

# Complementary responses of stream fish and benthic macroinvertebrate assemblages to environmental drivers in a shale-gas development area

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## Abstract

Shale-gas production could impact freshwater quality through contamination of the physical and chemical habitat (e.g., fracturing fluids, untreated or treated effluent) or development-related impacts. Despite environmental concerns, information is lacking to support biomonitoring as a diagnostic tool to assess impacts of shale-gas production. We characterized water quality and biota in areas of high shale gas potential (Early Carboniferous bedrock in New Brunswick, Canada) and surrounding geologic areas, and we assessed patterns in benthic macroinvertebrate (BMI) and fish assemblages. Early Carboniferous stations differed primarily based on water chemistry, and BMI were associated with a gradient in conductivity and temperature across geologic classes. Concordance analysis indicated similar classification of stations by both organism groups, though fish were more related to turbidity and nutrients. Concordance among fish and BMI was strongest at high conductivity, Early Carboniferous stations. These results suggest that geology plays a strong role in driving abiotic habitats and biotic communities of streams, even at small spatial scales. Furthermore, they suggest BMI and fish can provide complementary information for biomonitoring in shale-gas development areas, with BMI responding to increased ion concentrations from surface water contamination, and fish responding to changes in nutrients and turbidity resulting from development.

**Key words:** benthic macroinvertebrate, fish, concordance, water chemistry, freshwater habitat, hydraulic fracturing

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## Introduction

Hydraulic fracturing of oil shale deposits (unconventional gas production) offers high potential for the production of natural gas (Rivard et al. 2014), particularly with recent technological advances that have made the fracturing and extraction process more efficient (Vengosh et al. 2013; Vidic et al. 2013; Soeder and Engle 2014). Hydraulic fracturing involves horizontal drilling at depths >1 km into the shale and injection of large volumes of fluids at high pressure (slickwater that is laced with viscosity gels and chemical additives including hydrocarbons) to assist in the recovery of gas (Gregory et al. 2011; Rivard et al. 2014; Soeder and Engle 2014). Environmental concerns related to unconventional

gas activities include the potential for groundwater contamination through stray gases or hydraulic connectivity from fracturing activities (Vengosh et al. 2013, 2014; Lavoie et al. 2014), the fragmentation and alteration of habitats as a result of water withdrawal and infrastructure development to support fracturing activities (Entrekin et al. 2011; Brittingham et al. 2014), and the need for safe disposal of the large volumes of recovered fracking fluids, which could cause groundwater and surface water contamination if insufficiently treated or if a spill occurs (Entrekin et al. 2011; Gregory et al. 2011; Warner et al. 2013; Lavoie et al. 2014; Vengosh et al. 2014). Spills of untreated fracking fluids can release high concentrations of salts, organics, and metals into surrounding freshwaters (Vengosh et al. 2014). Furthermore, an analysis of treated wastewater effluent from fracking activities in Pennsylvania indicated that some contamination of surface waters is possible even after treatment if fluids are discharged into nearby streams (Warner et al. 2013). Despite the potential for environmental impacts, there remains a lack of information to support the development of diagnostic tools for environmental monitoring of these activities (Vengosh et al. 2013, 2014; Vidic et al. 2013; Leger et al. 2016a, 2016b), and significant gaps exist in the monitoring of shale-gas production areas (Entrekin et al. 2011; Kinchy et al. 2016).

Shale-gas formations have particular geological characteristics (bedrock age, formation, organic content, and depth) that determine the accessibility of the shale gas and the potential yield (Dyini 2003; Rivard et al. 2014). The geological characteristics that determine suitability for shale-gas development may also play a role in determining physical and chemical characteristics of overlying freshwater habitats. For example, water chemistry composition and substrate bed material of rivers have been shown to vary across geology types (Johnson et al. 1997; Dow et al. 2006). Specifically, Lento et al. (2019) found higher ion concentrations in streams underlain by Early Carboniferous bedrock (a formation with high shale-gas potential) than in surrounding streams. Several studies have noted that the variability in the chemical and physical habitat that is observed in freshwaters with different underlying geology is accompanied by strong differences in biotic communities across these systems, such as differences in species composition and dominant species (Leland and Porter 2000; Esselman et al. 2006; Yates and Bailey 2010), even when geological shifts occur over small spatial areas (Neff and Jackson 2011, 2012). Thus, it may be possible to predict the biota that would be present across natural differences in geology (including areas with and without shale-gas potential) based on known tolerances to the physical and chemical habitat characteristics associated with different types of bedrock geology. However, such prediction would require a better understanding of the characteristics of abiotic habitats and biotic communities found in freshwaters overlying geologic formations with high shale-gas potential. This information is necessary to describe the natural variation that may exist in these areas relative to other (potentially neighbouring) geological formations, but also to improve our ability to predict biotic and abiotic shifts within these systems as development and production increase.

Evaluation of the biotic communities that are characteristic of the chemical and physical habitat conditions of shale-gas-producing geologic areas and monitoring of changes within those areas relative to surrounding geological formations is reliant on the selection of appropriate taxonomic groups, focusing on those that might be expected to respond to the anthropogenic stressors of interest (Hering et al. 2006; Goodsell et al. 2009; Yates and Bailey 2010). In river systems, benthic macroinvertebrates (BMI) and fish are commonly used as bioindicators because they are widespread, easy to sample, and have well-documented tolerances to perturbation (Bonada et al. 2006; Resh 2008). In addition, each group has its own advantages: for example, BMI are relatively immobile and reflect local conditions, whereas fish are long-lived and have social and economic importance (Bonada et al. 2006; Resh 2008). However, each group of organisms also differs in its response to perturbation (Hering et al. 2006; Johnson et al. 2006), with a stronger response of BMI to local water chemistry and substrate size changes and a response of fish to physical habitat shifts, including temperature (Hering et al. 2006;

Yates and Bailey 2011; Bae et al. 2014). Selection of suitable indicator groups must involve a detailed evaluation of the response of target groups to relevant anthropogenic stressors (Goodsell et al. 2009; Yates and Bailey 2010). Where the full extent of potential stressors is unknown, the combined assessment of multiple groups may provide the best characterization of baseline conditions and biotic integrity in a system (Bonada et al. 2006; Hering et al. 2006). The use of multiple bioindicator groups in monitoring and assessment assumes that each biotic group has a complementary stressor response, i.e., that each group responds to different stressors, improving the diagnostic capacity of the suite of bioindicators and increasing the likelihood of detecting impacts (Hering et al. 2006).

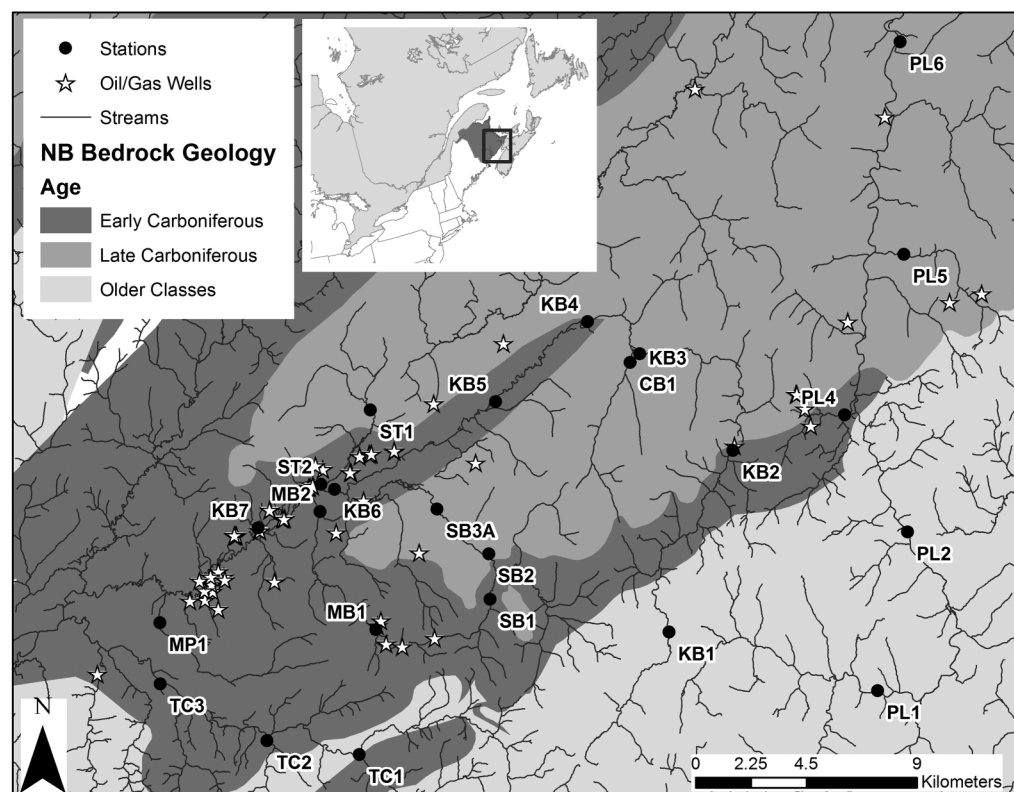
A measure of similarity might be expected in the characterization of rivers or lakes by different organism groups where there are large-scale environmental drivers acting on those freshwater systems, if all organisms respond in a similar manner (Paavola et al. 2006; Johnson et al. 2007). One way to assess the differences and similarities in the responses of groups of organisms is to evaluate community concordance, which measures the extent to which there is coherence or consistency among biotic assemblages, i.e., similarity in the characterization of stations based on the community structure of different groups of organisms (Jackson and Harvey 1993). Significant levels of community concordance have been found in assessments of BMI, fish, and other freshwater organism groups (e.g., diatoms, bryophytes, plankton, macrophytes) in both lakes and rivers (Kilgour and Barton 1999; Johnson and Hering 2010; Neff and Jackson 2013), though in some cases the strength of concordance was dependent on spatial or temporal scale of assessment (Allen et al. 1999; Heino 2002; Paavola et al. 2006; Bowman et al. 2008). At small spatial scales, researchers have noted a lack of concordance among communities where local-scale drivers dominate and have suggested that each organism group responded to different environmental and anthropogenic pressures (Paavola et al. 2003; Heino et al. 2005; Infante et al. 2009; Larsen et al. 2012). In contrast, the higher concordance among communities at regional scales has been suggested to result from large-scale environmental drivers acting in a similar fashion across broad spatial scales (e.g., Heino 2002; Paavola et al. 2006). If this is the case, then the unique physical and chemical habitat of stream systems in geological areas with shale-gas potential has the potential to drive concordance among stream assemblages, even if there are differing responses of organism groups to localized environmental drivers.

