INVERTEBRATE ABUNDANCE AND FOOD SOURCES WITHIN RIVERINE-ESTUARINE SYSTEMS ALONG THE WEST COAST OF THE ISLAND OF NEWFOUNDLAND

By

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Abstract

Understanding natural patterns and processes underlying the structure and function of biotic communities remains a central aim of ecology. However, there is a paucity of information related to the distribution patterns and community structure of biota that inhabit the transitional zone between rivers and estuaries in boreal estuarine systems. Moreover, the influence of climate change on these patterns is still poorly understood. To better elucidate these patterns this study focused on selected aquatic invertebrates within four boreal riverine-estuarine zones located along a south to north gradient in western Newfoundland, Canada. Samples of Ephemeroptera, Plecoptera and Trichoptera species were collected from each zone and identified to determine whether there was an expected decrease in number of species with both increasing latitude and increasing salinity. A decrease in the number of species with latitude was not observed. Within riverine-estuarine zones a negative correlation was evident between number of species and salinity. Aquatic insects and one amphipod species were collected from Salmon River and categorized into functional feeding groups. These invertebrates, along with biofilm, particulate organic matter, and various terrestrial and aquatic plants were analyzed for carbon, nitrogen, and hydrogen stable isotopes. Hydrogen isotope values were useful as a supplement to carbon in determining sources of energy being utilized by the functional feeding groups. However, the hydrogen isotope values were not distinct between functional groups and therefore, cannot be used in conjunction with nitrogen isotopes to determine trophic levels.

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Table of Contents

List of Tables

List of Figures

1 Introduction

1.1 General Introduction

Understanding natural patterns and processes underlying the structure and function of biotic communities remains a central aim of ecology. Patterns can exist at various scales in time and space, ranging from population abundances, through communities, ecosystems, biomes and the entire biosphere (Lawton 1996). In recent years, analysis of spatial trends has been used to combine pattern and scale as a means to better understand terrestrial and aquatic ecosystems. Moreover, the functional role of species within an ecosystem is identified as an important driver of ecosystem functioning and continues to receive increased attention.

Within aquatic systems much attention has been given to stream and river ecology. There is much less information related to the distribution patterns and community structure of biota that inhabit the transitional zone between rivers and estuaries in boreal estuarine systems. Complicating this lack of information is the strong potential for climate change effects. The influence of climate change on estuarine ecosystems is still poorly known but the need to assess the present state of ecosystems and to forecast future biodiversity scenarios under human impact and global climate change is pressing. Climate change effects, such as sea level rise, changes in precipitation patterns, and increasing temperatures, are predicted to result in an increase in salinity in estuaries and the freshwater rivers that feed into them (Prandle and Lane, 2015). In aquatic ecosystems,

salinity appears to be the environmental factor of utmost importance. According to de Jonge (1974), Michaelis et al. (1992), and Attrill (2002) extreme salinity values and long-term salinity variations define the heterogeneity of habitats and species richness of aquatic communities. The distribution of benthic fauna along a salinity gradient is dependent on the species' salinity tolerances. Smyth and Elliot (2016) proposed than an increase in salinity, driven by climate change, may result in a landward migration of estuarine benthic communities. As such, more attention to salinity as an important environmental factor in defining community structure and function in this transitional zone is warranted particularly given the uncertainty associated with climate change.

To better elucidate the distribution patterns and community structure of biota that inhabit estuarine systems in the boreal region of Canada, this study focused on four riverine-estuarine zones located in western Newfoundland. The study originated under the Humber River Basin Project (HRBP) established by a research partnership interested in expanding research in boreal ecosystems through advancement of research in the Humber River Basin (HRB). The study expands this research effort along a latitudinal gradient north and south of the HRB; the gradient, described in Chapter 2, was established to offer a means to promote climate change research in the western region of Newfoundland.

The following research questions were addressed:

(i) How do species diversity and species abundance of Ephemeroptera, Plecoptera, and Trichoptera (aquatic Orders of the Class Insecta) vary among estuarine systems across a south to north transect along the west coast of the island of Newfoundland? Determining

how Ephemeroptera, Plecoptera, and Trichoptera (EPT) groups vary with latitude will serve as a means to understand how they are impacted by changes in the abiotic factors across the gradient. Moreover, a latitudinal gradient could offer a basis for determining shifts in ecosystem structure under changing climatic conditions.

(ii) How do species diversity and species abundance of EPT change as salinity increases from the upstream reaches of rivers to the mouths of estuaries? Determining how aquatic insects in the EPT groups vary with salinity will serve as a means to understand how they may be impacted by alteration of salinity gradients due to climate change or other anthropogenic influences.

(iii) Can hydrogen isotopes be used to determine the trophic level of macroinvertebrates in streams and can these isotopes be used to assess the base of the food web fueling these groups along a salinity gradient? Hydrogen isotopes are only beginning to be utilized in food web studies. This study will advance this science and will test how hydrogen isotopes compare with carbon and nitrogen in determining the trophic level of functional groups of the macroinvertebrates.

Specifically, the objectives of this study were (1) to examine the distribution of selected aquatic insects within and among four boreal riverine-estuarine zones in western Newfoundland, (2) to investigate the influence of salinity on spatial distribution of EPT groups in riverine-estuarine systems, (3) to investigate the stable isotope signatures of a collection of aquatic invertebrates within one particular estuarine system and (4) to

determine whether hydrogen isotopes can be used to assess the base of the food web along a salinity gradient within an estuarine-riverine system.

1.2 Estuaries

There is considerable literature but little agreement on the definition of an 'estuary'. Elliott and McLusky (2002) note that despite intensive investigations into the estuarine, coastal and shelf ecology and related disciplines there is still a lack of comprehensive definition of an estuary. The various types of estuaries offer a number of different characterizations, partly determined by the upper limit of saltwater intrusion or by the maximum of any marine influence due to tidal action or traces of salinity. Based on features in temperate regions of the northern hemisphere, Pritchard (1967) defined an estuary as 'a semi-enclosed coastal body of water which has a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage'. Pritchard's definition has been followed in this study.

Despite various definitions reported in the literature, Telesh and Khlebovich (2010) point out that certain generalizations can be applied to estuaries. They have the predominant characteristic of an environmental gradient of conditions, one of which is salinity, from the marine region into the estuary and up into the river (Elliot and McLusky, 2002). As such, estuaries represent zones where freshwater flowing from rivers mixes with salt water from the ocean (Levinson, 2005) and are characterized by large shifts in hydrological, morphological and chemical conditions (Day et al., 1989). Several workers (Ysebaert et al., 1998; Cortelezzi et al., 2007) maintain that along the gradient of

environmental conditions, salinity is the key environmental factor and it plays a decisive role and defines structural and functional characteristics of aquatic biota in estuaries.

Given their unique properties at the interface of fresh and saline environments and the inherent instability of the chemical-physical parameters, most notably the salt concentration, estuaries are areas with varied ecosystems and communities. Sediment carried from the land by rivers and from the ocean by tides is commonly trapped within estuaries providing an area rich in nutrients. The rise and fall of the tide provides a constant mixing of these nutrients that in turn influences abundance and diversity of biota. The dynamic nature of estuaries makes them highly productive and therefore areas of great importance for many species.

Rich food supply and absence of predators also make estuaries ideal nurseries for invertebrate and vertebrate species (Kennedy, 1990). Estuaries are also ecologically important because they serve as migration sites for both freshwater and marine species as well as feeding areas for migrating water birds (Day et al., 1989). Because of this, estuaries are among the most productive aquatic systems on earth (Kennedy, 1990). The shallow, nutrient rich water of estuarine systems results in an abundance of phytoplankton, aquatic plants and algae. These primary producers form the basis of the riverine-estuarine food web. They become established, grow and reproduce on any submerged surface within all aquatic ecosystems (Merritt and Cummins, 2007). This community of algae, cyanobacteria and detritus is termed periphyton or biofilm. Many aquatic animals use autochthonous organic matter such as the biomass synthesized by

periphyton as their main energy source or as a variable portion of their diet (Merritt and Cummins, 2007). Plankton in the water column also contribute to the primary production at the base of the food web. For instance, by grazing on phytoplankton, zooplankton provides a carbon source to the food web through metabolic pathways or as detritus. A supplementary source of energy for primary consumers in estuaries is the organic matter that enters the rivers through runoff from the land. This allochthonous source provides a significant input of fixed carbon to the estuarine system (Williams and Feltmate, 1992; Merritt and Cummins, 2007).

Estuaries are often studied under the discipline of marine sciences. However, the ecology of the zone defined by the interaction of the estuary with the freshwaters of river systems is not widely examined (Rundle et al., 1998).

1.3 Aquatic Insects

Although the structure and function of aquatic insect communities has been the subject of much research in lake and river systems, few studies have addressed the transitional area from riverine to estuarine systems. In particular, little information exists on the faunal communities of this transitional zone in boreal ecosystems. Several authors indicate the distribution of estuarine species is driven mainly by salinity and salinity fluctuations (Diaz, 1989; Remane and Schlieper 1971; Ristich et al. 1977; Sanders et al. 1965; Verhoeven, 1980).

Aquatic insects represent a wide diversity of organisms living among stones, logs, sediments and aquatic plants on the bottom of streams, rivers, lakes and estuaries. Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), commonly designated as the EPT group, are among the most studied taxa, largely because they show a continuum of responses to environmental variables (Rosenberg and Resh 1993; Maddock 1999; Dohet et al. 2008). As such, the EPT taxa are commonly used for biological assessments of environmental change in lakes and streams (Barbour et al. 1996; Lammert and Allan 1999; Bonada et al. 2006).

Aquatic insects form a vital link in the aquatic food web where they play important roles in nutrient cycling (Righi-Cavallaro et al. 2010), in processing of coarse organic matter (Graça et al. 2001; Boyero et al. 2011), and in the diets of vertebrates and invertebrates (Ferro and Sites, 2007). In many systems, fish are the top predators feeding on aquatic insects which in turn have utilized the primary producers such as bacteria, algae, mosses, and plants as their food source.

Aquatic insects can be categorized into functional feeding groups (FFGs) based on their feeding mechanisms and the size of the particulate matter that they ingest (Williams and Feltmate, 1992). The food sources for invertebrates are divided into four categories: coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM), periphyton and prey (Voshell, 2002; Merritt and Cummins, 2007). CPOM is comprised of both woody and non-woody debris such as leaves, needles, flowers and twigs that fall from plants along the shoreline. The particles are generally larger than 1.0 mm in size

(Williams and Feltmate, 1992; Voshell, 2002). FPOM ranges from 0.5 μm to 1.0 mm in size and is comprised of fragments of CPOM, microbial cells and unattached algal cells (Williams and Feltmate, 1992; Voshell, 2002; Merritt and Cummins, 2007). Periphyton, as previously mentioned, is comprised mainly of algae and detritus that attaches itself to solid substrates within aquatic ecosystems (Voshell, 2002; Gaiser, 2009). Finally, prey consists of all the small invertebrates captured and consumed by the larger aquatic insects. They may be either small species or species in early stages of their development (Merritt and Cummins, 2007).

The four types of functional feeding groups in this study are shredders, collectors, scrapers and predators (Table 1.1). The shredders consume CPOM and have basic mouthparts including mandibles which are used for cutting and grinding and a labrum and labium which are used to keep the food inside the mouth (Voshell, 2002; Merritt and Cummins, 2007). The collectors feed on FPOM and use special straining mechanisms to acquire their food (Voshell, 2002). Some invertebrates, such as certain species of caddisflies, spin nets out of silk to collect FPOM as it flows past them in the stream. Others have fine hairs on their legs or heads that are used to collect the organic matter. Scrapers consume the periphyton and have mouthparts that are adapted to allow them to remove this from the substrate. For example, some mayflies have jaws with sharp, angular edges while snails have a rough tongue-like structure to loosen the periphyton (Voshell, 2002). Finally, predators feed on living insects and either ingest the whole organism or tear it into smaller pieces that are more easily consumed (Voshell, 2002).

As important links in the estuarine food chain, aquatic insects can be used as indicators of the system's overall productivity and can supply useful information about the structure of the ecosystem (Clarke and Scruton, 1997; Khan and Colbo, 2008).

Table 1-1 Classification of functional feeding groups for aquatic insects

Functional Feeding Groups	Food Source	Description	Particle Size (mm)
Shredders	CPOM	Leaves, needles, bark, woody and non-woody plant debris	> 1.0
Collectors	FPOM	Fragments of CPOM, unattached algae	< 1.0
Scrapers	Periphyton	Algae, phytoplankton, detritus	< 1.0
Predators	Living Animal Tissue	Whole or parts of small invertebrates	> 1.0

1.4 Stable Isotopes

One approach to understanding the complex trophic relationships among functional feeding groups mentioned above is through stable isotope analysis. Isotopes are forms of the same element that differ only in the number of neutrons (Fry, 2006). Since different isotopes of a given element have the same number of electrons, they share similar electronic structure and therefore exhibit nearly identical chemical behavior. However,

the lighter isotope usually reacts faster than the heavier isotope in the same molecule which leads to kinetic isotope fractionation.

Isotope ratio measurements in environmental samples are made relative to standards (Coplen, 1996). Results are expressed as delta (δ) values in parts per thousand (‰) difference between the sample ratio and the standard ratio (McKinney et al., 1950). The equation used to describe this difference is:

$$\delta = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R_{sample} is the isotope ratio for the sample and $R_{standard}$ is the isotope ratio for the standard where, in both cases, the heavy isotope is in the numerator and the light isotope is in the denominator. Therefore, a positive value within this notation denotes a sample in which the heavy isotope is more abundant than in the standard. A sample in which the heavy isotope is less abundant than in the standard will be represented by a negative value (Bianchi, 2007).

Stable isotope analyses have been used to better understand the sources of organic matter within food webs. Studies focusing on the natural abundances of carbon in estuarine systems have shown that it is possible to trace inputs of terrestrial and aquatic matter because organisms retain the isotopic signatures of the food they assimilate (Bianchi, 2007; Deines et al., 2009). Differences in $\delta^{13}C$ values between organisms will indicate that their diet consists of different primary producers. Carbon isotopes can also be used to determine shifts in diets, whether the shifts be due to seasonal, environmental, or climatic

changes. Nitrogen isotopes can be used to determine the trophic position of organisms. The differential reaction rates between heavy and light isotopes lead to selective metabolism of lighter isotopes during food assimilation and excretion. Thus, the organism becomes enriched in the heavier isotope. Organisms at a higher trophic level will be enriched in ^{15}N. Hydrogen isotopes have only recently been utilized in food web studies. On occasion there are overlaps in the carbon isotope ratios in aquatic food webs that make it difficult to determine the source of carbon. Hydrogen isotopes can be used in tandem with carbon isotopes to trace subsidies to aquatic food webs.

2 Abundance, Diversity and Distribution of Aquatic Insects Within and Among Riverine-Estuarine Systems

2.1 Introduction

Species abundance, diversity and distribution are important attributes that underpin the many complex community patterns evident in nature. Knowledge of the factors that influence species attributes is therefore important in understanding community organization. Despite the foundational works of Hutchinson (1957, 1959) and MacArthur (1955, 1965), and numerous works that followed, understanding the factors that influence the structure and function of biotic communities remains a major challenge to ecologists. It has been suggested that analyzing the relationships between the patterns of distribution of organisms and physical or biological factors is usually the first step towards this understanding (Hoffman and Blows, 1994).

Distribution of biota can be associated with a collection of environmental factors whether they are biogeographical, physical, chemical, or geological (Galbraith et al., 2008; Naddafi et al., 2011; Neff and Jackson, 2011). This study examined four locations in western Newfoundland to investigate two of these factors, the biogeographical influence of a latitudinal gradient and the chemical influence of salinity, on abundance and diversity of the aquatic insect Orders Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies).

2.1.1 Latitudinal Diversity Gradient

The latitudinal diversity gradient of high numbers of species near the equator to low numbers at higher latitudes is one of the most widely recognized patterns in ecology (Gaston, 2000; Hof et al., 2008; Scott et al., 2011). Latitudinal gradients have been observed at various scales in terrestrial, marine, and freshwater environments (Gaston, 2000).

A number of hypotheses have been proposed as to why this gradient exists (Hof et al., 2008). One hypothesis, the species-energy hypothesis, states that the amount of energy available limits species richness (Hawkins et al., 2003). At the lower latitudes there is a higher amount of solar energy which increases net primary productivity. An increase in net primary productivity increases the number of individuals that can be supported and thus the number of species in that area will increase. Related to this is the hypothesis that climatic conditions may have an influence on the gradient. Certain species may not be able to physiologically tolerate the harsher climate found at higher latitudes (Currie et al., 2004) or fluctuating climatic conditions may prevent species from inhabiting these areas. The climate in temperate regions varies more with season than in the tropics and this may prevent speciation (Currie et al., 2004).

A third hypothesis concerning latitudinal gradients originates from a historical perspective. It proposes that low species richness occurs at higher latitudes because of geological disturbances caused by glaciation (Willig et al., 2003). It has been suggested that Pleistocene glaciations can explain the low numbers of species in temperate zones in

that the diversity in these regions has not yet reached equilibrium and will, therefore, continue to increase.

In Newfoundland and Labrador, freshwater studies have focused on the distribution and diversity of aquatic insects in streams and lake outlets (Larson and Colbo, 1983; Clarke and Scruton, 1997; Lomond and Colbo, 2000). These studies have largely concentrated on local sites on the east coast of the island. With the exception of Smith et al. (2013) few studies have been carried out across broader spatial scales. This study attempts to define the abundance and diversity of EPT within riverine-estuarine systems in western Newfoundland and determine if there is evidence for a latitudinal gradient of EPT at a regional scale. Given that climatic conditions may influence the latitudinal gradient, it was expected that abundance and diversity of EPT species would decrease along the latitudinal gradient from south to north. Despite the small scale along the west coast of the island of Newfoundland, there is a climatic difference which could have had an influence on the distribution of species along the gradient.

2.1.2 Conductivity and Salinity

Biological communities within streams often show distinct gradients in terms of species abundance and composition in response to specific variables, notably temperature, pH, flow rates, and oxygen concentration. Aquatic insects found at the riverine-estuarine interface are particularly influenced by complex salinity gradients as a result of tidal intrusion (Magdych 1984; Williams and Williams, 1998; Teske and Wooldridge, 2003). Given that accelerated sea level rise is projected to substantially increase salinity in

estuaries (Nicholls et al., 2007), it is important to consider the influence of salinity on invertebrate communities. Conductivity of a solution is a measure of its ability to conduct an electrical current (Snoeyink and Jenkins, 1980). This is directly related to the concentration of ions in the solution. As the concentration of ions increases, the conductivity increases. In aquatic systems such as rivers, lakes, and estuaries these ions come from dissolved salts and inorganic materials such as alkalis, chlorides, sulfides, and carbonate compounds (Miller et al., 1988). Conductivity is measured in microsiemens per centimeter (μScm^{-1}) and is affected by the temperature of the water. The conductivity measurement of water at a standard temperature of 25°C is termed the specific conductance (Miller et al., 1988). The conductivity meter used for the research presented herein was corrected for temperature. Therefore, all reported conductivity measurements are in terms of specific conductance.

Salinity is a major component of a conductivity measurement and is defined as the total concentration of all dissolved salts in a solution (Wetzel, 2001). Therefore, salinity changes can be indirectly derived from conductivity measurements and expressed in terms of electrical conductivity (EC) at 25°C (Kefford et al., 2004). Conductivity measurements were used in this study as a means to track differences in salinity values among sites.

In most freshwater environments conductivity levels are attributed to the surrounding geology. Weathering of limestone rocks and clay will contribute to conductivity through dissolution while granite rocks withstand weathering and do not contribute significantly

(Olson, 2012). The conductivity values in estuaries are influenced by both the run-off from the freshwater sources, which can range in value from 20 to 600 μScm^{-1} (Kalff, 2002), and the influx of salt water from the ocean, which has a conductivity value of approximately 50,000 μScm^{-1} (Kalff, 2002).

Salinity levels in estuarine systems can vary from freshwater to seawater over a short distance and certain organisms will only be found at one end of the estuary or the other (Horne and Goldman, 1994). To survive the variable salinity within the riverine-estuarine system, eurylhaline organisms are equipped with physiological mechanisms to regulate salt (Horne and Goldman, 1994). They will absorb or excrete salt ions through osmotic processes as required in order to adapt to the ionic concentrations of the water. Most organisms, however, can only tolerate a specific salinity range as they do not have the capabilities to excrete the salt ions (Horne and Goldman, 1994).

Numerous studies have investigated the diversity, density, production, taxonomic composition or trait constitution of assemblages of freshwater insects (Bradt et al., 1999; Voelz et al., 2000; Metzeling et al., 2002; Nanami et al 2005; Beche and Resh, 2007; Costa and Melo, 2008). However, much less is known concerning aquatic insects inhabiting the transition zones between rivers and marine environments in boreal ecosystems.

2.1.3 Study Objectives

This study was designed to determine a) whether EPT diversity and abundance change in relation to a salinity gradient within the transitional zones between riverine and estuarine systems and b) whether EPT diversity and abundance changes with distance along a latitudinal gradient from south to north along the west coast of the island of Newfoundland. It was expected that EPT abundance and diversity will decrease from the freshwater sites into the more saline estuary as osmotic stress increases. It was also expected that a latitudinal gradient exists and EPT abundance and diversity will decrease from the south to north along this gradient due to climatic differences.

2.2 Methods

2.2.1 Study Locations, Geological Setting, and Climate Conditions

The island of Newfoundland ($51^{0}38'$-$46^{0}37'$N, $59^{0}24'$-$52^{0}37'$) has a land area of 111,390 km^2 (Government of Newfoundland and Labrador, 2016). The area of study was insular Newfoundland with the main focus on four riverine-estuarine systems on the west coast of the island: 1) Grand Codroy River; 2) Hughes Brook, a tributary of the Humber River; 3) Main River; and 4) Salmon River (Table 2.1). These four rivers represent significant discharge systems for the west coast.

These four rivers fall along a transect that spans almost 4 degrees of latitude along the west coast and are located within four different subregions of the Western Newfoundland Forest and Northern Peninsula Forest Ecoregions on the island (Figure 2.1).

Table 2-1 Name, location, latitude/longitude and climate data for the four sites sampled in this study.

Site Name	Site ID	Location	Latitude/ Longitude Coordinates	Mean Temperature (ºC)		Mean Annual Precipitation (mm)
				Summer	Winter	
Grand Codroy River	CV	Doyles/ Searston, NL	47.850520, -59.250310	15.0	-4.1	1504
Hughes Brook	HB	Hughes Brook, NL	48.998367, -57.893674	15.8	-4.7	1286
Main River	MR	Sop's Arm, NL	49.764138, -56.909069	14.1	-6.0	1002
Salmon River	SR	Main Brook, NL	51.176345, -56.016057	13.0	-8.7	1224

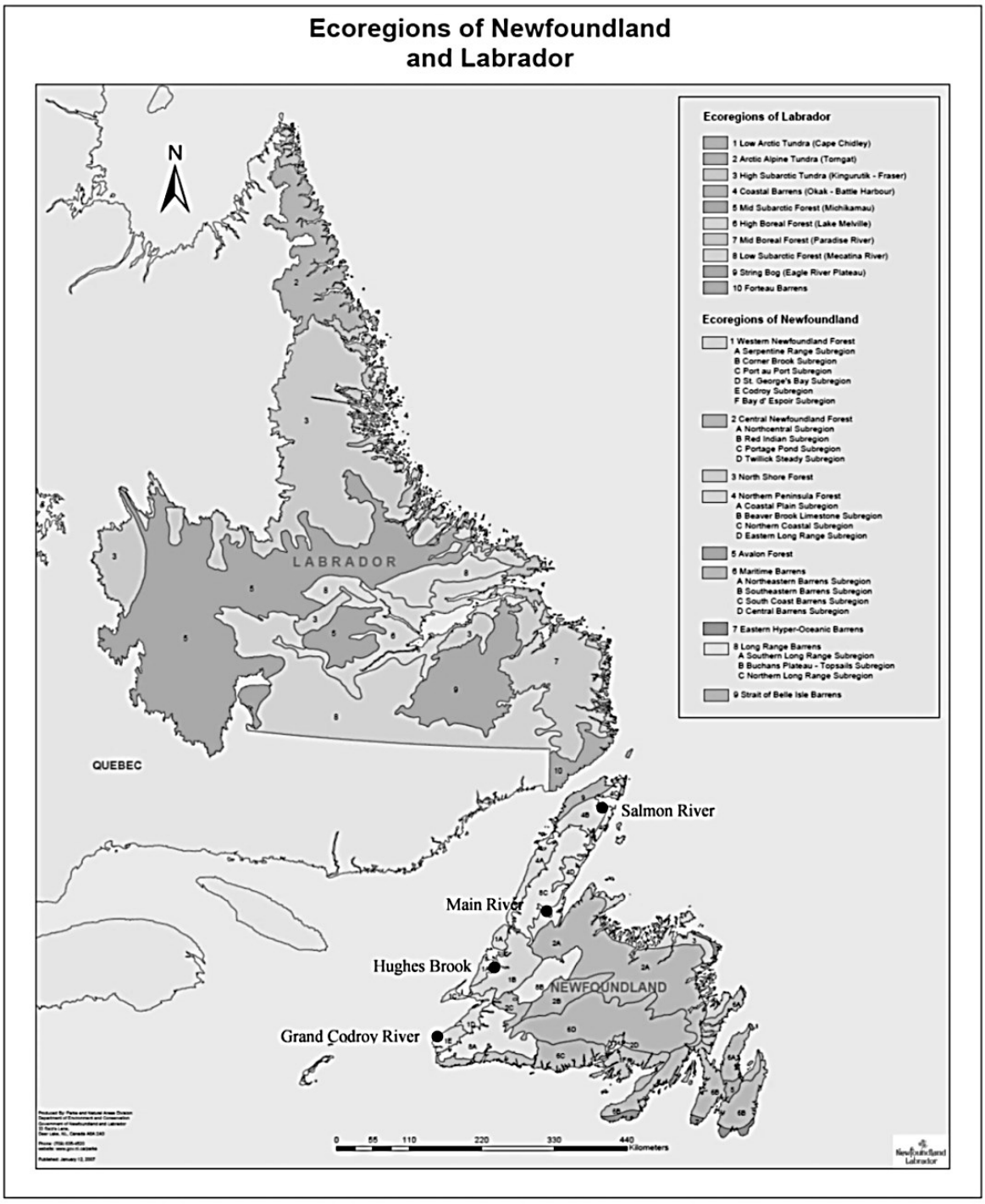


Figure 2-1 Map of ecoregions (including subregions) of Newfoundland and Labrador showing sampling areas (Adapted from Dept. of Environment and Conservation, Government of Newfoundland and Labrador, 2007)

The Newfoundland climate has been described as 'modified continental', one that is relatively cool and wet, with a short growing season (Banfield, 1983). Along the west coast, climate is largely influenced by the Long Range Mountains resulting in various zones. The West Coast Zone (Bonne Bay and south), which includes the Codroy River, Hughes Brook and Main River, is characterized by cold and snowy winters with occasional heavy snowfalls influenced by west to northwest airflows (Banfield, 1983). Annual sea level precipitation is 1300-1400 mm around Bonne Bay and the Bay of Islands (Banfield, 1983). Summers are moderately warm with maxima up to 30^0C in sheltered valleys and inlets. The Northern Peninsula Zone, which includes Salmon River, is progressively colder throughout the year especially further north where winters are longer.

The climatic conditions along the latitudinal gradient, combined with unique surface geology, largely determine and influence forest ecosystems in the four respective river basins. The Island of Newfoundland falls in the boreal forest biome (Rogerson, 1983), a landmass that was completely glaciated in the most recent ice age with most of the soil eroded away (South, 1981; Dyke et al., 2002).

2.2.1.1 *Grand Codroy River*

The Grand Codroy River is located on the southwest coast of the island in the Codroy subregion of the Western Newfoundland Forest Ecoregion (Figure 2.2). It is the most southerly estuary in this study. This area has rich soils that were formed from glacial deposits and runoffs. Its location, surrounded by the Long Range and Anguille Mountains

that provide shelter from northeasterly winds, provides the province's most favourable climate with relatively warm summers and the longest growing season on the island. The mean annual precipitation for this area is 1504 mm (Environment Canada, 2016) and the mean daily temperature during the summer months is 15.0^{0}C (Environment Canada, 2016). This combination has proven relatively advantageous for agriculture and much of the area surrounding the sampling sites was farmland. During the winter months the mean daily temperature falls to -4.1°C (Environment Canada, 2016). Forested areas in this subregion consist mostly of *Abies balsamea* (Balsam fir) and *Picea mariana* (Black spruce).

