Predators of aerial insects and riparian cross-boundary trophic dynamics: web-building spiders, dragonflies and damselflies

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LENIN DZIBAKWE CHARI

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Abstract

This thesis characterises the cross-boundary trophic interactions of a relatively small model ecosystem, the Kowie River (Eastern Cape of South Africa), to explore their epistemic implications for systems ecology. Using web-building spiders and odonates (dragonflies and damselflies) as model organisms, I sought to investigate whether the diets of predators of aerial insects could be used to assess the strength of the trophic connectivity between freshwater and terrestrial systems in relation to variables such as stream width, distance from the river and aquatic insect emergence rates and abundances. Predator diet composition was determined by using a combination of diet analysis tools: direct observations of cross-subsidies, naturally-abundant stable (carbon and nitrogen) isotope analysis and fatty acid analysis. I also sought to reveal feeding niches and guilds among riparian aerial predators and investigate how the environment influenced predators' access to aquatic prey subsidies.

As emergent aquatic insect abundances decreased with an increase in distance from the river, and increased with stream width and seasonal changes from winter to summer, stable isotope and fatty acid analyses revealed distinct changes in web-building spider diet composition. Examination of the fatty acid eicosapentaenoic acid, a component commonly used as an indicator of consumer reliance on aquatic nutritional subsidies, showed that aquatic subsidies extended further inland at the wider sections of the river. Spiders and odonates at the wider sections of the Kowie River generally received more subsidies (56 – 70%) than those at the narrower sections (25 – 60%). When terrestrial insect biomass was distinctly low in winter, the benefit of aquatic subsidisation to spiders was relatively lower at the narrower sections of the Kowie River relative to the wide sections. As such, riparian areas adjacent to wide parts of the river were more likely to support larger populations of aerial predators than those at the narrow sections.

Apart from the diet changes across time and space, there was evidence of inter-specific niche partitioning in both spiders and odonates, but no differences were observed between males and females of the same species. Results showed odonates of different sizes and hunting strategies had separate dietary niches, hence varied access to aquatic nutritional subsidies. The larger odonate taxa that frequently foraged mid-air had more varied diets and relied less on aquatic emergent insects than the smaller odonates that foraged from perches near the river. There was also evidence of niche partitioning amongst the spiders, as those that built horizontal webs captured more aquatic insects (40 – 78%) than the vertical orb-web builders (20 – 66%).

This study showed that the nature and extent of trophic cross-boundary linkages in riparian areas largely depended on the availability of subsidies that varied seasonally and spatially. The width of the stream and seasonal variability emerged as important predictors of emergent insect abundances/biomasses that influenced predator feeding niches. The high mobility of odonates made their reliance on aquatic nutritional subsidies different from the less mobile spiders. The link between the width of the river and the extent of trophic connectivity has implications for riparian area management and definition of riparian buffer zones. However, the variation in diet niches amongst terrestrial consumers makes the results area-specific, and more studies are required that incorporate additional terrestrial predators in other fluvial systems so that we can make some generalizations on the dynamics of riparian trophic cross-boundary links.

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Dedication

This thesis is dedicated to "the little guy", starting from the bottom and striving to make it to the top of the field of science. May your dreams and ambitions be your driving force.

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List of abbreviations

AA	Arachidonic acid
ALA	Alpha-linolenic acid
ATD	The ratio of aquatic biomass to terrestrial insect biomass
ASD	The ratio of aquatic insect biomass to spider biomass
BHT	Butylated hydroxytoluene
C	Carbon (element)
DHA	Docosahexaenoic acid
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
FAA	Fatty acid analysis
FAME	Fatty acid methyl ester
GLM	General linear model
HUFA	Highly unsaturated fatty acid
LA	Linoleic acid
LC-PUFA	Long-chain polyunsaturated fatty acids
MUFA	Mono-unsaturated fatty acid
N	Nitrogen (element)
NB	Negative binomial
SASS	South African Scoring System (for aquatic ecosystem health)
SEAc	Size-corrected standard ellipse area
SI	Stable isotope
SIA	Stable isotope analysis
SIAR	Stable Isotope Analysis in R (software)
SIBER	Stable isotope Bayesian ellipses in R (software)
PUFA	Polyunsaturated fatty acid
UFA	Unsaturated fatty acid

Chapter 1. INTRODUCTION AND OVERVIEW

1.1. **Food web ecology and ecosystem connectivity**

1.1.1. Ecological boundaries

For epistemic purposes, the universe is characteristically subdivided into collections of interacting entities termed systems, the boundaries of which are defined to occur where characteristic variables change discontinuously. In ecology, interfaces are defined to occur between environmental patches where structural or functional system properties change discontinuously in space and / or time (Naiman and Decamps 1997). If these discontinuities were absolute, the system that they define would be closed. Thus, while ecology has traditionally focussed on the internal dynamics of ecosystems, cross-boundary interactions can fuel those dynamics. This thesis investigates the cross-boundary trophic interactions of a relatively small model ecosystem to explore their epistemic implications for systems ecology.

Traditionally, boundaries of ecosystems have simply been defined spatially and as being system-specific, but more recent concepts acknowledge the role of processes, functions, and related biological, chemical, and physical links between ecosystems (Cadenasso et al. 2003, Strayer et al. 2003). Habitat boundaries may be physical or simply conceptual boundaries created by humans (Strayer et al. 2003), and movements of nutrients, detritus and animals across them provide mechanisms for habitats to interact and consequently influence biodiversity and productivity, and form larger systems. Therefore, adjacent ecosystems have generally been studied separately as they have been considered to be distinct, discontinuous entities. However, adjacent ecosystems are usually linked, as nutrients and energy are exchanged across the habitat boundaries (Polis and Hurd 1996, Fariña et al. 2003). Polis et al. (1997) described the movements of organisms across boundaries as cross-boundary subsidisation, since predators, prey, and nutrients are brought to a recipient community, a concept termed *allochthony*. The sharing of organic and inorganic nutrients therefore links ecosystems and make them interdependent to some degree, which is why Newton's 2nd Law of Thermodynamics alone does not describe contemporary ecosystems, and also why ecosystems can become organised despite that law.

The exchange of energy and nutrients between adjacent environments affects food webs and community structure. Within some habitats, nutrients are found in low and inadequate amounts and consumers have to rely on subsidizations from adjacent habitats that have high amounts of the nutrients (Gladyshev et al. 2009a). The presence of such subsidies allows adjacent habitats to support larger populations and communities of consumers (Richardson et al. 2010). A good example of food web links can be seen between detritus and grazing food chains in terrestrial habitats that are linked by detrital subsidies/infusion (e.g. Polis and Strong 1996, Shimazaki and Miyashita 2005), where detritivores (e.g. dung-flies and woodlice) from the detritus food chain provide subsidies to generalist predators (e.g. spiders) in above-ground food chains. The above-ground community structure is ultimately affected, as the increase in subsidy may result in increased success or abundances of its predators and possibly top-down effects on selected prey species. Freshwater and terrestrial systems are also linked trophically in many ways. Insects that emerge from freshwater systems provide food subsidies to terrestrial predators such as birds, spiders and dragonflies, whilst some aquatic predators prey on terrestrial insects, and diets of detritivores are subsidized through terrestrial leaf litter fall (e.g. Nakano and Murakami 2001, Lynch et al. 2002, Baxter et al. 2005, Richardson et al. 2010, Wesner 2010). Therefore, cross-habitat nutritional subsidies are ecologically important and therefore worth studying.

Until recently, lateral links among neighbouring ecosystems did not attract much attention. Research on the dynamics of spatially subsidised food webs was strongly fostered by Gary Polis (Polis and Hurd 1996), but only with the development of isotopic and molecular techniques has this topic gained some popularity. Nevertheless, the number of systems examined in this respect remains very low. Either none or very few studies on the role of aquatic subsidy in terrestrial food webs have been performed in Africa. Furthermore, there is a need to further understand and quantify the reliance of riparian terrestrial arthropod consumers on aquatic food resources in South African river systems.

1.1.2. Riparian ecotones

In fluvial systems, the term riparian zone refers to the area that lies between the water course and the adjacent terrestrial upland (or hill slope) and is sometimes referred to as the “buffer zone”, “buffer strip”, “riparian management zone”, “floodplain” or “streamside management zone” (Gregory et al. 1991; Wenger 1999; Lee et al. 2004). Depending on the perspective of the author and user, definitions may vary. Naiman and Decamps (1997) defined the riparian zone as the area in the stream channel between the low and high water marks, and that portion of the terrestrial landscape from the high water mark to where vegetation may be influenced by elevated water tables or flooding and by the ability of the soils to hold water. The boundaries of the riparian zone extend outward to the limits of flooding and upwards into the canopy of the streamside vegetation (Gregory et al. 1991). These definitions by Gregory et al. (1991) and Naiman and Decamps (1997) offer further support that there are structural or functional discontinuities along ecological boundaries such as between rivers and the adjacent lands.

Riparian zones are conceptualized as ecotones with characteristic biological, physical and chemical interactions between terrestrial and aquatic ecosystems (Swanson et al. 1982; Gregory et al. 1991), as they play a large part in mediating the flow and characteristics of nutrients, energy and other materials from the land to the water and vice versa (Décamps et al. 2004). Riparian habitats essentially join rivers and adjacent upland. These zones possesses some of the biosphere's most diverse, dynamic and ecologically complex systems (Naiman et al. 1993) and may carry higher densities of consumers compared to terrestrial systems that are far inland, or offer individuals better growth opportunities, as a result of resource exchange between aquatic and terrestrial habitats (Naiman et al. 1993, Polis and Hurd 1995, Naiman and Decamps 1997, Fagan et al. 1999, Nakano and Murakami 2001, Power 2001, Sabo and Power 2002a). Riparian zones represent important corridors for the transfer of organic or inorganic nutrients to subsidize consumers in potentially less productive adjacent habitats.

There are three defining components of a riparian ecosystem: (1) geomorphology, (2) hydrology, and (3) biota (Kauffman et al. 1997). Much is now known about geomorphology and hydrology yet a great many biotic interactions still remain to be studied and modelled (Mertes 1997, McGlynn and Seibert 2003, Laeser et al. 2005, Queheillalt and Morrison 2006, Weissteiner et al. 2013). The trophic connections between rivers and riparian areas have been well documented in some climatic regions, in particular the Sonoran desert in the USA (Jackson and Fisher 1986, Sanzone et al. 2003), Mediterranean areas in Europe (Paetzold et al. 2005) and the USA (Wallace et al. 1997, Rundio and Lindley 2012) and the temperate forests of Japan (Nakano and Murakami 2001, Iwata et al. 2003, Kato et al. 2003, 2004, Iwata 2006). There are distinct plant and animal communities in these different regions, but the role of allochthonous inputs (e.g. leaf litter, terrestrial invertebrate in-fall and flooding) as energy sources for aquatic biota in rivers is widely recognized (Fisher and Likens 1973, Mathooko et al. 2000, Nakano and Murakami 2001, Doucett et al. 2007b). Much less is known about aquatic transfers to terrestrial habitats (Henschel et al. 2001, Kato et al. 2003, 2004, Sanzone et al. 2003, Paetzold et al. 2005, Spiller et al. 2010). Few or no connectivity studies have been carried out in Africa. Invertebrates are highly abundant along riparian (Sabo and Power 2002a, Iwata et al. 2003, Kato et al. 2003) and coastal interfaces (Polis and Hurd 1995, 1996, Dugan et al. 2003, Heck et al. 2008). Consequently, there are large amounts of cross-habitat nutritional subsidies that support high consumer abundances in riparian ecotones (Kato et al. 2003, Krell et al. 2015).

1.1.3. Riparian buffer zones

A riparian buffer zone refers to the area within an objectively defined distance from a stream. This zone is important for both ecological (Murphy et al. 1986) and managerial reasons. Management reasons include protecting the river from polluted runoff, sewage, excess nutrients from near-by farms (fertilizer runoff), siltation or eutrophication. Traditionally, determination of buffer widths has been based largely on local hydrology, soil factors, slope and other physical or chemical variables, as these are factors that relate to water run-off and thus influence the levels of nitrates entering a stream (e.g. Wenger 1999). In a literature review of riparian buffer widths in Georgia, Wenger (1999) suggested that buffer widths between 30 and 15 m are proficient in controlling nitrogen concentrations entering a stream. Similarly, a review on provincial, territorial and state guidelines in the USA and Canada reported mean buffer widths between 15.1 and 29.0 m for a wide range of rivers (Lee et al. 2004). Scientists have acknowledged the importance of river buffer zones in South Africa (Rouget et al. 2006), but clear protocols for defining appropriate buffer widths remain absent (Herdien and Collins 2011). In South Africa, the buffer zone has been viewed as a barrier protecting streams from pollution, nutrient loading and sediment deposition from upland areas, and yet the biotic (food web and community) aspects have been largely ignored.

Despite the widespread acknowledgement of the importance of buffer zones and the management reasons for conserving them, determination of system- or region-specific minimum buffer widths is needed, especially in countries outside USA and Europe. Scientifically-based guidelines are important for developing riparian buffer protection plans, particularly if ecological and biotic factors are to be considered. The width of the riparian zone and the size of the water body determine the extent to which this zone influences the water body (Naiman and Decamps 1997). In this thesis I aim to determine the relationship between the width of a river and the trophic connectivity between the river and the adjacent terrestrial upland. The data can be used to define riparian zone buffer widths based on trophic processes.

1.2. **Vectors and recipients of aquatic subsidies**

1.2.1. Aquatic insects: vectors of aquatic subsidies

Many aquatic insects emerge from the water as winged subimagos (sexually immature) or adults in fresh water systems (Wallace and Anderson 1996). When they emerge, some insects disperse laterally into the terrestrial system (Lynch et al. 2002), and they can represent a quantitatively important nutritional component of terrestrial consumer diets (Gladyshev et al. 2009b). Occasionally, aquatic insects emerge in thousands instantaneously, and these events are called mass emergence (Edmunds and Edmunds 1980). These mass emergence events can enable the riparian ecotone to support a high density and biomass of predators (Power and Rainey 2000, Iwata et al. 2003, Kato et al. 2003, Baxter et al. 2005) relative to habitats further inland (Janzen and Schoener 1968, Catterall et al. 2001, Lynch et al. 2002).

1.2.2. Riparian predators: recipients of aquatic subsidies

Regions characterized by high abundances of aquatic and terrestrial insects can support a variety of terrestrial predators. Many consumers are reliant on aquatic subsides: spiders (Paetzold and Tockner 2005, Burdon and Harding 2008), adult odonates (Baird and May 1997, Rivera 2005), beetles (Paetzold et al. 2005), birds (Nakano and Murakami 2001), bats (Fukui et al. 2006) and reptiles (Sabo and Power 2002a). In addition to providing food sources for terrestrial predators, aquatic insects may provide essential nutrients that are low or non-existent in terrestrial systems (Gladyshev et al. 2009a). Many riparian predators congregate at the water's edges where aquatic insect abundance is highest (Hering and Plachter 1997). Some predators such as odonates and frogs use the river as a breeding resource, but still take advantage of the high prey availability (Cappo 1987, Corbet 1999). Two types of consumers that congregate at the river for different reasons were the focus of my research. Web-building spiders have terrestrial origins and congregate at the river in response to the high numbers of aquatic insects present (Akamatsu et al. 2004, Marczak and Richardson 2007, Tagwireyi and Sullivan 2015), whilst odonates have aquatic larval stages and come to the river to reproduce and deposit their eggs (Corbet 1999).

1.2.2.1. Web-building spiders

Spiders are typically generalist predators that are abundant in most terrestrial ecosystems (Nentwig 1986, Nyffeler 1999, Snyder and Wise 2001), and spider populations are important top-down controlling agents for terrestrial insect communities through predator/prey interactions (Young and Edwards 1990). Spider feeding guilds, including ground-dwelling, plant-wandering and web-building forms, all prey on both aquatic and terrestrial insects, and my study focused exclusively on web-building spiders. Web-building spiders are appropriate for monitoring organic nutrient fluxes from aquatic to terrestrial habitats because the webs are effective (although not unbiased) aerial sampling devices that have direct biological relevance. Web-building spiders are the first-stop consumers of aquatic exports and they may relay the nutrients to their habitat/ecosystem.

Web-building spiders are described as prey searchers (rather than hunters) because they devote a large amount of time and energy to the construction of any single web (Peakall and Witt 1976). The webs allow the spiders to expend minimal amounts of energy until their prey establish direct contact with the webs and are captured (Peakall and Witt 1976, Prestwich 1977). The use of a web increases the effective range of the sensory system and appendages of the foraging spider, thereby allowing the spider to capture prey more efficiently and effectively than wandering spiders (Turnbull 1973). Studies involving web observations over time revealed that web-building spiders are generalist feeders that are almost strictly insectivorous, and they consume almost any insect that is captured by their webs (Robinson and Robinson 1970, Rypstra 1982, Castillo and Eberhard 1983, Nentwig 1983, Nyffeler 1999, Shimazaki and Miyashita 2005), particularly small flies and other insects much smaller than the spider. Many studies reported the orders Coleoptera, Hymenoptera, Lepidoptera, and Diptera as the most common prey in spider webs (Castillo and Eberhard 1983, Meraz et al. 2012). Essentially, web-building spiders can prey on all flying insects (including alates of Hymenoptera and Isoptera) that are small enough to be subdued, although signs of prey-selectivity, biases or preference (Nyffeler et al. 1989, Nyffeler 1999, Ludy 2007) have been detected in some species. Wise (1995) described spiders as model terrestrial predators because they are abundant and ubiquitous in terrestrial systems. As such, a study that aims to describe the trophic ecology of terrestrial consumers located near water must include spiders. Also, the web-building species act as natural sampling traps that can be used to investigate levels of aquatic subsidisation.

As a consequence of their generalist feeding nature and high abundance in riparian areas, web-building spiders collectively represent a major biomass of predators in these habitats. Spiders can control different prey populations through top-down forces (Henschel et al. 2001), and they play an influential role in insect community structure in general (Polis et al. 1997). Given that some spiders in ecotones can subsist at least partially on aquatic insects, they can play an important trophic role as the receivers of nutrients originating from aquatic systems. The trophic role of web-building spiders has been studied in some regions of the world (Kato et al. 2003, 2004, Paetzold et al. 2005, Iwata 2006, Rundio and Lindley 2012), and my aim is to assess the strength of the trophic connectivity between freshwater and terrestrial systems in relation to variables such as stream width, distance from the river and temporal variations.

1.2.2.2. Dragonflies and damselflies (odonates)

Odonates (dragonflies and damselflies) have life cycles and feeding habits that link aquatic and aerial environments. The nymphs are aquatic predators of benthic invertebrates, tadpoles, small fish, mosquito larvae and small crustaceans, while adults are aerial predators of small flying invertebrates such as mosquitoes, blackflies, midges, horseflies and deerflies (Corbet 1999). In addition to being generalists, adult odonates are regarded as opportunistic feeders as they can adjust their diets over short periods to take advantage of large concentrations of prey such as occurs during mass emergence (Neal and Whitcomb 1972, Baird and May 1997, Corbet 1999). In this sense they are similar to web-building spiders.

Odonates represent potentially significant avenues of organic nutrient transfers across ecotones, particularly from the aquatic system to the terrestrial system. Given that odonates in many riparian areas are considerably taxonomically diverse, it is not known whether prey selectivity takes place (Corbet 1999, Rashed et al. 2005). Foraging behaviour has been described in detail for many species (Corbet 1999), but it is unclear how the foraging behaviour relates with any diet differentiation among odonate species. Furthermore, individuals within the same species may display differences in diet when they are spatially separated (Corbet 1999). There may be a correlation between body size and the size (and type) of prey that odonates consume (Olberg et al. 2005). Moreover, sexes within the same species may exhibit different foraging behaviour as the males often harass the females and force them to live and forage in habitats far from the areas of high insect abundance (Sherratt 2001, Happold 2009, Fincke 2015).

Like spiders, odonates are very abundant in riparian areas and they could represent model terrestrial predators for addressing connectivity questions. As a result, I have focused on spiders and odonates in this thesis to determine the influence of aquatic subsidies to terrestrial systems. Adult odonates are highly mobile predators whilst spiders are sedentary. As such, the two groups of consumers represent riparian predators of aerial insects with contrasting feeding methods. Unlike other riparian consumers, odonates are highly abundant and easy to collect and identify (Corbet 1999). I refer to web-building spiders and odonates as predators of aerial insects (Paetzold et al. 2005), as they both consume flying insects in the riparian area.

1.2.3. Niche partitioning

Living in riparian areas affords terrestrial invertebrate consumers with a great diversity of prey from terrestrial and aquatic habitats. The presence of high prey abundances and diversity supports greater abundances and diversities of invertebrate consumers. As a result, niche partitioning occurs amongst locally coexisting predator species, and in some instances predators that are morphologically or taxonomically similar develop different feeding strategies. For instance, some web-building spiders build horizontal webs that enable them to capture small and weakly flying insects (Yoshida 1989, Williams et al. 1995, Marczak and Richardson 2007), whilst others build webs that are structurally more suited for catching larger, faster and stronger flying terrestrial prey (Olive 1982). In some cases, individuals within the same species may display differences in diet when they are spatially or temporally separated (Corbet 1999). It is important that the nature of variations among riparian predator taxonomic groups and guilds are understood because the predators act as the natural sampling devices of aquatic subsidies in riparian areas. The dynamics of trophic connectivity largely depend on the predator species present, and any cross-boundary trophic study has to consider trophic niche partitioning and feeding guilds. A few studies have described invertebrate predator niche partitioning in riparian areas (e.g. Kato et al. 2003, Grant et al. 2014), but even fewer of these studies have been carried out in Africa. Apart from competition for food resources, other ecological interactions like local extinctions and immigrations can contribute to niche partitioning (Pulliam 2000), but in riparian areas prey availability and competition for prey are likely to be the most important determinants of niche partitioning.

1.3. **Diet determination**

In the study of food webs within any ecosystem, one aim is to establish trophic relationships, usually through the identification of sources and sinks of organic matter, and this conceptual approach can be adopted for cross-boundary transfers as well. These trophic relationships are determined using various techniques, namely direct observations, inspection of gut contents, immunological techniques, stable isotope analysis and biochemical analysis.

1.3.1. Traditional methods

The oldest method of diet determination is through direct observations of feeding under natural conditions. Although tedious, field observations have been successfully used to trace energy flows from plants to primary consumers, and primary consumers to predators (Robinson and Robinson 1970, Rypstra 1982, Castillo and Eberhard 1983, Corbet 1999, Nyffeler 1999, Shimazaki and Miyashita 2005). Some researchers have mimicked consumer natural habitats and environment conditions and have investigated their prey preferences under experimental conditions (e.g. Wise 1979; Nentwig 1983). Direct observational studies have been ineffective in determining the diets of highly mobile and fast predators such as dragonflies and damselflies. As a result of this difficulty, much of the foraging knowledge on dragonflies and damselflies is based on opportunistic sightings (Corbet 1999).

Over the past few decades, gut content analysis has been the most commonly used diet analysis tool in trophic studies, particularly for larger consumers such as fish, birds and frogs (Hyslop 1980, Zacharia and Abdurahiman 2004). Through analysing and identification of gut/stomach contents, it has been possible to determine what an organism has been consuming. More recently, DNA-based detection of prey species has been used to accurately identify prey species that constitute the gut contents of the consumers (Welch et al. 2014). DNA-based methods have been particularly advantageous for small invertebrates like spiders that digest externally, because when the form of the prey is lost the prey items become morphologically unidentifiable.

1.3.2. Tracer techniques

The most commonly used diet tracer techniques in ecology are fatty acid analysis (FAA) and stable isotope analysis (SIA), and increasing numbers of studies are incorporating more than one tracer technique to increase the scope and scale of information produced for a particular community. Fatty acid and SIA offer accurate and time-integrated estimations of consumer diets, hence producing more information than direct observations and gut content analysis methods (Dalsgaard et al. 2003, Budge et al. 2007, Parnell et al. 2010).

1.3.2.1. Fatty acid analysis

Much of the trophic research utilising fatty acid (FA) composition has focused on marine systems (Parrish et al. 2000). Freshwater systems are not as well studied, although organisms in these habitats can contain appreciable quantities of essential FAs (EFAs) of similar composition to marine organisms (Ackman 1999). However, over the past 20 years, lipid research in freshwater systems has seen increasing attention. The advantage of using lipids in trophic ecology lies in their heterogeneous nature, which allows information to be gathered by tracing the path of individual and groups of FAs through food webs (Parrish et al. 2000). Aquatic systems yield greater amounts of the physiologically important EFAs that tend to be limiting in the terrestrial environment (Gladyshev et al. 2009a). EFAs are not manufactured by many consumers, so they must be obtained largely from the diet and can therefore be traced from producers through to upper levels of food webs (Brett and Müller-Navarra 1997). Subsidies from aquatic habitats may be vital for nourishing life in terrestrial habitats, and FA content can represent an ecologically-relevant measure of flux by revealing the sources, sinks and trophic pathways of organic nutrients (Gladyshev et al. 2009b, Pollierer et al. 2010).

1.3.2.2. Stable isotope analysis

Isotopic compositions of natural materials change in predictable ways as elements cycle through the biosphere (Peterson and Fry 1987), and these variations have been exploited by ecologists to trace the path of nutrients in ecosystems. Nutrient ecology therefore represents a field in which SIA has found widespread applications (Thompson et al. 2005). Stable isotope analysis specifically looks at the relative enrichment of heavier isotopes in consumer tissues relative to their food. Like FAs, SI data can provide source-to-sink information on food webs (Peterson and Fry 1987) when the carbon sources have distinct isotopic signatures (Parnell et al. 2010). As such, isotopic signatures can be used to draw conclusions about carbon cycling and transfers of material within and between habitats.

Over the past two decades SI ratios, especially of those of carbon and nitrogen, have been increasingly used to trace the movement of organic nutrients between freshwater and terrestrial systems (e.g. Kato et al. 2004, Doucett et al. 2007, Seifert and Scheu 2012) and between marine and adjacent terrestrial systems (e.g. France 1995, Anderson and Polis 1998). The values of $\delta^{13}C$ are largely conserved in food chains, and provide information about the identity of the energy base, whereas the nitrogen signature of an organism provides a time-integrated value of its trophic position (Vander Zanden and Rasmussen 1999, Ponsard and Arditi 2000). However, there is evidence that different tissues can have different fractionation and turnover rates of stable carbon isotopes, thus different isotopic values (Tieszen et al. 1983).

More metabolically active tissues such as liver and fat have more rapid turnover rates than less metabolically active tissues such as hair (Tieszen et al. 1983). Several researchers have successfully utilised stable isotope techniques to demonstrate that aquatic and terrestrial habitats are connected through reciprocal movements of organic nutrients (Anderson and Polis 1998, Sanzone et al. 2003, Kato et al. 2004, Laeser et al. 2005, Paetzold et al. 2005, Doucett et al. 2007b). Geometric (e.g. Whitledge and Rabeni 1997, Szepanski et al. 1999) and Bayesian-based (Moore and Semmens 2008) mixing models have been developed to estimate the proportions of prey or food items in the diets of consumers (Hobson 1993, Koch et al. 1995, Iverson et al. 2004). Depending on the system examined and the basal signatures of different prey and consumers, species-specific details cannot always be produced, so it is becoming more common for SIA to be used in combination with other diet determination techniques. The narrow dynamic ranges of both C and N can be limiting, especially in situations when there is insufficient separation between basal sources. To a lesser extent, other isotopes such as hydrogen and sulphur have been used to investigate animal diets (McCutchan et al. 2003). Stable hydrogen isotope ratios can potentially be used to quantify water source use by measuring the isotopic composition of body water and the source end points (McKechnie et al. 2004).

Stable isotope and FA signatures serve as ideal complementary techniques in the investigation of food web dynamics (e.g. Allan et al. 2010, Bergamino et al. 2014). Due to biological and ecological complexities, some organisms may have different diets that are not distinguishable using either FA or SI indicators. For example, SI signatures in some insects shift between aquatic larval and terrestrial adult stages towards those of other terrestrial insects, hence these aquatic insects become isotopically indistinguishable from terrestrial insects (Alp et al. 2013). As such, predation of adults of such prey by fish cannot be distinguished from purely terrestrial prey (Alp et al. 2013). In these cases, the use of both FA and SI methods results in more reliable diet estimations. In this thesis, both methods were incorporated to increase the breadth of information derived from the samples collected.

1.4. **Questions**

The research questions of this study are as follows;

1. *Is there variation in access to aquatic subsidies and is niche partitioning evident amongst aerial predators?*
2. *Does the access of aerial riparian predators to aquatic subsidies vary with distance from the river?*
3. *Does the strength of freshwater-terrestrial trophic connectivity vary along gradients of physical characteristics and aquatic prey subsidies?*

1.5. **Thesis overview**

The objective of this thesis was to investigate the roles of aerial predators as recipients and indicators of cross-boundary subsidies to assess the strength of the trophic connectivity between freshwater and terrestrial systems in relation to variables such as stream width, and aquatic insect emergence rates and abundances. Many aspects of the river can affect insect productivity, but stream width (which is easier to measure) was singled out to show that different parts of the same river could have varied cross boundary interactions that are directly linked to aquatic insect productivity. Two common recipients of aquatic subsidies in riparian areas, web-building spiders and odonates (dragonflies and damselflies), were chosen to represent riparian aerial predators because they were highly abundant in the study area, the Kowie River catchment. Web-building spiders and odonates also represented biological sampling devices that were used to trace the movements of aquatic nutrients in the terrestrial system. However, they represent different sampling methods with different prey capturing strategies, as spiders catch insects passively during both night and day, whilst odonates catch insects only during the day. Spiders were used to determine buffer zone parameters of the riparian region, whilst odonates were used to investigate variations in riparian predator access to aquatic subsidies. It was not possible to use odonates to determine buffer zone parameters because they are highly mobile predators that are not always confined to the riparian area. Unlike odonates, web-building spiders are relatively sessile and can therefore exclusively represent riparian predators.

The ecological theory of cross-boundary subsidisation and literature on web-building spiders and odonates are reviewed in *Chapter 1*. Detailed descriptions of the study sites and methods are given in *Chapter 2*. Aquatic insect dispersal with increasing distance from the river was tracked using direct observations of cross-subsidies, as documented in *Chapter 3*. The relationship between the abundances of aquatic insects and web-building spiders were tested in *Chapter 3,* together with the roles of environmental and habitat conditions in structuring spider communities. This thesis was based on the assumption that there were diet-associated taxonomic variations among riparian predators and their potential prey. In *Chapter 4*, this assumption was tested by assessing variation in FA and isotopic signatures amongst web-building spider and insect prey species. Before effects of aquatic subsidies were investigated in later chapters, diet niche partitioning was investigated amongst two major web-building spider guilds, the horizontal and vertical web-building spiders, and between insects of aquatic and terrestrial origin. Fatty acid indicators of aquatic origin were determined through assessing FA compositions of aquatic and terrestrial insect groups. In *Chapters 5* and *6*, SI and FA indicators of cross-subsidies were used to investigate the spatial and temporal extents of aquatic subsidies to web-building spider diets at three sections of the river with varied stream widths. The relationships between the FA and SI indicators and empirical data of aquatic insect abundance (from *Chapter 3*) were investigated in the respective chapters. In *Chapter 7*, niche partitioning and the extent of cross-boundary dietary subsidies were described for odonate species and groups. Fatty acid and SI niche partitions were used to illustrate odonate diet niches. As such, the extent of aquatic subsidisation to each feeding guild was described. In *Chapter 8*, empirical findings from all the chapters were synthesized and conclusions made about cross-subsidies in the study area relative to established ecological concepts.

Chapter 2. STUDY SITES, SAMPLE COLLECTION AND LABORATORY METHODS

2.1. **Study Area**

2.1.1. The Kowie River

The Kowie River is situated in the Eastern Cape Province in South Africa and flows for approximately 70 km (Heydorn and Grindley 1982, Uys and O'Keeffe 1997) from the hills of Grahamstown Heights and into the Indian Ocean on the south-east coast of South Africa (Figure 2-1). The Kowie River tributaries drain the major part of the Bathurst region (Figure 2-1) and the major tributaries are the Bloukrans, Brakrivier and Lushington (or Torrens) Rivers. The estuary extends about 20 km upstream from the river mouth, where the river banks have been canalised (Watling and Watling 1983, Whitfield et al. 1994), and it is tidal throughout its entire length (Hill 1975, Whitfield et al. 1994). The river is a non-perennial, aseasonal, intermittent stream (Uys and O'Keeffe 1997), as the flow is unpredictable due to the heterogeneous temporal distribution of rainfall in the region. The climate in the Kowie region is temperate, although moderate droughts and floods are relatively common (Heydorn and Grindley 1982). Most of the rainfall occurs in spring and autumn and annual rainfall estimates range from 500 to 600 mm (Heydorn and Grindley 1982). Between 2006 and 2012, annual estimates from a single station (Bathurst) along the Kowie River ranged between 500 and 1000 mm (Department of Water Affairs 2013). A large part of the Kowie catchment is made of Bokkenveld shale and sandstone, which weather away relatively rapidly and allow the river course to cut deep into the rock, forming valleys (Heydorn and Grindley 1982). The river has a catchment area between 576 and 769 km^2 and the topography is one of a gently sloping plain with undulating hills (Heydorn and Grindley 1982, Schumann et al. 2001). Mean annual runoff is between 23 and 46 x 10^6 m^3, giving an average freshwater inflow, exclusive of floods, of less than 1 m^3s^{-1}. The flows are lowest during summer (Heydorn and Grindley 1982).

Terrestrial vegetation in the catchment has been described by Heydorn and Grindley (1982) and was classified into five main mapping units that include Hummock Dune Vegetation, Warm Temperate Coastal Forest, Sub-succulent Woodland, *Acacia karroo* Bushclump, and a vegetation complex between Coastal Woodland and Forest Scrub. Large plots of invasive lantana (*Lantana camara*) and wattle are common features of the landscape. Other alien species such as *Eucalyptus globulus* and *Acacia cyclops* are also common. The Kowie River generally has vegetation that is typical of other Eastern Cape systems (Heydorn and Grindley 1982). Much of the vegetation in the Bathurst area has arisen because the original thicket vegetation was cleared for pastures (Puttick et al. 2011).

The Kowie River catchment contains three towns: Grahamstown in the headwaters, Bathurst substantially downstream, and Port Alfred at the mouth (Figure 2-1). The Bathurst area has a long record of use dating back to 1825. It has been used for timber extraction, grazing, agriculture and human settlement (Puttick et al. 2011). Over the past few decades, much of the catchment has been made up of privately owned farms that produce pineapples, chicory, citrus, fodder crops, beef cattle or goats, but more recently there have been large tracts of land near the river designated for wildlife management, conservation and ecotourism (Zietsman and Van Der Merwe 1981, Stickler 2010).

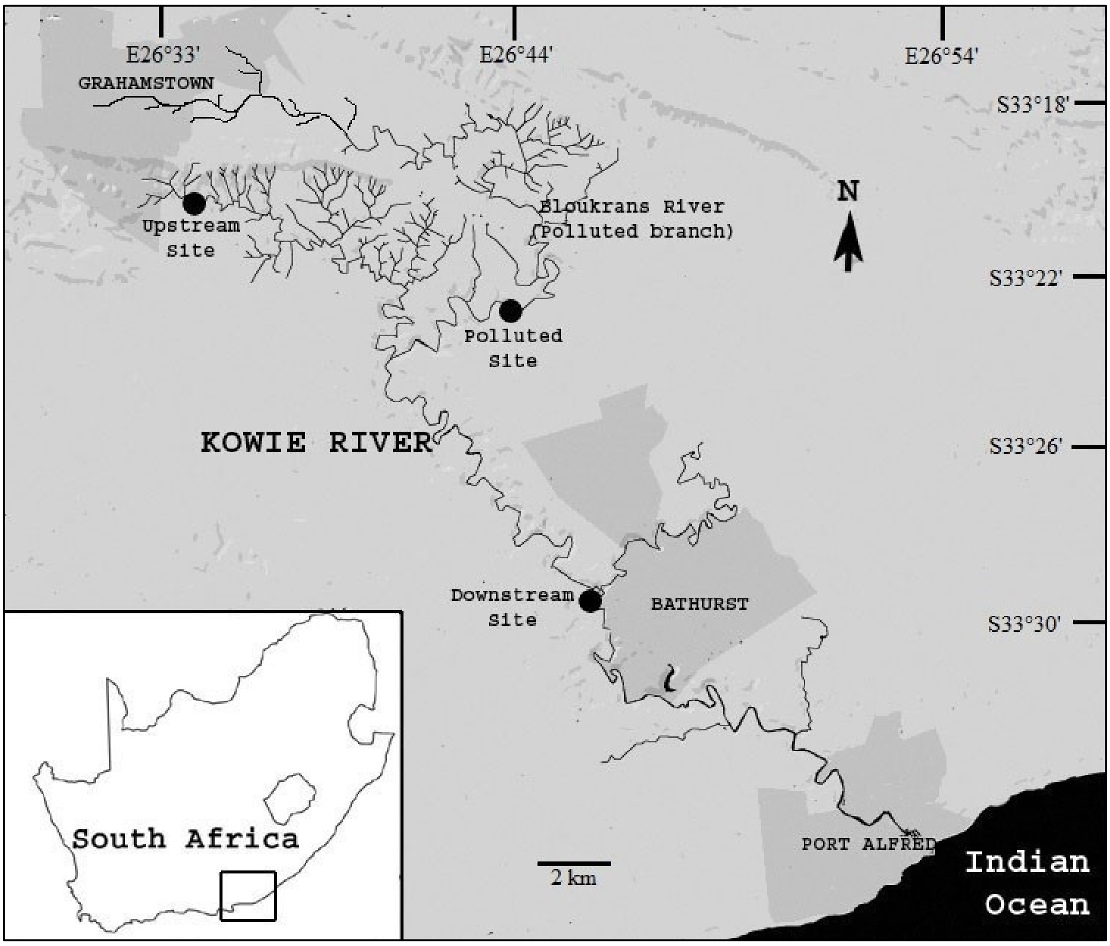


Figure 2-1: Study area map showing the Kowie River and its catchment. The large black dots represent the sampling sites.

2.1.2. Study sites

Three sites along the Kowie River were chosen in line with the research objectives, whilst taking into account physical accessibility. A major flood event took place in the Kowie catchment region between October and November 2012. All field collections took place after this event and the chosen sites were somewhat altered (signs of uprooted and broken vegetation) compared with pre-flooding conditions. The sites were located upstream (a narrow section of the river) and downstream (a wide section of the river) along the main Kowie channel, and an additional site was selected in the Bloukrans River (a tributary of the Kowie River, the intermediate site; Figure 2-1).

2.1.2.1. Upstream (narrow) site

The site that contained the narrow section of the river, the narrow site, was on a privately owned farm (33°20'57.32"S, 26°33'36.26"E) near Grahamstown. At this site, the river channel meandered and the water was usually brown and murky, with brown filamentous algae occurring on boulders in the shallowest parts. Pebbles of different sizes and occasional boulders formed the shallow river bed. The channel was relatively narrow (1.49 ± 0.36 m), shallow (10 - 45 cm) and covered overhead by a large amount of terrestrial vegetation canopy (71.92 ± 16.22%) (Figure 2-2).

Figure 2-2: The narrow, shallow, heavily-shaded channel at the narrow site, with dense cover of *Eucalyptus globulus* (photo taken February 2014). The river valley was relatively flat on the

left (SW) side, but the right side was bordered by a steep river cliff. Almost half of the riparian vegetation was made up of two invasive plant species, lantana (*Lantana camara)* and black wattle (*Acacia mearnsii*), and the rest of the vegetation was made up of a few indigenous species such as *Vachellia karroo.* The river was bordered by large blue gum trees (*Eucalyptus globulus*) and bushes of *L. camara* that formed a dense canopy cover over the stream. The area was appropriate for the investigation because it displayed properties of a typical upstream site (Mallik and Richardson 2009), with a narrow, shallow, heavily-shaded river channel.

2.1.2.2. Downstream (wide) site

The site that contained the wider section of the river, the wide site, was located in the Bathurst area (33°30'16.04"S, 26°44'41.06"E) and it was surrounded by game and cattle ranching farms. Wire fences ran parallel to the river on both sides at distances of about 80 m on the left (SW) and 40 to 50 m on the right (NE). A bridge at one end of the site impeded water flow, creating a large, deep pool upstream of the bridge and a fast flowing rapid after the bridge as water was channelled through concrete pipes. This section of the river was relatively straight and wide (stream width = 11.28 ± 4.90), with a deep channel (6 – 14 cm) open to the sky (canopy cover = 24.17 ± 32.07%) (Figure 2-3).

Figure 2-3: The wide, open and deep channel at the wide site (photo taken February 2014).

The river valley sides had gentle slopes that ran into the riparian area and on the right (north-east) side of the river there was a large hill about 50 to 60 m from the river. The riparian vegetation largely consisted of dense bushes and occasionally a tall tree. Some of the plant species present included Karoo Thorn (*Acacia karoo*), Pondo Turkeyberry *(Canthium vanwykii)* and Wild olive (*Olea europaea*). The left (south-west) side had very dense vegetation that was impenetrable, whilst the right (north-east) side had relatively less vegetation.

The river valley sides had gentle slopes that ran into the riparian area and on the right (NE) side of the river there was a large hill about 50 to 60 m from the river. Some of the plant species present included Karoo Thorn (*Vachellia karroo*), Pondo Turkeyberry *(Canthium vanwykii)* and Wild olive (*Olea europaea*). The left (SW) side had very dense vegetation that was impenetrable, whilst the right (NE) side had relatively less vegetation.

2.1.2.3. Polluted (intermediate width) Tributary site

The Bloukrans River is a polluted low-order tributary that flows into the Kowie River (Figure 2-1). This water is largely sourced from runoff from Grahamstown, and municipal waste water effluent is released into the head waters in addition to pollutants arising from local farms and mines (Roux and Oelofse 2010, Jacobs et al. 2012). The site (33°23'28.49"S, 26°42'28.37"E) was moderately wide (5.5 m) but shallow (45 – 95 m), with an intermediate amount of vegetation cover (43.67 ± 26.08%; measurements taken during the duration of the study). As at the narrow site, the river bed was made of numerous pebbles of different sizes. Much of the ground in the riparian area was covered densely with kikuyu grass (*Pennisetum clandestinum*) for a distance of about 50 m from the river channel. Cape Gooseberry (*Physalis peruviana),* Blue Kuni-bush (*Searsia glauca*), Karoo Thorn *(Vachellia karroo),* Pondo Turkeyberry (*Canthium vanwykii*) and Wild olive (*Olea europaea*) were present.

2.1.3. Study design

The study was designed to capture spatial and temporal variations in web-building spiders' diets, abundance, richness and community structure, as well as variations in odonates' diets. Due to time constraints it was not possible to replicate the study in other river systems, so three sites were thoroughly surveyed in the Kowie River. To quantify subsidies to terrestrial consumers, numerous invertebrate collections needed to be carried out four times during one year. Due to the diversity of invertebrate consumers and prey, collections had to be done using a diversity of sampling methods during different times of the day. Invertebrate samples were taxonomically keyed and processed for various diet analysis procedures. Consequently, some limitations in spatially replicating the study are likely to limit the transferability of the results beyond the Kowie River. The broader conclusions of this study are therefore largely based on correlative dependencies rather than statistically proven causal relationships. The intermediate sized site has a history of pollution input, but the effects of pollution on trophic connectivity could not be investigated due to the lack replication of this factor. The following was done to answer the research questions:

Question 1 (*Is there variation in access to aquatic subsidies and is niche partitioning evident amongst aerial predators?)* - The riparian areas at the study sites were surveyed intermittently (during calm and dry days) during the years 2012 - 2013 to determine seasonal changes in diets of the web-building spider fauna. Odonates were collected during the summer months of 2012 to 2013. Each site was defined as a river stretch 50 m long and its adjacent terrestrial habitat up to 100 m from the channel (Figure 2-4). The starting assumption of this study was that as an evolutionary mechanism of coexistence, there would be niche partitioning among aerial predator species. Fatty acid (FA) and stable isotope (SI) tracer techniques were used to investigate niche partitioning amongst odonate and web-building spider taxa, at all the study sites. Thereafter, the extent of diet subsidisation to predator groups or guilds was tested.

Question 2 (*Does the access of aerial riparian predators to aquatic subsidies vary with distance from the river?*) **-** To investigate the relationship between the levels of aquatic subsidisation to web-building spider diets and the distance from the river, web-building spiders and their potential prey were collected along a distance gradient away from the stream into the adjacent terrestrial upland. A transect-based approach was used to identify any trends in abundance and diet that occurred with increased distance from the stream. Increasing distance from the fresh water was hypothesized to coincide with gradual changes in prey availability. Previous studies on aquatic insect dispersal have identified distances of less than 50 m as the outermost limits of dispersal of many emergent insects (Sanzone et al. 2003, Jonsson and Wardle 2009), although more recent studies report distances up to five kilometres (Muehlbauer et al. 2014, Kautza and Sullivan 2016). Nevertheless, I assumed that 64 m was beyond the outermost limit of influence by aquatic subsidies because during preliminary inspections (the pilot study), aquatic insects were very rarely seen beyond this distance. Along each 64 m transect, seven smaller transects perpendicular to the stream were sampled using geometric progression, with increasing inter-transect distances away from the freshwater [Figure 2-4; 0 m, 2 m (2^1), 4 m (2^2), 8 m (2^3), 16 m (2^4), 32 m (2^5) and 64 m (2^6)]. Each transect was 2 m wide and 10 m long and consisted of twenty 1 m^2 plots. The criterion for selection of sampling transect locations was dependent on accessibility, so the transects were more or less scattered along the distance gradient (not in a straight line; Figure 2-4). The transects were placed on the most accessible side of the river.

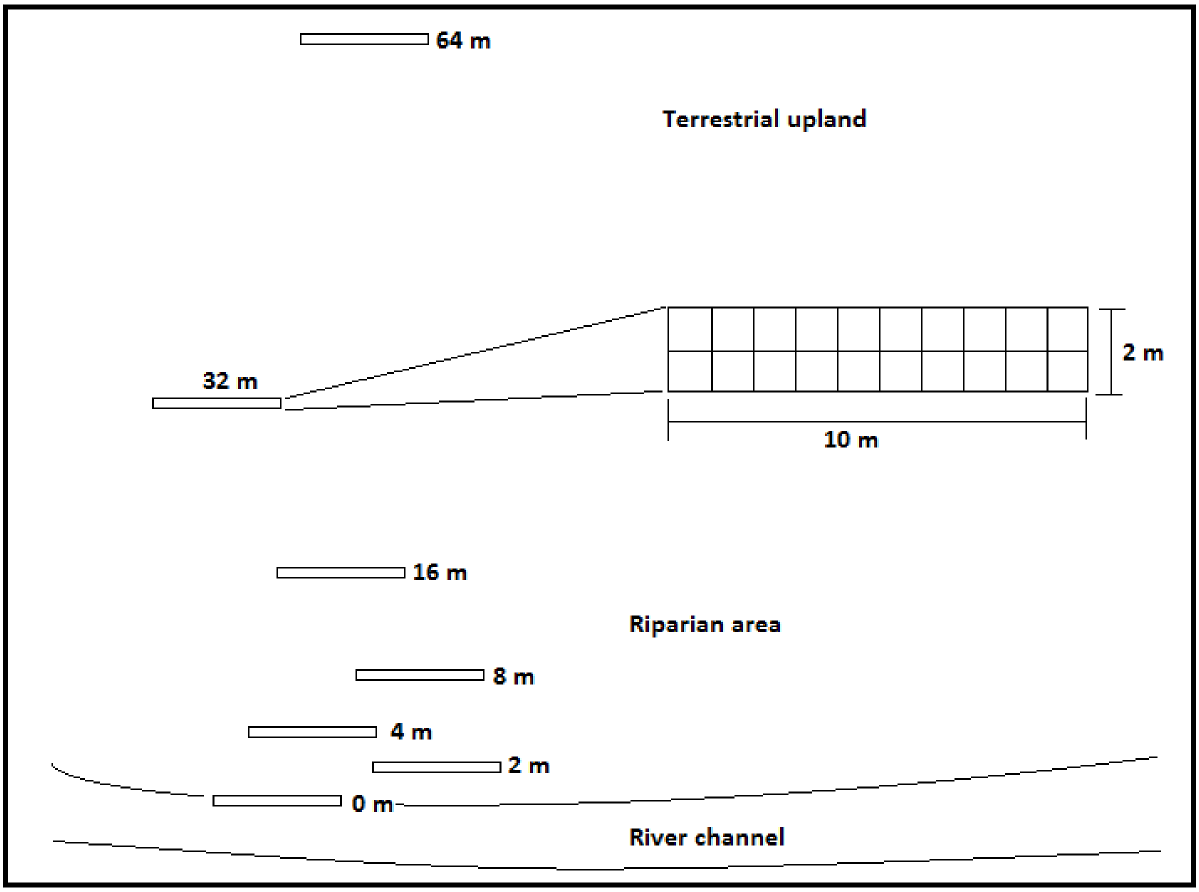


Figure 2-4: Representation of the layout of sampling transects. Each transect was 10 m x 2 m (20 m^2) and consisted of twenty 1 m^2 plots.

Question 3 (*Does the strength of freshwater-terrestrial trophic connectivity vary along gradients of physical characteristics and aquatic prey subsidies?*) - To investigate the influence of stream width on the extent of aquatic subsidisation to terrestrial consumers, sampling took place in three sites with different stream widths, a narrow, wide and intermediate sites on the Kowie River. To investigate temporal shifts in aquatic insect subsidisation, spiders and insects of aquatic and terrestrial origin were collected four times during the year: in November 2012 (summer) and March (autumn), June (winter) and October 2013 (spring). Thereafter, the relationships between aquatic insect abundances and FA/SI compositions were tested against those of web-building spiders. The strength of trophic connectivity was quantified by assessing the distance to which trophic subsidies moved into the terrestrial habitat and the relative dietary contributions of aquatic subsidies to riparian predators across sites with varied stream widths.

2.2. **Field Collections and observations**

2.2.1. Habitat characterisation

To define all available microhabitats, each site was surveyed and categorized into one of three zones: river, riparian or terrestrial upland. Spider microhabitats were defined in terms of micro-climate [temperature (°C), relative humidity (%) and wind speed (km/hr)] and habitat complexity [plant volume (m^3), plant density and number of web attachment structures (contacts)]. All measurements were carried out during the summer months of 2012-13.

2.2.1.1. Micro-climate

Quick-set push-button maximum - minimum thermometers (Brannan) were used to record air temperatures (°C). Each thermometer was tied to tree branches at a height of 1.5 m from the ground (to prevent reading ground temperature) and covered to prevent direct sun exposure. To provide replicates, three thermometers were setup in each transect, one at either end and one in the middle. Thermometers were left out in the field for 5 to 7 days during the period of invertebrate collections. Daily minima and maxima for each transect (3 replicates) were pooled and compared to successive transects with distance from the stream.

Due to the expense of meteorological instruments, a low-cost data logger (Major Tech - MT662) was used to record instantaneous humidity during times of the day when humidity was assumed to be at its highest (night) and lowest (midday). The data loggers were hung on tree branches 1.5 m above the ground, and left undisturbed for 10 minutes in each transect. A hand-held digital anemometer was used to measure instantaneous wind speed (Kestrel 3000 Pocket Weather Meter). The anemometer was tied to vegetation branches and left for five minutes to record the maximum wind speed during the five minutes. To get a single average value per transect, these measurements were repeated three times and averaged.

2.2.1.2. Habitat complexity

To estimate number of web attachment structures (contacts), calculator-generated random numbers were used to select 5 out of 20 1 m^2 plots (Figure 2 - 4) from each transect. At the centre of the selected plots, a 2 m stick was positioned vertically (perpendicular to the ground, except on slopes) and the number of plant leaves or any plant material contacting its length was recorded. The average number of web attachment structures (contacts) for each transect was then calculated to get a single value for each transect (Balfour and Rypstra 1998, McNett and Rypstra 2000).

Linear measurements of height and minimum and maximum plant width were recorded to determine the transect volume occupied by plants within the plots. The volume of plants less than 2 m in height was estimated using the formula of a basic ellipsoid volume (Thorne et al. 2002):

$$V = \frac{2}{3}\pi h(\frac{a}{2} * \frac{b}{2})$$

where **h** is the height of the plant from the base to the top, and **a** and **b** are the maximum (linear dimension on the major semi-axis) and minimum (linear dimension of the minor semi-axis) widths of the plant, respectively. The volume of large tree stems was estimated by treating the stem as a cylinder and using the formula of finding the volume of a cylinder:

$$V = \pi r^2 h$$

where **r** is the average radius of the stem and **h** is the height of the stem.

Vegetation density was measured and estimated at 10 cm and 80 cm from the ground. Density was represented by the vertical area occupied by any plant material (Diehl et al. 2013). In each transect, a white wooden board measuring 1 m x 1 m was placed vertically at each height and a photograph of the board was taken 2 m from it (Figure 2-5). The procedure was replicated twice in each transect, at each height, resulting in a total of six photographs per transect (3 at 10 cm and 3 at 80cm). The boards were placed equidistant from each other in each transect. The photographs were analysed by using image editing software, Photoshop 7 (Adobe, Systems, Munich, Germany), and the area of the board covered by any plant material was measured and recorded in pixels. Given that the area (in m^2) of the board was known, the area covered by plant material was converted to square meters, and the resultant value represented the vertical vegetation density. Average values were obtained for each transect.

2.2.1.3. Site characterization

One of the aims of the study was to investigate the effect of stream width on spider diets. A measuring tape was used to measure the distance across the river, from bank to bank and at bank-full height. Three measurements, 5 m apart, were taken and an average value was obtained at each site. A spherical densitometer was used to estimate the amount of vegetation cover over the stream. Vegetation cover was expressed as the percentage of the sky that was obscured by plant material.

Figure 2-5: A 1 m x 1 m white board raised to 80 cm from the water surface. The board was placed behind the vegetation to estimate plant density (photo taken in February 2013).

2.2.2. Spider collections

Spider collections were restricted to members of the web-building guild, particularly the aerial builder families Araneidae, Uloboridae, Tetragnathidae, Theridiidae and Linyphiidae. Since web-building spiders rarely crawl on the ground and are restricted to their webs and the super-strata, active searches (aerial hand collections) were the most appropriate and conservative sampling method (Green 1999). Active searching is considerably less destructive to invertebrate microhabitats relative to methods like beating (McCaffrey et al. 1984, Sutherland 2006) and fogging (New 1998) that disturb or kill non-target invertebrates (Sutherland 2006). Spiders were therefore collected using the hand-to-jar technique (Whitmore et al. 2002) and a mouth suction sampler [pooter; (Sutherland 2006)] was used for the smaller species such as those of the family Linyphiidae.

Searches were carried out on a catch-per-unit-area basis to gain information on different habitats. Time and effort were standardized in each transect, once each season and the same transects were used in subsequent sampling trips (each season) mainly due to the difficulty in finding physically accessible sampling transects in the dense vegetation. In each season, at each site, intensive searches for web-building spiders were done for 10 minutes by two collectors within each transect once during the day and once at night, making a total of 40 minutes in each transect. Night collections were necessary as many spiders are nocturnal (Leslie et al. 1990, Green 1999, Willett 2001) and a head torch was used to provide lighting. Day collections took place between 08h00 and 15h00, whilst night collections were carried out between 18h00 and 22h00. Due to the difficulty in collecting spiders in out-of-reach heights, searches were restricted to a maximum vertical height of 2 m. Collections were carried out on climatically stable days and nights, when most web-building spiders forage (Henschel et al. 2001).

A commercial hand-operated water sprayer (for ornamental plants) was used to increase the visibility of spider webs. The smaller spiders were collected into 2 ml microtubes whilst the larger individuals were collected into 25 ml glass vials, one per tube to prevent post-capture predation or cannibalism. Vials were labelled and stored on ice until they reached the lab where they were sorted, identified, and stored at -80°C pending lipid and SIA. Spiders were identified to the lowest taxonomic level possible, which was the genus level in many cases. Juveniles were excluded from the analyses due to difficulties in identification.

2.2.3. Insect collections

2.2.3.1. Qualitative sampling

To estimate predator diets using FAs and SIs, tracer data for all of the potential prey must be obtained. Consequently, all potential aerial prey of spiders and odonates were collected wherever possible. A portable backpack D-Vac (Dietrick 1961) suction sampler was used to collect flying insects that rested on plants, particularly small species that were difficult to observe with the naked eye (Figure 2-6; Sutherland 2006). The D-Vac sampler was equipped with a circular nozzle of diameter 17.5 cm, which was lowered over the area to be sampled. A sweep net with 0.5 m diameter and a 1 m handle was used to catch larger invertebrates such as many of the Odonata, Lepidoptera, Hymenoptera and Hemiptera. Many of the larval stages of aquatic flying insects were collected using SASS nets (Dickens and Graham 2002) during a parallel study (Moyo 2016). Due to diurnal variations in insect activity (e.g. Svensson and Petersson 1995, El-Moursy et al. 1999), collections were carried out three times a day (morning, afternoon and evening).

