NEWFOUNDLAND MACROINVERTEBRATE RIFFLE COMMUNITIES AND THEIR POTENTIAL FOR USE IN BIOASSESSMENT

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By

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Abstract

Macroinvertebrate communities were sampled from 65 Newfoundland riffles from three geographic regions (the Avalon Peninsula, Terra Nova, and Gros Morne) in three seasons (summer, fall, spring). A suite of physical, chemical and land use variables were also measured. Differences in community composition across regions were found as well as large differences among seasons between sites sampled. Macroinvertebrate richness and abundance data were cross-examined with associated environmental variables to detect which ones were most related to macroinvertebrate community differences. UTM Easting, % macrophytes, % igneous rock, % local forest, nitrates, total Nitrogen and alkalinity were all highly correlated with trends in the macroinvertebrate community data. Urban communities differed from rural and pristine communities, the latter two community types being virtually indistinguishable. Temporal effects were examined to tease apart seasonal versus non-seasonal factors affecting the invertebrate-environment relationship. Changes in the frequency of occurrence of a few key taxa between years strongly impacted regional differences.

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1 Introduction

Humans depend heavily on rivers for a broad range of services: drinking water, irrigation, industrial production, waste disposal, fishing for food and recreation. Therefore, maintaining healthy aquatic habitats should be a matter of great concern (Karr 1991; Niemi and McDonald 2004; Bonada et al. 2006). Ironically, many of humanity's activities have detrimental impacts on the Earth's watersheds. To assess these impacts and prompt mitigating efforts, if necessary, tools are needed that will permit the evaluation of the effects of human activity on the health of the river ecosystem. A healthy aquatic ecosystem is one that is stable, resilient, clear of pollutants and maintains high diversity and evenness in its biological systems (Haskell et al. 1992). Biologists have developed surrogates to serve as indicators of environmental health (Karr 1999; Niemi and McDonald 2004). These surrogates are known as *bioindicators*.

1.1 Invertebrates as bioindicators

The impact of humans on water quality has been studied for decades using physical, chemical and biological characteristics. Biological monitoring has gained popularity since the 1980s for many reasons (summarized by Rosenberg and Resh 1993). First, the biological realm can reflect the integrity of the physical and chemical conditions without expensive surveys *e.g.* toxicity tests (Karr 1991; Barbour et al. 1999). Second, organisms accumulate impacts over time and therefore provide more than just a single point measure of environmental status (Wallace and Webster 1996; Barbour et al. 1999). Third, biological organisms also react to synergistic effects that would be missed if the

chemistry and physical attributes of the stream were measured separately. Finally, using organisms enhances public interest and facilitates involvement in water chemistry studies and remedial measures (Barbour et al. 1999).

Macroinvertebrates have often been the organism of choice when studying stream health because they are intrinsically linked to ecosystem processes. Invertebrates can be important to the nutrient cycling within the stream, and so may be the first to indicate disturbance (Wallace and Webster 1996). For example, Richardson (1993) suggested that the amount of fish that can be sustained by a particular stream is limited by the abundance of benthic invertebrates, rather than the abundance of phytoplankton or piscivores. In addition, concurrently collected fish and invertebrate samples have been found to produce identical responses to some stresses, and the use of macroinvertebrates to evaluate the health of fish and other taxonomic groups has been encouraged (Ormerod et al. 1987; Kilgour and Barton 1999; Mebane 2001).

Invertebrates can change water flow patterns in small streams by their feeding and retreat building (Wallace and Webster 1996). These natural activities in turn re-suspend particles and retain organic matter, hence influencing downstream communities and processes. Grazers and shredders can increase the movement of toxins downstream as contaminants in the plant matter they feed on are released through egestion (Sallenave et al. 1994). However, filter-feeding insects can be bioaccumulators of some pollutants, effectively removing toxins from the ecosystem cycle until the filter-feeders themselves are consumed (Wallace and Webster 1996).

Several characteristics about benthic macroinvertebrates make them suitable for study. First of all, many species are widely distributed among habitats and regions, which aids in comparing habitats across regions (Hellawell 1978; Barbour et al. 1999). Secondly, there is a large number of species and therefore presumably more niche differentiation and specialization than in communities with fewer taxonomic entities, thereby increasing the sensitivity of richness-based tests (Wiggins and Mackay 1978; Merritt and Cummins 1996; Barbour et al. 1999). Thirdly, macroinvertebrates are generally sessile and relatively long-lived compared to algae and microcrustacea. Therefore, their composition and health can reflect the accumulated impact of perturbations in the area (Hellawell 1978). Other benefits include: ease of qualitative sampling and analysis (Hellawell 1978; Norris and Georges 1993; Resh and Jackson 1993; Barbour et al. 1999), well-documented taxonomy for many major groups (Morihara and McCafferty 1979; Merritt and Cummins 1996; Wiggins 1996), and new methods of data analysis that are built specifically for deriving explanations from biomonitoring studies (Barbour and Yoder 2000; Resh et al. 2000; Bowman and Somers 2006). All these characteristics make macroinvertebrates an obvious choice for rapid bioassessment techniques in streams.

Several studies have compared conclusions gained from macroinvertebrate analyses to those determined from other taxonomic groups such as diatoms, macrophytes and fish. Such studies found that macroinvertebrates are highly sensitive to disturbance (Griffith et al. 2005); their sensitivity was higher than that of periphyton (Mazor et al. 2006) and diatoms (Sonneman et al. 2001). Macroinvertebrates were also more sensitive

than periphyton at indicating metal pollution (Griffith et al. 2005) and were as efficient as diatoms (Hirst et al. 2002). Johnson et al. (2006a; 2006b) showed that macroinvertebrates and diatoms had lower error frequencies than fish and macrophytes, and macroinvertebrates had the strongest response to catchment land use, nutrient concentration and habitat quality. They also acted as good early warning indicators for lowland rivers (Johnson et al. 2006b).

1.2 Using macroinvertebrate community data

There are many ways to utilize macroinvertebrate data. One of the most popular methods is to condense abundance or count data of macroinvertebrate communities into a single, meaningful index or metric (Norris and Georges 1993; Lenat and Barbour 1994; Downes et al. 2002). There are three main types of indices: diversity, biotic and similarity (Washington 1984). Diversity indices generally represent the relationship between the number of taxa and the number of individuals in each taxon in a given community. They may be weighted to increase the sensitivity to rare species, or even utilize numbers of food web interactions to increase reliability of the index (Washington 1984). Possibly the most popular index of all is the diversity index developed concurrently by Shannon and Wiener, now known as the Shannon-Wiener index or H′ (Washington 1984; Resh and Jackson 1993; Resh and McElravy 1993). Strangely enough, this index is also considered one of the most dubious methods of describing a biotic community, due to the lack of knowledge of what H′ really means biologically (Washington 1984). Diversity indices as a whole are generally criticized due to their relative inability to indicate water quality and

the predisposition of researchers to simply use them as "magic bullets" in assessments (Washington 1984). However, simple richness metrics (as a subset of diversity measures) such as total taxa richness, Ephemeroptera Plecoptera Trichoptera (EPT) taxa richness and Chironomid taxa richness have proven useful and sensitive to water quality differences (Resh and Jackson 1993; Lenat and Barbour 1994).

Biotic indices are typically formed around specific taxa whose presence or abundance reflect different and specific types of pollutants or disturbance in a particular area (Washington 1984; Johnson et al. 1993). These indices are geographically limited in their application and should be used with extreme caution in areas outside of where they were developed. However, they are popular because they are effective at detecting impacts in their home regions (Washington 1984; Norris and Georges 1993; Downes et al. 2002). For example, the Hilsenhoff Biotic Index (Hilsenhoff 1977) and the Family Biotic Index (Hilsenhoff 1988) focus on organic pollution of rivers in the mid-western United States and therefore are applicable only to those locations unless modified, as they were from Chutter's South African index (Chutter 1972; Hilsenhoff 1987; Hilsenhoff 1988; Resh and Jackson 1993).

Similarity indices measure how closely related sites or samples are to one another. Their cousins, dissimilarity indices, work on the same principle; they are a measure of how different sites/samples are (Washington 1984). There are fewer commonly used similarity indices than diversity or biotic indices, though each type tends to be useful for certain types of data. For example, Jaccard's Index is predominantly used for presence/absence data (Dyer 1978; Washington 1984; Norris and Georges 1993; Lorenz

and Clarke 2006). Various authors have found Bray-Curtis and Percent Similarity indices to be very useful in separating niches in communities (Norris and Georges 1993). However, the Percent Similarity Index fails when the abundance between sites does not change, but the species present in those sites change with disturbance (Whittaker 1952; Dyer 1978). Euclidean measures of similarity are criticized for weighting predominant taxa unfairly. All similarity indices are subject to difficulties associated with appropriate transformation of the data (Washington 1984; Norris and Georges 1993).

Differences in the estimated value of metrics among sites can be assessed individually with ANOVA and other univariate analyses, but in bioassessment studies, they are usually taken as groups in what is known as multimetric analysis (Norris and Georges 1993; Bonada et al. 2006). The multimetric method involves two steps: first, selecting and calibrating metrics to work together with classes of sites to create a composite index, and second, creating index thresholds to assess water quality at sites and make a decision as to their health (Barbour et al. 1999; Smith et al. 2005). The creation of site classes or conditions is most often determined *a priori* in multimetric analysis, though that is not always the case (Barbour et al. 1999). The multimetric method is also simple to perform and easy to understand for those who are not well-versed in statistical analysis, for it condenses biological data into a single number, or at least fewer numbers than in multivariate analyses. The end index from the multimetric analysis can be used to establish biocriteria (Gerritsen 1995; Barbour et al. 1999; Lewis et al. 2001), which allows managers to easily set numerical boundaries for water quality assessments.

There are several concerns with the multimetric method, though it has been shown to correctly detect human impacts (Hering et al. 2004). Firstly, *a priori* site classification by environmental data can introduce bias, through classing sites in the wrong groups (Norris 1995). For example, ecoregions are typically used to separate sites prior to analysis, but there is little evidence that sites within ecoregions are closely related (Corkum 1991; Hughes et al. 1994). Also, the thresholds for each bioassessment index are created artificially by using quartiles to indicate which sites are significantly impacted (Gerritsen 1995; Hannaford and Resh 1995). These thresholds may or may not be ecologically significant for that region. Another problem is that condensing the number of metrics can cause important information to be lost. In addition, the ones retained may be redundant, which only confounds the results and compounds error. High amounts of variation in the data can also confound the interpretation of results (Reynoldson et al. 1997).

Most current studies that use biological data to separate sites into groups for purposes of water quality comparison tend to use multivariate analyses (Wright et al. 1984; Reynoldson et al. 1995; Rosenberg et al. 2000). Multivariate analyses are characterized by the use of changes in macroinvertebrate community composition and environmental variables to detect human impact on sites (Bonada et al. 2006). By this method, site groups are typically formed *a posteriori*, as opposed to the predominantly *a priori* methodology of multimetrics (Barbour et al. 1999; Bonada et al. 2006). An important benefit to multivariate statistical models is that variability among reference sites is accounted for through inclusion of all data as separate, independent metrics

(Reynoldson et al. 1997). In addition, there is great predictive power in the method as ordinations allow taxa to be placed alongside environmental gradients, making correlations between macroinvertebrates and their environment much clearer (Furse et al. 1984; Wright et al. 1984; Moss et al. 1987; Johnson et al. 1993; Hirst et al. 2002; Johnson et al. 2006b). New software is constantly being created to better respond to the needs of ecological studies and increase interpretation by non-statisticians (Reynoldson et al. 1997; Bowman and Somers 2006).

The main problem with multivariate analysis remains that the initial statistics are still very complex, so training and specialized statistical programs are required before successfully implementing multivariate analyses into a study (Gerritsen 1995; Norris 1995; Reynoldson et al. 1997). In addition, many models only utilize presence-absence data, causing information to be lost for lack of quantitative analysis (Reynoldson et al. 1997). The multivariate method tends to remove sites or variables where there are data missing (Norris 1995). Due to the assumption of the model that all data collected are pertinent to the separation of sites, many environmental parameters must be measured, then weeded through to see which ones actually indicate cleanliness in a region (Wright et al. 1984; Norris 1995). Multivariate analysis also does not account for the fact that a test site may be attributed to the wrong test group when utilizing a method with *a priori* classifications (Reynoldson et al. 1997; Mazor et al. 2006).

Using any of these methods is risky when one wishes to make some sort of statement about water quality or impact without a broad database encompassing a wide range of habitats with known characteristics from the region in question. Indices and

models used incorrectly can and will give spurious results, perhaps leading to erroneous conclusions. The only way to gain reliable results is to understand how each element of the data analysis works so as to utilize the best analysis for the data available.

1.3 Current biomonitoring systems

1.3.1 Rapid bioassessment method

The recommended approach of rapid bioassessment methods for wadeable streams is to gather environmental and habitat data from the sample site, and then collect invertebrates via kick-net or sweep sampling (Barbour et al. 1999; Reynoldson et al. 2003). A subsample of 100-300 macroinvertebrates is removed from the sample and identified to a defined taxonomic level. Both subsampling and identification may occur in the field or in the laboratory, but these elements (as well as the level of identification) have not been standardized (Barbour et al. 1999). The data collected are then used to form various metrics pertaining to the structure and function of the community (Hannaford and Resh 1995; Barbour et al. 1999; Reynoldson et al. 2003) or placed in multivariate matrices (Reynoldson et al. 1995; Wright 1995; Verdonschot 2006), both of which can be analyzed to reveal the relative environmental condition of the sample site.

There are many advantages to this method of sampling the stream community. Hannaford and Resh (1995) maintain that without improving the habitat surrounding the stream site, water quality cannot be improved. Therefore, by including habitat data in the analysis, disturbance in the surrounding area is accounted for and the predictive power of the model increases. Another advantage to this technique is the ease and low level of expertise required to collect invertebrate samples, which reduces the resources needed to

assess the stream. Therefore, more sites can feasibly be sampled due to the decreased effort in the field and in the lab (Resh and Jackson 1993; Hannaford and Resh 1995).

There are, however, many disadvantages and limits to the rapid bioassessment method. The first is a lack of quality assurance and/or quality control as only one sample may be taken at a site (Hannaford and Resh 1995). Seasonality of invertebrates and physical factors affects accuracy, which makes applying the results of a study to other regions or seasons difficult (Resh and Jackson 1993; Vinson and Hawkins 1998; Downes et al. 2002).

It is true that a broad-scale study will only show which rivers are in poorer conditions than others, but the method is not limited to large-scale, broad-ranged studies. Rapid bioassessment techniques can be used in studies attempting to rank streams, produce impact assessments for legal documentation, or as end points in water quality monitoring programs, but usually not all three at the same time (Hannaford and Resh 1995; Downes et al. 2002). Depending on the focus of the study, this technique can be used to perform any of these tasks as long as variability is acknowledged (Hannaford and Resh 1995). There are still other difficulties to overcome when choosing sites to represent the unimpacted condition against which test sites are compared. This collection of unimpacted sites is known as the reference condition.

1.3.2 The reference condition

The reference condition has been described as "the condition that is representative of a group of minimally disturbed sites organized by selected physical, chemical, and biological characteristics" (Reynoldson et al. 1997). Therefore, the reference condition is

considered a baseline against which a test site is compared. The reference condition methodology allows the researcher to study streams where point-source problems do not exist and where other methods, such as the Before After Control Impact (BACI) design (Stewart-Oaten and Bence 2001), may not be appropriate. Reference sites are selected based on low levels of physical, chemical and biological disturbance (Reynoldson et al. 1997). Each reference site is considered a replicate, instead of many samples within a site, which satisfies the complaint of Hurlbert (1984) on pseudoreplication in ecological studies (Reynoldson et al. 1997). In addition, this method can be used to determine the natural fluctuations in physical, chemical and biological parameters in different streams, the study region, and a broader inter-regional scale (e.g. global warming effects) (Hughes 1995; Reynoldson et al. 1997).

1.3.3 Current bioassessment protocols

There are almost as many protocols as there are countries practicing water quality assessment in the world today. Most have been developed after intensive studies have been performed to lay the framework for a set of methodologies that provide accurate results for that region (e.g. RIVPACS, Wright 1995). The following is a listing and short description of a few of the most prominent bioassessment systems currently in use.

The River InVertebrate Prediction And Classification System (RIVPACS) has been the main system for predicting water quality in the UK since its creation (Wright 1995). It utilized macroinvertebrate data from (initially) 268 sites and allowed the biota to separate sites using two-way indicator species analysis (TWINSPAN). Environmental variables were then correlated to multivariate ordination scores created by the

macroinvertebrates, by which a reduced number of environmental variables were found to relate to the classification of rivers (Wright et al. 1984). TWINSPAN proved to have difficulties with assigning new sites to groups, due to the fairly small number of species causing the differences between groups, therefore the procedures were updated in RIVPACS II (Wright 1995). The current program predicts the macroinvertebrate community of a new site using the new site's environmental data. If the site meets the expected composition, then the site is considered healthy, if not, then the site is classed as "stressed" (Wright 1995). The Australian River Assessment System (AUSRIVAS) in Australia (Coysh et al. 2000) and the PERLA system in Czech Republic (Kokes et al. 2006) are based on this system of bioassessment as well, each with their own regional adjustments.

The STAR-AQEM system is a standardized multimetric method of water quality assessment put into place by eight countries of the European Union for the purpose of meeting the European Union Water Framework Directive (WFD) (Hering et al. 2004; Clarke and Hering 2006). The method uses ecoregions and stream typologies to break the streams into groups prior to macroinvertebrate sampling, then uses the macroinvertebrates and/or abiotic factors to further classify the streams into specific types according to disturbance. Metrics that correlate with stream degradation are then used to calibrate assessment systems so as to be able to give a definition of "high, good, moderate, poor, and bad ecological status for the selected stream types" (Hering et al. 2004). However, the typology portion of the method in particular is under heavy scrutiny for being too restrictive and biased, as opposed to using reference conditions

(Davy-Bowker et al. 2006; Verdonschot 2006). This method is still under constant review by the authors, who seek to further reduce error and increase accuracy (Clarke and Hering 2006; Clarke et al. 2006a; Clarke et al. 2006b).

1.3.4 CABIN: the Canadian perspective

The purpose of the Canadian Aquatic Biomonitoring Network (CABIN) was to take long-term studies done in the Great Lakes and the Fraser River in British Columbia and develop a national reference condition for biological assessment using benthic macroinvertebrates (Reynoldson et al. 2003). The method uses the reference condition approach (Reynoldson et al. 1997) and a suite of diversity and biotic indices together with multivariate analysis (Reynoldson et al. 2003). Environment Canada's National Water Research Institute supports the methods set out by the CABIN protocols and invites the sharing of data via the internet by making space for researchers to post their data and findings for comparison to other studies (Reynoldson et al. 2003).

1.3.5 Biomonitoring in Newfoundland

There are concerns about the usefulness of monitoring benthic macroinvertebrates in Newfoundland streams due to the impoverished fauna of the Island (Larson and Colbo 1983). A few studies have addressed the question of whether conditions in Newfoundland are suitable for a biomonitoring program, however, all the studies came to the same conclusion: Newfoundland's biota is sufficient to detect impacts on the environment (Colbo 1993; Ryan et al. 1993; Colbo et al. 1999; Lomond 1997). Lomond (1997) found that Ephemeroptera, Plecoptera and Trichoptera groups alone were able to detect water

quality changes brought about by urbanization and Colbo et al. (1999) were able to correlate several taxa with levels of disturbance and impact. These reports are encouraging, but long-term monitoring is still required to answer more detailed questions about the factors influencing distribution of Newfoundland macroinvertebrate taxa (Ryan et al. 1993). Land use occurs throughout the province; most watersheds are affected by logging, mining, farming or residential and recreational uses. In addition, globally transported air pollutants and climate change influence all regions of the Earth. Given these larger, ever-changing environmental conditions, it is imperative the methodologies for monitoring are tested and modified (if required) for Newfoundland's unique fauna and environment to provide clearer data with which to monitor and assess the province's stream health over time.

CABIN sampling was performed in Newfoundland in 2002-2003 as part of a stream health survey initiated by Parks Canada in partnership with Memorial University of Newfoundland (Colbo et al. submitted). Findings indicated a separation of sites on the basis of human disturbance, not unlike previous studies. Therefore CABIN, a nationally standardized methodology, was deemed an appropriate method with which to further test the quality of Newfoundland's waters.

1.4 Study objectives

There were five main study objectives:

1) Define the taxa and ecological range of the macroinvertebrate community in Newfoundland riffles not associated with lake outlets.

2) Determine if patterns of occurrence and abundance within the macroinvertebrate riffle community exist and, if they do, relate these patterns to seasonal, physical and/or chemical stream parameters measured.
3) Determine the sensitivity of Newfoundland macroinvertebrate communities to human impacts.
4) Assess the ability of the CABIN protocol to use depauperate macroinvertebrate communities for biomonitoring.
5) Provide recommendations for the application of a benthic macroinvertebrate biomonitoring program in Newfoundland.

2 Overview of Methods

2.1 Study location and design

The study incorporated 58 streams and 65 stream sites across the Island of Newfoundland, which is the most easterly island in Canada (W 59°24`—W 52°37`/N 51°38`—N 46°37`). The Island falls in the boreal forest biome (Roberts 1983) and was completely glaciated in the most recent ice age event (Rogerson 1983). As a result, the Island obtained most of its flora and fauna post-glaciation via colonization and mediated introductions (South 1983). There is a great deal of variation in climate, geology, soil and vegetation which led to the designation of nine main ecoregions on the Island (Damman 1983) (Figure 2.1). Seven of the nine ecoregions were encompassed by this study; only the Avalon Forest and Strait of Belle Isle ecoregions did not have any site representatives.

The general climate of Newfoundland is cool and wet, with a shorter growing season than is observed on the mainland at this latitude. The west-central part of the Island tends to be colder and has more snow in the winter than the Avalon and experiences an earlier spring and warmer summer than the Avalon. Summers also are warmer and sunnier on the west and central parts of the Island. The Avalon Peninsula experiences a very cool summer compared to the other regions, aside from the Northern Peninsula (Banfield 1983).

Figure 2.1 The ecoregions of the Island of Newfoundland and their subdivisions. Roman numerals indicate the ecoregions: I = Western Newfoundland, II = Central Newfoundland, III = North Shore, IV = Northern Peninsula Forest, V = Avalon Forest, VI = Maritime Barrens, VII = Eastern Hyper-oceanic Barrens, VIII = Long Range Barrens, IX = Straight of Belle Isle. Capital letters indicate sub-regions.

Taken from Damman (1983).

The Island is made up of three tectonic plates of different origins. The western side of the Island came from the North American Appalachian continental plate, the centre from a raised oceanic plate and the eastern side from a European or North African continental plate. Therefore, geology in each of these three regions reflects their distinct

origins while evidence of past volcanic activity exists along the fault lines (Rogerson 1983). As different as the parentage may be, the soils across the Island are primarily recently derived from glacial till which is typically poorly-sorted, rocky, low in nutrients and acidic, though some areas of high pH do exist in conjunction with limestone and serpentine deposits on the west coast. Organic layers are typically present and are made up of either peat or boreal coniferous forest duff, therefore these layers are acidic (Roberts 1983).

Approximately one half of the Island is forested and the other half is made up of barrens, peatlands and lakes with residential and agricultural areas making up a very small proportion of the total landmass (Roberts 1983). The southern part of western Newfoundland is heavily forested with the percentage of bog and barrens increasing towards the north. The uplands of the Long Range Mountains are essentially barren, with patches of heath or stunted trees called tuckamore that transition to forested valleys. As one moves east, there is a general increase in the number of heathlands, bogs and fens. Within the Maritime Barrens region, wherein St. John's lies, there is a larger proportion of forests in the northern part of the ecoregion, decreasing to the south with the southern Avalon being primarily bog, ericaceous barrens or scrub forest. Forests are made up mostly of balsam fir (*Abies balsamea*), black spruce (*Picea mariana*) and white spruce (*Abies glauca*), with smaller proportions of tamarack (*Larix laricina*) and pine (*Pinus spp.*). There are also extensive stands of hardwood, particularly in logged or burned areas, dominated by birch (*Betula spp.*), aspen (*Populus tremuloides*) and alder (*Alnus spp.*) (Damman 1983).

The watersheds of Newfoundland tend to follow a glacial-fluvial morphology, with most streams being short and close to the ocean. Thus, they do not follow the classic smoothly graded river system profile, but rather tend to have headwaters on low plateaus and move in a step-wise fashion until they near the ocean, where the grade plunges steeply (Figure 2.2). As a result, the drainage system is often poorly defined where there are extensive bogs, fens and lakes which separate the sections of the stream that have deeply-carved channels of steep relief (Larson and Colbo 1983).

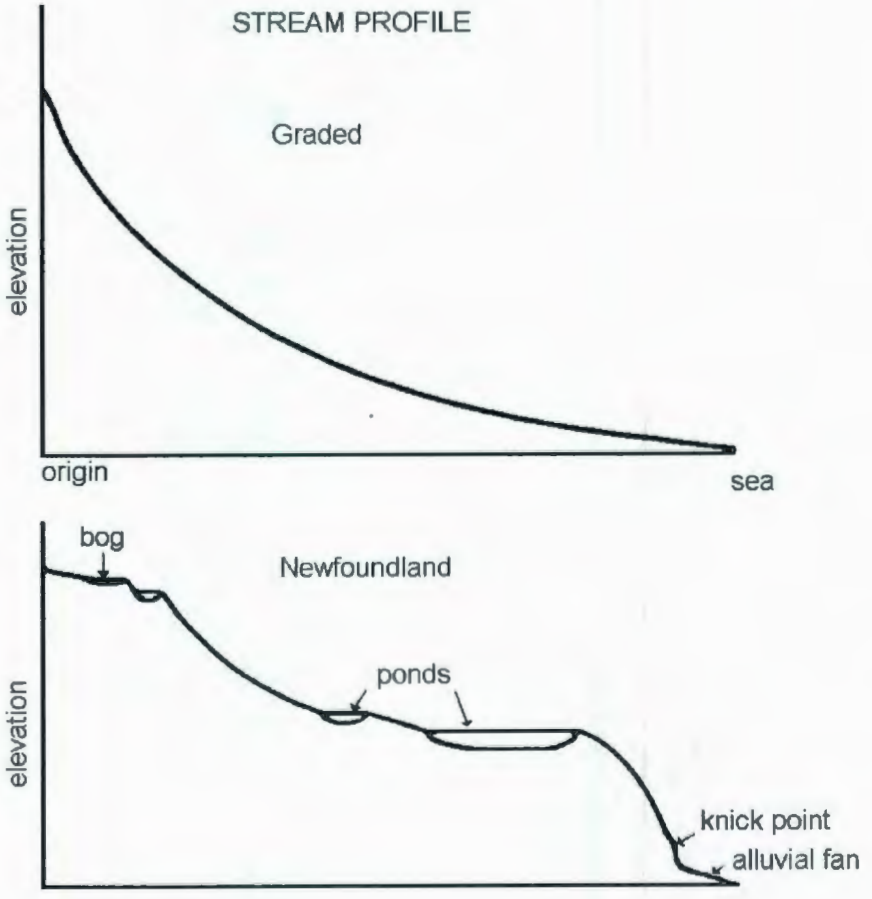
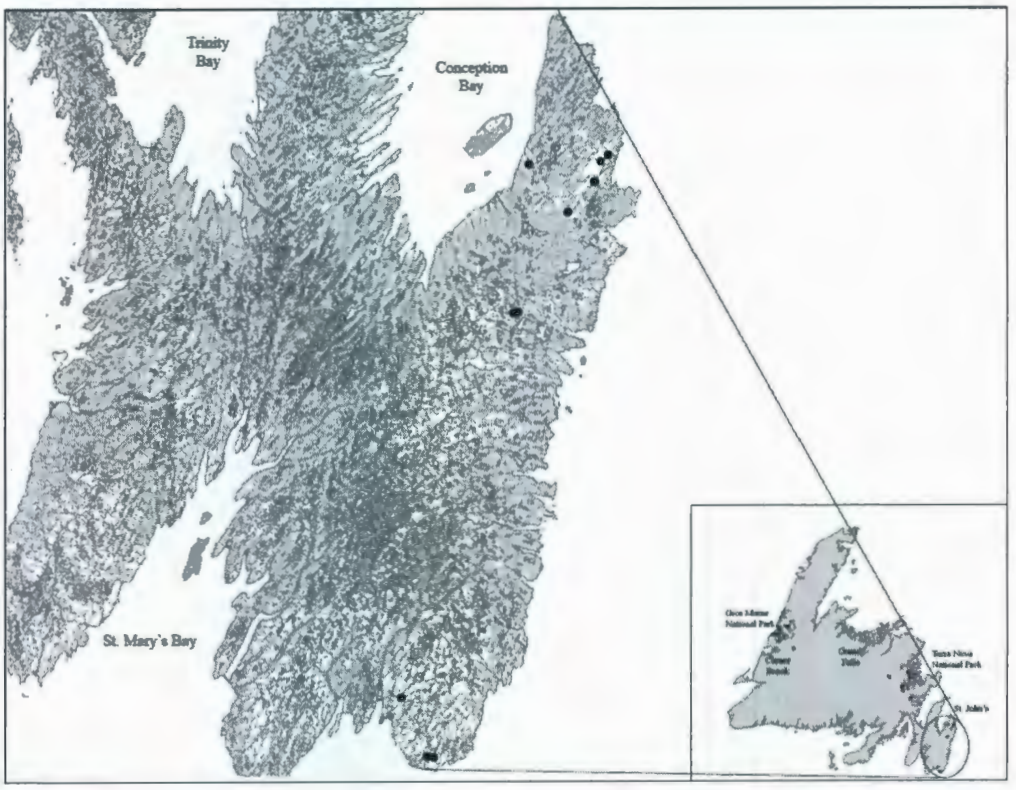
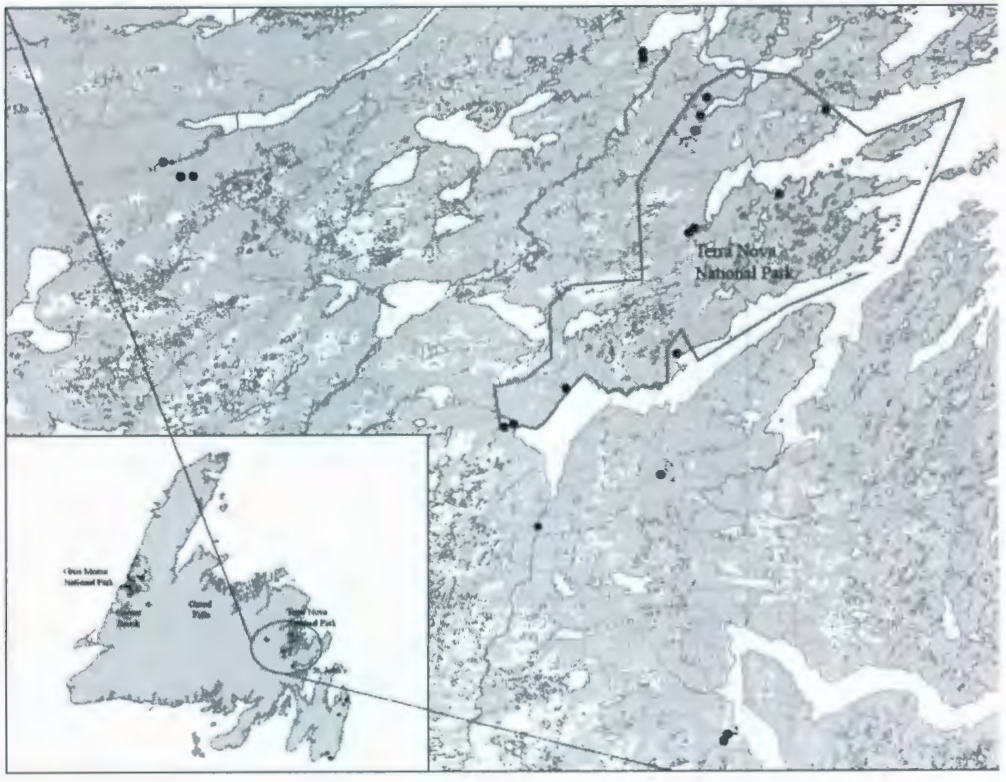


Figure 2.2 Generalized profiles of mainland streams (top) and Newfoundland streams (bottom). Taken from Larson and Colbo (1983).

Streams were selected from three general geographic locations (Figure 2.3). On the west coast, thirty-five sites were selected in and around Gros Morne National Park where three ecoregions converge. This area, as part of the Appalachian tectonic group, is the most geologically diverse area on the Island (Rogerson 1983). On the east coast, twenty sites in and near Terra Nova National Park were chosen; most of these sites were within the Central Newfoundland ecoregion, with two in the North Shore ecoregion. The third region, with ten sites, was on the Avalon Penninsula. Seven sites were in the Maritime Barrens ecoregion close to and within the city of St. John's and three more were on the South Avalon Burin Peninsula Barrens. Both the sites in Terra Nova and on the Avalon have relatively the same geological make up. Sampling was concentrated around both of Newfoundland's National Parks as one aim of the study was to support the development of a monitoring and management system for streams by Parks Canada. A list of sites with their coordinates is given in Appendix 1.


Trinity Bay
Conception Bay
St. Mary's Bay

a)


Terra Nova National Park

b)

c)

Figure 2.3 The Island of Newfoundland with study regions zoomed in to display placement of sites in each region. a) the Avalon Peninsula, b) Terra Nova, and c) Gros Morne.

St. John's sites on the Avalon Peninsula were chosen to provide streams in highly disturbed basins to compare to the relatively pristine nature of most of the National Parks' rivers, and the other Avalon sites were chosen to expand the range of natural variation in pristine habitats sampled across the Island. Individual river sites were picked on merit of their distance from a lake outlet, accessibility and habitat type. All sites were chosen to be at least 500 metres from a lake outlet to avoid the lake outlet influence on the riffle macroinvertebrate community in streams with lakes (Colbo et al. submitted; Lomond and Colbo 2000). In addition, rivers needed to be relatively accessible by road,

boat or short walk to make sampling feasible. However, six high-altitude sites on the Long Range Mountains in Gros Morne were added as helicopter transport was available. Within parks, rivers were chosen in a wide variety of habitats (i.e. forested, barrens, boggy), substrate types (i.e. sand, organic matter, boulder) and sizes to get an adequate representation of insect communities in the area. Some sites were chosen as repeat sites of sampling efforts in previous years to acquire temporal data.

In the summer (July 13-August 3, 2004), ten sites on the Avalon, twenty in Terra Nova National Park and surrounding area, and twenty-nine on the west coast, including Gros Morne National Park, were selected for sampling. In the fall sampling season (October 4-October 27, 2004), six more sites were added in the Long Range Mountains within Gros Morne park boundaries, bringing the total sites sampled to sixty-five. All the fall sites, except for two in Terra Nova, were sampled again in spring (April 28 -June 10, 2005). The two were not sampled due to unsafe high water conditions.

2.2 Field procedures

In-field procedures were adopted primarily from Canadian Aquatic BIomonitoring Network (CABIN) protocols developed by Reynoldson et al. (2003), with elements taken from other protocols (Fitzpatrick et al. 1998; Platts et al. 1983). All measured physical and chemical parameters are listed with their concordant unit measure in Table 2.1. At each site, a reach was chosen that was dominated by riffle habitat. Riffle communities have a greater variety of species than pools or runs (Wohl et al. 1995; Angradi 1999), and thus to maximize diversity measures and minimize variation, only the

riffle community was sampled. Reference photos were taken upstream, downstream and across from the middle of the reach, as well as the shore substrate. Latitude, longitude and altitude were recorded with a handheld GPS unit (Garmin E-Trex Legend). The positioning variables were transformed into Universal Transverse Mercator units from hours, minutes and seconds for ease of analysis. Water temperature, pH and conductivity were recorded with a YSI 63 meter on every sampling occasion, and water samples were procured in the fall and summer sampling sessions. Two water samples were taken from each site, one was left as is and the other was preserved with sulphuric acid (1:1). The fall samples were analyzed by Environment Canada for Nitrates, total Nitrogen, Phosphorus, colour, specific conductivity, pH, granular alkalinity and alkalinity pertaining to total Calcium carbonate. The spring samples were analyzed by Ryan Pretty at the Marine Institute of Memorial University of Newfoundland for nitrates, phosphorus, colour, and alkalinity.

Table 2.1 List of physical and chemical parameters and their respective units of measure.

Physical/Chemical Property	Units	Physical/Chemical Property	Units
UTM Easting	m	Sedimentary rock in watershed	%
UTM Northing	m	Metamorphic rock in watershed	%
Altitude	m	Non-glacial rock in watershed	%
Maximum summer wetted width	m	Glacial rock in watershed	%
Maximum bankfull width	m	Bedrock in watershed	%
Average velocity	m/s	Size of watershed	km^2
Maximum velocity	m/s	Forest in whole watershed	%
Canopy cover	%	Forest in local watershed	%
Macrophyte cover	%	Forest within 100m of reach	%
Riffle in reach	%	Nitrates	mg/L
Average vegetation overhang	cm	Total nitrogen	mg/L
Dominant substrate	scale	Total phosphorus	mg/L
2nd Dominant substrate	scale	Colour	Hazenuni
Surrounding substrate	scale	Conductivity	uS/cm
Embeddedness	scale	pH	pH scale
Average depth	m	Alkalinity	mg/L
Igneous rock in watershed	%	Granular Alkalinity (CaCO3)	mg/L

On the first sampling session for each site, substrate measures were taken to gauge substrate stability and riffle depth. This entailed a protocol for assessing size of the dominant substrate, second dominant substrate and nature of the surrounding substrate in the reach (Reynoldson et al. 2003) (Table 2.2). Substrate particle size and embeddedness (Table 2.3) were obtained from measures of ten randomly selected rocks. Depth was obtained from a measure at every second rock.

Table 2.2 Particle sizes, descriptions and their resulting substrate scores.

Particle Size (mm)	Description	Score
--	organic cover	0
<1	silt	1
1-2	sand	2
2-5	gravel	3
5-25	small pebbles	4
25-50	large pebbles	5
50-100	small cobble	6
100-250	large cobble	7
>250, < stream bed	boulders	8
entire stream bed	bedrock	9

Table 2.3 Embeddedness categories and scores.

Category	Score
completely embedded	1
3/4 embedded	2
1/2 embedded	3
1/4 embedded	4
unembedded	5

Stream wetted width was measured in all three seasons, while bankfull width was only measured on the first sampling session. In the summer, the width measurements were made at three different points in the reach and samplers located the widest, narrowest and medium width of the river; however, in the fall and spring, the potential bias of this method was replaced with measuring the width at the bottom, middle and top of the invertebrate sampling reach.

Velocity was assessed by measuring the speed of a tennis ball over 5 metres unless stream size or debris prevented this, requiring distance to be reduced accordingly. Velocity was measured on all sampling occasions using the procedures outlined by

Reynoldson et al. (2003). If the stream was over 5 metres wide, then the width was divided by six to obtain a set of five velocity measures taken at even distances from the shore. For example, if the river was 6 metres wide, then a velocity measure was conducted a metre out from shore, followed by another measurement at 2 metres, etc. for the five velocity measures. In streams smaller than 5 metres, only three measurements were taken: approximately a quarter, half, and three-quarters of the way across the stream. In very shallow streams, three tests were run in the thalweg, or fastest channel, instead. At each trial, depth was measured at the half-way mark. Maximum velocity was the highest velocity recorded and average velocity was calculated by averaging the velocity of all trials.

Canopy cover, macrophyte cover and the amount of riffle in the reach were estimated by all samplers present and the average value was recorded in summer and in spring. In the summer season, the presence or absence of grass, shrubs, deciduous trees and coniferous trees were recorded for the reach's riparian zone. In summer and spring, overhanging vegetation within 6 inches of the water's surface was measured in three places on each side of the stream, usually where width measurements had been made. Overhanging vegetation is another measure of shading and riparian growth, and may be used by some species for laying eggs and rearing young (Merritt and Cummins 1996).

Macroinvertebrate sampling was performed using a five minute kick-net sample with a D-frame net. Three streams were too small to kick for the full five minutes, so time was reduced to two or three minutes in those locations. In addition to kicking, large rocks were rubbed with the hand to disturb surfaces not done so by boot kicks. Upon

collection, the sample was brought to shore and large stones and sticks were washed and removed. The sample was then poured through a 250 micron screen, rinsed, then poured into a large zipper-locking bag and preserved with 90% ethanol. A label on water-proof paper was placed inside the bag prior to sealing.

2.3 Laboratory procedures

In the lab, samples were washed through a series of large-mesh sieves, ending in a 250 micron screen, to remove leaves, sticks and larger stones prior to sorting. Any large macroinvertebrates found were transferred to the sieved material. The sieved sample was divided among 100 cells using a Marchant box (Marchant 1989) whereupon cells were randomly selected and invertebrates removed. Cells were selected and processed until the cumulative number of invertebrates reached or exceeded 300 individuals. All invertebrates recovered were placed in a vial with 75% ethanol. This method allows for the estimation of species abundance and community composition within the sample. The entire sample was sorted in samples with low invertebrate numbers, as well as in cases where the presence of filamentous algae or large quantities of sand prevented the use of the Marchant box. The latter two kept the organisms from sorting evenly and sand also tended to destroy the soft-bodied invertebrates. Invertebrates were identified using a Wild dissecting microscope to the lowest feasible taxonomic entity using numerous keys, some specific to Newfoundland fauna (Larson unpublished data; Morihara and McCafferty 1979; Peckarsky et al. 1990; Merritt and Cummins 1996; Wiggins 1996; Adler et al. 2004;). Some organisms, such as blackflies and heptageniid mayflies, required parts to be

slide mounted and viewed under a compound microscope in order to ensure correct identification.

