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The response of insects to wastewater effluent in the Red Deer River: A spatial perspectiv

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The response of insects to wastewater effluent in the Red Deer River: A spatial perspective

by

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Abstract

Few studies document the spatial responses of biota to point-source nutrient enrichment in rivers. Even fewer identify the mechanisms acting to create these responses. This study addressed these research shortages by investigating spatial patterns of physicochemical variables, insect abundance, and periphyton abundance, in the Red Deer River, Alberta, Canada, downstream of wastewater input. Additionally, patterns in nitrogen isotope signatures driven by effluent input were used to estimate the scale of insect movements. Analyses of family assemblage spatial structure, and of across-scale explanators of local insect abundance, were used to infer whether movement helps structure the spatial response of insects to effluent addition. Notably, nutrient concentrations peaked downstream of effluent addition, and were significantly correlated with insect abundance. Although insects were estimated to have undergone downstream movements of ~1-5 km per month, broad-scale spatial patterns did not show obvious signs of being affected by downstream movement.

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Epigraph

…[T]he problem of pattern and scale is the central problem in ecology, unifying population biology and ecosystem science, and marrying basic and applied ecology.

Levin, S. A. 1992. The problem of pattern and scale in ecology. Ecology **73**:1943-1967

Chapter One: **Introduction**

1.1 General introduction and objectives

Rivers are increasingly subject to nitrogen and Phosphorus enrichment from the addition of wastewater effluent (Khan and Ansari 2005). These inputs cause changes to the structure and functioning of downstream aquatic ecosystems. These changes start at the base of aquatic food webs, as the growth of primary producers (such as periphyton, phytoplankton and macrophytes) is typically limited by the availability of nitrogen and Phosphorus, and thus growth constraints are relieved when nutrients are added, causing increased primary productivity downstream of wastewater treatment plants (WWTPs) (Teissier et al. 2002, Gucker et al. 2006, Askey et al. 2007, Gucker et al. 2011). This, in turn, affects higher trophic levels, instigating change in the community composition and productivity of downstream consumers (Walsh et al. 2005, Gucker et al. 2006, Gafner and Robinson 2007, Ortiz and Puig 2007, Singer and Battin 2007, Grantham et al. 2011). In order to best manage the use of freshwater resources, it is important to understand the nature of the changes in ecosystem structure and functioning that result from effluent input.

Because rivers are systems with a predominantly unidirectional flow of water, they are characterized by a continual transport of dissolved/suspended matter in a downstream direction. This transport can result in nutrients from WWTPs being spread far downstream of the point of input, causing them to have not only local, but regional effects on river ecosystems (Marti et al. 2004). Despite this knowledge, few studies

document the broad-scale spatial responses of important ecosystem components (especially biota) to these inputs. Studies that do report higher-level consequences of effluent input tend to simply compare the average downstream response at site(s) within a kilometre or so of input, to conditions upstream (i.e. Dyer and Wang 2002, Camargo et al. 2005, Ortiz et al. 2005, Gucker et al. 2006, Ortiz and Puig 2007, Spanhoff et al. 2007, Sanchez-Perez et al. 2009, Gucker et al. 2011). If they do consider higher trophic levels and larger downstream areas -in the order of tens of kilometres- they assess fewer sites, and exclude areas close to the nutrient source (i.e. Teissier et al. 2002). Very few empirical studies describe spatial patterns at small scales close to effluent input, and at larger scales, so as to consider implications in far downstream reaches (however, see Askey et al. 2007). This is surprising given the popularity of theoretical works such as the river continuum (Vannote et al. 1980) and discontinuum (Rice et al. 2001, Poole 2002) concepts, which promote a paradigm that views rivers as longitudinal entities. Thus, research that addresses the longitudinal consequences of effluent input, considering both the immediate downstream impacts of effluent, and how these impacts progress in a downstream direction, is needed. Such research can inform understanding of how disturbances affect rivers spatially, providing knowledge that could aid design of monitoring programs (Do et al. 2012) and prediction of how future disturbances will affect aquatic ecosystems (Anderson et al. 2006b).

There is also a shortage of empirical studies that succeed in deciphering the mechanisms that act to create broad-scale spatial responses to disturbances in rivers. This being said, recent process-oriented theoretical studies have addressed this matter, creating predictions about how populations should respond spatially to disturbance, considering

the demographic and dispersal characteristics of the populations (Anderson et al. 2005, Anderson et al. 2006a, Anderson et al. 2008). These studies emphasize the importance of the scale of long-term dispersal in structuring the spatial response of populations to disturbance, or more generally, environmental variation. Unfortunately, little is known about the scale of long-term dispersal of aquatic organisms, which is necessary to validate the predictions of these theoretical models. In particular, despite a large body of literature detailing small-scale movements of aquatic insect larvae in streams (reviewed in Brittain and Eikeland 1988), the long-term net movements of these organisms in rivers is virtually unknown.

The Red Deer River downstream of the Red Deer Wastewater Treatment Plant (RDWWTP) in Alberta, Canada, represents an ideal system to a) study the scale of long-term invertebrate movements, and how river ecosystems respond longitudinally to effluent input, and to b) assess whether the movement of organisms is an important mechanism structuring these large-scale responses. It is ideal because it is relatively unimpacted except for a municipal WWTP that discharges ~3 ML/day of tertiary treated effluent into the river (City of Red Deer 2007). Depending on discharge rates, this accounts for ~0.3-1.5 % of the river's volume, but the total nitrogen (TN) and total Phosphorus (TP) concentrations in the effluent are hundreds of times greater than the natural concentrations in the river (Hogberg 2004). Thus this WWTP represents a significant sustained point disturbance that could drive large-scale spatial patterns in various components of the river ecosystem. Thus, in this study, the Red Deer River is used as a study system to address the following objectives:

1) To document how abiotic conditions and biotic abundance vary longitudinally in relation to the point of effluent input from a WWTP.

2) To infer whether WWTP effluent drives changes in total insect abundance, and the abundance of a number of important groups.

3) To estimate the scale of long-term in-stream movement of select groups of insects with differing movement and feeding styles.

4) To assess whether movement may be affecting the spatial response of insect groups to WWTP effluent, or to variability in environmental conditions.

To meet these objectives, several complementary analyses were performed. To describe longitudinal trends in abiotic conditions and biotic abundance, LOESS regression (locally weighted scatter plot smoothing) was used to emphasize mid-scale spatial patterns without *a priori* conditions regarding the shape of these patterns (Jacoby 2000). To evaluate whether effluent from the RDWWTP drives changes in insect communities, generalized linear models (Nelder and Wedderburn 1972) were used to determine whether effluent-influenced variables (nitrate concentration and periphyton abundance) explained variation in insect abundance after accounting for variation related to hydraulic/habitat conditions. A similar analysis conducted with the same independent variables averaged over different spatial lags assessed whether consideration of conditions multiple kilometres beyond the sampling site improved the explanation of variation in insect abundance at the local scale, which would suggest that insects respond to these areas, perhaps because they have moved through them. This analysis used the reasoning that if local insect abundance was better explained by averages of environmental variables across sites rather than by site-level values of variables, this

could indicate that movement is an important factor structuring large scale abundance patterns. Thus, this analysis supported the fourth objective. It was also meant to complement analyses that estimated movement scales, based on the expectation that the best scale of analysis would correspond to the scale of movement, because organisms respond to their environment most strongly in the area through which they move. The scale of net movements was estimated independently by comparing gradients in nitrogen stable isotope signatures ($\delta^{15}N$) of insects and periphyton, and analysing spatial cross-correlations in the residuals of these gradients. Finally, spatial structure in family assemblages of insects was used as another way to evaluate whether the WWTP drives changes in insect communities, and whether movement structures insect distribution and abundance at scales larger than that of the sampling site.

1.2 Thesis outline

In this thesis I first present predictions for each study objective in the context of the research that informed these predictions (section 1.3). In Chapter 2, I further describe the study system, report field work and sample processing protocols (sections 2.1 through 2.3), and also detail the analytical approaches taken to meet the study objectives, including the statistical analyses (sections 2.4 through 2.9). Further background information that is necessary to understand and justify these approaches is also included. Some details regarding the datasets used are reported in Appendix A: Datasets, as noted in the text. Each analytical section in the methods includes an initial portion presenting general approaches and background, followed by (a) more detailed section(s) describing statistical analyses. For some objectives, multiple independent analyses were performed, and in some cases, these analyses served to support multiple objectives. Thus not all

analytical methods are presented under headings referencing specific objectives. Particularly, the analysis of environmental explanators of abundance across scales (section 2.6), which was concurrently meant to a) provide insight towards whether movement affects the spatial response of insects to their environment, and to b) evaluate whether estimates of the scale of insect movements were reasonable, is presented in its own section, after the description of the analogous procedure performed at the local scale to infer whether the WWTP drives changes in insect communities. Similarly, the analysis of spatial structure in insect families was distinct from other analyses because it dealt with multivariate data, and was used to supplement multiple objectives. It was thus considered separately throughout this thesis (sections 2.9, 3.4, and 4.4).

Chapter 3 presents the study results, beginning with descriptions of longitudinal patterns in abiotic and biotic variables, which serves to meet the first objective of this study. These sections correspond with sections in the approach (sections 2.4 through 2.9), but the results for environmental explanators of abundance across scales is combined with the results of the local-scale analysis.

Finally, in Chapter 4, the study results are synthesized and interpreted as they pertain to the study objectives. Again, the areas of discussion mostly correspond with sections laid out in Chapter 2. However, discussion of the analysis of abundance across scales is separated from discussion of the local-scale analysis, and is found in section 4.3. This section integrates various lines of evidence to address the final study objective, whether long-term movement affects the spatial response of insects to their environment, or, in other words, whether a consideration of long-term movement is necessary to understand the spatial patterns in insect distribution and abundance. Chapter 4 ends with

a discussion of the analysis of spatial structure in insect assemblages, followed by a general conclusion that summarizes and integrates all analyses.

1.3 Literature review and predictions

1.3.1 The effects of nutrient enrichment on primary and secondary productivity in rivers

The nature of changes in consumer communities as a result of nutrient input depends upon the trophic status of the system, and the amount and type of nutrient input (Sand-Jensen and Borum 1991, Sanchez-Perez et al. 2009). In some systems, nutrient enrichment tends to increase consumer biomass as a result of increased producer biomass that in turn relieves food limitations (Hart and Robinson 1990, Camargo et al. 2005, Gucker et al. 2006). In other systems nutrient enrichment decreases consumer biomass because of oxygen depletions caused by increased biomass of (and consequently respiration by) primary producers, heterotrophic organisms, such as bacteria, for which they provide substrate (Edwards 1968), and decomposers that eventually break down these organisms (Wilcock et al. 1998, Khan and Ansari 2005). Additionally, excessive growth of macrophytes can create calmer waters, which lowers physical aeration. Additionally, tall in-stream canopies, while providing increased substrate for heterotrophs, do not provide a comparable increase in oxygen-producing photosynthesis, because of shading of lower parts of the canopy (Edwards 1968). Thus increasing abundance of primary producers can cause periods of hypoxia, or even anoxia. These oxygen depletions can reduce consumer biomass, because different primary (and higher order) consumers require different levels of dissolved oxygen for growth, and as dissolved oxygen becomes depleted, taxa begin to disappear as their oxygen requirements

are not met, and anaerobic taxa, or those tolerant of low oxygen, persist (Khan and Ansari 2005). The most drastic example of this occurs in coastal areas receiving massive nutrient loads from large rivers and/or urban centres, where so-called “dead zones” are created by oxygen depletions resulting from increased respiration associated with excessive primary productivity (Karlson et al. 2002, Rabalais et al. 2002, Diaz and Rosenberg 2008). Thus, one might expect increases in primary productivity and secondary productivity as a response to nutrient enrichment until dissolved oxygen becomes depleted, after which secondary productivity would drop with nutrient addition and the growth of primary producers. Thus, predictions regarding the effect of nutrient additions on aquatic systems should depend on the pre-disturbance nutrient status of the water body, and the degree of enrichment.

Based on nutrient concentration standards suggested in Dodds et al. (1998), the Red Deer River upstream of the WWTP is oligotrophic or mildly mesotrophic (see sections 3.1.1.1 and 3.1.1.2). Additionally, the effluent from the RDWWTP, although being nutrient-rich compared to natural conditions, undergoes tertiary treatment. Thus, in this low-nutrient system with relatively well-treated effluent, it was predicted that nutrient addition from the WWTP would change downstream macroinvertebrate communities indirectly by increasing primary productivity (manifesting as increased periphyton abundance), which would alleviate food limitations on insects, and thus increase secondary productivity, as represented by insect biomass. It was not expected that the addition of effluent would push the system into a hyper-eutrophic state in which secondary productivity would be diminished as a result of oxygen depletion. Indeed, a positive relation between periphyton abundance and macroinvertebrate abundance has

been observed (across streams) with a range of dissolved nutrients similar to that measured in this study (Lewis and McCutchan 2010).

It may be erroneous to assume that the Red Deer River is nutrient limited, as meta-analyses report mixed responses of primary producers to experimental nutrient enrichments in lotic systems (Dodds and Welch 2000, Francoeur 2001, Tank and Dodds 2003), and many studies conclude that nutrients are not limiting in lotic systems (reviewed in Doi 2009). However, this may be a result of a bias towards studying nutrient limitation in small streams, which tend to be shaded, and thus light limited (reviewed in Doi 2009). Indeed, some studies report nutrient limitation in streams (Pringle 1987, Hill and Knight 1988, Chessman et al. 1992), particularly if canopy cover is removed (Hill and Knight 1988), and meta-analyses agree that algal biomass generally increases with the addition of nitrogen and phosphorus (Dodds and Welch 2000, Francoeur 2001, Tank and Dodds 2003). Also, considerable indirect evidence indicates that nutrient additions increase primary productivity in rivers (see above discussion regarding effluent inputs). Thus, given that the Red Deer River is a mid-order system with an open canopy, which should cause a dominance of autotrophy and an autochthonous base to food webs (Vannote et al. 1980), nutrient limitation was expected to be present. This should allow increased primary and secondary productivity in reaches downstream of effluent input.

1.3.2 Movements of river insects

For river macroinvertebrates, movement is of special interest because the predominantly unidirectional flow of water tends to lift/push (advect) them from the substrate in which they reside, and transport them downstream in the water column, a phenomenon called drift (Waters 1972). This downstream movement has led to questions

of how populations persist in upstream reaches (Muller 1954, 1982), and has implications for how these populations respond spatially to environmental variability (Anderson et al. 2005). Lotic macroinvertebrates also undergo benthic movements by crawling or swimming upstream or downstream over short distances, on the scale of a few metres per day (Elliott 1971b, 2003). However, little is known about the cumulative distance travelled over the lifetime of lotic macroinvertebrates, or the rate at which they move (Malmqvist 2002). This being said, a few studies report in-stream movement over larger scales of time and space, but these are typically reserved to cased caddisflies, which can be marked effectively (Neves 1979, Hart and Resh 1980, Erman 1986, Jackson et al. 1999). These studies report net long-term movement (on the scale of months) ranging from 60 m upstream to 1500 m downstream of release points.

Although long-term estimates of the net movement of river insects are lacking, there are estimates of the scales of individual drift events and upstream and downstream benthic movements, and of the frequency of drift versus benthic movements. These studies indicate that individual *drift* events are typically a few metres, but can be up to tens of metres (Elliott 1971a, Townsend and Hildrew 1976, Larkin and Mckone 1985, Otto and Sjostrom 1986, Lancaster et al. 1996). Estimates of the rate of *benthic* movements range from less than 10 m per day (Elliott 2003) to 10 m per hour (Hayden and Clifford 1974). These data could be combined and scaled up to provide estimates of net movement, and indeed, one such investigation estimated net downstream displacements of 0-10 km per generation for different insect taxa, with all but one group being estimated to move at least 2.7 km downstream (Hemsworth and Brooker 1979). However, whether this kind of extrapolation is accurate is unclear, particularly because

there is little information regarding the relative frequency of drift versus benthic movements (Humphries 2002). There is debate over whether the cumulative effect of multiple drift events causes downstream displacement from habitats, or just redistribution within habitat patches. Early studies concluded that drift does indeed represent downstream displacement, and not local random movements, as drifting individuals are often too great in number to be supplied only by local populations (Waters 1965). However, recent studies challenge this assertion, arguing that the observation of frequent downstream drift may have had too much influence on perspectives regarding the movements of immature aquatic insects. For instance, Lancaster et al. (2011) suggested that *Baetis rhodani*, an archetypal drifter, rarely even move downstream to adjacent riffles despite their propensity to drift. They suggested that downstream drift could later be compensated for by upstream swimming, that these organisms settle in slow areas between riffles, or that drifters have high mortality. They stressed the need to readdress assumptions about the in-stream dispersal of river invertebrates, and highlighted the lack of empirical studies that test these assumptions.

Despite recent challenges to the downstream-movement paradigm in lotic insect ecology, I have assumed that net downstream movement of insect larvae occurs in the Red Deer River for the following reasons. First of all, although Elliott (2003) found that (in exclusion of drift) upstream movement was preferred to downstream movement in all taxa (except for the cased caddisfly, which showed no directional preference), studies that consider net movement with an inclusion of drift agree that net downstream displacement should occur for the aquatic stages of river invertebrates. For instance, research examining the relative frequency of upstream versus downstream movements widely

concludes that upstream movements of insect larvae are not sufficient to compensate numerically for downstream drift (Bishop and Hynes 1969, Elliott 1971b, Bird and Hynes 1981, Benson and Pearson 1987, Williams and Williams 1993, Turner and Williams 2000). Exceptions in these studies include full compensation at isolated sites within rivers (but not within any river as a whole). Second, the vast majority of movement studies are conducted in slower, shallower streams or small rivers than the Red Deer River. In systems like the Red Deer, downstream movement should exceed that reported above because: a) drift distances increase (Elliott 1971a, Larkin and Mckone 1985, Lancaster et al. 2006), or equally, return rates decrease (Ciborowski 1983, Lancaster et al. 1996), with greater flow velocity; b) the frequency of downstream movements relative to upstream movements increases with greater flow velocity (Bird and Hynes 1981, Turner and Williams 2000); and c) in deeper waters, drifting individuals should experience less contact with the substrate that is initiated by random, passive movement, which is often necessary for resettlement of drifting individuals (Elliott 1971a, Madsen et al. 1973). Thus, it was predicted that, as estimated by Hemsworth and Brooker (1979), net downstream movement on the scale of multiple kilometres would be detected.

Whether detectable differences were present in the net movements of insects with different movement and feeding styles was also of interest in this study. Thus, 11 groups of insects were selected for analysis using some or all of the following criteria: cosmopolitan distribution, abundant, different feeding and/or movement behaviour than other groups, and large and easy to handle/identify.

Additionally, for some groups, different life stages were analyzed separately to assess whether movement changes with maturity. Ultimately, the chosen groups were:

"late" instar *Hydropsyche* larvae (Trichoptera: Hydropsychidae), *Hydropsyche* pupae, "late" instar *Brachycentrus* (Trichoptera: Brachycentridae) larvae, Chironomidae larvae (Diptera), "early" instar *Heptagenia* (Ephemeroptera: Heptageniidae) larvae, "mid" instar *Heptagenia* larvae, "late" instar *Heptagenia* larvae, "mid" instar *Baetis* (Ephemeroptera: Baetidae) larvae, "late" instar *Baetis* larvae, "mid" instar *Tricorythodes* (Ephemeroptera: Tricorythidae,) larvae, and "late" instar *Tricorythodes* larvae. I predicted that net movement would be downstream for all groups, and that the relative magnitude of net downstream movement would be the following:

Chironomidae > *Heptagenia* > *Hydropsyche* > *Baetis* > *Tricorythodes* > *Brachycentrus*.

This follows the results of Elliott (1971a), who investigated drift distances of a number of invertebrate groups including Chironomidae, *Rhithrogena* and *Ecdyonurus* (which are in the same family as *Heptagenia*), *Hydropsyche*, *Baetis*, and cased caddisflies (which were in different families than *Brachycentrus*). Estimates of movement rates of *Tricorythodes* have not been published, but Ramirez and Pringle (1998) show that their drift rate is disproportionately low (considering their high abundance), as compared to Chironomidae, *Hydropsyche*, and Baetidae, thus their net downstream displacement should be on the low range of the studied organisms. Note that Elliott (1971a) investigated short-term induced drift distances, and it is unclear whether it is legitimate to scale up these data through space and time to predict relative long-term movements between groups, as done here. For instance, if farther-drifting animals rarely leave the substrate naturally, or if insects with greater drift distances are also active upstream swimmers, they may not actually move the farthest.

I also predicted that the younger life stages would have greater downstream movement than older stages of the same genus, which has been observed in *Baetis*, *Cinygmula* (which is in the same family as *Heptagenia*) (Allan and Feifarek 1989), and Trichopterans (Otto 1976).

1.3.3 Expected longitudinal responses of primary producers and primary consumers to point source nutrient enrichment in rivers

Although increased productivity is expected downstream of the RDWWTP, there is a limited precedent of empirical research to guide predictions about the spatial manifestations of this increased productivity, in terms of the nonlinear longitudinal patterns in the abundance of both primary producers and consumers. Nonetheless, an understanding of the mobility of these organisms prompts several expectations.

Epilithic algae (i.e. periphyton), the dominant producers in the studied section of the Red Deer River (*personal observation*) are considered to be relatively stationary as they are attached to rocks and other substrates. Because these organisms absorb nutrients from the water column and retain them in their tissues (Sand-Jensen and Borum 1991), yet do not move downstream with the current, they deplete nutrient concentrations in a downstream direction after effluent input, if nutrients are limiting in the system. In turn, this should create a decline in periphyton biomass in downstream reaches. A small number of studies report such longitudinal decreases in nutrient concentrations downstream of effluent inputs, but this is not observed in all systems (Marti et al. 2004, Ogura et al. 2009), and these studies do not address consequences for higher trophic levels.

As for consumer biomass, a positive relation might be expected between primary and secondary productivity in the Red Deer River, but according to theoretical models the resultant abundance of consumers (in this case aquatic insects) could become spatially decoupled from the factors driving their abundance (presumably productivity or abundance or of periphyton) if they undergo net downstream displacement (Anderson et al. 2005, Anderson et al. 2006a). These models emphasize that the spatial response of a population to a disturbance, or to environmental variation in general, depends on the scale of movement relative to the scale of environmental variation. They propose the average lifetime dispersal distance of a population (its so-called response length) as a key parameter that interacts with the scale of environmental variation to determine abundance patterns through space. They predict that downstream movement of individuals can spread the response of population to environmental forcing factors through space, creating downstream lags (of up to ~ 1 response length) in the response of populations to local conditions if their response length is small in comparison to the scale of environmental variability, and the environmental variability affects demographic, rather than dispersal, rates. Similarly, they show that movement could average out a population's response to environmental variation that exists at a scale smaller than the response length. This reinforces the need to learn more about the net long-term movement of in-stream insects to, for instance, forecast how environmental impacts could affect lotic ecosystems.

Given the insight from the previous theoretical works, and the prediction that insects undergo lifetime downstream dispersal on the scale of a few kilometres (which was thought to be less than the scale of the impact of the WWTP), I expected that in the

Red Deer River, insect abundance would follow a similar pattern to periphyton abundance (i.e. increased abundance after effluent input, which decreases downstream), but that peak insect abundance would lag downstream of peak periphyton abundance by a few kilometres. However, I expected that factors other than nutrient concentration could be important determinants of periphyton and insect abundance, so this was a casual prediction, and not tested quantitatively. Consumer-resource interactions could further confuse relative spatial patterns of insect and periphyton abundance, and although some of the spatial consequences of these interaction have been addressed theoretically by Anderson et al. (2006a), their study did not address the inferred mechanisms linking resource and consumer abundance predicted in the present study of the Red Deer River (i.e. alleviations on growth constraints). Instead, they focused on how variation in resource abundance affects emigration rates. Thus, their predictions cannot be fully used to inform this study. Instead, emphasis will lie in indirect methods of inferring impacts of the RDWWTP and the potential consequences of downstream insect movement.

Chapter Two: **Approaches, materials, and methods**

2.1 Study system

The study examined a 185 km stretch of the Red Deer River between Normandeau (~5 km west of the city limits of Red Deer, AB, elevation 855 m) and Morrin Bridge (~30 km upstream of Drumheller, AB, elevation 670 m) (Figure 2.1). The Red Deer River originates in the Rocky Mountains and flows 724 km until it joins the South Saskatchewan River just east of the Alberta-Saskatchewan border. Its total watershed is 49 650 km^2 (*Aqua*lity Environmental Consulting Ltd. 2009). Upstream of the study extent, the river flows through forested subalpine regions and foothills. The portion included in this study flows through ecoregions classified as aspen parkland, in the upper reaches, and mixed grassland, in the middle and lower reaches (Cross 1991), although the entire area is dominated by agriculture. 43% of land in the basin can be considered cropland, 5% is summer fallowed, and 48% is pasture (*Aqua*lity Environmental Consulting Ltd. 2009). This stretch of the Red Deer River is a medium-sized, relatively shallow river, with an average yearly discharge of 70 m^3/s. In the upper reaches of the study area, the quartile ranges of base and peak flow respectively are ~20-30 m^3/s, and ~50-150 m^3/s (Government of Alberta 2012). Peak flows usually occur around late May to mid-July, when winter snowpack in the headwaters melt. The Red Deer River is an alkaline, hard water system, characterized by high concentrations of calcium and bicarbonate (Cross 1991). The City of Red Deer is located in the upper reaches of the study extent, being by far the largest urban centre in the area. Red Deer has a population of over 80 000 people, and its municipal WWTP discharges ~3 ML/day of

tertiary treated effluent into the river (City of Red Deer 2007). Depending on discharge rates, this accounts for ~0.3-1.5 % of the river's volume, but the TN and TP concentrations in the effluent are hundreds of times greater than the natural concentrations in the river (Hogberg 2004). Aside from existing in an agricultural catchment, the river upstream of Red Deer is relatively unimpacted, although it is dammed 50 km upstream of this study's furthest upstream site. Other notable impacts include chemical manufacturing plants and substantial oil and gas development in the catchment, which averages 2 wells/km^2 (*Aqua*lity Environmental Consulting Ltd. 2009).

2.2 Field sampling

Sampling took place August 10-20, 2010, at 41 sites on the Red Deer River (Figure 2.1). Sites were positioned in relation to the Red Deer Wastewater Treatment Plant (RDWWTP), which releases effluent into the left side of Red Deer River, when facing downstream. Three sites were located upstream of the effluent outfall: one site upstream of the limits of the City of Red Deer (14 km upstream of the RDWWTP by river), and one site on each bank of the river ~0.5 km upstream of the RDWWTP. Previous research (Hogberg 2004) suggested that full transverse mixing of effluent is achieved by 9-18 km downstream of the WWTP, and that at 13 km downstream of the WWTP the Blindman River, a tributary to the Red Deer, inputs additional nutrients, also on the left bank when facing downstream. Thus, within 20 km of the WWTP, sites were paired, with one site on each bank for each distance at which samples were taken (~0.5, 2, 4, 8, 11, 13, 15, 17, and 19 km downstream of the WWTP), in order to capture lateral heterogeneity in nutrient concentrations and other conditions. The remaining 20 sites

were located every few km until 80 km downstream of the WWTP, beyond which the last three sites were positioned 117, 146, and 170 km downstream of the WWTP. Sites were preselected, but at the time of sampling, some sites were relocated to accommodate sampling restrictions. Thus GPS locations were taken at each site, and distances along the river main-stem were calculated using the path tool in Google Earth (Google Inc. 2009), which allows a more ecologically relevant measure of distance between sites than geographic distance (Murphy and Davy-Bowker 2005, Landeiro et al. 2011).

2.2.1 Macroinvertebrate sampling

At each site, three samples of benthic macroinvertebrates were taken using a Surber net with a mesh size of 500 μm mounted on a 30 by 30 cm frame. The frame was placed on the substrate with the net tailing downstream, and within a 30 by 30 cm area upstream of the net, all rocks were removed individually and invertebrates were scrubbed/picked off, letting the flow of water carry them into the net. Once all larger particles were removed, the underlying substrate was disturbed to ~10 cm for 60 s with a trowel. The contents of the net were then rinsed to the bottom of the net, letting the water drain out, and the samples were transferred to glass jars and preserved in 75% ethanol. As the Surber sample was being collected, the substrate particle size distribution within the sample was estimated. This distribution was converted to one average particle-size metric following a modified Wentworth scale (Allan 1995), in which size classes are assigned an integer value (Table 2.1), and a weighted average of these integers are calculated, based on the proportion of each size class in the sample. Because of the nature of Surber sampling, samples were restricted to areas where the water depth was less than 30 cm, where boulders were absent, and where flow was sufficient to transport material

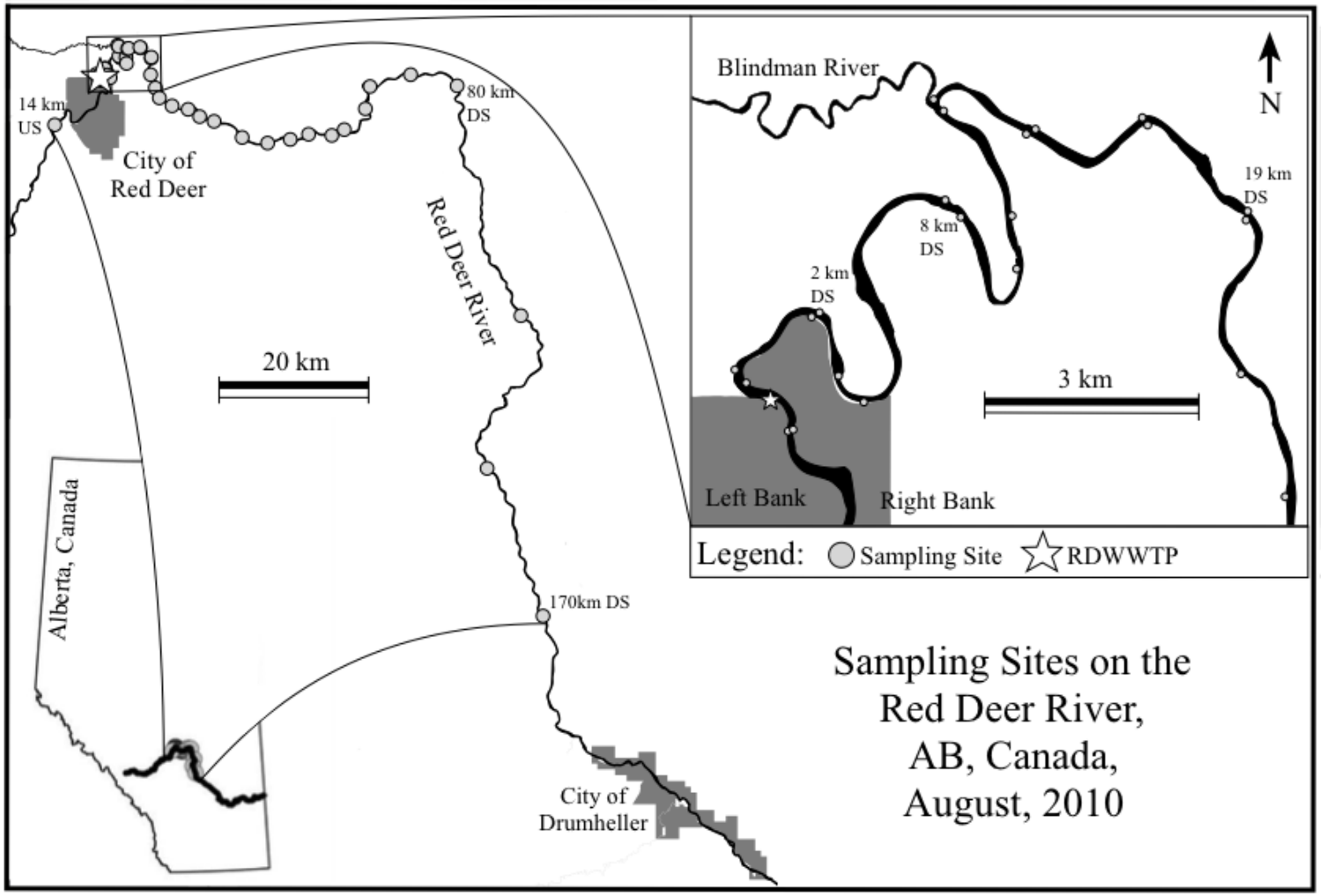


Figure 2.1: Map of study area. Select in-stream distances from the point of effluent input from the Red Deer Wastewater Treatment Plant (RDWWTP) are listed as x km US (upstream) or DS (Downstream). Sites near the WWTP are magnified in the top right corner.

into the net. Surber sites were selected randomly, with each sample being located upstream of the previous sample at the same site, to avoid disturbance to downstream samples. Although other studies argue that at least 4 Surber samples are required to capture family assemblages (Grenouillet et al. 2008), in this study, sampling more sites was prioritized with the potential cost of not fully characterizing the macroinvertebrate assemblages at each site.

Table 2.1: Modified Wentworth scale for substrate particle size. The smallest diameter of a given particle was used in measurement.

Integer Code	Description	Diameter (mm)
1	Sand	0.25-2
2	Fine gravel	2-6
3	Coarse gravel	6-16
4	Small Pebble	16-32
5	Large Pebble	32-64
6	Small Cobble	64-128
7	Large Cobble	128-256
8	Small Boulder	256-400
9	Large Boulder	>400

2.2.2 Periphyton sampling

Directly beside each Surber sample, a cobble-sized rock was selected, and two 12.56 cm^2 circles were traced out using a teasing needle and a section of a PVC tube with a 4 cm internal diameter. The periphyton samples delineated therein were scraped off using a scalpel and toothbrush, and rinsed with deionised water into glass jars. One sample was used for the analysis of biofilm and periphyton abundance (measured by ash-

free dry mass, AFDM, and Chl *a* respectively, see section 2.3.2), and one for analysis of biofilm $\delta^{15}N$ (hereafter referred to as periphyton $\delta^{15}N$). These samples were stored on ice packs in a cooler during the field day, then were stored in a 4°C refrigerator overnight, and were processed the following day.