The purpose of this study was to characterize stream fish and BMI assemblage structure in southeastern New Brunswick, Canada, an area underlain by different ages of bedrock (Early-Late Carboniferous and older-age sedimentary and nonsedimentary bedrock), identify dominant drivers for fish and BMI, and assess the level of community concordance among fish and BMI in these systems. Areas underlain by Early Carboniferous bedrock have a high potential for shale-gas extraction, yet characterization of the physical and chemical habitat and biotic communities of freshwaters in these areas has been limited (Kinchy et al. 2016). Therefore, the assessment of biotic and abiotic patterns within these systems and in relation to surrounding geological age classes has the potential to inform management and biomonitoring of freshwater systems in shale-gas development areas. Community concordance among fish and BMI assemblages was predicted to be high if (i) BMI and fish displayed similar patterns because they responded to the same environmental drivers or (ii) geological age class acted as a large-scale driver of community structure, leading to increased concordance among organism groups.

## Methods

### Study design

Sampling was conducted in the Kennebecasis and Pollett watersheds in southeastern New Brunswick, Canada (Fig. 1). The area is primarily forested, with some agriculture and a small percentage of land cover composed of settlements, infrastructure, and industry, including shale-gas exploration and



**Fig. 1.** Study area map, showing sample stations where biotic and abiotic data were collected in 2015, location of oil and gas wells (producing and nonproducing wells, as of 2014) and underlying bedrock geology of the area (geology classified by geological age as Early Carboniferous, Late Carboniferous, or Older Classes, which include Neoproterozoic and Devonian-Carboniferous). Inset map indicates the study location in eastern New Brunswick, Canada. Source for geology, stream, and oil and gas well map layers: Government of New Brunswick Department of Natural Resources. Map created in ArcMap (version 10.3, ESRI, St. Paul, Minnesota, USA).

extraction. Headwater regions of the Kennebecasis and Pollett rivers comprise morainal and colluvial surficial geology deposits, whereas the mid to lower reaches are dominated by morainal, glaciofluvial, and alluvial deposits (Rampton 1984). Bedrock geology in the western part of the study area is characterized by Early Carboniferous sedimentary bedrock (Mississippian era) underlain by the Lower Carboniferous Albert formation, which has potential for conventional natural gas and unconventional (shale gas) extraction (Macauley et al. 1984, 1985; Leblanc et al. 2011). The eastern part of the study area is dominated by Late Carboniferous sedimentary bedrock (Pennsylvanian era), which differs from oil shale bedrock with respect to mineral content and organic composition (Dyni 2003). Older classes of bedrock, including sedimentary Devonian-Carboniferous bedrock and volcanic and intrusive bedrock from the Neoproterozoic era, are found along the southern boundary of the study area (see Lento et al. 2019 for a full description of bedrock geology in the study area).

An extensive spatial survey was completed in late August and early September 2015 to characterize fish and BMI assemblage structure, water chemistry, and physical habitat in the Kennebecasis and Pollett watersheds ( $n = 18$  and 6 stations, respectively; Fig. 1). Selection of sampling areas utilized existing geographic information system (GIS) data for topography, geology, and land use information, complemented by local knowledge. Site selection followed a stratified random sampling design to

**Table 1.** Stations in the assessment of water quality and biota in the Kennebecasis and Pollett watersheds.

Station	Stream name	Latitude	Longitude	Catchment area (km <sup>2</sup> )	Geological age
CB1	Calamingo Brook	45.82396	−65.22416	18.33	Late Carboniferous
KB1	Kennebecasis River	45.72493	−65.20672	16.08	Older Classes
KB2	Kennebecasis River	45.79116	−65.17107	52.47	Early Carboniferous
KB3	Kennebecasis River	45.82706	−65.21902	77.73	Late Carboniferous
KB4	Kennebecasis River	45.83904	−65.24611	108.39	Late Carboniferous
KB5	Kennebecasis River	45.81035	−65.29384	119.82	Early Carboniferous
KB6	Kennebecasis River	45.77914	−65.37976	220.07	Early Carboniferous
KB7	Kennebecasis River	45.76548	−65.42001	292.73	Early Carboniferous
MB1	McLeod Brook	45.72736	−65.36009	7.25	Early Carboniferous
MB2	McLeod Brook	45.77112	−65.38745	24.17	Early Carboniferous
MP1	Millpond Brook	45.73120	−65.47202	13.39	Early Carboniferous
PL1	Pollett River	45.70245	−65.09772	72.37	Older Classes
PL2	Pollett River	45.75614	−65.07883	122.99	Older Classes
PL4	Lee Brook	45.80344	−65.11241	22.36	Early Carboniferous
PL5	Colpitts Brook	45.86169	−65.07990	12.61	Late Carboniferous
PL6	Pollett River	45.93900	−65.07997	247.8	Late Carboniferous
SB1	Negro Brook	45.73813	−65.29926	8.14	Early Carboniferous
SB2	South Branch	45.75476	−65.29966	46.37	Late Carboniferous
SB3A	South Branch	45.77139	−65.32639	55.61	Late Carboniferous
ST1	Stone Brook	45.80803	−65.36037	19.68	Late Carboniferous
ST2	Stone Brook	45.78099	−65.38686	27.84	Early Carboniferous
TC1	Cedar Camp	45.68205	−65.36880	36.43	Older Classes
TC2	Parlee Brook	45.68751	−65.41667	38.97	Early Carboniferous
TC3	Trout Creek	45.70886	−65.47225	150.16	Early Carboniferous

**Note:** Coordinates are presented in decimal degrees. Geological age is the dominant age class in a 1-km upstream catchment buffer (Early Carboniferous, Late Carboniferous, or Older Classes, including Neoproterozoic and Devonian-Carboniferous).

ensure coverage over a variety of catchment sizes across a natural habitat gradient. Mainstem and tributary stations were selected to cover a range of sub-catchment areas from 7.25 to 292.73 km<sup>2</sup> (Table 1). Stations were classified by underlying geological age as Early Carboniferous, Late Carboniferous, or Older Classes (Devonian-Carboniferous and Neoproterozoic) based on the dominant age of geology in a 1-km upstream catchment buffer (see Lento et al. 2019). Several of the Early Carboniferous stations were located in the vicinity of oil and gas wells (Fig. 1), though active fracking activities were halted in the area in 2014 after the provincial government imposed a moratorium, and many wells were out of operation prior to the moratorium. Groundwater methane levels measured in private wells in the area were elevated compared to surrounding areas, but there was no correlation with proximity to oil and gas wells, which indicated that these were naturally high methane levels (Loomer et al. 2016). Furthermore, spills associated with unconventional shale-gas production have not been reported for the area, and chloride levels in surface waters were not



correlated with proximity to oil and gas wells; thus, stations were considered to be least disturbed condition sites (sensu [Stoddard et al. 2006](#)).

## Sample collection and processing

Water grab samples were collected following provincial guidelines and were analyzed at the New Brunswick Department of Environment and Local Government's analytical lab. Samples were analyzed for a standard suite of metals, nutrients, and ions following standardized analytical procedures ([Table S1](#)). A habitat survey was conducted at each station following protocols of the national Canadian Aquatic Biomonitoring Network (CABIN; [Environment Canada 2012](#)), including measurement of stream width, depth, velocity, and bed slope; description of in-stream and riparian vegetation and periphyton coverage; and a rock walk ([Table S2](#)). During the rock walk, 100 particles were chosen at random from within the sampled habitat, and the length of the intermediate axis length was recorded in mm (after [Wolman 1954](#)). Substrate measurements were grouped by grain size (using the Wentworth scale; see [Environment Canada 2012](#)) to quantify the percent composition of different size classes (e.g., sand, gravel, cobble) and median particle size ( $D_{50}$ ).

BMI samples were collected following standard CABIN protocols ([Environment Canada 2012](#)). The operator held a 400- $\mu\text{m}$  mesh kick-net downstream while travelling through the stream channel in a zig-zag fashion and disturbing the substrate for a period of 3 min ([Environment Canada 2012](#)). BMI samples were randomly subsampled in the laboratory using the Marchant box method ([Environment Canada 2014](#)) until a minimum of 300 organisms was counted. Non-Chironomidae (midge) taxa were sent to an independent Society for Freshwater Science (SFS)-certified taxonomist for identification to genus level where possible, and Chironomidae taxa were sent to EcoAnalysts, Inc. to be identified to species by SFS-certified taxonomists (see [Lento et al. 2019](#) for more details). Samples contained large numbers of taxa that were too small (early instars) to identify to the level of genus and to avoid mixed-level taxonomy within an order (which may lead to misrepresentation of trends), data were rolled up to the level of subfamily for Chironomidae and the level of family or higher for all other organisms. [Bowman and Bailey \(1997\)](#) demonstrated that analysis of BMI communities at the level of genus did not improve the ability to detect patterns in the data.

