AQUATIC INSECTS AS AN ENERGETIC SUBSIDY TO RIPARIAN CONSUMERS IN THE OLDMAN RIVER BASIN, ALBERTA

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ABSTRACT

Freshwater and terrestrial ecosystems are connected through biomass exchanges such as the flow of predators, prey, nutrients and detritus between them. Emerging aquatic insects provide an alternate food source to riparian consumers, often termed an allochthonous subsidy. Stable isotope analyses of naturally occurring carbon and nitrogen is effective for tracing energetic food sources to consumers. This thesis evaluated whether stable isotope analysis would be effective in the Oldman River Basin, Alberta. Aquatic and riparian primary consumers are distinct in their isotopic signatures and valid for to use in stable isotope analysis. Stable isotope modelling was then used to evaluate the proportional contribution of aquatic insects to riparian spider and beetle diets. Carbon analysis showed an overall aquatic insect contribution of 25 % and 18 % for spiders and beetles, respectively; while nitrogen analysis showed an overall contribution of 36 % and 20 %, respectively. The spatial extent of the aquatic insect contribution upland from the river was shown to decrease from 50 – 55 % at 1 m to 0 % at 30 m for both consumers using carbon, and from 35 – 40 % at 1 m to 0 % at 40 m using nitrogen. Finally, regression modelling of the size of a river and the spatial scale of an aquatic insect dietary contribution showed a significant relationship, indicating larger water bodies support higher production of aquatic insects. A meta-analysis of published literature applied to this model also indicated a significant relationship between the size of the river and the extent of an aquatic insect contribution.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BS: Blazingstar, also known as Pearce Corner

CR: Castle River

CTW: Cottonwood

OMRB: Oldman River Basin

OMR: Oldman River

PA: Pavan

PC: Pincher Creek

PDB: Vienna Pee Dee Belemnite

STMR: St. Mary River

SV: Summerview

WWTP: Waste Water Treatment Plant

CHAPTER ONE

General introduction

1.1.1 The energetic link between freshwater and riparian ecosystems

Freshwater and riparian ecosystems are seemingly demarcated, and are viewed as distinct habitats. However, these habitats are connected by biomass exchanges occurring among them such as the movement of predators, prey, nutrients and detritus. However, quantifying such interactions is challenging, especially as they are seldom directly observable.

Stable isotope analyses of naturally occurring carbon (^{13}C) and nitrogen (^{15}N) have proven effective in the quantification of energetic movement of aquatic resources (Collier et al. 2002, Baxter et al. 2005, Milligan and Humphries 2010). As such, the objectives of the present study were to evaluate the effectiveness of stable isotope analyses in determining the diet of higher trophic level riparian consumers in the Oldman River Basin, Alberta (OMRB) (Figure 1.1); and secondly, to use stable isotope analyses to test the spatial scale at which emerging aquatic insects contributed to the diet of riparian consumers. Further, the relationship between the size of the river and the spatial scale of aquatic insect subsidy was investigated.
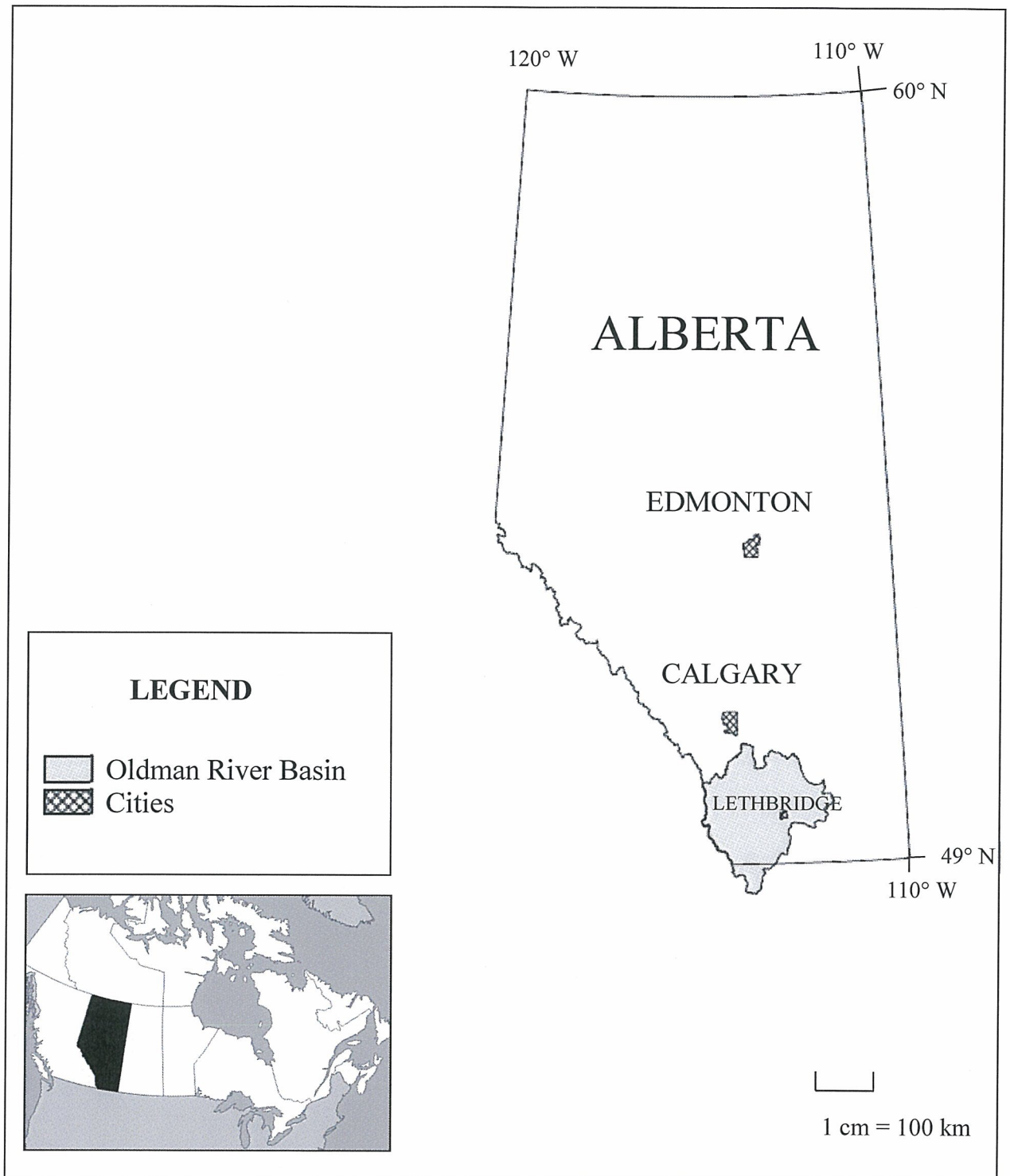
Figure 1.1: The location of the Oldman River Basin in Alberta. Inset: the location of Alberta within Canada.

1.1.2 Food-web dynamics

Freshwater and riparian ecosystems represent diverse physical environments that may seem to be functionally distinct with separate food webs. However, these food webs are linked by naturally occurring abiotic properties, and the diverse array of organisms that complete their life-cycles between them. Elton (1927) defined a food web as a complex society of feeding interactions between species in a community. The identification of these interactions provides a snapshot of the overall community dynamic by revealing the associations between consumers and resources (Elton 1927).

In order to simplify the food web, ecologists organize species into a position in the food web based on their feeding mode, referred to as a trophic level (Carpenter et al. 1985, Vander Zanden and Rasmussen 1999). Autotrophic organisms synthesize complete chemical compounds using light or chemical energy sources, and simple inorganic nutrients. On the other hand, heterotrophic organisms consume organic forms of carbon such as plant or animal tissue. Autotrophs or producers such as plants and algae form the first trophic level of a food web, followed by consumer organisms, including species that feed on decomposing material or detritus, non-living organic materials (Pimm et al. 1991, Campbell and Reece 2008). This trophic-dynamic viewpoint emphasizes a relationship between the biological components of an ecosystem with factors of landscape ecology such as the cycling of organic and inorganic compounds in order to establish a holistic depiction of the food web (Lindeman 1942).

However, the trophic-dynamic viewpoint, along with other early theories (Lindeman 1942, Hairston et al. 1960), failed to capture the complexity of the connections in an ecosystem as it was only a small representation of the full system. For instance, mixotrophic species such as carnivorous plants are not strictly autotrophic or heterotrophic as they obtain organic energy by both mechanisms of feeding. Further, generalist feeders such as omnivores consume both primary producers and higher-level consumers' and are especially important in large ecosystems (Thompson et al. 2007). The efficiency of energy transfer among trophic levels is not constant and varies between ecosystems (e.g., tropical forest vs. open ocean). Multi-trophic-level connections between species are diverse and simple linear food web models are unrealistic to define these community dynamics (Phillipson 1966).

Food webs can be conceptualized as open systems as they are not static and continuously interact with the surrounding community. Energy, in the form of living or dead biomass, is exchanged by predator - prey interactions across numerous food web links. Therefore, the food web is not necessarily a complete view of community structure as stated by Polis and Strong (1996). Spatial characteristics also influence the movement of materials across ecosystems influencing abiotic and biotic processes. The incorporation of spatial relationships between habitats with elements of food web dynamics improves the understanding of ecological processes (Polis et al. 1997, Marczak et al. 2007a).

1.1.3 Non-traditional energetic exchanges

Energetic and nutrient flows often cross into other physical habitats. Energy flow can be viewed in terms of a production gradient, energetic movement to areas of low

productivity from high productivity which assists in the stabilization of the food web (Huxel and McCann 1998, Marczak and Richardson 2007). However, this does not account for general food web exchange that naturally occurs in adjacent habitats. The contribution of nutrients and energy between environments is often referred to as an energetic subsidy (Polis and Strong 1996, Polis et al. 1997). This movement of energy across habitats establishes an additional accessible food source to the diet of consumers allowing them to maintain high population numbers although *in situ* resources may be depressed (Polis and Strong 1996, Henschel et al. 2001, Rip and McCann 2011).

According to Polis and Hurd (1996), energetic subsidies are significant and well recognized across ecotones of aquatic habitats such as littoral zones of lakes or riparian areas adjacent to freshwater streams and rivers. Recognizing the importance of these energetic contributions, the current study aims to enhance knowledge on aquatic energy subsidies to riparian consumers and to quantify their spatial scale.

In lotic (flowing) freshwater systems, such as streams and rivers, numerous studies have demonstrated that the addition of organic sediments and riparian detritus, known as an allochthonous subsidy, into the upper head-waters of an aquatic system significantly enhances secondary productivity (Mason and Macdonald 1982, Polis and Hurd 1996, France 1997, Nakano et al. 1999, Baxter et al. 2004, Bergfur et al. 2009). Further, as Doi (2009) asserted, allochthonous subsidies were particularly prevalent in headwater streams as *in situ* primary production is low due to shading from extensive canopy cover.

Early research by Petersen and Cummins (1974) showed that organic matter in the form of leaf detritus influenced the aquatic invertebrate community suggesting selective

colonization and feeding during optimal times such as autumn when foraging options were abundant. Many other examples of allochthonous subsidies have been expressed including the input of leaf litter into soil, and the movement of riparian insects into the aquatic habitat creating an alternative food source for fish (Baxter et al. 2005, Francis and Schindler 2009, Cole et al. 2011).

Alternatively, larger downstream rivers have increased primary production and less reliance on allochthonous subsidies as expressed by the river continuum concept (Vannote et al. 1980, Doi 2009, Rasmussen 2010). Conversely, deposition of algae and aquatic detritus has been shown to influence riparian herbivores in the adjacent riparian ecosystem (Bastow et al. 2002). Interconnections between riparian and freshwater food webs have been shown to occur at all trophic levels and thus, these food webs are not always distinct (Cederholm et al. 1999, Bastow et al. 2002, Baxter et al. 2005, Fukui et al. 2006).

1.1.4 Material input from aquatic habitats

While the contribution of energetic biomass from riparian to aquatic habitats is well known, the reverse input is less well understood. Aquatic ecosystems are often highly productive habitats due to their high growth rates for primary producers, great herbivory and higher densities of organisms (Cyr and Pace 1993, Rip and McCann 2011). It was initially assumed that material transfer from the land to the water was the dominant process (Power et al. 2004); however, it has been demonstrated that material input from the aquatic ecosystem is also important to the riparian ecosystem which may substantially

benefit from energetic input (Polis et al. 1997, Nakano and Murakami 2001, Iwata et al. 2003, Marczak and Richardson 2007).

Subsidies were originally recognized by researchers attempting to quantify the enrichment to forested ecosystems after major spawning runs of semelparous salmon that perish immediately after spawning (Kline et al. 1997). Salmon-derived nutrients enter the food web through uptake by primary producers, direct consumption of eggs, or the decomposition of carcasses (Cederholm et al. 1999). Francis et al. (2006) determined that emerging aquatic insects from streams with pacific salmon were enriched in nitrogen by 21 - 31 % compared with non-salmon streams, which manifested as an energetic subsidy to riparian consumers and acted as a link between the habitats.

There are numerous mechanisms for the transfer of aquatic energy to the riparian ecosystem aside from salmon-derived nutrients incorporated by bears and other biological vectors (Kline et al. 1997).

For example, beached detritus and algae is a major food source for riparian invertebrates such as beetles and grasshoppers, and represent a secondary pathway into the riparian food web (Bastow et al. 2002). More recently, it has been shown that subsidies of emergent aquatic insects from freshwater habitats support a variety of riparian consumers such as insectivorous birds (Murakami and Nakano 2002, Iwata et al. 2003), spiders (Collier et al. 2002, Kato et al. 2003, Kato et al. 2004, Briers et al. 2005, Marczak and Richardson 2007), beetles (Henschel 2004), bats (Fukui et al. 2006) as well as lizards (Sabo and Power 2002).

1.1.5 Aquatic insects as an energetic subsidy

The transport of energy via emerging aquatic insects enhances productivity and diversity of streamside consumers (Akamatsu et al. 2004, Baxter et al. 2005). Aquatic insects emerge from streams or rivers to complete their life-cycles, which may include completion of metamorphosis, swarming, feeding or mating (Collier and Smith 1997). The aquatic insects that emerge can be very numerous and represent a significant proportion of benthic production from the lotic habitat (Baxter et al. 2005). These adult insects are consumed by riparian insectivores, and as few as 3% actually return to the stream to oviposit (Iwata et al. 2003). In warmer climates aquatic insects develop into adults more quickly and therefore, the net flux is often greater than in cooler climates (Jackson and Fisher 1986).

In temperate zones, the emergence of aquatic insects varies seasonally, and thus the amount of energy transferred between ecosystems is seasonally dependent and is often asynchronous with riparian insect population peaks (Nakano and Murakami 2001, Kato et al. 2003, Marczak and Richardson 2008). It has been shown that fluxes of emergent aquatic insects coincide with periods of consumer breeding, provisioning, and migration stop-over periods (MacKenzie and Kaster 2004, Fukui et al. 2006). Aquatic insect emergence is high during the spring and early summer due to high in-stream productivity, and this subsides in the late summer during high riparian insect abundance, and peaks again in early fall when eggs have reached their peak incubation time. These large fluxes of aquatic insects are often overlooked as they occur quickly throughout the summer requiring researchers to sample the area frequently (Baxter et al. 2005). After their

emergence, aquatic insects move varying distances into the riparian zone in order to continue their life-cycles.

Aquatic insects provide an alternative energy source when other food sources, such as riparian insects, are less abundant. Even when riparian insects are abundant, consumers may preferentially feed on aquatic insects due to their high nutritional content as a result of high abundances of unsaturated fatty acids, required by zooplankton during rapid growth, moving through the food web (Perhar et al. 2012). Nakano and Murakami (2001) found that 50 - 90 % of a riparian bird's diet was aquatically derived in autumn when riparian insects were not readily available. Additionally, they found that 25.6 % of the annual diet for riparian birds was aquatically derived which suggested reliance on aquatic insects despite other food sources being available.

Similarly, riparian spiders have been found to have varying predation strategies specifically tailored to the seasonal influx of emerging aquatic insects and they contributed to over 50 % of a spider's annual diet in a riparian area (Kato et al. 2003, Baxter et al. 2005). Studies on both web-weaving and ground-dwelling spiders have shown that aquatically derived carbon and nitrogen constitute 48 - 100 % and 25 - 39 % of the consumer's diet, respectively (Baxter et al. 2005).

Predators adjacent to a water source are provided with an additional food source during aquatic insect emergence; however, the extent of this subsidy decreases with distance from the stream or river (Francis et al. 2006). Studies quantifying dispersal activity of

emerging aquatic insects are inconsistent in quantifying the decrease of aquatic insect dispersal.

A study by Briers et al. (2005), demonstrated that energetic subsidies to Lycosid spiders (wolf spiders) by aquatic insects were restricted to within 10 m of the stream. However, other studies found the spatial scale of aquatic subsidies to be variable leading researchers to conclude that the scale of subsidies differed among aquatic insect taxa and with the geographical characteristics of the habitat. Ground-dwelling and web-weaving spiders in New Zealand have been shown to be subsidized within 1 - 3 m from a stream (Collier et al. 2002), while others have noted that subsidization can occur up to 25 m from the stream edge from streams that are approximately 2 - 10 m in width (Iwata et al. 2003, Baxter et al. 2005).

The dispersal of aquatic insects following emergence occurs through either active or passive mechanisms, such as flying or being swept by wind, respectively. While the abundance and seasonality of aquatic insect emergence has been well studied, few studies have quantified the spatial aspects of aquatic insect emergence (Baxter et al. 2005). Dispersal rates and direction have been linked to several factors including post-mating behaviour, environmental conditions, habitat features, feeding activities and circadian rhythms (Collier and Smith 1997).

Dispersal by emerging aquatic insects and the subsequent penetration into upland habitat depends on the taxa and traits of aquatic insects (Jackson and Resh 1989, Power et al. 2004, Baxter et al. 2005). For example, an entire generation of adult mayflies will emerge and aerially mate within a short time period. These insects are spatially restricted

unless passively dispersed as they have been shown to swarm and aerially copulate above the surface of the water (Ward 1992). On the other hand, adult stoneflies have been observed to crawl or fly a substantial distance to mate after emergence (Clifford 1991, Wesner 2010). Behavioural differences in feeding and mating strategies influence insect taxa dispersal patterns (Wesner 2010). Aquatic insect taxa have variable dispersal traits and the taxa of emerging aquatic insects trapped at canopy-level have been shown to disperse upland further than those caught at stream height (Jackson and Resh 1989).

Landscape characteristics and variable geographic patterns have been suggested to influence aquatic insect dispersal (Kovats et al. 1996, Collier and Smith 1997). For example, emergence of aquatic insects from river pools is 4.5 times greater than that from riffles due to greater cover from predators, thus increasing the success of upland movement by aquatic insects (Iwata et al. 2003). Vegetative patches which provide shelter and swarming sites from unpredictable weather conditions and predation pressures are favoured habitats for aquatic insects, especially those that complete metamorphosis on land before mating (Ward 1992). A decline in the vegetative patchiness and cover with distance from the stream edge should therefore coincide with a reduction in aquatic insect abundance into upland habitats, unless facilitated by strong winds (Kovats et al. 1996).

Aquatic insect emergence has been shown to be greater in tributaries with high primary production in the channel from factors such as rich inorganic sedimentary layers, and high accessibility of nutrients. Large water bodies support higher *in situ* production due to their size and catchment, which fuels aquatic insect reproductive success (Kato et al. 2003, Henschel 2004). Baxter (2005) asserts that aquatic insect contributions will vary

11

with longitudinal gradients due, in part, to changes in river width and aspects of the aquatic-riparian interface. As per the River Continuum Concept, both Baxter (2005) and Power and Rainey (2000) predicted that emergence and lateral dispersal of adult aquatic insects will change predictably from headwaters to downstream runs as terrestrial detrital inputs lessens and channel widths increase supporting abundant algal growth.

A study by Gratton and Vander Zanden (2009) showed that average-sized lakes (10 ha) have estimated aquatic insect deposition rates to the riparian environment similar to deserts or low productivity ecosystems (~ 0.07 g C m^{-2} yr^{-1}) while large lakes (1300 ha) and streams (16 m width) have aquatic insect deposition rates similar to productive riparian ecosystems such as grasslands ($\sim$0.01-2.40 g C m^{-2} yr^{-1}). The relationship between river width and export distance by emerging aquatic insects has been previously considered, although empirical evidence on this subject is lacking (Henschel 2004, Gratton and Vander Zanden 2009).

Table 1.1 and Table 1.2 provide a summary of literature highlighting the importance of emerging aquatic insects to the diets of riparian consumers.

Table 1.1: Literature review collective of data showing the dietary importance of aquatic insects to riparian consumers in percent (%) from stable isotope analysis.

Location	Consumer	Dietary % of Aquatic Insects & Distance from Stream		Average Stream Width	Reference
		Nitrogen	Carbon		
River Severn, UK				3.0-4.0 m	Briers et al 2005
	Lycosid	40 at 1.0 m	*		
		< 1 at 20.0 m	*		
Waitekauri River & Wairere stream, New Zealand				6.0-8.0 m	Collier et al 2002
	Lycosid	58[‡] at 1.0 - 3.0 m	55 at 1.0 - 3.0 m		
	Tetragnathidae & Araneidae	58[‡] at 1.0 - 3.0 m	61 at 1.0 - 3.0 m		
Sycamore Creek, U.S.A				4.8 m	Sanzone et al 2003
	Lycosidae & Gnaphosidae	4 - 96 at 0.0 - 50.0 m	68 at 0.0 - 50.0 m		
	Agelenidae & some Gnaphosidae	3 - 10 at 0.0 - 50.0 m	37at 0.0 - 50.0 m		
	Araneidae & Tetragnathidae	5 - 68 at 0.0 - 50.0 m	100 at 0.0 - 50.0 m		
	Linyphiidae	7 - 14 at 0.0 - 50.0 m	69 at 0.0 - 50.0 m		
	Salticidae & Thomisidae	7- 24 at 0.0 - 50.0 m	73 at 0.0 - 50.0 m		
Tagliamento River, Italy				20.0 m	Paetzold et al 2005
	Lycosidae & Carabidae, Staphylinidae	*	48 - 100 at 1.0 m		

* Input was not measured.
‡ All species for all sites combined.

Table 1.2: Literature review collective of data showing the dietary importance of aquatic insects to riparian consumers in biomass (%).

Location	Consumer	Dietary Importance from Biomass (%)	Average Stream Width	Reference
Horonai stream, Japan	10 Passerine Bird Species	25.6 % of overall energy budget in 20m^2 grids within 40 m	2.0 - 5.0 m	Nakano & Murakami 2001
Wurzburg, Germany				Henschel et al 2001
	Opiliones & Araneae	53.6 % at 1.0 m	70.0 m	
		24.2 % at 30.0 m		
		27.1 % at 1.0 m		
		16.9 % at 30.0 m		
Tomakomai, Japan	Forest Birds	82.3 % at 0.0 - 5.0m	2.2 - 7.7 m	Iwata et al 2003
		19.2 % at 0.0 - 30.0 m		
		66.7 % at 0.0 - 5.0m		

14

Riparian zones are defined as the interface between the aquatic and riparian habitat, that are important ecological areas as they support diverse communities of invertebrate and vertebrate consumers (Iwata et al. 2003). Riparian areas are common roosting sites for bats and temporary shelters for migratory or breeding birds, as well as host to diverse invertebrate communities. The emergence and dispersal of aquatic insects into riparian habitats influences the feeding strategies and diets of local predators, especially during large flux periods (Baxter et al. 2005). Highly mobile predators are known to link habitats over a larger spatial scale than those that are less mobile, thereby acting as a vector for the energy transfer (McCann et al. 2005).

Quantifying the diet of riparian consumers is a useful approach for tracking the extent of aquatic subsidies into upland habitats. The limited spatial range and opportunistic feeding strategies of ground beetles restricts the contribution of aquatic subsidies to short distances inland near the water source. On the other hand, spiders have larger spatial ranges; as such aquatic nutrients may be identifiable in upland habitats. Furthermore, using the same approach on variable sized rivers, the relationship between river width and the spatial dispersal of aquatic insects into upland habitats can be evaluated.

1.2.1 Stable isotopes in ecological food web studies

Stable isotope analysis is a useful technique employed in ecological studies, due to its precision of measuring natural elements such as carbon, nitrogen, or oxygen. (Peterson and Fry 1987, Phillips 2001). Isotopes of an element have the same number of protons but a different number of neutrons in their nucleus. The difference in neutrons does not change the chemical reactivity of the element but, results in subtle differences in mass

leading to isotope splitting by natural processes, referred to as fractionation (Peterson and Fry 1987).

Carbon and nitrogen stable isotopes are often used to determine diet sources and the trophic level of an animal, rendering them important for ecological studies (Vander Zanden and Rasmussen 2001, Briers et al. 2005, Darling and Bayne 2010, Cole et al. 2011). Carbon and nitrogen have multiple stable isotopes and can be studied by comparing isotope ratios in samples of different materials such as plant or animal tissue. The natural abundance of stable isotopes is approximately 98.9 to 1.1 for carbon-12, and carbon-13, and 99.6 to 0.4 for nitrogen-14 and nitrogen 15, respectively (Peterson and Fry 1987).

Stable isotopes ratios are expressed as δ with the units per mil (‰) or parts per thousand (Rounick and Winterbourn 1986, Peterson and Fry 1987) using the following equation:

$$X = \left[\left(\frac{Rsample}{Rstandard}\right) - 1\right] * 1000 \qquad Equation\ 1.1$$

where X is $\delta^{13}C$ or $\delta^{15}N$ and the ratio of heavy to light isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) is represented by R (Peterson and Fry 1987). Carbon isotopic ratios within tissue are compared to Vienna PeeDee belemnite limestone (PDB), an international limestone standard; while nitrogen isotope ratios are compared to the international standard of atmospheric nitrogen gas (N_2) (Rounick and Winterbourn 1986). Equation 1.1 is also used for other stable isotopes such as hydrogen and oxygen. Stable isotope researchers make use of the δ notation (parts per thousand) to magnify the difference between the

samples and standards as they are frequently different by only a fraction of a percent (Rasmussen, personal communication).

