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Applying the Reference Condition Approach to bioassessment of Cape Breton Island streams

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APPLYING THE REFERENCE CONDITION APPROACH TO BIOASSESSMENT
OF CAPE BRETON ISLAND STREAMS

(Thesis format: Monograph)

By

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Graduate Program in Biology with Environment & Sustainability

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

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ABSTRACT AND KEYWORDS

The Reference Condition Approach (RCA) to bioassessment assesses the effect of human activity on ecosystems relative to sites that are relatively unexposed to such activity. This study uses the RCA to characterize the nature of relatively pristine streams on Cape Breton Island, Nova Scotia as a comparison to assess sites that have various degrees and types of exposure to human activities, including sites that have been remediated from acid mine drainage related to coal mining.

RCA models consisted of general linear models with environmental characteristics of reference sites as the predictor variables and biotic indices as the response variables. Findings suggest that the diverse natural environments of the island correlate poorly with stream biota (macroinvertebrates and fishes). Further research should be completed to enhance these predictive models. Results also show that healthy biological communities can be re-established at remediated sites but it may take a few years for full recovery. This baseline biological data will be used to track the progression of remediation programs in Cape Breton.

Keywords: Reference Condition Approach, bioassessment, benthic macroinvertebrates, fishes, remediation

DEDICATION

I dedicate this thesis to Bear.
Thank you for motivating me to be the best role model that I can be.

ACKNOWLEDGEMENTS

Finally I would like to thank my entire family for their support throughout the entire process. I need to especially thank my parents for constantly 'walking the walk'. Their ability to start a project and work on it continually to ensure success is absolutely inspiring. They will always be my biggest role models.

Lastly I would like to thank my husband, Dave for his patience and encouragement in all that I do. Without his support this thesis would not have been possible. What other husband would be supportive of their wife leaving for the field the day after their wedding! I can't possible thank you enough.

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LIST OF ABBREVIATIONS

ACAP	Atlantic Coastal Action Program
ACR	Atlantic Coastal Plain Region
AMD	Acid mine drainage
AUSRIVAS	Australian River Assessment System
BACI	Before-After, Control-Impact
BMI	Benthic macroinvertebrates
CABIN	Canadian Aquatic Biomonitoring Network
CB	Cape Breton
CBHNP	Cape Breton Highlands National Park
CYR	Canyon Region
D	Simpson's Diversity
DEM	Digital Elevation Model
ED	Simpson's Equitability
EPT	Ephemeroptera, Plecoptera, Trichoptera
GME	Geospatial Modeling Environment
HAG	Human Activity Gradient
HLR	Highland Region
LLR	Lowlands Region
MFR	Mountain Flank Region
P / F	Pass / Fail
PCA	Principal Component Analysis
RCA	Reference Condition Approach

INTRODUCTION

Bioassessment of Freshwater Ecosystems

Bioassessment is an evaluation of the condition of a freshwater ecosystem based on the composition of the biological communities living within the ecosystem (Barbour et al. 1999). The amount and type of human activities in a watershed has been demonstrated to affect the composition of aquatic communities (e.g. Allan 2004, Richards et al. 1996) thus bioassessment evaluates the impact of human activities on these ecosystems.

Many different approaches to bioassessment have evolved over the last century (Figure 1, see Bailey et al. 2004 for full history of bioassessment). Using biological indicators to assess pollution began in Europe in the early twentieth century with the Saprobic System (Kolkwitz and Marsson 1909). This system focused on water oxygen levels associated with the presence of plankton and periphyton. Soon after, bioassessments began to include benthic macroinvertebrates (BMI) in their assessments, leading to the development of biotic indices and scoring systems in the mid-1900s. These systems set expectations of 'scores' that a stream would receive if it were a healthy ecosystem. This method assumed that healthy ecosystems were consistent in biota and environmental features when in reality there was variation in the characteristics of healthy ecosystems. There was a need to develop more site-specific approaches to bioassessment. By the 1970s, the most commonly used method of bioassessment was the BACI (Before-After, Control-Impact) design developed by Green (1979). This design consisted of a comparison of biota upstream (control site) and downstream (impact site) of a point source of pollution, before and after the onset of the point source. Although
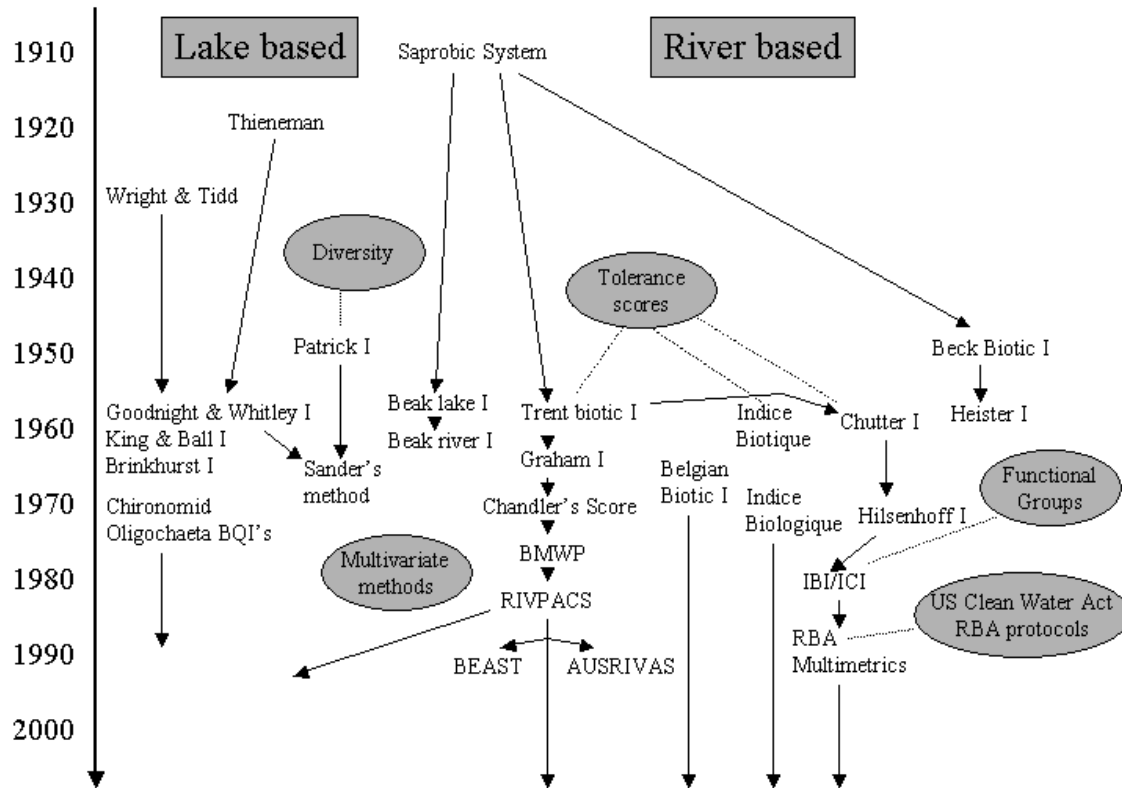
Figure 1. The evolution of bioassessment of freshwater ecosystems in the 20th century. Reprinted with permission from Bailey et al. 2004.

(Saprobic System: Kolkwitz and Marsson 1909; Thienemann Lake Classification: Thienemann 1925; Wright and Tidd: Wright 1955; Patrick I: Patrick, 1949; Beak Indices: Beak, T.W. 1965.; King and Ball I.: King and Ball 1964; Brinkhurst I: Brinkhurst 1966; Oligochaete and Chironomid BQIs: Milbrink 1973, Wiederholm 1980, Howmiller and Scott 1977; Beck Biotic I: Terrell and Perfetti 1996; Trent Biotic Index: Woodiwiss, 1964; Chandlers Score: Cook 1976; BMWP: ISO 1979: RIVPACS: Wright et al. 1984 ; BEAST: Reynoldson et al. 1995; AUSRIVAS: Davies 2000; Belgian Biotic I.: De Pauw et al 1979; Indice Biotique: Tuffery and Verneaux 1968; Indice Biologique: Verneaux et al 1982; Chutter I: Chutter 1972; Hilsenhoff I. : Hilsenhoff 1977;   IBI: Karr 1981; RBA and Multimetrics: Plafkin et al. 1989).

this design set site-specific expectations, it required resources (time and money) that were not available for most research projects. There was a need for rapid bioassessment protocols.

Predictive models relating the natural environment to the biological community were used to develop rapid bioassessments (Barbour et al. 1999). Variation in the natural environment can significantly alter the composition of aquatic communities (e.g. Hynes 1975, Townsend et al. 2003) so the natural environment can be used to predict the biota present. Predictive models are favored in bioassessment because they are relatively inexpensive tools for characterizing the status and trends of biological communities of large regions. Additionally, once developed, the models allow for quick turn-around of results for management decisions (Barbour et al. 1999). One of the most commonly used modeling bioassessment approaches is the Reference Condition Approach (Bailey et al. 2004).

Preferred Biota in Bioassessment

Benthic macroinvertebrates (BMI) are the most commonly used organisms in bioassessment (Bailey et al. 2004, Barbour et al. 1999). There are many benefits of using BMI in bioassessment. First, they are present in nearly all freshwater ecosystems and are relatively easy to sample and identify. Also, they are good indicators of site-specific conditions because they have a relatively sedentary lifestyle compared to fish which may have been exposed to a stressor many kilometres away. Because they live for approximately one to three years, BMI are also beneficial in detecting the cumulative impacts of multiple stressors over a long-time period compared to periphyton which have

very short life cycles. Finally, BMI communities are typically diverse with many families that respond differently to various stressors so provide a useful tool for judging the condition of an ecosystem (Bailey et al. 2004, Barbour et al. 1999). Fish are commonly used in conjunction with BMI in bioassessment because they good indicators of cumulative effects over a longer period of time than BMI (several years). Additionally, fish can provide additional information in regards to human activities occurring at a larger scale. Fish assemblages have been shown to be most strongly associated with human activities at the watershed scale whereas BMI communities are most strongly associated with activities at the reach scale (Yates and Bailey 2010c).

Reference Condition Approach to Bioassessment of Freshwater Ecosystems

The Reference Condition Approach (RCA) has been developed to assess the effect of human activity on aquatic ecosystems relative to sites that are relatively unexposed to such activity (Bailey et al. 2004). In the RCA, "reference" sites are minimally exposed to human activity, whereas "test" sites are or have been exposed to varying amounts and types of activity. Based on the natural environment of reference sites (e.g. geology of the site's catchment area), a model is constructed to predict the biota that should be present at a test site, given its natural environment, if it is in reference condition. The magnitude and nature of the deviation between the biota observed at a test site and that expected if it is in reference condition is a measure of the effect of the human activity on the ecosystem (Bailey et al. 2004).

RCA models have been successfully developed and implemented in Canada and internationally to assess the effects of mining, forestry, agriculture and urban

development on freshwater ecosystems. The first large-scale application of the RCA was developed in Great Britain in the 1980s as a nationwide biological assessment program (Natural Environment Research Council 2013, Wright 2000). Benthic macroinvertebrates from over 800 reference sites were sampled across Great Britain and used to develop a River Invertebrate Prediction and Classification System (RIVPACS) that could predict the reference biological community at other sites with similar natural environments (Wright 2000). Although modified and enhanced through the years, RIVPACS is still widely applied to evaluate the status of freshwater ecosystems in Great Britain.

Although there is no ongoing national program evaluating freshwater ecosystems in Canada, the RCA has been used in regional programs throughout the country. The RCA was first used in Canada in the early 1990's to evaluate sediment contamination in nearshore Great Lakes sites after water chemistry approaches were deemed inadequate or misleading (Reynoldson et al. 1995, Reynoldson et al. 2001). It was again employed in the Fraser River Basin to assess the effects of the growing urban population on freshwater resources (Reynoldson et al. 2000, Bailey et al. 2006). Since the 1990's, RCA has also been used in the Yukon River Basin to assess the impact of placer gold mining effluent on freshwater steams (e.g. Bailey et al. 1998). Data from these disparate studies have been incorporated into the Canadian Aquatic Biomonitoring Network (CABIN) database (Environment Canada 2013). CABIN is an RCA-based biological monitoring program managed by Environment Canada. Although CABIN has not developed a national reference model, it uses specific field collection and laboratory protocols to promote collaboration and data-sharing between different regions (Environment Canada 2013).