2.2.3 Water-column sampling

1-L water samples were collected in acid-washed polyethylene bottles that were rinsed three times each with tap, deionised, distilled, and finally (at the site of sampling) river water. Water samples were taken in deeper water than were Surber samples to avoid disturbing benthic matter, and the bottle lids were secured under water to ensure no large air bubbles were present in the samples. Samples were kept among ice packs in a cooler throughout the field day, then were stored in a 4°C refrigerator overnight, and were processed the following day. Average water velocity at each Surber sample was estimated by measuring water velocity at 0.6 depth using a Geopacks Flowmeter (MFP51).

2.3 Sample processing

2.3.1 Macroinvertebrate processing

To obtain an initial impression of the variety and abundance of macroinvertebrates within the study area, six samples representing different parts of the spatial extent were selected, and all macroinvertebrates were classified to genus following Clifford (1991), with the aid of Merritt et al. (2008), and enumerated. From these data, 11 insect groups were selected (as described in the introduction) to undergo stable-isotope analysis. For genera with targeted classes of larval instars, age designation was based on head width, as measured using a stage micrometer in a watch glass placed

over the insect. Although this may not have yielded "true" head widths, it was consistent across samples and thus sufficient to group individuals into the broad ages classes used in this study. Specific head widths used to categorize age classes are listed in Table 2.2, as are the movement and feeding behaviours of the different groups.

For all samples, macroinvertebrates were first separated from periphyton, sand, and other matter present in the samples. If a sample included many invertebrates, it was sub-sampled by evenly spreading the invertebrates in a Petri dish with marked quadrants, taking the invertebrates from one or two quadrants, and proceeding with these (completing further sub-samples if necessary). Individuals were identified to family (or genera (and) life stage if they belonged to the select groups) and counted. For stable isotope analysis, invertebrates of the select groups were taken from the sorted samples in sufficient number to obtain 1-4 mg samples (the mass required for $\delta^{15}N$ analysis), for a maximum of 5 samples (for invertebrates for which one individual was sufficient for one sample), or two samples (if multiple individuals had to be combined to form a sample). If individuals exceeded 4 mg, they were cut in half along the midsagittal plane. See Table 2.2 for the approximate number of individuals in each sample. The specific Surber sample (of a particular site) from which each stable isotope sample was taken was not of interest, as often individuals had to be combined from multiple Surber samples. These samples were dried at 55°C for at least 48 hrs, and weighed before stable-isotope analysis (see below). After removing the individuals for isotope analysis, the remainder of the samples (sorted to family or genera (and) life stage) were also dried, and weighed (with the mass of the removed individuals and the magnitude of sub-sampling being accounted for).

Table 2.2: Methodological and ecological features of insect groups selected for stable isotope analyses. Head widths were used to designate general age classes. Feeding and movement behaviours were obtained from Merritt et al. (2008). Collector-gatherers feed on loose detritus and algae, collector-filterers construct nets or other features to obtain suspended particles from the water column. Scrapers feed on attached algae (periphyton). Swimmers move actively by "fishlike" swimming, clingers are adapted for attachment to surfaces, sprawlers inhabit surfaces of fine sediment or plants, burrowers inhabit fine sediments or other soft substrates, or construct tubes.

Insect group	Head width (mm)	No. per $\delta^{15}N$ sample	Feeding behaviour	Movement behaviour
Baetis mid instar	0.5-0.7	20	Collector-gatherers, facultative scrapers	Swimmers, clingers
Baetis late instar	≥ 0.9	4-8		
Brachycentrus	≥ 1.0	1	Omnivorous collector-filterers, facultative scrapers	Clingers
Chironomidae	N/A	10-40	Collector-gatherers and filterers, predators	Burrowers, sprawlers, clingers
Heptagenia early instar	1.5-2.2	2-3	Scrapers, facultative collector-gatherers	Clingers, swimmers
Heptagenia mid instar	2.3-2.6	1		
Heptagenia late instar	≥ 2.7	0.5-1		
Hydropsyche larvae	≥ 1.3	0.5-1	Omnivorous collector-filterers, facultative scrapers, predators	Clingers
Hydropsyche pupae	N/A	0.5-1		
Tricorythodes mid instar	0.7-0.8	8-12	Collector-gatherers, facultative scrapers	Sprawlers, clingers
Tricorythodes late instar	≥ 0.9	2-3		

2.3.2 Periphyton processing

The periphyton samples taken for stable-isotope analysis were filtered onto VWR® grade 696 glass fibre filters, wrapped in tinfoil, and stored in a -20°C freezer until further analysis (see below). The periphyton samples taken for ash-free dry mass (AFDM, organic C) and Chl *a* analysis were filtered onto pre-combusted pre-weighed VWR® grade 696 glass fibre filters (1.2 µm pore size), which were subsequently cut in half. One half was dried at 55°C for at least 48 hours, weighed, combusted at (550°C) for 4 hrs, and weighed again to obtain an estimate of AFDM, which was measured as loss on ignition. The other half was wrapped in tinfoil and stored in a -20°C freezer until extraction into 100% methanol, and analysis of Chl *a* via spectrofluorometry using a SPECTRAmax® GEMINI-XS, following the methods of Thompson et al. (1999) without correction for phaeophytin content.

2.3.3 Water-sample processing

The day after collection, pH was immediately measured after opening sample bottles using a Fisher Accumet® pH Meter Model 140, and turbidity was measured using an Orbeco-Hellige portable turbidimeter, Model 968, which was calibrated with a 0 NTU blank, and a 40 NTU standard. From the 1-L water sample, a 50-mL sample was taken for analysis of total Phosphorus concentration (TP), and another 50-mL sample was filtered through a VWR® grade 696 glass fibre filter (1.2 µm pore size) for analysis of total dissolved Phosphorus concentration (TDP). These samples were digested with potassium persulfate, and P concentrations were measured by spectrophotometry using the molybdate-blue technique (Menzel and Corwin 1965, Prepas and Rigler 1982) on a Shimadzu UV Spectrophotometer (model UV-1800 120 V). For measurement of water-

column Ch *a* concentration, another 200-500 mL of river water was filtered through a VWR® grade 696 glass fibre filter (1.2 µm pore size), which was wrapped in tinfoil and frozen until extraction into 90% acetone, and fluorometric analysis of Chl *a* (Arar and Collins 1997), performed on a Quantech™ Fluorometer (model QNT WIDE 120V). An equal amount of water was filtered through a pre-combusted pre-weighed VWR® grade 696 glass fibre filter (1.2 µm pore size), which was analyzed for water column AFDM as for periphyton AFDM. 10 mL samples were frozen for analysis of total nitrogen (TN) and nitrate (NO_3) concentration, which was performed at the Pacific and Yukon Laboratory for Environmental Testing (PYLET), North Vancouver, BC, Canada, by persulphate digests and flow injection analysis (for TN) (Soo 2011b) and ion chromatography (for NO_3) (Soo 2011a).

2.3.4 $\delta^{15}N$ signatures

The measurement of insect and periphyton $\delta^{15}N$ signatures was conducted at the Isotope Science Lab at the University of Calgary, AB, Canada, using Continuous Flow-Elemental Analysis-Isotope Ratio Mass Spectrometry technology, in which a Finnigan Mat Delta Plus XL Mass Spectrometer is coupled with a Costech 4010 Elemental Analyzer. International standards (USGS 40 and USGS 41) and lab standards of keratin, peach leaves (for insect and periphyton stable-isotope analysis respectively), and caffeine were used to normalize the data to international standards, and to correct for instrumental drift. This analysis has a measurement uncertainty of ± 0.2‰ (University of Calgary 2013). Insect samples were prepared as above, as entire samples (i.e. whole or multiple individuals) could be packed into the analytical tin cups. Periphyton samples, which were stored frozen on glass-fibre filters, were thawed, dried at 55°C for at least 48 hrs,

removed from the glass fibre filters, and ground and homogenized with a ceramic mortar and pestle. For most sites there were a total of three samples per site (one associated with each Surber sample); however, at a few sites, the biomass of individual periphyton samples was insufficient to attain 10 mg (the mass required for $\delta^{15}N$ analysis of periphyton), so some or all samples at such sites had to be combined. It should be noted that although invertebrate samples were stored in ethanol, and periphyton samples were frozen, these techniques have no significant systematic effect on tissue $\delta^{15}N$ signatures (Kaehler and Pakhomov 2001, Sarakinos et al. 2002, Ventura and Jeppesen 2009), although ethanol preservation does tend to increase signature variance (Sarakinos et al. 2002, Ventura and Jeppesen 2009). Thus, the method of sample fixation was not deemed to be of concern in later comparisons between insect and periphyton signatures, at least in terms of creating bias in signatures.

2.4 Longitudinal patterns in abiotic and biotic variables

As longitudinal patterns had obvious nonlinearities, LOESS, or locally weighted regression (Cleveland 1979) was used to facilitate the description of general spatial patterns in biotic and abiotic variables. LOESS is a smoothing technique that uses polynomial regression to create smoothed points from raw data within a defined radius of each point. These calculations are weighted in the sense that each point of raw data within the radius is weighted to have less of an effect on the value of a smoothed point the farther it is located from the smoothed point (Cleveland 1979). In creating LOESS curves for variables, the raw data from every site excluding the reference site 14 km upstream of the WWTP and the left-bank site directly upstream of the WWTP were

included. This was done to best capture abrupt changes occurring after effluent input. Separate curves for left and right-bank data were created, with left or right-bank designation in this case extending beyond paired sites, to 60 km downstream, to better view where LOESS averages converged (which was not always within 20 km of the WWTP, where the paired sites were located). Smoothed points were located every ~4 km, with a sampling parameter of 0.3 (the proportion of the "nearest neighbour" raw data used to create a smoothed point), and a first-degree polynomial was chosen to fit the data, with tricube weighting. Slight modifications to these specifications were made if the data were sparse, as for some groups of insects. LOESS regression was performed to aid the description of longitudinal patterns in $\delta^{15}N$ signatures of periphyton and insect groups, water column variables (NO_3, TN, TP, TDP, and Phytoplankton Chl *a*, in µg/L, water column AFDM , in mg/L, turbidity, in NTU, and average water velocity, in m/s) and benthic variables (abundance of insect groups, in No./per Surber sample, periphyton Chl *a*, in $\mu g/cm^2$, total insect dry mass, in g/m^2, and average substrate particle size, in a modified Wentworth scale).

2.5 The effect of WWTP effluent on local insect abundance

2.5.1 General approach and justification

To infer whether the WWTP affected insect communities, generalized linear models were used to determine which of four "environmental" variables (periphyton abundance (mg/cm^2 Chl *a*), nitrate concentration (NO_3, mg/L), average substrate particle size (in terms of a modified Wentworth scale, Table 2.1), and average water velocity (m/s)) best explained total insect abundance, and the abundance of several important insect genera. Two of these variables, NO_3 and periphyton Chl *a*, were expected to be

affected by effluent input, and thus a significant influence either variable was interpreted as an insect response to changes driven by the WWTP's effluent. In contrast, average water velocity and substrate particle size were related to hydraulic habitat conditions, and were included in this analysis because primary productivity was not expected to be the only factor driving insect distribution abundance, and thus it was of interest to assess whether variables related to primary productivity were significantly related to insect abundance after accounting for variation in other factors. Thus relations of abundance to hydraulic / habitat variables will not be explicitly addressed in the discussion.

In this analysis, NO_3 concentration was included as a signal of effluent presence, and as a proxy for potential periphyton productivity. NO_3, rather than periphyton abundance, was granted this interpretation as it was expected that consumer-resource interactions could blur correlations between local periphyton standing crop and insect abundance. A measure of dissolved nitrogen, as opposed to dissolved phosphorus, was chosen because at most sites the ratio between TN:TP was less than 20, which, in the presence of nutrient limitation, would cause nitrogen limitation specifically (Guildford and Hecky 2000). Within 4 km of the WWTP the ratio of TN:TP was between 20 and 50, which would indicate co-limitation at these sites (Guildford and Hecky 2000), but given that TN and TP were both high at these sites (Figure 3.1), only nitrogen was included for consistency. Additionally, NO_3 concentration was chosen instead of TN because the two variables were highly correlated (r=0.98), and TN includes organic nitrogen, which would be less biologically available to primary producers than a dissolved inorganic form (Tank et al. 2010).

The hydraulic and habitat variables chosen for analysis were mean water velocity and mean particle size, both of which affect macroinvertebrate distribution and abundance (Lancaster and Hildrew 1993, Quinn and Hickey 1994, Quinn et al. 1996, Buffagni et al. 2000, Fairchild and Holomuzki 2002, Heino et al. 2004, Merigoux and Doledec 2004, Brooks et al. 2005, Effenberger et al. 2006, Ortiz et al. 2006, Principe et al. 2007, Pastuchova et al. 2008), and thus have become essential considerations in habitat-based approaches to river management (Milner et al. 1985, Harper et al. 1995). These variables interact to determine hydraulic forces and substrate stability (Gibbins et al. 2007), which can affect various aspects of insects' lives, from the ease of maintaining location, to the delivery of resources (Quinn and Hickey 1994, Brooks et al. 2005, Gibbins et al. 2007).

The two-way interactions between each of the four environmental variables were also included as potential effects in models of insect abundance, as it was expected that the effect of each variable may differ depending on the values of other variables. For example, a strong positive correlation between NO_3 and insect abundance (which would presumably occur via a positive effect of NO_3 on primary productivity) could be weakened where water velocities are low, as nutrient delivery is slower, given the development of thick nutrient-depleted diffusive boundary layers above periphyton that are otherwise diminished at greater velocities (Sand-Jensen and Borum 1991). Thus each full model consisted of four main effects and six interaction terms.

2.5.2 Statistical procedures

Separate analyses were conducted for the insect groups chosen for stable isotope analysis (except that late-instar *Tricorythodes* and *Baetis* were excluded to reduce effort,

see Table 2.2), and also for total insect abundance. Analyses were performed at the "site" level, rather than the "sample" level (Appendix A: Datasets). In the analysis of insect genera (number of individuals/site), a log link function and a negative binomial error distribution were chosen, as opposed to a Poisson distribution, as these were count data, and likely to be clumped, which would cause the data to be overdispersed (when the variance of the data is greater than the mean), making the Poisson distribution inappropriate (Hoffman 2004). In the analysis of total insect abundance (mg/m^2), which was a right-skewed continuous variable, a gamma distribution and a log link function were used. The suitability of these distributions was assessed by the ratio of model deviance to degrees of freedom, which should be less than 1.6-1.5 given that deviance asymptotically follows a χ^2 distribution with n-p degrees of freedom (Myers et al. 2002, p refers to the number of parameters in the model, n refers to sample size), and n was 29 for all models. Manual, backwards elimination of variables was employed in the following manner: using a type one error rate, α, of 0.05, non-significant main effects or interaction terms were eliminated by first removing the term with the highest p-value in the output of Type 3 likelihood-ratio tests, which assess the significance of each effect given all other effects in the model (SAS Institute Inc. 2011). Even if considered non-significant at $\alpha = 0.05$, main effects were not eliminated if they were included in an interaction term still present in the model. After an effect was removed, the model was re-run, and the process was repeated until all main effects or interaction terms left in the model were significant, if any.

2.6 Analysis of local abundance with independent variables averaged across sites

2.6.1 General approach and justification

To determine whether insects respond to conditions beyond those at the site of sampling, which could imply movement through these areas, the same backwards selection procedure as above was repeated, but using independent variables that were averaged over various upstream and/or downstream lags. This follows the results of Anderson et al. (2005), who showed, theoretically, that if the average lifetime dispersal distance of a population exceeds the scale of abundance-determining environmental variation, the resultant abundance at any point matches the average of the variation through space more closely than the local value of the environmental variable. Thus, if a) abundance-determining conditions vary meaningfully on a scale that corresponds to the distance between sites in this study, yet b) throughout their lifetime, insects move over an area that is greater than the distance between sites, then c) abundance at any one location may be representative of a pool of individuals that is best predicted by conditions averaged between nearby sites, as these sites would be unified by migration, and this movement could average out local responses to small-scale environmental variation.

To detect responses to upstream conditions in addition to local conditions (which could imply downstream movement on a scale larger than the distance between sites) models were run with independent variables comprising the averages of measurements at the local site and either next site upstream, or the next two sites upstream (Appendix A: Datasets). This analysis was repeated with independent variables that were averaged over the local and next *downstream* site (so that the possibility of net upstream movement, by upstream swimming or flights of adults, was not excluded), and with variables that were

averaged over the local and adjacent upstream and downstream sites (to assess bidirectional movement). The additional consideration of upstream flights of adults is distinct from inferences made from stable-isotope analyses (see section 2.7), as larval abundance, unlike stable isotope signatures, could be affected by downstream conditions as follows: larval abundance is affected by the spatial distribution of eggs, which are laid by terrestrial adult insects (Lancaster et al. 2011) that can move upstream considerable distances (Hershey et al. 1993, Macneale et al. 2005), and whose abundance could thus be expected to be affected by downstream conditions that determine adult emergence, for instance.

2.6.2 Analysing residual autocorrelation

The presence of spatial autocorrelation in the residuals of models of insect abundance was also assessed because a) it can bias significance tests by underestimating the error associated with parameters (Diniz et al. 2008), and b) it can be indicative of disparity in the scale of analysis used to model a population's response to its environment, and the scale at which these organisms actually respond to their environment (de Knegt et al. 2010). Specifically, analysis at a larger scale than a population's response causes spatial smoothing, and analysis at a smaller scale than its response causes a loss of landscape context. The presence of significant residual spatial autocorrelation was detected by reanalysing the models resulting from the above analysis with the inclusion of a spatial variance-covariance matrix to explicitly model residual spatial structure (as in Lichstein et al. 2002), and comparing this model, via a likelihood ratio test, to one assuming the independence of residuals. This procedure identifies whether residual covariance between sites changes in a predictable (declining) manner as

a function of distance between sites, and estimates the spatial extent to which significant autocovariance exists (the range). This analysis was performed twice: once assuming an exponential model of covariance (in which covariation between sites declines exponentially with increasing distance between sites, reaching zero asymptotically) and once more, assuming a spherical model, which is similar except that after a certain distance between sites, covariation between sites plateaus at zero (Littell et al. 2006). Other models of residual spatial autocorrelation (i.e. power, Gaussian etc.) were not considered, as inspection of empirical residual semivariograms (which depict spatial variance as a function of distance between sites) indicated that these were acceptable models, and major differences in spatial analysis depend primarily on the presence or absence of a reasonable spatial variance-covariance model, rather than in the specific model used (Littell et al. 2006). If these models gave similar results, the one that qualitatively best fit the semivariogram was chosen. If significant spatial covariance was present, indicating non-independence of observations, the significance of effects was reassessed using the Kenward-Roger method for degrees-of-freedom adjustment (Kenward and Roger 1997), and coefficients were re-reported. Otherwise, spatial variance-covariance matrices were not kept in the final reported models. Because of issues involving model convergence, some parameters had to be constrained, as described in Appendix B: Further details regarding generalized linear mixed models with spatial variance covariance matrices.

2.6.3 Comparing models

In summary, each analysis (i.e. for total insect abundance and for each insect group) considered five models that differed in the area through which their independent

variables were calculated. These models will be labelled as “local” (for the original analysis with site-level data), “1 Up” (for variables averaged over the local and next upstream site), “2 Up” (for variables averaged over the local and next two upstream sites), “1 Down” (for variables averaged over the local and next downstream site), and “1 Up, Down” (for variables averaged over the local and next upstream and downstream sites). To compare the relative fit of the various models for each group of insects, the Akaike information criterion (AIC) values were compared, with lower values indicating a better fit to the raw data. Any model whose AIC exceeded the best model by <6 units was considered to be equally good at explaining abundances (Richards 2005, 2008). Unfortunately, the models including spatial variance-covariance matrixes couldn’t be directly compared to models with variables of different lags (with or without residual spatial autocorrelation accounted for), as AIC values are not provided for generalized linear mixed models in the software used for this analysis, SAS/STAT of SAS 9.2 (PROC GLIMMIX, SAS Institute Inc., Cary, NC, USA).

Note that although sites were not evenly spaced, this was considered to be a preliminary analysis, and variables showed less variation in downstream areas, where sites were spaced farther apart. Furthermore, in a river with varying current speed and direction, distance between sites is an imprecise measure of functional separation. It should also be emphasized that the variables and interactions retained in each of these five models were not necessarily the same, as the possibility that insects could respond to different variables at different scales was not discounted. This being said, the purpose of this analysis was primarily to assess whether upstream (an/or downstream) conditions

explained local abundance better than local conditions, regardless of the specific variables or interaction retained in the models.

2.7 The use of stable-isotope signatures to estimate the scale of insect movements

2.7.1 General approach and background

One of the reasons for the lack of long-term movement studies of aquatic insect larvae is, simply, that it is a difficult undertaking. Long-term mark-recapture techniques tend to not be feasible for anything but cased caddisflies, and radio telemetry, despite being used in movement studies of large aquatic macroinvertebrates (Bubb et al. 2002), is not realistic for small ones. Thus, indirect techniques are becoming popular for inferring the movement tendencies of small aquatic macroinvertebrates in rivers. One such technique involves the use of stable isotopes as markers, which will be described below, before presenting the specific approach used in this study.

2.7.1.1 The use of stable-isotope signatures to infer animal movements

Stable isotopes are atoms of a given element that have different numbers of neutrons in their nucleus, and that do not radioactively decay. Although the global proportion of each isotope of an element is roughly constant, these proportions do change on other scales during various physical and (bio)chemical processes in which atomic mass affects the relative activity of different isotopes of an element, causing unequal allocation of isotopes in substrate(s) versus product(s), a phenomenon called isotope fractionation (Fry 2006). For instance, in situations of chemical equilibria, heavier isotopes tend to accumulate in the state with the highest bond strength. In more complicated reactions, kinetic effects tend to cause light isotopes to react faster than heavy ones, causing products to be enriched in light isotopes, and residual substrates to

be enriched in heavy isotopes (Peterson and Fry 1987, Fry 2006). The isotopic constitution of a given measured sample is represented with δ notation, which assigns a signature in units of parts per thousand (per mil) difference from a standard:

$$\delta^{x}X = [(R_{sample}/R_{standard})-1] \times 10^{3} ,$$

where ^{x}X represents the heavier isotope of an element (for example ^{15}N) and R represents the ratio of the heavy to the light isotope (for example $^{15}N/^{14}N$) in the sample or standard (for example nitrogen gas in the atmosphere). Positive $\delta^{x}X$ values mean the sample is more enriched in the heavier isotope than in the standard, and vice versa for negative values. Thus a sample with a $\delta^{x}X$ signature that is greater than that of another sample has a higher proportion of the heavier isotope (Peterson and Fry 1987, Fry 2006).

The discrepancy, or lack thereof, between stable-isotope signatures of animals and their diet can provide insight into their movement, based on the following rationale. When animals consume a food source –which will have a particular ratio of a given elemental isotopes in its tissues- their own tissues acquires a similar isotopic signature (Deniro and Epstein 1978). If a consumer is sedentary, it should be in isotopic equilibrium with -and thus have the same signature as- its local food source, shifted by a constant representing, for example, biased consumption or assimilation (Rasmussen et al. 2009). This shift represents a type of isotope fractionation that occurs in trophic interactions, and will be further discussed shortly. Thus, if an animal has an isotopic signature that is different than that of its local food source (after accounting for the aforementioned shift), one could assume that it has consumed resources from an area with a different resource signature, and moved to its current location. This is because an animal does not immediately acquire the isotope signature of local food resources, but

retains a signature that is an average of the resources used to build its current tissues, and thus reflects diet history (Peterson and Fry 1987). Furthermore, if the isotope signatures of food in other areas are distinct from one another and known, where the animal has moved from can be inferred (Fry 1981).

2.7.1.1.1 Use of $\delta^{15}N$ to infer river insect movements

Although the elements C, N, S, H, and O all have different naturally occurring isotopes (Peterson and Fry 1987), and have been used for the study of animal movements (Rubenstein and Hobson 2004), ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ seem to be the most commonly analysed isotopic ratios for studies of macroinvertebrate movements in rivers, and are thus most relevant to this study. For instance, ^{15}N added to aquatic ecosystems has been used as a marker to trace the flight patterns of lotic populations of adult *Callibaetis* mayflies (Caudill 2003), and lentic populations of adult stoneflies (Macneale et al. 2005) and *Baetis* mayflies (Hershey et al. 1993). However, the application of stable isotope tracers to the study of in-stream larval populations is limited.

2.7.1.2 General Approach

In this study, spatial gradients in the $\delta^{15}N$ signatures of insects and periphyton were used to assess the plausibility of previous net downstream movement. Additionally, the residuals of these gradients were used to estimate the *scale* of movement of these groups. In the Red Deer River, $\delta^{15}N$ gradients are driven by the input of wastewater effluent, which contains dissolved forms of nitrogen (such as nitrate, nitrite, and ammonia) that have an unusually high $\delta^{15}N$ signature as a result of the various biochemical processes involved in effluent treatment, including the hydrolysis of urea, subsequent volatilization of ammonia, and the denitrification of nitrate (which

counteracts a decrease in overall $\delta^{15}N$ caused by nitrification) (Heaton 1986). Addition of these nutrients thus causes the average $\delta^{15}N$ values of dissolved forms of nitrogen downstream of the WWTP to be higher than in upstream areas (Hogberg 2004). $\delta^{15}N$ signatures then decrease to upstream levels as distance downstream of the WWTP increases, likely as a result of dissolved nitrogen becoming diluted and incorporated into biomass, (and probably not as a result of being removed from the river by denitrification (Hogberg 2004)). A similar $\delta^{15}N$ gradient also occurs in the primary producers that acquire these nutrients from the water column (Hogberg 2004). In this study, it was expected that macroinvertebrates feeding on these primary producers would have a similar $\delta^{15}N$ gradient, but that their signatures would be altered in a manner that would reflect their movement behaviour, as follows. If insects are relatively stationary, they should have similar spatial $\delta^{15}N$ gradients to that of periphyton (whose $\delta^{15}N$ gradient was assumed to be representative of the resource gradient) with a possible directional shift in signatures resulting from biased consumption or assimilation (Rasmussen et al. 2009). In contrast, if insects were undergoing net downstream dispersal as presumed, organisms close to the WWTP should have lower $\delta^{15}N$ values than the local (relatively immobile) resource, as they would retain, at least partially, the signature of areas upstream of the WWTP, which were not enriched in ^{15}N compared to ^{14}N. At some point, $\delta^{15}N$ signatures should exceed that of the local resources, because they had not been exposed to sites upstream of the WWTP, and were thus moving from upstream areas with higher periphyton $\delta^{15}N$ values than their sampled downstream location. This would cause a decreased slope in the spatial $\delta^{15}N$ gradient of insects compared to periphyton (Figure

2.2). Based on these predictions, linear regression was used to determine whether insect $\delta^{15}N$ gradients differed (in either slope or intercept) from periphyton $\delta^{15}N$ gradients (section 2.7.2.1), with a lack of significant difference to periphyton signatures representing a lack of substantial directional movement, and a depressed slope of the signature gradient suggesting downstream movement. The estimates of movement gained from this study were inferred to reflect feeding habits from, roughly, the previous month, as half-lives for nitrogen in the tissues of macroinvertebrates are typically around 15-20 days (McIntyre and Flecker 2006, Kaufman et al. 2008, Larsen et al. 2011), but can range as low as 5 days (Larsen et al. 2011) and as high as 50 days (McIntyre and Flecker 2006).

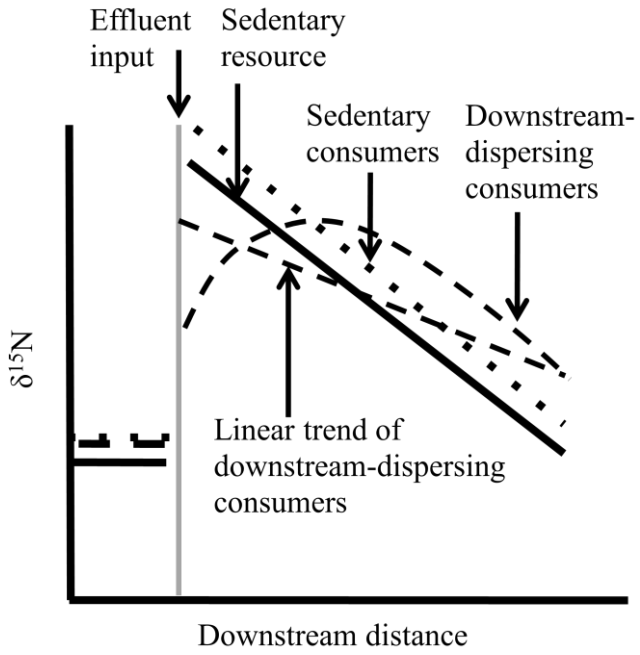


Figure 2.2: Expected spatial patterns in the $\delta^{15}N$ signatures of biota after WWTP effluent input in the Red Deer River, given different movement regimes, without correction for trophic fractionation.

As previously mentioned, in this analysis it is assumed that the isotopic signature gradient of periphyton scrapings is representative of that of the diet of all insect groups. This assumption is fair for some groups and less so for others, for whom it is a recognized limitation that will be discussed here, and in section 4.2.2.2.2. It is safe to assume that Collector-gatherers and scrapers in the Red Deer River would be feeding on local periphyton rather than externally-derived material, as autotrophic communities, as opposed to allochthonous inputs, tend form the base of open-canopy lotic food chains in summer months (Minshall 1978, Vannote et al. 1980). For filter feeders, on the other hand, the assumption that periphyton signatures can represent the signatures of their food source could be erroneous. If filter feeders were to solely feed on filtered particles - sourced from an indeterminate area upstream- signatures that indicate downstream movement of insects from upstream areas could equally as well represent the downstream movement of food resources, or the combined result of the two. Nonetheless, results concerning filter feeders are reported, because regardless of whether movement specifically can be inferred from this analysis, it does provide information about the spatial extent of trophic linkages. This can lend insight towards the upstream area from which resources affect downstream populations (Finlay et al. 2002), and fill a gap in knowledge that exists regarding the spatial scale of trophic interactions (Cooper et al. 1997). This could aid the understanding of population, community, and ecosystem functioning, and could be used in designing experimental investigations, and in conserving important habitats (Finlay et al. 2002).

Another limitation of this approach involves the isotopic fractionation, or enrichment in one isotope relative to another, that occurs in consumers relative to their

diet as a result of a feeding or assimilation bias, or because of the energetics of metabolic reactions (Rasmussen et al. 2009). In the latter case, for $\delta^{15}N$, biomass becomes more enriched in ^{15}N relative to ^{14}N with increasing trophic level, as the heavier isotope of nitrogen is preferentially retained in consumer tissues during certain metabolic processes, with the remaining lighter isotope being excreted in nitrogenous wastes(Peterson and Fry 1987). Hence the $\delta^{15}N$ signature of a consumer- or insect in this case- will always be higher than that of the primary producer on which it fed –assumed to be periphyton. Because of this phenomenon, comparing raw signatures of macroinvertebrates and periphyton in order to infer movement could lead to erroneous interpretations, as the incidence of a consumer signature that has a different $\delta^{15}N$ signature than the local resources could be attributed either to a recent movement from an area that is isotopically distinct, or to trophic fractionation, or to both. Thus, trophic fractionation must be accounted for before interpreting differences in nitrogen isotopic signatures. However, because this is a novel study, no previous examples of trophic correction can, rightfully, be applied. Furthermore, error in the trophic fractionation correction used could affect interpretations of movement. As an example, if correction was not great enough (and thus insect signatures were not sufficiently lowered to counteract trophic fractionation), this could detract from the ability to detect downstream movement near the WWTP, as signatures might be too similar to periphyton. Thus, the method conceived will receive special attention in this thesis.

2.7.1.3 Trophic fractionation correction

In order to make insect and periphyton signatures "trophically equivalent" so that differences in signatures could be attributed to movement, trophic fractionation was

accounted for, as follows. For each group, the mean macroinvertebrate $\delta^{15}N$ signature at the reference site 14 km upstream of the WWTP (where the $\delta^{15}N$ signature was assumed to be more consistent spatially) was subtracted from the mean periphyton signature at the same site, to obtain a trophic correction for each group of macroinvertebrates. This correction value was then subtracted from the raw data to make the macroinvertebrate signatures "trophically equivalent" to those of periphyton. Because of uncertainty in whether this correction would be accurate for downstream areas enriched in nutrients and ^{15}N, analyses relatively insensitive to the degree of fractionation were performed to complement analyses whose interpretation could change, depending on whether the trophic correction value was correct, as follows.

2.7.2 Statistical procedures

Two major analyses were performed on the site means (Appendix A: Datasets) of trophically corrected isotope signatures: an analysis of linear gradients, and an analysis of the cross correlation between the residuals of these linear trends, as described below, in sections 2.7.2.1 and 2.7.2.2 respectively.

2.7.2.1 Differences between linear trends in insect and periphyton $\delta^{15}N$ signatures

Analysis of covariance (ANCOVA) was used to assess whether linear trends in left-bank trophically-corrected insect $\delta^{15}N$ signatures differed significantly from that of periphyton (Appendix A: Datasets).

Before proceeding with the final ANCOVA, it was of interest to assess whether isotope signatures differed between life stages of genera that had multiple life stages represented, and if not, to combine these groups in order to decrease redundant analyses and interpretations. Thus separate ANCOVAs were performed between life stages of

macroinvertebrates within the same genus to determine whether their mean $\delta^{15}N$ signatures, or their spatial gradient in signatures differed. For life stages of a given genus that were not significantly different in either respect, the isotope signatures were combined into one category, and the site means were recalculated. This resulted in all three life stages of *Heptagenia* being combined, and the two life stages of *Hydropsyche* being combined.

In the final ANCOVA, to determine whether insect and periphyton $\delta^{15}N$ signatures differed, I first determined whether $\delta^{15}N$ signatures were affected by distance from the RDWWTP, group of organism (including insect groups and periphyton), and the interaction between these terms. Post-hoc analysis was used to determine which insects differed significantly from periphyton in terms of least-squared (LS) mean $\delta^{15}N$ signatures and/or their linear spatial gradient in $\delta^{15}N$ signatures. Insect groups were not compared, as the primary purpose of this analysis was to detect differences between insect and periphyton signatures, which could provide clues about movement. It was deemed satisfactory to compare insects in terms of their suspected degree of movement. Thus, for these two sets of analyses ($\delta^{15}N$ LS means and $\delta^{15}N$ gradients), the type I error rate for each comparison (α_c) was adjusted to maintain an experiment-wide error rate (α_e) of 0.05 using the Dunn-Sidak correction $\alpha_c = 1 - (1- \alpha_e)^{1/j}$ where j is the number of comparisons. Hence the type I error rate for these comparisons was $\alpha_8=1-(1-0.05)^{1/8} = 0.00639$. The residuals of this model were then assessed for normality using Shapiro-Wilk and Kolmogorov-Smirnov tests. These analyses were performed using the SAS/STAT software of SAS 9.2 for Windows (SAS Institute Inc., Cary, NC, USA). They were also evaluated for the presence of spatial autocorrelation using non-parametric

spline correlograms in the package *ncf* version 1.1-3 (Bjornstad 2009) in R (R Development Core Team 2012). Finally, spatial cross-correlations between the residuals of periphyton and each insect group were calculated, as described below.