The Grand Codroy River is approximately 51 kilometers in length and flows into the Atlantic Ocean at Searston, NL. The estuary is 7 kilometers long and varies between 1.0 and 1.5 kilometers in width. It includes 13 small islands (Rao et al., 2009). The estuary was listed as a Wetland of International Importance under the Ramsar Convention of Wetlands in 1987 and in 2016 the nature Conservancy of Canada announced it had expanded its reserve to include the much of property along the estuary

The sites that were sampled all had an open canopy with an abundance of sunlight. The substrate ranged from boulders to medium sand according to the Wentworth Scale of Grain Sizes (1922; Table 2.2).

Table 2-2 Wentworth Grain-Size Classification (Wentworth, 1922)

Wentworth Size Class	Millimeters (mm)	Micrometers (µm)
Boulder	256 – 4096	-----
Cobble	64 – 256	-----
Pebble	4 – 64	-----
Granule	2 – 4	-----
Very Coarse Sand	1 – 2	-----
Coarse Sand	0.50 – 1.00	-----
Medium Sand	0.25 – 0.50	250 – 500
Fine Sand	0.125 – 0.25	125 – 250
Very Fine Sand	0.0625 – 0.125	63 – 125
Coarse Silt	0.031 – 0.0625	31 – 63
Medium Silt	0.0156 – 0.0625	15.6 – 31
Fine Silt	0.0078 – 0.0156	7.8 – 15.6
Very Fine Silt	0.0039 – 0.0078	3.9 – 7.8
Clay	0.00006 – 0.0039	0.06 – 3.9

2.2.1.2 *Hughes Brook*

Hughes Brook is located in the Bay of Islands in the Corner Brook subregion of the Western Newfoundland Forest ecoregion (Figure 2.3). The geology in this subregion is characterized by a mixture of shale, marble and limestone. The climate in this region is also relatively favourable with a mean daily temperature during the summer months of 15.8^{0}C (Environment Canada, 2016) and a mean annual precipitation of 1286 mm (Environment Canada, 2016) which results in the most favourable growing conditions for forest vegetation on the island. The mean daily temperature during the winter is -4.7^{o}C

(Environment Canada, 2016). Similarly to the Codroy subregion, *A. balsamea* is the dominant softwood species and grows in association with *P. mariana* and *P. glauca* (White spruce).

Hughes Brook is approximately 15 kilometers in length. The lower portion flows through agricultural and residential areas while the upper portion is comprised of harvested forest stands. The estuary is 725 meters long and varies between 100 and 200 meters in width. It is characterized by a number of small sand bars and mud flats at low tide.

The sites that were sampled all had open canopies with the exception of sites 1 and 2, which had moderately closed canopies. The substrate ranged from boulders to medium sand.

2.2.1.3 ***Main River***

Main River is located on the eastern base of the Great Northern Peninsula and lies within the Eastern Long Range subregion of the Northern Peninsula Forest ecoregion. This is one of the coldest regions on the island and has the shortest growing season. This is a result of being bounded by the cold North Atlantic Ocean to the east and the highland plateau of the Northern Peninsula to the west. The mean daily summer temperature is 14.1^{o}C and the mean daily winter temperature is -6.0^{o}C. The mean annual precipitation is 1002 mm (Environment Canada, 2016).

Many species of trees, such as *Pinus strobus* (White pine) and *Acer rubrum* (Red maple), reach their northern limit just south of this ecoregion. However, *A. balsamea* is still the

dominant species except at higher altitudes where *P. mariana* is more common. In the Sop's Arm area (Figure 2.4), where Main River is located, the rocks are mainly granites and the soil cover is thin with many areas of exposed bedrock.

In 2001, Main River was designated a Canadian Heritage River. It is 57 kilometers in length and flows through an old growth forest comprised of 200-year-old black spruce. The estuary is comprised of a delta with many small islands and sandbars. The sampling sites all had open canopies and the substrate ranged from cobble to very coarse sand.

2.2.1.4 ***Salmon River***

Salmon River, located near the community of Main Brook on the northeastern tip of the Great Northern Peninsula, is the most northerly estuary in this study. It is located in the Beaver Brook Limestone subregion of the Northern Peninsula Forest ecoregion. As the name suggests the underlying rock in this subregion is limestone bedrock. The area has short cool summers and long cold winters with an average summer temperature of 13^0 C and -8.7°C in the winter. The average annual precipitation is 1224 mm (Environment Canada, 2016). The forests in this area are considered the most productive on the island. While *A. balsamea* is still the dominant species of tree, a number of species, such as *P. strobus* and *A. rubrum*, are absent in this area because of climatic conditions and the less acidic soil.

Salmon River flows through a forested area that was used for logging in the past. It is approximately 40 kilometers in length while the estuary is approximately 1 kilometer in

length and varies between 100 to 500 meters in width. There is a man-made breakwater at the mouth of the estuary where it flows into Hare Bay (Figure 2.5). The sampling sites all had open canopies and the substrate ranged from boulders to very coarse sand.

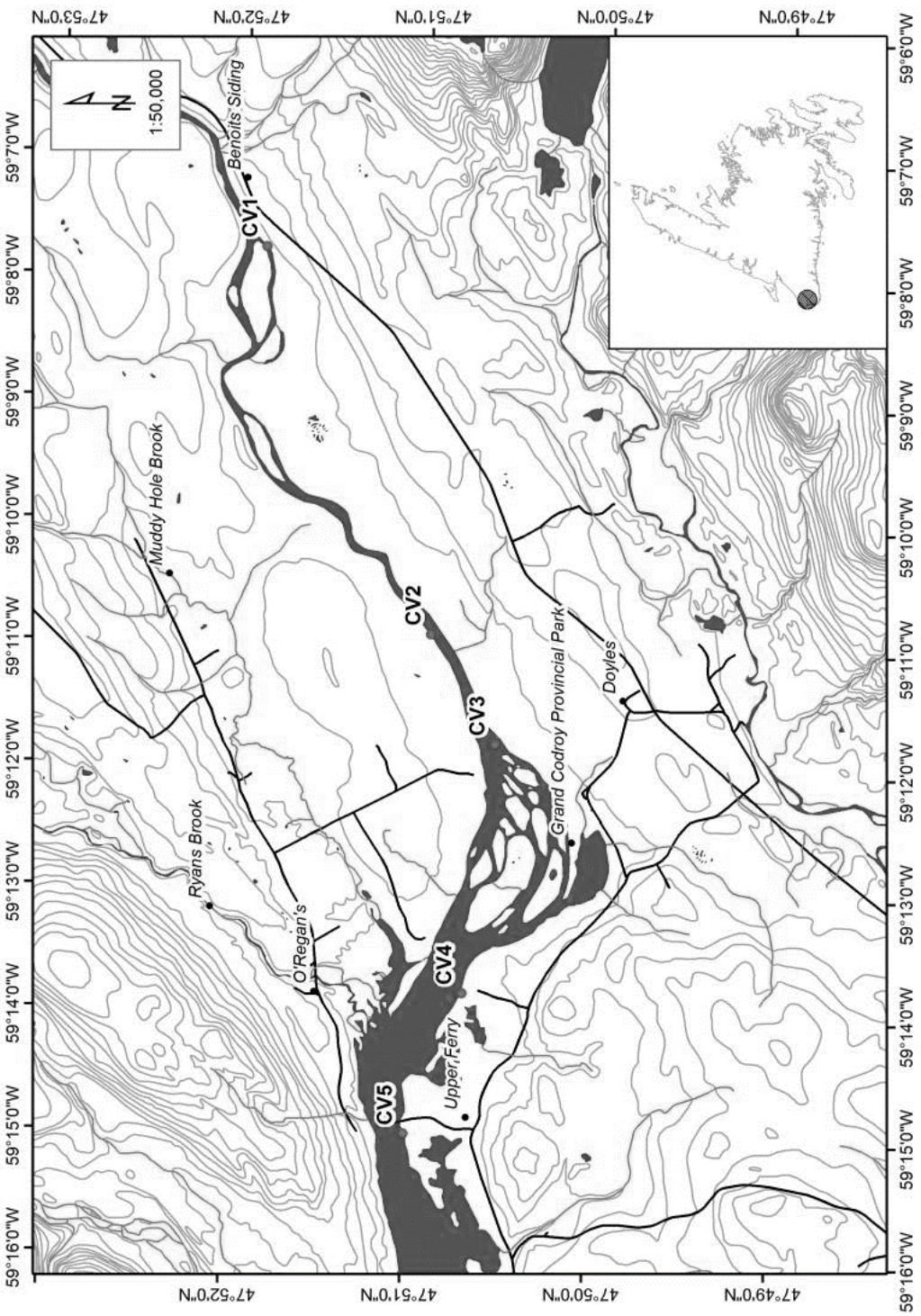


Figure 2-2 Sampling sites within the Grand Codroy River

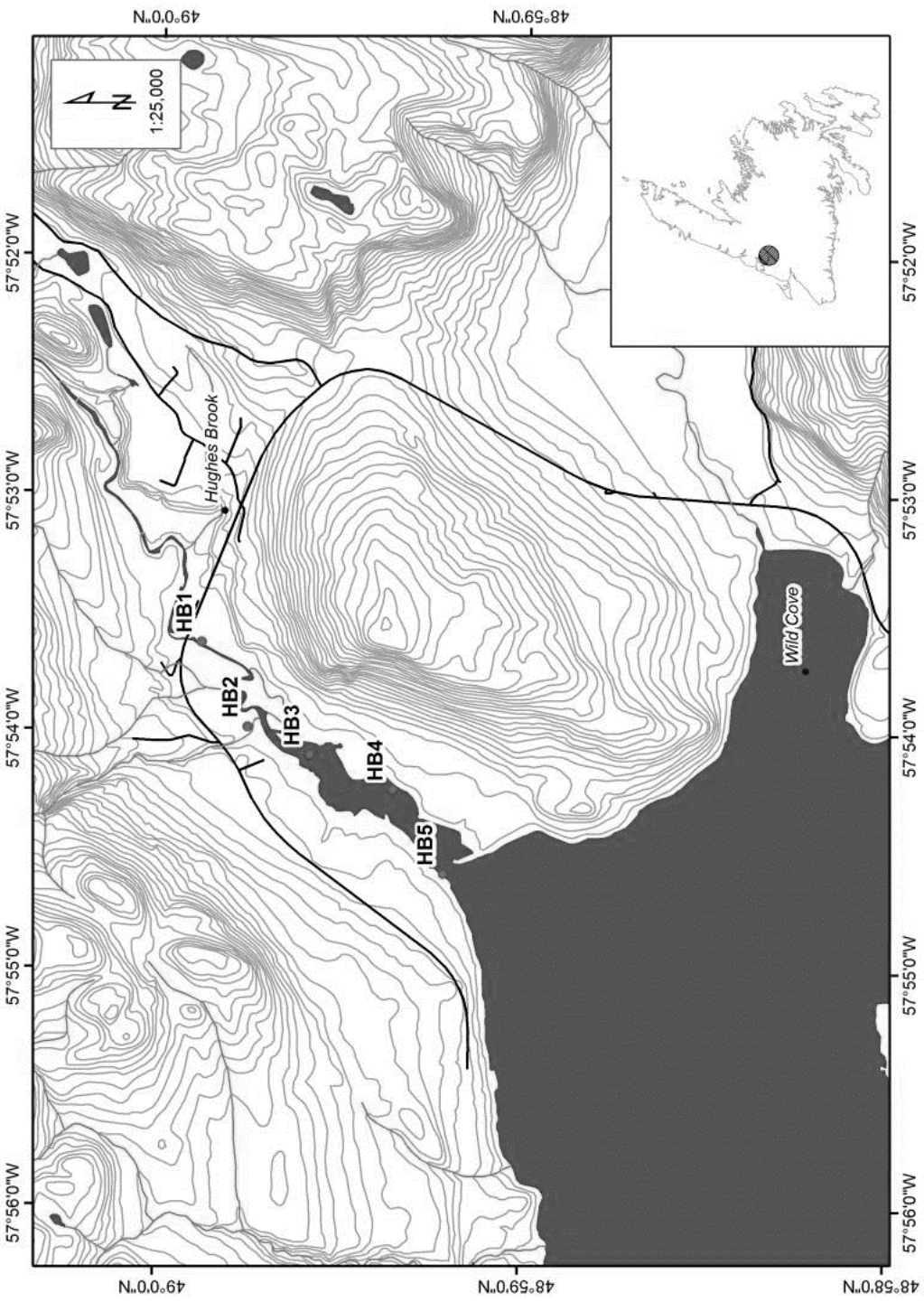


Figure 2-3 Sampling sites within Hughes Brook

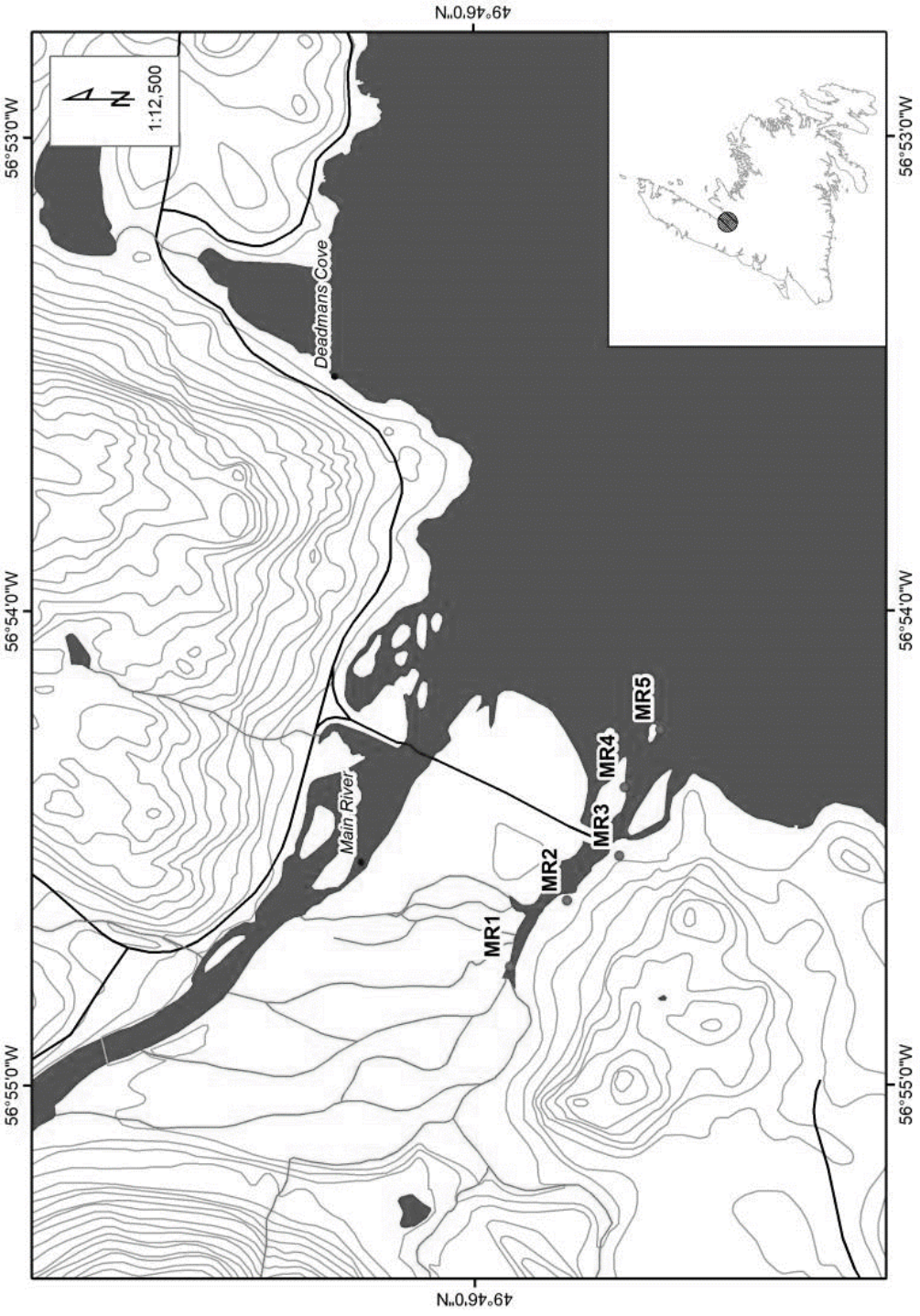


Figure 2-4 Sampling sites within Main River

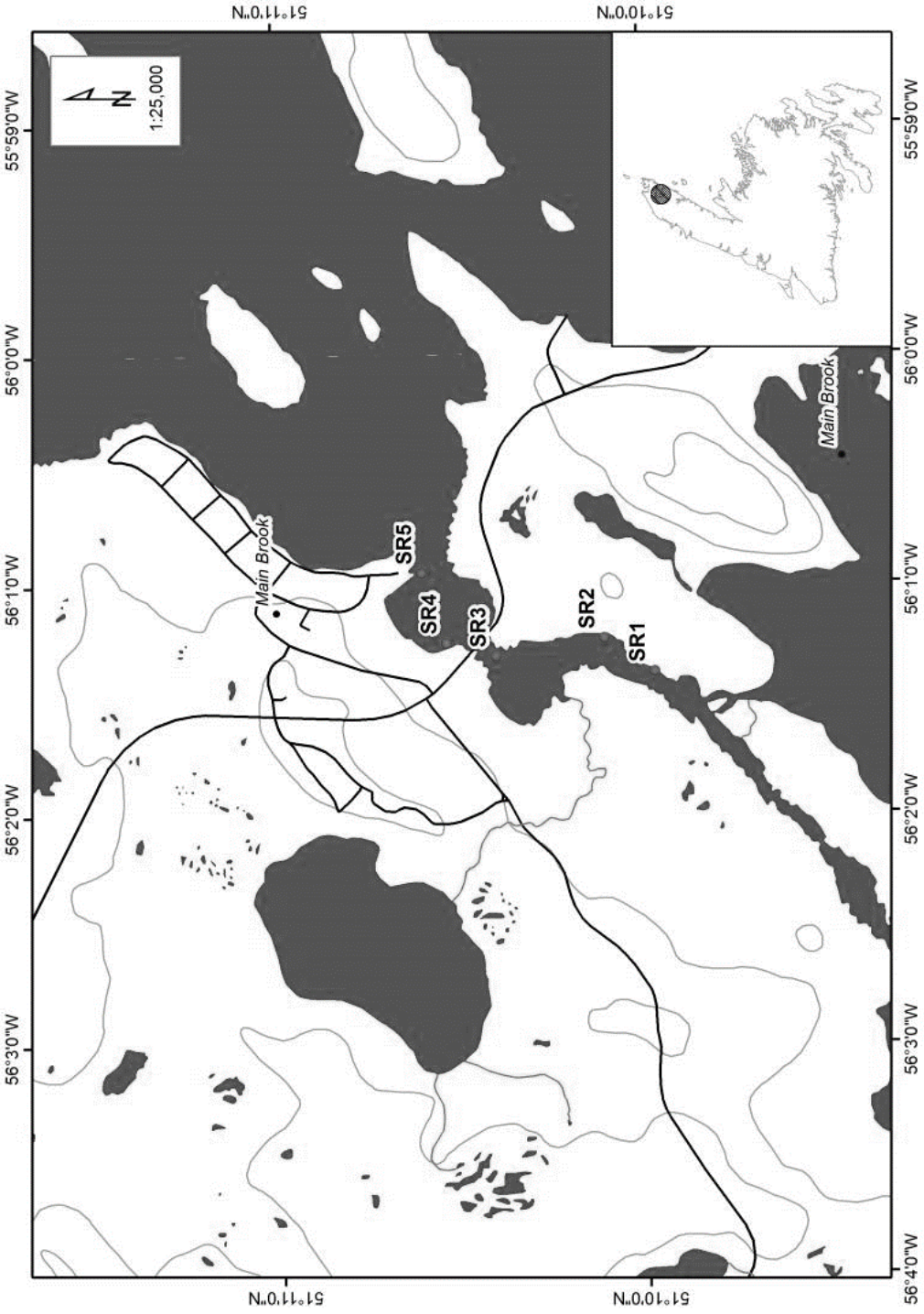


Figure 2-5 Sampling sites within Salmon River

2.2.2 Sampling Procedure

Samples were collected twice in the summer of 2010 during a 7-day period from June 2nd to the 9th and a 17-day period from August 8th to the 25th. Five sampling sites were chosen within each river (Figures 2.2 to 2.5). Sites within the rivers were chosen based on conductivity values at high tide with Site 1 having the lowest conductivity and Site 5 the highest. These measurements were taken at high tide so as to determine the maximum value of conductivity to which the insects were subjected. Conductivity readings were taken using a portable multi-parameter digital meter (HACH, HQ40d, Canada) with IntelliCAL Probes (HACH, CDC40105, Canada) that automatically recognized the testing parameter. The meter was calibrated using a calibration standard of 0.01M KCl (1413 µS/cm, 25^{0}C). The conductivity probe was accurate to +/- 0.5% of a conductivity range of 0.01µS/cm to 200mS/cm. The conductivity meter also provided water temperature measurements for each of the sites. These readings were taken at low tide.

Flow rates were measured using a propeller type current meter (Swoffer, Model 2100, USA). The propeller was placed at 0.6 of the depth of each site and the current was measured at this depth. Flow was measured at the midpoint of the river at each sampling site where possible. In Salmon River where the midpoint of the river at site 1 was too deep, flow was measured as close to the center of the river as possible. Three readings were taken at each site during low tide and the average flow was calculated from these readings.

Depth at each site was measured using a meter stick. Five depth measurements were taken at each sampling site at low tide and the average was calculated (Table 2.3).

Five samples were taken at each site within the rivers at low tide using a Surber bottom sampler (0.09 m^2 area, 800 μm mesh). Rocks within the sampler frame were scrubbed with a soft bristled brush and removed from the frame. Substrate that could not be removed was disturbed with the brush or by hand to a depth of approximately 5 centimeters, where possible, to remove any invertebrates that may have been buried within the sediment. The samples were held in river water in transparent, contaminant-free, non-reactive polyethylene bags with round wire enclosures and stored in a cooler until they were returned to the laboratory.

In the laboratory, all benthic invertebrates were removed from the debris. The samples were further sorted and specimens of the Orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) were preserved in 70% ethanol and identified to species where possible using taxonomic keys some of which were specific to Newfoundland and Labrador (Larson, unpublished; Merritt and Cummins, 1996; Clifford, 1991).

Table 2-3 Abiotic data for the five sites at each river for the two sampling dates

River	Site	Water Temperature (°C)		Average Flow (m/s) (n = 3)		Conductivity at High Tide (µS/cm)		Average Depth (cm) (n = 5)	
		June	**August**	**June**	**August**	**June**	**August**	**June**	**August**
Codroy	1	10.8	20.4	.426	.428	97.8	139.7	11.6	26.8
	2	9.8	20.3	.117	.013	97.9	133.4	19.2	23.6
	3	9.8	19.7	.077	.060	96.8	131.0	16.2	21.4
	4	11.5	20.2	.020	.010	195.9	2174.0	14.8	14.0
	5	10.0	19.4	.030	.000	10154.0	6200.0	22.6	5.6
Hughes	1	13.7	16.4	.770	.270	157.0	163.5	21.4	30.6
	2	12.7	16.9	.590	.510	163.3	164.4	22.8	27.4
	3	11.7	17.2	.280	.360	162.3	1170.0	30.4	27.6
	4	12.1	17.2	.050	.020	187.0	2830.0	32.6	13.8
	5	10.2	17.1	.220	.040	388.5	4000.0	22.6	9.4
Main	1	13.4	18.2	.420	.120	25.3	58.8	14.8	9.6
	2	13.0	21.1	.744	.283	27.0	1773.0	12.8	12.8
	3	10.3	14.0	.240	.000	200.0	39630.0	24.8	23.4
	4	10.1	14.8	.050	.077	333.0	40800.0	23.2	15.0
	5	10.0	14.2	.017	.000	1087.0	38200.0	18.6	19.4
Salmon	1	10.1	22.0	.594	.300	144.0	161.0	28.2	27.0
	2	11.4	21.8	.107	.200	148.6	165.0	33.6	24.6
	3	11.1	23.7	.443	.167	142.4	652.0	31.0	29.2
	4	10.5	23.2	.017	.000	959.0	13990.0	24.6	27.0
	5	10.3	19.9	.010	.120	3300.0	32390.0	17.2	44.2

2.2.3 Analytical Methods

Data were analyzed to (a) characterize and compare taxa composition and abundance within the four ecological systems; (b) compare selected environmental variables influencing the structure of EPT assemblages; and (c) examine evidence for a latitudinal gradient of EPT at a regional scale.

Relative species abundance was calculated for each insect Order within the four systems. The number of species of each Order was divided by the total number of species collected at each site. Relative species abundance per river was calculated for all four rivers. The total number of species collected at each river was divided by the total number of species collected at all four rivers to calculate this value. Relative species abundance per site was also calculated for each site within each river. The number of species at each site was divided by the total number of species collected at each river.

The Shannon-Wiener diversity index is commonly used to characterize species diversity in a community. It is a measurement that takes into account species richness and the proportion of each species within a community. It is defined by the formula below:

$$H = -\sum_{i=1}^{k} pi \ln(pi)$$

where p_i denotes the proportion of individuals found in species i. That is, $p_i = n_i/N$ where n_i is the number of individuals in species i and N is the total number of individuals in the sample (Krebs, 2001).

A high diversity index indicates a community with a large number of evenly distributed individuals whereas a low diversity index indicates a community with fewer species and the individuals are distributed less equitably.

Evenness can be calculated from the Shannon-Wiener diversity index and it measures how similar the abundance of different species is. When the proportions of all species are similar the evenness is one but when there are very different abundances of species the value decreases. Evenness is defined by the formula below:

$$E = \frac{H}{\ln(S)}$$

where *H* is the Shannon-Wiener diversity index and *S* is the species richness (Krebs, 2001).

Shannon-Weiner diversity indices and evenness values were calculated for each of the four rivers and for each site within the river-estuarine system (Magurran, 1988).

The slope of the regression line for the plot of the total number of individuals at each river against distance from the Grand Codroy River was also examined.

All statistical analyses described below were completed using IBM SPSS Statistics (version 23).

2.2.3.1 ***Univariate Analysis***

Smith (2009) reported seasonal differences in EPT populations. Therefore, data for June and August were analyzed separately.

2.2.3.1.1 Between Rivers

EPT data were examined to determine whether the mean abundances were the same for each river. However, the assumptions that the residuals are normal and homogeneous were not met. Following convention, data were $\log_{10}(x+1)$ transformed (Field, 2009) and the residuals were reexamined. The transformation did not improve the normality and homogeneity of the residuals. Because of this an ANOVA could not be used.

Therefore, the non-parametric counterpart to the analysis of variance (ANOVA), the Kruskal-Wallis test was used to test for significant differences in the abundances between rivers and between sites at each river. It tests the null hypothesis that all samples are taken from populations with the same median (Dytham, 2003). The Kruskal-Wallis test ranks all the data, irrespective of the site, from lowest to highest. Once the data is ranked, it is sorted back into the original groups (Quinn and Keough, 2001; Field, 2009). Then ranks within each site are added to give a value, R_i. The test statistic, H, is then calculated as:

$$H = \frac{12}{N(N-1)} \sum_{i=1}^{k} {R_i^2}/{n_i} - 3(N+1)$$

where R_i is the sum of the ranks of each group, N is the total sample size, and n_i is the sample size of a particular group (Quinn and Keough, 2001; Field, 2009). A chi-square distribution is used to calculate the p-value for the test statistic (Quinn and Keough, 2001). One assumption of the Kruskal-Wallis test is that the distributions for the different groups have the same shape (Quinn and Keough, 2001). This was not the case for the four rivers so this analysis could not be used.

It has been shown that the ANOVA test is robust to the assumption of normality when the sample sizes are large enough. If there are between 2-9 groups, the sample size for each group should be at least 15 (Schmider et al., 2010). This was the case for each river. The assumption of homogeneity of variances cannot be waived with large sample sizes. However, Welch's ANOVA does not assume equal variances (Field, 2009). This test adjusts F and the residual degrees of freedom to deal with violations of the homogeneity of variances assumption (Field, 2009). Therefore, this test was used to determine whether there were differences in the mean abundances between rivers.

2.2.3.1.2 Among Sites Within Each River

EPT data were examined to determine whether the mean abundances were the same for each site within each of the four rivers. Again the assumptions that the residuals were normal and homogeneous were not met for any of the four rivers. The data were then $\log_{10}(x+1)$ transformed. However, this did not improve the normality or homogeneity of the residuals.