Figure 2-6: A D-vac suction sampler used to collect flying insects resting on plants in the riparian landscape at the wide site (Photograph was taken May 2013).

To account for the nocturnal insect species, collections were also carried out at night using a light trap. Light traps are the most commonly used method for sampling moths as they collect the widest range of species of moths of any trapping technique (Oxbrough et al. 2012). They are also effective at attracting aquatic insects such as mayflies and caddisflies (Kovats et al. 1996). The traps consisted of one black and one white fluorescent light placed in front of a white sheet measuring 2 m by 2 m to increase visibility. The black light emitted ultraviolet light that attracted insects more than white light. White fluorescent light was used to pick out insects that were resting on the white sheet. To collect insects from both the river and the terrestrial upland, two light traps were simultaneously deployed at each site, one at the river edge and another at 40 m from the river. Trapping was carried out only on nights when there was no rainfall and wind, and the traps were deployed between 20h00 and 00h00.

The potential prey (mostly flying insects) of spiders and odonates were categorised as having a terrestrial or an aquatic origin. The term ‘‘aquatic prey’’ is used for taxa with aquatic larval and terrestrial adult stages (e.g. Paetzold et al. 2006) and "terrestrial prey" is used for taxa having larvae that develop in the terrestrial ecosystem. I assumed that most insects that emerged from the water did not feed after emergence into the terrestrial upland, and that the isotopic/FA signatures of the adults were not different from those of the larval stages. These are reasonable assumptions because adult mayflies, for example, do not feed in the terrestrial environment since they have non-functional mouthparts and they rely on reserves built up during their nymphal life stage (Brittain 1982). There are some reports of adult caddisflies (e.g. Petersson and Hasselrot 1994) and true flies (e.g. Burtt et al. 1986) feeding after emergence. However, recently emerged adults should resemble their larvae since isotope/FA signatures take time to turn over once a different diet is established (e.g. Alp et al. 2013), although metamorphosis in some taxa with complex life cycles may affect SI signatures through biochemical and physical processes which might lead to isotopic fractionation (Alp et al. 2013). Therefore, SI and FA data (Moyo 2016) of larvae were used instead of those of aquatic adults that are difficult to catch in sufficient numbers without using traps that often contain substances (e.g. alcohol) that alter the signature of the insect. Pyramidal emergence traps are commonly used with success and with no use of substances, but terrestrial insects are more difficult to catch without alcohol or other chemicals.

The aquatic insects collected (Moyo 2016) included the mayflies (Ephemeroptera), which consisted of the families Baetidae and Leptophlebiidae, the caddisflies (Trichoptera) of Hydropsychidae, the true flies (Diptera) of Chironomidae and Simulidae, and the damselflies (Odonata) of Coenagrionidae. The terrestrial insects collected included a diverse range of flying insects in the orders Diptera, Hymenoptera, Lepidoptera, Coleoptera, Hemiptera, Isoptera (= Psocodea), Orthoptera and Phasmida.

2.2.3.2. Quantitative sampling

Trapping effort for insects was standardised to enable temporal and spatial statistical comparisons (Sutherland 2006, Ludy 2007). To avoid sampling invertebrates not typically preyed on by web-building spiders, collections were made in roughly the same manner that the predators use to acquire prey. A Malaise trap (Owen and Chanter 1970) with a vertical barrier or flight interception of 1.80 m long and 1.40 m high (Campos et al. 2000) was used (Figure 2-7). A Malaise trap has the advantage that it does not use any attractants and so ensures that only individuals in the immediate area (transect) or habitat are targeted. A single trap was placed at the centre of each of the transects and left for seven days, after which the collecting unit was removed and the contents (insects in 70% ethanol) were poured into a labelled container.

Figure 2-7: One of the study transects showing a Malaise trap and a maximum-minimum thermometer suspended in shade.

The numbers of insect prey captured did not indicate the population sizes within the entire community, but rather a measure of change in the relative abundance of species over space and time.

There is no method that captures all insect families with the same efficiency during different times of the day. Although the Malaise trap was originally designed to sample diurnal insects, it has been used with success to sample some nocturnal species (e.g. Alexander 2000), probably because many insects exhibit negative geotropism (Carpenter 1905, Brues 1908, Jander 1963), which prompts them to move upwards when they encounter an obstruction.

2.3. **Laboratory methods**

2.3.1. Fatty acid analysis

On arrival to the laboratory, invertebrates (spiders, odonates and potential prey) were sorted, identified, stored at -80°C for at least 24 hours and then freeze-dried to remove water whilst retaining the nutritional quality (Tang and Pikal 2004). Samples were weighed, placed in labelled 2 ml screw-topped glass test tubes and homogenised using a glass rod. Chloroform containing 0.01% butylated hydroxytoluene (BHT) was added and the test tubes were sealed with teflon tape after flushing with nitrogen to prevent oxidation (Budge et al. 2006). Samples (in BHT) were stored at -20°C. Each sample type was replicated three times per site and collection period and each sample constituted at most 20 mg (dry mass) of animal matter. Body parts or whole individuals were used, and invertebrates taxa smaller than 1 mg were pooled (by taxa – order, family and genus) to reach 2 – 20mg.

Total lipids were extracted from each sample and fatty acid methyl esters (FAMEs) derived using a one-step method modified from Indarti et al. (2005). Two mL of an anhydrous methanol-sulphuric acid mixture were added to each sample followed by heating in a dry block heater at 100 °C for 30 minutes. One mL of ultra-distilled water was added to the samples after they cooled, followed by centrifugation at 3 000 rpm for three minutes. The resultant top aqueous layer was discarded and the bottom layer, containing the FAMEs, was transferred through a pipette with anhydrous sodium sulphate into a clean vial. FAMEs were concentrated by evaporating to dryness using a gentle nitrogen stream and then topped with hexane and injected into an Agilent 7890 gas chromatograph fitted with a ZB-WAXplus 320 column and a flame ionization detector. One µl of each FAME extract was auto-injected into the column at 250°C (injector temperature), with the oven set at 150°C and helium as the carrier gas. After 5 minutes, the oven temperature was raised to 225°C at 2.5°C/min. The resultant FAME peaks were visualized using ChemStation chromatography software, identified by comparison with retention times using external standards (marine PUFA no. 1, 37 component FAMEs mix; Supelco) and confirmed using mass spectrometry on an Agilent 7000A GC/MS-QQQ coupled with a NIST 08 MS library (column and methods as per the GC runs).

Each FA was measured as a proportion of the total FAs (%TFA). The nomenclature used for naming FAs was a:bωx, where a is the number of carbon atoms, b is the number of double bonds and x is the position of the first double bond from the methyl end of the molecule.

2.3.2. Stable isotope analysis

As for FAA, samples were sorted, frozen, freeze-dried and homogenised in a test tube. Approximately 1 mg of homogenized tissue was weighed into a tin capsule. Samples (in capsules) were sent for stable nitrogen and carbon analysis at IsoEnvironmental Laboratory in Grahamstown, South Africa. Samples were combusted in a Europa Scientific 20-20 isotope-ratio mass spectrometry (IRMS) linked to an ANCA SL Prep Unit. Internal standards consisting of beet sugar, ammonium sulphate and casein were calibrated against the International Atomic Energy reference materials (Vienna Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen). Isotope ratios were expressed in the δ-unit notation in the following equation: $\delta X = [(Rsample/Rstandard) - 1] \times 1000$, where X represents ^{13}C or ^{15}N and R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio, respectively.

Chapter 3. DIRECT EFFECTS OF CROSS-BOUNDARY TROPHIC SUBSIDISATION ON RIPARIAN COMMUNITIES OF WEB-BUILDING SPIDERS

3.1. **Introduction**

Riverine systems can produce large quantities of aquatic insects that serve as important prey for terrestrial predators such as spiders, lizards and birds (Henschel et al. 2001, Sabo and Power 2002b, Murakami and Nakano 2002, Kautza and Sullivan 2016). Aquatic insects can fuel terrestrial communities by serving directly as resources for predators and decomposers (Gratton et al. 2008) and having cascading effects on terrestrial herbivores (Henschel et al. 2001, Murakami and Nakano 2002, Knight et al. 2005). Kato *et al.* (2003) experimentally manipulated insect emergence from a river using a greenhouse-type covering and provided direct evidence that spider populations in the riparian area relied on aquatically-derived insect subsidies. Empirically, consumer abundance is related to prey abundance, but there is still a need to generalise this finding in the context of linked aquatic-terrestrial systems (but see Kennedy and Turner 2011).

Riparian web-building spiders offer an appealing tool for addressing questions of riparian ecosystem connectivity. Like most web-spiders, riparian web-spiders are generalist predators that forage in a largely opportunistic manner; consequently, their abundances are related to the local biomass of appropriately-sized flying insects (Briers et al. 2005, Burdon and Harding 2008, Gratton et al. 2008). They are ideally situated in the environment to demonstrate the significance of cross-boundary subsidies. If the subsidy is significant, that will be reflected in the spider community. In addition, their webs act as biologically-relevant trapping devices. In other words, they sample any potential subsidy in the same way as the beneficiaries of the subsidy. Thus, although spiders' webs are biased sampling devices, they perfectly reflect the nature of any potential subsidy to the spider community, almost certainly better than any standard entomological trap.

Aquatic subsidies in riparian landscapes are often characterized by a decreasing abundance of emerging aquatic insects with increasing distance from the river (Collier and Smith 1998, Griffith et al. 1998, Petersen et al. 1999, Delettre and Morvan 2000, Henschel et al. 2001). Existing evidence suggests that spiders are responsive to the gradient of prey abundance

(Murakami and Nakano 2002, Briers et al. 2005, Burdon and Harding 2008). Spider abundance is particularly high on the water's edges in riparian areas, especially during periods of high insect emergence (Henschel et al. 2001, Iwata 2006). The high abundance of insects at the water's edges causes high spider diversity and abundance (Briers et al. 2005, Burdon and Harding 2008, Gratton et al. 2008). At the headwater stream of the River Severn in UK, Briers et al. (2005) enriched aquatic insects with ^{15}N and they assessed the signatures of $\delta^{15}N$ in spider diets. Their results showed that the aquatic subsidies to spider diets decreased with increasing distance from the river. More studies are required to fully describe the influence of aquatic subsidies on spider success, particularly regarding changes in aquatic insect abundance that are associated with variations in habitats over space and through time.

In addition to evidence of clear links between spider abundance and prey availability, there is strong evidence that suggests habitat structure, habitat complexity and environment are equally if not more important in determining habitat preferences of spiders (Rypstra 1983, Greenstone 1984, Diehl et al. 2013), which ultimately determine the composition of spider communities. Web-building spiders anchor their webs to appropriate substrata and use thigmotactic (vibratory) cues to detect the environments which best suit them (Barth 1985, McNett and Rypstra 2000). It is therefore likely that complex habitats provide appropriate sites or attachment structures (Rypstra et al. 1999) for a greater range of sizes and types of webs (MacArthur and MacArthur 1961, Pianka 1966, Riechert and Gillespie 1986, Uetz 1991) and possibly influence the rate of prey capture (Diehl et al. 2013). Numerous studies repeatedly demonstrated relationships between the physical complexity and habitat structure of an environment and the abundance and diversity of spiders (Lowrie 1948, Barnes 1953, Hatley and Macmahon 1980, Dobel et al. 1990, Uetz 1991, Hurd and Fagan 1992, Balfour and Rypstra 1998, Langellotto and Denno 2004). Current evidence suggests differences in vegetation complexity can alter interactions between web-building spiders and their prey (Colebourn 1974, Dobel et al. 1990, Sunderland and Samu 2000, Tagwireyi and Sullivan 2015).

Spider abundances in riparian areas are largely influenced by the abundance of emergent insects. Areas with high aquatic insect productivity are thus likely to support large abundances of spiders. Factors that influence aquatic insect productivity are important determinants of cross-boundary trophic linkages between the river and the adjacent terrestrial habitats. Such factors may include river depth, sediment type, pollution, vegetation cover, water temperatures and river width, among many others (Cummins et al. 1989, Huryn and Wallace 2000, Sweeney et al. 2004). Although variations can occur, wider sections of many rivers are typically open to the sky (low vegetation cover), deeper and thus are likely to have warmer water temperatures than the narrower sections upstream that have high amounts of cover (Lammert and Allan 1999, Sweeney et al. 2004). Wider sections of rivers are also likely to present larger surface areas (stream bed) for emergence of aquatic insects than narrower areas. The Riverine Productivity Model (Thorp and Delong 1994) reflects that primary productivity is generally low in the head waters, highest in the middle reaches of a river, and remains high in wide areas. Given that headwaters are narrow and supposedly have lower primary productivity rates, I expected that wider sections of a river support more aquatic insect productivity than narrow sites and thus provide more aquatic subsidies to riparian predators like spiders. Therefore, the width of a river can potentially represent a useful yet imperfect (does not consider factors such as sediment type and pollution levels) surrogate for insect productivity when insect productivity data are unavailable.

Although it has been established that spider abundance varies with aquatic insect abundance (Dennis et al. 2015), it is unknown how seasonal and spatial variations in aquatic insect abundance affect spider abundance. Additionally, it is not known how the movement of aquatic subsidies into terrestrial landscapes varies across sections of a river channel with varied widths. In my study, I aimed to determine the effect of variations in prey abundance on spider abundance during different seasons at different sections of a river. Finally, I aimed to determine the effects of habitat complexity and the environmental conditions on web building spider communities.

The hypotheses of this study are as follows:

1) *aquatic insect biomass decreases with increasing distance from the river because the aquatic insects are preyed on by terrestrial predators or they rest or die along the way, and this relationship is dependent on emergence rates that are high in wider sections of the river and during warmer periods;*
2) *web-building spider abundance/biomass decreases with increasing distance from the stream because predator abundance/biomass is a factor of prey availability, and this relationship is dependent on emergence rates that are high in wider sections of the river and during warmer periods; and*

3) *the abundance/biomass of web-building spiders is positively related to aquatic insect biomass because aquatic insects constitute an important portion of riparian predator diets, and this relationship is dependent on factors that drive aquatic insect biomass: distance from the water, stream width and season*

3.2. **Methods**

3.2.1. Study design

Invertebrate collections took place during four different seasons at three sites with varying stream widths; *narrow*, *wide* and *intermediate* sites. The methods of collecting flying insects (prey) and web-building spiders are described in Chapter 2. Invertebrate samples were enumerated and dried at 50°C for three days. Biomass was expressed as the total dry mass per transect, per sampling location, per time period. A vegetation survey was conducted in an attempt to quantify the habitat architecture (complexity) available for web-building spiders and the methods are described in Chapter 2. The following was done to test the hypotheses of this study:

1) ***Hypothesis*** **1.** *Aquatic insect biomass decreases with increasing distance from the river because the aquatic insects are preyed on by terrestrial predators, and this relationship is dependent on emergence rates that are high in wider sections of the river and during warmer periods* – Aquatic insects were collected with increasing distance from the river, and their biomass estimates were compared across the distance gradient, sites and seasons. Correlation analysis was used to investigate any trends in insect biomass with increasing distance from the river. Effects of site (stream width) and season were tested using general linear models (GLMs). The ratio of aquatic to terrestrial insect biomass (ATB) was calculated by dividing the biomass of aquatic insects by that of terrestrial insects. The ATB ratio provided a measure of relative prey availability of either aquatic or terrestrial insects. The correlation between spider abundance and ATB was also estimated using correlation analysis (see statistical analysis below).

2) ***Hypothesis*** **2.** *Web-building spider abundance/biomass decreases with increasing distance from the stream because predator abundance/biomass is a factor of prey availability, and this relationship is dependent on emergence rates that are high in wider sections of the river and during warmer periods* – As with aquatic insects, values of web-building spider biomass

and abundance were obtained with increasing distance from the river and the effects of distance, site and season were tested using correlation analysis and GLM.

3) ***Hypothesis* 3.** *The abundance/biomass of web-building spiders is positively related to aquatic insect biomass because aquatic insects constitute an important portion of riparian predator diets, and this relationship is dependent on factors that drive aquatic insect biomass: distance from the water, stream width and season* - Correlation analysis was used to investigate the relationship between spider biomass and aquatic insect biomass (from malaise traps), with increasing distance from the river. The ratio of aquatic insect to spider biomass (ASB) was estimated as an indication of the relationship between the two invertebrate groups. Effects of site and season on the ASB were tested using GLMs.

3.2.2. Statistical analysis

3.2.2.1. Correlation analysis

Preliminary analysis revealed that spider abundance and biomass and insect biomass had exponential/hyperbolic relationships with distance from the river. Natural log (ln) transformations were carried out to linearize the relationships because non-monotonic relationships are not appropriate for linear analyses (Zuur et al. 2009). Spearman's rank correlation (Rho) coefficient was used to measure the strength of association between web-building spider biomass/abundance and aquatic insect biomass with increasing distance from the river. Correlation analyses were also carried out to investigate any habitat or environment gradients with increasing distance from the river. After the correlation analyses, one-way analyses of variance (ANOVA) were conducted to determine any differences in environment conditions and habitats among transects at the same sites. Despite being intervals, the distance transects were considered as categories to determine if habitat or environment varied in each site.

Using habitat and environmental variables as explanatory variables, multivariate analyses were attempted. The RELATE function in PRIMER V6 (Clarke and Warwick 2001) was used to investigate the presence of any relationship between spider biomass/abundance and the proposed explanatory variables (air temperature, humidity, plant density, number of attachment points, wind speed, plant height, prey abundance and prey biomass), but no relationships were detected (Rho = 0.016, p = 0.48). As such, intrinsic results of these analyses were not presented.

3.2.2.2. Effects of stream width and season

General linear models (GLM) were run with a combination of categorical and linear factors. The categorical factors were site and season and the linear factor was distance from the river. Spider abundance/biomass, aquatic insect biomass and ASB were the response variables. All statistical analyses were carried out using the software package Statistica (StatSoft Inc. 2011). Due to loss of some spider samples before biomass estimations I was unable to report spider biomass measurement for spring.

3.3. **Results**

3.3.1. Terrestrial and aquatic flying insects (Hypothesis 1)

There was variation in both aquatic and terrestrial flying insect biomass with increasing distance from the river, at all sites, and across all seasons (Figure 3-1).

3.3.1.1. Variation with distance

Aquatic insect biomass decreased significantly ($p < 0.05$) with increasing distance from the river at all the sites (Table 3-1). Correlation coefficients (r) were more than 0.7 during all the seasons at the wide and intermediate sites. The correlation between distance and aquatic insect biomass was statistically significant ($p < 0.05$) only during the summer at the narrow site.

The biomass of aquatic insects was always lower than terrestrial insects, except during summer, at all the sites (Figures 3-1). Aquatic insect biomass was highest at 0 m from the stream and lowest at the furthest distance, 64 m. However, there was a sharp decline in aquatic insect biomass between 0 to 4 m from the river at most sites. Subsequently, there was variation in aquatic biomass between 4 and 32 m and no clear decreasing or increasing trend could be observed across all the sites and seasons. Apart from a slight dip in biomass at 0 m, no patterns could be observed with the terrestrial insects. The ATB also varied with distance from the river (Figure 3-2). The highest ratios were observed at 0 m and there was a sharp decline between 0 and 2 m. The ratios between 4 m and 64 m basically remained the same.

Table 3-1: Spearman's rank correlation (Rho) coefficient for aquatic insect biomass with distance from the river. Correlations were run for each study site (narrow, wide and intermediate sites), in each season (summer, autumn and winter).

	Narrow		**Wide**		**Intermediate**	
SEASON	**r**	**p**	**r**	**p**	**r**	**P**
Summer	**-0.85**	**< 0.05**	**-0.71**	**< 0.05**	**-0.96**	**< 0.05**
Autumn	-0.58	> 0.05	**-0.86**	**< 0.05**	-0.75	> 0.05
Winter	-0.32	> 0.05	**-0.74**	**< 0.05**	**-0.90**	**< 0.05**

3.3.1.2. Variations across seasons and sites

A significant GLM model ($F = 100.38$, d.f. = 1, $p < 0.01$) showed that aquatic insect biomass was distinguishable based on distance from the river ($F = 46.93$, d.f. = 1, $p < 0.01$) and season ($F = 8.75$, d.f. = 2, $p < 0.01$). Multiple comparisons revealed significant differences between winter and the other two seasons (summer and autumn). Aquatic insect biomass could not be distinguished based on site ($F = 1.86$, d.f. = 2, $p > 0.05$) or the interaction of site and season ($F = 1.18$, d.f. = 4, $p > 0.05$). The biomasses of both terrestrial and aquatic insects were lowest during winter (Figure 3-1 c, g and k) at all sites, and highest during summer at the narrow and intermediate sites. At the wide site, aquatic insect biomass was highest during spring, but at the other two sites the lowest biomasses were in spring. Terrestrial insect biomass was lowest during winter at all the sites. The narrow site had the lowest biomasses of aquatic insects across the four seasons, followed by the wide and intermediate sites, respectively. There were no predictable trends in biomass of terrestrial insects across the sites or seasons. The ratios of aquatic-terrestrial insect biomass did not vary much across seasons, but were highest at 0 m at all times. The ratios fell to their lowest between 0 and 4 m from the stream. Between 4 and 64 m, the ratios were generally higher at the wide site, followed by the intermediate and narrow sites, respectively.

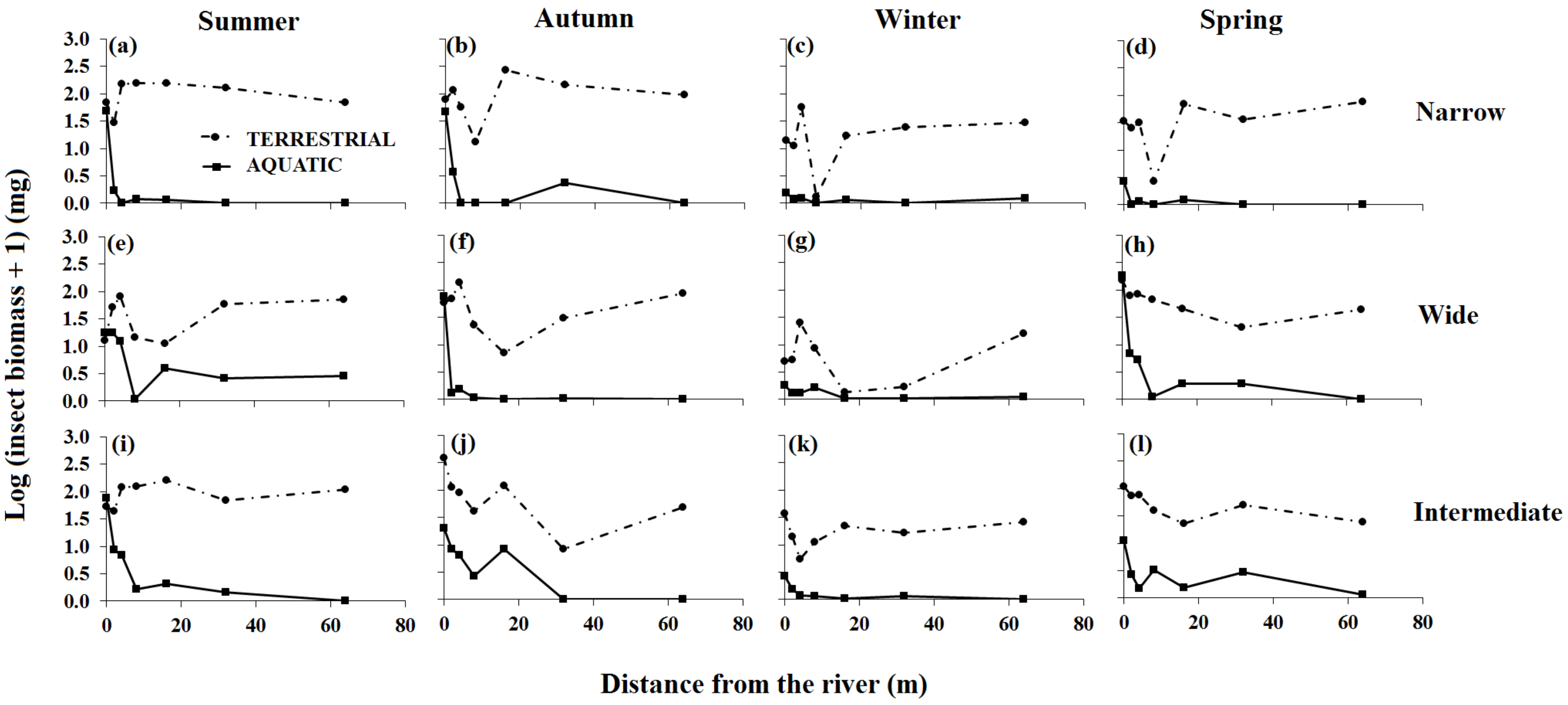


Figure 3-1: Total insect biomass at different distances (m) from the river, in four seasons at three sites along the Kowie River. Insects were collected using Malaise traps.

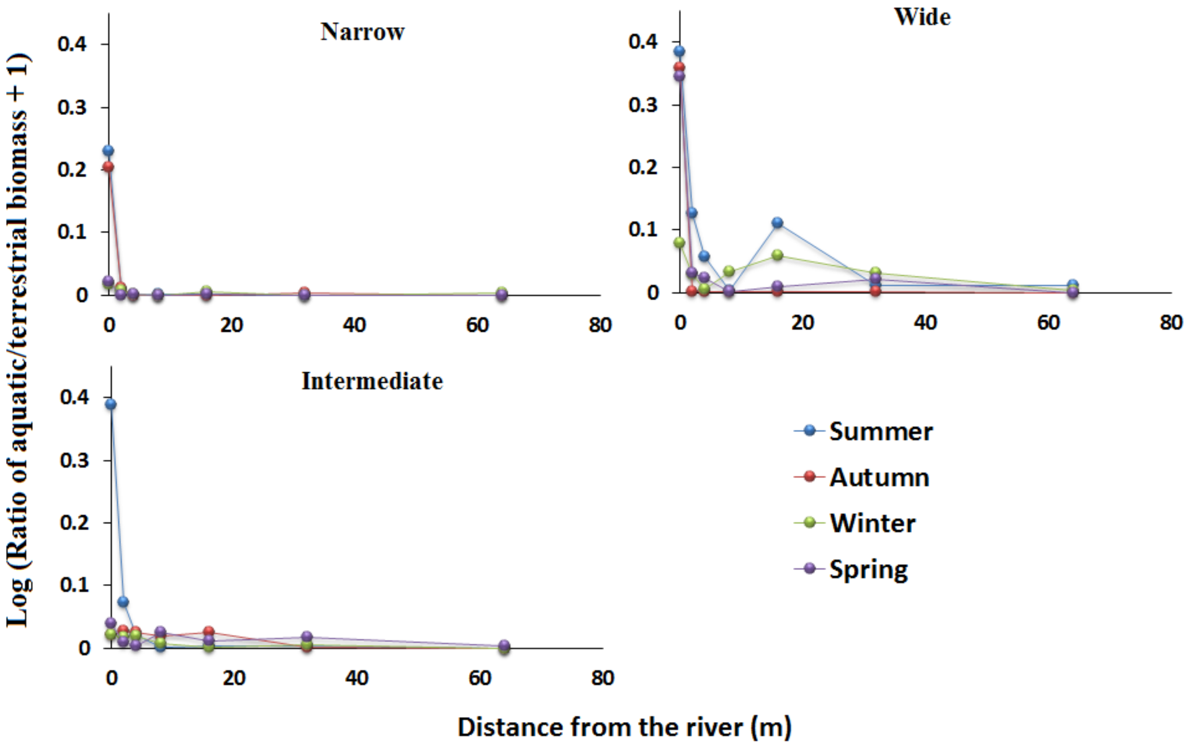


Figure 3-2: Log-transformed ratios of aquatic/terrestrial insect biomass (ATB) in four seasons at three sites along the Kowie River.

3.3.2. Spider communities (Hypothesis 2)

A total of 1113 spiders, representing more than nine families, was collected from the three sites during the four seasons. A diverse range of spiders of varying sizes and weights were collected at all the sites (Appendices 1 – 3). Due to loss of some spider samples prior to biomass analysis, spring biomass data were not obtained.

3.3.2.1. General trends in spider abundance

Generally, similar spider genera and families were found at all of the sites, and the families Araneidae and Tetragnathidae were the most abundant (Figure 3-3). The family Araneidae had relatively high abundances at all of the distances (Figure 3-3). Spiders from the Tetragnathidae were highly abundant in areas next to the river, but less abundant further away. For most families, there was a general decline in abundance with increasing distance from the river. The occurrences of the families Deinopidae, Nephilidae and Pholcidae were lower than other taxa.

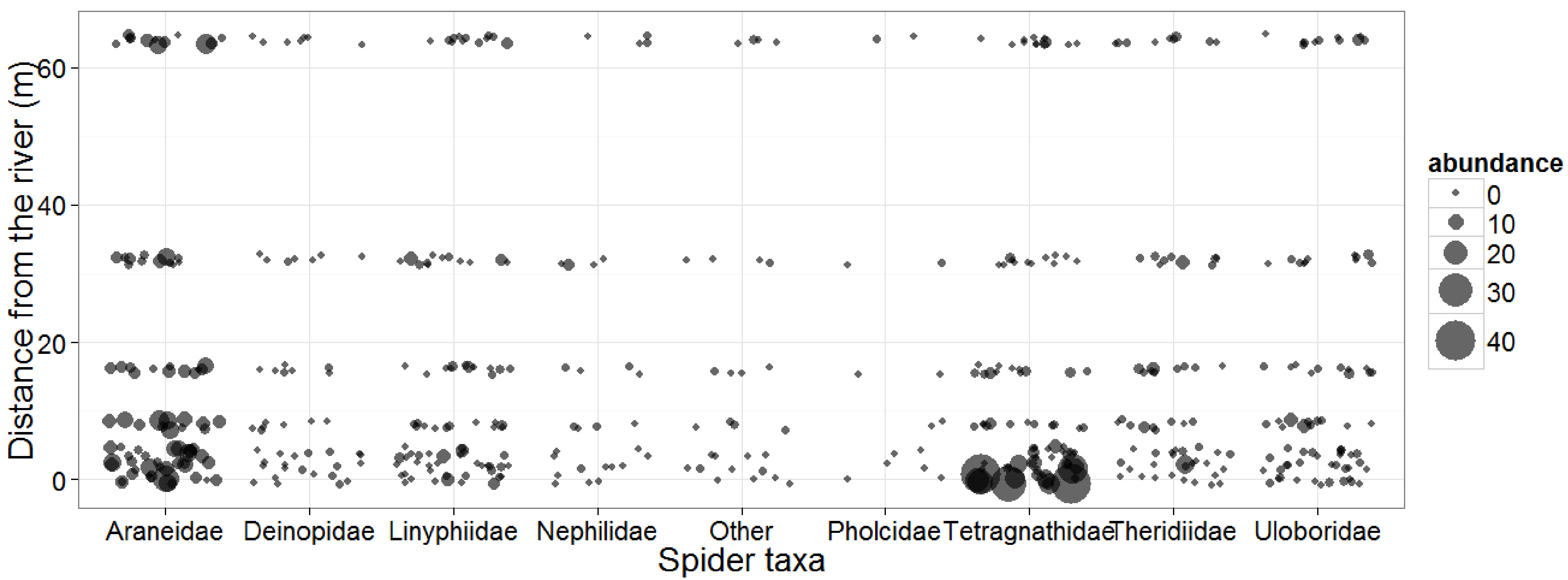


Figure 3-3: Bubble plot representing abundances of all spider families collected from the three sites during all the seasons (combined). Each bubble represents a single spider taxon, at a particular distance from the river at one of the three sites. The size of each bubble is an indication of the abundance at a time and place. A greater number of bubbles signifies higher occurrences. Abundance data from different times and places had to be pooled to present an overall picture of how spider abundance changed with distance from the river and how this change varied with spider taxon. The group “Other” includes individuals from the Amaurobiidae, Mimetidae and Theridiosomatidae.

3.3.2.2. Total spider abundance

There was a distinct decrease in spider abundance with increase in distance from the river during summer at the wide site and winter at the intermediate site (Table 3-2). There were no significant correlations between spider abundance and distance from the river during the other seasons and at the narrow site. A significant GLM ($F = 373.63$, d.f. = 1, $p < 0.01$) showed that total spider abundance was distinguishable based on distance from the river ($F = 19.86$, d.f. = 1, $p < 0.01$), season ($F = 2.68$, d.f. = 2, $p < 0.05$), site ($F = 5.36$, d.f. = 2, $p < 0.01$) and the interaction factor of site and season ($F = 3.96$, d.f. = 4, $p < 0.01$). Multiple comparisons revealed significant differences between winter and the other two seasons (summer and autumn) and between the narrow site and the other two sites. At each site, total spider abundance was highest at the stream edge (0 m) during summer, winter and spring (Figure 3-4). During autumn, spider abundance was highest at 2 - 4 m from the river. During the other three seasons there was a sharp decrease in abundance between 0 and 4 m from the stream. Subsequently there was a steady decrease in abundance from 4 to 64 m. Abundance was therefore at its lowest at 64 m during all seasons except summer at all the sites. Spider abundance was very low ($0\ m^{-2}$) during winter at the narrow site, but in other sites it remained as high as during other months.

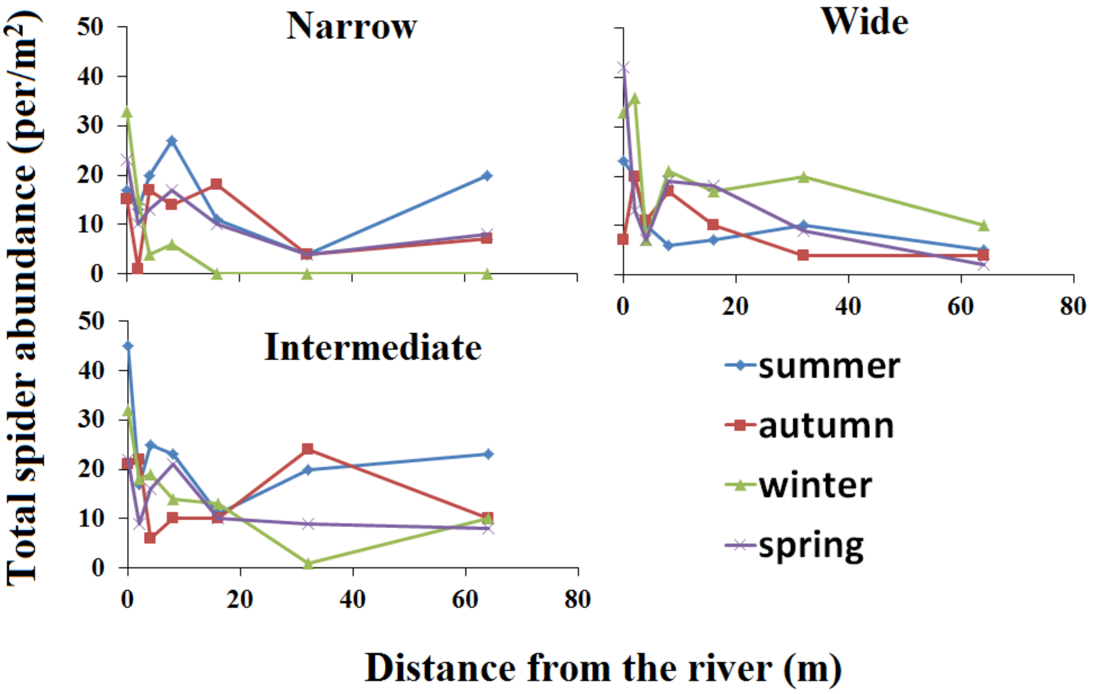


Figure 3-4: Total spider abundance (per/m^2) at different distances (m) from the river, across four seasons.

Table 3-2: Spearman's rank correlation (Rho) coefficients for spider abundance with distance from the river. Correlations were run for each study site (narrow, wide and intermediate sites), in each season (summer, autumn and winter).

SEASON	NARROW		WIDE		INTERMEDIATE	
	R	P	r	p	r	p
Summer	-0.14	> 0.05	**-0.85**	**<0.05**	0.27	> 0.05
Autumn	-0.38	> 0.05	0.63	> 0.05	-0.11	> 0.05
Winter	0.44	> 0.05	0.5	> 0.05	**-0.89**	**< 0.05**

3.3.2.3. Variation in spider biomass

The variations in spider biomass were not consistent with spider total abundances, except for spider biomass peaking at 0 m during some seasons (Figures 3–3 to 5). At all of the sites, spider biomass was generally highest at most of the distances during autumn. Spider biomass was lowest during the winter at the narrow and intermediate sites. At the wide site, spider biomass during winter was similar to the other months. Summer spider biomass at the intermediate site was distinctly higher than at the narrow and wide sites (Figure 3-5).

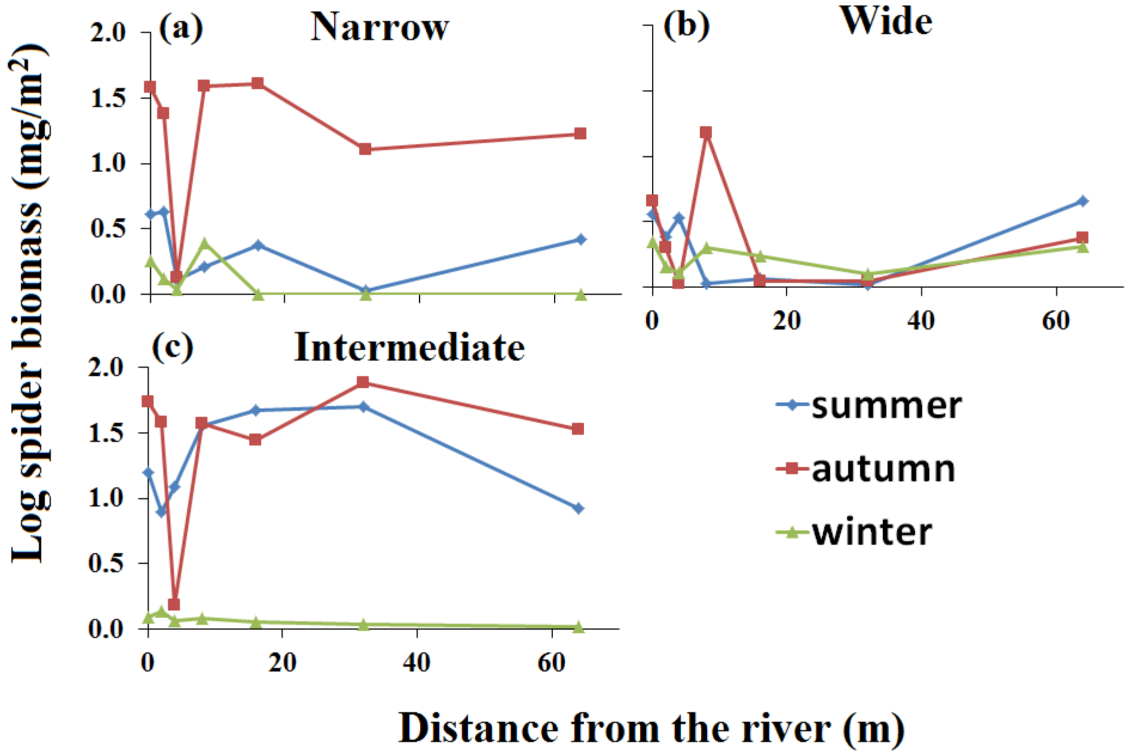


Figure 3-5: Total spider biomass (mg/m^2) at increasing distances from the river, across different seasons.

3.3.3. Relationships between spiders and prey (Hypothesis 3)

Spider abundance was positively correlated to both aquatic insect biomass and ATB in some instances (Table 3-3). The correlations with the ATB were generally higher than those with the aquatic insect biomass. All the correlations were generally weakest at the narrow site, but there was no evidence of statistical significance in all of them. Apart from the correlations during winter, spider abundance had stronger correlations with ATB at the wide site than at the intermediate site, and many of the correlation coefficients were statistically significant. At the intermediate site, the correlations with both aquatic insect biomass and ATB were very strong during winter, weak and not statistically significant during the other seasons.

Table 3-3: Spearman's rank correlation (Rho) non-parametric test for spider abundance with both aquatic insect biomass (from Malaise traps) and the ratio of aquatic to terrestrial insect biomass (ATB). Correlations were done for each study site (narrow, wide and intermediate sites), in each season (summer, autumn and winter).

	Aquatic insect biomass		**Aquatic/terrestrial biomass (ATB)**	
Season	**r**	**p**	**r**	**p**
NARROW				
Summer	0.56	> 0.05	-0.13	> 0.05
Autumn	0.32	> 0.05	-0.38	> 0.05
Winter	-0.04	> 0.05	0.44	> 0.05
WIDE				
Summer	0.50	> 0.05	**0.85**	**<0.05**
Autumn	0.18	> 0.05	0.63	> 0.05
Winter	**0.52**	**< 0.05**	**0.50**	**< 0.05**
INTERMEDIATE				
Summer	-0.29	> 0.05	0.27	> 0.05
Autumn	-0.14	> 0.05	-0.11	> 0.05
Winter	**0.79**	**< 0.05**	**0.89**	**< 0.05**

A significant GLM model ($F = 57.69$, d.f. = 1, $p < 0.01$) showed that total spider abundance was distinguishable based on distance from the river ($F = 17.96$, d.f. = 1, $p < 0.01$), season ($F = 3.43$, d.f. = 2, $p < 0.01$), site ($F = 6.58$, d.f. = 2, $p < 0.01$) and the interaction factor of site and season ($F = 4.24$, d.f. = 4, $p < 0.01$). Multiple comparisons revealed significant differences between winter and the other two seasons (summer and autumn) and between the wide site and the other two sites.

3.3.4. Environment and habitat conditions

Apart from the weak correlation between plant density at 10 cm and distance from the river at the narrow site, none of the other environmental and habitat variables were significantly correlated with distance from the river at all the sites ($p > 0.05$; Table 3-4). However, some haphazard differences were observed between transects. Air temperature did not differ significantly between the 0 m distance and increased distances inland at both the wide ($F = 1.550$, d.f. = 21, $p > 0.05$) and intermediate ($F = 2.190$, d.f. = 21, $p > 0.05$) sites. At the narrow site, significant differences in air temperature ($F = 7.356$, d.f. = 21, $p < 0.05$) were detected among the transects, but no pattern could be discerned.

Table 3-4: Spearman's rank correlation (Rho) coefficients for various environmental variables with increasing distance from the river. Correlations were run only using summer data from each study site (narrow, wide and intermediate sites). Only summer data is presented here because it was deemed unnecessary to continue collecting this data when there was no established relationship with spider abundances in summer.

VARIABLE	**NARROW**		**WIDE**		**INTERMEDIATE**	
	R	**p**	**R**	**p**	**R**	**p**
Maximum temperature	-0.36	> 0.05	-0.1	> 0.05	0.39	> 0.05
Minimum temperature	0.1	> 0.05	0.11	> 0.05	0.27	> 0.05
Number of contacts	0.19	> 0.05	0.32	> 0.05	0.2	> 0.05
Plant volume	0.15	> 0.05	0.16	> 0.05	-0.1	> 0.05
Plant density at 10cm	**-0.37**	**< 0.05**	-0.52	> 0.05	0.22	> 0.05
Plant density at 80cm	0.37	> 0.05	-0.01	> 0.05	-0.34	> 0.05

At both the wide and intermediate sites, there were no statistically significant differences amongst transects in terms of plant volume (wide site: $F = 7.10$, d.f. = 54, $p = 0.15$; intermediate site: $F = 0.197$, d.f. = 75, $p = 0.21$) and number of contacts (wide site: $F = 1.245$, d.f. = 34, $p = 0.09$; intermediate site: $F = 1.267$, d.f. = 33, $p = 0.33$). At the narrow site, only one of the transects at 8 m had an average plant volume significantly different ($F = 3.733$, d.f. = 58, $p < 0.05$) from the transects, and another at 64 m had an average number of contact points significantly different ($F = 3.444$, d.f. = 34, $p < 0.05$) from the rest. Again, no pattern could be detected with increase in distance from the river and a habitat gradient was not apparent.

Plant density yielded greater differentiation amongst the transects at the narrow and wide sites, although density at 10 cm (height) was not statistically different ($F = 1.16$, d.f. = 14, $p = 0.41$) across transects at the narrow site. At the height of 80 cm there were differences in plant density between two transects; 8 m and 64 m, and all the other transects ($F = 50.81$, d.f. = 14, $p < 0.05$) at the narrow site. At the wide site, differences were observed at both heights; 10 cm ($F = 32.54$, d.f. = 20, $p < 0.05$) and 80 cm ($F = 8.67$, d.f. = 20, $p < 0.05$), with more differences being observed at the 80 cm height. However, all the transects at the intermediate site were statistically indistinguishable at both the heights; 10 m ($F = 1.40$, d.f. = 20, $p = 0.12$) and 80 m ($F = 1.16$, d.f. = 20, $p = 0.21$). Overall, plant density, plant volume, number of contacts and air temperature varied between some transects at some sites, but there was no pattern of variation with increasing distance from the shore. Above all, there was no significant relationship between spider biomass/abundance and the proposed explanatory variables (Rho = 0.016, $p = 0.48$).

3.4. **Discussion**

The biomass of aquatic insects in the Kowie catchment was generally consistent with patterns observed in other studies on aquatic subsidies (Polis and Hurd 1996, Polis et al. 1997, Jonsson and Wardle 2009), and it was predictably related to the biomass and abundance of web-building spiders (Briers et al. 2005, Burdon and Harding 2008). There were indications that the correlations between spider biomass and aquatic insect biomass were dependent on the availability of aquatic emergent insect abundances. Therefore, correlations were strong in seasons and sites with higher aquatic abundances. However, there were no correlations in some instances, suggesting that other factors contributed to the variation in spider biomass with increasing distance from the river. Habitat and environmental factors were measured, but no statistically significant influence was detected with increasing distance from the river. Nevertheless, trends in spider and insect biomass/abundance provided indications of the extent of movements of aquatic subsidies into the terrestrial system at different sites and during different seasons.

3.4.1. Trends in aquatic and terrestrial insect biomass (Hypothesis 1)

I hypothesized that aquatic insect biomass would decrease with increasing distance from the river and the relationship would be dependent on emergence rates that would be high in wider sections of the river and during warmer periods. The results of the study support this hypothesis because aquatic insect abundance/biomass was significantly correlated to distance from the river and the relationship was stronger in summer and autumn (warmer months) and lowest in winter.

The relatively lower biomass of terrestrial insects at the stream edge than further away (Figure 3-1) was likely a result of a lack of vegetation cover on top of the stream, especially at the intermediate and wide sections. Typically there are strong associations between local vegetation and the abundance and biomass of insects because many insects are phytophagous (Strong et al. 1984). Phytophagous insects have limited scales of movements and often remain close to their host plants (Smith and Remington 1996). Vegetation characteristics such as species diversity (Knops et al. 1999), species richness (Hawkins and Porter 2003) and structural complexity (Lara et al. 2008) influence patterns of insects abundance (Ober and Hayes 2008). Therefore, quantity, quality, or distribution of vegetation determines abundance and biomass of insects (Ober and Hayes 2008). There are few plants that can grow from within the river channel, and the Kowie River supported lower numbers of terrestrial phytophagous insects than the terrestrial habitats that support greater abundance and diversity of plants. Based on the theory that web-building abundances are a factor of vegetation cover/structure (Dennis et al. 2015) it would be reasonable to expect that the stream edge would support lower abundances of web-building spiders. Instead, my results showed that spider abundance was highest closest to the river and it is likely that the aquatic subsidies caused these high spider abundances and possibly facilitated top down effects on the local terrestrial insect populations (e.g. Knight et al. 2005).

The relatively low biomass of terrestrial insects at the stream edge, compared to further distances (Figure 3-1), may also be due to high predation by the highly abundant shoreline spiders (Figure 3-3). Aquatic subsidisation encourages web-building spiders to congregate at stream edges, but when aquatic insect abundance is low, the formerly subsidized web-builders can impose strong top-down control of in situ terrestrial prey (Henschel et al. 2001, Helmus et al. 2013). In summer and autumn, the biomass of aquatic insects at all the sites was high, possibly because aquatic insect emergence is dependent on air and water temperature and can vary daily (Corbet 1964), but terrestrial biomass was still lowest at the edge (0 m). On the days that conditions were less suitable for emergence, the high density of spiders may have been supported by the terrestrial insects, as they do in winter. Therefore, the biomass of terrestrial prey at the stream edge was indirectly affected by aquatic insect biomass more than terrestrial prey that lie further from the river, which explains the drop in terrestrial insect biomass at stream edge (0 m) (Figure 3-1). The abundance/biomass of other non-spider predators of insects was not reported, whereas other predators may have played a large role in regulating aquatic and terrestrial flying insects in the study areas (e.g. Murakami and Nakano 2002; Sabo and Power 2002).

3.4.2. Relationships between terrestrial web-building spiders and their prey (Hypothesis 3)

There were indications that the differences in spider abundance with increasing distance were correlated with biomass of emerging insects (Table 3-3). The ideal free distribution (IFD) model is that the number of animals that aggregate in various patches is proportional to the amount of resources available in each patch, which minimizes resource competition and maximizes fitness (Fretwell and Lucas 1970, Kacelnik et al. 1992, Jurczyk et al. 2012). Considering that terrestrial insect abundance did not change with increasing distance from the Kowie River (Figure 3-1), aquatic insect abundance was the most likely cause of variation in spider abundance. Similarly, a study by Burdon and Harding (2008) in the forests of central South Island of New Zealand showed that web-building spider biomass was correlated with aquatic insect biomass. Their study was very extensive and spanned numerous sites along 37 forest streams, but aspects of temporality were not explored. Another study by Jonsson and Wardle (2009) showed that web-building spider biomass was positively related to the biomass of emerging aquatic insects, which changed with increasing distance from lakes in the boreal zone of northern Sweden. My findings add support to such studies, and they provide new information on the temporality of spider–prey relationships in riparian areas.

The large difference in spider abundance/biomass between 0 and 4 m from the stream (Figure 3-3 to 4) was likely an effect of spider colony formation at the stream edge (Rypstra 1986). Most spiders do not live in groups and often pay little regard to other spiders, even members of their own species. However some species such as *Metabus gravidus* (Cambridge) (Araneae: Araneidae) are considered colonial because they live in large groups, despite individuals functioning independently (Buskirk 1975). The formation of spider colonies is believed to be caused by the occurrence of high prey densities (Buskirk 1975, Rypstra 1986, Mestre and Lubin

2011) in an evolutionary pathway to group living that is referred to as the ‘parasocial-route’ (Whitehouse and Lubin 2005). It is possible that some form of group living may have occurred at the edges of the Kowie River for spiders in the families Tetragnathidae and Araneidae, especially at the wide sites where aquatic insect abundance was greater than all other sites (Figure 3-1). I observed high densities of horizontal webs at the water edges during sample collections, and most of the webs belonged to individuals of the genus *Tetragnatha* (personal observation). Several studies have demonstrated that spiders in the genus *Tetragnatha* are characteristically highly abundant at stream edges (Laeser et al. 2005, Tagwireyi and Sullivan 2015). These horizontal web-building spiders may well have been forming colonies to utilize the large amounts of aquatic subsidies that were present at the edge of the stream, especially during summer and autumn in all the sites.

3.4.3. Seasonal variation among and within sites (all hypotheses)

The emergence of aquatic insects can be highly variable over time (Corbet 1964), and the emergence of stream insects is normally seasonal, highly synchronized and peaks in summer in temperate zones (Sweeney and Vannote 1982). The results of my study showed that aquatic insect biomass in the terrestrial landscape was highest in summer and lowest during winter (Figure 3-1). Emergence data from a concurrent study in the Kowie River has also shown that emergence was highest during summer months and lowest during winter (Moyo 2016). These temporal trends in aquatic insect abundance and biomass are common in many riverine systems (Baptista et al. 2001, Kato et al. 2003, Scheibler and Debandi 2008). Since spider biomass is related to emergent aquatic insect biomass, the seasonal shifts in amounts of aquatic subsidies is bound to influence spider abundance and biomass in the terrestrial area. My study provided evidence of a seasonal effect on web-building spider success, and because spider biomass was highest in summer and autumn, months that had the highest emergent aquatic insect biomasses.

It is likely that stream width regulates the quantity of emergent aquatic subsidies, with wider parts having the greatest abundance and biomass. Other physical factors such as river depth, sediment type, pollution, vegetation cover, stream velocity and water temperature (Cummins et al. 1989, Huryn and Wallace 2000, Sweeney et al. 2004) may have contributed to variations in subsidies across sites. Due to time constraints, some of these variables could not be quantified and it was assumed that wider sections of the river would possess many of the factors that encourage high insect productivity, e.g. high solar irradiance (hence high water temperature) and a large river bed. Wider river channels provide greater surface area for emergence and produce higher abundances of emerging aquatic insects (e.g. Johnson et al. 2007). Spider biomass was generally low during winter at the narrow and intermediate sites of the Kowie River, but high at the wide site (Figure 3-5). The wide site had a larger surface area for insect emergence than the other sites, which enabled greater emergence (Moyo 2016) and therefore greater subsidisation to the adjacent terrestrial upland. Winter was characterized by low emergence, but the high biomass of emerging aquatic insects produced at the wide site were probably sufficient to maintain high abundances/biomasses of terrestrial spiders up to 64 m from the river. Similarly, the study by Jonsson and Wardle (2009) (see section 3.4.2) on two large freshwater lakes on islands showed that the smaller islands with higher perimeter to area ratio yielded greater subsidisation to the terrestrial system. In my study, the benefit of aquatic subsidisation to terrestrial consumers in winter was relatively lower at the sites with narrower channels, i.e. the narrow and intermediate sites. The benefits of aquatic subsidies to terrestrial consumer diets may therefore be vital during times when terrestrial biomass is low.

3.4.4. Environmental and habitat conditions

In addition to prey availability, habitat and environmental factors are important in determining spider success (Dennis et al. 2015). A few habitat and environmental factors were measured in my study, but there was very low variation in environmental factors across the relatively small spatial scale of the study (0 to 64 m). Additionally, the lack of trends in vegetation variables with increasing distance from the Kowie River was consistent with the lack of trends in terrestrial insects. It is plausible that 64 m may not have been a sufficiently long distance for changes in micro-climate to be detected. The relative contributions of abiotic and biotic factors to community structure are regulated by the extent of variability in a system (Poff and Ward 1989). Brosofske et al. (1997) examined microclimate across small streams in western Washington, USA, and reported similar results. They measured air and surface temperature and humidity with increasing distance from a river up to 300 m, and observed no trends. However, they observed significant changes in both temperature and humidity at about 200 m from the river (Brosofske et al. 1997). It is plausible that the point where the environment changes may represent a boundary between the riparian area and the adjacent terrestrial upland. My study provided temperature measurements up to 64 m from the river, and it is likely that the environment within the 64 m was homogeneous, thus resulted in the absence of environmental gradients. Factors with low or no variation may not be important in structuring animal communities, so the habitat and environmental factors that were estimated in this study were not dominant factors influencing terrestrial spider biomass.

On the other hand, micro-habitats are likely to vary within 64 m of the river's edge, but a lot of variation was observed among sampling units in the same transects (among replicates). For example, there were high standard deviations of plant volume and density among transects within the same sites, in the same season. In the past, other researchers have resorted to measuring numerous micro-habitat variables and using models to find the important variables (e.g. Stauffer and Best 1980). The major objectives of my thesis were to describe spider diets (see Chapter 1) and to trace movements of aquatic subsidies, and less time was available to measure micro-habitat variables that could have influenced the spider community structure. Other studies have also failed to detect significant trends in micro-habitats with increasing distance from a river (Henschel et al. 2001, Polis et al. 2004). There was no evidence to suggest that any of the habitat or environmental variables that were measured in the Kowie River could have influenced web-building spider biomass. However, correlations between prey biomass and spider abundance/biomass represented evidence that prey availability was an important factor in determining spider community structure.

3.5. **Conclusion**

Data from this study showed that web-building spider communities and abundances varied with distance from the main channel. Evidence of varying spider taxa at different distances added further justification for riparian zone management/conservation. My results confirmed that spider community structure was related to the biomass of emerging aquatic insects. The width of the river was likely an influential factor in determining the extent of aquatic subsidisation from the river to adjacent terrestrial landscapes, with wider parts providing the greatest subsidisation. Higher aquatic insect biomasses at the wider parts of the river may greatly assist in maintaining spider populations during the winter when aquatic insect emergence is generally lower. The results of this study are largely based on the assumption that all flying insects are potential prey for spiders, and I recommend that future studies incorporate detailed observations of actual diets of spiders.

