Aquatic–terrestrial trophic linkages via riverine invertebrates in a South African catchment

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ABSTRACT

Rivers play a vital role in human livelihoods and are likely to undergo substantial alteration due to climate and land use changes from an increasing human population. Mitigating the pressures facing rivers in the world requires scientists and environmental managers to understand the ecological mechanisms, and ultimately the strength, of connections between ecosystems. This understanding of connections between adjacent habitats will enable environmental managers to predict the consequences of perturbing these linkages in the future. In this thesis, aquatic-terrestrial linkages in rivers were investigated using ecologically meaningful variables including abundances, biomasses, stable isotopes and fatty acids. This study is part of a larger project entitled "Connectivity through allochthony: reciprocal links between adjacent aquatic and terrestrial ecosystems in South Africa", in which a team of researchers assessed a variety of pathways connecting riverine and estuarine systems to land within a catchment in the Eastern Cape, South Africa.

I conceptualised the flow of energy within a temperate southern hemisphere river (the Kowie River) within theoretical models of energy flow such as the River Continuum Concept (RCC; presents lotic systems as being longitudinally linked with food webs in shaded headwaters being principally driven by allochthonous energy, with the addition of autochthonous food as a minor carbon source in the lower reaches) and the Riverine Productivity Model (RPM; proposes consumers derive most of their energy from local production of phytoplankton, benthic algae and aquatic plants, as well as directly from riparian zones via terrestrial leaf litter). Using the RCC as a starting point, I collected macroinvertebrates (September 2012 to May 2013) along a longitudinal gradient and grouped them into functional feeding groups (FFGs). The results revealed that gatherers and filterers dominated in the Kowie River, and together represented 50 – 83% of the invertebrate assemblages. There was a general paucity of shredders (relative abundance was ≤ 10% across all sites and seasons). The changes in relative abundances of different FFGs did not follow predictions of the RCC along the longitudinal gradient, as there were no correlations of community structure with some physical attributes (stream width, canopy cover, distance of river) that changed along the river continuum. However, FFG abundances were related to water velocity, total dissolved solids and canopy cover. Broadly, the Kowie River data showed that changes in relative abundances of FFGs along the river continuum could not be

explained by changes in physical attributes alone, and may be highly influenced by the availability of food and the chemistry of the stream.

Analysis of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes was used to estimate the contributions of algal and land-based production to consumers over space (six sites) and time (November 2012 to September 2013). Carbon contributions determined by the use of mixing models (Stable Isotope Analysis in R) revealed that consumers in the headwater assimilated mainly terrestrially-derived organic matter, with consumers in the middle and lower reaches assimilating autochthonous basal resources (macrophytes and algae). The findings from this river supported aspects of the RCC (at the headwaters; terrestrial organic matter made up 41% of consumer diets), but overall the data supported the predictions of the RPM (local production made the highest contributions of 50 – 86% to all FFGs across all seasons). The carbon isotopes of consumers and their food sources changed substantially every season, indicating that samples of food sources and consumers should be analysed many times throughout the year to capture that variability and to ensure that ephemeral components of the food web are not missed. To validate the findings from the isotope data, fatty acids were used as complementary tracers to determine the contributions of algal versus terrestrial organic matter to the consumers. Fatty acid tracers for terrestrial (Σω3/Σω6; 18:2ω6; 18:3ω3) vs aquatic (Σω3/Σω6; 20:5ω3) sources corroborated the findings from the isotope data set, as the mean ratio of Σω3/Σω6 in consumers was less than one at the headwaters (indicating allochthony), while middle and lower reaches were associated with Σω3/Σω6 > 1 (indicating autochthony).

In addition to the tracer and FFG analyses for examining trophic connections between land and river, the bidirectional exchange of organisms between the riparian zone and the river was assessed using floating pyramidal traps (to measure emergence) and pan traps (for infalling invertebrates) placed at different sites in the river and the biomass in each trap was determined. The exchanges were variable over space and time, with emergence peaking in summer (169 to 1402 mg m^{-2} day^{-1}) and declining in winter (3 to 28 mg m^{-2} day^{-1}). Similarly, infalling invertebrates increased in summer (413 to 679 mg m^{-2} day^{-1}) and declined in winter (11 to 220 mg m^{-2} day^{-1}). Biomass measurements are indications of quantity that ignore nutritional quality, so I determined the bidirectional flow of invertebrates using absolute concentrations of physiologically important biochemical compounds (essential and polyunsaturated fatty acids). The fluxes of emergent and infalling arthropods peaked in summer (emergence = 0.3 to 18 mg m^{-2} day^{-1} and terrestrial infall = 0.3 to 3 mg m^{-2} day^{-1})

and declined in winter (emergence = 0.01 to 0.51 mg m^{-2} day^{-1} and terrestrial infall = 0.01 to 0.03 mg m^{-2} day^{-1}). However, during some seasons, no significant differences in polyunsaturated fatty acid flux in either direction were observed; this finding indicated the balance of reciprocal subsidisation via reciprocal flows of animals. Factors such as air temperature and algal productivity affected the reciprocal flows between adjacent habitats, with algal productivity being positively related to emergence while air temperature was positively correlated to infalling terrestrial invertebrates.

This research enhances the growing body of literature on the function of riverine systems and offers some invaluable information on the flow of energy and the role played by invertebrates in translocating nutrients from terrestrial systems to aquatic systems and vice versa. This study unifies the concepts of the RCC and RPM and shows that these concepts are not limited only to large rivers, but are applicable to small southern temperate rivers too. However, some tenets of the theoretical models were challenged. For example, it challenges the proposition by the RCC that the fine particulate organic matter leaked from upstream breakdown of coarse particulate organic matter is predominantly allochthonous. Additionally, this study showed that in the headwaters, the RPM underestimated the role of autochthony. Overall, the results showed that the Kowie River and its riparian area are intrinsically connected. Once we understand the mechanisms controlling connections and subsidies across ecotones, we can then start to predict the consequences of disruptions to these connections by climate change and/or land use changes. To make predictions about future perturbations to rivers and riparian zones, studies like this, which considers the form and magnitude of subsidies, are needed to provide baseline information. Algal resources (e.g. epiphyton), macrophytes, riparian plants, terrestrial organisms and aquatic organisms all contributed to aquatic and terrestrial linkages in the Kowie River; therefore, it is important to conserve the different components of these ecosystems.

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This project is dedicated to the memory of my mother Ms L. Ndlovu …….How I wish you were here.

DECLARATION

I declare that this thesis submitted for a degree at Rhodes University (Grahamstown, South Africa) has not been submitted to any other university. The work presented here is that of the author unless otherwise stated.

Sydney Moyo
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1 GENERAL INTRODUCTION

The *National Water Act* (1998) of South Africa highlights that water must be managed in a way that guarantees availability and accessibility of water for the general population. The act further provides that water should be allocated to meet the increased demand from agriculture, industry and other productive sectors, and must also satisfy the requirements of the aquatic ecosystems themselves (environmental sustainability). Several countries in southern Africa are presently classified as "water-scarce", and it is predicted that by 2025 a number of them will be "water-stressed", where less than 1500 cubic metres of water will be available per person annually (Abrams 2003, Fischlin et al. 2007, Malzbender and Earle 2007). With these projections, it is evident that water will continue to be a limited resource in the future. The increase in the demand for water resources is attributable to escalating human populations, industrialisation and greater demands for irrigation water (Fischlin et al. 2007). With the escalating demand for water resources, one of the most important ecological issues facing conservationists is to resolve how anthropogenic disturbances to river systems, such as the building of dams and eutrophication, will affect the transport of matter, energy and organisms within or between habitats (i.e. connectivity) (Power et al. 1996, Ward 1998, Pringle 2003, Freeman et al. 2007).

Connectivity in lotic systems can typically occur laterally (e.g. from stream to riparian area), longitudinally (e.g. head water to estuary) or vertically (e.g. between pelagic and benthic environments) (Wiens 2002). Lateral connectivity can be mediated by organisms such as aquatic insects that may emerge from rivers and reach riparian zones, thus constituting a subsidy for terrestrial consumers (Ward 1989). Longitudinal connectivity generally occurs from upstream to downstream and includes migrations of aquatic species and movements of organic and inorganic materials down the river (Ward 1989, 1998, Wiens 2002). Vertical connectivity concerns exchanges between river and ground water (Ward and Stanford 1995) and subsurface differentiation of habitats (such as surface or pelagic vs benthic environments; Allan and Castillo 2007). Research on food webs helps to answer questions about connectivity via organic nutrients between rivers and terrestrial habitats. Food web studies provide basic information on ecosystem structure and feeding relationships, and enable the prediction of the consequences of disruptions to habitat connections (Mills et al. 1993).

1.1 Macroinvertebrates in freshwater systems

Macroinvertebrates are ubiquitous in freshwater streams and live on and/or in rocks, logs, sediments, debris and aquatic plants (Cummins 1975). The macroinvertebrate community includes crustaceans (e.g. zooplankton, shrimps, isopods, amphipods and crabs), molluscs (clams and snails) and the naiads of aquatic insects (e.g. dragonflies) (Table 1.1). Invertebrates perform a variety of functions in freshwater food webs. First, they provide critical ecosystem services by accelerating leaf litter breakdown (Dudgeon and Gao 2011), as they can process 20 - 73% of riparian leaf litter inputs to headwater streams (Covich et al. 1999). Moreover, invertebrates release bound nutrients into solution through their feeding, excretion, and burrowing activities. These liberated nutrients are available for uptake by bacteria, fungi and algae (Wallace et al. 1997). Some aquatic invertebrates are predators (e.g. giant water bugs) that play critical roles in regulating populations of their prey (Thorp and Rogers 2014).

Table 1.1: A list of some of the invertebrate fauna found in freshwater systems. Bold text shows orders of insects.

Class	Common name	Family name/ Order
Malacostraca	freshwater shrimp	Atyidae
Malacostraca	crabs	Potamonautidae
Insecta	giant water bugs	Belostomatidae
Insecta	water boatmen	Corixidae
Insecta	predacious diving beetle	Dytiscidae
Insecta	pond skater	Gerridae
Insecta	marsh treaders	Hydrometridae
Insecta	back swimmers	Notonectidae
Insecta	mayflies	**Ephemeroptera**
Insecta	caddisflies	**Trichoptera**
Insecta	dragonflies	**Odonata**
Insecta	mosquitos	Culicidae
Insecta	midges	Chironomidae
Gastropoda	orb snails	Planorbidae

Apart from their ecological roles, macroinvertebrate assemblages in rivers reflect the overall biological integrity of a benthic community, and monitoring these assemblages is useful in assessing the environmental health of a water body (Gratwicke 1998, Dickens and Graham 2002). Macroinvertebrate communities respond differently to a wide array of stressors, hence it is often possible to determine the type of stress that has affected a riverine system (Bonada et al. 2006). Because many macroinvertebrates have relatively long life cycles of a year or more and are relatively immobile, macroinvertebrate community structure

is a function of past environmental conditions and can be successfully applied in biomonitoring (Dallas 2000, Dickens and Graham 2002).

1.2 Food sources in streams

Some food webs in streams and rivers can be contextualised within the River Continuum Concept (RCC; Vannote et al. 1980). The RCC places food sources into two categories: (i) allochthonous material produced from outside the system, e.g. terrestrial plants, and (ii) autochthonous material produced from within the system, e.g. benthic algae. According to the RCC, contributions from allochthonous and autochthonous materials to, and the consequent structure of, an aquatic invertebrate community vary along the length of a river or stream in accordance with changes in the physical and chemical factors of the environment (Vannote et al. 1980; Fig. 1.1a). As such, any study on lotic systems must address these environmental gradients.

The RCC postulates that headwaters of streams are typically heavily shaded, shallow, and cooler than downstream reaches. The riparian vegetation in headwater regions limits primary production and increases contributions of allochthonous coarse particulate organic matter (CPOM, organic matter > 1 mm), subsequently increasing the reliance of macroinvertebrate consumers on terrestrial detritus (Vannote et al. 1980). Consumers in streams that feed exclusively on CPOM consisting of mainly decaying leaf litter (Cummins 1973, Allan 1995) represent a functional feeding group (FFGs) called shredders (e.g. caddisflies) (Bland 1978, Vannote et al. 1980). The shredders (also known as detritivores) break down CPOM and make it available as fine particulate organic matter (FPOM, organic matter < 1 mm) to the collectors (e.g. dipterans such as blackflies) (Ciborowski et al. 1997). Predators such as dragonflies and damselflies (Odonata) feed on other invertebrates (Cummins 1973), and fish are usually the top predators in lotic systems (Cummins 1973, Vannote et al. 1980, Allan 1995).

The RCC hypothesises that shredders and collectors co-dominate in headwaters, whereas collectors are dominant in downstream regions. Essentially, the feeding activities of shredders breaking CPOM down to FPOM in upstream areas increase the transport of FPOM to the downstream areas, where collectors can dominate because of increased food availability. The RCC further predicts that the relative abundance of predatory invertebrates (e.g. dragonflies) changes very little along a river gradient, possibly because of ample prey availability along the entire longitudinal gradient (Vannote et al. 1980).

Studies on temperate lotic food webs generally support the RCC's predictions (Rounick et al. 1982, Naiman et al. 1987, Hicks 1997, Greathouse and Pringle 2006, Groff

2006, Jiang et al. 2011). For example, Rosi-Marshall and Wallace (2002) recorded the reliance of macroinvertebrate shredders on leaf detritus in the headwaters of a Tennessee river, while macroinvertebrates relied primarily on algae in the mid-reaches and FPOM in the downstream regions. Similarly, in an Appalachian stream, shredder populations were largest in the headwaters and declined downstream, whereas the relative abundance of scrapers and collectors increased in downstream regions (Grubaugh et al. 1997). Despite an abundance of published research that supports the RCC, some research has revealed that the RCC may overemphasise the role of allochthonous food sources and may not fully reflect the relative importance of autochthonous resources such as periphyton and green algae, which are important to consumers in some temperate streams (Bunn et al. 2003, Torres-Ruiz et al. 2007).

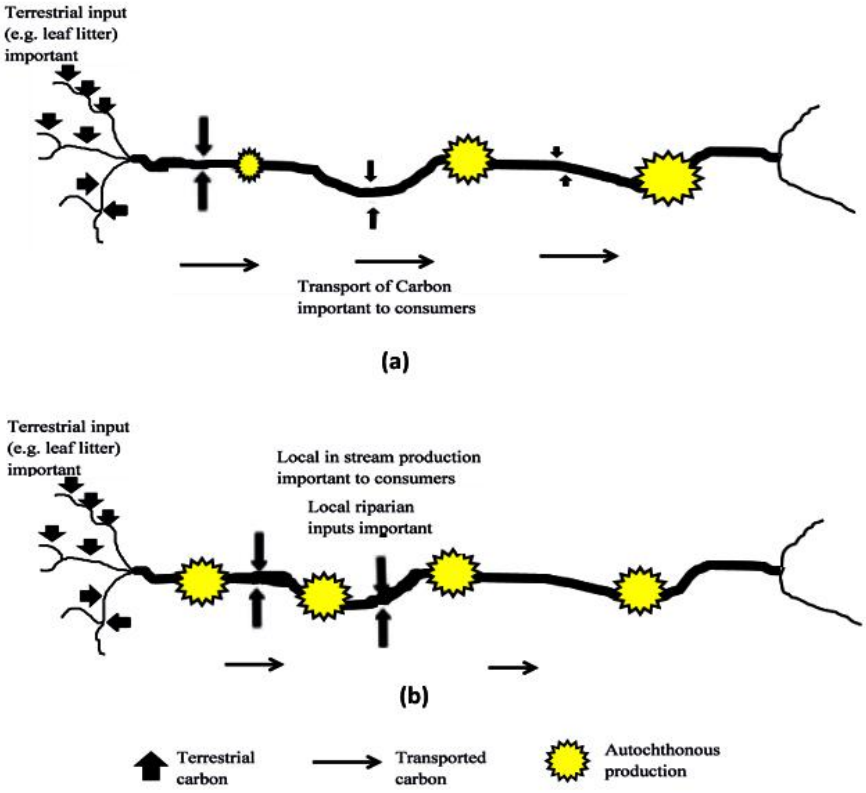

Figure 1.1: Two conceptual models for the role of autochthonous and allochthonous food sources across a river continuum (redrawn from Bunn and Davies 2007) (a) River Continuum Concept (RCC; Vannote et al. 1980) (b) Riverine Productivity Model (RPM; Thorp and Delong 2002).

An alternative to the RCC, the Riverine Productivity Model (RPM; Thorp and Delong 1994), emphasises the importance of local autochthonous production (phytoplankton, benthic algae and aquatic plants) in addition to direct carbon inputs from adjacent riparian land (Fig. 1.1b). The RPM emphasises the role of carbon from locally generated autochthonous algae

that are easier for organisms to assimilate, as opposed to processed organic matter transported from upstream (as suggested by the RCC; Vannote et al. 1980). The CPOM from upstream is often considered to be of low nutritional value in the RPM (Allan and Castillo 2007).

Evidence is accumulating in the literature that the role of autochthony has been generally underestimated in rivers (e.g. Minshall 1978, Minshall et al. 1983, McCutchan et al. 2003, McNeely et al. 2007). Non-shredding primary consumers examined in five shaded streams in Hong Kong derived approximately 61% of their biomass from autochthonous food sources, while in unshaded streams in the same region, macroinvertebrates derived up to 71% of their biomass from autochthonous sources (Li and Dudgeon 2008). Autochthonous sources are important to aquatic consumers in various tropical (March and Pringle 2003) and subtropical streams (Bunn et al. 2003) in addition to temperate lotic systems (Delong and Thorp 2006, Torres-Ruiz et al. 2007). Studies on lakes have also generated considerable debate on the role of allochthonous and autochthonous food to consumers (Jones et al. 1998, Cole et al. 2006, Brett et al. 2009, Galloway et al. 2014b). For example, Cole et al. (2006) estimated the terrestrial input to multiple trophic levels in four Indiana lakes and found that carbon flow to benthic invertebrates (33-70%) and fish (20-50%) was derived from terrestrial inputs. Contrary to the findings by Cole et al. (2006), Brett et al. (2006) and Galloway et al. (2014b) argued that autochthony (phytoplankton) has been underestimated (Table 1.2) and may contribute up to 94% to consumers' diets.

The flood pulse concept (FPC; Pingram et al. 2012), a third conceptual model, suggests that predictable seasonal flood pulses (especially in the tropics) amass organic matter from the riparian zones; this process results in stream consumers deriving most of their energy from terrestrial sources because of the floods (Pingram et al. 2012). The validity or applicability of the flood pulse concept may not be readily testable in South African temperate streams as flooding is stochastic and unpredictable in much of southern Africa.

Aquatic macrophytes are an additional potential food source for primary consumers, but they are rarely eaten fresh by aquatic herbivores due to their low digestibility and high levels of cellulose and lignin (Allan 1995, Allan and Castillo 2007). Macrophytes are considered relatively unimportant to food webs until they decay and enter the detrital pool (Allan 1995). However, some researchers have argued that direct consumption is more common and more important to ecosystem function than previously thought (Lodge 1991, Newman 1991). However, studies using stable isotope analysis have provided little evidence of any significant contributions from aquatic macrophyte carbon to aquatic consumers, either through direct herbivory or through detrital pathways (Hamilton et al. 1992, France 1996, Lewis et al. 2001, Delong and Thorp 2006).

There is some debate on whether macrophytes should be considered autochthonous or allochthonous materials in freshwater systems. Autochthonous production, as defined by Wetzel (1975) and McWilliam-Hughes et al. (2009), includes any producer indigenous to the stream. As such, filamentous algae, suspended algae, epiphyton, submerged macrophytes and emergent macrophytes were all considered autochthonous materials in this thesis.

Table 1.2: Potential autochthonous and allochthonous resources for food webs in various aquatic ecosystems and their potential contribution to consumers. "?" denotes systems in which contribution of organic matter sources was not reported.

Study area	Dominant carbon Source	Average contribution to diets	Publication
Tropical stream	Allochthonous - leaf litter	55-100%	Leigh et al. (2010)
Forested, tropical and piedmont streams	Allochthonous - terrestrial invertebrates	70 - 80% of fish diets	Cloe III and Garman (1996), Kawaguchi et al. (2003), Saunders and Fausch (2007)
Tropical stream	Allochthonous - CPOM and FPOM	55 - 78%	Li and Dudgeon (2008)
Austral temperate river	Autochthonous – benthic algae	50 - 90%	Pingram et al. (2014))
Temperate large rivers	Autochthonous - Autochthonous transported algal matter	48 - 82%	Delong and Thorp (2006)
Lakes (small, deep, oligotrophic)	Allochthonous - terrestrial particulate organic carbon (t-POC)	20 - 70%	Grey et al. (2001), Karlsson et al. (2003), Cole et al. (2011)
Boreal Lakes	Autochthonous – phytoplankton	86 - 94%	Galloway et al. (2014)

The energetic value of terrestrial invertebrates to stream consumers via fortuitous "infall" has been recognized (Cloe III and Garman 1996, Wipfli 1997, Kawaguchi et al. 2003), with terrestrial insects contributing 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 infall 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), values that almost match with those of aquatic insect emergence (Baxter et al. 2005). In a 14-month study on two adjacent Californian streams, Rundio and Lindley (2012) found that the annual flux of terrestrial invertebrates to both streams was between 7.9 and 8.6 g m^{-2} $year^{-1}$. Apart from the studies by Kawaguchi and Nakano (2001) and Rundio and Lindley (2012), research incorporating concomitant measures of reciprocal stream riparian invertebrate fluxes annually is rare, and there is little understanding of whether patterns differ among aquatic systems in various parts of the world.

Terrestrial invertebrate input to temperate aquatic habitats varies over time, typically peaking in the summer and approaching zero in the winter (Kawaguchi and Nakano 2001). In Japan, Kawaguchi and Nakano (2001) observed summer peaks in terrestrial invertebrate input corresponded with declines in aquatic prey, indicating that there was a seasonal asynchrony where one habitat contributed more organic nutrition when prey from the connected habitat was scarce. Fish diets reflected the observed increase in summer terrestrial invertebrate input, with 50 - 90% of fish diets derived from terrestrial invertebrates during summer and 0% during the winter (Kawaguchi and Nakano 2001). Terrestrial arthropods, a high quality food for stream fishes, are often preferred resources in forested headwater streams (Nakano et al. 1999a, 1999b, Kawaguchi and Nakano 2001, Saunders and Fausch 2007). When terrestrial invertebrate input to a Japanese stream was reduced using a greenhouse cover, fish biomass declined, suggesting that the distribution of fish was directly influenced by terrestrial inputs. Moreover, reduced terrestrial subsidies may result in trophic cascades, forcing fish to shift from terrestrially-derived subsidies to local aquatic prey, which may decrease aquatic insect emergence and have detrimental effects on riparian predators such as spiders (Baxter et al. 2005). Thus, seasonal variations in terrestrial invertebrate inputs to streams may be an important driver of ecosystem processes in temperate regions.

Aquatic consumers (predacious insects like giant water bugs) other than fish may also prey on terrestrial invertebrates (Ohba and Nakasuji 2006), but the extent to which predacious invertebrates consume terrestrially-derived prey is unknown and the body of literature on this subject is scanty (Baxter et al. 2005).

1.3 Habitat coupling: lateral connectivity

A great deal of food web connectivity research has focused on the unidirectional net transfer of material such as higher plant organic carbon from terrestrial to aquatic habitats, and its effect on ecosystem processes and food web interactions in streams (Schindler and Scheuerell 2002, Moore et al. 2004). This unidirectional view arises from the general assumption that energy is dominated by transfers from land to water (Schindler and

Scheuerell 2002). However, emerging insects can represent a significant transfer of organic matter in the other direction (water to land) and constitute an important dietary item for a wide range of predators (Baxter et al. 2005). Aquatic insects have complex life histories, and the larval stages typically occur in water. After the larval stage, the insects undergo metamorphosis and emerge to terrestrial habitats as volant adults (Bland 1978). The emergence of insects represents the final component of insect production, a measure of the amassed growth, natural mortality and predation during the life cycle, and a potential net export of insect material from an aquatic system (Davies 1984). Several researchers have documented the timing and rate of emergence of adult aquatic insects (Poepperl 2000; Table 1.3) and the flux of emerging insects can be large, averaging ~4.3 g dry weight of aquatic insects m^{-2} $year^{-1}$ globally (Gladyshev et al. 2009).

Table 1.3: Emergence rates for adult aquatic insect taxa in diverse ecosystems.

Taxa	Ecosystem, Region	Location	Stream Temp (^oC)	Emergence ($g\ m^{-2}year^{-1}$)	Reference
Ephemeroptera	Pick creek, South west Alaska	59°20′N,158°40′W	7.5 - 11.2	0.2478	Francis et al. (2006)
	Lake Belau, Germany	54°N, 10°E	6 - 17	0.0928	Poepperl (2000)
Plecoptera	Broadstone stream England		5 - 17	0.00399	Petersen et al. (1999)
	Pick creek, South west Alaska	59°20′N,158°40′W	7.5 - 11.2	0.0648	Francis et al. (2006)
Trichoptera	Pick creek, South west Alaska	59°20′N,158°40′W	7.5 - 11.2	0.0147	Francis et al. (2006)
	Two Rivers in the Philippines islands	$10^o09'$ N,118^o 49′ E	23.6 - 30.4	0.031[a]	Freitag (2004)
Diptera	Open water, of Wetland, Alabama	$32^o52'$ N, $87^o26'$W	7 - 29	0.46	Stagliano et al. (1998)
Chironomidae	Sycamore creek, Arizona	33°N, 111°W	9 - 25	13.79	Jackson and Fisher (1986)
Odonata	Peshtigo River, Lake Michigan	$44^o59'$N $87^o39'$E	18 - 20	2.44	MacKenzie and Kaster (2004)
	Subtropical blackwater river	32°09′ N, 81°25′ W	10 - 30	1.801[b]	Benke et al. (2001)

a enumerated using mean Trichopteran dry mass of 0.0041 mg (Stagliano et al. 1998).
b emergence average for two years.

Owing to the flux of energy during emergence, adult insects can act as a conduit of energy flow between aquatic and terrestrial habitats (Richardson et al. 2010). Emerging aquatic insects constitute an important dietary item for a host of consumers such as birds (Gray 1993, Kawaguchi and Nakano 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, Kato et al. 2003, Burdon and Harding 2008). For example, Paetzold et al. (2005) reported that emerging aquatic insects along braided rivers in Switzerland and Italy made up 80% of the diet of a rove beetle, and approximately 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 also increased the abundance of insectivorous birds in the riparian zones, with aquatic insects constituting 67 – 82% of bird diets. Another study on the distribution of spiders in a Japanese stream showed a positive correlation between aquatic insect emergence and the density of tetragnathid spiders (Iwata 2007). These studies clearly showed 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 a very small percentage of emergent aquatic insects return to the stream. Jackson and Fisher (1986) enumerated the return of adult aquatic insects to Sycamore Creek after their emergence and found that only 3.1% of the emerged biomass returned to the stream. Elsewhere, Gray (1989) found that less than 1% of aquatic insects in a prairie stream returned to the aquatic system, whereas other researchers documented larger (9.2% and 25%, respectively) returns by biomass in lake and wetland systems in North America (Stagliano et al. 1998, Francis et al. 2006). Adults that fail to return to the water represent a net loss of organic nutrients from the aquatic system and potential food for consumers in adjacent terrestrial ecosystems. Gratton et al. (2008) quantified aquatic insect (midge) export to terrestrial habitats adjacent to Icelandic lakes, and the values ranged from 1200 to 1500 kg midges ha^{-1} $year^{-1}$. Rates of midge infall to the terrestrial ecosystem varied among the lakes, declined with increased distance from the lakeshore, and near some lakes were large enough to have a fertilizing effect on terrestrial flora. Carbon isotope ratios revealed that many terrestrial consumers located near lakes with large midge populations were highly reliant upon midge-derived trophic subsidies (Gratton et al. 2008, Hoekman et al. 2012). A fraction of emergent insects ultimately return to the aquatic system in the form of insect carcasses and either sink, decompose, or become consumed. Many of the carcasses eventually become fodder for detritivores such as Collembola (Gratton et al. 2008).

Interface specialists, namely semi-aquatic insects, can mediate the flux of material from riverine to terrestrial landscapes (Ballinger and Lake 2006). A number of semi-aquatic

insects such as water striders (Gerridae) and water measurers (Hydrometridae) can translocate onto the terrestrial landscape, a feature that couples aquatic and terrestrial habitats. Because a number of the semi-aquatic insects are predacious (e.g. Hydrometridae) or feed on algae via the consumption of aquatic insects (e.g. water striders; Jardine et al. 2008), they may constitute a major pathway in the transport of nutrients to terrestrial consumers. Semi-aquatic insects may be consumed by terrestrial predators (or die while on the land), thereby adding nutrients to terrestrial systems. Terrestrial detritivores such as pygmy grasshoppers (Tetrigidae) can form an additional pathway for the transfer of energy by grazing river algae stranded by shorelines (Bastow et al. 2002).

1.4 Food web analysis

Polis and Strong (1996) suggested two potential scenarios of trophic forcing involving allochthony: bottom-up forces dominate when lower trophic levels receive allochthonous subsidies, whereas top-down forces dominate when upper trophic levels are recipients of allochthonous subsidies. Several studies have demonstrated that bottom-up effects result from the input of allochthonous resources (Rosemond et al. 2001). For instance, some lotic ecosystems are maintained by inputs of terrestrial organic matter such as leaf litter, which when excluded from entering the lotic systems can have a strong bottom-up effect that is propagated from detritivores through to predators and results in declines in biomass of several taxa (Wallace et al. 1997). Top down controls are created when abundance and distribution of predators increase. For instance, increases in *Daphnia* spp. can cause sharp declines in the biomass of phytoplankton (McQueen et al. 1986). Additionally, subsidies via emerging insects affect the abundance, fitness and growth of lizards (*Sceloporus occidentalis*) and ultimately may result in lizards having a large top down effect on ground dwelling arthropods (Sabo and Power 2002a).

Gut contents analysis can provide the first step for studying such trophic relationships (Taylor 1989, Hering and Plachter 1997, Hering 1998). Unfortunately, this method is biased towards recently ingested material and is contingent upon researchers having good taxonomic knowledge of ingested biota (Créach et al. 1997). Immunoassays of gut contents (such as precipitin tests) are an alternative way to resolve food web questions (Feller 1984, Feller et al. 1985, Venter et al. 1999, Nejstgaard et al. 2003). They involve developing antisera by injecting target prey proteins into a mammal followed by immune-diffusion tests of antiserum specificity. The antigens produced are usually taxon specific and help to identify partially digested stomach contents (Grisley and Boyle 1985). Unfortunately, in complex food webs where multiple monoclonal antibodies are required, the high expense and time-consuming

nature of their development make this method problematic (Chen et al. 2000). Other macromolecular techniques that show considerable promise in studying food web dynamics involve DNA (Agustí et al. 2000, Nejstgaard et al. 2003, Carreon-Martinez and Heath 2010), and these methods are predicated on the differentiation between unique pieces of DNA sequences from predator and prey species (Sheppard and Harwood 2005). Once sequences have been obtained and prey-specific primers have been designed and published, they can be used immediately by any scientist with the appropriate equipment (Symondson 2002). A drawback with DNA based markers is that development of primers is difficult and time-consuming, and specialized equipment and expertise are needed to perform polymerase chain reaction analyses (Feller et al. 1985, Symondson 2002). The shortcomings of the traditional methods (gut contents) and DNA techniques in studying food webs have led ecologists to turn to techniques that make use of naturally occurring tracers, such as the determination of fatty acid profiles and stable isotope signatures.

1.4.1 Stable isotope analysis

Stable isotope ratios of consumers reflect those of the foods they have assimilated (Fry 2006). Stable isotope analyses of carbon (^{13}C:^{12}C), nitrogen (^{15}N:^{14}N), sulphur (^{34}S:^{32}S), hydrogen (^{2}H:^{1}H), and oxygen (^{18}O:^{16}O) represent useful tools for characterising feeding habits of stream macroinvertebrates and inferring the relative importance of terrestrial versus aquatic sources (Peterson and Fry 1987, Michener and Kaufman 2007). Isotopes of carbon and nitrogen are the elements most often used in these types of analyses. Carbon and nitrogen each have more than one naturally occurring isotope (^{13}C/ ^{12}C and ^{15}N/^{14}N). Changes in the ratio of the heavy to light isotope (fractionation) between biological and the non-biological sources of these elements result in different isotopic compositions of autotrophs utilising different photosynthetic pathways (Peterson and Fry 1987, Fry 2006). Because the ^{13}C/^{12}C ratio ($\delta^{13}C$, when compared to a universal standard) changes little (around 1‰) with increasing trophic level relative to differences among autotrophs (Peterson and Fry 1987), stable carbon isotope analysis is useful to indicate which autotrophs are the most important sources of food for animals. In contrast, consumers tend to excrete ^{14}N preferentially over ^{15}N, which results in an enrichment of ^{15}N by 3 to 4‰ with each trophic level so that one can determine the number of trophic levels in a food web (Fry 1991). However, reviews suggested that ^{15}N enrichment is more variable than the previously envisaged range of 3 to 4‰ (Vanderklift and Ponsard 2003, McCutchan et al. 2003). The minor, yet sometimes predictable, isotopic differences between animals and their diet have allowed researchers to answer ecological questions about stream food webs.

One natural abundance stable isotope study by Delong and Thorp (2006) documented the significance of instream autotrophs in a braided river (Mississippi River). The use of a six-source mixing model using $\delta^{13}C$ and $\delta^{15}N$ indicated that autochthonous material was the major resource consumed by primary consumers. Results from the same study demonstrated that secondary consumers and higher trophic levels ultimately derived their energy from instream autotrophs (Delong and Thorp 2006). A $\delta^{13}C$ and $\delta^{15}N$ study by Zah et al. (2001) showed that aquatic insects of a Swiss glacial stream derived most of their food from autochthonous sources [filamentous gold algae (*Hydrurus foetidus*) and epilithic diatoms], an unexpected result based on previous findings (Hicks 1997). As such, stable isotopes have been used quite successfully to elucidate insect diets, which in turn can allow researchers to assess: (i) whether insect diets conform to the predictions of the RCC or other conceptual models, (ii) isotope fractionation for lotic invertebrates and, (iii) whether distinct FFGs fractionate diets differently (Jardine et al. 2005). For example, stable isotope data ($\delta^{15}N$) have been used to establish the trophic positions for predatory and non-predatory species of dragonflies, mayflies, stone flies and caddisflies in the Saint Lawrence River, North America (Anderson and Cabana 2007).

Although stable isotope analysis is an invaluable tool for delineating food web structure, it can lead to ambiguous conclusions in complex systems because the $\delta^{13}C$ values of food sources (e.g. terrestrial detritus and epilithic algae) frequently overlap and are highly variable through space and time (Finlay 2001). As a result, the importance of different food items to a consumer may be difficult to estimate. Moreover, the use of stable isotopes is complicated by poor understanding of the effects of eco-physiological factors, namely isotope fractionation and routing processes, on the isotopic signatures in consumers (Martínez del Rio et al. 2009). For these reasons, it is increasingly important for researchers to combine two or more methods to study food webs.

1.4.2 Fatty acid analysis

Fatty acid analysis represents an additional and complementary tool for assessing food web interactions. The fatty acid approach is predicated on the observation that primary producers synthesise certain fatty acids, especially polyunsaturated fatty acids (PUFAs; having more than one double bond), that may be transferred predictably to primary consumers and higher consumers (Dalsgaard et al. 2003, Budge et al. 2006). Along with the use of certain PUFAs as potential tracers in food web studies, several PUFAs [namely arachidonic acid (ARA;20:4ω6), docosahexaenoic acid (DHA; 22:6ω3) and eicosapentaenoic acid (EPA; 20:5ω3)] are of fundamental physiological importance to all organisms (Brett and

Müller-Navarra 1997, Müller-Navarra 2000). The highly required PUFAs are designated as essential fatty acids (EFAs) because most consumers do not possess the necessary enzymes to synthesize them in the required quantities, so they must obtain them from their diet. Essential fatty acids are required for maintaining cell membrane structure and function (Brett and Müller-Navarra 1997), regulating hormonal processes and preventing cardiovascular diseases (Arts et al. 2001). Primary producers in aquatic ecosystems (e.g. phytoplankton) are the primary sources of ω3 highly unsaturated fatty acids (HUFAs; EPA and DHA) for all aquatic and terrestrial animals (Hanson et al. 1985, Brett and Müller-Navarra 1997, Gladyshev et al. 2009). In their seminal work in various streams of the United States, Hanson et al. (1985) compared the lipid contents among various insects. They concluded that aquatic insects were higher in PUFAs (particularly ARA and EPA) than terrestrial insects (Hanson et al. 1985). Other studies on lipids in aquatic systems generally supported the premise that aquatic insects are richer in HUFAs (Bell et al. 1994, Ghioni et al. 1996, Torres-Ruiz et al. 2007). Aquatic insects lay their eggs in rivers, where the larvae then develop and accumulate HUFAs (Gladyshev et al. 2009), and because of their complex life cycles, aquatic insects can effect transfer of HUFAs to the terrestrial system when they emerge and fall prey to terrestrial predators (Burdon and Harding 2008). Preliminary estimates of HUFA export to the terrestrial landscape via insect emergence revealed values as low as 0.1kg km^{-2} $year^{-1}$ to as high as 672.2 kg km^{-2} $year^{-1}$ (Gladyshev et al. 2009). However, estimates of PUFA flux to terrestrial habitats through the emergence of insects have been based on the averaging of diverse data obtained from entirely different ecosystems (Gladyshev et al. 2009). Moreover, concentrations of the PUFAs in insect biomass are known only for larvae, whereas data for adult insects 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. 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.

Fatty acid analysis, like stable isotopes, has proven to be particularly useful in dietary studies. In a Swedish lake, Goedkoop et al. (2000) used fatty acid analysis to determine the food sources of profundal invertebrates and found that chironomid larvae tissues contained 16:1ω7 and EPA, implying that diatomaceous sediment constituted a dominant food source assimilated by consumers in this lake system. The fatty acid approach was particularly useful in that study because fatty acid concentrations could be used to assess not only the food choice of the invertebrates, but also how the food sources varied over time (Goedkoop et al. 2000). In addition, distinctions in fatty acid profiles in sediments at different locations

corresponded to shifts among chironomid larvae at those locations, and indicated changing contributions of autotrophic (phytoplankton) and heterotrophic (terrestrial detrital) sources to various chironomid species (Goedkoop et al. 2000). Similarly, Ephemeropteran larvae tissue can contain high abundances of 14:0, 16:1ω7, 20:5ω3 and 18:3ω3, indicating that mayflies rely on algal resources in freshwater streams (Torres-Ruiz et al. 2007). The fatty acid approach thus enables one to assess the seasonal importance of autochthonous and allochthonous food sources to macroinvertebrates (Torres-Ruiz et al. 2007). In their study of a New York stream, Torres-Ruiz et al. (2007) used fatty acid analysis to determine the primary diets of mayflies (*Ephemerella* spp.) and caddis larvae (*Hydropsyche* spp.). They noted that fatty acid concentrations in the insects varied seasonally, indicating that the food sources varied over time (Torres-Ruiz et al. 2007). Their study emphasised the need for trophic researchers to increase the temporal scale of sample collections. Limiting studies to particular parts of a year could be a major barrier to understanding stream food webs because temperate streams are dynamic systems in which trophic interactions and energy flow are greatly affected by seasonal changes in potential food sources (Torres-Ruiz et al. 2007).

Although fatty acid analysis is a powerful method for resolving food web questions, it does have inherent limitations. First, no single fatty acid can be assigned uniquely to any one species, and not even to any group of phytoplankton or bacteria (Dalsgaard et al. 2003). Second, the preferential retention and modification of fatty acids make it difficult to match the fatty acid profiles in a consumer with profiles of its diet (Galloway et al. 2014a). Additionally, temporally changing factors (e.g. light and temperature), metabolic conditions or reproductive states (De Lange and Van den Brink 2006) affect fatty acid concentrations in organisms. As such, lipid signatures usually do not have the precision to identify species-specific interactions (De Lange and Van den Brink 2006), but they can provide trophic information on the level of larger taxonomic groups.

1.4.3 Combined approach

The inherent limitations of stable isotope and fatty acid analyses justify the concurrent use of two or more techniques (Post 2002, Ballinger and Lake 2006). While each approach has specific applications, strengths and weaknesses, a dual tracer approach is an innovative and promising way of overcoming the limitations of individual methods (Pitt et al. 2009). A combination of the two methods has proven helpful in resolving several food web questions (Kharlamenko et al. 2001, 2008, Lebreton et al. 2011, Lau et al. 2014, Jardine et al. 2015). For instance, Hanson et al. (2010) used stable isotopes and fatty acids to differentiate among algal species on the west coast of Australia. They found that $\delta^{13}C$ signatures could not

differentiate between algal phyla (red and brown algae), whereas fatty acid profiles could readily distinguish these forms (Hanson et al. 2010). In an Australian coastal area, mixing models using stable isotopes yielded ambiguous results and indicated that brown algae and sea grasses were both feasible food sources for the amphipod *Allorchestes compressa* (Crawley et al. 2009). The initial interpretation of these data was that the amphipod consumed both brown algae and seagrass. However, the presence of different fatty acids such as ARA and 18:4ω3 in brown algae and the amphipod, combined with the isotope data, suggested that the amphipod was actively consuming brown algae but not seagrass (Crawley et al. 2009).

1.5 Rationale for studying connectivity in riverine food webs

There is a general need for ecologists to transcend from a single-population perspective (i.e. autecological) to a food web perspective (i.e. multi-trophic), especially when developing strategies for resource management or conservation (Wootton et al. 1996). My research incorporated a food web perspective to investigate the trophic linkages and subsidies involving invertebrates in a river system of South Africa using stable isotope and fatty acid approaches. Most studies on subsidy transfers between freshwater and terrestrial ecosystems have a geographical bias, as much of our knowledge is confined to the northern hemisphere (reviewed by Bartels et al. 2011). There is a need for investigations on trophic relations in different climate zones and regions of the world, especially those where vegetation is more sparse (Bartels et al. 2011). My study contributes new information on trophic subsidies in a poorly understood region of the world (southern Africa).

Several studies have been carried out to assess aquatic insects as potential subsidies for terrestrial systems (Sanzone 2001, Bastow et al. 2002, Collier et al. 2002, Lynch et al. 2002, Iwata et al. 2003, Kato et al. 2003, Paetzold and Tockner 2005, Wesner 2010). However, most of these studies have typically relied on abundance and biomass measurements of insects to determine their contributions to terrestrial systems. In contrast, my approach incorporated fatty acid and stable isotope tracers in conjunction with measures of insect biomass to assess and quantify connections via trophic subsidies across ecotones. A unique aspect of my study is the investigation of reciprocal transfers of organic material via invertebrates (to and from the aquatic habitat), as researchers typically focus on only one direction of transfer.

The International Panel for Climate Change (2007) predicts that by 2025, southern Africa will have experienced significant declines in precipitation and increases in temperature. Any decreases in precipitation are likely to negatively impact benthic

invertebrates and aquatic food webs in general (Kling et al. 2003). In response to warming waters, some insect species increase their growth rates, emerge earlier, are smaller at maturity, alter their sex ratios, or reduce their fecundity (Hogg and Williams 1996). Another potential impact on stream food webs and the biodiversity they support comes directly from increasing atmospheric CO_2 levels. Some studies indicated that plant leaves grown under elevated CO_2 have lower food value (Lindroth et al. 2001). The change in atmospheric CO_2 could slow microbial decomposition of plant material that falls into streams, a major source of energy and nutrients in many aquatic ecosystems, and also reduce growth and survival in some stream insects that feed on the leaves (Tuchman et al. 2002, 2003, Alto et al. 2005). Any such impacts would be magnified up the food web. Knowledge on the structure of food webs, and the trophic transfers between adjacent systems, is important for allowing us to predict the consequences of any of these linkages being altered through climate change or other sources of disruption.

1.6 Research objectives

By assessing the role of macroinvertebrates in trophically linking habitats in a South African River system, I addressed the following aims:

I. to investigate how location influences the relative importance of allochthonous and autochthonous energy to stream consumers as predicted by the RCC.

H1a: Consumption of allochthonous food sources is greatest in the headwaters where shading is high, with consumption of autochthonous resources increasing downstream due to increasing light.

H2: The diets of aquatic insects are generally reflective of their FFGs assignments (i.e. shredders primarily consume leaf detritus and grazers consume algae).

II. to determine the extent to which the aquatic and terrestrial habitats in a river riparian area exchange resources using invertebrates as vectors (data to include abundances, fatty acid profiles and isotopic signatures), and how these exchanges vary through time.

H1b: Consumption of autochthonous organic matter (middle and lower reaches) or allochthonous organic matter will persist in the diets of consumer regardless of season

H3a: Insect emergence is greatest in the summer months when water temperatures are at their highest. Insect emergence is lower in winter months when water temperatures are at their lowest.

H4a. Invertebrate fall-in rates peak in the summer months (higher temperatures) and drop to zero in the winter (when temperature is typically lower).
H5b. Invertebrate fall-in rates relative to emergence are greatest in the winter (because invertebrates subsidise streams at low emergence).

1.7 Thesis overview

The main theme of this thesis is to investigate the trophic relationships in a temperate stream of southern Africa. **Chapter 2** details collection methods and includes summaries of data sourced from concurrent studies in the Kowie River. **Chapter 3** provides a description of the FFGs and principally addresses whether relationships exist between physical attributes of the river and abundances of FFGs. Building on the FFG organization in the Kowie River, **Chapter 4** investigates the dependence of consumers on different basal resources using stable isotopes. **Chapter 5** describes the seasonal and spatial dynamics of fatty acids in consumers with the aim to further investigate diets of consumers. **Chapter 6** investigates reciprocal fluxes mediated by invertebrates across the riparian-stream interface using biomass and essential fatty acids. **Chapter 7** synthesises my findings of all data chapters and considers the importance of these findings in understanding linkages between aquatic and terrestrial ecosystems. **Chapter 7** also includes suggestions for future studies on aquatic linkages in riverine systems.

2 MATERIALS AND METHODS

2.1 Study area

The study was conducted at six sites along the Kowie River, South Africa, from its headwaters to the lower reaches (Fig. 2.1). The Kowie River and its main tributary, the Bloukrans River, originate in Grahamstown (approximately 70 kilometres from the coastline) and drain into the Indian Ocean. The catchment area is approximately 769 km^2 with an average annual run off of 23 x 10^6 m^3 (Heydorn and Grindley 1982). The Bloukrans River drains the suburban areas of Grahamstown and is exposed to various sources of pollutants such as sewage from a water treatment plant (Slaughter 2011). The farming practises in the Kowie catchment consist mainly of pineapple production and animal husbandry. The average annual rainfall in Grahamstown over the study period (September 2012 to October 2013) was approximately 750mm [Department of Water Affairs and Forestry (DWAF) database, South Africa http://www.dwaf.gov.za/hydrology]. Monthly average discharge in the Kowie River was 0.29 m^3/s in September 2012, 0.54 in December 2012, 0.55 in February 213 and 1.57 in October 2013 [Department of Water Affairs and Forestry (DWAF) database, South Africa https://www.dwaf.gov.za/hydrology/HyDataSets.aspx?Station=P4H001; Fig. 2.1]. The Kowie River experienced a major flooding event in October 2012 that resulted in the highest discharge (96.6 m^3/s) recorded in the Kowie River for the entire sampling period.

