AGRICULTURAL LAND USE IMPACTS ON COOL-SPRING FLORA AND FAUNA, WITH AN EMPHASIS ON FRESHWATER INVERTEBRATE DIVERSITY AND PHENOLOGY IN SPRING POOLS OF EASTERN PRINCE EDWARD ISLAND (CANADA)

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Abstract

Freshwater springs are focused discharge points from groundwater to surface water environments. Cool springs have consistent temperatures close to the mean annual temperature for the region and chemical composition that can vary with land use and local geology. Animal taxa inhabiting these springs must be able to tolerate nearly constant cool temperatures (<10^{o}C), so springs usually have lower numbers of species than reported in nearby surface waters. Agricultural activities adjacent to springs add nutrients to groundwater, and alter benthic sediment structure and adjacent riparian areas, all factors that affect populations of freshwater plants and invertebrates. High nutrients should increase invertebrate abundance, but habitat alterations such as sediment addition may depress abundance. Increased food availability can also affect growth and life history patterns such as insect emergence timing, which can be disrupted in cool springs due to lack of temperature cues to synchronize development. Agricultural impacts on springs were examined by comparing water quality and invertebrate community structure in rheo-limnocrene springs in forested and agricultural areas in Prince Edward Island, Canada. Twenty springs (10 surrounded by agricultural land and 10 forested) were monitored for water quality and nine of these (five agricultural and 4 forested) were further examined to explore invertebrate and aquatic plant patterns. Agricultural sites had open canopies, high nitrogen and sulphur levels, high amounts of fine sediment, and plant cover dominated by vascular plants. Forested sites had closed canopies, low nutrient levels, clean gravel substrates, and plant cover dominated by bryophytes. Invertebrate diversity and abundance were highest in forested springs and community structure differed between land-use types. Midges (Chironomidae) dominated the macroinvertebrate community in all sites, but several midge and mite (Hydrachnidiae) genera

were most abundant in forested sites. Mayflies (Ephemeroptera) were very rare in the springs, but stoneflies (Plecoptera) and caddisflies (Trichoptera) were most abundant and diverse in agricultural sites. Emergence timing was compared between agricultural and forested sites for the stoneflies, and although most showed the asynchronous emergence periods expected for constant temperature sites, at least two species began to emerge earlier in agricultural sites than forested ones. Reduction of the riparian canopy leading to increased light levels from open cover was a better predictor of plant and invertebrate species assemblages than either nutrients or sediment patterns in agriculturally impacted springs; the higher light levels increased the presence and cover of vascular vegetation which altered the overall invertebrate community.

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Chapter 1

Introduction and Literature Review

"Let the student be taught to collect and identify each object for himself. Thus he will form an intimate acquaintance with nature in all her beauty and richness, and learn the lofty art of investigating her secrets for himself".

Bain 1890: The Natural History of Prince Edward Island.

1.1 Introduction

Freshwater springs can be considered natural controlled-temperature laboratories since many have constant water temperatures (Odum 1957; Williams and Williams 1998). Further, they are ideal for examining anthropogenic land use influences on groundwater quality because they are fed predominantly by groundwater (van der Kamp 1995; Williams and Williams 1998). Since springs are the transition from groundwater to surface water habitats (Barquin and Scarsbrook 2008; Cantonati et al. 2012), they are also influenced by leaf litter and erosion from the terrestrial zone (Naiman et al. 2005; Barquin and Scarsbrook 2008). This combination of influences from many different biotic realms makes the influences on springs dynamic. Local temperatures and geology determine the thermal state and the dissolved ion composition in spring water (van Everdingen 1991). Springs with water temperature similar to the average air temperature of a region are called "cool springs" (these may also be referred to as ambient springs), whereas those that are warmer than the mean annual temperature are thermal springs (van Everdingen 1991) and include "hot springs" (Cantonati et al. 2012). Springs may be dominated by one or two ions ("mineral springs", e.g., certain salts or sulfur), or lack a dominating ion ("non-mineral springs"; Cantonati et al. 2012). Chemistry of non-mineral springs varies with geologic and anthropogenic influences (van der Kamp 1995; Barquin and Scarsbrook 2008; White 2010).

Agricultural activity alters natural landscapes and inputs into freshwater ecosystems in a variety of manners. Forest clearing in naturally forested landscapes reduces forest cover in the watershed and riparian areas (Vought et al. 1995; Naiman et al. 2005; Dunn et al. 2011). Alterations in streamside vegetation change the volume and type of litter entering a waterway, and may increase solar radiation on aquatic habitats if cover is lost (Naiman et al.

2005; Burrell et al. 2014; Mebane et al. 2014). Excess nutrients from fertilizer and manure leach into groundwater and or may run into surface water systems (Barquin and Scarsbrook 2008), increasing primary production and impacting native flora and fauna in many freshwater habitats (e.g., Griffith et al. 2009; Burrell et al. 2014; Mebane et al. 2014). In addition, erosion following loss of riparian cover and tillage from crop production can increase the amount of sediment entering aquatic systems from overland flow (Cairns 2002; Naiman et al. 2005; Griffith et al. 2009). These effects, in isolation or in combination, alter the plant and animal communities in aquatic habitats, often by reducing abundance or diversity, or altering the community of organisms (e.g., Griffith et al. 2009; Mebane et al. 2014).

The flora and fauna of temperate zone cool springs are unique due to the constant temperature conditions (Cantonati et al. 2012) which are cooler in summer and warmer in winter than most nearby flowing water habitats. Since many species need warmer summer temperatures than are found in cool-springs to complete life cycle development, the number of species adapted to such springs is limited. The unique fauna and threat of human disturbance makes springs "hot spots" for biodiversity (*sensu* Cantonati et al. 2006), with assemblages of many otherwise rarely seen species (Spitale 2012). Constant temperatures also remove temperature cues for life cycle synchronization, so spring organisms such as aquatic insects, either use other cues (e.g. photoperiod: Hynes 1970; Bottová et al. 2013) or show asynchronous life cycles (Dobrin and Giberson 2003). Food availability also influences growth and life cycle timing of organisms in springs (Williams 1991). Knowledge about the influence of anthropogenic activities on spring dwelling organisms is limited (e.g., Williams et al. 1997; Keleher and Rader 2008; Lencioni et al. 2012). Thus information is needed on

the effect of agricultural activities on the restricted set of organisms in this groundwater dependent habitat.

Springs are numerous on PEI (Somers et al. 1999; Dobrin and Giberson 2003), which makes them ideal sites to study the ecology of organisms living at the groundwater- surface water interface (Williams and Williams 1998). In addition, all rivers on the Island are spring-fed, with large areas affected by agricultural inputs (Cairns 2002; Klassen and Locke 2010). Prince Edward Island's generally uniform geology (van der Poll 1983), and potential for local influences on groundwater (Jiang and Somers 2009) provides an area that allows for researchers to test questions of agricultural land influences on spring habitats.

1.2 Study Objectives

Three major questions are addressed in this thesis:

1. How is surrounding agriculture affecting the water quality, vegetation, invertebrate habitat and biodiversity in Eastern PEI pool springs?
2. What are the species of Ephemeroptera, Plecoptera, and Trichoptera (EPT) in PEI springs and how do constant temperatures and habitat variation affect their populations and phenology?
3. What other arthropods and other invertebrates inhabit PEI Rheo-Limnocrene springs?

1.3 Literature Review

Springs are sites where groundwater erupts to the surface. This is an innocuous description of a habitat with many interesting properties. Freshwater springs are unique habitats within the traditional groupings of freshwater biomes. They may be considered lotic in that they are flowing, lentic if they form a pool, a wetland if they have a diffuse origin, or a combination of all depending on how they emerge from the ground. Some are even temporary, depending on the stability of the groundwater source (Williams 2006). Because of this unique and hybrid nature of aquatic ecology, the study of springs forms a sub-discipline of limnology known as crenobiology (*sensu* Botosaneanu 1998). Until recently, very little attention has been given to the ecology of springs (Cantonati et al. 2012), despite early ground-breaking studies highlighting their unique ecology (Odum 1957), or their long historical use by humans (See section 1.3.1). Major textbooks on freshwater ecology have given springs little space, for example Giller and Malmqvisit (1998) and Mitsch and Gosselink (2007) have one page or less each on springs. Older textbooks give springs a bit more attention with Hynes (1970) devoting four pages to the habitat. Many articles on springs are organized into journal special issues or books of collected papers, and include Danks and Williams (1991), Ferrington (1995), Botosaneau (1998), and Cantonati et al. (2007, 2011, 2012). No textbook on the ecology of springs exists, but an excellent text on the hydrogeology of springs worldwide synthesizes the dynamics of the groundwater-surface water transition (Kresic and Stevanovic 2010).

1.3.1 Brief history of human use of springs

The ancient Greeks believed springs to be the home of water nymphs; mystical spirits of nature that could turn into beautiful young women. In one such myth, Hylas, the companion of Hercules, was reported to be abducted by these "water nymphs" (Apollonius Rhodius cited from Seaton 1912). Springs have long held an aura of mysticism and there is a long history of people making offerings into springs. In one example, excavation of a Roman spring (when digging a new well) yielded coins dating to 2000 B.C., and deeper yet were older arrowheads (Chapelle 2005). This folk practice is still performed today with the habit of throwing coins into fountains. The first recorded mention of springs in a natural history setting was by Pliny the elder: "*Tales sunt aquae quales terrae per quas fluunt*", which can be roughly translated as: "The place where quality water flows from" (Cantonati al. 2006). This statement highlights another important historical use of springs, which is as an important source of drinking water. The consistent water supply provided by springs has influenced where towns and cities were situated, including well-known cities like Las Vegas (Chapelle 2005), and also influenced traveling routes and settlement patterns (e.g., PEI springs; Giberson et al. 2013). In medieval Croatia, a person caught contaminating the local spring would be punished by losing a hand (Kresic 2010a), a punishment that might make present day polluters take notice before contaminating local groundwater, if it were still in effect.

1.3.2 Springs hydrogeology

Springs are focused points of water discharge from groundwater sources and often possess unique physical and biological characteristics (van der Kamp 1995). Springs form

where the water table meets the land surface or through fractures in rock layers through which water under pressure is forced to the surface (van der Kamp 1995; Kresic 2010b). Many spring outflows show consistent temperatures with low variation and chemical regimes over time, due to the way they flow to the surface (van der Kamp 1995). Groundwater maintains a temperature approximately equal to the average regional air temperature (if no other factors are influencing temperature, e.g., volcanic activity) and the groundwater chemistry depends on a combination of local geology, topography, and land use (van Everdingen 1991; van der Kamp 1995; Cantonati et al. 2012). Depending on the topography, water discharging into springs can be a mix of older groundwater with high residence time in the aquifer, and more recently formed groundwater, which has spent little time in the deeper aquifer (van Everdingen 1991). Both temperature and water volume can fluctuate seasonally if the spring is fed by very recent sub-surface flow rather than the deeper groundwater (van der Kamp 1995; Hogg and Williams 1996; Kresic and Bonacci 2010). More recent subsurface flow is tied directly to recent rainfall, snowmelt, and also anthropogenic water abstraction, so discharge at a given spring can vary seasonally (Kresic and Bonacci 2010). This is most dramatic in temporary springs that they only flow for part of the year. Thermal springs" exceed the average air temperature of a region by >5^{o}C (with "hot springs" usually exceeding 37 - 39°C), and are generally fed by water influenced by geothermal heating or volcanic activity (van Everdingen 1991; Cantonati et al. 2012). "Cool springs" have temperatures approximating the mean annual temperature for the region, and the term "cold springs" should be reserved for springs that are below the average air temperature for a region (Cantonati et al. 2012). The temperatures of many cool springs are stable through the

year, with little fluctuation from the regional average temperature (Williams and Williams 1998; Cantonati et al. 2012).

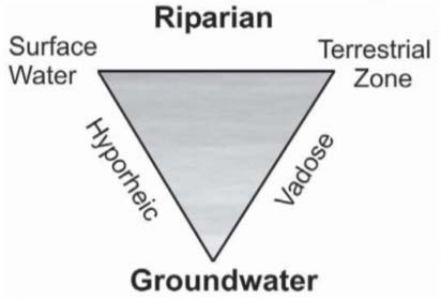

Fig. 1.1. Schematic showing the transitional nature of springs (adapted from Barquin and Scarsbrook 2008). 'Riparian' refers to the vegetated area around the spring outflow; 'Hyporheic' refers to the water flow within the substrates under and near the stream, and "Vadose" refers to the unsaturated soil zone, between the water table and the surface.

Local geology is critical to both the formation of springs and to the types of springs that will be found in an area. Much of the biological literature has focused on springs and springbrooks in aquifers with karst geology (e.g., Smith et al. 2003; Dumnicka et al. 2007; Wojtal and Sobczyk 2012). Karstic areas form in regions with soluble rock formations such as limestone or gypsum (Kresic 2010b; White 2010). Water flows underground in channels and reaches the surface in springs (Kresic 2010b). Underground water flow in sandstone aquifers (such as those on Prince Edward Island (PEI); van der Poll 1983) can also flow through channels, formed from breaks in the rock surface, however, water can also move through permeable sandstone (Jiang and Somers 2009; White 2010). Springs in sandstone aquifers are less common than karstic springs, tend to have smaller fractures, and be more stable in terms of flow (van der Kamp 1995).

Spring water chemistry relates to the chemical composition of the rock through which it flows, the residence time in the aquifer, and local land use (van Everdingen 1991; White 2010). Mineral springs are springs that have high concentrations of at least one ion, and can be hot, cool, or cold (Cantonati et al. 2012). Examples of mineral springs can include iron springs (Guasch et al. 2012), salt springs (Ring 1991), carbonate springs (White 2010) and sulfur springs (Pritchard 1991). Without a dominate ion, water chemistry can vary temporally and regionally, and will be influenced by local geologic and anthropogenic influences (van der Kamp 1995; Barquin and Scarsbrook 2008; White 2010). Many sandstones are composed of predominantly low-solubility silicate minerals, so should contain fewer dissolved compounds than highly soluble rocks such as limestone (White 2010). The presence and form of many ions are also influenced by dissolved oxygen, which varies between the groundwater and the surface water. Groundwater may be anoxic from oxidation processes (e.g. microbial breakdown of organic material in soil), but it mixes rapidly with atmospheric oxygen at the surface, leaving spring habitats with relatively high oxygen levels (van der Kamp 1995; Koperski et al. 2011). Nutrients such as nitrogen and phosphorous are usually present at low concentrations in most groundwater systems, but these may vary depending on the composition of the sub-surface rock and anthropogenic inputs into recharging water (van der Kamp 1995; Jiang and Somers 2009). Many groundwater sources and springs show elevated nutrients from anthropogenic sources (e.g., Williams et al. 1997; Lencioni et al. 2012), including Prince Edward Island (Priddle et al. 1989; Jiang and Somers 2009).

1.3.3 The Prince Edward Island landscape

Prince Edward Island has an area of 5685 km^2 and is located in the Gulf of St. Lawrence on the east coast of Canada (Fig. 2.1). The Island geology is predominantly permo-carboniferous sandstone overlain by quaternary glacial deposits (van de Poll 1983). Soil on the Island is primarily made up of highly erodible sandy-loam podzols underlain by fractured sandstone which is permeable to groundwater flow (MacDougall et al. 1988, Jiang and Somers 2009). Groundwater discharge into springs forms the base flow of all the lotic systems on PEI (Klassen and Locke 2010).

The landscape and watersheds of PEI have developed since the last glacial retreat (Miller 2010). Prince Edward Island became ice free ~10,000 years ago, and became an island ~5,000 years ago (Miller 2010). Pre-settlement vegetation was Acadian Forest, which included the following species: red spruce (*Picea rubens* Sarg.), white pine (*Pinus strobus* L.) eastern hemlock (*Tsuga canadensis* (L.)) American beech (*Fagus grandifolia* Ehrh.) yellow birch (*Betula alleghaniensis* Britt.) and sugar maple (*Acer saccharum* Marsh.) (Loo et al. 2010). Currently, nearly half of Prince Edward Island is under cultivation, with a combination of row crops (mainly potato), and pasture crops (Jiang and Somers 2009; PEI Department of Agriculture and Forestry 2013). Agricultural crops, especially potatoes, are the primary source of anthropogenic nutrient inputs (particularly nitrogen) into PEI waterways (Jiang and Somers 2009). Prince Edward Island has an undulating plain topography, with a maximum elevation of 139 m above sea level (MacDougall et al. 1988). The sandy soil and high agricultural intensities lead to high sedimentation rates into PEI waterways after heavy rains or rapid snowmelt, causing issues for freshwater life (Cairns 2002; Curry and MacNeill 2004). Recharge into PEI aquifers is dependent on precipitation

(Jiang and Somers 2009) and PEI receives an average of 890 mm of precipitation each year (PEI Government 2010).

1.3.4. The springs as aquatic habitat

Springs can be classified into three major types based on the habitat type into which they discharge. Springs that flow rapidly outward from hillsides are classified as Rheocrene (river-like), those which form a basin are classified as Limnocrene (pool-like), and those that seep into marshy areas are called Heleocrene (Danks and Williams 1991). These classification types may also overlap and may be present in a combination of forms, as outlined in Table 1.1. However, definition of what constitutes a spring habitat has been inconsistent in the literature, with many treating the spring source (springhead or "eucrenal zone") and the adjacent brook (springbrook or "hypocrenal zone") as one complete unit, and "spring" sampling locations in many references have been poorly described (Cantonati et al. 2006; von Fumetti et al. 2007). For example, inconsistencies in the definition of spring habitats can be seen in a recent technical manual for watershed groups on PEI (Harris et al. 2012). More precise definitions are based on thermal or spatial separation, with a change in temperature of more than 2^{o}C defining the difference between the springhead and springbrook, or cutting off the eucrenal zone at 5 m from the spring source (von Fumetti et al. 2007). Defining the habitat zones is especially important in macroinvertebrate studies, because different taxa will be found in the springhead than in the stream below (Williams and Hogg 1988; von Fumetti et al. 2007). The main invertebrate distribution pattern results from specialized habitat demands (especially tolerance to spring temperature patterns) and

limited dispersal of spring fauna, resulting in lower diversity in the spring source when compared to downstream reaches (Butler and Hobbs 1982; Barquin and Death 2011).

Table 1.1 Summary of some spring typology terminology, based on Danks and Williams (1991) and Gathmann and Williams (2006).

Spring Type	Description
Rheocrene	Discharges as a stream right at spring source
Limnocrene	Forms a large (pond or lake sized) pool at the spring source often with a springbrook as an outlet.
Rheo-limnocrene	Forms a small pool which then flows directly as a brook below the pool
Helocrene	Seepage area out of ground which forms a marshy area
Rheohelocrene	Seeping out of ground and coalescing to form- a brook afterward

Because many workers do not differentiate between types of springs, it can be difficult to compare results from different studies. For example, a recent Environmental Impact Assessment on PEI (Bonshaw Road Re-alignment; Stantec 2012) grouped springs and seeps into a single habitat type, but listed vegetation (ferns, sedges and goldenrod) suggesting a diffuse seepage area or a helocrene spring (Stantec 2012; K.M. Knysh, Personal Observation). Their results cannot be directly compared to studies in limnocrene springs since different types of springs support different plant and animal assemblages (e.g., Spitale et al. 2012). Clear habitat definitions are needed when studying springs.

1.3.5. Agricultural land use and springs

Surrounding land use affects aquatic systems through the chemicals (e.g., fertilizers and pesticides) and matter (e.g., sediment) transported in surface runoff or leaching to groundwater (Barton 1996; Williams et al. 1997; Probst et al. 2005). Disturbed lands such as agricultural fields have less vegetation to stabilize upper soil layers and absorb water when compared to forested sites (Vought et al. 1995; Dunn et al. 2011). The highest risk of runoff to surface waters occurs during high rainfall events (Liess et al. 1999), but since springs receive most of their recharge from groundwater rather than direct rainfall and have relatively small drainage areas, direct effects of runoff have relatively little effect. In contrast, springs are mainly affected by inputs to groundwater (van der Kamp 1995; Cantonati et al. 2006). Soluble nutrients like nitrates leach readily and are often higher in groundwater outflows than in surface water (Cantonati et al. 2006). However, transport of nutrients between groundwater and surface waters has been poorly studied (van der Kamp 1995). An early study on nitrate and pesticide leaching to groundwater from a Prince Edward Island potato field was one of the few studies directly attributing ground water sources to nitrate contamination in a spring (Priddle et al. 1989; van der Kamp 1995). Nutrient inputs affect surface water habitats by changing the algal and macrophyte communities, but though this is well documented in streams, lakes, and estuaries (e.g., Schindler et al. 2008; Schein et al. 2012, Mebane et al. 2014), less is known on the effects of nutrients in springs. Primary producer cover and type should also structure invertebrate communities in springs, so potential changes in macrophytes and attached algae should affect spring dwelling invertebrates (Barquin and Death 2004; Cantonati et al. 2006; Virtanen et al. 2009).

Very few studies have focused on the anthropogenic impacts on springs aside from conservation for direct water use (Cantonati et al. 2012). In Canada, examples of land-use effects on spring fauna are limited to urban-agricultural gradients in southern Ontario, and road salt contamination in springs in southern Ontario (Williams et al. 1997; Williams et al.1999). Outside of Canada, benthic invertebrate biomonitoring metrics have been used to assess springs affected by livestock trampling in Utah, USA (Keleher and Rader 2008) and water quality and land use in or around European springs have been correlated with Chironomidae (Diptera) species (Lencioni et al. 2012), diatom diversity (Angeli et al. 2010; Wojtal and Sobczyk 2012) and aquatic plants (Heino et al. 2005; Kapfer et al. 2012). Other research on springs has been predominantly descriptive to date, relating chemical and microhabitat variables across natural gradients (e.g., Virtanen et al. 2009; Barquin and Death 2009; Omelková et al. 2013). Therefore, many researchers have called for studies on drainage basin impacts on spring ecosystems (Danks and Williams 1991, van der Kamp 1995; Cantonati et al. 2012), especially in places such as Atlantic Canada (including Prince Edward Island) (Danks and Williams 1991; Dobrin and Giberson 2003; Smith 2010) where springs are critical and widespread aquatic habitat. Before assessing potential impacts of agricultural land use, however, basic floral, faunal, and habitat descriptions are required.

1.3.6 Organisms in springs

1.3.6.1 Plants in springs

Like most aquatic habitats, springs are inhabited by a number of primary-producers such as algae, bryophytes, and vascular plants, with many of the species restricted to spring habitats (Cantonati et al. 2006). Structurally, these groups are quite different in terms of their

size, attachment to structures, and absorption of nutrients. Vascular plants are often referred to as macrophytes, however this term can include all aquatic plants and sometimes plants and macroalgae (Bowden et al. 2006). Algae are important food sources for invertebrates and can use plants as a substrate (Cummins 1973; Wojtal and Sobczyk 2012). Algal groups that have been reported from springs include cyanobacteria, diatoms, green algae, yellow-green algae and red algae (Cantonati et al. 2006). Diatoms and cyanobacteria may be abundant in eutrophied systems (e.g., Griffith et al. 2009; Wojtal and Sobczyk 2012). Bryophytes, including mosses and liverworts, are important components of spring habitats, and provide cover for invertebrates (but poor quality food) and trap organic matter (Giller and Malmqvist 1998; Virtanen et al. 2009; Bottazzi et al. 2011). Bryophytes lack roots and an organized transport system, so absorb nutrients directly from the water through surface tissues (Bowden et al. 2006). Both mosses and liverworts require stable surfaces, like large stones, to attach to (Bowden et al. 2006). Bryophytes are shade tolerant, especially when compared to some algae and most vascular plants (Vanderpoorten and Goffinet 2009; Giller and Malmqvist 1998). Vascular plants are also common in springs, and can include ferns, grasses, and flowering plants (Cantonati et al. 2006). They can be free-floating at the water surface, rooted with leaves floating, submerged, or emergent from the water surface (Bowden et al. 2006). Submerged plants have all structures below the surface, though some can produce above-water reproductive structures (Bowden et al. 2006). Emergent plants occur mainly above water, but have roots and some leaves below the surface (Bowden et al. 2006). In any of these cases, most nutrients are absorbed through the root surface (Bowden et al. 2006; Lacoul and Freedman 2006). Vascular plants generally prefer fine sediments for root growth and absorption of available nutrients (Giller and Malmqvist 1998; Mebane et al. 2014). Most

aquatic secondary consumers feed on detritus rather than living plant material, so most nutrients are transferred to shredding invertebrates upon senescence (Hynes 1970; Bartodziej and Perry 1990; Giller and Malmqvist 1998).

1.3.6.2 Spring macroinvertebrates

The main factor affecting macroinvertebrate assemblages in temperate-zone springs is the constant, cool temperature regime (Cantonati et al. 2006). Since many aquatic invertebrates require a minimum temperature for development or use temperature cues to synchronize their life cycles (Danks 1987; Dobrin and Giberson 2003), springs generally have lower species richness than adjacent fluctuating-temperature stream habitats (Williams and Hogg 1988; Barquin and Death 2004). Some aquatic insects that inhabit both springs and surface water streams have flexible life cycles with long emergence periods, or may show less synchronous emergence in springs than in stream habitats (Dobrin and Giberson 2003). Other invertebrate taxa in these habitats may be cool- or cold-water specialists, so that they are not found in other aquatic habitats. Therefore, specialized and sometimes endemic organisms may be found in springs (see Table 1.2 for the categories of taxa that inhabit springs) (Danks and Williams 1991; Barquin and Death 2011). For example, the oribatid mite *Mucronothrus nasalis* (Willmann) lives only in cold, thermally stable habitats, and has been found in springs worldwide (Norton et al. 1988). In addition, different spring typologies have invertebrate assemblages that are characteristic of different spring types (e.g., Gathmann and Williams 2006; Spitale et al 2012). Because of the presence of specialized endemic taxa in springs, these habitats have been labelled as areas of conservation concern for biodiversity (Cantonati et al. 2006). In addition, monitoring the

diversity and abundance of the spring fauna can be a useful indicator of regional groundwater contamination (van der Kamp 1995; Williams and Williams 1998).

Table 1.2. Categories of spring water organisms in the literature, based on Cantonati et al. (2006).

Organism type	Description
Stygobiont	Hyporheic obligate species, restricted to groundwater
Stygophile-crenobiont	Groundwater organism found frequently in springs
Crenobiont	Species only found at spring source
Crenophile	Species preferring springs but can be found in other habitat types
Crenoxene	Species occasionally occurring in springs

1.3.6.3 Riparian influences on spring invertebrates

The surrounding terrestrial ecosystem also plays an important role in structuring aquatic communities, including those in springs (Barquin and Scarsbrook 2008). Riparian vegetation may shade the habitat and provide organic matter from litter fall and other dead material (Naiman et al. 2005). Shading has a dramatic influence on aquatic plant diversity, as light availability is one of the major determining factors of aquatic plant assemblages (Lacoul and Freedman 2005). Vascular emergent plants can exploit high light availability by growing above the surface water, whereas low light conditions favor shade-tolerant non-vascular bryophytes and submerged vegetation (Lacoul and Freedman 2005).

The presence and type of riparian trees influence the type and abundance of aquatic invertebrates both directly and indirectly, by influencing habitat and food resources. For example, detritus that falls as leaf litter from deciduous trees and shrubs is a high quality food resource for shredding detritivors in aquatic habitats, so presence of these riparian species can influence invertebrate abundance (Naiman et al. 2005; Taylor and Andrushchenko 2014). In contrast, conifer litter is more difficult to decompose and consume due to high lignin content, so does not provide as high quality a food resource as deciduous litter (Naiman et al. 2005). Aquatic macrophytes vary in their food quality to invertebrates as well, but also contribute to habitat structure. Bryophytes are a poor food source for most aquatic invertebrates, but provide shelter and trap organic matter for consumption by collector-gatherers or provide substrates for attached microscopic algae like diatoms that are consumed by scraping invertebrates (Vanderpoorten and Goffinet 2009; Bottazzi et al. 2011; von Fumetti and Nagel 2011). Few invertebrate taxa feed directly on living aquatic plants, but the plants provide a source of litter when they die. The volume of watercress (a vascular plant) in springs in Minnesota has been directly related to invertebrate abundance (Bartodziej and Perry 1990). Land uses that alter the riparian zone around springs should therefore influence the biota present in the springs. Agricultural activity may result in land clearing that can alter tree species composition or amount of shading. When areas are completely cleared for planting, light levels in the spring can increase dramatically, and any trees that are allowed to re-grow are often early succession species such as spruce. For example, >70% of the original Acadian forest on PEI (a mix of deciduous and coniferous species; Loo et al. 2010) has been cut at some time, with much of the re-growth in white spruce (Loo et al. 2010). Since legislation mandating a 15 m vegetated buffer area adjacent to agricultural land

is relatively recent (Implemented in 2008; Dunn et al. 2011), riparian zones in agricultural areas (as well as much of PEI) are now dominated by conifers (Loo et al. 2010). In addition, riparian buffers can reduce nutrient contaminants in surface waters (Vought et al. 1995; Naiman et al. 2005; Dunn et al. 2011) if they are extensive enough.

1.3.7 Importance of life history responses to invertebrate patterns in springs

Freshwater insects and many freshwater mites are dynamic in that they spend part of their life cycle in the water and part in terrestrial habitats (Lancaster and Downes 2013).The terrestrial dispersal stage allows them to move between isolated aquatic habitats. Adult freshwater mites are aquatic predators, but the juveniles are parasitic on winged adult stages of aquatic insects, allowing them to disperse among habitats (Smith et al. 2001). Most aquatic insects are winged as adults, so they can also disperse through terrestrial habitats to find suitable habitats to lay their eggs (Lancaster and Downes 2013). Aquatic insects show two main life cycle types, and these influence their ability to adapt to environmental conditions. Hemimetabolous groups, such as mayflies (Ephemeroptera), stoneflies (Plecoptera), dragonflies (Odonata) and true bugs (Hemiptera) have incomplete metamorphosis, where their immature stage is similar in appearance to the adult stage and wings develop externally as the insect grows (Lancaster and Downes 2013). Many hemimetabolous insects, including mayflies and stoneflies, also have indeterminate growth, where life cycle length and the number of larval moults can vary within species, depending on temperature and food resources (Butler 1984; Brittain 1990;). For example, the life cycle of the burrowing mayfly, *Hexagenia limbata*, in a lake in northern Manitoba varied from one to four years, depending on localized water temperatures (Giberson and Rosenberg 1992).

However, most mayflies cannot complete their development at low temperatures so are absent from cool-water habitats (Brittain 1990; 2008). In contrast, many stoneflies are cool-adapted, and are common in northern streams and lakes characterized by cool temperatures and short seasons (Brittain 1990).

In contrast to the hemimetabolous insects, holometabolous orders show complete metamorphosis, where their adult stage has a completely different appearance to the larval stage (Lancaster and Downes 2013).). Holometabolous aquatic insects include caddisflies (Trichoptera) and true flies (Diptera). Holometabolous species generally have a fixed number of instars (though instar number can vary depending on order, genus, or species), and possess a separate pupa stage where larval tissues are absorbed and adult tissues form (Butler 1984). All emerge from their pupa as fully formed adults. Although temperature does not cause the number of instars to vary, temperature can affect life history by affecting the rate of development in each stage, and for some species, it can provide important cues for onset of pupation or emergence, through mechanisms such as diapause (a resting stage, with limited metabolic function) (Danks 1987). For example, caddisflies in the genus *Neophylax* undergo an extended pupal diapause before emerging (Beam and Wiggins 1987).

Some life-history studies on aquatic insect larval growth have suggested that the absence of thermal cues can lead to asynchronous populations with individuals present in multiple size- and age-classes during much of the species' life cycle (Williams and Hogg 1988; Glazier 1991; Dobrin and Giberson 2003). These differences in size- and age-classes result in extended emergence periods over a wide range of environmental conditions compared to thermally fluctuating systems (Dobrin and Giberson 2003).

The other major factors that influence timing in freshwater insects include resource availability and photoperiod (Sweeney 1984; Williams 1991). In springs with constant water temperature, photoperiod has been assumed to provide the major environmental cue affecting developmental timing (Hynes 1970). However, growth rates can also increase with increasing food availability, resulting in larger body sizes and/or more rapid development (e.g., mayflies; Giberson and Rosenberg 1992), chironomids; Liber et al. 1996). Although the relationships between temperature or photoperiod with growth and development are well established through a combination of field and lab studies, most studies on the relationship between resource availability and emergence timing have been carried out in the laboratory where temperature can be controlled or in single water bodies without replication (Williams et al. 1995). One exception is a study by Kominoski et al. (2012) where emergence timing was related to food quality along a forest composition gradient. The constant temperature in springs makes them ideal habitats to test questions on life history timing of aquatic insects (Williams 1991).

1.3.8 Invertebrates in PEI springs

Because of the relatively recent emergence of PEI as an island, dispersal opportunities for freshwater species have been low, resulting in a diminished species pool (Klassen and Locke 2010), especially for fish (Cairns 2002) but presumably also for invertebrates. The small area of PEI also results in relatively low faunal species diversity. Most freshwater invertebrate species have not been assessed completely on PEI, but studies on some species (e.g., black flies, Adler et al. 2005; freshwater Coleoptera, Majka 2008; unionid mussels, Martel et al. 2010; and Odonata, Brunelle 2010) show lower faunal

diversity than on adjacent mainland areas. Habitat-specific freshwater invertebrate surveys on PEI are limited, since most studies have not identified their invertebrate specimens to species. Examples exist for ponds (Smith 1946; Meijering 1991; Giberson et al. 2007) and spring-fed streams (Dobrin and Giberson 2003). Further, no inventory of spring dwelling arthropods is available for most of Atlantic Canada (Smith 2010).

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Chapter 2

The Influence of Agricultural Land-use on Plant and Macroinvertebrate Communities in Springs in Eastern Prince Edward Island, Canada.

Abstract

Freshwater springs can be characterized by low variation in temperatures and chemical composition that depend on local conditions and land use. Agricultural activities may leach nutrients to the groundwater, add sediment and nutrients from overland flow, and change the cover of the riparian area surrounding springs; all factors that influence aquatic invertebrate and plant communities. Springs influenced by agriculture were expected to show higher nutrient and fine sediment levels and more open canopies than forested areas, which in turn should affect macrophyte and invertebrate abundance and diversity. Twenty rheo-limnocrene springs in Prince Edward Island, Canada (10 surrounded by and within ~20m of agricultural land, and 10 located in forested areas with <5% Agriculture within 1 km), were studied to determine effects of agricultural activities on invertebrate and plant community structure. Chemical, flow, sediment, and cover variables were examined in all 20 springs, and invertebrates and macrophytes were evaluated in a subset of four agricultural and four forested springs. Although nutrients (particularly nitrates and sulphates) were generally higher in agricultural springs than in forested ones, and plant communities differed between springs in the two land use types, light level (relating to the riparian canopy in the two land-use types) was a stronger predictor of aquatic plant community composition than nutrients. Plant diversity was highest in open agricultural sites. Overall invertebrate richness and abundance were higher in forested sites than agricultural sites, and invertebrate community composition differed between the two land-use types, which was primarily related to aquatic plant composition. Few taxa responded directly to elevated nutrients. The composition of the riparian area may be more important than direct inputs of nutrients and sedimentation when assessing agricultural impacts on PEI springs.

2.1 Introduction

Springs have long been considered as natural laboratories to examine questions of basic and applied ecology (Odum 1957) due to their clearly defined boundaries, constancy in many physical variables (e.g., temperature and water chemistry; van der Kamp 1995), and their relatively low species diversity (Williams and Williams 1998). Therefore, springs provide an ideal environment in which to examine the broader concepts of effects of anthropogenic stressors on aquatic habitats.

Springs are transition habitats between groundwater and surface water (Danks and Williams 1991; Cantonati et al. 2006; Barquin and Scarsbrook 2008), but are dominated by local groundwater conditions (van Everdingen 1991). The constant cool temperatures found in many cool-springs restrict species diversity since many species require warm temperatures for development, and greater fluctuating temperatures to cue emergence (Danks and Williams 1991). Most previous studies of spring organisms, including invertebrates, have focused on biodiversity and ecological differences between springs and their adjacent stream habitats (e.g., von Fumetti et al. 2007; Barquin and Death 2004; Barquin and Death 2011), or between different spring types (e.g., Gathmann and Williams 2006; Martin and Brunke 2012). Spring dwelling organisms should also be useful bioindicators of anthroprogenic contamination (Williams et al. 1997; Williams and Williams 1998; Keleher and Rader 2008), though little is known about they respond to habitat alteration or other anthropogenic stresses (Cantonati et al. 2006; Cantonati et al. 2012; Lencioni et al. 2012), especially in North America, (e.g., Colbo 1991; Williams et al. 1997; Keleher and Rader 2008).

High intensity agricultural activity is a major anthropogenic stressor that impacts water bodies through a combination of nutrient and sediment inputs, and riparian and in-

stream habitat alteration. These effects are well known in surface waters (e.g., Vought et al. 1995; Erman 2002; Barquin and Scarsbrook 2008), and should be similar in springs. For example, added nutrients could change plant and algal communities, changing food and habitat structure for invertebrates (Virtanen et al. 2009; Wojtal and Sobczyk 2012; Mebane et al. 2014). Activities that alter substrate composition (e.g., Polyakov et al. 2005; Dumnicka et al. 2007) and light penetration (Beierkuhnlein and Gräsle 1998; Mebane et al. 2014) also influence spring invertebrate communities and functional assemblages by changing habitat and food availability (von Fumetti and Nagel 2011; Cantonati et al. 2012). Therefore, understanding biological responses at the groundwater-land interface is not only important in understanding ecological questions, but is also important in understanding the impacts from, and how to manage agricultural land use.

Prince Edward Island (PEI), a large (5,685 km^2) island located off the east coast of Canada, provides an ideal laboratory for studying ecology and anthropogenic influences on springs. All PEI rivers are spring-fed from a generally uniform geological formation consisting of fractured Carbo-Permian sandstone bedrock overlain by Fero-Humic podzolic (sandy-loam) soil and glacial till (van der Poll 1983, Klassen and Locke 2010; Sanborn et al. 2011). However, sub-surface flow patterns are not uniform and are localized from the fractured geology (Jiang and Somers 2009). Streams in eastern PEI are a fed by a number of small rheo-limnocrene springs that arise in a combination of agricultural and forested sites. PEI is farmed intensively (~40% of land in agriculture; Jiang and Somers 2009) with heavy reliance on row crops with high fertilizer and pesticide inputs (Jiang and Somers 2009; Dunn et al. 2011). Frequent high rainfall events result in high sediment and dissolved material (nutrients, pesticides) runoff to surface waters (Dunn et al. 2011) and leaching to

groundwater (Benson et al. 2007; Jiang and Somers 2009). Negative impacts from agricultural activities have occurred on biotic communities in PEI rivers (Curry and MacNeill 2004; Purcell and Giberson 2007) and estuaries (Schein et al. 2012; Finley et al. 2013; Bugden et al. 2014), but no studies have been carried out on springs.

The goal of this study is to examine the influence of local land use (agricultural vs. forest) on plant and invertebrate diversity and community structure. Agricultural zone springs should show higher nutrient levels, more open riparian forest cover and higher levels of fine substrates than forested springs, leading, in turn, to differences in the macrophyte and invertebrate communities. To test these hypotheses, plant and animal diversity and abundances were compared among representative springs in eastern Prince Edward Island across sites with agricultural influences and more natural forest.

2.2 Methods

2.2.1 Study springs

Twenty rheo-limnocrene springs (small pool springs that flow into spring-brooks below) in eastern Prince Edward Island, Canada (Fig. 2.1), were studied between June 2011 and June 2012 to determine physical, chemical, and biological responses to agricultural activities. The percentage of forested and agricultural land within a 1 km diameter circle around each spring was quantified by analysing the 2010 PEI Land use data layer (PEI Department of Agriculture and Forestry 2010) summary statistics function in Arc GIS V.10.2 (ESRI Redlands, California, USA). Ten of the study springs were located in agricultural areas (with surrounding row crops, forage crops, or pasture within 20 m of the spring outflow) and ten were in forested areas (chosen to have < 5% agricultural activity within a 1

km radius of the spring and no visible forestry or agricultural activity adjacent to the spring. Eight springs (four forested and four agricultural) were further selected as "biodiversity sites" to assess diversity and community ecology patterns of the spring invertebrates. These biodiversity sites all had good year-round access, and were chosen to show clear differences in nitrate levels between land use categories (forested sites: <0.5 mg/L nitrate-nitrogen; agricultural sites: >0.7 mg/L nitrate-nitrogen), as well as land-use differences in qualitative habitat characteristics. The riparian vegetation around the agricultural springs was dominated by white spruce (*Picea glauca* (Moench) Voss), balsam fir (*Abies balsamea* (L.) Mill), trembling aspen (*Populus tremuloides* Michx.) and white birch (*Betula papyrifera* Marsh.) (Giberson et al. 2013; see list in Appendix 2.2). In contrast, the riparian area around forested springs had a wider mix of deciduous and coniferous species which were dominated by red maple (*Acer rubrum* L.) and balsam fir (Giberson et al. 2013; Appendix 2.2). Another major difference between agricultural and forested sites was the amount of understory vegetation, with forested sites heavily shaded by a number of deciduous shrubs such as speckled alder (*Alnus incana* (L.) Moench) and wild raisin (*Viburnum nudum* L.), compared to agricultural sites which had relatively open understories with few shrubs (Giberson et al. 2013, and see list of species in Appendix 2.2, Table A.2.2.1).