Nitrogen and carbon stable isotopes have been used for modeling bioaccumulation in fish species (Cabana and Rasmussen 1994), tracking movement and migration of animals (Rubenstein and Hobson 2004) and, more prominently, in food web investigations, where diet compositions (Branstrator et al. 2000), spatial energy movement (Briers et al. 2005) and general food web systems have been studied (Cabana and Rasmussen 1996, Post 2002).

The ability to trace nutrients is possible because of the difference of isotopic signatures of sources from the base of the food web in an animal's diet. For example, plants perform varying types of photosynthesis depending on their habitat, and this creates differing $\delta^{13}C$ signatures that can be traced through the food web (Oleary 1981, Keeley and Sandquist 1992). Photosynthesizing riparian producers have distinct metabolic pathways that have allowed discrimination into separate groups including C_3, C_4 and crassulacean acid metabolism (CAM). These plants have different isotopic signatures based on the way they incorporate atmospheric carbon (CO_2), the carbon source for riparian producers during photosynthesis, for plant metabolism (Rounick and Winterbourn 1986).

Plants that fix carbon by C_3 fixation are the most dominant on the landscape worldwide and abundant in locations such as the prairies of southern Alberta with high temperatures and sun exposure. These plants, known as Calvin pathway plants, fractionate CO_2 during photosynthesis by approximately 20.00 ‰, and therefore, have an isotopic value of -28.00 ‰, however this ranges from -32.00 ‰ to -22.00 ‰ in value (Oleary 1981,

Rounick and Winterbourn 1986, Schidlowski 1995). Carbon fixation in C_4 plants is more elaborate than for C_3 plants due to a physiological mechanism that allows survival in tropical, dry climates. In fact, carbon uptake by C_4 plants is such an efficient mechanism, it has been proposed that an increase in C_4 plant abundance may help sequester the increasing levels of global CO_2 (Osborne and Beerling 2006). The fractionation of carbon in C_4 plants is only 5.00 ‰, and thus, they have an isotopic value near -13.00 ‰, which has been shown to range between -9.00 ‰ and -23.00 ‰ (Oleary 1981, Rounick and Winterbourn 1986). The final group of vegetation, CAM, has more variable isotopic values due to their rapid response to extreme conditions. CAM plants are found in arid climates such as the desert, and have been shown to shift their carbon fixation method in response to the environmental conditions. Their isotope values lie between -9.00 ‰ and -32.00 ‰, in the ranges for C_3 and C_4 plants (Oleary 1981).

During the metabolic processes of assimilation, fractionation accounts for minor variation of the isotopic signature between the sample and the source. For example, metabolically active areas such as the liver or fat tissue of animals have more rapid turnover rates, compared to less metabolically active areas such as bone which causes variation in isotopic signatures (Tieszen et al. 1983). Fractionation between prey and consumer is minimal, and therefore, easily traced. Animals that consume and assimilate foods from different sources have these differences reflected in the $\delta^{13}C$ and $\delta^{15}N$ ratios in their tissues.

In order to accurately determine the dietary components of an animal, various tissues are sampled to establish which has an isotopic composition that reflects the diet most closely (Deniro and Epstein 1978). Fractionation factors are easily incorporated, and numerous

researchers have determined dietary records for various animal tissues, such as feathers, hair, bone and muscle (Tieszen et al. 1983, Rubenstein and Hobson 2004, Sponheimer et al. 2006).

Primary producers, such as aquatic and riparian vegetation, and detritus form the endpoints of a food web. These endpoints are important to the ecology of the food web as they are the key drivers of production. However, if they are not distinct in isotopic signatures, it is difficult to assess the relative importance of aquatic and riparian food sources to an animal consumer (Rasmussen 2010). While the isotopic composition for algae and detritus has been established, the isotopic composition of riparian and freshwater plants is less well understood (Rounick and Winterbourn 1986, France 1995a).

Carbon isotopic compositions for riparian producers range from -9.00 to -32.00 ‰ depending on the mechanism by which they perform photosynthesis (Oleary 1981, Rounick and Winterbourn 1986, Schidlowski 1995). Unlike riparian producers, the carbon ranges for aquatic plants are more variable and are not indicative of the photosynthetic pathway, with values reported from -50.00 ‰ to -10.00 ‰ (Rounick and Winterbourn 1986, Keeley and Sandquist 1992, France 1995a). Freshwater plants do not derive their carbon from a single source, but from multiple sources such as limestone ($CaCO_3$), CO_2, mineral springs or autochthonous or allochthonous derived material (Fry 2006). Additionally, other abiotic factors may affect the isotopic signatures of freshwater plants including water velocity and concentrations of dissolved inorganic carbon (DIC), which creates variation in their isotopic composition (Trudeau and Rasmussen 2003, Rasmussen and Trudeau 2007, 2010).

Aquatic producers tend to exhibit a downstream gradient in isotopic signatures where they are depleted in upstream reaches compared to riparian producers and become more enriched downstream. Riparian producers do not generally exhibit gradients in their isotopic signatures. Aside from isotopic signature gradients, autochthonous processes are also useful for distinguishing aquatic and riparian producers. Autochthonous food sources are more important in downstream reaches as shading decreases, and aquatic production increases compared with heavily shaded head-water stream habitats (Rasmussen 2010). Of note is that literature values of primary production are not always useful and it is important to sample the isotopic signature of local plants local primary productivity when conducting a field based isotope study (France 1995b, Dawson et al. 2002).

1.3.1 Riparian forest communities

Riparian forest communities are transition zones between freshwater and upland riparian habitats and thus, their boundaries are difficult to identify. Riparian areas are defined as moist habitats between land and water bodies such as rivers, streams, sloughs or wetlands, which are often subject to flooding. As such, the unique soil structure and plant community of riparian habitats reflect the presence of free-moving water (Reclamation Criteria Advisory Group 2010).

Riparian forests are often characterized by their high water tables, frequent inundation by the aquatic ecosystem and nutrient-rich soils (Davis et al. 2006). They also have variable topographic conditions, including drier slopes, and intermittent wetland depressions, which enhances plant species biodiversity. These habitats improve water quality through filtration of sediment, nutrients and pollution (Poff et al. 2011) and provide flood control,

and bank stabilization, which supports aquatic and riparian biodiversity (Fitch and Adams 1998). As such, riparian areas are often used as an indicator of ecosystem health and overall water quality.

The constant exchange of nutrients, organisms, and detritus between aquatic and riparian ecosystems is facilitated by transitional riparian forest communities. Riparian vegetation has been shown to influence a number of abiotic properties such as air and water temperatures, as well as light penetration (Poff et al. 2011). Debris and litter from riparian vegetation enhances fish habitat and provides an additional food source to aquatic invertebrates (Petersen and Cummins 1974, Gregory et al. 1991). On the other hand, large-order rivers (i.e. containing a number of contributing tributaries) with high *in situ* aquatic primary production from high amounts of sun penetration provides an additional food source to herbivorous riparian consumers (Vannote et al. 1980, Bastow et al. 2002). The productivity of riparian forests is directly linked to aquatic ecosystem productivity. For example, *Populus* species (cottonwoods) are dominant tree species in riparian forests of Southern Alberta and colonization and establishment by cottonwood seedlings is directly reliant on the natural processes of the freshwater ecosystem (i.e. flood disturbance) (Rood et al. 2007).

The diverse plant community of riparian forests offers prime habitat for animals. Frequent flooding improves riparian plant production by deposition of detritus and other essential organic nutrients and thus, herbivorous insect abundance is enhanced, thereby supporting higher-level riparian consumers. Varied plant structure provides shelter from climatic conditions and predators. Riparian forests are frequently used by migrating birds during rest periods, and roosting habitats for bats (Baxter et al. 2005).

21

It has been recognized that anthropogenic alterations of the landscape impact the overall health and biodiversity of the riparian and aquatic ecosystems. Essential landscape features such as buffer strips, hedgerows and wetland swales have declined due to the modernization of agricultural practices. Plant species richness and overall habitat diversity have deteriorated from several intense agricultural operations such as the use of pesticides and herbicides, increased livestock grazing and densities, and simplified crop rotations with increased reliance on fertilizer (McCracken et al. 2012).

The development and use of these agricultural tools produces homogenous upland habitats with low biodiversity. For example, studies have shown that there is a higher density of several taxonomic families of riparian invertebrates (Heteroptera, Cicadellidae, Opiliones, Delphacidae, and Arionidae) in fenced heterogeneous riparian margins compared with homogenous grassland habitats used for grazing (Cole et al. 2012).

Riparian forest community structure in the Oldman River Basin (OMRB) is diverse and includes a variety of mosses, and sedges, a range of forbs including the rare Western Blue Flag (iris), as well as willow and cottonwood species. Drier, south-facing slopes support the production of pasture and prairie sages, as well as rose species. However, in Southern Alberta, agricultural based land-uses such as farming and ranching are prevalent due to rich soils and high insolation rates, transforming upland areas into low diversity landscapes. For example, 2.5 million head of cattle were slaughtered in Western Canada in 2010, and Alberta contributed 98 % of those cattle (Alberta Agriculture and Rural Development 2011). Furthermore, census Canada reported that in 2006 there were approximately 947 farms over 2240 acres in size in Lethbridge County, which is a large area in the OMRB.

With a great proportion of land-use in the OMRB directed towards the agricultural industry (Brinkmann and Rasmussen 2012), pockets of riparian forest communities may support the flora and fauna of this semi-arid region. High river productivity and influxes of nutrients, detritus and organisms may subsidize the riparian food web considerably and may be more relevant in biological systems where upland habitats are composed mainly of homogenous grass or croplands such as in the OMRB.

There are still considerable questions in the field of energetic ecology that need to be addressed. Research on the significance of energetic flows to riparian consumers has been overlooked in arid, grassland ecosystems. Quantifying energetic subsidization in the OMRB will improve understanding of the local trophic community and serve as a platform for similar research to riparian ecosystems. The overarching aim of this study will heighten awareness of ecosystem connectivity leading to increased conservation of water systems.

1.4.1 Research objectives

In this thesis, the contribution of aquatic and riparian insects to the diet of riparian spiders and beetles was quantified to enhance knowledge of energetic subsidies in riparian ecosystems. In the second chapter, the use of stable isotope analysis was tested for viability in the OMRB by characterizing the isotopic patterns of both aquatic and riparian producers and primary consumers. In the third chapter, stable isotopic mixing model analysis was performed on riparian spiders and beetles using putative endpoints established in chapter 2 and the distance from the river to which these riparian consumers' diets are subsidized by emerging aquatic insects were quantified. Finally, the

relationship between the size of the river (width) and the extent of aquatic insect subsidization to riparian consumers in upland habitats was measured.

CHAPTER TWO

Differentiation between Aquatic and Riparian Signatures of Stable Carbon and Nitrogen

Isotopes for Producers and Primary Consumers in the Oldman River Basin, Alberta

2.1.0 Introduction

Freshwater and riparian ecosystems are often considered as an important intermediary zone of energetic exchange. The movement of energy and nutrients across habitat boundaries influences trophic linkages and impacts the overall food web (Polis et al. 1997, Marczak et al. 2007a). Energetic exchange among habitats, often referred to as an allochthonous subsidy, provides alternative food sources to consumers (Huxel and McCann 1998, Marczak et al. 2007b). Energetic exchanges between aquatic and riparian ecosystems stem from a variety of sources such as predator and prey movement, and deposition of primary production such as leaf litter, aquatic autotrophs or detritus (Petersen and Cummins 1974, Baxter et al. 2005, Bergfur et al. 2009). However, it is difficult to trace energetic flow through a food web as interactions are seldom directly observed and measured (Rasmussen 2010).

Stable isotope analysis of ^{13}C and ^{15}N is effective for tracing food web interactions as they provide a natural quantitative estimate of dietary patterns, and are time-integrated rather than providing a single glance of consumer diet as with gut-content analysis (Tieszen 1991, France 1995a, France and Peters 1997, Vander Zanden and Rasmussen 2001).

Applications of stable isotope analysis require critical *a priori* measurements of signatures to establish representative food sources in the food web. Generally, the carbon and nitrogen isotopic signatures of basal food web biota such as producers are initially established to standardize the base of the food web to common reference as fractionation differences that occur are not exclusively based on organism physiology and can, in part, be a function of abiotic and geographical properties (Woodland et al. 2012). Establishing isotopic signatures at the base of the food web is also useful in discriminating among several food webs across a landscape such as a river basin, and often enables researchers the ability to track mobile consumers.

Partitioning food sources to the diet of a consumer using stable isotope signatures is feasible if the source signatures are distinct. However, distinctness of source signatures differs because of local variability in isotopic signatures (Finlay and Kendall 2007). Geographic gradients such as elevation and anthropogenic impacts such as water storage facilities cause variability in isotopic signatures, and thus, it is important to assess the baseline of the food web at specific locations.

Riparian producers are depleted in ^{13}C compared to atmospheric CO_2 from physiological processes that discriminate in favor of the isotope ^{12}C. Differential photosynthetic pathways of producers (C_3, C_4 and Crassulacean acid metabolism) variably discriminate against ^{13}C, and thus, producer isotopic signatures are often different. Nitrogen isotopes, on the other hand, become enriched between predator and prey, and thus, can also be traced through the food web while providing an indicator of trophic level (Vander Zanden and Rasmussen 2001).

Producer nitrogen signatures are often determined by nitrogen source, differential assimilation, fractionation and uptake mechanisms, and thus, plants often exhibit differing signatures that can assist in food source differentiation (Evans 2001). Nitrogen signatures of riparian producers may show among-site variability from differential inputs (sewage, and agricultural), as well as nitrogen cycling of dominant plant species.

Spatial differences in the carbon signatures of riparian producers, however, is minimal and tends only to vary with moisture regimes, agricultural influence, and sewage inputs (Cabana and Rasmussen 1996, Anderson and Cabana 2009, Brinkmann and Rasmussen 2012). Riparian producer carbon signatures often fall within an isoscape, meaning they are invariable in isotopic signature across a landscape if those plants utilize the same photosynthetic pathway.

The differentiation of isotopic signatures in freshwater ecosystems is more complex due to the presence of spatial gradients in producer signatures, and differential nutrient pools such as dissolved inorganic carbon and nitrogen (Rasmussen 2010, Woodland et al. 2012). For example, $\delta^{13}C$ signatures of *in situ* or autochthonous production are generally depleted in upstream reaches compared to riparian producer signatures, whereas riparian producer signatures exhibit no gradient. Variable isotopic signatures at the base of the freshwater food web often transfers to riparian consumers as an allochthonous subsidy and thus, food source signatures of freshwater and riparian origin can be distinguished. If potential food source signatures are distinct with respect to $\delta^{13}C$ and $\delta^{15}N$, then redundancy can be achieved by incorporating both isotopes into analysis providing a robust estimate of consumer diet.

The assimilation of food through metabolic processes by consumers results in fractionation in isotopic signature from source to consumer. It is commonly assumed that fractionation values of carbon and nitrogen are redundant; however, there is considerable variability around these fractionation estimates. Isotopic signatures at the base of a food web are conserved through the food web thus generating variation among ecosystems (Vander Zanden and Rasmussen 1999, Vander Zanden and Rasmussen 2001).

Isotopic model outputs generate error when variation in isotopic fractionation is not accounted for, however, if observed variation is included in model analysis then the resultant error is minor even when mean or assumed fractionation values are used (Vander Zanden and Rasmussen 2001). Further, research has shown that mean trophic fractionation values and associated variability are substantial at the plant-herbivore level compared with carnivores; however, modelling error is negligible when primary consumers are used as a baseline and mixing model end points are sufficiently distinct (Phillips and Gregg 2001, Vander Zanden and Rasmussen 2001).

Quantitative estimates of energy flow across aquatic-riparian boundaries on prairie landscapes characterized by large floodplains, is limited; although, river-floodplain interactions of large order rivers are considerably more productive than adjacent upland zones (Johnson et al. 1995). In order to quantify the importance of allochthonous subsidies to consumers in riparian ecosystems, the isotopic distinctness of aquatic and riparian food sources needs to be verified for each site studied.

2.2.0 Objectives

The overall objective of this chapter was to evaluate whether $\delta^{13}C$ and $\delta^{15}N$ signatures could be used to differentiate between aquatic and riparian biota (producers and primary consumers) that have the potential to serve as food sources for higher consumers, such as spiders and beetles, in riparian flood plain communities in the Oldman River Basin (OMRB). If aquatic and riparian food sources are distinct in their isotopic signatures, they can be applied to mixing model analysis to assess the relative contribution of each to riparian consumers. Variability in food source signatures depends on several abiotic and biotic properties in the OMRB such as elevation, water storage facilities, local floodplain factors and variation along the river continuum.

Isotopic signatures have been shown to vary along downstream elevation gradients in rivers (Rasmussen and Trudeau 2007, Rasmussen 2010) and with local differences in water velocity associated with the river meander (Finlay et al. 2002, Trudeau and Rasmussen 2003, Rasmussen and Trudeau 2007, 2010). Thus we might expect to see along-stream isotopic signature gradients; however, by standardizing aquatic sampling to riffles with similar water velocity, local variability resulting from this factor would likely be minimized.

Dams and reservoirs disrupt along stream patterns by affecting water chemistry, organic matter cycling, reducing sediment loading and simplifying downstream habitat, resulting in variable isotopic signatures of riverine biota (Angradi 1994). Flow in the OMRB is regulated by several large storage facilities such as the St. Mary River and Oldman dam and reservoirs. Flow regulation may influence the stable isotopic signatures of riverine

and riparian biota, and the contribution of this factor to among-site variability in aquatic primary consumer signatures will be evaluated by comparing $\delta^{13}C$, and $\delta^{15}N$ signatures from sites in tail water reaches below dams to sites upstream of reservoirs. Tail water reaches influenced by dams might be expected to lead to more depleted $\delta^{13}C$ signatures and enriched $\delta^{15}N$ signatures (Vander Zanden and Rasmussen 1999).

Riparian isotopic signatures of both producers and consumers are generally thought to be less spatially variable than their aquatic counterparts since the ultimate carbon and nitrogen sources acquired by riparian biota are well-mixed atmospheric gases, in contrast to dissolved solutes in a dense aquatic medium (Marshall et al. 2007). Fractionation of stable carbon isotopes are, however, known to vary with the moisture regime for a number of riparian plants; as stomata spend more time closed in increasingly dry environments, isotopic fractionation is reduced (Ehleringer and Cooper 1988, Schuster et al. 1992). Thus it is possible that more moist sites farther upstream at higher elevations might yield more depleted $\delta^{13}C$ signatures.

Spatial signature variation in $\delta^{15}N$ arise from differences in agricultural influence (Anderson and Cabana 2009, Brinkmann and Rasmussen 2012), and domestic sewage inputs (Cabana and Rasmussen 1996), both of which might be expected to increase downstream. Differences in the relative dominance of plants such as Alder and legumes, that harbour N-fixing nodules on their roots have also been known to affect nitrogen signatures (Virginia et al. 1989, Bedard-Haughn et al. 2003) and might be a possible contributor to between-site variability.

This analysis will be able to test for effects of a long-stream gradient by regressing $\delta^{13}C$ and $\delta^{15}N$ signatures of producers and primary consumers, from both aquatic and riparian sources, to elevation, and stream width, reflecting their position along the river continuum. In addition, effects of distance upslope from the river will be tested using regression analyses against perpendicular distance from the river shoreline. Local factors, unrelated to along stream gradients, would be reflected in 'Site' effects included in the general linear model, although this would of course yield little insight as to cause.

By establishing the distinctness in $\delta^{13}C$ and $\delta^{15}N$ signatures of aquatic and riparian biota in the OMRB, proportional estimates of aquatic and riparian dietary sources to riparian consumers can be made. Specifically, aquatic and riparian producer signatures will be compared to establish the baseline for the river basin, while primary consumer signatures from the aquatic and riparian ecosystems will be analyzed for distinctness for use in future food web analysis.

The consideration of river basin properties such as elevation, water storage facilities, and site factors ensures that dietary analysis of consumers will be based on local information accounting for site-specific variation and effectively eliminating uncertainty in food web estimates. This study is novel in that it examines site-specific differentiation of carbon and nitrogen signatures of aquatic and riparian biota to assess their potential for food web analysis in the OMRB.

2.3.0 Methods

2.3.1 Study area

The study was conducted at 7 sites in the Oldman River Basin located in the Southwest portion of Alberta, Canada and the Northern part of Montana, USA (Figure 2.1). The OMRB has an area of 28,200 km^2 and is a sub-basin of the South Saskatchewan River basin which drains into Hudson Bay.

The headwaters of the Oldman River are located within the Rocky Mountains and the system contains several sub-basins. The Oldman River Dam, constructed in 1990, is located near the confluence of the Upper Oldman, Castle and Crowsnest Rivers and forms the Oldman River Reservoir. Of interest is that most of the tributaries in the system are dammed and flow controlled.

The OMRB is located within the mixed grass natural sub-region of Alberta, which has a mean annual temperature of + 3 °C; mean annual precipitation of 410 mm and annual insolation of 4600-4800 MJ/m^2.

The upper basin is cooler in temperature, and is prime habitat for *Salvelinus confluentus* (bull trout) and other salmonid species, while the lower basin is warmer, supporting *Sander vitreus* (walleye) and *Esox lucius* (pike) fisheries. The Oldman River is the largest tributary in the basin, and has an annual mean discharge of 101 m^3/s with over 80% of the

water allocated to irrigation (Koning et al. 2006). The rivers and creeks included in the study were the Oldman River, the Castle River, the St. Mary River, and Pincher Creek.

Freshwater algae is mainly filamentous (*Cladophora*), or planktonic (*Aphanizomenon*), with some macrophyte species represented (*Chara*). The diatom, *Didymosphenia*, is also found in abundance at several sites. Aquatic invertebrate larvae include but are not limited to caddisflies (Trichoptera), stoneflies (Plecoptera) and mayflies (Ephemeroptera).

The adjacent riparian habitat is patchy, and is variable in width (less than 1 km) before transitioning into agricultural based upland habitats. Agricultural uplands are often used for growing cereal crops or for ranching of livestock including cattle and horses. Oil and gas development is common on both private and crown land, and areas of the upper basin (near Castle River) have been approved for forestry operations.

Riparian vegetation is dominated by cottonwoods (*Populus* spp) and common shrubs such as rose (*Rosa* spp), snowberry (*Symphoricarpos occidentalis*) and willow (*Salix* spp) and may include rare plant species such as the Western Blue Flag (iris).

SAMPLING SITES	LEGEND
CR (1): Castle River; 49° 29' 20" N, 114° 05' 21" W	• Town
PC (2): Pincher Creek; 49° 29' 40" N, 113° 55' 34" W	
SV (3): Summerview; 49° 33' 28" N, 113° 49' 06" W	— Hypolimnic release dam
BS (4): Blazingstar; 49° 51' 06" N, 113° 15' 11" W	
CTW (5): Cottonwood; 49° 38' 08" N, 112° 52' 00" W	
STMR (6): St. Mary River; 49° 35' 17" N, 112° 52' 59" W	★ Sampling Site
PA (7): Pavan; 49° 44' 11" N, 112° 51' 35" W	

Figure 2.1: Location of sampling sites in the Oldman River Basin, Alberta showing sampled water bodies, reference towns, and hypolimnic release dams. Sampling sites are labelled from upstream to downstream. GPS coordinates were taken in 10TM NAD83.

34

These areas also have a wide array of grass species such as wheat grasses (*Agropyron* spp), and invasive grasses (*Bromus tectorum, Poa pratensis)*. Extensive research on the cottonwood forests in the OMRB have indicated that the species may include balsam poplar *(Populus balsamifera)*, narrowleaf cottonwood *(Populus augustifolia)*, and sparse amounts of Plains cottonwood *(Populus deltoids)* as well as a hybridization of any of these species. The Castle River site hosts the cooler species trembling aspen *(Populus tremuloides)* (Samuelson and Rood 2004, Willms 2005, Willms et al. 2006).

Riparian sites are rich in insect and arthropod fauna such as emergent aquatic insects, leafhoppers (Cicadellidae), European ground beetles (Carabidae) and spiders (Lycosidae, Salticidae*)* including harvestmen (Opiliones).

The sample sites were chosen based on the relative ease of access as well as their familiarity within the Rasmussen Laboratory. Most of the sites complement the Master's and Doctoral work of Lars Brinkmann, and thus, they have been qualitatively analysed for species compositions, mercury concentrations and bioenergetic factors (Brinkmann 2009).

Extensive literature on regional river hydrology, riparian woodlands and other relevant topics are available for the OMRB (See Hyland et al. 2003, Byrne et al. 2006, Rock and Mayer 2006, 2007, Rood et al. 2008, Birks and Gibson 2009, Rock and Mayer 2009, Brinkmann and Rasmussen 2010, Poirier and De Loe 2011, Warnock et al. 2011, Brinkmann and Rasmussen 2012).

2.3.2 Sample Collections

All sample collections occurred between March and November of 2009 and 2010. Riparian producers were haphazardly surveyed by manual clipping of leaves and aquatic producers were sampled by brushing material off of covered rocks and filtering with distilled water. Aquatic primary consumers were haphazardly from riffles, which are areas of similar velocity, using a D-frame aquatic net.