In the mid-1990s, a RIVPACS-like model was developed in Australia to standardize methods for assessing rivers on a national scale (Davies 2000). To develop the reference model, over 2000 sites were sampled throughout Australia to assess the variability of sites in reference condition. The variability in environment variables was then used to build a model that could predict the reference communities. The success of the program led to the development of the Australian River Assessment System (AUSRIVAS) where thousands of sites are sampled annually and applied to the reference model to monitor the health of freshwater systems (Davies 2000, Bailey et al. 2004).

Although the RCA has frequently been used for environmental impact assessments and "state of the environment" programs, and ultimately to inform management decisions, it has not previously been used to evaluate either residual negative effects of past exposure to industrial activity or the positive effects of remediation of previously exposed ecosystems.

Natural Variation in Cape Breton Island Streams

Cape Breton Island encompasses a wide diversity of landscapes and waterscapes condensed into a relatively small area (10,000 km^2). It is a mosaic of rolling mountains, glacial valleys, fertile lowlands and rocky shores. Although this natural variation has been characterized from hydrological and geological perspectives, the variation among stream ecosystems has not been captured in a broader scale, RCA study. Some monitoring programs focus on particular environmental impact assessments, but these programs sample chemical parameters (e.g. pH, electrical conductivity and mineralization), whereas benthic macroinvertebrates have only been used in a few of

these assessments. For example, Ogden (2010) assessed the diversity and responses in stoneflies (Plecoptera) to forest harvest practices. There are 30 - 35 Canadian Aquatic Biomonitoring Network (CABIN) streams sites in the Cape Breton Highlands National Park (CBHNP) dating back to 2005 that have sampled benthic macroinvertebrates (BMI). These sites are sampled in order to evaluate and monitor the status of freshwater ecosystems in the Park (Environment Canada 2013, CBHNP 2011). Since 2003, the Atlantic Coastal Action Program (ACAP) has been sampling BMI at sites in industrial Cape Breton, particularly concentrated around Sydney. These 25-30 sites were sampled to monitor human impacts associated with urbanization (e.g. urban runoff, road salt, riparian zone clearing) (Environment Canada 2013, ACAP 2013). Although these data contribute to the national CABIN database, no sampling program has been developed for the entire island. Cape Breton Island is in need of a large-scale bioassessment program to characterize the status and trends of the biotic communities and abiotic environment to evaluate freshwater ecosystem health.

History of Coal Mining & Remediation

Although most freshwater ecosystems on Cape Breton Island presently have relatively low exposure to human activity, some areas have endured centuries of intense industrial activity, including coal mining, steel production, forestry and fishing. The major economic driver on the island was coal mining. The Sydney coalfield is the oldest mined coalfield in North America, with underground mining occurring from the early 1700s to the early 2000s (Kwong et al. 2006, Shea 2009). It is also the largest in Atlantic Canada with over 100 mines over a 60 km stretch of land (Millward 1984). The last

underground coal mine on Cape Breton Island was closed over a decade ago, but the vacant tunnels provide void space for more than 190 million m^3 of acid mine drainage (Shea 2009). Acid mine drainage (AMD) is formed when pyrite (iron sulfide) from exposed coal surfaces reacts with air and water to form sulfuric acid and dissolved iron (Ziemkiewicz 2009). This minewater is acidic so when it leaches into streams it can cause serious harm. Effects of AMD on BMI communities include a reduction in species richness, diversity, and abundance, a relative reduction in intolerant taxa (Ephemeroptera, Plecoptera, and Trichoptera) and a relative increase in tolerant taxa (Chironomidae) (Peplow and Edmonds 2005, Gray and Delaney 2010). Therefore, minewater drainage from former coal mining and processing sites is a significant threat to freshwater ecosystem health (Ziemkiewicz 2009).

Since 2001, various remediation programs have been developed and managed as part of a former mine site closure program responsible for cleaning up over 4450 hectares of industrial sites on Cape Breton Island, including waste rock piles, minewater discharge, sinkholes, soil contamination, pollution of ground water (Public Works and Government Services Canada 2010). Remediation has been completed for several of these former coal mining sites with many others that are currently in various stages of cleanup. Currently, no attempt has been made to assess the effects of this remediation on the biotic community. Therefore, Cape Breton Island presents a unique opportunity to study the effects of former industrial activity in a context with many reference ecosystems in a wide range of geological, hydrological, and climatic conditions. The RCA can assess whether a legacy of past coal mining remains, and evaluate the effectiveness of remediation on the biotic communities.

Research Objectives & Significance

The primary objective of my research was to characterize the variability in the environment and biota of Cape Breton Island streams relatively unexposed to human activity (i.e. reference sites), and then build a preliminary RCA predictive model with these data. To accomplish this goal, a GIS study of the island was completed that characterized the landscape-scale natural environment for all of the watersheds across Cape Breton (CB), as well as the extent of human activity within in each of these watersheds. A boundary that divided all of the watersheds across CB into either "reference" (limited human activity) or "test" (varying degrees and types of human activities) was determined. The relationship between the natural environment and biota at 36 reference sites were then used to create a model that could predict the biota at a test site, given its natural environment, if it were in reference condition. To capture the variability in natural environments around the island, the sampling effort was stratified among six hydrological regions in CB (Baechler and Baechler 2009). My null hypothesis was that variation in stream biota would not correspond to the different hydrological regions. I then built a model to predict the biota at test sites from the properties of the natural environment at reference sites, with the null hypothesis that the benthic invertebrate and fish communities were not predictable from the natural environment of reference streams.

The second objective of my study was to use the predictive models to evaluate the effect of various forms of human activities on the biological communities of freshwater ecosystems. This was accomplished by applying the reference condition models to test sites that had various degrees and types of exposure to human activities (e.g. urban,

agriculture, silviculture). I used the same models to evaluate test sites from MacLeod (2013), where several remediated (from acid mine drainage related to coal mining) streams were sampled.

This research provides the first, albeit preliminary, sampling of a variety of reference stream ecosystems in Cape Breton Island, the development of reference condition models from this sampling, and the application of these models for assessment of test sites. This study, and others building on it, will assist in management decisions for further remediation throughout Cape Breton Island and other areas around the world exposed to similar industrial activity or remediation strategies.

MATERIALS & METHODS

Study Area

This study was conducted on Cape Breton Island (CB), Nova Scotia, Canada. The island has an area of 10,416 km^2 and 11,504 km of stream channel ultimately draining into the Atlantic Ocean (Figure **2**). It is part of the temperate Acadian Forest ecozone (Neily et al. 2003) and the Scotia - Fundy freshwater ecoregion (Abell et al. 2000). Cape Breton has cooler summers and milder winters than the rest of the ecoregion because it is completely surrounded by the Gulf of St. Lawrence and the Atlantic Ocean. CB encompasses a wide range of natural habitats with elevations from sea level to 586 metres. The dominant bedrock geology is clastic and organic or intrusive rock overlaid primarily by ground moraine surficial deposits. The landcover is variable but consists primarily of large expanses of coniferous forests with deciduous sections intermixed. The population of CB is 136,000 people, consisting of multiple small urban areas scattered throughout the island, but concentrated in Cape Breton Regional Municipality (2,433 km^2), which includes Sydney, Glace Bay and surrounding towns (97,400 people) (Statistics Canada 2011). Small-scale farms can be found throughout the island but primarily are found in the lowland regions. Former coal mining sites are concentrated on the southeastern coast of the island.

Cape Breton is composed of six distinct freshwater hydrological regions as defined by Baechler and Baechler (2009) (Figure 3). The regions are defined primarily based on the physical and chemical aspects of water and sediment, the bedrock geology, climate, topographic relief and the primary vegetation:
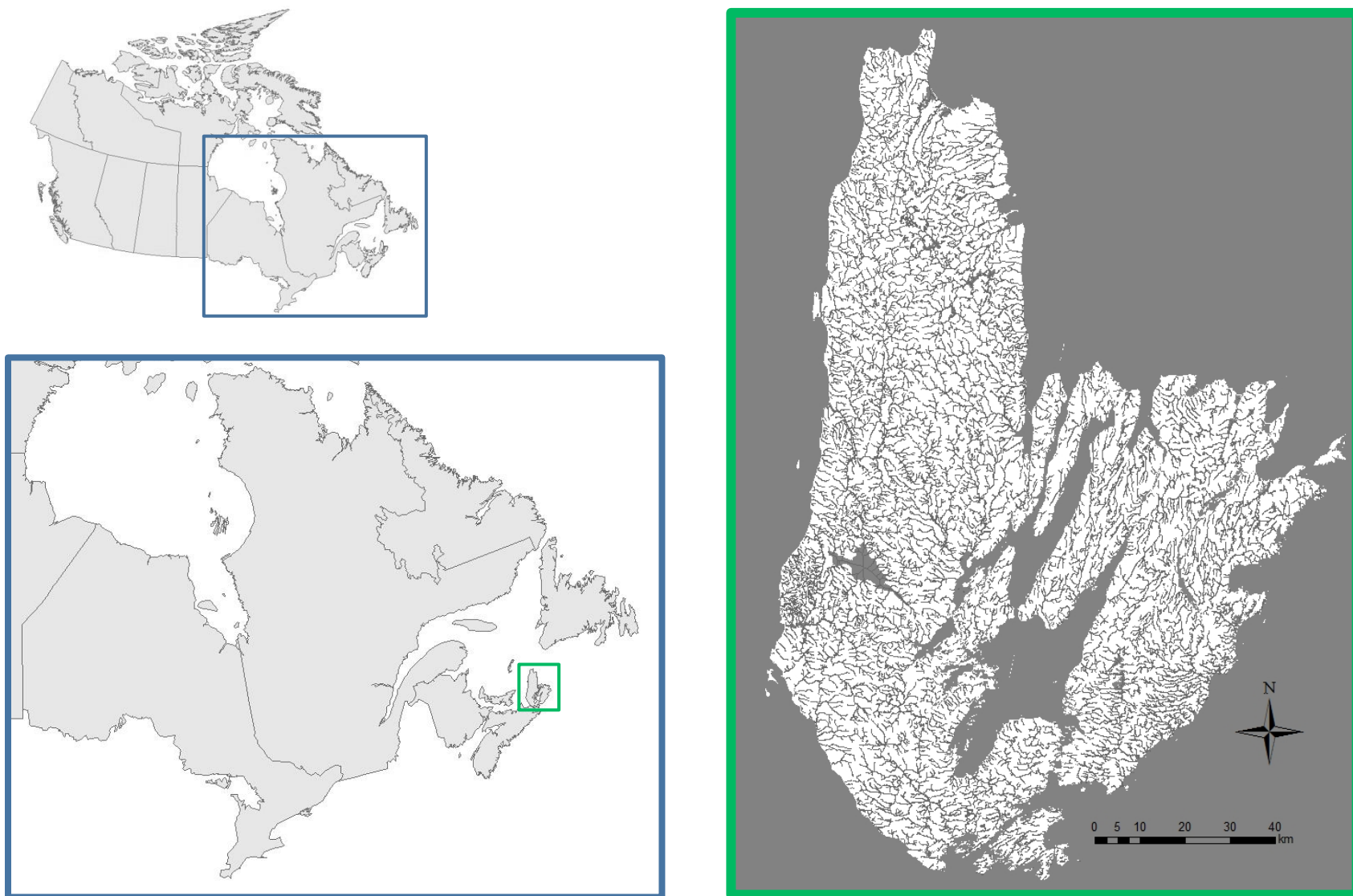
Figure 2. Location of Cape Breton Island in Canada.

1) Highland Region (HLR)

HLR (3,210 km^2) is the largest freshwater region, covering the majority of the northern portion of the island known as the Cape Breton Highlands. HLR also includes isolated patches that extend above the Lowlands. It is underlain primarily by igneous and metamorphic bedrock with only a thin layer of glacial deposits. Vegetative cover is dominated by wetlands and boreal forest.

2) Mountain Flank Region (MFR)

MFR (1,440 km^2) consists of the steep slopes that form the transition between areas of high and low topographic relief. The higher relief areas are composed of exposed igneous and metamorphic bedrock with occasional covering by a thin layer of glacial deposits. The lower relief areas are characterized by sedimentary rock covered with thick glacial tills. Deciduous forests dominate the region.

3) Foothills Region (FHR)

FHR includes moderate elevation regions (1,010 km^2) as the land gradually rises into the Highlands. The region is underlain by conglomerates and sandstones, which are covered by a continuous layer of sandy glacial till. The vegetative cover is primarily mixed coniferous and deciduous forest.

4) Lowlands Region (LLR)

LLR (2,790 km^2) is composed of gently undulating plain underlain by sedimentary bedrock covered by a thick layer of glacial till. Mixed coniferous and deciduous forests dominate the landscape.