2.2.2 Environmental variables

2.2.2.1 Water quality

Water chemistry variables, flow, and overhead cover were measured in all 20 study springs. Water samples were collected monthly from June 2011-November 2011 and from

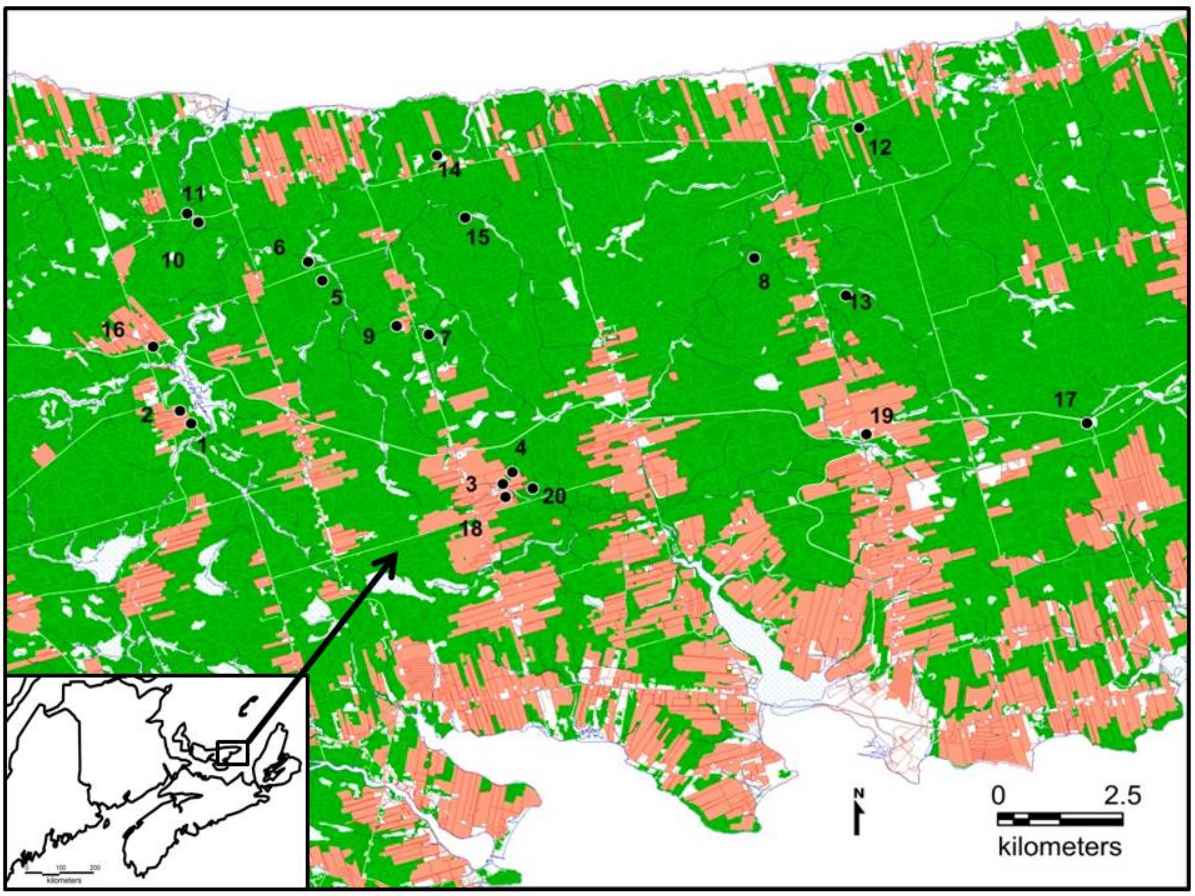

Figure 2.1. Locations of study springs in major watersheds in eastern Prince Edward Island (PEI), with inset maps showing location of Prince Edward Island in the Maritime Provinces of Canada. Numbers correspond to springs listed in Appendix 1.5, Table A.1.5.1. Green areas indicate forest, and red indicate agricultural land use.

March 2012-June 2012 from all 20 sites to assess water chemistry and determine seasonal variability of the spring water. Winter samples were collected from a subset of the sites only; due to difficulties in accessing some sites during winter (see Appendix 1.5, Table A.1.5.1. for sampling dates and Appendix 2.1 and 3.1for more detailed information on winter temperatures and environmental variables). Conductivity, dissolved oxygen, pH, and spot-temperature readings were measured for all 20 springs during every visit to the springs using a YSI 550 or a YSI Professional Plus Multimeter. Continuous temperature data were obtained from HOBO© tidbit or HOBO© Water Temp Pro 2 temperature data loggers deployed in the eight biodiversity sampling sites between July 2011 and July 2012, and set to measure temperature every 3 hours to get a daily average. Water samples were collected for laboratory analysis by immersing a 125 ml bottle below the water surface and filling it, then transporting it in a cooler to the laboratory. Samples were stored at -20^{o}C prior to laboratory analysis. Samples were analysed using suppressed anion chromatography for nitrate-nitrogen, total nitrogen, phosphate, sulphate, and chloride following the methods in Schein et al. (2012) (see Appendix 1.1 for the full methodology for each chemical parameter).

2.2.2.2 Discharge and spring-brook slope

Water velocity and discharge were measured concurrently with water chemistry sampling (See Appendix 1.5 and Appendix 2.1 for details on which parameters were measured at each site on each sampling date) at the outlets to each of the 20 spring pools to determine the flow from each spring. Mean velocity (v) was measured over a transect across each outflow stream with a HYDRO-PROP stream flow-meter (Great Atlantic Flow Meters, Bank Square, Penzance, Cornwall, UK) at 60% of stream depth above the bed. Stream width

(w) and mean depth (d) were calculated from depth measurements taken across the transect using a meter stick to determine cross-sectional area ($A = d \cdot w$). Discharge (Q) was determined from the product of mean velocity and cross-sectional area ($Q = v \cdot A$). Slope of the spring-brook immediately below the spring pool was measured at the eight biodiversity sites only, using a survey level and a graduated rod.

2.2.2.3 Overhead cover and riparian habitat

Overhead cover (a proxy for light availability) was measured monthly during summer water sampling trips in all 20 springs (see Table A.1.5.1 in Appendix 1.5 for sampling dates for cover analysis and Fig A.2.1.3 in Appendix 2.1 for detailed seasonal patterns). Cover was estimated with a spherical densiometer which projects the overhead cover onto a gridded spherical mirror surface so that it can be quantified (Lemmon 1957). Four readings were taken at each site and sample date (at north, south, east and west directions) and averaged to get an estimate of the percent of open or unshaded area.

Riparian trees and shrubs were identified and quantified to compare riparian vegetation surrounding forested and agricultural springs. All trees and shrubs were assessed in three 5 x 15 m quadrats adjacent to each of the eight biodiversity springs (summarized in Giberson et al. 2013; see Appendix 2.2 for the full list of species and Fig A.1.3.1 for the orientation of the quadrats). The distance of 15 m from the spring was chosen to quantify patterns in the entire vegetated buffer required around water bodies in PEI (Dunn et al. 2011). Riparian soil conditions were compared between forested and agricultural sites to assess the potential for sediment erosion (Carter et al. 1998) into the springs. Three soil cores were taken 2.5 m from the shore around each of the 20 spring pools, at approximately equal

distances around the spring pool. The depth of organic and humic material (LFH horizon) within the core was recorded and averaged for each site.

2.2.2.4 Substrate

Substrate particles sizes were assessed for the eight biodiversity spring sites to assess substrate heterogeneity in the invertebrate habitat. Substrate composition was assessed from overhead photographs of each pool, with a meter stick placed in the pool to provide a scale. Photos were analysed using ImageJ® image analysis software (Rasband 2013) by tracing each substrate particle or region with specified particle categories (e.g., sand or rocks) to determine the total pixels in the image for each substrate category. Pixels were then converted to area measurements using the scale from the meter stick. Particle categories were defined by size: "Rocks" were any particles of >6.4 cm diameter or 40.96 cm^2 area (equivalent to cobble [6.4 to 25.6 cm diameter] or boulder [>25.6 cm diameter] on the Wentworth class scale). "Gravel" (including both coarse and medium gravel) consisted of particles with 0.8-6.4 cm diameter or 0.64-40.96 cm^2 area. Particles that measured <0.8 cm diameter (0.64 cm^2) were placed into the "Fines" category, which included fine gravel, sand, and silt. In addition to quantitative substrate analysis, fine sediments were assessed qualitatively for each spring pool, based on the photographs and field notes on whether clouds of sediment were disturbed during sampling.

2.2.3 Aquatic plants

Aquatic plant species and cover and plant detritus cover were assessed in each of the eight biodiversity springs between 15 and 25 September 2011 to provide a further measure of

habitat structure and food resources for invertebrates at the biodiversity sites. Plants were assessed in the late summer since this is when biomass of aquatic plants in springs is most representative (Varza and Covich 1995; Beierkuhnlein and Gräsle 1998). Cover was not assessed in remaining sites, but presence or absence of each identified taxon was noted for the full set of 20 sites.

Cover patterns were estimated by dividing each spring pool into a series of 50 cm x 50 cm quadrats, then assigning cover classes for each plant taxon in each quadrat, to map out the overall vegetation cover in the pool. Non-vascular plants were categorized as Moss (Bryophyta: Musci: Bryopsida) or Leafy Liverwort (Bryophyta: Marchantiophyta: Jungermanniales) whereas vascular plants were identified to family or genus (See Table 2.1 for taxonomic references). Seasonal differences in plant structure were not quantified in this study, but did not appear change throughout the summer. However, above-water material did die back in the winter (Fig 2.2). The cover of coarse particulate organic matter in the pool (CPOM) at each site was quantified concurrently with the vegetative cover. Cover classes were based on a Braun-Blanquet cover scale (modified from Bowden et al. 2006) giving the following cover class designations for each plant taxon: Categories 1: <5% cover (midpoint: 2.5%), Category 2: 5-10% cover (midpoint: 5%), Category 3: 10-25% cover (midpoint: 17.5%), Category 4: 25-50% cover (midpoint: 37.5%), and Category 5: 50-75% cover (midpoint: 62.5%), and 6: 75-100% cover (midpoint: 87.5%). Cover classes were quantified

Table 2.1 Summary of the major taxonomic references and ecological references used in this study. *primary classification references used.

Taxa	Main Reference	Supplementary References
Plants	Crow and Hellquist 2000 (Vascular Plants)	Ireland 1982 (Mosses) and Lincolin 2008 (Liverworts)
Ephemeroptera	Waltz and Burian 2008*	Peckarsky et al. 1990
Plecoptera	Stewart and Stark 2008*	Hitchcock 1974
Trichoptera	Wiggins 1996	Morse and Holzenthal 2008*
Coleoptera	White and Roughley 2008*	Larson et al. 2000 (Dytiscidae)
Diptera	Courtney and Merritt 2008*	Cook 1981 (Chaoboridae); Peters 1981 (Dixidae); and Peckarsky et al. 1990
Diptera: Tipuloidea	Byers and Gelhaus 2008*	Alexander and Byers 1981
Diptera: Chironomidae	Ferrington et al. 2008*	Brundin 1983 (Podominae); Cranston et al. 1983 (Orthocladiinae); Oliver 1983 (Diamesinae); Oliver and Roussel 1983; Bode 1990; Epler 1995; Cranston 2010[1]
Acarina	Smith 1990	Smith et al. 2001*, and Smith 2010
Crustacea	Peckarsky et al. 1990	Delorme 2001(Ostracoda)*; Dodson and Frey 2001 (Cladocera)*; Williamson and Ried 2001 (Copepoda)*
Non-Arthropoda	Peckarsky et al. 1990	Clark 1981 (Mollusca)*; Brinkhurst and Gelder 2001 (Oligochaeta)*.

[1]Website: http://Chirokey.Skullisland.info

A)

B)

Fig 2.2 .Winter (Feb. 2012) (A) and summer (June. 2011) (B) picture of sample spring at Site 4 (the headwaters of the Souris River, in the community of Bear River). The winter picture was taken upstream of the spring whereas the summer picture was taken from the left side (relative to the orientation of the winter picture) of the spring. Photos: K. Knysh

for the entire pool by averaging the midpoint % cover values for each species over all squares to get an estimate of the overall vegetative cover and diversity within the entire spring pool.

2.2.4 Benthic invertebrates

Aquatic invertebrates were collected in June 2011 from two spring micro-habitat types in each of the eight biodiversity springs: one in a vegetated section and one in a non-vegetated section of the pool. Samples were collected using a Hess sampler (250 μm mesh; 0.07 m^2 area) deployed from above, with a harness, to disturb as little of the spring pool habitat as possible while sampling (Fig. 2.3). Samples were preserved in 70% ethanol and processed in the lab under a dissecting microscope. Each sample was subsampled using a gridded sorting tray (divided into 20 sections) to reduce processing time. The amount of sample to be processed was determined by sorting three full samples, and determining the number of subsamples needed to obtain 90% of the diversity and abundance in each sample (determined by constructing species accumulation curves, and sorting enough grids to obtain at least 90% of taxa and abundance).

High variability among sample grids meant that at least 50% of subsamples needed to be processed, so 75% of each sample was processed for all taxa (15 of 20 subsamples) to ensure adequate sorting for comparison among samples. Chironomid midges were much more abundant and diverse than other invertebrate taxa, so a separate subsample analysis was carried out for this group. Only six subsamples (30% of the total sample) were needed to reach 90% of diversity and obtain 90% precision in abundance estimates for the chironomids. Therefore, invertebrate identifications for each sample consisted of identifications from 15 subsamples for the non-chironomid taxa and 6 subsamples for Chironomidae. An additional

Fig 2.3. Deploying the Hess Sampler in a study spring. Photo: M. Jang

test of the subsampling procedure was carried out by including random subsamples from the full sort (obtained by identifying grids with a random number generator) to compare the fully sorted samples to samples from the sites that were not fully processed.

Abundance for each taxon was estimated from subsamples by converting the numbers from subsamples to the total sample based on the proportion of the sample sorted. Taxa were identified in the laboratory to the lowest practical level (LPL), following the keys listed in Table 2.1. Most insect taxa were identified to genus, except for water beetles in the subfamily Hydroporinae (Dytiscidae), and muscoid flies (Brachycera: Muscomorpha) which were identified to subfamily and infraorder respectively. Most non-insect taxa (oribatid mites, copepods, ostracods, and oligochaetes) were identified to order; however, water mites ("Hydrachidia") were identified to genus. Primary feeding groups (i.e., shredder, scraper, collector-gatherer or predator) and life-habit groups (i.e., sprawler, clinger, or swimmer) for aquatic insects were determined from the taxonomic chapters of Merritt et al. (2008) (see Table 2.1 for specific chapters consulted). Equivalents of the feeding and life-habit groups for non-insect taxa were determined from ecological descriptions in the taxonomic chapters of Thorp and Covich (2001) (see Table 2.1 for group specific chapters). Numbers from each Hess sample were combined for the two habitats in each pool for site specific analyses.

2.2.5 Statistical analysis

2.2.5.1 Water quality and physical habitat

Water chemistry and habitat variables were compared using both univariate and multivariate techniques. All data were transformed as necessary to meet assumptions of linear univariate and multivariate tests. Homogeneity of variance was tested with Bartlett's

test, and normality of residuals was assessed from normal probability plots generated in STATISTICA v.8 (Stat Soft 2007, Tulsa, Oklahoma, USA). Continuous measurements such as concentrations and lengths were all log transformed (except pH which is the log of hydrogen ion concentration), whereas values with fixed lower bounds such as percentages and densities were square root transformed.

Differences in individual environmental variables between land use types (the 10 forested vs. the 10 agricultural springs) were assessed by comparing values averaged over the study period for each spring using one-way ANOVA (STATISTICA v.8). Variables that violated assumptions (even after transformation) of parametric models were compared using a Mann-Whitney U-test (STATISICA, v.8). Among-site relationships between land uses for all 20 sites were explored to identify variables that corresponded to land use and site to site variation using principle components analysis (PCA) and correlations in a correlation matrix (PRIMER v.6, Plymouth, UK). Multivariate patterns in environmental data were further assessed for all of the sites (n=20), and for the subset of biodiversity sites when considering invertebrate patterns (n=8) using a number of distance based similarity measures (PRIMER v.6 with PERMANOVA + v.1, Plymouth, UK). Distance-based tests for homogeneity of multivariate dispersions (PERMDISP) were performed to see if there was a difference in heterogeneity between land use variables (Anderson 2006). Permutational MANOVA (PERMANOVA; Euclidian distance matrix) was used to test whether the centroids of the environmental data clouds (the positions of the data points on multivariate ordination plots) differed between land uses, as a measure of the overall differences between land use types (Anderson 2001a, 2001b). The measurements of % agriculture and % forest were removed for these tests as land-use categories were chosen on this basis. The analysis options for each

test (e.g., type of distance measure, number of permutations [9999]) were set based on Anderson (2001a) and Anderson et al. (2008) as being appropriate for the sample size used and the type of environmental data being assessed. With a small sample size ($n \leq 10$) the lowest type I error estimate that can be obtained with a permutation in most cases is $p=0.05$ (Anderson 2001b), so p-values that are near 0.05 were considered to be important variables in structuring communities. Significant differences ($p \leq 0.05$) have been noted in the text.

2.2.5.2 Biota

Biotic diversity and composition were compared between land-use types from both a taxonomic perspective and a functional perspective. Richness (the number of taxa) was estimated using rarefaction and a Chao 1 or Chao2 estimator (EstimateS, v.9.1.0, Colwell 2013) to compare the number of taxa (genera and LPL) present at sites within each land use category. Examining site accumulation curves from rarefaction analysis gives information on whether enough sites were sampled to accurately estimate the number of species present within each category. The Chao 1 estimator is a good indicator of species richness using abundance data with many rare species and the Chao 2 Estimator is a good measure of richness using presence or absence of a species (Maurer and McGill 2011). Community parameters such as taxa evenness and Shannon diversity index (exponential index) were also calculated for each site (EstimateS, v.9.1.0). Total numbers of taxa, densities of individual taxa, densities in functional and life habit groupings, and total invertebrate densities were compared between land use types using a One-Way ANOVA (STATISTICA, v.8).

Comparisons were also made between multivariate data clouds for the two land use categories for plant taxa, invertebrate taxa and functional group assemblages as described

above for the land use variables (PERMDISP and PERMANOVA, PRIMER v.6; PERMANOVA + v.1). However, the resemblance matrix for the biotic analyses was based on a Bray-Curtis distance measure for taxa cover and density (rather than Euclidian distance as used with environmental data), since Bray-Curtis is the most common distance measure used for community data (e.g., Legendre and Anderson 1999). The resemblance matrix for presence/absence data for the plant taxa at all 20 sites was based on Sorensen distances, as this is equivalent to Bray-Curtis for binary data. Within the multivariate data clouds for each land use, taxa that contributed more to the relative differences between land uses than others were identified using the Similarity of Percentages (SIMPER) procedure in PRIMER v.6 (Clarke 1993). This procedure identified the taxa that were most similar in the collection of springs in each land use type and which taxa were most dissimilar, so that lists of most similar or dissimilar species could be compared between land use types. The top ten taxa contributing to similarity and dissimilarity were chosen for comparison in this study.

2.2.5.3 Interactions between biotic assemblages and environmental variables

Interactions between biotic assemblages and environmental variables were explored using a combination of multivariate and bivariate techniques. The environmental variables that best explained the variation in biotic assemblages in the eight biodiversity springs were determined with a distance-based linear model (DISTLM; PERMANOVA+; PRIMER v.6), then the correlations between invertebrate taxa and the environmental data were assessed using a distance based redundancy analysis (db-RDA).

The distance-based linear model (DISTLM) is a non-parametric multivariate multiple linear regression (McArdle and Anderson 2001). The non-parametric model was chosen

since large numbers of zero values from specific taxon absences meant data did not meet assumptions of traditional multivariate-linear models (Legendre and Anderson 1999). Also, the number of environmental variables and invertebrate variables far exceeded the number of sampling sites, so meeting the required degrees of freedom to test models was difficult using parametric methods (Legendre and Anderson 1999). Total numbers of variables used in the models were reduced by removing variables that were highly inter-correlated ($\rho \geq 7$; determined from the previous PCA and correlation matrixes). Model selection was carried out using forward selection based on adjusted-R^2 criteria (Anderson et al. 2008). The distance-based redundancy analysis was carried out using the variables identified with DISTLM plus total nitrogen, a variable that was an indicator of agricultural land use determined by previous PCA. This procedure is a redundancy analysis using principle coordinates axes (Legendre and Anderson 1999; McArdle and Anderson 2001). The result from this analysis was plotted as a constrained ordination of the invertebrate matrix, and strengths of correlations of both biota and environmental to the db-RDA axis were reported as Pearson's Correlation (ρ). Correlations for taxa groups where $\rho \geq 0.7$ and which occurred at $\geq$4 sites were selected as important for consideration of patterns.

2.3. Results

2.3.1. Patterns from Physical and Chemical variables

Forested and agricultural spring sites formed two distinct groups in the PCA ordination plot (Fig. 2.4), and were statistically different based on measured chemical and physical habitat variables (PERMANOVA, with variables % agriculture and % forest

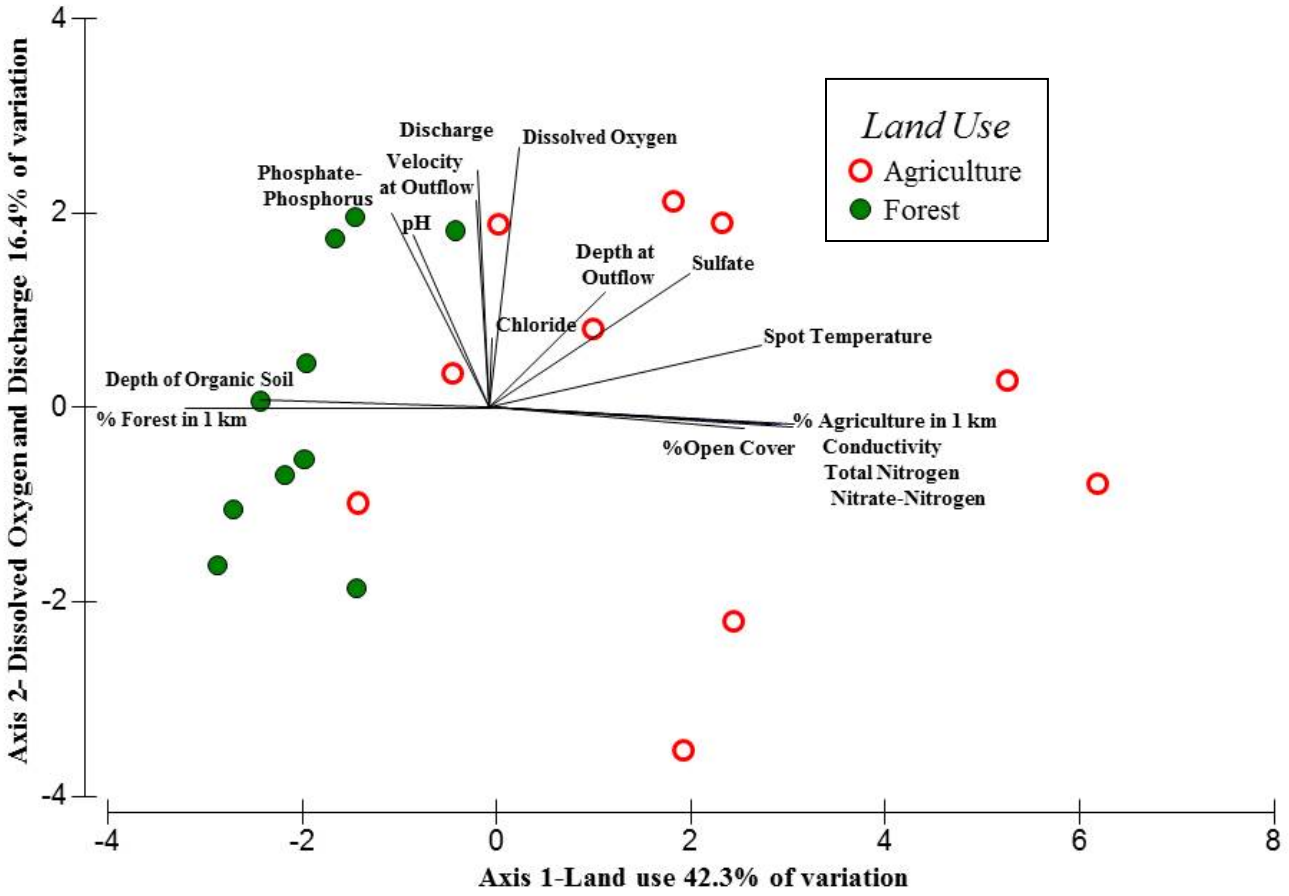

Fig. 2.4. Groupings of agricultural (open circles) and forested spring sites (closed circles) with respect to chemical and physical patterns at each site using principle components analysis (Axis 1 and 2). "Land use" on the first axis refers to the combination of variables (Nitrates, conductivity, riparian cover patterns and organic layer depth in soil) found to correlate strongly with the proportion of agriculture or forest land within a 1-km diameter of the spring.

removed for statistical testing, as the variables were used for initial site selection. The proportion of agricultural (or forest) land use in the immediate area of the springs accounted for nearly half the variability among sites (PC1; Table 2.2, Fig. 2.4). However, agricultural sites were significantly more variable in most physical and chemical parameters than the forested sites (PERMDISP), showing much more scatter along the land use axis of the PCA ordination (Fig 2.4). Nitrate, conductivity, and spot-measured water temperature were all highest in the sites with the highest proportion of agricultural land adjacent to the spring (Fig. 2.4, Table 2.2). Agricultural sites also had more open canopy than forested sites (Table 2.2), so received more direct sunlight. The depth of the organic layer in soils around the springs was highest in forested springs, also showing a strong correlation to land use. Sulphate was also positively correlated to the amount of agriculture in the area around the spring, but more weakly than the other agriculture variables (Fig. 2.4, Table 2.2). All variables identified as being correlated with agriculture in the principle components analysis also showed significant differences between agricultural and forested springs when analysed individually (Table 2.2, and see Appendix 2.1, Table A.2.1.1 for the correlation matrix and Table A.2.1.2 for values). Spring flow volume (discharge) influenced site groupings as well (explaining about 16% of the variability in the sites; PC axis 2, Fig. 2.4, Table 2.2) but was not related to land use immediately adjacent to the study spring (Table 2.2). Springs with the highest discharge levels also showed the highest dissolved oxygen and phosphate levels, though phosphate did not correlate as strongly as dissolved oxygen (Table 2.2).

Table 2.2. Comparison of chemical and habitat variables (median, mean ± 1 Std. Error) between the 10 forested and 10 agricultural study springs in eastern PEI. Pearson correlations show the relationship of each variable to principle components axes for the analysis shown in Fig. 2.4. Numbers in bold text denote the most important correlation for that variable. Comparisons were made using site averages using a one-way ANOVA or a Mann-Whitney U test (ǂ). Statistically significant land-use comparisons are denoted by asterisks: *p≤0.05, ** p≤0.01, *** p≤0.001.

	Forest (n=10)			Agriculture (n=10)				Principle Components Pearson Correlations (ρ)			
	Median	Mean	±SE	Median	Mean	±SE	Sig. level	PC1= 42.3%	PC2= 16.4%	PC3= 11.2%	PC4= 7.9%
%Agriculture in 1km diam.	3.88	3.57	0.92	26.52	29.06	5.12	***	**0.9**	-0.1	0.1	-0.2
% Forest in1 km diam.	89.87	89.62	1.45	54.72	56.57	5.44	***	**-1.0**	0.0	0.0	0.0
Average Soil Organic Depth (cm)	4.92	5.88	1.17	1.17	1.85	0.48	***	**-0.7**	0.0	0.2	0.4
Average Nitrate-Nitrogen (mg/L)	0.21	0.30	0.07	1.29	2.02	0.60	***	**0.9**	-0.1	0.1	-0.1
Average Total Nitrogen (mg/L)	0.17	0.31	0.07	1.39	2.22	0.65	***	**0.9**	-0.1	0.1	-0.1
Average Phosphate-Phosphorus (µg /L)	41.3	41.0	0.00	33.3	37.3	0.00		-0.3	0.6	0.3	-0.3
Average N:P Ratio	5.7	7.9	2.00	37.4	63.7	21.46	***	**0.9**	-0.1	0.1	0.1
Average Chloride (mg/L)	15.6	16.0	0.60	14.69	15.20	0.59		0.0	0.2	-0.2	**-0.7**
Average Sulphate (mg /L)	4.92	5.10	0.28	6.57	10.69	ǂ2.68	**	0.6	0.4	-0.3	0.5

Continued

Table 2.2, Continued

	Forest (n=10)			Agriculture (n=10)				Principle Components Pearson Correlations (ρ)			
	Median	Mean	±SE	Median	Mean	±SE	Sig. level	PC1= 42.3%	PC2= 16.4%	PC3= 11.2%	PC4= 7.9%
Average Spot Temperature (°C)	7.09	7.11	0.04	7.29	7.36	0.07	**	**0.8**	0.2	-0.1	0.2
Average Conductivity (µS/cm)	236.0	233.6	5.5	279.2	275.4	11.1	**	**0.9**	-0.1	-0.4	0.0
Average pH	7.58	7.58	0.06	7.59	7.55	0.07		-0.2	0.5	**-0.8**	-0.1
Average Dissolved Oxygen (mg/L)	10.01	9.64	0.26	9.97	9.61	0.44		0.1	**0.8**	-0.3	-0.0
Average Water Velocity at Outflow (m/s)	0.17	0.19	0.03	0.21	0.22	0.04		-0.0	0.6	0.0	-0.1
Average Water Depth at Outflow (cm)	8.98	9.97	1.29	10.51	10.72	1.17		0.4	0.4	**0.7**	-0.1
Average Water Discharge at Outflow (m^3/s)	0.01	0.02	0.01	0.02	0.02	0.00		-0.0	**0.7**	0.5	0.3
Average Open Cover (%)	16.88	17.48	1.52	27.42	38.93	∓8.80	**	**0.8**	-0.1	0.1	0.1

Note: Average Sulphate and Average Open Cover variables were not normally distributed; SE values are listed for comparison of deviations around estimated mean.

2.3.2. Plant community analysis in all sites and Biodiversity sites

More plant taxa were observed in agricultural sites than in forested sites, whether total richness (all sites; presence/absence data (12 taxa in agricultural sites compared to seven in forested sites) or estimated richness (11 and four taxa respectively; biodiversity sites; within-spring cover data) was considered (Fig. 2.5, Table 2.3)). The presence or absence of vascular plants was the main separator of the macrophyte communities between land use categories (Fig. 2.6). Nitrogen and light availability corresponded to the first db-RDA axis (Fig. 2.6). Surprisingly, the second db-RDA axis also corresponded with light availability, in addition to pH (Fig. 2.6). The species assemblage present in agricultural sites was significantly different from that in forested sites (PERMANOVA, Fig. 2.6); however the total number of taxa per spring did not differ significantly between land uses (One-Way ANOVA; 3.0 ± 0.8 present per site in agricultural versus 2.2 ± 0.4 present in forested springs (average ± Std. Error)). Agricultural sites had higher numbers of vascular plant species (2.2 ± 0.8 taxa) than forested sites (0.7 ± 0.2). Bryophytes were indicative of forested sites. Liverworts were present at 70% of forested sites and mosses were present at 80%. Only 30% of agricultural sites held liverworts and 50% had mosses.

More intensive sampling in the subset of biodiversity sites provided the opportunity to explore detailed diversity patterns. The subset (n=8) was a good estimator of the species richness (Fig. 2.5) and the biodiversity sites were representative of the full suite of sites sampled (Fig. 2.6). Agricultural sites had a more even plant community,

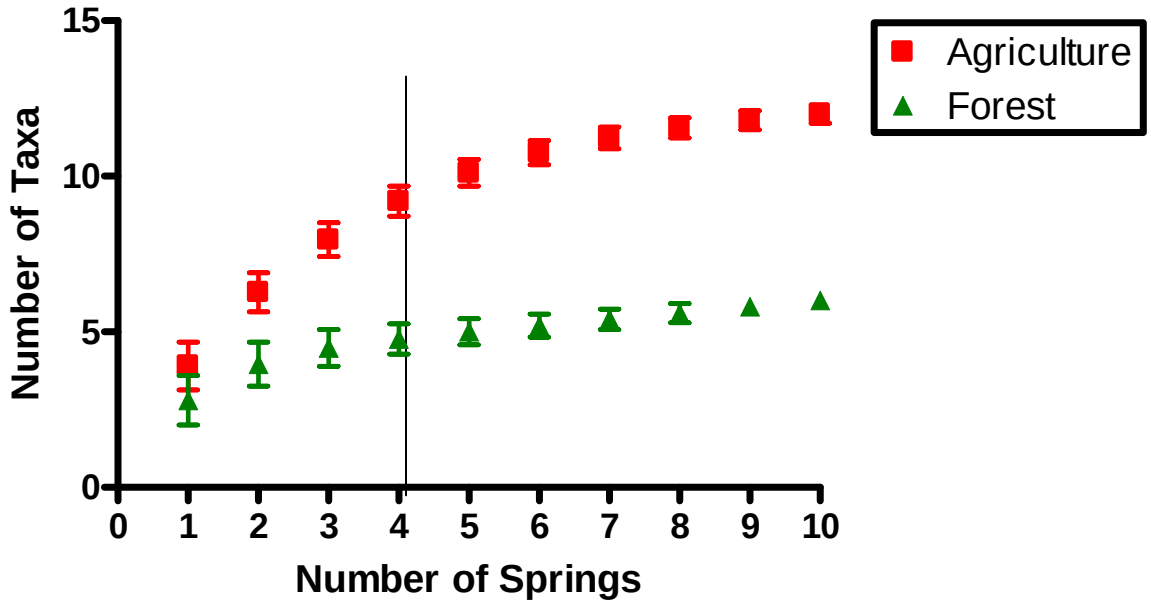

Fig 2.5. Comparison of plant taxa richness in agricultural and forested springs. Estimated species richness (±SE) for all sites in each land use (n=10) from presence-absence data (n=20) The vertical line indicates the number of sites in each land use category used for cover estimates (n=8).

Table 2.3. Diversity and structural comparisons of plant communities between springs in the two land use types. Species Richness Estimates of botanical diversity were calculated in EstimateS. Differences between land use were compared using a one-way ANOVA (DF=1,7) or Mann-Whitney U-test (ǂ). *p<0.05

	Forested springs			Agricultural springs			
Variable	Median	Mean	SE	Median	Mean	SE	
Taxa Richness (n=8)	2.5	2.5	0.29	4.5	5.0	1.47	
Taxa Richness (n=20)	3	2.8	0.36	3.5	3.9	0.80	
Chao 1 Richness (n=8)	N/A	4	0.24	N/A	11	1.13	N/A
Chao 2 Richness (n=20)	N/A	7	0.64	N/A	12	0.27	N/A
Evenness (n=8)	0.69	0.69	0.03	0.83	0.82	0.01	ǂ *
Shannon (Exponential) (n=8)	2.27	2.24	0.07	6.08	5.70	0.65	*
Bryophytes (%Cover, n=8)	57.6	58.7	8.21	26.6	32.8	15.34	
Vascular Plants (%Cover, n=8)	0.2	4.5	4.37	60.4	22.7	6.91	*

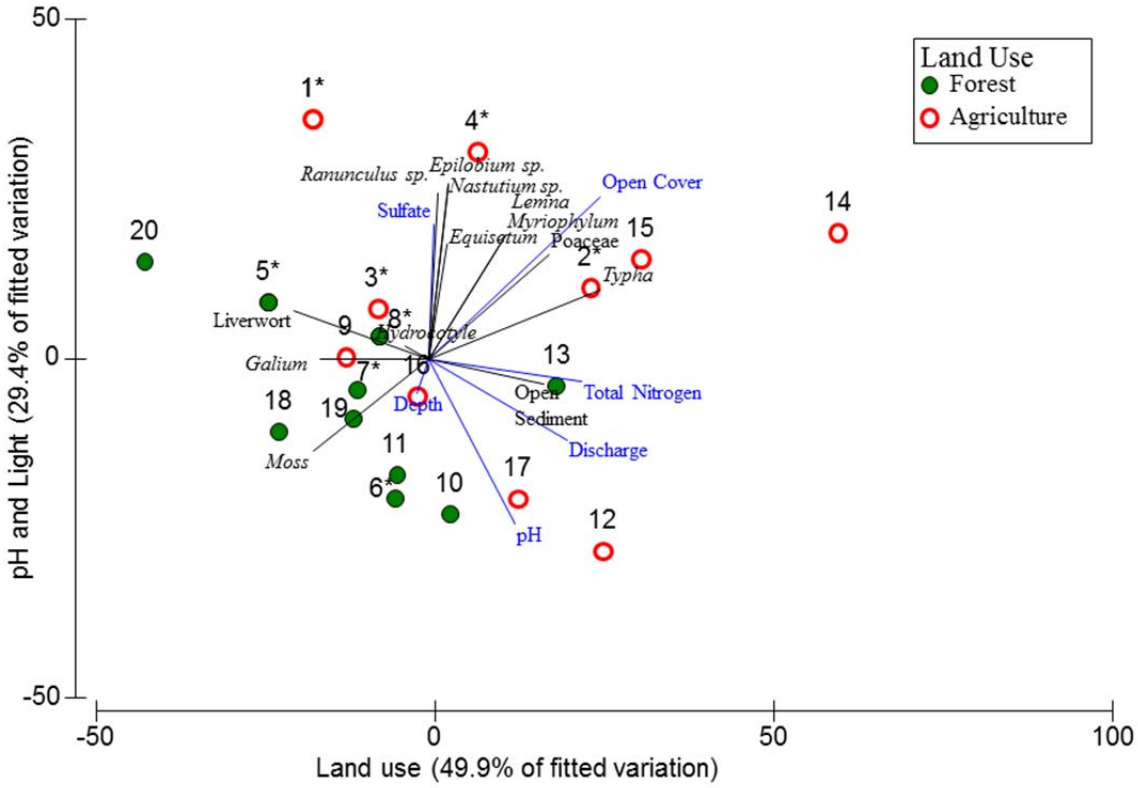

Fig 2.6. Distance based redundancy analysis ordination by-plot (db-RDA) of plant presence or absence in the 20 spring sites (n=10 for each land use type). Plant taxa vectors correspond to more frequent presence in a group of sites of the specified taxa and vectors of environmental variables correspond to sites with higher values of that variable. The numbers refer to the site numbers, and their locations in respect to taxa and environmental variable. Sites used for more detailed biodiversity analysis are starred * and circled. See Table A.2.1.1 for correlations of environmental variables.

whereas forested sites were dominated by liverworts (especially *Chiloscyphus* sp., Geocalycaceae), within the bryophyte taxa group (Table 2.3). Emergent plants (all of which were vascular plants) had their highest cover in agriculture-dominated spring pools, and submerged plants (primarily non-vascular liverworts) had their highest cover at forested sites (Tables 2.3 and Fig. 2.7). However, cover values for individual plant taxa did not differ among forested and agricultural springs (Appendix 2.3, Table A.2.3.2). Taxonomically, the forested biodiversity sites all had very similar species composition (71.5% similarity), whereas the agricultural sites were much more variable (37.4% similarity). Liverworts contributed most to the site similarity within forested sites (SIMPER: 81.42%), whereas watercress (*Nasturtium*) contributed most to the agricultural sites (SIMPER: 43.27%), indicating that these two taxa corresponded with land use type. This pattern was seen again when considering taxa that contributed most to the dissimilarity of forested vs agricultural sites (SIMPER: 23.4% and 30.1% respectively) with higher liverwort cover in forest springs, and higher *Nasturtium* cover in agricultural sites. Overall, the vegetation patterns between the sites in the two land use categories were 61.73% dissimilar.