Chapter 4. DIET-ASSOCIATED TAXONOMIC VARIATIONS OF RIPARIAN WEB-BUILDING SPIDERS AND THEIR POTENTIAL PREY: A COMBINED FATTY ACID AND STABLE ISOTOPE APPROACH

4.1. **Introduction**

Riparian zones are transitional areas between aquatic and terrestrial ecosystems and they are characterised by a high diversity and abundance of flying insects (Muehlbauer et al. 2014). The aquatically-derived insects transfer some organic nutrients from the aquatic system onto land, hence subsidizing terrestrial landscapes. Many aquatic insects feed on terrestrially-derived plant material (Doucett et al. 2007a), so only a proportion of the nutritional subsidies exported to the riparian zone are truly aquatically-derived. Many insects with aquatic life cycles do not feed as adults and their bodies are largely constituted of nutrients consumed by immature life stages in the water. Even though insects are generally taxonomically diverse, aquatic and terrestrial insects are likely to be compositionally distinct. The nutritional (compositional) distinction between the insects in aquatic and terrestrial environments must be documented to investigate the degree of terrestrial dependency on aquatic ecosystems. The stable isotope (SI) signatures of the two insect groups can be distinct (Collier et al. 2002, Bennett and Hobson 2009, Speir et al. 2014), but few studies have focused on the compositional differences between the two insect groups (Fontaneto et al. 2011, Hixson et al. 2015), and information on African insects is particularly scarce. Moreover, regarding body composition, there is a lack of knowledge on the aquatic-terrestrial insect dichotomy with varying stream width, from the headwaters to the mouth of a river.

Riparian areas are ideal for generalist consumers such as spiders that can shift their diets to the most readily available prey. The high insect abundance in riparian areas supports a high abundance and diversity of web-building spiders (Nakano et al. 1999). It is assumed that riparian consumers have developed varied diet niches in response to the wide variation of prey (both aquatic and terrestrial; Nakano et al. 1999). In particular, web-building spiders have a wide variety of foraging tactics and body sizes that can lead to varying success rates when capturing particular insect taxonomic groups (Higgins and Buskirk 1992). Despite web-building spiders being largely considered as generalist consumers, spider taxonomic groups have varied diets and some spiders occupy specific feeding guilds (Uetz et al. 1999).

A good example of feeding guilds in web-building spiders can be provided by two commonly contrasting guilds, horizontal and vertical web builders. Horizontal web-builders are adapted to capture small weakly flying insects whilst vertical web-builders (Araneidae) construct large vertical webs that are structurally more suited to catching large and fast-flying terrestrial insects (Olive 1982, Kato et al. 2003). Horizontal web-builders have shown a strong response to aquatic insect subsidisation (Speir et al. 2014, Tagwireyi and Sullivan 2015) or experimental reduction (Marczak and Richardson 2007). It is important that the nature of trophic variations among riparian web-building spider taxonomic groups and guilds are understood because web-building spiders act as the natural sampling devices of aquatic subsidies.

As a consequence of spiders' external digestion and generalist predation, it is a challenge to investigate their diets through conventional methods such as gut content analysis. Fatty acid (FA) and SI tracer techniques have been used to describe variations in consumer diets, but the taxonomic variation remains to be adequately described using these techniques. Differences in FA profiles and proportions of individual FAs can give indications of dietary sources e.g. highly unsaturated FAs (HUFAs) are plentiful in aquatic habitats (Gladyshev et al. 2009a). Previous studies have identified groups of FAs that are biologically important and can be used as indicators of dietary origins, e.g. the ratio of ω3/ω6 or the sum of ω3 FAs can indicate levels of aquatic contributions (Hixson et al. 2015). Furthermore, the natural distribution of $\delta^{13}C$ and $\delta^{15}N$ values reflects an integrated history (over several months) of physical and metabolic processes within ecosystems. In trophic studies, carbon isotopes can identify the ultimate sources of the organic matter, whilst $\delta^{15}N$ values can give an estimation of the trophic level (Peterson and Fry 1987, Belivanov and Hambäck 2015). Fertilization, pollution and other stream ecosystem perturbations can alter the flow of elements such as nitrogen (Peterson and Fry 1987). It is therefore possible that rivers have $\delta^{15}N$ values (in resident organisms) that are different from those in the adjacent terrestrial habitats.

The objective of my study was to describe isotopic and FA diet variations amongst taxonomic groups of consumers, with web-building spiders as the target organisms. Additionally, the variation in dietary niches between two common web-building spider guilds, horizontal and vertical web-builders, was evaluated. Finally, I sought to describe compositional variations amongst taxonomic groups of prey insects and contrast the isotope signatures of aquatic- and terrestrially-derived insects. The hypotheses of this study are as follows:

***Hypothesis* 1** – *Aquatic and terrestrial insect taxonomic groups can be distinguished using FA and SI tracer techniques due to their different food sources because aquatic habitats have higher quantities of highly unsaturated fatty acids (HUFAs) and different nitrogen sources than adjacent terrestrial habitats*

***Hypothesis* 2** – *There is inter-specific niche partitioning among web-building spider taxa because larger spiders feed on larger prey whilst spider foraging behaviour is specialized to capture particular groups of insects. As such, spider taxa can be distinguished using FA tracer techniques.*

***Hypothesis* 3** – *Webs of different spiders are biased towards the capture of particular groups of insects and the significance of inter-guild differences (in determining the sharing of available resources) is reflected in the differences in FA and SI dietary niches between horizontal and vertical web-building spiders.*

4.2. **Methods**

Invertebrate collections took place during four different seasons at three sites with varying stream widths; narrow, wide and intermediate sites. The methods of collecting flying insects (prey) and web-building spiders were described in Chapter 2. Data for aquatic insects was obtained from a concurrent study in the Kowie River (Moyo 2016). The following was done to test the hypotheses of this study:

***Hypothesis* 1.** *Aquatic and terrestrial insect taxonomic groups can be distinguished using FA and SI tracer techniques due to their different food sources because aquatic habitats have higher quantities of highly unsaturated fatty acids (HUFAs) and different nitrogen sources than adjacent terrestrial habitats* - The total fatty acid (TFA) composition of each insect was expressed as FA relative proportions of the FAs present. Comparisons of insects from different taxonomic groups were made based on entire FA profiles. Similarity percentage analysis (SIMPER) was used to determine the relative contribution of each FA to the total variation. Resemblance matrices were calculated on untransformed data using Euclidean distances. Fatty acids with the highest contributions were considered as major FAs; mostly these were FAs contributing more than 5% of the total variation. A dominance curve, based on rank abundance and frequency, was also used to determine the major and minor FAs. Non-metric multi-dimensional scaling (nMDS) was used to visualize the TFA profiles of the insects, and the influential FAs were fitted as the vectors in a biplot (see Gower et al. 2010). Like all multivariate ordination or clustering methods, nMDS preserves taxon-specific information and is generally more sensitive in detecting changing community patterns (Warwick et al. 1990). The length of the each of the radial lines (vectors) represented the relative degree of influence exerted by each FA on the TFA composition of insects (points) in the direction of the line.

Fatty acids that contributed more than 5% to variation amongst aquatic and terrestrial insect groups, in addition to biologically important groups (e.g. ω3 FAs, essential FAs, polyunsaturated FAs, etc.) were selected for detailed analyses. T-tests were conducted to assess the significance of differences in FA proportions between terrestrial and aquatic insects.

Fatty acid analysis on aquatic and terrestrial insects was done for each season and site, but for the purposes of comparing insects from the aquatic and terrestrial environments, in many cases only the summer data were presented and data from all the sites were combined. Variations across sites and seasons were not focussed on because the objective was to determine whether the two insect groups could be distinguished using FAs, regardless of spatial variability.

Due to the wide variation in nitrogen source contributions to the river, pooling of aquatic and terrestrial insect groups across sites was avoided, but instead, isotopic values of aquatic and terrestrial insect groups of each site were analysed separately. Only the summer isotope data were presented because seasonality was important in addressing this hypothesis. T-tests were used to investigate significance of differences in isotope values between aquatic and terrestrial insect groups.

***Hypothesis* 2.** *There is inter-specific niche partitioning among web-building spider taxa because larger spiders feed on larger prey whilst spider foraging behaviour is specialized to capture particular groups of insects. As such, spider taxa can be distinguished using FA tracer techniques* - The TFA profile of each spider was expressed as FA relative proportions of the TFAs present. Similarity percentage analysis (SIMPER) and nMDS were used as with the insects (see above). To investigate differences in FA profiles between web-building taxonomic groups, all spiders in the same taxonomic group were considered as replicates. Analysis of similarity (ANOSIM) was run to test for statistically significant differences among spider groups (see above). Post ANOSIM multiple comparisons were run to determine the spider taxa that were statistically different from each other.

***Hypothesis* 3.** *Webs of different spiders are biased towards the capture of particular groups of insects and the significance of inter-guild differences (in determining the sharing of available resources) is reflected in the differences in Fa and SI dietary niches between horizontal and vertical web-building spiders* - Isotopic niche widths within spider communities were calculated using SIBER (Stable Isotope Bayesian Ellipses in R) metrics in R Version 26 2.12.0. Bayesian ellipses (relatively unbiased with respect to sample size; Jackson et al. 2011) encompassing at least 40% of the data were drawn in isotopic space. All spiders were categorized according to two of the web types, vertical and horizontal webs. The concept of 'the niche' is typically applied in population analysis, but SIA metrics are considered informative for characterizing patterns in food web structure (Layman et al. 2007a, 2007b), and therefore can be used to analyse many individuals across diverse taxonomic groups for which direct diet analysis may be difficult (Layman and Post 2008). The method of pooling individuals by web guilds predictably results in poor taxonomic resolution, but it provides an integrated measure of isotopic niche width representing trophic niche at the guild level (Jackson et al. 2011). The trophic niche widths of different web guilds were represented by the areas within the ellipses and expressed as sample size corrected standard ellipse areas (SEAc). The extent of niche overlap was estimated by dividing the SEAc of the overlap by the SEAc of the ellipse, and expressed as a percentage.

As an additional measure of trophic niche, the SIBER routine was adopted to analyse niche partitioning among the two spider guilds using the x and y coordinates of the nMDS analysis of FA profiles of each individual. The areas of the ellipses represented the calculated FA niche widths, parallel to the isotopic niche (Antonio and Richoux 2014).

4.3. **Results**

4.3.1. Variations amongst aquatic and terrestrial prey

4.3.1.1. Fatty acid variations

The FA profiles of aquatic insects were distinct from those of terrestrial insects (Global $R = 0.614$, $p < 0.001$). The FAs 18:1ω9 (14%), 18:2ω6 (12%), 16:0 (11%), 16:1ω7 (11%), 20:5ω3 (8%), 18:1ω7 (8%), 18:3ω3 (7%) and 18:0 (6%) were responsible for 77% of the variation in FAs profiles between aquatic and terrestrial insects. The nMDS analysis (Figure 4-1) showed that 18:1ω9, 18:2ω6 and 18:0 were the main drivers of FA profiles of terrestrial insects whilst 18:1ω7, 20:5ω3, 16:1ω7, 18:3ω3 and 16:0 were the main drivers for aquatic insect FA profiles.

The levels of most major fatty acids (FAs), except 16:0 and 18:3ω3, were distinct across the aquatic and terrestrial insect groups (Table 4-1). The proportions of 18:0, 18:1ω9 and 18:2ω6 were highest in the terrestrial insects whilst 16:1ω7, 18:1ω7, and 20:5ω3 were highest in the aquatic insects. The proportions of the ω3, ω3/ω6 and EFA groups were significantly higher ($p < 0.05$) in the aquatic insects than in the terrestrial insects.

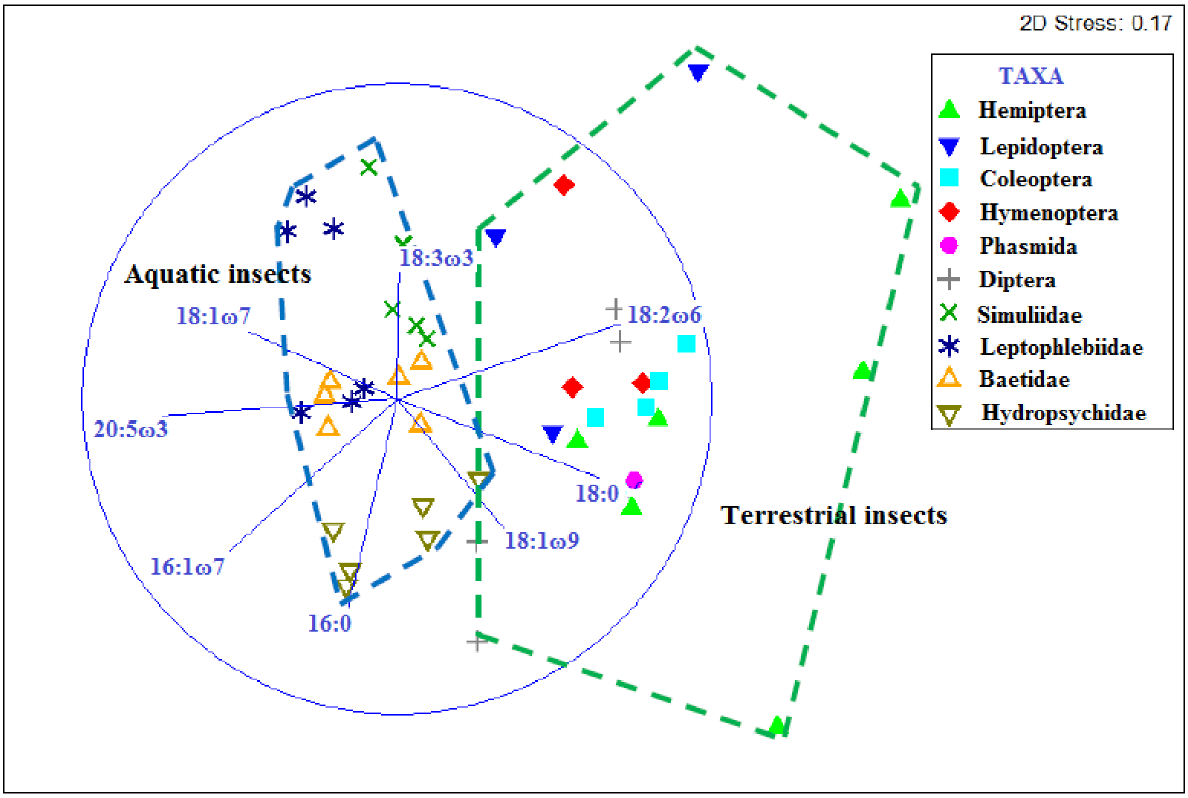


Figure 4-1: Non metric multidimensional scaling representation of aquatic and terrestrial insect TFA composition during summer (November 2012 to February 2013) in the Kowie River catchment. Data represent insects collected from three sites of varying width in the Kowie River. Data were ordinated by TFA composition and each colour represents an insect taxonomic group (family or order). Aquatic insect data were obtained from a concurrent study in the Kowie River (Moyo 2016).

Table 4-1: Mean (with standard deviation) proportions of selected FAs (and groups) found in the aquatic and terrestrial insects collected from the Kowie River from November 2012 to February 2013. Data for aquatic insects were obtained from a parallel study (Moyo 2016). T-test results of comparisons between aquatic and terrestrial insects were also presented. A star (*) represents statistically significant differences ($p < 0.05$).

	Terrestrial insects			**Aquatic insects**			**t-test**		
Fatty acid	**Mean**	**s.d.**	**N**	**Mean**	**s.d.**	**N**	**t-value**	**df**	**p**
16:0	20.9	10.9	21	23.3	9.5	24	-0.8	43	0.43
18:0	11.0	8.9	21	6.0	1.8	24	2.7	43	0.01*
16:1ω7	5.9	9.8	21	13.4	9.4	24	-2.6	43	0.01*
18:1ω7	3.5	6.4	21	8.2	5.9	24	-2.6	43	0.01*
18:1ω9	23.1	11.6	21	13.8	6.5	24	3.4	43	<0.01*
18:2ω6	18.0	14.1	21	7.5	3.9	24	3.5	43	<0.01*
18:3ω3	7.7	8.9	21	7.2	4.2	24	0.3	43	0.80
20:5ω3	0.5	0.7	21	9.1	3.2	24	-12.1	43	<0.01*
ω3	8.3	9.3	21	18.1	7.4	24	-3.9	43	<0.01*
ω3/ω6	0.6	0.9	21	2.0	0.9	24	-5.0	43	<0.01*
Sum PUFA	28.2	16.3	21	27.9	8.8	24	0.1	43	0.95
Sum EFA	1.0	1.4	21	10.8	3.1	24	-13.4	43	<0.01*
Sum MUFA	33.3	16.2	21	38.8	13.6	24	-1.2	43	0.22

4.3.1.2. Isotopic variations

The $\delta^{15}N$ isotope values of aquatic and terrestrial insects were distinct at the wide ($t = 13.8$, d.f. $= 54.3$, $p < 0.001$) and intermediate ($t = 12.8$, d.f. $= 59.6$, $p < 0.001$) sites, but no significant differences were observed at the narrow site ($t = -0.6$, d.f. $= 75.6$, $p = 0.5$). The $\delta^{13}C$ isotope values of aquatic and terrestrial insects were distinct at all the three sites; narrow ($t = -4.8$, d.f. $= 66.4$, $p < 0.001$), wide ($t = -6.1$, d.f. $= 43.6$, $p < 0.001$) and intermediate ($t = -6.7$, d.f. $= 49.4$, $p < 0.001$) sites. There was relatively higher variability in $\delta^{13}C$ and $\delta^{15}N$ values among terrestrial than aquatic insects at all the sites (Table 4-2, Figure 4-2). Most terrestrial insects had $\delta^{13}C$ values that were between -20 and -28 ‰, but some individuals in the Orthoptera, Diptera and Hemiptera orders had values between -10 and -20 ‰ (Table 4-2).The $\delta^{15}N$ isotope values of all insects at the narrow site were much lower than insects at the wide and intermediate sites. The $\delta^{15}N$ isotope values of terrestrial insects at the wide site were not significantly different from those at the intermediate site ($t = 1.8$, d.f. = 86, $p = 0.08$), but the aquatic insect values were distinct between the two sites ($t = 4.3$, d.f. = 196, $p < 0.001$).

Table 4-2: A summary of mean [with standard deviation (s.d.)] carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values of terrestrial and aquatic insects collected at the narrow (n), wide (w) and intermediate (i) sites along the Kowie River. The insects were categorized into three major foraging groups: herbivores (H), predators (P) and mixed feeders (M).

Foraging group	Insect orders	Site	N	$\delta^{15}N$ (‰) Mean	s.d.	$\delta^{13}C$ (‰) Mean	s.d.
Aquatic Insects							
H	Ephemeroptera	w	30	17.1	1.7	-26.7	2.3
H	Ephemeroptera	n	26	5.7	1	-27.7	2.2
H	Ephemeroptera	i	33	16.3	1.5	-26.9	2.3
H	Trichoptera	w	12	18.1	1.7	-26.6	1.9
H	Trichoptera	n	12	6.8	0.6	-27.1	1.1
H	Trichoptera	i	18	17	0.9	-26.8	1.8
M	Diptera	w	29	17.7	2.2	-27.2	2.4
M	Diptera	n	25	4.6	1.8	-26.9	1.2
M	Diptera	i	26	17.4	1.9	-26.3	1.4
P	Odonata	w	24	18.3	1.6	-26.9	1.9
P	Odonata	n	31	7	0.8	-27.1	1.3
P	Odonata	i	30	17.1	0.8	-26.1	1
Terrestrial insects							
H	Blattodea	w	1	5	-	-23.7	-
H	Lepidoptera	w	6	11.7	2.3	-27.9	1.3
H	Lepidoptera	i	8	10.7	3	-21.6	5.8
H	Lepidoptera	n	13	5.2	4.4	-26.2	3.6
H	Orthoptera	w	4	11	0.3	-12.7	0.8
H	Orthoptera	i	3	14	0.6	-14	0.3
H	Orthoptera	n	2	4.5	1.9	-26	0.7
M	Coleoptera	w	9	9.1	1.9	-20.9	6.5
M	Coleoptera	i	7	7	2.4	-25.1	6.1
M	Coleoptera	n	10	5.1	2.5	-23.7	3.5
M	Diptera	w	4	14.2	3.9	-24.1	0.5
M	Diptera	i	6	11.3	2.9	-19.5	3.6
M	Diptera	n	14	9.6	3.4	-23.1	4.4
M	Hemiptera	w	8	8.1	3.4	-17.6	8.4
M	Hemiptera	i	7	8	3.8	-15.8	5
M	Hemiptera	n	7	5.3	1.2	-20.7	7
P	Hymenoptera	w	7	8.9	3.6	-24.1	1.2
P	Hymenoptera	i	14	6.5	3	-23.2	3.2
P	Hymenoptera	n	14	6.8	1.7	-23.8	4.4
P	Mantodea	w	2	11.8	2.4	-21.4	2.4
P	Mantodea	n	1	7.9	-	-24.9	-
P	Neuroptera	w	1	14.3	-	-27.1	-

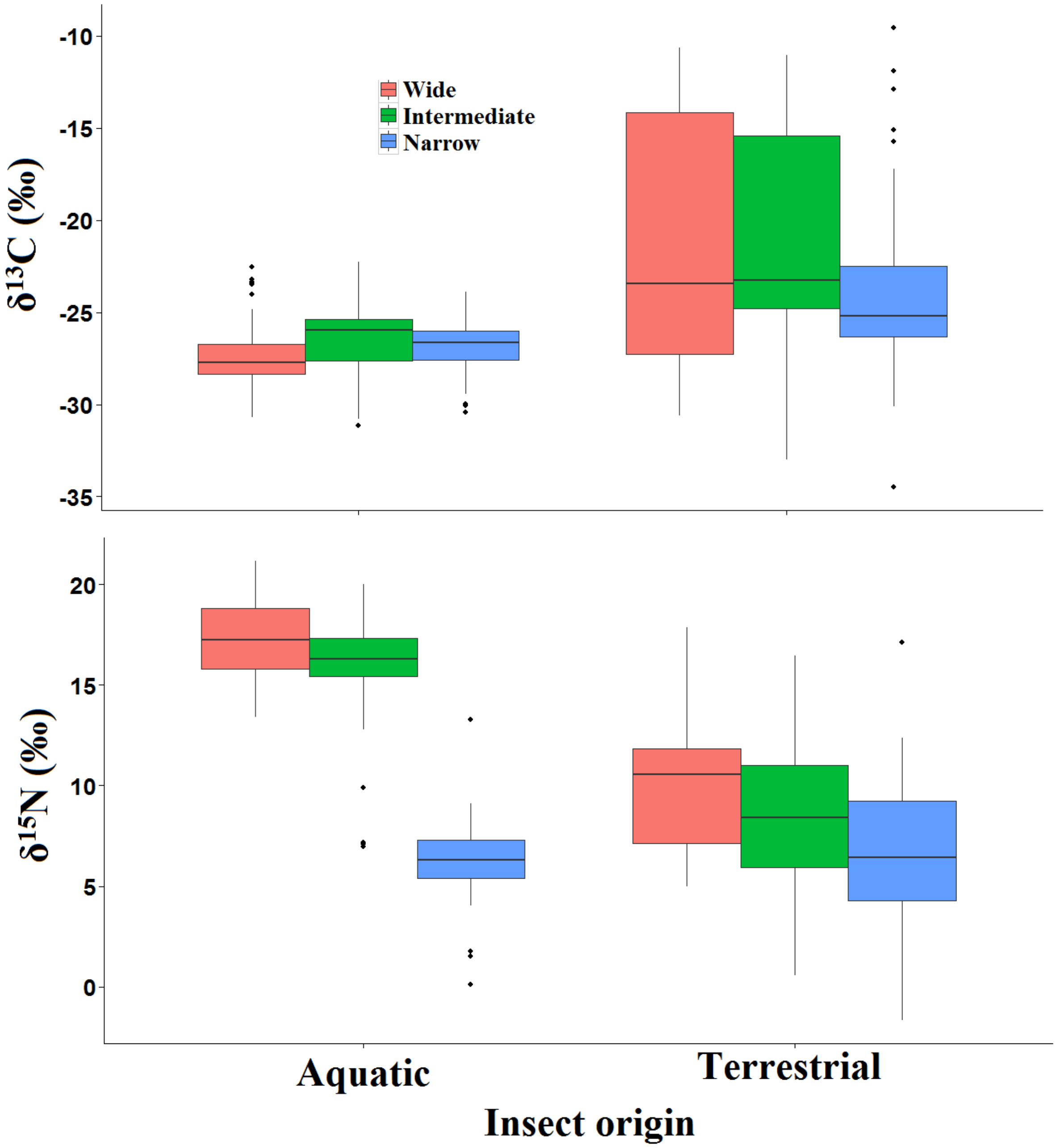


Figure 4-2: δ^{13}C (a) and δ^{15}N (b) values (‰) of terrestrial and aquatic (Moyo 2016) insects collected from the three study sites in the Kowie River during four seasons (annual). Graph shows medians with quartiles and outliers (dots).

4.3.2. Web-building spider diet by taxonomic group

Fatty acid profiles of spiders were ordinated by taxonomic grouping (regardless of distance at which the spiders were collected) and taxon-specific groupings were observed (Figure 4-3). The ANOSIM results showed that there were significant differences in FA profiles among the taxonomic groups at the narrow (Global R = 0.163, $p < 0.001$), wide (Global R = 0.426, $p < 0.001$) and intermediate (Global R = 0.289, $p < 0.001$) sites.

At the narrow site, multiple comparisons (pairwise tests) showed that the TFA compositions of the genera *Tetragnatha* and *Leucauge* were significantly ($p < 0.05$) different from most of the other spider taxa. Spiders of the genus *Tetragnatha* were significantly different from those of *Leucauge* ($R = 0.68$, $p = 0.001$), *Caerostris* ($R = 0.10$, $p = 0.04$), *Cyrtophora* ($R = 0.38$, $p = 0.074$), *Nephila* ($R = 0.51$, $p = 0.008$) and those of the family Deinopidae ($R = 0.5$, $p = 0.003$); whilst spiders in the *Leucauge* genus had TFAs that were significantly different from those in *Caerostris* (Global $R = 0.20$, $p = 0.03$), *Cyrtophora* ($R = 0.25$, $p = 0.003$), *Nephila* ($R = 0.54$, $p = 0.002$) and the families Uloboridae ($R = 0.80$, $p = 0.02$) and Deinopidae ($R = 0.6$, $p = 0.004$). There was no evidence of significant differences ($p > 0.05$) between any of the other taxa.

At the wide site, multiple comparisons showed that the TFA profiles of the genus *Tetragnatha* were significantly different from those of *Araneus* (Global $R = 0.67$, $p < 0.001$), *Argiope* (Global $R = 0.95$, $p = 0.007$), *Caerostris* (Global $R = 0.89$, $p = 0.001$) and *Neoscona* (Global $R = 0.61$, $p < 0.001$). There was no evidence of significant differences ($p > 0.05$) between *Tetragnatha* and Deinopidae and Uloboridae, or amongst all the other spider taxa except between *Neoscona* and *Araneus* (Global $R = 0.10$, $p = 0.04$).

At the intermediate site, multiple comparisons (pairwise tests) showed that the TFA profiles of the genus *Tetragnatha* were significantly different from all the other spider taxa, including *Araneus* (Global $R = 0.61$, $p = 0.009$), *Argiope* (Global $R = 0.86$, $p = 0.02$), *Caerostris* (Global $R = 0.20$, $p = 0.02$), *Gasteracantha* (Global $R = 0.58$, $p = 0.006$), *Leucauge* (Global $R = 0.6$, $p = 0.001$), *Nephila* (Global $R = 0.68$, $p = 0.001$) and the family Theridiidae (Global $R = 0.46$, $p = 0.001$). The family Theridiidae was also significantly different from *Argiope* (Global $R = 0.50$, $p = 0.04$), *Caerostris* (Global $R = 0.2$, $p = 0.02$), *Leucauge* (Global $R = 0.5$, $p = 0.001$) and *Nephila* (Global $R = 0.6$, $p = 0.001$). Female *Nephila* TFA profiles were significantly different from those of *Caerostris* (Global $R = 0.18$, $p = 0.003$) and male *Nephila* (Global $R = 0.63$, $p = 0.03$). There was no evidence of any significant different amongst the other spider taxa that were collected.

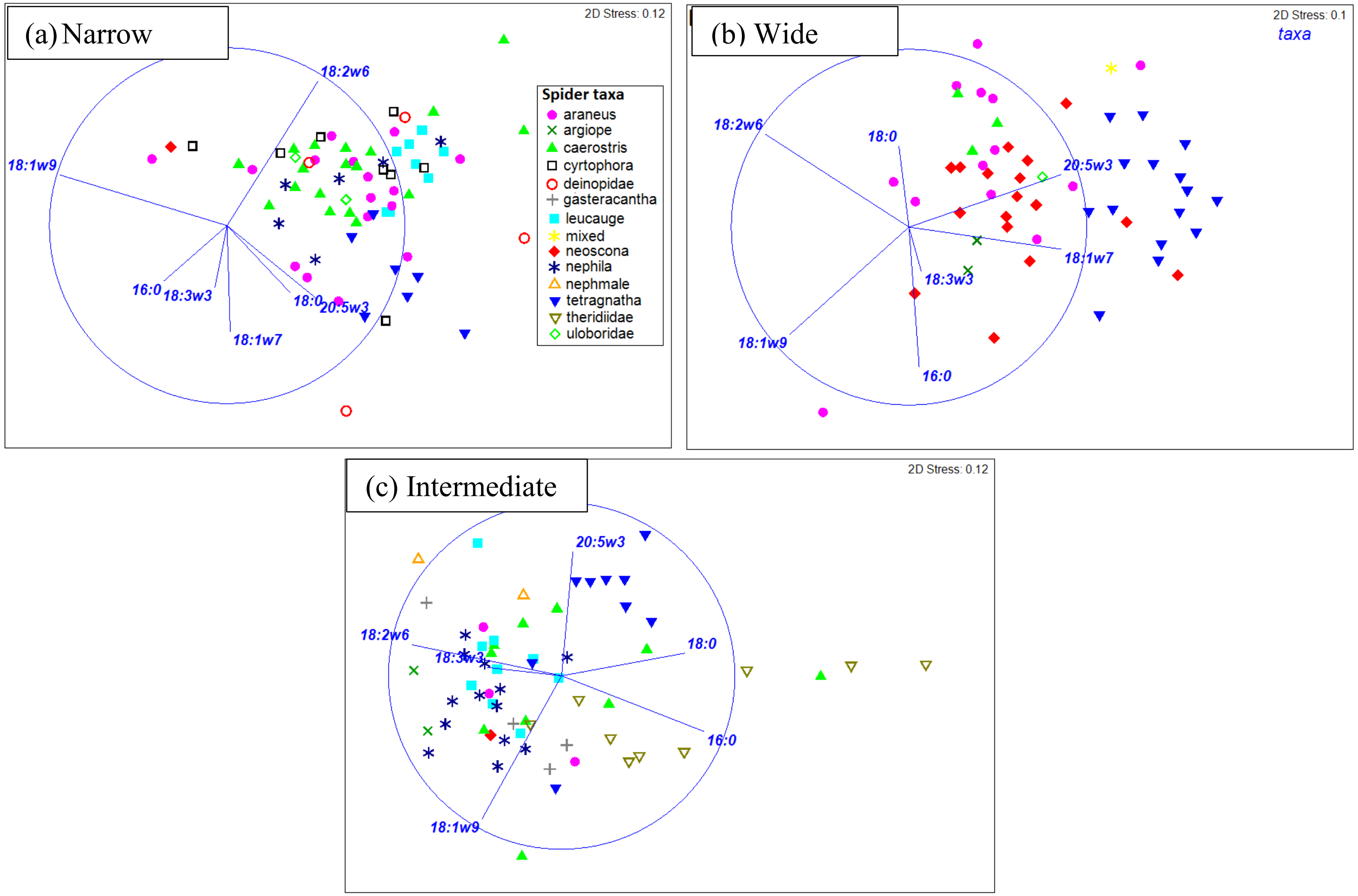


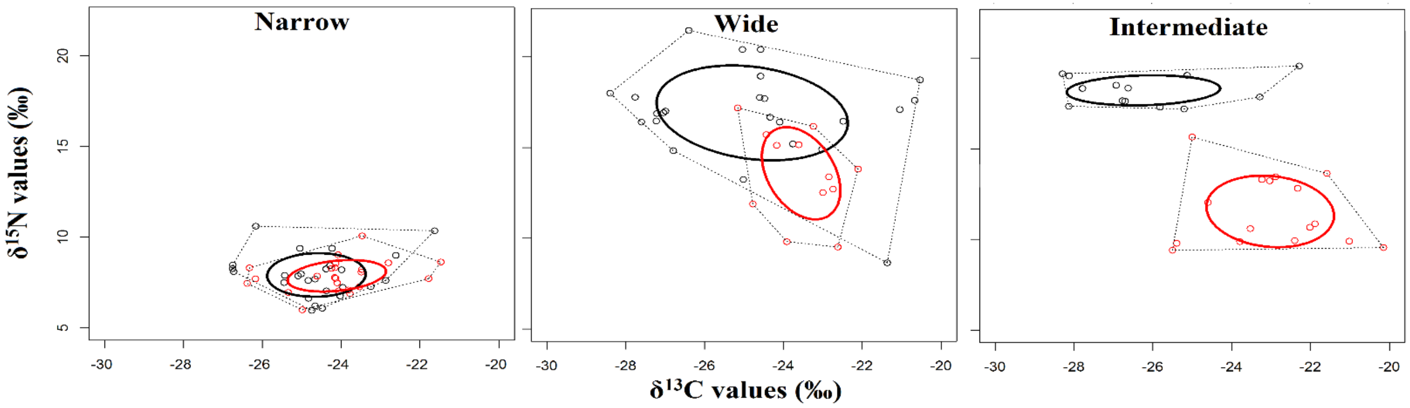
Figure 4-3: Non metric multidimensional scaling representation of annual TFA composition of web building spiders (represented by different colours) collected from the a) narrow, b) wide and c) intermediate sites along the Kowie River. Data were ordinated by spider taxa.

4.3.3. Variation in diet niches between horizontal and vertical web-building spiders

Spider genera and families were selected for diet niche analysis (using SIBER) based on the hunting strategies indicated by the web orientation. Two major web orientation types were horizontal and vertical (Figure 4-4). Based on field observations, *Tetragnatha*, *Leucauge, Cyrtophora, Uloboris* and *Deinopis* were categorized as horizontal web builders, whilst *Caerostris*, *Araneus*, *Nephila*, *Neoscona* and *Argiope* were categorized as vertical web builders. The two web guilds occurred together at high frequencies only at distances close to the river, so only the data from the first two transects (0 m and 2 m) were used in the spider niche analysis. The two spider web guilds were assessed for their isotopic and FA niche widths using standard ellipse areas (Figure 4-4).

There was separation of isotopic and FA niches between vertical and horizontal web-building spiders, at all the three sites (Figure 4-4). The horizontal web guild had wider isotopic and FA niche widths than the vertical guild at the narrow (*horizontal isotopic SEAc = 4.7*, *vertical isotopic SEAc = 3.3*, horizontal FA SEAc = 0.043, vertical FA SEAc = 0.041) and wide (*horizontal isotopic SEAc = 19.5*, *vertical isotopic SEAc = 7.5*, horizontal FA SEAc = 0.26, vertical FA SEAc = 0.053) sites. At the intermediate site, the vertical web guild (SEAc = 9.9) had a wider isotopic niche width than the horizontal web guild (SEAc = 5.1), whilst FA niches were similar (SEAc = 0.078). Niche widths were generally larger at the wide and intermediate sites than at the narrow site. The degree of niche overlap between the two guilds very highest at the narrow site (53% and 20.8% of the horizontal web guild, based on SI and FA, respectively). The degree of overlap was much lesser at the wide site (10 and 0 % of the horizontal web guild). In the absence of spring data, there was little to no niche overlap between the two web guilds at the intermediate site (0 and 1.2 % of the horizontal web guild).

a) Isotopic feeding niche



b) Fatty acid feeding niche

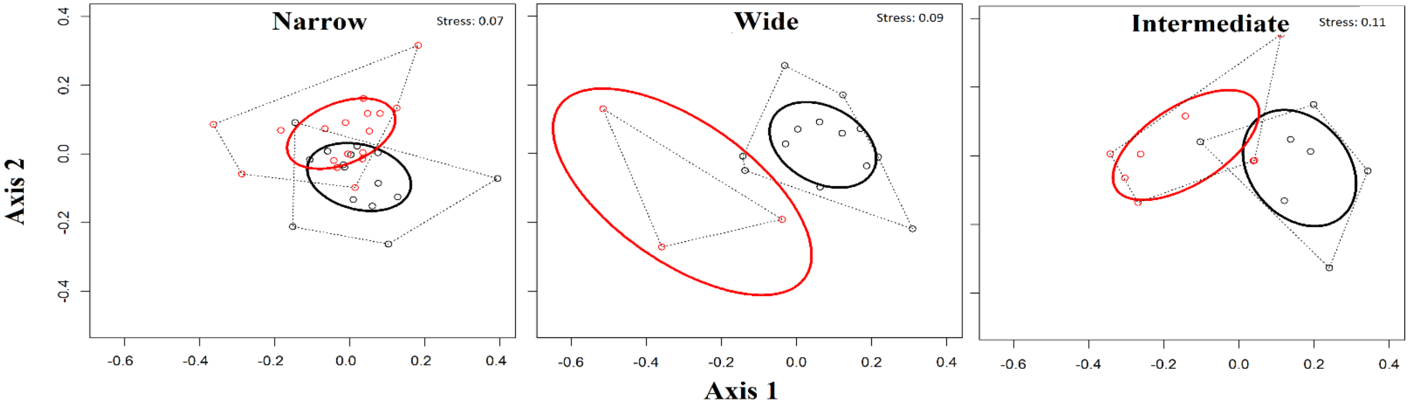


Figure 4-4: A dual a) isotope ($\delta^{13}C$ and $\delta^{15}N$, ‰) and b) FA (nMDS x-y ordinates) plots representing the annual niche widths of horizontal (black line) and vertical (red line) web building spiders at the narrow, wide and intermediate sections of the Kowie River. Spiders were not collected during spring at the intermediate site, hence the niche spaces represent spider diets only during summer, autumn and winter months. The plots were created using SIBER and the ellipses (solid lines) represent sample size corrected niche widths and the dotted lines are convex hulls.

4.4. **Discussion**

4.4.1. Variations amongst aquatic and terrestrial prey (Hypothesis 1)

4.4.1.1. Fatty acid variations

As hypothesized (hypothesis 1), results of my study showed that the FA profiles of aquatic and terrestrial insects were indeed distinct. A few studies have explored the compositional differences between aquatic and terrestrial insect groups and their results show clear distinctions between these prey groups (Fontaneto et al. 2011, Hixson et al. 2015). In a world-wide meta-analysis of studies conducted on a variety of aquatic and terrestrial insect taxa, Fontaneto et al. (2011) reported significant differences in proportions of important long-chain polyunsaturated FAs (LC-PUFA) between aquatic and terrestrial insects. With the use of linear models, their study showed that terrestrial insects were high in ω6 PUFAs whilst aquatic insect were high in ω3 PUFAs. Similarly, in my study, 18:2ω6 was among the FAs driving terrestrial insect FA profiles whilst 20:5ω3 and 18:3ω3 were among those driving FA profiles in aquatic insects. Hixson et al. (2015) followed a similar approach to Fontaneto et al. (2011), where they collected FA data from the primary, peer-reviewed, scientific literature and from the authors' unpublished sources. Their review showed that aquatic and terrestrial insects had distinct proportions of individual ω3 or ω6 LC-PUFAs or groups of FAs (total MUFAs and PUFAs) in both aquatic and terrestrial organisms. The presence of measureable differences in FAs profiles of aquatic and terrestrial insects in my study is well in accordance with the results obtained from the two meta-analyses.

Although aquatic and terrestrial insect FA profiles were distinct, there was high variability within terrestrial and aquatic insect groups, potentially due to confounding effects like stream width, trophic level and physiology that I did not include in my analysis. In addition to habitat (aquatic or terrestrial), the trophic level is an important factor in determining the proportions of ω3 or ω6 LC-PUFAs or groups of FAs (total MUFAs and PUFAs) in both aquatic and terrestrial organisms (Hixson et al. 2015). The nature of inter- and intra-taxonomic variation in FA composition remains poorly documented. Most FA studies on aquatic and terrestrial insect groups have been descriptive and they have been used to evaluate the usefulness of insects as potential sources of PUFAs for human beings in countries such as Thailand (Yang et al. 2006, Raksakantong et al. 2010), Japan (Fontaneto et al. 2011), Cameroon (Womeni et al. 2009), Belize (Wang et al. 2006), Zaire (Bukkens and Paoletti 2005) and Venezuela (Paoletti et al. 2003). My study shows that in insects in southern Africa, the FAs 18:0, 18:1ω9 and 18:2ω6 can be indicative of terrestrial consumption whilst 16:1ω7, 18:1ω7, 20:5ω3 together with ω3, ω3/ω6 and EFAs, can be indicative of aquatic consumption.

4.4.1.2. Isotopic variations

As hypothesized (hypothesis 1), my study showed that the SI signatures of the two insect groups were distinct at all the sites. These results are consistent with other studies that have compared aquatic and terrestrial insect isotopic values (Collier et al. 2002, Bennett and Hobson 2009, Speir et al. 2014). Nevertheless, there were higher variations in terrestrial insect $\delta^{13}C$ and $\delta^{15}N$ values than with the aquatic insects. It is likely that the terrestrial insects were more diverse and had a higher diversity of food sources than those in the aquatic environment. The differences in aquatic insect $\delta^{15}N$ values across the three sites were likely a result of different nitrogen sources. Fertilization, pollution and other stream ecosystem perturbations can alter the flow of elements such as nitrogen (Peterson and Fry 1987). In my study, the values of $\delta^{15}N$ were higher at the wide and intermediate sites and distinctly lower at the narrow (Figure 4-2). The narrow site was relatively pristine when compared to the other two sites that were downstream of several farms and human populated areas that constituted several sources of $\delta^{15}N$ (Chapter 2). Some of the tributaries drain cattle grazing areas that probably provide a specific source of nitrogen from cattle dung (Roux and Oelofse 2010, Jacobs et al. 2012). Additionally, the municipal runoff of Grahamstown and waste water effluent is released into the head waters together with pollutants from local farms and mines (Roux and Oelofse 2010, Jacobs et al. 2012). Caution is needed when interpreting results on $\delta^{15}N$ values because the $\delta^{15}N$ values of riparian spiders are likely to include both anthropogenic nitrogen and isotope discrimination during the feeding process (e.g. Akamatsu and Toda 2011). Therefore higher N values do not necessarily mean total nitrogen exported at the site increases.

Due to variations in fixation in the carbon cycle, the $\delta^{13}C$ values of primary producers in freshwater (algae and macrophytes) are expected to be different to those in terrestrial landscapes (Peterson and Fry 1987). In my study, the differences in $\delta^{13}C$ values between aquatic and terrestrial insects suggested that the two insect groups had different sources of food. The high variability in $\delta^{13}C$ values among terrestrial insects indicates that terrestrial insects had higher trophic diversity than the aquatic insects. Terrestrial herbivorous insects are exposed to a high diversity of plants. Higher plants fall into three categories, C3, C4 and CAM plants. Plants possessing the C3 photosynthetic pathway fix carbon differently from C4 plants (Smith and Epstein 1971). The $\delta^{13}C$ values of C4 plants range from -24 to -34 ‰ whilst those of C3 have a much wider range of -6 to -19 ‰ (Smith and Epstein 1971, Deniro and Epstein 1978). The large differences in $\delta^{13}C$ values between C3 and C4 plants should be reflected in the carbon isotopic composition of consumers, which derive their carbon predominantly from plants of one or the other photosynthetic type (Belivanov and Hambäck 2015). My study did not include detailed species specific comparative analysis of isotopic values of terrestrial plants, but both C3 and C4 plants were observed in the study area. It is highly probable that the terrestrial herbivorous insects were using both types of plants as carbon sources. Also, some polyphagous insects could have been consuming both C3 and C4 plants, resulting in intermediate isotopic values and high variations in $\delta^{13}C$ values.

4.4.2. Diet variations between taxonomic groups of web-building spiders (Hypothesis 2)

I hypothesized that web-building spider taxonomic groups have varied diets and FA results show wide variations in FA profiles amongst the spiders. The high variation in FA profiles among spider taxonomic groups was also consistent with their generalist feeding nature (Figure 4-3). Web-building spiders are typically passive and they consume almost any insect that is captured by their webs (Robinson and Robinson 1970, Rypstra 1982, Castillo and Eberhard 1983, Nentwig 1983, Nyffeler 1999, 2009, Shimazaki and Miyashita 2005). Despite the high variation in FA profiles, a few taxonomic groups had similar FA profiles, especially at the wide site. The groupings suggested that some taxonomic groups had specialised diets or prey biases. Signs of prey selectivity or preference (Nyffeler et al. 1989, Nyffeler 1999, Ludy 2007) have been detected in some species of spiders through direct observations.

While spiders are generalist predators, they have developed varied web types to feed on different insect groups (Olive 1980, Gregorič et al. 2015). The nMDS revealed distinct groupings based on spider taxa, particularly between the genera *Tetragnatha* and *Leucauge* and the other families/genera (Figure 4-3). Other studies have shown that *Tetragnatha* feeds mainly on aquatic insects that its horizontal web type is specialized to catch (Williams et al. 1995, Gillespie 2003, Kato et al. 2004, Krell et al. 2015, Tagwireyi and Sullivan 2015). Similar to *Tetragnatha*, individuals of the genus *Leucauge* also build horizontal orb webs that are suited for capturing emerging insects (Eberhard 1990, Bishop and Connolly 1992). A more horizontal orientation allows spiders to build closer to microhabitats with abundant prey (Eberhard 1990, Bishop and Connolly 1992), or to utilize limited support structures for web-building above the river (Buskirk 1975, Riechert and Gillespie 1986).

In the Kowie River, the genera *Tetragnatha*, *Leucauge* and other potential horizontal web-building spiders such as those of the family Uloboridae were mostly found between 0 and 2 m from the river, where the abundance of aquatic insects was high (Chapter 3). Web-building spiders may select habitats as a response to prey activity (Welch et al. 2013). The high aquatic insect abundance at the stream edges may be an important attractant of horizontal web-building spiders such as *Tetragnatha* and *Leucauge* that build webs that are suited to maximize the use of the high abundance of emerging insects. Therefore, although all spiders are generalists, their webs can be considered as selective traps for particular groups or types of insects. The size, orientation and placement of the webs may be the most important factors affecting their diets (Nentwig 1986, Nyffeler 1999, Snyder and Wise 2001). The placement of large horizontal webs over the stream is likely to enable *Tetragnatha* to feed on largely aquatic insects.

The variability among web-building spider taxa may be influenced by the width of the river or other habitat factors that vary across sites (see Chapters 5 and 6). Based on FA profiles, nMDS revealed more taxonomic groupings at the wide and intermediate sites than the narrow site (Figure 4-3). The narrow site had a narrower channel (1.49 ± 0.36 m) and consequently lower emergence rates (Moyo 2016), whilst the wide (11.28 ± 4.90 m) and intermediate (5.5 m) sites had wider channels and higher emergence rates (Moyo 2016). The sites with higher aquatic emergence rates probably enabled some spiders, such as the horizontal web-builders, to become temporary specialist feeders on highly abundant species of aquatic insects. Other riparian generalist predators such as dragonflies display a similar foraging strategy of becoming temporary specialists on the most abundant prey species (Corbet 1999).

4.4.3. Diet niches in horizontal and vertical web-building spiders (Hypothesis 3)

I hypothesized (hypothesis 3) that diet niches of horizontal and vertical web-building spiders are different and the SIBER models showed the two web guilds (horizontal and vertical web builders) had separate isotopic and FA niches that overlapped at the narrow and wide sites (Figure 4-4). Fitting spiders to functional groups (or guilds) according to their web orientation (horizontal/vertical) resulted in mostly separated niches, which indicated that web orientation was meaningful for separating functional entities in riparian web-building spiders. My results are consistent with generalizations about the preferred diets of the two guilds (Figure 4-5). Horizontal web-building spiders have webs that are suited for capturing small or weakly flying aquatic insects such as true flies (Chironomidae) and mayflies (Ephemeroptera) (Yoshida 1989, Williams et al. 1995, Marczak and Richardson 2007). Vertical web-building spiders have webs that are structurally more suited for catching larger, faster and stronger flying terrestrial prey (Olive 1982).

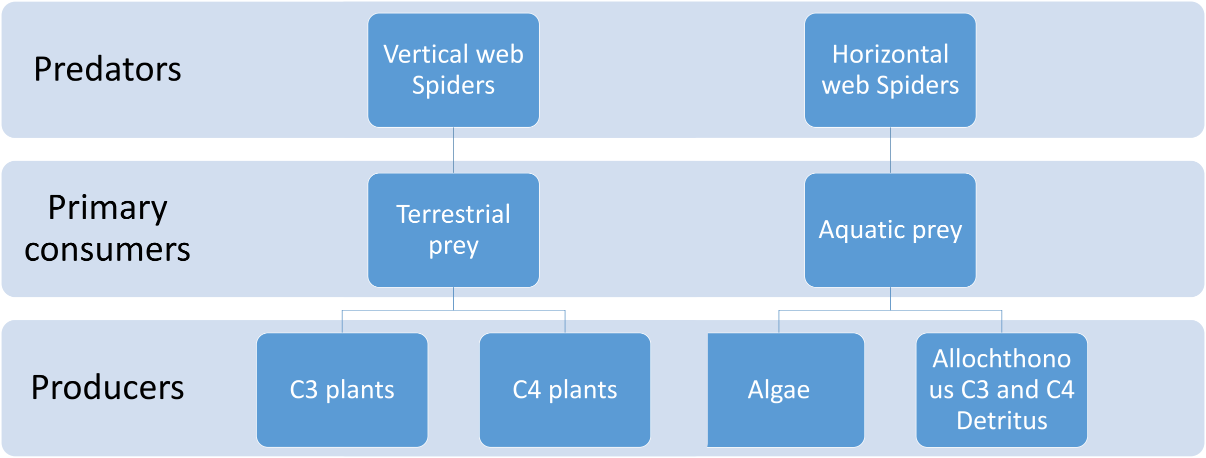


Figure 4-5: Generalized food chains illustrating trophic pathways of two major spider web-building guilds, vertical and horizontal web-builders, in the Kowie River. Web orientation biases each guild to one of two types of prey, aquatic or terrestrial, that have different food sources. Note: algae are typical C3 plants, but some may possess enzymes that are present in C4 plants.

The high dietary overlap at the narrow site (isotopic = 55.3%, FA = 20.8%) as compared to the low overlap at the wide site (isotopic = 10%, FA = no overlap), suggested that horizontal and vertical web-building spiders largely fed on the similar resources. However, caution is needed in interpreting the high dietary overlap at the narrow section because $\delta^{15}N$ isotope values of aquatic and terrestrial insects were indistinguishable (t = -0.6, d.f. = 75.6, p = 0.5). Stable isotope analysis may not be able to reliably estimate spider dietary niches in such situations and other diet analysis tools may be necessary to confirm the results. The lack of isotopic niche overlap at the intermediate site suggested that the two web guilds fed on different insect groups or taxa. Stable isotope mixing models (Chapter 5) confirmed that both guilds consumed aquatic and terrestrial material. In addition to the terrestrial insects that vertical web-building spiders are suited to catch, the vertical builders could have been consuming the faster (and stronger) flying aquatic insects that could have evaded the horizontal webs or insects that crawled out of the stream rather than emerging vertically. The horizontal web builders could have been consuming the smaller and weaker flying terrestrial insects such as small flies (Diptera; Cyclorrhapha, Drosophilidae and Phoridae), bugs (Heteroptera and Homoptera) and wasps

(Hymenoptera) (Yoshimoto and Gressitt 1964, Compton et al. 2000, Kirk-Spriggs and McGregor 2009), in addition to the weaker flying aquatic insects that most vertical webs are not suited to catch (Yoshida 1989, Williams et al. 1995, Marczak and Richardson 2007).

The characteristically low aquatic insect emergence rates at the narrow site (Chapter 3, Moyo 2016) may have required the riparian spiders to remain primarily consumers of the readily available small and weak-flying terrestrial insects. This claim supports the notion that riparian spiders feed primarily in the terrestrial habitats but opportunistically consume allochthonous material (Ostfeld and Keesing 2000, Ballinger and Lake 2006). Aquatic insect emergence was higher at the wide and intermediate sites and this possibly caused greater separation between the two guilds as the horizontal web builders were able to respond opportunistically to the larger pulses of aquatic insects whilst the vertical web-builders remained as terrestrial insect consumers.

It is possible that these web morphologies may have also been evolved by different spider taxa as a result of competitive interactions. Being opportunistic feeders, web-building spiders frequently switch their diets in response to available prey. To date, these diet shifts are poorly understood (Belivanov and Hambäck 2015). My study shows that there are guild specific (vertical and horizontal web-builders) responses to varying levels of insect emergence across sites. Species compositions of predator diets are not always possible to determine using SI techniques (e.g. Hobson 1993, Koch et al. 1995, Iverson et al. 2004), but the contrasting isotopic niches of co-occurring web-builders in the Kowie River have provided valuable new information about the partitioning of nutrients in this region.

4.5. **Conclusion**

In spite of the taxonomic variations within aquatic and terrestrial environments, my study showed that FA and SI indicators can be used to distinguish aquatic from terrestrial insects. Stable isotope and FA indicators also proved to be useful tracer methods in distinguishing web-building spider groups. Despite the observed FA and isotopic differences across taxonomic groups it remains to be seen how collective (pooled) samples of spiders can be used to trace the movement of aquatic subsidies from the river into the adjacent terrestrial landscape. The following two chapters (Chapters 5 and 6) assess the use of pooled samples to trace movement of aquatic subsidies across the aquatic-terrestrial interface and further into terrestrial habitats.

Chapter 5. TEMPORAL AND SPATIAL VARIATIONS IN CROSS-BOUNDARY TROPHIC SUBSIDISATIONS FOR RIPARIAN COMMUNITIES: A STABLE ISOTOPE MARKER ANALYSIS

5.1. Introduction

Web-building spiders are major predators in riparian areas and they play an important role in linking adjacent aquatic and terrestrial ecosystems (e.g. Speir et al. 2014). Aquatic nutrients in the form of emerging insects pass into terrestrial ecosystems through riparian web-building spider predation. The availability of prey drives spider communities (Uetz 1979) and the presence of high abundances of insects in riparian areas supports high abundances of web-building spiders and other predators (Henschel et al. 2001, Sabo and Power 2002b, Baxter et al. 2005). Experimental (e.g. Kato et al. 2003) and observational (e.g. Nyffeler 2009) studies have been carried out to investigate the importance of aquatic subsidies in structuring spider communities. More recently, SIA tools (Collier et al. 2002, Kato et al. 2003, 2004, Sanzone et al. 2003, Akamatsu et al. 2004, Briers et al. 2005, Paetzold et al. 2006, Marczak and Richardson 2007, Alp et al. 2013, Speir et al. 2014) have been used to quantify the dietary contributions of aquatic and terrestrial prey to spider diets.

Web-building spiders are highly abundant at riversides (Power and Rainey 2000, Nakano and Murakami 2001) and it is expected that their diets largely consist of aquatic prey that are also highly abundant at stream edges (Power and Rainey 2000). Evidence of high aquatic prey contributions to spider diets has been provided by directly collecting and identifying insect prey caught in spider webs and consequently estimating spider diet composition (e.g. Nentwig 1985, Ludy 2007, Nyffeler 2009). Additionally, Kato et al. (2003) used an experimental prey exclusion technique to determine spider dietary responses under different prey availability conditions. Stable-isotope-aided studies also suggest that the diets of spiders living at stream edges largely consist of aquatic insects. Such studies determined the diets of web-building spiders at the edges of numerous water bodies in varying climatic and habitat conditions, for example; 1) Waitekauri River in the subtropical North Islands of New Zealand (Collier et al. 2002); 2) an intermittent Sonoran desert stream in Arizona, USA (Sanzone et al. 2003); 3) ten experimental ponds near Fort Worth, Texas, USA and 4) Shinano River in the warm temperate town of Sakaki, Japan (Akamatsu et al. 2004). Thus far, SI mixing models (Phillips et al. 2005) have provided the most dependable estimates of consumer diets, although there remains some uncertainty around the results because the δ values of resources frequently overlap and are highly variable (Phillips and Gregg 2001).

A few scientists have described the diets of web-building spiders in relation to their proximity to water bodies (Henschel et al. 2001, Akamatsu et al. 2004, Briers et al. 2005). However, little attention has been focussed on the temporal or spatial variability in aquatic subsidies to terrestrial predators such as spiders (but see Kato et al. 2003). Considering that insect productivity in rivers or terrestrial systems changes seasonally (e.g. Denlinger 1980, Nakano and Murakami 2001), it is important to investigate how spiders' diets respond on a seasonal basis. Flow regime of a river is another important factor that drives seasonal changes in aquatic insect abundances (e.g. Greenwood and Booker 2015). Little is known about whether this trophic connectivity (between the aquatic and terrestrial systems) varies at different parts of the same river system (Kautza and Sullivan 2015).