5) Atlantic Coastal Plain Region (ACR)

ACR (1,010 km^2) forms most of the southeastern coastline of CB. It is composed of igneous and metamorphic bedrock covered by thick glacial till. Vegetative cover is dominated by wetlands.

6) Canyon Region (CYR)

CYR (940 km^2) is the smallest freshwater region on the island. This region consists of large river systems that are deeply incised into the HLR. The upper parts of the canyons are formed by exposed igneous and metamorphic bedrock, whereas alluvial deposits cover the valley floors. Forest type transitions from boreal at the higher elevations to deciduous as the elevation drops.

Landscape-Scale Data Collection & Analysis

Description of Human Activity

Watersheds across the island were delineated in ArcGIS 10 and ArcHydro 2.0 (ESRI 2013) by using a digital river network and a 30-meter resolution Digital Elevation Model (DEM) (Table **1**). This process generated 6,020 watersheds greater than 1 km^2 which were then intersected with numerous GIS layers describing the human activities occurring within the watershed (e.g. agriculture, urban, silviculture; Table **1**). These intersections provided the area of each type of human activity occurring in each watershed, which were then transformed into relative areas in the watershed. Road density was expressed as the length of road (meters) per hectare of watershed.

Using Primer 6 (Primer-E 2009), a Principal Component Analysis (PCA) was conducted on the correlation matrix of the human activity variables to determine gradients of human activities across CB. This analysis resulted in a single score for each
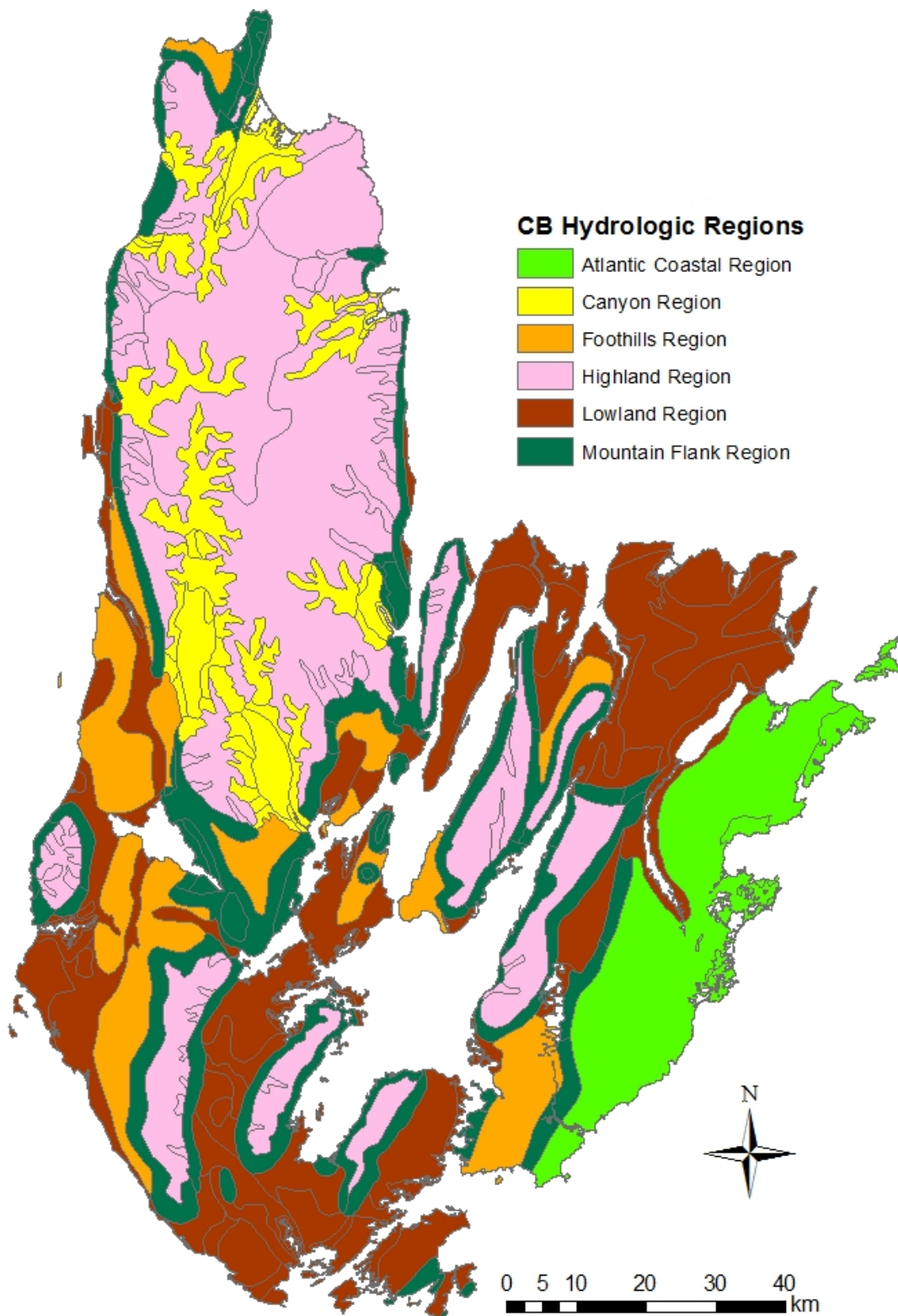
Figure 3. Hydrologic regions of Cape Breton. Modified with permission from Baechler and Baechler 2009.

watershed describing the amount of human activity occurring in that watershed. These scores were used to generate a Human Activity Gradient (HAG) ranging from the watersheds with the most overall human activity to those that are the least disturbed. This HAG was used to create a boundary that separated reference sites (least disturbed) from test sites by maximizing the difference in the median values of the reference sites compared to the median value of the test sites (Yates and Bailey 2010b).

Description of Natural Environment

The delineated watersheds were also intersected with GIS layers describing the landscape-scale natural environment of the watersheds (e.g. surficial geology, climate, vegetation; Table **1**). These intersections provided the area of land in each watershed associated with each of the different environmental characteristics. These values were then converted to the relative proportion of each watershed characterized by the different environmental characteristics. Geospatial Modeling Environment (GME) (Geospatial Modeling Environment 2012) was used to describe the regional climate in each watershed by determining the average annual maximum and minimum temperatures and average annual precipitation occurring in each watershed. GME was also used to calculate the mean, minimum and maximum elevations in each watershed.

A PCA was conducted on the correlation matrix of natural environmental characteristics to efficiently describe the variation in natural environments among the watersheds. Natural environment PC scores were then used in RCA models to predict the biota present at a site if it is in reference condition.

Table 1. Landscape-scale natural environment and human activity descriptors of each watershed.

Layer	Primary Descriptors	Source	Description
Natural Environment			
River Network		Nova Scotia Department of Natural Resources	Vector data of the Nova Scotia river network
Digital Elevation Model	Mean, Minimum and Maximum Elevations	ASTER, NASA	Raster data of the elevation of 30 meter resolution cells
Climate	Total Precipitation, Maximum Temperature, Minimum Temperature	Natural Resources Canada	Annual average temperatures and total precipitation (1971-2001)
Surficial Geology	Alluvial, Colluvial, Glaciolacustrine, Glaciofluvial, Ground Moraine, Organic Deposits, Lakes	Nova Scotia Department of Natural Resources	Proportion of land occupied by various types of surficial geology
Bedrock Geology	Clastic & Organic, Evaporites, Intrusive, Undivided, Volcanic	Nova Scotia Department of Natural Resources	Proportion of land occupied by various types of bedrock geology
Forestry	Coniferous, Deciduous, Mixed, Shrubs, Wetlands, Herbaceous, Grassland	Nova Scotia Department of Natural Resources	Proportion of land occupied by various types of vegetative cover
Human Activity			
Forestry	Silviculture, Clear Cut, Urban	Nova Scotia Department of Natural Resources	Proportion of land occupied by various types of human activities
Land Cover	Developed, Agriculture, Landfill, Miscellaneous Human Activities	Natural Resources Canada	Proportion of land occupied by various types of human activities
Restricted and Limited Use Land	National Parks	Nova Scotia Department of Natural Resources	Proportion of land within Cape Breton Highlands National Park
Road Network	Paved, Unpaved	Natural Resources Canada	Length of road per hectare

Reference Site Selection

The 40 tentative reference sites (Figure 4) for this study were randomly selected by considering accessible sites around the island exposed to limited amounts of human activity. All stream networks were intersected with road crossings on a map of CB to ensure that the sites were accessible. Sampling effort was stratified by hydrological region (Baechler and Baechler 2009) to ensure that reference sites covered a wide range of natural conditions across the island. The amount of human activity in the watershed was preliminarily assessed using Google Earth, and sites with obvious disturbances in the watershed were eliminated.

The 40 tentative reference sites were later scored on the HAG to determine if they were indeed reference sites. Four of these tentative reference sites (ACR-26, LLR-23, MFR-11, MFR-14) fell above the reference boundary so were deemed to be test sites.

Field Data Collection & Analysis

Biota (benthic macroinvertebrates, fish), water chemistry and physical site attributes were measured at each of the 40 tentative reference sites (including the four eliminated sites) between 26 July and 11 August 2011. Benthic macroinvertebrates (BMI) were collected and processed following the standardized CABIN protocol (Environment Canada 2010). BMI were sampled by a 3-minute kick sample with a 500μm net. Samples were taken along a diagonal sweep to cover all available habitats. Captured BMI were preserved in 70% ethanol and transported to the lab for processing. In the lab, samples were washed using a 500μm sieve to remove excess sand and silt. Large woody debris was also inspected and removed from the sample. Washed samples were then evenly spread across a gridded pan for sub-sampling. Each cell in the pan was
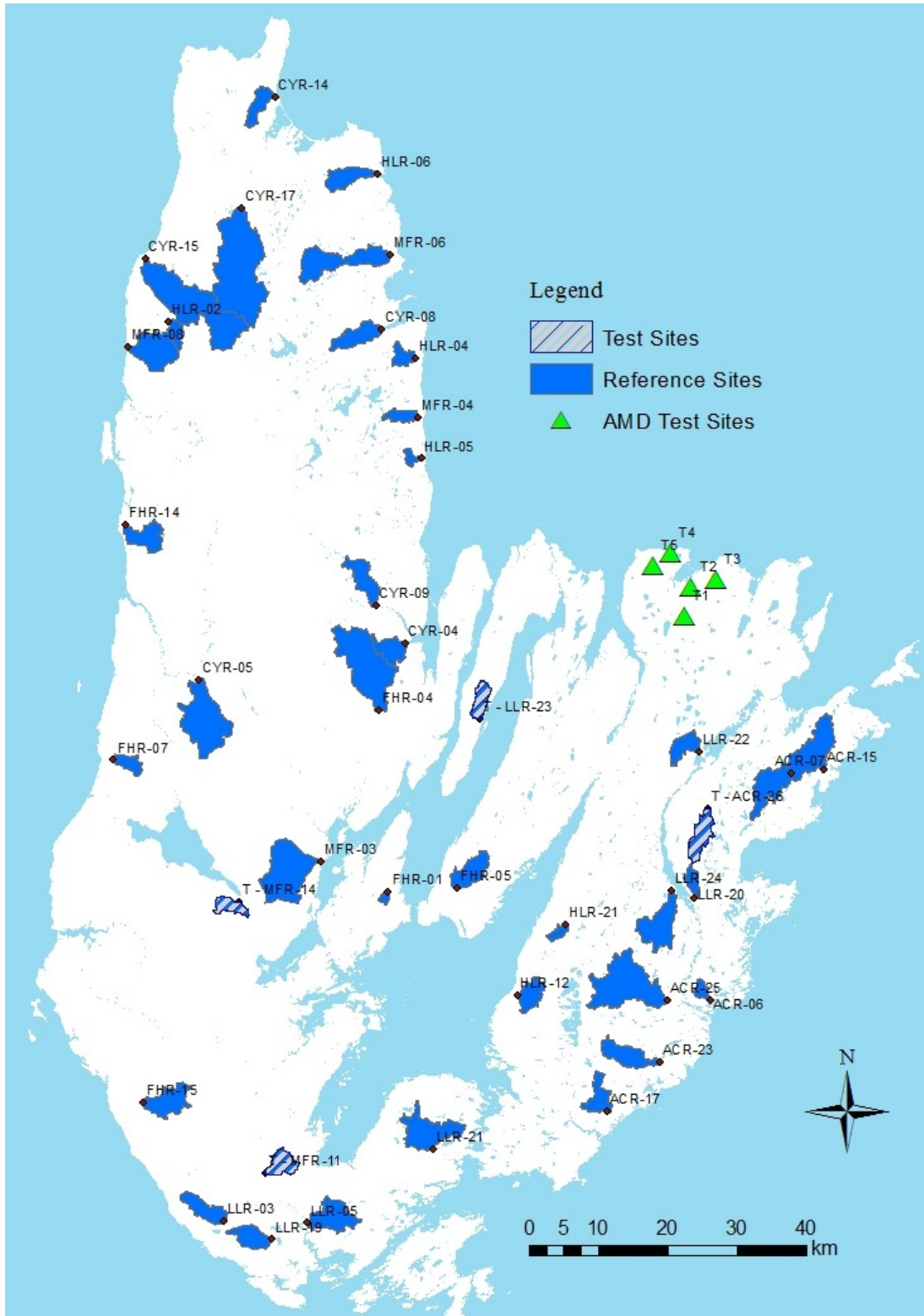
Figure 4. Reference (n = 36) and test (n = 4) stream sites sampled on Cape Breton Island. "AMD Test Sites" (n = 5) were sites sampled by MacLeod (2013).

numbered from 1 to 60 (10 x 6) and individual cells were randomly selected for sampling. All of the contents from a selected cell were removed and placed under a dissecting microscope for analysis. All BMI were removed from the contents and identified to family level where possible.   Sub-sampling continued until a minimum of 300 individuals was reached. This sub-sampling process was repeated for each sample.