Further physical environmental calculations were made using Geographic Information Systems (GIS) (ArcGIS v.9.2 support provided by Tracy Harvey of Parks Canada). ArcGIS was used to determine the size of the watershed upstream of each site, as well as how much of the watershed was made up of various geologic and vegetative parameters. The amounts of igneous, sedimentary and metamorphic rock making up the bedrock geology, as well as the amount of glacial till, non-glacial till and bedrock making up the surficial geology in the watershed were taken from the GIS data for further statistical analysis. Simple measures of the amount of forest in the entire watershed, the local watershed (1 kilometre upstream of the site) and the immediate watershed (100 metres surrounding the site) were made and added to the list of physical parameters.

All data (biological and environmental) were entered into Microsoft Excel spreadsheets and transformed and analyzed using PRIMER 6. Minitab 14 was used for univariate analyses. Specific transformations and statistical approaches are detailed in the methods section of each subsequent chapter.

3 Regional and Seasonal Variation of Macroinvertebrate Community Composition in Newfoundland Riffles

3.1 *Introduction*

The Island Biogeography Theory predicts that islands have a much more limited fauna than comparable land masses on the adjacent mainlands (MacArthur 1967). Newfoundland's aquatic insect fauna, not surprisingly, was also found to be impoverished compared to other regions on the Atlantic North American mainland (Larson and Colbo 1983; Lomond 1997). The most intensive studies of macroinvertebrates have been primarily on the Avalon Peninsula and the central to north-eastern regions of the island, including Terra Nova National Park (Ryan et al. 1993; Lomond 1997; Colbo et al. submitted). Despite the reduced diversity of taxa, differences in macroinvertebrate lake-outlet communities were detected among locations in Eastern Newfoundland (Lomond 1997; Lomond and Colbo 2000). Riffle macroinvertebrate communities differ from lake-outlet communities. Therefore, it is unknown whether or not this community would be able to achieve the same level of regional detection as seen in Lomond and Colbo (2000). The western portion of the province, which is the most diverse geologically and topographically, has had little research on lotic benthic invertebrates. Therefore there is an information gap in the understanding of regional variation within the province. To have an accurate picture of the faunal composition of the entire island, a representation of all the regions within Newfoundland should be examined as regional factors have been shown to explain many of the differences in macroinvertebrate communities (Vinson and Hawkins 1998; Lomond and Colbo 2000).

In addition to a lack of spatial representation, previous studies were limited temporally as well. All of the previous studies on the macroinvertebrates of Newfoundland were conducted in spring and summer, with little analysis of seasonality as a factor influencing the faunal composition among sites and regions. Seasonality has been shown to be a primary factor in defining differences between macroinvertebrate communities largely due to the ability of the researcher to identify the specimens (Linke et al. 1999; Gibbins et al. 2001; Reece et al. 2001; Sporka et al. 2006). The national reference database, and subsequently the Canadian Aquatic BIomonitoring Network (CABIN), has based their analyses on fall seasonal sampling, and in general it is recommended that sample comparisons be made with data from samples all taken in the same season (Reece et al. 2001). However, an understanding of seasonal variation of community structure has also been encouraged, not only to have data available for each season individually, but also for evaluating the information resulting from pooling seasonal data (Furse et al. 1984; Reece et al. 2001; Clarke et al. 2002; Sporka et al. 2006).

This chapter is an overview of the diversity of Newfoundland riffle macroinvertebrate fauna based on a geographically broad sampling program. The analysis includes regional taxa comparisons of the total stream riffle benthic macroinvertebrate fauna among the Avalon Peninsula, Terra Nova National Park (and area), and Gros Morne National Park (and area) sites. Any regional and seasonal differences will be explored to elucidate the taxa that define Newfoundland's stream communities. The main goals of this chapter are: 1) to define the taxa and ecological range of the macroinvertebrate community in Newfoundland riffles, 2) to determine if

patterns of occurrence and abundance within the macroinvertebrate riffle community exist and, if they do, relate these patterns to season and region, and 3) to identify the best season to sample for monitoring purposes.

3.2 *Methods*

Between fifty-nine and sixty-five sites were sampled in each of three seasons from three study regions of the Island of Newfoundland: the Avalon Peninsula, Terra Nova National Park and Gros Morne National Park. All ten sites on the Avalon Peninsula were sampled in all three seasons, of the twenty Terra Nova sites only eighteen were sampled in the spring season, and in Gros Morne only twenty-nine of the thirty-five sites were sampled in the summer season. All sixty-five sites were sampled in the fall season. Details on site descriptions and macroinvertebrate sampling and processing were provided in Chapter 2.

The raw macroinvertebrate abundance data underwent three transformations for use in several different analyses. Presence/absence transformation was used to calculate the number of taxa in each region. The seasons were combined and taxa that had lower levels of identification under them were removed (e.g. *Heptageniidae* spp. was removed when *Heptagenia pulla* was also present at a particular site) to give the most conservative measure of taxonomic richness. Mean number of taxa at each site was calculated for each region and ANalysis Of VAriance (ANOVA) was performed using Minitab 14. AVOVA tables were interpreted with an alpha of 0.05.

The second transformation used on the macroinvertebrate abundance data

expressed the abundance of each taxon as a proportion of the total number sampled. These were then totalled for each of the major taxonomic groups (e.g. Ephemeroptera, Diptera). Excel was used to create stacked columns for each of the three geographic regions, showing proportions of the taxonomic groups in each region.

The third transformation expressed the raw macroinvertebrate abundance data as a more quantitative measure of "insects per minute" to standardize effort among sites. It is the estimated number of each taxon in the entire sample collected in each minute of the kick-net sample. At this point, rarefactions of the taxa present in each site were performed. Rarefaction uses the abundance of each taxon in a "random-grab" analysis, through which the number of taxa expected to be present at increasing sample sizes are obtained (Clarke and Gorley 2006). Rarefactions create plots known as "species-area curves" and are useful for comparing numbers of taxa between samples taken over different amounts of area (Preston 1960). The rarefaction increments were obtained with PRIMER 6 and MS Excel was used to create the species-area curves. The maximum number of individuals used in the test was 10,000, as that was the number of individuals collected from the Avalon (the region with the lowest number of sample sites).

The "insects per minute" abundance measure was transformed using $\log_{10}(X+1)$ to decrease the probability of over-emphasizing common species and under-emphasizing rare species (Clarke and Gorley 2006). This particular transformation was used as it works best with the chosen resemblance matrix, Bray-Curtis, which does not employ weighting on its own (Bray and Curtis 1957; Hruby 1987; Clarke and Gorley 2006). Nijboer et al. (2005) found that logarithmic transformations reduced classification error,

particularly when compared with presence/absence data. Transformation, the formation of resemblance matrices and the following three analyses were all performed with the PRIMER 6 statistical package.

The number of taxa makes it difficult to understand and/or visualize the community differences between the three seasons and the three regions. The non-metric Multi-Dimensional Scaling (MDS) ordination was used with the Bray-Curtis resemblance matrix to order the sites from most to least similar in two to three dimensions (as opposed to a dimensional space equal to the number of taxa in the analysis which would be impossible to interpret) (Clarke and Gorley 2006). Sites closest to one another in the resulting plot are sites whose macroinvertebrate communities are the most alike (high Bray-Curtis similarity score, low Bray-Curtis dissimilarity score) and sites furthest from one another are the most different in community composition (low Bray-Curtis similarity, high Bray-Curtis dissimilarity) (Clarke and Gorley 2006). MDS ordinations (1000 iterations) were conducted to ensure that the resulting plot was the best conformation (lowest stress).

Analysis of Similarity (ANOSIM) tests were also run with a Bray-Curtis resemblance matrix to test for statistical differences between sample groups. These tests are multivariate analogs to ANOVA tests. Factors applied to the data *a priori* (e.g. region, season) can be analyzed in either a one-way or a two-way layout, where a two-way ANOSIM takes a second factor into account when looking for differences between groups of a primary factor (Clarke and Gorley 2006). As the sites were sampled over three seasons, two-way analyses were performed as "crossed with replicates". The results

of ANOSIM are in the form of rho values, or R statistics. The statistic ranges from -1 to +1; an R statistic equal to 0 means there is no difference between the groups being compared (i.e. there are no groups) and rho = 1 means that the groups are completely different. A p-value is also produced with every R statistic (Clarke and Gorley 2006).

Often paired with ANOSIM is the "similarity percentages" (SIMPER) analysis, which interprets the ANOSIM results by showing which macroinvertebrate taxa contribute to the differences between groups as well as which taxa contribute to the similarity within groups (Clarke and Gorley 2006). The average Bray-Curtis dissimilarity between pairs of samples within or between groups is broken down into percent contributions of each taxon. The original log transformed abundance data was used for this analysis (Clarke and Gorley 2006).

3.3 *Results*

3.3.1 Macroinvertebrate community structure of Newfoundland riffles

A total of 148 taxa was recorded from all 65 sites over all three sampling seasons (Table 3.1). A full taxonomic list can be found in Appendix 2. Of the total taxa, three taxa were exclusive to the Avalon, thirteen were restricted to Terra Nova and twenty-one taxa were found only in Gros Morne. Many others were found in only two of the three regions. Six taxa were new records either for the province or for the Island, but none of the new records occurred on the Avalon Peninsula (see Appendix 2 for details).

Plecoptera were the least diverse of all the taxonomic groups, while the Trichoptera group was the most diverse. However, many dipterans were not identified to levels lower than family (e.g. Chironomidae and Ceratopogonidae) due to the difficulties

associated with identification. Therefore, the relative number of taxa for this particular taxonomic group is artificially low. Although no one region contained all taxa, all the Ephemeroptera taxa found in this study were recorded in Gros Morne. In all cases, the number of taxa recorded increased from the Avalon to Terra Nova to Gros Morne (Table 3.1). However, this staged increase may be attributed to the species-area curve where, with greater areas sampled, there is increased likelihood of greater numbers of taxa collected (Figure 3.1). A rarefaction analysis indicated that Terra Nova and Gros Morne have similar levels of taxonomic richness, though regional differences in the composition of taxa do not entirely disappear.

Table 3.1 Taxonomic richness and mean number of taxa per site for the Avalon Peninsula, Terra Nova and Gros Morne regions over all three seasons. Standard deviations are in parentheses.

Taxa	Data	Avalon (n = 10)	Terra Nova (n = 20)	Gros Morne (n = 35)	All Sites (n = 65)
Total Taxa	Total taxa	79	120	129	148
	Mean taxa/site	33.6 (5.2)	44.4 (7.4)	34.5 (8.4)	37.3 (8.9)
Ephemeroptera	Total taxa	17	21	25	25
	Mean taxa/site	7.6 (3.0)	10.6 (2.5)	8.7 (2.5)	9.1 (2.8)
Plecoptera	Total taxa	6	7	8	9
	Mean taxa/site	2.2 (1.3)	2.9 (0.7)	2.9 (1.1)	2.8 (1.1)
Trichoptera	Total taxa	22	32	34	40
	Mean taxa/site	7.9 (3.0)	12.2 (3.3)	9.5 (2.9)	10.1 (3.3)
Diptera	Total taxa	17	29	30	34
	Mean taxa/site	6.8 (0.9)	8.8 (2.9)	7.6 (2.3)	7.8 (2.4)
Coleoptera	Total taxa	4	10	13	17
	Mean taxa/site	1.9 (1.2)	3.4 (1.2)	2.1 (1.2)	2.5 (1.4)
Non-insects	Total taxa	11	14	13	14
	Mean taxa/site	6.7 (2.3)	5.2 (1.3)	3.1 (2.1)	4.3 (2.3)

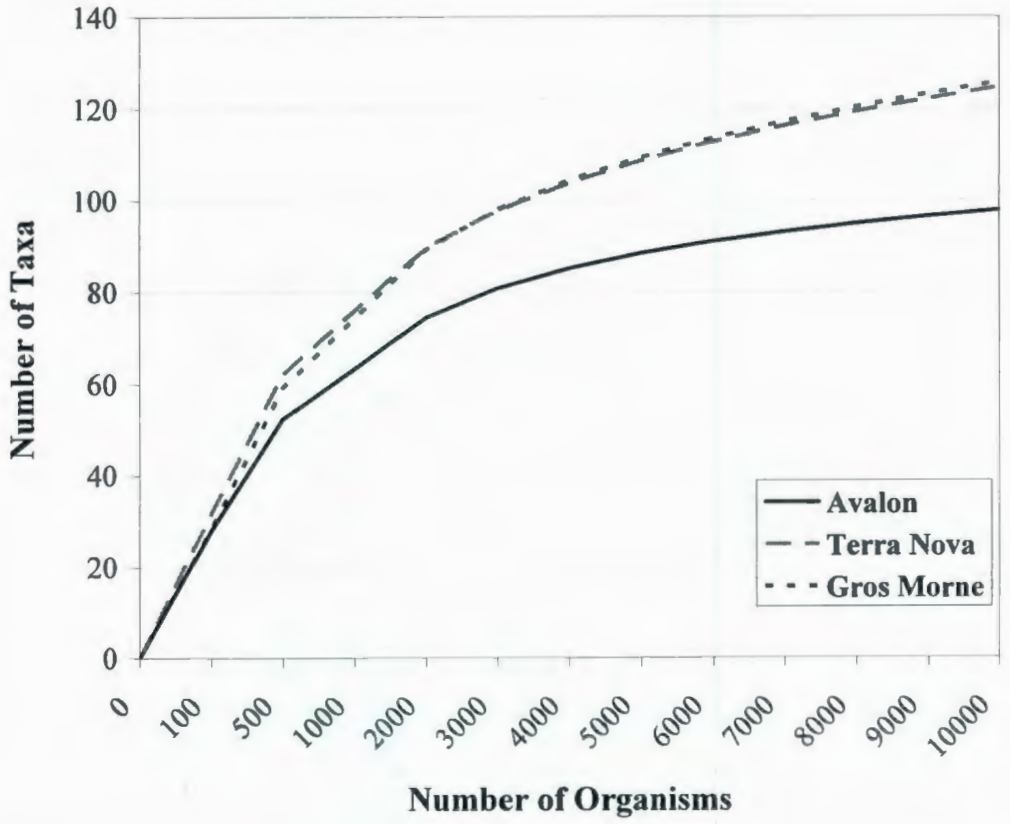


Figure 3.1 Accumulation of taxa as number of organisms sampled increases for the Avalon Peninsula, Terra Nova and Gros Morne regions (rarefaction curves).

In most cases, Terra Nova had the highest mean number of taxa per site, except for Plecoptera, where it was even with Gros Morne, and for non-insects, where the Avalon had approximately 1.5 more taxa per site than Terra Nova (Table 3.1). Analysis of Variance (ANOVA) tests detected significant differences among the three regions for total taxa, Ephemeroptera, Trichoptera, Coleoptera and non-insects (p between <0.001 and 0.008) (Table 3.2). Mean numbers of Diptera and Plecoptera per site did not differ significantly between regions. The Avalon and Gros Morne had identical mean numbers of total taxa in their streams.

Table 3.2 Analysis of Variance (ANOVA) tables comparing the taxonomic richness of sites in the Avalon Peninsula, Terra Nova and Gros Morne.

	Source	Degrees Freedom	Sum of Squares	Mean Squares	F-statistic	P-value
Total Taxa	Factors	2	1401.90	701.00	11.73	**<0.001**
	Error	62	3703.70	59.70		
	Total	64	5105.60			
Ephemeroptera	Factors	2	70.55	35.28	5.25	**0.008**
	Error	62	416.89	6.72		
	Total	64	487.45			
Plecoptera	Factors	2	3.85	1.93	1.76	**0.180**
	Error	62	67.69	1.09		
	Total	64	71.54			
Trichoptera	Factors	2	149.77	74.89	8.25	**0.001**
	Error	62	562.84	9.08		
	Total	64	712.62			
Diptera	Factors	2	29.39	14.69	2.63	**0.080**
	Error	62	345.75	5.58		
	Total	64	375.14			
Coleoptera	Factors	2	23.87	11.93	7.86	**0.001**
	Error	62	94.19	1.52		
	Total	64	118.06			
Non-insects	Factors	2	125.40	62.70	17.51	**<0.001**
	Error	62	222.04	3.58		
	Total	64	347.45			

The relative abundance of Plecoptera, Trichoptera and Coleoptera was nearly the same in the communities of all three regions, while Ephemeroptera, Diptera and non-insects varied (Figure 3.2). The mayfly individuals formed almost half of the community in Gros Morne, while on the Avalon, mayflies only contributed to about a quarter of the stream individuals. The Avalon had the highest percentage of flies and non-insects of the three regions. Despite inter-regional differences, all streams were predominantly a mix of

Diptera (34.9-44.39%) and Ephemeroptera (24.15-41.51%) with the other four main taxonomic groups together making up between 23.4 and 31.32% of the community.

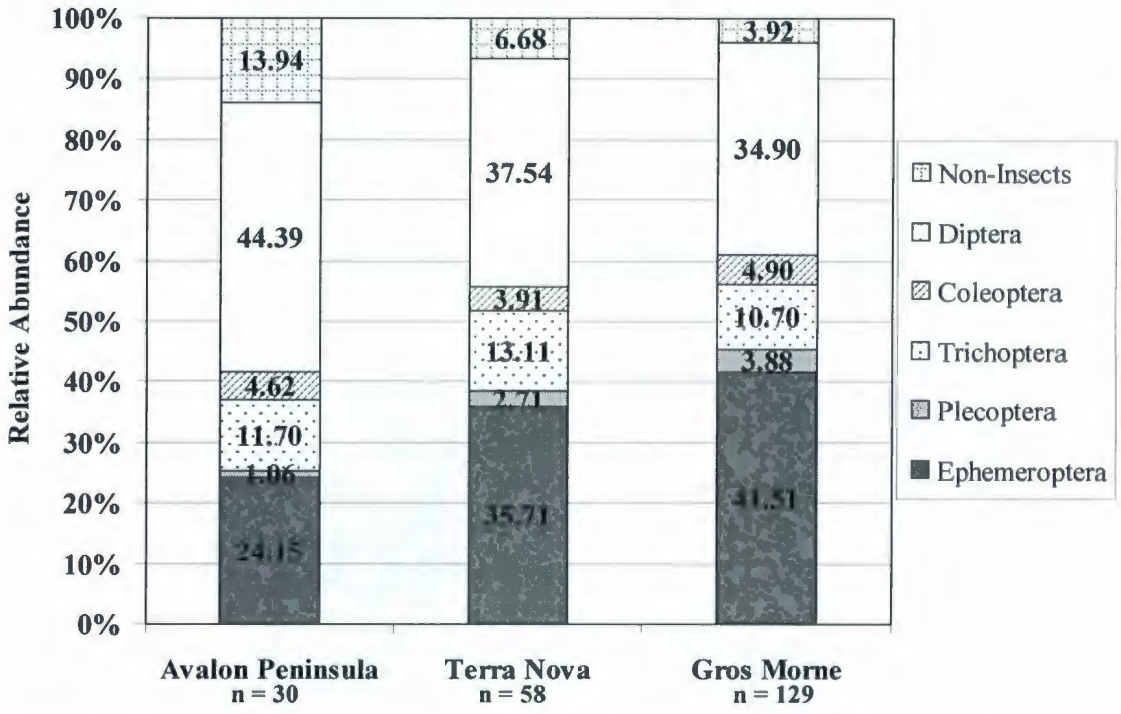


Figure 3.2 The relative abundance of individuals from the major taxonomic groups contributing to the total macroinvertebrate community in sites from the Avalon Peninsula, Terra Nova and Gros Morne.

3.3.2 Ordination of Newfoundland macroinvertebrate communities – all samples

Plotting the macroinvertebrate abundance at each site revealed a basic pattern where the Avalon sites were on one "end" of the data "cloud", Terra Nova sites plotted in the centre, and Gros Morne sites largely plotted towards the opposite "end" of the "cloud" from the Avalon (Figure 3.3). Terra Nova and Gros Morne sites had an especially large amount of overlap in the plot, indicating greater similarity in the macroinvertebrate community structure.

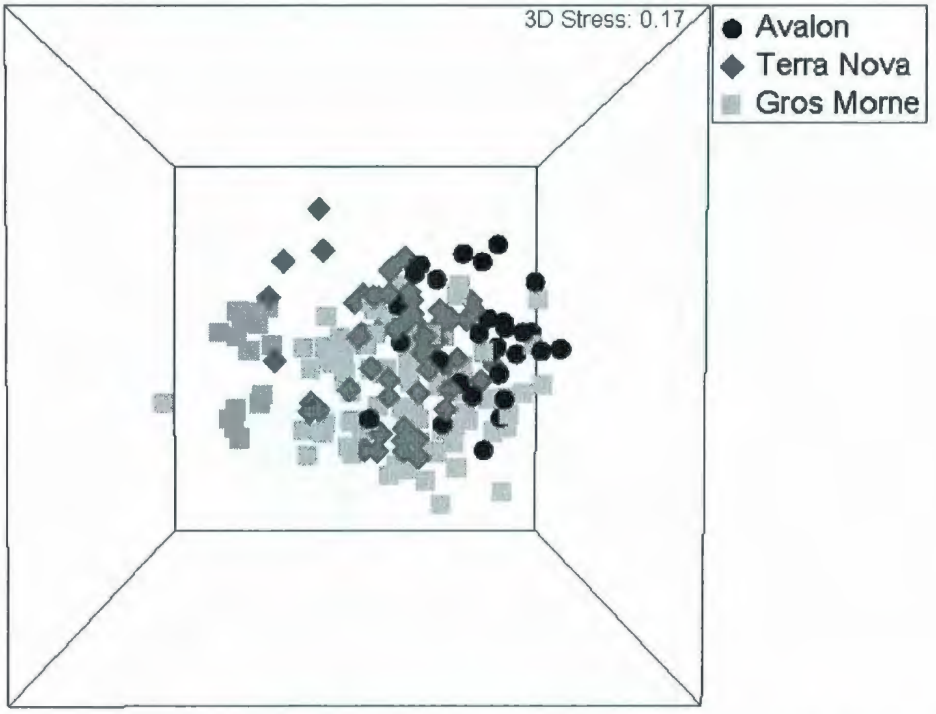


Figure 3.3 3-dimensional MDS plot of macroinvertebrate abundance data from all sites and seasons, coded for region.

An ANOSIM test for differences between regions gave a global R statistic of 0.165 ($p = 0.001$), which indicated that 16.5% of the total variation in the data was assigned to the regional grouping (Table 3.3). A larger proportion of the macroinvertebrate community variation was explained by the pairwise tests between the Avalon and Terra Nova ($R = 0.201$, $p = 0.001$) and the Avalon and Gros Morne ($R = 0.243$, $p = 0.001$). Less of the variation between Terra Nova and Gros Morne sites was explained by the regional label, where the pairwise test had the lowest R statistic ($R = 0.119$), though the difference between the groups was still significant ($p = 0.001$).

A greater portion of the variation in the macroinvertebrate community composition was explained when season was accounted for in the ANOSIM test (Table 3.3). The global R statistic increased from 0.165 to 0.225. Though both values were

significant to the level of 0.001, a larger R statistic indicates an increase in the amount of variation explained by the analysis. When season was accounted for, the difference between the Avalon and Terra Nova became the largest regional difference of the three with an R statistic of 0.390. However, Terra Nova and Gros Morne sites were still the most similar of the regional pairs with a comparatively small R statistic of 0.191. All pairs of regions were significantly different with a p-value of 0.001.

Table 3.3 ANOSIM tests for strengths of regional differences between sites sampled on the Avalon Peninsula, Terra Nova and Gros Morne with all sites tested regardless of season (one-way analysis) and accounting for season (two-way analysis).

	One-way		**Two-way crossed with season**	
	R	p	R	p
Global R	0.165	0.001	0.255	0.001
Avalon vs. Terra Nova	0.201	0.001	0.390	0.001
Avalon vs. Gros Morne	0.243	0.001	0.344	0.001
Terra Nova vs. Gros Morne	0.119	0.001	0.191	0.001

The macroinvertebrate taxa responsible for the differences between the regions are elucidated in Table 3.4. The Avalon Peninsula and Gros Morne were the most dissimilar with an average Bray-Curtis dissimilarity of 71.83. However, unlike the ANOSIM, Terra Nova and Gros Morne were more dissimilar than the Avalon and Terra Nova. The taxa causing the dissimilarity between the Avalon and Terra Nova and the Avalon and Gros Morne were the same three: Chironomidae (dipteran midges), *Hydropsyche slossonae* (caddisfly) and Acariformes (mites). The Avalon consistently had more of these three taxa than the other two regions. Terra Nova and Gros Morne's

average dissimilarity of 69.10 was partially due to four taxa: Chironomidae, *Baetis flavistriga* (mayfly), *B. tricaudatus* (mayfly), and *Oulimnius latiusculus* (elmid beetle).

Table 3.4 Bray-Curtis dissimilarities of macroinvertebrate abundance between regions and taxa responsible for at least 3% of the total dissimilarity.

Average dissimilarity = 68.32	**Average Abundance**		**% Contribution**
Taxon	**Avalon**	**Terra Nova**	**to Total Dissimilarity**
Chironomidae	5.69	3.72	3.49
Hydropsyche slossonae	2.23	0.44	3.4
Acariformes	2.73	1.54	3.1
Average dissimilarity = 71.83	**Average Abundance**		**% Contribution**
Taxon	**Avalon**	**Gros Morne**	**to Total Dissimilarity**
Chironomidae	5.69	3.77	3.78
Hydropsyche slossonae	2.23	0.35	3.43
Acariformes	2.73	1.49	3.06
Average dissimilarity = 69.10	**Average Abundance**		**% Contribution**
Taxon	**Terra Nova**	**Gros Morne**	**to Total Dissimilarity**
Chironomidae	3.72	3.77	3.57
Baetis flavistriga	0.75	1.24	3.16
Baetis tricaudatus	3.2	3.35	3.07
Oulimnius latiusculus	1.25	1.35	3.03

Separations in the plot of all macroinvertebrate abundance samples were seen when the axes were rotated 90 degrees from their position in Figure 3.3. These separations did not reflect regional differences (Figure 3.4A), but rather seasonal ones (Figure 3.4B and Figure 3.5). As expected from the MDS plot, season explained a large portion of the macroinvertebrate community differences, ($R = 0.482$, $p = 0.001$) (Table 3.5). Again, pairwise examinations revealed a greater explanation of the data's variation where summer and fall differences totalled 58.7% of the variation, and summer and

spring, 55.2%. Fall and spring were the most similar, explaining only 31.6% of the variation. All seasons were significantly different from one another ($p = 0.001$).

Accounting for region in a 2-way ANOSIM analysis improved the amount of macroinvertebrate community variation explained by season (Table 3.5). The global R statistic increased from 0.482 to 0.516, summer and fall's differences explained 66.3% of the variation and fall and spring explained 33.5%. The differences between communities in the summer versus those in spring decreased marginally when region was accounted for as the R statistic dropped from 0.552 to 0.540.

The macroinvertebrate taxa most responsible for the differences between the seasons seen in the MDS plot and in the ANOSIM results are listed in Table 3.6. Average Bray-Curtis dissimilarities were high, with the highest difference between summer and fall (72.47). Four taxa were influential in causing the dissimilarity: *Baetis flavistriga*, Chironomidae, *Simulium venustum/verecundum* (blackfly) and *Lepidostoma* (caddisfly). Summer and spring were the next most dissimilar, with six taxa contributing at least three percent of the total dissimilarity, three of which were the same as in the summer and fall dissimilarity. Fall and spring were the most similar of the three pairs, though the dissimilarity was still 70.44.

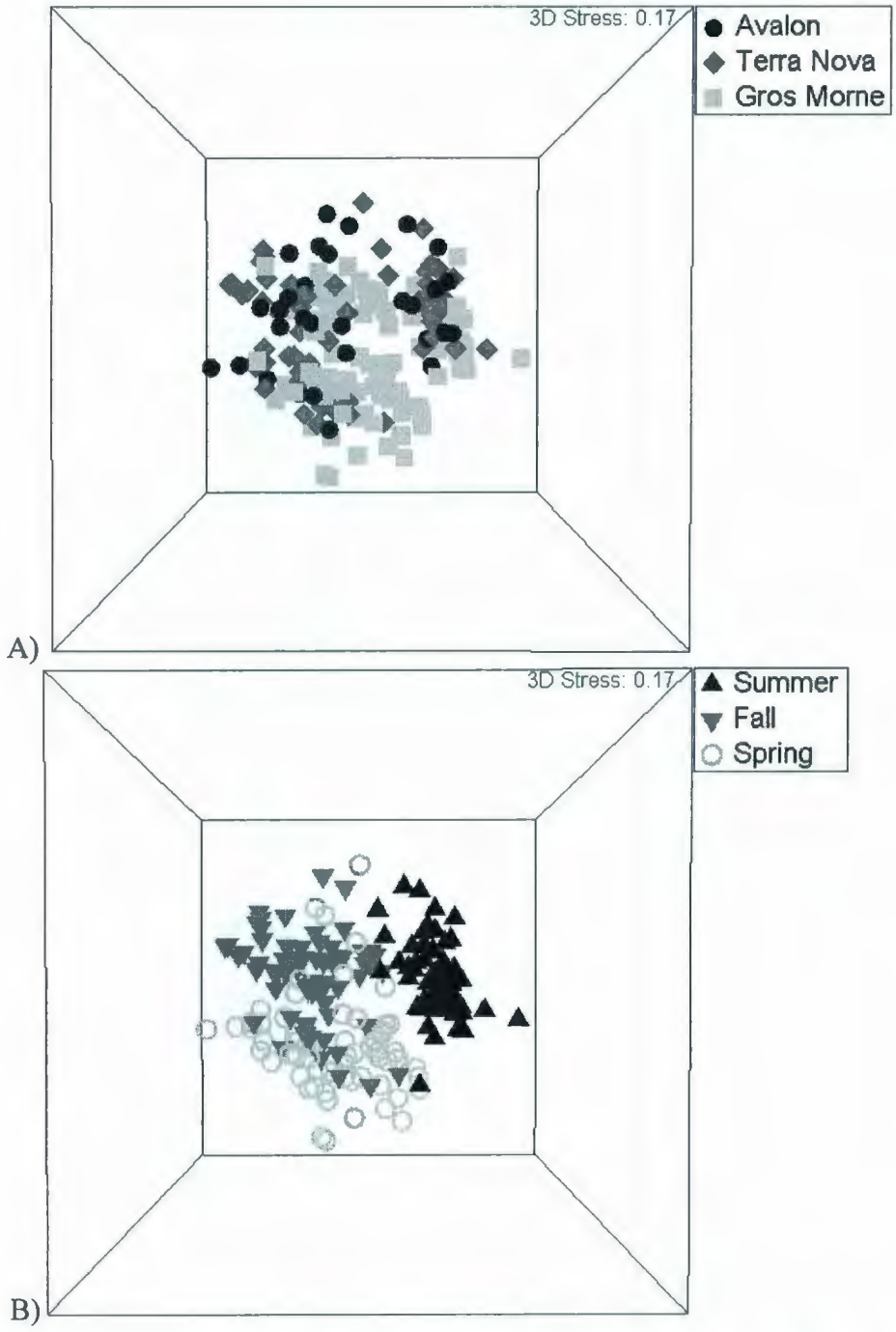


Figure 3.4 3-dimensional MDS plot of macroinvertebrate abundance data from all sites and seasons, turned 90 degrees to the left of Figure 3.3. A) sites coded for region, B) sites coded for season.

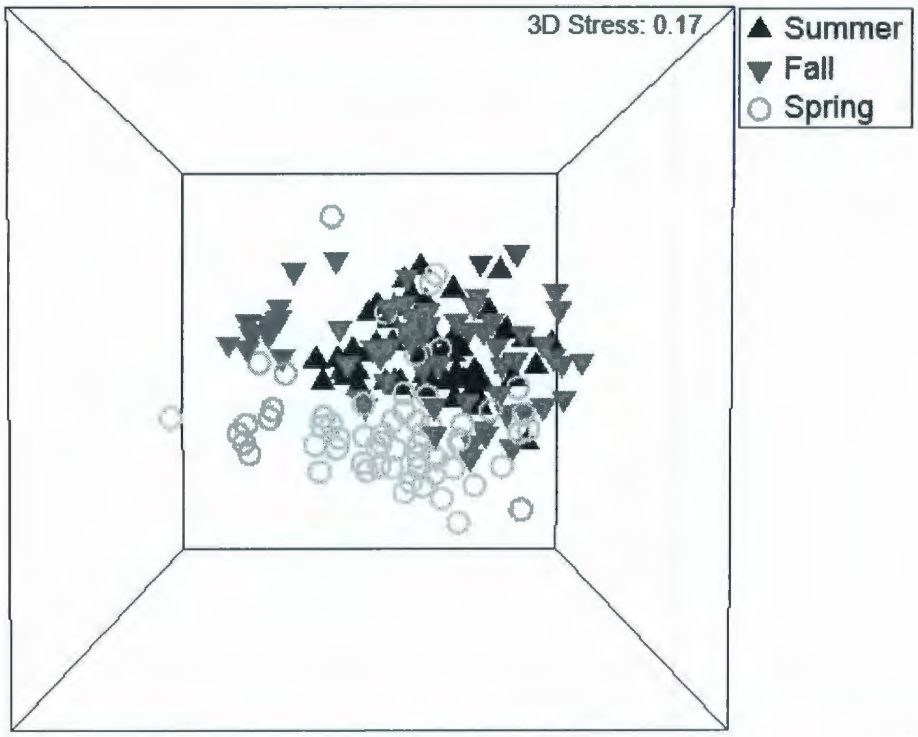


Figure 3.5 3-dimensional MDS plot of macroinvertebrate abundance data from all sites and seasons, turned 90 degrees to the right of Figure 3.4B, sites coded for season.

Table 3.5 ANOSIM tests for strengths of seasonal differences between sites sampled in summer, fall and winter, with all samples tested regardless of region (one-way analysis) and accounting for region (two-way analysis).

	One-way		**Two-way crossed with region**	
	R	p	R	p
Global R	0.482	0.001	0.516	0.001
Summer vs. Fall	0.587	0.001	0.663	0.001
Summer vs. Spring	0.552	0.001	0.540	0.001
Fall vs. Spring	0.316	0.001	0.355	0.001

Table 3.6 Bray-Curtis dissimilarities of macroinvertebrate abundance between seasons and taxa responsible for at least 3% of the total dissimilarity.

Average dissimilarity = 72.47	Average Abundance		% Contribution
Taxon	**Summer**	**Fall**	**to Total Dissimilarity**
Baetis flavistriga	2.97	0.10	5.38
Chironomidae	4.90	3.42	4.17
Simulium venustum/verecundum	2.14	0.22	3.62
Lepidostoma	0.22	1.95	3.11
Average dissimilarity = 71.62	Average Abundance		% Contribution
Taxon	**Summer**	**Spring**	**to Total Dissimilarity**
Baetis flavistriga	2.97	0.24	5.51
Simulium venustum/verecundum	2.14	0.73	3.55
Acariformes	2.57	0.95	3.50
Drunella cornutella	0.00	1.95	3.48
Chironomidae	4.90	3.95	3.25
Epeorus pleuralis	0.75	1.40	3.01
Average dissimilarity = 70.44	Average Abundance		% Contribution
Taxon	**Fall**	**Spring**	**to Total Dissimilarity**
Chironomidae	3.42	3.95	3.75
Drunella cornutella	0.00	1.95	3.66
Baetis tricaudatus	3.53	3.14	3.10

3.3.3 Ordination of Newfoundland macroinvertebrate communities - seasons

Obvious seasonal differences prompted individual consideration of each season to determine if regional distinctions could be seen in the data. Regional separation in summer was best seen in three dimensions in an MDS plot (Figure 3.6). All sites from a particular region sorted together and the three regional data "clouds" were almost entirely segregated. An ANOSIM test revealed a global R of 0.382 (p-value = 0.001), where the amount of variation in the macroinvertebrate dataset explained between the Avalon and Terra Nova was 51.3%, 53.7% by the Avalon and Gros Morne, and 26.9% by Terra Nova and Gros Morne (Table 3.7). The ordering of the variations from most to least amount

explained was the same as in the one-way ANOSIM test, where the Avalon and Gros Morne were the most dissimilar of the three region pairs. These values were substantially higher than the R-statistics for both the one-way and two-way ANOSIM tests run with all the seasons together, as well as those of the other two seasons (Table 3.3, Table 3.7). All the region pairs were significantly different to p = 0.001.

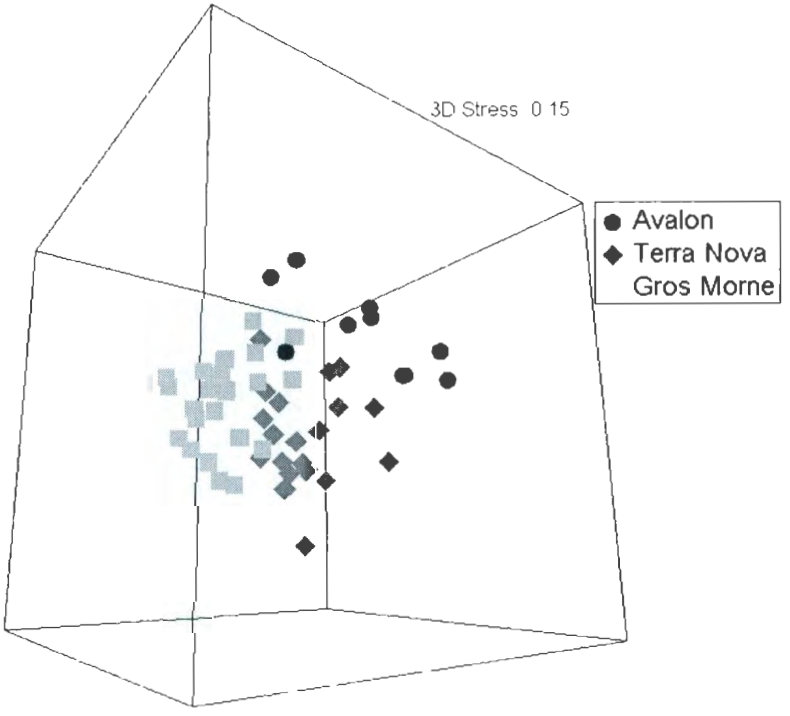


Figure 3.6 3-dimensional MDS plot of summer macroinvertebrate abundance, coded for region.

Table 3.7 ANOSIM tests for strengths of regional differences between sites sampled on the Avalon Peninsula, Terra Nova and Gros Morne in summer, fall and spring, separately.

	Summer		Fall		Spring	
	R	p	R	p	R	p
Global R	0.382	0.001	0.224	0.001	0.189	0.001
Avalon vs. Terra Nova	0.513	0.001	0.400	0.001	0.229	0.006
Avalon vs. Gros Morne	0.537	0.001	0.274	0.001	0.284	0.001
Terra Nova vs. Gros Morne	0.259	0.001	0.173	0.003	0.149	0.008

The list of macroinvertebrate taxa causing the dissimilarities between regions was much larger for summer than the list provided by SIMPER for all the seasons together (Table 3.8). As a result, a larger proportion of the total dissimilarity was explained by a few species. Two taxa from the list of 9 prominent taxa making up the difference between the Avalon and Gros Morne supplied over 4% each to the cumulative dissimilarity: Chironomidae (4.27%) and Acariformes (mites, 4.20%). These two taxa groups were major contributors to the dissimilarity between the Avalon and Terra Nova. *Hydropsyche slossonae* also contributed to the dissimilarity between the first two regional sets (Avalon and Terra Nova, Avalon and Gros Morne), as in the SIMPER analysis for all the seasons together. The caddisfly was most abundant in the Avalon Peninsula sites.

Baetis tricaudatus aided in the segregation of the Avalon from Gros Morne and Terra Nova from Gros Morne; average abundance of the taxon on the Avalon did not differ enough from the abundance in Terra Nova to be included by SIMPER in the dissimilarity measure between those regions. An identical pattern was seen with *Promoresia tardella* (Elmidae), where Terra Nova and Gros Morne did not have sufficiently different abundances of the taxon. Terra Nova and Gros Morne were the least dissimilar (as in the ANOSIM test), but over 5% of the dissimilarity was explained by one taxon: *Baetis flavistriga. Epeorus pleuralis* (mayfly) was the second highest contributor in the set. *Simulium venustum/verecundum* and *Oulimnius latiusculus* were also major contributors to region dissimilarity between all three regions.

Table 3.8 Bray-Curtis dissimilarities of macroinvertebrate abundance between regions in summer and taxa responsible for at least 3% of the total dissimilarity.

Average dissimilarity = 60.66	Average Abundance		% Contribution
Taxon	**Avalon**	**Terra Nova**	**to Total Dissimilarity**
Hydropsyche slossonae	2.31	0.19	3.91
Acariformes	4.17	2.46	3.87
Chironomidae	6.82	4.67	3.85
Naididae	2.40	0.63	3.69
Promoresia tardella	2.40	0.73	3.55
Baetis flavistriga	3.04	1.99	3.50
Lumbriculidae	2.06	0.61	3.29
Simulium venustum/verecundum	2.96	2.05	3.22
Oulimnius latiusculus	1.79	1.02	3.15

Average dissimilarity = 63.60	Average Abundance		% Contribution
Taxon	**Avalon**	**Gros Morne**	**to Total Dissimilarity**
Chironomidae	6.82	4.39	4.27
Acariformes	4.17	2.10	4.20
Hydropsyche slossonae	2.31	0.14	3.80
Naididae	2.40	0.37	3.72
Promoresia tardella	2.40	0.59	3.70
Baetis tricaudatus	4.75	3.00	3.51
Simulium venustum/verecundum	2.96	1.92	3.44
Lumbriculidae	2.06	0.53	3.30
Oulimnius latiusculus	1.79	1.55	3.14

Average dissimilarity = 57.54	Average Abundance		% Contribution
Taxon	**Terra Nova**	**Gros Morne**	**to Total Dissimilarity**
Baetis flavistriga	1.99	3.62	5.47
Epeorus pleuralis	0.00	1.53	4.13
Dolophilodes distinctus	1.65	1.80	3.91
Simulium venustum/verecundum	2.05	1.92	3.78
Simulium tuberosum	1.61	1.48	3.63
Oulimnius latiusculus	1.02	1.55	3.61
Baetis tricaudatus	3.69	3.00	3.36
Chironomidae	4.67	4.39	3.09
Leptoceridae (immatures)	1.23	0.20	3.02

Fall was the only season in which all 65 sites were sampled. The regions were not as obviously separated as in the summer MDS, and as such required two views to portray separation of site clusters (Figure 3.7). Avalon sites clustered quite tightly on the plot, whereas Gros Morne sites were widely scattered throughout the space. Approximately half of the Terra Nova sites lay within the Gros Morne site "cloud". The global R-statistic was also lower in this season compared to the all season two-way ANOSIM and the summer R-statistics (R = 0.224) (Table 3.3, Table 3.7). The amount of variation explained by the first two season pairs (Avalon and Terra Nova = 40%, Avalon and Gros Morne = 27.4%) was higher than that explained by all the seasons together. Terra Nova and Gros Morne explained less of the macroinvertebrate variation, where the resulting R statistic was 0.173. Fall was the only season to have a higher R-statistic for the Avalon and Terra Nova than for the Avalon and Gros Morne. This was reflected in Figure 3.7 B, which showed that Terra Nova points extended off into a different axis from the other two regions. All comparisons were statistically significant to p = 0.001, except for the pairwise analysis of Terra Nova and Gros Morne which was significant to p = 0.003.