Rivers generally increase in width and the amount of vegetation cover decreases from the headwaters to the downstream areas and, as described by the River Continuum Concept (Vannote et al. 1980), there are predictable changes in aquatic insect communities along this gradient. Aquatic ecologists have reported changes in dominant macroinvertebrate functional feeding groups from typical shredders (leaf-eaters) such as caddisflies in headwaters, to filter feeders such as black flies wide (e.g. Cummins and Klug 1979, Covich et al. 1999). The Riverine Productivity Model (Thorp and Delong 1994) reflects that primary productivity is generally low in the headwaters, highest in the middle reaches of a river, and remains high in wide areas. Given that headwaters are narrow and supposedly support less aquatic insect productivity than wide sites, trophic connectivity with the terrestrial area is expected to be stronger in the wide sites. However, trophic connectivity is also highly dependent on the receiving community and changes in vegetation structure covering the stream (e.g. Kautza and Sullivan 2015). We currently lack empirical evidence that addresses these expectations.

The objective of this study was to evaluate the contributions of aquatic and terrestrial prey to terrestrial spiders at varying distances from three locations in a river and across different seasons. I hypothesized that *the contribution of aquatic prey subsidies to spider diets is determined by aquatic insect abundances that decrease further inland, increase at wider sections of the river that have larger surface area (stream bed area) for insect emergence, and increase during periods (seasons) of higher emergence rates*.

5.2. **Methods**

The study sites, SI methods and spider and insect collection methods are described in **Chapter 2**. The narrow, wide and intermediate sites represented three sections of the river with different widths.

5.2.1. Variation in isotope values

A preliminary evaluation of the variation in spider isotopic values was made to determine whether there were any differences across seasons and with increasing distance from the river at all the three sites. Two-way factorial ANOVA procedures were used to test for the effects of different factors on the isotope values. The $\delta^{13}C$ and $\delta^{15}N$ values were treated as the dependent variables whilst the factors for spider models were site, season and distance from the river and those for insect models were site, season and insect origin (aquatic or terrestrial).

5.2.2. Determination of prey contributions to spider diets

Stable Isotope Analysis in R (SIAR) mixing models were run in R Version 26 2.12.0 (The R Foundation for Statistical Computing 2010) to determine the percentage contributions of aquatic and terrestrial food sources to the diets of spiders living at different distances from the river, and at different times of the year. Due to the high variability in $\delta^{15}N$ isotopic values of invertebrates (spider prey; Chapter 4; Vander Zanden and Rasmussen 2001, McCutchan et al. 2003), a decision was made not to use insects as the food sources in the models. Instead, the producers that the herbivorous invertebrates feed on were used to infer diet contributions of aquatic- and terrestrially-derived food sources. Additionally, insect $\delta^{13}C$ isotopic values were also very variable in many polyphagous terrestrial insects that consumed both C3 and C4 plants (personal observation). Terrestrial food sources were therefore represented by C3 and C4 plants, whilst aquatic sources were represented by macrophytes and different types of algal forms (epiphyton, benthic algae and periphyton). Producers with similar isotopic compositions were grouped to minimize the number of sources and simplify and narrow the range of possible solutions (Phillips et al. 2005, 2014). Isotope values of all the producers were obtained from concurrent studies (Dalu et al. 2014, Moyo 2016).

Trophic fractionations of 2.3 ‰ (SD = 0.24) for $\delta^{15}N$ and 0.5 ‰ (SD = 0.19) for $\delta^{13}C$ were adopted between invertebrates and producers and between invertebrate prey and spiders (Mcnabb et al. 2001, Vander Zanden and Rasmussen 2001, Post 2002, Oelbermann and Scheu

2002, McCutchan et al. 2003, Paetzold et al. 2005). I assumed that most spiders preyed on herbivorous insects and that few preyed on invertebrate predators, as flying invertebrates tend to avoid spider webs (Nentwig 1982, 1987, Riechert and Lawrence 1997), therefore resulting in a trophic level between 3 and 4. A trophic level of 3.5 was thus used for most web-building spiders, but in some cases this value had to be adjusted slightly so that the corrected isotopic values would fall within the convex hull bordered by the possible sources (Phillips et al. 2014), allowing for a SIAR model to be utilised. Fractionation from the producers to the spiders was therefore adjusted to 5.75 ‰ (SD = 1) for $\delta^{15}N$ and 1.25 ‰ (SD = 0.19) for $\delta^{13}C$ after factoring in the trophic level of 3.5. The proportions of the different producers in the diets of spiders, as estimated by SIAR, were grouped as terrestrial or aquatic food sources. Proportions of aquatic food in the diets of spiders were then assessed with increasing distance from the river, at all the sites and in different seasons.

Simple linear regression was used to measure the significance and strength of association (p and R^2, respectively) between the aquatic dietary contributions (%) and distance from the river (0 to 64 m), and between aquatic dietary contributions and aquatic insect biomass. Natural logarithm transformations were performed on both the dependent (aquatic contribution) and independent (distance from the river) variables to improve linearity:

$$\text{In}(y+1) = a + \ln(x+1)b$$

where **y** = aquatic dietary proportion at distance **x**, **b** = slope of the line and **a** = y intercept. The regression analysis was also used to make predictions of how far the aquatic subsidies stretch into the terrestrial upland.

5.3. **Results**

5.3.1. Spatial and temporal variations in $\delta^{13}C$ and $\delta^{15}N$ values of spiders

There were significant variations in $\delta^{13}C$ and $\delta^{15}N$ values of spiders among the sites (F = 24.87, d.f. = 4, $p < 0.001$), seasons (F = 7.1, d.f. = 4, $p < 0.001$) and the distances from the river (F = 18.3, d.f. = 2, $p < 0.001$), but there were no significant interaction effects (F = 0.83, d.f. = 8, p = 0.576). Carbon ($\delta^{13}C$) values were distinctly low whilst $\delta^{15}N$ values were highest at distances closest to the river at the wide and intermediate sites (Figure 5-1). There was also high variability in $\delta^{13}C$ and $\delta^{15}N$ values among potential insect prey from aquatic vs. terrestrial habitats (see Chapter 4) and among sites (F = 133.20, d.f. = 4, $p < 0.001$). There were no significant differences in $\delta^{13}C$ and $\delta^{15}N$ values of insects across seasons (F = 1.81, d.f. = 4, p =

0.12), but all two and three way interactions between season, site and insect origin produced significant effects ($F = 15577.46$, d.f. = 2, $p < 0.001$).

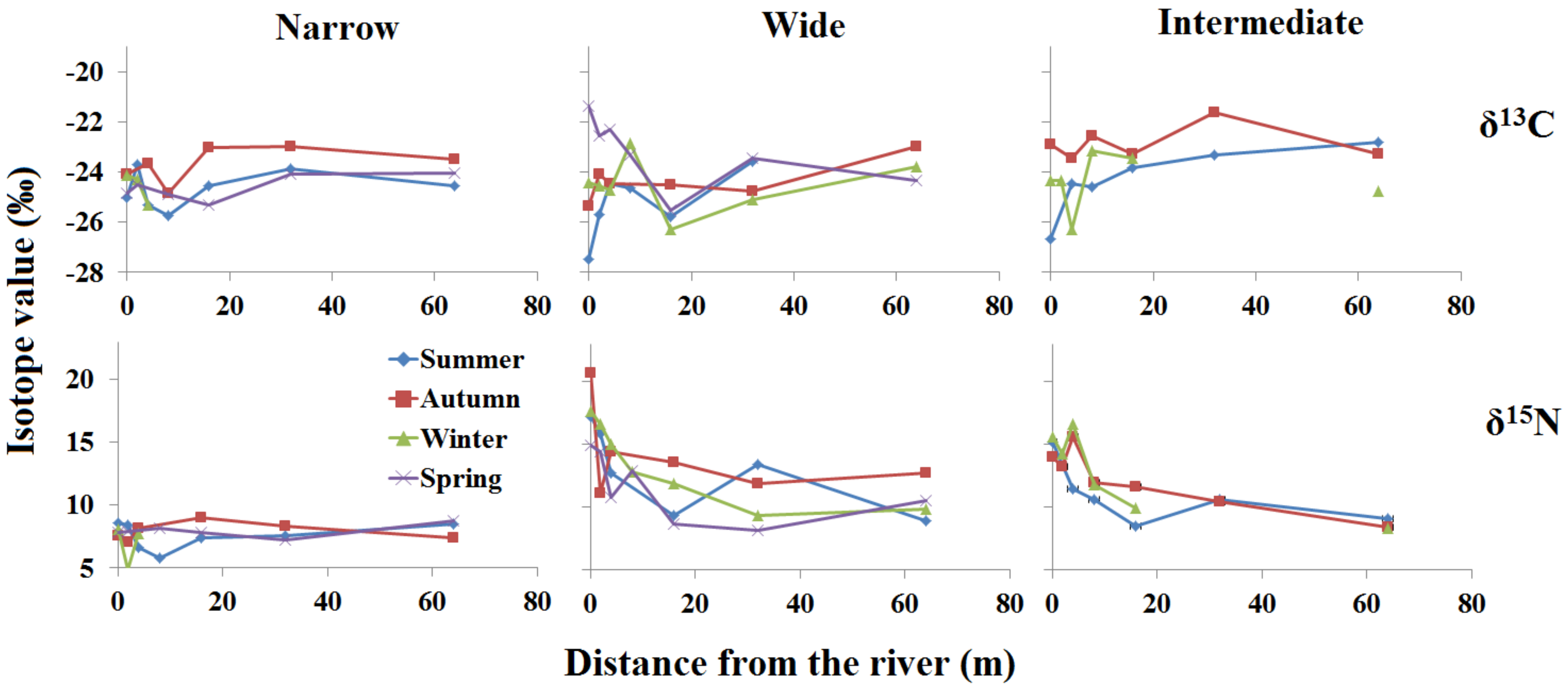


Figure 5-1: Mean $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values (‰) for web-building spiders collected at differing distances from the river channel, during four seasons from the narrow, wide and intermediate sites on the Kowie River. Due to sample losses, no spiders from the intermediate site were analysed for SIs in spring.

5.3.2. Spider diet composition at varying distances from the river

When SIAR results for individual spiders (Appendix 4) were pooled based on their proximity to the river, a general decline in the dietary contributions of aquatic food sources to spiders occurred with increasing distance at both the wide and intermediate sites during most seasons. No patterns could be detected across the distance gradient at the narrow site. Linear regression analysis (Table 5-1) showed that there was a statistically significant ($p < 0.05$) relationship between proximity to the river and the contribution of aquatic material to spiders at the wide ($R^2 = 0.71 – 0.98$) and intermediate ($R^2 = 0.85 – 0.97$) sites in all seasons [except autumn in the intermediate ($R^2 = 0.58$, $p > 0.05$) site]. The highest coefficient of determination (R^2) values were observed in summer and winter at both sites (wide and intermediate). Distance (proximity to the river) was not significantly (Table 4-4, $p > 0.05$) linearly related to the proportion of aquatic food sources in spider diets at the narrow site except during spring. During spring in the narrow site, the R^2 value of the relationship was relatively low ($R^2 = 0.48$) when compared to other seasons in different sites (Table 5-1).

Regression analyses (Table 5-2) were performed between aquatic insect biomass (data from Chapter 3) and the SIAR generated proportions of aquatic food sources in spider diets. The coefficient of determination (R^2) was relatively low (0.04 – 0.29) at the narrow site in all seasons except winter (0.59), but none of the linear regression models were statistically significant ($p < 0.05$). At the wide site, R^2 values were high in summer and autumn (0.82 and 0.91, respectively) and relatively low in winter and spring (0.48 and 0.36, respectively). All the linear regression models from the wide site were statistically significant ($p < 0.05$) except during spring ($p > 0.05$). All the models at the intermediate site had high values of R^2 (0.57 – 0.92) and they were all statistically significant ($p < 0.05$).

None of the spiders showed total dependence (100%) on either nutritional subsidies derived from aquatic primary producers or terrestrial food sources at any of the sites (Appendix 4). SIAR results from the most frequently encountered genera (*Tetragnatha, Leucauge, Caerostris* and *Araneus)* were selected for representation of any trends across seasons and along the distance from river gradient (Figure 5-2). Taxon-specific SIAR results (Figure 5-2) showed that different spider genera (groups) consumed different proportions of aquatic food sources with increasing distances from the river.

Table 5-1: Results of linear regression analyses for proportions of aquatic contributions to spider diets with distance from the river, y = ax + b; where y = aquatic contribution at distance x, b = slope of the line and a = y intercept. $P < 0.05$ signifies statistical significance of the regression model **(bold** = statistically significant).

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

The SIAR results from the narrow site showed no discernible trends with increased distance from the river except during spring, when the genera *Tetragnatha* and *Araneus* at 0 m consumed greater proportions (between 60 and 70%) of aquatic prey compared with spiders at further distances (between 20 and 50% of aquatic prey contributions; Figure 5-2). In contrast, SIAR results of the wide and intermediate sites showed general declines in aquatic contributions to spider diets with increased distance from the river (in all spider genera during at least one season). Individuals in the genus *Tetragnatha* (Figure 5-2b, c) had relatively high proportions of aquatic material in their diets when compared to those in the other three genera: *Leucauge* (Figure 5-2e, f), *Caerostris* (Figure 5-2h, i) and *Araneus* (Figure 5-2k, l) at similar distances, particularly in summer and winter. However, aquatic contributions to spiders at the shores (0 m) of the river at the wide and intermediate sites were greatest (> 60%) during summer.

Table 5-2: Results of linear regression analyses of proportions of aquatic material contributing to spider diets with the biomass of aquatic insects (potential prey availability), y = ax + b; where y = aquatic dietary proportion at distance x, b = slope of the line and a = y intercept (bold = statistically significant)

Site	Season	R^2	F	p-value	b	a
Narrow	Summer	0.04	1.27	0.31	0.39	0.03
	Autumn	0.2	1.25	0.31	0.25	-0.01
	Winter	0.58	1.36	0.45	0.25	0.03
	Spring	0.29	2.08	0.21	0.4	0.4
Wide	**Summer**	**0.82**	**18.02**	**0.01**	**0.17**	**0.02**
	Autumn	**0.91**	**41.94**	**<0.01**	**0.52**	**0.12**
	Winter	**0.48**	**4.61**	**0.05**	**0.33**	**0.36**
	Spring	0.36	2.87	0.15	-1.42	0.05
Intermediate	**Summer**	**0.93**	**51.86**	**<0.01**	**-0.41**	**0.03**
	Autumn	**0.57**	**6.72**	**0.05**	**0.04**	**-0.93**
	Winter	**0.72**	**10.35**	**0.03**	**-0.56**	**0.27**

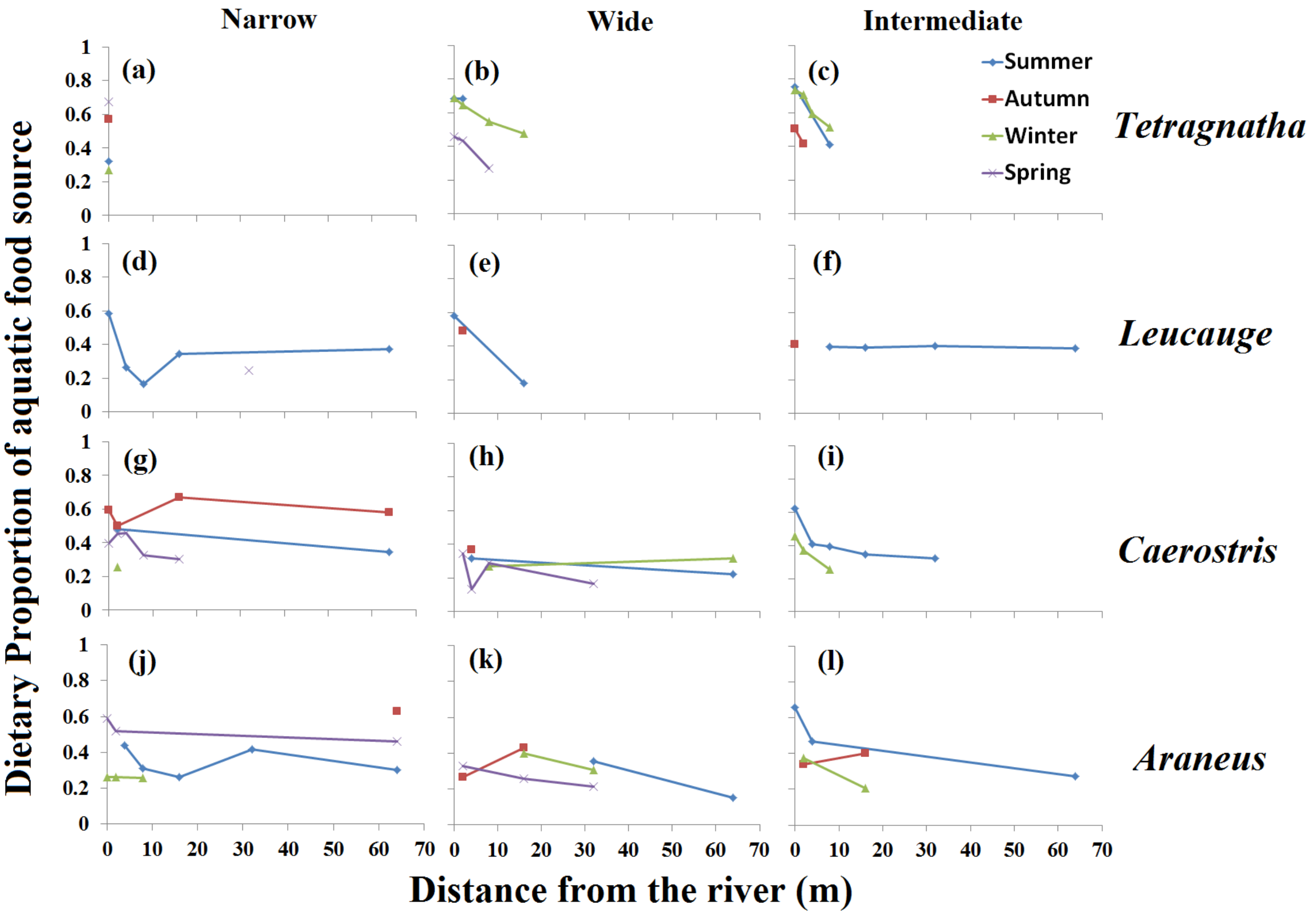


Figure 5-2: Lateral and longitudinal variations in cross-boundary subsidies received by different guilds of web-building spiders. Relative contributions (SIAR generated) of aquatic food sources to web-building spiders at varying distances from the river during four seasons at three study sites in the Kowie River catchment. The letters (a) – (c) represent the spider genus *Tetragnatha*, (d) – (f) represent *Leucauge*, (g) – (i) represent *Caerostris* and (j) – (l) represent *Araneus*. The different colours represent seasons.

5.4. **Discussion**

5.4.1. Variations in spider diet composition

I hypothesized that the contribution of aquatic subsidies to spider diets is determined by aquatic insect abundances and the results of this study support this hypothesis because variations in aquatic insect biomass/abundance were significantly correlated with isotopic indicators of aquatic contribution, along temporal and spatial scales (at the wide and intermediate sites).

5.4.1.1. Lateral and longitudinal trends in diet

Regression analysis (on SIAR results) showed that at the wide and intermediate sized sections of the Kowie River, the proportions of aquatic food sources in spider diets was linearly related to proximity to the river and the biomass of aquatic insects, but the strength of the relationship varied across seasons (stronger in summer). These relationships were not present at the narrow section of the river. Regardless of site, the extent of aquatic material contributed to spider diets was taxon-specific, e.g. individuals from the genus *Tetragnatha* had the highest proportions of aquatic food sources in their diets when compared to other genera (see Chapter 4).

Insect prey biomass values in Chapter 3 (Figure 3-4) showed that aquatic insect biomass was highest at the distances closest to the river, corresponding to where aquatic food contributed the most to spider diets (Figure 4-2). Various authors (Power and Rainey 2000, Kato et al. 2003, Akamatsu et al. 2004, Baxter et al. 2005, Iwata 2006, Burdon and Harding 2008) have linked high insect abundances emerging from the rivers with high contributions of nutritional subsidies derived from aquatic primary producers to spider diets, but how far inland the insect abundance (or biomass) influences spider diet composition remains understudied. Aquatic-based dietary contributions were significantly related with distance from the river in spring only at the narrow section of the river. However, similar to the other river sections (wide and intermediate sized), aquatic-based dietary contributions were at their highest at the stream edge (0 to 2 m) during all the seasons. Therefore, the influence of aquatic subsidies stretched further into the land at the sites with larger stream widths (wide and intermediate sites), whilst the influence was pronounced only at the immediate stream edge (0 to 2 m) at the narrow section of the river.

Overall, the data derived from the Kowie River indicated that stream width regulated the quantity of aquatic subsidies available for consumption by terrestrial consumers, with the wider sections producing more subsidies (see Chapter 3). Wider river channels provide greater surface area and stream bed area for emergence and produce higher abundances of aquatic insects (e.g. Johnson et al. 2007; Chapter 3). The wide site had a wider stream width (11 ± 5 m) and therefore larger surface area for insect emergence than the other sites (narrow = 2 ± 0.4 m, intermediate = 6 ± 0.5 m). The larger surface area enabled greater emergence (Moyo 2016) and therefore greater subsidisation to the adjacent terrestrial upland. A study by Jonsson and Wardle (2009) on islands in two large freshwater lakes in the boreal zone of northern Sweden showed that the extent of subsidisation to the terrestrial system was dependent on the perimeter to area ratio of the islands. The smaller islands yielded greater subsidisation because they had higher surface area perimeter to area ratios. Similarly, my study showed that the extent of subsidisation was greater at the widest section of the Kowie River, which had the greatest stream bed and water surface areas for emergence.

Besides the width of the river, there are additional factors that could potentially affect the extent of aquatic subsidies to land-based consumers. For example, the three sites that were chosen for this study had varied vegetation types and structures (see Chapter 2). It is possible that the amount of vegetation cover could have been a determinant of the aquatic insect flux rates (e.g. Nakano and Murakami 2001). The three sites in this study had varied vegetation cover and it is possible that cover could have been a determinant of emergence and therefore affecting diets of terrestrial consumers. Temperature changes have been identified as additional drivers of aquatic insect emergence in rivers (Petchey et al. 1999, Jonsson et al. 2015). Higher water temperatures result in greater emergence of aquatic insects due to higher autotrophic production (Petchey et al. 1999, Jonsson et al. 2015). Similarly, rivers or sections of rivers that are more shaded have reduced primary production, thereby influencing the rate of emergence. Additional studies that incorporate measurements across numerous rivers will assist in describing the numerous potential biotic and abiotic effectors of aquatic subsidisation to land.

The relationships between spider diet composition (i.e. aquatic vs. terrestrial contributions) and aquatic insect biomass (or the distance from the water) were very weak at the narrow section of the river, but the percentage contributions of aquatic-based food to spider diets remained as high as those observed in the wider sections with increasing distance from the water (Figure 5-2). Given that the biomass of aquatic insects decreased with distance (Chapter 3), it appears that web-building spiders maintain high proportions of the aquatic insects in their diets up to 64 m from the Kowie River regardless of the low aquatic biomass at such distances. It may be that their webs are more specialized for the capture of small weakly flying insects (Yoshida

1989, Williams et al. 1995, Marczak and Richardson 2007) that happen to be abundant near aquatic systems. It is possible that web-building spiders practice a degree of active prey selectivity (e.g. Nyffeler et al. 1989, Nyffeler 1999, Ludy 2007), although these spiders typically are passive and generalist feeders and the size, orientation and placement of the webs are considered the most important factors affecting their diets (Nentwig 1986, Nyffeler 1999, Snyder and Wise 2001). Controlled field studies would help to determine whether web-building spiders are selective regarding what prey are captured within their webs. Such studies would require release of known flying insect taxa into an area with web-building spiders, but where all possible natural prey has been intentionally removed.

5.4.1.2. Diet variations across seasons

In the Kowie River, aquatic food contributions to spider diets were highly variable among spider taxa, hence it was difficult to detect seasonal shifts. However, insect biomass (aquatic and terrestrial) was at its highest in summer and autumn at the narrow and intermediate sites and during spring at the wide site, but was always at its lowest during the winter season at all the sites (Chapter 3 - Figure 3-3). Results from a concurrent study also showed that aquatic insect emergence was highest in summer and lowest in winter at all the three sites; narrow (summer = 169 ± 13, winter = 4 ± 2 mg m^{-2} day^{-1}), wide (summer = 1402 ± 152, winter = 28 ± 24 mg m^{-2} day^{-1}) and intermediate (summer = 939 ± 59, winter = 7 ± 1 mg m^{-2} day^{-1}; Moyo 2016). The spider diets at the wide site were therefore strongly affected by insect biomass ($R^2 = 0.82 – 0.91$) during seasons when aquatic insect biomass was high (i.e. during autumn and summer). It is not clear why the correlation was strong in winter ($R^2 = 0.72$) at the intermediate site when aquatic insect biomass was relatively low, but perhaps it was because the insect community was more reliant on algal sources during the winter. The high correlation during winter could be due to the fact that both aquatic dietary contributions (Figure 5-2) and aquatic insect biomass (Chapter 3 - Figure 3-3) were higher at the intermediate than both the narrow and wide sites at the stream edge (0 to 2m). Through experimentation, Kato et al. (2003) provided direct evidence that there is a temporal limitation on spider distribution by emerging aquatic insect fluxes. Their study was carried out in a headwater stream in Northern Japan where they used green-house type coverings to exclude emerging aquatic insects and determine the influence of their absence on spider success and community structure (Kato et al. 2003). Their results showed that when aquatic insects were scarce, there was no measurable influence on the abundances of web-building spiders (Kato et al. 2003). I expect that in seasons such as winter when low emergent aquatic insect abundance (and biomass) occurs in a narrow section of the river (that already has low emergence rates), the importance of aquatic insect abundance to spider diets is lower than in wider sections of the river. The low correlation between the percentage of aquatic insects in spider diets and insect biomass in winter provides evidence for this assertion.

Overall, the biomass of aquatic insects was always much lower than the biomass of terrestrial insects, even during seasons with the highest emergence rates (Moyo 2016; Chapter 3, see also previous paragraph). A similar study was carried out with a focus on birds in a cool–temperate riparian deciduous forest in the Horonai Stream (Japan), but the authors reported different seasonal dynamics in emergent insect biomass and bird diet compositions (Nakano and Murakami 2001). In that study, emergent aquatic insect biomass was far greater than terrestrial insect biomass during winter (aquatic = 5 – 15, terrestrial = 0 – 5 mg m^{-2} day^{-1}), spring (aquatic = 70 – 190, terrestrial = 0 – 50 mg m^{-2} day^{-1}) and autumn (aquatic = 10 – 30, terrestrial = 0 – 20 mg m^{-2} day^{-1}), and the birds consumed large quantities of allochthonous (aquatic) prey during these periods (Nakano and Murakami 2001). Other studies in temperate (e.g. Uesugi and Murakami 2007) and Mediterranean climates (e.g. Rundio and Lindley 2012) have also recorded aquatic insect flux rates that were higher than terrestrial biomass for most of the year. Since the Kowie River is characterised by relatively low aquatic insect flux relative to terrestrial biomass on an annual basis compared with other rivers in temperate regions (e.g. Nakano and Murakami 2001), there was decreased influence of allochthonous material on the diets of web-building spiders (Chapter 3). Therefore, trophic connectivity between freshwater and terrestrial systems is ecosystem and area specific, and the flux and productivity of emerging aquatic insects is a major determining factor in diet determination under specific environmental circumstances. The methods used in other studies were very different from those used in the Kowie River, and this variability certainly contributed to variations in findings among studies. Research on several additional predator populations within the same riparian areas of contrasting ecosystem types will help to clarify such questions and explain the dynamics involved.

In the Kowie River, diet proportions were estimated with indirect SIA methods using plant material as the basal resources. Due to reasons explained in the methods (Section 4.2.2.), fractionation-corrected isotopic values of plants (i.e. primary producers) were used as proxies for the insect prey that web-building spiders consumed. As such, the proportions of aquatic or terrestrial food sources in spider diets could have been underestimated or overestimated. SIAR models have to factor in trophic fractionation because every time a consumer assimilates prey, preferential retention of heavier isotopes takes place in the consumer's body and this retention varies amongst organisms (Peterson and Fry 1987, McCutchan et al. 2003). Inclusion of fractionation values at multiple levels undoubtedly added some error to the model outputs, but the broad analysis format was necessary for determining the aquatic contributions for such a wide range of spider taxonomic groups. Additional verification of fluctuating levels of aquatic contributions to terrestrial consumers is needed through the completion of further field studies, and perhaps incorporating additional diet analysis tools [e.g. FAA (Chapter 6) and DNA techniques].

5.4.2. Guild-specific variations in proportions of aquatic insects contributing to the diets of horizontal and vertical web-building spiders

Horizontal web-building spiders (*Tetragnatha* and *Leucauge*) tended to consume more aquatic than terrestrial insects at distances closest to the stream (Figure 5-2). At the riverside (0 m), the horizontal web builders always had the highest proportions of aquatic prey in their diets, and even when they were living at distances further in the terrestrial upland, they had distinctly higher dietary aquatic contributions when compared to the vertical web builders (Figure 5-2). The proportions of aquatic food in the diets of *Tetragnatha* spiders collected immediately adjacent to streams at the wide and intermediate sites of the Kowie River during summer (60 - 80%) were similar to those reported in Akamatsu et al. (2004; 70 – 90%). In their study, Akamatsu et al. (2004) investigated web-building spider diets using SIA in three zones based on height above the river surface. Their study was carried out in a large section of the Shinano River (Japan), with a width (300 m) much wider than the largest width (16 m) of the sites in the Kowie River. Similar to my study, Akamatsu et al. (2004) reported smaller proportions (56 %) of aquatic insects in the diets of *Tetragnatha* spiders at increased distances into the adjacent forest from the Shinano River (between 30 and 60 m from the river), than on the stream edge (70 – 90%). Spiders of the family Tetragnathidae (including the genera *Tetragnatha* and *Leucauge*) specialize in capturing adult insects emerging from streams by building horizontal orb webs, often suspended over streams (Henschel et al. 2001, Kato et al. 2003). Web-building spiders have varying feeding strategies (Yoshida 1989) and they use different web morphologies (including web orientation) to capture their preferred prey. It is possible that these web morphologies evolved in different spider taxa as a result of competitive interactions.

Spiders from the genus *Tetragnatha* (horizontal builders) consumed greater proportions of aquatic material and those from *Caerostris* and *Araneus* (the vertical web builders) that generally had higher proportions of terrestrial material in their diets at large distances from the river (Figure 5-2). Unlike the horizontal web builders, the vertical web builders did not seem specialized for capturing either aquatic or terrestrial prey types, although their webs are typically suited for catching fast-flying terrestrial insects (Olive 1982). When aquatic insect biomass was low during winter (Chapter 3), the vertical web-builders (*Caerostris* and *Araneus*) generally consumed greater proportions of terrestrial material (Figure 5-2) compared with the summer period. It was therefore evident that diets of vertical web building spiders were influenced by prey availability more than those of horizontal web-builders. Despite the general decline in aquatic predation with distance, my study showed that spider diet niches had varied use of aquatic subsidies. In generalist predators, the size and position of the trophic niche is likely determined by traits such as hunting mode, the stratum they occur in and their body size (Sanders et al. 2015). Traits such as web orientation (vertical/horizontal) may determine the extent of use of aquatic subsidies by riparian spiders. My results indicated that web designs or orientations are useful in distinguishing closely related species or species with similar feeding strategies.

5.4.3. Conclusion

My findings suggested that aquatic-derived carbon (and nitrogen) provided a significant source of nutrition for riparian spiders, and that variations in insect biomass influenced the extent of the aquatic influence. Consequently, as insect biomass decreased with increasing distance from the river, varied across different river sections, and fluctuated across seasons, diets of riparian spiders also changed. Additionally, I established that different spider taxa responded differently to the spatial and temporal changes in prey availability, largely because web-building guilds are specialized for capture of particular insect groups. In particular, the horizontal web builders displayed some specialization for aquatic food, whilst the vertical web builders did not. The different feeding guilds varied in the extents to which they exploited aquatic and terrestrial materials, and the isotopic niches of the different spider guilds were distinct during some seasons. My study represents one of the first estimations of aquatic subsidies into riparian areas of Southern Africa (possibly Africa) using SIA, and it indicated that SIAR models show promise for examining the spatial and temporal shifts in the diets of terrestrial predators.

Chapter 6. TEMPORAL AND SPATIAL VARIATIONS IN CROSS-BOUNDARY TROPHIC SUBSIDISATION IN RIPARIAN COMMUNITIES: A FATTY ACID MARKER ANALYSIS

6.1. **Introduction**

Consumption of prey derived from aquatic sources can provide significant nutrition for spiders living in riparian areas, and stable isotope (SI) mixing models have shown that 20 to 90% of a riparian spider's diet constitutes insects of aquatic origin (e.g. Chapter 4; Collier et al., 2002; Akamatsu et al., 2004). The high insect abundances typical of water edges constitute an important source of essential nutrients for terrestrial consumers. However, it is not known how far the aquatic subsidy disperses into the adjacent uplands and how this supply influences predator populations (abundances and composition) in different seasons, as insect emergence rates change (Nakano and Murakami 2001). Additionally, it is not known whether changes in terrestrial consumers can be tracked through changes in fatty acid (FA) quantities or composition.

The FA composition of predators is partly determined by what they eat because some assimilated FAs are directly deposited in the adipose tissue (Budge et al. 2006). Some dietary FAs are modified before deposition, whilst others are endogenously derived (inside the predator) through *de novo* synthesis (Budge et al. 2006). The high diversity of FAs and the somewhat specific origins of some of them have enabled their use as tools for assessing predator diets (Iverson et al. 2004). The physiologically important FAs that predators can attain only through consumption are referred to as essential fatty acids (EFAs). *De novo* synthesis in predators is not sufficient to produce the necessary amounts of EFAs, hence predators have to rely on EFAs attained from their diets (Brett and Müller-Navarra 1997, Arts et al. 2009). Autotrophs produce many of the long chain polyunsaturated fatty acids (PUFAs) and highly unsaturated fatty acids (HUFAs) that are important to predators.

Generally, there is low abundance of HUFAs in terrestrial plants (Wolff et al. 2001, Simopoulos 2004, Gladyshev et al. 2009a) and most of the terrestrial vegetative matter that falls into streams and lakes does not contain HUFAs (Mills et al. 2001). The majority of unsaturated fatty acids (UFAs) in higher plants are the 18-carbon chain (C 18) compounds: oleic, linoleic, and alpha-linolenic acids (Harwood 1996). Higher plants generally cannot convert C 18 PUFAs to HUFAs (Tocher et al. 1998). In contrast, lower plants such as some groups of algae and a few fungus species possess the enzymatic systems for producing significant amounts of HUFAs (Tocher et al. 1998, Gladyshev et al. 2009b). Aquatic systems are thus the producers and providers of HUFAs to terrestrial organisms (Gladyshev et al. 2009a). As a consequence of EFAs being obtained through feeding, they can act as trophic biomarkers that can be traced from producers through to upper levels of food webs (Brett and Müller-Navarra 1997, Parrish et al. 2000, Dalsgaard et al. 2003). The best biomarkers are unique to a food source in a specific environment and are transferred without modification into higher trophic levels (Dalsgaard et al. 2003). Subsidies from aquatic habitats may be vital for nourishing life in terrestrial habitats, and FA content can represent an ecologically-relevant measure of fluxes by revealing the sources, sinks and trophic pathways of organic nutrients.

The high amounts of PUFAs and HUFAs that aquatic systems produce are vital for both aquatic and terrestrial consumers and yet they are limiting in terrestrial systems (Choi et al. 1987, Sterner and Schulz 1998, Park et al. 2002, Crawley et al. 2009, Gladyshev et al. 2009a, Lau et al. 2009). Once synthesized at the level of primary producers, HUFAs are transferred and accumulate at progressively higher trophic levels in the biomass of aquatic organisms. Insects from the orders Ephemeroptera (mayflies), Diptera (midges, black flies), Trichoptera (caddisflies), and Plecoptera (stoneflies) lay their eggs in rivers, ponds, and lakes where the larvae subsequently develop whilst accumulating HUFAs (Bell et al. 1994, Ghioni et al. 1996, Barroso et al. 2014, Henry et al. 2015). When these insects emerge as adults they enter the riparian zone and adjacent regions where mating occurs (Delettre and Morvan 2000, Briers et al. 2005, Walters et al. 2008, Wesner 2010). It is during this flight period that they constitute prey for terrestrial predators and thus transfer the EFAs to terrestrial systems (Wesner 2010). Eicosapentaenoic acid (EPA, 20:5ω3), docosahexaenoic acid (DHA, 22:2ω6) and arachidonic acid (AA, 20:4ω6) have been identified as the major aquatic biomarkers in terrestrial systems (Gladyshev et al. 2009b). Arts et al. (2009) suggested that, except for particular locations in the Pacific Rim where bears intensively consume salmon, the main pathways of HUFA export from aquatic to terrestrial systems are emerging insects and water birds.

Using web-building spiders as model organisms, I investigated how the seasonal variations in aquatically derived EFAs affected terrestrial consumer diets and determined how far the influence stretches into the adjacent terrestrial habitat. Considering that insect emergence is an important determinant of the distribution of web-building spiders (Jackson and Fisher 1986,

Henschel et al. 2001), this study makes use of FA biomarkers to investigate the reliance of terrestrial predators on aquatic subsidies with increasing distance from the source (river). Additionally, I sought to identify important FA tracers and investigate their extent of diffusion at river sites with varying widths and aquatic insect abundances and emergence rates.
I hypothesized that:

1) *there is a negative relationship between proportions of FA indicators of aquatic feeding in spider diets and the distance from the river because as aquatic insect abundance decreases further inland, the consumption of aquatic insects by web-building spiders decreases*
2) *nutritional subsidies from aquatic primary productivity are more abundant and travel further inland at wider sections of the river because the surface area (stream bed area) for insect emergence is greater at wide sites, hence there is a positive relationship between river width and the proportions of FA indicators of aquatic feeding in spider diets,*
3) *seasonal variations in aquatic insect emergence rates cause increases in the content of aquatic insects in spider diets during warmer seasons, which can be reflected though increases in proportions of FA indicators of aquatic feeding in spider diets.*

6.2. **Methods**

6.2.1. Study design

The study sites, fatty acid protocols and spider and insect collection methods are described in Chapter 2. Questions on overall differences in FA content at taxa level were addressed using total fatty acid (TFA) proportions. To investigate variations in major FAs with increasing distance from the river, proportions of selected FA were utilized.

The hypotheses of this study were tested using the following methods:

***Hypothesis* 1.** T*here is a negative relationship between proportions of FA indicators of aquatic feeding in spider diets and the distance from the river because as aquatic insect abundance decreases further inland, the consumption of aquatic insects by web-building spiders decreases* - For the tracer analysis, individual aquatic FAs (Gladyshev et al. 2009a) were used to investigate the extent of contribution of aquatic subsidies to spiders at different distances from the river. Detailed analyses were run using FAs (and groups of FAs) that both (1) distinguished aquatic from terrestrial insect groups (Chapter 4) and (2) contributed > 5% to the variation among spider species. These FAs or FA groups included linoleic acid (LA, 18:2ω6), eicosapentaenoic acid (EPA, 20:5ω3), the sum of ω3 FAs (sum ω3), the sum of essential FAs [EFA, arachidonic acid (AA, 20:4ω6) + 20:5ω3] and the ratio of ω3/ω6 FAs (ω3/ω6). Linoleic acid was considered as an indicator of the consumption of terrestrial material in this study because it has been measured in elevated amounts in terrestrial systems (Koussoroplis et al. 2008) and is considered an indicator of terrestrial food sources (Malainey et al. 1999). Eicosapentaenoic acid, EFAs, sum ω3 and ω3/ω6 were used as indicators of aquatic contributions to spider diets (see Chapter 4).

***Hypothesis* 2.** A*quatic subsidies are more abundant and travel further inland at wider sections of the river because the surface area (stream bed area) for insect emergence is greater at wide sites, hence there is a positive relationship between river width and the proportions of FA indicators of aquatic feeding in spider diets* – Methods for Hypothesis 1 (see above) were replicated in the three study sites that had varying stream widths (Chapter 2 - Section 2.2.1.). General comparisons were made across the sites and some statistical inferences were made to determine significant differences across sites (see below).

***Hypothesis* 3.** *Seasonal variations in aquatic insect emergence rates cause increases in the content of aquatic insects in spider diets during warmer seasons, which can be reflected though increases in proportions of FA indicators of aquatic feeding in spider diets* - Methods for Hypothesis 1 (see above) were replicated in the four seasons and general comparisons were made across seasons and some statistical inferences were made to determine significant differences across sites (see below).

6.2.2. Statistical analysis

6.2.2.1. Spatial and temporal variations in spider FA profiles

The total FA (TFA) composition of each spider was expressed as proportions of FAs relative to the TFA present. Subsequently, comparisons of spiders at varying distances from the river at three contrasting sites during four seasons were made based on FA composition. Similarity percentage analysis (SIMPER) was used to determine the relative contribution of each FA to the total variation. Resemblance matrices were calculated on untransformed data using Euclidean distances. The FAs with the highest contributions were considered as major FAs;

mostly those contributing more than 5% to the total variation. A dominance curve, based on rank abundance and frequency, was also used to determine the major and minor FAs. Non-metric multi-dimensional scaling (nMDS) was used to present TFA composition at each site during each season, and the major FAs (those with the greatest influence) were fitted as the vectors in a biplot (Gower et al. 2011). The length of the each of the radial lines (vectors) represented the relative degree of influence exerted by each FA on the TFA composition of spiders (points) in the direction of the line. Non-metric multi-dimensional scaling was appropriate because it preserves taxon-specific information and can reveal changing community patterns in spider diets (Warwick et al. 1990).

To investigate seasonal differences in FA profiles, two-way analysis of similarity (ANOSIM) tests were run with distance and season as the factors, at each site. To investigate differences in TFA profiles across sites, two-way ANOSIM tests were run with site and distance as the factors. Distance from the river was an important factor because it was expected that spiders living at different distances would have varied access to the aquatic subsidies.

6.2.2.2. Variation of major FAs with increasing distance from the river

One of the aims of this study was to determine the variation in proportions of EFA with increasing distance from the river and identify appropriate effective aquatic FA tracers. For many taxonomic groups, sufficient replicates could not be collected at each distance category from the river in each season, in all the sites. To test the effect of distance on FA proportions, spiders collected at the same distance from the stream were considered as replicates. The Spearman's rank correlation (Rho) non-parametric test was used to measure the strength of association between distance from the river (0 to 64 m) and the relative proportion (%) of FAs in spider diets.

6.2.2.3. Modelling FA proportions

Based on the results of the SIMPER and correlation analyses, an intricate analysis was done on the variation of an aquatic tracer with increasing distance from the river. EPA, AA and DHA are well known aquatic indicators (Gladyshev et al. 2009b), but I used only EPA because AA was highly variable and DHA was absent. Other potential aquatic indicators like the sum of ω3 FAs and the ratio of ω3/ω6 had similar correlations to EPA, but EPA was preferred because the ratio of ω3/ω6 includes FAs that are present in large amounts in terrestrial ecosystems. The

FAs causing variation between groups of spiders consuming varying levels of aquatic subsidies were determined in Chapter 4.

Negative binomial (NB) general linear models (GLM) were used to model the proportions of EPA in spiders with increasing distance from the river. The model factors were site and distance from the river whilst the proportions of EPA represented the response variable. The NB distribution produced the best fit to the spider FA data, and no patterns were observed in the residuals. A log link function was chosen because it linearised the models appropriately and returned realistic non-negative results that were biologically meaningful. The validity of the models was assessed through residual and dispersion analysis and it was expected that if model assumptions were satisfied, the residuals would vary randomly around zero and the spread of the residuals would be about the same throughout the residual plot (Zuur et al. 2009, Hilbe 2011). Residual analysis is important to ensure the model is appropriate for the data used and helps to explains as much variation as possible and the random pattern indicates that a linear model provides a decent fit to the data (Zuur et al. 2009). The residuals were plotted against spider taxa to determine whether taxa could help in explaining variations in FA proportions.

The dispersion parameter targeted for the NB GLM was 1, and any model with a dispersion below 1.5 and above 0.9 was considered good. Simple linear regression and mixed effects models were not used because they produced biologically improbable EFA estimates that were either totally absent or negative at distances far from the river. Ecologists frequently use the negative binomial distribution for data when the variance is greater than the mean, as often is the case for count and proportion data (Zuur et al. 2009). The negative binomial models were used because they acted as over-dispersed Poisson distributions, modelling proportion data without having the variance tied directly to the mean (Zuur et al. 2009, Hilbe 2011). Capturing this over-dispersion was important for the highly variable and noisy spider EPA data. Dispersion was calculated as:

$$Dispersion = \boldsymbol{A}/\boldsymbol{B}$$

where **A** = $\sum$ (residuals^2) and **B** = number of residuals – (number of model coefficients + 1) (Zuur et al. 2009). Statistical analyses were completed using various statistical programmes; PRIMER V6 (Clarke and Warwick 2001) for all ordination analysis, PAST (Hammer Ø et al. 2001) for SIMPER and ANOSIM, Statistica (StatSoft Inc. 2011) for Spearman's rank correlations and R (R Core Team 2015) for NB GLMs.

6.3. **Results**

6.3.1. Spatial and temporal variation

6.3.1.1. Fatty acid profiles

During the summer and autumn months, spiders living at different sites and distances from the river formed distinct groupings based on their FA profiles (Table 6-1). During winter and spring, spider FA profiles did not vary with distance from the river, but profiles were distinctly different across sites (Table 6-1). The FAs 16:0, 16:1ω7, 18:0, 18:1ω7, 18:1ω9, 18:2ω6, 18:3ω3 and 20:5ω3 each contributed more than 5% to the total variation in spider TFA profiles at all the sites during all seasons (Table 6-2). These FAs were therefore labelled as the major FAs. The PUFA 18:2ω6 and the MUFA 18:1ω9 contributed the most to variation in spider FA profiles across different distance categories, but in some instances the contribution of the PUFA 20:5ω3 was more than 10% (Table 6-2). Distinct spider groupings could not be identified in any of the nMDS plots for all the sites and seasons, but spiders collected from the same or similar distances from the river tended to group together, especially those collected between 0 and 2 m from the stream (Figures 6-1 to 3, also see Chapter 4). Different patterns in TFAs were observed across the sites and seasons, as well as the influence of some major FAs on spider distribution.

Table 6-1: Two-way ANOSIM test results for web-building spider FA profiles amongst sites (narrow, wide and intermediate sites) and transects (0, 2, 4, 8, 16, 32 and 64 m) in the Kowie River catchment. Bolded p values are statistically different ($p < 0.05$)

	Summer		Autumn		Winter		Spring	
	Site	Transect	Site	Transect	Site	Transect	Site	Transect
Global R	0.218	0.156	0.175	0.166	0.199	0.018	0.372	-0.019
p	**0.008**	**0.004**	**0.048**	**0.045**	**0.05**	0.442	**0.02**	0.518

Non metric multidimensional scaling showed that there were more differences in TFA profiles between spider taxa at distances closer to the river (Figure 6-1 to 3). At further distances from the river, there were fewer differences in TFA profiles among spider taxa. The MUFA 18:1ω7 and the PUFA 20:5ω3 were mainly associated with spiders of the genus *Tetragnatha* that were collected on the stream's edge, and the genera *Neoscona* and *Araneus* at 4 to 8 m from the river (Figure 6-1 to 3). The PUFA 18:2ω6 was associated with individuals from the genera *Araneus* and *Neoscona* at various distances but of relatively fewer numbers when compared to 18:2ω6 and 20:5ω3. Similar to the narrow site, at the wide site the SFAs 16:0 and 18:0 were associated with one or two spider taxa except during autumn when 16:0 was highly associated with spiders of the genera *Neoscona* between 4 and 16 m from the river.

Table 6-2: Results of SIMPER analyses indicating the contributions (%) of the major FAs to observed differences between distance (from the river) transects 0, 2, 4, 8, 16, 32 and 64 m, at each site, during each season. Major FAs were those contributing more than 5% to variation amongst groups. FAs with less than 5% were included if they were more than 5% in other seasons and sites.

	Fatty acid							
	16:0	**16:1ω7**	**18:0**	**18:1ω7**	**18:1ω9**	**18:2ω6**	**18:3ω3**	**20:5ω3**
Narrow								
Summer	8.72	6.77	7.43	4.62	19.47	18.56	10.73	5.18
Autumn	9.93	5.74	10.61	8.06	18.04	15.6	6.69	6.65
Winter	6.67	6.82	6.6	5.83	23.66	12.46	4.85	13.7
Spring	6.97	8.27	5.3	6.31	23.89	19.98	5.26	6.46
Wide								
Summer	10.18	5.24	7.81	9.94	15.68	19.86	6.71	12.65
Autumn	11.77	4.07	9.93	8.58	14.41	17.84	6.48	12.8
Winter	7.01	6.89	4.31	9.33	14.91	24.19	7.95	13.52
Spring	8.24	4.82	5.66	12.32	16.83	16.05	6.56	11.31
Intermediate								
Summer	11.39	3.73	9.75	6.43	16.29	13.66	6.75	8.64
Autumn	14.81	3.31	11.84	5.04	16.38	18.61	7.83	6.27
Winter	8.76	1.16	1.96	7.16	15.96	3.69	7.69	9.06

There were notable differences in TFA profiles across the three sites (Figure 6-1 to 3). Different FAs were important in distinguishing individual spiders with increasing distance from the river. The FAs 18:1ω9 18:2ω6 and 20:5ω3 contributed the most to variation in spider FA profiles during all of the seasons at the wide and intermediate sites (Table 6-2), particularly in spiders that were collected directly above the stream (e.g. *Tetragnatha,* Figure 6-1 to 3). The contributions of 20:5ω3 were always highest during winter at all the sites. The nMDS plots showed that 20:5ω3 was important in distinguishing FA profiles of *Tetragnatha* spiders at 0 to 8 m from the river (Figure 6-2 to 3) at the wide and intermediate sites. At the narrow site, the relative contribution of 20:5ω3 was low during all seasons except winter (Table 6-2). Similar to the other two sites, the contributions of 18:1ω9 and 18:2ω6 were the higher than other FAs at the narrow site.

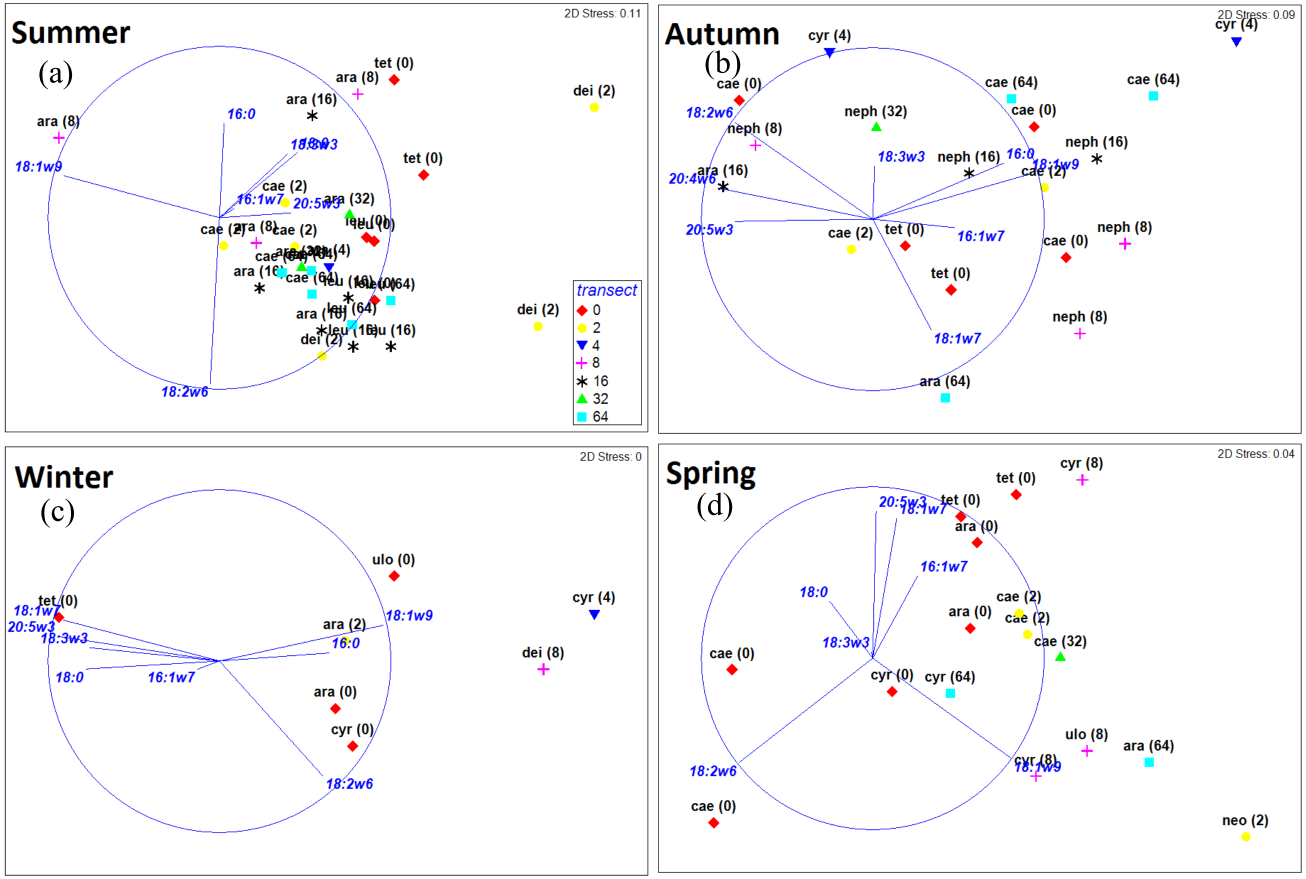


Figure 6-1: Non metric multidimensional representation of spider TFA composition during a) summer, b) autumn, c) winter and d) spring seasons at the *narrow site* of the Kowie River. The different colours are the distances (m) at which the spiders were collected from the river. Each point represents an individual spider, denoted by an abbreviation of the taxon name (genus or family) followed by the distance from the river. The taxa are: *Araneus* (ara), *Caerostris* (cae), *Cyrtophora* (cyr), Deinopidae (dei), *Leucauge* (leu), *Neoscona* (neo), *Nephila* (neph), *Nephila* male (nephmale), *Tetragnatha* (tet), Uloboridae (ulo).

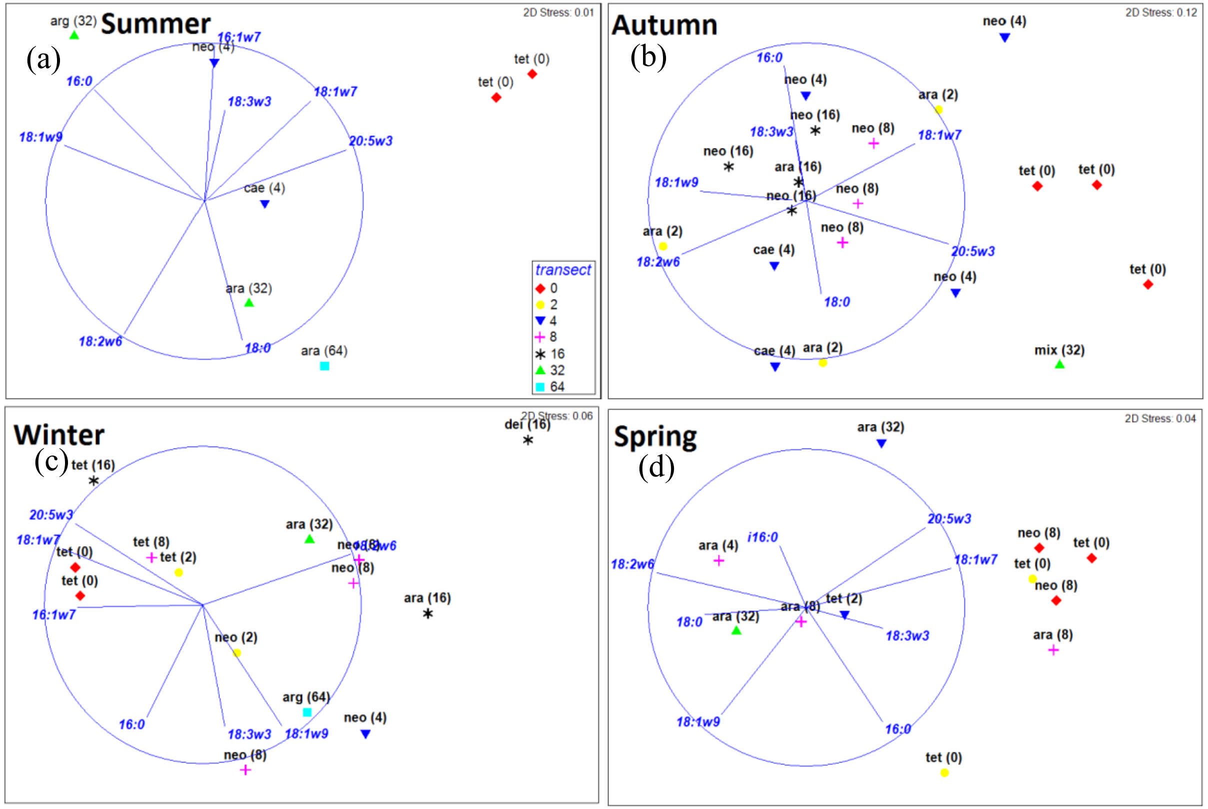


Figure 6-2: Non metric multidimensional representation of spider TFA composition during a) summer, b) autumn, c) winter and d) spring seasons at the *wide site* of the Kowie River. The different colours are the distances (m) at which the spiders were collected from the river. Each point represents an individual spider, denoted by an abbreviation of the taxon name (genus or family) followed by the distance from the river. The taxa are: *Araneus* (ara), *Caerostris* (cae), *Cyrtophora* (cyr), Deinopidae (dei), *Leucauge* (leu), *Neoscona* (neo), *Nephila* (neph), *Nephila* male (nephmale), *Tetragnatha* (tet), Uloboridae (ulo).