The streambed of the Kowie River is heterogeneous (Table 2.1) and consists primarily of cobbles (> 64 - 256 mm), with lesser amounts of gravel (> 2 - 64 mm) and sand (0.06 - 2 mm) (personal observations, 2012). All sites except FW1 were fringed with beds of emergent macrophytes. The most dominant aquatic macrophyte was *Cyperus eragrotis* at all sites except FW5, where the stream periphery was colonized by *Juncus effusus, Potamogeton pectinatus, Phragmites australis* and *Schoenoplectus brachycerus*. The riparian vegetation was dominated by *Eucalyptus globulus, Searsia* spp. and *Olea africana.*

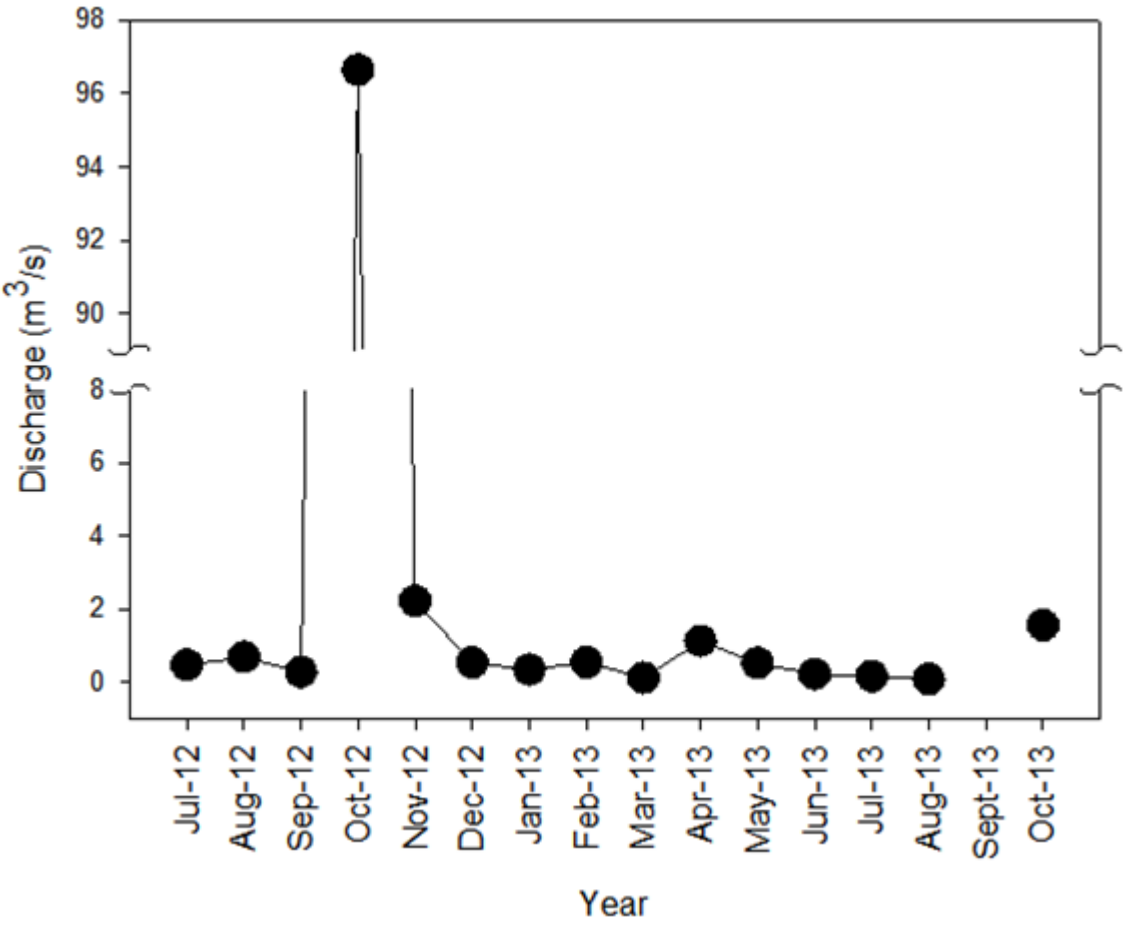

Figure 2.1: Monthly average values of the Kowie River discharge (m^3/s) from September 2012 to September 2013. Data obtained from the Department of Water Affairs and Forestry database, https://www.dwaf.gov.za/hydrology/HyDataSets.aspx?Station=P4H001.

Figure 2.2: Pictorial view of selected downstream and upstream sites in the Kowie River, South Africa.

Table 2.1: Physical characteristics of study sites along the Kowie River.

Site	Mean depth (m)	Mean width[a] (m)	Elevation (m)	Canopy cover [b] (%)	Major habitats
FW1	0.34	1.49	390	72	sand pools, cobble riffles
FW2	0.38	4.10	234	27	sand pools, cobble riffles
FW3	0.44	17.80	148	26	boulder pools, boulder–bedrock riffles
FW4	0.65	11.28	52	24	cobble pool, cobble riffles
FW5	0.61	12.04	11	11	small cobble pools, submerged macrophytes[c], cobble riffles
FW6	0.26	5.50	230	44	boulder pools, boulder–bedrock riffles

[a] Stream width measurements (mean of three measurements per site).
[b] Spherical densiometer measurements (mean of five measurements per site).
[c] Macrophytes were submerged *Potamogeton pectinatus* in riffles and pools.

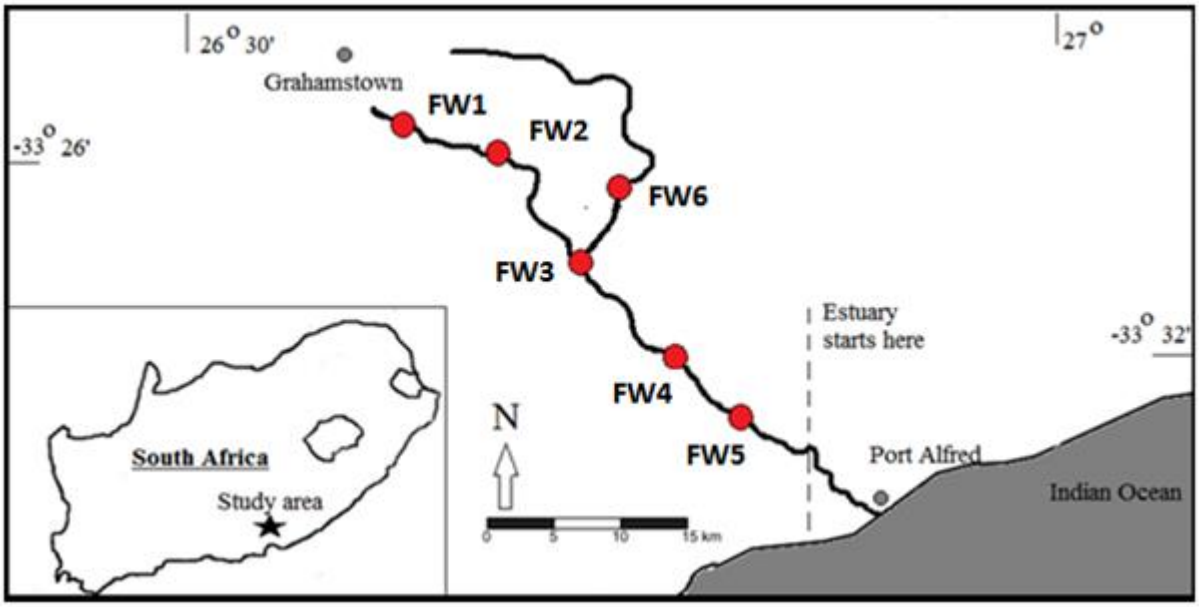

Figure 2.3: The Kowie River (red circles show study sites). The lower insert shows the location of the Kowie River in relation to South Africa. Latitude and longitude coordinates of sites are included (also see Table 2.-1 for site numbers). Dashed line denotes the approximate boundary between the estuary and river sections of the Kowie River.

2.2 Sample collection

Samples of plants (aquatic macrophytes and terrestrial plants), algae and invertebrates were collected on four occasions (Table 2.2) representing late spring (November), late summer (February), winter (May) and spring (September). Emergent insects and terrestrial invertebrates were collected on four occasions in summer (December), autumn (March), winter (June) and spring (September).

Table 2.2: Sampling occasions for plants (P), aquatic macroinvertebrates (M), insect emergence (E) and "infalling" terrestrial invertebrates (I) at each site.

Sampling occasion	**Site name**					
	FW1	FW2	FW3	FW4	FW5	FW6
28 September† - 11 December 2012	P M E I	P M	P M	P M E I	P M	P M E I
21 February - 23 March 2013	P M E I	P M	P M	P M E I	P M	P M E I
13 May - 30 June 2013	P M E I	P M	P M	P M E I	P M	P M E I
13 September – 1 October 2013	P M E I	P M	P M	P M E I	P M	P M E I

† 28 September 2012 only macroinvertebrates for community analysis were collected.

2.2.1 Invertebrate sampling

2.2.1.1 Invertebrate sampling for community analysis

Invertebrates were sampled on four occasions between September 2012 and May 2013. Kick samples of macroinvertebrates were collected on four sampling occasions bimonthly using a South African Scoring System (SASS; 0.8mm mesh) net. Three ×3 minute kick samples (an area of 1 m x 1m for each sample) were taken on each sampling visit to include various habitat types (riffles, pools, vegetation). The three samples were combined in the field to produce one sample per site. Where stream sections (namely FW5) had large beds of macrophytes, invertebrates were sampled by brushing and washing plants into the net, or by placing them in a bucket for washing and picking out the invertebrates using forceps. Kick sampling disturbed the substrate, so the net was moved progressively upstream during a site visit. Macroinvertebrate samples were washed with distilled water to remove attached algae, and all the animals were enumerated and identified to the lowest practical taxon under a dissecting microscope and using regional keys (Day et al. 1999, 2001a, 2001b, Gerber and Gabriel 2002, De Moor et al. 2003).

2.2.1.2 Invertebrate sampling for food web analysis

Invertebrates for food web analysis were collected concurrently with samples for community analysis. A suite of stream insects, representing all major functional feeding groups, were selected for collection from each site along the Kowie River. At each site, three replicate samples were collected for analyses. Invertebrates were kept alive in plastic containers for 6 h to let their guts clear. All samples were placed into labelled aluminium foil envelopes and stored at -80^{o}C until further analysis.

2.2.2 Emergence and terrestrial infall

Floating pyramidal emergence traps (0.37 m^2 base) were used to quantify the rate of emergence of aquatic insects (Davies 1984). Traps were constructed from polyvinyl chloride pipes, foam and screening (0.08 mm mesh). The foams were used to float the traps at the water surface, and the bases of the traps were anchored into the stream substrate using bricks. Three emergence traps were deployed per site for a period of three days, four times per year. Insects were identified and counted under a dissecting microscope to determine abundances of emerging insects (mg m^{-2} day^{-1}; De Moor et al. 2003).

Pan traps (40cm x 55cm x 13cm; designed from plastic) were used to quantify terrestrial invertebrate "infall" into the river. Each pan was filled with water (2 cm depth) mixed with a few drops of a surfactant (detergent) to reduce the surface tension of the water (Nakano and Murakami 2001). Three pan traps were deployed per site for a period of three days, four times per year. Insects were identified and counted under a dissecting microscope to determine abundances of terrestrial infall (mg m^{-2} day^{-1}). Pan trap contents were sieved through a 500µm mesh. One limitation of pan traps is that they can collect only those terrestrial invertebrates entering from above and they do not capture invertebrates delivered laterally across the stream bank (Cloe III and Garman 1996, Wipfli 1997, Nakano et al. 1999a). Body lengths of animals were measured (to the nearest 0.05mm from the anterior part of the head to the posterior end of the abdomen) using Vernier callipers (for specimens > 10 mm) or an ocular micrometre fitted to a dissecting binocular microscope (for specimens < 10 mm). Appendages extending beyond these points (wings, ovipositors, caudal cerci, styles, etc.) were disregarded.

2.2.3 Terrestrial and aquatic organic matter sources

At each riverine site, leaves and roots of dominant aquatic macrophytes (e.g. *Phragmites australis*, *Juncus effesus*, *Potamogeton pectinatus*) and conspicuous fringing trees were collected using a scalpel. Triplicate samples were collected at all sites per season. All macrophytes were washed with distilled water to remove epiphytic algae (Thorp et al.

1998). After collection, all plant samples were immediately placed onto ice in a cooler box and transported to the laboratory for further processing.

Additional organic matter sources for all sites along the Kowie River [i.e. epiphyton (algae attached to plants), benthic algae (algae growing on the surficial sediment), periphyton (algae growing on rocks), filamentous algae (algal mats) and fine particulate organic matter] were obtained from a separate study (Dalu et al. 2014a, 2015b)

2.2.4 Stable isotope analysis

In the laboratory, all samples intended for stable isotope analysis were placed in foil envelopes and lyophilized at -60^{o}C (Virtis Benchtop 2K) for 24 to 48 hours. After lyophilization, solid material was ground to a fine, homogeneous powder and weighed into 6 x 4 mm tin capsules. Stable isotope analysis of carbon and nitrogen was performed on a Europa Scientific 20-20 isotope ratio mass spectrometer coupled to an elemental analyser (ANCA SL Prep Unit, all analyses completed at IsoEnvironmental cc, Grahamstown). The $\delta^{13}C$ and $\delta^{15}N$ values of the plant and animal samples were determined by comparisons with beet sugar and ammonium sulphate standards that were calibrated against International Atomic Energy Agency standards. The ratio of stable isotopes was expressed by convention in delta (δ) notation:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000,$$

where δ denotes the isotope ratio of the sample relative to the standard, and R_{sample} and $R_{standard}$ are the ratios of heavy to light isotopes in the sample and the standard, respectively. One is deducted from the $R_{sample}/R_{standard}$ fraction so that samples with lower ratios of heavy isotopes than the standard are assigned a negative value and those with higher ratios of heavy isotopes than the standard have positive values. The resultant value is then multiplied by 1000 so that the ratio values are expressed in units of parts per thousand (‰).

2.2.5 Fatty acid analysis

Lipids were extracted from freeze-dried samples with a modified one-step method (Indarti et al. 2005). Sample preparation by the Indarti method is much simpler and can yield results with superior precision when compared to traditional methods such as the Folch (see Folch et al. 1957) chloroform/methanol extraction method (Ulberth and Henninger 2006). Invertebrates and plants were ground to a fine powder with a stainless steel rod or a mortar and pestle and subsamples weighed (1 to 200 mg depending on availability and lipid content) prior to extraction. An internal 19:0 fatty acid standard (10 - 40µL of 6 to 8 mg of standard per 10ml of chloroform) was introduced to each sample, and the samples were covered with

chloroform ($CHCl_3$) containing 0.01% butylated hydroxytoluene followed by the addition of a mixture of sulphuric acid and methanol. The samples were topped with nitrogen gas and Teflon caps, vortexed and sonicated (sonication in an ice bath). The mixtures were heated at 100 ºC for 30 minutes, cooled, and ultrapure (milliQ) water was added and the solution centrifuged at 3000 rpm for 3 minutes. The upper layer of the stratified sample containing the fatty acid methyl esters (FAMEs) was separated, dried with sodium sulphate, and suspended in hexane. Analysis was completed by gas chromatography (GC) on an Agilent 7890 equipped with a ZB-WAXplus 320 column (30 m long ×0.32 mm internal diameter) and a flame ionization detector. The settings for the GC oven temperature were set as follows: 70 ºC for 1 min, to 170 ºC at 40°C/min for 3 min, and then to 250 ºC for 4.5 minutes (total run time was 40 minutes).

Peak identifications were accomplished by calibrating and integrating peaks with Chemstation (version B.04.02). The identity of the peaks was confirmed on representative samples (for each species) by gas chromatography/mass spectrometry (GC/MS; Agilent Technologies 7000 GCMS-QQQ using Masshunter version 5.00 and NIST 08 MS library). The same column type and protocols as the GC analyses were utilised to run the GC/MS confirmations. FAME peaks were quantified by comparing peak areas with those of the internal standard. Each fatty acid was measured qualitatively as a proportion of the total fatty acids (% TFA), and quantitatively as the fatty acid weight per mg dry mass. Fatty acids are reported in the shorthand form A: BωX, where A indicates the number of carbon atoms, B is the number of double bonds and X indicates the location of the first double bond relative to the methyl end of the molecule.

2.3 Data sourced from concurrent studies

The present study was part of a large project on the Kowie River investigating reciprocal subsidies laterally and longitudinally in a South African catchment. Some of the data used in this thesis, particularly the physical and chemical data and fatty acids for terrestrial insects, were obtained from other researchers.

2.3.1 Physico-chemical parameters

All physico-chemical data (used in Chapter 3 and Chapter 6) were obtained from Dalu et al. 2014 (summarised in Table 2.3). Air temperature data used in Chapter 6 were collected from a concurrent study (Table 2.3; Chari 2016).

Table 2.3: Mean of physico-chemical data for sites along the Kowie River (mean for all seasons). All data were obtained from Dalu et al. 2014b, 2015a. Maximum (max) and minimum (min) air temperatures (mean and standard deviations for all seasons) at the Kowie River were obtained from Chari (2016).

	FW1	FW2	FW3	FW4	FW5	FW6
Dissolved oxygen (mg L^{-1})	7.3 (1.1)	5.4 (0.9)	6.8 (1.6)	6.2 (0.4)	6.5(0.9)	7.0(0.9)
Conductivity (ms cm^{-1})	0.9 (1.0)	1.3 (0.7)	2.4 (0.8)	2.7(0.4)	2.8(0.2)	1.5(0.2)
Total dissolved solids (ppt)	0.6 (0.7)	1.4(0.7)	1.6 (0.6)	1.8(0.3)	3.4 (2.9)	1.0(0.2)
pH	6.8 (0.7)	6.9 (0.3)	7.8 (0.4)	8.1(0.7)	8.2(0.3)	7.8(0.6)
Chlorophyll-a (mg L^{-1})	1.2 (1.3)	2.0(1.2)	6.9(5.4)	7.2(6.7)	6.0 (3.2)	2.3(2.8)
Temperature (^{o}C)	17.0(4.7)	17.4(3.7)	19.9(4.9)	19.1(4.7)	20.0(4.7)	16.7(2.9)
Water velocity (m s^{-1})	0.3 (0.3)	0.1 (0.1)	0.4 (0.3)	0.3 (0.3)	0.4(0.2)	0.6(0.3)
Ammonia (mg L^{-1})	0.20(0.2)	0.20(0.1)	0.2(0.2)	0.3(0.3)	0.2(0.1)	0.3(0.2)
Phosphate (mg $L^{-1)}$	1.9 (1.3)	3.2 (4.1)	2.2(0.8)	0.7 (0.5)	1.7 (2.1)	2.4(1.7)
Nitrate (mg $L^{-1)}$	1.3(1.4)	0.4(0.5)	4.2(4.3)	0.4(0.5)	0.3(0.5)	2.1 (1.6)
Max temperature	24.9(0.9)	-	-	27.7(3.0)	-	34.7(6.6)
Min temperature	15.0(4.3)	-	-	9.1(73.0)	-	9.3(5.9)

2.4 Fatty acid data

Lipid data for terrestrial insects (used in Chapter 6) were obtained from a concurrent study investigating the diets of aerial predators in the Kowie catchment (Table 2.5; Chari 2016).

Table 2.4: Mean contents of arachidonic acid (ARA) and eicosapentaenoic acid (EPA) (mg g^{-1} of dry weight) of terrestrial insects falling into the Kowie River (mean for all seasons; data extracted from Chari 2016). Values in parentheses show standard deviations. * indicate sites where particular taxa were not collected.

	FW1		FW4		FW6	
	ARA	EPA	ARA	EPA	ARA	EPA
Araneae	1.9 (0.9)	3.6 (2.3)	1.3 (0.4)	8.5(3.2)	1.3(0.8)	6.9 (5.5)
Coleoptera	1.4 (1.8)	1.1 (1.8)	0.3 (0.2)	0.3 (0.2)	0.1 (0.1)	0.0 (0.0)
Diptera	0.7 (0.6)	0.4 (0.3)	0.6 (0.6)	1.5 (2.2)	0.8 (0.9)	0.4 (0.2)
Hemiptera	0.2 (0.2)	0.1 (0.1)	0.2 (0.3)	0.6 (1.6)	0.0 (0.0)	0.0 (0.0)
Hymenoptera	0.7 (0.8)	0.7 (1.27)	0.1 (0.1)	0.1 (0.2)	0.3 (0.6)	0.3 (0.4)
Lepidopetra	0.1 (0.1)	1.3(2.3)	0.1 (0.1)	0.7 (0.4)	0.2 (0.0)	0.6 (0.0)
Orthoptera	0.5 (0.0)	0.5 (0.0)	0.2 (0.1)	0.3 (0.6)	0.2 (0.0)	0.1 (0.0)
Neuroptera	*	*	0.0 (0.0)	1.3 (0.0)	*	*
Mecoptera	*	*	0.8 (0.0)	0.2 (0.0)	*	*

3 Macroinvertebrate functional organisation in the Kowie River

3.1 Introduction

Macroinvertebrate assemblages are essential for the functioning of riverine ecosystems (Vannote et al. 1980). Macroinvertebrates are an important resource for fish, frogs, birds and other vertebrates (Huryn and Wallace 2000), and as such are a vital link between primary producers and higher trophic levels. Additionally, macroinvertebrates play a crucial role as ecosystem engineers. Macroinvertebrates aid in the decomposition of organic matter by breaking down the leaf litter that falls into stream (20 -73% leaf litter is processed by benthic invertebrates; Wallace et al. 1997). Due to invertebrates' central role in the flow of energy through aquatic ecosystems, their community composition can be useful in understanding riverine systems as these invertebrate assemblages typically change with land use and environmental conditions (Allan 2004). Macroinvertebrate assemblages can be described in a variety of ways, including their species composition (taxonomy) and their functional feeding groups (FFGs), i.e. the relative abundance of species in different trophic groups (Allan and Castillo 2007). The grouping of macroinvertebrates into FFGs (their roles in a stream), compared to the taxonomical approach (that gives a description of what organisms are present in a system), offers substantial insights into the functioning of a riverine ecosystem (e.g. river health; Yoshimura et al. 2006) and typically requires less taxonomic effort compared to the taxonomical approach (Cummins et al. 2005).

The River Continuum Concept (RCC) provides a framework to predict the changes of FFGs along a longitudinal gradient. The RCC states that FFGs follow an expected pattern along the longitudinal gradient of a stream (Cummins 1975, Vannote et al. 1980). River geomorphology changes longitudinally and is closely linked to changes in organic matter flow, canopy cover, stream width and the relationship between primary production and respiration (Vannote et al. 1980, Naiman and Bilby 1998, Allan and Castillo 2007). Moreover, the RCC proposes that community composition along the longitudinal gradient, particularly the relative abundance of different FFGs, should shift from allochthnonous structured communities in the headwaters to autochthonous structured communities downstream as local primary production increases with increasing stream size (e.g. stream width; Allan and Castillo 2007). Increasing river size results in a switch in the organization of invertebrate assemblages from collector and shredder dominated communities in the headwaters to collector-gatherer and grazer dominated assemblages in the lower reaches

(Vannote et al. 1980). According to the RCC, shredders will be concentrated in headwater sections owing to their high dependence on allochthonous organic matter from terrestrial leaves. In middle reaches and lower reaches of rivers, grazers can represent the dominant macroinvertebrate FFGs due to the decreased detritus particle size and increased algal primary production. It is generally expected that as a stream transcends from an allochthonous dependent system to an autochthonous dependent system, FFGs will also change in line with the shifts in basal resources (Allan and Castillo 2007). In downstream reaches, collectors are anticipated to have the greatest dominance while other groups (e.g. shredders) become relatively rare (Vannote et al. 1980). According to the RCC, predator abundances are expected to remain relatively constant along the continuum.

Data from temperate streams support the shifts in FFGs predicted by the RCC (Hawkins and Sedell 1981, Grubaugh et al. 1996, Rosi-Marshall and Wallace 2002). However, several datasets from other regions of the world document that the longitudinal distribution of FFGs fits only partially to the RCC (e.g. in Asia: Dudgeon 1989, Jiang et al. 2011; in Finland: Heino et al. 2005; in South America: Miserendino and Masi 2010; in Puerto Rico: Greathouse and Pringle 2006). Therefore, the global applicability of the RCC remains tentative and the factors potentially responsible for the discrepancies reported in the literature have not been thoroughly explored. Additionally, because the development of the RCC was initially based on data collected from pristine and forested streams of North America (e.g. Minshall et al. 1985, 1992), and was further substantiated from other studies of temperate streams and rivers in North America (Tennessee River; Rosi-Marshall and Wallace 2002), its relevance in the temperate streams and rivers of the southern hemisphere is largely untested (Palmer et al. 1993b).

In this study, I provide a description of the longitudinal and seasonal shifts in FFG abundances. The objectives of this study are to: (1) describe the macroinvertebrate fauna in terms of abundance and FFGs in the Kowie River, (2) determine how the FFGs of macroinvertebrates change along a longitudinal stream gradient and compare these patterns to the generalizations formulated by the RCC, and (3) use multivariate analysis to assess the relationships between FFG structure and environmental variables in the Kowie River. To this end and building from the listed research questions and published data showing variable applicability of the RCC to pristine streams and large rivers, I postulated that the RCC cannot fully explain FFG assemblages in the Kowie River (a small river that is relatively pristine), and that FFGs do not conform entirely to the classic RCC.

3.2 Material and methods

3.2.1 Study area and sample collection

Samples were collected at six sites along the Kowie River (see Section 2.1 for detailed site descriptions). Macroinvertebrates were collected by kick sampling along the river (see Section 2.2.1 for details of sampling procedure). Physico-chemical parameters (dissolved oxygen, conductivity, total dissolved solids, pH, chlorophyll-*a*, temperature, water velocity, total nitrogen, nitrates, total phosphorus and phosphates) were measured using multi-meter probes (Dalu et al. 2014b). Canopy cover (measured using a spherical densiometer), stream width and depth were measured to describe the physical attributes at each site.

3.2.2 Classification of functional feeding groups

To answer **objective 1 (**describe the macroinvertebrate fauna of the Kowie River in terms of abundance and FFGs), the trophic structure of the macroinvertebrate communities was examined by grouping taxa into FFGs categories based on published criteria (Palmer and O'Keeffe 1992, Palmer et al. 1993a, 1993b, Merritt et al. 1996, Dobson 2004, Cummins et al. 2005). Where individuals classified into more than one FFG (for example, some groups of baetids could be considered grazers or gatherers), their abundances were divided equally (e.g. 3 baetids was equal to 1.5 grazers and 1.5 gatherers) among the potential FFGs before enumerating the FFG proportions (Dudgeon 1994). Functional feeding group proportions in this study were based on abundance data instead of biomass. Although some studies testing the RCC are based on biomass measures rather than abundance (*c.f.* Grubaugh et al. 1996), a number of studies have demonstrated that the applicability of the RCC can readily be tested using abundance data (e.g. Delong and Brusven 1998, Doisy and Rabeni 2001, Jiang et al.

2011). FFG analysis is predicated on knowing the relationships between a number of feeding adaptations in freshwater invertebrates and their available food resources (Cummins 1973). This approach involves identification of distinct morphological and behavioural features of invertebrate taxa, as opposed to species identity (Cummins et al. 2005). Invertebrate taxa were grouped into shredders, which feed primarily on coarse particulate organic matter (CPOM), grazers that harvest periphyton and material attached to substrate surfaces, filterers and gatherers that rely on fine particulate organic matter (FPOM), and predators that capture live prey. The advantage of using FFGs over a taxonomic approach is that the FFGs are directly linked to ecosystem function and stability (Cummins et al. 2005). Any changes in the nutritional base of river systems will be reflected in the proportions of FFGs (because different FFGs depend on different basal resources); hence, important information about ecosystem function (relating to changes in basal resources) can be captured using FFG ratios as surrogates (Wagner 2001, Merritt et al. 2002, Cummins et al. 2005). To this end, I used FFG ratios to estimate ecosystem parameters in the Kowie River, including the amount of autochthony versus allochthony [(Σgrazers)/(Σshredders, Σfilterers and gatherers)], the strength of the linkage between streams and riparian vegetation (CPOM/FPOM; shredders/ Σfilterers and gatherers), and the predator prey balance (Σpredators/Σprey). There are no published ratios for southern African streams or rivers, so my interpretations of these data were based on studies from other regions (Merritt et al. 2002, Cummins et al. 2005). Index values for autochthony versus allochthony > 0.75 designate autochthony, while all values < 0.75 designate allochthony, values for CPOM/FPOM > 0.25 designate normal shredder association linked to a functioning riparian zone, and predator/prey ratios between 0.1 and 0.2 show a normal predator-to-prey balance, whereas values > 0.2 are indicative of an overabundance of predators.

3.2.3 Data analysis

To further address **objective 1**, differences in the macroinvertebrate assemblages among sites were tested using analysis of similarity (ANOSIM), which was based on a resemblance matrix of Bray-Curtis resemblance measures calculated from square root transformed taxon abundance data (square root transformations were carried out to down weigh the dominant taxa; Clarke and Gorley 2006). In ANOSIM, the R-value provides an absolute measure of between-group separation (Clarke 1993), where values > 0.75 indicate that groups are well separated, values between 0.5 and 0.75 indicate overlapping but clearly different groups, while values below 0.25 show that there is much overlap among groups

(Clarke 1993). Additionally, the Shannon-Weiner diversity index was calculated for all sites for the entire sampling period.

To answer **objective 2 (**FFGs change along a longitudinal gradient in the stream, as generalized by the RCC), I ran Spearman rank correlations on FFGs against morphometric variables of rivers that are predicted to change according to the RCC. The variables considered here were distance (headwater to river mouth, measured in km), canopy cover (determined from spherical densiometer readings) and stream width (measured in metres).

To address **objective 3** (assess the relationships between FFG organization and environmental variables in the Kowie River), I ran Spearman rank correlations of FFGs against physico-chemical variables. Additionally, constrained ordination was used to evaluate the relationships between environmental variables and FFG abundances. Detrended correspondence analysis (DCA) was used to determine the adequate type of model for direct gradient analysis (ter Braak and Smilauer 2002, Lepš and Šmilauer 2003). DCA showed that a linear model (gradient length of less than 2 standard units) was appropriate for the data, and hence redundancy analysis (RDA) was used instead of canonical correspondence analysis (CCA) (Lepš and Šmilauer 2003). All ordinations were run with CANOCO 4.5 using 'inter-species (metrics) correlations' and 'species scores divided by standard deviation' for RDA. These procedures ensured the use of standardised metrics and correlations in the ordination plot rather than co-variances (ter Braak and Smilauer 2002). In all cases, species abundances were square root transformed to reduce the influence of the most dominant species (Clarke and Gorley 2006). Investigations into the combination of environmental factors having potential influence on FFG abundances was first carried out using BVSTEP (a function that analyses relationships between biological and environmental data; Clarke and Ainsworth 1993) in the Plymouth Routines in Multivariate Ecological Research (PRIMER v 6) statistical programme. The analysis was then re-run with non-colinear environmental variables using CANOCO's forward selection procedure (9999 permutations) to analyse the contribution of environmental variables to trophic structure.

The two approaches (BVSTEP and CCA/RDA) examine environment-taxa relationships in contrasting ways and were thus used to provide a more thorough inspection of the potential relationships between biota and environmental variables. The BVSTEP procedure relates the ranked similarities of pairwise comparisons of biotic assemblages and environmental variables and selects the best subset of environmental data that explains the biological data (Clarke and Ainsworth 1993). The BVSTEP method makes few assumptions about the responses of taxa to environmental variables (Quinn and Keough 2002), making it an appropriate test for elucidating species-environment relationships. Conversely, CCA and

RDA are ordination procedures in which ordination axes are constrained to be linear combinations of environmental variables (ter Braak 1986, ter Braak and Smilauer 2002). CCA and RDA assume unimodal responses of taxa to environmental variables and each method concurrently explores the relationships among environmental variables, samples and taxa (Quinn and Keough 2002) and provides insights into taxon-environment relationships.

3.3 Results

3.3.1 Macroinvertebrate faunal abundance and FFGs in the Kowie River (objective 1)

Fifty-six taxa (see Appendix 8-1) with 24863 invertebrates were collected and identified. The taxa collected were primarily insects, but there were also specimens from the phyla Mollusca, Crustacea, and Oligocheata. The families Baetidae, Simuliidae (*Simulium* spp.) and Coengrionidae (*Pseudagrion* spp.) were represented at all sites. ANOSIM showed that the macroinvertebrate assemblages at the six sites were significantly different, with little overlap among some sites (Global R = 0.56, p = 0.001; Fig. 3.1). Pairwise comparisons revealed that there were significant differences across all sites except between the following: FW2 vs FW3, FW2 vs FW4, FW3 vs FW6, FW3 vs FW5 and FW4 vs FW6. Headwaters were dominated by *Simulium* spp., middle regions were dominated by Oligocheata and *Simulium* spp., while Oligocheata dominated downstream reaches. Mean Shannon diversity values (H') ranged from 1.81 to 2.20 across all sites (Appendix 8-1). The lowest H' value was recorded at FW6 (which is the site that receives hight nitrate input from agricultural activities upstream) while the highest H' was recorded at FW3 (Appendix 8-1).

ANOSIM revealed that there were no significant differences among FFG abundances, with considerable overlap among sites (Global R = 0.10, p = 0.1408). Pairwise comparisons revealed that differences in FFG abundances mainly occurred between FW1 and FW6. Field counts of the invertebrate FFG ratios were enumerated as proxies for three ecosystem attributes (autochthony vs allochthony, CPOM/FPOM, and predator prey balance). Adopting the suggested thresholds for autochthony vs allochthony, all sites were characterised as being allochthonous dependent (the dominant base of the food chain for invertebrate communities at all the sites originated from allochthonous resources). FW3 was the most allochthonous driven site (mean 0.01), while FW5 was the less allochthonous driven site (with a mean value of 0.45). While all the sites showed high levels of allochthony, high values of autochthony were recorded in early spring at FW2, FW4 and FW5, and at FW1 during late summer. The related FFG proxy for CPOM/FPOM (which indicates the shredder populations that depend on terrestrial leaves) ranged from 0.02 to 0.16. Generally, the values < 0.25 showed that the

shredders were underrepresented at all the sites and that there was a poor link between shredders and riparian zones. The FFG surrogate for abundance of predators was higher than the threshold of 0.20 at all sites except FW6, which showed even numbers of predators and their prey. The change in predator prey balance did not follow an obvious trend from the headwaters to the downstream site.

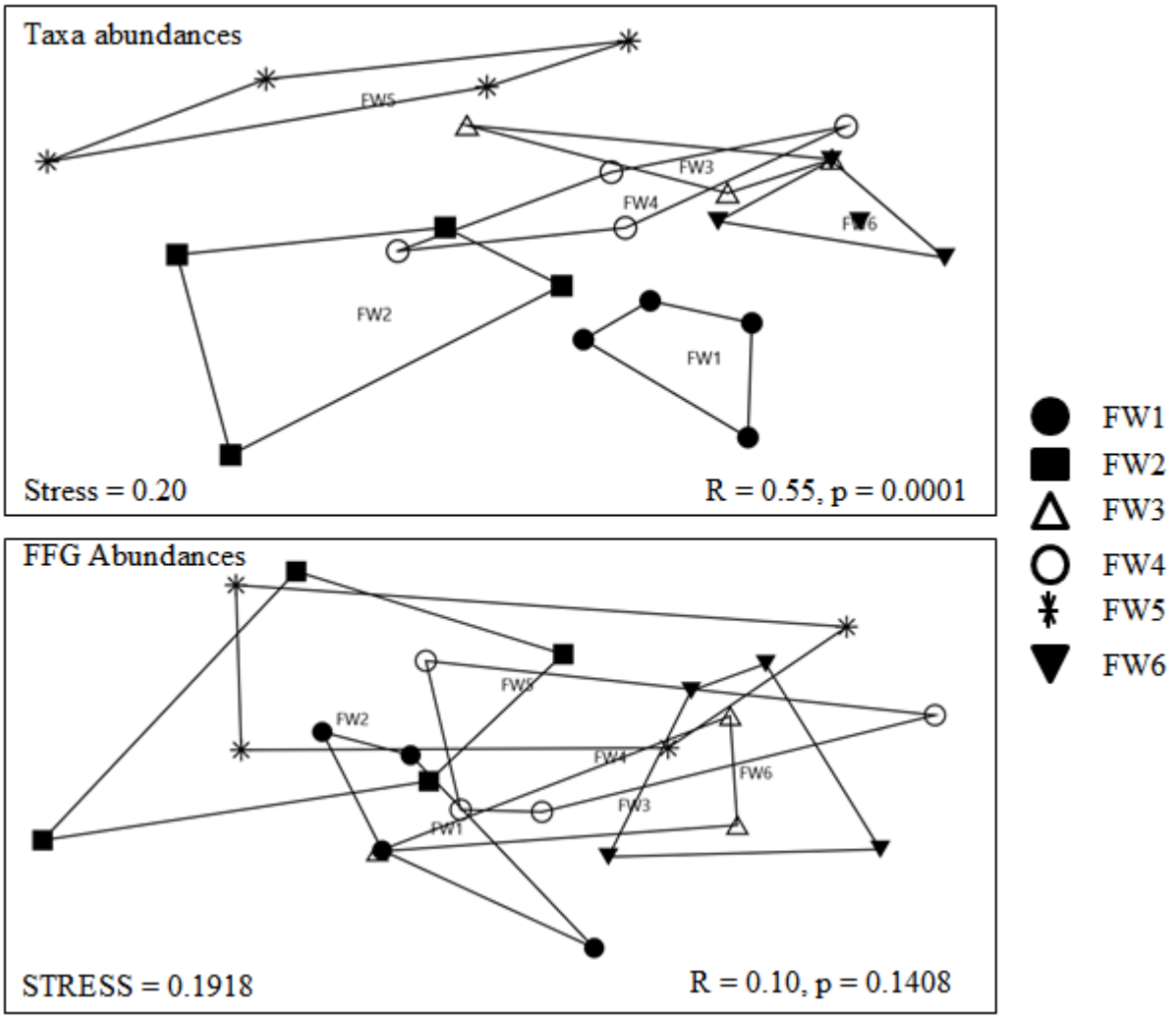

Figure 3.1: Two-dimensional non-metric multi-dimensional scaling ordination of abundances by taxon and FFG from six sites along the Kowie River. Axes are dimensionless. R and p values reported are outputs of the ANOSIM. Points represent four sampling periods (early spring, late spring, late summer and winter).

Table 3.1: Mean values of stream ecosystem attributes derived from ratios of macroinvertebrate functional feeding groups (FFGs) in the Kowie River. Ratios are based on numerical abundance of FFGs. Autochthony to allochthony was calculated as the ratio of grazers/(shredders + total collectors). Coarse particulate organic matter (CPOM)/fine particulate organic matter (FPOM) is the ratio of shredders to total collectors. Predator/prey is the ratio of predators to all other FFGs. Bolded values indicate autochthony, a functioning riparian zone, or an over-abundance of predators. 'NS' shows sites where no samples were collected.

Site	Early spring	Late spring	Late summer	Winter
	Autochthony vs allochthony			
FW1	0.01	0.03	**0.92**	0.07
FW2	**0.93**	0.16	0.36	0.1
FW6	0.03	0.04	0.08	0.12
FW3	NS	0.01	0	0.02
FW4	**1.09**	0.03	0.07	0.14
FW5	**1.75**	0.01	0.02	0.01
	CPOM/FPOM			
FW1	0.05	0.01	0.08	0.16
FW2	0.05	0.05	0.12	0.02
FW6	0.01	0.01	0.04	0.02
FW3	NS	0.01	0.1	0.02
FW4	0.03	0.03	0.1	0.03
FW5	0.05	0.00	0.04	0.01
	Predator/Prey			
FW1	**0.27**	0.11	**0.31**	**0.33**
FW2	**0.60**	**0.42**	**0.45**	**0.22**
FW6	0.04	0.02	0.06	0.12
FW3	NS	0.03	**0.33**	0.06
FW4	**0.36**	**0.36**	**0.33**	0.05
FW5	**0.37**	0.02	**0.46**	**0.32**

3.3.2 FFGs change along a longitudinal gradient in the Kowie River (objective 2)

The relative importance of each FFG to an entire community was determined by examining abundances of each FFG relative to the total invertebrate abundance. There was a general paucity of shredders across all sites, with the highest number (10%) of shredders recorded during the winter months at FW1 (Fig. 3.1). Seasonal shifts in the relative abundance of each group occurred to varying degrees at all sites (Fig. 3.1). Gatherers (mainly

Caenidae and Oligocheata) were relatively unimportant in downstream reaches but gradually increased in abundance in upstream regions during the winter. Filterers (mainly *Simulium* spp. and Hydropsychidae larvae) were relatively important in the Kowie River and reached maximum dominance at FW1 (83%) and FW6 (81%) during late spring. Grazers were relatively unimportant in the entire system during late spring and winter, but were abundant during the early spring (47% of the total invertebrate population at FW5) and late summer months (37% of total invertebrate population at FW1). Predator (mainly Gyrinidae and Odonata) abundances were relatively consistent among the sites, except for FW6 and FW3 during the summer season when the relative abundance of the predators was low (< 3%). The cumulative FFG organisation (based on combined data for all sampling occasions, Fig. 3.3) showed that the gatherers were relatively abundant in the headwaters, but were less so in the lower reaches (FW4) and increased in abundance further downstream (FW5). Filterers were abundant at all sites (except FW2) and often co-dominated with gatherers. Similar to the patterns observed in FFG abundances seasonally (Fig. 3.2), grazers and shredders were rare across all sites (Fig. 3.3). Grazers did not follow an obvious trend from upstream to downstream sites, but high abundances of grazers were recorded at FW2, FW4 and FW6.

The correlations between morphometric features (stream width, canopy cover, distance) and FFGs in the Kowie River revealed that there were a few significant relationships between morphometric features and the FFGs (Table 3.3). Significant correlations ($N = 6$ for each FFG; $p < 0.05$) occurred only in gatherers during early spring (distance vs gatherers; $r = 0.89$, stream width vs gatherers; $r = 0.89$ and canopy cover vs gatherers; $r = - 0.94$) and grazers during early spring (distance vs grazers; $r = 0.90$ and canopy cover vs grazers; $r = - 1.00$), late summer (distance vs grazers; $r = - 0.83$) and winter (distance vs grazers; $r = - 0.83$ and stream width vs grazers; $r = - 0.83$).

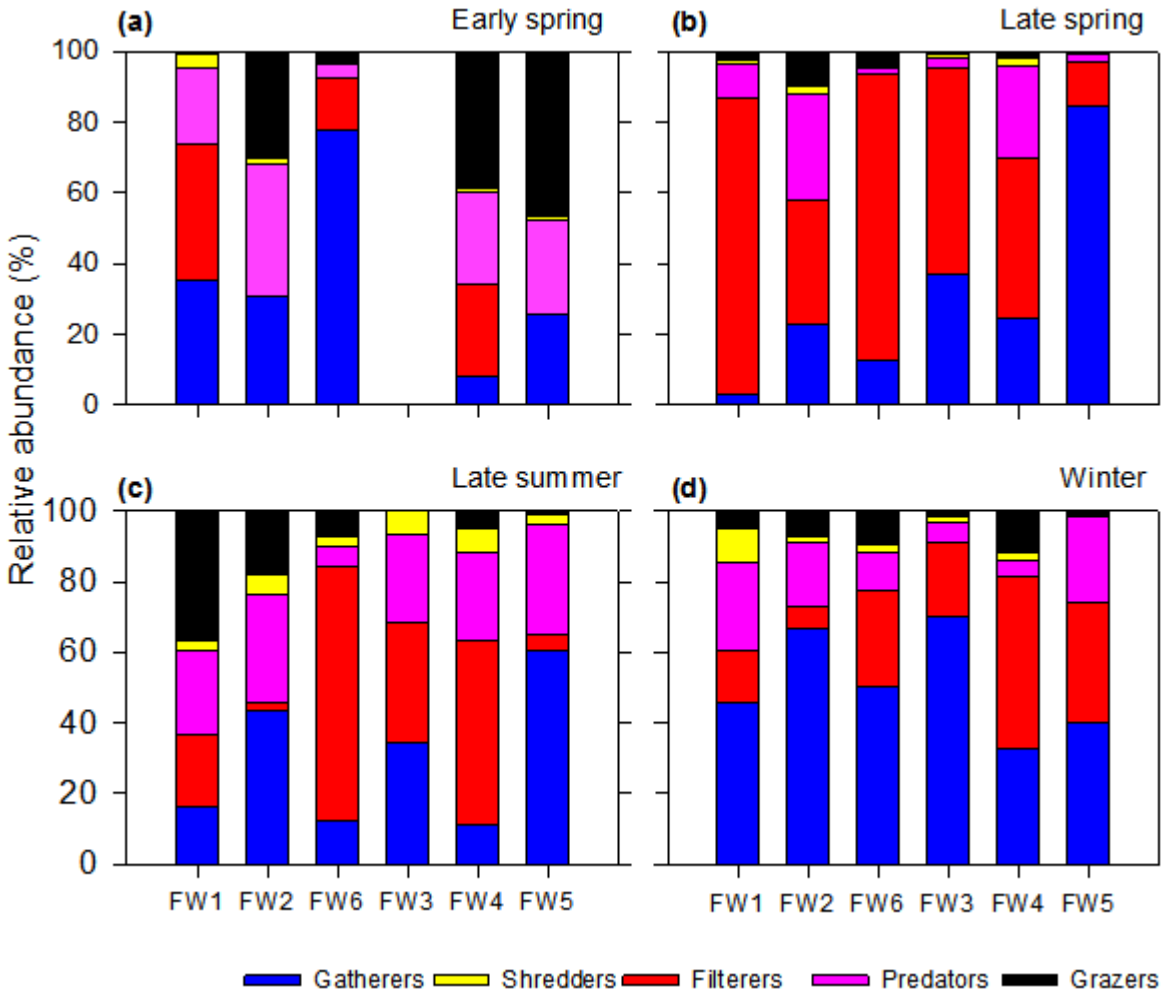

Figure 3.2: Seasonal relative abundance of functional feeding groups of macroinvertebrates along the Kowie River during four sampling seasons from the headwaters (FW1) to the mouth of the river (FW5). Site FW6 is included at the confluence of the main and tributary channels, although it was located several km upstream within the tributary.

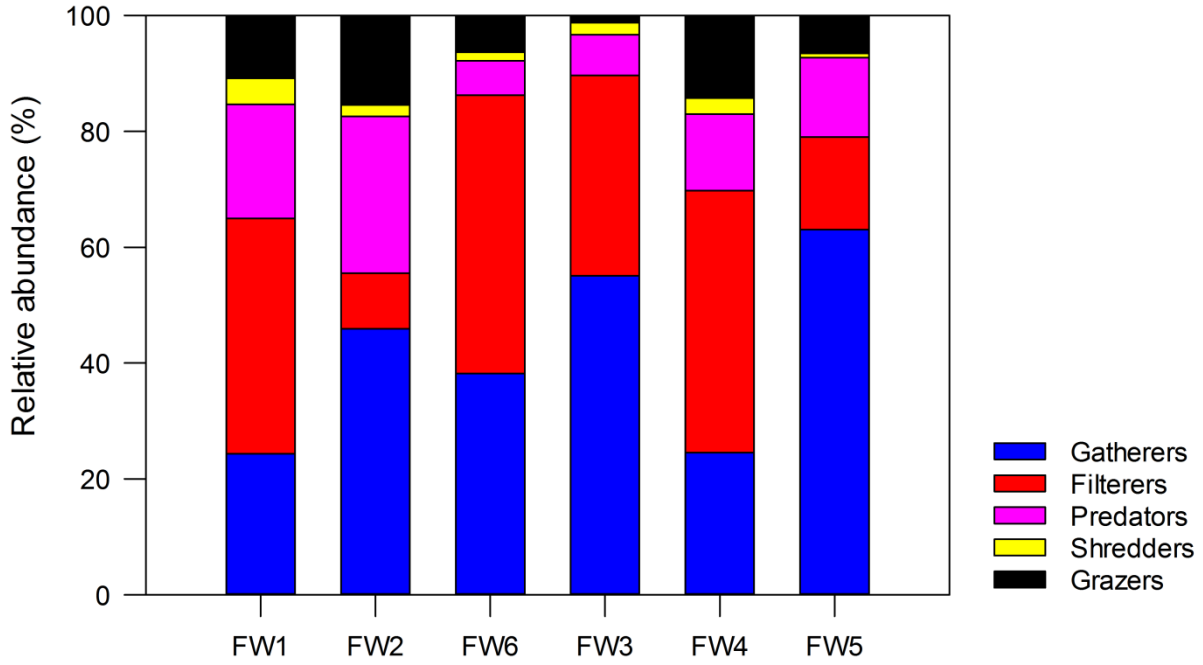

Figure 3.3: Relative abundance of functional feeding groups of macroinvertebrates for six sites (all seasons combined). Site FW6 is included at the confluence of the main and tributary channels, although it was located several km upstream within the tributary.

Table 3.2: Spearman rank correlations of FFGs against morphometric features (distance measured from FW1, stream width and canopy cover) that change according to the RCC. All bolded values indicate statistically significant correlations; $p < 0.05$ ($N = 6$ for each FFG).

