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Establishing baseline biological conditions and monitoring metrics for stream benthic macroinvertebrates
and fish in an area of potential shale gas development

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Abstract

Potential for shale gas production is linked to regional geology, which influences water chemistry of freshwater systems. However, there has been little work to establish baseline ecological conditions of rivers within areas of shale gas development. In this study, water chemistry and monitoring metrics for fish and benthic macroinvertebrates were compared among varying-sized streams in New Brunswick, Canada with different underlying geology. Water chemistry and biotic community structure differed strongly among geological age classes. Early Carboniferous stations, with highest potential for shale gas production, had the highest ions, invertebrate abundances, and richness of invertebrates and fish, with strongest differences between Early Carboniferous and older bedrock classes. A reference condition model indicated numerous sites deviated from normal, but this was not specific to geological classes, and reflected the lack of model reference site coverage. These results highlight the importance of selecting appropriate reference sites for bioassessment in areas of resource development to ensure that response to perturbation is differentiated from natural variability along environmental gradients in geology and resource-dependent variables that potentially influence aquatic ecosystem composition.

Introduction

Monitoring and metrics of water quality and biotic community structure are necessary tools for meaningful assessment of potential impacts on freshwater ecosystems (Bonada et al. 2006; Arciszewski et al. 2017). In the absence of disturbance, water quality and biotic monitoring provide the baseline information about what is “normal” or “natural” for a region, which enhances our ability to detect any future changes when they do occur (Stoddard et al. 2006; Munkittrick and Arciszewski 2017). However, variability in local conditions, including large-scale drivers such as geology and climate patterns, can affect water chemistry levels and habitat characteristics of an area, as well as the types of biotic communities that might be expected in an undisturbed system (Frissell et al. 1986; Allan and Johnson 1997; Poff 1997; Allan 2004; Herlihy et al. 2008). Moreover, in areas where some development has already occurred, the current “natural” state is most probably different than a hypothesized pristine condition, thus necessitating an assessment of the current state of the environment and of realistic expectations for critical effects levels in monitoring and management (Stoddard et al. 2006; Munkittrick et al. 2009; Hawkins et al. 2010).

Determining how freshwater quality varies across natural gradients and in relation to pre-existing perturbations is particularly important when planning land use activities that are spatially predetermined, e.g., resource extraction, and potentially with limited impact zones. For example, mining and energy resource development will be focused on areas with specific surface deposits and underlying bedrock formations (Dyni 2003; Rivard et al. 2014). Geology also plays a large role in driving the water chemistry of freshwater systems (Johnson et al. 1997; Dow et al. 2006), which may in turn affect the composition of biotic communities (Leland and Porter 2000; Esselman et al. 2006; Neff and Jackson 2011). Reference condition models that are developed to evaluate freshwater quality typically rely on the characterization of ecological responses to natural environmental gradients, including geomorphic typology, to predict reference communities (Hawkins et al. 2010; Armanini et al. 2013). However, models that make use of geology to characterize reference communities often do so at an extremely coarse level (e.g., using broad

geological type settings, such as sedimentary, metamorphic, and intrusive, as in the Atlantic Reference Model developed by Armanini et al. 2013) to allow for wide-scale model application. Because the exploitation of resources can be limited spatially to more specific geological classes and the same geologic constraints can create differences in water quality and biotic community structure, meaningful monitoring and metrics during resource development activities may vary within a system, e.g., across surface and bedrock formations.

Natural gas production from oil shale deposits through hydraulic fracturing (unconventional gas) has gained in popularity over the last 15 years as a potential alternative to conventional oil and gas deposits (Vidic et al. 2013; Rivard et al. 2014). Shale gas production is the extraction of natural gas from fine-grained sedimentary bedrock containing high amounts of organic matter (Dyni 2003), and is considered an unconventional source because the low permeability of the bedrock requires the injection of hydraulic fracturing to allow the gas to flow (Vidic et al. 2013). Potential environmental concerns to water resources from shale gas production include contamination of groundwater from hydrologic connectivity to fractured areas and improper handling/disposal of fracturing fluids and wastewater, which could lead to spills in nearby surface waters (Vengosh et al. 2013; Vidic et al. 2013; Vengosh et al. 2014). Despite the potential for environmental impacts, there remains a lack of information to support environmental monitoring for these activities (Vengosh et al. 2013; Vidic et al. 2013; Vengosh et al. 2014; Leger et al. 2016a, b). Monitoring of surface water quality will be required in most jurisdictions (e.g., as reviewed in Vidic et al. 2013; Brantley et al. 2014; and Rivard et al. 2014; but see review of gaps in monitoring in Kinchy et al. 2016), but there is currently insufficient information about the abiotic and biotic characteristics of freshwater systems underlain by the distinctive bedrock geology of areas with high shale gas potential. Such knowledge is important for meaningful management and assessment planning for freshwater systems in these cases of geology-specific and driven resource development, i.e., understanding in-river variability as it relates to surface and underlying geology to establish spatially-appropriate metrics and monitoring programmes.

In this study, we evaluated abiotic and biotic measures of river water quality in an area of Early-Late Carboniferous and older age sedimentary and non-sedimentary bedrock. The Early Carboniferous area has high potential for shale gas production and has already been subject to hydraulic fracturing activities. We compared the current state of surface water quality with neighbouring areas of different bedrock age to evaluate potential differences, and used a standard reference condition model that incorporates only coarse-level geological classification to determine whether the results of the model were biased by fine-scale geological differences. The community structure and composition of benthic macroinvertebrates and fish (e.g., taxa abundance, richness and diversity, and % key species) were assessed across geologic zones based on bedrock age to characterize the systems. Such a direct assessment of the relationship between stream community composition and underlying geology has not been conducted in areas of unconventional gas development to our knowledge. Our goal was to present an informed decision-making framework for the selection of biomonitoring sites in areas of shale gas production.

Materials and Methods

Study area

Sampling was conducted in the Kennebecasis and Pollett River watersheds near Sussex in southeastern New Brunswick, Canada (Figure 1). The watersheds are located in the New Brunswick Highlands sub-region of the Appalachian physiographic region (Bostock 2014). The area is classified as part of the New England-Acadian Forests ecoregion (Olson et al. 2001) and is largely forested (>90%), with some agriculture (5-7%) and a small percent of the land area used for settlements, infrastructure, and industry, including areas of shale gas exploration and extraction. Surficial geology in the area is characterized by morainal and colluvial deposits in the headwaters and morainal, glaciofluvial, and alluvial deposits in the mid- to lower reaches of the rivers (Rampton 1984).