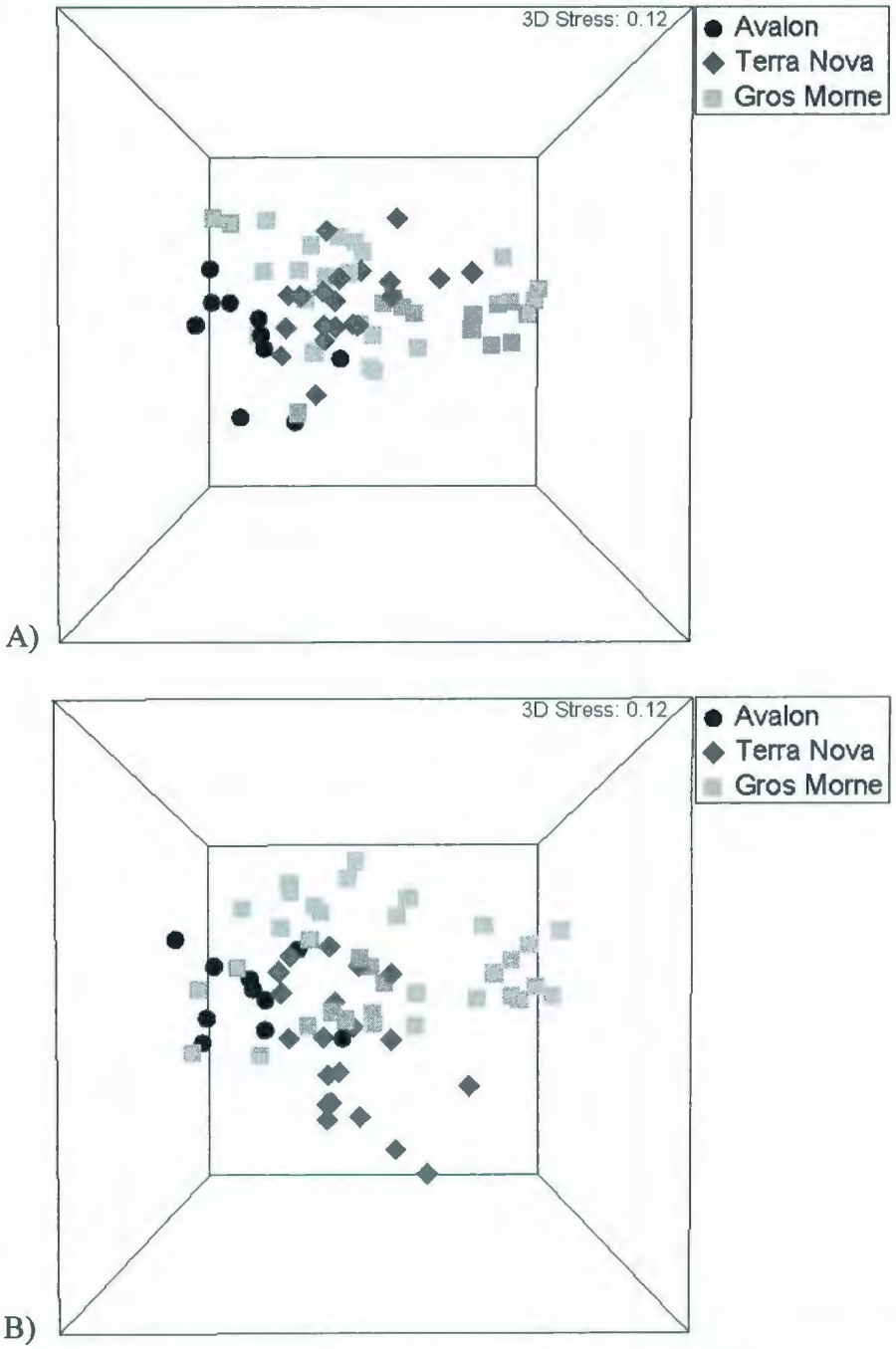


Figure 3.7 3-dimensional MDS plot of fall macroinvertebrate abundance, coded for region. A) initial plot, B) view when axes in A) rotated 90 degrees downward.

Despite the ANOSIM tests indicating that fall macroinvertebrates were less able to define regions than in the summer, the SIMPER test revealed that regions were more dissimilar in fall than in summer (Table 3.9). The Avalon and Gros Morne were the most different regional pair, with a Bray-Curtis dissimilarity of 69.04. Chironomidae and *Acerpenna pygmaeus* (mayfly) were highly influential in all three pairwise comparisons of regions. In both the Avalon and Terra Nova test and the Avalon and Gros Morne test, the family Chironomidae contributed at least 4% to the total dissimilarity. For Terra Nova and Gros Morne, *Acerpenna pygmaeus* and *Baetis tricaudatus* contributed 4.89 and 4.17% respectively to the dissimilarity between the two regions. As in the all season and summer analyses, *Hydropsyche slossonae* was selected by SIMPER as a top contributor to the dissimilarity between the Avalon and Terra Nova and also the Avalon and Gros Morne. Naididae, a group of oligochaete worms, also contributed to the dissimilarity between the two region sets. *Ephemerella subvaria* was only effective in distinguishing the Avalon from Gros Morne; *Lepidostoma* (caddisfly) and Heptageniidae (immatures) were only contributors to the dissimilarity between Terra Nova and Gros Morne.

Table 3.9 Bray-Curtis dissimilarities of macroinvertebrate abundance between regions in fall and taxa responsible for at least 3% of the total dissimilarity.

Average dissimilarity = 63.43	Average Abundance		% Contribution
Taxon	**Avalon**	**Terra Nova**	**to Total Dissimilarity**
Chironomidae	5.34	2.82	4.10
Hydropsyche slossonae	2.70	0.77	3.67
Naididae	2.72	0.50	3.60
Acerpenna pygmaeus	2.51	2.51	3.00
Average dissimilarity = 69.04	**Average Abundance**		**% Contribution**
Taxon	**Avalon**	**Gros Morne**	**to Total Dissimilarity**
Chironomidae	5.34	3.21	4.00
Hydropsyche slossonae	2.70	0.64	3.63
Naididae	2.72	0.37	3.56
Ephemerella subvaria	2.59	0.30	3.47
Acerpenna pygmaeus	2.51	0.97	3.25
Average dissimilarity = 65.01	**Average Abundance**		**% Contribution**
Taxon	**Terra Nova**	**Gros Morne**	**to Total Dissimilarity**
Acerpenna pygmaeus	2.51	0.97	4.89
Baetis tricaudatus	2.99	3.87	4.17
Chironomidae	2.82	3.21	3.93
Lepidostoma	2.53	1.57	3.62
Oulimnius latiusculus	1.42	1.20	3.26
Heptageniidae	1.52	1.40	3.09

Spring macroinvertebrate communities ordinated much the same as fall communities (Figure 3.8). Terra Nova sites clustered tightly together, while Avalon sites were more spread out but still gathered to one "end" of the ordination. Gros Morne sites spread throughout the ordination plot. According to the ANOSIM tests, the global R (percent variation explained by region) was the lowest out of all the seasons with an R-statistic of 0.189, though not as low as the one-way all-seasons global R (Table 3.3, Table 3.7). All regions differed significantly from one another: the Avalon and Terra Nova ($R = 0.229$, $p = 0.006$), the Avalon and Gros Morne ($R = 0.284$, $p = 0.001$), and

Terra Nova and Gros Morne (R = 0.149, p = 0.008).

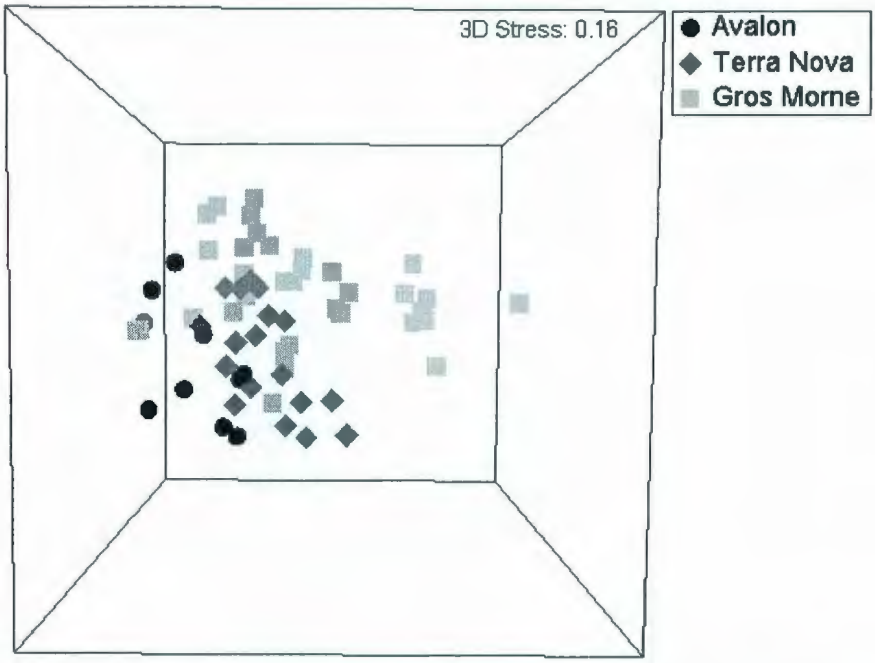


Figure 3.8 3-dimensional MDS plot of spring macroinvertebrate abundance, coded for region.

The average dissimilarity of region pairs in spring was high; in all but one pair (Avalon and Terra Nova) the dissimilarities were the highest of the three seasons (Table 3.10). Several different species became important in this season. Where summer and fall were mainly influenced by organisms that were ubiquitous in distribution through most regions and all seasons (such as *Hydropsyche slossonae*, Naididae and elmid beetles), spring dissimilarity studies also contained season-specific taxa (such as *Prosimulium mixtum* (blackfly), *Drunella cornutella* (mayfly) and *Simulium murmanum*). The Avalon Peninsula and Gros Morne were the most distinct from each other, though no one taxon

had a substantially higher contribution to the total dissimilarity. *P. mixtum* provided the highest amount of dissimilarity in both the Avalon and Terra Nova and Terra Nova and Gros Morne regional pairs (4.27 and 4.57%, respectively). *Epeorus pleuralis* also distinguished Terra Nova from Gros Morne, where its contribution was 4.05%.

Table 3.10 Bray-Curtis dissimilarities of macroinvertebrate abundance between regions in spring and taxa responsible for at least 3% of the total dissimilarity.

Average dissimilarity = 62.37	**Average Abundance**		**% Contribution**
Taxon	**Avalon**	**Terra Nova**	**to Total Dissimilarity**
Prosimulium mixtum	1.69	2.34	4.27
Drunella cornutella	2.37	2.00	3.87
Baetis tricaudatus	3.69	2.88	3.47
Hydropsyche slossonae	1.67	0.36	3.42
Oulimnius latiusculus	1.45	1.32	3.33
Simulium murmanum	1.03	1.04	3.00
Average dissimilarity = 69.20	**Average Abundance**		**% Contribution**
Taxon	**Avalon**	**Gros Morne**	**to Total Dissimilarity**
Epeorus pleuralis	0.28	1.83	3.53
Drunella cornutella	2.37	1.80	3.49
Hydropsyche slossonae	1.67	0.24	3.41
Prosimulium mixtum	1.69	0.80	3.34
Chironomidae	4.91	3.82	3.33
Oulimnius latiusculus	1.45	1.34	3.06
Promoresia tardella	1.68	1.04	3.01
Acerpenna pygmaeus	1.69	0.31	3.00
Baetis tricaudatus	3.69	3.12	3.00
Average dissimilarity = 65.57	**Average Abundance**		**% Contribution**
Taxon	**Terra Nova**	**Gros Morne**	**to Total Dissimilarity**
Prosimulium mixtum	2.34	0.80	4.57
Epeorus pleuralis	1.20	1.83	4.05
Oulimnius latiusculus	1.32	1.34	3.31
Chironomidae	3.67	3.82	3.24
Simulium murmanum	1.04	1.05	3.23
Drunella cornutella	2.00	1.80	3.20
Leucrocuta hebe	1.38	0.09	3.00

3.4 *Discussion*

The Gros Morne region initially appeared to have the greatest diversity of the three regions based on number of taxa found. However, the species-area curve analysis showed that for similar sample sizes, Terra Nova and Gros Morne had a nearly equal diversity of the taxa identified. This relative equality of richness between Gros Morne and Terra Nova was not expected given the greater geological and topographical diversity of the Gros Morne region (examples of habitat diversity resulting in increased richness: Townsend et al. 1983; Lillehammer 1985; Vinson and Hawkins 1998; Lomond and Colbo 2000). Most Terra Nova sites were in forested areas with cobble substrates, where Gros Morne sites ranged from forested to barren and sand substrate to bedrock, and exhibited a wide range of pH and conductivity values. However, species-area analysis merely shows that an equal sample of individuals from the two areas produced an essentially equal diversity. Therefore, in this study, the number of taxa filling the niches did not change in spite of differences in habitat between regions. Despite this, Terra Nova sites tended to be richer than Gros Morne sites when analyzed on a per-site basis (mean number of taxa per site), this may reflect the more adverse sites present in Gros Morne (e.g. pH extremes, low organic input), which tended to have lower macroinvertebrate abundance (and thus richness) than buffered forested sites. While there were more novel taxa in Gros Morne, there were still a number of taxa in Terra Nova that were not shared with Gros Morne or the Avalon. This was again unexpected as all regions had examples of riffle habitat resembling those in Terra Nova.

The six new records for the island are largely a reflection of the increased

sampling effort of this study compared to studies of the past. Therefore, two types of survey are supported for future study. One is a more intense survey of the established sampling sites to increase the chance of collecting rare specimens. The second is a broader survey including more portions of Newfoundland (the Strait of Belle Isle ecoregion, for example) which may reveal novel taxa occurring in restricted or specialized habitats.

The Avalon Peninsula had the fewest taxa as well as the lowest mean number of taxa per site. This is unlikely to be wholly due to the number of sites sampled as the rarefactions indicated there were fewer taxa for the same sample size compared to the other two regions. Also, Avalon sites encompassed a range of stream sizes and samples were taken from riffles just as they were in the other regions, decreasing the chance of sampling bias between regions. The low taxonomic richness may, however, been influenced by the large proportion of urban sites in the area. Urbanization causes a decrease in sensitive taxa and overall species richness, as well as an increase in pollution-tolerant taxa (Colbo 1993; Walsh et al. 2001). The effects of human activities will be discussed in greater detail in a following chapter.

The proportions of major taxonomic groups in Newfoundland streams are of significant interest. Oswood's (1989) survey of Alaskan streams led to the confirmation that numbers of Ephemeroptera, Plecoptera and Trichoptera taxa decrease with increasing latitude while numbers of Chironomidae increase. Proportions of the same six major groups cited in this paper (Figure 3.2) were elucidated and then compared to a high and a low-altitude Rocky Mountain study, a northern prairie study and the Alaskan study as

displayed in Oswood (1989). The other three regions' streams were made up of no more than 25% Diptera, whereas Alaskan streams averaged about 60%. The relative abundance of Diptera in Newfoundland riffles was between 35 and 45%. As the latitude of the island is not as high as Alaska, but more akin to the latitudes of the other sites, this was an interesting finding. However, similarities between Newfoundland and Alaska may explain the resemblances in the macroinvertebrate composition in the two areas. Oswood (1989) cited biogeography and tolerance of what can be considered "adverse ecological conditions" by individual taxa as possible reasons for the distribution patterns he saw. Newfoundland has biogeographical similarities to Alaska with the latter's psuedo-island effects caused by watershed isolation and its high-Arctic location (Damman 1983; Oswood 1989). Many streams on the island are affected by one or many environmental factors that can stress and therefore exclude macroinvertebrate taxa, such as extreme pH, low nutrients, and high disturbance. Thus, as dipterans are better suited to some environmental extremes than other taxa, this makes them more successful in places like the high arctic and boreal, mid-Atlantic islands. What these environmental factors may be will be discussed in Chapter 4.

Converse to the pattern seen in the Diptera, proportions of Ephemeroptera on the Island are in the mid-range between Alaska and the Rockies where the Rockies have a much higher proportions of mayflies (Oswood 1989). The proportion of Ephemeroptera in the Avalon streams mirrored the northern prairie streams (24% to 20%, respectively). The mayflies drastically increased in relative abundance moving west across Newfoundland where Gros Morne streams were composed of nearly 42% mayflies,

which was comparable to low-elevation Rocky Mountain streams in Idaho at about 45% (Andrews and Minshall 1979; Oswood 1989). This tendency towards increased mayfly dominance in Gros Morne streams compared to Avalon streams may be influenced either by physical/chemical variables associated with the different types of habitats present in the two regions or urbanization effects, which are also physical-chemical in nature. A direct tie of mayfly abundance to urbanization could be a simple and useful measure for bioassessments. Therefore, the likelihood and strength of this relationship will be further examined in Chapters 4 and 5.

Macroinvertebrate data showed more variation with seasons than among regions as evidenced by the much larger amounts of variation explained by season with ANOSIM. Summer and fall were always the most divergent, followed by summer and spring, then fall and spring. Many other studies have also described this disparity between the macroinvertebrate communities among sites sampled in different seasons (Minshall et al. 1985; Gibbins et al. 2001; Lorenz and Clarke 2006; Sporka et al. 2006). Lorenz and Clarke (2006) found that macroinvertebrate communities sampled using different collection methods still ordinated 100% to the season they were sampled in. The study performed by Sporka et al. (2006) involved sampling a fixed number of sites every two months. When the macroinvertebrate communities were plotted using Principal Component Analysis, three groups formed: samples taken in April, those taken in June and August and sites sampled in October, December and Februrary. Season is obviously a large driving factor for macroinvertebrate community structure and distribution due to the effects of climate on stream physical and chemical parameters (temperature,

precipitation, etc.) (Lenat 1988; Sporka et al. 2006).

The difference in macroinvertebrate community structure between the seasons is largely due to life history (Hynes 1970; Reice et al. 1990). Many benthic insects in cold waters emerge over the summer and for many species, the eggs hatch soon after oviposition in the summer (Hynes 1970; Larson and Colbo 1983), therefore a large proportion of the organisms sampled in the summer are immature (Merritt and Cummins 1996). Immature individuals are difficult to identify to lower levels of taxonomy and often only family-level identifications were possible for several groups in the summer samples. These insects would then be in their third to fourth instar in the fall, and sufficiently mature to improve the level of identification, and even more advanced by the spring sample period. However, some species have their larval stages confined to the summer, which also contributes to disparity among seasons (Larson and Colbo 1983).

Taking the different growth strategies into account, it would appear that spring would be the best time to sample the macroinvertebrate community, as the greatest number of species would have fully developed preimaginal stages present. It would then be assumed that more reliable identifications would be better able to segregate regional communities. However, this work did not indicate an increase in the ability of spring multivariate analyses to detect differences amongst regions. Spring had the lowest R statistics of the three seasons both globally and for each season pair. Possible reasons for spring's decreased ability to explain variation in macroinvertebrate communities between regions are as follows. One, sampling in Gros Morne in particular was strongly affected by torrential spring rains. Many of Gros Morne's sites do not have hydrological buffering

capacity as there are few fen/pond step-wise type streams, especially in the Tablelands. Therefore, spates were more destructive in those areas, as evidenced by the severely reduced abundance of organisms in those samples. Gibbins et al. (2001) also found the spring season to be the most variable due to high precipitation and resultant drift of macroinvertebrates. Two, "spring" described as the season just prior to pupation/hatching, can be difficult to identify. One week too late and many of the nymphs could have already left the stream bed, reducing the number of those taxa sampled or completely removing them from the taxonomic list for that season (Hynes 1970). Three, the spring season seemed to be dominated by blackflies, which are ubiquitous in their distribution (Larson and Colbo 1983). Species of blackfly, unique to Gros Morne in habitats sampled, also tended to be rare. Therefore those taxa did little to distinguish Gros Morne from the other regions.

Though the regions were all statistically different from one another, the R values associated with those p-values indicate that the differences between regions may not be biologically significant. R statistics may be positive, negative or zero, with zero meaning there is no difference between the groups being compared (Clarke and Gorley 2006). Most of the R values produced from the regional analyses were small: between 0.1 and 0.3. Therefore, the regions with small values would be considered only slightly different from one another. For example, Terra Nova and Gros Morne's R statistic was almost always below 0.2 (except in summer where it was 0.269). However, the Avalon Peninsula was very different from the other two regions; comparisons with the Avalon gave the highest R statistics in all the ANOSIM tests especially in summer where the R

values for those two comparisons were over 0.5. Therefore, there is generally little difference between the macroinvertebrate communities of Newfoundland, but the Avalon Peninsula communities tend to significantly differentiate from the other two main regions depending on the season. P-values in ANOSIM are highly susceptible to differences in sample size between groups, which explains the highly significant values paired with low R statistics (Clarke and Gorley 2006).

The observed differences within "regions" may also be limited by the fact that the three regions used were not true ecoregions; the sites gathered into "regions" due to similarities in geographic location and geological history. An analysis of differences between macroinvertebrate communities in the various "true" ecoregions of Newfoundland may reveal a greater segregation between communities by reducing intra-regional variation, thereby increasing inter-regional dissimilarity. Classification of macroinvertebrate communities by ecoregion has been met with varying success. In some studies, there was an excellent concordance of community differences and ecoregions (Feminella 2000; Rabeni and Doisy 2000). In others, a more conservative view of the ecoregion method of *a priori* site sorting has led to using clustering to adjust terrestrial ecoregions to suit aquatic biological data (Gerritsen et al. 2000; Sandin and Johnson 2000), and still others find ecoregions to be of no use whatsoever for aquatic community classification (Marchant et al. 2000). Should the ecoregion method of classification prove useful for Newfoundland macroinvertebrates it would mean that *a priori* classifications could preclude the initial exploratory multivariate analysis. The ecoregion view was not tested in this study due to uneven numbers of sites sampled in each region.

The SIMPER analysis pairs well with the ANOSIM analysis as it picks out the macroinvertebrate taxa that drive observed differences among groups (Clarke and Gorley 2006). Here it becomes a little clearer how the regions of Newfoundland have a high degree of similarity between them. The dissimilarity that was observed between seasons and between regions was often provided by the same taxa. Chironomidae supplied at least 3% of the total dissimilarity to all regions pairs over all seasons except for the Avalon and Terra Nova pair in spring. *Oulimnius latiusculus*, an elmid beetle was also useful for dividing the seasons in seven out of nine instances. Taxa such as *Simulium venustum/verecundum*, *Acerpenna pygmaeus* and *Prosimulium mixtum* were more "within season" descriptors of regional disparity. SIMPER essentially provides a list of "core" macroinvertebrate taxa that could be used as bioindicators in some systems (Clarke and Gorley 2006). However, as most of the "core taxa" are the same among the regions due to Newfoundland's depauperate fauna, the abundance of the taxa becomes of greater importance. The absence of a taxon or group of taxa is certainly a strong indicator of differences between sites and regions, but the differences in abundance of a certain taxon can indicate habitat preference or the onset of degrading conditions. Therefore, abundance may give an earlier warning of stress in the habitat before the taxon is completely lost. This possible reliance on abundance for preventative measures presents the need for a sampling method that is relatively insensitive to spatial variation (Merritt and Cummins 1996; Carter and Resh 2001; Gebler 2004).

3.5 *Conclusions*

In addressing objectives one and two of this chapter, significant differences in composition between macroinvertebrate communities in the three geographic regions studied were found. This study supports the review of Vinson and Hawkins (1998) who suggested that the analysis of regional compatibility of data collected over a broad geographic area must be addressed prior to making comparisons and conclusions. Terra Nova and Gros Morne were more taxonomically rich than the Avalon, even when accounting for sample size. Differences in the relative abundance of some taxonomic groups composing the regional communities were found. Avalon sites were dominated by Diptera, while Gros Morne sites had a greater proportion of mayflies, but there was little difference between the regions in proportions of beetles, caddisflies and stoneflies. However, the R values for comparisons among regions were low, indicating that the macroinvertebrate communities were composed of many of the same taxa. The spread of the sites observed in the MDS plots indicate considerable variation within regions and suggest non-regional environmental gradients may exert greater influence on macroinvertebrate communities. Environmental variables often affect patterns in benthic macroinvertebrate communities (Boulton and Lake 1992; Gibbins et al. 2001; Sporka et al. 2006). This line of reasoning will be pursued in the following chapter.

In addressing the second and third chapter objectives, clear differences between seasons were seen which encourages the use of a standardized sampling period for site comparisons and biomonitoring. The current baseline data provided a rationale for suggesting the fall as the most appropriate season for monitoring studies. The summer is

often plagued with low water levels and immature specimens, and spring precipitation and snow melt cause spates that disturb macroinvertebrate communities and impair sampling.

4 Environmental Effects on Macroinvertebrate Community Structure in Newfoundland Riffles

4.1 Introduction

Understanding the interaction of organisms with their environment has been the aim of ecologists for decades, if not centuries (*vis* Vinson and Hawkins 1998; Bonada et al. 2006). Ultimately, the relationship between macroinvertebrates and their environment must be elucidated before the taxa can be of use in biomonitoring (Vinson and Hawkins 1998). Many abiotic and biotic factors influence the make-up of a community. Therefore, changes in one or more environmental factors will affect the members of the community as a whole. Recognizable changes in the community can, with this knowledge, lead to the identification of the stressor and thereby amelioration of the conditions causing the change (Bonada et al. 2006).

Poff (1997) reasoned how an understanding of the interactions of stream organisms with their environment could be accomplished: "…understanding patterns of distribution and abundance of lotic species requires that we test theoretical predictions about functional relationships between species and their environments across a range of spatial and temporal scales." (Poff 1997 p. 392). The author indicates that the relationship between macroinvertebrates and their environment may change across scales of space and time. This theory is confirmed by the contradictory results of studies employing the same methods on the same types of habitats, but in different areas of the world (Vinson and Hawkins 1998). The implied differences between the results of foreign studies and those conducted in Newfoundland are compounded by the fact that Newfoundland's

fauna is greatly reduced by island effects and its recent glaciation (Larson and Colbo 1983; Preston 1962). A reduction in fauna tends to lead to an increase in the breadth of each taxon's niche (Preston 1980). Therefore, even though the mainland may be geographically close to Newfoundland, a differential response of Newfoundland's macroinvertebrates to their environment may be expected merely due to the Island's reduced diversity and thus inter-specific interactions.

Despite differences in interactions between varying locations, all organisms require resources: suitable habitat and nourishment, for example. The fact that species have ranges is indicative of the ability of external factors to limit macroinvertebrate presence and abundance. Also, it could then be expected that species within taxonomic groups will respond to environmental cues across time and space in the same way, due to similar environmental requirements. There are nearly as many studies in agreement as not over the effects of environmental factors on invertebrates (Vinson and Hawkins 1998). The presence of corroborating conclusions validates the formation of some general hypotheses from the results of other studies, particularly when the sites have similar physical and chemical properties (Pennak 1970). For example, New Zealand streams have similar hydrology to most Newfoundland streams in that they tend to form on plateaus and then fall to the ocean quite rapidly (Death and Joy 2004). Therefore, some inference about how hydrology-mediated processes affect macroinvertebrates may be drawn from those studies, even though the species complement differs.

Richards et al. (1997) discovered that large-scale environmental parameters (i.e. bedrock geology) influenced macroinvertebrates at the community level, while

small-scale gradients affected invertebrates at a finer scale (i.e. species traits). Therefore, it is expected that the composition of a macroinvertebrate community will be more strongly related to large spatial-scale environmental factors (e.g. watershed-level differences vs. site-level differences). In contrast, individual taxa should show a greater response to smaller-scale gradients (e.g. substrate size).

There are two pieces of information required from each environmental parameter in this study: does it correlate with macroinvertebrate communities or individual taxa richness and/or abundance, and if so, is the relationship positive or negative. A lack of environmental correlations with the biota indicates a lack of biologically important parameters in the test, while the direction of the relationship can be used for biomonitoring and site prediction. Most studies can agree on a parameter's importance, though directionality of the response tends to vary as widely as the study locations (Vinson and Hawkins 1998). Newfoundland macroinvertebrates are expected to respond to the same contingent of environmental parameters as invertebrate communities in other studies, though the direction of the relationship may differ due to the effects of the Island's unique geographic location (island effects), recent glaciation (later colonization, fewer established taxa) and stream form (see Figure 2.2).

Longitude and latitude were considered prime factors influencing invertebrate community distribution patterns in northern Europe (Heino 2001) and world-wide (Fischer 1960; Giller and Malmqvist 1998). These large-scale "location" variables often correlate with climate, geology and geological history, and are typically equated with terrestrial ecoregions. Ecoregions are considered driving forces in invertebrate

colonization and persistence, often overriding the effects of smaller-scale variables (Corkum 1991; Tate and Heiny 1995; Verdonschot 2006). Geomorphic processes such as glaciations have an effect on the benthos as they dictate when colonization of the region can begin (Giller and Malmqvist 1998; Wohl et al. 1995). Geologic structure is important as it dictates the type of rock making up the region and thus, the potential mineral and nutrient inputs into the stream. In Newfoundland, a study of lake outlet Ephemeroptera, Plecoptera, and Trichoptera (EPT) communities observed a significant difference in diversity and abundance between relatively close geographical regions (Lomond and Colbo 2000). Therefore, regional differences in diversity and abundance can also be expected to occur between the riffle communities of the full complement of macroinvertebrate taxa.

Related to geology is substrate size, which is also an important factor affecting the colonization of macroinvertebrates (Reice 1980; Scarsbrook and Townsend 1993; Wohl et al. 1995; Giller and Malmqvist 1998). Generally, a cobble-size substratum and medium to high embeddedness produce the highest diversity and abundance of macroinvertebrates (Williams and Mundie 1978; Hawkins et al. 1982; Cobb et al. 1992; Giller and Malmqvist 1998). Channel width, both in the bankfull (maximum flow) stage and average (wetted width during sampling) flow stage are linked to macroinvertebrate richness, abundance and location within a stream (Bronmark et al. 1984; Jenkins et al. 1984; Kilgour and Barton 1999; Malmqvist and Hoffsten 2000). Areas of stream described as riffles, which are sections of fast-moving, choppy water, have been recorded as having the highest macroinvertebrate abundance in a stream (Brussock and Brown

1991; Halwas et al. 2005). Velocity has a variety of effects on invertebrate communities in the literature, ranging from no effect (Quinn and Hickey 1990; Sylvestre and Bailey 2005), to a positive effect on diversity and abundance (Rabeni and Minshall 1977; Hawkins et al. 1982), to a negative effect on richness and abundance (Erman and Mahoney 1983; Brooks et al. 2005).

The simple measure of watershed size has also been found to correlate with invertebrate community composition (Bronmark et al. 1984; Kilgour and Barton 1999; Malmqvist and Hoffsten 2000). In many ways altitude is linked to watershed size, primarily through stream order (*c.v.* River Continuum Concept Vannote et al. 1980). In many studies, increased altitude was linked to macroinvertebrate composition (Furse et al. 1984; Malmqvist and Hoffsten 2000; Heino 2001; Sanderson et al. 2005), though others have found little effect or conflicting results (Hawkins et al. 1997; Verdonschot 2006). Correlated with altitude is riparian vegetation, as higher altitudes have less vegetation than lower altitudes (Meades 1983). The type and amount of vegetation impacts the amount of nutrients and tannins that enter the river during rain events. In turn, the vegetation dictates the types and abundance of herbivores that may colonize the area (Woodall and Wallace 1972; Hawkins et al. 1982; Giller and Malmqvist 1998; Black et al. 2004). Also, riparian vegetation can cause shading effects, which may increase or decrease taxonomic richness (Clenaghan et al. 1998; Malmqvist and Hoffsten 2000). Vegetation may also grow directly in the stream in the form of macrophytes. Macrophytes have been shown to increase macroinvertebrate richness and density in several studies (Clenaghan et al. 1998; Malmqvist and Hoffsten 2000; Heino 2005).

Some others found reductions in Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa due to decreased oxygen levels and a possible increase in the number of predators in macrophyte beds (Carpenter and Lodge 1986; Collier et al. 1998).

Water chemistry is strongly influenced by the climate, underlying geology and land use in the stream's catchment (Tate and Heiny 1995; Walsh et al. 2001; Huryn et al. 2002; Doledec et al. 2006). Many studies have declared the importance of chemical parameters such as nutrients, conductivity and pH to macroinvertebrate communities, yet did not record the relationships between the variables and the community (Malmqvist and Maki 1994; Paavola et al. 2003; Sylvestre and Bailey 2005). As chemical variables are so highly influenced by larger, over-arching variables such as geology and land use, the effects of individual water chemistry parameters can be masked as they may act in concert with other variables (Tate and Heiny 1995; Walsh et al. 2001; Huryn et al. 2002; Slavik et al. 2004). For example, the chemical measure of colour of the water is directly related to the amount of coniferous forest, peat beds and humus in the soil (Colbo *pers. comm.*; Hoff 1957; Roberts 1983). Macroinvertebrates may then respond to any one of those land-based variables, or several at once, as measured through the "colour" variable. Therefore, it is important to be aware of the broad-scale effects and relationships of water chemistry parameters with the physical habitat.

Nutrients are a primary restrictor of any community in what is known as the "bottom-up effect". Reduced nitrogen and phosphorus availability limits algal growth. This in turn limits invertebrate growth and production, which then limits fish growth and production (Peterson et al. 1993; Slavik et al. 2004). All streams on the Island of

Newfoundland are nutrient limited except for those receiving inputs from urban and agricultural land use (South 1983; Roberts 1983). Increases in nutrients leads to increased macroinvertebrate production and richness (Heino et al. 2003; Doledec et al. 2006). Therefore, the amount of nitrates, total nitrogen and phosphorus should be the most important commodities in Newfoundland stream ecosystems and should result in higher abundance and richness in areas with higher amounts of nutrients.

Conductivity tends to decrease with an increase in the presence of forest in the watershed; an increase in conductivity often indicates the presence of urban land use. An increase in conductivity often coincides with a loss of EPT taxa and a decrease in their abundance (Huryn et al. 2002; Black et al. 2004). Gibson and Colbo (2001) found urban St. John's streams had a much lower proportion of the community made up of EPT taxa. Therefore, streams with higher conductivity would be expected to have lower abundances of EPT taxa and a lower proportion of EPT in the macroinvertebrate community.

Streams with low pH tend to have low species diversity and abundance (Townsend et al. 1983; Clenaghan et al. 1998; Malmqvist and Hoffsten 2000). However, some streams in Newfoundland tended to have a large selection of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa present in low pH streams, but not in high pH streams (Colbo et al. 1999). Studies from hydrologically similar New Zealand streams found that EPT taxonomic richness was limited by high pH as well (Death and Joy 2004). Therefore, it is expected that low pH streams will have a positive relationship with EPT species richness.

In this chapter, two main hypotheses are tested. 1) There are differences between the three geographic regions that are distinguishable based on the environmental variables sampled. 2) Macroinvertebrate abundance and occurrence is linked to these environmental variables in a predictable manner.

4.2 Methods

Two datasets were used in this chapter. The environmental dataset was made up of physical variables that were considered relatively stable in the temporal scale of the macroinvertebrates and those that were more temporally dynamic (e.g. wetted width, velocity and water chemistry). Data from the fall sampling season was generally used for the latter variables as fall was the season considered to be the best for invertebrate monitoring (Chapter 3). For a list of variables and their units, see Table 2.1 in Chapter 2. Physical variables included vegetation and macrophyte measures due to their part in forming the macroinvertebrate habitat. Draftsman plots were created to detect correlates and evaluate the need for transformation of non-normal variables (Clarke and Gorley 2006). Pairs of variables with correlations of 0.95 had one of the correlates removed; the one removed was selected based on the highest correlations with other variables. $Log_{10}(x+1)$ transformation was applied to the following variables: altitude, maximum summer wetted width, maximum bankfull width and average vegetation overhang. Average velocity, maximum velocity, pH, conductivity, nitrates and alkalinity were $log_{10}(x)$ transformed. These transformations linearized the variables in order to normalize the data for analysis. Draftsman plots of dominant substrate, 2nd dominant substrate and

surrounding substrate measures also indicated non-normal curves, so some classes were lumped together. Bedrock was given a class of 5, boulder a class of 4, large and small cobble became 3, large and small pebbles a 2, and gravel, sand, silt and organic cover were all classed as 1.

The environmental dataset was split into physical and chemical variables for normalization. The chemical dataset required the removal of the Northwest River site (NWR) as it lacked water chemistry data. Likewise, when the physical and chemical variables were re-joined, NWR had to be removed prior to normalization of the entire dataset. UTM co-ordinates were removed for all sites in analyses involving the environmental dataset only, but added to later comparisons with the macroinvertebrate datasets. All environmental resemblance matrices were measured with Euclidian distances. Analysis of Similarity (ANOSIM) tests were performed on the environmental data to test for differences between the regional groups depicted with the macroinvertebrate data in Chapter 3. Refer to the methods section of Chapter 3 and Clarke and Gorley (2006) for details of this analysis.

Principal Component Analysis (PCA) was used to ordinate the sites according to their environmental attributes. The analysis takes all of the variables, which would customarily make their own dimension in a regular ordination, and condenses them into a smaller number of dimensions (Clarke and Gorley 2006). The resulting two or three dimensions explain as much of the variation in the dataset as possible, and this is reflected in the eigenvalues of the Principal Component (PC) axes (Clarke and Gorley 2006). The PC axes are merely linear rotations of the original dimensions; therefore, the

relationship between the sites does not change, but is simply re-expressed on the new axes (Kenkel 2004). Each variable used in the analysis has an eigenvector associated with it, which is the cosine of the angle between the original dimension and the new PC axis. Eigenvectors are produced on the plot as a measure of the strength of the correlation between the variable and the PC axis (Kenkel 2004; Clarke and Gorley 2006). The PCA analysis used here was performed with the transformed environmental dataset. All eigenvectors were reported, despite goodness of fit. Axes were not rotated, but were left in their original position as determined by the PRIMER program.

The second dataset used in this chapter was the fall macroinvertebrate abundance data with the units being individuals/minute. The macroinvertebrate dataset was $\log_{10}(x+1)$ transformed as in Chapter 3. All macroinvertebrate resemblance matrices were calculated using Bray-Curtis similarity (Bray and Curtis 1957). Justification for the choices of transformation and similarity matrix was given in the methods of Chapter 3.

The other prominent analysis utilized in this chapter was the BVSTEP algorithm from Primer v.6. BVSTEP tests for correlations between a normal dataset and resemblance matrix. It uses a "forward-stepping and backward-elimination stepwise procedure" to distil a subset of variables that provide the highest correlation to a selected resemblance matrix (e.g. invertebrate data Bray-Curtis similarity matrix) (Clarke and Gorley 2006). The methodology involves a random selection of variables in the first run that correlates to the secondary dataset (e.g. an environmental similarity matrix). Then, variables are added to the first group. They remain if the correlation increases, and are removed if they do not increase the correlation. The variables in the final set are those

that give the highest correlation with the resemblance matrix (Clarke and Gorley 2006). The Spearman rank correlation was used and resulted in correlations being expressed as Rho values (between 0 and 1).

Two different BVSTEP analyses were used in this chapter. First, BVSTEP was run on the macroinvertebrate matrix against the environmental datasets (physical, chemical, physical and chemical combined). A random selection of 6 variables for the first run with 100 repetitions was used. This analysis provided a subset of environmental variables that maintained a similar relationship to macroinvertebrate data. The second BVSTEP analysis was run using the environmental Euclidean distance matrix against the fall macroinvertebrate abundance dataset. A starting random selection of 6 taxa and 100 repetitions was used. This analysis determined which taxa were consistently associated with particular environmental variables.

A BIOENV analysis, also from the Primer v.6 package, was performed on the environmental data to determine how it related to the macroinvertebrate community structure. BIOENV performs the same task as BVSTEP, though it does not use a forward-backward method. It starts with the first variable and adds each subsequent variable in turn, removing those that do not add to the overall correlation, and it runs methodically through all possible combinations of variables (Clarke and Gorley 2006). The end result is the individual correlations of each of the environmental variables to the macroinvertebrate community as a whole. This enables an understanding of exactly how (positively or negatively) each variable relates to the macroinvertebrate community.

The final analysis, used to identify the link between macroinvertebrates and their environment, was linear, univariate regression. The second form of BVSTEP analysis mentioned above resulted in a list of macroinvertebrate taxa that, either by themselves or combined with others, were correlated with the pattern of sites in the environmental data. The environmental data was split into physical and chemical variables due to the loss of Northwest River in the chemical samples; BVSTEP was performed on these two environmental datasets separately. All the taxa combinations resulting in a correlation of 0.5 or more were examined to find taxa common to all, or all but one, of the combinations. These "common" taxa were then individually regressed with each water chemistry parameter to examine the strength of the correlations using the fall insects/minute abundance data for the invertebrates. Taxa that were not present at five or more sites ("rare" taxa) were excluded. Linear, univariate regressions were performed using MINITAB 14. Regressions were also performed with several community measures: estimated number of organisms, number of taxa, number of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa, percent EPT, percent Simulidae (blackflies) and percent Chironomidae (midges). Metrics involving percentages were arcsin transformed before regressing against the chemical variables in order to reduce skewing in the proportional data (Wheater and Cook 2000).