		Gatherers	Filterers	Shredders	Predators	Grazers
Early spring						
	distance	-0.60	-0.46	-0.60	0.20	**0.90**
	width	-0.70	-0.21	-0.70	0.10	0.80
	cover	0.80	0.62	0.30	-0.60	**-1.00**
Late spring						
	distance	**0.89**	-0.66	-0.31	-0.31	-0.77
	width	**0.89**	-0.43	-0.20	-0.37	-0.77
	cover	**-0.94**	**0.83**	0.09	0.03	0.71
Late summer						
	distance	0.14	0.14	-0.09	0.09	**-0.83**
	width	0.20	0.20	0.26	-0.03	-1.00
	cover	-0.31	0.14	-0.03	-0.31	0.77
Winter						
	distance	-0.43	0.14	-0.09	0.09	**-0.83**
	width	-0.26	0.43	0.43	-0.31	**-0.83**
	cover	0.37	0.14	-0.03	-0.31	0.77

3.3.3 Relationships between FFG organization and environmental variables in the Kowie River (objective 3)

Spearman rank correlations of FFGs against environmental variables [pH, depth, dissolved oxygen (DO), conductivity, total dissolved solids (TDS), chlorophyll-*a*, temperature, water velocity, ammonia, phosphorus, nitrates] revealed that filterers were positively correlated to water velocity during early spring, late spring and late summer (early spring, $r = 0.95$; late spring, $r = 0.89$, late summer, $r = 0.81$; all $p < 0.05$). In winter, the correlation between filterers and water velocity was non-significant ($r = 0.49$, $p = 0.36$). Filterers were negatively correlated with nitrates during the late summer months ($r = - 0.89$, $p < 0.05$). No significant correlations were observed between shredders and environmental variables across most seasons; positive significant correlations between shredders and nitrates were recorded in the winter months ($r = 0.90$, $p < 0.05$). Predators were negatively correlated with nitrates in late spring ($r = - 0.97$, $p < 0.05$) and positively correlated in the winter months ($r = 0.83$, $p < 0.05$).

RDA ordination of pooled samples (Fig. 3.4) indicated that variation in FFG structure was most strongly associated with four environmental variables: canopy cover, total dissolved solids (TDS), pH and water velocity. The first axis accounted for 72% (eigen value 0.33), while the second axis accounted for an additional 26% of the variation (eigen value 0.118). In the RDA ordination of FFG structure and environmental variables, the gradients separated sites with high abundances of grazers and predators (which were associated with TDS) from sites with high abundances of filterers (which were associated with water velocity). Shredders were negatively correlated with pH and positively with TDS. Sites with greater abundances of gatherers were not associated with any of the environmental variables but were clearly negatively correlated to canopy cover (Fig. 3.4).

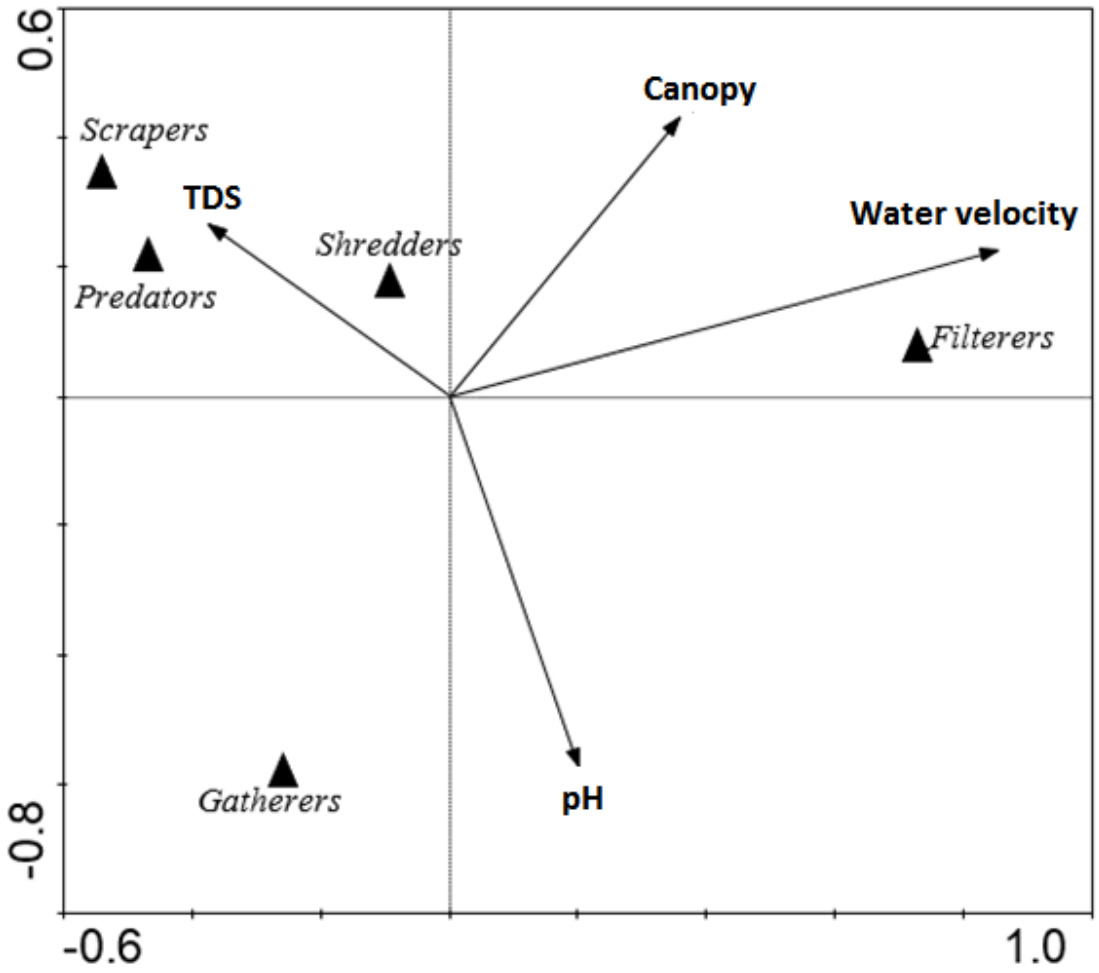

Figure 3.4: Redundancy analysis ordination (axes 1 and 2) of invertebrate feeding guilds (triangles) and environmental variables (arrows with lines). Significant environmental variables ($p < 0.05$) were canopy cover, water velocity, total dissolved solids and pH.

3.4 Discussion

In an effort to describe the fauna of the Kowie River in relation to predictions of the RCC, macroinvertebrates were collected and analysed to assess the trophic organisation of the fauna (**objectives 1 and 2**). Furthermore, the changes in the FFGs in relation to environmental variables were investigated (**objective 3**). A diverse macroinvertebrate fauna occurred within the Kowie River, represented by 56 taxa in total from six sites across four sampling occasions (Appendix 8-1). This level of diversity is similar to diversity measures recorded in other temperate rivers of South Africa (Eastern Cape; 37 – 69 taxa in Buffalo River, Palmer et al. 1994, and 104 taxa in Umzimvubu River, Madikizela and Dye 2003), in both numbers and taxa present, and may be greater than diversity recorded in other eastern parts of South Africa (16 taxa in Bushman's River; Grab 2014). Considering that the level of

taxonomic resolution often differs among studies, researchers need to be cognisant of the different collection methods when comparing diversity between studies.

I hypothesised that the FFGs in the Kowie River would, at best, only partially fit the predictions of the RCC because the RCC was mainly developed in near pristine streams of the northern hemisphere. In the Kowie River, the predictions of the RCC were partially supported (**objective 2**; hypothesis supported). First, the RCC predicts that with changing stream size (stream width), the stream widens and deepens; this prediction of the RCC is concordant with the patterns observed in the Kowie River (Chapter 2, Table 2.1). Additionally, the RCC predicts that with the widening of the stream there will be corresponding decreases in overstory vegetation; a finding supported by results in the Kowie (shown by significant negative correlations between stream width and canopy cover in the Kowie River). Second, the RCC predicts dominance by filterers in flowing rivers with relatively low contributions from shredders and grazers; filterers were more abundant at most sites in the Kowie River, and generally tended to dominate over grazers at most sites. Third, as predicted by the RCC, predators were present and abundant in all macroinvertebrate samples in the Kowie River. However, some RCC predictions were not fully supported. During most seasons, there were no significant correlations between stream physical attributes (stream width, canopy cover and distance) and FFGs (Table 3.3); this indicates that the changes in these physical attributes did not fully explain the changes in FFG composition.

Considering the FFG compositions across all sites, gatherers and filterers were the most dominant group (Fig. 3.2 and Fig. 3.3). These findings were consistent with those from North America (Salmon River, Idaho; Minshall et al. 1992), southeast of China (Fu et al. 2015) and Finland (Heino et al. 2005). The predominance of gatherers and filterers may be due to a large supply of FPOM in the sediment or in the water column (Minshall et al. 1985, Allan and Castillo 2007). The gatherer/filterer dominance in the Kowie River contrasted those from other studies, whereby grazers dominated relative to other FFGs (Delong and Brusven 1998). Such discrepancies between studies is probably because grazers dominate in agriculturally impacted streams (Delong and Brusven 1998). The original RCC did not make separate predictions for filterers and gatherers, as gatherers are typically grouped with filterers into a single collector category, and collectors are predicted to remain constant from headwaters to lower reaches. In their revision of the RCC, Minshall et al. (1985) characterized gatherers as decreasing slightly from headwaters to middle reaches in line with changes in canopy cover. This trend was partially supported in the Kowie River, as there were negative correlations between canopy cover and gatherer abundance (Table 3.3). Filterers were the one FFG in the Kowie River that clearly did not conform to the original or

revised RCC. Filterers declined in relative dominance in the downstream direction of the Kowie system, but the RCC predicted that contributions of filterers either do not change substantially (Vannote et al. 1980, Grubaugh et al. 1997) or increase with increasing distance along the longitudinal gradient (Minshall et al. 1992).

According to the RCC, predator relative abundances change little with increasing stream width (Vannote et al. 1980) because the number of predators is dependent on the relative abundance of their prey (other FFGs). Consequently, no matter how the grazers, shredders, gatherers and filterers change in response to shifting basal resources, the relative predator population remains the same (Vannote et al. 1980). There were no significant correlations between predators and stream attributes, so the predators recorded in the Kowie River supported the predictions of the RCC. The number of predatory invertebrates in the Kowie River was lower (6 to 27% across all seasons) than values recorded in streams of Zimbabwe (28.9 to 29.2%; Chakona and Marshall 2008) and Mpumalanga, South Africa (20 to 41%; Farrell et al. 2015). In the Kowie River, greater abundances of predators were found in the headwater sections (Fig. 3.2 and Fig. 3.3).

Grazers are the primary consumers of autotrophs in lotic systems, and they feed on attached algal communities (periphyton) and biofilm (Vannote et al. 1980, Allan and Castillo 2007). Because grazers are confined to stream reaches where production of periphyton and/or biofilm occurs, they tend to be most abundant in middle to lower stream reaches where light is not limiting and periphyton biomass is high (Vannote et al. 1980). However, in the Kowie River the grazers did not conform to the predictions of the RCC, as the grazers had higher abundances at the headwater sites and decreased in abundance at downstream sites. In support of the findings in the Kowie River, data collected by other researchers did not support the RCC prediction that grazer abundance increases with increasing stream width (Hawkins and Sedell 1981, Sterling and Campbell 2006). For instance, Sterling and Campbell (2006) studied the abundance of grazers in relation to quantity of food sources (algal biomass) and stream size among different south-eastern Australian streams, and they found that grazer abundance and biomass were unrelated to stream size and algal biomass.

The RCC predicts that shredders decrease in abundance from headwaters to the mouth of the river (Allan 1995), in accordance with changes in terrestrial inputs (vegetation cover decreases with increasing stream width). In the Kowie River, there was no significant relationship between shredders and distance measured from the headwaters (Table 3.3). However, all sites were characterised by a low representation of shredders (4.5% in the Kowie River vs 8 – 15% in Wilge River, South Africa; Farrell et al. 2015) at the headwater sites (Fig. 3.2 and Fig. 3.3). Low shredder abundance is characteristic of tropical streams in

Africa (0-6% of the entire community; Tumwesigye et al. 2000, Dobson et al. 2002), Asia (2.7% of the entire community, Jiang et al. 2011), the neotropics (0.3% of entire community; Brasil et al. 2014) and south-temperate streams (12-14% of the entire community; Winterbourn et al. 1981, Lake et al. 1986) but *cf*. Cheshire et al. (2005). In the Kowie River, shredders (mainly Potamonautidae and Tipulidae) are supplanted largely by grazers, gatherers and filterers at most sites. Several hypotheses have been proposed to explain the paucity of shredders in certain systems including low leaf palatability and nutrition caused by the defensive compounds produced by leaves (Irons et al. 1994), and the low supply and retention of terrestrial leaves in riverine systems (Minshall et al. 1985, Allan and Castillo 2007). While none of these hypotheses were directly tested in the Kowie River, it is plausible that the quality and retention of leaf litter both affected the shredder populations. In African rivers, large amounts of CPOM do not reach the stream as they are typically broken down by terrestrial detritivores during the dry seasons, and when the rains occur the CPOM is reduced to FPOM (Minshall et al. 1985).

In addition to running correlations between FFGs and physical attributes of the Kowie River, the relationships between FFGs and environmental variables were also considered (Objective 3). The RDA and the spearman correlations showed that the abundance of filterers was positively correlated to water velocity (Fig. 3.4), in accordance with findings in other studies (Riseng et al. 2004). Filterers depend on organic matter suspended in the water column (Whiles and Dodds 2002), which includes phytoplankton and FPOM. Abundant filterers are associated with high water velocity because they can collect more food by filtering more water in the water column (Riseng et al. 2004). This trend may be due to filtering being more profitable at higher velocities as more food can be captured per unit of time (Wallace et al. 1977). Filterers can be at an advantage over other FFGs in this situation as their passive feeding provides them with a greater energetic return per unit energy invested (Huryn and Wallace 2000). This relationship allows them to attain greater densities than other groups in situations of high velocity (Kobayashi et al. 2013). The aforementioned reasons may explain why filterers were associated with flow in the Kowie River.

In late spring, canopy cover was negatively correlated with gatherers in the Kowie River (Fig. 3.4 and Table 3.3). Autotrophic (epilithon and pure algae) productivity increases in response to decreasing canopy cover (increase in light availability; England and Rosemond 2004). Invertebrates that depend on biofilm-dependent taxa (i.e. gatherers) will thus thrive in areas with lower canopy cover (Benstead and Pringle 2004, England and Rosemond 2004). The decline of gatherers with changing canopy cover was supported in the Kowie River, in accordance with the conclusions made by England and Rosemond (2004), who found that

deforestation was associated with reductions in stream food web dependence on terrestrial subsidies by consumers.

The RDA revealed that the abundances of grazers and predators were positively correlated with total dissolved solids (Fig. 3.4). Total dissolved solids are a mixture of salts (e.g. ions of sodium, potassium, chlorides, magnesium, sulphates and carbonates; Allan and Castillo 2007) and were associated positively with phytoplankton biomass in the Kowie River (Dalu et al. 2014b). Therefore, the positive correlation between grazers and TDS may be indirectly linked to the changes to the phytoplankton communities with changing TDS. Surprisingly, the predators, which are often cited as dependent on abundances of their prey (Allan and Castillo 2007), were strongly related to TDS in the Kowie River (Fig. 3.4). The influence of TDS on FFGs has been observed in mining-influenced streams, where elevated levels of conductivity/TDS (> 700 mg/L) resulted in loss of Ephemeroptera (Clements 1994, Pond et al. 2008). Most of the studies that have investigated the effect of TDS on aquatic consumers have been experimental (e.g. Pond et al. 2008), typically carried out at very high concentrations (> 700 mg/L vs 0.57 to 3.36 mg/L in the Kowie River), therefore the effects of TDS on FFGs may be more conservative in the Kowie River than what is proposed by experimental work. However, it is likely that at higher concentrations of TDS, there would be corresponding decreases in the predators and grazers in the Kowie River.

Shredders in the Kowie River were negatively related to pH (Fig. 3.4). Corroboration from other streams suggests that acidic conditions reduce the breakdown of leaf litter by inhibiting the activity of microbes and making leaves less palatable for shredders (Dangles et al. 2004), hence resulting in reduction of shredder abundances. Shifts in shredder abundances in rivers may be linked to changes in food resources with changing pH (Rosemond et al. 1992). Algal resources increase rapidly with rising pH (Hildrew 2009), and hence promote the proliferation of autochthonous-dependent organisms such as grazers. Other authors have noted the disappearance of some shredders in favour of specialist grazers at low pH (Layer et al. 2013). For the Kowie River, the relationship between pH and shredders may be more conservative, especially considering that the pH levels recorded in this river fell within a narrow range.

In spite of changes in stream width and canopy cover, there were little overall differences in FFGs of the stream invertebrates (Fig. 3.1), possibly indicating that the organization of FFGs may not be strongly linked to the physical attributes but rather to food resources. The ratio of grazers to detrital-dependent organisms was used to assess the dependence of the stream on autochthony vs allochthony (Table 3.1, Cummins et al. 2005). Based on the recommended thresholds (> 0.75 indicates autochthony), most of the sites were

designated as allochthonous dependent (six out of six sites had values less than 0.75). The autochthony vs allochthony values recorded in the Kowie River fell within the ranges of those reported elsewhere (Merritt et al. 2002, Cummins et al. 2005, Masese et al. 2014). Merritt et al. (2002) worked on a temperate stream in the United States of America (Florida) and reported autochthony vs allochthony values between 0.01 and 0.20. Similarly, Cummins et al. (2005) reported autochthony vs allochthony values between 0.04 and 0.56 in rivers in southern Brazil, while Masese et al. (2014) reported values between 0.13 and 1.61 for a tropical stream in Kenya. The findings from autochthony vs allochthony in the Kowie River showed that the system was dependent on allochthonous organic matter, in support of one of the central tenets of the RCC. Given that the other studies were from entirely different systems and few studies are available in southern Africa for comparison, the threshold values for autochthony vs allochthony should be evaluated in other South African systems. Additionally, considering that the autochthony vs allochthony ratio was developed with the assumption that the FPOM in streams is derived from detritus processed upstream brings this idea into question. In the Kowie River, FPOM is a matrix of macrophytes, phytoplankton and terrestrial leaves (a mixture of terrestrial and aquatic autotrophs; Dalu et al. 2015b) and hence the ratio may need adjustments to consider this composition in South African rivers (i.e., FPOM is not entirely made of allochthonous material).

The ratio depicting CPOM and FPOM in streams was determined with total abundance of shredders relative to the collectors. The ratio of CPOM/FPOM in the Kowie River ranged from 0.01 to 0.16 (Table 3.1). Using the recommended threshold (> 0.25 shows a strong riparian link between shredders and the stream), the CPOM/FPOM ratios in the Kowie River showed that there was a weak shredder to riparian vegetation link (as all values were typically below the threshold). All sites were clearly endowed with large amounts of FPOM. The CPOM/FPOM ratios from the Kowie River were within the range of a Kenyan steam (0.01 to 0.29; Masese et al. 2014) and an American stream (0.01 to 0.17; Merritt et al. 1996). According to the RCC, the quantity and quality of FPOM increases downstream because of processing and breakdown of CPOM. The present findings do not entirely follow the RCC, as CPOM/FPOM ratios were constant along the longitudinal gradient (Table 3.1).

The predator vs prey balance in the Kowie River ranged from 0.02 to 0.60 (Table 3.1). The proposed threshold for predator prey balance is 0.20, with values > 0.20 indicative of an overabundance of predators and values < 0.20 showing a normal predator community (Merritt et al. 2002). During some seasons, and at some sites, the values were < 0.20 indicating that there was a balance between predators and their prey. Values > 0.20 were also recorded during some seasons (at most sites during early spring and late summer), showing

that there was an overabundance of predators during those seasons. The predator/prey ratio recorded in the Kowie River was in accordance with those from Appalachian streams in the eastern United States of America (0.01 to 0.34; Wagner 2001) and in Kenyan streams (0.01 to 0.43; Masese et al. 2014). The RCC predicts that predators will be relatively constant along a river gradient. Changes in predator prey balance along the Kowie River may result from the changes in the abundances of the potential prey rather than the predators themselves. Additionally, predators may change seasonally due to fish predation. In the Kowie River, insect predators (e.g. Belostomatidae, Aeshnidae, Libellulidae) contribute to the diets of largemouth bass (*Micropterus salmoides*; Magoro et al. 2015) and Cape stumpnose (*Rhabdosargus holubi*; Carassou et al. 2016) across all seasons. The contribution of insects to the diets of largemouth bass and Cape stumpnose suggests that these predators may exert a top down effect on insect predators, which may contribute to the seasonal changes in insect predators.

The FFG approach assumes that each taxon conforms to its feeding group designation (e.g. shredders only feed on terrestrial leaves). However, there is evidence that some invertebrates fall into different designations depending on factors such as food availability (MacNeil et al. 1997, Kelly et al. 2002). The feeding plasticity of invertebrates suggests the facultative nature of FFGs, as opposed to the obligate view of FFGs. The designation in this study did not account for the facultative nature of some FFGs. However, where clear plasticity of FGGs was known, I separated FFGs accordingly (see section 3.2.2). While there are questions regarding the efficacy of FFG designations, FFGs still provide useful information about the functioning of an ecosystem (Cummins et al. 2005). By identifying feeding behaviours and consumer mouthparts, macroinvertebrate FFGs indicate which food sources are most likely ingested rather than those assimilated by consumers. This practice creates the potential for substantial mismatch in the perceived importance of different food sources between studies based on FFGs alone. However, potential mismatches of FFGs in this study do not imply that processing roles of macroinvertebrate within the Kowie River are redundant. For instance, even though taxa traditionally regarded as shredders may rely heavily on algal sources for their organic carbon, they can still play an important role in breaking down leaf litter, as has been observed in neotropical island streams (Lancaster and Waldron 2001, March and Pringle 2003, Tomanova et al. 2006).

3.5 Conclusions

In summary, macroinvertebrate patterns in the Kowie River were in accordance with other temperate rivers and agreed with some of the *a priori* predictions of the RCC. River

systems are complex and experience large variations across a time-space continuum. The dynamic nature of riverine systems means that conceptual models may not always adequately represent ecosystem function. The patterns observed in the Kowie River do not fully support or negate the applicability of the RCC, and the longitudinal links in function and diversity of macroinvertebrates in the Kowie River were not as proposed by the RCC. Data from the Kowie River, together with others from different areas of the world, highlight the complexity of riverine ecosystems across space and time.

This study of the Kowie River suggested that the pattern of FFG composition may mirror food resource distribution, and the findings have contributed towards our understanding of organic matter processing in temperate streams of southern Africa. However, when attempting to link FFG distribution to water quality variables (as in this study), it is possible that these links can be indirect, as physico-chemical variables only partly determine what types of food are available (Palmer et al. 1996). The data obtained in the Kowie River suggested that stream width, canopy cover and depth (i.e., parameters describing the longitudinal river gradient) may not be sufficient to accurately predict FFG structure, and that the influence of other environmental factors such as temperature and pH (related to vegetation changes, leaf litter decomposition) should be accounted for.

Allochthonous food sources were the most important along the Kowie continuum based on FFG ratios. Researchers using stable isotope analyses in temperate streams have discovered that autochthonous energy sources are more important to all primary consumers than allochthonous sources (Delong and Thorp 2006). Investigating energy sources supporting southern African streams in relation to streams in the rest of the world would test this hypothesis. The next step is to perform stable isotope analysis to determine the assimilation importance of autochthonous versus allochthonous energy sources in the Kowie River (see Chapter 4) to validate the FFG approach.

4 Spatial and temporal changes in the food web structure of a small temperate river

4.1 Introduction

Rivers play a crucial role in human livelihoods by providing ecosystem services. These ecosystems services include water supply for domestic, industrial, agricultural, waste disposal and recreational purposes (e.g. angling; Malmqvist and Rundle 2002). The increase in human populations has led to an increase in the demand for freshwater resources (Malmqvist and Rundle 2002). The rapid increase in human populations has thus created an urgent need to ensure sufficient freshwater for the well-being of the human populous, while at the same time maintaining the integrity of biological diversity of freshwater systems (Strayer 2006). Aquatic ecologists realise that to conserve, manage and restore lotic ecosystems, we need to understand how these systems function, particularly in relation to energy flow and their interactions with riparian areas (Delong and Thorp 2006).

In an effort to understand the functioning of lotic systems, three general concepts of ecosystem functioning are widely cited, each emphasizing different processes that affect the dominant organic matter sources reaching consumers: the River Continuum Concept (RCC; Vannote et al. 1980), the Flood Pulse Concept (FPC; Junk et al. 1989) and the Riverine Productivity Model (Thorp and Delong 1994, 2002). Pertinent details relating to the RCC, RPM and FPC are all extensively described in Chapter 1. The Riverine Ecosystem Synthesis (RES; Thorp et al. 2006) represents the most recent model for riverine food webs, and expounds on the tenets proposed by the RPM and suggests that autochthonous production depends on hydrogeomorphological characteristics of the river (e.g. lateral connectivity and geomorphic complexity). All four concepts have merit, but when and where each one is most applicable remains to be better understood.

Great strides have been made in tracer methodologies for studying food webs (e.g. fatty acid and DNA-based analyses), thus allowing for qualitative or quantitative evaluations of these conceptual models (Sheppard and Harwood 2005). One such method is stable isotope analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), which has been successfully employed in aquatic systems to untangle the importance of different organic matter sources in river food webs (Zah et al. 2001, Zeug and Winemiller 2008). Stable isotope ratios in consumers reflect assimilated food, and thus offer an advantage over gut content data that indicate ingestion only (Chapter 1, Section 1.4). Upon differentiation of the organic matter sources, mixing

models can then be used to elucidate the feasible contributions of sources to consumers (Parnell et al. 2013). These models can allow researchers to quantify nutrient flow and ultimately determine the sources of carbon and nitrogen supporting consumers (Wang et al. 2014).

Our understanding of organic matter sources supporting lotic consumers arises mostly from studies in North America (Delong and Thorp 2006, Zeug and Winemiller 2008), Australia (Bunn and Boon 1993, Bunn et al. 2003, Reid et al. 2008), South America (Jepsen and Winemiller 2002, Hoeinghaus et al. 2007), New Zealand (Pingram et al. 2014) and Asia (Huang et al. 2007, Li and Dudgeon 2008, Wang et al. 2014). Many of the aforementioned studies have described food web issues such as the importance of autochthonous carbon sources in temperate streams (Pingram et al. 2014), the importance of fine transported matter (Thorp et al. 1998), and details about consumer trophic interactions (Zah et al. 2001). However, owing to the limited food web data from African locations, it remains an enigma whether findings of food web structure in temperate and tropical streams apply to rivers in southern Africa.

The aim of this study was to identify the principal carbon sources supporting macroinvertebrate consumers in the Kowie River, South Africa. I determined whether the riverine concepts (namely RCC, FPC and RPM) developed for large temperate rivers hold true in the context of a small temperate river in the southern hemisphere. A vast array of evidence using stable isotope analysis across a range of riverine systems suggests that autochthonous carbon is the primary source supporting riverine food webs. Based on the known dependence of aquatic consumers on autochthonous carbon, I hypothesised that the carbon fuelling consumers is primarily derived from autochthonous food sources across all seasons. Furthermore, I hypothesised that the contribution of autochthonous basal resources to consumers increases in importance as the stream widens (from headwaters to downstream reaches). To address these hypotheses, I (i) used stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to differentiate among organic matter sources, (ii) determined spatial and temporal differences in stable isotope values of basal sources and consumers along the longitudinal river gradient, (iii) estimated the contributions of different organic matter sources to the primary consumers in several locations and periods, and (iv) estimated the potential contributions of organic matter sources to secondary consumers.

4.2 Material and methods

4.2.1 Study area and sample collection

Samples for nitrogen and carbon isotope analysis were collected between September 2012 and September 2013 from six sites along the Kowie River. All sites were part of a larger study investigating energy flow in a small temperate river. At each stream reach, basal resources were sampled when present (Table 4.1): (i) coarse particulate organic matter (CPOM) (ii) abscised C_3 riparian plants, (iii) fine particulate organic matter (FPOM), iv) periphyton (PERI), (v) benthic algae (Benth), (vi) filamentous algae (FA), (vii) macrophytes and (vii) epiphyton (EPI). All basal resources, with the exception of C_3 terrestrial leaves and macrophytes, were sourced from a concurrent study (see Dalu et al. 2014a for a detailed description of sampling methods).

Table 4.1: Basal autochthonous and allochthonous resources sampled for carbon and nitrogen isotope analyses. Codes refer to the organic matter sources (see Figs. 4.1 and 4.2). 'x' denotes sites of collection and '–' denotes where basal resources were absent/not collected.

Organic matter source	code	FW1	FW2	FW3	FW4	FW5	FW6
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 effesus	MACRO1	-	-	-	-	x	-

Invertebrates were collected using a SASS net from November 2012 to September 2013 (Table 4.2; see Chapter 2, Section 2.21). Black fly larvae (*Simulium* spp.) and caddisfly larvae (*Hydropsyche* spp. and *Cheumatopsyche* spp.) represented filter-collectors. Freshwater crabs (*Potamonautes* spp.) represented shredders. Snails (*Physella* spp.), midges (Chironomidae), some groups of mayflies (Baetidae) and cased caddisflies (*Leptocerus* spp.) represented grazers. Small square gill mayflies (*Caenis* spp.*)* and the freshwater shrimp

(*Caridina nilotica*) represented gatherer-collectors. Damselfly nymphs (Coenagrionidae and Chlorolestidae), dragonfly nymphs [Libellulidae, Aeshnidae and Gomphidae (*Paragomphus spp.*)], horsefly larvae (*Tabanus* spp.) and giant water bugs (*Belostoma* spp. and *Apassus* spp.) represented predatory invertebrates.

Table 4.2: Functional feeding groups and taxonomic names of invertebrates collected from the Kowie River from November 2012 to September 2013. x denotes that representative animals were found at the site.

Functional feeding group	Taxon	FW1	FW2	FW3	FW4	FW5	FW6
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	*Potamonautes* spp.	**x**	**x**	**x**	-	-	**x**
	Potamonautes 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**	-

4.2.2 Stable isotope analysis

All materials were ground to a fine homogenous powder, and 0.5 to 1.5 mg of each sample was transferred into small tin capsules (see Chapter 2.2.4 in general methods section for a detailed description of stable isotope analyses). Because the invertebrates were very small, 1 to 3 whole individuals from each of the dominant taxa were combined to create each sample.

4.2.3 Data analysis

All statistical analyses were performed using the Plymouth Routines in Multivariate Ecological Research (PRIMER v 6) statistical programme (Clarke and Gorley 2006). Permutational multivariate analysis of variance (PERMANOVA version 1.03) with Monte Carlo p values was used to test for differences in isotope signatures among food sources (dependent variables were $\delta^{13}C$ and $\delta^{15}N$ values of organic matter sources). Subsequent pairwise comparisons were made for the appropriate factors (source, season, time, and all interaction terms). Additionally, a three way PERMANOVA was used to assess differences in $\delta^{13}C$ and $\delta^{15}N$ values of consumers with functional group, season and time included as main fixed factors. The associated interactions among the three factors were also examined.

To determine the proportional contribution of each carbon source to consumers, I used the package Stable Isotope Analysis in R (SIAR; Parnell et al. 2013). This model is based on a series of related linear equations that utilize Bayesian statistical techniques to identify proportional contributions of source pools (Moore and Semmens 2008, Ward et al. 2010). In contrast to other statistical tools used in stable isotope analysis (e.g., IsoSource), outputs from the Bayesian models are true probability distributions, not just summaries of all feasible solutions (Layman et al. 2012). The mean trophic fractionation used for nitrogen was 2.3‰ (SD = 0.18‰; McCutchan et al. 2003) and for carbon was 0.5‰ (SD = 0.13‰; McCutchan et al. 2003). Although the fractionation factors used here are smaller than those often cited in food web studies (3.4‰ for nitrogen and 1‰ for carbon isotopes), the smaller values correspond to average values extracted from literature specific to stream and riverine systems (Anderson and Cabana 2007, Cremona et al. 2010).

Following the approach recommended by Phillips et al. (2005), I aggregated species according to published functional feeding groups (FFGs; Merritt and Cummins 1996) and similarities of isotopic values (see Table 4.2). To determine the sources to include in each mixing model, I initially graphed source values and consumer isotope values together and discarded sources with very distant values from the consumers (e.g. sources more enriched in ^{15}N than consumers and sources more enriched in ^{13}C than consumers were disregarded;

Phillips et al. 2014). This method ensured that each model was reduced to a three or four source mixing model. Simplifying mixing problems to two-source or three-source problems that can be solved unambiguously with two tracers (Phillips et al. 2005, Newsome et al. 2007) ensured that the models were not underdetermined but that unique and feasible solutions were possible. I excluded FPOM from the mixing models for three reasons: firstly, FPOM is ultimately made up of different components such as epiphyton, terrestrial plants and macrophytes (Hamilton et al. 2005), so it was logical to include only those original components in the models. Secondly, there is evidence that macroinvertebrate taxa can selectively feed on particular components of FPOM (Thorp et al. 1998, Raikow and Hamilton 2001, Clapcott and Bunn 2003), so including it as one category would ignore this selectivity. Thirdly, since the objective of this study was to identify the importance of allochthonous versus autochthonous food (*in situ*) in a temperate river food web, it was important to include sources that fit into one category rather than both. This approach of including only pure sources as end members in mixing models has been advocated for in other studies (*sensu* Hadwen and Bunn 2004, Hadwen et al. 2007, Hadwen and Arthington 2007).

The SIAR model was further used to determine the contributions of organic matter sources to secondary consumers. Predator models were run using the primary consumers (filter-collectors, gatherer-collectors and grazers) as food sources. Because predators consume and assimilate these aquatic prey (different FFGs having different diets themselves), the proportional contributions of allochthonous and autochthonous materials into the predators were re-calculated based on the diets of the herbivores they consumed.

4.3 Results

4.3.1 Isotope values of potential food sources

Stable isotope values of basal resources varied seasonally and among sampling stations, the extent of which was greater for $\delta^{15}N$ than for $\delta^{13}C$ (Table 4.1). No clear seasonal patterns of variation in $\delta^{15}N$ and $\delta^{13}C$ of the different basal resources were observed (Fig. 4.1 and Fig. 4.2). The variation in $\delta^{13}C$ and $\delta^{15}N$ values of the primary producers reinforced the need to collect food sources every season and across sites. Biplots of $\delta^{13}C$ and $\delta^{15}N$ revealed clear separation among the major forms of organic matter collected. For example, riparian leaves were easily distinguishable from aquatic macrophytes, which were substantially more ^{15}N-enriched than riparian leaves. Overall, $\delta^{13}C$ values were all within the same ranges at all the sites, but $\delta^{15}N$ values were lowest in the upstream sites.

The $\delta^{13}C$ values of C_3 riparian leaves ranged from -31.10‰ to -25.63‰. Generally, riparian leaves were lower in $\delta^{13}C$ than all other basal resources across all seasons. The $\delta^{15}N$

values of riparian leaves (range: 1.37‰ to 7.12‰) varied considerably among sites and seasons. Conversely, macrophyte $\delta^{13}C$ (namely *Cyperus eragrotis)* ranged from -31.44‰ to -19.05‰. The $\delta^{15}N$ values of macrophytes (range: 4.50 to 20.11‰) also varied among sites.

A three-way PERMANOVA indicated that values of $\delta^{13}C$ and $\delta^{15}N$ differed significantly among sources (PERMANOVA, N = 528, $p < 0.01$; Table 4.3). There were significant differences in the $\delta^{13}C$ values among all sources, but there were no significant differences between $\delta^{13}C$ of riparian leaves and macrophytes (PERMANOVA post-hoc pairwise test: $t = 0.96$, $p = 0.34$) across sites. Similarly, there were significant differences of $\delta^{15}N$ among basal resources. Relevant pairwise comparisons indicated that organic matter sources differed in $\delta^{13}N$ values across sites and time, with samples collected in early spring and winter months of 2013 being more similar than those collected across the other seasons. There were significant interactions among the different sources, sites and seasons (Table 4.3). Overall, aquatic sources (epiphyton, benthic algae, periphyton and aquatic macrophytes) were more variable over space and time than terrestrial sources (riparian leaves).

Table 4.3: Permutational ANOVA on stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of basal resources collected at the Kowie River, September 2012 to September 2013. Asterisks show statistically significant effects (* $p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$). The factors were site (six sampling sites), source (six basal resources) and time (four seasons).

Source of variations	Permanova							
	$\delta^{13}C$				$\delta^{15}N$			
	df	SS	MS	Pseudo-F	df	SS	MS	Pseudo-F
source [a]	5	1430.40	286.08	211.85***	5	1772.40	354.48	638.26**
site[b]	5	66.10	13.22	9.79**	5	2867.50	573.50	1032.60**
time[c]	3	35.20	11.74	8.69**	3	26.59	8.86	15.96**
source x site	23	569.84	24.78	18.35**	23	495.86	21.56	38.82**
source x time	15	430.62	28.71	21.26**	15	411.86	27.46	49.44**
site x time	15	219.46	14.63	10.83**	15	299.66	19.98	35.97**
source x site x time	69	1001.10	14.51	10.74**	69	1174.30	17.02	30.64**
residual	298	402.41	1.35		298	165.51	0.56	
total	433	4175.20			433	8198.20		

[a] source: epiphyton, benthic algae, periphyton, riparian leaves, FPOM, aquatic macrophytes, [b] site: FW1, FW2, FW3, FW4, FW5, FW6 [c]time: late spring, late summer, winter, early spring

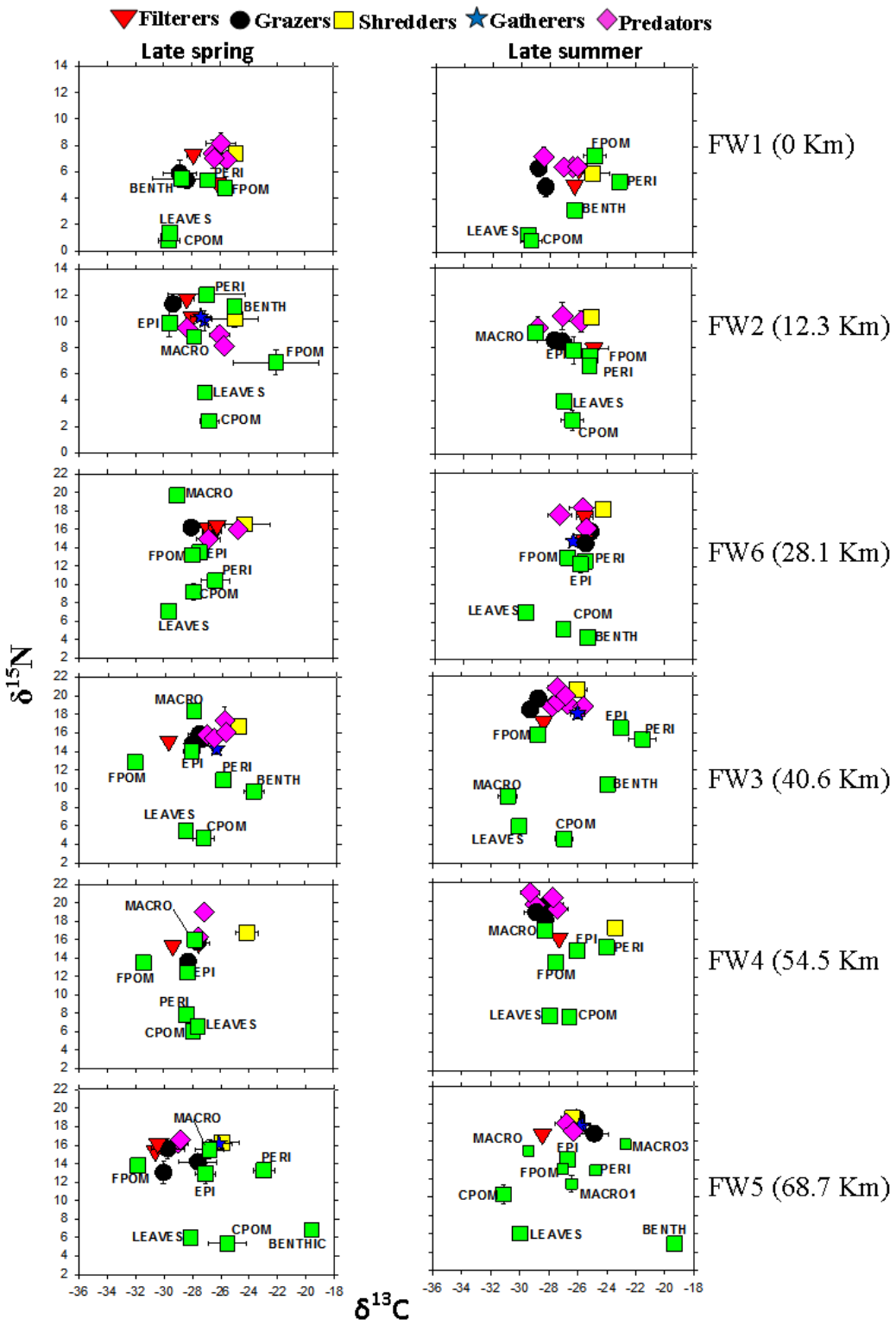

Figure 4.1: Mean stable isotopic ($\delta^{13}C$ and $\delta^{15}N$) values (‰; error bars denote standard deviations) of potential food sources (green boxes) and consumers from the Kowie River, November 2012 to December 2013. 'Km' denotes the distance in kilometres of the stream measured from the headwaters. Consumers include filterer-collectors, gatherer-collectors, grazers, shredders and predators. Note: FW1 and FW2 are drawn to a different scale on the $\delta^{15}N$ axis.

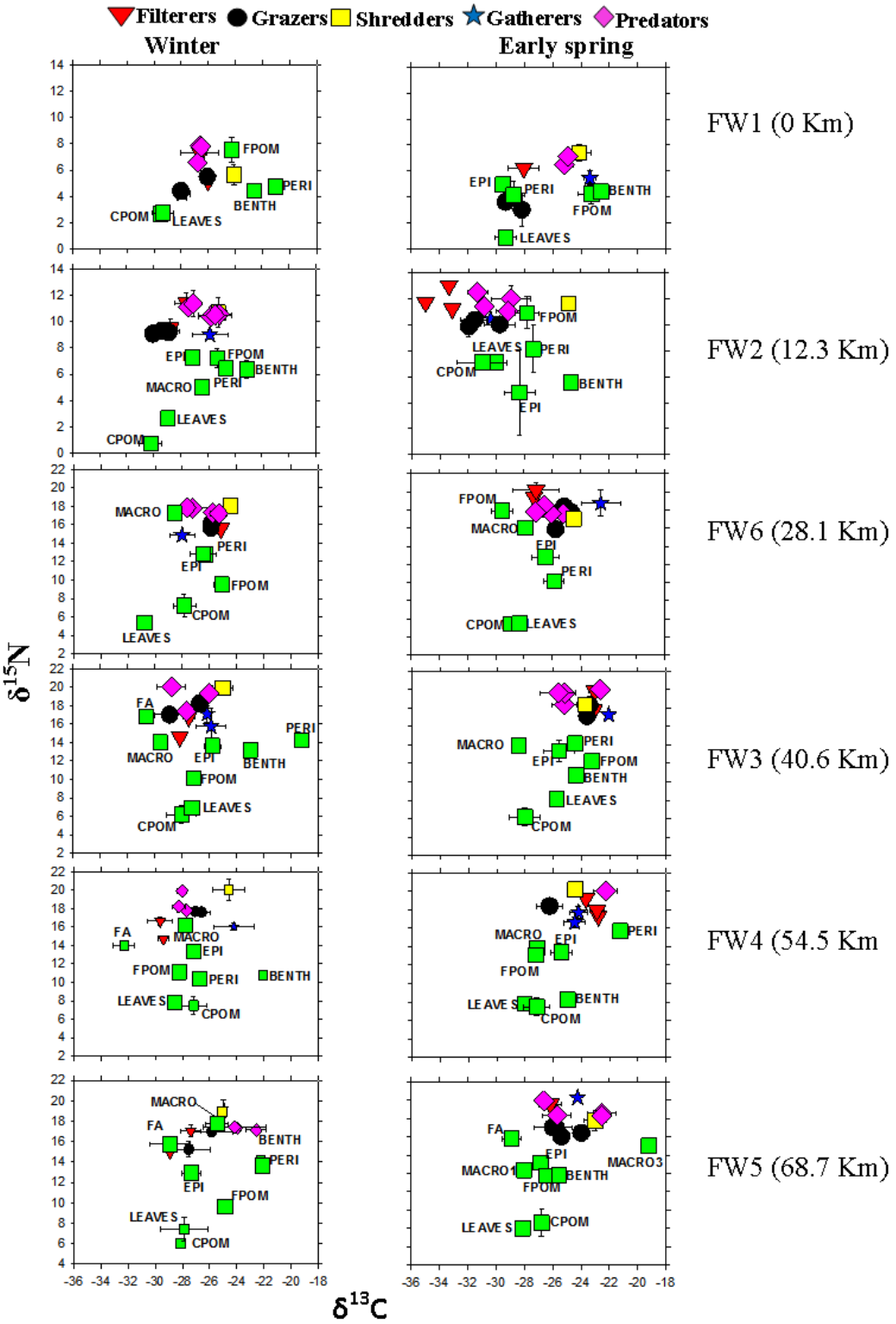

Figure 4.2: Mean stable isotopic ($\delta^{13}C$ and $\delta^{15}N$) values (‰; error bars denote standard deviations) of potential food sources (green boxes) and consumers from the Kowie River, November 2012 to December 2013. 'Km' denotes the distance in kilometres of the stream measured from the headwaters .Consumers include filterer-collectors, gatherer-collectors, grazers, shredders and predators. Note: FW1 and FW2 are drawn to a different scale on the $\delta^{15}N$ axis.

4.3.2 Isotope values of consumers

Invertebrate isotope values were variable among species, but encompassed narrow ranges overall, relative to those of primary producers. At stations FW1 and FW2 (which represent the headwater reaches of the stream), consumers exhibited notably lower values in $\delta^{13}C$ (range: -35.05 to -23.38‰) and $\delta^{15}N$ (range: 3.04 to 12.54‰) than those recorded downstream. Most consumers exhibited considerable variation in both $\delta^{13}C$ and $\delta^{15}N$ (e.g. baetid grazers) over space and time. Conversely, caddisfly larvae (*Cheumatopsyche afra*) at both sites did not show considerable variation in $\delta^{13}C$ and $\delta^{15}N$ across all seasons. While isotopic values in most of the consumers in the upper reaches fell well within the ranges of the food sources, filter feeders in early spring at FW2 (range: -35.05 to -33.14‰) fell outside the carbon range of the sources. At station FW3, the range of $\delta^{13}C$ was -30.11 to -22.07‰ for all invertebrate consumers, whereas $\delta^{15}N$ ranged from 14.77 to 20.77‰. The horsefly (*Tabanus* spp.) exhibited a broader range in $\delta^{15}N$, whereas a narrow range of $\delta^{15}N$ values characterized the filtering black fly (*Simulium* spp.) across all seasons. At station FW4, the $\delta^{13}C$ values of the consumers spanned a wide range of values among species (range: -29.63 to -21.32‰). The $\delta^{13}C$ values, however, were similar in most organisms across seasons. Similarly, the $\delta^{15}N$ of consumers spanned a range from 13.60 to 20.95‰. The predator Tabanidae (*Tabanus* spp.) showed the highest $\delta^{15}N$ values, while the grazing mayfly (*Castanophlebia calida*) showed lower $\delta^{15}N$ (13.60‰). At station FW5 (downstream site), $\delta^{13}C$ values of most invertebrate taxa were in the range of -29.74‰ to -22.10‰. The freshwater crab (*Potamonautes sydneyi*) showed the highest value (average -25.08‰ ± 1.60) during the winter and early spring months of 2013, while the filtering black fly (*Simulium* spp.*)* showed the lowest $\delta^{13}C$ values (average -28.50 ± 1.66). At FW5, the $\delta^{15}N$ of consumers showed a wide range of values among species (range: 13.03 to 20.27‰). Some taxa such as amphipods and the freshwater shrimp (*Caridina nilotica*) had wide ranges of $\delta^{15}N$ values that varied over the sampling period. The values of $\delta^{13}C$ and $\delta^{15}N$ at FW6 varied broadly across seasons. For example, the caddisfly (*Cheumatopsyche afra*) was depleted in ^{13}C and ^{15}N during late spring to winter months and more enriched in early spring. At station FW6 (the site in the main tributary that drains into the Kowie River that was similar to FW3 in the main Kowie channel), consumers exhibited $\delta^{13}C$ values ranging from -30.30 to -22.55‰, while $\delta^{15}N$ values ranged from 14.44 to 20.18‰ for all consumers

Carbon values were influenced by season, site, and functional group ($p < 0.001$; $N = 676$; Table 4.4). Functional feeding group, with the greatest mean square, was the variable that explained the greatest variation in $\delta^{13}C$ amongst consumers. There were significant effects of functional feeding groups in $\delta^{13}C$ values at each site, but not between filterers and

grazers (PERMANOVA post-hoc pairwise test: t = 1.54, p = 0.25). The second most influential factor was season, and $\delta^{13}C$ values in consumers varied with season ($p < 0.05$). Subsequent pairwise comparisons indicated that there were no significant differences between late summer and winter (t = 0.15, p = 0.88). Site was the third most influential variable on isotope values in consumers. Pairwise comparisons showed that there were no significant differences between FW4 and FW5 (t = 0.77, p = 0.46), FW3 and FW5 (t = 0.74, p = 0.75), FW1 and FW5 (t = 0.74, p = 0.75), FW3 and FW4 (t = 0.43, p = 0.66), FW3 and FW6 (t = 1.29, p = 0.21). There were significant interactions among all the factors (Table 4.4), indicating that these factors shifted together. Nitrogen values in consumers by season, site, functional group and all corresponding interactions were all significant (PERMANOVA, N= 676, $p < 0.001$, Table 4.4), with site explaining the highest degree of variation overall. The $\delta^{15}N$ values of consumers varied by sampling site, and specimens at FW1 were significantly lower in $\delta^{15}N$ than all other sites (FW1 = 2.06 to 9.10‰). There were no significant differences in consumer $\delta^{15}N$ values between FW4 and FW5 (PERMANOVA post-hoc pairwise test: t = 1.42, p = 0.16). Nitrogen values also varied by season, but there were no significant differences between consumers in late summer and winter (t = 0.04, p = 0.97). The functional group was the third most influential variable on the $\delta^{15}N$ isotope values of consumers. Pairwise tests of the factor "functional feeding group" on $\delta^{15}N$ showed that there were significant differences among functional groups, but no significant differences in $\delta^{15}N$ between predators and shredders (t = 1.08, p = 0.28), or grazers and gatherers (t = 1.36, p = 0.18).

