State of Aquatic Ecosystems on the Island of Newfoundland

2023

ASSESSMENT OF WADEABLE STREAMS IN NEWFOUNDLAND USING BIOMONITORING DATA FOR 2006-2019

Newfoundland Labrador

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Photo credit: Kyla Brake, WRMD-ECCNL

Executive Summary

This baseline report presents results for the period from 2006 to 2019. Biomonitoring data were collected at 93 sampling sites across the island of Newfoundland: 77 potential reference sites and 16 test sites. These sites are distributed amongst the nine ecoregions of the island and were all sampled using the standardized CABIN protocol. Out of the 77 potential reference sites, three of them consist of long-term reference sites, all of which contain 10 years of data. Sites in Labrador were not used due to differences in habitat.

Samples included in this analysis have been collected by different partners: Newfoundland and Labrador's Department of Environment and Climate Change – Water Resources Management Division, Environment and Climate Change Canada, and Parks Canada (Terra Nova and Gros Morne National Parks).

This report aimed at answering three main questions:

1. **Are benthic communities in reference areas different across ecoregions on the island of Newfoundland?** Our analysis showed some differences in communities by ecoregion with some ecoregions showing greater variability in the relative abundances of EPT and Chironomidae taxa. We observed some differences in dominant taxa between ecoregions when looking at the taxa groups individually. Dominant taxa are Chironomidae (non-biting midges) and Baetidae (small minnow mayfly) for most ecoregions, with the exception of Pisidiidae (peaclam) for the Strait of Belle Isle and Northeastern Newfoundland ecoregions.

2. **Are there changes in benthic communities at three long-term reference sites over time?** Long-term trends were detected in all three long-term biomonitoring reference sites; though Careless Brook and Northeast River showed many more trends than Pinchgut Brook. Results suggest that the benthic community is shifting to one with greater tolerance to organic pollutants. Overall, it is difficult to say which factors may be driving these trends in the ARM results and benthic metrics as the benthic community is influenced by numerous environmental conditions.

3. **Can we detect impacts to biological communities using reference models and baseline metrics? If so, can we relate these impacts to stressors?** We observed that key metrics differed between reference and test sites across the island of Newfoundland. Test sites generally had numerous divergent ARM and benthic metric results, though they do tend to vary from site to site. Investigations of potential impacts at individual watersheds as well as the PCA analysis of water quality highlight the need to consider all possible disturbances when evaluating the condition of a site.

Finally, we are presenting some recommendations in order to improve the biomonitoring program in Newfoundland, notably: 1. increasing the number of sampling sites in under-represented ecoregions, 2. adding temperature loggers and flow sites at the long-term reference sites and 3. repeating this analysis in five years, in a simplified way.

Sommaire exécutif

Ce premier rapport présente les résultats pour la période de 2006 à 2019. Les données de biosurveillance ont été recueillies dans 93 sites d'échantillonnage à travers l'île de Terre-Neuve : 77 sites de référence potentiels et 16 sites d'étude. Ces sites sont répartis dans les neuf écorégions de l'île et ont tous été échantillonnés selon le protocole standardisé du Réseau canadien de biosurveillance aquatique (RCBA). Sur les 77 sites de référence potentiels, trois d'entre eux sont des sites de référence à long terme, qui contiennent tous 10 ans de données. Les sites du Labrador n'ont pas été utilisés en raison des différences d'habitat.

Les échantillons inclus dans cette analyse ont été recueillis par différents partenaires : le ministère de l'Environnement et du Changement climatique de Terre-Neuve-et-Labrador - Division de la gestion des ressources en eau, Environnement et Changement climatique Canada, ainsi que Parcs Canada (parcs nationaux de Terra Nova et de Gros-Morne).

Ce rapport visait à répondre à trois questions principales :

1. **Les communautés benthiques des zones de référence sont-elles différentes selon les écorégions de l'île de Terre-Neuve ?** Notre analyse a montré certaines différences dans les communautés par écorégion, certaines écorégions montrant une plus grande variabilité dans l'abondance relative des taxons EPT et Chironomidae. Nous avons observé certaines différences dans les taxons dominants entre les écorégions lorsque nous examinons les groupes de taxons individuellement. Les taxons dominants sont les Chironomidae (moucherons non piqueurs) et les Baetidae (petit éphémère) pour la plupart des écorégions, à l'exception des Pisidiidae (pisidies ou petites palourdes) pour les écorégions du détroit de Belle Isle et du nord-est de Terre-Neuve.

2. **Y a-t-il des changements dans les communautés benthiques aux trois sites de référence à long terme au fil du temps ?** Des tendances à long terme ont été détectées dans les trois sites de référence de biosurveillance à long terme, bien que Careless Brook et Northeast River aient montré beaucoup plus de tendances que Pinchgut Brook. Les résultats suggèrent que la communauté benthique évolue vers une communauté plus tolérante aux polluants organiques. Dans l'ensemble, il est difficile de dire quels facteurs peuvent être à l'origine de ces tendances dans les résultats issus de l’analyse du modèle de référence (ARM) et des variables benthiques, car la communauté benthique est influencée par de nombreuses conditions environnementales.

3. **Pouvons-nous détecter les impacts sur les communautés biologiques à l'aide du modèle de référence et des variables de base ? Si oui, pouvons-nous relier ces impacts à des facteurs de stress ?** Nous avons observé que les mesures clés différaient entre les sites de référence et les sites d’étude sur l'île de Terre-Neuve. Les sites d'étude ont généralement obtenu de nombreux résultats divergents tant pour l’analyse avec le modèle ARM que pour les variables benthiques, bien que les résultats aient tendance à varier d'un site à l'autre. La recherche des impacts potentiels sur les bassins hydrographiques individuels ainsi que l'analyse par composantes principales de la qualité de l'eau soulignent la nécessité de prendre en compte toutes les perturbations possibles lors de l'évaluation de l'état d'un site.

Finalement, nous présentons quelques recommandations afin d'améliorer le programme de biosurveillance à Terre-Neuve, notamment : 1. augmenter le nombre de sites d'échantillonnage dans les écorégions sous-représentées, 2. ajouter des enregistreurs de température et de débit aux sites de référence à long terme et 3. répéter cette analyse dans cinq ans, de manière simplifiée.

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List of Acronyms

ANOSIM	Analysis of similarities
ARM	Atlantic Reference Model
CABIN	Canadian Aquatic Biomonitoring Network
CANAL	Canada-Newfoundland/Labrador AquaLink
CCME	Canadian Council of Ministers of the Environment
CEFI	Canadian Ecological Flow Index
DFO	Department of Fisheries and Oceans of Canada
ECCC	Environment and Climate Change Canada
EPT	Ephemeroptera-Plecoptera-Trichoptera
GIS	Geographical Information System
GOID	Gastropods, Oligochaeta, Isopods, Diptera
HBI	Hilsenhoff Biotic Index
LWD	Large wooden debris
NB	New Brunswick
NL	Newfoundland
NMDS	Nonmetric Multi-dimensional Scaling
NP	National Park
NS	Nova Scotia
PCA	Principal Component Analysis
RCA	Reference Condition Approach
TOC	Total Organic Carbon
WRMD	Water Resources Management Division
WRMD-ECCNL	Water Resources Management Division of the Department of Environment & Climate Change of Newfoundland and Labrador

Introduction

What is Aquatic Biomonitoring?

Traditionally, water quality monitoring programs have relied upon chemical and physical measurements to assess water quality and aquatic ecosystem condition. Measurements are compared to environmental water quality guidelines developed for the protection of aquatic life (CCME, 2012). Measurement of chemical and physical parameters in water provides valuable information on the state of water quality in an aquatic ecosystem and allows us to detect trends or changes that may impact the biota inhabiting the ecosystem. Aquatic biomonitoring, on the other hand, directly measures changes in biotic communities (e.g., fish, benthic macroinvertebrates, and algae), in order to assess the health of aquatic ecosystems.

Biomonitoring serves a number of purposes, such as the verification of assessments based on chemical monitoring and identification of effects that were not detected by traditional monitoring. In addition, biomonitoring can measure impacts of cumulative stressors, including impacts from chemical interactions, contaminant pulses, or unknown contaminants that are difficult to capture with routine chemical sampling. Other stressors that may be indicated by measuring the biotic communities include the presence of exotic species, habitat degradation in the water body or surrounding land, climate change, and fluctuations in water quantity (e.g. water level and flow). Ultimately, the biological effects of environmental stressors can only be determined by measuring the biota themselves. A monitoring program that uses both traditional chemical/physical and biological monitoring provides complimentary information resulting in an integrated understanding of aquatic ecosystem health; the biotic community being a measurement of effect while physical and chemical measurements aid identifying the potential cause of the effect.

Biomonitoring may include the use of any aquatic biota such as fish, algae, zooplankton, phytoplankton, and macrophytes. Benthic macroinvertebrates are, however, the most widely used indicator group for current aquatic biomonitoring programs (Rosenberg and Resh, 1993) and are the current focus of national standard development for use in the CABIN program.

What is CABIN?

The Canadian Aquatic Biomonitoring Network (CABIN) is a national aquatic biological monitoring program led by Environment and Climate Change Canada (ECCC), which assesses the biological condition of freshwater habitats across Canada. Monitoring biological communities such as fish, invertebrates, and algae can provide an indicator of ecosystem health. Biological indicators of aquatic ecosystem health can complement indicators of water quality because aquatic life can be affected by factors not incorporated into the water quality indicator such as:

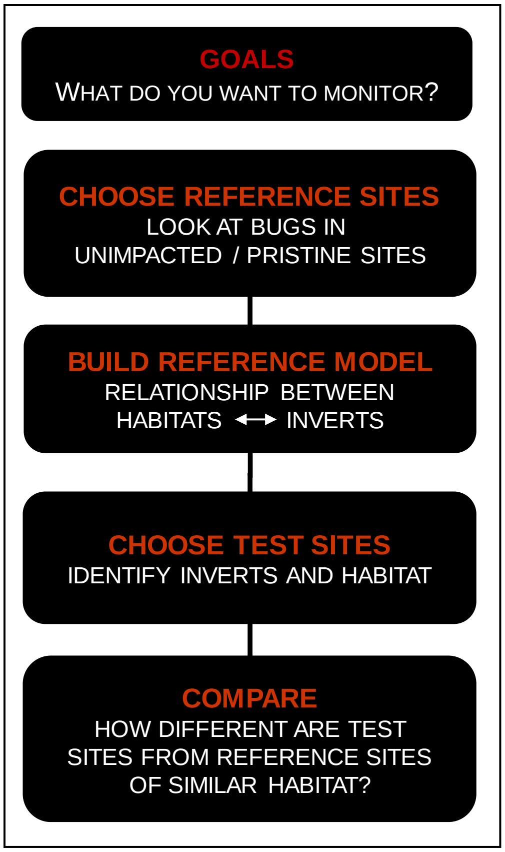
- the effects of chemicals interacting with each other;
- contaminant releases not detected at the time of sample collection;
- unknown contaminants in the water;

- introduction of exotic species;
- habitat degradation in the water or surrounding land;
- climate change;
- changes in water levels, flows, and timing (ice formation and spring thaw).

CABIN collects benthic macroinvertebrates in streams, rivers, lakes and wetlands using standardized data collection methods. Benthic macroinvertebrates are aquatic, bottom-dwelling animals without backbones that are generally visible to the naked eye. They include worms, crustaceans, molluscs and the larval stages of many insects. Macroinvertebrate communities have many advantages as biological indicators:

- They reflect conditions at specific locations and also show cumulative impacts;
- They are sensitive to a variety of disturbances;
- They are present in all fresh water ecosystems;
- They are a key part of aquatic food webs;
- Their assessment methods are well-developed.

CABIN provides standardized protocols, a national web accessible database, online analysis and reporting tools, and a training and certification program. CABIN data collection is growing across the country with extensive data in some areas and gaps in others. Data gathered by CABIN participants (government and non-government organizations, Indigenous organisations, and academia) are entered into the national database. They are shared among the network to achieve consistent and comparable data collection and reporting on freshwater aquatic ecosystem conditions in Canada. CABIN data are publicly available on the Open Data Portal from the Government of Canada.



To analyse data and assess sites, the CABIN program uses the well-established Reference Condition Approach (RCA) for consistent interpretation and assessment of aquatic ecosystem condition (Bailey *et al*., 2004). Two types of sites are identified using RCA; reference sites and test sites. Reference sites represent the best available conditions or minimally disturbed by human impact within a region, and are used to establish a baseline of expected variability within benthic macroinvertebrate communities. In CABIN, the data from these sites are used to develop predictive bioassessment models to determine if a test site (a site exposed to environmental stressors) is similar or divergent from reference. Metrics calculated at test sites can also be compared against metrics calculated at reference sites to assess ecosystem conditions, in regions where no RCA model is available.

In Atlantic Canada, the preliminary RCA model included the extensive taxonomic dataset from New Brunswick (N.B.), as well as other reference sites collected throughout Atlantic Canada (N.B., Nova Scotia, and the island portion of Newfoundland) up to 2010 using the standard CABIN protocol (Armanini et al. 2013).

Review of Benthic Macroinvertebrate Research in Newfoundland

Water quality research involving benthic macroinvertebrates has been conducted on the island of Newfoundland by various agencies since the 1970’s (Pickavance, 1971; Colbo, 1979). The direction of the research and target species has chiefly been dependant on the principal interests of the project’s coordinating agency, which has included Memorial University researchers, Department of Fisheries and Oceans (DFO), Environment and Climate Change Canada, Parks Canada, community groups and private consulting firms. How the invertebrate data was used within the studies varies greatly depending on the specific research objectives. These studies have covered a wide range of aquatic habitats inhabited by benthic macroinvertebrates: ponds and lakes (Ryan et al., 1993; Khan, 2003; Noordhof, 2002; Pelley, 2006; O’Connell & Walsh, 2007; Moore, 1999; Muzaffer, 1998; Muzzafer & Colbo, 2002; Higgins, 1995; Humber, 2005; Christie, 1966; Clarke, 1995; Clarke & Knoechel, 2008; Cutler, 1997), bogs and wetlands (Larson & House, 1990; Rideout & Colbo, 1999), streams and rivers (Meade, 1993; Yetman, 1998; Rideout, 1999; Colbo, 1985; O’Connell & Andrews, 1996; Lomond & Colbo, 1996; Johnson, 1999; Gabriel, 2008; Gibson, 2001; Clemens, 1985; Rolls, 2017), and riffles specifically (Smith, 2009; Thonney et al., 1987; Clarke & Scruton, 1997). The majority of research has been conducted in the urban area surrounding St. John’s, the experimental ponds research area in central Newfoundland, and within Terra Nova and Gros Morne National Parks.

Initial surveys of benthic macroinvertebrates on the island of Newfoundland determined that the recent glaciation of the island, in combination with it’s isolation from the mainland, has made it difficult for new taxa to migrate and establish themselves within the province. As an example, Colbo et al. 1997 has pointed out that on the island of Newfoundland, there are 34 species of Odonata, compared to the 132 species found throughout the Maritimes. Colbo et al. 1997 then studied Ephemoptera, Plecoptera, Trichoptera and Simuliidae at 23 sites in eastern Newfoundland, concluding that impoverished fauna on the island is adequate to provide an accurate measure of water quality, though correct interpretation of such information requires a thorough knowledge of the natural and anthropogenic history of the sites and region. Perez 1999, using the same data collected from the 23 sites between Bonavista and St. John’s, attempted to determine the sensitivity of the impoverished fauna to different environmental gradients, and thus its usefulness for biomonitoring programs. Through analysis of EPT diversity and abundance, an index of three highly pollution sensitive orders (Ephemeroptera, Plecoptera, Trichoptera), they concluded that impoverished EPT fauna are suitable indicators of water quality, with the high water pollution in urban areas markedly reducing diversity while infrequent physical disturbance enhances diversity.

Benthic macroinvertebrates have been used to gauge the effects of urbanization and associated pollution on stream health and water quality in Newfoundland. Rennie’s River, which weaves its way through St. John’s before entering Quidi Vidi Lake, was assessed in 1971 (Pickavance), 1993 (Meade) and 1999 (Rideout). The most recent study compared the communities found with those of the previous studies, finding that Rennie’s River had improved markedly from 1969 to 1992, while improvements from 1992 to 1998 were not as conspicuous. Several pollution sensitive species identified in the 1999 study were previously absent from these sites, indicating that the water quality may have indeed improved over time.

Surveys by Clemens (1985) and Johnson (1999) focused fully or partially on the Waterford River System, including the South Brook tributary. Clemens (1985) used rock bags as artificial substrate in areas co-located with provincial water quality grab sampling sites so that benthic data could be combined with available chemical and physical data. It was concluded that though Waterford River runs through Mount Pearl and St. John's, the sites located downstream of Bowring Park were in a 'fair condition', similar to that of South Brook upstream of urbanization. This is likely due to the influx of relatively good water from the South Brook system as it enters the Waterford River upstream of this station. Johnson (1999) compared the diversity of benthic macroinvertebrate assemblages in natural and urbanized stream sections in the St. John's/Mount Pearl area. Rivers surveyed included: South Brook, Leary's Brook, Kitty Gaul's Brook and Virginia River. It was concluded that urbanization resulted in a decrease of taxon richness. In urbanized areas, tolerant organisms became abundant while intolerant taxa were eliminated.

In 2008, a project by Gabriel, Clarke and Campbell (2010) in Compensation Creek, a man-made flow controlled stream in south-central Newfoundland, looked at the influence of large woody debris (LWD) on benthic invertebrate communities. Communities found within the LWD were compared to benthic communities in Compensation Creek as well as from a nearby natural reference stream. It was found that taxa composition between the natural and man-made streams were similar, however, taxa richness was higher in the benthic environment than within the LWD, likely the product of more complex refugia and accumulation of fine detritus.

Other research conducted in NL using benthic macroinvertebrates looked at areas such as baseline stream community composition (Lomond & Colbo, 2000; O'Connell & Andrews, 1996), the impact of physical disturbances (Khan & Colbo, 2008), boat traffic (Humber, 2005), sediment addition from logging roads (Yetman, 1998), sampling techniques on the community characterization (Muzaffar & Colbo, 2002), and the effect of whole-lake enrichment on communities (Clarke, 1995; Moore, 1999; Pelley, 2006).

Graduate research out of Memorial University and in association with Parks Canada, was the first survey of Newfoundland found in the literature which was conducted using CABIN protocols (Smith, 2009). Again, the issue of Newfoundland's impoverished fauna and its usefulness as a water quality indicator were questioned. Sixty-five riffle communities on the Avalon Peninsula, Terra Nova Park, and Gros Morne Park were sampled from 2002-2004 using CABIN protocols through partnerships between Parks Canada and Memorial University. It was found that the majority of macroinvertebrate communities on the island were more highly correlated with physical than chemical variables, while a few were sensitive to both physical and chemical conditions. The correlations between macroinvertebrates and environmental variables indicated that CABIN protocols are suitable for biomonitoring in Newfoundland. It was recommended that in order to produce meaningful trend results, sites be sampled repeatedly over time. Further analysis determined that despite the impoverished taxonomic diversity, benthic macroinvertebrate communities on the island of Newfoundland are adequately diverse and show sufficient variation to be useful in determining community changes due to anthropogenic effects (Smith et al. 2013).

The most recent publication was by associates of Terra Nova National Park (MacDonald & Cote 2013) who analyzed data collected in the park using CABIN methods between 2006 and 2011 to examine the variability of sites in Reference Condition over time. Consequently, a major hurricane affected the study area in 2010, allowing this phenomena's effect on temporal variation to also be examined. The study concluded that annual sampling was important to account for natural year-to-year variability of reference condition sites.

Water quality research using benthic macroinvertebrates is not new in Newfoundland, but study areas have traditionally been small and concentrated in one region of the province or another. Sampling techniques have also been varied across the years, with the CABIN program gaining traction first with Parks Canada followed by ECCC and the province in 2008. Future research may or may not choose to sample using CABIN protocols, but either way, previous researchers have shown that despite the challenges of working with limited taxa, benthic macroinvertebrate communities in Newfoundland can provide a wealth of water quality information.

Current Biomonitoring Activities

The Water Quality and Monitoring Division of Environment and Climate Change Canada (ECCC) and the Water Resources Management Division of the Department of Environment & Climate Change of Newfoundland and Labrador (WRMD-ECCNL) began implementing a joint aquatic biological monitoring program in 2008, under the Canada-Newfoundland and Labrador Water Quality Monitoring Agreement. The objectives of this program were to:

1. validate the results of water chemistry analyses at long-term monitoring sites with a direct measure of biological response;
2. develop a monitoring tool that could be used to detect new problem areas for further monitoring, and;
3. develop a cost-effective tool that could be used for monitoring remote areas by requiring fewer visits.

Initially, implementation of the CABIN program in Newfoundland focused on collecting sufficient baseline data at reference sites, mostly on the island, to develop a reliable and accurate reference condition model to enable assessment. This focus has changed in recent years to begin collecting at long-term water chemistry monitoring sites, where feasible, and to operationalize the first version of the reference condition approach (RCA) model to enable assessments.

Objectives of This Report

This report aims to answer the following questions:

1. Are benthic communities in reference areas different across ecoregions on the island of Newfoundland?
2. Are there changes in reference communities over time?
3. Can we detect impacts to biological communities using reference models and baseline metrics? If so, can we relate these impacts to stressors?

Therefore, in the coming pages, we will:

- present the CABIN data collected on the island of Newfoundland for the period 2006-2019;
- describe the baseline data collected thus far;
- provide a trend analysis of the three long-term reference sites to detect any changes;
- review the program to date and its implementation in Newfoundland and Labrador;
- provide direction for future monitoring.

Note that results for the Labrador sites are not included in this report as the Atlantic Reference Model cannot be used for analyzing those sites since the geography is vastly different from the island of Newfoundland. Further work is required to determine the best course of action for building a model for Labrador, including potential variants on the established wadeable streams protocol, such as large river or riverine wetland derivations.

Data Collection

Summary of the CABIN Protocol

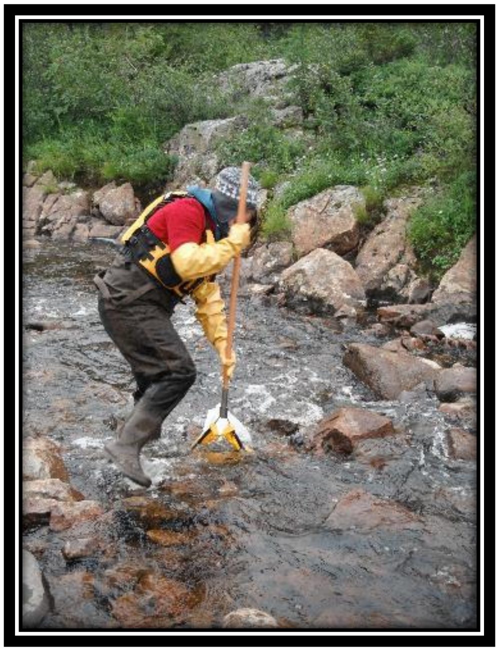


Photo credit: Kyla Brake, WRMD-ECCNL

The invertebrate samples were collected at all sampling sites using the standardized Canadian Aquatic Biomonitoring Network field manual - wadeable streams protocol (ECCC, 2012). Invertebrates were collected with a kick net of 400 µm mesh size, using a zigzag pattern, over a period of exactly three minutes.

The samples were transferred to a jar where a preservative agent was added (ethanol or 10% buffered formalin) The samples were then sent to a certified taxonomist for identification. Identification of macroinvertebrates was made to the Standard Taxonomic Effort as recommended in the Appendix A of the CABIN laboratory methods : processing, taxonomy, and quality control of benthic macroinvertebrate samples (ECCC, 2021).