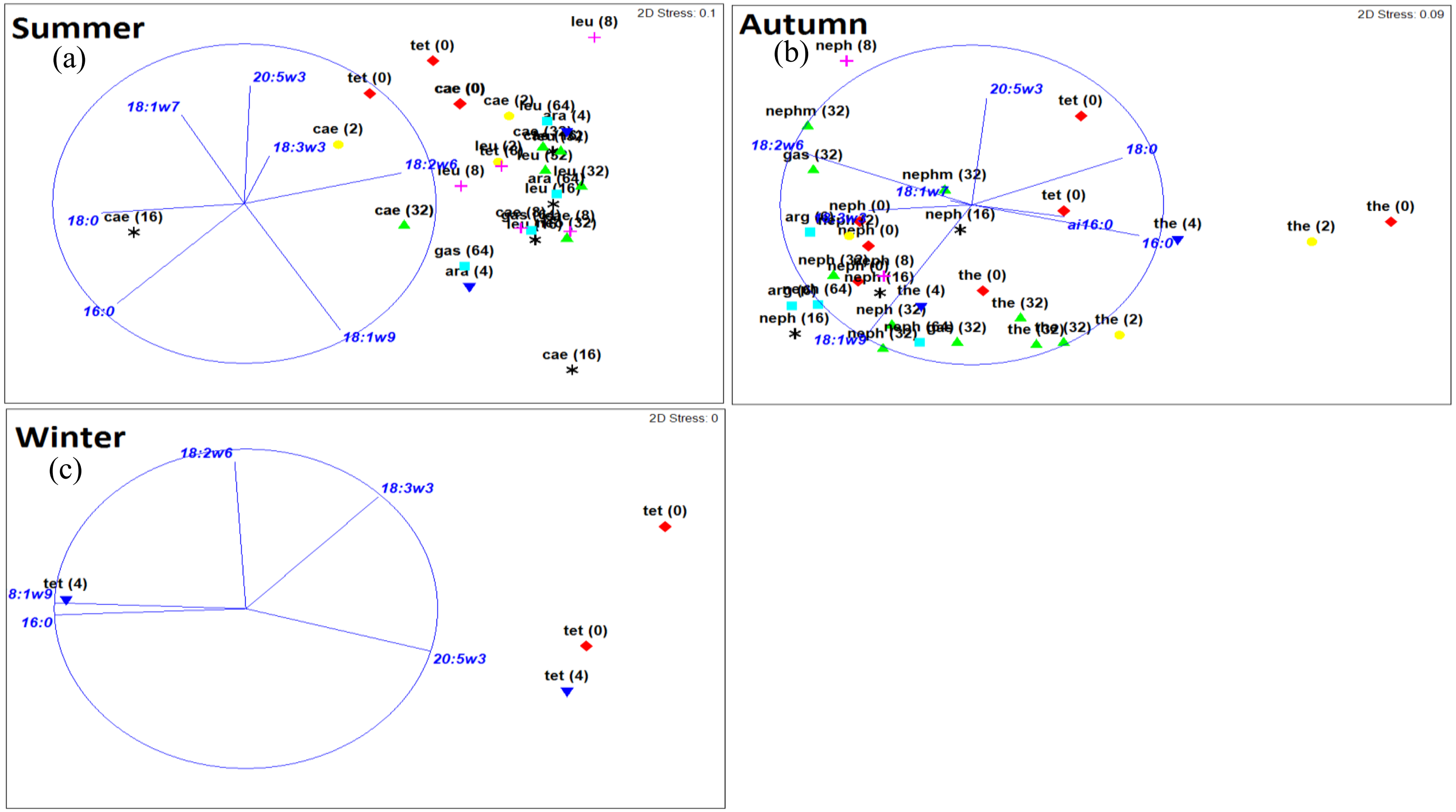


Figure 6-3: Non metric multidimensional representation of spider TFA composition during a) summer, b) autumn and c) winter seasons at the *intermediate site* of the Kowie River. Data were ordinated by distance (colour coded) from the river. Each point represents an individual spider, denoted by an abbreviation of the taxon name (genus or family) followed by the distance from the river. The taxa are: *Araneus* (ara), *Caerostris* (cae), *Cyrtophora* (cyr), Deinopidae (dei), *Leucauge* (leu), *Neoscona* (neo), *Nephila* (neph), *Nephila* male (nephmale), *Tetragnatha* (tet), Uloboridae (ulo).

6.3.1.2. Variations in major fatty acids

There were several positive and negative correlations between FA proportions in spiders and distance from the river, particularly at the wide and intermediate sites. Results of the Spearman's correlations showed that the levels of unsaturated fatty acids (UFAs) did not change significantly ($p < 0.05$) with increased distance (Table 6-3). The proportions of the MUFAs 18:1ω7 and 16:1ω7 were negatively correlated with distance from the river, with 18:1ω7 having particularly strong correlations at the wide site during all seasons except winter. Additionally, there was a negative correlation between distance from the river and 20:5ω3, the ratio of ω3/ω6 FAs and the sum of ω3 FAs. Correlations with 18:2ω6 were largely positive except at the narrow site, where there were no correlations ($p > 0.05$) during autumn and spring. Generally, the strongest correlations between PUFAs and the distance gradient were observed in the wide site, whilst the narrow site had the weakest correlations. Moreover, most of the correlations between distance and most of the major PUFAs were statistically significant at the wide site.

At the narrow site, only a few trends could be identified when proportions of selected major FAs (and groups of FAs) were plotted against distance from the river (Figure 6-4). In contrast, at both the wide and intermediate sites, clear decreases or increases in FA proportions could be observed with increasing distance from the river (Figure 6-4). At the wide and intermediate sites, substantial decreases in the sums of EFAs and ω3 FAs and the ratio of ω3/ω6 FAs were observed between 0 and 4 m from the river (Figure 6-4). Thereafter, only slight decreases in levels of these FAs were observed. An opposite trend was observed with 18:2ω6 at the wide and intermediate sites, where the FA proportions increased markedly from 0 to 4 m from the river and thereafter only increased slightly (Figure 6-4).

Differences in FA proportions were observed across sites. Proportions of EFAs and ω3 FAs and the ratio of ω3/ω6 FAs were generally lower at the narrow site when compared to the other two sites, particularly between 0 and 4 m from the river. Inversely, the proportion of 18:2ω6 between 0 and 2 m from the river was noticeably lower at the wide and intermediate when compared to the narrow site (Figure 6-4).

Table 6-3: Results of Spearman's rank correlation (Rho) non-parametric tests for relative FA proportions in spider tissues with increasing distance from the river. PUFA = polyunsaturated FAs, ω3 = sum of ω3 FAs, P/S = ratio of polyunsaturated/saturated FAs and ω3/ω6 = ratio of ω3 FAs/ ω6 FAs. * signifies statistical significance.

Season	**Fatty acid**												
	16:0	**16:1ω7**	**18:0**	**18:1ω9**	**18:1ω7**	**18:2ω6**	**18:3ω3**	**20:4ω6**	**20:5ω3**	**PUFA**	ω3	**P/S**	**ω3/ω6**
NARROW													
Summer	-0.27	-0.22	-0.10	0.22	-0.25	0.40*	-0.25	0.00	-0.26	0.11	-0.51*	0.24	-0.52*
Autumn	0.17	0.32	-0.22	0.03	-0.19	-0.07	0.44	-0.22	-0.29	-0.10	0.04	0.07	0.16
Winter	-0.09	-0.58	-0.22	0.76*	-0.76*	0.18	0.04	0.13	-0.76*	-0.58	-0.76*	-0.09	-0.76*
Spring	-0.28	0.01	-0.30	0.75*	-0.05	-0.09	0.29	-0.52*	-0.17	-0.31	-0.11	-0.04	-0.02
WIDE													
Summer	-0.24	-0.46	0.33	0.33	-0.97*	0.84*	-0.37	0.24	-0.84*	-0.29	-0.83*	-0.39	-0.83*
Autumn	0.27	-0.23	-0.10	0.35	-0.55*	0.28	-0.04	-0.12	-0.44	0.01	-0.47*	-0.09	-0.37
Winter	-0.21	-0.52	-0.04	-0.01	-0.47	0.62*	0.47	-0.05	-0.46	0.85*	-0.18	0.82	-0.51
Spring	0.05	-0.58*	0.13	0.53	-0.70*	0.73*	-0.38	0.04	-0.75*	0.04	-0.76*	-0.16	-0.80*
INTERMEDIATE													
Summer	0.13	-0.37*	0.04	0.47*	-0.52*	0.35	-0.07	-0.28	-0.65*	-0.17	-0.50*	-0.12	-0.51*
Autumn	-0.18	-0.11	-0.24	0.50*	-0.27	0.32	0.15	-0.06	-0.47*	0.14	-0.08	0.18	-0.43*
Winter	0.89	-0.45	-0.45	0.89	-0.45	-0.45	-0.89	0.00	0.00	-0.45	-0.89	-0.89	0.00

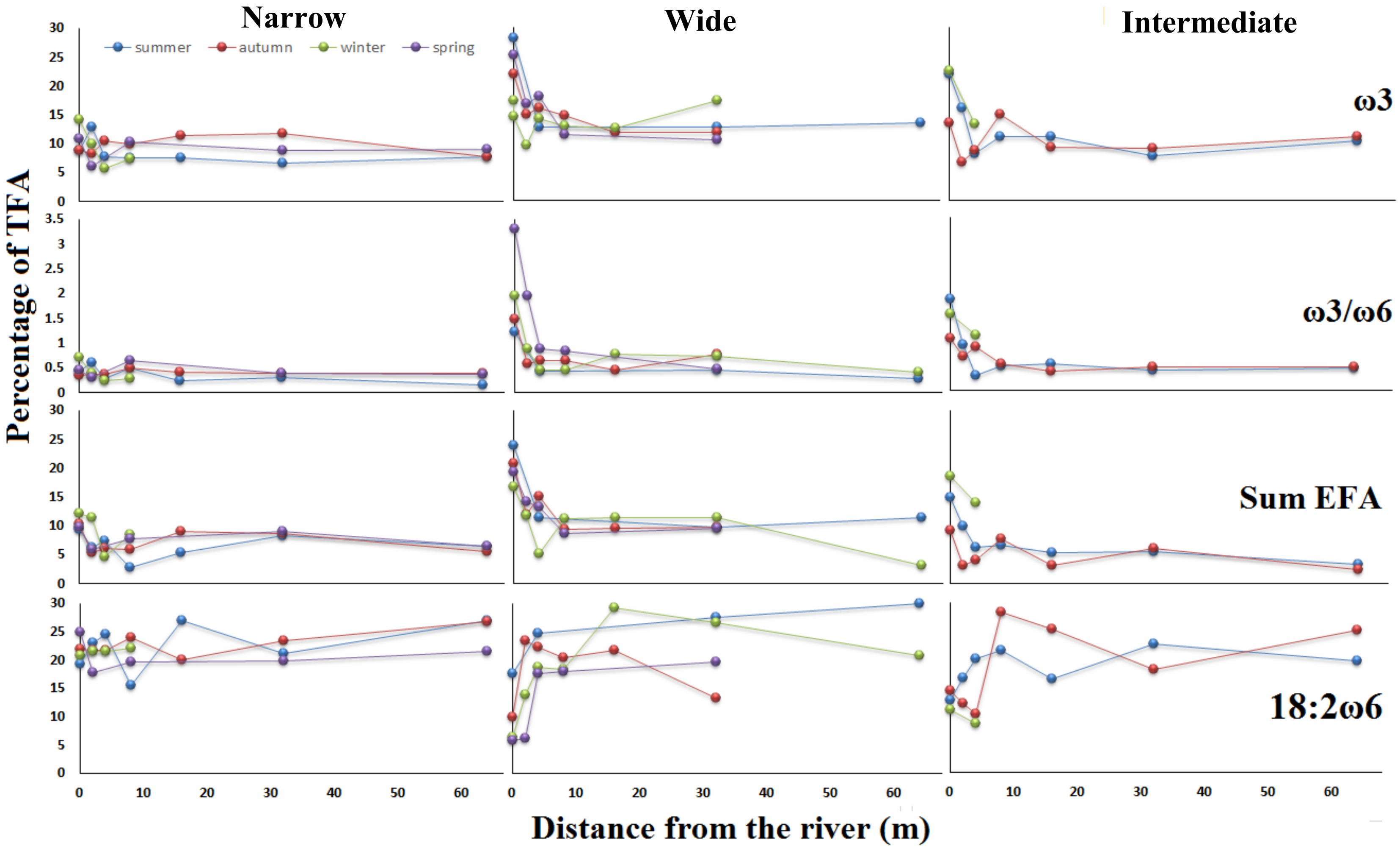


Figure 6-4: Relative proportions of FAs (% TFA) in spiders from varying distances from the river during different seasons, and at three study sites in the Kowie River catchment. The FAs include 18:2ω6, the sum of 20:4ω6 and 20:5ω3, the sum of all ω3 FA and the ratio of ω3/ω6 FA. The different colours represent seasons.

6.3.2. Modelling proportions of 20:5ω3 (EPA)

Correlations between distance from the river and the FA 20:5ω3 were similar to those with either ω3 or ω3/ω6 (Table 6-3). However, a decision was made to model only 20:5ω3 because both ω3 and ω3/ω6 included some FAs that that explained less than 5% variation in spider FA profiles (Table 6-2). Results of the NB regressions showed that all of the models fit the data well ($p < 0.05$). The proportions of 20:5ω3 were modelled against distance from the river and showed that 20:5ω3 decreased with increasing distance from the river during all seasons at the wide and intermediate sites (Figure 6-5). At the narrow site, proportions of 20:5ω3 decreased with distance during winter. All the coefficients (distance from the river and the sites) in the final NB models for all the sites were significantly different from each other and the model slopes were therefore different as well ($p < 0.05$), except in winter.

Residuals were plotted against spider taxa and there was heterogeneity in the residuals among taxa (Figure 6-6). The NB GLM models were not well suited for describing the data from all taxa, as *Tetragnatha*, *Neoscona* and *Nephila* had residuals that were far from the zero line. A three dimensional presentation of 20:5ω3 proportions showed that EPA values varied across taxa at similar distances from the river (Figure 6-7). The most apparent trend was that the genus *Tetragnatha* almost always had the highest levels of 20:5ω3, but it was largely confined to the immediate stream edge. Taxa such as *Araneus*, *Caerostris*, *Leucauge* and *Nephila* were collected from more than two distances, and proportions of 20:5ω3 were often lower further from the river. Other taxa such as Deinopidae, *Neoscona* and Uloboridae were under-represented.

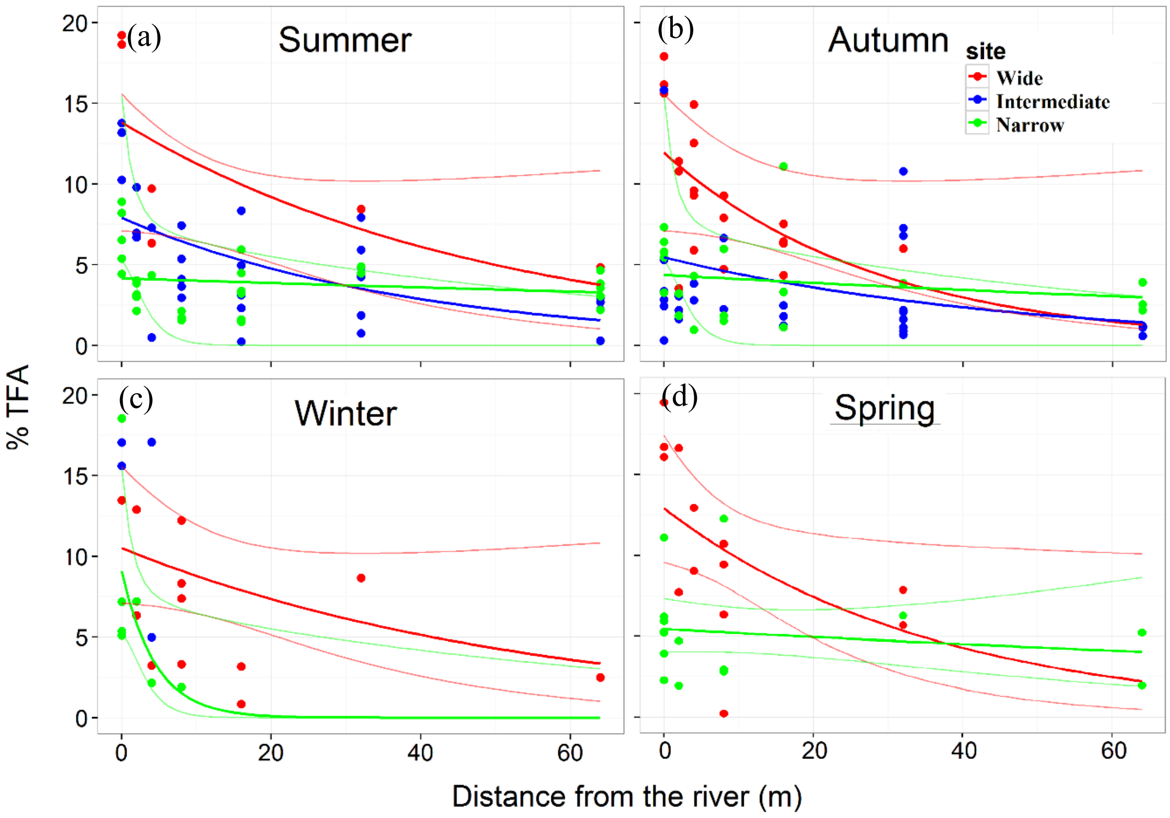


Figure 6-5: Negative binomial models showing the expected relative proportions of 20:5ω3 (EPA) in web building spiders with increasing distance from the river (m) at each of the study sites; wide (red), narrow (green) and intermediate (blue). The a) summer, b) autumn, c) winter and d) spring seasons were modelled, and 95 percent confidence intervals (thin lines) included.

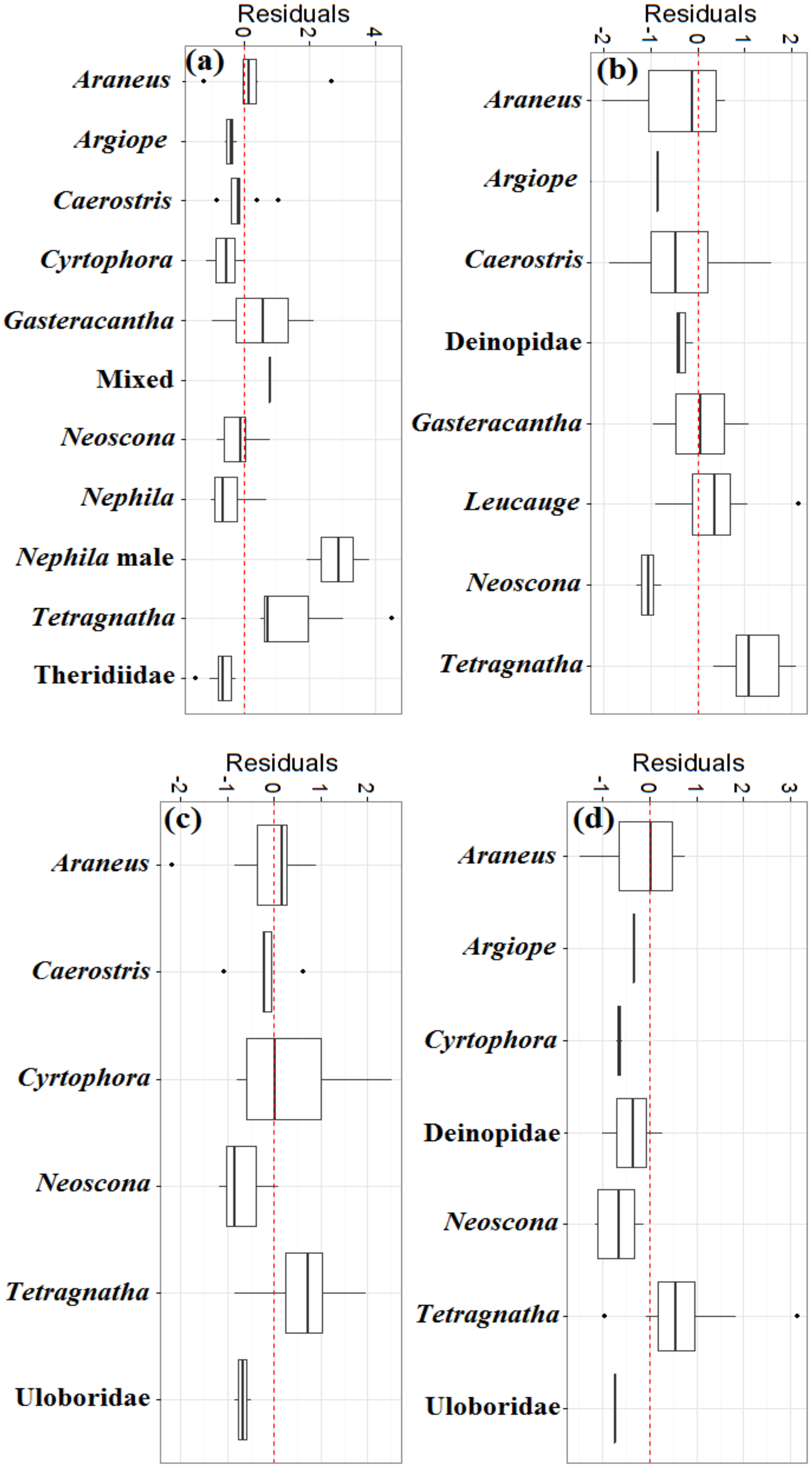


Figure 6-6: Box plots of NB GLM residuals plotted against spider taxonomic groupings during summer (a), autumn (b), winter (c) and spring (d) seasons at three sites along the Kowie River.

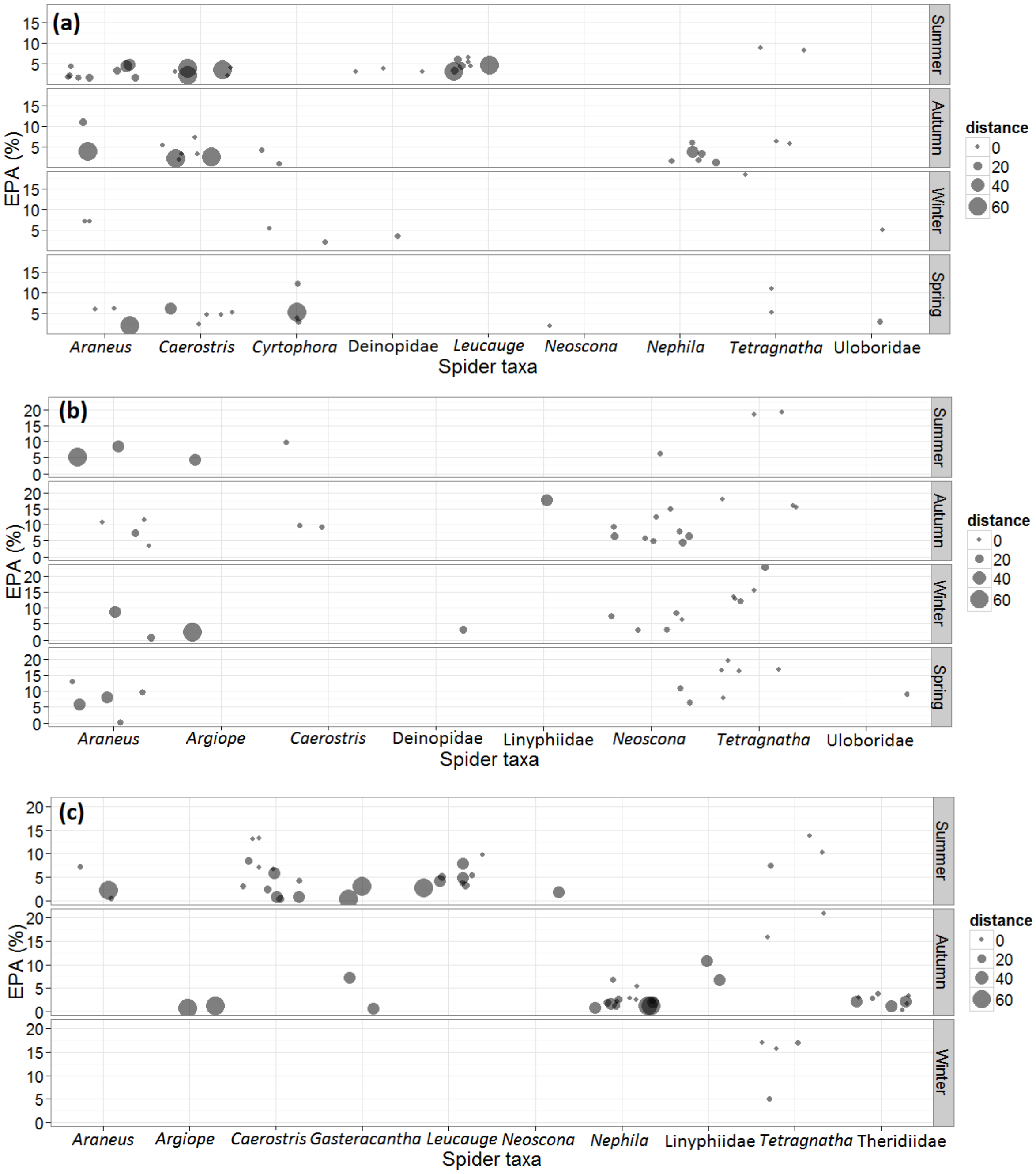


Figure 6-7: Bubble plot illustrating the relative proportions of 20:5ω3 (EPA) in web-building spider taxa at varying distances from the river. The plot presents EPA proportions for spiders from the narrow (a), wide (b) and intermediate sites (c), during four different seasons.

6.4. **Discussion**

There were measurable differences in FA compositions of spiders with increasing distance from the river, across sites and amongst four seasons. However, there was large variability even within the same sites or at similar distances from the river. The high variability probably results from the high diversity within the web-building group, and the associated variety of foraging tactics and body sizes involved (e.g. Higgins and Buskirk 1992, Wallace and Anderson 1996, see Chapter 4). Despite the high diversity within this group, statistically significant effects of space and time on spider FA compositions were apparent, and potential confounding factors such as body size, behaviour, maturity and web type (Gregorič et al. 2015) could not be included in the analyses owing to limited data on these aspects.

6.4.1. Diet variations with increasing distance from the river (Hypothesis 1)

6.4.1.1. Fatty acids profiles

Fatty acid profiles of web building spiders changed with increasing distance from the river. As spider taxonomic groups had varied FA profiles (Chapter 4), the change in FA profiles was likely an indication of the change in spider genus/family composition with distance. At further distances from the river, the abundance of aquatic insects became limiting and there were less spiders that specialized in capturing aquatic insects (horizontal web-builders), resulting in fewer distinction in TFA profiles among spider taxa. Additionally, the differences in TFA profiles between spider taxa were more apparent at distances closer to the river, which suggests that there was greater prey variety close to the river.

Eicosapentaenoic acid (EPA, 20:5ω3) is an aquatic marker made by algae in large quantities (Brett and Müller-Navarra 1997, Gladyshev et al. 2009b). Decreasing proportions of EPA with increasing distance from the river confirmed that aquatic nutrients were dispersing into the terrestrial system and spiders were feeding on them (Figure 6-5). Similar to EPA, the ω3/ω6 ratio is used as an indicator of material of aquatic origin (Koussoroplis et al. 2008, Taipale et al. 2015). Taipale et al. (2015) conducted feeding experiments on *Daphnia* (zooplankton) and discovered that a lower ω3/ω6 ratio indicated a more terrestrial diet and a higher ratio an aquatic one (Taipale et al. 2015). In the Kowie River, both ω3/ω6 and EPA were negatively correlated with distance from the river at the wide site (Table 6-4). Therefore, the ω3/ω6 ratio could equally be used in predicting spider (or other predators) diets, but EPA was chosen for intrinsic analysis in this study because the ω3/ω6 ratio included FAs that were present in large amounts in terrestrial habitats along the Kowie River (see methods section 6.3.3).

Docosahexaenoic acid (DHA, 22:6ω3) and arachidonic acid (AA, 20:4ω6) were recognized as aquatic biomarkers in several studies (Brett and Müller-Navarra 1997, Koussoroplis et al. 2008, Gladyshev et al. 2009a), but they were not appropriate tracers in the Kowie River system.

Docosahexaenoic was not detected in any of the spiders or terrestrial insects that were collected, and only rarely in the aquatic insects collected from a parallel study in the Kowie River (Moyo 2016). Docosahexaenoic is normally a minor FA in diatoms (Napolitano 1994, Bergamino et al. 2014), but is a dominant constituent in the lipids of dinoflagellates (Budge and Parrish 1998, Scholz and Liebezeit 2013, Cnudde et al. 2015) and some species of freshwater Chrysophyceae (Ahlgren et al. 1992). Low proportions of DHA in some consumers may indicate the decreased influence of dinoflagellates in the Kowie River relative to other primary producers (Bergamino et al. 2014). Arachidonic acid was present in most spiders, but it contributed less than 5% to the variation in spider FA profiles at all the sites during all seasons and was therefore not a useful tracer.

Long chain FAs (> 24 carbons) are characteristic of cuticular waxes of terrestrial plants and are often used as terrestrial plant indicators (Colombo et al. 2005, Panetta and Gélinas 2009). However, long chain FAs were not encountered in the spiders collected in this study. The FAs linoleic acid (LA, 18:2ω6) and alpha-linolenic acid (ALA, 18:3ω3) are additional terrestrial indicators, as they have been measured in elevated amounts in terrestrial systems (Koussoroplis et al. 2008). The proportions of LA, but not ALA, in spiders were strongly and positively correlated with distance from the Kowie River in many instances (Table 6-3). Fewer terrestrial plants contain significant amounts of ALA, whilst many synthesize and store large amounts of LA (Malainey et al. 1999). Detailed studies on the FA composition of the Kowie River vegetation will help to resolve the origin and nature of the terrestrial markers detected in the spiders.

The low proportions of terrestrial marker LA, and high quantities of aquatic markers EPA and ω3/ω6 between 0 and 2 m from the stream, suggested that spiders living at these distances consumed greater quantities of the aquatic organisms than spiders more distant from the water (Figure 6-4). These results agreed with the SIAR estimates that showed a high dependence of spiders living near the stream edge on aquatic food (see Chapter 4). According to Budge and Parrish (1998), samples with more than 2.5% LA can be considered to have terrestrial material as a significant source of organic matter. In the Kowie River, all the web-building spiders had more than 2.5% LA, hence they all fed on mixtures of aquatic and terrestrial prey.

6.4.2. Influence of stream width on extent of subsidisation (Hypothesis 2)

I hypothesised that aquatic subsidies in the Kowie River would be more abundant and travel further inland at wider sections of the river because the surface area (stream bed area) for insect emergence is greater at wide sites. Results generally showed that subsidisation was indeed highest at the widest section of the river and extended further into the terrestrial habitat at the wide sections.

Some herbivores and omnivores can convert ALA to EPA and DHA, but the conversion is inefficient and organisms grow better when provided with direct sources of EPA and DHA (Brett and Müller-Navarra 1997). Evidently, the lack of DHA or insignificant amounts of it, suggest that very little conversion was taking place in spiders of the Kowie River. It is likely that most of the EPA was being obtained from consuming aquatic subsidies. The very low proportions of EPA in spiders at the narrow section of the Kowie River (Figure 6-5) probably resulted from the low rates of aquatic insect emergence rates (Moyo 2016) and low abundances of aquatic adult insects in the riparian area (Chapter 3). Given that relatively low spider abundances and biomasses were observed in winter at the narrow section of the river (Chapter 3), it is plausible that the low aquatic insect abundance provided decreased amounts of EFAs that could support only a small community of riparian web-building spiders. In support of this claim, researchers have found that, irrespective of phytoplankton biomass, low production marine systems support low zooplankton and fish biomass when compared with highly productive systems such as upwelling sites (Dortch and Packard 1989, Minas and Minas 1992, Rossberg 2011). Brett and Muller-Navarra (1997) suggested that the levels of HUFAs are associated with the efficiency at which phytoplankton is converted to zooplankton biomass. In the same way, the amounts of EFAs in the Kowie River could be driving terrestrial predator biomass. The transference of EFAs to terrestrial systems is therefore important, if not vital, in maintaining terrestrial predator communities.

Correlation analysis revealed no significant relationships between EFA proportions in spiders and distance from the river at the narrow section of the Kowie River, in all seasons except winter (Table 6-3). It is likely that the low terrestrial insect abundance during winter made the aquatic subsidies more important in spider diets. The NB model also predicted relatively unchanging EPA proportions with distance, except in winter (Figure 6-5). On the contrary, it was evident that EPA levels in spiders decreased with increasing distance at the wider sections of the Kowie River in all seasons (Figure 6-5). The point at which the prediction curve levels off probably represents the at which the influence of aquatic subsidisation ended (Figure 6-5). Therefore, EPA always dispersed further inland at the wide than the narrow section of the river. As such, the width of a stream probably determines the amount of subsidies that the adjacent land receives, and wider streams extend their influence further inland. If a narrow river like the Kowie (16 m at the widest measured point) can influence spiders that are 64 m away, I expect that larger rivers such as the Congo (up to 12 km wide; Oberg et al. 2009), can have even greater influences on terrestrial communities. Additional studies in large and small rivers will improve our understanding of the relative importance of rivers to terrestrial food webs, and the trophic implications of human or climate induced changes to these connections.

Without considering the relatively wide confidence intervals, NB GLM results indicated that at far distances from the river, EPA levels were higher at the narrow than the wide section of the Kowie River (Figure 6-5). It is likely that this anomaly was a result of inter-taxon differences in EPA proportions that arose from differences in spider diets (Figures 6-6 to 7). Alternatively, EPA deficiencies in terrestrial predator diets may have stimulated some bioconversion of C 18 to C 20 FAs. Such an an adaptive mechanism has not been described in web-building spiders, but it has been observed in marine fish fed on EPA-deficient diets (Seiliez et al. 2003, Robin and Skalli 2007, Henry et al. 2015). Invertebrate predators along the Kowie River that did not have access to aquatic insects may have had adequate amounts of EPA in their diets, particularly in the narrow site where aquatic emergence is low, but the source of their EPA remains to be described. The experimental reduction of aquatic insect flux, such as that done by Kato et al. (2003), could be used to investigate potential dietary sources of EPA. Kato et al. (2003) used greenhouse coverings to cover stretches of up to 1.2 km of a river channel. Insect emergence could be blocked from a large section of the river for several months and spiders would be collected intermitently to investigate if levels of EPA change with aquatic insect deprivation.

Predictions of EPA proportions in spiders past the sampled distances was not feasable, as the different taxa had varied proportions of EPA at the same distances from the river (Figure 6-6 to 7). The lack of sufficient representation from each genera or family made it impossible to analyse trends of EPA with distance for each taxon. Future studies may be designed to target a single species that occurs at varying distances from the river, but web-building spider communities change with increasing distance from a river (Chapter 3), so targeting species is probably not a viable option. Nonetheless, the NB model was useful for describing the movements of EPA from a river into the terrestrial upland at a coarse level.

6.4.3. Temporal variation in web-building spider diets (Hypothesis 3)

6.4.3.1. Fatty acid profiles

The high variation in TFA profiles was maintained across the seasons, and there were distinct temporal patterns in spider FA profiles (Table 6-1). Eicosapentaenoic acid (EPA) contributed the most to variation in TFA profiles at all sites during winter. The FA EPA was the major driver of FA profiles during winter at all the sites and this demonstrated the importance of EPA in the diets of terrestrial consumers (Figure 6-1 to 3). Winter is generally characterized by low abundances of both aquatic and terrestrial insects (Baptista et al. 2001, Kato et al. 2003, Scheibler and Debandi 2008) and results from Chapter 3 illustrate this trend in the Kowie River. A study done by Nakano and Murakami (2001) showed that aquatic prey biomass was high in winter when terrestrial insect abundance was low. In that study, they reported that the aquatic prey provided between 50 – 90% of the energy budget of half of the bird species in their study area, and they concluded that the aquatic subsidies terrestrial consumers were significant in all seasons. Rundio and Lindley (2012) observed similar flux changes and showed that terrestrial insect biomasses peaked 1 – 2 months earlier than aquatic insect emergence. In the Kowie River, both aquatic emergence and terrestrial biomass were low during winter (Chapter 3), but the importance of EPA as a driver of FA profiles suggested aquatic subsidisation was present even though emergence was low. When terrestrial biomass is at its lowest, aquatic subsidies become more important in sustaining spider communities. The differences in patterns of subsidisation between my study and the two previous ones (Nakano and Murakami 2001, Rundio and Lindley 2012) is that there were differences in the emergence seasonal patterns. Alternatively, temporal patterns in the diets of web-building spiders suggested that spiders did not feed extensively in winter when the number of aquatic adult insects decreased and when lower air temperatures lead to lower food requirements by spiders (e.g. Williams 1979, Williams et al. 1995).

6.5. **Conclusion**

Anthropogenic impacts and global warming may cause decreases in EFA or HUFA production in rivers, but at present it is not possible to assess the potential ecological risks associated with these changes owing to the lack of global or even regional estimates of the amounts of HUFA exported from aquatic ecosystems (Gladyshev et al. 2009a). My study showed that the diets of terrestrial invertebrate predators are influenced by EPA produced in adjacent aquatic systems. Fatty acid patterns in spiders showed a gradual transition from aquatic to terrestrial environments, and these patterns varied with stream width and season. Despite the high diversity of spiders in riparian areas (Chapter 4), FAs can be used as indicators of cross subsidies in the aquatic/terrestrial interface. The following chapter (Chapter 7) illustrates the extent of intra- and inter- specific variability among aerial predator species and guilds.

Chapter 7. DIET AND FORAGING BEHAVIOUR IN ODONATA: NICHE PARTITIONING AND CROSS-BOUNDARY DIETARY SUBSIDIES

7.1. Introduction

Most adult odonates are opportunistic generalist aerial feeders that may adjust their diets over short periods to take advantage of shifting concentrations of prey (Baird and May 1997, Meraz et al. 2012). Most odonates live near water bodies and their diets constitute flying insects from both the terrestrial and aquatic habitats (Corbet 1999). High mobility and agility has made it difficult to observe prey selection by odonates, such that most of our knowledge has been derived from opportunistic sightings and direct observations, particularly of the larger prey items consumed by sit-and-wait predators launching from perches in open spaces (Corbet, 1999). Additionally, foraging behaviour is difficult to observe in dense vegetation or at great heights. Temporal patterns of foraging have been well documented in some odonate species, but individuals (particularly of one sex) could be foraging far from the study sites at other times (e.g. Martens and Rehfeldt 1989; Rehfeldt 1991).

There is phylogenetic variation in foraging behaviour amongst odonate species, but there is no direct link between phylogenetic distinctiveness and foraging behaviour. Foraging behaviour has been described in detail for many species (Baird and May 1997, May and Baird 2002, Olberg et al. 2005), but it is not known how the foraging behaviour relates with any diet differentiation among or within species. Individuals within the same species may display differences in diet when they are spatially separated (Corbet 1999). Furthermore, sexes within the same species may exhibit different foraging behaviour as the males often harass the females and force them to live and forage in habitats far from the areas of high insect abundance (Grant et al. 2014). With such variation, it is difficult to assign odonates to feeding guilds or functional feeding groups.

It is expected that most females live on the hillsides above streams, away from the stream, to avoid being attacked by males that live on the stream (Sherratt 2001, Happold 2009, Fincke 2015). Females have to resort to foraging far from the areas with high prey abundance because a high density of males often exists at the river. According to Pilastro et al. (2003) male sexual activity or aggression reduces female foraging efficiency in the eastern mosquitofish (*Gambusia holbrooki*). Male sexual activity in odonates may similarly reduce female foraging efficiency by driving females further from the river where aquatic insect abundance is likely to be low and result in low proportions of aquatic food in female Odonata diets. It is common that male and female odonates occupy different habitats for a significant time during their lives, apart from the time they are together mating (Corbet 1999).

The type of prey available to odonates, and hence their foraging behaviour, may relate to thermoregulation strategies. The principle thermoregulation mechanism for all odonates is ectothermy, as regulation of body temperature depends on external sources, such as sunlight or heated rock surfaces (Heinrich and Casey 1978, May 1981). However, some of the big odonates produce a lot of heat from flying and are thus prone to overheating. These big odonates are referred to as “fliers” because they spend most of their time flying and therefore forage mid-air and perform reproductive activities in flight (Corbet and May 2008). The fliers have developed additional behaviours to deal with overheating and they control their body temperatures by modulating the loss of body-generated heat (heterothermy). The smaller odonates that are entirely ectothermic are called “perchers”, as they spend most of their time on a perch and make only short flights to capture prey or engage females or males during mating and fighting, respectively. Fliers and perchers are therefore likely to have different diets because of their differences in foraging methods. Present knowledge suggests that odonate body sizes and foraging methods are correlated with thermoregulation strategies (Corbet 1999). However, the extent of diet partitioning by body size or foraging method remains undescribed. Additionally, it is not known how variations in body size and foraging method category affect the access of aquatic insects to odonates.

Living in riparian areas affords odonates with opportunities to subsist on the often highly abundant emerging aquatic insects, but the extent of use of this subsidy remains largely undefined. Through aquatic subsidisation, insects that emerge from the river subsidize the diets of adult odonates in terrestrial habitats (Figure 7-1). Odonates of different sizes, sexes and feeding behaviours are likely to have varied access to the subsidies. It is not known how the importance of this subsidy may change from narrow areas that typically have smaller channel widths and lower emergence rates to wide areas with wider channels and higher emergence rates. A few studies have made use of gut content analysis (Baird and May 1997), serological analysis (Sukhacheva 1996, Pfitzner et al. 2015), and recently, SIA (Bennett and Hobson 2009, Grant et al. 2014, Kautza and Sullivan 2016) to describe variations in odonate diets, but to my knowledge, none of these studies have assessed the levels of aquatic subsidisation amongst odonate guilds. Biomarker techniques such as SIA can be used to trace odonate diets across spatial scales because they provide time integrated signals that represent what the odonate has been consuming over several months (Peterson and Fry 1987). Fatty acid analysis (FAA) can be used to investigate odonate diet through assessing nutritional composition of odonates and presumed prey through revealing the sources, sinks and trophic pathways of organic nutrients. A few studies have made use of SIA to describe species-specific odonate diet partitioning and determine their diets (e.g. Bearhop et al. 2004; Grant et al. 2014), but to my knowledge, no study has used fatty acids (FA) to describe odonate food sources and niche partitioning.

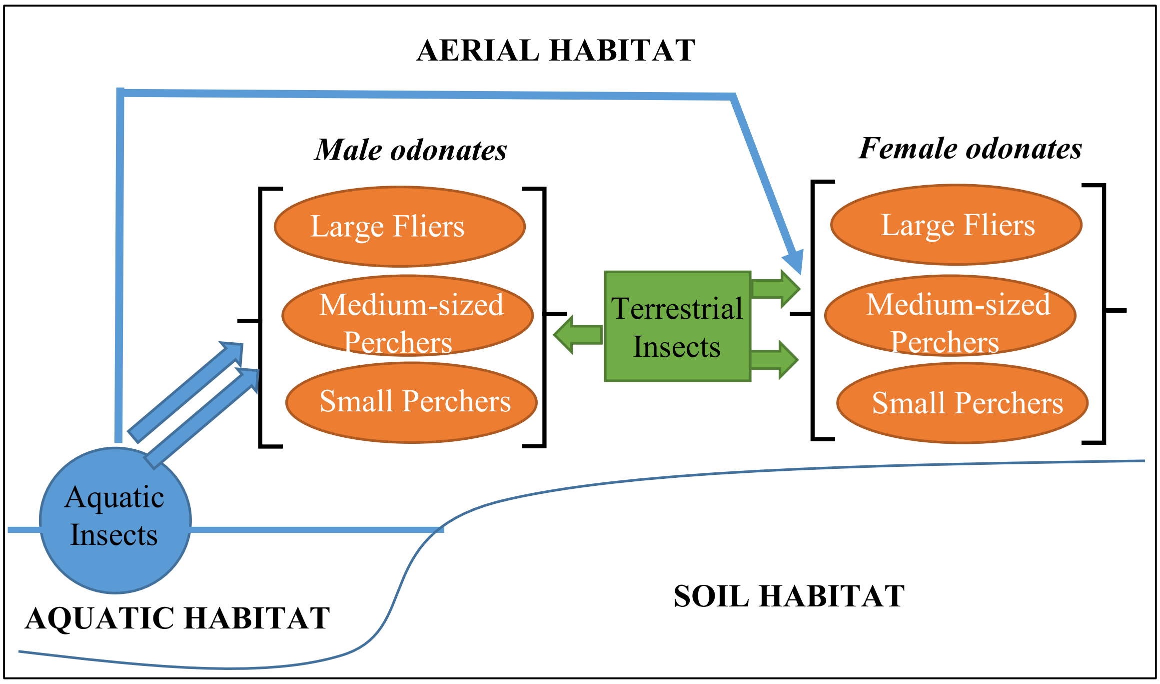


Figure 7-1: Conceptual diagram illustrating the inter-partition transfer (between habitats) of organic nutrients from the aquatic habitat to odonates in the terrestrial habitat. The diagram also shows intra-partition transfer (within the same habitat) of organic nutrients within the terrestrial habitat, from terrestrial insect prey to the odonates. Male and female odonates have different access to aquatic and prey.

In this chapter, I used FA and SI tools to investigate the amount of dietary opportunism/generalism among riparian predators. Odonates were the target organism because their identification shows much more resolution than that of spiders, which better controls for behavioural variables. I investigated the relationships between diet composition and two aspects of Odonata biology, body size and hunting strategy. Additionally, I investigated niche partitioning among groups of Odonata. Finally, I investigated the effect of environmental variation on dietary opportunism.

I hypothesized that:

1. *Odonata body size determines diet composition because bigger odonates can subdue and eat bigger insects than smaller odonates.*
2. *Odonata foraging behaviour (fliers and perchers) influences their overall diet composition because each hunting tactic exposes them to aquatic insects at different degrees.*
3. *There is sexual niche partitioning among odonates because females are often harassed by males and driven to forage in areas further from the stream where aquatic insect abundance is low.*
4. *Differences in feeding niche (based on size, foraging method, sex) are reflected in contributions of aquatic food sources to odonate diets because some odonate guilds (smaller odonates, perchers, and males) have better access to aquatic prey.*
5. *Odonates inhabiting wide sections of a river incorporate greater aquatic insects (relative to terrestrial insects) into their diets than those at narrow sections because the surface area (stream bed area) for insect emergence is greater at wide sites.*

7.2. **Materials and Methods**

7.2.1. Study sites

Odonates were collected from two sites in the Kowie River. The two sites consisted of a narrow and a wide section of the river. A detailed description of the sites is provided in Chapter 2.

7.2.2. Odonate samples

A sweep net with a 0.5 m diameter mouth and a 1 m handle was used to catch adult odonates resting on their perches or flying in mid-air in the Kowie River area. Odonata samples were collected from both sites during December, January and February (2013-2014). Newly-emerged adult odonates were avoided because their isotopic signatures would reflect their diets as aquatic larvae (Seifert and Scheu 2012). Newly-emerged, often with pale and undeveloped body colours, were identified using identification guides (Samways 2008). Collections were carried out three times a day (morning, afternoon and evening) to account for diurnal variations in odonate activity (Higashi 1978, Baird and May 1997). To minimize disturbance to the ecosystem, only 3-5 representatives of each species were collected at each site. Field guides were used to identify specimens in the field (Samways 2008) and samples were placed on ice until they reached the laboratory, where they were stored at -80°C.

Odonata species were grouped according to size and foraging strategy. Based on body length and sub-orders, there were three size classes that consisted of 1) damselflies (Zygoptera) all < 50 mm, 2) medium-sized dragonflies (Anisoptera) < 50 mm and 3) large dragonflies (Anisoptera) > 50 mm in length. A study by Knight et al. (2005) used similar size classes to investigate the effects of fish predation on odonate abundances in a number of ponds at the Katharine Ordway Preserve, Florida. Damselflies and dragonflies of the same body length were assigned to different size classes because damselflies are generally more slender, and hence smaller than dragonflies of the same length. The foraging strategy categories were fliers and perchers.

Due to expected intra-specific variation, males and females of the same species were considered as separate entities in most analyses. Both female and male categories were not represented in most species groupings (i.e. some species did not have both genders represented in the samples), hence to investigate the effect of gender (as a factor) in explaining variation in odonate diets all odonates samples were pooled according to gender at each site. Male odonates were used to test the factors of foraging behaviour, size and spatial variation.

7.2.3. Prey samples

Potential terrestrial and aquatic prey of odonates were consistent with those for the riparian web-building spiders (see Chapter 2).

7.2.4. Dietary analyses

Stable isotope analysis (SIA) and fatty acid analysis (FAA) were carried out as described in Chapter 2. The hypotheses of this study were tested using the following methods:

7.2.4.1. Hypothesis 1. Odonata body size determines diet composition because bigger odonates can subdue and eat bigger insects than smaller odonates.

The initial assumption was that diets vary and there is niche partitioning amongst odonate species. To investigate this assumption, isotopic- and FA-based feeding niches were assessed.

All odonates were categorized by species and isotopic niche widths of each species were calculated using SIBER (Stable Isotope Bayesian Ellipses in R) metrics in R Version 26 2.12.0 (see Chapter 4). Bayesian ellipses (Jackson et al. 2011) encompassing at least 40% of the data were drawn in isotopic space. Niche analysis was done at two levels, with (1) individual species, and (2) size categories as the niche groups. The trophic niche widths of odonate groups were represented by the areas within the ellipses and expressed as sample-size-corrected standard ellipse areas (SEAc). The extent of niche overlap was estimated as the ratio of the SEAc of the overlap to the SEAc of the ellipse, expressed as a percentage.

The FA profile of each odonate was expressed as FA relative proportions of the total FAs (TFA) present. Similarity percentage analysis (SIMPER) was used to determine the relative contribution of each FA to the total variation. Fatty acids with the highest contributions (> 5% of the total variation) were considered as major components. Non-metric multi-dimensional scaling (nMDS) was used to present TFA composition of odonate species at each site, and the major FAs were overlaid in the biplots (Gower et al. 2011). The major FAs were fitted as the vectors in a biplot (see Chapter 4). As an additional measure of trophic niche, the SIBER routine was adopted to analyse niche partitioning among the Odonata species using the x and y coordinates of the nMDS analysis of FA profiles of each individual. The areas of the ellipses represented the calculated FA niche widths, parallel to the isotopic niche (Antonio and Richoux 2014). To test the influence of body size on niche partitioning, males from each Odonata species were grouped according to the three body size categories (damselflies, medium-sized dragonflies and large dragonflies) and niche partitioning of sizes was analysed using SIBER and nMDS (as above).

7.2.4.2. Hypothesis 2. Odonata foraging behaviour (fliers and perchers) influences their overall diet composition because each hunting tactic exposes them to aquatic insects at different degrees.

To test the influence of foraging behaviour on niche partitioning, males from each odonate species were grouped into the percher and flier categories, and niche partitioning of foraging groups was analysed using SIBER and nMDS (as above).

7.2.4.3. Hypothesis 3. There is gender-based niche partitioning among odonates because females are often harassed by males and driven to forage in areas further from the stream where aquatic insect abundance is low.

To investigate gender-based niche partitioning among odonates, males and females were grouped separately, and the trophic niche of each gender was analysed using SIBER and nMDS (as above). Most of the odonate species that were collected were not represented by both sexes, hence to investigate niche partitioning based on gender it was necessary to pool all individuals of the same sex at each site.

7.2.4.4. Hypothesis 4. Differences in feeding niche (based on size, foraging method and sex) are reflected in contributions of aquatic food sources to odonate diets because some odonate guilds (smaller odonates, perchers and males) have better access to aquatic prey.

At each site, the contribution of aquatic food sources to diets of odonate groups (based on size, foraging method and gender) was estimated using Stable Isotope Analysis in R (SIAR) mixing models and FA indicators of aquatic/terrestrial contribution. The isotopic mixing models provided proportions of aquatic food sources in odonate diets, whilst FA indicators provided information on possible food sources of odonates.

a) Nutritional contribution of the primary producers to odonates

Stable Isotope Analysis in R (SIAR) mixing models were run in R Version 26 2.12.0 (The R Foundation for Statistical Computing 2010) to determine the percentage contributions of aquatic and terrestrial food sources to the diets of Odonata species. Due to the high variability in $\delta^{15}N$ isotopic values of invertebrate prey (Vander Zanden and Rasmussen 2001, McCutchan et al. 2003), I created the SIAR models using primary producers (the basal sources of carbon in the food web) that the invertebrates fed on to infer diet contributions of aquatic- and terrestrially-derived food to odonate predators. Insect $\delta^{13}C$ isotopic values are also highly variable in many polyphagous terrestrial insects that consume both C3 and C4 plants (personal observation). Terrestrial food sources were therefore represented by C3 and C4 plants, whilst aquatic sources were represented by macrophytes and different types of algal forms (epiphyton, benthic algae and periphyton). Producers with similar isotopic compositions were grouped to minimize the number of sources and narrow the range of possible solutions (Phillips et al. 2005, 2014). Isotope values of all the producers were obtained from concurrent studies (Dalu et al. 2014, Moyo 2016).

Trophic fractionations of 2.3 ‰ (SD = 0.24) for $\delta^{15}N$ and 0.5 ‰ (SD = 0.19) for $\delta^{13}C$ were adopted between invertebrates and producers and between invertebrate prey and Odonata (McCutchan et al. 2003). I assumed that most odonates preyed on herbivorous insects and that few preyed on invertebrate predators, therefore resulting in a trophic level between 3 and 4. A trophic level of 3.5 was thus used for most odonates, but in some cases this value had to be adjusted slightly so that the corrected isotopic values would fall within the convex hull bordered by the possible sources (Phillips et al. 2014), allowing for a SIAR model to be utilised. Fractionation from the producers to the odonates was therefore adjusted to 5.75 ‰ (SD = 1) for $\delta^{15}N$ and 1.25 ‰ (SD = 0.19) for $\delta^{13}C$ after factoring in the trophic level of 3.5.

The proportions of the different producers in the diets of odonates, as estimated by SIAR, were grouped as terrestrial or aquatic food sources. Proportions of aquatic food in the diets of odonates were compared across two sites with different stream widths (narrow and wide). Student t-tests were used to test for significant differences in proportions of aquatic insects between the narrow and wide sites. SIAR outputs of males and females were analysed separately to illuminate possible variation between gender categories.

b) FA indicators of aquatic contribution

Fatty acids associated with aquatic systems (Gladyshev et al. 2009a) were used to investigate the contributions of aquatic subsidies to different consumer species. Detailed analyses incorporated the FAs (and FA groups) that (1) distinguished aquatic or terrestrial insect groups (Chapter 4), and (2) contributed > 5% to the variation among odonate species. These FA tracers included oleic acid (ALA 18:1ω9), linoleic acid (LA, 18:2ω6), eicosapentaenoic acid (EPA, 20:5ω3), the sum of ω3 FAs (sum ω3), the sum of essential FAs [EFA, arachidonic acid (AA, 20:4ω6) + 20:5ω3] and the ratio of ω3/ω6 FAs (ω3/ω6). Oleic acid and linoleic acid were considered as indicators of terrestrial contributions. In odonates from the Kowie River, oleic acid represented the major FA in most cases, and its proportions in terrestrial insects were significantly higher than in the aquatic insect tissues (Chapters 4; Moyo 2016). The high proportions of oleic acid in odonates probably originated in their food, and as such this FA was used to indicate relative consumption of terrestrial food sources. Oleic acid is a major end product of FA biosynthesis in vertebrates and invertebrates (e.g. Hagen et al. 2000, Iverson et al. 2002). Oleic acid is often non-specific, but can be used as a tracer for carnivorous (Garrido 2007), fungal (Joergensen and Wichern 2008), plant (Ruess et al. 2007, Ruess and Chamberlain 2010) or algae feeding by consumers. Linoleic acid has been measured in elevated amounts in terrestrial systems (Koussoroplis et al. 2008) and is considered an indicator of terrestrial food sources (Malainey et al. 1999). Eicosapentaenoic acid, EFAs, sum ω3 and ω3/ω6 were used as indicators of aquatic contribution (see Chapter 4).