2.3.3. Invertebrate community patterns

2.3.3.1. Diversity patterns

Observed total invertebrate richness (number of taxa) and density (number of organisms/m^2) did not vary significantly between the forested and agricultural springs in the biodiversity sites (Table 2.4). However, richness measures estimated using rarefaction analysis (to provide a more standardized comparison among land use types) showed

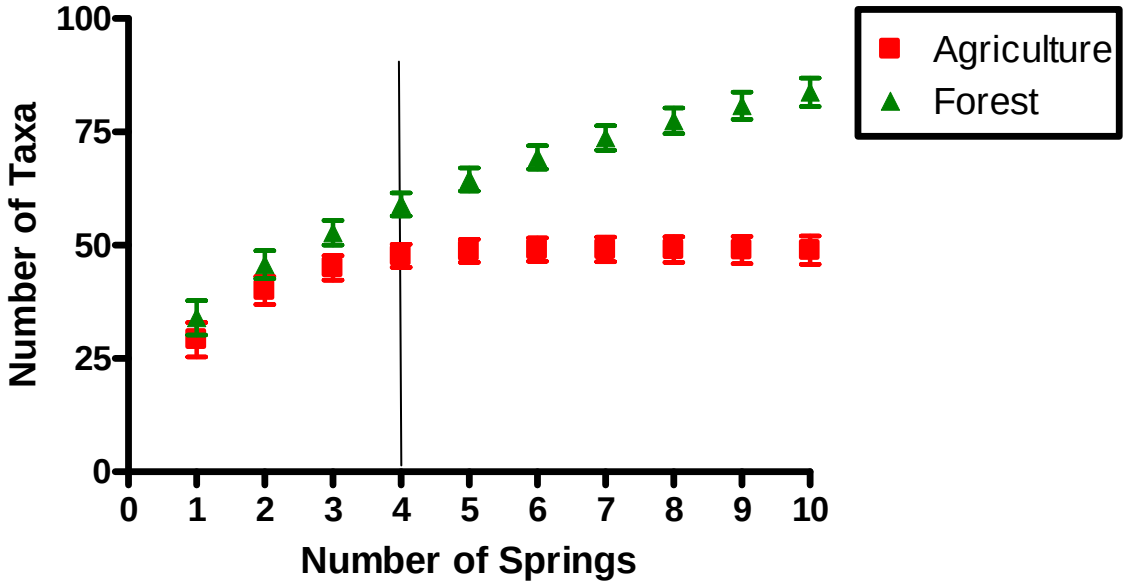

Fig. 2.7. Comparison of invertebrate taxa richness (±SE) in agricultural and forested springs extrapolated using rarefaction analysis. The vertical line indicates the number of springs sampled to generate estimates in each land use category.

Table 2.4. Comparisons of community patterns, feeding group patterns and life-habit patterns between the two land use categories (n=4, 4). Densities are presented as number of invertebrates/m^2 for the two samples for each spring. Taxa richness estimates of invertebrate diversity were calculated in EstimateS. Differences between land use were compared using a one-way ANOVA (DF=1,7) or Mann-Whitney U-test (ǂ). *p<0.05

	Forest sites (n=4)			Agriculture sites (n=4)			
Variable	Median	Mean	SE	Median	Mean	SE	*p*
Taxa Richness	34.0	32.5	3.2	33.5	33.0	1.5	
Chao 1	N/A	59.0	N/A	N/A	51.0	N/A	N/A
Evenness (J')	0.6	0.6	0.01	0.7	0.7	0.01	*
Shannon (H' Exponential)	10.7	10.3	1.10	12.5	12.4	0.87	
Densities of:							
Invertebrates	55908.3	46212.3	11059.3	24777.9	31089.7	11322.0	
Shredders	778.6	1481.4	807.9	1875.3	2020.2	259.9	
Scrapers	328.0	3350.2	3080.4	404.8	657.1	395.6	
Predators	4479.2	5638.7	2080.9	1419.6	3190.8	2040.3	
Collector-Gatherers	40871.4	35446.9	8687.9	18822.3	24151.6	9469.7	
Collector-Filterers	124.8	289.1	217.0	314.6	1070.0	852.3	
Burrowers	436.6	802.2	430.94	1129.7	2359.8	1307.1	
Clinger	255.4	317.2	145.8	246.5	458.2	251.3	
Climbers	8256.0	8382.6	2605.1	3451.3	11423.9	8446.7	
Sprawlers	36360.1	31994.9	8146.04	8099.7	7138.2	1694.92	*
Swimmers	51.8	54.5	23.5	58.7	102.0	82.1	
Planktonics	9.5	4661.0	4654.7ǂ	10263.9	9589.6	4421.1ǂ	

ǂ denotes a variable that was not normally distributed; SE is listed for comparison of deviations around means.

higher diversity (richness and the Chao 1 estimator) in the forested sites than the agricultural sites (Table 2.4). The sample size of four sites per land use category was too small to provide accurate richness estimates (especially for the forested sites), since new taxa continued to be added to the taxa list with each spring examined, and no asymptote was reached (rarefaction analysis, Fig 2.7). The small number of spring sites may also explain why the overall invertebrate community distribution (calculated from absolute densities) did not differ significantly between land uses categories (PERMANOVA).

The groups of species in each land use category showed even dispersion of taxa (PERMDISP); like the plants, the invertebrate community was more even in agricultural sites, whereas forested sites were dominated by particular taxa (Table 2.4). For example, the orthoclad midge *Thienemanniella* had significantly higher densities for forested springs (One-way ANOVA; Appendix 2.2). *Thienemanniella* was the greatest contributing taxa to the dissimilarity between springs between the two land use categories (SIMPER: 10.27%) and to the similarity within forested sites (SIMPER: 22.78%). The next taxon that differed significantly in density between land uses was the mite genus *Sperchon*, which was more abundant in the forested sites than the agricultural ones (One-way ANOVA; Appendix 2.4, Table A.2.4.2). However, *Sperchon* was only the 9th most important contributor to the dissimilarity between springs in the different land use types, since its overall abundance was quite low (Table 2.5; Appendix 2.4: Table A.2.4.2). The only other taxon whose density differed significantly between springs in land use categories was the pediciid cranefly *Pedicia* (One-way ANOVA; Appendix 2.4: Table A.2.4.2) with highest densities in agricultural sites. However, the relative abundance of

Table 2.5. Top ten taxa that contributed to the similarity within and dissimilarity between springs in different land use categories (SIMPER analysis). Taxa shown in bold type indicate those whose highest average densities occurred in the land use shown. (For a full list of SIMPER contributions and taxonomic classifications refer to Appendix 2.4. Table A.2.4.1. For a full list of average densities for each taxon see Table A.2.4.2).

	Forest Similarity= 46.84%			**Agriculture** Similarity = 42.19%			**Contrasting Taxa** Dissimilarity = 57.99%	
	Taxon	Average Density	Contribution %	Taxon	Average Density	Contribution %	Taxon	Contribution %
1	***Thienemanniella***	14864.3	**22.78**	**Tanytarsini**	8083.3	**10.16**	*Thienemanniella*	10.27
2	***Sperchon***	2854.5	**9.76**	**Ostracoda**	6296.7	**9.60**	Ostracoda	8.05
3	***Panisopsis***	1582.6	**7.35**	*Thienemanniella*	1357.1	6.77	Tanytarsini	5.97
4	Tanytarsini	2053.8	6.35	*Sperchon*	361.6	5.17	*Orthocladius/ Cricotopus*[1]	4.97
5	***Orthocladius/ Cricotopus***[1]	4469.2	**6.26**	***Metriocnemus***	702.4	**4.86**	*Parochlus*	4.71
6	***Corynoneura***	1739.3	**5.50**	***Sweltsa***	367.0	**4.55**	*Panisopsis*	4.24
7	***Rheocricotopus***	1236.9	**4.35**	*Lepidostoma*	487.6	4.44	*Hydrobaenus*	4.11
8	***Parochlus***	3172.6	**3.94**	***Nemoura***	423.4	**4.16**	Cyclopoida	4.00
9	***Hydrobaenus***	3236.2	**3.26**	***Pisidium***	1070.0	**3.99**	*Sperchon*	3.96
10	*Nemoura*	232.1	2.74	***Heterotrissocladius***	285.7	**3.51**	*Eukiefferiella*	3.64

[1]This taxon can also include *Paratrichocladius* (Ferrington et al. 2008), and may be present in this study (Appendix 4, Table A.4.2.1).

this taxon was not a great contributor to the similarity or dissimilarity between land use (SIMPER Dissimilarity: 0.55% ; Table 2.5).

2.3.3.3. Community functional roles

Abundance patterns for invertebrates grouped by functional roles in the community showed little difference between land use categories. Numbers in the different functional feeding groups (shredders, scrapers, collectors, predators) and in most life-habit groups (e.g., burrowers, swimmers, etc.) did not differ between springs in the two land use categories (PERMANOVA; Table 2.4). However, sprawler density was higher in agricultural sites than forested ones, and was the only community factor to differ significantly between land use categories (One-Way ANOVA; Table 2.4).

2.3.3.4. Community structure patterns

Relationships between densities of individual invertebrate taxa and habitat variables were more clearly illustrated through distance-based redundancy analysis (db-RDA), with nearly two-thirds of variation in the invertebrate community explained by the first two axes (Fig. 2.8). Invertebrate taxa patterns related most strongly to vegetation patterns, both in the surrounding riparian zone and macrophytes within the spring pools The first axis (explaining 39.1% of the variation in community structure) corresponded primarily to deciduous tree densities, with the second axis (explaining 22.7% of variation) corresponding to relative bryophyte (mainly liverwort) or vascular plant cover (Fig. 2.9). Several invertebrate taxa, including the stonefly *Nemoura* (ρ=0.8), the midges *Corynoneura* (ρ=-0.9), *Heterotrissocladius* (ρ=0.8), and *Platysmittia/Psilometriocnemus*

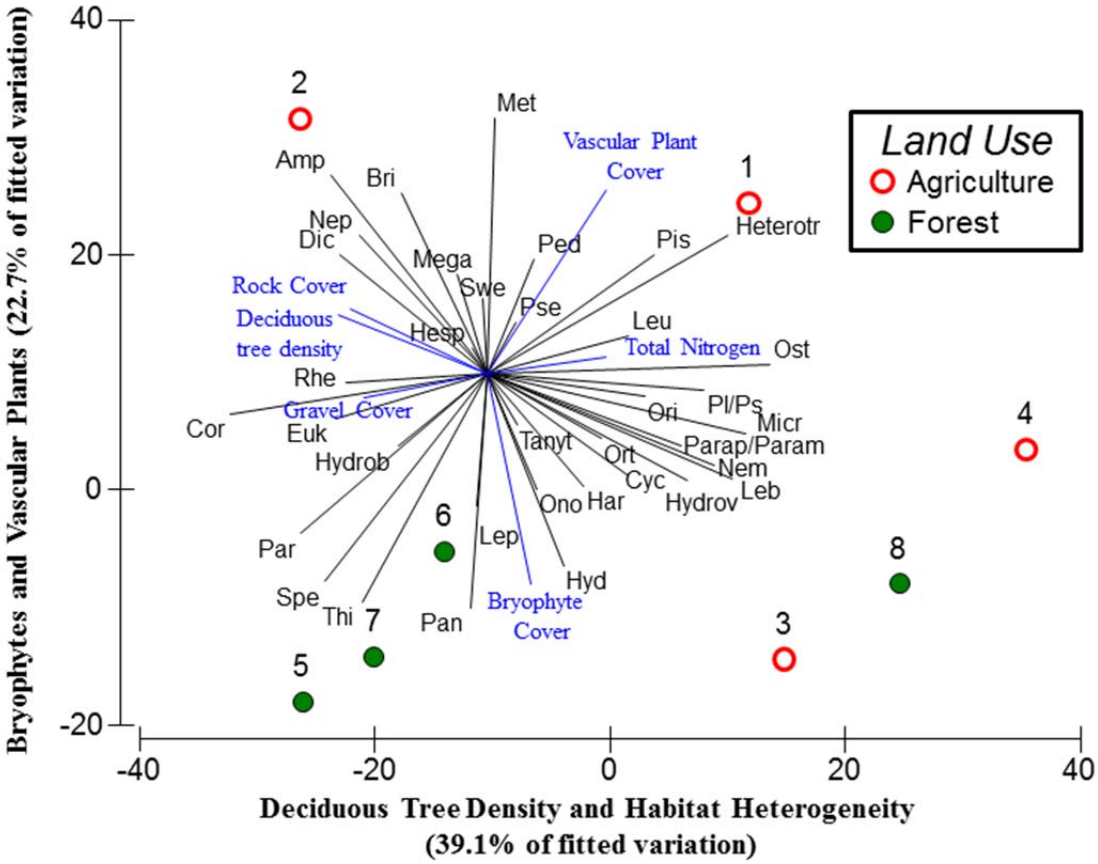

Fig 2.8. Results of distance-based Redundancy analysis of invertebrate taxa (present at ≥4 sites) with environmental variables. Vectors of taxa correspond to higher densities. Environmental variables pointing in the same direction represent a positive correlation with that variable, and longer lines represent stronger explanatory relationships with the axis. Abbreviations for individual taxa are defined in Table A.2.4.1.

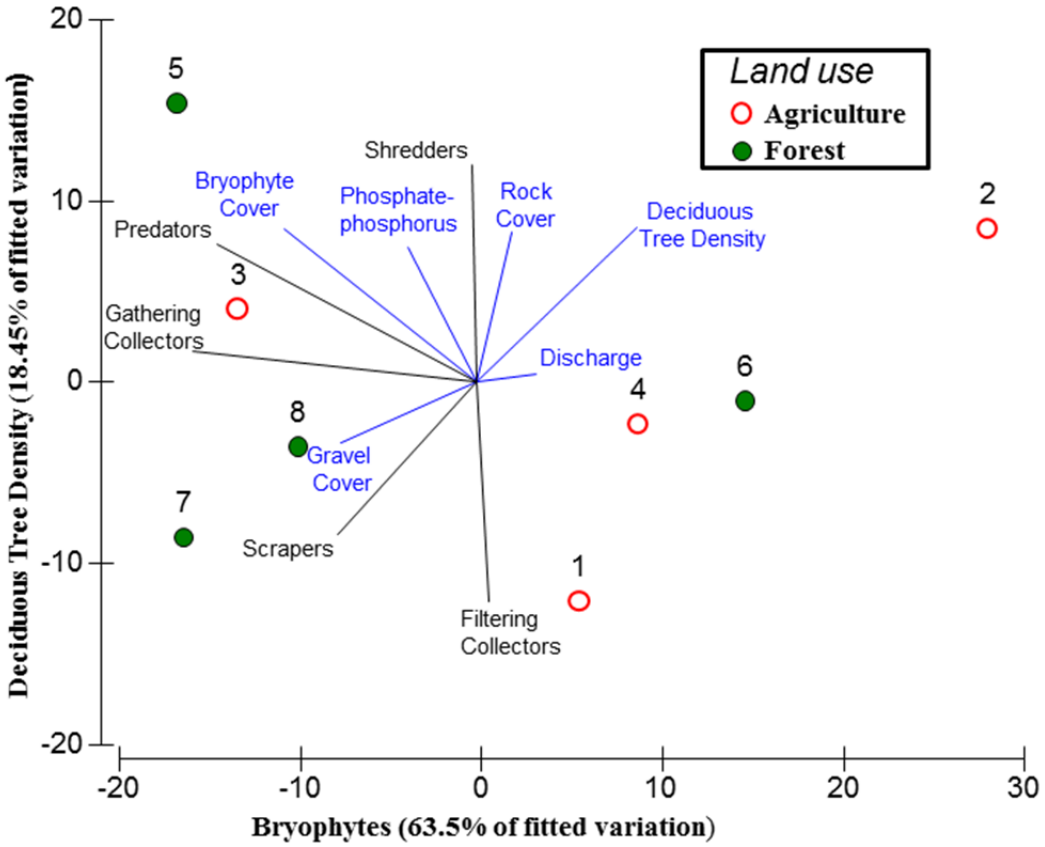

Fig. 2.9. Results of distance-based redundancy analysis of functional feeding group relationships and measured environmental variables that best explain the variation of the assemblages.

(ρ=0.7), the water mite *Lebertia* (ρ=0.8), the Ostracoda (ρ=1.0), and the oligochaete group, Microdriles (ρ=0.9) were associated with deciduous tree density and habitat heterogeneity (mainly substrate variability and Nitrogen), so were highly correlated with the first axis. In contrast, the midges *Metriocnemus* (ρ=0.9) and *Thienemanniella* (ρ=-0.8), and the water mites *Panisopsis* (ρ=-0.8) and *Sperchon* (ρ=-0.7) were associated with bryophyte cover, so were correlated to the second axis. The third db-RDA Axis explained 14.4% of the variation and was primarily related to variability in gravel cover. The taxa that correlated with this axis included the stonefly *Leuctra* (ρ=-0.7), the caddisfly *Neophylax* (ρ=-0.7), and the midges *Eukiefferiella* (ρ= 0.7) and *Hydrobaenus* (ρ=-0.7).

Functional feeding group patterns with habitat variables showed stronger patterns than those for individual taxa (explaining nearly 82% of variation in fitted data in the first two axis), though the main habitat variables driving abundance patterns were the same as for the individual taxa (Fig. 2.9). Bryophytes were associated strongly with the first axis (64.1 % of the total variation) whereas the second axis was strongly associated with deciduous tree density (18.63% of the variation in the assemblage). Collector-gatherers (ρ=-1.0), and predators (ρ=-0.9) had highest densities in springs with high bryophyte cover, so their densities correlated with the first axis. In contrast, shredder (ρ=0.7), and collector-filterer (ρ=-0.7) densities correlated with the second axis (deciduous vegetation).

Life-habit type also showed strong patterns with habitat variables (85.8% of the fitted variation explained in the first two axes (Fig. 2.10), but the most important variable affecting life-habit was substrate type, rather than vegetation. The first axis (52.4% of the variation) was strongly associated with the cover of gravel, and bryophytes correlated

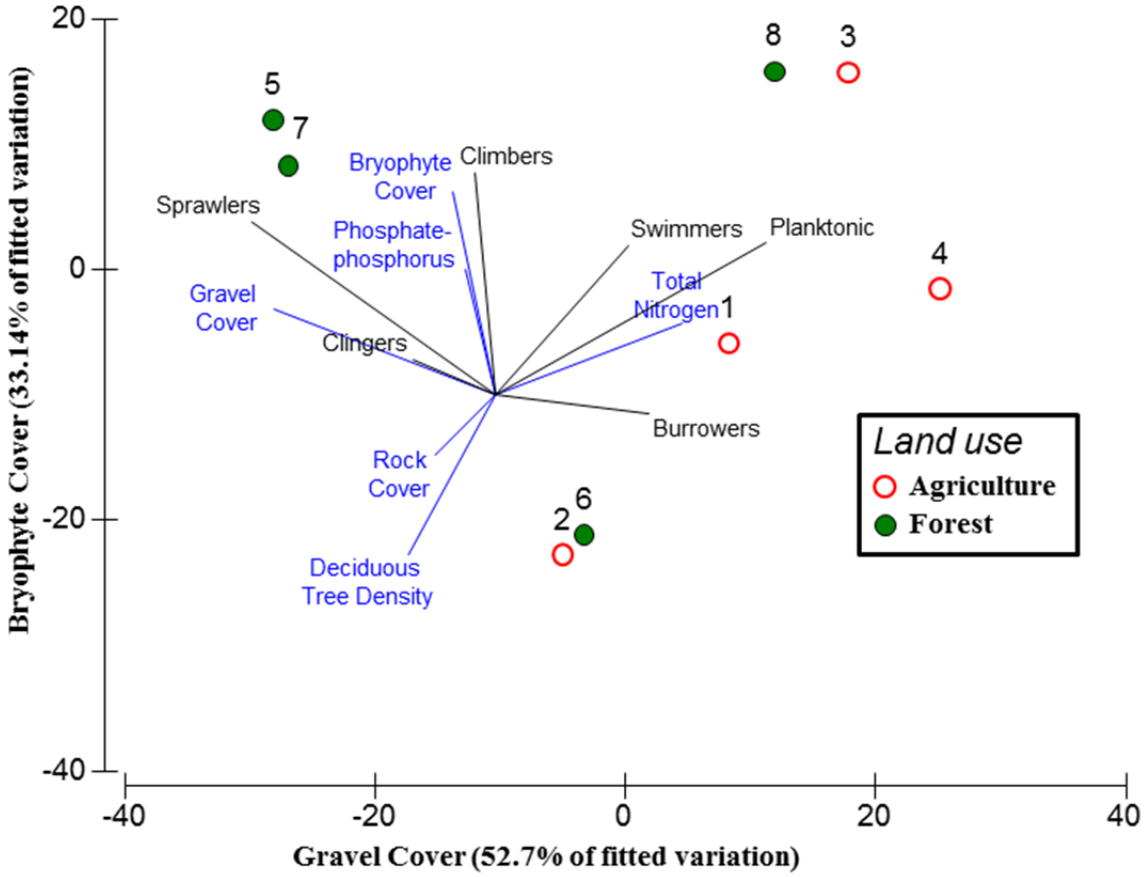

Fig 2.10. Results of distance-based redundancy analysis of life-habit group relationships compared to environmental variables that best explain the distribution of habit assemblage from DISTLM.

strongly with the second axis (34.2% of the variation). Densities of the sprawler group increased with higher gravel cover (ρ=-0.8) and densities of planktonic taxa declined with increasing proportions of gravel (ρ=0.9), so their densities were correlated with the first axis. Climber taxa (ρ=0.7) had their highest densities in sites with high cover of bryophytes, so their densities correlated with the second axis.

2.3.3.5. Summary of overall invertebrate community patterns with environmental variables

Taxonomic and community patterns in the springs could be predicted by relatively few habitat variables, though most of these were only indirectly related to agricultural activities. Aquatic plant type (dominance of bryophytes vs vascular plants) played a major role in structuring the invertebrate communities, and although aquatic plants differed among land use categories, plant-type related more to light availability, and in turn riparian cover, than to water quality patterns. Substrate variables were also important, especially the presence of clean gravel. Other important factors related to the riparian zone around the springs. The depth of the organic soil layer (which was itself related to forest type) was a good predictor of invertebrate community composition, and densities of riparian deciduous trees predicted patterns for feeding and life-habit group assemblages as well (Fig 2.9, 2.10). In contrast, nitrogen (total nitrogen and nitrate) was strongly associated with the amount of agriculture around the spring and was also expected to show relationships with invertebrate patterns, but was not as strongly correlated as expected. The forward-stepping procedure (where variables were added if they contributed significantly to the model) excluded nitrogen concentration variables at

the analysis stage, suggesting that nitrogen was not as predictive as other variables, or that it may have been correlated with other variables (e.g., plant type). Since nitrogen variables correlated with some life-habit assemblages (e.g., abundance of planktonic or swimming taxa), it is likely that the invertebrates were responding more to the habitat structure (e.g., light availability) than to the presence of nitrogen directly (Fig 2.10).

2.4 Discussion

The aim of this study was to examine the influences of surrounding agricultural land use on the biota of cool springs. Agricultural activities, especially nutrient and sediment additions, were expected to affect biota by changing plant communities and habitat structure. Springs in agricultural areas did show different plant communities compared to forested areas, and this in turn altered the structure of the invertebrate community. However, the main factor that correlated with the change was a difference in the structure of the riparian area that allowed greater light penetration to the spring pool and the direct effects of nutrient addition were not as clear. This pattern may relate to the type of nutrients available in these springs, or other limiting factors.

Nitrate and phosphate are the two main agricultural nutrients associated with eutrophication of fresh waters (van der Kamp 1995; Vought et al. 1995; Barquin and Scarsbrook 2008), but neither nutrient appeared to be limiting in the PEI springs. Nitrate was the strongest chemical predictor of the extent of agricultural activity around the study springs, supporting previous study demonstrating the association between agriculture and nitrate concentrations in PEI streams and groundwater (Jiang and Somers 2009; Bugden et al. 2014). In contrast, relatively high levels of phosphate (average

concentrations of 40 µg/L) were found in all study springs, with no differences between springs in forested or agricultural areas. Phosphate usually binds to the iron-rich soil in PEI, rather than remain in soluble forms in groundwater (Somers et al. 1999), though this pattern can be influenced by the relative solubility of different forms of iron with oxygen (Hupfer and Lewandowski 2008). The tendency to adsorb to soil particles may explain why there was no relationship between land use and dissolved phosphate concentrations in this study. The only phosphate pattern noted was a weak relationship between phosphate, oxygen and discharge. Other factors that influence dissolved phosphorus release include bacterial and algal uptake, sulphate and aluminum levels (Hupfer and Lewandowski 2008); only sulphate was measured in this study and no correlation was seen with phosphate. Since nitrogen is clearly higher in agricultural streams, and phosphorus did not appear to be limiting, it was surprising that neither plant growth nor invertebrate patterns related to nitrogen concentrations. Even in the presence of high nutrients, primary producers can be limited by shading (Burrell et al. 2014).

In contrast to nutrients, light limitation, specifically the amount of canopy shading on the spring pool, showed a much clearer relationship to the types and cover of aquatic plants present, with bryophytes dominating in shaded forest springs and vascular plants in the more open agricultural springs. Low light is known to limit the vascular plant diversity in springs in other locations (Beierkuhnlein and Gräsle 1998; Spitale et al. 2009). Light competition is a major driver in botanical communities in freshwaters, affecting both diversity and morphology of the plant community (Beierkuhnlein and Gräsle 1998; Lacoul and Freedman 2006). Emergent plants (the vascular plants in this study) that can grow above the water have a light advantage over bryophytes such as

liverworts that cannot grow above the water, so the vascular plants may limit other plant growth where they are present (Beierkuhnlein and Gräsle 1998). Bryophytes are better adapted to low light conditions, with physiological adaptations to photosynthesize with limited light (Marschall and Proctor 2004).

The differences in plant community strongly affected the invertebrate community, likely through a combination of food availability and habitat complexity. Bryophyte presence was one of the best predictors of overall invertebrate patterns in these study springs, and invertebrate density and diversity was highest in the bryophytes. The bryophytes provide a complex leafy aquatic habitat for clinging and sprawling invertebrates (Lacoul and Freedman 2006; Ilmonen and Paasivirta 2005), and many mite (Smith 1991) and midge species (Virtanen et al. 2009; Lencioni et al. 2012) strongly select this microhabitat type. Both mites and midges were important in the bryophyte habitat in this study. In contrast, emergent vascular plants have relatively low habitat complexity below the water surface (mainly stems), and may shade the spring-bed as well, limiting micro-algal (diatom) growth and also the type and diversity of invertebrates selecting this habitat (Lacoul and Freedman 2006; Wojtal and Sobczyk 2012). Diatoms are an important food source for scraper invertebrates (Cummins 1973), and diatom abundance is related strongly to light, nutrients, and the availability of a clean substrate on which to grow (Wojtal and Sobczyk 2012). In springs, bryophyte leaflets can trap organic matter (Bottazzi et al. 2011), and the leaflets and clean gravel are both important substrates for algal growth (Wojtal and Sobczyk 2012), so their presence increases food availability for a variety of groups. Diatoms were not measured in this study, but gravel cover was related to abundance of one of the dominant scrapers, the midge *Hydrobaenus*.

Ostracoda presence and abundance (a group that feeds on pelagic and epiphytic algae; Delorme 2001) also correlated to deciduous tree density, light, and substrate variability. Erman (2002) found that caddisfly species richness was higher in shaded springs, so the effects of shading are not limited to smaller sized taxa. Where light is not limiting, for example in spring systems that naturally have little cover, such as spring fens, water chemistry can be major driver of diversity (Omelková et al. 2013).

Although light limitation may have obscured any direct effects of nutrient levels in the study springs, nutrients may still play a role in plant biomass. Nitrogen can be a strong predictor of macrophyte biomass in springs (Beierkuhnlein and Gräsle 1998; Mebane et al. 2014). Watercress (*Nasturtium*), the primary vascular plant species in these springs, grows faster and larger under high nutrient conditions (Fernandez-Going et al. 2013) as well as needing high light conditions for growth (Going et al. 2008). In the present study, Tanytarsini (chironomid midges, mainly consisting of *Microspectra*) densities were highest in high nitrogen springs. *Microspectra* is a potential indicator of nutrient enrichment in Ontario spring-brooks (Oliver and Dillon 1994), and other Tanytarsini species have been variously reported as indicators of natural springs and springs impacted by livestock (Keleher and Radner 2008; Lencioni et al. 2012). Further taxonomic resolution would be needed to define which Tanytarsini genera are driving the correlation and which factors were responsible. Reported nutrient effects on European springs have been conflicting, with some studies reporting declines in both bryophyte and vascular plant diversity with increased nutrients (Spitale et al. 2009; nutrients inferred from conductivity readings) and others finding clear relationships between conductivity and the plant communities (e.g., Kapfer et al. 2012). These differences may relate to the

interactions between nutrients and light availability, or to the types of nutrients present. For example, sulphate levels appear to have affected plant community structure in this study, and sulphate is not a commonly measured nutrient in stream studies, even though it is another commonly elevated anion in eutrophic environments (Lammers et al. 2002). Elevated sulphate levels (e.g., 100 mg/L) can be toxic to some wetland plants (e.g., Lammers et al. 1998) and aquatic mosses (e.g., Davies 2007) so can help structure wetland plant communities (Lammers et al. 1998), although sulphate levels did not approach toxic concentrations in the study springs. Very fine sediment was not quantified separately in this study; however, vascular plants need finer sediments in which roots can grow and draw nutrients (Giller and Malmqvist 1998). Agricultural springs had visibly higher amounts of fine sediment covering substrate materials, took longer for the water to clear when disturbed, had higher numbers of vascular plants, and more burrowing taxa, such as *Pedcia* than forested springs. Increased numbers of burrowing taxa is one response to increased sedimentation (e.g., Griffith et al. 2009). The connection between the plant communities and the invertebrates, as discussed above, was primarily structural, providing habitat for the invertebrates. However, the combination of nutrients and light availability may also affect other food resources in the spring, such as the microscopic algal community, including diatoms (Griffith et al. 2009; Wojtal and Sobczyk 2012).

This study has demonstrated that measuring nutrients or sediment addition alone would have provided an incomplete picture of agricultural impacts on springs. The primary "agricultural" factors affecting the springs in this study were those that affected light availability to the spring, and potential impacts from commonly measured nutrients (nitrate, phosphate) or sediment (e.g., Williams et al. 1997; Barquin and Scarsbrook

2008; Griffith et al. 2009) were obscured by the larger effects of light limitation. Other agricultural contaminants (e.g., sulphate) may need to be investigated in greater detail (excluding mineral springs where sulphates are naturally high, e.g., Omelková et al. 2013). The extent of the riparian area is widely known to be important in maintaining spring health (Erman 2002; Griffith et al. 2009; Barquin and Scarsbrook 2008); this study shows that the species composition within the riparian zone is also critically important, specifically relating to providing shade to the spring pool. Future studies could evaluate whether planting of shrubs to stabilize soil and shade the spring bottoms could return springs in agricultural areas to a more natural habitat. Shading has mitigated effects on primary production in other eutrophied landscapes (Burrell et al. 2014).

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Chapter 3

Factors Affecting Species Composition, Emergence Phenology and Habitat Preferences of Ephemeroptera, Plecoptera and Trichoptera in Rheo-limnocrene Springs.

Abstract

Temperate zone cool-springs have been poorly studied in many regions, despite their importance as "biodiversity hotspots" and indicators of groundwater conditions. Their low (usually <10^{o}C) and constant temperatures limit the diversity of aquatic insects by affecting developmental timing and life cycle synchrony. Therefore, cool-spring taxa are cold stenotherms that are often regionally rare, with asynchronous life cycles. Other habitat factors, such as food availability, also influence diversity, abundance, and life cycle timing, but their effects are often difficult to distinguish from temperature responses. Life cycle, diversity, and abundance patterns of emerging aquatic insects (Ephemeroptera, Plecoptera, Trichoptera; "EPT" species) were examined in nine rheo-limnocrene springs in eastern Prince Edward Island (Canada) from May-October 2011 to assess species responses to habitat and resource availability in these constant temperature environments. Adults were collected from emergence traps and riparian sweep samples along with environmental measurements in the nine spring sites: five surrounded by agricultural land and four surrounded by forest. Twenty-four EPT species were recorded around the springs, 17 of which could be definitively associated with the spring pool habitats. Ephemeroptera were too rare to assess abundance patterns (although the study produced several new provincial distribution records), but most Plecoptera and two Trichoptera species showed asynchronous emergence patterns. Diversity and abundance of all Plecoptera and most Trichoptera were highest in springs influenced by agriculture, with only one species group showing negative impacts of agriculture-related habitat alteration.

3.1 Introduction

Springs occur at the interface between groundwater, surface water and terrestrial systems, have generally stable temperature and hydrological patterns (van der Kamp 1995; Cantonati et al. 2012), and chemical and physical properties that depend on local geology, climate, and land use (van Everdingen 1991; Barquin and Scarsbrook 2008; Chapter 2). These factors influence the diversity and type of organisms that live in springs, as well as their life history characteristics (e.g., Barquin and Death 2009; Barquin and Death 2011; Lencioni et al. 2012; Bottová et al. 2013. Although many springs are small relative to other aquatic habitats, they are important biodiversity "hot spots" and may support a number of rare species (Cantonati et al. 2012).

Cool springs, where water temperatures approximate the mean annual temperature for the region, are widely distributed (van Everdingen 1991; Cantonati et al. 2012), but have not been as well studied as other running water habitats (Danks and Williams 1991; Cantonati et al. 2012). Recent ecologically-based studies in Europe and New Zealand have provided considerable information on the links between spring characteristics and biotic responses, but despite the importance of local geology and land use on these responses, little is known about these questions in other regions (Williams 1991; Gathmann and Williams 2006; Smith 2010). In Prince Edward Island (PEI), Canada, all rivers and streams are fed by groundwater springs (Somers et al 1999; Klassen and Locke 2010) that maintain a near-constant water temperature of ~7^{o}C (Chapter 2). Nearly 40% of the total land surface (of ~5660 km^{2}) is agricultural land use (PEI Department of Agriculture and Forestry 2013) leading to localized differences in groundwater chemistry and habitat conditions among springs (Chapter 2).

Aquatic insect life histories (e.g., length of growing period, emergence timing) are strongly influenced by environmental cues such as temperature (Sweeney 1984), day length (Hynes 1970), and food availability (Williams 1991), so these factors influence species composition and diversity patterns. Constant low temperatures common in temperate zone cool-springs restrict which species can survive and develop (Williams 1991; Williams and Williams 1998), and also remove needed environmental cues for emergence (Dobrin and Giberson 2003). Therefore, cool springs may have impoverished, cold-tolerant insect communities (von Fumetti et al. 2007; Barquin and Death 2011) with flexible life cycles and extended emergence periods (Williams et al. 1995; Dobrin and Giberson 2003). Life cycle patterns and abundance may also be affected by the type and amount of food available (Sweeney 1984; Williams 1991), but food responses can be difficult to distinguish from temperature ones unless temperature can be controlled. The objective of this study is to determine the species composition, diversity and emergence patterns of the mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddisflies (Trichoptera) ("EPT" groups) in nine PEI cool-springs that differ in surrounding land use, and relate EPT diversity, abundance, and life history patterns to measured habitat factors. Species in constant temperature springs should have extended emergence timing compared to fluctuating temperature habitats, and their abundance and diversity should relate to surrounding land use variables that alter food availability or habitat structure. Overall abundance should be higher in high-nutrient than low-nutrient springs due to increased food availability, and diversity should also be highest in springs with high substrate and habitat heterogeneity.

3.2 Methods

Adult mayflies, stoneflies and caddisflies were collected concurrently with habitat data from nine rheo-limnocrene springs (small pool-type springs with outflows) in eastern Prince Edward Island, Canada (Chapter 2; Fig. 2.1: Sites: 1-9). Springs were chosen based on surrounding land use (to provide an approximately equal number of forested and agricultural springs for a concurrent study on land use effects; Chapter 2) and year-round accessibility.

3.2.1 Study Area

Prince Edward Island is in the Atlantic Maritime Ecozone (Miller 2010; Eco-Province 130) with historical forest cover consisting of mixed Acadian Forest (primarily *Abies balsamea* (L.) Mill (balsam fir) and *Acer rubrum* L. (red maple)). The present-day forest is now dominated by secondary succession species such as *Picea glauca* (Moench) Voss (white spruce) and *Populus tremuloides* Michx. (trembling aspen) (Loo et al. 2010; PEI Department of Agriculture and Forestry 2013). Overall, ~44% of the land area of PEI is forested and ~40% under agricultural production (Jiang and Somers 2009; PEI Department of Agriculture and Forestry 2013), but eastern PEI is generally more forested than the central parts of the province. All study springs had at least a 15 m forested zone around the spring, but the riparian forest type varied depending on surrounding land use. Agricultural springs were defined as those with agricultural activity within 20 m of the spring outflow and ≥ 7% agriculture within a 1 km diameter surrounding the spring. Cool-spring riparian zones in agricultural areas were dominated by white spruce and fir with little understory vegetation in the agricultural sites, and by fir and deciduous species

with high amounts of understory vegetation in forested sites (See Chapter 2 and Appendix 2.2 for tree species cover surrounding sites). The average spring water temperature in the study springs is 7.17 $^{\circ}$C ± 0.24 (mean ± standard deviation; Chapter 2).

3.2.2 Environmental Variables

Chemical and physical parameters were assessed during regular visits to the sites, and most parameters were measured approximately monthly between June 2011 and June 2012 (except for winter, when sampling occurred at ~2 mo intervals; see Chapter 2 and Appendix 1.5 for specific details). Water temperatures were monitored continuously (3-hr intervals) using HOBO© tidbit or Water-temp pro V.2 dataloggers between July 2011-July 2012. Air temperatures were obtained from Environment Canada weather station data (http://climate.weather.gc.ca/) at two weather stations located just west and east of the study sites in St. Peters and East Point, PEI and averaged for the two stations. Some chemical parameters were measured on site (pH, dissolved oxygen, and conductivity) using a YSI 550 multimeter or a YSI Professional Plus multimeter, and water samples were collected for laboratory analysis of nitrate (NO_3-N), ortho-phosphate (PO_3^-P), total nitrogen (Total-N), chloride (Cl), sulphate (SO_4) using supressed ion Chromatography (Chapter 2). Discharge (Q) was calculated from the relationship between mean velocity (v) and cross-sectional area (A) of flow (Q=vA). Velocity was measured at 0.6 m depth at regular intervals across the outflow using a HYDRO-PROP stream flow-meter (Great Atlantic Flow Meters. Bank Sq, Penzance, Cornwall, UK) and averaged across the flow. Overhead cover (a surrogate for light penetration) was measured during each site visit using a spherical densitometer (Lemmon 1957). Physical habitat variables measured only

once during the study included pool area, slope of the brook just downstream of the spring pool (determined with a surveyor's level), tree/shrub species composition and density, the type and cover of aquatic vegetation in the pool (percent aquatic bryophyte and percent emergent vegetation), and the substrate type (percent Rocks >6.4 cm diameter, Gravel, and Fines <0.8 cm diameter) (see Chapter 2 and Appendix 1.5 for methods and details of timing).

3.2.3 Insect Collection

Adult Ephemeroptera, Plecoptera, and Trichoptera were collected by a combination of emergence trapping and sweeping and beating riparian vegetation. Four cone-shaped suspended emergence traps were deployed in each spring (300 µm mesh, basal area of 0.07 m^2; Appendix 1.2; Fig A.1.2.2); three were set up above the spring pool and one was set 5 m downstream in the spring-brook. Emerging insects were preserved in 80% ethanol, and traps were emptied every two to three weeks between 19 May and 1 November 2011. Riparian collections consisted of sweeping the vegetation around the spring pool with a sweep net and using a beating sheet under trees and shrubs. Adult collections were compared to larval EPT collections in benthic samples (Chapter 2; Appendix 4) to confirm that they were spring inhabitants. All taxa were identified to genus using keys in Merritt et al. 2008, then identified to species using the most current regional keys for each genus, following the current taxonomy reported in the Mayflies of North America species list (http://www.entm.purdue.edu/mayfly/), the Plecoptera Species File (http://plecoptera.speciesfile.org/) and the Trichoptera World Checklist (http://www.clemson.edu/cafls/departments/esps/database/trichopt/) as of August 2014.

Voucher specimens for each taxon were verified by S.Burian (mayflies), or through examination of verified vouchers in the UPEI insect collection. New record designations were based on checklists for the orders and publications of PEI fauna. Vouchers are presently deposited in the University of Prince Edward Island invertebrate collection.

3.2.4 Statistical Analysis

Habitat preferences (whether species were associated with habitat variables) were assessed for the most common species (>40 individuals and occurrence at ≥5 sites) collected in the emergence traps (or species group for *Lepidostoma* spp., where species could not be separated for adult females). Only emergence trap data were used in this analysis to ensure that the individuals were associated with that spring. Seven species (five Plecoptera and two Trichoptera) were abundant enough for habitat analysis. Relationships among the total number of individuals in each site and the environmental variables in the aquatic habitat immediately below the traps was explored through multivariate ordination (PRIMER V.6 with PERMANOVA+ V1.06; Plymouth, UK). Environmental and abundance variables were transformed as necessary meet the assumptions of multivariate linear models (see Chapter 2 for details on environmental data transformations; total number of individuals was log+1 transformed). Site groupings and inter-correlations among variables were explored through principle components analysis (PCA) and a correlation matrix (Draftsman Plot, PRIMER V.6), and highly inter-correlated variables were removed from the analysis to reduce the number of variables. For example, total nitrogen and nitrate were highly correlated, so only total nitrogen was retained for the habitat preference analysis (see Chapter 2 for more detail on

which variables explained the most variation in a larger group of eastern PEI springs). Variables were further reduced by determining which variables contributed the most to variation in abundance, by comparing total emergence numbers at different levels of the habitat variables using distance based linear modeling (DISTLM) with forward selection (adding variables until there is no improvement to the explained variation), an adjusted R^2 criterion and 9999 permutations (PRIMER V.6 with PERMANOVA+; Anderson et al. 2006). This can be thought of as a series of non-parametric multivariate multiple linear regressions (considering multiple responding variables and each species simultaneously or a multiple linear regression if looking at a single dependent variable in the case of individual species). Variables that did not contribute to the model were removed from further analysis. The relationships between emergence for each species and the remaining habitat variables were then explored through distance based redundancy analysis (db-RDA), which provides a constrained ordination of all species and test variables simultaneously and examines how much variability an environmental variable explains in determine the biotic community (PERMANOVA+).