4.3 Results

4.3.1 Environmental description of the regions

The Global rho value of an Analysis of Similarity (ANOSIM) test between regions indicated that there was little to distinguish regions from one another using only physical data (Table 4.1) (Global R = 0.115, p-value = 0.015). The Avalon and Terra Nova regions were significantly different (p = 0.003), and though the rho value was the largest of the four given by the ANOSIM analysis, the relationship was still weak (R = 0.269). The Avalon and Gros Morne pair and the Terra Nova and Gros Morne pair were not statistically different from one another and had R statistics that approached zero.

The variable vector plots appeared to adequately describe each region and reflected the ANOSIM results (Figure 4.1). The cumulative amount of variation explained by the first three Principal Component (PC) axes was 47.4%; where 20.4%, 15.5%, and 11.5% of the variation was explained by the first, second, and third PC axes, respectively. Avalon sites never formed a distinct data cluster from the other regions in any of the three PCA views, and were generally relegated to the negative side of PC axis two and the positive side of PC axis three. Velocity and watershed size were most strongly related to the negative side of PC axis two, whereas % riffle and % igneous rock in the underlying geology were indicative of the positive portion of PC axis three (Table 4.2). Percent macrophytes and % glacial till also described the Avalon according to the variable vector plots, though the two variables were not strongly related to any of the three PC axes. Terra Nova sites overlapped with Avalon sites and some of the Gros

Morne sites (Figure 4.1). Terra Nova sites were typically widespread across PC axis one and three, but on the negative side of PC axis two. Therefore, Terra Nova sites were largely described by the same group of environmental variables as the Avalon. Gros Morne sites were widely scattered across all the PC axes, denoting a broad spectrum within each physical variable for this group of thirty-five sites. However, a larger proportion of the Gros Morne sites sorted to the positive side of PC axes one and two. Altitude and width in particular vectored in those areas of the PCA plot, as well as some geological variables (Table 4.2).

Table 4.1 ANOSIM tests for strengths of regional differences between sites sampled on the Avalon Peninsula, Terra Nova and Gros Morne using only physical data, only chemical data, and both physical and chemical data.

	Physical Only		**Chemical Only**		**Physical + Chemical**	
	R	p	R	p	R	p
Global R	0.115	0.015	0.333	0.001	0.227	0.001
Avalon vs. Terra Nova	0.269	0.003	0.489	0.001	0.546	0.001
Avalon vs. Gros Morne	0.142	0.064	0.312	0.002	0.236	0.013
Terra Nova vs. Gros Morne	0.086	0.058	0.321	0.001	0.185	0.002

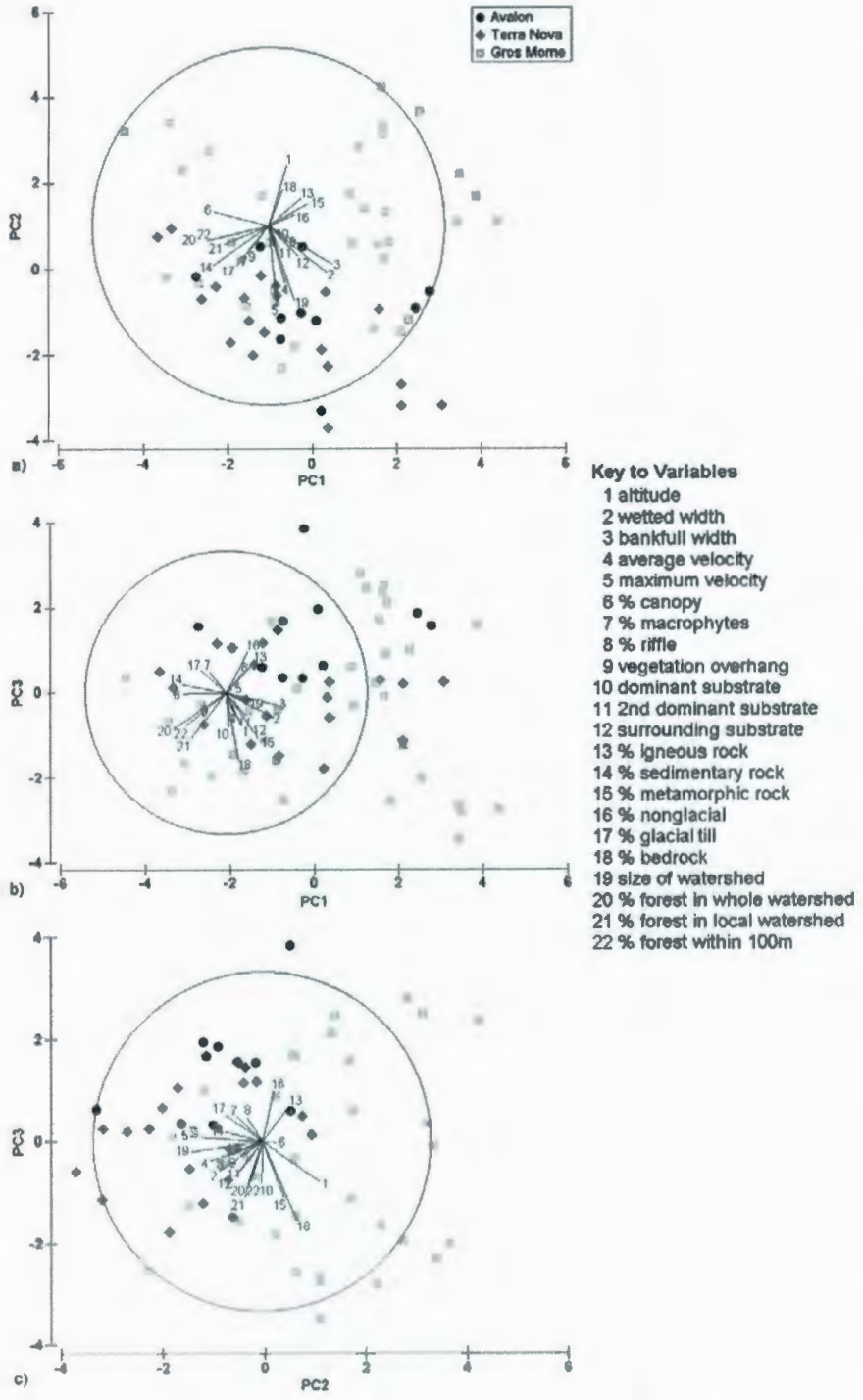


Figure 4.1 2-dimensional PCA biplots of the a) first vs. second, b) first vs. third, and c) second vs. third Principal Component axes for the physical environmental variables. Points represent sample sites, labelled by region. Length of lines represent the correlation of variables with the principal components.

Table 4.2 Eigenvector strengths corresponding with the physical environment variable vector plots in Figure 4.1. Asterisks mark the five eigenvectors that are most strongly related to each axis.

Variable	PC1	PC2	PC3
altitude	0.103	0.340*	-0.234
wetted width	0.333*	-0.260*	-0.176
bankfull width	0.362*	-0.212	-0.088
average velocity	0.095	-0.320*	-0.116
maximum velocity	0.047	-0.429*	0.029
% canopy	-0.312*	0.082	-0.006
% macrophytes	-0.124	-0.157	0.153
% riffle	0.112	-0.092	0.143
vegetation overhang	-0.113	-0.135	-0.077
dominant substrate	0.018	-0.004	-0.243
2nd dominant substrate	0.061	-0.124	-0.158
surrounding substrate	0.167	-0.166	-0.206
% igneous rock	0.187	0.157	0.208
% sedimentary rock	-0.314*	-0.219	0.056
% metamorphic rock	0.227	0.127	-0.328*
% nonglacial	0.150	0.065	0.293*
% glacial till	-0.186	-0.225	0.163
% bedrock	0.081	0.207	-0.465*
watershed size	0.149	-0.418*	-0.059
% forest in whole watershed	-0.377*	-0.088	-0.253*
% forest in local watershed	-0.260	-0.107	-0.330*
% forest within 100m	-0.294	-0.070	-0.236

Water chemistry variables segregated the three regions more clearly than the physical variables (Table 4.1). All the rho statistics were statistically significant and were in most cases twice as large as those produced by the physical ANOSIM test (Global R = 0.333, p = 0.001). The Avalon and Terra Nova region groups were again the most well defined groups in the test, with a rho value approaching 0.5. The Avalon and Gros Morne regions differed by an R statistic of 0.312 (p = 0.002), while Terra Nova and Gros Morne were significantly distinct groups (R = 0.321, p = 0.001).

The analysis of the water chemistry parameters at all of the fall sites produced a highly defined series of regional clusters (Figure 4.2). The cumulative amount of variation explained by the first three PC axes was 84.5%, where 51.5%, 18.1%, and 15.0% was explained by the first, second and third PC axes, respectively. The Avalon sites were scattered across PC axis 1, though three of the sites were distinctly at the highest point of the nitrates vector, which was most prominent on PC axis three (Table 4.3). Two Avalon sites were strongly associated with high Phosphorus and conductivity, whose largest eigenvectors were on PC axis two and one, respectively. Terra Nova sites were associated with higher total nitrogen and colour as well as phosphorus, but the latter only showed up on PC axis three as a weaker eigenvector. Gros Morne sites, except for the six upper Long Range Mountain sites, were associated with higher conductivity, pH, alkalinity and total nitrogen.

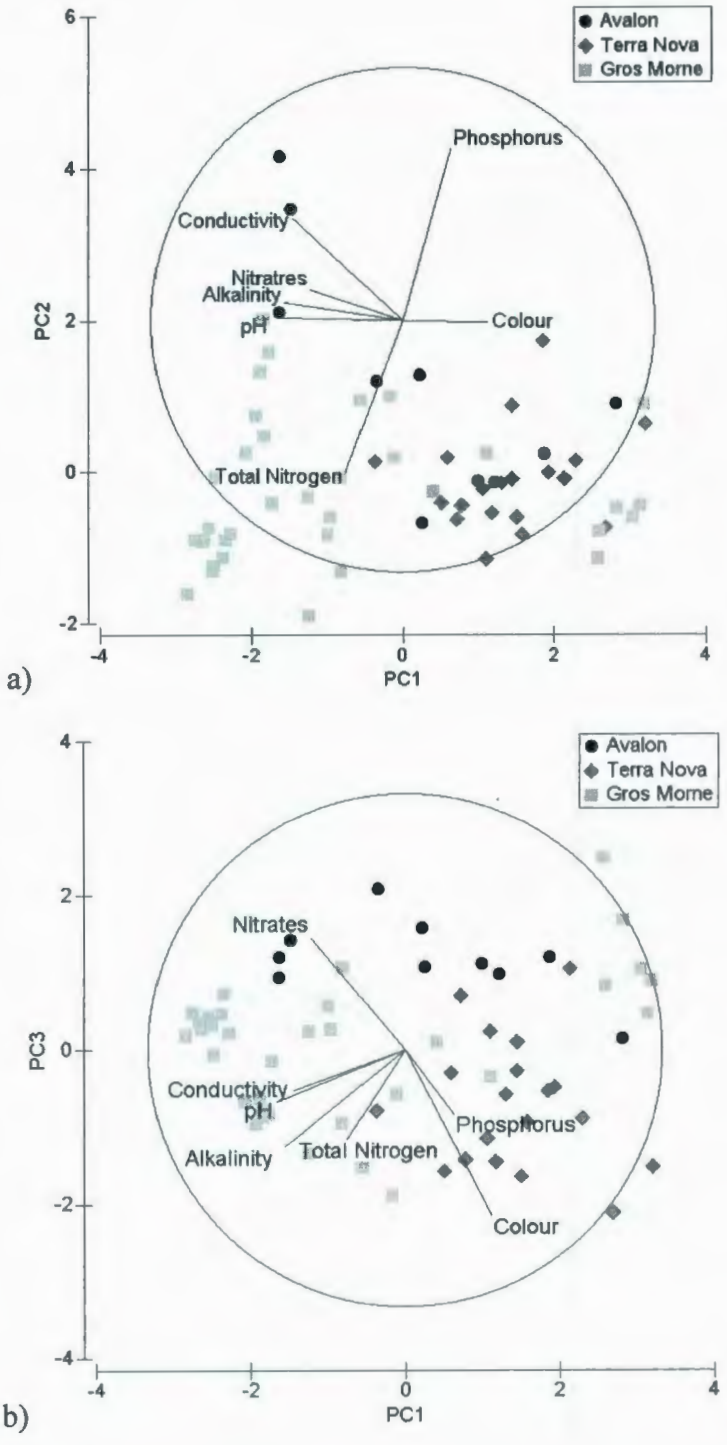


Figure 4.2 2-dimensional PCA biplots of the a) first vs. second and b) first vs. third Principal Component axes for the chemical environmental variables. Points represent sample sites, labelled by region. Length of lines represent the correlation of variables with the principal components.

Table 4.3 Eigenvector strengths corresponding with the water chemistry variable vector plots in Figure 4.2. Asterisks mark the two eigenvectors that are most strongly related to each axis.

Variable	PC1	PC2	PC3
nitrates	-0.368	0.123	0.436*
total Nitrogen	-0.226	-0.594*	-0.346
Phosphorus	0.191	0.680*	-0.252
colour	0.336	-0.008	-0.647*
conductivity	-0.438	0.405	-0.159
pH	-0.501*	0.011	-0.201
alkalinity	-0.470*	0.071	-0.377

When the physical and chemical variables were combined, the amount of variation between the regions explained by the combined environmental datasets decreased from what was explained by the water chemistry alone in all but one region pair (the Avalon and Terra Nova, Table 4.1).

4.3.2 Linking the environment and macroinvertebrate community structure

The maximum correlation of the environment with the macroinvertebrate community was 0.509 using 11 of the 31 physical and chemical variables (Table 4.4). The average amount of overhanging vegetation, amount of igneous rock in the watershed and the amount of forest in the local watershed were consistently included in the BVSTEP analysis results as key factors in connecting the physical and macroinvertebrate datasets. The Easting map units and the amount of macrophytes in the sample reach were also included in most of the high-correlation results.

The physical data on its own had a maximum correlation of 0.454 with the macroinvertebrate abundance data using five variables (Table 4.4). The three highest correlating sets of variables included seven of twenty-four variables, four of which were in all three sets: UTM Easting, average vegetation overhang, % igneous rock and % forest in the local watershed. Wetted and bankfull widths as well as watershed size were each in one of the three results.

The chemical variables had the least amount of correlation with the macroinvertebrate data, where the correlation was 0.353 using three of seven water chemistry parameters (Table 4.4). Nitrates, total nitrogen and alkalinity were also often included in the sets of variables chosen by the BVSTEP algorithm in the joint physical-chemical analysis. No other combinations of chemical variables gave a higher correlation, nor were additions of the other four variables to the prime three able to increase the amount of agreement between the two datasets.

Individual correlations of environmental variables to the abundance data revealed similar results to those deciphered using the BVSTEP algorithm (Table 4.5). UTM Easting co-ordinates, amount of igneous rock in the catchment, nitrates, pH and alkalinity were the most highly correlated with fall macroinvertebrate abundance. All five variables were positively correlated with the macroinvertebrate data. Total nitrogen and UTM Northing co-ordinates were also related to the macroinvertebrates, with correlations of 0.223 and 0.200, respectively.

Table 4.4 Environmental variables contributing to a maximum correlation with the fall macroinvertebrate abundance data. Variables segregated into only physical variables, only chemical variables and both physical and chemical variables. # Observed is the number of times that arrangement of variables occurred out of 100 random starts of BVSTEP.

Fall Macroinvertebrate Abundance		
# Observed	**Correlation**	**Variables**
Physical Variables Only		
73	0.454	1,6,8,10,11
17	0.453	1,4,6,8,11
10	0.445	1,3,6,8,11
Chemical Variables Only		
100	0.353	12,13,16
Physical and Chemical Variables		
19	0.509	1,5-13,16
5	0.508	1,5-13,15
44	0.505	1,4,6-8,11-13,16
12	0.505	1,4,6-8,11-13,15
11	0.486	2,6-8,10-13,16
9	0.476	1,4,6,8,11,13,14

Key to Variables
1 UTM Easting
2 UTM Northing
3 Maximum summer wetted width
4 Maximum bankfull width
5 % Macrophytes in reach
6 Average vegetation overhang
7 Dominant substrate
8 % Igneous rock in watershed
9 % Sedimentary rock in watershed
10 Size of watershed
11 % Forest in local watershed
12 Nitrates
13 Total Nitrogen
14 Conductivity
15 pH
16 Alkalinity

Table 4.5 Individual correlations of each of the environmental variables with fall macroinvertebrate abundance data. Asterisks mark the top five correlates.

Variables	Correlations
UTM Easting	0.258*
UTM Northing	0.200
Altitude	0.091
Maximum summer wetted width	0.060
Maximum bankfull width	0.105
Average velocity	0.112
Maximum velocity	0.105
% Canopy cover	0.008
% Macrophyte cover	0.127
% Riffle in reach	0.071
Average vegetation overhang	0.169
Dominant substrate	0.069
2nd dominant substrate	-0.002
Surrounding substrate	-0.023
% igneous rock	0.331*
% sedimentary rock	0.181
% metamorphic rock	-0.038
% non-glacial	0.134
% glacial till	0.054
% bedrock	-0.023
Size of watershed	0.109
% Forest in whole watershed	0.051
% Forest in local watershed	0.125
% Forest within 100m of site	0.012
Nitrates	0.312*
Total nitrogen	0.223
Phosphorus	0.166
Colour	0.062
Conductivity	0.145
pH	0.224*
Alkalinity	0.277*

The BVSTEP analysis was previously run in such a way as to compare the correlation of environmental variables to the fall abundance macroinvertebrate data (Table 4.4). Here, the fall macroinvertebrate abundance was correlated with the environment to associate taxa with the patterns seen in Figure 4.1 and Figure 4.2. The highest correlation of macroinvertebrates with the physical environmental variables was 0.552 with 25 of 127 taxa. Of the twenty-four taxa that were in all, or all but one, of the combinations that correlated with the physical data at 0.5, only nine were not considered "rare" (present at five or more sites). Of these nine, one taxon was not correlated with any of the physical parameters: *Isogenoides fontalis*. Also, seven physical variables were not correlated with any of the nine species: bankfull width, wetted width, average velocity, % canopy cover, % riffle, vegetation overhang and dominant substrate.

Most taxa were strongly correlated with more than one environmental variable (Table 4.6). *Ceraclea sp.* and Chironomidae were positively correlated with UTM Easting, whereas *Gyralis sp.*, *Ephemerella sp.* and Chironomidae were negatively associated with the UTM Northing variable. Substrate size was an important factor for *Heptagenia pulla* and *Apatania sp.*, where both were positively related to larger second dominant and surrounding substrates, respectively. *Epheremerella sp.*, *Hydroptila sp.* and Chironomidae were positively related to the amount of macrophytes on site, and the latter two were also negatively correlated with presence of forest in the catchment. Chironomidae showed the strongest relationship with the base geology of the stream, where their relationship with igneous rock is strongly negative, but positive with streams having sedimentary and/or metamorphic rock. Seven of the eight taxa had a significant

relationship with any one of the three substrate type variables – non-glacial, glacial till and bedrock. *Apatania sp.* alone showed a preference for streams with larger catchments.

For the community measures, only % canopy cover at the sampling site was not correlated with any of the metrics. The total number of organisms estimated at each site was related to 8 of the 23 available physical variables; the metric was positively correlated with macrophytes and non-glacial type substrate in the catchment (Table 4.7). Total number of organisms at a site was negatively related to the UTM Northing, bankfull width, surrounding substrate size, percent of the watershed containing glacial debris and igneous rock and forestation in the local watershed. The total number of taxa at each site was positively correlated with twelve variables, and was only negatively correlated with igneous rock. UTM Northing, altitude, stream width, macrophytes, amount of riffle, dominant substrate size, metamorphic rock and the glacial till in the catchment had no significant effect on taxonomic richness.

The total number of EPT taxa per site was correlated with five fewer variables than the total-taxa-per-site metric (Table 4.7). The number of EPT taxa at a site was positively correlated with catchment size, forest cover in the watershed and on site, average velocity, as well as second dominant substrate and surrounding substrate size. Interestingly, the physical variables having the strongest correlations with number of EPT taxa were not the variables that had the strongest correlations with percentage of the sample made up of EPT taxa. Higher abundances of EPT organisms were positively associated with sites that were further north, had a higher amount of riffle in the reach, were based on igneous rock with glacial debris and whose local watershed was largely

forested. Lower proportions of EPT organisms were found at sites where there was a high percentage of macrophytes and overhanging vegetation and in catchments dominated by metamorphic bedrock.

The proportion of the sample made up of blackflies was negatively correlated with the bankfull width and the amount of the catchment that contained glacial till, but was positively correlated with dominant substrate and bedrock (Table 4.7). The percentage of chironomids in the sample was not always correlated with the same physical variables as the overall abundance of Chironomidae in the sample (Table 4.6), though the shared variables did have the same relationship with the two metrics. A greater proportion of chironomids was found in samples that were higher in altitude, had a greater wetted width and average velocity and were in catchments dominated by metamorphic bedrock. Percentages of chironomids dropped at sites in igneous rock with glacial till and large amounts of forest at all three levels.

Table 4.6 The relationship of specific macroinvertebrate taxa to physical environmental variables. If the relationship was not significant, neither the p-value nor the direction of the relationship was reported. Key to shorthand: dir = direction of relationship, sub = substrate, WW = whole watershed, LW = local watershed.

	Gyralis sp.		*Heptagenia pulla*		*Ephemerella sp.*		*Ameletus sp.*		*Hydroptila sp.*		*Ceraclea sp.*		*Apatania sp.*		Chironomidae	
	dir	p	dir	p	dir	p	dir	p	dir	p	dir	p	dir	p	dir	p
UTM Easting											+	0.013			+	0.047
UTM Northing	-	0.010			-	0.006									-	0.015
Altitude							+	0.004			-	0.041				
Maximum velocity											+	0.004				
% Macrophytes					+	<0.001			+	<0.001					+	0.040
2nd Dominant sub.			+	0.049												
Surrounding sub.													+	0.027		
% Igneous															-	<0.001
% Sedimentary							-	0.038							+	0.033
% Metamorphic			+	0.003			+	<0.001							+	0.017
% Nonglacial					+	0.004			+	<0.001						
% Glacial till			-	0.001					-	0.037	+	0.033	+	0.044	-	0.024
% Bedrock			+	0.018			+	0.035								
Size of watershed													+	0.047		
WW % forest									-	0.024						
LW % forest									-	0.006					-	0.003
100m % forest													+	0.008		

Table 4.7 The relationship of macroinvertebrate community measures to physical environmental variables. If the relationship was not significant, neither the p-value nor the direction of the relationship was reported. Key to shorthand: dir = direction of relationship, sub = substrate, WW = whole watershed, LW = local watershed.

	Total # /site		Total # taxa/site		Total # EPT taxa/site		% EPT		% Simulidae		% Chironomidae	
	dir	p	dir	p	dir	p	dir	p	dir	p	dir	p
UTM Easting			+	0.009								
UTM Northing	-	0.004					+	0.031				
Altitude											+	0.002
Bankfull width	-	0.023							-	0.021		
Wetted width											+	0.011
Average velocity			+	0.015	+	0.041					+	0.050
Maximum velocity			+	0.012								
% Macrophytes	+	<0.001					-	0.045				
% Riffle							+	0.044				
Vegetation overhang			+	0.002			-	0.036				
Dominant substrate									+	0.020		
2nd Dominant sub.			+	0.008	+	0.017						
Surrounding sub.	-	0.020	+	0.013	+	0.024						
% Igneous	-	0.011	-	0.001			+	<0.001			-	<0.001
% Sedimentary			+	0.017								
% Metamorphic							-	0.003			+	<0.001
% Nonglacial	+	0.012										
% Glacial till	-	0.032					+	0.049	-	0.004	-	0.001
% Bedrock							-	0.024	+	0.002	+	<0.001
Size of watershed			+	0.020	+	0.023						
WW % forest			+	0.001	+	0.006					-	0.016
LW % forest	-	0.002	+	0.002	+	0.001	+	0.008			-	<0.001
100m % forest			+	<0.001	+	0.001					-	0.004

The fall macroinvertebrate abundance data was also correlated with the water chemistry information in such a way as to associate taxa with the water chemistry PCA plot (Figure 4.2). The best correlation of macroinvertebrates with the water chemistry data was 0.529 using 25 of 127 taxa. Of the twenty-one taxa that were in all, or all but one, of the combinations that correlated with the physical data at a level of 0.5 or more, only fifteen were not considered "rare" (present at five or more sites). Two of the common taxa were not significantly related to any of the water chemistry parameters:

Rhyacophila ignorata and *Ephemerella aurivilli*. Most of the other fifteen prominent taxa were related to several parameters (Table 4.8). Ten taxa were associated with pH and alkalinity, nine of which were negatively related to these variables. Nine taxa were strongly related to total nitrogen and conductivity; eight of the nine taxa displaying a negative relationship. Eight taxa were associated with nitrates, six negatively. Phosphorus and colour were correlated with the least number of taxa: five and three, respectively. Of these, only *Rithrogenia undulata* had a negative relationship with the two variables. Of the fifteen "common" taxa, *Acerpenna pygmaeus* and *Rithrogenia undulata* related to the highest number of water chemistry parameters; both taxa only lacked an association with conductivity.

The total number of organisms present at each site was negatively related to total nitrogen, pH and alkalinity (Table 4.8). Total number of taxa per site increased with increasing phosphorus and colour, but decreased with increasing nitrates, total nitrogen and pH. This pattern of relational directions was identical to that for *Acerpenna pygmaeus*, a baetid mayfly. The total number of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa per sample did not have the same pattern as the total number of all taxa as there was no significant relationship between the metric and total nitrogen and Phosphorus, but instead a significant negative relationship with conductivity. The percentage of each sample that was made up of EPT taxa was very strongly and positively correlated with nitrates, pH and alkalinity, but negatively associated with Phosphorus. The percent Simulidae (blackflies) in the sample was not significantly related to any water chemistry parameters. The proportion of Chironomidae present was negatively related to total nitrogen, conductivity, pH and alkalinity.

Table 4.8 The relationship of specific macroinvertebrate taxa and community measures to water chemistry variables. If the relationship was not significant, neither the p-value nor the direction of the relationship was reported.

	Nitrates	Total Nitrogen	Phosphorus	Colour	Conductivity	pH	Alkalinity
Lumbriculidae	+	-	+		+		
p-value	0.043	0.002	0.001		0.001		
Acerpenna pygmaeus	-	-	+	+		-	-
p-value	<0.001	0.002	0.032	<0.001		0.004	0.029
Heptageniidae	-						
p-value	0.028						
Heptagenia pulla					-	-	-
p-value					0.041	0.014	0.007
Leucrocuta hebe		-			-	-	-
p-value		0.008			0.038	0.024	0.023
Rithrogenia undulata	+	+	-	-		+	+
p-value	0.007	0.003	<0.001	0.010		<0.001	<0.001
Stenonema vicarium	-	-			-	-	-
p-value	0.005	0.013			0.026	0.004	0.015
Eurylophella sp.		-			-	-	-
p-value		0.023			0.028	0.003	0.002
Ameletus sp.					-	-	-
p-value					0.014	0.017	0.008
Paracapnia opis	-	-			-	-	-
p-value	0.001	0.004			0.003	0.001	<0.001
Isoperla transmarina	-	-			-	-	-
p-value	0.040	0.031			0.012	0.017	0.008
Oxyethira sp.		-			-	-	-
p-value		0.048			0.003	<0.001	<0.001
Hydatophylax argus			+				
p-value			0.007				
Rhyacophila minora				+			
p-value				0.001			
Prosimulium mixtum	-		+				
p-value	0.016		0.039				
Community Measures							
Total # organisms/site		-				-	-
p-value		0.026				0.044	0.010
Total # taxa/site	-	-	+	+		-	
p-value	0.003	0.026	0.011	0.001		0.027	
Total # EPT taxa/site	-			+	-	-	
p-value	0.001			0.002	0.027	0.041	
% EPT		+	-			+	+
p-value		<0.001	0.027			<0.001	<0.001
% Chironomidae		-			-	-	-
p-value		<0.001			0.001	<0.001	<0.001

4.4 Discussion

4.4.1 Environmental description of the regions

The ordination of the environmental data indicated that there were small, but consistent and significant differences in the environmental attributes of the three regions, which mirrored findings from the analysis of the macroinvertebrate data (Chapter 3). The Global R statistics associated with the physical variables in particular were close to zero, indicating that all the regions are physically similar. The statistical significance matters little when the rho values are low as p-values are strongly affected by sample size (Clarke and Gorley 2006). Part of the lack of significant differences between regions on the Island of Newfoundland is related to the fact that it is completely contained within the Boreal Shield ecozone (Damman 1983). Ecozones are identified by similarities in climate, geology and vegetation (Damman 1983). Therefore, sites within an ecozone would be expected to have the same, or at least a broadly similar, physical description. Thus analysis of sites by specific ecoregion may have revealed a greater segregation of groups than the analysis of sites by region. However, testing of ecoregion effects was not possible here as sites were not evenly spread among the nine ecoregions on the Island, which negatively influences sample-size dependent measures (Clarke and Gorley 2006).

The water chemistry exhibited some power to distinguish regions as the Global R was higher than 0.3, though still not close to 0.4: the general rule of thumb in deciding whether or not the R statistic is ecologically significant (Clarke and Gorley 2006). The region pairs indicated a consistent difference between the Avalon and Terra Nova regions in particular. In the water chemistry ANOSIM test, the pair had the highest R statistic of the three (0.489), and in the combined physical and chemical test, the pair had the highest

R statistic of all the pairs in all the tests (0.546). As the rho value was quite low in the physical test, the large R statistic in the latter is more related to the water chemistry than the physical differences between the regions. Chemical differences between the Avalon and the other regions likely come from the large urban component of sample sites from this eastern region. Effects of urbanization on water chemistry are discussed later. The physical similarities are understandable as these eastern regions share the same basic geological morphology due to their shared glacial history (Rogerson 1983).

Ordinations and associated variable vector plots of physical environmental data mirrored the ANOSIM results. Regions did not form distinct, tight clusters, probably for the reasons noted above. The regions often lay on specific sides of various axes, so their presence or lack thereof in quadrants of the plot made it possible to describe them. The variable vectors associated with the axes related well to what is already known of the regions from past studies (*vis* South 1983) and to the raw environmental data collected in this study. For example, many Gros Morne sites were at higher altitudes, had large amounts of bedrock in the surficial geography, and had lower amounts of vegetative cover, which was supported by the ordination of a large number of Gros Morne sites in the positive ends of PC axes 1 and 2 (Figure 4.1). However, not all Gros Morne sites followed this description, as evidenced by dispersion of Gros Morne points throughout the other region "clouds" in the PC plot. An ecoregion approach may improve the results, for example, there are barrens both in the Long Range Mountain portion of Gros Morne and around the streams near Cape Race on the Avalon. The environmental similarities of these two regions resulted in these sites ordinating together, rather than with sites belonging to their won regions. If these ecoregions had been segregated from the others

in their general regions, the sorting of Avalon sites with Gros Morne sites may have been better explained.

In the regionally well-defined chemical PCA plot, it became clear that Terra Nova sites were associated with greater colour and increased total amounts of phosphorus. The inference from the raw data implied that Avalon sites would be most strongly associated with phosphorus as Virginia River and Waterford River had the highest concentrations of phosphorus (Appendix 3). However, phosphorus concentrations at these two sites were higher than others by only a few μg/litre. Phosphorus, when available, is quickly removed from stream systems by algae and riparian vegetation. This immediate consumption in the nutrient-poor streams of the island masks the record of the true amount of phosphorus entering the stream system. Phosphorus input cannot be measured by the simple water samples taken in this study and therefore the values should be treated with a measure of caution. The nitrates vector is also commonly associated with urbanization and nutrient enrichment in general (Huryn et al. 2002), which was the case in the St. John's region. However, total nitrogen did not have such a specific regional eigenvector, perhaps because the amounts of nitrogen were low and stable across the island, except on the Avalon where the readings were below detection. Using accumulators of nutrients (e.g. algae, stream-side plants) could eliminate the problems with single water samples and give a more accurate reading of actual nutrient inputs. The vectors associated with Gros Morne were expected due to the high pH and alkalinity in the Tablelands area, as well as the extremely low pH and alkalinity in the high-elevation Long Range Mountain sites. Conductivity was generally greater not only in certain Gros Morne sites, but also in the Avalon city sites, which was reflected by vector direction.

Surprisingly, the Avalon had more in common with Gros Morne than with Terra Nova. The eigenvectors explained part of the reason for this small degree of similarity between some of the Avalon and Gros Morne sites: low pH, conductivity, alkalinity and colour as well as high amounts of nitrates. Sites with these specifications were the barrens sites of the southern Avalon Peninsula and the high-altitude Long Range Mountain sites of Gros Morne. The city sites on the Avalon as well as the streams draining the sites with basic rock in Gros Morne tended towards more elevated levels of conductivity, pH, alkalinity and colour (Appendix 3).

4.4.2 Broad linkages between macroinvertebrates and their environment

Though knowing the extent of the environmental differences among regions is important and informative, it is arguably more important to know how those differences relate to the distribution of macroinvertebrates. That is, are the differences or similarities imposed on the macroinvertebrates by the environment biologically relevant? The aquatic macroinvertebrates of Newfoundland tend to be habitat generalists (Larson and Colbo 1983), which can cause a lack of visible response of invertebrates to environmental variation (Batzer et al. 2004). However, in this study, the maximum correlation of the environmental data with the macroinvertebrate data was Rho = 0.509, with eleven of the thirty-one variables available (Table 4.4). A study of seasonal woodland ponds in Minnesota achieved an overall explained variation of 31.6% on the first three CCA axes for an entire community (Batzer et al. 2004). Heino's (2001) study on river benthos in northern Europe described the correlation of climate and geography with stoneflies as the environment having explained 25% of the variation, dragonflies 37.3%, and dytiscid beetles 40.5%. Richards et al. (1996) managed a 46% correlation of stream

macroinvertebrates with a series of environmental variables. By comparison, the level of correlation between macroinvertebrates and their environment reached in this study is high enough to infer a definite relationship between the environmental variables sampled and the macroinvertebrate communities.

In the analysis of the environment's connection to the macroinvertebrate data, seven physical variables were included in most or all of the results from the BVSTEP algorithm: Easting map co-ordinates, the amount of macrophytes, wetted width, bankfull width, average vegetation overhang, % igneous rock in the watershed and amount of forest in the local watershed. Of these seven, UTM Easting and % igneous rock had the highest individual correlations with the macroinvertebrate data. Three chemical variables, nitrates, total nitrogen and alkalinity were also pulled out in the BVSTEP analyses as parameters best explaining macroinvertebrate community structure. Nitrates, alkalinity and pH had the highest independent relationships with the macroinvertebrate data. The highest correlation of the physical data with the macroinvertebrate pattern was 0.454, but the highest correlation afforded by the chemical data was 0.353. This supports the theory that macroinvertebrate communities are foremost influenced by the physical parameters of the island, mirroring the sampling focus of most monitoring programs (Reynoldson et al. 1997; Vinson and Hawkins 1998; Barbour et al. 1999; Hering et al. 2004). More specific responses of macroinvertebrates to individual variables, as well as interaction between environmental variables, will be discussed later on in the chapter.

When the macroinvertebrate data were compared to the patterns existing in the environmental data, instead of the environmental data with the patterns in the macroinvertebrate community, much higher correlations between macroinvertebrates and

their habitat resulted. This change is understandable as the resulting correlation uses a subset of taxa that was selected specifically to maximize the correlation with the environmental data. Reducing 127 taxa to 25 "important" taxa increases the likelihood of detecting correlations due to decreased "noise" and variation in the dataset. Twenty-five taxa had a maximum correlation of 0.552 with the physical variables, while another subset of twenty-five taxa had a slightly lower correlation of 0.529 with the chemical variables. Therefore the study showed that some freshwater macroinvertebrate taxa in Newfoundland can be associated with both physical and chemical measures of their environment.

In contrast with the low number of variables considered to be well correlated with macroinvertebrate community structure, most of the thirty-one environmental variables were either positively or negatively correlated with both individual "common" taxa and community metrics. The increase in highly correlated variables is because the whole dataset of 127 taxa was pared down to a reduced number using their correlation with the environmental variables. Only the community metrics were associated with stream width, average velocity, % riffle, vegetation overhang, dominant substrate and % sedimentary rock in the watershed. It is understandable that community-based metrics would correlate with the same variables as the entire dataset, as they are merely a simplified invertebrate community descriptor.

4.4.3 Specific linkages between macroinvertebrates and their environment

The most basic components of any habitat will influence the organisms that live there: from simple geographic location, to climate, to vegetation patterns. It has been proposed that even the evolutionary history of a watershed affects macroinvertebrate

community structure (Wohl et al. 1995). Verdonschot (2006) recorded that ecoregion, as influenced by climate and geology, was a driving force in macroinvertebrate communities. In a study of Northern Europe, longitude was implicated in ultimately describing community composition (Heino 2001). In Newfoundland, the UTM Easting (longitude) directly corresponds with the ecoregional divisions of the Island (Damman 1983). Therefore, according to the definition of an ecoregion, the base geology type and origin are also intrinsically related to the map Easting co-ordinates. Rogerson (1983) confirmed this with his study of the geological origins of the Island of Newfoundland and the coinciding topography. The six geological variables in this study were not significantly correlated (<0.95) with each other or the UTM Easting variable, but their theoretical relationship is undeniable.

All tests relating macroinvertebrate communities to the Easting map unit co-ordinates resulted in a positive correlation. The univariate analysis indicated by a positive relationship that the taxonomic richness at each site may have been part of what related the macroinvertebrate community to this variable. This appears to contradict the findings of Chapter 3, where Terra Nova had the highest number of taxa at its sites and not the most easterly Avalon as suggested here. The likely reason is that the large easterly shift between Gros Morne (with a lower number of taxa per site) and Terra Nova and the comparably small easterly shift from there to the few Avalon sites. In fact, the taxonomic richness per site on the Avalon and Gros Morne were nearly equal. Therefore the univariate result does not indicate that the further east a site is on the Island, the more taxa it will have. This stresses the importance of having a broad knowledge of geographical characteristics, geological history and associated environmental variables of

sites sampled before attempting to interpret the statistical analyses. The positive correlations of abundances of *Ceraclea sp.* and Chironomidae are less controversial. There are generally higher abundances of these taxa in the eastern portion of the province, for example, Gros Morne only had one specimen of *Ceraclea* at one site. Larson and Colbo (1983) and results here also showed that other taxa were limited to the western side of the province.

As stated above, geological type and origin is related to ecoregion. However, of the geological variables, only the amount of igneous rock was indicated in the BVSTEP and BEST analyses as being highly correlated with macroinvertebrate community structure. The community metrics were often mixed in their response to a particular geological factor. The total estimated abundance was negatively correlated with the amount of igneous rock and the amount of glacial deposits. The Avalon sites had the highest abundances on average, in agreement with previous research on St. John's stream macroinvertebrate production (Gibson and Colbo 2001), and also have no igneous rock in their basins. However, the northern portion of the Avalon has large amounts of glacial till in its watersheds. Therefore, the correlation of macroinvertebrates with % glacial till does not relate to UTM Easting in the same way as their correlation with % igneous rock. Longitude is not the only large-scale factor affecting macroinvertebrate distribution. In areas such as the Avalon Peninsula where there are distinct north-south ecoregional separations, the Northing map co-ordinates must have an indirect effect. Gros Morne has north-south separations as well, the granite highland sites being confined to the northern side of the park and the Tablelands strictly residing on the southern side (*vis.* Chapter 2,

Figure 2.1). Thus, geographic positioning of the site is not the direct factor, but rather the correlation of co-ordinates with an environmental variable.

Substrate size is an important factor determining colonization of macroinvertebrates (Reice 1980; Scarsbrook and Townsend 1993; Wohl et al. 1995; Giller and Malmqvist 1998). First, an increasing dominant substrate size indicates an increase in substrate stability. Stability affects colonization and the resulting macroinvertebrate community composition, partly due to the loss of organisms in spates (Boulton and Lake 1992; Death 1995; Miyake and Nakano 2002). Some macroinvertebrates, such as black flies, require a stable substrate to anchor to while they filter feed (Merritt and Cummins 1996). In this study, the proportion of simulids in the community did increase with increasing dominant substrate size, which presumably relates to increased stability. Also, black fly presence was negatively correlated with bankfull width, which is also connected to substrate stability. The smaller the bankfull width compared to wetted width, the less likely it is that the stream floods or flashes. If blackflies simply preferred smaller rivers, there would have been a corresponding negative correlation with wetted width.

Another factor indicated by a larger substrate size is a lower amount of inorganic particulate matter in the stream (Rabeni and Minshall 1977; Parker 1989). Taxa with external gills tend to be sensitive to fine particles such as sand and silt (Johnson et al. 2005; Doledec et al. 2006). Richards et al. (1997) found that abundances of all macroinvertebrates, except for burrowers, decreased with an increased amount of fine particulates. The abundance of the caddisfly *Apatania* was positively correlated with surrounding substrate size, and *Heptagenia pulla* was positively correlated with the size

of the 2nd most dominant substrate. *Ceraclea sp.* was associated with faster-moving waters, but not with surrounding substrate, which is interesting as several of these taxa require sand grains to build their external cases. However, larger rocks tend to form depositional zones immediately downstream where sand, detritus and organic matter may collect (Parker 1989). This may explain why *Ceraclea sp.* did not appear to be associated with surrounding substrate as small depositional zones behind boulders would not have been considered extensive enough to identify as the surrounding substrate.