Fish community surveys were conducted by using a backpack electrofishing unit (Smith-Root LR-24, Vancouver, WA, USA) to fish an extent of the stream site containing at least one riffle and one pool or run in a single upstream pass (stream area ranged from 50 to 830  $\text{m}^2$ ). The effort was standardized by maintaining power within a range of 65–75 W (voltage varied depending on conductivity of the water) and sampling time ranged from 419 to 3009 s. All fish were identified to species level.

Temperature loggers (Onset HOBO UA-001-08, UA-002-64, and U24-001, Bourne, MA, USA) were deployed in stream sites in May 2015 to capture longer-term temperature trends. Loggers were anchored by rebar driven into the substrate and were housed in light gray PVC pipe to avoid effects from solar radiation and shield them from damage caused by shifting substrate. The loggers recorded data every 30 min from May to November. Logger data were examined for records that indicated the logger was out of water (e.g., spikes in temperature or light levels, or decrease in conductivity to near 0 for the combination temperature/conductivity loggers). These records were removed, and remaining data were used to calculate summary temperature metrics for the summer period (June–August), including the number of degree days above 19 °C and the coefficient of variation (CV) of temperature. Catchment area upstream of each station was delineated by using the Hydrology tools in the Spatial Analyst toolbox to analyze a 30 m resolution Digital Elevation Model (DEM; Canada Digital Elevation Data; [open.canada.ca/data](https://open.canada.ca/data)) in ArcGIS (version 10.3, ESRI, St. Paul, Minnesota, USA). Additional geospatial variables were considered for analysis, including catchment vegetation and slope, but in-stream measures were retained to reduce the number of variables for the analysis.

Surficial geology was also characterized from available geospatial layers but was correlated with the bedrock geology classes and therefore removed from the analysis to avoid masking differences in water chemistry and physical habitat variables among geologic age classes.

## Data analysis

### Characterization of stations

Multivariate data analysis was used to characterize the abiotic environment of the stream stations. Principal components analysis (PCA) was run with water quality and physical habitat data to assess similarities among stations. Data were pretreated by replacing water chemistry values that were below the limit of quantification (LOQ) with a value of half the LOQ. Prior to performing the PCAs, water quality parameters with extremely low detection frequency (defined as a water quality parameter that was only measured above the LOQ for fewer than two stations) were removed. The resulting subset of water quality and physical habitat variables was  $\log_{10}$ - or logit-transformed as appropriate to lessen the effect of extreme values and was analyzed in a PCA of the correlation matrix (centering and standardizing by variable scores) to control for the range of measurement scales in the abiotic variables. PCA biplots with station symbols coloured to reflect dominant underlying geological age were examined to evaluate associations of sample stations in multidimensional space, reflecting similarity in water quality or habitat conditions.

Stations were also characterized with respect to their biotic communities through multivariate analysis. Analysis was conducted separately on BMI data (at the level of subfamily for Chironomidae and family or higher for other taxa) and on fish data (at the species level; see [Tables 2](#) and [3](#) for taxon names and abbreviations). Both sets of data were converted to relative abundances to focus on compositional differences, and data were transformed using  $\log_{10}(x + 1)$  to dampen the effect of extreme values and reduce asymmetry ([Legendre and Legendre 2012](#)). Transformed BMI data were adjusted by adding 0.1 to further downweight the effect of rare taxa. PCA was run separately for BMI and fish data, in both cases post-transforming taxa scores to represent standardized scores on the resultant biplot. PCA biplots with station symbols coloured to reflect dominant underlying geological age were visually assessed to evaluate similarities and differences among stations with respect to assemblage composition.

### Analysis of community concordance

Procrustes analysis was used as a test of community concordance to determine whether similar gradients were evident when stations were assessed using different biotic assemblages. Procrustes analysis compares the spatial arrangement of stations in multivariate space between two ordinations (one target ordination, one rotational ordination) by translating and rotating the rotational ordination to match the target ordination as closely as possible. Residual vectors describe deviations of sample points from their location in the target ordination to their location in the rotational ordination, and the sum of squared residuals ( $m_{12}^2$ ) provides an estimate of the fit of the two ordinations, with a high  $m_{12}^2$  statistic being indicative of strong differences between the two ordinations.

To test for community concordance, Procrustes analysis was used to compare the PCA ordinations of stations based on BMI relative abundance and fish relative abundance. For the analysis, the fish PCA was the target ordination and the benthic macroinvertebrate PCA was the rotational ordination. A randomization test was used as part of the analysis to test the significance of  $m_{12}^2$  by comparing 9999 random configurations of the sample points with the target ordination; a significant  $p$ -value (at  $\alpha = 0.05$ ) was an indication that the target ordination and rotational ordination were more similar than could be obtained by chance ([Jackson 1995](#)).

**Table 2.** Abbreviations of benthic macroinvertebrate taxa used in multivariate analysis of Kennebecasis and Pollett watershed stations.

Class/order	Family/subfamily	Abbreviation
Coleoptera	Elmidae	C_Elm
Diptera	Athericidae	D_Ath
	Ceratopogonidae	D_Cerat
	Chironomidae Chironominae	D_C_Chin
	Chironomidae Diamesinae	D_C_Dia
	Chironomidae Orthocladiinae	D_C_Orth
	Chironomidae Tanypodinae	D_C_Tany
	Ephydriidae	D_Eph
	Muscidae	D_Musc
	Sciomyzidae	D_Scio
	Simuliidae	D_Simu
	Tabanidae	D_Tab
	Tipulidae	D_Tipu
Ephemeroptera	Baetidae	E_Bae
	Baetiscidae	E_Baesc
	Ephemerellidae	E_Ephe
	Heptageniidae	E_Hept
	Isonychiidae	E_Iso
	Leptohyphidae	E_Lepthy
	Leptophlebiidae	E_Lepto
Megaloptera	Corydalidae	M_Cory
Odonata	Gomphidae	O_Gomp
Plecoptera	Capniidae	P_Cap
	Chloroperlidae	P_Chlor
	Leuctridae	P_Leu
	Nemouridae	P_Nem
	Perlidae	P_Perl
	Perlodidae	P_Perlo
	Pteronarcyidae	P_Ptero
	Taeniopterygidae	P_Taen
Trichoptera	Apataniidae	T_Apa
	Brachycentridae	T_Bra
	Goeridae	T_Goer
	Glossosomatidae	T_Glos
	Helicopsychidae	T_Hel

(continued)



Table 2. (concluded)

Class/order	Family/subfamily	Abbreviation
	Hydropsychidae	T_Hpsy
	Hydroptilidae	T_Hpti
	Lepidostomatidae	T_Lepi
	Leptoceridae	T_Lept
	Limnephilidae	T_Limn
	Odontoceridae	T_Odont
	Philopotamidae	T_Phil
	Polycentropodidae	T_Poly
	Psychomiidae	T_Psy
	Rhyacophilidae	T_Rhya
	Uenoidae	T_Ueno
Acari	Trombidiformes (6 families)	Acari
Amphipoda	Gammaridae and unknown	Amph
Bivalvia	Pisidiidae	Bivalvia
Hirudinea	Hirudinea unknown	Hiru
Oligochaeta	Enchytraeidae	Oli_Ench
	Lumbriculidae	Oli_Lumb
	Naididae	Oli_Naid

Table 3. Abbreviations of fish species names used in multivariate analysis of Kennebecasis and Pollett watershed stations.

Species	Abbreviation
American Eel	EEL
Atlantic Salmon	ATS
Blacknose Dace	BND
Blacknose Shiner	BNS
Brook Trout	BKT
Burbot	BUR
Common Shiner	CSH
Golden Shiner	GSH
Lake Chub	LKC
Ninespine Stickleback	9SB
Sea Lamprey	SLP
Slimy Sculpin	SLS
Threespine Stickleback	3SB
White Sucker	WHS

### Determination of baseline criteria

Water quality and biotic data were summarized as metrics and 95% confidence intervals were calculated to further characterize differences among geological age classes. The purpose of this analysis was to quantify the normal range of water quality and biotic metrics to establish baseline criteria for differentiating among geologic age classes, with a focus on metrics that differentiated Early Carboniferous stations. Metrics were chosen based on parameters that characterized Early Carboniferous stations in the abiotic and biotic PCAs and also included metrics that were used to differentiate among geologic age classes in [Lento et al. \(2019\)](#). For water quality, this included conductivity, nitrate, total phosphorus (TP), turbidity, and total organic carbon (TOC). For BMI, biotic metrics included: abundance; genus richness; % Chironomidae; % Ephemeroptera, Plecoptera, and Trichoptera (EPT); % noninsects; % Mollusca; and % Oligochaeta. For fish, biotic metrics included catch per unit effort; species richness; % Brook Trout; and % Slimy Sculpin. Confidence intervals that did not overlap between geologic age classes, and thus indicated a significant difference in the normal range, were used to determine baseline normal ranges for metrics.