Table 4.4: Permutational ANOVA on stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of functional feeding groups (FFGs) collected in the Kowie River, November 2012 to September 2013. Asterisks show statistically significant effects (* $p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$). The factors were six sampling stations (site), FFGs, and four seasons (time).

Source of variations	Permanova 2							
	$\delta^{13}C$				$\delta^{15}N$			
	df	SS	MS	Pseudo-F	df	SS	MS	Pseudo-F
FFG[a]	4	482.37	120.59	110.06***	4	221.23	55.307	60.636***
site[b]	5	163.11	32.622	29.773***	5	8666.6	1733.3	1900.3***
time[c]	3	116.27	38.755	35.371**	3	194.14	64.713	70.948***
FFG x site	20	115.43	5.7716	5.2675**	20	162	8.1002	8.8806**
FFG x time	12	65.434	5.4529	4.9766**	12	96.064	8.0054	8.7767**
site x time	15	555.9	37.06	33.823**	15	263.85	17.59	19.285**
FFG x site x time	54	481.95	8.9251	8.1456**	54	192.69	3.5683	3.9122**
residual	562	615.78	1.0957		562	512.61	0.91212	
total	675	3140			675	14403		

[a] functional group: gatherers, grazers, filterers, shredders, predators, [b]site: FW1, FW2, FW6, FW3, FW4, FW5, [c]time: late spring, late summer, winter, early spring

4.3.3 Carbon sources supporting primary consumers

Estimates derived from mixing models suggested there were important contributions of autochthonous carbon sources (epiphyton and macrophytes) to the diets of grazers (Table 4.5). Epiphyton contributed up to 42% of grazer diets at FW5 during the late spring of 2012. Highest contributions of epiphyton to grazers (57%) occurred during late summer. At site FW4, the contribution of macrophytes (*Cyperus eragrotis*) to grazers increased in the winter and late summer months, with macrophytes contributing up to 31 to 88% of grazer diets.

Periphyton and benthic algae did not contribute to the diets of most grazers. One exception was at the headwaters, where grazers assimilated benthic algae and periphyton (range 2 to 40%) throughout the sampling period. While terrestrial carbon was not important to grazers in the lower reaches, contributions were greater in the late summer months at FW1, FW2 and FW3. Terrestrial contributions to consumers showed a gradual shift from the headwater (FW1) to the downstream reaches (FW5). In the headwaters, which were characterised by high canopy cover (72%), leaves contributed substantially (≥ 50%) to grazer diets. The highest consumption of terrestrial organic matter occurred in grazers at the headwaters, which consumed up to 96% of terrestrially-derived organic matter. Filamentous algae found only during the winter and early spring months of 2013, contributed to the diets of grazers at FW3 (53% in winter) and FW5 (ranging from 44% during winter and 53% in early spring; Table 4.5).

Table 4.5: Mean percentage contributions (95% credibility intervals displayed in parentheses) of organic matter sources to grazers in the Kowie River derived from Stable isotope in R (SIAR) models. The major basal resources with lower 95% confidence intervals greater than 1% are bolded. "No solution" indicates that no solution was possible as the consumers fell outside the convex hull implied by the sources.

		Terrestrial	Macrophyte	Epiphyton	Benthic algae	Periphyton	Filamentous algae
FW1							
	Late spring	**43 (13-70)**			33 (0-63)	24 (0-48)	
	Late summer	**39(21-56)**			31(0-66)	**30(11-47)**	
	Winter			**NO SOLUTION**			
	Early spring	**24 (1-47)**			27(0-56)	**49 (15-87)**	
FW2							
	Late spring	**16(1-30)**	**32(3-56)**	**52 (33-71)**			
	Late summer	**44 (23-64)**	9 (0-23)	16 (0-37)		**32 (1-56)**	
	Winter	**33 (1-56)**	23 (0-49)	**43 (2-83)**			
	Early spring			**NO SOLUTION**			
FW6							
	Late spring	11(0-36)		**73(32-100)**		16(0-44)	
	Late summer	19 (0-52)		**73 (33-100)**	8 (0-28)		
	Winter	4 (0-14)	24 (0-47)	**71 (41-98)**			
	Early spring	11 (0-36)	**66 (29-99)**	23 (0-51)			
FW3							
	Late spring	17 (0-37)		**62 (38-83)**		21 (0-44)	
	Late summer			**NO SOLUTION**			
	Winter	10 (0-23)	**60 (45-74)**	**30 (16-43)**			
	Early spring	9 (0-30)	28 (0-62)	**63 (26-99)**			
FW4							
	Late spring	13 (0-26)	**42 (20-66)**	**45 (8-79)**			
	Late summer	**30 (20-38)**	**54 (29-71)**	16 (0-45)			
	Winter	16 (0-30)	20 (0-39)	18 (0-39)			**46 (30-60)**
	Early spring	6 (0-19)		**47 (27-63)**		**47 (36-58)**	
FW5							
	Late spring	10 (0-20)	**47 (21-76)**	**42 (5-74)**			
	Late summer	**24 (2-44)**	**43(10-76)**	**33 (15-50)**			
	Winter	17(0-33)		30(0-56)			**53(31-76)**
	Early spring	19 (0-44)	14 (0-29)	28 (0-54)			**38 (8-70)**

Autochthonous carbon (namely epiphyton and macrophytes) also contributed to filterer diets. However, riparian organic carbon was of some importance to filter-collectors (caddisfly and black fly larvae; see Table 4.6) in the upper reaches (FW1 and FW2; 35% to 96% across all seasons). The model results suggested that autochthonous carbon (macrophytes and epiphyton) made major contributions to filter-collectors at downstream sites (FW4 and FW5), middle reaches (FW3) and in the main tributary (FW6) over all the

seasons. Autochthonous carbon contributions were more than 70% of filterer diets at the downstream and middle reaches. Filamentous algae, which was collected only during two months, was also clearly assimilated by the downstream filterers (53% assimilated in winter and 38% in spring).

Based on calculations from the SIAR model (Table 4.7), terrestrial organic matter made relatively small contributions to gatherer-collector diets in the downstream (4-17%) and middle reaches (FW3; 14-22%). Terrestrial organic matter was more important in the headwater reaches (FW2), with the highest contribution occurring in the spring months (30% of gatherer diets). Gatherer collectors were not found at FW1 in most seasons, except for spring when benthic algae was the major energy source for gatherers (69% of the total diet). Autochthonous carbon was the main energy source (contributing more than 74% towards consumer diets across all seasons) fuelling gathering consumers in the tributary (FW6). Generally, autochthony was important at all sites, although there was a shift from a high dependence on terrestrial organic matter in the headwaters and autochthony increasing in importance in the downstream direction.

Model estimates showed that shredders also derived most of their energy from autochthonous sources at all the sites (Table 4.8). Terrestrial carbon was important to shredders in the headwaters (contributions of 10 – 32%), with the highest contributions recorded at FW1 (winter and late summer) and FW2 (late spring). While no solutions could be generated for some of the sites, the general pattern showed the importance of autochthony to shredders, with terrestrial organic matter playing a major role at the headwater sites.

Table 4.6: Mean percentage contributions (95% credibility intervals displayed in parentheses) of organic matter sources to filterers in the Kowie River from Stable isotope in R (SIAR) models. The major basal resources with a lower 95% confidence interval greater than 1% are shown in bold.

		Terrestrial	Macrophyte	Epiphyton	Benthic algae	Periphyton	Filamentous algae
FW1							
	Late spring	**59 (54-65)**			**23 (2-40)**	18 (0-37)	
	Late summer	**77 (43-96)**			15 (0-41)	8 (0-22)	
	Winter	**96 (90-100)**			2 (0-6)	2 (0-7)	
	Early spring	**35 (1-65)**			25 (0-47)	**40 (1-76)**	
FW2							
	Late spring	10 (0-25)	37 (0-73)	**52 (18-92)**			
	Late summer	**51 (41-59)**	**30 (11-46)**	19 (0-43)			
	Winter	5 (0-11)	8 (0-18)	**87 (78-95)**			
	Early spring	**88 (72-100)**	7 (0-19)	5 (0-16)			
FW6							
	Late spring	18 (0-49)		**73 (36-100)**		9 (0-29)	
	Late summer	16 (0-43)		**67 (22-100)**	17 (0-47)		
	Winter	5 (0-14)	**24 (4-64)**	**71 (41-94)**			
	Early spring	4 (0-11)	**70 (49-90)**	**26 (1-48)**			
FW3							
	Late spring	10 (0-20)		**79 (66-91)**		12 (0-24)	
	Late summer	**38 (1-69)**	**48 (18-48)**	**14 (8-21)**			
	Winter	15 (0-32)	32 (0-59)				**53 (31-77)**
	Early spring	17 (0-46)	35 (0-69)	**48 (8-92)***			
FW4							
	Late spring	21 (0-38)	**31 (2-54)**	**48 (11-91)**			
	Late summer	5 (0-11)	**88 (74-99)**	7 (0-21)			
	Winter	4 (0-10)	**76 (62-91)**	**20 (1-36)**			
	Early spring	20 (0-48)	**45 (4-86)**	**35 (1-62)**			
FW5							
	Late spring	10 (0-20)	**47 (21-77)**	**42 (4-74)**			
	Late summer	11 (0-34)	32 (0-68)	**57 (17-98)**			
	Winter	12 (0-28)		**43 (3-77)**			**44 (15-74)**
	Early spring	4 (0-10)	13 (0-32)	**30 (1-54)**			**53 (34-74)**

*periphyton and epiphyton combined

Table 4.7: Mean percentage contributions (95% credibility intervals displayed in parentheses) of organic matter sources to gatherers in the Kowie River from Stable isotopes in R (SIAR) models. The major basal resources with a lower 95% confidence interval greater than 1% are shown in bold "No sample" indicates that there were no taxa that fell into the designation of gatherer-collector.

		Terrestrial	Macrophyte	Epiphyton	Benthic algae	Periphyton
FW1						
	Late spring		**NO SAMPLE**			
	Late summer		**NO SAMPLE**			
	Winter		**NO SOLUTION**			
	Early spring	14 (0-46)			**69(22-100)**	17 (0-50)
FW2						
	Late spring	**30 (15-44)**	**49 (20-80)**	21 (0-41)		
	Late summer		**NO SAMPLE**			
	Winter	10(0-31)	26(0-56)	**64(27-93)**		
	Early spring	**65 (19-99)**	15 (0-48)	20 (0-52)		
FW6						
	Late spring		**NO SAMPLE**			
	Late summer	**22 (5-45)**	**18 (4-34)**	**60 (23-85)**		
	Winter	**26(14-40)**	**38(18-59)**	**36(5-63)**		
	Early spring	8(0-26)	**57(23-90)**	34(0-64)		
FW3						
	Late spring	10 (0-27)		**44 (23-65)**		**46 (21-68)**
	Late summer	22 (0-47)	22 (0-44)	**56 (38-73)**		
	Winter	14 (0-40)	23 (0-46)	**64 (35-89)**		
	Early spring	17(0-45)	32(0-65)			**52(13-95)**
FW4						
	Late spring		**NO SAMPLE**			
	Late summer		**NO SAMPLE**			
	Winter	17(0-32)	**43 (15-71)**	**40 (1-76)**		
	Early spring	11 (0-35)	**37 (0-74)**	**52 (13-96)**		
FW5						
	Late spring	13 (0-35)	**40 (8-70)**	**47 (11-83)**		
	Late summer	4 (0-12)	33 (0-72)	**63 (24-100)**		
	Winter	14 (0-43)	**64 (23-94)**	22 (0-51)		
	Early spring		**NO SAMPLE**			

Table 4.8: Mean percentage contributions (95% credibility intervals displayed in parentheses) of organic matter sources to shredders in the Kowie River from Stable isotopes in R (SIAR) models. The major basal resources with a lower 95% confidence interval greater than 1% are shown in bold. "No solution" indicates that no solution was possible as the consumers fell outside the convex hull implied by the sources.

		Terrestrial	Macrophyte	Epiphyton	Benthic algae	Periphyton	Filamentous algae
FW1							
	Late spring	10 (0-21)			**39 (1-72)**	**51 (15-93)**	
	Late summer	**23 (2-41)**			**37 (2-70)**	**40 (22-59)**	
	Winter	**28 (18-41)**			**41 (12-71)**	31 (0-60)	
	Early spring			**NO SOLUTION**			
FW2							
	Late spring	**32 (10-52)**	**36 (1-68)**	**33 (1-58)**			
	Late summer	16 (0-39)	16 (0-41)	28 (0-54)		**40 (3-77)**	
	Winter			**NO SOLUTION**			
	Early spring	24 (0-55)	**50 (10-96)**	26 (0-57)			
FW6							
	Late spring	28 (0-58)		**43 (2-83)**		29 (0-60)	
	Late summer			**NO SOLUTION**			
	Winter	26 (0-52)	**35 (1-63)**			**39 (1-75)**	
	Early spring	8 (0-26)	**57 (23-90)**	34 (0-64)			
FW3							
	Late spring	13 (0-32)	**38 (23-53)**	25 (0-48)		**24 (1-45)**	
	Late summer	25 (0-49)	24 (0-47)	**50 (30-69)**			
	Winter			**NO SOLUTION**			
	Early spring	11 (0-38)	20 (0-54)			**69 (27-100)***	
FW4							
	Late spring	9(0-21)	**64(40-86)**	27(0-50)			
	Late summer	3 (0-9)	9 (0-27)	26 (0-58)		**61 (27-97)**	
	Winter			**NO SOLUTION**			
	Early spring			**NO SOLUTION**			
FW5							
	Late spring	13 (0-37)	**43 (11-73)**	**44 (7-76)**			
	Late summer	19 (0-47)	32 (0-66)	**49 (8-93)**			
	Winter	14 (0-39)	**44 (7-83)**	19 (0-44)			22 (0-47)
	Early spring	19 (0-45)	**44 (26-63)**	**37 (6-61)**			

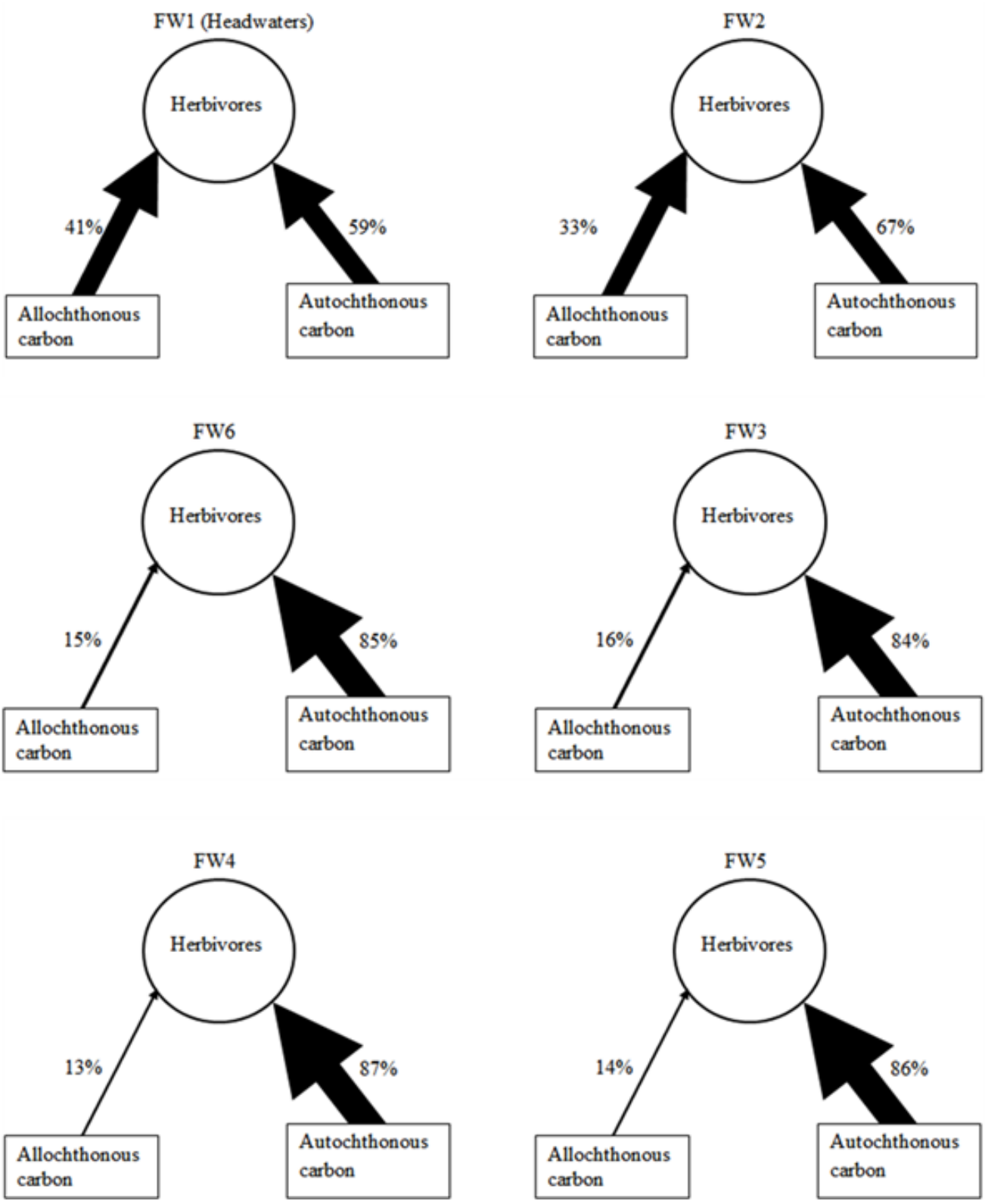

Figure 4.3: Schematic of organic matter sources supporting primary consumers in the Kowie River between November 2012 and September 2013. Contributions are derived from averaging mean contributions of all primary consumers.

4.3.4 Carbon sources supporting secondary consumers

Autochthonous carbon (adjusted using primary consumer diets) was important for all secondary consumers in the Kowie River (Table 4.10 and Fig. 4.4). Terrestrial carbon was important in the headwater site (Table 4.10, Fig. 4.4 contribution of 43%), while autochthony increased in importance in the downstream direction (Fig. 4.4, contribution of 88%). Most consumers fed on grazers that were dependent on autochthonous carbon sources (Table 4.9). The substantial contributions of autochthonous carbon to secondary consumers were notable throughout all the seasons, with a gradual increase in assimilation of autochthonous carbon from headwaters (57%) to the downstream sites (88%). Terrestrial carbon increased in importance during late summer, particularly in the upstream reaches (FW1 and FW2).

Table 4.9: Mean percentage contributions (95% credibility intervals displayed in parentheses) of prey to the diets of predacious insects in the Kowie River from Stable isotope in R (SIAR) models. The major basal resources with a lower 95% confidence interval greater than 1% are shown in bold.

		Filterers	Grazers	Gatherers
FW1				
	Late spring 2012	31 (0-59)	**69 (41-100)**	
	Late summer 2013	**54 (36-72)**	**46 (28-64)**	
	Winter 2013	37 (0-78)	**63 (22-100)**	
	Early spring 2013	7 (0-22)	**93 (78-100)**	
FW2				
	Late spring 2012	34 (0-67)	**66 (33-100)**	
	Late summer 2013	**44 (23-63)**	**56 (37-77)**	
	Winter 2013	**65 (51-80)**	**28 (7-47)**	7 (0-17)
	Early spring 2013	**24 (1-47)**	**40 (14-64)**	**36 (18-53)**
FW6				
	Late spring 2012	**59 (16-100)**	41 (0-84)	
	Late summer 2013	**52 (28-77)**	**48 (23-72)**	
	Winter 2013	20 (0-43)	24 (0-48)	**56 (37-76)**
	Early spring 2013	12 (0-35)	**88 (65-100)**	
FW3				
	Late spring 2012	13 (0-31)	28 (0-56)	**59 (30-89)**
	Late summer 2013	**51 (23-81)**	**49 (19-77)**	
	Winter 2013	18 (0-41)	**52(23-83)**	30 (0-56)
	Early spring 2013	**47 (7-85)**	**53 (15-83)**	
FW4				
	Late spring 2012	27 (0-58)	16 (0-43)	**57 (20-97)**
	Late summer 2013	**42 (8-71)**	**58 (29-92)**	
	Winter 2013	12 (0-30)	21 (0-49)	**67(33-98)**
	Early spring 2013	**4 (0-13)**	**59 (21-98)**	37 (0-73)
FW5				
	Late spring 2012	**86 (62-100)**	14 (0-38)	
	Late summer 2013	**65 (46-84)**	**35 (16-54)**	
	Winter 2013	16 (0-33)	**45 (18-72)**	**39 (19-59)**
	Early spring 2013	**41 (21-61)**	**59 (39-79)**	

Table 4.10: Mean percentage carbon contributions to predatory consumer biomass estimated using SIAR models of consumed prey. No solution" indicates that no solution was possible.

		Terrestrial	Macrophyte	Epiphyton	Benthic algae	Periphyton	Filamentous algae
FW1							
	Late spring	45.24		31.6		23.16	
	Late summer	52.3		25.4		22.3	
	Winter	**NO SOLUTION**					
	Early spring	30.49		25.82		43.69	
FW2							
	Late spring	22.36	43.04	34.33			
	Late summer	46.94	17.82	17.26		17.98	
	Winter	14.23	23.21	62.35			
	Early spring	**NO SOLUTION**					
FW6							
	Late spring	13.87		13.13		73	
	Late summer	17.56		12.32	70.12		
	Winter	16.56	31.84	51.4			
	Early spring	4.84	69.52	25.64			
FW3							
	Late spring	10.91		56.14			33.23
	Late summer	**NO SOLUTION**					
	Winter	13.8	34.34	24.6		27.56	
	Early spring	13.24	31.71	55.05			
FW4							
	Late spring	18.28	34.74	46.98			
	Late summer	16	73.04	10.96			
	Winter	12.71	37.29	20.1			29.9
	Early spring	13.4	42.6	44			
FW5							
	Late spring	10	47	43			
	Late summer	16.98	37.06	45.96			
	Winter	15.15	18.9	49.3			16.28
	Early spring	17.95	13.93	28.14			39.05

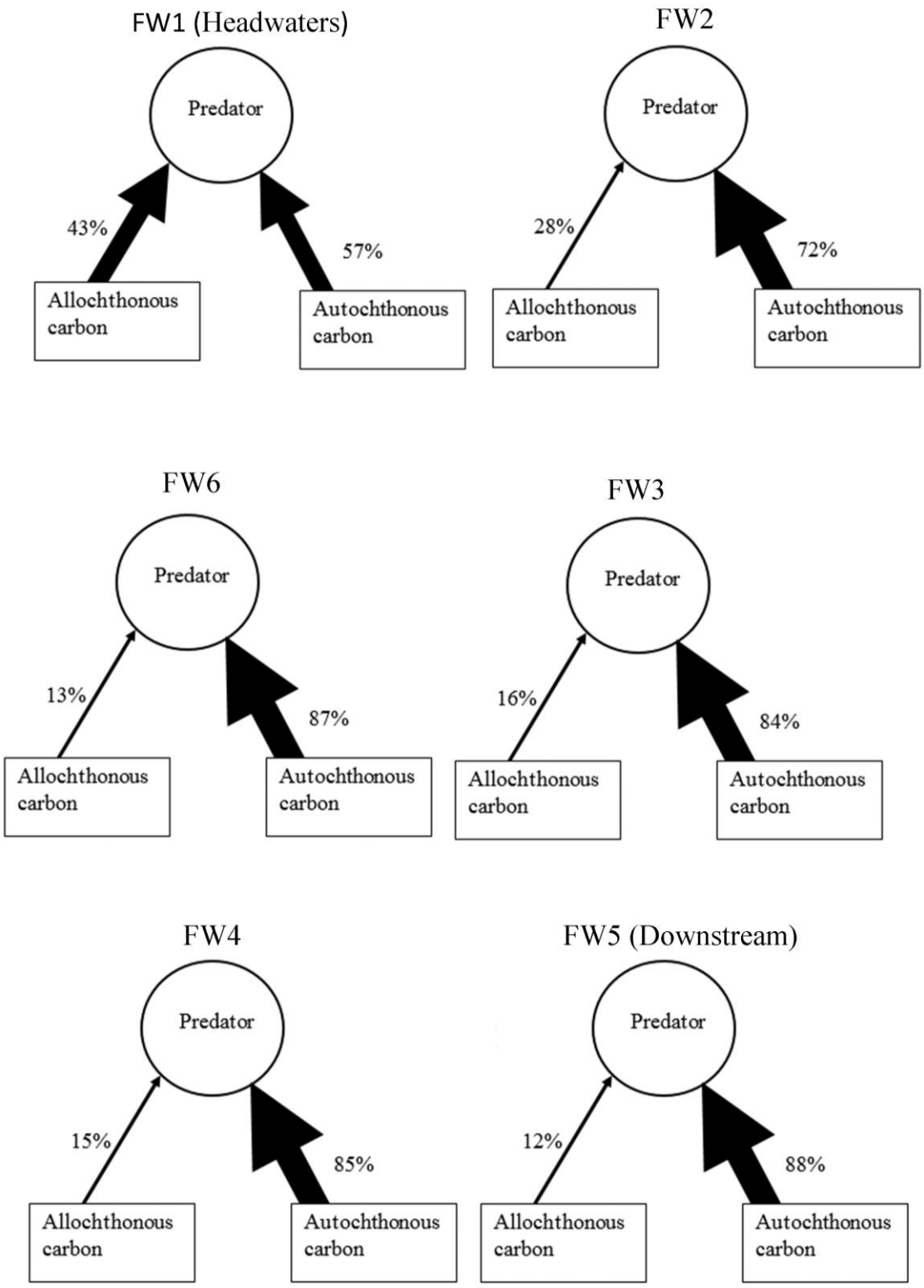

Figure 4.4: Schematic of organic matter sources supporting secondary consumers in the Kowie River between November 2012 and September 2013. Contributions are derived from adjusted SIAR models of primary consumers.

4.4 Discussion

In this study, trophic relationships of benthic invertebrates within the Kowie River were examined. Carbon and nitrogen values were interpreted within the context of the FFGs, collection times and locations. Temporal patterns in carbon and nitrogen values of primary producers and the contributions of these primary producers to primary and secondary consumers were also considered. While both $\delta^{13}C$ and $\delta^{15}N$ values of basal resources varied widely among sites and seasons, it was possible to discriminate between allochthonous and autochthonous food sources using a site-oriented approach. The $\delta^{13}C$ signatures of invertebrates analysed were usually within the range of potential food sources at their respective sites. While the SIAR mixing models identified autochthonous (mainly macrophytes and attached algae) organic matter as the major food source fuelling consumers at most sites, allochthonous organic matter was an important food source in the headwater reaches (Tables 4.5 to 4.8). There was a gradual shift from assimilation of allochthonous to autochthonous food from the headwater region to downstream sites in all of the FFGs.

4.4.1 Temporal and spatial changes in isotopic values of basal resources

First, I considered the effect of basal resource type, site and season on the carbon and nitrogen values recorded in the Kowie River. Basal resources collected in the Kowie River varied isotopically over time and among sites (Table 4.3). Carbon isotope values of riparian vegetation (-31.10‰ to -25.63‰) were within the range of values recorded in temperate large rivers from the Americas (~ -31 to -27; Fry 1991, Thorp et al. 1998) and in New Zealand (~ -29.7‰ to -25.1‰; Hicks 1997). Similarly, aquatic macrophytes (range: -31.44‰ to -19.05‰) fell within the ranges derived from other freshwater systems (e.g. Thorp et al. 1998). Similarities in range of carbon values between aquatic macrophytes and riparian plants are typical, as these are both plants that utilise the C_3 photosynthetic pathway (Fry 2006). While aquatic macrophytes are considered autochthonous producers in rivers (by some researchers), they are able to utilise both dissolved and atmospheric carbon dioxide (Madsen and Sand-Jensen 1991). As such, it was not surprising that there was overlap in carbon isotope values in the riparian and aquatic macrophytes in the Kowie system. The differences that did occur between macrophytes and terrestrial leaves at some of the sites may have been due to shifting environmental factors that had variable effects on the physiology of the primary producers. The environmental factors involved probably included flow velocity, water turbidity and temperature (Finlay et al. 1999, Finlay 2001), although the first two would affect only aquatic plants. Furthermore, such factors change with season and

catchment area (Finlay et al. 2002), thus contributing to the temporal and spatial variability in plant isotope signatures observed in the Kowie River and other lotic systems.

Aquatic macrophytes were elevated (range: 4.50‰ to 20.11‰) in $\delta^{15}N$ at all the sites compared with terrestrial plants (range: 0.67‰ to 12.43‰), congruent to findings in large rivers (e.g. Thorp et al. 1998). Emergent aquatic macrophytes (especially in downstream regions) showed high $\delta^{15}N$ values (> 10‰) that varied spatially and temporally, while terrestrial leaves were not as varied among seasons. High values of $\delta^{15}N$ above 10‰ in aquatic plants are typically associated with rivers that have greater supplies of nitrogen from agricultural activities (Chang et al. 2002, Anderson and Cabana 2005). In the Kowie, values of most basal resources (except for terrestrial leaves) were higher than 10‰, indicating that the nitrogen in the streams is possibly arising from agricultural inputs. The depleted nitrogen values in riparian plants enabled the differentiation between terrestrial and aquatic sources (isotope values of algal sources are discussed in Dalu et al. 2015b).

4.4.2 Temporal and spatial changes in isotope values of consumers

Second, I examined the effects of FFG, site and season on the carbon and nitrogen values in the Kowie River. Isotope values of consumers changed seasonally and among sites in the Kowie River (Table 4.4). Similar types of variations have been observed in other studies on riverine food webs (e.g. Reid et al. 2008). The changes in isotopic values resulted from variation in the relative use of different basal resources by consumers (Fry 2006). The $\delta^{13}C$ value of consumers showed significant differences among areas within the Kowie River, indicating that different carbon sources supported the consumers living along the length of the system. Consumers assimilated basal resources collected in the Kowie River, as evidenced by the consumers having similar values to the basal resources collected. However, during the spring months (at FW3), isotopic values of the filterers fell outside the range of the basal resources (-33.14 ‰ to -35.05 ‰ for filterers versus - 31 ‰ to -22 ‰ for basal resources). This mismatch between consumer and food suggested that there was a food source that was not collected, or there was a lag time in incorporation of carbon into the tissues of the filterers (Martínez del Rio et al. 2009).

Site explained most of the variation in nitrogen signatures in consumers inhabiting the Kowie River (Table 4.4). There were significant (< 1‰ within site and > 4‰ among some sites) 'within site' differences in $\delta^{15}N$ of consumers during the sampling period. Consumers showed a more conservative enrichment (< 2.3‰ between most FFGs and their food; Figs 4.1 and 4.2) compared to the widely cited enrichment factor of 3.4‰ (Post 2002). The discrepancies in the fractionation factors could arise from the differences in physiology of consumers and their diets (Jardine et al. 2014). For instance, McCutchan et al. (2003) noted

that fractionation factors of 3.4‰ are common in fish that are normally fed on a high protein diet.

Generally, nitrogen values in consumers in the Kowie River increased from headwaters to downstream (range: 3.54 to 21.26‰; Fig 4.1, Fig 4.2). The nitrogen value of consumers at a particular site is related to the nitrogen loading and may point to the source of pollution (Anderson and Cabana 2005), which is most likely from agricultural run-off in the Kowie River (Dalu et al. 2015b); this loading increased the $\delta^{15}N$ of basal resources downstream and ultimately the values of consumers at each site.

Season also had an effect on the $\delta^{15}N$ of consumers in the Kowie River, with significant differences revealed except between late summer and winter. The change in nitrogen values could be reflective of trophic shifts in consumers over time (Anderson and Cabana 2007) or changes in abiotic and biotic factors like denitrification (Diebel and Vander Zanden 2009), temperature or primary production (Woodland et al. 2012) that affect fractionation. Temperature increases cause an elevation in respiration of aquatic insects (Dallas and Ross-Gillespie 2015), and results in loss of the lighter isotopes of nitrogen (^{14}N). Furthermore, the denitrification of inorganic fertilizers from agricultural run-off also causes the loss of the lighter isotope (Diebel and Vander Zanden 2009). Use and application of nitrogen containing fertilizers (inorganic and organic) in the Kowie River changes seasonally; typically peaking during the pineapple season (July and December of every year) when fertiliser applications increase (Hill et al. 2012, Agri Eastern Cape 2015). This change in application of fertilisers consequently translates to the seasonal variation in $\delta^{15}N$ consumers in the Kowie River. The effect of changes in fertiliser application and its effect on consumers has also been observed in other studies (Diebel and Vander Zanden 2009). Changes in food sources, temperature and biochemical process like denitrification contribute to the seasonal variability in the isotopic values in consumers in the Kowie River.

Functional feeding group also had an effect on $\delta^{15}N$ values of consumers in the Kowie River. Since FFGs collected in the Kowie represent different organisms with alternate modes of acquiring their food, changes in nitrogen values are reflective of the differences in trophic levels. The effect of FFG on $\delta^{15}N$ has not been thoroughly investigated in freshwater, though the data available suggest that the manner in which consumers acquire basal resources affects their nitrogen values (Cremona et al. 2010). For instance, the relative mobility of the different species within each FFG can influence their diet composition (Lancaster and Waldron 2001). For example, predators (e.g. Odonata) have high variation in their nitrogen values as they typically encounter a large range of food sources owing to their high mobility, whereas a

sessile filterer (*Simulium* spp.) has less overall variation in its nitrogen values as it encounters fewer food types (Lancaster and Waldron 2001).

4.4.3 Carbon flow in the Kowie River

Primary consumers (gatherers, filterers, grazers and shredders) consumed 59 to 86% of autochthonous carbon, while terrestrial organic matter contributed only 13 to 41% to primary consumer diets (Fig. 4.3); there was a gradual shift from partly autochthonous in the headwater to predominantly autochthonous at other sites. The results of FFG diets in the Kowie River were supported by findings from other studies that found that FFGs depended on both autochthonous and allochthonous basal resources, and the contributions of autochthonous vs allochthonous resources changed with stream size and canopy cover (e.g. Collins et al. 2015).

I had postulated that carbon derived from local production would make up the bulk of organic matter supporting consumers (*sensu* Thorp and Delong 1994). My results supported findings from several other studies that demonstrated that autochthonous carbon (macrophytes and algae) was important for consumers in lakes (Batt et al. 2012), tropical streams (March and Pringle 2003) and large temperate rivers (Delong and Thorp 2006). For example, Hoeinghaus et al. (2007) recognized C_3 aquatic macrophytes and phytoplankton as important sources of energy for consumers in large river food webs in the upper Parana River. Similarly, Jepsen and Winemiller (2007), in their study of food webs in Venezuelan rivers, found little evidence of terrestrial organic matter sources being important to consumers, whilst a combination of algae and aquatic macrophytes provided the major food source for consumers. Notable contributions of aquatic macrophytes to the diets of consumers in the Kowie River was a finding that supported conclusions from elsewhere that showed the trophic importance of aquatic macrophytes in sections of large rivers (Jepsen and Winemiller 2002, Hoeinghaus et al. 2007). Contrary to the results obtained for the Kowie River, other studies have noted that terrestrial organic matter made up the bulk of organic matter supporting riverine consumers (Huryn et al. 2001, Zeug and Winemiller 2008, Reid et al. 2008). The rivers differ in their geographical characteristics with some occurring in temperate regions (e.g. Zeug and Winemiller 2008) while others are located in Neotropical streams (e.g. Jepsen and Winemiller 2002). The differences in biotic and abiotic characteristics in these streams may account for the differences in basal resource contribution to consumers. Differences in the relative importance of different basal resources to consumers in different streams pose the following questions: (1) what factors influence the differences in

contribution of different basal resources to consumers? (2) What factors may affect the contribution of basal resources to the Kowie River?

In riverine systems, the potential importance of terrestrial or algal carbon is influenced by factors such as turbidity, hydrology, land use characteristics, catchment size and morphology, catchment vegetation (Hoeinghaus et al. 2007, Pingram et al. 2012, Roach et al. 2015) and climate (Roach 2013). For example, in some studies that documented the importance of terrestrial organic matter to consumers, high flow and high turbidity caused by sediment resuspension limited autochthonous primary production and resulted in consumers deriving energy from terrestrial organic matter (Roach et al. 2015). Concurrent studies by Dalu et al. (2015b) and Richoux et al. (in prep) document that the contributions of terrestrial plants, algae and aquatic macrophytes to detritus and FPOM changes along the continuum. For example, these two studies in the Kowie River reveal that the dense aquatic macrophytes that colonise the middle and lower reaches of the Kowie promote the deposition of macrophytes into the detritus and FPOM pools. These shifts in the make up of the organic material in the Kowie River result from changes in hydrology (flow conditions and water depth) and total dissolved solids (Dalu et al. 2015a, 2015b). In the Kowie River, water velocity and water depth were positively related to the contribution of terrestrial plant matter to the detritus matrix (Dalu et al. 2015b) and hence these factors affect the quantity of terrestrial organic matter available in the detritus for consumers. Additionally, total dissolved solids and water velocity also affect the development of benthic algae within the river (Dalu et al. 2015), and ultimately affect the quantity of resources assimilated by consumers. The differences in environmental conditions (flow regimes, water depth, vegetation, climate, geomorphology and physico-chemistry) between the Kowie River and those of other studies could explain the contrasting findings between rivers regarding the influence of allochthonous vs autochthonous materials.

The connections between secondary consumers and basal resources were determined indirectly by examining which primary consumers were preyed upon, and using the diets of these prey to estimate the transport of the basal sources up the food web (Table 4.10; Fig 4.4). In the Kowie River, linkages between organic matter sources and secondary consumers showed that aquatic macrophytes and algae were the main basal resources supporting secondary consumers (Table 4.10). The dependence of secondary consumers on autochthonous carbon in the Kowie River was in agreement with other studies. For example, damselfly nymphs in the Waikato River (a large river in New Zealand) incorporated large amounts of autochthonous carbon into their diet (Pingram et al. 2014). Similarly, predators (dragonfly and backswimmers) in the Ovens River (south eastern Australia) also primarily

consumed autochthonous carbon (Hladyz et al. 2012). In comparison, predatory invertebrates in upstream locations of the Kowie River were dominated by terrestrial sources in some parts of the year (Table 4.1), via the consumption of herbivores that derived their nutrition from terrestrial organic matter. There was a possibility that the increase in terrestrial assimilation by predators at upstream sites could have resulted from increased consumption of terrestrial insect infall; this is highly likely considering that terrestrial infall is high at headwaters of the Kowie River when compared to downstream sites (Chapter 6). Further studies are needed to validate the possibility of secondary consumers assimilating terrestrial insects. Gut content analysis coupled with emerging techniques of using DNA may be useful in investigating this possibility (Sheppard and Harwood 2005). The diet estimates for secondary consumers were derived by assuming that predators consume only herbivores; however, it is plausible that some of the predators were omnivores that consumed some basal resources directly. Although direct herbivory by predators could not be evaluated in the Kowie River, direct consumption of basal resources has been observed elsewhere (Palmer et al. 1993b, Miyasaka and Genkai-Kato 2009), where insects such as stone flies (predators) shifted their diet and assimilated up to 55% of algae (Lancaster et al. 2005). The shift from carnivory to herbivory occurs when food is scarce (which commonly occurs during harsh weather conditions) or when insect predators are competitively excluded by other predators (Hellmann et al. 2013, Blanchette et al. 2014).

4.4.4 Conceptual models for energy flow in small rivers

I determined whether the riverine concepts developed primarily for large temperate rivers would be valid within the context of a small temperate river in the southern hemisphere. The FPC suggests that river food webs are dependent on organic matter from terrestrial sources because of periodic floods (Junk et al. 1989). There is limited support for the FPC in the Kowie River as there are no floodplains as represented in the original FPC model systems. The conclusions that the FPC does not apply to the Kowie River does not imply that the floods may be unimportant to other aspects of aquatic ecosystem function, but in this current study the influence of flooding remains undetermined. Floods in the Kowie River are rare and ephemeral (typically lasting only a few days) when compared to the rivers used in formulating the original FPC, so flooding may not provide the time needed for decomposition of any floodplain material, which can then enter the metazoan food web. However, one idea from the FPC that may be applicable to the Kowie River is its emphasis on the importance of lateral connectivity between a stream and its riparian area. For example,

nutrients or organisms may move from the terrestrial habitat into the river and thus contribute to consumer diets (*sensu* Junk and Wantzen 2004).

The RPM differs from the FPC in that it emphasizes the importance of algal grazer pathways in fuelling consumers in large rivers. Using the RPM as a framework, Thorp and Delong (1994) proposed that combinations of *in situ* production and riparian zone inputs during periods not limited to flood pulses are the major sources of carbon supporting consumers. In the Kowie River, the macrophytes and attached algae contributed significantly to the diets of consumers, an interesting outcome in this study. Aquatic macrophytes have been described as potential food sources for primary consumers, but these plants are rarely grazed directly by aquatic herbivores due to their low digestibility and high levels of cellulose and lignin (Allan 1995, Allan and Castillo 2007). Macrophytes are relatively unimportant in food webs until they decay and enter the detrital pool (Allan 1995). 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 diets of Ephemeroptera (*Atalophlebia albiterminata)* and the case-building Trichopterans (*Lectrides varians*, *Notalina bifaria* and *Triplectides similis*; Watson and Barmuta 2011). The RPM argues that the labile nature of autochthonous organic matter sources makes them preferred food choices rather than their recalcitrant allochthonous counterparts (Thorp and Delong 2002). I argue that the consumption of detrital macrophytes probably explains how macrophytes are assimilated into consumer diets, as most consumers in the Kowie River derived nutrition from FPOM that ultimately contained autochthonous matter (*sensu* Dalu et al. 2015b). Furthermore, the paucity of studies recording observations of direct herbivory brings questions regarding the relative prevalence of this behaviour in river communities. Overall, the data from the Kowie River support the tenets proposed by the RPM; however, the RPM may underestimate the relative importance of direct riparian inputs at the headwaters.

One striking outcome from the Kowie River data is evidence for the dependence of primary consumers on food sources not typically linked with their FFGs. For example, why would grazers such as the family Baetidae and Leptophlebiidae (*Castanophlebia calida* and *Adenophlebia auriculata*) consume and assimilate epiphyton instead of periphyton, and why would some shredders assimilate periphyton instead of terrestrial leaves. One plausible explanation is that phytoplankton, periphyton and epiphyton in rivers share some common taxa (Reynolds et al. 1994, Dalu et al. 2014b, 2014a) that alternate between planktonic and

benthic existences, hence the consumption of either one of the resources may result in consumers having isotope values that corresponded with the one of the food sources. Additionally, the consumers may be opportunistic and feed on the resources that are most abundant (Leigh et al. 2010).

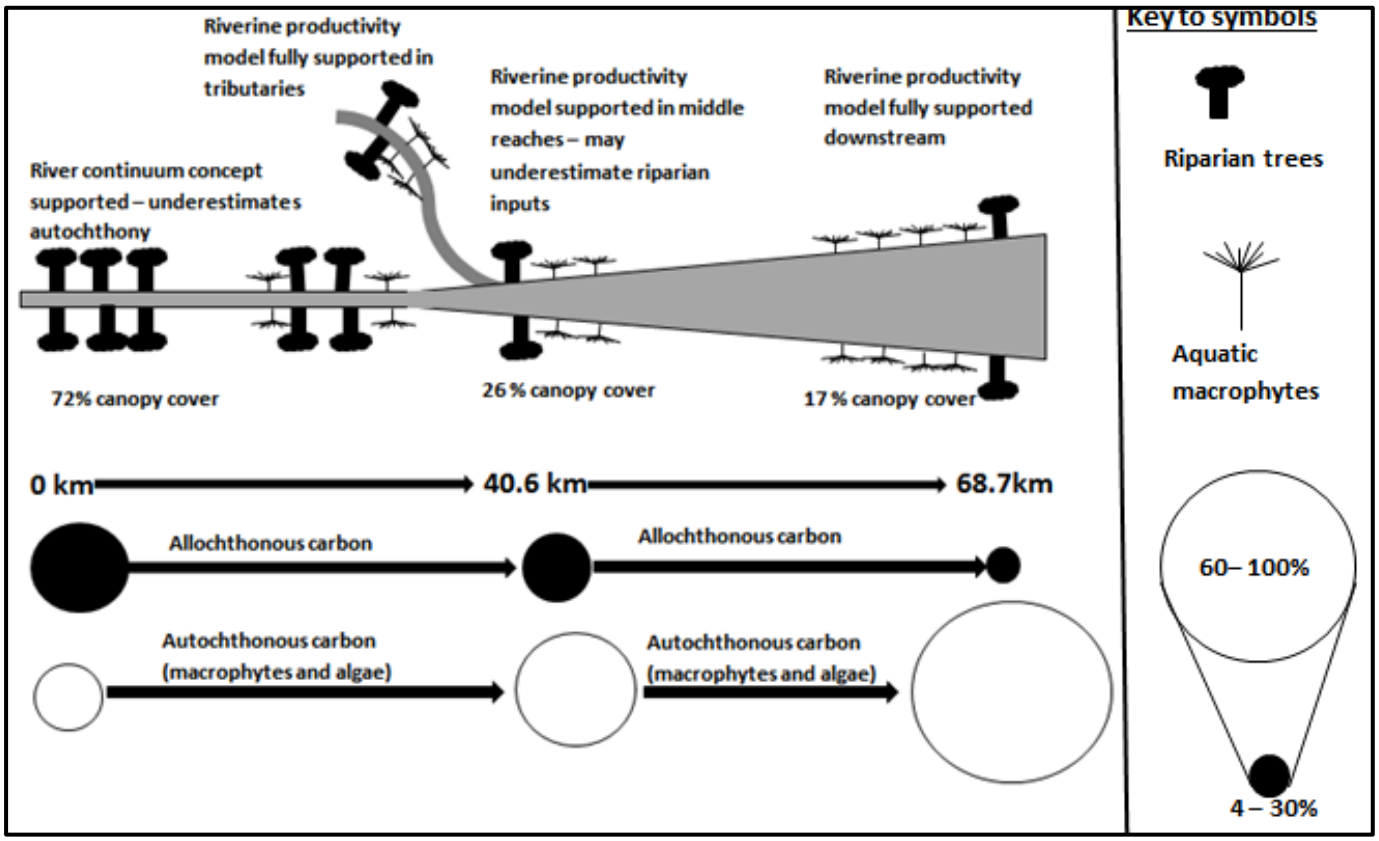

Figure 4.5: Conceptual diagram illustrating the contribution of organic matter sources supporting consumers at different reaches of the Kowie River. The relevant energy flow concepts (RPM, RCC, ad FPC) are also highlighted. Only carbon sources that contributed to consumer diets are included in the diagram.