The study area is located in the Moncton geological sub-basin and the Caledonia Uplift outcrop of the Upper Paleozoic Maritimes Basin (Wilson and White 2006). Bedrock geology is sedimentary in the

mid- to lower reaches of the Kennebecasis and Pollett rivers (within the Moncton sub-basin), and volcanic or intrusive in the headwater areas that fall on the Caledonia Uplift outcrop to the south (Geological Survey of Canada). The western part of the study area is characterized by Early Carboniferous sedimentary bedrock (Mississippian era), with deposits in the Mabou, Sussex, and Windsor groups (Wilson and White 2006). The Lower Carboniferous Albert formation underlies this Early Carboniferous bedrock, and contains the Hiram Brook and Frederick Brook members, which have potential for conventional natural gas and unconventional (shale gas) extraction, respectively (Macauley et al. 1984; Macauley et al. 1985; Leblanc et al. 2011). The McCully gas field, which was the first commercial location for shale gas extraction in the province (Leblanc et al. 2011), is located in this area, and houses a number of wells for natural gas production and exploration (Wilson and White 2006; Leblanc et al. 2011); however, a moratorium on hydraulic fracturing that was issued by the Government of New Brunswick in December 2014 has halted the fracturing activities required to stimulate production in these wells. In the eastern part of the study area is Late Carboniferous sedimentary bedrock (Pennsylvanian era), with deposits in the Cumberland and Pictou groups. This typically coal-producing bedrock differs from oil shale bedrock with respect to mineral content and composition of organic material (Dyni 2003). Along the southern boundary of the study area are older classes of bedrock, including sedimentary Devonian-Carboniferous bedrock and volcanic and intrusive bedrock from the Neoproterozoic era (Geological Survey of Canada).

Study design

An extensive spatial survey of biotic and abiotic ecosystem components was completed to characterize community structure and water quality in the Kennebecasis and Pollett River watersheds, $n = 20$ and 8 sites, respectively (Figure 1). Sample stations were located on the mainstem and on tributaries, which provided varied catchment sizes to assess (sub-catchment areas ranged from 7.25 km² to 305.06 km²; Table 1). Stations were primarily located on Early and Late Carboniferous bedrock, with additional stations sampled in areas with older bedrock age classes (Devonian-Carboniferous and Neoproterozoic)

(Figure 1). Several of the Early Carboniferous stations were located in the vicinity of oil or gas wells (Figure 1), though active fracking activities were halted in the area in 2014, and some wells were out of operation prior to the moratorium. Spills associated with unconventional shale gas production have not been reported for the area, and though methane levels in groundwater wells are higher in this region than surrounding areas, Loomer et al. (2016) found that methane concentrations in groundwater were not correlated with proximity to oil and natural gas wells. Moreover, Cl levels in our study systems were 4-5 orders of magnitude less than has been found for unconventional and conventional gas-produced waters (Wilson et al. 2014), and there was no correlation between SO_4/Cl ratios in our systems and the number of upstream oil/gas wells. Thus, stations were considered to be at least disturbed state (*sensu* Stoddard et al. 2006), providing a baseline for future comparison with potentially impacted stations.

Stream sampling took place in late August and early September 2015. Water chemistry and benthic macroinvertebrate (BMI) community structure were sampled at 26 stations (Table 1). Fish community surveys were conducted at 27 stations, including two stations that were not sampled for BMI or water chemistry (Table 1). The lowest BMI station on the Kennebecasis River (KB8) was excluded from fish sampling as it was too deep to safely conduct a backpack electrofishing survey.

Sample collection and processing

Water grab samples were collected following provincial guidelines and were kept cool until delivery within 30 hours to the New Brunswick Department of Environment and Local Government's (NBDELG)'s analytical lab in Marysville, NB. The samples were analyzed for a standard suite of metals, nutrients, and ions following standardized analytical procedures (see Table S1 for details on measured parameters and analytical procedures).

BMI samples were collected following the standard protocol of the national Canadian Aquatic Biomonitoring Network (CABIN; Environment Canada 2012). Benthic samples were collected at each station using a time-limited travelling-kick method, in which the operator held a 400- μm -mesh kick-net downstream while travelling through the stream channel in a zig-zag fashion and disturbing the substrate

for a period of 3 minutes (Environment Canada 2012). Samples were preserved in the field with 10% formalin and transferred to 90% ethanol in the lab after one week to preserve sample integrity.

Macroinvertebrate samples were randomly subsampled in the laboratory using the Marchant box method (Environment Canada 2010) until a minimum of 300 organisms was counted. Organisms were sent to a Society for Freshwater Science (SFS)-certified taxonomist for identification to genus level where possible. Whole-sample abundances were estimated by adjusting counts based on the percent of the sample that was processed to reach 300 organisms. Chironomidae (midge) taxa were sent to EcoAnalysts, Inc. to be identified to species by SFS-certified taxonomists. Due to high abundance, Chironomidae were sub-sampled using a smaller-scale method similar to the Marchant box, until at least 150 individuals were identified. Remaining chironomids in the sample were enumerated and a subsampling correction factor was applied to unidentified chironomids.

Fish community surveys were conducted by using electrofishing techniques (Murphy and Willis 1996) in which a backpack electrofishing unit (Smith-Root LR-24) was used 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 m²). The effort was standardized by maintaining power within a range of 65-75W (voltage varied depending on conductivity of the water) and sampling time ranged from 419 to 3009 seconds. All fish were identified to species level, and catch per unit effort (CPUE) was calculated for each site at which fish were sampled (number of fish/100 seconds sampling time).

Data Pre-treatment

Stations were grouped by the age of underlying bedrock geology for statistical analysis of station differences to examine the relationship of geology with water chemistry and community structure of streams. Focus was placed on geology within an extended reach upstream of the stations by intersecting the NB provincial bedrock substratum layer (Service New Brunswick-GeoNB) with a 1 km buffer (using only the upstream catchment) for each station. The area of each geological age class within the buffers was calculated in ArcMap (Version 10.3) to estimate relative proportions and determine the dominant age

class. Catchment buffers were generally dominated by a single geological age class that made up >70% of the buffer area, with the exception of two stations that had dominant age classes at 57% (SB2) and 62% (TC2) of the buffer area. 19 of the stations had only one geological age class within the upstream catchment buffer. Dominant geological age classes for the full set of 28 stations included Early Carboniferous (14 stations), Late Carboniferous (9 stations), Devonian-Carboniferous (2 stations), and Middle Neoproterozoic (3 stations). For the purpose of analysis, Devonian-Carboniferous and Middle Neoproterozoic were combined into one class to represent stations with underlying geology older than Early Carboniferous (termed Older Classes; see Table 1 for classification of stations). To account for differences in system size in statistical analyses, stations were binned into three catchment size classes based on geometrical intervals, with groups defined as small catchment (area up to 27 km²; 9 stations), medium catchment (area of 27-93 km²; 8 stations), or large catchment (area of 93 km² and larger; 9 stations). Each geological classification included systems with small, medium, and large catchment sizes.

Water chemistry data included a suite of major ions, nutrients, physicals, and metals, and correlations among water chemistry parameters were assessed prior to analysis to identify redundancy among parameters and reduce the set of water chemistry variables for analysis (see Table 2 for list of chosen variables). Where estimated values for a parameter were below the Limit of Quantification (LOQ; Table S1), values were corrected to be half the LOQ for calculation of summary statistics and analysis. BMI and fish data were summarized by metrics that described abundance, diversity, and composition of communities (Table S1). Metrics included descriptors that are commonly used in bioassessment (Barbour et al. 1999).