7.2.4.5. Hypothesis 5. Dragonflies inhabiting wide sections of a river incorporate greater aquatic insects (relative to terrestrial insects) into their diets than those at narrow sections because the surface area (stream bed area) for insect emergence is greater at wide sections.
The dietary contributions of aquatic food sources to odonates were compared between the two sites with different stream widths. Dietary contributions were quantified through SIAR mixing models and FA levels (see above). Student's t-tests were used to determine statistical differences in diet contributions between the two sites.

Analysis of combined effects of body size, gender, foraging method, and stream width on the ratio of aquatic/terrestrial insects in Odonata diets

The proportions of aquatic dietary contributions in odonates was statistically analysed for differences between Odonata groups. To investigate the influence of body size or foraging method on Odonata diet, a factorial 4-way permutational analysis of variance (PERMANOVA; Anderson 2001) was run with Euclidean distances calculated from the raw data. Site (narrow and wide), body size (Zygoptera, Anisoptera less than 50 mm and Anisoptera > 50 mm), foraging method (flier and percher) and gender (male and female) were considered as the main factors in a Euclidean based PERMANOVA. A factorial design was chosen because results would account for any shared variance between factors and allow identification of significant interaction effects. Each term in the analysis was tested using 9999 permutations of the correct relevant permutable units.

Initially, a full factorial model was run to check for all main effects (factors) and interactions. Subsequently, a stepwise deletion of insignificant terms and interactions was done by assessing mean squares (MS) and significance levels. Terms with the lowest MS were the first to be considered for deletion because they had the lowest contributions to variation in the model. Some non-significant terms were not deleted because they were part of the factorial model and their deletion influenced the significant terms (Lindstrom and Bates 1990).

Statistical analyses were completed using various statistical programmes; R (R Core Team 2015) for SIBER and SIAR, PAST (Hammer Ø et al. 2001) for SIMPER, Statistica (StatSoft

Inc. 2011) for Student's t-tests and ANOVA analyses and PRIMER V6 (Clarke and Warwick 2001) for PERMANOVA.

7.3. **Results**

A total of 21 species of Odonata were observed in the two study areas, 10 in the narrow site and 16 at the wide site (Table 7-1). A larger number of perchers than flier species was collected (Table 7-1). Based on field collections, odonates were grouped according to size (body length) categories, which constituted damselflies (all were less than 50 mm in body length), medium-sized sized dragonflies (< 50 mm) and large dragonflies (> 50 mm). The 50 mm cut off was chosen because many of the odonates that are generally considered (Olberg et al. 2005) to be large have body lengths greater than 50 mm. Damselfly and dragonflies < 50 mm were assigned to different size classes because the dragonflies generally had wider bodies and greater biomasses than damselflies of the same body length.

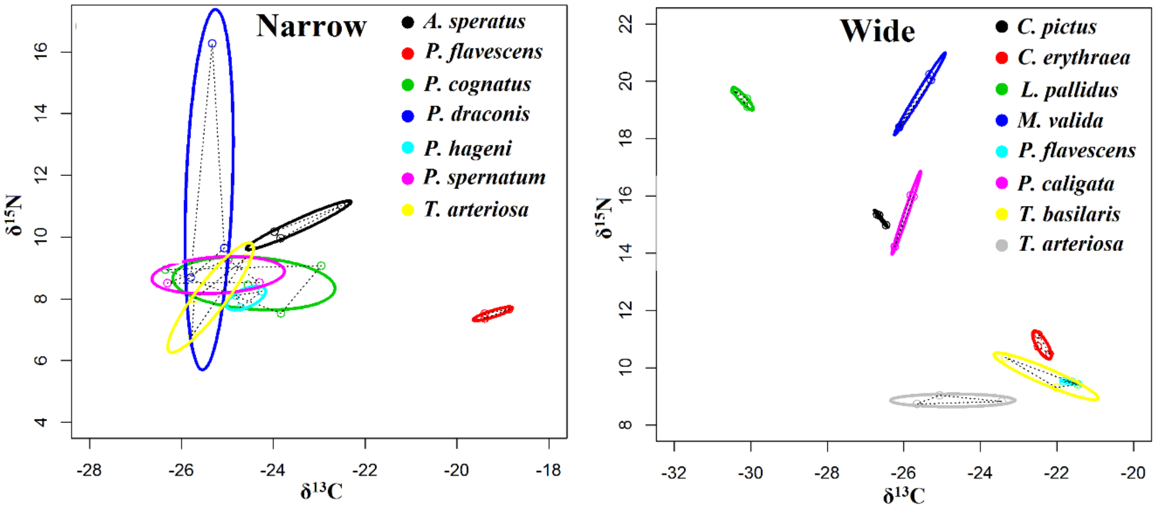
7.3.1. Dietary specialization and niche partitioning

There was generally more isotopic niche overlap between odonate species at the narrow than wide site (Figure 7-2a). Isotopic niches of odonates were highly distinct (and small; all with SEAc < 1.0) at the wide site (Figure 7-2a). Fatty acid niches of odonates were not visibly different across the two sites, but some species [e.g. *Pantala flavescens* (SEAc = 0.1)*, Tramea basilaris* (SEAc = 0.3) and *Crocothemis erythraea* (SEAc = 1.1)] at the wide site had small niches (Figure 7-2b). At the narrow site only one species, *Trithemis arteriosa*, had a very small FA niche (SEAc = 0.07, Figure 7-2b).

Table 7-1: Odonata species of two suborders, Zygoptera (damselflies) and Anisoptera (dragonflies), captured from the study sites. Body length measurements were obtained from literature (Corbet 1999, Samways 2008). The letters f (female) and m (male) indicate the gender of the captured odonate specimens whilst the (*) indicates that an odonate species was absent at a site.

	Hunting guild	Species	Presence		Body size (mm)
			Narrow	Wide	
Anisoptera	Fliers	*Tramea basilaris*	*	m	45-49
		Pantala flavescens	m	m	47-50
		Aeshna subpupillata	*	f	58-60
		Anax speratus	m	*	76-78
	Perchers	*Trithemis arteriosa*	m/f	mf	32-36
		Trithemis dorsalis	m/f	*	37.5-38.5
		Crocothermis erythraea	*	m/f	39-40
		Nesciothemis farinosa	*	m	40-46
		Paragomphus cognatus	m	*	42-44
		Orthetrum julia	m	*	45-49
		Ceratogomphus pictus	*	m	53-54
		Orthetrum trinacria	*	m	53-58
Zygoptera	Perchers	*Ischnura senegalensis*	*	m	29-30
		Pseudagrion spernatum	m	*	38-43
		Platycypha caligata	*	m/f	31-33
		Pseudagrion massaicum	*	m	31-35
		Pseudagrion draconis	m	m	31-36
		Pseudagrion hageni	m	m	36-43
		Pseudagrion kersteni	m/f	m/f	38-41
		Metacnemis valida	*	m/f	39-40
		Lestes pallidus	*	m	40-42

a) Isotopic feeding niche



b) Fatty acid feeding niche

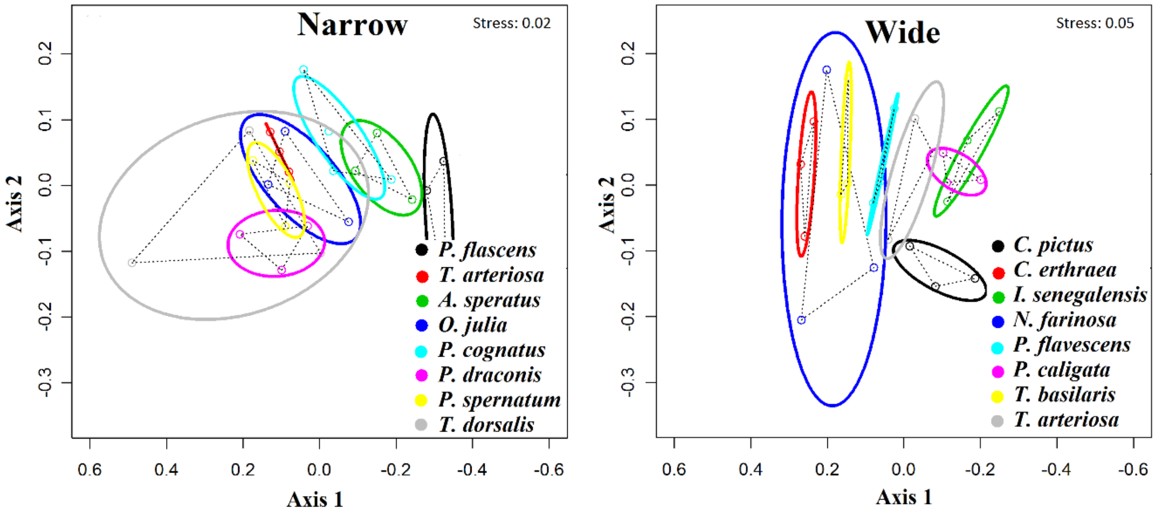
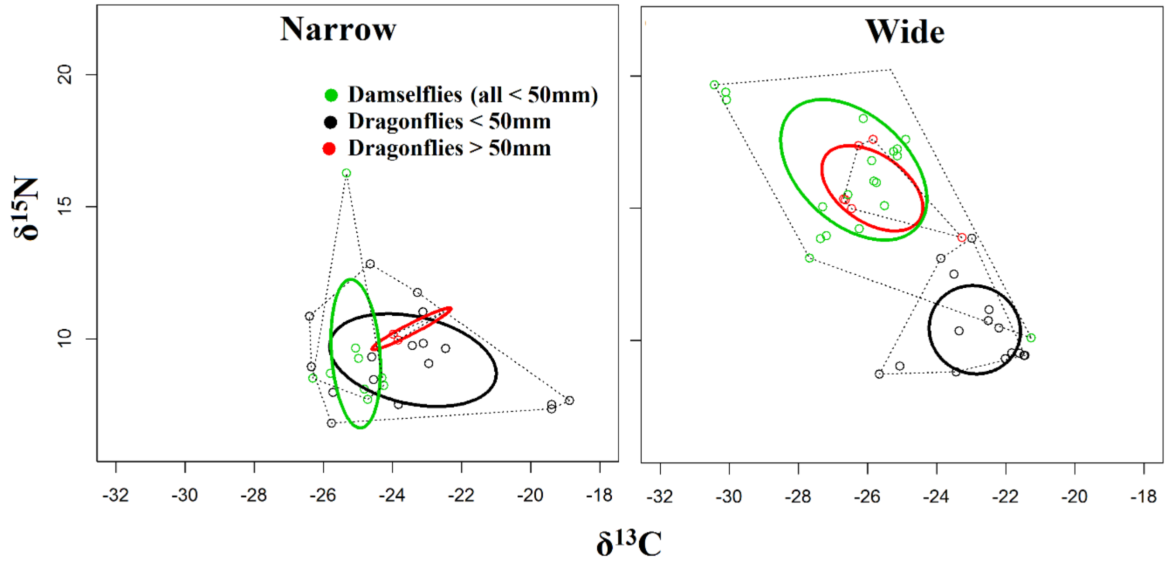


Figure 7-2: Dual a) isotopic ($\delta^{13}C$ and $\delta^{15}N$, ‰) and b) FA (nMDS x-y ordinates) plots representing niche widths of male odonates (different species represented by different colours) at the narrow and wide sites. The plots were created using SIBER and the ellipses (solid lines) represent sample size-corrected niche widths and the dotted lines are convex hulls. **NOTE**: change in the isotope axes scales between the two sites.

7.3.1.1. The effect of body size on niche partitioning

There was separation of isotopic and FA niches amongst odonates of different sizes (body length; Figure 7-3). There was more isotopic and FA niche overlap at the narrow than wide site. At the narrow site, the medium-sized dragonflies had larger isotopic (SEAc = 10.7) and FA (SEAc = 14.3) niche widths than the damselflies (isotopic SEAc = 5.4, FA SEAc = 4.7) and large dragonflies (isotopic SEAc = 0.4, FA SEAc = 4.9), but there was no niche (isotopic and FA) overlap between the large dragonflies and the damselflies (Figure 7-3). At the wide site, there was no FA niche overlap between any of the size classes, but the isotopic niches of large dragonflies and damselflies overlapped (100% of the large dragonfly niche space, Figure 7-3).

a) Isotopic feeding niche



b) Fatty acid feeding niche

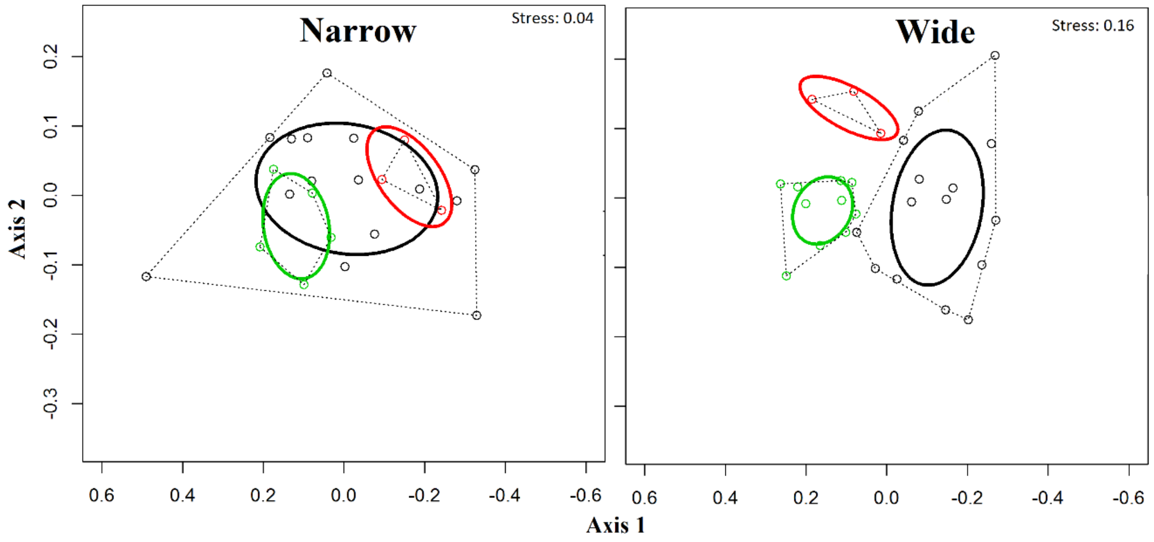
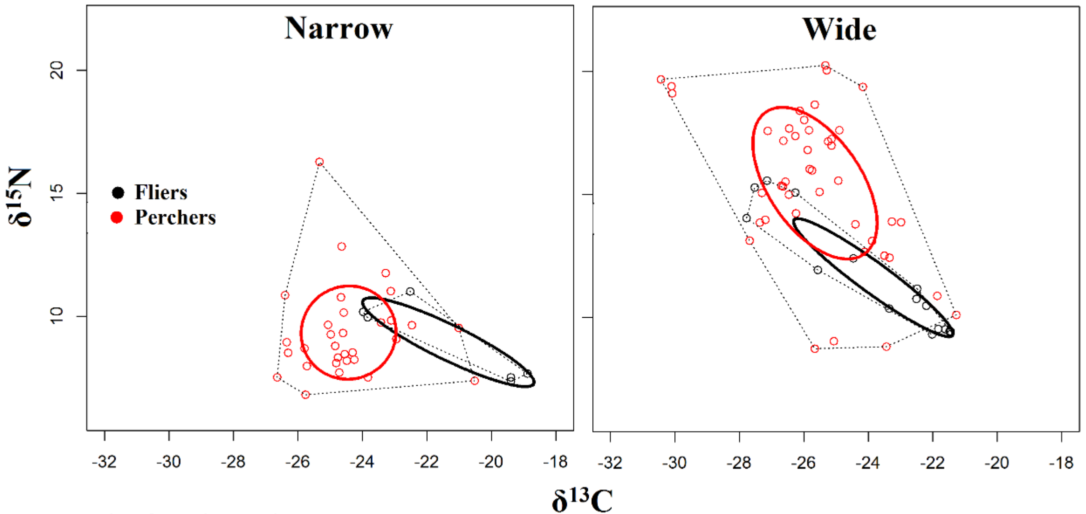


Figure 7-3: Dual a) isotopic ($\delta^{13}C$ and $\delta^{15}N$, ‰) and b) FA (nMDS x-y ordinates) plots representing niche widths of male odonates grouped by size classes (damselflies, medium and large sized dragonflies) at the narrow and wide sites. The plots were created using SIBER and the ellipses (solid lines) represent sample size-corrected niche widths and the dotted lines are convex hulls.

7.3.1.2. The role of hunting strategies in diet partitioning

Fliers and perchers largely had separate isotopic and FA niches at both sites, with minimal overlap (5 - 25% of percher niche space) except for FA niches at the narrow site that did not overlap (Figure 7-4). The niche width of the perchers at the wide site (isotopic SEAc = 15.5, FA SEAc = 14.1) was much larger than that at the narrow site (isotopic SEAc = 8.5, FA SEAc = 10.1), and the relative differences between niche widths of perchers and fliers were greater at the wide (isotopic SEAc = 10, FA SEAc = 6.4) than the narrow site (SEAc = 3.2, FA SEAc = 0.9).

a) Isotopic feeding niche



b) Fatty acid feeding niche

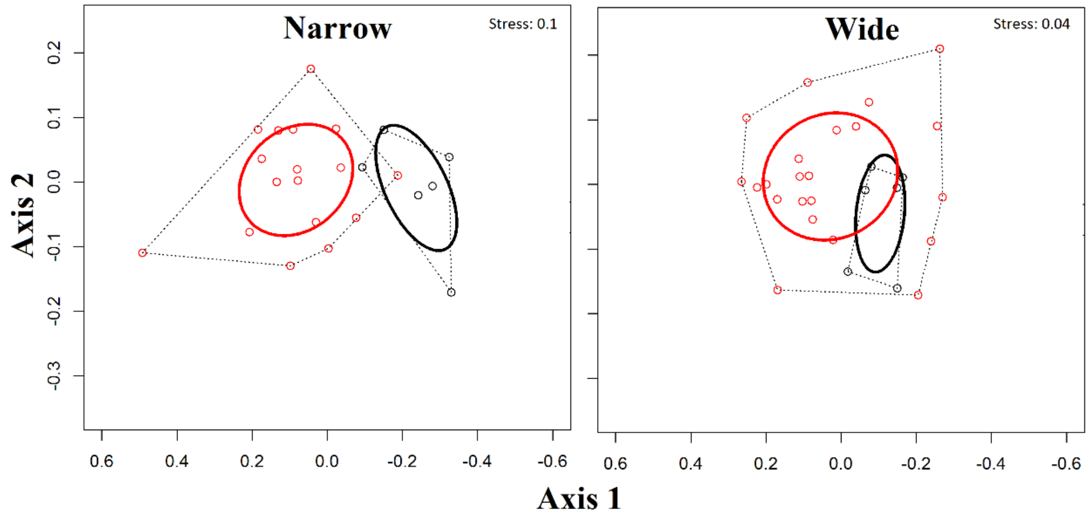
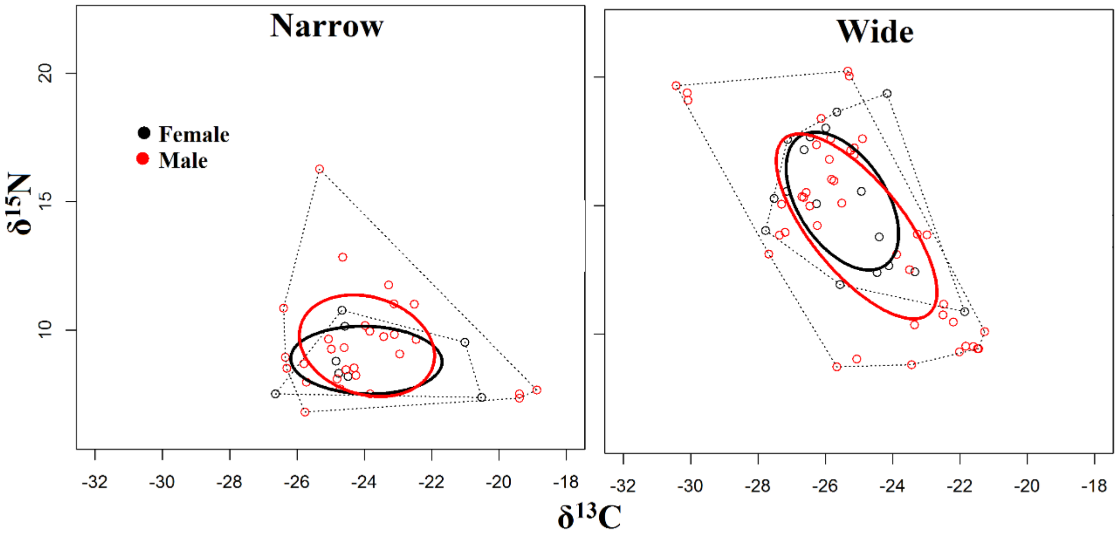


Figure 7-4: Dual a) isotopic ($\delta^{13}C$ and $\delta^{15}N$, ‰) and b) FA (nMDS x-y ordinates) plots representing niche widths of male odonates based on foraging groups (fliers and perchers) at the narrow and wide sites. The plots were created using SIBER and the ellipses (solid lines) represent sample size-corrected niche widths and the dotted lines are convex hulls.

7.3.1.3. Niche partitioning based on gender

There was appreciable isotopic and FA niche overlap between male and female odonates at both sites (Figure 7-5; 50 – 90% of female odonate niche space). Male odonates had larger isotopic and FA niche widths than females at the narrow (*Isotopic niche*, male SEAc = 8.7, female SEAc = 6.9; *FA niche*, male SEAc = 11.4, female SEAc = 9.4) and wide sites (*Isotopic niche*, male SEAc = 15.9, female SEAc = 11.2; *FA niche*, male SEAc = 9.0, female SEAc = 8.8; Figure 7-5).

a) Isotopic feeding niche



b) Fatty acid feeding niche

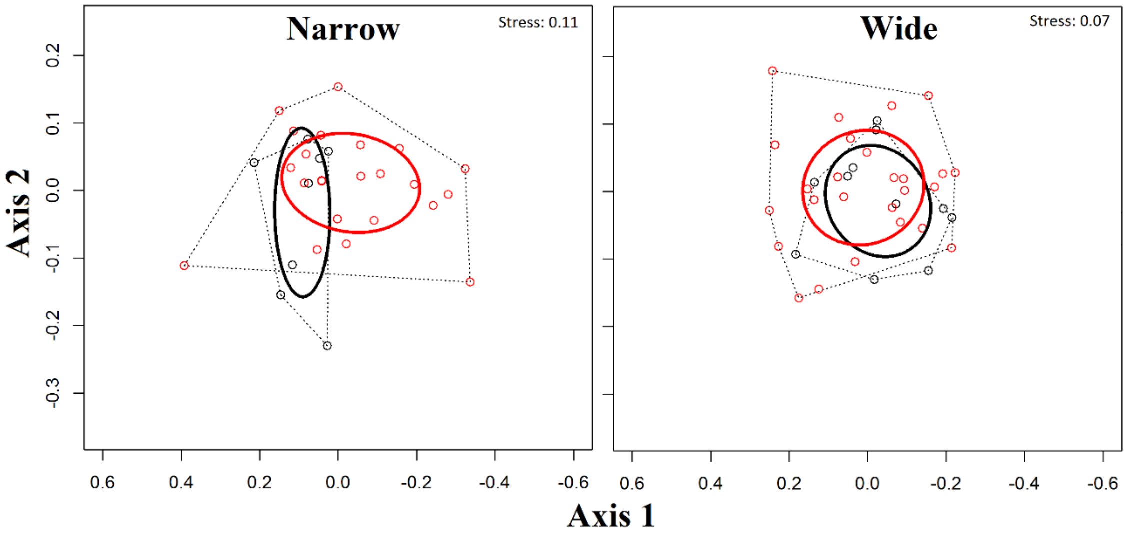


Figure 7-5: Dual a) isotopic ($\delta^{13}C$ and $\delta^{15}N$, ‰) and b) FA (nMDS x-y ordinates) plots representing niche widths of male and female odonates at the narrow and wide sites. The plots were created using SIBER and the ellipses (solid lines) represent sample size-corrected niche widths and the dotted lines are convex hulls.

7.3.1.4. *Niche partitioning based on site*

Odonata niches at the narrow site were largely separate from those at the wide site. Isotopic niche analysis revealed no niche overlap between odonates at the two sites (Figure 7-6a), but FA niche analysis showed considerable overlap (48% of the narrow site odonates niche space; Figure 7-6a). Isotope and FA results produced conflicting results. The isotopic niche width of narrow site odonates (SEAc = 12.1; Figure 7-6a) was larger than that of wide (SEAc = 8.9), but the FA niche width of narrow site odonates (SEAc = 2.7; Figure 7-6b) was distinctly smaller than those at the wide section (SEAc = 7.3).

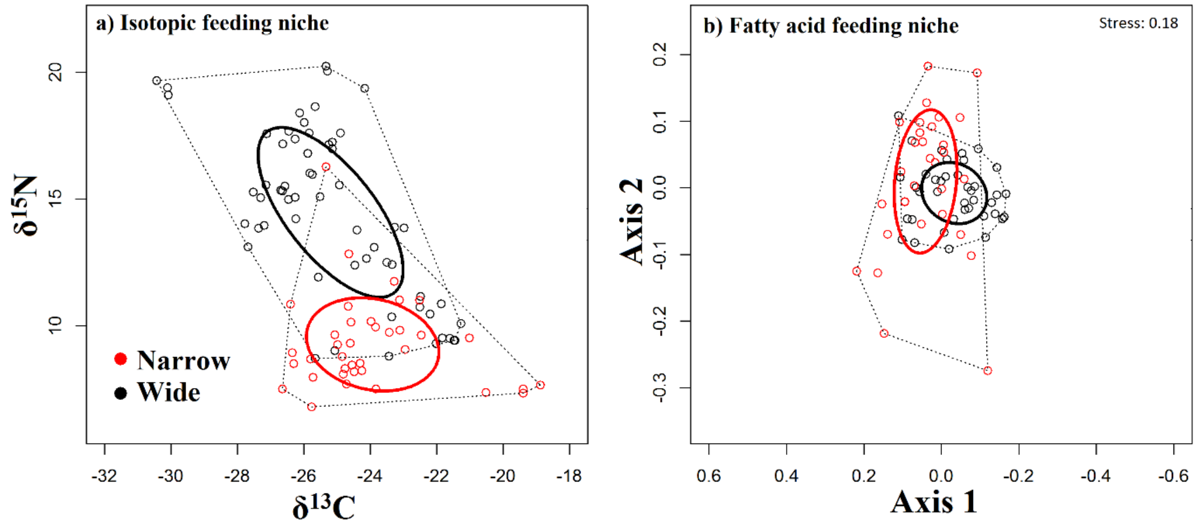


Figure 7-6: Dual a) isotopic ($\delta^{13}C$ and $\delta^{15}N$, ‰) and b) FA (nMDS x-y ordinates) plots representing niche widths of all odonates at the narrow and wide sites. The plots were created using SIBER and the ellipses (solid lines) represent sample size-corrected niche widths and the dotted lines are convex hulls.

7.3.2. *Variations in use of cross-boundary subsidies*

7.3.2.1. *Odonate diet proportions*

The contributions of aquatic food in the diets of odonates varied between and within species at each site (Figures 7-7 and 7-8). Additionally, there was variation between males and females of some species, e.g. *Crocothermis erythraea* (Figure 7-8), but there were generally no significant differences in the proportions of aquatic food between male and female groups at both sites (narrow site, $t = -0.35$, d.f. = 10, $p = 0.74$; wide site, $t = 0.46$, d.f. = 20, $p = 0.64$). Irrespective of the high variability, the proportion of aquatic food in odonate diets was generally higher in perchers than fliers at the wide site (Figure 7-8). At the wide site, the relative contribution of the aquatic food was significantly lower in the fliers (*Pantala flavescens*, *Aeshna subpupillata* and *Tramea basilaris;* 34.1% on average) than the perchers (mean = 63.0%; t-test, t = 2.728, d.f. = 19, p = 0.01). However, no significant differences could be detected in contributions of the aquatic food to fliers (*Anax speratus* and *Pantala flavescens*; 31.0%) and to perchers (mean = 32.3%) at the narrow site (t-test, t = 0.111, d.f. = 10, p = 0.91).

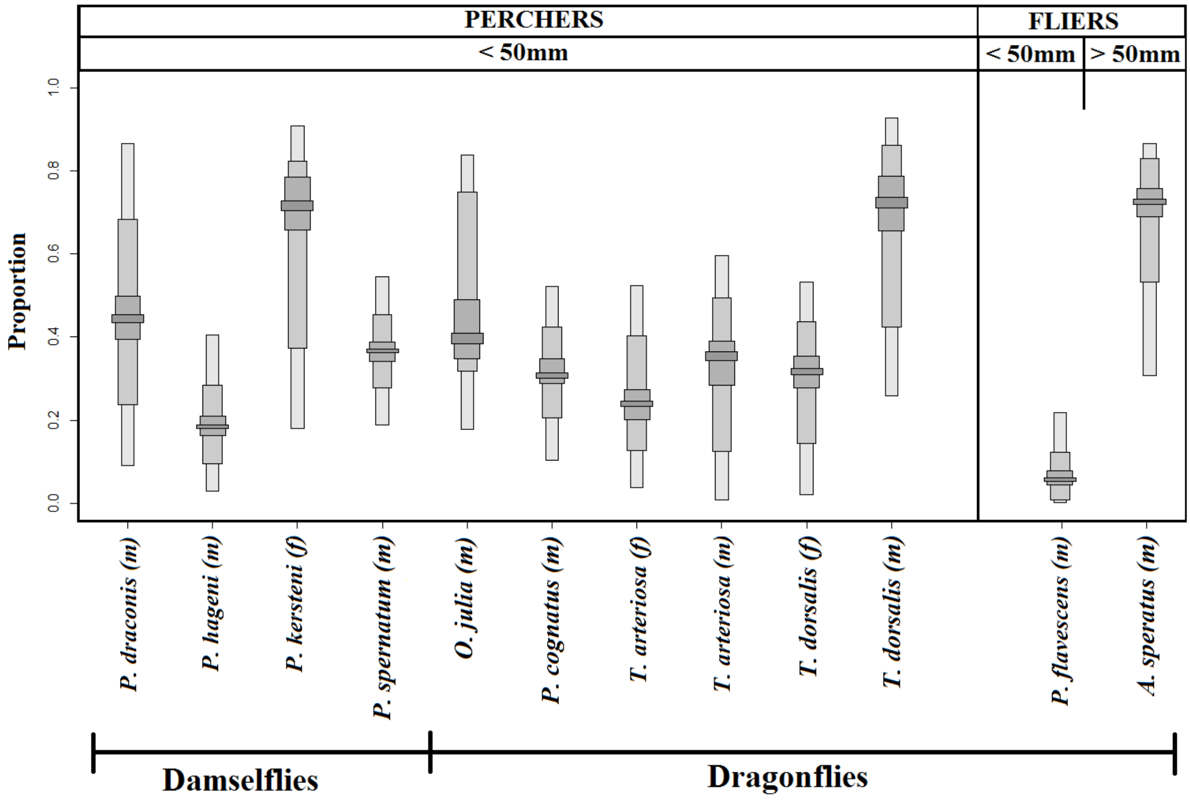


Figure 7-7: Contributions of combined aquatic food sources to diets of Odonata species at the narrow site. The letters m and f represent male and female specimens, respectively. The boxplots represent the probability densities in credibility intervals of 95, 75 and 25% for the aquatic food sources.

The relative contributions of aquatic food were distinct amongst the three size classes at the wide site (F = 5.5, d.f. = 2, p = 0.02). The damselflies and large dragonflies had significantly higher proportions of aquatic food in their diets (p < 0.05; means = 57.9 and 47.7%, respectively) than the medium-sized dragonflies (mean = 21.6%). At the narrow site, the contributions of aquatic food sources were not significantly different amongst the damselfly (29.4%), small dragonfly (32.0%) and large dragonfly (48.4%) size classes (F = 0.48, d.f. = 2, p = 0.64).

The proportions of aquatic food sources in odonate diets were generally low (most species < 40% aquatic diet) at the narrow site, but species such as *Anax speratus*, *Pseudagrion kersteni* and *Trithermis dorsalis* had proportions of aquatic food as high as 70 to 80% (Figure 7-7). In contrast, the proportions of aquatic food sources consumed were considerably higher (many species with more than 60%) at the wide site, although a few species such as *Crocothermis erythraea, Pantala flavescens*, *Tramea basilaris* and *Trithemis arteriosa* had aquatic contributions less than 40% (Figure 7-8). Generally, odonates at the narrow site (32.1%) had distinctly lower proportions of aquatic food in their diets than those at the wide site (47.6%; t = 2.34, d.f. = 22, p = 0.03).

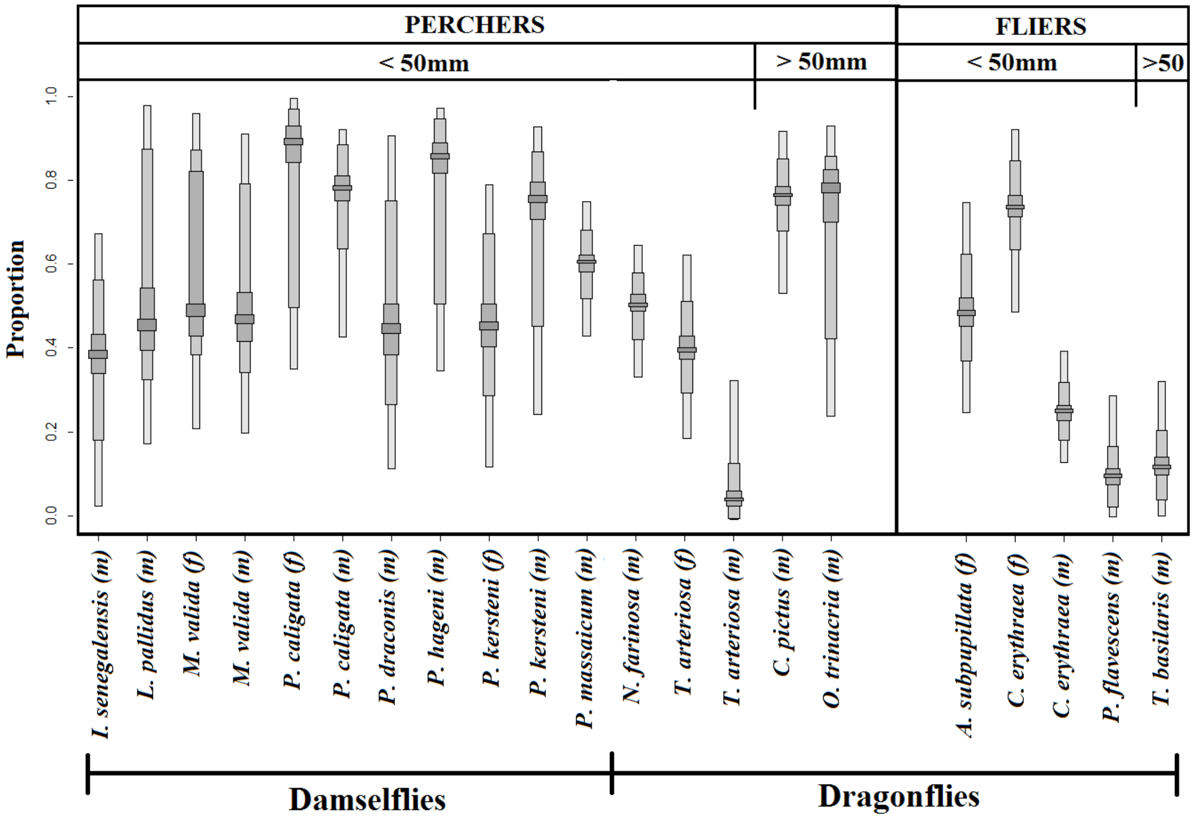


Figure 7-8: Contributions of combined aquatic food sources to diets of Odonata species at the wide site. The letters m and f represent male and female specimens. The boxplots represent the probability densities in credibility intervals of 95, 75 and 25% for the aquatic food sources.

Multivariate analysis (Table 7-2; PERMANOVA) showed that the factors body size and foraging method were significant (P(perm) < 0.05) determinants of odonate diet composition. The factors site and gender were not statistically significant drivers of aquatic subsidies in

Odonata diets (P(perm) < 0.05). However, the two or three way interactions between size and foraging method; size and site; and size, site and gender were statistically significant contributions to variation in aquatic food in Odonata diets (P(perm) < 0.05).

Table 7-2: PERMANOVA results showing factors that influenced aquatic food proportions in Odonata at the Kowie River. The * indicates statistically significant effects.

Factor	Df	MS	Pseudo-F	P(perm)
Size	2	0.079	4.31	0.02*
Foraging method	1	0.164	9.01	< 0.01*
Site	1	0.062	3.43	0.09
Gender	1	0.010	0.54	0.45
Size x Foraging method	1	0.078	4.27	0.05*
Size x Site	2	0.078	4.28	0.03*
Size x Gender	1	0.025	1.37	0.25
Size x Site x Gender	1	0.178	9.79	< 0.01*

7.3.2.2. Fatty acid indicators of aquatic contributions

SIMPER showed that the FAs 18:1ω9 (40%), 18:2ω6 (20%), 18:0 (11%), 16:0 (8%) and 20:5ω3 (6%) were responsible for discrimination among Odonata species at the narrow site (up to ~ 84% of the cumulative profiles), while 18:1ω9 (34%), 18:2ω6 (19%), 18:0 (12%), 18:1ω7 (9%), 20:5ω3 (8%) and 16:1ω7 (6%) were responsible for discrimination among species at the wide site (up to ~ 88% of the cumulative profiles).

There was wide variation in proportions of FAs amongst odonate species at both sites (Figures 7-9 to 7-10) and there were significant (ANOVA, $p < 0.05$) differences in FA proportions amongst different species, but no differences between male and female groups (Table 7-4 and 7-5). Fatty acids that were both indicators of aquatic contributions (Chapter 4) and major contributors (> 5%) to variation amongst overall FA profiles, in addition to biologically important groups (ω3 FAs, essential FAs and the ω3/ω6 ratio), were selected for detailed analyses. At both sites, 18:1ω9, 18:2ω6 and 20:5ω3 were reported, together with the sums of ω3 FAs and all EFAs (sum EFA; 20:5ω3 + 20:4ω6) and the ratio of ω3/ω6 FAs (Figures 7-9 and 7-10). The FA 18:1ω9 had considerably more variation amongst odonate species than the other FAs and FA groups (Figures 7-9 and 7-10). The variation of proportions of 20:5ω3 was similar to that of sum EFA and ω3/ω6, where most odonates had similar proportions apart from *C. erythraea* males that had distinctly low values when compared to the other species (Figures 7-9 and 7-10).

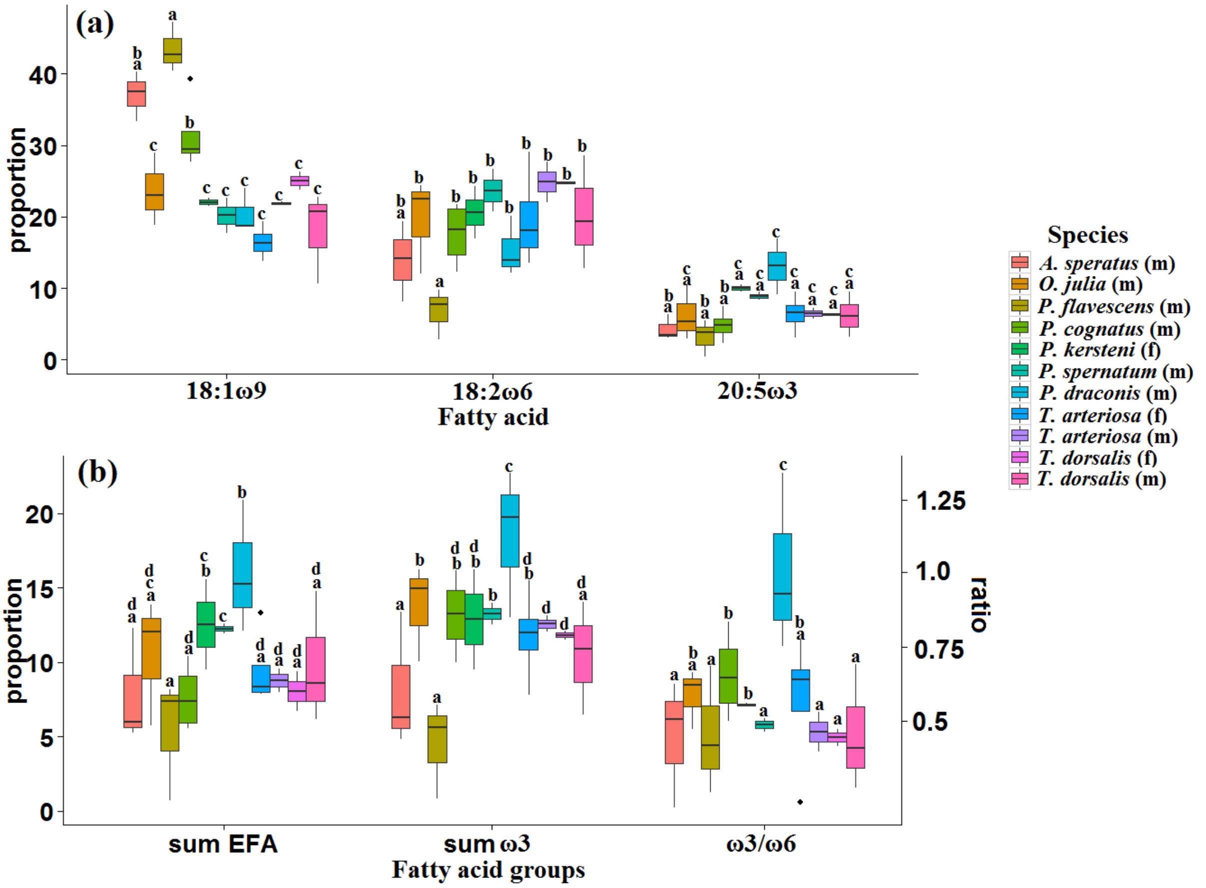


Figure 7-9: Box plots representing a) proportions (%TFA) of major FAs and b) proportions of FA groups in odonates at a narrow site along the Kowie River. The different colours represent Odonata species. The letters on top of the box plots were generated from Tukey post-hoc tests and any species with the same letter had statistically indistinguishable FA levels. The boxes represent 50% of the data and the horizontal line inside each box is the median value. The axis on the right side applies to the ω3/ω6 ratio.

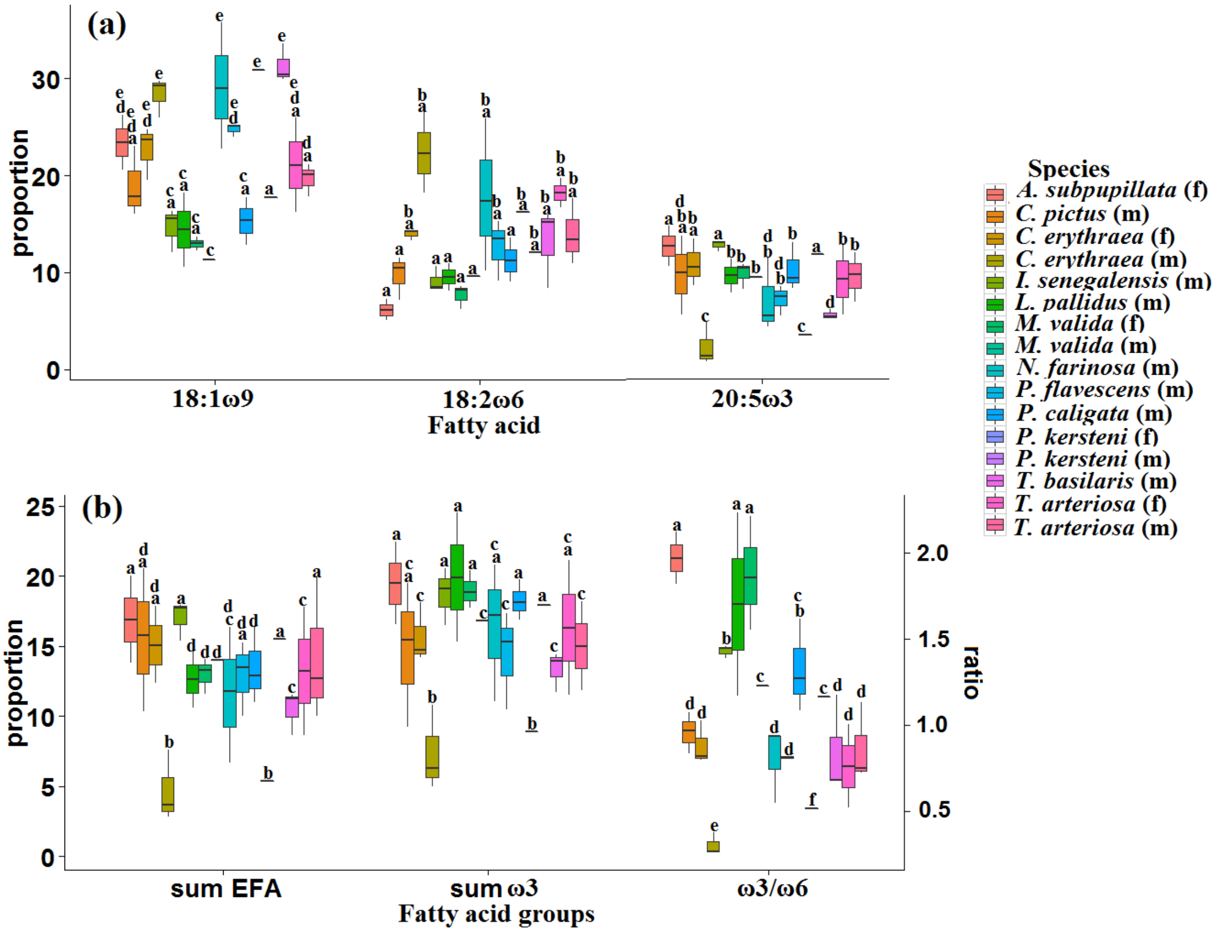


Figure 7-10: Box plots representing a) proportions (%TFA) of major FAs and b) proportions of FA groups in odonates at a wide site along the Kowie River. The different colours represent Odonata species. The letters on top of the box plots were generated from Tukey post-hoc tests and any species with the same letter had statistically indistinguishable FA levels. The boxes represent 50% of the data and the horizontal line inside each box is the median value. The axis on the right side only applies for the ω3/ω6 ratio.

Odonates that occurred at both sites (e.g. *P. flavescens, T. arteriosa*) often had lower proportions of 20:5ω3 and higher proportions of 18:1ω9 at the narrow than they did at the wide site (Figures 7-9 and 7-10). Odonates at the wide site had distinctly higher levels of 20:5ω3 (mean = 9.8; t-test, t = 3.7, d.f. = 68, $p < 0.001$), sums of EFA (mean = 12.9; t-test, t = 3.2, d.f. = 68, p = 0.002) and ω3 (mean = 15.7; t-test, t = 3.6, d.f. = 68, $p < 0.001$) FAs and the ω3/ω6 ratio (mean = 1.1; t-test, t = 5.2, d.f. = 68, $p < 0.001$) than odonates at the narrow site (means; 20:5ω3 = 6.7, sum EFA = 9.7, sum ω3 = 11.8 and ω3/ω6 = 0.6). Proportions of the FAs 18:1ω9 (t = -2.3, d.f. = 68, p = 0.02) and 18:2ω6 (t = -3.8, d.f. = 68, $p < 0.001$) were distinctly higher in odonates at the narrow than the wide site.

Odonata size categories had varying proportions of selected FAs at the two sites (Table 7-3 to 7-4). The damselflies had distinctly higher proportions of the FA 20:5ω3 and the ω3, EFA and ω3/ω6 groups than the dragonflies (Table 7-3 to 7-4, both size classes) at both sites, whilst large dragonflies had lower proportions of these FAs and groups than the medium-sized dragonflies. At the narrow site, the proportions of the FA 18:1ω9 were distinctly ($p < 0.05$) higher in large dragonflies than both medium-sized dragonflies and damselflies (Table 7-3 to 7-4). At the wide site 18:1ω9 and 18:2ω6 proportions were highest in medium-sized dragonflies, but post hoc tests revealed no significant differences between large dragonflies and damselflies ($p > 0.05$).

Flier and percher foraging groups had different ($p < 0.05$) proportions of many FAs at the narrow site. At the narrow site, the levels of 20:5ω3, ω3 and EFA were distinctly higher in the perchers and lower in the fliers, whilst proportions of 18:1ω9 were higher in fliers and 18:2ω6 was higher in the perchers. At the wide site, there were no significant differences between fliers and perchers in proportions of all the FAs except 18:1ω9 (Table 7-3 to 7-4). The proportions of 18:1ω9 were distinctly higher in fliers than perchers at the wide site (Table 7-3 to 7-4).

Table 7-3: Results of ANOVA and t-tests of individuals and groups of FAs belonging to Odonata groups from the narrow site of the Kowie River. The letters z, a1 and a2 represent Zygoptera < 50 mm (damselfly), medium-sized Anisoptera < 50 mm (dragonflies) and large Anisoptera > 50 mm (dragonflies) odonate size classes, respectively.

		Size			**Hunting**		**Gender**	
FA		**z**	**a1**	**a2**	**Fliers**	**Perchers**	**Females**	**Males**
18:1ω9	*mean*	20.9	25.8	37.1	40.3	22.3	20.0	27.7
	stat	F=3.9, df=28, p=0.03			t=6.9, df=29, p<0.001		t=-2.2,df=29,p=0.4	
18:2ω6	*mean*	19.2	18.5	13.9	10.3	20.1	21.2	17.2
	stat	F=0.7, df=28, p=0.50			t=-3.9,df=29, p<0.001		t=1.5,df=29, p=0.4	
20:5ω3	*mean*	11.0	5.6	4.3	3.8	7.4	7.3	6.5
	stat	F=12.8,df=28, p<0.01			t=-2.5, df=29, p=0.02		t=0.5,df=29, p=0.6	
ω3	*mean*	15.4	11.2	8.2	6.4	13.2	12.1	11.8
	stat	F=4.1, df=28, p=0.02			t=-4.1,df=29, p<0.001		t=0.2,df=29, p=0.8	
EFA	*mean*	14	8.6	7.9	6.6	10.4	9.9	9.7
	stat	F=7.3,df=28, p=0.002			t=-2.2, df=29, p=0.03		t=0.1,df=29, p=0.9	
ω3/ω6	*mean*	0.7	0.5	0.4	0.5	0.6	0.5	0.6
	stat	F=2.8, df=28, p=0.08			t=-1.5, df=29, p=0.2		t=-0.5,df=29,p=0.6	

Table 7-4: Results of ANOVA and t-tests of individuals and groups of FAs belonging to Odonata groups from the wide site of the Kowie River. The letters z, a1 and a2 represent Zygoptera < 50 mm (damselfly), medium-sized Anisoptera < 50 mm (dragonflies) and large Anisoptera > 50 mm (dragonflies) odonate size classes, respectively.

		Size			**Hunting**		**Gender**	
FA		**z**	**a1**	**a2**	**Fliers**	**Perchers**	**Females**	**Males**
18:1ω9	*mean*	15.5	25.5	20.7	26.9	19.9	20.6	21.6
	stat	F=16, df=36,p<0.01			t=2.9, df=37,p=0.007		t=-0.4,df=37,p=0.69	
18:2ω6	*mean*	10.1	15.9	8.3	11.2	13.3	11.8	13.3
	stat	F=12,df=36,p<0.001			t=-1.0,df=37, p=0.31		t=-0.8,df=37, p=0.43	
20:5ω3	*mean*	11.1	8.3	11.8	8.9	10.0	10.9	9.3
	stat	F=4.3,df=36,p=0.03			t=-0.7,df=37,p=0.50		t=1.3, df=37, p=0.21	
ω3	*mean*	18.0	14.0	16.6	15.3	15.9	16.8	15.3
	stat	F=4.1,df=36,p=0.03			t=-0.3,df=37, p=0.74		t=0.9, df=37, p=0.36	
EFA	*mean*	13.6	11.7	16.1	13.0	12.9	13.6	12.7
	stat	F=2.6,df=36,p=0.09			t=0.0, df=37, p=0.96		t=0.6, df=37, p=0.54	
ω3/ω6	*mean*	1.4	0.8	1.4	1.1	1.1	1.3	1.0
	stat	F=15,df=36,p<0.001			t=0.2, df=37, p=0.83		t=1.7, df=37, p=0.09	

7.4. **Discussion**

7.4.1. Dietary specialization and niche partitioning among odonate species (Hypotheses 1 - 3)

7.4.1.1. Interspecific and spatial niche partitioning

The initial assumption of this study was that odonate diets would vary and there would be niche partitioning amongst odonate species. Fatty acid and isotopic niches of many odonate species in the Kowie River were largely distinct although there was considerable niche overlap amongst some species, hence there was niche partitioning. Casual observations by other researchers have showed that odonates frequently compete with each other at their breeding and foraging areas via intra- and inter-specific competition (Moore 1964, Corbet 1999). Animals can reduce the competition for a limiting resource through spatial and temporal segregation, whereby individuals exploit the same resource at different places and times (Alanärä et al. 2001). Fatty acid and SI indicators provided evidence of niche partitioning among the odonate species in the Kowie River, which was likely due to competition for prey. Additionally, there was high variation in FA and isotopic niches amongst the species in the Kowie River, contributing to the evidence that most odonates are generalist feeders with varied diets (Corbet 1999).

Feeding niches varied more amongst odonates resident in the wider section of the Kowie River (wide site, stream width = 11.28 ± 4.90) than those at the narrower section (narrow site, 1.49 ± 0.36 m, Figures 7-2 to 7-6). Due to differences in water surface area or stream bed area, the differences in width of the river resulted in the differences in aquatic insect emergence (see Chapter 3). The aquatic insect emergence rates and other differences (habitat and environmental factors) across the two sites were discussed in detail in Chapter 3. The high amount of FA and SI niche overlap at the narrower section (narrow site) suggested that there was a high degree of diet resource sharing at that site (Figure 7-2). At the narrower section of the Kowie River, different species had more similar (less distinct) diet niches than at the wider section because aquatic subsidisation was low and odonates were presented with less prey choice at the narrower site (Figure 7-2). Results from a concurrent study in the Kowie River showed that there was a higher diversity of aquatic insects emerging from the wider section (Shannon index = 1.97) than at the narrower section (Shannon index = 1.81; Moyo 2016). It is therefore possible that the odonates at the wider section of the river had greater variation in prey types than those at the narrow section. The high insect emergence and diversity at the wider river section were the likeliest sources of greater prey variation. A study by Dudgeon (1989) on resource partitioning among the larvae of four odonate species in the Tai Po Kau Forest Stream, Hong Kong, showed that differences in micro sites/habitats resulted in differences in diet. He concluded that odonates in more extreme (less favourable) conditions had more specialized diets and there was more dietary overlap amongst these species than those in more favourable conditions (Dudgeon 1989). Similarly, the large niche overlap amongst odonate species at the narrow section of the Kowie River could be attributed to the lower prey abundances (less favourable conditions) and diversity at this site.

The idea that odonates in areas of higher aquatic insect abundance (such as the wider sections of a river) have greater niche widths and consume a greater variety of prey is contrary to the belief that when emergence is high, odonates become temporary specialists of one of the highly abundant prey species (Corbet 1999). However, it was not possible to determine diurnal patterns of insect emergence over a sustained period in the Kowie River. Stable isotope signatures and FA profiles are integrated over time and represent what the individual has been consuming over days to weeks or months (Peterson and Fry 1987). As such, the feeding niches described in this study represent odonate diets over a long period in which aquatic insect species composition could have changed with shifts in environmental conditions.

7.4.1.2. Effects of body size on feeding niche (Hypothesis 1)

The odonate size classes had varied FA and SI niches with considerable overlap among the size classes at both sites (Figure 7-3). The overlap meant that different odonata sizes were selecting similar prey, even though foraging strategies may have differed between fliers and perchers. The isotopic niches of all the size classes were less distinct at the narrow than at the wider section, which indicated that there was greater prey variety at the wide site, as confirmed by the higher insect diversity at the wider section of the Kowie River (Figure 7-3; Moyo 2016). The large dragonflies at the narrow section were represented by only one species, *Anax speratus*, hence the distinctly small niche width (Figure 7-3). It is possible that the high overlap at the narrow section was a result of larger odonates preying on smaller odonates, as this is very common behaviour with large dragonflies (Corbet 1999).