Riparian primary consumers were sampled along transects established at each site that runs perpendicular to the shore. Transects extended to distances twice the width of the river and sampling points were spaced at equal intervals along transects. Riparian primary consumers were collected using a standard sweep net, as well as with pit fall traps in order to collect non-sedentary ground dwelling insects and nocturnal insects. Samples were then stored in coolers and frozen within 4 hours of collection until sorting and identification to family (Clifford 1991, Wise 1993, MacRae 2008).

2.3.3 Stable Isotope Analyses

All samples collected were oven dried at 60°C for approximately 3 days. They were used whole and ground into a homogenous powder using a mortar and pestle and packaged into tin capsules for isotopic analysis. Isotopic analyses were conducted using a Costech 4010 Elemental Combustion System and Thermo Delta V Advantage (with Conflo 4 interface) (Brinkmann 2009). The homogenous powders were sub-sampled with 0.6 mg of tissue for analysis of animals and 3 mg and 0.4 mg for carbon and nitrogen plant tissue analysis, respectively. The isotopic signatures were expressed in δ, delta notation, as a

ratio relative to a Vienna PeeDee Belemnite (carbon) and atmospheric N_2 (nitrogen) using the following equation:

$$X = \left[\left(\frac{Rsample}{Rstandard}\right) - 1\right] * 1000 \qquad \textit{Equation 2.1}$$

Where X is carbon or nitrogen, and R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Samples with higher heavy isotope ratios from standards were termed "enriched" whereas those with lower heavy isotope ratios were termed "depleted".

2.3.4 Statistical Analyses

Statistical computations included general linear models such as one-way and two-way analysis of variance (ANOVA), analysis of covariance (ANCOVA), linear regression and, independent groups' t-test. All statistical analysis for general linear modelling was performed in JMP version 9.0.2 (SAS Institute Inc, Cary, North Carolina). Variables were examined for skewness, kurtosis and outliers using IBM SPSS Statistics 19. Descriptive statistics were also performed in JMP, IBM SPSS and Microsoft excel to assess patterns.

Stable isotope $\delta^{13}C$ and $\delta^{15}N$ data were transformed ($\log_{10}$ and square root) to normalize data and reduce heteroscedasticity, when necessary. Variance equality was tested using Levene's test followed by a Welch's ANOVA if variance was deemed unequal. Post-hoc analysis was conducted with Tukey's Honestly Significantly Different multiple comparisons. Residuals were tested with Shapiro-Wilk W for normal distribution, and with analysis of Q-Q plots (Whitlock and Schluter 2009, Sheskin 2011, Williams 2011).

Interaction terms were tested and if found to have no significant effect, they were removed in order to increase power in the model. Alternatively, if the interaction term was significant, than this term was reported.

Outliers were removed when deemed to be caused from technical error during mass spectrometry analysis.

2.4.0 Results

Overall, $\delta^{15}N$ and $\delta^{13}C$ mean signatures of aquatic and riparian producers, and primary consumers by site and elevation (m) are summarized in Figure 2.1 (A –D) and Figure 2.2 (A –D), respectively.

The $\delta^{15}N$ and $\delta^{13}C$ signatures of all biota categories (aquatic and riparian producers and consumers) were analysed for indications of along stream gradients against either elevation (m) or river width (m) for descriptive purposes (i.e. in order to identify patterns) (See Appendix A).

Aquatic and riparian producers and primary consumers were analysed descriptively by biota type, as well as by site. Aquatic and riparian biota categories are also compared to each other to analysis the base of the food web, as well as endpoints for mixing model analysis in the future.
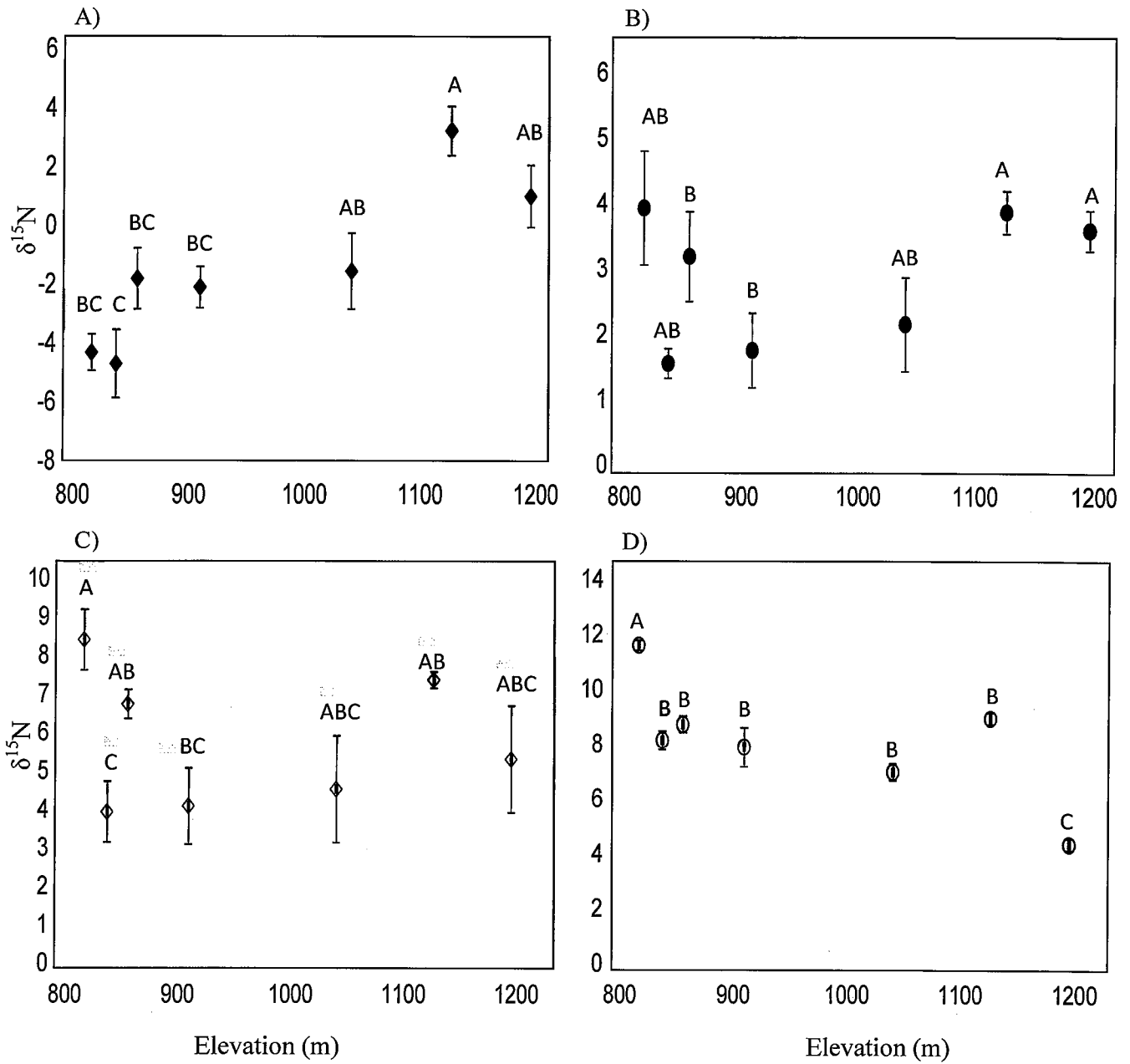
Figure 2.2: Nitrogen signatures ($\delta^{15}N$) of aquatic and riparian biota as a function of elevation (m). A) Riparian producers are denoted by shaded diamonds (♦), B) Riparian consumers are denoted by shaded circles (●) C) Aquatic producers are denoted by open diamonds (◊) and D) Aquatic primary consumers are denoted by open circles (o). Error bars denote the standard error of the mean. Levels not connected by the same letter are significantly different.
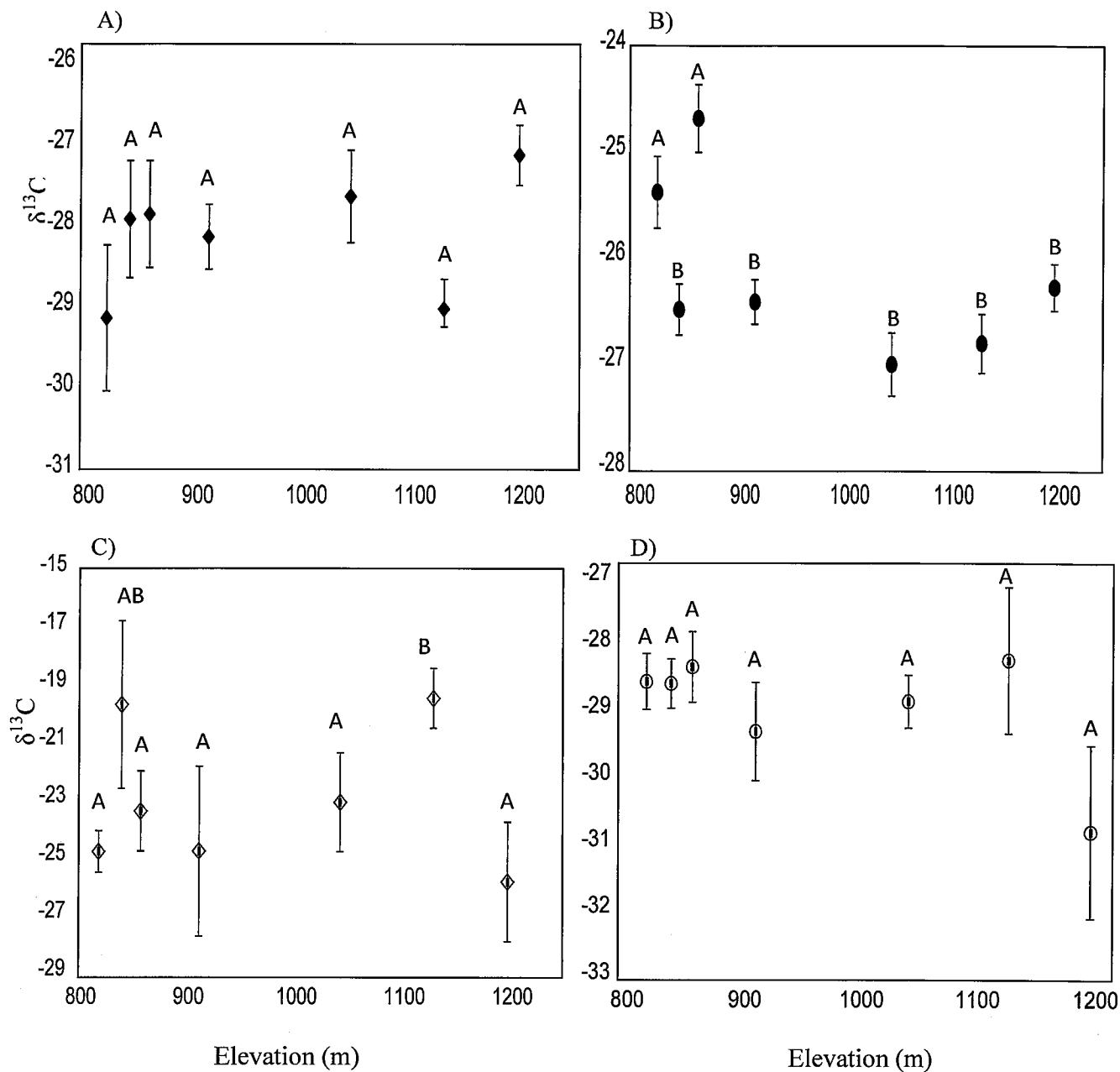
Figure 2.3: Carbon signatures (δ^{13}C) of aquatic and riparian biota as a function of elevation (m). A) Riparian producers are denoted by shaded diamonds (♦), B) Riparian consumers are denoted by shaded circles (●) C) Aquatic producers are denoted by open diamonds (◊) and D) Aquatic primary consumers are denoted by open circles (o). Error bars denote the standard error of the mean. Levels not connected by the same letter are significantly different.

2.4.1 Aquatic producer and primary consumer $\delta^{15}N$ and $\delta^{13}C$ signatures

Aquatic producer (n = 43) signatures ranged from -1.48 to 10.82 ‰ for $\delta^{15}N$ and -36.08 to -16.34 ‰ for $\delta^{13}C$ and site means ranged from 3.93 to 8.40 ‰ for $\delta^{15}N$ and − 26.02 to − 19.67 ‰ for $\delta^{13}C$ and significantly differed for nitrogen ($\delta^{15}N$: $F_{(6, 13)} = 5.18$, $p < 0.05$; $\delta^{13}C$: $F_{(6, 13)} = 2.84$, $p = 0.06$).

Aquatic primary consumers (n = 148) mean signatures ranged from 2.59 to 15.15 ‰ for $\delta^{15}N$ and -34.66 to -23.34 ‰ for $\delta^{13}C$. Aquatic primary consumer site means ranged from 4.36 to 11.60 ‰ for $\delta^{15}N$ and -30.90 to -28.32 ‰ for $\delta^{13}C$ and significantly differed for nitrogen ($\delta^{15}N$: $F_{(6,33)} = 111.73$, $p < 0.0001$; $\delta^{13}C$: $F_{(6, 141)} = 1.02$, $p = 0.42$).

The pooled mean signatures of aquatic primary consumer were enriched in $\delta^{15}N$ by 2.90 ‰ ± 0.52 and depleted in $\delta^{13}C$ by 5.20 ± 0.78 ‰ relative to aquatic producer pooled mean signatures. Despite overall mean isotopic signatures demonstrating signature overlap, aquatic producers and primary consumers differed significantly in their isotopic signatures.

Stable nitrogen signatures of aquatic primary consumers (mean $\delta^{15}N$ = 8.72 ± 0.21) were significantly greater than those of aquatic producers (mean $\delta^{15}N$ = 5.84 ± 0.47). On the other hand, stable carbon signatures of aquatic primary consumers (mean $\delta^{13}C$ = -28.84 ± 0.20) were consistently depleted compared to aquatic producer signatures (mean $\delta^{13}C$ = -23.59 ± 0.75).

Aquatic producer and primary consumer nitrogen and carbon signatures varied significantly from each other within sites. Further, a significant interactive effect of biota

type and site was observed for nitrogen, however, this interactive effect was not observed for carbon (Figure 2.4 A and B).

2.4.2 Above and below dam $\delta^{15}N$ and $\delta^{13}C$ signatures in the Oldman River Basin

Hypolimnic release dams deplete carbon signatures and enrich nitrogen signatures in downstream reaches. Isotopic signatures of aquatic insects at the Castle River site (n = 5), located above the Oldman Dam, were compared with those of the Summerview site (n = 42), located downstream of the Oldman Dam.

Tail water (Summerview) aquatic insect $\delta^{15}N$ and $\delta^{13}C$ signatures were enriched by 2.63 and 1.96 ‰, respectively, compared with above dam consumer signatures. Despite the slight enrichment of carbon signatures in the tail waters of the dams, carbon signatures of aquatic insects above and below dams were not distinct. However, aquatic insect signatures in the tail waters of the dam were significantly enriched in nitrogen. ($\delta^{15}N$: $t_{(45)}$ = 7.20, $p < 0.05$; $\delta^{13}C$: $t_{(45)}$ = 1.63, $p = 0.11$).

A)

$\delta^{15}N \pm SE$

- ─◉─ Aquatic Primary Consumers
- ─◇─ Aquatic Producers

BCD

DE

BCDE

BCDE

*

Above Dam

BCDE

Below Dam

A

E

B

A

CDE

BCDE

CDE

BC

A

| Castle River 1195 m 30 m | Pincher Creek 1126 m 20 m | Summerview 1040 m 95 m | Blazingstar 909 m 94 m | St.Mary River 855 m 35 m | Cottonwood 837 m 113 m | Pavan 816 m 120 m |

B)

$\delta^{13}C \pm SE$

- ─◉─ Aquatic Primary Consumers
- ─◇─ Aquatic Producers

ABCD

ABD

A

AB

ACD

C

B

A

A

A

A

A

A

Below Dam

Above Dam

A

A

| Castle River 1195 m 30 m | Pincher Creek 1126 m 20 m | Summerview 1040 m 95 m | Blazingstar 909 m 94 m | St.Mary River 855 m 35 m | Cottonwood 837 m 113 m | Pavan 816 m 120 m |

Figure 2.4: A) Nitrogen ($\delta^{15}N$) and B) Carbon signatures ($\delta^{13}C$) of aquatic producers and aquatic primary consumers as a function of elevation (m) and site with river width (m). Aquatic primary consumers are denoted by open circles (o) and aquatic producers are denoted by open diamonds (◊). Error bars denote the standard error of the mean. Levels not connected by the same letter are significantly different. Letters for A) are a result of post-hoc analysis of a significant interaction term whereas, letters for B) are a result of post-hoc analysis of site parameter differences. Italics indicate aquatic primary consumers above and below the Oldman Dam, and (*) represents a significant difference. ($\delta^{15}N$: $F_{(6, 177)} = 3.03$, $p < 0.05$; $\delta^{13}C$: $F_{(13, 177)} = 4.92$, $p < 0.0001$).

43

2.4.3: Riparian producer and primary consumer $\delta^{15}N$ and $\delta^{13}C$ signatures

Riparian producer (n = 54) $\delta^{15}N$ signatures ranged from - 9.81 to 8.89 ‰ and – 31.91 to - 24.08 ‰ for $\delta^{13}C$, and site means ranged from - 4.71 to 3.21 ‰ for $\delta^{15}N$ and – 29.20 to - 27.19 ‰ for $\delta^{13}C$ and significantly differed for nitrogen ($\delta^{15}N$: $F_{(6, 47)} = 4.83$, $p < 0.05$; $\delta^{13}C$: $F_{(6, 47)} = 1.44$, $p = 0.22$).

Riparian primary consumers (n = 191) signatures ranged -3.57 to 10.54 ‰ for $\delta^{15}N$ and - 33.69 to -22.83 ‰ for $\delta^{13}C$ and site means ranged from 1.55 to 3.93 ‰ for $\delta^{15}N$ and – 27.07 to – 24.74 ‰ for $\delta^{13}C$ and significantly differed for both nitrogen and carbon ($\delta^{15}N$: $F_{(6, 53)} = 8.17$, $p < 0.0001$; $\delta^{13}C$: $F_{(6, 184)} = 5.47$, $p < 0.0001$).

Riparian primary consumers were enriched in both $\delta^{15}N$ and $\delta^{13}C$ relative to riparian producers by 4.03 ± 0.29 and 1.64 ± 0.20 ‰, respectively.

Stable nitrogen signatures of riparian primary consumers (mean $\delta^{15}N$ = 2.75 ± 0.19) were significantly greater than those of riparian producers (mean $\delta^{15}N$ = -1.30 ± 0.53). Similarly, stable carbon signatures of riparian primary consumers (mean $\delta^{13}C$ = -26.44 ± 0.11) were consistently enriched compared to riparian producers signatures (mean $\delta^{13}C$ = -28.08 ± 0.22). Further, a significant interactive effect of biota type and site was observed for nitrogen and for carbon. (Figure 2.5 A and B).

Effects of distance upslope from the shoreline were examined for $\delta^{15}N$ and $\delta^{13}C$ signatures of riparian primary consumers. Neither $\delta^{15}N$ ($F_{(1,183)} = 0.48$, $p = 0.49$) nor $\delta^{13}C$ ($F_{(1,183)} = 0.99$, $p = 0.32$) signatures of riparian primary consumers showed any significant trend in isotopic signature with distance upland from the river. There was no interactive effect observed for site and distance.
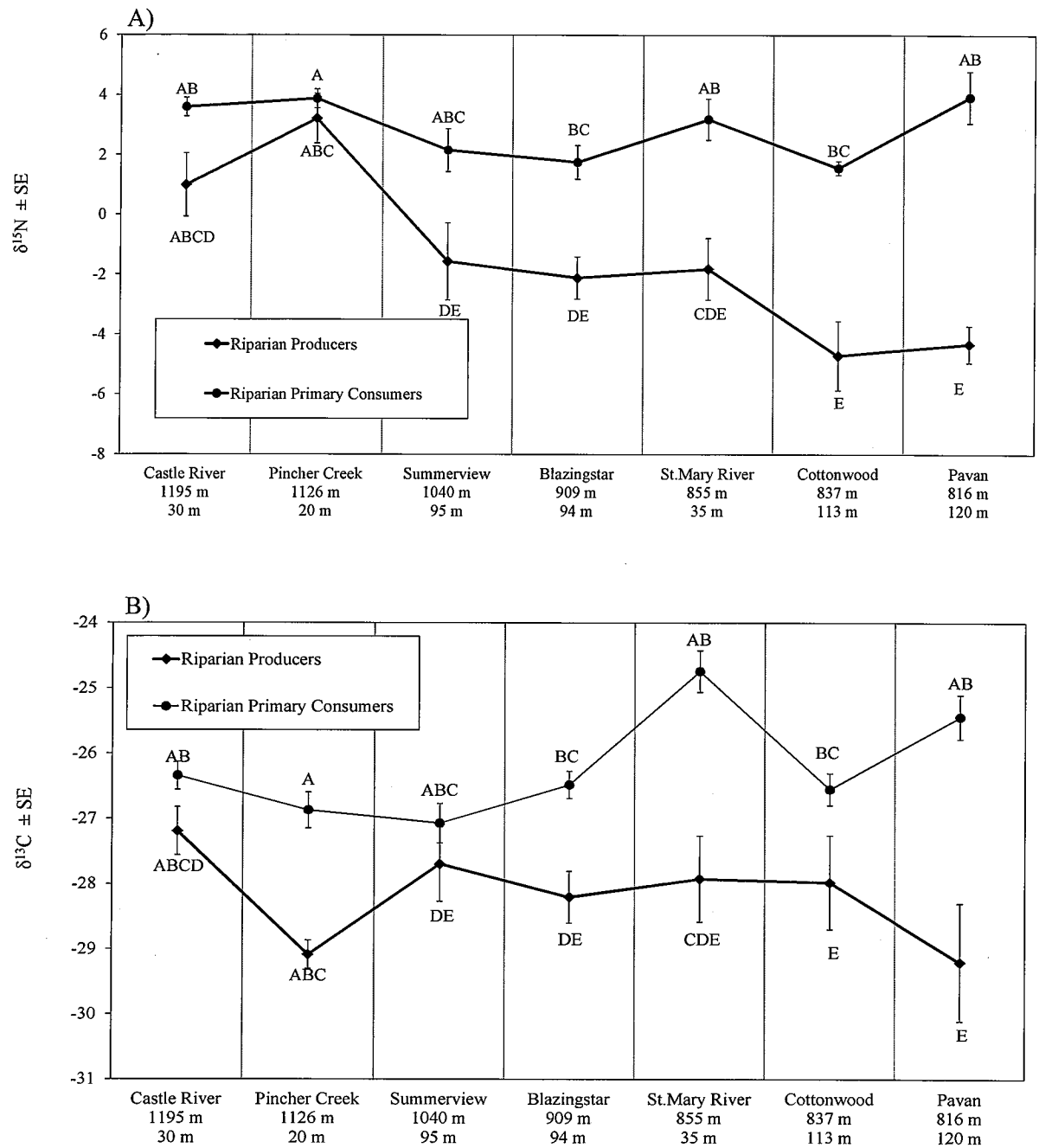
Figure 2.5: A) Nitrogen ($\delta^{15}N$) and B) Carbon signatures ($\delta^{13}C$) of riparian producers and riparian primary consumers as a function of elevation (m) and site with river width (m). Riparian primary consumers are denoted by shaded circles (●) and riparian producers are denoted by shaded diamonds (♦). Error bars denote the standard error of the mean. Levels not connected by the same letter are significantly different. Letters are a result of post-hoc analysis of a significant interaction term. ($\delta^{15}N$: $F_{(6, 231)}$ = 4.12, p < 0.05; $\delta^{13}C$: $F_{(6, 231)}$ = 3.13, p < 0.05).

2.4.4: Validity of mixing model analysis

In order to perform dietary modelling, putative food sources or endpoints must be distinct. Food sources to riparian secondary consumers included aquatic and riparian primary consumers. Aquatic and riparian producer signatures were also compared. Site specific comparisons of $\delta^{15}N$ and $\delta^{13}C$ mean isotopic signatures of aquatic and riparian producers, and primary consumers were conducted to evaluate signature distinctness among sites for similar biota.

Aquatic producers were significantly enriched in both $\delta^{15}N$ and $\delta^{13}C$ compared with riparian producers. There was no interactive effect observed for either carbon or nitrogen. (Figure 2.6 A and B).

