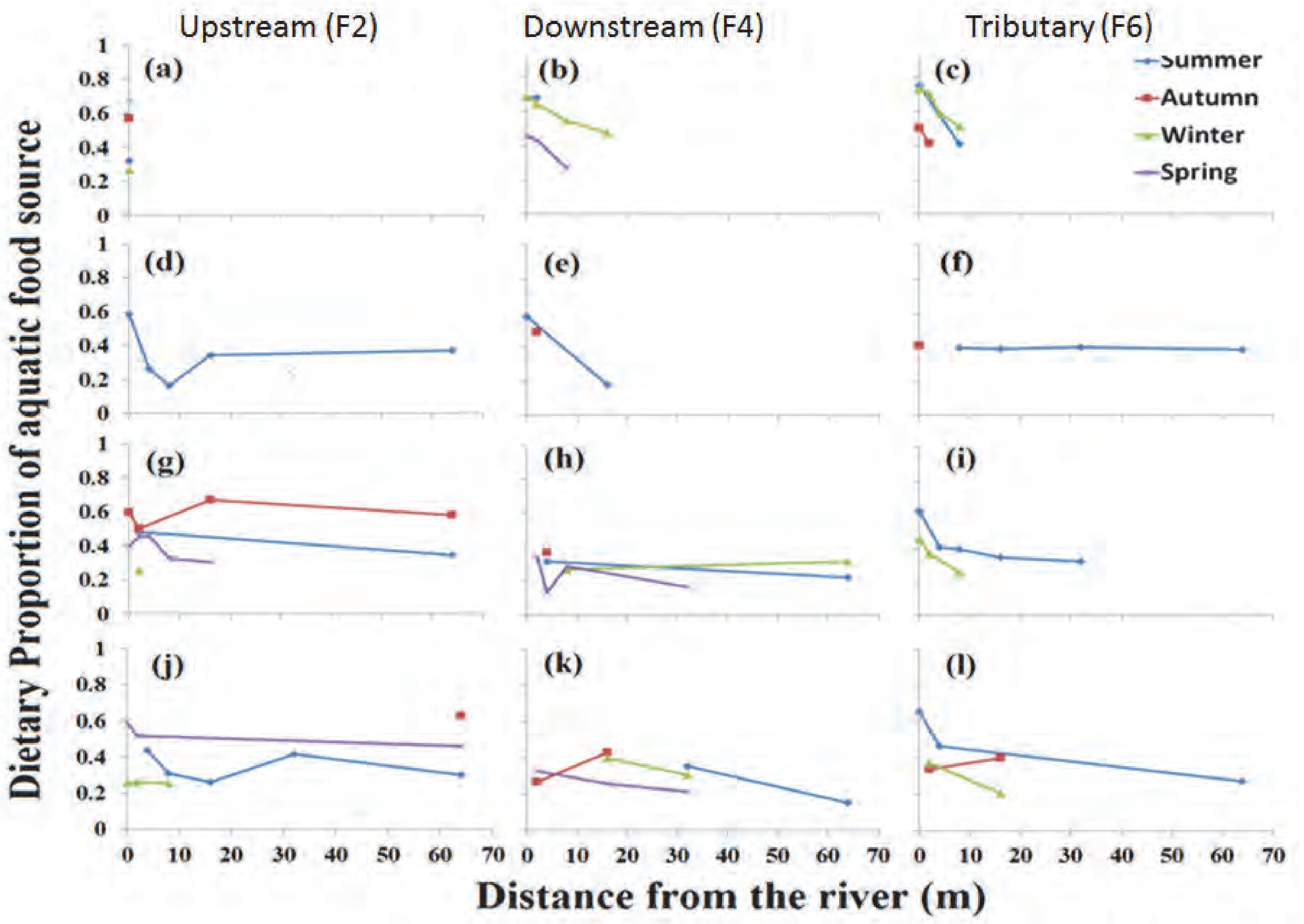

Figure 25. SIAR generated contributions of aquatic insects to the food sources of web building spiders at varying distances from the Kowie River during different seasons at three study sites. The letters (a)-(c) represent the spider genus *Tetragnatha*, (d)-(f) represent *Leucauge*, (g)-(i) represent *Caerostris* and (j)-(l) represent *Araneus*

Table 19: Regression analyses for proportions of aquatic contributions to spider diets with distance from the river, y = ax + b; where y = aquatic dietary proportion at distance x, b = slope of the line and a = y intercept

Site	**Season**	**R^2**	**F**	**p-value**	**b**	**a**
Upstream	Summer	0.17	0.13	0.36	-0.018	0.35
(F2)	Autumn	0.27	1.86	0.23	0.012	0.43
	Winter	0.41	0.70	0.56	-0.004	0.24
	Spring	0.48	4.62	0.08	-0.023	0.40
Downstream	Summer	0.83	20.10	0.01	-0.074	0.46
(F4)	Autumn	0.71	9.84	0.03	-0.074	0.50
	Winter	0.98	96.90	<0.001	-0.096	0.52
	Spring	0.76	6.71	0.05	-0.027	0.28
Tributary	Summer	0.97	69.91	<0.001	-0.038	0.43
(F6)	Autumn	0.58	2.56	0.17	-0.020	0.36
	Winter	0.85	10.82	0.03	-0.073	0.48

Aquatic insect biomass available as prey for aerial invertebrate predators was relatively low when compared to terrestrial biomass, but certainly aquatic biomass was greater closest to the river (0 m; **Figure 26**). The lowest insect biomasses were observed in the winter, when the biomass of aquatic insects was exessively small, even at 0 m.

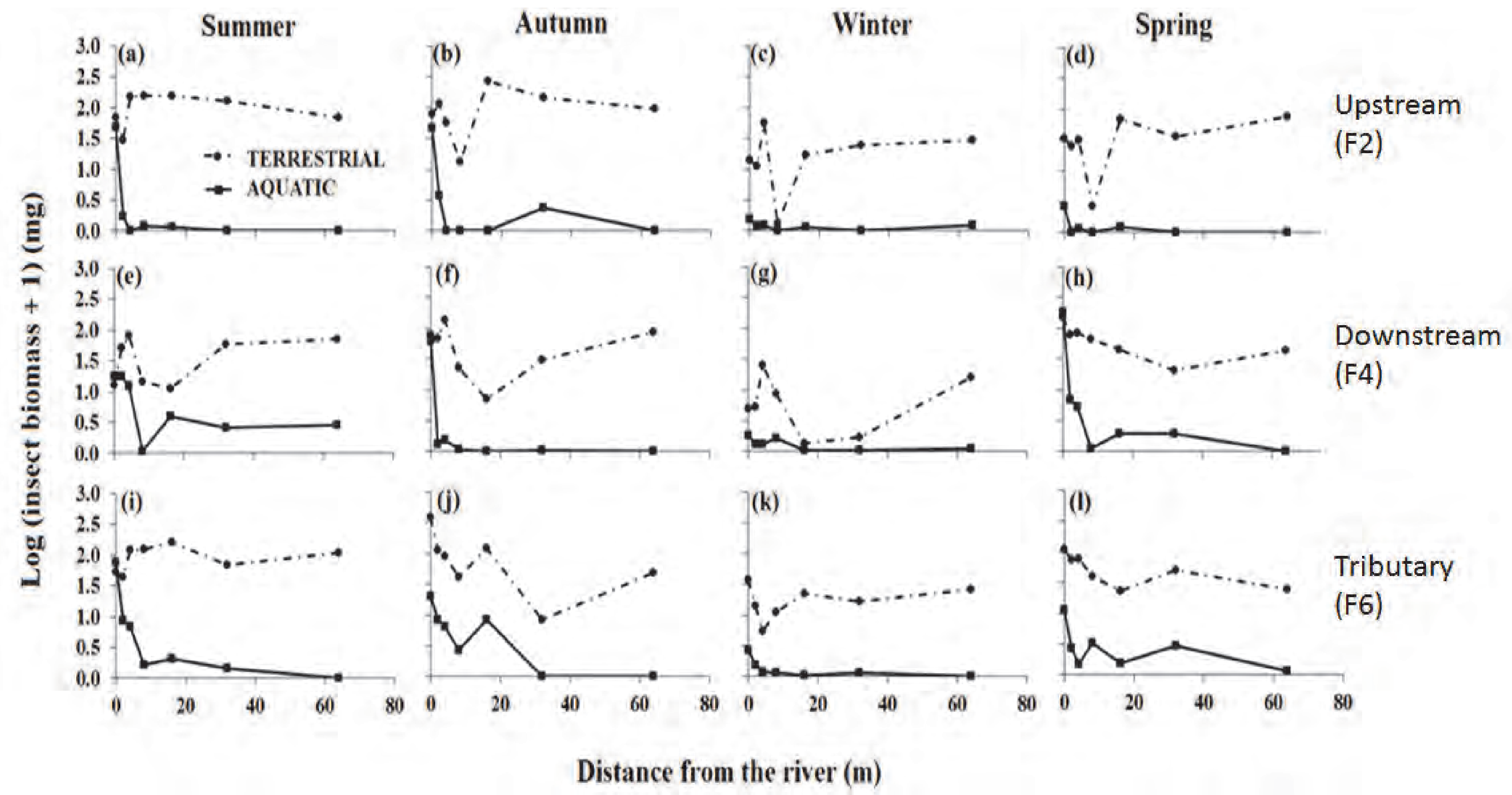

Figure 26. Total insect prey biomass at different distances (m) from the river during different seasons at the downstream site F4. Insects were collected using Malaise traps.

7.4.1 Spatial variations

We evaluated the contributions of aquatic and terrestrial prey to terrestrial spiders at varying distances from three locations in the Kowie River across several seasons. Regression analysis on the SIAR model outputs showed that in the two more downstream sites (F4 and F6), the proportions of aquatic food sources in spider diets corresponded to proximity to the river and the biomass of aquatic insects, but the strength of the relationship varied across seasons (more pronounced relationship during summer). These relationships were not strong at the upstream site F2. Regardless of type of site, the extent of aquatic contributions to spider diets was taxa specific, e.g. the genus *Tetragnatha* had the highest proportions of aquatic food in their diets when compared to other genera.

The greatest availability of aquatic insect prey biomass occurred at distances closest to the river (**Figure 26**), corresponding to where aquatic food contributed the most to spider diets (**Figure 25**). Other researchers have found links between local insect abundances at the riverside and contributions of aquatic food to spider diets (e.g. Baxter et al., 2005; Iwata, 2007; Burdon and Harding, 2008), but how far inland the insect abundance (or biomass) influences spider diet composition remains poorly studied. We found a larger influence of aquatic subsidies onto land at the sites with larger stream widths (downstream sites F4 and F6), as the influence was only from 0 to 2 m into the land at the upstream F2 site. Certainly the stream width affects insect emergence, with larger areas allowing for increased emergence (see **Section 7.3.1**).

An interesting result (and unexpected) was that percentage contributions of aquatic prey to spider diets remained very high with increasing distance from the river in some casees (**Figure 25**). Given that the biomass of aquatic insects decreased with distance (**Figure 26**), it appears that some web building spiders can consume more aquatic insects regardless of their shifts in availability. It may be that some webs are more specialized for the capture of small weakly flying insects (Yoshida, 1989; Williams et al., 1995; Marczak and Richardson, 2007), or that some spiders practice a degree of active prey selectivity (e.g. Nyffeler et al., 1989; Nyffeler, 1999; Ludy 2007). Although web-building spiders are largely passive generalist feeders, and the size, orientation and placement of the webs are considered highly important factors affecting their diets (Nentwig, 1986; Nyffeler, 1999; Snyder and Wise, 2001), controlled field studies would help to determine whether web-building spiders are selective regarding what prey are captured within their webs.

7.4.2 Temporal variations

Contributions of aquatic food to spider diets were highly variable among the spider taxa, therefore it was difficult to detect seasonal changes in diets. We expected seasonal differences, as emergence was much greater during summer compared to winter, for example. However, the seasonal aspects in the spider diets were complex and aquatic subsidies to their diets was not always greatest in summer. Additional factors (e.g. habitat complexity, microhabitats, etc.) are certainly influencing spider diets besides distance from the river and insect emergence. Kato et al. (2003) provided direct evidence of temporal variability in spider distributions related to emerging aquatic insect fluxes. In the Kowie River, the spiders at the downstream site F4 were strongly affected by insect biomass during seasons when aquatic insect biomass was high (i.e. during autumn and summer). However, overall the biomass of aquatic insects was always much lower than the biomass of terrestrial insects available, even during seasons and at sites with the high emergence rates. Since the Kowie River is characterised by relatively low aquatic insect flux on an annual basis compared with other rivers in temperate regions (e.g. Nakano and Murakami, 2001), there is probably generally less influence of aquatic-derived material on the diets of web-building spiders (and other local predators). A stream study was carried out with a focus on birds in a cool temperate deciduous forest in Japan, and the authors reported different seasonal dynamics occurred in insect biomass and bird diet compositions (Nakano and Murakami, 2001). Aquatic insect biomass was far greater than terrestrial insect biomass during winter, spring and autumn, and the birds consumed large quantities of aquatic prey during these periods (Nakano and Murakami, 2001). As such, trophic connectivity between freshwater and terrestrial systems is ecosystem- and area-specific, and the flux and productivity of local insects is a major determining factor in diet determination of aerial predators, and hence the size of the riparian buffer zone. Research on several additional predator populations within the same riparian areas of contrasting ecosystem types will help to clarify such questions.

7.5 Estuarine invertebrates

7.5.1 Community

Over 400 estuarine animals were identified, represented by 21 species. Species richness and abundance of taxa varied among site and period, although some groups were abundant everywhere at all sampling times. Taxa common in all areas of the estuary included the goby

Glossogobius callidus, the soleidae *Solea bleekeri*, the epibenthic crab *Hymenosoma* sp.*,* the isopod *Exosphaeroma hylecoetes*, and the amphipod *Grandidierella lignorum*. The salt marsh crabs *Sesarma catenata* (abundance peak during summer) and *Scylla serrata,* and the caridean shrimp *Palaemon peringueyi* (peak during spring) dominated (by abundance) the mid and lower sites. Mudprawns *Upogebia africana,* the hermit crab *Diogenes brevirostris*, and crab larvae were numerically abundant during summer at E3.

7.5.2 Isotopic composition of consumers

Invertebrate δ^{13}C ratios differed among sites and times. Consumers at E3 had significantly higher δ^{13}C values than those from E2 and E1 during all sampling periods, and the lowest δ^{13}C ratios values were measured in Feb at the three sites (**Table 20**). Annual means (±SD) at E1 ranged from -27 ± 0.1‰ in the amphipod *G. lignorum* to -23 ± 0.1‰ in the prawn *Penaeus monodon*. At E2, the mugilid *Liza dumerilii* had the highest δ^{13}C values (-18.8 ± 0.1‰), whereas copepods had the lowest (-27.2 ± 1.2‰). At E3, copepods had the lowest δ^{13}C signatures (-24.6 ± 0.2‰), whereas the prawn *U. africana* and the hermit crab *D. brevirostris*, together with the flathead mullet *Mugil cephalus*, had the highest δ^{13}C values among consumers (ranged from -12 to -15‰).

The δ^{15}N ratios of the benthic consumers differed among sites and times (**Table 20**). Consumers at E1 and E2 had significantly higher δ^{15}N values compared with those at E3 during all sampling times, and the highest values occurred in Nov and Feb. Consumer δ^{15}N values in E1 ranged from 20.2 ± 0.7‰ (*Glossogobius callidus*) to 19.6 ± 1.2‰ (isopod *Exosphaeroma hylecoetes*). At E2, the goby *G. callidus* had the highest δ^{15}N values (19.0 ± 0.4‰), while the crabs *Sesarma catenata* had the lowest δ^{15}N (16.0 ± 1.1‰). At E3 the signatures ranged from 16.3 ± 0.7‰ in *Solea bleekeri* to 12.5 ± 1.0‰ in the crab *S. catenata* and crab zoea.

Table 20. Two-way factorial ANOVA of isotopic data from pooled benthos (*Hymenosoma* sp., *Exosphaeroma hylecoetes*; *Grandidierella lignorum*, *Palaemon peringueyi*, *Glossogobius callidus*, *Solea bleekeri*, and *Sesarma catenata*) from E1, E2 and E3, with site and time as main fixed factors.

	df	MS	*F*	*p*	Tukey's post hoc
$\delta^{13}C$					
Site	2	327.34	100.00	<0.001	E1 > E2 > E3
Time	3	16.17	4.94	<0.01	June > Feb; Sept > Feb
Interaction	6	15.12	4.62	<0.001	
Residual	84	3.27			
$\delta^{15}N$					
Site	2	92.86	55.23	<0.001	E1 > E3; E2 > E3
Time	3	18.72	11.14	<0.001	Sept > June, Feb; Nov > June, Feb
Interaction	6	6.77	4.03	<0.01	
Residual	141	1.68			

7.5.3 Food source contributions to consumers

The goby *G. callidus* utilized copepods as a principal food source at all three sites (**Table 21**), and the amphipod *G. lignorum* contributed significantly to goby diets at E2 and E3. *G. lignorum* and copepods were large contributors to the diet of the sole *S. bleekeri* at E1 and E2, and mysids were the main prey in at E3. At E1 and E2, the crab *Hymenosoma* sp. fed mainly on SPM, whereas *Spartina maritima* and benthic invertebrates made important dietary contributions to the crabs at E3. *Spartina* detritus contributed substantially to the diets of the mudprawn *U. africana,* the hermit crab *D. brevirostris* and the brachyuran crab *S. catenata* at E3. This marsh grass food source combined with SPM occurred in high proportions in the diets of the mugillids *Liza dumerilii, Liza richardsonii* and *Mugil cephalus* (**Table 21**).

Table 21. Mean percentage contributions (95% credibility interval; determined using SIAR models) of organic matter sources to the diets of benthic consumers at three sites in the Kowie Estuary. Abbreviations of species/groups see **Table 22**. Reproduced from Bergamino and Richoux (2015) with permission.

Consumers	**Food sources**							
	POM	COP	EXO	GRA	MPB	SPA	CRA	SOM
Upper								
GRA	**57 (14-83)**				21 (0-48)			25 (0-53)
EXO	**83 (68-98)**				8 (0-20)			8 (0-20)
HYM	**16 (7-25)**	12 (0-32)	**29 (13-44)**	11 (0-31)				
GLO	5 (0-16)	**75 (45-100)**		22 (0-48)				
SOL		30 (0-56)	10 (0-28)	**43 (13-83)**			12 (0-32)	
Middle								
GRA	**44 (8-82)**				30 (0-57)			28 (0-60)
EXO	**83 (68-95)**				8 (0-21)			9 (0-23)
HYM	**36 (18-36)**	1 (0-27)	16 (0-34)	**36 (8-64)**				
GLO	6 (1-11)	**52 (41-61)**		41 (27-54)				
SOL		8 (0-21)	11 (0-31)	**57 (27-77)**			27 (0-52)	
SCY	33 (9-53)		22 (4-42)	**52 (26-80)**				
SES	17 (0-35)		12 (0-30)	20 (0-40)	**50 (30-70)**			
Lower								
GRA	**64 (30-97)**				23 (0-54)	12 (0-30)		
HYM	18 (0-38)	23 (0-45)	21 (0-39)	22 (0-44)		**37 (11-63)**		
GLO	22 (12-32)	29 (0-51)		**48 (24-77)**				
SOL		14 (0-32)		**40 (14-64)**			**45 (22-70)**	
SES	41 (13-63)				7 (0-23)	**41 (33-49)**		
UPO	10 (0-27)				17 (0-42)	**66 (56-74)**		
DIO	2 (0-7)				4 (0-13)	**92 (80-99)**		
SCY	**30 (12-47)**		31 (12-48)	20 (0-40)		**18 (6-30)**		
DUM	**37 (7-60)**				13 (0-36)	**50 (31-67)**		
CEP	10 (0-22)				7 (0-16)	**82 (63-64)**		
RIC	**31 (0-61)**				**32 (0-64)**	**36 (0-68)**		

Table 22. Food sources, consumers and their acronyms for the estuary of the Kowie system

Species/Group	Acronyms	Description/Size (cm)
ORGANIC MATTER SOURCES		
Phragmites australis	PHR	macrophytes
Schoenoplectus brachyceras	SCH	macrophytes
Chenolea diffusa	CHE	saltmarsh plant
Sarcocornia perennis	SAR	saltmarsh plant
Spartina maritima	SPA	marsh grass
suspended particulate matter	SPM	detritus/phytoplankton
epiphyton	EPI	algae attached on plants
terrestrial leaves	LEA	senescent leaves
sediment organic matter	SOM	microalgae/detritus/bacteria
microphytobenthos	MPB	microalgae
CONSUMERS		
Pseudodiaptomus hessei	COP	copepod (<0.02)
Acartia longipatella	COP	copepod (<0.02)
Mysidacea	MYS	mysids <2
Polychaeta	POL	polychaetes 4-6 cm
Exosphaeroma hylecoetes	ISO	isopoda (1-2)
Grandidierella lignorum	AMP	amphipods (1-2)
Hymenosoma orbiculare	HYM	brachyuran crabs (0.5-1.5)
Scylla serrata	SCY	brachyuran crabs (13-10)
Sesarma catenata	SES	brachyuran crabs (1-3)
Cleistostoma edwardsii	CLE	brachyuran crabs (1-2)
crab zoea	CRA	crustacean larvae
Palaemon peringueyi	PAL	caridean shrimp (2-3)
Penaeus monodon	PEN	tiger prawn (4-6)
Upogebia africana	UPO	mudprawn (6.5-7.5)
Diogenes brevirostris	DIO	hermit crab (4-6)
Glossogobius callidus	GLO	Gobiidae (2-4)
Liza dumerilii	DUM	Mugilidae (19-22)
Liza richardsonii	RICH	Mugilidae (23-27)
Mugil cephalus	CEP	Mugilidae 18
Solea bleekeri	SOL	Soleidae (5-8)
Syngnathus acus	SYN	pipefish (12-14)
Gilchristella aestuaria	GIL	Clupeidae (1.5-2.5)

7.5.4 Fatty acid composition of consumers

The fatty acid profiles of the dominant benthic consumers showed differences among the three estuarine sites. Seventeen fatty acids were detected at proportions >1% TFA in the consumers (**Table 23**). The consumers had high levels of 16:0, 18:0 (ubiquitous components; Volkman et al., 1998) and the polyunsaturated fatty acids (PUFAs) 20:5ω3 and 22:6ω3. The brachyuran crabs *S. catenata and S. serrata,* the mudprawn *U. africana,* and the hermit crab *D.*

brevirostris from E3 had high levels of 18:2ω6 and 18:3ω3. Among all sites, monounsaturated fatty acids (MUFAs) were the least dominant compared with PUFAs and saturated fatty acids (SFAs), and the most abundant MUFAs were 18:1ω9, 18:1ω7 and 16:1ω7 (**Table 23**).

The nMDS ordination (all dates pooled) and ANOSIM (Global R 0.12; $p < 0.05$) indicated that fatty acid profiles in the dominant benthic consumers were separable based on site (**Figure 27**). SIMPER together with PCA loadings showed that 20:5ω3 (41%); 22:6ω3 (40%), 22:5ω3 (6%) and 18:2ω6 (5%) contributed significantly to distinguishing the benthic invertebrates by site. Additional nMDS ordinations based on fatty acid profiles of the co-ocurring species (species pooled) discriminated among times within each site (**Figure 28**). At E1, the fatty acid profiles in consumers during Sept were similar to those in June, but they differed significantly from Nov and Feb (Global R 0.33; $p < 0.05$). Three fatty acids contributed ~60% to the discrimination among times at E1: 22:6ω3 (26%), 20:5ω3 (25%) and 16:0 (10%). At E2, ANOSIM results showed that the fatty acid profiles in consumers differed among times (Global R 0.31; $p < 0.05$). Pairwise comparisons revealed significant differences between Nov and the other times, and June was different from all the other time periods ($p < 0.05$). The temporal differences in consumers were driven mainly by 16:0 (36%), 20:5w3 (22%) and 18:0 (13%; **Figure 28**). At E3, the fatty acid composition of consumers in June differed from the other sampling times (Global R 0.562; $p < 0.05$; Fig. 3.5), and the same pattern was identified in Nov (Global R 0.562; $p < 0.05$), but no differences were identified between Feb and Sept ($p > 0.05$). SIMPER (and PCA loadings) showed that the fatty acids responsible for discriminating among times (up to ~70% of the cumulative profiles) at E3 were 16:0 (39%), 22:6w3 (15%) and 20:5ω3 (12%; **Figure 28**).

Table 23. Fatty acid composition (% ± SD) of dominant benthic consumers from 3 sites in the Kowie Estuary. SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids. Abbreviations of species/groups see **Table 22.** Reproduced from Bergamino and Richoux (2015) with permission.

	E1			**E2**					**E3**									
	HYM	GLO	SOL	HYM	GLO	SOL	SES	SCY	HYM	GLO	SOL	SES	DIO	UPO	SCY	DUM	CEP	RIC
14:0	1.7 (0.4)	5.3 (5.5)	4.43	1.3 (1.0)	3.2 (0.6)	2.6 (0.7)	1.02	0.6 (5.6)	4.55	1.9 (1.5)	2.5 (1.1)	2.4 (1.22)	7.5 (12)	1.7 0.6)	1.2 (0.4)	8.8	5.7	5.6
16:0	16.5 (2.4)	21.2 (3.6)	19.72	14.9 (4.8)	19.7 (8.7)	19.3(1.7)	44.60	14.5 (10.7)	28.62	23.2 (9.7)	16.4 (1.0)	30 (13.7)	18.3 (0.30)	17.45 (1.6)	15 (1.6)	38.6	46.8	10.8
17:0	1.9 (0.5)	1.6 (0.1)	1.34	2.4 (1.3)	1.3 (0.4)	3.7 (0.7	6.01	1.2 (0.8)	5.42	1.2 (0.5)	1.0 (1.0)	1.4 (1.3)	2.2 (0.2)	2.4 (0.3)	1.6 (0.0)	3.6	3.8	0.8
15:0	1.0 (0.17)	1.5 (0.6)	1.63	1.1 (0.2)	2.5 (2.1)	1.6 (0.9)	2.72	0.7 (1.2)	1.31	1.0 (0.3)	2.5 (2.3)	1.3 (0.4)	1.7 (0.3)	0.9 (0.1)	0.9 (0.4)	0.0	0.0	1.2
18:0	11.9 (0.9)	10.4 (3.0)	8.35	8.9 (1.2)	12.1 (6.1)	13.1 (0.7)	30.91	10.4 (3.5)	16.13	15.2 (6.4)	11.4 (2.4)	13.3 (7.9)	9.8 (1.6)	13.4 (2.5)	9.6 (2.1)	11.8	15.4	3.5
SFAS	34.1 (2.0)	38 (6.5)	36.25	34.7 (2.3)	40.5 (15.1)	41.3 (3.3)	86.68	28.3 (22)	57.8	42.8 (16)	36.4 (7.7)	50.8 (24)	40.3 (14.8)	38 (5.4)	28.8 (0.6)	62.7	71.7	22.0
16:1ω7	3.9 (1.3)	7.3 (2.2)	7.44	6.6 (2.1)	9.0 (5.2)	6.0 (0.9)	0.00	5.3 (25.8)	0.00	5.0 (2.7)	3.6 (2.3)	5.2 (5.7)	6.2 (2.6)	5.9 (1.8)	4.5 (1.0)	0.0	0.0	25.8
17:1ω7	0.6 (1.1)	0.8 (0.9)	1.35	1.3 (0.5)	0.7 (0.5)	1.6 (0.7)	0.00	3.5 (0.3)	2.87	0.2 (0.3)	1.8 (2.6)	0.3 (0.6)	1.4 (1.0)	0.0 (0.00	1.7 (2.5)	0.0	0.0	0.4
18:1ω9	6.8 (1.2)	8.0 (2.3)	10.21	7.8 (2.2)	7.9 (2.5)	7.4 (2.8)	0.00	11.2 (10.4)	2.87	6.5 (2.6)	6.1 (2.5)	6.0 (3.0)	5.0 (1.1)	6.0 (0.2)	10.5 (3.5)	0.0	0.0	10.4
18:1ω7	7.2 (2.4)	6.2 (2.4)	4.05	5.8 (0.1)	6.4 (3.7)	6.6 (4.4)	0.00	3.4 (0.8)	0.00	6.2 (3.9)	2.4 (0.9)	3.8 (2.9)	6.4 (1.6)	4.7 (0.2)	3.8 (1.6)	0.0	0.0	0.9
MUFAS	18.8 (2.8)	26.2 (12.3)	23.89	22.2 (0.9)	25.0 (7.9)	24.5 (5.6)	0.00	24.5 (37.4)	7.08	19.5 (6.2)	16.4 (1.6)	15.5 (10.6)	19. (6.3)	19 (2.6)	22.3 (3.7)	0.0	0.0	37.4
18:2ω6	1.3 (0.2)	1.7 (1.4)	0.96	1.4 (0.2)	1.2 (0.7)	1.0 (0.1)	0.00	0.9 (1.5)	0.00	0.8 (0.6)	0.5 (0.4)	3.8 (2.9)	2.0 (1.6)	2.6 (0.8)	4.1 (4.1)	0.0	0.0	1.6
18:3ω3	05 (0.5)	0.6 (0.8)	0.78	0.9 (0.9)	0.9 (0.7)	0.3 (0.4)	0.00	0.3 (0.9)	0.00	0.3 (0.3)	0.7 (0.6)	2.8 (2.5)	4.3 (3.3)	1.7 (0.7)	1.1 (0.6)	0.0	0.0	0.9
18:4ω3	0.6 (0.4)	0.9 (0.7)	1.59	0.3 (0.5)	0.9 (1.0)	0.6 (0.3)	0.00	0.5 (1.5)	0.00	0.1 (0.2)	1.3 (1.0)	0.3 (0.5)	0.6 (0.3)	0.6 (0.4)	0.5 (0.1)	2.5	1.9	1.6
20:4ω6	5.9 (0.4)	3.2 (2.2)	3.33	2.1 (3.0)	3.3 (1.4)	3.0 (0.05)	0.24	6.8 (2.3)	1.62	4.7 (3.0)	3.8 (0.7)	3.1 (3.0)	4.7 (1.3)	4.9 (1.0)	6.8 (0.9)	3.7	2.4	2.3
20:5ω3	23.5 (4.0)	12.2 (4.6)	7.89	21.5 (1.8)	11.2 (7.1)	8.5 (3.0)	0.00	21.1 (11.9)	9.40	11.5 (2.5)	6.1 (3.1)	12.4 (8.5)	19 (4.4)	23 (0.9)	19.0 (6.8)	3.1	6.3	12.0
22:4ω6	0.2 (0.4)	0.3 (0.4)	0.93	0.3 (0.3)	0.4 (0.4)	0.6(0.2)	1.09	0.7 (0.2)	0.00	1.0 (0.9)	2.7 (2.6)	0.07 (0.13)	0.00	0.0 (0.)	0.9 (0.6)	2.9	0.0	0.2
22:5ω3	1.3 (0.3)	3.9 (2.0)	7.98	1.4 (0.4)	3.0 (2.3)	7.4 (0.8)	0.00	1.7 (3.2)	3.65	4.6 (3.2)	5.8 (2.9)	0.2 (0.4)	1.7 (0.6)	0.8 (0.0)	2.3 (1.0)	5.9	6.7	3.2
22:6ω3	11.3 (1.5)	10.9 (10.6)	13.70	10.2 (1.9)	11.0 (8.4)	10.6 (2.5)	8.02	0.0	14.48	12.5 (9.7)	19.8 (11.4)	7.3 (3.6)	10.7 (1.6)	0.0	10.7 (3.6)	13.7	9.6	15.4
PUFAS	45.6 (4.5)	34.5 (18.6)	38.27	38.5 (5.2)	33.0 (19)	32.4 (0.07)	9.35	32.1 (38)	29.48	36.9 (20.7)	46.0 (8.2)	30.4 (17.25)	42.3 (13)	26.4 (4.0)	46.7 (5.0)	33.9	26.9	38

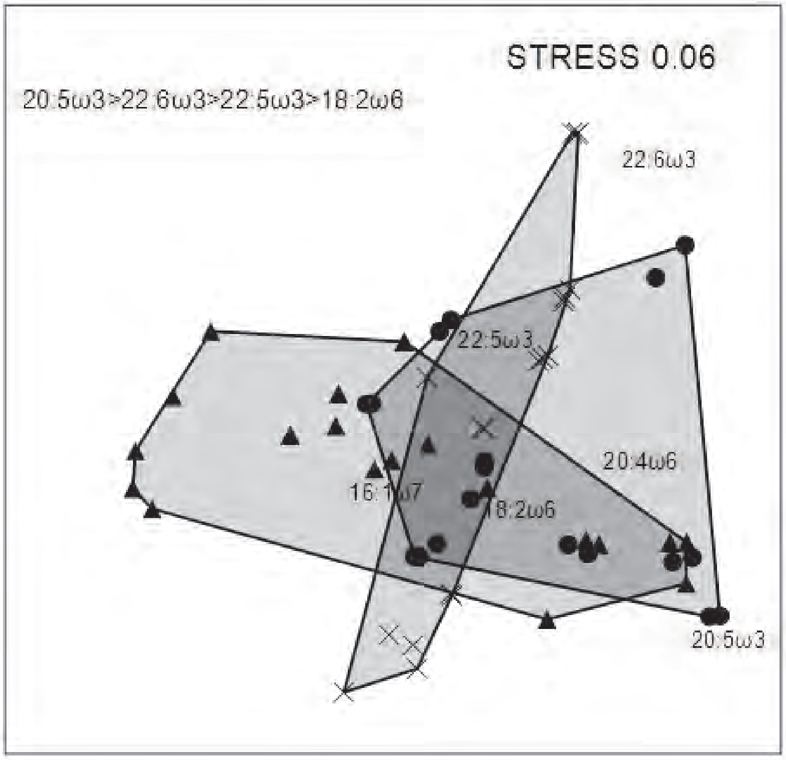

Figure 27. Two-dimensional non-metric multi-dimensional scaling of the fatty acid profiles in the dominant benthic consumers from 3 sites in the Kowie Estuary (all dates pooled): E1 (●); E2 (▲); E3 (x). Axes are dimensionless. Major fatty acids contributing to the separation across sites (derived from SIMPER and PCA) shown in decreasing order. Reproduced from Bergamino and Richoux (2015) with permission.

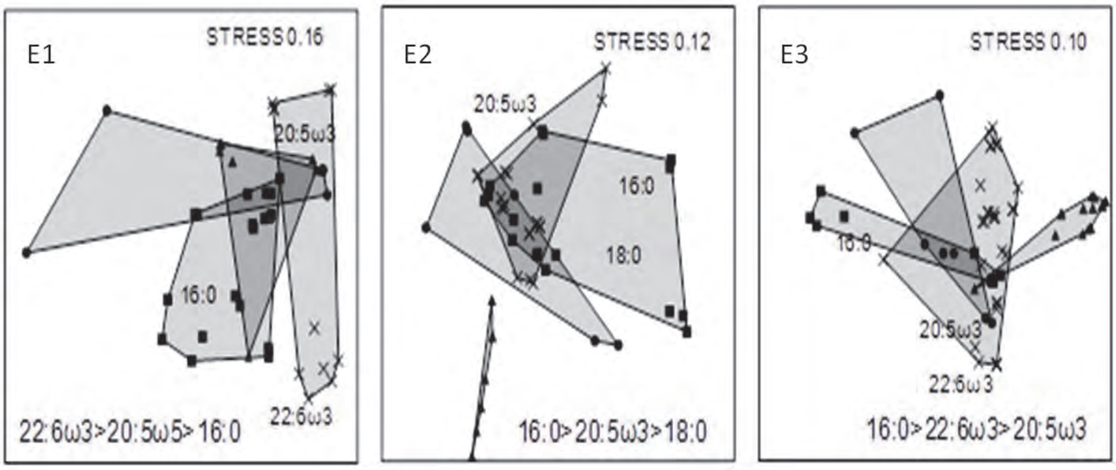

Figure 28. Two-dimensional ordination for fatty acid profiles of benthic consumers at three sites and among four periods (● Sept; ▲ Nov; x June; ■ Feb). Axes are dimensionless. Major fatty acids contributing to the separation among times (derived from SIMPER and PCA) are shown in decreasing order. Reproduced from Bergamino and Richoux (2015) with permission.

7.5.5 Estuarine food web

The benthic food webs were measurably different among the three sites of the small temperate Kowie Estuary, and trophic interactions within each site varied with season. A variety of potential carbon sources for invertebrates and fishes were considered including phytoplankton, epiphyton, microphytobenthos, sediment organic matter, live and senescent plants. The $\delta^{13}C$ values in consumers showed differences among sites, suggesting that different carbon sources supported the food web in different reaches. Consumers assimilated carbon sources produced locally, as these consumers had similar $\delta^{13}C$ values to those of the local resources where they resided (such as SPM, microphytobenthos or salt marsh grass). The models demonstrated that SPM was highly utilised by consumers at E1, whereas large contributions of marsh grass detritus were detected in the diets of consumers at E3 (**Table 21**). These results were supported by fatty acid and stable isotope data of sediment organic matter in the system, which indicated that the marsh grass *Spartina maritima* contributed substantially to the sediments in the lower reaches of the estuary (Bergamino et al., 2014a).