Fish were sampled by using single-pass backpack electrofishing with a Smith-Root Model 12B at a rate of approximately 10 sec/m^2 over a minimum site length of 30 metres for a minimum shocking time of 600 seconds. The sampling was completed in diagonal transects across the stream to ensure that banks and macrohabitats, such as woody debris, were sampled. All collected fishes were identified to species, counted and released.

Habitat data (e.g. stream width, substrate type, riparian vegetation) were also collected at each site (Appendix 1).

Variation in Reference Condition

The variation in the natural environment of reference sites was compared to the realm of natural environments of all watersheds across CB to determine if selecting reference sites by hydrological region accurately captured the environmental variation of CB freshwater ecosystems. This was completed by considering the PC scores of each watershed from the Principal Component Analysis of the natural environmental characteristics. A scatterplot of PC1 scores versus PC2 scores was completed to view the distribution of reference scores within the variation for all watersheds across the island. Boxplots were also created using these scores to compare the range of reference sites

scores to the range of scores for all watersheds in CB to determine the proportion of total variation that was captured by the reference sites.

The variation in biotic communities sampled at reference sites was also analyzed. Biotic indices were calculated to describe the biological communities at each site. BMI communities for each site were described by abundance, richness, Simpson's Diversity, Simpson's Equitability, Ephemeroptera Plecoptera Trichoptera (EPT) richness, percent EPT and percent Chironomidae (Barbour et al. 1999). Fish communities were described by abundance and species richness. Additionally, a Cluster Analysis using the Bray-Curtis distance matrix of BMI reference communities was conducted using Systat 13 (SYSTAT Software, Inc. 2009) to describe variability among reference site biota.

Developing the Reference Models

The RCA predictive model was developed by using general linear models with environmental characteristics of the reference sites as the candidate predictor variables and the biotic indices as the response variables. These data were used to develop predictive models that relate the environmental characteristics of the reference sites to the observed biota (as described by a variety of biotic indices) at each site. The predictors used in the model consisted of the most important principal component scores that resulted from the PCA of the natural environmental characteristics. For each biotic index, the natural environment PC's that best predicted the index were used in the reference condition model. The equations derived from these regressions were used to predict the biotic index at each test site based on their natural environment. If no

significant relationship with the natural environment of any of the five PC's was found

for a biotic index, the best prediction was the mean of that index across all reference sites.

Applying the Reference Models

Based on the environmental characteristics of each test site, the reference

condition models determined a predicted value for each biotic index, which was the value

of the index expected if that site were in reference condition. The distribution of

reference condition model residuals was used to decide if a test site passed or failed for

each biotic index. If the residual value from the test site fell outside of the middle 95% of

the reference model residuals, then the test site failed for that biotic index. When the

natural environment as described with the PC's did not predict the biotic index at

reference sites, the predicted value for a test site was simply the average of all reference

values for each biotic index, and the residual for each reference site was just the

difference between the overall mean and the value for that site. For each of the indices,

test sites could fail by falling too far above or below the predicted value. Thus the Pass /

Fail (P/F) boundaries were placed at 2.5% (lower P/F boundary) and 97.5% (upper P/F

boundary) of the reference site distribution.

Test Site Descriptions

Four sites sampled fell above the reference boundary on the HAG and were

evaluated as test sites using the reference condition model:

1) *ACR-26*

The watershed of ACR-26 is 1528 hectares, with coniferous forest covering the

majority of that area (62%). However, based on the HAG, ACR-26 was in the top 21%

of the sites exposed to the most overall human activity in CB. There are various types of

human activities occurring in the watershed, but mostly in small amounts (e.g. urban,

<2%; agriculture, <5%). The activities that eliminated ACR-26 from reference condition

were the above average amounts of silviculture (13% compared to an average of 5%),

paved roads (2 meters / hectare compared to 0.6 m/ha) and unpaved roads (8 m/ha

compared to a mean of 3 m/ha).

2) LLR-23

Coniferous forest (47%) and deciduous forest (23%) dominate the watershed of LLR-

23 (949 hectares). LLR-23 scored in the upper 15% of watersheds on the HAG because

of the high degree of agriculture, covering 10% of the land compared to the average of

0.8% agricultural land per watershed. Other types of human activities were present in

small amounts in the watershed, including clearcut land (5%) and urban areas (<1%).

3) MFR-11

The watershed of MFR-11 (1144 hectares) is primarily covered by coniferous, mixed

and deciduous forests, amounting to over 90% of the area. This site fell in the upper 28%

of watersheds around the island, just outside of reference condition. No single human

activity in the watershed was significantly above average, but MFR-11 was eliminated

from reference because of the combination of clearcut land, developed land, landfills and

unpaved roads occurring in the watershed.

4) MFR-14

The dominant land cover in the watershed of MFR-14 (830 hectares) is deciduous

forest (51%) and coniferous forest (28%). Small amounts of urban areas (<1%) and

agriculture (<2%) can be found in this watershed, but MFR-14 fell in the upper 21% on

the HAG because of clearcutting. The amount of clearcut land in this watershed (22%) is significantly higher than the mean amount occurring in the other watersheds around CB (3%).

In addition to these four test sites, MacLeod (2013) sampled five sites in industrial Cape Breton (Figure 4) between 28 October and 04 November 2012 with the same CABIN field and lab protocols (Environment Canada 2010) as used in this study, and each of these five test sites were assessed with the reference condition models. Four of these sites were previously exposed to acid mine drainage (AMD) from coal mining, but have been remediated within the last 5 years. These test sites were placed in the reference model to assess the residual effect of mining and the effectiveness of remediation on the biological communities.

1) Veres Brook (receptor for No. 25 Passive Treatment System discharge)

The Gardiner Mine operated intermittently from 1870 until it was abandoned after flooding in 1893. In 1941, the Dominion Coal Company decided to re-work the Gardiner seam, opening No. 25 Colliery until it flooded once more in 1959. By the early 1960s, this colliery had become completely flooded allowing AMD to flow onto private lands. This prompted the construction of the No. 25 Passive Treatment System in 2005 / 2006, which was completed in 2010 (Shea 2012). The sampling for the test site at this location was completed just downstream from where the treatment system drains into Veres Brook.

2) Cadegan's Brook (receptor for Neville Street Passive Treatment System discharge)

The 1B mine pool consists of a series of 10 interconnected mines located under the towns of Dominion, Reserve Mines, and Glace Bay. In 1985, this mine pool flooded

because the last operating mine in the group was closed and the associated mine water pumps were shut off. In anticipation of the overflow of AMD into the environment, a passive treatment system was constructed in 2003 and deemed successfully complete in January of 2009 (Shea 2012). Sampling at this test site occurred just downstream from where the treatment system drains into Cadegan's Brook.

3) *Gracie's Brook (receptor for Lingan remediated waste rock pile discharge)*

The Harbour coal seam was mined at the Lingan Colliery from 1970 to 1992. Like many other mining sites in Cape Breton, waste rock was disposed of on site, leaving piles of waste rock exposed to the elements. Remediation of the rock pile was deemed necessary to control acidic run off from entering nearby freshwater systems. In 2010, the rock pile was capped with soil and vegetation (Parsons and MacDonald 2010). Gracie's Brook runs adjacent to the remediated waste rock pile and receives runoff from the cap. Sampling at this test site occurred just downstream from the remediated waste rock pile.

4) *Irish Brook (receptor for the Scotchtown Summit remediated waste rock pile drainage)*

Waste rock taken from three collieries in Dominion was disposed of in a large waste rock pile at Scotchtown Summit from 1911 to the early 1970s. Between 1949 and 1950, a berm was constructed to direct acidic runoff from the pile toward Irish Brook. This was done in order to direct the AMD from Waterford Lake, which was the potable water supply for nearby towns. In the 1990s, an attempt was made to reclaim the pile but the developed system was shut down because it was not able to handle the higher than expected flow of AMD. In 2010, a high-density polyethylene cap was placed over the pile in attempt to finally control the run off. All drainage from the site was directed to

Irish Brook (Parsons and MacDonald 2010). Remediation activities were announced as complete in July of 2012. The test site in this stream was sampled just downstream from where the remediated waste rock pile drains into Irish Brook.

5) Southwest Brook

This stream was neither exposed to AMD nor remediated, but is exposed to some human activity. This site is just downstream from Cape Breton University, so is subject to runoff from nearby buildings and parking lots.

RESULTS

Development of HAG and Reference Boundary

The PCA of the correlation matrix of human activity resulted in two important principal components that explained over 40% of the total variation in human activity among Cape Breton stream watersheds (Table 2). The first principal component (PC1) accounted for 21% of the total variation, and represented a gradient that was driven by the amount of urban development, silviculture and other miscellaneous human activities. The second principal component (PC2) accounted for nearly 20% of the total variation and was primarily defined by the amount of agriculture, roads and developed land (e.g. urban, industrial, mines), as well as the inverse effect of the amount of area of the watershed found within the Cape Breton Highlands National Park boundary. Because PC2 included a broader range of human activities, it was deemed as a more comprehensive gradient of human activity potentially detrimental to stream ecosystems, and was thus selected as the Human Activity Gradient (HAG). The sites with high amounts of human activities are at one extreme of the HAG, whereas the other extreme consists of sites with little to no such activities.

Using Yates and Bailey (2010b), the boundary between reference and test sites along the HAG was placed at a point where 70% of sites with the least amount of human activity were considered to be in reference condition. This boundary was created by maximizing the difference in the median value of the reference sites to the median value of the test sites, as well as by considering the limited amount of human activity occurring throughout CB. When the 40 sampled reference sites were placed on the HAG, it was found that four of the sites fell outside of reference condition (ACR-26, LLR-23,

MFR-11 and MFR-14), and therefore not used in the RCA predictive modeling.

Description of Natural Environment

The first five PC's from the PCA conducted on the correlation matrix of natural environmental characteristics were used to describe the variation in natural environments among all of the watersheds across CB (Table 3). The first PC was primarily driven by elevation and temperature. PC2 focused on the variation in the proportion of deciduous forest and the composition of surficial geology. The amount of coniferous forest, ocean and exposed land (e.g. river and lake sediments, exposed soils, beaches) explained the majority of variation in PC3. PC4 was driven by the proportion of lakes in the watershed as well as by size of the watershed. PC5 was controlled by the size of watershed and proportion of alluvial deposits.

Variation in Reference Condition

There was significant variation in the landscape-scale natural environments among the reference sites (Table 4). Watershed size ranged from just over 200 hectares to nearly 9000 hectares. Average elevation of a watershed ranged from just over sea level to over 400m above sea level. Figure 5 shows the distribution of reference scores within the variation for all watersheds across the island. The plot demonstrates that reference sites are randomly scattered throughout the distribution of all watersheds, suggesting that the sampled references sites covered a broad range of natural variation around CB. Further, the boxplots (Figure 6) of PC scores compare the range of natural environments of reference sites to the range for all watersheds in CB. The boxplots demonstrate that although the reference sites did not cover the entire spectrum of natural

Table 2. Characteristics of the first two principal components from the Principal Component Analysis of the correlation matrix of human activity within the watershed (n = 6020). Bold font denotes eigenvectors contributing the most variation for each principal component.