Some taxa were removed from the samples (such as Cladocera, Copepoda, Ostracoda, etc.) because of some difficulties in their numbering or because they are not considered benthic. See the CABIN Lab Manual for more details (ECCC, 2021).

Data were finally entered in the CABIN national database and made available by the Open Data Portal.

Physical and chemical water quality measures were also taken at the sampling site, as prescribed by the CABIN protocol, as well as information about the habitat in and around the stream; substrate characteristics, channel measurements such as bankfull width, wetted width, velocity, depth and slope. This information is useful in interpreting the results from the morphological identification.

Overview of the Sites Sampled

THE PHYSICAL SETTING OF NEWFOUNDLAND

Sitting on the easternmost edge of the large North American landmass, surrounded by the cold Labrador Current and the warmer Gulf Stream, Newfoundland and Labrador has many unique landforms shaped in the distant past. Many of the characteristic landforms that we see today, such as the great fjords and U-shaped valleys of the island's west coast are the result of glacial activity. Around 8.4% of the province (405,730 km^2) is covered in water[1].

Newfoundland is divided into nine ecoregions: from the Island's rugged South Coast and Maritime Barrens, through the islands of Witless Bay and Baccalieu and the Tablelands of Gros Morne, the variety of natural environment is spectacular and rich. Each of these ecoregions consist of an area that has distinctive and repeating patterns of vegetation and soil development, which are determined and controlled by regional climate. Ecoregions can be distinguished from each other by their plant communities, landscapes, geology, and other features. These characteristics, in turn, influence the kinds of wildlife that can find suitable habitat within each ecoregion.

The nine ecoregions are (refer to **Figure 1**):

1. Western Newfoundland Forest
2. Central Newfoundland Forest
3. North Shore Forest
4. Northern Peninsula Forest
5. Avalon Forest
6. Maritime Barrens
7. Eastern Hyper – Oceanic Barrens
8. Long Range Barrens
9. Strait of Belle Isle Barrens

You can find a detailed description of each of the ecoregions here: https://www.gov.nl.ca/ecc/natural-areas/publications/#brochures.

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[1] See the brochure "Understanding and Protecting our Natural Heritage": https://www.gov.nl.ca/ecc/files/publications-parks-intro1-understanding-2007.pdf

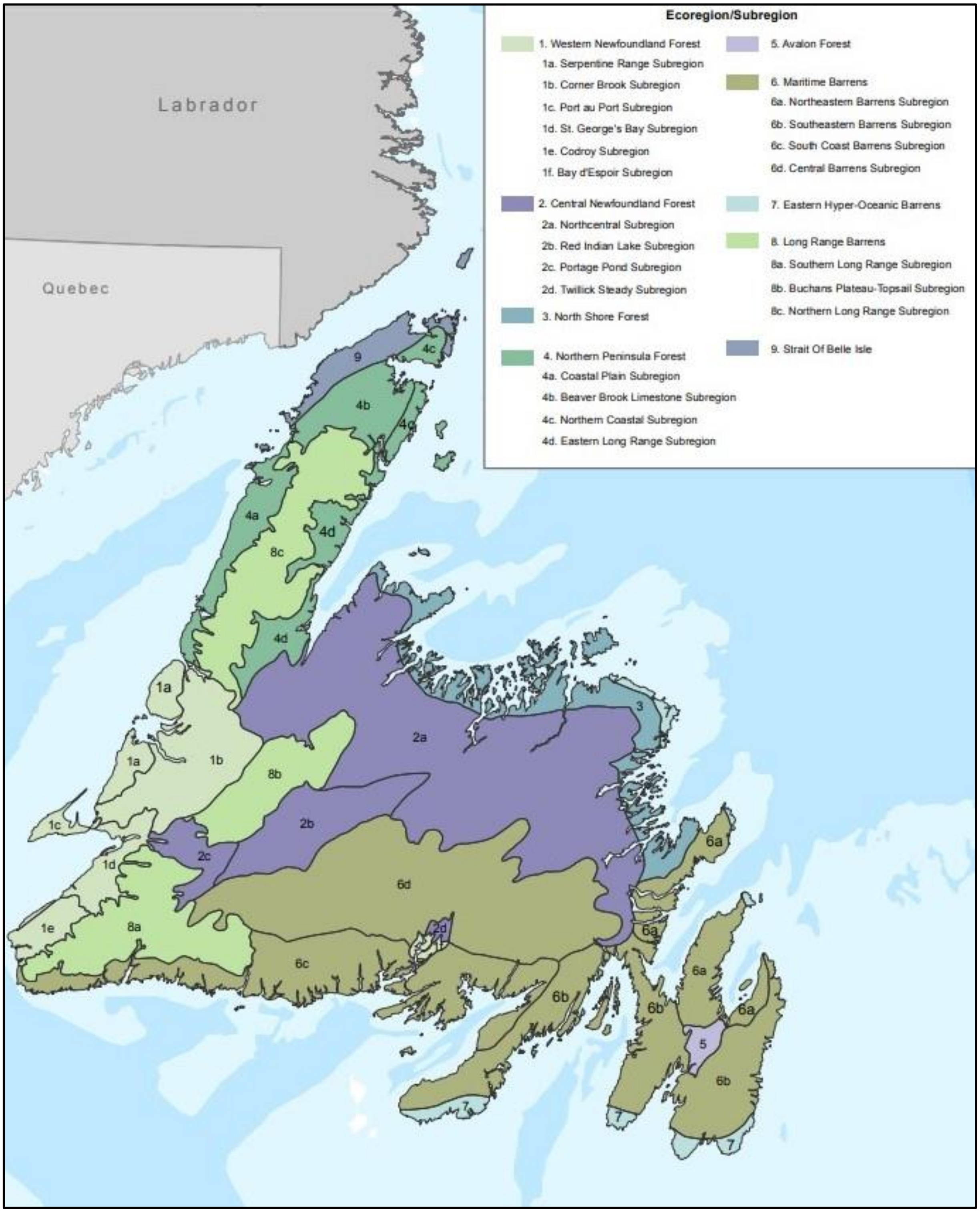


FIGURE 1. MAP OF THE ECOREGIONS OF NEWFOUNDLAND REF.(natural-areas-maps-ecoregion-subregion-nl.pdf (gov.nl.ca))

SAMPLING SITES

Biomonitoring data were collected at 93 sampling sites across the island of Newfoundland (sampling sites in Labrador are not presented here). These sampling sites are distributed amongst the nine ecoregions (**Table 1** and **Figure 2**).

TABLE 1. DISTRIBUTION OF CABIN SAMPLING SITES ACROSS ECOREGIONS

Ecoregion	**Number of sites**	**Site type**		**Name of the CABIN studies containing the data**
		Potential Reference	**Test**	
Avalon Forest	1	1	0	NL Province - WRMD
Central Newfoundland	27	24	3	Atlantic CABIN, NL Province - WRMD, Terra Nova NP
Long Range Mountains	5	5	0	Atlantic CABIN, NL Province - WRMD, Gros Morne NP
Maritime Barrens	19	11	8	NL Province - WRMD
Northeastern Newfoundland	5	4	1	Atlantic CABIN, NL Province - WRMD
Northern Peninsula	12	12	0	Atlantic CABIN, NL Province - WRMD, Gros Morne NP
South Avalon-Burin Oceanic Barren	2	1	1	NL Province - WRMD
Southwestern Newfoundland	18	15	3	Atlantic CABIN, NL Province - WRMD, Gros Morne NP
Strait of Belle Isle	4	4	0	Atlantic CABIN
Total number:	**93**	**77**	**16**	

As presented in the table above, samples have been collected by partners for different studies. The study Atlantic CABIN includes samples taken by ECCC as part as their biomonitoring network. Study NL Province-WRMD regroup data taken by the provincial government of Newfoundland and Labrador. Sites from the two studies Terra Nova National Park (NP) and Gros Morne NP were sampled by Parks Canada staff. Data from the Northeast Avalon CABIN study were not included into this analysis for some proprietary reasons.

Although the CABIN program was implemented in 2002 in Newfoundland, the temporal scale of data used for this analysis range from 2006 to 2019 (see the explanations in next section). The next graph shows the temporal distribution of the sampling effort (**Figure 2**). It shows that the sampling effort has been variable throughout the years, with a more extensive sampling effort in 2009 and 2010 as both the government of NL and ECCC increased efforts to expand the collection of reference sites for model building.

Out of the 77 potential reference sites, three of them consist of long-term reference sites, all of which contain at least 10 years of data:

- **NF02YJ0004**: sampled from 2008 to 2019, no sampling in 2009 nor 2010;
- **NF02YQ0072**: sampled from 2010 to 2019;
- **NF02ZK0005**: sampled from 2009 to 2019, no sampling in 2010.

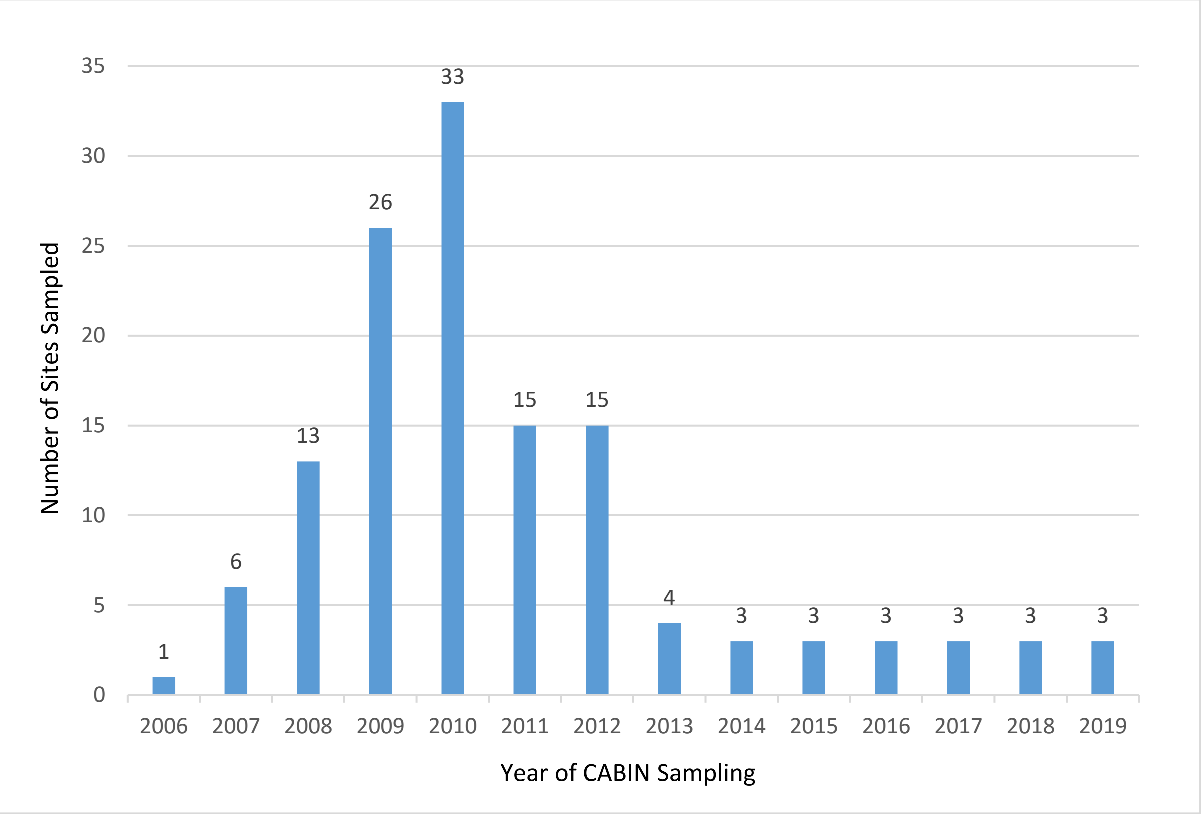
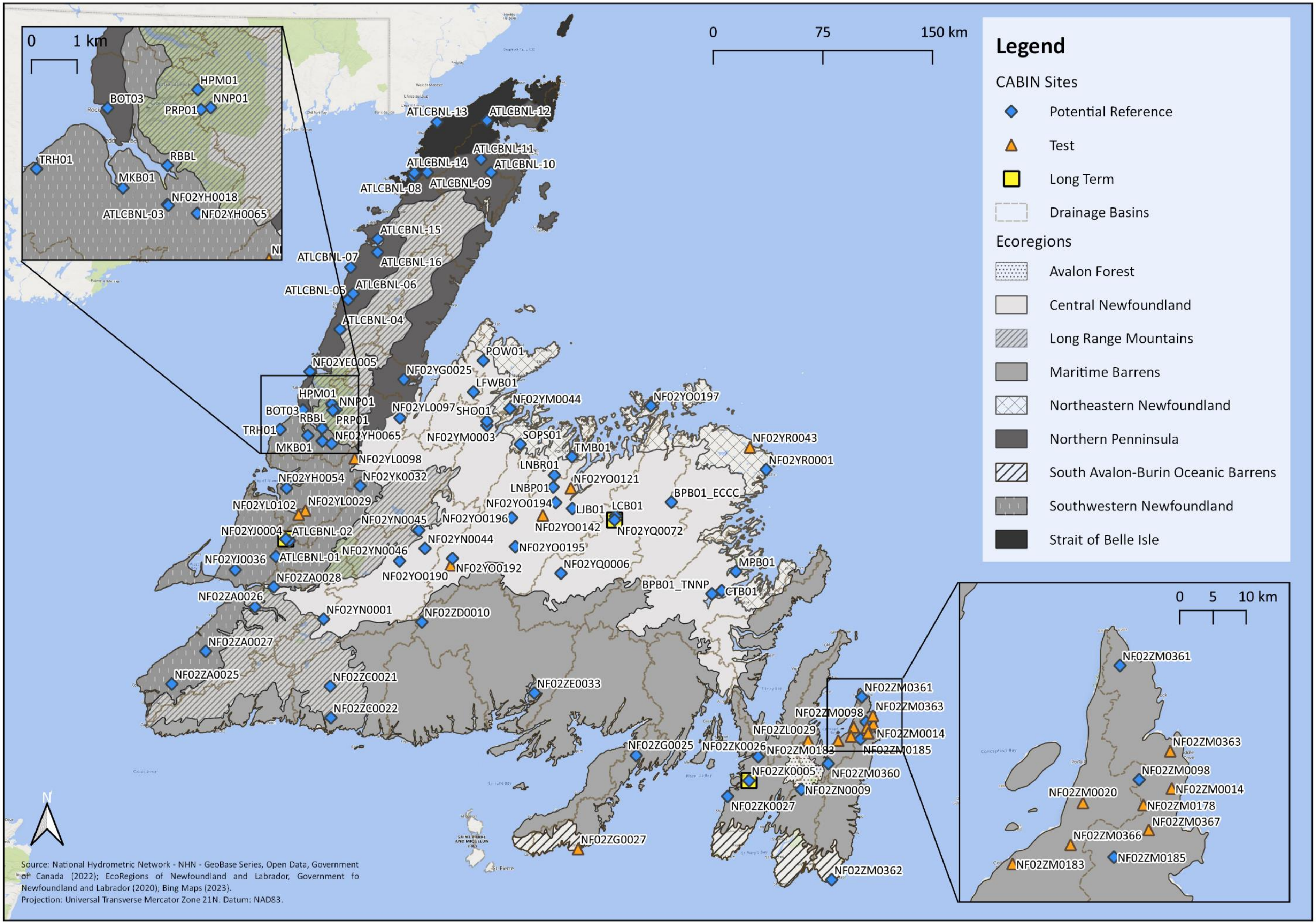


FIGURE 2. DISTRIBUTION OF THE SAMPLING EFFORT THROUGHOUT THE YEARS

For the complete list of sites along with their geographic coordinates, refer to **Appendix 1**.

DATA GAPS

From **Table 1** and **Figure 3**, it appears that the total number of biomonitoring sampling sites is not equally distributed throughout the nine ecoregions. Most sites in the Maritime Barrens ecoregion are concentrated around St. John's and the Avalon peninsula; other parts of the ecoregion could use more data. Four ecoregions have five or less sampling sites including Avalon Forest, Northeastern Newfoundland, South Avalon-Burin Oceanic Barren and Strait of Belle Isle. To ensure a better portrait of the biomonitoring results on the island, we would recommend increasing the number of sampling sites in these ecoregions.



Source: National Hydrometric Network - NHN - GeoBase Series, Open Data, Government of Canada (2022); EcoRegions of Newfoundland and Labrador, Government fo Newfoundland and Labrador (2020); Bing Maps (2023).
Projection: Universal Transverse Mercator Zone 21N. Datum: NAD83.

FIGURE 3. MAP OF CABIN SITES SAMPLED IN NEWFOUNDLAND AND INCLUDED IN THIS ANALYSIS.

SAMPLING SITE PROFILES

Each sampling site has their own characteristics. The Water Resources Management Division has produced a profile for each of the 93 sites sampled since 2002. These profiles are available via the WRMD website so members of the public can access information about each of these sites. Information provided includes:

- photo of the site
- map
- site description
- channel data
- reach data
- water chemistry
- and substrate data.

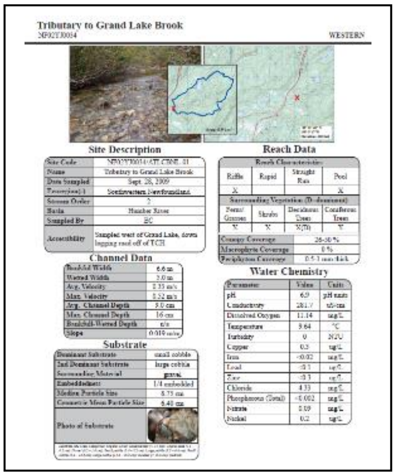
Site profiles are gathered in the second volume of this study called **"Newfoundland Sampling Sites Profiles"**.

In addition, the Water Resources Management Division of the Department of Environment & Climate Change of Newfoundland and Labrador created a Web page, the Canada-Newfoundland/Labrador AquaLink (CANAL)[2], where all CABIN sites in Newfoundland and Labrador are listed and described with more extensive information about the site, including land cover summaries, geology, soils and cover, development, urbanization and recreational activities within the watershed.

Methodology and Data Analysis

1. Data Extraction and Validation

Data for all reference and potential reference sites from the four studies listed above were extracted from the CABIN database. Although the CABIN program was implemented in 2002 in Newfoundland, the temporal scale of data used for this analysis range from 2006 to 2019. Several samples in the original 2002-2019 data export were excluded from the final dataset due to benthic collection using kick-nets with mesh sizes other than the 400 µm CABIN standardization requirement, or incomplete data (missing taxonomy and/or habitat data). As a result, all samples collected prior to 2006 were removed and certain sites no longer remain in the dataset including BBA01 and TNR01.



Tributary to Grand Lake Brook

WESTERN

Site Description

Reach Data

Channel Data

Water Chemistry

Substrate

Data was extracted at lowest practical level. As recommended in the CABIN Laboratory Methods (Environment and Climate Change Canada, 2021), the following taxa were excluded from the analysis: Nemata, Ostracoda, Platyhelminthes, Maxillopoda, Lumbricidae (family not aquatic), Branchiopoda (class not benthic). On the other hand, the family Enchytraeidae was

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[2] CABIN sites in Newfoundland and Labrador (Canada-Newfoundland/Labrador AquaLink (CANAL): https://www.gov.nl.ca/ecc/waterres/watermonitoring/CABIN

retained in the dataset, even though not all species are considered aquatic, because Enchytraeidae contributes towards the calculation of the metric %GOID (see after for the description of this metric).

The dataset was read into R Studio (version 1.1.419). In order for site codes to be unique alphanumeric combinations, sites with the same code but different geographic locations were identified and renamed. More specifically, there was two sites with the same code, but in different stream and study (BPB01). So we changed the site codes to “BPB01_ECCC” and “BPB01_TNNP”.

Many sites in the dataset contain multiple taxa count entries for a single taxon of the same family class. This is because when the original taxonomy report was conducted to the lowest possible taxa group, some could be identified to genus/species while others could not, so they are counted in different rows. Since the taxonomic resolution established for the national indicator is currently at the family level, these rows were summed so there would be a single taxon count for each family group within the same site code and sample id.

Water quality data was extracted from the ECCC water quality database, and included all measured parameters. Sites in the database are named using an internal identifier and did not always match the CABIN names, therefore manual matching of the CABIN site names to the identifiers was necessary. Non-detects in water quality values were excluded and a subset of parameters with valid values at most sites were included. Where some sites had multiple years of water quality data, the median value for each parameter was taken.

2. Atlantic Reference Model (ARM)

CABIN promotes the use of the Reference Condition Approach (RCA) and the development of predictive models to conduct site assessments. For assessing test sites in Newfoundland, we used the Atlantic Reference Model (ARM) developed for CABIN test sites that fall within the geographical extents of Nova Scotia, New Brunswick, Prince Edward Island, Newfoundland (not including Labrador) and the Gaspe Peninsula. The current model uses data up to 2010 collected with the standard CABIN protocol. The procedure is detailed in Armanini et al. (2013). At the time of writing this report, researchers were working on updating the model with most recent data.

By comparison with other CABIN models, the Atlantic Reference Model is not housed within the CABIN Analytical tools. This is explained by the fact that the ARM uses a different RCA methodology than the “standard” CABIN models.

HOW DOES THE ARM WORK?

The Atlantic reference condition model (ARM) is based on the River Invertebrate Prediction and Classification System (RIVPACS) approach with regional-scale applicability. Biological monitoring data collected from wadeable streams across Atlantic Canada together with freely available, nationally consistent geographic information system (GIS) environmental data layers make up the model. The Atlantic Reference Model (ARM) is run in GenGIS using R software.

- Model development requires us to sample from a wide range of reference sites that capture the variation that occurs in a given watershed or region. We need biological and habitat information to develop a model.

- Once we have sufficient reference data, we look at the biological data and we try to partition the reference communities into subsets or groups.

- The next step is to try to match the different groups with habitat features that drive the differences between the groups. These are the **predictor variables** for the model. These must be habitat features that are **NOT** affected by human disturbance.
  Four geospatial habitat variables are derived for each CABIN site:
    1. long-term annual air temperature range
    2. percentage intrusive bedrock
    3. percentage sedimentary bedrock
    4. percentage sedimentary and volcanic bedrock

- When we want to assess a test site, we run the benthic data and required habitat features (predictor variables) through the model. The model generates an output of how similar (or dissimilar) the observed benthic assemblages are at test sites compared to the expected reference condition. The ARM documentation provides ranges for each metric to aid in interpretation of the outputs as either Normal, Divergent, or Strongly Divergent. If one or more metrics for a test site falls in the Divergent or Strongly Divergent categories, it may be an indication that the site is experiencing environmental stress.

INTERPRETATION OF THE RCA MODEL OUTPUT

The numbers produced in the output represent the (Observed) values measured at test sites divided by the values predicted (Expected) for the sites by the Atlantic Reference model (Observed/Expected).

Currently, there are five metrics calculated for each site (Taxon Richness, Shannon-Wiener Diversity Index, Berger- Parker Dominance Index, Pielou Evenness and Simpson Diversity Index). The predicted values depend on the habitat variables (i.e. temperature and bedrock geology) that you calculated at each of your sites. These determine the assemblage (benthic community) that is expected to be found at that site in the absence of human pressures. The lower the Observed/Expected ratio the more your sites differ (or diverge) from reference conditions.