Aquatic primary consumers were enriched in $\delta^{15}N$ by a mean of 5.98 ‰ ± 0.22 relative to riparian primary consumers. However, stable carbon isotopic signatures of aquatic primary consumers are significantly depleted by a mean of 2.40 ± 0.24 ‰ relative to riparian primary consumers. There was no significant interactive effect of biota type and site observed for carbon whereas, an effect was observed for nitrogen (Figure 2.7 A and B). See Appendix A.
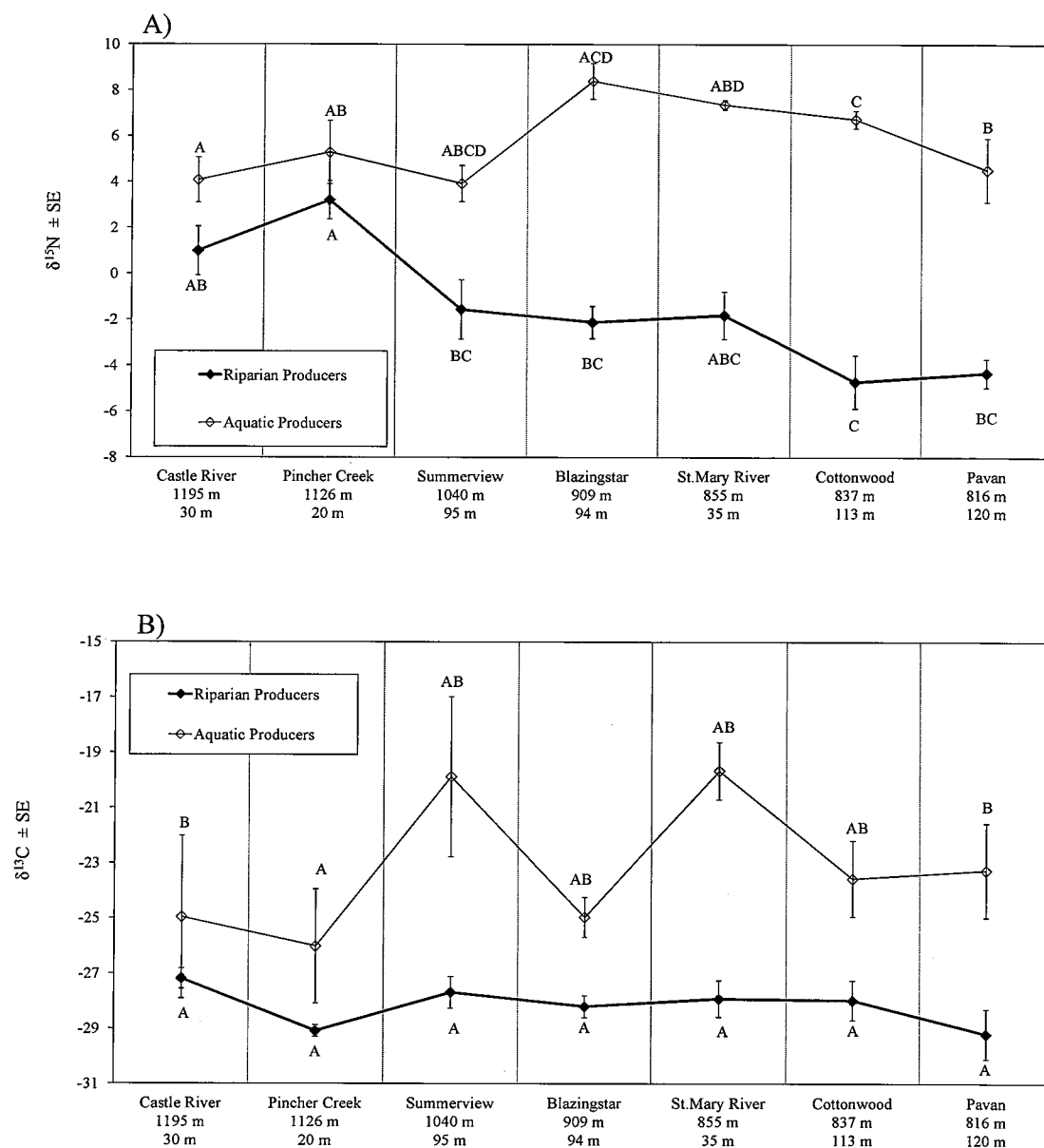
Figure 2.6: A) Nitrogen ($\delta^{15}N$) and B) Carbon signatures ($\delta^{13}C$) of riparian producers and aquatic producers as a function of elevation (m) and site with river width (m). Riparian producers are denoted by shaded diamonds (♦) and aquatic producers are denoted by open diamonds (◊). Error bars denote the standard error of the mean. Levels not connected by the same letter are significantly different. Letters are a result of post-hoc analysis of site parameter differences. ($\delta^{15}N$: $F_{(7, 89)}$ = 49.10, $p < 0.0001$; $\delta^{13}C$: $F_{(7, 89)}$ = 2.30, $p < 0.05$).

A)

δ¹⁵N ± SE values:

- —○— Aquatic Primary Consumers
- —●— Riparian Primary Consumers

y-axis: $\delta^{15}N \pm SE$ (0, 2, 4, 6, 8, 10, 12)

Data labels (Aquatic): BCDEF, ABC, BC, ABC, ABC, AB, A
Data labels (Riparian): DF, D, EFG, G, DEF, EG, CDEF

x-axis:
| Castle River 1195 m 30 m | Pincher Creek 1126 m 20 m | Summerview 1040 m 95 m | Blazingstar 909 m 94 m | St.Marys River 855 m 35 m | Cottonwood 837 m 113 m | Pavan 816 m 120 m |

B)

y-axis: $\delta^{13}C \pm SE$ (-24, -25, -26, -27, -28, -29, -30, -31, -32, -33)

Data labels (Riparian): A, ABD, ABCD, A, A, A, AB
Data labels (Aquatic): E, ABCDE, E, CDE, BCDE, BCDE, CE

- —○— Aquatic Primary Consumers
- —●— Riparian Primary Consumers

x-axis:
| Castle River 1195 m 30 m | Pincher Creek 1126 m 20 m | Summerview 1040 m 95 m | Blazingstar 909 m 94 m | St.Marys River 855 m 35 m | Cottonwood 837 m 113 m | Pavan 816 m 120 m |

Figure 2.7: A) Nitrogen ($\delta^{15}N$) and B) Carbon signatures ($\delta^{13}C$) of riparian primary consumers and aquatic primary consumers as a function of elevation (m) and site with river width (m). Riparian primary consumers are denoted by shaded circles (●) and aquatic primary consumers are denoted by open circles (○). Error bars denote the standard error of the mean. Levels not connected by the same letter are significantly different. Letters are a result of post-hoc analysis of a significant interaction term. ($\delta^{15}N$: $F_{(6, 231)} = 4.12$, $p < 0.05$; $\delta^{13}C$: $F_{(6, 231)} = 3.13$, $p < 0.05$).

48

2.5.0 Discussion

2.5.1 Aquatic - Riparian $\delta^{15}N$ and $\delta^{13}C$ Signatures

Stable isotopes of carbon and nitrogen are often applied to quantify contributions of food sources to consumer diets (Phillips and Gregg 2001, Rasmussen 2010). This technique is highly effective due to the predictability of stable isotopes, as well as their time-integrated incorporation into a consumer diet (Moore and Semmens 2008, Rasmussen 2010). However, there is the potential for error when food sources or endpoints are not isotopically distinct leading to uncertainty in dietary calculations (Moore and Semmens 2008; France 1995a).

By evaluating the $\delta^{15}N$ and $\delta^{13}C$ signatures of potential food sources and considering signature variability along the river continuum (elevation, and river width) as well as local site factors, our research has shown that stable isotope analysis in the OMRB is a valid method for determining food sources to the diets of consumers. Aquatic and riparian producer $\delta^{15}N$ and $\delta^{13}C$ signatures were distinct enough to be used as endpoints in mixing model analysis; however, large variation around source signals may introduce unnecessary modelling error. Aquatic and riparian primary consumer $\delta^{15}N$ and $\delta^{13}C$ signatures were distinct for either carbon or nitrogen at all sites, and displayed a small amount of error and thus, can be used as endpoints in mixing model analysis.

2.5.2: Aquatic producer and primary consumer $\delta^{15}N$ and $\delta^{13}C$ signatures

Isotopic signatures of aquatic producers often vary along a river continuum, shifting locally as a result of abiotic impacts of differential velocity in relation to river meanders

49

(Finlay et al. 2002, Rasmussen 2010, Rasmussen and Trudeau 2010) and along elevation gradients (Rasmussen and Trudeau 2007). For example, carbon signatures of aquatic algae often exhibit a downstream gradient being more depleted at the headwaters and gradually becoming enriched downstream (Rasmussen 2010), while nitrogen signatures are often indicative of anthropogenic land-use.

Isotopic $\delta^{13}C$ signatures of aquatic producers did vary significantly among sites, however, they did not show a gradient with elevation (m) or river width (m) and thus do not support the expectation of a signature gradient. However, $\delta^{15}N$ did vary among sites as expected, although there was no indication of a long-stream gradient.

In river systems, differential velocity affects the $\delta^{15}N$ and $\delta^{13}C$ signatures of aquatic producers by increasing fractionation under high velocity from enhanced gas exchange at the boundary layer (Finlay et al. 2002, Trudeau and Rasmussen 2003, Rasmussen and Trudeau 2007). Local variability of aquatic producer signatures was likely minimized by confining collections to riffle areas of similar velocity. Further, periphyton may have been included in samples and have been shown to exhibit a weaker downstream gradient as it is generally a mixture of autochthonous and allochthonous matter such as attached algae, detritus and bacteria (Rasmussen 2010).

As this study focused predominately on middle order sections of the river (1195 m to 816 m above sea level [a.s.l]), the inclusion of headwater reaches may have shown the expected gradient more distinctively. For example, researchers sampling the Chikuma River from headwater to downstream sections near the river mouth (1978 m a.s.l to 407 m a.s.l) found both $\delta^{15}N$ and $\delta^{13}C$ signatures of periphyton, and aquatic insects are

50

significantly depleted in both carbon and nitrogen in head water sections (Akamatsu and Toda 2011).

Aquatic producer $\delta^{15}N$ and $\delta^{13}C$ signatures were within the range of literature values of $-$ 15.00 to 20.00 ‰ for $\delta^{15}N$ and $-$ 50.00 to $-$ 8.00 ‰ for $\delta^{13}C$, respectively (France 1995b, Fry 2006, Finlay and Kendall 2007). However, literature based-ranges are expansive and may not be representative when considering values of a specific river basin (Finlay and Kendall 2007). For example, in a Swiss glacier stream with elevational ranges from 1768 m to 4049 m, mean aquatic algae $\delta^{13}C$ signatures vary from $-$ 37.00 to -18.00 ‰, and - 8.00 to 2.00 ‰ for $\delta^{15}N$, respectively (Zah et al. 2001). While $\delta^{13}C$ isotopic signatures on the Toyo River, Japan ranged from $-$ 18.60 to $-$ 13.10 to ‰ from mountain (1172 m) to lowland sections (600 m); they did not report $\delta^{15}N$ signatures (Kobayashi et al. 2011).

Variation in aquatic producer $\delta^{15}N$ signatures is often associated with differential nitrogen sources and anthropogenic inputs. The $\delta^{15}N$ signatures of aquatic producers were at the high end of the literature values (enriched), which may reflect the influence of agricultural activity, some of it consisting of intensive animal production, combined with domestic inputs (Brinkmann and Rasmussen 2012). For example, benthic nitrate respiration has been shown to increase with the increasing enrichment of nitrate and dissolved organic matter which is often linked to agricultural inputs (Brinkmann and Rasmussen 2012).

Aquatic primary consumer carbon isotopic signatures varied slightly (although not significantly) across all sampling sites, a reflection of carbon source and uptake mechanisms, and further there was no effects of along-stream gradients. The nitrogen

51

isotopic signatures for aquatic primary consumers did vary across sites. The effects of an along-stream gradient were apparent in the $\delta^{15}N$ signatures of aquatic primary consumers and there was an interaction between site elevation (m) and river width (m). This pattern is a consequence of a higher volume of ^{15}N enriched loadings contributing to the watershed with increased anthropogenic influence downstream where the river widens and elevation decreases.

Overall, nitrogen signatures of aquatic primary consumers were enriched compared to aquatic producers, which generally reflects their uptake of anthropogenic nutrients associated with high intensity farming and waste water inputs. Aquatic primary consumers from the Pavan site were relatively enriched in nitrogen (11.60 ‰) compared with all other sites. This is due to its location downstream (816 m elevation) of the Lethbridge Waste Water Treatment Plant (Brinkmann and Rasmussen 2012). This is the widest river width (120 m) for all the sampling locations, and thus, a high order stream (by the Strahler stream order classification method) in which all basin tributaries feed. The Castle River site, on the other hand, is relatively depleted in both N and C it is closest to the head waters (1195 m elevation) of the Oldman River Basin (4.36 ‰ and -30.90‰, respectively). For example, the closest carbon average value at the Blazingstar site is over 95 % more enriched in carbon than the Castle River site.

The $\delta^{13}C$ isotopic signatures of aquatic primary consumers did not resemble the signatures of aquatic producers at several locations, which is a significant departure from the expectation. Typically, aquatic primary consumers are isotopically enriched

compared to both riparian primary consumers and aquatic producers whereas, our result differ from this pattern.

Riverine food webs are highly complex systems, both spatially and temporally (Finlay and Kendall 2007). Temporal variation likely did not play a role in the departure of isotopic signatures from normal expectations as both aquatic primary consumers and aquatic producers were sampled simultaneously and further, producer signatures were also recorded during winter; thus it is unlikely that the discrepancy between aquatic primary consumer and producer signatures is due to temporal bias in averaging. Further, the longitudinal gradient from headwaters to downstream environments described first by the River Continuum Concept (Vannote et al. 1980), and by isotopic signature analysis (Rasmussen 2010), also should not play a large role in variation of isotopic signatures due to the small spatial scale of the study.

Aquatic primary consumer carbon signatures were depleted compared with aquatic producer signatures, which is a departure from the typical expectation. Algal growth in the OMRB is fairly dense, and thus, heavy signatures may be reflective of carbon limitation in these dense growths. There is strong support that increased chlorophyll standing stock enriches carbon signatures by increasing nutrient uptake (Hill and Middleton 2006, Rasmussen and Trudeau 2007, Woodland et al. 2012). For example, the $\delta^{13}C$ signatures of dense algal mats are more enriched compared to thin diatom films under similar velocities (Rasmussen and Trudeau 2007). Further research has shown that periphyton $\delta^{13}C$ signatures became enriched as chlorophyll standing stock increases over time (Hill and Middleton 2006). Moreover, invertebrate grazers are unable to feed on

dense algal mats, and thus, algal signatures may not accurately reflect invertebrate food sources. For example, the dominant aquatic invertebrate collected was a net spinning Trichoptera (*Hydropsychidae*) which may, in fact, feed on suspended river seston, which was not included in our analysis of putative food sources. River seston is particulate suspended matter that can be made up of inorganic materials and organic materials such as plankton, and detritus. Dense algal growth and increased chlorophyll standing stock is likely responsible for the observed magnitude of difference in aquatic primary consumer and producer signatures.

Anthropogenic disturbance from excessive nutrient inputs associated with agricultural land-use, fertilizers and animals waste products have been recognized within the Oldman River Basin (Rock and Mayer 2007). A study conducted by Brinkmann & Rasmussen (2012) found an increase in chlorophyll *a* concentrations with the downstream gradient in the OMRB from 0.72 ± 0.39 $\mu g/$ L^{-1} at the Summerview site to 2.32 ± 1.59 $\mu g/$ L^{-1} near the Pavan site coinciding with increased nutrient loadings. The authors concluded that increased algal production is occurring at lower elevations in the OMRB; thus, decreasing the ability of invertebrate grazers to consume increasingly dense algal blooms and likely causing enrichment in aquatic producer $\delta^{13}C$ signatures.

Discerning the dietary importance of local producer food sources to consumers is often difficult and based primarily on assumptions. A study conducted in Plum Island Estuary, Massachusetts found that phytoplankton was more enriched in carbon than *Manayunkia aestuarina,* a suspension feeding polychaete known to consume phytoplankton based on previous isotopic and gut content analysis. The authors concluded that mixing model

analysis could not be conducted without further resolution of food sources and asserted that using isotopic additions in combination with natural stable isotopes could further decrease source uncertainty error (Galvan et al. 2008, Galvan et al. 2011) However, the use of multiple stable isotopes such and carbon and nitrogen often negates the uncertainty associated with a single isotope model and provides more accurate source proportion estimates than isotopic addition alone (Galvan et al. 2011).

2.5.3 Above and below dam $\delta^{15}N$ and $\delta^{13}C$ signatures in the Oldman River Basin

Water storage facilities that control riverine flows in arid landscapes such as in the South-West United States and the plains of Canada, have been shown to considerably alter ecosystem conditions in downstream tail waters of lotic habitats. Dams modify physicochemical conditions, temperature regimes, sediment and nutrient loadings and flow variability, ultimately impacting downstream biotic communities (Blinn et al. 1998, Saito et al. 2001).

The Oldman River Basin is flow controlled, and delineated by several water storage facilities with a great proportion of the water allocated to irrigation. Hypolimnion release dams, such as those located in the OMRB, discharge water from the bottom layer of a thermally stratified reservoir (i.e. they do not circulate). Hypolimnion release dams have implications on the riverine ecosystem as the discharged water remains at a constant temperature (thus, warm in the winter, and cold in the summer relative to surface waters) of approximately + 4°C, which impacts diel and seasonal water temperature regimes. Water released from the bottom layer of a reservoir is oxygen-poor, with depleted

amounts of inorganic particles, as well as fine and course particulate matter from increased settling time.

Physiochemical properties and nutrient cycling regimes become modified impacting both downstream floral and faunal communities and their evolutionary interactions (Blinn et al. 1998). For example, benthic algae growth increases below dams from increased sunlight penetration as released water is clearer from a decrease in suspended sediment and the substrate is more stable. Subsequently, the downstream habitat recovers from these impacts with increasing spatial distance from the water storage facility. Natural flow restoration experiments on the Spol River, Switzerland found that debris flow occurs at 250 m below the dam, and sediment scouring is minimal further downstream from this point (Uehlinger et al. 2003). The downstream distance at which 100 % recovery occurs is variable, and has been shown to range from 4 to 125 km from a review examining 21 dams constructed in the United States (Willliams and Wolman 1984).

Typically, $\delta^{13}C$ signatures show depletion in the tail waters of such a facility as exchange with atmospheric CO_2 will have decreased considerably during settling time in the reservoir; while $\delta^{15}N$ signatures are often enriched as ammonium is deposited and fixed by ammonificating bacteria creating biologically accessible nitrogen compounds (Vander Zanden and Rasmussen 1999, Tonno et al. 2005).

Our results do not support the expectation that carbon isotopic signatures would be more depleted downstream of a hypolimnion release dam. However, the expected enrichment of nitrogen isotopic signatures was observed in the tail waters below the dam, thus partially supporting our hypothesis.

Sampling years coincided with high flow events, and data from the Water Survey of Canada shows that the volume of the Oldman Reservoir was normal at 309,443 dam^3 (63 % of capacity) in 2008, while the volume reached 1,022,000 dam^3 in 2010 (200 % capacity). Furthermore, overall daily discharge of the Oldman River near Brocket (station #: 05AA024) increased from 34 m^3/s in 2008, to 40 m^3/s in 2010, with a total discharge increase from 12, 500 m^3/s to 14, 500 m^3/s, respectively.

The increase in discharge corresponds to an increase in spillway release events, which might be expected to reduce the impact of hypolimnic water downstream of the dam. The Oldman Reservoir is also rather oligotrophic (Mitchell 2001) and often poorly stratified as a result of high water throughput. The conditions favouring hypolimnic accumulation of CO_2 and NH_3 from organic matter decomposition were not likely present (Vander Zanden and Rasmussen 1999).

A limitation to testing the hypothesis of depletion of carbon signatures and enrichment of nitrogen signatures in tail waters is the uneven sample sizes decreasing the power to detect statistical significance, after accounting for variances. However, group means show that carbon signature differs only slightly by 1.96 ‰, and thus, the likelihood of a type II error is low. Also, nitrogen testing may be considered more conservative as it is difficult to find statistical significance with such low power.

2.5.4 Riparian producer and primary consumer $\delta^{15}N$ and $\delta^{13}C$ signatures

The $\delta^{15}N$ signature of riparian producers is a reflection of the source of nitrogen, which may differ based on climate, geographic origin or land use. Overall, the $\delta^{15}N$ signatures

of riparian producers in the OMRB differed among sites and with elevation (m) which may reflect differences in agricultural influence which increases nutrient availability and loading (Brinkmann and Rasmussen 2012), and/or the influences of plants that support N-fixing nodules (Virginia et al. 1989, Bedard-Haughn et al. 2003). The $\delta^{15}N$ signatures of C_3 riparian producer samples collected in the OMRB were within the global literature range (- 3.00 to 7.00 ‰) for C_3 riparian vegetation (Finlay and Kendall 2007).

The carbon signatures of riparian producers collected along the river gradient in the OMRB did not vary among sites, as expected, and exhibit no along stream gradient or other detectable patterns. The overall similarity of the riparian $\delta^{13}C$ signatures indicate that photosynthetic pathways and water use efficiencies are likely similar across the study area despite the elevational and climatic gradient present from the foothills to the prairies. The median literature value for Calvin Cycle (C_3) plants is between -28.00 to -27.10 ‰ which is comparable to the plants in this study (-28.06 ± 0.22 ‰) suggesting that isotopic signatures for riparian producers in the OMRB are similar to global average values for C_3 plants (Tieszen and Boutton 1989, Finlay and Kendall 2007).

Riparian primary consumer $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures differed among sampling sites indicating variation in isotopic signatures of food sources. Nitrogen signatures of riparian primary consumers were significantly affected by river width (m). Soil $\delta^{15}N$, which determines producer isotopic composition, has several inputs (nitrogen fixation, atmospheric deposition, and anthropogenic influence such as fertilizers) and may go through multiple fractionation events such as nitrification, NO_3^- immobilization and denitrification (Evans 2007).

Spatial variation among sampling sites in riparian primary consumer δ^{15}N signatures is likely due to the intensification of agricultural operations and increased sewer inputs in downstream reaches, characterized by larger river widths, and capacity increases to accommodate additional catchment tributaries (Anderson and Cabana 2009, Brinkmann and Rasmussen 2012). The Blazingstar and Cottonwood sites mean δ^{15}N signatures of riparian primary consumers were depleted compared to other sites (1.10 and 1.55 ‰, respectively); however, both sites were likely not impacted by differential sources of nitrogen in their metabolic tissues as with other sampling sites

Variability in C signatures of riparian primary consumers indicates differences in food source signatures among sites and was significant with river width (m) and site elevation (m). Nitrogen and carbon signature variability due to diet-tissue discrimination is directly related to animal metabolic physiology, assimilation vs. ingestion, food source and animal life stage (Ben-David and Flaherty 2012, Phillips 2012).

Overall, riparian primary consumer nitrogen and carbon isotopic signatures were enriched relative to riparian producer isotopic signatures. The trophic enrichment of nitrogen and carbon from diet to consumer tissues is often assumed to be ~ 3.0 and ~ 1.0 ‰, respectively. However, variation in such estimates occurs among environments, taxa, diet, and physiology; and further, only a small percentage of fractionation values actually fall within the assumed fractionation range (McCutchan et al. 2003, Phillips 2012).

Trophic enrichment estimates of δ^{15}N and δ^{13}C by primary consumers based on literature and experimental values have been shown to range from - 0.77 to 6.59 ‰ for δ^{15}N and − 3.47 to 4.28 ‰ for δ^{13}C (McCutchan et al. 2003, Spence and Rosenheim 2005, Wehi

and Hicks 2010). Our mean fractionation values from diet to consumer (i.e. plant tissue to herbivorous insect) falls within this range at 1.30 ± 1.14 ‰ for $\delta^{15}N$ and 1.97 ‰ ± 1.08 ‰ for $\delta^{13}C$.

There was no effect of distance upslope from the river in the $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures for riparian primary consumers with perpendicular distance from the river shoreline and there were no interactive effects observed with site and distance. This indicates local factors have no significant effect on isotopic signatures of riparian primary consumers.

2.5.5 Validity of mixing model analysis

In order to perform dietary modelling, putative food sources or endpoints must be distinct. Available food sources included aquatic and riparian primary consumers as well as aquatic and riparian producers. Site specific comparisons of $\delta^{15}N$ and $\delta^{13}C$ mean isotopic signatures of aquatic and riparian producers, and consumers were conducted to evaluate signature patterns among sites for similar biota.

Aquatic producers were significantly enriched in both $\delta^{15}N$ and $\delta^{13}C$ compared with riparian producers. Aquatic primary consumers were slightly enriched in $\delta^{15}N$ relative to riparian primary consumers; although carbon isotopic signatures of aquatic consumers are significantly depleted relative to riparian primary consumers.

Primary producers may not be explicitly useful in mixing model analysis due to limiting site and along stream factors which increased variation around the source signature. Isotopic signatures of riparian producers are influenced by several factors including soil

moisture, temperature, photosynthetic rates, stomatal conductance and water source creating signature variation (Ben-David and Flaherty 2012). Aquatic producers and detritus have been shown to display a high amount of temporal variation in $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures and thus, their use as baseline indicators is often prone to error (Cabana and Rasmussen 1996, Post 2002). A primary producer baseline is insufficient for mixing model analysis due to variability from differential carbon and nitrogen sources, and fractionation for this study.

On the other hand, aquatic and riparian primary consumers were sufficiently distinct for either carbon or nitrogen at all sites due to their source signature differences. Therefore, mixing model analysis can be performed using these endpoints without source uncertainty.

2.6.0 Conclusion

Evaluation of aquatic and riparian biota in the OMRB has implications for food web studies involving higher consumers in riparian communities. Distinct isotopic signatures depend upon local factors, as well as factors along the river continuum.

Unexpectedly, aquatic producer signatures did not show variability in $\delta^{13}C$ among sites, or show any significance with along stream gradients. This is partly due to the standardization of samples by collecting from areas of constant velocity, and the focus of sampling in the middle sections of the river basin. Aquatic primary consumer $\delta^{13}C$ isotopic signatures were similar among sampling sites as the spatial scale over which sites were located was quite small reflecting analogous carbon sources and uptake.

However, aquatic producer and primary consumer N signatures varied among sites and consumer nitrogen signatures were affected by a long-stream gradient. The site elevation (m) and river width (m) interaction is due to the geographic changes over the river gradient, where higher elevations with smaller river widths are less likely to be affected by anthropogenic influences. Downstream at lower elevations intensive livestock operation, sewage outflows and anthropogenic developments become more intense as river width increases to accommodate an increase in tributaries entering the river.

Aquatic producers and primary consumers differed significantly in isotopic signature; however, aquatic primary consumer carbon signatures were depleted relative to aquatic producers. This unexpected result indicates that aquatic primary consumers may rely on a secondary food source that was not identified.