Differences in factors such as local vegetation (Bouillon et al., 2004; Richoux and Froneman, 2007), riverbank slope and floodplain width (Hoeinghaus et al., 2007) can be important in contributing to variations in the organic matter source contributing to consumers in estuaries. The lower reaches of the Kowie Estuary are colonised extensively by marsh grasses that may contribute largely to the production and accumulation of detritus in the region. These dense patches of saltmarsh plants and other vegetation in the shallow waters at E3 may promote the deposition of detritus by reducing hydrodynamic forces and leading to calm physical conditions (Bouma et al., 2005). Such physical differences in upper and lower estuarine regions support the idea that food webs based on detritus are dominant in the downstream regions of the estuary, in contrast with the deeper upstream regions where phytoplankton supports the estuarine food web (see also Odum and Heald, 1972). In addition to these hydrodynamic differences along an estuarine gradient, absolute salinity and variations in salinity both affect the spatial distribution of organisms having different salinity tolerances (Attrill, 2002; Whitfield et al., 2012). The salinity gradient along the length of an estuary may similarly affect the prey availability within the estuary (Gning et al., 2010). A combination of conditions (i.e. water depth, vegetation type and salinity regime) and biological features (i.e. feeding behaviour of consumers) may all contribute to spatial changes in community and food web dynamics within the Kowie Estuary.

Both the stable isotope and fatty acid data indicated enhanced assimilation of *Spartina* detritus by consumers at E3. Assuming fractionation of ~1‰ in $\delta^{13}C$ from food to consumer, the epibenthic crab *Hymenosoma* sp. utilized large proportions of SPM and small invertebrates at E1 and E2. Plant detritus was an important additional food for *Hymenosoma* sp. At E3 (**Table 21**). Stomach content analysis of *Hymenosoma* sp. also identified detritus and benthic invertebrates as key food items (Whitfield, 1989). The low nitrogen values for *Hymenosoma* sp. confirmed that this species occupies a primary consumer trophic position. A clear trophic association between *Spartina* detritus and the crabs *S. catenata, S. serrata* and *D. brevirostris,* and the mudprawn *U. africana* from E3 during Feb and June. The high δ^{13}C values in the mugilids *L. dumerilii*, *L. richardsonii* and *M. cephalus* suggested that their dietary carbon was derived at least partially from *Spartina* detritus. A diet including *S. maritima* was expected in the mugilid fishes from E3 since stomach content analysis of the same species indicated diets of SPM, unicellular algae and benthic plant material (Whitfield, 1988). Furthermore, given the high mobility of mugilid fishes together with their ability to utilize a variety of food sources, these fishes may be important habitat couplers, as has been observed in other systems (Schindler and Scheuerell, 2002). Either way, the dominance of detritivores in the lower estuarine reaches of the Kowie system suggests a donor control dynamic (Pimm, 2002) in which the rate of detrital input is a major factor influencing the trophic interactions within the macrobenthic community. The SIAR models can potentially overestimate the importance of marsh grasses because some potential food sources were missing (e.g. filamentous algae colonizing *Ruppia cirrhosa* and *Zostera capensis* that are periodically abundant in littoral areas of the Kowie Estuary; Whitfield et al., 1994). Epiphytes have been noted in the stomachs of some estuarine benthic invertebrates including amphipods, brachyuran crabs and isopods (Whitfield, 1989).

Consistent with the stable isotope data, the fatty acid profiles of the dominant benthic consumers were distinct among sites. Differences in the ω3 and ω6 PUFAs (particularly 20:5ω3 and 22:6ω3) in the gobies and sole collected from different sites confirmed spatial variations in the quality of their food sources (**Figure 27**). These fatty acids were dominant in benthic consumers from other temperate estuaries (Alfaro et al., 2006; Richoux and Froneman, 2008). Primary producers (primarily aquatic forms) are the sources of 20:5ω3 and 22:6ω3, EFAs that are key components needed for consumer growth, reproduction and survival (Arts et al., 2001). Furthermore, 20:5ω3 and 16:1ω7 are major components in the microphytobenthos and SPM in the Kowie Estuary (Bergamino et al., 2014a; **Section 7.2.2**), so we can use them as indicators of these food sources. These EFAs are associated with diatoms in marine environments (Dunstan et al., 1993; Parrish et al., 2000), so relatively high levels of 20:5ω3 and 16:1ω7 in the profiles of the benthic consumers may indicate a dominance of diatoms (benthic and/or pelagic) at the base of the Kowie food web during all periods. The PUFAs 18:2ω6 and 18:3ω3 are dominant in estuarine vascular plants like *S. maritima* and they can also be incorporated into consumer tissues (Richoux and Froneman, 2008). These components were present in significant amounts only in the brachyuran crabs (mostly in the *S. catenata* and the small mud crab *S. serrata*), mudprawns *U. africana* and hermit crabs from E3. Consistent with evidence from the stable isotope ratios, these fatty acid results indicate that the consumers assimilate plant detritus. However, the fatty acid profiles in *Hymenosoma* sp. and the mugilids *L. dumerilii* and *M. cephalus* differed from the other primary consumers from E3 and did not reflect assimilation of marsh grasses, so there are clear differences in diet among species.

The spatial variations that we observed in the relative importance of organic matter sources supporting estuarine consumers within the Kowie Estuary were consistent with other findings (e.g. Deegan and Garritt, 1997; Richoux and Froneman, 2007; Hoeinghaus et al., 2011; Claudino et al., 2013). These works showed that consumers assimilated organic carbon produced locally. However, certainly the contributions of saltmarsh grasses to the diets of estuarine animals vary with the region of study and the associated local environmental conditions (Kwak and Zedler, 1997; Riera et al., 1999; Kang et al., 2003; Hoeinghaus and Davis, 2007). For example, the relative importance of saltmarsh contributions to consumer diets is associated with the size of saltmarsh patches (Guest and Connolly, 2006). Our research has indicated that further investigations on the role of saltmarsh material in estuarine food webs are necessary to determine how extensive (at large scales) such contributions are in estuaries having different environmental features.

Our data represent evidence of seasonal variability in the isotopic signatures of consumers within each estuarine region. These seasonal changes may be induced by annual variability in environmental features, perhaps mainly determined by seasonal pulses of freshwater discharge (Riera and Richard, 1997; McLeod et al., 2010; Antonio et al., 2012; Olin et al., 2013). The highest freshwater discharge in October and November 2012 through the Kowie River (**Figure 6**) invariably brought in large amounts of nutrients, phytoplankton productivity (e.g. Froneman, 2001b) and terrestrial detritus. River phytoplankton and terrestrial C_3 plants have low $\delta^{13}C$ signatures that range from -23 to -30‰ (Fry and Sherr, 1984), so the low $\delta^{13}C$ signatures in consumers relative to SPM during Nov and Feb at the three sites likely resulted from increased assimilation of highly abundant low-$\delta^{13}C$ components during these periods. Additionally, during periods of low freshwater discharge, the estuary could be dominated by marine phytoplankton entering the mouth, resulting in higher $\delta^{13}C$ signatures that were reflected in consumers. The higher values of $\delta^{15}N$ that occurred during Nov and Sept may have resulted from high rates of denitrification promoted by the elevated temperatures during summer that lead to loss of isotopically light ^{14}N, resulting in the enrichment of the remaining N pools (Baeta et al., 2009). Overall, a combination of shifts in abiotic variables such as river discharge, and biochemical processes such as denitrification, may have contributed to the seasonal variability in the isotopic values in consumers and food sources in the Kowie Estuary.

7.6 Amphibians

7.6.1 Stomach contents

Among 82 frogs collected, seven had stomachs up to 25% full, ten were 50% full, 12 were partially full (75%), and 23 specimens had full stomachs (100%). Twelve individuals had no prey in their stomachs, and nine specimens had food items that were not identifiable. A total of 147 prey items belonging to 12 prey orders was identified (**Tables 24 and 25**). Diet composition varied among the four species. *Xenopus laevis* collected upstream at F1 consumed dipterans (Chirinomidae and Simuliidae), Hemiptera, and Ephemenoptera (mayfly larvae), with mayflies, aquatic hemipterans, chirinomids and blackflies constituting the most important food items in the diet (IAi = 6.4; 5.2; 4.2, and 3.7, respectively). Downstream at F4, *X. laevis* specimens had Ephemenoptera, Simuliidae and Chirinomidae as the most important prey in their diet (IAi = 9.6; 7.1 and 5.0, respectively); aquatic hemipterans and coleopterans, and Baetidae were also noticeable prey in the diet (IAi: 2.3; 2.1, and 1.3, respectively). *Amietophrynus rangeri* fed mostly on terrestrial insects, with Formicidae the most frequent food item in the stomach contents (*%F* = 30.0) followed by Hymenoptera (non-Formicidae) (*%F* = 20.0) and beetles (*%F* = 16.7), and the alimentary index indicated these food items were important for this species (IAi: 8.3; 3.4, and 2.2, respectively). Ingestion of plant material (such as twigs, small leaves, grass, etc.) was recorded in 21 stomachs (8 at F4 and 13 at F1) of *A. angolensis* and *S. grayii*, but the material made up a small percentage of each stomach (~2-4%). In *X. laevis*, some stomachs had traces of sediment (<5%) and algae (<3%).

Table 24. Alimentary Index (IAi) of the stomach contents of the frogs collected upstream at F1 along the Kowie River. SVL= Snout vent-length

		A. angolensis			*S. grayii*			*X. laevis*		
n		15			9			10		
Mean SVL (mm)		49.14			35.00			42.29		
Mean mass (g)		15.81			6.52			11.56		
Prey items	*Common names*	*%F*	*%N*	IAi	*%F*	*%N*	IAi	*%F*	*%N*	IAi
Araneae	Spiders^t	16.21	12.50	2.03	22.30	7.14	1.59	0.00	0.00	0.00
Coleoptera-A	Beetles^a	11.20	25.00	2.80	0.00	0.00	0.00	16.25	15.63	2.54
Coleoptera-B	Beetles^t	10.25	4.16	0.43	20.05	28.57	5.73	0.00	0.00	0.00
Diptera	Flies	15.32	4.17	0.64	9.30	7.14	0.66	0.00	0.00	0.00
Chironomidae^a		0.00	0.00	0.00	0.00	0.00	0.00	10.23	8.96	4.23
Simulidae^a	Blackflies	0.00	0.00	0.00	0.00	0.00	0.00	11.32	6.63	3.69
Hemiptera-A	Bugs^a	9.09	4.17	0.38	7.21	7.15	0.52	19.63	26.27	5.16
Hemiptera-B	Bugs^t	10.21	8.33	0.85	11.72	7.14	1.67	0.00	0.00	0.00
Hymenoptera		9.09	12.50	1.14	5.40	6.35	0.68	0.00	0.00	0.00
Formicidae	Ants^t	2.58	4.17	0.11	10.82	14.29	1.55	0.00	0.00	0.00
Lepidoptera	Moths^t	0.00	0.00	0.00	2.30	7.14	0.16	0.00	0.00	0.00
Odonata	Damselflies^t	8.62	12.50	1.08	11.60	7.15	0.83	0.00	0.00	0.00
Orthoptera										
Gryllacridae	Grasshoppers^t	7.43	12.50	0.93	4.70	6.14	0.34	0.00	0.00	0.00
Ephemenoptera	Mayflies	0.00	0.00	0.00	0.00	0.00	0.00	27.23	23.56	6.42
Baetidae	Small minnow mayflies^a	0.00	0.00	0.00	0.00	0.00	0.00	16.66	13.65	2.27
Blattodea	Cockroaches^t	7.14	8.54	1.85	0.00	0.00	0.00	0.00	0.00	0.00
Collembola	Springtails	6.14	3.59	0.85	0.00	0.00	0.00	0.00	0.00	0.00
Diplura		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Jaygidae	Diplurans^t	8.09	4.15	0.65	0.00	0.00	0.00	0.00	0.00	0.00

Table 25. Alimentary Index (IAi) of stomach contents of the anurans collected downstream at F4 along the Kowie River. SVL= Snout vent-length

		A. angolensis			*S. grayii*			*X. laevis*			*Am. rangeri*		
n		9			10			7			4		
Mean SVL (mm)		50.95			35.90			44.43			37.00		
Mean mass (g)		20.73			7.73			14.14			6.62		
Prey items	*Common names*	*%F*	*%N*	IAi	*%F*	*%N*	IAi	*%F*	*%N*	IAi	*%F*	*%N*	IAi
Araneae	Spiders^{t}	12.51	15.29	1.91	11.11	8.96	1.45	0.00	0.00	0.00	0.00	0.00	0.00
Coleoptera-A	Beetles^{a}	8.26	13.69	1.13	13.89	7.41	1.50	11.88	17.50	2.08	0.00	0.00	0.00
Coleoptera-B	Beetles^{t}	14.32	5.00	0.72	21.05	12.50	3.84	0.00	0.00	0.00	16.67	18.75	3.44
Diptera	Flies	13.15	10.95	1.44	15.21	9.26	2.06	0.00	0.00	0.00	0.00	0.00	0.00
Chironomidae^{a}	Chironomids	0.00	0.00	0.00	0.00	0.00	0.00	12.77	7.97	5.01	0.00	0.00	0.00
Simulidae^{a}	Blackflies	0.00	0.00	0.00	0.00	0.00	0.00	23.77	29.06	7.12	0.00	0.00	0.00
Hemiptera-A	Bugs^{a}	5.86	8.50	0.50	6.67	5.56	0.54	16.67	13.95	2.33	0.00	0.00	0.00
Hemiptera-B	Bugs^{t}	11.43	10.01	1.14	10.25	8.33	1.25	0.00	0.00	0.00	0.00	0.00	0.00
Hymenoptera		9.65	10.35	1.05	5.68	4.51	0.35	0.00	0.00	0.00	20.00	9.99	2.20
Formicidae	Ant^{t}	6.13	5.01	0.31	4.25	3.70	0.25	0.00	0.00	0.00	30.01	25.00	8.25
Lepidoptera	Moths^{t}	5.59	4.95	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Odonata	Damselflies^{t}	0.00	0.00	0.00	12.11	9.09	1.61	0.00	0.00	0.00	5.00	5.43	0.30
Orthoptera		6.98	10.92	0.76	0.00	0.00	0.00	0.00	0.00	0.00	8.33	6.70	0.61
Gryllacridae	Grasshoppers^{t}	6.12	5.30	0.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ephemenoptera	Mayflies	0.00	0.00	0.00	0.00	0.00	0.00	35.43	26.99	9.62	0.00	0.00	0.00
Baetidae^{a}		0.00	0.00	0.00	0.00	0.00	0.00	12.25	10.60	1.30	0.00	0.00	0.00
Blattodea	Cockroaches^{t}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Collembola	Springtails	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Diplura	Diplurans^{a}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Jaygidae		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Values of diet overlap varied from 0, when no food type was shared, to 1, when there was the same proportional use of all food resources. Although there is no critical level with which overlap values can be compared, Labropoulou and Eleftheriou (1997) suggested that values higher than 0.6 are biologically significant. The niche overlap between *A. angolensis* and *S. grayii* was high at F1 ($\hat{O}_{jk}$ = 0.46) and F4 ($\hat{O}_{jk}$ = 0.66), where the highest prey diversity occurred in diets. At F4, the trophic niche overlap was low for *Am. rangeri* and *X. laevis* ($\hat{O}_{jk}$ = 0.21), while upstream the niche overlap was low for *S. grayii* and *X. laevis* ($\hat{O}_{jk}$ = 0.21). *Xenopus laevis* and *Am. rangeri* had the lowest niche overlap. A very low degree of overlap existed between *X. laevis* and the other three anuran species, *A. angolensis, S. grayii* and *Am. rangeri*.

The trophic niche breadth of *A. angolensis* (Levin's measure) was 7.7 (B_{st} = 0.84) at F4, and 7.6 (B_{st} = 0.82) at F1; these niche breadth and standardized niche breadth measures were larger than those of all other species from both sites (**Table 26**). The species that consumed fewer prey such as *X. laevis* and *Am. rangeri* had smaller trophic niche breadths (**Table 26**).

Table 26. Trophic niche breadth of four anuran species from the Kowie River. B = Levin's measure of niche breadth and B_{st} = Levin's standardized niche breadth.

Species	**Downstream F4**		**Upstream F1**	
	B	**Bst**	**B**	**Bst**
A. angolensis	7.68	0.84	7.58	0.82
S. grayii	4.17	0.79	8.93	0.99
X. laevis	2.53	0.76	4.05	0.35
Am. Rangeri	2.36	0.68	-	-

Interpretations

Amietia angolensis and *Strongylopus grayii* in the Kowie River had a wide diet diversity made up of both aquatic and terrestrial invertebrates, while *Xenopus laevis* had stomach contents consisting only of aquatic invertebrates, and *Amietophyrnus rangeri* fed only on terrestrial invertebrates. Anuran species consume invertebrate prey with notable variations in behaviour (cryptic or conspicuous; slow moving or highly mobile insects) and developmental stage (larval or adult; França et al., 2004; Santos et al., 2004; Dietl et al., 2009). Such variations in prey selection suggest differences in the surrounding habitat, which is the case for *A. angolensis* and *S. grayii* in the Kowie River. These anurans were observed and collected on the water edges and banks of the stream, and they were associated with the vegetation, water (since aquatic insects were identified in the stomach contents) and soil (given the ingestion of terrestrial hemipterans). These findings support those from previous studies (França et al., 2004; Santos et al., 2004) that identified large ranges in prey type in several amphibians. França et al. (2004) found a large range of prey (including subaquatic and terrestrial organisms) used by two coexisting species of anurans (*Leptodactylus ocellatus* and *Leptodacytlus labryinthicu*). These authors also detected differences in microhabitat utilization by the two species, which might have been enough to allow coexistence. *Leptodactylus ocellatus* was found in a greater variety of habitats, whereas *Leptodacytlus labryinthicu* was restricted to water bodies with standing water and hence had a narrow trophic breadth (França et al., 2004). According to Forstner et al. (1998), there is a relationship between diet composition of a frog species and the microhabitat used, as different environments shelter various types of prey.

A relatively wide niche breadth was measured in *A. angolensis* in the Kowie River*,* reflecting a broader diet range compared to other species which consumed fewer numbers of prey. The observed differences in trophic niche breadth among the frog populations in the Kowie River may be related to their spatial distribution. *Amietia angolensis* occurred in a greater variety of habitats, and had a wide trophic niche (7.58 at F1 and 7.68 at F4), whereas *X. laevis* was restricted to water bodies and standing water and had a narrower trophic niche (4.05 at F1 and 2.50 at F4). The similarity in diets among some populations (such as *A. angolensis* and *S. grayii* sp.) may be a result of a wide variety of prey available in the Kowie River and to the coarse food categories used in the dietary analysis. Differences between the microhabitat utilized by the consumer, type of prey exploited, and time of activity have provided evidence that competition for food can be avoided in some habitats (Lima and Magnusson, 1998). In our study of anurans in the Kowie River, microhabitat variations among the species could occur at relatively small spatial scales even though they fed within a few meters from each other. For example, Van Sluys and Rocha (1998) reported two syntopic frog species (*Hyla minuta* and *Pseudopaludicula* sp.) in the Amazon varied in their feeding habits as a result of their body size, microhabitat use and activity time. The researchers observed that when active, individuals of *Pseudopaludicula* sp. were found partially submerged at the lake borders, whereas *H. minuta* were found predominantly on *Nymphae* sp. leaves on the banks of the lake (Van Sluys and Rocha, 1998).

From the insect orders identified as prey, Diptera and Hemiptera were consumed by almost all of the anuran species. Other invertebrates such as Araneae, Coleoptera and Hymenoptera were also consumed frequently. Some prey items could not be identified; as a result, some insect orders may have been underestimated as prey items. Several studies have identified zoobenthos and zooplankton to make up a principal part of the diet of some frogs, especially *X. laevis* (McCoid and Fritts, 1980; Measey, 1998; Bwong and Measey, 2010). In the Kowie River, these prey categories were not identified in the stomach contents of *X. laevis. Amietophrynus rangeri* appears to fit into the category defined by Toft (1980) as an “ant-specialist” given the high intake of ants by all individuals examined. Researchers have described terrestrial and aquatic insects as preferential prey items for frog genera such as *Hyla, Phyllomedusa, Leptodactylus* and *Physalaemus* (Toft, 1980; 1981; Van Sluys and Rocha, 1998; Anderson et al., 1999; Cogalniceanu et al., 2001; Santos et al., 2004). Hothem et al. (2009) evaluated the diets of three sympatric anuran species (*Pseudacris regilla*, *Rana boylii*, and *Lithobates catesbeianus*), and based on the stomach content analysis of all the species, *L. catesbeianus* was the only one having equal preferences for both terrestrial and aquatic prey. In another study, Stojanova and Mollov (2008) looked at the diet and trophic niche overlap of *Rana arvalis* (the moor frog) and *Rana temporaria* (the common frog) from Poland and identified the most important prey categories as Coleoptera, Hemiptera, Hymenoptera, Diptera, and Arachnida. The authors observed that both frogs consumed terrestrial prey almost exclusively (Stojanova and Mollov, 2008).

The vegetation material found in some adult frog stomachs in the Kowie River was probably unintentionally consumed while the animals were foraging (for example, see Hirai and Matsui, 1999; Solé et al., 2005; Dietl et al., 2009). Still, the idea that frogs may possibly select plant resources as food items should be considered. According to Anderson et al. (1999), anurans may actively select vegetation because it aids in the removal of intestinal parasites, makes available roughage to assist in crushing up arthropod exoskeletons, or provides nutrients and added sources of water. More information on the ingestion of vegetation by amphibians will enhance our understanding of behavioural patterns. For example, the occurrence of stamens, seeds and leaves in the guts of some specimens from the Kowie River showed that vegetated areas are used as foraging territories in addition to potential reproductive sites. Herbivory in frogs (especially in tropical frogs) has become more frequently reported in recent years (Santos et al., 2004).

Prey availability in the environment (a variable that was beyond the scope of this study) was not incorporated in any of the parameters used here. Resource availability is not easy to measure and has rarely been incorporated into anuran diet studies (but see Cogalniceanu et al., 2001; Whitfield and Donnelly, 2006). Researchers have recently illustrated that small insectivores can be very selective, discriminating actively among prey taxa (Simon and Toft, 1991). Furthermore, anurans can change their diet as they grow (Schriever and Williams, 2013). Mites and collembolans are usually the smallest arthropods available to anurans, prey of intermediate sizes include ants, beetles, bugs and termites, whereas the largest arthropod items are orthopterans, spiders, and lepidopterans (Lima and Magnusson, 1998; Santos et al., 2004). The most important prey categories in general include coleopterans, hemipterans and dipterans, and these were consumed frequently by almost all the frogs collected in the Kowie River. The coleopterans and dipterans are relatively abundant and occur in a wide range of habitats, and as such are readily available for predators (Stojanova and Mollov, 2008). The importance of these two insect groups as dominant prey for frogs in the Kowie River was confirmed by their frequent occurrence in stomach contents. Additional important prey included non-insect invertebrates such as Arachnida.

7.6.2 Stable isotope ratios

Adult *Amietia angolensis* averaged 8.3 ± 1.5‰ ($\delta^{15}N$) and -23.9 ± 1.3‰ ($\delta^{13}C$) upstream at F1, similar to the isotopic values measured downstream at F4 (**Table 27**). *Amietia angolensis* tadpoles had the lowest $\delta^{13}C$ values among consumers at both sites (-27.1 ± 2.1‰ upstream and -26.8 ± 2.0‰ downstream; **Figures 29c and 30c; Table 27**). The raucous toad *Amietophrynus rangeri* at F4 was the most ^{13}C-enriched consumer (-20.9 ± 1.1‰), followed by the clicking stream frog *Strongylopus grayii* (-23.1 ± 1.8‰). Common platanna *Xenopus laevis* exhibited similar $\delta^{13}C$ values upstream and downstream (**Table 27**). *Amietia angolensis* tadpoles had $\delta^{15}N$ values (8.9‰ F1 and 8.5‰ F4) similar to that of the adults (8.3‰ F1 and 8.4‰ F4). The clicking stream frog *S. grayii* had much higher $\delta^{15}N$ values than tadpoles of the same species at both sites (1.8‰ higher F1 and 2.5‰ higher F4). *Xenopus laevis* was highest in $\delta^{15}N$ values (12.3 ± 2.2‰) of all the consumers collected.

Trophic positions (TP) of the anurans were estimated using nitrogen isotope ratios, and they revealed clear differences between tadpoles and adults (**Table 27**). Tadpoles occupied a lower trophic position (*A. angolensis* tadpoles; TP = 2.3 upstream and 1.6 downstream; *S. grayii* tadpoles; TP= 2.3 upstream at F1 and 1.6 downstream at F4), whereas adults shifted to higher trophic positions. Adults within the same species maintained relatively constant trophic positions across sites. *Xenopus laevis* and *S. grayii* occupied the highest trophic position (3.1 and 3.0, respectively), followed by *A. angolensis* (2.5 downstream; **Table 27**). Ontogenetic diet shifts were evident in *A. angolensis* and *S. grayii* based on their trophic position.

SIAR models indicated that aquatic derived food sources contributed the majority of the tadpole diets (**Figures 31 and 32**). For *X. laevis*, the models suggested that aquatic sources contributed the majority of the diet (55% upstream at F1 and 72% downstream at F4), with little contributions from terrestrial sources (**Figures 33 and 34**). In contrast, dietary items from terrestrial sources made up the largest proportions of the diet of *Amietophyrnus rangeri* (**Figure 34**). Terrestrially-derived resources had relatively greater nutritional contributions to *A. angolensis* than aquatic-derived food sources (**Figures 33 and 34**). For

Strongylopus, the equal contributions of aquatic (48% upstream and 49% downstream) and terrestrial (52% upstream and 51% downstream) sources occurred in their diet.

Table 27. Stable isotope signatures (‰; mean ± SD) in adult frogs, tadpoles and potential food sources from upstream (F1) and downstream (F4) locations in the Kowie River. TP = trophic position, F4 isotope values in parentheses

	TP	**δ^{15}N**	**δ^{13}C**
amphibians			
A. angolensis	2.3	8.29 ± 1.84	-23.86 ± 1.30
	(2.5)	(8.43 ± 2.10)	(-23.40 ± 0.81)
S. grayii	3.0	9.51 ± 2.29	-24.99 ± 2.37
	(2.8)	(10.23 ± 3.12)	(-23.10 ± 1.79)
X. laevis	3.1	9.56 ± 2.66	-25.66 ± 2.13
	(3.1)	(12.34 ± 2.18)	(-25.43 ± 0.50)
Am. rangeri	(2.6)	(10.14 ± 1.52)	(-20.93 ± 1.14)
A. angolensis tadpoles	2.3	8.85 ± 1.59	-27.10 ± 2.14
	(1.6)	(8.52 ± 1.54)	(-26.77 ± 2.02)
S. grayii tadpoles	2.3	7.69 ± 0.81	-25.72 ± 1.57
	(1.6)	(7.73 ± 0.75)	(-24.32 ± 1.08)
aquatic sources			
Baetidae		6.95 ± 0.55	-28.09 ± 1.58
		(17.76 ± 1.33)	(-27.22 ± 2.80)
Coengrionidae		7.00 ± 0.71	-26.46 ± 0.43
		(18.92 ± 0.93)	(-27.22 ± 0.84)
Hydropsychidae		6.67 ± 0.57	-26.41 ± 0.38
		(18.49 ± 1.11)	(-27.38 ± 0.80)
Simulidae		5.14 ± 0.23	-26.17 ± 0.20
		(15.41 ± 0.77)	(-28.35 ± 1.19)
periphyton		5.61 ± 0.76	-24.99 ± 2.94
		(3.61 ± 1.37)	(-23.86 ± 1.63)
SPM		6.29 ± 1.49	-25.00 ± 0.66
		(5.82 ± 1.11)	(-29.60 ± 2.81)
benthic algae		4.00 ± 1.47	-19.22 ± 0.61
		(4.22 ± 1.68)	(-18.21 ± 1.32)
terrestrial sources			
Araneae		7.59 ± 0.80	-24.75 ± 0.23
		(13.65 ± 0.77)	(-21.24 ± 0.50)
Hymenoptera		5.69 ± 0.84	-26.62 ± 0.12
		(7.59 ± 2.41)	(-22.81 ± 0.96)
Formicidae		5.17 ± 0.24	-25.07 ± 0.74
Orthoptera		4.54 ± 1.32	-26.02 ± 0.49
		(7.38 ± 2.71)	(-20.64 ± 3.33)
leaves		2.55 ± 0.89	-28.61 ± 1.19
		(6.41 ± 1.99)	(-28.37 ± 0.30)

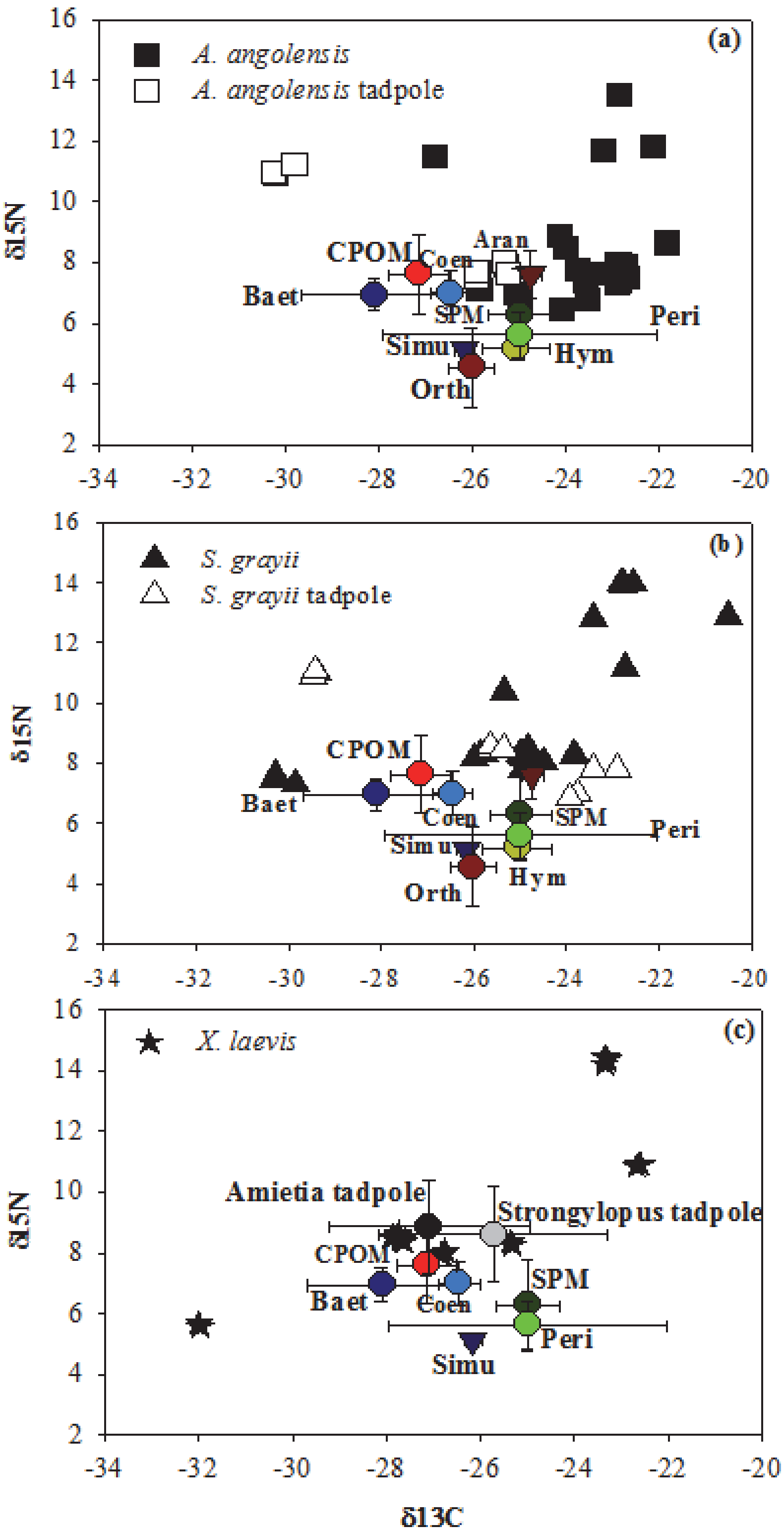

Figure 29. Stable isotope biplots representing adult anurans and tadpoles from F1, and mean values of prey items. Prey items are SPM suspended particulate matter, Peri periphyton, Aran Araneae, Baet Baetidae, Simu Simuliidae, Coen Coengrionidae, Orth Orthoptera, Hym Hymenoptera

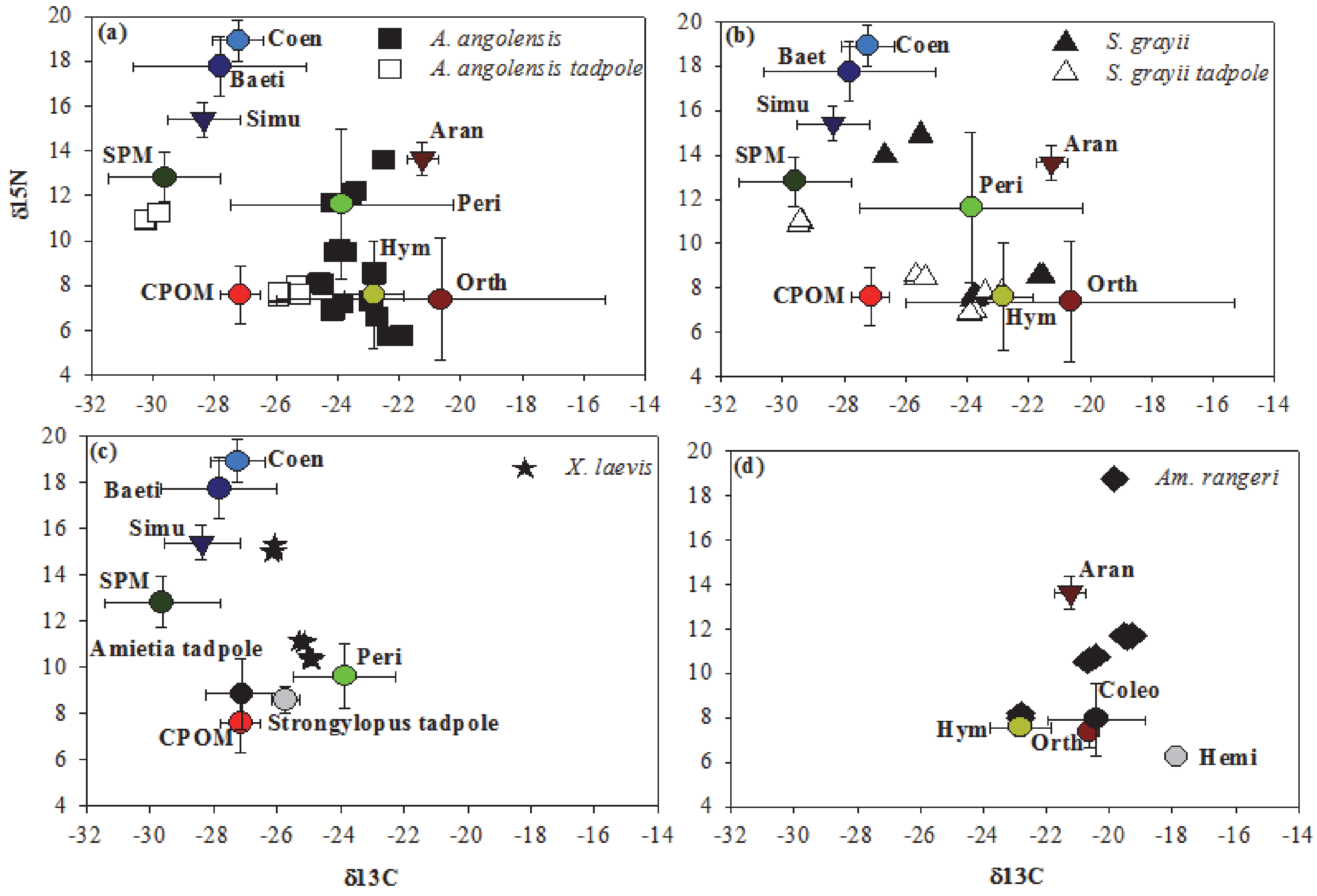

Figure 30. Stable isotope biplots of adult anurans and tadpoles from F4, and mean values of prey items. Abbreviations of prey are in **Figure 29**

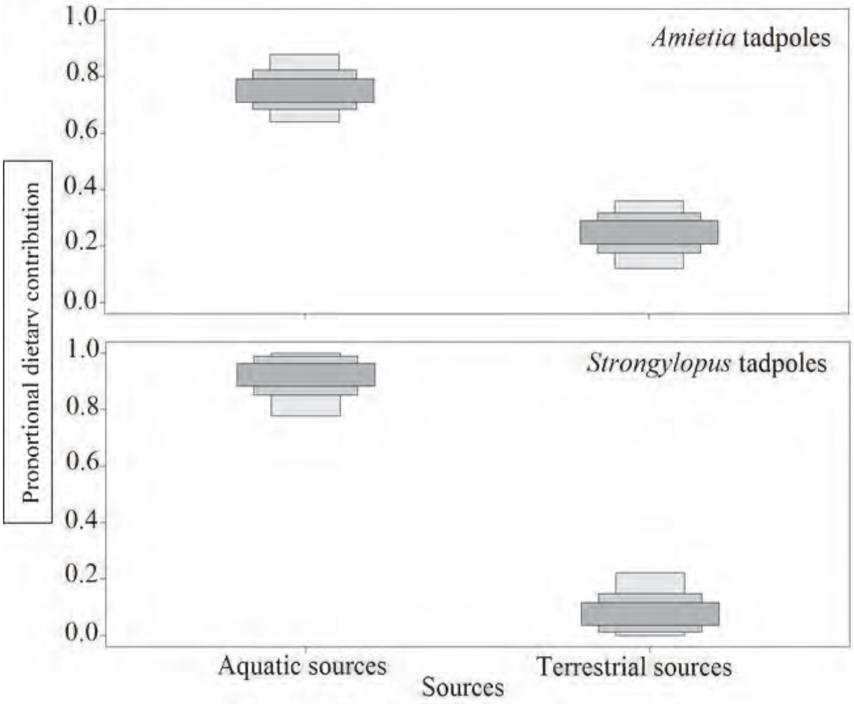

Figure 31. SIAR model output showing the dietary contributions of potential prey sources to the diets of the tadpoles collected from F1. The dietary proportions indicate the credibility intervals at 95, 75 and 25%.