Each of the five metrics measures a different aspect of the benthic assemblages that can be affected by human pressures:

- **Taxon Richness** is a measure of the number of expected taxonomic groups present.
- **Shannon-Wiener Diversity** Index is a measure of taxonomic richness and the equitability in abundance within the assemblage. Lower values are expected with increased disturbance.
- **Berger-Parker Dominance** Index measures the dominance based on the proportional abundance of the most abundant taxon. Increased disturbance is expected to result in higher abundance of the most tolerant taxa.
- **Pielou's Evenness** measures the equitability in abundance within the assemblage. Increased disturbance is expected to result in lower evenness.
- **Simpson Diversity** Index is a measure of diversity. It is used to quantify the biodiversity of a habitat. It takes into account the number of species present as well as the abundance of each species. The greater the value, the greater the sample diversity. Increased disturbance will result in lower diversity, meaning fewer species.

There are also two other metrics that are used to describe benthic assemblages:

- **Canadian Ecological Flow Index (CEFI)** measures the response of benthic assemblages to flow alterations based on the flow preference of each taxa. Increased flow regime disturbance results in lower CEFI scores (Armanini et al., 2011).
- **Hilsenhoff Biotic Index (HBI)** is based on tolerance of individual taxa to organic pollution. Increasing disturbances result in decreasing HBI scores (Hilsenhoff, 1982).

Below in **Table 2** are the ecological status classes (normal, divergent, highly divergent) and their related boundaries for all seven metrics.

TABLE 2. ARM OBSERVED VS EXPECTED VALUE THRESHOLDS FOR ECOLOGICAL STATUS CLASSES.

Metric (O/E = Observed vs Expected ratio)	Normal	Divergent	Highly divergent
O/E Taxon Richness (R)	>0.95	0.95-0.47	<0.47
O/E Shannon-Wiener Diversity (H)	>0.91	0.91-0.45	<0.45
O/E Simpson Diversity Index (S)	>0.96	0.96-0.48	<0.48
O/E Pielou's Evenness Index	>0.92	0.92-0.46	<0.46
O/E Berger-Parker dominance (D)	>0.77	0.77-0.38	<0.38
O/E Canadian Ecological Index (CEFI)	>0.97	0.97-0.48	<0.48
O/E Hilsenhoff Biotic Index (HBI)	>0.96	0.96-0.48	<0.48

3. Metric Calculations

In addition to the analysis done with the ARM, we calculated some other biological metrics. Calculations were done at the family level, using R software. When multiple years of sampling occurred at a same site, the median of the years was calculated. Here are the 20 metrics calculated on Newfoundland CABIN data:

1. Total Abundance
2. Total Richness
3. Dominance (calculated as the sum of the two most dominant taxon)
4. Total EPT Taxa (Ephemeroptera, Plecoptera, Trichoptera)
5. Percent (%) EPT
6. Total Chironomidae Taxa
7. Percent (%) Chironomidae
8. Total GOID Count (Gastropods, Oligochaeta, Isopods, Diptera)[3].
9. Percent (%) GOID (Gastropods, Oligochaeta, Isopods, Diptera).
10. Percent (%) Oligochaeta
11. EPT / Chironomids + EPT
12. Percent (%) Trichoptera that are Hydrophychidae

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[3] Only worms were included as part of the Oligochaeta subclass for calculation of %GOID (leeches were excluded), in keeping with findings from the literature (Prygiel et al, 2014; Collier et al, 2014).

13. Percent (%) Ephemeroptera that are Baetidae
14. Simpson's Diversity (D)
15. Shannon-Wiener Diversity (H)
16. Simpson's Evenness (E)
17. Pielou's Evenness (j)
18. Bray-Curtis Dissimilarity
19. Canadian Ecological Flow Index (CEFI)
20. Hilsenhoff Biotic Index (HBI)

Please refer to **Appendix 2** for individual descriptions of each metric. All metric values that were calculated can be found in a comma separated values file that is included with this report.

Some of the metrics that were calculated were also included in the ARM outputs (Richness, Shannon-Weiner Diversity, Simpson’s Diversity, Pielou’s Evenness, CEFI, and HBI). We retained these results because the ARM results are reported as observed over expected ratios while here we report the metric values.

For the purpose of the report, we generally focused our discussion on the following most significant, yet meaningful, metrics. In general, healthy streams have greater Richness and Percent EPT as sensitive taxa, primarily EPT taxa, disappear with reduced water quality. Chironomidae are tolerant taxa and tend to take over the benthic community in impacted streams. Diversity and Evenness metrics summarize the general make-up of the benthic community, and the CEFI and HBI metrics describe the benthic community in terms of their tolerance to changing flow regimes and nutrient enrichment. By looking at a wider range of metrics, we can investigate potential impacts that are causing changes in the benthic community.

- Total Richness
- Percent (%) EPT
- Percent (%) Chironomidae
- Shannon-Wiener Diversity
- Pielou's Evenness
- Canadian Ecological Flow Index (CEFI)
- Hilsenhoff Biotic Index (HBI)

4. Atlantic Benthic Normal Ranges

In order to have some basis for comparison, the full list of metrics above (except for the Bray Curtis Dissimilarity Index, Percent (%) Oligochaeta, Percent Trichoptera that are Hydrophychidae and Percent Ephemeroptera that are Baetidae as normal ranges do not exist for these metrics) were calculated for all reference and potential reference sites from 26 CABIN studies within the Atlantic Provinces (ECCC, 2022). Data from 2002 to 2021 were used and several percentiles were calculated (5, 10, 25, 50, 75, 90 and 95%). These reference values, also called normal ranges (Table 2), are used below to compare the metrics values calculated for the Newfoundland benthic communities. Metrics from a site are classified based on which percentile range they belong to. A metric value is considered **normal** if the value falls between the 25th and 75th percentiles. Values from 10th - 25th and from 75th – 90th are **potentially divergent**. Values below 10th or greater than the 90th percentile are deemed **divergent**, and values below 5% or above 95% are further categorized as **highly divergent**. You will find below a table with the values.

Table 3. Benthic Normal Ranges Calculated for the Atlantic Provinces

Percentile	**5th**	**10th**	**25th**	**50th**	**75th**	**90th**	**95th**
			Normal Range				
Total Abundance	232.05	366.68	**870.32**	**1962.50**	**4539.10**	9316.50	14748.90
Richness	12.00	15.00	**19.00**	**22.00**	**25.00**	29.00	31.00
Dominance	33.54	36.17	**41.98**	**51.24**	**62.71**	75.00	82.34
Total EPT	140.00	230.29	**528.89**	**1170.68**	**2607.48**	4839.33	7497.00
Percent EPT	22.74	33.92	**49.70**	**65.79**	**79.99**	90.11	93.37
Total Chironomidae	10.00	20.00	**88.38**	**327.75**	**1041.48**	2737.75	4795.83
Percent Chironomidae	1.93	3.13	**7.74**	**16.47**	**29.27**	49.27	61.07
Total GOID	18.00	33.00	**128.00**	**431.99**	**1283.35**	3149.97	5357.50
Percent GOID	3.43	5.65	**11.99**	**22.32**	**35.65**	56.51	67.11
EPT/EPT+Chironomidae	0.29	0.42	**0.65**	**0.80**	**0.91**	0.97	0.98
Simpson's Diversity	0.53	0.62	**0.75**	**0.83**	**0.87**	0.89	0.90
Simpson's Evenness	0.13	0.15	**0.19**	**0.26**	**0.32**	0.38	0.43
Shannon-Wiener Diversity	1.21	1.48	**1.88**	**2.17**	**2.38**	2.55	2.63
Pielou's Evenness	0.46	0.54	**0.63**	**0.70**	**0.76**	0.79	0.81
CEFI	0.31	0.32	**0.34**	**0.36**	**0.39**	0.42	0.43
HBI	1.71	1.96	**2.38**	**3.04**	**3.77**	4.74	5.50

5. Trend Analysis

We used the Observed/Expected outputs from the Atlantic Reference Model (ARM) as well as all of previously calculated metrics for three long-term CABIN sites in Newfoundland. The three sites are listed below, each having 10 years of CABIN samples.

- NF02YJ0004 – Pinchgut Brook
- NF02YQ0072 – Careless Cove Brook
- NF02ZK0005 – Northeast River

Long-term trends in the benthic metrics were calculated for the three long-term sites using the Mann-Kendall trend test, a non-parametric test for monotonic trends over time. The analyses were performed in the R environment (version 4.0.0), using the *rkt* package. The same tests were also performed on environmental parameters such as air and water temperature, hydrological flow, and water quality variables. More detailed descriptions of the trend tests for environmental parameters can be found in the results section.

The test is performed at a significance level of 0.1 for a two-tailed test, therefore a statistically significant trend is detected if the p value is lower than 0.1. The direction of the trend is determined by the tau value – a positive tau indicates an increasing trend while a negative tau value indicates a decreasing trend.

6. Nonmetric Multi-dimensional Scaling (NMDS)

The NMDS ordinations were performed using the *vegan* package in the R environment (version 4.0.0). The *metaMDS* function performs ordinations using Bray-Curtis dissimilarities to fit sites along 2 axes, while minimizing stress within each of the axes. The ordinations were run with auto-transformation to account for large abundance values (square root and Wisconsin double standardization). A maximum of 1000 runs were performed to find convergence among the runs, based on the Procrustes method. Stress values ranged from 0.23 to 0.29 in all runs which is relatively high for this type of analysis. Lower stress is preferred where stress below 0.2 is ideal, although stress between 0.2 and 0.3 can still be interpreted. The high stress values can be attributed to the similarities among the communities.

Analysis of similarities (ANOSIM) were performed to compare the similarity of assemblages at sites from different ecoregions as well as the different types of samples (test/reference). The *anosim* function from the *vegan* package was used with the benthic communities dataset as the input. The Bray-Curtis dissimilarity matrix from the NMDS plot was used to define distances between samples and 1000 permutations were performed for the p value approximation.

The null hypothesis for this test states that there is no difference in the similarities of communities between the categories, while the alternate hypothesis states that there exists differences in the similarities of communities between the categories.

7. Principal Components Analysis (PCA)

Habitat data were extracted from the CABIN database and included geology, stream characteristics, and climate data. These data are unverified and in some cases are incomplete; we retained the more reliable values that are derived from GIS work such as bedrock composition, precipitation, and temperature.

Water quality data was extracted from the ECCC water quality database, and included all measured parameters. Sites in the database are named according to their Envirodat codes and did not always match the CABIN names, therefore manual matching of the CABIN site names to the Envirodat codes was necessary. Non-detects in water quality values were excluded and a subset of parameters with valid values at most sites were included. Where some sites had multiple years of water quality data, the median value for each parameter was taken.

Principal components analyses (PCA) were performed in R (version 4.0.0) using the *vegan* package. Data were normalized prior to input into the PCA. The results were displayed in a PCA biplot which plots the distribution of each site within the first two principal components, along with vectors denoting the influence of the input parameters. Only the sites with complete data were retained for the PCA as this analysis does not handle missing data.

As a result, 63 sites of a possible 93 sites were included for the habitat PCA analysis and 70 of a possible 85 matched sites were included for the water quality PCA analysis.

Results

Descriptive Summary

Samples used in this report were collected during the period of 2006-2019. Average abundance was 2134 organisms; with 50% of samples in the range of 700 to 2500 organisms per sample. However, abundance varied substantially among samples, ranging from as few as 45 individuals at NF02ZC0022 (Seal Brook) in 2012 to almost 20,000 at SHO01 (Shoal Brook) in 2010.

Among the 93 samples there were a total of 23 Orders, 90 Families, and 203 genera observed consisting primarily of Insects. Collectively, EPT taxa made up 53.4% of all taxa counted among potential reference and test sites. The most abundant orders overall were Ephemeroptera, Diptera, and Trichoptera (**Figure 4**).

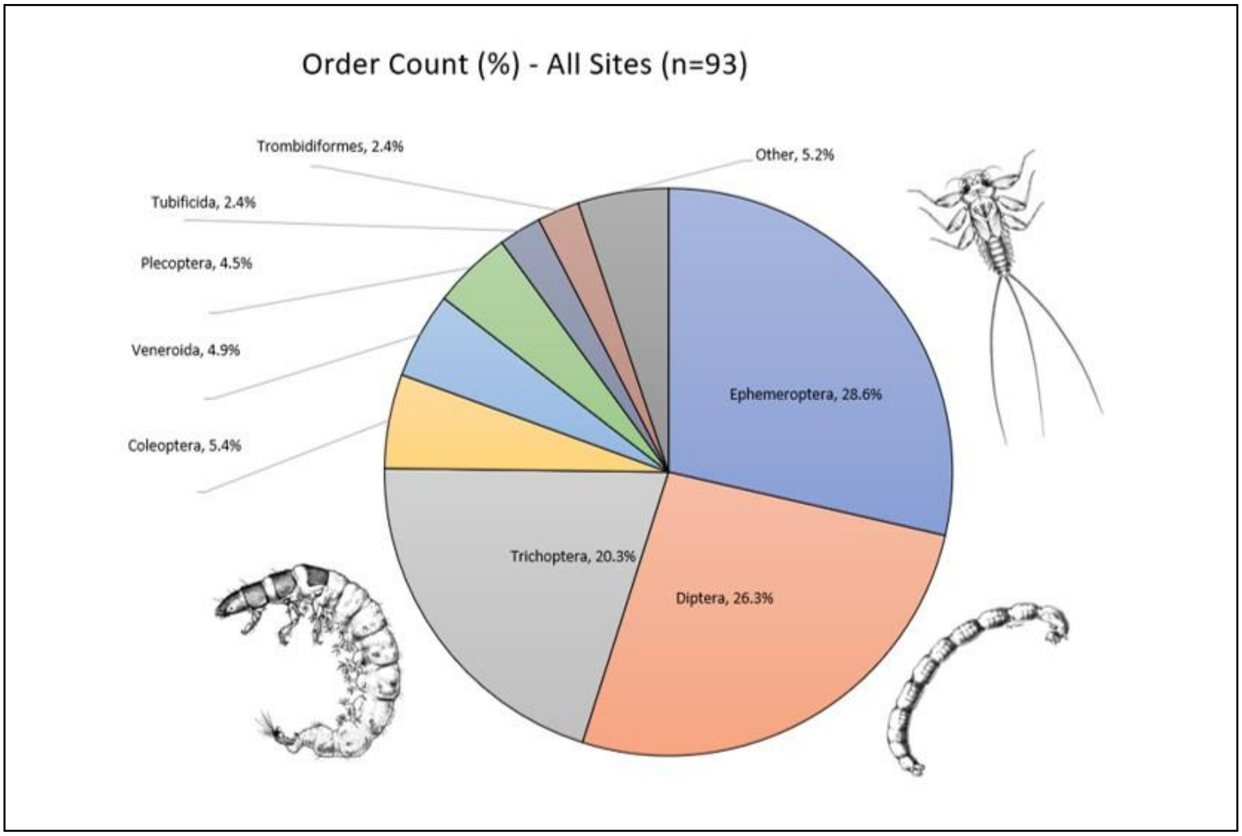


FIGURE 4. MOST ABUNDANT TAXA FOR ALL CABIN SAMPLING SITES (POOLED) IN NEWFOUNDLAND. ORDERS MAKING UP LESS THAN 2% OF TOTAL TAXA COUNT ARE COMBINED INTO "OTHER" CATEGORY. (IMAGES FROM WWW.MACROINVERTEBRATES.ORG)

Ephemeroptera is comprised mostly of a mix of Baetidae (small minnow mayflies), Leptophlebiidae (prong-gilled mayflies) and Heptageniidae (flat-head mayflies); Chironomidae (non-biting midges) accounting for most Diptera; and a range of taxa making up Trichopterans including Lepidostomatidae (a case-making caddis fly), Hydropsychidae (net-spinning caddisfly), and Philopotamidae (finger-net caddisfly). In terms of families, Chironomidae were the most abundant family (22.9%) followed by Baetidae (11.8%).

Commonly occurring taxa (found at >70% of all sites) include Chironomidae, Elmidae (riffle beetles), and a variety of EPT taxa (**Table 4**) reflecting a range of tolerances, functional feeding groups, and adaptations. Conversely, there were 43 taxa found at 5% or fewer of all sites; considered rare for the purposes of this report. Many of these rare taxa are generally uncommon in wadeable streams CABIN samples across Canada in part due to the targeted erosional habitat and timing of sampling. Therefore the low frequency of many of the rare taxa in the Newfoundland samples is typical, including Odonata (dragonflies and damselflies), Hirudinea (leeches), winter stoneflies (Nemouridae), Collembola (springtails) as well as a variety of Trichoptera, Coleoptera, and Diptera. However there were a few notable taxa that are observed more commonly in other provinces in the Atlantic Region that were identified as rare in this report (**Figure 5**). These findings support previous benthic research findings on the island of Newfoundland that concluded a lower diversity of taxa (Colbo eta l. 1997; Smith, 2009).

Table 4. List of common taxa found in report dataset (found at >70% of all sites)

Common Taxa		
Family	**Common name**	**Image**
Baetidae	Small minnow mayflies	
Leptophlebiidae	Prong-gilled mayflies	
Heptageniidae	Flat-head mayflies	
Rhyacophilidae	Free-living caddisflies	
Lepidostomatidae	Case-making caddisflies	
Hydropsychidae	Net-spinning caddisflies	
Philopotamidae	Finger-net caddisflies	
Chironomidae	Midges	
Empididae	Dance flies	
Elmidae	Riffle beetles	

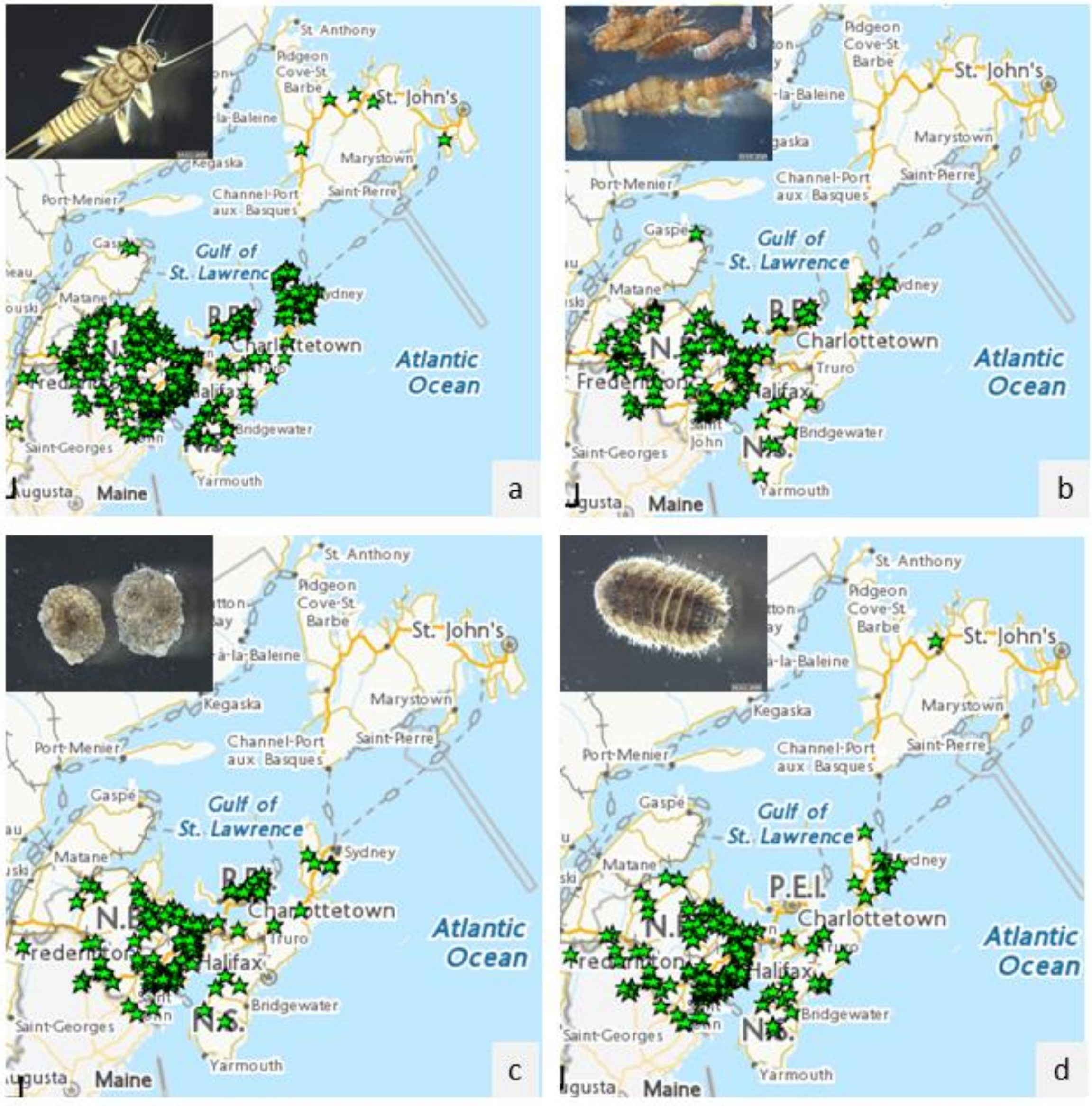


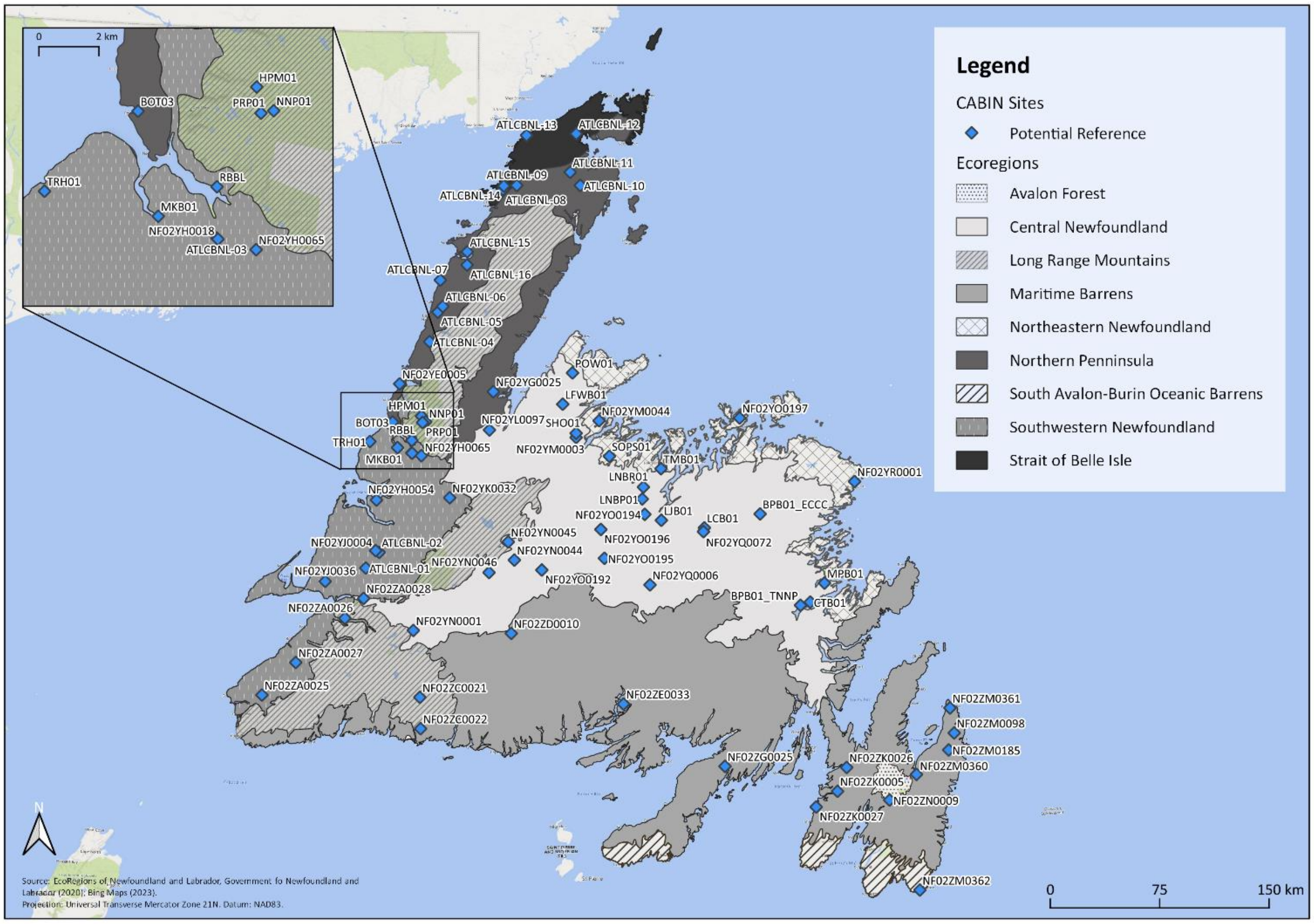
Figure 5. Distribution of select taxa demonstrating differences in assemblages between the island of Newfoundland and the rest of Atlantic Canada; a) Perlidae, b) Athericidae, c) Helicopsychidae, and d) Psephenidae. Maps retrieved from CABIN database, photo credits A. Martens (National CABIN Taxonomist).