Data Analysis

Characterization of stream stations by geology and size

Two-way orthogonal ANOVAs were used to assess differences in abiotic and biotic variables among geological age classes and catchment size classes. For each of the chosen water chemistry parameters, BMI metrics, and fish metrics (Table 2), differences in means among the geological classes

and catchment size classes were assessed by testing the Model 1 ANOVA model: $Y = \text{Geology} + \text{Size} + \text{Geology} * \text{Size}$, where Y was the chosen water chemistry parameter or biological metric, Geology tested for mean differences due to the geological age class of the station, Size tested for mean differences due to the catchment size class of the station, and Geology * Size tested for an interaction between the two orthogonal factors. Because there were missing values due to unequal replication of size classes in each geological class (water chemistry and benthic macroinvertebrates were missing two medium Early Carboniferous stations, one large Late Carboniferous station, and one small Older Classes station; fish were missing two medium Early Carboniferous stations and one small Older Classes station), the ANOVAs were adjusted for unequal replication. Average values were calculated for each of the geology-size combinations that were missing values, and those averages were included as additional dummy samples in the analysis of the ANOVA model to create a proportional, though still unbalanced model (5 Early Carboniferous samples, 3 Late Carboniferous, and 2 Older Classes samples of each catchment size class). To remove the effect of the dummy samples, the total degrees of freedom (DF) and the error DF calculated in the ANOVA were adjusted to remove the added samples, and all F -ratios were recalculated using the adjusted error Mean Square (Zar 1999). Because separate ANOVAs were run for each of the chosen abiotic and biotic variables, there was an increased risk of Type I error. To account for this, we applied a false discovery rate (FDR) correction (Benjamini and Hochberg 1995) to the p -values from the water chemistry, BMI, and fish ANOVAs.

If the interaction term in an ANOVA model was not significant (at the FDR-adjusted alpha level), differences among levels of any significant terms in the model were analyzed using a Tukey test. Tukey tests compared the original group means without the addition of the dummy sample values, and were adjusted for unequal sample sizes among groups, to account for the differences in replication for each geological age group and catchment size class (Zar 1999). Dependent variables were log-transformed or logit-transformed (as appropriate for the data type) prior to analysis to meet the underlying assumptions

of the ANOVA with respect to normality and homogeneity of residual variances. Analyses were conducted in Systat 12 (Version 12.02).

Assessment of stream quality

Stream quality was assessed in the study area by comparing water chemistry parameters and BMI assemblages with targets that have been designed to indicate samples that deviate from what is considered “normal” for an unperturbed system. Water chemistry parameters were compared with the Canadian Council of Minister’s (CCME) Water Quality Guidelines for the Protection of Aquatic Life (<http://sts.ccme.ca/en/index.html>) to identify any exceedances in parameter values. Data were assessed for the measured parameters that had written guidelines for short-term or long-term exposure, which included metals and a selection of ions and nutrients.

Stream quality assessment with BMI data relied on the Atlantic Reference Model (ARM), which is a Reference Condition Approach (RCA) model created using data collected from Atlantic Canada streams through the CABIN program, and was designed to select reference conditions for comparison with test sites after classification of stations using coarse bedrock geology type settings (e.g., sedimentary, intrusive, volcanic, etc.) and temperature range data (Armanini et al. 2013). Though geology is incorporated into this model, it is at a coarse level that does not distinguish shale gas potential areas (e.g., both Early Carboniferous and Late Carboniferous bedrock are classified as sedimentary bedrock) and therefore does not provide the same level of site characterization. Through the model, test sites are compared with reference sites in a River Invertebrate Prediction and Classification System (RIVPACS) approach to obtain estimates of observed over expected (O/E) for a number of biological metrics. O/E values for metrics for each station are compared with an acceptable range to determine whether the station is classified as normal, divergent, or highly divergent. O/E ranges for each station classification differ by metric, as there is variability among reference sites for each biological metric.

Comparison of the study stations with the reference model provided an opportunity to test the effectiveness of the large-scale Atlantic model in an area that had minimal sample coverage for model

development (see gaps in coverage in Armanini et al. 2013), and determine whether results of the RCA classification were biased by the finer-scale geological differences among stations (e.g., geological age). BMI community data from the study stations in the Kennebecasis and Pollett Rivers, along with the percent coverage of coarse bedrock geology classifications (e.g., sedimentary, intrusive, volcanic, etc.; from a national bedrock geospatial layer provided by CABIN) and the average temperature range within the catchment (from a long-term average temperature layer provided by CABIN) were input into the program GenGIS 2.5.0 to run RCA analysis using the ARM. O/E values for richness and diversity (Simpson's Index) metrics were compared with reference ranges and plotted to assess stream health based on BMI community structure. Contingency tables were used to evaluate whether the observed number of divergent stations was higher than expected for any of the geological age classes or catchment size classes, to determine in particular whether results of the ARM were biased by finer-scale geological differences. In this analysis, the χ^2 statistic evaluated deviations from expected distributions in two 3x2 contingency tables (each with three classes of geology or size versus two RCA classes: normal or divergent) at $\alpha = 0.05$.

Results

Water chemistry

Water chemistry differed across geological age classes, but did not appear to differ by catchment size ($p > 0.05$ for the size terms and size x geology interaction terms in the ANOVA models; Table 3a). The clearest difference in water chemistry among geological age classes was related to ion levels. Conductivity levels differed significantly among the three geological age classes ($p < 0.001$; Table 3a), with Early Carboniferous stations displaying higher conductivity (mean = 167.91 mg/L) than the other two age classes (mean = 65.90 and 41.62 mg/L for Late Carboniferous and Older Classes, respectively; Figure 2A and Table 4). Examination of related parameters indicated that there was a general pattern for conductivity, alkalinity, and major ions of highest average values in Early Carboniferous stations, lower average values in Late Carboniferous stations, and lowest average values in Older Class stations, although

for a small number of ions the difference between geological classes appeared minimal (e.g., magnesium and potassium; Table 4). Among the major ions, calcium, chloride, and sulphate showed the strongest differences between the three geological age classes, following the pattern of highest concentrations in Early Carboniferous stations and lowest concentrations in Older Class stations (Table 4).

Differences in water chemistry among geological age classes were weaker or less consistent for other water chemistry parameters. Turbidity levels were significantly different among the geological age classes ($p = 0.013$; Table 3a) and appeared to show higher levels in Early Carboniferous stations (Figure 2E), but when the Tukey test was adjusted for unequal sample sizes, there was insufficient power to detect a difference among geological age classes. Similarly, total phosphorous appeared higher in both Early and Late Carboniferous stations than in Older Class stations (Figure 2B), but the results of the ANOVA were non-significant ($p > 0.05$ for all terms; Table 3a). There were significant differences among geological age classes for both nitrate ($p = 0.008$; Table 3a) and total organic carbon ($p = 0.012$; Table 3a), but these other nutrients did not display a similar pattern of lowest levels in Older Class stations. Nitrate levels were significantly lower in Late Carboniferous than in Early Carboniferous stations, but did not differ significantly from the other two classes in Older Class stations (Figure 2C). In contrast, total organic carbon was significantly higher in Older Class stations than in Late Carboniferous stations (not significantly different from Early Carboniferous; Figure 2D), which was contrary to the patterns in other water chemistry variables.