Variable	Description	PC1	PC2
Eigenvalue	-	2.76	2.54
%Variation	-	21.2	19.5
Eigenvectors:			
Park Area	Proportion of watershed within Cape Breton Highlands National Park boundaries	-0.154	**-0.341**
Silviculture	Any treated area of forest, including plantation, Christmas trees or sugarbush	**0.543**	0.132
Clear Cut	Any forestry stand that has been completely cut and any residuals make up less than 25% crown closure and with little or no indication of regeneration.	-0.041	0.165
Urban	Any area used primarily as residential and related structures such as streets, sidewalks, parking lots, etc.	**0.395**	0.084
Miscellaneous	Miscellaneous human activities (e.g. old mill site, rifle range, tower site, observation site, quarry, mining activity, airstrips, etc.)	**0.543**	0.132
Landfill	Areas used by municipalities for disposal of garbage by means of burying the material	-0.019	0.031
Corridor	Lands with limited uses due to powerlines, roads or rails.	-0.17	**0.323**
Developed	Developed areas, including buildings and paved surfaces, urban areas, industrial sites, mine structures and farmsteads.	-0.166	**0.327**
Agriculture	Cultivated cropland including annual field crops, vegetables, summer fallow, orchards and vineyards.	-0.183	**0.364**
Paved Roads	Length of paved roads in watershed	-0.22	**0.39**
Unpaved Roads	Length of unpaved roads in watershed	0.215	**0.314**

Table 3. Characteristics of the first five principal components from the Principal Component Analysis of the correlation matrix of natural environmental characteristics (n = 6020). Bold font denotes eigenvectors contributing the most variation for each principal component.

PC	PC1	PC2	PC3	PC4	PC5
Eigenvalues	8.16	3.33	2.35	1.58	1.57
%Variation	20.9	8.5	6.0	4.1	4.0
Eigenvectors:					
Watershed Area	-0.008	-0.060	-0.040	**0.359**	**0.357**
Clastic and Organic	0.210	-0.048	-0.169	-0.065	0.149
Evaporites	0.089	-0.001	-0.089	-0.179	0.195
Intrusive	-0.159	-0.019	0.340	0.132	-0.006
Undivided	-0.205	-0.076	-0.218	-0.047	-0.066
Volcanic	0.081	0.192	0.083	0.067	-0.265
Max Temperature	**0.322**	-0.037	-0.004	-0.023	0.003
Min Temperature	**0.315**	0.096	-0.030	-0.003	-0.049
Total Precipitation	-0.217	0.265	0.068	0.066	-0.037
Alluvial	0.040	-0.160	-0.037	-0.026	**0.368**
Colluvial	-0.045	**-0.402**	-0.007	0.099	-0.122
Glaciofluvial	0.038	-0.072	-0.042	-0.064	0.230
Glaciolacustrine	0.019	-0.042	-0.002	0.015	0.163
Ground Moraine	0.186	**0.346**	-0.106	-0.089	0.054
Lakes	0.040	0.073	-0.060	**0.571**	0.253
Organic	-0.136	0.133	-0.197	-0.063	-0.001
Average Elevation	**-0.327**	-0.102	0.075	0.042	-0.021
Min Elevation	**-0.298**	0.069	0.074	-0.019	-0.009
Max Elevation	**-0.299**	-0.197	0.053	0.085	0.058
Coniferous	0.023	0.330	**0.317**	-0.039	-0.078
Deciduous	0.089	**-0.394**	-0.089	0.107	-0.137
Mixed	0.123	-0.228	0.028	-0.112	0.076
Shrubs	-0.083	0.096	0.126	-0.102	0.100
Dead	-0.188	0.007	-0.266	-0.025	-0.061
Lichens	-0.086	0.083	-0.057	-0.157	0.220
Wetland	-0.216	0.144	-0.191	-0.003	0.034
Herb	-0.039	-0.024	0.202	-0.121	0.265
Grassland	-0.154	0.007	-0.259	-0.059	-0.045
Beaver	0.011	-0.001	0.000	-0.030	0.031
Ocean	-0.087	0.001	**0.328**	-0.146	0.291
Barren Rock	-0.153	0.097	-0.104	0.069	-0.033
Beach	0.078	-0.102	-0.086	-0.029	0.193
Barren	0.013	0.013	-0.008	-0.114	0.080
Rubble	-0.136	0.064	-0.156	-0.140	0.196
Exposed Land	-0.200	0.106	**-0.308**	-0.130	0.141

variation across the island, they did cover a large proportion of the different environmental conditions.

There was also significant variation in the biotic communities observed at the 36 reference sites sampled (Table 5). BMI abundance varied from under 400 individuals to over 5000 individuals, whereas richness ranged from 12 to 23 different families. As few as 3 fishes were sampled at one reference site, whereas there were over 30 fishes collected from another.

Although there was substantial variation in the biotic community among reference sites, this variation was not aligned with different hydrologic regions. Cluster analysis showed a lack of correspondence between groups of sites defined by their similar benthic invertebrate communities and the hydrological regions (Figure 7, Table 6). These clusters were mainly divided by the total abundance of BMI as well as the percent of the sample consisting of either chironomids or Ephemeroptera Plecoptera and Trichoptera (EPT).

Development of Reference Condition Predictive Models

The first five PC's resulting from the PCA were used as candidate predictor variables for each of the biotic indices to determine which PC best predicted the biota for each individual biotic index (Table 7). The only two indices that could be predicted by the natural environment were BMI abundance and EPT percent. PC1 was the only significant predictor for BMI abundance (p=0.028). PC2 was the only significant predictor for EPT percent (p=0.002). For other biotic indices, no relationship with the natural environment as described by the five PC's was found, so the best prediction of these index values at each test site was the mean of the index across all reference sites.

Table 4. Landscape-scale natural environment of reference sites (n = 36).

Category	Sub-Category	Mean	Median	Minimum	Maximum
Area (ha)	Area	2343.4	1607.1	207.7	8896
Bedrock Geology (relative proportion)	Clastic and Organic	0.320	0.162	0.000	1.000
	Evaporites	0.014	0.000	0.000	0.157
	Intrusive	0.353	0.235	0.000	1.000
	Undivided	0.135	0.000	0.000	0.902
	Volcanic	0.178	0.000	0.000	1.000
Climate	Max Annual Average Temperature (°C)	9.4	9.6	7.6	10.2
	Min Annual Average Temperature (°C)	1.2	1.3	-0.2	2.0
	Annual Average Total Precipitation (mm)	1519.3	1522.5	1373.1	1632.1
Surficial Geology (relative proportion)	Alluvial	0.018	0.000	0.000	0.186
	Colluvial	0.106	0.014	0.000	0.602
	Glaciofluvial	0.013	0.000	0.000	0.092
	Glaciolacustrine	0.000	0.000	0.000	0.000
	Ground Moraine	0.527	0.454	0.000	1.000
	Lakes	0.006	0.000	0.000	0.047
	Organic	0.021	0.000	0.000	0.158
Elevation (m)	Average Elevation	191.4	204.1	18.1	435.7
	Min Elevation	30.0	10.5	0.0	372.0
	Max Elevation	295.7	306.5	42.0	564.0
Vegetation (relative proportion)	Coniferous	0.568	0.589	0.275	0.872
	Deciduous	0.115	0.069	0.000	0.519
	Mixed	0.153	0.131	0.000	0.431
	Shrubs	0.003	0.000	0.000	0.049
	Dead	0.024	0.001	0.000	0.223
	Lichens	0.001	0.000	0.000	0.006
	Wetland	0.046	0.023	0.000	0.244
	Herb	0.000	0.000	0.000	0.001
	Grassland	0.005	0.000	0.000	0.082
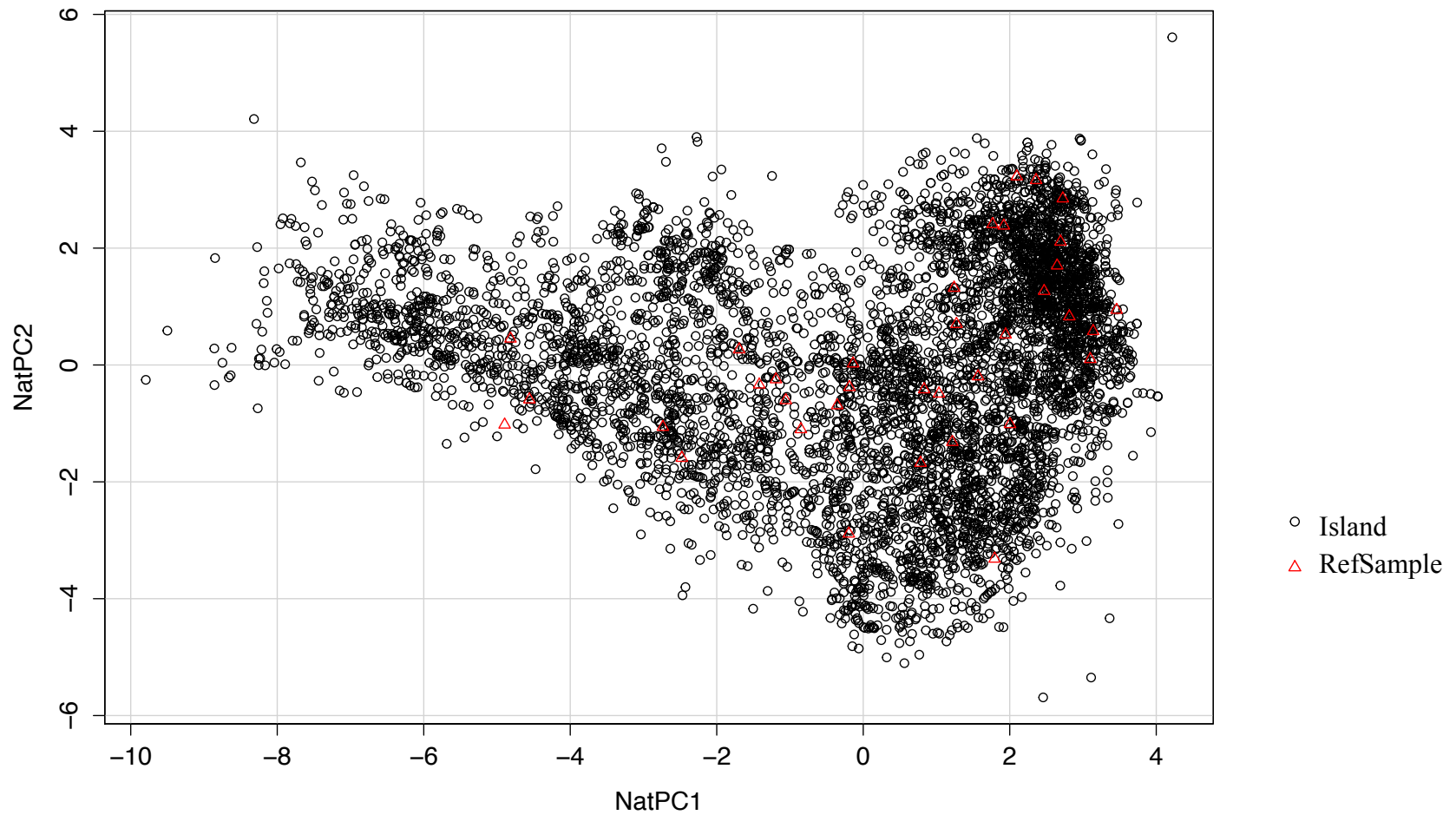
Figure 5. PC1 versus PC2 scores from the Principal Component Analysis of the natural environment variables. Watersheds from across Cape Breton Island (n = 6020) are denoted with a **O**. Reference sites where biota were sampled (n = 36) denoted with a **Δ**.
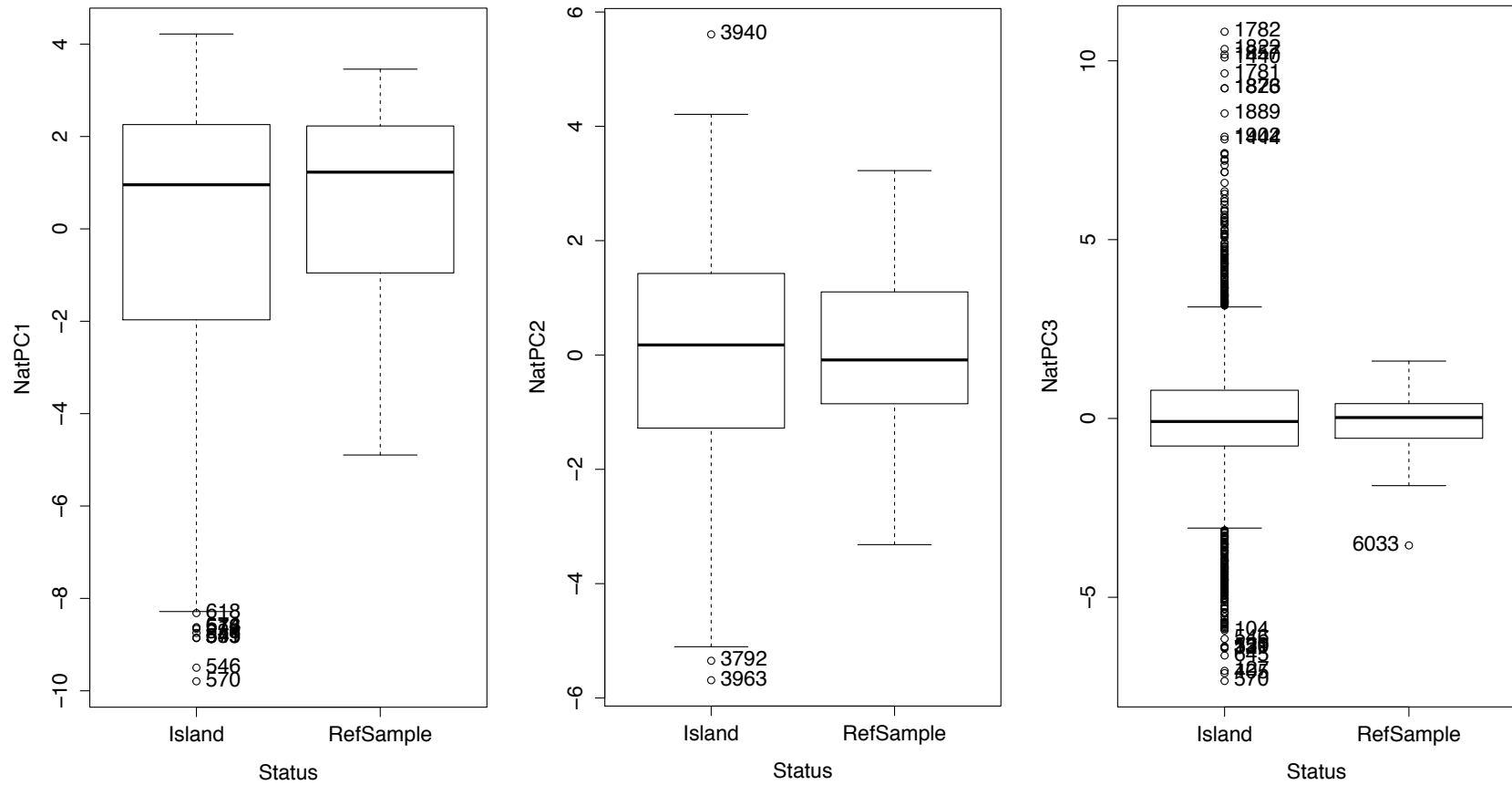
Figure 6. PC scores for the first three PC's from the Principal Component Analysis of the natural environment variables.