A Kruskal-Wallis test could not be used due to the fact that the data distributions for each river did not have the same shape.

Because of the small sample sizes at the sites with higher conductivity measurements, the assumption that the residuals were normal could not be discounted and Welch's ANOVA could not be used.

As an alternative, individual relative abundance was calculated and examined for each site within the four rivers. This number was calculated by dividing the total number of individual aquatic insects at each site by the total number of aquatic insects in each individual river.

2.2.3.2 *Ordination*

Non-metric ordination was used to elucidate patterns in species composition. Canonical correspondence analysis (CCA) was employed because it is appropriate where data is collected over a habitat range for species showing nonlinear, non-monotonic relationships with environmental variables (ter Braak 1986). CCA is a multivariate method designed to extract environmental gradients from noisy ecological data and is particularly wide-spread in aquatic sciences (ter Braak, 1986; ter Braak and Verdonschot, 1995). It is a direct gradient analysis that assumes a unimodal model for the relationships between the response of each species to the environmental gradients, and that the ordination axes are linear combinations of the environmental variables (ter Braak, 1986). It can be used to

determine the type, whether negative or positive, and magnitude of the relationship between species response and the environmental factors (ter Braak, 1986).

The environmental variables used in the ordination for each sampling date were conductivity at high tide, flow at low tide, water temperature at low tide, and sampling depth at low tide. The species abundance data was $\log_{10}(x+1)$ transformed to give less weight to the larger values that would otherwise act as outliers. Sites in which no individuals were collected at all four rivers were omitted from the analysis due to the limitations of the statistical package.

The canonical correspondence analysis was completed using the PC-Ord statistical package (version 5.10; McCune and Mefford, 2006).

2.3 Results

2.3.1 Richness and Abundance of EPT Taxa

In the course of the two sampling periods, 1937 individuals were collected from 66 different species of EPT. Seven species occurred in all four riverine-estuarine systems during the June sampling period – 4 Ephemeroptera - *Baetis tricaudatus, Drunella cornuta, Ephemerella subvaria, Leucrocuta hebe,* 1 Plecoptera -*Isoperla transmarina* and 2 Trichoptera - *Lepidostoma* sp.*,* and *Hydropsyche betteni*. During the August sampling period, only two species of Trichoptera, *H. betteni* and *H. sparna*, were common in all four systems.

The number of species at each river differed. There was no detectable gradient in number of species per Order from the south at the Grand Codroy River to the north at Salmon River (Table 2.4). The number of Ephemeroptera and Trichoptera species differed at each river. However, the number of Plecoptera species was consistent across the transect.

In both sampling periods, 12 species were exclusive to the Grand Codroy River – *Callibaetis skokianus* (1), *Ephemerella aurivillii* (1), *Ameletus* sp. (2), *Siphlonurus* sp. (10), *Paraleuctra sara* (3), *Oecitis* sp. (15), *Glyphopsyche irrorata* (1), *Wormaldia moesta* (2), *Polycentropus aureolus* (8), *Rhyacophila carolina* (1), *R. melita* (2), *Neophylax* sp. (5).

One species, *Lype diversa* (1), was unique to Hughes Brook and only appeared in the June sampling period.

Ten species were exclusive to Salmon River – *Acentrella laponicus* (1), *Caenis amica* (1), *Ephemerella rotunda* (2), *Eurylophella funeralis* (2), *Eurylopella bicolor* (17), *Paracapnia opis* (3), *Limnephilus* sp. (26), *Pseudostenophylax sparsus* (3), *Chimarra* sp. (5), *Ptilostomis* sp. (1).

The most abundant species in the Grand Codroy River for both sampling periods combined were *Tricorythodes allectus* (81), *Lepidostoma* sp. (44), and *Leucrocuta hebe* (41).

The most abundant species in Hughes Brook for both sampling periods combined were *Tricorythodes allectus* (117), *Lepidostoma* sp. (30), and *Mystacides sepulchralis* (30).

The most abundant species in Main River for both sampling periods combined were *Glossosoma* sp. (276), *Drunella cornuta* (98), and *Rhithrogena undulata* (33).

The most abundant species in Salmon River for both sampling periods were *Cheumatopsyche pettiti* (146), *Ephemerella subvaria* (138), and *Glossosoma* sp. (134).

Similar proportions of Trichoptera were observed across the four rivers. However, the proportions of Plecoptera were higher in Hughes Brook and Main River than in the other two estuarine systems. The proportion of Ephemeroptera in the Grand Codroy River was slightly higher than the other three systems (Figure 2.6).

Table 2-4 Number of species of each Order collected at each river from north to south for two sampling periods, June and August 2010. The number of species that are common between sampling periods are shown in parenthesis.

River	Ephemeroptera		Plecoptera		Trichoptera	
	June	August	June	August	June	August
Grand Codroy River	15	9(5)	3	1(1)	9	18(8)
Hughes Brook	8	3(2)	3	0(0)	7	7(2)
Main River	9	8(4)	3	2(1)	9	11(5)
Salmon River	12	9(5)	3	1(1)	14	14(9)

Table 2-5 Common species between June and August 2010 for each river system.

River	Order	Species
Codroy	Ephemeroptera	*Ameletus* sp. *Baetis flavistriga* *Heptagenia pulla* *Leucrocuta hebe* *Stenonema vicarium*
	Plecoptera	*Isoperla transmarina*
	Trichoptera	*Hydropsyche betteni* *Hy. morosa* *Hy. sparna* *Lepidostoma* sp. *Mystacides sepulchralis* *Oecitis* sp. *Polycentropus aureolus* *Rhyacophila melita*
Hughes	Ephemeroptera	*B. tricaudatus* *Ephemerella subvaria*
	Trichoptera	*Hy. betteni* *M. sepulchralis*
Main	Ephemeroptera	*B. tricaudatus* *L. hebe* *Paraleptophlebia adoptive* *Rhithrogena undulata*
	Plecoptera	*Alloperla* sp.
	Trichoptera	*Arctopsyche ladogensis* *Hy. betteni* *Hy. slossonae* *Dolophilodes distinctus* *Glossosoma* sp.
Salmon River	Ephemeroptera	*Ep. subvaria* *Eurylophella bicolor* *L. hebe* *S. femoratum* *S. vicarium*
	Plecoptera	*I. transmarina*
	Trichoptera	*Apatania* sp. *Ceraclea* sp. *Cheumatopsyche pettiti* *Glossosoma* sp. *Helicopsyche borealis* *Hy. slossonae* *Lepidostoma* sp. *Neureclipsis* sp. *Psilotreta frontalis*

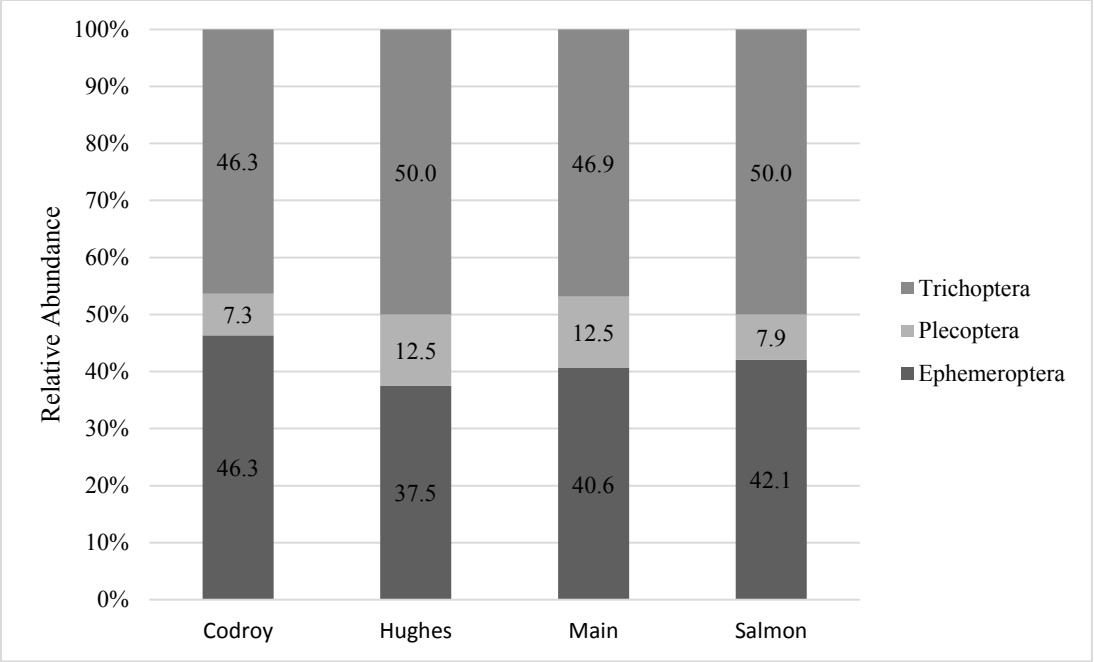


Figure 2-6 Relative abundance from the EPT groups for the combined sampling periods for the four riverine-estuarine systems

2.3.2 Diversity

The Shannon-Wiener Diversity Index and Evenness values were calculated for each site within the four rivers and for each individual river for both sampling periods.

2.3.2.1 *Diversity Between Riverine-Estuarine Systems*

The Shannon-Wiener diversity index was highest at Salmon River with a value of 2.81 and lowest at Main River with a value of 2.01 for the June, 2010 sampling period (Table 2.6). The same trend was observed for the evenness values with Salmon River having a value of 0.83 and Main River having a value of 0.66. Salmon River had a much more

evenly distributed population of EPT than either of the other Rivers while Main River had one species, *Drunella cornuta*, with a large number of individuals in comparison to the other species. The results for June showed no evidence of a south to north gradient in species richness or diversity.

Table 2-6 Shannon-Wiener Index, Relative Species Abundance per River, Number of Species, and Evenness values for four riverine-estuarine systems for June and August 2010.

River	Shannon-Wiener Index		Species Richness		Evenness		Relative Species Abundance (%)	
	June	Aug	June	Aug	June	Aug	June	Aug
Codroy	2.68	2.38	27	28	0.81	0.71	22.0	17.8
Hughes	2.28	1.02	18	10	0.79	0.44	10.9	13.7
Main	2.01	1.24	21	21	0.66	0.41	29.8	28.0
Salmon	2.81	2.01	29	24	0.83	0.63	37.2	40.5
Total							99.9	100.0

For the August, 2010 sampling period the Shannon-Wiener diversity index values are much different in comparison to the June sampling period (Table 2.6). Salmon River and the Grand Codroy River have the highest values with 2.38 and 2.01 respectively while Main River and Hughes Brook have indices that are much lower at 1.24 and 1.02. the Grand Codroy River had a higher number of species and a more evenly distributed population which accounts for the evenness value of 0.71. The high number of *Glossosoma* sp. in comparison to the other species collected at Main River is the reason

for the low diversity and evenness values found there. Hughes Brook also had a low evenness value and that is a result of the low number of species and the high number of individuals of the species *Tricorythodes allectus* collected there. Again, these results showed no evidence of a south to north gradient in species richness or diversity.

Relative species abundance for both June and August showed a similar pattern. The values decreased from the Grand Codroy River to Hughes Brook but then increased along the south to north gradient from Main Brook to Salmon River (Table 2.6).

The total number of individuals at each river were plotted against distance from the Grand Codroy River for each sampling date. The slope of the regression line was examined to determine whether there was a change in the number of individuals of each species as distance from the Grand Codroy River increased. The regression of total number of individuals at each river against distance from the Grand Codroy River was not significant. There was no change in abundance with distance in either June (t_{127} = 0.0003, p = 0.50) or August (t_{107} = 0.0033, p = 0.50)

2.3.2.2 ***Diversity Within Riverine-Estuarine Systems***

During the June, 2010 sampling period, as conductivity increased along the riverine-estuarine systems from site 1 to site 5, the Shannon-Wiener diversity index decreased (Table 2.7). This indicates that there were a greater number of insect species collected and individuals were distributed more equitably in the freshwater sites as compared to the more saline sites. The only exception to this trend was site 3 at Salmon River where the

diversity index increased before decreasing again at site 5. The reason for the increase in diversity at this site was that there was a slight increase in number of species from eleven at site 2 to twelve at site 3.

During the August, 2010 sampling period, the same diversity patterns were observed; as conductivity increased, the diversity index decreased (Table 2.8). Again, Salmon River was the exception to this trend. Diversity increased between site 1 and 2 and then decreased up to site 5. While there were a higher number of species collected at site 1 than at site 2, there were three species collected at site 1, *Ephemerella subvaria*, *Glossosoma* sp., and *Cheumatopsyche pettiti*, with much larger numbers of individuals than the other species.

Table 2-7 Relative Species Abundance of EPT per Site, Shannon-Wiener Index, Species Richness and Evenness values for sampling sites within four rivers for June 2010.

River	Grand Codroy River	Hughes Brook	Main River	Salmon River
Site	**Relative Abundance (%)**			
1	66.9	45.7	83.7	60.9
2	21.5	44.4	16.3	21.4
3	11.0	2.5	0.0	15.6
4	0.6	7.5	0.0	0.7
5	0.0	0.0	0.0	1.4
	Shannon-Wiener Index			
1	2.53	2.14	1.92	2.23
2	1.83	1.45	1.27	1.90
3	1.69	0.00	0.00	2.06
4	0.00	0.69	0.00	0.00
5	0.00	0.00	0.00	0.69
	Species Richness			
1	21	12	19	17
2	8	9	7	11
3	7	1	0	12
4	1	2	0	1
5	0	0	0	2
	Evenness			
1	0.83	0.86	0.65	0.79
2	0.88	0.66	0.65	0.79
3	0.87	0.00	N/A	0.83
4	0.00	1.00	N/A	N/A
5	N/A	N/A	N/A	1.00

Table 2-8 Relative Species Abundance of EPT per Site, Shannon-Wiener Index, Species Richness and Evenness values for sites within four rivers for August 2010.

River	Grand Codroy River	Hughes Brook	Main River	Salmon River
Site	**Relative Abundance (%)**			
1	34.3	11.0	56.7	75.6
2	44.6	71.3	43.3	23.8
3	20.2	1.2	0.0	0.6
4	0.9	16.5	0.0	0.0
5	0.0	0.0	0.0	0.0
	Shannon-Wiener Index			
1	2.44	1.96	1.86	1.70
2	1.43	0.14	0.00	2.29
3	1.24	0.00	0.00	1.10
4	0.69	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00
	Species Richness			
1	18	9	21	22
2	9	3	1	14
3	7	1	0	3
4	2	1	0	0
5	0	0	0	0
	Evenness			
1	0.85	0.89	0.61	0.55
2	0.65	0.12	N/A	0.87
3	0.64	N/A	N/A	N/A
4	1.00	N/A	N/A	N/A
5	N/A	N/A	N/A	N/A

2.3.3 Univariate Analysis

2.3.3.1 *Differences Between Riverine-Estuarine Systems*

The residuals for the comparison of each riverine-estuarine system with respect to number of individuals were examined to determine whether they met the assumptions of the ANOVA. First, the assumption of normal residuals was checked by observing the histograms for both sampling dates. The frequency of the residuals was skewed to the left and did not show a normal distribution (Figure 2.7). To further validate this assumption, Q-Q probability plots were examined for each date (Figure 2.8). The residuals did not fall on the diagonal line for either date. The assumption of normal residuals was not met.

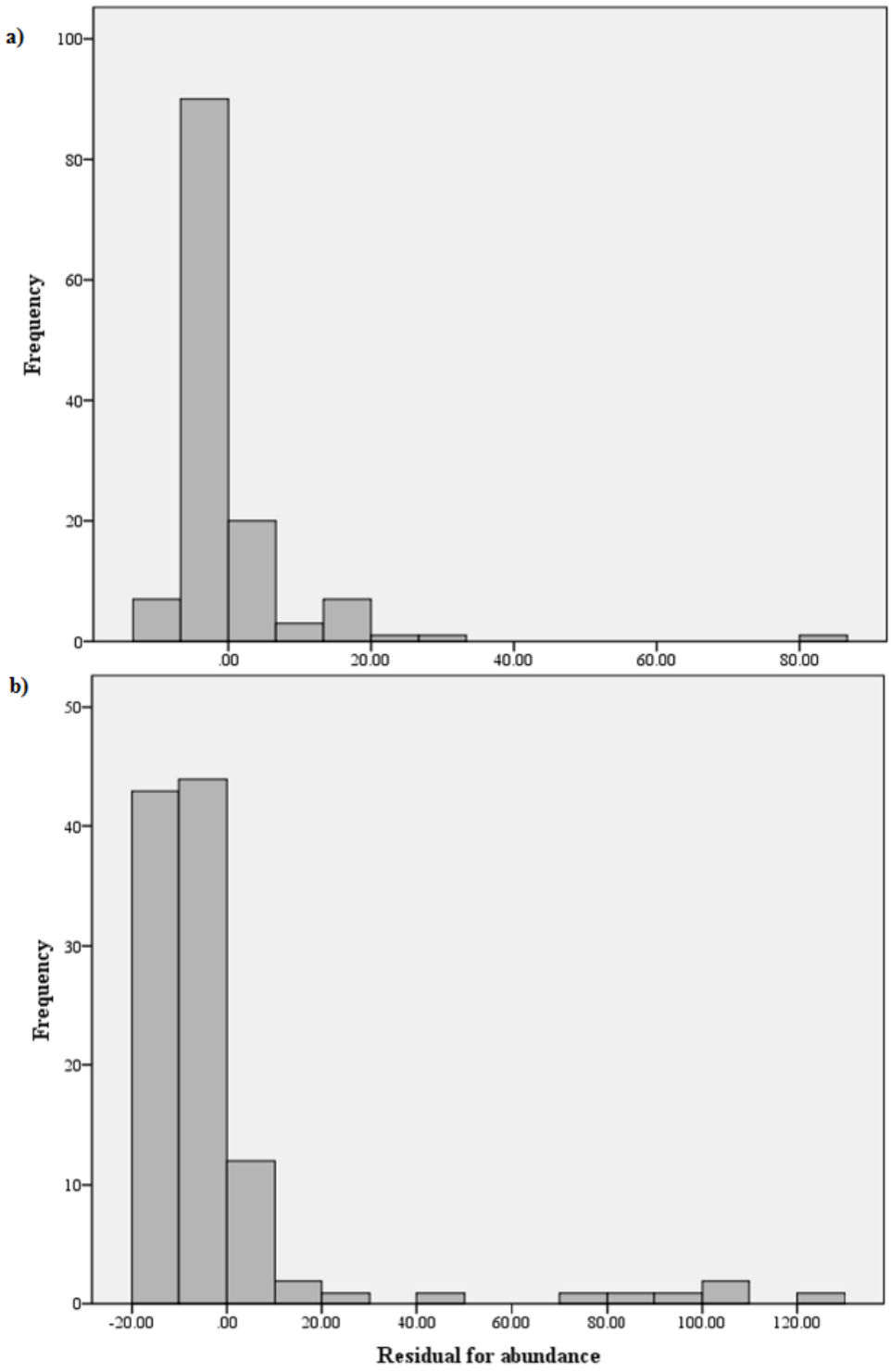


Figure 2-7 Histograms of residuals for a) June and b) August sampling dates

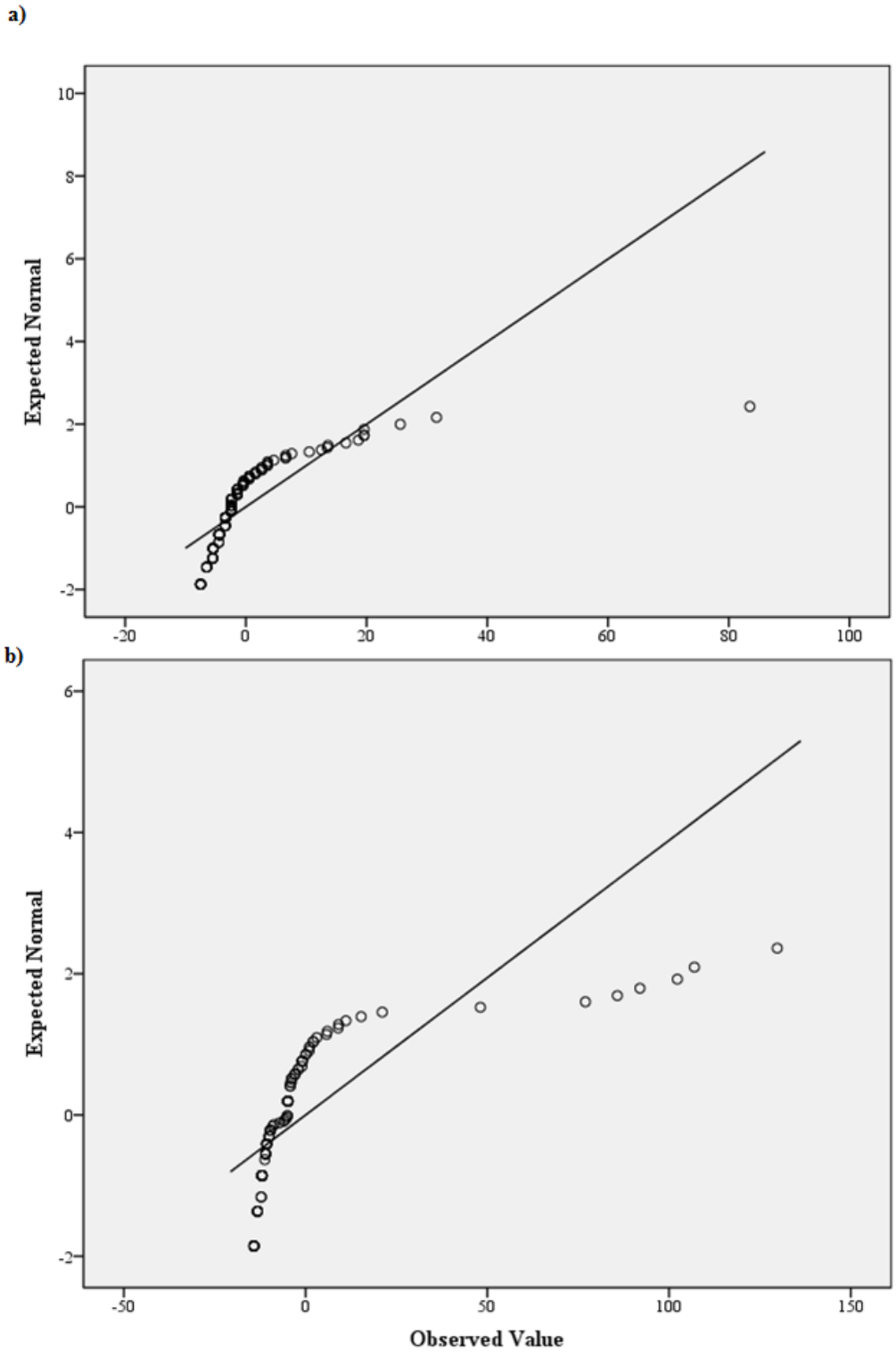


Figure 2-8 Q-Q plots of residuals for a) June and b) August sampling dates.

The assumption of homogeneity of variance for the residuals was checked by examining the plot of residuals versus predicted values (Figure 2.9).

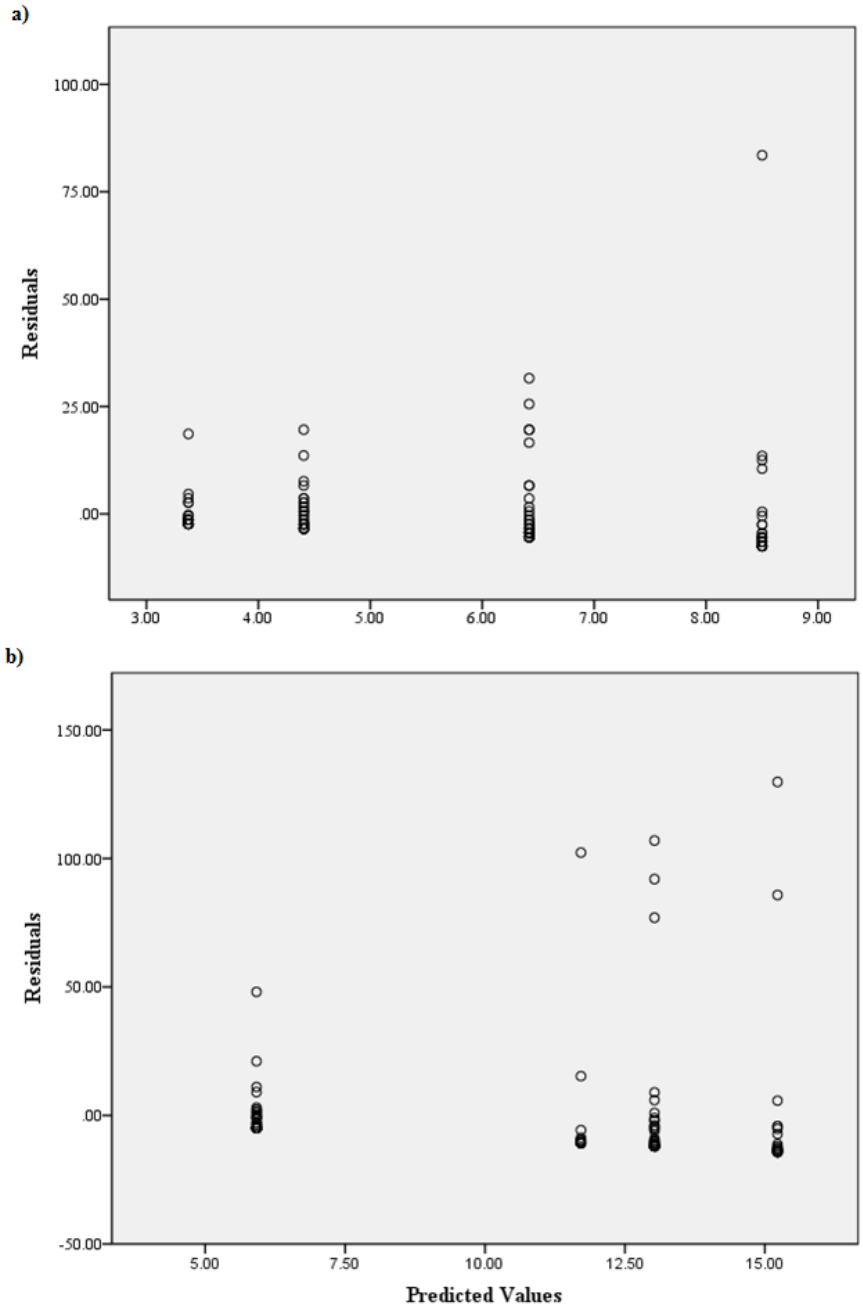


Figure 2-9 Residuals versus predicted values for a) June and b) August sampling dates

A distinctive cone shape from left to right was observed for both sampling dates so the assumption of homogeneous residuals was not met. Since the assumptions that the residuals are normal and homogeneous were not met the results of the ANOVA could not be accepted.

The data was then $\log_{10}(x+1)$ transformed but this did not improve the residuals. The assumptions of normality and homogeneity of variances for the residuals were not met.

Welch's ANOVA on the untransformed data indicated no significant difference in mean abundance among the four rivers in June, $F_{(3, 61.93)} = 1.555$, $p = 0.209$ or August, $F_{(3, 35.97)} = 1.149$, $p = 0.343$.

2.3.3.2 *Differences Within Riverine-Estuarine Systems*

Due to the high variability of the benthic invertebrate data for the sites at each river, neither parametric nor non-parametric univariate analyses could be used. Relative abundance of individuals for each site in the four rivers was calculated to determine whether any trends were apparent in the data. The June sampling data displays a downward trend in relative abundance as the conductivity increases with site (Figure 2.10). The August sampling data, however, for the Grand Codroy River (Figure 2.11a) and Hughes Brook (Figure 2.11b) do not display this trend, while the trends in Main Brook and Salmon River (Figures 2.11c and d) are similar to those found in June.