The RCC proposed that the main source of carbon driving consumers is FPOM derived from upstream, and upriver CPOM of terrestrial origin is broken down into FPOM with increasing distance from the headwaters (Vannote et al. 1980). In the Kowie River, CPOM was derived primarily from riparian leaves (Fig 4.1; Dalu et al. 2015b); however, in contrast with predictions of the RCC, FPOM transported downriver in the Kowie was not of mainly terrestrial origin. For upstream derived FPOM to be considered purely allochthnonous, FPOM carbon isotopic values must be aligned with those of riparian plants. Instead, FPOM at all sites sampled had $\delta^{13}C$ values that were more similar to the autochthonous sources, suggesting that the FPOM was predominantly composed of autochthonous materials. A dominance of autochthonous sources in mixed organic pools in temperate steams has been documented, and phytoplankton was often the major constituent

of FPOM (Thorp et al. 1998, Delong and Thorp 2006, Dalu et al. 2015b). Autochthonous carbon dominated FPOM in southeastern Brazilian rivers (Martinelli et al. 1999), rivers in Columbia (Kendall et al. 2001) and the upper Mississippi River (Delong and Thorp 2006). Whilst FPOM composition can vary seasonally, the dominance of autochthonous organic carbon can extend for long periods, particularly during periods of low flow conditions in spring and summer when phytoplankton blooms occur (Kendall et al. 2001). Considering that FPOM is a complex matrix of allochthonous and autochthonous material, the RCC is insufficient for explaining the organic matter sources fuelling consumers in the Kowie River. Allochthonous resources were consumed in the greatest amounts at the headwater site (an observation which corresponds with the predictions of the RCC), but consumption of allochthonous materials was not as pronounced as expected from the RCC, especially given that the dominant shredding crab (*Potamonautes* spp.) was highly dependent on algal resources (whereas according to the RCC they should consume terrestrial leaves). Overall, the RPM was the model that best predicted feeding relationships in the Kowie River (Fig. 4.5). However, the other models provided some complementary ideas that helped to explain the energy flow in a small temperate river like the Kowie. All three models emphasize that connectivity is important for riverine functions, with the RCC emphasizing longitudinal connectivity, the FPC emphasizing lateral connectivity and the RPM emphasising *in situ* connectivity; in turn, all forms of connectivity are affected by flow patterns and food web processes. All of the models are based on the bottom up view of connectivity within riverine systems, whereby the availability, type and quality of basal resources control consumers and the entire aquatic food web. An amalgamation of the three concepts that view river connectivity laterally, locally and longitudinally helps to explain the flow of energy in the Kowie River and probably other small temperate systems. The amalgamation of the three main concepts has been suggested by the River Wave Concept (RWC; Humphries et al. 2014). The RWC (a recently proposed integrated model) conceptualises rivers as behaving like waves (with amplitudes, troughs and crests) and posits that each of the riverine models will apply depending on the flow regime of the river. While the RWC has not been tested empirically (Roach et al. 2015), it does provide a unified view of the three models of ecosystem function.

4.5 Conclusions

Food web studies using stable isotopes are often limited to one season or one habitat (e.g. Delong et al. 2001, Brito et al. 2006). While these studies have provided valuable information on the flow of carbon in riverine food webs, they may have missed ecologically

relevant spatial and temporal variations in the contributions of different organic matter sources. Food webs are dynamic over both time and space (Polis et al. 1997), and by sampling multiple food sources across sites and seasons, I was able to elucidate the complexity of energy sources in the Kowie River.

The next logical step in this research is to incorporate hydrogeomorphological features in the context of the ecosystem concepts (RCC, FPC and RPM), an approach that will offer a holistic understanding of ecosystem processes in the Kowie River. One promising model that may help in this regard 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 is needed to evaluate the roles of hydrogeomorphology in relation to the carbon sources fuelling consumers in temperate rivers.

This is the first study, to my knowledge, to document energy flow in a small temperate river of southern Africa. Additional studies in dissimilar systems would unravel some of the remaining questions about the carbon sources that fuel riverine food webs in southern Africa. The findings from this study provide a baseline of energy flow in one system that can be used as a foundation for further study. Additional tracer data such as that from fatty acid analyses (see Chapter 5) will enhance our knowledge on the carbon flow in small rivers, which is critical for effective management of freshwater systems.

5 Spatial and temporal dynamics of fatty acids in macroinvertebrate consumers in a stream food web

5.1 Introduction

Consumers in temperate streams obtain energy from a variety of basal food sources. In aquatic environments, sources of basal resources may be autochthonous (e.g. macrophytes, phytoplankton) or allochthonous (e.g. terrestrial plants; Richardson and Sato 2015). Understanding the sources and fates of these basal resources is essential for sustainably managing riverine environments (Allan 1995). Traditionally, the relative importance of energy resources in freshwater food webs has been assessed by direct observation (impractical or difficult for small freshwater invertebrates) and gut content analyses (Mihuc and Toetz 1994), techniques often more biased towards the quantity (not quality) of the resource ingested rather than what is assimilated (Altig et al. 2007).

Assimilation-based techniques such as fatty acids and stable isotope analysis have become promising tools in the examination of trophic linkages (e.g. Chapter 4, Torres-Ruiz et al. 2007, Pingram et al. 2014). Interpretations of stable isotope data can be ambiguous when isotope values of different food sources overlap (Crawley et al. 2009). Fatty acid data can be more specific to certain dietary sources relative to stable isotope ratios, removing some of the ambiguities that arise from using only one technique (reviewed in Chapter 1).

Lipid (fatty acid) requirements of invertebrates have been extensively studied in marine (reviewed by Dalsgaard et al. 2003) and lake ecosystems (reviewed by Brett and Müller-Navarra 1997), but less research has been conducted in river systems (Sushchik et al. 2003, Torres-Ruiz et al. 2007). As with most marine animals, freshwater invertebrates are unable to synthesise polyunsaturated fatty acids (PUFAs) in sufficient quantities to meet their physiological needs and must therefore derive these from their food. The most important PUFAs, commonly termed essential fatty acids [EFAs; namely 18:2ω6, 18:3ω3, arachidonic acid (ARA; 20:4 ω6), docosahexaenoic acid (DHA; 22:6ω3) and eicosapentaenoic acid (EPA; 20:5ω3)], play key roles in the health and function of organisms by influencing their survival, growth, and reproduction (Cargill et al. 1985, Arts et al. 2001). Although the roles of EFAs (18:2ω6, 18:3ω3, ARA, EPA and DHA) in marine and lake systems have been emphasized, few studies have related lotic macroinvertebrate fatty acid profiles with their food. There is evidence that EPA and DHA in marine and lake invertebrates are directly derived from algae (Scott et al. 2002, Brett et al. 2009), while 18:2ω6 and 18:3ω3 are derived

from terrestrial plants (Napolitano et al. 1997, Budge et al. 2001). The existing data affirm that long-chain PUFAs, and in particular EPA, are abundant among aquatic insects in temperate streams (Hanson et al. 1985, Ghioni et al. 1996, Sushchik et al. 2003, Torres-Ruiz et al. 2007).

Consumers ingest fatty acids from primary producers and these fatty acids are often incorporated relatively unchanged into their tissues. Based on this phenomenon, fatty acids are useful as biomarkers to infer diets of aquatic organisms (Goedkoop et al. 2000, Alfaro et al. 2006). For instance, the fatty acids EPA and 16 carbon PUFAs are generally considered as diatom markers (Viso and Marty 1993), DHA and 18:4ω3 are markers of dinoflagellates (Viso and Marty 1993), and the ratio of the sums of ω3 and ω6 (Σω3:Σω6) is a marker for the dependence of organisms on allochthonous (< 1) or autochthonous (> 1) derived lipids (Pollero et al. 1981, Desvilettes et al. 1994). The usefulness of fatty acid tracers in aquatic systems is well established, so this is a promising approach for assessing consumer diets and movements of organic matter along a river continuum. For example, a field study utilising fatty acid analysis in a large temperate stream in France confirmed that autochthonous sources (as evidenced by high proportions of diatom specific fatty acids) provided the major basal resource for consumers in all reaches of the river (Descroix et al. 2010). In the Muscoot River (New York), fatty acid analysis revealed that mayflies and caddisflies consumed autochthonous sources (green algae and diatoms) all year round, even when shading was high (Torres-Ruiz et al. 2007). In a Swedish study, Lau et al. (2011) studied the fatty acid compositions of mayflies and zooplankton and showed that the consumers depended on diatom rich basal resources. In addition, Lau et al. (2011) found strong evidence that functional feeding groups (FFGs) influenced fatty acid compositions more than collection times and location combined. With these studies in mind, additional studies are needed to further describe the spatial and temporal variability in the sources and quality of food for riverine consumers.

I examined the seasonal patterns in the fatty acids of consumers along a river continuum in South Africa. Based on studies that have shown the importance of autochthonous sources to consumers (Chapter 4; Sushchik et al. 2003, 2012), my main hypothesis was that shifts in the supply of organic matter sources along the river continuum cause shifts in the diets of invertebrates (i.e. autochthony increases in the downstream direction). Along with this hypothesis, three complementary questions were posed: (i) are variations in physiologically important fatty acid proportions explained better by trophic groups (FFGs), temporal or spatial factors? [**Corresponding prediction 1**: variations in physiologically important fatty acid proportions are explained more by FFGs rather than

collection times (season) and site, due to the differences in feeding mechanisms and physiological characteristics of FFGs], (ii) how do seasonal changes in fatty acid proportions in FFGs relate to those of their of their major basal resources [**Corresponding prediction 2**: given that the fatty acid profiles of consumers are reflective of their diets, FFGs will be similar to the food sources they consume], and (iii) do the shifts in consumer diets along the river continuum correspond to the changes in trophic markers for freshwater systems? [**Corresponding predictions 3:** changes in trophic markers will be indicated by decreases in terrestrial markers and increases in markers common to the aquatic primary producers in the consumer tissues. Specifically, 18:2ω6 and 18:3ω3 will decrease with increasing distance from the headwaters (**prediction 3A**) while aquatic markers (20:4ω6 and 20:5ω3) will increase with increasing distance **prediction 3B**].

5.2 Material and methods

5.2.1 Study area and sample collection

Sampling was carried out at six sites along the Kowie River from the headwaters to the downstream regions. Primary producers were collected as described in preceding chapters. Briefly, samples of fine particulate organic matter (FPOM), periphyton, benthic algae, epiphyton, aquatic macrophytes, terrestrial leaves and detritus (CPOM in Chapter 4) were collected following methods described in Dalu et al. (2015b), and analysis of lipids in basal resources were described in Richoux et al. (in prep). Organisms were collected using a SASS net. At each site, several specimens were collected that belonged to five FFGs: filterer1 (*Simulium* spp.), filterer2 (*Hydropsyche* spp.), shredders (*Potamonautes* spp.), gatherers (*Caenis* spp. and *Caridina nilotica)*, grazers and predators (see Table 4.2). Filterers were placed into separate categories because of their distinct fatty acid profiles (with the *Hydropsyche spp*. consistently having high proportions of 12:0 and 14:0 compared to the *Simulium* spp.).

Detailed descriptions of the analyses of fatty acids of all the samples (animals and organic matter sources) were provided in preceding sections (see Chapter 2.2.5).

5.2.2 Data analysis

I performed all analyses using the proportional fatty acid data (percentage of the total fatty acids; TFA), as these data allowed for standardized comparisons among samples with very dissimilar lipid contents (Napolitano et al. 1997).

To address **question 1 (**variations in physiologically important fatty acid proportions are explained better by FFG, temporal or spatial factors), three way permutational

multivariate analyses of variance (PERMANOVA; Clarke and Gorley 2006) were used to test for effects of the three factors on EFA proportions (factors = season, site, FFG, season × site, season × FFG, site× FFG, season × site × FFG). Post-hoc pairwise comparisons of the appropriate factors were performed following identification of any significant effects.

To address **question 2** (how do seasonal changes in fatty acid proportions in FFGs relate to those of their of their major basal resources), non-metric multidimensional scaling (nMDS) was used to compare the fatty acid compositions (untransformed proportional data) among the different FFGs, and among the FFGs with their potential food sources. Similarity percentages (SIMPER) were compared with the loadings results following principal components analyses (PCA) of the same data. The results from the SIMPER and PCA were overlaid on the nMDS plot to determine the fatty acids responsible for any separation of consumer or food groups.

To address **question 3 (**do the shifts in consumer diets along the river continuum correspond to the changes in trophic markers for freshwater systems), relationships between increasing distance (from the headwaters) and specific EFAs in each FFG were examined using simple linear regressions. Separate linear regressions were performed for each FFG across all seasons. Distance was used as a predictor variable as it correlated strongly with other physical attributes such as stream width and canopy cover. One of the weaknesses when using fatty acid analysis is that most fatty acids are not associated with only one basal resource and can be synthesized by different plants (e.g. 18:3ω3 can be synthesised by terrestrial plants and algae). Moreover, some insects may be capable of modifying ingested fatty acids; however, the metabolism of lipids in stream invertebrates remains poorly studied. Thus EFAs, known to be diagnostic or highly characteristic of specific carbon sources (autochthonous vs allochthonous), were chosen as response variables in the regression models.

In addition to the individual EFAs used in the regressions, fatty acid metrics that are indicative of different sources were also calculated. The following groups of fatty acids were used to infer diet: (i) the ratio Σω3/Σω6, an indicator for the reliance of consumers on autochthonous versus allochthonous organic matter (Pollero et al. 1981, Desvilettes et al. 1994, Torres-Ruiz et al. 2007), (ii) a diatom marker [(14:0 + 16:1ω7 + 16:2ω4 + 16:3ω4 + 16:4ω1)/16:0] (Léveillé et al. 1997), (iii) bacterial indicators (sum of odd-chain, *iso* (*i*) and *anteiso* (*ai*) fatty acids) (Viso and Marty 1993, Budge and Parrish 1998), and (iv) a green algae marker (16:2ω6 +16:3ω6 +16:3ω3; Sushchik et al. 2003). Bacterial fatty acids were used in the Kowie River because they represent a potential pathway of terrestrial organic matter consumption by consumers. There are propositions that consumers can consume

terrestrial organic matter via direct herbivory or by grazing on the bacteria that grow on the leaf litter (*sensu* France 2011).

5.3 Results

The complete fatty acid profiles of all consumers from all sites are detailed in Appendices 8-2 to 8-6, and the results of analyses using the EFAs are documented here. For clarity, I have divided the results section into three main parts. The first section describes the proportions of four EFAs (18:2ω6, 18:3ω3, ARA and EPA) in consumers in relation to potential food sources that could provide these EFAs to the invertebrates. While DHA is an EFA, it is not presented here, as it occurred in very small quantities or was undetected in most consumers. The second part aimed at showing how fatty acids linked members of the food web using a multivariate approach. The third section is dedicated to comparing trophic marker fatty acids among all consumers.

5.3.1 Are variations in physiologically important fatty acid proportions explained better by FFGs, temporal or spatial factors? (question 1)

PERMANOVA results across FFGs, seasons and sites indicated that these factors influenced 18:2ω6 ($N = 549$, $p < 0.05$, Table 5.1), and interactions among the factors were also highly significant. For 18:2ω6 (as estimated from components of variation), FFG explained the most variation while site and season, respectively, accounted for less variation compared to FFG (**prediction 1 supported**). Relevant pairwise comparisons of the fixed factor 'season' revealed that sites were significantly different except during the late spring and early spring months (PERMANOVA post-hoc pairwise test: $t = 1.68$, $p = 0.07$). FFG significantly influenced 18:2ω6, and pairwise comparisons of fixed factor 'FFG' showed that there were no significant differences between filterer2 and grazers (PERMANOVA post-hoc pairwise test: $t = 1.40$, $p = 0.14$) and between grazers and gatherers (PERMANOVA post-hoc pairwise test: $t = 1.60$, $p = 0.09$). Subsequent comparisons on the factor 'site' showed that there were significant differences among sites with the exceptions of FW2 vs FW4 (PERMANOVA post-hoc pairwise test: $t = 1.21$, $p = 0.22$), FW4 vs FW6 (PERMANOVA post-hoc pairwise test: $t = 1.23$, $p = 0.21$) and FW2 vs FW6 (PERMANOVA post-hoc pairwise test: $t = 1.67$, $p = 0.06$).

Season had the greatest influence on 18:3ω3, while FFG and site accounted for smaller degrees of influence (contrary to **prediction 1,** as season had the greatest influence on 18:3ω3)**.** There were no significant differences in proportions of 18:3ω3 between FW1 vs FW5 (PERMANOVA post-hoc pairwise test: $t = 1.09$, $p = 0.28$) and between FW1 vs FW6 (PERMANOVA post-hoc pairwise test: $t = 1.75$, $p = 0.05$). FFG pairwise wise comparisons

with site indicated that there were significant differences between all sites and FFGs (PERMANOVA post-hoc pairwise, $p < 0.05$).

With respect to the effects of three fixed factors (FFG, site and season) on ARA, all effects were significant ($p < 0.05$; Table 5.1), with FFG being the most influential on ARA (in support of **prediction 1**). The second most influential factor on ARA was season, while site accounted for a much smaller amount of the total variation compared to FFG and site. Follow up pairwise comparisons on the different fixed factors indicated that there were no significant differences between FW4 vs FW5 (PERMANOVA post-hoc pairwise test: $t = 0.98$, $p = 0.35$), FW2 vs FW5 (PERMANOVA post-hoc pairwise test: $t = 0.53$, $p = 0.75$) and FW5 vs. FW6 (PERMANOVA post-hoc pairwise test: $t = 1.43$, $p = 0.12$).

Season had the most influential effect on EPA (**prediction 1** not supported as season was the most influential), while site was the second most influential factor on EPA ($p < 0.05$; Table 5.1). FFG was the third most important factor affecting EPA. There were significant pairwise differences among FFGs, but there were no significant differences between predators and shredders (PERMANOVA post-hoc pairwise test: $t = 1.77$, $p = 0.06$).

Table 5.1: Results of a three factor PERMANOVA (Pseudo F- values presented) to determine the differences in EFAs of invertebrates by site, season and feeding group (FFG), and the interactions of these three factors. Asterisks show significant differences *p< 0.05 ** p < 0.01. N = 549.

EFA	site	season	FFG	season x site	season x FFG	site x FFG	season x site x FFG
18:2ω6	33.24**	20.01**	49.82**	17.06**	6.67**	5.74**	2.97**
18:3ω3	23.10**	133.67**	32.12**	34.79*	6.01**	5.70**	4.61**
ARA	17.53**	73.96**	74.20**	25.00**	4.45**	4.06**	3.02*
EPA	75.63**	204.99**	42.52**	46.17*	14.95**	7.25*	8.32**

5.3.2 How do seasonal changes in fatty acid proportions in FFGs relate to those of their major basal resources (question 2)

Figures 5.1 and 5.2 represent fatty acid proportions of consumers and their potential food sources. Means of autochthonous basal sources plotted (with the exception of aquatic plants) closer to animals in the nMDS than terrestrial leaves, which in some cases had moderate variation. Overall, the nMDS plots indicated a consistency in the fatty acid profiles within each FFG and food source category across all seasons and sites. An nMDS analysis was performed for each season and site. Most consumers plotted close to algal sources based on common fatty acids that are typically of autochthonous origin (16:1ω7, 20:5ω3, 22:6ω3), although sometimes the consumer profiles were very different from potential food sources

(e.g. late summer FW4). Several fatty acids were identified as important to all consumers: 12:0, 14:0, 16:0, 16:1ω7, 18:1ω7, 18:1ω9, 18:2ω6, 18:3ω3, 20:4ω6 and 22:6ω3. The fatty acids 12:0 and 14:0 were almost always associated with filterer 2 (*Hydropsyche* spp.), while ARA (and DHA at FW6 in late spring) was consistently found in shredders (*Potamonautes* spp.). Aquatic plants and terrestrial leaves had very distinct profiles compared with all of the consumers. There was consistent overlap between macrophytes and aquatic plants (owing to high proportions of 18:2ω6 and 18:3ω3); however, detritus had similar profiles to the consumers during some seasons.

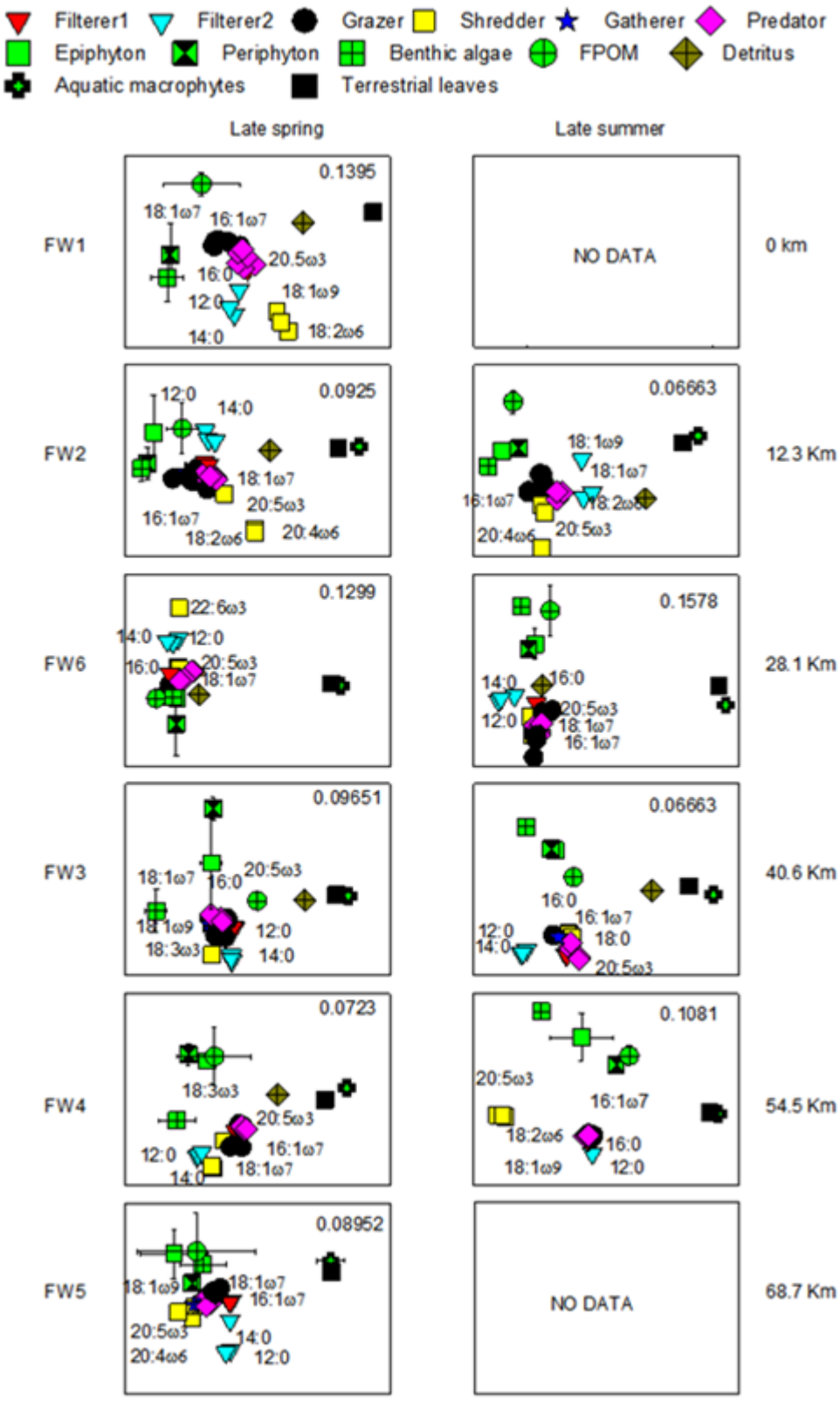

Figure 5.1: Non-metric multidimensional scaling plots of proportional fatty acids of basal resources and macroinvertebrate consumers at six sites along the Kowie River between late spring and late summer. Axes are dimensionless and stress levels are presented in the top right corner. Major fatty acids contributing to the consumers (resulting from SIMPER and PCA) are shown on each plot. Error bars for basal resources denote standard deviations.

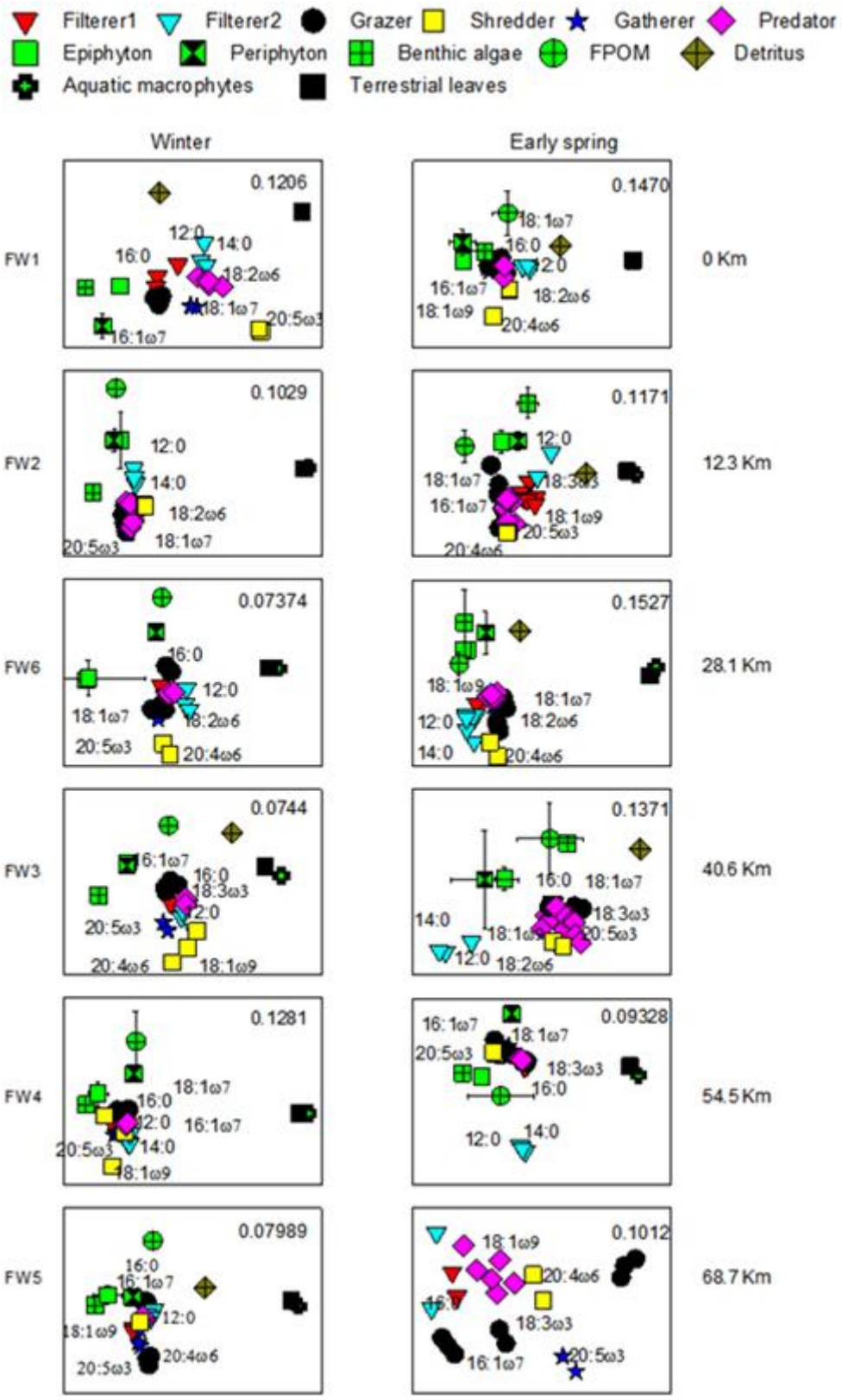

Figure 5.2**:** Non-metric multidimensional scaling plots of proportional fatty acids of basal resources and macroinvertebrate consumers at six sites along the Kowie River between winter and early spring. Axes are dimensionless and stress levels are presented in the top right corner. Major fatty acids contributing to the consumers (resulting from SIMPER and PCA) are shown on each plot. Error bars for basal resources denote standard deviations.

5.3.3 Do shifts in consumer diets along the river continuum correspond to the changes in trophic markers for freshwater systems? (question 3)

Essential fatty acids (18:2ω6, 18:3ω3, 20:4ω6 and 20:5ω3) of the organisms studied are presented in Fig. 5.3 and Fig. 5.4. Figure 5.3 shows scatter plots for 18:2ω6 and 18:3ω3 in FFGs with season and Figure 5.4 shows the scatter plots of ARA and EPA of FFGs with season.

Linoleic acid (18:2ω6), the principal ω6 PUFA, was present in all consumers throughout the seasons (range of 1.21 – 36.16% TFA). Linoleic acid was highest in shredders (36.16% TFA) compared with all the other FFGs, while filterer2 contained the lowest proportions across all season and sites (1.21% TFA). Linear regressions of 18:2ω6 proportions in some FFGs showed decreased proportions from upstream to downstream sites, in winter and early spring (linear regressions; $p < 0.05$; Fig. 5.5 and Table 5.1); however, in the late summer and late spring proportions increased from headwaters to the downstream (Fig. 5.5). **Prediction 3A** was partially supported because 18:2ω6 decreased with increasing distance during some seasons.

Linolenic acid (18:3ω3) was detected in all consumers (range from 0 - 22.5% TFA). Low proportions of 18:3ω3 were recorded in all FFGs in late spring and late summer (range 0 - 0.08% TFA). The highest proportions of 18:3ω3 were recorded in grazers (22.5% TFA). Filterers and grazers were higher in 18:3ω3 than all the other consumers studied. Broadly, 18:3ω3 proportions decreased with increasing distance downstream across all seasons (linear regressions; $p < 0.05$; Fig. 5.5 and Table 5.1). The proportions of 18:3ω3 decreased with distance in all FFGs in the summer (linear regression $p < 0.05$). There was no obvious trend in 18:3ω3 with distance during early spring in all FFGs (linear regressions; $p > 0.05$; Table 5.1). **Prediction 3A** was supported because obvious trends occurred in some FFGs (18:3ω3 decreased with increasing distance).

Levels of ARA (20:4ω6) in filterers, predators and grazers were low (range of 0.3 to 10% TFA), while the highest ARA proportions were recorded in shredders (20%). In contrast, shredders and gatherers had markedly higher percentages of ARA, with maxima occurring at FW2 (summer and autumn; 19.96 % TFA) and FW6 (winter 7.44% TFA). The levels of ARA decreased with increasing distance during the winter and late summer (linear regressions; $p < 0.05$, Table 5.1); however, in some FFGs there was clearly no trend in ARA with increasing distance. In predators during the late spring the levels of 20:4ω6 increased with increasing distance (Fig. 5.5 and Table 5.1**)**. **Prediction 3B** was not supported as proportions of ARA tended to decrease with increasing distance.

Eicosapentaenoic acid was the dominant EFA among all the consumers, ranging between 0.2 to 27.1% TFA (though smaller proportions of EPA were recorded in the autumn compared to the other seasons; range 0.2 to 18.8 % TFA in late summer). EPA proportions were small in shredders at the headwaters and the middle reaches. EPA in shredders was highest during the winter and spring, with peaks occurring in animals from the middle reaches and the tributary. In all FFGs, maximum EPA proportions were recorded in the winter (maximum values in the range of 7.4 to 27.1% TFA). It is interesting to note that low levels of 18:3ω3 in the winter were associated with higher levels of EPA in most consumers during most of the seasons (i.e. 18:3ω3 decreased and EPA increased). Spatially, EPA levels of FFGs increased with increasing distance in the winter, but in the late summer EPA proportion decreased with increasing distance. EPA levels in shredders did not change predictably with increasing distance across all seasons. EPA proportions in predators increased with increasing distance in the winter, but decreased with increasing distance in the late summer, late spring and early spring. **Prediction 3B** was partially supported because EPA did not change predictably in all FFGs.

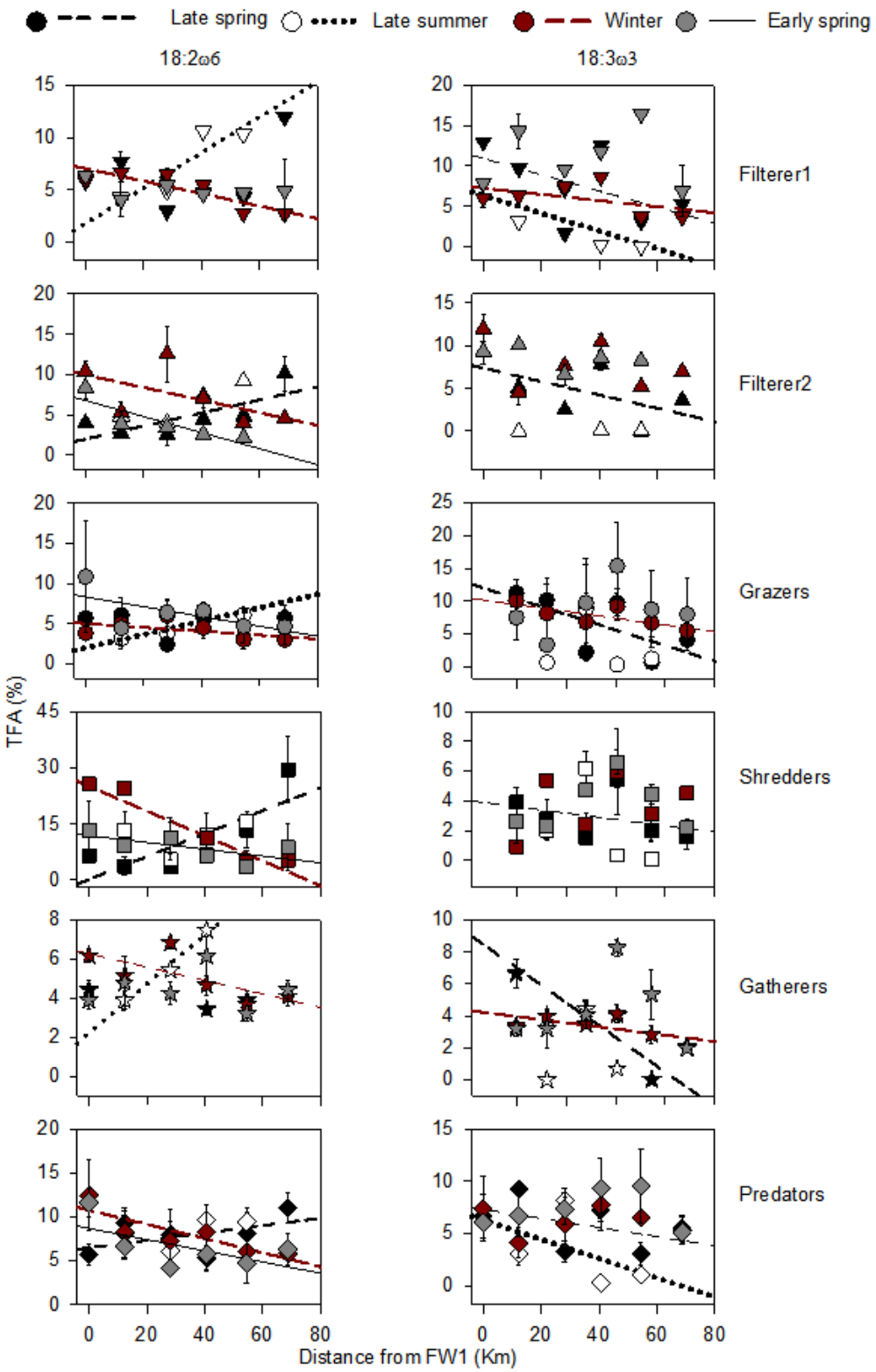

Figure 5.3: Simple linear regressions between distance (measured from headwater FW1) and EFAs (% TFA) in FFGs collected in the Kowie River. Each coloured symbol represents a different season. Error bars show standard deviations above and below the mean. Regression lines are shown for statistically significant relationships ($p < 0.05$).

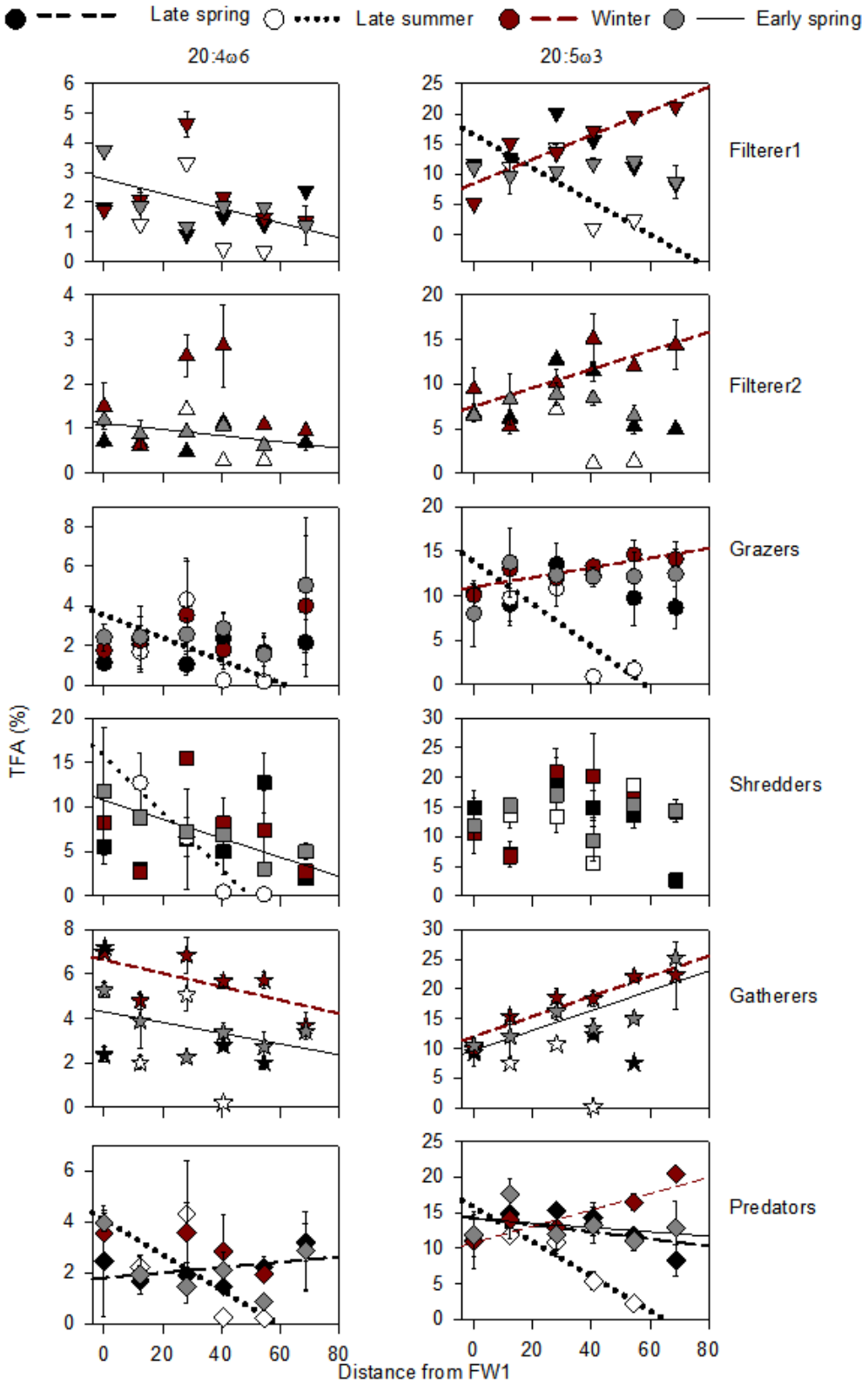

Figure 5.4: Simple linear regressions between distance (measured from headwater FW1) and of EFAs (% TFA) in FFGs collected in the Kowie River. Each coloured symbol represents different seasons. Error bars show standard deviations above and below the mean. Regressions lines are shown for statistically significant relationships ($p < 0.05$).

Table 5.2: R^2 and p values for linear regressions of specific fatty acids (18:2ω6, 18:3ω3, 20:4ω6 and 20:5ω3; % total fatty acids) against stream distance (measured from the headwaters) in FFGs in the Kowie River; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Late spring		**Late summer**		**Winter**		**Early spring**	
	R^2	p	R^2	p	R^2	p	R^2	P
18:2ω6								
Filterer1	0.13	0.22	0.80	*	0.73	***	0.15	0.65
Filterer2	0.57	***	0.76	0.05	0.34	*	0.75	***
Grazer	0.03	0.78	0.81	***	0.27	***	0.42	**
Shredders	0.63	***	0.12	0.22	0.89	***	0.45	*
Gatherers	0.55	0.32	0.98	***	0.53	**	0.04	0.81
Predators	0.25	*	0.31	0.21	0.73	***	0.38	*
18:3ω3								
Filterer1	0.28	**	0.35	*	0.24	*	0.01	0.59
Filterer2	0.35	***	0.04	0.53	0.13	0.16	0.23	0.16
Grazer	0.45	***	0.03	0.67	0.61	*	0.12	0.40
Shredders	0.13	*	0.24	0.11	0.16	0.19	0.04	0.26
Gatherers	0.85	**	0.03	0.58	0.43	**	0.01	0.71
Predators	0.22	*	0.22	*	0.03	0.97	0.11	0.95
20:4ω6								
Filterer1	6.82	0.93	0.24	0.11	0.06	0.32	0.47	*
Filterer2	5.44	0.23	0.23	0.56	0.00	0.84	0.49	***
Grazer	0.27	0.69	0.28	*	0.15	0.36	0.24	0.19
Shredders	0.02	0.53	0.91	***	0.02	0.44	0.83	**
Gatherers	0.04	0.33	0.04	0.05	0.39	**	0.36	*
Predators	0.18	**	0.40	***	0.08	0.45	0.15	0.75
20:5ω3								
Filterer1	0.11	0.35	0.58	**	0.81	***	0.02	0.67
Filterer2	0.04	0.49	0.56	0.09	0.57	*	0.03	0.99
Grazer	0.04	0.45	0.68	***	0.73	***	0.21	0.18
Shredders	0.16	0.22	0.04	0.58	0.22	0.08	0.01	0.78
Gatherers	0.01	0.33	0.40	0.44	0.89	**	0.68	0.05
Predators	0.22	***	0.92	***	0.78	***	0.11	*

The indicator for aquatic versus terrestrial material, Σω3/Σω6, was compared among all FFGs. Values of Σω3/Σω6 were greater than 1.0 in all macroinvertebrate consumers (on most sampling dates), with the lowest values recorded in late summer (< 1, Fig. 5.4). Values of Σω3/Σω6 increased in shredders from 0.4 at the headwaters to 2.2 downstream. In general,

shredders shifted from an allochthonous diet upstream to a greater reliance on autochthonous food downstream (Fig. 5.4). Values of Σω3/Σω6 were relatively low (ranged from 1 to 3) in predators. Similar to shredders, predators showed a gradual shift from an allochthonous diet to an autochthonous diet in the downstream direction. The highest Σω3/Σω6 ratios were recorded in filter feeding consumers (ranged from 0.8 to 7), and ratios for gatherers and grazers were usually above one, indicating that these consumers were possibly more reliant on autochthonous sources.

There were seasonal and spatial changes in the diatom marker in consumers from the Kowie River (Fig. 5.6). Diatom marker ratios were higher in the late spring and early spring (0.2 to 2.1) amongst all consumers, with the lowest ratios recorded during the autumn (0.2 to 1.7). Caddisflies (filterer2) were usually consistently high in their diatom indicator levels (range 0.3 to 2.1 across all season and sites); whereas the black flies (filterer1) had lower values (0.2 to 1.3 across all seasons and sites) than their filtering counterparts. There was no obvious spatial trend in the diatom marker ratio for predators. However, diatom marker ratios for predators seemed closely aligned with those of the grazers (i.e. when diatom values of grazer were low, the values of predators were also low). Gatherers were not collected at all sites, but high values of the diatom ratio occurred in the gatherers during winter and late spring (0.6 to 1.6). Diatom ratios in shredders were low (0.2 to 1.8) at the headwaters, but generally increased downstream (Fig. 5.6). Peaks in diatom ratios for all consumers were recorded at FW4, particularly in the late spring and early spring (Fig. 5.6).

Bacterial-derived fatty acids in shredders were low (range 0.9 to 7.0% TFA; Fig. 5.5) relative to other groups of consumers across all seasons (with the exceptions of shredders from FW4 and FW5 in winter). Filterer1 consumers had the highest amounts of bacterial fatty acids (1.8 to 10.4 % TFA) amongst all the groups. There was no obvious trend in bacterial derived fatty acids across sites in most consumers. Predators had consistently low proportions of bacterial fatty acids at all sites and during all seasons (0.9 to 5.1% TFA). In gatherers, bacterial derived fatty acids ranged between 1.2 to 5.9% TFA; however, they decreased in the downstream direction in winter and early spring (Fig. 5.5).

Proportions of green algae marker fatty acids (Fig. 5.5) were low or absent in consumers across all sites and seasons (range 0.0 to 4.3% TFA). The highest proportions of the green algae indicator occurred in grazers at FW3 and FW4 (2.3 to 3.0% TFA) during the autumn and at FW4 in filterer2 during early spring (4.3% TFA; Fig. 5.5). All filterers (filterer1 and filterer2) had greater levels of green algal markers than all the other animals. Gatherers, where present, consumed the lowest proportions of green algae in the late spring (0.0 to 0.9% TFA), with higher proportions consumed in the early spring (0.0 to 1.2% TFA).

Predators contained the lowest proportions of green algae in their diet (0.0 to 1.8% TFA) compared to other FFGs. In the headwaters, except in winter, green algae markers were very low in all consumers, with maxima occurring in the middle and lower reaches of the Kowie River (Fig. 5.5).

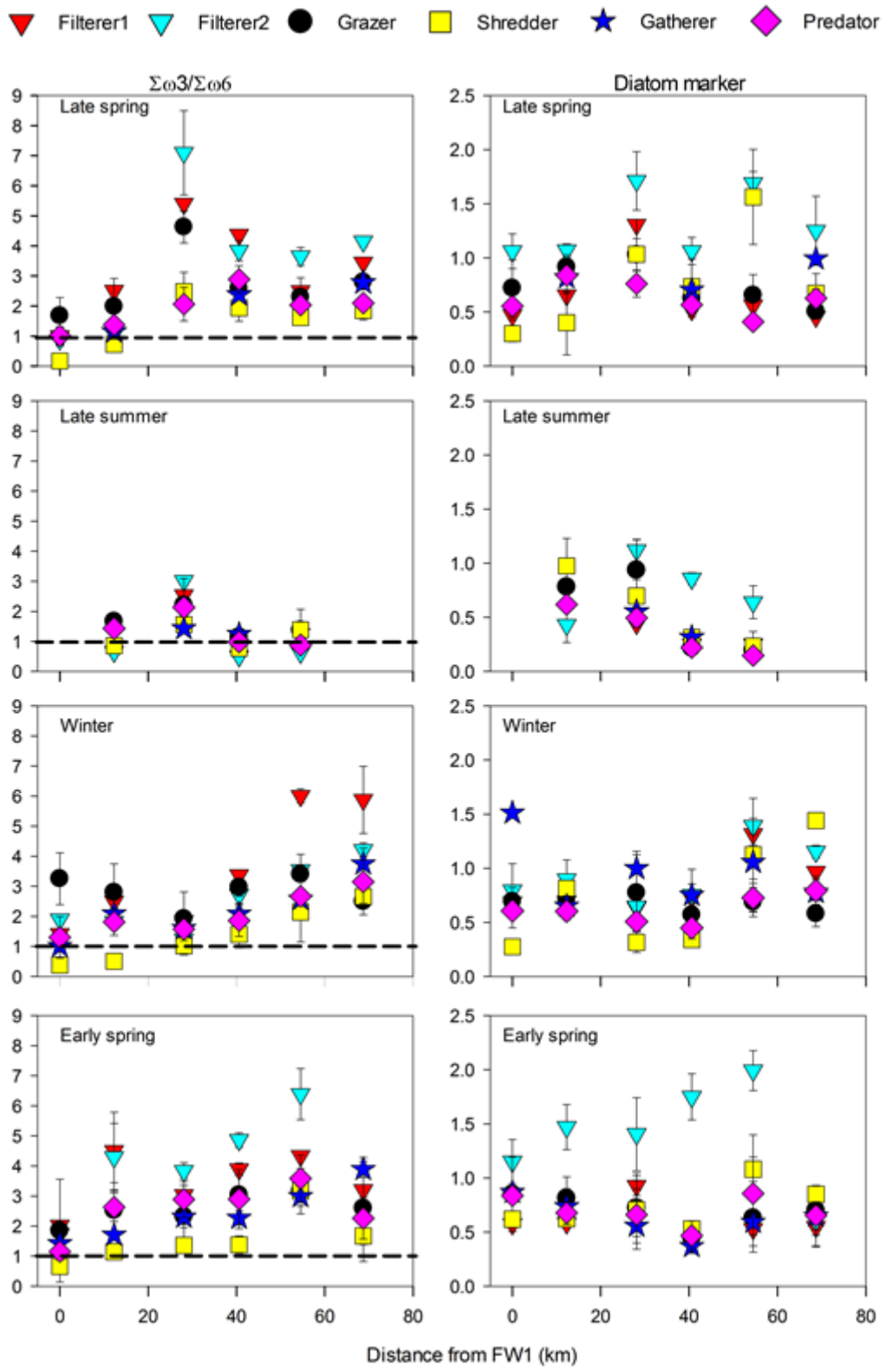

Figure 5.5: Mean (± SD) of Σω3/Σω6 fatty acid ratios and diatom marker ratios (14:0 + 16:1ω7 + 16:2ω4 + 16:3ω4 + 16:4ω1/16:0) along the Kowie River between November 2012 to September 2013 from the headwaters to the river mouth. The dashed line on the Σω3/Σω6 scale shows the threshold that indicates terrestrial- (values less than one) and aquatic-based (greater than one) diets of consumers.