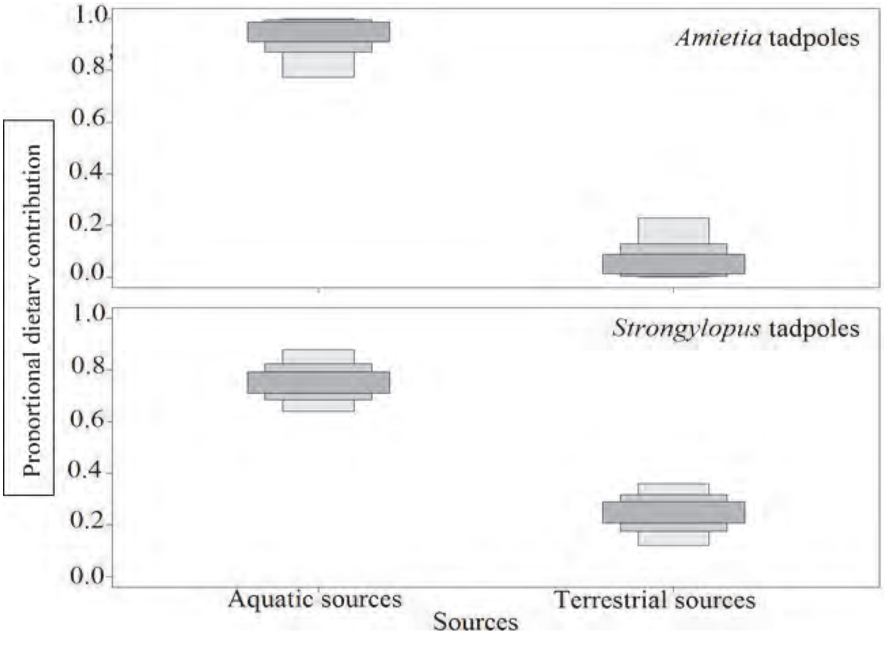

Figure 32. Boxplots from SIAR models showing the dietary contributions of potential prey sources to the diets of tadpoles collected from the downstream site F4. The dietary proportions indicate the credibility intervals at 95, 75 and 25%.

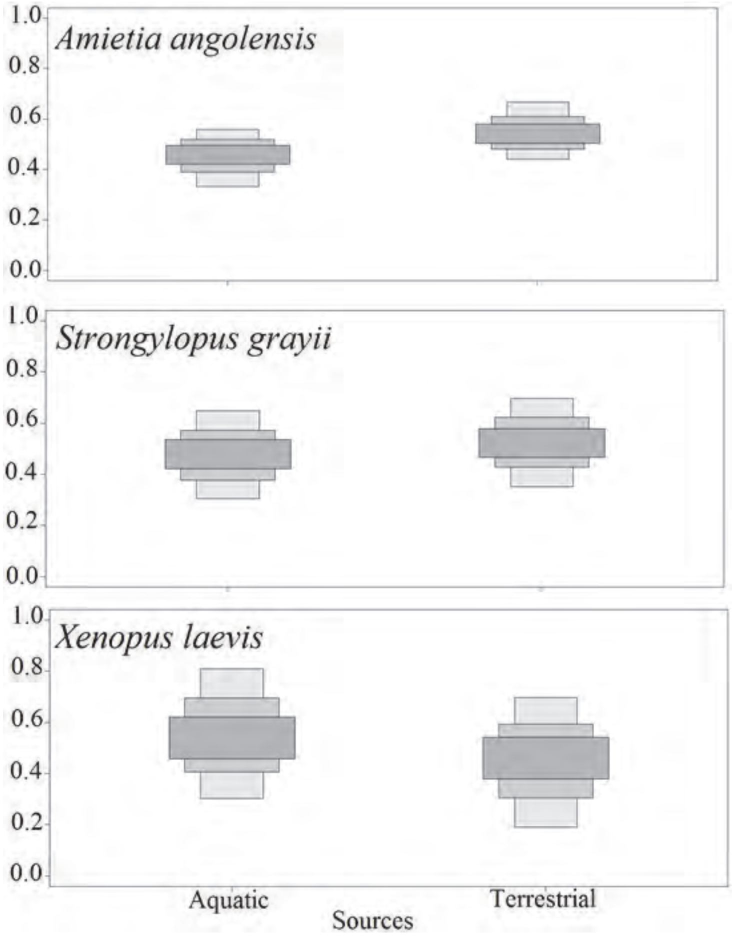

Figure 33. Boxplots from SIAR models showing the dietary contributions of potential prey sources to the diets of frogs collected from F1 in the Kowie River. The dietary proportions indicate the credibility intervals at 95, 75 and 25%.

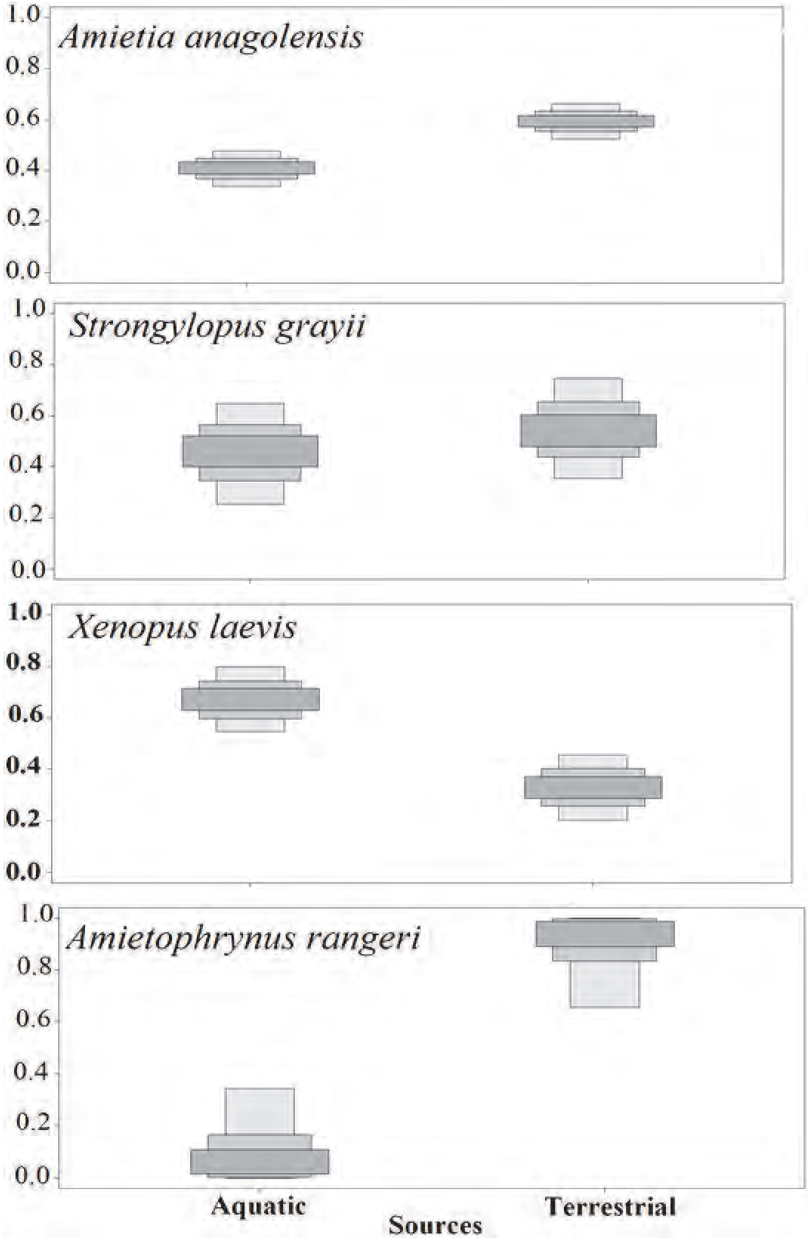

Figure 34. Boxplots from SIAR models showing the dietary contributions of potential prey sources to the diets of frogs collected from F4 in the Kowie River. The dietary proportions indicate the credibility intervals at 95, 75 and 25%

Interpretations

We investigated ontogenetic diet shifts of four anurans in the Kowie River based on stable isotope (δ^{13}C and δ^{15}N) analysis. Stable isotopic models showed that aquatic-derived sources had greater contributions to *Xenopus laevis* than terrestrial sources, while for *Amietia angolensis* and *Strongylopus grayii* the models revealed equal nutritional contributions of aquatic and terrestrial food sources. In contrast, for *Amietophrynus rangeri* the model indicated that terrestrially-derived sources made up major portions of its diet.

Higher trophic positions were measured in *A. angolensis* and *S. grayii* adults (2.5 and 3.0, respectively) compared to the tadpoles (1.6 and 1.6, respectively). Based on the isotopic compositions of the consumers, anurans from the upstream site F1 had more variable (intraspecific variability) diets compared to those from the downstream site F4. Interestingly, potential food sources at F4 were more enriched in nitrogen compared to F1 (see **Section 7.2.1**).

Isotopic data have demonstrated that the diet composition among and within frog species is more variable than previously thought based on stomach content data (Trakimas et al., 2011). If different frogs from the same population were all feeding on similar proportions of the same prey, one would expect much less variation in $\delta^{13}C$ values within the population (Gillespie, 2013). Interestingly, $\delta^{13}C$ values of frogs in the Kowie River showed some variation among individuals of the same species at both sites (**Figures 29 and 30**). Extreme $\delta^{13}C$ values for some individual frogs and tadpoles indicated that these individuals may have been eating a very different combination of prey than much of the population (**Figure 29** shows individuals with extremely low $\delta^{13}C$ values, **Figure 30** shows some extremely high $\delta^{13}C$). This result of wide dietary variations within a population is similar to that from a study by Araújo et al. (2009), who reported evidence of intraspecific diet variation in four frog species of Brazilian frogs. Furthermore, Schriever and Williams (2013) observed intraspecific dietary variation in amphibian larvae (wood frog tadpoles, *Lithobates sylvaticus,* and blue-spotted salamander larvae, *Ambystoma laterale*). *Lithobates sylvaticus* had a wide range of isotopic values ($\delta^{13}C$ = -37.6 to -27.2‰ and $\delta^{15}N$ = 3.8 to 5.0‰), suggesting generalist feeding (Schriever and Williams, 2013). In the Kowie River populations, a similar pattern was also observed where *A. angolensis* and *S. grayii* showed wide ranges of isotopic values ($\delta^{13}C$ = -26.8 to -21.8‰ and $\delta^{15}N$ = 5.8 to 13.63‰; $\delta^{13}C$ = -30.3 to -20.5‰ and $\delta^{15}N$‰ = 7.3 to 14.9‰, respectively), confirming generalist feeding behaviors and in agreement with gut content findings.

Our data have further identified the changes in diet with frog development in the species analyzed. Throughout their life history cycles, anurans go through ontogenetic habitat (aquatic-terrestrial) and dietary (herbivory-carnivory) shifts. Associated with these changes during amphibian development are changes in their isotopic signatures, as primary aquatic and terrestrial production tends to vary in $\delta^{13}C$ (e.g. Rau, 1980), while stepwise changes in trophic position are revealed by $\delta^{15}N$ (Minagawa and Wada, 1984). We found that $\delta^{13}C$ values in *A. angolensis* adults were markedly enriched in comparison with tadpoles, suggesting differences in their diets. Changes in anuran diet in the Kowie River were reflected in changing trophic positions of frogs, especially a marked shift between tadpoles that were ranked as primary consumers and post-metamorphic stages that were classified as tertiary consumers. Changes in diet to a more insectivorous diet for *A.*

angolensis and *S. grayii* through developmental stages (from tadpoles to adult form) at both sampled locations were reflected in their isotopic values and mixing models. Schriever and Williams (2013) also found ontogenetic diet shifts in amphibian larvae (*Lithobates sylvaticus*) based on both isotopic signature and gut content data. These researchers observed that *L. sylvaticus* switched from dependence on primarily non-filamentous algae and detritus as tadpoles, followed by an abrupt increase in the consumption of insects as adults (Schriever and Williams, 2013). Furthermore, Trakimas et al. (2011) found ontogenetic dietary shifts in the European common frog (*Rana temporaria*). These authors found that δ^{13}C values in *R. temporaria* tadpoles were significantly depleted in comparison to adults, signifying a terrestrial to aquatic shift in energy sources.

The models suggested that prey from aquatic sources made up the largest proportion of the potential *X. laevis* diet within the sampled community. The high contribution of aquatic sources to the diet of *Xenopus* was consistent with findings in previous studies (Measey, 1998; Bwong and Measey, 2010). On the other hand, terrestrial sources made up the largest proportions of the diets for the other species within the Kowie River community. Though stable isotope analysis does not give the taxonomic resolution of stomach content analysis, it did suggest that terrestrial prey constitute consistently significant portions of the diets of *Amietiophyrus* and *Amietia* sp. Other authors have noted that terrestrial material plays an important role in the aquatic environment. For instance, observations indicate that not only prey associated with aquatic vegetation, but insects that fall on the surface of water bodies make important contributions to the diet composition of some amphibian species (Dure and Kehr, 2001). In contrast, our results suggested that some amphibians such as the *Xenopus* are strongly dependent on carbon sources originating in aquatic systems.

7.6.3 Fatty acid composition

Thirty-six fatty acids were detected at concentrations > 1% of the total fatty acids (TFA) in at least three individuals of the sampled anurans (**Tables 28 and 29**). Fatty acid profiles were significantly different among anurans collected from upstream F1 (R = 0.35) and downstream F4 (R = 0.76) (**Table 30**). Major fatty acids in the individuals from F1 included the monounsaturated fatty acids (MUFAs) 18:1ω9 and 16:1ω7; the saturated fatty acids (SFAs) 16:0 and 18:0, and the polyunsaturated fatty acids (PUFAs) 18:3ω3, 18:3ω6, 20:4ω6, 20:5ω3, 22:5ω3 and 22:6ω3. At F4, the major fatty acids included the MUFAs 18:1ω9, 18:1ω7 and 16:1ω7, the SFAs 16:0 and 18:0, and the PUFAs 18:3ω3, 18:3ω6, 18:2ω6, 20:3ω6 and 20:4ω6.

Proportions of SFAs ranged from 32.2 to 50.0% at F4, with the highest proportions found in *Strongylopus* tadpoles, *Am. rangeri* and *A. angolensis*, and the lowest levels found in *A. angolensis* tadpoles (**Table 29**). At F1 there was relatively little variation in SFA proportions (ranged between 30.6 and 36.0%), with *S. grayii* tadpoles having the highest proportions (36.0%) and *S. grayii* having the lowest. The fatty acid 16:0 was the most common SFA identified in all species at both sites, followed by 18:0, 17:0, and 14:0, respectively. Of all the MUFAs identified, 18:1ω9 was the most dominant in all species, followed by 16:1ω7 and 18:1ω7 at both sites (**Tables 28 and 29**). The highest levels of long-chained MUFAs (i.e. 17:1ω7, 20:1ω8 and 20:1ω9) occurred in *X. laevis* at F4. The diatom-associated 16:1ω7 occurred in substantial proportions in tadpoles at both F1 and F4.

The proportions of PUFAs were high at both sites in different species (F1: *A. angolensis* tadpole, *S. grayii* tadpole and *X. laevis*, and F4: *S. grayii* tadpole and *Am. rangeri*) having low MUFA and/or SFA content. Levels ranged from 39.1% in *X. laevis* at F1 to 50.0% in *S. grayii* tadpoles at F4. The most prominent PUFAs were 20:5ω3, 22:5ω3 and 22:6ω3 in all species at both locations. However, the essential fatty acid (EFA) 22:6ω3 was found in small proportions in *Am. rangeri* (2.4 ± 0.4%) and *X. laevis* (3.5 ± 1.3%) at F4. While the proportions of ω3 PUFAs in anurans from F4 showed relatively little variation among species (14.3-18.9%), proportions in anurans at F1 were highly variable, with values ranging from 14.7% in *S. grayii* to 23.7 and 23.0% in *S. grayii* tadpoles and *A. angolensis* tadpoles, respectively. The proportions of EFAs in specimens from F1 were considerably high (ranged from 13.8 to 20.0%), whereas at F4 EFAs were moderate and showed more variability. There were also moderate proportions of bacterial fatty acids (BAFAs; *i*15:0, *ai*15:0, *i*17:0 and 17:0), with *A. angolensis* tadpoles and *S. grayii* tadpoles having high proportions of these fatty acids followed by *X. laevis* at F1. At F4, *A. angolensis* tadpoles and the adult form had similar proportions of BAFAs, and the remaining species showed high levels of these fatty acidds. The BAFA 17:0 occurred consistently in substantial concentrations in all the different species at both sampled regions.

SIMPER analysis (>50% cumulative percent contribution) revealed the fatty acids that greatly influenced the differences among the four species (**Figure 35**). Ordination by n-MDS suggested differentiation among the species at each site (**Figure 35**). Pairwise comparisons using the fatty acid dataset showed that *S. grayii* tadpoles were completely similar to *A. angolensis* tadpoles at the upstream site F1, and the remaining species were statistically dissimilar (**Table 31**). Downstream at F4, the pairwise comparisons using fatty acid profiles indicated complete dissimilarities of *Am. rangeri* and *X. laevis* to the other species (**Table 32**).

Table 28. Fatty acid composition (mean % TFA ± SD) of three anurans from the upstream site F1 in the Kowie River

FA	*Amietia* tadpole	*A. angolensis*	*Strongylopus* tadpole	*S. grayii*	*X. laevis*
14:0	1.00 ± 0.39	0.45 ± 0.27	1.30 ± 0.57	0.46 ± 0.40	0.79 ± 0.38
i-15:0	0.00 ± 0.00	0.03 ± 0.10	0.07 ± 0.20	0.11 ± 0.21	0.11± 0.20
ai-15:0	0.05 ± 0.12	0.05 ± 0.13	0.13 ± 0.27	0.07 ± 0.13	0.11 ± 0.18
15:0	0.68 ± 0.24	0.26 ± 0.20	0.64 ± 0.19	0.38± 0.23	0.38 ± 0.21
16:0	20.51 ± 1.97	17.97 ± 2.60	21.43 ± 1.18	19.10 ± 1.82	19.18 ± 2.74
i-17:0	0.93 ± 0.45	0.01 ± 0.04	0.15 ± 0.45	0.01 ± 0.03	0.32 ± 0.23
ai-17:0	1.00 ± 0.56	0.20 ± 0.49	0.46 ± 1.00	0.18 ± 0.37	0.37 ± 0.30
17:0	1.81 ± 0.94	0.71 ± 0.44	1.94 ± 0.57	1.14 ± 0.71	1.00 ± 0.59
18:0	8.91 ± 1.84	10.03 ± 2.36	10.00 ± 0.90	9.68 ± 2.71	9.67 ± 2.52
20:0	0.43 ± 0.22	0.60 ± 0.52	0.38 ± 0.31	0.54 ± 0.81	0.33 ± 0.28
22:0	0.42 ± 0.40	0.66 ± 0.61	0.31 ± 0.46	0.36 ± 0.36	0.34 ± 0.54
23:0	0.17 ± 0.46	0.12 ± 0.49	0.38 ± 1.14	0.04 ± 0.10	0.00 ± 0.00
24:0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16:1ω7	7.31 ± 1.94	1.78 ± 1.60	7.05 ± 1.84	4.36 ± 2.64	4.26 ± 1.33
17:1ω7	0.21 ± 0.24	0.04 ± 0.10	0.00 ± 0.00	0.08 ± 0.26	0.22 ± 0.22
18:1ω7	6.79 ± 1.53	3.82 ± 1.93	5.78 ± 1.75	3.71 ± 1.61	7.11 ± 1.78
18:1ω9	11.78 ± 3.58	24.38 ± 7.23	12.85 ± 6.11	25.33 ± 8.66	16.29 ± 5.11
18:2ω6	3.38 ± 1.46	16.74 ± 6.52	4.10 ± 2.60	12.56 ± 4.65	8.48 ± 1.74
18:3ω3	0.00 ± 0.00	3.09 ± 1.32	0.00 ± 0.00	2.64 ± 1.05	3.80 ± 1.67
18:3ω6	3.80 ± 1.14	0.00 ± 0.00	2.41 ± 1.87	0.00 ± 0.00	0.00 ± 0.00
18:4ω3	0.00 ± 0.00	0.02 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20.2ω7	0.27 ± 0.73	0.00 ± 0.00	0.00 ± 0.00	0.37 ± 0.25	0.00 ± 0.00
20:1ω8	0.00 ± 0.00	0.02 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.35 ± 0.76
20:1ω9	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:2ω9	0.00 ± 0.00	0.65 ± 1.24	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.35
20:3ω6	0.22 ± 0.59	0.41 ± 0.53	0.00 ± 0.00	0.62 ± 0.33	0.45 ± 0.32
20:3ω7	1.28 ± 1.80	0.66 ± 1.99	1.09 ± 0.80	0.47 ± 0.903	0.59 ± 1.57
20:3ω9	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:4ω3	1.29 ± 1.76	0.39 ± 0.97	1.10± 1.49	0.02 ± 0.07	0.93 ± 1.70
20:4ω6	5.27 ± 2.34	4.74 ± 2.48	6.34 ± 1.01	5.15 ± 2.08	7.06 ± 3.09
20:5ω3	8.44 ± 2.33	2.61 ± 2.17	9.39 ± 1.42	3.41 ± 1.67	5.10 ± 2.77
22:4ω6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.14
22:5ω3	6.58 ± 1.17	2.80 ± 2.32	4.87 ± 3.03	3.15 ± 1.67	4.23 ± 1.20
22:5ω6	1.01 ± 1.47	0.34 ± 0.89	0.19 ± 0.58	1.03 ± 0.93	0.58 ± 0.36
22:6ω3	6.32 ± 1.55	6.408 ± 3.24	7.656 ± 2.46	5.01 ± 2.80	7.64 ± 2.38
ΣPUFA	**38.00 ± 5.46**	**38.34 ± 8.20**	**37.22 ± 2.98**	**34.61 ± 11.45**	**39.14 ± 5.32**
ΣEFA	**20.02 ± 3.51**	**13.75 ± 5.83**	**22.57 ± 4.11**	**13.59 ± 7.26**	**19.80 ± 5.21**
ΣSFA	**33.93 ± 4.29**	**30.84 ± 4.36**	**35.95 ± 2.09**	**30.62 ± 4.25**	**31.79 ± 4.28**
ΣMUFA	**26.10 ± 2.84**	**30.41 ± 7.72**	**25.68 ± 4.09**	**34.55 ± 12.39**	**27.88 ± 3.82**
ΣBAFA	**24.98 ± 3.26**	**19.33 ± 2.79**	**24.34 ± 1.67**	**20.59 ± 2.31**	**21.47 ± 2.80**
ΣHPFA	**3.61 ± 1.86**	**18.85 ± 8.23**	**4.78 ± 4.46**	**14.65± 5.72**	**12.28 ± 1.70**
Σω3	**23.01 ± 3.87**	**15.07 ± 6.01**	**23.69 ± 4.77**	**14.77 ± 6.33**	**21.74 ± 5.01**
ω3/ω6	**1.76 ± 0.45**	**0.79 ± 0.51**	**2.273 ± 1.62**	**0.82 ± 0.37**	**1.34 ± 0.39**

ΣSFA: sum of all saturated fatty acids, Σ*MUFA*: monounsaturated fatty acids, sum of fatty acids with one double bond; Σ*PUFA*: polyunsaturated fatty acids, sum of fatty acids with two or more double bonds; Σ*EFA*: essential fatty acids (20:4ω6+20:5ω3+22:6ω6); Σ*BAFA*: bacterial fatty acids (*i*-14:0+*i*-15:0+*ai*-15:0+15:0+*i*-16:0+*i*-17:0+*ai*-17:0+17:0); *ΣHPFA*: higher plant fatty acids (18:2ω6+18:3ω3+24:0+25:0+26:0+28:0).

Table 29. Fatty acid composition (mean % TFA ± SD) of the four anurans from the downstream site F4. See **Table 28** for abbreviations

FA	*Amietia* tadpole	*A. angolensis*	*Strongylopus* tadpole	*S. grayii*	*Am. Rangeri*	*X. laevis*
14:0	0.86 ± 0.523	0.89 ± 0.92	1.15 ± 0.74	1.18 ± 1.05	1.35 ± 0.81	2.51 ± 1.97
i-15:0	0.12 ± 0.21	0.00 ± 0.00	0.00 ± 0.00	0.35 ± 0.77	0.00 ± 0.00	0.55 ± 0.64
a-15:0	0.13 ± 0.23	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.55	0.2 ± 0.28	0.32 ± 0.47
15:0	0.84 ± 0.39	0.62 ± 0.85	0.48 ± 0.33	0.52 ± 0.38	0.66 ± 0.38	0.90 ± 0.83
16:0	17.42 ± 4.78	17.74 ± 1.41	20.06 ± 3.10	21.79 ± 3.69	13.62 ± 0.81	21.30 ± 1.32
i-17:0	0.31 ± 0.54	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.99 ± 0.72
ai-17:0	0.65 ± 0.15	0.00 ± 0.00	0.49 ± 0.85	0.25 ± 0.61	0.00 ± 0.00	1.04 ± 0.71
17:0	1.51 ± 0.54	1.45 ± 0.69	0.37 ± 0.28	1.39 ± 0.43	1.14± 0.42	1.92 ± 0.56
18:0	8.79 ± 1.11	15.57± 2.05	11.31 ± 1.18	10.73 ± 1.02	10.03 ± 2.81	7.85 ± 1.16
20:0	0.56 ± 0.54	0.98 ± 0.61	0.42 ± 0.24	0.26 ± 0.16	0.59 ± 0.45	0.26 ± 0.31
22:0	1.00 ± 0.28	1.78± 0.65	0.79 ± 0.63	0.20 ± 0.19	0.74 ± 0.49	0.54 ± 0.85
23:0	0.00 ± 0.00	0.38 ± 0.65	0.00 ± 0.00	0.29 ± 0.28	0.25 ± 0.35	0.12 ± 0.31
24:0	0.17 ± 0.29	0.39 ± 0.68	0.00 ± 0.00	0.00 ± 0.00	0.39 ± 0.55	0.13 ± 0.35
16:1ω7	7.02 ± 0.88	1.99 ± 2.21	5.15 ± 2.44	3.95 ± 3.01	3.95 ± 1.82	6.85 ± 3.05
17:1ω7	0.54 ± 0.70	0.44 ± 0.75	0.00 ± 0.00	0.00 ± 0.00	0.46 ± 0.09	1.22 ± 0.42
18:1ω7	6.42 ± 0.29	3.39 ± 0.95	3.40± 0.39	3.96 ± 0.45	2.72 ± 0.16	7.79 ± 1.97
18:1ω9	11.13 ± 3.42	23.79 ± 6.24	14.87 ± 0.62	24.08 ± 5.71	21.61 ± 3.13	12.96 ± 2.46
18:2ω6	3.97 ± 0.83	11.98 ± 4.23	11.72 ± 1.02	8.14 ± 4.37	19.82 ± 3.75	5.79 ± 1.38
18:3ω3	1.02 ± 1.77	3.64 ± 0.65	3.71 ± 0.51	0.44 ± 1.07	2.51 ± 1.03	0.59 ± 1.05
18:3ω6	4.10 ± 3.44	0.00 ± 0.00	0.00 ± 0.00	2.85 ± 1.60	0.31 ± 0.44	6.39 ± 0.98
18:4ω3	0.14 ± 0.25	0 .00± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20.2ω7	0.15 ± 0.23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.16 ± 0.19
20:1ω8	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.43 ± 0.77
20:1ω9	0.18 ± 0.32	0.11 ± 0.30	0.00 ± 0.00	0.00 ± 0.00	0.49 ± 0.08	0.06 ± 0.16
20:2ω9	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.32 ± 0.45	0.16 ± 0.31
20:3ω6	4.12 ± 2.92	0.19 ± 0.33	6.95 ± 0.51	0.00 ± 0.00	0.74 ± 0.27	3.19 ± 1.24
20:3ω7	0.18 ± 0.31	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.13 ± 0.18	0.00 ± 0.00
20:3ω9	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:4ω3	0.89 ± 0.38	0.10 ± 0.26	2.89 ± 0.69	1.38 ± 0.93	0.00 ± 0.00	6.03 ± 1.53
20:4ω6	2.45 ± 3.37	3.97 ± 0.90	0.64 ± 0.35	1.75 ± 1.34	9.91 ± 1.52	0.72 ± 0.24
20:5ω3	9.13 ± 1.73	2.69 ± 1.95	0.14 ± 0.24	6.11 ± 1.88	2.69 ± 0.35	1.27 ± 0.75
22:4ω6	0.12 ± 0.21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.55 ± 0.18	0.38 ± 0.38
22:5ω3	6.83 ± 1.00	2.73 ± 1.28	5.89 ± 0.66	2.79 ± 0.81	1.72 ± 0.45	3.18 ± 1.83
22:5ω6	1.22 ± 0.38	0.00 ± 0.00	1.29 ± 0.19	0.00 ± 0.00	0.39 ± 0.11	0.61 ± 0.46
22:6ω3	6.66 ± 4.44	4.61 ± 1.47	8.28 ± 0.073	7.06 ± 2.13	2.39 ± 0.35	3.46 ± 1.31
ΣPUFA	**32.61 ± 5.22**	**37.51 ± 18.64**	**50.01± 16.46**	**33.25 ± 5.81**	**42.21 ± 0.53**	**33.29 ± 8.45**
ΣEFA	**14.16 ± 6.40**	**3.64 ± 2.39**	**5.03 ± 3.12**	**5.63 ± 3.73**	**8.77 ± 2.78**	**5.91 ± 3.28**

FA	*Amietia* tadpole	*A. angolensis*	*Strongylopus* tadpole	*S. grayii*	*Am. Rangeri*	*X. laevis*
ΣSFA	**37.05 ± 3.83**	**30.77 ± 7.51**	**22.83 ± 12.74**	**33.24 ± 11.69**	**31.16 ± 1.60**	**31.70 ± 6.89**
ΣMUFA	**30.34 ± 5.83**	**30.42 ± 18.39**	**24.18 ± 5.69**	**32.55 ± 7.55**	**25.92 ± 1.12**	**33.74 ± 11.72**
ΣBAFA	**16.02 ± 7.49**	**16.96± 13.46**	**10.97 ± 11.79**	**19.76 ± 11.55**	**19.66 ± 1.44**	**19.79 ± 7.13**
ΣHPFA	**15.09 ± 8.00**	**7.76± 9.12**	**0.76 ± 0.69**	**5.76 ± 6.15**	**3.57 ± 0.54**	**2.92 ± 2.45**
Σω3	**16.22 ± 6.78**	**14.34 ± 8.80**	**15.39 ± 12.09**	**14.37 ± 4.04**	**18.92 ± 8.42**	**15.71 ± 8.27**
ω3/ω6	**1.26 ± 1.06**	**2.47 ± 1.99**	**2.99 ± 2.54**	**1.50 ± 1.81**	**0.97 ± 0.73**	**0.90 ± 0.45**

Table 30. One-way ANOSIM outputs for the significant differences in the fatty acid profiles among anurans collected from the F1 and F4 in the Kowie River.

		ANOSIM output		
FACTOR	R	mean rank within	mean rank between	p
species	0.37	1079	1655	< 0.0001
site	0.07	1488	1602	< 0.0261

Table 31. Follow up pair-wise ANOSIM comparisons to determine how fatty acid signatures differed among the anurans sampled at the upstream site F1 in the Kowie River.

	Amietia tadpoles	*A. angolensis*	*Strongylopus* tadpoles	*S. grayii*	*X. laevis*
Amietia tadpoles		**<0.05**	0.35	**<0.05**	**<0.05**
A. angolensis	**0.69**		**<0.05**	0.24	**<0.05**
Strongylopus tadpoles	0.00	**0.65**		**<0.05**	**<0.05**
S. grayii	**0.59**	0.03	**0.55**		0.06
X. laevis	**0.49**	**0.29**	**0.34**	0.18	

**Lower left: R values, upper right p-values.*

Table 32. Follow up pair-wise ANOSIM comparison to determine how fatty acid signatures differed among the anurans sampled at the downstream site F4 in the Kowie River.

	Amietia tadpoles	*A. angolensis*	*Strongylopus* tadpoles	*S. grayii*	*Am. rangeri*	*X. laevis*
Amietia tadpoles		**<0.05**	0.10	**<0.05**	0.10	**<0.05**
A. angolensis	0.81		**<0.05**	**<0.05**	0.06	**<0.05**
Strongylopus tadpoles	0.70	0.64		**<0.05**	0.10	**<0.05**
S. grayii	0.65	0.43	0.57		0.03	**<0.05**
Am. rangeri	**1.00**	0.51	**1.00**	0.82		**<0.05**
X. laevis	0.75	**0.91**	**0.99**	0.84	**1.00**	

**Lower left: R values, upper right p-values.*

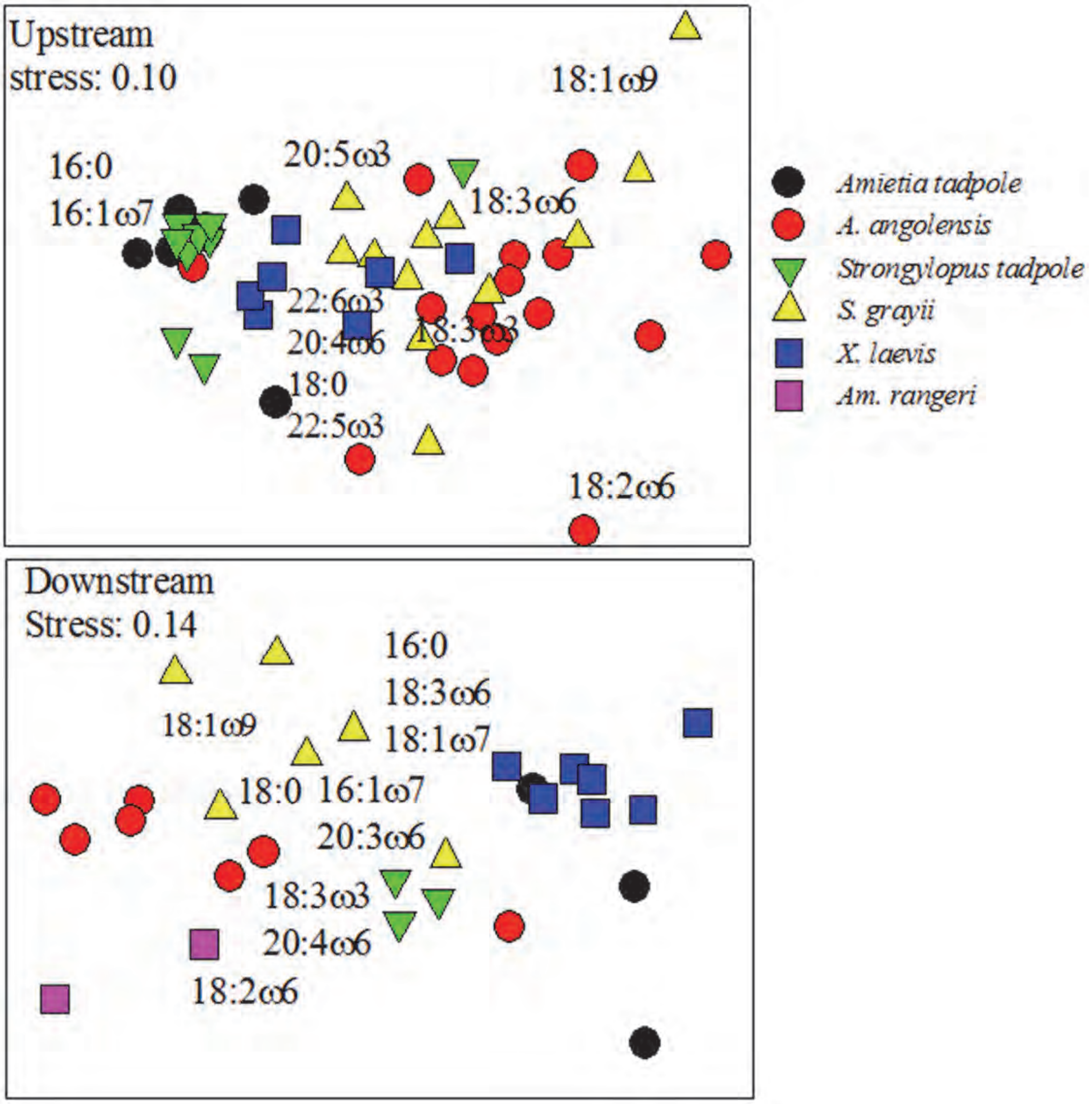

Figure 35. Ordination (n-MDS) output using fatty acid profiles of *A. angolensis, Am. rangeri, S. grayii* and *X. laevis* from both sites (A = upstream F1 and B = downstream F4). Fatty acids responsible for separating the four species (derived from SIMPER and PCA) are overlaid in the plots.

Interpretations

Trophic relationships in omnivores are complex because their lipid profiles originate from a variety of dietary sources (Dalsgaard and St. John, 2004), and it is not often possible to determine whether fatty acids were transferred to consumers directly or via lower-order consumers (Iverson, 2009; Pitt et al., 2009). Nevertheless, fatty acid composition techniques have been used to assess diets of aquatic consumers (e.g. Desvilettes et al., 1997; Koussoroplis et al., 2008). Here, we used fatty acids to elucidate the diets of four anurans in the Kowie River, South Africa. The anurans analysed were characterized by different compositions which varied with location and among species. Fatty acid profiles were more similar among species from the upstream region, while downstream specimens were much more spread out from each other. The fatty acid profiles for the anurans did not show great distinctions based on life stage. The fatty acids 16:0, 20:5ω3 and 22:6ω3 were always present in high proportions in both the adults and tadpoles (**Tables 28 and 29**).