2.7.2.2 Spatial cross-correlation in residual insect and periphyton $\delta^{15}N$ signatures

Because of potential error in trophic correction, spatial analyses were performed on the residuals of the above ANCOVA, which forced all data to be centered on 0, regardless of the trophic correction value used. Conveniently, computing residuals also removed large-scale linear spatial trends in the data, helping to ensure stationarity, which is the assumption in most spatial analyses that the mean and spatial covariance of the variable of interest remain constant over the spatial extent (Legendre and Legendre 1998). The specific analysis performed was the computation of nonparametric spline cross-correlograms (Bjornstad and Falck 2001), which assesses the correlation of two variables (in this case the residual stable isotope signatures of periphyton and an insect group) at different spatial lags. A nonparametric spline-smoothed analysis was chosen because sites were not equally spaced, a requirement for the computation of classical cross-correlation functions. This analysis was performed following the logic that if insects were not moving, the residuals of linear regressions of insect and periphyton signatures would be highly correlated within sites (i.e. at a lag of zero). On the other hand, if considerable downstream movement was occurring, local insect $\delta^{15}N$ signatures would be correlated to periphyton $\delta^{15}N$ signatures at upstream sites. This analysis estimates cross-correlation as a continuous function of distance, producing cross-correlograms with bootstrapped 95% confidence intervals (calculated from 10000 resamples of the data) (Bjornstad and Falck 2001), and was performed using the function

spline.correlog in the package *ncf* version 1.1-3 (Bjornstad 2009) in R (R Development Core Team 2012). In these graphs, the "zero line" represents the average similarity of the extent, and thus a significant estimate of cross-correlation at a given separation distance means that samples separated by this distance are more similar than expected by chance, given the base similarity in the study extent.

Autocorrelation in residual isotope signatures was also evaluated similarly to determine whether cross-correlation between insect and periphyton signatures could be induced by local correlation to periphyton signatures that were themselves, spatially autocorrelated.

2.8 Movement as a potential mechanism structuring the large-scale spatial response of insects to environmental variability

The fourth and final objective of this study was approached by comparing insights from a number of observations and analyses. The first was a consideration of whether insect abundance was lagged downstream of periphyton abundance, as would be predicted theoretically, in the absence of ecological complexities. The other analyses were conducted to infer whether movement may be important in spatially structuring insect abundance by assessing whether consideration of movement is necessary to explain abundance through space. These analyses were concurrently used to evaluate the plausibility of the scales of movement estimated by other means. The first of these analyses is described in section 2.6, and involved assessing whether local insect abundance was best explained by variables measured at the sampling site (in the case of movement at a scale less than the distance between sites), or by averages of variables over larger scales, implying larger-scale movement. In a second supplementary analysis,

Mantel correlograms (Oden and Sokal 1986, Sokal 1986) were used to describe the spatial autocorrelation structure in insect family assemblages. This analysis differs from the previous in that it uses multivariate data, so it will be allotted a distinct examination in the remaining sections, starting below.

2.9 Analysing spatial structure in insect family assemblages

2.9.1 General approach

The possibility that spatial structure in insect assemblages can be used to infer how movement affects their spatial distribution is founded under the expectation that downstream flow and upstream flights enhance connectivity along river channels, which should cause spatial autocorrelation in assemblage compositions (Lloyd et al. 2006). Furthermore, this autocorrelation is suggested to exist on a scale similar to that of the overall movement of invertebrates, including downstream drift and upstream flights (Lloyd et al. 2006, de Knegt et al. 2010). Although the discussion so far has focused on in-stream movements, the movements of adult terrestrial stages of insects are equally important, and the scale of adult flights should influence spatial concordance between invertebrate assemblages both longitudinally within rivers/streams, (Lloyd et al. 2006) and between them (Sanderson et al. 2005, Mykra et al. 2007). Arguably, measurements of the scale of upstream/overland flights are becoming more common than for instream movements (for examples see Caudill (2003), Macneale et al. (2005) and Hershey et al.(1993)), but both sets of information are needed, and the analysis of spatial structure in assemblages can provide a larger picture of the spatial concordance between communities, which may be partially explained by movement scales (Lloyd et al. 2006).

This type of analysis can also provide insight into how spatial autocorrelation in environmental variables can cause spatial autocorrelation in community assemblages via spatial dependence (Legendre and Legendre 1998, Lloyd et al. 2005, Grenouillet et al. 2008), so it was also used to support conclusions regarding the effect of the WWTP on insect communities (as WWTP effluent was predicted to create spatial structure in environmental conditions and consequently insect assemblages). Indeed, many physicochemical variables are spatially structured, and thus investigating spatial structure in general could bring unexplained patterns to light, account for unmeasured variables, etc. (Murphy and Davy-Bowker 2005).

2.9.2 Statistical procedures

This analysis loosely followed the procedures of Borcard et al. (2011) for computing Mantel correlograms. The data representing community assemblages were the summed number of individuals of each family from all three Surber samples in a site, i.e. there was one value of abundance in a given family per site. Although more samples would have been ideal to fully characterize community composition, this number was considered sufficient, as rare species are generally eliminated from this type of analysis anyways (Lloyd et al. 2006). For the same reason, families found at only one site were eliminated prior to analysis. For family abundance data, the family assemblage distance matrix was created from Hellinger-transformed abundance data. In a Hellinger transformation, abundances are divided by the total site abundance, and then the square root of this value is taken. This reduces the importance of high abundances (as compared to, for example, Bray-Curtis dissimilarity, in which a difference in abundance of x has the same meaning whether there are tens or thousands of individuals) (Borcard et al. 2011).

This was appropriate given the extensive differences in the range of abundances for different families.

Because spatial autocorrelation can be generated by spatial dependence on spatially structured external processes, or by internal processes such as movement, and the two can be difficult to tell apart (Fortin and Dale 2005, Legendre and Legendre 2012), additional Mantel correlograms were constructed for which a) the original assemblage data had been linearly detrended, as trends can indicate spatial dependence, and b) the spatial structure in environmental variables had been partialled out. These two analyses served the same purpose, the difference being that the former incorporated no assumption about the cause of spatial dependence, whereas the latter was designed to assess whether the environmental variables specifically measured in this study could themselves be spatially structured and thus induce structure in insect assemblages. In the former analysis, linear detrending also promoted compliance with the assumption of second-order stationarity. This investigation was not restricted to detrended data because similar studies do not perform this step despite trends evident from positive autocorrelation grading to negative at larger scales (see Lloyd et al. 2005, 2006, Grenouillet et al. 2008). In the detrending procedure, the presence of significant spatial linear trends in the Hellinger-transformed abundance data was tested using redundancy analysis. Redundancy analysis is essentially multiple regression for multivariate data (Legendre and Legendre 1998), in this case with data being regressed on X-Y coordinates. Significant trends were present, and thus the residuals of this analysis were used in the construction of the Hellinger distance matrix, which was used in the computation of the detrended correlograms (see method in Borcard et al. 2011).

In the analysis that partialled out spatial structure in environmental conditions, partial Mantel correlograms, described by Matesanz et al. (2011), were used to recalculate the original family assemblage correlograms while accounting for environmental differences. These differences were presented in an "environmental distance" matrix, which represented how different sites were in terms of their environmental conditions. Canberra distances (Lance and Williams 1967) were used to standardize the contribution of each variable, which had very different ranges and magnitudes given their different units (Lloyd et al. 2005). The environmental variables used were TN, TP, turbidity, pH, water column ash free dry mass, water velocity, mean substrate particle size, and phytoplankton and periphyton abundance. Mantel correlograms visualizing spatial structure in these environmental conditions were also created.

During the production of all correlograms, the following ranges of geographic-distance-between sites were considered as classes:1) 0-900m (this class only captured corresponding left/right-bank pairs of sites, as the width of the river was considered, as a simplification, to be 100m, and longitudinally, the two closest sites were 940m apart), 2) 900 m –3 km, 3) 3-6 km, 4) 6-10 km, 5) 10-15 km 6) 20-30 km, 7) 30-40 km, … 23) 180-190 km. As suggested by Legendre and Legendre (1998) and Matesanz et al. (2011), a progressive Bonferroni correction (Hewitt et al. 1997, Legendre and Legendre 1998) was used to account for the multiple tests using the same data that were performed to compute the correlograms. 10 000 permutations were used to calculate of the reference distribution for r_{Md}. Because nutrient inputs from the WWTP on the left bank could drive large differences in community assemblages over a small scale (between the left and right

banks near the WWTP), Mantel correlograms with either the left-bank or the right-bank sites excluded (within 20 km of the WWTP, where sites were paired) were also created. The correlograms with left-bank sites excluded (referred to as the right-bank correlograms) were considered to depict spatial structure in assemblages in the absence of, or with reduced influence of, WWTP effluent. The correlograms with right-bank sites excluded (the left-bank correlograms) were designed to capture any impact on assemblage spatial structure driven by WWTP effluent. These analyses necessarily had one less distance class (0-900 m), and were performed to aid interpretation of the analysis including all sites. Separate right bank, left-bank, and all-site datasets were used to construct Mantel correlograms for detrended data and non-detrended data, and to construct partial Mantel correlograms, so a total of nine family-assemblage Mantel correlograms were produced in this investigation, in addition to the three Mantel correlograms showing spatial structure in environmental conditions. For further information regarding Mantel correlograms and the Bonferroni correction, see Appendix C: Mantel correlograms and partial mantel correlograms- formulation and interpretation.

Chapter Three: **Results**

3.1 . Longitudinal patterns in abiotic and biotic variables, in relation to effluent inputs from the RDWWTP

3.1.1 Longitudinal patterns- water column

3.1.1.1 NO_3 and TN

The dominant pattern in both NO_3 and TN during August 2010 was a peak in concentration on the left bank after input of effluent from the RDWWTP (1400 and 2220 µg/L respectively), followed by a rapid downstream decline in concentration (Figure 3.1). Concentrations were considerably lower (~10 and 200 µg/L respectively) at sites upstream of the WWTP, and on the right bank of the river near the WWTP. 20 km downstream of the WWTP, left bank NO_3 concentrations gained parity with right-bank values, which increased to 320 µg/L by 8 km downstream of the WWTP, and then decreased. Beyond 34 km downstream of the WWTP, TN concentrations remained quite stable at about 260 µg/L. For NO_3, stability was reached around 60km downstream of the WWTP, beyond which concentrations stayed lower than 20 µg/L. In these downstream reaches, NO_3 values were comparable to those sites upstream of the WWTP, whereas TN concentrations were slightly higher.

3.1.1.2 TP and TDP

The longitudinal patterns of TP and TDP concentrations (Figure 3.1) were similar to that of NO_3 and TN in that they peaked just downstream of the point of effluent outfall,

reaching 39 and 57 µg/L for TDP and TP, respectively. Another peak of similar magnitude (47 and 85 µg/L) was observed 13 km downstream of the WWTP, where a small tributary, the Blindman River, enters the Red Deer River. Downstream of these peaks, left-bank concentrations declined rapidly, reaching levels equivalent to upstream sites (12-20 µg/L for TDP and 24-28 µg/L for TP) around 27 km downstream of the WWTP, after which they remained fairly low. Right-bank concentrations did not show the clear pattern observed with NO_3 and TN, but were generally lower than equivalent left-bank sites, with a couple of exceptions. Interestingly, TDP and TP concentrations on the right bank above the WWTP were much higher than at the other two sites upstream of the WWTP. Elevated concentrations of TDP and TP also occurred at an isolated site 80km downstream of the WWTP, which was excluded from the LOESS averaging as it disproportionately affected local trends.

3.1.1.3 Phytoplankton abundance (Chlorophyll *a*)

Relative to upstream sites, a slight increase was observed in phytoplankton Chl *a* directly below the WWTP, but right and left-bank values in this vicinity were fairly similar to both upstream sites and to each other (~0.2-0.5 µg/L, Figure 3.2). Downstream of this point, left-bank Chl *a* concentration gradually increased until immediately downstream of the mouth of the Blindman River, where it increased sharply along the left bank, to the maximum measured value, 1.3 µg/L. Further downstream, Chl *a* concentration decreased, although another local peak occurred 27 km downstream of the WWTP. Concentrations remained fairly high on average (>300 µg/L) until 49 km downstream of the WWTP, beyond which point were only slightly higher than at sites

upstream of the WWTP. Right-bank values were consistently lower than left-bank values, but followed similar patterns to those on the left bank, increasing throughout the area 11 km downstream of the WWTP, and then dropping to upstream levels, beyond which they increased towards left-bank values.

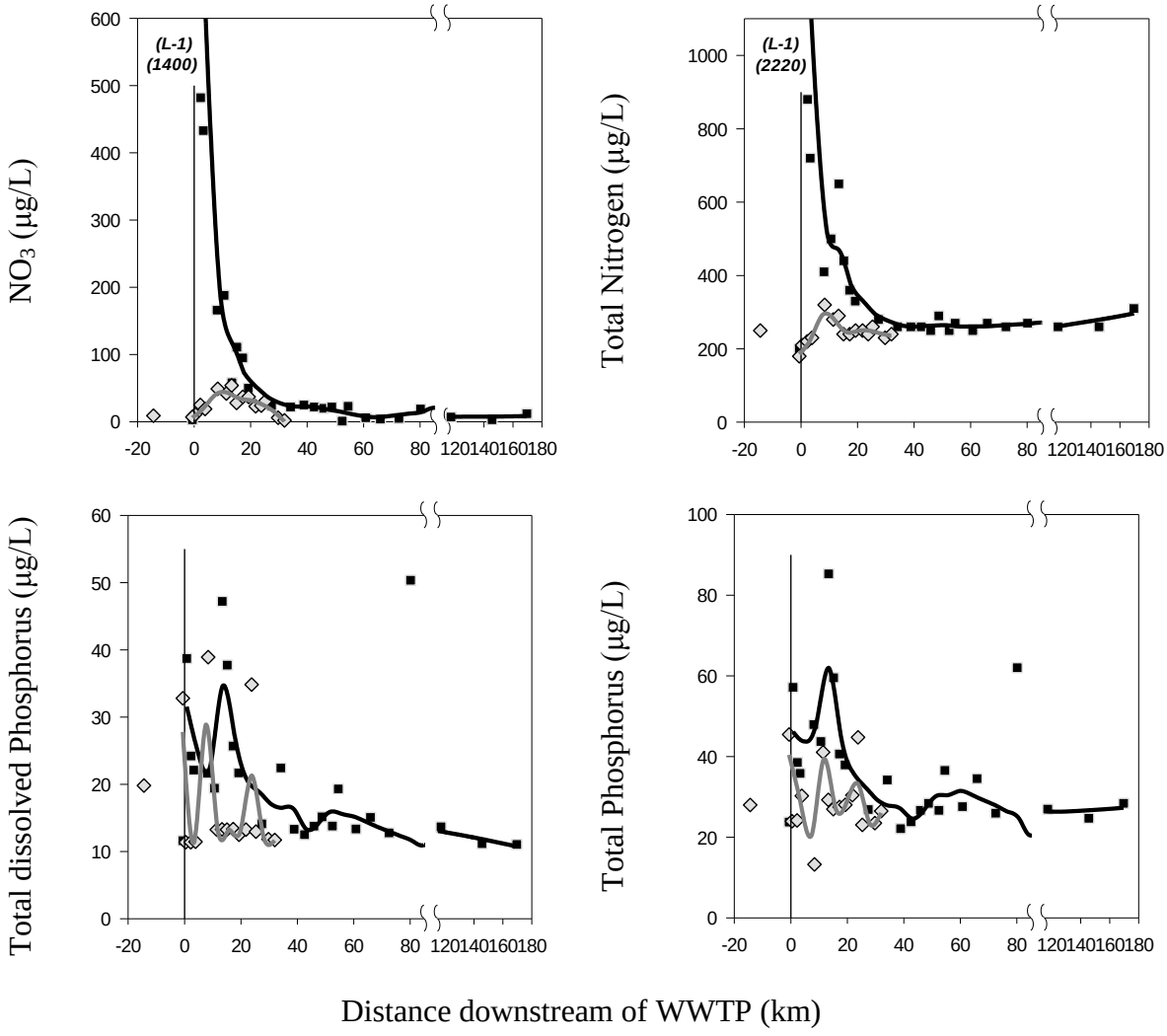


Figure 3.1: Longitudinal patterns in dissolved nutrients in the Red Deer River, August, 2010. Right-bank raw data and trends are represented in grey, and left-bank raw data and trends in black. Trends were determined by LOESS regression. The Red Deer Wastewater Treatment Plant (WWTP) is represented by the solid vertical line at distance = 0 km. Extreme values not pictured are represented by ***(bank- distance) (value)***.

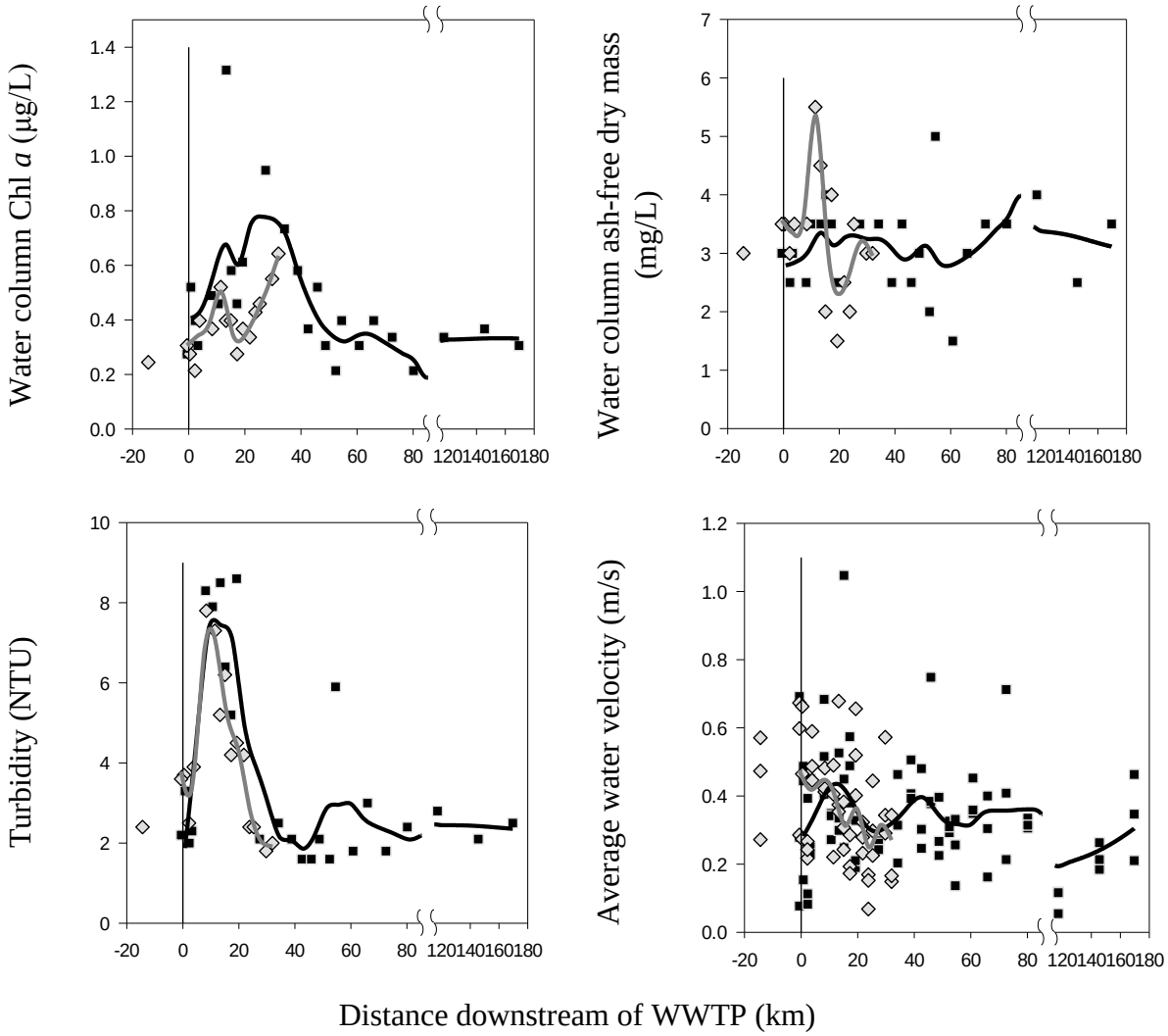


Figure 3.2: Longitudinal patterns in water velocity, and in measures of suspended substances in the water column of the Red Deer River in August, 2010. Right-bank raw data and trends are represented in grey, and left-bank raw data and trends in black. Trends were determined by LOESS regression. The Red Deer Wastewater Treatment Plant (WWTP) is represented by the solid vertical line at distance = 0 km.

3.1.1.4 Water column AFDM

Water column AFDM lacked a strong spatial pattern. Values were typically around 3-3.5 mg/L on the left bank (Figure 3.2). On the right bank, there was a noticeable peak in AFDM 11 km below the WWTP (5.5 mg/L), after which concentrations declined to very low levels by 20 km downstream of the WWTP, before increasing to values equivalent to those on the left bank.

3.1.1.5 Turbidity

Patterns of turbidity were similar on the left and right banks. Turbidity was generally low, ~2-4 NTU, but was elevated 8 km downstream of the WWTP (~8NTU), remained high for a few km, and returned to low levels by 24/34 km downstream of the WWTP (Figure 3.2).

3.1.1.6 Average water velocity

There was considerable variation in average water velocity throughout the study extent, but there appeared to be an overall downstream decline in *maximum* velocity on both the left and right banks. On the right bank, the LOESS average of water velocity declined from ~0.48 m/s near the WWTP to ~0.26 m/s 32 km downstream (Figure 3.2). On the left bank, the LOESS average resembled dampened oscillations with velocity fluctuating around a value of ~0.36 m/s. The LOESS average of velocity on this bank was ~0.28 m/s just downstream of the WWTP, increased to ~0.43 m/s 13 km downstream of the WWTP, and continued to fluctuate with lower local maximum values for the rest of the study extent.

3.1.2 Longitudinal patterns- benthos

3.1.2.1 Average substrate particle size

The LOESS average of average substrate particle size was highest just downstream of the WWTP on the left bank, where it could be described as large pebble and small cobble (Figure 3.3, Table 2.1). Sites upstream of this had smaller substrates (small to large pebble), particularly on the right bank. Substrate particle size continued to be lower on the right bank than on the left, and size decreased on both banks for the next 8-11 km downstream of the WWTP, reaching values similar to those upstream of the WWTP. Substrate size then increased until 25-27 km downstream of the WWTP, again being defined as large pebble to small cobble, after which it declined steadily in a downstream direction, becoming smaller at the last three sites than at any sites upstream, falling in the range of coarse gravel to small pebble.

3.1.2.2 Periphyton standing crop (Chlorophyll *a*)

Periphyton Chl *a* was low (less than 101 µg/cm^2) at sites above the RDWWTP (Figure 3.3). There was a moderate local peak in average Chl *a* on the left bank just below the WWTP (~150 µg/cm^2), then Chl *a* dropped to low values by 8 km downstream, and increased again, with its greatest average abundance (just over 200 µg/cm^2) occurring 15-27 km downstream of the WWTP. Chl *a* decreased and increased again to moderately high levels between 52 and 73 km downstream of the WWTP, beyond which it was low. Right-bank values were quite similar to those on the left bank, but were lower than left-bank values until 8 km downstream of the WWTP.

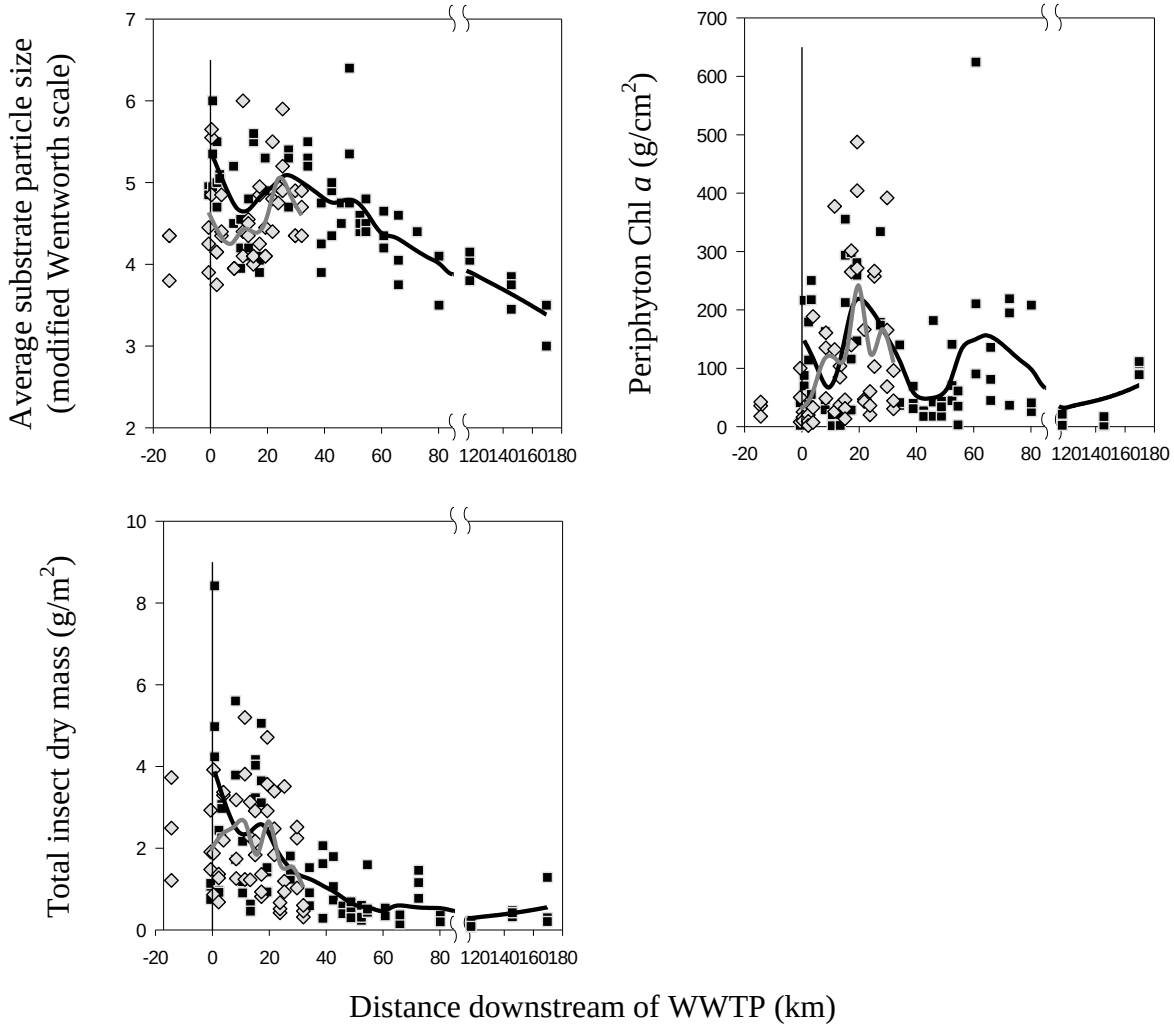


Figure 3.3: Longitudinal patterns in benthic characteristics of the Red Deer River in August, 2010. Data are associated with each of three Surber samples taken in shallow waters at the edges of the river at each site. Right-bank raw data and trends are represented in grey, and left-bank raw data and trends in black. Trends were determined by LOESS regression. The Red Deer Wastewater Treatment Plant (WWTP) is represented by the solid vertical line at distance = 0 km.

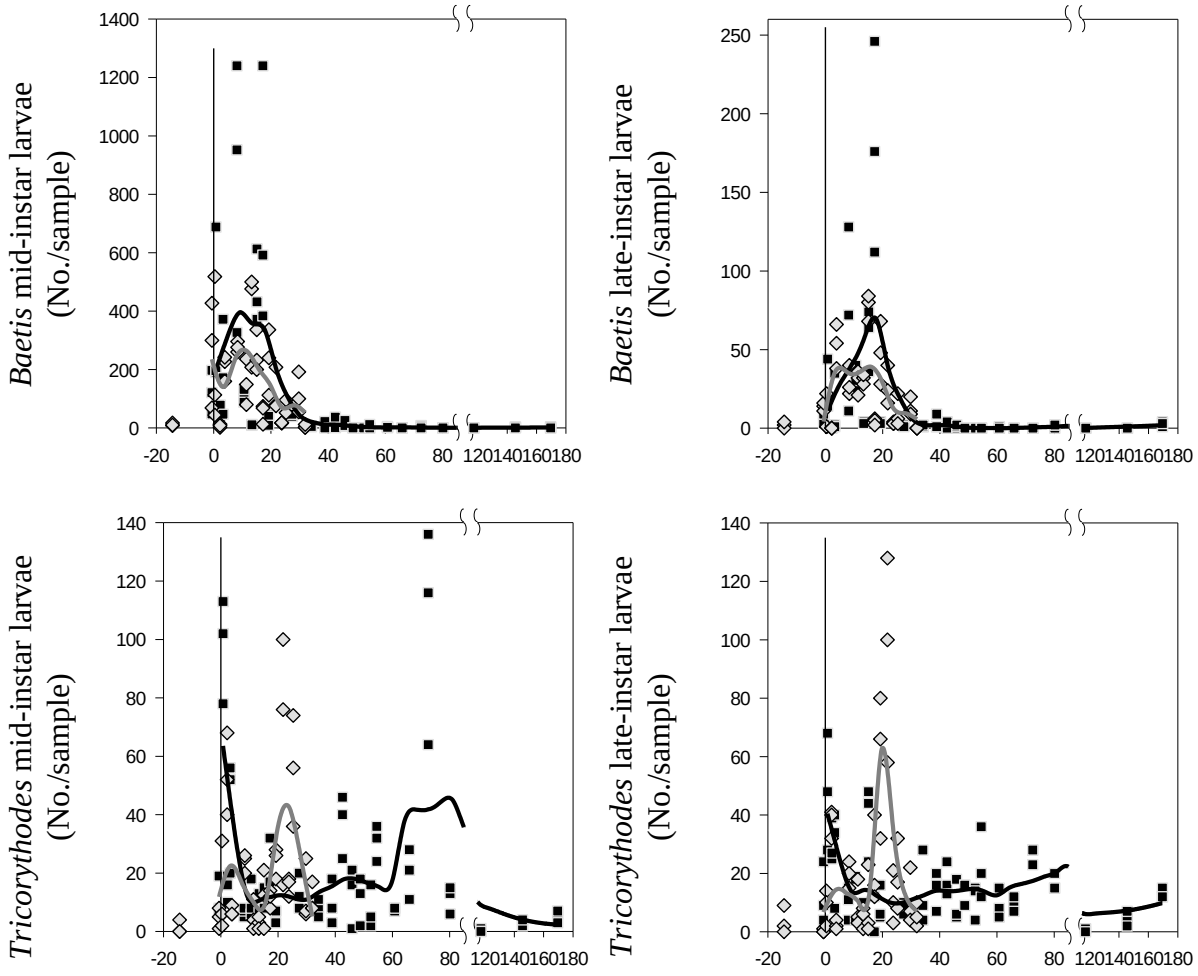


Figure 3.4: Longitudinal patterns in the abundance of *Baetis* and *Tricorythodes* mid and late-instar larvae on the Red Deer River in August, 2010. Abundance was determined from three Surber samples taken in shallow waters at the edges of the river at each site. Right-bank raw data and trends are represented in grey, and left-bank raw data and trends in black. Trends were determined by LOESS regression. The Red Deer Wastewater Treatment Plant (WWTP) is represented by the solid vertical line at distance = 0 km.

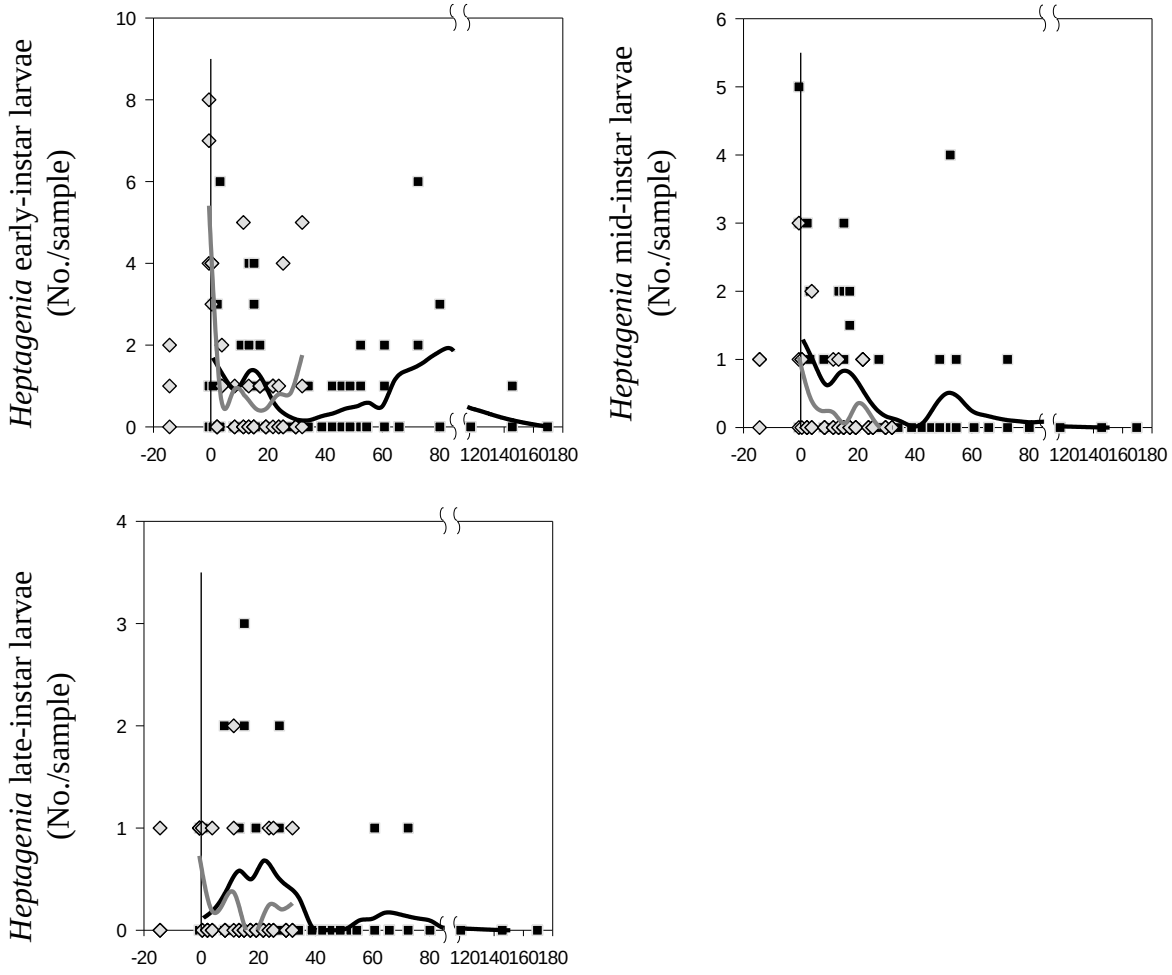


Figure 3.5: Longitudinal patterns in the abundance of *Heptagenia* early, mid, and late-instar larvae in the Red Deer River, August, 2010. Abundance was determined from three Surber samples taken in shallow waters at the edges of the river at each site. Right-bank raw data and trends are represented in grey, and left-bank raw data and trends in black. Trends were determined by LOESS regression. The Red Deer Wastewater Treatment Plant (WWTP) is represented by the solid vertical line at distance = 0 km.