Water storage facilities often affect isotopic signatures of riverine biota; however, our hypothesis was only partially supported. Our results did not support the expectation of $\delta^{13}C$ depletion downstream of a hypolimnion release dam, however, expected enrichment of $\delta^{15}N$ isotopic signatures was observed. The unexpected $\delta^{13}C$ results is likely due to a combination of increased spillway release events reducing dam impacts and a decrease in abiotic factors that favour hypolimnic accumulation.

Riparian producer $\delta^{13}C$ isotopic signatures supported the expectation of little variation among sites or over a gradient due to similar photosynthetic uptake mechanisms. Riparian producer $\delta^{15}N$ signatures, however, showed variation among sites and with site elevation (m), which is likely caused by anthropogenic inputs along the river gradients as with aquatic algae $\delta^{15}N$ signatures.

The δ^{15}N and δ^{13}C isotopic signatures of riparian primary consumers differed among sampling sites indicating variation in isotopic signatures of food sources; however, their signatures showed no significant difference inland from the shoreline indicating no presence of limiting site factors.

Both riparian and aquatic producers and riparian and aquatic primary consumers were compared on a site-by-site basis. Producers have limited capability as mixing model endpoints as the fractionation, and overall carbon and nitrogen source variability throughout the river basin may generate excessive uncertainty in mixing model calculations.

However, the site-specific analysis of isotopic signatures of riparian and aquatic primary consumers showed a consistent distinction for either C or N at all locations. This study reveals the potential for an applied food web analysis of riparian consumers in the OMRB using aquatic and riparian primary consumers as baseline indicators (Phillips and Gregg 2003).

CHAPTER THREE

Aquatic Insects as an Energetic Subsidy to Riparian Consumers: the Scale of the Effect

and its Relationship to River Size

3.1.0 Introduction

Energetic inputs from adjacent habitats, such as resource fluxes, can enhance the structure and dynamics of the food web (Polis and Strong 1996, Sabo and Power 2002, Marczak et al. 2007a). Energy in the form of nutrients, organisms and detritus influences the local food web by providing an alternate food source to predators and prey, thereby, increasing stability in the food web (Huxel and McCann 1998, McCann et al. 2005, Marczak et al. 2007b). The model developed by Polis and Strong (1996) highlighted the theoretical idea that resources can be acquired by a food web through alternative pathways such as energetic exchanges.

Allochthonous inputs can be important in a variety of habitats, and may involve several biological vectors. Riparian inputs to lotic or flowing systems include leaf litter in forested watersheds (Petersen and Cummins 1974, Vannote et al. 1980), riparian invertebrate deposition, or movement of prey species (Bergfur et al. 2009). At the same time, aquatic input to the riparian ecosystem in the form of algae, dry deposition of detritus (Bastow et al. 2002), or predator-prey movement (Huxel and McCann 1998, Baxter et al. 2005) may also be important.

Understanding energetic inputs has only recently become a major focus of food web studies. The flux of prey from an aquatic ecosystem can be a major energetic input to the riparian ecosystem. For example, emerging aquatic insects are an important biological

vector for energetic transfer (Collier and Smith 1997, Briers et al. 2005, Fukui et al. 2006). Adult aquatic insects emerge in large numbers in order to complete their life-cycles in the riparian ecosystem, which includes swarming and mating (Baxter et al. 2005). These aquatic insects subsidize a number of riparian predators such as birds (Nakano and Murakami 2001, Iwata et al. 2003), lizards (Sabo and Power 2002), bats (Fukui et al. 2006), predatory beetles (Paetzold et al. 2005) and spiders (Collier et al. 2002, Sanzone et al. 2003, Kato et al. 2004, Briers et al. 2005).

Stable carbon and nitrogen isotopes have been used to quantify the contribution of aquatic insects to riparian consumers. These studies have involved both tracer addition as well as natural abundance levels of stable isotopes (Power and Rainey 2000, Henschel et al. 2001, Collier et al. 2002, Sanzone et al. 2003, Briers et al. 2005).

A study conducted on the North Island of New Zealand using natural abundance isotopes showed that web-weaving spiders and ground-dwelling spiders derived 61 % and 55 % of their dietary carbon from aquatic insects, respectively (Collier et al. 2002). Sanzone et al. (2003) used labelled $^{15}N\text{-}NH_4$ to trace the dietary proportion to the diet of web-weaving and ground-dwelling spiders over a 6 week period in the Sonoran desert, Arizona. They found that emerging aquatic insects comprised 100 % of dietary carbon, and 39 % of dietary nitrogen to web-weaving spiders; while ground-dwelling spiders derived 68 % of carbon and 25 % nitrogen from aquatic insects.

Although aquatic contributions to consumers immediately adjacent to streams have been quantified, the spatial extent of a contribution has received less attention. That is, how far away from the river does the aquatic insect contribution extend?

Power and Rainey (2000) concluded that web-weaving spiders in northern California derived about half of their dietary carbon from aquatic insects, even hundreds of meters away from the 30 m wide river (Power and Rainey 2000). Using natural abundance isotopes Sanzone et al. (2003) found that the spatial distribution of spiders was dependent on the availability of aquatic prey, with higher abundance and biomass of consumers within 50 m of the stream edge which decreased more than three-fold by 25 m from the shoreline. Further, they found spiders collected in riparian (10 m) and upland (25 - 50 m) areas were subsidized less by aquatic insects than spiders collected at the shoreline. Briers et al. (2005) used a stable isotope enrichment of ^{15}N to quantify the proportion of emerging aquatic insects in the diet of riparian spiders in an unproductive upland habitat. Using tracer addition of ^{15}NH$_4$Cl on a 4 m wide headwater stream they found 40 % of the diet of riparian spiders was composed of aquatic insects adjacent to the stream which declined to 1 % at 20 m from the stream edge.

Although energetic inputs strongly impact riparian consumers immediately adjacent to the lotic system, this influence may be spatially restricted as aquatic insect supply decreases with distance from the river (Baxter et al. 2005). The degree of penetration of aquatic insects into the riparian forest after emergence may be considerably variable depending on the taxa of aquatic insect and the pattern of movement after emergence (Jackson and Resh 1989, Power et al. 2004). Adult stoneflies, for example, emerge onto land and may crawl or fly a substantial distance to mate, whereas mayflies will swarm within a few meters of the water surface to perform aerial copulation and thus, are spatially restricted unless passively dispersed (Clifford 1991, Wesner 2010).

Several studies have indicated that the abundance of aquatic insects decreased significantly within 20 - 25 m from the edge of the streams that are approximately 2 - 10 m in width (Iwata et al. 2003, Baxter et al. 2005). Further, taxonomic compositions of organism-based subsidies may alter the dispersal of aquatic insect adults. A study by Jackson and Resh (1989) found that the taxa of emerging aquatic insects caught at stream height did not disperse upland as much as taxa trapped at the canopy-level. Wesner (2010) asserts that aquatic insect consumer's and non-consumer's behavioural differences further affect their dispersal from the stream edge.

Geographic and landscape characteristics influences the extent of dispersal by aquatic insects upland from a water body (Kovats et al. 1996, Collier and Smith 1997). Vegetative type and cover may influence dispersal patterns of aquatic insects. For example, riparian forests are productive habitats which offer a refuge from predation pressure and meteorological conditions such as high wind and insolation. Long distance dispersal of mayflies and caddisflies (> 5 km) have been reported, however, this has been suggested to be facilitated by wind and a lack of vegetative cover (Kovats et al. 1996).

Further, aquatic insect emergence is greater from larger tributaries with high primary production in the channel from factors such as rich sedimentary layers, and high accessibility of nutrients. Power et al. (2004) showed that emergence by aquatic insects is 3 to 6 times greater from floating algal mats compared with submerged algae or bare gravel. Algal mats offer minimal exposure of ovipositing females, protection from aquatic predators, optimal temperatures and a rich food source. They concluded that landscape features which offer protection from predators enhances emergence and

abundance thus increasing the biomass of aquatic insects into upland habitats (Power et al. 2004).

Aquatic - riparian exchange via emerging aquatic insects should be dependent on ecosystem geometry, that is, the size of the lotic habitat; however, empirical evidence to develop the general framework for understanding the relationship between river width and aquatic insect emergence is lacking. It has been suggested that area to perimeter ratios, a large lotic habitat with less shoreline, generally produce larger amounts of emerging aquatic insects and thus, the potential for further dispersal upland (Henschel 2004, Gratton and Vander Zanden 2009).

Gratton and Vander Zanden (2009) simulated aquatic insect fluxes from data collected from literature to model the insect deposition rate into riparian habitat from lakes and streams. Large lakes (1300 ha) and streams (16 m width) have high insect deposition rates ($\sim$0.01-2.4 g Cm^{-2} yr^{-1}) comparable to productive grassland ecosystems; while average sized lakes (10 ha) reflect insect deposition rates of low productivity ecosystems such as deserts ($\sim$ 0.07 g C m^{-2} yr^{-1}), and insect flux rates for average sized streams (4 m width) is negligible (0.002 g C m^{-2} yr^{-1}). They concluded that the ecosystem size (stream half-width or lake radius) to shoreline is a determining factor in total export of emerging aquatic insects to riparian areas. Others have speculated on the relationship between river width and spatial dispersal by aquatic insects (Henschel 2004), however, empirical evidence has not been reported.

Headwater streams are often characterized by small channel widths, low *in situ* primary production due to lack of sunlight penetration, and a larger reliance on the input of

organic matter from the riparian ecosystem. According to the river continuum concept, as stream size and relative channel width increase further downstream, *in situ* primary production becomes more significant than input from riparian vegetation and riparian habitat boundaries are more difficult to define (Vannote et al. 1980). Larger order streams, defined as those that include other tributaries, often branch into braided portions, and delineate the landscape through a series of meanders, thereby increasing the overall area of riparian habitat.

In the Oldman River Basin, rivers flow through foothill - prairie transition zones and forests in these areas tend to be confined to river valleys. Further, high insolation and rich soils often support modernized agricultural operations in upland habitats which have caused a decline in landscape features such as buffer strips, hedgerows and wetland swales. Riparian invertebrates from several taxonomic families (Heteroptera, Cicadellidae, Opiliones, Delphacidae, and Arionidae) have decreased density in grazed homogenous grassland habitats compared to fenced riparian margins with high biodiversity (Cole et al. 2012).

Confined valley riparian forest communities in the OMRB may significantly support the flora and fauna of the region as a great proportion of land-use is directed towards the agricultural industry (Brinkmann and Rasmussen 2012). Riparian forest communities support the influx of nutrients, detritus and organisms from high river productivity and may significantly subsidize the food web. As such, riparian forest habitats may be considerably more important in the OMRB and other semi-arid grassland regions, where adjacent upland habitats have low overall biodiversity being composed of homogenous grass or croplands.

Estimates of energy flow across aquatic - riparian boundaries are limited in riparian floodplain habitats, although interactions along riparian river gradients are considerably more productive than adjacent upland habitats.

3.2.0 Objectives

In this study, the spatial variability in aquatic insect subsidization to riparian spiders and beetles from the shoreline to upland habitat is examined and related to stream size in an attempt to answer the question: is the scale over which an aquatic subsidy occur a function of the size of the lotic habitat in semi-arid grassland ecosystems?

In foothill-grassland transition zones, river width can vary considerably from headwaters to downstream reaches. (Vannote et al. 1980, Rasmussen 2010). Large rivers support benthos production as upstream processing of organic matter and debris enhances resource availability and increased light attenuation improves algal and macrophytic growth (Vannote et al. 1980, Benke and Huryn 2010). Riparian spiders and beetles should be subsidized by aquatic insects further upland adjacent to large water bodies compared with smaller streams as increased benthos production should produce a higher number of dispersing emerging adult aquatic insects. Our analysis will be able to test for a relationship between river width and the extent of subsidization by regressing 10% threshold of aquatic influence to river width using both $\delta^{15}N$ and $\delta^{13}C$ inverse predictions of aquatic insect subsidization with distance from the river.

Using a classic two endpoint mixing model, treating carbon and nitrogen independently, we quantified the contribution of aquatic and riparian food sources to riparian spiders and beetles at several distances from the shoreline. Riparian spiders and beetles are likely

subsidized by aquatic insects within the 5 – 10 m of the water source with a decline with distance from the shoreline (Iwata et al. 2003, Power et al. 2004, Baxter et al. 2005) . Riparian beetles may show a more rapid decline in aquatic derived diets as they are less mobile than riparian spiders. However, the mobility of the emerging aquatic insects will largely determine the extent of an aquatic contribution.

We tested for effects of spider guild (web-weaving vs. ground-dwelling) on isotopic signatures to infer feeding and prey capture strategy. Although capture strategies of these guilds are dissimilar, riparian spiders are strictly carnivorous predators feeding on invertebrate insects and other arthropods (Wise 1993).

Orographic lifting on the windward slopes of the Rocky Mountains produce warm, dry and often turbulent winds on the leeward side called Chinooks in southern Alberta. High occurrence of Chinook winds facilitating dispersal of aquatic insects will likely increase web-weaving spiders ability to capture prey at greater extents, and thus, we might expect that δ^{13}C and δ^{15}N signatures of riparian spiders will not markedly differ from each other, although this will not account for among-site variability that may occur from differential sources of carbon and nitrogen (i.e. atmospheric gases, agricultural influence, sewage inputs etc.) and fractionation.

This study is novel in that it attempts to quantify an energetic subsidy to spider and beetle consumers in the OMRB, and uses a comparative approach to model the relationship between river width and subsidization.

3.3.0 Methods

3.3.1 Study Area

Field work was conducted during the summer of 2009 and 2010 (early June to late November) at 7 sites in the OMRB, an area of 26,700 km^2 in the South-West corner of Alberta, Canada (See figure 2.1). These sites are located within the mixed grass natural sub-region of Alberta with an annual precipitation of 410 mm, a mean annual temperature of +3 °C and an annual insolation of 4600-4800 MJ/m^2. Spring snowmelt from headwater reaches is regulated by the Oldman Dam and the St. Mary River dam. The substrate of the river beds in the Oldman River basin consist mainly of gravel and cobble, with frequent substrate rearrangement from high annual flows.

The riparian cottonwood (*Populus* spp) forests adjacent to the sites are confined to river valleys, and extend approximately 1 km into upland habitat and are located approximately 1 – 3 m above the level of the river. The cottonwood forests in the OMRB may include such species as balsam poplar *(Populus balsamifera)*, narrowleaf cottonwood *(Populus augustifolia)*, and sparse amounts of Plains cottonwood *(Populus deltoids)* as well as a hybridization of any of these species. The Castle River site hosts the cooler species trembling aspen *(Populus tremuloides)* (Samuelson and Rood 2004, Willms 2005, Willms et al. 2006).

The forest understory is dominated by common shrubs such as rose (*Rosa* spp), snowberry (*Symphoricarpos occidentalis*) and willow (*Salix* spp). These areas also contain of a wide array of grass species such as wheat grasses (*Agropyron* spp), and invasive grasses (*Bromus tectorum, Poa pratensis)*.

Land use is mainly agricultural with intensive livestock operations such as feedlots. Previous research has indicated substantial organic loadings from discharging sewage effluent and animal waste (Brinkmann and Rasmussen 2012). Riparian sites consist of insect and arthropod taxa including but not limited to European ground beetles (Carabidae), leaf hoppers (Homoptera) and spiders (Lycosidae, Salticidae) including harvestmen (Opiliones). Emergent aquatic insects included but were not limited to, caddisflies (Trichoptera), stoneflies (Plecoptera) and mayflies (Ephemeroptera).

Extensive literature on regional river hydrology, riparian woodlands and other relevant topics are available for the OMRB (Hyland et al. 2003, Byrne et al. 2006, Rock and Mayer 2006, 2007, Rood et al. 2008, Birks and Gibson 2009, Rock and Mayer 2009, Brinkmann and Rasmussen 2010, Poirier and De Loe 2011, Warnock et al. 2011, Brinkmann and Rasmussen 2012).

Aquatic and riparian insects (primary consumers) as well as riparian arthropods (spiders) were collected in 2009 and 2010. Aquatic invertebrates were haphazardly sampled from riffles, which are areas of similar velocity, among the sampling sites using hand-held dip nets.

Riparian insects were sampled along transects established at each site that runs perpendicular to the shore using a standard sweep net, and pit fall traps. Transects extended to distances twice the width of the river and sampling points were spaced at equal intervals.

Whole samples were stored in coolers and frozen within 4 hours of collection until sorting and identification into family (Clifford 1991, Wise 1993, MacRae 2008) All

spider and beetle samples included up to 30 individuals collected from the same site, at the same distance from the river, on the same day.

3.3.2 Stable isotope analysis

Whole samples were oven dried at 60 °C for 3 days and ground into a homogenous powder using a mortar and pestle. Sample material was packaged into 4 x 6 mm tin capsules for isotopic analysis. Stable carbon and nitrogen isotopic analyses were performed on the same sample using a Costech 4010 Elemental Combustion System and Thermo Delta V Advantage (with Conflo 4 interface) (Brinkmann 2009).

Stable isotope ratios are expressed in δ, delta notation, as a ratio relative to a standard. The internationally recognized standard for carbon is Vienna PeeDee Belemnite and, atmospheric N_2 for nitrogen, which were expressed as:

$$X = \left[\left(\frac{Rsample}{Rstandard}\right) - 1\right] * 1000 \qquad \textit{Equation 3.1}$$

Where X is carbon or nitrogen, and R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Samples with higher heavy isotope ratios than standards were termed "enriched" whereas those with lower heavy isotope ratios were termed "depleted". Mass spectrometry and quality assurance methods are described in Brinkmann 2009.

3.3.3 Dietary Analysis

The composition of mixed diets was determined using mixing models based on weighted averaging (Vander Zanden and Rasmussen 2001, Phillips and Gregg 2003). The relative

proportions of food sources to the diet of riparian spiders and beetles were calculated using the formula:

$$P_T = \frac{Y_C - f - Y_A}{Y_T - Y_A}$$ *Equation* 3.2

where P_T is the proportion of riparian insects to a diet, Y_C is the signature of the consumer (mixture), Y_T and Y_A are the signatures of the food sources and f represents the fractionation in the signature (Rasmussen 2010). Error from the mixing model was estimated by generating normally distributed operators with a mean of 0 and a standard deviation equal to the standard error estimate and averaging the simulations. The generated values were within 1 standard error and 1 standard deviation of the mean of the mixing model values that did not include random operator functions. This method, developed by Rasmussen (2010), considers both source and mixture variability. Mixing model analysis was performed at several distances from the river in order to establish the spatial effect of aquatic insects on consumers.

3.3.4 Statistical Analyses

Statistical computations (general linear model including one-way and two-way ANOVA, ANCOVA, and linear regression) were performed in JMP version 9.0.2 (SAS Institute Inc, Cary, North Carolina). Descriptive statistics were also performed in JMP, IBM SPSS and Microsoft Excel to assess patterns. Variables were examined for skewness, kurtosis and outliers using IBM SPSS Statistics 19. Data were transformed (log_{10} and squared) to normalize data and reduce heteroscedasticity when necessary. Variance equality was tested using Levene's test followed by a Welch's ANOVA if variance was deemed

75

unequal. Post-hoc analysis was conducted with Tukey's Honestly Significantly Different multiple comparisons. Residuals were tested with Shapiro-Wilk W and Q-Q plots for normal distribution (Whitlock and Schluter 2009, Williams 2011, Sheskin 2011). Interaction terms with no significant effects were removed to increase power in the model. If the interaction term was significant, this term was reported. Outliers were removed when caused from technical error during mass spectrometry analysis.

3.4.0 Results

3.4.1 Riparian spider and beetle δ^{15}N and δ^{13}C signatures

Riparian spiders (n = 176) were represented by 2 common guilds including web-weaving (28 %), ground-dwelling (63 %) and unknown spider samples (9 %). Web-weaving spider (n = 50) signatures ranged from 3.98 to 11.59 ‰ for δ^{15}N and -27.86 to -24.75 ‰ for δ^{13}C; similarly ground-dwelling spider (n = 111) signatures ranged from 3.99 to 11.80 ‰ for δ^{15}N and -28.44 to - 24.10 ‰. Unknown spider sample (n = 15) signatures ranged from 3.54 to 9.72 ‰ for δ^{15}N and - 28.32 to - 24.59 ‰ for δ^{13}C. Overall means for pooled spider signatures among sites ranged from 3.53 to 11.80 ‰ for δ^{15}N and - 28.44 to - 24.10 ‰ for δ^{13}C.

Web-weaving, ground-dwelling, and unknown spider δ^{15}N and δ^{13}C signatures did not significantly differ (δ^{15}N: $F_{(2, 167)}$ = 2.71, p = 0.10; δ^{13}C: $F_{(2,167)}$ = 1.51, p = 0.23). Spider signatures varied among sites (δ^{15}N: $F_{(6, 167)}$ = 3.95, p <0.05; δ^{13}C: $F_{(6, 167)}$ = 8.75, p <0.0001).

Riparian spider signatures did not significantly vary with distance from the river for δ^{15}N ($F_{(1,168)}$ = 0.53, p = 0.47), and there was no interactive effect between site and distance.

76

Signatures varied with distance from the river for $\delta^{13}C$ ($F_{(1, 162)} = 4.28$, $p < 0.05$); there was an interaction between distance from the river, and site for $\delta^{13}C$ signatures ($F_{(6, 162)} = 2.69$, $p < 0.05$).

Riparian beetles were exclusively represented by the nocturnal Carabidae family with signatures ranging from 0.29 to 9.47 ‰ for $\delta^{15}N$ and -30.20 to -22.98‰ for $\delta^{13}C$. Overall means for pooled beetle signatures among sites ranged from 2.77 to 8.22 ‰ for $\delta^{15}N$ and -27.09 to -24.25 ‰ for $\delta^{13}C$. Beetle $\delta^{15}N$ and $\delta^{13}C$ signatures varied significantly among sites ($\delta^{15}N$: $F_{(5, 35)} = 14.41$, $p < 0.0001$; $\delta^{13}C$: $F_{(5, 46)} = 4.20$, $p < 0.05$) and with distance from the river ($\delta^{15}N$: $F_{(1, 35)} = 6.86$, $p < 0.05$; $\delta^{13}C$: $F_{(1, 46)} = 4.37$, $p < 0.05$). There was an interaction between site and distance from the river for $\delta^{15}N$ ($F_{(5, 35)} = 3.17$, $p < 0.05$).

The $\delta^{15}N$ signatures of riparian beetles and spiders differed overall ($t_{(57)} = 5.11$, $p < 0.0001$), while the $\delta^{13}C$ signatures did not differ ($t_{(66)} = 0.95$, $p = 0.34$). Riparian spiders were enriched in $\delta^{15}N$ compared with riparian beetles by an average of 2.63 ± 0.24, and slightly depleted in $\delta^{13}C$ by 0.33 ± 0.10.

3.4.2 Dietary modelling of riparian spider and beetle $\delta^{15}N$ and $\delta^{13}C$ signatures

Isotopic mixing model analysis was used to identify the contribution of food sources to the diets of riparian spiders and beetles. Single isotope, two source mixing models assess the proportional contribution of food sources to animal tissue while accounting for dietary fractionation from source to consumer. Source signatures, often termed endpoints, must be distinct from one another in order to be considered as a model input. As established in chapter 2, aquatic and riparian primary consumer signatures were sufficiently distinct for mixing model analysis, with the exception of nitrogen at the

Castle river site, and carbon at the Pincher Creek site. Fractionation estimates for the model were based on average values cited from literature sources that resulted in partitioning estimates that fell mostly between aquatic and riparian reference values. At sites where the partitioning estimates were shifted out of this aquatic/riparian frame, the trophic fractionation estimates were adjusted so as to remove bias.

Overall, isotopic mixing model results indicate that aquatic insects subsidize riparian spider and beetle diets (Table 3.1). The overall mean contribution of aquatic insects to riparian spiders is 0.36 ± 0.09 for $\delta^{15}N$ and 0.25 ± 0.09 for $\delta^{13}C$; while the overall mean contribution of aquatic insect to riparian beetles is 0.20 ± 0.14 for $\delta^{15}N$ and 0.18 ± 0.12 $\delta^{13}C$.

Table 3.1: Riparian spider and beetle isotopic mixing model results averaged by site and isotope ± SE where PA is the proportion of aquatic insects to the diet of consumers. "-"represents no available data due to non-distinct isotopic signatures of food sources.

Riparian Spiders				Riparian Beetles			
Site	Type	PA	SE	Site	Type	PA	SE
Castle River	Nitrogen	-	-	Castle River	Nitrogen	-	-
	Carbon	0.14	0.09		Carbon	0.02	0.05
Pincher Creek	Nitrogen	0.26	0.05	Pincher Creek	Nitrogen	0.36	0.18
	Carbon	-	-		Carbon	-	-
Summerview	Nitrogen	0.41	0.06	Summerview	Nitrogen	0.11	0.2
	Carbon	0.25	0.08		Carbon	0.49	0.11
Blazingstar	Nitrogen	0.39	0.14	Blazingstar	Nitrogen	0.13	0.14
	Carbon	0.16	0.08		Carbon	0.03	0.12
Cottonwood	Nitrogen	0.56	0.07	Cottonwood	Nitrogen	0.22	0.15
	Carbon	0.31	0.14		Carbon	0.2	0.18
St. Mary River	Nitrogen	0.32	0.12	St. Mary River	Nitrogen	0.38	0.1
	Carbon	0.25	0.08		Carbon	0.04	0.11
Pavan	Nitrogen	0.26	0.15	Pavan	Nitrogen	0.02	0.09
	Carbon	0.41	0.11		Carbon	0.3	0.14

3.4.3 Dietary changes with distance from the river

The spatial extent of the aquatic insect contribution upland from the river decreased from 50 – 55 % at 1 m to 0 % at 30 m for both consumers using carbon, and from 35 – 40 % at 1 m to 0 % at 40 m using nitrogen.