High levels of SFAs 16:0 and 18:0 were expected in the tissue muscles of the analysed samples as these components represent the most common FA in nature and they form an important part of cellular membrane components (Garret and Grisham, 1999). According to Freites et al. (2002), detritus is recognized as a source of SFAs with 14 to 18 carbons. High levels of these fatty acids could indicate significant inputs of detritus and bacterial material in the diets of consumers. All four anuran species in the Kowie River had substantial levels of BFAs (upstream; 19.3-24.9% and downstream; 10.9-19.8%, reflecting detrital contributions to the diets of these consumers. The tadpoles (*A. angolensis* and *S. grayii*) had greater fractions of BFAs (i.e. 15:0, *ai*-17:0 and 17:0; *A. angolensis* = 0.7, 1.0, 1.8% and *S. grayii* tadpole = 0.6, 0.5, 1.9%, respectively) at both sites. Similarly, Whiles et al. (2010) used FA to examine tadpole diets from ponds in southern Illinois and found that tadpoles consumed substantial amounts of detritus.

Many animals can produce 18:1ω9 from the desaturation of 18:0, the modification of 18:1ω7, or the elongation of 16:1ω7, which is algal or bacterial in origin (Graeve et al., 1997). As a result, proportionally high levels of 18:1ω9 can be used as an indicator of carnivorous feeding in some cases (Graeve et al., 1997; Falk-Petersen et al., 2002; Drazen et al., 2008). The levels of 18:1ω9 were elevated in the anurans from the Kowie River (11.8-25.3% upstream and 11.1-24.1% downstream), supporting high levels of carnivory and/or omnivory in the species' diets. Whiles et al. (2010) reported high degrees of omnivory and dietary plasticity by analysing fatty acid compositions in pond dwelling tadpoles in southern Illinois, USA. Furthermore, Gillespie (2013), who used stable isotope analysis (which also accounts for prey assimilation) to study the foraging ecology of a highly endangered amphibian, *Eurycea sosorum*, in Eliza Spring Texas, USA, found that this amphibian demonstrated high levels of carnivory. Several researchers have shown that anurans feed on a variety of food sources in general (Altig et al., 2007; Whiles et al., 2010; Caut et al., 2012; Schriever and Williams, 2013). The summation of 18:2ω6 and 18:3ω3 represents the higher plant fatty acids (HPFAs) and these represent a terrestrial input in consumer diets (Dalsgaard et al., 2003). HPFAs were found in low concentrations in *X. laevis, A. angolensis* tadpole and *S. grayii* tadpole, suggesting that terrestrial organic matter does not constitute an important part of these consumer's diets.

In higher trophic level consumers, the PUFAs 20:5ω3 and 22:6ω3 are very important for growth and survival and these components must be acquired mainly from feeding

(González-Félix et al., 2003). Generally, high levels of PUFAs correspond with high quantity and quality food (i.e. good nutritional condition in the field) (Narváez et al., 2008). Based on the profiles in anurans from the Kowie River, the proportions of PUFAs were high at both upstream and downstream locations (upstream; 34.1-39.1% and downstream; 32.6-50.0%) and relatively similar among some of the species (*A. angolensis* tadpole, *S. grayii* tadpole, *A. angolensis, S. grayii* and *X. laevis*). The frogs and tadpoles at the upstream site showed large proportions of PUFAs and EFAs (PUFAs; 34.6-39.1 and EFAs; 13.6-22.6%), reflecting a good quality of food as consumers were acquiring sufficient lipids to meet their dietary needs. Interestingly, variations in EFA levels were reflected amongst species in the downstream (3.6-14.2%) suggesting a large variation in the food sources available.

The fatty acid compositions of aquatic animals mostly depend on the diets consumed in their habitats and the specific requirements related to physiological adaptations to the environment (Ackman, 1995). Fatty acid profiles differed between upstream and downstream sites, with a greater overall similarity in profiles among species in the upstream region, while downstream the species were overall much more distinct from each other (**Figure 35**). These results suggested that there may be more opportunity for diet differentiations (perhaps a larger food spectrum available) in the downstream site. Additionally, the intraspecific dietary differences across sites may have been linked to the differences in the relative availabilities and nutritional values of the food sources, which in turn may be a function of physical differences between the sites.

Fatty acid analysis proved a useful tool in assessing trophic relationships within the Kowie River. In most cases, information derived from interpreting the fatty acid profiles was in agreement with the results obtained using gut content and stable isotope data. While stable isotopes were able to provide information on the contributions of potential food sources within each location, fatty acid profiles could identify the overall nutritional quality of the food consumed, and the greater variability of food available at F4 (also show in greater isotopic variability at F4). The SIAR results illustrated that aquatic-derived food sources constituted most of the diet of *X. laevis, A. angolensis* tadpoles and *S. grayii* tadpoles, indirectly agreeing with fatty acid results that highlighted that terrestrial-derived sources did not constitute an important part of the diet of these species. Stomach content and stable isotope data were not able to provide any evidence on the bacterial sources consumed. However, fatty acids indicated that the four species had consistent BFAs levels at both sites, and high proportions of SFAs with 18 carbons, suggesting that bacterial and detritus food sources played a significant role in their diets.

7.7 Aquatic vertebrates – indigenous fish

7.7.1 Body sizes and stomach contents

The average size [standard length (SL), cm] of fish collected was similar at all sampling times (**Figure 36a**; *p* > 0.05), but varied among sites (**Figure 36b**; *p* < 0.05). The smallest fish were collected in the most upstream site (FW1). Fish increased in size along a river-estuary mouth gradient, and largest specimens were collected at sea (**Figure 36b**).

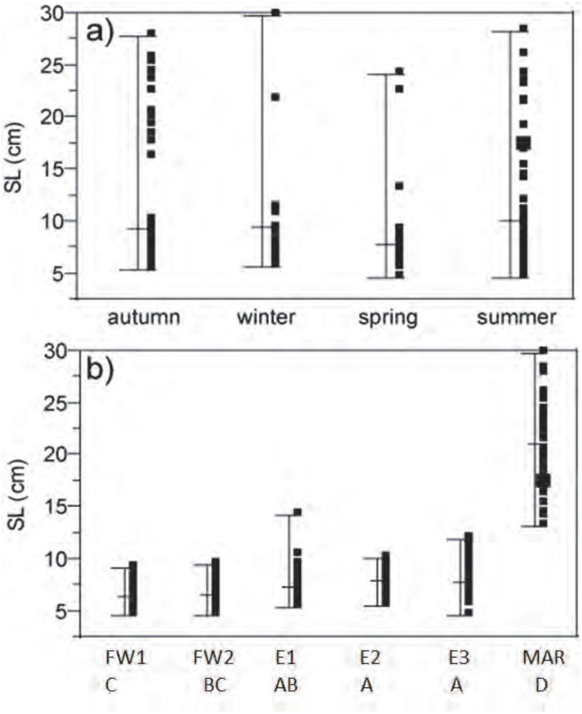

Figure 36. Average and range (min-max) of *R. holubi* sizes (SL, cm) observed at a) each season and b) at each site.

Stomach contents indicated that filamentous algae, amphipods, SPM and macrophytes were the dominant food items for *R. holubi*, with occurrences > 20% (**Table 33**). The proportions of prey in *R. holubi* stomachs significantly varied among habitats (R = 0.258, *p* = 0.001), salinity classes (R = 0.258, *p* = 0.001) and fish size classes (R = 0.460, *p* = 0.001), but not among seasons (R = 0.019, *p* = 0.123). Patterns observed for habitats, salinity classes and fish sizes classes were very similar, and corresponding n-MDS analyses provided very similar statistical performances and visual representations (**Figure 37**).

Prey contributing to differences between habitats, salinity groups and/or fish size classes varied depended on the factors and levels examined (**Table 34**). Overall, amphipods, barnacles, filamentous algae, gastropods and macrophytes were the most often responsible for differences between habitats, salinity groups and fish sizes. Barnacles and gastropods were more abundant in stomachs of fish collected at high salinity sites, i.e., large fish from the marine environment (**Figure 38**). Conversely, filamentous algae were dominant in stomachs of the smallest fish collected in freshwaters in the river. Amphipods were more abundant in stomachs of fish collected at low and medium salinity values, i.e., very small to medium sized fish from the river and the estuary. Finally, macrophytes were found mostly in stomachs of fish collected in medium salinity values, i.e., small to medium sized-fish from the estuary (**Figure 38**).

Table 33. Total frequency of occurrence (% of total number of fish dissected) of prey found in *R. holubi* stomachs (N = 170).

Prey	Occurrence (%)
Filamentous algae	68.8
Crustaceans (Amphipods)	32.4
suspended particulate matter	24.7
Macrophytes	21.8
Crustaceans (Isopods)	12.9
Crustaceans (unidentified)	12.9
Molluscs (Gastropods)	12.9
Annelida (Polychaetes)	10.0
Molluscs (Bivalves)	10.0
Insects (Chironomids)	8.8
Crustaceans (barnacles)	7.6
Decapods (Brachyura)	4.7
Decapods (Macrura)	2.9
Echinoderms (Holothurids)	2.4
Cladocerans (Daphnia)	1.8
Cnidarians (Hydrozoa)	1.8
Insects (aquatic breeding flies)	1.8
Crustaceans (Decapods)	1.2
Echinoderms (Echinoidae)	1.2
Echinoderms (Ophiuridae)	1.2
Insects (Dolichopodidae)	1.2
Insects (Stratiomyidae)	1.2
Sand	1.2
Unidentified	1.2
Arachnidae	0.6
Crustaceans(Copepods)	0.6
Diatoms	0.6
Insects (ants)	0.6
Insects (small bug or damselfly)	0.6
Molluscs	0.6
Plant seeds	0.6

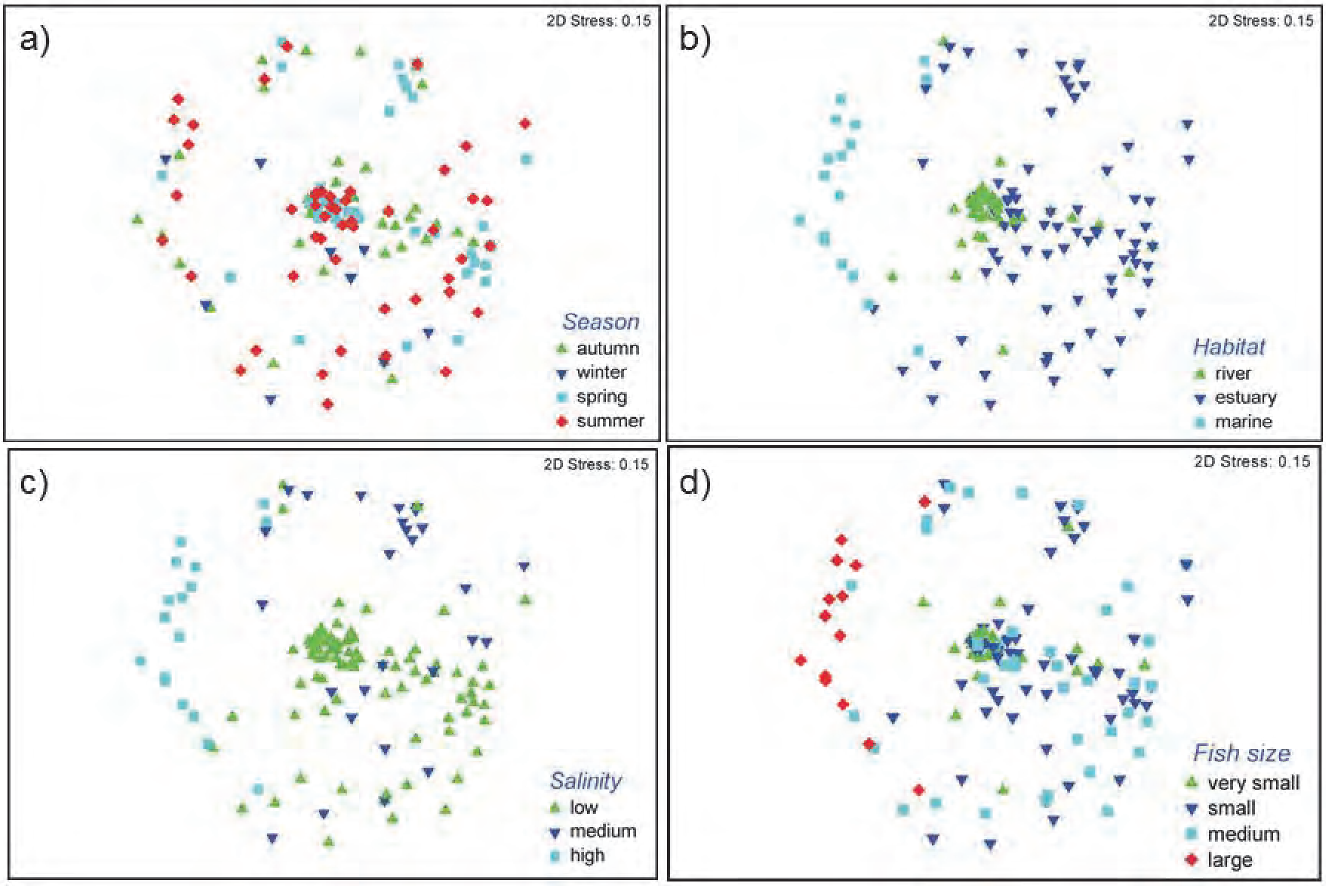

Figure 37. Multidimensional scaling analysis of stomach content data (prey proportion, %) as a function of a) season, b) habitat, c) salinity group, d) fish size class. Variations were significant for habitats, salinity groups and fish size classes only.

Table 34. Prey contributions to the dissimilarity (SIMPER) between habitats (river, estuary, marine), salinity groups (low, medium, high), and fish size classes (very small, small, medium, large) as observed in *R. holubi* stomachs. For clarity, only prey contributing to more than 5% of the dissimilarity for each levels tested are reported.

Factor	Levels	Prey	Contribution (%)
Habitat	River vs. estuary	filamentous algae	33.90
		amphipods	17.14
		macrophytes	14.72
		SPM	8.70
		isopods	5.31
	River vs. marine	filamentous algae	35.17
		gastropods	20.42
		barnacles	15.06
		bivalves	5.15
	Estuary vs. marine	gastropods	19.08
		barnacles	14.44
		amphipods	11.85
		macrophytes	10.98
		filamentous algae	7.54
		SPM	6.54
		bivalves	5.41
Salinity	Low vs. medium	filamentous algae	22.95
		macrophytes	21.78
		amphipods	13.62
		SPM	11.76
		unidentified crustaceans	6.03
	Low vs. high	filamentous algae	20.18
		gastropods	19.45
		barnacles	13.88
		amphipods	10.12
		bivalves	5.02
	Medium vs. high	gastropods	19.73
		macrophytes	18.70
		barnacles	14.19
		SPM	8.30
		unidentified crustaceans	6.48
		bivalves	5.38
Fish size	Very small vs. small	filamentous algae	31.78
		macrophytes	19.41
		amphipods	17.73
		SPM	7.00
		isopods	6.82
	Very small vs. medium	filamentous algae	32.55
		amphipods	16.53
		SPM	9.29
		unidentified crustaceans	6.09
		isopods	5.92
		decapods (Macrura)	5.59
		macrophytes	5.46

Table 34, continued

Factor	Levels	Prey	Contribution (%)
Fish size classes	Small vs. medium	filamentous algae	19.76
		amphipods	17.11
		macrophytes	15.96
		SPM	11.45
		unidentified crustaceans	6.47
		decapods (Macrura)	5.40
	Very small vs. large	filamentous algae	33.00
		gastropods	20.91
		barnacles	16.94
		amphipods	5.08
	Small vs. large	gastropods	20.91
		barnacles	16.88
		filamentous algae	14.92
		macrophytes	11.61
		amphipods	9.01
	Medium vs. large	gastropods	20.59
		barnacles	16.69
		amphipods	11.34
		filamentous algae	8.95
		SPM	7.15
		decapods (Macrura)	6.67

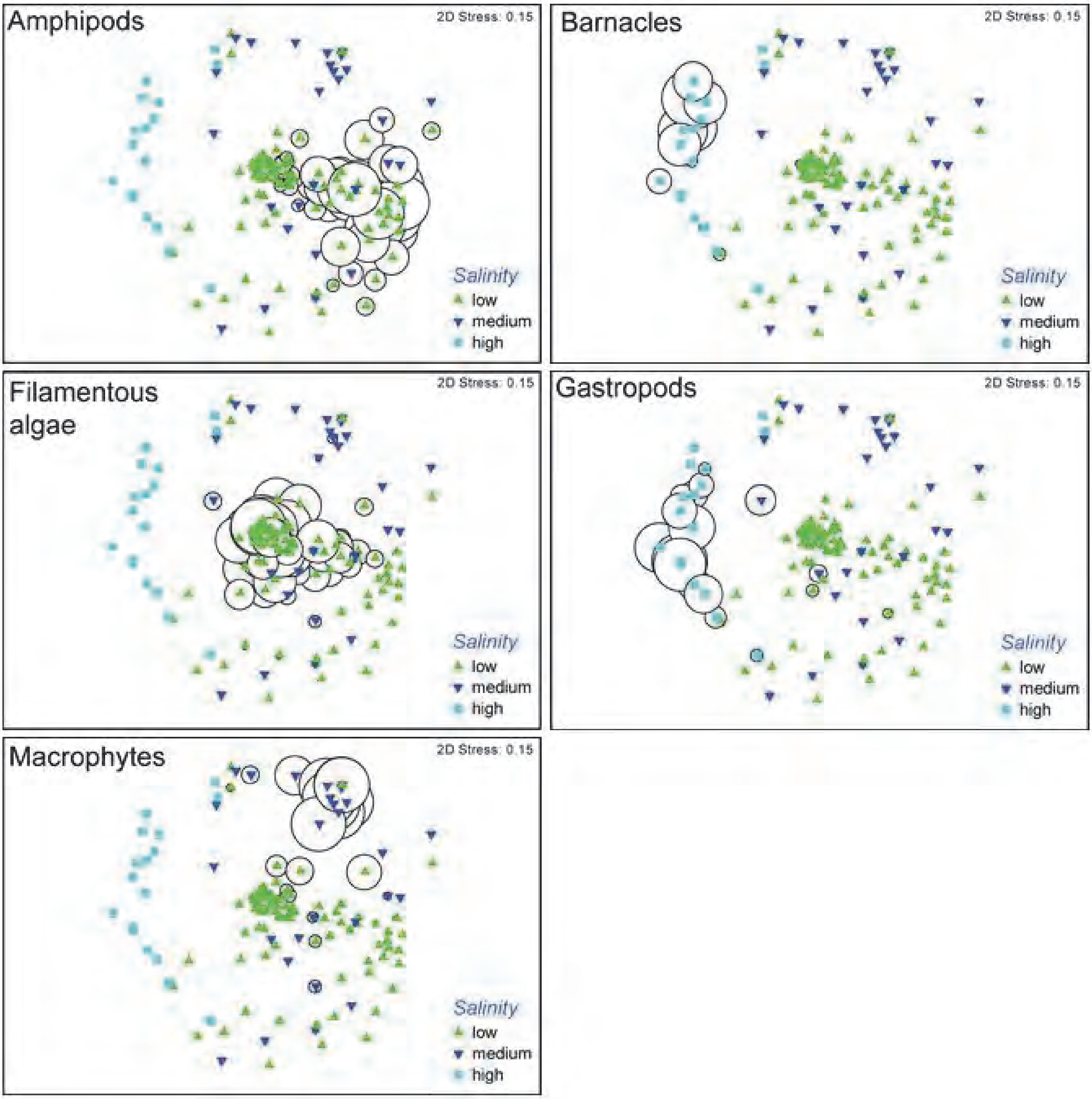

Figure 38. Bubble plots overlaid on nMDS projections (from **Figure 37**) illustrating the relative proportion of the 5 prey types contributing the most to differences between habitats, salinity groups and fish sizes classes in *R. holubi* stomachs. Only charts for salinity groups are presented herein, as patterns observed were very similar for the three factors tested.

7.7.2 Stable isotope ratios

Stable isotope (δ^{13}C and δ^{15}N) composition measured in *R. holubi* dorsal muscles varied significantly among seasons (R = 0.046, *p* = 0.012), habitats (R = 0.533, *p* = 0.001), salinity groups (R = 0.713, *p* = 0.001), and fish size classes (R = 0.342, *p* = 0.001). Patterns observed between habitats, salinity groups and fish size classes were similar (**Figure 39b-d**), with decreasing δ^{15}N and increasing δ^{13}C values from very small and small fish collected in freshwaters from the river, to medium fish collected in medium salinities from the estuary, to large fish collected in high salinities from the marine environment (**Figure 40b-d**). Conversely, seasonal variations were less evident on nMDS plots (**Figure 39a**), although samples collected in winter had significantly lower δ^{15}N and higher δ^{13}C values (**Figure 40a**).

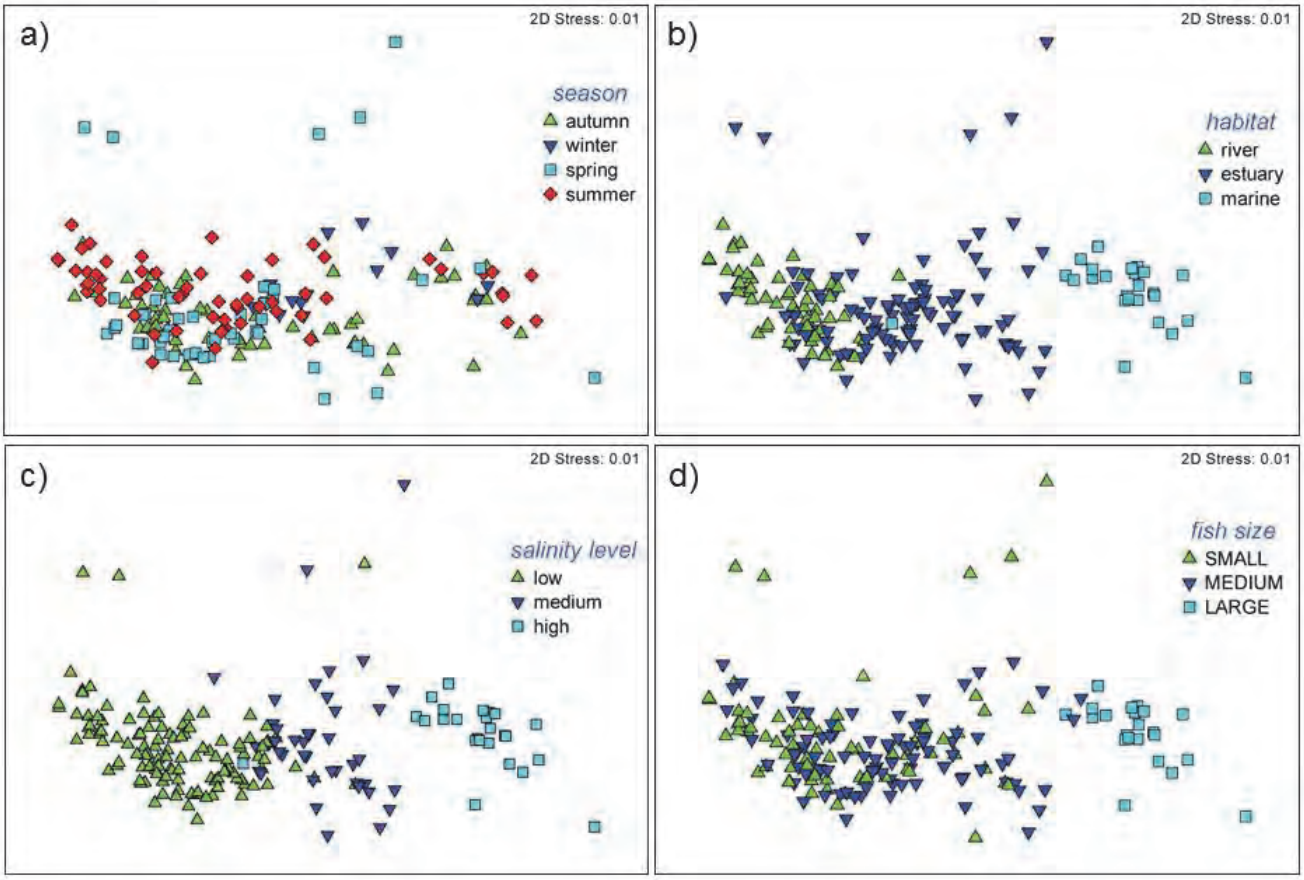

Figure 39. nMDS of stable isotopes data (δ^{13}C and δ^{15}N) as a function of a) seasons, b) habitats, c) salinity groups, d) fish size classes. All factors had a significant effect on stable isotope composition.

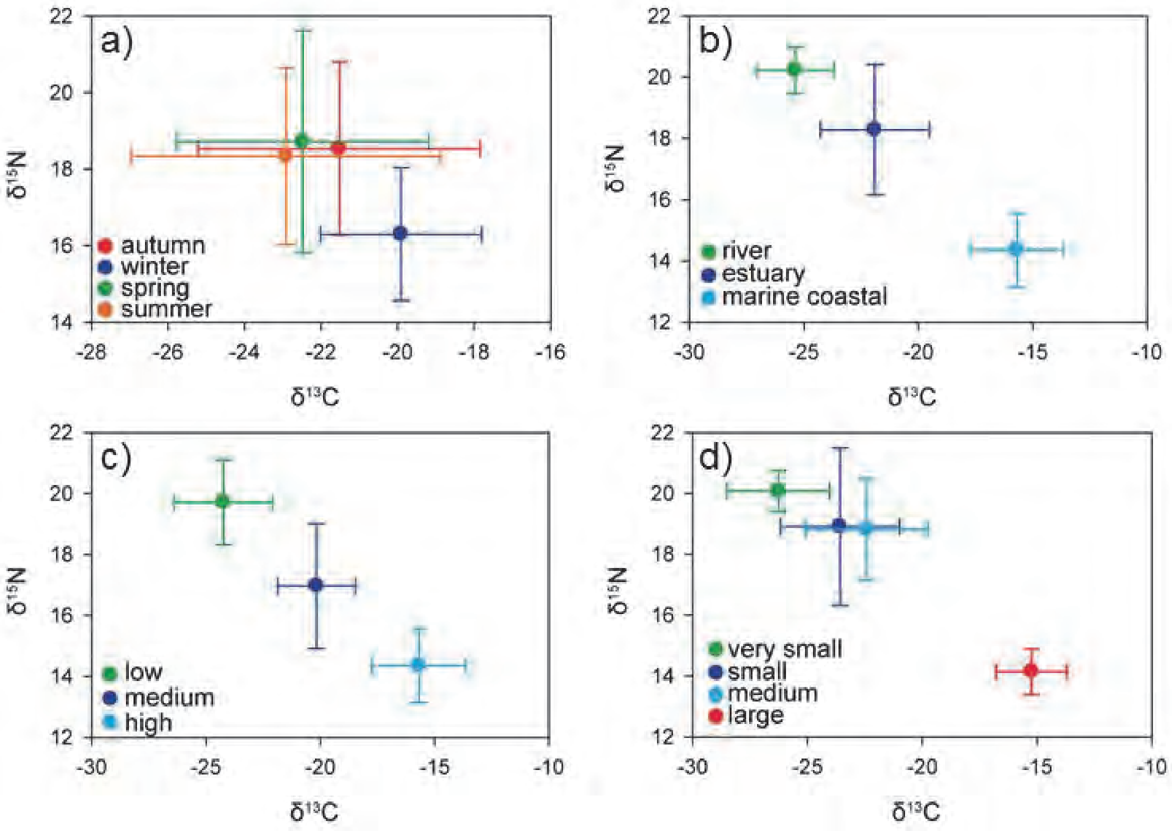

Figure 40. δ^{13}C and δ^{15}N values measured in *R holubi* dorsal muscles at each season (a), habitat (b), within each salinity group (c) and for each fish size class (d). Circles represent average values, and bars standard deviations.

7.7.3 Fatty acid profiles

A total of 48 fatty acids was identified in *R. holubi* dorsal muscles, among which 16 were SFAs, 11 were MUFAs and 21 were PUFAs. MUFAs and PUFAs were always dominant (% TFA) regardless of season, habitat, salinity group or fish size (**Figure 41**). The overall fatty acid composition (proportional data) of *R. holubi* dorsal muscles varied significantly among seasons (R = 0.123, *p* = 0.001), habitats (R = 0.185, *p* = 0.001), and salinity groups (R = 0.164, *p* = 0.009), but not among fish size classes (R = 0.016, *p* = 0.331; **Table 35**). Habitat was the factor that affected the variability the greatest (see high R statistic). Patterns observed between habitats and salinity groups were logically very similar (**Figure 42**). Seasonal patterns consisted mostly of strong contrasts between winter and summer samples (**Figure 42**).

Eight fatty acids, including one SFA, two MUFA, and five PUFA, contributed to most of the observed seasonal and spatial differences: 22:6ω3, 18:3ω3, 24:1ω9 were the most frequently involved, followed by 18:2ω6, 18:3ω6, and 16:1ω7, 16:2ω6 and 14:0 (**Table 36**). Among these eight fatty acids, 22:6ω3, generally considered in the literature as an indicator of diatom consumption, had higher proportions in winter, and in the medium saline waters from the estuary (**Figures 43, 44 and 45**). Differences were also observed between autumn and spring, autumn and summer, and spring and summer. The fatty acid 24:1ω9 was clearly more abundant in fish tissues in spring, and lowest in summer, while the inverse was true for 18:3ω3 (**Figure 43**). Patterns of seasonal variations were less consistent for 18:2ω6, which aslo contributed to at least 4% of significant seasonal variations (**Table 35; Figure 43**). Proportions of 18:3ω3, 18:3ω6 and 18:2ω6 were higher in fish collected in the river than in those from the estuary, while the inverse was true for 24:1ω9 and 22:6ω3 (**Figure 44**). The fatty acid 18:3ω3, generally considered as indicative of the consumption of macrophytes, was more represented in fish tissues during in autumn, and in freshwater from the river (**Figure 45**).

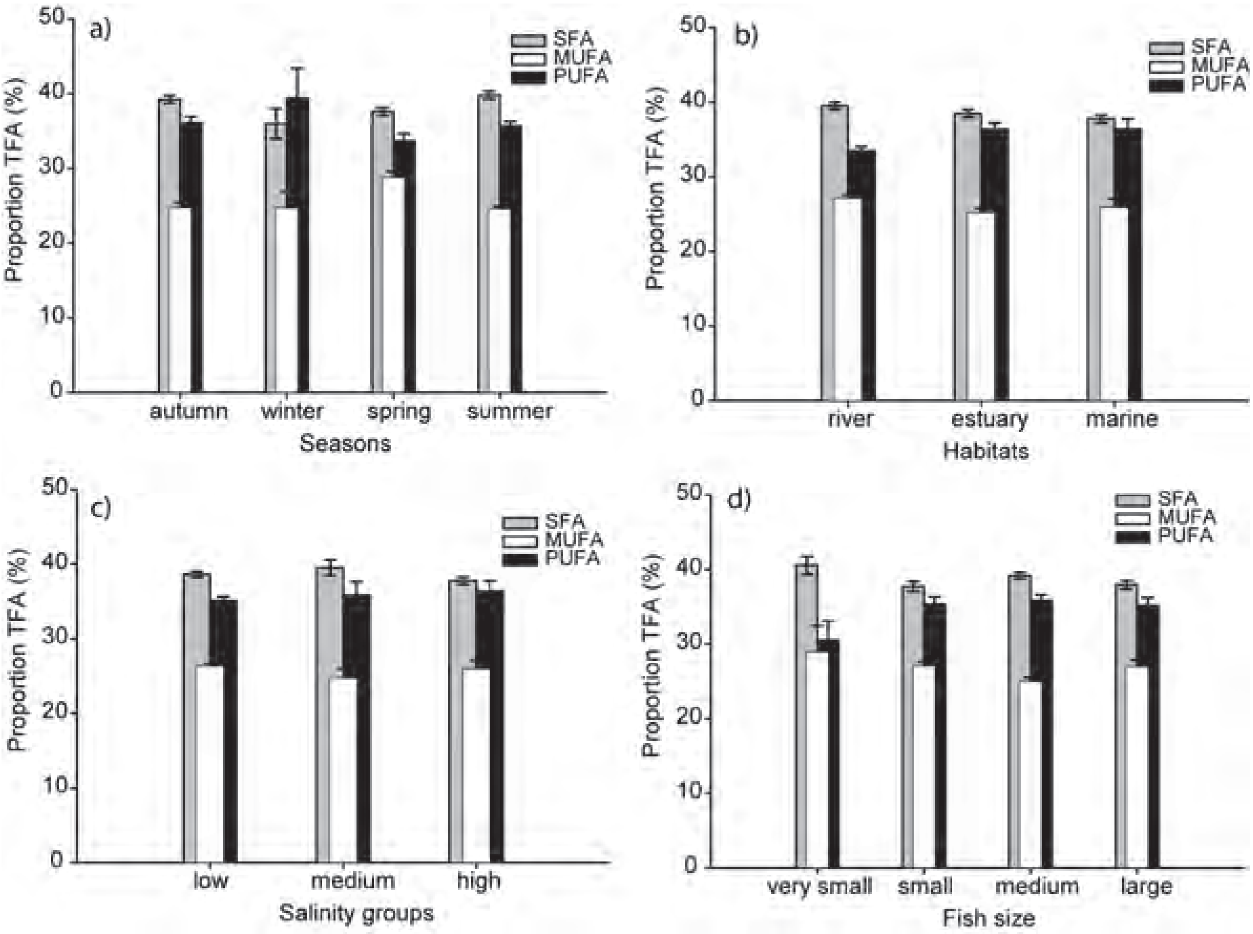

Figure 41. Proportions of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in *R. holubi* dorsal muscles as a function of seasons (a), habitats (b), salinity groups (c) and fish sizes (d). Vertical bars represented means, and error bars are standard errors.

Table 35. Results from ANOSIM and SIMPER analyses of differences between seasons and habitats in *R. holubi* fatty acids composition. Only fatty acids contributing to at least 4% of significant differences are listed, for clarity. Differences are considered significant if *p* < 0.05.

	Global ANOSIM		Pairwise ANOSIM			SIMPER analysis	
Factor	R	*P*	Pair	R	*P*	Fatty acids	Contribution (%)
Season	0.123	0.001	autumn × winter	0.043	0.311	-	-
			autumn × spring	0.082	0.004	C24:1ω9	5.7
						C18:3ω3	5.3
						C22:6ω3	4.6
						C18:2ω6	4.1
			autumn × summer	0.095	0.003	C18:3ω3	4.7
						C22:6ω3	4.3
			winter × spring	0.088	0.223	-	-
			winter × summer	0.254	0.040	C22:6ω3	6.6
			spring × summer	0.192	0.001	C24:1ω9	5.4
						C22:6ω3	4.6
						C18:3ω3	4.3
Habitat	0.185	0.001	river × estuary	0.219	0.001	C22:6ω3	6.0
						C18:3ω3	5.3
						C24:1ω9	4.6
						C18:3ω6	4.1
			river × marine	0.463	0.001	C18:2ω6	6.2
						C18:3ω3	6.1
						C22:6ω3	5.3
			estuary × marine	0.005	0.444	-	-
Fish size	0.016	0.331	-	-	-	-	-

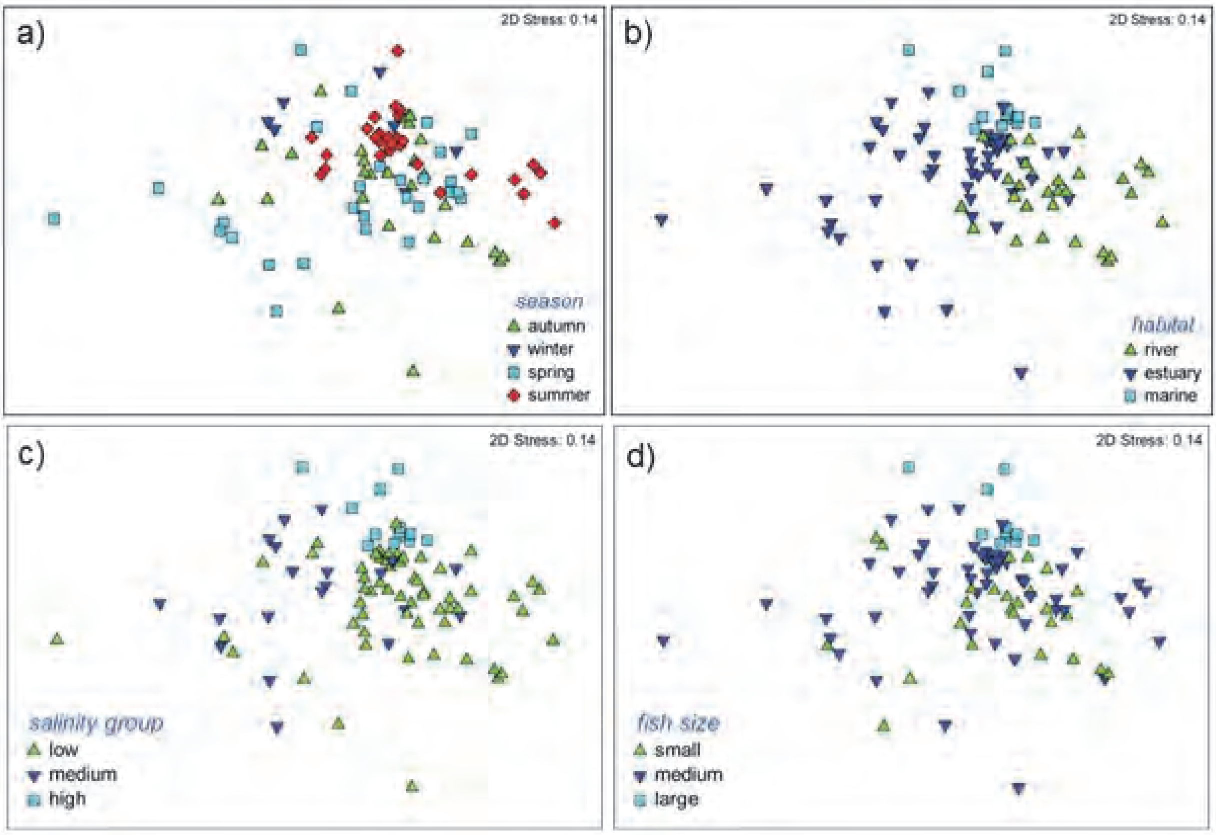

Figure 42. Multidimensional scaling analysis of fatty acid proportional data (% total fatty acids) measured in *R. holubi* dorsal muscles as a function of a) season, b) habitat, c) salinity groups, d) fish size class. Significant variations were detected for seasons, habitats and salinity groups only.