Benthic macroinvertebrates

BMI communities in the study area were taxonomically rich, with a total of 110 genera identified in 44 families of insects and 13 families/classes of non-insects collected across the 26 stations (see Table S2 for list of taxa). Taxonomic richness at the genera level was on average ($\pm$ SE) 35.2 ± 1.1 across the 26 stations. The most diverse groups were Diptera, Ephemeroptera, Plecoptera, and Trichoptera, though Trichoptera was the most diverse with 23 genera in 16 families found across the study area. Abundances of benthic macroinvertebrates were generally high, but differed among stations by an order of magnitude,

ranging from 4,200 to 66,440 individuals per sample. Sites tended to be fairly diverse, with Simpson's Index (1-D) ranging from 0.738 to 0.928, but they were generally numerically dominated by Ephemeroptera, Plecoptera, Trichoptera, and Chironomidae.

Similar to water chemistry, benthic macroinvertebrate metrics differed in response to geological age class but not to catchment size ($p > 0.05$ for size and size x geology terms in all ANOVA models; Table 3b). Average abundance of benthic macroinvertebrates differed among geological age classes ($p = 0.003$; Table 3b), and was significantly higher at Early and Late Carboniferous stations (mean = 25046 and 25797, respectively) than at Older Class stations (mean = 8534; Figure 3A). Genus richness also appeared to differ among geological age classes, with higher richness in Early Carboniferous stations (mean = 37.9) than in either Late Carboniferous or Older Class stations (mean = 33.9 and 30.2, respectively; Figure 3B), but the differences among geological age classes were not significant when the FDR correction was applied ($p = 0.034$; Table 3b). In contrast, Simpson's Index indicated similar diversity among the stations grouped by geology, and did not differ significantly among geological age classes ($p > 0.05$; Table 3b; Figure 3E).

Despite similarities in diversity among geological age classes, there was evidence of a shift in the relative proportions of major taxonomic groups, with a clear increase in dominance of EPT and slightly lower abundance of Chironomidae in Older Class stations relative to Early or Late Carboniferous stations (Figure 3C, 3D). The percent Chironomidae declined from a mean of 36.5% in Early Carboniferous stations and 31.3% in Late Carboniferous stations to a mean of 18.6% in Older Class stations (Figure 3C), though differences among geological age classes were not significant at the FDR-corrected alpha level ($p = 0.045$; Table 3b). There was stronger evidence of the compositional difference when the percent EPT was examined, as the percent EPT differed among geological age classes ($p = 0.002$; Table 3b) and was significantly higher in Older Class stations (mean = 73.5%) than in Early or Late Carboniferous stations (mean = 47.3% and 55.8%, respectively; Figure 3D).

Fish

Fish community monitoring in 27 stations resulted in the collection of 14 different species (see Table S3 for list of taxa), with Brook Trout (*Salvelinus fontinalis*) and Slimy Sculpin (*Cottus cognatus*) captured at the highest number of stations (22 and 21 stations, respectively). Other species including Atlantic Salmon (*Salmo salar*), Blacknose Shiner (*Notropis heterolepis*), and Golden Shiner (*Notemigonus crysoleucas*) were more rare and only collected at 1-2 stations. Total catch per unit effort (CPUE; abundance standardized per 100 seconds) at a station was variable, ranging from 1.39 to 36.53; average CPUE was highest for Slimy Sculpin (CPUE = 11.39), Blacknose Dace (*Rhinichthys atratulus*) (CPUE = 2.54), and Brook Trout (CPUE = 2.05), and was much lower for remaining species. Richness was low across many stations, and ranged from one species per station (PL2) to a maximum of nine species at a station (KB6, KB7, and PL4). Simpson's (1-D) Index indicated moderately to highly uneven communities, with 14 stations having an index value below 0.50. The percent Slimy Sculpin at a station provided clear evidence of the uneven distribution of species within some communities; this single species made up 90% to 97.5% of the assemblage at five stations, and over 50% of the assemblage at 14 of the 27 stations.

In contrast to what was found for water chemistry and benthic macroinvertebrates, catchment size appeared to be more strongly related to some measures of fish community structure than was geology, although geological age class remained more important for two metrics (and there was no evidence of an interaction between the two factors; $p > 0.05$ for interaction terms in all models; Table 3c). The strongest effect of catchment size was evident for the percent Brook Trout at a station ($p < 0.001$; Table 3c), as there was a significantly higher percent Brook Trout in small catchments (mean = 35.5%) than in either medium or large catchments (mean = 8.4% and 3.7%, respectively; Figure 4A). The percent composition of the other dominant species, Slimy Sculpin, appeared to be higher on average in medium-sized catchments (mean = 74.5%) than in large or small catchments (mean = 32.9% and 48.8%, respectively; Figure 4B), but means were highly variable within size categories and differences were not significant when the FDR correction was applied ($p = 0.019$; Table 3c). Similarly, Simpson's (1-D) Index did not

differ significantly among catchment sizes when compared with the FDR-corrected alpha ($p = 0.009$; Table 3c), but diversity was higher in small catchments than in medium-sized catchments, where Slimy Sculpin dominated (Figure 4C). Although median values for each of these fish metrics appeared to be lower for Late Carboniferous and Older Class stations than for Early Carboniferous stations (Figure 4A-C), the relationships were variable and there were no significant differences in the % Brook Trout, % Slimy Sculpin, or diversity in relation to geological age class ($p > 0.05$; Table 3c).

General measures of abundance and taxonomic composition appeared to be more related to geological age class than to catchment size, although no fish parameters differed significantly among geological age classes when the FDR correction was applied. Species richness ($p = 0.023$; Table 3c) was higher for Early Carboniferous stations (mean = 5.5) than for Older Class stations (mean = 3; Figure 5D), similar to the patterns that were noted for benthic macroinvertebrates, and there was no evidence of patterns in richness in relation to catchment size ($p = 0.976$; Table 3c; Figure 4D). CPUE ($p = 0.047$; Table 3c) appeared to show slightly higher abundance in Late Carboniferous stations than Older Classes (Figure 5E), and similarly showed no patterns in relation to catchment size ($p = 0.366$; Table 3c; Figure 4E).

Stream quality

Water chemistry values for measured parameters were generally within recommended short-term and long-term exposure guidelines as outlined in the CCME Water Quality Guidelines for the Protection of Aquatic Life (Table S4). The fluoride guideline of 0.120 mg/L was exceeded at station MP1 (Early Carboniferous), where the measured value was 0.153 mg/L, but this was the only exceedance noted across the 26 water chemistry stations. Examination of water chemistry monitoring data from other streams in the region with data from 2014-2015 (not shown) also provided little evidence of exceedances for the parameters listed in Table S4, and confirmed that water quality in the study area was generally within acceptable limits.