Emergence patterns were assessed for common species (>40 specimens) to determine whether life history patterns or species composition were influenced by spring habitat variables. Effects of the constant temperature regime on emergence timing was assessed by determining whether emergence timing was synchronous or occurred over a long period, and comparing the emergence patterns to literature information on emergence period in non-spring habitats. The total numbers emerging in each site were compared for each of the nine sampling periods through the emergence season using two-way ANOVA (STATISICA V. 7; Stat Soft 2007); based on a Poisson (Log-linear)

distribution as this distribution is best for species count data (Krebs 1999). Emergence patterns were also assessed visually using graphs of cumulative total individuals over time.

3.3 Results

3.3.1 Environmental variables

Habitat variables varied among springs and many were related to surrounding land use. Nitrogen, sulphate, and specific conductivity (range ~216-289 µS/cm) were generally highest in springs in agricultural areas (Fig. 3.1; see Appendix 2.1, Table A.2.1.2. for average values, and Chapter 2 for discussion of patterns). Dissolved oxygen (range: 7.2-11.3 mg/L) and pH (range: 7.2-7.8) did not relate to land use or nutrient concentrations, but did relate to pool discharge and spring-brook velocity (Fig. 3.1, and see Chapter 2). Several within-pool physical habitat variables also related to land use. Agricultural sites had high amounts of fine substrate (silt and sand) and either primarily sand substrates or fine sediments covering large rocks (thought to have been placed in the springs to stabilize the bed; F.Cheverie, coordinator, Souris and Area Watershed Group, PEI, Personal Communication), whereas forested sites were dominated by gravel with little fine sediment. Aquatic plants varied based on the type and extent of riparian canopy cover; dense canopy cover in forested sites resulted in dominance by aquatic shade-tolerant bryophytes, and vascular plants were dominant in more open agricultural sites (Chapter 2). Water temperature remained very constant year-round at 7.17 ± 0.01 standard error, although springs were warmest in October (based on average maximum temperature) and coldest in April (based on average minimum temperature) (Fig 3.2). The annual average air temperature was 7.3^{o}C (Fig. 3.2).

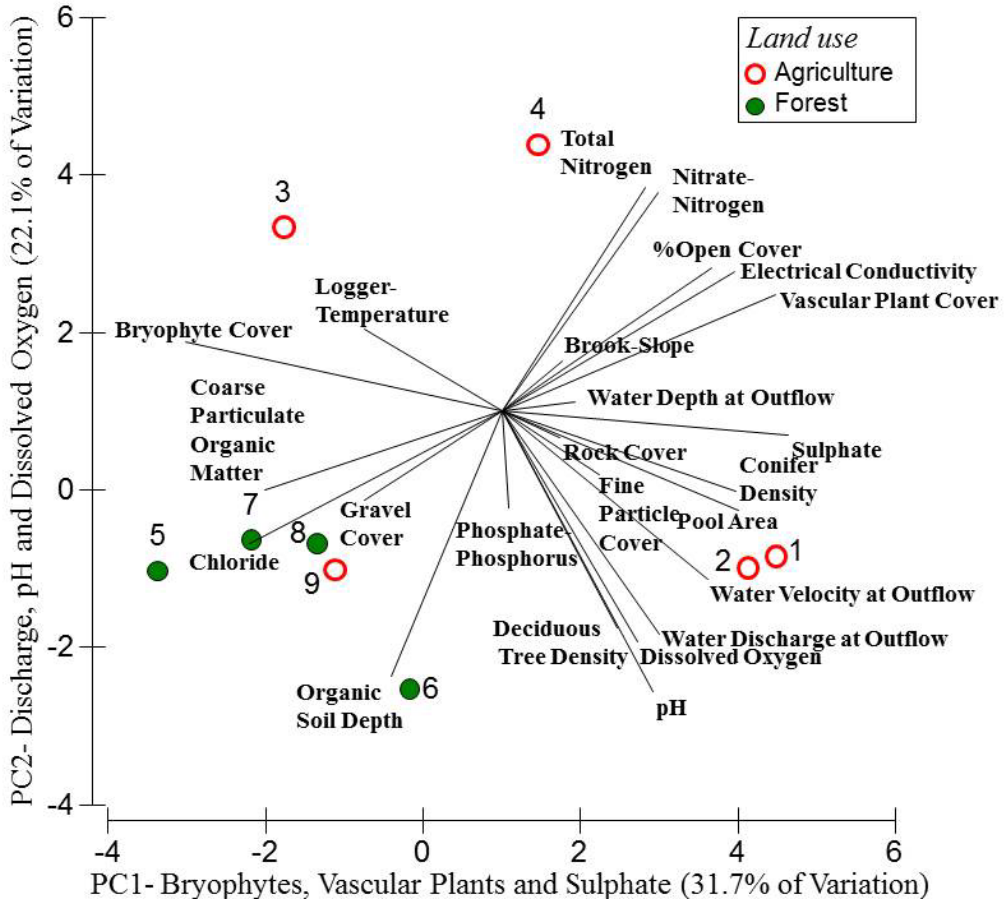


Fig 3.1. Results of the first two axes of Principle Components Analysis (PCA) relating environmental variables for the nine study sites. The variables showing the highest correlation to each axis are identified, along with the percent of variation explained by that axis. Numbers indicate site numbers (see Fig. 2.1 for locations). Closed circles indicate sites that were adjacent to forested land, and open circles are springs that were adjacent to agricultural land.

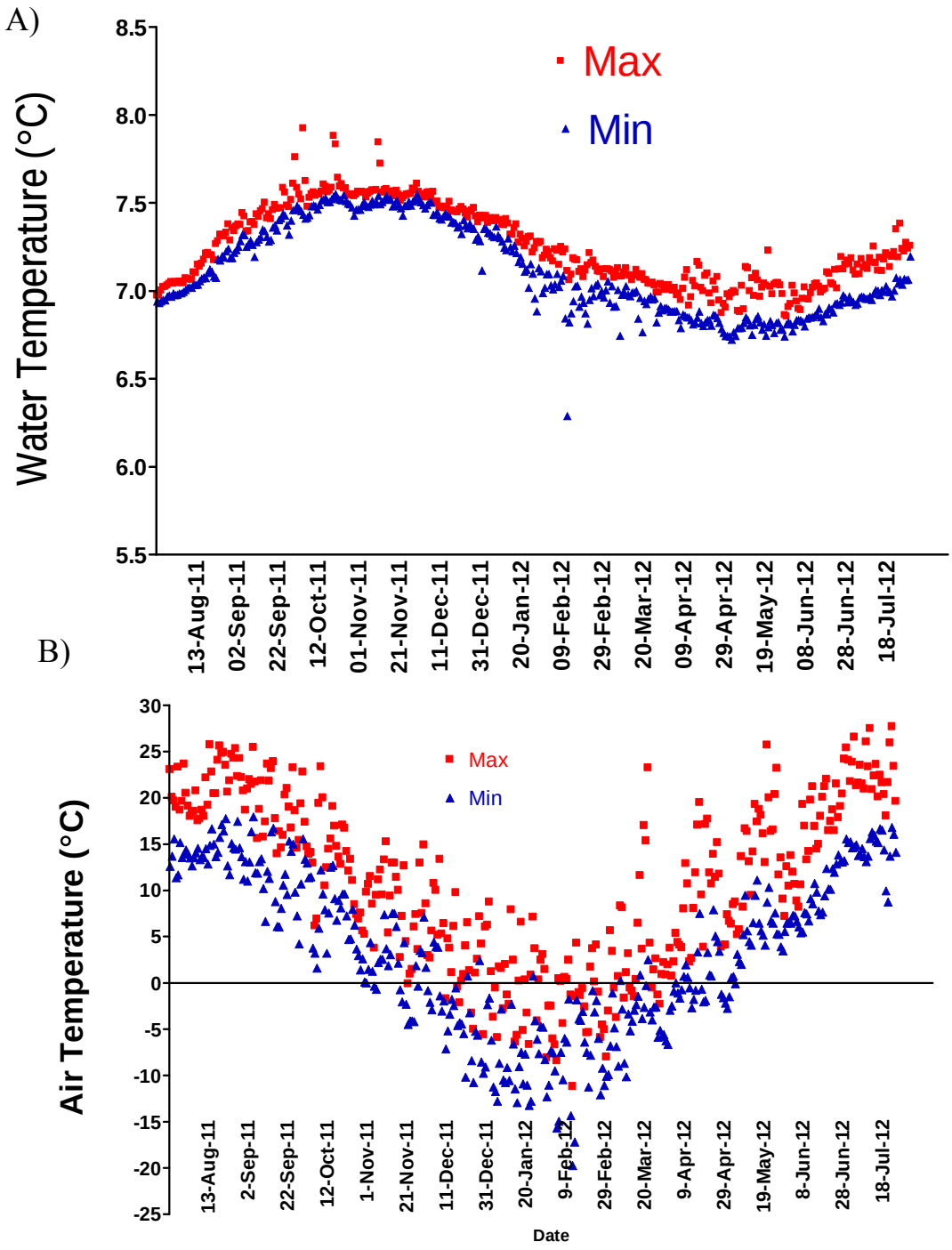


Figure 3.2. Temperature patterns in and around eastern PEI springs between 25 July 2011 and 30 July 2012. Maximum temperatures are shown as red dots, and minimum temperatures as blue dots. A) Average maximum and average minimum water temperature from continuous data-loggers in nine PEI spring pools, and B) the average maximum and minimum air temperatures in eastern PEI (average of two stations located just east and west of the study sites).

3.3.2 Species composition and Emergence Phenology of Ephemeroptera, Plecoptera, and Trichoptera

3.3.2.1 Overall species patterns:

Seventeen of the EPT species collected in adult sampling could be associated with larvae from benthic samples (Chapter 2; Appendix 4.2), so were assumed to have originated from the spring pools (Table 3.1). Seven additional species were collected rarely and could not be confirmed as spring inhabitants since they were not collected as larvae in the spring pools (Table 3.1). Caddisflies made up 47% (54% if all 24 species are included) of the total number of species, followed by the stoneflies with 35% (29% with the additional species), and the mayflies with 18% (17% with additional species). Nine of the 24 species (38%) collected in this study are newly recorded for PEI (Table 3.1). Diversity patterns showed a clear relationship with land-use pattern in the springs. Agricultural springs (n=5) had significantly higher diversity than forested springs (n=4), with an average of 11.2 and 7.5 species from emergence traps in agricultural and forested springs, respectively (one-way ANOVA).

3.3.2.2 Ephemeroptera:

Adult sampling yielded four mayfly species in three families, and three of the species were associated with larvae in the spring pools (Table 3.1). A single specimen of the wetland species *Callibaetis fluctuans* was collected from a riparian sample at one site (Site 2 on 6 July 2011, Table 3.1). The mayflies were generally very rare with only 12 specimens collected over all sites and dates. Two of the species, *Ameletus lineatus* and *Callibaetis fluctuans,* are new provincial records. Although mayflies were collected in

Table 3.1. EPT species recorded from the nine spring sites sampled in eastern PEI, including primary larval ecology, flight period, and number of generations/year reported from the literature. *Denotes new provincial record. A: Agricultural springs; F: forested springs.

Order/Family	Species and authority	Spring type	Larval Ecology	Reported Flight Period	Life cycle length	Notes	Reference source(s)
Ephemeroptera							
Ameletidae	***Ameletus lineatus* Traver, 1932**	A,F	Swimmer Scraper	May (April-June)[1]	1 yr?[1]	Parthenogenetic [1]Life Cycle is based on sister species *A. ludens* Needham, 1905	Burian and Gibbs (1991)
Baetidae	*Baetis tricaudatus* Dodds, 1923	A,F	Swimmer Collector	May-Oct	<1yr		Dobrin and Giberson (2003)
	**Callibaetis fluctuans* (Walsh, 1862)	A	Swimmer Collector	Mar-Aug	<1yr.	Unconfirmed in PEI springs; Open water wetlands.	Johnson et al. (2000)
Ephemerellidae	*Ephemerella invaria* (Walker, 1853)	A,F	Clinger Collector	June-July	1 yr.		Harper (1990)
Plecoptera							
Chloroperlidae	*Sweltsa naica* (Provancher, 1876)	A,F	Clinger Predator	June-July	2 yr.		Dobrin and Giberson (2003)
Perlodidae	*Isoperla* cf. *transmarina (Newman 1838)*	F	Clinger Predator	May-June	1 yr.	Unconfirmed in PEI springs; stream inhabitant.	Harper 1973; Giberson and Garnett (1996)
Leuctridae	*Leuctra ferruginea* (Walker, 1851)	A,F	Sprawler Shredder	May- Dec	1-2 yrs.		Dobrin and Giberson (2003)
Nemouridae	*Amphinemura wui* (Claassen, 1936)	A,F	Sprawler Shredder	May-Aug	1 yr.		Harper and Magnin (1969); Markarian (1980)
	Amphinemura nigritta (Provancher, 1876)	A,F	Sprawler Shredder	May-Aug	1 yr.		Dobrin and Giberson (2003)

Table 3.1. Continued

Order/Family	Species and authority	Spring type	Larval Ecology	Reported Flight Period	Life cycle length	Notes	Reference source(s)
	Nemoura trispinosa Claassen, 1923	A,F	Sprawler Shredder	May-Aug	1 yr.		Harper 1973; Dobrin and Giberson (2003)
	***Soyedina washingtoni* (Claassen, 1923)	A	Sprawler? Shredder?	April-June	1 yr.		Singh et al. (1984)
Trichoptera							
Hydroptilidae	*Palaegapetus celsus* (Ross, 1938)	F	Sprawler Shredder?	May-July?	?	Found only as larvae	Ito et al. 2014
Rhyacophilidae	*Rhyacophila brunnea* Banks, 1911	A	Clinger Shredder?	June-Sept	2 yrs.		Dobrin and Giberson (2003)
Philopotamidae	**Dolophilodes distinctus* (Walker, 1852)	F	Clinger Filtering-Collector	April-Sept	< 1 yr.	Unconfirmed in PEI springs; stream inhabitant.	Swegman et al. (1981); Williams and Williams (1981).
Lepidostomatidae	*Lepidostoma vernale* (Banks, 1897)	A,F	Climber Shredder	May-July	1 yr.		Hogg and Williams (1996)
	** Lepidostoma sommermanae* Ross, 1946	A,F	Climber Shredder	May- Sept	1 yr?		Flint et al. (2008)
Limnephilidae	*Onocosmoecus unicolor* (Banks, 1897)	A,F	Sprawler Shredder	June-Oct	1 yr.		Wiggins and Richardson (1986)
	Hesperophylax designatus (Walker, 1852)	A,F	Sprawler Shredder	May-Sept	1 yr.		Parker and Wiggins (1985)
	**Limnephilus sericeus* (Say, 1824)	A	Clinger Shredder	Aug-Sept	1 yr?	Unconfirmed in PEI springs; Pools in peat bogs.	Swegman et al. (1981); Waringer et al. (2012)
	**Limnephilus moestus* Banks, 1908	A	Clinger Shredder	May- Sept	1 yr?	Unconfirmed in PEI springs; Ponds with sedges.	Nimmo (1971); Swegman et al. (1981)

Table 3.1, Continued

Order/Family	Species and authority	Spring type	Larval Ecology	Reported Flight Period	Life cycle length	Notes	Reference source(s)
	**L. indivisus* Walker, 1852	A	Clinger Shredder	Aug- Oct	1 yr.	Unconfirmed in PEI springs; Temporary pool inhabitant.	Richardson and Mackay (1984); Swegman et al. (1981).
	Psychoglypha subborealis (Banks, 1924)	A,F	Sprawler Shredder	All year?	1 yr.	Adults emerge in fall and overwinter as adults	Denning (1970); Schmid (1998)
	Pycnopsyche gentilis (McLachlan, 1871)	F	Sprawler Scraper	July- Oct	1 yr.		Flint et al. (2008); Betten 1950
Leptoceridae	**Triaenodes tardus* Milne, 1934	A	Climber Shredder	May-Oct	?	Unconfirmed in PEI springs; Aquatic plants, in ponds and lakes.	Glover (1996); Flint et al. (2008)
Uenoidae	*Neophylax aniqua* Ross, 1947	A,F	Sprawler Scrapers	Sept-Oct	1 yr.		Beam and Wiggins (1987)

five of the nine springs (including emergence and riparian sampling together), their numbers were too low to statistically assess phenology or habitat patterns. Adult mayflies were collected from emergence samples only at sites 1, 2 (*Baetis tricaudatus* only), 4 (Both *B. tricaudatus and A. lineatus*), and 5 (*A. lineatus* only), and from the riparian samples at sites 6 (*Ephemerella invaria* and *B. tricaudatus*) and 9 (*E. invaria* only).

3.3.2.3 Plecoptera:

Seven species of stonefly in four families were collected in and around eastern PEI springs (Table 3.1). All were associated with larvae from the spring pool benthic samples except a single specimen of *Isoperla* sp. (cf. *I. transmarina* based on Hitchcock 1974) (Perlodidae) collected from a spring brook trap at site 2 on 26 July 2011. Stoneflies were common in the spring pools, with 1,233 specimens collected from the combination of emergence trapping and riparian sampling. All spring-dwelling stoneflies that were captured in the riparian area were also captured in the emergence traps, and all species were abundant enough to assess pheonology except *Soyedina washingtoni* (Nemouridae). *Soyedina washingtoni* was the rarest of the cool-spring stonefly species, and is also a new provincial record for PEI.

Most of the stoneflies emerged throughout the summer, showing extended emergence periods (Table 3.2), with two exceptions. *Sweltsa naica* showed a synchronous emergence period with a clear peak in early June (Table 3.2). Though very few individuals of *Soyedina washingtoni* were collected, this species also showed a similar emergence pattern to *Sweltsa naica*. Stoneflies with extended emergence

Table 3.2. Emergence phenology for Ephemeroptera, Plecoptera, and Trichoptera confirmed in eastern PEI springs. Shading indicates intervals when the species was found in traps or sweep samples (total numbers from 36 traps (four in each of nine springs) and from riparian sweep samples (in parentheses) when traps were emptied). Traps were deployed on May 19, 2011, and emptied on the dates indicated. Asterisks denote the sampling period with the highest number of individuals: * highest number in one of the methods; **highest number in both methods; ǂ species where totals were high enough for subsequent statistical analyses.

Date	May 19	June 3	June 23	July 6	July 26	Aug.13	Aug.26	Sep.14	Oct.10	Nov.1	Total in traps (total in sweeps)	Number of sites with adults
Ephemeroptera												
Ephemerella invaria	0(4)										0(4)	2
Baetis tricaudatus	3 (0)	0(1)	1(0)								4(1)	4
Ameletus lineatus		1 (0)		1(0)							2(0)	2
Plecoptera												
Soyedina washingtoni	6(0)	8(5)**	4 (1)								18(6)	5
ǂ *Sweltsa naica*	12(0)	68(6)**	16 (6)	5(0)	1(0)						102 (36)	9
ǂ *Amphinemura wui*	21(0)	77 (66)*	39 (35)	27 (22)	34 (43)	34 (30)	28 (16)	28 (79)*	41 (0)		329 (291)	9
ǂ *Nemoura trispinosa*	31 (0)	178 (22)**	104 (12)	34 (10)	43 (10)	26 (2)	26 (0)	12 (6)	4 (0)		458 (52)	9
ǂ *Leuctra ferruginea*	2(0)	5(0)	1 (0)	3(0)	6 (2)	9 (1)	29 (2)*	16 (9)*	11 (0)		82 (14)	5
ǂ *Amphinemura nigritta*		11(23)*	5(1)	1 (3)	8(1)	16 (3)	24 (5)*	11 (2)	20 (0)		96 (38)	6
Trichoptera												
ǂ *Hesperophylax designatus*	7 (0)	2(1)		1(1)	2(5)*	6(1)	15(1)*	11 (0)			44(9)	8
Rhyacophila brunnea		0 (1)	0 (1)	1 (0)		2(0)	2 (1)				5(1)	4
ǂ *Lepidostoma vernale and L. sommermanae*		4 (9)	6 (0)	4 (0)	10 (2)	3 (3)*	22(1)*	4(1)			53(16)	9
Onocosmoecus unicolor		1 (0)					1(0)	0 (2)*	6 (0)*		8(2)	3
Psychoglypha subborealis		1 (0)							11 (0)*		12(0)	5
Pycnopsyche gentilis					0(1)	0(1)	2 (0)	1(0)			3(2)	2
Neophylax aniqua							0(3)	4 (39)*	13 (0)*		17 (42)	7

periods emerged throughout most or all of the summer, though most showed a smaller peak at some point during the season. For example, *Nemoura trispinosa* (the most common stonefly collected) emerged throughout the summer and fall, but had a peak in early June (Table 3.2). In contrast, *Amphinemura nigritta* and *Leuctra ferruginea* were most abundant later in the season, with late season numbers significantly higher than early season numbers for both species (Table 3.2). There was no significant peak for *Amphinemura wui*, the second most abundant stonefly in the springs (Table 3.2).

3.3.2.4 Trichoptera:

Thirteen caddisfly species in six families were collected within and around eastern PEI springs. Five of these species were collected from riparian samples and emergence traps but could not be confirmed as spring-inhabitants by associating with larvae from benthic samples, and they are not known to occur in springs as larvae (Table 3.1). The unconfirmed species were all rare, and are all new provincial records. Single specimens of the net spinning caddisfly, *Dolophilodes distinctus* (Philopotamidae) and long-horned caddisfly *Triaenodes tardus* (Leptoceridae) were captured in a spring-brook trap (3 June 2011) and a pool emergence trap (26 July 2011) respectively. For the Limnephilidae (northern tube-case caddisflies), six specimens of *Limnephilus sericeus* were collected on 21 June 2011 during riparian sampling, six specimens of *Limnephilus moestus* were collected from a combination of riparian and spring pool emergence traps from two sites and dates (late June to early July, 2011), and 1 specimen of *Limnephilus indivisus* was from a spring pool trap in one site on 6 July 2011. Although these three limnephilid caddisflies could not be directly associated with larvae from spring pool benthic samples, they may have emerged from the spring pool, since

a number of small limnephilid larvae (too small to identify) were captured from these sites (Chapter 2, Limnephilidae,not determined)

Caddisflies were the most diverse group inhabiting the springs, but most species were relatively rare, and only 214 were collected (Table 3.2). Only three species or species-groups were numerous enough to assess phenology in detail, and two of these had extended emergence periods. *Lepidostoma vernale/sommermanae* (data for the two species were pooled since females could not be differentiated) emerged from June until September with a small peak in late-August. *Hesperophylax designatus* were found in emergence traps from May until September (except late-June), and peaked in late August. In contrast, *Neophylax aniqua* showed a more synchronous emergence, beginning at the end of August, and peaking in late September/early October.

3.3.3. Site and Habitat influence on Total Emergence

Species patterns in emergence traps varied significantly among springs for all species that had high enough abundance for analysis. Both numbers of individuals (Fig 3.4, two-way ANOVA) and emergence timing (two-way ANOVA, interaction) varied by species among spring sites. A significant interaction term in the two-way ANOVA indicated that species (abundance or emergence timing) responded differently in the different springs. Species that were common in some springs were rare or absent in others and they frequently showed different timings for the peak emergence among springs (Fig. 3.4). These patterns appeared to be associated with nutrients and other habitat factors (Fig. 3.5). For example, *Nemoura trispinosa* was extremely common in high nutrient agricultural sites but rarer in low nutrient sites, and began to emerge earlier in all high nutrient sites compared to low nutrient

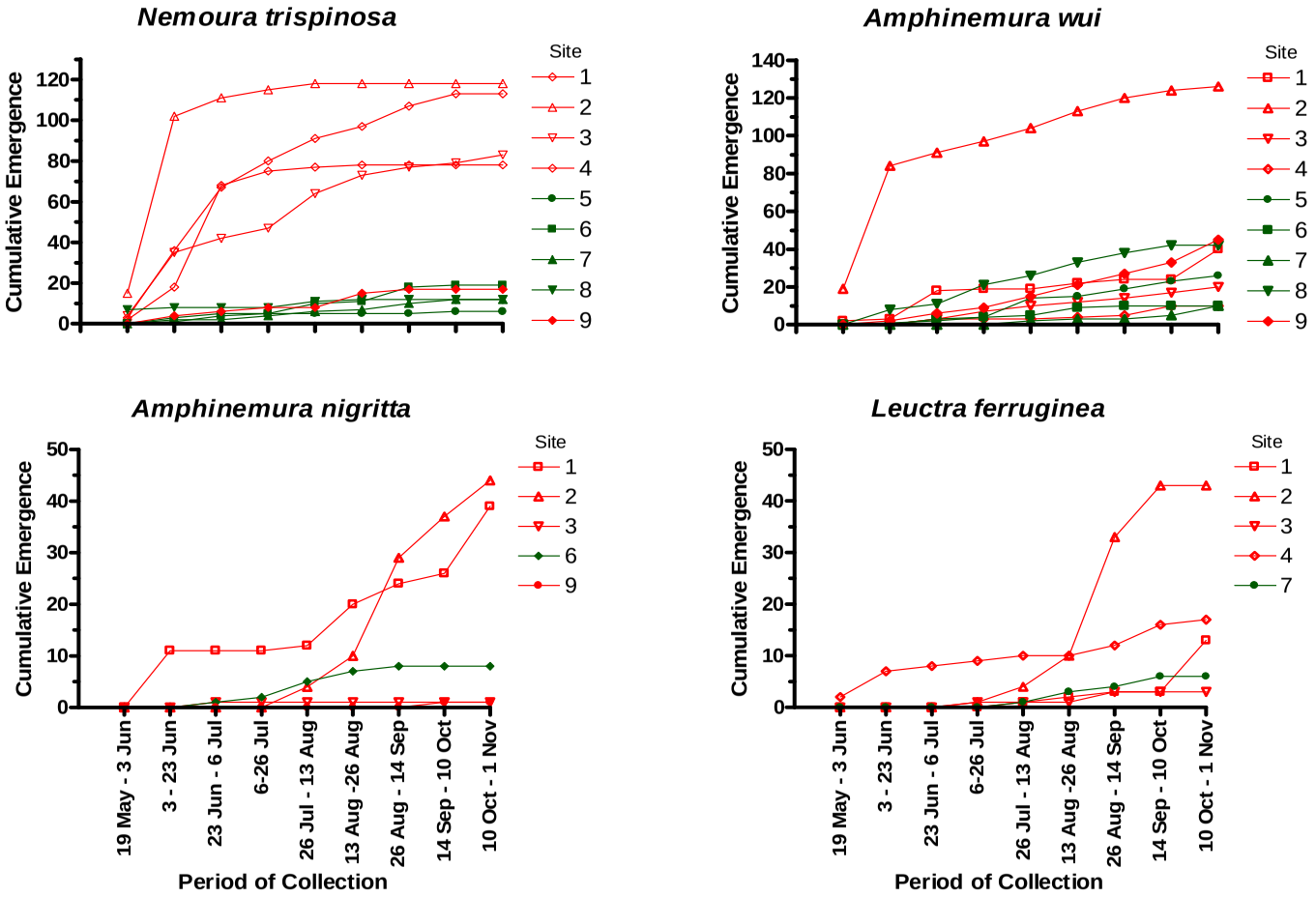
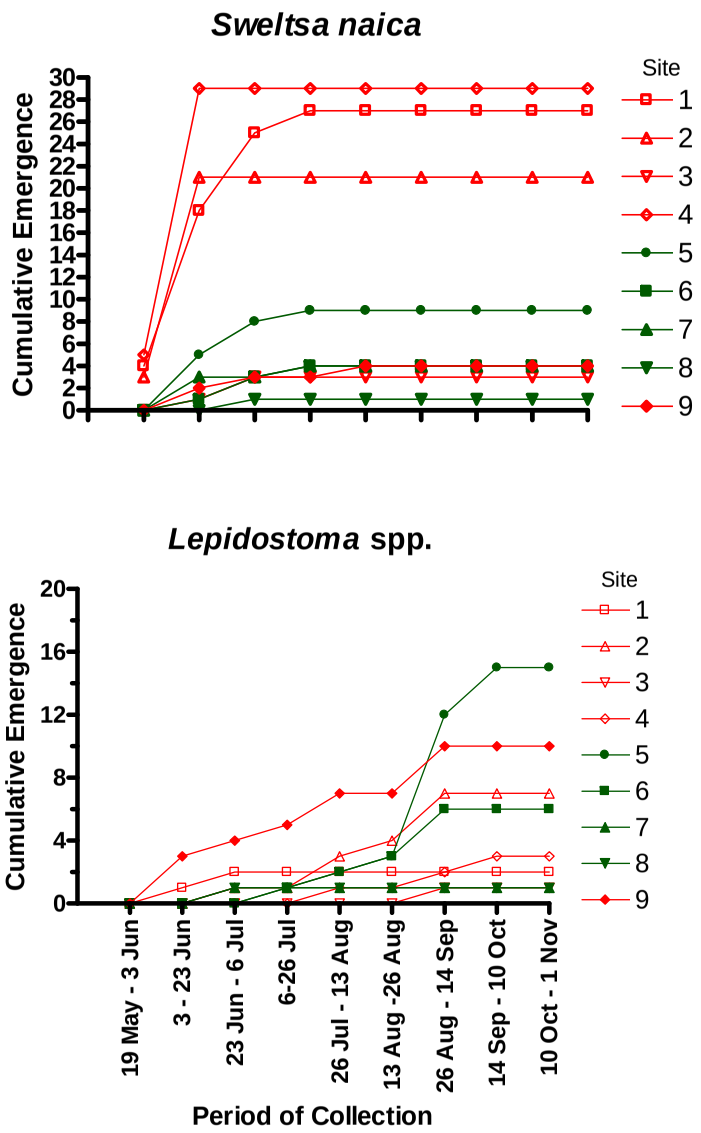


Fig 3.4a. Cumulative emergence (total number of individual from four emergence traps in the nine study springs) for species with >40 individuals captured in emergence traps. Site numbers correspond to map locations (Fig 2.1; habitat conditions can be seen in Fig. 3.1). See Table 3.2 for total numbers in each sampling interval. Open points are sites with high nutrient loads; closed points have low nutrient loads. Red labels and lines indicate sites adjacent to agricultural land; green labels/lines are adjacent to forested land.



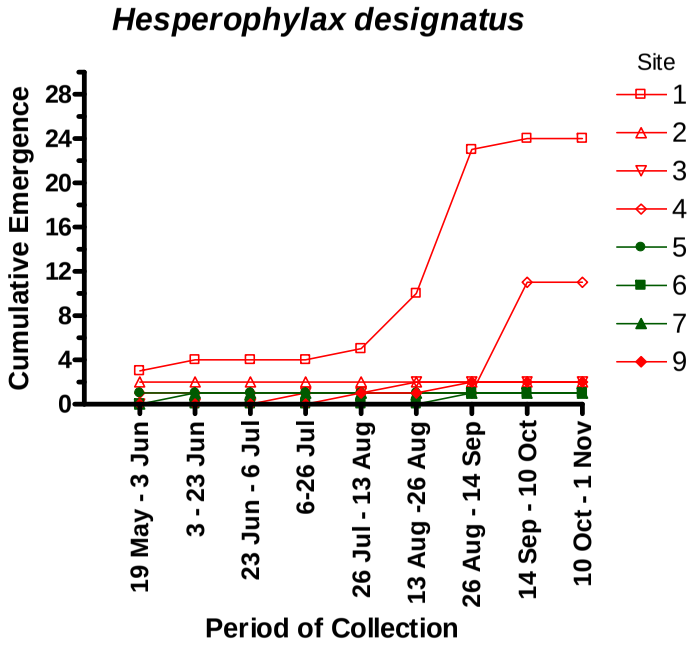


Fig 3.4b. Cumulative emergence (total number of individual from four emergence traps in the nine study springs) for species with >40 individuals captured in emergence traps. Site numbers correspond to map locations (Fig 2.1; habitat conditions can be seen in Fig. 3.2). See Table 3.2 for total numbers in each sampling interval. Open points are sites with high nutrient loads; closed points have low nutrient loads. Red labels and lines indicate sites adjacent to agricultural land; green labels/lines are adjacent to forested land.

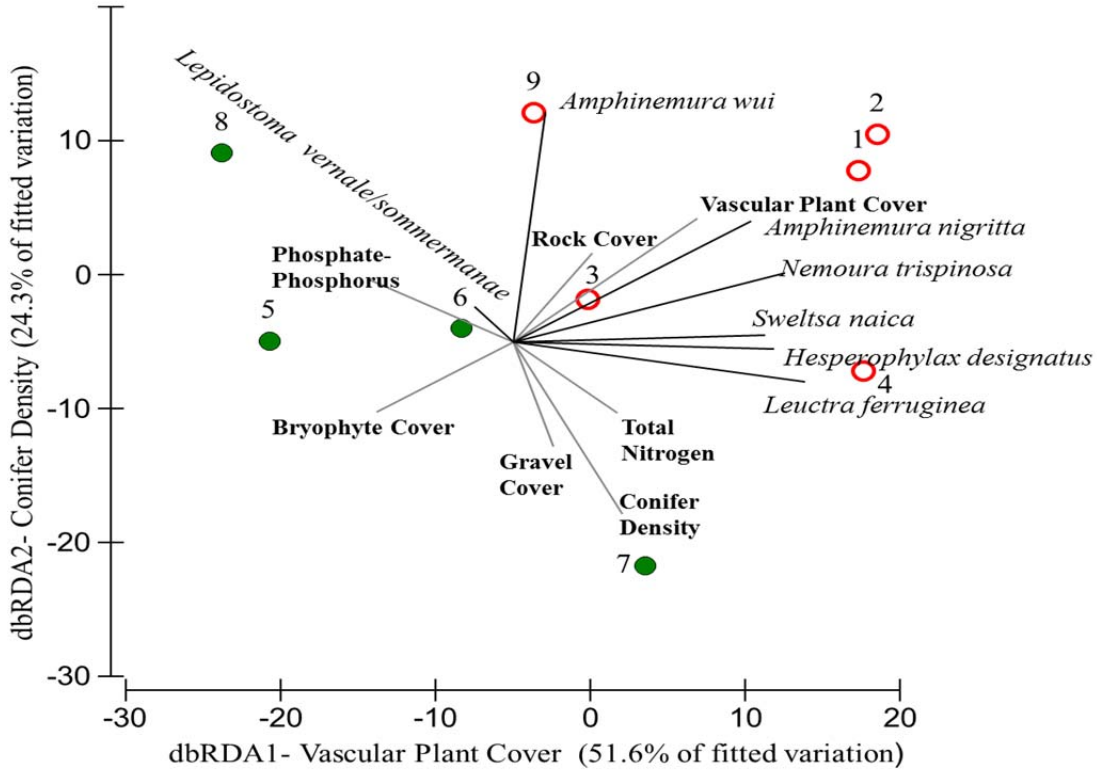


Fig 3.5. Relationships between total Plecoptera and Trichoptera abundances and measured habitat variables identified with the distance based linear model for species that occurred at ≥5 sites. Open circles indicate sites that are near agricultural fields, whereas closed circles indicate springs from forested areas, numbers correspond to site location, see Fig 2.1, 3.1 and Table 1.1 for more site details.

sites (Fig. 3.4a). Similarly, the stonefly *Sweltsa naica* began to emerge in May in three of the four high nutrient sites, whereas specimens were not detected in the low nutrient sites until June or early July (Fig.3.4b).

The overall stonefly and caddisfly community was influenced by within-pool vegetation variables (vascular plant vs. bryophyte cover), riparian conifer density (which also affected macrophyte type and cover; see chapter 2), the concentrations of nutrients (nitrogen and phosphate) and the substrate variables (gravel and rocks) (DISTLM, using all high abundance species). However, vascular plant cover was the only statistically significant variable in explaining the distribution of the common Plecoptera and Trichoptera in the community (DISTLM). Aquatic plant type (whether pools were dominated by vascular plants or bryophytes) explained more than half of the variation in stonefly and caddisfly species abundances (db-RDA, Fig. 3.5) and species richness (DISTLM, using species richness only). Highest abundances and richness values were found in sites dominated by vascular plants, and lowest richness in bryophyte dominated pools. Phosphate concentrations showed little variation among sites (Chapter 2), but were highest in the forested sites dominated by bryophytes. Conifer density in the riparian zone (which was negatively related to shading of the spring pool and densities of deciduous trees; Chapter 2), was the strongest predictor of the second db-RDA axis, accounting for another 24% of the variation in abundances (Fig. 3.5). Total nitrogen was highest in the agricultural sites (sites which were also dominated by vascular plants) (Fig. 3.5) and was the strongest predictor of the third dbRDA axis, explaining 17% of variation in species abundances. All stonefly species except *A. wui* ($\rho>0.7$; Fig. 3.5), and the caddisfly *Hesperophylax designatus* ($\rho=0.8$, Fig. 3.5) were

primarily associated with the first db-RDA axis, showing highest emergence numbers in sites with high cover of vascular plants. *Amphinemura wui* correlated positively with the second axis (Riparian vegetation) (ρ=0.8, Fig. 3.5), and was more abundant in sites with high conifer density and little shading. However, *Amphinemura wui* was also correlated with phosphate and discharge, with highest numbers in the sites with highest phosphate concentrations and pool discharges (DISTLM, assessing only *A. wui*). *Hesperophylax designatus* and *Sweltsa naica* were also significantly correlated with conifer density (DISTLM, assessing only *H. designatus* or *S. naica*) as well as bryophyte cover, and conifer density and bryophyte cover were strongly inter-correlated (ρ=-0.8). *Lepidostoma* spp. correlated negatively with the third db-RDA axis (ρ=-0.9, Fig. 3.5) with lower numbers collected at sites with the highest nitrogen levels, whereas *Nemoura trispinosa* correlated positively with nitrogen (Fig 3.4a; DISTLM, assessing only *N. trispinosa*). Overall species richness also correlated positively with nitrogen, and was highest in agricultural sites with high nitrogen levels (DISTLM, assessing only species richness). The two remaining common stonefly species were not analysed using the DISTLM procedure, as they were encountered at only five sites. Both species were most common in agricultural sites (Fig 3.4), and were only collected at a single forested site each.

3.4 Discussion

Ephemeroptera, Plecoptera, and Trichoptera diversity and abundance clearly related to habitat conditions within the springs, especially agricultural land use variables affecting macrophytes and nutrient loading. As expected, most species responded to higher nutrients in

the agricultural springs with higher diversity and abundances; a pattern that is likely due to increased resources (food and habitat) in pool microhabitats (Merten et al. 2014).

Several habitat variables contributed to the pattern for higher diversity and abundance in agricultural springs. Agricultural springs showed greater substrate variability (frequently a high proportion of large rocks) than forested sites, as well as higher light levels, which in concert with high nitrogen levels, led to a dominance by vascular plants within the pools (Chapter 2). The combination of variable rock sizes and vascular plant dominance provided heterogeneous habitat and potential food resources for the EPT communities. However, although the spring-associated EPT community was dominated by shredders (usually associated with high inputs of allochthonous deciduous tree litter; e.g., Taylor and Andrushchenko 2014), most shredder taxa were related to the presence or absence of vascular plants within the pool rather than to deciduous trees or shrubs in the riparian zone. Aquatic vascular plants can also provide high quality detritus for shredders (Bartodziej and Perry 1990), and high nitrogen levels can increase the microbial decomposition rate of leaf matter (e.g., Bartodziej and Perry 1990), so the vascular macrophytes may be taking the place of allochthonous leaf litter in these springs. For example, decaying watercress (*Nasturtium* spp., the dominant aquatic vascular plant in this study; Chapter 2) is preferentially consumed over live watercress for *Hesperophylax designatus* and *Limnephilus* sp. in an experimental setting (Newman et al. 1992).

In contrast to their high abundance and diversity in agricultural springs, few EPT taxa were associated only with the forested streams. Those that were associated with forested streams (e.g., *Lepidostoma* spp.) may feed on fine detritus that collects on gravel and

bryophyte leaflets (Wiggins 1996; Eedy and Giberson 2007; Morse and Holzenthal 2008; Bottazzi et al. 2011). Bryophytes can also be an important direct food resource for some Trichoptera; for example, the microcaddisfly *Palaegapetus celsus* Ross, 1938 (Hydroptilidae) is endemic to and feeds on liverworts (Ito et al. 2014). This species was found in the PEI springs, but only as larvae, so they were not considered in this analysis since none were detected in emergence traps or riparian sampling (Appendix 4.2).