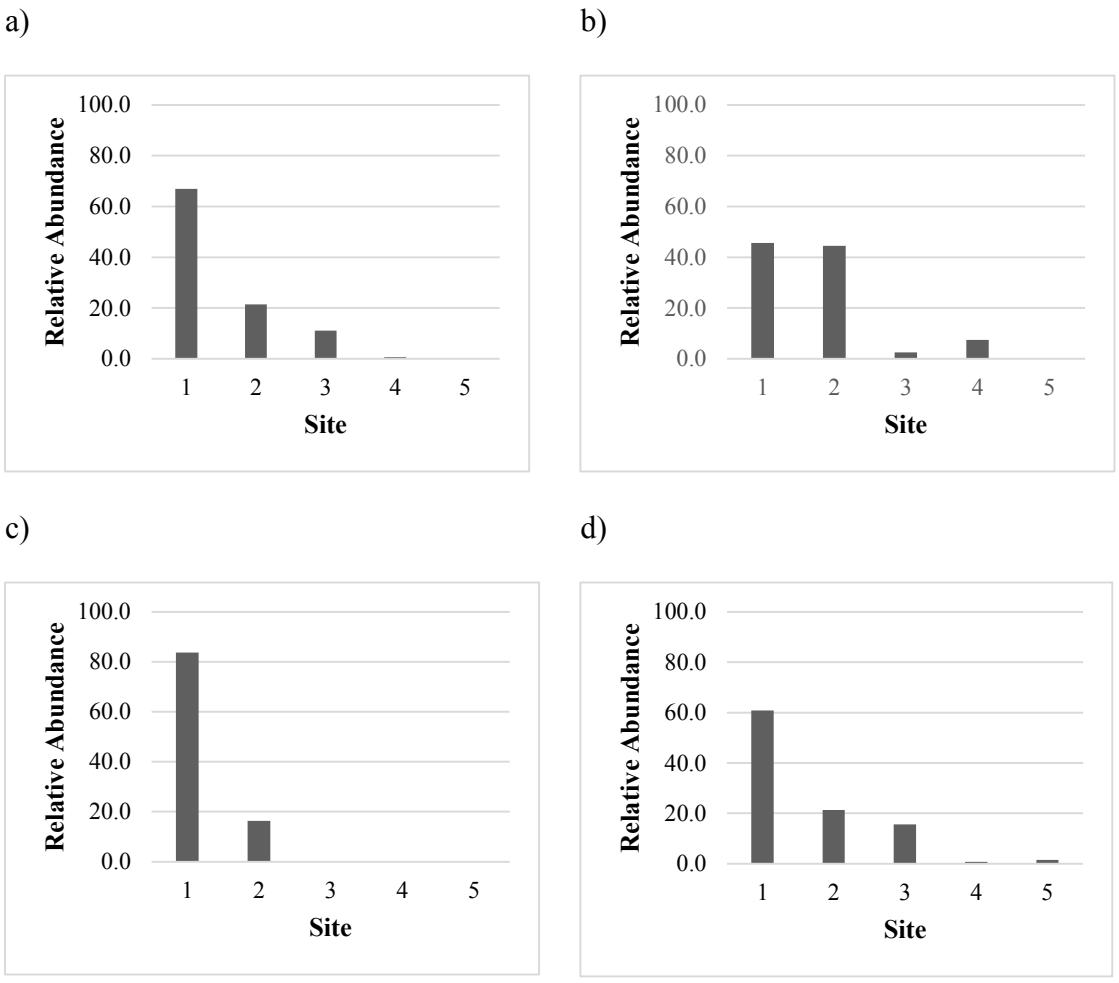
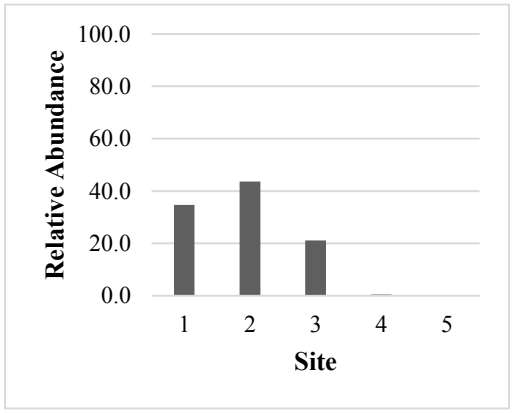
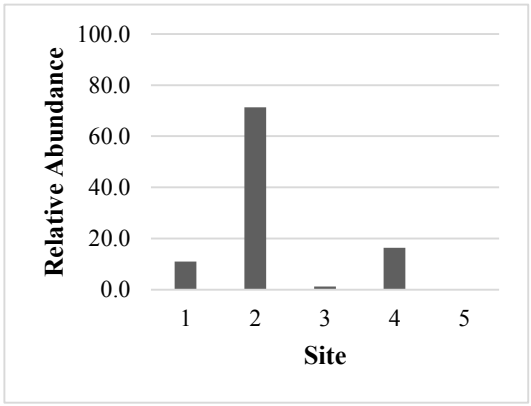


Figure 2-10 Relative species abundance of individual insects per site for the five sites in a) Grand Codroy River, b) Hughes Brook, c) Main River, and d) Salmon River in June 2010

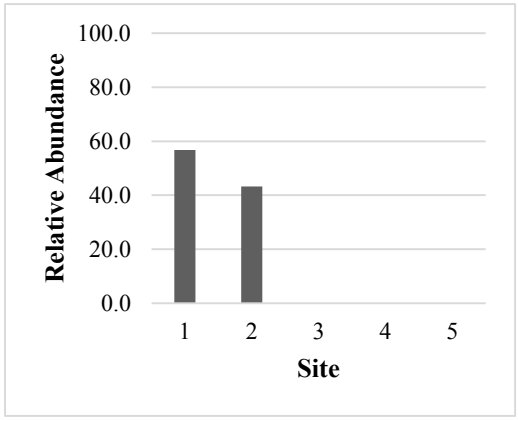
a)



b)



c)



d)

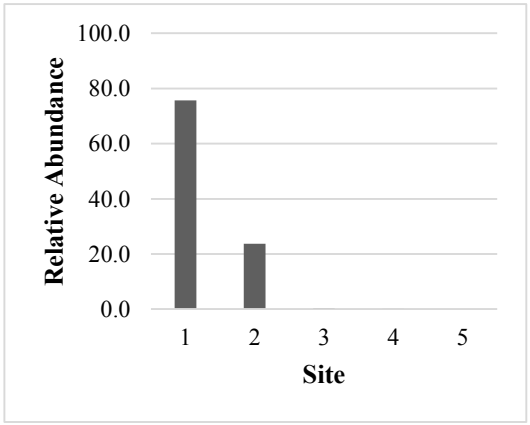


Figure 2-11 Relative species abundance of individual insects per site for the five sites in a) Grand Codroy River, b) Hughes Brook, c) Main River, and d) Salmon River in August 2010

2.3.4 Ordination – Canonical Correspondence Analysis

CCA ordination showed a strong relationship between macroinvertebrate species distribution and the environmental variables for both June and August. The Pearson species-environmental correlations, which are measures of how well the extracted variation in community composition can be explained by the environmental variables, were 0.949, and 0.949 for the first and second axis respectively, for June and 0.914, and 0.937 for August (Table 2.9). These values suggest a close relationship between the environmental variables selected. For June, 23.4% of the variation in the species data was explained by the environmental variables whereas in August the environmental variables accounted for 26.5% of this variation (Table 2.9).

Table 2-9 Correlation of environmental variables with the axes of canonical correspondence analysis (CCA) for macroinvertebrate taxa in four riverine-estuarine systems for the two sampling periods

Variable	Axis 1		Axis 2	
	June	**August**	**June**	**August**
Eigenvalue	0.546	0.676	0.412	0.439
Pearson Species-Environmental Correlation	0.949	0.914	0.949	0.937
Percent Variance	13.3	16.0	10.1	10.4
Cumulative Percent Variance	13.3	16.0	23.4	26.5
Temperature	-0.311	-0.336	0.783	0.646
Flow	-0.658	-0.386	0.588	0.198
Conductivity	0.378	0.895	-0.106	0.009
Depth	0.786	-0.221	0.305	0.901

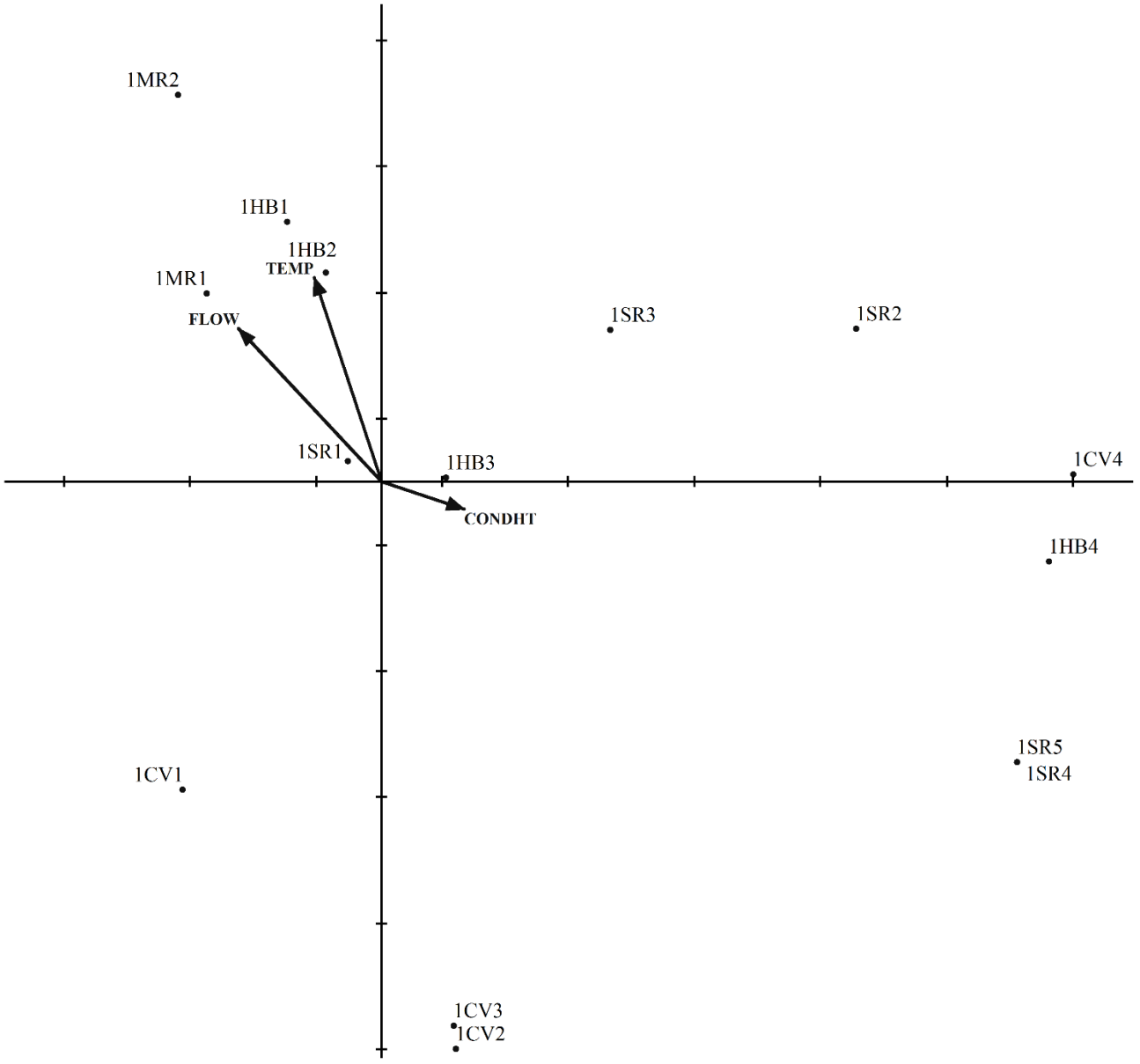


Figure 2-12 Biplot of canonical correspondence analysis with sites (•) and abiotic variables (arrows) for the four riverine-estuarine systems sampled, June 2010. Abundance values were Log10(x + 1) transformed. FLOW = flow at low tide (m/s), CONDHT = conductivity at high tide (µS/cm), TEMP = water temperature at low tide (°C). Codes for sites in Table 2.10

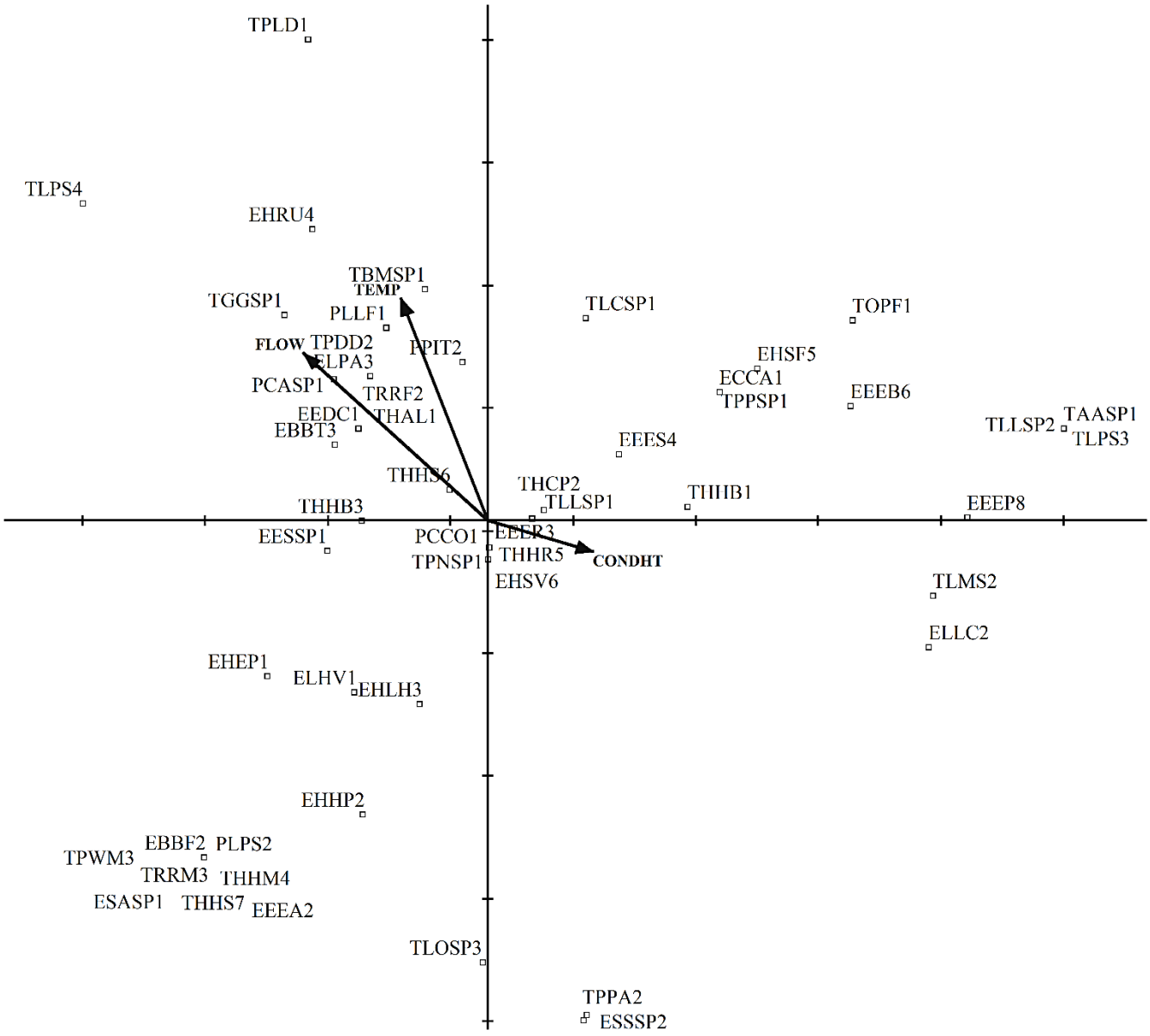


Figure 2-13 Biplot of canonical correspondence analysis with species (□) and abiotic variables (arrows) for the four riverine-estuarine systems sampled, June 2010. Abundance values were Log10(x + 1) transformed. FLOW = flow at low tide (m/s), CONDHT = conductivity at high tide (µS/cm), TEMP = water temperature at low tide (°C). Codes for species in Table 2.11

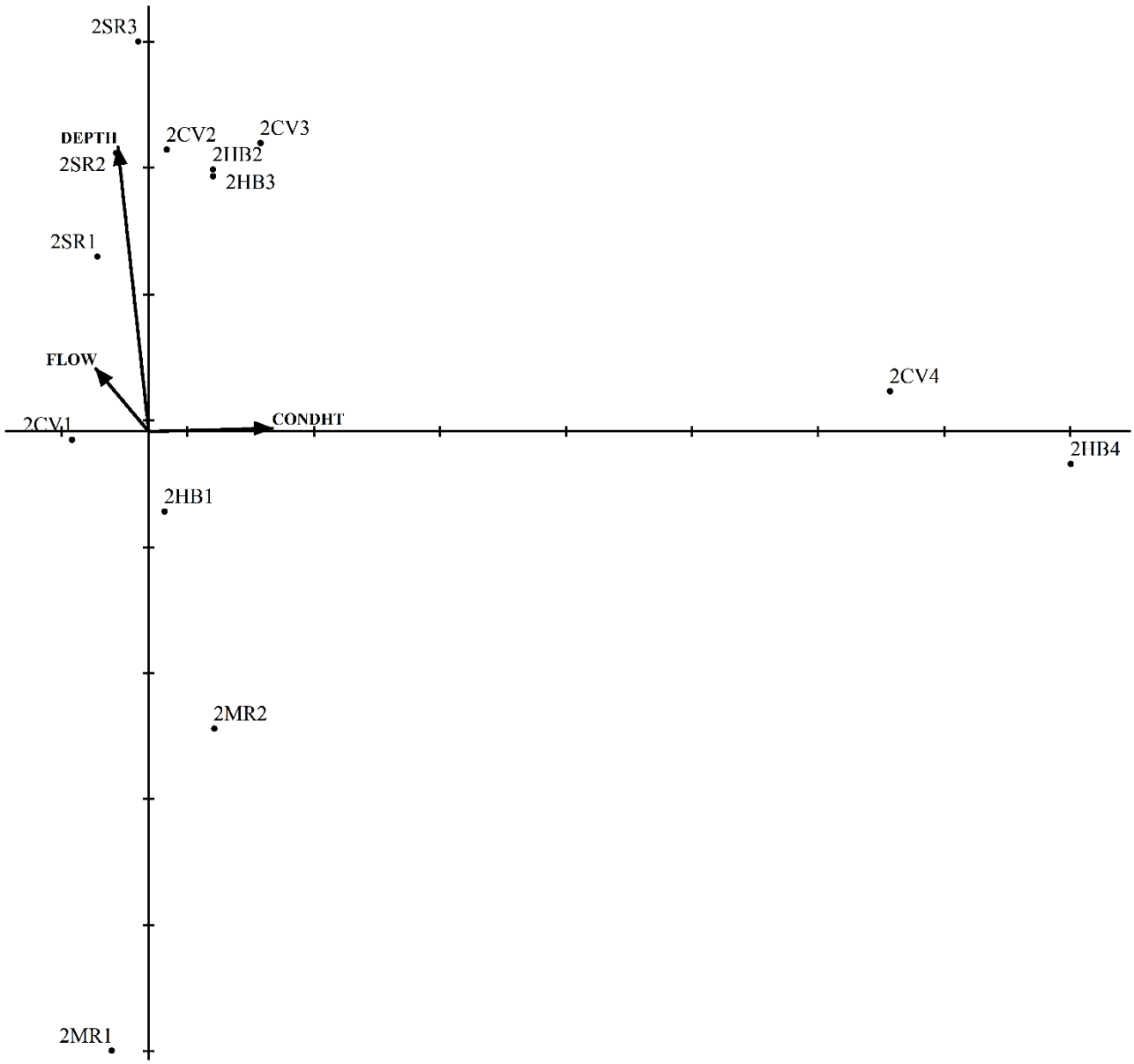


Figure 2-14 Biplot of canonical correspondence analysis with sites (•) and abiotic variables (arrows) for the four riverine-estuarine systems sampled, August 2010. Abundance values were Log10(x + 1) transformed. FLOW = flow at low tide (m/s), CONDHT = conductivity at high tide (µS/cm), DEPTH = depth at sampling site at low tide (cm). Codes for sites in Table 2.10

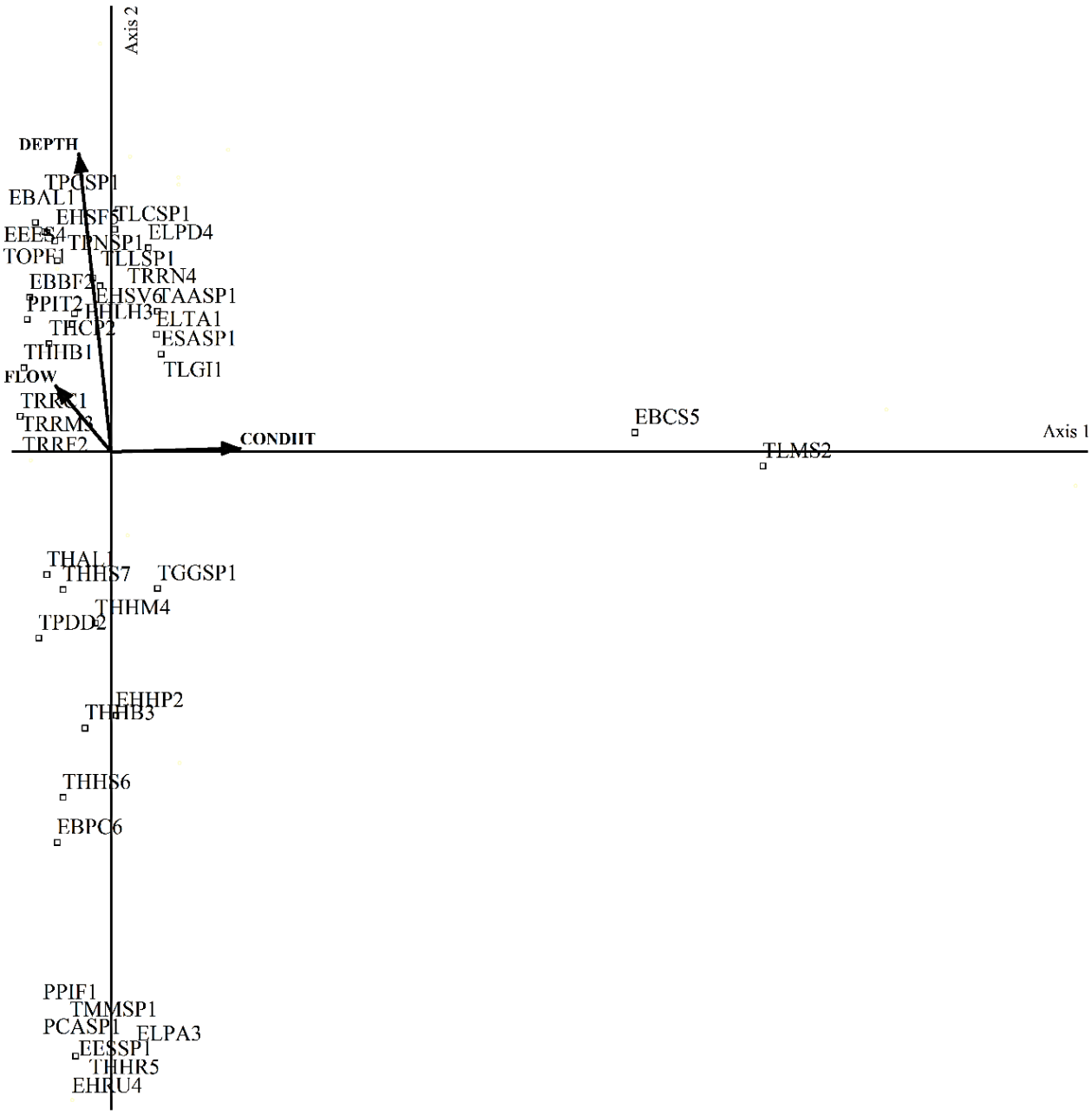


Figure 2-15 Biplot of canonical correspondence analysis with species (□) and abiotic variables (arrows) for the four riverine-estuarine systems sampled, August 2010. Abundance values were Log10(x + 1) transformed. FLOW = flow at low tide (m/s), CONDHT = conductivity at high tide (µS/cm), DEPTH = depth at sampling site at low tide (cm). Codes for species in Table 2.11

Table 2-10 Site codes used in canonical correspondence analysis biplots.

Date	River	Site	Code
June	Grand Codroy River	Site 1	1CV1
		Site 2	1CV2
		Site 3	1CV3
		Site 4	1CV4
	Hughes Brook	Site 1	1HB1
		Site 2	1HB2
		Site 3	1HB3
		Site 4	1HB4
	Main River	Site 1	1MR1
		Sitev2	1MR2
	Salmon River	Site 1	1SR1
		Site 2	1SR2
		Site 3	1SR3
		Site 4	1SR4
		Site 5	1SR5
August	Grand Codroy River	Site 1	2SR1
		Site 2	2SR2
		Site 3	2SR3
		Site 4	2SR4
	Hughes Brook	Site 1	2HB1
		Site 2	2HB2
		Site 3	2HB3
		Site 4	2HB4
	Main River	Site 1	2MR1
		Site 2	2MR2
	Salmon	Site 1	2SR1
		Site 2	2SR2
		Site 3	2SR3

Table 2-11 Species codes used in canonical correspondence analysis biplots

Order	Species	Code
Ephemeroptera	*Acentrella laponicus*	EBAL1
	Baetis flavistriga	EBBF2
	B. tricaudatus	EBBT3
	Callibaetis skokianus	EBCS5
	Ephemerella rotunda	EEER3
	Ep. subvaria	EEES4
	Ep. aurivillii	EEEA2
	Eurylophella bicolor	EEEB6
	Eu. funeralis	EEEF7
	Eu. prudentalis	EEEP8
	Serratella sp.	EESSP1
	Epeorus pleuralis	EHEP1
	Heptagenia pulla	EHHP2
	Leucrocuta hebe	EHLH3
	Rhithrogena undulata	EHRU4
	Stenonema femoratum	EHSF5
	S. vicarium	EHSV6
	Leptophlebia cupida	ELLC2
	Paraleptophlebia adoptive	ELPA3
	P. debilis	ELPD4
	Ameletus sp.	ESASP1
	Siplonurus sp.	ESSSP2
	Procleon convexum	EBPC6
	Caenis amica	ECCA1
	Drunella cornuta	EEDC1
	Tricorythodes allectus	ELTA1
	Habrophlebia vibrans	ELHV1
Plecoptera	*Isogenoides frontalis*	PPIF1
	Isoperla transmarina	PPIT2
	Paracapnia opis	PCCO1
	Alloperla sp.	PCASP1
	Leuctra ferruginea	PLLF1
	Paraleuctra sara	PLPS2
Trichoptera	*Apatania* sp.	TAASP1
	Micrasema sp.	TBMSP1
	Glossosoma sp.	TGGSP1
	Helicopsyche borealis	THHB1
	Arctopsyche ladogensis	THAL1
	Cheumatopsyche pettiti	THCP2
	Hydropsyche betteni	THHB3

	Hy. morosa	THHM4
	Hy. recurvata	THHR5
	Hy. slossonae	THHS6
	Hy. sparna	THHS7
	Lepidostoma sp.	TLLSP1
	Ceraclea sp.	TLCSP1
	Mystacides sepulchralis	TLMS2
	Oecitis sp.	TLOSP3
	Glyphopsyche irrorata	TLGI1
	Limnephilus sp.	TLLSP2
	Pseudostenophylax sparsus	TLPS3
	Psychoglypha subborealis	TLPS4
	Molanna sp.	TMMSP1
	Psilotreta frontalis	TOPF1
	Chimarra sp.	TPCSP1
	Dolophilodes distinctus	TPDD2
	Wormaldia moesta	TPWM3
	Ptilostomis sp.	TPPSP1
	Neureclipsis sp.	TPNSP1
	Polycentropus aureolus	TPPA2
	Lype diversa	TPLD1
	Rhyacophila carolina	TRRC1
	R. fuscula	TRRF2
	R. melita	TRRM3
	R. nigrita	TRRN4
	Neophylax sp.	TUU1

The biplots for the CCA ordination for June and August are presented in Figures 2.12 – 2.15. Arrows represent the environmental variables and point in the direction of maximum change in the value of associated variable. The arrow length is proportional to the maximum rate of change (ter Braak and Verdonschot, 1995). In June, axis 1, the horizontal axis, which represents the main source of variance is positively correlated with conductivity at high tide. Sites in the right quadrants closest to the axis, such as 1CV4 and 1HB4, are thus positively correlated with high conductivity and sites in the left

quadrants, such as 1SR1 and 1MR1, are positively correlated to low conductivity. Water temperature at low tide and flow at low tide are both positively correlated with axis 2, the vertical axis. Sites in the upper quadrants have higher water temperatures and higher flow than sites in the lower quadrants (Figure 2.12).