The differences between FA and SI niches at the wider section of the Kowie River suggested that damselflies and large dragonflies were consuming insect prey with similar carbon and nitrogen sources but with differences in nutritional quality (Figure 7-3). It is likely that the damselflies and the large dragonflies did not consume the same groups of insects. As such, there may not have been any dietary niche overlap between the odonate size classes at the wide section of the Kowie River. In an experiment to investigate prey size selection by perching dragonflies, Olberg et al. (2005) used glass beads of different sizes as artificial prey. In their study, dragonflies were highly selective for bead size, hence prey size (Olberg et al. 2005). It is plausible that based on their body sizes, the odonates at the wide section of the Kowie River were highly selective of their prey. The large dragonflies likely fed on the bigger insect species whilst the damselflies fed on the smaller insects.

7.4.1.3. The role of hunting strategies in diet partitioning (Hypothesis 2)

Fliers and perchers have contrasting foraging methods and behaviours. Many of the fliers fly in open areas far from the water or over tree tops whilst most perchers stay close to the water's edge (Corbet 1999). As such, the fliers and perchers had separate isotope and FA niches in the Kowie River, but there was always niche overlap (Figure 7-4). The isotopic and FA niche widths for the fliers were relatively similar across sites, but the perchers at the narrow site had much smaller niche widths than those at the wide site (Figure 7-4). The smaller niche widths at the narrower section of the river suggested that the perchers had lower prey variety than those at the wider section of the river. Populations that consume a wide range of prey species will exhibit wider variation in their tissue isotopic signatures than those consuming a narrow range of prey items (Bearhop et al. 2004). Results from a concurrent study in the Kowie River support the claim that there was greater prey variety at the wider section of the river, as aquatic insect diversity and abundance were highest at the wider section (Moyo 2016; see Chapter 6 discussion).

7.4.1.4. Gender based feeding niches (Hypothesis 3)

There was a high amount of overlap between male and female odonate FA and SI niches in the Kowie River, suggesting that males and females had similar diets (Figure 7-5). Male odonates in the Kowie River had relatively larger niche widths than females, suggesting that male odonates had higher prey variety (Figure 7-5). Female odonates often forage far from the river to avoid being sexually harassed by males that usually forage near the river (Sherratt 2001, Happold 2009, Fincke 2015). As such, it is likely that male odonates in the Kowie River had higher access to insects emerging from the river and hence higher diet variety that was reflected in the wider isotopic and FA niche widths. Similarly, a study by Grant et al. (2014) in northern Michigan revealed large intraspecific niche overlaps of the damselfly *Enallagma hageni.* They also reported broader isotopic niche areas among the males (Grant et al. 2014). Despite the similarities between the two studies, the results of the Kowie River were based on comparisons of males and females pooled from several species, whilst those from northern Michigan were based on intraspecific comparisons. It is possible that this pooling masked potentially highly variable niches of male and female odonates within the various species. Alternatively, perhaps my study challenges findings of earlier studies that show such foraging segregation. The presence of niche partitioning between size categories and foraging groups provides further evidence of the high niche variability within the odonate community of the Kowie River. Future studies should invest in making intraspecific comparisons of many odonate species.

7.4.2. Variations in use of cross-boundary subsidies (Hypotheses 4 and 5)

Most odonates are described as opportunistic consumers that have diets that vary with taxonomic group (Corbet 1999), and this generality was evident from the high variability in the aquatic food source indicators (Figures 7-9 and 7-10) and proportions of aquatic food in the diets of the odonates from the Kowie River (Figures 7-7 and 7-8). Despite the variability, there were indications of linkages between odonate feeding niche and the extent of use of aquatic subsidies. Multivariate analysis showed that size and foraging method were significant determinants of the proportion of aquatic food in odonate diets (Table 7-2). Additionally, the combination of size, site and gender was also important in determining contributions of aquatic subsidies.

7.4.2.1. Variation of aquatic subsidy use with body size

The differences in body sizes (length) of odonates in the Kowie River contributed to the segregation in amount of aquatic subsidies (Figures 7-7 to 7-10). My results were consistent with the findings of Bennett and Hobson (2009) in the boreal forest at Prince Albert National Park in Canada. Using SIs of carbon and nitrogen, they reported that the smaller damselflies consumed greater proportions of aquatic insects than the larger dragonflies (Bennett and Hobson 2009). Stable isotope results showed that damselflies (Zygoptera) in the Kowie River consumed greater proportions of aquatic food than the dragonflies (Anisoptera, medium and large), whilst medium-sized dragonflies consumed greater proportions than larger dragonflies (Table 7-7 and 7-8). Similarly, the levels of FA aquatic indicators (20:5ω3, sum ω3, ratio of ω3/ω6 and EFA) were highest in the damselflies and lowest in the dragonflies (medium and large), although the medium sized dragonflies had lower levels than the large dragonflies (Figures 7-9 and 7-10).

Olberg et al. (2005) ran an experiment with suspended beads to determine if dragonflies were selective for prey size. Their results showed that smaller dragonflies targeted smaller prey than the larger dragonflies (Olberg et al. 2005). Also, larger odonates are generally faster, more agile and suited to capture terrestrial prey that are characteristically larger, faster and stronger flyers than their aquatic counterparts (Olive 1982). Smaller odonates are smaller and weaker fliers more suited for capturing relatively small, weak flying aquatic insects such as true flies (Chironomidae) and mayflies (Ephemeroptera) (Marczak and Richardson 2007). Given that in some areas larger insects are found in terrestrial than in aquatic systems (Sabo et al. 2002), it is plausible that larger odonates feed more on terrestrial than aquatic insects. Nevertheless, my results should be taken with caution because damselflies and dragonflies are phylogenetically distinct, as are all odonate species (Corbet 1999). My results showed that there was wide variation in FA and isotopic indicators amongst odonate species, but the overall differences in aquatic subsidy use across the size categories needs to be studied further.

There were differences in proportions of oleic acid (18:1ω9) across the odonate size classes at both sites (Figure 7-9 and 7-10). At the narrow section of the Kowie River, the damselfly and medium-sized dragonfly groups had lower proportions of oleic acid than the larger dragonflies (Figure 7-9), thus possibly indicating higher proportions of aquatic food in their diets. High levels of oleic acid in large anisopterans at the narrow site were potentially indicative of lower aquatic subsidisation. For instance, high amounts of monounsaturated FAs (MUFA) in human diets have been linked to deficiencies in essential fatty acids (EFA) (Geerling et al. 1999). Deficiencies in EFAs can be compensated for by production of more MUFAs, as the EFAs are replaced by MUFAs in an attempt to maintain membrane fluidity (Siguel and Lerman 1996). At the wide section of the Kowie River, the large dragonflies had higher proportions of oleic acid than the other two size groups and thus lowest aquatic food source contributions (Figure 7-10). Results of oleic acid proportions supported those of the SI mixing models and the FA aquatic indicators (20:5ω3, sum ω3, ratio of ω3/ω6 and EFA), which showed that the large dragonflies consumed less aquatic food than the damselflies.

7.4.2.2. Aquatic subsidies and foraging behaviour

Odonate foraging behaviours in the Kowie River contributed to the extent of use of aquatic subsidies. Perchers and fliers had different levels of aquatic food in their diets, probably because they naturally have different access to aquatic resources. The differences in diets of fliers and perchers were consistent with their foraging behaviours. Perchers spend several hours at their encounter sites every day and often forage nearby (Corbet and May 2008). However, fliers spend relatively short periods of time (about 30 minutes) at their encounter sites and spend the rest of the day foraging in distant areas (Kaiser 1982, Corbet and May 2008). Flight is the most energetically costly activity that dragonflies undertake (May 1995), and fliers must acquire energy by feeding for longer daily periods than perchers because they expend a lot of energy on flight for most of the day (Corbet and May 2008). The SIAR results indicated the fliers *Tramea basilaris* and *Pantala flavescens* at the wider section of the Kowie River generally had lower proportions of aquatic food in their diets when compared to perchers (34% and 63%, respectively). The proportions of FA aquatic indicators (20:5ω3, ω3, ω3/ω6 and EFA) were distinctly higher in the perchers and lower in the fliers, whilst the terrestrial indicator proportions (18:1ω9 and 18:2ω6) were higher in the fliers (especially *Tramea basilaris* and *Pantala flavescens,* Figures 7-9 and 7-10). It is plausible that the longer periods of time that the perchers spent at the river may have enabled them to have more access to aquatic food.

The contrasting foraging behaviour between fliers and perchers in the Kowie River is comparable to some findings in a study by Pfitzner *et al.* (2015). These researchers studied the incorporation of mosquitoes into the diets of odonates on the flood plains of the River Rhine in south-western Germany, and although there were high densities of mosquitoes in their study area, apart from *Aeshna cyanea*, all the large anisopterans (dragonflies) did not consume large amounts of the aquatic subsidy (Pfitzner *et al.* 2015). Their results were attributed to contrasting foraging behaviours between *Aeshna cyanea,* which exclusively patrolled the river for most of the day, and the rest of the large anisopterans that patrolled roadsides and other open areas far from the river (Pfitzner *et al.* 2015). In the Kowie River, *A. speratus* and *A. subpupillata* were the only large dragonflies that spent most of their foraging effort patrolling along the river at both sites, whilst the other large dragonflies foraged far from the river (personal observations; Samways 2008). Odonates that spend more time foraging along the river, where aquatic insects are more abundant that terrestrial, are likely to consume more aquatic insects than terrestrial. As such, their increased use of aquatic resources compared to fliers that forage far from the river was consistent with their foraging behaviour.

Among the perchers in the Kowie River, SIAR results showed that there were odonate species that had smaller proportions (less than 50%) of aquatic diet contributions, and their known behavioural patterns were consistent with these small contributions. For instance, one of these species, *Crocothemis erythraea,* is a typical percher, and usually when perch sites are plentiful and there is a low arrival rate of females to encounter (mating) sites, males prefer foraging from a plant perch and make short darting flights to capture insects that fly close by (Rehfeldt 1991, Corbet 1999). However, when the arrival rates of females to the encounter sites are high and the number of perch sites is limited, *C. erythraea* males tend to exhibit flier behavioural traits (Rehfeldt 1991). The SIAR results from the Kowie River showed that *C. erythraea* males had low percentages of aquatic food sources in their diet (10 – 35%), possibly suggesting that in the flier mode, *C. erythraea* foraging patterns become more similar to those of the typical fliers that also had low aquatic food source proportions. Similar to some fliers such as *P. flavescens* and *Anax tristis*, *C. erythraea* has been observed migrating long distances (Dumont 1967, Khrokalo 2010), and it is possible that it spends less time at the encounter sites and more time foraging far from the river, where terrestrial insects are more abundant that aquatic insects.

Despite general distinctions in aquatic dietary contributions between perchers and fliers in the Kowie River, there were exceptions to this observation. Similar to the fliers, the perchers *P. cognatus* and *N. farinosa* consumed large amounts of terrestrial food at the narrow and wide sections of the Kowie River, respectively (Figures 7-7 and 7-8). Similar to the fliers, the perchers *P. cognatus* and *N. farinosa* had higher proportions of the terrestrial marker oleic acid than most perchers (Figures 7-9 and 7-10), and thus higher proportions of terrestrial food sources. It is not clear why the perchers *P. cognatus* and *N. farinosa* had diets similar to the fliers, but *P. cognatus* could have resorted to consuming many terrestrial insects when the aquatic insect abundance and emergence were low at the narrow section of the Kowie River (Moyo 2016; Chapter 2). It is possible that aquatic insect abundance was lowest during the times that *P. cognatus* foraged, because the foraging times of different odonates vary diurnally (Higashi 1978, Baird and May 1997). Odonates that forage in the percher mode cannot raise their body temperature endothermically by flying (Corbet 1999). They depend on external heat to fly, and ground perchers such as *P. cognatus* rely on the heat from the rocks. The flights of *P. cognatus* are likely to be different from the plant perchers, hence their foraging times may vary resulting in differences in access to aquatic subsidies. Nevertheless, most fliers generally consumed less aquatic food resources than the perchers in the Kowie River.

7.4.2.3. Aquatic subsidies to male and female odonates

Odonate isotope results from the Kowie River showed that gender alone was not a significant determinant of diet composition (PERMANOVA, $p > 0.05$, Table 7-2). Mixing model and FA indicator methods showed that there was no significant difference between all male and female odonates in their usage of aquatic food sources in the Kowie River (Table 7-2). Similarly, in a study conducted in the floodplains of the western Khentey Mountains, Mongolia, male and female odonates had similar diets (Seifert and Scheu 2012). The researchers investigated Odonata diet shifts from larvae to adults using isotopes, but did not find any significant differences between males and females (Seifert and Scheu 2012). They suggested that although males stayed mostly close to the water source and females used the water source for mating and egg laying only, their diets remained similar (Seifert and Scheu 2012). The Odonata species they collected included *Aeshna crenata*, *Leucorrhnia orientalis*, *Aeshna juncea*, *Coenagrion johanssoni*, *Sympetrum danae*, *Sympetrum flaveolum*, *Lestes dryas*, and *Lestes sponsa,* but they used only one species, *Leucorrhnia orientalis*, to examine changes based on gender. In the Kowie River, SIAR models revealed that some males and females had different proportions of aquatic food in their diets and the differences between males and females were variable across species (Figures 7-7 and 7-8). It is possible that the other species that Seifert and Scheu (2012) did not examine also had diets that varied with gender. My study examined a wider range of odonate species but lacked sufficient replication of both genders to make definitive statements about the effects of gender in all the species that were present. To determine gender-based dietary differences, future studies should focus on increased samples sizes of fewer species.

Contrary to the evidence that males have greater access to aquatic insects than females (Corbet 1999), some of the females in the Kowie River had similar or higher proportions of aquatic food in their diets than the males (Figures 7-7 and 7-8). In particular, *T. arteriosa* and *C. erythraea* females had higher proportions of aquatic food than the males, and the FA profiles of *C. erythraea* males and females were different from each other. Females that had similar or greater proportions of aquatic food in their diets may have had less aggressive males in the population. It is also possible that the density of males at the river may be low, hence allowing females to go undetected when they are foraging at the river. For example, unlike many Odonata species, *Platycypha caligata* females readily move up and down the stream and perch on plants along the bank because they are largely ignored by the males (Robertson 1982). The males move in only to intercept the females when they fly low and slowly into their respective territories (Robertson 1982, Martens and Rehfeldt 1989). The similarity in proportions of aquatic food contribution in males and females that was observed in the Kowie River was consistent with such behaviour.

7.4.2.4. Spatial variation in use of aquatic subsidies

Fatty acid and SI indicators both showed that odonates at the wider parts of the Kowie River had distinctly higher aquatic contributions to their diets that those at narrower parts (t-test, $p < 0.05$). The high insect emergence (Moyo 2016) and abundance (Chapter 3) at the wide site were the likeliest source of greater aquatic contribution. The effect of stream width on aquatic insect emergence was discussed in detail in Chapter 3 (Section 3.4.3). Odonates inhabiting wider parts of a river were therefore more likely to have higher access to aquatic food sources than those at the narrower sections and/or perhaps simply spent more time foraging in the open habitat above the stream in larger systems. Odonate population sizes in the Kowie River were not estimated, but it is likely that the wider parts of the river supported higher abundances of odonates than narrower sections. In support of this claim, there was greater odonate richness and higher numbers of odonates flying around at the wider sections of the Kowie River compared with the narrow regions during the course of the study (Table 7-1; personal observation). Prey availability seemed less likely to control the distribution of adult odonates around water bodies because they are mobile, generalist predators, but aquatic insect abundances (or emergence rates) could be driving the abundance/diversity of odonates frequenting the river.

The amounts of shade and vegetation structure are the biggest determinants of odonate abundance (Remsburg et al. 2008). Riparian vegetation could affect Odonata abundances if it were correlated with terrestrial prey densities (Baird and May 1997, Garono and Kooser 2001). The results from the Kowie River indicated that the abundance of aquatic insects (or emergence rates) may influence odonate success. The results of my study must be taken with caution because the two sites (narrow and wide) had varying vegetation types and quantities and it is likely that the stream width was not the only factor influencing odonate diets and species richness. For instance, a high amount of shade over a stream can reduce odonate abundance (Remsburg et al. 2008) and species diversity (Remsburg and Turner 2009). Future studies should be carried out to determine the direct effects of aquatic insect emergence on odonate abundance in a variety of habitat types.

7.5. **Conclusion**

Both diet methods (FA and SI analyses) produced data that indicated high levels of diet variation amongst Odonata species but distinct feeding niches among odonate guilds. The data supported the claim that the biases in access to aquatic subsidies were a factor body size, hunting strategy and stream width, but there was no evidence of differences across gender. Figure 11 conceptualizes the access of aquatic subsidies to odonates in the Kowie River.

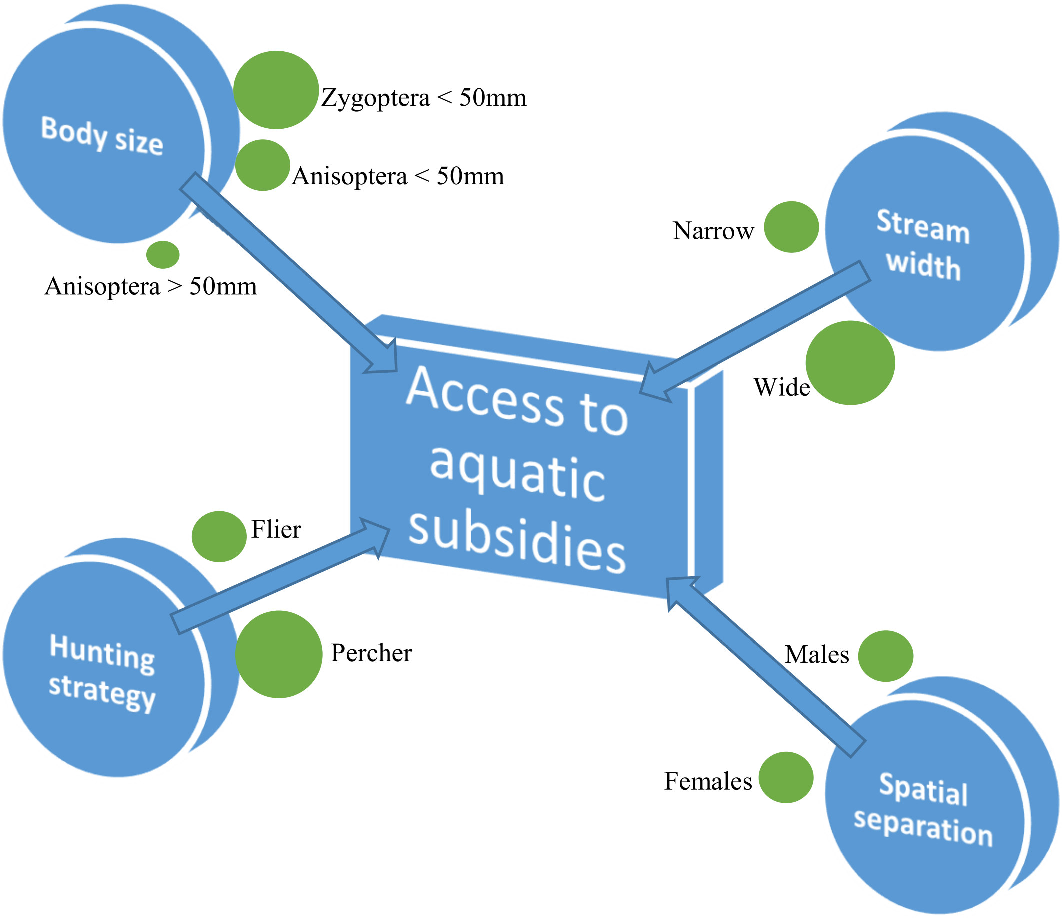


Figure 7-11: Conceptualized determinants of access by odonates to aquatic subsidies in the Kowie River. The relative sizes of the green circles represent the different proportions of aquatic food in the odonates' diet.

Chapter 8. GENERAL DISCUSSION

8.1. Introduction

I set out to explore dietary cross-subsidisation between freshwater and terrestrial habitats, and I identified web-building spiders as significant indicators of spatial and temporal changes in aquatic subsidies and measured the contribution of aquatic subsidies to riparian predator guilds (spiders and odonates). Cross-subsidisation in riparian areas is important because some essential nutrients that are lacking in terrestrial habitats, are highly abundant in aquatic habitats (Gladyshev et al. 2009b). The high biomass of flying insects that often emerge from rivers acts as an important subsidy for terrestrial consumers in periods or places of low terrestrial insect abundance. Riparian ecotones may carry higher densities of consumers compared to terrestrial systems that are far inland, or offer individuals better growth opportunities, as a result of resource exchange between aquatic and terrestrial habitats (Naiman et al. 1993, Polis and Hurd 1995, Naiman and Decamps 1997, Fagan et al. 1999, Nakano and Murakami 2001, Power 2001, Sabo and Power 2002a).

The conceptual diagram (Figure 8-1) summarises the theoretical basis of this study. It was assumed that aerial predator success (biomass, abundance, richness and composition) was influenced directly by terrestrial prey and aquatic subsidies, but indirectly by vegetation complexity, habitat and micro-climatic conditions. The few studies on riparian cross-boundary subsidies (e.g. Polis et al. 1997, Alp et al. 2013), specifically in the context of Africa (Rouget et al. 2006), were inconclusive on several vital questions within a much broader context. Many similar studies have not addressed potential spatial and temporal variations concurrently, while others reported on a few predator and aquatic insect species. As such, I sought to investigate the roles of predators of aerial insects as recipients of cross-boundary subsidies, and whether these predators could be used to assess the strength of the trophic connectivity between freshwater and terrestrial systems in relation to variables such as stream width, distance from the river and aquatic insect emergence rates and abundances. I also sought to reveal guilds among predators of aerial insects and investigate how the environment and attributes such as behaviour, size and gender influenced predators' access to aquatic subsidies. The predominant focus of this study was on trophic interactions between terrestrial and freshwater habitats, hence influences of habitat and micro-climatic factors were secondary concerns.

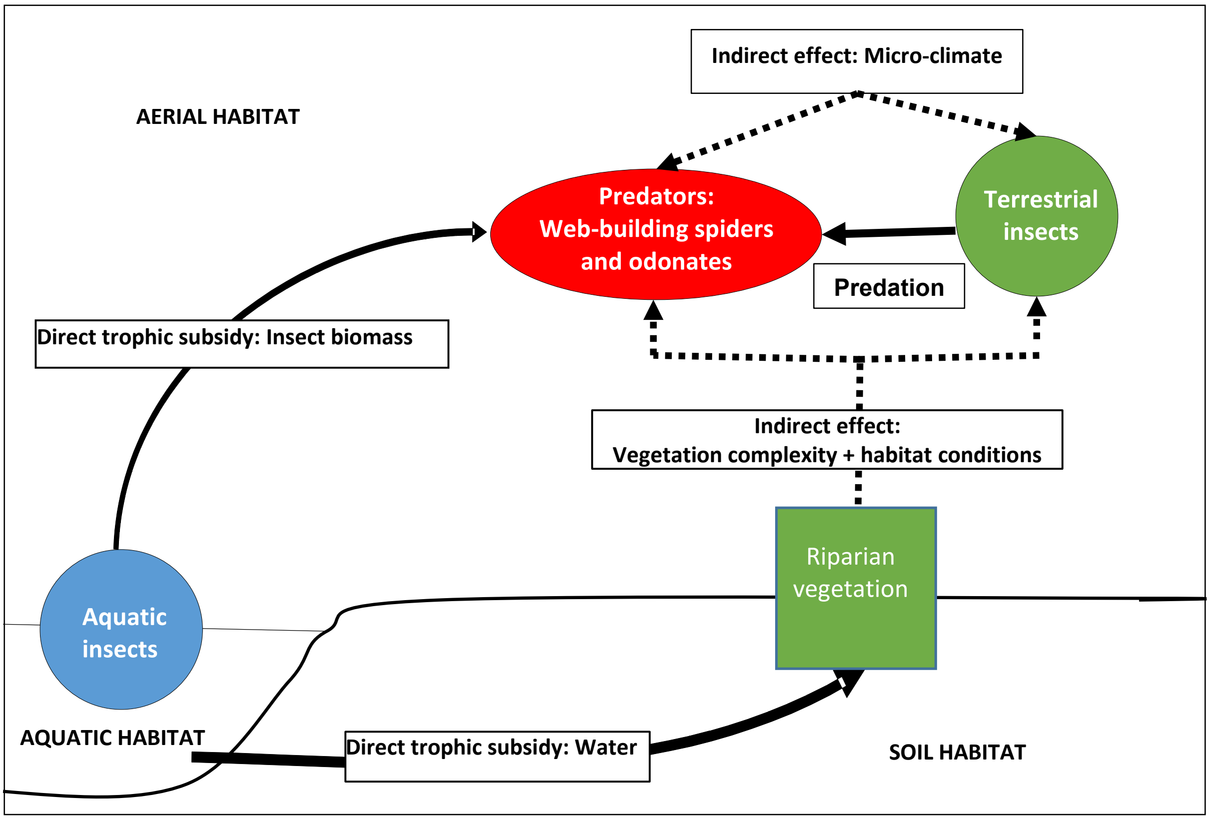


Figure 8-1: An illustration of the role of aquatic subsidies and other effects in shaping the trophic ecology of riparian predators in the Kowie River.

8.2. **Empirical findings**

The main empirical findings of this study are chapter-specific and were summarized in the Chapters 3 to 7. This chapter synthesizes the empirical findings to address the study's three main questions:

1. *Is there variation in access to aquatic subsidies and is niche partitioning evident amongst aerial predators*?
   - **a) Niche partitioning amongst species (Chapters 4 and 7)**: The starting assumption was that there would be inter- and intra-specific niche partitioning among the aerial predator and insect prey taxa. There was high compositional variation amongst insect prey taxa but there were distinct differences between aquatic and terrestrial prey groups, largely due to their different food sources. As such, the predators preying on different insect groups had different body compositions which were reflected in their FA and SI signatures. Results of FAA and SIA were considerably aligned, but in some cases FAA potrayed less diet niche overlaps than SIA, suggesting that these odonates were feeding

on different prey species that possesed similar nutritional/isotopic content. The presence of distinct dietary niches suggested that spiders and odonates were not entirely generalist feeders and may have had prey biases, hence varied feeding niches.

b) **Riparian predator guilds have different access to aquatic subsidies (Chapters 5 to 7)**: It is plausable that the differences in predator diet niches were a result of variation in access to prey types, especially between prey of aquatic and terrestrial origin. There were distinct differences in access to aquatic subsidies between spiders and odonates with different feeding methods that were probably a result of evolutionary adaptations. Web-building spiders were largely separated by web orientation, as spiders with horizontal webs had greater access to aquatic subsidies than the vertical web-builders (Chapter 5). The diversification of web architectures has played a key role in the evolutionary success and ecological dominance of spiders as predators of insects (Blackledge et al. 2009). Odonates in the Kowie River were separated based on their foraging method, hence those that spent most of their time flying while foraging in mid-air and travelling long distances from the river (the fliers) had less access to aquatic subsidies than those that spent most of the day resting on perches at the river (the perchers, Chapter 7). The differences in foraging methods between fliers and perchers are mostly adaptive strategies of thermoregulation (Corbet and May 2008), but these strategies ultimately resulted in differences in access to aquatic subsidies.

Due to the ease of identification of odonate species, it was possible to further assess the accessibility of aquatic subsidies to several trait-based aerial predator groups (Chapter 7). The study showed differences in access to subsidies between size groups, but there was no evidence of any intra-specific differences. The presence of inter- and intra-specific niche partitioning among aerial predators suggested that estimates of the significance of the subsidy may be affected by the sampling device.

2. *Does the access of aerial riparian predators to aquatic subsidies vary with distance from the river?*
   a) **Web-building spider biomass was correlated with aquatic insect biomass (Chapter 3)**: there were indications that aquatic subsidies drove web-building spider communities in the Kowie River. As aquatic insect biomass increased, so did spider biomass, but there

were seasonal and spatial variations in these correlations, which were stronger at the wider sections of the river.

b) **Aquatic subsidies extend further from the river in summer (Chapter 3)**: Seasonal variations in aquatic insect abundance and emergence rates were reflected in the spiders' diets. Although community and SIAs did not clearly show how far aquatic subsidies moved inland during different seasons, the levels of the FA eicosapentaenoic acid in spiders' diets showed that aquatic subsidies travelled inland furthest (past 64 m) during summer and least during winter.

3. *Does the strength of freshwater-terrestrial trophic connectivity vary along gradients of physical characteristics and aquatic prey subsidies?*
   a) **Trophic connectivity was stronger at wider sections of the Kowie River (Chapters 5 and 6)**: Stream width regulated the quantity of aquatic subsidies available for consumption by terrestrial consumers, with the wider parts producing more subsidies. Spiders and odonates at the wider section of the river exhibited higher amounts of aquatic subsidies than those at the narrower section. Larger stream bed surface area and hence higher emergence rates were responsible for the improved connectivity between the two habitats.
   b) **There are seasonal variations in trophic connectivity (Chapters 5 and 6)**: The study highlighted the presence of distinct temporal patterns in the extent of subsidisation, mainly between winter and summer. Aquatic insect abundances and emergence rates (Moyo 2016) were both higher in summer than winter, which was consistent with the pattern of aquatic subsidisation to web-building spider diets. Despite the lower aquatic insect abundance in winter, the wider section of the river had sufficient aquatic insect biomass to assist in maintaining spider populations during winter. Figure 8-2 illustrates the concept that subsidisation is highest in the summer at the wider section of the river.

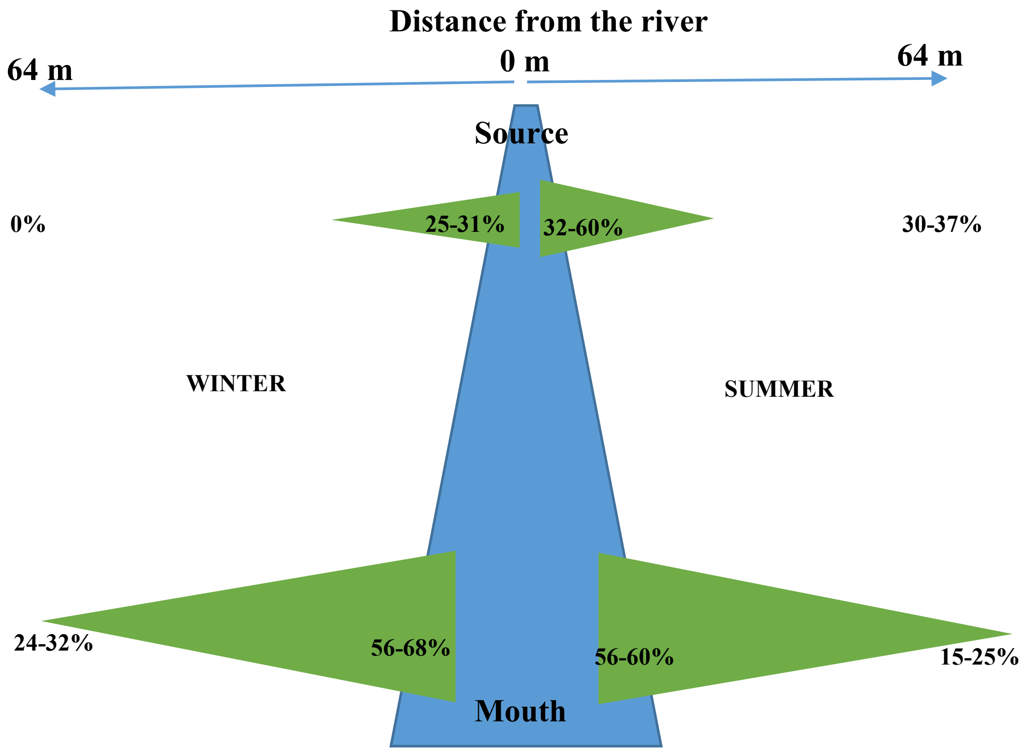


Figure 8-2: The extent of cross-boundary subsidisation at narrow and wide sections of the river (shaded area), during summer and winter. The triangles (green) represent proportions of aquatic subsidies to riparian spiders (from SIAR, Chapter 5); their horizontal length represents how far they extend from the river and their vertical width represents the relative biomass in the gradient. Subsidies were generally higher closer to the river but there was no evidence of a linear decrease with distance.

8.3. **Theoretical implications**

While ecology has traditionally focussed on the internal dynamics of ecosystems, the cross-boundary interactions are vital for some of those dynamics. This thesis characterises the cross-boundary trophic interactions of a relatively small model ecosystem to explore their implications for systems ecology. Knowledge of the trophic dynamics of web-building spiders in the Kowie River will further assist in understanding of the theory of cross-boundary subsidisation in riparian areas. The trophic dynamics of invertebrates needs to be revisited to further understand the theory of cross-boundary subsidisation in riparian areas. Ecosystem connectivity suggests that adjacent ecosystems are linked because nutrients and energy are exchanged across the habitat boundaries (Polis and Hurd 1996, Fariña et al. 2003). However, this study shows that the nature and extent of these linkages in riparian areas varies seasonally and spatially.

Despite considerable absolute variation in temporal patterns in insect emergence rates across the world, high insect abundances emerging from the rivers are linked with high contributions of aquatic food to the diets of riparian spiders (Power and Rainey 2000, Kato et al. 2003, Akamatsu et al. 2004, Baxter et al. 2005, Iwata 2006, Burdon and Harding 2008). Due to the importance of seasonal and spatial variations in aquatic subsidies demonstrated in this study, it is imperative that any study on cross-boundary trophic dynamics factors them in. In addition to demonstrating temporal shifts in aquatic subsidies, this study offers a new perspective on how far inland the aquatic subsidies can influence predator diet composition, and hence has management implications for defining riparian buffer zones. To my knowledge, apart from a study in the Shinano River (Akamatsu et al 2004), the Kowie River study is the only other to have investigated the proportion of aquatic subsidies in web-building spider diets at different distances from the river. As in the Kowie River, aquatic subsidisation to spider diets in the Shinano River study (Japan) was greatest at the river and lowest further from the river (Akamatsu et al 2004).

In South Africa, the buffer zone has been viewed as a barrier protecting streams (conceived primarily as natural resources for humans) from pollution, nutrient loading and sedimentation, and the biotic (food web and community) aspects have been largely overlooked (Rouget et al. 2006). This study adds empirical data for the development of scientifically-based, quantitative guidelines for riparian buffer protection plans, particularly if ecological and biotic factors are to be considered. A distinct link was established between the Kowie River and the adjacent terrestrial upland, where aquatic subsidies travelled further inland at wider sections of the river. Not only does the width of the riparian zone and the size of the water body determine the extent to which the riparian zone influences the water body (Naiman and Decamps 1997), but also aspects of the river such as emergence and stream width can influence the communities in the adjacent habitat (Chapter 3). My study has provided new information about predator–prey trophic dynamics in riverine areas.

8.4. **Future direction and direction of further research**

Stable isotope analyses often lead to ambiguous conclusions in complex ecosystems because the δ-values of different resources frequently overlap and are highly variable (Peterson and Fry 1987). Consumption of both C3 and C4 plants in the Kowie River area (see Chapters 4 and 5) resulted in a great deal of isotopic overlap amongst terrestrial insect taxa, and hence in their predators (Chapter 4). Although FA and stable isotope indicators could differentiate aquatic and terrestrial prey groups, they could not be used to distinguish insect species within feeding guilds. Fatty acid profiles may provide useful dietary information for specific groups with known and unequivocal lipid biomarkers (Shin et al. 2014), but the composition of many groups remains undescribed. As such, it is imperative that direct feeding observations and taxon-specific compositional analyses are made to aid diagnosis of trophic relationships with more precision. Alternatively, it may be beneficial to conduct similar studies in areas with less food source variation, such as areas devoid of C4 plants. Nevertheless, as evidenced from this study, the use of a combination of analytical tools can prove useful in describing riparian cross-boundary trophic dynamics. Similar studies utilising one or more biomarker approaches may help to further elucidate trophic dynamics in other riparian areas. However, since there was a basic assumption that all flying insects were potential prey for generalist aerial predators, I recommend that future studies incorporate detailed observations of actual diets of spiders (e.g. Nentwig 1983, 1985, Nyffeler 2009) and odonates (e.g. Knight et al. 2005, Pfitzner et al. 2015) wherever possible.

If a narrow river like the Kowie (16 m at the widest measured point) can influence spiders that are four times that distance away, I expect that larger rivers can have even greater influences on terrestrial communities. Additional studies in larger rivers will improve our understanding of the importance of rivers for terrestrial food webs, and the trophic implications of anthropogenic or climate-induced changes to these river catchments. Present knowledge suggests that trophic dynamics in riparian areas vary across regions with different climates and biomes. The results of from the Kowie River can be considered specific to relatively small streams in temperate regions of Southern Africa. As such, there is a need for similar studies in other river systems for results of trophic subsidies to have generalised applications hence assisting in management of riparian areas. The data collected from this study can be used as part of a process to define riparian zone buffer widths based on trophic processes, but more studies are needed to convince environment managers of the importance of trophic subsidies.

A parallel study on frog diets in the Kowie River showed that anurans (frogs and toads) at wider sections of the river had higher proportions of aquatic subsidies in their diets than those at narrower sections (Sikutshwa 2015). There was also higher variation in anuran diet niches at the wider than narrower sections of the Kowie River (Sikutshwa 2015). The similarities in the results of the two studies is further evidence of the link between the size of a river and the extent of cross-boundary subsidisation. Furthermore, parallel studies in the Kowie River reported productivity (primary and secondary), stream depth, water temperatures, vegetation cover etc., all factors that could be used to accurately characterise the sites used in my study, rather than using stream width as a surrogate and assuming productivity would be high at wide sections. I recommend that in future, fluvial systems (and their catchments) be conceptualized as natural laboratories where diet analyses can be concurrently conducted on multiple organisms, hence developing predictive models on reciprocal transfers. Additionally, controlled field studies would help to determine selectivity and generalism among riparian predators.

REFERENCES

Ackman, R. G. 1999. Lipids in freshwater ecosystems. Pages 263–298 *in* M. T. Arts and B. C. Wainman, editors. Lipids in freshwater ecosystems. Springer-Verlag, New York.

Ahlgren, G., I.-B. Gustafsson, and M. Boberg. 1992. Fatty acid content and chemical composition of freshwater microalgae. Journal of Phycology 28:37–50.

Akamatsu, F., and H. Toda. 2011. Aquatic subsidies transport anthropogenic nitrogen to riparian spiders. Environmental Pollution 159:1390–1397.

Akamatsu, F., H. Toda, and T. Okino. 2004. Food source of riparian spiders analyzed by using stable isotope ratios. Ecological Research 19:655–662.

Alanärä, A., M. D. Burns, and N. B. Metcalfe. 2001. Intraspecific resource partitioning in brown trout: the temporal distribution of foraging is determined by social rank. Journal of Animal Ecology 70:980–986.

Alexander, B. 2000. Sampling methods for phlebotomine sandfies. Medical and Veterinary Entomology 14:109–122.

Allan, E. L., S. T. Ambrose, N. B. Richoux, and P. W. Froneman. 2010. Determining spatial changes in the diet of nearshore suspension-feeders along the South African coastline: stable isotope and fatty acid signatures. Estuarine, Coastal and Shelf Science 87:463–471.

Alp, M., B. L. Peckarsky, S. M. Bernasconi, and C. T. Robinson. 2013. Shifts in isotopic signatures of animals with complex life-cycles can complicate conclusions on cross-boundary trophic links. Aquatic Sciences 75:595–606.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26:32– 46.

Anderson, W. B., and G. A. Polis. 1998. Marine subsidies of island communities in the Gulf of California: evidence from stable carbon and nitrogen isotopes. Oikos 81:75–80.

Antonio, E. S., and N. B. Richoux. 2014. Trophodynamics of three decapod crustaceans in a temperate estuary using stable isotope and fatty acid analyses. Marine Ecology Progress Series 504:193–205.

Arts, M. T., M. T. Brett, and M. J. Kainz. 2009. Lipids in aquatic ecosystems. (M. Kainz, M. T. Brett, and M. T. Arts, Eds.). Springer New York, New York, NY.

Baird, J. M., and M. L. May. 1997. Foraging behavior of *Pachydiplax longipennis* (Odonata: Libellulidae). Journal of Insect Behavior 10:655–678.

Balfour, R. A., and A. L. Rypstra. 1998. The influence of habitat structure on spider density in a no-till soybean agroecosystem. Journal of Arachnology 26:221–226.

Ballinger, A., and P. S. Lake. 2006. Energy and nutrient fluxes from rivers and streams into terrestrial food webs. Marine and Freshwater Research 57:15–28.

Baptista, D. F., L. F. Dorvillé, D. F. Buss, and J. L. Nessiamian. 2001. Spatial and temporal organization of aquatic insects assemblages in the longitudinal gradient of a tropical river. Brazilian Journal of Biology 61:295–304.

Barnes, R. D. 1953. The ecological distribution of spiders in non-forest maritime communities at Beaufort, North Carolina. Ecological Monographs 23:315–337.

Barroso, F. G., C. de Haro, M. J. Sánchez-Muros, E. Venegas, A. Martínez-Sánchez, and C. Pérez-Bañón. 2014. The potential of various insect species for use as food for fish. Aquaculture 422-423:193–201.

Barth, F. G., editor. 1985. Neurobiology of arachnids. Springer Berlin Heidelberg, Berlin, Heidelberg.

Baxter, C. V., K. D. Fausch, and W. Carl Saunders. 2005. Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biology 50:201–220.

Bearhop, S., C. E. Adams, S. Waldron, R. A. Fuller, and H. Macleod. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. Journal of Animal Ecology 73:1007–1012.

Belivanov, Y. K., and P. A. Hambäck. 2015. The time scale of isotope signals in spiders: molting the remains of a previous diet. Entomologia Experimentalis et Applicata 156:271–278.

Bell, J. G., C. Ghioni, and J. R. Sargent. 1994. Fatty acid compositions of 10 freshwater invertebrates which are natural food organisms of Atlantic salmon parr (*Salmo salar*): a comparison with commercial diets. Aquaculture 128:301–313.

Bennett, P. M., and K. A. Hobson. 2009. Trophic structure of a boreal forest arthropod community revealed by stable isotope (δ^{13}C, δ^{15}N) analyses. Entomological Science 12:17–24.

Bergamino, L., T. Dalu, and N. B. Richoux. 2014. Evidence of spatial and temporal changes in sources of organic matter in estuarine sediments: stable isotope and fatty acid analyses. Hydrobiologia 732:133–145.

Bishop, L., and S. R. Connolly. 1992. Web orientation , thermoregulation , and prey capture efficiency in a tropical forest spider. Journal of Arachnology 20:173–178.

Blackledge, T. A., N. Scharff, J. A. Coddington, T. Szüts, J. W. Wenzel, C. Y. Hayashi, and I. Agnarsson. 2009. Reconstructing web evolution and spider diversification in the molecular era. Proceedings of the National Academy of Sciences of the United States of America 106:5229–34.

Brett, M. T., and D. C. Müller-Navarra. 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38:483–499.

Briers, R. A., H. M. Cariss, R. Geoghegan, and J. H. R. Gee. 2005. The lateral extent of the subsidy from an upland stream to riparian lycosid spiders. Ecography 28:165–170.

Brittain, J. E. 1982. Biology of mayflies. Annual Review of Entomology 27:119–147.

Brosofske, K. D., J. Chen, R. J. Naiman, and J. F. Franklin. 1997. Harvesting effects on microclimatic gradients from small streams to uplands in Western Washington. Ecological Applications 7:1188–1200.

Brues, C. T. 1908. On the interpretation of certain tropisms of insects. The American Naturalist 42:297–302.

Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Marine Mammal Science 22:759–801.

Budge, S. M., and C. C. Parrish. 1998. Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. Organic Geochemistry 29:1531–1545.

Budge, S. M., A. M. Springer, S. J. Iverson, and G. Sheffield. 2007. Fatty acid biomarkers reveal niche separation in an Arctic benthic food web. Marine Ecology Progress Series 336:305–309.

Bukkens, S. G. F., and M. G. Paoletti. 2005. Insects in the human diet: nutritional aspects. Pages 545–577 *in* M. G. Paoletti, editor. Ecological implications of minilivestock: potential of insects, rodents, frogs and snails. Science Publishers, Inc., Enfield, USA.

Burdon, F. J., and J. S. Harding. 2008. The linkage between riparian predators and aquatic insects across a stream-resource spectrum. Freshwater Biology 53:330–346.

Burtt, E. T., R. J. O. Perry, and A. J. McLachlan. 1986. Feeding and sexual dimorphism in adult midges (Diptera: Chironomidae). Holarctic Ecology 9:27–32.

Buskirk, R. E. 1975. Coloniality, activity patterns and feeding in a tropical orb-weaving spider. Ecology 56:1314–1328.

Cadenasso, M. L., S. T. A. Pickett, K. C. Weathers, and C. G. Jones. 2003. A Framework for a theory of ecological boundaries. BioScience 53:750–758.

Campos, W. G., D. B. S. Pereira, and J. H. Schoereder. 2000. Comparison of the efficiency of flight-interception trap models for sampling Hymenoptera and other insects. Anais da Sociedade Entomológica do Brasil 29:381–389.

Cappo, M. 1987. Frogs as predators of organisms of aquatic origin in the Magela Creek system, Northern Territory (Masters Thesis). University of Adelaide.

Carpenter, F. W. 1905. The reactions of the Pomace Fly (*Drosophila ampelophila Loew*) to light, gravity, and mechanical stimulation. The American Naturalist 39:157–171.

Castillo, J. A., and W. G. Eberhard. 1983. Use of artificial webs to determine prey available to orb weaving spiders. Ecology 64:1655–1658.

Catterall, C. P., S. D. Piper, S. E. Bunn, and J. M. Arthur. 2001. Flora and fauna assemblages vary with local topography in a subtropical eucalypt forest. Austral Ecology 26:56–69.

Choi, K. J., Z. Nakhost, V. J. Krukonis, and M. Karel. 1987. Supercritical fluid extraction and characterization of lipids from algae *Scenedesmus obliquus*. Food Biotechnology 1:263–81.

Clarke, K. R., and R. M. Warwick. 2001. Change in marine communities: an approach to statistical analysis and interpretation. PRIMER-E, Plymouth, UK.

Cnudde, C., E. Werbrouck, G. Lepoint, D. Vangansbeke, T. Moens, and M. De Troch. 2015. Trophodynamics of estuarine intertidal harpacticoid copepods based on stable isotope composition and fatty acid profiles. Marine Ecology Progress Series 524:225–239.

Colebourn, P. H. 1974. The influence of habitat structure on the distribution of *Araneus diadematus clerck*. Journal of Animal Ecology 43:401–409.

Collier, K. J., S. J. Bury, and M. Gibbs. 2002. A stable isotope study of linkages between stream and terrestrial food webs through spider predation. Freshwater Biology 47:1651–1659.

Collier, K. J., and B. J. Smith. 1998. Dispersal of adult caddisflies (Trichoptera) into forests alongside three New Zealand streams. Hydrobiologia 361:53–65.

Colombo, J. C., N. Cappelletti, A. Barreda, M. C. Migoya, and C. N. Skorupka. 2005. Vertical fluxes and accumulation of PCBs in coastal sediments of the Rio de la Plata estuary, Argentina. Chemosphere 61:1345–1357.

Compton, S. G., M. D. F. Ellwood, A. J. Davis, and K. Welch. 2000. The flight heights of chalcid wasps (Hymenoptera, chalcidoidea) in a lowland bornean rain forest: Fig wasps are the high fliers. Biotropica 32:515–522.

Corbet, P. 1999. Dragonflies: behaviour and ecology of Odonata. (P. Corbet, Ed.). Harley Books, Colchester, UK.

Corbet, P. S. 1964. Temporal patterns of emergence in aquatic insects. The Canadian Entomologist 96:264–279.

Corbet, P. S., and M. L. May. 2008. Fliers and perchers among Odonata: dichotomy or multidimensional continuum? A provisional reappraisal. International Journal of Odonatology 11:155–171.

Covich, A. P., M. A. Palmer, and T. A. Crowl. 1999. The role of benthic invertebrate species in freshwater ecosystems: zoobenthic species influence energy flows and nutrient cycling. BioScience 49:119–127.

Crawley, K. R., G. A. Hyndes, M. A. Vanderklift, A. T. Revill, and P. D. Nichols. 2009. Allochthonous brown algae are the primary food source for consumers in a temperate, coastal environment. Marine Ecology Progress Series 376:33–44.

Cummins, K. W., and M. J. Klug. 1979. Feeding ecology of stream invertebrates. Annual Review of Ecology and Systematics 10:147–172.

Cummins, K. W., M. A. Wilzbach, D. M. Gates, J. B. Perry, and B. Taliaferro. 1989. Shredders and riparian vegetation. BioScience 39:24–30.

Dalsgaard, J., M. St. John, G. Kattner, D. Müller-Navarra, and W. Hagen. 2003. Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology 46:225–340.

Dalu, T., P. Froneman, and N. Richoux. 2014. Using multivariate analysis and stable isotopes to assess the effects of substrate type on phytobenthos communities. Inland Waters 4:397–412.

Décamps, H., G. Pinay, R. J. Naiman, G. E. Petts, M. E. Mcclain, A. Hillbricht-ilkowska, T. A. Hanley, R. M. Holmes, J. Quinn, J. Gibert, A. P. Tabacchi, F. Schiemer, and E. Tabacchi. 2004. Riparian zones: where biogeochemistry meets biodiversity in management practice. Polish Journal of Ecology 52:3–18.

Delettre, Y. R., and N. Morvan. 2000. Dispersal of adult aquatic Chironomidae (Diptera) in agricultural landscapes. Freshwater Biology 44:399–411.

Deniro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta 42:495–506.

Denlinger, D. L. 1980. Seasonal and annual variation of insect abundance in the Nairobi National Park, Kenya. Biotropica 12:100–106.

Dennis, P., J. Skartveit, A. Kunaver, and D. I. Mccracken. 2015. The response of spider

(Araneae) assemblages to structural heterogeneity and prey abundance in sub-montane vegetation modified by conservation grazing. Global Ecology and Conservation 3:715–728.

Department of water affairs. 2013. Kowie River, Bathurst station P4H001. http://www.dwa.gov.za/hydrology/ HyDataSets.aspx?Station=P4H001.

Dickens, C. W. S., and P. M. Graham. 2002. The South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers. African Journal of Aquatic Science 27:1–10.

Diehl, E., V. L. Mader, V. Wolters, and K. Birkhofer. 2013. Management intensity and vegetation complexity affect web-building spiders and their prey. Oecologia 173:579–89.

Dietrick, E. J. 1961. An improved backpack motor fan for suction sampling of insect populations. Journal of Economic Entomology 54:394–395.

Dobel, H. G., R. F. Denno, and J. A. Coddington. 1990. Spider (Araneae) community structure in an intertidal salt marsh: effects of vegetation structure and tidal flooding. Evnvironmental Entomology 19:1356–1370.

Dortch, Q., and T. T. Packard. 1989. Differences in biomass structure between oligotrophic and eutrophic marine ecosystems. Deep Sea Research Part A. Oceanographic Research Papers 36:223–240.

Doucett, R. R., J. C. Marks, D. W. Blinn, M. Caron, and B. A. Hungate. 2007a. Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. Ecology 88:1587–92.

Doucett, R. R., J. C. Marks, D. W. Blinn, M. Caron, and B. A. Hungate. 2007b. Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. Ecology 88:1587–92.

Dudgeon, D. 1989. Resource partitioning among Odonata (Insecta: Anisoptera and Zygoptera) larvae in a Hong Kong forest stream. Journal of Zoology 217:381–402.

Dugan, J. E., D. M. Hubbard, M. D. McCrary, and M. O. Pierson. 2003. The response of macrofauna communities and shorebirds to macrophyte wrack subsidies on exposed sandy beaches of southern California. Estuarine, Coastal and Shelf Science 58:25–40.

Dumont, H. J. 1967. A possible scheme of the migration of *Crocothemis erythraea* (Brullé) - populations from the Camargue (Odonata: Libellulidae). Biological Sciences 35:222–227.

Eberhard, W. G. 1990. Function and phylogeny of spider webs. Annual Review of Ecology and Systematics 21:341–372.

Edmunds, G. F. J., and C. H. Edmunds. 1980. Advances in Ephemeroptera biology. Pages 277–285 *in* J. F. Flannagan and K. E. Marshall, editors. Advances in Ephemeroptera Biology. Springer US, Boston, MA.

El-Moursy, A., F. Gilbert, S. Zalat, and M. El-Hawagry. 1999. Foraging behaviour of anthracine flies (Diptera: Bombyliidae) in Southern Sinai, Egypt. Egyption Journal of Biology 1:87–95.

Fagan, W. F., R. S. Cantrell, and C. Cosner. 1999. How habitat edges change species interactions. The American Naturalist 153:165–182.

Fariña, J. M., S. Salazar, K. P. Wallem, J. D. Witman, and J. C. Ellis. 2003. Nutrient exchanges between marine and terrestrial ecosystems: the case of the galapagos sea lion *Zalophus wollebaecki*. Journal of Animal Ecology 72:873–887.

Fincke, O. M. 2015. Trade-offs in female signal apparency to males offer alternative anti-harassment strategies for colour polymorphic females. Journal of Evolutionary Biology 28:931–943.

Fisher, S. G., and G. E. Likens. 1973. Energy flow in Bear Brook , New Hampshire: an integrative approach to stream ecosystem metabolism. Ecological Monographs 43:421–439.

Fontaneto, D., M. Tommaseo-Ponzetta, C. Galli, P. Risé, R. H. Glew, and M. G. Paoletti. 2011. Differences in fatty acid composition between aquatic and terrestrial insects used as food in human nutrition. Ecology of Food and Nutrition 50:351–67.

France, R. L. 1995. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnology and Oceanography 40:1310–1313.

Fretwell, S. D., and H. L. Lucas. 1970. On territorial behavior and other factors influencing habitat distribution in birds. Acta Biotheoretica 19:16–36.

Fukui, D., M. Murakami, S. Nakano, and T. Aoi. 2006. Effect of emergent aquatic insects on bat foraging in a riparian forest. The Journal of Animal Ecology 75:1252–1258.

Garono, R. J., and J. G. Kooser. 2001. The relationship between patterns in flying adult insect assemblages and vegetation structure in wetlands of Ohio and Texas. The Ohio Journal of Science 101:12–21.

Garrido, C. S. F. 2007. Trophic ecology of the Atlanto-Iberian sardine (*Sardina pilchardus*) (Doctoral thesis). University of the Algarve.

Geerling, B. J., A. C. V Houwelingen, A. Badart-Smook, R. W. Stockbrügger, and R. J. Brummer. 1999. The relation between antioxidant status and alterations in fatty acid profile in patients with Crohn disease and controls. Scandinavian Journal of Gastroenterology 34:1108–1116.

Ghioni, C., J. G. Bell, and J. R. Sargent. 1996. Polyunsaturated fatty acids in neutral lipids and phospholipids of some freshwater insects. Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology 114:161–170.

Gillespie, R. G. 2003. Spiders of the genus *Tetragnatha* (Araneae, Tetragnathidae) in the Society Islands. Journal of Arachnology 31:157–172.

Gladyshev, M. I., M. T. Arts, and N. N. Sushchik. 2009a. Lipids in aquatic ecosystems. Pages 179–210. Springer New York.

Gladyshev, M. I., M. T. Arts, and N. N. Sushchik. 2009b. Preliminary estimates of the export of omega-3 highly unsaturated fatty acids (EPA + DHA) from aquatic to terrestrial ecosystems. Pages 179–209 Lipids in aquatic ecosystems. Springer New York.

Gower, J., S. Lubbe, and N. Le Roux. 2011. Understanding biplots. 1st edition. John Wiley & Sons, Ltd, West Sussex.

Grant, M., A. Robison, and O. M. Fincke. 2014. Use of stable isotopes to assess the intraspecific foraging niche of males and female colour morphs of the damselfly

Enallagma hageni. Ecological Entomology 39:109–117.

Gratton, C., J. Donaldson, and M. J. Vander Zanden. 2008. Ecosystem linkages between lakes and the surrounding terrestrial landscape in Northeast Iceland. Ecosystems 11:764–774.

Green, J. 1999. Sampling method and time determines composition of spider collections. The Journal of Arachnology 27:176–182.

Greenstone, M. H. 1984. Determinants of web spider species diversity: vegetation structural diversity vs. prey availability. Oecologia 62:299–304.

Greenwood, M. J., and D. J. Booker. 2015. The influence of antecedent floods on aquatic invertebrate diversity, abundance and community composition. Ecohydrology 8:188–203.