Overall EPT diversity patterns were similar to those reported in other springs. Trichoptera generally show the highest diversity in springs, though with low overall numbers, followed by Plecoptera, then Ephemeroptera (e.g., Dobrin and Giberson, 2003; Gathmann and Williams 2006; Maiolini et al. 2011). Also as expected, the cool-springs had low Ephemeroptera, Plecoptera, and Trichoptera (EPT) diversity compared to studies of other running-water habitats on or near PEI. Only 17 EPT species (in 16 genera) were confirmed as spring-inhabitants in the nine springs, compared to 22 EPT species (in 22 genera) in a small PEI spring-brook (Dobrin and Giberson 2003), and 42 EPT genera in a study of several PEI warm water streams (Curry et al. 2006). Thirty-one stonefly species were collected from 12 small spring-brooks in the highlands of Cape Breton Island (Ogden 2012). Peterson and Eeckhaute (1992) also noted that small-neutral spring-fed brooks in southern Nova Scotia and New Brunswick possessed separate EPT assemblages compared to larger river systems. The pattern for relatively low EPT diversity in cool springs and spring-fed streams relates to the cold, stable temperatures and the relatively low habitat heterogeneity compared to nearby flowing-water habitats.

Low temperatures limit the number of species that can complete development in cool springs, but the constant temperature in springs can also affect life cycles, by removing temperature cues needed to synchronize emergence timing (Sweeney 1984; Danks 1987; Dobrin and Giberson 2003; Bottová et al. 2013). The mechanisms by which these cues act may vary among taxa with different life cycle types, and relate to other habitat features. Developmental rates and timing strongly relate to temperature accumulation during the life cycle (degree-days above the developmental threshold for growth; Markarian 1980, Wilson and Barnett 1983; Sweeney 1984). If temperatures are below or near the growth threshold, little or no growth will occur, and even if recruitment occurs, larvae will not complete development. Therefore, emergence from these habitats will be restricted to species that can complete development at low temperatures. Many stoneflies are cold tolerant (Hynes 1976), and several of the spring-associated species found in this study have wide temperature tolerances and grow well at 17-18°C in other Atlantic Canadian streams (Peterson and Eeckhaute 1992).

Synchronization of emergence timing in aquatic insects is common, and believed to be related to mate-finding and predator avoidance (Sweeney and Vannote 1982; Sweeney 1984). Temperature cues are important in many species for synchronizing life cycle stages (Danks 1987), so springs are often dominated by species showing asynchronous life cycles (e.g., Dobrin and Giberson 2003). However, food availability and photoperiod can also provide developmental cues or influence development rates of aquatic insects (Hynes 1970; Liber et al. 1996). For example, *Neophylax aniqua* (Beam and Wiggins 1987) uses diapause, an obligatory "resting" stage, to synchronize its life cycle. *Neophylax aniqua* showed

synchronous emergence in the spring pools, despite the lack of temperature cues, so may rely on light cues instead. Photoperiod may be important in the life cycle of many spring-dwelling aquatic insects (Hynes 1970); recently growth of two *Leuctra* spp.in Slovakia was positively correlated with photoperiod in a constant temperature stream (Bottová et al. 2013).

The pattern for the majority of spring species to show asynchronous life cycles may be due to spring-associated species already possessing this life cycle pattern (Dobrin and Giberson 2003) or to life cycle flexibility (Sweeney 1984). For example, *Nemoura trispinosa*, *Amphinemura nigritta,* and *Lepidistoma vernale*, all species with extended emergence in the PEI spring pools, showed much more synchronous emergence periods in streams with warmer and fluctuating temperatures (e.g., Williams et al. 1995; Hogg and Williams 1996; Dobrin and Giberson 2003). Even in these species with extended emergence in the cool-springs, however, there is usually a minor peak in the emergence period that is similar to reported stream patterns (e.g., *Nemoura trispinosa* in Ontario streams; Hogg and Williams 1996), suggesting that other factors may also play a role.

Although temperature and photoperiod are the most commonly cited predictors for growth and development, food availability can affect developmental timing (Williams 1991; Giberson and Rosenberg 1992), with both increased growth and more rapid life cycle development when more food is available (Anderson and Cummings 1978). The importance of food resources on life cycles has been shown in lab studies (e.g., Giberson and Rosenberg 1992: burrowing mayflies; Liber et al. 1996: chironomid midges; Ito 2005: *Lepidostoma* caddisflies), but few studies have investigated field patterns over a range of sites. At least two common stonefly species in the PEI springs, *Nemoura trispinosa* and *Sweltsa naica,*

showed earlier emergence in high nutrient springs than in low nutrient ones suggesting that increased food can alter emergence patterns in natural systems. Spring habitats are useful natural laboratories for addressing these ecological questions, because of their constant temperature and chemical regimes, and their replicability (Odum 1957; Williams and Williams 1998). In this study, agricultural activity produced changes in EPT populations that were driven by changes in food availability (e.g., increased vascular plant abundance), and resources not only played a role in total numbers of individuals, but influenced life cycle timing, diversity, and size of the population.

In Europe and parts of the United States, springs are considered to be highly endangered habitats (Cantonati et al. 2012), and many spring species are listed as threatened or endangered (e.g., the red listed *Crunoecia irroata* (Curtis, 1834) (Lepidostomatidae) in Europe; Ilmonen 2008). Conservation status of aquatic insects in Canadian springs is generally unstudied, however, so the extent of threats either to the habitat or to individual species is unknown. Two spring-associated invertebrate species are presently listed as endangered under COSEWIC (Committee on the status of endangered wildlife in Canada): *Sanfilippodytes bertae* Larson, Alarie and Roughley, 2000 (Coleoptera: Dytiscidae) and the Banff hot-springs snail (*Physella johnsoni* (Clench, 1926) (Mollusca: Gastropoda: Physidae), though anthropogenic water use is the main threat to these species (COSEWIC 2008; 2009). Two lepidostomatid species in this study (*Lepidostoma vernale* and *Lepidostomata sommermanae*) showed negative responses to increased agricultural land use, suggesting that species status information in this habitat needs future study.

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Chapter 4

Conclusions, Recommendations and Future Directions

4.1 Summary

4.1.1 Impacts of agricultural land use on Prince Edward Island springs

Aquatic plants and invertebrate abundance and diversity differed markedly between agricultural and forested springs, but differences were more strongly related to the type of riparian vegetation than to nutrient or sediment differences. Conifers with few shrubs, low amounts of soil organic material, and little overhanging vegetation dominated agricultural springs, leading to high light levels, high amounts of fine sediment deposition, and low deciduous leaf litter inputs. Springs in forested areas generally had clean gravel substrates and a mixed riparian zone with deciduous trees and shrubs providing shade during summer and a good source of allochthonous leaf litter. Agricultural springs also had much higher levels of nitrates and sulphates than forested ones, probably though leaching from agricultural fields to the groundwater. In contrast, phosphate did not differ significantly between land use types, suggesting that phosphate may be binding to iron or clay soils and not leaching readily to the groundwater. The relationship between water discharge and phosphate concentration, however, needs further study. Differences in light level and nitrogen availability led to profound differences in the aquatic plant communities, which in turn, drove differences in the invertebrate community. Shade-tolerant bryophytes in the forested springs were associated with higher overall invertebrate densities than in agricultural springs. In contrast, the vascular plant dominated agricultural springs had lower invertebrate abundance and diversity. The collector-gathering midges (Chironomidae) which feed on deposited detritus on bryophyte leaflets and gravel were the main drivers of these patterns.

4.1.2 The diversity and emergence of Ephemeroptera, Plecoptera and Trichoptera in Prince Edward Island springs under the influence of agricultural land use

Twenty-five EPT species (24 as adults) were identified from samples collected in or around the PEI springs; 18 (17 plus *Palaegapetus celsus* (Ross, 1938) collected as a larva) of these could be directly associated with the spring-pool habitat. This is the first study to assess species diversity in PEI springs, so it is not surprizing that a high proportion of these (40%) are newly recorded for PEI. The Trichoptera (caddisflies) were the most diverse of the taxa identified, followed by stoneflies (Plecoptera) and mayflies (Ephemeroptera) which were relatively rare. Stoneflies were the most abundant, followed by the caddisflies and mayflies. Most of the EPT taxa showed higher abundance and diversity in the agricultural springs, and most appeared to be directly related to the abundance of vascular plants or correlated nitrogen and/or riparian patterns in the agricultural sites. Only one EPT group (the two species of *Lepidostoma*) was most strongly associated with the forested springs. The combination of benthic sampling and adult collections allowed assessment of patterns at the species level, and provided information on habitat associations and responses. The observed patterns for low diversity and extended emergence periods in PEI cool-springs supports patterns reported for other studies in cool springs.

4.2 Relevance and Watershed Recommendations

Few studies worldwide have looked at anthropogenic disturbance on the species living in spring habitats (Cantonati et al. 2012), especially effects of agricultural land use (Williams et al. 1997; Keleher and Rader 2008; Lencioni et al. 2012). Confusion over how to define spring habitats and little information on their ecology means current management practices are outdated (e.g., von Fumetti et al. 2007; Barquin and Scarsbrook 2008;

Cantonati et al. 2012; Harris et al. 2012) though there is a growing and widespread interest in monitoring and protecting springs (Cantonati et al. 2012; Harris et al. 2012). Data from this study suggest that watershed managers could mitigate many of the agricultural impacts on springs by planting deciduous trees and shrubs around agricultural springs and headwater streams to increase shading. This should limit light to favour bryophyte communities and limit growth of aquatic vascular plants such as the invasive watercress (*Nasturtium*). Although the biota of springs can be indicators of groundwater quality (van der Kamp 1995; Williams and Williams 1998), other important variables to consider when monitoring effects on springs include riparian composition, discharge volumes, and benthic sediment structure. Biotic monitoring should include both plant and animal diversity and be carried out at taxonomic resolutions appropriate to the questions being asked (See Appendix 5 for an example of how taxonomic resolution can affect interpretation of biotic indices). Simple monitoring programs on PEI could be carried out at the family taxonomic level, for example, since springs affected by agriculture should have lower numbers of Chironomidae and higher numbers of Plecoptera than forested springs. However, determination of specific effects of particular variables will require species- or genus-level identification. Further floral and faunal surveys in springs and headwater streams are needed to identify the species restricted to this unique habitat, and to monitor populations.

4.3 Future Research

A major finding of thesis is the role of the species composition in the riparian area in structuring spring biotic communities. Riparian areas on Prince Edward Island have been considered two-dimensionally, as a minimum distance of undisturbed land to filter and intercept materials in runoff from agricultural or forestry activities (Dunn et al. 2011). Stream

shading can reduce water temperatures in non-spring-influenced streams (e.g., Johnson 2004) and mitigate nutrient input responses in a variety of stream types (Burrell et al. 2014). Wide buffer strips can be effective in removing sediment from runoff water (Vought et al. 1995; Naiman et al. 2005) and wide protection areas around springs can remove contaminants from subsurface flow (Goldscheider 2010), but it is not clear how much surface and subsurface flow runs into and affects headwater springs with low drainage basin areas. Further research should be undertaken to see if increasing deciduous vegetation and/or allowing natural overhanging vegetation to persist over springs limits vascular plant growth and allows the spring to return to a more natural bryophyte community. Research is also needed to determine the relative importance of chemical and sediment inputs from groundwater and surface runoff sources.

Spring habitats have been confirmed as unique habitats on PEI in this study, with unique communities, and several species not known to occur in other PEI habitats. Therefore, any activity that threatens springs could threaten these species. Water abstraction (for urban use or irrigation) is a newly identified threat to springs and headwater streams, and the extent to which it should be allowed is presently being hotly debated on Prince Edward Island. Water abstraction has led to spring habitat loss worldwide (Barquin and Scarsbrook 2008), since it reduces discharge and can cause some springs to dry completely at certain times of the year (e.g., Erman and Erman 1995; Williams 2006; Barquin and Scarsbrook 2008). Temporary springs have different fauna and flora than permanent ones, so activities that cause current permanent springs to dry, even in some seasons, should negatively affect the spring communities. Research is needed on PEI on the effects of seasonal drying on formerly permanent habitats.

This study focused on small pool-like (rheo-limnocrene) springs, but the fauna of other common PEI spring types (e.g., rheocrenes and helocrenes) was not investigated in any detail and can have very different flora and fauna (e.g., Spitale et al. 2012). The biota of these spring types needs to be investigated to get a full picture of the biota present in PEI and other eastern Canadian springs, and potential impacts of agricultural land use on all spring types.

This study identified several variables associated with agricultural land use that had direct or indirect effects on spring biota, but did not consider implications to higher trophic levels, such as stream fish. Springbrooks are important nurseries for brook trout (*Salvelinus fontinalis* (Mitchill,1814)) in PEI, where the young trout feed predominantly on orthoclad midges (MacInnis 1994). Midges in the subfamily Orthocladiine (especially in the genus *Thienemanniella*) showed dramatically lower abundance in agricultural springs than in forested ones, suggesting that agricultural activities may interfere with an important food source for developing trout. Future study could identify whether feeding patterns and growth rates are different in brooks discharging from agricultural and forested springs, to determine whether land use affects other species dependent on the spring habitats.

This thesis has opened a window into the dynamics of springs on Prince Edward Island, identified many species previous unknown to occur on the Island or the region, and detected effects of agricultural land use influencing those species. Future research needs find out more about the species that inhabit this unique habitat and determine methods to help return affected springs to places where quality water flows.

"... the interior is intersected with rivers which meander through the richest natural forest in every district; while springs, and streams of the purest water, everywhere abound."

S.S. Hill, A Short Account of Prince Edward Island, 1839

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Appendix 1.

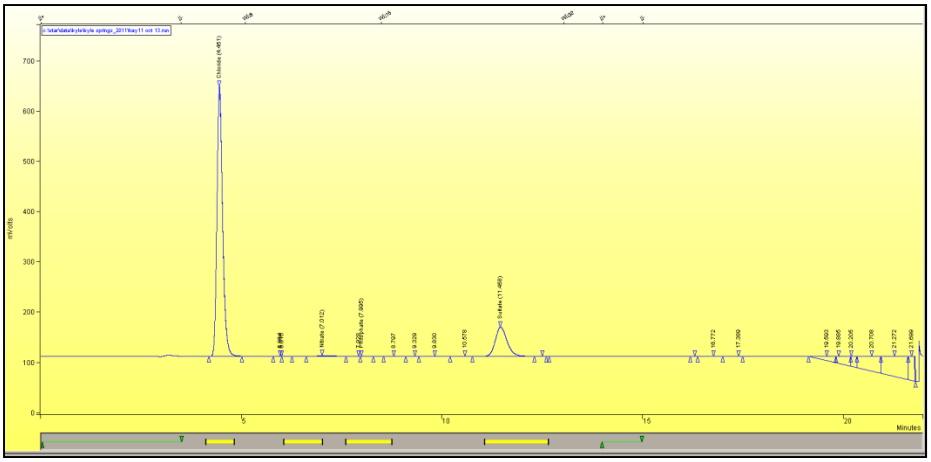
Details of Water Chemistry and Sampling Methods

Appendix 1.1. Suppressed Anion Chromatography

(The following methods are summarized from Schein et al. (2012))

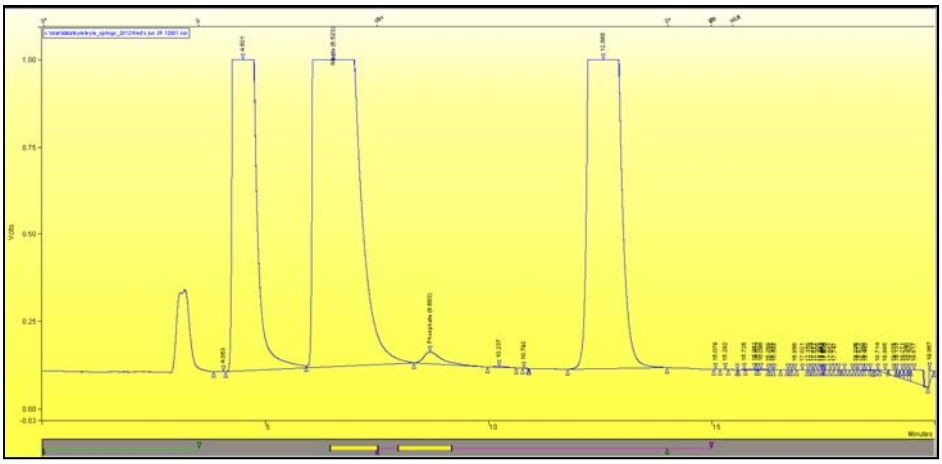
Anion concentrations (Nitrate-Nitrogen (NO_3-N), Phosphate-Phosporus (PO_4-P), Chloride (Cl^-), and Sulphate (SO_4^{2-})) were measured using suppressed anion chromatography in the Aquatics Laboratory at the University of Prince Edward Island. Chromatography was set up with a Varian model 240 pump, a model 410 auto sampler, and an Alltech model 650 conductivity detector attached to a model 641 suppressor. The column that was used was a Varian AN300 anion exchange column which has a 5µm particle size space. The eluent that was mixed with water samples was an isocratic 2.5 mM Sodium Carbonate ($NaCO_3$) solution, and the mixture moved through the column at 35°C, heated with the column oven. Water samples were filtered through a High Performance Liquid Chromatograpy (HPLC) grade filter prior to analysis, so measurements represent dissolved concentrations of the chemical parameters. Total nitrogen was obtained by converting all nitrogenous compounds in 5mL of sample water into dissolved Nitrate-Nitrogen through a persulphate reaction at high temperatures (~120°C), and analyzed in a separate run as described above. The persulphate solution was made by mixing 0.676g of Potassium persulphate ($K_2S_2O_8$) with 0.25 g of Sodium hydroxide (NaOH) and 100 mL of HPLC grade water (near pure-H_2O). The sample water (5mL) was mixed with 400µL of persulphate solution in a covered test tube and autoclaved at temperatures >120°C for 1 hour. Total Phosphorus could not be determined through this method since the point at which Phosphate would come out on the chromatogram was overshadowed by the nitrate and sulphate peaks for many sites, Total Phosphorus was not determined. Therefore, dissolved phosphate is the only measure of phosphorus used in this study.

a) Typical Forest Output



b) Agricultural Spring

Low Concentrations



c) Agricultural Spring

High Concentrations

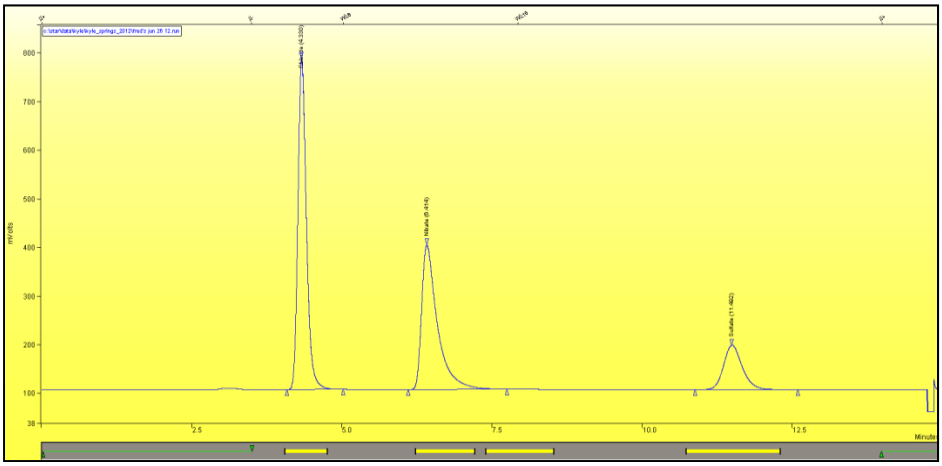


Fig A.1.1.1 Screen Shots of representive chromatograms. Samples for most agricutural springs were run twice, on low and high sentivity. All water samples were run on low sensitivity.

Appendix 1.2 Hess and Emergence Trap Methods

Benthic Sampling: The Hess Sampler

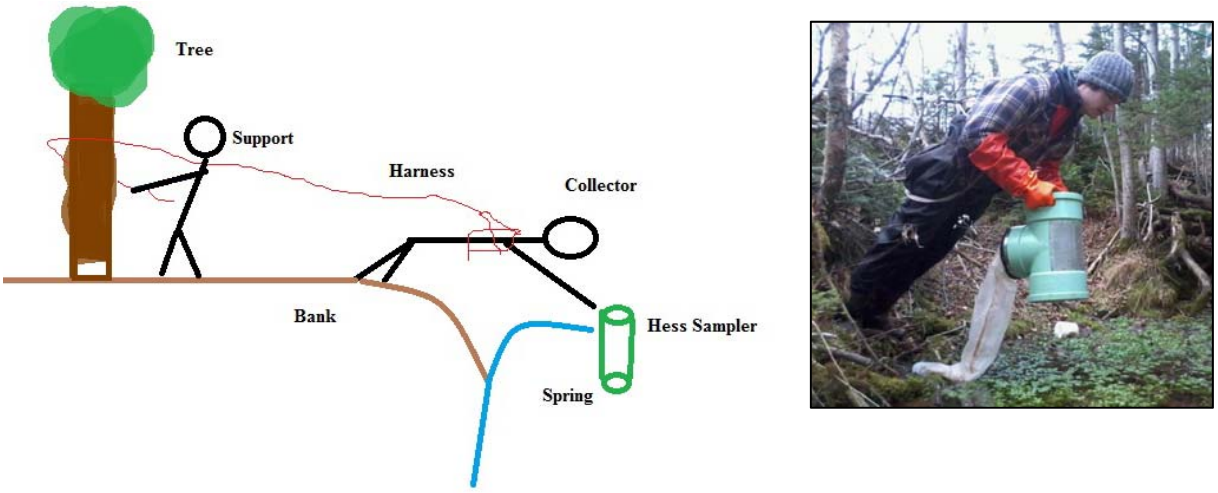


Fig A.1.2.1. Schematic of harness system used for Hess sampling.

The Hess sampler is a type of "box sampler" that encloses an area of substrate so that the substrate can be disturbed inside the enclosure, and invertebrates within that area of substrate collected. The Hess sampler used in this study had an area of 0.07 m^2 and a mesh size of 200 µm. All Hess samples were collected from above, using a harness system to lower the operator into place, due to the presence of soft substrates that would be disturbed by walking into the spring (all sites had trees that were close enough and large enough to support the weight of the collector). All hess samples were collected by a single operator to provide consistent sampling among springs, since the sampling location could be affected by the height and reach of the operator. The specific location where the Hess sampler was placed was therefore determined by the distance from shore using the harness, but was generally random within the reach of the operator. Substrates were disturbed within the Hess sampler for two minutes, and floating material, plants, and surface sediments were directed into the net bag of the sampler. Larger rocks and sticks were washed in the field and returned to the spring; remaining sediment and plant matter was brought back to the lab for sorting and identification as descibed in Chapter 2.
See http://www.theguardian.pe.ca/Living/2012-04-14/article-2954476/The-signs-of-springs/1 for a video of the process.

Emergence Sampling: Emergence Trap

Fig A.1.2.2. Emergence traps used for adult sampling.

Four emergence traps were deployed in each spring; three in the spring pool and one in the brook immediately below the spring. Traps consisted of a cone-shaped 300 µm mesh net, enclosing an area of 0.07 m^2, and tapering to a collecting bottle with a pvc pipe insert; each bottle contained about 5 cm of 80% ethanol as a preservative. Traps were suspended above the water with ropes, and weighted with fishing weights attached to the base to reduce movement. Emerging insects were directed upwards by the cone shape, and through the pvc pipe into the collecting jar, where they drowned in the ethanol, and were preserved between collecting intervals. Traps were emptied approximately every two weeks through the summer. See chapter 3 for other details.

Appendix 1.3. Riparian Area Sampling.

Tree species and soil organic matter were assessed in the riparian area around the springs, as shown above. Trees were identified and counted in adjacent 5 m x 5 m quadrats in three transects around each biodiversity spring. Soil Cores were were taken with a standard soil corer (2.2 cm, diameter) within 2 m of the bank (shown as dots in the schematic above) at each spring and assessed by measuring the depth of the litter and organic soil layers. Overall site values were generated by averaging the density values from each transect for all the deciduous and and all the coniferous trees, and averaging the litter and organic measurements for each transect within a spring site.

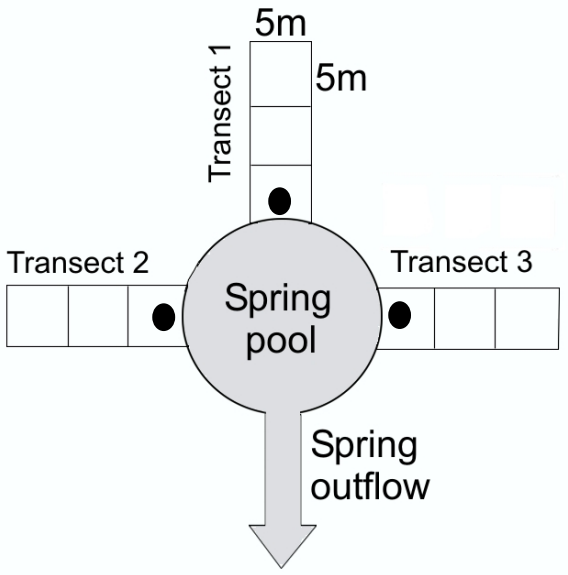


Fig. A.1.3.1. Sampling schematic for riparian sampling.

Appendix 1.4 Spring-pool Sediment Characterization

Fig A.1.4.1. Example of an image used to characterize sediment. The image is from site 9.

The sediment in the spring pools was characterized by assessing digital photographs taken over a grid from above the spring. A meter stick was placed in each grid for scale, and the proportion of the bottom covered by each sediment class (as described in Chapter 2) determined by counting pixels using the image analysis software program 'Image J'.

Appendix 1.5. Sampling Sites and Date Summary.

Table A.1.5.1. Summary of sites and sample dates for measurements carried out in this study. [1] 'Agr' = Agriculture; 'For' = forested. [2] 'BIO' = sites where all parameters (benthic invertebrates, adults, plants, water chemistry, riparian assessment) were measured; 'WAT' = sites where water samples were collected, but regular biotic sampling did not occur; 'TEMP' = sites with temperature loggers. [3] Supplemental sampling refers to aditonal water chemisty samples that were limited due to accessiblity. Site 9 only Ephemeroptera, Plecoptera, and Trichoptera larvae presence was noted to compare to adults (Chapter 3). See Table 3.2 for adult sampling intervals.

Site	Site Code	Longitude/ Latitude/	[1]Land Use	[2]Type of data collected	Main Water Chemistry Sample Dates	[3]Suppl. Sampling Dates	Benthic Sampling Dates	Plant Sampling Dates
1	NAF04	62°25'42.27" 46°24'42.91"	Agr	BIO, WAT, TEMP	2011: 6/21, 7/27, 8/25, 10/2, 11/23 2012: 4/7, 5/29, 6/29	2012-2-23	2011-06-22	2011-09-24
2	NAF05	62°25'42.24" 46°24'44.06"	Agr	BIO, WAT, TEMP	2011: 6/21, 7/27, 8/26, 10/2, 11/23 2012: 4/7, 5/29, 6/29	2012-2-23	2011-06-20	2011-09-15
3	SOU01	62°20'40.99" 46°23'54.52"	Agr	BIO, WAT, TEMP	2011: 6/15, 7/27, 8/25, 10/8, 11/27 2012: 3/29, 6/1, 6/29	2011-2-16 2012-2-23	2011-06-15	2011-09-25
4	SOU02	62°20'41.60" 46°23'56.79"	Agr	BIO, WAT, TEMP	2011: 6/15, 7/26, 8/25, 10/8, 11/27 2012: 3/29, 6/1, 6/29	2011-2-16 2012-2-23	2011-06-15	2011-09-25
5	BAR01	62°23' 33.43" 46°26'14.60"	For	BIO, WAT, TEMP	2011: 6/22, 7/27, 8/24, 10/8, 11/20 2012: 4/7, 5/29, 6/29	2012-2-23	2011-06-22	2011-09-24
6	BAR02	62°23'38.9" 46°26'21.04"	For	BIO, WAT, TEMP	2011: 6/22, 7/27, 8/24, 10/8, 11/20 2012: 4/7, 5/29, 6/29.	2012-2-23	2011-06-22	2011-09-24
7	HAY02	62°22'3.18" 46°25'37.3"	For	BIO, WAT, TEMP	2011: 6/15, 7/27, 8/25, 10/10, 11/28 2012: 3/29, 5/29, 6/29	2012-2-23	2011-06-16	2011-09-25
8	CRS17	62°16'49.62" 46°26'22.84"	For	BIO, WAT, TEMP	2011: 6/12, 7/31, 8/24, 10/8, 11/27 2012: 4/16, 5/30, 6/26		2011-06-12	2011-09-15
9	HAY07	62°22'11.6" 46°25'41.26"	Agr	BIO (Adults only) , WAT, TEMP	2011: 6/15, 7/27, 8/25, 10/10, 11/28 2012: 3/29, 5/29, 6/29	2012-23-12	2011-06-16	2011-09-25

Table A.1.5.1. Continued

Site	Site Code	Longitude/ Latitude/	[1]Land Use	[2]Type of data collected	Main Water Chemistry Sample Dates	[3]Suppl. Sampling Dates	Benthic Sampling Dates	Plant Sampling Dates
10	NAF01	62°25'28.99" 46°26'49.55"	For	WAT, TEMP	2011: 6/18, 9/27, 8/26, 10/13, 11/28 2012: 4/7, 5/29, 6/29	2011-2-16		
11	NAF02	62°25'29.45" 46°26'50.12"	For	WAT, TEMP	2011: 6/18, 9/27, 8/26, 10/13, 11/28 2012: 4/7, 5/29, 6/29	2011-2-16		
12	CRS05	62°15'10.40" 46°27'49.14"	Agr	WAT	2011: 6/10, 7/31, 8/30, 10/13, 11/27 2012: 4/16, 5/30, 6/26			
13	CRS21	62°15'24.19" 46°25'57.43"	For	WAT,TEMP	2011: 6/12, 7/27, 8/30, 10/13, 11/27 2012: 6/1, 6/29			
14	HAY01	62°21'45.36" 46°27'33.01"	For	WAT,TEMP	2011: 6/7, 7/31, 8/26, 10/10, 11/29 2012: 4/16, 5/30, 6/26	2011-2-16		
15	HAY11	62°21'19.4" 46°26'51.43"	For	WAT	2011: 6/23, 7/31, 8/30, 10/13, 11/29 2012: 4/16, 5/30, 6/26			
16	NAF03	62°26'12.01" 46°25'26.93"	Agr	WAT	2011: 6/21, 11/28 2012: 4/7	2012-2-23		
17	NOL01	62°11'40.41" 46°24'30.67"	For	WAT, TEMP	2011: 6/10, 7/27, 8/30, 10/10, 11/27 2012: 5/30, 6/26			
18	SOU03	62°20'46.17" 46°23'53.73"	Agr	WAT	2011: 6/23, 7/27, 8/25, 10/13, 11/27 2012: 6/1, 6/29	2012-2-23		
19	SOU04	62°15'06.30" 46°24'24.76"	Agr	WAT, TEMP	2011: 6/12, 7/27, 8/24, 10/10, 11/27 2012: 4/16, 5/30, 6/26			
20	SOU08	62°20'24.57" 46°23'50.46"	Agr	WAT	2011:6/26, 7/27, 8/30, 10/13, 11/28 2012: 6/1, 6/29			

Appendix 2

Supplemental Data and Comparison of Biota and Water Quality Between Land use types

Appendix 2.1. Chemical and Phsical Habitat Comparisons for sites in each land use type.

These following figures show the how patterns in chemical and physical habitat variables changed through the seasons in study springs in each of the two land use types (Agriculture vs. Forested). Time and land use were compared using a Two-Way ANOVA with sampling month and land use as factors, except where specified.

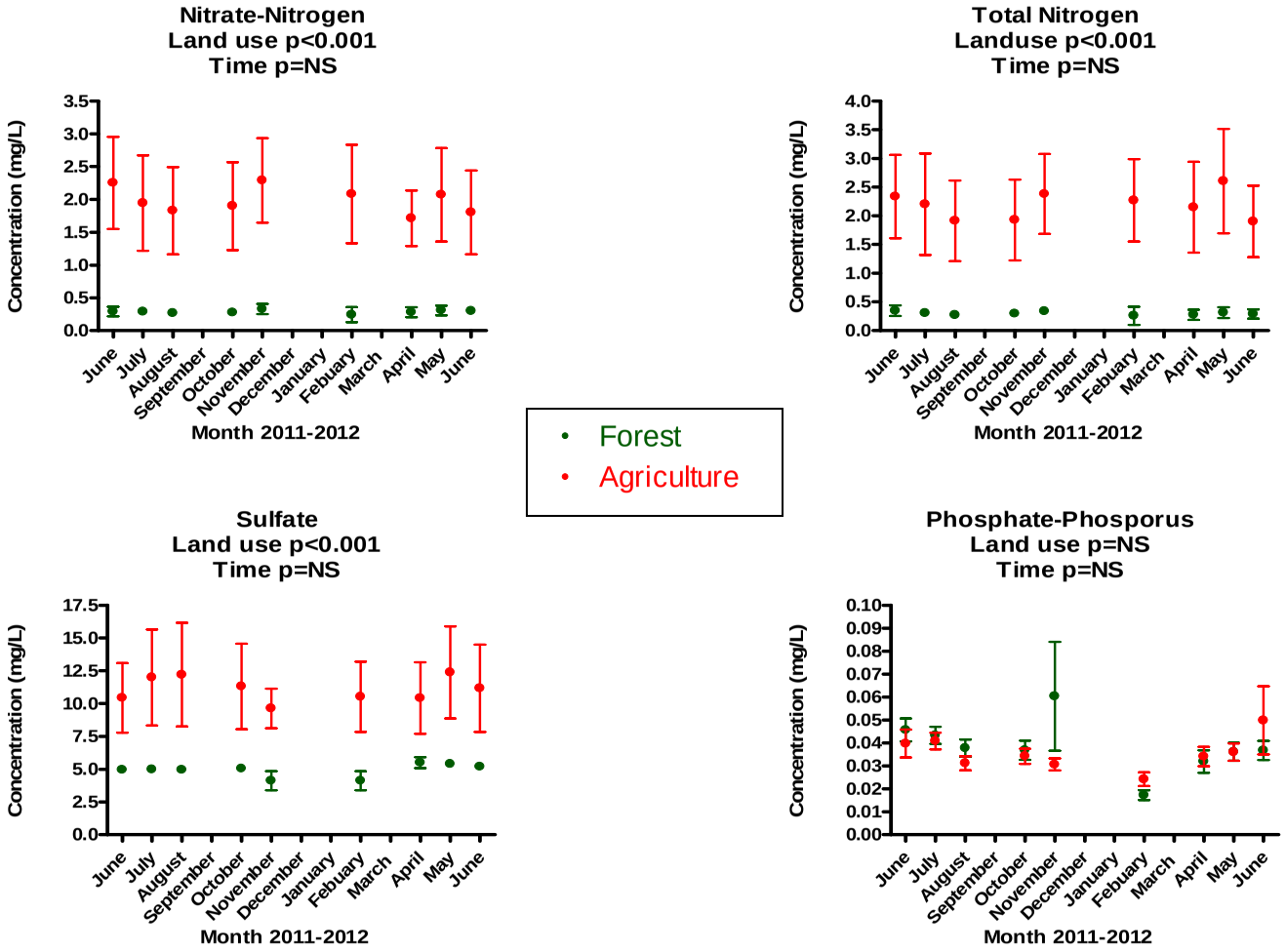


Fig A.2.1.1. Average nutrient concentrations (±Std. Error) in spring water in the two land use types from all sites (n=20) over time.

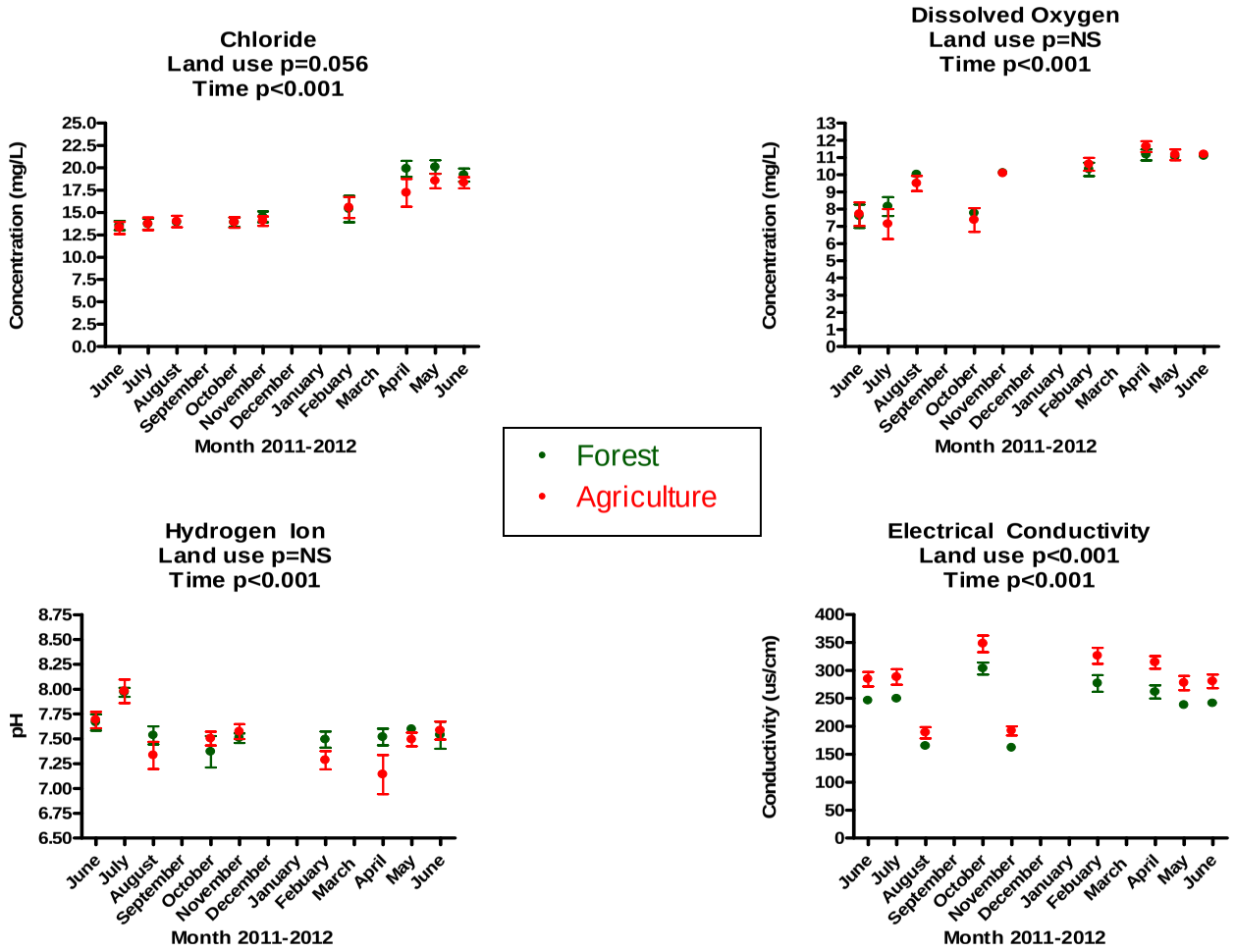


Fig A.2.1.2. Average chemical concentrations and conductivity (±Std. Error) in spring water in the two land use types from all sites (n=20) over time.

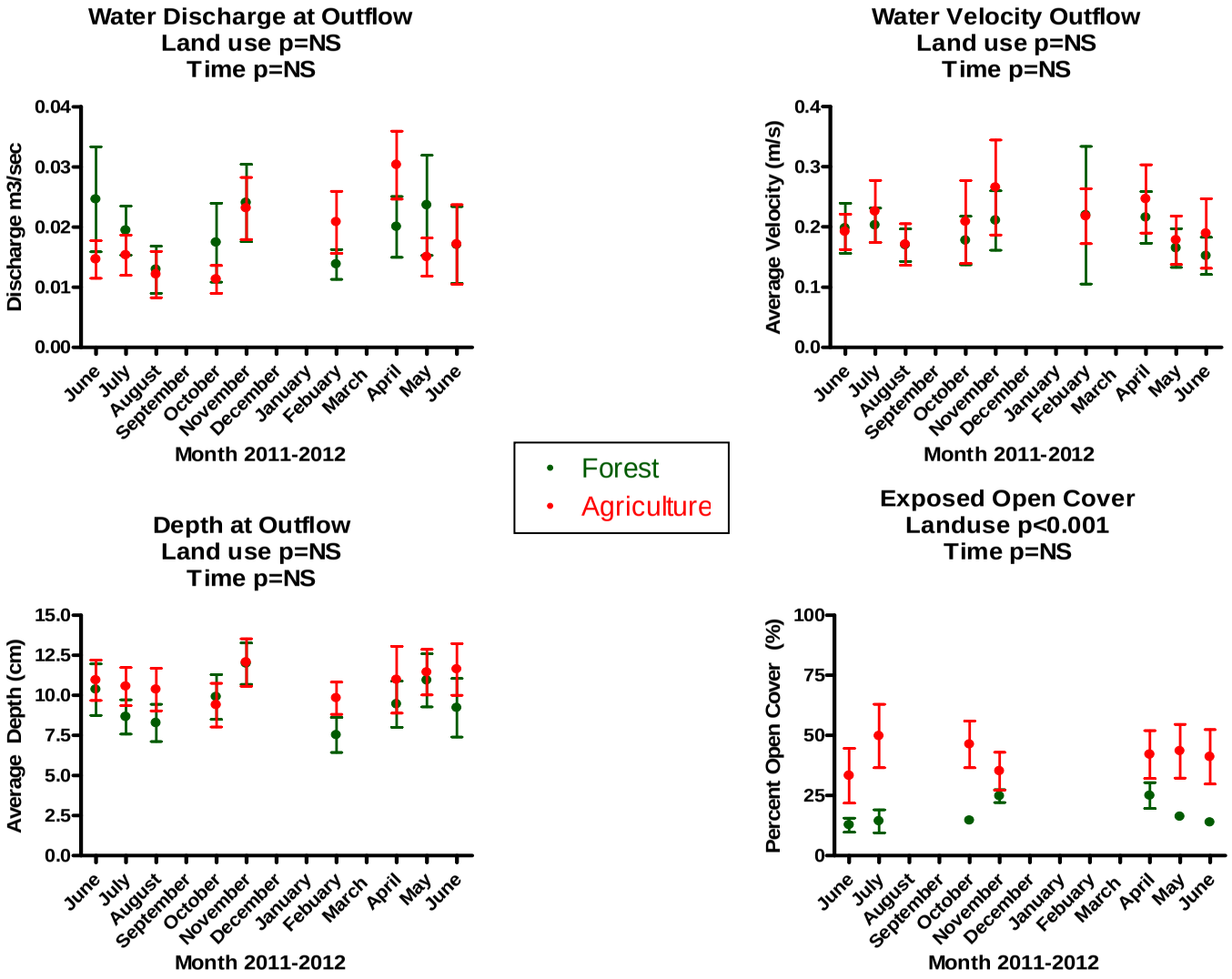


Fig A.2.1.3. Average physical habitat variables (±Std. Error) in spring pools in the two land use types from all sites (n=20) over time.