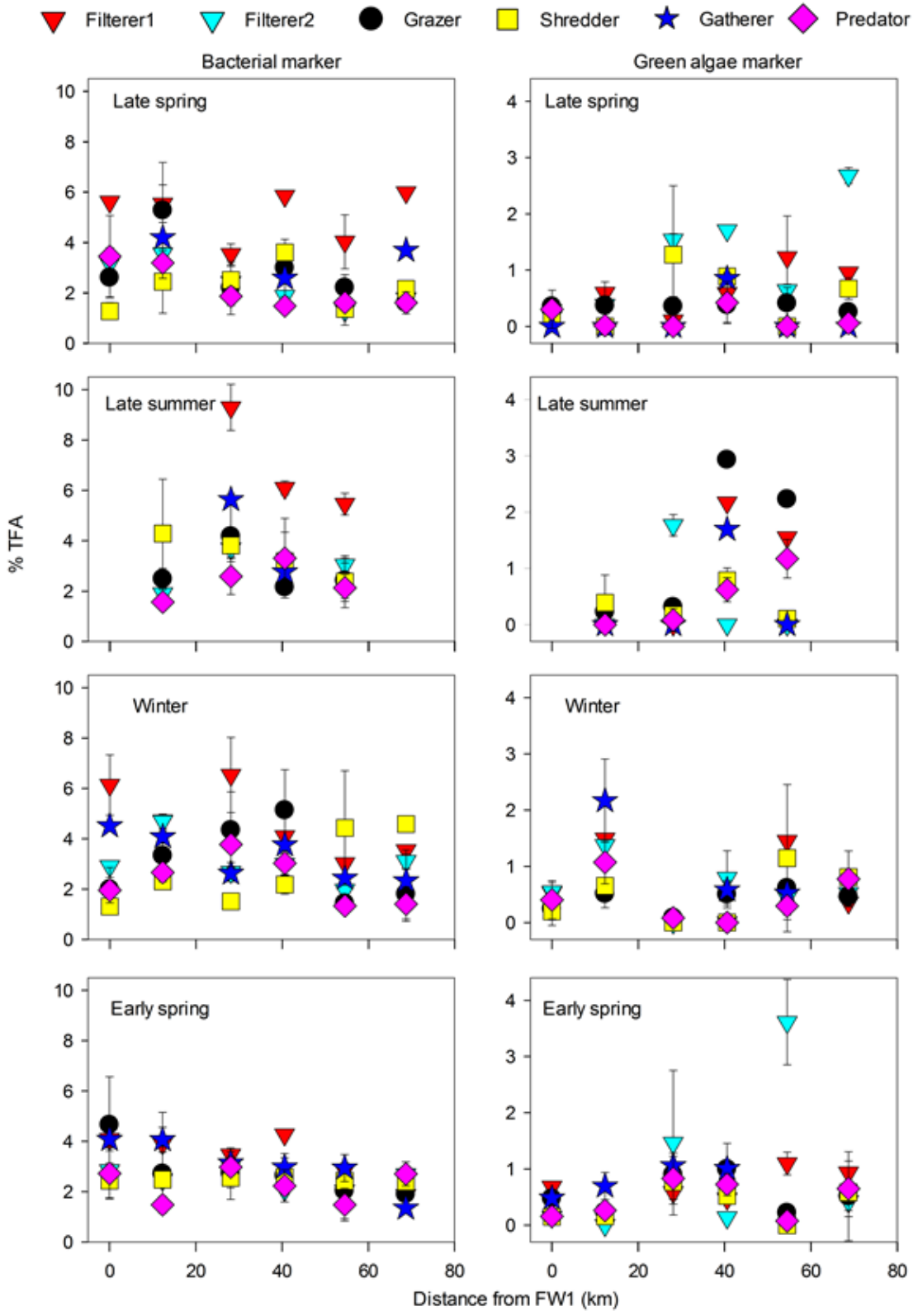

Figure 5.6: Proportions (% of total fatty acids; means and standard deviations) of specific bacterial (sum of odd-chain, *iso* (*i*) and *anteiso* (*ai*) fatty acids) and green algal (16:2ω6 +16:3ω6 +16:3ω3) markers in consumers along the Kowie River between November 2012 to September 2013.

5.4 Discussion

The primary objectives of this study were to examine the seasonal dynamics of fatty acids in consumers along a river continuum and to infer the diets of these consumers. To address the seasonal dynamics in fatty acids, diagnostic EFAs (18:2ω6, 18:3ω3, 20:4ω6, 20:5ω3) for terrestrial vs aquatic organic matter and a multivariate approach were both used in tandem to assess diet shifts in consumers. There are few studies in streams that have investigated the dynamics of fatty acids in consumers (e.g. Torres-Ruiz et al. 2007 and Descroix et al. 2010a), but this study represents the first attempt to characterise the flow of organic matter to consumers in a riverine system in southern Africa using a fatty acid approach. By covering different groups of consumers (FFGs), four sampling seasons and six sites along a riverine gradient, this study of the Kowie River represents an original contribution towards a better understanding of the dynamics of fatty acids in stream consumers. Specifically, my results indicated the utility of fatty acids in understanding the temporal and spatial changes in consumer diets, which would not be possible using traditional methods such as gut contents. Furthermore, the fatty acid data obtained from the Kowie River have revealed the usefulness of diagnostic fatty acids (specific EFAs and fatty acid ratios; Fig. 5.5 and Fig. 5.6) to determine the trophic links in the Kowie River. Traditionally, rivers were considered to be mainly dependent on sources from terrestrial organic matter, largely because terrestrial systems contributed more organic matter to streams while primary producers in streams contributed little and were seasonally light-limited (Vannote et al. 1980). The data obtained from the Kowie River suggested that autochthonous sources contributed to the lipids of FFGs across most seasons except in the late summer, when terrestrial organic matter made greater contributions to consumer diets (Fig. 5.5) relative to other seasons. The ratio for allochthonous vs autochthonous reliance of consumers increased in the downstream direction (except in the late summer; $\Sigma\omega3/\Sigma\omega6 < 1$ for most consumers), suggesting that the dependence of consumers on different basal resources changed seasonally and spatially.

5.4.1 Are variations in physiologically important fatty acid proportions explained better by FFG, temporal or spatial factors? (question 1)

Consumers lack the necessary enzymes to synthesise or elongate physiologically important fatty acids and must obtain them from their diet (Hixson et al. 2015); this makes the spatial and temporal changes of these fatty acids in riverine systems very important for inferring diets. In light of the physiological importance of EFAs, I posed a question about whether FFG influences EFAs more than time or location. Partially confirming *a priori*

predictions, FFG was the most influential factor on 18:2ω6 and ARA (**prediction 1 supported**). Contrary to **prediction 1**, 18:3ω3 and EPA were more influenced by season (**prediction 1 not supported**). However, FFG, site and collection time all had significant effects on the EFAs in consumers (Table 5.1).

Functional feeding group was an important variable in explaining EFAs in the Kowie River (Table 5.1). The effects of FFG on fatty acids is in agreement with many studies that have shown that FFG, physiological characteristics (such as the ability to store lipids or to use lipids for growth and reproduction and metabolic requirements) and phylogeny of consumers can affect their lipids (Makhutova et al. 2011). Considering that the FFGs in the Kowie River represented diverse taxa (Chapter 3), which were in their larval stages (except for crabs), the use of lipids for growth and reproduction would most likely differ among consumers (as different FFGs have different lipid needs for growth). For instance, Volk and Kiffney (2012) investigated the factors that affect the variability of fatty acids and reported that trophic level explained the most variation in physiologically important fatty acids owing to differences in metabolic requirements of consumers (e.g. predatory fish and herbivorous insects).

Collection time (season) was the second most influential variable on EFAs in the Kowie River (Table 5.1). The availability and consequently the quality of basal resources in the Kowie River changed seasonally, possibly due to shifting environmental conditions such as rainfall and water velocity (Dalu et al. 2015b). The seasonal changes (e.g. in periphyton EFAs increased in early spring and were lowest in late spring) in basal resources results in changes in the nutritional quality of the resource (Richoux et al.; in prep), which in turn causes seasonal changes in EFAs. The seasonal changes in EFAs are in accordance with findings by other researchers that investigated the seasonal changes of fatty acids in stream (Guler et al. 2011) and lake food webs (Goedkoop et al. 2000). Dynamics of EFAs are affected by temperature and light in streams (which change seasonally; McMeans et al. 2015). Temperature changes from season to season and can potentially affect EFAs by causing consumers to retain or utilise EFAs in their tissue (Masclaux et al. 2012). For example, low temperatures may cause the production of more unsaturated fatty acids by consumers (Ahlgren et al. 2009). However, freezing temperatures were not recorded in the Kowie River (Dalu et al. 2014b), hence this effect was unlikely. In the Kowie River, phytoplankton abundance and community structure change seasonally, which can cause changes in the nutritional supply of EFAs (Dalu et al. 2014b). Light increases fatty acid synthesis by affecting the chloroplasts in algae (Guschina and Harwood 2009). Luminous intensity in the Eastern Cape changes seasonally (with high intensity measured in summer

and spring months; Brooks et al. 2015), and these changes affect the quality of basal resources, which in turn affect the EFAs recorded in consumers.

Site also had a significant influence on EFAs in consumers (Table 5.2). Different sites within a riverine system have differing overstory vegetation and are exposed to alternate climatic conditions and land use activities (Chapter 4, Boëchat et al. 2011, Larson et al. 2013). Agricultural activities (agricultural intensity) and urban developments in different portions of a river influence the nutrient loading, so areas with increased nutrients (phosphorus and nitrogen) in turn increase algal production (Boëchat et al. 2011). Additionally, geomorphology (elevation and substrate type) at different sites can affect EFAs in consumers (Smits et al. 2015). These factors may all have contributed to the effects of site on EFAs in consumers in the Kowie River. Overall, the site, FFG and season factors influenced the consumer EFAs (confirmed by the significant interactions among all the exploratory variables; Table 5.2).

5.4.2 How do seasonal changes in fatty acid proportions in FFGs relate to those of their major basal resources? (question 2)

A question was posed on the seasonal changes in fatty acid proportions amongst different FFGs and how they relate to those of their major basal resources. I postulated that carbon derived from local production would make up the bulk of lipids in consumer tissues. The nMDS analysis (using all fatty acids of basal resources) of the Kowie River food web linked all macroinvertebrates with autochthonous primary producers (Figs. 5.1, 5.2, 5.5), in support of my hypothesis. A comparison of the EFAs in macroinvertebrates and the primary producers showed that autochthonous food sources (Richoux et al. in prep) and macroinvertebrates had markedly higher total EFAs than terrestrial plants and detritus. ARA and EPA in macrophytes, terrestrial plants and detritus (where samples were available) occurred in very low proportions or were undetected, with the most abundant EFA being 18:3ω3 (summarised in Table 5.3). The relative importance of different organic matter sources in supporting riverine consumers in the Kowie River was consistent with other studies in freshwater environments using fatty acids to assess diets (Torres-Ruiz et al. 2007, Descroix et al. 2010a, Galloway et al. 2014b). The aforementioned works (e.g. Torres-Ruiz et al. 2007) showed that consumers assimilated food sources from autochthonous production. The consumption of more autochthonous sources in the Kowie River could be due to many reasons. For instance, the unpalatability and recalcitrant nature of the terrestrial leaves may explain consumers not utilising terrestrial plants as basal resources (Thorp and Delong 2002). Moreover, consumers may selectively assimilate fractions of autochthonous material (e.g.

Mulholland et al. 2000), which is a higher nutritional source. The fatty acid profiles of aquatic plants and terrestrial leaves were distinct from the animals, suggesting that most of them consumed autochthonous algal food sources. However, in some seasons, detritus had similar profiles to the consumers, suggesting consumption of detritus. Leaves act as substrates upon which nutritious microbes grow (Allan and Castillo 2007), and colonized leaves are rapidly consumed by stream invertebrates rather than fresh leaf material (Torres-Ruiz and Wehr 2010). In the Kowie River, it is possible that the detritus was consumed because of the colonisation by microbes and benthic algae, as the detritus is composed of a rich matrix of benthic algae, macrophytes and terrestrial leaves (Dalu et al. 2015b). During some seasons, the food sources had fatty acid profiles that were dissimilar to fatty acid profiles of consumers (nMDS plot for FW4 in late summer; Fig. 5.5). Such differences between food sources and the consumers may imply a lag time in the incorporation of fatty acids into the consumer tissues. It is unlikely that the differences between food source and consumer EFAs arose from elongation of other fatty acid components, as experimental studies in stream food webs have indicated that few stream invertebrates are able to elongate shorter chains (Torres-Ruiz et al. 2010); however, data on how fatty acids are incorporated into consumer tissues remain scarce. In Chapter 4, the consumption of macrophytes via direct herbivory was considered but concluded as unlikely. The distinct fatty acid profiles of the macrophytes and terrestrial leaves compared with the consumers strengthened that conclusion. Combining the findings from Chapter 4 with this part of the study shows that while macrophytes are high in 18:2ω6, they are not consumed until they enter the detrital pool, as confirmed by other researchers (e.g. Pingram et al. 2014).

Table 5.3: Mean (± SD) essential fatty acid proportions recorded in basal resources of the Kowie River (summarised from Richoux et al. in prep). Asterisks show EFAs that were undetected.

group	18:2ω6	18:3ω3	ARA	EPA
Aquatic plant	14.1 (3.7)	53.1(7.2)	*	*
Detritus	10.8 (7.9)	18.0 (8.6)	0.6(0.5)	2.2(3.0)
Benthic algae	2.1 (1.4)	4.0 (4.9)	1.7 (0.8)	10.1 (5.7)
Epiphyton	3.1 (1.4)	3.6(2.5)	1.4 (0.8)	9.1(4.9)
Periphyton	3.4 (1.6)	4.9 (3.7)	1.5(0.9)	7.73(4.6)
POM	3.0 (2.1)	4.0(6.2)	0.4 (0.4)	4.49 (3.5)
Terrestrial plant	12.5 (5.5)	53.2(8.0)	*	*

5.4.3 Do shifts in consumer diets along the river continuum correspond to changes in trophic markers for freshwater systems? (question 3)

Considering that the fatty acid profiles of consumers are linked to their diets (e.g. Ahlgren et al. 2009), I investigated the efficacy of trophic markers in inferring the diets of consumers, and postulated that carbon derived from local production made up the bulk of organic matter supporting consumers and that the relative importance of autochthonous markers increased in the downstream direction. My data indicated that EFAs changed with increasing distance from the headwaters (Figs. 5.5 and 5.6). Changes in the ω3 and ω6 PUFAs (particularly 18:2ω6, 18:3ω3, 20:5ω3) of consumers at different sections of the Kowie River showed that the quality of their food sources differed spatially (Figs. 5.5 and 5.6).

EPA was important in the tissues of most consumers in the Kowie River (0.2 to 27.1%TFA). EPA is a major component in algal pools (benthic algae, epiphyton and periphyton) in the Kowie River (Dalu et al. 2015b), and was therefore a suitable indicator of consumption of algae (particularly diatoms). As such, relatively high levels of EPA in the profiles of the stream invertebrates may indicate a dominance of diatoms at the base of the Kowie River food web during all seasons. Generally, the consumption of diatoms increased in the downstream direction (Fig. 5.6), while in the late summer the consumption of diatoms decreased with increased distance (Fig. 5.6). Predators generally decreased in EPA with increasing distance from the headwaters (contrary to **prediction 3B**; Fig. 5.6), probably because predators do not consume diatoms directly but obtain their PUFAs via consumption of their prey (primary consumers).

The fatty acids 18:2ω6 and 18:3ω3 are typically considered to arise primarily from terrestrial plants (Napolitano 1999). The potential of 18:2ω6 and 18:3ω3 as terrestrial indicators was supported by the high proportions of these fatty acids in terrestrial leaves in the Kowie River (Richoux et al. in prep). Using 18:2ω6 and 18:3ω3 as tracers in the Kowie River revealed that the consumption of terrestrial organic matter by invertebrates was highest in the headwaters and decreased in the downstream direction (except for 18:2ω6, which increased in proportions with increasing distance in some FFGs), in partial support of **prediction 3A**. The lack of obvious trends (in some seasons) of the terrestrial markers in animals could have been caused by occurrences of 18:2ω6 and 18:3ω3 in the autochthonous basal resources. Linoleic acid (18:2ω6) and 18:3ω3 do occur in macrophytes and algae (Honeyfield and Maloney 2015, Richoux et al. in prep), hence these tracer proportions in consumers cannot be attributed to terrestrial consumption alone.

Proportions of ARA in consumers decreased with increasing distance during the winter and late summer (Fig. 5.6), in contradiction to my original predictions (**prediction 3B not supported**). While the role of 20:4ω6 as a marker for inferring diets is not well understood in freshwater systems, my study showed that ARA was generally low in consumers in the Kowie River [in agreement with ARA proportions obtained in other studies (0 – 7.2%TFA, Hanson et al. 1985, Torres-Ruiz et al. 2007); but *cf.* 11 - 18%TFA in freshwater bivalves, Newton et al. 2013)]. Riverine consumers can obtain ARA directly from their diet or via the lengthening of 18:2ω6 (Bell and Tocher 2009). While there is evidence from lakes that some species of zooplankton can elongate 18:2ω6 to ARA (Ahlgren et al. 2009), one river study showed that freshwater invertebrates were unable to make such elongations (Torres-Ruiz et al. 2010). As such, it is highly unlikely that the ARA originated through elongation in the Kowie River consumers.

Confirming the initial predictions (**prediction 3 supported**), the Σω3/Σω6 ratio in consumers changed along the continuum and generally increased with increasing distance from the headwaters towards the river mouth. The values recorded for the Σω3/Σω6 ratio in animals from all sites in the Kowie River were characteristic of published values for freshwater invertebrates (Desvilettes et al. 1994) and indicative of which group of basal resources was supporting each consumer group. Often the values of Σω3/Σω6 for all invertebrates were greater than one across all seasons, which indicates consumption of autochthonous organic matter, as previously reported by other researchers (Descroix et al. 2010a). No studies have documented longitudinal changes of Σω3/Σω6 in riverine consumers, but the Σω3/Σω6 ratios derived from the Kowie River corroborated the major finding from Chapter 4 that contributions of autochthonous organic matter into the food web increased from upriver to downriver.

There was no obvious trend in the trophic marker indicative of consumption of green algae (16:2ω6 +16:3ω6 +16:3ω3) in the Kowie River (Fig. 5.6), contrary to my initial prediction that aquatic markers would increase in consumers with increasing distance from the headwaters. The tracer for green algae was highest in filterers and grazers (2.3 to 4.3% TFA). The presence of green algae in the diets of grazer and filterers has been demonstrated in other riverine systems (Sushchik et al. 2003), and the high ω3 content in green algae makes it a preferred food choice for consumers. However, the proportion of green algae in the diets of the Kowie River consumers (0 to 4.3% TFA) fell within the ranges recorded in the Yenisei River in Russia (0 to 4.5% TFA). The low values may indicate the low abundances of green algae (Chlorophyta) relative to diatoms in riverine systems (*sensu* Dalu et al. 2014b), which explains the lack of an obvious trend in the Kowie River.

There was no obvious trend in bacterial fatty acids in consumers (Fig. 5.6), suggesting that the consumption of bacteria did not vary with distance from the headwaters. I would have expected the bacterial fatty acids to follow longitudinal trends of terrestrial markers (18:2ω6 and 18:3ω3), as bacteria typically colonise detritus in streams (France 2011). Similarly bacterial diversity and abundance can decrease with increasing distance from the headwaters (Savio et al. 2015). In the Kowie River, the sums of bacterial fatty acids were lower (< 10% TFA; Fig. 5.6) compared to those recorded in other riverine studies (~ 20% in a North Carolina stream; Hall and Meyer 1998). The low values may not necessarily imply that the contributions of bacterial fatty acids to invertebrates were insignificant in the Kowie River. Unlike the long chain fatty acids, bacterial fatty acids do not have a structural function in diets of consumers and tend to be catabolized for energy rather than stored in tissues (Taipale et al. 2012). The catabolisation of bacterial fatty acids by consumers may explain why bacterial fatty acids did not follow an obvious trend in the Kowie River.

The nMDS and SIMPER were used to identify groupings and influential fatty acids in the Kowie River consumers. The identified fatty acids were then matched to those cited in the literature (Table 5.4). Arachidonic acid (ARA) was usually associated with the shredders, while 12:0 was always associated with filterer1 (*Hydropsyche* spp.). The presence of 12:0 in Hydropsychidae has been cited in other riverine studies (Descroix et al. 2010). For instance, Descroix et al. (2010) found that 12:0 was absent in three families (Baetidae, Chironomidae and Simuliidae) and occurred only in the tissues of Hydropsychidae (12%TFA in caddisflies from Allier River, France). A search in the literature did not reveal the possible organic matter source for 12:0 and an experimental approach would be needed to determine whether 12:0 is a useful trophic marker in caddisfly diets.

Table 5.4: Fatty acid trophic tracers from the literature and the Kowie River. '?' denotes uncertainty about the potential tracer.

Trophic tracer	Fatty acid (s)	Calculated by:	References
Bacterial			
	Branch-chain odd FA (e.g. *iso* and *anteiso* of 15:0 and 16:0)	Summing all	Najdek et al. (2002)
	18:1ω7	Raw data	Napolitano 1999, Bühring et al. (2005)
Diatoms			
	16:1ω7	Raw data	Dunstan et al. (1993), Napolitano et al. (1997)
	16:2ω4, 16:3ω4	Summing both	Léveillé et al. (1997), Napolitano (1999)
	20:5ω3	Raw data	Ahlgren et al. (1992), Demott and Müller-Navarra (1997), Scott et al. (1999)
Terrestrial matter, fungi, some algae	18:2ω6	Raw data	Napolitano (1999)
Green algae or terrestrial plants	18:3ω3	Raw data	Desvilettes et al. (1994), Léveillé et al. (1997), Napolitano et al. (1997)
Animal matter or algae	18:1 ω9	Raw data	Napolitano (1999)
?	20:4ω6	?	Kowie River
Diatoms?	12:0	?	Kowie River

5.4.4 What do fatty acids contribute to theoretical models of energy flow in small rivers?

The RPM suggests that consumers are fuelled by *in situ* primary production in large rivers (Thorp and Delong 1994). The support for the RPM has been verified by studies including Delong and Thorp (2006) and Pingram et al. (2014). Unfortunately, less work using fatty acids has been done in streams to assess the validity of the energy flow models. One fatty acid study has tested the validity of the theoretical models in the Amazon River. In this study, Mortillaro et al. (2012) found strong correlations between suspended particulate organic matter pools and fatty acid markers reflective of allochthony, suggesting limited support for the RPM in the river. The present study (Kowie River) is incongruent to those findings and extends the notion discussed in Chapter 4 and in Thorp and Delong (1994) that *in situ* carbon makes up the bulk of food assimilated by consumers. The fatty acid data from

the Kowie River also supported some of aspects of the RCC in that allochthonous material contributed to consumer diets, especially at the headwaters. On the whole, the fatty acid data supported the RPM showing that *in situ* production fuels consumers across all seasons and at most sites and validated conclusions made in Chapter 4 and those made by other authors (Mulholland et al. 2000, Lewis et al. 2001, Thorp and Delong 2002) that autochthony may play a major role in riverine systems.

5.5 Conclusions

The key findings from this chapter are that: (1) FFG, site and season all influenced the EFAs in riverine consumers; (2) autochthonous sources (and detritus to a lesser extent) made up the bulk of organic matter supporting consumers (hypothesis supported); and (3) the consumption of autochthonous organic matter increased with increased distance from the headwaters during some seasons and in some FFGs (hypothesis partially supported).

Amalgamating the results from this chapter and those of preceding chapters reveals similarities and differences in the contributions of different basal resources to consumers of a small temperate river. The fatty acid data obtained from the Kowie River provided strong evidence for the dependence of different consumers on autochthonous sources, and autochthonous materials were the most likely sources of EFAs in consumers. According to the RCC, allochthonous materials are a more important carbon source for invertebrates (Vannote et al. 1980), a finding supported in the Kowie River according to invertebrate community metrics of autochthony versus allochthony (allochthony was common at most sites except in early spring; Chapter 3, Table 3.1). The FFG metrics for autochthony vs allochthony in Chapter 3 were incongruent to the results from stable isotope analysis (Chapters 4) and fatty acid analysis (Chapter 5), whereby the tracer methods showed that autochthony was dominant in the middle reaches and lower reaches of the Kowie River. In Chapter 3, these findings were questioned as the allochthony versus autochthony metrics were developed for the northern hemisphere and assumed that FPOM was predominantly allochthonous. This assumption in the metrics of autochthony versus allochthony may explain the differences between Chapter 3 and the tracer data (stable isotopes and fatty acids).

Results in this chapter and the preceding isotope chapter also highlighted the importance of considering spatial and temporal approaches in food web studies of dynamic environments such as streams and rivers. Future studies are needed on the specific EFA requirements of different invertebrate consumers, and their ability to synthesize and elongate other fatty acids. Further studies will also determine the role of EFAs in energy transfer within riverine food webs and their adjacent habitats.

6 Terrestrial-aquatic linkages: reciprocal flows of invertebrate prey couple streams and riparian areas in a temperate river

6.1 Introduction

Rivers are coupled to adjacent riparian habitats through spatial subsidies. Spatial subsidies occur when resources (e.g. detritus and invertebrates) move from one habitat (donor) to another (recipient), thereby influencing the productivity of the recipient system (Polis et al. 1997). Whenever subsidies occur, depending on the magnitude of the subsidy, they may substantially increase resources above those produced in the recipient system (Nakano and Murakami 2001). Several researchers proposed that the general flow of energy is from more productive to less productive systems (Vannote et al. 1980, Polis and Hurd 1996, Polis et al. 1997). Owing to this unidirectional view, most studies have focused on the "more productive" systems and have addressed terrestrial inputs to aquatic food webs. Recently, ecologists have documented the flow of energy from aquatic to terrestrial systems (Paetzold et al. 2005). Such investigations of flow from aquatic to terrestrial systems have changed the prevalent view of energy flow from a somewhat unidirectional one to a bi-directional aquatic-terrestrial flow. Materials that may potentially move from the stream to the riparian zone include emerging insects, remains of stream animals and algae that is washed to the riverside (Richardson and Sato 2015). Materials that may flow from the riparian area to the stream include leaf litter, pollen grains, fruits, seeds and falling terrestrial invertebrates (Richardson and Sato 2015).

Asynchronous seasonal changes in productivity between a stream and its riparian forest can result in reciprocal and alternate aquatic–terrestrial subsidies (Nakano and Murakami 2001, Rundio and Lindley 2012). Such freshwater-terrestrial linkages are dynamic over time and space. For example, terrestrial inputs fluctuate daily and annually, but follow regular seasonal patterns in temperate zones, with inputs highest in summer and lowest in winter (Nakano and Murakami 2001). Similarly, emergence of insects has seasonal peaks that coincide with high solar inputs and algal production (Kawaguchi and Nakano 2001). These types of cross-ecosystem food web linkages have important implications for the structure, function, and trophic dynamics of recipient and donor ecosystems (Iwata 2007). Although cross-habitat connections are viewed as important by ecologists, research on such linkages is generally conducted using dry weight and/or abundance measures (Kautza and Sullivan 2015). While these measures portray the possible contributions of resources to habitats, they

tend to be biased towards the magnitude of the subsidy rather than the nutritional quality or the ecological impact of the subsidy (Marcarelli et al. 2011).

When insects move from one habitat to another, they represent energy for the recipient habitat in the form of biochemical compounds needed to support consumers. Some of these biochemical compounds, such as essential fatty acids, are important for the physiological and biochemical functioning of brain, nervous and cardiovascular systems of organisms (Arts et al. 2001). Lipids (fatty acids) have been used to determine trophic relationships and identify food web connections within aquatic and terrestrial systems (Chapter 5; Lam et al. 2013), and so are useful for tracing trophic transfers between adjacent systems. Particular polyunsaturated fatty acids (PUFAs) such as arachidonic acid (ARA; 20:4ω6), eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic (DHA; 22:6ω3) can indicate the nature of food subsidies, and total fatty acid concentrations relative to dry mass can provide information about the potential nutritional quality of the subsidies (Brett et al. 2006). Incorporating fatty acids into reciprocal subsidy studies may provide valuable information on the quality of resources shared across ecotones (Marcarelli et al. 2011). To date, there is one key study that provided estimates of the export of PUFAs to land via emerging insects in a suite of different ecosystems (Gladyshev et al. 2009). Appropriately, the authors suggested that PUFA export be determined in additional ecosystems (where data are not available) to increase our general knowledge about ecosystem connectivity (Gladyshev et al. 2009).

The available information on trophic aquatic-terrestrial linkages is mainly restricted to headwater streams in most parts of the world (Bartels et al. 2011). Therefore, my goal was to expand the perspective to include a southern African river, where invertebrate flux between the aquatic and terrestrial systems is unknown. I examined the flow of energy across the stream-riparian ecotone at three sites along a South African temperate stream over four seasons. The primary objective was to quantify the reciprocal fluxes of invertebrates in southern Africa by assessing the movements and biomasses of terrestrial and emergent insects. The second objective was to incorporate physiologically important PUFAs in invertebrates to estimate the flow of these components across the stream-riparian interface.

6.2 Material and methods

6.2.1 Study area and sample collection

Samples of terrestrial invertebrates and emerging insects were collected between November 2012 and September 2013 from three sites along the Kowie River (Fig. 6.1). Collection times for emerging and infalling invertebrates coincided with a concurrent study

by Chari (2016), therefore the season designations used herein differed from preceding chapters (see Chapter 2 for detailed collection dates). Floating pyramidal emergence traps (0.37m^2 base) were used to quantify the rates of emergence of aquatic insects (Davies 1984). Pan traps with shallow clear plastic trays (filled with a few drops of liquid soap) were used to quantify terrestrial inputs through fortuitous infall (all sampling protocols described extensively in Chapter 2, Section 2.1.2). All traps were arranged perpendicular to the river's edge (Fig. 6.1).

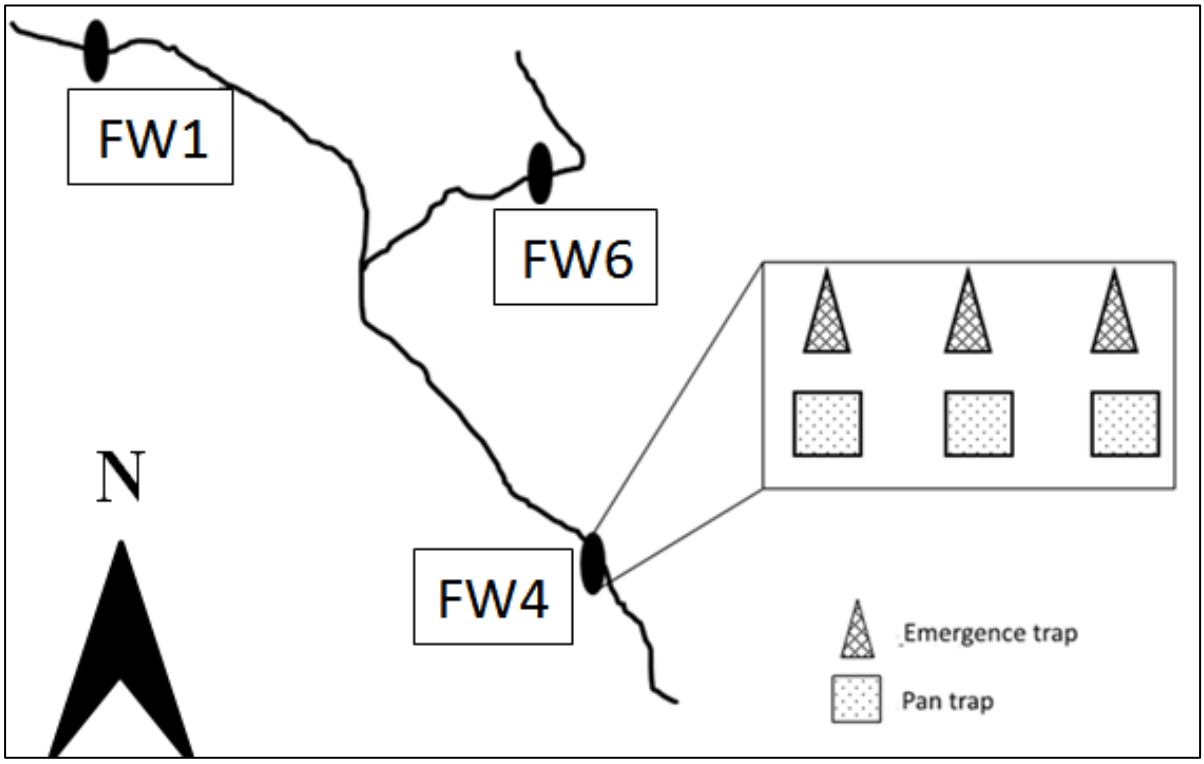

Figure 6.1: Location of the study sites and the insect trap arrangements (arranged perpendicular to the river's edge) in the Kowie River (not drawn to scale), South Africa. Triangles and squares show the arrangement of traps at each study site.

6.2.2 Sample processing

Invertebrates were identified and counted under a stereo microscope. Adult aquatic insects were usually identified to family, while most terrestrial invertebrates were identified to order, suborder (some Diptera), or family. Dry mass of invertebrates was estimated from length-mass regressions following Sample et al. (1993), Towers et al. (1994), Ganihar (1997), Benke et al. (1999), Johnston and Cunjak (1999), Sabo et al. (2002), Gruner (2003) and Höfer and Ott (2009).

6.2.3 Data analysis

Daily aquatic invertebrate biomass flux (mg m^{-2} day^{-1}) for each of the three replicates from emergence traps, and daily terrestrial invertebrate biomass flux from each pan trap (mg m^{-2} day^{-1}), were calculated over four seasons. The data were log transformed to balance the effects of dominant and less dominant taxa. A two way analysis of variance (ANOVA) was used to assess the effects of site and season on terrestrial or aquatic fluxes. Multiple regression analysis was used to assess the influence of habitat characteristics and stream productivity on terrestrial invertebrate inputs to the river and emergence of invertebrates to the land. The six independent variables were: (1) stream and air temperature, (2) overstory cover on the stream, (3) stream width, (4) rainfall (from November 2012 to September 2013), (5) water velocity and (6) algal biomass. Data for these factors were obtained from concurrent studies on the Kowie River (summarised in Chapter 2, Section 2.2). Prior to running the multiple regression analysis, correlations among these independent variables were determined to assess multicollinearity. All regressions and ANOVA were implemented using Microsoft® Excel 2010/XLSTAT© (Version 2014.5.03, Addinsoft, Inc., Brooklyn, NY, USA). In each season, unpaired t-tests were used to determine whether terrestrial inputs equalled emergence (in mg m^{-2} day^{-1}). Unpaired t-tests were conducted in SYSTAT 13 (SYSTAT Software Inc., Chicago, Illinois, USA).

PUFA flow via aquatic invertebrate flux was estimated by calculating fatty acid concentrations (mg g^{-1}) in larval insects [from fatty acid peak areas relative to the area of an internal standard 19:0 (10 -15 µl of 6 to 8 mg standard solution) added to each sample]. Similarly, PUFAs supplied by terrestrial arthropods into the stream were estimated by calculating fatty acid concentrations in terrestrial arthropods at order level (see Table 2.5 for PUFAs summaries). All PUFA values of terrestrial arthropods were obtained from a concurrent study investigating the diets of aerial predators (Chari 2016). Bidirectional total flow at each study reach (mg m^{-2} day^{-1}) was estimated by multiplying the mean dry weight daily input (calculated from individual traps) with the PUFA concentrations in insects. In Chapter 5, qualitative fatty acid data (proportional fatty acids) were used as they allow for comparisons between samples with dissimilar lipid contents (*sensu* Napolitano et al. 1997). However, to quantify the movements of organisms across an ecotone requires the use of quantitative lipid contents (*sensu* Hixson et al. 2015) as these provide biomass values of exported nutrients, therefore quantitative data (mg g^{-1}) were used to obtain biomass measurements of PUFA flow.

Annual bidirectional flow by biomass (g m^{-2} $year^{-1}$) and PUFA (mg m^{-2} $year^{-1}$) of terrestrial arthropods and insect emergence at each site were calculated by multiplying the

daily mean for the whole study by 365 days. Estimating annual flows in this way provided a standard scale for comparing the Kowie River results with those from Hokkaido, Japan (Nakano and Murakami 2001), California, USA (Rundio and Lindley 2012). Estimating annual flow of PUFA also allowed for PUFA flow in the Kowie River to be comparable to estimates for riverine ecosystems recorded in Gladyshev et al. (2009).

6.3 Results

Terrestrial inputs to the river and emergence fluxes from the river changed among sites and seasons (two way ANOVA, N = 36, $p < 0.01$; Table 6.1). Terrestrial inputs and emergence followed similar seasonal patterns at all sites (i.e. emergence was high when terrestrial inputs were high and vice versa), typically peaking in the summer months and declining in the winter (Fig. 6.2). For clarity, the results section is divided into three main parts. Firstly, a description of the seasonal patterns in aquatic emergence across all sites is presented. Secondly, seasonal patterns in terrestrial invertebrate inputs to the stream across all sites are described. Finally, estimations of the reciprocal flows in the context of physiologically essential PUFAs (ARA, EPA and DHA) are presented.

6.3.1 Emergence of aquatic insects

Aquatic invertebrate biomass flux from the river showed similar seasonal patterns across all sites, with emergence peaking in the summer (FW6 > FW4 > FW1; Fig. 6.2, declining in the winter (FW4 > FW6 > FW1) and increasing slightly during the spring months (FW6 > FW4 > FW1). The highest emergence rates by number and biomass were recorded in the summer at all three sites (ranging from 169 to 1402 mg m^{-2} day^{-1}; Fig. 6.2). During the winter, emergence rates declined to between 3 and 28 mg m^{-2} day^{-1} at all sites. Aquatic emergence varied significantly among sites and seasons, with significant interactions occurring between site and time (two way ANOVA, Table 6.1). Season explained the largest source of variation in emergence, whilst site accounted for a smaller component of the total variation.

The assemblage of emergent aquatic insects was composed of Diptera, Ephemeroptera, Coleoptera, Trichoptera and Odonata, and included at least 18 families. Overall, composition of the insects in emergence traps was relatively similar between sites and was dominated numerically by Diptera (primarily the families Simuliidae and Chironomidae, Table 6.2). Odonata (families Libellulidae and Coengrionidae), which were collected only from FW4 and FW6 during the summer months, were fewer numerically compared to other taxa, but by mass Odonata contributed substantially to the insects emerging at FW4 and FW6 (Table 6.4; 17 - 27% by mass).

Multiple linear regression of emergence rates against algal biomass (determined from chlorophyll-*a* measurements), canopy cover and stream width showed that emergence rates correlated strongly with chlorophyll-*a* ($r = 0.79$, $p < 0.0001$; Table 6.3), and weakly with stream cover and canopy cover (cover: $r = -0.42$, stream width: $r = 0.38$; $p < 0.05$). The regression model and the habitat variables explained 75% of the variation ($R^2 = 75\%$; Table 6.3).

6.3.2 Stream inputs of terrestrial invertebrates

Total inputs of terrestrial arthropods to the river varied significantly among seasons and sites (Tables 6.1 and 6.2). Similar to emergence, terrestrial inputs were high in the summer (range = 413 to 679 mg m^{-2} day^{-1}; FW1 > FW6 > FW4; Fig. 6.2), with the lowest values recorded in the winter (range = 11 to 220 mg m^{-2} day^{-1}; FW1 > FW6 > FW4; Fig. 6.2) and significant peaks recorded in the spring at all sites (range = 101 to 250 mg m^{-2} day^{-1}; FW6 > FW4 > FW1; Fig. 6.2). Numbers of individuals varied among the three sites, with higher inputs collected in the shaded part of the stream, at FW1 (FW1 > FW6 > FW4; Table 6.2; Table 6.4). A similar pattern was observed in biomass of terrestrial inputs, and those inputs were statistically different among all sites (Table 6.1). Season accounted for the highest source of variation in terrestrial inputs, whilst site accounted for a smaller component of variation when compared to season (Table 6.1).

Terrestrial invertebrates collected over two seasons represented 14 orders. Generally, invertebrate inputs were comprised of small-bodied taxa such as Diptera, Hemiptera, and Thysanopetra (which were most numerous). The larger-bodied taxa such as Araneae, Hymenoptera, Lepidoptera, Neuroptera and Orthoptera were numerically fewer than the smaller taxa but contributed the most by mass (Table 6.4). Although some seasons were dominated by a single taxon, two to five orders contributed to inputs by mass or number across the study period.

Table 6.1: Two-way analysis of variance to determine differences in dry mass of aquatic invertebrate emergence (mg m^{-2} day^{-1}) and terrestrial invertebrate infall (mg m^{-2} day^{-1}) in the Kowie River. Asterisks show statistically significant effects (* $p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$).

Factor	df	sum of squares	Mean square	F	p
Aquatic invertebrates (mg m^{-2} d^{-1})					
Site	2	3.15	1.57	95.17	**
Season	3	16.04	5.35	323.23	**
site x time	6	0.67	0.11	6.80	**
Error	24	0.40	0.02		
Terrestrial invertebrate inputs (mg m^{-2} day^{-1})					
Site	2	0.45	0.23	18.30	**
Season	3	8.30	2.77	222.96	**
site x time	6	0.48	0.08	6.41	**
Error	24	0.29	0.01		

Table 6.2: Two-way analysis of variance to determine differences in abundances of aquatic invertebrate emergence (individuals m^{-2} day^{-1}) and terrestrial invertebrate infall (individuals m^{-2} day^{-1}) in the Kowie River. Asterisks show statistically significant effects (* $p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$).

Factor	df	sum of squares	Mean square	F	p
Aquatic invertebrates (ind m^{-2} d^{-1})					
site	2	1.09	0.54	48.64	***
season	3	12.07	4.02	359.65	***
site x time	6	0.65	0.11	9.63	***
Error	24	0.27	0.01		
Terrestrial invertebrate inputs (ind m^{-2} day^{-1})					
site	2.00	0.31	0.16	14.84	**
season	3.00	1.12	0.37	35.82	***
site x time	6.00	0.47	0.08	7.50	**
error	24.00	0.25	0.01		

Multiple linear regressions of air temperature, canopy cover, stream width, flow and rainfall on terrestrial infall revealed a strong positive relationship with water velocity ($r = 0.58$, $p < 0.001$; Table 6.3), a weak negative relationship with stream width ($r = -0.14$, $p = 0.01$; Table 6.3), a positive relationship with air temperature ($r = 0.39$, $p = 0.02$), a weak positive relationship with rainfall ($r = 0.1$, $p = 0.01$) and a weak positive relationship with canopy cover ($r = 0.1$, $p = 0.04$). The habitat variables explained 51% of the variation in the regression model ($R^2 = 51\%$; Table 6.3).

Table 6.3: Linear regressions of environmental factors and their relationships with emergence (mg m^{-2} day^{-1}) and terrestrial invertebrate infall (mg m^{-2} day^{-1}) in the Kowie River. Asterisks show statistically significant effects (* p < 0.05, ** p < 0.01 and ***p < 0.001).

Source	t	p	r	R^2	N
Emergence (mg m^{-2} d^{-1})					
stream width	-3.35	**	0.38	0.75	36
cover	-3.24	**	-0.42		
chlorophyll-a	7.89	***	0.79		
Terrestrial inputs(mg m^{-2} day^{-1})					
stream width	-2.62	*	-0.14	0.51	36
cover	-2.69	*	0.1		
air temperature	3.2	*	0.39		
water velocity	5.03	***	0.58		
rainfall	2.85	*	0.1		

6.3.3 Seasonality of cross ecosystem exchanges via invertebrates

Terrestrial inputs and emergence were significantly different at FW1 across all seasons (summer: $t = 6.99$, $p < 0.05$; autumn: $t = 1.32$, $p < 0.05$; winter: $t = 4.62$, $p < 0.05$; spring: $t = 2.76$, $p < 0.05$; all n = 6 for each season), although terrestrial inputs were always greater than emergence. Terrestrial inputs and emergence did not differ significantly at FW4 across autumn, winter and spring seasons (autumn: $t = 4.96$, $p > 0.05$; winter: $t = 1.20$, $p > 0.05$, spring: $t = 2.78$, $p > 0.05$; Fig. 6.2), but during the summer emergence was three times the value of terrestrial infall (summer: $t = 10.24$, $p < 0.05$). A similar pattern was observed in reciprocal flows of emerging and terrestrial invertebrates at FW6, but those inputs were statistically different only between summer and winter (summer: $t = 6.769$, $p < 0.05$; autumn: $t = 0.69$, $p > 0.05$; winter: $t = 10.44$, $p < 0.05$; spring: $t = 1.47$; $p > 0.05$; Fig. 6.2).

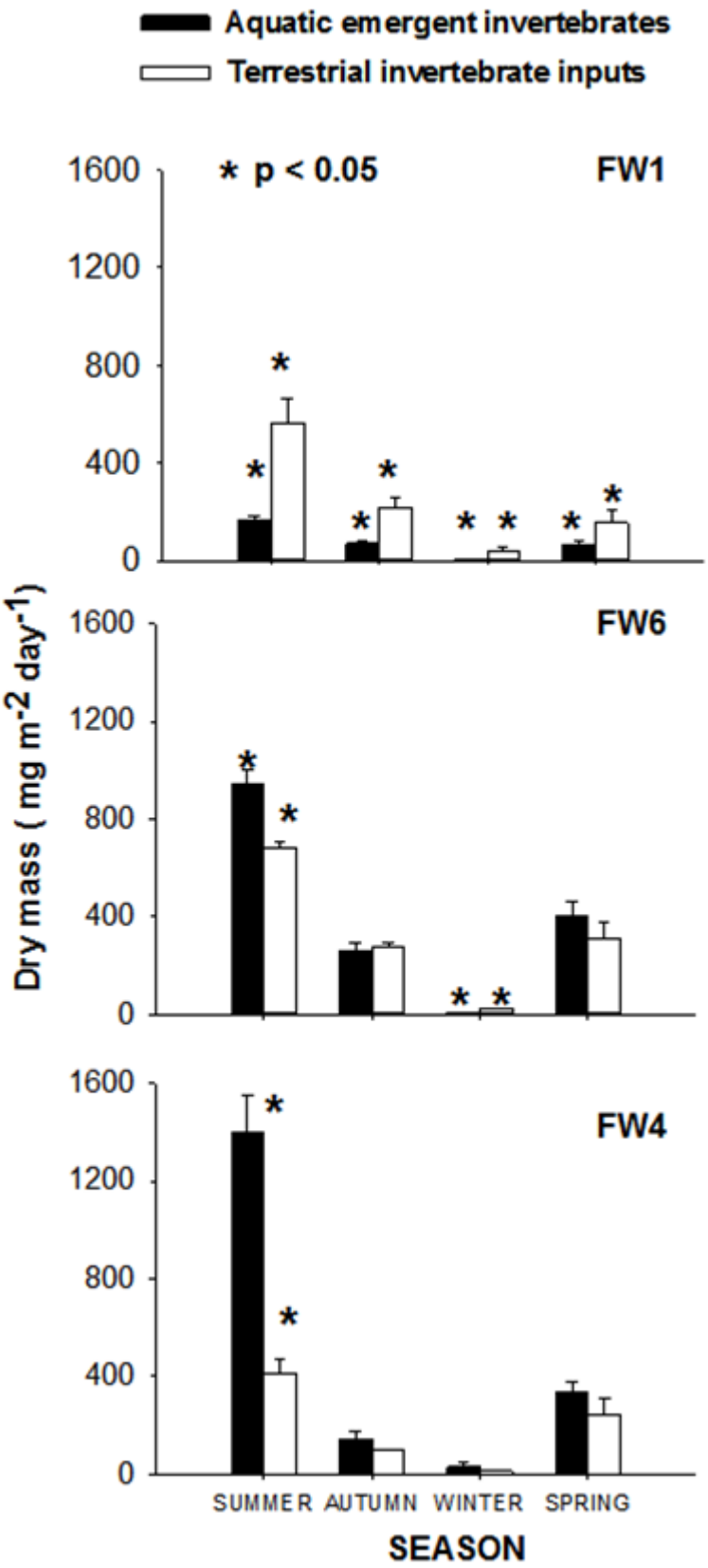

Figure 6.2: Biomass of aquatic and terrestrial invertebrates (mg m^{-2} day^{-1}) from the Kowie River in summer, autumn, winter and spring in 2012 to 2013. Asterisks show significant differences between emergence and terrestrial inputs at each site within seasons. Error bars denote standard deviations.