### Assessment of environmental drivers

Biotic–abiotic associations were explored through multivariate analysis for both BMI and fish. Redundancy analysis (RDA) was run separately for BMI and fish data, constraining assemblage data to a subset of water quality and habitat variables to identify the dominant drivers of assemblage composition in the stream systems. The RDA was run with forward selection, limiting the final set of environmental variables to a maximum of nine to avoid over-fitting (as there were only 14 fish species). However, the same initial set of environmental variables was used to create the model for BMI and fish to assess the relative importance of environmental variables to both BMI and fish composition. Prior to analysis, the full suite of environmental variables was reduced by testing Pearson correlations among drivers to remove highly redundant variables (those with  $|r| > 0.6$ ). The final subset of environmental variables used to build the RDA models is indicated in [Tables S1](#) and [S2](#). Because environmental variables were measured at different scales, the RDAs were run on the correlation matrix by centering and standardizing by species. For each RDA, the variance explained by each axis was compared with the total variance in the biotic community (unconstrained variance) to assess the strength of the chosen variables to explain patterns in community similarities among stations. The significance of each environmental variable included in the RDA was assessed using a Monte Carlo permutation test with 999 permutations. Biplots were used to visually assess biotic–abiotic associations among stations, with station symbols coloured by underlying geological age.

The subset of abiotic variables used in the RDAs was further assessed by relating it to a concordance gradient in the stations (as in [Paavola et al. 2006](#)). For this analysis, the station residuals from the Procrustes analysis of biotic data were considered to represent a gradient in community concordance across stations. This concordance gradient was compared with each environmental variable used in the water chemistry and habitat PCA through correlation analysis (Pearson product-moment correlations) to determine which abiotic variables were associated with changes in the level of concordance between fish and BMI.

Multivariate analyses (PCAs, RDAs, and significance tests) were run in Canoco version 4.55 ([ter Braak and Šmilauer 2002](#)). Procrustes analysis was run in R version 3.2.3 ([R Development Core Team 2015](#)) using the vegan package ([Oksanen et al. 2015](#)). Correlation of environmental variables and residuals was run in Systat 12 (version 12.02).

Results

Abiotic characterization

The PCA of water chemistry and habitat variables identified clear gradients in the data that were driven by a contrast between stations with high levels of ions, nutrients, and suspended sediment particles and stations that were more strongly associated with habitat descriptors. The first axis of the PCA, which explained 31.9% of the variance among stations, separated most Early Carboniferous stations from those underlain by Late Carboniferous or older classes of bedrock, as Early Carboniferous stations were generally positively correlated with conductivity, alkalinity, hardness, and ions (including Cl, Na, K, Ca, and SO<sub>4</sub>) as well as turbidity and the habitat variable bankfull depth (Fig. 2). On the opposite end of the first axis gradient, stations underlain by Late Carboniferous or older classes of bedrock were associated with a number of habitat variables, including higher D<sub>50</sub> (large median particle size), larger bankfull width, presence of coniferous trees, more variable temperature (higher CV), increased dominance of deciduous trees, and higher cover of periphyton and boulders (Fig. 2).

The second axis of the PCA explained 15.8% of the variance among stations and contributed to separation within each bedrock geology age class (Fig. 2). The positive end of the gradient was associated with degree days above 19 °C, average depth, catchment area, nutrients (TN, TOC, nitrate), colour, and aluminum (Fig. 2). Several Early Carboniferous stations (ST2, KB7, KB6, and PL4) followed a gradient in water chemistry along this axis. On the negative end of the gradient, stations were associated with high slope, dominance of coniferous vegetation, and % silt/clay (Fig. 2). Stations such as TC1, TC2, and KB4 (Older Class, Early Carboniferous, and Late Carboniferous, respectively) were

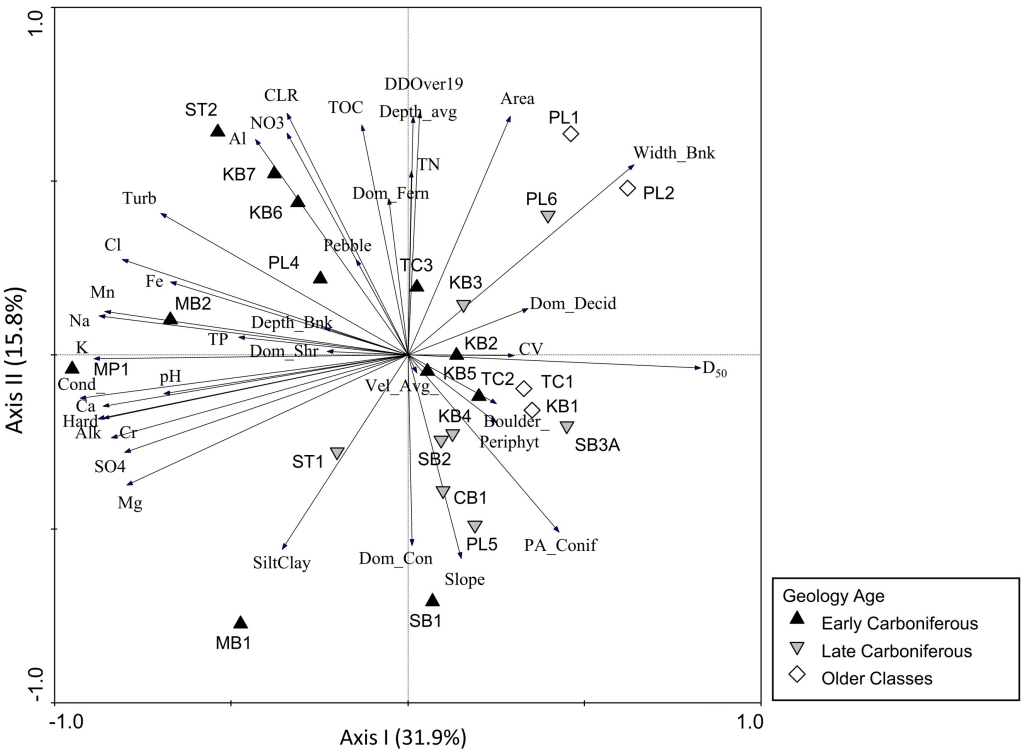


Fig. 2. Principal components analysis biplot of the 24 stations based on water quality and habitat survey data, with station symbols indicating the age of underlying bedrock geology. Station abbreviations are listed in Table 1 and variable abbreviations are listed in Tables S1 and S2.

negatively correlated with parameters such as TN, TOC, TP, colour, and turbidity and associated total metals such as Al and Fe (Fig. 2). In contrast, some stations on the Pollett River (PL1, PL2, and PL6) were positively associated with TN, TOC, and colour along the second axis while still reflecting a negative association with ions along the first axis. The third axis (not shown), which explained 11.2% of the variance among stations, was positively correlated with TP (associated with Early Carboniferous stations) and periphyton cover (associated with Late Carboniferous stations).