As stated by Death (1995), instability may not affect colonization of a site, but it does affect the relative success of macroinvertebrates attempting to colonize. Futhermore, Death and Winterbourn (1995) also found that the highest diversity in stream habitats occurred with low levels of disturbance. Moderate levels of deposition (stability) can lead to increased species richness in some streams (Miyake and Nakano 2002). However, when stability leads to complete embeddedness, macroinvertebrate productivity has been shown to decrease (Giller and Malmqvist 1998). In this current study, the total number of taxa per site and the total number of EPT taxa per site were positively correlated with 2nd dominant substrate (the second most common size of substrate) and surrounding substrate (interstitial substrate) size. The dominant substrate was generally the largest sized substrate in the stream bed, though sometimes the 2nd dominant substrate exceeded the size of the dominant. As a result, the dominant substrate was most likely to have the highest size class, and therefore the highest stability. The 2nd dominant substrate size was most frequently recorded as either large or small cobble. The surrounding substrate size rarely exceeded 50-100mm or "small cobble" and was registered to be as miniscule as "silt" (Appendix 3). Certain macroinvertebrate taxa have been found to prefer cobble

substrates (though abundance does not usually reflect this preference) but preferences are also related to substrate availability (Reice 1980).

It has been suggested that substrate stability and size are negatively associated with macrophytes (Collier 1995). Understandably, substrates such as bedrock and large boulders would be poor for growing macrophytes due to a lack of rooting material. Several studies have indicated an increase in macrophytes led to an increase in taxonomic richness, and in some cases, density (Clenaghan et al. 1998; Malmqvist and Hoffsten 2000; Heino 2005). The density, or abundance, of macroinvertebrates was positively correlated with macrophytes density in this study. *Ephemerella sp.*, *Hydroptila sp.* and Chironomidae were all positively correlated with macrophytes in the sample reach. The *Hydroptila sp.* feed on filamentous algae (Wiggins 1996) and immature ephemerellids and midges may use macrophytes for refuge from predators. However, the proportion of EPT taxa in the stream was negatively correlated with macrophytes. Collier et al. (1998) found this to be the case in New Zealand streams as well. They cite dissolved oxygen variation, decreased velocity and increased predation by some fish species within the macrophyte beds as possible reasons for the reduction of EPT taxa in this habitat (Carpenter and Lodge 1986; Collier et al. 1998).

Macroinvertebrate abundance tends to be higher in riffles than in pools, runs and chutes (Brussock and Brown 1991; Halwas et al. 2005). However, in these literature studies, community structure was not affected by this finer level of habitat specification. Some invertebrate families, such as Heptageniidae, Leptophlebiidae and Chloroperlidae, were more abundant in riffles than in other habitats, but the overall composition of the community did not differ (Halwas et al. 2005). Likewise, Scarsbrook and Townsend

(1993) found no difference in species diversity between riffles and pools, though riffles were reported to have a greater amount of refuge for macroinvertebrates. However, studies performed in the Southern Appalachians denoted the cobble-riffle habitat as having the highest diversity, but the lowest productivity in the stream (Wohl et al. 1995; Angradi 1996). The current study found a higher proportion of EPT taxa at streams sites with a greater amount of riffle habitat, much as Halwas et al. (2005) reported. Neither total nor EPT taxa numbers correlated with % riffle, which indicates an agreement with findings of the first three studies, but not with Wohl et al. (1995). In this study, there was also no relationship found between % riffle and macroinvertebrate abundance, which mirrors the results of the Appalachian studies to an extent, though a negative correlation would have been a stronger corroborating result.

Velocity has shown varying degrees of importance in describing macroinvertebrate communities. Some studies have found little or no correlation between flow and macroinvertebrate communities (Quinn and Hickey 1990; Sylvestre and Bailey 2005). Others have found a negative correlation for community richness and abundance (Erman and Mahoney 1983; Brooks et al. 2005). While yet other studies have found a positive relationship between macroinvertebrate diversity and abundance with increasing velocity (Rabeni and Minshall 1977; Hawkins et al. 1982). In the current study, there were several significantly positive relationships between community measures and velocity. The total number of taxa, the number of EPT taxa and the proportion of Chironomidae were all positively correlated with average velocity, and only total richness was positively related with maximum velocity. The one fact agreed upon by all studies is that as velocity increases, the amount of silt decreases (Rabeni and Minshall 1977;

Hawkins et al. 1982; Erman and Mahoney 1983). As mentioned earlier, silting is detrimental to macroinvertebrates with delicate external gills, limiting respiratory efficiency. However, Brooks et al. (2005) also found that increased velocity led to increased "roughness" that decreased the abundance of young mayflies in particular. In their study, they postulated that the high turbulence required a greater amount of energy from the macroinvertebrates than was available in food (Brooks et al. 2005). Newfoundland streams are generally very nutrient-poor and extremely rocky (Larson and Colbo 1983). Therefore, as the same types of habitats were sampled across the island, turbulence and available food may not have been significantly different between sites. An exception to these observations occurred in the upstream site of Winter House Brook where an exponentially high number of mayflies inhabited the rocky, flashy brook (Appendix 4). Despite the physical conditions, abundant algal growth provided a high-quality food source for the grazers (Colbo, *pers. comm.*). As a result, the mayflies could probably access enough energy to withstand the high velocity and turbulence of Winter House Brook. A reduction in silting is therefore the most plausible explanation for a significantly positive correlation of community richness to velocity.

Many studies have found the width of the stream to influence macroinvertebrate community structure (Bronmark et al. 1984; Jenkins et al. 1984; Grubaugh et al. 1996; Kilgour and Barton 1999; Malmqvist and Hoffsten 2000). Some have postulated that the effects of stream size are a simple matter of larger streams having a greater area of habitat available, including a greater number of microhabitats (Bronmark et al. 1984; Jenkins et al. 1984). The presence of more microhabitats in a stream indicates an increase in heterogeneity, which increases macroinvertebrate diversity (Giller and Malmqvist 1998).

The results of this study are not congruous with this idea. Macroinvertebrate abundance decreased with increasing bankfull width and there was no correlation between either of the richness variables and the width variables.

Stream order is closely tied to stream size and has been shown to affect community composition, abundance and biomass going from low to high order streams (Brussock and Brown 1991; Grubaugh et al. 1996). This change in the invertebrate community with size was predicted by Vannote et al. (1980) in the "River Continuum Concept", or RCC. They explained changes in community composition partly in regards to changing organic inputs from leaves in low-order headwaters to periphyton and plankton in larger orders in "typical" streams. In agreement with Vannote et al. (1980), studies have found the highest taxonomic richness to occur in mid-order streams (Minshall et al. 1985; Grubaugh et al. 1996). However, in others, macroinvertebrate abundance and richness has been discovered to increase the further downstream the sample is taken (the increased distance also presumably increasing the order of the stream *c.v.* Vannote et al. 1980) (Furse et al. 1984; Jenkins et al. 1984; Brussock and Brown 1991; Clenaghan et al. 1998; Vinson and Hawkins 1998). Still others have found little to no support for the RCC, citing instead that physical channel morphology drives macroinvertebrate communities (Brussock and Brown 1991). Stream order was not directly considered in this study. However, the topographic nature of the Island of Newfoundland means that the vast majority of streams are low- to mid-order streams (Larson and Colbo 1983) which were the range sampled here. Stream width measures were considered an acceptable surrogate in this study.

Using measures of stream width as a function of stream order, the BVSTEP algorithm pulled out both wetted and bankfull width as factors influencing community composition. Wetted width was positively correlated with the proportion of chironomids inhabiting the stream. However, bankfull width was negatively correlated with the total abundance of invertebrates as well as the amount of Simulidae taxa making up the community. Erman and Mahoney (1983) found Shannon diversity and evenness to decrease with an increase in the bankfull width. Bankfull width increased with drainage area, which in turn was positively correlated with velocity. High velocity led to a decrease in diversity and evenness (Erman and Mahoney 1983). Previously, the argument was presented that high velocity (linked to substrate instability) affected the ability of simulids to colonize rocks and generally cost all organisms more energy (Brooks et al. 2005). In joint sedimentation/substrate/velocity experiments, Lenat et al. (1981) discovered that under high flow conditions, sediment falling on rocky substrate merely reduced available habitat. The community composition was basically the same, but abundance decreased (Lenat et al. 1981) and so community composition was relatively unaffected (Bradt and Wieland 1981; Maier 2001; Death 2002). The negative correlation between macroinvertebrate abundance and bankfull width is proposed to be due to increased velocity, instability of substrate and increased movement of sediments downstream during spates.

Many studies have found macroinvertebrate composition to be correlated with watershed size itself (Bronmark et al. 1984; Kilgour and Barton 1999; Malmqvist and Hoffsten 2000), which also relates to stream order. In this study, the total number of taxa at a site, the number of EPT taxa at a site and *Apatania sp.* were positively correlated

with watershed size. If watershed size is correlated with stream order, we can presume that a positive correlation of richness with watershed size indicates a positive correlation of richness with mid-order streams in the range of stream sizes studied here. Stream order should be incorporated into the list of physical variables in future studies.

There is much debate on the influence of altitude on macroinvertebrate community structure (Vinson and Hawkins 1998). Altitude was correlated with invertebrate community structure in some studies (Furse et al. 1984; Malmqvist and Hoffsten 2000; Heino 2001; Sanderson et al. 2005), but has also had no effect in others (Hawkins et al. 1997; Verdonschot 2006). The effects of altitude may have been connected to differences in pH between high and low altitudes (Clenaghan et al. 1998; Sanderson et al. 2005). In Gros Morne, the high altitude Long Range Mountain plateau sites were on acidic granite, but on the Tablelands side they were basic, therefore pH is not necessarily confounded with altitude in this study. In addition, all high sites were barren, whereas for the low-altitude sites only three on the south Avalon were barren.

In theory, the RCC is also connected to altitude, as low-order streams are generally from highlands and high-order streams in lowland valleys (Vannote et al. 1980). However, as noted previously, the recent glaciations have altered the nature of the drainage system profiles on the Island. Thus, the many complex variables along stream gradients made the effects of altitude difficult to decipher. As a result, it was not a significant factor in the BVSTEP and BEST analyses in this study. At the taxon level, *Ameletus sp.* and the amount of chironomids constituting the community were positively correlated with altitude. *Ameletus*, a siphlonurid mayfly, is typically found in montane regions across North America (Merritt and Cummins 1996) as is the case in

Newfoundland. Chironomidae are a highly diverse group with many taxa that range over a wide spectrum of environmental variables (Merritt and Cummins 1996). Therefore, family level dominance for the high-altitude granite regions of Gros Morne may be taxa quite different from lowland sites.

Riparian vegetation affects invertebrate community composition by the amount and type of vegetation in the catchment, which dictates the terrestrial organic inputs, which in turn influences invertebrate community composition (Woodall and Wallace 1972; Hawkins et al. 1982; Molles 1982; Black et al. 2004). Black et al. (2004) found that heptageniid and ephemerellid mayflies, as well as chloroperlid stoneflies, were the most consistently correlated with increasing forest cover. Heptageniidae and Ephemerellidae were both indicative of a high amount of forest cover in the local watershed, whereas Ephemerellidae and Chloroperlidae were considered indicators of 70-80% forestation in the whole watershed. Amphipods, nematodes and chironomids were correlated with a much lower amount of forest in both the local and whole watershed (Black et al. 2004). Kilgour and Barton (1999) found that with increased forest cover, there was an increase in the insect families Perlodidae, Tricorythidae and Nemouridae, while watersheds with low forest cover were typified by Erpobdellidae, Tubificida, and Planorbidae. However, in Kilgour and Barton's (1999) study, un-forested areas were coincident with agricultural areas, thus stream eutrophication rather than forest cover may have been indicated by the taxa they found.

The current study found Chironomidae occurrence and the proportion of chironomids making up the community were negatively correlated with the amount of forest at whole, local and immediate watershed levels. Conversely, the total number of

taxa per site, the total number of EPT taxa per site and the proportion of EPT taxa in the sample were all positively correlated with varying levels of watershed forestation. Kilgour and Barton (1999) and Black et al. (2004) found EPT families were strongly related to high amounts of forestation. Inputs of leaves and logs, aside from providing food, also increase the number of available microhabitats. Microhabitats increase macroinvertebrate abundance and taxonomic richness (Giller and Malmqvist 1998; Brooks et al. 2005). EPT taxa tend to prefer wood substrates and leaf packs, microhabitats associated with forest in the stream catchment (Collier et al. 1998; Woodall and Wallace 1972). Velocity also decreases in these kinds of complex environments (Carpenter and Lodge 1986). However, in the current study, one high-velocity brook (Winterhouse Brook, upstream) with no overhanging vegetation was completely dominated by an EPT taxonomic group: the grazer-type mayflies. As this site was unique, it had little influence on the overall analysis, but illustrates the need to evaluate the statistically significant findings against the underlying biology of the taxa.

The type of forest contributing to the stream catchment also has an effect on macroinvertebrate community structure and abundance (Woodall and Wallace 1972; Hawkins et al. 1982; Molles 1982; Kilgour and Barton 1999). One study found the type of forest cover directly affected taxonomic richness (Black et al. 2004), while the others did not (Woodall and Wallace 1972; Hawkins et al. 1982; Molles 1982). This may be due to the inclusion of urban and suburban watershed in the study by Black et al. (2004), as opposed to the others that tended to compare less impacted watersheds with one another, or simply watersheds with different types of trees. Woodall and Wallace (1972) found no difference in the taxa present between the sites, but found a change in the importance of

various feeding groups depending on whether the stream was canopied with deciduous or coniferous trees. The vegetation data from this study was incomplete; therefore a comparison between all sites in regards to types of land cover was not feasible.

The amount of shading caused by riparian vegetation can affect macroinvertebrates through the amount of light reaching the stream surface, and temperature increases associated with irradiation (Collier 1995; Kreutzweiser et al. 2005). This was especially the case with the negative correlation of *Hydroptila sp.* with the amount of forest in the whole and local watersheds. This caddisfly genus feeds on filamentous algae (Wiggins 1996), and a decrease in incident light, coupled with low nutrients produced by forest uptake, decreases the amount of primary production (Giller and Malmqvist 1998; Woodall and Wallace 1972). For these reasons, *Hydroptila* is less likely to inhabit heavily shaded streams (Hughes 1966).

Shading of streams has been identified as a factor causing decreases in species richness (Malmqvist and Hoffsten 2000), yet another study found it increased diversity and evenness (Clenaghan et al. 1998), and others showed decreases in abundance and biomass (*vis.* Kreutzweiser et al. 2005). Still others found no significant difference in the taxonomic richness with varying degrees of shading, though preferences by certain taxa (causing shifts in dominance and guild structure) were noted (Woodall and Wallace 1972; Hawkins et al. 1982; Molles 1982). Thus, a complex set of relations have been reported. Presently, none of the common species or community metrics were correlated with the amount of canopy over the sample site, nor was this parameter considered useful by the BVSTEP and BEST algorithms in this study. This supports the findings of Hawkins et al. (1982), Molles (1982) and Woodall and Wallace (1972). However, total taxonomic

richness was positively correlated with the average amount of overhanging vegetation, while the proportion of EPT taxa in the stream decreased with increasing overhang. Clenaghan et al. (1998) noted this was possibly because shading is related to terrestrial stream inputs and increased detritus. These factors were discussed earlier on. Considering the above, a better measurement of vegetation overhang would be the resulting proportion of the stream that was shaded, as smaller streams would be more shaded than large streams with the same amount of overhang.

Newfoundland's streams are nutrient poor (Larson and Colbo 1983; Roberts 1983), so it may be expected that macroinvertebrates would generally respond positively at sites with elevated levels of nutrients. However, most of the individual and community metrics were negatively correlated with concentrations of total nitrogen, nitrates and phosphorus. The two types of nutrients, nitrogen and phosphorus, together are considered a measure to assess eutrophication (Giller and Malmqvist 1998; Schindler 2006). Levels of nitrates and phosphorus were extremely low at most sites, though the phosphorus levels at seven sites indicated mesotrophy, and at two others, mild eutrophy (Pelechata et al. 2006; also Appendix 3). Therefore, the negative relationship between the invertebrate metrics and phosphorus concentration may indicate a community difference between sites with low phosphorus concentrations and sites with higher concentrations. The effects of phosphorus levels on macroinvertebrates as seen in this study will be more closely examined later in the chapter.

A long term study of nutrient additions to a nutrient-poor arctic stream indicated that as some taxa increased in response to fertilization, others decreased (Slavik et al. 2004). In the arctic study, nutrient enriched areas exhibited a switch in their dominant

primary producer from algae to moss, which affected not only the food source for invertebrates, but also the substrate of the stream. Up until the primary producer change, some taxa flourished, only to be replaced by others when the moss became dominant (Slavik et al. 2004). Moss was present in several of the streams in the current study, however its presence does not necessarily indicate nutrient enrichment as the vegetative succession of Newfoundland streams has not been tested with controlled nutrient additions.

Nitrates and phosphorus did appear to be correlated to the position of St. John's sites in the PCA plot. In addition, the nitrate vector also lay over some Gros Morne sites and Phosphorus had higher readings in some Terra Nova sites (Figure 4.2, Appendix 3). The negative associations of most of the other "important" taxa may be a negative relationship with urbanization or flooding. The positive correlation of *Rithrogenia undulata* with nitrates appears to be related to areas of higher nitrate concentrations in Gros Morne. *Acerpenna pygmaeus*, *Hydatophylax argus* and *Prosimulium mixtum* abundances were all positively correlated with phosphorus, but not with nitrates. These species, particularly the first two, are not considered strongly pollution-tolerant taxa (Hilsenhoff 1988; Klemm et al. 2002). The finding could be resultant of a covariant relation with the Terra Nova region as there was also a positive correlation of total number of taxa per site with phosphorus. Whether the increased phosphorus and colour in the Terra Nova region resulted in the greater taxonomic richness will require further study.

The total nitrogen vector in the PCA analysis lay in the complete opposite direction from phosphorus and was associated with sites outside of St. John's, especially

those in Gros Morne. The association of Gros Morne with total nitrogen may explain the negative correlation of total abundance and total richness with total nitrogen as Gros Morne streams had some of the lowest macroinvertebrate abundances and taxonomic richness of the three regions. However, other studies showed that total nitrogen increases led to increased taxonomic richness, particularly in nutrient-poor regions (Peterson et al. 1993; Heino et al. 2003; Doledec et al. 2006). Total nitrogen was unlikely to be linked to agriculture in Newfoundland as only one stream near St. John's had agricultural activity in its watershed. The link between total nitrogen and Gros Morne is likely related to geological factors, which are difficult to tease apart from the other variables here.

Conductivity can also be related to anthropogenic activities: often increasing in response to urbanization or agriculture (Huryn et al. 2002; Black et al. 2004). Conductivity decreases with an increase in the presence of forest in the watershed (Huryn et al. 2002; Black et al. 2004). A typical response of the macroinvertebrate community to an increase in conductivity is the loss of EPT taxa and individuals (Collier et al. 1998; Huryn et al. 2002; Black et al. 2004) and a concordant increase in Chironomidae (Woodall and Wallace 1972). In this study, the conductivity readings were generally very low (Appendix 3). However, both the number of EPT taxa and percent chironomid measures were negatively correlated with conductivity. It would be expected that Chironomidae would increase with increasing conductivity if the variable were directly related to urbanization as the streams in urban St. John's are largely made up of this taxonomic group (Gibson and Colbo 2001). Nevertheless, all samples in this study contained a large amount of chironomids except for the streams from the Tablelands in Gros Morne (Appendix 4). However, the Chironomidae are a diverse group

taxonomically and ecologically. Different taxa may have inhabited each region, thus family-level identification reduces their usefulness as indicators of environmental relationships.

The variables pH and alkalinity are at first glance, the same measure. However, there is a slight difference between the two: pH measures the amount of free hydrogen ions (cations) in the water, while alkalinity quantifies the amount of carbonate (a dominant anion). As there is limestone present in some areas of the Island, both measures were included in the tests. There is a general consensus that pH (and/or alkalinity) is important to macroinvertebrate community composition (Peterson and Van Eeckhaute 1992; Malmqvist and Maki 1994; Malmqvist and Eriksson 1995; Clenaghan et al. 1998; Paavola et al. 2003). That increasing pH tends to increase taxonomic richness is also well-agreed upon (Townsend et al. 1983; Jenkins et al. 1984; Peterson and Van Eeckhaute 1992; Clenaghan et al. 1998). However, there are also studies that have found evidence to the contrary, particularly for specific taxonomic groups (Huryn et al. 2002; Death and Joy 2004). Taxonomic richness, abundance and most of the individual taxa were negatively correlated with pH and alkalinity in this study, which is in direct opposition to the findings of the majority of studies. The streams with the highest pH were those of the Tablelands region, which are also known to be flashy (prone to spates) and have barren watersheds. As taxonomic richness was positively correlated with forest, and abundance was negatively associated with bankfull width (flashiness), it becomes apparent that the negative association with pH and alkalinity was likely produced via the confounding influences of the physical variables associated with these sites. If these basic sites were removed, then perhaps the relationship of acidity/alkalinity with

macroinvertebrates could be more accurately measured. Huryn et al. (2002) documented similar conclusions as this study, as they found pH decreased with increasing forestation and forested sites had higher taxonomic richness.

The proportion of EPT taxa in the sample increased with increasing pH and alkalinity in this study. Death and Joy (2004) found EPT taxa were more prevalent in acidic streams and were replaced by molluscs, crustaceans and chironomids at higher alkalinities. However these alkaline streams also tended to be low gradient streams with decreased velocity. In Newfoundland, the streams with the highest proportion of EPT taxa were in the Tablelands in Gros Morne: streams known for high-velocity flashiness and barren watersheds as well as ultra-basic springs. Velocity has been cited as having an important positive correlation with EPT taxa in this study, therefore the findings of Death and Joy (2004) linking high pH with low EPT richness may not be applicable to Newfoundland. *Rithrogenia undulata* was one of the taxa in high abundance in these basic streams. As a result of this unique alkaline nature of the Tablelands, *R. undulata* and % EPT were positively correlated with pH and alkalinity.

Colour is also related to the amount forest in stream catchments. Coniferous trees leak tannins into streams and humus leaks from the soil with rainfall, increasing acidity and colour (Colbo *pers. comm.*; Hoff 1957; Roberts 1983). Terra Nova sites were consistently associated with high amounts of colour according to the PCA plots. All sites from Terra Nova had watersheds that were almost entirely bog or forested, therefore the leeching of tannins into the water seems a likely reason for this relationship (Appendix 3). The link of this region with colour explains the positive correlations between total and EPT richness, as Terra Nova was the most taxonomically rich region, as well as the

positive relationship of *Acerpenna pygmaeus*. *A. pygmaeus* was a common species in Terra Nova, but quite rare in the other regions. Therefore the link between this baetid mayfly and colour may be related to its distinct relationship with Terra Nova as a region. *Rhyacophila minora* was found primarily in wooded streams across the province and so was more likely to be related directly to colour from humus and tannins rather than geographical effects. A more detailed study of riparian vegetation and its relationship to colour would benefit future studies by pinpointing how much colour belongs to tannins and how much belongs to humus and peat bogs. This in turn would lead to further divergence of regional effects from more local water chemistry effects.

4.5 Conclusions and Recommendations

1) Physical variables were unable to consistently distinguish the three geographic regions, though water chemistry consistently segregated Avalon Peninsula and Terra Nova sites. The environmental ordination plots suggested that the sites sampled might be better distinguished on the basis of ecoregion than by geographic region.
2) Variation in macroinvertebrate community composition was correlated with several of the environmental variables. Community composition was most highly influenced by physical habitat and to a lesser degree by water chemistry.
3) The UTM Easting co-ordinates, % macrophytes, % igneous rock, % forest in the local watershed, nitrates, total nitrogen and alkalinity variables were the most consistently correlated with macroinvertebrates at the community and individual taxa level. However, UTM Northing co-ordinates, stream width, vegetation overhang, and pH showed detectible but weaker correlations with macroinvertebrate communities.

4) Physical and chemical variables were highly inter-linked at several locations, but as this differed among sample locations, they were not significantly correlated.

5) Newfoundland's geography, ecoregions, and land use are generally split along an east-west gradient, causing difficulty in identifying structural vs. regional effects. More sites on the Avalon Peninsula with reduced urban influences, may improve the clarity of distinction (or lack thereof) between community structure caused by physical surroundings and/or regional segregations.

6) Macroinvertebrate richness was greatest at sites characterized by larger substrate sizes and higher velocities, which implied that they were moderately stable stream beds with little suspended silt. A direct measure of turbidity in future studies is recommended.

7) It is suggested that stream order be measured in future studies to reduce variation in the watershed size variable and test the usefulness of the River Continuum Concept (RCC) in Newfoundland.

8) More refined measurement of land use, e.g. urbanization, and more specific classification of vegetation cover is recommended to improve detection of the interactions between degree and type of vegetation cover, which may be a result of natural or anthropogenic disturbance.

9) A more precise measure of stream shading is required to judge the impact of this parameter on stream fauna.

10) The links between macroinvertebrate communities and water chemistry were clouded by confounding geographical and regional effects. However, community metrics and individual taxa showed that there were few positive relationships between them and

nutrient concentrations, which was unexpected. Total EPT had a negative relationship with conductivity and pH as hypothesized.

11) The Island's impoverished freshwater macroinvertebrate fauna did show patterns that related to the environmental variables sampled. However, the known correlations of invertebrate metrics are contradictory and emphasize the need for local understanding.

5 Biomonitoring Applications

5.1 *Introduction*

Biomonitoring uses biological variables to indicate environmental health, where health may include stability, resilience, low pollution levels and high biological richness (Haskell et al. 1992; Bonada et al. 2006). These biological variables are often referred to as ecological indicators and may take form on varying levels of detail. Niemi and McDonald (2004) define ecological indicators as: "measurable characteristics of the structure (e.g. genetic, population, habitat, and landscape pattern), composition (e.g. genes, species, populations, communities, and landscape types), or function (e.g. genetic, demographic/life history, ecosystem, and landscape disturbance processes) of ecological systems."

There are many goals biomonitoring programs are designed to achieve (Niemeijer 2002; Niemi and McDonald 2004). Ecological indicators are often applied to create an early warning system for ecosystems or human health (Karr 1999; Niemi and McDonald 2004). Defining the cause of a shift in the environment is also a common goal, where Before-After-Control-Impact (BACI) designs provide concise answers to precisely-set problems (Vieira et al. 2004). Predicting changes in the future of a region's environment is another important role that ecological indicators can fulfill. Long-term studies are particularly useful for answering this question, primarily because it provides researchers with sufficient power to identify changes and/or trends in the indicator(s) over time (Niemi and McDonald 2004; Jackson and Füreder 2006).

Biomonitoring programs are in place all over the world (RIVPACS in the United Kingdom, AUSRIVAS in Australia, PERLA in the Czech Republic, STAR-AQEM in Europe), a large proportion of those being in North America (CABIN and BEAST in Canada, NRI and EMAP in the United States and numerous state programs). Despite the methodological contributions from each program, each tends to only work in the region(s) in which it was developed (Washington 1984; Urquhart et al. 1998; Niemi and McDonald 2004; Maloney and Feminella 2006). Therefore, the application of existing programs must be evaluated in each new geographical and ecological region.

Indicators of anthropogenic impacts for the Island of Newfoundland, a species-poor region, have yet to be defined. In Newfoundland, the Canadian Biomonitoring Network (CABIN) biomonitoring program has shown promise (Colbo et al. submitted). In the current study use of the CABIN method was expanded: the effects of seasonality, regions, environmental variables (including anthropogenic effects) and temporal variation among years were examined.

Urban streams tend to have increased amounts of nutrients and heavy metals, as well as disturbance due to higher amounts of impervious surfaces in urban environments causing high amounts of runoff (Morse 2001; Paul and Meyer 2001). In addition, increased conductivity, ammonium, suspended solids and pH have also been recorded in urban areas (Garie and McIntosh 1986; Paul and Meyer 2001). While additional nutrients can bring about higher densities of invertebrates, when coupled with other urban effects, reduced taxonomic richness and the reduction or loss of taxonomic groups tends to occur (Lenat and Crawford 1994; Colbo et al. 1999; Miserendino 2008). The mayflies in

particular are sensitive to urban influences, while the Chironomidae flourish (Pratt et al. 1981; Garie and McIntosh 1986; Lenat and Crawford 1994; Colbo et al. 1999; Brisbois et al. 2008; Miserendino 2008). Less urban, or "rural", invertebrate communities tend to differ very little from urban communities (Pedersen and Perkins 1986), unless these rural areas are agriculturally based (Lenat and Crawford 1994). As there was very little agricultural activity in the regions sampled, detecting impacts of agriculture is unlikely.

Long-term studies have indicated that there are variations in macroinvertebrate communities between years which are often unexplainable (Hynes 1970; Colbo 1985; Slavik et al. 2004). However, Townsend et al. (1987) states that "Overall, persistence was greatest at low discharge, upstream sites with cool summer temperature regimes and low, stable pH." (Townsend et al. 1987 p.597). These conditions adequately describe the state of most Newfoundland streams (Larson and Colbo 1983).

In this chapter, objectives 3 to 5 of Chapter 1 are evaluated. The goal of objective 3 was to determine the sensitivity of Newfoundland macroinvertebrate communities to human impacts. Objective 4 was to test the usefulness of the CABIN protocol for biomonitoring. The aim of objective 5 was to provide recommendations for the application of a biomonitoring system in Newfoundland. In addition to these goals, a series of hypotheses based on the arguments in the above introduction will be tested. They are as follows: 1) the abundance and richness of ephemeropteran and dipteran orders are good indicators of levels of urbanization in Newfoundland, with severely impaired areas generally having fewer mayflies and more chironomids, 2) rural areas (suburban) are not biologically different from urban communities, 3) chemically, urban

sites are expected to have a higher conductivity and a higher pH than pristine sites, and 4) Newfoundland invertebrate community structure and the relationships between sites are expected to persist between years.

5.2 *Methods*

The goals of this chapter have been split into two sections: the first identifies the sensitivity of Newfoundland invertebrates to land use. Urbanization on the Island of Newfoundland is concentrated on the Avalon Peninsula. Thus it was the region of focus for studying the effects of human impacts on macroinvertebrate communities. Three levels of land use were identified: the "urban" rating denoted a site that was urbanized for 3-5 km upstream, "rural" did not have the level of urbanization as the previous rating, nor the extent, and may include agricultural areas. "Pristine" was defined as an un-urbanized site with little to no anthropogenic impact in the surrounding area. There were three urban sites (Rennies River, Virginia River and Waterford River), two rural sites (Broad Cove Brook and South Brook) and five pristine sites (Beaver Brook, Bristol Brook, Peyton Brook, Portugal Cove Brook and Wattern Brook).

Land use sensitivity was first assessed with the seasonally amalgamated data from Chapter 3, transformed into proportions. The proportion of each taxon was calculated from the combined seasonal abundance of that taxon at each site, and then a group average was performed for each land use type. Major taxonomic groups were tallied and graphed in MS Excel as described in Chapter 3. The amalgamated data was also transformed into presence/absence for easy assessment of taxonomic richness. Taxa with

lower taxonomic entities present were removed from analysis for each site where that occurred (e.g. if *Heptagenia pulla* was present, then a corresponding mark for presence at the Heptageniidae level was removed). Analysis of variance (ANOVA) was also carried out using MS Excel. The Waterford River is a distinct community, having an unusually high abundance of salmonids, unlike other less urbanized sites in the area (Gibson and Colbo 2001). Due to its unique nature, this site was removed from some tests.

A second dataset consisting of fall macroinvertebrate abundance data was also used to assess land use sensitivity. The latter dataset was $\log_{10}(x+1)$ transformed in PRIMER6. Principal Component Analysis (PCA) was utilized to ordinate sites, as described in detail in the methods section of Chapter 4 and also by Clarke and Gorley (2006). A cluster analysis was performed on the macroinvertebrate abundance data with the intent of overlaying the clusters on the PCA plot. The cluster analysis used a Bray-Curtis similarity matrix, performed a group average test on the sites, and measured the level of similarity between the sites. In addition, Analysis of Similarity (ANOSIM – see Chapter 3; Clarke and Gorley 2006) was performed with the land use factor, resulting in a Rho value: a correlation coefficient valued between 0 and 1 (Clarke and Gorley 2006). A third dataset of the fall physical and chemical data was used to interpret macroinvertebrate abundance. ANOSIM was used on the transformed variables (list of transformations in Chapter 4), and the dataset was split to further analyse the connections of water chemistry to land use. PCA and ANOSIM were performed on the chemical dataset.

The second goal of this chapter examines the sensitivity of Newfoundland invertebrates to temporal effects. The datasets examined in this area of the chapter were

presence-absence fall macroinvertebrate data from the Avalon Peninsula and Terra Nova in the current study, and presence-absence data previously collected in those regions in the fall of 2002 and 2003 (Colbo et al. submitted). Presence/absence was used to more easily amalgamate data from different sources. PCA and ANOSIM were utilized to measure differences between the two regions and between years within a region. PCA was also used to track changes in repeat sites over time.

5.3 *Results*

5.3.1 Sensitivity of Newfoundland invertebrates to land use

An analysis of the community composition of the three land use types revealed a few key differences in several of the taxonomic orders (Figure 5.1). Urban communities were largely populated by Ephemeroptera and had the lowest abundance of beetles of the three land use types. Rural sites had the highest proportions of beetles, stoneflies and caddisflies, and the lowest of non-insect macroinvertebrates. Pristine sites were dominated by Diptera, this taxon made up nearly half the community. Non-insect fauna were most abundant in pristine streams, while mayflies were the least abundant there.

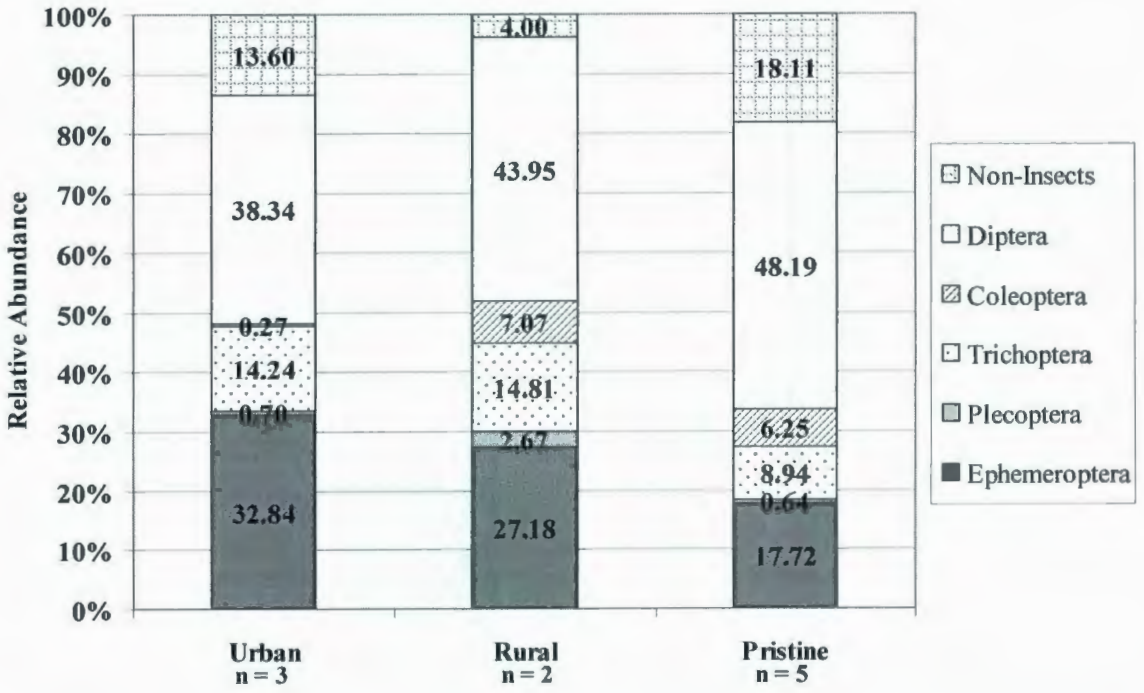


Figure 5.1 The proportion of individuals from the major taxonomic groups contributing to the macroinvertebrate community in samples from urban, rural and pristine sites on the Avalon Peninsula.

Ephemeroptera were expected to be indicators of urbanization, therefore an identical figure was created using the proportion each mayfly family contributed to the total mayfly population (Figure 5.2). Urban sites lack heptageniid mayflies and have the lowest abundances of Ephemerellidae of the three land use types. Baetids make up over 85% of the mayfly population in urban streams, but just over 50% in the other two land use types. Leptophlebiid mayflies are found at relatively similar levels in all areas, though are slightly more abundant at pristine sites. Heptageniids are equally prevalent at rural and pristine sites, while ephemerellids are slightly more common in rural areas.

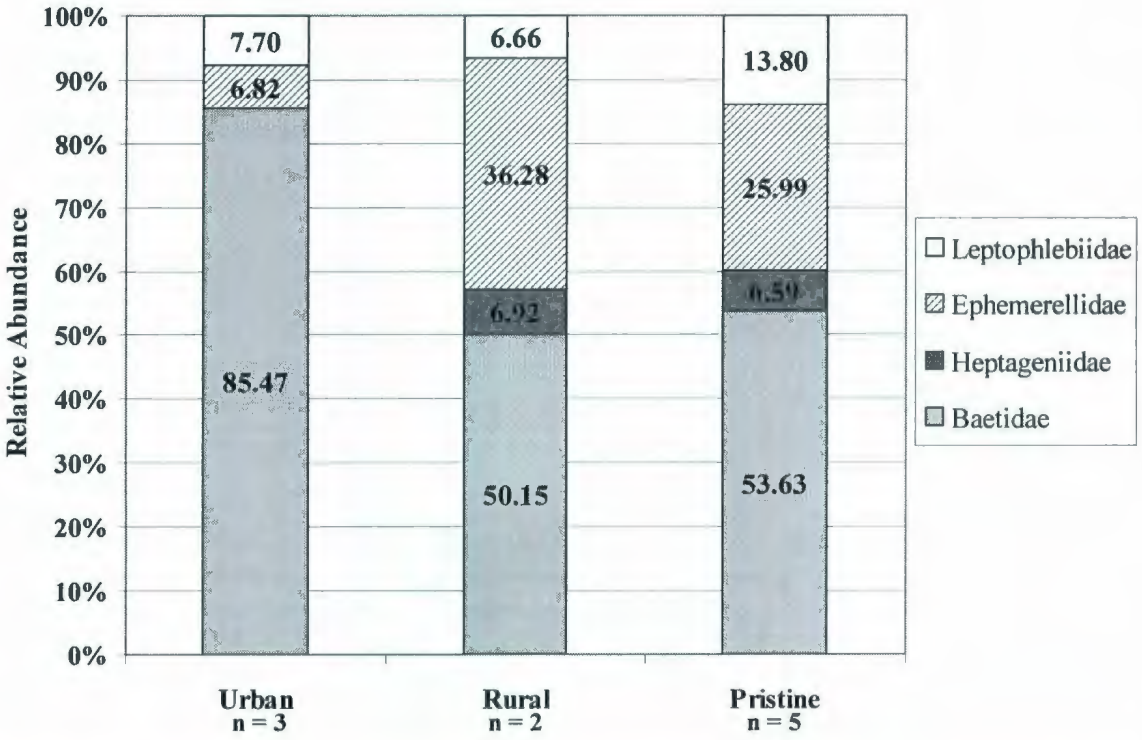


Figure 5.2 The proportion of individuals from the families of the order Ephemeroptera in urban, rural and pristine sites on the Avalon Peninsula.

The number of taxa making up the macroinvertebrate community was variable between sites, irrespective of land use type (Table 5.1). Of the urban sites, Rennies River and Virginia River both had much lower richness than Waterford River, where the Waterford had over ten more taxa than the other two. Among the pristine sites, Peyton Brook had the lowest overall richness (eight less taxa than the highest) while Beaver Brook had five less mayfly taxa than the richest pristine site. Analysis of variance (ANOVA) tests indicated that there was no difference in total and mayfly richness between land use types (Table 5.2). However, when the Waterford River site was removed from the analysis, both richness tests were significant where the total richness ANOVA had a p-value of 0.012 and the mayfly richness ANOVA had a p-value of 0.018.

Table 5.1 Total taxonomic richness and richness of Ephemeroptera in sampled Avalon streams.

Land use	Brook Name	Total	Ephemeroptera
Urban	Rennies River	18	4
	Virginia River	19	3
	Waterford River	33	8
Rural	Broad Cove Brook	33	10
	South Brook	28	7
Pristine	Beaver Brook	27	7
	Peyton Brook	22	9
	Bristol Brook	30	9
	Portugal Cove Brook	27	12
	Watern Brook	27	10

Table 5.2 ANOVA tables of land use comparisons with and without the Waterford River for A) total richness and B) Ephemeroptera richness. P-values less than $\alpha = 0.05$ are in bold type.