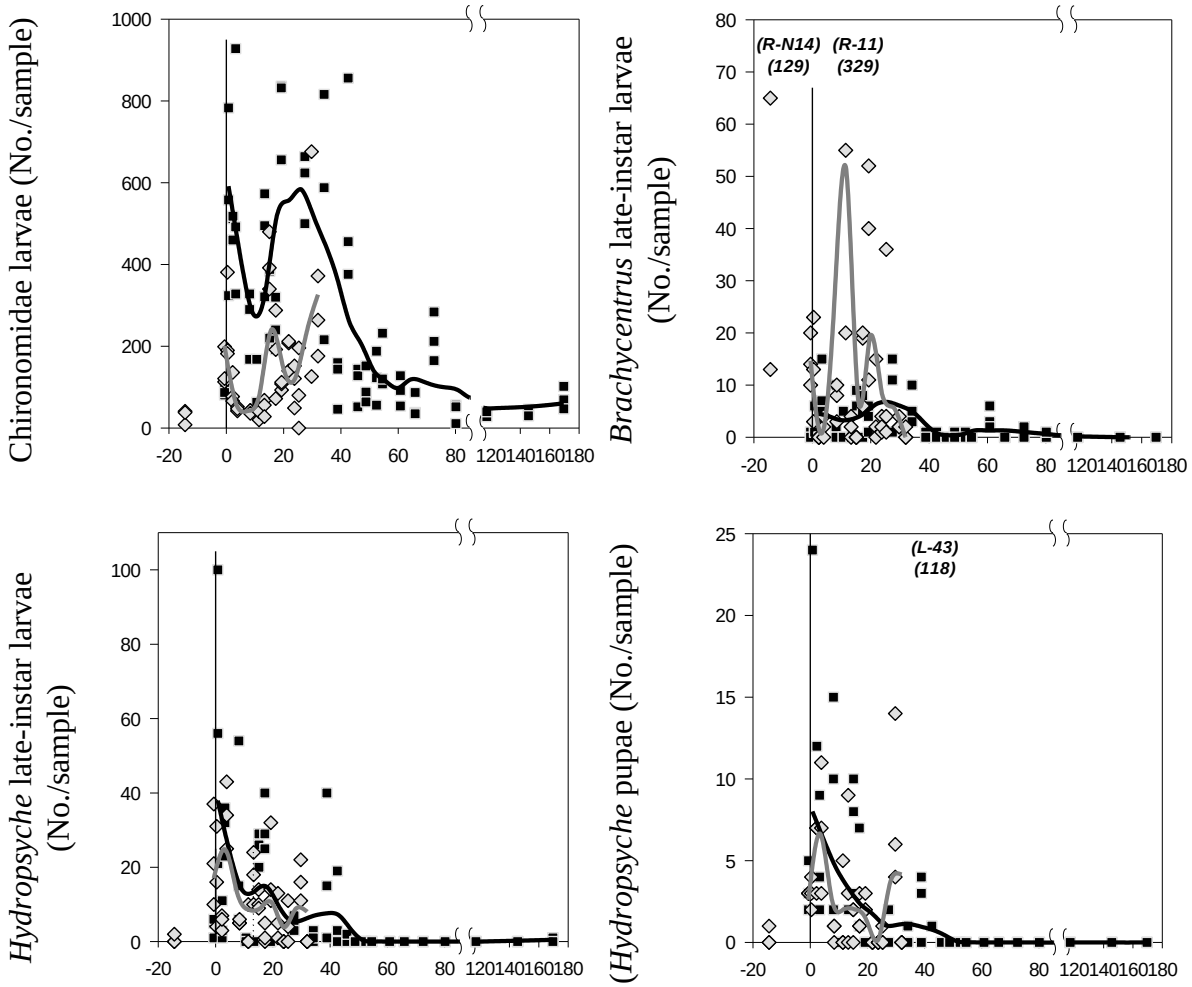


Figure 3.6: Longitudinal patterns in the abundance of Chironomidae larvae, late-instar*Brachycentrus* larvae, and *Hydropsyche* pupae and late-instar larvae on the Red Deer River in August, 2010. Abundance was determined from three Surber samples taken in shallow waters at the edges of the river at each site. Right-bank raw data and trends are represented in grey, and left-bank raw data and trends in black. Trends were determined by LOESS regression. The Red Deer Wastewater Treatment Plant (WWTP) is represented by the solid vertical line at distance = 0 km. Extreme values not pictured are represented by ***(bank-distance) (value)***.

3.1.2.3 Total insect dry mass

Total insect dry mass (referred to as total insect abundance) was greatest directly below the WWTP on the left bank (maximum 8.8 g/m^2), and declined consistently in a downstream direction, but for a small local peak in abundance17 km downstream of the WWTP (Figure 3.3). Right-bank abundance was similar to left-bank abundance, except at sites near the RDWWTP, where it was lower than on the left bank. Interesting, dry mass was fairly high 14 km upstream of the WWTP (with a maximum value of 3.7 g/m^2), and 22 km below the WWTP, maximum abundance dropped below this value and did not increase above it again.

3.1.2.4 *Baetis* abundance

The abundance of both mid- and late-instar *Baetis* larvae was very low 14 km upstream of the WWTP (<20/sample, Figure 3.4). Near to the WWTP abundance increased, with average abundance of mid-instar larvae peaking at ~400/sample on the left bank and 300/sample on the right bank 8 km downstream of the WWTP. Abundance of mid-instar larvae stayed relatively high until 17 km downstream of the WWTP on the left bank, and 15 km on the right. The abundance of late-instar larvae peaked (~180/sample) further downstream of the WWTP, at 17 on the left bank and at 4 and 15 km on the right bank (~80/sample). Counts of both mid- and late-instar larvae diminished to very low levels by 30 km downstream of the WWTP, and, for the most part, were higher on the left bank than on the right.

3.1.2.5 *Tricorythodes* abundance

The abundance of late and mid-instar *Tricorythodes* larvae had very similar spatial patterns (Figure 3.4). Abundance was quite low (<25/sample) at sites above the WWTP. On the left bank, directly below the WWTP, abundance of both stages peaked (to ~ 100 or 50/ sample for mid- and late-instar respectively), sharply declined by 8 km downstream of the WWTP, and stayed at a similar average magnitude for the remainder of the study area, although high numbers of mid-instar larvae occurred 73 km downstream of the WWTP. Abundance on the right bank peaked 2 km downstream of the WWTP (~40-50/sample), then decreased to low levels by 13 km downstream of the WWTP. In this area abundance on the right bank was lower than on the left bank. Beyond this point, right-bank abundance began to increase to levels equivalent to or greater than those observed on the left bank, displaying a large peak 22-25 km downstream of the WWTP for mid-instar larvae (~50-70/sample), and 19-22 km downstream for late-instar larvae (~70-100/sample), after which point abundance decreased again.

3.1.2.6 *Heptagenia* abundance

Heptagenia larvae of all age classes had a patchy distribution in the sense that individuals were not present in all samples or at all sites (Figure 3.5). A maximum of 8 individuals was observed in any one sample, which occurred for early-instar larvae on the right bank just upstream of the WWTP. This site also had the highest average abundance, with the equivalent left-bank site having no more than one individual per sample. At all other sites, for all stages of *Heptagenia,* the LOESS average of abundance was no more

than 2 individuals per sample. In individual samples, higher abundances tended to occur within 20 km downstream of the WWTP, and abundances were particularly low around 40 km downstream of the WWTP, and after 80 km downstream.

3.1.2.7 Chironomidae abundance

The most striking pattern in chironomid abundance was that average left-bank abundance consistently exceeded that on the right bank, except at sites above the RDWWTP, where abundance was lower than 200 individuals per sample (Figure 3.6). Average chironomid abundance peaked just downstream of the WWTP on the left bank, decreased to moderate levels (~300 individuals) by 11 km downstream, and increased again between 20 to 43 km downstream of the WWTP. After this point, abundance dropped to levels equivalent to sites just above the WWTP. On the right bank, abundance was low directly upstream of the WWTP, and continued to decrease to very low levels until 15 km downstream of the WWTP, where average abundance increased to levels close to those on the left bank (with overlap occurring among individual samples). Abundance then deceased until 22 km downstream of the WWTP, and increased to its highest average levels by 32 km downstream of the WWTP.

3.1.2.8 Brachycentrus abundance

Brachycentrus larvae had more extreme abundances than other groups, with some samples having no individuals, and others having close to or over 100, including two samples at the reference site 14 km upstream of the WWTP (Figure 3.6). In general, the LOESS average of abundance was higher on the right bank than the left bank, but it fluctuated between ~0-50 individuals within 35 km downstream of the WWTP. Left bank

average abundance remained more consistently around ~3-8 individuals per sample. After 35 km downstream of the WWTP, abundance remained consistently very low.

3.1.2.9 *Hydropsyche* abundance

Hydropsyche larvae had a very similar pattern of abundance on the left and right banks of the Red Deer River, although the left bank had slightly higher average values (Figure 3.6). Abundance peaked on the left bank just downstream of the WWTP, and decreased virtually to zero by 50 km downstream of the WWTP, with small local peaks in abundance ~17 and 40 km downstream of the WWTP. Pupae had a very similar spatial distribution to larvae, but without local peaks in LOESS average abundance on the left bank, and moderately high abundance 30 km downstream of the WWTP on the right bank.

3.1.3 Longitudinal patterns- $\delta^{15}N$ signatures

Considering large scale left-banks trends, $\delta^{15}N$ signatures of periphyton were low 14 km upstream of the WWTP (~2.1-3.3 ‰). They increased markedly downstream of the point of effluent input from the RDWWTP (up to 11.5-12.9 ‰), and declined gradually in a downstream direction, becoming increasingly variable, and reaching their lowest values, ~0.2-0.8 ‰, at the furthest downstream site (Figure 3.7). Insect $\delta^{15}N$ signatures were also greatest just downstream of the WWTP, and followed the same general trend as periphyton. However, they tended to be lower than periphyton $\delta^{15}N$ signatures in areas within 20-30 km downstream of the WWTP, if not throughout the entire study area (Figure 3.8, Figure 3.9, Figure 3.10). On the right bank, invertebrate $\delta^{15}N$ signatures were more similar to that of local periphyton than was observed on the

left bank, although differences existed between groups. At the site-level, insect signatures varied in whether they fell within the range of periphyton signatures (implying local residency) or not (implying they have moved to the site of sampling from elsewhere). Figure 3.11 gives a site-by-site representation of the overlap of insect signatures with that of local periphyton, whereas a group-level description of $\delta^{15}N$ spatial trends follows in sections 3.1.3.1 to 3.1.3.7.

3.1.3.1 Periphyton $\delta^{15}N$

The dominant pattern in periphyton $\delta^{15}N$ signatures on the left bank was an increase in $\delta^{15}N$ (to 11.5-12.9 ‰) directly below the RDWWTP (as compared to 2.4-7.1 ‰ on the left bank just upstream of the WWTP), followed by a fairly steady downstream decline in $\delta^{15}N$ signatures (Figure 3.7). This pattern was in evident contrast to right-bank periphyton, for which samples near the WWTP had low $\delta^{15}N$ values (2.0 to 6.3 ‰) that were similar to those upstream of the WWTP (1.8-8.9 ‰). Right-bank $\delta^{15}N$ signatures then steadily increased in a downstream direction, reaching parity with left-bank signatures around 26 km downstream of the WWTP (considering LOESS averages), where $\delta^{15}N$ signatures were still relatively high (~7.1-10.9 ‰). Considerable variation was observed in signatures throughout the study area, particularly within and between sites in the range of 43 to 66 km downstream of the WWTP, where the range of $\delta^{15}N$ values within a site was as high as 6 ‰, although more typically 1-4 ‰.

3.1.3.2 *Baetis* $\delta^{15}N$

Below the WWTP on the left bank, late-instar *Baetis* larvae had similar average $\delta^{15}N$ values to that of periphyton, with some small differences, as follows. In the area 2-8

km downstream of the WWTP late-instar *Baetis* signatures were lower than that of local periphyton, having $\delta^{15}N$ values around 7.6-8.1 ‰ (Figure 3.8). 25 km downstream of the WWTP, at 8.1-9.1 ‰, they became greater than the LOESS average (but within the range) of periphyton $\delta^{15}N$ values until 50 km downstream of the WWTP, where they dropped below that of the LOESS average of periphyton $\delta^{15}N$. Mid-instar larvae followed a similar pattern to those of late-instar larvae, but had $\delta^{15}N$ values that were consistently ~2 ‰ lower, being intermediate to left and right-bank periphyton $\delta^{15}N$ until about 25 km downstream of the WWTP, at which point they dropped below both right and left-bank average periphyton values, and remained in the lower range of periphyton $\delta^{15}N$. On the right bank, late-instar *Baetis* larvae had $\delta^{15}N$ values with a similar spatial pattern to that of periphyton $\delta^{15}N$, but with values ~0.5-2.5 ‰ greater. Mid-instar larvae had $\delta^{15}N$ signatures that were typically <1 ‰ greater than periphyton $\delta^{15}N$ until 22 km downstream of the WWTP, at which point they dropped slightly below average periphyton $\delta^{15}N$, but continued to follow the same trend.

3.1.3.3 *Tricorythodes* $\delta^{15}N$

Just downstream of the WWTP on the left bank, late-instar *Tricorythodes* larvae had similar $\delta^{15}N$ values to periphyton (~11 ‰, Figure 3.8). Downstream of this point, $\delta^{15}N$ signatures dropped below that of average periphyton and were quite consistent (~8 ‰) until 46 km downstream of the WWTP. This was in contrast to periphyton $\delta^{15}N$ signatures, which decreased in this area. After this point, late-instar larvae had a very similar average $\delta^{15}N$ signature to that of periphyton, except at the last three sites, where signatures were higher by about 1-1.5 ‰. Left-bank mid-instar larvae had similar $\delta^{15}N$

signatures to late-instar larvae, but with lower values in general. On the right bank, late-instar larvae had a similar $\delta^{15}N$ trend to right-bank periphyton, but their average signatures were ~0.5-2 ‰ higher than the average local periphyton $\delta^{15}N$ signature until about 22 km downstream of the WWTP, at which point they dropped below periphtyon $\delta^{15}N$, but remained similar. Mid-instar larvae had a very similar $\delta^{15}N$ trend to late-instar larvae on the right bank, but with lower values. They tended to have higher $\delta^{15}N$ than local periphyton until 11 km downstream of the WWTP, then lower average values.

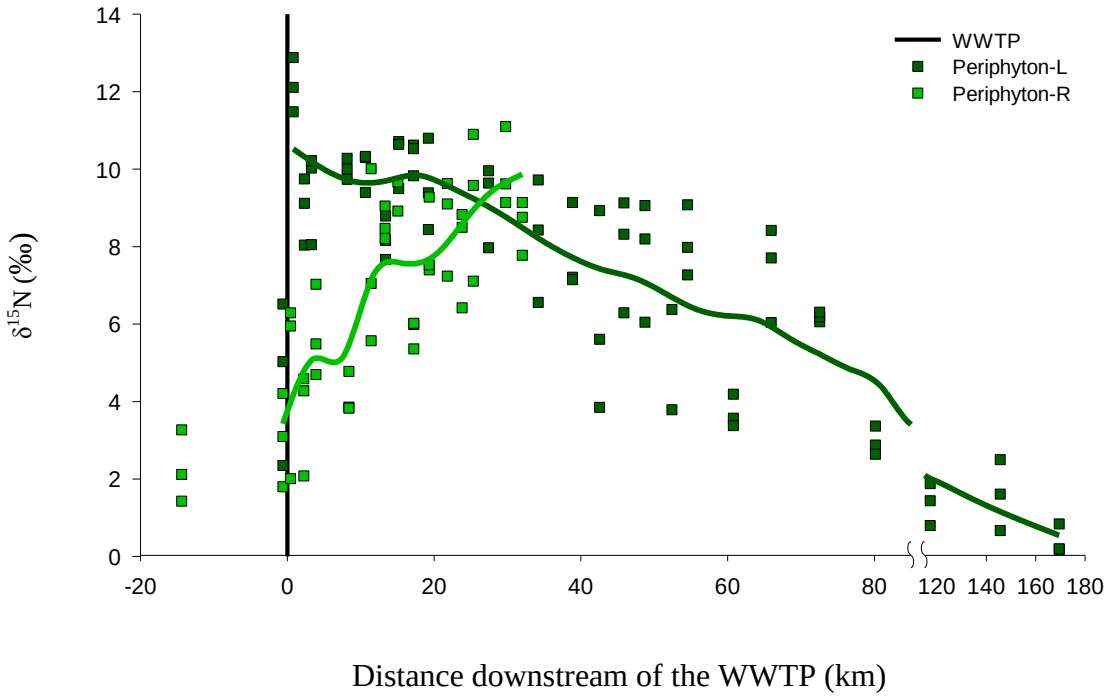


Figure 3.7: Spatial patterns in $\delta^{15}N$ signatures of periphyton on the left (L) and right (R) banks of the Red Deer River, AB, Canada, in reference to the Red Deer Wastewater Treatment Plant (WWTP). Trends were determined by LOESS regression.

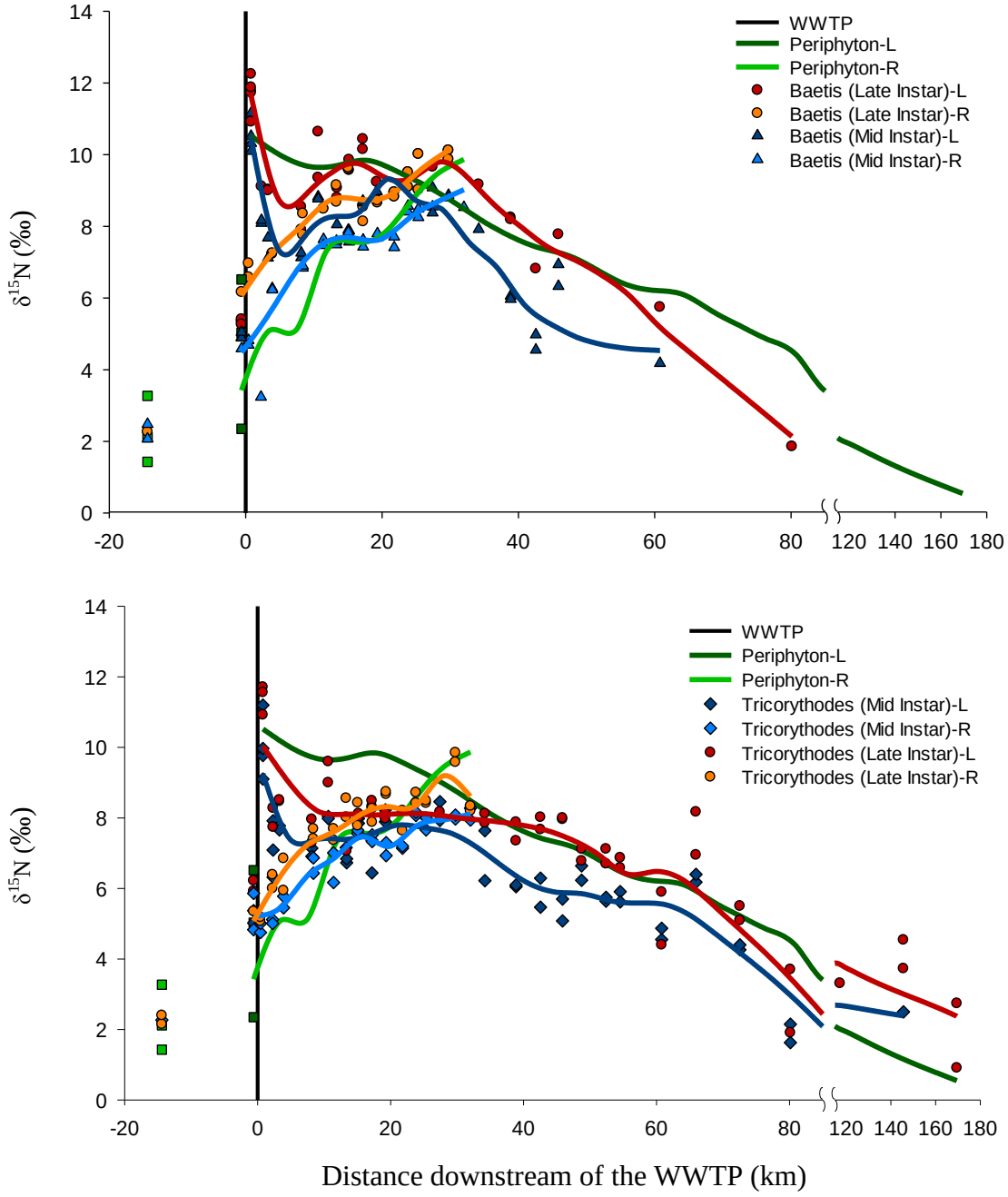


Figure 3.8: Spatial patterns in $\delta^{15}N$ signatures of periphyton, and mid and late-instar *Baetis* and *Tricorythodes* on the left (L) and right (R) banks of the Red Deer River, AB, Canada, in reference to the Red Deer Wastewater Treatment Plant (WWTP). Trends were determined by LOESS regression.

3.1.3.4 Chironomidae $\delta^{15}N$

Directly downstream of the WWTP, left-bank chironomid $\delta^{15}N$ values were lower than local left-bank periphyton $\delta^{15}N$ by ~2-3.5 ‰ (Figure 3.9). They were highest (8.8-8.9 ‰) just downstream of the WWTP, decreased to ~6 ‰ by 2 km downstream of the WWTP, and increased to ~8 ‰ by 27 km downstream, beyond which they decreased again. Chironomid $\delta^{15}N$ values remained, on average, lower than local left-bank periphyton values until 117 km downstream of the WWTP (although beyond 34 km downstream of the WWTP chironomid $\delta^{15}N$ values were, for the most part, within the range of periphyton $\delta^{15}N$). Chironomids sampled on the right bank had similar $\delta^{15}N$ values to right-bank periphyton for 11 km downstream of the WWTP, then fell below average periphyton $\delta^{15}N$, but still existed within the range of periphyton values. Interestingly, after 8 km downstream of the WWTP, chironomids sampled on the right bank had very similar $\delta^{15}N$ values to those on the left bank.

3.1.3.5 *Heptagenia* $\delta^{15}N$

On the left bank directly downstream of the WWTP, late-instar *Heptagenia* had $\delta^{15}N$ values on the lower end of the range of local periphyton, at 11.3 ‰ (Figure 3.9). By 8km downstream -the next site at which they were present in samples- $\delta^{15}N$ values were quite depressed compared to local periphyton (~7 ‰), after which they remained low, but tended to increase, following a more similar trend to right bank than left-bank periphyton $\delta^{15}N$ signatures. By 27 km downstream, left-bank late-instar *Heptagenia* $\delta^{15}N$ followed left-bank periphyton trends, but with average values (~7.8 ‰) slightly under average periphyton $\delta^{15}N$. Left-bank mid-instar larvae had similar $\delta^{15}N$ spatial patterns to older

larvae, although they had average $\delta^{15}N$ values ~1 ‰ lower until about 27 km downstream of the WWTP. Compared to the other age groups, early-instar larvae had quite low $\delta^{15}N$ just downstream of the WWTP (8.3 ‰). Values continued to drop, to ~2 ‰, till 17 km downstream of the WWTP, at which point they were well below the average periphyton $\delta^{15}N$ on either bank. $\delta^{15}N$ values then increased to resemble that of other age groups, with all groups having similar average $\delta^{15}N$ values to periphyton for the remainder of downstream sites. Samples taken on the right bank followed right-bank periphyton $\delta^{15}N$ trends very closely, however *Heptagenia* signatures dropped below local periphyton signatures at 25 km downstream of the WWTP for late-instar larvae, and 11 km for early and mid-instar larvae.

3.1.3.6 *Hydropsyche* $\delta^{15}N$

On the left bank, $\delta^{15}N$ signatures of *Hydropsyche* pupae and larvae were very similar, and were always lower than the LOESS average of left-bank periphyton $\delta^{15}N$ signatures, even dropping below that of right-bank periphyton beyond 15 km downstream of the WWTP (Figure 3.10). $\delta^{15}N$ signatures were, nevertheless, highest just downstream of the WWTP (8.7-9.8 ‰ for larvae, 6.9-8.8 ‰ for pupae). They then decreased more steeply than that of periphyton for the next 8 km, after which they maintained an overall negative trend that was less steep than that of periphyton, with values being near 7 ‰ on average. Right-bank *Hydropsyche* had similar $\delta^{15}N$ spatial patterns to that of right-bank periphyton, but had slightly higher $\delta^{15}N$ values than right-bank periphyton until 11 km downstream of the WWTP, after which point they stayed lower than local periphyton $\delta^{15}N$.

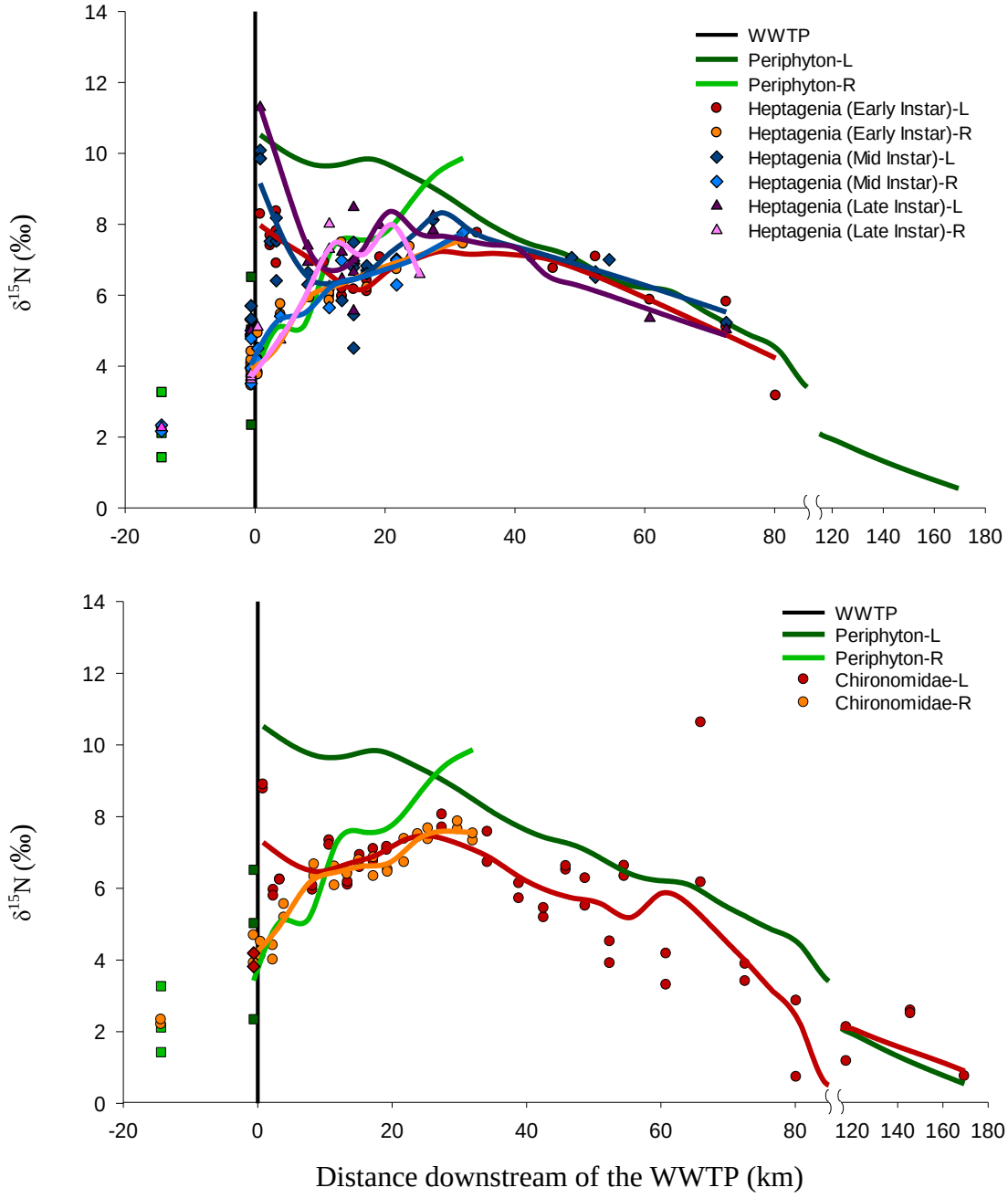


Figure 3.9: Spatial patterns in $\delta^{15}N$ signatures of periphyton, early, mid, and late-instar *Heptagenia*, and Chironomidae on the left (L) and right (R) banks of the Red Deer River, AB, Canada, in reference to the Red Deer Wastewater Treatment Plant (WWTP). Trends were determined by LOESS regression.

3.1.3.7 *Brachycentrus* $\delta^{15}N$

Overall, the downstream gradient of *Brachycentrus* $\delta^{15}N$ signatures on the left bank was quite flat (Figure 3.10). Like other groups, left-bank $\delta^{15}N$ signatures were highest just downstream of the WWTP (7.3-9.3 ‰), but dropped sharply in the next 8 km, and maintained a fairly steady average signature, fluctuating around ~6.5 ‰, until 50 km downstream of the WWTP, beyond which they decreased. On both the right and left banks, *Brachycentrus* $\delta^{15}N$ dropped below right-bank periphyton $\delta^{15}N$ around 11 km downstream of the WWTP, and remained low, with individuals from both banks displaying considerable overlap in their $\delta^{15}N$ signatures, and following very similar downstream trajectories.