Research Objective #1:

Are benthic communities in reference areas different across ecoregions on the island of Newfoundland?

In order to be able to determine if benthic communities from reference areas are different across ecoregions of Newfoundland, we performed:

1. a brief comparison of the taxonomic composition;
2. non-metric multidimensional scaling (NMDS) ordinations;
3. principal components analyses (PCA) on the habitat and water quality data to determine if differences exist between sites of different ecoregions.



Source: EcoRegions of Newfoundland and Labrador, Government fo Newfoundland and Labrador (2020); Bing Maps (2023).
Projection: Universal Transverse Mercator Zone 21N. Datum: NAD83.

FIGURE 6. MAP OF CABIN REFERENCE SITES AND NEWFOUNDLAND ECOREGIONS.

COMPARISON OF THE TAXONOMIC COMPOSITION ACROSS ECOREGIONS

The dominant taxa at reference sites for most ecoregions across Newfoundland is either Chironomidae or Baetidae. This is consistent with the overall dominant taxa for the entire island discussed in the previous section (see **Figure 4**). Using the metrics that we calculated we first take a broad look at the taxonomic composition of reference sites across ecoregions. We look at two specific metrics, % EPT and % Chironomidae (PercentEPT and PercentChiro in **Figure 7**). We find that % EPT varies across the different ecoregions, with the Long Range Mountains and

Southwestern Newfoundland having the highest median relative abundance of EPT taxa, and Northeastern Newfoundland, Northern Peninsula, and the Strait of Belle Isle ecoregions having the lowest % EPT. Some ecoregions had high variability among its sites, for example Central Newfoundland had sites with % EPT ranging from under 25% to just over 90%. Generally, as EPT and Chironomidae taxa were the most dominant taxa across all ecoregions, where % EPT was lower, % Chironomidae tends to be elevated. This can be seen in the example of the Maritime Barrens, which had lower % EPT but higher % Chironomidae relative to other ecoregions.

However, there were a few exceptions that can be seen in the boxplots. The Northeastern Newfoundland and Strait of Belle Isle ecoregions had the lowest median % EPT values, but also did not have elevated % Chironomidae values. Instead, the communities at certain sites in these ecoregions were dominated by Pisidiidae (pea clams). The relative abundances of Pisiididae in Northeastern Newfoundland was 27% and in Strait of Belle Isle was 24.3%. The Northern Peninsula also had high relative abundance of Pisidiidae (11.3 %).

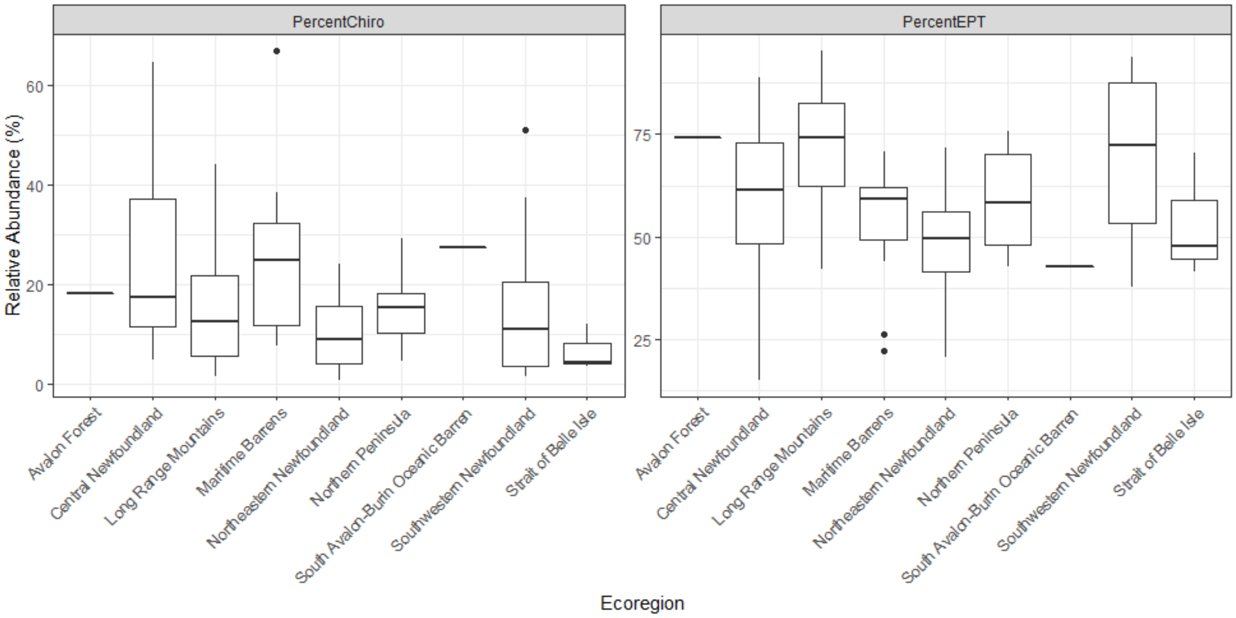


FIGURE 7. BOXPLOTS OF % EPT AND % CHIRONOMIDAE TAXA AT SITES, GROUPED BY THEIR ECOREGIONS.

Pisidiidae prefer softer substrate or sediment (Maine Department of Environmental Protection, 2019), typically found more abundantly in slower moving waters, such as ponds, and lakes or slow areas of streams. Several of the sites in the Northeastern Newfoundland and Strait of Belle Isle ecoregions appear to be located close to the outflow of ponds, lakes, and in wetland areas which may explain the high abundance of Pisidiidae. PCA analysis (see below) also shows that sites in the above ecoregions have lower slopes overall, which tend to be slower moving lower gradient streams where sediment is more likely to accumulate. This is likely due to the site selection process. The landscape in the Northern Peninsula of the island of Newfoundland (encompassing the Northern Peninsula and Strait of Belle Isle ecoregions) consists mainly of low lying areas where slow-flowing waters are likely to contain more Pisidiidae. Meanwhile, one site in the Northeastern Newfoundland ecoregion drove the elevated relative abundance of Pisidiidae, where this taxa made up over 60% of the community there.

Looking into specific families, the more northern ecoregions (Long Range Mountains, Northeastern Newfoundland, Northern Peninsula, and Strait of Belle Isle) all had around approximately 4-5 % relative abundance of Leuctridae (a type of winter stonefly), while all other ecoregions had even fewer (or none) found in samples. Conversely, apparent similarities in community composition are observable in southern ecoregions. Central Newfoundland, Maritime Barrens, and Southwestern Newfoundland reference sites have similar compositional breakdowns of Chironomidae as dominant, followed by Leptophlebiidae/Baetidae, Heptageniidae, and Lepidostomatidae.

NON-METRIC MULTIDIMENSIONAL SCALING ORDINATIONS RESULTS

Non-metric multidimensional scaling (NMDS) ordinations were created using the benthic community data at potential reference sites to determine if there are differences in the overall communities between sites belonging to the different ecoregions.

The ordination shows that most of the potential reference sites fall within one main cluster, with a few sites lying further from the main cluster (see **Figure 8**). For example, the site LNBR01 (Little New Bay River) lies further from the main cluster due to its higher abundance of the family Leuctridae, which is much higher than any other reference site included in this analysis. Other similarities can be noted, for example there is a slight grouping of Northern Peninsula and Strait of Belle Isle sites in the left half of the plot. As noted in the previous section, the benthic communities in these ecoregions are similar as they both have higher abundances of Pisidiiae and Leuctridae.

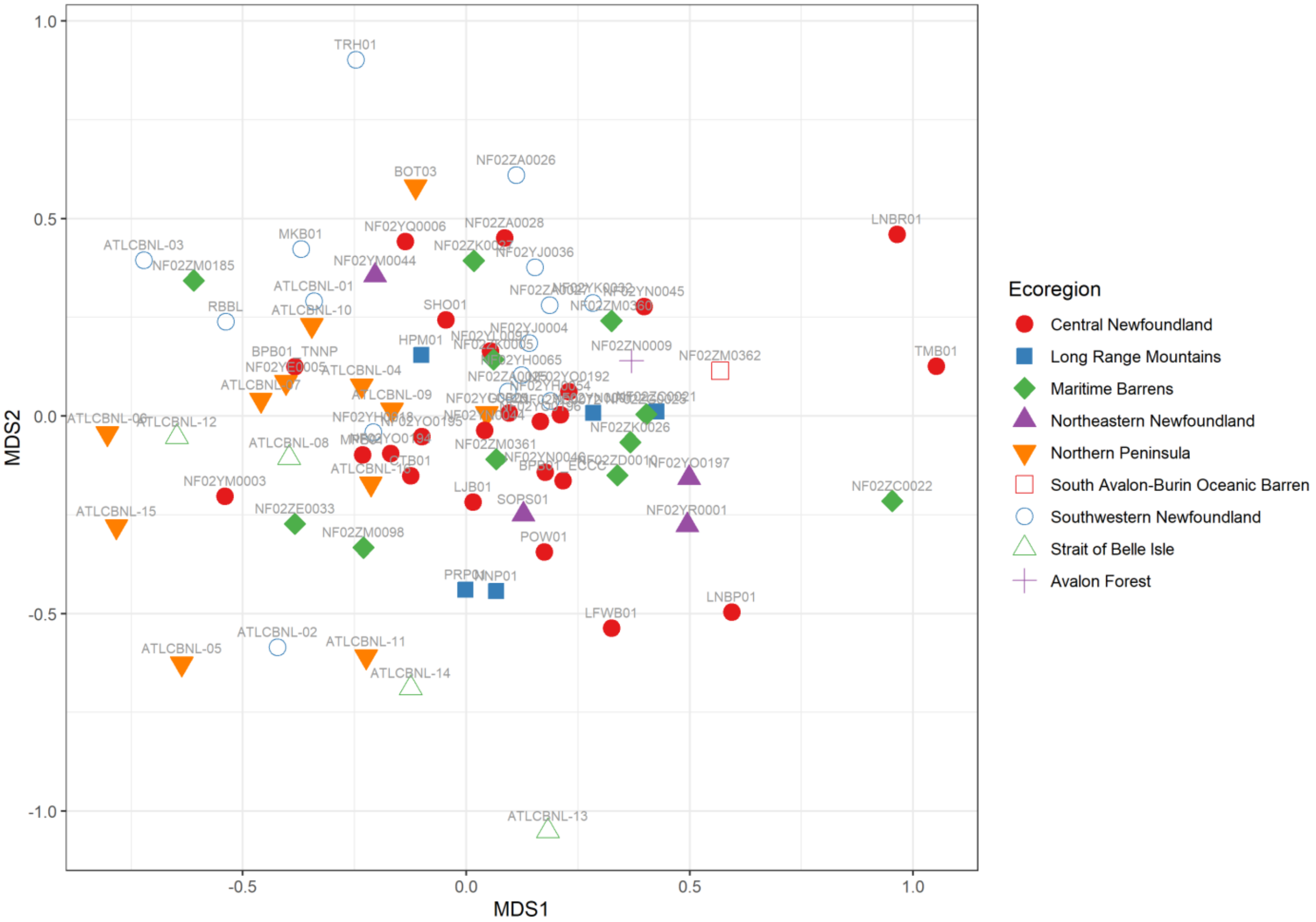


FIGURE 8. NMDS ORDINATION PLOT FOR POTENTIAL REFERENCES SITES. SYMBOLS ARE USED TO DISTINGUISH SITES BY ECOREGION.

The analysis of similarities (ANOSIM) result (p = 0.057) compares how similar the sites in each Ecoregion against how similar they are to sites in other Ecoregions. This can help identify Ecoregions where all sites have fairly distinct communities from other Ecoregions, or Ecoregions where sites have very different communities from each other. The results show that there does not exist a significant difference in the similarities of sites within each Ecoregion at the 0.05 significance level. Nonetheless, there does exist some variability in the similarity rankings between the various ecoregions.

The Northeastern Newfoundland sites appear to be more different from each other than would be expected when compared against the between-group similarities (**Figure 9**). Looking more closely at the communities at sites in this ecoregion, we see that one of the Northeastern Newfoundland sites had high proportions of Pisidiidae while the remaining sites had very low abundances of this family. Due to a relatively small sample size (four reference sites), this difference likely explains the greater dissimilarities among sites within this ecoregion. Analyses in the future would benefit from more potential reference samples in the Northeastern Newfoundland ecoregion as well as in other ecoregions with few reference sites to avoid undue influence from outliers.

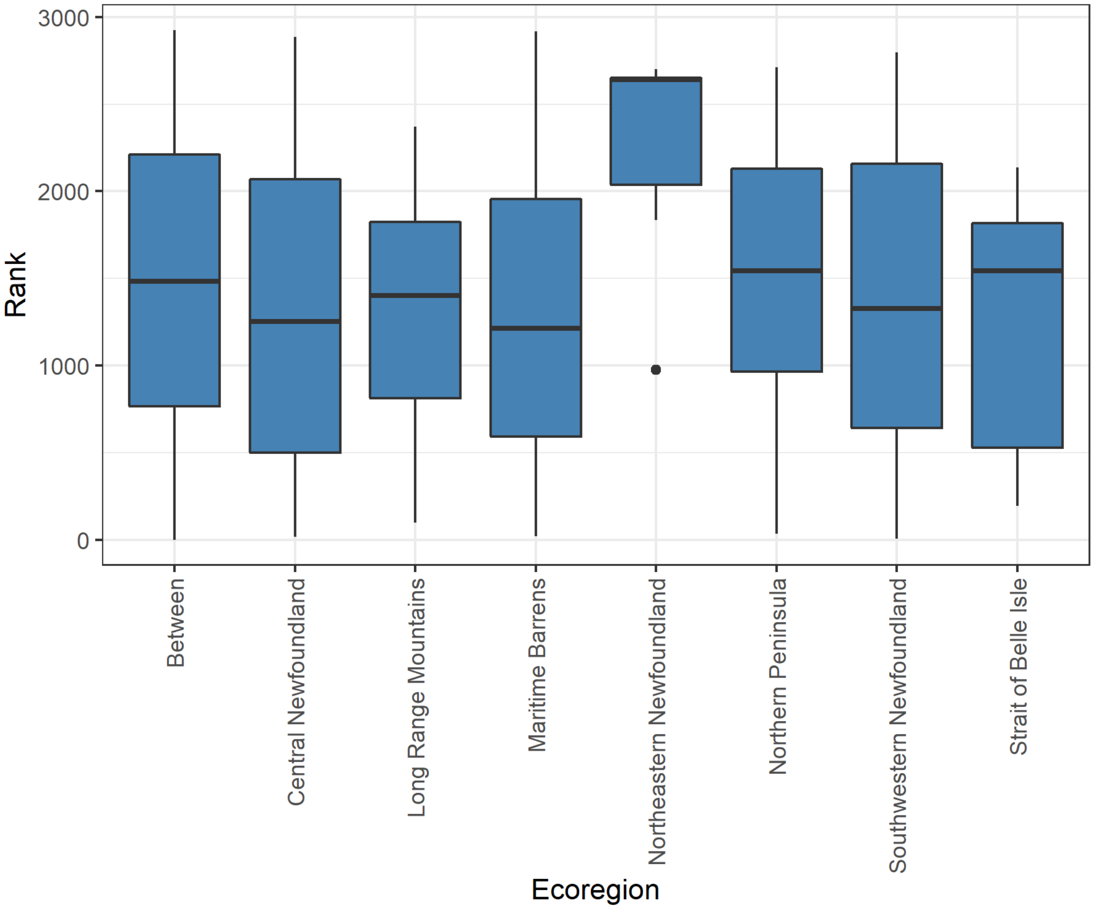


FIGURE 9. BOXPLOTS OF BRAY-CURTIS DISSIMILARITY RANKS BETWEEN SITES OF EACH OF THE ECOREGIONS.

PRINCIPAL COMPONENTS ANALYSES (PCA) RESULTS

Do variations in habitat and water quality agree with differences in communities across ecoregions? We performed principal components analyses (PCA) on the habitat and water quality data at the potential reference sites to investigate whether there are notable differences in habitat and water quality variables between sites of different ecoregions. Subsets of parameters were taken from the habitat and water quality datasets which represent the most relevant and complete parameters. Completeness of the data was important as the PCA method is unable to handle missing values (refer to Methodology and Data Analysis section for more details).

This first PCA plots reference sites on the bi-plot according to a subset of habitat data at each site (**Figure 10**). The first PCA dimension (x-axis) is defined mainly by the geology and slope of the site, while the second dimension (y-axis) is defined primarily by the mean long-term temperature and mean annual precipitation at the sites. Keep in mind that the arrows point towards increasing values of the labelled parameter. We can see that Central Newfoundland sites tend to plot in the upper quadrants and are defined by higher long-term temperatures and lower mean precipitation. Conversely, the Maritime Barrens sites are plotted in the lower quadrant with greater mean precipitation and lower long-term temperatures. Sites tend to cluster with others in their ecoregion and distinct clusters are present, indicating that sites within each ecoregion tend to have similar habitat variables.

One interesting result from the PCA of habitat variables is that some of the Northern Peninsula and Strait of Belle Isle sites cluster together closely. These sites generally have lower slopes than many other sites and similar climate variables. Based on information from these sites, it is known that the sites are generally located in regions of gentle relief where there exist many ponds and wetland areas. As previously noted, the shallower slopes at these sites may explain the greater dominance of Pisidiidae (pea clams) as they are more frequently found in ponds, wetland areas, and slower flowing sections of rivers.

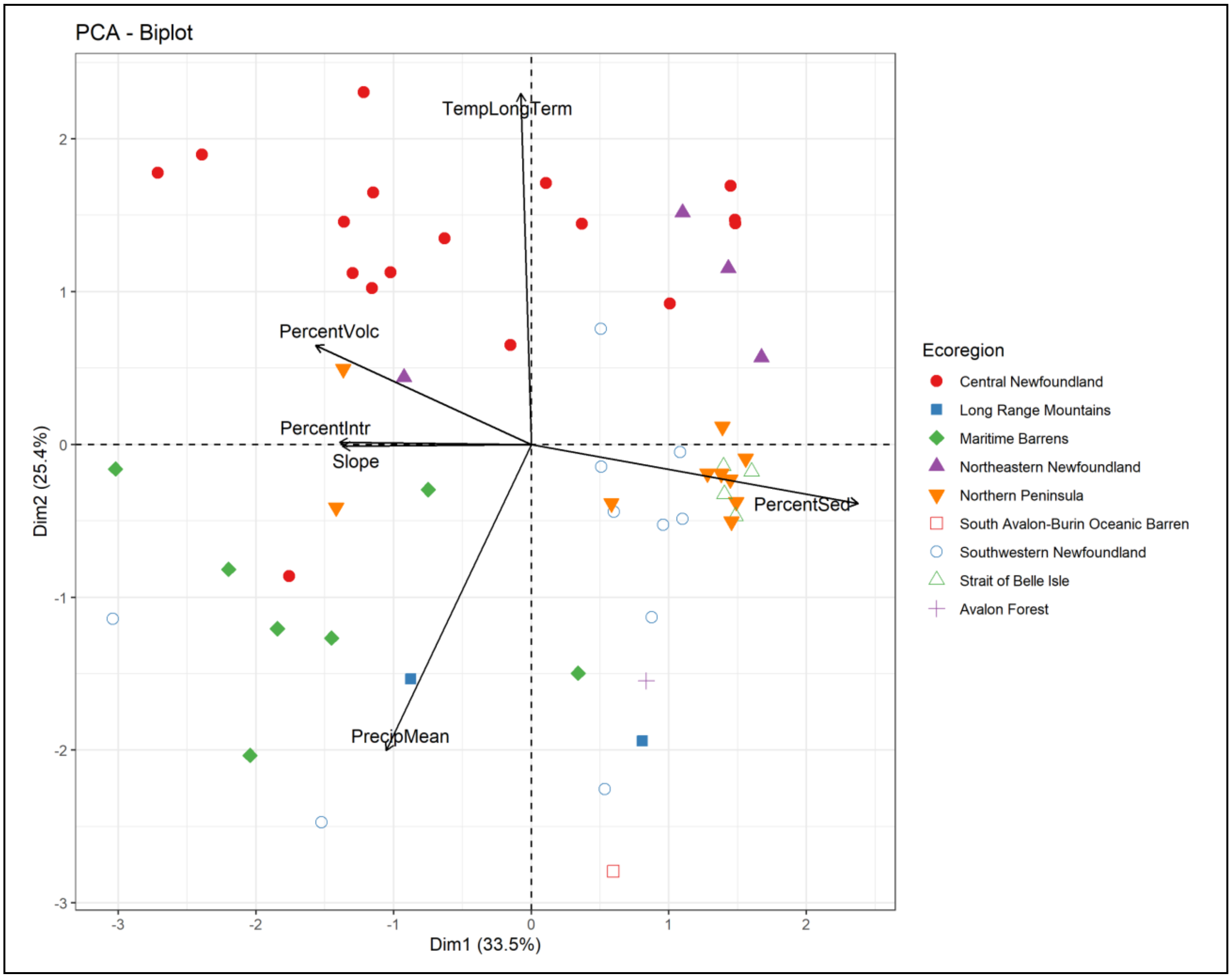


FIGURE 10. PCA BIPLOT OF A SUBSET OF HABITAT PARAMETERS AT POTENTIAL REFERENCE CABIN SITES.

The second PCA (**Figure 11**) plots sites in the bi-plot space using a subset of their water quality parameters. The first PCA dimension (x-axis) is defined mainly by major ions (chloride, calcium, magnesium), while the second PCA dimension (y-axis) is defined primarily by total nitrogen, total phosphorus, total organic carbon, and copper. As expected, these reference sites plot mainly in the upper half of the bi-plot, indicating lower concentrations of almost all parameters. Of the sites that plot within the lower quadrants, they consisted mainly of sites from the Long Range Mountains, Northern Peninsula, and Strait of Belle Isle. These sites may have more distinct water chemistries due to their sedimentary geology as seen in the above habitat plot. The two sites which are found near the bottom of the plot area, POW01 (Central Newfoundland) and ATLCBNL-15 (Northern Peninsula), have higher concentrations in several water quality parameters including total nitrogen and total phosphorus. Despite the distinct water quality profiles at these two sites there are no notable differences in their benthic communities when compared to other sites from within their respective ecoregions.