A) Total richness with Waterford River included

Source of Variation	*Sum of Squares*	*Degrees Freedom*	*Mean Squares*	*F*	*P-value*	*F critical*
Between Groups	62.0	2	31.0	1.165	0.366	4.737
Within Groups	186.4	7	26.6			
Total	248.4	9				

Total richness without Waterford River

Source of Variation	*Sum of Squares*	*Degrees Freedom*	*Mean Squares*	*F*	*P-value*	*F critical*
Between Groups	153.8	2	76.9	9.987	**0.012**	5.143
Within Groups	46.2	6	7.7			
Total	200	8				

B) Ephemeroptera richness with Waterford River included

Source of Variation	*Sum of Squares*	*Degrees Freedom*	*Mean Squares*	*F*	*P-value*	*F critical*
Between Groups	37.2	2	18.6	4.107	0.066	4.737
Within Groups	31.7	7	4.5			
Total	68.9	9				

Ephemeroptera richness without Waterford River

Source of Variation	*Sum of Squares*	*Degrees Freedom*	*Mean Squares*	*F*	*P-value*	*F critical*
Between Groups	50.7	2	25.3	8.355	**0.018**	5.143
Within Groups	18.2	6	3.0			
Total	68.9	8				

The Principal Component Analysis (PCA) explained a cumulative total of 64.2% of the total variation in the macroinvertebrate dataset in the first three axes. 30.2% of the total variation was explained by the first Principal Component (PC) axis, 18.5% by the second, and 15.5% by the third. This first axis segregated the sites according to land use type, with urban sites sorting to the positive end of PC1 and pristine sites sorting towards the negative end (Figure 5.3). All strong eigenvectors were related to the negative part of the first PC axis (Table 5.3). Two hydroptilid caddisflies, *Hydroptila* and *Oxyethira*, as well as a baetid mayfly were the taxa most directly related to that axis. All urban sites sorted to the negative side of the second PC axis, which was correlated with the presence of Sphaeridae. Immature ephemerllid mayflies, Lepidostoma sp. and Oulimnius latiusculus had large positive eigenvalues for the second PC axis.

Macroinvertebrate communities at all Avalon sites were at least 20% similar to one another, as seen by the clusters overlaid on the PCA plot (Figure 5.3). All urban sites had a Bray-Curtis similarity equivalent to 40%, while rural sites were included with the pristine sites at this level of similarity. At the 60% similarity level, the rural sites were in the same cluster as two pristine sites.

The segregation of sites on a gradient of land use was significant in Analysis of Similarity (ANOSIM) tests where the Global Rho value was 0.59 ($p = 0.011$) (Table 5.4). The R-statistics for urban vs. pristine and urban vs. rural sites approached 1 ($R = 0.877$, $p = 0.018$; $R = 0.917$, $p = 0.1$ respectively). Pristine sites did not differ from rural sites, where the R value for the pair was 0.073 and the p-value was 0.619.

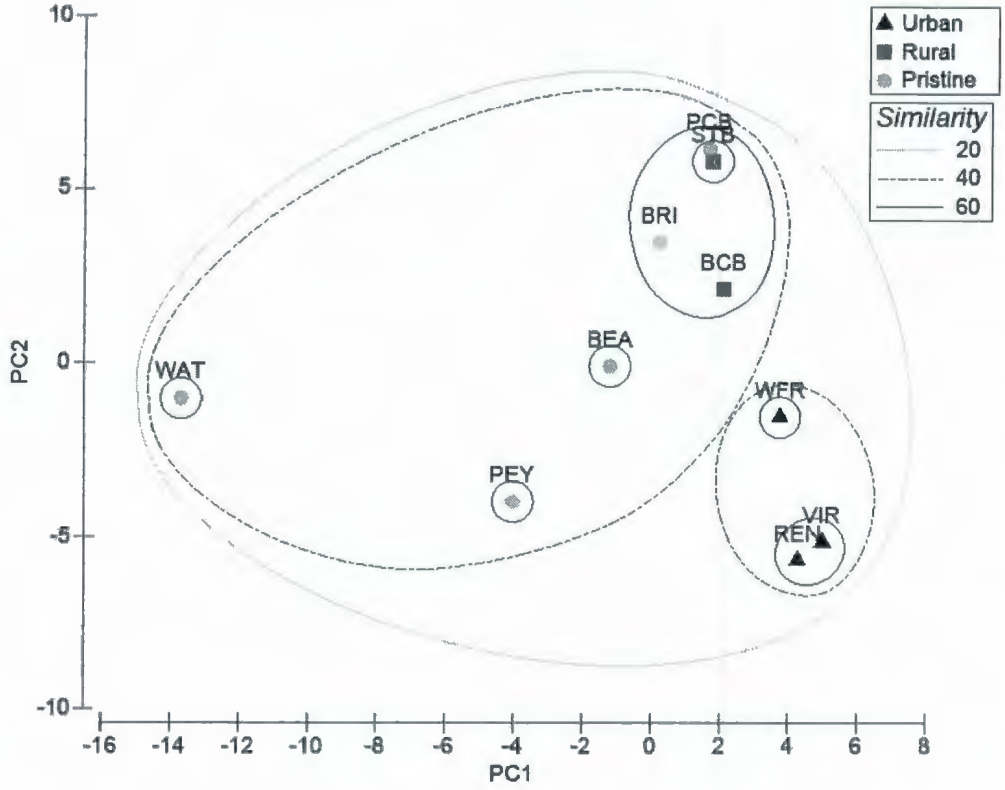


Figure 5.3 2-dimensional PCA biplot of the first vs. second Principal Component axes for the Avalon fall macroinvertebrate abundance data. Points represent sample sites, labelled by land use type. Similarity clusters are overlaid.

Table 5.3 Eigenvector strengths corresponding with the Principal Component axes of Figure 5.1. Only eigenvectors equalling or exceeding 0.250 are listed.

	PC1	PC2
Sphaeridae	-0.285	-0.305
Hyalella azteca	-0.259	
Acerpenna pygmaeus	-0.288	
Ephemerellidae		0.362
Hydroptila sp.	-0.324	
Oxyethira sp.	-0.292	
Lepidostoma sp.		0.310
Simulium venustum/verecundum	-0.284	
Oulimnius latiusculus		0.364

Table 5.4 ANOSIM tests for strengths of land use differences between sites sampled on the Avalon Peninsula, using fall macroinvertebrate abundance data.

	R	p
Global R	0.590	0.011
Urban vs. Pristine	0.877	0.018
Urban vs. Rural	0.917	0.100
Pristine vs. Rural	0.073	0.619

The CABIN protocols specify physical and chemical data to be recorded as well. Physical variables did not sort sites along a gradient of land use, where the Global Rho for the ANOSIM test was 0 with a p-value of 0.485. However, the water chemistry did sort sites along a gradient similar to that seen in the macroinvertebrate data (Figure 5.4). 95.3% of the variation in the data was explained in the first three PC axes, 76.9% in the first, 12.3% in the second, and 6.0% in the third. Urban sites sorted to the negative end of PC axis 1, rural sites centred on the zero of both axes, and pristine sites were restricted to the positive side of PC axis 1. Most eigenvalues were large, resulting in large variable vectors. According to the variable vectors in both plot views, conductivity, pH and alkalinity were best correlated with the urban sites. Nitrates were also strong on PC axis 1, though the strength of the variable on other axes pulled the vector from the urban site cloud in Figure 5.4 b).

The Global Rho value was very high for the land-use segregations of the water chemistry dataset, at 0.88 ($p = 0.001$). Both pairs containing urban sites had a Rho of 1.00, indicating perfect difference between land use pairs (Table 5.5). Interestingly, pristine and rural site water chemistry was statistically different ($R = 0.6$, $p = 0.048$), unlike the results for the macroinvertebrate communities (Table 5.4).

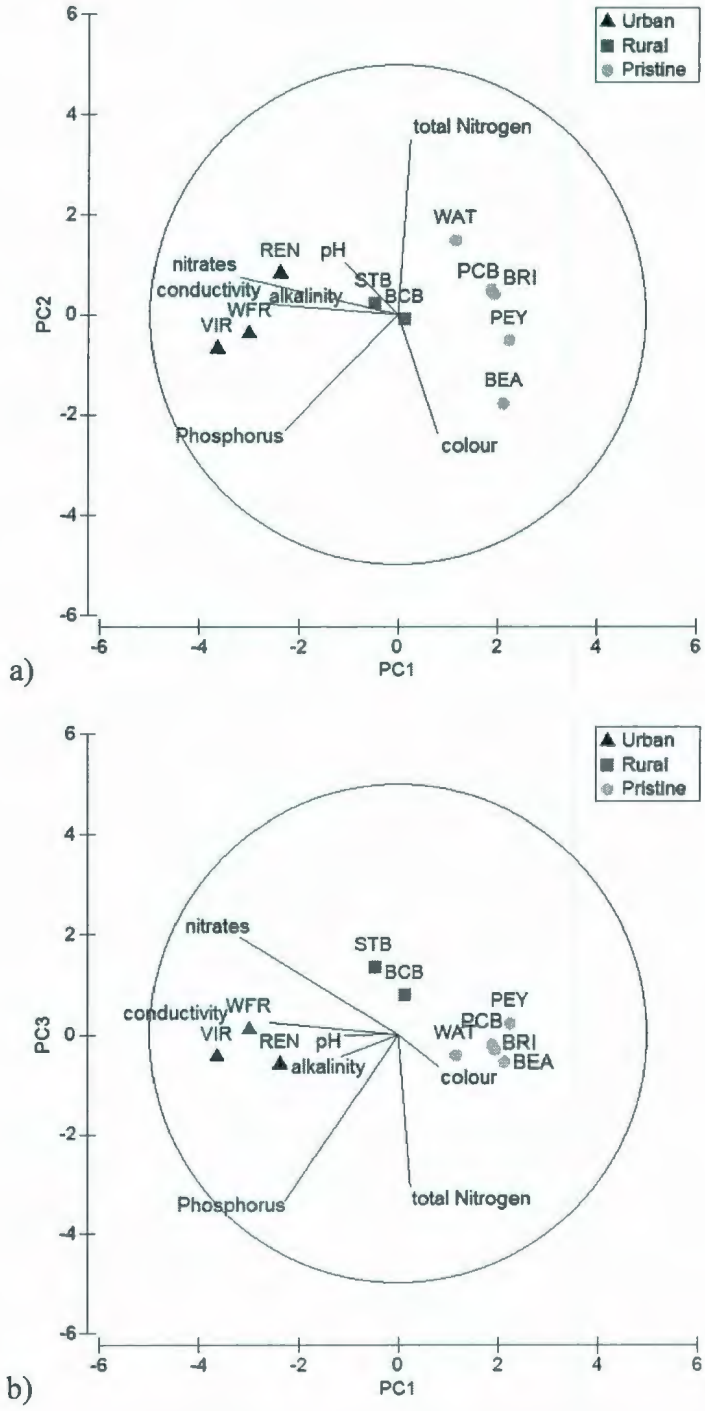


Figure 5.4 2-dimensional PCA biplots of the a) first vs. second and b) first vs. third Principal Component axes for the Avalon water chemistry data. Points represent sample sites, labelled by land use type. Length of lines represent the correlation of variables with the principal components.

Table 5.5 ANOSIM tests for strengths of land use differences between sites sampled on the Avalon Peninsula, using fall water chemistry data.

	R	p
Global R	0.88	0.001
Urban vs. Pristine	1.00	0.018
Urban vs. Rural	1.00	0.100
Pristine vs. Rural	0.60	0.048

5.3.2 Sensitivity of Newfoundland invertebrates to temporal effects

The fall macroinvertebrate presence-absence data from 2002, 2003 and 2004, when combined in a PCA plot, indicated that there were clear differences between the Avalon Peninsula and Terra Nova (Figure 5.5). However, only 19.2% of the variation in the dataset was explained by the first two PC axes. Avalon sites tended to lie on the negative side of the second PC axis, though some sites were scattered in among the sites in the Terra Nova "cloud". *Hydropsyche slossonae* was associated with the negative side of PC axis 2 (Table 5.6). Immature leptophlebiids were related to the positive side of the second PC axis where the majority of the Terra Nova sites lay. ANOSIM proved the separation of the two regions with an R-statistic of 0.308, p-value = 0.001.

Using the same macroinvertebrate plot, but labelling it by year, revealed another pattern in the data (Figure 5.6). Sites from the 2004 sampling season tended to sort together on the negative side of PC axis 1, while 2002 and 2003 clustered on the positive side. All strong eigenvectors for PC axis 1 were negative associations, indicating a lack of these taxa in 2002 and 2003 samples (Table 5.6). The clearest regional separation was seen in the 2002 sites across PC axis 2, as indicated in Figure 5.5.

ANOSIM was also used to search for significant differences between each region within and between years (Table 5.7). The only regional/temporal pair that was not significantly different was the 2004 Avalon and 2004 Terra Nova pair, which had an R-statistic of 0.122 and a p-value of 0.064. Every other pair was statistically significant to $p = 0.001$ with the exception of 2002 and 2003 Terra Nova, which had a p-value of 0.004. The Rho value for the 2002 and 2003 Terra Nova pair was low, recorded as 0.249.

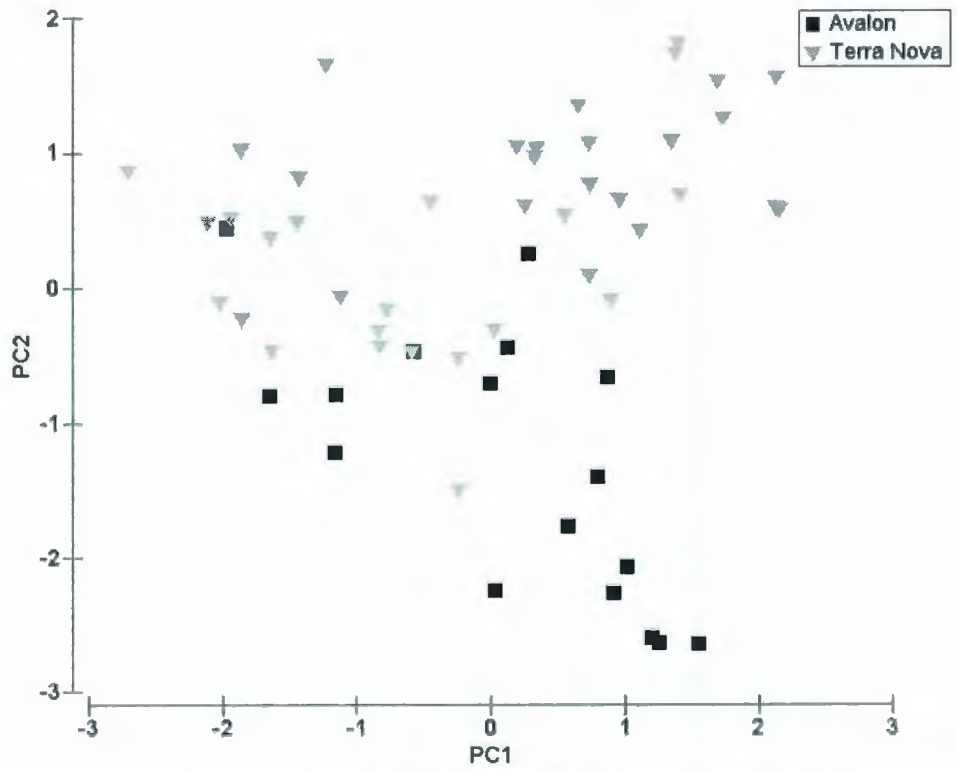


Figure 5.5 2-dimensional PCA biplot of the first vs. second Principal Component axes for the 2002, 2003 and 2004 fall macroinvertebrate presence/absence data. Points represent sample sites, labelled by region.

Table 5.6 Eigenvector strengths corresponding with the Principal Component axes of Figure 5.5 and Figure 5.6. Only eigenvectors equalling or exceeding 0.225 are listed.

Axis	Taxon	Eigenvector
PC 1	*Paraleptophlebia adoptiva*	-0.268
	Paracapnia opis	-0.333
	Isoperla transmarina	-0.264
PC 2	Leptophlebiidae (imm.)	0.248
	Hydropsyche slossonae	-0.275

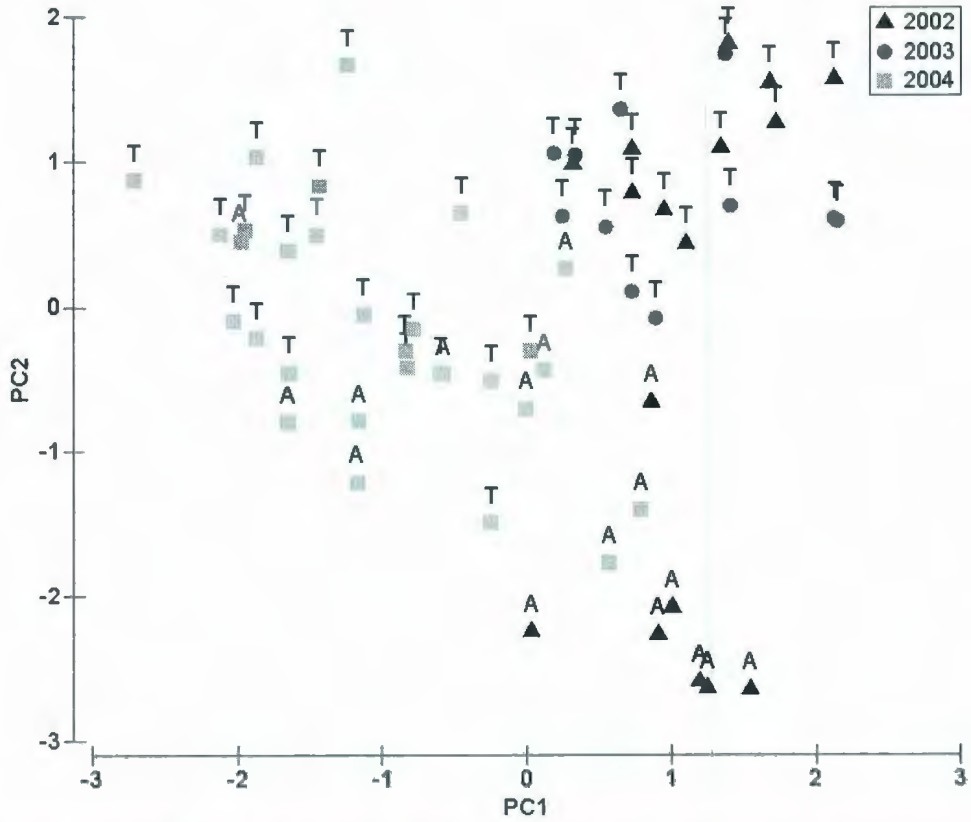


Figure 5.6 2-dimensional PCA biplot of the first vs. second Principal Component axes for the 2002, 2003 and 2004 fall macroinvertebrate presence/absence data. Points represent sample sites, which are labelled with an A for Avalon Peninsula sites and a T for Terra Nova sites, and symbolically labelled by year.

Table 5.7 R-statistic and p-values for the ANOSIM analysis of the combined temporal and regional effects on the combined 2002-2004 fall macroinvertebrate presence/absence data from the Avalon Peninsula and Terra Nova.

	R-statistic	p-value
2004 Avalon vs. 2004 Terra Nova	0.122	0.064
2004 Avalon vs. 2002 Avalon	0.726	0.001
2004 Terra Nova vs. 2002 Terra Nova	0.644	0.001
2004 Terra Nova vs. 2003 Terra Nova	0.540	0.001
2002 Terra Nova vs. 2003 Terra Nova	0.249	0.004
2002 Terra Nova vs. 2002 Avalon	0.948	0.001

The PCA of the repeat sites explained more variation than the 2002-2004 dataset together, where 42.4% of the variation was explained in the first three axes. 17.1% of the variation was explained by PC axis 1, 14.1% by PC axis 2, and 11.2% by PC axis 3. In Figure 5.7a), there were obvious changes in the communities between years, though those shifts did not cause sites to change in the same way. Subtly, Terra Nova sites tended to shift towards the positive side of PC axis 2, while Avalon sites shifted toward the negative. PC axis 2 was associated with Lumbriculidae on its negative axis, as well as with *Acerpenna pygmaeus* and *Oulimnius latiusculus* on the positive (Table 5.8). However, when viewed as the third PC axis against the first, a definite directional shift in macroinvertebrate communities between 2002 and 2004 took place at each repeat site. All 2004 sites were further to the negative end of PC axis 3 than in previous years, which was associated with increases in the taxa *Paracapnia opis*, *Isoperla transmarina* and *Apatania sp.* (Table 5.8). Despite differences in directional shifts of sites belonging to different regions, none of the lines between repeat sites crossed each other.

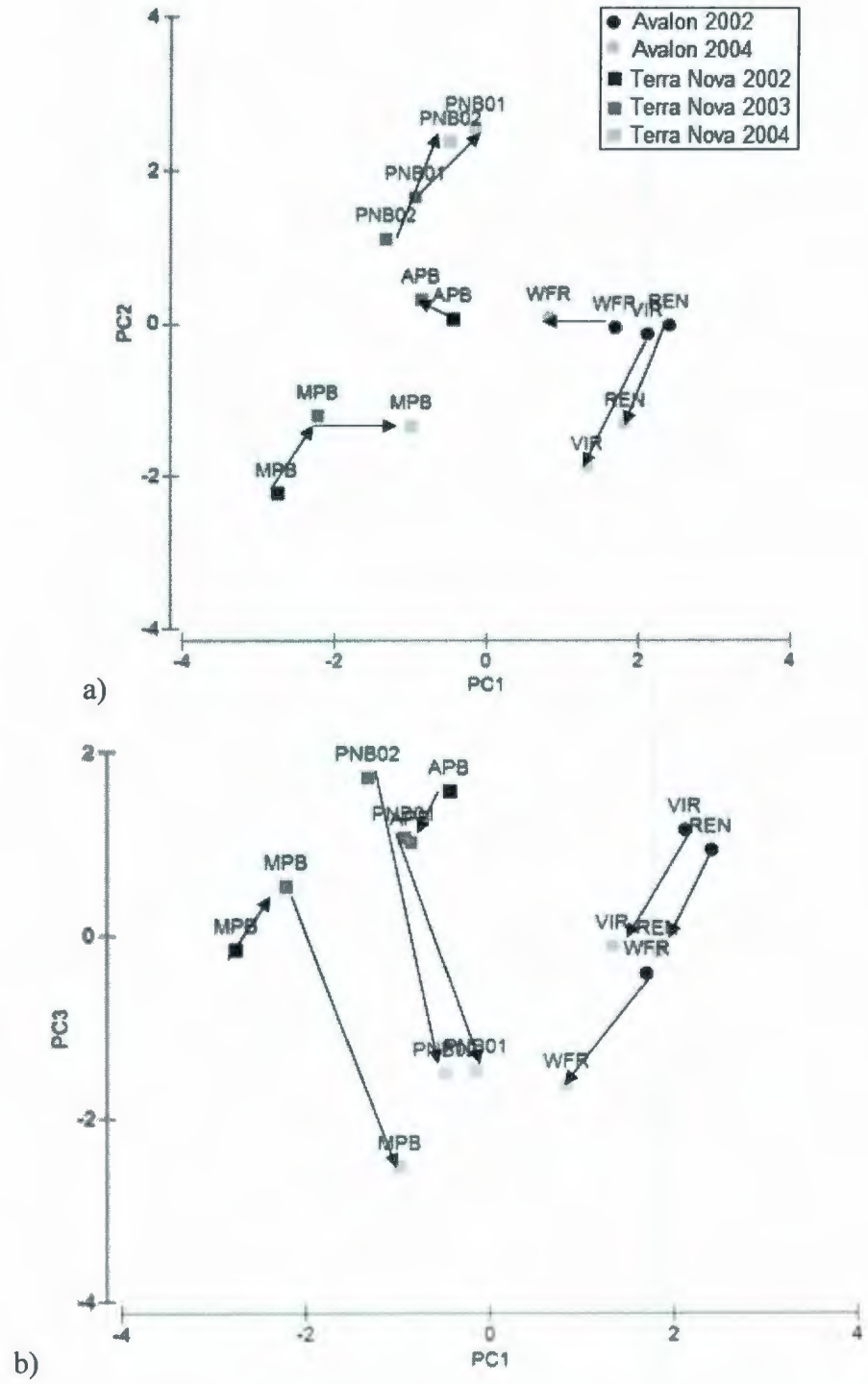


Figure 5.7 2-dimensional PCA biplots of the a) first vs. second and b) first vs. third Principal Component axes for the fall macroinvertebrate repeat sampling sites. Points represent sample sites, labelled by site code. Symbols denote both region and year, arrows track yearly site movement.

Table 5.8 Eigenvector strengths corresponding with the Principal Component axes of Figure 5.7. Only eigenvectors equalling or exceeding 0.225 are listed.

Axis	Taxon	Eigenvector
PC 1	*Helobdella stagnalis*	0.244
	Stenonema vicarium	-0.245
	Dolophilodes distinctus	-0.241
	Hydropsyche slossonae	0.228
PC 2	Lumbriculidae	-0.252
	Acerpenna pygmaeus	0.232
	Oulimnius latiusculus	0.229
PC 3	*Paracapnia opis*	-0.317
	Isoperla transmarina	-0.256
	Apatania sp.	-0.231

5.4 *Discussion*

5.4.1 Sensitivity of Newfoundland invertebrates to land use

Differences in macroinvertebrate communities among watersheds of varying levels of human impact were evident. Colbo et al. (1999) and Lomond and Colbo (2000) found that highly urbanized sites had decreased richness and abundance of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa. Many papers have found that the density, diversity and abundance of macroinvertebrates decreases in urban settings, especially for EPT taxa. Conversely, the density and abundance of Diptera, particularly Chironomidae, and oligochaetes increase in urban communities (Pratt et al. 1981; Garie and McIntosh 1986; Lenat and Crawford 1994; Paul and Meyer 2001). However, in the current study, the urban sites had the highest proportion of mayflies in their communities, stoneflies were in short supply over the entire Avalon Peninsula, and caddisflies generally made up the same proportion in all communities. Pristine sites had the lowest

proportion of EPT taxa, comprising approximately 30% of the population, whereas urban and rural communities were approximately 50% EPT taxa. Pristine sites also had the largest proportions of Diptera and non-insects (including Oligochaeta) while urban communities had 10% less of the former order than the pristine sites. However, Colbo et al. (1999) reported that heptageniid mayflies were absent from the most urban sites, a result confirmed by this study. Therefore, though mayflies made up a larger proportion of the community, the taxa doing so changed in the urban environment compared to the other environments. Despite the above findings that were contrary to the literature, the taxonomic richness of Avalon streams did decrease with increasing urbanization for the most part. The Waterford River is an anomaly, as it had the highest taxonomic richness of all the sites sampled (except for Broad Cove Brook, which had the same richness) while Rennies and Virginia rivers had the lowest. Ephemeroptera richness was lower in the Waterford stream, presumably reflecting the loss of the heptageniid group, but was still richer than the other urban streams. The precise factors making Waterford River unique are unknown, but possibilities will be discussed later on.

The macroinvertebrates visibly followed a land use gradient when the site information was plotted using Principal Component Analysis (PCA). Water chemistry produced an identical plot, suggesting that chemical variables related to urban sites are also those acting on macroinvertebrates at those sites. High conductivity and pH were correlated with urbanization, as noted in other studies (Garie and McIntosh 1986; Paul and Meyer 2001). A consistent pattern emerged when the results of the eigenvector analyses and proportional analyses between the invertebrates and water chemistry were

combined. Heptageniidae and Hydroptilidae can be considered intolerant of urban conditions due to their absence or severe depletion in urban streams. Coleoptera (and elmid beetles, by inclusion), *Acerpenna pygmaeus* and *Lepidostoma sp.* were less abundant in urban streams, indicating a sensitivity to urban conditions. Baetid mayflies, *Baetis tricaudatus* to be specific (Appendix 4) and Sphaeridae may not prefer urban conditions, but seem to be more tolerant of them as they are more abundant in those streams compared to most others.

St. John's streams are cold and relatively fast-moving, eliminating dissolved oxygen as a limiting factor in this environment (Larson and Colbo 1983). However, moving water displaces sediments from river banks and ponds, adding suspended solids to the water. High amounts of suspended sediments are particularly common in urban areas due to the large amount of human disturbance and spates caused by an increase in impervious surfaces and storm drains (Morse 1996; Paul and Meyer 2001; Lomond and Colbo 2000; Miserendino et al 2008). The east-coast provinces experience a large amount of rain events, causing frequent spates (Colbo 1985). Coupled with increased sedimentation in urban streams, stream invertebrates would be constantly bombarded with inputs of suspended solids.

Colbo et al. (1999) cited high physical disturbance and storm drains with their input of silt and pollutants as key inhibitors of EPT colonization. In the present study, ephemeropterans such as Heptageniidae and Ephemerellidae were either absent or rare in urban sites, indicating difficulties with colonization despite the high abundances of baetids. In contrast, high amounts of suspended sediments released from a dam instantly

reduced the chironomid population by 90% in Wyoming rivers, while Ephemeroptera and oligochaetes increased in density (Gray and Ward 1982). Therefore, if the flushing of sediments occurred more frequently, then the Chironomidae would potentially remain at a reduced density, as they did in the current study. The genus *Baetis* is also tolerant of high-flow episodes and silting (Gray and Ward 1982; Wallace and Anderson 1996), which explains the prevalence of this taxon in urban streams. Coleoptera too, particularly the elmids, are known for low tolerance to storm water runoff (Lenat et al. 1981; Pratt et al. 1981; Garie and McIntosh 1986; Lenat and Crawford 1994). Therefore, as "urban conditions" tend to produce results akin to those seen in this study, it is suggested that the sensitive taxa in Newfoundland respond to physical disturbance. Conductivity and pH may play a part, either as indicators of some conditions or as factors directly affecting taxa. However, the extent to which these environmental factors individually affect the macroinvertebrate community is un-testable with the current data. The current results imply that proportions of mayfly families and coleopteran abundance could be used to test for impacts of fire and logging as higher velocity and sedimentation are intrinsically connected to loss of forest (Kerr 1995).

A recent study by Townsend et al. (2008) pursued the question of segregating sedimentation and nutrient interactions with stream macroinvertebrates. They found that taxonomic richness and EPT richness were lowest when sedimentation and nutrient levels were at their highest, but that richness increased exponentially when sediment levels were low and nutrient levels were high. This effect could explain the high richness in the Waterford River compared to the other two urban streams. It is possible that Rennies

River and Virginia River have higher concentrations of suspended solids which counteract the more beneficial aspects of nutrient introduction. Furthermore, these latter two sites did not have clean water tributaries entering the stream near the sampling site, unlike the Waterford River. Clean water tributaries may also allow recolonization of impacted lower reaches. Accurate measures of turbidity would enable testing of the sedimentation hypothesis in the future.

Analysis of Similarity (ANOSIM) tests confirmed that macroinvertebrate communities in different land use areas were significantly different. Urban sites were strongly divergent from pristine and rural sites according to the R-statistics. In contrast, the corresponding p-values was not significant. Similar results were found in the ANOSIM test of land use differences in water chemistry where the highest possible Rho values (R = 1) were coupled with less significant p-values than would be expected. In situations where this occurs, Clarke and Gorley (2006) recommend the use of R-statistics for decision making as Rho is a measure of differences between two (or more) groups and is not affected by the number of sites, unlike p-values. Consequently, it is concluded that 1) urban, rural and pristine sites are chemically distinct, and 2) invertebrates respond to the urban level of land use, but community composition does not drastically differ between rural and pristine sites. The second conclusion is supported by the cluster analysis, which grouped the two rural and two of the pristine sites together at 60% similarity.

A measure of the percentage of the basin that is residential would be useful for future studies of land use effects, particularly in defining "urban" versus "rural" sites.

Morse (2001) found that a threshold of 6% impervious surfaces in the basin must be exceeded before a change in taxonomic composition and richness would occur in the stream. A threshold similar to this may exist on the Avalon Peninsula as indicated by the lack of segregation between pristine and rural sites. Also, direct measures of turbidity taken over a full season would increase knowledge of silting events caused by storm runoff and would improve understanding of the conditions urban invertebrates tolerate.

5.4.2 Sensitivity of Newfoundland invertebrates to temporal effects

The macroinvertebrate communities of the Avalon Peninsula and Terra Nova have been shown to be significantly different (Chapter 3). Changing the abundance information to presence/absence and adding two additional years of data did not alter this result, though the Rho value decreased from 0.400 ($p = 0.001$) with 2004's fall macroinvertebrate data to 0.308 ($p = 0.001$) in the combined dataset. Presence/absence data also decreases the size of some individual eigenvectors, while raising the size of others (Clarke and Gorley 2006), reducing the probability of locating an indicator species for either region or year.

The temporal aspect of the combined study delivered some interesting results: one being that the Avalon and Terra Nova sites were not statistically different in 2004, but were so in 2002. In fact, macroinvertebrate communities at Avalon sites from 2004 and 2002 differed significantly and the Terra Nova sites from 2004 differed from samples taken in 2002 and 2003. This would seem to indicate either a) a shift in the invertebrate community that occurred over two years, or b) a difference in the sampling and/or laboratory regime between studies. As no drastic visible changes occurred at or near any

of these sites in those two years (e.g. new building complex upstream, drought, fire), then b) seems to be the cause. This conclusion is fortified by the close clustering of Terra Nova sites between 2002 and 2003, both of which studies were performed by the same person.

In some cases different field sampling and sub-sampling techniques/methods have not affected models or sampling standard deviations (Clarke et al. 2002; Ostermiller and Hawkins 2004) , though others caution against differences in sampling and sub-sampling effort (Cao et al. 2005), and some refute the validity of using sub-sampling at all (Doberstein et al. 2000). However, in the current study, nearly identical methods were used. All invertebrates were sampled in-field according to protocols outlined in the Canadian Aquatic Biomonitoring Network manual (Reynoldson et al. 2003) and sub-sampled using a Marchant box (Marchant 1989). No information regarding the number of cells used to produce the 300-individual count was located, hence the use of presence/absence data. If this information had been available, the data would have been standardized in the same manner as the 2004 data. Still, there may not have been any difference in the conclusions reached by the presence/absence data from what may have been expected from the abundance data (Wright et al. 1995). Therefore, the hypothesis that differences in sampling/identifying techniques caused the variation between the 2002/2003 and 2004 datasets is falsified and hypothesis a) appears to be supported by the evidence.

A closer examination of the taxonomic information reveals some differences between the types of taxa sampled and counted each year. Data from 2002 and 2003

contained more pond taxa (i.e. aeshnid dragonflies, planarians, cladocerans), some of which were not identified in 2004. Lake outlet stream communities frequently differ from those in riffle habitats (*vis* Richardson and Mackay 1991; Lomond and Colbo 2000; Colbo et al. submitted). Therefore, if some of the sites sampled in 2002 and 2003 were in fact from lake outlets, the presence of pond taxa would result, and potentially differentiate between these communities and the other streams sampled from 2002-2004. Not all 2002 and 2003 sites were at different locations from those in 2004; there were several sites that were repeated in the exact same location. The main reason for the split between 2004 and 2002-2003 in the plots was due to the fact that some taxa that were common in 2004 were rarely sampled in 2002 and 2003 (*Paraleptophlebia adoptiva*, *Paracapnia opis* and *Isoperla transmarina*).

The repeat sites generally agreed with the overall findings of the compiled datasets, with a few additional conclusions. The fact that there are differences between communities at identical sites over several years indicates a loss of predictive power due to natural interannual variation. However, it also follows that the more data collected, the less small, intra-annual variation affects the end results (e.g. temperature variation data becoming viewed as seasonal maxima and minima instead of daily highs and lows). Continuing to sample the repeat sites would better define the limits of natural variation and increase the possibility of using directional site movement within a plot to indicate potential degradation. It is also encouraging that the temporal lines connecting the site pairs did not cross, which indicates that site interrelationships remained identical among years (Brady et al. 2007).

As in the Avalon and Terra Nova PCA plots, *Paracapnia opis* and *Isoperla transmarina* were representative of the separation of 2004 sites from 2002 and 2003 sites. The reduced occurrence of these taxa in 2002 and 2003 may be explained by the adoption of a semi- or multi-voltine life cycle by these taxa due to cold temperatures and limited nutrients (Edmunds and Waltz 1996; Stewart and Harper 1996; Wallace and Anderson 1996). As a result, the loss of a cohort would have effects in subsequent years, but might not show up in the collected data every year the community was sampled. An intensive study would reveal if these particular taxa have a less than one life cycle a year which would be critical for protecting Newfoundland taxa in the future.

Differences in taxonomic assemblages among years are not uncommon, though few studies have been dedicated to long-term experiments (Hynes 1970; Drake 1982; Colbo 1985; Slavik et al. 2004). Many of these studies have been relatively unsuccessful, where success is defined as: if inter-annual variation did occur, it was accounted for by some measurable term (Hynes 1970; Colbo 1985). Complicated models by which populations may be predicted have been devised, but still require many years' worth of data to create (Urquhart et al. 1998). Generally, inconsistency across temporally diverse data is to be expected, and may be considered a symbol of a healthy invertebrate community responding to natural changes in the local environment. Also to be gained from this and other studies is the understanding that many sites should be revisited to avoid identifying local fluctuations as overall trends.

One result that may not be affected by the between-year differences is the change in distance between the Avalon sites and the Terra Nova sites between years. In 2004, the

two regions were much more similar than in 2002, as evidenced not only in the PCA plot, but also by close-to-1 Rho value of the 2004 samples in the ANOSIM results. These results indicate an increased likeness of the macroinvertebrate communities in these two regions between 2002 and 2004. This may be, at least partially, due to the addition of several "pristine" sites to the Avalon dataset in 2004. If the similarity was totally due to the addition of pristine sites, then the urban St. John's sites would have been expected to segregate from the rest of the sites in an identically discreet manner as 2002.

5.5 *Conclusions and Recommendations*

1) Newfoundland macroinvertebrate communities in heavily urbanized areas differ from communities in rural and pristine environments. Rural and pristine invertebrate communities are similar.
2) Impacted urban communities are characterized by a large *Baetis tricaudatus* population, an absence of Heptageniidae, and few beetles. They tend to have lower taxonomic and Ephemeroptera richness than rural or pristine sites.
3) An abundance of spate events and resulting sedimentation are suspected potent agents of change associated with urbanization in St. John's.
4) Chemically, sites classed as urban, rural and pristine, were distinct. Urban sites had higher conductivity and pH than rural and pristine sites.
5) Accurate measures of the density of urbanization (e.g. % impervious substrates, % residential area) in the basin would increase the amount of explained variation.

6) The macroinvertebrate community can change over time in response to an unknown combination of environmental variables. This temporal variability has implications for biomonitoring program design and power.
7) Samples taken in 2004 differed from samples taken in 2002 and 2003 due to a greater number of occurrences of *Paracapnia opis* and *Isoperla transmarina* in 2004.
8) Terra Nova and the Avalon Peninsula invertebrate communities were found to be more similar since the 2002 study, indicating variation in the strength of region-based relationships. This may be due to annual population changes of the taxa in these communities. Thus, spatially and temporally expanded data bases are recommended prior to establishing a biomonitoring system.
9) The diversity of the island fauna sampled with the CABIN protocol can provide data to biomonitor the Island's rivers.

6 Summary

A total of 148 taxa was collected from 65 riffle sites in the Avalon Peninsula, Terra Nova National Park and Gros Morne National Park regions over three sampling seasons (summer 2004, fall 2004, spring 2005). Newfoundland macroinvertebrate riffle community richness was lowest in the Avalon Peninsula region. Gros Morne and Terra Nova had identical taxa accumulation curves, though highest mean richness per site varied across taxonomic groups (orders). The proportion of the community made up by beetles, caddisflies and stoneflies did not vary drastically between regions, but non-insects and flies contributed more to Avalon populations than the other two regions. Mayflies were the richest taxonomic family in Gros Morne communities, and they contributed more individuals to sites in that region than any other. Regional differences were more pronounced when the effects of season were taken into account. Season was responsible for large amounts of variation in the macroinvertebrate community dataset. Based on examinations of the three seasons, fall was chosen for subsequent analyses. Specimens collected in spring and fall were generally more mature than summer-collected specimens, and spring has a higher occurrence of spate events than summer and fall. Therefore, due to increased certainty of identification and safety for the sampler, fall sampling is recommended for future studies.

Physical environmental variables did not differ consistently among geographic regions, although Avalon Peninsula sites taken as a group consistently differed in water chemistry from Terra Nova sites. Macroinvertebrate community structure was more

highly correlated with physical than with chemical variables. UTM Easting, % macrophytes, % igneous rock, % forest in the local watershed, nitrates, total Nitrogen and alkalinity were the variables most highly correlated with the macroinvertebrate community data. However, descriptions of structural and regional effects often did not follow the same pattern, causing difficulty in determining why invertebrates responded to their environment as observed (e.g. positive relationship between number of taxa present at a site and UTM Easting co-ordinates, though lower numbers of taxa were present at sites with the highest Easting co-ordinates). In general, streams with low sediment loads (as indicated by middling substrate size and velocity as well as the presence of macrophytes) positively influenced invertebrate richness and abundance.

Within the Avalon Peninsula, "urban", "rural" and "pristine" land use classes exhibited significant differences in water chemistry. Urban sites had consistently higher conductivity and pH than rural and pristine sites. The invertebrate community did not differ between rural and pristine sites, but urban communities were significantly different from communities found in the other two land use zones. This suggests that anthropogenic effects are negligible outside of urban centres, likely due to Newfoundland's limited agriculture, cool temperatures and turbulent streams. Urban communities were largely populated by *Baetis tricaudatus* and completely lacked the mayfly family Heptageniidae. In general, urban sites had the lowest taxonomic and mayfly richness of all sites.

Relative abundances of dominant macroinvertebrates within the sites varied among years, but not enough to affect the ordering of sites relative to one another.

Continued monitoring, particularly with repeat sites, is strongly advised in order to measure the amount of natural variation in Newfoundland communities in time and space. Despite the few years available for analysis, a reduction in the difference between Avalon Peninsula and Terra Nova sites was observed from comparing a 2002 to 2004 dataset. Changes in the frequency of occurrence in two key species between these sample dates were the apparent cause of this merging of regions. Again, continued sampling may determine if this reduction in regional difference is persistent through time, or if it is a result of cyclical, more global environmental effects rather than more local land use changes.

In summary, this research defined the macroinvertebrate community of riffles across the island, which also revealed some differences in composition among geographic regions. The macroinvertebrate communities of Newfoundland appeared to be sensitive to physical and chemical conditions, including land use patterns. Human land use influences were only detected in St. John's urban sites. Correlations between macroinvertebrates and the environmental variables indicated that the CABIN protocol is a suitable method for biomonitoring in Newfoundland, despite the island's depauperate species assemblage. Macroinvertebrates were good indicators of stream characteristics, but to detect meaningful gradual trends at local or global levels, data collected over a long time series will be required.