3.2 Environmental explanators of insect abundance- local and lagged analyses

Based on deviance to degrees of freedom ratios (dev/df), which ranged from 0.3558 to 1.6375, with most between 1.1 to 1.3, the fit of most models, including the chosen error distributions, was good to reasonable, given that the degrees of freedom for each model was less than 29 (for n=29, an acceptable dev/df is <1.6-1.5, Myers et al. 2002). In all but two models, insect abundance varied significantly with at least one of the four variables (periphyton abundance, *Peri*, dissolved nitrate, *NO3*, average substrate particle size, *Part*, and average water velocity, *Flow*), either as a main effect, or as part of a two-way interaction with another variable. The two models that reduced to the intercept were the analysis of *Heptagenia* abundance using the average of local variables

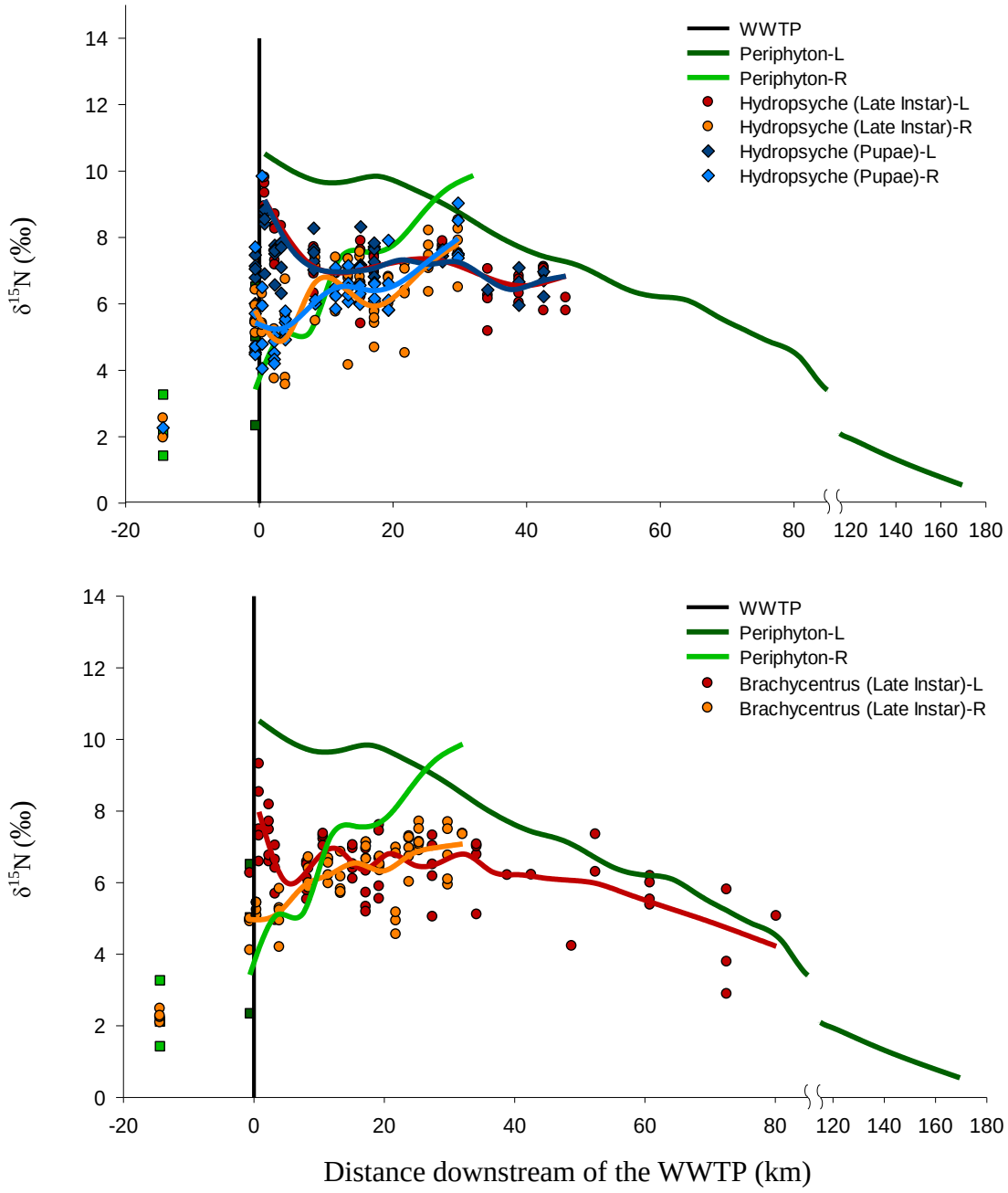
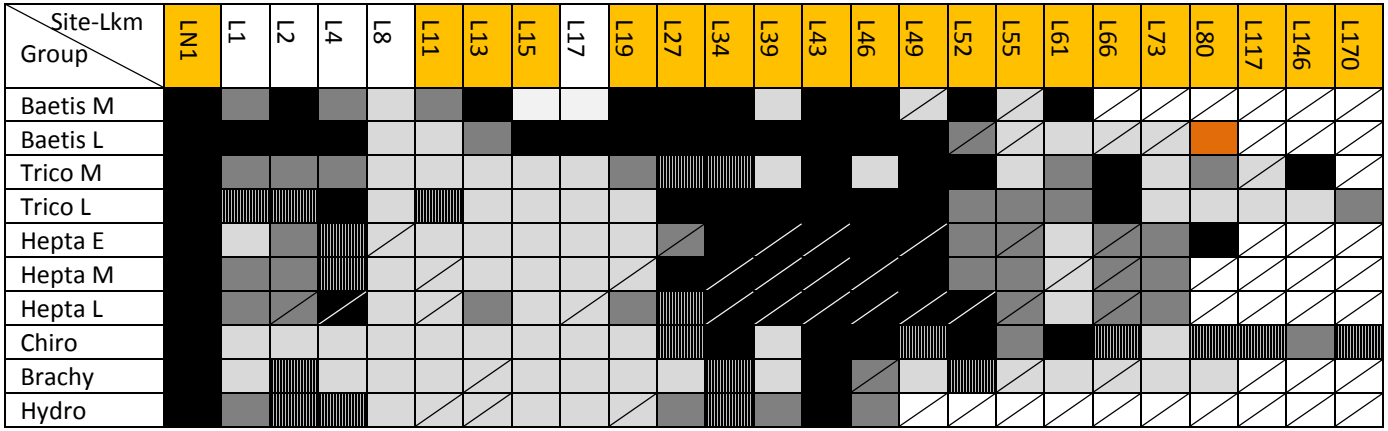
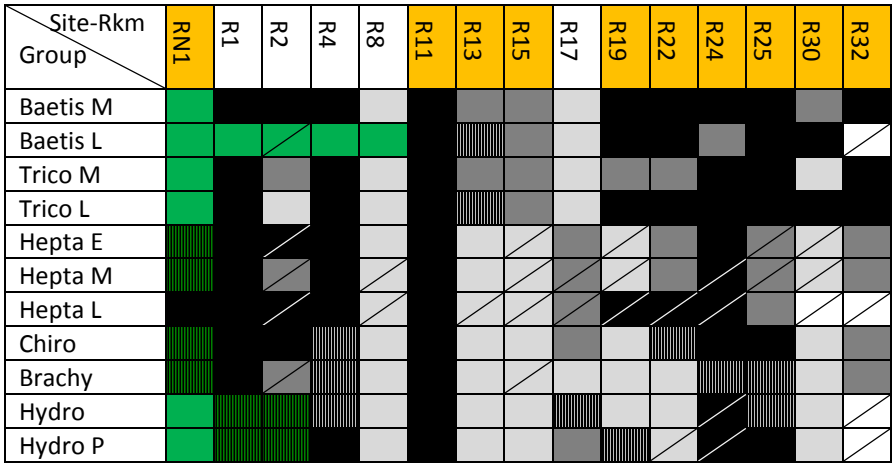


Figure 3.10: Spatial patterns in $\delta^{15}N$ signatures of periphyton, *Hydropsyche* pupae and late-instar larvae, and *Brachycentrus* late-instar larvae on the left (L) and right (R) banks of the Red Deer River, AB, Canada, in reference to the Red Deer Wastewater Treatment Plant (WWTP). Trends were determined by LOESS regression.





See caption on following page.

	All insect samples within periphyton range. Local residency.
	Some samples within periphyton range. Others moved up, across, or downriver to sampling location.
	Some samples within periphyton range. Others moved up or across river to sampling location.
	Movement down, across, or upriver to sampling location. Signatures indicate lengthy residency at site.
	Movement down, across, or upriver to sampling location, signatures indicate recent arrival.
	Movement upriver to sampling location.
	Movement up or across-river to sampling location.
	No sample, LOESS average used in interpretation.
	Periphyton $\delta^{15}N$ not significantly different between R and L banks, or this is assumed (R22R to L170R).

Figure 3.11: Site-level summary of insect $\delta^{15}N$ signatures as compared to periphyton $\delta^{15}N$ signatures, on the left (L) and right (R) banks of the Red Deer River, at various distances upstream (L/RNkm) or downstream (L/Rkm) of the Red Deer Wastewater Treatment Plant. Interpretation of movement behaviour required to produce insect $\delta^{15}N$ signatures is also included, assuming that signatures are appropriately corrected for trophic fractionation. L = late instar, M = mid instar, E = early instar, P = pupae, Trico = *Tricorythodes*, Hepta = *Heptagenia*, Chiro = Chironomidae, Hydro = *Hydropsyche*, Brachy = *Brachycentrus*.

and those from one site upstream, and the analysis of *Hydropsyche* abundance using variables averaged over the local and next downstream site. Significant residual spatial autocorrelation (as diagnosed when the inclusion of a spatial variance-covariance matrix improved model performance) was not common. It was detected, however, in the analysis of total insect abundance using local variables, in the model for which variables were averaged over the local and next downstream site, and in the model for which variables were averaged over the local, next upstream, and next downstream site. Interestingly, although the spherical and exponential models for covariance structure typically gave very similar results, for the local model of total benthic abundance, a spherical model did not significantly improve the model, whereas an exponential model did, thus results are presented for the exponential model. Significant residual spatial autocorrelation was only detected in two other models: the local models for mid-instar *Baetis* abundance, and for *Hydropsyche* abundance. For these models, because different covariance structures gave similar results, the one that seemed to best suit the semivariogram was reported.

Using $\Delta AIC = 6$ as the discriminating value for model equivalence (Richards 2005, 2008), total insect abundance was described best by variables averaged over the

local, next upstream, and next downstream site ("1 Up, Down", Table 3.1) this model included *NO3*, *Part*, and *Peri* and interaction term between *NO3* and *Part*). When accounting for residual spatial autocorrelation, which was estimated to occur up to an extent of 18.74 km, the effect of *Part* became non-significant, but was still retained because the significant interaction between *NO3* and *Part* remained. The abundance of mid-instar *Baetis* larvae was best described by the "1 Up" model. This model included all main effects, although *NO3*, *Flow*, and *Part* were non-significant, being retained because the following interaction terms were significant: *NO3*Flow*, *NO3*Part*, and *Peri*Part*. Late-instar*Brachycentrus* abundance was explained equally well by the local model and the "1 Up" model, which was reduced to simply *Peri* and *Part*. The local model included all main effects plus the following three interaction terms: *NO3*Flow*, *NO3*Part*, and *Flow*Part*. Chironomid abundance was explained equally well by all models except for "1 Up". The local model reduced to *Part*, *Flow*, and their interaction, whereas the "2 Up" model did not include an interaction between *Part* and *Flow*, but included *Peri*, and *Peri*Part*, but *Part* itself was non-significant. The "1 Down" and "1 Up, Down" models both included only *Flow* and *Part*. *Heptagenia* abundance was also explained equally well by all models except "1 Up". In this case, the local and "1 Down" models reduced to *Part* and *Flow*, and the "2 Up" model additionally included *NO3* and the interaction between *Part* and *Flow*. The "1 Up, Down" model also included these terms, as well as the interaction between *NO3* and *Flow*, and *NO3* and *Part*. Late-instar *Hydropsyche* abundance was explained equally well by models except "1 Down". The local model only included *Flow* and *Part* as main effects, and was improved by the addition of an exponential spatial variance-covariance matrix, with a practical range of 7.59 km. The "1

Up" and "2 Up" models also included *Peri*, plus an interaction between *Peri* and *Part* that left *Part* as a main effect non-significant. The "1 Up, Down" model included only *Part*. Mid-instar *Tricorythodes* abundance was equally well explained by all models. The local, "1 Up", and "1 Down" models only included *Part*, *Flow*, and their interaction, but the "2 Up" model included only *Peri*, *Part*, and their interaction. The "1 Up, Down" model was much more complicated, including all main effects (with *Peri* being non-significant), and the following three interactions: *NO3*Flow NO3*Peri NO3*Part Flow*Part*.

In these models, because a log link function was used, the intercept, and coefficients of variables as estimated by maximum likelihood, come together to represent an exponent with a base of *e* (see Table 3.1) . This means that the terms have a multiplicative effect on abundance, but simply, a positive coefficient represents a positive relationship between the given independent variable and abundance, taking into account all other terms, while a negative coefficient represents a negative relationship. The coefficients of interaction terms are less straightforward to interpret, as a significant interaction terms indicates that the coefficient of one variable changes depending on the value of the other, and vice versa (Aiken and West 1991). A positive interaction means that the effect on the dependent variable of a given variable in the interaction becomes more positive as the value of the other variable in the interaction increases. The opposite is true for a negative interaction. As can be inferred from above, the main effects that were most frequently included in models were *Part* and *Flow*, which were always included as positive effects (although *Part* was included as a non-significant negative effect in the presence of significant interaction terms in some models). *Peri* was often

included in models, but not consistently as a positive or negative effect. *NO3*, when retained (in about 35% of the models) was mostly a positive effect, but was negative in some N1 and B1 models. In terms of interaction effects, an interaction between *NO3* and *Peri* and between *Peri* and *Flow*, were retained only once. An interaction between *NO3* and *Flow*, and between *Part* and *Flow*, were retained occasionally, always as positive and negative effects respectively. An interaction between *NO3* and *Part* and between *Peri* and *Part* were occasionally retained, typically as negative and positive effects respectively, but not always. Again, the details of these results are presented in Table 3.1.

Table 3.1: Explanators of benthic insect abundance, as analysed with independent variables averaged over various lags, and defined in the text. Generalized linear models with a log link function and a gamma (for total abundance) or negative binomial (for counts of select insect groups) error distribution were used in analysis. Model residuals were evaluated for the presence of spatial autocorrelation by including an exponential (exp) or spherical (sph) spatial variance-covariance matrix (vcm), as judged by the shape of a residual semivariogram. The presence of significant residual spatial autocorrelation is indicated by *, and the model as changed with the addition of a spatial variance-covariance matrix is presented below that without. The practical range, *a*, of spatial autocorrelation is presented for models with significant residual spatial autocorrelation, and, for the case of an exponential variance covariance matrix, was estimated by multiplying SAS' output range parameter by 3. ϕ represents the scale parameter of the variance function for models with a gamma error distribution (i.e. var(x) = $\phi\mu^2$), while *k* represents a similar scale parameter (the dispersion parameter) for the variance function of the negative binomial error distribution (var(x) = $\mu + k\mu^2$) (SAS Institute Inc. 2008). For each insect group, the best model, as determined by the lowest AIC, and those considered equivalent based on ΔAIC = 6, are indicated by shaded-grey AIC values. Dev = deviance.

Group	**Model**	**AIC**	**Dev/df**
Total Abundance (g)			
Local*	*e*^(22.45NO3 +0.67Part +3.90Flow -3.98NO3*Part + 2.38); ϕ = 3.55	459.51	0.3558
Local + exp vcm	*e*^(5.90Flow +5.08); var=1.13, *a* = 20.28	N/A	
1 Up	*e*^(1.33NO3 +3.92Flow -61.68Peri -0.28*Part* +13.65Peri*Part +6.56); ϕ = 3.65	460.71	0.3615
2 Up	*e*^(56.08NO3 -8.72*Flow* -26.93*Peri* +1.38Part -10.71NO3*Part +89.27Flow*Peri +2.85); ϕ = 3.26	466.31	0.4255
1 Down*	*e*^(-6.62NO3 +2.38Part +42.68Flow -41.08Peri +27.37NO3*Flow -8.86Flow*Part +9.35Peri*Part -5.*02*)	441.93	0.1625
1 Down + sph vcm	*e*^(1.27NO3 +3.94Flow +5.44); *a*=16.64, var=0.42	N/A	

Group	Model	AIC	Dev/df
Total Abundance (g)- *Continued*			
1 Up, Down*	*e*^(59.95NO3 +0.74Part +4.49Peri -11.37NO3*Part +2.80); ϕ = 10.58	433.56	0.1160
1 Up, Down + sph vcm	*e*^(50.16NO3 +0.32*Part* +3.08Peri -9.34NO3*Part +4.74); *a* = 18.74 var=0.27	N/A	
Baetis- mid-instar larvae			
Local*	*e*^(85.57NO3 +1.98Part +8.89Flow -15.64NO3*Part -8.15); *k* = 1.77	333.49	1.4498
Local + sph vcm	*e*^(95.25NO3 +1.25*Part* +10.36Flow -17.24NO3*Part -*3.48*); *k* = 1.77*, var = 2.03, *a* = 5.56	N/A	
1 Up	*e*^(72.27*NO3* +3.62*Flow* -186.09Peri -0.42*Part* 41.06NO3*Flow -16.08NO3*Part +41.44Peri*Part +2.99); *k* = 0.84	317.61	1.6375
2 Up	*e*^(132.80NO3 +11.20Flow -232.68Peri -1.48*Part* -25.56NO3*Part +52.37Peri*Part + *5.33*); *k* = 1.49	331.80	1.5634
1 Down	*e*^(185.99NO3 +2.26Part +10.94Peri -35.17NO3*Part -7.86); *k* = 1.88	335.44	1.4563
1 Up, Down	*e*^(-54.01NO3 -5.11*Flow* +2.30Part +10.82Peri +187.06NO3*Flow -6.59); *k* = 1.37	327.56	1.5069
Brachycentrus- late-instar larvae			
Local	*e*^(44.59NO3 +31.18Flow +8.43Peri +4.31Part +30.75NO3*Flow -10.32NO3*Part - 7.00Flow*Part -19.34), *k* = 0.22	158.60	1.5047
1 Up	*e*^(9.84Peri +2.68Part -12.07); *k* = 0.48	160.71	1.2031
2 Up	*e*^(13.69Peri +2.73Part -12.61); *k* = 0.83	170.73	1.1864
1 Down	*e*^(62.25NO3 +8.03Peri +1.85Part -12.02NO3*Part -7.88); *k* = 0.80	176.10	1.3731
1 Up, Down	*e*^(9.13Peri +1.89Part -8.45); *k* = 0.91	174.49	1.2584
Chironomidae larvae			
Local	*e*^(25.68Flow +2.84Part -5.20Part*Flow -7.17); *k* = 0.35	431.17	1.2233
1 Up	*e*^(1.07Part +*1.61*); *k* = 0.53	441.13	1.1656
2 Up	*e*^(4.95Flow -59.28Peri +0.31*Part* +12.86Peri*Part +*3.19*); *k* = 0.34	432.61	1.2745
1 Down	*e*^(3.03Flow +1.29Part -0.45); *k* = 0.36	430.16	1.1787
1 Up, Down	*e*^(4.23Flow +1.21Part -*0.48*); *k* = 0.42	435.50	1.1906
Heptagenia- early, mid, and late-instar larvae			
Local	*e*^(0.68Part +3.35Flow -*2.92*); *k* = 0.60	150.64	1.2620

Group	Model	AIC	Dev/df
Heptagenia- early, mid, and late-instar larvae- *Continued*			
1 Up	*e*^(1.50); *k* = 0.92	154.99	1.1760
2 Up	*e*^(1.74NO3 +7.44Part +117.71Flow -23.70Part*Flow -36.00); *k* = 0.35	146.57	1.4014
1 Down	*e*^(4.19Flow +0.86Part -4.03); k = 0.57	150.00	1.2774
1 Up, Down	*e*^(-340.58NO3 +7.44Part +115.32Flow +246.27NO3*Flow +51.96NO3*Part -25.35Flow*Part -32.47); *k* = 0.22	145.90	1.5912
Hydropsyche- late-instar larvae			
Local*	*e*^(8.66Part +102.10Flow -18.47Part*Flow -43.62); *k* = 3.10	188.51	1.1104
Local + exp vcm	*e*^(7.61Part +95.46Flow -16.32Part*Flow -39.81); *k* = 3.10*, var= 0.67, *a* = 7.59		
1 Up	-334.08Peri -1.84*Part* +13.45Flow +70.05Peri*Part +*6.27*); *k* = 2.91	189.32	1.1603
2 Up	*e*^(-336.54Peri -1.99*Part* +20.17Flow +68.74Peri*Part +*6.02*); *k* = 3.51	192.94	1.1472
1 Down	*e*^(3.20); *k* = 5.73	196.65	1.0061
1 Up, Down	*e*^(3.09Part -11.71); *k* = 4.37	192.63	1.0515
Tricorythodes- mid-instar larvae			
Local	*e*^(3.70Part +42.56Flow -8.54Part*Flow -14.03); *k* = 0.64	300.88	1.2547
1 Up	*e*^(5.31Part +71.49Flow -15.07Part*Flow -21.00); *k* = 0.67	302.49	1.2728
2 Up	*e*^(120.19Peri +3.05Part -25.22Peri*Part -10.33); *k* = 0.65	301.67	1.2786
1 Down	*e*^(5.34Part +70.37Flow -14.94Part*Flow -20.98); *k* = 0.64	300.77	1.2632
1 Up, Down	*e*^(-382.91NO3 +161.85Flow +1.59*Peri* +10.18Part +218.91NO3*Flow -63.86NO3*Peri +63.16NO3*Part -35.36Flow*Part -42.36); *k* = 0.29	286.84	1.5131

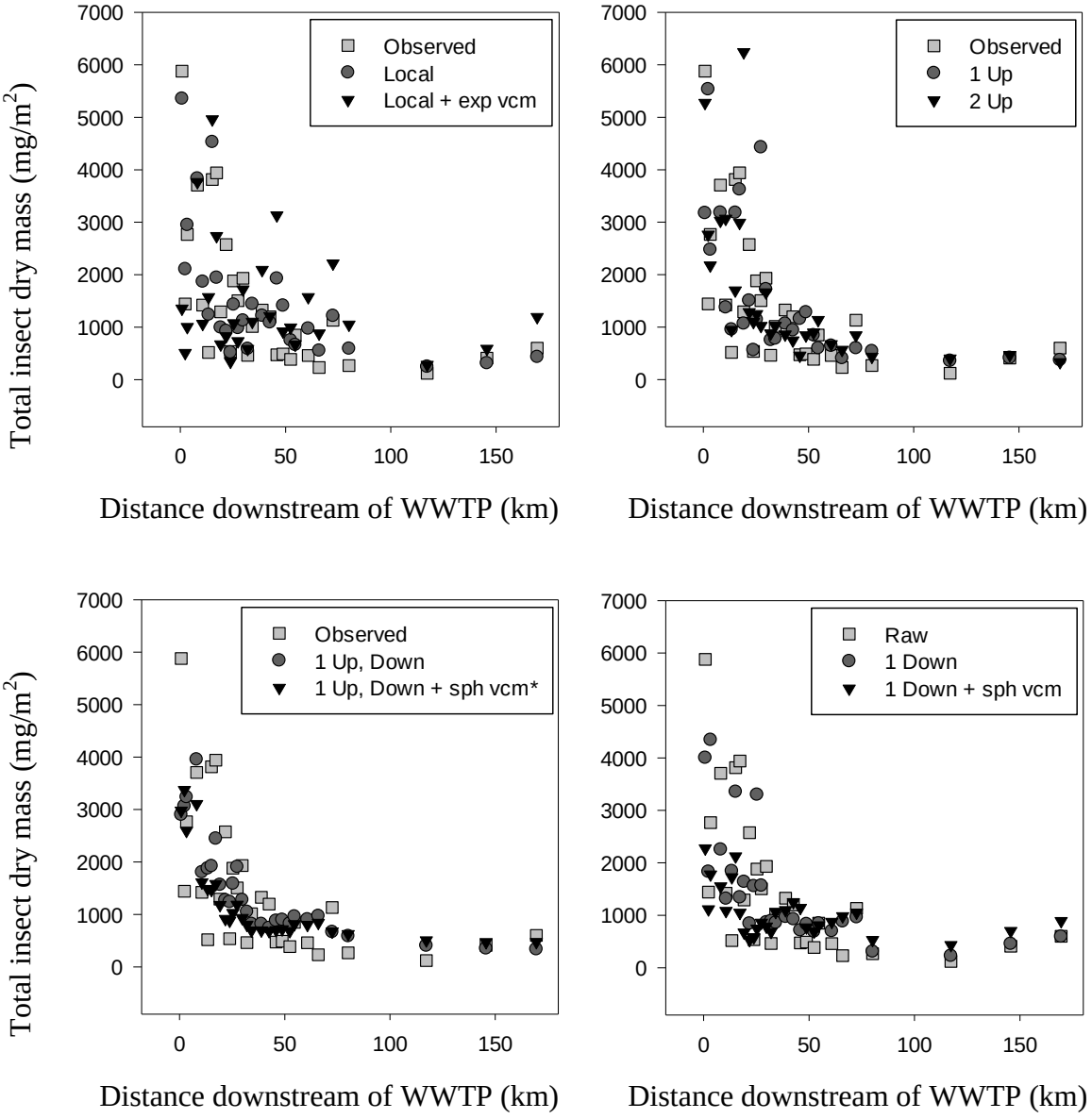
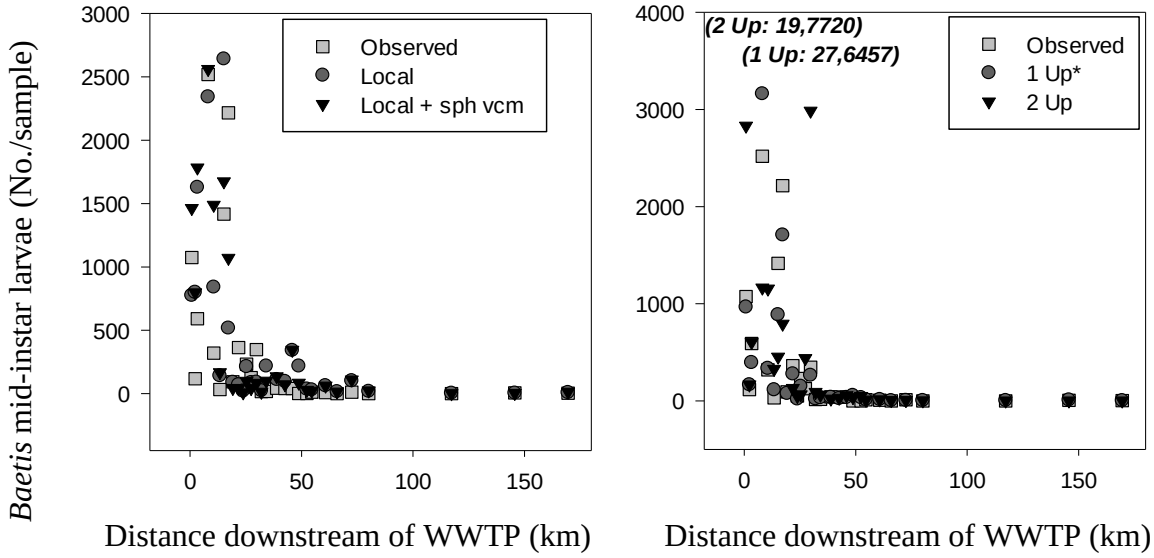
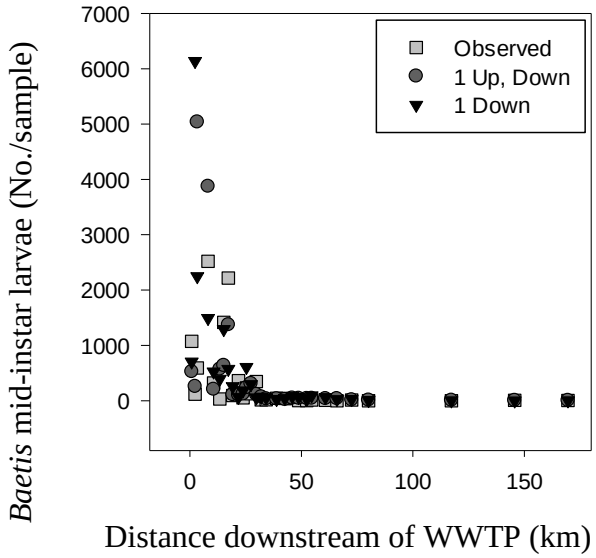


Figure 3.12: Insect abundance as modelled statistically by explanatory environmental variables averaged over various lags. Observed data are also shown for reference, and model labels are described in the text. The best model as determined by having the lowest AIC, or those that are equivalent to the best model, are marked by asterisks. Extreme predicted values not pictured are indicated as follows: ***(model: distance, value)***. Model details can be found in **Table 3.1**. Figure continued on next 6 pages.





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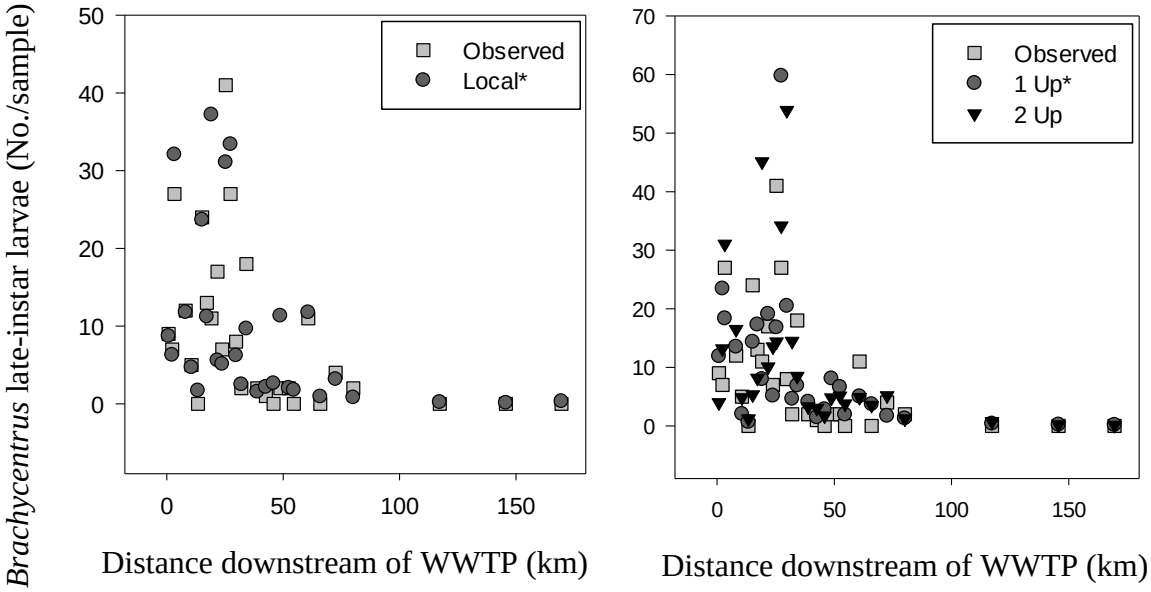

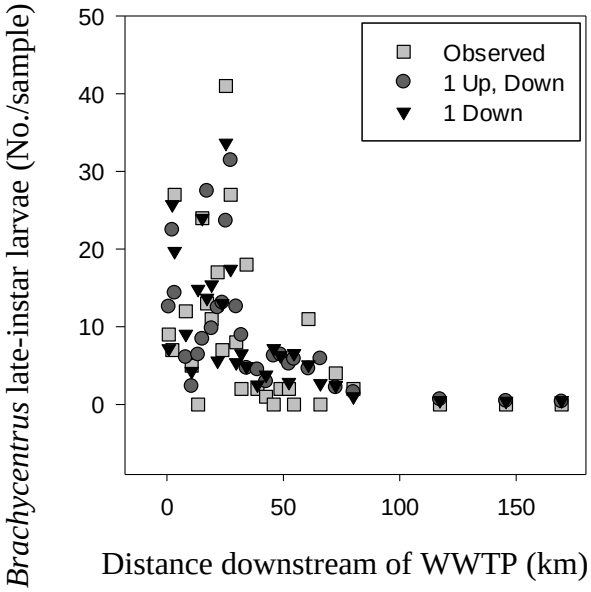

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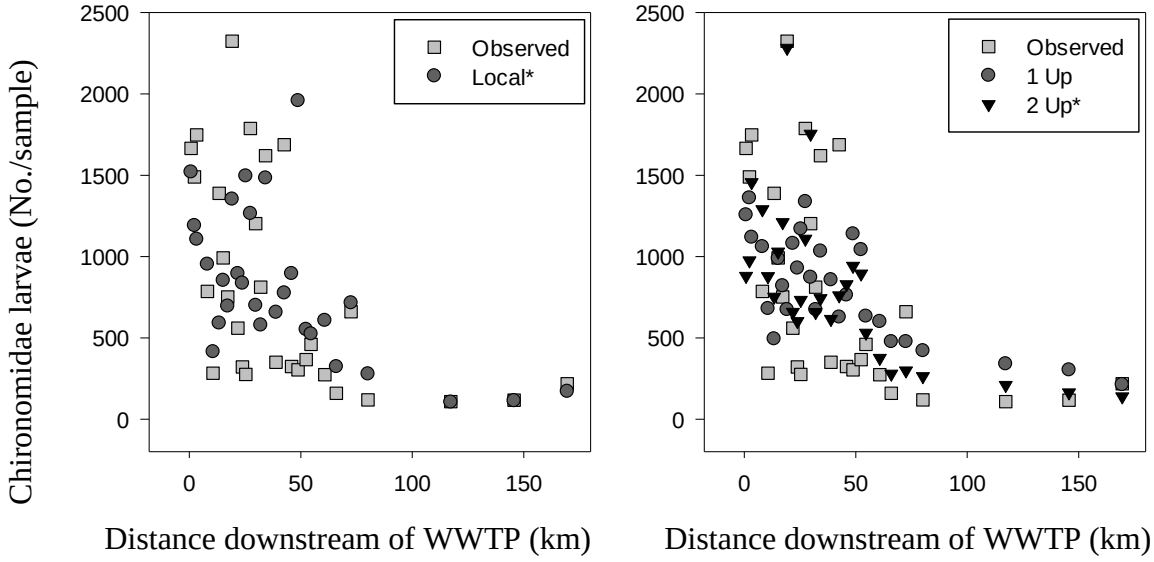

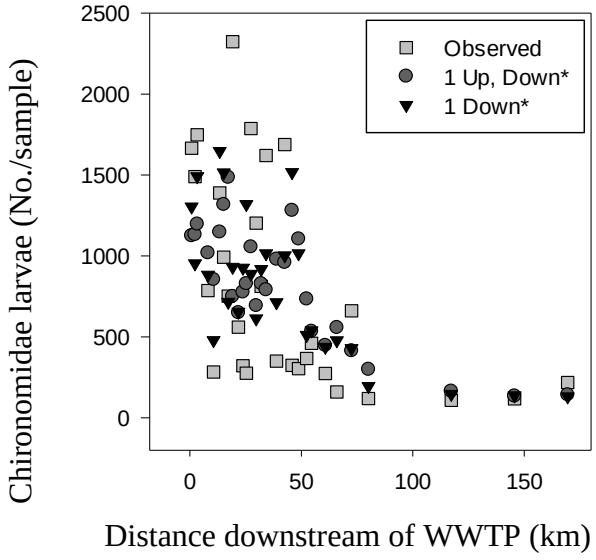

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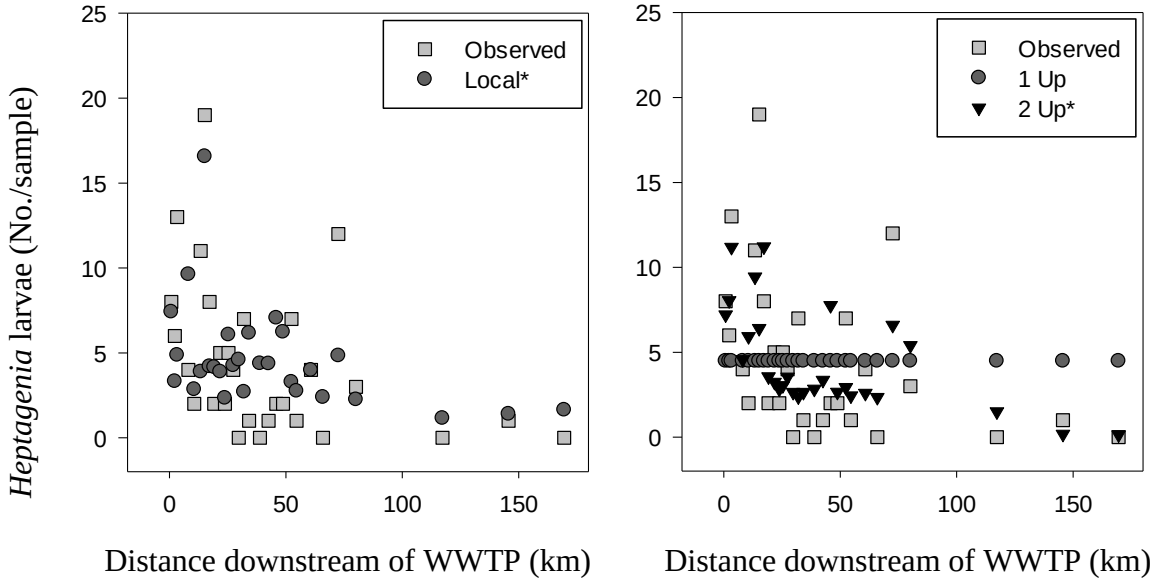