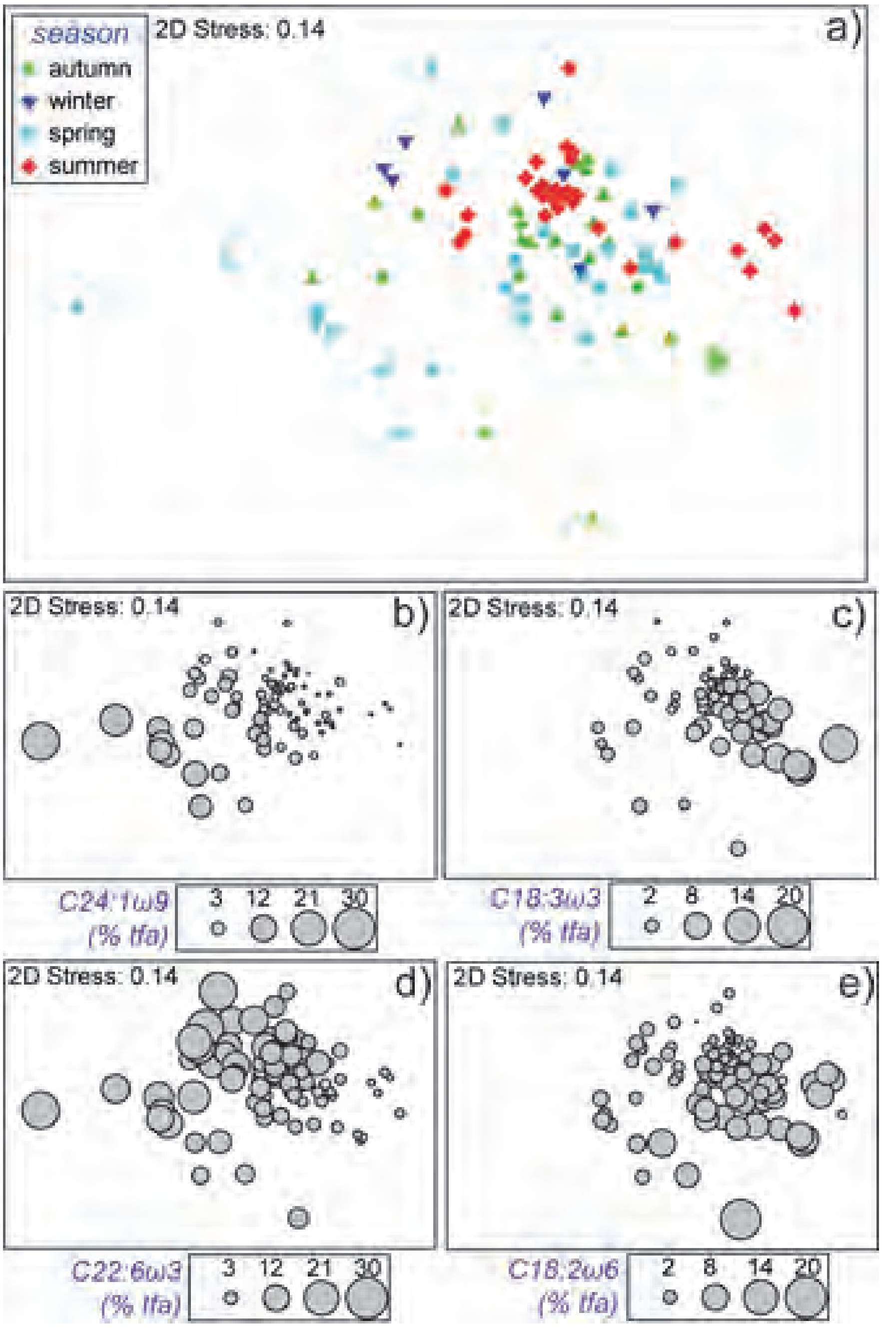

Figure 43. a) n-MDS analysis of fatty acid composition (% total fatty acids) of *Rhabdosargus holubi* collected at four seasons in the Kowie system and (b, c, d, e) corresponding proportions of fatty acids shown to contribute to at least 4% of differences detected between seasons (**Table 35**).

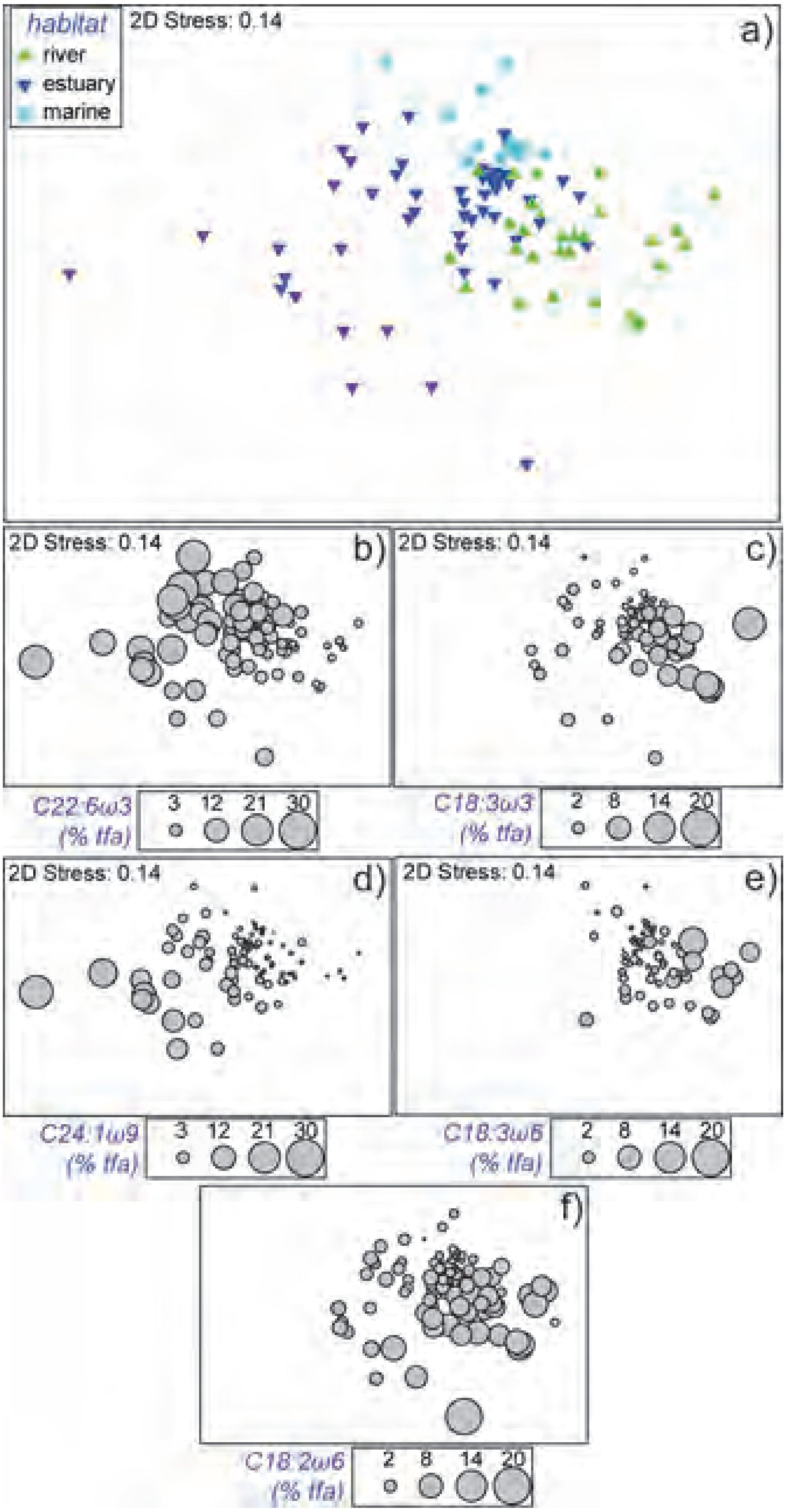

Figure 44. a) n-MDS analysis of fatty acid composition (% total fatty acids) of *Rhabdosargus holubi* collected at three habitats in the Kowie system and (b c, d, e, f) corresponding proportions of fatty acids shown to contribute to at least 4% of differences detected between habitats (**Table 35**).

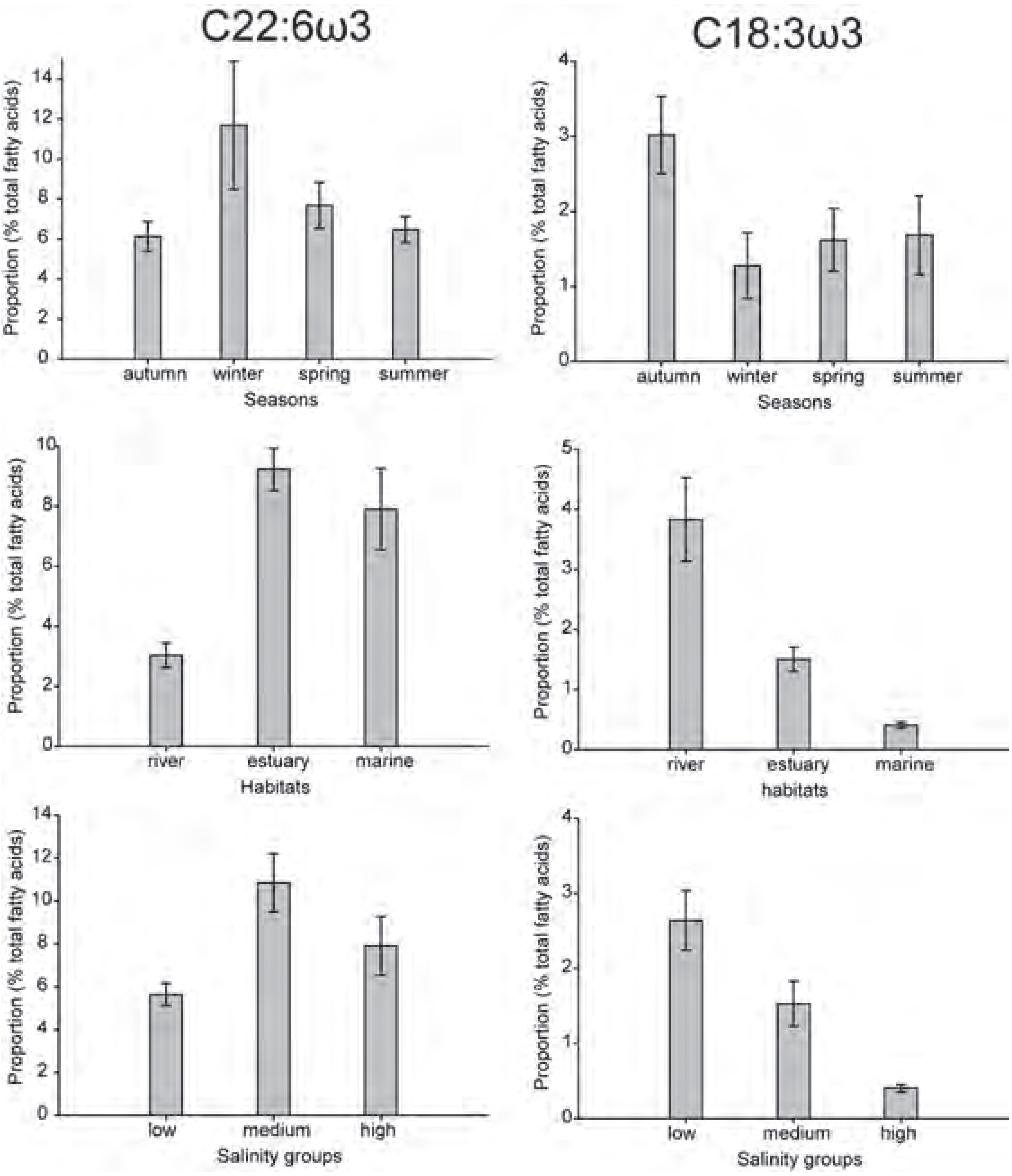

Figure 45. Proportions of 22:6ω3 and 18:3ω3 (the two fatty acids contributing most to spatial and temporal differences in relative fatty acid composition in *R. holubi* dorsal muscles; see **Table 36**) as a function of seasons, habitats and salinity values.

Table 36. Fatty acid contributions to the dissimilarity (SIMPER) between habitats (river, estuary, marine), salinity groups (low, medium, high), and fish size classes (very small, small, medium, large) as measured in *R. holubi* dorsal muscles. Only fatty acids contributing to more than 5% of the dissimilarity for each levels tested are reported, for clarity.

Factor	Levels	Fatty acids	Contribution (%)
Season	Autumn vs. winter	22:6ω3	14.73
		16:1ω7	6.07
		18:3ω3	5.76
		16:2ω6	5.11
	Autumn vs. spring	24:1ω9	12.44
		18:3ω3	9.38
		22:6ω3	7.64
		18:2ω6	6.17
	Winter vs. spring	22:6ω3	13.33
		24:1ω9	11.01
		18:3ω3	5.27
	Autumn vs. summer	18:3ω3	8.85
		22:6ω3	7.01
		18:2ω6	6.67
	Winter vs. summer	22:6ω3	15.80
		18:3ω6	5.75
	Spring vs. summer	24:1ω9	12.45
		22:6ω3	8.00
		18:3ω3	7.56
		18:2ω6	5.46
		18:3ω6	5.06
Habitat	River vs. estuary	22:6ω3	11.62
		18:3ω3	9.44
		24:1ω9	8.78
		18:3ω6	6.31
	River vs. marine	18:3ω3	11.73
		18:2ω6	11.45
		22:6ω3	10.33
		18:3ω6	6.10
	Estuary vs. marine	24:1ω9	12.03
		22:6ω3	6.73
		18:2ω6	6.63
Salinity	Low vs. medium	22:6ω3	11.12
		24:1ω9	10.53
		18:3ω3	6.40
	Low vs. high	18:2ω6	9.96
		18:3ω3	8.04
		22:6ω3	7.97
		24:1ω9	6.22
	Medium vs. high	24:1ω9	13.25
		22:6ω3	8.46
		14:0	5.03

Each method used herein provided different insights on the trophic dynamics of the indigenous fish species. The level of variability detected by each method can be compared using the value of R statistics provided by ANOSIM. Seasonal variations in *R. holubi* diet were detected for stable isotope and fatty acid data only, while fish size related variations were detected by stomach content and stable isotope analysis (**Table 37**). Spatial variations, either defined based on habitat, defined a priori or by the ranges of salinity values characterizing the study sites, were always significant for all three methods (**Table 37**). Differences in the patterns observed can be attributed to the variable temporal scales that are investigated using each method (characteristics of each method have been extensively described in previous deliverables): stomach contents provided information about food consumed by fish just before they were captured, while fatty acid and stable isotope composition in fish dorsal muscles provided insights about food assimilated by fish over periods ranging from several weeks to months. Consequently, the spatial and temporal resolution of data obtained with those three methods was highly complementary, and their association allowed for a comprehensive understanding of the trophic dynamics of the targeted species in the Kowie River system.

Table 37. Summary of the level of variability (R statistics) in the diet of *R. holubi* revealed by the three methods used.

Temporal scale	Method	Information provided	Level of variability in diet detected (ANOSIM R statistics)		
			Season	Habitat	Fish size
Short (previous meal)	Stomach contents	Nature of food sources		0.258	0.184
Medium (a few weeks)	Fatty acids	Nature and origin of food sources	0.123	0.185	
High (a few months)	Stable isotopes		0.046	0.533	0.342

Interpretations

Rhabdosargus holubi fed on different food sources in linked aquatic habitats of the Kowie system during its life cycle. We confirmed the migration of fish among the marine, estuarine and lower riverine areas from the differing fish size classes occurring in these habitats. Consumption of food resources originating from the different regions was clear from the shifts in stomach contents, stable isotope ratios and fatty acid profiles. Our results imply that *R. holubi* practices site fidelity behaviour at differences stages of its life cycle within the Kowie system.

All three datasets indicated large variability in the diet of *R. holubi* as it developed and traveled along the salinity gradient, with invertebrate prey increasing with fish size from the river to the sea. By utilising different food resources as it grows and migrates, the Cape stumpnose contributes to the transfer of energy among the river, estuary and sea. Our stomach content data were consistent with results from other studies of similar estuarine (Blaber, 1974; Whitfield, 1984; De Wet and Marais, 1990; Sheppard et al., 2012) and coastal marine systems (Buxton and Kok, 1983). In general, adult Cape stumpnose feed on invertebrates (mainly gastropods and barnacles) in the ocean, and juveniles feed on filamentous algae, macrophytes and invertebrates in estuaries. Although the role of macrophytes in the diets of estuarine *R. holubi* has been challenged (Blaber, 1974; Paterson and Whitfield, 1997; Sheppard et al., 2012), our data represent evidence that some macrophyte material is digested by fish within the riverine habitat [e.g. note the depleted carbon signatures (**Figure 40**) and the high levels of higher plant fatty acid 18:3ω3 (**Figure 45**) in the riverine fish.]

Overall, *R. holubi* showed a typical omnivorous diet in the Kowie system, as revealed by the diversity of plant and animal items found in the stomach contents. Changes in trophic position were not evident between the river and estuary, but the type of food consumed across the habitats differed considerably. This differentiation was particularly noticeable between the larval, juvenile and adult life stages. The food consumed in a particular site tended to originate from the local habitat (similar to our results of the estuarine invertebrates, see **Section 7.5**), as indicated by similarities in $\delta^{13}C$ values of fish and food within each site and season. We infer that these results indicate site fidelity behaviour by *R. holubi*.

By shifting its diet and migrating among adjacent habitats during its life cycle, the Cape stumpnose contributes to connectivity within the Kowie system, mainly through transport of biomass and energy among the marine, estuarine and riverine regions. In turn, juveniles are consumed by piscivorous fish and some birds in estuaries (Blaber, 1973b; Whitfield and Blaber, 1978), this biomass is further transported within and outside the system. Immigration of postlarvae from the ocean into the estuary, and later in the life cycle the downstream migration of juveniles back to the ocean, all contribute to longitudinal energy transfers. These are all important conduits for connecting the neighboring systems. Our findings from stomach contents, stable isotope ratios and fatty acid profiles all emphasize the advantages of combining dietary approaches when addressing questions about fish trophic ecology and connectivity.

7.8 Aquatic vertebrates – alien fish

7.8.1 Counts and stomach contents

A total of 67 *M. salmoides* was sampled from the four freshwater sites, including 20 small fish (≤150 mm TL; mean TL = 102 mm), 18 medium fish (151-300 mm TL; mean TL = 249) and 29 large fish (301-420 mm TL; mean TL = 342; **Figure 46**). Most fish (41%) were collected at site FW1, and most large fish at sites FW1 and FW2 (**Table 38**). A total of 10 fish was collected during April 2012, 19 during August 2012, 5 during November 2012 and 33 during February 2012 (**Table 38**).

Small and medium fish had the lowest (14%) and highest (40%) percentage of empty stomachs, respectively. There was no significant variation in mean stomach fullness among the four sampling seasons (ANOVA: F = 0.77579, p > 0.05). Fish collected at site F4 had the highest stomach fullness when compared to the other sites (ANOVA: F= 3.0862, P < 0.05; **Figure 47**).

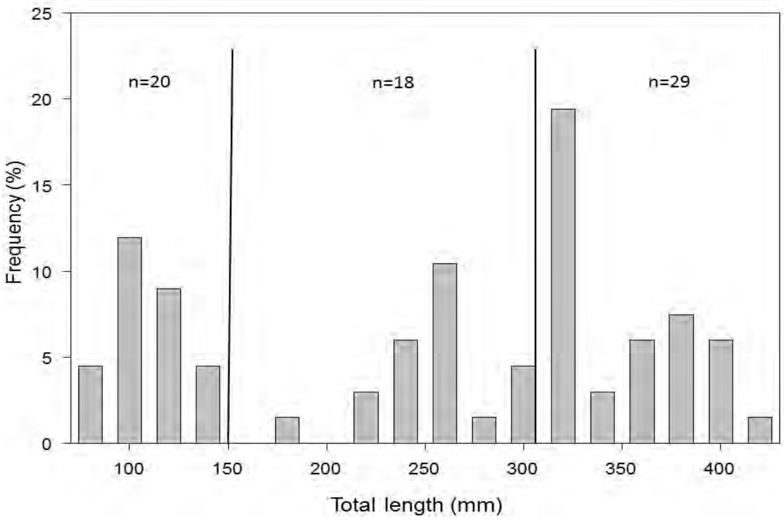

Figure 46. Length frequency distribution of *M. salmoides* in the Kowie River. Fish were divided into small (≤150 mm TL), medium (151-300 mm TL) and large (301-420 mm TL).

Table 38. Number of fish collected at each site per sampling session and the minimum and maximum total length (mm).

		F4	**FW1**	**FW2**	**FW3**
April 2012	Number of fish	0	2	8	0
	Minimum TL	0	135	219	0
	Maximum TL	0	205	337	0
August 2012	Number of fish	0	18	1	0
	Minimum TL	0	253	313	0
	Maximum TL	0	400	313	0
November 2012	Number of fish	0	0	3	2
	Minimum TL	0	0	110	107
	Maximum TL	0	0	175	115
February 2013	Number of fish	8	9	9	7
	Minimum TL	71	228	295	79
	Maximum TL	138	355	402	128
Total fish		8	29	21	9

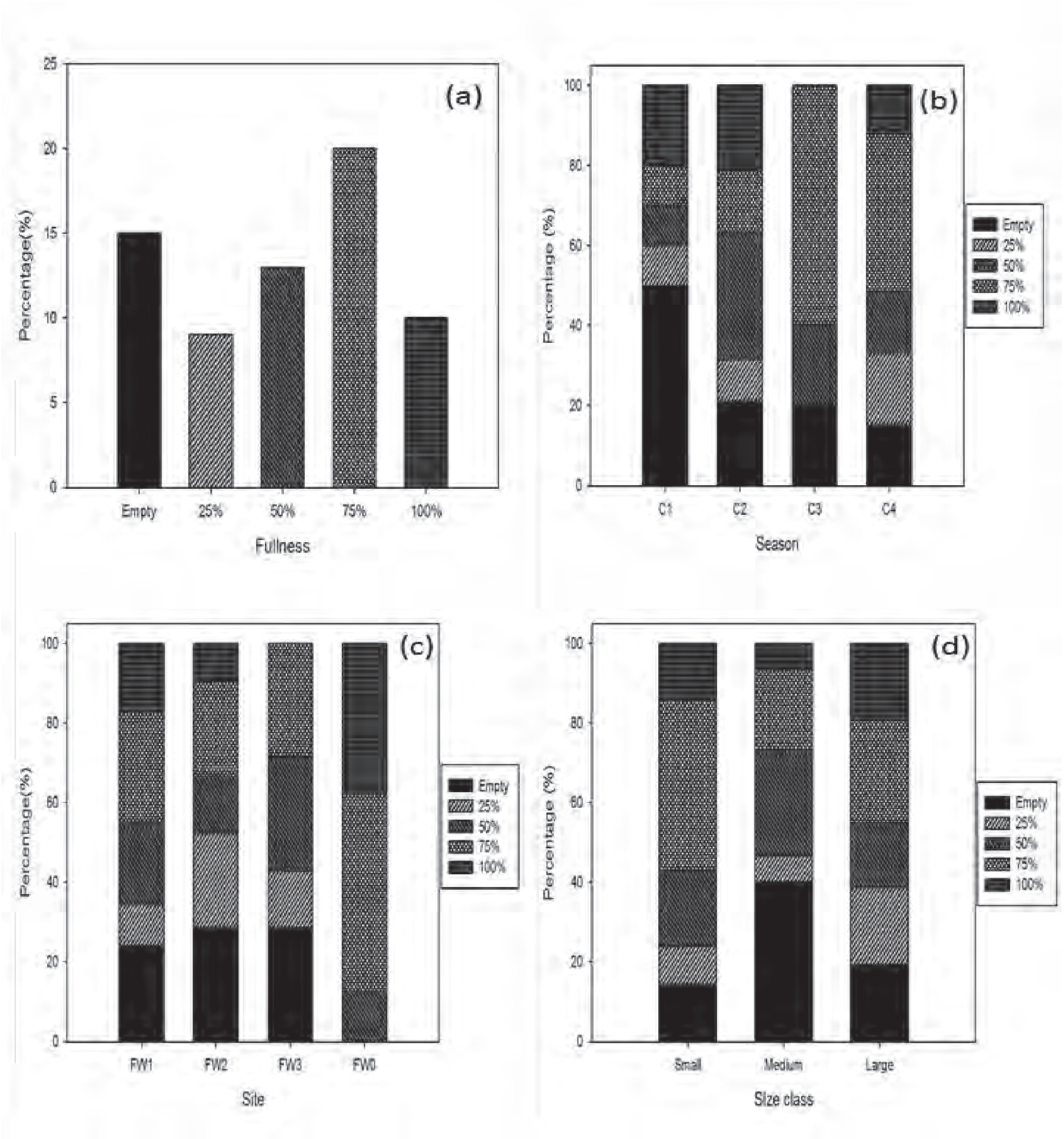

Figure 47. Stomach fullness index of all *Micropterus salmoides* (a), *M. salmoides* collected at each season (C1 autumn, C2 winter, C3 spring, C4 summer) (b), each site (c), and per fish size class (d).

A total of 17 dietary taxa was recorded during the stomach contents analysis. Based on the %IRI, the freshwater crab *Potamonautes sidneyi* was the dominant prey item of *M. salmoides* (**Table 39**), with insects from the family Aeshnidae dominating in terms of both %N and percentage frequency of occurrence. The second most important item in the diet of the largemouth bass was the Cape moony (*Monodactylus falciformis*).

Diet composition comparisons among the three size classes of *M. salmoides* indicated that the freshwater mullet dominated the diet of small specimens when compared to the other two size classes (**Table 40**). A total of 12 prey taxa was recorded in the stomachs of small sized largemouth bass. Medium sized fish had only five recorded prey taxa and displayed increased cannibalism compared to the other two groups, with their diets dominated by juvenile *M. salmoides*. Out of the 12 prey taxa detected in the stomach contents of large sized *M. salmoides*, the crab *P. sidneyi* was the most important. Only *M. capensis, M. falciformis* and *P. sidneyi* were common prey in the stomachs of fish belonging to all the size classes.

Seasonally, *M. falciformis* dominated the diet of fish collected during April 2012, whereas *P. sidneyi* was the most important prey item in August 2012. Freshwater mullet *M. capensis* was dominant during November 2012, while *Caffrogobius* spp. were the most important prey item during February 2013. Crabs were found in 78.9% of fish collected during spring, a period following a severe flood. Fish dominated the diets of *M. salmoides* collected from FW2 and FW3. This result was expected as these sites are located far downstream where estuarine-associated fish species are most abundant. Insects (Aeshnidae) dominated the diet of fish collected at F4. The diet of *M. salmoides* collected at FW1 was dominated by *P. sidneyi.* The crustacean *P. sidneyi* was not recorded in the stomachs of fish found at the uppermost (F4) and lowermost (FW3) sites. They were most prevalent in the stomachs of *M. salmoides* collected from FW1 and only three were found in the stomachs of individuals from FW2. Cannibalism was observed only in *M. salmoides* individuals collected at FW1 and F4. The freshwater mullet *M. capensis* was the only estuary-associated fish species that was recorded in the stomach contents of individuals from all four sampling sites.

Table 39. Pooled stomach contents analysis data for 67 *Micropterus salmoides* collected between April 2012 and February 2013 in the lower Kowie River. %N = the number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of stomachs containing a particular food item expressed as a percentage of all stomachs sampled; %M = the mass of each food category expressed as a percentage of the total mass of all food categories; %V = the volume of each food category expressed as a percentage of the total volume of all food items; %IRI = index of relative importance, as a proportion of the total IRI of all prey items. Insect prey were larval forms.

Prey category				**Prey abundance, occurrence and importance**					
Class	**Order**	**Family**	**Species**	**N**	**%N**	**%F**	**%M**	**%V**	**%IRI**
Actinopterygii	Mugiliformes	Mugilidae	*Myxus capensis*	8	3.4	11.9	1.5	0.9	0.88
	Perciformes	Centrachidae	*Micropterus salmoides*	6	2.6	6.0	5.9	8.8	2.75
		Gobiidae	*Caffrogobius* sp.	2	0.9	3.0	0.6	2.9	0.33
			Glossogobius callidus	3	1.3	4.5	0.8	4.7	0.76
		Monodactylidae	*Monodactylus falciformis*	10	4.3	13.4	11.9	11.7	9.96
		Sparidae	*Rhabdosargus holubi*	1	0.4	1.5	0.8	4.7	0.26
Amphibia	Anura			1	0.4	1.5	4.5	8.4	0.60
Insecta	Hemiptera	Belostomatidae		1	0.4	1.5	0.0	0.2	0.01
		Naucoridae		3	1.3	3.0	0.1	0.3	0.04
	Odonata	Aeshnidae		54	23.2	10.4	0.6	1.5	0.69
		Coenagrionidae		1	0.4	1.5	0.0	0.0	0.002
		Libellulidae		3	1.3	3.0	0.3	1.1	0.13
Malacostraca	Decaopoda	Potamonautidae	*Potamonautes sidneyi*	32	13.7	26.9	47.7	49.9	82.21
Other			Fish remains	1	0.4	1.5	0.2	0.3	0.02
			Plant leaves	4	1.7	7.5	0.4	0.8	0.29
			Stones	2	0.9	1.5	0.4	0.6	0.05
			Unidentified organic matter	-	-	4.5	0.4	1.3	0.24

Table 40. Index of relative importance (%IRI) of prey observed in *M. salmoides* stomachs collected in the lower Kowie River between April 2012 and February 2013.

	Months				Size classes			Sites			
Prey type	**April**	**August**	**November**	**February**	**Small**	**Medium**	**Large**	**FW0**	**FW1**	**FW3**	**FW4**
Myxus capensis	-	-	82.01	1.37	41.01	1.89	0.09	2.48	0.07	0.81	73.78
Micropterus salmoides	-	-	-	12.11	1.27	33.08	-	4.01	2.94	-	-
Caffrogobius sp.	-	-	-	0.25	-	-	11.35	-	0.23	58.90	-
Glossogobius callidus	-	-	-	53.31	5.63	-	-	17.63	-	-	-
Monodactylus falciformis	68.99	-	-	25.91	6.06	24.66	6.12	-	2.26	23.29	25.25
Rhabdosargus holubi	19.25	-	-	0.03	-	-	0.24	-	0.36	1.37	-
Anura	-	0.91	-	-	-	-	1.01	-	-	-	-
Belostomatidae	1.72	-	-	-	1.14	-	-	-	0.02	-	-
Naucoridae	3.44	-	-	-	1.14	-	0.01	-	0.02	0.08	-
Aeshnidae	6.59	-	-	3.30	29.57	-	0.07	74.57	-	1.43	-
Coenagrionidae	-	-	-	-	0.16	-	-	-	-	-	0.60
Libellulidae	-	0.02	-	0.02	-	9.01	0.02	-	0.02	0.84	-
Potamonautes sidneyi	-	98.54	-	3.59	11.13	31.36	80.64	-	93.57	13.22	-
Fish remains	-	-	17.99	-	2.40	-	-	-	-	-	-
Plant leaves	-	0.22	-	0.10	0.39	-	0.13	1.30	0.21	0.06	-
Stones	-	0.07	-	-	-	-	0.08	-	0.07	-	-
Unidentified organic matter	-	0.23	-	0.01	0.09	-	0.25	-	0.22	-	0.37

7.8.2 Stable isotope ratios

The comparison between lipid-extracted and non-lipid-extracted isotope samples revealed no significant differences in isotope values (F = 1.80, p = 0.20). Based on this result, only bulk (non-lipid extracted) isotopes samples were analysed further. When all the data were pooled, *M. salmoides* had the most enriched δ^{15}N value compared to its potential food sources (mean ± SD; 20.7 ± 1.6‰; **Figure 48**). *M. salmoides* possessed the most enriched δ^{15}N values at sites FW2 ($\bar{x}$ = 20.5 ± 1.2‰), FW3 ($\bar{x}$ = 21.2 ± 0.5‰) and F4 ($\bar{x}$ = 23.2 ± 1.1; **Figure 49**). Amongst the sites, the δ^{13}C value of *M. salmoides* was most enriched at FW1 (-25.9 ± 1.2‰). The δ^{13}C values of *M. salmoides* (**Figure 50a**) increased with their size, while both the δ^{15}N values and C:N ratios (**Figure 50)** showed decreasing trends with increasing size. Two-way ANOVA revealed no significant synergistic variation between season and site for δ^{15}N (F = 0.011, p = 0.989). One-way ANOVA revealed a significant difference in mean δ^{15}N of *M.* salmoides values among seasons (F= 7.56, $p < 0.05$), size classes (F = 15.78, $p < 0.05$), and sites (F = 19.54, $p < 0.05$).

Although significantly different, the mean δ^{15}N values for *M. salmoides* did not exhibit any discernible trend among the seasons. There was also a significant variation in mean δ^{13}C values among season (F = 8.66, $p < 0.05$), size class (F = 14.34, $p < 0.05$) and sites (F = 8.91, $p < 0.05$). The mean δ^{13}C values exhibited a decreasing trend from one sampling season to the next. The C:N ratio did not vary significantly among sites (F = 1.7, p = 0.175) or seasons (F = 0.7, p = 0.54) but slightly among the three size classes (F = 6.0, $p < 0.05$).

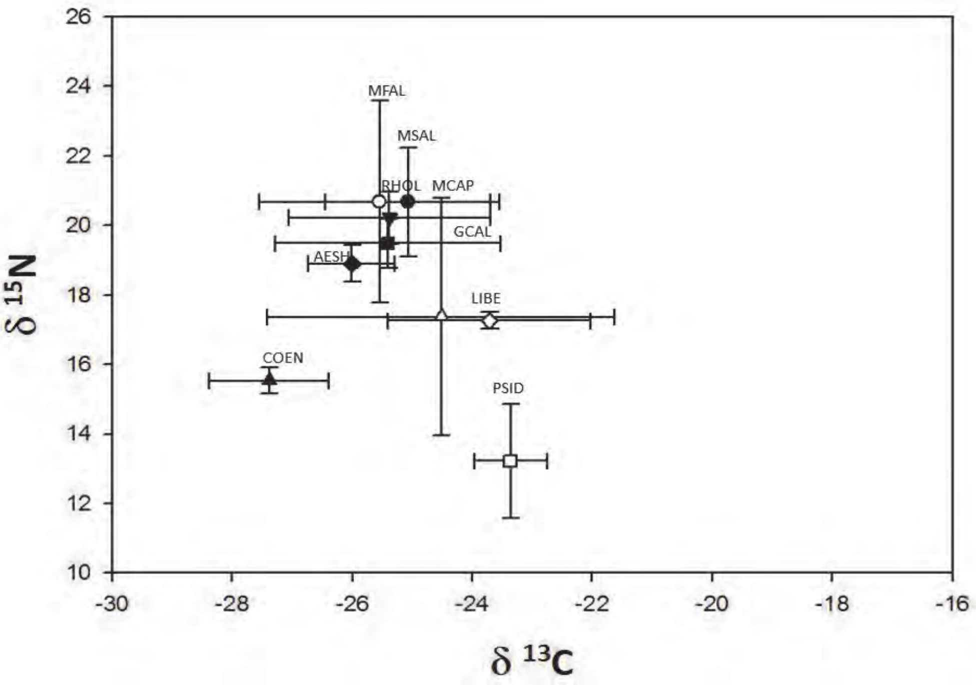

Figure 48. Mean (± SD) values of δ^{13}C and δ^{15}N for pooled *M. salmoides* and potential main prey items collected between April 2012 and February 2013 in the lower reaches of the Kowie River (MSAL: *Micropterus salmoides*; MFAL: *Monodactylus falciformis*; MCAP: *Myxus capensis*; RHOL: *Rhabdosargus holubi*; GCAL: *Glossogobius callidus*; AESH: Aeshnidae; COEN: Coenagrionidae; LIBE: Libellulidae; PSID: *Potamonautes sidneyi*).