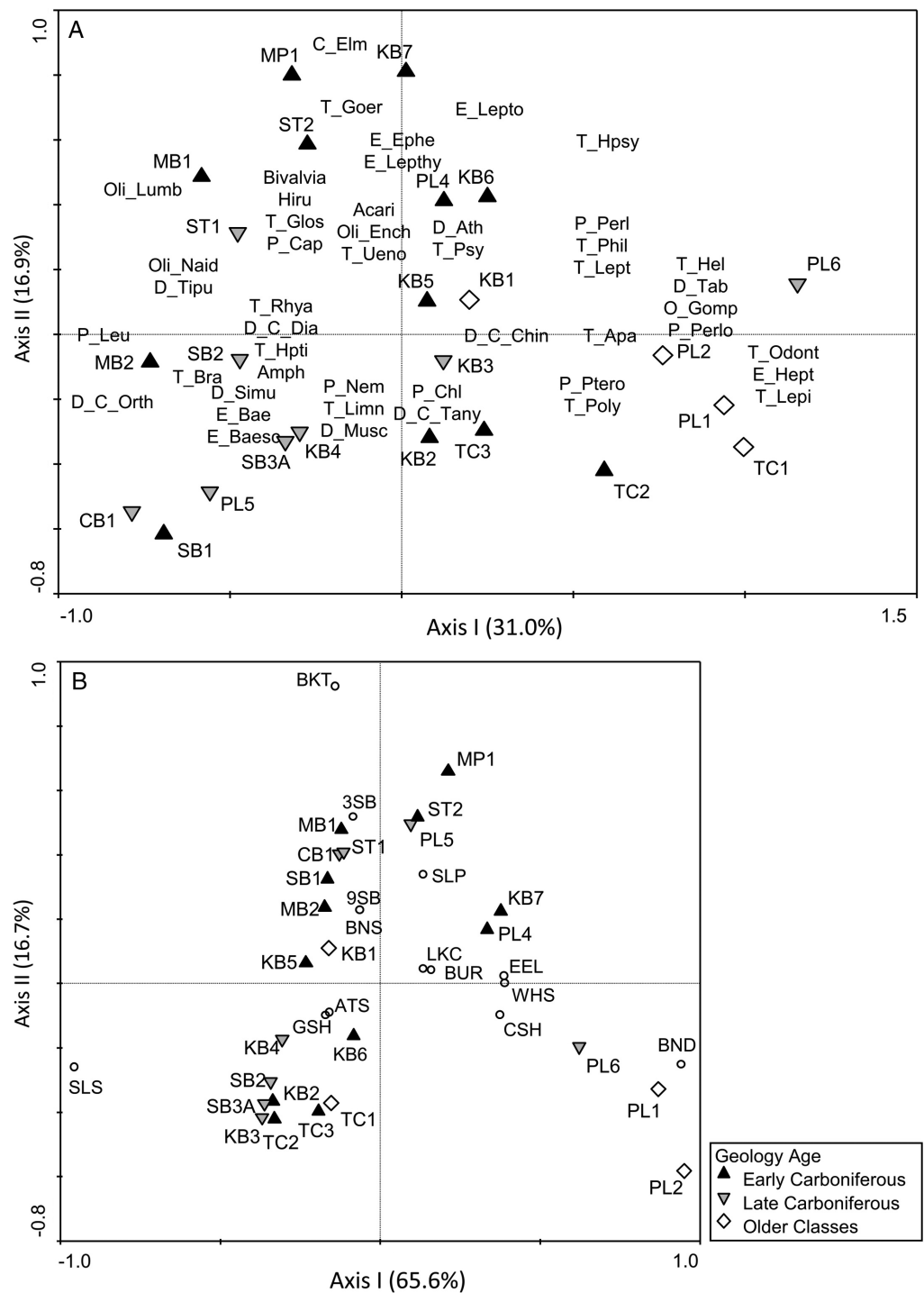
## Biotic characterization

Multivariate assessment of BMI community structure indicated a strong separation of stations along the first and second axes that was similar to patterns in the water quality and habitat PCA. The first axis, which explained 31% of the variance in BMI samples, separated the Older Class stations and a small number of Early and Late Carboniferous stations with higher bankfull width and larger substrate size (stations PL1, PL2, PL6, TC1, and TC2) from the majority of other stations (Fig. 3A). Stations that were positively correlated with the first axis were associated with Odonata and nine families of Trichoptera (Apataniidae, Helicopsychidae, Hydropsychidae, Lepidostomatidae, Leptoceridae, Odontoceridae, Philopotamidae, Polycentropidae, and Psychodidae) whereas only four Trichoptera families were strongly associated with the negative end of the gradient (Brachycentridae, Glossosomatidae, Hydroptilidae, and Rhyacophilidae; Fig. 3A). Plecoptera families appeared evenly distributed along the length of the first axis (including Cloroperlidae, Perlidae, Perlodidae, and Pteronarcyidae on the positive end and Capniidae, Leuctridae, and Nemouridae on the negative end; Fig. 3A), suggesting a wide range of preferences to the underlying environmental gradient. The second PCA axis, which described 16.9% of community variance, was positively correlated with a group of predominantly Early Carboniferous stations that had high conductivity and high concentrations of ions, and was negatively correlated with most Late Carboniferous stations (Fig. 3A). Along the second axis, a large number of taxa were positively associated with the Early Carboniferous stations, including Coleoptera (Elmidae), several families of Ephemeroptera (including Ephemerellidae, Leptohyphidae, and Leptophlebiidae) and Trichoptera (Goeridae, Glossosomatidae, Hydropsychidae, Psychomiidae, and Uenoidae), and most noninsect groups, such as Oligochaeta, Bivalvia, Hirudinea, and Acari (Fig. 3A).

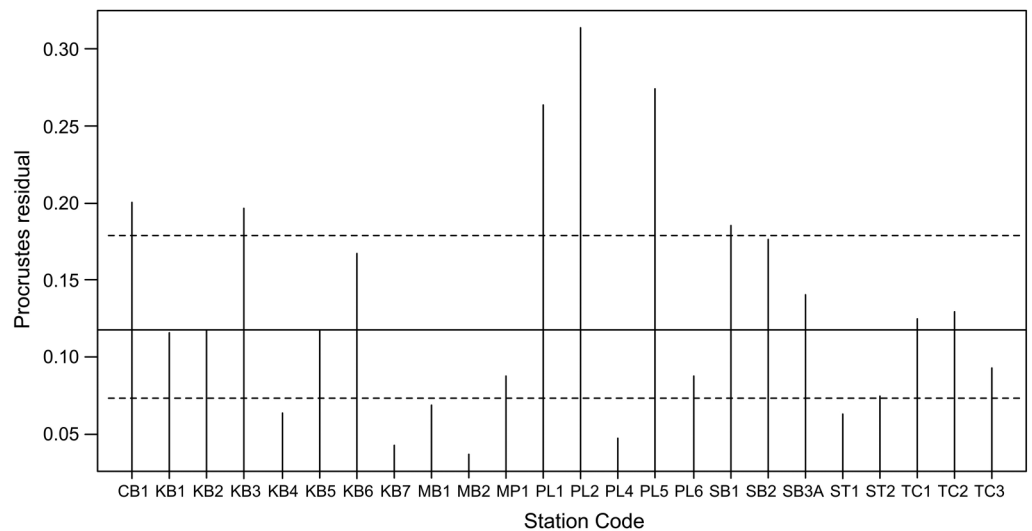
The PCA based on fish relative abundance explained a great deal of variation in the community data, but did not appear to distinguish strongly among bedrock geology age classes (Fig. 3B). The first axis, which explained 65.6% of the variance in the fish community, was positively associated with Pollett River stations PL1, PL2, and PL6, and more weakly correlated with the remaining stations (Fig. 3B). This axis was primarily driven by a negative association between Slimy Sculpin (*Cottus cognatus*; on the negative end of the gradient) and Blacknose Dace (*Rhinichthys atratulus*; on the positive end of the gradient), though other stations on the positive end of the gradient were also positively correlated with taxa such as American Eel (*Anguilla rostrata*), White Sucker (*Catostomus commersonii*), and Common Shiner (*Luxilus cornutus*; Fig. 3B). The second axis explained much less community variance (16.7%) but represented a gradient in Brook Trout (*Salvelinus fontinalis*) abundance along which most stations differed. Brook Trout were positively correlated with Threespine Stickleback (*Gasterosteus aculeatus*) but were weakly or uncorrelated with other fish species (Fig. 3B).

## Community concordance

Procrustes analysis indicated that the fit of the BMI PCA (rotational ordination) to the fish PCA (target ordination) was more similar than could be obtained by chance ( $m^2_{12} = 0.56$ ;  $p = 0.001$  after 999 permutations). The largest residual difference was found for station PL2 (residual vector length = 0.313), and two other stations had moderately long residual vectors (e.g., PL1 and PL5; Fig. 4), suggesting that the similarity of these stations to other stations differed depending on whether



**Fig. 3.** Principal components analysis (PCA) biplot of the 24 stations based on (A) benthic macroinvertebrate (BMI) data and (B) fish data, with station symbols indicating the age of underlying bedrock geology. The BMI plot includes a subset of taxa for ease of visual interpretation, grouped in clusters based on approximate location in the PCA. Station abbreviations are listed in [Table 1](#) and taxonomic abbreviations are listed in [Tables 2 and 3](#).



**Fig. 4.** Residuals from Procrustes analysis of fish (target) and benthic macroinvertebrate (rotational) principal components analysis ordinations, indicating the residual distance between a site's location in the target ordination and its location in the rotational ordination after translation and rotation. The solid and dotted horizontal lines represent type 7 quartiles of the residuals.

fish or BMI data were considered. However, residual vectors were short for most stations, indicating significant community concordance that was evident as a strong overall similarity between the fish ordination and the BMI ordination.

### Baseline criteria

Baseline criteria were established through analysis of confidence intervals in water quality and biotic metrics, with the differences predominantly found between Early Carboniferous and Older Class stations. Conductivity, which was strongly associated with Early Carboniferous stations in the abiotic PCA, was higher on average in Early Carboniferous stations (mean = 167.9  $\mu\text{S}/\text{cm}$ ) than in Older Class stations (mean = 41.62  $\mu\text{S}/\text{cm}$ ), and 95% confidence intervals for these geologic age classes did not overlap (Table 4). Similarly, TP was higher on average in Early Carboniferous stations (mean = 0.013 mg/L) than in Older Class stations (mean = 0.003 mg/L) and confidence intervals did not overlap (Table 4). Nitrate differed significantly between Early and Late Carboniferous stations (results not shown), but the 95% confidence intervals of Older Class stations overlapped with both other classes. Estimates of the normal range for other water quality parameters overlapped between Early Carboniferous, Late Carboniferous, and Older Class stations, which indicated that there was insufficient distinction among geologic classes to allow their use for establishing baseline criteria from the normal range.

There were a number of BMI metrics for which it was possible to develop baseline criteria from the normal range, with all differences found between Early Carboniferous and Older Class stations. Total abundance of BMI was highest on average in Early Carboniferous stations (mean = 25 046.2) and the 95% confidence interval for this geologic class did not overlap with Older Class stations (mean = 8534; Table 4). Taxonomic richness was also higher on average in Early Carboniferous stations (mean = 37.9) than in other geologic classes, and the 95% confidence interval did not overlap with that for Older Class stations (mean = 30.2; Table 4). The remaining metrics that were used to establish baseline criteria reflected the stronger association of Early Carboniferous stations with



**Table 4.** Means and 95% confidence intervals (lower and upper CI) for water quality metrics and biotic metrics that provide baseline distinction between sites underlain by Early Carboniferous bedrock geology and sites underlain by Older Classes of bedrock geology (which includes Neoproterozoic and Devonian-Carboniferous).