The declining contribution of aquatic insects to the diets of riparian spiders and riparian beetles indicates the presence of a dietary trend with distance (Figure 3.1 A) & B) and Figure 3.2 A) & B), respectively). See appendix A.

3.4.4 Relationship between stream size and aquatic insect subsidization

The prediction of a value of the independent (X) variable based on a dependent (Y) variable can be effective for quantifying the relationship between the variables as regressing X on Y is not possible since it violates the primary assumptions of a regression analysis. These prediction methods are often used in clinical applications to detect a threshold value or concentration level of the target variable (Mandrekar and Allmer 2008). This is referred to as an inverse prediction in JMP software version 9.0.2 (SAS Institute Inc, Cary, North Carolina)

In this analysis, the distance away from the river that 10 % of a riparian consumer diet was made up of aquatic insects was projected using an inverse prediction. The 10 % threshold value was used to provide a fair estimate that would not occur through random chance (i.e. anything less than 10 % could have been a response to anomalous capture of aquatic insects contributing to the diet while a higher threshold value would be close to the river's edge where aquatic insects are expected to be captured).
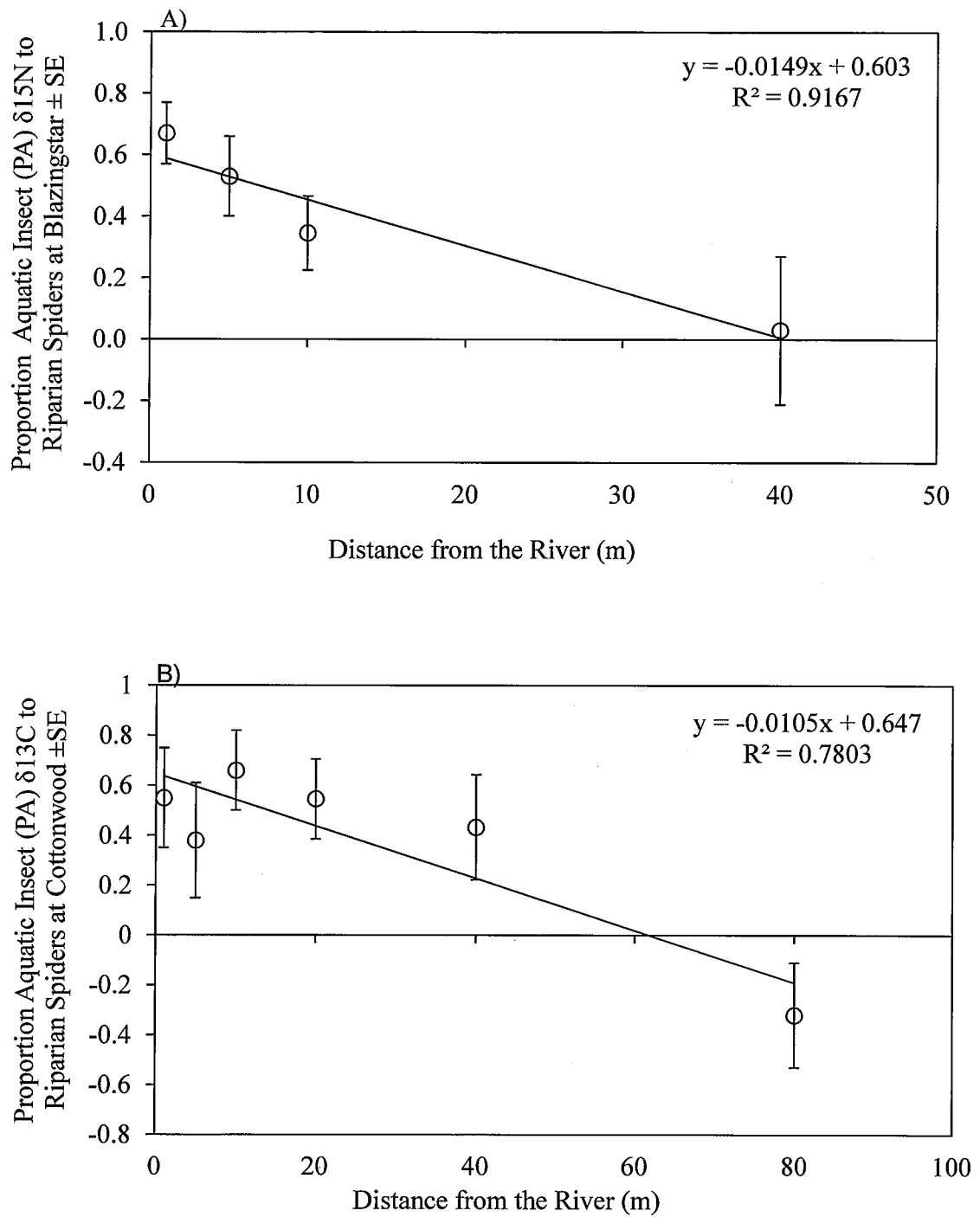
Figure 3.1: An example of isotopic mixing model results A) Nitrogen ($\delta^{15}N$) signatures and B) Carbon signatures ($\delta^{13}C$) of riparian spiders at the Blazingstar and Cottonwood sites, respectively as a function of distance from the river (m). Riparian spiders are denoted by open circles (o). Error bars denote the standard error of the mean. The overall ANCOVA for the declining contribution of aquatic insects to riparian spider diets was significant ($F_{(10, 44)} = 3.98, p < 0.05$).
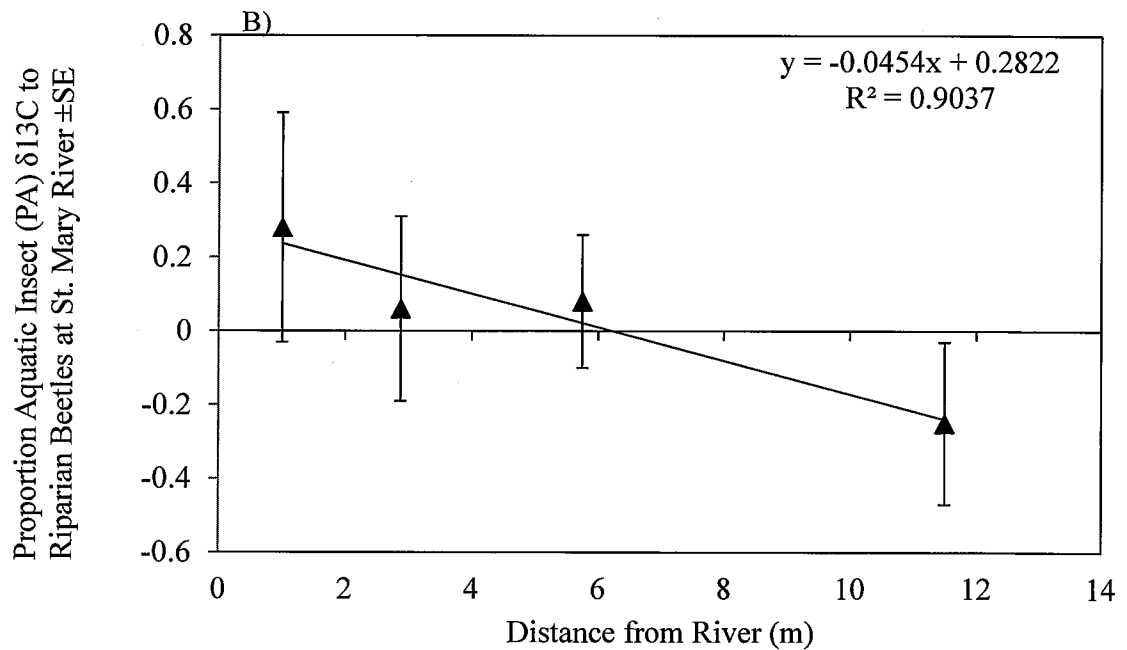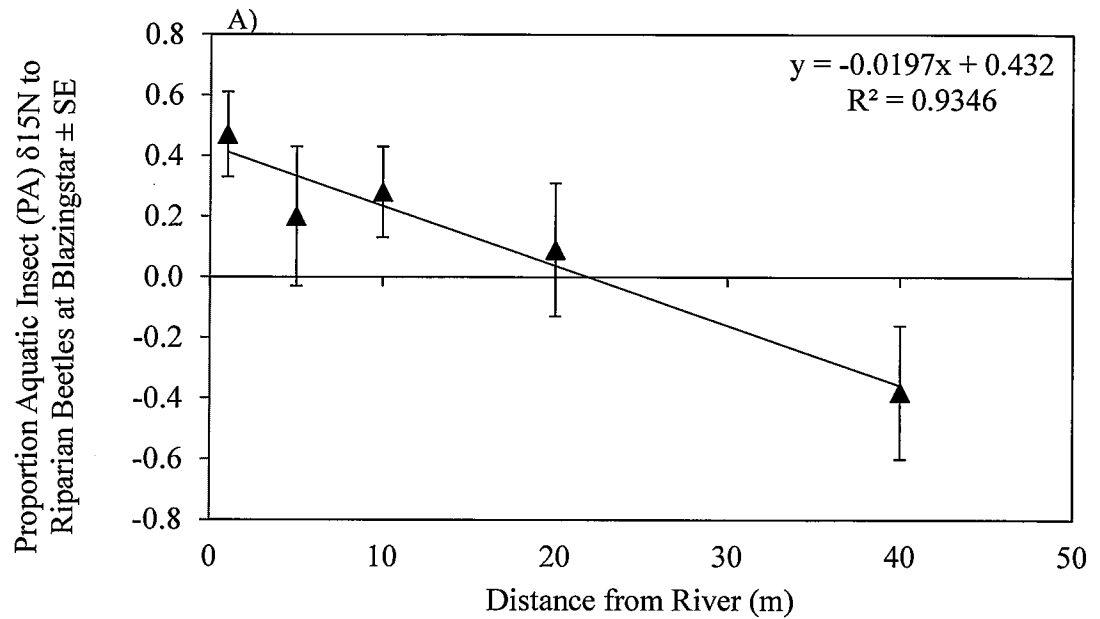
Figure 3.2: An example of isotopic mixing model results A) Nitrogen (δ^{15}N) signatures and B) Carbon signatures (δ^{13}C) of riparian beetles at the Blazingstar and St. Mary River sites, respectively as a function of distance from the river (m). Riparian beetles are denoted by shaded diamonds (♦). Error bars denote the standard error of the mean. The overall ANCOVA for the declining contribution of aquatic insects to riparian beetle diets was significant (F $_{(4, 14)}$ = 4.38, $p < 0.05$).

Inverse prediction results were termed "10 % distance threshold of aquatic influence (m)" and were regressed by the width of the river (m) at a specific site. The model included a covariate of consumer type to test for differences in riparian spiders and beetles.

The overall model (ANCOVA) was significant (F $_{(2, 11)}$ = 20.82, p < 0.001. There was a significant relationship between distance threshold of 10 % aquatic insect influence and river width (m) (Figure 3.3). Riparian consumer type, spiders and beetles respectively, significantly differed in their 10 % distance threshold of aquatic influence value (m) (F $_{(1, 11)}$ = 23.08, p < 0.001) and post-hoc analysis showed that riparian spiders and beetles significantly differed in predicted 10% threshold distance (t $_{(11)}$ = 4.80. p < 0.001).

General linear modelling provides an estimate of intercept, which is the value of Y when X is 0. The estimate of the intercept for proportion of aquatic insects to consumers at each site as a function of distance from the river (m) is equivalent to the % or proportion of aquatic influence at the shoreline. The estimated aquatic influence (%) at the shoreline was regressed by the width of the river (m) to test for difference among sites with differing river sizes.

Regression analysis showed a significant relationship between the intercept at the shoreline of aquatic influence (%) and river width (m) (Figure 3.4).
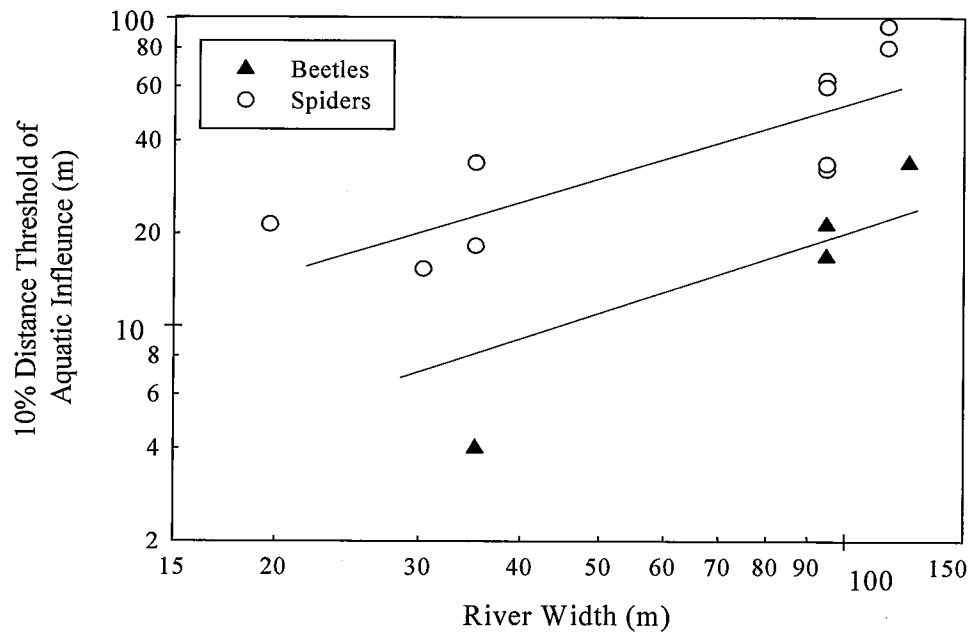
Figure 3.3: The 10% distance threshold of aquatic influence to riparian spiders and beetles produced from an inverse prediction of a general linear model (ANCOVA) of proportional dietary estimates regressed from the river width (m) of the sampling sites riparian spiders are represented by open circles (o), and riparian beetles are represented by shaded triangles (♦). Lines represent regression trends (line of best fit as produced by SigmaPlot version 12 on log scales). (F $_{(1,11)}$ = 26.28 p < 0.001).
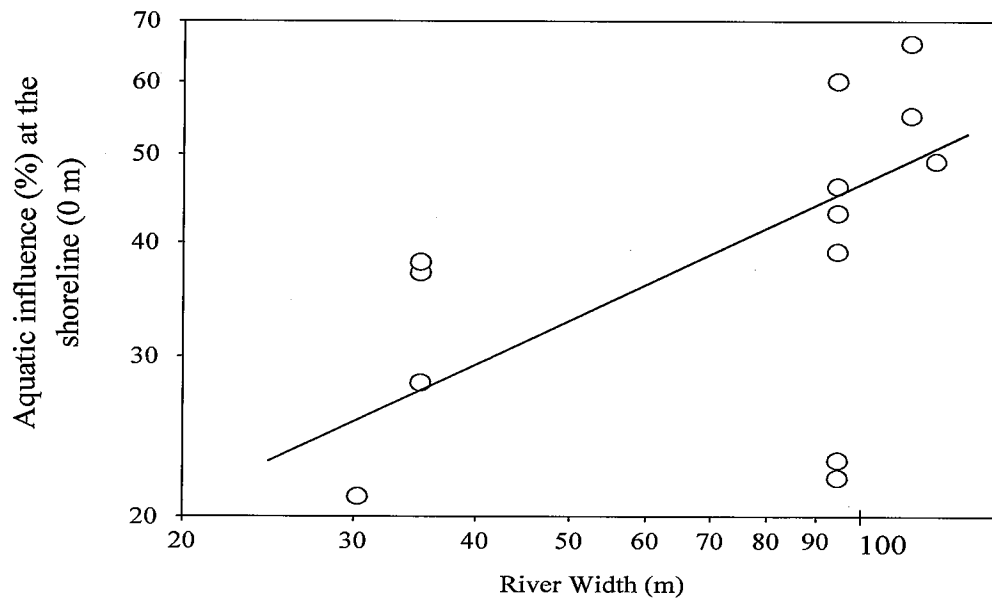
Figure 3.4: The aquatic influence (%) to riparian consumer diets at the shoreline calculated from general linear model intercepts from proportional dietary estimates regressed from river width (m) of sample sites. Riparian consumers are denoted by open circle (o). The line represents regression trends (line of best fit as produced by SigmaPlot version 12 on log scales). (F $_{(1, 12)}$ = 6.28, p < 0.05).

3.5.0 Discussion

Using stable isotopic mixing model analysis of $\delta^{15}N$ and $\delta^{13}C$, the contribution of aquatic insects to the diet of riparian spiders and beetles was quantified. Riparian spiders and beetles were observed to be more enriched in $\delta^{15}N$ than both putative food sources indicating they foraged on aquatic and riparian insects. Spider and beetle $\delta^{13}C$ closely resembled those of primary consumers demonstrating a positive indication of food sources. The results suggest that riparian consumers exploit aquatic insect emergence in peripheral freshwater-riparian habitats for nutritional subsidization.

Riparian consumer diets are composed mainly of aquatic insects near the river, which declines with distance from the river into upland habitat (Henschel et al. 2001, Collier et al. 2002, Sanzone et al. 2003, Briers et al. 2005). The isotopic signatures of riparian consumers resembled those of aquatic insects within 1 m of the river edge, and gradually shifted to 0 % between 30 – 40 m from carbon and nitrogen analysis, respectively.

These results are consistent with similar studies of aquatic insect subsidization. For example, Briers et al. (2005) found an aquatic insect subsidy, mainly by the species *L.inermis*, declined to < 1 % further than 10 m from the 3 - 4 m wide stream channel. An earlier study also estimated that 90 % of emerging aquatic insects were confined to within 10 m of the channel (Briers et al. 2002).

The proportion of aquatic insects in consumer diets directly alongside streams (1 m) was approximately 35 – 40 % for $\delta^{15}N$ and 50 – 55 % at for $\delta^{13}C$ for both consumers. Other studies have shown different estimates of aquatic insect contribution to riparian consumers immediately adjacent to streams, which indicates that the potential for aquatic

84

insect contribution varies between ecosystems (Sanzone et al. 2003, Briers et al. 2005, Mellbrand and Hamback 2010).

Mellbrand and Hamback (2010) conducted dietary mixing models on coastal predators along the shoreline of the Baltic Sea and found an overall marine contribution (Chironomid and Collembola) of 80 % to spider diets and 40 % to carabid beetle diets. On the other hand, Briers et al. (2002) showed a 40 % proportion of aquatic insects to the diet of lycosid spiders collected directly beside the River Severn in the United Kingdom. Yet, Sanzone et al. (2003) reported a smaller contribution to wandering and sit-and-wait ground spiders of 20 % and 5 %, respectively, at the intermittent stream channel near Phoenix, Arizona. They showed that wandering and sit-and wait ground spiders were less subsidized at the riparian edge (10 m) with an aquatic contribution of 5 % and 3 %, respectively.

Unlike similar studies, the mixing model performed was not based on a single type of aquatic or riparian insect prey source, thus eliminating uncertainty in isotopic estimates as riparian spiders and beetles are generalist predators. Briers et al. (2005) asserts mixing model errors are reduced when a single prey type is used as a food source as there is a decrease in the variability of the signature.

However, distinct isotopic endpoints (food sources), whether from a single prey type or a weighted average of several prey types, effectively eliminate error as the model will output a more precise estimation of dietary proportions (Phillips 2012). Error propagation calculations have been performed using the free mixing model software (www.epa.gov/wed/pages/models.htm) called IsoError (Phillips and Gregg 2001). The

researchers found that uncertainty in proportion estimation was lessened when the sources were more distinct, and analytical error is small when standard deviations are small (or sampling populations are large).

3.5.1 Dietary changes with distance from the river

The spatial extent of the aquatic insect contribution upland from the river was shown to decrease from 50 – 55 % at 1 m to 0 % at 30 m for both consumers using carbon, and from 35 – 40 % at 1 m to 0 % at 40 m using nitrogen.

Comparable studies have quantified the extent of an aquatic insect subsidy to riparian consumers, although there has been no well described pattern of decline. For example, Briers et al. (2005), describes the decline in $\delta^{15}N$ enrichment of spiders as a negative exponential function, while their interpretation of Sanzone et al. (2003) indicates a gradual decline often associated with an inverse power function. Similarly, Power and Rainey (2000) found the decline in dietary $\delta^{13}C$ for web-weaving spiders was more gradual as they were subsidized even hundreds of meter away from the 30 m wide river.

Although energetic contributions strongly impact riparian consumers immediately adjacent to the lotic system, this influence may be spatially restricted as aquatic insect supply decreases with distance from the river. The degree of penetration into the riparian forest after emergence may be considerably variable depending on the taxa of aquatic insect and the pattern of movement after emergence (Jackson and Resh 1989, Power et al. 2004). Adult stoneflies, for example, emerge onto land and may crawl or fly a substantial distance to mate, whereas mayflies will swarm within a few meters of the water surface

to perform aerial copulation and thus, are spatially restricted unless passively dispersed (Clifford 1991, Wesner 2010).

Several studies have indicated that the abundance of aquatic insects decreased significantly within 25 m from the edge of the streams that are approximately 2 - 10 m in width (Iwata et al. 2003, Baxter et al. 2005). Further, taxonomic compositions of organism-based subsidies may alter the dispersal of aquatic insect adults. Jackson and Resh (1989) found that the taxa of emerging aquatic insects caught at stream height did not disperse upland as much as taxa trapped at the canopy-level, while Wesner (2010) asserts that aquatic insect feeding differences (consumer vs. non – consumer) affect their dispersal from the stream edge.

Consumers along stream edges or gravel bars have diets that consist mainly of aquatic insects as they are readily available upon emergence, however, geographical mechanisms may alter the distance to which aquatic insects disperse. Dispersal patterns of emerging aquatic insects could be affected by the high wind speed from orographic lift occurring over the Rocky Mountains in South Western Alberta. Wind assistance has been shown to directly influence passive dispersal of emerging aquatic insects, especially taxa such as stoneflies that have weak flying abilities (Briers et al. 2004). Passive dispersal by wind has also been suggested to be further intensified in fragmented habitats where the forest canopy is sparse or irregular such as the patchy cottonwood forests of Southern Alberta (Macneale et al. 2005). However, the spatial variability in consumer diets is also influenced by the life history traits of the consumer, such as feeding strategies.

3.5.2 Riparian consumer feeding strategies

The current study showed that riparian spiders and beetles fed heavily on aquatic insects near the stream and this declines spatially into upland habitat. The $\delta^{15}N$ signatures of riparian spiders were enriched compared to riparian beetles, while the $\delta^{13}C$ signatures did not differ. Similar isotopic studies have shown that riparian insect predators were generally depleted in nitrogen compared with spiders (Mellbrand and Hamback 2010), suggesting that riparian consumer taxa utilized emerging aquatic insect subsidies to different degrees.

Beetles have been observed to expand their range to seasonally utilize sand bar micro-habitats in order to feed on emerging aquatic insects (Horn and Ulyshen 2009). Riparian ground beetles are mostly nocturnal, or crepuscular, emerging during twilight, as such they do not rely on vision for prey capture (Lovei and Sunderland 1996). Further, they have been shown to feed opportunistically and locate their prey through random search (Lovei and Sunderland 1996).

Riparian beetles feed on dead, injured or immobile prey such as stoneflies in sheltered habitat (Hering and Plachter 1997). It has been suggested that riparian prey is less important to beetle diets within the water zone as they preferentially consume dead or injured aquatic insect wash up (Hering and Plachter 1997). Although ground beetles may have flight capabilities, they spend most of their life on the ground, and use flight only for dispersal (Jahnig et al. 2009).

Similar studies have shown that riparian ground beetles feed predominately on immobile aquatic insects (Hering and Plachter 1997, Paetzold et al. 2006, Jahnig et al. 2009). A

controlled subsidy reduction of emerging aquatic insects showed a 46 % decrease in ground beetle abundance in the reduced-subsidy plot compared to the control plot and a 10 % increase in an increased subsidy plot suggesting beetles mainly feed on aquatic insects (Paeztold et al 2006). Gut-content analysis of the ground beetle *Nebria picicornis,* along the Isar flood plain, Germany, showed that 51 % of the items were identified as aquatic and the most important prey source were emerging stoneflies that settle on nearby gravel and rocks (Hering and Plachter 1997).

Aquatic insects may not be a substantial contributor to beetle diets upland of the river due to their decreased abundance, and the opportunistic nature of prey capture by riparian beetles. Further, it has been suggested that ground beetle communities generally prefer sand bar habitats which influences their distribution to shore areas and makes them excellent indicator species of riverine health (Manderbach and Hering 2001).

On the other hand, riparian spiders are visual predators and ground-dwelling guilds are highly mobile. Riparian spiders have been shown to obtain between 40 - 60 % of their dietary nitrogen and carbon from emerging aquatic insects within a short distance from the river (5- 10 m) (Collier et al. 2002, Sanzone et al. 2003, Paetzold et al. 2005) and are still subsidized by 24 % at 30 m from the river (Henschel 2004). Mobile spiders often move into upland habitats and are good indicators of the spatial extent of an aquatic contribution (Manderbach and Hering 2001).

3.5.3 River width and the scale of subsidization

Riparian consumers rely on aquatic insects, in part, to meet their dietary needs. Studies have identified that the aquatic contribution to consumers tends to be spatial restricted to within the confines of the riparian area immediately adjacent to the water source (Henschel et al. 2001, Collier et al. 2002, Sanzone et al. 2003, Briers et al. 2005). However, previous studies have overlooked the pattern of decline in dietary proportional estimates of aquatic insects to riparian consumers.

Landscape characteristics, such as stream size, have been linked to aquatic insect emergence. Larger rivers produce a larger proportion of emerging aquatic insects. A study by Gratton and Vander Zanden (2009) that simulated the flux of emerging aquatic insects using empirical data from literature showed that productive lakes and rivers that are larger in size have the potential to export a greater number of aquatic insects into the riparian landscape.