A larger number of species were located at sites with faster flowing, warmer freshwater. However, a number of species were also located in sites with lower flow and cooler water temperatures. Species located at sites with higher conductivity and lower flow rates were less abundant (Figure 2.13).

In August, the horizontal axis which again represents the main source of variance is positively correlated with conductivity at high tide and is negatively correlated with flow at low tide. Depth at low tide is also negatively correlated with axis 1. Axis 2 is positively correlated with depth at low tide. Sites found in the far right quadrant are positively correlated to high conductivity while sites found in the upper right quadrants are positively related to depth at low tide. Sites are negatively related to conductivity but positively related to flow at low tide (Figure 2.14).

Most of the EPT species were located at sites with a faster flow and a greater depth. However, a number of species were also located at sites with shallower, slower moving water. Very few insect species were located at sites with higher conductivity values (Figure 2.15).

2.4 Discussion

The aquatic macroinvertebrate diversity in freshwater streams and rivers on the island of Newfoundland has been described as impoverished relative to the mainland areas of Labrador and Nova Scotia (Larson and Colbo, 1983). The EPT fauna on the island appear to follow this pattern. On the island of Newfoundland, Larson and Colbo (1983) identified 30 species of Ephemeroptera from 8 different families. As a comparison, there are 120 species representing 13 families in the province of Alberta (Clifford, 1991). Plecoptera, which have the greatest diversity in cool streams, have 215 species which represent 9 families in Alberta (Clifford, 1991) while in Newfoundland there are only 12 known species representing 6 families (Larson and Colbo, 1983). Trichoptera are the most diverse Order in both Alberta and on the island. There are 219 species representing 15 families in Alberta but only 116 species from 14 families on the island (Clifford, 1991; Larson and Colbo, 1983). Larson and Colbo (1983) noted that there are many gaps in the information on insect diversity on the island but western Newfoundland, which is divided into northern and southern regions, is richer in fauna than the rest of the island.

In the current study limited to western Newfoundland the numbers of EPT species collected were similar to those reported by Larson and Colbo (1983). There were 30 species of Ephemeroptera, 6 species of Plecoptera and 36 species of Trichoptera collected overall. Several explanations may account for the numbers of EPT in Newfoundland.

According to island biogeography theory (MacArthur and Wilson, 1967), increasing isolation decreases immigration rates. The west coast of Newfoundland is isolated from

the mainland by the Gulf of St. Lawrence and the east coast is bounded by the Atlantic Ocean, which isolates it from Europe. This distance creates a dispersal barrier for most insect species (South, 1983). Adler et al. (2005) concluded that open waters of more than 100 kilometers would reduce colonization by flying insects originating on the mainland. It is possible that the distance between the southern part of the island and the mainland may create a dispersal barrier. However, the distance from Labrador to the northern region of the island is less than 20 kilometers and is small enough that the strait could be crossed by flying insects (Adler et al., 2005).

Another explanation for the low macroinvertebrate diversity is that the most recent glaciation on the island was less 10,000 y BP. According to South (1983) most of the flora and fauna was established post-glaciation with colonization and human mediated introductions. The fauna that are present may have a stronger ability to disperse than other insects that are common in the rest of North America (South, 1983).

2.4.1 Latitudinal Gradient

It has been noted that at a larger scale there is a much higher diversity of species in tropical as opposed to temperate regions (Pearson and Boyero, 2009). Several latitudinal gradient studies involving freshwater taxa, however, have provided varied results. Some researchers (Benson and Pearson, 1987; Jacobsen et al., 1997) have reported that there is a higher diversity of species in tropical regions while others (Stanford and Ward, 1983; Zwick, 1986; Flowers, 1991) have reported lower macroinvertebrate richness in tropical

regions. Hillebrand (2004) reported that trends across latitudinal gradients are less robust in freshwater environments than in marine or terrestrial environments.

In the current study, due to the climatic differences associated with the warmer temperatures in the south and the cooler temperatures in the north, it was expected that diversity would be higher in the Grand Codroy River than in Salmon River. However, this was not the case. In June and August, the Shannon-Wiener diversity index was highest in both Codroy and Salmon Rivers with Main River and Hughes Brook maintaining a lower diversity index. The results of Welch's ANOVA and the regression analysis also confirmed that there was no statistically significant difference between the mean abundances at each river along the gradient.

There are several explanations for why there does not appear to be a latitudinal gradient. The most apparent explanation is related to scale; the distance is not large enough for a differentiation in diversity to be observed. There is only 4 degrees of latitude (47.850520 – 51.176345) separating the most northerly and southerly sites in the transect. Researchers with a much larger mean range in latitude of 37 degrees have observed no change in diversity of freshwater insects (Hillebrand, 2004). However, Bowden and Buddle (2010) did report an increase in abundance of spider assemblages across a south to north gradient of a mere 3 degrees of latitude.

Another factor which could have influenced the results of this study is that EPT were the only invertebrates studied. Including a larger number of benthic invertebrate Orders, such as Odonata and Coleoptera, may have given a better representation of the richness and

diversity along the gradient. The patterns detected in latitudinal studies often depend on which groups are examined (Scott et al., 2011). Vinson and Hawkins (2003) found that species richness of Plecoptera increased from south to north while species richness of Trichoptera decreased with latitude. When the EPT Orders were investigated individually in the current study this trend was not observed. Again, the small spatial scale may be the reason for this.

2.4.2 Salinity Gradient

Insects are one of the largest classes with over 30000 species in the freshwater environment alone (Williams and Williams, 1998). The number of insect species that can be considered marine however is much smaller at only several hundred (Williams and Feltmate, 1992). The majority of these marine insects are associated with the transitional estuarine habitat (Axtell, 1976). According to Williams and Williams (1998) there is a salinity gradient along estuaries that results in a longitudinal zonation of biota. Marine derived taxa are found in the area nearest the ocean while freshwater taxa inhabit the area where rivers enter the estuary (Cunha and Moreira, 1995).

Freshwater invertebrate species are typically isolated to the upper portion of the estuary where conductivity values are relatively low. Freshwater invertebrates, especially insects, are highly sensitive to elevated salt levels (Williams and Feltmate, 1992; Cañedo-Arguelles et al., 2012). It has been shown that when conductivity values are below 1500 μScm^{-1} there is little negative impact on the freshwater insects (Hart et al., 1991; Marshall and Bailey, 2004). Aquatic insects have to maintain a proper salt and water

balance through osmoregulation. Their body fluids usually contain a much higher salt concentration than the surrounding water so the water moves into the haemolymph which has a higher osmotic pressure (Wallace and Anderson, 1996). If the surrounding water is higher in salt concentration than the insect's body fluid, osmoregulation will be reversed and this will adversely affect the insect. The ability of a freshwater insect to tolerate saltwater is based on its ability to change its osmotic capabilities (Williams and Williams, 1998).

As expected, diversity of the EPT Orders decreased with increasing conductivity values within each of the riverine-estuarine systems during both June and August sampling periods. This result agrees with current literature that suggests tidal areas support low species diversity of aquatic insects (Metzeling, 1993; Marchant, 1999) and their diversity decreases with increased salinity (Ysebaert et al., 1993).

Relative abundance of individuals was also used to examine the patterns of species abundance. As expected, there was a downward trend in relative abundance for all rivers during both sampling periods.

During the June sampling period five different species were collected from sites with higher conductivity values at the Grand Codroy River, Hughes Brook and Salmon River. Four of the five species were from the Ephemeroptera Order. This is in contrast to other studies that reported, of all aquatic insects, Ephemeroptera is the freshwater taxon most adversely affected by high salt levels (Williams and Williams, 1998; Piscart et al., 2005b). A possible explanation for the presence of these insects in the more saline sites is

that during the June sampling period there was a higher run-off associated with the spring thaw. These insects may have drifted into these areas as a result of the higher than normal flow. The numbers of each species were low with only 1 or 2 found at each site which would indicate that drift may be the probable explanation. The other species, *Mystacides sepulchralis*, is from the Trichoptera Order and was found only in Hughes Brook at site 4 but it was collected in both June and August in higher numbers. Piscart et al. (2005a) found that Trichoptera were among the most salt tolerant Orders of aquatic insects so it is possible that this species is able to temporarily survive higher than average salt concentrations.

2.4.3 Ordination – Canonical Correspondence Analysis

Salinity is most likely the reason for the patterns of distribution observed within the riverine-estuarine sites in this study. However, there are other variables that could affect the distribution of EPT. For this reason, canonical correspondence analysis was used to visualize the relationships between these other variables and the aquatic insects collected.

CCA analysis supports the assumption that species abundance is affected by high salinity values. The conductivity variable was correlated to axis 1, which shows the highest species variance, in the analysis. In Figures 2.13 and 2.15 there are only 2 species located in the quadrant that correspond to high conductivity. The species *M. sepulchralis*, which was found exclusively at the sites with higher conductivity values in Hughes Brook, appears in both figures and is correlated with high conductivity.

The CCA biplots also show a negative correlation between both water temperature and flow rate and conductivity. While higher temperatures are usually linked to higher conductivity levels (Miller et al., 1988), this does not appear to be the case in this study. It is possible that the water temperatures measured during sampling are not necessarily indicative of the long term averages for each riverine-estuarine system and this would affect the correlation.

The negative correlation between flow rates and conductivity was expected since the inflow in all the estuaries was a freshwater source. In the June sampling periods this inflow was increased and so there was a larger negative correlation in the biplot for June. Most of the insects collected were located in the quadrants that correlated with higher water temperatures and higher flow rates.

2.5 Conclusions and Recommendations

The EPT fauna on the island portion of the province of Newfoundland and Labrador is impoverished in comparison to the mainland and the rest of North America (Larson and Colbo, 1983). The low number of species collected in this study further validate this finding.

Based on the finding in this study, there does not appear to be a latitudinal gradient along the west coast of Newfoundland that reflects trends in species diversity or abundance. Had sites on the mainland portion of the province, Labrador, been included in the study to increase geological/ecological scale there may have been evidence of a latitudinal

gradient. Future studies could include this part of the province as well as more sites within the study area along the west coast of the island.

In contrast, it is clear that the aquatic insect community shows a decrease in both diversity and abundance along the salinity gradients within the four estuarine systems sampled. The low Shannon-Weiner diversity indices for the sites with high conductivity values and the CCA biplots are evidence of this finding.

An effort could be made in future work to sample sites with similar conductivity values to compare species diversity and abundances. For example, site 4 in the Grand Codroy River had a different conductivity reading than site 4 in Hughes Brook. It was therefore difficult to compare the number of species collected at each of the two sites. Had the conductivity values been similar the comparisons between the sites would have been more effective.

Future work could also include more abiotic data at each site. Tests such as pH, alkalinity, dissolved oxygen and other chemical analyses could have been performed on the water samples to improve the results of the ordination. Such data when combined with trophic information discussed in chapter three would provide a better means to elucidate the ecology of EPT in riverine-estuary systems within boreal regions in Newfoundland and elsewhere.

3 Stable Isotope Analysis of Trophic Levels Within an Aquatic Food Web

3.1 Introduction

Food webs are often used to depict energy flow through the trophic levels of primary producers, primary consumers, secondary consumers, and tertiary consumers in an ecosystem (Schmidt et al., 2007). In riverine systems, aquatic macroinvertebrates provide the link between primary producers and higher organisms such as fish, amphibians and other invertebrates. Unlike terrestrial communities, aquatic invertebrates cannot be classified by the food they eat. Freshwater aquatic invertebrates have been found to be mostly omnivorous in that they cannot discriminate the food they ingest (Merritt and Cummins, 2007). For instance, if an insect in the riverine-estuarine transition zone consumes the periphyton from the surface of a rock, it not only ingests algae but whatever bacteria are associated with the periphyton, diatoms and smaller macroinvertebrates (Cummins, 1973). For this reason, studying the trophic levels of aquatic macroinvertebrates does not provide consistent information about the relationships between the insect and its food resources (Merritt and Cummins, 1996). As such, aquatic macroinvertebrates are categorized into functional feeding groups (FFGs) based not on the food they ingest but on the mode of feeding (Cummins, 1973; Merritt and Cummins, 1996).

There are four main functional groups of freshwater aquatic invertebrates – shredders, collectors, scrapers, and predators. Shredders have mandibles for cutting and grinding

food (Voshell, 2002). The dominant food for shredders is living vascular plant tissue and course particulate organic matter (CPOM) which is comprised of decomposing plant matter with particles greater than 1 millimeter (mm) in size (Merritt and Cummins, 1996). Shredder feeding enhances the release of fine particulate organic matter (FPOM) of less than 1 mm and dissolved organic matter (DOM) which consists of soluble organic materials less than 0.5 μm in size (Meyer and O'Hop, 1983). Collectors can be further divided into collector-gatherers and collector-filterers based on the mechanism they use to acquire food. Collector-gatherers collect FPOM or fine detritus that has settled out of suspension and is lying on the bottom of rivers (Merritt and Cummins, 1996; Voshell, 2002). They have no specialized mechanism for collecting food; they either eat the FPOM from the bottom or they burrow into the soft substrate and swallow the sediment and detritus as they move (Voshell, 2002). Collector-filterers, conversely, use special mechanisms to filter FPOM or detritus that is suspended in the water. Some invertebrates use the fine hairs on their forelegs or labral fans to strain the particles from the water while others spin nets from silk to collect detritus and FPOM as it floats by (Voshell, 2002). Collector-filterers with labral fans, such as Simuliidae, utilize the DOM in the rivers as well (Cibrowski et al., 1997). Scrapers have mandibles with sharp, angular edges that can be used to shear periphyton from the river substrate (Merritt and Cummins, 1996; Merritt and Cummins, 2007; Voshell, 2002). Finally, predators consume living animal tissue such as other macroinvertebrates (Merritt and Cummins, 2007). They either ingest whole organisms or tear them into smaller pieces that are easier to consume.

Some predators have mouthparts designed to pierce the prey and then consume the body fluids.

Benthic food webs are supported by two sources of energy – autochthonous and allochthonous production. Autochthonous materials are produced in-stream in the form of biofilm, macroalgae and macrophytes. Allochthonous production consists of terrestrial input from either plant detritus such as leaves, needles, fruit, and flowers or DOM transported to the riverine-estuarine systems from soils via surface run-off (Hayden et al. 2016; Power and Dietrich, 2002).

Productivity within the riverine-estuarine interface is influenced by the amount of allochthonous materials transported there from the river. In boreal ecosystems this amount is dependent upon hydrological, biogeochemical, and ecological processes (Benoy et al., 2007). These processes interact to transport dissolved and particulate organic matter from the terrestrial environment into aquatic systems. Climate change is expected to have a pronounced effect on the boreal ecosystem in part through increased summer temperatures and precipitation amounts (Amiro et al., 2001). This will impact the hydrological and biogeochemical processes influencing the transport of allochthonous materials (Benoy et al., 2007). It is, therefore, important to interpret the ecology and food web dynamics within these aquatic zones in an effort to better assess these climatic changes.

According to the River Continuum Concept (RCC) proposed by Vannote et al. (1980) the distribution of allochthonous and autochthonous matter is modified from upstream to

downstream and as a result the distribution of functional feeding groups changes as well in order to make the most efficient use of the resources available. Vannote et al. (1980) suggested that at the headwaters of rivers the riparian vegetation overhangs the stream and resultant shading reduces primary production. The river in this area is thus nourished by leaves and other riparian detritus. As such, the dominant FFG at the headwaters would be the shredders who utilize the leaf litter and break it down to FPOM which makes its way downstream to the estuary to be utilized by the collectors. As the river increases in size as it approaches the estuary there is less shading and more photosynthesis can occur, resulting in an increase in the amount of periphyton and biofilm in the river. Shredders would still be found in this area; however, grazers would become dominant as well to feed on the algae and biofilm. Turbidity increases in the lower reaches of the river as it enters the estuary and, as a result, the amount of light available for photosynthesis decreases. The fine particulate organic matter increases and collectors become more abundant.

The Riverine Ecosystem Synthesis (RES) concept (Thorp et al., 2006) provides a contrasting view to the RCC. It proposes that instead of a continuous gradient from headwaters into the riverine systems, there exists hydrogeomorphic patches that are formed by hydrologic patterns, geomorphic structure of the channel bed, climate and riparian conditions (Flotemersch et al., 2010). In terms of food webs, the RES proposes that autochthonous matter produced by autotrophs provides the trophic basis for the majority of the productivity in a river network as a whole. However, in areas such as shallow, heavily canopied headwaters where photosynthesis is limited due to low light,

allochthonous organic matter may be more important to some species. The inorganic materials in the system are provided by a decomposer food pathway that processes most of the allochthonous and autochthonous carbon (Thorp et al., 2006).

Raymond et al. (2016) recently extended this idea and proposed the Pulse-Shunt Concept (PSC) that states allochthonous matter is pulsed from the landscape via surface and subsurface run-off during the high discharge that is normally associated with heavy rainfall and snowmelt. Instead of being processed by organisms in the headwaters of the river, this allochthonous DOM is shunted downstream due to the increase in velocity of the stream. This large export of unprocessed DOM from headwaters downstream into the larger river disrupts the energy flow in the food web.

Investigating functional feeding groups and differences between trophic levels within a boreal system offers a means to understand the changes in source materials at a regional level. Moreover, it allows researchers to assess any future shifts in sources of organic materials in light of the uncertainty associated with climate change. Longitudinal shifts in trophic structure may be altered as salt water intrusion increases, water temperatures changes, and water levels increase or decrease.

3.1.1 Stable Isotopes

One way to determine the food sources being utilized by functional feeding groups is through gut content analysis. However, the presence of a type of food in the digestive tract of an invertebrate does not necessarily mean it is being utilized as an energy source

(Cummins, 1973). Also, rates of digestion of food items may hinder the interpretation of what foods are most important to the diet. Differences in rates of digestion will result in non-representative proportions in the diet (Cummins, 1973). An alternative method that can be utilized to study the complex interactions among the different functional feeding groups in food webs is stable isotope analysis. Once a food is assimilated by the invertebrate the isotopic signature of that food is retained by the invertebrate.

Isotopes are forms of the same element that differ in the number of neutrons (Fry, 2006). Stable isotopes do not decay into other elements in the same way that unstable, radioactive isotopes do. Since the sum of the proton and neutron masses is equivalent to the atomic mass of the element, isotopes with a higher number of neutrons will have slightly higher atomic masses. These heavier isotopes tend to be less abundant in nature than the lighter isotopes (Fry, 2006).

Isotope ratio measurements in environmental samples are made relative to an international standard and results are reported as delta (δ) values expressed as parts per thousand (‰) (McKinney et al., 1950). The equation used to calculate these values is given as:

$$\delta = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R_{sample} is the ratio of heavy isotope to light isotope in the sample and $R_{standard}$ is the ratio of heavy isotope to light isotope in the standard reference material. Each element has a primary international standard with a value of 0‰. Consequently, any change in δ

towards a negative or positive value implies enrichment or depletion in the heavy isotope with respect to the standard (Fry, 2006).

Chemical reactions are controlled by the number of electrons in the outer shell of an atom, and not the proton: neutron distribution in the nucleus. Therefore, the chemical behavior of two isotopes of the same element will be functionally similar in that they will undergo the same reactions. However, the mass difference between the two isotopes will cause their physical behavior to be different (Peterson and Fry, 1987; Sulzman, 2007). This can be attributed differences in kinetic and vibrational energies.

The kinetic energy (KE) for an atom is constant if the environmental surroundings are fixed. Based on the formula for kinetic energy, $KE = \frac{1}{2} mv^2$ where m is mass and v is velocity of the atom, if the kinetic energy is fixed, an atom with a higher mass must have a slower velocity (Peterson and Fry, 1987; Sulzman, 2007). This means that in a chemical reaction the heavier isotope will move at a slower rate than the lighter isotope and will result in the lighter isotope accumulating in the product (Hershey et al., 2007; Sulzman, 2007).

The vibrational energy of a molecule determines the strength of the bonds between atoms within the molecule. Atoms that vibrate slowly have lower vibrational energy and therefore form stronger, more stable bonds. The heavier the atom, the more slowly it will vibrate so isotopes that are slightly heavier will form stronger bonds (Peterson and Fry, 1987; Sulzman, 2007).

The net effects of these differences in kinetic and vibrational energies lead to fractionation, which can be defined as the separation of isotopes of an element during naturally occurring processes due of the mass differences between their nuclei (Fry, 2006; Hershey et al., 2007; Peterson and Fry, 1987). It is because of fractionation that stable isotopes can be used to trace the transfer of energy between trophic levels. The stable isotopes of carbon and nitrogen have customarily been used to determine both the pathways of organic matter transfer and trophic structure in ecosystems. Studies focusing on the natural abundances of isotopes of these elements in estuarine and riverine systems have shown that it is possible to trace the source of terrestrial and aquatic matter to these systems because organisms retain the isotopic signatures of the food they assimilate (Bianchi, 2007; Deines et al., 2009). However, carbon and nitrogen isotope analyses can sometimes produce ambiguous results. In some instances, stable isotope values of carbon from terrestrial sources overlap the carbon isotope values from aquatic sources making it difficult to determine the actual energy source being used by consumers (Jardine et al., 2009). Stable hydrogen isotopes may prove useful as a complement to these other isotopes (Doucett et al, 2007).

3.1.1.1 *Carbon*

Two isotopes of carbon, ^{12}C and ^{13}C, occur naturally in a proportion of approximately 98.89:1.11, respectively (Penzias, 1979). The primary international standard for carbon is Pee Dee Belemnite (PDB). However, over time this standard has become depleted and other secondary standards such as carbonatite, marble, and limestone have come into use

(Hoefs, 2009). The delta values for carbon remain the same but the secondary standards have non-zero delta values. Therefore, it is important to state the primary international standard being used when reporting $\delta^{13}C$ values.

Measurement of ^{13}C cannot be used to differentiate trophic levels since there is only an average of 0.3-0.5 per mil (‰) enrichment in animal tissues relative to their food (DeNiro and Epstein, 1978). However, it can be used to follow the transfer of carbon from plants and detrital sources to primary and secondary consumers (Hershey et al., 2007). In aquatic ecosystems, the isotopic ratio of carbon ($\delta^{13}C$) can be used to distinguish between allochthonous versus autochthonous inputs (Rau, 1980).

Terrestrial plants are depleted in ^{13}C in comparison to the atmospheric carbon dioxide that they use for photosynthesis (Marshall et al., 2007). The degree of fractionation between terrestrial plants and atmospheric CO_2 depends on which photosynthetic pathway is utilized. Plants that undergo the Calvin cycle, or C3 plants, have an average $\delta^{13}C$ value of approximately -27‰ while plants that undergo the Hatch-Slack cycle, or C4 plants, have an average $\delta^{13}C$ value of approximately -14‰ (Marshall et al., 2007). Riparian trees are among the C3 plants and are the main source of allochthonous inputs, whereas C4 plants are mainly grasses. Once carbon is fixed by plants there are very few changes during decomposition (Finlay and Kendall, 2007). The terrestrial detritus that comes from these plants becomes a source of energy at the base of aquatic food webs, and will have the roughly same delta values as the parent plant.

Algae and aquatic macrophytes are the main sources of autochthonous organic carbon in aquatic food webs and have a wide range of $\delta^{13}C$ values because of the large range in the concentration of inorganic carbon utilized by the macrophytes and its isotopic composition (Finlay and Kendall, 2007).

As a result of the variations between terrestrial and aquatic primary producers, the source of carbon that feeds the base of the food chain can be determined. However, occasionally there are overlaps in the values of autochthonous and allochthonous carbon. Because of this, other isotopes such as N and H are used in conjunction with carbon.

3.1.1.2 *Nitrogen*

Nitrogen also consists of two naturally occurring stable isotopes, ^{14}N and ^{15}N, which occur in a proportion of approximately 99.64:0.36 (Penzias, 1979). The largest pool of nitrogen on or near Earth's surface is atmospheric nitrogen, N_2 (Hoefs, 2009). This is the least reactive form of nitrogen and it is for this reason that it is used as the international standard for $\delta^{15}N$. Atmospheric nitrogen can be converted to more useful forms of nitrogen such as ammonia by fixation (Hoefs, 2009). Nitrogen-fixing bacteria use nitrogenase enzyme to cause the reaction of atmospheric nitrogen with hydrogen, breaking the N_2 triple bond, to produce ammonia, the most useful form of nitrogen for plants (Hoefs, 2009).

Nitrification, the process by which bacteria convert ammonia to nitrates, and denitrification, the reduction of nitrates back into atmospheric nitrogen, are two processes

that contribute to nitrogen fractionation (Hershey et al., 2007). The nitrate formed from the oxidation of ammonia may reduce $\delta^{15}N$ up to 35‰ relative to ammonia, whereas denitrification of that nitrate back to N_2 typically causes $\delta^{15}N$ of the nitrate remaining to increase by up to -20‰ (Newton, 2010).

Nitrogen sources in aquatic systems may be natural or anthropogenic. Nitrogen is a by-product of many natural biogeochemical processes that occur in rivers and streams including decomposition of plants and organisms. Anthropogenic sources include agricultural and industrial inputs. These inputs are utilized as energy sources for both the invertebrates and larger organisms within the system.

As a result of fractionation, nitrogen stable isotopes can be used to estimate trophic position in aquatic food webs. The tissues of consumers are enriched in ^{15}N by an average of 3.4‰ relative to their food source (Finlay and Kendall, 2007; Hershey et al., 2007). This results from kinetic isotope fractionation of nitrogen during metabolism within the organism (McClelland et al., 1997). For example, an increase in the heavy nitrogen isotope can be explained by the tendency for organisms to excrete more of the light nitrogen isotope in their waste.

3.1.1.3 ***Hydrogen***

As mentioned previously, on occasion there are overlaps in the carbon isotope ratios in aquatic food webs which make it difficult to determine the source of the carbon. To overcome this problem, hydrogen isotopes can be used in tandem with carbon to trace

subsidies to aquatic food webs (Estep and Dabrowski, 1980; Doucett et al., 2007; Finlay et al., 2010). Hydrogen has two naturally occurring isotopes, hydrogen or protium, ^{1}H and deuterium, ^{2}H. The average abundances of the stable hydrogen isotopes are 99.984:0.016 (Penzias, 1979) and the international standard for hydrogen is Vienna Standard Mean Ocean Water (V-SMOW). Since the nucleus of the hydrogen atom ^{1}H contains a single proton and no neutrons, the mass of the deuterium atom ^{2}H, containing one proton and one neutron, is twice that of hydrogen. Because of this hydrogen has the largest variation in stable isotope ratios compared to any of the other elements (Fry, 2006; Hoefs, 2009; Solomon et al, 2009).

The largest source of hydrogen isotopes is water. As part of the hydrologic cycle the hydrogen atoms in water molecules are moved from one reservoir to another via evaporation and precipitation. These transfers result in fractionation. The vapor pressure of $^{2}H_2O$ is lower than that of $^{1}H_2O$ because the vibrational energy of the heavier isotopes is lower resulting in stronger bonds (Sulzman, 2007). Evaporation will lead to vapor with a larger number of light isotopes and deuterium rich water left behind. Water vapor that has evaporated from the ocean has $\delta^{2}H$ values between -10 to -20‰ (Fry, 2006). As water vapor moves inland or upwards, precipitation occurs and the heavy isotopes preferentially fall out as rain or snow resulting in a further depletion of ^{2}H. As such, there is a gradual decrease in the heavy isotope concentration in precipitation moving from the coast inland and with increasing altitude (Seigenthaler, 1979). This difference in hydrogen isotope values between coastal water and inland water can be used to differentiate between marine and freshwater plant and animal species.

The source of hydrogen in plant tissues is water. This water is not fractionated as it is taken up by the plant, so that δ^2H for the plant is the same as the δ^2H for the source water (Newton, 2010; Solomon et al., 2009; Sulzman, 2007). The water in the xylem of the plant should have the same isotopic signature as the soil water available to the plant. In terrestrial plants when the water reaches the leaves and evapotranspiration occurs, the hydrogen isotopes are fractionated and the heavier isotope is left behind. This evapotranspiration does not occur in most submerged aquatic plants, and as a result they have a much lower δ^2H value than terrestrial plants from the same area (Solomon et al., 2009). For this reason, hydrogen stable isotopes have the potential to differentiate between allochthonous and autochthonous organic matter in the diet of aquatic consumers.