Table 5. Biotic indices for benthic macroinvertebrate and fish at reference sites (n = 36).

	Mean	Median	Minimum	Maximum
BMI				
Abundance	1764	1570	390	5040
Richness	18	18	12	23
Chironomids Percent	25.1	21.1	3.4	62.8
EPT Percent	52.6	52.1	18.6	92.1
EPT Richness	10.1	10.0	6	13
Simpson's Diversity	5.10	4.66	1.57	10.4
Simpson's Equitability	0.29	0.28	0.12	0.52
Fish				
Abundance	13.6	11.5	3	34
Species Richness	1.9	2.0	1	4
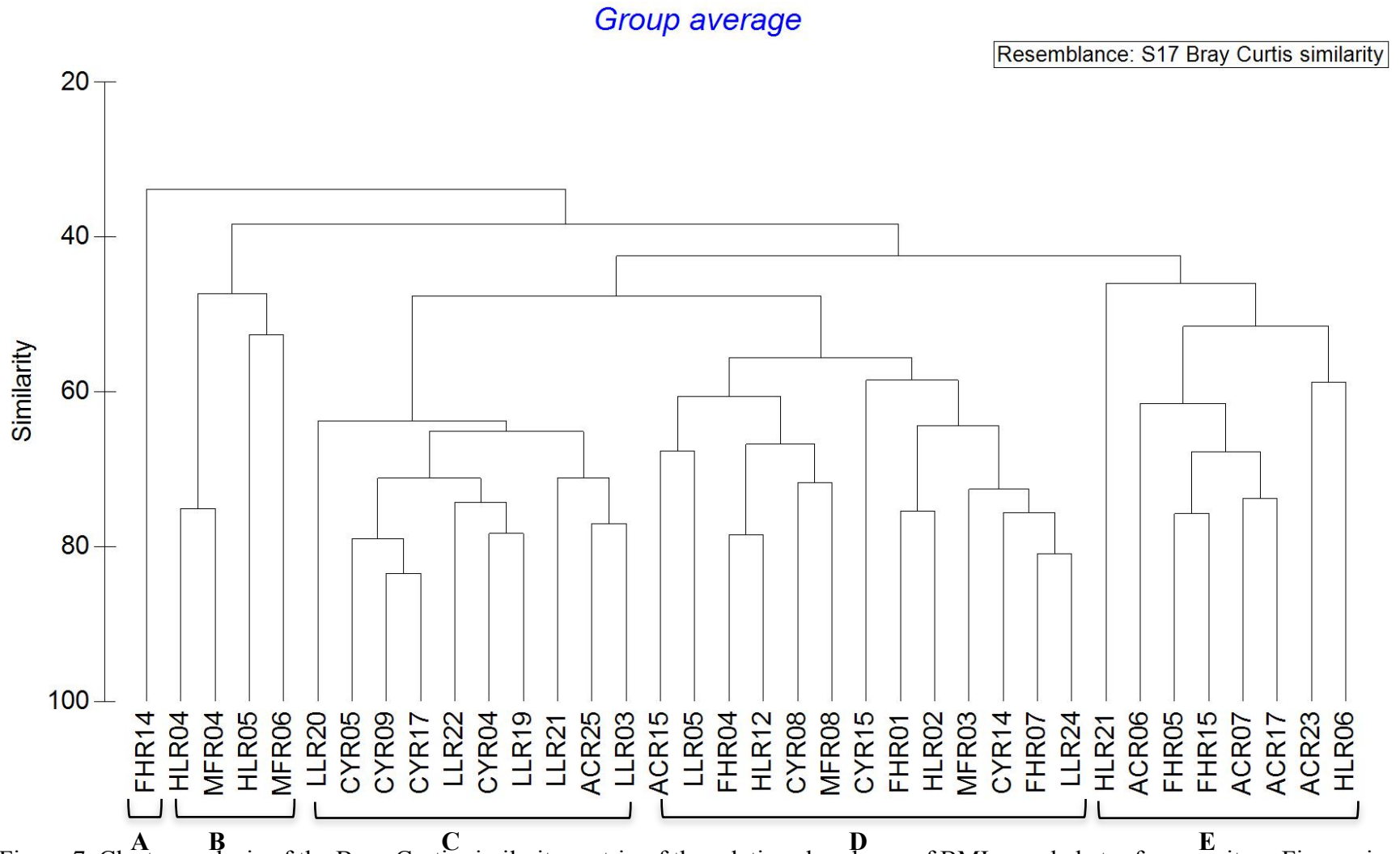
Figure 7. Cluster analysis of the Bray-Curtis similarity matrix of the relative abundance of BMI sampled at reference sites. Five main groups are labeled "A" to "E".

Table 6. Properties of the five groups resulting from cluster analysis of benthic macroinvertebrate communities at reference sites (n = 36).

Cluster	# sites	Properties of Biota	Regions	Sites
A	1	High BMI abundance	FHR	FHR14
B	4	Low BMI abundance & Low chironomids percent	HLR	HLR04, HLR05
			MFR	MFR04, MFR06
C	10	High chironomids percent	ACR	ACR25
			CYR	CYR04, CYR05, CYR09, CYR17
			LLR	LLR03, LLR19, LLR20, LLR21, LLR22
D	13	High EPT percent	ACR	ACR15
			CYR	CYR08, CYR14, CYR15
			FHR	FHR01, FHR04, FHR07
			HLR	HLR02, HLR12
			LLR	LLR05, LLR24
			MFR	MFR03, MFR08
E	8	Moderate EPT percent & High Simpson's equitability	ACR	ACR06, ACR07, ACR17, ACR23
			FHR	FHR05, FHR15
			HLR	HLR06, HLR21

Application of Models to Test Sites

The models for each of the biotic indices were applied to each test site to determine if the test site passed or failed for each biotic index, as well as the direction of failure (Table 8).

There was a significant relationship between BMI abundance and the environment, so residuals were calculated based on the deviation of observed abundance from the abundance predicted from the regression equation. Based on the distribution of reference residuals, the passing range was from -1711 to +3146 individuals. Veres Brook (-1885 individuals), Irish Brook (-1851 individuals), Gracie's Brook (-1837 individuals) and Southwest Brook (-1755 individuals) each failed due to abundances that were significantly lower than expected values. Veres Brook had the largest residual value indicating the greatest magnitude of failure. The remaining five test sites passed for the abundance model because they each had residuals that fell within 95% of the reference site residuals.

Taxon richness of the benthic macroinvertebrates was not correlated with the environment, so the predicted value for each test site was the average richness of all reference sites (18 families). Based on deviation from the mean, passing residuals ranged from -5.4 to +5.4 families. Irish Brook failed due to a residual richness (-5.5 families) that fell below the lower P/F boundary. MFR11 (+6.5 families) and Veres (+7.5 families) also failed, but fell above the upper P/F boundary. The remaining six sites all passed the reference model for richness.

The percent of chironomids (family Chironomidae) was not correlated with the environment, so the predicted value for each test site was the average value of all

Table 7. Multiple regressions of each biotic index with natural environment PC scores as candidate predictors. Predictors were left blank if they did not have a significant relationship (p > 0.05) with the given biotic index. "+/-" denotes a significant (p < 0.05) positive (+) or negative (-) relationship. "Passing range" indicates the range of residuals considered to pass the reference condition assessment.

			Predictors						
	PC1	PC2	PC3	PC4	PC5	p	R^2	Predicted value	Passing Range
BMI									
Abundance	+					0.028	0.134	$1{,}671.5 + 182.7(PC1)$	-1711.4 to 3146.3
Richness								17.5	-5.4 to 5.4
Chironomids Percent								25.1	-21.6 to 37.6
EPT Percent		+				0.002	0.244	$53.6 + 6.4(PC2)$	-33.4 to 34.6
EPT Richness								10.1	-4.0 to 2.8
Simpson's Diversity								5.11	-3.53 to 5.30
Simpson's Equitability								0.29	-0.16 to 0.22
Fish									
Fish Abundance								13.6	-10.5 to 20.3
Fish Richness								1.86	-0.85 to 2.13

Table 8. Residual values for each biotic index at each test site. 'F-' indicates that the site failed by having a value that fell below the reference passing range, whereas 'F+' indicates that the value was above the reference passing range.

Test Site	ACR26	LLR23	MFR11	MFR14	SWB	Irish	Cadegan	Veres	Gracie
BMI									
Abundance	1249.5	-444.7	-87.7	2070.7	**-1754.8 (F-)**	**-1850.9 (F-)**	-728.4	**-1885.4 (F-)**	**-1837.3 (F-)**
Richness	-0.5	1.5	**6.5 (F+)**	0.5	-0.5	**-5.5 (F-)**	0.5	**7.5 (F+)**	1.5
Chironimids Percent	11.0	-4.1	13.3	2.6	-18.3	**40.5 (F+)**	3.1	20.2	-9.5
EPT Percent	-13.7	-2.3	-4.6	-11.5	**45.7 (F+)**	-29.3	-0.5	-27.7	-5.8
EPT Richness	1.9	0.9	1.9	0.9	0.9	**-6.1 (F-)**	-3.1	-1.1	-2.1
Simpson's Diversity	-0.23	2.44	-0.22	1.34	2.47	-2.95	-0.69	-0.94	0.49
Simpson's Equitability	0.00	0.11	-0.09	0.07	0.16	-0.11	-0.04	-0.12	0.00
Fish									
Fish Abundance	**25.4 (F+)**	**24.4 (F+)**	10.4	13.4	N/A	N/A	N/A	N/A	N/A
Fish Richness	1.14	0.14	1.14	0.14	N/A	N/A	N/A	N/A	N/A

reference sites (25% chironomids). Based on deviation from the mean, passing residuals ranged from -22% to +38% chironomids. Irish Brook was the only test site that failed this reference model. The residual for this site fell above the upper P/F boundary with +41% chironomids. The remaining test sites all fell within the 95% boundary with residual values ranging from -18% to +20% chironomids.