Recommendations for the application of a benthic macroinvertebrate biomonitoring program in Newfoundland are as follows:

1) Samples should be collected in the fall.

2) Sample sites should be chosen in such a way as to give equal numbers of sites in each ecoregion. At the very least, there should be five sites in a region for statistical power; a number generally accepted by statisticians.

3) The accuracy of physical data collection is important due to the high correlation of physical variables with macroinvertebrates. The inclusion of forest type in the watershed is highly recommended.

4) Alkalinity and nutrients should continue to be monitored closely and turbidity should be added to water chemistry protocols. Conductivity and pH also become important within a region where human impacts are expected.

5) Abundances of the taxa *Baetis tricaudatus*, Heptageniidae and Coleoptera are clear indicators of urbanization in Newfoundland. Continued use of abundance/proportion data as opposed to presence/absence data is encouraged.

6) Repeated sampling of past sites is highly recommended for long-term monitoring.

Topics for future study that will increase the effectiveness of the biomonitoring system include:

1) Describing the effects of sedimentation on Newfoundland invertebrates to separate these effects from those of urbanization.

2) Measuring nutrient levels (Phosphorus, nitrates, total Nitrogen) from plant matter and water samples to assess overall nutrient inputs (bioaccumulated in plants) to compare to values of single point collections (water chemistry).

3) Determining the threshold of land use change (including impervious surfaces) that, when exceeded, causes change in invertebrate communities. This will result in refining the definition of "rural" and "urban" sites, as pertaining to agriculture, logging and residential activities.

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Appendix 1. Listing of sample sites, codes and respective latitude and longitude for each brook sampled at some point in the summer and fall 2004 and spring 2005.

St. John's and Avalon Peninsula

River Name	Site Code	Latitude	Longitude
Watern Brook	WAT01	46° 38' 52.0"	53° 09' 55.8"
Bristol Brook	BRI01	46° 38' 59.1"	53° 10' 54.5"
Portugal Cove Brook	PCB01	46° 44' 41.7"	53° 14' 03.7"
Rennies River	REN01	47° 34' 28.7"	52° 42' 28.9"
Virginia River	VIR01	47° 35' 04.1"	52° 41' 24.2"
Waterford River	WFR01	47° 32' 36.5"	52° 43' 26.8"
South Brook	STB01	47° 29' 50.6"	52° 47' 25.7"
Beaver Brook	BEA01	47° 20' 40.3"	52° 55' 09.1"
Peyton Brook	PEY01	47° 20' 38.1"	52° 55' 46.0"
Broad Cove Brook	BCB02	47° 34' 34.8"	52° 52' 32.2"

Terra Nova National Park

River Name	Site Code	Latitude	Longitude
Square Pond Brook	SQP02	48° 38' 10.5"	53° 57' 36.0"
Arnolds Pond Brook	APB02	48° 37' 21.9"	53° 58' 7.12"
Southwest Brook	SWB01	48° 36' 41.5"	53° 58' 30.0"
Upper Southwest Brook	USW01	48° 21' 02.1"	54° 01' 48.0"
Southwest River	SWR01	48° 18' 53.6"	54° 10' 21.9"
Salmon Brook	SAB01	48° 23' 30.2"	54° 12' 25.8"
Big Brook	BBA02	48° 32' 12.4"	53° 58' 49.5"
Terra Nova Brook	TNB03	48° 32' 01.1"	53° 59' 11.2"
Charlottetown Brook	CTB02	48° 26' 30.6"	54° 00' 21.4"
Northwest River	NWR03	48° 23' 36.5"	54° 11' 46.6"
Cobblers Brook	CBB02	48° 25' 08.9"	54° 08' 04.8"
Rocky Brook	RPB01	48° 35' 30.6"	54° 33' 11.1"
Bloody Brook	BBB01	48° 35' 30.6"	54° 34' 01.0"
Triton Brook	TRI01	48° 36' 12.1"	54° 35' 11.9"
Penneys Brook	PNB02	48° 40' 02.8"	54° 01' 52.9"
Penneys Brook	PNB01	48° 40' 22.0"	54° 01' 54.4"
Minchins Pond Brook	MPB01	48° 33' 36.72"	53° 52' 52.64"
North Broad Cove Brook	NBC01	48° 37' 21.8"	53° 49' 26.9"
Little Shoal Harbour River	LSR01	48° 08' 49.5"	53° 58' 15.7"
Little Shoal Harbour River	LSR02	48° 09' 07.4"	53° 57' 57.3"

Gros Morne National Park

River Name	Site Code	Latitude	Longitude
Slants Brook	SLB01	49° 57' 06.1"	57° 45' 04.4"
Western Brook	WEB01	49° 49' 44.9"	57° 51' 17.5"
Bakers Brook	BAB01	49° 39' 20.6"	57° 57' 18.9"
Eels Brook	EEL02	49° 37' 20.9"	57° 55' 37.7"
Eels Brook	EEL01	49° 37' 9.8"	57° 55' 22.9"
Rocky Harbour Pond Brook	RHP01	49° 34' 25.5"	57° 53' 03.3"
Deer Brook	DEE01	49° 34' 05.0"	57° 50' 19.0"
Trout River Tributary	TRT01	49° 21' 03.1"	57° 53' 15.2"
Trout River Brook	TRH01	49° 21' 06.4"	57° 53' 14.5"
Clear Cut Brook	CCB01	49° 21' 06.5"	57° 53' 14.5"
Martina Steger Brook	MSB01	49° 23' 03.5"	57° 49' 51.2"
McKenzies Brook	MKB01	49° 24' 40.2"	57° 50' 38.2"
Nichols Brook	NIC01	49° 33' 34.4"	57° 49' 54.9"
Nichols Brook	NIC02	49° 11' 26.2"	57° 26' 56.9"
Bottom Brook	BOT01	49° 34' 53.1"	57° 54' 20.6"
Bottom Brook	BOT02	49° 35' 20.1"	57° 55' 01.0"
Feeder Brook	FED01	49° 28' 11.7"	58° 06' 54.9"
Feeder Brook	FED02	49° 28' 07.0"	58° 07' 31.0"
Wallace Brook	WAL01	49° 29' 29.3"	58° 00' 53.6"
Manuels Brook	MAN01	49° 29' 20.7"	58° 04' 46.9"
Wallace Brook Tributary 2	WBT01	49° 29' 19.3"	58° 00' 47.6"
Wallace Brook Tributary 1	WBO01	49° 28' 34.0"	57° 57' 57.9"
Winter House Brook	WHB01	49° 28' 05.6"	57° 57' 24.0"
Winter House Brook	WHB02	49° 29' 13.7"	57° 55' 38.2"
Shoal Brook	SHO01	49° 28' 24.6"	57° 55' 18.2"
Sellars Brook	SEL01	49° 26' 01.0"	57° 53' 43.1"
Lomond River	LOM01	49° 24' 18.8"	57° 43' 42.6"
Mitchells Brook	MIT01	49° 33' 31.2"	57° 49' 49.9"
Southeast Brook	SEB01	49° 27' 37.9"	57° 40' 22.4"
Birch Rine Woods Brook	BRW01	49° 38' 50.5"	57° 42' 48.5"
Pilgrims Pond Brook	PPB01	49° 35' 29.6"	57° 35' 29.5"
Middle Barrens Brook	MBB01	49° 37' 30.4"	57° 38' 20.8"
Sloping Rock Brook	SRO01	49° 43' 40.3"	57° 31' 38.8"
Glander Gulch Brook	GGB01	49° 39' 20.5"	57° 35' 19.7"
No Name Brook	NNB01	49° 35' 26.9"	57° 36' 01.8"

Appendix 2. Taxonomic list obtained from all three sets of sorted samples and geographical location of each taxon. Key: A = Avalon Peninsula, T = Terra Nova National Park and area, G = Gros Morne National Park and area. An asterisk (*) marks those species that are new records for either the province or the Island. Common names listed in parentheses where appropriate.

Mollusca
- **Gastropoda (snails)**
  - Ancylidae
    - *Ferissia rivularis* A, T
  - Limnaeidae G
    - *Stagnicola elodes* T
  - Physidae A, T, G
  - Planorbidae
    - *Helisoma* A, T, G
    - *Gyraulus* A, T, G
  - Valvatidae
    - *Valvata sp.* T, G
- **Bivalvia (clams)**
  - Sphaeriidae A, T, G
  - Unionidae T, G

Annelida
- **Oligochaeta (worms)**
  - **Tubificida**
    - Naididae A, T, G
    - Enchytraeidae A, T, G
  - **Lumbriculida**
    - Lumbriculidae A, T, G
- **Hirudinea (leeches)**
  - **Rhynchobdellida**
    - Glossiphoniidae
      - *Helobdella stagnalis* A, T, G
  - **Arhynchobdellida**
    - Erpobdellidae
      - *Erpobdella punctata* A, T, G

Arthropoda
- **Crustacea**
  - **Amphipoda**
    - Hyalellidae
      - *Hyalella azteca* A, T, G
- **Arachnida**
  - Acariformes
    - Mites (not classified) A, T, G

Insecta

Collembola (springtails) A, T, G

Ephemeroptera (mayflies)

Baetidae

Acentrella lapponicus G
Acerpenna pygmaeus A, T, G
Baetis flavistriga A, T, G
B. macdunnoughi G
B. tricaudatus A, T, G
Procloeon convexum T

Heptageniidae

Epeorus pleuralis A, T, G
Heptagenia pulla A, T, G
Leucrocuta hebe A, T, G
Rithrogena undulata T, G
Stenonema femoratum G
Stenonema vicarium A, T, G

Ephemerellidae

Drunella cornuta A, T, G
Drunella cornutella A, T, G
Ephemerella aurivillii. A, T, G
Ephemerella subvaria A, T, G
Eurylophella sp. A, T, G
Eurylophella funeralis A, T, G
Eurylophella temporalis A, T, G
* *Serratella sp.* T, G

Caenidae

Caenis amica G

Tricorythidae

Tricorythodes allectus T, G

Leptophlebiidae

Habrophlebia vibrans A, T, G
Leptophlebia sp. A, T, G
Paraleptophlebia adoptiva A, T, G
Paraleptophlebia debilis T, G

Siphlonuridae

Ameletus sp. T, G

Odonata (dragonflies and damselflies)

Calopterygidae

Calopteryx aequabilis T

Gomphidae

Ophiogomphus colubrinus A, T, G

Aeshnidae

Aeshna umbrosa A, G

Plecoptera (stoneflies)

Nemouridae

Podmosta macdunnoughi A, T, G

Leuctridae

Leuctra ferruginea A, T, G

Capniidae

Allocapnia minima A

Capnia sp. T, G

Paracapnia opis A, T, G

Perlodidae

Isogenoides fontalis T, G

Isoperla bilineata T, G

Isoperla transmarina A, T, G

Chloroperlidae

Alloperla sp. A, T, G

Hemiptera

Gerridae

Gerris comatus A, T

G. remigis T

Trichoptera (caddisflies)

Glossosomatidae

Glossosoma nigrior A, T, G

Hydroptilidae

Hydroptila metoecea A, T, G

* *Neotrichia sp.* T, G

Oxyethira sp. A, T, G

Helicopsychidae

Helicopsyche borealis T

Rhyacophilidae

Rhyacophilia atrata G

R. carolina A, T, G

* *R. carpenteri* G

R. fuscula A, T, G

R. ignorata A, G

R. invaria T, G

R. melita T, G

R. minora A, T, G

R. torva A, T, G

R. vibox G

Hydropsychidae

Arctopsyche ladogensis A, T, G

Parapsyche apicalis T, G

* *Hydropsyche alternans* T, G

H. slossonae A, T, G

H. sparna A, T, G
Philopotamidae
Chimarra sp. A, T, G
Dolophilodes sp. A, T, G
Wormaldia sp. G
Polycentropodidae
Neureclipsis T, G
Ncytiophylax T, G
Polycentropus A, T, G
Apatanidae
Apatania sp. A, T, G
Brachycentridae
Micrasema sp. A, T, G
Lepidostomatidae
Lepidostoma sp. A, T, G
Leptoceridae
Ceraclea sp. A, T, G
Mystacides sepulchralis A, T
Oecetis sp. A, T
Triaenodes sp. T
Odontoceridae
Psilotreta sp. G
Limnephilidae
Hydatophylax argus A, T, G
Limnephilus T
Pseudostenophylax sp. G
Psychoglypha sp. G
Molannidae
Molanna sp. A
Uenoidae
Neophylax sp. A, T, G

Lepidoptera (butterflies)
Pryalidae T
Cossidae
Prionoxystus sp. T, G
Tortricidae
Archips sp. T, G

Diptera (true flies)
Blephariceridae
Blepharicera sp. G
Tipulidae (crane flies)
Antocha sp. A, T, G
Dicranota sp. A, T, G
Hexatoma sp. A, T, G

Limnophila sp. T, G
Limonia sp. A, T, G
Molophius T, G
Tipula sp. A, T, G

Dixidae
Dixa sp. G

Psychodidae
Pericoma sp. T, G

Culicidae T

Simuliidae (black flies)
Helodon pleuralis T, G
Prosimulium approximatum T, G
Prosimulium mixtum A, T, G
Stegopterna mutata A, T, G
Simulium annulus T, G
* *S. violator* T, G
S.craigi T, G
S. silvestre T, G
S. vittatum sp. complex A, T, G
S. murmanum A, T, G
* *S. parnassum* T
S. tuberosum sp. complex A, T, G
S. venustum/verecundum A, T, G

Ceratopogonidae A, T, G

Chironomidae A, T, G

Nymphomyiidae
Nymphomyia walkerii A, T, G

Empididae
Hemerodromia sp. A, T, G

Muscidae
Limnophora A

Tabanidae G

Sciomyzidae G

Coleoptera (beetles)

Dytiscidae

Colymbetinae
Agabus sp. G
A. thompsoni G
A. tristis G
Colymbetes sp. G

Dytiscinae
Hydaticus aruspex G
Liodessus affinis T

Hydroporinae T, G

Nebrioporus rotundatus T

Georyssidae

Georyssus sp. G

Gyrinidae

Gyrinus sp. T

Hydrophilidae

Helophorus sp. G

Laccobius agilis G

Elmidae

Optioservus sp. T, G

Oulimnius latiusculus A, T, G

Promoresia tardella A, T, G

Stenelmis crenata A, T, G

Curculionidae T

Staphylinidae G

Appendix 3. Physical and chemical data from fall sampling season. Please reference Appendix 1 for location of sites.

	altitude	summer wetted width	bankfull width	canopy	macrophytes	riffle	vegetation overhang	dominant substrate
units	m	m	m	%	%	%	cm	scale
VIR01	6	6.4	15.5	80	2	85	85.00	3
WFR02	6	7.5	17.3	0	1	70	48.33	3
PCB01	61	21.0	23.0	0	1	90	27.50	4
STB01	40	3.6	5.7	50	3	45	53.33	3
BRI01	15	17.0	17.0	0	1	95	51.67	4
PEY01	46	13.8	15.6	0	3	25	0.00	3
BCB02	26	6.5	7.0	10	1	85	29.17	3
REN01	6	9.0	10.5	30	2	95	2.67	3
BEA01	46	4.7	6.4	10	1	40	13.83	4
WAT01	30	3.4	3.4	5	5	90	29.17	3
LSR01	17	6.9	9.6	5	2	70	75.33	4
LSR02	2	12.5	16.2	0	2	75	27.50	3
TNB03	8	3.5	4.8	30	1	80	69.67	3
BBA02	1	6.7	9.2	5	1	85	41.33	3
NBC01	2	7.3	8.7	15	2	70	32.83	5
CTB02	1	7.2	11.2	70	1	85	61.67	2
SQP02	44	3.0	3.6	65	2	90	7.17	4
SWB01	13	9.6	10.7	5	2	80	0.00	3
USW01	88	7.4	9.1	5	2	90	28.00	5
APB02	14	2.8	3.0	10	2	80	13.67	3
MPB01	3	3.6	6.6	50	2	70	21.00	4
SWR01	9	39.6	53.7	0	2	75	5.67	4
NWR03	2	50.0	50.0	0	2	60	0.00	5
PNB01	2	5.2	7.0	25	2	60	59.17	3
PNB02	15	5.0	9.0	25	2	75	61.17	4
BBB01	23	11.8	17.2	5	2	90	19.33	4
RPB01	27	12.8	14.2	0	1	90	38.67	4
TRI01	15	19.5	23.6	0	2	95	101.67	3
CBB02	25	6.4	7.4	90	1	70	101.17	3
SAB01	23	19.7	22.7	5	2	90	50.17	3
NIC01	36	5.9	7.5	90	2	75	18.00	3
EEL01	76	4.0	5.5	70	1	80	152.67	4

	2nd dominant substrate	surrounding substrate	igneous	sedimentary	metamorphic	nonglacial	glacial
units	scale	scale	%	%	%	%	%
VIR01	3	2	0.0	100.0	0.0	0.0	93.1
WFR02	3	1	0.0	100.0	0.0	1.5	79.8
PCB01	3	2	0.0	100.0	0.0	100.0	0.0
STB01	2	1	0.0	100.0	0.0	0.0	98.4
BRI01	3	2	0.0	100.0	0.0	100.0	0.0
PEY01	4	2	0.0	100.0	0.0	1.0	99.0
BCB02	4	2	0.0	100.0	0.0	0.0	96.8
REN01	3	2	0.0	100.0	0.0	0.0	93.5
BEA01	3	1	0.0	100.0	0.0	33.0	67.0
WAT01	3	1	0.0	100.0	0.0	100.0	0.0
LSR01	5	3	44.6	0.0	55.4	0.1	92.6
LSR02	3	2	44.6	0.0	55.4	0.1	92.9
TNB03	2	1	0.0	89.6	10.4	2.7	95.9
BBA02	3	1	0.0	89.2	10.8	2.5	96.6
NBC01	3	2	0.0	100.0	0.0	5.5	17.4
CTB02	3	1	0.0	89.6	10.4	0.1	99.0
SQP02	3	2	0.0	89.3	10.7	0.0	100.0
SWB01	4	3	0.0	88.9	11.1	1.9	98.0
USW01	3	2	2.3	77.0	20.7	0.5	97.3
APB02	3	1	0.0	89.3	10.7	0.0	100.0
MPB01	3	1	0.0	100.0	0.0	1.4	45.5
SWR01	3	3	29.6	38.5	31.9	2.2	91.7
NWR03	4	2	36.1	58.2	5.7	3.7	90.7
PNB01	4	2	19.9	74.0	6.2	0.4	99.4
PNB02	3	1	19.9	74.0	6.2	0.4	99.4
BBB01	3	3	14.8	85.3	0.0	1.8	98.2
RPB01	3	3	100.0	0.0	0.0	3.3	96.7
TRI01	3	4	12.6	86.2	1.3	7.8	92.2
CBB02	3	2	4.8	62.7	32.6	0.9	98.3
SAB01	3	4	20.9	52.1	27.1	1.3	96.6
NIC01	3	1	0.0	100.0	0.0	0.0	71.1
EEL01	3	2	0.0	100.0	0.0	0.0	40.3

	bedrock	size of watershed	forest in whole watershed	forest in local watershed	forest within 100m of reach	nitrates	total Nitrogen
units	%	sq. km	%	%	%	mg/L	mg/L
VIR01	6.9	6.35E+06	6.32	15.00	0.00	1.21	0.00
WFR02	18.7	5.68E+07	31.74	44.36	0.00	1.04	0.00
PCB01	0.0	3.30E+07	0.00	0.00	0.00	0.01	0.06
STB01	1.6	8.26E+06	57.98	27.00	17.00	0.42	0.00
BRI01	0.0	3.81E+07	0.00	0.00	0.00	0.01	0.07
PEY01	0.0	1.56E+07	12.94	50.82	42.00	0.01	0.00
BCB02	3.2	2.88E+07	55.72	44.04	8.00	0.08	0.00
REN01	6.5	1.01E+07	15.47	0.00	0.00	0.69	0.11
BEA01	0.0	6.87E+06	36.23	54.00	22.00	0.01	0.00
WAT01	0.0	5.31E+06	0.00	0.00	0.00	0.05	0.15
LSR01	7.3	4.40E+07	60.70	71.70	64.00	0.03	0.00
LSR02	7.0	2.35E+07	52.04	77.79	41.00	0.01	0.23
TNB03	1.4	1.67E+07	39.32	35.05	88.57	0.01	0.13
BBA02	0.9	5.94E+07	46.30	42.27	100.00	0.01	0.00
NBC01	77.1	1.90E+07	71.17	62.24	0.59	0.01	0.14
CTB02	0.9	1.53E+07	36.47	62.47	64.06	0.01	0.11
SQP02	0.0	1.68E+06	53.10	70.14	100.00	0.01	0.12
SWB01	0.1	3.87E+07	64.67	70.67	100.00	0.01	0.16
USW01	2.2	2.09E+07	58.76	87.33	100.00	0.01	0.15
APB02	0.0	1.50E+06	76.69	78.47	100.00	0.01	0.15
MPB01	53.1	2.03E+07	50.95	69.11	100.00	0.01	0.09
SWR01	6.1	4.62E+08	12.13	74.57	86.69	0.01	0.12
NWR03	5.6	7.63E+08	12.13	2.46	0.00	N/A	N/A
PNB01	0.1	2.20E+07	35.03	12.13	0.00	0.01	0.17
PNB02	0.1	2.17E+07	35.48	20.14	43.83	0.01	0.15
BBB01	0.0	3.39E+07	33.47	58.89	15.03	0.01	0.12
RPB01	0.0	8.37E+07	8.36	71.12	46.82	0.01	0.13
TRI01	0.0	2.28E+08	47.00	73.68	77.28	0.01	0.14
CBB02	0.9	2.37E+07	24.24	67.15	62.30	0.01	0.18
SAB01	2.2	1.16E+08	9.84	28.95	11.99	0.01	0.00
NIC01	28.9	1.14E+07	83.58	98.00	72.00	0.01	0.05
EEL01	59.7	7.41E+05	72.62	79.08	68.80	0.05	0.16

	total Phosphorus	colour	conductivity	pH	alkalinity	alkalinity (CaCO3)
units	mg/L	Hazenuni	uS/cm	pH scale	mg/L	mg/L
VIR01	0.026	11	400.0	7.22	17.00	18.1
WFR02	0.021	19	353.0	7.13	11.99	13.6
PCB01	0.008	22	34.4	6.64	3.16	4.5
STB01	0.008	37	99.7	6.59	4.92	5.9
BRI01	0.008	37	41.3	6.35	2.53	3.9
PEY01	0.008	57	26.5	6.17	2.61	3.7
BCB02	0.009	40	132.9	6.42	3.45	4.5
REN01	0.018	23	321.0	7.02	10.19	11.6
BEA01	0.013	138	32.5	5.68	2.25	3.4
WAT01	0.007	32	49.6	6.38	3.51	4.6
LSR01	0.016	170	66.7	6.16	5.63	5.9
LSR02	0.016	147	31.0	6.39	5.62	6.1
TNB03	0.010	147	53.4	6.67	8.18	8.4
BBA02	0.012	96	33.5	6.76	7.03	7.4
NBC01	0.005	76	33.8	6.32	3.94	4.7
CTB02	0.012	71	51.9	6.86	8.88	9.4
SQP02	0.007	95	213.0	7.09	11.82	12.8
SWB01	0.009	147	33.3	6.23	5.20	5.4
USW01	0.014	95	32.5	6.42	6.04	6.5
APB02	0.009	171	50.7	6.60	8.12	8.2
MPB01	0.006	39	30.9	6.79	5.30	6.1
SWR01	0.013	91	28.0	6.46	5.08	5.7
NWR03	N/A	N/A	N/A	N/A	N/A	N/A
PNB01	0.009	155	96.0	6.84	9.51	10.3
PNB02	0.008	162	82.5	6.72	8.27	8.8
BBB01	0.020	202	24.4	5.49	3.35	3.8
RPB01	0.017	140	20.8	6.09	4.19	4.7
TRI01	0.016	103	22.7	6.09	4.45	4.9
CBB02	0.012	244	28.8	5.71	4.33	4.6
SAB01	0.006	80	21.6	5.89	3.43	4.2
NIC01	0.010	26	271.0	8.14	109.00	109.7
EEL01	0.006	137	126.2	7.83	42.36	42.7

	altitude	summer wetted width	bankfull width	canopy	macrophytes	riffle	vegetation overhang	dominant substrate
units	m	m	m	%	%	%	cm	scale
SLB01	6	3.3	6.1	80	2	80	0.00	3
DEE01	6	22.9	24.7	5	1	80	98.33	3
EEL02	75	2.1	3.4	85	2	40	27.50	3
WHB01	222	11.8	55.5	0	1	95	0.00	4
WHB02	2	7.5	13.0	0	1	80	0.00	4
SEB01	93	16.5	19.2	2	1	95	34.17	4
RHP01	38	7.1	7.9	17	1	65	125.50	3
WEB01	13	27.6	29.9	5	1	70	98.67	4
MIT01	8	6.1	13.0	95	1	80	21.67	4
WBO01	186	3.1	10.8	2	1	75	13.33	4
SEL01	12	8.5	20.8	5	1	50	0.00	4
LOM01	42	29.1	30.7	5	2	40	63.00	4
CCB01	258	3.0	5.2	60	1	70	37.50	4
MSB01	335	2.6	3.0	30	1	40	20.33	5
SHO01	10	5.3	8.0	0	1	85	0.00	4
TRT01	206	4.8	6.5	25	1	40	16.33	5
FED01	25	6.8	20.0	2	1	95	0.00	4
MAN01	212	3.3	10.3	0	1	80	0.00	3
FED02	7	11.3	19.5	5	1	80	30.83	3
MKB01	96	10.8	14.8	15	1	70	0.00	4
TRH01	197	11.0	15.8	30	1	70	3.33	4
WAL01	130	16.0	18.1	5	1	40	58.83	2
NIC02	16	4.3	6.7	25	1	80	96.17	3
BOT02	2	8.4	12.8	10	2	90	37.33	5
BOT01	30	9.2	10.5	5	2	80	52.50	5
WBT01	137	3.8	6.9	5	1	90	18.17	2
BAB01	14	32.6	34.2	0	1	90	29.00	3
BRW01	399	10.2	10.2	0	1	70	0.00	3
PPB01	607	7.3	7.3	0	1	80	11.67	3
MBB01	469	25.1	25.1	5	1	75	25.00	4
SRO01	402	14.0	15.2	0	1	60	29.67	3
GGB01	469	36.0	38.5	0	1	90	5.00	4
NNB01	604	15.1	20.6	0	1	50	20.50	4

	2nd dominant substrate	surrounding substrate	igneous	sedimentary	metamorphic	nonglacial	glacial
units	scale	scale	%	%	%	%	%
SLB01	4	2	0.0	100.0	0.0	65.6	22.0
DEE01	4	3	35.8	31.8	32.4	33.1	30.5
EEL02	3	2	0.0	100.0	0.0	16.4	81.1
WHB01	3	3	100.0	0.0	0.0	45.2	54.8
WHB02	2	1	88.3	11.7	0.0	34.5	62.7
SEB01	3	2	64.2	11.0	24.8	7.6	2.8
RHP01	3	2	0.0	100.0	0.0	17.1	70.0
WEB01	3	2	0.0	100.0	0.0	80.7	10.2
MIT01	3	2	100.0	0.0	0.0	1.7	40.5
WBO01	3	2	97.8	2.2	0.0	59.8	40.2
SEL01	3	2	56.9	35.7	7.4	24.4	46.0
LOM01	3	3	0.0	100.0	0.0	0.0	67.3
CCB01	3	2	0.0	100.0	0.0	8.4	18.5
MSB01	3	2	0.0	100.0	0.0	0.0	44.7
SHO01	3	1	72.4	12.5	15.1	19.6	40.3
TRT01	3	2	0.0	100.0	0.0	39.0	45.2
FED01	3	2	94.2	5.8	0.0	7.4	78.8
MAN01	3	2	99.3	0.7	0.0	24.6	75.4
FED02	3	1	94.2	5.8	0.0	7.4	78.8
MKB01	3	2	0.0	100.0	0.0	0.0	67.3
TRH01	3	2	0.0	100.0	0.0	0.0	75.1
WAL01	2	1	86.8	13.2	0.0	33.0	65.4
NIC02	3	1	0.0	100.0	0.0	0.0	78.2
BOT02	3	2	0.0	100.0	0.0	22.9	57.7
BOT01	3	2	0.0	100.0	0.0	24.9	58.3
WBT01	3	2	88.9	11.1	0.0	23.6	76.4
BAB01	4	3	0.0	100.0	0.0	49.8	50.2
BRW01	2	1	0.0	0.0	100.0	1.5	67.8
PPB01	4	1	0.0	0.0	100.0	0.0	1.3
MBB01	3	2	0.0	0.0	100.0	10.5	19.7
SRO01	4	2	0.0	0.0	100.0	8.8	2.3
GGB01	3	3	0.0	0.0	100.0	9.1	21.3
NNB01	3	3	0.0	0.0	100.0	0.0	19.1

	bedrock	size of watershed	forest in whole watershed	forest in local watershed	forest within 100m of reach	nitrates	total Nitrogen
units	%	sq. km	%	%	%	mg/L	mg/L
SLB01	12.4	9.24E+06	23.69	28.41	34.78	0.01	0.13
DEE01	36.5	9.26E+07	23.14	81.51	34.27	0.11	0.17
EEL02	2.5	8.07E+05	67.29	63.38	84.00	0.03	0.10
WHB01	0.0	7.44E+06	0.00	0.00	0.00	0.16	0.23
WHB02	2.8	1.43E+07	7.24	46.58	7.88	0.15	0.20
SEB01	89.6	6.06E+07	22.66	88.80	81.61	0.01	0.09
RHP01	13.0	2.09E+07	58.47	74.53	60.33	0.04	0.13
WEB01	9.1	1.28E+07	9.19	1.09	10.18	0.14	0.20
M1T01	57.8	3.67E+06	46.60	93.04	93.78	0.01	0.09
WBO01	0.0	8.21E+05	0.00	0.00	0.00	0.15	0.19
SEL01	29.6	1.84E+07	24.40	81.93	35.13	0.13	0.19
LOM01	32.7	1.13E+07	76.42	89.26	89.26	0.07	0.14
CCB01	73.1	2.86E+06	69.39	86.87	40.00	0.01	0.23
MSB01	55.3	4.77E+05	78.00	78.00	85.00	0.01	0.08
SHO01	40.0	9.51E+06	21.58	62.60	15.26	0.14	0.20
TRT01	15.8	3.56E+06	48.78	89.00	94.00	0.04	0.15
FED01	13.8	2.16E+07	3.57	41.79	61.09	0.18	0.21
MAN01	0.0	1.43E+06	0.00	0.00	0.00	0.23	0.28
FED02	13.8	2.21E+07	4.38	43.80	0.00	0.19	0.24
MKB01	32.7	1.84E+07	82.75	93.90	37.00	0.03	0.12
TRH01	24.9	1.27E+07	72.63	97.00	96.00	0.01	0.10
WAL01	1.5	1.84E+07	6.07	15.22	0.00	0.17	0.18
N1C02	21.8	1.32E+07	85.02	80.00	54.00	0.01	0.06
BOT02	19.4	3.34E+07	60.75	56.07	0.00	0.04	0.15
BOT01	16.8	3.03E+07	59.63	21.99	87.26	0.03	0.13
WBT01	0.0	8.70E+05	0.00	0.00	0.00	0.19	0.20
BAB01	0.0	7.32E+05	32.83	44.32	31.92	0.12	0.28
BRW01	30.7	2.66E+05	2.52	2.52	31.92	0.03	0.08
PPB01	98.7	6.68E+06	0.52	2.28	0.00	0.01	0.07
MBB01	69.8	2.21E+07	12.56	25.76	11.76	0.01	0.09
SRO01	88.9	7.99E+06	10.05	23.84	6.47	0.01	0.10
GGB01	69.6	3.08E+07	6.10	29.66	0.00	0.01	0.06
NNB01	80.9	5.86E+06	0.62	0.03	0.00	0.01	0.10

	total Phosphorus	colour	conductivity	pH	alkalinity	alkalinity (CaCO3)
units	mg/L	Hazenuni	uS/cm	pH scale	mg/L	mg/L
SLB01	0.016	91	114.5	7.73	38.29	38.8
DEE01	0.006	42	47.8	7.36	13.88	15.1
EEL02	0.019	28	227.0	8.06	96.67	97.0
WHB01	0.003	12	90.6	7.93	38.30	39.3
WHB02	0.003	13	119.7	8.02	48.55	49.3
SEB01	0.009	82	75.0	7.49	16.87	17.8
RHP01	0.007	59	193.1	8.02	80.53	81.0
WEB01	0.005	22	37.2	7.05	7.47	8.2
MIT01	0.013	91	32.3	6.88	7.24	7.8
WBO01	0.004	10	111.9	8.00	47.41	48.2
SEL01	0.004	29	101.6	7.92	42.04	43.3
LOM01	0.005	28	168.2	8.12	77.54	78.0
CCB01	0.025	68	76.8	7.26	28.32	29.1
MSB01	0.007	64	41.9	7.07	10.37	11.1
SHO01	0.003	18	125.1	8.01	49.81	50.7
TRT01	0.005	40	112.4	7.74	46.87	47.2
FED01	0.003	19	89.9	7.86	35.60	36.3
MAN01	0.003	14	103.3	7.94	41.10	42.4
FED02	0.004	15	92.9	7.87	36.21	37.0
MKB01	0.004	43	66.1	7.54	21.38	21.9
TRH01	0.006	90	101.1	7.77	43.72	44.4
WAL01	0.003	7	88.0	7.88	36.16	37.1
NIC02	0.013	29	262.0	8.14	106.80	107.4
BOT02	0.012	60	217.0	7.99	66.68	67.3
BOT01	0.009	59	199.8	7.95	64.65	65.0
WBT01	0.003	8	119.2	8.01	50.12	51.1
BAB01	0.005	39	43.5	7.28	10.97	12.2
BRW01	0.005	29	12.3	5.48	0.35	2.0
PPB01	0.021	56	15.2	5.56	0.74	2.3
MBB01	0.010	80	14.6	5.31	0.87	2.1
SRO01	0.009	79	14.4	5.60	1.46	2.6
GGB01	0.009	42	14.7	5.42	0.78	2.2
NNB01	0.012	111	14.6	5.42	1.02	2.4

Appendix 4. Estimated fall abundance of macroinvertebrates in the entire sample per minute sampled. Taxonomic list is in three parts.

	Order	Family	Genus	Species	VIR01	WFR02	PCB01
1	Gastropoda	fam.	gen.	sp.	0	0.2	0
2	Gastropoda	Ancylidae	Ferissia	rivularis	4.3	0	0
3	Gastropoda	Physidae	gen.	sp.	5.7	0	3.3
4	Gastropoda	Planorbidae	Heliosoma	sp.	1.4	0	0
5	Gastropoda	Planorbidae	Gyralis	sp.	1.4	1.4	13.3
6	Gastropoda	Valvatidae	gen.	sp.	0	0	0
7	Gastropoda	Valvatidae	Valvata	sp.	0	0	0
8	Bivalva	Sphaeriidae	gen.	sp.	1.4	0	0
9	Oligochaeta	Naididae	gen.	sp.	4.3	0	30.0
10	Oligochaeta	Enchytraeidae	gen.	sp.	20.0	4.8	0
11	Oligochaeta	Lumbriculidae	gen.	sp.	60.0	8.8	0
12	Hirudinea	Glossiphonidae	Helobdella	stagnalis	0	0.2	0
13	Hirudinea	Erpobdellidae	Erpobdella	punctata	0	0	0
14	Amphipoda	Hyalellidae	Hyalella	azteca	2.9	0.6	3.3
15	Acariformes	fam.	gen.	sp.	2.9	1	70.0
16	Collembola	fam.	gen.	sp.	0	0	0
17	Ephemeroptera	fam.	gen.	sp.	0	0	0
18	Ephemeroptera	Baetidae	gen.	sp.	0	0	0
19	Ephemeroptera	Baetidae	Acerpenna	pygmaeus	0	0.2	10.0
20	Ephemeroptera	Baetidae	Baetis	flavistriga	0	0.4	0
21	Ephemeroptera	Baetidae	Baetis	tricaudatus	125.7	15.8	180.0
22	Ephemeroptera	Heptageniidae	gen.	sp.	0	0	20.0
23	Ephemeroptera	Heptageniidae	Epeorus	pleuralis	0	0	0
24	Ephemeroptera	Heptageniidae	Heptagenia	pulla	0	0	0
25	Ephemeroptera	Heptageniidae	Leucrocuta	hebe	0	0	73.3
26	Ephemeroptera	Heptageniidae	Rithrogena	undulata	0	0	0
27	Ephemeroptera	Heptageniidae	Stenonema	vicarium	0	0	3.3
28	Ephemeroptera	Heptageniidae	Stenonema	femoratum	0	0	0
29	Ephemeroptera	Ephemerellidae	gen.	sp.	0	0	150.0
30	Ephemeroptera	Ephemerellidae	Ephemerella	sp.	0	0	0
31	Ephemeroptera	Ephemerellidae	Ephemerella	aurivilli	0	0	0
32	Ephemeroptera	Ephemerellidae	Ephemerella	subvaria	0	8.4	33.3
33	Ephemeroptera	Ephemerellidae	Eurylophella	sp.	0	0.4	0
34	Ephemeroptera	Ephemerellidae	Eurylophella	funeralis	0	0	0
35	Ephemeroptera	Ephemerellidae	Eurylophella	temporalis	0	0	0
36	Ephemeroptera	Tricorythidae	Tricorythodes	allectus	0	0	0
37	Ephemeroptera	Leptophlebiidae	gen.	sp.	1.4	1.4	16.7
38	Ephemeroptera	Leptophlebiidae	Habrophlebia	vibrans	0	0	0
39	Ephemeroptera	Leptophlebiidae	Leptophlebia	sp.	0	0.4	0
40	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	adoptiva	0	0	60.0
41	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	debilis	0	0	0
42	Ephemeroptera	Siphlonuridae	Ameletus	sp.	0	0	0

	STB01	BRI01	PEY01	BCB02	REN01	BEA01	WAT01	LSR01	LSR02	TNB03	BBA02
1	4.0	0	0	0	0	0	0	0.2	0	0	0
2	0	0	0	0	20.0	0	0	0.2	0	0	0
3	0	1.8	0	1.5	17.5	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0.4	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
8	0	0	428.0	0	17.5	2.0	153.3	0	0	0	0
9	12.0	14.5	108.0	3.1	60.0	14.0	46.7	0.6	0.2	0	0
10	32.0	3.6	16.0	0	2.5	10.0	6.7	0.2	0.8	2.0	0
11	20.0	5.5	0	1.5	50.0	0	0	1.0	0	8.0	1.7
12	0	0	0	0	2.5	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0
14	0	21.8	20.0	0	0	4.0	146.7	0.8	0	0	0
15	32.0	10.9	8.0	9.2	5.0	18.0	266.7	4.8	0.2	18.0	9.2
16	0	0	0	1.5	0	2.0	0	0.6	0.2	0	0
17	0	0	12.0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0.8	0	0	0
19	8.0	16.4	156.0	16.9	0	90.0	146.7	43.2	4.4	42.0	3.3
20	0	0	0	0	0	0	0	1.4	0	0	0.8
21	8.0	81.8	4.0	30.8	62.5	4.0	33.3	4.8	14.0	150.0	75.0
22	0	0	20.0	0	0	0	6.7	6.2	0.4	54.0	15.0
23	0	0	0	0	0	0	0	0	0	0	0
24	0	9.1	0	0	0	0	0	1.8	0	0	0
25	0	0	0	63.1	0	0	0	5.8	0.4	0	17.5
26	0	0	0	0	0	0	0	0	0	0	0.8
27	0	0	0	3.1	0	10.0	0	0.4	0	0	8.3
28	0	0	0	0	0	0	0	0	0	0	0
29	36.0	10.9	0	0	0	4.0	0	0	0.2	18.0	0
30	0	0	0	0	0	0	40.0	0	0	0	0
31	0	0	0	0	0	0	93.3	0.2	0	0	0
32	12.0	20.0	8.0	21.5	20.0	4.0	93.3	0.4	0.8	8.0	6.7
33	0	0	68.0	21.5	0	0	0	0	0.2	0	0
34	0	0	0	0	0	0	6.7	0	0	0	0
35	0	0	0	1.5	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0
37	16.0	0	0	0	60.0	0	20.0	1.0	0.2	26.0	0
38	0	0	0	3.1	0	34.0	6.7	0.6	0	0	0
39	8.0	0	0	0	37.5	0	0	2.6	0	0	0
40	0	18.2	12.0	40.0	0	0	0	2.4	0.4	4.0	10.0
41	0	0	0	0	0	0	0	1.0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0

	NBC01	CTB02	SQP02	SWB01	USW01	APB02	MPB01	SWR01	NWR03	PNB01	PNB02
1	0	0	0.4	0	0	0	0	0	0	0.2	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0.7	0	0	0	0
4	0	0	0	0	0.2	0	1.5	0	0	0	0
5	0	5.3	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0.2	0	0	0.7	0	0	0	0
8	0	2.7	0.2	0	0.4	0	0	0	3.2	0	0
9	6.7	13.3	0.4	0.2	0.4	0	0	1.8	0.2	0.6	0
10	1.7	2.7	0.6	0	0	0	0	0.4	0	0	0
11	0	4.0	0.2	0.6	0	5.6	4.4	1.2	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0
14	1.7	0	0.2	0.2	2.6	0	0	0	0	0	0.6
15	3.3	12.0	1.6	3.4	1	4.4	0	2.0	0.2	0.8	1.8
16	0	1.3	0.6	0	0	4.4	0	0	0.4	0.2	0.2
17	0	0	0.4	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0
19	36.7	57.3	2.6	8.2	16.2	31.1	0	7.2	4.6	8.8	13.4
20	0	1.3	0	0	0	0	0	2.0	0	0	0
21	121.7	33.3	77.6	4.2	1.6	82.2	14.1	1.6	1.6	5.4	3.2
22	0	5.3	7.2	12.2	3.4	38.9	40.7	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0.4	0	0	0
25	0	0	0	15.8	0.2	0	9.6	28.2	1.4	0	0
26	0	0	0	0	0	0	0	0.2	0	0	0
27	0	0	0	3.8	2.4	0	3.7	2.0	0	0.4	9.0
28	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	1.2	0	0	0	0.2	0	2.0	3.0
30	0	0	0	0	0	0	0	0	0	0	0
31	0	0	5.2	0	0	6.7	0	0	0	0	0
32	0	2.7	0	5.4	0.8	0	6.7	1.0	0.8	1.2	5.4
33	0	0	0	0	0.2	0	0	0	0.2	1.0	0
34	0	0	0.2	0	0	4.4	0	0	0	0.4	0
35	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0
37	0	16.0	0.2	3.8	5.0	13.3	14.1	0.2	0	0	2.2
38	0	0	0	1.4	1.0	0	0.7	0.4	0	17.8	1.2
39	0	0	0	3.2	1.4	0	0	0	0	4.2	4.0
40	0	0	0.2	6.6	3.0	15.6	3.7	1.6	0.4	0	0
41	0	0	0	0	0	0	0	0	0	0	3.8
42	0	0	0	0	0.2	0	0	0.2	0	0	0