The Reference Condition Approach that can be used to assess stream health based on BMI generated different classifications of stream quality depending on whether benthic macroinvertebrate stations were assessed on the basis of taxonomic richness or diversity (Simpson's Diversity index, incorporating richness and evenness). O/E values for richness were below the normal range for reference condition in over half of the stations (Figure 6A), indicating that the variation in taxonomic richness among stations was not fully captured by the model. Deviations from reference condition did not appear to be related to underlying geological age, though deviations were largest in Older Class and Late Carboniferous stations. CB1 provided the strongest evidence for deviations from reference condition, with the lowest O/E at 0.69. However, O/E values for the remaining divergent stations were generally close to the threshold value of 0.95, with eight stations above 0.90 (Figure 6A). Assessment of reference condition using diversity (Simpson's Index; Figure 6B) resulted in a higher proportion of samples being classified as within normal reference condition range. The samples that were found to be divergent included CB1, KB8, SB3A, and TC3, although all O/E values were close to the threshold of 0.96. Chi-square analysis with contingency tables indicated that classification in the normal range for either richness or diversity was not dependent on underlying geology age ($p > 0.05$ for both contingency tables), and thus did not appear to be driven by fine-scale geological differences.

Discussion

Potential for shale gas production is linked to geological composition and age (Macauley et al. 1985), and geology is known to influence water chemistry of freshwater systems (Snelder et al. 2004; Dow et al. 2006). In this study, we found evidence of differences in water chemistry and biotic community structure among stream sites classified into different geological age classes. Early Carboniferous stations, which had the highest potential for shale gas production and in some cases, were located in areas of hydraulic fracturing activity, were found to have the highest ions levels, highest abundance and genus richness of invertebrates, and highest species richness of fish, with the strongest differences noted between Early Carboniferous and older classes of bedrock. The results highlight the

importance of selecting appropriate reference sites for bioassessment of stations in areas of resource development to ensure that response to perturbation can be differentiated from natural variability due to environmental gradients in geology or other resource-dependent environmental variables.

Geological influence

Geology plays a significant role in the determination of groundwater and surface water chemistry (Johnson et al. 1997; Thornton and Dise 1998), affecting pH, ionic composition, and acid neutralizing capacity (Clair et al. 1995; Reimann et al. 2009). Within the study area, Early Carboniferous bedrock stations were characterized by higher solute levels than stations on Late Carboniferous or older age classes of geology, particularly with respect to calcium, chloride, sodium, and sulphate. Where surrounding landuse is dominated by intensive industry or agriculture, both of which have the potential to alter surface water chemistry, geology may play a smaller role in controlling water quality (e.g., Johnson et al. 1997). In particular, increases in agriculture may contribute significantly to nutrient levels in a system (e.g., nitrate) and shift the importance of geological influences (Reimann et al. 2009). The strong association of water chemistry with bedrock geology classifications suggests that geology plays a more dominant role in determining water quality in the stream systems of our study region.

The processes by which the solute levels of freshwaters are affected by geology are complex, and depend on various aspects of the form and composition of geological minerals, and the degree to which surface water and groundwater make contact with those minerals (Hem 1985; Drever 1997). Loomer et al. (2016) found that groundwater from wells in the vicinity of the Early and Late Carboniferous bedrock in this region is predominantly of calcium-bicarbonate composition, though a number of wells were dominated by sulphate (where deposits of gypsum, limestone, and potash were common) or by combinations of sodium, calcium, chloride, and sulphate, particularly where methane was highest. High levels of calcium, sodium, and sulphate have been previously noted in groundwater of the Carboniferous basin of New Brunswick (Stapinsky et al. 2002), suggesting that elevated levels of these ions are characteristic of freshwaters in this geological area. The increased solute levels that were found in Early

Carboniferous compared with Late Carboniferous stations could reflect differences in mineral levels among the geological formations found in each age class. The difference could also be due to differing levels of connectivity of surface waters to groundwater in these areas (see Lento et al. 2017 for description of surface water-groundwater connections in the study area). Although the influence of existing oil and gas wells cannot be ruled out, Loomer et al. (2016) noted that methane levels in groundwater wells were not correlated with proximity to oil and gas wells, and found lower methane levels than have been reported in cases of known contamination from hydraulic fracturing (e.g., Osborn et al. 2011). Moreover, chloride levels in our study systems were well below average values reported for inland waters and several orders of magnitude lower than what has been recorded in gas-produced waters (Wilson et al. 2014).

Benthic invertebrate assemblages also varied across geologic age classes. The clearest differences were evident between the Early Carboniferous and Older Class stations, with some variability in the Late Carboniferous stations. Geology has been examined as a potential driver of benthic invertebrate community composition (Cannan and Armitage 1999; Kratzer et al. 2006; Dudgeon 2012) and geology can play a significant role in determining assemblage structure in the absence of anthropogenic drivers such as nutrient enrichment from land use (Johnson et al. 1997; Neff and Jackson 2011). However, it can be difficult to differentiate the effects of geology from those of the other potential drivers to which it is linked, such as water chemistry, habitat structure, and altitude (Cannan and Armitage 1999). The strong association between benthic macroinvertebrates and water chemistry is well-documented (e.g., Sandin and Johnson 2004; Hering et al. 2006; Lento et al. 2008; Neff and Jackson 2011), including evidence of variability in composition in response to conductivity levels (Kratzer et al. 2006) and temporal shifts in community composition following trends in ion levels (Lento et al. 2012). Given the relationship between geology and water chemistry that we observed, the differences in benthic macroinvertebrate metrics across geological age classes were not surprising and indicate that geology acts at a large scale to influence invertebrate assemblages through variation in water chemistry. Moreover, these relationships

suggest that in other areas of geology-driven resource extraction, invertebrate assemblages might be expected to differ in a predictable manner in response to underlying geology and to its effects on the chemical composition of a system.

Fish species richness appeared to be related to geological age class, but compositional metrics such as the percent Brook Trout or Slimy Sculpin were more strongly linked to system size, indicating a weaker control of geology on fish assemblages. Fish community structure has been suggested to be the product of variables acting at multiple scales, with regional factors affecting the species pool and local factors having a stronger effect at the assemblage level, leading to smaller-scale compositional differences (Jackson et al. 2001; Hoeinghaus et al. 2007). In our study systems, the relationship between richness and geology may have reflected these large-scale biogeographic controls on taxonomy, whereas the other compositional metrics, which focused on abundance, may have reflected species- or assemblage-level responses to local-scale drivers such as temperature. Brook Trout and Slimy Sculpin are both noted to be temperature-sensitive, preferring smaller streams with cooler temperatures (Coker et al. 2001), and the dominance of these species in some streams determined not only differences in the compositional metrics for these two species, but also the measure of diversity that incorporated evenness. Biotic interactions within these systems may have played a large role in driving diversity such that Brook Trout and Slimy Sculpin were out-competing other fish species (Jackson et al. 2001). However, it is also possible that geology and water chemistry exert less control over fish assemblages than other physical habitat parameters (Neff and Jackson 2013). Esselman et al. (2006) examined the relationship between geology and fish assemblage composition in Mesoamerica and found little evidence that geology was a driving factor of fish assemblages in the river systems they sampled. Moreover, whereas benthic invertebrates have been shown to relate to ion levels in freshwater, there is evidence to suggest little correlation between fish condition and conductivity levels (Copp 2003). Assemblages in our study streams may be less impacted by the geological influence than water chemistry and benthic invertebrate assemblages, but the dominance of Brook Trout and Slimy Sculpin suggests that geological control is expressed via a

different pathway of effect, i.e., the presence of groundwater discharging to the stream in localized areas (Curry and Noakes 1995; Brasfield 2007).