Gregorič, M., M. Kuntner, and T. A. Blackledge. 2015. Does body size predict foraging effort? Patterns of material investment in spider orb webs. Journal of Zoology 296:67–78.

Gregory, S. V., F. J. Swanson, A. W. McKee, and K. W. Cummins. 1991. An ecosystem perspective of riparian zones: focus on links between land and water. BioScience 41:540–551.

Griffith, M. B., E. M. Barrows, and S. A. Perry. 1998. Lateral dispersal of adult aquatic insects (Plecoptera, Trichoptera) following emergence from headwater streams in forested Appalachian catchments. Annals of the Entomological Society of America 91:195–201.

Hagen, W., G. Kattner, and C. Friedrich. 2000. The lipid compositions of high-Antarctic notothenioid fish species with different life strategies. Polar Biology 23:785–791.

Hammer Ø, D. Harper, and P. Ryan. 2001. PAST: palaeontological statistics software package for education and data analysis. Palaeontologia Electronica.

Happold, D. C. D. 2009. Dragonflies of Jebel Marra, Sudan. Proceedings of the Royal Entomological Society of London. Series A, General Entomology 41:87–91.

Harwood, J. L. 1996. Recent advances in the biosynthesis of plant fatty acids. Biochimica et Biophysica Acta 1301:7–56.

Hatley, C. L., and J. A. Macmahon. 1980. Spider community organization: seasonal variation and the role of vegetation architecture. Environmental Entomology 9:632–639.

Hawkins, B. A., and E. E. Porter. 2003. Does herbivore diversity depend on plant diversity? The case of California butterflies. The American Naturalist 161:40–49.

Heck, K. L., T. J. B. Carruthers, C. M. Duarte, A. R. Hughes, G. Kendrick, R. J. Orth, and S. W. Williams. 2008. Trophic transfers from seagrass meadows subsidize diverse marine and terrestrial consumers. Ecosystems 11:1198–1210.

Heinrich, B., and T. M. Casey. 1978. Heat transfer in dragonflies: “fliers” and “perchers.” Journal of Experimental Biology 74:17–36.

Helmus, M. R., N. Mercado-Silva, and M. J. Vander Zanden. 2013. Subsidies to predators, apparent competition and the phylogenetic structure of prey communities. Oecologia 173:997–1007.

Henry, M., L. Gasco, G. Piccolo, and E. Fountoulaki. 2015. Review on the use of insects in the diet of farmed fish: past and future. Animal Feed Science and Technology 203:1–22.

Henschel, J. R., D. Mahsberg, and H. Stumpf. 2001. Allochthonous aquatic insects increase predation and decrease herbivory in river shore food webs. Oikos 93:429–438.

Herdien, E., and T. Collins. 2011. Wetland and riparian resource status quo delineation and sensitivity assessment for the proposed construction of a 132kV powerline from Bosloop to Gumeni, Mpumalanga province (SSI Environmental). Mpumulanga South Africa.

Hering, D., and H. Plachter. 1997. Riparian ground beetles (Coeloptera, Carabidae) preying on aquatic invertebrates: a feeding strategy in alpine floodplains. Oecologia 111:261–270.

Heydorn, A., and J. Grindley. 1982. Estuaries of the Cape: Kowie (National Research Institute for Oceanology). Stellenbosch.

Higashi, K. 1978. Daily food consumption of Sympetrum frequens selys (Odonata: Libellulidae). JIBP Synth 18:199–207.

Higgins, L. E., and R. E. Buskirk. 1992. A trap-building predator exhibits different tactics for different aspects of foraging behaviour. Animal Behaviour 44:485–499.

Hilbe, J. M. 2011. Negative Binomial Regression. Second Edition Cambridge University Press, New York.

Hill, B. J. 1975. Abundance, breeding and growth of the crab *Scylla serrata* in two South African estuaries. Marine Biology 32:119–126.

Hixson, S. M., B. Sharma, M. J. Kainz, A. Wacker, and M. T. Arts. 2015. Production, distribution, and abundance of long-chain omega-3 polyunsaturated fatty acids: a fundamental dichotomy between freshwater and terrestrial ecosystems. Environmental Reviews 23:1–11.

Hobson, K. A. 1993. Trophic relationships among high Arctic seabirds: insights from tissue-dependent stable-isotope models. Marine Ecology Progress Series 95:7–18.

Hurd, L. E., and W. F. Fagan. 1992. Cursorial spiders and succession: age or habitat structure? Oecologia 92:215–221.

Huryn, A. D., and J. B. Wallace. 2000. Life history and production of stream insects. Annual Review of Entomology 45:83–110.

Hyslop, E. J. 1980. Stomach contents analysis-a review of methods and their application. Journal of Fish Biology 17:411–429.

Indarti, E., M. I. A. Majid, R. Hashim, and A. Chong. 2005. Direct FAME synthesis for rapid total lipid analysis fromfish oil and cod liver oil. Journal of Food Composition and Analysis 18:161–170.

Iverson, S. J., C. Field, W. D. Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecological Monographs 74:211–235.

Iverson, S. J., K. J. Frost, and L. F. Lowry. 2002. Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. Marine Ecology Progress Series 151:255–271.

Iwata, T. 2006. Linking stream habitats and spider distribution: spatial variations in trophic transfer across a forest–stream boundary. Ecological Research 22:619–628.

Iwata, T., S. Nakano, and M. Murakami. 2003. Stream meanders increase insectivorous bird abundance in riparian deciduous forests. Ecography 26:325–337.

Jackson, A. L., R. Inger, A. C. Parnell, and S. Bearhop. 2011. Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. The Journal of Animal Ecology 80:595–602.

Jackson, J. K., and S. G. Fisher. 1986. Secondary production, emergence, and export of aquatic insects of a Sonoran Desert stream. Ecology 67:629–638.

Jacobs, H. M., D. A. Hughes, and A. R. Slaughter. 2012. An assesment of processes affecting water quality in a low order stream impacted by point and diffuse nutrient sources. 16th SANCIAHS National Hydrology Symposium, 1-3 October. Pretoria.

Jander, R. 1963. Insect Orientation. Annual Review of Entomology 8:95–114.

Janzen, D. H., and T. W. Schoener. 1968. Differences in insect abundance and diversity between wetter and drier sites during a tropical dry season. Ecology 49:96–110.

Joergensen, R. G., and F. Wichern. 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biology and Biochemistry 40:2977–2991.

Johnson, R. K., M. T. Furse, D. Hering, and L. Sandin. 2007. Ecological relationships between stream communities and spatial scale: Implications for designing catchment-level monitoring programmes. Freshwater Biology 52:939–958.

Jonsson, M., P. Hedström, K. Stenroth, E. R. Hotchkiss, F. R. Vasconcelos, J. Karlsson, and P. Byström. 2015. Climate change modifies the size structure of assemblages of emerging aquatic insects. Freshwater Biology 60:78–88.

Jonsson, M., and D. A. Wardle. 2009. The influence of freshwater-lake subsidies on invertebrates occupying terrestrial vegetation. Acta Oecologica 35:698–704.

Jurczyk, M., V. Wolters, and K. Birkhofer. 2012. Utilization of prey-rich patches leads to reproductive advantages for clustered individuals of a web-building spider. Ecoscience 19:170–176.

Kacelnik, A., J. R. Krebs, and C. Bernstein. 1992. The ideal free distribution and predator-prey populations. Trends in Ecology and Evolution 7:50–55.

Kaiser, H. 1982. Do Cordulegaster males defend territories? A preliminary investigation of mating strategies in *Cordulegaster boltoni* (Donovan) (Anisoptera: Cordulegasteridae). Odonatologica 11:139–152.

Kato, C., T. Iwata, S. Nakano, and D. Kishi. 2003. Dynamics of aquatic insect flux affects distribution of riparian web-building spiders. Oikos 103:113–120.

Kato, C., T. Iwata, and E. Wada. 2004. Prey use by web-building spiders: stable isotope analyses of trophic flow at a forest-stream ecotone. Ecological Research 19:633–643.

Kauffman, J. B., R. L. Beschta, N. Otting, and D. Lytjen. 1997. An ecological perspective of riparian and stream restoration in the Western United States. Fisheries 22:12–24.

Kautza, A., and S. M. P. Sullivan. 2016. The energetic contributions of aquatic primary producers to terrestrial food webs in a mid-size river system. Ecology DOI: 10.1890/15-

1095.

Kautza, A., and S. M. P. Sullivan. 2015. Shifts in reciprocal river-riparian arthropod fluxes along an urban-rural landscape gradient. Freshwater Biology 50:2156-2168.

Kennedy, T. L., and T. F. Turner. 2011. River channelization reduces nutrient flow and macroinvertebrate diversity at the aquatic terrestrial transition zone. Ecosphere 2:1-13.

Khrokalo, L. 2010. Expansion of *Crocothemis erythraea* in Ukraine. BIORISK – Biodiversity and Ecosystem Risk Assessment 5:211–223.

Kirk-Spriggs, A. H., and G. McGregor. 2009. Disjunctions in the Diptera (Insecta) fauna of the Mediterranean Province and southern Africa and a discussion of biogeographical considerations. Transactions of the Royal Society of South Africa 64:32–52.

Knight, T. M., M. W. McCoy, J. M. Chase, K. a McCoy, and R. D. Holt. 2005. Trophic cascades across ecosystems. Nature 437:880–3.

Knops, M. H. J., D. Tilman, N. M. Haddad, S. Naeem, C. E. Mitchell, J. Haarstad, M. E. Ritchie, K. M. Howe, P. B. Reich, E. Siemann, and J. Groth. 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. Ecology Letters 2:286–293.

Koch, P. L., J. C. Zachos, and D. L. Dettman. 1995. Stable isotope stratigraphy and paleoclimatology of the Paleogene Bighorn Basin (Wyoming, USA). Palaeogeography, Palaeoclimatology, Palaeoecology 115:61–89.

Koussoroplis, A.-M., C. Lemarchand, A. Bec, C. Desvilettes, C. Amblard, C. Fournier, P. Berny, and G. Bourdier. 2008. From aquatic to terrestrial food webs: decrease of the docosahexaenoic acid/linoleic acid ratio. Lipids 43:461–6.

Kovats, Z., J. Ciborowski, and L. Corkum. 1996. Inland dispersal of adult aquatic insects. Freshwater Biology 36:265–276.

Krell, B., N. Röder, M. Link, R. Gergs, M. H. Entling, and R. B. Schäfer. 2015. Aquatic prey subsidies to riparian spiders in a stream with different land use types. Limnologica - Ecology and Management of Inland Waters 51:1–7.

Laeser, S. R., C. V. Baxter, and K. D. Fausch. 2005. Riparian vegetation loss, stream channelization, and web-weaving spiders in northern Japan. Ecological Research 20:646–651.

Lammert, M., and J. Allan. 1999. Environmental auditing: assessing biotic integrity of streams: effects of scale in measuring the influence of land use/cover and habitat structure on fish and macroinvertebrates. Environmental management 23:257–270.

Langellotto, G. A., and R. F. Denno. 2004. Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. Oecologia 139:1–10.

Lara, D. P., L. A. Oliveira, I. F. P. Azevedo, M. F. Xavier, F. A. O. Silveira, M. A. A. Carneiro, and G. W. Fernandes. 2008. Relationships between host plant architecture and gall abundance and survival. Revista Brasileira de entomologia 52:757–763.

Lau, D. C. P., K. M. Y. Leung, and D. Dudgeon. 2009. Are autochthonous foods more important than allochthonous resources to benthic consumers in tropical headwater streams? Journal of the North American Benthological Society 28:426–439.

Layman, C. A., D. A. Arrington, C. G. Montana, and D. M. Post. 2007a. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88:42–48.

Layman, C. A., and D. M. Post. 2008. Can stable isotope ratios provide for community-wide measures of trophic structure? Reply. Ecology 89:2353–2357.

Layman, C. A., J. P. Quattrochi, C. M. Peyer, and J. E. Allgeier. 2007b. Niche width collapse in a resilient top predator following ecosystem fragmentation. Ecology Letters 10:937–44.

Lee, P., C. Smyth, and S. Boutin. 2004. Quantitative review of riparian buffer width guidelines from Canada and the United States. Journal of Environmental Management 70:165–180.

Leslie, J., H. A. Yesi, and T. C. Lockley. 1990. Prey and nocturnal activity of wolf spiders (Araneae: Lycosidae) in cotton fields in the Delta Region of Mississippi. Environmental Entomology 19:1512–1518.

Lindstrom, M. J., and D. M. Bates. 1990. Nonlinear mixed effects models for repeated measures data. Biometrics 46:673–687.

Lowrie, D. C. 1948. The ecological succession of spiders of the Chicago area. Ecology 29:334–351.

Ludy, C. 2007. Prey selection of orb-web spiders (Araneidae) on field margins. Agriculture, Ecosystems and Environment 119:368–372.

Lynch, R. J., S. E. Bunn, and C. P. Catterall. 2002. Adult aquatic insects: potential contributors to riparian food webs in Australia's wet-dry tropics. Austral Ecology 27:515–526.

MacArthur, R. H., and J. W. MacArthur. 1961. On bird species diversity. Ecology 42:594–598.

Malainey, M. E., R. Przybylski, and B. L. Sherriff. 1999. The fatty acid composition of native food plants and animals of Western Canada. Journal of Archaeological Science 26:83–94.

Mallik, A. U., and J. S. Richardson. 2009. Riparian vegetation change in upstream and downstream reaches of three temperate rivers dammed for hydroelectric generation in British Columbia, Canada. Ecological Engineering 35:810–819.

Marczak, L. B., and J. S. Richardson. 2007. Spiders and subsidies: results from the riparian zone of a coastal temperate rainforest. The Journal of Animal Ecology 76:687–94.

Martens, A., and G. Rehfeldt. 1989. Female aggregation in *Platycypha caligata* (Odonata: chlorocyphidae): a tactic to evade male interference during oviposition. Animal Behaviour 38:369–374.

Mathooko, J. M., C. M. M. Erimba, and M. Leichtfried. 2000. Decomposition of leaf litter of *Dombeya goetzenii* in the Njoro River , Kenya. Hydrobiologia 418:147–152.

May, M. 1995. Dependence of flight behavior and heat production on air temperature in the green darner dragonfly *Anax junius* (Odonata: Aeshnidae). The Journal of Experimental Biology 198:2385–92.

May, M. L. 1981. Wingstroke frequency of dragonflies (Odonata: Anisoptera) in relation of temperature and body size. Journal of Comparative Physiology B 144:229–240.

May, M. L., and J. M. Baird. 2002. A comparison of foraging behavior in two “percher” dragonflies, *Pachydiplax longipennis* and *Erythemis simplicicollis* (Odonata: Libellulidae). Journal of Insect Behavior 15:765–778.

McCaffrey, J. P., M. P. Parrella, and R. L. Horsburgh. 1984. Evaluation of the limb-beating sampling method for estimating spider (Araneae) populations on apple tress. The Journal of Arachnology 11:363–368.

McCutchan, J. J. H., W. J. M. Lewis, C. Kendall, and C. C. Mcgrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 2:378–390.

McGlynn, B. L., and J. Seibert. 2003. Distributed assessment of contributing area and riparian buffering along stream networks. Water Resources Research 39:2–7.

McKechnie, A. E., B. O. Wolf, and C. Martínez del Rio. 2004. Deuterium stable isotope ratios as tracers of water resource use: an experimental test with rock doves. Oecologia 140:191–200.

Mcnabb, D. M., J. Halaj, and D. H. Wise. 2001. Inferring trophic positions of generalist predators and their linkage to the detrital food web in agroecosystems: a stable isotope analysis. Pedobiologia 45:289–297.

McNett, B. J., and A. L. Rypstra. 2000. Habitat selection in a large orb-weaving spider: vegetational complexity determines site selection and distribution. Ecological Entomology 25:423–432.

Meraz, L. C., Y. Hénaut, and L. Legal. 2012. Prey selection in a nocturnal web-building spider, *Eriophora edax* (Araneae Araneidae). Ethology, Ecology and Evolution 24:1–13.

Mertes, L. A. K. 1997. Documentation and significance of the perirheic zone on inundated floodplains. Water Resources Research 33:1749–1762.

Mestre, L., and Y. Lubin. 2011. Settling where the food is: prey abundance promotes colony formation and increases group size in a web-building spider. Animal Behaviour 81:741–748.

Mills, G. L., J. V. Mcarthur, C. Wolfe, J. M. Aho, and R. B. Rader. 2001. Changes in fatty acid and hydrocarbon composition of leaves during decomposition in a southeastern blackwater stream. Archiv fur Hydrobiologie 152:315–328.

Minas, H. J., and M. Minas. 1992. Net community production in high nutrient-low chlorophyll waters of the tropical and antarctic oceans-grazing vs iron hypothesis. Oceanologica Acta 15:145–162.

Moore, J. W., and B. X. Semmens. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecology Letters 11:470–80.

Moore, N. W. 1964. Intra- and inter-specific competition among dragonflies (Odonata). The Journal of Animal Ecology 33:49–71.

Moyo, S. 2016. Aquatic - terrestrial trophic linkages via riverine invertebrates in a South African catchment (Doctoral Thesis). Rhodes University.

Muehlbauer, J. D., S. F. Collins, M. W. Doyle, and K. Tockner. 2014. How wide is a stream? Spatial extent of the potential “stream signature” in terrestrial food webs using meta-analysis. Ecology 95:44–55.

Murakami, M., and S. Nakano. 2002. Indirect effect of aquatic insect emergence on a terrestrial insect population through by birds predation. Ecology Letters 5:333–337.

Murphy, M. L., J. Heifetz, S. W. Johnson, K. V. Koski, and J. F. Thedinga. 1986. Effects of clear-cut logging with and without buffer strips on juvenile salmonids in Alaskan streams. Canadian Journal of Fisheries and Aquatic Sciences 43:1521–1533.

Naiman, J., and H. Decamps. 1997. The ecology of interfaces: riparian zones. Annual Review of Ecology and Systematics 28:621–658.

Naiman, R. J., H. Decamps, and M. Pollock. 1993. The role of riparian corridors in maintaining regional biodiversity. Ecological Applications 3:209–212.

Nakano, S., K. D. Fausch, and S. Kitano. 1999. Flexible niche partitioning via a foraging mode shift: a proposed mechanism for coexistence in stream-dwelling charrs. Journal of Animal Ecology 68:1079–1092.

Nakano, S., and M. Murakami. 2001. Reciprocal subsidies: dynamic interdependence between terrestrial and aquatic food webs. Proceedings of the National Academy of Sciences of the United States of America 98:166–170.

Napolitano, G. E. 1994. The relationship of lipids with light and chlorophyl measurements in freshwater algae and periphyton. Journal of Phycology 30:943–950.

Neal, T. M., and W. H. Whitcomb. 1972. Odonata in the Florida soybean agroecosystem. The Florida Entomologist 55:107.

Nentwig, W. 1982. Why do only certain insects escape from a spider’s web? Oecologia 53:412–417.

Nentwig, W. 1983. The prey of web-building spiders compared with feeding experiments (Araneae: Araneidae, Linyphiidae, Pholcidae, Agelenidae). Oecologia 56:132–139.

Nentwig, W. 1985. Prey analysis of four species of tropical orb-weaving spiders (Araneae: Araneidae) and a comparison with araneids of the temperate zone. Oecologia 66:580–594.

Nentwig, W. 1986. Non-webbuilding spiders: prey specialists or generalists? Oecologia 69:571–576.

Nentwig, W., editor. 1987. Ecophysiology of spiders. Springer Berlin Heidelberg, Berlin, Heidelberg.

New, T. R. 1998. Invertebrate surveys for conservation. Oxford University Press, Oxford, UK.

Nyffeler, M. 1999. Prey selection of spiders in the field. The Journal of Arachnology 27:317–324.

Nyffeler, M. 2009. Estimate of the daily catch of prey by the wasp spider *Argiope bruennichi* (Scopoli) in the field: original data and minireview. Contributions to Natural History 12:1007–1020.

Nyffeler, M., D. A. Dean, and W. L. Sterling. 1989. Prey selection and predatory importance of orb-weaving spiders (Araneae : Araneidae, Uloboridae) in Texas cotton. Environmental Entomology 18:373–380.

Ober, H. K., and J. P. Hayes. 2008. Influence of forest riparian vegetation on abundance and biomass of nocturnal flying insects. Forest Ecology and Management 256:1124–1132.

Oberg, K., J. M. Shelton, N. Gardiner, and P. R. Jackson. 2009. Discharge and other hydraulic measurements for characterizing the hydraulics of Lower Congo River, July 2008. Pages 1–8 The International Association for Hydraulic Research Congress.

Oelbermann, K., and S. Scheu. 2002. Stable isotope enrichment (δ^{15}N and δ^{13}C) in a generalist predator (*Pardosa lugubris*, Araneae: Lycosidae): effects of prey quality. Oecologia 130:337–344.

Olberg, R. M., A. H. Worthington, J. L. Fox, C. E. Bessette, and M. P. Loosemore. 2005. Prey size selection and distance estimation in foraging adult dragonflies. Journal of Comparative Physiology A 191:791–7.

Olive, C. W. 1980. Foraging specialization in orb-weaving spiders. Ecology 61:1133–1144.

Olive, C. W. 1982. Behavioral response of a sit-and-wait predator to spatial variation in foraging gain. Ecology 63:912–920.

Ostfeld, R. S., and F. Keesing. 2000. Pulsed resources and community dynamics of consumers in terrestrial ecosystems. Trends in Ecology and Evolution 15:232–237.

Owen, D. F., and D. O. Chanter. 1970. Species diversity and seasonal abundance in tropical Ichneumonidae. Oikos 21:142–144.

Oxbrough, A., V. French, S. Irwin, T. C. Kelly, P. Smiddy, and J. O'Halloran. 2012. Can mixed species stands enhance arthropod diversity in plantation forests? Forest Ecology and Management 270:11–18.

Paetzold, A., J. F. Bernet, and K. Tockner. 2006. Consumer-specific responses to riverine subsidy pulses in a riparian arthropod assemblage. Freshwater Biology 51:1103–1115.

Paetzold, A., C. J. Schubert, and K. Tockner. 2005. Aquatic terrestrial linkages along a braided-river: riparian arthropods feeding on aquatic insects. Ecosystems 8:748–759.

Paetzold, A., and K. Tockner. 2005. Effects of riparian arthropod predation on the biomass and abundance of aquatic insect emergence. Journal of the North American Benthological Society 24:395–402.

Panetta, R. J., and Y. Gélinas. 2009. Expressing biomarker data in stoichiometric terms: shifts in distribution and biogeochemical interpretation. Limnology and Oceanography: Methods 7:269–276.

Paoletti, M. G., E. Buscardo, D. J. VanderJagt, A. Pastuszyn, L. Pizzoferrato, Y.-S. Huang, L.-T. Chuang, M. Millson, H. Cerda, F. Torres, and R. H. Glew. 2003. Nutrient content of earthworms consumed by Ye'Kuana Amerindians of the Alto Orinoco of Venezuela. Proceedings of the Royal Society of London B: Biological Sciences 270:249–257.

Park, S., M. T. Brett, D. C. Müller-Navarra, and C. R. Goldman. 2002. Essential fatty acid content and the phosphorus to carbon ratio in cultured algae as indicators of food quality for Daphnia. Freshwater Biology 47:1377–1390.

Parnell, A. C., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Source partitioning using stable isotopes: coping with too much variation. PloS one 5:1–5 (e9672).

Parrish, C. C., T. A. Abrajano, S. M. Budge, R. J. Helleur, E. D. Hudson, K. Pulchan, and C. Ramos. 2000. Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. Pages 193–223 *in* Wangersky P, editor. The Handbook of Environmental chemistry Vol 5 Part D Marine Chemistry. Springer-Verlag Berlin Heidelberg, Berlin.

Peakall, D. B., and P. N. Witt. 1976. The energy budget of an orb web-building spider. Comparative Biochemistry and Physiology Part A: Physiology 54:187–190.

Petchey, O. L., P. T. McPhearson, T. M. Casey, and P. J. Morin. 1999. Environmental warming alters food-web structure and ecosystem function. Nature 402:69–72.

Petersen, I., J. H. Winterbottom, S. Orton, N. Friberg, A. G. Hildrew, D. C. Spiers, and W. S. C. Gurney. 1999. Emergence and lateral dispersal of adult Plecoptera and Trichoptera from Broadstone Stream, U.K. Freshwater Biology 42:401–416.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293–320.

Petersson, E., and A. T. Hasselrot. 1994. Mating and nectar feeding in the psychomyiid caddis fly *Tinodes waeneri*. Aquatic Insects 16:177–187.

Pfitzner, W. P., M. Beck, T. Weitzel, and N. Becker. 2015. The role of mosquitoes in the diet of adult dragon and damselflies (Odonata). Journal of the American Mosquito Control Association 31:187–189.

Phillips, D. L., and J. W. Gregg. 2001. Uncertainty in source partitioning using stable isotopes. Oecologia 127:171–9.

Phillips, D. L., R. Inger, S. Bearhop, A. L. Jackson, J. W. Moore, A. C. Parnell, B. X. Semmens, and E. J. Ward. 2014. Best practices for use of stable isotope mixing models in food-web studies. Canadian Journal of Zoology 92:823–835.

Phillips, D. L., S. D. Newsome, and J. W. Gregg. 2005. Combining sources in stable isotope mixing models: alternative methods. Oecologia 144:520–527.

Pianka, E. R. 1966. Latitudinal gradients in species diversity: a review of concepts. The American Naturalist 100:33–46.

Pilastro, A., S. Benetton, and A. Bisazza. 2003. Female aggregation and male competition reduce costs of sexual harassment in the mosquitofish *Gambusia holbrooki*. Animal Behaviour 65:1161–1167.

Poff, N. L., and J. V. Ward. 1989. Implications of streamflow variability and predictability for lotic community structure: a regional analysis of streamflow patterns. Canadian Journal of Fisheries and Aquatic Sciences 46:1805–1818.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an intergration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics 28:289–316.

Polis, G. A., and S. D. Hurd. 1995. Extraordinarily high spider densities on islands: flow of energy from the marine to terrestrial food webs and the absence of predation. Proceedings of the National Academy of Sciences of the United States of America 92:4382–6.

Polis, G. A., and S. D. Hurd. 1996. Linking marine and terrestrial food webs: allochthonous input from the ocean supports high secondary productivity on small islands and coastal land communities. The American Naturalist 147:396–423.

Polis, G. A., M. E. Power, and G. R. Huxel. 2004. Food webs at the landscape level. University of Chicago Press, Chicago, IL.

Polis, G. A., and D. R. Strong. 1996. Food web complexity and community dynamics. The

American Naturalist 147:813–846.

Pollierer, M. M., S. Scheu, and D. Haubert. 2010. Taking it to the next level: trophic transfer of marker fatty acids from basal resource to predators. Soil Biology and Biochemistry 42:919–925.

Ponsard, S., and R. Arditi. 2000. What can stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) tell about the food web of soil macro-invertebrates? Ecology 81:852–864.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718.

Power, M. E. 2001. Prey exchange between a stream and its forested watershed elevates predator densities in both habitats. Proceedings of the National Academy of Sciences of the United States of America 98:14–5.

Power, M. E., and W. E. Rainey. 2000. Food webs and resource sheds: towards spatially delimiting trophic interactions. Pages 291–314 *in* M. J. Hutchings, E. A. John, and A. J. A. Stewart, editors. Ecological Consequences of Habitat Heterogeneity. Blackwell Scientific, Oxford, UK.

Prestwich, K. N. 1977. The energetics of web-building in spiders. Comparative Biochemistry and Physiology Part A: Physiology 57:321–326.

Pulliam, H. R. 2000. On the relationship between niche and distribution. Ecology Letters 3:349–361.

Puttick, J. R., M. T. Hoffman, and J. Gambiza. 2011. Historical and recent land-use impacts on the vegetation of Bathurst, a municipal commonage in the Eastern Cape, South Africa. African Journal of Range and Forage Science 28:9–20.

Queheillalt, D. M., and M. L. Morrison. 2006. Vertebrate use of a restored riparian site: a case study on the central coast of california. Journal of Wildlife Management 70:859–866.

R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raksakantong, P., N. Meeso, J. Kubola, and S. Siriamornpun. 2010. Fatty acids and proximate composition of eight Thai edible terricolous insects. Food Research International 43:350–355.

Rashed, A., C. D. Beatty, M. R. Forbes, and T. N. Sherratt. 2005. Prey selection by dragonflies in relation to prey size and wasp-like colours and patterns. Animal Behaviour 70:1195–1202.

Rehfeldt, G. E. 1991. Site-specific mate-finding strategies and oviposition behavior in *Crocothemis erythraea* (Brullé) (Odonata: Libellulidae). Journal of Insect Behavior 4:293–303.

Remsburg, A. J., A. C. Olson, and M. J. Samways. 2008. Shade alone reduces adult dragonfly (Odonata: Libellulidae) abundance. Journal of Insect Behavior 21:460–468.

Remsburg, A. J., and M. G. Turner. 2009. Aquatic and terrestrial drivers of dragonfly (Odonata) assemblages within and among north-temperate lakes. Journal of the North American Benthological Society 28:44–56.

Richardson, J. S., Y. Zhang, and L. B. Marczak. 2010. Resource subsidies across the land-freshwater interface and responses in recipient communities. River Research and Applications 66:55–66.

Riechert, S. E., and R. Gillespie. 1986. Habitat choice and utilization in web building spiders. Pages 23–48 Spider webs, Behavior, and Evolution. Stanford University Press.

Riechert, S. E., and K. Lawrence. 1997. Test for predation effects of single versus multiple species of generalist predators: spiders and their insect prey. Entomologia Experimentalis et Applicata 84:147–155.

Rivera, A. C. (ed). 2005. Forests and Dragonflies. Pages 1–300 *in* A. C. Rivera, editor. Fourth WDA International Symposium of Odonatology, Pontevedra (Spain), July 2005. Pensoft Publishers, Sofia-Bulgaria.

Robertson, H. M. 1982. Mating behaviour and its relationship to territoriality in *Platycypha Caligata* (Selys) (Odonata: Chlorocyphidae). Behaviour 79:11–26.

Robin, J. H., and A. Skalli. 2007. Incorporation of dietary fatty acid in European sea bass (*Dicentrarchus labrax*) - a methodological approach evidencing losses of highly unsaturated fatty acids. Aquaculture 263:227–237.

Robinson, M. H., and B. Robinson. 1970. Prey caught by a sample population of the spider *Argiope argentata* (Araneae: Araneidae) in Panama: a year’s census data. Zoological Journal of the Linnean Society 49:345–358.

Rossberg, A. G. 2011. A complete analytic theory for structure and dynamics of populations and communities spanning wide ranges in body size. Advances in Ecological Research 46:429–522.

Rouget, M., R. M. Cowling, A. T. Lombard, A. T. Knight, and G. I. H. Kerley. 2006. Designing large-scale conservation corridors for pattern and process. Conservation Biology 20:549–561.

Roux, S. P., and S. H. H. Oelofse. 2010. The rising costs of both sewage treatment and the production of potable water associated with increasing levels of pollution in a portion of the Crocodile-West Marico water management area (A Case Study). Conference Proceedings, Water Institute of South Arfica. Durban.

Ruess, L., and P. M. Chamberlain. 2010. The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biology and Biochemistry 42:1898–1910.

Ruess, L., K. Schütz, S. Migge-Kleian, M. M. Häggblom, E. Kandeler, and S. Scheu. 2007. Lipid composition of Collembola and their food resources in deciduous forest stands-Implications for feeding strategies. Soil Biology and Biochemistry 39:1990–2000.

Rundio, D. E., and S. T. Lindley. 2012. Reciprocal fluxes of stream and riparian invertebrates in a coastal California basin with Mediterranean climate. Ecological Research 27:539–550.

Rypstra, A. L. 1982. Building a better insect trap; an experimental investigation of prey capture in a variety of spider webs. Oecologia 52:31–36.

Rypstra, A. L. 1983. The importance of food and space in limiting web-spider densities; a test using field enclosures. Oecologia 59:312–316.

Rypstra, A. L. 1986. High prey abundance and a reduction in cannibalism: the first step to sociality in spiders (Arachnida). Journal of Arachnology 14:193–200.

Rypstra, A. L., P. E. Carter, R. A. Balfour, and S. D. Marshall. 1999. Architectural features of agricultural habitats and their impact on the spider inhabitants. Journal of Arachnology 27:371–377.

Sabo, J. L., J. L. Bastow, and M. E. Power. 2002. Length-mass relationships for adult aquatic and terrestrial invertebrates in a California watershed. Journal of the North American Benthological Society 21:336–343.

Sabo, J. L., and M. E. Power. 2002a. Numerical response of lizards to aquatic insects and short-term consequences for terrestrial prey. Ecology 83:3023–3036.

Sabo, J. L., and M. E. Power. 2002b. River – watershed exchange: effects of riverine subsidies on riparian lizards and their terrestrial prey. Ecological Society of America 83:1860–1869.

Samways, M. J. 2008. Dragonflies and damselflies of South Africa. Sofia: Pensoft.

Sanders, D., E. Vogel, and E. Knop. 2015. Individual and species-specific traits explain niche size and functional role in spiders as generalist predators. Journal of Animal Ecology 84:134–142.

Sanzone, D. M., J. L. Meyer, E. Marti, E. P. Gardiner, J. L. Tank, and N. B. Grimm. 2003. Carbon and nitrogen transfer from a desert stream to riparian predators. Oecologia 134:238–50.

Scheibler, E. E., and G. O. Debandi. 2008. Spatial and temporal patterns in the aquatic insect community of a high altitude Andean stream (Mendoza, Argentina). Aquatic Insects 30:145–161.

Scholz, B., and G. Liebezeit. 2013. Biochemical characterisation and fatty acid profiles of 25 benthic marine diatoms isolated from the Solthorn tidal flat (southern North Sea). Journal of Applied Phycology 25:453–465.

Schumann, E. H., R. R. W. Gray, and R. W. Shone. 2001. Tidal flow structures and sedimentation in the lower Kowie estuary, Port Alfred, South Africa. Transactions of the Royal Society of South Africa 56:11–24.

Seifert, L. I., and S. Scheu. 2012. Linking aquatic and terrestrial food webs - Odonata in boreal systems. Freshwater Biology 57:1449–1457.

Seiliez, I., S. Panserat, G. Corraze, S. Kaushik, and P. Bergot. 2003. Cloning and nutritional regulation of a Δ6-desaturase-like enzyme in the marine teleost gilthead seabream (*Sparus aurata*). Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology 135:449–460.

Sherratt, T. N. 2001. The evolution of female-limited polymorphisms in damselflies: a signal detection model. Ecology Letters 4:22–29.

Shimazaki, A., and T. Miyashita. 2005. Variable dependence on detrital and grazing food webs by generalist predators: aerial insects and web spiders. Ecography 28:485–494.

Shin, W.-S., M. Fujibayashi, and O. Nishimura. 2014. Contribution of potential organic matter sources to the macrobenthos nutrition in the Natori estuarine tidal flats, Japan. Russian Journal of Marine Biology 40:396–404.

Siguel, E. N., and R. H. Lerman. 1996. Prevalence of essential fatty acid deficiency in patients with chronic gastrointestinal disorders. Metabolism 45:12–23.

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

Simopoulos, A. P. 2004. Omega-3 fatty acids and antioxidants in edible wild plants. Biological Research 37:263–77.

Smith, B. N., and S. Epstein. 1971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. Plant Physiology 47:380–384.

Smith, H. R., and C. L. Remington. 1996. Food specificity in interspecies competition. BioScience 46:436–447.

Snyder, W. E., and D. H. Wise. 2001. Contrasting trophic cascades generated by a community of generalist predators. Ecology 82:1571–1583.

Speir, S. L., M. M. Chumchal, R. W. Drenner, W. G. Cocke, M. E. Lewis, and H. J. Whitt. 2014. Methyl mercury and stable isotopes of nitrogen reveal that a terrestrial spider has a diet of emergent aquatic insects. Environmental Toxicology and Chemistry 33:2506–9.

Spiller, D. A., J. Piovia-Scorr, A. N. Wright, L. H. Yang, G. Takimoto, T. W. Schoener, and T. Iwata. 2010. Marine subsidies have multiple effects on coastal food webs. Ecology 91:1424–34.

StatSoft Inc. 2011. STATISTICA (data analysis software system).

Stauffer, F., and L. B. Best. 1980. Habitat selection by birds of riparian communities: evaluating effects of habitat alterations. Journal of Wildlife Management 44:1–15.

Sterner, R. W., and K. L. Schulz. 1998. Zooplankton nutrition: recent progress and a reality check. Aquatic Ecology 32:261–279.

Stickler, M. M. 2010. A comparison of ecosystem health and services provided by subtropical thicket in and around the Bathurst Commonage (Masters Thesis). Rhodes University.

Strayer, D. L., M. E. Power, W. F. Fagan, S. T. A. Pickett, and J. Belnap. 2003. A classification of ecological boundaries. BioScience 53:723–729.

Strong, D. R., J. H. Lawton, and S. R. Southwood. 1984. Insects on plants. Community patterns and mechanisms. Science:168–171.

Sukhacheva, G. A. 1996. Study of the natural diet of adult dragonflies using an immunological method. Odonatologica 25:397–403.

Sunderland, K., and F. Samu. 2000. Effects of agricultural diversification on the abundance, distribution, and pest control potential of spiders: a review. Entomologia Experimentalis et Applicata 95:1–13.

Sutherland, W. J., editor. 2006. Ecological census techniques: a handbook. 2nd edition. Cambridge University Press.

Svensson, B. G., and E. Petersson. 1995. Diurnal and seasonal variations in swarming and mating behaviour of the dance fly *Empis borealis* (Diptera;Empididae). Annales Zoologici Fennici 32:403–409.

Swanson, F. J., S. V Gregory, J. R. Sedell, and A. G. Campbell. 1982. Land-Water

Interactions : the Riparian Zone. Pages 267–291 *in* R. L. Edmonds, editor. Analysis of coniferous forest ecosystems in the western United States. Hutchinson Ross Publishers, Stroudsburg.

Sweeney, B. W., T. L. Bott, J. K. Jackson, L. A. Kaplan, J. D. Newbold, L. J. Standley, W. C. Hession, R. J. Horwitz, and G. M. Wolman. 2004. Riparian deforestation, stream narrowing, and loss of stream ecosystem services. Proceedings of the National Academy of Sciences of the United States of America 101:14132–14137.

Sweeney, B. W., and R. L. Vannote. 1982. Population synchrony in mayflies: a predator satiation hypothesis. Evolution 36:810–821.

Szepanski, M. M., M. Ben-David, and V. Van Ballenberghe. 1999. Assessment of anadromous salmon resources in the diet of the Alexander Archipelago wolf using stable isotope analysis. Oecologia 120:327–335.

Tagwireyi, P., and S. M. P. Sullivan. 2015. Distribution and trophic dynamics of riparian tetragnathid spiders in a large river system. Marine and Freshwater Research 67: 309-318.

Taipale, S. J., M. J. Kainz, and M. T. Brett. 2015. A low ω-3:ω-6 ratio in *Daphnia* indicates terrestrial resource utilization and poor nutritional condition. Journal of Plankton Research 37:596–610.

Tang, X. C., and M. J. Pikal. 2004. Design of freeze-drying processes for pharmaceuticals: practical advice. Pharmaceutical Research 21:191 – 200.

Thompson, D. R., S. J. Bury, K. A. Hobson, L. I. Wassenaar, and J. P. Shannon. 2005. Stable isotopes in ecological studies. Oecologia 144:517–9.

Thorne, M. S., Q. D. Skinner, M. A. Smith, J. D. Rodgers, A. Laycock, S. A. Cerekci, and A. William. 2002. Evaluation of a technique for measuring canopy volume of shrubs. Journal of Range Management 55:235–241.

Thorp, J. H., and M. D. Delong. 1994. The riverine productivity model: an heuristic of carbon in large river and organic processing ecosystems concept. Oikos 70:305–308.

Tieszen, L. L., T. W. Boutton, K. G. Tesdahl, and N. A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for δ^{13}C analysis of diet. Oecologia 57:32–37.

Tocher, D. R., M. J. Leaver, and P. A. Hodgson. 1998. Recent advances in the biochemistry and molecular biology of fatty acyl desaturases. Progress in Lipid Research 37:73–117.

Turnbull, A. L. 1973. Ecology of the true spiders (Araneomorphae). Annual Review of Entomology 18:305–348.

Uesugi, A., and M. Murakami. 2007. Do seasonally fluctuating aquatic subsidies influence the distribution pattern of birds between riparian and upland forests? Ecological Research 22:274–281.

Uetz, G. W. 1979. The influence of variation in litter habitats on spider communities. Oecologia 40:29–42.

Uetz, G. W. 1991. Habitat structure and spider foraging. Pages 325–348 *in* S. S. Bell, E. D. McCoy, and H. R. Mushinsky, editors. Habitat structure. Springer Netherlands, Dordrecht.

Uetz, W., J. Halaj, and A. B. Cady. 1999. Guild structure of spiders in major crops. Journal of Arachnology 27:270–280.

Uys, M. C., and J. H. O'Keeffe. 1997. Variability in the invertebrate fauna of an intermittent stream over three consecutive midsummer periods. South African Journal of Aquatic Science 23:55–79.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130–137.

Wallace, J. B., and N. H. Anderson. 1996. Habitat, life history, and behavioral adaptations of aquatic insects. Pages 41–73 *in* R. Merritt and K. Cummins, editors. An introduction to the aquatic insects of North America. 3rd edition. Kendall Hunt Pub Co, Iowa.

Wallace, J. B., S. L. Eggert, J. L. Meyer, and J. R. Webster. 1997. Multiple trophic levels of a forest stream linked to terrestrial litter inputs. Science 277:102–104.

Walters, D. M., K. M. Fritz, and R. R. Otter. 2008. The dark side of subsidies: adult stream insects export organic contaminants to riparian predators. Ecological Society of America 18:1835–1841.

Wang, Y., D. S. Lin, L. Bolewicz, and W. E. Connor. 2006. The predominance of polyunsaturated fatty acids in the butterfly *Morpho peleides* before and after metamorphosis. Journal of Lipid Research 47:530–536.

Warwick, R. M., K. R. Clarke, and Suharsono. 1990. A statistical analysis of coral community responses to the 1982-83 El Nio in the Thousand Islands, Indonesia. Coral Reefs 8:171–179.

Watling, R. J., and H. R. Watling. 1983. Metal surveys in South Africa estuaries VII Bushmans, Kariega, Kowie and Great Fish Rivers. Water SA 9:66–70.

Weissteiner, C. J., F. Bouraoui, and A. Aloe. 2013. Reduction of nitrogen and phosphorus loads to European rivers by riparian buffer zones. Knowledge and Management of Aquatic Ecosystems 408:08.

Welch, K. D., K. F. Haynes, and J. D. Harwood. 2013. Prey-specific foraging tactics in a web-building spider. Agricultural and Forest Entomology 15:375–381.

Welch, K. D., M. R. Schofield, E. G. Chapman, and J. D. Harwood. 2014. Comparing rates of springtail predation by web-building spiders using Bayesian inference. Molecular Ecology 23:3814–3825.

Wenger, S. 1999. A review of the scientific literature on riparian buffer width, extent and vegetation. Georgia.

Wesner, J. S. 2010. Aquatic predation alters a terrestrial prey subsidy. Ecology 91:1435–44.

Whitehouse, M. E. A., and Y. Lubin. 2005. The functions of societies and the evolution of group living: spider societies as a test case. Biological reviews of the Cambridge Philosophical Society 80:347–361.

Whitfield, A. K., A. W. Paterson, A. H. Bok, and H. M. Kok. 1994. A comparison of the ichthyofaunas in two permanently open eastern Cape estuari. Southern African Journal of Zoology 29:175–185.

Whitledge, G. W., and C. F. Rabeni. 1997. Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analysis. Canadian Journal of Fisheries and Aquatic Sciences 54:2555–2563.

Whitmore, C., R. Slotow, T. E. Crouch, and A. S. Dippenaar-Schoeman. 2002. Diversity of Spiders (Araneae) in a Savanna Reserve, Northern Province, South Africa. Journal of Arachnology 30:344–356.

Willett, T. R. 2001. Spiders and other arthropods as indicators in old-growth versus logged redwood stands. Restoration Ecology 9:410–420.

Williams, D. D., L. G. Ambrose, and L. N. Browning. 1995. Trophic dynamics of two sympatric species of riparian spider (Araneae: Tetragnathidae). Canadian Journal of Zoology 73:1545–1553.

Williams, D. S. 1979. The feeding behaviour of New Zealand *Dolomedes* species (Araneae: Pisauridae). New Zealand Journal of Zoology 6:95–105.

Wise, D. H. 1979. Effects of an experimental increase in prey abundance upon the reproductive rates of two orb-weaving spider species (Araneae: Araneidae). Oecologia 41:289–300.

Wise, D. H. 1995. Spiders in ecological webs. Cambridge University Press, Cambridge.

Wolff, R. L., O. Lavialle, F. Pédrono, E. Pasquier, L. G. Deluc, A. M. Marpeau, and K. Aitzetmüller. 2001. Fatty acid composition of Pinaceae as taxonomic markers. Lipids 36:439–51.

Womeni, H. M., M. Linder, B. Tiencheu, F. T. Mbiapo, P. Villeneuve, J. Fanni, and M. Parmentier. 2009. Oils of insects and larvae consumed in Africa: potential sources of polyunsaturated fatty acids. OCL - Oleagineux Corps Gras Lipides 16:230–235.

Yang, L. F., S. Siriamornpun, and D. Li. 2006. Polyunsaturated fatty acid content of edible insects in Thailand. Journal of Food Lipids 13:277–285.

Yoshida, M. 1989. Predatory behavior of three Japanese species of *Metleucauge* (Araneae, Tetragnathidae). Journal of Arachnology 17:15–25.

Yoshimoto, C. M., and J. L. Gressitt. 1964. Dispersal studies on Aphididae, Agromyzidae and Cynipoidea. Pacific Insects 6:525–531.

Young, O. P., and G. B. Edwards. 1990. Spiders in United States field crops and their potential effect on crop pests. Journal of Arachnology 18:1–27.

Zacharia, P. U., and K. P. Abdurahiman. 2004. Methods of stomach content analysis of fishes. Pages 148–200 Winter School on Towards Ecosystem Based Management of Marine Fisheries. Cochin, Kerala.

Vander Zanden, M. J., and J. B. Rasmussen. 2001. Variation in δ^{15}N and δ^{13}C trophic fractionation: implications for aquatic food web studies. Limnology and Oceanography 46:2061–2066.

Zanden, M., and J. Rasmussen. 1999. Primary consumer δ^{13}C and δ^{15}N and the trophic position of aquatic consumers. Ecology 80:1395–1404.

Zietsman, H. L., and I. J. Van Der Merwe. 1981. Economic atlas of South Africa (University of Stellenbosh, Institute for cartographic analysis). Stellenbosch.

Zuur, A., E. N. Ieno, N. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer Science and Business Media, California.

APPENDICES

Appendix 1: Total biomass of spider taxonomic groups (families and genera) collected from different distances from the narrow section of the Kowie River, during different seasons. At each distance category, value of contribution by biomass was estimated for each taxonomic group.

Season	Distance from the river (m)	Family	Relative biomass (mg)	Contribution by biomass (%)
Summer	0	*Tetragnatha* Uloboridae	61.9 0.2	99.7 0.4
	2	Araneidae Deinopidae	53.1 13.4	79.8 20.2
	4	Araneidae	5.8	100.0
	8	*Tetragnatha* Araneidae	2.0 10.6	15.9 84.1
	16	*Tetragnatha* Araneidae	5.7 21.8	20.6 79.4
	64	*Tetragnatha* Araneidae	7.4 25.4	22.6 77.4
Autumn	0	Araneidae Linyphiidae Nephilidae *Tetragnatha*	305.2 0.2 439.3 8.7	40.5 0.0 58.3 1.2
	2	Araneidae	461.9	100.0
	4	Araneidae Linyphiidae	5.3 0.3	95.5 4.5
	8	Nephilidae	767.0	100.0
	16	Araneidae Linyphiidae Nephilidae	233.5 0.1 565.2	29.2 0.0 70.8
	32	Linyphiidae Nephilidae	0.2 235.5	0.1 99.9
	64	Araneidae	317.3	100.0
Winter	0	Araneidae Deinopidae *Tetragnatha* Uloboridae	8.9 1.8 2.9 2.9	54.1 10.8 17.5 17.6
	2	Araneidae Deinopidae	5.3 1.2	81.0 19.0
	8	Araneidae Deinopidae	11.8 18.2	39.4 60.6

Season	**Distance from the river (m)**	**Family**	**Relative biomass (mg)**	**Contribution by biomass (%)**
Spring	0	Araneidae	9.2	33.4
		Tetragnathidae	15.8	57.2
		Uloboridae	2.6	9.4
	2	Araneidae	16.1	89.3
		Uloboridae	1.9	10.7
	4	Araneidae	1.0	65.8
Spring	4	Uloboridae	0.5	34.2
	8	Araneidae	9.7	78.3
		Uloboridae	2.7	21.7
	16	Araneidae	1.7	43.9
		Uloboridae	2.2	56.1
	32	Araneidae	3.3	58.5
		Tetragnathidae	1.9	33.7
		Uloboridae	0.4	7.8
	64	Araneidae	11.7	89.2
		Uloboridae	1.4	10.8

Appendix 2: Total biomass of spider taxonomic groups (families and genera) collected from different distances from the wide section of the Kowie River, during different seasons. At each distance category, value of contribution by biomass was estimated for each taxonomic group.

Season	Distance from the river (m)	Family/ genera	Relative biomass (mg)	Contribution by biomass (%)
Summer	0	*Tetragnatha*	52.1	100.0
	2	*Tetragnatha*	2.0	7.1
		Araneidae	26.0	92.2
		Linyphiidae	0.2	0.7
	4	Araneidae	48.3	100.0
	16	*Tetragnatha*	3.1	100.0
	64	Araneidae	71.7	100.0
Autumn	0	*Tetragnatha*	70.4	100.0
	2	*Tetragnatha*	0.3	1.4
		Araneidae	19.9	98.6
	8	Araneidae	282.3	99.8
		Theridiidae	0.7	0.2
	64	Araneidae	27.8	100.0
Season	**Distance from the river (m)**	**Family**	**Relative biomass (mg)**	**Contribution by biomass (%)**
Winter	0	*Tetragnatha*	24.5	100.0
	2	*Tetragnatha*	1.9	21.3
		Araneidae	6.8	77.2
		Linyphiidae	0.1	1.5
	4	Araneidae	5.5	100.0
	8	*Tetragnatha*	3.7	18.1
		Araneidae	16.6	81.9
	16	*Tetragnatha*	3.2	22.3
		Araneidae	2.0	14.0
		Deinopidae	9.1	63.6
	32	Araneidae	1.5	30.9
		Deinopidae	1.0	19.9
		Theridiidae	1.4	28.1
		Uloboridae	1.1	21.1
	64	Araneidae	21.1	100.0
Spring	0	Araneidae	0.5	0.9
		Tetragnatha	56.9	99.1
Spring	2	Araneidae	4.2	26.1
		Tetragnatha	12.0	73.9
	4	Araneidae	7.4	89.2
		Uloboridae	0.9	10.8

Season	**Distance from the river (m)**	**Family/ genera**	**Relative biomass (mg)**	**Contribution by biomass (%)**
	8	Araneidae	21.1	94.8
		Tetragnatha	0.7	3.0
		Uloboridae	0.5	2.1
		Linyphiidae	0.0	0.1
		Theridiidae	0.0	0.0
	16	Araneidae	6.7	61.3
		Uloboridae	2.2	20.3
		Linyphiidae	1.9	17.5
		Theridiidae	0.1	0.9
	32	Araneidae	13.8	96.4
		Theridiidae	0.5	3.6
	64	Linyphiidae	0.1	33.3
		Theridiidae	0.1	66.7

Appendix 3: Total biomass of spider taxonomic groups (families and genera) collected from different distances from the intermediate site of the Kowie River, during different seasons. At each distance category, value of contribution by biomass was estimated for each taxonomic group.

Season	**Distance from the river (m)**	**Family**	**Relative biomass (mg)**	**Contribution by biomass (%)**
Summer	0	Araneidae *Tetragnatha*	87.1 211.2	29.2 70.8
	2	Araneidae Uloboridae	134.4 3.0	97.8 2.2
	4	Araneidae *Tetragnatha* Uloboridae	134.1 86.2 3.0	60.1 38.6 1.3
	8	Araneidae	644.5	92.0
		Tetragnatha	55.8	8.0
	16	Araneidae *Tetragnatha*	922.1 11.7	98.7 1.3
	32	Araneidae *Tetragnatha* Uloboridae	953.3 37.5 1.5	96.1 3.8 0.2
	64	Araneidae *Tetragnatha* Theridiidae	137.9 6.7 2.9	93.5 4.5 1.9
Autumn	0	Nephilidae *Tetragnatha* Theridiidae	1037.3 34.4 10.0	95.9 3.2 0.9
	2	Nephilidae	739.1	99.7
	4	*Tetragnatha* Araneidae	0.8 1.5	0.1 0.2
Autumn	4	Theridiidae	10.5	100.0
	8	Nephilidae Theridiidae	729.9 0.9	99.9 0.1
	16	Nephilidae Theridiidae Araneidae	535.1 2.0 1.1	99.4 0.4 0.2
	32	Nephilidae Theridiidae Araneidae Linyphiidae	1491.4 2.8 34.6 1.1	97.5 0.2 2.3 0.1
	64	Nephilidae Araneidae	243.7 406.3	37.5 62.5

Season	**Distance from the river (m)**	**Family**	**Relative biomass (mg)**	**Contribution by biomass (%)**
Spring	0	Araneidae	2.9	60.3
		Linyphiidae	0.3	6.2
		Tetragnatha	1.6	33.5
	2	Araneidae	3.9	53.2
		Tetragnatha	3.0	41.8
		Uloboridae	0.4	5.1
	4	*Tetragnatha*	3.3	100.0
	8	Araneidae	2.3	55.2
		Tetragnatha	1.8	44.8
	16	Araneidae	1.2	46.6
		Tetragnatha	1.3	53.4
	32	Araneidae	1.7	100.0
	64	Araneidae	1.0	100.0

Appendix 4: Relative contribution by aquatic food sources to the diets of selected (the most common) web-building spider genera inhabiting the riparian areas of three sites in the Kowie River catchment during different seasons. The letter x represents distances where the respective spider genera were not found.

	Distance from the river (m)																				
	Narrow Site							**Wide Site**							**Intermediate Site**						
Spider Genera	**0**	**2**	**4**	**8**	**16**	**32**	**64**	**0**	**2**	**4**	**8**	**16**	**32**	**64**	**0**	**2**	**4**	**8**	**16**	**32**	**64**
Summer																					
Araneus	x	x	0.4	0.3	0.3	0.4	0.3	x	x	x	x	x	0.4	0.2	0.7	x	0.5	x	x	x	0.3
Caerostris	x	0.5	x	x	x	x	0.3	x	x	0.3	x	x	x	0.2	0.6	x	0.4	0.4	0.3	0.3	x
Leucauge	0.6	x	0.3	0.2	0.3	x	0.4	0.6	x	x	x	0.2	x	x	x	x	x	0.4	0.4	0.4	0.4
Tetragnatha	0.3	x	x	x	x	x	x	0.7	0.7	x	x	x	x	x	0.8	x	x	0.4	x	x	x
Autumn																					
Araneus	x	x	x	x	x	x	0.6	x	0.3	x	x	0.4	x	x	x	0.3	x	x	0.4	x	x
Caerostris	0.6	0.5	x	x	0.7	x	0.6	x	x	0.4	x	x	x	x	x	x	x	x	x	x	x
Leucauge	x	x	x	x	x	x	x	x	0.5	x	x	x	x	x	0.4	x	x	x	x	x	x
Tetragnatha	0.6	x	x	x	x	x	x	0.8	x	x	x	x	x	x	0.5	0.4	x	x	x	x	x
Winter																					
Araneus	0.3	0.3	x	0.3	x	x	x	x	x	x	x	0.4	0.3	x	x	0.4	x	x	0.2	x	x
Caerostris	x	0.3	x	x	x	x	x	x	x	x	0.3	x	x	0.3	0.5	0.4	x	0.3	x	x	x
Tetragnatha	0.3	x	x	x	x	x	x	0.7	0.6	x	0.5	0.5	x	x	0.7	0.7	0.6	0.5	x	x	x
Spring																					
Araneus	0.6	0.5	x	x	x	x	0.5	x	0.3	x	x	0.3	0.2	x	x	x	x	x	x	x	x
Caerostris	0.4	0.5	0.5	0.3	0.3	x	x	x	0.3	0.1	0.3	x	0.2	x	x	x	x	x	x	x	x
Leucauge	x	x	x	x	x	0.2	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Neoscona	x	x	x	0.4	x	x	x	x	x	0.3	0.3	0.2	x	x	x	x	x	x	x	x	x
Tetragnatha	0.7	x	x	x	x	x	x	0.5	0.4	x	0.3	x	x	x	x	x	x	x	x	x	x