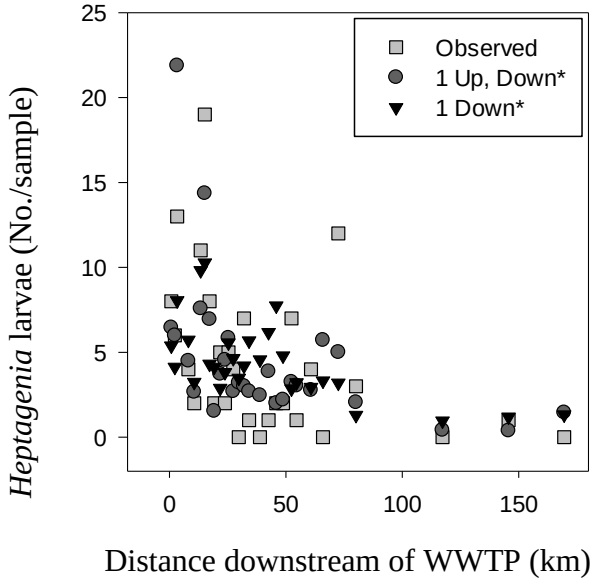

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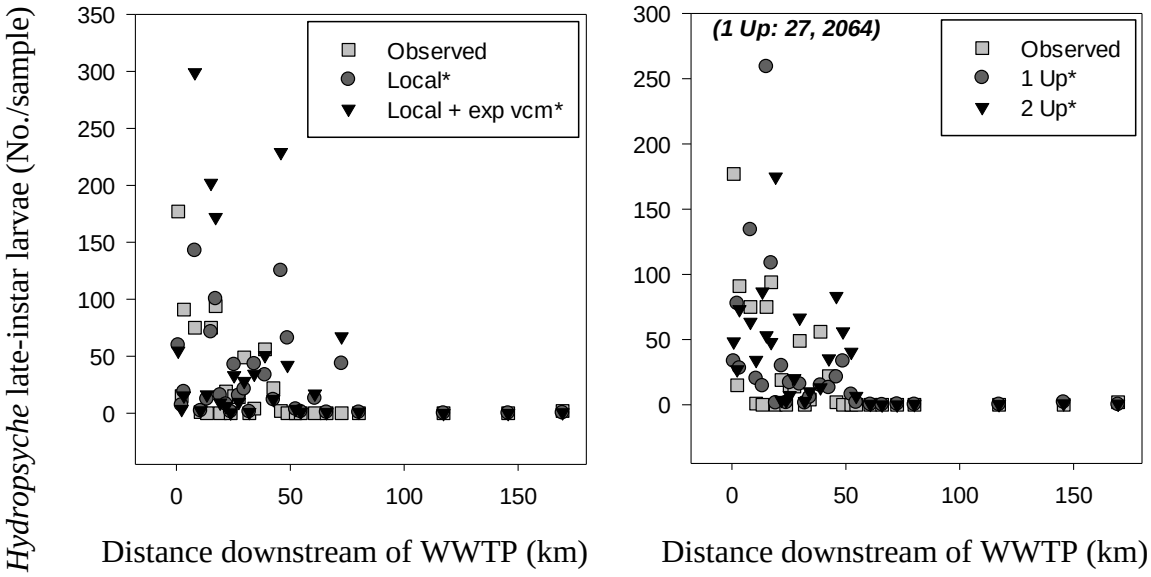

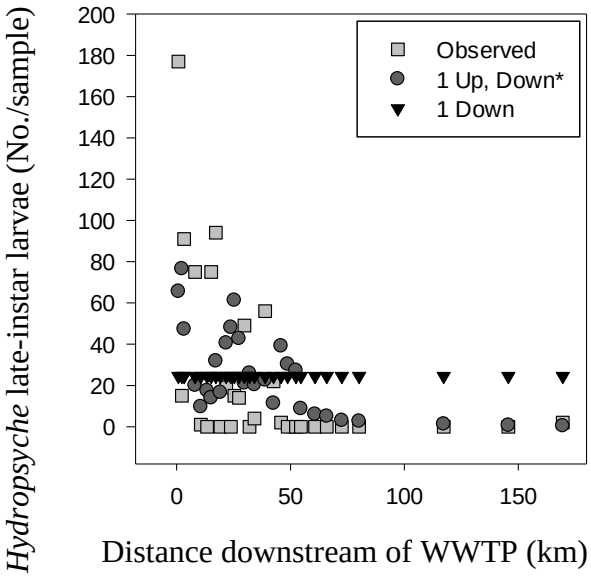

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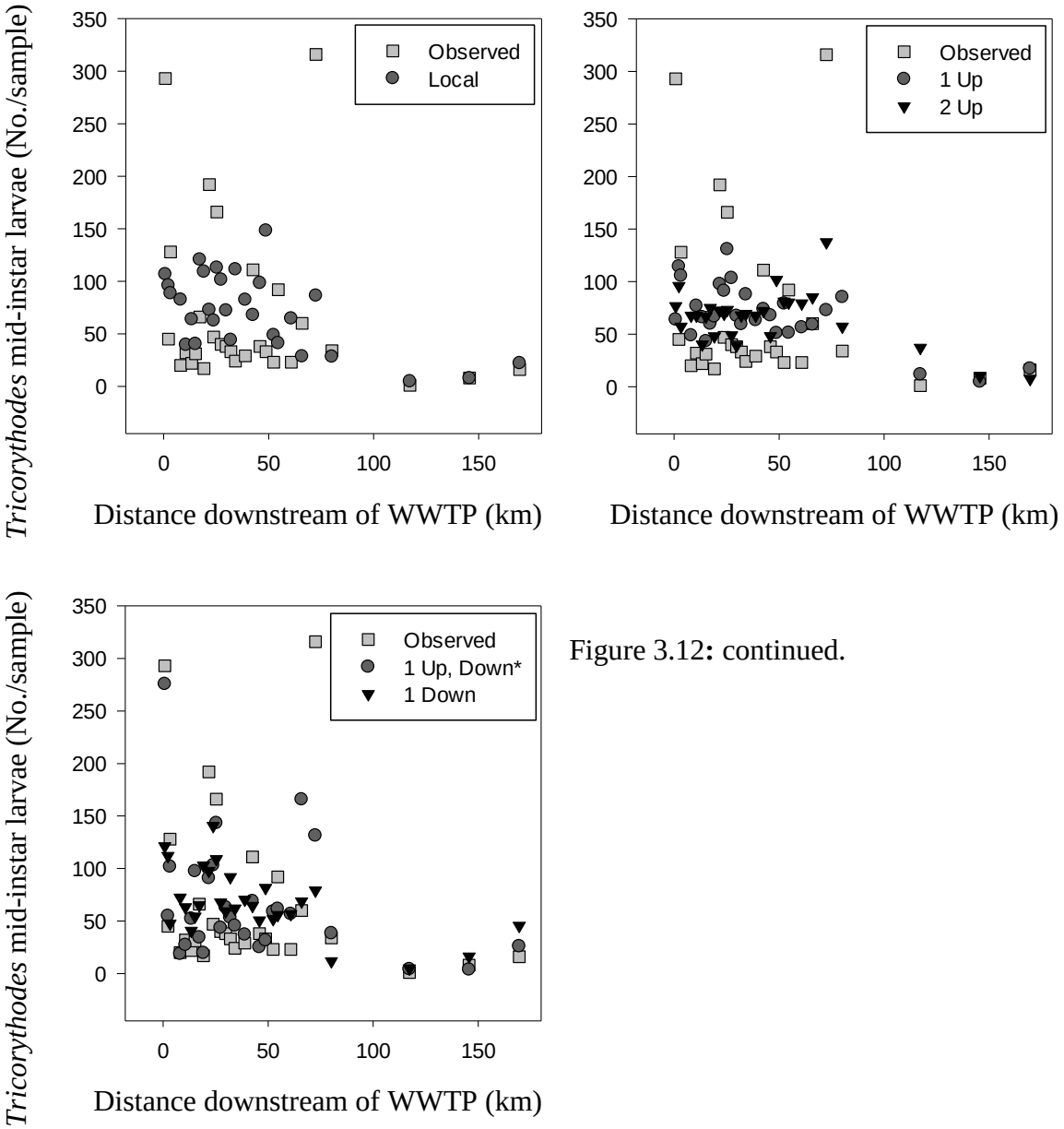

Figure 3.12**:** continued.

3.3 Stable isotope analysis

3.3.1 Trophic fractionation correction

Trophic fractionation correction values, as estimated from the reference site 14 km upstream of the WWTP, were 2.7 and 2.2 ‰ respectively for mid and late-instar *Baetis*. Mid- and late-instar *Tricorythodes* had similar corrections of 2.4 and 2.2 ‰, and *Heptagenia* had slightly higher values of 2.6, 2.6, and 3.1 ‰ for early, mid, and late-instarrespectively. *Brachycentrus*, Chironomidae, and *Hydropsyche* late-instar larvae and pupae had even higher trophic correction values of 4.2, 4.2, 4.3, and 5.7 ‰ respectively.

3.3.2 Linear regressions

The overall general linear model relating average left-bank $\delta^{15}N$ signatures to distance downstream of the RDWWTP and group (insect group or periphyton) was highly significant ($df_{model}=17$, $df_{error}=185$, $F= 21.60$, $P < 0.0001 < \alpha = 0.05$), and explained 66.5% of the variation in $\delta^{15}N$ signatures ($r^2=0.665$). Distance downstream of the WWTP, group, and the interaction between these effects were all highly significantly related to $\delta^{15}N$ signatures ($P < 0.0001$, $P < 0.0001$, $P = 0.0016$ respectively), meaning that there was a real gradient in isotope signatures through space, the mean signatures differed between some or all groups of insects and/or periphyton, and that the spatial gradient of $\delta^{15}N$ (i.e. the slope of the relationship between $\delta^{15}N$ and distance downstream of the WWTP) also differed between some or all of these groups. Periphyton had the greatest mean $\delta^{15}N$ signature (8.4 ± 0.2 ‰), followed by late-instar *Baetis* (8.3 ± 0.2 ‰), late-instar *Tricorythodes* (7.8 ± 0.2‰), mid-instar *Baetis* (7.4 ± 0.3 ‰), mid-instar *Tricorythodes* (6.9 ± 0.2 ‰), *Heptagenia* (6.9 ± 0.2 ‰), *Hydropsyche* (6.9 ± 0.3‰)

Chironomidae (6.5 ± 0.2 ‰), and *Brachycentrus* (6.3 ± 0.2 ‰) (Table 3.2). Periphyton had a spatial gradient in $\delta^{15}N$ of -0.077 ‰/km. Late-instar *Baetis* was the only group that had a steeper gradient (-0.085 ‰/km) than periphyton. Of the groups with shallower gradients than periphyton (i.e. reduced slopes compared to periphyton), mid-instar *Baetis* had the steepest (-0.064 ‰/km), followed by mid-instar *Tricorythodes* (-0.059 ‰/km), late-instar *Tricorythodes* (-0.054 ‰/km), Chironomidae (-0.042 ‰/km), *Heptagenia* and *Hydropsyche* (-0.035 ‰/km), and *Brachycentrus* (-0.028 ‰/km) (Table 3.2). In post-hoc analysis, using the Dunn-Sidak correction for type 1 error rates given experiment-wise error rate of α_e=0.05 and 8 comparisons (α_8=1-(1-0.05)$^{1/8}$ = 0.00639), it was shown that only *Brachycentrus* and *Heptagenia* had significantly different spatial $\delta^{15}N$ gradients than periphyton (P = 0.0005 and 0.0026 respectively), also having significantly lower mean $\delta^{15}N$ signatures than periphyton (P<0.0001 for both). Chironomidae had a $\delta^{15}N$ gradient that was almost significantly different than that of periphyton (P = 0.0084) and a mean $\delta^{15}N$ signature that was significantly lower (P = <0.0001). *Hydropsyche* and mid-instar *Baetis* and *Tricorythodes* had mean $\delta^{15}N$ signatures that were significantly lower than periphyton (P = <0.0001, 0.0014, and <0.0001 respectively) but gradients that were not (P = 0.0512, 0.4761, and 0.1758 respectively). Finally, late-instar *Tricorythodes* and *Baetis* had both mean $\delta^{15}N$ signatures and $\delta^{15}N$ gradient that were not significantly different from those of periphyton (P = 0.0479 and 0.7695 for means and 0.0790 and 0.5942 for gradients respectively). Pair-wise comparisons were not performed to uncover differences between specific insect groups, but it can be inferred that differences existed. Despite apparent non-linearities (Figure 3.13), the residuals of this analysis were found to be normal for each group, and significant autocorrelation was not detected for any group.

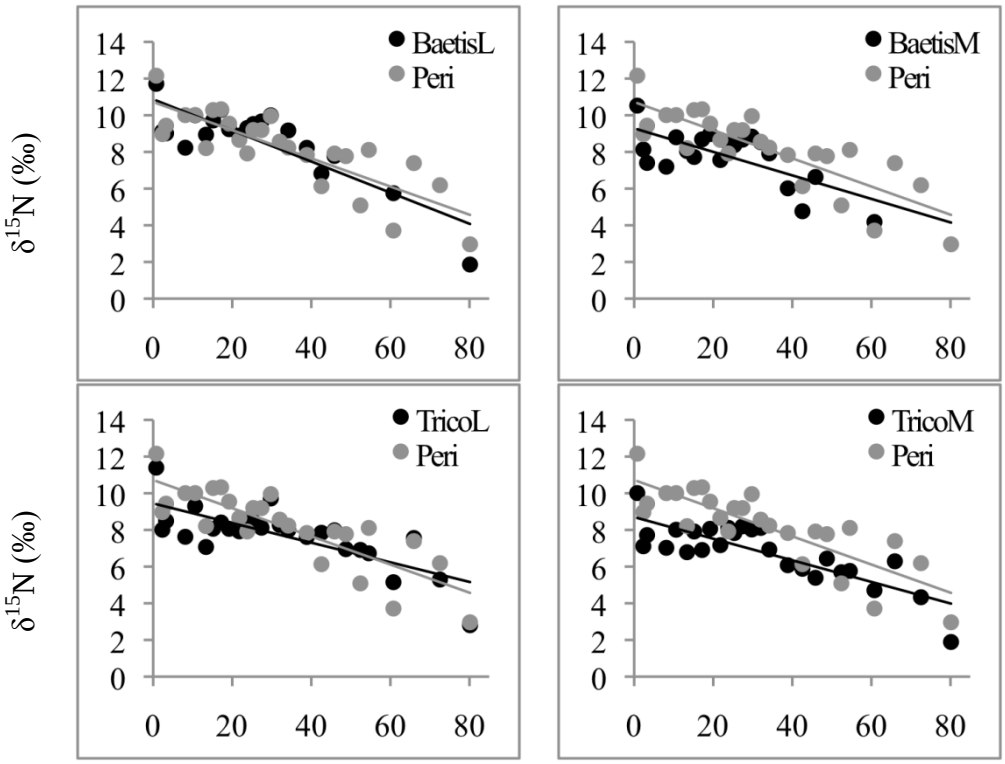

Figure 3.13: Linear spatial trends in site averages of insect and periphyton $\delta^{15}N$ signatures. WWTP = Wastewater Treatment Plant, Peri = periphyton BaetisL = late-instar *Baetis*, BaetisM = mid-instar *Baetis*, TricoL = late-instar *Tricorythodes*, TricoM = mid-instar *Tricorythodes*, Hepta = *Heptagenia*, Chiro = Chironomidae, Hydro = *Hydropsyche*, Brachy = *Brachycentrus*. Figure continued on next page.

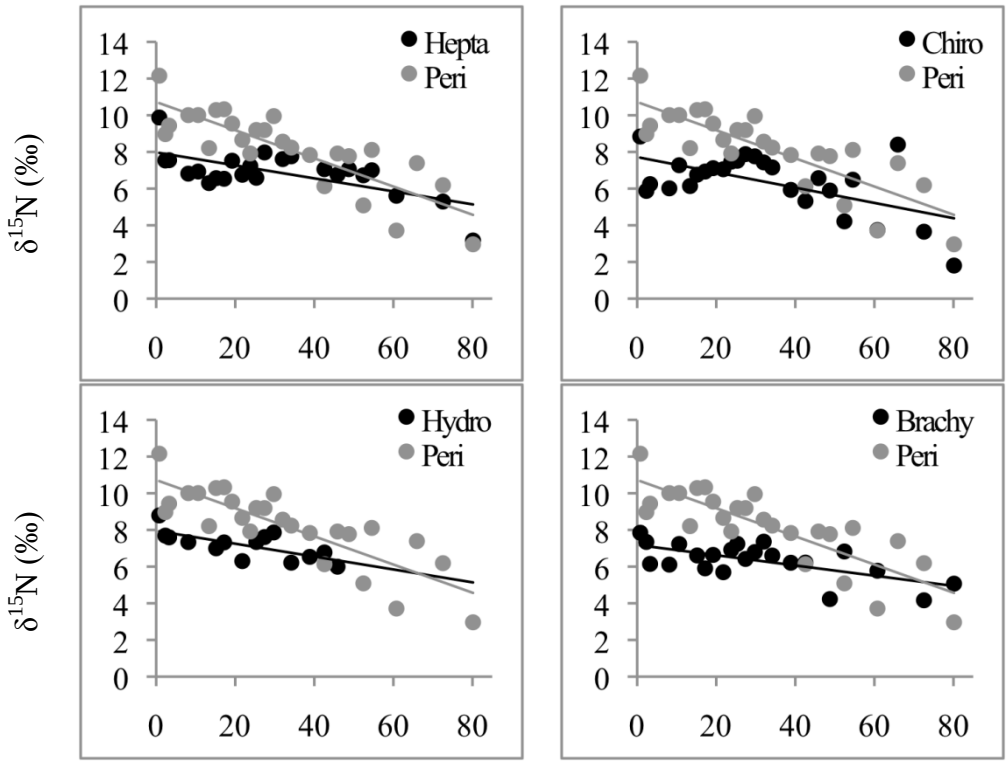

Figure 3.13**:** Continued.

Table 3.2: Least squared mean $\delta^{15}N$ signatures and change in $\delta^{15}N$ through space (‰/km, slope), of insect groups and periphyton, and results of post-hoc analysis of pre-planned comparisons between mean signatures and slope of signatures of each insect group versus that of periphyton. Type I error rate was adjusted using the Dunn-Sidak procedure for 8 comparisons ($\alpha_8=1-(1-0.05)^{1/8} = 0.00639$). P-values deemed significant at this level are shaded grey. Peri = periphyton, BaetisL = late-instar *Baetis*, BaetisM = mid-instar *Baetis*, TricoL = late-instar *Tricorythodes*, TricoM = mid-instar *Tricorythodes*, Hepta = *Heptagenia*, Chiro = Chironomidae, Hydro = *Hydropsyche*, Brachy = *Brachycentrus*.

Group	LS Mean, μ, ‰	SE of μ, ‰	P ($\mu_{insect} = \mu_{peri}$) α_8=0.00639	Slope, β, ‰/km	P ($\beta_{insect} = \beta_{peri}$) α_8= 0.00639	n
Peri	8.42	0.2		-0.077		26
BaetisM	7.37	0.3	0.0014	-0.064	0.4761	20
BaetisL	8.33	0.2	0.7695	-0.085	0.5942	20
Brachy	6.35	0.2	<.0001	-0.028	0.0005	22
Chiro	6.48	0.2	<.0001	-0.042	0.0084	26
Hepta	6.92	0.2	<.0001	-0.035	0.0026	23
Hydro	6.90	0.3	<.0001	-0.035	0.0512	14
TricoM	6.94	0.2	<.0001	-0.059	0.1758	26
TricoL	7.85	0.2	0.0479	-0.054	0.0790	26

3.3.3 Residual cross-correlation- nonparametric spline cross-correlograms

The detrended (residual) $\delta^{15}N$ signatures of some but not all insect groups were significantly positively cross-correlated to periphyton residual $\delta^{15}N$ signatures at small lags, and no significant negative cross-correlation was detected for any group at any lag. All of the spline cross-correlograms had broad confidence intervals that flared substantially after lags of 40-50 km (Figure 3.14). Those groups with significant cross-correlation included mid and late-instar *Baetis* and *Tricorythodes*, and Chironomidae. Of these groups, Chironomidae had the longest "correlation length" (4.6 km), the smallest lag at which significant cross correlation disappears (Bjornstad and Falck 2001), followed

by mid-instar *Tricorythodes* (3.8 km), late-instar *Baetis* (3.5 km), mid-instar *Baetis* (2.9 km), and late-instar *Tricorythodes* (1.3 km). *Brachycentrus*, *Hydropsyche*, and *Heptagenia* residual signatures were not significantly cross correlated to that of periphyton at any lag, although *Hydropsyche*, and *Heptagenia* had cross-correlograms that resembled that of the other groups in that the highest degree of cross-correlation was observed at a lag of zero (Figure 3.14). *Brachycentrus*, in contrast to all other groups, had a very reduced estimate of cross-correlation at a lag of zero. See Table 3.3 for select details of this analysis, including estimates of cross-correlation at a lag of zero and the degree of positive cross-correlation, with bootstrapped 95% confidence intervals.

Table 3.3: Select results of spline cross-correlation analysis between residual insect and periphyton $\delta^{15}N$ signatures. The estimate (Est) of cross-correlation at a lag of zero ("Y-int"), and the estimate of the extent of positive cross-correlation ("X-int") with upper (UCL) and lower (LCL) bootstrapped 95% confidence limits are reported. The LCL of the X-int, i.e. the correlation length of the correlogram, is marked with an asterisk. Groups for which significant cross correlation was not observed have, thus, illogical (negative) correlation lengths, and are shaded grey. BaetisL = late-instar *Baetis*, BaetisM = mid-instar *Baetis*, TricoL = late-instar *Tricorythodes*, TricoM = mid-instar *Tricorythodes*, Hepta = *Heptagenia*, Chiro = Chironomidae, Hydro = *Hydropsyche*, Brachy = *Brachycentrus*.

Insect Group	Y-int (cross-corr)			X-int (km)		
	LCL	Est	UCL	LCL*	Est	UCL
BaetisL	0.07	0.25	0.83	3.5*	10.3	46.9
BaetisM	0.04	0.25	0.78	2.9*	8.8	43.0
Brachy	-0.34	0.04	0.27	-53.3	14.0	38.1
Chiro	0.08	0.23	0.83	4.6*	9.1	46.8
Hepta	-0.13	0.11	0.63	-19.1	8.9	40.1
Hydro	0.11	-0.10	0.67	-28.7	7.5	45.9
TricoL	0.01	0.18	0.74	1.3*	8.8	45.5
TricoM	0.04	0.23	0.74	3.8*	8.9	44.2

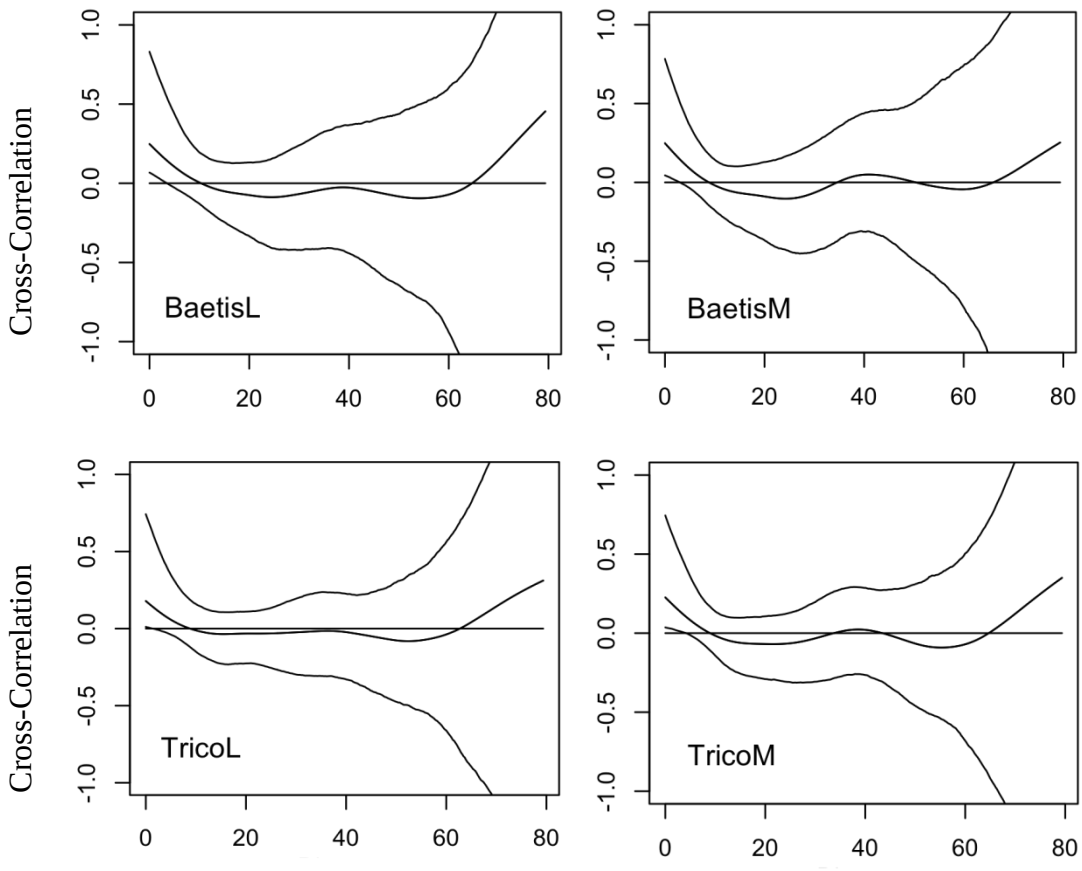

Figure 3.14: Non-parametric spline cross-correlograms representing cross-correlation (plus bootstrapped 95% confidence intervals) between linearly detrended insect and periphyton $\delta^{15}N$ signatures. BaetisL = late-instar *Baetis*, BaetisM = mid-instar *Baetis*, TricoL = late-instar *Tricorythodes*, TricoM = mid-instar *Tricorythodes*, Hepta = *Heptagenia*, Chiro = Chironomidae, Hydro = *Hydropsyche*, Brachy = *Brachycentrus*. Figure continued on next page.

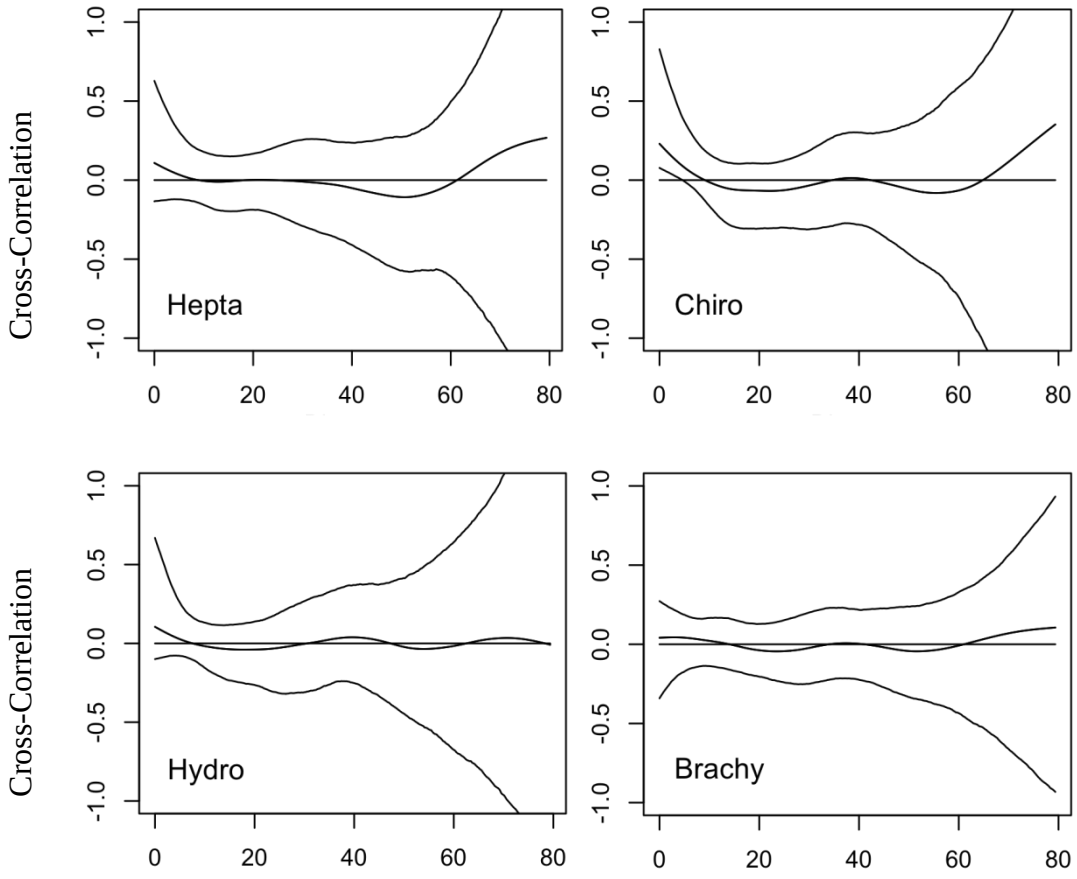

Figure 3.14**:** Continued.

3.4 Spatial structure in insect family assemblages

A total of 42 families of insects were recorded. Homopterans were only identified to order owing to the absence of adequate keys, as were Hymenopterans, which were rare. The Hellinger-transformed abundance of the 20 most common families at each site is displayed in Figure 3.15. The rest of the families had insubstantial abundance in comparison to these 20, but were still used in the construction of Mantel correlograms, minus those groups that were observed at only one site. Thus the Hellinger-transformed abundances of 35 families were used in the analysis of insect assemblage spatial

structure, which comprised 9 Mantel correlograms. These Mantel correlograms, to recollect, revealed spatial structure in family assemblages when both left and right bank sites were included, and when one or the other was excluded. These same data were analysed before and after linear detrending, and with spatial structure in environmental variables accounted for.

The Mantel correlogram that included both left and right-bank sites did not show significant autocorrelation in the first distance class (0-900 m) designed to assess L/R bank autocorrelation. Significant positive autocorrelation was found, however, in the classes up to and including 15-20 km. For sites separated by 20-30 km, non-significant positive spatial autocorrelation existed, and significant negative autocorrelation was found for classes 30-40 km, 60-70 km, and all in between. Beyond this, no significant results were found, but r_{Md} gradually increased towards zero, remaining negative. The use of linearly detrended similarity data resulted in a reduced magnitude of autocorrelation in general, and caused significant positive spatial autocorrelation to be detected only in the 3-6 km and 10-6 km distance classes (Figure 3.16). The Mantel correlograms that omitted either left or right-bank sites within 20 km of the WWTP had very similar shapes to the all-site correlogram, but differed in the detection of significant autocorrelation. When right-bank sites were excluded, non-significant negative autocorrelation occurred for sites separated by 30-40 km, and negative autocorrelation occurred for classes 40-50km, 70-80 km, and for all sites in between. When detrended assemblage similarities were used for this left-bank data, a very similar Mantel correlogram was produced, but significance was lost in the following distance classes, which were of lower significance when raw data was used: 15-20 km, 40-50 km, and 70-

80 km (Figure 3.16). The Mantel correlogram that omitted left-bank sites within 20km of the WWTP showed positive autocorrelation for all classes up to and including 10-15 km, non-significant decreasing correlation in the 15-20, 20-30, and 30-40 km classes, and significant negative correlation in the 40-50, 50-60, and 60-70 km classes When detrended data was used for these right-bank data, no significant spatial autocorrelation was found at any lag (Figure 3.17).

3.4.1 Partialling out Spatial Structure in Environmental Conditions

Despite expectation, there was very little spatial structure detected in environmental conditions. For the analysis including all sites, no significant spatial autocorrelation was found for any distance class (Figure 3.18). When either left or right-bank sites were excluded, however, significant positive autocorrelation was found in the first class (0.9-3 km), and –when left-bank sites were excluded- the second distance class (3-6 km) (Figure 3.18). Thus partialling out the effects of "environmental distance" between sites created partial Mantel correlograms for family assemblages that appeared almost identical to the original Mantel correlograms, with the following notable changes. In the all-site correlogram, the positive correlation in the L/R distance class (0-900m) became significant and greater, and in the left-bank-excluded correlogram, significant positive autocorrelation was detected in the 15-20 and 20-30 km classes, where it was formally smaller and non-significant. In general, if positive autocorrelation was observed in a given distance class of the environmental correlogram, then the autocorrelation in the same distance class of the corresponding partial correlogram became smaller, and vice versa (Figure 3.16, Figure 3.17).