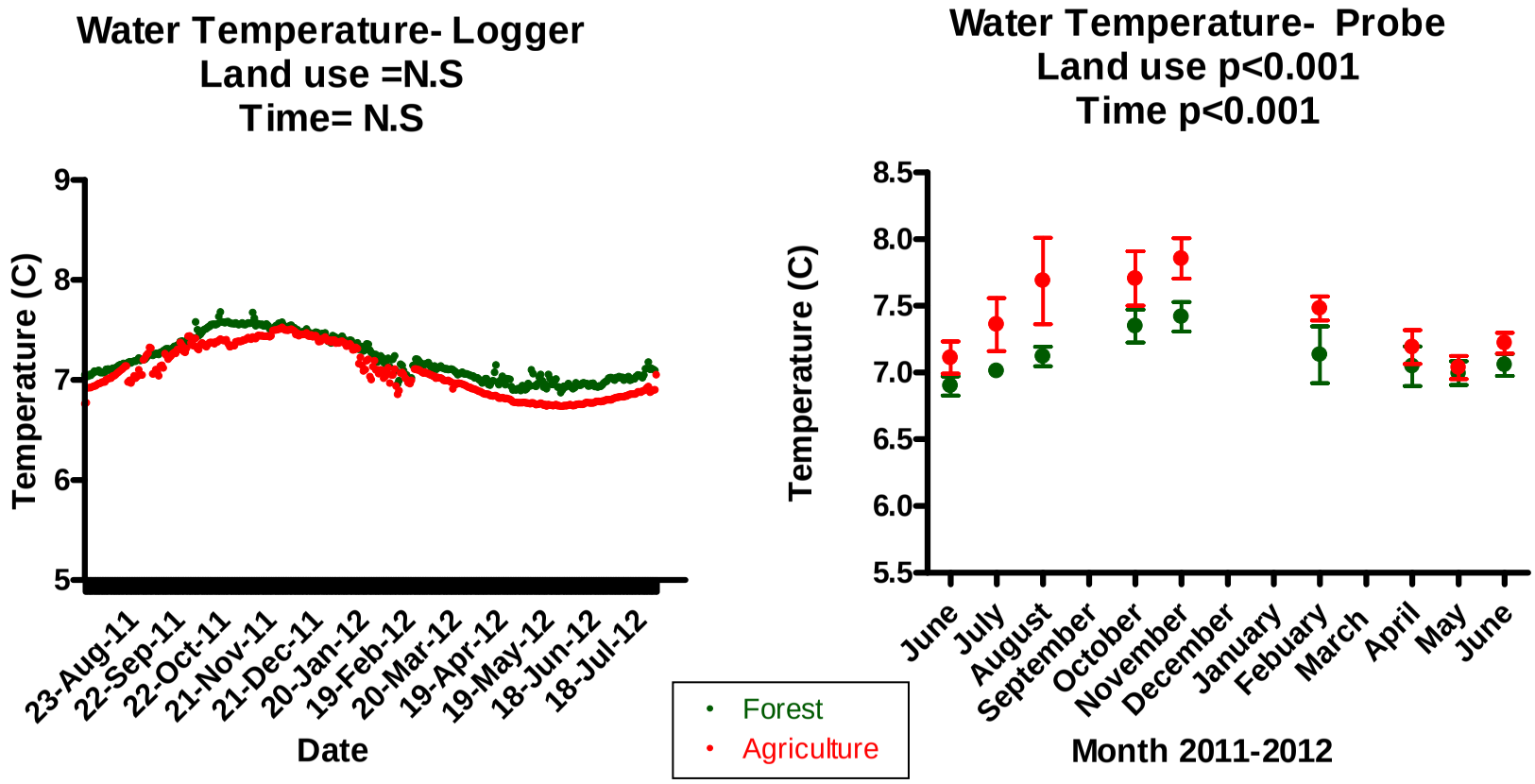


Fig A.2.1.4. Annual temperature patterns in forested and agricultural springs. Left panel: Average temperature (±Std. Error) in spring pools in each of the two land use types based on continuously recording temperature loggers in biodiversity sites (n=8). Right panel: spot temperature readings using a YSI multimeter temperature probe in all sites (n=20) over time. Time and land use were compared using a Two-Way ANOVA with sampling month and land use as factors for probe spot tempratures, and a repeated measures ANOVA for logger temperatures.

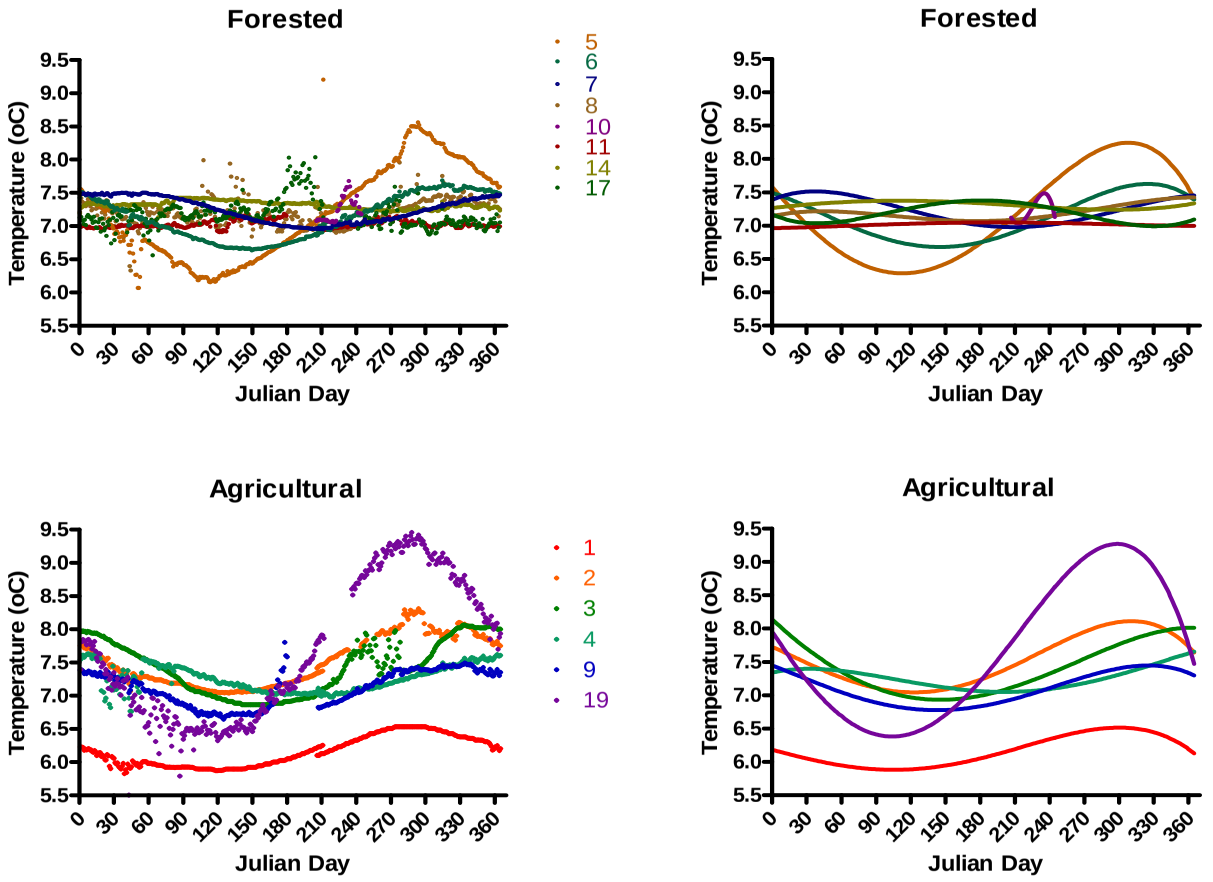


Fig. A.2.1.5 Site-specific temperature logger profiles at all sites with data loggers (n=14). Left panels are raw, temperature points, right panels are forth-order polynomial regression representations of points. Numbers refer to sites, see Fig. 2.1 and Table A.1.5.1. No significant predictor relationship was found between the change in temperature over time and land use, % of open area around the spring, discharge or water depth (Multiple Linear regression, $p>0.05$). A One-way ANCOVA with water depth and land use also showed no significant relationship between change in water temperature and land use.

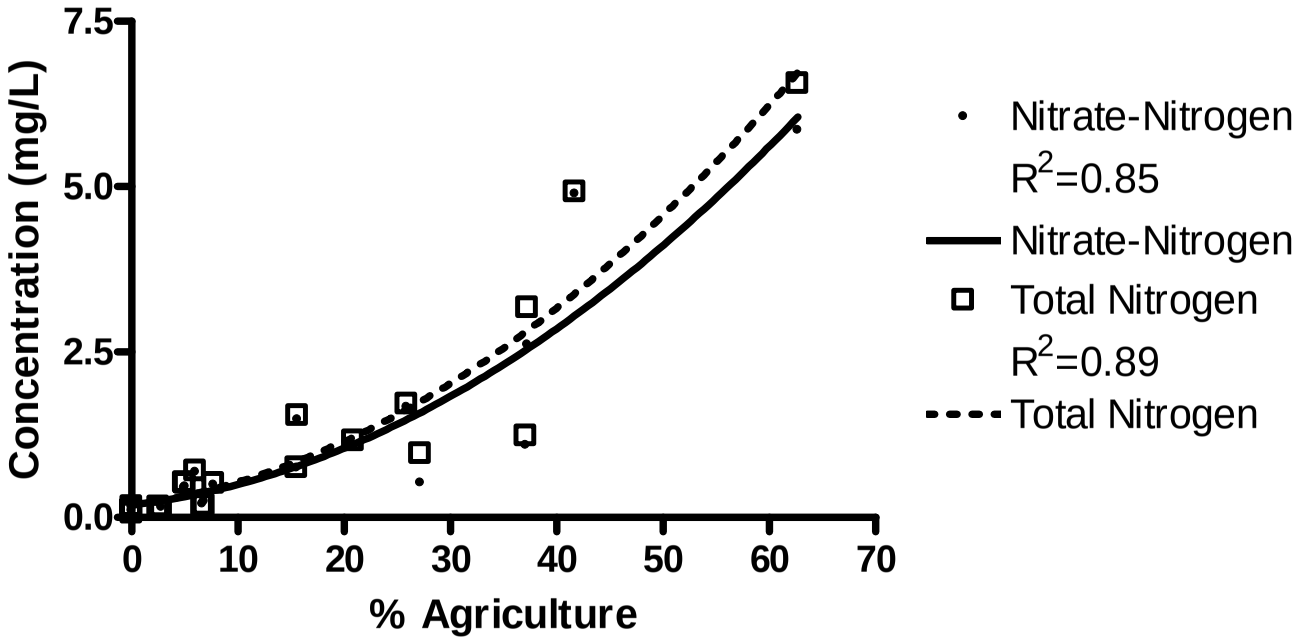


Fig. A.2.1.6. Relationship between nitrogen concentrations (Nitrate-Nitrogen and Total Nitrogen) in the spring pools and percent agriculture within a 1 km radius around the springs for all springs (n=20) using a second order polynomial regression.

Table A.2.1.1 Correlation Matrix for variables measured at all sites (n=20). Data were log or square root transformed as described in Chapter 2. Numbers in bold text indicate important correlations.

	%Agr	%For	Soil	NO_3	TN	PO_4	Cl	SO_4	Temp	Cond	pH	DO	V	Depth	Q	%Open
% Agr																
% For	**-0.9**															
Soil	**-0.7**	0.7														
NO_3	**0.9**	**-0.9**	-0.6													
TN	**0.9**	**-0.9**	**-0.6**	**1.0**												
PO_4	-0.1	0.2	0.0	-0.3	-0.2											
Cl	0.0	0.0	-0.1	0.0	0.0	0.1										
SO_4	0.4	-0.6	-0.2	0.5	0.4	-0.1	-0.1									
Temp	**0.7**	**-0.8**	-0.4	**0.7**	**0.7**	-0.2	0.1	**0.8**								
Cond	**0.7**	**-0.8**	**-0.7**	**0.8**	**0.8**	-0.5	0.1	0.6	**0.7**							
pH	-0.3	0.2	0.0	-0.3	-0.3	0.2	0.2	0.2	-0.1	0.0						
DO	0.0	-0.1	0.0	0.0	0.0	0.4	0.3	0.4	0.3	0.1	0.5					
V	0.0	0.1	-0.1	0.0	0.0	0.3	0.0	0.1	-0.1	-0.1	0.4	0.2				
Depth	0.3	-0.3	-0.1	0.4	0.4	0.2	0.2	0.2	0.4	0.1	-0.4	0.1	0.1			
Q	-0.1	0.1	0.2	0.0	-0.1	0.4	-0.1	0.3	0.1	-0.2	0.0	0.4	0.5	0.5		
%Open	**0.7**	-0.7	-0.5	**0.7**	**0.7**	-0.3	0.0	0.4	**0.7**	0.6	-0.3	0.0	0.0	0.3	0.0	

Key:

% Agr: % of land within 1km radius in Agriculture
% For: % of land within 1 km radius in Forest
Soil: Depth of organic soil layer
NO_3: Nitrate-Nitrogen Concentration
TN: Total Nitrogen Concentration
$P0_4$: Phosphate concentration
Cl: Chloride Concentration
SO_4: Sulphate Concentration
Temp: Spot Water Temperature
Cond: Electrical Conductivity
pH: log[Hydrogen Ion Concentration]
DO: Dissolved Oxygen Concentration
V: Mean velocity at Spring Outflow
Depth: Water Depth at Spring Outflow
Q: Water Discharge at Spring Outflow
%Open: % of open area of open canopy

Table A.2.1.2. Site-specific values of measured physical and chemical variables over time, summarized as means (Mean), median (Med) and Standard Error (SE) value of measured variables from June 2011- June 2012. Variables include Percent Agriculture within 1 km (%Ag), Percent Forest within 1 km (%For). Agr: Agriculture; For: Forested. Other land use within the area include wetlands, roadways, and residential. Note that all "Agricultural sites" were completely surrounded by open agriculture (row crop or pasture) though some (e.g., site 9) may have had forest surrounding the fields.

Site	Site			Total Nitrogen (mg/L)			Nitrate (mg/L)			Phosphate (µg/L)		
No.	Classif.	%Agr	%For	Mean	SE	Med	Mean	SE	Med	Mean	SE	Med
1	Agr	15.54	51.18	0.76	0.06	0.79	0.74	0.05	0.78	36.3	2.9	35.9
2	Agr	15.63	50.86	1.54	0.03	1.51	1.48	0.04	1.48	38.7	1.6	37.3
3	Agr	37.22	56.16	3.17	0.64	3.25	2.61	0.37	2.61	28.9	2.31	27.6
4	Agr	37.07	56.17	1.24	0.12	1.13	1.09	0.11	1.01	23.4	1.6	25.3
5	For	0.00	93.39	0.11	0.02	0.10	0.10	0.01	0.11	55.1	27.2	29.3
6	For	0.01	93.57	0.17	0.01	0.18	0.16	0.01	0.15	24.5	2.3	26.1
7	For	5.05	84.15	0.51	0.05	0.56	0.46	0.04	0.49	21.3	2.4	24.3
8	For	4.96	92.29	0.53	0.04	0.53	0.45	0.03	0.41	36.8	2.7	36.8
9	Agr	6.81	84.04	0.18	0.04	0.15	0.24	0.04	0.21	28.1	1.9	28.2
10	For	2.53	90.12	0.16	0.02	0.14	0.22	0.01	0.22	43.9	2.1	44.7
11	For	2.81	89.62	0.13	0.02	0.10	0.15	0.01	0.15	38.7	2.4	39.1
12	Agr	27.16	66.37	0.97	0.41	0.59	0.52	0.08	0.49	57.0	2.8	57.3
13	For	6.66	84.99	0.18	0.02	0.18	0.20	0.01	0.20	56.1	1.8	55.4
14	For	7.70	82.97	0.52	0.03	0.52	0.49	0.03	0.49	47.2	2.5	46.6
15	For	0.00	96.96	0.06	0.01	0.06	0.04	<0.01	0.03	30.6	1.9	31.4
16	Agr	25.88	53.28	1.72	0.16	1.77	1.67	0.16	1.62	45.4	12.2	36.5
17	For	6.00	88.15	0.70	0.06	0.76	0.68	0.04	0.68	56.1	3.0	53.8
18	Agr	41.72	50.72	4.92	0.18	4.78	4.89	0.20	4.83	28.0	2.4	28.8
19	Agr	62.68	20.39	6.56	0.32	6.43	5.85	0.36	6.01	30.2	3.3	33.2
20	Agr	20.85	76.56	1.16	0.15	1.18	1.07	0.15	0.91	56.5	18.5	40.5

Table A.2.1.2 (cont). Site-specific values of measured physical and chemical variables over time, summarized as means (Mean), median (Med) and Standard Error (SE) value of measured variables from June 2011- June 2012.

Site	Site	Chloride (mg/L)			Sulphate (mg/L)			Spot-measured Temp. (^{o}C)		
No.	Classif.	Mean	SE	Med	Mean	SE	Med	Mean	SE	Med
1	Agr	13.84	0.76	12.39	30.37	3.44	34.92	7.78	0.06	7.81
2	Agr	14.23	0.76	12.92	20.81	2.23	19.32	7.41	0.10	7.35
3	Agr	15.40	0.79	14.33	7.03	0.42	7.81	7.28	0.12	7.25
4	Agr	13.58	0.87	12.92	5.60	0.40	5.83	7.29	0.06	7.28
5	For	15.49	1.08	13.80	4.27	0.21	4.45	7.11	0.24	6.84
6	For	15.69	0.94	13.86	4.62	0.10	4.67	6.98	0.12	6.80
7	For	14.98	0.83	14.18	5.32	0.16	5.45	7.26	0.07	7.20
8	For	14.90	1.04	13.38	5.58	0.10	5.62	7.14	0.04	7.11
9	Agr	15.14	0.94	13.20	4.57	0.15	4.53	7.05	0.08	6.99
10	For	16.57	1.11	14.95	5.04	0.12	4.95	7.06	0.06	7.06
11	For	16.35	0.84	15.11	4.80	0.11	4.73	6.97	0.02	6.98
12	Agr	19.26	1.33	17.33	5.95	0.21	5.85	7.24	0.05	7.19
13	For	14.56	1.27	12.89	4.24	0.09	4.29	7.16	0.17	6.90
14	For	20.85	1.27	18.89	7.32	0.17	7.25	7.39	0.03	7.40
15	For	16.16	1.11	14.15	4.62	0.11	4.61	7.01	0.04	6.97
16	Agr	13.97	1.33	13.67	6.11	0.40	5.88	7.20	0.03	7.18
17	For	14.03	1.17	12.61	5.23	0.15	5.15	7.07	0.03	7.05
18	Agr	16.44	0.82	15.43	12.79	0.41	12.37	7.60	0.05	7.57
19	Agr	16.92	1.19	17.18	7.84	0.38	8.11	7.63	0.38	7.32
20	Agr	13.26	1.00	12.84	5.85	0.37	5.37	7.16	0.18	6.96

Table A.2.1.2 cont. Site-specific values of measured physical and chemical variables over time, summarized as means (Mean), median (Med) and Standard Error (SE) value of measured variables from June 2011- June 2012. . Variables include Electrical Conductivity (µS/cm, Cond), Hydrogen Ion Concentration (pH), Dissolved Oxygen (mg/L, DO), Water Discharge at outflow (m^3/second, Q).

Site	Site	Conductivity (µS/cm)			pH			Dissolved Oxygen (mg/L)			Discharge (m^3/s)		
No.	Classif.	Mean	SE	Med	Mean	SE	Med	Ave DO	SE DO	Med DO	Mean	SE	Med
1	Agr	288.23	17.94	299.45	7.74	0.10	7.67	11.31	0.35	11.18	0.027	0.004	0.027
2	Agr	288.69	18.79	296.60	7.71	0.19	7.80	10.44	0.64	10.79	0.040	0.005	0.035
3	Agr	288.35	21.61	307.90	7.21	0.14	7.33	8.24	0.95	9.62	0.010	0.002	0.008
4	Agr	270.20	17.67	278.00	7.30	0.08	7.33	7.16	0.96	8.27	0.003	0.000	0.003
5	For	223.67	14.66	233.20	7.44	0.08	7.35	9.13	0.70	9.59	0.008	0.002	0.005
6	For	245.91	15.77	255.00	7.79	0.06	7.77	9.22	0.72	9.97	0.019	0.002	0.019
7	For	244.88	16.75	259.10	7.58	0.07	7.54	8.52	0.67	9.31	0.010	0.001	0.009
8	For	216.50	15.23	224.90	7.58	0.09	7.59	10.56	0.50	10.74	0.008	0.001	0.007
9	Agr	238.40	15.82	244.80	7.53	0.08	7.50	9.17	0.68	9.70	0.020	0.004	0.019
10	For	244.49	16.23	259.00	7.79	0.06	7.74	10.43	0.49	10.73	0.033	0.004	0.033
11	For	258.90	18.17	267.50	7.85	0.06	7.82	10.02	0.55	10.50	0.002	0.000	0.002
12	Agr	242.24	16.73	253.00	7.80	0.09	7.81	10.79	0.43	10.82	0.016	0.004	0.013
13	For	216.34	30.23	210.00	7.56	0.08	7.50	9.99	0.52	10.16	0.022	0.003	0.021
14	For	250.40	17.89	260.50	7.66	0.07	7.63	10.20	0.46	10.82	0.030	0.006	0.025
15	For	227.44	16.68	235.25	7.35	0.18	7.44	8.24	0.71	8.92	0.008	0.001	0.008
16	Agr	268.29	36.29	284.00	7.75	0.10	7.81	10.90	0.49	10.90	0.017	0.007	0.015
17	For	207.17	15.13	224.00	7.21	0.16	7.22	10.08	0.51	10.50	0.066	0.007	0.070
18	Agr	334.63	24.37	351.65	7.59	0.07	7.56	9.86	0.58	10.25	0.017	0.001	0.018
19	Agr	314.09	21.63	314.05	7.26	0.18	7.46	10.08	0.59	10.53	0.010	0.001	0.009
20	Agr	220.79	21.20	234.00	7.59	0.08	7.64	8.17	0.93	8.46	0.013	0.003	0.009

Table A.2.1.2 cont. Site-specific values of measured physical and chemical variables over time, summarized as means (Mean), median (Med) and Standard Error (SE) value of measured variables from June 2011- June 2012. Variables include Average water velocity at outflow (m/s, V), Water depth at outflow (cm, Depth), Percent unshaded, open cover (% Open), Depth of organic soil layer(cm).

Site	Site	Velocity (m/s)			Depth at outflow (cm)			% open cover (unshaded)			Depth of org layer
Num.	Classif.	Mean	SE	Med	Mean	SE	Med	Mean	SE	Med	(cm) Mean
1	Agr	0.15	0.02	0.15	10.98	0.56	11.30	21.41	4.01	19.09	3.00
2	Agr	0.35	0.03	0.32	8.22	1.03	9.25	20.31	4.20	17.42	3.33
3	Agr	0.07	0.01	0.06	12.76	1.07	12.80	8.84	1.40	9.10	1.17
4	Agr	0.10	0.01	0.10	6.53	0.72	6.00	33.74	12.33	24.70	0.50
5	For	0.07	0.01	0.06	6.62	0.88	6.10	9.87	4.36	5.77	4.50
6	For	0.34	0.03	0.34	9.17	0.37	9.00	11.04	2.20	9.57	5.33
7	For	0.10	0.01	0.11	6.89	0.50	6.80	8.49	1.94	8.27	10.33
8	For	0.11	0.01	0.12	7.23	1.29	5.80	12.55	4.96	9.04	13.50
9	Agr	0.13	0.02	0.12	10.24	0.84	10.20	17.37	3.68	18.66	4.17
10	For	0.30	0.05	0.31	12.11	1.03	11.90	19.88	4.11	17.18	2.17
11	For	0.15	0.02	0.14	3.98	0.38	4.00	5.23	2.02	3.90	1.50
12	Agr	0.32	0.05	0.30	9.08	0.66	9.17	15.92	3.98	11.22	1.17
13	For	0.11	0.02	0.10	13.22	1.31	13.60	13.20	4.73	9.05	4.17
14	For	0.20	0.04	0.16	15.72	0.52	16.31	18.14	2.64	16.64	3.83
15	For	0.20	0.01	0.20	8.79	0.41	8.63	9.09	3.02	6.24	7.83
16	Agr	0.28	0.07	0.29	6.87	1.35	6.23	11.44	2.84	12.47	0.50
17	For	0.29	0.03	0.32	15.98	1.06	15.83	24.36	3.47	24.70	5.67
18	Agr	0.27	0.02	0.27	10.77	0.78	10.37	60.06	18.75	99.84	0.50
19	Agr	0.10	0.03	0.07	19.38	0.67	18.83	32.70	11.80	11.70	0.50
20	Agr	0.43	0.10	0.41	12.39	1.00	11.67	14.56	5.21	13.99	3.67

Table A.2.1.3. Site specific values for the variables that were only collected at biodiversity sites in chapter 2, and for site 9, which was assessed for adult EPT emergence for chapter 3. CPOM= Coarse Particulate Organic Matter. Note, very fine sediment amounts were assessed qualitatively from photos of bed materials (e.g., Fig A.1.4.1) and field notes about whether "clouds" of fine sediment were released when working in the spring that resulted in very turbid water that did not clear readily.

Site	Site Class.	Brook Slope (%)	Pool Area (m^2)	Conifer Density (no./m^2)	Deciduous Density (no./m^2)	Rock Cover %	Gravel Cover %	Fines Cover %	Very fine sediments present	Bryophyte Cover %	Vascular Plant Cover %	CPOM Cover %	Vertebrates Observed	Adjacent Agriculture
1	Agr	2.46	6.75	0.69	0.16	18.18	2.99	79.51	Yes	5.65	54.91	23.24	Fish, Frogs	Row Crop, Livestock
2	Agr	3.35	10.00	0.51	0.60	29.72	6.51	63.77	Yes	11.88	65.94	15.25	Fish, Frogs	Row Crop, Livestock
3	Agr	2.57	4.00	0.13	0.00	26.39	6.61	61.12	Yes	72.50	4.53	41.88	Frogs	Row Crop
4	Agr	2.67	6.00	0.39	0.09	1.56	0.00	75.23	Yes	41.25	68.44	10.42	Frogs	Row Crop
5	For	3.16	3.00	0.36	0.16	16.30	13.66	69.92	No	78.54	0.42	40.21	Fish	None
6	For	3.22	7.75	0.42	0.26	3.62	0.60	95.78	Some	38.79	0.00	28.15	Fish, Frogs	None
7	For	3.20	5.00	0.31	0.36	11.80	41.37	46.83	No	64.38	0.00	29.25	Fish, Frogs	None
8	For	0.92	6.00	0.12	0.12	0.37	0.65	75.78	No	50.83	17.60	34.90	Fish, Frogs	None
9	Agr	0.02	7.50	0.17	0.23	19.99	14.50	62.11	Yes	58.83	12.00	25.08	Fish	Livestock, Row Crop

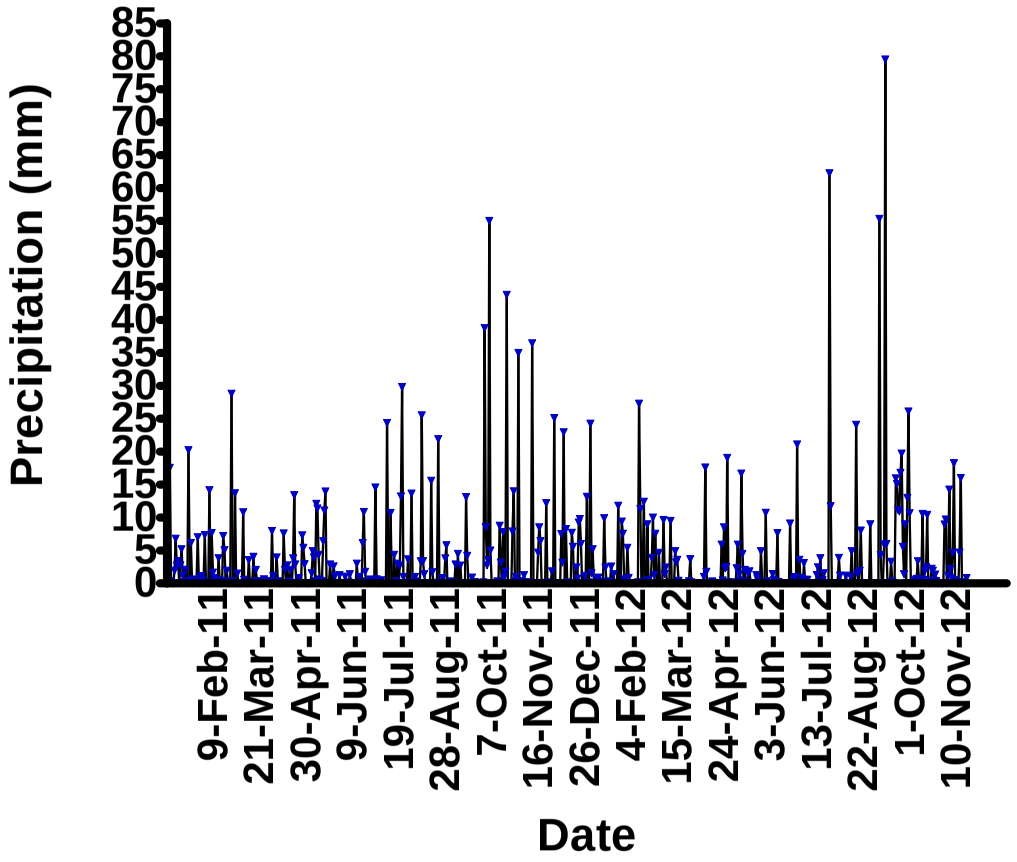


Fig. A.2.1.7. Average precipitation (n=2) in eastern Prince Edward Island from January 2011-December 2012. Data from Environment Canada weather stations in East Point and St. Peters PEI. See Chapter 2 for further details on data acquisition.

Table A.2.1.4. Eigenvectors coefficients that explain the weight of a variable contributing to the principle components axis from PCA in Chapter 2 (Fig. 2.4). The principle components analysis explained 89.7 % of the variation in six axes. Bolded numbers indicate important variables in explaining the axis.

Variable	PC1	PC2	PC3	PC4	PC5	PC6
	42.3%	16.4%	11.2%	7.9%	7%	4.9%
Sqrt(%Agriculture)	**0.347**	-0.032	0.064	-0.151	0.176	0.170
Sqrt(%Forested)	**-0.364**	-0.002	0.018	0.008	-0.031	-0.177
Log(Soil Organic Depth)	-0.272	0.015	0.147	0.389	-0.299	-0.132
Log(Nitrate)	**0.363**	-0.034	0.098	-0.066	0.095	-0.022
Log(Total Nitrogen)	**0.362**	-0.039	0.097	-0.106	0.093	-0.002
Log(Phosphate)	-0.116	0.370	0.221	-0.285	0.190	**0.533**
Log(Chloride)	0.004	0.131	-0.139	**-0.615**	-0.535	-0.321
Log(Sulphate)	0.239	0.256	-0.185	0.475	-0.111	0.022
Log(Spot-Temperature)	0.324	0.117	-0.060	0.216	-0.280	0.122
Log(Conductivity)	0.336	-0.034	-0.285	0.028	-0.062	-0.170
pH	-0.090	0.327	**-0.566**	-0.046	0.114	-0.089
Log(Dissolved Oxygen)	0.037	**0.496**	-0.197	-0.030	-0.237	0.310
Log(Water Velocity at Outflow)	-0.014	0.395	0.005	-0.060	**0.551**	-0.512
Log(Water depth at outflow)	0.139	0.218	**0.528**	-0.118	-0.267	-0.184
Log(Discharge)	-0.014	**0.451**	0.349	0.234	-0.005	-0.150
Sqrt(%Open Cover)	0.304	-0.041	0.089	0.051	0.015	-0.259

Appendix 2.2 Variation in Tree Composition in Riparian Zones Between Springs in Agricultural and Forested Land Use

(See A.1.3. Riparian Area Sampling for methods).

Table A.2.2.1. Comparison of tree and shrub species found around the forested and agricultural 'Biodiversity' springs (average of four springs in each land use category). See Appendix 1.3 for methods of data collection. Data from Giberson et al. 2013[1]

Tree Species	**Tree Class**	**% Abundance around forest springs**	**% Abundance around agriculture springs**
Juniperus communis L.	Conifer Shrub	0.3	0.3
Abies balsamea (L.) Mill	Conifer	34.9	28.0
Picea glauca (Moench) Voss	Conifer	0.6	23.1
Picea rubens Sarg.	Conifer	0.6	5.0
Alnus incana (L.) Moench	Deciduous Shrub	16.8	1.7
Amelanchier spp.	Deciduous Shrub	2.1	3.3
Corylus cornuta Marshall	Deciduous Shrub	0.9	1.2
Myrica pensylvanica Mirbel	Deciduous Shrub	0	0.3
Rosa spp.	Deciduous Shrub	0.4	0
Salix spp.	Deciduous Shrub	0.1	0.3
Sorbus americana Marshall	Deciduous Shrub	0.7	1.6
Viburnum nudum L.	Deciduous Shrub	4.8	3.1
Acer rubrum L	Deciduous	12.3	4.2
Acer spicatum Lam.	Deciduous	0.6	0
Betula papyrifera Marsh.	Deciduous	3.6	4.5
Betula populifolia Marsh.	Deciduous	0	1.2
Malus sp.	Deciduous	0	5.6
Populus tremuloides Michx	Deciduous	0.3	4.7
Prunus pensylvanica L.f.	Deciduous	0	0.3
Prunus virginiana L.	Deciduous	2.5	0
Snag	Dead Tree	17.9	10.8

[1]Giberson, D.J., K. Knysh, and G.E.MacDonald. 2013. Springs of the Clearest and Purest Water. The Island Magazine 73 (Spring/Summer 2013): 2-11.

Appendix 2.3 Macrophyte Differences and Model Details

Table A.2.3.1. Plant species found at Biodiversity sites (n = 8) and their contribution to explaining the differences between the two land use types using Similarity of Percentages (SIMPER) in PRIMER-E. Note: *Typha latifolia* L.or *T. angustifolia* L. (Poales: Typhaceae) was seen at one of the sites assessed only for water chemistry, but is not included here since it was not found at the intensively studied 'Biodiversity' sites.

Order	Family	*Genus species*	Taxon	% Contribution Similarity Forested sites	% Contribution Similarity Agricultural sites	% Contribution Dissimilarity
Jungermanniales	Geocalycaceae	*Chiloscyphus* sp.	Liverwort	**81.42**	**32.07**	**23.38**
Bryopsida		Unknown; 2-4 spp.	Moss	18.58	5.66	**10.07**
Equisetales	Equisetaceae	*Equisetum fluviatile* L.	*Equisetum*	0	0	0.50
Poales	Poaceae	Unknown; 1 or 2 spp.	Poaceae	0	3.53	4.64
Saxifragales	Haloragaceae	*Myriophyllum* sp.	*Myriophyllum*	0	0	0.35
Apiales	Araliaceae	*Hydrocotyle americana* L.	*Hydrocotyle*	0	0	6.09
Gentianales	Rubiaceae	*Galium* sp.	*Galium*	0	5.10	8.15
Myrtales	Onagraceae	*Epilobium ciliatum* Raf. ?	*Epilobium*	0	8.34	7.77
Brassicales	Brassicaceae	*Nasturtium officinale* and/or *N. microphyllum*	*Nasturtium*	0	**43.27**	**30.07**
Ranunculales	Ranunculaceae	*Ranunculus* spp.	*Ranunculus*	0	3.60	6.45
Alismatales	Araceae	*Lemna minor* L.	*Lemna*	0	0	2.52

Table A.2.3.2. Average and median cover percentages for in forested springs (n=4) compared to agricultural springs (n=4). Comparisons were made with a one-way ANOVA except those indicated with ǂ which were compared using Mann-Whitney U-test. No significant differences in cover were found for any species between agricultural and forested springs. Standard errors for non-normal data are reported as a measure of variation.

	Forested Springs			Agricultural Springs		
	Median	Mean	SE	Median	Mean	SE
Liverwort	55.8	52.4	10.55	25.0	30.6	16.53
Moss	57.6	58.1	8.58	26.6	32.8	15.34
Equisetum ǂ	0.0	0.0	-	0.0	0.1	0.05
Poaceae ǂ	0.0	0.0	-	1.4	1.5	0.86
Myriophyllum ǂ	0.0	0.0	-	0.0	0.0	0.03
Hydrocotyle ǂ	0.0	4.4	4.40	0.0	0.0	-
Galium ǂ	0.0	0.1	0.10	2.3	4.5	3.17
Epilobium ǂ	0.0	0.0	-	1.3	4.2	3.36
Nasturtium ǂ	0.0	0.0	-	35.7	33.4	14.09
Ranunculus ǂ	0.0	0.0	-	1.3	3.4	2.67
Lemna ǂ	0.0	0.0	-	0.0	1.3	1.33

Table A.2.3.3. Axis loadings (multiple partial correlations from the distance-based redundancy analysis) for each dbRDA axis for explaining plant taxa distribution using cover estimates at biodiversity sites (n=8) with environmental variables. These represent the relationships between measured variables and the dbRDA coordinate axes. Log= Natural Logarithm transformation, Sqrt= Square Root transformation.

Variable	dbRDA1	dbRDA2	dbRDA3	dbRDA4
Log(Total Nitrogen)	0.015	**0.484**	0.334	-0.42
Sqrt(Conifer Density)	0.24	0.064	-0.424	-0.254
pH	0.212	-0.443	-0.185	-0.77
Sqrt(%Open Cover)	**0.573**	0.391	0.432	-0.114
Log(Sulphate)	**0.736**	-0.33	0.003	0.382
Log(Soil Organic Depth)	-0.163	**-0.551**	**0.698**	-0.089

Table A.2.3.4. Pearson (ρ) correlations between plant taxon cover and each of the first two db-RDA Axes (n=8). The variables that are listed in the column headings contributed the most to building the axis and the percentage value represents the amount of fitted variation explained by that axis (92.42% of fitted variation, 92.22% of total variation).

Taxon	Number of Sites Present (For, Agr)	Sulphate and % Open (70.7 %)	Land use (21.7 %)
Liverwort	7 (4,3)	**-0.91**	0.34
Moss	6 (4,2)	0.14	**-0.89**
Equisetum	1 (0,1)	0.23	0.77
Poaceae	2 (0,2)	0.51	0.49
Myriophyllum	1 (0,1)	0.23	0.77
Hydrocotyle	1 (1,0)	-0.25	-0.21
Galium	3 (2,1)	-0.03	**0.94**
Epilobium	3 (0,3)	0.59	**0.67**
Nasturtium	3 (0,3)	**0.95**	0.13
Ranunculus	2 (0,2)	0.55	0.64
Lemna	1 (0,1)	0.23	0.77

Table A.2.3.5. Model building using DISTLM for plant cover estimates for db-RDA (n=8, Fig. A.2.3.1) with Total Nitrogen forced into the model. Model was forward selected with no other starting terms, Total Sum of Squares (trace)=12411 with df=6. Marginal test variables were selected to test; Sequential test variables were used in model. Final adjusted-R^2=0.98, R^2=1.0 with bolded values statistically significant ($p \leq 0.05$). Sqr=Square root transformation, Log= Natural Logarithm transformation.