Table 6.4: Annual percent composition (by number and mass) of terrestrial and aquatic invertebrates collected in traps (emergence and pan traps) from three sites along the Kowie River, 2012–2013 (samples pooled for entire study).

	FW1		FW6		FW4	
Taxon	% Number	%Mass	% Number	%Mass	% Number	%Mass
Aquatic emergent insects						
Diptera	95	83	52	28	42	15
Ephemeroptera	3	9	40	32	33	20
Trichoptera	1	7	8	23	25	39
Odonata	0	0	0	17	0	27
Coleoptera	0	0	1	1	0	0
Terrestrial infall						
Araneae	4	10	10	39	6	27
Coleoptera	14	13	11	6	8	7
Collembola	6	0	1	0	1	0
Diptera	31	21	43	14	52	19
Hemiptera	7	1	4	2	4	0
Hymenoptera	7	14	13	25	7	20
Isopoda	9	1	2	1	0	0
Lepidopetra	1	4	1	8	3	22
Mecoptera	0	0	0	1	0	4
Neuroptera	1	33	0	0	0	0
Orthoptera	0	1	2	5	0	0
Parasitiformes	0	0	0	0	1	0
Psocoptera	0	0	2	0	0	0
Thysanopetra	20	1	10	0	17	0

6.3.4 Estimate of the reciprocal flow of polyunsaturated fatty acids (ARA, EPA and DHA) across the stream-riparian ecotone

The PUFA concentrations in aquatic insects at sites FW1, FW4 and FW6 were dominated by EPA (range 1.43 to 19.17 mg g^{-1}), followed by ARA (range 0.09 to 3.31 mg g^{-1}; Table 6.6). EPA in aquatic insects was dominant in all seasons (Table 6.6), with higher EPA content recorded at FW4 and FW6. DHA occurred in small amounts in aquatic insects at all sites and across all seasons (range ~0 to 1.69 mg g^{-1}). Overall, EPA and DHA concentrations were higher in aquatic insects in summer compared to other seasons.

The flow of biologically important PUFAs varied among sites and seasons. Season and site all had significant effects on terrestrial input and emergent insects (two way ANOVA, $p < 0.05$; Table 6.5), with season explaining most of the variation observed in the flow of invertebrates in both directions. The interaction ‘site x time’ was highly significant

(two way ANOVA, $p < 0.01$; Table 6.5) for emergence and terrestrial inputs of invertebrates, but there was no significant interaction between 'season x time' (two way ANOVA, $p > 0.05$) for ARA in terrestrial inputs. At the headwater site (FW1), ARA concentrations in emergent and infalling invertebrates were highest in the summer (0.27 and 0.33 mg m^{-2} day^{-1}, respectively), with the lowest ARA contents recorded in the winter (0.01 and 0.03 mg m^{-2} day^{-1} in emergent and infalling invertebrates respectively) across all sites. ARA of infalling and emergent insects did not differ across seasons (summer: $t = 2.13$, $p = 0.12$; autumn: $t = 1.68$, $p = 0.17$; spring: $t = 0.32$, $p = 0.77$; Fig. 6.3). In contrast, terrestrial inputs of ARA differed significantly from emergent insects in the winter ($t = 5.13$, $p = 0.01$). Highly unsaturated fatty acids (HUFA = DHA + EPA) at FW1 were highest in the summer for both emergent and terrestrial invertebrates (~ 1.01 ± 0.09 mg m^{-2} day^{-1} for emergence; ~0.58 ± 0.31 mg m^{-2} day^{-1} for infall). Terrestrial input and emergent insects at FW1 did not differ significantly in the summer and winter (summer: $t = 2.41$, $p = 0.10$ and winter: $t = 0.49$, $p = 0.65$). In contrast, HUFA contents in terrestrial and emergent invertebrates were significantly different in autumn and spring (FW1; autumn: $t = 9.60$, $p = 0.01$ and spring: $t = 3.79$, $p = 0.02$; Fig. 6.3).

At FW4, high flows of ARA and HUFAs via emerging insects (ARA = 1.76 ± 0.18 mg m^{-2} day^{-1}; HUFA = 15.21 ± 1.88 mg m^{-2} day^{-1}) and terrestrial inputs (ARA = 0.33 ± 0.04 mg m^{-2} day^{-1}; HUFA = 1.19 ± 0.03 mg m^{-2} day^{-1}) were recorded during the summer. Low ARA and HUFA were recorded in emergent and terrestrial invertebrates at FW4 in the autumn and winter (Fig. 6.3). The reciprocal flows of ARA at FW4 differed significantly in the summer and spring (summer: $t = 13.62$, $p = 0.01$; spring: $t = 5.67$, $p = 0.01$), but not in the remaining times of the year (autumn: $t = 2.64$, $p = 0.06$ and winter = 1.36; $p = 0.31$). The inputs and exports of HUFA differed significantly in the summer and spring (summer: $t = 13.03$, $p = 0.01$; spring: $t = 6.09$, $p = 0.004$), but not during autumn and winter (autumn: $t = 1.69$, $p = 0.74$ and winter = 1.85, $p = 0.21$).

Similar to the sites FW1 and FW4, HUFA and ARA flows at FW6 were highest in the summer for emergent insects (ARA = 1.20 ± 0.18 mg m^{-2} day^{-1}; HUFA = 18.30 ± 5.30 mg m^{-2} day^{-1}) and terrestrial inputs (ARA = 0.69 ± 0.28 mg m^{-2} day^{-1}; HUFA = 3.25 ± 1.43 mg m^{-2} day^{-1}), and declined in the autumn months and approached zero in the winter. Reciprocal flows of ARA differed significantly in autumn only ($t = 8.347$, $p = 0.012$) while significant differences in cross directional flows of HUFA were recorded in the summer, autumn and winter (summer: $t = 4.69$, $p = 0.01$; autumn: $t = 8.42$, $p = 0.01$; winter: $t = 3.87$, $p = 0.02$).

Table 6.5: Two-way analysis of variance to determine differences in polyunsaturated fatty acids (ARA, EPA +DHA) of aquatic invertebrates (mg m^{-2} day^{-1}), dry mass of terrestrial invertebrates (mg m^{-2} day^{-1}) inputs in the Kowie River. Asterisks show statistically significant effects (* $p < 0.05$, ** $p < 0.01$ and ***$p < 0.001$).

	ARA					EPA + DHA				
	df	SS	MS	F	p	df	SS	MS	F	p
Aquatic invertebrates (mg m^{-2} day^{-1})										
site	2	0.107	0.053	151.891	***	2	1.493	0.746	164.039	***
season	3	0.392	0.131	372.424	***	3	3.660	1.220	268.134	***
site x time	6	0.158	0.026	75.226	***	6	1.115	0.186	40.842	***
error	24	0.008	0.000			24	0.109	0.005		
Terrestrial invertebrate inputs (mg m^{-2} day^{-1})										
site	2	0.017	0.009	6.238	**	2	0.256	0.128	4.241	*
season	3	0.081	0.027	19.717	***	3	0.931	0.310	10.293	***
site x time	6	0.014	0.002	1.762	0.150	6	0.460	0.077	2.540	*
error	24	0.033	0.001			24	0.724	0.030		

Table 6.6: Mean contents of arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) (mg g^{-1} of dry weight) of amphibiotic insects emerging from the three sites (FW1, FW4, FW6) in the Kowie River. Values in parentheses show standard deviations.

	FW1			FW6			FW4		
	ARA	EPA	DHA	ARA	EPA	DHA	ARA	EPA	DHA
Summer									
Diptera	1.7 (0.26)	6.0 (0.91)	0.1 (0.01)	0.8 (0.02)	16.6 (0.36)	0.8 (0.02)	1.8 (0.37)	10.7 (0.55)	0.5 (0.07)
Ephemeroptera	2.0 (0.54)	9.3 (4.32)	0.00	1.3 (0.74)	16.2 (2.36)	0.00	2.1 (1.16)	10.4 (4.02)	0.00
Trichoptera	1.0 (0.26)	7.3 (0.40)	0.3 (0.02)	0.7 (0.08)	18.8 (3.71)	1.7 (1.40)	1.1 (0.09)	9.6 (0.37)	0.3 (0.15)
Odonata	1.4 (0.26)	3.6 (0.75)	0.1 (0.09)	1.5 (0.36)	12.3 (1.95)	0.2 (0.13)	1.3 (0.12)	11.3 (0.72)	0.1 (0.00)
Coleoptera	1.6 (1.18)	5.4 (3.66)	0.2 (0.12)	1.4 (0.28)	13.0 (1.26)	0.1 (0.01)	2.7 (0.27)	12.5 (1.21)	0.1 (0.01)
Autumn									
Diptera	1.7 (0.02)	4.7 (1.5)	0.00	1.8 (0.57)	7.2 (2.38)	0.0 (0.06)	0.3 (0.08)	1.9 (0.68)	0.6 (0.77)
Ephemeroptera	3.8 (1.34)	5.4 (1.1)	0.00	3.3 (1.80)	8.0 (3.64)	0.00	0.2 (0.04)	1.9 (1.03)	0.1 (0.08)
Trichoptera	0.7 (0.21)	4.6 (0.97)	0.1 (0.01)	2.2 (1.08)	10.0 (5.71)	0.8 (0.90)	0.3 (0.02)	1.5 (0.20)	0.00
Odonata	-	-	-	1.8 (0.68)	7.97 (2.98)	0.1 (0.08)	0.1 (0.04)	1.4 (0.34)	0.1 (0.10)
Coleoptera	-	-	-	1.7 (0.20)	6.20 (1.08)	0.1 (0.04)	0.1 (0.02)	2.0 (0.65)	0.00
Winter									
Diptera	1.3 (0.62)	3.4 (1.94)	0.00	1.6 (0.06)	4.2 (0.72)	0.1 (0.04)	1.1 (0.43)	15.2 (5.99)	1.7 (0.67)
Ephemeroptera	2.6 (1.25)	8.0 (3.03)	0.00	1.3 (0.60)	4.6 (1.29)	0.00			
Trichoptera	0.7 (0.10)	4.6 (0.36)	0.1 (0.02)	0.9 (0.05)	3.3 (0.09)	0.0 (0.00)	1.0 (0.13)	14.5 (2.29)	0.4 (0.10)
Odonata	1.3 (0.19)	4.0 (1.75)	0.0 (0.05)	1.0 (0.26)	3.7 (1.20)	0.0 (0.01)	1.4 (0.23)	12.2 (5.66)	0.2 (0.24)
Coleoptera	1.7 (0.29)	4.9 (0.94)	0.00	4.8 (0.57)	19.2 (4.73)	0.1 (0.01)	1.1 (0.28)	12.1 (2.63)	0.1 (0.01)
Spring									
Diptera	1.3 (0.53)	3.1 (2.04)	0.1 (0.09)	1.3 (0.65)	9.7 (3.66)	0.5 (0.32)	0.8 (0.12)	8.6 (2.94)	0.1 (0.10)
Ephemeroptera	2.4 (0.94)	8.0 (3.80)	0.00	1.6 (0.33)	9.1 (2.30)	0.00	1.5 (0.81)	9.4 (1.82)	0.00
Trichoptera	1.2 (0.22)	6.6 (2.07)	0.1 (0.05)	1.0 (0.29)	9.9 (2.57)	0.7 (0.74)	0.7 (0.04)	7.6 (1.56)	0.1 (0.02)
Odonata	1.6 (0.06)	5.1 (0.88)	0.00	1.5 (0.58)	13.6 (5.35)	0.1 (0.05)	1.2 (0.22)	15.7 (2.88)	0.1 (0.05)
Coleoptera	2.2 (0.39)	7.8 (2.77)	0.00	1.6 (0.19)	12.1 (1.88)	0.00	1.4 (0.13)	12.8 (1.90)	0.1 (0.05)

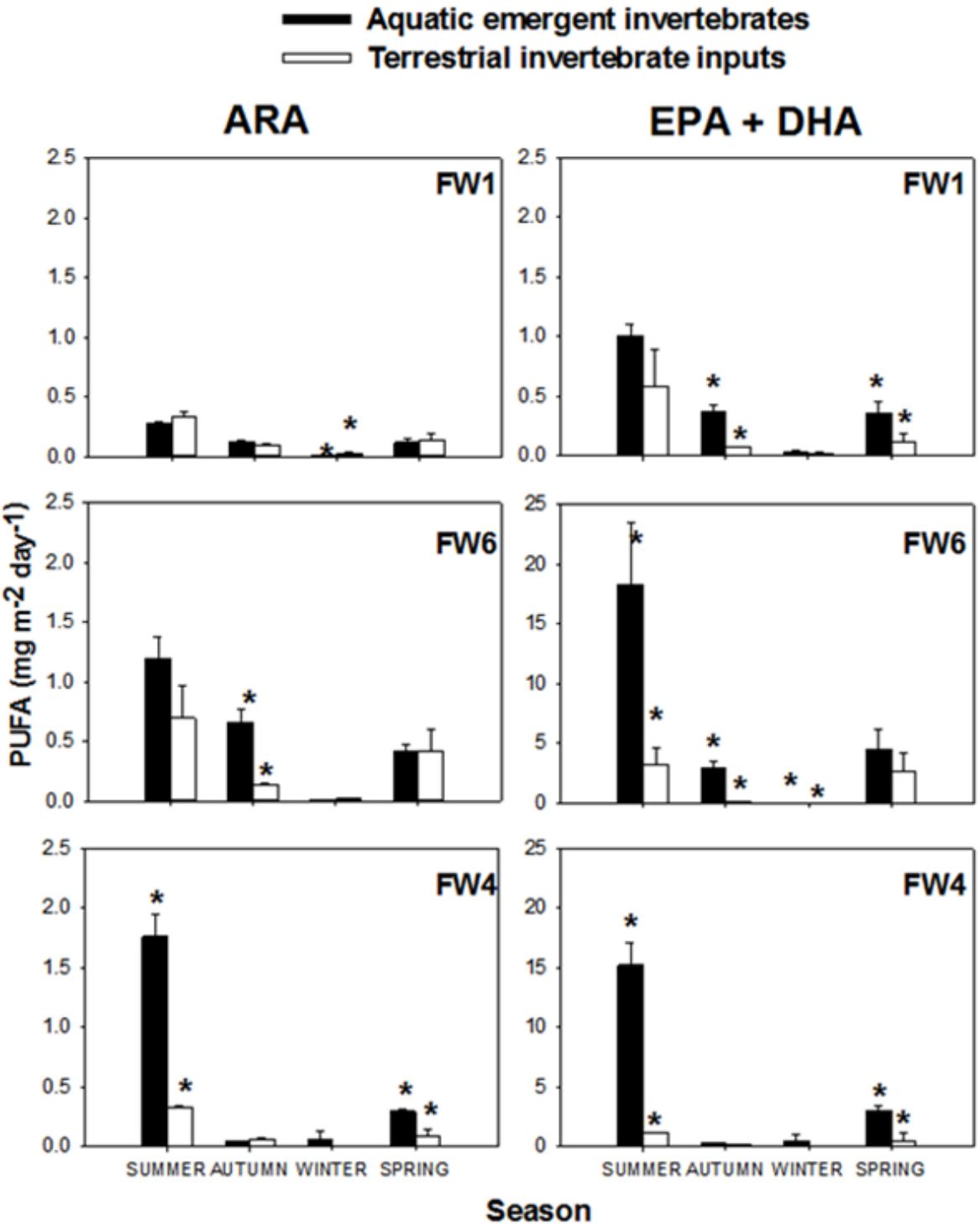

Figure 6.3: Reciprocal flows of physiologically important polyunsaturated fatty acids (PUFA) in aquatic and terrestrial invertebrates (mg m^{-2} day^{-1}) from the Kowie River in summer, autumn, winter and spring in 2012 to 2013. Asterisks show significant differences between emergence and terrestrial inputs with season. Note changes in scale for DHA + EPA at FW4 and FW6.

6.4 Discussion

The intent of this study was to quantify the reciprocal fluxes of invertebrates by examining the movements of terrestrial and emergent invertebrates. I examined the seasonal changes of terrestrial and emerging insects at three sites in the Kowie River, and found that the reciprocal fluxes changed seasonally and at different locations (Table 6.1). The second intent was to incorporate physiologically important PUFAs (ARA, EPA and DHA) in invertebrates to estimate the flow of these components across the stream-riparian interface. My study provides a unique contribution to the understanding of reciprocal subsidies because it incorporated the nutritional quality of subsides, as called for by many researchers (e.g. Marcarelli et al. 2011). I found that physiologically important PUFAs in consumers changed with season and location (Table 6.7) in that they increased in the summer and declined in the winter (Fig. 6.3). Assessing reciprocal flows into and out of streams using fatty acids is a promising approach not commonly incorporated into food web studies, and this approach adds to our understanding of food web structure and dynamics. My data, combined with the results obtained by other researchers, reiterates the importance of considering both the quality and quantity of subsidies across adjacent habitats.

6.4.1 Reciprocal flows of invertebrates

The goal was to quantify the reciprocal fluxes of invertebrates in southern Africa by assessing the movements of terrestrial and emergent insects. In the Kowie River, emergence and terrestrial inputs peaked in the summer and declined in the winter (Fig. 6.2). The terrestrial infall was lower than emergence at the downstream and tributary sites, while at the headwater site terrestrial inputs substantially exceeded emergence (Fig. 6.2). These data collected in the Kowie River corroborated key findings by Nakano and Murakami (2001) and Rundio and Lindley (2012), who found that reciprocal flows between terrestrial and aquatic systems fluctuated seasonally. Additionally, results observed for the reciprocal fluxes in the Kowie River were in accordance with the predictions by Paetzold et al. (2008a) about the timing of terrestrial and aquatic productivity in temperate streams, whereby headwaters are associated with higher terrestrial infall than emergence, whilst downstream sites are associated with higher emergence than terrestrial infall. Researchers have proposed that subsidies can stabilise food webs by increasing resources when production in an adjacent system is low (Nakano and Murakami 2001, Takimoto et al. 2002). The findings in the Kowie River are congruent with the findings by Nakano and Murakami (2001) in Japan: terrestrial inputs were higher when emergence was low at the headwaters, typically subsidising the low emergence from the Kowie River. Subsidies fluctuated seasonally in both

systems, but subsides were higher in the Kowie River than those recorded in the Japanese stream (Nakano and Murakami 2001).

The seasonally shifting bidirectional flows of invertebrates at different sites in the Kowie River were similar in some ways to those in Japanese and Californian streams. For instance, emergence quantities were higher in the Kowie River than in the Japanese and Californian streams (Table 6.7). Productivity differences between adjacent ecosystems, the area of donor habitat (boundary permeability) and boundary length have been identified as factors that influence the magnitude and direction of energy flows (Polis et al. 1997, Iwata et al. 2003, Witman et al. 2004). In general, factors that have been identified as influencing either aquatic or terrestrial exchanges include catchment geomorphology (Iwata et al. 2003, Iwata 2007), vegetation cover (Edwards and Huryn 1996), climate (Freitag 2004, Boulton et al. 2008) and water flow (Power et al. 2004). The variability in reciprocal flows among different study regions probably reflects changes in boundary permeability and boundary length, both of which are more closely related to catchment size and stream width (Table 6.7). Emergence depends on the primary productivity of the riverine system in question, with higher productivity resulting in higher emergence (Power and Rainey 2000). Emergence in the Kowie River was higher in the summer and lowest in the winter. The high algal productivity in the summer (10 – 16 mg chlorophyll-*a* m^{-3} downstream and tributary site with 4 mg chlorophyll-*a* m^{-3} for headwaters; Dalu et al. 2014b) compared to winter (0.10 to 3.3 mg chlorophyll-*a* m^{-3} downstream and tributary and 0.14 mg chlorophyll a m^{-3} for headwaters) probably caused emergence peaks in the summer. In the Japanese stream, the peaks in emergence in summer were attributed to high solar inputs and algal production coupled with a long leafless period (Nakano and Murakami 2001). Solar radiation was not measured in this study, but solar radiation in the eastern part of South Africa (where the Kowie River is situated) is higher in the summer (7.5 – 8.0 Kwh m^{-2}; Fluri 2009) compared to the winter (~ 4.5 to 5.9 Kwh m^{-2}; Fluri 2009), and may explain the increased emergence in the summer.

Table 6.7: Comparisons of physical attributes between two streams (California and Japan) and the Kowie River, including estimates of emergence and terrestrial invertebrate infall. "-" denotes data that were not available.

	Honorai Stream (Japan)	Big Creek (California)	Kowie River
Catchment area (km^2)	15.4	58*	769
Length of stream (m)	14	10.8*	69.8
Stream width (m)	2 to 5	5 to 5.5*	1.5 to 12
Canopy cover (%)	97$^{\varphi}$	-	11,44,72 (FW1, FW6, FW4)
Main activities around Catchment	Scientific experiments	Nature reserve	Pristine with agricultural activities
Emergence† (g m^{-2} $year^{-1}$**)**	15	5.3 to 7.8	28 to 174
Terrestrial infall (g m^{-2} $year^{-1}$)	8.7	7.9 to 8.6	90 to 117 (Table 6.8)

†calculated from Fig. 1 in Nakano and Murakami (2001) and Table 1 in Rundio and Rindley (2012)
*data obtained from Table 1 in Rundio and Lindley (2008)
$^{\varphi}$data available only for the headwater part of the stream

Along with the differences in seasonal patterns in reciprocal fluxes in the Kowie River, the annual reciprocal fluxes (g m^{-2} $year^{-1}$) also differed amongst sites, with larger fluxes occurring at the downstream and tributary sites (FW4 and FW6; Table 6.5). At the headwater site, annual terrestrial input substantially exceeded emergence (28 g m^{-2} $year^{-1}$ for emergence and 90 g m^{-2} $year^{-1}$ for terrestrial inputs). At the downstream sites, annual emergence substantially exceeded terrestrial inputs (146 – 174 g m^{-2} $year^{-1}$ for emergence and 70 – 117 g m^{-2} $year^{-1}$ for terrestrial inputs; Table 6.8). The annual reciprocal flows in the Kowie River exceeded those of the Japanese and Californian streams (Table 6.7), as the different areas of the donor habitats (permeability) and the boundary lengths varied among the three streams. Additionally, the colder temperatures in Japan and California (6 - 18^{o}C versus 15 - 23^{o}C in the Kowie River) and the absence of Odonata in Japan and California most likely reduced emergence. Temperature affects the metabolic rates of insects and their growth rates; warmer temperatures increase growth rates and cause increases in emergence (Harper and Peckarsky 2006). The differences in annual emergence could also be due to the influences of climate conditions and canopy cover on primary production (Power and Rainey 2000). The cover recorded in the headwaters of the Kowie River was 72% (which coincided with lower emergence). Emergence correlated positively with chlorophyll-*a* (r = 0.79), indicating that emergence was greater when algal productivity was high. The positive

correlation between emergence and chlorophyll-a is representative of the copious supply of diatoms, which are of a higher nutritional quality (Richoux et al., in prep).

As discussed in preceding chapters and other studies (see Chapter 4; Chapter 5; Vannote et al. 1980), headwaters are associated with consumption of terrestrial organic matter by consumers (a less nutritious source of carbon), which may in turn have caused the low emergence recorded at that site. The other two sites with lower percentage cover (24 – 44%) than the headwaters were associated with higher emergence rates and were associated with consumers utilising *in situ* carbon sources that were more nutritious, hence explaining the higher emergence at the downstream sites. The relationship between chlorophyll-*a* and emergence of insects has been documented in other studies (Polis et al. 2004, Power et al. 2009). For example, Polis et al (2004) recorded that sites with high algal productivity were associated with high insect emergence in the South Fork Eel of California River. Differences in terrestrial inputs between Kowie River and the Honorai and Californian streams (Nakano and Murakami 2001, Rundio and Lindley 2012) could also be due to differences in stream and air temperatures. Increasing air temperature increases flying activity of terrestrial insects, while smaller stream size, high overstory vegetation increases the number of terrestrial invertebrates that fall into a stream because of a shorter boundary length between the riparian zone and the stream edge (Edwards and Huryn 1995). Additionally water flow and rainfall increase the terrestrial inputs possibly by washing invertebrates into the river from the river edge (*sensu* De Jong and Canton 2014). In the Kowie River, rainfall, air temperature, stream size and overstory cover were weakly correlated with terrestrial inputs while water flow strongly related to terrestrial infall (Table 6.3). These above factors (water flow, rainfall, air temperature, stream size and overstory cover) explained only 51% of the variation of observed terrestrial inputs (Table 6.3). It is possible that factors other than those previously mentioned affected terrestrial inputs. Changes in air temperature coupled with shifts in humidity and wind speed could all affect terrestrial insect activity and their infall into streams (Mason and Macdonald 1982, Edwards and Huryn 1995, Allan et al. 2003). These factors were not measured in the Kowie River, so it is not possible to comment on these here.

Table 6.8: Estimates of annual fluxes (g m^{-2} $year^{-1}$) and fatty acids (mg m^{-2} $year^{-1}$) of terrestrial invertebrates and emerging adult insects across the riparian interface in study reaches along the Kowie River between December 2012 and September 2013.

Site	Biomass (g m^{-2} $year^{-1}$)		ARA (mg m^{-2} $year^{-1}$)		DHA+EPA (mg m^{-2} $year^{-1}$)	
	Terrestrial infall	Aquatic emergence	Terrestrial	Emergence	Terrestrial	Emergence
FW1	90	28	54	47	72	161
FW6	117	146	116	208	553	2354
FW4	70	174	45	197	169	1736

The taxonomic composition of reciprocally subsidised invertebrates varied seasonally in the Kowie River. The diversity (and proportions) of aquatic insects generally increased in the summer and spring months when air and water temperatures were high ,and decreased in winter when air and water temperatures were low (Dalu et al. 2014; Chari 2016). Emerging adult odonates (dragonflies and damsel flies) were present at the downstream (FW4) and tributary sites (FW6) during the summer, but were not collected in emergence traps across all the other seasons. Taxonomic composition of infalling and emergent arthropods was not reported by Nakano and Murakami (2001), though terrestrial inputs were examined by Rundio and Lindley (2012) in a Californian stream and Diptera, Hemiptera, Thysanopetra and Araneae made up the bulk of insects that fell into pan traps. In the Kowie River, inputs were predominantly composed of Diptera, Coleoptera and Thysanopetra. Spiders also made major contributions in the summer months at all three sites. The inputs of wingless invertebrates (e.g. spiders; Araneae) are thought to be most strongly influenced by the length of stream edge, proportion of fringing vegetation and changes in flow (Allan et al. 2003, Dineen et al. 2007). Kawaguchi and Nakano (2001) recorded that well developed overhanging vegetation in the forest reaches enhanced inputs of invertebrates with no flying ability, and this vegetation could be important as habitat for spiders to build webs on. These studies suggested that terrestrial invertebrate infall of wingless invertebrates should be greater in the headwaters (with higher vegetation) of any riverine system (Kawaguchi and Nakano 2001). This pattern was not evident in the Kowie River; however, it is worth noting that wingless invertebrates such as Collembola, Isopoda and some ants were abundant in some seasons at the headwater site. In the Kowie River, the seasonal variation in the taxonomic composition likely had an effect on the quantity of the reciprocally subsidised invertebrates, where emergence for many taxa (e.g. odonates) occurred only during a small portion of the year, typically in the summer months. The occurrence of odonates in the summer is congruent to findings in other studies (Merritt and Cummins 1996, Malison and Baxter 2010).

Seasonality in temperate regions changes the composition of insects available for predators (Kawaguchi and Nakano 2001). Reciprocal subsidies in the Kowie River, in agreement with other studies in temperate streams, also document the seasonality in cross ecosystem exchanges in the southern hemisphere.

6.4.2 PUFA flow at the stream-riparian interface

In the Kowie River, PUFA subsidies via invertebrates were estimated to examine the nutritional quality of subsidies. Changes in physiologically active PUFAs (ARA, EPA and DHA) followed similar patterns in line with those recorded for dry mass, as they typically peaked in the summer (1- 20 mg m^{-2} day^{-1}; Fig. 6.3) and dropped to nearly zero in the winter. The HUFA values obtained for aquatic insects were within the range of those recorded in other parts of the world (e.g. Yenisei River, EPA = 2 to 8 mg g^{-1}; Sushchik et al. 2006), and the average global HUFA content for aquatic insects was estimated at 9.3 mg g^{-1} dry weight (Gladyshev et al. 2009). ARA content in aquatic insects was low at all sites, a finding consistent with other studies on streams (0.01 to 0.6 mg g^{-1}; Sushchik et al. 2006). In the Kowie River, terrestrial insects, as expected, were very low in EPA and DHA (range: 0.04 to 1.71 mg g^{-1} for ARA and 0.09 to 4.75 mg g^{-1} for EPA; Chari 2016). The low DHA content in insects was not surprising, as terrestrial organisms are unable to synthesise HUFA in amounts needed to meet their metabolic needs (Hixson et al. 2015). A number of these organisms are phytophagous (feeding on terrestrial plants), which may explain the generally low HUFA contents in their tissues relative to aquatic insects. However, spiders were high in EPA and DHA (5.14 to 9.76 mg g^{-1}), primarily because spiders incorporate between 50 - 85 % of aquatic insects in their diets (Chari 2016).

Resource subsidies moving across ecosystems leads to changes in productivity that may eventually lead back to the donor system, such as leaf litter falling into freshwater enhancing productivity of aquatic insects, which at emergence are then consumed by spiders (Paetzold et al. 2005). Reciprocal flows from one ecosystem to the other can occur, although the potential feedback mechanisms depend on the magnitude and duration of the subsidy (Leroux and Loreau 2008). Spiders can contribute largely to the nutrients falling into the streams (Hayes and Rutledge 1991). While the contributions of spiders to diets of aquatic consumers were not investigated in the Kowie River, it is possible that a feedback mechanism exists whereby spiders consume high HUFAs from aquatic insects and via fortuitous infall transfer the same resources back into the stream system during emergence. So while emergence may contribute to terrestrial systems, the same resource possibly returns to the stream via insects in a feedback mechanism. This speculation that a feedback path exists is

based on the premise that terrestrial invertebrates are low in HUFA content. Preliminary research provided some evidence to support the existence of a feedback mechanism, as spiders (Araneae) made up a large component of the diets of frogs (anurans) in the Kowie River (~ 16 to 22 % of frog diets; Sikutshwa 2015). In tropical streams, spiders make up a large of component of terrestrial insects in the diets of fishes (~ 35 – 43% of fish; Chan et al. 2008). The existence of this feedback mechanism, whereby terrestrial organisms consume aquatic insects and fall into the stream, may explain why there were no significant differences between PUFA flows among some seasons.

Insect emergence from areas rich in primary production may supply a high-quality resource such as HUFA to riparian consumers (Gladyshev et al. 2013). These PUFAs are biologically important, and are supplied in greater quantity through aquatic primary production than terrestrial production (Chapter 5, Hixson et al. 2015). An estimate of the annual average export of HUFA from aquatic to the terrestrial environments from insect emergence in the Kowie River ranged from 161 to 2354 mg m^{-2} $year^{-1}$ (Table 6.5); the range for flux of insects from the stream to the land in other ecosystems is estimated to be between 0.1 to 672 mg $m^{-2}year^{-1}$ (Gladyshev et al. 2009). The variance in the estimates in the Kowie River and those of Gladyshev et al. (2009) probably arise from errors involved in the approximation of lipid content in the emergent insects by Gladyshev et al (2009), and variability in emergence flux across systems. The lipid fluxes from the Kowie River are greater than the conservative estimates of 0.1 to 672 mg m^{-2} $year^{-1}$ given by Gladyshev et al. (2009), and suggest that rivers in temperate South Africa may represent a rich supply of lipids for the terrestrial system. The estimate by Gladyshev et al. (2009) did not incorporate odonates, and it was based on means calculated from aquatic insect studies across different ecosystems.

In the Kowie River, ARA exported from land to the stream via insect emergence ranged from 47 to 208 mg m^{-2} $year^{-1}$ (Table 6.5). There is no literature on the flows of ARA from water to land or vice versa; however, in the Kowie River ARA fluxes were small (0.69 to 1.20 mg m^{-2} day^{-1}). This finding was not surprising, as ARA is typically found in low quantities in freshwater consumers (Torres-Ruiz et al. 2007). ARA concentrations in terrestrial insects and aquatic insects were generally low in the Kowie River, although aquatic insects were higher in ARA than terrestrial insects. The synthesis of ARA involves the use of Δ5 and Δ6 desaturase to convert 18:2ω6 to ARA in plants (Sprecher 2000). There is also an additional pathway with Δ17 desaturase that converts ARA to EPA in algae (Sprecher 2000). Only aquatic plants have the necessary enzymes to carry out the conversion of 18:2ω6 to ARA; thus, ARA arises from aquatic sources.

Considering subsidies using quantities of exported materials does not take into account that the strength of a subsidy depends not only on its quantity, but also on the quality of the resource (Bartels et al. 2011). Quality of a subsidy can be determined by measuring the amount of energy (measured in joules), protein (amino acids), prey size and essential fatty acids (*sensu* Marcarelli et al. 2011). There is need to concurrently measure quality and quantity of subsidies to derive accurate depictions of ecosystem exchanges (Bartels et al. 2011). Comparing the annual and daily exchanges at the headwaters of the Kowie River showed that terrestrial invertebrates by quantity exceeded emergence across all seasons (significant difference between emergence and infalling invertebrates; Fig. 6.1). When the quality (using essential fatty acids) of the exchanges at the headwaters was considered, it was evident that there were no differences between the contributions of emergence and infalling invertebrates (no significant differences between emergence and infalling invertebrates; Fig. 6.2 and Table 6.5). The results of the Kowie River corroborated one of the key findings by Marcarelli et al. (2011) that the quantity of the subsidy may be misleading, but quantity combined with quality may give a clearer picture of cross ecosystem exchanges. For example, consumers tend to select high quality food, as evidenced by some aquatic animals seen to consume *in situ* sources regardless of high detrital inputs (Delong and Thorp 2006); if only detrital inputs are considered they may not give an accurate picture of what consumers are depending on.

Reciprocal flows in the Kowie River were investigated using pan and emergence traps with similar designs to those used in other studies (e.g. Baxter et al. 2004, Meyer and Sullivan 2013). There is some speculation that trap designs may overestimate or underestimate fluxes (e.g. Edwards and Huryn 1996, Wipfli 1997). Pan traps are intended for capturing invertebrates that fall into the river via fortuitous infall. The addition of a surfactant to the pan traps may have introduced a collection bias, capturing terrestrial invertebrate that would normally be able to escape after contact with river water. Additionally, the arrangement of pan traps can affect the composition and quantity of arthropods that fall into the traps (e.g.Wipfli 1997, Allan et al. 2003). Similarly, emergence traps may underestimate the fluxes of odonates out of streams, as some odonates crawl rather than fly out (Merritt and Cummins 1996, Malison et al. 2010). For example, adult odonates were abundant in the terrestrial systems and were often sighted flying near the stream (LD Chari, pers. Comm.), but were rare in emergence traps. Odonates have very high biomasses, and their contributions to outward subsidies may have been underestimated in the Kowie River. While there are potential biases in any trap, I assumed that the biases would be minor; however, quantification of the full complement of emergence and infall via invertebrates (including

crawling insects) would be highly advantageous towards refining existing estimates of terrestrial and aquatic invertebrate exchange at riparian interfaces.

6.5 Conclusions

In conclusion, reciprocal flows measured in a river illustrated the importance of the interactions between invertebrates and their adjacent habitats. Within the Kowie River, reciprocal flows were dependent on biotic and abiotic factors (e.g. canopy cover, algal productivity). The management of streams has typically not incorporated exchange of resources across the riparian-stream interface (Baxter et al. 2005). Results from the Kowie River help to illustrate how reciprocal subsidies vary with scale, and how the quality (and quantity) of the resource supply changes over space and time. The differences in flux between the Kowie River and other locations showed the geographical variability of cross-ecosystem connections. The array of direct and indirect effects of resource flow across ecotones increases the complexity of management decisions. For example, opening the stream canopy could potentially increase algal productivity and insect emergence, but these actions may affect riparian spiders. Such a modification of the riparian zone may actually have a negative effect on stream biota because other essential stream parameters (e.g., water temperature) would also change the timing and duration of emergence. More studies investigating connections between stream and riparian habitats will provide further insights into variability in the quality and quantity of subsidies exported across ecotones.

7 Synoptic discussion

This study utilised a combination of both stable isotope and fatty acid analyses to provide a time-integrated depiction of the assimilated basal resources eaten by macroinvertebrate consumers in a South African catchment. This work fills gaps in knowledge on the trophic ecology of freshwater macroinvertebrates and spatial subsidies linking aquatic and terrestrial ecosystems in southern African catchments. First, effects of physico-chemical factor variation on aquatic invertebrate assemblages were investigated (**Chapter 3**). Second, food web structure across a longitudinal gradient (**Chapters 4** and **5**) over four seasons was investigated, an approach seldom used in the food web literature where data are typically collected over one season. Finally, I investigated reciprocal subsidies in a temperate river over four seasons (**Chapter 6**). Using the Kowie River as a model system, this dissertation provided information useful for improving the management of river systems to aid the conservation of water resources in southern Africa and the rest of the world.

In this chapter, I summarise the information discussed in the preceding chapters in relation to the objectives presented in **Chapter 1** (Section 1.6), discuss the implications of the results, and provide recommendations for future research.

7.1 Longitudinal changes in macroinvertebrates and trophic connections in the Kowie River

Rivers are dynamic environments with spatial and temporal complexities affected by changes in abiotic conditions such as geology and flow (Thorp et al. 2008). Change in abiotic conditions in turn causes changes in the biotic community of rivers (Vannote et al. 1980). In **Chapter 3**, the invertebrate distribution and abundance in relation to physico-chemical parameters were examined along the river continuum, using the River Continuum Concept (RCC) as a conceptual framework. Distributions and abundances of invertebrates did not follow the patterns predicted by the RCC, but some tenets of the RCC were supported. One tenet that was clearly supported was the presence of a larger proportion of filterers and gatherers compared to shredders and grazers. The partial fit of FFGs to the RCC was consistent with results obtained in other studies (e.g. Fu et al. 2015). Furthermore, I used metrics of FFGs to determine the energy sources that made up their diets. The index for autochthony vs allochthony showed that most consumers depended on allochthonous organic matter in accordance with metric measures from an east African River (Masese et al. 2014). In discussing the metrics used in Chapter 3, the shortcomings of using metrics was

highlighted and the need for more robust and established methods to assess organic matter flow in riverine systems was emphasised. After obtaining some general information on the functional organisation of the invertebrate assemblages in the Kowie River using abundances and proposed metrics for ecosystem function, I considered the dependence of the different FFGs on available organic matter resources using tracer techniques (Chapters 4 and 5).

It is important to consider the multidimensional nature of riverine systems, whereby they change spatially and temporally, to understand riverine ecosystem function (Pingram et al. 2012). Over the last few decades, studies have focused on understanding the main basal resources that support consumers in rivers (Roach et al. 2015). While these studies have contributed much information to the functioning of ecosystems, they have mainly been confined to the northern hemisphere (Pingram et al. 2012). As such, there is much that stream ecologists can learn from carbon flow in temperate rivers of the southern hemisphere.

The literature reviewed in **Chapter 1** showed the importance of autochthonous and allochthonous carbon to food webs in rivers across a wide range of climates and freshwater systems (Table 1.2). The review showed that there are contrasting findings on the contributions of different organic matter sources to the diets of consumers. Some authors suggested that terrestrial organic matter fuels food webs (e.g. Zeug and Winemiller 2008, Wang et al. 2014), while others argued that aquatic sources are more important (e.g. Mulholland et al. 2000). Evaluation of the production base supporting aquatic food webs in large rivers has been advanced greatly by stable isotope analysis (Middelburg 2014) and to a lesser extent, fatty acid analysis (e.g. Descroix et al. 2010a). Previous works investigating the basal resources supporting consumers using those markers suggested that autochthonous production is important for both primary and secondary consumers (Dangles 2002, Delong and Thorp 2006, Torres-Ruiz et al. 2007).

To address the question of the basal resources supporting consumers in the Kowie River, I deployed a cross-validating strategy that used both markers (**Chapters 4** and **5**). Autochthonous sources (macrophytes and epiphyton) supported consumers in the Kowie River, irrespective of site and season, supporting hypotheses H1a and H1b that food webs in the Kowie River were founded on autochthonous algal carbon (**Chapters 4** and **5**). However, a notable contribution of allochthonous carbon occurred mainly at the headwaters, with autochthonous contributions increasing downstream (further supporting hypothesis H1a). This (Chapters 4 and 5) confirmed the suggestions of previous studies that invertebrates in temperate streams depend on autochthonous production. Additionally, I had hypothesised that consumers consume only basal resources that coincide with their FFG designation; this

hypothesis (H2) was not supported within the Kowie River, as shredders also consumed algal resources.

In **Chapters 4** and **5,** I investigated factors that affect stable isotopes and physiologically important fatty acids in the Kowie River. FFG, season and site influenced the stable isotope and fatty acid values, which was consistent with other studies that have shown effects of FFG, site and season on tracers in consumers (Cremona et al. 2010, Volk and Kiffney 2012). The importance of different organic matter sources was influenced by factors that include water flow, FFG, vegetation type and primary productivity, all of which changed with season and site (Fig. 7.1). These factors mandated the need to sample animals and food sources over space and time. For example, filamentous algae was prevalent only during certain seasons (and certain sites), but constituted part of the diet of consumers when and where they were present. This food source would have been missed if sampling had been confined to a single period.

The changes in isotopic values of food sources over space and time justified sampling food sources on every sampling occasion. The changes in the isotopic values of basal resources with season and site in the Kowie River demonstrated the need to quantify temporal and spatial patterns to draw robust conclusions about carbon flow in riverine food webs. The Kowie River study, along with other studies that examined both temporal and spatial variability of basal resources (e.g. Zeug and Winemiller 2008), has improved our understanding of energy flows in aquatic ecosystems, and future studies should address and recognize the influence of temporal variability of tracers in producers and consumers.

7.2 Lateral connectivity

With information on the dependence of the macroinvertebrates obtained through the use of metrics (Chapter 3), stable isotopes (Chapter 4) and fatty acids (Chapter 5), I considered the bidirectional flow of resources between the Kowie River and its riparian corridor in Chapter 6.

Ecological focus on the importance of cross-habitat energy flow has increased (Baxter et al. 2005). Most habitats are open to multiple forms of resource subsidies, but most studies quantify only one direction of energy flow (Richardson and Sato 2015). In **Chapter 6**, I considered the reciprocal flow of invertebrates between the Kowie River and its neighbouring riparian habitat using emergent and infalling arthropods. My data showed that arthropod infall and emergence were both high in the summer (in support of hypothesis H3a and H4a) and decreased in the winter, and the values of infall did not decline to zero as was proposed in the initial hypothesis (hypothesis H4a partially supported). In the headwater sites, low

emergence corresponded to high infalling invertebrates, indicating that invertebrates may subsidise streams at low emergence at the headwaters (Hypothesis H5b supported at the headwaters).

Reciprocal subsidies shaped taxonomic composition in the Kowie River, with some groups (e.g., Odonata) occurring only in certain seasons (Chapter 6). The taxonomic composition of emergent insects relates to the benthic invertebrates found in the stream (Jackson and Fisher 1986). The taxonomic composition of emergent insects in the Kowie River correlated with the numbers and types of FFGs documented in Chapter 3. Filterers (Simuliidae and Hydropsychidae) were the dominant groups in the Kowie River (Chapter 3) and similarly filtering consumers (particularly simuliids) often dominated in emergence traps. Similarly, in the terrestrial system the order Diptera was often recorded in malaise traps (Chari 2016) and explains why they commonly occurred in high abundances in pan traps. The seasonal changes of the taxa in the Kowie River and the riparian area affected the magnitude of the subsidies, supporting the notion that taxon composition is important for assessing ecosystem function (Hooper et al. 2005).

To unravel the complexities of subsidies across the riparian-river interface (quantity and quality) involves several steps that include: (1) estimating the flux of organisms across ecotones and assessing these flux changes over space and time (Power et al. 2004, Gladyshev et al. 2009); (2) calculating the composition of eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) in aquatic insects (Gladyshev et al. 2009); (3) calculating biomass along with EPA and DHA requirements of terrestrial consumers (Gladyshev et al. 2013, Twining et al. 2015); (4) calculating the EPA and DHA exported by emergent insects and amphibians (Gladyshev et al. 2009); and (5) determining the environmental factors that affect the strength of subsidies (Baxter et al. 2005). I addressed steps 1, 2, 4 and 5 in Chapter 6 and thus showed that emergence density changes seasonally and the quality of resources changes over spatial and temporal scales, as reported by other authors (Nakano and Murakami 2001, Rundio and Lindley 2012). My study also recorded that emergence was affected by algal productivity, while terrestrial inputs were more affected by flow and air temperature, consistent with other studies (Edwards and Huryn 1996). Considering the quality and quantity of subsidies gives very accurate depictions of subsidy exchanges across ecotones, as has been demonstrated in this thesis and in previous studies (Bartels et al. 2011). To my knowledge, this is the first study to incorporate fatty acids to study reciprocal flows in African rivers, and the data have contributed to the understanding of the coupling that occurs in riparian areas and rivers by showing that exchanges varied over space and time.

7.3 Conceptual model of the Kowie River

Considering my thesis in a conceptual framework, the data generally supported the underlying patterns proposed in the River Continuum Concept (RCC; Vannote et al. 1980) and the Riverine Productivity Model (RPM; Thorp and Delong 1994), and contributed to the understanding of ecosystem function in southern temperate rivers.

Amalgamating the insights from Chapters 3, 4, 5 and 6, the following model of energy flow applies to the Kowie River. Factors such as light, flow, stream width, temperature affect the algal productivity of the Kowie River (Fig. 7.1). At the headwaters, riparian trees reduce autochthonous production owing to their large canopy cover (72%) shading the water, and this causes consumers to partly depend on allochthonous riparian nutrients transferred in leaves. However, autochthony still contributed substantially to the diets of consumers in the headwaters (Chapter 4), where insects consume a nutritionally lower-quality resource (evidenced by low EFA in riparian leaves), hence they had lower emergence rates (Chapter 6). At the sites where consumer diets were predominantly autochthonous, the consumers were provided with more nutritious EFAs. The consumption of a rich source of EFAs resulted in higher emergence and higher export of EPA and DHA from the water. Similarly, changes in air temperature, water flow, rainfall, and stream width affected terrestrial insect supply into the river (an increase in these factors was associated with increased terrestrial supply to the river; Fig. 7.1). Terrestrial arthropod infall therefore tends to be higher at the headwaters (typically subsidising the low emergence).

On the whole, the data from the Kowie River validated the findings by other researchers that habitats are connected and dependent on each other (Baxter et al. 2005). This study supported the notion that the sharing of resources that occurs between the riparian zone and river is dynamic over space and time (Nakano and Murakami 2001) and is affected by environmental conditions such as vegetation type, canopy cover, temperature and algal productivity (Paetzold et al. 2008b). My study represents an important step in integrating river science with landscape ecology as called for by many authors (e.g. Wiens 2002), and provides baseline information for riverine landscape conservation in the future. Information on the coupling between the Kowie River and its riparian area provides environmental managers with tools to determine the effect of future perturbations to the river and riparian areas due to climate and/or land use changes.

Presently, the Kowie River is classified as pristine (Department Of Water Affairs 2013). The effects of climate and land use changes on the flow of energy in the future are presently unclear (green boxes; Fig. 7.1), and therefore the model of energy flow proposed above may very well change in future. These changes in energy flow are particularly likely

considering that temperatures in southern Africa are expected to rise to 1.5-5.8°C by 2100 (Department of Environmental Affairs 2013). Climate change is set to increase the number of droughts and extreme flooding events (Kling et al. 2003), which will ultimately affect flow in rivers. The changes in land use and temperature have already been identified as causing shifts in reciprocal flows in the Scioto River in North America (lower reciprocal subsidies recorded in highly urbanized sites; Kautza and Sullivan 2015).