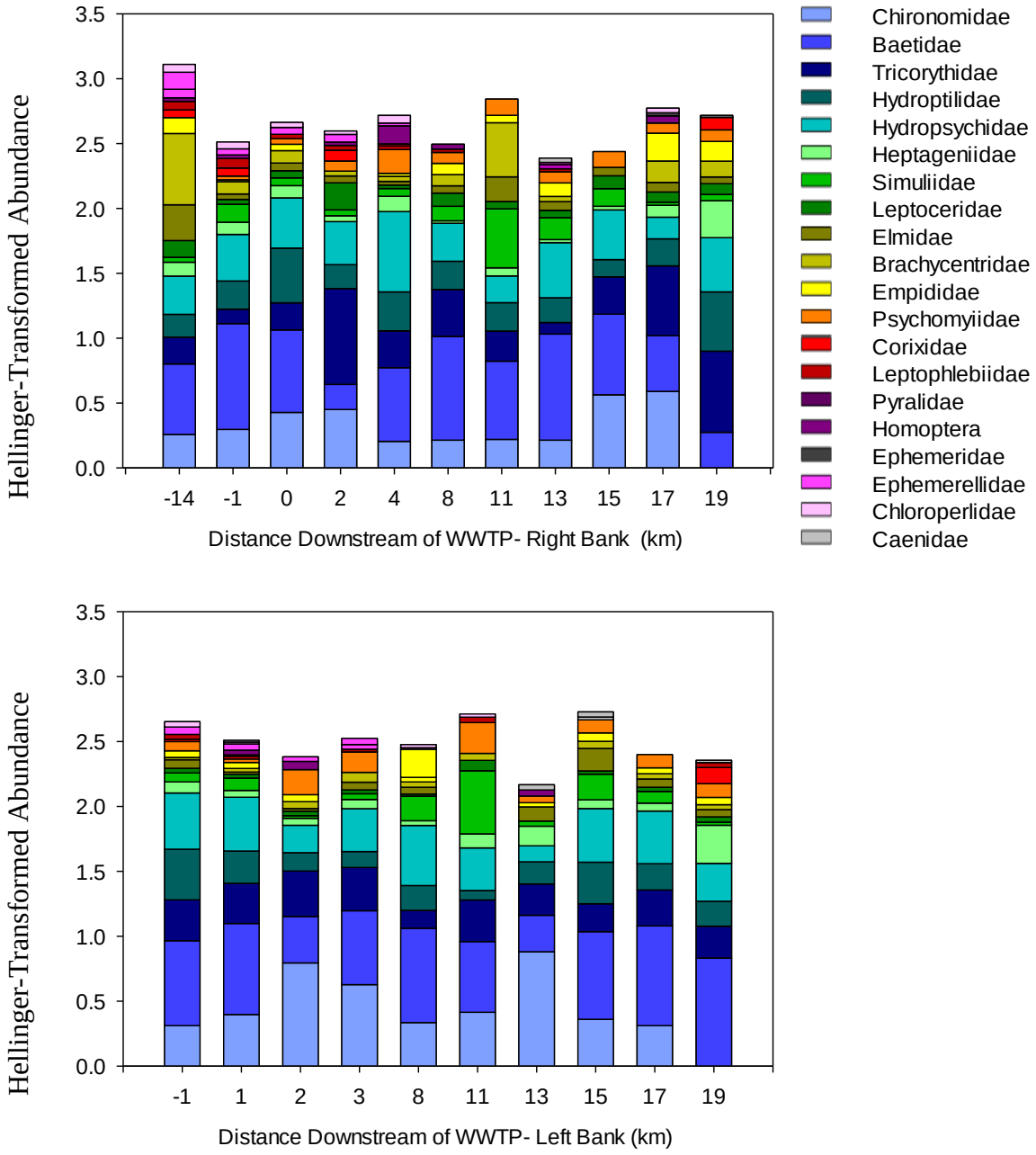

Figure 3.15: Abundance at each site of the most common 20 insect families. Hellinger-transformed abundance, as used to construct the family assemblage similarity matrices for spatial analysis, is depicted. In this transformation, abundances are divided by the total site abundance, and then the square root of this value is taken. Figure is continued on next page.

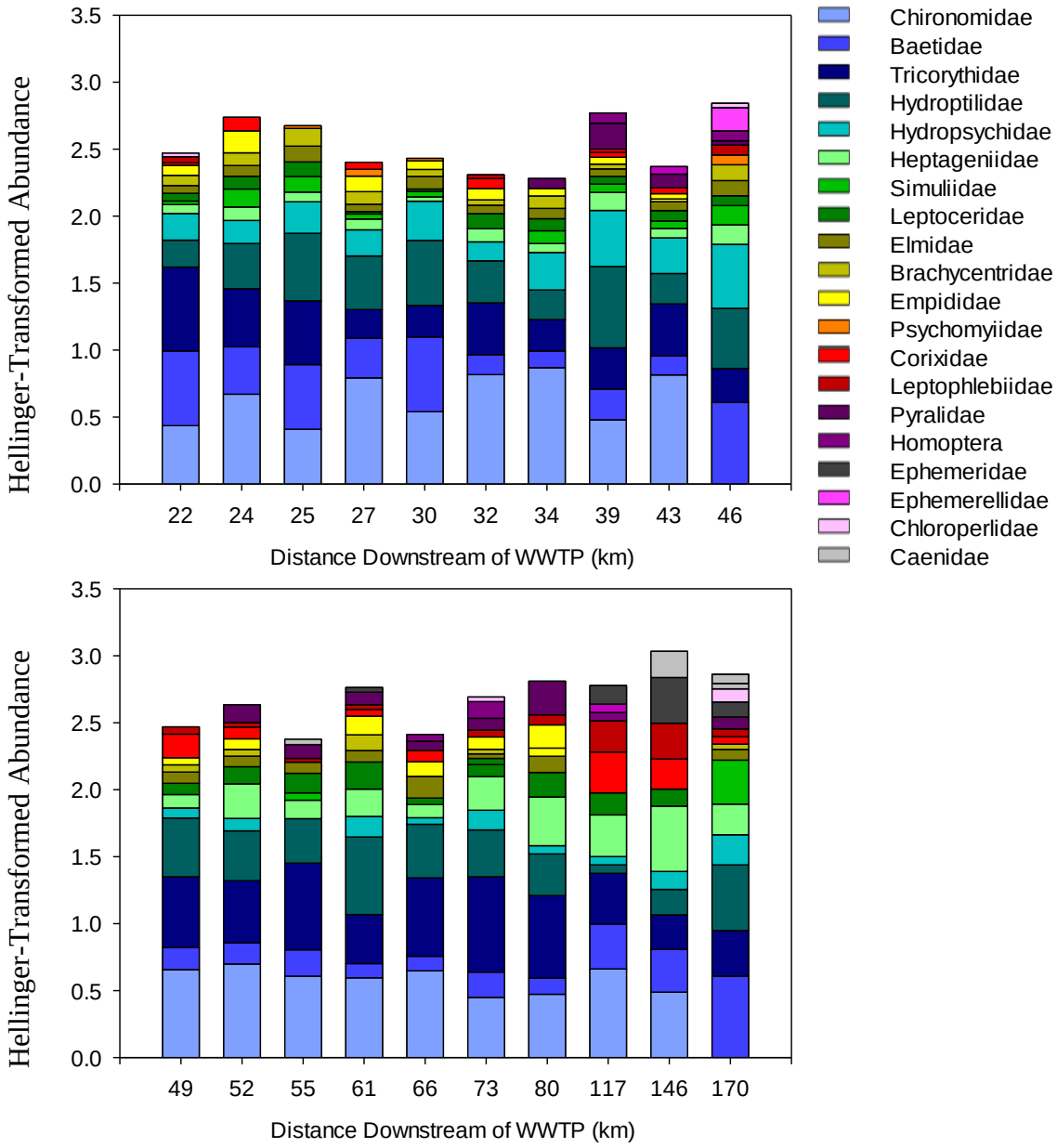

Figure 3.15**:** Continued.

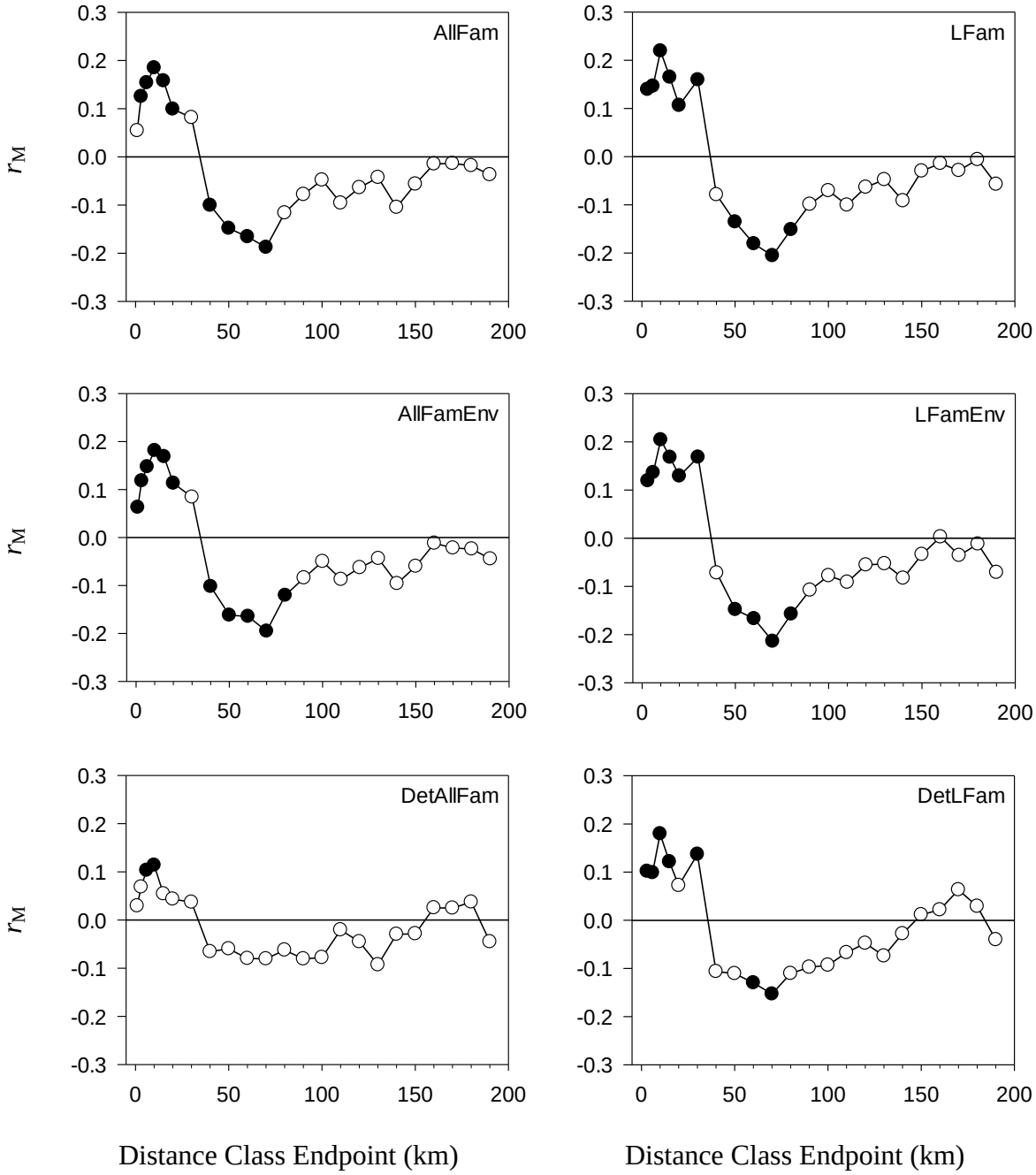

Figure 3.16: Mantel correlograms depicting spatial autocorrelation in family assemblages (Fam, top row), assemblages with autocorrelation in environmental variables partialled out (FamEnv middle row), and after detrending of the assemblage distance matrix (Det, bottom row). Results calculated with both left and right-bank data (All), and left-bank data (L) are shown. Significant r_M values, given a progressive Bonferroni correction, are shown by solid circles, non-significant values are open.

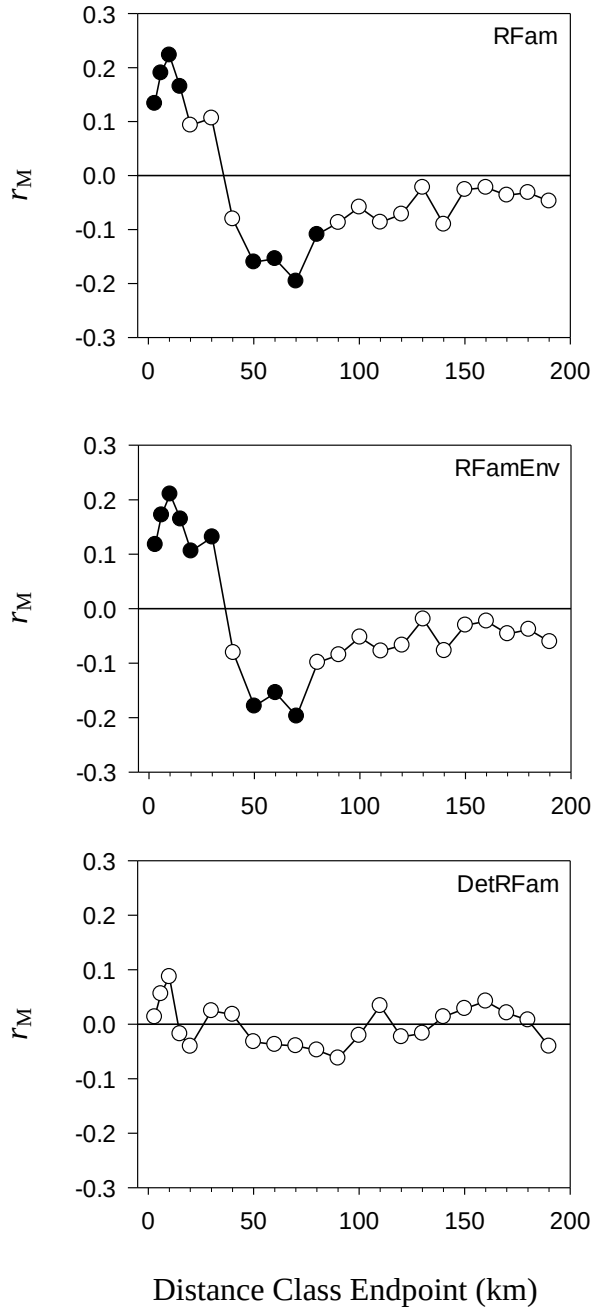

Figure 3.17: Mantel correlograms depicting spatial autocorrelation in family assemblages (Fam, top row), assemblages with autocorrelation in environmental variables partialled out (FamEnv middle row), and after detrending of the assemblage distance matrix (Det, bottom row). Results calculated right-bank data (R) are shown. Significant r_M values, given a progressive Bonferroni correction, are shown by solid circles, non-significant values are open.

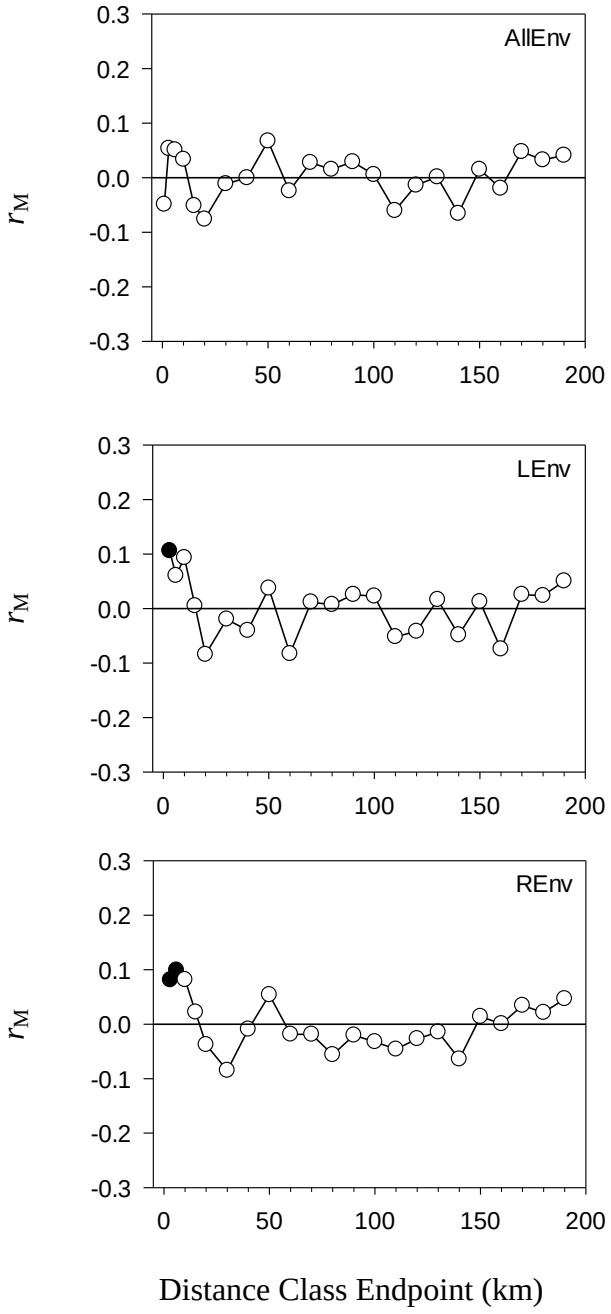

Figure 3.18: Mantel correlograms depicting spatial autocorrelation in environmental variables (Env) Results calculated with right-bank data (R), left-bank data (L), and both (All) are shown. Significant r_M values, given a progressive Bonferroni correction, are shown by solid circles, non-significant values are open.

Chapter Four: **Discussion**

Chapter four will interpret the observational and statistical results of this study in a manner corresponding loosely to sections 2.5 through 2.9 in Chapter 2. The objectives, hypotheses, and predictions of the study will be addressed as relevant, given that there is some degree of result synthesis required to answer certain objectives. To aid cross-referencing, links to the most relevant sections of other chapters are present in the text.

4.1 The response of insect abundance to WWTP effluent

The hypothesis that nutrient enrichment from the RDWWTP leads ultimately to increased consumer biomass in the Red Deer River was largely supported by the results of this study. As elaborated upon in this discussion, the observed spatial patterns of nutrient concentrations, and of insect and periphyton abundance, are in concordance with this idea. Statistical analyses relating benthic insect abundance to potential environmental explanatory variables also point towards nutrient status a potential driver of abundance.

Greatly elevated concentrations of nitrogen and phosphorous directly below the input of effluent from the RDWWTP suggest that it is a considerable contributor of nutrients to the Red Deer River. Downstream of this point, the sharp decline in nutrient concentrations implies that these nutrients are rapidly assimilated by primary producers (Ogura et al. 2009). This decline is also likely a result of dilution, but it is already known that WWTP-derived nitrogen is used extensively by periphyton in this river (Hogberg 2004), contributing to primary productivity.

The observation that insect biomass was highest directly downstream of the WWTP, and that it declined in reaches where nutrient concentrations were lower,

supports the prediction that WWTP-derived nutrients increase secondary productivity. The consistent positive association between NO_3 concentration and total insect biomass in generalized linear models containing local (and) upstream variables (Table 3.1) statistically corroborated this relationship. As outlined in section 2.5.1, this relationship, although correlative, was taken as an indication that WWTP-derived effluent could be augmenting secondary productivity.

Although maximum nutrient concentrations were coincident with maximum insect abundance directly downstream of the WWTP, the absence of maximum periphyton standing biomass in this area, and the lack of a significant relationship between periphyton and total insect abundance, could confound the assertion that nutrient enrichment increases consumer biomass by first augmenting primary productivity. However, a weak response of periphyton abundance to nutrient enrichment is consistent with consumer-resource studies undertaken in lotic systems. This literature testifies that invertebrates feeding on epilithon in turn reduce epilithon biomass (Jordan and Lake 1996, Barbee 2005, Wellnitz and Poff 2006, Donato-Rondon et al. 2010), even in conditions with elevated nutrient concentrations (Donato-Rondon et al. 2010, Sturt et al. 2011), and thus it is not uncommon for differences in nutrient enrichment to be more strongly expressed in invertebrate abundance than in periphyton abundance (Askey et al. 2007, Gafner and Robinson 2007, Sanchez-Perez et al. 2009). Because of these consumer-resource interactions, a measurement of the growth *rate* of periphyton, as opposed to the standing biomass, would have been ideal. All of this being said, in the absence of other variables, periphyton abundance was significantly positively related to total insect abundance, this term was merely not statistically significant in the presence of

other, stronger explanators of abundance, like NO_3. Thus the hypothesized link between nutrient enrichment and increased insect biomass remains plausible.

The observation that the abundance of individual insect groups were variously positively, negatively, or not significantly related to NO_3 concentration (Table 3.1) shows that, despite being positively associated with total abundance, nutrient enrichment may not always be associated with increased abundance of individual groups. This is not surprising if NO_3 is viewed as a proxy for potential primary productivity, as invertebrate communities might be expected to respond most strongly to primary productivity changes at the level of total secondary productivity. For individual insect groups, abundance could be affected by a number of interspecific interactions that could override associations with primary productivity.

The fact that the abundance of individual insect groups was not consistently related to periphyton abundance is also not surprising. There are many examples of, or reasons for, both positive and negative correlations between periphyton abundance and the abundance of most of the genera studied here. For instance, positive associations between periphyton abundance and insect abundance have been reported for *Baetis* (Richards and Minshall 1988) and *Tricorythodes* (Donato-Rondon et al. 2010), for which periphyton abundance is directly reflective of food resource availability. A positive association with periphyton could be expected for *Heptagenia* for the same reason. However, periphyton grazers such as *Baetis* (Roll et al. 2005), *Tricorythodes* (Donato-Rondon et al. 2010), and Chironomids (Gafner and Robinson 2007) can experience increased densities in response to increased periphyton growth while concomitantly decreasing periphyton accumulation, which could dampen positive relationships between

standing crops of insects and periphyton, or even cause negative associations. Mixed expectations for the relation between insect and periphyton abundance could also exist for *Hydropsyche*. Positive associations could theoretically exist between periphyton and *Hydropsyche* abundance, as *Hydropsyche* drift less from areas with more periphyton (Kerans 1996). On the other hand, negative relationships could exist, as *Hydropsyche* can indirectly eliminate the response of algal biomass to nutrient enrichment by building retreats that alter flow conditions and leave less space for algae to reside, ultimately reducing algal growth (Pan and Lowe 1995). In consideration of the grazer-collector hypothesis (Heard and Buchanan 2004), even filter feeder abundance might be expected to be positively related to periphyton abundance at the scales of analysis considered in this study. The premise of this association would be that greater periphyton abundance could support greater grazer abundance, and thus increased production of fine particulate organic matter (a grazing by-product), which could aid the growth and/or survival of collector gatherer/filterers downstream. Such a mechanism could explain why *Brachycentrus*, of all groups, was consistently positively related to periphyton abundance (Table 3.1), even across scales of analysis. Alternatively, this result could be explained if *Brachycentrus* were relatively weak competitors who tracked resource abundance as set by other primary consumers (Roll et al. 2005).

Although there are mixed relationships between the abundance of insects and periphyton, they need not be troubling, as through space, various realizations of consumer-resource and competitive interactions could be occurring at any one time, and this could obscure the detection of positive or negative relationships between individual groups and periphyton. Of course, the simplifying assumption that periphyton Chl *a* can

represent the abundance of food resources for all groups may have been a misjudgement. A consideration of detrital abundance, for example, may have revealed significant relationships between insect variables and food resources, as in Braccia and Voshell (2006). This differentiation could be useful in future studies. It could also be beneficial to consider additional sites upstream of the RDWWTP to better understand the correlates of insect abundance in the Red Deer River in the absence of a significant nutrient point-source.

In summary, in answer to the first objective of this study, a positive correlation between total insect abundance and NO_3 concentration could indicate that effluent additions allow increased insect biomass in the Red Deer River. The lack of relation of insect abundance to periphyton abundance, on the other hand, was consistent with consumer-resource theory. For select insect groups, variable associations with NO_3 and periphyton showed that at this level, explanators of abundance differed from those of total insect abundance. These groups had stronger relationships with mean water velocity and/or substrate particle size (Table 3.1).

4.2 Stable isotope analysis

4.2.1 Trophic fractionation correction

The trophic correction values calculated for the Ephemeropteran groups (2.2-3.1 ‰, section 3.3.1) are consistent with estimates of trophic fractionation for primary consumers obtained by meta-analyses, and are thus defensible. Such estimates range from +2.2±0.30 ‰ for consumers raised on algal diets (reviewed in McCutchan et al. 2003), 2.52 ± 2.50 ‰ for aquatic herbivores (Vander Zanden and Rasmussen 2001), and +3.4 ‰ (sd= 1%) for varied consumers (Post 2002). The greater trophic correction values

calculated for the remaining larval groups (*Hydropsyche*, *Brachycentrus*, and Chironomidae) are also reasonable given the omnivorous diets of *Hydropsyche* and *Brachycentrus*, and the fact that the family Chironomidae includes members of varied feeding strategies. The dietary addition of animal parts that in themselves, have higher $\delta^{15}N$ values than periphyton, would indeed warrant higher trophic correction values, remembering that trophic corrections were calculated by comparison to periphyton rather than "true" food sources. The fact that *Hydropsyche* pupae had a fractionation correction that was greater than that of their larval counterpart is also believable and expected, because during metamorphosis, insects become increasingly enriched in ^{15}N compared to ^{14}N (Tibbets et al. 2008).

Despite the credibility of the employed trophic correction values, there is concern that the same correction would not be equally applicable throughout the entire study extent because of spatial variability in factors that affect diet-tissue fractionation. Notably, the nutrient content of periphyton varied throughout the study area (Figure 4.1), and Adams and Sterner (2000) have shown that the degree of trophic fractionation in $\delta^{15}N$ between a consumer (the microcrustacean *Daphnia magna*) and its food source (*Scenedesmus acutus*, a green algae) is highly affected by the C:N ratio in the tissues of this food source, with greater nitrogen content being associated with smaller fractionation values. This could present a concern in this study system, as the nitrogen content of periphyton at the reference site, where samples were used to calculate fractionation values, was lower than that just below effluent output. This would create a potential overestimation of the trophic correction values in the high-nitrogen areas downstream of the WWTP, which could hypothetically contribute to the low insect $\delta^{15}N$ values in that

area. However, the concern that $\delta^{15}N$ trophic fractionation could change drastically given different nitrogen contents of periphyton is somewhat lessened considering the results of a study by Jardine et al. (2005). They found that for Hydropsychidae, Brachycentridae, and Heptageniidae sampled from rivers in New Brunswick, Canada, there is essentially no relationship between the diet-tissue fractionation of $\delta^{15}N$ and the % N in their gut contents, implying little differences in physiologically-induced fractionation (0.35±0.82‰ for non-predators) as a result of the nitrogen content in food. This does not mean, however, that there couldn't be observed "fractionation" resulting from feeding discrimination, as their study suggests that these insects may select high quality foods, as their gut contents had higher %N than is typical of algae in such systems, and the %N of insect tissue was very similar to that of their gut contents. Thus spatial variation in trophic fractionation remains a potential limitation in this study, and would be worth further investigation. Also, in future studies, the consideration of gradients of isotopes with lower fractionation values (particularly $\delta^{13}C$, as in Rasmussen et al. (2009)) would provide a valuable complement to inferences made from $\delta^{15}N$ gradients.

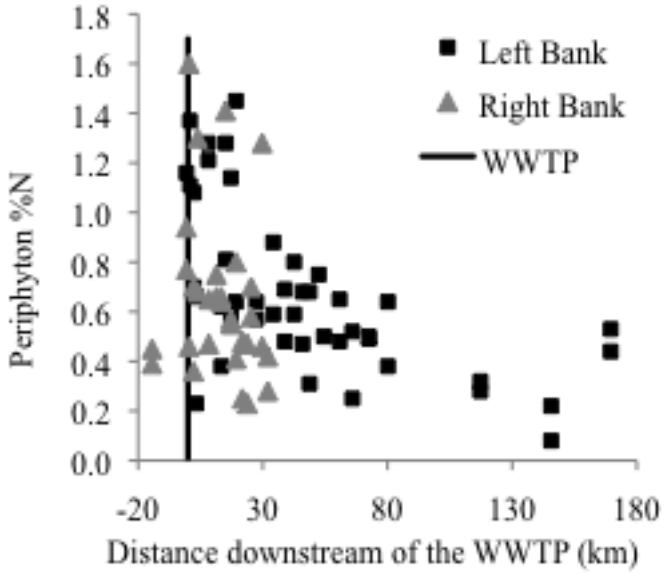

Figure 4.1: %N in samples of periphyton analysed for $\delta^{15}N$, in reference to the Red Deer Wastewater Treatment Plant (WWTP).

4.2.2 Using stable isotopes to understand downstream insect movements

Insight regarding in-stream insect movements can be had from both the general patterns of $\delta^{15}N$ signatures through space, and from the analysis of cross-correlation between the residuals of these patterns. Such insight includes an evaluation of the assumption of net downstream movement, and speculation on the extent of movement, both absolutely, and as a comparison between insect groups. The former will be discussed in section 4.2.2.1, followed by the latter in section 4.2.2.2.

4.2.2.1 Evaluating the assumption of net downstream movement

The fact that differences existed between the spatial patterns of the $\delta^{15}N$ signatures of periphyton and trophically-corrected insects (Figure 3.8 through Figure 3.10) suggests that in-stream movements of insects may be occurring, as rationalized in

sections 2.7.1.1 and 2.7.1.2. It has been assumed that the cause of these deviations is net downstream movement, which is more likely than net upstream movement in this system (section 1.3.2). As outlined in section 2.7.1.2, the observation that insect $\delta^{15}N$ signatures are lower than periphyton $\delta^{15}N$ signatures in areas downstream of the WWTP support this assumption (Figure 2.2). Across-bank movement could also explain depressed signatures downstream of the WWTP, as periphyton signatures on the right bank are similar to upstream values before effluent is fully mixed. However, substantial bank-to-bank movement is also unlikely in this system (or would be coincident with downstream movement), as Nakano and Nakamura (2008) suggest that in rivers the size of the Red Deer, invertebrates reside primarily in the shallow areas near river margins, as shear stress it too great in deeper, mid-stream areas. Thus, even if insects were to initiate across-river movement, they would likely be entrained in the drift and transported downstream (although potentially across stream if the water column was rotating). Furthermore, Bird and Hynes (1981) suggest that across-stream movement is insignificant compared to downstream movement. Thus the assumption of net downstream movement remains reasonable. This being said, there are areas in the river where across and/or upstream movement would seem to better explain local insect $\delta^{15}N$ signatures than would downstream movement (Figure 3.11).

4.2.2.2 Using stable isotope analysis to estimate the (relative) scale of downstream insect movements

The presence of differences in signature gradients between groups of insects suggests that these groups may have different movement behaviours. Furthermore, these differences can be used to categorize these groups, and/or to rank them by their relative

degree of movement in order to answer predictions made in this regard. Considering the analysis of cross-correlation between detrended stable isotope signatures of insects and periphyton, two groups of insect can be defined: those who had significant positive cross correlation at small scales (*Baetis, Tricorythodes,* and Chironomidae), and those who had no significant cross-correlation at any lag (*Heptagenia, Hydropsyche* and *Brachycentrus*). These two groups will be addressed separately.

4.2.2.2.1 Insects whose residual $\delta^{15}N$ signatures were significantly cross-correlated to periphyton residual $\delta^{15}N$ values.

In response to the third objective of this study (section 1.1), the positive cross-correlation between periphyton $\delta^{15}N$ signatures and that of Chironomidae and mid and late-instar *Baetis* and *Tricorythodes* could suggest that downstream movement at a rate of a few kilometres per month had occurred in these groups, as interpreted by the magnitude of correlation lengths (section 3.3.3). The lack of significant spatial *autocorrelation* in detrended periphyton signatures makes this interpretation robust against the possibility that cross-correlation between insect and periphyton $\delta^{15}N$ signatures was actually a result of local correlation to autocorrelated periphyton $\delta^{15}N$ signatures.

The fact that, for these groups, the highest cross-correlation occurred at a lag of 0 (Figure 3.14) additionally indicates that local residency may have been a dominant behaviour in the month before sampling. Because only one value of $\delta^{15}N$ for periphyton and for each insect group was used per site, the shape of the cross-correlograms, which indicates declining cross-correlation as distance between sites increases, may represent limited movement by insects at most sites, but, at a few sites, immigration from areas a few kilometres away. Spatial variation in movement histories could also explain why the

95% confidence intervals for the y-intercepts of the cross-correlograms are quite wide, an observation that suggests a high similarity between local insect and periphyton signatures at some sites, but not others. The implication that downstream movement may vary depending on location in the river agrees with qualitative interpretations of isotope signatures (summarized in Figure 3.11), which suggest that some local insect signatures, barring error in trophic fractionation, can best be explained by movement from elsewhere, whereas others fall within the range of local periphyton signatures, implying local residency. This is illustrated well when looking at, as an example, raw Chironomid $\delta^{15}N$ signatures (Figure 3.6). In upstream reaches, the low between-site variability of chironomid signature as compared to periphyton signatures could imply movement and feeding through these areas, and thus averaging of periphyton signatures. In contrast, in downstream reaches, the local similarity of chironomid and periphyton signatures could imply local retention of these insects.

Interestingly, the groups whose $\delta^{15}N$ signatures were significantly cross-correlated with periphyton signatures were those whose $\delta^{15}N$ signatures, as modelled by linear gradients, were most similar to that of periphyton in terms of slope and mean signature (Figure 3.13, Table 3.2). Because it was expected that downstream movement would cause a reduced slope in insect $\delta^{15}N$ gradients as compared to that of periphyton (Figure 2.2), the lack of significant difference between the slope of periphyton $\delta^{15}N$ gradients and that of *Baetis* and *Tricorythodes* suggests that little or no movement has occurred for these groups. This differs from the interpretation of cross-correlation analysis, above.

The analysis of linear gradients in isotope signatures also served to create a distinction between Chironomidae and the other groups with significant cross-correlation to periphyton signatures: the significantly reduced slope of chironomid signatures as compared to that of periphyton could suggest downstream movement. This supports the results of cross-correlation analysis that suggest that, having a longer correlation length, chironomids could be moving more than *Baetis* and *Tricorythodes*. Further differences between groups are discussed below.

4.2.2.2.1.1 Comparisons among groups

Among the groups for which significant positive cross-correlation with periphyton isotope signatures was found, predictions about which groups would have greater downstream movement (see section 1.3.2) were generally supported. Chironomids were expected to have the greatest net downstream displacement, and this expectation was fulfilled by the fact that they had a longer correlation length than *Baetis* and *Tricorythodes*. It is logical that chironomids would top this list, as they are recognized as organisms that are easily dislodged from the benthos, and are passive movers with little ability to regain the substrate once in the drift (Elliott 1971a, Brooker and Hemsworth 1978, Scullion and Sinton 1983). *Baetis* larvae, on the other hand, are strong swimmers (Campbell 1985, Otto and Sjostrom 1986, Oldmeadow et al. 2010), actively leave the drift (Otto and Sjostrom 1986, Allan and Feifarek 1989), readily cling to the substrate, have shorter drift distances than chironomids (Elliott 1971a), and likely seek refuge when flows are high to avoid dislodgement (reviewed in Brittain and Eikeland 1988). There have been far fewer studies that discuss the movement behaviour of *Tricorythodes* or closely related insects, but they may be more likely than *Baetis* to undergo upstream

movements that compensate for downstream drift (Bergey and Ward 1989), and less inclined (or less able) to undergo long distance drift through deep pools (Vinikour 1981). Such behaviour would theoretically result in less net downstream movement than *Baetis*, as was predicted. This prediction was supported by the observed correlation length for late-instar *Tricorythodes* which was shorter than that of *Baetis*, but not for mid-instar *Tricorythodes*. Nonetheless, they had similar correlation lengths. It was also interpreted that younger *Tricorythodes* move farther than more mature larvae, as predicted. This was not true for *Baetis*, which disagrees with studies that report young *Baetis* larvae to drift twice as far as mature ones (Allan and Feifarek 1989). No studies exist that make such a comparison for *Tricorythodes*.