MARGINAL TESTS

Variable	SS(trace)	Pseudo-F	P-Value	Proportion
Log(Total Nitrogen)	1520.60	0.84	0.45	0.12
Log(Phosphate)	609.35	0.31	0.79	0.05
Log(Sulphate)	6379.20	6.35	**0.02**	0.51
pH	3560.50	2.41	0.11	0.29
Log(Discharge)	3035.20	1.94	0.19	0.24
Sqrt(%Open Cover)	4847.00	3.85	**0.04**	0.39
Sqrt(Conifer Density)	6111.10	5.82	0.01	0.49
Sqrt(Deciduous Cover)	1904.80	1.09	0.38	0.15
Log(Soil Organic Depth)	2864.50	1.80	0.18	0.23
Sqrt(Rock Cover)	701.23	0.36	0.73	0.06
Sqrt(Gravel Cover)	1332.90	0.72	0.53	0.11
Sqrt(Coarse Particulate Organic Matter)	4857.70	3.86	**0.05**	0.39

SEQUENTIAL TESTS

Variable	Adj R^2	SS(trace)	Pseudo-F	P- Value	Proportion	Cumulative Variation	res.df
Sqr(Conifer Density)	0.55	6896.50	8.63	**0.01**	0.56	0.68	5
pH	0.67	1621.20	2.73	0.08	0.13	0.81	4
Srq(%Open)	0.80	1298.90	3.63	0.08	0.10	0.91	3
Log(Sulphate)	0.93	828.37	6.76	0.06	0.07	0.98	2
Log(Soil Organic Depth)	0.98	218.01	8.07	0.16	0.02	1.00	1

Table A.2.3.6. Correlation Matrix showing correlation coefficients for linear correlation between individual Plant Environmental Variables in 'Biodiversity' springs (n=8). Data are log or square root transformed as described in Chapter 2. Bolded values indicate important correlations

	%Ag	%For	TEMP	NO_3	TN	PO_4	Cl	SO_4	pH	COND	DO	Q	V	Depth	Slope	Area	%Open	Con	Des	Soil	Cob	Peb	Fines
%Ag																							
%For	**-0.9**																						
TEMP	0.0	0.2																					
NO_3	**0.9**	**-0.8**	0.1																				
TN	**0.9**	**-0.8**	0.2	**1.0**																			
PO_4	-0.3	0.1	-0.1	-0.3	-0.3																		
Cl	-0.6	**0.7**	0.4	-0.5	-0.5	0.1																	
SO_4	0.4	**-0.8**	-0.5	0.5	0.5	0.2	-0.6																
pH	-0.5	0.2	-0.4	-0.4	-0.4	0.1	0.0	0.4															
COND	**0.7**	**-0.9**	-0.2	**0.8**	**0.7**	-0.2	-0.5	**0.7**	-0.1														
DO	-0.3	0.0	-0.5	-0.1	-0.2	0.6	0.0	0.6	0.7	0.0													
Q	-0.2	-0.2	-0.3	0.1	0.0	0.3	0.1	**0.7**	0.8	0.4	**0.8**												
V	-0.2	-0.1	0.0	0.0	-0.1	-0.1	-0.1	0.4	**0.8**	0.3	0.4	**0.7**											
Depth	0.4	-0.5	-0.3	0.5	0.5	0.0	0.1	0.5	0.0	0.6	0.3	0.5	0.1										
Slope	0.0	-0.2	0.1	-0.1	-0.1	-0.2	0.0	0.1	0.1	0.4	-0.3	0.2	0.3	0.1									
Area	0.1	-0.3	-0.1	0.3	0.2	-0.3	-0.4	0.5	**0.7**	0.4	0.4	0.6	**0.9**	0.1	0.0								
%Open	0.6	-0.6	-0.2	0.4	0.3	-0.1	**-0.9**	0.3	-0.2	0.3	-0.2	-0.3	0.1	-0.2	-0.1	0.3							
Con	-0.1	-0.4	-0.6	-0.1	-0.2	0.1	-0.5	0.6	0.6	0.4	0.3	0.5	0.5	0.0	0.6	0.4	0.4						
Des	-0.4	0.1	0.1	-0.3	-0.3	0.0	-0.2	0.3	**0.7**	-0.1	0.4	0.5	**0.7**	-0.4	0.3	0.6	0.0	0.6					
Soil	**-0.8**	**0.7**	0.0	-0.6	-0.6	0.2	0.5	-0.2	0.6	**-0.7**	0.5	0.3	0.2	-0.3	-0.3	0.0	-0.6	-0.2	0.5				
Cob	0.2	-0.5	0.0	0.4	0.3	0.3	0.0	0.5	0.0	0.6	0.2	0.6	0.0	0.5	0.6	-0.1	-0.3	0.3	0.1	-0.2			
Peb	-0.2	0.2	0.1	-0.1	-0.1	0.0	0.3	-0.1	0.0	-0.1	0.0	0.1	-0.3	-0.1	0.4	-0.4	-0.6	0.0	0.3	0.4	0.5		
Fines	-0.3	0.1	-0.4	-0.4	-0.4	0.1	0.0	0.0	0.4	-0.1	0.2	0.1	0.4	0.2	-0.2	0.3	0.3	0.3	-0.1	-0.1	-0.5	-0.8	
CPOM	-0.4	0.6	0.0	-0.3	-0.3	0.3	**0.8**	-0.4	-0.2	-0.5	0.2	0.0	-0.5	0.3	-0.2	**-0.7**	**-0.8**	-0.6	-0.4	0.4	0.1	0.4	-0.1

Key:

% Agr: % of land within 1km radius in Agriculture
% For: % of land within 1 km radius in Forest
Temp: Spot Water Temperature
NO_3: Nitrate-Nitrogen Concentration
TN: Total Nitrogen Concentration
PO_4: Phosphate Concentration
Cl: Chloride Concentration
SO_4: Sulphate Concentration
pH: Hydrogen Ion Concentration
Cond: Electrical Conductivity
DO: Dissolved Oxygen Concentration
Q: Water Discharge at Spring Outflow
V: Water velocity at Spring Outflow
D: Water Depth at Spring Outflow
S: Slope of bed at Spring Outflow
Area: Area of the spring pool
%Open: % of open area of open canopy
Con: Conifer density
Dec: Deciduous tree density
Soil: Depth of organic soil layer
Cob: Rocks in Cobble size class
Peb: Pebble sized gravel
Fine: Fines, including sand/silt
Bry: Bryophyte cover
Macro: Vascular Macrophytes
CPOM: Coarse Particulate Organic Matter

Aquatic Plant Analysis in Biodiversity sites

(assessed using actual cover values rather than the presence/absence data used in the text)

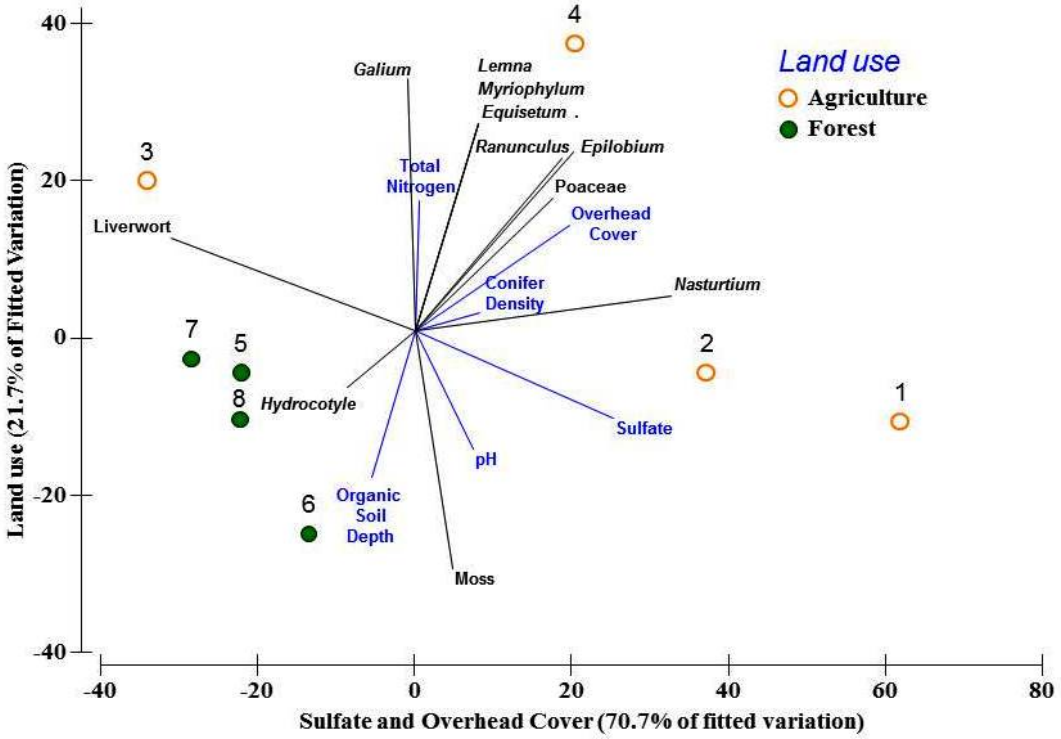


Fig. A.2.3.1 Distance based redundancy analysis ordination biplot of plant taxa cover estimates in the eight Biodiversity spring sites (n=4 for each land use type). Plant taxa vectors correspond to increased cover of the specified taxa and vectors of environmental variables correspond to sites with higher values of that variable. The numbers refer to the site numbers, and their locations in respect to taxa and environmental variable.

Correlations using distance-based redundancy analysis between cover values for individual plant taxa and measured habitat variables were explored for the two land use categories in the intensively studied Biodiversity springs (shown here) for direct comparison with benthic data in addition to the full suite of 20 sites shown in the text (Fig 2.6); this analysis assesses all taxa and habitat variables simultaneously through a constrained ordination. In the biodiversity subset based on cover the first two axes explained 92.42 % of the fitted variation observed. The first axis, was mainly associated with sulphate concentration and surounding overhead cover. The second axis was associated with soil organic depth (which correlated to forest type and amount) and nitrogen (the agricultural variable which was forced into the model). Liverwort cover correlated highly with the first axis (ρ = -0.9) and moss cover correlated with the second axis (ρ= -0.9). The majority of individual plant taxa were not encountered enough to from cover estimates draw conclusions about their land use patterns; however, many of the vascular plants were only encountered at sites with an open canopy (Fig 2.6; Appendix 2.2). (For the same analysis based on the presence/absence of plants, the first two db-rda axis explain 79% of the fitted variation: Axis 1was primarily explained by surounding overhead cover and nitrogen concentrations; Axis 2 was primarily explained by pH and light; Axis 3 was primarly associated with sulphate.)

Table A.2.3.7. Axis loadings (multiple partial correlations from the distance-based redundancy analysis) for each db-RDA axis for explaining plant taxon presence/absence (n=20) from Chapter 2, Fig. 2.6. These represent the relationships between measured variables and the db-RDA coordinate axes; bolded numbers represent important contributes to the axis. Sqrt=Square root transformation, Log= Natural Logarithm transformation.

Variable	dbRDA1	dbRDA2	dbRDA3
Log(Total Nitrogen)	**-0.542**	0.082	-0.44
pH	-0.305	**0.584**	**0.613**
Log(Sulphate)	-0.021	-0.477	**0.635**
Log(Discharge)	-0.49	0.287	0.039
Sqrt(%Open Cover)	**-0.608**	**-0.573**	0.02
Log(Water Depth)	0.042	0.12	-0.159

Table A.2.3.8. Correlations between plant taxon presence/absence and each of the first three db-RDA Axes (n=20). The variables that are listed in the column headings contributed the most to building the axis and the percentage value represents the amount of fitted variation explained by that axis (100% of fitted variation, 57.7% of total variation).

Taxon	Number of Sites Present (For, Ag)	Land Use (49.9%)	pH and Open Cover (23.4%)	Sulphate (23%)
Liverwort	(7,3)	0.48	-0.17	-0.17
Moss	(8,5)	0.41	0.32	0.61
Equisetum	(0,1)	-0.06	-0.41	-0.16
Poaceae	(1,3)	-0.43	-0.37	0.23
Myriophylum	(0,2)	-0.27	-0.44	-0.39
Hydrocotyle	(0,1)	0.08	-0.04	0.01
Galium	(5,3)	0.38	0.00	-0.24
Epilobium	(0,3)	-0.07	-0.62	0.47
Nastutium	(0,3)	-0.07	-0.62	0.47
Ranunculus	(0,4)	-0.03	-0.59	0.01
Lemna	(0,2)	-0.27	-0.44	-0.39
Open Sediment	(6,9)	-0.41	0.09	-0.08
Typha	(0,1)	-0.61	-0.25	0.11

Table A.2.3.9..Model building using DISTLM for plant presence or absence for db-RDA (n=20, Chapter 2, Fig.2.6) with Total Nitrogen forced into the model. The model was forward selected with no other starting terms, Total Sum of Squares (trace)=35705 with df=6. Marginal test variables were selected to test; Sequential test variables were used in model. Final adjusted-R^2=0.36, R^2=0.56 with bolded values statistically significant ($p \leq 0.05$). Sqrt=Square root transformation, Log= Natural Logarithm transformation.

MARGINAL TESTS

Variable	SS(trace)	Pseudo-F	P-Value	Proportion
Log(Total Nitrogen)	6723.30	4.18	**0.01**	0.19
pH	4570.70	2.64	0.06	0.13
Log(Dissolved Oxygen)	2601.70	1.41	0.26	0.07
Log(Water Depth)	453.23	0.23	0.84	0.01
Log(Discharge)	351.61	0.18	0.87	0.01
Sqrt(%Open)	6995.00	4.39	**0.01**	0.20
Log(Soil Organic Depth)	6181.60	3.77	**0.02**	0.17
Log(Phosphate)	1890.10	1.01	0.42	0.05
Log(Sulphate)	4313.30	2.47	0.08	0.12

SEQUENTIAL TESTS

Variable	Adj R^2	SS(trace)	Pseudo-F	P-Value	Proportion	Cumulative Variation	res.df
pH	0.23	4537.00	3.16	**0.04**	0.13	0.32	17
Log (Sulphate)	0.32	4027.90	3.16	0.06	0.11	0.43	16
LOG(Discharge)	0.35	1964.80	1.60	0.23	0.06	0.48	15
Sqrt(%Open Cover)	0.36	1540.90	1.28	0.32	0.04	0.53	14
LOG(Water Depth)	0.36	1359.30	1.14	0.36	0.04	0.56	13

Appendix 2.4 Invertebrate Summary

Table A.2.4.1. Benthic invertebrate taxa found at Biodiversity sites and their contribution to explaining the differences between the two land use types using Similarity of Percentages analysis (SIMPER) in PRIMER-E. Taxa that made a contribution of less than 0.5% are indicated by a dash (-), bolded values indicated the value is one of the ten highest contributors to similarity or dissimilarity.

Order	Family: Sub-family	Taxon	Code	% Similarity Forest sites	% Similarity Agr sites	% Dissimilarity
Ephemeroptera	Baetidae	*Baetis*	Bae	-	-	-
Ephemeroptera	Ameletidae	*Ameletus*	Aml	-	-	-
Ephemeroptera	Ephemerellidae	*Ephemerella*	Eph	-	-	-
Plecoptera	Nemouridae	*Nemoura*	Nem	**2.74**	**4.16**	1.14
Plecoptera	Nemouridae	*Amphinemura*	Amp	1.31	1.88	2.15
Plecoptera	Leuctridae	*Leuctra*	Leu	-	1.62	0.93
Plecoptera	Chloroperlidae	*Sweltsa*	Swe	2.55	**4.55**	1.08
Trichoptera	Rhyacophilidae	*Rhyacophila*	Rhy	-	0.49	0.51
Trichoptera	Lepidostomidae	*Lepidostoma*	Lep	2.12	**4.44**	2.42
Trichoptera	Uenoidae	*Neophylax*	Nep	0.6	0.64	0.71
Trichoptera	Limnephilidae	Limnephilidae n.det 1	Limn	-	0.45	0.59
Trichoptera	Limnephilidae	*Hesperophylax*	Hesp	1.04	2.68	0.64
Trichoptera	Limnephilidae	*Onocosmoecus*	Ono	0.64	-	-
Trichoptera	Limnephilidae	*Psychoglypha*	Psy	-	-	-
Trichoptera	Limnephilidae	*Pycnopsyche*	Pyc	-	-	-
Coleoptera	Dytiscidae	Hydroporonae n.det	Hyd	0.75	-	0.64
Coleoptera	Dytiscidae	*Agabus*	Aga	-	-	0.58
Coleoptera	Scirtidae	Scirtidae n.det	Sci	-	-	-
Diptera	Limoniidae	*Limnophila*	Lim	-	-	-
Diptera	Pediciidae	*Dicronata*	Dic	-	1.02	0.79
Diptera	Pediciidae	*Pedicia*	Ped	-	1.83	0.55
Diptera	Ceratopogonidae	*Probezzia/Mallchoerea*	Cera	-	-	-
Diptera	Chaoboridae	*Eucorethra*	Euo	-	-	-
Diptera	Dixidae	*Dixella*	Dix	-	-	-
Diptera	Psychodidae	*Pericoma*	Per	-	-	-
Diptera	Chironomidae: Podonominae	*Parochlus*	Par	**3.94**	0.97	**4.71**
Diptera	Chironomidae: Podonominae	*Boreochlus*	Bor	-	-	-
Diptera	Chironomidae: Tanypodinae	Tanypodinae n.det	Tanyp	-	-	-
Diptera	Chironomidae: Chironominae	Tanytarsini n.det	Tanyt	**6.35**	**10.16**	**5.97**
Diptera	Chironomidae: Diamesinae	*Diamesa*	Dia	-	-	0.6

Table A.2.4.1 continued

Order	Family: Sub-family	Taxon	Code	% Similarity Forest sites	% Similarity Agr sites	% Dissimilarity
Diptera	*Chironomidae: Diamesinae*	*Protanypus*	Pro	-	-	-
Diptera	Chironomidae: Diamesinae	*Pseudodiamesa*	Pse	0.99	-	0.71
Diptera	Chironomidae: Diamesinae	Diamesinae n. det 1	Pot	-	-	-
Diptera	Chironomidae: Orthocladiinae	*Brillia*	Bri	0.91	1.01	1.11
Diptera	Chironomidae: Orthocladiinae	*Corynoneura*	Cor	**5.5**	2.06	3.2
Diptera	Chironomidae: Orthocladiinae	*Diplocladius*	Dip	-	-	1.41
Diptera	Chironomidae: Orthocladiinae	*Eukiefferiella*	Euk	2.52	1.87	**3.64**
Diptera	Chironomidae: Orthocladiinae	*Heterotanytarsus*	Heterota	-	-	0.59
Diptera	Chironomidae: Orthocladiinae	*Heterotrissocladius*	Heterotr	0.48	**3.51**	1.42
Diptera	Chironomidae: Orthocladiinae	*Hydrobaenus*	Hydrob	**3.26**	2.02	**4.11**
Diptera	Chironomidae: Orthocladiinae	*Metriocnemus*	Met	1.5	**4.86**	1.93
Diptera	Chironomidae: Orthocladiinae	*Orthocladius/ Cricotopus*	Ort	**6.26**	3.17	**4.97**
Diptera	Chironomidae: Orthocladiinae	*Paraphaenocladius/Parametriocnemus*	Parap/Param	-	1.82	2.25
Diptera	Chironomidae: Orthocladiinae	Orthocladiinae n. det 1	Parak	-	-	0.6
Diptera	Chironomidae: Orthocladiinae	*Platysmittia/Psilometriocnemus*	Pl/Ps	0.84	1.81	0.87
Diptera	Chironomidae: Orthocladiinae	*Rheocricotopus*	Rhe	**4.35**	1.91	2.82
Diptera	Chironomidae: Orthocladiinae	*Orthocladius (Symposiocladius)*	O(Sym)	-	-	-
Diptera	Chironomidae: Orthocladiinae	*Thienemanniella*	Thi	**22.78**	**6.77**	**10.27**
Diptera	Chironomidae: Orthocladiinae	*nr. Unniella*	Unn	-	-	-
Diptera	-	Muscomorpha	Mus	-	-	-
Diptera	Syrphidae	*Eristalis*	Eri	-	-	-
Trombidiformes	Arrenuridae	*Laversia*	Lav	-	-	-
Trombidiformes	Hydryphantidae	*Panisopsis*	Pan	**7.35**	1.13	**4.24**
Trombidiformes	Hydrovolziidae	*Hydrovolzia*	Hydrov	1.82	2.61	1.93
Trombidiformes	Lebertiidae	*Lebertia*	Leb	1.72	1.65	0.67
Trombidiformes	Sperchonidae	*Sperchon*	Spe	**9.76**	**5.17**	**3.96**
Trombidiformes	Hygrobatidae	*Hygrobates*	Hygro	-	-	-
Trombidiformes	Feltriidae	*Feltria*	Fel	-	-	-
Oribatida	-	Oribatida	Ori	2.67	3.43	0.85
Copepoda	-	Cyclopoida	Cyc	-	1.17	**4**
Copepoda	-	Harpacticoida	Har	0.82	0.56	1.26
Ostracoda	-	Ostracoda	Ost	-	**9.6**	**8.05**
Cladocera	Chydoridae	*Chydorus*	Chy	-	-	-
Bivalvia	Sphaeriidae	*Pisidium*	Pis	1.61	**3.99**	2.34
Oligochaeta	-	Megadriles	Mega	1.05	1.18	0.97
Oligochaeta	-	Microdriles	Micr	0.8	1.92	-

Table A.2.4.2 Mean and median densities of invertebrate taxa in forested springs (n=4) and agricultural springs (n=4). Land-use comparisons were made with a one-way ANOVA or a Mann-Whitney U test. P-values shown as a dash (-) indicate no significant difference for that taxon between forested and agricultural sites.

		Forested Sites		Agricultural Sites		
Taxon	Code	Mean	Median	Mean	Median	P-Value
Baetis	Bae	0.00	0.00	14.29	0.00	-
Ameletus	Aml	4.76	0.00	0.00	0.00	-
Ephemerella	Eph	4.76	0.00	0.00	0.00	-
Nemoura	Nem	232.14	180.95	423.40	315.79	-
Amphinemura	Amp	88.99	25.60	646.94	158.11	-
Leuctra	Leu	19.05	0.00	107.19	109.62	-
Sweltsa	Swe	269.83	179.76	367.01	168.76	-
Rhyacophila	Rhy	0.00	0.00	29.32	28.57	-
Lepidostoma	Lep	961.80	163.69	487.63	516.10	-
Neophylax	Nep	42.56	27.98	61.90	47.62	-
Limnephilidae n.det 1	Limn	0.00	0.00	45.24	23.81	-
Hesperophylax	Hesp	82.21	66.67	83.63	94.24	-
Onocosmoecus	Ono	26.08	23.59	4.76	0.00	-
Psychoglypha	Psy	4.46	0.00	37.30	7.94	-
Pycnopsyche	Pyc	4.76	0.00	0.00	0.00	-
Hydroporonae n.det	Hyd	35.42	32.74	47.93	7.52	-
Agabus	Aga	0.00	0.00	57.80	22.56	-
Scirtidae n.det	Sci	4.76	0.00	0.00	0.00	-
Limnophila	Lim	4.46	0.00	0.00	0.00	-
Dicronata	Dic	28.57	0.00	59.81	36.90	-
Pedicia	Ped	4.76	0.00	28.94	30.49	0.010
Probezzia/Mallchoerea	Cera	4.46	0.00	14.29	0.00	-
Eucorethra	Euo	13.69	9.52	0.00	0.00	-
Dixella	Dix	14.29	9.52	0.00	0.00	-
Pericoma	Per	0.00	0.00	9.52	0.00	-
Parochlus	Par	3172.62	2464.29	285.71	142.86	-
Boreochlus	Bor	23.81	0.00	0.00	0.00	-
Tanypodinae n.det	Tanyp	11.90	0.00	11.90	0.00	-
Tanytarsini n.det	Tanyt	2053.76	1178.95	8083.33	2928.57	-
Diamesa	Dia	3.57	0.00	59.52	47.62	-
Protanypus	Pro	23.81	0.00	0.00	0.00	-
Pseudodiamesa	Pse	35.09	46.37	35.71	0.00	-
Diamesinae n.det 1	Pot	0.00	0.00	47.62	0.00	-
Brillia	Bri	66.67	61.90	154.76	119.05	-
Corynoneura	Cor	1739.29	1154.76	476.19	238.10	-

Table A.2.4.2. Continued

Taxon	Code	Forested Sites		Agricultural Sites		P-Value
		Mean	Median	Mean	Median	
Diplocladius	Dip	11.90	0.00	440.48	23.81	-
Eukiefferiella	Euk	1529.76	523.81	142.86	142.86	-
Heterotanytarsus	Heterota	120.24	0.00	0.00	0.00	-
Heterotrissocladius	Heterotr	99.00	43.23	285.71	285.71	-
Hydrobaenus	Hydrob	3236.22	186.72	583.33	357.14	-
Metriocnemus	Met	86.90	102.38	702.38	476.19	-
Orthocladius/ Cricotopus	Ort	4469.24	2674.19	940.48	452.38	-
Paraphaenocladius/ Parametriocnemus	Parap/ Param	705.95	23.81	357.14	166.67	-
Orthocladiinae n.det 1	Parak	107.14	0.00	0.00	0.00	-
Platysmittia/ Psilometriocnemus	Pl/Ps	39.29	47.62	119.05	142.86	-
Rheocricotopus	Rhe	1236.90	1330.95	428.57	119.05	-
Symposiocladius	O(Sym)	3.57	0.00	11.90	0.00	-
Thienemanniella	Thi	14864.29	12919.05	1357.14	523.81	0.002
Unniella	Unn	11.90	0.00	0.00	0.00	-
Muscomorpha	Mus	0.00	0.00	3.76	0.00	-
Eristalis	Eri	3.57	0.00	0.00	0.00	-
Laversia	Lav	17.86	0.00	25.27	8.93	-
Panisopsis	Pan	1582.60	1010.71	1750.08	85.21	-
Hydrovolzia	Hydrov	708.86	122.48	316.87	245.11	-
Lebertia	Leb	66.64	70.18	105.37	112.00	-
Sperchon	Spe	2854.51	1913.78	361.60	226.07	0.051
Hygrobates	Hygro	25.00	21.43	0.00	0.00	-
Feltria	Fel	7.14	0.00	40.18	0.00	-
Oribatida	Ori	299.53	152.38	156.92	141.19	-
Cyclopoida	Cyc	638.39	0.00	3292.89	64.16	-
Harpacticoida	Har	99.70	76.19	253.56	76.19	-
Ostracoda	Ost	3955.36	0.00	6296.73	3723.28	-
Chydorus	Chy	53.57	0.00	0.00	0.00	-
Pisidium	Pis	298.12	124.81	1069.98	314.57	-
Megadriles	Mega	42.61	47.62	186.39	77.54	-
Microdriles	Micr	54.21	38.78	179.43	183.43	-

Table A.2.4.3. Axis loadings (multiple partial correlations from the distance-based redundancy analysis) for each db-RDA axis for explaining invertebrate taxa community from Chapter 2, Fig. 2.8. These represent the relationships between measured variables and the dbRDA coordinate axes, with bolded numbers indicating important contributes to the axis. Sqrt=Square root transformation, Log= Natural Logarithm transformation.

Variable	dbRDA1	dbRDA2	dbRDA3	dbRDA4	dbRDA5	dbRDA6
Log(Total Nitrogen)	**0.402**	0.052	-0.097	0.502	0.381	0.655
Sqrt(Rock Cover)	**-0.466**	0.218	-0.18	**0.724**	-0.416	-0.072
Sqrt(Bryophyte Cover)	0.147	**-0.718**	-0.046	-0.013	-0.599	0.318
Sqrt(Deciduous Cover)	**-0.511**	0.196	0.523	-0.241	-0.075	0.603
Sqrt(Gravel Cover)	-0.42	-0.083	**-0.787**	-0.299	0.201	0.26
Sqrt(Vascular Plant Cover)	**0.404**	**0.623**	-0.251	-0.275	-0.526	0.182

Table A.2.4.4. Correlations between individual invertebrate taxa and each of the first four db-RDA Axes. The variables that are listed in the column headings contributed the most to building the axis and the percentage value represents the amount of fitted variation explained by that axis (86.34% of fitted variation, 79.90% of total variation).

Taxon	Code	Number of Sites Present (For, Ag)	Deciduous Trees and Heterogeneity 39.13 %	Plant Cover 22.65 %	Gravel Cover 14.40%	Rock Cover 10.15%
Baetis	Bae	1 (0, 1)	0.19	0.53	-0.42	0.12
Ameletus	Aml	1 (1, 0)	-0.43	-0.39	-0.11	-0.13
Ephemerella	Eph	1 (1, 0)	-0.33	-0.31	-0.54	-0.29
Nemoura	Nem	8 (4, 4)	**0.77**	-0.31	0.12	0.45
Amphinemura	Amp	7 (4, 3)	-0.54	0.68	-0.02	0.16
Leuctra	Leu	4 (1, 3)	0.48	0.13	**-0.65**	0.32
Sweltsa	Swe	8 (4, 4)	-0.02	0.25	-0.58	-0.04
Rhyacophila	Rhy	2 (0, 2)	-0.19	0.93	-0.21	0.14
Lepidostoma	Lep	8 (4, 4)	-0.04	-0.45	-0.32	0.01
Neophylax	Nep	5 (3, 2)	-0.44	0.47	**-0.65**	-0.23
Limnephilidae n.det 1	Limn	2 (0, 2)	-0.06	0.86	-0.31	0.14
Hesperophylax	Hesp	7 (3, 4)	-0.06	0.09	0.06	0.22
Onocosmoecus	Ono	4 (3, 1)	0.17	-0.39	0.03	-0.64
Psychoglypha	Psy	3 (1, 2)	0.19	0.70	-0.30	0.01
Pycnopsyche	Pyc	1 (1, 0)	-0.43	-0.39	-0.11	-0.13
Hydroporonae n.det	Hyd	5 (3, 2)	0.26	**-0.66**	0.19	0.52
Agabus	Aga	2 (0, 2)	0.51	-0.26	-0.06	0.67
Scirtidae n.det	Sci	1 (1, 0)	-0.23	-0.11	0.77	0.06
Limnophila	Lim	1 (1, 0)	0.41	-0.17	0.19	-0.36
Dicronata	Dic	4 (1, 3)	-0.51	0.41	-0.18	0.37
Pedicia	Ped	5 (1, 4)	0.16	0.39	-0.23	0.48

Table A.2.4.4. Continued

Taxon	Code	Number of Sites Present (For, Ag)	Deciduous Trees and Heterogeneity 39.13 %	Plant Cover 22.65 %	Gravel Cover 14.40%	Rock Cover 10.15%
Probezzia/Mallchoerea	Cera	2 (1,1)	0.76	-0.02	0.16	-0.51
Eucorethra	Euo	2 (2, 0)	0.21	-0.22	**0.65**	-0.27
Dixella	Dix	2 (2, 0)	-0.47	-0.34	0.61	-0.02
Pericoma	Per	1 (0, 1)	0.58	0.08	0.07	-0.35
Parochlus	Par	5 (3, 2)	-0.63	-0.54	-0.51	-0.08
Boreochlus	Bor	1 (1, 0)	-0.33	-0.31	-0.54	-0.29
Tanypodinae n.det	Tanyp	2 (1, 1)	-0.59	0.29	-0.31	-0.18
Tanytarsini n.det	Tanyt	8 (4, 4)	0.10	-0.17	-0.37	**0.80**
Diamesa	Dia	3 (1, 2)	0.45	0.19	-0.38	0.62
Protanypus	Pro	1 (1, 0)	0.41	-0.17	0.19	-0.36
Pseudodiamesa	Pse	4 (3, 1)	0.10	0.17	-0.17	-0.20
Diamesinae n.det 1	Pot	1 (0, 1)	0.19	0.53	-0.42	0.12
Brillia	Bri	5 (3, 2)	-0.30	0.61	-0.54	-0.20
Corynoneura	Cor	7 (4, 3)	**-0.88**	-0.14	-0.25	-0.19
Diplocladius	Dip	3 (1, 2)	0.37	0.53	-0.38	0.00
Eukiefferiella	Euk	6 (3, 3)	-0.52	-0.15	**0.67**	0.19
Heterotanytarsus	Heterota	1 (1, 0)	0.41	-0.17	0.19	-0.36
Heterotrissocladius	Heterotr	6 (2, 4)	**0.82**	0.47	0.11	-0.17
Hydrobaenus	Hydrob	7 (4, 3)	-0.31	-0.25	**-0.68**	-0.10
Metriocnemus	Met	7 (3, 4)	0.02	0.87	-0.10	0.21
Orthocladius/ Cricotopus	Ort	7 (4, 3)	0.39	-0.22	-0.21	-0.36
Paraphaenocladius/ Parametriocnemus	Parap/Param	5 (2, 3)	**0.66**	-0.24	0.00	0.15
Orthocladiinae n.det 1	Parak	1 (1, 0)	-0.33	-0.31	-0.54	-0.29
Platysmittia/ Psilometriocnemus	Pl/Ps	6 (3, 3)	**0.74**	-0.05	-0.58	-0.02
Rheocricotopus	Rhe	6 (3, 3)	-0.48	-0.03	-0.31	-0.51

Table A.2.4.4. Continued

Taxon	Code	Number of Sites Present (For, Ag)	Deciduous Trees and Heterogeneity 39.13 %	Plant Cover 22.65 %	Gravel Cover 14.40%	Rock Cover 10.15%
Symposiocladius	O(Sym)	2 (1, 1)	0.75	-0.02	0.16	-0.51
Thienemanniella	Thi	8 (4, 4)	-0.42	**-0.78**	-0.20	-0.24
Unniella	Unn	1 (1, 0)	-0.33	-0.31	-0.54	-0.29
Muscomorpha	Mus	1 (0, 1)	-0.43	0.69	0.14	0.06
Eristalis	Eri	1 (1, 0)	0.41	-0.17	0.19	-0.36
Laversia	Lav	3 (1, 2)	0.86	-0.18	0.16	-0.22
Panisopsis	Pan	7 (4, 3)	-0.06	**-0.80**	-0.17	0.52
Hydrovolzia	Hydrov	8 (4, 4)	0.68	-0.36	0.05	-0.10
Lebertia	Leb	7 (4, 3)	**0.83**	-0.36	-0.23	-0.04
Sperchon	Spe	8 (4, 4)	-0.55	-0.71	-0.14	-0.15
Hygrobates	Hygro	2 (2, 0)	0.02	-0.37	-0.31	-0.49
Feltria	Fel	2 (1, 1)	0.40	-0.37	-0.02	0.71
Oribatida	Ori	8 (4, 4)	0.54	-0.08	0.03	-0.59
Cyclopoida	Cyc	4 (1, 3)	0.48	-0.35	-0.04	**0.69**
Harpacticoida	Har	5 (3, 2)	0.33	-0.38	-0.30	0.65
Ostracoda	Ost	4 (1, 3)	**0.96**	0.03	0.00	-0.20
Chydorus	Chy	1 (1, 0)	0.41	-0.17	0.19	-0.36
Pisidium	Pis	7 (3, 4)	0.56	0.41	-0.30	0.07
Megadriles	Mega	6 (3, 3)	-0.10	0.34	-0.37	0.50
Microdriles	Micr	6 (3, 3)	**0.88**	-0.20	0.02	0.22

Table A.2.4.5. Model Building using DISTLM for invertebrate taxa community for db-RDA (Chapter 2, Fig.2.8) with Total Nitrogen forced into the model. The model was forward selected with no other starting terms, Total Sum of Squares (trace)=11520 with df=6. Marginal test variables were selected to test; Sequential test variables were used in model. Final adjusted-R^2=0.48, R^2=0.93 with bolded values statistically significant ($p \leq 0.05$). Sqrt=Square root transformation, Log= Natural Logarithm transformation.

MARGINAL TESTS

Variable	SS(trace)	Pseudo-F	P-Value	Proportion
Log(Total Nitrogen)	1775.30	1.09	0.36	0.15
Log(Phosphate)	1098.80	0.63	0.84	0.09
Log(Discharge)	2467.40	1.62	0.10	0.21
Sqrt(Conifer Density)	1929.60	1.20	0.28	0.17
Sqrt(Deciduous tree density)	2768.30	1.88	**0.04**	0.24
Log(Soil Organic Depth)	1941.30	1.21	0.26	0.17
Sqrt(Rock Cover)	2108.50	1.34	0.20	0.18
Sqrt(Gravel Cover)	2660.10	1.79	**0.05**	0.23
Sqrt(Bryophyte Cover)	2298.80	1.49	0.15	0.20
Sqrt(Vascular Plant Cover)	2621.10	1.76	0.08	0.23

SEQUENTIAL TESTS

Variable	Adj R^2	SS(trace)	Pseudo-F	P-Value	Proportion	Cumulative Variation	res.df
Sqrt(Rock Cover)	0.15	2758.00	1.96	**0.05**	0.24	0.39	5
Sqrt(Bryophyte Cover)	0.25	2059.80	1.65	0.12	0.18	0.57	4
Sqrt(Deciduous Cover)	0.30	1536.20	1.34	0.31	0.13	0.70	3
Sqrt(Gravel Cover)	0.37	1366.50	1.31	0.34	0.12	0.82	2
Sqrt(Vascular Plant Cover)	0.48	1221.30	1.41	0.40	0.11	0.93	1

Table A. 2.4.6. Correlation Matrix of environmental variables in all multivariate invertebrate analyses in chapter 2 (Fig. 2.8-10). Data were log or square root transformed as described in chapter 2. Numbers in bold text indicate important correlations (≥0.7).

	%Ag	%For	Temp	NO_3	TN	PO_4	Cl	SO_4	pH	Cond	DO	Q	V	D	S	Area	% Open	Con	Des	Soil	Cob	Peb	Fine	Bry	Macro
%Ag																									
%For	**-0.9**																								
Temp	0.0	0.2																							
NO_3	**0.9**	**-0.8**	0.1																						
TN	**0.9**	**-0.8**	0.2	1.0																					
PO_4	-0.3	0.1	-0.1	-0.3	-0.3																				
Cl	-0.6	**0.7**	0.4	-0.5	-0.5	0.1																			
SO_4	0.4	**-0.8**	-0.5	0.5	0.5	0.2	-0.6																		
pH	-0.5	0.2	-0.4	-0.4	-0.4	0.1	<0.1	0.4																	
Cond	**0.7**	**-0.9**	-0.2	**0.8**	**0.7**	-0.2	-0.5	**0.7**	-0.1																
DO	-0.3	0.0	-0.5	-0.1	-0.2	0.6	0.0	0.6	**0.7**	<0.1															
Q	-0.2	-0.2	-0.3	0.1	<0.1	0.3	0.1	**0.7**	**0.8**	0.4	**0.8**														
V	-0.2	-0.1	<0.1	0.0	-0.1	-0.1	-0.1	0.4	**0.8**	0.3	0.4	**0.7**													
D	0.4	-0.5	-0.3	0.5	0.5	<0.1	0.1	0.5	0.0	0.6	0.3	0.5	0.1												
S	0.0	-0.2	0.1	-0.1	-0.1	-0.2	<0.1	0.1	0.1	0.4	-0.3	0.2	0.3	0.1											
Area	0.1	-0.3	-0.1	0.3	0.2	-0.3	-0.4	0.5	**0.7**	0.4	0.4	0.6	**0.9**	0.1	<0.1										
%Open	0.6	-0.6	-0.2	0.4	0.3	-0.1	-0.9	0.3	-0.2	0.3	-0.2	-0.3	0.1	-0.2	-0.1	0.3									
Con	-0.1	-0.4	-0.6	-0.1	-0.2	0.1	-0.5	0.6	0.6	0.4	0.3	0.5	0.5	0.0	0.6	0.4	0.4								
Des	-0.4	0.1	0.1	-0.3	-0.3	<0.1	-0.2	0.3	**0.7**	-0.1	0.4	0.5	**0.7**	-0.4	0.3	0.6	0.0	0.6							
Soil	**-0.8**	**0.7**	0.0	-0.6	-0.6	0.2	0.5	-0.2	0.6	**-0.7**	0.5	0.3	0.2	-0.3	-0.3	<0.1	-0.6	-0.2	0.5						
Cob	0.2	-0.5	0.0	0.4	0.3	0.3	<0.1	0.5	<0.1	0.6	0.2	0.6	<0.1	0.5	0.6	-0.1	-0.3	0.3	0.1	-0.2					
Peb	-0.2	0.2	0.1	-0.1	-0.1	0.0	0.3	-0.1	<0.1	-0.1	<0.1	0.1	-0.3	-0.1	0.4	-0.4	-0.6	0.0	0.3	0.4	0.5				
Fine	-0.3	0.1	-0.4	-0.4	-0.4	0.1	<0.1	0.0	0.4	-0.1	0.2	0.1	0.4	0.2	-0.2	0.3	0.3	0.3	-0.1	-0.1	-0.5	**-0.8**			
Bry	-0.2	0.6	0.6	-0.3	-0.2	-0.1	0.7	**-0.9**	-0.6	-0.6	-0.6	**-0.7**	**-0.7**	-0.3	-0.1	-0.8	-0.5	**-0.8**	-0.5	0.1	-0.2	0.3	-0.3		
Macro	0.6	**-0.8**	-0.2	0.6	0.6	0.1	-0.9	**0.7**	0.0	0.6	0.2	0.1	0.2	<0.1	-0.1	0.5	**0.9**	0.4	0.1	-0.5	<0.1	-0.5	0.1	**-0.7**	
CPOM	-0.4	0.6	0.0	-0.3	-0.3	0.3	0.8	-0.4	-0.2	-0.5	0.2	<0.1	-0.5	0.3	-0.2	**-0.7**	**-0.8**	-0.6	-0.4	0.4	0.1	0.4	-0.1	0.6	**-0.8**

Key:

% Agr: % of land within 1km radius in Agriculture
% For: % of land within 1 km radius in Forest
Temp: Spot Water Temperature
NO_3: Nitrate-Nitrogen Concentration
TN: Total Nitrogen Concentration
PO_4: Phosphate Concentration
Cl: Chloride Concentration
SO_4: Sulphate Concentration
pH: Hydrogen Ion Concentration
Cond: Electrical Conductivity
DO: Dissolved Oxygen Concentration
Q: Water Discharge at Spring Outflow
V: Water velocity at Spring Outflow
D: Water Depth at Spring Outflow
S: Slope of bed at Spring Outflow
Area: Area of the spring pool
%Open: % of open area of open canopy
Con: Conifer density
Dec: Deciduous tree density
Soil: Depth of organic soil layer
Cob: Rocks in Cobble size class
Peb: Pebble sized gravel
Fine: Fines, including sand/silt
Bry: Bryophyte cover
Macro: Vascular Macrophytes
CPOM: Coarse Particulate Organic Matter

Table A.2.4.7. Axis loadings for environmental variables (multiple partial correlations from the distance-based redundancy analysis) for each db-RDA axis for explaining functional feeding group assemblages from Chapter 2, Fig. 2.19 These represent the relationships between measured variables and the dbRDA coordinate axes, with bolded numbers important contributes to the axis. Sqrt=Square root transformation, Log= Natural Logarithm transformation.