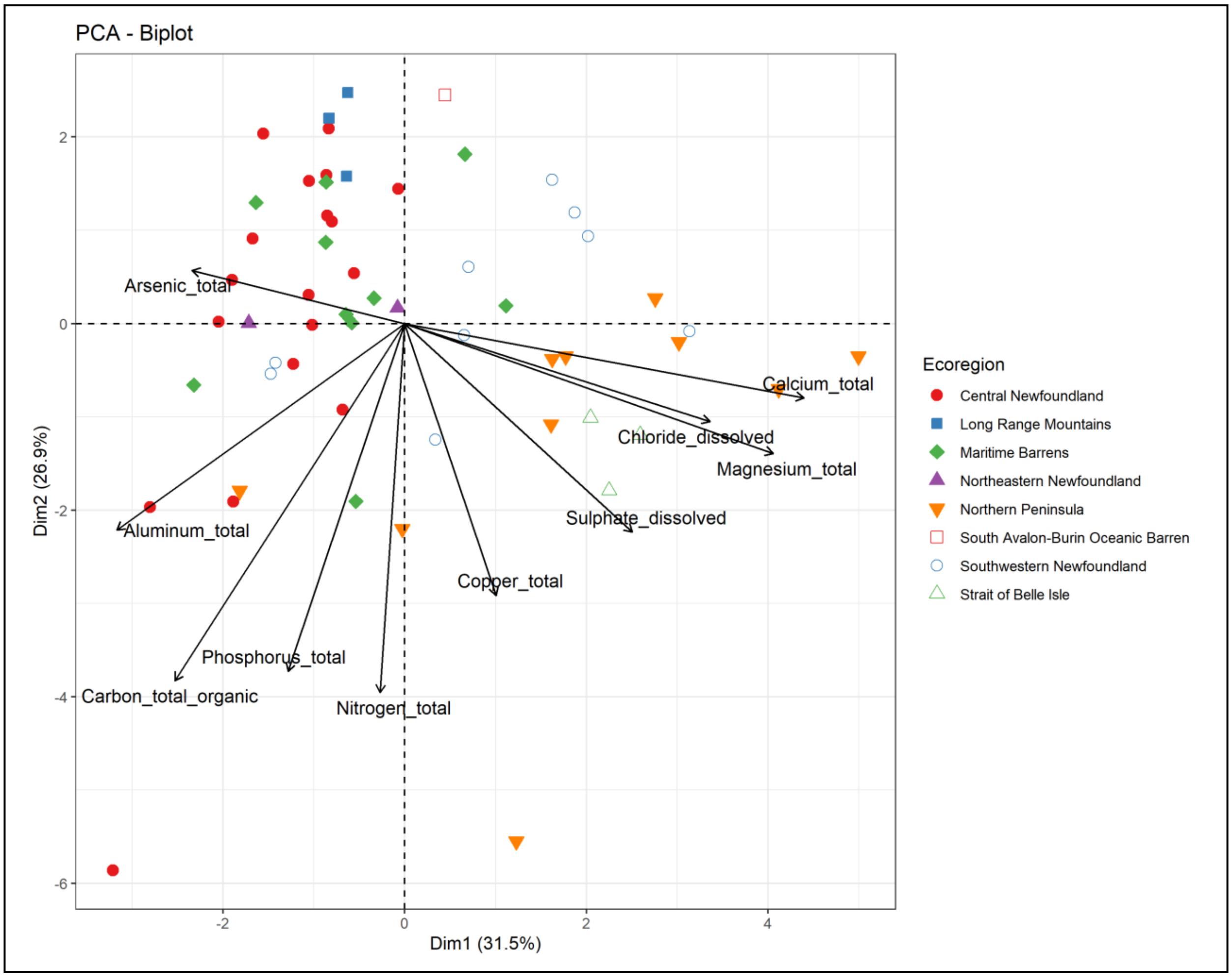


FIGURE 11. PCA BIPLOT OF A SUBSET OF WATER QUALITY PARAMETER VALUES AT POTENTIAL REFERENCE SITES.

RESEARCH OBJECTIVE #1: TAKE-HOME MESSAGES

Are benthic communities in reference areas different across ecoregions on the island of Newfoundland?

Our analysis showed some differences in dominant taxa between ecoregions when looking at the taxa groups individually. Dominant taxa are Chironomidae (non-biting midges) and Baetidae (small minnow mayfly) for most ecoregions, exception of Pisidiidae (pea clam) for Belle Isle and Northeastern Newfoundland. This was supported by the interpretations of the % EPT and % Chironomidae metric results for reference sites. Sites in most ecoregions showed a range of values for % EPT and % Chironomidae. We also noted some similarities:

- Comparable EPT taxa relative abundance between Central Newfoundland, Maritime Barrens, and Southwestern Newfoundland;
- and slightly higher relative abundance of the winter stonefly Leuctridae in more northern ecoregions.

The non-metric multidimensional scaling analysis showed only some weak grouping. However, one interesting finding is that benthic communities from the Northern Peninsula and Strait of Belle Isle showed similarities in their benthic community. In addition, the principal components analyses found that sites from these ecoregions are fairly similar in both habitat parameters and water quality conditions. Notably, Pisidiidae (pea clams) were more abundant in the Northern Peninsula and Strait of Belle Isle and likely relate to the conditions at these sites as the relief of the region is relatively gentle and ponds and wetland areas are common.

CABIN SAMPLING SITE UNNAMED RIVER NORTH OF SECOND SALMON POND (NF02YB0014 / ATLCBNL-11) IN STRAIT OF BELLE ISLE REGION (CREDIT: ECCC)

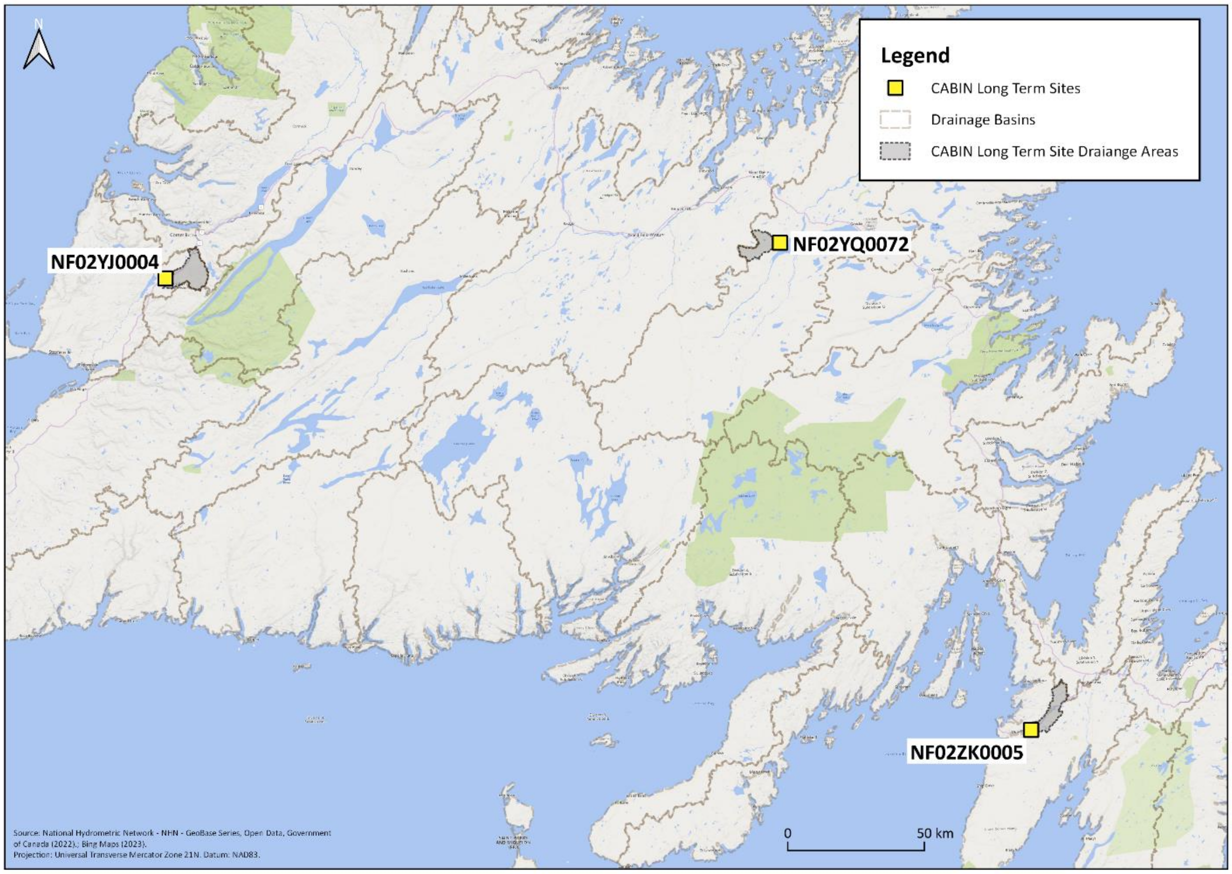
Research Objective #2:

Are there changes in benthic communities at three long-term reference sites over time?

This research objective focuses on the exploration of changes in the benthic communities of long-term CABIN sites over time. The aim here is to identify whether there are long-term trends in the ARM results and benthic metrics, as well as any trends in environmental parameters such as air temperature, water quality, and/or hydrological flow at these sites. The results of trend analysis in environmental parameters at these sites may help support the interpretation of benthic community results.

Trend analyses were performed on the ARM results and benthic metrics as well as environmental parameters for the three long-term biomonitoring sites (see **Figure 12** below). Below are some key facts about each site:

- **Pinchgut Brook** (NF02YJ0004): Located in Western Newfoundland, this site was sampled from 2008 to 2019 with a gap in 2009-2010. The watershed is 130.5 km^2 in area and the watershed is primarily forested. The main human activity in the watershed is recreational use such as ATVs, snowmobiles, hunting, and fishing. There is a small number (~50) of year-round residents at Pinchgut Lake, upstream of the site, although the number of residents is increasing. There are no agricultural or industrial activities and only 2 small quarries in the watershed.
- **Careless Brook** (NF02YQ0072): Located in Central Newfoundland, this site was sampled from 2010 to 2019. The watershed is 78.9 km^2 in area and is minimally disturbed. Forest access roads and several small quarries represent the greatest impacts to this site.
- **Northeast River** (NF02ZK0005): Located in Eastern Newfoundland, this site was sampled from 2009 to 2019 with a gap in 2010. The watershed is 93 km^2 in area and the main watershed activities include industrial activities with an active asphalt and cement plant at the mouth of the watershed, and recreational, with a private RV park and numerous cottages located in the watershed, and small amounts of historical logging and quarrying.



Source: National Hydrometric Network - NHN - GeoBase Series, Open Data, Government of Canada (2022).; Bing Maps (2023).
Projection: Universal Transverse Mercator Zone 21N. Datum: NAD83.

FIGURE 12. MAP OF THE THREE LONG-TERM BIOMONITORING REFERENCE SITES IN NEWFOUNDLAND.

TREND ANALYSIS FOR BENTHIC COMMUNITY METRICS

The following are the Mann-Kendall trend test results for the ARM results and benthic metrics at each of the three long-term sites. See **Appendix 3** for detailed trend test parameters for all metrics. At each site, detailed visual examination of trends in the main taxonomic families are also included, along with a brief interpretation of those trends.

Here we focus on several key metrics, namely Richness, EPT taxa, Chironomidae taxa, GOID taxa, and the Diversity, Evenness, CEFI, and HBI metrics. A brief description of the purpose of these metrics can be found in the methodology section and a full description of each metric can be found in **Appendix 2**.

Pinchgut Brook (NF02YJ0004) exhibited a trend for only two of the benthic metrics: an increase in Total Abundance and Total EPT counts (**Table 5**). Increases in EPT abundance and total abundance could be indicative of increasing nutrient inputs. In oligotrophic (nutrient poor) rivers and streams, nutrient enrichment can lead to elevated benthic macroinvertebrate abundance though may result in loss of diversity of EPT taxa (Minshall et al., 2014). Trend analyses of water quality (later in this section) identified an increasing trend in total nitrogen. Furthermore, while no trends were detected for % GOID, it is worth noting that the abundance of oligochaetes (worms) were comparatively high in 2017 (count of 283) and 2019 (count of 2010) (**Figure 13**). Future samples may reveal whether the relative abundance of oligochaetes continues to increase.

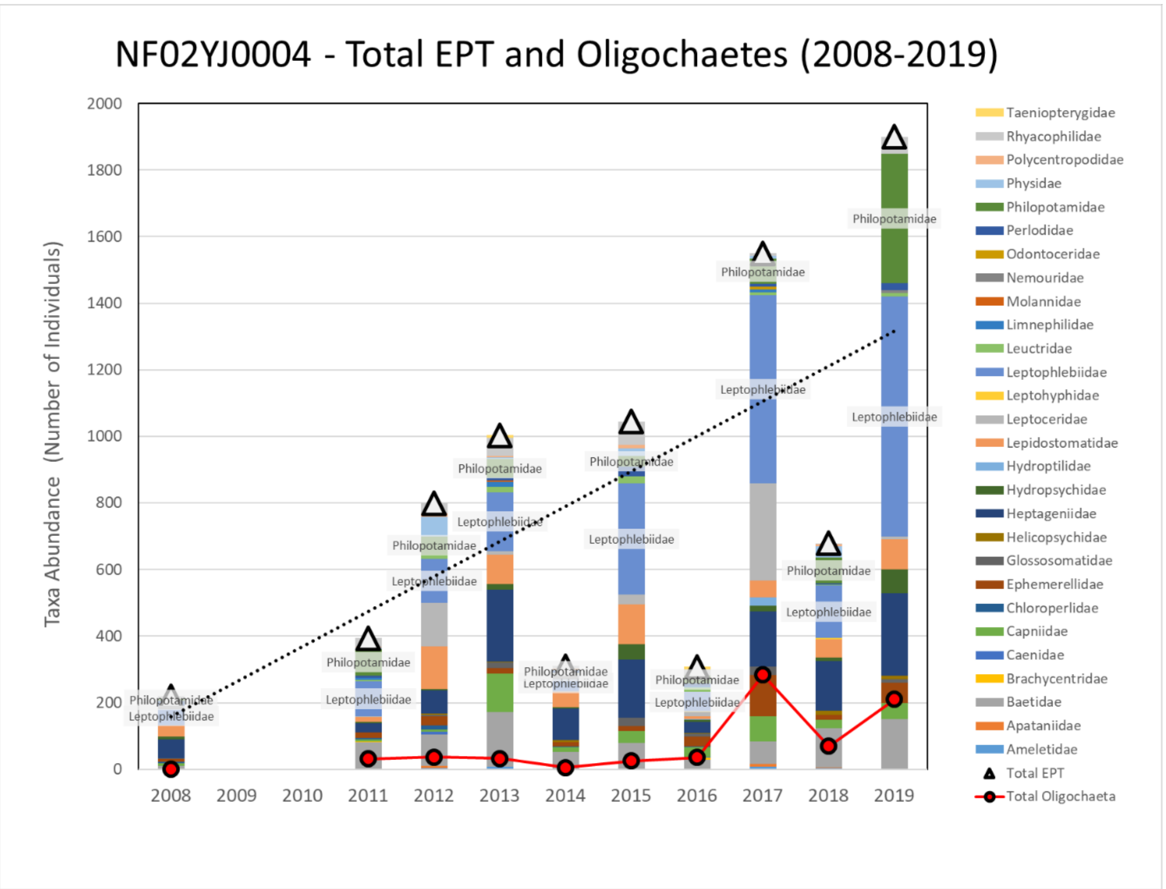


FIGURE 13. TRENDS IN EPT AND OLIGOCHAETE ABUNDANCES IN CABIN SAMPLES FROM PINCHGUT BROOK.

Careless Brook (NF02YQ0072) showed numerous trends in the ARM results and metrics. This site exhibited a decrease in the HBI ARM result, as well as decreases in the Dominance, % EPT, and EPT/Chironomidae + EPT metrics (**Table 5**). At the same time, both Chironomidae metrics (total and %), both GOID metrics (total and %), and all Diversity and Evenness metrics increased over the 10 year period. The metric calculations for HBI, distinct from the ARM HBI results, also showed an increasing trend. Note that due to modifications made for the ARM calculations, the decreasing HBI ARM result and increasing HBI benthic metric come to the same conclusion.

The benthic metric trends appear to highlight a distinct shift where the community is changing from one of highly dominated by a few EPT taxa (i.e. Lepidostomatidae and Leptophlebiidae) towards one that has greater amounts of chironomid and GOID taxa as well as greater overall diversity and evenness. The detailed figure (**Figure 14**) shows a consistent change from year to year in the relative abundances of EPT and Chironomidae taxa. The trends in the HBI results suggest that the benthic community is shifting to one with greater tolerance to organic pollutants as the taxa used in this index are weighted according to their tolerances.

The results are interesting as human disturbances in the watershed appear to be minimal. It may be that there are disturbances that have yet been undetected, or that changes may be due to shifts in the regional climate.

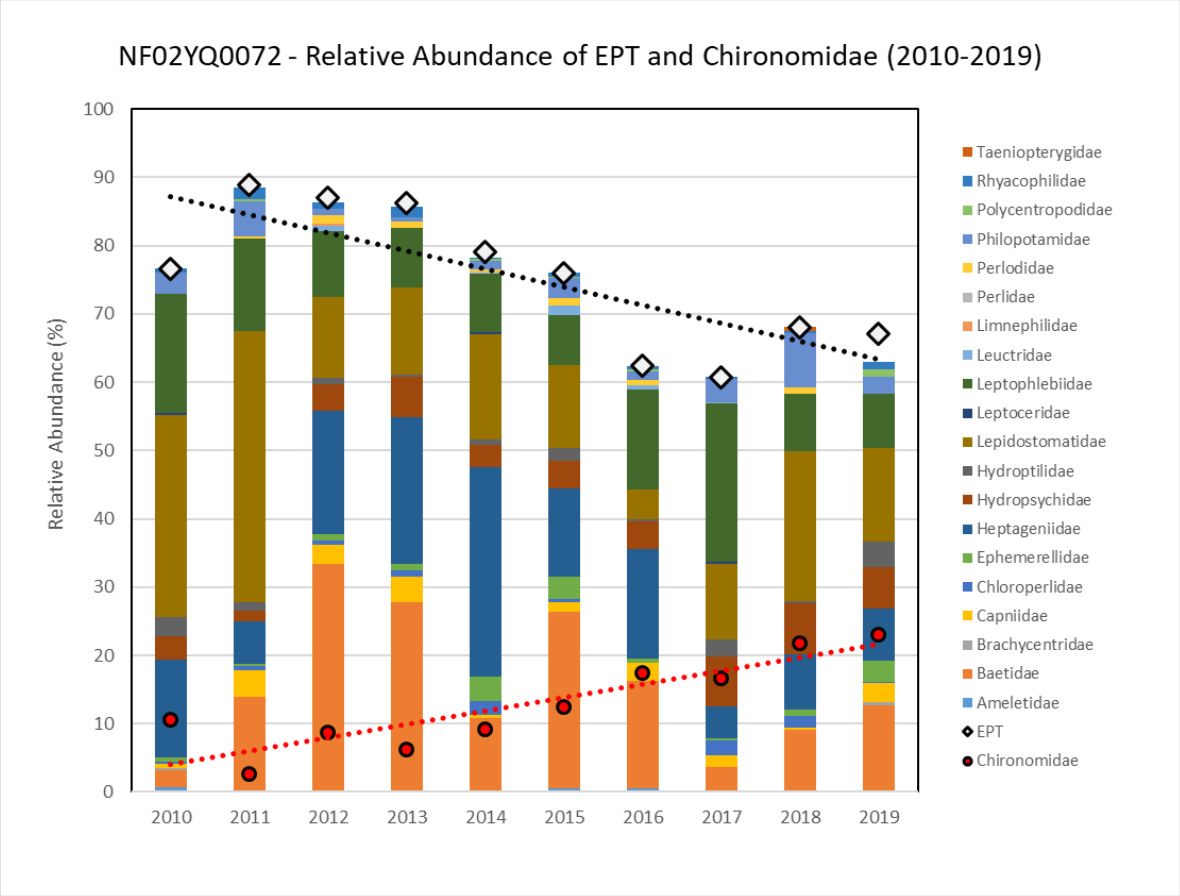


FIGURE 14. TRENDS IN EPT AND OLIGOCHAETE RELATIVE ABUNDANCES IN CABIN SAMPLES FROM CARELESS BROOK.

Northeast River (NF02ZK0005) showed increases in four of the seven ARM results: Shannon-Wiener Diversity, Simpson's Diversity, Evenness, and Berger-Parker dominance. Similar trends in the benthic metrics results emerged, with decreasing Dominance and % EPT while both GOID metrics (total and %) and all Diversity and Evenness metrics increased over the past 10 years (**Table 5**). In 2009, the EPT families Lepidostomatidae, Heptageniidae, and Baetidae dominated the sample and lacked abundance of several other EPT taxa were observed in higher proportion throughout the remainder of the data series. EPT taxa appear variable over time, however Lepidostomatidae was particularly high in 2009 compared to other years and may account for some of the decline in % EPT (**Figure 15**). The decreasing trend in EPT coincides with an increasing trend in % GOID due to higher proportions of the worms Enchytraidae in 2016 (9.2%), and Lumbriculidae in 2018 (12.3%) and 2019 (9.4%).

Similar to Careless Brook, the benthic community at Northeast River appears to be undergoing a change from one that was dominated by EPT taxa towards greater diversity and evenness.