3.1.2 Study Objectives

This study provides a preliminary survey of the carbon, nitrogen, and hydrogen stable isotope values of invertebrates within a longitudinal section of a riverine-estuarine system on the northern tip of the island of Newfoundland. The objective of this study is aimed at testing how hydrogen isotopes, in combination with carbon and nitrogen, contribute to our ability to predict the trophic status of macroinvertebrates in boreal riverine-estuarine transition zones. Specifically, the survey will be used to determine if hydrogen isotopes can be employed to assess the base of the food web fueling invertebrate trophic groups, parsing out allochthonous, autochthonous, and marine sources of energy along a salinity gradient.

3.2 Methods

3.2.1 Study Location

The area of study for the stable isotope analysis was Salmon River in Main Brook on the northern peninsula of Newfoundland (Figure 2.1). This river-estuary system is one of four used to investigate the abundance and diversity of macroinvertebrates in western Newfoundland riverine-estuarine transition zones.

3.2.2 Sampling Procedure

Samples were collected in June 2010. Three sites (1, 3, and 5) were chosen from the five sites previously sampled for Chapter 2 based on measurements of conductivity at high tide. Site 5 was within the estuary where the conductivity was highest and site 1 was located up river where the conductivity was lowest (Figure 2.5). Conductivity readings were taken using a portable multi-parameter digital meter (HACH, HQ40d, Canada) with IntelliCAL Probes (HACH, CDC40105, Canada) that automatically recognize the testing parameter. The meter was calibrated using a calibration standard of 0.01M KCl (1413 µS/cm, 25°C). The conductivity probe is accurate to +/- 0.5% of a conductivity range of 0.01µS/cm to 200mS/cm. Samples for isotopic analyses were collected at low tide using a kick net (48 X 22 cm frame, 900µm mesh). The net was held downstream with the mouth facing upstream. The substrate was disturbed by kicking the rocks to dislodge the invertebrates which were then carried into the net by the current. Samples were collected from the net by hand and held in river water from the collection site in transparent,

contaminant-free, non-reactive polyethylene bags with round wire enclosures. Because the sampling was intended to be qualitative rather than quantitative, invertebrates were also collected by hand from larger rocks and areas closest to the shore which contained woody debris. These were combined with the other invertebrates in the sample bags. The sample bags were then placed in a cooler with ice until they were returned to the laboratory. Predators such as Odonata (dragonfly) nymphs and Tabanidae (horse fly) larvae were kept in separate bags to avoid the consumption of potential prey. In the lab, samples were identified to the lowest taxonomic level possible, using various taxonomic keys, some of which were specific to Newfoundland and Labrador (Larson, unpublished; Merritt and Cummins, 1996; Clifford, 1991). The invertebrates were further divided into FFGs as described in Voshell (2002). In some instances, the cases of Trichopteran larvae were used for identification. These cases were then removed before the larvae were freeze-dried. The invertebrates were placed in vials which were labelled with species and functional feeding group. These vials were then frozen and later freeze-dried in a shelf type freeze drier.

Invertebrate samples were then individually crushed to a fine powder with a mortar and pestle to ensure homogeneity. Larger invertebrates, such as *Limnephilus*, were crushed individually, while smaller invertebrates of the same species or genus, depending on identification level, were combined before being crushed following the method of Bennett and Hobson (2009) (Table 3.2). Samples for carbon and nitrogen analysis were weighed on a microbalance into tin cups (3.5 x 5 mm) while samples for hydrogen analysis were weighed into silver cups (3.5 x 5 mm) (Table 3.1). These cups were then

folded into tiny balls and placed in separate 96-well trays – one for hydrogen, and one for carbon/nitrogen. The sample name, weight, and tray ID were recorded.

Biofilm was also collected from rocks at the three sites. Rocks were scrubbed with a soft bristle brush into a plastic tub and washed with distilled water until most of the biofilm was removed. This solution was then poured into a 2 litre brown high density polyethylene (HDPE) bottle and placed in a cooler. These samples were also frozen until they could be freeze-dried. The biofilm samples were shell-frozen using liquid nitrogen and freeze-dried in a LabConco freeze drier. After freeze-drying, a portion of biofilm from each site was acid fumigated with concentrated hydrochloric acid for four hours to remove inorganic carbon such as carbonate minerals before $\delta^{13}C$ analysis following the method of Hedges and Stern (1984).

Particulate organic matter (POM) was collected from the river at all three sites. An 18.9 litre (5 gallon) carboy fitted with a 0.500 mm sieve was placed in the stream flow and filled with water. When the carboys were returned to the lab the water was mixed well and 2 litres of water were filtered through a precombusted 47 mm glass microfiber filter (GF/F) with a 0.45μm mesh size using a glass vacuum filter apparatus and PVC hand operated vacuum pump. Three samples were filtered for each site. The filters were then individually wrapped in pre-combusted envelopes of aluminum foil and frozen and then later freeze-dried. After freeze-drying, a portion of the filters from each site was acid fumigated similarly to the biofilm samples prior to the analysis for $\delta^{13}C$.

Samples of plants, algae, and moss that were present at each of the sites were also collected. These samples were rinsed with deionized water and dried before being frozen and then later freeze-dried in a shelf type freeze drier. Whole water samples were collected in 4 ml clear glass vials with polypropylene screw-thread caps that contained a silicon/polytetrafluoroethylene (PTFE) septa for the analysis of water δ^2H and $\delta^{18}O$. Two samples were taken at each site. The sample vial was submersed in the river until it was completely filled and then capped underwater to prevent the presence of air bubbles.

The biofilm, plant, algae, and moss materials were crushed to a fine powder with a mortar and pestle and weighed on a microbalance in a similar manner to the invertebrate samples.

Table 3-1 Sample sizes required for carbon, nitrogen, and hydrogen stable isotope analysis

	Sample Size (µg)	
Sample	**H/O**	**C/N**
Invertebrates	~200	~500
Biofilm	~200	~1000-1500
Plants	~200	~600-800
Algae	~200	~1000-1500
Moss	~200	~600-800

All samples were packaged for shipping and sent to the Geophysical Laboratory at the Carnegie Institution for Science in Washington, DC where carbon, nitrogen, and hydrogen isotope analyses were performed. All stable isotope values are reported in δ notation.

3.3 Results

A total of 150 organisms were collected from the Amphipoda, Coleoptera, Diptera, Ephemeroptera, Odonata, Plecoptera and Trichoptera Orders at the three sites within Salmon River. These invertebrates were divided into 5 feeding guilds – collector-gatherers, collector-filterers, shredders, scrapers, and predators (Table 3.2).

3.3.1 Primary Producers

3.3.1.1 *Particulate Organic Matter*

A number of different types of primary producers were collected to determine their role in the aquatic freshwater-estuarine food web. Particulate organic matter (POM) was collected at the three sites; however, these filter samples could not be analyzed for hydrogen isotopes because of interference associated with the glass fiber upon pyrolysis. The nitrogen and carbon isotope composition of the POM, however, reflected the differences in salinity values at each site with the more saline or marine site (site 5) having the highest ratios and the freshwater site (site 1) the lowest. The nitrogen isotope ratios for POM ranged in value from 1.8‰ to 3.1‰ over the three sites. The $\delta^{13}C$ values for POM varied from -30.4‰ to -28.5‰ (Table 3.3).

Table 3-2 Invertebrates and their functional feeding guilds (FFG) collected at Salmon River, NL, June 2010 (C-G = collector-gatherer, C-F = collector-filterer, Pred = predator, Scrap = scraper, Shred = shredder)

Order	Family	Genus	FFG	n
Amphipoda	Hyalellidae	N/A	C-G	17
Coleoptera	Dytiscidae	N/A	Pred	1
Diptera	Tipulidae	N/A	Pred	1
Diptera	Tipulidae	N/A	Pred	1
Diptera	Tabanidae	N/A	Pred	1
Ephemeroptera	Leptophlebiidae	*Leptophlebia*	C-G	4
Ephemeroptera	Leptophlebiidae	*Leptophlebia*	C-G	1
Ephemeroptera	Leptophlebiidae	*Leptophlebia*	C-G	1
Ephemeroptera	Leptophlebiidae	*Leptophlebia*	C-G	15
Ephemeroptera	Ephemerellidae	*Eurylophella*	C-G	4
Ephemeroptera	Ephemerellidae	*Drunella*	Scrap	18
Ephemeroptera	Ephemerellidae	*Ephemerella*	Scrap	3
Ephemeroptera	Ephemerellidae	*Ephemerella*	Scrap	1
Ephemeroptera	Ephemerellidae	*Ephemerella*	Scrap	3
Ephemeroptera	Ephemerellidae	*Ephemerella*	Scrap	6
Ephemeroptera	Heptageniidae	*Stenonema*	Scrap	6
Ephemeroptera	Heptageniidae	*Stenonema*	Scrap	9
Odonata	Gomphidae	*Ophiogomphus*	Pred	1
Odonata	Gomphidae	*Ophiogomphus*	Pred	1
Odonata	Gomphidae	*Progomphus*	Pred	1
Plecoptera	Perlodidae	*Isoperla*	Pred	2
Trichoptera	Hydropsychidae	*Cheumatopsyche*	C-F	3
Trichoptera	Hydropsychidae	*Cheumatopsyche*	C-F	9
Trichoptera	Hydropsychidae	*Cheumatopsyche*	C-F	2
Trichoptera	Hydropsychidae	*Hydropsyche*	C-F	3
Trichoptera	Hydropsychidae	*Hydropsyche*	C-F	11
Trichoptera	Lepidostomatidae	*Lepidostoma*	Shred	19
Trichoptera	Limnephilidae	*Limnephilus*	Scrap	1
Trichoptera	Limnephilidae	*Limnephilus*	Scrap	1
Trichoptera	Limnephilidae	*Limnephilus*	Scrap	1
Trichoptera	Limnephilidae	*Limnephilus*	Scrap	1
Trichoptera	Glossosomatidae	*Glossosoma*	Scrap	2

Table 3-3 Average values of $\delta^{15}N$ and $\delta^{13}C$ stable isotopes for samples of particulate organic matter (POM) collected along a salinity gradient from three sites in Salmon River, NL, June 2010 where site 1 is freshwater and site 5 is marine (n = 2; SD in parentheses)

Site	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
1	1.8 (+/- 0.3)	-30.4 (+/- 0.1)
3	2.0 (+/- 0.3)	-29.8
5	3.1 (+/- 0.6)	-28.5 (+/- 0.1)

3.3.1.2 *Biofilm*

The values of $\delta^{15}N$ for the biofilm varied slightly from 3.1‰ to 4.1‰ among the three sites. However, there was no pattern detected in the biofilm isotope values with respect to the conductivity gradient between marine and freshwater sites. The values for $\delta^{13}C$ did vary with salinity having ranged from -22.3‰ to -17.5‰ but displaying a pattern opposite to that of the POM values. The most negative value was recorded at site 5 where salinity was highest and the least negative value was from site 1 in the freshwater end of the estuary. The hydrogen isotope composition of the biofilm ranged in value from -158.0‰ to -131.0‰ with no clear pattern in terms of the change in salinity (Table 3.4).

Table 3-4 Average values of $\delta^{15}N$, $\delta^{13}C$, and $\delta^{2}H$ stable isotopes for samples of biofilm collected along a salinity gradient from three sites in Salmon River, NL, June 2010 where site 1 is freshwater and site 5 is marine (n = 2; SD in parentheses)

Site	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)
1	3.5 (+/- 0.1)	-17.5 (+/- 0.2)	-131.0 (+/- 4.9)
3	4.1 (+/- 0.1)	-18.8 (+/- 0.3)	-158.0 (+/- 0.9)
5	3.1 (+/- 0.3)	-22.3 (+/- 0.8)	-147.7 (+/- 3.5)

3.3.1.3 ***Plant Materials***

The other producer species collected at the three sites include *Spirogyra* sp. - a type of green algae, the cyanobacteria *Rivularia* sp., a bladderwort – *Utricularia* sp., a plant – *Caltha palustrus* and an unidentified aquatic moss. The stable isotope ratios of $\delta^{15}N$, $\delta^{13}C$, and $\delta^{2}H$ for these producers varied across sites (Table 3.5).

Table 3-5 Average values of $\delta^{15}N$, $\delta^{13}C$, and $\delta^{2}H$ stable isotopes for primary producers collected along a salinity gradient from three sites in Salmon River, NL, June 2010 where site 1 is freshwater and site 5 is marine (SD in parentheses)

Site	Primary Producers	Plant Type	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)
1	Aquatic Moss	N/A	3.3 (+/- 0.3)	-34.2 (+/- 0.1)	-154.8 (+/- 1.3)
1	*Caltha palustrus*	Herbaceous Plant	-0.3 (+/- 0.1)	-25.9	-127.6 (+/- 0.5)
3	*Utricularia* sp.	Bladderwort	4.3	-33.3 (+/- 0.1)	-118.0 (+/- 3.7)
3	*Rivularia* sp.	Cyanobacteria	0.6	-15.7 (+/- 0.1)	-191.3 (+/- 3.5)
5	*Spirogyra* sp.	Green Algae	3.9 (+/- 0.6)	-32.7 (+/- 1.5)	-159.8 (+/- 2.1)

3.3.2 Functional Feeding Groups

The $\delta^{15}N$ values for the functional feeding groups were in agreement with what was expected, with the predator group being enriched in the heavy isotope relative to the other functional groups (Figure 3.1a). The enrichment between the predators and the collector-gatherers ($t(11) = 2.30$, $p = 0.0200$ and predators and the scrapers ($t(15) = 6.118$, $p= 0.001$) was statistically significant. However, this enrichment was not statistically significant when the predator group was compared with the collector-filterer group ($t(8) = 1.759$, $p = 0.117$). With only one sample from the shredder group, sampling error could not be dismissed as a source of the difference between this group and the predator group. The $\delta^{15}N$ values for the collector-gatherers ranged from 2.8‰ to 6.2‰ across all sites with a total range of 3.4‰ and a mean of 4.0‰ (+/- 1.2, n = 6). The collector-filterers exhibited a much smaller range in $\delta^{15}N$, having spanned 5.2‰ to 6.3‰ and exhibited a mean of 5.7‰ (+/- 0.4, n = 5). As expected the $\delta^{15}N$ values of both of these functional groups were enriched with respect to the POM (Figure 3.2c). This enrichment was statistically significant for both the collector-gatherers ($t(8) = 3.279$, $p = 0.005$) and the collector-filterers ($t(3) = 7.668$, $p = 0.003$). The $\delta^{15}N$ values for the predator group ranged from 5.2‰ to 7.5‰ with a mean of 6.5‰ (+/- 0.9, n = 7). Only one species of shredder was collected, which had an isotope value for nitrogen of 4.5‰. This was higher than the $\delta^{15}N$ values for the plant and bladderwort collected at the same site. However, because only one sample was collected sampling error could not be dismissed as a reason for this increase. Finally, $\delta^{15}N$ values for the scraper group had a range of 2.6‰ to 4.7‰ with an average of 3.8‰ (+/- 0.7, n = 12) (Table 3.6)

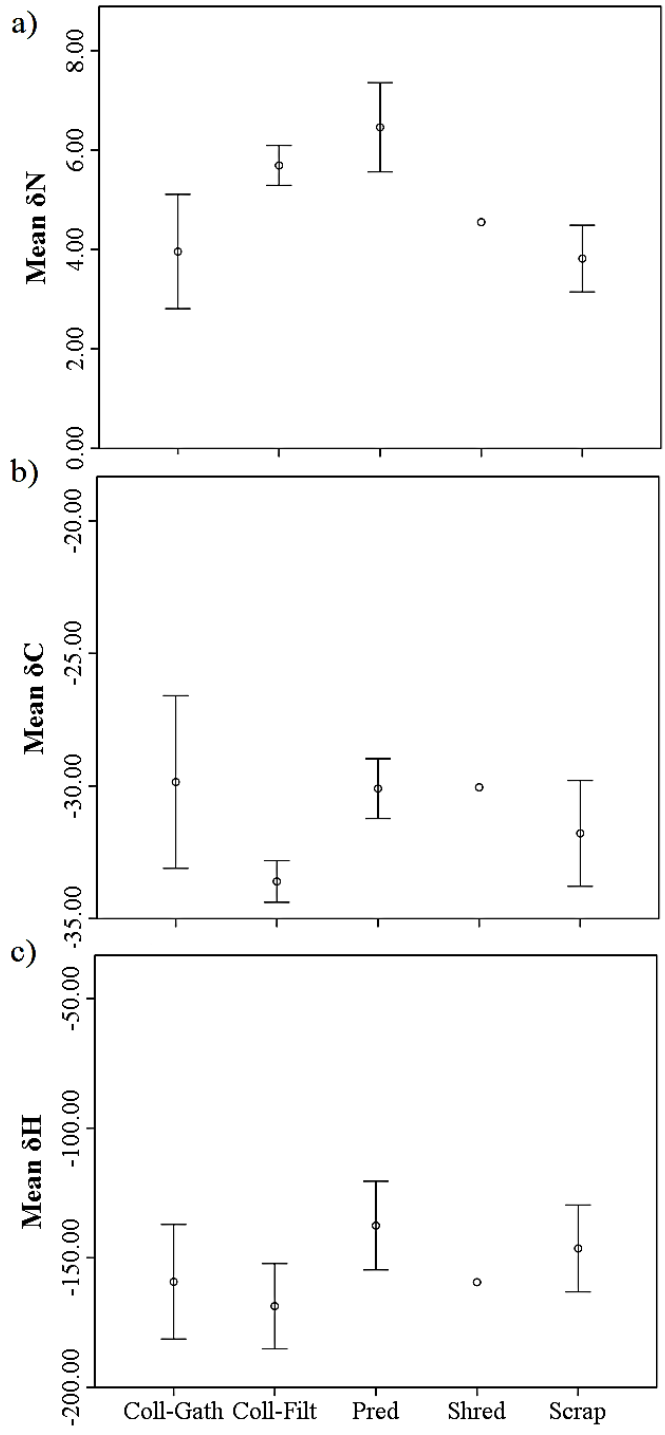


Figure 3-1 The average a) δN, b) δC, c) δH ratio values for each functional feeding group collected from Salmon River, NL, June 2010 (bars represent +/- SD)

Table 3-6 $\delta^{15}N$, $\delta^{13}C$, and $\delta^{2}H$ values for specific invertebrates collected at three different sites in Salmon River, NL, June 2010

Site	Conductivity at low tide (µS/cm)	Invertebrate	Functional feeding group	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)
5	3300	Hyalellidae	Coll-Gath	6.2	-23.4	-137.7
3	142.4	*Leptophlebia cupida*	Coll-Gath	3.5	-31.4	-170.7
3	142.4	*Leptophlebia cupida* (A)	Coll-Gath	4.0	-30.9	-143.3
3	142.4	*Leptophlebia cupida* (B)	Coll-Gath	3.5	-31.2	-152.8
3	142.4	*Eurylophella* spp.	Coll-Gath	2.8	-30.2	-191.8
1	144	*Leptophlebia cupida*	Coll-Gath	3.9	-32.2	---
3	142.4	*Hydropsyche* spp.	Coll-Filt	6.3	-32.5	-187.6
1	144	*Hydropsyche* spp.	Coll-Filt	5.9	-33.3	---
3	142.4	*Cheumatopsyche pettiti*	Coll-Filt	5.6	-33.5	---
1	144	*Cheumatopsyche pettiti*	Coll-Filt	5.2	-34.3	-158.6
1	144	*Cheumatopsyche pettiti*	Coll-Filt	5.5	-34.5	-159.7
5	3300	*Isoperla* spp.	Pred	7.3	-28.6	-142.3
3	142.4	*Ophiogomphus columbrinus*	Pred	6.1	-31.1	-144.3
1	144	*Ophiogomphus* sp.	Pred	6.4	-30.7	-143.8
3	142.4	Tipulidae	Pred	7.5	-28.9	-155.9
1	144	Tipulidae	Pred	5.6	-31.6	-141.4
1	144	Tabanidae	Pred	7.2	-30.1	-101.8
1	144	Progomphus	Pred	5.2	-29.9	-133.6
3	142.4	*Lepidostoma* sp.	Shred	4.6	-30.1	-159.4
3	142.4	*Ephemerella aurivillii*	Scrap	2.6	-34.3	---
3	142.4	*Ephemerella subvaria*	Scrap	2.95	-32.4	-166.4
3	142.4	*Ephemerella rotunda*	Scrap	3.0	-32.4	-177.1
1	144	*Ephemerella* sp.	Scrap	3.6	-34.0	---
1	144	*Drunella* sp.	Scrap	4.7	-32.8	-147.6
1	144	*Limnephilus* sp. (D)	Scrap	4.0	-28.5	-133.1
1	144	*Limnephilus* sp. (C)	Scrap	4.2	-29.8	-139.6
1	144	*Limnephilus* sp. (A)	Scrap	3.7	-29.3	-136.8
1	144	*Limnephilus* sp. (B)	Scrap	4.4	-29.9	-126.0
3	142.4	*Glossosoma* sp.	Scrap	4.3	-34.3	-162.9
1	144	*Stenonema vicarium*	Scrap	4.2	-32.0	-139.4
1	144	*Stenonema vicarium*	Scrap	4.3	-32.0	-135.2

The mean $\delta^{13}C$ values for the functional groups were relatively similar among groups (Figure 3.1b). The ratios for the collector-gatherer and collector-filterer groups reflected the values for the POM as expected. There was no significant difference between the collector-gatherers ($t(9) = 3.30$, $p = 0.749$) and POM or the collector-filterers ($t(2) = -2.013$, $p = 0.084$) and POM. However, the $\delta^{13}C$ values for the scraper group ($t(13) = -9.168$, $p = 0.000$) were significantly different from the values for the biofilm with an average ratio of -31.8‰ in comparison to the -19.5‰ average $\delta^{13}C$ value of the biofilm. (Table 3.6).

The hydrogen isotope values were similar among functional groups (Figure 3.1c). The $\delta^{2}H$ ratio for the shredder found at site 3 had a value of -159.4‰ which was lower than the value of the plant material found at that site. However, the $\delta^{2}H$ values for the scraper group ranged from -177.1‰ to -126.0‰ with a mean value of -146.4‰ (+/- 16.7, n = 10). These values were similar to the $\delta^{2}H$ values of the biofilm found at the corresponding sites (Table 3.6).

Dual isotope graphs were plotted for carbon versus nitrogen, hydrogen versus nitrogen, and carbon versus hydrogen for individual invertebrates. Clear patterns emerged with respect to functional feeding groups in the $\delta^{13}C$ versus $\delta^{15}N$ plot. Each group was clumped together with the exception of one collector-gatherer, Hyalellidae, whose carbon signature was much higher than all of the other invertebrates (Figure 3.2).

The $\delta^{13}C$ values between the scrapers at site 3 and *Utricularia* sp., a species of bladderwort, were similar. The carbon signature for the aquatic moss found at site 1

appeared to be similar to the collector-filterers found at site 1. The C3 plant, *Caltha palustrus*, which was found at site 1, also had a higher carbon ratio than any of the invertebrates (Figure 3.2a).

Since the $\delta^{15}N$ ratios of consumers are enriched by an average value of 3.4‰ relative to their food source it is possible to make inferences as to which functional group is consuming which producer (Finlay and Kendall, 2007; Hershey et al., 2007). In this case, there was some overlap with the collector-gatherers at site 3 and *Rivularia* sp. The predators at site 1 showed the expected enrichment over the moss collected at that site although it is unlikely that the predators were directly consuming moss. There did not appear to be any relationship between the predators and the other producers (Figure 3.2a).

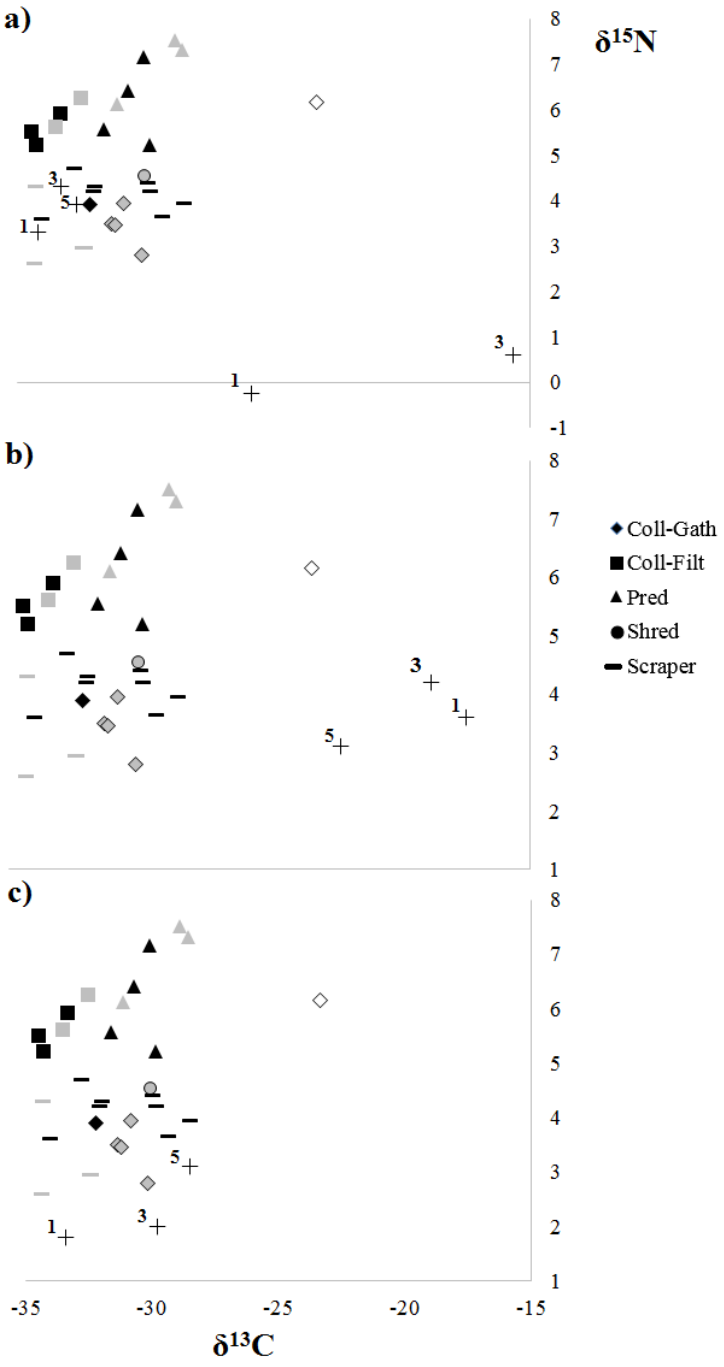


Figure 3-2 Dual isotope plot of $\delta^{13}C$ versus $\delta^{15}N$ for the functional feeding groups (FFGs) replotted to provide comparison with a) primary producers, b) biofilm, and c) POM collected from the three sites in Salmon River, NL, June 2010. The sites are represented by the numbers above the crosses (+) and the FFGs labels are distinguished by using color (black markers = site 1, grey markers = site 3, white markers = site 5)

The $\delta^{13}C$ values for the biofilm were higher than the values for the functional groups with no overlap occurring between the values (Figure 3.2b). The carbon and nitrogen values for the biofilm for site 5 were lower than those of sites 1 and 3. The *Hyalellidae*, the lone collector-gatherer, found at site 5 had a nitrogen ratio value that was 3.5‰ higher than the nitrogen isotope value for the biofilm at site 5. It was expected $\delta^{15}N$ values for the scrapers at each site would be linked to the biofilm values; however, that was not the case.

The POM carbon isotope values for site 1 were similar to the collector-filterer values at that site. The POM carbon values for site 3 were similar to the values for the collector-gatherers and the lone shredder at that site. Finally, the POM carbon value for site 5, which was higher than the other two sites, was similar to the value of the lone predator at site 5. In terms of the $\delta^{15}N$ values, the POM at site 5 had a higher value than the other two sites. Both the collector-gatherer and the predator collected at site 5 had nitrogen isotope values which were 3.5‰ greater than the nitrogen value of the POM collected there. The collector-filterers found at site 3 had nitrogen values that were 3.5‰ higher than the nitrogen values for POM collected at that site (Figure 3.2c).

The dual isotope plot of $\delta^{15}N$ versus $\delta^{2}H$ did not show any clear patterns in terms of functional feeding groups (Figure 3.3). The functional groups were still clumped but the spread of values for $\delta^{2}H$ was wider.

Among sites the collector-gatherers collected from site 3 had a larger spread of hydrogen isotope values than the other invertebrates collected at that site. The scrapers and the

collector-filterers collected from site 1 had higher hydrogen isotope values than those collected from site 3. However, the predators collected from all three sites had similar hydrogen isotope values (Figure 3.3).