Metric type	Metric	Geologic class	Mean	Lower CI	Upper CI
Water quality	Conductivity	Early Carboniferous	167.9	77.1	258.7
		Older Classes	41.6	21.2	62.0
	Total phosphorus (TP)	Early Carboniferous	0.013	0.007	0.020
		Older Classes	0.003	0.001	0.005
Benthic macroinvertebrate	Abundance	Early Carboniferous	25 046.2	16 472.5	33 619.8
		Older Classes	8534	2996.3	14 071.7
	Genus richness	Early Carboniferous	37.9	34.9	40.9
		Older Classes	30.2	26.5	33.9
	% Chironomidae	Early Carboniferous	34.5	27.6	41.4
		Older Classes	18.6	10.0	27.2
	% Ephemeroptera, Plecoptera, Trichoptera (EPT)	Early Carboniferous	47.4	40.8	54.0
		Older Classes	73.5	66.3	80.8
	% Oligochaeta	Early Carboniferous	1.25	0.48	2.03
		Older Classes	0.19	−0.05	0.44

**Note:** Only metrics that differentiate between these geological age classes (i.e., confidence intervals don't overlap) are presented.

noninsect taxa and weaker association with EPT taxa in the BMI PCA. The % EPT was higher on average in Older Class stations (mean = 73.5%) than other geologic age classes, particularly Early Carboniferous (mean = 47.4%), and confidence intervals for Early Carboniferous and Older Class stations did not overlap (Table 4). In contrast, the % Chironomidae (the other dominant taxonomic group in these systems) was significantly higher on average in Early Carboniferous stations (mean = 34.5%) than Older Class stations (mean = 18.6%), as was the % Oligochaeta (Early Carboniferous mean = 1.25% and Older Class mean = 0.19%; Table 4).

In contrast to the BMI, there were no fish metrics that differed significantly among geologic age classes, which supported the generally weaker differentiation of Early Carboniferous stations that was evident in the fish PCA. Although species richness was higher in Early Carboniferous stations than Older Class stations, the 95% confidence intervals for these two geologic age classes indicated no significant differences (Early Carboniferous confidence interval range: 4.1–6.8; Older Class confidence interval range: 0.7–5.3). It was therefore not possible to establish baseline criteria for fish metrics to distinguish among geologic age classes and characterize a distinct normal range for Early Carboniferous stations.

### Biotic–abiotic associations

Forward selection of variables for Redundancy Analysis resulted in similar sets of environmental variables for analysis of BMI and fish (8 of the 9 final variables were the same in both RDAs), though the relative importance of those variables differed. Notably, the set of variables that was significant in the RDA model differed, as BMI were significantly ( $p < 0.05$ ) associated with (in order of importance) temperature (degree days over 19 °C), conductivity, and catchment area, whereas fish were

**Table 5.** Effects of environmental variables in redundancy analyses of BMI and fish, including Lambda-A (conditional effects) and the results of permutational significance tests of Lambda-A.

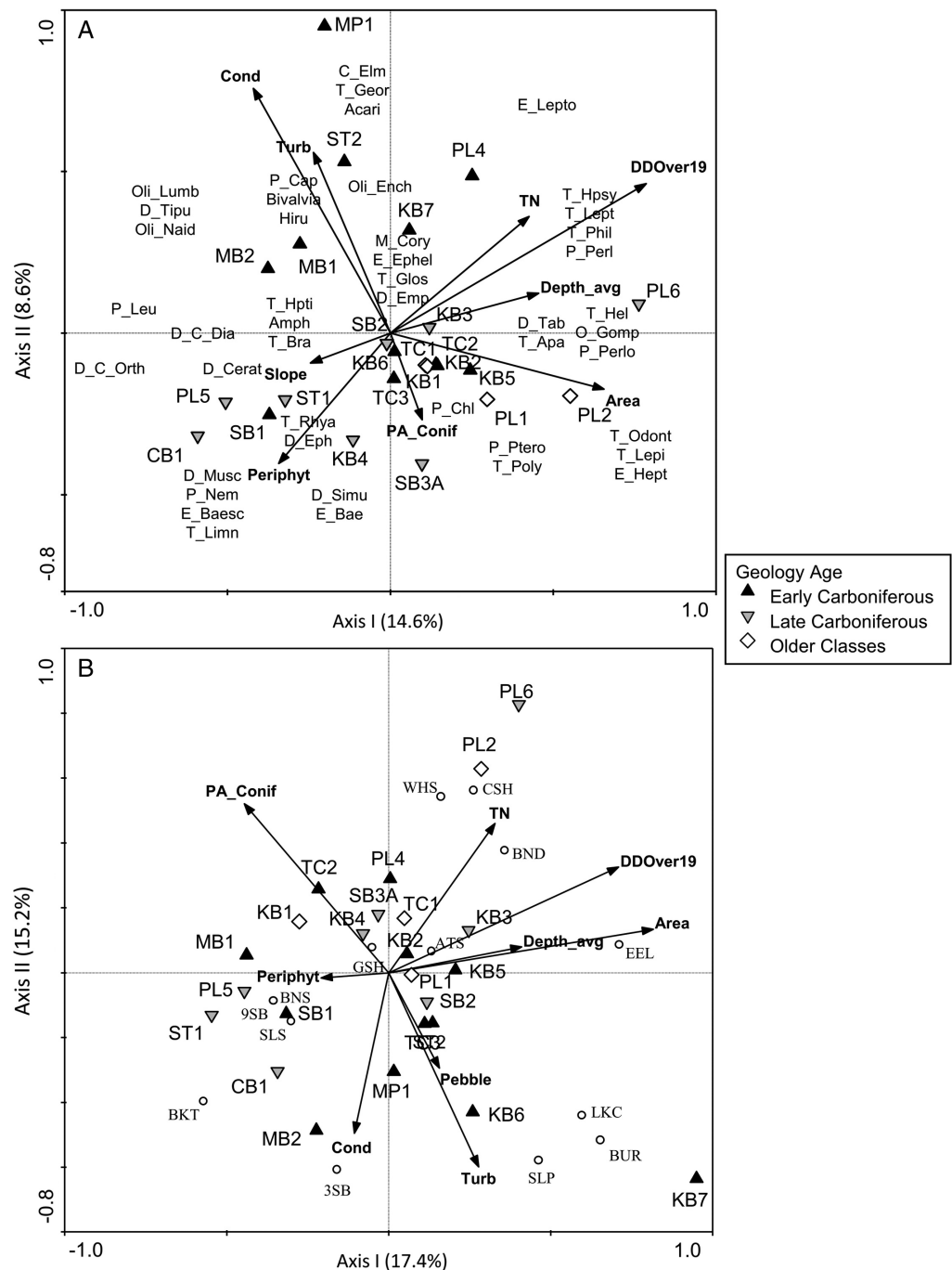
BMI				Fish			
Variable	Lambda-A	F	p	Variable	Lambda-A	F	p
DDOver19	0.12	2.92	<b>0.002</b>	Area	0.13	3.39	<b>0.001</b>
Cond	0.08	2.27	<b>0.006</b>	Turb	0.11	3.00	<b>0.008</b>
Area	0.06	1.50	<b>0.032</b>	TN	0.09	2.73	<b>0.014</b>
Turb	0.05	1.40	0.092	Depth_avg	0.04	1.36	0.240
TN	0.05	1.33	0.106	PA_Conif	0.05	1.34	0.228
PA_Conif	0.04	1.33	0.130	DDOver19	0.04	1.23	0.263
Depth_avg	0.05	1.25	0.120	Periphyt	0.04	1.22	0.282
Slope	0.04	1.17	0.258	Cond	0.03	1.05	0.406
Periphyt	0.04	1.09	0.294	Pebble	0.03	0.98	0.447

**Note:** Variables are listed in order of decreasing *F*-ratio for BMI and fish. *p*-values in bold indicate significance of variable effects at  $\alpha = 0.05$ . See [Tables S1](#) and [S2](#) for variable abbreviations. BMI, benthic macroinvertebrate.

significantly associated with catchment area, turbidity, and TN ([Table 5](#)). Conductivity, which was strongly associated with Early Carboniferous stations and significant in the BMI RDA, was included in the final fish model, but was one of the weakest variables in the model ([Table 5](#)). Average velocity and TP were not included in the final model for either BMI or fish, and only the fish RDA included a substrate size variable (% pebble), whereas slope was only selected in the BMI RDA ([Table 5](#)).

The spatial arrangement of stations and BMI taxa in constrained ordination space was similar between the RDA biplot and the BMI PCA, which was indicative of the strong contribution of the selected water chemistry and habitat variables to the environmental gradient underlying patterns in BMI community composition. The first axis of the RDA explained 14.6% of the unconstrained variance in BMI assemblage composition, and the second axis explained an additional 8.6% of the unconstrained assemblage variance ([Fig. 5A](#)). Several stations in the Pollett River catchment (PL1, PL2, PL4, and PL6) were positively associated with higher temperatures, larger catchment area, higher average depth, and higher TN along the first axis of the RDA ([Fig. 5A](#)). A large number of Late Carboniferous stations were negatively correlated with this gradient and with the second axis, and these stations were associated with lower temperatures, smaller catchment area and depth, lower TN, high periphyton coverage and high slope (stations CB1, PL5, ST1, KB4, and SB3A; [Fig. 5A](#)). In contrast, six of the Early Carboniferous stations were associated with high conductivity and turbidity along the second axis gradient. Plecoptera and Trichoptera families were distributed along the first axis, as in the BMI PCA, whereas the Early Carboniferous stations on the positive end of the second axis gradient (associated with high conductivity and turbidity) were still positively associated with noninsect groups and a small number of insect families ([Fig. 5A](#)).