EPT (Ephemeroptera Plecoptera Trichoptera) percent had a significant relationship with the natural environment so residual values were calculated based on the difference between observed values and values predicted from the regression equation. Passing residuals ranged from -33% to +35% EPT. Southwest Brook was the only test site to fail for this reference model with a residual value +45.7% placing it above the upper P/F boundary. The remaining eight test sites passed the EPT percent reference model with residuals ranging from -29% to -1%.

EPT richness was not correlated with the environment, so the predicted value for each test site was the average EPT richness of all reference sites (10 EPT families). Based on deviation from the mean, passing residuals ranged from -4 to +3 EPT families. Irish Brook was the only test site to fail this model with a residual of -6 EPT families, thus falling below the lower P/F boundary. The remaining eight test sites fell within the 95% boundary with between -1 and +2 EPT families.

Simpson's Diversity (D) was not correlated with the environment, so the predicted value for each test site was the average diversity of all reference sites (D = 5.5). Based on deviation from the mean, passing residuals ranged from -3.5 to +5.3. Due to the high variation in reference, all test site residuals fell within the 95% boundary thereby passing for this reference model.

Simpson's Equitability (E_D) was not correlated with the environment, so the predicted value for each test site was the average equitability of all reference sites (E_D = 0.29). Test site residuals ranged -0.12 to +0.16 whereas the passing residuals ranged from -0.16 to +0.22 so all test sites passed for this model.

Fishes were only sampled at four of the test sites (ACR26, LLR23, MFR11, MFR14). Fish abundance did not correlate with the natural environment so the predicted value for each test site was the average abundance of all reference sites (14 fishes). Passing residuals ranged from -11 to +20 fishes. LLR23 (+24 fishes) and ACR26 (+25 fishes) both failed this model with abundances over the upper P/F boundary. MFR11 and MFR14 both passed for this reference model with +10 and +13 fishes, respectively.

Fish richness did not correlate with the natural environment so the predicted value for each test site was the average richness of all reference sites (1.9 species). Passing residuals ranged from -0.9 to 2.1 species. Although this is a small range, the test site residuals only ranged from +0.1 to +1.1 species so all four test sites fell passed for this model.

DISCUSSION

This research provides a preliminary reference condition model for Cape Breton Island streams. There are a small number of reference sites included in this model as compared to previous RCA models such as those developed in Great Britain (Wright 2000), Australia (Davies 2000) or Canada (Great Lakes: Reynoldson et al. 1995; Fraser River Basin: Reynoldson et al. 2000; Yukon River Basin: Bailey et al. 1998). However, these previous RCA models have been developed over many years with continuous effort to add to the model. Similarly, research is ongoing in Cape Breton. Additional reference sites will be added to the RCA model over many sampling seasons to enhance the Cape Breton RCA model and increase its effectiveness in assessing test sites.

Overall Variation Captured in Reference

This study characterized over 6000 watersheds in Cape Breton with various elevations, climates, geology compositions and vegetation. The results show very diverse natural environments around the island and relatively little human activity. The natural environments of the 36 reference sites where biota was sampled covered a broad range of the natural variation among watersheds in Cape Breton Island. Previous RCA studies have been implemented in a similar fashion where the first priority of the study is to capture the natural variation of the study area (Davies 2000, Reynoldson et al. 2000, Reynoldson et al. 2001). This ensures a comprehensive reference model that is able to predict the biota of test sites of a range of natural environments. This study is the first effort to characterize the natural variability of freshwater ecosystems in Cape Breton. The next step in the RCA predictive model for CB will be to expand the number of

reference sites to capture a greater proportion of the natural variation and to develop a more precise reference condition model.

To capture the variability in natural environments around the island, the reference sites were stratified based on the six hydrological regions in CB (Baechler and Baechler 2009). The six hydrological regions varied greatly in their natural environments (e.g. bedrock geology, climate, topographic relief and the primary vegetation) so it seemed logical to allocate sampling effort based on these regions to cover the greatest amount of variation. Previous bioassessment programs have successfully used similar regional divisions (e.g. biome, ecoregion) to stratify reference sites (see Hawkins et al. 2000). For example, reference sites were dispersed by ecodistrict in the Great Lakes (Reynoldson et al. 1995, Reynoldson et al. 2000) and the Fraser River biomonitoring program used stream order and ecoregion to allocate their sampling effort (Reynoldson et al. 2001).

Relationship Between Hydrological Region and Biota

Although the sampling design of the study captured substantial variability in the landscape-scale natural environments of reference sites, variation in the benthic macroinvertebrate and fish assemblages was not strongly correlated with this variation. These results contradict the prediction that the sampled biota would correlate with the natural environment associated with the different hydrological regions. Several studies have shown that fish and BMI communities correspond to ecoregion, but many other studies have not seen this pattern (see Hawkins and Norris 2000, Hawkins et al. 2000). There are many reasons that could explain why the biota did not correlate with the natural environment of these regions. First, perhaps the distribution of biological communities is

more highly correlated with the small-scale differences in natural environment rather than the region in which they fall. Corkum (1992b) completed a study designed to test the biome dependency hypothesis, which states that similar assemblages of BMI occur along rivers both within and among drainage basins if the basins occupy the same biome. Corkum concluded that although there were significant differences in biota among biomes, macroinvertebrate composition was more strongly associated with local, site-specific factors like riparian vegetation (Corkum 1992b) or current velocity (Corkum 1992a).

Secondly, the landscape-scale description of the natural environment of these regions could have been too coarse to control the biota in the associated streams. Landscape classifications are frequently used in bioassessment based on the idea that larger landscape features at least partially control the conditions of a site which in turn affect the stream biota. The relationships between site-specific environment features and the resulting biotic communities have been well documented. For example, water velocity and depth (Brooks et al. 2005, Beauger et al. 2006), substrate size (Erman and Erman 1984) and substrate heterogeneity (Beisel et al. 2000) have been shown to affect the biotic communities at a site. However, the relationship between landscape features and the biological community sampled at a site is poorly known (Hawkins et al. 2000). Graça et al. (2004) found that microhabitats (e.g. water temperature, substrate) were far more correlated to BMI communities than the large-scale differences in the watersheds. Richards et al. (1997) found that some catchment features (e.g. surficial geology) influence BMI communities, but that reach-scale features still had a stronger relationship with these communities.

Finally, the correlation of BMI to the different hydrological regions could have been evident at a different taxonomic level. Family level identification has been shown to be adequate for describing BMI communities, but identifications down to genus or species level could provide additional information (Bailey et al. 2001, Lenat and Resh 2001). When testing the correspondence between landscape classifications and BMI communities, Hawkins and Vinson (2000) found that genus-level data produced stronger results than family-level data. Therefore, there may have been a relationship between the hydrological regions and the BMI at a lower taxonomic level.

Prediction of biota from natural environmental conditions

The Reference Condition Approach to bioassessment is sensitized by a strong relationship between the natural environment and biota among reference sites. Fish assemblage composition has been shown to correlate with climate and altitude (Ferreira et al. 2007) as well as watershed size, geology and soils (Hawkes et al. 1986). BMI community composition has been proven to vary with geology and altitude (Chaves et al. 2005) as well as watershed area (Richards et al. 1996) and surficial geology (Richards et al. 1997). However, these environment-biota relationships vary depending on the area in question so must be established in each bioassessment study (Yates and Bailey 2010a).

In Cape Breton, the landscape-scale natural environment did a poor job of predicting biological communities as characterized by the biotic indices used in this study. Of the nine different biotic indices, only BMI abundance and EPT percent had a significant relationship with the natural environment of the watershed. Although

significant, the natural environment still had a poor correlation with the biota (abundance $R^2=0.134$; EPT percent $R^2=0.244$). There are many reasons why the natural environment could have been a poor predictor of biota. First, perhaps the landscape-scale descriptors were too coarse to accurately predict the biota. As already noted, there have been mixed results when studying the relationship between landscape features and the biological community sampled at a site (Hawkins et al. 2000). The diverse natural environments of Cape Breton watersheds may not directly control the site characteristics, thus using smaller-scale environmental features (e.g. water velocity, substrate) may have been more successful.

Additionally, the biotic indices that were used to summarize the biological communities may have masked the relationship between the natural environment and the biota. Yates and Bailey (2010c) found that different methods of describing BMI and fish communities (e.g. different indices, presence/absence, relative abundance) resulted in different conclusions regarding the relationships between the biota and human activities at both watershed and reach-scales. Additionally, there may have been a stronger correlation of biota to the environment at a lower taxonomical level (Hawkins and Vinson 2000, Bailey et al. 2001) or to specific traits of the biota (e.g. tolerance, habitat or feeding preferences; Yates and Bailey 2010a).

Evaluation of Test Sites

The second objective of this study was to use the predictive model to evaluate the effect of various forms of human activities on Cape Breton stream ecosystems. This was accomplished by applying the developed reference models to test sites that had various

degrees of exposure to human activities (e.g. urban, agriculture, silviculture). The four test sites under consideration did not have any obvious reach-scale human activity, but had various amounts of human activities within their watersheds.

ACR-26 and LLR-23 both passed all reference models with the exception of fish abundance due to a higher than expected number of fishes. The majority of both these watersheds are forested, but when scored on the HAG they were classified as test sites primarily because of the amount of silviculture (ACR-26) or clearcut land (LLR-23). These watershed-scale human activities may be more prone to altering the distribution of fishes because fish assemblages have been shown to be most strongly associated with human activities at the watershed scale whereas BMI communities are most strongly associated with activities at the reach scale (Yates and Bailey 2010c).

MFR-11 passed all bioassessment evaluations except for richness because of a higher than expected number of BMI families. Over 90% of this watershed is forested, but it failed to be in reference condition because of the combination of clearcut land, developed land, landfills and unpaved roads occurring in the watershed. The increased biodiversity could be the result of mild organic enrichment from the developed land and landfills, however testing this possibility is beyond the scope of this research.

MFR-14 passed all nine predictive models. Although the majority of the watershed is forested, over 22% of the land is clearcut. Many studies have shown that providing a buffer strip along the riparian zone reduces or eliminates these impacts of clearcutting on the biological communities (Newbold et al. 1980, Davies and Nelson 1994). In Cape Breton, buffer strips are left along all stream boundaries according to

Wildlife Habitat and Watercourses Protection Regulations (Government of Nova Scotia 2011) so it is not surprising that this site passed all reference models.

Overall, the reference models only detected minor deviations of these sites from reference condition. This is in part due to the small Type I error rate (α) of 5% that was selected for this study. The Pass / Fail boundary for reference condition was placed at 95%, meaning that 5% of reference sites would mistakenly fail the reference models. Other studies (e.g. Linke et al. 1999) use a Type I error rate of 25% so that the reference models are more prone to failing test sites.

The reference model was also used to evaluate test sites from MacLeod (2013), where several streams were sampled that were remediated from acid mine drainage related to coal mining. These samples do not have fish data so only the seven BMI predictive models were considered. The results from these predictive models provide insight into the health of the biological communities at these sites; however, it must be considered that the results may be confounded by temporal variation. The reference sites for the predictive models were sampled in the summer (end of July to mid August) of 2011, whereas these five test site samples were collected in autumn (late October to early November) of 2012. BMI communities are known to vary based on the season in which they were sampled (Bailey et al. 2004, Sporka et al. 2006). There is no standard season to sample BMI communities because of various opinions on the best time of year to sample, as well as practical constraints (e.g. student volunteers only available in summer). Therefore, ideally reference models would include data from multiple seasons, over multiple years. Sampling season could then be used as a predictor in this multi-

season reference model for a more accurate prediction of biological communities (Linke et al. 1999). As the CB reference model continues to be expanded, reference sites over different seasons and different years will be incorporated to eliminate these temporal concerns.

Southwest Brook had lower than expected abundance but higher than expected EPT percent. This site was not exposed to AMD or remediation, but is subject to runoff from nearby buildings and parking lots from the Cape Breton University campus.