TABLE 5. RESULTS OF MANN-KENDALL TREND TESTS FOR METRICS AT LONG-TERM SITES

	Pinchgut Brook NF02YJ0004	**Careless Brook** NF02YQ0072	**Northeast River** NF02ZK0005
ARM Metrics	**Observed Trend**		
Richness	No trend detected	No trend detected	No trend detected
Shannon-Wiener Diversity	No trend detected	No trend detected	**Increase**
Simpson’s Diversity	No trend detected	No trend detected	**Increase**
Peilou’s Evenness	No trend detected	No trend detected	**Increase**
Berger-Parker Dominance	No trend detected	No trend detected	**Increase**
CEFI	No trend detected	No trend detected	No trend detected
HBI	No trend detected	**Decrease**	No trend detected

Benthic Metrics	**Observed Trend**		
Total Abundance	**Increase**	No trend detected	No trend detected
Total Richness	No trend detected	No trend detected	No trend detected
Dominance	No trend detected	**Decrease**	**Decrease**
Total EPT	**Increase**	No trend detected	**Increase**
% EPT	No trend detected	**Decrease**	**Decrease**
Total Chironomidae	No trend detected	**Increase**	No trend detected
% Chironomidae	No trend detected	**Increase**	No trend detected
Total GOID	No trend detected	**Increase**	**Increase**
% GOID	No trend detected	**Increase**	**Increase**
EPT/ Chironomidae + EPT	No trend detected	**Decrease**	No trend detected
Simpson’s Diversity	No trend detected	**Increase**	**Increase**
Simpson’s Evenness	No trend detected	**Increase**	**Increase**
Shannon-Weiner Diversity	No trend detected	**Increase**	**Increase**
Pielou’s Evenness	No trend detected	**Increase**	**Increase**
HBI	No trend detected	**Increase**	No trend detected
CEFI	No trend detected	No trend detected	No trend detected

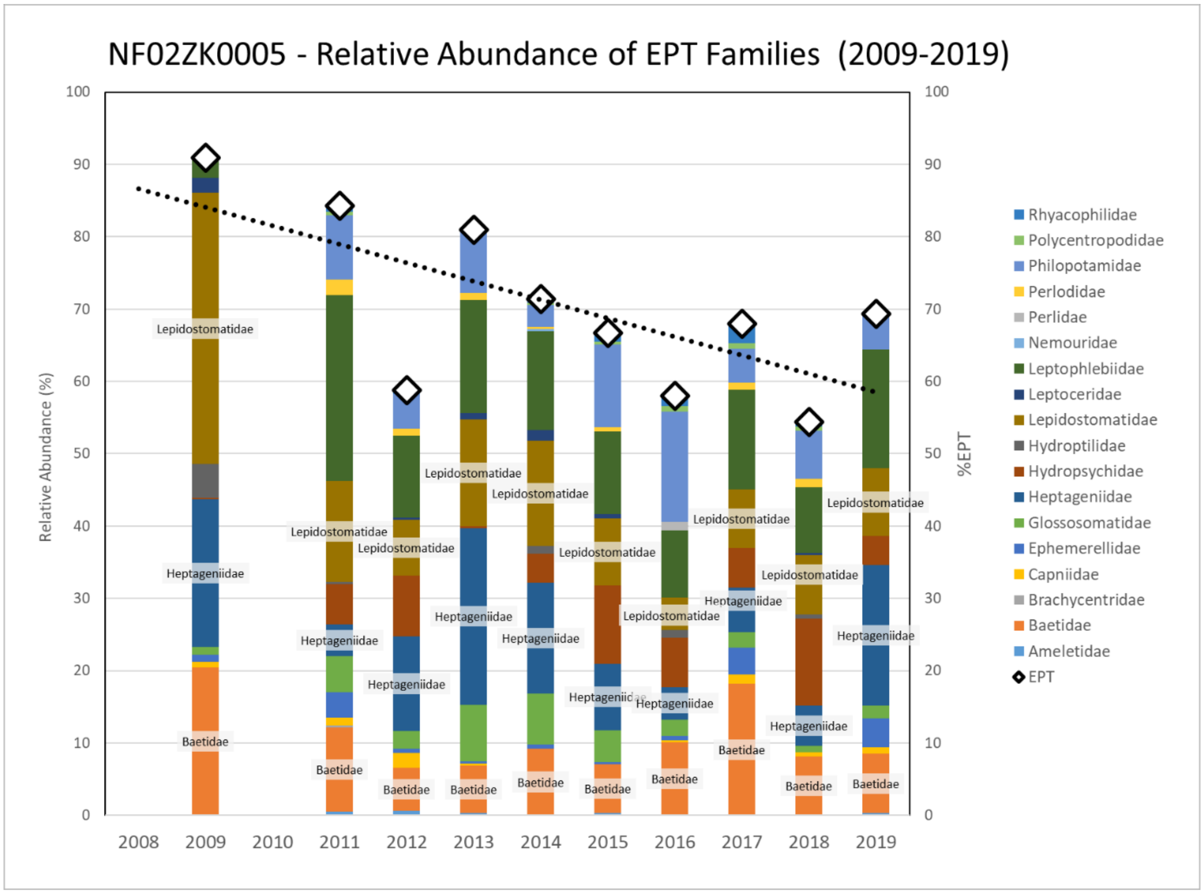


FIGURE 15. TRENDS IN EPT RELATIVE ABUNDANCES IN CABIN SAMPLES FROM NORTHEAST RIVER.

TREND ANALYSIS FOR ENVIRONMENTAL DATA

Mann-Kendall trend tests were performed on environmental data to determine whether changing environmental conditions may correspond to changes observed in the benthic macroinvertebrate communities. The environmental parameters that were selected include hydrological flow measurements, water quality from grab samples, and air temperature from nearby climate stations.

TRENDS IN FLOW DATA

In order to examine the potential influence of changing environmental conditions, a Seasonal trend test was performed on monthly and annual mean flow data at Northeast River (NF02ZK0005). This was the only site where a hydrometric station was co-located with the CABIN site and recorded flow data over the same period during which CABIN samples were collected. The Seasonal trend analysis did not detect a trend in the mean annual flow or any of the mean monthly flows at a significance level of p = 0.1.

TRENDS IN WATER QUALITY DATA

Trend tests on water quality parameters were performed at each site on a set of parameters consisting of major ions, nutrients, and metals. Both Pinchgut Brook and Careless Brook showed several trends in water quality parameter over the period of CABIN sampling while no trends were detected at Northeast River (**Table 6**). Increases in chloride concentrations were common to both sites with trends, while Pinchgut Brook showed decreases in TOC and increases in total nitrogen and Careless Brook had a decrease in lead concentrations. Interestingly, total abundances of the benthic community at Pinchgut Brook have been increasing in recent years and may be related to increases in nitrogen concentrations at this site which may be increasing productivity.

It is possible that human activity within the watersheds of these sites are changing, leading to changes in the observed water quality. The watershed of Pinchgut Brook appears to show increasing recreational use which may explain the trends in water quality parameters, although Careless Brook appears to have very minimal human impacts. It's unclear whether the trends in water quality parameters at Careless Brook relate to human activity.

TABLE 6. RESULTS OF SEASONAL MANN-KENDALL TREND TESTS WATER QUALITY

	Pinchgut Brook NF02YJ0004	**Careless Brook** NF02YQ0072	**Northeast River** NF02ZK0005
Water Quality Parameter	**Observed Trend**		
Aluminum, total	No trend detected	No trend detected	No trend detected
Copper, total	No trend detected	No trend detected	No trend detected
Lead, total	No trend detected	**Decrease**	No trend detected
Calcium, total	No trend detected	No trend detected	No trend detected
Chloride, total	**Increase**	**Increase**	No trend detected
Carbon, total organic	**Decrease**	No trend detected	No trend detected
Nitrogen, total	**Increase**	No trend detected	No trend detected
Phosphorus, total	No trend detected	No trend detected	No trend detected

TREND IN AIR AND WATER TEMPERATURE DATA

Trend analysis was performed using air temperature data from nearby Environment and Climate Change Canada weather stations. Weather station data was obtained by using the *weathercan* R package which gathers data from the ECCC website and imports it to R (LaZerte and Albers, 2018). Results are presented in the following table (**Table 7**). The Seasonal Mann-Kendall test was used for the air temperature data with each month considered a season.

Mean monthly air temperature follows a statistically significant decreasing trend for the Pinchgut Brook (NF02YJ0004) and Careless Brook (NF02YQ0072) sites while no trend was detected at the Northeast River site (NF0ZK0005). Thiel-Sen slopes, which estimate the rate of change in air temperature, showed decreases of 0.064 and 0.080 degrees Celsius per year for Pinchgut Brook and Careless Brook respectively. The two sites where air temperature trends were detected are both located on the west coast and central regions, and it's possible that these changes are more pronounced in these parts of the island due to regional factors.

The trends do not appear to align with the trends in benthic metrics as Pinchgut Brook showed little change in the metrics while Careless Brook showed multiple trends in its benthic metrics. Although air temperature has an influence on water temperature in the stream, local factors can affect this relationship and the two decreasing trends at Pinchgut and Careless Brook may not necessarily translate to the same effect in the stream.

TABLE 7. RESULTS OF SEASONAL MANN-KENDALL TREND TESTS FOR AIR TEMPERATURE

Site	Data Range	Nearest Weather Station (Distance)	Trend
NF02YJ0004 (Pinchgut Brook)	2008-2019	Corner Brook (18 km)	**Decreasing**
NF02YQ0072 (Careless Brook)	2010-2019	Gander (31 km)	**Decreasing**
NF02ZK0005 (Northeast River)	2009-2019	Argentia (11 km)	**No Trend Detected**

Water temperature data at these sites consisted of field measurements and could not be reliably used to calculate trends as they only represent conditions at a given point in time, usually when the CABIN sample was taken. We recommend that temperature loggers be placed at the long-term sites to capture water temperature data at more frequent intervals.

RESEARCH OBJECTIVE #2: TAKE-HOME MESSAGES

Are there changes in benthic communities at three long-term reference sites over time?

Trends in ARM results and benthic metrics were detected in all three long-term biomonitoring reference sites. Two of the sites (Careless Brook and Northeast River) showed the majority of the detected trends. These sites generally showed a decrease in % EPT and increases in % Chironomidae and % GOID, as well as increases in Diversity and Evenness measures. The HBI metric, a descriptor of community tolerance to nutrient enrichment, also increased at Careless Brook. A closer examination of the changes in taxa found a steady decline in the relative abundance of EPT taxa as well as corresponding increases in the relative abundances of Chironomid and GOID taxa at both sites.

We hypothesize that these sites are changing from a highly EPT dominated state to one that has greater abundances of other taxa (such as Chironomidae and GOID taxa). The benthic community at those two sites appear to be undergoing a change towards greater diversity and evenness, although the trends in the HBI results suggest that the benthic community is shifting to one with greater tolerance to nutrient enrichment.

The third site, Pinchgut Brook, also showed fewer trends. Specifically, the total abundances of benthic invertebrates have increased sharply over the 10-year record and abundances of EPT taxa are driving this change.

Trends in regional air temperature and water quality were observed at Pinchgut Brook and Careless Brook, while no trends were detected at Northeast River. In addition, those two sites showed an increase in total chloride concentration. Interestingly, Pinchgut Brook had multiple trends in environmental parameters, yet it exhibited much fewer trends in its ARM results and benthic metrics than the other two sites. **Overall, it is difficult to identify specific factors that may be driving these trends in the ARM results and benthic metrics as the benthic community is influenced by numerous environmental conditions.** However, it is possible that changes in human activity of various types in the watersheds are contributing to the observed changes.

Maintaining the biomonitoring at these three long-term sites would be ideal to assess further the changes in benthic communities over time. Adding one or two other long-term sites in the northern and/or southern part of the island would be valuable. We also recommend that temperature loggers be placed at each long-term sites to capture water temperature data at more frequent intervals. Finally, in order to have a better monitoring of climate change impacts over time at the three main long-term reference sites, we recommend adding a hydrological flow site at the two sites where no such data were available (Careless Brook and Pinchgut Brook).

Research Objective #3:

Can we detect impacts to benthic communities using reference models and baseline metrics? If so, can we relate these impacts to stressors?

In this last objective, we aimed to compare the quality of the benthic community at the biomonitoring tests sites compared to the reference sites. To accomplish this we compared the main benthic metrics between test and reference sites to see how they differ between the site classifications. If test sites were impaired, we may expect to see significant differences in the metrics.

We also investigated the divergent ARM results and benthic metrics at each test site to determine how communities at each test sites differs from the expected conditions of the ARM and benthic normal ranges. Divergences in the ARM and benthic metric results may indicate a community that has experienced impacts to water quality.

Finally, we performed PCA analyses on habitat parameters from both reference and test sites to determine if these habitat parameters are different at test sites when compared to reference sites.

BENTHIC COMPOSITION DESCRIPTION:

At the island scale, general differences between the benthic community at potential reference and test sites can be observed. In **Figure 16** below, six key metrics were selected to compare for differences between reference and test sites. Looking at the group of three relative abundance metrics (% EPT, % Chironimidae, % GOID taxa), it is evident that test sites have lower % EPT and corresponding elevated % GOID. % Chironimdae only appears slightly higher at test sites. These differences suggest that test sites may face greater disturbances such as impaired water quality. EPT taxa are generally less tolerant to disturbances, while Chironimidae and GOID taxa become more pronounced in the community when water quality is impaired. The HBI scores at test sites also appear to be elevated, which matches the differences in the key taxa as a higher HBI score indicates greater community tolerance to nutrient pollution. Richness and Diversity also appear to be lower at test sites.

As these comparisons are made with sites grouped by their site status, below we expand on these results at the individual site level and investigate potential impacts at key sites.

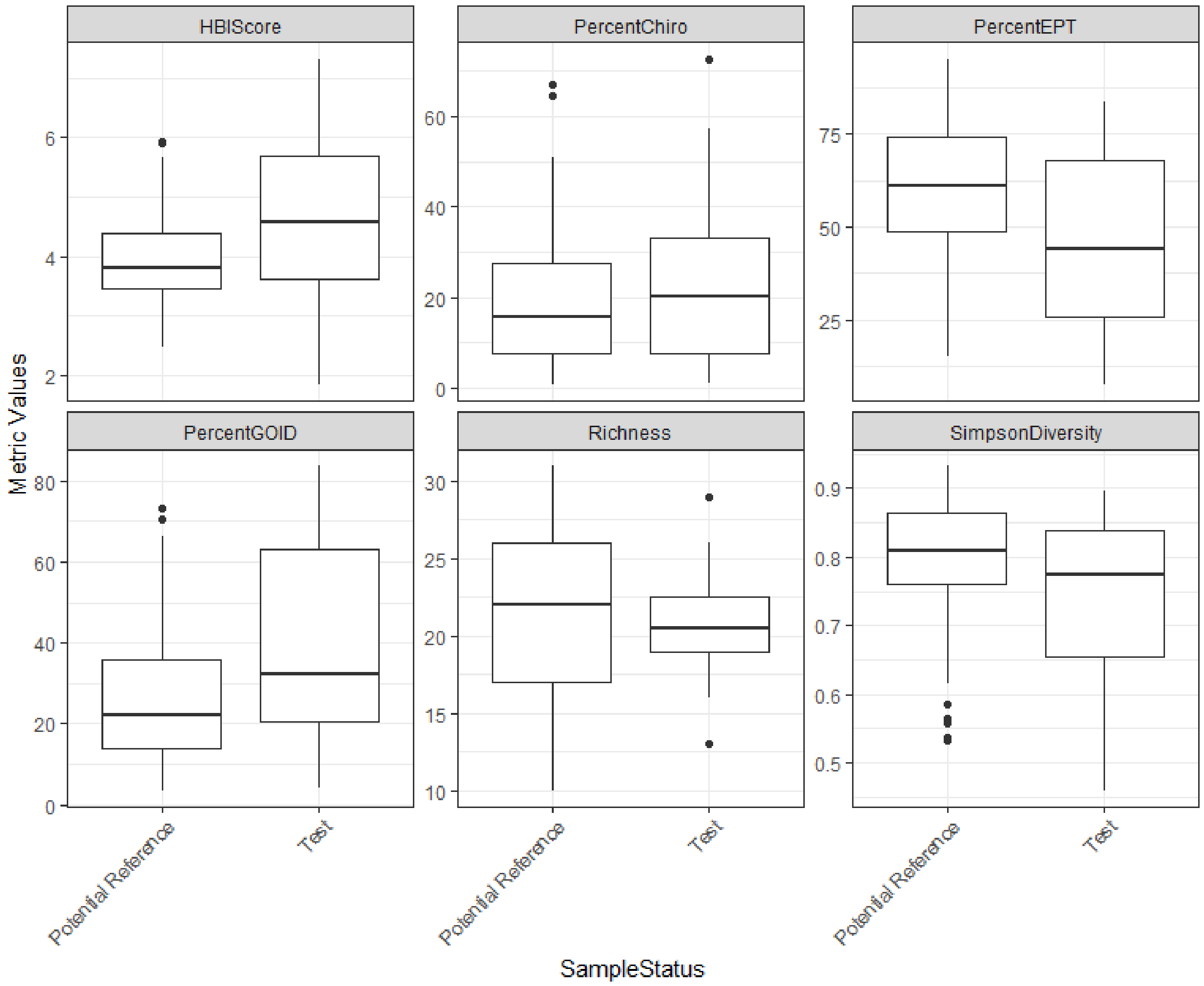


Figure 16. Comparison of benthic metric results between Potential Reference and Test sites.

ARM RESULTS FOR TEST SITES ON THE ISLAND OF NEWFOUNDLAND

Results from the Atlantic Reference Model (ARM) for the Newfoundland test sites are shown in **Table 8** below. The test sites in Newfoundland displayed some variability in their ARM results. Of the 16 sites, all but two were divergent for richness. However, for HBI some sites had very high observed over expected values while others were divergent, and in the case of one site, highly divergent. Several sites were divergent or highly divergent in all the ARM metrics (NF02ZM0183 and NF02YL0029) and several were normal in all the metrics (NF02YO0121 and NF02YL0098). In combination with the benthic metrics presented below they can be used to determine the level of habitat impairment at a CABIN site.

TABLE 8. ARM OUTPUTS (OBSERVED OVER EXPECTED VALUES) FOR EACH OF SEVEN METRICS FOR ALL TEST SAMPLES (N = 16). GREEN INDICATES A VALUE THAT IS IN THE NORMAL CATEGORY, YELLOW INDICATES DIVERGENT VALUES, AND RED INDICATES HIGHLY DIVERGENT VALUES. FOR THE TABLE OF RANGES SEE THE ARM METHODS SECTION.

		Observed over Expected (OoverE) Values						
Site	**Year**	**Richness (R)**	**Shannon -Wiener Diversity (H)**	**Simpson's Diversity (S)**	**Pielou's Evenness (J)**	**Berger Parker Dominance (D)**	**Canadian Ecological Flow Index (CEFI)**	**Hilsenhoff Biotic Index (HBI)**
NF02ZM0367	2012	0.69	0.88	1.01	0.96	1.08	0.93	0.82
NF02ZM0366	2012	0.85	1.20	1.13	1.18	1.25	0.89	0.88
NF02ZM0363	2013	0.85	1.24	1.22	1.23	1.32	1.19	1.49
NF02ZM0183	2011	0.93	0.85	0.89	0.88	0.76	0.87	0.81
NF02ZM0178	2011	0.85	0.97	1.07	1.00	1.07	0.97	0.68
NF02ZM0020	2008	0.70	0.64	0.67	0.69	0.55	1.02	0.86
NF02ZM0014	2010	0.66	0.86	0.96	0.89	0.93	1.03	0.91
NF02ZL0029	2009	0.76	0.95	0.91	0.93	0.80	1.06	1.65
NF02ZG0027	2012	0.78	0.92	0.98	0.89	0.98	0.98	0.87
NF02YR0043	2012	0.93	1.04	1.05	1.05	1.06	0.91	1.13
NF02YO0190	2011	0.65	0.78	0.82	0.84	0.66	1.03	1.00
NF02YO0142	2011	0.74	1.19	1.19	1.18	1.30	0.90	0.68
NF02YO0121	2012	1.14	1.18	1.14	1.10	1.22	0.98	1.13
NF02YL0102	2012	0.93	1.18	1.13	1.19	1.27	0.92	0.85
NF02YL0098	2011	1.02	1.26	1.21	1.23	1.37	1.14	1.38
NF02YL0029	2012	0.47	0.60	0.72	0.70	0.59	0.71	0.46

COMPARISON BETWEEN METRICS AT TEST SITES ON THE ISLAND NEWFOUNDLAND AND ATLANTIC BENTHIC NORMAL RANGES

Comparisons of the benthic metrics from test sites were made against the Atlantic benthic normal ranges. Each metric result at a given test site was compared against the normal range of that metric and was then reported as being normal, potentially divergent, divergent, or highly divergent. A direction category was also reported to indicate whether the metric was greater or lower than the normal range. Note that the colours and symbols used in the below figure do not necessarily denote “positive” or “negative” results as such. Detailed interpretation at each site is recommended in order to fully explore the status of the benthic communities.

From **Figure 17**, we can see that most test sites on the island of Newfoundland had a relatively high proportion of divergent or highly divergent metrics. Interestingly, several test sites had relatively few divergent metrics such as Outer Cove Brook at Salvage Creek (NF02ZM0363; 2 divergent metrics) and Shoal Cove Brook (NF02ZG0027; 2 divergent metrics). There are similarities to the ARM results, although Shoal Cove Brook had several divergent ARM results. These results help us identify sites on which to focus our analyses, and give us an idea of what kind of differences in community composition may be occurring at these sites.

We are also able to identify clusters of sites based on their metric results. For example several urban sites on the Avalon Peninsula (sites denoted by site codes NF02ZM-) had low Diversity and Evenness metrics as well as low proportions of EPT taxa. Notable sites include Broad Cove Brook (NF02ZM0020) where all of the diversity and evenness metrics were divergent and lower than the normal ranges while percent Chironomidae, percent GOID taxa as well as the HBI score were highly divergent and much higher than the normal ranges. These results at Broad Cove Brook suggest that the benthic community is potentially impacted by nutrients and other human influences. Overall the divergences at these sites may be related to the highly urbanized nature of the watershed on the Avalon Peninsula.

More detailed analyses of the benthic results and land use history at each site are presented below in **Table 9**.

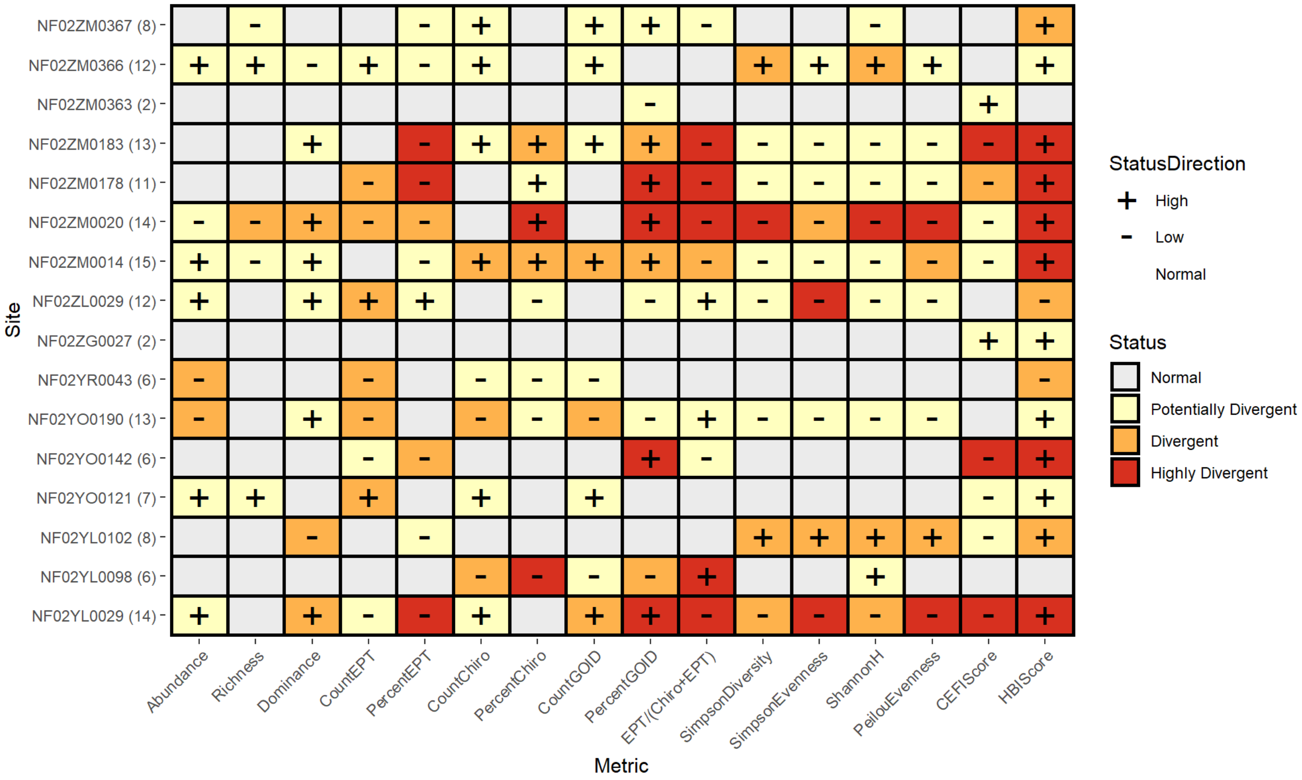


FIGURE 17. THE STATUS OF METRICS FOR EACH TEST SITE. THE RESULTS ARE CATEGORIZED AS NORMAL, POTENTIALLY DIVERGENT, DIVERGENT, OR HIGHLY DIVERGENT BASED ON COMPARISONS WITH THE ATLANTIC BENTHIC NORMAL RANGES AND A DIRECTION STATUS IS ASSIGNED TO INDICATE WHETHER THE METRIC WAS HIGHER OR LOWER THAN THE NORMAL RANGE.