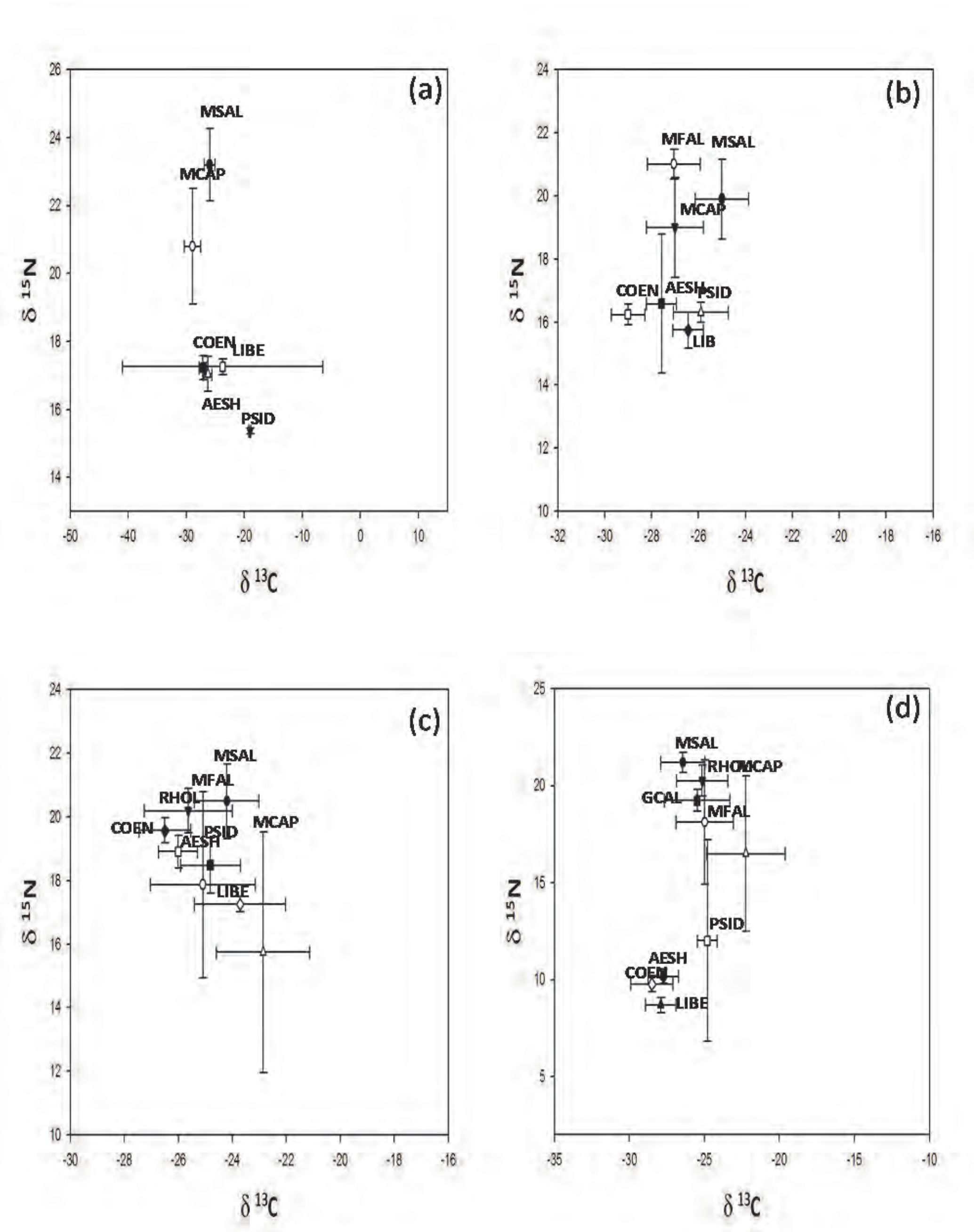

Figure 49. Mean (±SD) values of δ^{13}C and δ^{15}N for samples collected at sites F4 (a), FW1 (b), FW2 (c) and FW3 (d) between April 2012 and February 2013 in the Kowie River (MSAL: *Micropterus salmoides,* MFAL: *Monodactylus falciformis*, MCAP: *Myxus capensis*, RHOL: *Rhabdosargus holubi*, GCAL: *Glossogobius callidus*, PSID: *Potamonautes sidneyi*, AESH: Aeshnidae, COEN: Coenagronidae, LIB: Libellulidae).

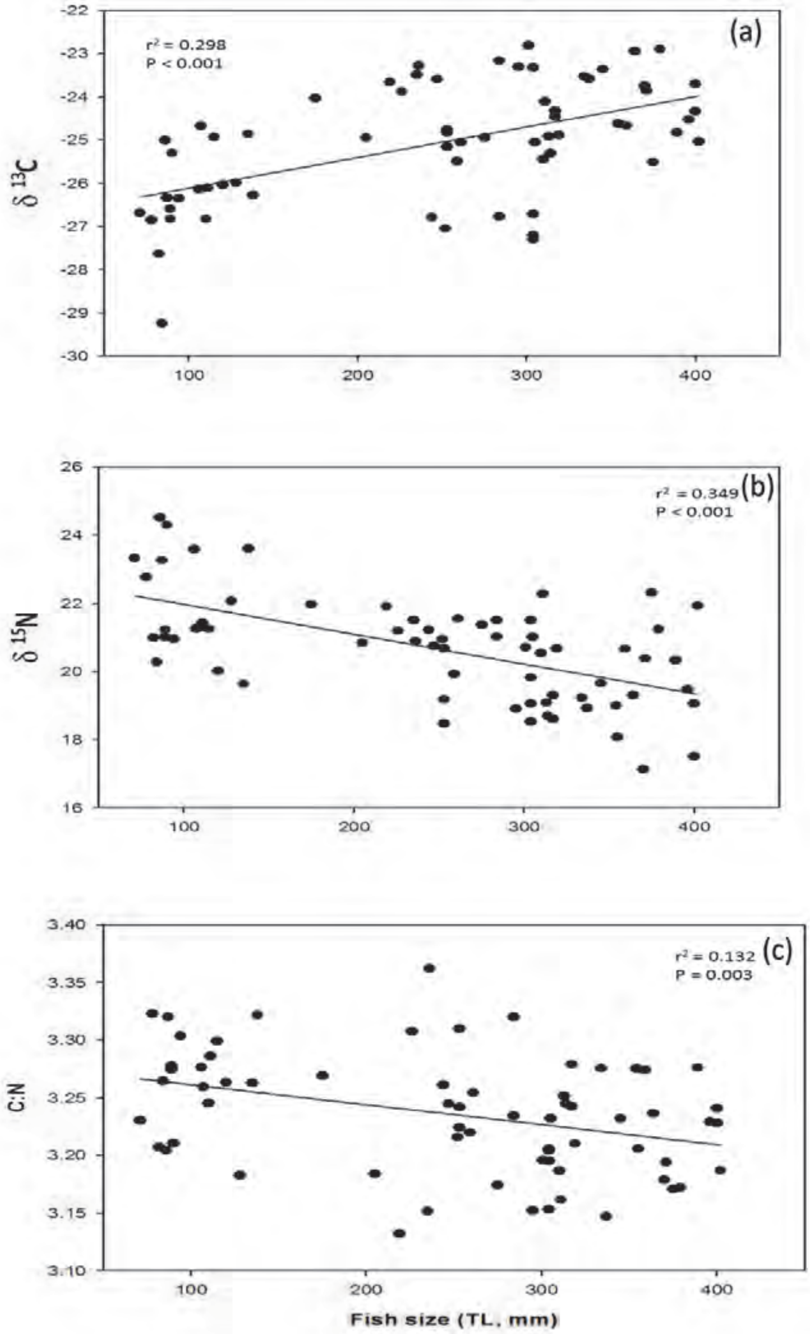

Figure 50. Linear regression plots illustrating the relationships of fish size to $\delta^{13}C$ (a), $\delta^{15}N$ (b) and the C:N ratio (c) for *M. salmoides* collected in the lower Kowie River.

Results of the SIAR model using pooled data (**Figure 51**) suggested that Odonata formed the highest proportion of the diet for *M. salmoides* (52.1%), followed by *M. falciformis* (17.9%) and *R. holubi* (12.4%). Data collected during the first sampling season (April 2012; **Figure 52a**) suggested that *G. callidus* dominated the diet (43.4%), followed by Odonata (27.8%). *M. capensis* contributed 55.3% of the diet during the second sampling season (August 2012; **Figure 52b**), with Odonata and *R. holubi* contributing 32.7% and 30.5%, respectively. Gobies *G. callidus* (24.7%), Odonata (19.4%) and *R. holubi* (18.9%) were the three highest contributors to the diet of *M. salmoides* collected during the third season (November 2012; **Figure 52c**). The diet of fish collected during the fourth season (February 2013; **Figure 52d**) was dominated by Odonata (34.7%) and *M. capensis* (31.6%). *Rhabdosargus holubi* (32.2%), *G. callidus* (24.9%) and *M. falciformis* (21.1%) were the major contributors to the diet of *M. salmoides* collected at F4 (**Figure 53a**). A fish and a crab species, *M. capensis* and *P. sidneyi,* were the highest contributors (38.9% and 37.4%, respectively) to the diet of *M. salmoides* from FW1 (**Figure 53b**) while *P. sidneyi* (25.4%), Odonata (23.3%) and *M. falciformis* (18.3%) made the highest contributions in fish from FW2 (**Figure 53c**). The diet of fish from FW3 (**Figure 53d**) was dominated by *R. holubi* (29.6%) and *G. callidus* (28.7%).

The diet of small sized *M. salmoides* comprised mainly *M. falciformis* (22.6%), *R. holubi* (22.1%) *G. callidus* (20.8%) and Odonata (18.7%) (**Figure 54**). Odonates contributed the highest proportion (32.9%) to the diet of medium sized *M. salmoides* (figure 16), followed by *M. falciformis* (16.7%) and *G. callidus* (15.7%). The model also suggested that 61% of the diet of large sized *M. salmoides* consisted of Odonata.

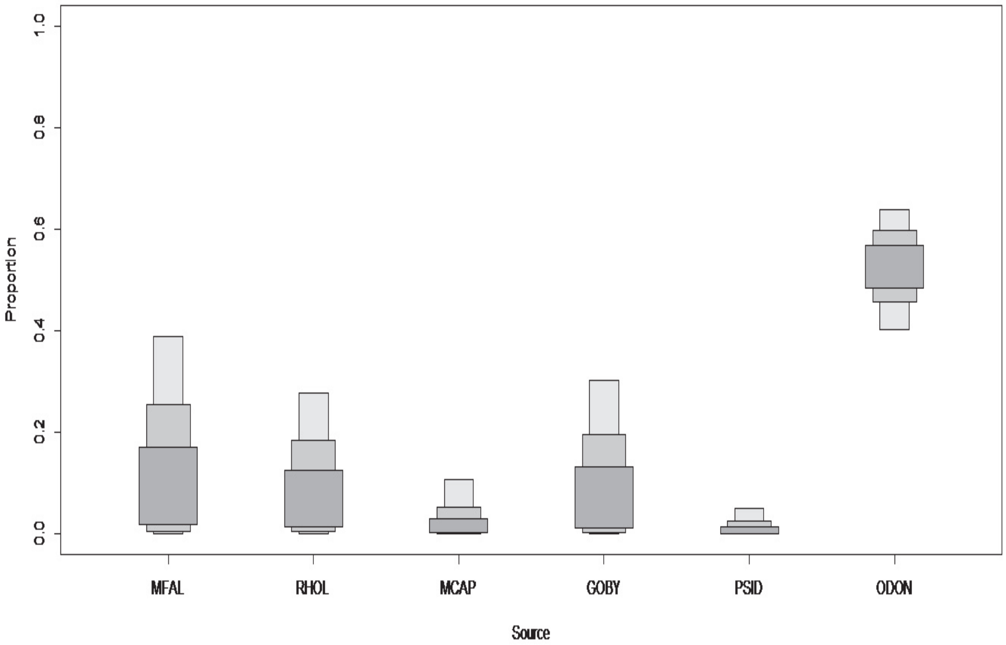

Figure 51. SIAR boxplot showing estimated proportions of source contributions to the diet of *M. salmoides* collected from April 2012 to February 2013 in the lower Kowie River. The widths of the bars show the 95, 75 and 50% credibility intervals (MFAL: *Micropterus salmoides*; RHOL: *Rhabdosargus holubi*; MCAP: *Myxus capensis*; GOBY: *Glossogobius callidus*; PSID: *Potamonautes sidneyi*; ODON: Odonata).

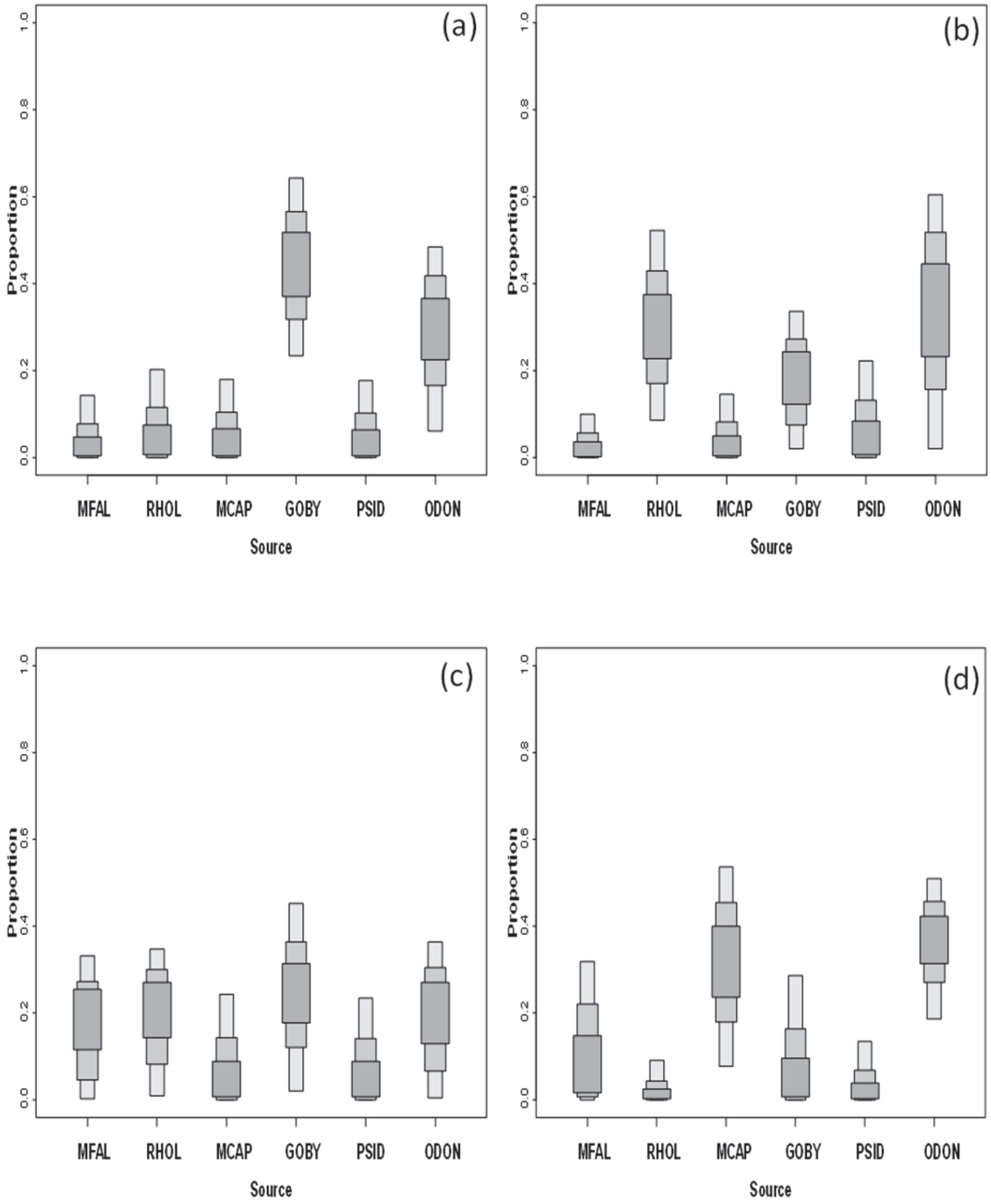

Figure 52. SIAR boxplot showing estimated proportions of source contributions to the diet of *M. salmoides* collected during April 2012 (a), August 2012(b), November 2012 (c) and February 2013 (d) in the lower Kowie River, Easter Cape, South Africa. Widths of bars show the 95, 75 and 50% credibility intervals. MFAL: *Micropterus* salmoides; RHOL: *Rhabdosargus holubi*; MCAP: *Myxus capensis*; GOBY: *Glossogobius callidus*; PSID: *Potamonautes sidneyi*; ODON: Odonata.

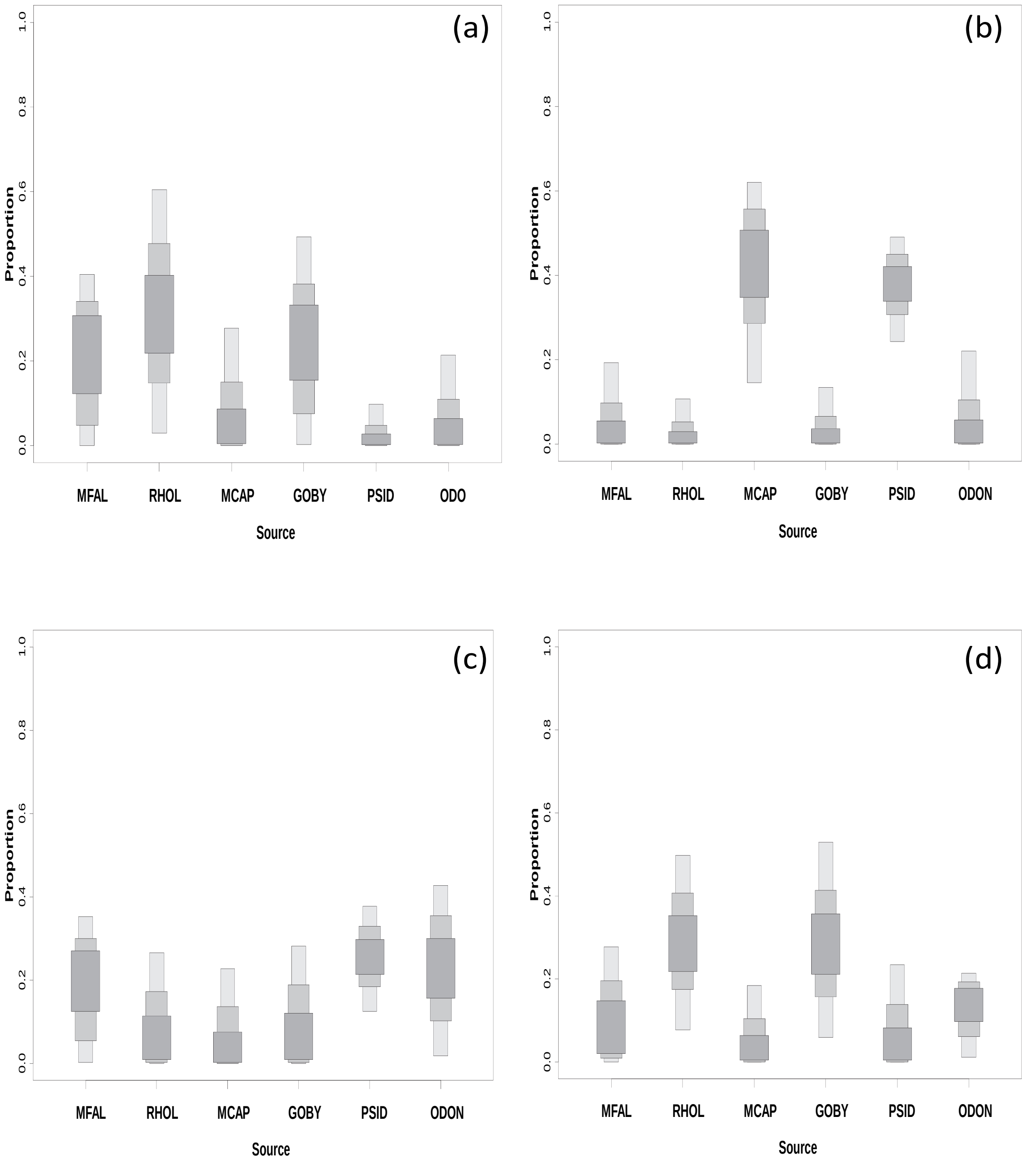

Figure 53. SIAR boxplot showing estimated proportions of source contributions to the diet of *M. salmoides* collected at sites F4 (a), FW1 (b), FW2 (c) and FW3 (d) between April 2012 and February 2013 in the lower Kowie River, Easter Cape, South Africa. Widths of bars show the 95, 75 and 50% credibility intervals. MFAL: *Micropterus* salmoides; RHOL: *Rhabdosargus holubi*; MCAP: *Myxus capensis*; GOBY: *Glossogobius callidus*; PSID: *Potamonautes sidneyi*; ODON: Odonata.

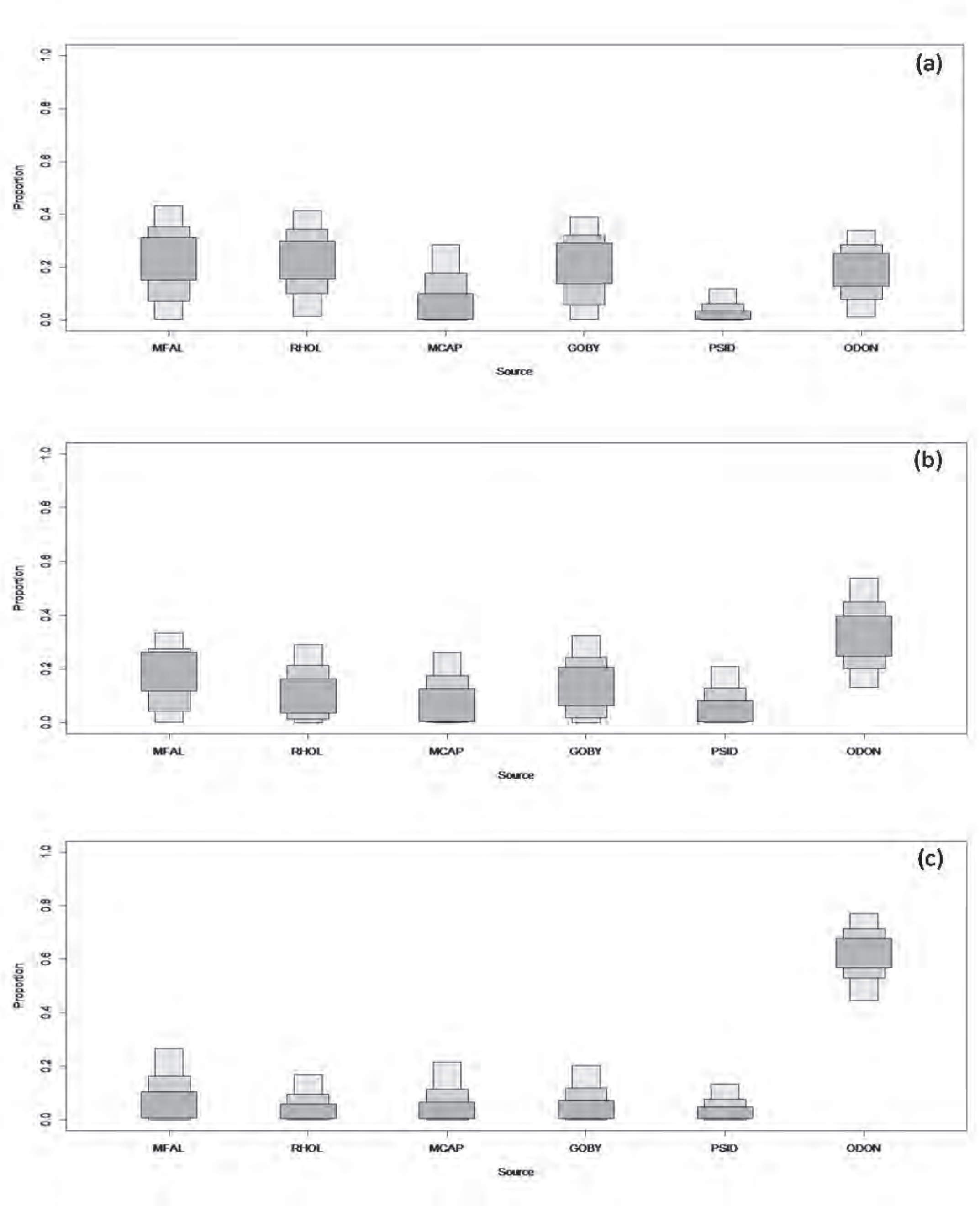

Figure 54. SIAR boxplot showing estimated proportions of source contributions to the diet of small (a), medium (b) and large (c) size-class *Micropterus salmoides* collected between April 2012 and February 2013 in the lower Kowie River, Easter Cape, South Africa. Widths of bars show the 95, 75 and 50% credibility intervals. MFAL: *Micropterus* salmoides; RHOL: *Rhabdosargus holubi*; MCAP: *Myxus capensis*; GOBY: *Glossogobius callidus*; PSID: *Potamonautes sidneyi*; ODON: Odonata.

Interpretations

Stomach content analysis (%IRI) from the pooled *M. salmoides* data suggested that this predatory fish had a particular preference for the freshwater crab, *P. sidneyi*. The dominance of *P. sidneyi* in the diet of largemouth bass is consistent with the results obtained by Wasserman et al. (2011) in the same river system. Based on stomach content analyses, the three estuary-associated fish species, *M. capensis, M. falciformis* and *R. holubi* together contributed only 11% to the diet of largemouth bass. The mid-water swimming Cape moony was the most important prey fish, suggesting that the population of *M. falciformis* was more impacted by largemouth bass relative to the other estuary-associated fish. In its native range of North America, fish (especially bluegill *Lepomis macrochirus*) form an integral part of the diet of largemouth bass (Olson et al., 1995; Olson, 1996; Keast, 1985). A study by Clady (1974) in a northern Michigan, USA, lentic system showed that fish form an important portion of large sized *M. salmoides*. Although fish form the dominant portion of its diet, it is not a strict piscivore as insects and aquatic invertebrates are also an integral part of the diet of young *M. salmoides* (García-Berthou, 2002; Wheeler and Allen, 2003).

The gobies *G. callidus* and *Caffrogobius* sp. constituted only 1.1% of the largemouth bass diet, which suggested that these benthic cryptic fish species were not targeted as prey. Based on number of individuals counted, the Aeshnidae were the most abundant prey item of *M. salmoides*. Due to their small size and weight, Aeshnidae %IRI scores were not reflective of such numerical dominance. The biggest portion (98%) of these insects was found in the stomachs of small sized individuals. In Eastern Africa, Hickley et al.(1994) found that insects were an important item in the diet of small *M. salmoides* while similar results have been reported in South-Western Europe (García-Berthou, 2002) and North America (Schramm and Maceina, 1986).

Stomach contents data clearly indicated that the diet of large sized *M. salmoides* was substantially dominated by *P. sidneyi,* with *Caffrogobius* sp. and *M. falciformis* being the most dominant fish species. Both Weyl and Lewis (2006) and Wasserman et al. (2011) reported the dominance of *P. sidneyi* in the stomachs of large sized *M. salmoides* in the lower Kowie River. The dominance of invertebrates in *M. salmoides* diet is not unique to this particular system. Indigenous invertebrates were recorded in the stomachs of *M. salmoides* sampled in the Wit River, Eastern Cape (Weyl et al., 2010). Several authors have also reported the presence of crustaceans such as crayfish in the stomachs of *M. salmoides* (Keast, 1985; Schramm and Maceina, 1986; García-Berthou, 2002; Wheeler and Allen, 2003; Braun and Walser, 2011). We found a single frog in the stomach of a large-sized *M.* salmoides; however, this could have been a rare opportunistic event.

The stomach contents of small *M. salmoides* were dominated by fish, with early juvenile *M. capensis* the dominant prey. Juvenile *M. capensis* migrate from the estuary to the freshwater areas as part of their life cycle (Bok, 1979; Beckley, 1984), rendering them vulnerable to predation by largemouth bass. The Aeshnidae and *P. sidneyi* also showed substantial %IRI scores. Wheeler and Allen (2003) showed that fish were the primary prey of small (age-0) largemouth bass in Chipola River, Florida, USA. In the Mediterranean region, small fish formed an important portion of the food of small and medium sized *M. salmoides* (García-Berthou, 2002).

The most dominant prey items in the stomachs of medium sized largemouth bass were *M. salmoides* and *P. sidneyi*, both of which had nearly identical %IRI values. The Cape moony *M. falciformis* was the third most important prey item, with a %IRI value that was close to both *M. salmoides* and *P. sidneyi*. Throughout the study, cannibalistic feeding on juvenile sized *M. salmoides* was detected in six samples, contributing 2.8% to the overall diet. Cannibalism was apparent only in small and medium sized *M. salmoides*. Clady (1974) similarly detected that juvenile largemouth bass exhibited substantially higher levels of cannibalism than adults living in two Northern Michigan lakes. The consumption of young largemouth bass individuals of a bigger size can negatively affect the population through a reduction in recruitment rates (Crass, 1964). Adult male largemouth bass may guard nests, thus lowering the occurrence of intra-cohort cannibalism while also protecting newly spawned individuals from predation until they reach a 15-30mm TL (Johnson and Post, 1999; Post, 2003). Nest guarding behaviour can thus also explain the low frequency of cannibalism in large sized *M. salmoides*.

The changes in diet preferences with size can be viewed as a shift in trophic levels, resulting in young bass changing from being potential prey to competing with large sized bass as they mature (Olson, 1996). The species specific timing of a shift from feeding on invertebrates to fish can influence the growth rate and alter the degree of fitness of facultative piscivorous fish. An early shift to piscivory is generally a favourable life history characteristic as it gives individuals from a cohort an advantage over their peers through increased growth rates and a decreased probability of being a victim of cannibalism and thus increasing their chances of survival till adulthood (Keast, 1985; Post, 2003). However, if too many individuals from a cohort survive past age-0, this will exert a strain on the population by increasing intraspecific population and thus stunting growth (Crass, 1964; McDowall, 1968). The stomach contents results suggested that this particular population of largemouth bass undergoes an early switch (at <150mm TL) from an insectivorous to a semi-piscivorous diet, perhaps further highlighting their status as successfully established aliens in the system. Stable isotopes also confirm this finding, as they indicated that 60% of the diet of small-sized bass consisted of fish. Differences in habitat fidelity between different sizes of largemouth bass have been reported (Schramm and Maceina, 1986; Olson et al., 1995), although some authors have reported that all size classes are evenly distributed across all habitats (Wheeler and Allen, 2003). There is need for an assessment of the patterns distribution of the different sizes of largemouth bass and the possible driving factors, such as salinity, temperature or habitat preferences and so forth, in the Kowie system.

The stomach contents showed variation in feeding preferences among the four sampling sessions. Fish were the most important prey items during April 2012 (*M. falciformis* and *R. holubi*), November 2012 (*M. capensis*) and February 2013 (*M. salmoides, M. falciformis*, *G. callidus* and *Caffrogobius* sp.) while *P. sidneyi* were the most important during August 2012. During August 2012, there was a dense prevalence of various species of crabs including *P. sidneyi* along the banks of both the lower river and the upper and middle reaches of the Kowie Estuary. This sampling was conducted following a substantial flood event which may have contributed to the sudden dramatic prevalence of crabs along the banks. There was an increase in consumption of estuary-associated fish from sites F4 to FW3. Aeshnidae and *G. callidus* dominated the diet at F4, while *M. capensis* and *M. falciformis* were the most important prey at FW3.

According to the SIAR models, Odonata made the largest contribution to the overall diet of *M. salmoides*, suggesting that largemouth bass have a primarily insectivorous diet in the Kowie River. Among the bass fish prey, *M. falciformis, R. holubi* and *G. callidus* made the highest contributions. The high contribution of *R. holubi* to largemouth bass diet was not expected, as this species was detected only in one stomach. However, stable isotope ratios reconstruct diet over a longer time scale (Vander Zanden and Rasmussen, 2002). We speculate that largemouth bass consumed significant amounts of *R. holubi* in the months preceding our study and the floods that occurred in 2012 reduced the abundance of this prey in the freshwater section of the Kowie River.

Estuary-associated fish made significant contributions to the diet of *M. salmoides* collected from all four sites. The dominance of *M. falciformis* in the diet of largemouth bass collected from FW2 agreed with the results from the stomach contents analysis. The dominance of fish in the diet of *M. salmoides* at FW1 also supported the stomach content results of Weyl and Lewis (2006). Both the stomach content and stable isotope data indicated that the population of largemouth bass which inhabits a section of the river downstream of the weir (i.e. FW2 and FW3) consume high quantities of the three aforementioned estuary-associated fish species. Largemouth bass occupying FW3 consumed the highest percentage of the indigenous fish.

The results of the SIAR mixing model for isotope data of small sized bass were congruent with our stomach content results and those reported by Weyl and Lewis (2006), with both studies showing a preference for feeding on fish (*M. capensis, G. callidus* and *M. falciformis*). Braun and Walser (2011) reported piscivory in small sized largemouth bass in the lower Boise River, USA. The SIAR results for large sized *M. salmoides* were congruent with those reported by Wasserman et al. (2011); however, there was a marked difference with the stomach contents results obtained during our study, as these showed that *P. sidneyi* was the most important prey. There was agreement only between stable isotope and stomach content analysis in terms of the significant contribution of *M. falciformis* to the diet of medium sized largemouth bass.

Seasonally, Odonata made the most important contribution to the diet of *M. salmoides*. In April 2012, *G. callidus* and Odonata were the most important prey, while during August 2012 *M. capensis*, Odonata and *R. holubi* were the most important prey. During November 2012, fish (*G. callidus* and *R. holubi*) were the most important prey, with Odonata also making significant contributions to the diet. The importance of fish in largemouth bass diet was congruent with the results obtained using stomach contents analysis. García-Berthou (2002) also found variation in bass diet with season, with fish forming part of largemouth bass diet during the summer season. However, the turnover rate of stable isotopes in muscle tissue is slow, so the models do not necessarily reflect the feeding habits during the season that the sampling was conducted. In February 2013, both *M. capensis* and Odonata were equally important prey items.

The results of our study demonstrate the generalist feeding behaviour of largemouth bass in the Kowie River, highlighting its ability to consume a wide array of food items depending on prey size and availability (Savino and Stein, 1982; Sanderson et al., 2009). For example, we observed the opportunistic feeding of bass on *P. sidneyi,* which was visibly prevalent along the river banks after a flooding event. The generalist feeding of largemouth bass can also affect, through competition for food sources, indigenous fish species such as *M. falciformis* primarily feeding on invertebrates (Whitfield, 1984; Lorenzoni et al., 2002). Consumption of estuary-associated fish by bass can readily affect the connectivity among the marine, estuarine and freshwater ecosystems through exchange of organic matter.

Our results illustrate that fish formed an important part of the diet of *M. salmoides* in the lower Kowie River. The prevalence of indigenous fish in the bass diet is cause for concern, as several authors have illustrated potential threats posed by the presence of *M. salmoides.*

Gratwicke and Marshall (2001) attributed altered distribution and density of *Barbus* spp. in forty two Zimbabwean streams to alien largemouth bass and *Serranochromis robustus* predation. Jackson (2002) stated that the presence of largemouth bass in Canadian lakes has a negative effect on the fish community structure by reducing the abundance of small-bodied fish and altering the flow of energy between trophic levels. The distribution of small bodied fish changes in response to the introduction of a new predator, confining these species to a certain portion of the water body and thereby increasing interspecific competition among the indigenous species. Introduction of predatory fish can result in trophic cascades as reduction of planktivorous fish may cause a change in the community structures of both zooplankton and phytoplankton Jackson (2002). In the western United States, introduced largemouth bass have reduced the abundance of anadromous coho salmon (*Oncorhynchus kisutch*) through high levels of predation. Largemouth bass were responsible for 98% of the predation on coho salmon relative to nine other introduced fishes and two native species (Bonar et al., 2005).

7.9 Wading birds

7.9.1 Stable isotope ratios

SIAR mixing models showed that the diet of Cape Shoveller in winter 2013 was dominated by micro-invertebrates (33%), while crabs contributed the least to winter diets (19%; **Table 41, Figure 55**). The diet of Cape Shoveller during the spring incorporated more plant matter compared to winter, with *Spartina maritima* comprising 33% of their diet, and crabs contributing 26% (**Figure 55**). In summer there was evidence of specialised feeding amongst individuals of the Cape Shoveller population. Of the four individuals captured, two were feeding solely on marsh plants, while the other two had diets similar to those individuals captured in spring (**Table 41**). Cape Shoveller diet in autumn was dominated by plant material (*S. maritima* = 36%), while crabs, *P. peringuyii* and micro-invertebrates contributed 27, 21 and 16%, respectively.