This research showed that the spatial extent of an aquatic insect contribution was greater adjacent to water bodies of greater width for both riparian spiders and beetles. The predicted distance at which the proportion of aquatic insects to consumer diets comprises 10 % of the total dietary estimate was on average over 300 % greater for river reaches over 90 m in width compared to those less than 40 m in width. An interpretation of the 10 % threshold of aquatic influence using data from Collier et al. (2002), Sanzone et al. (2003) and Briers et al. (2005) showed a significant relationship between aquatic insect subsidization and stream width (m) (Table 3.2, Figure 3.5).

Table 3.2: Meta-analysis of interpretation of 10 % threshold distance of aquatic influence (m) calculated from a literature review and added to model data from this thesis. Average stream width (m) was taken from methods of literature papers. "-"indicates unavailable data.

Authors	Consumer	Isotope	Average Stream Width (m)	10 % Threshold Distance (m)
Sanzone et al 2003	Overall	Nitrogen	5	6.98
Ground Collections	Wandering Spider	Nitrogen	5	12.53
	Sit and Wait Spider	Nitrogen	5	-
Vegetation Collections	Orb Weaver	Nitrogen	5	7.99
	Sit and Wait Spider	Nitrogen	5	8.88
	Sheet Weaving Spider	Nitrogen	5	5.48
Briers et al 2005	Spider	Nitrogen	4.0	13.35
Becker et al 2012 (thesis)	Spider Web Building and Ground Dwelling	Nitrogen	94	33.65
			113	80.23
			20	21.42
			35	34.04
			95	59.91
		Carbon	95	32.31
			113	94.26
			35	18.25
			95	63.27
Becker et al 2012 (thesis)	Carabid Beetle	Nitrogen	94	16.82
		Carbon	94	21.37
			120	33.82
			35	4.01
Collier et al 2002	Web Building	Carbon	7	7.00
	Free living	Carbon	7	6.50
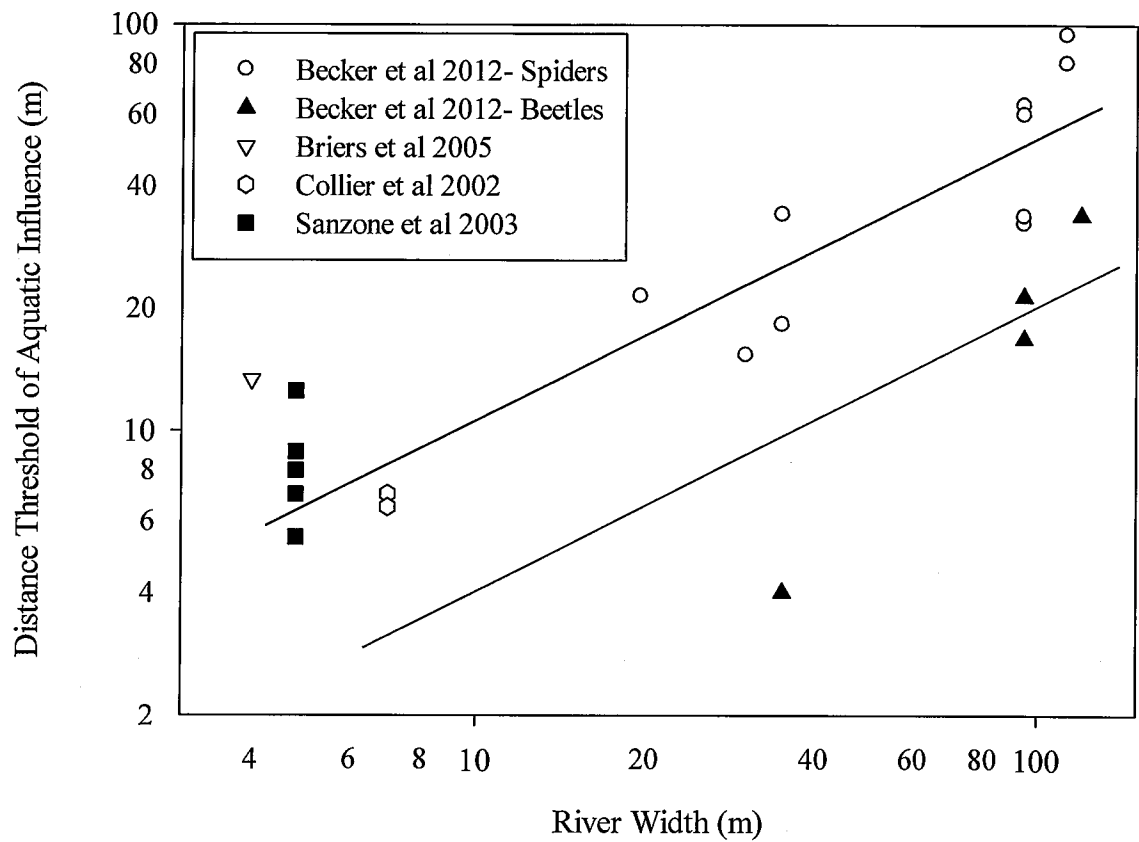
Figure 3.5: Meta-analysis of interpretation of 10 % threshold distance of aquatic influence (m) calculated from a literature review and added to model data from this thesis. Average stream width (m) was taken from methods of literature papers. "-"indicates unavailable data. The line represents regression trends (line of best fit as produced by SigmaPlot version 12 on log scales). ($F_{(1, 20)}$ = 30.36, $p < 0.0001$).

Riparian predator abundance and mobility may be a determinant of the spatial extent of an aquatic insect contribution since these predators differentially exploit resource fluxes of aquatic insects. For example, research has shown that web-weaving and ground-dwelling spiders differ in their ability to capture aquatic prey, resulting in a higher proportion of aquatic derived material in the diet of web-weaving spiders (Collier et al. 2002, Sanzone et al. 2003). This study did not find a difference in the isotopic values of web-weaving and ground-dwelling guilds of spiders, which is likely caused by high occurrence of Chinook winds driving aquatic dispersal and thus, standardizing isotopic signatures of riparian spiders.

The comparisons between research presented in this thesis and other aquatic subsidization based studies focus on both a single aquatic prey species source (Briers et al. 2005), and multiple prey species sources (Sanzone et al. 2003) indicating that the spatial extent of aquatic subsidization may not be highly dependent on dispersal characteristics of aquatic taxa as previously thought (Nakano and Murakami 2001, Sabo and Power 2002); rather, the abundance of aquatic insects dispersing the most important factor in the spatial extent of aquatic dietary contributions.

Habitat characteristics that connect the aquatic and riparian ecosystem are also important factors in aquatic derived inputs to the riparian ecosystem. Small headwater streams are often shaded by forested riparian canopies, decreasing penetration of sunlight which inhibits the growth of *in situ* primary production. These low productivity habitats are dependent primarily on riparian leaves as a food base, where benthic production is mainly from the breakdown of fine particulates into organic matter and the input of detritus from the Riparian ecosystem (Vannote et al. 1980). Research has shown that there is a decrease

93

in aquatic insect and periphyton production in forested sections of the stream, compared with open canopies (Progar and Moldenke 2009).

Such a decrease in aquatic insect abundance relates to a decrease in the spatial extent of an aquatic subsidy as there are less emerging aquatic insects available for dispersal. Further, it has been suggested that riparian contributions negates the importance of aquatic primary production in smaller streams as the material transport from the riparian ecosystem dominates as the interface between these ecosystems is maximized (Vannote et al. 1980). Larger streams receive processed upstream fine particulate organic matter (FPOM), detritus, woody debris, and ample amounts of sunlight, increasing *in situ* primary production. These highly productive ecosystems provide ample habitat and foraging opportunities thus, producing a high abundance and diversity of emerging aquatic insects.

Rundio and Lindley (2012) sampled Big Creek (58 km^2 drainage area) basin in central California and compared annual aquatic insect fluxes with the results from Nakano and Murakami (2001), which was conducted in the Hornai Stream, Japan (15.4 km^2 drainage area). They found that the total annual flux (g m^{-2} year^{-1}) of emerging aquatic insects of Big Creek basin at 2 sampling locations (mean stream width of 5 m) was 400 % and 650 % greater than the flux recorded by Nakano and Murakami in the temperate Horonai Stream (stream width of 2 - 5 m). The authors assert that temperature differences in the contrasting river basins likely decreased reproductive rates; however, they did not imply that habitat characteristics such as drainage and stream size may be important factors in aquatic insect emergence by providing foraging, and protective habitat (Rundio and Lindley 2012).

The comparison between our research and others has shown that the extent of aquatic insect subsidies is less variable between streams of similar size. For example, Briers et al. (2005) and Sanzone et al. (2003) had comparable estimates of 10 % threshold of aquatic influence to wandering spiders of 13.35 m and 12.53 m for streams with an average width of 4.00 m and 4.80 m, respectively. Further, the addition of literature data shows that smaller stream widths significantly fit into the model and enhance the data on larger stream widths.

3.6.0 Conclusion

An alternative food source positively impacts recipient consumers by decreasing the amount of energy spent on foraging however, until now; the extent of an aquatic insect contribution to riparian forests has not been described. This research has demonstrated that stream size exhibits a strong relationship with the extent of an aquatic insect contributions suggesting larger, productive habitats increase the amount of aquatic insects available for dispersal and thus, aquatic insect abundance may be the most limiting factor in aquatic contributions to riparian forests.

CHAPTER FOUR

General Conclusion

4.1.0 The importance of freshwater- terrestrial Linkages

The exchange of materials across ecosystem boundaries have long been recognized by ecologists (Elton 1927), and thus, quantifying the degree of such contributions has been an academic pursuit for food web ecologists. The history of subsidization is well known (Likens and Bormann 1974, Petersen and Cummins 1974, Vannote et al. 1980), however, the mechanisms guiding it are still puzzling (Baxter et al. 2005). Food webs are often viewed as singular features which are separate from other habitats. However, the most crucial ecosystem relationships are largely subsidized from fluxes of energy and nutrients. Constant inputs of solar energy, for example, stimulate primary productivity and thus, the equilibrium of the whole food web (Vanni et al. 2004).

The late Gary Polis pioneered the idea that energy, materials, and even organisms track between habitats influencing food web structure and dynamics. Through research supported by field evidence, he was able to show that resources moving from one habitat to another increases and stabilizes the population of the recipient system (Polis and Hurd 1996, Polis and Strong 1996, Polis et al. 1997). This research spurred interest in the interactions across habitats and brought up further questions in the field of food web ecology.

In this thesis, riparian spiders and beetle feeding patterns were studied using stable isotope analyses to identify relationships between cross-habitat exchanges in riparian ecosystems. A literature review was conducted to develop understanding and methodology for mixing model analysis focusing on aquatic subsidization to the terrestrial ecosystem (Chapter 1). To perform dietary analysis on riparian consumers, the recognized food sources in the river basin were established while considering along-stream gradients, and local site factors (Chapter 2). Finally, mixing model analysis (as defined in chapter 3) was performed to show the proportion of food sources to the diets of riparian consumers. Inverse prediction modelling was used to predict the distance threshold of aquatic influence (m) to riparian consumers and this was regressed against river width (m) to identify a spatial relationship of aquatic subsidization (Chapter 3).

4.2.0 Thesis Overview

In this study, aquatic producer $\delta^{13}C$ did not differ along the river continuum, possibly due to the standardization of collections from riffles and the small spatial scale of the river basin. On the other hand, $\delta^{15}N$ signatures of aquatic producers varied along the stream gradient, and were affected by local site factors, which were tested as a categorical input in the model. Intensive livestock operations, sewage outflows and other anthropogenic inputs are abundant in the OMRB, and account for these differences in site. Aquatic producer nitrogen signatures were reflected in aquatic primary consumer nitrogen signatures, which also varied among sites and over the river gradient. However, aquatic primary consumer carbon signatures did not reflect primary producer signatures thus indicating that they might not be a food source or that another factor was causing variation in signatures. River seston is suspended particulate matter containing plankton,

organic detritus and other inorganic materials and is an additional food source to aquatic primary consumers. However, field sampling did not include river seston which is likely the main food source to aquatic primary consumers, and future studies in the OMRB should consider isotopic analysis of river seston.

Isotopic signatures of riverine biota are often affected by hypolimnion release water storage facilities by depleting carbon signatures and enriching nitrogen signatures downstream of the release. Hypolimnion release dams discharge water from the bottom layer of a thermally stratified reservoir (i.e. they remain cold since they do not circulate). Again, carbon signatures did not meet the expectation of depletion which could be a result of increased spillway events and abiotic factors favouring hypolimnic accumulation. On the other hand, nitrogen signatures were enriched.

Terrestrial producer carbon signatures did not differ throughout the basin, which is expected due to similar photosynthetic pathways; nitrogen signatures did vary throughout the basin due to anthropogenic effects. Both the nitrogen and carbon signatures of terrestrial primary consumers differed between sampling sites, although their signatures did not vary with distance upland from the shoreline.

An overall comparison of terrestrial and aquatic producers yielded overlap in signatures leading to uncertain mixing model calculations and thus, had limited capability to act as endpoints for mixing model analysis. On the other hand, terrestrial and aquatic primary consumers were consistently distinct in isotopic signatures in either C or N at all locations, and therefore, their signatures were applied as baseline indicators for mixing model analysis.

Mixing model analysis on both riparian spiders and beetles was performed using the established endpoints, terrestrial and aquatic consumers. Differing guilds of riparian spiders did not show a significant difference in isotopic signature and thus, they were combined. The proportion of aquatic insects in the diet of riparian consumers was greatest near the river and declined with distance away from the river. This decline in the proportion of aquatic insects to diets was steeper for riparian beetles which are less mobile and often opportunistically capture prey. Using inverse prediction modelling, the distance threshold for aquatic influence was established and regressed against river width. There is a strong relationship between the size of the water body at which aquatic insects emerge and the spatial extent of an aquatic subsidy. This study has quantified the spatial extent of an aquatic insect contribution while considering local site factors such as river width (m), and along stream gradients such as elevation (m) for an entire river basin.

4.3.0 Limitations of this study

4.3.1 Stable isotopes and mountain bluebirds

It has been long recognized that there is variability in isotopic signatures, which compounded by sampling error, could lead to inaccurate data analysis (Phillips and Gregg 2001). Primary production is the base of the food web, and thus, the isotopic variability in plants, algae and detritus determines the effectiveness of stable isotope dietary analysis. For example, overlapping isotopic signatures at the base of the food web reduces the ability of mixing model analysis to correctly partition food sources. However, by adding a random number variable with a mean of 0 and a variance that is equal to the SE, it is possible to estimate the standard error of a proportion estimate to a

diet, and thus, provides an estimation of the uncertainty surrounding the proportion calculation (Rasmussen 2010).

This is especially important when considering dietary analysis of animals with large spatial ranges or those that are migratory. Initially, this study included stable isotope analysis of Mountain Bluebirds *(Sialia currcoides)*. In order to account for their migratory nature, our samples included only chicks that were hatched in Southern Alberta. The samples were collected from bird boxes at various locations in Southern Alberta; however a major caveat to these collections is that the boxes were located within flying distance to multiple water sources. As such, it was not possible to partition food sources of Mountain Bluebirds as the base of the food web could not be delineated within reasonable error.

Future studies considering migratory bird species would benefit from establishing boxes at several sites at intervals upland from the water source. This would not deter the migratory birds from travelling to other water bodies; however, it is unlikely that they would if a rich food source was located adjacent to their nesting site. On the other hand, researchers may consider sampling year round residents and comparing results with migratory species on an annual basis or the addition of tracers, which would magnify the isotopic signatures of the birds.

4.3.2 Flood disturbance, anthropogenic influence and wind factors

Flooding disturbance occurred during the sampling years of this study. Flooding impacts the riparian community by altering aquatic invertebrate patterns, increasing the connectivity with the terrestrial zone, and modifying productivity. The Water Survey of

Canada showed that the Oldman Reservoir was at 200 % (1,022,000 dam^3) capacity in 2010, compared with 63% (309,443 dam^3) capacity in 2008 and daily water levels in the reservoir had increase by 100 % within this time frame. Further, data from Alberta Environment showed that from May to September 2010 the % normal precipitation for the Oldman River Basin was "Much Above Normal" (+ 130 %), while from May to September 2008 the % normal precipitation ranged from "Normal to Above Normal" (90-110%). Furthermore, overall daily discharge of the Oldman River near Brocket (station #: 05AA024) increased from 34 m^3/s in 2008, to 40 m^3/s in 2010, with a total discharge increase from 12, 500 m^3/s to 14, 500 m^3/s, respectively.

High flow events likely impacted isotopic signatures of both aquatic and terrestrial producer and consumer samples. For example, scouring floods may have washed away invertebrate communities, thus decreasing the time of energetic accumulation of food sources. Increased precipitation or snowmelt may have caused an increase in spillway release events of local dams and thus, hypolimnic accumulation from organic matter decomposition was not present which affects both downstream aquatic carbon and nitrogen signatures. As environmental and climate events cannot be avoided, this research showed that it is important to conduct reconnaissance sampling when working with stable isotope analysis to ensure all possible impacts have been identified.

Although not analyzed in this study, it is important to recognize that riparian ecosystems of the OMRB are affected by increasing anthropogenic influences such as population growth, sewage inputs, fertilizers, and intensive livestock farming. Excessive nutrient inputs in the OMRB from fertilizer, animal waste and other agricultural related industries is recognizable in isotopic signatures of $\delta^{15}N$. In fact, the input of excessive nitrogen

causing spatial variation over the river gradient has been recognized by Brinkmann & Rasmussen (2012). The authors, along with others, have found that enrichment by N could potentially lead to hypoxia and increased nitrate respiration as levels of nitrate and dissolved organic matter increases. An allochthonous subsidy of nitrogen from anthropogenic influences will cause an enrichment of nitrogen signatures throughout the food web, which will not impact preferred food sources of consumers; however, signature variation at sampling sites was altered by these additions. Nitrogen enrichment was accounted for in mixing model estimates, and thus, the model analysis was conducted separately at every sampling location rather than combining sites with similar endpoint values.

High winds, known as Chinooks in Southern Alberta, can occur due to orographic lifting on the windward slopes of the Rocky Mountains which produce warm, dry and often turbulent winds on the leeward side of the mountain. Near the city of Lethbridge, the mean annual wind speed (6.48 m/s) is over 100 % greater than the mean annual wind speed at Manitoba, Winnipeg (5.51 m/s).

Winds aid in the passive dispersal of both terrestrial and aquatic insects, and may alter habitats by influencing the spatial distribution of vegetation (Briers et al. 2004, Vander Zanden and Gratton 2011). Emerging aquatic insects, which are generally poor fliers, may have been aided in their dispersal by the Chinook winds of southern Alberta. The high winds may have caused standardization of riparian spider diets as differing guilds often show distinct isotopic signatures. Further, these winds may have also increased the distance of an overall aquatic insect subsidization, especially to riparian beetles. Riparian beetles are opportunistic feeders, and do not actively search prey and thus, the overall

dietary trend may have shown a steeper decline in subsidization with distance from the water body, although the overall spatial relationship with river width is not affected.

4.4.0 Conclusion

This study is the first to quantify the relationship between the sizes of a lotic water bodies and the spatial scale at which aquatic insects contribute to a terrestrial food web. The findings suggest that riparian consumers differentially rely on aquatic insects as a food source in consideration of their life history traits. Both web-weaving and ground-dwelling riparian spiders are agile predators, and are able to capture prey in the upland habitats as efficiently as they are near the shore where the aquatic insects are more abundant. Riparian beetles, on the other hand, are predators that find food through random search and thus, they are not capable of capturing aquatic insect prey efficiently in upland habitats, although they may have been aided by high winds. This research is the first of its kind to show that the larger the water body, the greater the effect of aquatic insects to the diet of riparian beetles.

The ability to identify the importance of emerging aquatic insects to the diet of terrestrial animals has the potential to be important to the field of ecology. Current studies focus on the subsidy from the freshwater to terrestrial ecosystems but are limited by their lack of quantification of the spatial extent of this subsidy. This study is important as it can be used as an example for similar studies in ecosystems where riparian predators such as birds, bats, lizards, and insects rely on aquatic insects as a secondary available food source.

Furthermore, the potential of aquatic insects as an important food source for riparian consumers has implications for the field of water conservation. For example, water storage facilities such as dams may alter channel processes such as velocities and discharge rates, river width, friction and roughness of the stream bed, and sediment loading. The abiotic processes that shape the stream bed impact aquatic producers and consumers. Dams alter riparian habitat as they capture sediment, and slow stream velocities often allowing riparian vegetation to encroach, or alternatively, modifying growing conditions to the point of becoming inadequate for vegetative survival. These abiotic factors have the potential to alter aquatic insect communities by decreasing the availability of species and their reproductive capabilities. The riparian community, which relies heavily on aquatic insect emergence in arid ecosystems, will be impacted by the decreased availability of food sources which could potentially affect higher trophic level species.

4.5.0 Future Considerations

Future research in this area should consider the addition of a nitrogen tracer to determine the whole food web of the aquatic ecosystem. By increasing the magnitude of nitrogen by a known amount and sampling above and below the entry point of nitrogen, an aquatic subsidy would be magnified and thus easier to quantify. Another consideration is quantifying the biomass and abundance of emerging aquatic insects coupled with dietary isotopic analysis with multiple predator species. This combination of biomass and abundance with isotopic analysis would ensure robust results.

There is considerable variation in the method of prey capture among predators and thus, it would be useful to compare similar predators in different habitats; although, this would be difficult with birds as they feed at multiple locations and are often migratory. A comparison of multiple predators coupled with food source biomass and abundance estimates at a single location could shed insight into the role of terrestrial and aquatic insects as a dietary source for a single habitat, which then could be compared with other similar habitats. Another opportunistic consideration would be detailing the food web before and after riverine alterations to assess if there is a change in the aquatic insect community and how this change has affected the diets and abundance of riparian consumers.

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APPENDIX A

A.) One-way ANOVA on $\delta^{15}N$ and $\delta^{13}C$ signatures of biota (Aquatic producers and primary consumers and Riparian producers and primary consumers) among Sites. Dependent variable: $\delta^{15}N$ and $\delta^{13}C$ signatures, Independent variable: Site.

Biota Type	Isotope	Levene's	f	p
Aquatic Producers	$\delta^{15}N$	6.11, p <0.05	5.18 $_{(6,13)}$*	<0.05
	$\delta^{13}C$	4.42, p <0.05	2.48 $_{(6,13)}$*	0.06
Aquatic Primary Consumers	$\delta^{15}N$	2.47, p <0.05	111.73 $_{(6,33)}$*	<1.00E-04
	$\delta^{13}C$	1.04, p = 0.40	1.02 $_{(6,141)}$	0.42
Riparian Producers	$\delta^{15}N$	1.45, p= 0.22	4.83 $_{(6,47)}$	<0.05
	$\delta^{13}C$	1.30, p= 0.28	1.44 $_{(6,47)}$	0.22
Riparian Primary Consumers	$\delta^{15}N$	5.33, p <1.00E-04	8.17 $_{(6,53)}$*	<1.00E-04
	$\delta^{13}C$	1.20, p = 0.31	5.47 $_{(6,184)}$	<1.00E-04

* Levene's test for equal variance significant, Welch's ANOVA reported.

B.) Multiple regression analysis of $\delta^{15}N$ and $\delta^{13}C$ signatures of biota by river width (m) and elevation (m). Dependent variable: $\delta^{15}N$ and $\delta^{13}C$ signatures, Independent variable: river width (m), elevation (m), and interactions where significant.

Aquatic Producers $\delta^{15}N$	Df	Sum Sq.	Mean Sq.	F	p
River Width (m)	1	19.10	19.10	2.08	0.20
Elevation (m)	1	15.34	15.34	1.67	0.16
Parameter Estimates	**Estimate**	**SE**	**t**	p	**R²**
River Width (m)	-2.00E-02	1.60E-02	-1.29	0.20	0.28
Elevation (m)	-6.00E-03	4.40E-03	-1.44	0.16	-
Intercept	13.71	5.30	2.58	<0.05	-
Aquatic Producers $\delta^{13}C$	**Df**	**Sum Sq.**	**Mean Sq.**	**F**	p
River Width (m)	1	9.38	9.38	0.37	0.54
Elevation (m)	1	4.53	4.53	0.18	0.67
Parameter Estimates	**Estimate**	**SE**	**t**	p	**R²**
River Width (m)	-1.65E-02	7.36E-03	-0.61	0.54	0.20
Elevation (m)	-3.00E-03	7.00E-03	-0.42	0.67	-
Intercept	-19.21	8.76	-2.21	<0.05	-
Aquatic Primary Consumer $\delta^{15}N$	**Df**	**Sum Sq.**	**Mean Sq.**	**F**	p
River Width (m)	1	2.70	2.70	0.64	4.30E-01
Elevation (m)	1	242.64	2.42.64	57.42	1.00E-04
Interaction (RW * E)	1	45.49	45.49	10.76	1.30E-03
Parameter Estimates	**Estimate**	**SE**	**t**	p	**R²**
Elevation (m)	-1.45E-02	1.90E-03	-7.58	1.00E-04	0.59
River Width (m)	5.80E-03	7.00E-03	0.80	0.43	-
Intercept	21.25	2.21	9.61	1.00E-04	-
Interaction (RW * E)	-1.39E-04	4.24E-05	-3.28	1.30E-03	-
Aquatic Primary Consumer $\delta^{13}C$	**Df**	**Sum Sq.**	**Mean Sq.**	**F**	p
River Width (m)	1	7.75	7.75	1.28	0.26
Elevation (m)	1	0.34	0.34	0.06	0.81
Parameter Estimates	**Estimate**	**SE**	**t**	p	**R²**
River Width (m)	-2.00E-03	8.65E-03	-0.24	0.81	0.04
Elevation (m)	-2.50E-03	2.20E-03	-1.13	0.26	-
Intercept	-26.34	2.62	-10.04	1.00E-04	-

p-value (Bonferroni correction) = 0.0125

115

Continued from previous.