With respect to the producers, only *Rivularia* sp. and the aquatic moss showed any relationship with the functional groups (Figure 3.3a). The δ^2H value for *Rivularia* sp. at site 3 was similar to one of the collector-gatherers from that site. The moss found at site 1 had a δ^2H value that were similar to the two collector filterers found there.

Unlike the carbon isotope ratios for biofilm, the values of δ^2H were within the range of invertebrate values (Figure 3.3b). There was a slight overlap between the hydrogen isotope values of the biofilm and the scrapers at site 1. There was also a slight overlap between the hydrogen isotope values of the scrapers and biofilm at site 3.

The dual isotope plot was created for $\delta^{13}C$ versus δ^2H showed no obvious patterns in terms of functional feeding groups (Figure 3.4). However, when the data were plotted based on site alone (Figure 3.5), a division between the invertebrates found at sites 1 and 3 was noted, with site 5 falling between sites 1 and 3. In terms of the δ^2H values, the invertebrates found at site 1, the freshwater site, appeared to be more depleted in ^2H than site 3. There was also a larger spread of values for the invertebrates found at site 3 in comparison to the values for site 1. In terms of the $\delta^{13}C$ values, there did not appear to be any separation between the two sites.

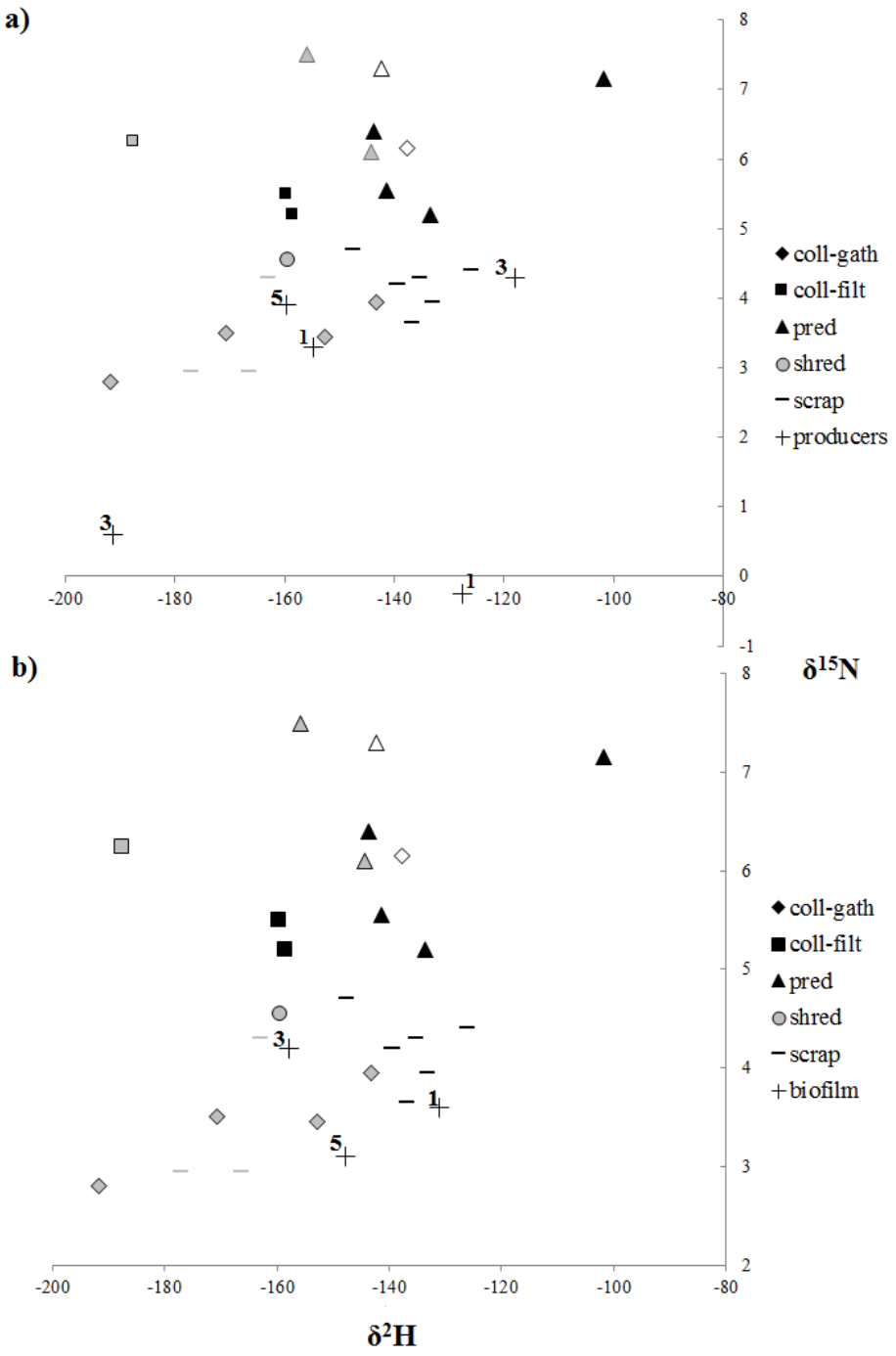


Figure 3-3 Dual isotope plot of δ^2H versus $\delta^{15}N$ for the functional feeding groups (FFGs) and a)primary producers, and b) biofilm collected from the three sites in Salmon River, NL, June 2010. The sites are represented by numbers above the crosses (+) and the FFGs labels are distinguished using color (black markers = site 1, grey markers = site 3, white markers = site 5)

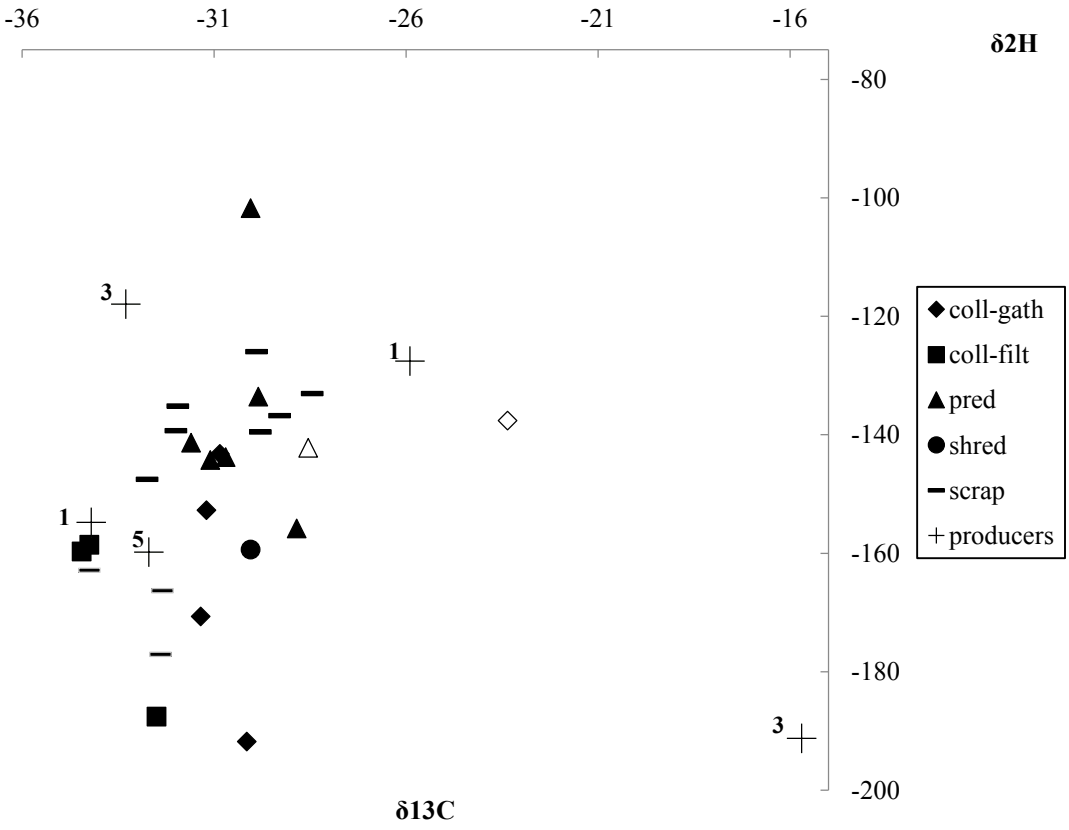


Figure 3-4 Dual isotope plot of $\delta^{13}C$ versus $\delta^{2}H$ for the functional feeding groups (FFGs) and primary producers collected from the three sites in Salmon River, NL, June 2010. The sites for primary producers are represented by numbers above the crosses (+) and the FFGs labels are distinguished using color (black markers = site 1, grey markers = site 3, white markers = site 5)

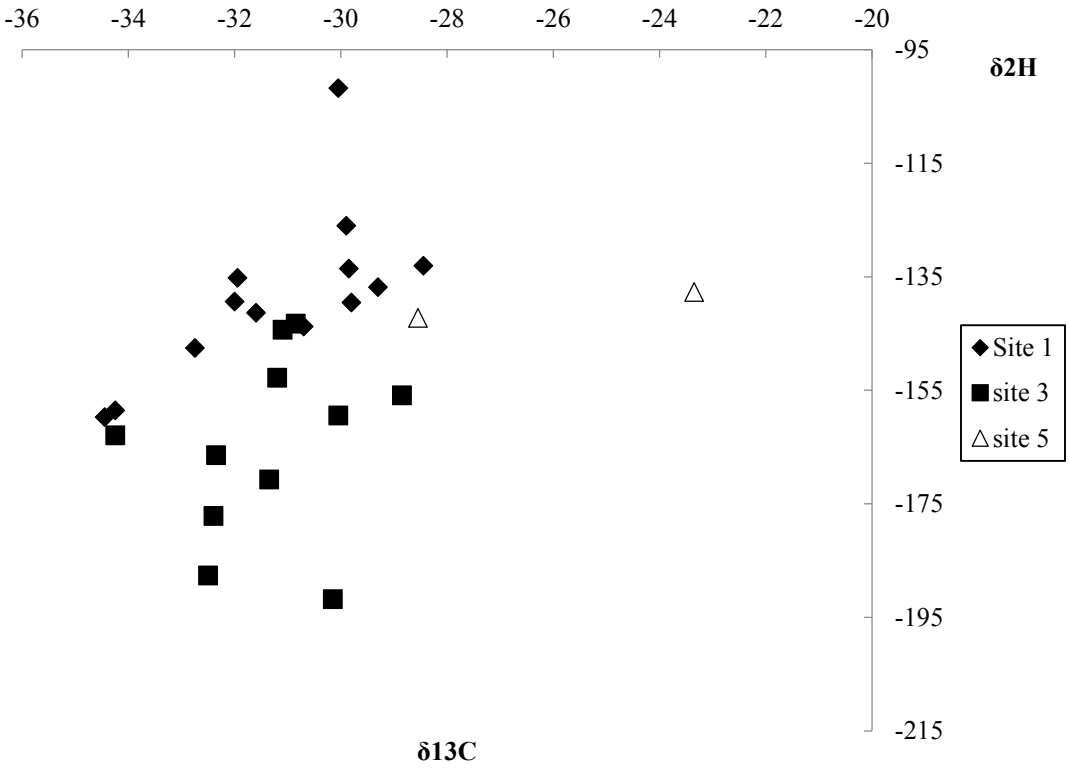


Figure 3-5 Dual isotope plot of $\delta^{13}C$ versus $\delta^{2}H$ for the invertebrates found at three sites in Salmon River, NL, June 2010

3.4 Discussion

The stable carbon, nitrogen and hydrogen isotope signatures of the invertebrates collected at Salmon River provided insight into the trophic dynamics at the base of the food web. The expected relationships between functional feeding groups and their food sources at each trophic level were observed in some cases but not in others. The samples collected at the marine sites within the riverine-estuarine system were more depleted in both carbon and nitrogen isotopes than the samples collected at the freshwater sites. This was not the case for the hydrogen isotope signatures. It was also established that hydrogen can be used, along with carbon, to determine the sources of organic matter within the riverine-estuarine system.

It was expected that the scraper group would utilize the biofilm as its main source of energy. Scrapers are specifically adapted to remove biofilm from the substrate and it was anticipated that this would be the energy source most commonly utilized. However, based on the isotope values for the scraper group this was not the case. There was no enrichment in the $\delta^{15}N$ isotope values from the biofilm, at the base of the food web, to the scraper group. The $\delta^{13}C$ values for the biofilm were depleted in ^{13}C in comparison to the values for the scrapers. There also did not appear to be any significant enrichment in the $\delta^{2}H$ values from the biofilm to the scraper group. Biofilm is comprised of benthic algae, bacteria, fungi, and protozoa. If the scrapers are omnivorous then the nitrogen isotope values of these invertebrates should be enriched by an average of 3.4‰ over the biofilm nitrogen values. However, if the scrapers are selectively consuming, for instance, the

algal parts of the biofilm, then the isotopic signature of the insects may not be enriched in comparison to the biofilm. Many primary consumers are selective feeders that do not feed randomly on biofilm (Costas and Pardo, 2015). This selectivity has been observed in shredders that consume the microbes found on leaf litter rather than the leaves (Arsuffi and Suberkropp, 1989).

It was expected that the results would show that the collector-gatherer and the collector-filterer groups were utilizing the particulate organic matter, as a source of energy. These groups collect and filter fine detritus within the water column and that which settles on the bottom among the biofilm. Both groups had $\delta^{15}N$ values that were enriched over the values of $\delta^{15}N$ for the POM and the carbon signatures of both groups showed little fractionation over the POM values, indicating that these groups were utilizing POM as a food source.

The predators, at the top of the functional feeding group food web, were expected to have the highest $\delta^{15}N$ values. This was the case with average values for sites 1, 3, and 5 of 6.1‰, 6.8‰, and 7.3‰ respectively. Predators feed on living organisms and based on the average nitrogen isotope values, there appears to be a nitrogen isotope enrichment from both the collector-gatherers and the scrapers to the predators. However, there did not appear to be any enrichment between these prey groups and the predator groups in terms of the $\delta^{2}H$ values. The predator group at all three sites was depleted in ^{2}H over all the other groups with the exception of the collector-gatherers at site 5. If fractionation was occurring between these feeding groups, it would be expected that the predators would be

enriched in ^{2}H over their prey. It is difficult to utilize hydrogen isotopes as food web tracers in aquatic systems because the hydrogen within consumer tissues comes from two different sources – diet and environmental water (Vander Zanden et al., 2016). It is possible that the predator group may have a higher contribution of hydrogen from the environmental water than the other invertebrates (Jardine et al., 2009). The $\delta^{13}C$ values for the predators were within the expected range observed for the prey groups and support the outcome of the nitrogen isotope analysis.

It was expected that there would be longitudinal changes in the values of the stable isotopes of carbon, nitrogen, and hydrogen for primary producers from the freshwater sites into the estuary. This would lead to a subsequent change in isotope signatures for the invertebrates along the gradient. However, the carbon and nitrogen isotope signatures for biofilm changed very little along the longitudinal gradient, especially from site 1 to 3. These two sites were similar in both conductivity values and the fact that there was no riparian vegetation overhanging the river. In this case, photosynthesis in the river would increase and the main source of carbon to both sites would be autochthonous (Vannote, 1980). The biofilm found at site 5, within the estuary, was slightly more depleted in both ^{13}C and ^{15}N than at the two freshwater sites. This is contrary to the findings of Garcia et al. (2007) and Winemiller et al. (2011) who stated that the isotope signatures of basal energy sources typically become more enriched from freshwater into the estuary. Both of these studies, however, were conducted in areas with subtropical climates unlike that of Salmon River. The different climate may have influenced the isotope signatures in this study.

The hydrogen isotope values showed no pattern with respect to biofilm among the three sites. The biofilm was more depleted in ^{2}H at site 3 than at site 1 but more enriched at site 3 than at site 5.

The patterns observed for the carbon and nitrogen signatures of the invertebrates matched those of the biofilm. There was little variation between the carbon and nitrogen signatures of the invertebrates at sites 1 and 3. The invertebrates at these sites appeared to be utilizing the autochthonous energy sources. However, the invertebrates collected at site 5 were again more depleted in ^{13}C and ^{15}N than the other two sites. It is possible that because of the high nutrient exchange in the estuary, the sources of carbon and nitrogen being utilized by the invertebrates would be different than those in the freshwater sites. With the influence of the tide in this area, the source of carbon and nitrogen may come from the marine, as opposed to the freshwater, environment.

There was no clear longitudinal pattern between the $\delta^{2}H$ values for the invertebrates at either of the three sites. There was, however, an interesting difference in the $\delta^{2}H$ values of the invertebrates between sites 1 and 3. Since the conductivity values between the two sites are similar, this difference cannot be explained by the variation in freshwater and marine sources. Nor can it be explained by the River Continuum Concept since both sites had an open canopy. This difference appears to be a habitat driven change. In the area slightly above site 1 there was a small stream flowing into the river from a nearby pond. POM with a different hydrogen signature from that at site 3 may have been carried from the pond into the river via the stream and deposited at site 1. This could explain the

apparent hydrogen isotope depletion observed at this site. Costas and Pardo (2015) observed a similar effect on nitrogen isotope ratios in a river caused by two tributaries entering the river near the sampling site. They theorized that the tributaries had a "rejuvenation and dilution" effect on the site. Another possibility is that the water flowing from the pond may have a different hydrogen isotope signature than the water at site 3. While there is still some disagreement as to whether the environmental water has an effect on the invertebrates living in it, this could be the case.

Carbon isotopes have been used to determine the source of organic matter within riverine-estuarine systems as there is a separation in carbon isotope values between terrestrial and aquatic sources. However, at times there is a lack of separation between the carbon isotope values and it is difficult to identify the sources. In this study there was an overlap between the lone terrestrial plant collected, *Caltha palustrus*, which had a carbon isotope value of -25.9‰ and the isotope values of the autochthonous matter collected which ranged from -34.2‰ to -15.7‰. This makes it difficult to determine whether the primary consumers are utilizing allochthonous of autochthonous energy sources within the riverine-estuarine system. The hydrogen isotope values for the allochthonous and autochthonous sources did show a separation. This was expected due to the fact that water enriched in ^{2}H is assimilated into terrestrial plants during the process of photosynthesis which does not affect aquatic plants (Smith and Ziegler, 1990).

3.5 Conclusion

In this study the results of the nitrogen and carbon isotope analyses were as expected with the exception of a relationship between the primary consumers, the scrapers, at the base of the food web and the biofilm values. The hydrogen isotope ratios confirmed this lack of a relationship. It can, therefore, be concluded that these insects are either selectively consuming parts of the biofilm or are utilizing another source of energy, possibly POM. Future work in this area could include collecting and analyzing other potential sources of primary production which could be possible components of the biofilm, such as algae or fungi. It would also be beneficial to collect more invertebrates at each site. For instance, with only one shredder species collected it was difficult to make inferences about the food sources utilized by the insect.

The expected differences in the carbon, nitrogen, and hydrogen isotope signatures of both the primary producers and consumers along the longitudinal transect from freshwater into the estuary were not not observed. However, a difference in δ^2H values of biofilm and invertebrate species between the two freshwater sites was observed that could be explored. Water samples from each site could be collected to determine if there is a difference between the hydrogen isotope values that could explain the differences observed.

Based on the results of the study, hydrogen isotopes could potentially be useful in determining whether the energy sources within riverine-estuarine systems are allochthonous or autochthonous. These sources were separated by the hydrogen isotopes

whereas the carbon isotope values overlapped. However, with only one terrestrial plant species collected the results may not be significant. Future work could involve the collection of more riparian plant species as well as more aquatic plant species.

It does not appear that hydrogen could be used to determine trophic levels as there were conflicting results between the fractionation of nitrogen and hydrogen isotopes for each functional group.

These results provide insight into the potential utilization of hydrogen isotopes to improve the study of trophic dynamics at the base of the boreal riverine-estuarine food web. In combination, carbon, nitrogen, and hydrogen isotope analyses may potentially be useful in the detection and assessment of the possible impacts of climate change.

4 Summary

Understanding the factors that influence the structure and function of biotic communities remains a major challenge to ecologists. Analyzing the relationships between the patterns of distribution of organisms and environmental factors is an important step towards this understanding. There is a paucity of information related to the distribution patterns and community structure of biota that inhabit the transitional zones between rivers and estuaries in boreal estuarine ecosystems. Complicating this lack of information is the strong potential for climate change effects. Climate change events, such as sea level rise, changes in precipitation patterns, and increasing temperatures, are predicted to result in an increase in salinity in estuaries and the freshwater rivers that feed into them (Prandle and Lane, 2015). Changes in precipitation levels will also impact the hydrological and biogeochemical processes influencing the transport of allochthonous materials into riverine estuarine systems (Benoy et al., 2007).

The objectives of this study were (1) to examine the distribution of Ephemeroptera, Plecoptera, and Trichoptera species within and among four boreal riverine-estuarine systems in western Newfoundland, (2) to investigate the influence of salinity on spatial distribution of EPT groups in riverine-estuarine systems, (3) to investigate the stable isotope signatures of a collection of aquatic invertebrates within one particular riverine-estuarine system and (4) to determine whether hydrogen isotope can be used to assess the base of the food web along a salinity gradient within that riverine-estuarine system.

Latitudinal gradients have been observed at various scales in terrestrial, marine and freshwater environments. As temperatures decrease from south to north, populations often decrease as well. However, numerous relationships between latitude and abundance have been observed, including positive, negative, hump-shaped and neutral responses of species to changes in latitude (Bowden and Buddle, 2010). Temperature increase due to climatic change has the potential to shift populations northward.

It was hypothesized that aquatic insect species abundance is negatively correlated with increasing latitude. This was tested by collecting and identifying EPT species in four riverine-estuarine systems across a latitudinal gradient from the Grand Codroy River in the south to Salmon River in the north. The number of species collected from each riverine-estuarine system were compared from south to north.

Contrary to the expectation that the species abundance would decrease from south to north, there was no significant change in the number of species collected at each river.

These results were not consistent with a number of other studies that showed a decrease in species abundance with increasing latitude (Benson and Pearson, 1987; Jacobsen et al., 1997). One apparent explanation for this difference may be related to scale. The distance from the Grand Codroy River to Salmon River may not provide a latitudinal scale that allows for enough sensitivity to discern significant changes in species abundance.

Biological communities within streams often show distinct gradients in terms of species abundance and composition in response to specific variables. Aquatic insects found at the

riverine-estuarine interface are particularly influenced by complex salinity gradients as a result of tidal intrusion. Given that accelerated sea level rise is projected to substantially increase salinity in estuaries (Nicholls et al., 2007), it is important to consider the influence of salinity on invertebrate communities. Salinity levels in estuarine systems can vary from freshwater to seawater over a short distance and certain organisms will only be found at one end of the estuary or the other. Most can only tolerate a specific salinity range as they do not have the capabilities to excrete the salt ions and maintain the proper salt and water balance through osmoregulation.

It was hypothesized that species abundance would decrease with increasing salinity. This was tested by measuring conductivity, as a representative measurement of salinity, at five sites along each of the four riverine-estuarine systems and then collecting and identifying EPT species at those sites. The number of species at each site was compared from the freshwater into the more saline estuary.

As hypothesized, the species abundance decreased with increasing salinity at each of the four riverine-estuarine systems.

The results were consistent with a number of other studies (Metzeling, 1993; Ysebaert et al., 1993 Marchant, 1999). Freshwater invertebrate species have typically been confined to the upper portion of estuaries where conductivity values are relatively low. Freshwater invertebrates, especially insects, are highly sensitive to elevated salt levels and therefore are restricted to the upper end of the riverine-estuarine system.

Studies focusing on the natural abundances of isotopes of carbon and nitrogen in estuarine and riverine systems have shown that it is possible to trace the source of terrestrial and aquatic matter to these systems, as well as, distinguish between trophic levels. However, carbon and nitrogen isotope analyses can sometimes produce ambiguous results. In some instances, stable isotope values of carbon from terrestrial sources overlap the carbon isotope values from aquatic sources making it difficult to determine the actual energy source being used by consumers (Jardine et al., 2009). Stable hydrogen isotopes may prove useful as a complement to these other isotopes.

It was hypothesized that hydrogen isotopes could be used to complement carbon and nitrogen isotope analysis in assessing the base of the food web fueling aquatic invertebrate trophic groups. Aquatic invertebrates were collected from three sites along a salinity gradient in Salmon River, identified, and sorted into functional feeding groups. Biofilm, particulate organic matter, and various aquatic and terrestrial plants were also collected from those three sites. These samples were sent for stable isotope analyses. The carbon, nitrogen, and hydrogen isotope values were compared to determine whether hydrogen values showed a separation between allochthonous and autochthonous sources and whether fractionation occurred between trophic levels.

It was determined through this study that hydrogen isotope analysis could be used along with carbon isotope values to distinguish between allochthonous and autochthonous input fueling the base of the food web. There was no distinction in hydrogen isotope

composition observed among trophic levels. Therefore, it could not be used to complement the results of the nitrogen isotope analysis.

This research helps build knowledge on boreal estuarine systems by offering insight into species abundance, their distribution patterns and C, N, and H isotope composition ranges. The results suggest the combined C, N, and H isotope analyses may provide a means to improve the study of trophic dynamics at the base of the boreal riverine-estuarine food web. By informing these new directions, this study may have significance for helping in the design of the detection and assessment of potential climate change through the identification of potential indicators of change.

5 References

Adler, P.H., Giberson, D.J., and Purcell, L.A. 2005. Insular black flies (Diptera: Simuliidae) of North America: Tests of colonization hypothesis. Journal of Biogeography, **32**: 211-220.

Amiro, B.D., Sobek, S., Bergström, A.K., Ågren, A., Tranvik, L.J., and Jansson, M. 2001. Roles of lakes for organic carbon cycling in the boreal zone. Global Change Biology, **10**: 141-147.

Arsuffi, T.L., and Suberkropp, K. 1989. Selective feeding by shredders on leaf-colonizing fungi: comparison of macroinvertebrate taxa. Oecologia, **79**(1): 30-37.

Attrill, M. 2002. A testable linear model for diversity trends in estuaries. Journal of Animal Ecology, **71**: 262-269

Axtell, R.C. 1976. Coastal horse flies and deer flies (Diptera: Tabanidae). *In* Marine Insects. *Edited by* L. Cheng. North Holland Publishers, Amsterdam. Pp. 415-446.

Badger, M.R., and Price, G.D. 2003. CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. Journal of Experimental Botany, **54**: 609-633.

Banfield, C.E. 1983. Climate. *In* Biogeography and Ecology of the Island of Newfoundland. *Edited by* G.R. South. Dr. W. Junk Publishers, The Hague. Pp. 37-106

Barbour, M.T., Gerritsen, J., Griffith, G.E., Frydenborg, R., McCarron, E., White, J.S., and Bastian, M.L. 1996. A framework for biological criteria for Florida streams using benthic macroinvertebrates. Journal of the North American Benthological Society, **15**: 185-211.

Bêche, L.A., and Resh, V.H. 2007. Short-term climatic trends affect the temporal variability of macroinvertebrates in California 'Mediterranean' streams. Freshwater Biology, **52**(12): 2317-2339.

Bennett, P., and Hobson, K. 2009. Trophic structure of a boreal forest arthropod community revealed by stable isotope ($\delta^{13}C$, $\delta^{15}N$) analysis. Entomological Science, **12**: 17-24.

Benoy, G., Cash, K., McCauley, E., and Wrona, F. 2007. Carbon dynamics in lakes of the boreal forest under changing climate. Environmental Reviews, **15**:175-189.

Benson, L.J., and Pearson, R.G. 1987. Drift and upstream movements of macroinvertebrates in a tropical Australian stream. Hydrobiologia, **153**: 225-239.

Bianchi, T. 2007. Biogeochemistry of Estuaries. Oxford University Press, London, UK. 720 pp.

Bonada, N., Prat, N., Resh, V.H., and Statzner, B. 2006. Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. Annual Review of Entomology, **51**: 495-523.

Bowden, J.J., and Buddle, C.M. 2010. Spider assemblages across elevational and latitudinal gradients in the Yukon Territory, Canada. Arctic, **63**(3): 261-272.

Boyero, L. 2002. Insect biodiversity in freshwater ecosystems: is there any latitudinal gradient? Marine Freshwater Research, **53**: 753-755.

Bradt, P., Urban, M., Goodman, N., Bissell, S., and Spiegel, I. 1999. Stability and resilience in benthic macroinvertebrate assemblages. Hydrobiologia, **403**: 123-133.