Table 41. SIAR results of diet contributions to wading birds in the Kowie Estuary.

species	potential prey	winter	spring	summer	autumn
Cape Shoveller	marsh plants	0	0	**76 (33-92)**	0
	S. maritima	6 (0-14)	**37 (17-60)**	24 (0-38)	32 (0-13)
	micro-invertebrates	**50 (26-74)**	30 (6-53)	0	**47 (35-78)**
	Mysidacea	20 (0-39)	9 (0-23)	0	6 (0-36)
	P. peringueyi	17 (0-38)	13 (0-33)	0	9 (0-32)
	crabs	7 (0-17)	11 (0-29)	0	6 (0-15)
Cape Teal	micro-invertebrates	**39 (19-60)**	**38 (18-59)**	23 (0-44)	8 (0-18)
	Mysidacea	28 (9-46)	32 (14-50)	**31 (3-55)**	5 (0-12)
	P. peringueyi	20 (0-38)	21 (0-40)	26 (0-50)	14 (0-28)
	crabs	13 (0-32)	9 (0-22)	21 (0-38)	**73 (60-86)**
Yellow-bill Duck	micro-invertebrates	24 (1-45)	11 (0-34)	12 (0-33)	**34 (2-63)**
	Mysidacea	**40 (15-67)**	**71 (37-96)**	**57 (30-82)**	16 (0-37)
	P. peringueyi	23 (0-45)	12 (0-36)	20 (0-43)	23 (0-46)
	crabs	13 (0-32)	6 (0-18)	12 (0-28)	27 (1-49)
Ruff	Mysidacea	0	19 (0-39)	20 (0-40)	0
	Amphipoda	0	**34 (5-61)**	**29 (3-53)**	0
	Copepoda	0	13 (0-32)	13 (0-31)	0
	Isopoda	0	11 (0-30)	15 (0-33)	0
	crab zoea	0	23 (0-44)	23 (0-42)	0
Little Egret	*M. cephalus*	**91 (79-98)**	**30 (5-56)**	**86 (61-92)**	**48 (11-91)**
	M. capensis	1 (0-3)	23 (0-27)	7 (0-32)	6 (0-23)
	S. bleekeri	2 (0-5)	19 (0-38)	2 (0-22)	11 (0-32)
	P. peringueyi	2 (0-6)	17 (0-35)	3 (0-10)	13 (0-35)
	crabs	4 (0-12)	12 (0-44)	1 (0-3)	21 (0-45)

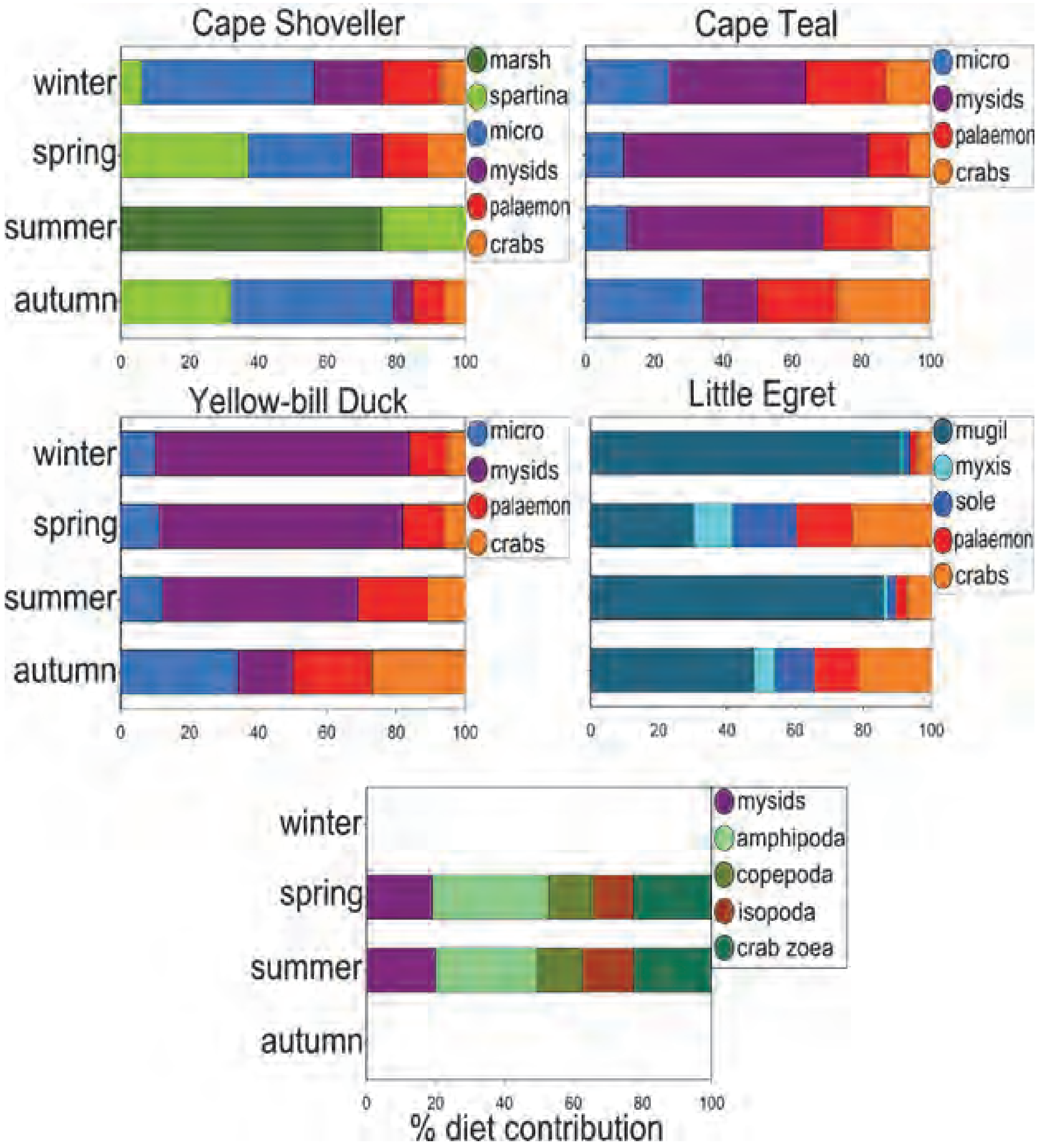

Figure 55. SIAR mixing model output for a) Cape Shoveller, b) Cape Teal, c) Yellow-bill Duck, d) Little Egret and e) Ruff. Prey items are presented as calculated percentages of total diet per season. Palaemon *Palaemon peringuyii*, Spartina *Spartina maritima*, Micro micro-invertebrates, Mugil *Mugil cephalus*, Myxus *Myxus capensis*, Sole *Sole blekerii*

Cape Teal fed primarily on micro-invertebrates and *S. maritima* during winter and spring 2013 (**Figure 55b**). Cape Shoveller diet was not dominated by one particular diet item, with all diet items used in the model contributing approximately the same to the summer diet (24-26%).

Micro-invertebrates (40%) were the dominant dietary source of Cape Teal during autumn 2014; however, *P. peringuyii* constituted 25% of their autumn diet (**Table 41, Figure 55b**). Yellow-bill Duck did not show a preference for any dietary item during winter, spring or summer, with their diet being evenly distributed among *S. maritima*, Polychaeta, *P. peringuyii* and micro-invertebrates (21-29%; **Table 41**; **Figure 55c**). *Spartina maritima* was the preferred food source of Yellow-bill Duck in autumn 2014 (35%), while Polychaeta constituted the smaller proportion of their diets in autumn (18%; **Figure 55c**). *Mugil cephalus* was the dominant diet item for Little Egret every season (**Table 41**; **Figure 55d**), contributing 91 and 88% in winter and spring, respectively. *Myxus capensis* contributed 18% to the diet of Little Egret during spring (19%), while crabs made up 21% of the Little Egret diet during autumn 2014 (**Figure 55d**). The diet of Ruff was comprised solely of micro-invertebrates, with amphipods being the preferred food item in spring (33%) and summer (29%; **Table 41, Figure 55e**). Mysidacea also contributed significantly to the diet of Ruff, making up 24 and 22% of the spring and summer diet, respectively.

Isotopic niche

The isotopic niche space of all species varied significantly between summer and winter (**Figure 56**). The isotopic niche of Yellow-bill Duck was not significantly different in size to Cape Shoveller ($p = 0.849$) or Cape Teal ($p = 0.997$). The isotopic niche of Cape Shoveller was significantly larger than Cape Teal ($p = 0.044$), Little Egret ($p < 0.001$) and Ruff ($p < 0.001$). The isotopic niche of Cape Shoveller overlapped with Cape Teal (by 8.8%), Yellow-bill Duck (by 49.2%) and Little Egret (by 30.8%). The isotopic niche of Cape Teal was significantly larger than that of Ruff ($p = 0.006$) and Little Egret ($p = 0.005$), but the niche of Cape Teal did not overlap with other species (**Figure 56**). The isotopic niches of Little Egret and Ruff were not significantly different.

Cape Shoveller, Cape Teal and Yellow-bill Duck displayed a significant reduction in their isotopic niche widths in winter compared to summer. Cape Shoveller and Cape Teal, in particular, both displayed a 90° shift in the shape of their isotopic niches in winter compared to summer. The niche occupied by Cape Shoveller in winter was not significantly different to the niche occupied by Cape Teal ($p = 0.154$) or Yellow-bill Duck ($p = 0.831$), but was significantly larger than that of the Ruff ($p = 0.048$) and Little Egret ($p = 0.005$). Cape Shoveller displayed niche overlap with Cape Teal (16.6%) and Yellow-bill Duck (11.9%) only during winter, while Cape Teal exhibited overlap of its isotopic niche with Yellow-bill duck (29.8%) and Ruff (49.8%). The arrows displayed in **Figure 56** show the clustering of Cape Shoveller individuals during winter, whereby these individuals exhibited feeding specialisation (see previous section). These individuals were feeding exclusively on aquatic marsh plants, and were ^{13}C-depleted compared to other individuals of the Cape Shoveller population.

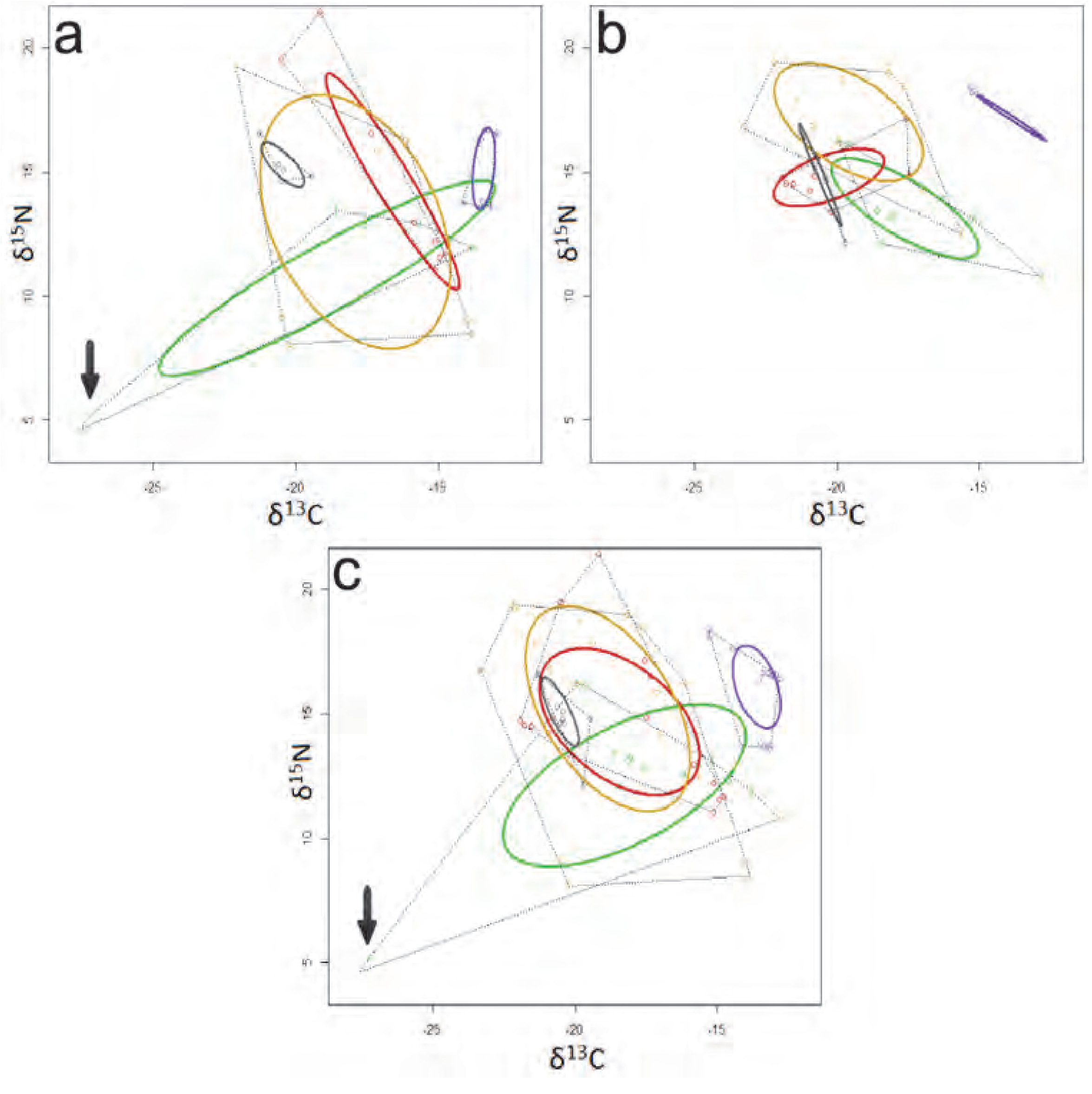

Figure 56. Isotopic niche of Cape Shoveller (green), Cape Teal, (red), Yellow-bill Duck (gold), Ruff (grey) and Little Egret (blue) in isotopic space. September 2013-February 2014 = summer (**a**); June-August 2013 + March-May 2014 = winter (**b**), June 2013-May 2014 = study year (**c**). The arrows show the clustering of Cape Shoveller specialists

Seasonal changes in $\delta^{13}C$ and $\delta^{15}N$ values of blood

With the exception of Ruff, all other species exhibited significant changes in $\delta^{13}C$ and $\delta^{15}N$ values between seasons. There was a significant shift in the mean $\delta^{13}C$ value of Cape Shoveller from spring (-16.3 ± 2.5‰) to summer (-20.8 ± 6.8‰; Q = 4.596, p = 0.011), and from summer (-20.8 ± 6.8‰) to autumn (-17.0 ± 1.2‰; Q = 3.742, p = 0.052, **Figure 57**). The mean $\delta^{15}N$ value of Cape Shoveller displayed a significant shift from spring (12.6 ± 1.5‰) to summer (8.5 ± 3.8‰; Q=6.821, p = 0.0001), and from summer (8.5 ± 3.8‰) to autumn (13.0 ± 0.4‰; Q = 7.641, p = 0.0001, **Figures 57 and 58)**. Cape Teal exhibited a shift in $\delta^{13}C$ value from summer (-17.9 ± 2.1‰) to autumn (-15.2 ± 0.4‰; Q = 4.498, p = 0.014). Similarly, the $\delta^{15}N$ values of Cape Teal blood were significantly different when the seasons changed from summer (17.2 ± 4.2‰) to autumn (12.1 ± 0.6‰; Q = 6.817, p = 0.0002, **Figure 57**). Yellow-bill Duck did not exhibit a significant shift in their $\delta^{13}C$ values between seasons, but did display a significant change in $\delta^{15}N$ values between summer (17.3 ± 1.4‰) and autumn (8.7 ± 0.5‰; Q = 16.88, p = 0.0001). The $\delta^{13}C$ values of Little Egret changed significantly from winter (-13.1 ± 0.2‰) to spring (-15.1 ± 0.7‰; Q = 11.51, p = 0.0001, **Figure 59**), and from spring (-15.1 ± 0.7‰) to summer (-13.5 ± 0.4‰; Q = 9.911, p = 0.0001, **Figure 58**). The $\delta^{13}C$ and $\delta^{15}N$ values of blood cells in Ruff did not show any significant changes during the time that they spent in South Africa.

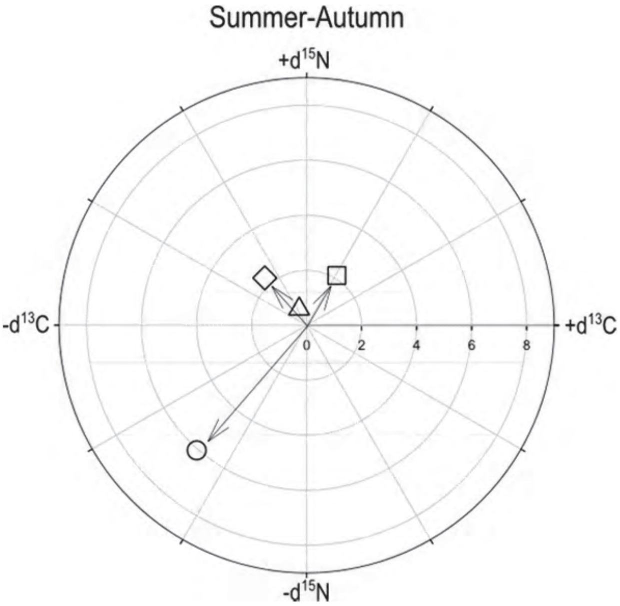

Figure 57. Direction and magnitude of change of $\delta^{13}C$ and $\delta^{15}N$ stable isotope values from Summer 2014 to Autumn 2014 for Cape Shoveller (circle), Cape Teal (square), Yellow-bill Duck (triangle) and Little Egret (diamond). Values in the right and left hemispheres from the origin are ^{13}C-enriched or depleted, respectively. Values in the top and bottom hemispheres from the origin are ^{15}N-enriched or depleted, respectively

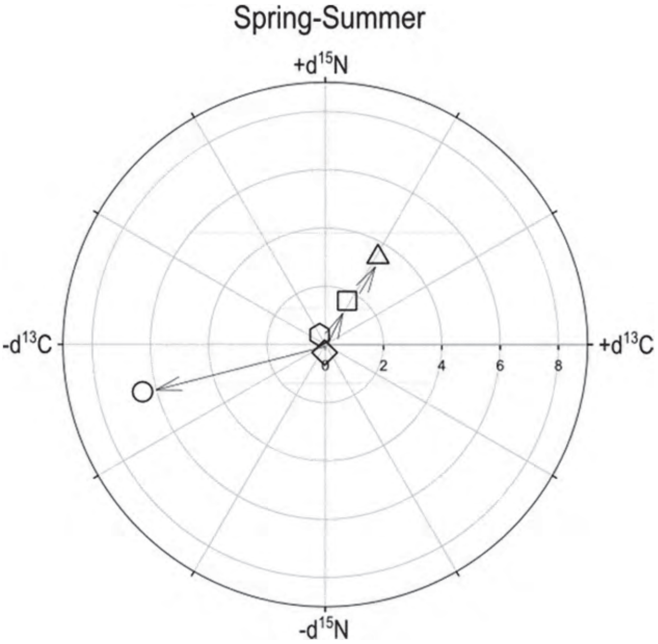

Figure 58. Direction and magnitude of change of δ^{13}C and δ^{15}N stable isotope values from Spring 2013 to Summer 2014 for Cape Shoveller (circle), Cape Teal (square), Yellow-bill Duck (triangle), Ruff (hexagon) and Little Egret (diamond)

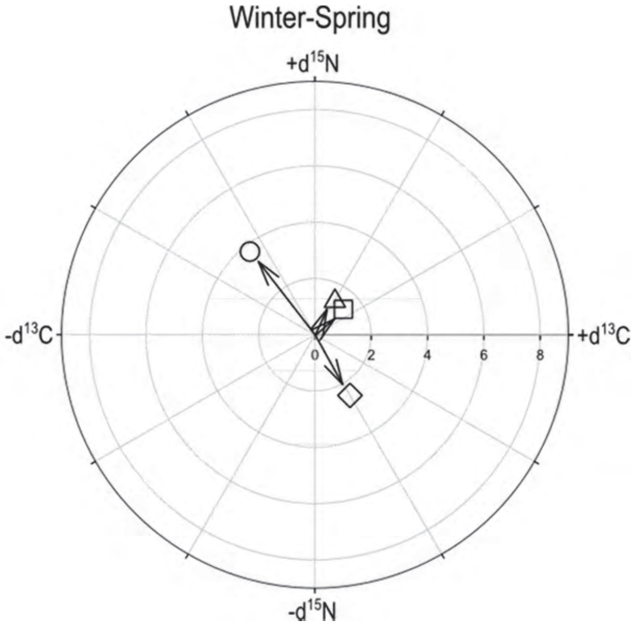

Figure 59. Direction and magnitude of change of δ^{13}C and δ^{15}N stable isotope values from Winter 2013 to Spring 2013 for Cape Shoveller (circle), Cape Teal (square), Yellow-bill Duck (triangle) and Little Egret (diamond)

Interpretations

There appeared to be a great demand for similar dietary resources by Cape Shoveller, Cape Teal and Yellow-bill Duck, particularly during spring and summer. Micro-invertebrates were a prominent food source amongst these consumers, while the interspecific competition for micro-invertebrate resources increased with the arrival of Ruff. The output from the SIAR models suggested that the Cape Shoveller, Cape Teal and Yellow-bill Duck populations were composed of generalist feeders, while Ruff and Little Egret were specialist feeders. Additionally, Little Egret display an “opportunistic” specialist feeding regime, whereby *Mugil cephalus* was the preferred food source, but the egrets can feed opportunistically on other food sources in the estuary such as freshwater mullet (*Myxus capensis*), particularly in spring (Fishbase, 2014). The degree of overlap of the isotopic niches of Cape Shoveller, Cape Teal and Yellow-bill Duck was high (see **Figure 56c**), particularly when the yearly isotopic niche of each species was calculated. Of particular interest was the degree of overlap between Cape Teal and Yellow-bill duck (approximately 98% for the year). Yellow-bill Duck are described in the literatures as herbivorous, yet our models showed that this species was feeding on invertebrates to a large extent. The extent of the isotopic niche overlap between Cape Teal and Yellow-bill duck suggested that these species are in direct competition with one another for dietary resources. However, if the seasonal trophic niches are considered, we see that these two species, along with Cape Shoveller, partition the resources within their environment and display various degrees of specialist and generalist behaviours. Additionally, our data showed that all species were feeding on organisms from several trophic levels, particularly during the summer (see **Figure 56a**). This flexibility represents evidence that the co-occurring birds are partitioning the available resources.

Analysis of $\delta^{13}C$ and $\delta^{15}N$ values of individuals within each species showed that individuals were significantly different from one another, although this result does not necessarily support specialist feeding. However, in the summer months, individuals from the Cape Shoveller and Yellow-bill Duck populations exhibited distinct patterns of feeding segregation (see arrows in **Figure 56a and b**), whereby the $\delta^{13}C$ and/or $\delta^{15}N$ values of individuals were highly distinct ($\Delta\delta^{13}C$ = 13.0‰, $\Delta\delta^{15}N$ = 7.3‰). Similarly, the same trophic specialisation was seen in Yellow-bill duck in summer in two individuals feeding at a significantly higher trophic level ($\Delta\delta^{13}C$ = 5.9‰, $\Delta\delta^{15}N$ = 4.5‰). The top-down pressure that these avian consumers exert on the prey community in the lower reaches of the Kowie Estuary is likely significant, especially when we consider the abundances of these bird species. Little Egret was feeding specifically on flathead mullet (*Mugil cephalus*), especially during winter and summer

(**Figure 55**). Coupled with the population size of Little Egret in summer (mean = 102 individuals), the predatory role that these birds play is likely significant. Similarly, the combined predatory pressure that Cape Shoveller, Cape Teal, Yellow-bill Duck and Ruff exert on aquatic micro-invertebrates is great considering that the combined abundance of these species peaked at 115 individuals at one time. These data provide evidence for the importance of cross-habitat subsidies in supporting consumers in upper trophic levels. The seasonal mixing model data may have underestimated the source contributions to the diet of each species, as the sample size of each species is small and likely under-represents the variation in the entire populations. Similarly, Bayesian ellipse size and isotopic niche sizes and shapes can be affected by this under-representation. However, the combined “yearly” Bayesian ellipses and isotopic niche widths may be more accurate representations of the bird community because the sample size is larger. Although the seasonal replication was relatively low (n=8 in most instances), examples of intraspecific trophic specialisation were observed and provide support that the data represent the natural variations in $\delta^{13}C$ and $\delta^{15}N$ values amongst members of the bird community in the Kowie Estuary.

7.10 Bloukrans River community

7.10.1 Channel morphology and environmental parameters

On average, the levels of dissolved oxygen, electrical conductivity and pH increased from site B1 to Site B4, with a decrease from upstream to downstream areas in total dissolved solutes over the four seasons (**Table 42**). Water depth was greatest (43.5 cm) at B3, and B2 was wider (5.9 m) than the other three sites, with the lowest water velocity (0.5 ms^{-1}). Site B1 had the narrowest channel with temperatures higher (20.3 °C) than the other three sites. The highest levels of ammonia and lowest amounts of phosphates occurred at B1. Nitrate concentrations were higher at B2 and B3 relative to B1 and B4. There were some significant differences in phosphate and nitrate concentrations (not ammonia) among the sites, and significant effects of all the other physico-chemical parameters (except for substrate) across the sites.

Table 42. Environmental variables (mean ± standard deviation) measured in four sites (B1 to B4) at the Bloukrans River between April 2013 and January 2014. *P*-values indicate statistical level of significance based on Kruskal-Wallis tests, with significant differences indicated in bold. Abbreviations: DO – dissolved oxygen, TDS – total dissolved solutes

Parameters	**B1**	**B2**	**B3**	**B4**	***p***
DO (mg L^{-1})	4.6±2.6	5.8±0.9	6.5±1.3	7.1±1.9	**< 0.001**
conductivity (mS cm^{-1})	1.3±0.1	1.2±0.1	1.2±0.4	1.5±0.3	**0.010**
TDS (ppt)	0.9±0.1	0.8±0.1	0.7±0.2	0.7±0.4	**< 0.001**
salts (ppt)	0.6	0.6	0.5±0.1	0.7±0.1	**< 0.001**
pH	7.9±0.6	7.8±0.5	8.0±0.5	8.2±0.9	**< 0.001**
temperature (°C)	20.3±4.4	15.8± 4.4	15.9±5.0	17.1±5.5	**< 0.001**
water velocity (ms^{-1})	0.6±0.5	0.5±0.8	1.2±1.1	0.8±1.0	**< 0.001**
depth (cm)	35.3±13.9	38.8± 3.3	43.5±4.4	35.5±16.1	**< 0.001**
width (m)	3.4±1.1	5.9±2.3	4.5±0.5	5.4±1.4	**< 0.001**
substratum	2.0±0.8	2.0±0.8	2.0±0.8	2.0±0.8	**-**
Ammonia (mgL^{-1})					
NH_3-N	8.2±1.6	2.0±1.2	0.5±0.6	0.1±0.1	0.1792
NH_3	10.0±2.0	2.5±1.4	0.7±0.7	0.1±0.1	0.3342
NH_4^+	10.6±2.1	2.6±1.5	0.7±0.8	0.1±0.1	0.3739
Phosphates (mgL^{-1})					
PO_3^{-4}	7.0±5.9	4.5±2.4	4.1±1.1	4.7±2.7	**0.002**
P	2.3±1.9	1.5±0.8	1.3±0.3	1.5±0.9	**0.019**
P_2O_5	5.2±4.4	3.4±1.7	3.1±0.8	3.4±2.0	0.050
Nitrates (mgL^{-1})					
NO_3^- -N	2.5±2.6	6.5±4.5	5.2±5.5	0.9±1.0	0.894
NO_3^-	10.4±12.5	28.7±20.1	23.0±24.2	3.8±4.4	**0.016**

The river bed (**Table 43**) at B2 and B3 was characterised by similar but unevenly distributed substrates (cobble, pebble, gravel and sand). Sites B1 and B4 had distinct riverbed substrates, with some silt, mud or clay at B1 and bedrocks at B4.

Table 43. Abundance scores and percentage degree of embeddedness of the substratum at B1 to B4 between April 2013 and January 2014 at the Bloukrans tributary.

Material (mm)	**B1**	**B2**	**B3**	**B4**	**Degree of embeddedness of substratum (%)**
bedrock				2	47
boulder (> 256)				3	30
cobble (100-256)		3	3		60
pebble (16-100)		2	3		50
gravel (2-16)		2	2		70
sand (0.06-2)	3	3	2		78
silt/ mud/ clay (<0.06)	2				80

The habitat in the riparian areas of B2, B3 and B4 was in class C of the habitat integrity criteria except for site B1, which was class D (**Table 44**). The in-stream habitat of B3 and B4 was class D, and B1 and B2 were class E and D, respectively.

Table 44. Environmental variables (mean ± standard deviation) at the Bloukrans River between April 2013 and January 2014. Abbreviations: M – mean; S – Standard deviation; HI – habitat integrity.

Sites	**Criterion**	**M, S**	**HI class**	**Description of Habitat**
B1	In-stream	21.9 ± 0.8	E	Critically modified, complete loss of natural habitat and ecosystem functioning
	Riparian	48.0	D	Largely modified, loss of natural habitat and basic ecosystem functioning
B2	In-stream	68.3 ± 3.8	C	Moderately modified. A loss of natural habitat and biota but basic ecosystem functioning appears to be unchanged
	Riparian	71.2 ± 4.9	C	Moderately modified. A loss of natural habitat and biota but basic ecosystem functioning appears to be unchanged
B3	In-stream	59.8 ± 14.3	D	Largely modified, loss of natural habitat and basic ecosystem functioning
	Riparian	79.1 ± 0.1	C	Moderately modified. A loss of natural habitat and biota but basic ecosystem functioning appears to be unchanged
B4	In-stream	57.8 ± 24.5	D	Largely modified, loss of natural habitat and basic ecosystem functioning
	Riparian	65.6 ± 18.2	C	Moderately modified. A loss of natural habitat and biota but basic ecosystem functioning appears to be unchanged

7.10.2 Macroinvertebrates

We collected 4076 individual macroinvertebrates from 43 taxa between April 2013 and January 2014. On average, the number of macroinvertebrate taxa increased from B1 to B4, with significant differences ($p < 0.05$) in the distribution of Hydropsychidae, Aeshnidae, Chironomidae and Potamonautidae, but not with all the other taxa across the sites (**Table 45**).

The distribution of macroinvertebrate communities along the Bloukrans River fluctuated across the sites (**Figure 60**). The community labelled “others” represents a percentage abundance of the sum of all taxa with a maximum of 9 individuals per taxa. Site B1 had fewer taxa compared to the other three sites. This area of the river was dominated by the family Chironomidae, which contributed over 85% to the overall community. Lower contributions of the families Oligochaeta and Physidae were apparent. Dominance in community abundances shifted from Chironomidae to Baetidae (47% of the total community at site B2; **Figure 60b**). Chironomid abundance decreased to 21% of the total community at B2, and several additional families appeared including Potamonautidae, Coenagrionidae, Hirudinae and a few others. The presence of Baetidae remained high (31%) at B3 (**Figure 60c**), and the number of families increased compared with B1 and B2 (families added included Corixidae, Caenidae, Gomphidae and Notonectidae). There was a large number of dominant families identified at B4 (**Figure 60d**), including Baetidae, Corixidae, Simulidae and Leptophlebiidae. New families found at this site included Heptageniidae and Amphipoda, and no chironomids were present.

There was a significant difference ($p < 0.05$) in the distribution of functional feeding groups across the sites. On average, gathering collectors were the most abundant group from all four sites, with the highest numbers found at B4 (**Figure 61**), followed by filtering collectors, predators, and shredders.

Table 45. Macroinvertebrate taxa collected from the Bloukrans River between April 2013 and January 2014. *P*-values indicate statistical level of significance based on one-way ANOVA, with significant differences indicated in bold

Taxa	B1	B2	B3	B4	p
Baetidae		112.5 ± 125.6	55.5 ± 48.7	142.3 ± 187.1	0.353
Caenidae		1.0 ± 2.0	11.5 ± 7.6	18.3 ± 26.4	0.237
Leptophlebiidae				38.3 ± 44.4	0.074
Heptageniidae				4.3 ± 8.5	0.426
Hydropsychidae		4.5 ± 5.7	1.5 ± 1.9	62.5 ± 63.0	**< 0.043**
Philiopotamidae	1.3 ± 2.5			7.3 ± 9.5	0.168
Ecnomidae	1.3 ± 2.5		1.3 ± 2.5		0.588
Gyrinidae		0.8 ± 1.5		11.5 ± 23.0	0.444
Elmidae				1.3 ± 1.5	0.086
Hydraenidae	0.3 ± 0.5			0.5± 1.0	0.551
Psephenidae	0.3 ± 0.5				0.426
Dytiscidae	0.3 ± 0.5				0.426
Corixidae	0.5 ± 1.0	0.8 ± 1.5	18.3 ± 32.0	45.5 ± 91.0	0.531
Gerridae		0.3 ± 0.5	0.3 ± 0.5		0.588
Notonectidae	0.3 ± 0.5	0.5 ± 1.0	2.5 ± 2.6		0.105
Belostomatidae	0.5 ± 1.0		0.5 ± 0.6		0.426
Libellulidae				0.8 ± 1.5	0.426
Aeshnidae			0.3 ± 0.5	4.8 ± 3.9	**< 0.012**
Gomphidae	0.3 ± 0.5	0.5 ± 0.6	7.8 ± 8.7	10.0 ± 18.7	0.453
Chlorolestidae		2.3 ± 4.5	1.5 ± 1.7	1.3 ± 1.9	0.675
Coenagrionidae	0.5 ± 1.0	6.3 ± 9.2	13.5 ± 10.1	8.5 ± 14.5	0.360
Platycnemidae			0.8 ± 1.5	0.5 ± 1.0	0.574
Lestidae			0.5 ± 1.0	0.3 ± 0.5	0.551
Ceratopogonidae			1.3 ± 2.5		0.426
Chironomidae	150.0 ± 74.0	50.3 ± 31.6	9.8 ± 7.4	4.5 ± 5.3	**< 0.001**
Culicidae	0.5 ± 1.0				0.426
Simulidae		29.5 ± 17.5	21.5 ± 29.8	36.8 ± 41.5	0.294
Syrphidae	0.5 ± 1.0				0.426
Tabanidae			0.5 ± 1.0	4.3 ± 4.4	0.058
Tipulidae		0.3 ± 0.5	0.3 ± 0.5		0.588
Psychodidae	0.5 ± 1.0				0.426
Potamonautidae		11.3 ± 5.4	6.3 ± 4.8	12.0 ± 9.1	**< 0.045**
Lumbricidae	0.5 ± 1.0	4.5 ± 9.0	2.3 ± 4.5		0.599
Oligochaeta	11.5 ± 18.1	4.8 ± 4.9	1.5 ± 1.7	1.0 ± 2.0	0.407
Hirudinae	0.5 ± 0.6	6.3 ± 6.1	5.0 ± 3.9	2.3 ± 4.5	0.269
Ancylidae	0.5 ± 1.0	2.5 ± 2.1	11.0 ± 16.1		0.246
Physidae	5.5 ± 8.4		4.0 ± 5.7		0.340
Vespoidae	0.5 ± 1.0				0.426
Argyronetidae		0.3 ± 0.5			0.426
Amphipoda				5.3 ± 10.5	0.426
Oxyopidae	0.5 ± 1.0			0.5 ± 1.0	0.588
Anura	0.3 ± 0.5				0.426
Tetragnata				0.3 ± 0.5	0.426

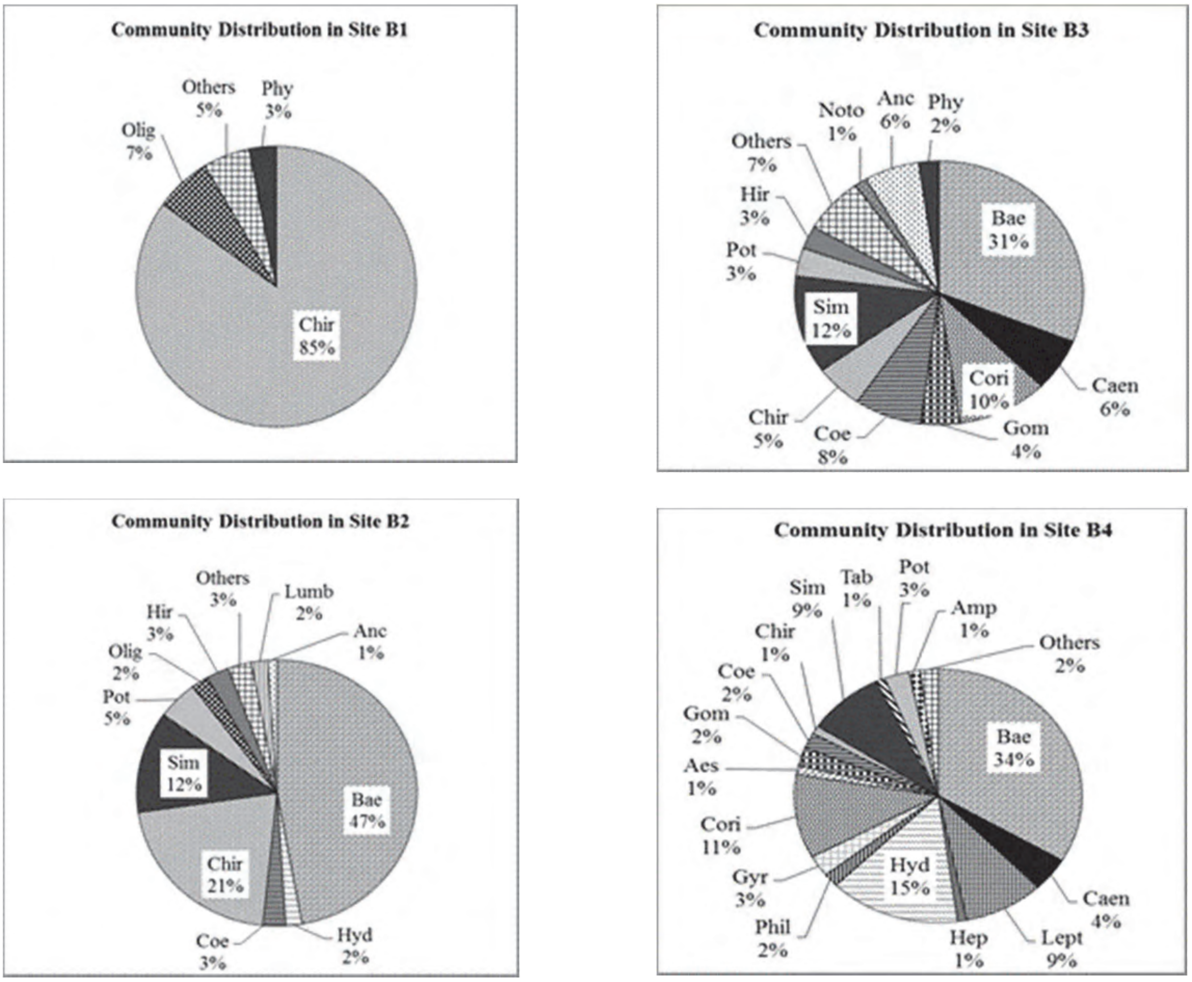

Figure 60 (a to d). Change in macroinvertebrate community structure from site B1 to B4 at the Bloukrans River between April 2013 and January 2014

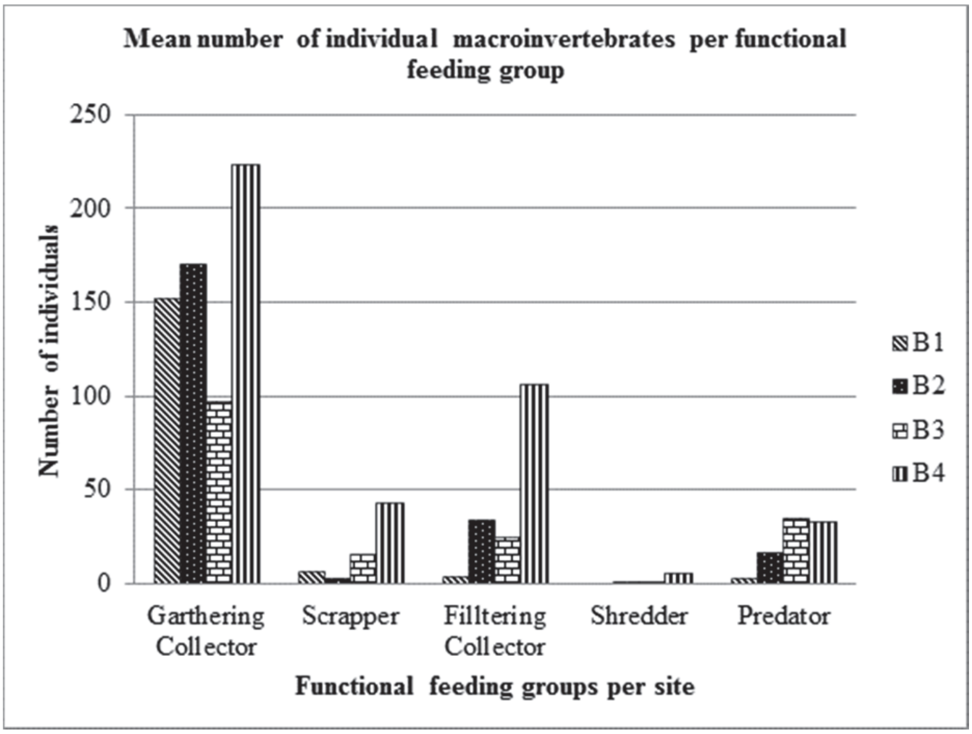

Figure 61. Mean number of individual macroinvertebrates per functional feeding group from sites B1 to B4 between April 2013 and January 2014 at the Bloukrans River.