	BBB01	RPB01	TRI01	CBB02	SAB01	NIC01	EEL01	SLB01	DEE01	EEL02	WHB01
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0.2	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	2.2	0
6	0	0	0	0.8	0	0	0	0	0	0	0
7	0.2	0	0	0	0	0	0	0	0	0	0
8	0	0.2	0	0	7.4	0	0	0	0	2.2	0
9	0	0	0.6	0	4.2	0	0	8.9	0.4	3.2	0
10	0	0.4	0	0	2.1	0	0	4.4	0.6	6.5	0
11	0	0.2	0.6	6.9	0	2.2	0	6.7	0	3.2	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	0.2	0	0	0	0	0	0	0	0	0
14	0.2	0	0	0.8	0	0	0	0	0	0	0
15	0.8	1.4	0.4	1.5	4.2	11.1	6.9	6.7	5.6	10.8	3.1
16	0	0	0	1.5	0	0	2.8	0	0.2	4.3	0
17	0	0.2	0	0	2.1	0	1.4	0	0	3.2	0
18	0	0	0	0	0	0	0	0	0	0	0
19	11.0	15.2	1.4	43.1	25.3	53.3	44.4	26.7	2.2	15.1	0
20	0	0	0.2	0	0	0	0	0	0	0	0
21	66.6	60.0	4.4	77.7	53.7	171.1	191.7	188.9	9.8	49.5	470.8
22	2.4	2.4	12.0	1.5	0	2.2	1.4	2.2	6.8	0	13.8
23	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0
25	2.8	4.2	5.4	0	15.8	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	4.6	0	1.5
27	1.4	0	3.2	0	3.2	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0
30	0	0.2	1.0	0	2.1	0	0	0	0	0	0
31	0.2	0	0	1.5	0	11.1	1.4	2.2	1.8	16.1	0
32	1.0	1.6	1.0	0	4.2	0	0	0	3.8	0	0
33	0	0	2.2	1.5	0	0	0	2.2	0	0	0
34	0.4	0	0	0.8	0	0	1.4	0	0	20.4	0
35	0	0	0	0	0	0	1.4	0	0	0	0
36	0	0	0.2	0	0	0	0	0	0	0	0
37	0	0.8	0	0.8	0	2.2	0	44.4	0	5.4	0
38	0	0	0	0	0	0	0	0	0	0	0
39	0.6	0.2	30.2	0	0	0	0	0	0	0	0
40	1.0	4.8	2.2	0	16.8	0	0	0	4.8	0	3.1
41	0	0.2	0	0	0	0	0	0	0	0	0
42	0	0	1.2	0	0	2.2	0	0	0.2	0	0

	WHB02	SEB01	RHP01	WEB01	MIT01	WBO01	SEL01	LOM01	CCB01	MSB01	SHO01
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0.6	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0.2	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0.4	0	2.1	0
9	0	0	0	3.0	0	0	0	0	0	9.4	0
10	0	0	0	0	0	0	0	0	0	2.1	0
11	0	0.2	5.7	1.5	1.0	0	0	0.2	0	3.1	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	5.2	0	0	0	0.2	0	3.1	0
15	0.4	1.8	11.4	0.7	4.0	0.4	0	1.8	1.5	9.4	0.4
16	0	0	0	0	0.8	0	0	0	0	1.0	0
17	0	0	0	0	0.2	0	0.2	0	0	0	0.2
18	0	0	0	0	0	0	0	0	0	0	0
19	0	0.2	0	0	0	0	0	0.4	3.8	53.1	0
20	0.4	0	0	0	0	0	0	0.2	0	0	0.2
21	30.0	27.8	282.9	2.2	130.8	46.4	54.0	16.4	76.2	0	62.6
22	1.0	5.8	77.1	3.7	33.2	1.0	4.2	5.4	18.5	0	1.0
23	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	1.5	0	0
25	0	0	0	0	0	0	0	0	0	0	0
26	3.0	3.6	8.6	0.7	0	0.6	4.8	4.2	0	0	6.2
27	0	0.2	0	0	0	0	0	0.2	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	3.1	0	0
30	0	0	0	0	0	0	0	0.2	0	0	0
31	0	1.4	5.7	0.7	6.4	0	0.4	0.2	7.7	1.0	0.2
32	0	0.2	8.6	8.1	0	0	0	4.0	0	0	0
33	0	0	0	0	0	0	0	0	0.8	2.1	0
34	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0.8	0	0
36	0	0	0	0	0	0	0	0	0	0	0
37	0	0	40.0	0	0	0	0	4.8	1.5	2.1	0
38	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	3.0	0	0	0
40	0	1.0	5.7	44.4	0	0	0	1.8	2.3	0	0
41	0	0	0	0	0	0	0	0	0	3.1	0
42	0.2	0	0	0	0	0	0	0	0.8	0	0.2

	TRT01	FED01	MAN01	FED02	MKB01	TRH01	WAL01	NIC02	BOT02	BOT01	WBT01
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0.7	90.0	0
9	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	3.3	0	0	0
11	0	0	0	0	0.2	1.9	0	1.7	1.4	6.7	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	3.3	0
15	0.4	1.8	1.2	3.2	0.4	1.9	4.2	35.0	15.7	26.7	1.1
16	0.2	0	0	0	0	0	0	1.7	0	0	0
17	0	0	0	0	0	0	4.7	0	0	0	1.1
18	0	0	0	0	0	0	0	0	0	0	0
19	0.6	0	0	0	0.6	5.7	0	20.0	0.7	0	0
20	0	0	0	0.2	0.2	0	0	0	4.3	0	0
21	59.2	89.4	42.0	13.2	39.0	176.2	68.4	173.3	103.6	50.0	103.3
22	0.8	2.0	0	0.6	2.2	21.0	3.2	3.3	2.1	53.3	1.1
23	0	0	0	0	0.4	0	0	0	0	0	0
24	0	0	0	0	0.2	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0
26	0	2.8	0	1.4	4.6	4.8	2.1	0	1.4	0	0
27	0	0	0	0	0	0	0	0	2.1	10.0	0
28	0	0	0	0	0	0	0	0	0	0	0
29	1.2	0	0	0	0	0	0	0	1.4	13.3	0
30	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0.2	1.9	0	1.7	0.7	0	1.1
32	0.4	0	0	0	0	0	0	0	0	6.7	0
33	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	1.0	0	0	0	153.3	2.2
38	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0
40	0	0.2	0	0	1.0	0	0	0	8.6	10.0	0
41	0.2	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0.5	0	0	0	0

	BAB01	BRW01	PPB01	MBB01	SRO01	GGB01	NNB01
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0
8	0.2	0	0	0	0	3.3	0
9	0.2	0	0	0	6.0	5.0	2.9
10	0	0	0	1.0	0	1.7	5.7
11	0	0	0	0	0.2	0.8	0
12	0	0	0	0	0.4	0	0
13	0	0	0	0	0.2	0	0
14	0	0	0	0	0	0	0
15	1.6	15.0	75.0	6.6	2.4	9.2	14.3
16	0.2	0	0	0	0	0	0
17	0	0	0	4.4	8.4	0	0
18	0	0	0	0	0	0	0
19	0	0	115.0	0.4	0	0	2.9
20	0	0	0	0	0	0	0
21	9.8	620.0	45.0	23.2	37.4	9.2	2.9
22	0.2	15.0	0	0.6	2.2	0	2.9
23	0	0	0	0	0	0	0
24	0	0	5.0	0.2	0.6	1.7	0
25	0	0	0	0	0	0	0
26	5.4	0	0	0	0	0	0
27	0	0	0	0.8	5.6	5.0	0
28	0	0	0	1.6	0	0	0
29	0	0	0	0	0	4.2	0
30	0	0	0	0	0	0	0
31	0.4	0	10.0	0.2	0	0	0
32	0	0	0	0.2	0	0	0
33	0	0	20.0	0	2.0	0.8	11.4
34	0	0	5.0	0	0	0	0
35	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0
37	0.6	0	10.0	0.6	0	0	5.7
38	0	0	0	0	5.0	0	0
39	0	0	5.0	0.2	0	0.8	20.0
40	0.4	5.0	0	0.2	0	0	0
41	0	0	0	0	0	0	0
42	0.2	10.0	0	0.4	1.2	5.8	0

	Order	Family	Genus	Species	VIR01	WFR02	PCB01
43	Odonata	Calopterygidae	Calopteryx	aequabilis	0	0	0
44	Odonata	Gomphidae	Ophiogomphus	colubrinus	0	0	0
45	Plecoptera	fam.	gen.	sp.	0	0.4	3.3
46	Plecoptera	Leuctricidae	Leuctra	ferruginea	0	0	0
47	Plecoptera	Capniidae	gen.	sp.	0	0	0
48	Plecoptera	Capniidae	Allocapnia	minima	0	0	0
49	Plecoptera	Capniidae	Paracapnia	opis	0	4.0	16.7
50	Plecoptera	Perlodidae	gen.	sp.	0	0	0
51	Plecoptera	Perlodidae	Isogenoides	fontalis	0	0	0
52	Plecoptera	Perlodidae	Isoperla	transmarina	0	0.4	16.7
53	Plecoptera	Perlodidae	Alloperla	sp.	0	0.2	0
54	Trichoptera	fam.	gen.	sp.	0	0	0
55	Trichoptera	Hydropsychidae	gen.	sp.	0	0	0
56	Trichoptera	Hydropsychidae	Arctopsyche	lagdogensis	0	0	0
57	Trichoptera	Hydropsychidae	Parapsyche	apicalis	0	0	0
58	Trichoptera	Hydropsychidae	Hydropsyche	sp.	10.0	0	0
59	Trichoptera	Hydropsychidae	Hydropsyche	alternans	0	0	0
60	Trichoptera	Hydropsychidae	Hydropsyche	slossonae	118.6	6.4	10.0
61	Trichoptera	Hydropsychidae	Hydropsyche	sparna	11.4	0.6	10.0
62	Trichoptera	Polycentropodidae	Neureclipsis	sp.	0	0	0
63	Trichoptera	Polycentropodidae	Nyctiophylax	sp.	0	0	0
64	Trichoptera	Polycentropodidae	Polycentropus	sp.	0	0	0
65	Trichoptera	Glossosomatidae	Glossosoma	nigrior	0	0	0
66	Trichoptera	Hydroptilidae	Hydroptila	sp.	0	0	0
67	Trichoptera	Hydroptilidae	Oxyethira	sp.	0	0	0
68	Trichoptera	Leptoceridae	gen.	sp.	0	0	0
69	Trichoptera	Leptoceridae	Ceraclea	sp.	0	0.8	0
70	Trichoptera	Leptoceridae	Mystacides	sepulchralis	0	0	0
71	Trichoptera	Leptoceridae	Oecetis	sp.	0	0	0
72	Trichoptera	Leptoceridae	Triaenodes	sp.	0	0	0
73	Trichoptera	Odontoceridae	Psilotreta	sp.	0	0	0
74	Trichoptera	Bracycentridae	Micrasema	sp.	0	0.4	0
75	Trichoptera	Lepidostomatidae	Lepidostoma	sp.	0	11.0	16.7
76	Trichoptera	Limnephilidae	Apatania	sp.	0	0	0
77	Trichoptera	Limnephilidae	Hydatophylax	argus	0	0	0
78	Trichoptera	Limnephilidae	Limnephilus	sp.	0	0	0
79	Trichoptera	Limnephilidae	Psychoglypha	sp.	0	0	0
80	Trichoptera	Philopotomadae	gen.	sp.	0	0	0
81	Trichoptera	Philopotomadae	Chimarra	sp.	0	0	0
82	Trichoptera	Philopotomadae	Dolophilodes	distinctus	0	0.4	16.7
83	Trichoptera	Philopotomadae	Wormaldia	sp.	0	0	0
84	Trichoptera	Rhyacophilidae	Rhyacophila	sp.	0	0	0
85	Trichoptera	Rhyacophilidae	Rhyacophila	atrata	0	0	0
86	Trichoptera	Rhyacophilidae	Rhyacophila	carolina	0	0	0
87	Trichoptera	Rhyacophilidae	Rhyacophila	fuscula	0	0.4	13.3

	STB01	BRI01	PEY01	BCB02	REN01	BEA01	WAT01	LSR01	LSR02	TNB03	BBA02
43	0	0	0	0	0	0	0	0	0	0	0
44	0	0	12.0	3.1	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0.4	0	4.0	0.8
46	0	0	0	1.5	0	0	0	0.2	0	0	0
47	60.0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	2.0	0	0	0	0	0
49	32.0	7.3	4.0	10.8	0	0	6.7	2.6	2.0	8.0	5.0
50	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	2.0	0
52	0	3.6	0	4.6	0	4.0	0	0.6	0	0	3.3
53	4.0	0	0	0	0	0	0	0	0.2	4.0	0
54	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0
56	0	1.8	0	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0	0	0	0
60	28.0	5.5	4.0	32.3	115.0	14.0	0	1.0	0.4	0	3.3
61	4.0	0	4.0	6.2	17.5	28.0	0	0.2	0.4	10.0	5.8
62	0	0	0	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0
65	20.0	3.6	0	0	0	0	0	0.4	0	6.0	2.5
66	0	0	0	0	0	0	633.3	0.2	0	0	0
67	4.0	9.1	96.0	4.6	0	4.0	126.7	0.8	0.2	16.0	0
68	0	0	0	0	0	0	0	0.2	0	2.0	0
69	0	0	0	1.5	0	0	0	0.2	0	0	0
70	20.0	0	0	0	0	0	0	0.8	0	0	0
71	0	0	4.0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0	0	0	0
74	136.0	1.8	0	3.1	0	0	0	0	0	2.0	2.5
75	48.0	12.7	0	53.8	0	24.0	6.7	12.4	0.6	38.0	30.0
76	0	0	0	0	0	0	0	0.6	0	0	0
77	0	0	0	1.5	0	0	0	1.2	0	2.0	0
78	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	28.0	6.7	0	0	0	0
82	0	36.4	4.0	13.8	0	0	6.7	0.4	0	2.0	0.8
83	0	0	0	0	0	0	0	0	0	0	0
84	4.0	0	0	3.1	0	2.0	33.3	0	0.2	12.0	1.7
85	0	0	0	0	0	0	0	0	0	0	0
86	0	1.8	0	0	0	0	0	0	0	0	0
87	0	1.8	0	1.5	0	0	0	0.4	0	0	2.5

	NBC01	CTB02	SQP02	SWB01	USW01	APB02	MPB01	SWR01	NWR03	PNB01	PNB02
43	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0.6	0	0	0	0.4	0	0	0
45	0	0	0	0	0.2	1.1	0	0	0	1.4	2.2
46	0	0	1.4	0.2	0.2	4.4	3.7	0	0	0	0.2
47	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0
49	1.7	22.7	1.4	3.0	5.6	10.0	3.0	2.0	0.6	1.6	0.4
50	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0.2	0	0
52	1.7	1.3	0	2.8	5.6	1.1	17.8	1.4	0	0	0.2
53	0	1.3	4.6	0	0	8.9	0	0	0	0	0
54	0	0	0	0	0	1.1	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0	0
57	0	0	1.0	0	0	1.1	0	0	0	0	0
58	13.3	0	0	0	0	3.3	0	0	0	0	0
59	0	0	0	0	0	0	0	0	0	0	0
60	16.7	6.7	0.2	2.8	0	1.1	13.3	0	0	0	0
61	58.3	0	2.6	2.8	2.8	4.4	14.8	2.4	0.2	0.4	1.2
62	0	0	0.4	0	0.2	0	2.2	1.0	0.4	0	0
63	0	0	0	0	0	0	0	0.2	0	0	0
64	0	0	0.2	0	0	0	0	0	0	0	0
65	0	0	0	0	0	0	2.2	1.2	0.4	0	0
66	0	0	0	0.4	0	0	0	0	0	0	0
67	0	0	0	0	1.6	0	0	0.4	0	0	0.2
68	1.7	1.3	0	0	0	0	0	0	0.4	0	0
69	0	4.0	0	0.6	0.4	0	0	0.8	0	0	0.6
70	0	0	0	0	0	0	0	0.2	0	1.2	0
71	0	0	0	0	0	0	0	0.2	0	0	0
72	0	0	0	0	0	0	0	0	0.2	0	0
73	0	0	0	0	0	0	0	0	0	0	0
74	0	0	0	0.4	0	0	0.7	0.4	0	0	0
75	15.0	117.3	6.2	40.4	7.4	14.4	31.1	8.2	0.8	1.2	1.4
76	0	0	0	4.4	6.0	0	0.7	0	0	0.2	1.2
77	0	1.3	0	0.2	0.2	1.1	0	0	0	0	0
78	0	0	0	0	0.2	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0.2	0.2
81	5.0	0	0	0	0	0	2.2	0	0.2	0	0
82	0	0	3.0	0	1.0	5.6	19.3	1.0	0	0	0
83	0	0	0	0	0	0	0	0	0	0	0
84	1.7	0	0	0.2	0	5.6	0	0.2	0.2	0	0
85	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	0	0	0
87	0	1.3	0.2	0.4	0.2	1.1	0.7	0.2	0	0	0

	BBB01	RPB01	TRI01	CBB02	SAB01	NIC01	EEL01	SLB01	DEE01	EEL02	WHB01
43	0	0	0.2	0	0	0	0	0	0	0	0
44	0.2	0.4	0.2	0	1.1	0	0	0	0	0	0
45	0	0	0	0	0	11.1	0	6.7	0	0	0
46	0	0	0	2.3	0	22.2	27.8	33.3	0	7.5	0
47	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0
49	3.8	2.6	9.4	3.8	4.2	0	0	28.9	0.8	0	0
50	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0.4	0	1.5
52	0.4	1.0	0.6	0	1.1	0	0	0	2.0	0	0
53	0	0	0	4.6	0	17.8	1.4	6.7	0.2	9.7	3.1
54	0.8	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0.6	0	0
57	0	0	0	0	0	0	1.4	0	0	14.0	0
58	0	0	0	0	0	0	0	0	0	3.2	0
59	0	0.4	0	0	0	0	0	0	0	0	0
60	2.2	1.4	0.4	0	1.1	11.1	0	0	3.6	0	0
61	0.6	15.2	0.6	2.3	6.3	4.4	0	8.9	2.6	0	0
62	0	0	0.2	0	0	0	0	0	0	1.1	0
63	0	0	0	0	0	0	0	0	0	1.1	0
64	0	0	0	0	0	0	0	0	0	4.3	0
65	0	0	0	0	0	26.7	0	0	17.8	0	0
66	0	0.2	0	0	1.1	0	0	0	0	0	0
67	1.2	0	0.4	0.8	2.1	0	0	2.2	0	0	0
68	0	0.6	0	0	0	0	4.2	2.2	0	0	0
69	0.2	0.2	0	0	0	0	0	0	0	0	0
70	0	0	0.2	0	0	0	0	0	0	0	0
71	0	0	0	0	1.1	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	2.2	0	0	0
74	0.2	1.0	0	0	0	0	0	0	0	1.1	0
75	13.6	14.6	7.6	23.1	40.0	8.9	1.4	6.7	23.0	26.9	0
76	0	0	9.2	0	0	0	0	0	0	0	0
77	0	0	0.2	0	0	0	0	2.2	0	2.2	0
78	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	2.2	6.9	0	0	0	0
81	0	0.4	0.2	0	6.3	0	0	0	0	0	0
82	0	8.8	0	0	13.7	0	5.6	0	1.0	1.1	0
83	0	0	0	0	0	0	18.1	8.9	0	0	0
84	0.6	0	0	3.1	0	15.6	6.9	44.4	0.4	18.3	0
85	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	0	1.1	0
87	0	0.2	0	0	0	0	0	0	0.4	0	0

	WHB02	SEB01	RHP01	WEB01	MIT01	WBO01	SEL01	LOM01	CCB01	MSB01	SHO01
43	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0.2	0	0	0
45	0.6	0	5.7	0	0	0	0.2	0.6	1.5	9.4	0
46	0	0	20.0	0.7	3.0	0.4	0	0	3.1	0	0.8
47	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0
49	0	5.8	5.7	0.7	0.4	0	0	1.8	4.6	34.4	0
50	0	0	0	0	0	0	0	0.8	0	0	0
51	0	0.4	0	0	0	0.4	0.2	0.6	0.8	0	0
52	0	0	17.1	2.2	0	0	0	0	0	0	0
53	1.0	4.4	0	0	1.2	0.4	0.2	0	9.2	1.0	1.4
54	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0.6	0	0	0
57	0	0	0	0	2.8	0	0	0	0	0	0.2
58	0	0	5.7	0	0	0	0.4	0.6	0	0	0
59	0	0	0	6.7	0	0	0	0.4	0	0	0
60	0	1.8	8.6	16.3	1.0	0	0.4	4.8	0	0	0
61	0.8	0.8	34.3	15.6	0.2	0	2	1.4	2.3	0	0.8
62	0	0	2.9	4.4	0	0	0	0	0	3.1	0
63	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0
65	0	1.2	5.7	0	2.6	0	0	0.4	0	0	0
66	0	0	0	13.3	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0	0.8	1.0	0
68	0	0	0	0	0	0	0	0	0	0	0
69	0	0	0	0.7	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0	0	0	0
74	0	0	0	3.7	0	0	0	0	0	0	0
75	0.2	3.6	82.9	8.1	12.8	0	0.4	5.8	16.9	4.2	0.4
76	0	0	0	0	0	0	0	0	0	0	0
77	0	0	0	0	0	0	0	0	1.5	0	0
78	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	1.0	0
80	0	0	0	0	0	0	0	0.8	0	0	0
81	0	0	0	0	0	0	0	1.6	0	0	0
82	0.4	0.4	37.1	0	0	0.4	0	8.0	0	0	0.2
83	0	0	0	0	1.8	0	0.2	0	1.5	0	0.2
84	0.2	0	0	0	8.6	1.2	0.4	0.2	1.5	2.1	0.8
85	0.4	0	0	0	0	0	0	0	0	0	0
86	0	0.4	5.7	0.7	4.0	0	0	0	0	0	0
87	0.2	0.4	0	0	0	0	0.2	0	0	0	0

	TRT01	FED01	MAN01	FED02	MKB01	TRH01	WAL01	NIC02	BOT02	BOT01	WBT01
43	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	20.0	0
45	0	1.6	0.4	2.2	0.2	0	4.7	46.7	0	0	0
46	0	0	0	0	0	0	1.6	8.3	0	3.3	16.7
47	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0
49	0.4	0	0	0	0.4	8.6	0	1.7	0	3.3	0
50	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0.2	0	0	0	0	0	0
52	0	0	0	0	0	0	0.5	0	0	0	0
53	1.0	1.8	0.2	1.0	4.6	8.6	0	18.3	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0.2	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0	0
58	0	0	0.6	0	0	0	3.7	0	0	26.7	2.2
59	0	0	0	0	0	0	0	0	0	0	0
60	0	0	2.4	0	1.8	1.0	0	8.3	0	0	10.0
61	0	0.2	0	0.6	0.2	0	0	0	10.7	0	1.1
62	0	0	0	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0	0	0	0	0
65	0	0	0	0.4	0.2	0	8.9	16.7	6.4	23.3	0
66	0	0	0.2	0	0	0	0	0	0	0	38.9
67	0	0	0	0	0	0	0	0	0	3.3	53.3
68	0	0	0	0	0	0	0	1.7	0	6.7	0
69	0	0	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0	0	0	0	0
74	0	0	0	0	0	1.0	0	0	0	0	0
75	1.0	0.6	0.6	0.2	4.2	23.8	1.1	1.7	5.0	0	0
76	0	0	0	0	0	0	0	0	0	0	0
77	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0.2	0	0	0	0	0	0
82	0	0.2	0.2	0	0.2	0	0	1.7	2.1	0	5.6
83	0	0	0	0	0	0	0	0	0	0	0
84	0.2	0.4	0.6	0	0.6	0	7.9	13.3	0	10.0	25.6
85	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	1.7	0	0	3.3
87	0	0	0	0	0.2	0	0	0	0	3.3	0

	BAB01	BRW01	PPB01	MBB01	SRO01	GGB01	NNB01
43	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0
45	0.2	0	0	0.2	0	0	0
46	0	5.0	5.0	0.4	0	0	0
47	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0
49	4.2	15.0	5.0	0.4	7.0	2.5	5.7
50	0	0	0	0	0	0	0
51	0.2	0	0	0	0	0	0
52	0	0	0	2.4	0	1.7	2.9
53	0.4	0	0	0.2	0.8	0	0
54	0	0	0	0	0.2	0	8.6
55	0.2	0	0	0	0	0	0
56	0	5.0	0	0.2	0.2	0.8	0
57	0	5.0	0	0	0	0	0
58	0	5.0	0	0	0	0	0
59	0.2	0	0	0	0	0	0
60	1.6	0	0	0	1.4	0	0
61	2.2	0	0	1.2	3.8	0.8	0
62	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0
64	0	0	0	0	0	0	0
65	4.8	0	5.0	0	0	0	0
66	0.2	0	0	0	0.4	0	2.9
67	0	5.0	340.0	4.8	0.2	5.0	240.0
68	0	0	0	0	0	0	0
69	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0
71	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0
73	0	0	0	0	0	0	0
74	0.8	0	5.0	0.6	0	3.3	0
75	8.8	40.0	5.0	6.2	13.2	14.2	0
76	0	0	0	0	0	0	0
77	0	0	0	0	0.6	0	2.9
78	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0
82	3.4	0	0	0	0.4	2.5	8.6
83	0	0	0	0	0	0	0
84	0	60.0	0	0.2	0.6	0	0
85	0	0	0	0	0.4	0	0
86	0.4	15.0	0	0	0	0	0
87	0	0	0	0.6	0.4	0	0

	Order	Family	Genus	Species	VIR01	WFR02	PCB01
88	Trichoptera	Rhyacophilidae	Rhyacophila	ignorata	0	0	0
89	Trichoptera	Rhyacophilidae	Rhyacophila	melita	0	0	0
90	Trichoptera	Rhyacophilidae	Rhyacophila	minora	0	0	0
91	Trichoptera	Rhyacophilidae	Rhyacophila	torva	0	0	0
92	Trichoptera	Rhyacophilidae	Rhyacophila	vibox	0	0	0
93	Lepidoptera	Pryalidae	gen.	sp.	0	0	0
94	Lepidoptera	Tortricidae	Archips	sp.	0	0	0
95	Diptera	fam.	gen.	sp.	0	0.2	0
96	Diptera	Tipulidae	gen.	sp.	0	0	0
97	Diptera	Tipulidae	Antocha	sp.	4.3	1.0	6.7
98	Diptera	Tipulidae	Dicranota	sp.	0	0	0
99	Diptera	Tipulidae	Hexatoma	sp.	0	0.2	0
100	Diptera	Tipulidae	Limnophila	sp.	0	0	0
101	Diptera	Tipulidae	Limonia	sp.	0	0	0
102	Diptera	Tipulidae	Tipula	sp.	0	0.2	0
103	Diptera	Psychodidae	gen.	sp.	0	0	0
104	Diptera	Psychodidae	Pericoma	sp.	0	0	0
105	Diptera	Dixidae	Dixa	sp.	0	0	0
106	Diptera	Simulidae	Prosimulium	sp.	0	0	0
107	Diptera	Simulidae	Prosimulium	mixtum	0	0	0
108	Diptera	Simulidae	Simulium	sp.	1.4	0	0
109	Diptera	Simulidae	Simulium	silvestre	0	0	0
110	Diptera	Simulidae	Simulium	tuberosum	0	0	0
111	Diptera	Simulidae	Simulium	v/v	0	0	0
112	Diptera	Ceratopogonidae	gen.	sp.	0	0	0
113	Diptera	Chironomidae	gen.	sp.	84.3	34.2	200.0
114	Diptera	Nymphomyiidae	Nymphomyia	walkeri	0	0	0
115	Diptera	Empididae	Hemerodromia	sp.	12.9	2.4	10.0
116	Diptera	Ephydridae	Discocerina	sp.	0	0	0
117	Diptera	Muscidae	Limnophora	sp.	0	0	0
118	Coleoptera	fam.	gen.	sp.	0	0	0
119	Coleoptera	Dytiscidae	Liodessus	affinis	0	0	0
120	Coleoptera	Hydrophilidae	gen.	sp.	0	0	0
121	Coleoptera	Hydrophilidae	Laccobius	agilis	0	0	0
122	Coleoptera	Elmidae	gen.	sp.	0	0	0
123	Coleoptera	Elmidae	Optioservus	sp.	0	0	0
124	Coleoptera	Elmidae	Oulimnius	latiusculus	0	0.8	36.7
125	Coleoptera	Elmidae	Promoresia	tardella	0	1.0	3.3
126	Coleoptera	Elmidae	Stenelmis	crenata	0	0	0
127	Coleoptera	Curculionidae	gen.	sp.	0	0	0
			Total invertebrates examined		332	545	308

	STB01	BRI01	PEY01	BCB02	REN01	BEA01	WAT01	LSR01	LSR02	TNB03	BBA02
88	0	0	0	0	0	0	6.7	0	0	0	0
89	0	0	0	0	0	0	0	0	0	0	0
90	0	0	0	1.5	0	0	0	0.2	0	14.0	0.8
91	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0.2	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0
95	0	0	4.0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	0
97	0	1.8	0	7.7	20.0	4.0	0	0	0	0	0
98	0	0	0	0	0	0	6.7	0	0	0	0
99	4.0	0	0	0	0	0	0	0	0	0	0.8
100	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	2.5	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	1.5	0	16.0	0	0	0	4.0	0
108	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	286.7	0	0	0	1.7
112	4.0	0	0	0	0	2.0	0	0	0	2.0	0
113	492.0	214.5	408.0	104.6	240.0	238.0	973.3	7.8	1.8	54.0	29.2
114	0	1.8	0	0	0	0	0	0	0	0	0
115	4.0	12.7	4.0	0	17.5	14.0	46.7	0	0.2	0	2.5
116	0	0	0	0	0	0	0	0	0	0	0
117	0	1.8	0	0	0	0	0	0	0	0	0
118	0	0	0	0	0	0	0	0	0	0	0
119	0	0	0	0	0	0	0	0.2	0	0	0
120	0	0	0	0	0	0	0	0	0	0	0
121	0	0	0	0	0	0	0	0	0	0	0
122	0	0	0	0	0	0	0	0	0	0	0
123	0	0	0	0	0	0	0	0	0	0	0
124	108.0	30.9	0	0	0	0	0	6.8	0	90.0	17.5
125	100.0	16.4	0	1.5	0	48.0	166.7	0.2	0.2	12.0	1.7
126	0	0	0	0	0	2.0	0	0	0	0	0
127	0	0	0	0	0	0	0	0	0	0	0
tot.	315	319	351	310	307	313	506	597	143	307	313

	NBC01	CTB02	SQP02	SWB01	USW01	APB02	MPB01	SWR01	NWR03	PNB01	PNB02
88	0	0	0	0	0	0	0	0	0	0	0
89	1.7	0	0	0.2	0	0	0	0	0	0	0
90	0	0	1.6	0.2	0.2	13.3	0	0.2	0	0	0
91	0	0	0.4	0	0.2	0	0.7	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0.2
95	0	0	1.0	0	0	0	0	0	0	0	0
96	0	0	0	0	0.2	0	0	0	0	0	0
97	18.3	0	0	0.2	0	0	0	0.4	0.2	0.2	0
98	0	0	0.2	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0.2	0
100	0	2.7	0.6	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0
104	0	1.3	1.0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0.4	0.2	0.2	1.1	0	0	0	0	0
108	0	0	0	0	0	0	0	0	0.2	0	0
109	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0.2	0	0	0	0.2	0	0	0
111	50.0	0	0	0	1.2	0	0	0	0	0.2	0.4
112	0	1.3	0	0	0	0	0	0.2	0	0	0
113	148.3	92.0	14.4	7.6	5.0	23.3	19.3	18.4	6.8	4.0	12.8
114	0	0	0	0	0	1.1	0	0	0	0	0
115	25.0	0	0	0.6	0.4	0	0	0.4	0.4	0.6	3.2
116	0	0	0	0	0	0	0	0	0	0	0
117	0	0	0	0	0	0	0	0	0	0	0
118	0	0	0	0	0	0	0	0	0	0.2	0
119	0	0	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	0	0
121	0	0	0	0	0	0	0	0	0	0	0
122	0	0	0	0	0	0	0	0	0	0	0
123	0	0	0	0	0	0	0	2.4	0	0	0
124	0	13.3	12.4	2.0	0.4	22.2	1.5	0.2	0	0.4	3.8
125	25.0	0	0.8	0	0.6	1.1	0	0	0	0	0.2
126	0	1.3	0	0.2	0.2	0	0	0.2	0.2	0.2	0.6
127	0	0	0	0	0	0	0	0	0	0.4	0
tot.	333	320	759	705	401	300	316	467	122	278	384

	BBB01	RPB01	TRI01	CBB02	SAB01	NIC01	EEL01	SLB01	DEE01	EEL02	WHB01
88	0	0	0	0	0	8.9	1.4	2.2	0	1.1	1.5
89	0	0	0	0	0	0	0	0	0	0	0
90	2.6	0.2	0	6.9	0	0	0	8.9	0	0	0
91	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0.2	0	0	0	0	0	0	1.1	0
96	0	0	0	0	0	0	0	0	0	0	0
97	0	0.8	0.4	0	1.1	0	0	0	0.6	0	0
98	0	0	0	0	0	0	0	0	0	8.6	0
99	0	0	0.2	0	0	0	0	0	0.6	0	0
100	0	0	0	0	0	0	0	0	0	1.1	0
101	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	1.4	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	1.4	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0	2.2	0	2.2	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0.2	0	0	0	0	0	0	0	0
111	0	0	0	0	1.1	0	0	0	0	0	0
112	0	0	0	0	0	0	0	6.7	0.4	1.1	0
113	5.4	21.2	6.6	13.1	84.2	53.3	22.2	177.8	9.2	48.4	3.1
114	0.2	0	0	0.8	0	0	29.2	8.9	0.4	0	0
115	0.2	1.0	0.2	0	4.2	0	8.3	2.2	0.8	0	0
116	0	0	0	0	0	0	0	0	0	0	0
117	0	0	0	0	0	0	0	0	0	0	0
118	0	0	0	0	0	0	0	0	0	0	0
119	0	0	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	0	0
121	0	0	0	0	0	0	0	0	0	0	0
122	0	0	0	0	0	0	0	0	0	0	0
123	0	0	0	0	0	0	0	0	0	0	0
124	1.6	1.0	2.0	27.7	0	73.3	63.9	80.0	2.8	31.2	0
125	0.2	0.8	0	2.3	1.1	162.2	1.4	8.9	0.8	0	0
126	0	0	0	0	0	0	0	0	0	0	0
127	0	0	0	0	0	0	0	0	0	0	0
tot.	613	821	528	298	295	304	328	335	546	303	326

	WHB02	SEB01	RHP01	WEB01	MIT01	WBO01	SEL01	LOM01	CCB01	MSB01	SHO01
88	0	0	0	0	1.2	0.2	0	0	5.4	0	0.2
89	0.2	0	0	0	0	0	0.2	0.2	0	0	0
90	0	0.2	2.9	0.7	3.2	0.2	0	0.2	0	0	0
91	0.4	0.4	0	0	0	0	0	0.2	0	0	0.2
92	0	0	0	0.7	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0.2	0	0	0	0	0	0
95	0	0	0	0	0.2	0	0	0	0	2.1	0
96	0	0	0	0	0	0	0	0	0	0	0
97	0	0	2.9	9.6	0	0	0	0.8	0	0	0
98	0	0	0	0	1.0	0	0.2	0	3.8	0	0
99	0	0	5.7	0	0	0	0	0.2	0.8	0	0
100	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0	0	0	0	0
102	0	0	0	0	0	0	0	0	0	0	0
103	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0
106	0	0	0	0	0	0	0	0	0	0	0
107	0	1.8	0	0	0	0	0	0	22.3	19.8	0
108	0	0	0	0	0	0	0	0	0	5.2	0
109	0	0	0	0	0.6	0	0	0	0	0	0
110	0	0.2	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0	4.2	0	0	0
112	0	0	2.9	0	0	0	0	1.2	0	4.2	0.2
113	2.0	15.2	157.1	59.3	16.2	0.4	4.6	25.8	21.5	127.1	0.8
114	0	0	0	0	0	0	0	0	3.8	0	0
115	0	0	2.9	0.7	0.6	0	0	0.6	0	10.4	0.2
116	0	0	0	0	0	0	0	0	0	0	0
117	0	0	0	0	0	0	0	0	0	0	0
118	0	0	0	0	0	0	0	0	0	0	0
119	0	0	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	1.0	0
121	0	0	0	0	0	0	0	0	0	1.0	0
122	0	0	0	0	0	0	0	0	0	0	0
123	0.2	0	0	0	0	0	0	7.2	0	0	0
124	0	0.4	28.6	4.4	0.2	0	0	0.6	7.7	0	0
125	0	2.6	0	0.7	0	0	0	0.2	1.5	8.3	0
126	0	0	0	0	0	0	0	0	0	0	0
127	0	0	0	0	0	0	0	0	0	0	0
tot.	208	411	308	297	1191	260	366	571	298	314	387

	TRT01	FED01	MAN01	FED02	MKB01	TRH01	WAL01	NIC02	BOT02	BOT01	WBT01
88	0.2	0	0	0	0	0	0	3.3	0	0	1.1
89	0	0	0	0	0.2	0	0	0	0	0	1.1
90	0	0	0.2	0.2	0	0	2.1	1.7	0	0	1.1
91	0	0.2	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0	0	0.7	0	0
93	0	0	0	0	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0.7	0	0
96	0	0	0	0	0	0	0	3.3	0	0	0
97	0	0	0	0	0	0	0	0	0.7	0	0
98	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0.2	0.2	0	0.5	5.0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0
101	0	0	0	0	0	0	1.1	0	0	3.3	0
102	0	0	0	0	0	0	0	0	0	0	1.1
103	0	0	0	0	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0	0	0
106	0.2	0	0	0	0	0	0	0	0	0	0
107	0	0	0	0	0.2	0	0	6.7	0	0	0
108	0	0	0	0	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0	0	0
111	0	0	0	0	0.2	0	0	0	0	0	0
112	0	0	0	0	0	0	6.3	1.7	10.0	10	0
113	0.8	1.6	2.4	0.6	1.4	19.0	20.0	31.7	27.1	296.7	34.4
114	0	0	0	0	0	0	0	0	0	0	0
115	0	0	0	0	0	2.9	1.1	6.7	0	20.0	0
116	0	0.2	0	0	0	0	0	0	0	0	0
117	0	0	0	0	0	0	0	0	0	0	0
118	0	0	0	0	0	0	0	0	0	0	0
119	0	0	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0	0	0
121	0	0	0	0	0	0	0	0	0	0	0
122	0	0	0	0	0	0	0	0	0	0	5.6
123	0	0.2	0	0	0	0	0	0	7.1	120.0	0
124	1.4	0	0.2	0.4	0.4	1.0	17.4	65.0	0	20.0	20.0
125	0.2	0	0	0	0	1.0	0	21.7	5.0	6.7	0
126	0	0	0	0	0	0	0	0	0	6.7	0
127	0	0	0	0	0	0	0	0	0	0	0
tot.	342	516	256	122	324	295	304	304	306	302	298

	BAB01	BRW01	PPB01	MBB01	SRO01	GGB01	NNB01
88	0	5.0	0	0	0	0	0
89	0.4	0	0	0.6	0	0.8	0
90	0.2	0	0	0	0.4	0	0
91	0	0	5.0	0	0	0	2.9
92	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0
97	0.2	0	0	0	0	0	0
98	0	0	0	0	0	0	0
99	0.4	0	0	0	0	0	0
100	0	0	0	0	0	0	0
101	0	0	0	0	0	0	0
102	0	0	0	0.6	0	0	0
103	0	0	0	0	0	0	0
104	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0
106	0	0	0	0	0	0	5.7
107	0	20.0	25.0	4.4	0.2	0	0
108	0	0	0	0	0	0	0
109	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0
111	0	0	0	0	0	0	0
112	0.2	0	0	0	0	0	0
113	9.4	840.0	890.0	121.8	91.2	157.5	468.6
114	0	0	0	0.2	0	0	0
115	0.6	40.0	5.0	3.0	3.4	14.2	0
116	0	0	0	0	0	0	0
117	0	0	0	0	0	0	0
118	0	0	0	0	0	0	0
119	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0
121	0	0	0	0	0	0	0
122	0	0	0	0	0	0	0
123	0	0	0	0	0	0	0
124	0.8	0	0	0	0	0	0
125	0	10.0	85.0	13.8	3.4	2.5	62.9
126	0	0	0	0	0	0	0
127	0	0	0	0	0	0	0
tot.	295	347	333	1012	999	304	308