Irish Brook had the highest degree of failure of all sites, failing four of the seven reference models. Observed values of abundance, richness and EPT richness were lower than predicted, while chironomids percent was higher than expected. These results suggest that the remediation at this site has not been successful but time since remediation must be considered. Remediation activities at Irish Brook were deemed complete in July of 2012, only four months before the BMI community was sampled. Gunn et al. (2010) suggest that it could take over 8 years for a BMI community to recover after remediation from AMD exposure. A longer recovery time must be provided before decisions are made in regards to the success of this remediation attempt.

Cadegan's Brook was the only remediated test site to pass all reference models in this study. Remediation at this site was completed in early 2009, making it the first site in this study to have completed remediation activities. Cadegan's Brook had nearly four years of recovery time before the BMI community was sampled. These results suggest that remediation of the biological communities at this site has been successful.

Remediation activities at both Gracie's Brook and Veres Brook were complete in 2010. Both sites had lower than expected abundance. Veres also had higher than expected richness. These results suggest the BMI communities at both sites are beginning to recover, but that Gracie's Brook is potentially recovering at a faster rate. Both sites should be sampled again to ensure continuing improvement.

CONCLUSIONS & SIGNIFICANCE

This research provides the first reference condition model for Cape Breton streams, as well as baseline data on benthic invertebrate and fish information from relatively unperturbed streams on Cape Breton Island. The predictive model incorporates a broad range of natural environments but requires additional reference sites over multiple sampling seasons for proper comparison of various test sites. Further research should be completed to include characteristics of the natural environment that will have a higher correlation with the biological community.

This study has also provides insight into the effectiveness of various remediation programs for streams exposed to AMD. Healthy biological communities can be re-established at remediated sites but it may take a few years for full recovery. This baseline biological data will be used to track the progression of remediation programs in Cape Breton.

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Appendix 1. Field data collected at each site (n = 40).

Cape Breton RCA Bioassessment Study
FIELD DATA SHEET

	LOCATION DESCRIPTION
SITE CODE _____ ___ _ _____ ___ LAT _____ _____ ___ LONG ___ _____ Elevation _____ GPS Waypoint _____	

Potential Reference ❑ Test ❑   Stream Name _____ Stream Order _____

DATE ddMMyy _____ TIME _____ _ AM PM

WEATHER CONDITIONS	**Now**		**Past 24 hours**	**Has there been a heavy rain in the last 7 days?**
	❑ ❑ ❑ ___%❑ ❑	Storm (heavy rain) Rain (steady rain) Showers (intermittent) % cloud cover Clear / sunny	❑ ❑ ❑ ❑ ___% ❑	❑ Yes ❑ No Air Temperature_____ ⁰ C Other_____

SITE LOCATION DRAWING

PHOTOS

Field sheet _____ _____ U/S _____ D/S _____ Substrate _____

Others

WATER QUALITY   Time _____ _____ Water chemistry collected ❑ Yes ❑ No

Air Temp _____   Water Temp _____   pH _____ Specific Conductance (µS/cm) ____ ___

DO (mg/L)_____ ___ DO % _____   Turbidity (cm) _____   Water Colour ❑ Clear ❑ Stained

OBVIOUS WATERSHED FEATURES

Surrounding Land Use   - Check those present   - Circle dominant land use

❑ Forest ❑ Field/Pasture ❑ Agriculture   ❑ Residential/Urban

❑ Logging ❑ Mining ❑ Commercial/Industrial   ❑ Other ___ ___ _____

REACH DATA (represents 6x bankfull width)	
RIPARIAN VEGETATION (30 meter buffer)	- Check those present - Circle dominant ❑ Deciduous Trees ❑ Coniferous Trees ❑ Shrubs ❑ Ferns / Grasses
INSTREAM FEATURES	**Fish obstructions between site and outflow:** ❑ None ❑ Few ❑ Moderate ❑ Impassable **Dam Present** ❑ Yes ❑ No **Proportion of Reach Represented by Stream Morphology Types** Riffle _____% Run _____% Pool _____%_____Rapids _____% **Canopy Cover** ❑ 0% ❑ 1 – 25% ❑ 26 – 50% ❑ 51 – 75% ❑ 76 – 100% **Macrophyte Coverage** (i.e. not algae) ❑ 0% ❑ 1 – 25% ❑ 26 – 50% ❑ 51 – 75% ❑ 76 – 100% **Periphyton Coverage on Substrate** (benthic algae) ❑ 1 - Rocks not slippery, no obvious colour (thin layer < 0.5 mm thick) ❑ 2 - Rocks slightly slippery, yellow-brown to light green colour (0.5-1 mm thick) ❑ 3 - Rocks have a noticeable slippery feel (footing is slippery), with patches of thicker green to brown algae (1-5 mm thick) ❑ 4 - Rocks are very slippery (algae can be removed with thumbnail), numerous large clumps of green to dark brown algae (5 mm -20 mm thick) ❑ 5 - Rocks mostly obscured by algal mat, extensive green, brown to black algal mass may have long strands (> 20 mm thick)

SUBSTRATE DATA	Substrate Type	Size	% Composition	Substrate Type	Characteristic	% Composition
	Bedrock			Detritus	sticks, wood, coarse plant materials (CPOM)	
	Boulder	> 256 mm				
	Cobble	64-256 mm		Muck-Mud	black, very fine organic (FPOM)	
	Gravel	2-64 mm				
	Sand	0.06-2 mm		Marl	grey, shell fragments	
	Silt	0.004-0.06 mm				
	Clay	< 0.004 mm				

CHANNEL DATA (represents 6x bankfull width)				
WIDTH / DEPTH / VELOCITY (Starting from D/S)	Distance from D/S	Velocity	Wetted width	Depth

BMI SAMPLE

# of jars _____

Comments _____

ELECTROFISHING DATA

Electrofisher Operator:		Netter:	
Settings:	**Voltage:**		**Sampling Time (seconds)**

FISH COLLECTED

Species	Number	Comments

FISH VOUCHERS

Sample #	Number of fish in sample	Comments

Appendix 2. List of benthic macroinvertebrate taxa collected and the number and proportion of the 36 reference sites at which each family was found.

Order	Family	# of Sites Present	% of Sites Present
Amphipoda	Hyalellidae	7	19
Coleoptera	Chrysomelidae	2	6
Coleoptera	Curculionidae	3	8
Coleoptera	Dytiscidae	6	17
Coleoptera	Elmidae	35	97
Coleoptera	Hydraenidae	2	6
Coleoptera	Hydrophilidae	3	8
Coleoptera	Psephenidae	3	8
Diptera	Athericidae	8	22
Diptera	Ceratopagonidae	14	39
Diptera	Chironomidae	36	100
Diptera	Dixidae	0	0
Diptera	Empididae	4	11
Diptera	Simuliidae	32	89
Diptera	Tipulidae	18	50
Ephemeroptera	Baetidae	34	94
Ephemeroptera	Ephemerellidae	36	100
Ephemeroptera	Heptageniidae	34	94
Ephemeroptera	Leptophlebiidae	29	81
Gastropoda	Hydrobiidae	4	11
Gastropoda	Physidae	2	6
Gastropoda	Planorbidae	2	6
Hemiptera	Gerridae	1	3
Hirudinea	Erpobdellidae	1	3
Hydrachnidia		32	89
Isopoda	Asellidae	1	3
Lepidoptera	Pyralidae	1	3
Megaloptera	Corydalidae	7	19
Megaloptera	Sialidae	2	6
Odonata	Aeshnidae	1	3
Odonata	Calopterygidae	1	3
Odonata	Coenagrionidae	1	3
Odonata	Gomphidae	11	31
Oligochaeta		20	56
Pelecypoda	Sphaeriidae	6	17
Plecoptera	Chloroperlidae	26	72
Plecoptera	Leuctridae / Capniidae	33	92
Plecoptera	Nemouridae	10	28
Plecoptera	Perlidae	20	56

Plecoptera	Perlodidae	4	11
Trichoptera	Brachycentridae	5	14
Trichoptera	Glossosomatidae	21	58
Trichoptera	Hydropsychidae	23	64
Trichoptera	Hydroptilidae	6	17
Trichoptera	Lepidostomatidae	9	25
Trichoptera	Leptoceridae	5	14
Trichoptera	Limnephilidae	10	28
Trichoptera	Odontoceridae	4	11
Trichoptera	Philopotamidae	24	67
Trichoptera	Phryganeidae	1	3
Trichoptera	Polycentropodidae	10	28
Trichoptera	Rhyacophilidae	18	50

Appendix 3. List of fish species collected and the total number of individuals captured of each species, as well as the number and proportion of the 36 reference sites at which each species was found

Species	Total Captured	# of Sites Present	% of Sites Present
American Eel	126	21	58
Atlantic Salmon	217	22	61
Brook Trout	269	30	83
Threespine Stickleback	2	2	6
White Sucker	3	2	6

Appendix 4. Animal care protocol approval

CAPE BRETON UNIVERSITY

September 8, 2011

Dr. Robert Bailey
VP Academic
Cape Breton University

Dear Bob:

<u>**Re: ACC protocol ACC1011-09.**</u>

The submission of the DFO Permit 7-000140-46 completes the file for the protocol entitled *"Applying the reference Condition Approach to Bioassessment of Cape Breton Streams"* and the Cape Breton University, Animal Care Committee (ACC) has approved this research project.

ACC approvals are granted for a period of one (1) year. Principal researchers, of ongoing projects, are required to submit an annual renewal/ review form. Any change(s) to the approved protocol must be reviewed and approved by the ACC prior to implementation.

The submission of Annual Review/Renewal forms for Tri-council (NSERC, SSHRC, CIHR) funded research is mandatory to keep your research account active.

Good luck with your research.

Charles A. (Sander) Taylor
Research Officer

C. Kari Everett

P.O. Box 5300, 1250 Grand Lake Road, Sydney, NS Canada B1P 6L2 | WWW.CBU.CA

Appendix 5. Curriculum vitae

KARI A. MORELAND

EDUCATION

M.Sc. *(Biology and Environment & Sustainability)*   2010 – Present
Western University, London, Ontario
Thesis: Applying the Reference Condition Approach to bioassessment of Cape Breton
Island streams

B.Sc. *(Biology, Honours)*   2006 – 2010
Cape Breton University, Sydney, Nova Scotia

Thesis: The intraseasonality of fish assemblages in headwater streams of Southwestern
Ontario

TEACHING EXPERIENCE

Professor, Biology & Chemistry   2012 – Present
School of Language and Liberal Studies, Fanshawe College, London, ON

Certificate of University Teaching and Learning
Teaching Support Centre, Western University, London, ON   2010 – Present

Graduate Student Teaching Assistant
Department of Biology, Western University, London, ON   2010 – 2012

Biology Teacher
Let's Talk Science, Western University, London, ON   2010 – 2012

Guest Lecturer
Department of Biology, Western University, London, ON   2010

SCHOLARSHIPS & AWARDS

Ontario Graduate Scholarship	2011 – 2012
Western University, Department of Biology Teaching Award	2011 – 2012
Western University, Environment & Sustainability Travel Award	2011 – 2012
NSERC Alexander Graham Bell Canadian Graduate Scholarship	2010 – 2011
Western University, Graduate Research Scholarship	2010 – 2011
Western University, Society of Graduate Students Teaching Award	2010 – 2011
James Bayer Memorial Award	2009 – 2011
Cape Breton University Caper Award in Leadership	2008 – 2011
NSERC Undergraduate Student Research Awards	2009 – 2010
Cape Breton University Chancellor's Scholarship	2006 – 2010
Capers Athletic Scholarship	2006 – 2010
Cape Breton University Dean's List	2006 – 2010
Academic All-Canadian	2006 – 2010
Cape Breton University President's Award	2008 – 2009
Cape Breton University Chemistry Book Prize	2008 – 2009

RELATED VOLUNTEER EXPERIENCE

SOGS Sustainability Committee	2010 – 2012
Environment & Sustainability Society	2010 – 2012
Earth Day Colloquium Organizing Committee	2010 – 2012
Field assistant – Ausable Bayfield Conservation Authority	2009 – 2011

PROFESSIONAL EXPERIENCE

Basketball Coach – Fanshawe Falcons
Athletics Department, Fanshawe College, London, ON   2011 – Present

Entrepreneur / Photographer
Moreland Photography, London, ON   2008 – Present

Research Assistant
Department of Biology, Cape Breton University, Sydney, NS   2009

Manager
Utopia U-pick Strawberry & Raspberry Farm, Stilesville, NB   2004 – 2008