Variable	dbRDA1	dbRDA2	dbRDA3	dbRDA4	dbRDA5
Sqrt(Bryophyte Cover)	**-0.641**	0.504	-0.408	0.298	-0.147
Log(Phosphate)	-0.229	0.443	0.397	0.036	0.411
Sqrt(Gravel Cover)	-0.455	-0.202	-0.233	**-0.823**	0.047
Sqrt(Deciduous Cover)	0.527	**0.511**	**-0.466**	-0.249	-0.286
Sqrt(Rock Cover)	0.116	0.497	**0.466**	-0.413	0.05
Log(Discharge)	0.196	0.026	**-0.433**	0.02	**0.850**

Table A.2.4.8. Pearson correlation coefficients (ρ) between functional feeding groups and environmental variables in the db-RDA axes found in Chapter 2 (Fig. 2.9). The variables listed in the column headers contributed the most to building the axis and the percentage represents the amount of fitted variation explained by that axis (4 of 5 axis shown; 98.6 % of fitted variation, 99.5% of total variation). Bolded correlations indicate important correlation with db-RDA axis and life-habit group.

	Bryophyte Cover 63.45%	Deciduous Tree Density 18.45%	Heterogeneity 10.57%	Gravel 6.11 %
Shredders	-0.02	**0.72**	0.62	-0.30
Scrapers	-0.46	-0.50	-0.26	**-0.68**
Predators	**-0.87**	0.45	0.02	0.09
Collector-Gatherers	**-0.94**	0.10	0.07	0.16
Collector-Filterers	0.04	**-0.73**	0.57	0.18

Table A.2.4.9. Model Building using DISTLM for functional feeding group assemblage for db-RDA (Chapter 2, Fig.2.9) with Total Nitrogen forced into the model. Model was forward selected with Total Nitrogen forced into the model with no other starting terms, Total Sum of Squares (trace)= 3023.7 with df=6. Marginal test variables were selected to test; Sequential test variables were used in model. Final adjusted-R^2=1.0, R^2=1.0 with bolded values statistically significant ($p \leq 0.05$). Sqrt=Square root transformation, Log= Natural Logarithm transformation.

MARGINAL TESTS

Variable	SS(trace)	Pseudo-F	P-Value	Proportion.
Log(Phosphate)	296.24	0.65	0.58	0.10
Log(Discharge)	534.42	1.29	0.29	0.18
Sqrt(Conifer Density)	1010.40	3.01	0.07	0.33
Sqrt(Deciduous Density)	650.62	1.65	0.21	0.22
Log(Soil Organic Depth)	271.07	0.59	0.62	0.09
Sqrt(Rock Cover)	208.99	0.45	0.75	0.07
Sqrt(Gravel Cover)	624.70	1.56	0.22	0.21
Sqrt(Bryophyte Cover)	1281.30	4.41	**0.02**	0.42
Sqrt(Vascular Plant Cover)	734.11	1.92	0.15	0.24
Log(Total Nitrogen)	62.95	0.13	0.96	0.02

SEQUENTIAL TESTS

Variable	Adj R^2	SS(trace)	Pseudo-F	P	Proportion.	Cumulative Variation	res.df
Sqrt(Bryophyte Cover)	0.33	1281.30	4.41	**0.02**	0.42	0.42	6
Sqrt(Phosphate)	0.35	341.99	1.22	0.34	0.11	0.54	5
Sqrt(Gravel Cover)	0.36	293.84	1.06	0.37	0.10	0.63	4
Sqrt(Deciduous Density)	0.52	486.86	2.36	0.20	0.16	0.80	3
Sqrt(Rock Cover)	0.91	538.42	13.25	**0.04**	0.18	0.97	2
Log(Discharge)	1.00	110.81	-3.75	0.87	0.04	1.01	1

Table A.2.4.10. Axis loadings (multiple partial correlations from the distance-based redundancy analysis) for each db-RDA axis for explaining life-habit distribution from chapter 2, Fig. 2.10 These represent the relationships between measured variables and the dbRDA coordinate axes, with bolded numbers indicate important contributions to the axis. Sqrt=Square root transformation, Log= Natural Logarithm transformation.

Variable	dbRDA1	dbRDA2	dbRDA3	dbRDA4
Sqrt(Gravel Cover)	**-0.708**	0.271	0.210	0.104
Sqrt(Deciduous Density)	-0.277	-0.509	-0.334	**0.644**
Log(Total Nitrogen)	0.598	0.23	-0.163	0.235
Sqrt(Rock Cover)	-0.192	-0.194	**-0.511**	**-0.697**
Log(Phosphate)	-0.094	0.398	0.348	-0.015
Sqrt(Bryophyte Cover)	-0.134	**0.647**	**-0.660**	0.181

Table A.2.4.11. Pearson correlation coefficients (ρ) between life-habit groups and environmental variables in the db-RDA axes found in Chapter 2. The variables listed in the column headers contributed the most to building the axis and the percentage represents the amount of fitted variation explained by that axis (3 of 4 axis shown; 93.49% of fitted variation, 96.62% of total variation). Bolded correlations indicate important correlation with db-RDA axis and life-habit group.

Life-Habit Group	Gravel 52.66%	Bryophyte Cover 33.14 %	Heterogeneity 7.69%
Burrower	0.49	-0.06	**0.72**
Clingers	-0.26	0.11	0.61
Climbers	-0.07	**0.71**	-0.50
Sprawler	**-0.78**	0.55	0.20
Swimmers	0.42	0.48	-0.44
Planktonic	**0.87**	0.48	0.05

Table A.2.4.12. Model Building using DISTLM for life-habit group assemblage for db-RDA (Chapter 2, Fig.2.10) (in this analysis, Total Nitrogen was not forced into the model as in previous analyses). The model was forward-selected with no starting terms. Total Sum of Squares (trace)= 4993.1 with df=6. Marginal test variables were selected to test; Sequential test variables were used in model. Final adjusted-R^2=1.0, R^2=1.0 with bolded values statistically significant ($p \leq 0.05$). Sqrt=Square root transformation, Log= Natural Logarithm transformation.

MARGINAL TESTS

Variable	SS(trace)	Pseudo-F	P-Value	Proportion
Log(Phosphate)	201.54	0.25	0.87	0.04
Log(Discharge)	934.16	1.38	0.28	0.19
Sqrt(Conifer Density)	988.69	1.48	0.28	0.20
Sqrt(Deciduous Density)	1672.60	3.02	**0.04**	0.33
Log(Soil Organic Depth)	1545.30	2.69	0.07	0.31
Sqrt(Rock Cover)	471.62	0.63	0.59	0.09
Sqrt(Gravel Cover)	1836.30	3.49	**0.03**	0.37
Sqrt(Bryophyte Cover)	1178.30	1.85	0.17	0.24
Sqrt(Vascular Plant Cover)	1557.10	2.72	0.10	0.31
Log(Total Nitrogen)	1183.60	1.86	0.18	0.24

SEQUENTIAL TESTS

Variable	Adj R^2	SS(trace)	Pseudo-F	P-Value	Proportion	Cumulative Variation	res.df
Sqrt(Gravel Cover)	0.26	1836.30	3.49	**0.03**	0.37	0.37	6
Sqrt(Deciduous Density)	0.52	1433.50	4.16	**0.02**	0.29	0.65	5
Log(Total Nitrogen)	0.64	698.60	2.73	0.10	0.14	0.79	4
Sqrt(Rock Cover)	0.74	476.29	2.61	0.17	0.10	0.89	3
Log(Phosphate)	0.86	355.03	3.67	0.14	0.07	0.96	2
Sqrt(Bryophyte Cover)	1.00	360.26	-2.16	0.79	0.07	1.03	1

Table A.2.4.13. Axis loadings (multiple partial correlations from the distance-based redundancy analysis) for each dbRDA axis for explaining stonefly and caddisfly species distribution from chapter 3, These represent the relationships between measured variables and the dbRDA coordinate axes. Sqrt=Square root transformation, Log= Natural Logarithm transformation.

Variable	dbRDA1	dbRDA2	dbRDA3	dbRDA4
Sqrt(Vascular Plant Cover)	**0.570**	0.443	-0.134	0.384
Log(Total Nitrogen)	0.323	-0.256	**0.862**	-0.124
Sqrt(Conifer Density)	0.340	**-0.619**	-0.461	-0.138
Sqrt(Bryophyte Cover)	-0.425	-0.253	-0.061	-0.024
Log(Phosphate)	-0.446	0.222	0.108	0.379
Sqrt(Rock Cover)	0.246	0.319	-0.107	-0.278
Sqrt(Gravel Cover)	0.124	-0.374	0.029	**0.773**

Table A.2.4.14. Pearson correlation coefficients (ρ) between individual stonefly and caddisfly species and the four db-RDA axes found in Chapter 3. The variables listed in the column headers contributed the most to building the axis and the percentage represents the amount of fitted variation explained by that axis (99.7% of fitted variation, 96.7% of total variation). *****Indicate species with <40 individual collected from emergence traps and not used in discussion, correlations with db-RDA axis are for potential trends.

Species	Vascular Plants 51.6 %	Conifer Density 24.3 %	Total Nitrogen 17.2 %	Gravel Cover 6.5 %
Nemoura trispinosa	**0.84**	0.25	0.26	-0.28
Amphinemura wui	0.10	**0.82**	0.08	0.37
*Soyedina washingtoni**	**0.84**	0.23	-0.14	0.43
Amphinemura nigritta	**0.74**	0.43	-0.11	-0.39
Sweltsa naica	**0.78**	0.02	-0.48	0.04
Leuctra ferruginea	**0.91**	-0.14	0.26	0.16
*Rhyacophila brunnea**	**0.75**	0.51	-0.34	0.17
*Neophylax aniqua**	0.10	**0.72**	-0.46	0.14
Lepidostoma spp.	-0.12	0.13	**-0.86**	0.01
Hesperophylax designatus	**0.81**	-0.03	-0.36	-0.20
*Psychoglypha subborealis**	0.23	0.81	0.37	0.09
*Onocosmoecus unicolor**	0.61	-0.29	-0.07	0.27
*Pycnopsyche gentilis**	-0.40	0.23	**-0.65**	0.16

Table A.2.4.15 Model Building using DISTLM for stonefly and caddisfly assemblage for db-RDA (Chapter 3, Fig.3.5). Model was forward selected with no starting terms, Total Sum of Squares (trace)= 4075.7 with df=7. Marginal test variables were selected to test; Sequential test variables were used in model. Final adjusted-R^2=0.78, R^2=0.97. Sqtr=Square root transformation, Log= Natural Logarithm transformation.

Marginal Tests

Variable	SS(trace)	Pseudo-F	p-value	Proportion
Log(Total Nitrogen)	1069.00	2.49	0.06	0.26
Log(Phosphate)	459.77	0.89	0.50	0.11
Log(Discharge)	290.62	0.54	0.74	0.07
Log(Depth)	420.89	0.81	0.55	0.10
Sqrt(Conifer Density)	891.85	1.96	0.13	0.22
Sqrt(Descidous Density)	187.28	0.34	0.89	0.05
Log(Soil Organic Depth)	801.34	1.71	0.17	0.20
Sqrt(Rock Cover)	338.26	0.63	0.65	0.08
Sqrt(Gravel Cover)	258.57	0.47	0.79	0.06
Sqrt(Bryophyte Cover)	1145.10	2.74	**0.04**	0.28
Sqrt(Vascular Plant Cover)	1207.30	2.95	**0.04**	0.30

Sequential Tests

Variable	Adj R^2	SS(trace)	Pseudo-F	p-value	Proportion	Cumulative Variation	res.df
Sqrt(Vascular Plant Cover)	0.20	1207.30	2.95	**0.05**	0.30	0.30	7
Log(Total Nitrogen)	0.30	726.13	2.03	0.12	0.18	0.47	6
Sqrt(Conifer Density)	0.40	618.23	2.03	0.13	0.15	0.63	5
Sqrt(Bryophyte Cover)	0.44	376.66	1.31	0.30	0.09	0.72	4
Log(Phosphate)	0.48	350.18	1.32	0.33	0.09	0.80	3
Sqrt(Rock Cover)	0.58	371.47	1.75	0.26	0.09	0.90	2
Sqrt(Gravel Cover)	0.78	316.03	2.88	0.29	0.08	0.97	1

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Table A.2.4.16. Correlation Matrix of variables measured in sites for chapter 3 (n=9). Data were log or square root transformed as described in chapter 3. Numbers in bold text indicate important correlations. See Table A. 2.4.6 for a key to the variables.

	Temp	NO_3	TN	PO_4	Cl	SO_4	pH	Cond	DO	Q	V	D	S	A	%Open	Con	Des	Soil	Cob	Peb	Fines	Bry	Macro	CPOM
Temp																								
NO_3	0.1																							
TN	0.2	**1.0**																						
PO_4	-0.1	-0.2	-0.2																					
Cl	0.3	-0.5	-0.5	0.1																				
SO_4	-0.5	0.5	0.5	0.3	-0.6																			
pH	-0.4	-0.4	-0.4	0.1	0.0	0.4																		
Cond	-0.2	**0.8**	**0.8**	-0.2	-0.5	0.7	-0.1																	
DO	-0.5	-0.1	-0.1	0.6	0.0	0.6	0.7	0.0																
Q	-0.3	0.0	-0.1	0.2	0.1	0.6	0.7	0.3	**0.8**															
V	0.0	0.0	0.0	-0.1	-0.1	0.4	0.8	0.3	0.4	**0.7**														
D	-0.3	0.4	0.3	-0.1	0.2	0.4	0.0	0.5	0.2	0.5	0.1													
S	0.0	0.2	0.3	0.1	-0.2	0.3	0.0	0.3	-0.1	-0.2	0.1	-0.3												
A	-0.1	0.2	0.1	-0.3	-0.4	0.4	0.6	0.3	0.4	0.6	**0.8**	0.2	-0.2											
%Open	-0.2	0.3	0.3	0.0	**-0.9**	0.3	-0.1	0.3	-0.1	-0.2	0.1	-0.2	-0.1	0.4										
Con	-0.5	0.0	0.0	0.1	-0.5	0.6	0.5	0.5	0.3	0.4	0.5	-0.1	0.5	0.3	0.3									
Des	0.1	-0.3	-0.3	0.0	-0.1	0.2	**0.7**	-0.1	0.4	0.5	0.7	-0.4	0.0	0.6	0.0	0.5								
Soil	0.0	-0.6	-0.6	0.2	0.5	-0.2	0.6	**-0.7**	0.5	0.3	0.2	-0.3	-0.2	0.1	-0.6	-0.2	0.5							
Cob	0.0	0.3	0.2	0.3	0.1	0.4	0.0	0.5	0.2	0.6	0.0	0.5	-0.1	0.0	-0.3	0.2	0.1	-0.2						
Peb	0.1	-0.2	-0.2	0.0	0.4	-0.1	0.0	-0.2	0.0	0.2	-0.3	-0.1	-0.2	-0.3	-0.6	-0.1	0.3	0.4	0.5					
Fines	-0.4	-0.3	-0.3	0.1	0.0	0.1	0.4	-0.1	0.2	0.0	0.4	0.1	0.2	0.3	0.3	0.3	-0.1	-0.1	-0.5	**-0.8**				
Bry	0.5	-0.3	-0.3	-0.1	**0.7**	**-0.9**	-0.6	-0.6	-0.6	-0.6	-0.7	-0.2	-0.2	**-0.7**	-0.5	**-0.8**	-0.4	0.1	-0.2	0.4	-0.4			
Macro	-0.2	0.6	0.5	0.1	**-0.9**	**0.7**	0.0	0.6	0.2	0.1	0.2	0.0	0.0	0.5	**0.9**	0.4	0.1	-0.5	0.0	-0.5	0.1	**-0.7**		
CPOM	0.0	-0.3	-0.3	0.3	0.8	-0.4	-0.2	-0.4	0.2	0.0	-0.5	0.2	0.0	**-0.7**	**-0.8**	-0.5	-0.4	0.4	0.1	0.3	-0.1	0.6	**-0.8**	

Table A.2.4.17. Probability values for individual multiple linear regressions using Distance Based Linear Modeling (DISTLM). Statistically significant values ($p \leq 0.05$) are bolded. Direction of responding variables relationship to forward selected predictor variables is reported for $p < 0.10$. Positive (+) p-values indicate that variable values increased as the predictor variable increased; negative (-) p-values indicate that variable values decreased as the predictor variable increased. Dashes indicate that the variable was not selected in model building.

Variable	Number of Species (Adj R^2=0.94, R^2=0.98)	Total Abundance (Adj R^2 =1.00, R^2=1.00)	*Nemoura trispinosa* (Adj R^2 = 0.99, R^2=1.0)	*Amphinemura wui* (Adj R^2 = 1.00, R^2=1.0)	*Sweltsa naica* (Adj R^2 = 0.97, R^2=1.0)	*Lepidostoma* spp. (Adj R^2 = 0.95, R^2=0.99)	*Hesperophylax designatus* (Adj R^2 = 0.86, R^2=0.98)
Vascular Plant Cover	**+0.036**	**+0.001**	-	0.104	0.141	0.194	+0.058
Bryophyte Cover	-	0.158	-0.057	-	0.328	-	-
Rock Cover	0.106	**+0.004**	0.256	-	-	0.151	+0.053
Gravel Cover	-	**-0.028**	0.192	-0.056	-0.094	0.142	-
Deciduous Density	-	0.244	-	+0.073		0.298	0.382
Conifer Density	-		-	**+0.012**	**+0.005**		**+0.016**
Phosphate	0.105	-	0.166	+0.057	-	0.272	
Total Nitrogen	**+0.026**	**+0.037**	**+0.010**	0.322	0.115	-0.063	0.430
Soil Organic Depth	0.210	-	**-0.014**	-	**-0.018**	-	-
Discharge at Outflow	-	0.140	0.176	+0.080	0.324	-	0.288
Dissolved Oxygen	-	-	-	-	-	0.132	0.122

Table A.2.4.18. Eigenvector coefficients that explain the weight of a variable contributing to the principle components axis for PCA in Chapter 3. The principle components analysis explained 100% of the variation in eight axis. Bolded numbers indicate important variables in explaining the axis.

Variable	PC1- 31.8%	PC2- 21.5&	PC3- 14.1%	PC4- 9.6 %	PC5- 7.7%	PC6- 6.4 %	PC7- 4.9%	PC8- 3.9%
Log(TEMP)	0.153	-0.109	-0.001	-0.374	0.094	-0.168	0.424	-0.424
Log(NO3)	-0.171	-0.294	-0.231	-0.085	0.067	-0.072	0.272	0.178
Log(TN)	-0.157	**-0.301**	-0.217	-0.062	0.035	-0.131	0.308	0.196
Log(PO4)	-0.007	0.129	-0.067	0.339	-0.356	0.199	0.348	-0.46
Log(Cl)	0.28	0.178	-0.129	0.061	0.217	-0.184	0.028	-0.189
Log(SO4)	**-0.315**	0.032	-0.207	0.109	-0.153	0.021	0.08	0.112
pH	-0.165	**0.376**	0.06	-0.033	0.034	-0.104	-0.018	0.164
Log(COND)	-0.256	-0.188	-0.255	-0.021	0.044	-0.193	-0.158	-0.073
Log(DO)	-0.148	**0.310**	-0.113	0.222	-0.046	0.163	0.31	0.156
Log(Q)	-0.173	0.299	-0.274	-0.025	0.14	0.035	0.018	-0.12
Log(V)	-0.226	0.226	0.068	-0.168	0.245	-0.27	0.071	-0.199
Log(Depth)	-0.079	-0.013	-0.36	0.238	0.432	0.036	-0.174	0.008
Log(Slope)	-0.065	-0.067	0.005	0.095	-0.361	-0.652	0.135	0.076
Log(Area)	-0.26	0.133	0.083	-0.244	0.341	0.061	0.09	0.021
Log(%Open)	-0.23	-0.192	0.267	0.019	-0.037	0.311	-0.021	-0.096
Sqr(Con)	-0.257	0.106	0.055	0.044	-0.31	-0.227	-0.364	-0.11
Sqr(Des)	-0.127	0.291	0.073	-0.395	-0.168	-0.005	0.014	-0.109
Log(Soil)	0.123	**0.356**	0.019	-0.095	-0.05	0.048	0.277	0.341
Sqr(COB)	-0.063	0.035	-0.47	-0.06	-0.102	0.105	-0.16	-0.385
Sqr(PEB)	0.153	0.118	-0.298	-0.31	-0.265	0.112	-0.227	0.135
Sqr(FINES)	-0.107	0.085	0.304	0.395	0.234	-0.21	-0.027	-0.182
Sqr(BRY)	**0.350**	-0.092	0.003	-0.063	0.036	0.018	-0.007	-0.09
Sqr(MACRO)	**-0.300**	-0.156	0.082	-0.004	-0.069	0.27	0.179	-0.064
Sqr(CPOM)	0.263	0.105	-0.222	0.297	0.012	-0.075	0.142	0.112

Appendix 3

Physiochemical Patterns during Winter Sampling

Appendix 3.1 Winter Water Chemistry Data

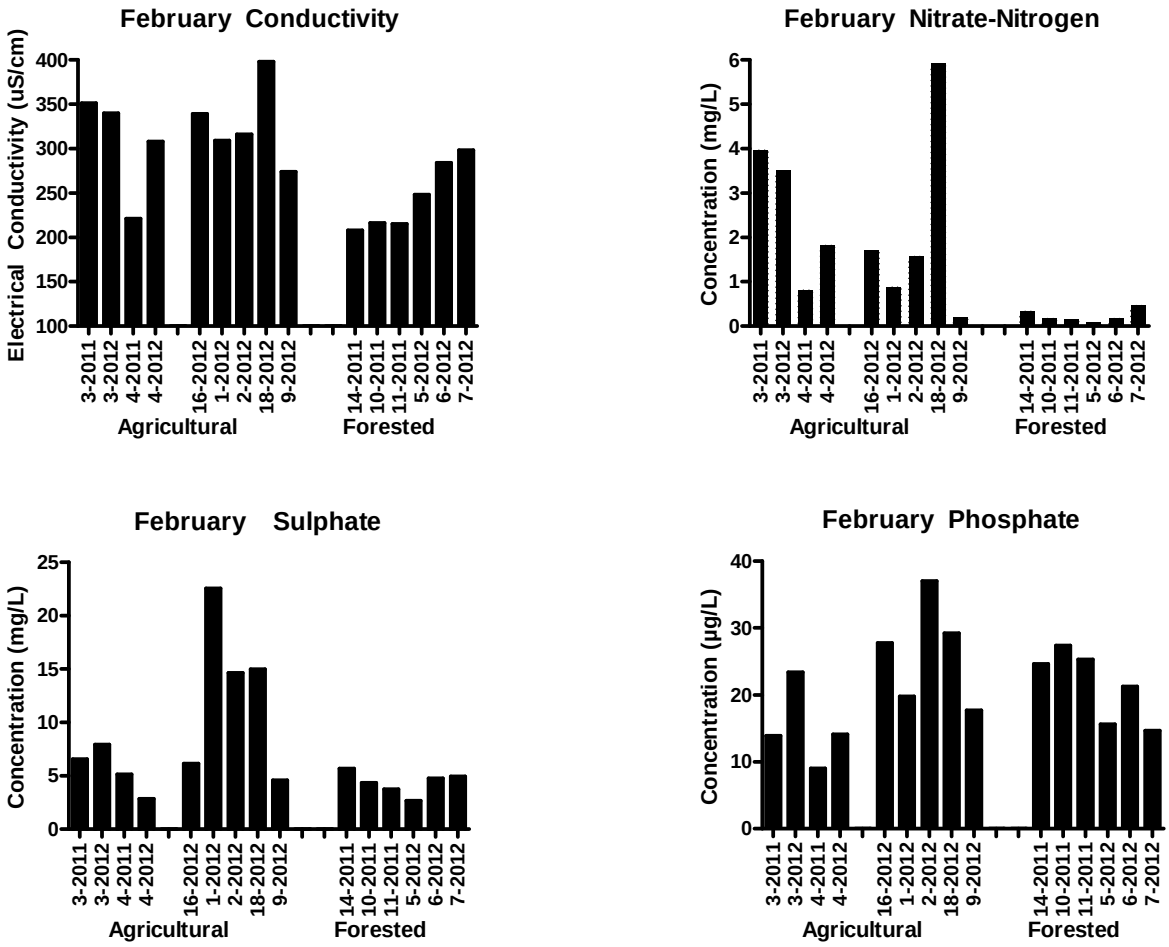

Fig. A.3.1.1a. Physical/chemical data from samples collected during February 2011. Numbers refer to site codes, e.g., SOU01= Site 3. See Table A.1.5.1 for a key to the code locations and specific sampling dates; locations are also shown in Fig 2.1.

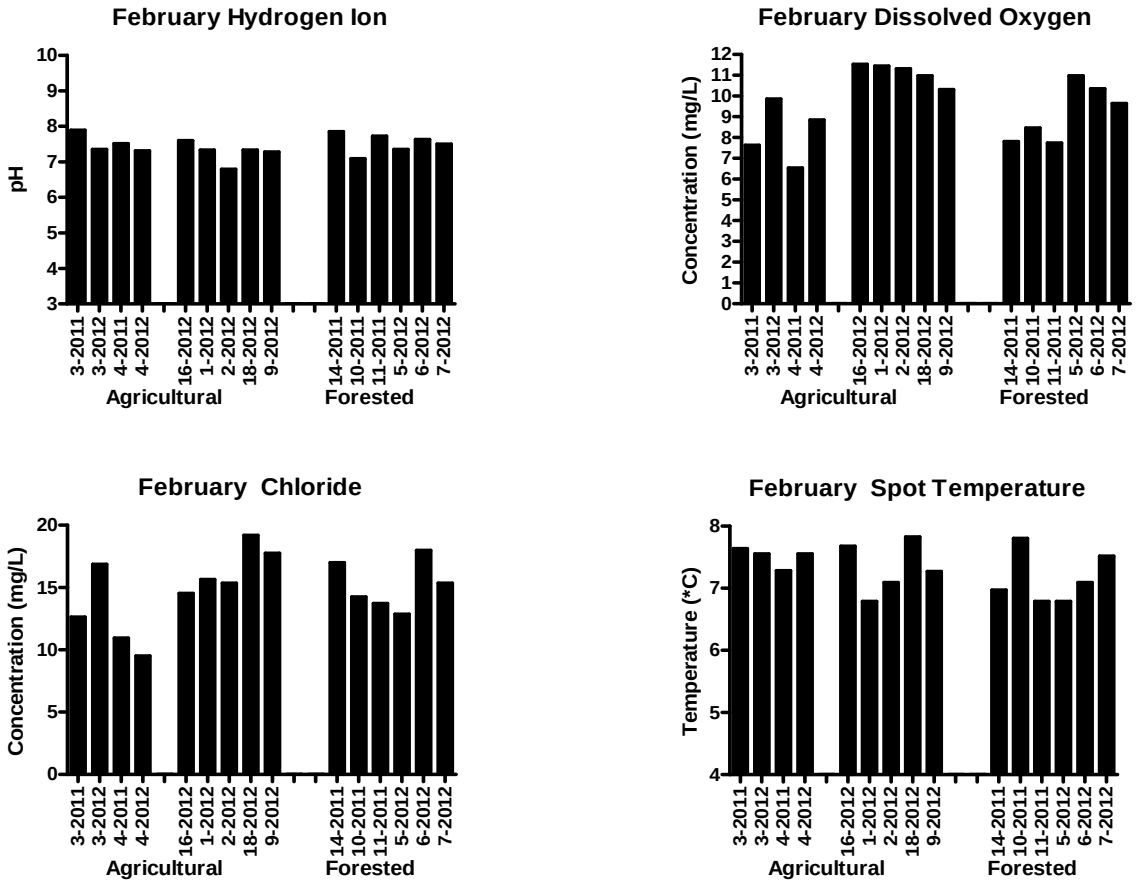

Fig. A.3.1.1b. Physical/chemical data from samples collected during February 2011. Numbers refer to site codes, e.g., SOU01= Site 3. See Table A.1.5.1 for a key to the code locations and specific sampling dates; locations are also shown in Fig 2.1.

Appendix 4

Animal taxa recorded from the rheo-limnocrene study springs in eastern Prince Edward Island (Canada), with information on feeding groups, life-habit and known occurrence in spring habitats.

Appendix 4.1 Non-arthropod Invertebrates

Table A.4.1.1. Non-arthropod invertebrate taxa observed in spring pools in eastern PEI. Life-habit and functional feeding group information was determined from descriptions in Thorp and Covich (2001)[1], dashes indicate unclear habits or level of taxonomic distinction is not adequate to place into a group.

Taxon			Life-Habit Category	Functional Feeding Group Category
Mollusca	Bivalvia	Pisidiidae *Pisidium*	Burrower	Collector Filterer
Annelida	Oligochaeta:	Naididae, Tubificidae and Lumbricidae	Burrower	Collector Gatherer
Nematoda				
Platyhelminthes		Tricladida	-	-
Cnidaria		Anthomedusae, Hydridae, *Hydra*	-	Predator

[1]Thorp, J.H., and A.P. Covich. 2001. Ecology and Classification of North American Freshwater Invertebrates, 2nd Edition. Academic Press, San Diego, California

Appendix 4.2. Arthropod taxa encountered in the study springs

Table A.4.2.1. Taxa list and biological information for arthropod taxa from the study springs in eastern Prince Edward Island.
* before the species name indicates new provincial record; L: found as larva in the spring pools; A: found as adult, either in the emergence trap or in riparian sampling. Life habit and functional feeding groups were determined in the references given in chapter 2 and 3 in tables 2.1 and 3.1.

Taxon	Species (where known)	Life stage recorded	Life-Habit	Functional Feeding Group
Hexapoda: Collembola				
Isotomidae		A	Skater	Collector-Gatherer
Sminthuridae		A	Skater	Collector-Gatherer
Hypogastridae		A	Skater	Collector-Gatherer
Entombrionidae		A	Skater	Collector-Gatherer
Hexapoda: Insecta				
Ephemeroptera				
Ameletidae	**Ameletus lineatus* Traver, 1932	L, A	Swimmer	Scraper
Baetidae	*Baetis tricaudatus* Dodds, 1923	L, A	Swimmer	Collector-Gatherer
	**Callibaetis fluctuans* (Walsh, 1862)	A	Swimmer	Collector-Gatherer
Leptophlebiidae	*Paraleptophlebia debilis* (Walker, 1853)	A	Swimmer	Collector-Gatherer
Ephemerellidae	*Ephemerella invaria* (Walker, 1853)	L, A	Clinger	Collector-Gatherer
Plecoptera				
Chloroperlidae	*Sweltsa naica* (Provancher, 1876)	L, A	Clinger	Predator
Perlodidae	*Isoperla transmarina* (Newman, 1838)	A	Clinger	Predator
Leuctridae	*Leuctra ferruginea* (Walker, 1851)	L, A	Sprawler	Shredder
Nemouridae	*Amphinemura wui* (Claassen, 1936)	L, A	Sprawler	Shredder
	Amphinemura nigritta (Provancher, 1876)	L, A		
	Nemoura trispinosa Claassen, 1923	L, A	Sprawler	Shredder
	** Soyedina washingtoni* (Claassen, 1923)	L, A	Sprawler	Shredder
Hemiptera				
Gerridae	*Aquarius remigis* (Say, 1832)	A	Skater	Predator

Table A.4.2.1. Continued

Taxon	Species (where known)	Life stage recorded	Life-Habit	Functional Feeding Group
Trichoptera				
Rhyacophilidae	*Rhyacophila brunnea* Banks, 1911	L, A	Clinger	Shredder
Hydroptilidae	**Palaegapetus celsus* (Ross, 1938)	L	Sprawler	Shredder
Philopotamidae	**Dolophilodes distinctus* (Walker, 1852)	A	Clinger	Collector Filterer
Lepidostomatidae	*Lepidostoma vernale* (Banks, 1897)	L, A	Climber	Shredder
	**Lepidostoma sommermanae* Ross, 1946	L, A		
Limnephilidae	*Onocosmoecus unicolor* (Banks, 1897)	L, A	Sprawler	Shredder
	* *Frenesia missa* (Milne, 1935)	A	Sprawler	Shredder
	Hesperophylax designatus (Walker, 1852)	L, A	Sprawler	Shredder
	* *Limnephilus sericeus* (Say, 1824)	A	Clinger	Shredder
	* *Limnephilus moestus* Banks, 1908	A		
	* *Limnephilus indivisus* Walker, 1852	A		
	Psychoglypha subborealis (Banks, 1924)	L, A	Clinger	Shredder
	Pycnopsyche gentilis (McLachlan, 1871)	L, A	Clinger	Shredder
Uenoidae	*Neophylax aniqua* Ross, 1947	L, A	Clinger	Scraper
Leptoceridae	**Triaenodes tardus* Milne, 1934	A	Climber	Shredder
Coleoptera				
Dytiscidae				
Colymbetinae	*Agabus* spp.	L, A	Swimmer	Predator
Hydroporinae	*Sanfilippodyte?*	L, A	Swimmer	Predator
Scirtidae		A	Climber	Scraper
Diptera				
Ceratopogonidae	*Probezzia/Mallchoerea*	L, ?A	Burrower	Predator
Chaoboridae	**Eucorethra underwoodi* Underwood, 1903	L, A	Swimmer	Predator
Chironomidae				
Chironominae				
Tanytarsini	*Micropsectra* spp.	L, A	Climber	Collector-Gatherer
Podonominae	**Parochlus kiefferi* (Garrett, 1925)	L, A	Sprawler	Collector-Gatherer
	Boreochlus	L	Sprawler	Collector-Gatherer
Orthocladiinae	**Brillia parva* Joahnnsen, 1934	L, A	Sprawler	Shredder
	* *Corynoneura* n.r. *lobata* Edwards, 1924?	L, A	Sprawler	Collector-Gatherer
	* *Diplocladius cultriger* Kieffer, 1908	L	Sprawler	Collector-Gatherer

Table A.4.2.1. Continued

Taxon	Species (where known)	Life stage recorded	Life-Habit	Functional Feeding Group
	Eukiefferiella	L	Sprawler	Collector-Gatherer
	Heterotanytarsus sp.	L	Sprawler	Collector-Gatherer
	**Heterotrissocladius marcidus* (Walker, 1856)	L, A	Sprawler	Collector-Gatherer
	**Hydrobaenus conformis* (Holmgren, 1869)?	L, A	Sprawler	Scraper
	**Limnophyes hastulatus* Saether, 1975	A	Sprawler	Collector-Gatherer
	Metriocnemus sp.	L, A	Burrower	Collector-Gatherer
	Orthocladius/ Cricotopus/ Paratrichocladius	L, A	Sprawler	Collector-Gatherer
	Paratrichocladius nr. *skirwithensis* (Edwards, 1929)	?L, A		
	* *Orthocladius (Symposiocladius) lignicola* Kieffer, 1915	L	Burrower	Scraper
	Parametriocnemus/ Paraphaenocladius	L, ?A	Sprawler	Collector-Gatherer
	**Paraphaenocladius pseudirritus* Strenzke, 1950?	?L, A		
	*Parakiefferiella?*or *Stilocladius?*	L	Sprawler	Collector-Gatherer
	Psilometriocnemus/ Platysmittia	L	Sprawler	Collector-Gatherer
	Rheocricotopus spp.	L, A	Sprawler	Collector-Gatherer
	nr. Unniella	L	Sprawler	Collector-Gatherer
Diamesinae	*Diamesa*	L	Sprawler	Collector-Gatherer
	Protanypus	L	Burrower	Collector-Gatherer
	Potthastia?	L	Sprawler	Collector-Gatherer
	Pseudodiamesa	L	Sprawler	Collector-Gatherer
Prodiamesinae	**Prodiamesa olivacea* (Meigen, 1818)	A	Burrower	Collector-Gatherer
Tanypodinae	*Nilotanypus?*	L	Sprawler	Predator
	Zavrelimyia thryptica group	?L,A	Sprawler	Predator
Dixidae	*Dixella*	L	Swimmer	Collector-Gatherer
Psychodidae	*Pericoma*	L, A	Burrower	Collector-Gatherer
Ptychopteridae	**Bittacomorpha clavipes* (Fabricius, 1781)	A	Burrower	Collector-Gatherer
Tipulidae	*Tipula*	A	Burrower	Shredder
Limoniidae	*Limnophila*	L, ?A	Burrower	Predator
Pediciidae	**Pedicia margarita* Alexander, 1929	L, A	Burrower	Predator
	Dicranota	L, A	Burrower	Predator
Dolichopodidae		A	Sprawler	Predator
Empididae		A	Sprawler	Predator
Syrphidae	*Eristalis*	L, ?A	Burrower	Collector-Gatherer
Muscomorpha		L	Sprawler	Predator

Appendix 4.3: Vertebrates

Table A.4.3.1.Vertebrates observed within eastern Prince Edward Island spring pools. All are considered predators but were not quantified in the thesis.

Observed Species	Common name
Salvelinus fontinalis (Mitchill, 1814)	Brook Trout
Lithobates clamitans (Latreille, 1801)	Green Frog
Lithobates sylvaticus (LeConte, 1825)	Wood Frog
Ambystoma maculatum (Shaw, 1802)	Yellow-spotted Salamander
Ambystoma laterale Hallowell, 1856	Blue-spotted Salamander
Phalacrocorax auritus (Lesson, 1831)	Double-crested Cormorant
Anas rubripes (Brewster, 1902)	American Black Duck

Appendix 5 Family Biotic Index Scores

Table A.5.1.1. Family Biotic Index (FBI) scores for sampled springs (n=8) using the Hilsenhoff (1988)[1] index for organic pollution. Good: Some Organic Pollution Probable, Fair: Fairly Substantial Organic Pollution, Fairly Poor: Some Organic Pollution Probable.

Site	Land Use	FBI Score	FBI Assessment of Organic Pollution	Total Nitrogen (mean) (mg/L)	Phosphate (mean) (µg/L)
1	Agricultural	5.92	Fairly Poor	0.76	36.3
2	Agricultural	5.27	Fair	1.54	38.7
3	Agricultural	5.81	Fairly Poor	3.17	28.9
4	Agricultural	4.94	Good	1.24	23.4
5	Forested	5.74	Fairly Poor	0.11	55.1
6	Forested	5.94	Fairly Poor	0.17	24.5
7	Forested	5.96	Fairly Poor	0.51	21.3
8	Forested	5.97	Fairly Poor	0.53	36.8

The Hilsenhoff index is a family-level index intended to provide a rapid index of stress from organic pollution (usually excess nutrients) in streams. This index of organic pollution was a poor indicator of eutrophication in the study springs, with no relationship between the FBI score and either Total Nitrogen or Phosphate concentrations, or with Land-use Classification. In this analysis, the forested sites were assessed by the index as "fairly poor", and more stressed by organic pollution than two of the agricultural sites. This index has not previously been applied to springs, and the high proportion of Chironomidae (a taxon usually identified as an indicator of high stress) may obscure responses. Also, this index is calibrated to the Great Lakes region, and may not be as applicable in the Maritimes. Overall, the family level index could not detect effects of agricultural land use, and should not be used in Prince Edward Island springs.

[1]Hilsenhoff, W.L. 1988. Rapid field assessment of organic pollution with a family-level biotic index. Journal of the North American Benthological Society. 7: 65-68.