Implications for monitoring and assessment

Characterization of the chemical composition that might be expected in lakes and rivers with differing underlying geology provides a baseline from which to assess impacts of landuse and perturbation, both of which may override the effects of geology and exert dominant control over the chemical composition of freshwaters (Johnson et al. 1997). In the case of shale gas exploitation, there is a risk for contamination of surface waters and groundwaters from fracturing fluids and flowback waters (Vengosh et al. 2013; Rivard et al. 2014; Vengosh et al. 2014). Fracturing fluids contain a large number of chemical constituents including gelling agents, surfactants, friction inhibitors, and biocides, with the exact composition of the fluids generally not made known by industry (Brantley et al. 2014; Rivard et al. 2014). Flowback waters may contain contaminants from fracturing fluids as well as high levels of chloride and other cations such as calcium and magnesium resulting from increased contact with the mineral-rich shale (Barbot et al. 2013). A review by Brantley et al. (2014) of water quality records associated with shale gas production in Pennsylvania revealed minimal evidence of contamination from spills of fracturing fluids or flowback waters, but the authors noted that it may be difficult to detect impacts in waters that are naturally high in the same ions found in these fluids. Furthermore, there is also potential for increased solutes in surface waters with high groundwater inputs if the process of hydraulic fracturing increases connectivity of groundwater with mineral-rich bedrock (Vengosh et al. 2014). Carboniferous stations in our study both inside and outside the area of shale gas exploration had higher ion levels than stations on older classes of bedrock. Assessment of water chemistry parameters against water quality guidelines detected only a single guideline exceedance (fluoride in one station), indicating adequate water quality across the study area, but comparison among stations would indicate quite high variability if geological groupings of stations were not considered. Thus, for effective monitoring of water

quality in areas of shale gas production, it is important to be able to differentiate increased ion levels due to perturbation from naturally-elevated ion levels (Brantley et al. 2014).

Researchers have examined methods of classifying rivers for the development of biological reference models (see review in Hawkins et al. 2010) to ensure test stations are assessed against systems that display comparable ecological characteristics (e.g., geology, climate, ecoregion, etc.; Reynoldson et al. 1997; Snelder et al. 2004). For example, the Eastern Canadian Diatom Index (IDEC; Lavoie et al. 2014) was split into sub-indices to reflect natural variation in water chemistry due to underlying geology, as it was found to be a significant driver of diatom community composition (Grenier et al. 2006). Similarly, large-scale reference condition models for benthic macroinvertebrates in the US and Canada rely on catchment characteristics such as geology and climate to select appropriate reference sites for monitoring and assessment (e.g., see Herlihy et al. 2008; Armanini et al. 2013). The use of environmental descriptors in the development of large-scale reference models recognizes the importance of these variables as natural drivers of water chemistry and biotic communities; however, these models may rely on coarse-level environmental descriptors that do not fully capture the fine-scale characteristics that determine resource potential. In our study, benthic macroinvertebrate assemblages with different underlying bedrock age were compared with a reference model that was built using undisturbed stations from Atlantic Canada. A number of stations within our study area were classified as deviant from the reference model, indicating that composition (particularly taxonomic richness) at those stations was outside the range of the model. However, these deviations were not limited to particular geological classes, and the frequency of deviations indicated that the model may not fully capture the natural variability in this region. For example, the spring-fed station CB1 was found to deviate the most from the reference model for both taxonomic richness and diversity, and could potentially contribute to expanding the bounds of the model to include groundwater-dominated systems. Despite significant differences in BMI composition across geological age classes, there was no evidence that deviation from reference condition within the model was associated with geological age, which suggested that the inclusion of

coarse geological classification in the model played a role in facilitating the assessment of water quality (through the reference condition approach) by controlling for natural variability among stations. At a broad scale, the coarse characterization of bedrock geology may be sufficient to act as a general flagging tool for the region. However, this analysis makes clear the importance of ensuring adequate coverage of representative reference stations in such models, as the classification of over half the stations as deviant from reference condition indicates that incorporation of additional samples is necessary to ensure the natural variability in BMI composition is captured for this geologically complex area.

Smaller-scale biotic assessments of test sites and reference sites (e.g., where extensive monitoring of reference stations for development of a reference model is not possible, or where geology-specific resource extraction necessitates finer-scale classification and assessment) must also consider environmental factors such as geology in the development of study design and site selection. Biotic metrics such as richness and abundance differed by geological age class for benthic invertebrates, and to a lesser extent, fish, and compositional metrics for benthic invertebrates also differed across geology age classes. Such differences in composition might suggest a measure of impairment in test sites, even though differences are due to the natural gradient in geology and water chemistry. For example, samples were numerically dominated by Ephemeroptera, Plecoptera, Trichoptera, and Chironomidae, but the abundance of each group differed across geology types, with the highest proportion of EPT in Older Class stations and the highest proportion of Chironomidae in Carboniferous (Early and Late) stations. Although tolerances and preferences differ across and within families, the orders Ephemeroptera, Plecoptera, and Trichoptera are generally considered to be sensitive to perturbation within stream systems due to generalized low tolerances to pollution, and low abundance or richness of these groups is taken as an indicator of impaired water quality (Barbour et al. 2001). In contrast, the family Chironomidae includes a number of genera that are tolerant of perturbation, and thus is used as a general indicator of potential impairment (Barbour et al. 2001). Applying these general principles to the assessment of the study sites without information about environmental variability might suggest that some stations were of lower water

quality than others, when in fact the difference in relative proportions of EPT and Chironomidae appears to have been driven by natural differences in geology. Assessment with fish provides an additional challenge, as fish composition appeared to be more strongly associated with system size than geology type for some compositional measures (particularly the percent of Brook Trout and Slimy Sculpin). However, there did appear to be a difference in species richness of fish among geology age classes (albeit not significant after correction for multiple testing), which indicated the importance of this geological classification for some measures of fish diversity.

The results of this study indicate that rivers and streams in areas of Carboniferous bedrock with the potential for resource extraction have higher conductivity and ion levels and higher taxonomic richness of benthic invertebrates and fish than in areas with older classes of sedimentary and non-sedimentary bedrock. Streams in such areas can be expected to have naturally different water chemistry and composition of benthic invertebrates (with shifts in proportions of Chironomidae and EPT), though fish composition may be more linked to system size. Assessment of water quality in areas with high shale gas potential must consider the natural differences in water quality and community structure that occur in freshwaters as a result of geology type. Moreover, this idea can be extended to other forms of resource exploration/exploitation that are restricted to particular habitats, as biotic and abiotic characteristics of surrounding freshwaters may not be directly comparable with systems outside those habitat types. Managers should consider geology (e.g., type and age) and other important environmental drivers (e.g., system size, flow, and temperature, especially as it relates to fish and fish resources) when selecting stations for inclusion in a monitoring plan and when planning future development and resource exploration, to ensure accuracy in water quality and biotic assessments.

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Table 1. Stations included in the characterization of water quality and biotic communities in the Kennebecasis and Pollett watersheds, with details on station codes, stream names, coordinates (latitude and longitude in decimal degrees), data collected at each station, upstream catchment area (in km²), classification of catchment size based on catchment area (small, medium, or large), and classification of geological age class determined by the dominant age class within a 1 km upstream catchment buffer (Early Carboniferous, Late Carboniferous, or Older Classes, which includes Neoproterozoic and Devonian-Carboniferous).