Separation of stations in the fish RDA showed a weaker grouping of geological age classes, with most classes distributed across the ordination ([Fig. 5B](#)). The first axis, which explained 17.4% of the constrained variance, was primarily driven by a gradient in temperature, catchment area, depth, and TN, as in the BMI RDA. However, Pollett River stations were largely uncorrelated with this axis, and the gradient was driven by a negative association of these variables with stations such as ST1, PL5, SB1, MB1, and CB1 ([Fig. 5B](#)). Brook Trout, and to a lesser extent Slimy Sculpin, Ninespine

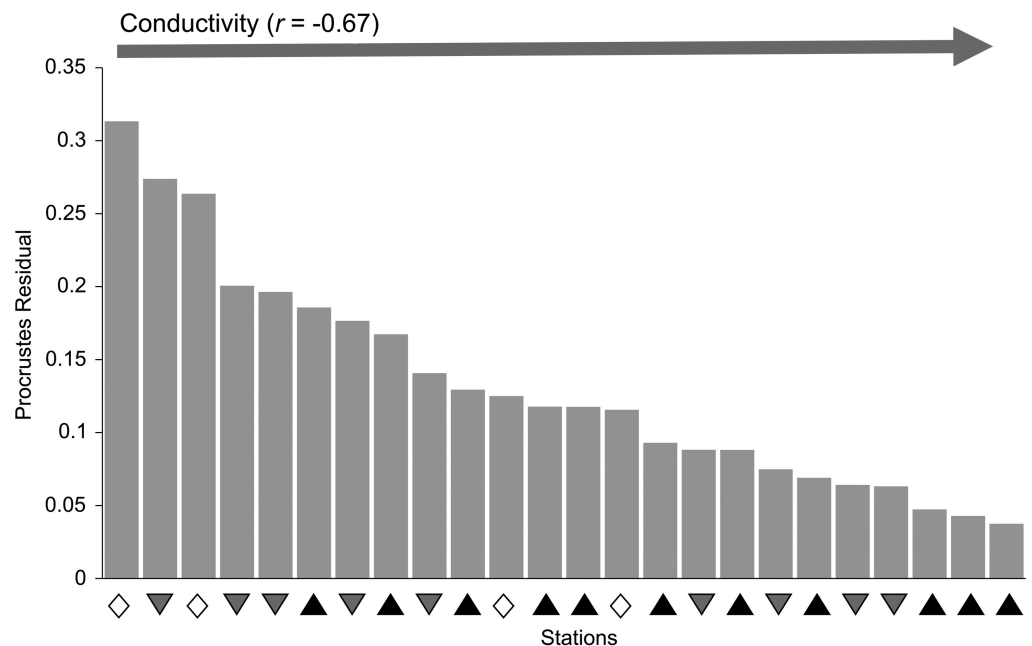


**Fig. 5.** Redundancy analysis (RDA) biplot of the 24 stations based on (A) benthic macroinvertebrate (BMI) data and (B) fish data, with both sets of data constrained by water chemistry and habitat survey variables. Station symbols indicate the age of underlying bedrock geology. The BMI plot includes a subset of taxa for ease of visual interpretation, grouped in clusters based on approximate location in the RDA. Station abbreviations are listed in Table 1, abiotic variable abbreviations in Tables S1 and S2, and taxonomic abbreviations are listed in Tables 2 and 3.

Stickleback (*Pungitius pungitius*), and Blacknose Shiner (*Notropis heterolepis*) were negatively associated with system size and temperature along the first axis gradient. The second axis indicated a gradient in conductivity and turbidity, both of which were positively associated with a number of Early Carboniferous stations (Fig. 5B). On the other end of that axis, stations in all geological age classes were positively associated with the presence of coniferous vegetation or TN. Brook Trout and Threespine Stickleback were positively correlated with conductivity along the second axis, whereas Burbot (*Lota lota*), Lake Chub (*Couesius plumbeus*), and Sea Lamprey (*Petromyzon marinus*) were positively associated with turbidity (Fig. 5B).

### Concordance gradient

A concordance gradient was created by aligning stations in order of decreasing Procrustes residuals, using the results of the analysis of fish and BMI concordance (i.e., as plotted in Fig. 4). Geology age classes were generally spread across the gradient, although the highest residuals were found in Older Class and Late Carboniferous stations, whereas the lowest residuals were found in Early Carboniferous stations (Fig. 6), indicating stronger similarity in station characterization based on fish and BMI for Early Carboniferous stations. The strongest correlation of environmental variables with the concordance gradient was a negative correlation of residual values with conductivity ( $r = -0.67$ ) that indicated that concordance between fish and BMI increased as conductivity increased (Fig. 6). Other environmental variables were generally weakly correlated with the concordance gradient ( $|r| < 0.30$ ; results not shown), but there was evidence of a positive correlation with  $D_{50}$  ( $r = 0.43$ ) and bankfull width ( $r = 0.38$ ) that indicated a decline in concordance (increasing Procrustes residuals) with an increase in substrate size and system size.



**Fig. 6.** Gradient of concordance residuals from the Procrustes analysis of fish and benthic macroinvertebrate principal components analyses, ordered by decreasing residual value and with symbols to indicate bedrock geology age underlying stations (black triangles = Early Carboniferous, grey upside-down triangles = Late Carboniferous, and white diamonds = Older Classes, which include Neoproterozoic and Devonian-Carboniferous). The correlation coefficient of conductivity with the concordance gradient is shown above the plot.

## Discussion

Monitoring and assessment to detect the potential impacts of shale-gas activities relies on the identification of appropriate response variables, including the selection of monitoring endpoints that will respond to relevant anthropogenic stressors. In our study, BMI assemblage structure was strongly related to water chemistry variables such as conductivity, which characterized areas with shale-gas potential, and the normal range of conductivity as well as several BMI metrics in Early Carboniferous stations were distinct from stations without shale-gas potential. Conductivity, in particular, would be expected to change in response to spills or other potential impacts of unconventional gas production, such as groundwater contamination, overland runoff, or release of treated effluents, which implies a strong potential for BMI abundance and composition to be used as a diagnostic tool to detect shale-gas impacts. In contrast, fish assemblage composition had a stronger association with a gradient in nutrients that was independent of geologic age, and with turbidity, which was highest in stations with high shale-gas potential. Despite the difference in driving variables, BMI and fish provided similar characterization of study sites, as evidenced by significant concordance. The response of BMI and fish taxa to differing environmental drivers suggests that these two taxonomic groups could be utilized in concert to detect and identify community shifts in response to a number of different environmental and anthropogenic pressures.

## Biotic associations across environmental gradients

Stations underlain by different geological age classes were characterized by environmental variables that have the potential to drive gradients in biotic assemblages (e.g., [Snelder et al. 2004](#)). Early Carboniferous stations, which have high shale-gas potential, were strongly characterized by high levels of several water chemistry variables (e.g., ions, conductivity, turbidity, and metals), which may be driven by underlying geologic composition ([Johnson et al. 1997](#); [Reimann et al. 2009](#)). In contrast, stations underlain by Late Carboniferous or older classes of bedrock were more strongly associated with a range of habitat variables that were indicative of gradients in stream width, periphyton cover, and riparian vegetation. These results supported the idea that surface and groundwaters in distinctive geological regions might display characteristic abiotic conditions with unique chemical habitats ([Stapinsky et al. 2002](#); [Reimann et al. 2009](#); [Neff and Jackson 2011, 2012](#)). Furthermore, differences in conductivity and TP among geologic age classes in our study allowed the development of baseline criteria for monitoring based on the normal range for stations underlain by Early Carboniferous bedrock.

There was clearly a stronger association of BMI with conductivity, which was a defining feature of stations underlain by Early Carboniferous bedrock geology, whereas fish were more strongly associated with system size (catchment area and depth) and a gradient in nutrients that was not specific to a particular geologic age class. [Neff and Jackson \(2013\)](#) noted similar patterns when comparing stream systems on the Precambrian Shield and off-Shield, with BMI responding more strongly to water chemistry differences across geology types and fish predominantly responding to physical habitat variables. Conductivity and alkalinity played a dominant role in distinguishing between Shield and off-Shield stations in their study, and [Neff and Jackson \(2011\)](#) found an association of several noninsect groups (e.g., Gastropoda, Amphipoda, mites, Hirudinea, and Isopoda) with the higher ion levels in off-Shield stations, similar to our results. Furthermore, [Johnson et al. \(2015\)](#) sampled streams in active shale-gas development areas in the southern United States and found their BMI assemblages to be dominated by Oligochaeta, Chironomidae, and tolerant genera of Ephemeroptera, which corresponds to the BMI patterns seen in our study for Early Carboniferous stations. The predominance of noninsect groups in high conductivity waters underlain by Early Carboniferous bedrock may relate to the dependence of taxa such as gastropods and bivalves on uptake of calcium from the water to maintain shell growth ([Havas and Rosseland 1995](#); [Neff and Jackson 2013](#)). Though conductivity appeared to

be a less dominant driver for fish, it was positively correlated with both Brook Trout and Threespine Stickleback. Conductivity has been found to correlate with abundance (Scarnecchia and Bergersen 1987) and size (as outlined in Copp 2003) of some fish species including trout, with high conductivity stations equated to more productive systems. The positive association of Brook Trout relative abundance with both conductivity and periphyton in these streams appears to support this idea.