There was a significant difference in taxa diversity ($p < 0.001$), SASS scores ($p < 0.05$), ASPT ($p < 0.001$), EPT Richness ($p < 0.001$), and filtering collectors + scrapers: total abundance ($p < 0.05$) per site between April 2013 and January 2014. The biotic indices revealed that higher abundances of macroinvertebrates occurred at B4 in April and July, and at B1 in October and January. The number of taxa was higher (17) at B4 in April and July, followed by B3 (14) in October and B2 (13) in January. The lowest number of taxa (4) occurred at B4 in October. Site B1 had lower diversity than the other sites. The diversity generally increased from B1 to B4. High SASS score and ASPT were measured at B4 in April and July. The lowest SASS scores and ASPT occurred at B1 in October and at B1 and B4 in January. EPT richness and the ratio of EPT to Chironomidae increased from B1 to B4. The ratio of filtering collectors and scrapers to the total abundance was high at B1 in October and January, at B1 and B2 in April, and at B2 and B4 in July (**Table 46**).

Table 46. Metrics used to identify macroinvertebrate community structure at sites B1 to B4 between April 2013 and January 2014 at the Bloukrans River. *P*-values indicate statistical level of significance based on one-way ANOVA, with significant differences indicated in bold. Abbreviations: ASPT – Average scores per taxon; EPT – Ephemeroptera, Plecoptera and Trichoptera; FC – filtering collector; SC – scrapers and Tot Ab – total abundance.

Metric	**April**				**July**				**October**				**January**				
	B1	**B2**	**B3**	**B4**	**B1**	**B2**	**B3**	**B4**	**B1**	**B2**	**B3**	**B4**	**B1**	**B2**	**B3**	**B4**	***P***
Total abundance	117	425	270	1024	130	245	319	490	220	172	75	64	239	116	51	119	0.459
Taxa Richness	10	11	15	17	10	10	16	17	5	9	14	8	4	13	12	8	0.057
Diversity index *(H')*	0.7	1.1	2.0	2.0	1.2	1.5	2.2	1.9	0.1	1.6	2.4	1.7	0.4	2.2	2.1	1.2	**< 0.001**
SASS Scores	42	37	63	104	33	39	63	88	14	34	62	52	6.0	51	57	47	**0.006**
ASPT	4.2	3.4	4.2	5.8	3.7	3.9	3.9	5.5	2.8	3.8	4.4	6.5	2.0	3.9	4.8	6.7	**< 0.001**
EPT Richness	1.0	2.0	4.0	5.0	1.0	1.0	2.0	4.0	0.0	2.0	2.0	4.0	0.0	2.0	3.0	3.0	**< 0.001**
EPT : Chironomidae	0.1	8.8	12.5	60.1	0.1	1.2	6.3	43.8	0.0	0.5	1.5	0.0	0.0	2.5	0.0	0.0	0.136
FC & SC : Tot Ab	0.9	0.9	0.5	0.6	0.6	0.9	0.7	0.9	1.0	0.8	0.5	0.6	1.0	0.5	0.4	0.8	**0.045**

Of the physico-chemical parameters measured, the macroinvertebrate communities of the Bloukrans River responded to nitrate levels, water velocity, water depth, ammonia levels, pH, channel width and the substratum of the riverbed (**Figure 62**).

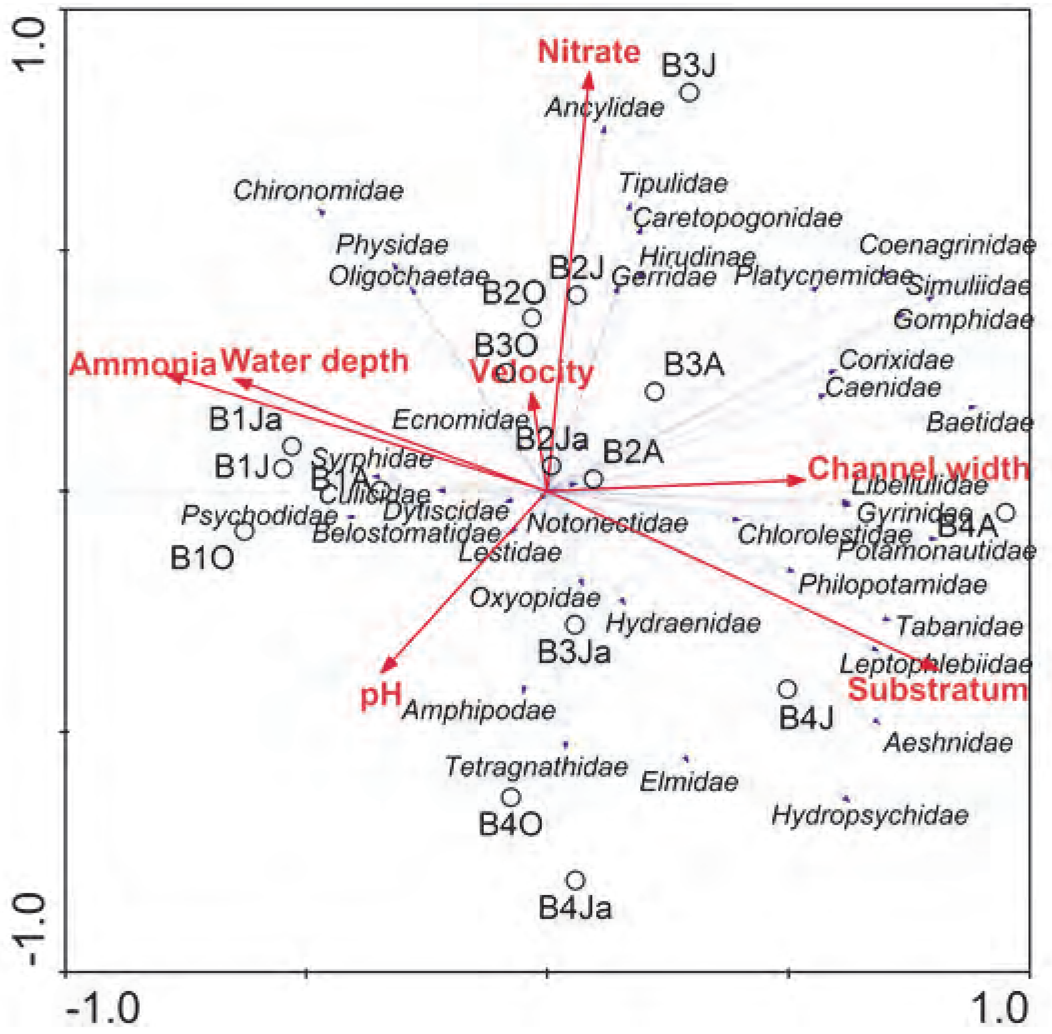

Figure 62. Redundancy analysis showing the distribution of macroinvertebrate taxa in relation to selected environmental parameters measured in the Bloukrans River in four seasons between April 2013 and January 2014. A – autumn, J – winter, O – spring, Ja – summer, B1 to B4-sites.

Interpretations

Our study indicated that water quality was severely altered upstream in the Bloukrans River, mainly as a result of local land use rather than natural climatic conditions. Poor water quality at B1 was clearly shown by decreased oxygen and increased TDS compared to downstream sites. The increase in TDS was associated with the high levels of ammonium ions and elevated phosphates. The most upstream site was highly impacted by urbanization, and other studies (e.g. Collier, 1995; Ndaruga et al., 2004; Miserendino et al., 2011) have reported clear cases of poor water quality from rivers located within urban areas. High nitrate levels at B2 can be attributed to point source pollution entering the stream from sewage works adjacent to the site. Site B3 was impacted by agricultural land use related to alterations in channel morphology by damming, leading to deeper channel and high nitrate levels at this site. Site B4 was high in dissolved oxygen, conductivity, salts and pH compared to all the other sites, likely because primary producers were able to colonize this location. No riparian species were observed at B1 except for the abundant *Pennisetum clandestinum* (kikuyu) grass, whereas downstream sites were partly covered by riparian trees that allowed some sunlight to pass through into the water.

We aimed to determine how the macroinvertebrate community structure changed along the Bloukrans River. The habitat integrity analysis indicated that the riparian habitat was largely modified by overgrazing and degradation by sand extraction at B1. Critical modification of instream habitat was also indicated at the site, which was located next to urban settlements. Basically there was a complete loss of natural habitat and basic ecosystem functioning at B1. During April and July, the streambed was heavily affected by inputs of garbage including litter and undegradable material such as broken glasses, bricks and metal pieces deposited through direct runoff after a few days of moderate rain. There was a major deterioration in water quality as indicated by SASS scores of < 50 and ASPT < 6 across all the seasons. The state of the habitat and water quality favoured the high abundances of tolerant macroinvertebrate communities at B1 (**Figure 60**). Ordination analysis (**Figure 62**) indicated that ammonia and water depth were the most important environmental parameters influencing community structure at B1. There was a strong negative correlation from the first axis between water depth and ammonia and the communities in this site, indicating that at increased levels of ammonia the number of organisms decreased. Taxa diversity, EPT richness and the ratio of EPT to Chironomidae were very low at B1 due to absence of EPT taxa. Other researchers have reported decreased abundances of EPT taxa in sites heavily impacted by pollution (Palmer et al., 1996; Compin and Céréghino, 2007; Jun et al., 2011).

The habitat at B2 was more moderately impacted than B1. This site is subject to occasional sewage effluent discharges which alter the state of water for prolonged periods.

In July 2013 effluent discharge was observed during sample collection. The water was critically impacted by partially treated waste which increased nitrate levels. The SASS scores and ASPT indicated that water quality was low (**Table 44**); however, diversity of the animal taxa was higher compared to B1. We propose that certain habitat characteristics, such as substrate diversity, were more suitable for macroinvertebrates at B2. Other freshwater researchers have discovered that habitats with a larger variety of substrates support more diverse communities of macroinvertebrates (Quinn and Hickey, 1990; O'Connor, 1991; Baptista et al., 2001). Furthermore, the presence of cobbles, pebbles, gravels and sand allowed for increased circulation of the available oxygen for fauna (Lowell and Culp, 1999).

Sites B3 and B4 were largely modified in the channel and moderately impacted in the riparian zone, resulting in some loss of natural habitat and basic ecosystem functioning. The impact was clearly indicated by the lower total abundances of taxa collected in October 2013 and January 2014. Periods of flooding occurred two days prior to sampling in October, which probably related to the decreased richness at B4 during that period. In January 2014, both the sites were impounded for irrigation of crop lands, and B4 nearly dried out. EPT richness was high at B3 and B4, indicating the ability of these sites to support sensitive taxa regardless of the state of water quality. Higher water velocity at B3 probably helped promote a fairly high diversity. Flow regimes can be very influential on macroinvertebrate community structure (Lods-Crozet et al., 2001; Bunn and Arthington, 2002; Bonada et al., 2006; Monk et al., 2006). Site B4 had large riffle areas and stones that provided suitable habitat for a diverse macroinvertebrate community. As such, the RDA (**Figure 62**) indicated the importance of substratum and channel width to the macroinvertebrate communities.

Macroinvertebrates were assigned to groups based on their stage of development (e.g. larvae) and feeding habits. Five functional feeding groups were identified as gather-collectors, scrapers, filter-collectors, shredders and predators. Gather-collectors were the most abundant group across all sites, followed by filter-collectors, predators and scrapers. There is some evidence that gather-collectors can thrive in urban streams because they have ample food even in impacted locations (Suren and McMurtrie, 2005). Shredders were observed only at B4, probably because the site had an abundance of leaf litter input. Site B4 was the most preferred site by all the functional feeding groups. Scraper and predator abundances were reduced in site B1 and B2, as scrapers require healthy periphyton for food, which can be heavily impacted in highly polluted areas (Jun *et al.,* 2011). Predators increased in abundance in the downstream direction, probably a result of limited prey availability at upstream sites.

Environmentally stressful conditions were identified by shifts in macroinvertebrate communities, from more diverse communities at sites located downstream to low diversity upstream due to water pollution. The macroinvertebrate communities in the Bloukrans River were negatively impacted by organic nutrient pollutants from urbanization and sewage effluents. The mean SASS Scores and ASPT for the whole tributary were relatively low compared to the standard reference scores that indicate an undisturbed stream in South Africa (SASS score = 49.5, ASPT = 4.3). These findings indicated major deterioration in water quality of the stream and a need for management and conservation interventions to restore its intrinsic natural value.

7.11 Flux model

Model Construction

In accordance with our proposed modeling plan, we have created a preliminary food web structure for freshwater sites F1 and F4, approximating conditions from September to January. Using *Ecopath with Ecosim* (*EwE*), we have established a basic food web using data collected in the field or borrowed from the literature. Our reliance on literature values to drive the model may lessen if additional empirical data from the Kowie River eventually become available. For the initial model framework, we delineated organisms found at F4 into functional groups (**Table 47**). *EwE* is a relatively powerful modeling platform requiring a variety of datasets to drive a linear governing equation to balance food web energetics and production. **Eq. (1)** expresses the steady-state model for each ecosystem component:

$$B_i \left(\frac{P_i}{B_i}\right) EE_i = Y_i + \sum_{j=1}^{n} B_j \left(\frac{Q}{B}\right)_j DC_{ji} \qquad \textbf{Eq. (1)}$$

where B_i is the biomass of group i during the study period. The ratio $(P/B)_i$ is the production/biomass of functional group i, and is equal to total mortality under the equilibrium assumption. EE_i is the ecotrophic efficiency, i.e., the fraction of production consumed, and Y_i is group i's yield. B_j is the biomass of the consumer j, while the fraction $(Q/B)_j$ is the consumption/biomass ratio of consumer j. Finally, DC_{ji} is the fraction of group i in the diet of group j. The first dataset required is biomass: we approximated functional group biomass values from collected samples (temporal variation in fish captures was approximated). Second, *EwE* requires P/B, Q/B, and ecotrophic efficiency values for each functional group. We have approximated these from the literature (**Table 47**). Lastly, *EwE* requires a diet matrix, outlining the diet composition of each functional group. Our diet matrix is based on primary data from the Kowie River (**Table 48**).

Table 47. Ecopath model basic inputs for F4 in the Kowie River.

Groups	Biomass (g/m^2)	P/B (y^{-1})	Q/B (y^{-1})	Ecotrophic Efficiency
Producers (phytoplankton)	0.145[a]	133.3[j]	--	8.314[x]
Algae (epiphyton, epipelon, periphyton, filter-collectors)	8.770[b]	5.200[k]	33.43[r]	0.950[y]
Smaller fish (juvenile largemouth bass, *Rhabdosargus holubi*, *Myxus capensis*, *Monodactylus falciformis*)	1.020[c]	3.600[l]	20.80[s]	0.512[z]
Larger fish (adult largemouth bass)	44.64[d]	0.320[m]	6.220[t]	0.492[x]
Odonates and spiders	5.398[e]	2.700[n]	7.710[u]	0.530[&]
Lower trophic level insects (shredders and grazers)	17.95[f]	4.200[o]	30.00[v]	0.950[^]
Potamonautes sidneyi (crabs)	6.608[g]	2.800[p]	8.500[w]	0.000[x]
Terrestrial plants	160.974[h]	9.014[q]	--	0.017[#]
Detritus	0.001[i]	--	--	0.058[x]

a – producers biomass calculated based on phytoplankton collections
b – algae biomass calculated based on sum of collections of benthic algae and filter collectors
c – smaller fish biomass estimate based on mass of total *Rhabdosargus holubi* caught and seine net size (**time aspect missing so this value is definitely wrong**)
d – adult largemouth bass biomass estimate based on mass of total *Micropterus salmoides* caught and seine net size (**time aspect missing so this value is definitely wrong**)
e – Odonates and spiders biomass estimate taken from predators biomass measured
f – lower trophic level insects calculated based on sum of biomass of shredders, grazers
g – crab biomass obtained from the biomass estimates
h – biomass calculated by Ecopath with other existing data inputs
i – detritus biomass calculated based on drift collections
j – production per biomass estimate taken from the 1990s value in the Shannon et al. paper on the Benguela Current, located at a similar latitude as the study site (2003).
k – production per biomass estimate calculated from the sum of the 1990s values of meiobenthos and macrobenthos in Shannon et al. study on the Benguela Current (2003)
l – production per biomass estimate taken from the 1990s value of a species of a similar trophic level in Shannon et al. study on the Benguela Current (2003)
m – production per biomass estimate taken from Hossain et al. paper on Hamilton Harbour (2012)
n – production per biomass estimate of Odonates and spiders obtained from Poepperl's values for Odonates in a northern German stream (2003)
o – lower insects production per biomass estimate calculated from Hossain et al. paper on Lake Toya in Japan (2010).
p – production per biomass estimate obtained from Okey et al. value for large crabs in the West Florida shelf (2004)
q – production per biomass estimate of terrestrial plants obtained from Okey et al. value for seagrasses in the West Florida shelf (2004)
r – consumption per biomass estimate calculated from production per biomass and production/consumption values of meiobenthos and macrobenthos in Shannon et al. (2003)
s – consumption per biomass calculated from 1990s production per biomass and production/consumption values of a species of a similar trophic level in Shannon et al. study
t – consumption per biomass estimate taken from Hossain et al. paper on Hamilton Harbour (2012)
u – consumption per biomass of Odonates and spiders calculated from Poepperl's values for production per biomass and production/consumption ratios of Odonates in a northern German stream (2003)

v – consumption per biomass value calculated from Hossain et al. values of production and production/consumption ratio in Lake Toya (2010).
w – consumption per biomass estimate obtained from Okey et al. value for large crabs in the West Florida shelf (2004)
x – Ecotrophic efficiency calculated by Ecopath with other existing data inputs
y – Ecotrophic efficiency value taken from 1990s ecotrophic efficiencies of various sized zooplankton and benthos in Shannon et al. paper on the Benguela Current (2003)
z – Ecotrophic efficiency obtained from Okey et al. value for mullet fish in the West Florida shelf (2004)
& – Ecotrophic efficiency obtained from Poepprl et al. (2003)
^ – Ecotrophic efficiency obtained from Hossain et al. (2010)
# – Ecotrophic efficiency obtained from Okey et al. value for seagrasses in the West Florida shelf (2004)

Bass migrate between freshwater and estuary sites and we would like to incorporate these migrations into the model so that we can provide more accurate estimates of the energy and biomass flow. Other *EwE* outputs are shown in **Figure 63**, and **Tables 49 to 51**. **Figure 63** illustrates the flow of matter across trophic levels, whereby the first and third trophic levels are considerably larger than the second. **Table 49** shows accumulation rate and net efficiencies across functional groups as calculated by *EwE*, **Table 50** shows predatory electivity as calculated by *EwE*, and **Table 51** shows predatory search rates as calculated by *EwE*. Using the most recent SIAR and biomass data, we were able to update our model for the F4, adding two more trophic groups, *Potamonautes sidneyi* (crabs) and terrestrial plants. Biomass and production per biomass estimates could not be obtained for terrestrial plants from a similar system, as Ecopath studies are typically done for aquatic systems. The most comparable trophic group in the literature was the seagrass group in the West Florida Shelf. The high calculated ecotrophic efficiency value for phytoplankton suggests that it is highly utilized as a food source.

Electivity values were calculated by Ecopath and described a predator's preference for a prey (**Table 50**). According to the electivity values for *Potamonautes sidenyi*, crabs demonstrate avoidance for terrestrial plants and larger fish such as *Micropterus salmoides*, whilst demonstrating almost complete preference for detritus. Algae and smaller fish such as *Rhabdosargus holubi* showed almost complete preference for detritus; smaller fish and lower trophic level insects show near complete avoidance for algae. Lower trophic level insects had a complete preference for detritus. Based on search rates, the most effort goes into searching for detritus across all predators (**Table 51**).

Table 48. Diet composition of food web groups in F4. Totals in columns add up to 1

	Prey\Predator	2^a	3^a	4^b	5^c	6^a	
1	Producers	0.500	0.333	0	0	0	0.125
2	Algae (epiphyton, epipelon, periphyton, filter-collectors)	--	0.333	0	0.576	0.500	0.125
3	Smaller fish	0	--	0.921	0	0	0.125
4	Larger fish (adult largemouth bass)	0	0	--	0	0	0.125
5	Odonates and Spiders	0	0	0.790	--	0	0.125
6	Lower trophic level insects (shredders and grazers)	0	0	0	0	--	0.125
7	*Potamonautes sidneyi*	0	0	0	0	0	--
8	Terrestrial plants	0	0	0	0.424	0	0.125
9	Detritus	0.500	0.333	0	0	0.500	0.125

a – diet matrix estimated from literature information on diet; numbers were divided equally across different food types
b – diet composition of adult largemouth bass based on gut content analysis
c – diet composition based on SIAR

Table 49. Key Ecopath model outputs for F4

	Biomass accumulation (g/m^2/year)	Biomass accumulation rate (year^{-1})	Net migration (g/m^2/year)	Flow to detritus (g/m^2/year)	Net efficiency	Omnivory index
Producers			This is identified as an area that requires further research. Fish are known to migrate and these can have important implications on the model.	-141.370		
Algae (epiphyton, epipelon, periphyton, filter-collectors)	-263.922	-30.094		60.92	0.194	
Smaller fish	-260.922	-255.806		6.035	2.16	0.222
Larger fish (adult largemouth bass)				62.80	0.0643	0.00429
Odonates and Spiders	-21.232	-3.933		15.17	0.438	0244
Lower trophic level insects (shredders and grazers)	64.58	3.599		111.4	0.175	0.250
Crabs				29.74	0.412	0.689
Terrestrial plants				1426		
Detritus						0.108

Table 50. Electivity as calculated by Ecopath at F4. Electivity describes a predator's preference for prey and ranges from -1 to 1 where -1 indicates complete avoidance of prey and 1 indicates a complete preference (Bakus 2007)

	Prey \ predator	2	3	4	5	6	7
1	Producers	-0.894	-0.894				-0.895
2	Algae		-0.998		0.990	-0.998	-0.998
3	Smaller fish			0.996			-0.984
4	Larger fish (adult largemouth bass)						-1.000
5	Odonates and spiders			-0.770			-0.997
6	Lower trophic level insects						-0.999
7	Crabs						
8	Terrestrial plants				-0.514		-1.00
9	Detritus	0.998	0.998			1.000	0.998

Table 51. Search rates as calculated by Ecopath, describing the amount of volume searched per unit time by a predator seeking a given prey (Christensen and Walters 2004)

	Prey \ predator	2	3	4	5	6	7
1	Producers	115.3	47.82				7.328
2	Algae		0.791		0.506	1.710	0.121
3	Smaller fish			5.618			1.042
4	Larger fish (adult largemouth bass)						0.0238
5	Odonates and spiders			0.0910			0.197
6	Lower trophic level insects						0.0592
7	Crabs						
8	Terrestrial plants				0.0203		0.00660
9	Detritus	16556	6867			14856	1052

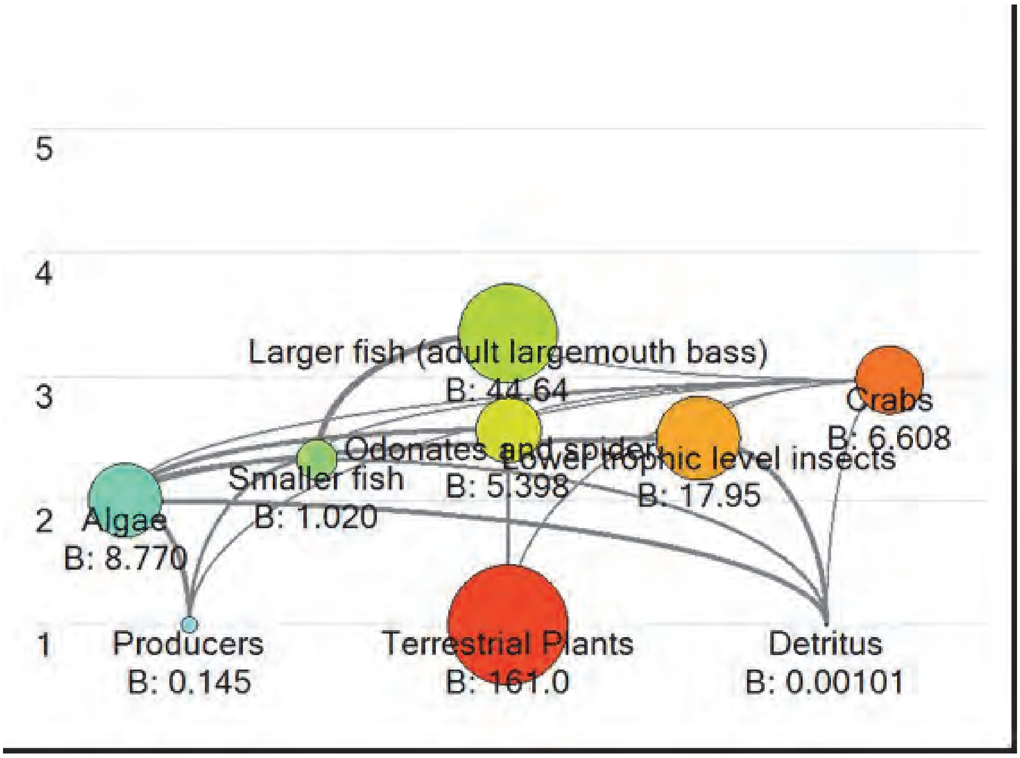

Figure 63. Flow diagram of F4. Numbers under group title indicate biomass in units of g m^{-2}

Fish were not captured at F1, so they are not reflected in the model. The current model for F1 includes trophic groups for phytoplankton, terrestrial plants, algae, crabs, odonates and spiders, lower trophic level insects, and detritus (**Tables 52, 53 and 54**). As per the terrestrial plants in F4, biomass and production per biomass estimates were taken from estimates of seagrasses in the West Florida Shelf. The ecotrophic efficiencies assume that the crabs experience high predation pressure while the terrestrial plants experience low predation pressure. However, our diet matrix does not reflect any predation upon the crab trophic group. Future iterations of the model will require additional data to balance the model.

Electivity values (**Table 55**) crabs *Potamonautes sidenyi* crabs at F1 demonstrated their avoidance of terrestrial plants and algae, whilst demonstrating their almost complete preference for detritus. Algae also shows almost complete preference for detritus; lower trophic level insects showed near complete avoidance for algae. Lower trophic level insects had a complete preference for detritus. Odonates and spiders demonstrated a strong preference for algae. Based on search rates, the most effort goes into searching for detritus across all predators (**Table 56**).

Table 52. Ecopath model basic inputs for F1 in the Kowie River.

Groups	Biomass (g/m^2)	P/B (y^{-1})	Q/B (y^{-1})	Ecotrophic Efficiency
Phytoplankton	0.030[a]	133.3[h]	--	0.636[r]
Algae (epiphyton, epipelon, periphyton, filter-collectors)	5.399[b]	5.200[i]	33.43[n]	0.950[s]
Potamonautes sidneyi (crabs)	5.208[c]	2.800[j]	8.500[o]	0.990[t]
Odonates and spiders	3.872[d]	2.700[k]	7.710[p]	0.530[u]
Lower trophic level insects (shredders and grazers)	2.091[e]	4.200[l]	30.00[q]	0.950[v]
Terrestrial plants	180.662[f]	9.014[m]	--	0.017[w]
Detritus	0.001[g]	--	--	0.058[r]

a – producers biomass calculated based on phytoplankton collections
b – algae biomass calculated based on sum of collections of benthic algae and filter collectors
c – biomass of crabs based on biomass measurements
d – Odonates and spiders biomass estimate taken from spider biomass
e – lower trophic level insects calculated based on sum of biomass of shredders, grazers
f – biomass calculated by Ecopath with other existing data inputs
g – detritus biomass calculated based on drift collections
h – production per biomass estimate taken from the 1990s value in the Shannon et al. paper on the Benguela Current, located at a similar latitude as the study site (2003).
i – production per biomass estimate calculated from the sum of the 1990s values of meiobenthos and macrobenthos in Shannon et al. study on the Benguela Current (2003)
j – production per biomass estimate obtained from Okey et al. value for large crabs in the West Florida shelf (2004)
k – production per biomass estimate of Odonates and spiders obtained from Poepperl's values for Odonates in a northern German stream (2003)
l – lower insects production per biomass estimate calculated from Hossain et al. paper on Lake Toya in Japan (2010).
m – production per biomass estimate of terrestrial plants obtained from Okey et al. value for seagrasses in the West Florida shelf (2004)
n – consumption per biomass of algae obtained from Shannon et al. study on the Benguela Current (2003)
o – consumption per biomass estimate obtained from Okey et al. value for large crabs in the West Florida shelf (2004)
p – consumption per biomass of Odonates and spiders calculated from Poepperl's values for production per biomass and production/consumption ratios of Odonates in a northern German stream (2003)
q – consumption per biomass value calculated from Hossain et al. values of production and production/consumption ratio in Lake Toya (2010).
r – Ecotrophic efficiency value taken from 1990s value for phytoplankton in Shannon et al. (2003)
s – Ecotrophic efficiency value taken from 1990s ecotrophic efficiencies of various sized zooplankton and benthos in Shannon et al. paper on the Benguela Current (2003)
t – Ecotrophic efficiency obtained from Okey et al. value for large crabs in the West Florida shelf (2004)
u – Ecotrophic efficiency obtained from Poepprl et al. (2003)
v – Ecotrophic efficiency obtained from Hossain et al. (2010)
w – Ecotrophic efficiency obtained from Okey et al. value for seagrasses in the West Florida shelf (2004)

Table 53. Diet composition of food web groups in F1. Totals in columns add up to 1

	Prey\Predator	2[a]	3[a]	4[b]	5[c]
1	Phytoplankton	0.500	0.167	0	0
2	Algae (epiphyton, epipelon, periphyton, filter-collectors)	--	0.167	0.32	0.751
3	*Potamonautes sidneyi* (crabs)	0	--	0	0
4	Odonates and spiders	0	0.167	--	0
5	Lower trophic level insects (shredders and grazers)	0	0.167	0	--
6	Terrestrial plants	0	0.167	0.68	0
7	Detritus	0.500	0.167	0	0.245

a – diet matrix estimated from literature information on diet; numbers were divided equally across different food types

b – diet composition of adult largemouth bass based on gut content analysis

c – diet composition based on SIAR results of *Simulium spp*., *Aulonogyrus spp*., Leptophlebiidae, Baetidae, and *Hydropsyche spp*.

Table 54. Key Ecopath model outputs for F1

	Biomass accumulation (g/m^2/year)	Biomass accumulation rate (year^{-1})	Net migration (g/m^2/year)	Flow to detritus (g/m^2/year)	Net efficiency	Omnivory index
Producers	-95.088	-3169.597		1.456		
Algae (epiphyton, epipelon, periphyton, filter-collectors)	-37.339	-6.916		37.51	0.194	
Crab	14.44	2.772		8.999	0.412	0.508
Odonates and Spiders	-1.837	-0.474		10.88	0.438	0.218
Lower trophic level insects (shredders and grazers)	0.965	0.462		12.99	0.175	0.187
Terrestrial plants				1600		
Detritus						0.0698

Table 55. Electivity as calculated by Ecopath at F1. Electivity describes a predator's preference for prey and ranges from -1 to 1 where -1 indicates complete avoidance of prey and 1 indicates a complete preference (Bakus 2007)

	Prey \ predator	2	3	4	5
1	Producers	-0.667	-0.667		
2	Algae		-0.998	0.979	-0.993
3	Crab				
4	Odonates and spiders		-0.997		
5	Lower trophic level insects		-0.994		
6	Terrestrial plants		-1.000	-0.448	
7	Detritus	0.989	0.989		1.000

Table 56. Search rates as calculated by Ecopath at F1, describing the amount of volume searched per unit time by a predator seeking a given prey (Christensen and Walters 2004)

	Prey \ predator	2	3	4	5	6
1	Producers	0.1152238	0.04778925	0	0	0
2	Algae	0	0.782403	0	0.2900157	1.692716
3	Smaller fish	0	0	5.617514	0	0
4	Larger fish (adult largemouth bass)	0	0	0	0	0
5	Odonates and spiders	0	0	0.09103002	0	0
6	Lower trophic level insects	0	0	0	0.2864215	0
7	Detritus	16555.86	6866.568	0	0	14855.71

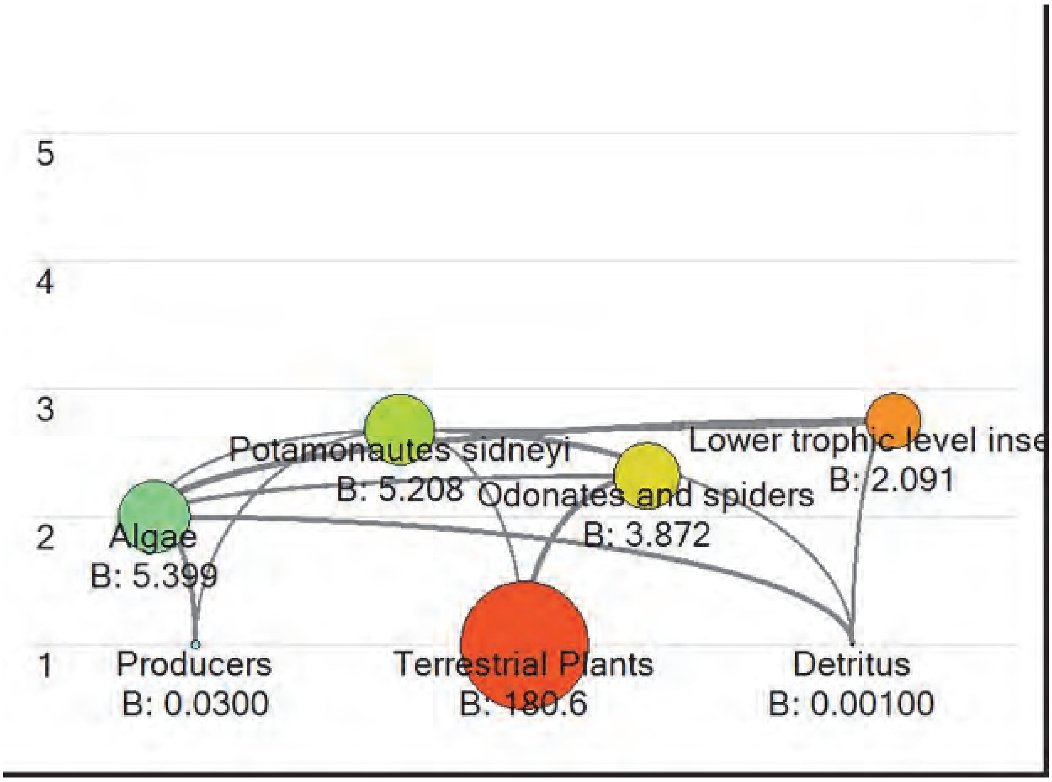

Figure 64. Flow diagram of F1 site. Numbers under group title indicate biomass in units of g/m^2.

8 SUMMARY

It is clear from the flux model results that further model refinements are necessary so that the empirical dietary data of some consumers more closely match what the model is calculating overall for a site. We have seen that sometimes small adjustments to the model parameters can have large influences on the general outcome.

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