Cañedo-Argüelles, M., Grantham, T.E., Perrée, I., Rieradevall, M., Céspedes-Sánchez, R., and Prat, N. 2012. Response of stream invertebrates to short-term salinization: a mesocosm approach. Environmental Pollution, **166**: 144-151.

Cibrowski, J.H., Craig, D.A., and Fry, K.M. 1997. Dissolved organic matter as food for black fly larvae (Diptera: Simuliidae). Journal of the North American Benthological Society, **16**(4): 771-780.

Clarke, K., and Scruton, D. 1997. The benthic community of stream riffles in Newfoundland, Canada and its relationship to selected physical and chemical parameters. Journal of Freshwater Ecology, **12**: 113-121.

Clifford, H. 1991. Aquatic Invertebrates of Alberta. The University of Alberta Press, Edmonton, Alberta, Canada. 550 pp.

Coplen, T.B. 1996. New guidelines for reporting stable hydrogen, carbon, and oxygen isotope-ratio data. Geochimica et Cosmochimica Acta, **60**: 3359-3360.

Cortelezzi, A., Capítulo, A.R., Boccardi, L., and Arocena, R. 2007. Benthic assemblages of a temperate estuarine system in South America: transition from a freshwater to an estuarine zone. Journal of Marine Systems, **68**: 569-580.

Costa, S.S., and Melo, A.S. 2008. Beta diversity in stream macroinvertebrate assemblages: among-site and among-microhabitat components. Hydrobiologia, **598**: 131-138.

Costas, N., and Pardo, I. 2015. Isotopic variability in a stream longitudinal gradient: implications for trophic ecology. Aquatic Ecology, **77**: 231-260.

Cummins, K. 1973. Trophic relations of aquatic insects. Annual Review of Entomology, **18**: 183-206.

Cunha, M.R., and Moreira, M.H. 1995. Macrobenthos of *Potamageton* and *Myriophyllum* beds in the upper reaches of Canal de Mira (Ria de Aveiro, NW Portugal): Community structure and environmental factors. Netherlands Journal of Aquatic Ecology, **29**(3-4): 377-390.

Currie, D.J., Mittelbach, G.G., Cornell, H.V., Field, R., Guégan, J.F., Hawkins, B.A., Kaufman, D.M., Kerr, J.T., Oberdorff, T., O'Brien, E., and Turner, J.R.G. 2004. Predictions and tests of climate based hypotheses of broad-scale variation in taxonomic richness. Ecology Letters, **7**(12): 1121-1134.

Day, J., Hall, A., Kemp, W., and Yáñez-Arancibia, A. 1989. Estuarine Ecology. Wiley-Interscience, New York, USA. 576 pp.

Deines, P., Wooller, M., and Grey, J. 2009. Unravelling complexities in benthic food webs using a dual stable isotope (hydrogen and carbon) approach. Freshwater Biology, **54**: 2243-2251.

de Jong, V. 1974. Classification of brackish coastal inland waters. Hydrobiological Bulletin, **8**: 29-39.

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

Diaz, R.J. 1989. Pollution and tidal benthic communities of the James River Estuary, Virginia. Hydrobiologia, **180**: 195-211.

Díaz, S., and Cabibo, M. 2001. Vive la différence: plant functional diversity matters to ecosystem processes. Trends in Ecology and Evolution, **16**(11): 646-655.

Dohet, A., Ector, L., Cauchie, H., and Hoffmann, L. 2008. Identification of benthic invertebrates and diatom indicator taxa that distinguish different stream types as well as degraded from reference conditions in Luxembourg. Animal Biology, **58**:419-472.

Doucett, R.R., Marks, J., Blinn, D., Caron, M., and Hungate, B. 2007. Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. Ecology, **88**(6): 1587-1592.

Dyke, A.S., Andrews, J.T., Clark, P.U., England, J.H., Miller, G.H., Shaw, J., and Weillette, J.J. 2002. The Laurentide and Innuitian sheets during the last glacial maximum. Quaternary Science Reviews, **21**(1-3): 9-31.

Dytham, C. 2003. Choosing and Using Statistics: A Biologists Guide, 2nd Edition. Blackwell Publishing, Oxford, UK. 248 pp.

Elliot, M., and McLusky, D. 2002. The need for definitions in understanding estuaries. Estuarine, Coastal and Shelf Science, **55**: 815-827.

Environment Canada. 2016. 1981-2010 Climate Normals. http://climate.weather.gc.ca/climate_normals/results_1981_2010_e.html.

Estep, M., and Dabrowski, H. 1980. Tracing food webs with stable hydrogen isotopes. Science, **209**: 1537-1538.

Ferro, M.L., and Sites, R.W. 2007. The Ephemera, Plecoptera, and Trichoptera of Missouri State Parks, with notes on biomonitoring, mesohabitat associations, and distribution. Journal of the Kansas Entomological Society, **80**: 105-129.

Field, A. 2009. Discovering Statistics Using SPSS, 3rd Edition. Sage Publications, London, UK. 822 pp.

Finlay, J., and Kendall, C. 2007. Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems. *In* Stable Isotopes in Ecology and Environmental Science, 2nd Edition. *Edited by* R. Michener and K. Lajtha. Blackwell Publishing Ltd, Oxford, UK. Pp. 283-333.

Finlay, J., Doucett, R., and McNeely, C. 2010. Tracing energy flow in stream food webs using stable isotopes of hydrogen. Freshwater Biology, **55**: 941-951.

Flotemersch, J.E., Thorp, J.H., and Williams, B.S. 2010. Application of the Riverine Ecosystem Synthesis (RES) and the Functional Process Zone (FPZ) approach to EPA Environmental Mission Tasks for Rivers. U.S. Environmental Protection Agency, Washington, DC. EPA/600/R-11/089.

Flowers, R.W. 1991. Diversity of stream insects in Northwestern Panama. Freshwater Science, **10**(3): 322-334.

France, R. 1994. Nitrogen isotopic composition of marine and freshwater invertebrates. Marine Ecology Progress Series, **115**: 205-207.

Fry, B. 2006. Stable Isotope. Ecology. Springer-Verlag, New York, USA. 308 pp.

Fuller, B., Maildner, G., Van Neer, W., Ervynek, A., and Richards, M. 2012. Carbon and nitrogen isotope ratio analysis of freshwater, brackish, and marine fish from

Belgium archaeological sites (1st and 2nd millennium A.D.). Journal of Analytical Atomic Spectroscopy, **27**(5): 807-820.

Gaiser, E. 2009. Periphyton as an indicator of restoration in the Florida Everglades. Ecological Indicators, **9**(6): S37-S45.

Galbraith, H.S., Vaughn, C.C., and Meier, C.K. 2008. Environmental variables interact across spatial scales to structure Trichopteran assemblages in Ouachita Mountain rivers. Hydrobiologia, **596**: 401-411.

Garcia, A.M., Hoeinghaus, D.J., Vieira, J.P., and Winemiller, K.O. 2007. Isotopic variation of fishes in freshwater and estuarine zones of a large subtropical coastal lagoon. Estuarine and Coastal Shelf Science, **73**: 399-408.

Gaston, G., Rakocinski, C., Brown, S., and Cleveland, C. 1998. Trophic function in estuaries: response of macrobenthos to natural and contaminant gradients. Marine and Freshwater Research, **48**: 833-846.

Gaston, K.J. 2000. Global patterns in biodiversity. Nature, **405**: 220-227.

Government of Newfoundland and Labrador. 2016. http://www.gov.nl.ca/aboutnl/area.html. (accessed November 12, 2016).

Graça, M.A.S., Ferreira, R.C.F., and Coimbra, C.N. 2001. Litter processing along a stream gradient: the role of invertebrates and decomposers. Journal of the North American Benthological Society, **20**: 408-420.

Hart, B.T., Bailey, P., Edwards, R., Hortle, K., James, K., McMahon, A., Meredith, C., and Swadling, K. 1991. A review of the salt sensitivity of the Australian freshwater biota. Hydrobiologia, **210**: 105-144.

Hawkins, B.A., Field, R., Cornell, H.V., Currie, D.J., Guégan, J.F., Kaufmann, D.M., Kerr, J.T., Mittelbach, G.G., Oberdorff, T., O'Brien, E.M., Porter, E.E., and Turner, J.R.G. 2003. Energy, water, and broad-scale geographic patterns in species richness. Ecology, **84**(12): 3105-3117.

Hayden, B., McWilliam-Hughes, S., and Cunjak, R. 2016. Evidence for limited trophic transfer of allochthonous energy in temperate river food webs. Freshwater Science, **35**(2): 544-558.

Hedges, J.I., and Stern, J.H. 1984. Carbon and nitrogen determinations of carbonate-containing solids. Limnology and Oceanography, **29**(3): 657-663.

Hershey, A., Forting, K., Peterson, B., and Ulster, A. 2007. Stream food webs. *In* Methods in Stream Ecology. *Edited by* F. Hauer and G. Lamberti. Elsevier Inc., London, UK. Pp. 637 – 659.

Hillebrand, H. 2004. On the generality of the latitudinal diversity gradient. The American Naturalist **163**: 192-211.

Hoefs, J. 2009. Stable Isotope Geochemistry, 6th Edition. Springer-Verlag, Berlin, Heidelberg. 286 pp.

Hof, C., Brändle, M., and Brandl, R. 2008. Latitudinal variation of diversity in European freshwater animals not concordant across habitat types. Global Ecology and Biogeography **17**: 539-546.

Hoffman, A.A., and Blows, M.W. 1994. Species borders: ecological and evolutionary perspectives. Trends in Ecology and Evolution, **9**: 223-227.

Hondula, K., Pace, M., Cole, J., and Batt, R. 2014. Hydrogen isotope discrimination in aquatic primary producers: implications for aquatic food web studies. Aquatic Sciences, **76**: 217-229.

Horne, A.J., and Goldman, C.R. 1994. Limnology, 2nd Edition. McGraw-Hill Inc., New York, USA. 480 pp.

Hutchinson, G.E. 1957. Concluding Remarks. Cold Spring Harbor Symposia on Quantitative Biology, **22**: 415-427.

Hutchinson, G.E. 1959. Homage to Santa Rosalia, or Why are there so many kinds of animals? The American Naturalist, **93**(870): 145-159.

Jacobsen, D., Schultz, R., and Encalada, A. 1997. Structure and diversity of stream invertebrate assemblages: the influence of temperature with altitude and latitude. Freshwater Biology, **38**(2): 247-261.

Jardine, T., Kidd, K., and Cunjak, R. 2009. An evaluation of deuterium as a food tracer in temperate streams across eastern Canada. Journal of the North American Benthological Society, **28**(4): 885-893.

Kalff, J. 2002. Limnology: Inland Water Ecosystems. Prentice-Hall Inc., New Jersey, USA. 592 pp.

Kefford, B.J., Papas, P.J., Metzeling, L., and Nugegoda, D. 2004. Do laboratory salinity tolerances of freshwater animals correspond with their field salinity? Environmental Pollution, **129**: 355-362.

Kennedy, V. 1990. Anticipated effects of climate change on estuarine and coastal fisheries. Fisheries, **15**: 16-24.

Krebs, C.J. 2001. Ecology: The Experimental Analysis of Distribution and Abundance. 5th Edition. Benjamin Cummings, San Francisco, USA. 695 pp.

Khan, B., and Colbo, M. 2008. The impact of physical disturbance on stream communities: lessons from road culverts. Hydrobiologia, **600**: 229-235.

Lammert, M., and Allan, J.D. 1999. Assessing biotic integrity of streams: effects of scale in measuring the influence of land use/cover and habitat structure on fish and macroinvertebrates. Environmental Management, **23**: 257-270.

Larson, D.L., and Colbo, M.H. 1983. The aquatic insects: some biological considerations. *In* Biogeography and Ecology of the Island of Newfoundland. *Edited by* G.R. South. Dr. W. Junk Publishers, The Hague. Pp. 593-677.

Lawton, J. (1996). Patterns in ecology. Oikos, **75**(2): 145-147.

Levinson, B. (2005). Remote sensing and the EPA Estuarine and Great Lakes Research Program. International Journal of Remote Sensing, **26**(23): 5343-5346.

Lomond, T.M., and Colbo, M.H. 2000. Variations in lake-outlet Ephemeroptera, Plecoptera, and Trichoptera communities amongst regions of eastern Newfoundland, Canada. Canadian Journal of Zoology, **78**: 1536-1543.

MacArthur, R.H. 1955. Fluctuations of animal populations and a measure of community stability. Ecology, **36**: 533-536.

MacArthur, R.H. 1965. Patterns of species diversity. Biological Reviews, **40**: 510-533.

MacArthur, R.H., and Wilson, E.O. 1967. The Theory of Island Biogeography. Princeton University Press, New York, USA. 203 pp.

Macko, S., Lee, W., and Parker, P. 1982. Nitrogen and carbon isotope fractionation by two species of marine amphipods: laboratory and field studies. Journal of Experimental Marine Biology and Ecology, **63**: 145-149.

Macko, S., Estep, M., and Lee, W. 1983. Stable hydrogen isotope analysis of food webs on laboratory and field populations of marine amphipods. Journal of Experimental and Marine Biology and Ecology, **72**: 243-249.

Maddock, I. 1999. The importance of physical habitat assessment for evaluating river health. Freshwater Biology, **41**: 373-391.

Magdych, W.P. 1988. Salinity stresses along a complex river continuum: Effects on mayfly (Ephemeroptera) distributions. Ecology, **65**: 1662-1672

Magurran, A.E. 1988. Ecological Diversity and Its Measurement. Princeton University Press, Princeton, New Jersey, USA. 179 pp.

Marshall, J., Brooks, J., and Lajtha, K. 2007. Sources of variation in the stable isotopic composition of plants. *In* Stable Isotopes in Ecology and Environmental Science, 2nd Edition. *Edited by* R. Michener and K. Lajtha. Blackwell Publishing Ltd., Oxford, UK, Pp. 22-60.

Marshall, N.A., and Bailey, P.C.E. 2004. Impact of secondary salinization on freshwater ecosystems: effects of contrasting experimental, short-term releases of saline wastewater on macroinvertebrates in a lowland stream. Marine and Freshwater Research, **55**: 509-523.

McClelland, J., Valiela, I., and Michener, R. 1997. Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal wetlands. Limnology and Oceanography, **42**(5): 930-937.

McCune, B., and Mefford, M.J. 2006. PC-ORD. Multivariate Analysis of Ecological Data. Version 5.10. MjM Software, Gleneden Beach, Oregon, USA.

McKinney, C.R., McCrea, J.M., Epstein, S., Allen, H.A., and Urey, H.C. 1950. Improvements in mass spectrometers for the measurement of small differences in isotope abundance ratios. Review of Scientific Instruments, **21**: 724-730.

Merritt, R., and Cummins, K. 1996. An Introduction to the Aquatic Insects of North America. 3rd Edition. Kendal/Hunt Publishing Co., Dubuque, Iowa, USA. 862 pp.

Merritt, R., and Cummins, K. 2007. Trophic relationships of macroinvertebrates. *In* Methods in Stream Ecology. *Edited by* F. Hauer and G. Lamberti. Elsevier Inc., London, UK. Pp. 585 – 609.

Metzeling, L. 1993. Benthic macroinvertebrate community structure in streams of different salinities. Australian Journal of Marine and Freshwater Research, **44**: 335-351.

Metzeling, L., Robinson, D., Perriss, S., and Marchant, R. 2002. Temporal persistence of benthic invertebrate communities in south-eastern Australian streams: taxonomic resolution and implications for the use of predictive models. Marine and Freshwater Research, **53**: 1223-1234.

Meyer, J.L., and O'Hop, J. 1983. Leaf-shredding insects as a source of dissolved organic carbon in headwater streams. American Midland Naturalist, **109**: 175-183.

Michaelis, H., Fock, H., Grotjahn, M., and Post, D. 1992. The status of the intertidal zoobenthic brackish-water species in estuaries of the German Bight. Netherlands Journal of Sea Research, **30**: 201-207.

Miller, R.L., Bradford, W.L., and Peters, N.E. 1988. Specific conductance: Theoretical considerations and application to analytical quality control. U.S. Geological Survey Water-Supply Papers, **2311**.

Minagawa, M., and Wada, E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta, **48**: 1135-1140.

Naddafi, R., Blenckner, T., Eklöv, P., and Pettersson, K. 2011. Physical and chemical properties determine zebra mussel invasion success in lakes. Hydrobiologia, **669**: 227-236.

Nanami, A., Saito, H., Akita, T., and Kuwahara, H. 2005. Spatial distribution and assemblage structure of macrobenthic invertebrates in a brackish lake in relation to environmental variables. Estuarine, Coastal and Shelf Science, **63**(1): 167-176.

Neff, M.R., and Jackson, D.A. 2011. Effects of broad-scale geological changes on patterns in macroinvertebrate assemblages. Journal of the North American Benthological Society, **30**(2): 459-473.

Newton, J. 2010. Stable Isotope Ecology. *In* Encyclopedia of Life Sciences. John Wiley & Sons Ltd, Chichester. http://www.els.net [doi: 10.1002/9780470015902.a0021231] (accessed June 12, 2015).

Nicholls, R.J., Wong, P.P., Burkett, V.R., Codignotto, J.O., Hay, J.E., McLean, R.F., Ragoonaden, S., and Woodroffe, C.D. 2007. Coastal systems and low-lying areas. *In* Climate Change 2007: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. *Edited by* M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden, and C.E. Hanson. Cambridge University Press, Cambridge, UK. Pp. 315-356.

Olson, J.R., 2012. The influence of geology and other environmental factors on stream water chemistry and benthic invertebrate assemblages. Utah State University, Logan, Utah. All Graduate Theses and Dissertations. Paper 1327. http://digitalcommons.usu.edu/ (accessed October 12, 2016).

Pearson, R.G., and Boyero, L. 2009. Gradients in regional diversity of freshwater taxa. Journal of the North American Benthological Society, **28**: 504-514.

Penzias, A.A. 1979. The origin of the elements. Science, **205**:549-554.

Peterson, B., and Fry, B. 1987. Stable Isotopes in Ecosystem Studies. Annual Review of Ecology and Systematics, **18**: 293-320.

Piscart, C., Lecerf, A., Usseglio-Polatera, P., Moreteau, J.C., and Beisel, J.N. 2005a. Biodiversity patterns along a salinity gradient: the case of the net-spinning caddisflies. Biodiversity and Conservation **14**: 2235-2249.

Piscart, C., Moreteau, J.C., and Beisel, J.N. 2005b. Biodiversity and structure of macroinvertebrate communities along a small permanent salinity gradient (Meurthe River, France). Hydrobiologia, **551**: 227-236.

Post, D. 2002. Using stable isotopes to estimate trophic position: Model, methods, and assumptions. Ecology, **83**(3): 703-718.

Power, M., and Dietrich, W. 2002. Food webs in river networks. Ecological Research, **17**: 451-471.

Prandle, D., and Lane, A. 2015. Sensitivity of estuaries to sea level rise: vulnerability indices. Estuarine, Coastal and Shelf Science, **160**: 60-68.

Pritchard, D. 1967. What is an estuary: physical viewpoint. *In* Estuaries. *Edited by* G. Lauff. American Association for the Advancement of Science. Publication Number 83, Washington, D.C. Pp 3-5.

Quinn, G.P., and Keough, M.J. 2009. Experimental Design and Data Analysis for Biologists, 9th Edition. Cambridge University Press, Cambridge. 537 pp.

Rao, A., Outhouse, L., and Gregory, D. 2009. Special marine areas in Newfoundland and Labrador. Canadian Parks and Wilderness Society – Newfoundland and Labrador Chapter, St. John's, Newfoundland.

Rau, G.H. 1980. Carbon-13/Carbon-12 variation in subalpine lake aquatic insects: food source implications. Canadian Journal of Fisheries and Aquatic Sciences, **37**:742-746.

Raymond, P.A., Saiers, J.E., and Sobczak, W.V. 2016. Hydrological and biochemical controls on watershed dissolved organic matter transport: pulse-shunt concept. Ecology, **97**(1): 5-16.

Remane, A., and Schlieper, C. 1970. The Biology of Brackish Waters. Wiley Interscience, New York, USA. 372 pp.

Righi-Cavallaro, K.O., Spies, M.R., and Siegloch, A.E. 2010. Ephemeroptera, Plecoptera and Trichoptera assemblages in Miranda Basin, Mato Grosso do Sul State, Brazil. Biota Neotropica, **10**(2): 253-261.

Ristich, S.S., Crandall, M., and Fortier, J. 1977. Benthic and epibenthic macroinvertebrates of the Hudson River. I. Distribution, natural history and community structure. Estuarine and Coastal Marine Science, **5**(2): 255-266.

Robson, H., Andersen, S., Clarke, L., Craig, O., Gron, K., Jones, A., Karston, P., Milner, N., Price, T., Ritchie, K., Zabilskia-Kunek, M., and Heron, C. 2015. Carbon and nitrogen stable isotope values in freshwater, brackish and marine fish bone collagen from Mesolithic and Neolithic sites in central and northern Europe. Environmental Archaeology, **21**(2): 105-118.

Rogerson, R.J. 1983. Geological Evolution. *In* Biogeography and Ecology of the Island of Newfoundland. *Edited by* G.R. South. Dr. W. Junk Publishers, The Hague. Pp. 5-35.

Rosenberg, D.M., and Resh, V.H. 1993. Introduction to freshwater biomonitoring and benthic macroinvertebrates. *In* Freshwater Biomonitoring and Benthic Macroinvertebrates. *Edited by* Rosenberg, D.M., and Resh, V.H. Chapman/Hill, New York, USA. Pp. 1-9.

Rundle, S.D., Attrill, M.J., and Arshad, A. 1998. Seasonality in macroinvertebrate community composition across a neglected ecological boundary, the freshwater-estuarine transition zone. Aquatic Ecology, **32**: 211-216.

Sanders, H.L., Mangelsdorf, P.C., and Hampson, G.R. 1965. Salinity and faunal distribution in the Pocasset River, Massachusetts. Limnology and Oceanography, **10**: R216-R229.

Schmider, E., Ziegler, M., Danay, E., Beyer, L., and Bühner, M. 2010. Reinvestigating the robustness of ANOVA against violations of the normal distribution assumption. Methodology, **6**(4): 147-151.

Schmidt, S., Olden, J., Solomon, C., and Vander Zanden, M. 2007. Quantitative approaches to the analysis of stable isotope food web data. Ecology, **88**(11): 2793-2802.

Schoeninger, M., and DeNiro, M. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochimica et Cosmochimica Acta, **48**, 625-639.

Scott, R.W., Barton, D.R., Evans, M., and Keating, J. 2011. Latitudinal gradients and local control of aquatic insect richness in a large river system in northern Canada. Journal of the North American Benthological Society, **30**(3): 621-634.

Siegenthaler, U. 1979. Stable hydrogen and oxygen isotopes in the water cycle. *In* Lectures in Isotope Geology. *Edited by* E. Jäger and J. Hunziker. Springer-Verlag, Berlin, Heidelberg. Pp. 264-273.

Smith, E. 2009. Newfoundland macroinvertebrate riffle communities and their potential for use in bioassessment. Master of Science Thesis, Memorial University of Newfoundland. http://research.library.mun.ca/9281/1/Smith_EricaL.pdf (accessed June 21, 2016).

Smith, E., Coté, D., and Colbo, M. 2013. An impoverished benthic community shows regional distinctions. Northeastern Naturalist, **20**(1): 91-102.

Smith, B.N. and Ziegler, H. 1990. Isotopic fractionation of hydrogen in plants. Botanica Acta, **103**(4): 335-342.

Smyth, K., and Elliott, M. 2016. Effects of changing salinity on the ecology of the marine environment. *In* Stressors in the Marine Environment: Physiological and Ecological Responses; Societal Implications. *Edited by* M. Solan and N. Whiteley. Oxford University Press, Oxford, UK. Pp. 161-174.

Snoeyink, V.L., and Jenkins, D. 1980. Water Chemistry. John Wiley & Sons, New York, USA. 463 pp.

Solomon, C.T., Cole, J.J., Doucett, R.R., Pace, M.L., Preston, N.D., Smith. L.E., and Weidel, B.C. 2009. The influence of environmental water on the hydrogen stable isotope ratio in aquatic consumers. Oecologia, **161**: 313-324.

South, G.R. 1983. Biogeography and Ecology of the Island of Newfoundland. Dr. W. Junk Publishers, The Hague. 723 pp.

Stanford, J.A., and Ward, J.V. 1983. Insect species diversity as a function of environmental variability and disturbance in stream systems. *In* Stream Ecology. Application and Testing of Ecological Theory. *Edited by* J.R. Barnes and G.W. Minshall. Plenum Press, New York, USA. Pp. 265-278.

Sulzman, E. 2007. Stable isotope chemistry and measurement: a primer. *In* Stable Isotopes in Ecology and Environmental Science, 2nd Edition. *Edited by* R. Michener and K. Lajtha. Blackwell Publishing, Oxford, UK. Pp. 1-21.

Telesh, I., and Khlebovich, V. 2010. Principal processes within the estuarine salinity gradient: a review. Marine Pollution Bulletin, **61**: 149-155.

ter Braak, C.J.F. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology, **67**: 1167-1179.

ter Braak, C.J.F., and Verdonschot, P.F.M. 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquatic Sciences, **57**: 255-289.

Teske, P.R., and Wooldridge, T.H. 2003. What limits the distribution of subtidal macrobenthos in permanently open and temporarily open/closed South African estuaries? Salinity vs. sediment particle size. Estuarine, Coastal and Shelf Science, **57**: 225-238.

Thorp, J.H., Thoms, M.C., and Delong, M.D. 2006. The Riverine Ecosystem Synthesis: biocomplexity in river networks across space and time. River Research and Applications, **22**: 123-147.

Vander Zanden, H.B., Soto, D.X., Bowen, G.J., and Hobson, K.A. 2016. Expanding the isotopic toolbox: Applications of hydrogen and oxygen stable isotope ratios to food web studies. Frontiers in Ecology and Evolution, **4**:20. Doi: 10.3389/fevo.2016.00020.

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

Verhoeven, J.T.A. 1980. The ecology of *Ruppia*-dominated communities in western Europe. III. Aspects of production, consumption and decomposition. Aquatic Botany, **8**: 209-253.

Vinson, M.R., and Hawkins, C.P. 2003. Broad-scale geographic patterns in local stream insect genera richness. Ecography, **26**(6): 751-767.

Voelz, N.J., Shieh, S-H., and Ward, J.V. 2000. Long-term monitoring on benthic macroinvertebrate community structure: a perspective from a Colorado River. Aquatic Ecology, **34**: 261-278.

Voshell, J. 2002. A Guide to Common Freshwater Invertebrates of North America. McDonald and Woodland Publishing Co., Blacksburg, Virginia. 442 pp.

Wallace, B.J., and Anderson, N.H. 1996. Habitat, life history, and behavioral adaptations of aquatic insects. *In* An Introduction to the Aquatic Insects of North America. *Edited by* R.W. Merritt and K.W. Cummins. Kendall/Hunt, Dubuque, Iowa, USA. Pp. 41-73.

Wentworth, C.K. 1922. A scale grade and class terms for clastic sediments. The Journal of Geology, **30**(5): 377-392.

Wetzel, R.G. 2001. Limnology: Lakes and River Ecosystems. 3rd Edition. Academic Press, San Diego, California, USA. 1006 pp.

Williams, D., and Feltmate, B. 1992. Aquatic Insects. C.A.B. International, Wallingford, Oxfordshire, UK. 358 pp.

Williams, D.D., and Williams, N.E. 1998. Aquatic insects in an estuarine environment: densities, distribution and salinity tolerance. Freshwater Biology, **39**: 411-421.

Willig, M.R., Kaufmann, D.M., and Stevens, R.D. 2003. Latitudinal gradients of biodiversity: pattern, process, scale and synthesis. Annual Review of Ecology, Evolution and Systematics, **34**: 273-309.

Winemiller, K., Hoeinghaus, D., Pease, A., Esselman, P., Honeycut, R., Gbanaador, D., Carrera, E., and Payne, J. 2011. Stable isotope analysis reveals food web structure and watershed impacts along the fluvial gradient of a mesoamerican coastal river. River Research and Applications, **27**: 791-803.

Ysebaert, T., Meire, P., Maes, D., and Buijs, J. 1993. The benthic macrofauna along the estuarine gradient of the Schelde Estuary. Netherlands Journal of Aquatic Ecology, **27**: 327-341.

Zwick, P. 1986. The Bornean species of the stonefly genus *Neoperla* (Plecoptera: Perlidae). Aquatic Insects, **8**: 1-53.