Physical habitat differences among stations were reflected by taxonomic associations of both BMI and fish with gradients in temperature and system size. For example, stations that were cooler were associated with Plecoptera taxa such as Leuctridae, Nemouridae, and Chloroperlidae, which prefer small cool-water streams (McCafferty 1998). Brook Trout were similarly associated with small cool-water streams, consistent with their preferred temperature of 16 °C (Coker et al. 2001). The strongest gradient in the fish PCA was a negative relationship along the first axis between Slimy Sculpin, which have a cool-water preference (11–16 °C; Lyons 1990), and stations PL1, PL2, and PL6, which were large, warm systems, with higher maximum summer temperatures than other systems (ranging from 26 to 27.5 °C). These stations had more degree days above 19 °C, and they were positively correlated with Pteronarcyidae, Perlidae, and Perlodidae, which inhabit larger systems and are more tolerant of warm temperatures (McCafferty 1998). Blacknose Dace and American Eel were the fish species that were most strongly associated with these warm-water stations, and these species have preferred temperatures of 24.6 °C and 19 °C, respectively (Coker et al. 2001). Temperature in our study was not specifically related to particular geological age classes, though O'Sullivan et al. (2019) found that surficial geology, slope, and areas of geologic contact could be used to predict stream temperatures. The Pollett River stations, which had the highest degree days above 19 °C and the most variable temperatures in our study, were large stations that also had low percent cover of riparian vegetation and low percent forest in the upstream catchment. Johnson (2004) found evidence that shading affected maximum temperatures in small stream systems, and the lack of riparian vegetation at the Pollett River stations may have contributed to increased temperatures, driving differences in BMI and fish assemblages.

## Community concordance in relation to environmental drivers

The detection of significant community concordance among BMI and fish has two potential implications: (i) that the current range of environmental drivers in the study systems acts on BMI and fish in a similar manner, resulting in similar groupings of stations regardless of the organism examined and (ii) that there is redundancy in the evaluation of both BMI and fish assemblage structures within the same system, as they both lead to the same conclusion (Jackson and Harvey 1993). In the case of the Kennebecasis and Pollett River watersheds, the evaluation of both BMI and fish assemblages cannot be considered fully redundant as these groups of organisms appeared to respond most strongly to different environmental drivers. Other studies have noted significant concordance among organism groups despite evidence of differing responses to localized drivers (e.g., Jackson and Harvey 1993; Dolph et al. 2011; Bae et al. 2014). In particular, Neff and Jackson (2013) found significant community concordance between fish and BMI assemblages in Shield and off-Shield stream systems despite a stronger association of BMI with water chemistry variables and fish with physical habitat variables. Similar to our study, Neff and Jackson (2013) assessed streams within a localized region where there were distinct geological differences that had a significant effect on the physical and chemical habitat of the stream systems. Where the importance of drivers differs among groups that are concordant (e.g., fish and BMI), each assemblage could be expected to respond differently to future perturbation, which indicates that they provide complementary information about ecosystem condition.

Previous studies that have noted weaker concordance at smaller spatial scales (e.g., within drainage basins or within ecoregions) have attributed the difference in response of organism groups to strong localized differences in small-scale environmental drivers such as water chemistry and physical



habitat (Paavola et al. 2003, 2006; Larsen et al. 2012). However, Paavola et al. (2006) suggested that concordance might be evident in small-scale assessments if there is a single, strong environmental gradient driving assemblages in a similar way. In our study, the geological topologies characterized by Early Carboniferous, Late Carboniferous, and older classes of bedrock may have acted in such a manner. The concordance gradient that we identified across stations indicated that the strongest concordance or consistency among fish and BMI was evident in small systems with small substrate size and high conductivity. This association with system size is contrary to the findings of Paavola et al. (2006), who found increased concordance in larger systems in their broadscale regional assessment of streams. However, this may speak to the strength of the geological gradient in the current study and the important distinction of Early Carboniferous (shale-gas producing) areas from those underlain by other geological formations, providing further evidence of the dominant role geology can play in characterizing freshwater habitats (e.g., Neff and Jackson 2011, 2012, 2013).

### Monitoring surface waters in shale-gas formations

Though differences in system size, substrate size, and temperature were evident within and among geology age classes, water chemistry was the most important set of variables for characterizing Early Carboniferous stations. In a PCA including only water chemistry data (excluding physical habitat data), the first two axes explained 71.8% of the variance among sites (results not shown) further indicating the importance of water chemistry to distinguish among geology classes. Water chemistry also represents one of the characteristics of surface waters that is most likely to be impacted by potential spills, contamination by untreated flowback fluids (hydraulic fracturing fluids that return to the surface), or the release of treated effluent from shale -gas production (Entrekin et al. 2011; Vengosh et al. 2014). In their review of the potential impacts of hydraulic fracturing activities, Vengosh et al. (2014) noted that surface spills in the United States have caused high levels of organics, metalloids (Se, As), radionuclides (Ra), methane, pH, salts (total dissolved salts, and Cl and Br in particular), and specific conductivity. Wilson et al. (2014) found Cl levels in produced water from shale gas activities to be on average 133 733 mg/L, several orders of magnitude higher than the average Cl levels in the Early Carboniferous stations in the current study (8.38 mg/L), which were elevated compared to Cl levels in Late Carboniferous and Older Class stations (2.34 and 1.63 mg/L, respectively). Lento et al. (2019) found a number of ions had elevated levels in Early Carboniferous stations, consistent with the strong positive association of these stations with conductivity and ions in the present study. Given the already elevated levels of Cl and conductivity in the Early Carboniferous (high shale-gas potential) stations relative to other geological formations in the current study, this suggests the potential for even stronger geological distinction of stations if shale-gas production were to cause impacts to surface waters through wastewater spills. To support monitoring and detection of such impacts, baseline criteria can be developed by identifying the range of values outside which there may be evidence of impairment (normal range; Munkittrick et al. 2009). In our study, confidence intervals for conductivity provided evidence of the distinction among geologic age classes with respect to this chemical parameter, but also indicated the normal range for Early Carboniferous stations within which 95% of the conductivity values fell. With additional monitoring of streams in areas of potential shale gas development, these baseline criteria could be further refined to allow greater accuracy in the detection of impacts.

The stronger association of BMI with conductivity has important implications for biomonitoring in areas with high shale-gas potential, as it suggests that any changes to ion levels that might result from shale-gas production through spills or overland runoff may be most evident as impacts on BMI assemblages. Noninsect taxa and some families of Ephemeroptera and Trichoptera were highly associated with ion levels and could act as indicators of shifts in conductivity, whereas many Plecoptera in particular were more related to temperature gradients. BMI abundance, taxonomic richness, and the percent composition of Chironomidae, EPT, and Oligochaeta were all significantly different in stations underlain by Early Carboniferous bedrock relative to older classes of bedrock geology, similar

to the patterns noted by [Lento et al. \(2019\)](#), and the baseline criteria developed for these metrics can be used to monitor how test stations compare with the expected normal range in the absence of impacts from shale-gas development. Fish assemblages provided complementary information by strongly reflecting other physical and chemical habitat characteristics that might be altered with shale-gas development (e.g., increased turbidity from development activities; [Entrekin et al. 2011](#)) and natural gradients (e.g., nutrients) that did not relate as strongly to geologic age. Shifts in fish assemblages may be expected to reflect impacts to the system that are related to more general land use and development changes, such as changes to nutrient and sediment regimes or habitat fragmentation caused by clearing the land for construction of wells and development of supporting infrastructure ([Entrekin et al. 2011](#); [Brittingham et al. 2014](#)). Thus, monitoring of both BMI and fish may provide a measure of the relative strength of different anthropogenic impacts, including multiple aspects of shale-gas development, as taxonomic preferences and tolerance levels inform assessment of community shifts.

This study characterized the abiotic habitat and biotic communities of streams in geologic areas with high shale-gas potential, contrasting with streams on neighbouring geologic formations. There was clear evidence of differences in the abiotic habitat across geological age classes, and these differences were associated with variability in BMI and fish assemblage structure, with BMI more strongly reflecting temperature and conductivity and fish relating more to system size and gradients in nutrients and turbidity. Despite the differences in dominant drivers across organism groups, there was evidence of significant community concordance among BMI and fish assemblages, and this concordance increased with increasing conductivity and decreasing system size and substrate size. Geological age, differentiating Early Carboniferous, Late Carboniferous, and older classes of bedrock, appeared to act as a large-scale driver in these systems, contributing to concordance within the biotic community despite differences in localized environmental drivers (i.e., physical and chemical habitat variables), and the results of this study suggest that BMI and fish provide complementary information about ecosystem condition across the range of geology types examined. Effective environmental monitoring in areas of shale-gas development should therefore include assessment of both BMI and fish assemblages to allow for the detection of impacts related to shale-gas extraction as well as general perturbation related to development, with deviations from concordance used to detect assemblage-specific driver–response relationships. Importantly, baseline criteria developed in this paper provide estimates of the normal range for chemical and biotic metrics that might be affected by shale-gas development, thereby supporting the development of diagnostic tools for monitoring. This study highlights the importance of examining biotic response to environmental drivers in multiple assemblages to understand and interpret baseline conditions and ultimately measure meaningful change to adequately, and appropriately, inform management and biomonitoring of freshwater systems.

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## Author contributions

MAG and RAC conceived and designed the study. AJF performed the experiments/collected the data. JL analyzed and interpreted the data. MAG and RAC contributed resources. JL, MAG, AJF, and RAC drafted or revised the manuscript.

## Competing interests

The authors have declared that no competing interests exist.

## Data availability statement

All relevant data are within the paper and Supplementary Material.

## Supplementary material

The following Supplementary Material is available with the article through the journal website at doi:[10.1139/facets-2019-0024](https://doi.org/10.1139/facets-2019-0024).

Supplementary Material 1

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