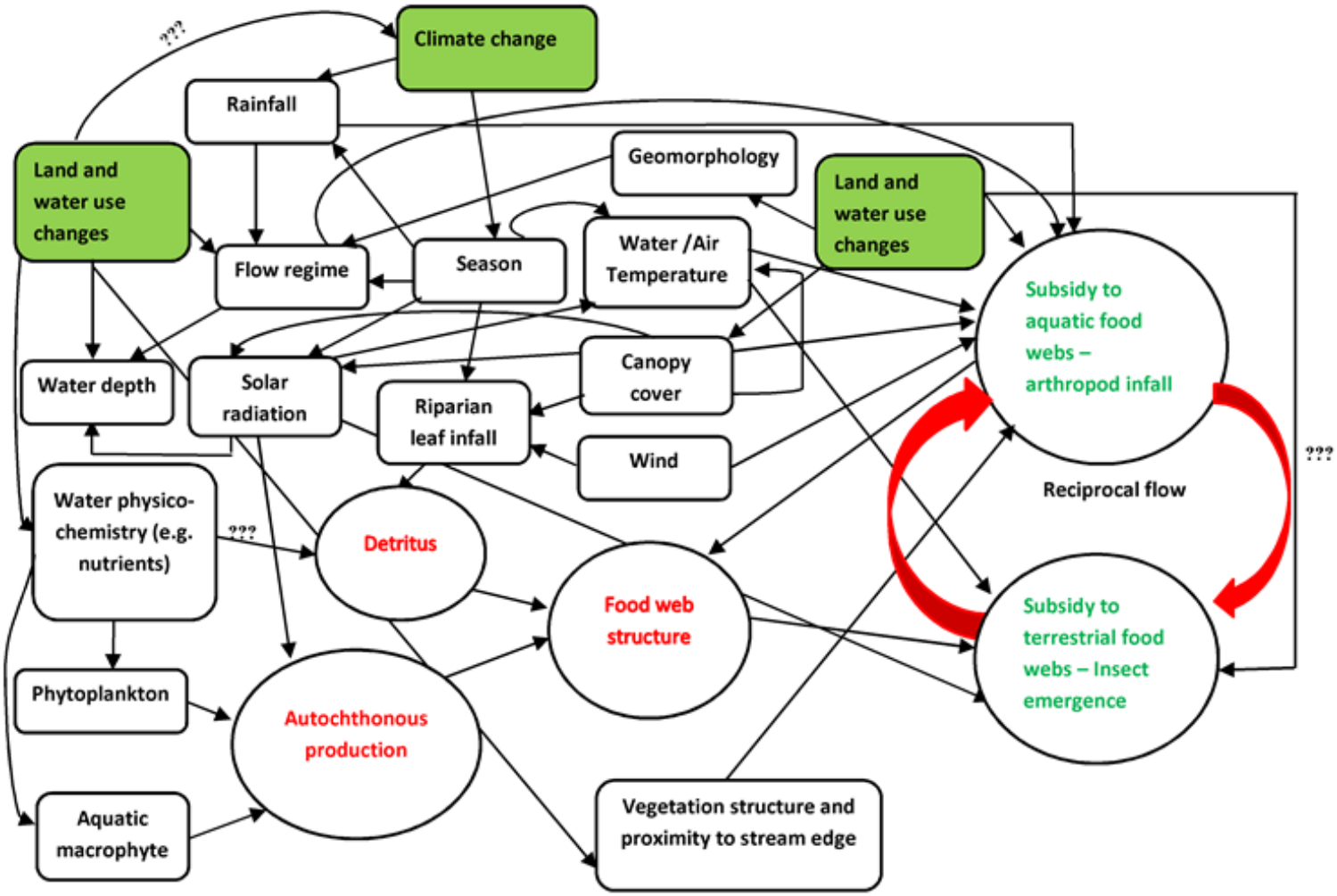

Figure 7.1: Conceptual model of the factors affecting the energy flow within the Kowie River across spatial and temporal scales. Arrows represent factors discussed in previous chapters. Green coloured boxes and arrows with question marks refer to factors that may affect energy flow in the future.

7.4 Recommendations

The Kowie River is of social and ecological importance. The Kowie River is important for anglers as it provides them with a diverse number of fish like the Largemouth bass (*Micropterus salmoides*; Magoro et al. 2015) and the Cape Stumpnose (*Rhabdosargus holubi*; Carassou et al. 2016). The river is also important for agricultural practises that consist mainly of pineapple production and animal husbandry. Sections of the river represent habitat for the endangered Eastern Cape Rocky (*Sandelia bainsii*; Cambray 2007). Additionally, the Kowie River is important socially as it is used for religious and spiritual ceremonies by the native people. The ecosystem services provided by the Kowie River make knowledge of the lateral and longitudinal connections within the system important for environmental managers and the general populous.

7.4.1 Management considerations

Several recommendations for management of streams and their riparian zones can be derived from this thesis. This thesis has shown that autochthonous carbon, terrestrial carbon, terrestrial insects and aquatic invertebrates are important in the functioning of the Kowie River. Therefore, there is need for conserving the different components in the Kowie catchment to maintain this ecosystem's function.

Riparian plant composition needs to be maintained at all sites (especially the headwaters). This conservation will maintain important aquatic-terrestrial links and subsidies. In Chapters 4 and 5, I demonstrated that terrestrial organic matter from terrestrial leaves was important to the diets of invertebrates in the headwaters, whilst in Chapter 6 I demonstrated that riparian trees affect the quantity of terrestrial invertebrates that fall into the Kowie River. Removal of riparian trees may ultimately alter the exchanges that occur between the Kowie River and the riparian area. Hence, I recommend the conservation of indigenous riparian trees, particularly in the headwater region. The Kowie River is colonised by a dense population of aquatic macrophytes. Macrophytes provide sites for epiphytes to grow (Dalu et al. 2014a) and once they have entered the detrital cycle they represent a food source for consumers (Chapter 4). Removal of macrophytes (which are sometimes considered weeds) affects the diets of consumers; therefore, it is important to conserve macrophytes and terrestrial plants, as they are very important for ecosystem function.

In Chapter 3, the results revealed that water velocity was positively associated with the abundance of filterers in the Kowie River. Similarly, in Chapter 6, flow was positively correlated with terrestrial inputs. Increasing pressures of abstraction of water resources and damming of rivers (Shen et al. 2014) will affect water flow in the future (Bunn and

Arthington 2002). Consequently, damming will negatively affect the biodiversity in freshwaters at a broader scale. The change in flow conditions will result in changes in composition of resources that enter or are exported from streams. As such, there is cause for managers to create solutions that will ensure that these factors that support riverine functions are not compromised.

Putting the aforementioned recommendations into practise will require the collaboration of multiple stakeholders such as the local municipality, farmers and residents. This process will ensure that the recommendations laid out in the *National Water Act* (1998) for managing catchments and water resources are suitably realised.

7.4.2 Future studies

Terrestrial arthropods represent a potential pathway of terrestrial carbon contributions to aquatic systems, especially in the headwaters where terrestrial insects contribute significantly to fishes' diets (reviewed in Chapter 1; Nakano et al. 1999a). Infall data in the Kowie River (Chapter 6) showed that terrestrial insect infall was greater in the headwaters, which coincided with higher consumption of terrestrially-derived organic matter by invertebrates (Chapter 4). A pathway where terrestrial organic matter is assimilated by aquatic secondary consumers via consumption of terrestrial insects may be possible especially considering that the isotopic value in terrestrial insects falls within the range of predatory terrestrial insects in the headwaters (Chari 2016). Future studies should incorporate data on terrestrial arthropod contributions to the diets of predators (see Fig. 7.3).

In Chapter 4, stable isotope mixing models were used to estimate the contributions of several organic matter sources to consumers. To run these mixing models, I used fractionation factors (*sensu* McCutchan et al. 2003) generated from freshwater systems from different ecosystems. To improve estimates of food source contributions to consumers in the Kowie River, controlled experiments are needed to determine the local fractionation values of nitrogen and carbon (*sensu* Perkins et al. 2014). Experiments investigating fractionation in southern African temperate rivers offer a promising area for further research, and will help to improve estimates of carbon flow to consumers. In mixing model estimates in this thesis, the number of food sources was limited to three or four in all models owing to the use of only two isotopes (carbon and nitrogen). Using more isotopes (e.g. hydrogen and sulphur) will allow the inclusion of more sources in mixing models and will help to untangle some of the uncertainties that arise from using only two isotopes (e.g. Cole et al. 2011). Using more than one isotope allows for the addition of sources into the mixing models (Phillips et al. 2014). The use of several isotopes has been advocated by many authors (Phillips 2001). In chapter 4,

the need to separate fine particulate organic matter (FPOM) into its different components was highlighted. Separating FPOM into its various components is a daunting task (but see Delong and Thorp 2006). Future studies in the Kowie River should incorporate attempts to separate FPOM into its fractions. Such an approach will further elucidate the food web structure in southern African temperate rivers and the possible connections with their riparian zones.

In Chapter 5, potential markers were used to determine the diets of consumers in the Kowie River. Future studies using an experimental approach are needed to identify additional diet tracers for consumers in freshwater systems. For example, 12:0 was consistently recorded in high proportions in the diets of caddis flies, while arachidonic acid occurred in the diets of shredders. An experimental approach involving feeding of caddis flies on known diets could help unravel the utility of these components as source tracers.

In light of land use and climate changes related to an escalating human population (Ormerod et al. 2010), holistic research is needed that combines stake holders from different levels of management of rivers and riparian areas. Priority must be given to research that is more centred on the drivers of environmental change (e.g. sewage and fertilizer inputs by farmer around catchments) as opposed to the resultant factors. In this regard, a multidisciplinary approach that brings together government, ecologists and social scientists (e.g. assessing farmer activities) may be very important in managing ecosystems. The multi-disciplinary approach will aid South Africa and the other nations in the world in being able to achieve the goals set out for integrated water resource management (Rahaman and Varis 2005).

Further studies in the Kowie River that include additional sites in comparison with other riverine systems in South Africa are recommended. Such extensions will enable further testing of my conclusions across a larger spatial scale. Ultimately, this process will increase the depth of our understanding of river function, and assist in the long term aim of protecting ecologically significant ecosystems.

This thesis is part of a larger project investigating the reciprocal links between adjacent aquatic and terrestrial ecosystems in South Africa (summarised in Fig. 7.2). My study and those of other researchers within the Kowie have addressed several pathways. A few pathways still provide opportunities for future research; for example, how much do snakes and birds rely on aquatic insects and amphibians, and do the insects and amphibians provide them with adequate polyunsaturated fatty acids for their dietary needs? These areas provide some missing links that could be investigated. Finally, one further opportunity for research is the transfer of polyunsaturated fatty acids to humans via the consumption of fish.

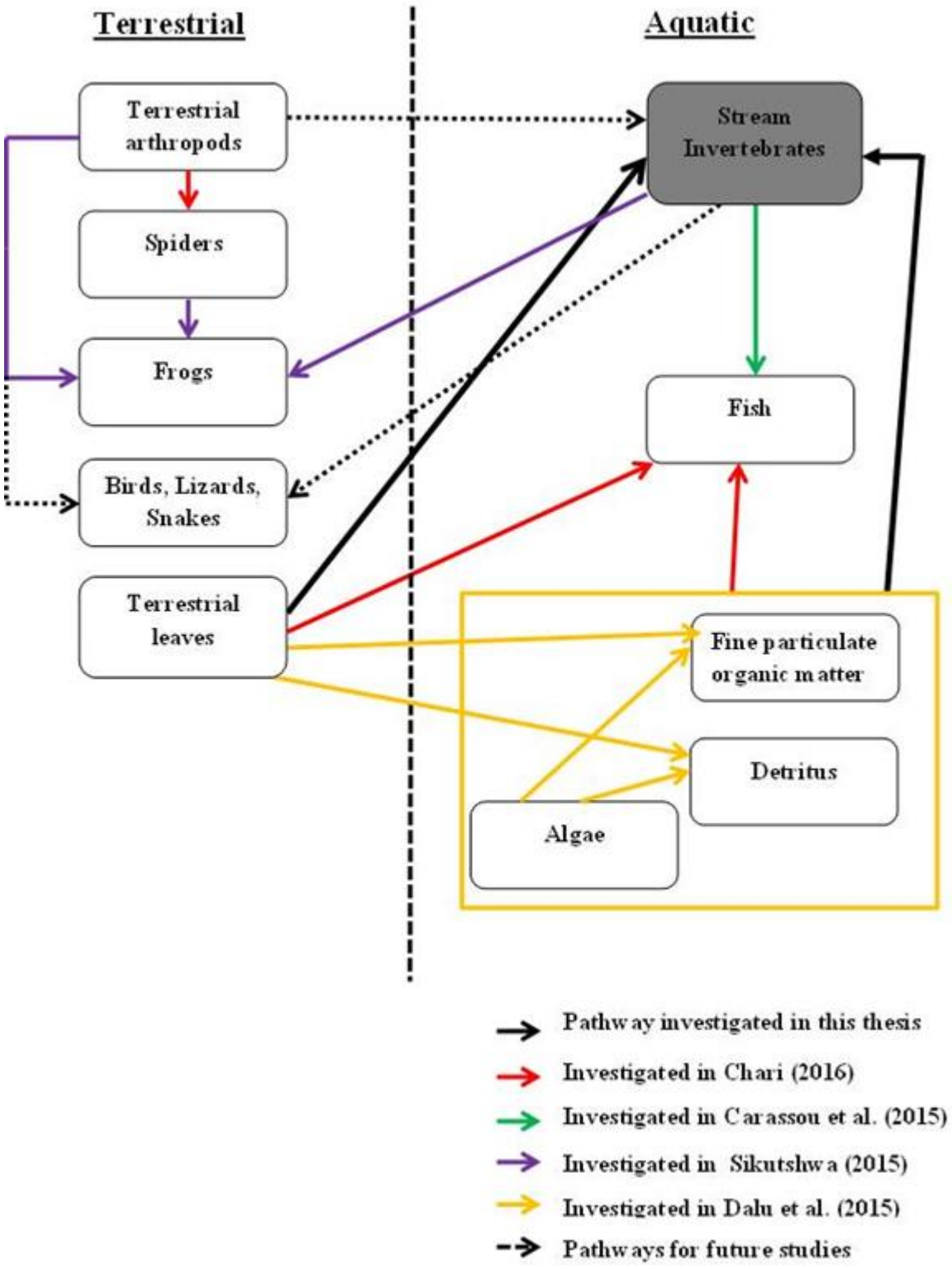

Figure 7.2: Simplified view of aquatic–terrestrial subsidy pathways. Coloured arrows show pathways that have already been investigated in the Kowie River. Dashed lines with arrows show pathways that are recommended for future studies.

7.5 Conclusions

To conclude, this thesis has contributed to a greater understanding of the ecology of small temperate rivers by showing that:

1. in the Kowie River, the occurrence and abundance of the different functional feeding groups was affected by many factors including water velocity (which affects filterers), total dissolved solids, canopy cover. Physical attributes (stream width and distance from headwaters) do not fully explain the organization of functional feeding groups. Functional organization partially did follow predictable patterns in the Kowie River, which is similar to findings from other studies (Jiang et al. 2011).
2. fatty acids and stable isotopes in the Kowie River suggests most macroinvertebrates depend on an autochthonous diet, although there a greater contribution of terrestrial organic matter in the headwater. The results in the Kowie support findings from other researchers that have documented the dependence of consumers on autochthonous sources (Delong and Thorp 2006, Torres-Ruiz et al. 2007). The dual approach of concurrently using fatty acid and stable isotope analysis is important for our understanding of riverine systems (Pingram et al. 2012; Chapter 4 and 5).
3. functional feeding groups, collection time and location influence the physiologically important fatty acids and isotope values. The effects of functional feeding group, collection time and site in the Kowie is in accordance with findings by other researchers (Cremona et al. 2010, Makhutova et al. 2011).
4. the Kowie River is tightly coupled to its neighbouring riparian area and there are exchanges that are constrained over spatial temporal scales. Generally, reciprocal flows are higher in the summer and decline in the winter. Additionally, aquatic insects are the major source of physiologically important fatty acids. The findings of the tight coupling between rivers and their riparian areas are in accordance with those of Nakano and Murakami (2001) and Rundio and Lindley (2012).

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8 Appendices

Appendix 8-1: Cumulative number of individuals for macroinvertebrate taxa collected at six sites along the Kowie River.

	FW1	FW2	FW6	FW3	FW4	FW5
Shannon-Wiener Diversity (H')	1.81	2.18	1.77	2.2	1.97	1.84
Evenness (E)	0.67	0.72	0.65	0.76	0.75	0.64
Baetidae sp 1	836	418	225	169	150	99
Baetidae sp2	890	445	280	148	292	5
Baetidae sp3	342	171	244	171	-	-
Simulium spp.	2544	1272	869	341	683	248
Aulonogyrus spp.	216	108	33	14	67	27
Tabanus spp.	120	60	12	15	28	17
Macrostermum capense	76	38	147	-	38	-
Hydropsyche spp.	624	312	191	6	239	67
Cheumatopsyche afra	198	99	30	99	-	-
Potamonautes spp.	210	105	31	26	64	15
Aeshnidae	56	28	15	2	11	15
Castanophlebia calida	406	203	-	-	201	2
Adenophlebia auriculata	28	14	161	14	-	-
Caenis spp.	98	49	68	26	23	-
Chironomidae	230	115	10	16	38	61
Chironomidae sp 1	26	13	-	5	8	-
Ecnomidae	60	30	-	24	6	-
Pseudagrion spp.	318	159	21	25	93	41
Pseudagrion spp. 2	152	76	13	10	-	66
Ischnura spp.	110	55	-	-	55	-
Tipulidae	10	1	-	-	-	-
Veliidae	2	-	-	-	-	-
Ancylidae	8	4	-	-	4	-
Gerridae	4	2	-	-	-	2
Libellulidae	40	20	3	5	-	15
Chlorolestidae	-	-	4	-	-	-

Leptocerus spp.	458	229	-	-	102	127
Athericidae	3	-	-	-	-	-
Notonectidae	6	3	-	-	2	1
Paragomphus spp.	10	5	22	5	-	-
Nepidae	6	3	-	-	1	2
Hydaticus bivittatus	64	32	-	-	12	20
Gyrinidae-larvae	4	2	-	-	2	-
Apassus spp.	44	22	-	-	-	22
Belostoma spp.	20	10	-	4	6	-
Caridina nilotica	64	32	-	-	-	32
Lestes spp.	6	3	-	-	3	-
Amphipoda	208	104	-	-	-	104
Corixidae	2	1	45	-	-	1
Hydracarina spp.	16	8	150	-	-	8
Chlorocyphidae	18	9	-	5	3	1
Oligocheata	2386	1193	17	120	-	1073
Ceratopogonidae	5	-	-	-	-	-
Laccophilus spp.	-	19	-	-	-	-
Physidae	148	74	-	-	-	74
Cucilidae	-	8	-	-	-	-
Hydrometra spp.	4	2	-	-	-	2
Prosopistomatidae	-	-	-	-	-	2
Helodidae	-	1	-	-	-	-
Philopotamidae	-	-	20	-	-	-
Psephenidae	2	1	-	1	-	-
Platycnemidae	2	1	10	1	-	-
Pleidae	60	30	-	-	-	30
Heptageniidae	-	-	2	-	-	4
Oligoneuridae	-	-	1	2	-	3
Platyhelminthes	-	-	4	-	-	-

Annual Mean percents (± SD) of dominant fatty acids recorded in macroinvertebrates: Filterer1 (Appendix 82), Filterer 2 (Appendix 8-3), Grazer (Appendix 8-4), Shredder (Appendix 8-5), gatherer (Appendix 8-6) and Predators (Appendix 8-7) in the seasonal and spatial study of the Kowie River 2012-2013.

Appendix 8-2

	FW1		**FW2**		**FW3**		**FW4**		**FW5**		**FW6**	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
12:0	0.15	0.20	0.08	0.04	0.07	0.01	0.12	0.08	0.22	0.09	0.10	0.05
14:0	1.11	0.25	1.32	0.47	1.84	1.20	1.48	0.42	1.14	0.18	1.08	0.28
15:0	0.35	0.15	0.38	0.11	0.40	0.09	0.83	0.38	0.43	0.19	0.47	0.12
16:0	19.41	3.35	19.14	2.91	20.23	1.77	19.76	2.67	19.24	2.18	19.11	1.95
17:0	0.86	0.23	0.99	0.58	1.27	0.51	1.41	0.53	1.12	0.13	0.73	0.40
18:0	5.73	1.20	6.28	0.57	5.72	0.71	6.61	1.07	5.21	0.49	5.51	0.46
20:0	0.48	0.25	0.45	0.14	0.27	0.05	0.63	0.46	0.63	0.20	0.29	0.08
14:1ω5	2.80	2.59	2.37	2.48	1.65	1.07	5.54	2.29	10.16	0.65	3.04	0.47
16:1ω5	0.65	0.15	0.51	0.32	0.49	0.38	0.76	0.35	1.37	0.61	1.07	0.66
16:1ω7	8.96	2.40	7.21	4.24	5.69	3.18	8.12	2.14	9.03	2.74	11.26	4.27
16:1ω9	0.44	0.34	3.20	4.75	3.56	5.63	0.26	0.28	0.51	0.16	0.46	0.29
16:2ω4	0.70	0.93	1.16	1.09	0.37	0.23	0.57	0.71	0.36	0.20	2.02	1.41
16:3ω3	0.44	0.32	1.24	0.44	0.66	0.93	0.45	0.60	0.33	0.33	0.15	0.27
16:3ω4	0.93	0.40	0.45	0.30	0.37	0.26	0.98	0.98	0.26	0.25	0.89	0.71
16:4ω3	0.80	0.67	0.28	0.11	0.46	0.18	0.77	0.58	0.15	0.08	0.12	0.10
18:1ω7	4.10	1.60	3.07	1.97	3.25	2.20	4.86	0.74	5.61	0.94	4.94	1.01
18:1ω9	12.90	2.25	10.36	4.43	9.97	3.80	11.92	2.05	11.23	2.29	11.04	1.95
18:2ω6cis	5.06	2.07	6.38	3.03	6.37	2.58	4.63	1.55	8.59	3.16	4.95	1.36
18:2ω6trans	0.02	0.04	0.78	1.31	0.66	0.97	0.15	0.11	0.05	0.06	0.12	0.02
18:3ω3	8.61	4.36	7.44	6.50	8.28	5.12	9.34	5.55	5.94	1.16	6.46	3.09
18:3ω6	0.30	0.09	1.30	1.82	2.27	3.63	0.47	0.15	0.31	0.19	0.52	0.14
18:4ω3	2.15	0.35	1.23	0.60	1.69	1.29	1.16	0.56	1.15	0.71	1.44	0.72
20:1ω9	0.12	0.04	0.12	0.10	0.15	0.06	0.21	0.09	0.17	0.14	0.10	0.05
20:2ω6	0.30	0.49	0.19	0.07	0.15	0.04	0.15	0.11	0.94	2.21	0.10	0.04
20:3ω3	0.07	0.07	0.04	0.08	0.10	0.07	0.13	0.11	0.01	0.04	0.08	0.06
20:3ω6	0.14	0.04	0.10	0.06	0.12	0.08	0.16	0.05	0.20	0.18	0.12	0.03
20:4ω3	0.54	0.09	0.29	0.09	0.34	0.14	0.24	0.15	0.27	0.32	0.24	0.09
20:4ω6	1.63	0.22	1.43	0.72	1.49	0.68	1.72	0.45	2.30	0.72	2.51	1.62
20:5ω3	14.37	5.90	11.69	6.43	11.39	6.60	11.22	2.97	7.36	2.37	14.60	3.72
22:5ω3	0.24	0.08	0.10	0.09	0.11	0.08	0.02	0.04	0.03	0.05	0.08	0.05
22:5ω6	0.22	0.12	0.11	0.09	0.18	0.07	0.04	0.07	0.05	0.05	0.10	0.07
22:6ω3	1.26	0.76	1.07	0.77	0.50	0.26	0.52	0.48	0.09	0.12	0.49	0.35
Σ PUFA	37.88	1.88	39.48	1.88	39.21	1.98	32.72	5.71	28.39	4.28	34.99	1.79
Σ MUFA	30.34	3.76	28.22	5.37	26.28	2.83	32.85	2.18	38.72	2.33	32.44	3.12
ΣSAFA	31.79	2.64	32.30	3.92	34.51	1.64	34.42	4.97	32.89	2.65	32.57	4.36
ΣBACTERIAL	4.04	1.76	3.84	1.19	5.08	0.96	4.56	1.17	5.62	1.01	5.71	2.64

Appendix 8-3

	FW1		FW2		FW3		FW4		FW5		FW6	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
12:0	11.33	9.37	16.35	4.54	15.49	6.40	12.72	5.10	11.17	5.48	11.34	5.90
14:0	9.63	4.24	10.98	2.88	9.89	3.84	9.61	3.27	7.60	3.02	8.80	3.24
15:0	0.73	0.19	0.59	0.25	0.58	0.13	0.93	0.33	0.58	0.20	0.56	0.19
16:0	19.38	5.00	16.14	2.88	14.85	1.92	18.47	3.91	15.93	1.13	16.00	1.44
17:0	0.77	0.24	0.69	0.41	0.94	0.31	1.44	0.46	1.17	0.32	0.46	0.31
18:0	5.11	1.59	3.78	0.62	5.05	2.19	6.38	1.91	5.21	1.21	5.00	1.28
20:0	0.34	0.20	0.18	0.08	0.21	0.11	0.33	0.20	0.39	0.27	0.21	0.07
14:1ω5	1.37	0.55	1.44	1.24	1.17	1.10	2.04	0.33	2.18	0.88	2.18	0.70
16:1ω5	0.27	0.09	0.25	0.16	0.25	0.18	0.52	0.20	0.53	0.25	0.46	0.21
16:1ω7	8.06	4.78	8.70	7.95	3.54	2.01	7.87	2.06	6.15	1.09	7.12	3.08
16:1ω9	0.39	0.22	2.11	3.34	1.48	1.93	0.40	0.15	0.41	0.12	0.31	0.20
16:2ω4	0.59	0.82	0.39	0.56	0.15	0.12	0.69	0.70	0.30	0.38	0.92	1.22
16:3ω3	1.10	0.86	1.13	1.54	0.60	0.65	0.52	0.60	0.29	0.22	1.21	1.00
16:3ω4	0.57	0.60	0.47	0.54	1.48	1.17	1.14	1.27	1.40	1.09	1.82	1.54
16:4ω3	0.51	0.24	0.30	0.25	0.37	0.23	0.47	0.56	0.15	0.08	0.25	0.23
18:1ω7	2.21	1.25	1.64	1.09	2.01	1.14	3.60	0.70	3.51	4.40	2.30	0.85
18:1ω9	10.49	2.34	7.99	4.24	8.49	5.08	10.63	3.77	12.23	5.07	12.49	3.14
18:2ω6cis	3.76	0.97	4.47	2.93	5.33	2.14	4.69	1.05	9.61	1.73	5.18	4.10
18:2ω6trans	0.11	0.10	1.01	1.68	1.00	1.67	0.16	0.06	0.07	0.05	0.14	0.09
18:3ω3	7.09	2.26	4.67	3.10	6.75	4.13	4.18	4.56	8.31	3.79	6.23	2.13
18:3ω6	0.13	0.12	1.71	2.81	2.33	3.92	0.28	0.09	0.17	0.10	0.23	0.10
18:4ω3	0.96	0.17	0.70	0.38	0.62	0.45	0.51	0.15	0.54	0.13	1.21	0.82
20:1ω9	0.15	0.07	0.08	0.06	0.08	0.07	0.16	0.09	0.07	0.04	0.10	0.09
20:2ω6	0.10	0.04	0.08	0.02	0.10	0.04	0.09	0.02	0.07	0.03	0.09	0.06
20:3ω3	0.13	0.05	0.09	0.06	0.08	0.03	0.08	0.07	0.06	0.03	0.06	0.02
20:3ω6	0.16	0.15	0.07	0.04	0.09	0.06	0.11	0.03	0.07	0.02	0.08	0.03
20:4ω3	0.35	0.09	0.16	0.07	0.20	0.09	0.18	0.06	0.12	0.04	0.20	0.07
20:4ω6	0.79	0.24	0.66	0.30	1.33	1.07	0.69	0.19	1.13	0.46	1.27	0.79
20:5ω3	10.24	4.25	6.46	4.02	9.03	5.52	6.14	1.96	6.95	2.37	9.56	2.17
22:5ω3	0.11	0.09	0.09	0.09	0.14	0.27	0.02	0.04	0.06	0.03	0.07	0.04
22:5ω6	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.03	0.05
22:6ω3	0.31	0.18	0.12	0.09	0.25	0.33	0.21	0.15	0.12	0.05	0.46	0.55
Σ PUFA	27.02	6.06	25.19	6.81	32.01	5.54	20.17	6.84	29.41	7.15	29.03	5.54
Σ MUFA	23.11	7.42	23.53	9.41	17.83	5.51	26.20	7.11	25.98	3.04	25.23	3.54
ΣSAFA	49.87	8.98	51.28	5.51	50.17	8.68	53.63	8.58	44.61	6.69	45.74	7.68
ΣBACTERIAL	2.51	0.65	2.09	0.76	2.50	0.59	3.57	0.92	2.97	0.38	2.96	0.53

Appendix 8-4

	FW1		FW2		FW3		FW4		FW5		FW6	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
12:0	0.13	0.04	0.09	0.04	0.09	0.03	0.10	0.04	0.09	0.06	0.11	0.08
14:0	2.53	1.06	1.73	0.95	1.35	0.66	1.71	1.02	1.63	0.71	1.57	1.46
15:0	0.49	0.13	0.50	0.22	0.60	0.17	0.63	0.27	0.71	0.65	0.67	0.43
16:0	19.79	6.39	24.92	4.17	22.49	4.94	21.43	5.33	23.97	6.07	20.85	6.16
17:0	0.53	0.19	0.80	0.46	1.04	0.28	1.09	0.60	1.05	0.57	0.96	0.48
18:0	6.74	2.01	5.15	0.96	6.37	1.47	6.25	1.32	5.98	1.41	6.45	1.67
20:0	1.04	1.72	0.25	0.09	0.23	0.12	0.25	0.12	0.52	0.40	0.36	0.28
14:1ω5	0.11	0.32	0.01	0.03	0.09	0.17	0.00	0.00	0.00	0.01	0.03	0.05
16:1ω5	0.47	0.20	0.91	0.93	0.70	0.48	0.87	0.59	0.88	0.60	0.94	0.68
16:1ω7	7.96	4.01	8.47	4.81	7.28	3.81	12.82	3.11	13.01	2.51	11.90	4.33
16:1ω9	0.29	0.31	3.74	5.93	2.00	3.56	0.49	0.29	0.46	0.17	0.38	0.25
16:2ω4	0.52	0.49	1.06	1.09	0.51	0.48	1.21	0.89	1.09	0.70	1.48	1.35
16:3ω3	0.45	0.54	0.74	0.88	0.96	0.95	0.30	0.22	0.31	0.21	0.38	0.32
16:3ω4	0.79	0.57	0.62	0.38	0.33	0.23	0.64	0.36	0.72	0.53	0.67	0.51
16:4ω3	0.13	0.40	0.22	0.22	0.30	0.53	0.18	0.20	0.09	0.13	0.16	0.29
18:1ω7	9.66	1.93	8.89	5.27	8.93	4.63	13.81	3.28	11.94	4.87	12.31	4.49
18:1ω9	6.42	2.08	9.39	2.74	8.12	2.00	7.92	2.39	6.94	2.03	7.15	2.32
18:2ω6cis	4.24	1.86	4.96	1.98	5.81	1.48	4.47	1.52	6.91	4.85	5.57	3.40
18:2ω6trans	0.90	0.47	1.25	1.17	1.16	0.92	0.66	0.24	0.61	0.51	0.82	0.85
18:3ω3	7.66	4.28	6.84	4.83	9.87	6.16	4.45	3.96	6.79	3.62	6.90	5.05
18:3ω6	1.08	1.09	1.39	1.46	1.39	2.46	1.05	1.28	0.42	0.28	0.46	0.46
18:4ω3	1.31	0.73	1.26	0.78	1.93	1.05	0.94	0.37	1.19	0.42	1.21	0.75
20:1ω9	0.29	0.33	0.07	0.09	0.07	0.09	0.16	0.15	0.09	0.06	0.08	0.09
20:2ω6	0.67	0.82	0.11	0.04	0.06	0.05	0.13	0.07	0.10	0.09	0.08	0.06
20:3ω3	0.38	0.31	0.08	0.07	0.10	0.07	0.10	0.06	0.06	0.04	0.07	0.04
20:3ω6	0.38	0.30	0.09	0.04	0.13	0.06	0.12	0.04	0.17	0.11	0.11	0.09
20:4ω3	0.37	0.17	0.19	0.06	0.19	0.08	0.18	0.06	0.17	0.05	0.16	0.06
20:4ω6	3.98	4.39	1.34	1.17	2.04	1.25	2.27	1.01	2.13	0.89	2.53	1.69
20:5ω3	12.65	2.55	9.59	5.16	10.79	4.36	11.90	2.83	8.79	2.63	10.68	3.13
22:5ω3	1.74	2.29	0.03	0.05	0.03	0.07	0.02	0.03	0.00	0.00	0.00	0.02
22:5ω6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.02
22:6ω3	0.79	0.92	0.06	0.12	0.03	0.10	0.01	0.02	0.00	0.01	0.01	0.05
Σ PUFA	38.45	5.45	32.56	4.16	37.37	5.71	28.62	7.48	29.54	5.44	31.07	7.72
Σ MUFA	25.84	5.81	32.08	6.61	27.58	6.26	36.75	6.43	34.02	7.29	33.54	8.95
ΣSAFA	35.71	1.46	35.36	3.90	35.05	4.49	34.63	5.93	36.44	4.61	33.46	6.90
ΣBACTERIAL	1.81	0.75	2.02	0.79	3.29	1.51	3.48	1.56	3.12	2.04	3.21	1.64

Appendix 8-5

	FW1		FW2		FW3		FW4		FW5		FW6	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
12:0	0.66	0.87	0.95	1.63	0.66	0.60	0.18	0.11	0.33	0.49	0.93	1.14
14:0	2.78	0.73	2.04	2.17	1.73	1.19	1.20	0.80	0.82	0.65	1.68	1.60
15:0	0.29	0.14	0.31	0.14	0.54	0.22	0.77	0.33	0.33	0.15	0.47	0.18
16:0	15.60	1.71	13.73	5.17	15.71	3.18	13.20	2.49	12.60	2.27	12.75	3.32
17:0	0.70	1.03	1.28	1.44	1.01	0.40	1.30	0.91	0.72	0.29	1.13	0.41
18:0	7.99	1.31	7.54	2.65	7.67	1.24	7.42	1.57	6.56	1.74	7.96	1.50
20:0	0.61	0.23	0.63	0.45	0.58	0.41	0.62	0.26	0.65	0.20	0.51	0.11
14:1ω5	0.21	0.31	0.34	0.40	0.14	0.20	0.01	0.02	0.11	0.18	0.32	0.53
16:1ω5	0.50	0.17	0.39	0.34	0.54	0.59	0.36	0.13	0.73	0.45	0.57	0.35
16:1ω7	8.41	3.03	12.02	12.90	4.13	3.64	6.51	4.15	3.49	1.54	6.21	5.04
16:1ω9	0.31	0.16	0.51	0.40	2.08	3.40	0.20	0.14	0.20	0.09	0.22	0.14
16:2ω4	0.90	0.83	0.89	1.18	0.46	0.34	0.62	0.49	0.23	0.10	0.79	0.49
16:3ω3	0.67	0.18	0.34	0.78	0.55	0.37	0.27	0.33	0.17	0.10	0.55	0.75
16:3ω4	0.43	0.51	1.01	0.76	0.66	0.50	1.00	0.49	0.36	0.35	0.20	0.27
16:4ω3	0.15	0.38	0.17	0.24	0.11	0.21	0.12	0.15	0.05	0.10	0.05	0.08
18:1ω7	6.19	1.68	3.16	2.22	4.58	2.74	6.38	1.73	8.29	7.45	5.41	1.79
18:1ω9	11.29	1.38	10.57	5.95	11.46	3.38	12.05	3.78	17.65	13.26	12.60	1.66
18:2ω6cis	7.07	3.26	7.28	5.65	9.18	4.04	13.40	6.51	22.48	9.90	7.91	4.66
18:2ω6trans	0.22	0.11	1.88	2.98	1.93	2.39	0.33	0.21	0.13	0.09	0.28	0.12
18:3ω3	3.42	1.19	2.45	1.94	4.56	2.90	2.60	1.45	1.80	1.14	3.69	2.01
18:3ω6	0.27	0.05	0.53	0.53	1.63	2.57	0.39	0.28	0.16	0.10	0.19	0.10
18:4ω3	0.61	0.21	0.48	0.31	0.52	0.22	0.35	0.13	0.44	0.42	0.84	0.47
20:1ω9	0.47	0.27	0.31	0.31	0.55	0.44	0.55	0.29	0.44	0.13	0.27	0.19
20:2ω6	0.63	0.26	0.39	0.35	0.62	0.29	1.04	0.36	1.19	0.58	0.92	0.59
20:3ω3	0.55	0.18	0.18	0.15	0.43	0.08	0.29	0.10	0.15	0.10	0.34	0.12
20:3ω6	0.13	0.17	0.24	0.12	0.30	0.23	0.34	0.11	0.16	0.15	0.09	0.09
20:4ω3	0.37	0.14	0.20	0.12	0.26	0.19	0.23	0.03	0.11	0.06	0.26	0.10
20:4ω6	4.89	1.74	3.41	3.50	5.10	3.47	8.98	4.02	7.19	5.95	8.91	4.76
20:5ω3	14.58	2.00	14.30	4.93	12.49	6.81	13.29	4.02	8.05	5.17	17.63	4.07
22:5ω3	0.83	0.43	0.60	0.30	0.66	0.76	0.94	1.23	0.32	0.15	0.35	0.31
22:5ω6	0.36	0.10	0.48	0.94	0.55	0.40	0.36	0.25	0.37	0.11	0.59	0.52
22:6ω3	3.27	0.80	2.65	1.74	1.39	0.81	1.57	1.74	1.19	0.99	2.32	1.27
Σ PUFA	39.53	3.77	43.04	20.84	44.42	6.69	46.10	5.63	44.55	7.83	45.94	10.77
Σ MUFA	28.24	3.34	28.08	17.91	24.38	5.95	26.58	3.72	31.21	7.93	26.10	6.75
ΣSAFA	32.23	2.60	28.88	5.50	31.20	4.68	27.32	3.74	24.24	1.87	27.96	5.68
ΣBACTERIAL	2.64	0.96	2.73	1.65	2.89	0.78	2.93	1.42	1.72	0.73	2.60	1.00

Appendix 8-6

	FW1		FW2		FW3		FW4		FW5		FW6	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
12:0	0.13	0.09	0.16	0.05	0.18	0.06	0.15	0.03	0.12	0.04	0.27	0.15
14:0	2.26	0.77	1.90	0.37	1.87	0.50	1.73	0.58	2.14	0.28	1.67	0.90
15:0	0.54	0.33	0.83	0.27	1.06	0.15	1.38	0.53	1.21	0.22	0.94	0.18
16:0	16.22	2.29	16.26	3.37	19.49	3.87	20.57	4.92	16.02	4.49	16.10	4.73
17:0	0.93	0.23	1.36	0.19	1.54	0.18	2.29	0.40	1.83	0.64	0.57	0.42
18:0	8.29	2.74	7.07	0.89	6.12	1.53	6.70	0.72	6.42	1.43	7.25	1.76
20:0	0.57	0.32	0.57	0.31	0.31	0.10	0.44	0.22	0.72	0.24	0.39	0.13
14:1ω5	0.07	0.06	0.00	0.00	0.19	0.32	0.07	0.12	0.00	0.00	0.15	0.09
16:1ω5	0.44	0.13	1.18	0.37	0.85	0.36	1.18	0.23	1.46	0.28	1.07	0.16
16:1ω7	8.09	3.67	7.22	0.82	6.49	3.22	10.08	4.10	12.48	0.94	6.14	0.64
16:1ω9	0.22	0.18	0.49	0.12	1.23	2.60	0.42	0.08	0.39	0.22	0.33	0.17
16:2ω4	0.49	0.42	1.85	0.89	0.80	0.85	1.22	1.29	1.01	0.61	1.43	0.50
16:3ω3	0.00	0.00	0.26	0.41	0.90	0.34	0.95	1.04	0.24	0.27	0.35	0.54
16:3ω4	2.01	1.04	1.24	0.24	0.79	0.59	1.01	0.87	1.87	0.79	0.64	0.55
16:4ω3	0.55	1.05	0.19	0.21	0.13	0.24	0.16	0.25	0.00	0.00	0.10	0.15
18:1ω7	7.37	1.28	15.62	2.76	14.53	5.40	15.54	2.09	14.83	1.05	15.29	2.11
18:1ω9	8.64	2.26	8.27	0.90	9.25	2.09	8.48	1.63	7.95	0.61	10.56	2.43
18:2ω6cis	4.32	0.44	3.44	0.42	5.02	1.52	4.61	0.93	5.04	1.30	5.51	1.18
18:2ω6trans	0.23	0.19	0.36	0.40	0.92	1.02	0.59	0.20	0.43	0.11	0.51	0.34
18:3ω3	3.56	2.36	4.08	1.73	5.00	2.51	2.39	1.94	3.28	0.32	3.99	0.67
18:3ω6	0.31	0.13	0.51	0.19	0.93	1.46	0.44	0.18	0.39	0.23	1.00	1.14
18:4ω3	0.53	0.26	0.75	0.17	0.83	0.17	0.61	0.15	0.56	0.11	0.84	0.43
20:1ω9	0.21	0.11	0.04	0.04	0.05	0.05	0.10	0.08	0.13	0.20	0.02	0.03
20:2ω6	0.33	0.06	0.14	0.07	0.09	0.09	0.04	0.07	0.10	0.04	0.09	0.09
20:3ω3	0.46	0.32	0.12	0.04	0.10	0.05	0.08	0.07	0.09	0.05	0.08	0.09
20:3ω6	0.17	0.07	0.16	0.06	0.14	0.06	0.06	0.09	0.13	0.03	0.09	0.08
20:4ω3	0.39	0.14	0.18	0.11	0.50	1.14	0.11	0.10	0.12	0.06	0.08	0.07
20:4ω6	3.15	0.71	4.22	1.70	3.56	1.74	3.56	1.38	6.11	0.96	4.71	2.07
20:5ω3	18.94	8.00	18.53	3.91	13.25	5.40	11.64	3.92	10.19	0.94	15.18	3.65
22:5ω3	0.51	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:5ω6	0.47	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:6ω3	5.97	1.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Σ PUFA	42.67	6.24	36.03	5.63	34.25	4.49	27.47	7.87	29.56	3.20	34.62	6.04
Σ MUFA	25.80	4.94	33.36	3.70	33.09	3.90	36.64	2.44	38.33	0.65	34.28	2.13
ΣSAFA	31.53	1.35	30.61	2.00	32.66	2.69	35.89	5.58	32.12	2.77	31.10	4.76
ΣBACTERIAL	2.45	1.19	2.69	0.44	3.07	0.57	4.11	0.53	4.29	0.38	3.80	1.42

Appendix 8-7

	FW1		FW2		FW3		FW4		FW5		FW6	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
12:0	0.48	0.63	1.61	3.47	0.61	0.93	0.22	0.06	0.28	0.19	0.81	1.32
14:0	2.38	0.75	2.49	3.14	1.80	1.22	1.79	0.63	1.38	0.58	2.24	1.69
15:0	0.43	0.21	0.40	0.10	0.55	0.13	0.66	0.26	0.41	0.24	0.49	0.19
16:0	19.57	3.72	20.48	2.41	20.02	4.09	18.56	3.18	16.79	2.50	19.82	2.54
17:0	0.75	0.32	0.83	0.31	1.11	0.59	1.20	0.45	1.25	0.49	1.09	0.61
18:0	7.87	1.73	7.47	1.81	8.07	2.06	8.50	1.75	9.50	2.40	7.86	1.67
20:0	0.65	0.51	0.49	0.26	0.39	0.26	0.50	0.21	1.03	0.52	0.37	0.16
14:1ω5	0.42	0.34	0.51	0.64	0.14	0.22	0.23	0.37	0.47	0.53	0.37	0.48
16:1ω5	0.44	0.23	0.49	0.34	0.53	0.34	0.49	0.12	0.58	0.25	0.68	0.21
16:1ω7	8.75	1.78	6.23	3.85	5.36	2.95	8.65	2.37	7.80	2.13	7.83	2.26
16:1ω9	0.20	0.16	2.35	3.60	1.48	2.47	0.23	0.08	0.30	0.09	0.28	0.15
16:2ω4	0.81	0.64	0.93	0.66	0.29	0.45	0.85	0.90	0.58	0.44	0.58	0.69
16:3ω3	0.50	0.48	0.41	0.50	0.60	0.44	0.50	0.52	0.32	0.31	0.35	0.47
16:3ω4	0.58	0.28	0.87	0.82	0.45	0.47	0.75	0.63	0.59	0.38	0.75	0.76
16:4ω3	0.23	0.17	0.16	0.13	0.19	0.20	0.20	0.19	0.01	0.04	0.14	0.16
18:1ω7	7.55	2.83	6.58	4.94	8.44	5.24	11.99	2.16	9.23	1.95	10.52	3.62
18:1ω9	12.19	3.94	11.29	3.07	11.87	4.25	10.62	2.36	13.04	3.54	12.00	4.13
18:2ω6cis	6.12	1.50	6.84	2.58	6.87	2.46	7.56	2.25	11.80	2.94	6.02	2.35
18:2ω6trans	0.45	0.41	2.06	2.74	1.95	2.47	0.65	0.22	0.31	0.16	1.17	0.70
18:3ω3	5.44	1.29	6.50	4.09	7.11	4.04	4.80	2.20	6.47	2.36	6.34	2.35
18:3ω6	0.52	0.15	2.01	2.31	1.51	2.58	0.62	0.23	0.27	0.20	0.33	0.28
18:4ω3	1.17	0.37	1.08	0.59	1.07	0.43	0.70	0.21	0.55	0.21	0.97	0.48
20:1ω9	0.29	0.32	0.06	0.05	0.11	0.10	0.19	0.12	0.14	0.12	0.17	0.26
20:2ω6	0.20	0.13	0.16	0.14	0.18	0.17	0.14	0.07	0.16	0.09	0.17	0.20
20:3ω3	0.15	0.09	0.08	0.06	0.16	0.12	0.07	0.07	0.09	0.07	0.09	0.06
20:3ω6	0.18	0.07	0.09	0.05	0.12	0.11	0.15	0.04	0.23	0.13	0.14	0.12
20:4ω3	0.31	0.07	0.18	0.11	0.24	0.07	0.21	0.05	0.16	0.07	0.21	0.07
20:4ω6	2.66	1.48	1.07	0.70	1.86	1.16	2.00	0.54	3.49	0.78	2.34	1.16
20:5ω3	15.02	4.40	10.65	5.66	11.73	4.20	14.82	3.14	10.18	3.38	12.93	2.62
21:5ω3	0.16	0.27	3.65	6.31	2.89	6.34	0.00	0.00	0.00	0.00	0.00	0.00
22:1ω11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:1ω9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:2ω13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:2ω6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:2ω9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:4ω6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:5ω3	0.22	0.34	0.01	0.05	0.00	0.00	0.01	0.03	0.00	0.00	0.02	0.05
22:5ω6	0.04	0.10	0.00	0.00	0.01	0.02	0.03	0.07	0.00	0.00	0.00	0.00
22:6ω3	0.65	0.87	0.12	0.10	0.04	0.06	0.01	0.03	0.10	0.16	0.13	0.18
Σ PUFA	35.41	4.39	36.88	5.48	37.26	6.46	34.06	4.77	35.32	5.15	32.68	4.15
Σ MUFA	30.32	2.42	27.87	4.89	28.39	5.71	32.87	3.56	32.14	4.19	32.37	3.46
ΣSAFA	34.27	2.67	35.25	4.79	34.35	3.14	33.07	3.17	32.54	3.86	34.95	2.14
ΣBACTERIAL	2.02	0.80	1.63	0.55	2.36	0.91	2.35	0.83	2.62	1.24	2.92	0.88