NOTABLE ARM AND BENTHIC METRIC RESULTS AND THEIR INTERPRETATION AT SELECT TEST SITES

Upstream land use for each watersheds and the potential impacts on the watershed were examined for each site to see whether they relate to the ARM and Metric results. The table below highlights a subset of the sites, specifically those sites that are part of the Canada-NL Water Quality Agreement and the long-term CABIN test sites. Generally, the results find that divergent metrics tend to be in Chironomidae, GOID, and the Hilsenhoff Biotic Index and tend to relate to impacts from urbanization and other human activities. While the HBI relates to increases in nutrient enrichment, it’s not possible to identify specific impacts that may be driving these changes as they are quite general – a wide variety of disturbances (physical, chemical, etc.) could make conditions more favourable for tolerant taxa.

TABLE 9. INTERPRETATION OF ARM AND METRIC RESULTS, SUPPORTED BY INFORMATION ABOUT LAND USE AND POTENTIAL HUMAN IMPACTS.

Site Name/Site Code	Upstream Land Use	Potential Impacts	ARM Results	Metrics Results	Notes
NF02ZM0183 Kelligrew's River	Industrial, some agriculture and some residential land use.	Industrial operations, waste management.	Divergent across all metrics.	EPT highly divergent and below normal, richness and diversity metrics divergent and below normal. Chironomidae, GOID, and HBI divergent and elevated.	All ARM results are divergent, metrics such as Chironomidae, GOID, and HBI point towards the influence of nutrient input.
NF02ZM0178 Leary's Brook	Heavily urban, some industrial land use.	Stream runs directly through main urban areas.	HBI and Richness divergent.	EPT highly divergent and below normal, richness and diversity metrics divergent and below normal. Chironomidae, GOID, and HBI divergent and elevated.	ARM and Metric results point towards impairment through urban impacts, notably nutrient input as suggested by divergent HBI and GOID metrics.
NF02ZM0020 Broad Cove Brook	Forest, residential, and minor industrial land use.	Likely residential impacts and recreational use of parks.	Divergent in all metrics except CEFI.	Abundance, Richness, EPT divergent and below normal. Dominance, GOID, Chironomidae, and HBI divergent and elevated. All evenness and diversity metrics divergent and below normal.	ARM and Metric results point towards dominance by non-EPT taxa, possibly influenced by residential activities. Divergent HBI metric suggests nutrient enrichment.
NF02ZM0014 Virginia River	Primarily urban with some forest some industrial land use.	Urban and some light industrial activity.	Richness, Evenness, and HBI divergent.	Abundance and dominance potentially divergent and elevated. Chironomidae and GOID divergent and elevated. Diversity and Evenness divergent and below normal. HBI highly divergent and elevated.	ARM and Metrics indicate dominance by non-EPT taxa. Water quality has multiple exceedances in WQI calculations. Both are likely influenced by urban activity.
NF02ZL0029 Goulds Brook	Primarily forest with some urban and some agriculture. ATV trail network in watershed.	Likely recreational impacts from ATV trails and some residential and agricultural activity.	Richness and Diversity divergent.	Richness and dominance potentially divergent and elevated. EPT potentially divergent and elevated, Chironomidae and GOID potentially divergent and below normal.	ARM and Metrics indicate community dominated by EPT taxa, possibly the state in less disturbed streams in NL.
NF02YO0142 Corduroy Brook	Forest, urban and industrial.	Impacts from urban activities, some industrial.	Richness, CEFI, HBI divergent.	EPT divergent and below normal. GOID, CEFI, and HBI highly divergent and elevated.	Site is likely impacted by a combination of urban and industrial impacts. High in worms at site, leading to GOID and HBI being highly divergent. WQI indicates multiple water quality exceedances.

Site Name/Site Code	Upstream Land Use	Potential Impacts	ARM Results	Metrics Results	Notes
NF02YL0029 Wild Cove Brook	Quarries, waste management.	Impacts from waste management facility.	All metrics divergent, HBI highly divergent.	Abundance and dominance divergent and elevated. EPT highly divergent and below normal. HBI and GOID highly divergent and elevated.	Nutrients frequently exceed guidelines here. GOID and HBI metrics suggest nutrient enrichment driving the community changes.

PCA RESULTS – HABITAT AND WATER QUALITY DATA OF TEST SITES:

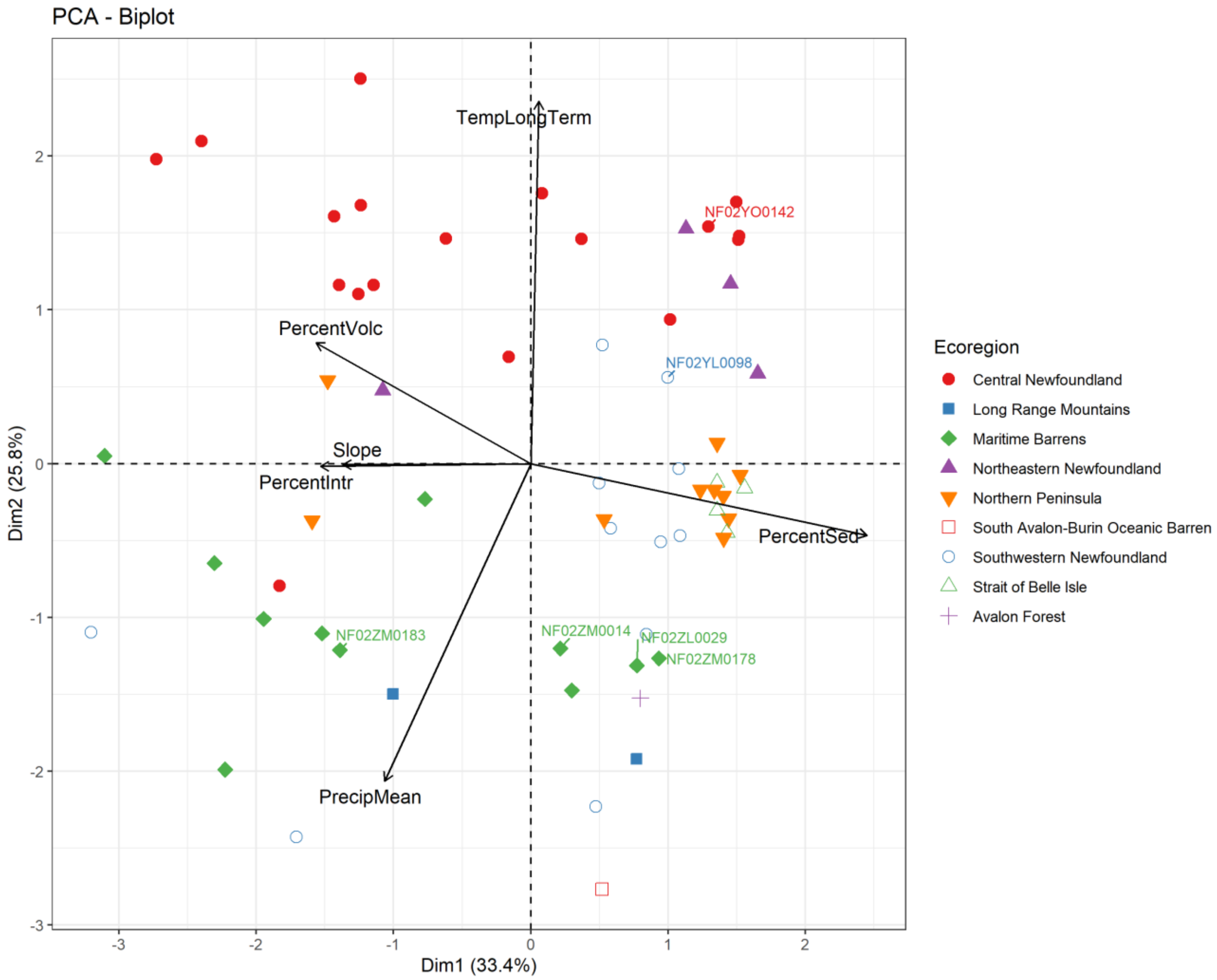


FIGURE 18. PCA BIPLOT OF HABITAT PARAMETER VALUES FOR BOTH TEST AND POTENTIAL REFERENCE SITES. TEST SITES ARE LABELLED FOR EASE OF COMPARISON WITH THE REST OF THE SITES.

This PCA plots test and reference sites on the biplot according to the habitat data at each site and adds on to the previous habitat PCA (**Figure 10,** in Objective 1) by including test sites that have sufficient habitat data. In general test sites (labelled with their site codes) are located within the same cluster as the other sites in each ecoregion. This indicates that the test sites have similar habitat variables to those of the reference sites in each ecoregion, suggesting that differences between test and reference sites of a given ecoregion are more likely to be influenced by other factors.

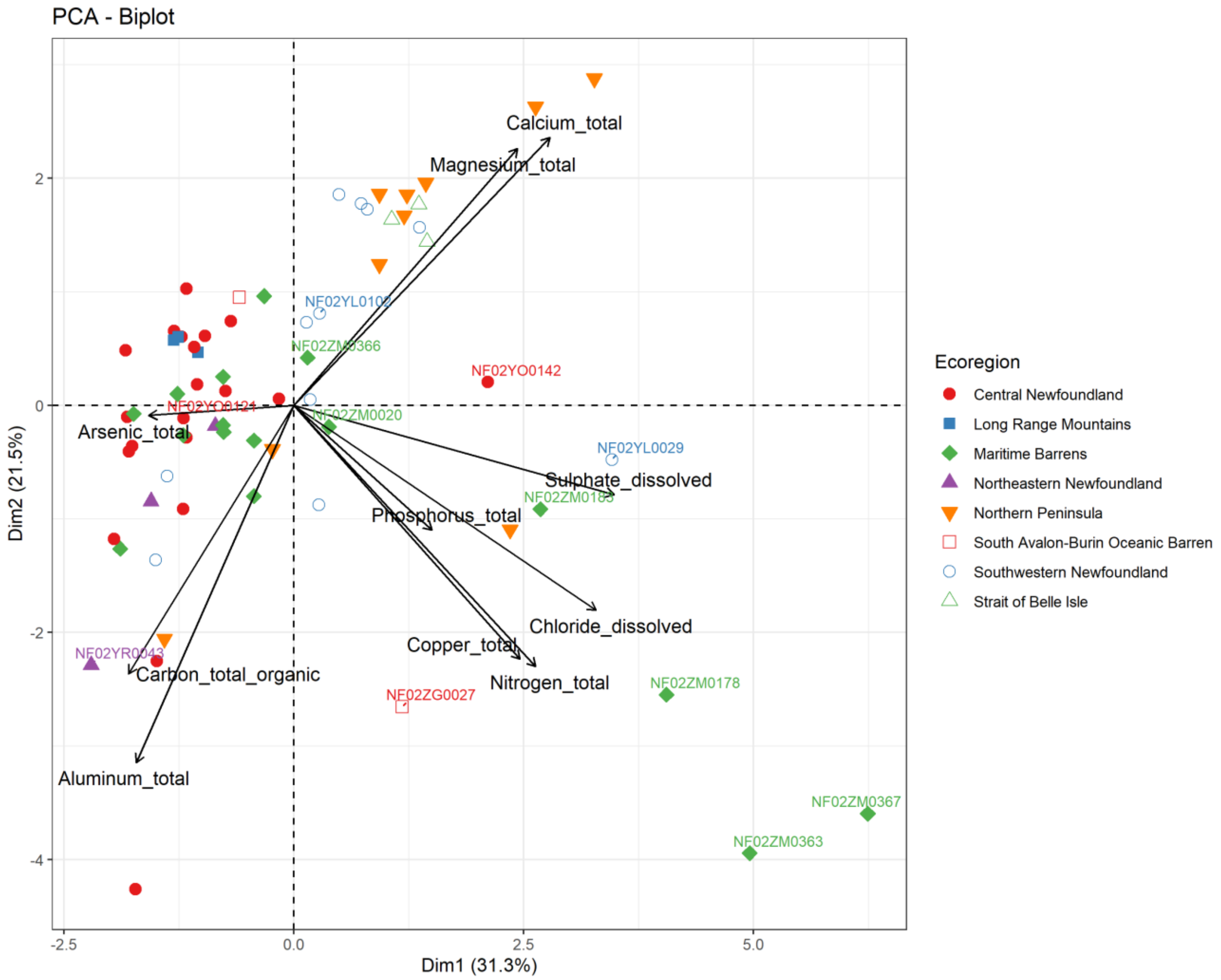


FIGURE 19. PCA BIPLOT OF WATER QUALITY PARAMETER VALUES FOR BOTH TEST AND POTENTIAL REFERENCE SITES. TEST SITES ARE LABELLED FOR EASE OF COMPARISON WITH THE REST OF THE SITES.

The second PCA (**Figure 19**) plots sites in the biplot by their water quality parameters and adds in the test sites. The dimensions have changed slightly compared to Figure X (above), and the first PCA dimension (x-axis) is defined mainly by major ions (chloride, sulphate) and total nitrogen, while the second PCA dimension (y-axis) is defined mainly by aluminum, total organic carbon, calcium, and magnesium.

Several test sites fell within the bottom right quadrant and generally have higher chloride, copper, sulphate, and nitrogen concentrations. These sites were primarily within urban areas on the Avalon Peninsula and are potentially impacted by run-off from roads and urban development, for example Leary's Brook and Waterford River. However, the observed divergences in the benthic metrics vary even among the urban sites with run-off impacts. For example, Leary's Brook (NF02ZM0178), Waterford River (NF02ZM0367), and Outer Cove Brook (NF01ZM0363) all plot in the bottom right quadrant and have elevated water quality parameters. Despite the similarities in their water quality profiles their benthic metrics differ. Where Leary's Brook and Waterford River are both divergent in multiple benthic metrics and show decreased EPT taxa and increased Chironomidae and GOID taxa, Outer Cove Brook showed the fewest divergent metrics of any test site (**Figure 17**).

A number of the other test sites were closer or within the main cluster, showing that their water quality parameters are generally similar to the reference sites. In particular, the Peter’s River test site (NF02YO0121) plots within the main cluster of Central Newfoundland reference sites while the Corduroy Brook test site (NF02YO0142) plots outside the cluster. Although both are test sites, Peter’s River appears to be minimally influenced by upstream agricultural operations while Corduroy Brook is situated within the urban center of Grand Falls and is highly influenced by road run-off and other urban impacts. These differences are also reflected in the ARM results and benthic metrics where Peter’s River had no divergent ARM results and few potentially divergent and divergent benthic metrics (Richness, % Chironomidae, and CEFI). Comparatively, Corduroy River had multiple divergent ARM results (CEFI, HBI, and Richness) and divergent and highly divergent benthic metrics in % EPT, % GOID taxa, CEFI, and HBI.

RESEARCH OBJECTIVE #3: TAKE-HOME MESSAGES

Can we detect impacts to benthic communities using reference models and baseline metrics? If so, can we relate these impacts to stressors?

When examining test and reference sites as a group, there are distinct differences in the benthic communities at test and reference sites. The ARM and benthic metrics results allowed us to investigate possible impairment at each test site. Most test sites showed some degree of impairment, although the number of divergent metrics varied between the sites with some having relatively few divergent metrics while others having nearly all metrics divergent to some degree. We were also able to identify clusters of sites with similar results such as the urban sites on the Avalon Peninsula, likely due to having similar environmental conditions as well as similar disturbances in their watersheds. There were some surprising results as well. For example, some sites with similar water quality profiles showed fairly different ARM and benthic metrics results.

Overall, we can conclude that these divergences in the ARM results and benthic metrics are due to a combination of impacts on the benthic communities. An analytical approach to synthesize the different impacts may be able to reveal how specific impacts are affecting different taxa or communities.

Conclusion

This baseline report presents results for the period from 2006 to 2019. Biomonitoring data were collected at 93 sampling sites across the island of Newfoundland: 77 potential reference sites and 16 test sites. These sites are distributed amongst the nine ecoregions of the island and were all sampled using the standardized CABIN protocol. Out of the 77 potential reference sites, three of them consist of long-term reference sites, all of which contain 10 years of data. Sites in Labrador were not used due to differences in habitat.

Samples included in this analysis have been collected by different partners: Newfoundland and Labrador's Department of Environment and Climate Change – Water Resources Management Division, Environment and Climate Change Canada, and Parks Canada (Terra Nova and Gros Morne National Parks).

This report aimed at answering three main questions:

1. **Are benthic communities in reference areas different across ecoregions on the island of Newfoundland?** Our analysis showed some differences in communities by ecoregion with some ecoregions showing greater variability in the relative abundances of EPT and Chironomidae taxa. We observed some differences in dominant taxa between ecoregions when looking at the taxa groups individually. Dominant taxa are Chironomidae (non-biting midges) and Baetidae (small minnow mayfly) for most ecoregions, with the exception of Pisidiidae (peaclam) for the Strait of Belle Isle and Northeastern Newfoundland ecoregions.

2. **Are there changes in benthic communities at three long-term reference sites over time?** Long-term trends were detected in all three long-term biomonitoring reference sites; though Careless Brook and Northeast River showed many more trends than Pinchgut Brook. Results suggest that the benthic community is shifting to one with greater tolerance to organic pollutants. Overall, it is difficult to say which factors may be driving these trends in the ARM results and benthic metrics as the benthic community is influenced by numerous environmental conditions.

3. **Can we detect impacts to biological communities using reference models and baseline metrics? If so, can we relate these impacts to stressors?** We observed that key metrics differed between reference and test sites across the island of Newfoundland. Test sites generally had numerous divergent ARM and benthic metric results, though they do tend to vary from site to site. Investigations of potential impacts at individual watersheds as well as the PCA analysis of water quality highlight the need to consider all possible disturbances when evaluating the condition of a site.

Recommendations

1. To ensure a better portrait of the biomonitoring results on the island, we would recommend increasing the number of occasional sampling sites (for example, sampled every five years) in these ecoregions, with varying types of terrain if possible:
    a. Avalon Forest
    b. Northeastern Newfoundland
    c. South Avalon-Burin Oceanic Barren
    d. Strait of Belle Isle

2. Particularly to obtain a better understanding of the climate change impacts on the island, we recommend adding one or two long-term biomonitoring reference sites in northern and southern Newfoundland.

3. In order to have a better monitoring of climate change impacts over time at the three main long-term reference sites, we recommend adding a flow site for the two sites where no such data were available (NF02YQ0072, NF02YJ0004).

4. As water temperature data consisted of only field measurements, and could not reliably be used to calculate trends, we recommend that temperature loggers be placed at the long-term sites (NF02YQ0072, NF02YJ0004, NF02ZK0005) to capture water temperature data at more frequent intervals.

5. Co-locating future CABIN sites with sites for other projects would be beneficial. Collecting benthic macroinvertebrate samples at sites that are part of the long-term water quality network would allow better linkages between the two types of monitoring to support reporting for both programs. Currently, the three long term sites are co-located with water quality monitoring, and the Northeast River site is co-located with a hydrometric site. These efforts should be pursued.

6. We recommend conducting such a report in five years from now in order to assess the changes in benthic communities across the island of Newfoundland, provided that additional data has been collected.