Station	Stream Name	Lat	Long	Data Collected	Catchment Area km ²	Catchment Size	Geological Age
CB1	Calamingo Brook	45.82396	-65.22416	WQ, BMI, Fish	18.33	Small	Late Carboniferous
KB1	Kennebecasis R.	45.72493	-65.20672	WQ, BMI, Fish	16.08	Small	Older Classes
KB2	Kennebecasis R.	45.79116	-65.17107	WQ, BMI, Fish	52.47	Medium	Early Carboniferous
KB3	Kennebecasis R.	45.82706	-65.21902	WQ, BMI, Fish	77.73	Medium	Late Carboniferous
KB4	Kennebecasis R.	45.83904	-65.24611	WQ, BMI, Fish	108.39	Large	Late Carboniferous
KB5	Kennebecasis R.	45.81035	-65.29384	WQ, BMI, Fish	119.82	Large	Early Carboniferous
KB6	Kennebecasis R.	45.77914	-65.37976	WQ, BMI, Fish	220.07	Large	Early Carboniferous
KB7	Kennebecasis R.	45.76548	-65.42001	WQ, BMI, Fish	292.73	Large	Early Carboniferous
KB8	Kennebecasis R.	45.74291	-65.44499	WQ, BMI	308.70	Large	Early Carboniferous
MB1	McLeod Brook	45.72736	-65.36009	WQ, BMI, Fish	7.25	Small	Early Carboniferous
MB2	McLeod Brook	45.77112	-65.38745	WQ, BMI, Fish	24.17	Small	Early Carboniferous
MP1	Millpond Brook	45.73120	-65.47202	WQ, BMI, Fish	13.39	Small	Early Carboniferous
PL1	Pollett River	45.70245	-65.09772	WQ, BMI, Fish	72.37	Medium	Older Classes

PL2	Pollett River	45.75614	-65.07883	WQ, BMI, Fish	122.99	Large	Older Classes
PL3	Pollett River	45.79697	-65.10211	WQ, BMI, Fish	148.30	Large	Older Classes
PL4	Lee Brook	45.80344	-65.11241	WQ, BMI, Fish	22.36	Small	Early Carboniferous
PL4A	Pollett River	45.81056	-65.10694	Fish	181.71	Large	Early Carboniferous
PL5	Colpitts Brook	45.86169	-65.07990	WQ, BMI, Fish	12.61	Small	Late Carboniferous
PL6	Pollett River	45.93900	-65.07997	WQ, BMI, Fish	247.8	Large	Late Carboniferous
PL7	Pollett River	45.97603	-65.08706	Fish	303.92	Large	Late Carboniferous
SB1	Negro Brook	45.73813	-65.29926	WQ, BMI, Fish	8.14	Small	Early Carboniferous
SB2	South Branch	45.75476	-65.29966	WQ, BMI, Fish	46.37	Medium	Late Carboniferous
SB3A	South Branch	45.77139	-65.32639	WQ, BMI, Fish	55.61	Medium	Late Carboniferous
ST1	Stone Brook	45.80803	-65.36037	WQ, BMI, Fish	19.68	Small	Late Carboniferous
ST2	Stone Brook	45.78099	-65.38686	WQ, BMI, Fish	27.84	Medium	Early Carboniferous
TC1	Cedar Camp	45.68205	-65.36880	WQ, BMI, Fish	36.43	Medium	Older Classes
TC2	Parlee Brook	45.68751	-65.41667	WQ, BMI, Fish	38.97	Medium	Early Carboniferous
TC3	Trout Creek	45.70886	-65.47225	WQ, BMI, Fish	150.16	Large	Early Carboniferous

778 Table 2. Variables selected for inclusion in data analyses for water quality, benthic macroinvertebrates,
 779 and fish.

Water chemistry	Benthic Macroinvertebrates	Fish
Conductivity ($\mu\text{S}/\text{cm}$)	Abundance	Catch per unit effort (CPUE; per 100s)
Turbidity (NTU)	Genus richness	Species richness
Nitrate (NO_3 ; mg/L)	Simpson's 1-D Index	Simpson's 1-D Index
Total phosphorus (TP; mg/L)	% Chironomidae	% Brook Trout
Total organic carbon (TOC; mg/L)	% Ephemeroptera, Plecoptera, and Trichoptera (EPT)	% Slimy Sculpin

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Table 3. Results of the two-way ANOVA models testing the effects of geological age class (Geology), catchment size (Size), and the interaction between the two (Geology * Size) on (a) water chemistry parameters, (b) BMI metrics, and (c) fish metrics. Table presents the mean square and degrees of freedom (df) for each model term, the *F*-ratio, and *p*-value. Denominator df is 17 for all *F*-ratios. *p*-values in bold indicate significance at $\alpha = 0.05$, and * indicates significance at the FDR-corrected alpha level.

a)

Parameter	Model term	Mean square	df	<i>F</i>	<i>p</i>
log ₁₀ Conductivity	Geology	0.601	2	13.00	< 0.001*
	Size	0.068	2	1.47	0.258
	Geology * Size	0.037	4	0.79	0.545
log ₁₀ Turbidity	Geology	1.102	2	5.69	0.013*
	Size	0.066	2	0.34	0.716
	Geology * Size	0.079	4	0.41	0.802
log ₁₀ Total phosphorus	Geology	0.521	2	2.82	0.088
	Size	0.186	2	1.01	0.385
	Geology * Size	0.154	4	0.83	0.523
log ₁₀ Nitrate	Geology	0.653	2	6.60	0.007*
	Size	0.079	2	0.80	0.466
	Geology * Size	0.064	4	0.65	0.633
log ₁₀ Total organic carbon	Geology	0.296	2	5.76	0.012*
	Size	0.023	2	0.45	0.647
	Geology * Size	0.016	4	0.31	0.867

b)

Parameter	Model term	Mean square	Degrees of freedom	<i>F</i>	<i>p</i>
$\log_{10}$ Abundance	Geology	0.616	2	8.12	0.003*
	Size	0.085	2	1.12	0.349
	Geology * Size	0.061	4	0.80	0.541
$\log_{10}$ Genus richness	Geology	0.020	2	4.15	0.034
	Size	0.005	2	1.01	0.384
	Geology * Size	0.001	4	0.26	0.904
Simpson's (1-D) index	Geology	0.004	2	1.82	0.192
	Size	0.0005	2	0.20	0.819
	Geology * Size	0.003	4	1.11	0.383
logit % Chironomidae	Geology	0.002	2	3.74	0.045
	Size	0.001	2	1.01	0.386
	Geology * Size	0.001	4	1.03	0.420
% EPT	Geology	1313.5	2	9.14	0.002*
	Size	348.4	2	2.42	0.118
	Geology * Size	175.2	4	1.22	0.340

789 c)