It is difficult to critically assess whether the estimated scales of the long-term movements of chironomids, *Baetis*, and *Tricorythodes* agree with previously published values, as next to none exist. However, Hershey et al. (1993) estimated that one third to one half of *Baetis* larvae in an arctic river drift at least 2.1 km downstream over summer periods of ~30 days. This agrees very well with interpretations for *Baetis* larvae in this study. To the contrary, Finlay et al. (2002) found a very high concordance between $\delta^{13}C$ signatures of scrapers/collector-gatherers and that of local resources in a small river (R^2=0.97). They interpreted this to mean that these insects were feeding on local resources instead of isotopically different resources originating in adjacent upstream pools. Their study did not, however, address the possibility that downstream movement of insects could be occurring.

4.2.2.2.2 Insects lacking significant cross correlation of residual $\delta^{15}N$ signatures to periphyton residual $\delta^{15}N$ signatures

In the groups (*Hydropsyche*, *Brachycentrus*, and *Heptagenia*) for which there was no observation of significant cross-correlation between insect and periphyton residual $\delta^{15}N$ signatures, estimates of the scale of insect movements cannot be made. This result may indicate that these insects are moving more than those groups for which significant cross-correlations were observed, although issues of diet complicate interpretations.

For *Hydropsyche* and *Brachycentrus*, a lack of relationship to periphyton signatures could have occurred if these organisms were not directly eating periphyton. Although they are categorized as facultative scrapers (Merritt et al. 2008), these Trichopterans are considered to be omnivorous filter feeders, screening out and consuming particles suspended in the water column (Wallace and Merritt 1980, Merritt et al. 2008). Thus it is unlikely that these organisms were exclusively consuming periphyton. Furthermore, any signal representing downstream movement of these insects could just as well have represented the filtration of particles originating from upstream areas. For these groups, the use of local periphyton signatures as a proxy for local resource signatures was, admittedly, a simplification. Although seston signatures could be similar to periphyton signatures if seston represented a by-product of the local grazing of periphyton, this cannot be said for sure. Future study could address this problem by sampling seston directly above the substrate.

For *Heptagenia*, which are scrapers and facultative collector gatherers (Merritt et al. 2008), it is more surprising that a significant cross-correlation to periphyton was not observed. In this case, such a result could represent frequent movement and low residency time sufficient to average-out the variation in local periphyton signatures, thus limiting strong cross-correlation at any lag. Considerable downstream movement could

also explain the observations that *Heptagenia* $\delta^{15}N$ signatures were a) lower than those of periphyton near the WWTP, and b) similar to periphyton average signatures in downstream reaches, but with much less variation (Figure 3.9, Figure 3.13), implying spatial averaging of signatures. This supports the prediction that *Heptagenia* would have among the greatest net downstream movement of the insects studied.

The three groups lacking significant cross-correlation with periphyton $\delta^{15}N$ signatures (and Chironomidae, which had the longest correlation length) also had the lowest mean $\delta^{15}N$ signatures compared to periphyton, and the greatest reductions in slope of spatial $\delta^{15}N$ gradients (Figure 3.13, Table 3.2). This observation is consistent with the idea that a lack of significant cross-correlation could be a result of downstream movement of food or organisms. It also supports the predictions made regarding the relative movement of insects, as Chironomidae, *Heptagenia*, and *Hydropsyche* were expected to have the greatest downstream displacement. It does not, however, support the prediction that *Brachycentrus* was expected to move the least (although, again, filtration of food particles originating in upstream areas could be confounded with downstream movement here). That being said, it remains difficult to say where the insects whose signatures were not cross-correlated to periphyton signatures fit in terms of relative net downstream movement, a topic that is further discussed below.

4.2.2.2.2.1 Comparisons among groups

The suggestion that *Heptagenia* experience greater net downstream movement than other insects is very believable, given the description of lotic *Heptagenia* by Madsen (1968) as passive drifters who only regain the substrate after random contact. However, other accounts rank *Heptagenia* as among the more active swimmers of a number of

Ephemeropteran, Plecopteran, and Trichopteran species (Otto and Sjostrom 1986), and an ability to swim upstream could compensate for downstream drift.

For *Hydropsyche*, who had a very similar cross-correlogram to *Heptagenia*, and for *Brachycentrus*, who had the flattest cross-correlogram (Figure 3.14), a combination of both recent movement from other sites, and the consumption of food that originated from other sites, could explain the lack of significant cross-correlation with periphyton signatures. There is little research examining the movement behaviour of *Brachycentrus* specifically, but there is a multitude for cased caddisflies in general, which may provide a reasonable comparison given that the physical constraints of a case may carry across groups. For instance, because of the weight of their case, *Brachycentrus americanus* and other cased caddsiflies, unlike all other groups, were unable to drift through a deep, low velocity 500 m pool owing to the fact that the weight of their case caused them to rapidly sink to the substrate (Vinikour 1981). Cased caddisflies also tend not to drift as far as other groups (Elliott 1971a), and have been shown in some cases to move only a few metres to tens of metres over weeks/months (Hart and Resh 1980, Erman 1986, Jackson et al. 1999). Thus, unlike *Heptagenia*, it is doubtful that *Brachycentrus* larvae would have been moving at greater downstream rates than the groups for which significant cross-correlation with periphyton signatures was observed. However, their low signatures may not be solely attributed to the filtration and consumption of matter from upstream areas, as other studies show a pronounced downstream movement bias in cased caddisflies (Lancaster et al. 2006), and a net downstream movement of up to 1500 m in ~1 month (Neves 1979). Thus it is maintained that these isotopic patterns are probably a combined

result of downstream movement, and a diet comprised of both local periphyton scrapings, and filtration of material derived from upstream areas.

This brings us, lastly, to a discussion of *Hydropsyche*. Simply considering behaviour while in the drift, it makes sense that *Hydropsyche* would move further distances downstream than other groups (which could account for the lack of cross-correlation with periphyton $\delta^{15}N$ signatures), as they are poor swimmers (Kerans 1992) who passively exit the drift (Otto and Sjostrom 1986), causing, in one study, shorter drift distances than dead invertebrates, live chironomids, and *Rhithrogena* (Heptageniidae), but longer drift distances than *Baetis* and cased caddisflies (Elliott 1971a). They are also not expected to wholly compensate for downstream drift by upstream crawling (Bergey and Ward 1989), and have been estimated to undergo net downstream movement of ~3 km per generation (Hemsworth and Brooker 1979). This being said, despite having $\delta^{15}N$ signatures that might imply greater movement than chironomids, for example, *Hydropsyche* are unlikely to be greater downstream movers because they cling very well to substrates (*personal observation*), and being large and compact, sink faster than chironomids (Elliott 1971a). Thus in this case, as for *Brachycentrus*, the potential varied diet of *Hydropsyche* may explain why this group lacked strong cross-correlation to periphyton signatures at any lag.

The interpretations of filter feeder isotopic signatures agree in some ways, but not in others, with the findings of a somewhat similar study by Finlay et al. (2002), who used spatial variation in carbon stable isotope signatures to suggest that *Hydropsyche* filter material from adjacent upstream pools. The interpretations presented in this study support their results in the suggestion that *Hydropsyche* may filter material derived from

upstream areas. However, their study assumed *Hydropsyche* to be stationary and did not raise the possibility of movement from upstream areas, which appears likely in the Red Deer River, at least in some areas. This emphasizes that interpretations of isotope signatures can vary depending on the assumptions of the study.

4.2.3 Recommendations for future study

From these results, a number of suggestions can be made regarding the future implementation of similar studies. First of all, the use of stable isotopes to infer aquatic insect movements appears to be a promising method, but limitations such as trophic fractionation need to be better addressed. In-stream quantification of trophic fractionation, when movement is prevented using enclosures, would be very valuable, as would a more stringent consideration of insect diet, as discussed earlier. Additionally, finer scale sampling in areas with high variation in resource signatures is highly recommended. This follows the suggestion that sampling designed to capture a given process by spatial analysis should include many sites within the scale at which the process operates (Fortin and Dale 2005). In this study, the average distance between sites turned out to be similar to the estimated scale of monthly movements obtained by cross-correlation analysis, which is not ideal given the previous logic. Also, across-river transects would be valuable in order to explore the possibility of lateral movement. If such recommendations were made, there exists great potential to use the method employed in this study to further explore the movement of aquatic insects, particularly in rivers, where other approaches are difficult.

4.2.4 Summary of stable isotope analysis

Overall, the patterns in the $\delta^{15}N$ signatures of insects as compared to periphyton seem to suggest that net downstream movement on the scale of a couple of kilometres is plausible, but that net movement is variable through space, and between different groups of insects. The relative estimates of net movement agreed fairly well with predictions, and seemed logical in the context of the literature regarding small-scale movement behaviour. This interpretation differs from a meta-analysis done by Rasmussen et al. (2009) who analysed natural large-scale gradients in $\delta^{13}C$ signatures of different trophic levels, and found no indication of movement by invertebrates. Their study may have been less sensitive than the present study, however, as it pooled data from multiple studies in different rivers.

4.3 Evaluating movement as a potential mechanism structuring the large-scale spatial response of insects to environmental variability

The three analyses conducted in support of this fourth objective (section 1.1) are addressed here in sections 4.3.1 and 4.3.2. Section 4.4 also adds to this discussion. This objective can alternatively be described as an indirect assessment of whether a consideration of long-term movement is necessary to understand how insect populations and communities relate spatially to environmental variation. These analyses are also used to gauge the plausibility of the scales of movement estimated by stable isotope analysis.

4.3.1 The analysis of local abundance with independent variables averaged across sites

For mid-instar *Baetis* and *Tricorythodes* density, and total insect mass, the fact that variation in local abundance was best explained by independent variables averaged over multiple sampling sites (Table 3.1) suggests that these groups may be responding to

upstream (and downstream conditions in the case of *Tricorythodes* density, and total insect mass) in addition to local conditions. For mid-instar *Baetis* and total insect abundance, the presence of significant residual spatial autocorrelation in the local model further supports this claim by implying that when local variables were used, a mismatch existed between the scale of analysis and the scale at which these organisms were related to the chosen environmental variables. Movement through space causing averaging of numerical responses to environmental variability is one possible explanation for these results. For *Baetis*, the inferred scale of response to upstream conditions is equivalent to the distance between adjacent sites (~2.5-4 km), given the superiority of the "1 Up" model (section 3.2). This distance is consistent with the length of cross-correlation between *Baetis* and periphyton $\delta^{15}N$ signatures (section 3.3.3).

For mid-instar *Tricorythodes* and total insect abundance, the superiority of "1 Up, Down" models would, extending the above logic, point towards a scale of bidirectional - rather than specifically downstream- movement. A response to downstream conditions could be caused by upstream swimming, or potentially by adult flights, which have an upstream component (Madsen et al. 1973, Coutant 1982, Muller 1982, Hershey et al. 1993, Williams and Williams 1993, Turner and Williams 2000, Macneale et al. 2005). Indeed, unlike inferences made from stable isotopes, larvae abundance is likely to be affected by residual patterns from the spatial distribution of eggs laid by terrestrial adult insects (Lancaster et al. 2011), which have been observed to fly upstream multiple kilometres, at least in the case of *Baetis* mayflies (Hershey et al. 1993). This could help link local abundances to conditions elsewhere that affect adult abundance, and therefore egg abundance, and is consistent with the scale of movement implied in this analysis.

For the remaining groups (Chironomidae, *Heptagenia*, *Brachycentrus*, and *Hydropsyche*, the lack of one best scale of analysis (i.e. the equivalence of multiple models, Table 3.1) is difficult to interpret. Although this result doesn't suggest that local abundance is better explained by variables averaged across sites than by models using local variables, it also doesn't exclude the possibility. Thus for these groups, it is hard to say one way or the other whether this finding points to long-term movement as an important driver of large-scale patterns of abundance. Nor does this analysis offer much insight towards potential scales of movement. Interestingly, these are the same groups that exhibited very low isotope signatures near the WWTP, and whose signatures, excepting those of Chironomidae, were not significantly cross-correlated to periphyton signatures, which also precluded insight towards potential scales of movement.

There are a number of reasons why, for these latter groups, multiple equivalent models were found. It could be that these groups were responding most strongly to some other variable that was not considered, and thus, given that the variables with various scales of spatial averaging are correlated, they could explain variation in abundance equally well in the absence of more important abundance-determining variables. This explanation is likely, and unavoidable given the correlative nature of these analyses. Following the theme of this discussion, if movement is relied upon for explanation, this result could be caused by spatial variation in movement behaviour. For example, in the case of chironomids, inspection of model residuals revealed that when using local variables, abundance in far downstream reaches was better estimated than in models with variables averaged across sites. However, local analysis grossly under/overestimated abundance at certain sites within 20 km of the WWTP. For analysis at larger scales, the

opposite was true. This is consistent with the interpretations of the stable isotope data for chironomids, which may indicate substantial movement in upstream reaches, and less movement in downstream reaches (section 4.2.2.2.1).

There appear to be no studies that use a similar approach (i.e. analysis across scales) to infer how movement could affect insect *abundances* over large longitudinal extents in river main-stems. However, some comparisons can be made to studies that consider spatial autocorrelation in *assemblage composition* to infer the impact of dispersal on structuring these assemblages. Some among-stream analyses agree that variation in insect assemblages is best explained by both local environmental variables, and spatial autocorrelation with adjacent assemblages, and that the relative importance of these factors changes with the dispersal ability of the organisms (Sanderson et al. 2005, Thompson and Townsend 2006). Assemblage studies within single river stems (see section 4.4 for further discussion) tend to agree (Lloyd et al. 2005, Grenouillet et al. 2008). To the contrary, other studies find no evidence for spatial structuring of assemblages, with (spatially unstructured) local variables best explaining assemblages (Heino and Mykra 2008, Diggins and Newman 2009). Although caution must be taken in comparing single-stem studies of abundance, as in this study, to (especially among-stream) studies of insect assemblages, the results of this study agree with the results of the above studies, even in the disagreement regarding whether factors external to the sampling site affect local invertebrates. To elaborate, the implication that insects could be responding to conditions beyond the area in which they were sampled agrees with those studies that find significant spatial autocorrelation in assemblages. This being said the fact that local models were often on par with other models, and that residual spatial

autocorrelation was only found in a few local models, suggests that local conditions may be sufficient to explain patterns of abundance, at least in some groups. This is a similar inference to those studies that do not find spatial structure in assemblages.

4.3.2 Insights from large-scale abundance patterns

The absence of a downstream lag in peak total insect abundance as compared to either periphyton standing crop or NO_3 could suggest that large-scale net downstream movement was not occurring. This goes against the prediction that considerable downstream movement of insects would displace their peak abundance downstream of high productivity areas that would be expected to support high recruitment and/or survival (see section 1.3.3). It must be considered that other factors could have been at play that delegitimized the predictions made assuming such a simplistic system. For instance, the observed patterns are likely indicative of a consumer-resource interaction, with high insect abundance suppressing periphyton biomass where its productivity was expected to be the greatest. This suggests that insects may resist downstream movement in these presumably high productivity habitats, a phenomenon that has been previously reported (Kohler 1985, Hershey et al. 1993, Kerans 1996, Roll et al. 2005). With this insight, the observed pattern of total insect abundance could be broadly explained by a) reduced emigration rates in high productivity areas promoting a retention of insects in the area a few km downstream of the RDWWTP, b) emigration rates increasing as productivity decreases in downstream areas, thus spreading elevated abundances downstream, with c) added variation from a multitude of other factors such as hydraulic conditions. Such a hypothesis would be worth further investigation.

4.4 Spatial structure in insect family assemblages

Following descriptions from Legendre and Legendre (2012), the shape of the family assemblage correlograms (Figure 3.16, Figure 3.17) indicates a dominant spatial structure in these data that can best be described as a "single bump" or patch that occupies a large portion (70-80 km, as judged by the furthest extent of significant negative autocorrelation) of the study area. Such structure could be created by induced spatial dependence on spatially structured environmental factors, or by an internal process, such as movement, that creates true autocorrelation (Fortin and Dale 2005). The latter has been offered as an explanation for assemblage autocorrelation structure in other rivers (Lloyd et al. 2006). Here it is proposed the former may be at work, as a result of impacts of the RDWWTP. Thus the following exploration of this idea is in support of the second objective of this study.

The changes in shape of correlograms in response to linear detrending implies spatial dependence on external gradients. This is most evident in the right-bank correlograms (Figure 3.17), because the complete disappearance of significant spatial autocorrelation with detrending is symptomatic of such spatial dependence (Legendre and Legendre 2012). The linear trend that was removed could be reflective of a "river-continuum" style upstream-downstream gradient in assemblages. Such a gradient would be expected in this system given the gradual changes that occur in the physical characteristics of rivers from headwaters to mouth (Vannote et al. 1980). For example, the negative spatial trend in substrate particle size in the study extent (Figure 3.3) could create gradients in stability (Cobb et al. 1992) and habitat suitabilities (Fairchild and Holomuzki 2002), and thus drive trends in community structure.

The decrease in the extent of autocorrelation observed in the left-bank and all-site correlograms as a result of linear detrending also indicates dependence on external gradients. However, the fact that the general shape of these correlograms remained indicates that some factor that was not active on the right bank could be creating spatial structure on the left-bank (and this effect was still detected when all sites were included in analysis). This could indicate an effect of effluent addition from the RDWWTP, which creates large-scale humped/peaked patterns in nutrient concentrations on the left side of the river that are not present on the right (Figure 3.1). Other analyses suggest that this nutrient addition may increase insect abundance (section 4.1), in which case it would likely affect assemblage composition as well. Furthermore, the hypothetical single bump has a "range of influence", or extent to which significant negative correlation is detected in the assemblage correlograms (Legendre and Legendre 1998) that corresponds well to the impact of the WWTP as inferred from stable isotopes: periphyton $\delta^{15}N$ signatures are elevated, as compared to upstream values, for ~80 km (Figure 3.7, Figure 3.16). This strengthens the case of effluent addition as a factor affecting insect assemblages. Also, after the 60-70 km distance class, assemblages start becoming less dissimilar as the distance between sites increases. This could represent where impacts of the WWTP are lessened, and assemblages return to a state more similar to that upstream of the WWTP.

Although a number of observations give indirect support for the WWTP as a driver of spatial structure in insect assemblages, there exist inconsistencies in these interpretations. The first is that weak spatial structure in environmental variables as a whole (Figure 3.18) meant that when this spatial structure was partialled out, insect assemblage correlograms barely changed (Figure 3.16, Figure 3.17). This would seem to

counter the suggestion that assemblage spatial structure was simply reflecting environmental conditions. However, it can be argued that the use of all measured environmental variables in calculating the environmental distance matrix may have diluted the importance of individual variables, masking the effect of those (spatially structured) variables that may have strongly affected (or were strongly correlated to) assemblage composition. Analyses with targeted explanatory variables could clarify this issue. The second inconsistency is that, before detrending, the right-bank correlogram had a similar shape to the left-bank and all-site correlograms. This could imply that some other factor, independent of the effects of the WWTP, and applicable to both banks, was inducing this shape. One possible explanation for this phenomenon is that across-bank movements (by larvae or, more likely, by flying adults) could be inducing similarity in across-bank communities, and thus the effects of the WWTP could be translated across-river faster than would occur simply by passive mixing. This idea is supported by a consideration of the 0-900m distance class in the all-site correlogram (which was created specifically to assess similarity between across-bank pairs of sites). In the unaltered all-site correlogram, this distance class indicates that assemblages at across-bank sites are positively, but not significantly, related, indicating that despite being close, they are not relatively similar. As an aside, this is consistent with the idea that the WWTP contributes to across-bank differences in insect assemblages. However, when the effects of environmental differences were partialled out, these assemblages became significantly positively autocorrelated, although less-so than longitudinally close sites on the same bank (Figure 3.16). This indicates that the assemblages are more similar than their environmental characteristics would dictate, which could suggest that movement is

promoting greater left and right-bank similarity, thus accounting for the similar, but weaker spatial structure in the right-bank correlogram as compared to the others. This is an interesting finding because the possibility of lateral movement, particularly in rivers, is rarely addressed in the literature, as most studies focus on small systems, in which lateral movement would not be unexpected.

Internal processes could also create the patterns observed in assemblage correlograms. For instance, Lloyd et al. (2006) suggest positive correlation in assemblages separated by 0-6 km could be explained by upstream flights and downstream larval movements. The reverse logic could imply that autocorrelation structures could be used to infer movement scales, catering to one of the objectives of this study. However, movement as an explanation for autocorrelation structure is lacking here because a) one would not necessarily expect movement to differ greatly between banks, as suggested by the lack of autocorrelation in detrended right-bank data, and b) the degree of movement necessary to create, by itself, positive autocorrelation up 30 km (considering the left-bank correlogram) would be unlikely for either downstream drifting larvae (section 4.2.2) or adults flying upstream (Hershey et al. 1993, Macneale et al. 2005). Also, if movement on the scale of a few kilometres was creating spatial concordance in assemblages as proposed in Lloyd et al. (2006), small scale autocorrelation in detrended right-bank data should have been present, yet it was not.

In addition to dependence on spatially structured environmental variables, the shape of the Mantel correlograms suggest that assemblages have a patchy spatial structure, as revealed by relatively fine-scale fluctuations in r_M. This could represent random noise, but the presence of patchiness, which can cause nearby locations to have

less similar assemblages than more distance sites with more similar patch characteristics (Legendre and Legendre 2012), would not be an unexpected finding. This is because rivers are notoriously patchy systems, being composed of varying sequences of habitats such as rapids, riffles, runs, and pools (Malmqvist 2002), and at least two of these patch types (riffles and runs) were sampled. Other studies have found similar spatial structure in benthic macroinvertebrate assemblages in lotic systems. Grenouillet et al. (2008) found significant positive spatial autocorrelation to be present in (non-detrended) assemblages separated by small distances (0-8 km), with r_M declining to negative values beyond 38 km (and negative autocorrelation being significant in the 38-54 km and 69-84 km classes). They described this structure as representing patchy assemblages, but as proposed here, it could represent patchiness superimposed upon a larger-scale assemblage gradient, which they did find for fish and diatom assemblages. Their study suggested a much smaller scale of positive autocorrelation than observed here, but the shape of autocorrelation remained somewhat similar. In their study they also attempted to define whether environmental characteristics were creating spatial structuring along the spatial gradient. Unlike this study, they found that environmental distances had a very similar spatial structure to that of invertebrate assemblages, however, using partial Mantel tests (rather than partial Mantel correlograms, as in this study), they found no relationship between invertebrate assemblages and distance between sites (i.e. no spatial autocorrelation in assemblages) when the effect of environmental differences were partialled out. In this study, and in one river in a similar study by Lloyd et al. (2005), significant spatial structure remained after partialling out environmental distances. This appears to suggest that in the study system of Grenouillet et al. (2008), local

environmental variables determined assemblages. However, in their study, invertebrate assemblage dissimilarities were not in fact related to environmental distances when geographic distances were partialled out. They were, however related to fish assemblage dissimilarity, which itself was spatially structured, even with environmental distances partialled out, implying that fish assemblage structure could contribute to spatial structure in invertebrate assemblages. A consideration of fish assemblage spatial structure in the Red Deer River would be useful, because fish assemblages tend to be autocorrelated at scales that could induce the broad-scale autocorrelation in insect assemblages observed in the Red Deer River (Wilkinson and Edds 2001, Grenouillet et al. 2008).

In conclusion, Mantel correlograms suggest that large-scale spatial structure exists in insect assemblages. It is proposed that this autocorrelation structure is driven by nutrient enrichment from the RDWWTP. There are also indications of patchy distributions superimposed upon this broad-scale assemblage trend. Little insight into movement scales can be gained from this analysis because of the dominant broad-scale trend present in assemblages, but the detrended right-bank correlograms, in which significant autocorrelation was not detected at any lag, do not lend support for the idea that movement could be occurring on the scale of a few kilometres, creating spatial concordance in assemblages. To remove large scale trends, and thus bring attention to any smaller-scale autocorrelation caused by internal processes such as movement, a nonlinear detrending method may have been more effective. Additionally, because different spatial autocorrelation patterns are revealed depending on sampling resolution and extent (Cooper et al. 1997), it is suspected that finer sampling in a smaller spatial

extent might allow better detection of movement occurring on the scale of a few kilometres.

4.5 General conclusions

This study represents one of only a handful of those that report quantitative longitudinal patterns in abiotic and biotic variables downstream of effluent inputs from a WWTP. It appears that in the Red Deer River this input drives changes that propagate tens of kilometres downstream. Importantly, this study provides strong support for the idea that the RDWWTP is a significant driver of insect distribution and abundance in the Red Deer River above and beyond the effect of hydrological/habitat variables. This was substantiated by the frequent retention of nitrate concentration in empirical models explaining the total abundance of insects, and that of select insect groups. The hypothesized mechanism for the WWTP's influence -nutrient enrichment that increases primary and consequently secondary productivity -was also largely supported by this result.

Analysis of residual cross-correlation between insect and periphyton $\delta^{15}N$ signatures suggested that movement on the scale of ~1-5 km per ~ 1 month could be occurring for *Baetis*, *Tricorythodes*, and Chironomidae, but was inconclusive for *Heptagenia*, *Hydropsyche*, and *Brachycentrus*. For these three groups it was proposed that they had either undergone considerable movement sufficient to blur correlations between insect and periphyton $\delta^{15}N$ signatures at any lag, or that the use of periphyton signature as a proxy for resource signature was inaccurate. This latter suggestion applied specifically to *Hydropsyche* and *Brachycentrus*, who were likely filtering out and

consuming material derived from upstream areas. Considerable deviation from periphyton $\delta^{15}N$ signatures within 20-30 km of the WWTP seemed to support these propositions. Despite these mixed results, and issues regarding correcting for trophic fractionation, it is maintained that the approach used to estimate long-term insect movements is promising. However, this method could be improved with a consideration of separate resource pools (i.e. benthic detritus versus benthic periphyton versus suspended resources etc.), and finer-scale, perhaps two-dimensional, sampling. It is suspected for all groups that the scale of downstream movement is not consistent through space, an inference that is also worth further investigation.

Despite stable isotope support for the hypothesis that large-scale in-stream insect movements occur in the Red Deer River, other analyses and observations did not entirely confirm these interpretations. Thus it still remains unclear whether a consideration of movement is necessary to adequately explain broad-scale spatial patterns. For instance, in empirical models of benthic abundance, analyses considering environmental conditions in areas larger than that of the local site did not always do a better job of explaining benthic abundance than local scale analyses. That being said, in no group was the local model better than all others in explaining abundance. This could support the case for movement on the scale of a few of kilometres by implying that local abundance is affected by upstream (and) downstream conditions, but it is at best indirect support. Analysis of spatial structure in insect families also did not indicate movement as a potential mechanism for structuring insect distribution and abundance spatially. Instead, Mantel correlograms indicated that large-scale gradients in assemblages were present, likely driven by environmental factors. It was proposed that broad-scale spatial patterns in

nutrient concentrations driven by WWTP effluent could be inducing the observed spatial structure in insect assemblages. Finally, there was no observation that peak insect abundance lagged downstream of peak periphyton abundance, as would be theoretically predicted if significant net downstream movement of insects was occurring. The opposite was, in fact, observed, possibly because of a consumer-resource interaction.

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APPENDIX A: DATASETS

Table A.1: Comments regarding datasets used in statistical analyses, with reasoning for modifications to the raw data. Cross links to the relevant methods sections are present.

Analysis (corresponding section in methods)	Dataset comments
2.5.2 Statistical Procedures (for generalized linear models of abundance using local independent variables)	1) Right-bank sites within 20 km of the WWTP and sites above the WWTP were excluded. 2) Mean of variable at each site was used
Reasoning 1) To simplify the analysis by focusing on the area directly impacted by effluent 2) I.e. the averages of variables across the three replicate samples per site (or the sum in the case of counts of insects) was used instead of the raw values, as interest lay in between-site rather than within-site variation, and only one value of NO_3 concentration per site existed.	
2.6 Analysis of environmental explanators of abundance across scales	1) Although not included in local analyses, the site directly upstream of the WWTP was included in computing the variables for the "1 Up" models, and both sites upstream of the WWTP were included in the "2 Up" models 2) For the "1 Down" and "1 Up, Down" models, values at the last downstream site were left as were
Reasoning 1) No reasoning necessary. Note: The absence of more than two sites upstream of the WWTP precluded considering greater lags. 2) In these reaches there was less spatial variation in environmental conditions.	
2.7.2 Statistical Procedures (for stable isotope analysis)	1) Mean signatures of each group (insects or periphyton) at each site were used.
Reasoning A) Different groups had different numbers of samples per site (and different numbers of individuals per sample), thus using means made sample sizes per group, and thus power to detect significant relationships, more similar (although some groups were not found at all sites, so this was not entirely effective) B) Reasonable evidence exists for short-term movement on the scale that would be considered "within-site" in this study, i.e. a few tens of metres (Waters 1965, Larkin and Mckone 1985, Elliott 2003), thus averaging values at a site acted as a guard against potential pseudoreplication caused by having multiple samples per site. This promoted an analytical focus on movement between, rather than within, sites. C) Taking site averages served to reduce the presence of outliers.	

Continued on next page.

Analysis (corresponding section in methods)	Dataset comments
2.7.2.1 Assessing differences between linear trends in insect and periphyton $\delta^{15}N$ signatures	1) Right-bank sites within 20 km of the WWTP and sites above the WWTP were excluded. 2) The last three sites were excluded.
Reasoning 1) To simplify analysis by focusing on the area directly impacted by effluent 2) Because most insect groups were not present in large enough quantities for stable isotope analysis.	

APPENDIX B: FURTHER DETAILS REGARDING GENERALIZED LINEAR MIXED MODELS WITH SPATIAL VARIANCE COVARIANCE MATRICES

See corresponding section 2.6.2. For models with a spatial variance covariance matrix, a nugget effect was not included, as the 95% confidence intervals for semivariance in the smallest lag always ranged close to zero. Semivariogram inspection was also used to place upper bounds on covariance parameter estimates, which was often necessary to ensure model convergence (Littell et al. 2006). Model convergence was additionally aided by restricting scale parameter estimates to a maximum value of that estimated in the analyses preceding the addition of spatial variance-covariance structures, which did not overly affect estimates of the range parameter.

APPENDIX C: MANTEL CORRELOGRAMS AND PARTIAL MANTEL CORRELOGRAMS-FORMULATION AND INTERPRETATION

The computation of Mantel correlograms was first proposed by Sokal (1986) and Oden and Sokal (1986). As described in Legendre and Legendre (1998), Mantel correlograms can be used to assess the spatial autocorrelation of multivariate data, such as community compositions, by first condensing these data into a matrix representing the normalized (dis)similarities or "distances" between each pair of sites, and then cross correlating this matrix to a series of corresponding matrices, each representing whether pairs of sites are part of a discrete geographic-distance-between-sites class, *d*, (given a value of 1 in the matrix) or not (given a value of zero). The result is a correlogram comprising a series of normalized Mantel statistics (r_{Md}), which can be interpreted in a similar manner to Pearson's correlation coefficient, plotted against the different distance classes. Each r_{Md} is tested by randomly permutating the rows of one matrix and recalculating the (dis)similarity or distance matrix and subsequent r_{Md} thousands of times to obtain a distribution for the test statistic under the condition of no spatial structure. Thus no assumption is made about the shape of the distribution of the test statistic. If r_{Md} falls within the upper or lower tail of this distribution, as designated by the type I error rate (α) of the test for a given distance class, the test it is considered significant. The exact p-value or α that defines the cut-off for significance depends on what method, if any, is used to correct for the fact that multiple tests using the same data are undertaken to produce the correlogram, which inflates the chance of a type I error.

It should be noted the computation of Mantel statistics for a correlogram differs from that of a classic (normalized) Mantel statistic (Mantel 1967) in that for the classic statistic, the

(standardized) (dis)similarity matrix is cross-correlated to the *actual values* of (standardized) distances between sites, summarized in one matrix, as opposed to the series of distance classes described above. As follows, the interpretation of the Mantel statistic produced also differs. In a classic Mantel test using a *dissimilarity* matrix and quantitative distances, a positive value means dissimilarity increases as distance between sites increases, which would be expected. A negative value means dissimilarity decreases as distance between sites increases (Lloyd et al. 2006). In the case of a correlogram, a positive r_{Md} indicates that positive autocorrelation exists in that distance class, i.e. assemblages separated by that distance are more similar than pairs not in that distance class, as compared to the randomly permutated dataset. A significant negative r_{Md} means communities separated by that distance are less similar than those separated by other distances, as compared to the randomly permutated dataset (Borcard et al. 2011). The sign of the statistic does not provide information regarding how (dis)similarity changes as a function of separation distance, but this information could be gleaned from the shape of the correlogram. Additionally, The correlogram is useful in that it enables the determination of the *scale* (corresponding to the distance class) at which assemblages are autocorrelated, whereas a classic Mantel statistic, using all of the data within a spatial extent, will produce just one value, describing the linear relationship between (dis)similarity or distance between communities at two sites, and the geographic distance between them.

Bonferroni Correction

The Bonferroni correction uses a significance level of α/d for each r_{Md} statistic calculated, with d=1 being the smallest distance class, d=2 being the second smallest, etc. This correction lends greater allowance to the detection of autocorrelation for close sites, where it is assumed to be greatest. It becomes increasingly stringent for subsequent distance classes. It is appropriate for

this study, as there is greater interest in understanding autocorrelation in sites that are close together, and it does not overly penalize the dataset for having a large spatial extent, with a large number of potential lags.