Riparian Producer $\delta^{15}N$	Df	Sum Sq.	Mean Sq.	F	*p*
River Width (m)	1	10.90	10.90	1.06	0.31
Elevation (m)	1	102.27	102.27	9.96	<0.05

Parameter Estimates	Estimate	SE	t	*p*	R^2
River Width (m)	-1.64E-02	1.59E-02	-1.03	0.31	0.41
Elevation (m)	1.39E-02	4.40E-03	3.16	2.70E-03	-
Intercept	-34.10	2.62	-13.01	1.00E-04	-

Riparian Producer $\delta^{13}C$	Df	Sum Sq.	Mean Sq.	F	*p*
River Width (m)	1	7.66	7.66	3.02	0.08
Elevation (m)	1	13.28	13.28	5.24	0.03

Parameter Estimates	Estimate	SE	t	*p*	R^2
River Width (m)	1.37E-02	7.00E-03	1.74	0.09	0.20
Elevation (m)	5.00E-03	2.00E-03	2.29	<0.05	-
Intercept	-34.10	2.62	-13.01	1.00E-04	-

Riparian Primary Consumer $\delta^{15}N$	Df	Sum Sq.	Mean Sq.	F	*p*
River Width (m)	1	43.51	43.51	7.25	<0.05
Elevation (m)	1	0.34	0.34	0.06	8.10E-01

Parameter Estimates	Estimate	SE	t	*p*	R^2
Elevation (m)	4.80E-04	2.00E-03	0.24	8.10E-01	0.15
River Width (m)	-1.90E-02	7.00E-03	-2.69	<0.05	-
Intercept	3.58	2.45	1.46	1.50E-01	-

Riparian Primary Consumer $\delta^{13}C$	Df	Sum Sq.	Mean Sq.	F	*p*
River Width (m)	1	24.94	24.94	11.05	<0.05
Elevation (m)	1	37.54	37.54	16.64	1.00E-04

Parameter Estimates	Estimate	SE	t	*p*	R^2
River Width (m)	-1.47E-02	4.00E-03	-3.32	<0.05	0.15
Elevation (m)	-5.00E-03	1.20E-04	-4.08	1.00E-04	-
Intercept	-20.34	1.50	-13.53	1.00E-04	-

p-value (Bonferroni correction) = 0.0125

C.) Two-Way ANOVA of $\delta^{15}N$ and $\delta^{13}C$ signatures of aquatic primary consumers and aquatic producers (type 1 & 2) and site. Dependent variable: $\delta^{15}N$ and $\delta^{13}C$ signatures, Independent variables: Site, Type and Site * Type Interaction.

Aquatic Producers and Primary Consumers $\delta^{15}N$					
Source	Df	Sum Sq.	Mean Sq.	F	p
Model	13	1011.61	77.82	22.20	1.00E-04
Error	177	621.66	3.51		
C. Total	190	1633.27			
Effect Test	Df	Sum Sq.	Mean Sq.	F	p
Site	6	337.91	56.32	16.04	1.00E-04
Type	1	120.33	120.33	34.26	1.00E-04
Interaction: Type * Site	6	63.81	10.64	3.03	7.60E-03

Least Squares Means Table				
Site	Type	LSM	SE	Level
Castle River	1	2.97	0.84	D,E
	2	4.21	0.66	B,C,D,E
Pincher Creek	1	7.29	0.77	A
	2	5.65	0.77	B,C,D,E
Summerview	1	5.40	0.29	B,C,D,E
	2	3.62	0.66	C,D,E
Blazingstar	1	6.34	0.57	B,C,D
	2	2.93	0.77	E
Cottonwood	1	6.53	0.33	B
	2	2.68	1.08	C,D,E
St. Mary River	1	7.08	0.59	A
	2	5.05	0.84	B,C,D,E
Pavan	1	10.44	0.29	A
	2	6.82	0.71	B,C

Aquatic Producers and Primary Consumers $\delta^{13}C$					
Source	Df	Sum Sq.	Mean Sq.	F	p
Model	13	5.88	0.45	4.92	1.00E-04
Error	177	16.25	0.09		
C. Total	190	22.12			
Effect Test	Df	Sum Sq.	Mean Sq.	F	p
Site	6	1.57	0.26	2.85	1.12E-02
Type	1	4.15	4.15	45.16	1.00E-04

No interactive effect observed.

D.) Two-Way ANOVA of δ^{15}N and δ^{13}C signatures of riparian primary consumers and riparian producers (type 1 & 2) and site. Dependent variable: δ^{15}N and δ^{13}C signatures, Independent variables: Site, Type and Site * Type Interaction.

Riparian Producers and Primary Consumers δ^{15}N					
Source	Df	Sum Sq.	Mean Sq.	F	p
Model	13	1185.91	91.22	13.40	1.00E-04
Error	231	1573.077	6.81		
C. Total	244	2758.99			
Effect Test	Df	Sum Sq.	Mean Sq.	F	p
Site	6	406.42	67.74	9.95	1.00E-04
Type	1	662.17	662.17	97.24	1.00E-04
Interaction: Type * Site	6	168.42	28.07	4.12	6.00E-04

Least Squares Means Table				
Site	Type	LSM	SE	Level
Castle River	1	3.59	0.48	A,B
	2	0.99	0.92	A,B,C,D
Pincher Creek	1	3.87	0.39	A
	2	3.21	0.99	A,B,C
Summerview	1	2.15	0.57	A,B,C
	2	-1.57	0.7	D,E
Blazingstar	1	1.75	0.45	B,C
	2	-2.12	0.83	D,E
Cottonwood	1	1.55	0.42	B,C
	2	-4.71	1.07	E
St. Mary River	1	3.19	0.67	A,B
	2	-1.82	1.3	C,D,E
Pavan	1	3.93	0.87	A.B
	2	-4.33	1.17	E

Riparian Producers and Primary Consumers δ^{13}C (Squared data)					
Source	Df	Sum Sq.	Mean Sq.	F	p
Model	13	40258.43	3096.80	7.63	1.00E-04
Error	231	93775.66	405.96		
C. Total	244	134034.09			
Effect Test	Df	Sum Sq.	Mean Sq.	F	p
Site	6	5497.74	916.29	2.26	3.89E-02
Type	1	25078.28	25078.28	61.78	1.00E-04
Interaction: Type * Site	6	7634.58	1272.43	3.13	5.70E-03

Least Squares Means Table				
Site	Type	LSM	SE	Level
Castle River	1	7.74	1.93	A,B
	2	6.88	2.67	A,B,C,D
Pincher Creek	1	7.37	1.73	A
	2	4.94	2.76	A,B,C
Summerview	1	7.06	2.10	A,B,C
	2	6.63	2.32	D,E
Blazingstar	1	7.61	1.87	B,C
	2	5.92	2.52	D,E
Cottonwood	1	7.60	1.79	B,C
	2	6.23	2.87	E
St. Mary River	1	9.34	2.28	A,B
	2	6.18	3.17	C,D,E
Pavan	1	8.61	2.59	A,B
	2	5.13	3.00	E

E.) Two-Way ANOVA of $\delta^{15}N$ and $\delta^{13}C$ signatures of riparian producers and aquatic producers (type 4 & 3) and site. Dependent variable: $\delta^{15}N$ and $\delta^{13}C$ signatures, Independent variables: Site, and Type.

Riparian Producers and Aquatic Producers $\delta^{15}N$ ($\log_{10}$)					
Source	Df	Sum Sq.	Mean Sq.	F	p
Model	7	3.02	0.43	9.10	1.00E-04
Error	89	4.22	0.05		
C. Total	96	7.32			
Effect Test	Df	Sum Sq.	Mean Sq.	F	p
Type	1	2.05	2.05	43.25	1.00E-04
Site	6	0.67	0.11	2.34	0.04

Riparian Producers and Aquatic Producers $\delta^{13}C$ ($\log_{10}$)					
Source	Df	Sum Sq.	Mean Sq.	F	p
Model	7	0.58	0.08	2.30	3.37E-02
Error	89	3.20	0.04		
C. Total	96	3.78			
Effect Test	Df	Sum Sq.	Mean Sq.	F	p
Type	1	0.16	0.03	0.75	0.61
Site	6	0.43	0.43	12.07	1.00E-04

No interactive effect observed.

F.) Two-Way ANOVA of $\delta^{15}N$ and $\delta^{13}C$ signatures of riparian primary consumers and aquatic primary consumers (type 1 & 2) and site. Dependent variable: $\delta^{15}N$ and $\delta^{13}C$ signatures, Independent variables: Site, and Type and Site * Type Interaction for $\delta^{15}N$.

Riparian Primary Consumers and Aquatic Primary Consumers $\delta^{15}N$ ($\log_{10}$)

Source	Df	Sum Sq.	Mean Sq.	F	p
Model	13	10.11	0.78	31.48	1.00E-04
Error	325	8.02	0.02		
C. Total	328	18.12			
Effect Test	**Df**	**Sum Sq.**	**Mean Sq.**	**F**	**p**
Type	1	3.87	3.87	156.67	1.00E-04
Site	6	0.80	0.13	5.40	1.00E-04
Interaction: Type * Site	6	0.44	0.07	2.98	<0.05

Least Squares Means Table

Site	Type	LSM	SE	Level
Castle River	1	0.87	0.03	D,F
	2	0.92	0.07	B,C,D,E,F
Pincher Creek	1	0.88	0.02	D
	2	1.11	0.06	A,B,C
Summerview	1	0.73	0.03	E,F,G
	2	1.03	0.02	B,C
Blazingstar	1	0.65	0.03	G
	2	1.07	0.05	A,B,C
Cottonwood	1	0.73	0.03	E,G
	2	1.08	0.03	A,B
St. Mary River	1	0.83	0.04	D,E,F
	2	1.1	0.05	A,B,C
Pavan	1	0.88	0.05	C,D,E,F
	2	1.19	0.02	A

Riparian Primary Consumers and Aquatic Primary Consumers $\delta^{13}C$ ($\log_{10}$)

Source	Df	Sum Sq.	Mean Sq.	F	p
Model	7	3.11	0.44	13.24	1.00E-04
Error	331	11.11	0.03		
C. Total	338	14.22			
Effect Test	**Df**	**Sum Sq.**	**Mean Sq.**	**F**	**p**
Site	1	2.37	2.37	70.53	1.00E-04
Type	6	0.24	0.04	1.21	0.30

No interactive effect observed.

G.) Two-Way ANOVA of δ^{15}N and δ^{13}C signatures of riparian spiders (guild ID) and site. Dependent variable: δ^{15}N and δ^{13}C signatures, Independent variables: Site, and Guild ID. Levels not connected by the same letter are significantly different.

Spiders δ^{15}N					
Source	**Df**	**Sum Sq.**	**Mean Sq.**	**F**	***p***
Model	8	78.55	9.82	3.76	4.00E-04
Error	167	436.00	2.61		
C. Total	175	514.55			
Effect Test	**Df**	**Sum Sq.**	**Mean Sq.**	**F**	***p***
Guild ID	2	14.13	7.06	2.71	7.00E-02
Site	6	61.92	10.32	3.95	1.00E-03
Least Squares Means Table Guild ID					
Level	**LSM**	**SE**	**Level**	-	-
Ground-dwelling	7.56	0.16	A	-	-
Unknown	7.24	0.44	A	-	-
Web-weaving	8.13	0.24	A	-	-
Least Squares Means Table Site					
Site	**LSM**	**SE**	**Level**	-	-
Castle River	6.84	0.48	B	-	-
Pincher Creek	7.88	0.31	A,B	-	-
Summerview	7.25	0.25	B	-	-
Blazingstar	7.08	0.40	B	-	-
Cottonwood	7.82	0.39	A,B	-	-
St. Mary River	7.66	0.35	A,B	-	-
Pavan	8.96	0.36	A	-	-

No interactive effect observed.

Spiders δ^{13}C					
Source	**Df**	**Sum Sq.**	**Mean Sq.**	**F**	***p***
Model	8	41.47	5.18	7.13	1.00E-04
Error	167	121.38	0.73		
C. Total	175	162.85			
Effect Test	**Df**	**Sum Sq.**	**Mean Sq.**	**F**	***p***
Guild ID	2	2.19	1.09	1.51	2.25E-01
Site	6	38.15	6.36	8.75	1.00E-04
Least Squares Means Table Guild ID					
Level	**LSM**	**SE**	**Level**	-	-
Ground-dwelling	-26.04	0.08	A	-	-
Unknown	-25.99	0.23	A	-	-
Web-weaving	-26.28	0.13	A	-	-
Least Squares Means Table Site					
Site	**LSM**	**SE**	**Level**	-	-
Castle River	-26.00	0.25	A,B	-	-
Pincher Creek	-25.87	0.16	A,B	-	-
Summerview	-26.84	0.13	C	-	-
Blazingstar	-25.85	0.21	A,B	-	-

Continued from previous.					
Cottonwood	-26.47	0.21	B,C	-	-
St. Mary River	-25.49	0.19	A	-	-
Pavan	-26.19	0.19	A,B,C	-	-

H.) ANCOVA of δ^{15}N and δ^{13}C signatures of riparian spiders, site and distance from the river (m). Dependent variable: δ^{15}N and δ^{13}C signatures, Independent variables: Site, and Distance from River (m). Levels not connected by the same letter are significantly different.

Riparian Spider ANCOVA δ^{15}N					
Effect Test	**Df**	**Sum Sq.**	**Mean Sq.**	**F**	**_p_**
Site	6	65.65	10.94	4.10	<0.05
Distance from River (m)	1	1.43	1.43	0.53	0.47
Residuals	168	448.7	2.67		
Model Parameters	**Estimate**	**SE**	**t**	**_p_**	**-**
Intercept	7.76	0.16	48.14	1.00E-04	-
Distance from River (m)	0.00	0.00	-0.73	4.66E-01	-
Castle River	-0.83	0.41	-2.05	4.22E-02	-
Pincher Creek	0.26	0.28	0.94	3.48E-01	-
Blazingstar	-0.67	0.35	-1.92	5.62E-02	-
Cottonwood	0.33	0.34	0.94	3.46E-01	-
Pavan	1.29	0.32	3.99	1.00E-04	-
Least Squares Means Table Site					
Site	**LSM**	**SE**	**Level**	**-**	**-**
Castle River	6.86	0.46	B	-	-
Pincher Creek	7.96	0.29	A,B	-	-
Summerview	7.28	0.24	B	-	-
Blazingstar	7.02	0.38	B	-	-
Cottonwood	8.02	0.37	A,B	-	-
St. Mary River	7.73	0.34	A,B	-	-
Pavan	8.98	0.35	A	-	-

No interactive effect observed.

Riparian Spider ANCOVA δ^{13}C					
Effect Test	**Df**	**Sum Sq.**	**Mean Sq.**	**F**	**_p_**
Site	6	1.26	0.21	8.48	1.00E-04
Distance from River (m)	1	0.11	0.11	4.28	<0.05
Distance from River (m) * Site	6	0.4	0.07	2.69	<0.05
Residuals	162	4.03	0.02	-	-
Model Parameters	**Estimate**	**SE**	**t**	**_p_**	**-**
Intercept	0.41	0.02	23.4	1.00E-04	-
Castle River	0.12	0.05	2.25	2.59E-02	-
Pincher Creek	-0.01	0.04	-0.21	8.30E-01	-
Blazingstar	0.09	0.07	1.3	1.95E-01	-
Cottonwood	-0.08	0.04	-2.34	2.07E-02	-
St. Mary River	0.06	0.03	1.86	6.48E-02	-
Pavan	-0.01	0.03	-0.37	7.11E-01	-

Continued from previous.					
Distance from River (m)	0.00	0.00	2.07	4.02E-02	-
(DFR -23.7436)*Castle River	0.01	0.00	3.47	7.00E-04	-
(DFR -23.7436)*Pincher Creek	0.00	0.00	-2.02	4.55E-02	-
(DFR -23.7436)*Blazingstar	0.00	0.00	0.38	7.08E-01	-
(DFR -23.7436)*Cottonwood	0.00	0.00	-1.56	1.21E-01	-
(DFR -23.7436)*Pavan	0.00	0.00	-2.52	1.26E-02	-

Least Squares Means Table Site					
Site	LSM	SE	Level	-	-
Castle River	3.77	0.14	A,B	-	-
Pincher Creek	2.81	0.09	A,B	-	-
Summerview	1.95	0.13	C	-	-
Blazingstar	3.52	0.18	A,B	-	-
Cottonwood	2.36	0.09	B,C	-	-
St. Mary River	3.31	0.08	A	-	-
Pavan	2.78	0.08	A,B,C	-	-

I.) ANCOVA of $\delta^{15}N$ and $\delta^{13}C$ signatures of riparian beetles, site and distance from the river (m). Dependent variable: $\delta^{15}N$ and $\delta^{13}C$ signatures, Independent variables: Site, and Distance from River (m). Levels not connected by the same letter are significantly different.

Riparian Beetle ANCOVA $\delta^{15}N$					
Effect Test	Df	Sum Sq.	Mean Sq.	F	p
Site	5	18.05	18.05	6.86	<0.05
Distance from River (m)	1	189.43	37.89	14.41	1.00E-04
Distance from River (m) *Site	5	41.62	8.32	3.17	<0.05
Residuals	35	92.05	2.63		
Model Parameters	Estimate	SE	t	p	-
Intercept	6.33	0.38	16.86	1.00E-04	-
Distance from River (m)	-0.05	0.02	-2.62	1.29E-02	-
Pincher Creek	2.81	0.66	4.28	1.00E-04	-
Blazingstar	-1.03	0.61	-1.70	9.87E-02	-
Cottonwood	-2.57	0.53	-4.86	1.00E-04	-
St. Mary River	2.88	0.64	4.51	1.00E-04	-
Pavan	1.16	0.55	2.12	4.12E-02	-
DFR-18.508*Pincher Creek	0.06	0.05	1.33	1.91E-01	-
DFR-18.508*Blazingstar	-0.09	0.04	-2.12	4.09E-02	-
DFR-18.508*Cottonwood	0.05	0.03	1.78	8.40E-02	-
DFR-18.508*St. Mary River	0.1	0.04	2.55	1.52E-02	-
DFR-18.508*Pavan	0.04	0.02	1.70	9.83E-02	-

Least Squares Means Table Site					
Site	LSM	SE	Level	-	-
Pincher Creek	8.17	0.72	A	-	-
Summerview	2.12	0.99	C	-	-
Blazingstar	4.32	0.66	B,C	-	-
Cottonwood	2.78	0.54	C	-	-
St. Mary River	8.23	0.70	A	-	-
Pavan	6.52	0.57	A,B	-	-

Riparian Beetle ANCOVA $\delta^{13}C$					
Effect Test	Df	Sum Sq.	Mean Sq.	F	p
Site	5	7.07	7.07	4.37	<0.05

Continued from previous.				
Distance from River (m)	1	33.95	6.79	4.20	<0.05
Residuals	46	74.39	1.62	-	-

Model Parameters	Estimate	SE	t	p	-
Intercept	-26.18	0.22	-121.69	1.00E-04	-
Castle River	-0.08	0.5	-0.16	8.70E-01	-
Blazingstar	-0.06	0.33	-0.18	8.57E-01	-
Cottonwood	-0.13	0.42	-0.31	7.57E-01	-
St. Mary River	1.90	0.47	4.02	2.00E-04	-
Pavan	-0.39	0.44	-0.90	3.75E-01	-
Distance from River (m)	0.01	0.00	2.09	4.20E-02	-

Least Squares Means Table Site					
Site	LSM	SE	Level	-	-
Castle River	-25.99	0.57	A,B	-	-
Summerview	-27.14	0.45	B	-	-
Blazingstar	-25.97	0.31	B	-	-
Cottonwood	-26.04	0.46	A,B	-	-
St. Mary River	-24.01	0.53	A	-	-
Pavan	-26.31	0.49	B	-	-

J.) ANCOVA of δ^{15}N and δ^{13}C proportional aquatic insect estimates to the diet of riparian spiders and beetles with site and distance from the river (m). Dependent variable: δ^{15}N and δ^{13}C proportional estimates. Independent variables: Site, and Distance from River (m). Levels not connected by the same letter are significantly different.

Riparian Spider ANCOVA					
Effect Test	Df	Sum Sq.	Mean Sq.	F	p
Site	9	0.87	0.87	22.34	<0.05
Distance from River (m)	1	0.89	0.10	2.53	1.00E-04
Residuals	44	1.72	0.04		

Model Parameters	Estimate	SE	t	p	-
Intercept	0.39	3.30E-02	11.92	1.00E-04	-
Distance from River (m)	-0.01	1.14E-03	-4.73	1.00E-04	-
Castle River Carbon	-0.18	7.73E-02	-2.36	2.30E-02	-
Pincher Creek Nitrogen	-0.10	7.81E-02	-1.28	2.06E-01	-
Summerview Carbon	0.00	7.80E-02	-0.06	9.51E-01	-
Blazingstar Carbon	-0.16	9.26E-02	-1.73	9.00E-02	-
Blazingstar Nitrogen	0.08	9.26E-02	0.82	4.16E-01	-
Cottonwood Carbon	0.16	7.93E-02	2.03	4.83E-02	-
Cottonwood Nitrogen	0.24	8.37E-02	2.92	5.60E-03	-
St. Mary River Carbon	-0.10	7.78E-02	-1.26	2.15E-01	-
St. Mary River Nitrogen	0.00	7.23E-02	-0.05	9.64E-01	-

Least Squares Means Table Site					
Site	LSM	SE	Level	-	-
Castle River Carbon	0.12	0.08	B	-	-
Pincher Creek Nitrogen	0.20	0.08	A,B	-	-

Continued from previous.					
Summerview Carbon	0.30	0.08	A,B	-	-
Summerview Nitrogen	0.37	0.10	A,B	-	-
Blazingstar Carbon	0.14	0.10	A,B	-	-
Blazingstar Nitrogen	0.38	0.10	A,B	-	-
Cottonwood Carbon	0.46	0.08	A,B	-	-
Cottonwood Nitrogen	0.54	0.08	A	-	-
St. Mary River Carbon	0.20	0.08	A,B	-	-
St. Mary River Nitrogen	0.30	0.07	A,B	-	-

No interactive effect observed.

Riparian Beetle ANCOVA (Log$_{10}$)					
Effect Test	**Df**	**Sum Sq.**	**Mean Sq.**	**F**	***p***
Site	3	0.36	0.12	1.44	0.27
Distance from River (m)	1	0.95	0.95	11.22	4.80E-03
Residuals	14	1.18	0.08		
Model Parameters	**Estimate**	**SE**	**t**	***p***	-
Intercept	-0.02	1.21E-01	-0.15	8.85E-01	-
Distance from River (m)	-0.38	1.15E-01	-3.35	4.80E-03	-
Blazingstar Carbon	-0.07	1.12E-01	-0.65	5.28E-01	-
Blazingstar Nitrogen	0.10	1.24E-01	0.82	4.27E-01	-
Pavan Carbon	0.17	1.24E-01	1.41	1.79E-01	-

No interactive effect observed.

K.) ANCOVA of $\delta^{15}N$ and $\delta^{13}C$ proportional aquatic insect estimates to the diet of riparian spiders and beetles with site and distance from the river (m). Dependent variable: $\delta^{15}N$ and $\delta^{13}C$ proportional estimates. Independent variables: Site, and Distance from River (m). Levels not connected by the same letter are significantly different.

10% Distance Threshold ANCOVA For Riparian Spiders and Beetles					
Effect Test	**Df**	**Sum Sq.**	**Mean Sq.**	**F**	***p***
River Width (m)	1	4.32	4.32	26.28	1.00E-03
Type	1	3.80	3.80	23.08	1.00E-03
Residuals	11	0.34	0.03		
Model Parameters	**Estimate**	**SE**	**t**	***p***	-
Intercept	-0.37	3.40E-01	-1.08	3.10E-01	-
River Width (m)	0.95	1.85E-01	5.13	3.00E-04	-
Type	-0.25	5.00E-02	-4.80	5.00E-04	-
Least Squares Means Table Type					
Site	**LSM**	**SE**	**Level**	**Df**	**t**
Riparian Beetle	1.10	0.09	A	11	4.80
Riparian Spider	1.60	0.06	B	***p***	1.00E-04

L.) Regression of $\delta^{15}N$ and $\delta^{13}C$ proportional aquatic insect intercepts at the shoreline and river width (m). Dependent variable: Intercept at the shoreline (%). Independent variables: River width (m).

Aquatic Influence at the Shoreline	Df	Sum Sq.	Mean Sq.	F	p
River Width (m)	1	0.09	0.09	6.28	2.76E-02
Error	12	0.18	0.01		
C. Total	13	0.27			

Parameter Estimates	Estimate	SE	t	p	R^2
Intercept	0.22	0.08	2.82	0.02	0.58
River Width (m)	2.32E-03	9.27E-04	2.51	0.03	-

M.) Regression of relationship between the 10 % threshold of aquatic influence interpreted from several literature studies and combined with model data of this study. Dependent variable: 10% threshold distance of aquatic influence (m) Independent variables: Average stream width (m).

10 % Distance Threshold of Aquatic Influence	Df	Sum Sq.	Mean Sq.	F	p
River Width (m)	1	2.09	2.09	30.36	1.00E-04
Error	20	1.38	0.07		
C. Total	21	3.47			

Parameter Estimates	Estimate	SE	t	p	R^2
Intercept	0.49	0.14	3.31	3.50E-02	0.83
River Width (m)	0.54	0.10	5.51	1.00E-04	-