Parameter	Model term	Mean square	Degrees of freedom	<i>F</i>	<i>p</i>

			freedom		
logit % Brook Trout	Geology	0.472	2	2.37	0.122
	Size	4.156	2	20.81	< 0.001*
	Geology * Size	0.074	4	0.372	0.826
logit % Slimy Sculpin	Geology	0.714	2	1.26	0.306
	Size	2.826	2	5.00	0.019
	Geology * Size	0.816	4	1.44	0.260
Simpson's (1-D) index	Geology	0.074	2	1.78	0.198
	Size	0.260	2	6.24	0.009
	Geology * Size	0.024	4	0.57	0.688
log ₁₀ Species richness	Geology	0.190	2	4.70	0.023
	Size	0.001	2	0.02	0.976
	Geology * Size	0.102	4	2.53	0.076
log ₁₀ CPUE	Geology	0.470	2	3.63	0.047
	Size	0.138	2	1.06	0.366
	Geology * Size	0.038	4	0.295	0.877

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Table 4. Mean ($\pm$ standard error) conductivity ($\mu\text{S}/\text{cm}$), alkalinity (mg/L), and concentrations of major ions (mg/L) for stream stations grouped by geological age class determined by the dominant age class within a 1 km upstream catchment buffer (Early Carboniferous, Late Carboniferous, or Older Classes, which includes Neoproterozoic and Devonian-Carboniferous).

Water chemistry parameter	Early Carboniferous ($n = 13$)	Late Carboniferous ($n = 8$)	Older Classes ($n = 5$)
Conductivity ($\mu\text{S}/\text{cm}$)	167.91 ± 41.67	65.90 ± 6.42	41.62 ± 7.35
Alkalinity (mg/L)	42.35 ± 4.89	22.69 ± 2.55	15.98 ± 3.90
Calcium (mg/L)	23.56 ± 8.16	6.97 ± 1.05	4.54 ± 1.44
Chloride (mg/L)	8.38 ± 2.15	2.34 ± 0.32	1.63 ± 0.13
Magnesium (mg/L)	1.76 ± 0.15	1.36 ± 0.14	0.96 ± 0.26
Potassium (mg/L)	0.90 ± 0.12	0.51 ± 0.03	0.25 ± 0.02
Sodium (mg/L)	7.83 ± 1.52	3.48 ± 0.36	2.31 ± 0.14
Sulphate (mg/L)	28.96 ± 16.98	6.06 ± 0.83	2.12 ± 0.27

Figure Captions

Figure 1. Map of the study area, showing sample locations, location of oil and gas wells (producing and non-producing wells, as of 2014) and underlying bedrock geology of the area (with 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/gas well map layers: Government of New Brunswick Department of Natural Resources.

Figure 2. Water chemistry data summarized for each of the three geological age classes (with geology classified by geological age as Early Carboniferous, Late Carboniferous, or Older Classes, which include Neoproterozoic and Devonian-Carboniferous) for (A) conductivity ($\mu\text{S}/\text{cm}$), (B) total phosphorous (mg/L), (C) nitrate (mg/L), (D) total organic carbon (mg/L), and (E) turbidity (NTU). Lower-case letters on each plot indicate significant differences (at $\alpha = 0.05$) among geological age classes, as determined by Tukey tests adjusted for unequal sample sizes. In each box plot, the median (central horizontal line) is in a box bounded by the 25th and 75th percentiles, with whiskers indicating 10th and 90th percentiles and points for statistical outliers.

Figure 3. Benthic macroinvertebrate community metric values summarized for each of the three geological age classes (with geology classified by geological age as Early Carboniferous, Late Carboniferous, or Older Classes, which include Neoproterozoic and Devonian-Carboniferous), including (A) abundance, (B) genus richness, (C) percent Chironomidae, (D) percent EPT, and (E) diversity (Simpson's 1-D Index). Lower-case letters on each plot indicate significant differences (at $\alpha = 0.05$) among geological age classes, as determined by Tukey tests adjusted for unequal sample sizes. In each box plot, the median (central horizontal line) is in a box bounded by the 25th and 75th percentiles, with whiskers indicating 10th and 90th percentiles and points for statistical outliers.

822

823 Figure 4. Fish community metric values summarized for each of the three catchment size classes (small,
824 medium, and large), including (A) percent Brook Trout, (B) percent Slimy Sculpin, (C) diversity
825 measured by Simpson's (1-D) Index, (D) species richness, and (E) catch per unit effort (CPUE) per 100s.
826 Lower-case letters on each plot indicate significant differences (at $\alpha = 0.05$) among geological age
827 classes, as determined by Tukey tests adjusted for unequal sample sizes. In each box plot, the median
828 (central horizontal line) is in a box bounded by the 25th and 75th percentiles, with whiskers indicating 10th
829 and 90th percentiles and points for statistical outliers.

830

831 Figure 5. Fish community metric values summarized for each of the three geological age classes (with
832 geology classified by geological age as Early Carboniferous, Late Carboniferous, or Older Classes, which
833 include Neoproterozoic and Devonian-Carboniferous), including (A) percent Brook Trout, (B) percent
834 Slimy Sculpin, (C) diversity (Simpson's 1-D Index), (D) species richness, and (E) catch per unit effort
835 (CPUE) per 100s. Lower-case letters on each plot indicate significant differences (at $\alpha = 0.05$) among
836 geological age classes, as determined by Tukey tests adjusted for unequal sample sizes. In each box plot,
837 the median (central horizontal line) is in a box bounded by the 25th and 75th percentiles, with whiskers
838 indicating 10th and 90th percentiles and points for statistical outliers.

839

840 Figure 6. Observed/Expected (O/E) (A) richness and (B) diversity (Simpson's index) of benthic
841 macroinvertebrate data from 26 stations assessed using the Atlantic Reference Model, with symbols
842 indicating underlying bedrock geology age (white diamonds = Older Classes, including Neoproterozoic
843 and Devonian-Carboniferous, grey upside-down triangles = Late Carboniferous, and black triangles =
844 Early Carboniferous). The upper dashed line indicates the breakpoint between normal samples (> 0.95 for
845 richness and > 0.96 for diversity) and divergent samples (0.47-0.95 for richness and 0.48-0.96 for

846 diversity), and the lower limit of the divergent class and upper limit of the highly divergent class is
847 indicated by the lower dashed line. Samples that fall within the divergent range are coloured in dark grey.

Draft

Figure 1 Map of the study area, showing sample locations, location of oil and gas wells (producing and non-producing wells, as of 2014) and underlying bedrock geology of the area (with 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/gas well map layers: Government of New Brunswick Department of Natural Resources.

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Figure 6. Observed/Expected (O/E) (A) richness and (B) diversity (Simpson's index) of benthic macroinvertebrate data from 26 stations assessed using the Atlantic Reference Model, with symbols indicating underlying bedrock geology age (white diamonds = Older Classes, including Neoproterozoic and Devonian-Carboniferous, grey upside-down triangles = Late Carboniferous, and black triangles = Early Carboniferous). The upper dashed line indicates the breakpoint between normal samples (> 0.95 for richness and > 0.96 for diversity) and divergent samples (0.47-0.95 for richness and 0.48-0.96 for diversity), and the lower limit of the divergent class and upper limit of the highly divergent class is indicated by the lower dashed line. Samples that fall within the divergent range are coloured in dark grey.