7. Finally, we recommend the use of the updated version of the ARM (when available) for the next edition of this report.

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Appendices

1. Summary table of sampling sites included in the analysis

Study	Site	Name	Latitude	Longitude	Ecoregion*	Sample Status
Atlantic CABIN	ATLCBNL-01	Grand Lake	48.68944	-58.1517	Southwestern NL	Potential Reference
	ATLCBNL-02	Big Gull Pond Brook	48.7875	-58.0286	Southwestern NL	Potential Reference
	ATLCBNL-03	Three Tom Brook	49.40361	-57.7331	Southwestern NL	Potential Reference
	ATLCBNL-04	Tributary to Greavett Brook	50.09083	-57.5719	Northern Peninsula	Potential Reference
	ATLCBNL-05	Tributary to Bowing Brook	50.27361	-57.4953	Northern Peninsula	Potential Reference
	ATLCBNL-06	Bound Brook	50.30917	-57.4481	Northern Peninsula	Potential Reference
	ATLCBNL-07	Unnamed stream south of River of Ponds	50.47194	-57.4739	Northern Peninsula	Potential Reference
	ATLCBNL-08	East Cove Brook	51.03194	-56.8658	Strait of Belle Isle	Potential Reference
	ATLCBNL-09	Kelly's Brook	51.05667	-56.7303	Northern Peninsula	Potential Reference
	ATLCBNL-10	Southwest Brook	51.05333	-56.1083	Northern Peninsula	Potential Reference
	ATLCBNL-11	Unnamed river north of Second Salmon Pond	51.13556	-56.2061	Northern Peninsula	Potential Reference
	ATLCBNL-12	Unnamed river west of St.Anthony Airport	51.37222	-56.1422	Strait of Belle Isle	Potential Reference
	ATLCBNL-13	Unnamed stream south of Pines Cove	51.36556	-56.6325	Strait of Belle Isle	Potential Reference
	ATLCBNL-14	Brig Bay Brook	51.05389	-56.8575	Strait of Belle Isle	Potential Reference
	ATLCBNL-15	Gilmore's Pond Brook	50.64722	-57.2133	Northern Peninsula	Potential Reference
	ATLCBNL-16	Little Brook North of Island Pond	50.56556	-57.2147	Northern Peninsula	Potential Reference
	BPB01	Boot Pond Brook	49.00111	-54.4583	Central NL	Potential Reference
	LCB01	Little Careless Brook	48.92583	-54.9844	Central NL	Potential Reference
	LFWB01	Little Flat Water Brook	49.70444	-56.3006	Central NL	Potential Reference
	LJB01	Little Jumper Brook	48.97861	-55.3858	Central NL	Potential Reference
	LNBP01	Little New Bay Pond Brook	49.11222	-55.5614	Central NL	Potential Reference
	LNBR01	Little New Bay River	49.18556	-55.5481	Central NL	Potential Reference
	POW01	Powder House Pond Brook	49.89695	-56.2047	Central NL	Potential Reference
	SHO01	Little Shoal Brook	49.52417	-56.1761	Central NL	Potential Reference

Study	Site	Name	Latitude	Longitude	Ecoregion*	Sample Status
	SOPS01	SOPS Pond Brook	49.37972	-55.8641	Northeastern NL	Potential Reference
	TMB01	Two Mile Brook	49.29667	-55.3789	Central NL	Potential Reference
Gros Morne NP	BOT03	Bottom Brook	49.59269	-57.9176	Northern Peninsula	Potential Reference
	HPM01	Hardings Pond Middle Barrens	49.63083	-57.6439	Long Range Mountains	Potential Reference
	MKB01	McKenzies Brook	49.43533	-57.8682	Southwestern NL	Potential Reference
	NNP01	No Name Pond	49.59544	-57.6043	Long Range Mountains	Potential Reference
	PRP01	Pilgrim Rock Pond	49.59163	-57.6337	Long Range Mountains	Potential Reference
	RBBL	Rocky Barachois Brook Lower	49.48056	-57.7336	Southwestern NL	Potential Reference
	TRH01	Trout River Brook	49.47067	-58.1307	Southwestern NL	Potential Reference
NL Province - WRMD	NF02YE0005	Western Brook at Route 430	49.82894	-57.855	Northern Peninsula	Potential Reference
	NF02YG0025	Little Brook tributary to Main River	49.78389	-56.9613	Northern Peninsula	Potential Reference
	NF02YH0018	Lomond River at Route 431	49.40194	-57.7303	Southwestern NL	Potential Reference
	NF02YH0054	Cox’s Brook southeast of Cox’s Cove	49.11003	-58.061	Southwestern NL	Potential Reference
	NF02YH0065	Line Brook above Bonne Bay little pond	49.38667	-57.6422	Southwestern NL	Potential Reference
	NF02YJ0004	Pinchgut Brook at TCH	48.7975	-58.0619	Southwestern NL	Potential Reference
	NF02YJ0036	Cold Brook off Cold Brook Road	48.60253	-58.5277	Southwestern NL	Potential Reference
	NF02YK0032	Tributary to Glide Brook below Glide Lake	49.12831	-57.3719	Southwestern NL	Potential Reference
	NF02YL0097	Gales Brook at Route 420	49.54722	-56.9983	Central NL	Potential Reference
	NF02YM0003	Indian Brook at Route 390	49.49806	-56.1764	Central NL	Potential Reference
	NF02YM0044	First Pond Brook at Little Bay	49.59969	-55.9569	Northeastern NL	Potential Reference
	NF02YN0001	Lloyds River at Route 480	48.30778	-57.7028	Long Range Mountains	Potential Reference
	NF02YN0044	Sutherlands Pond outflow at forest access road	48.74422	-56.7658	Central NL	Potential Reference
	NF02YN0045	Tributary to Buchans Brook	48.85639	-56.8222	Central NL	Potential Reference
	NF02YN0046	Unnamed stream on Halfway Mountain access road	48.66719	-57.0023	Central NL	Potential Reference
	NF02YO0192	East Pond Brook below East Pond	48.68197	-56.51	Central NL	Potential Reference
	NF02YO0194	Tributary to Peters River	49.01722	-55.5403	Central NL	Potential Reference

Study	Site	Name	Latitude	Longitude	Ecoregion*	Sample Status
	NF02YO0195	Tributary to Diversion Lake	48.75028	-55.9244	Central NL	Potential Reference
	NF02YO0196	Tom Joe Brook at access road	48.92861	-55.9542	Central NL	Potential Reference
	NF02YO0197	Burnt Arm Brook at Route 346	49.59672	-54.622	Northeastern NL	Potential Reference
	NF02YQ0006	Northwest Gander River at highway bridge	48.58178	-55.5041	Central NL	Potential Reference
	NF02YQ0072	Careless Brook at Resource Road steel bridge	48.90222	-54.9939	Central NL	Potential Reference
	NF02YR0001	Pound Cove Brook at Route 330	49.17814	-53.5591	Northeastern NL	Potential Reference
	NF02ZA0025	Mollichignick Brook at TCH	47.892	-59.0873	Southwestern NL	Potential Reference
	NF02ZA0026	Hell’s Gulch on Steel Mountain Road	48.37722	-58.3381	Southwestern NL	Potential Reference
	NF02ZA0027	Rainy Brook East at TCH	48.09822	-58.7841	Southwestern NL	Potential Reference
	NF02ZA0028	Unnamed Stream 9km south of TCH on Route 480	48.50194	-58.1669	Central NL	Potential Reference
	NF02ZC0021	Top Pond Brook at Route 480	47.89519	-57.6373	Long Range Mountains	Potential Reference
	NF02ZC0022	Seal Brook at Route 480	47.70157	-57.6284	Maritime Barrens	Potential Reference
	NF02ZD0010	Tributary to unnamed pond west of Meelpaeg Lake	48.29139	-56.7961	Maritime Barrens	Potential Reference
	NF02ZE0033	Southwest Brook below Southwest Pond	47.84897	-55.7679	Maritime Barrens	Potential Reference
	NF02ZG0025	Rattle Brook at South Branch	47.45086	-54.8536	Maritime Barrens	Potential Reference
	NF02ZK0005	Northeast River near Placentia	47.27306	-53.8396	Maritime Barrens	Potential Reference
	NF02ZK0026	Tributary to Rattling Brook Big Pond	47.41778	-53.7476	Maritime Barrens	Potential Reference
	NF02ZK0027	Little Barachois River near Placentia	47.18139	-54.0381	Maritime Barrens	Potential Reference
	NF02ZM0098	Virginia River at headwaters	47.59833	-52.7545	Maritime Barrens	Potential Reference
	NF02ZM0185	South Brook at headwaters	47.4956	-52.8133	Maritime Barrens	Potential Reference
	NF02ZM0360	South River near Holyrood	47.35528	-53.1167	Maritime Barrens	Potential Reference
	NF02ZM0361	Pouch Cove Brook near Pouch Cove	47.75389	-52.7804	Maritime Barrens	Potential Reference
	NF02ZM0362	Long Beach River at Long Beach	46.64139	-53.1378	SA-B Oceanic Barren	Potential Reference
	NF02ZN0009	Back River at Salmonier Line	47.20511	-53.3712	Avalon Forest	Potential Reference
	NF02YL0029	Wild Cove Brook at Route 440	48.974	-57.8836	Southwestern NL	Test
	NF02YL0098	Rocky Brook East Branch	49.29706	-57.4244	Southwestern NL	Test
	NF02YL0102	Corner Brook Stream at Brook Street	48.94903	-57.9455	Southwestern NL	Test

Study	Site	Name	Latitude	Longitude	Ecoregion*	Sample Status
	NF02YO0121	Peters River near Botwood	49.104	-55.3961	Central NL	Test
	NF02YO0142	Corduroy Brook near Centennial Park	48.94	-55.6629	Central NL	Test
	NF02YO0190	Tributary to Gills Pond Brook	48.64008	-56.5271	Central NL	Test
	NF02YR0043	Southern Brook at Deadman's Bay	49.31919	-53.7018	Northeastern NL	Test
	NF02ZG0027	Shoal Cove Brook below Shoal Cove Pond	46.88489	-55.3987	SA-B Oceanic Barren	Test
	NF02ZL0029	Goulds Brook near Makinsons	47.50428	-53.2889	Maritime Barrens	Test
	NF02ZM0014	Virginia River at the Boulevard	47.58406	-52.6903	Maritime Barrens	Test
	NF02ZM0020	Broad Cove Brook near St. Phillips	47.57123	-52.8695	Maritime Barrens	Test
	NF02ZM0178	Leary's Brook at Prince Philip Drive	47.56389	-52.7488	Maritime Barrens	Test
	NF02ZM0183	Kelligrews River at Kelliview Crescent	47.49378	-53.0164	Maritime Barrens	Test
	NF02ZM0363	Outer Cove Brook at Savage Creek	47.63453	-52.6892	Maritime Barrens	Test
	NF02ZM0366	Three Island Pond outflow at Buckingham Drive	47.51542	-52.8985	Maritime Barrens	Test
	NF02ZM0367	Waterford River at Bay Bulls Road	47.52981	-52.7409	Maritime Barrens	Test
Terra Nova NP	BPB01	Blind Pond Brook	48.42917	-54.1122	Central NL	Potential Reference
	CTB01	Charlottetown Brook	48.44611	-54.0208	Central NL	Potential Reference
	MPB01	Minchin's Brook	48.5602	-53.8813	Central NL	Potential Reference

* Note: SA-B Oceanic Barren = South Avalon-Burin Oceanic Barren

2. Description of Individual Metrics

Metrics	Description
Total Abundance	The abundance of benthic macroinvertebrates in a stream changes according to many factors. Abundance will often decrease with toxic water conditions but may increase in nutrient enriched conditions. Abundance is calculated by summing the total number of individuals in a sample.
Total Richness (total number of taxa)	Stream biodiversity may decline as water quality deteriorates. When this occurs, there is usually an increase of tolerant taxa and a decrease in intolerant taxa. Total richness is calculated by summing the number of individual taxa (differentiated by family level) in a sample.
Dominance (% top 2 dominant taxa)	Dominance is calculated as the abundance of the two most dominant taxa. As diversity declines, a few taxa tend to dominate within the sample. As water quality declines, more tolerant taxa usually dominate.
Total EPT Taxa	Total number of Ephemeroptera, Plecoptera, Trichoptera taxa at the Family level. Calculated as the sum of all EPT taxa within a sample. These taxa are typically considered to be intolerant to unfavorable conditions. The EPT population typically declines as water quality decreases.
Percent (%) EPT	The percentage of the sample that are EPT taxa. It is calculated as the total number of EPT individuals divided by the total number of individuals in the sample. Percent EPT typically decreases along with water quality.
Total Chironomidae Taxa	Total number of Chironomidae taxa identified to the Family level. Calculated as the sum of all Chironomidae taxa within a sample. These taxa are typically considered to be tolerant to unfavorable conditions. The chironomid population typically increases as water quality decreases.
Percent (%) Chironomidae	The percentage of the sample that are Chironomidae. It is calculated as the total number of Chironomids divided by the total number of individuals in the sample. Percent Chironomidae typically increases as water quality decreases.
Percent (%) Oligochaeta	Total number of Oligochaeta identified to the Family level. Calculated as the sum of all Oligochaetes within a sample. These taxa are typically considered to be tolerant to unfavorable conditions. The oligochaete population typically increases as water quality decreases.
Total GOID Taxa (Gastropods, Oligochaeta, Isopods, Diptera)	Gastropods, Oligochaetes, Isopods and Diptera are all pollution tolerant species. As diversity declines, these may tend to dominate the community. Total GOID is calculated by summing the total number of individuals within these groups, at the family level.
Percent (%) GOID (Gastropods, Oligochaeta, Isopods, Diptera)	Percent GOID is calculated as the proportion of GOID individuals within the entire sample.

Metrics	Description
EPT / Chironomidae + EPT	EPT tend to decline in the presence of most anthropogenic influences to stream conditions. In contrast, Chironomidae tend to be pollution tolerant. The metrics calculates the proportion of EPT taxa within the overall population of EPT and Chironomidae within a sample.
Percent (%) Trichoptera that are Hydrophychidae	Hydropsychidae tend to be more tolerant than other families of Trichoptera. The proportion of Hydropsychidae within the total Trichoptera community is calculated.
Percent (%) Ephemeroptera that are Baetidae	Baetidae tend to be more tolerant than other families of Ephemeroptera. The proportion of Baetidae within the total Ephemeroptera community is calculated.
Simpson's Diversity (D)	Measures the relative abundance and distribution of taxa present in the sample. Simpson's Diversity gives results between 0 and 1, where 0 indicates no diversity and 1 indicates complete diversity in the sample.
Shannon-Wiener Diversity (H)	Measures the relative abundance and distribution of taxa present in the sample. As these factors increase, so does Shannon-Wiener Diversity (H).
Simpson's Evenness (E)	Measures the relative distribution of species in a sample based on species richness. Values range between 0 and 1, with 0 meaning there is no equitability in the distribution of individuals among taxa groups and 1 meaning complete equitability. The calculation utilizes Simpson's Diversity to calculate evenness.
Pielou's Evenness (j)	Measures the relative distribution of species in a sample based on species richness. Values range between 0 and 1, with 0 meaning there is no equitability in the distribution of individuals among taxa groups and 1 meaning complete equitability. The calculation utilizes Shannon-Wiener Diversity to determine evenness.
Bray-Curtis Dissimilarity Index	The Bray-Curtis (B-C) Index is a distance coefficient that reaches a maximum value of 1 for two sites that are entirely different and a minimum value of 0 for two sites that possess identical descriptors. Distance coefficients measure the amount of association between sites. The distance statistic is calculated as below: $$B-C = \frac{\sum_{i=1}^{n} \lvert y_{i1} - y_{i2} \rvert}{\sum_{i=1}^{n} (y_{i1} + y_{i2})}$$ where: B-C = Bray-Curtis distance between sites 1 and 2 Y_{i1} = count for taxon i at site 1 Y_{i2} = count for taxon i at site 2 n = total number of taxa present at the two sites This index summarizes the overall difference in community structure between reference and exposed sites in a single number.

Metrics	Description
	(Taken from Environment Canada, 2012[4]).
Hilsenhoff Biotic Index (Mid-Atlantic) - HBI	This index takes into account tolerance values for each family, and the abundance of those families. Tolerance values are largely based on the response to organic pollution, with sensitive species having low scores and tolerant species having high scores. According to the HBI, a value of 0 indicates excellent water quality, while a value of 10 indicates very poor water quality.
Canadian Ecological Flow Index (CEFI)	This index is sensitive to change in hydrological conditions (e.g., timing, intensity of flows). The CEFI is calculated as follows: $$\mathrm{CEFI} = \frac{\sum_{i=1}^{n} F_i * R_i * V_i * W_i}{\sum_{i=1}^{n} F_i * R_i * W_i}$$ where F_i is relative frequency class of ith taxon, R_i is relative abundance of ith taxon in the sample, V_i is optimum of the ith taxon (current velocity preference), W_i is indicator weight score of ith taxon (Armanini *et al*., 2011).

Adapted from the CABIN Online Training Program.

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[4] Environment Canada, 2012. *Metal Mining Technical Guidance for Environmental Effects Monitoring*, http://www.ec.gc.ca/esee-eem/AEC7C481-D66F-4B9B-BA08-A5DC960CDE5E/COM-1434---Tec-Guide-for-Metal-Mining-Env-Effects-Monitoring_En_02[1].pdf.

3. Mann-Kendall Trend Test Results

	NF02YJ0004 (n = 10)			NF02YQ0072 (n = 10)			NF02ZK0005 (n = 10)		
ARM Metric	**p value**	**tau**	**Trend**	**p value**	**tau**	**Trend**	**p value**	**tau**	**Trend**
Richness	0.385	0.222	No trend	0.850	-0.067	No trend	0.213	-0.289	No trend
Shannon-Wiener Diversity	1.000	0.022	No trend	0.210	0.333	No trend	0.074	0.467	**Increase**
Simpson's Diversity	0.592	0.156	No trend	0.474	0.200	No trend	0.032	0.556	**Increase**
Evenness	0.721	0.111	No trend	0.210	0.333	No trend	0.032	0.556	**Increase**
Berger-Parker Dominance	0.592	0.156	No trend	0.371	0.244	No trend	0.074	0.467	**Increase**
CEFI	0.858	-0.067	No trend	0.152	-0.378	No trend	1.000	0.022	No trend
HBI	1.000	-0.022	No trend	0.002	-0.778	**Decrease**	0.107	-0.422	No trend

Benthic Metrics	**p value**	**tau**	**Trend**	**p value**	**tau**	**Trend**	**p value**	**tau**	**Trend**
Total Abundance	0.107	0.422	No trend	0.107	0.422	No trend	0.107	0.422	No trend
Total Richness	0.419	-0.225	No trend	0.367	0.250	No trend	0.410	0.236	No trend
Dominance	0.858	-0.067	No trend	0.020	-0.600	**Decrease**	0.012	-0.644	**Decrease**
Total EPT	0.059	0.494	**Increase**	0.152	0.378	No trend	0.073	0.467	**Increase**
% EPT	1.000	0.022	No trend	0.012	-0.644	**Decrease**	0.049	-0.511	**Decrease**
Total Chironomidae	0.211	0.333	No trend	0.074	0.467	**Increase**	0.152	0.378	No trend
% Chironomidae	1.000	0.022	No trend	0.004	0.733	**Increase**	0.474	0.2	No trend
Total GOID	0.152	0.378	No trend	0.074	0.467	**Increase**	0.049	0.511	**Increase**
% GOID	1.000	0.022	No trend	0.012	0.644	**Increase**	0.049	0.511	**Increase**
EPT/ Chironomidae + EPT	1.000	-0.022	No trend	0.004	-0.733	**Decrease**	0.210	-0.333	No trend
Simpson's Diversity	0.721	0.111	No trend	0.007	0.689	**Increase**	0.031	0.556	**Increase**
Simpson's Evenness	0.721	0.111	No trend	0.007	0.689	**Increase**	0.004	0.733	**Increase**
Shannon-Weiner Diversity	0.858	-0.067	No trend	0.012	0.644	**Increase**	0.073	0.467	**Increase**
Pielou's Evenness	0.371	0.244	No trend	0.004	0.733	**Increase**	0.012	0.644	**Increase**
HBI	0.858	-0.067	No trend	0.007	0.689	**Increase**	0.211	0.333	No trend
CEFI	1.000	-0.022	No trend	0.371	-0.244	No trend	0.858	-0.067	No trend

4. Interpretation of ARM and Metrics Results for Test Sites

Site Name/Site Code	Upstream Land Use	Potential Impacts	ARM Results	Metrics Results	Notes
NF02ZM0367 Waterford River	Primarily urban, some agricultural land use.	Impacts from urban areas and agricultural activity.	Richness, CEFI, and HBI divergent.	Richness and EPT mildly divergent and below normal. Chironomidae, GOID and HBI divergent and above normal.	Baetidae and Hydropsychidae taxa are more prevalent in the sample. Along with high HBI value, these suggest influence of nutrient input.
NF02ZM0366 Three Island Pond Outflow	Primarily forested with some residential and industrial land use. Dams upstream in the watershed.	Agricultural, industrial, and dam impacts.	Richness, CEFI, and HBI divergent.	Abundance and richness slightly divergent and above normal. Diversity, evenness, and HBI metrics divergent and above normal.	Nutrient input may be driving slightly higher abundance of all taxa.
NF02ZM0363 Outer Cove Brook	Forested with some urban and commercial land use. There is a major airport upstream.	Urban and commercial impacts, potentially the airport as well. This site is also downstream of a large golf course and the river winds through the middle of it.	Richness divergent.	Only GOID mildly divergent and below normal, CEFI mildly divergent and above normal.	ARM and Metrics are generally in the normal category. Less human activity in the watershed than other sites.
NF02ZM0183 Kelligrew's River	Industrial, some agriculture and some residential land use.	Industrial operations, waste management.	Divergent across all metrics.	EPT highly divergent and below normal, richness and diversity metrics divergent and below normal. Chironomidae, GOID, and HBI divergent and elevated.	All ARM results are divergent, metrics such as Chironomidae, GOID, and HBI point towards the influence of nutrient input.
NF02ZM0178 Leary's Brook	Heavily urban, some industrial land use.	Stream runs directly through main urban areas.	HBI and Richness divergent.	EPT highly divergent and below normal, richness and diversity metrics divergent and below normal. Chironomidae, GOID, and HBI divergent and elevated.	ARM and Metric results point towards impairment through urban impacts, notably nutrient input as suggested by divergent HBI and GOID metrics.
NF02ZM0020 Broad Cove Brook	Forest, residential, and minor industrial land use.	Likely residential impacts and recreational use of parks.	Divergent in all metrics except CEFI.	Abundance, Richness, EPT divergent and below normal. Dominance, GOID, Chironomidae, and HBI divergent and elevated. All evenness and diversity metrics divergent and below normal.	ARM and Metric results point towards dominance by non-EPT taxa, possibly influenced by residential activities. Divergent HBI metric suggests nutrient enrichment.
NF02ZM0014 Virginia River	Primarily urban with some forest some industrial land use.	Urban and some light industrial activity.	Richness, Evenness, and HBI divergent.	Abundance and dominance potentially divergent and elevated. Chironomidae and GOID divergent and elevated. Diversity and	ARM and Metrics indicate dominance by non-EPT taxa. Water quality has multiple exceedances in WQI calculations. Both are likely influenced by urban activity.

Site Name/Site Code	Upstream Land Use	Potential Impacts	ARM Results	Metrics Results	Notes
				Evenness divergent and below normal. HBI highly divergent and elevated.	
NF02ZL0029 Goulds Brook	Primarily forest with some urban and some agriculture. ATV trail network in watershed.	Likely recreational impacts from ATV trails and some residential and agricultural activity.	Richness and Diversity divergent.	Richness and dominance potentially divergent and elevated. EPT potentially divergent and elevated, Chironomidae and GOID potentially divergent and below normal.	ARM and Metrics indicate community dominated by EPT taxa, possibly the state in less disturbed streams in NL.
NF02ZG0027 Shoal Cove Brook	Mining and forest.	Likely residual mining impacts in this very small watershed. Historical tailings present upstream.	Richness, Evenness, and HBI divergent.	CEFI and HBI potentially divergent and elevated.	ARM and Metrics generally indicate a fairly normal benthic community.
NF02YR0043 Southern Brook	Mainly forest and agriculture.	Impacts from cranberry farming operation.	Richness and CEFI divergent.	Abundance, EPT, Chironomidae, and GOID potentially divergent and below normal.	Low abundance in counts for all taxa metrics may relate to granitic geology and acidic soils leading to a nutrient poor region and low productivity.
NF02YO0190 Tributary to Gills Pond Brook	Heavy mining activity. Tailings ponds upstream in watershed.	Impacts from tailings ponds.	Richness, Evenness, and all Diversity metrics divergent.	Abundance and nearly all taxa count metrics divergent and below normal. All diversity and evenness metrics potentially divergent and below normal.	Possibly impacted by mining activities but low abundance may be related to the acidic and nutrient poor geology.
NF02YO0142 Corduroy Brook	Forest, urban and industrial.	Impacts from urban activities, some industrial.	Richness, CEFI, HBI divergent.	EPT divergent and below normal. GOID, CEFI, and HBI highly divergent and elevated.	Site is likely impacted by a combination of urban and industrial impacts. High in worms at site, leading to GOID and HBI being highly divergent. WQI indicates multiple water quality exceedances.
NF02YO0121 Peter's River	Mix of agriculture, forest, and residential.	Impacts from agricultural activities.	No divergent metrics.	Abundance and all taxa count metrics potentially divergent and elevated. HBI potentially divergent and elevated.	Site is possibly impacted by nutrients from agricultural activities. Abundances are very high at this site
NF02YL0102 Corner Brook Stream	Urban and industrial with some forest. Dams in watershed upstream.	Urban and industrial impacts. Possibly influences from upstream dams.	Abundance, CEFI, and HBI divergent.	Dominance and EPT divergent and below normal, while all diversity and evenness metrics divergent and elevated. HBI divergent and elevated.	Fairly diverse benthic community. Likely minor impacts from urban and industrial activities providing opportunities for a variety of more tolerant taxa to become established in the community.
NF02YL0098 Rocky Brook East Branch	Heavy agriculture land use, some forestry.	Primarily agricultural impacts.	No divergent metrics.	Chironomidae and GOID divergent and below normal.	EPT taxa dominate here, surprising as there is agricultural impacts. Possible that these

Site Name/Site Code	Upstream Land Use	Potential Impacts	ARM Results	Metrics Results	Notes
					impacts are not increasing nutrient input.
NF02YL0029 Wild Cove Brook	Quarries, waste management.	Impacts from waste management facility.	All metrics divergent, HBI highly divergent.	Abundance and dominance divergent and elevated. EPT highly divergent and below normal. HBI and GOID highly divergent and elevated.	Nutrients frequently exceed guidelines here. GOID and HBI metrics suggest nutrient enrichment driving the community changes.