MACROINVERTEBRATE ASSEMBLAGE RESPONSES TO ANTHROPOGENIC STRESSORS: A BIOASSESSMENT SCORING TOOL FOR MANAGING STREAM ECOSYSTEMS IN EASTERN ONTARIO

Élysabeth Théberge

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Department of Biology

Faculty of Sciences

University of Ottawa

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STATEMENT OF THESIS CONTRIBUTION

This project opportunity was made possible through a collaboration with the Stream Monitoring and Research Team in Eastern Region (SMARTER) network, who collected the biological data, provided funding, and feedback throughout the completion of this work. This particular thesis was completed concurrently with a similar Master's project which used fish monitoring information rather than macroinvertebrate data. As a result, many of the same information and equivalent analyses have been used for both projects. Both thesis results are meant for complementary use as decision support tools for management by members of the SMARTER network.

This thesis manuscript has been written in the following format with the intention of submitting it for journal publication. Therefore, I used the pronoun 'we' rather than 'I' throughout the manuscript as it will be submitted with more than one author.

ABSTRACT

Using site-specific macroinvertebrate monitoring information and catchment-scale predictors, we developed multiple linear regression models to assess stream ecological status in eastern Ontario. With the developed equations, we hindcasted metric reference conditions for each site by setting anthropogenic activity variables to zero. Observed metric values were then scored based on their deviation from their expected reference value. These deviation scores were subsequently scaled by an estimate of the regional natural variability (i.e., distribution of residuals) observed for each metric. Scaled scores were then used to construct a summary index incorporating multiple, now compatible metrics. Although our simplified models were highly statistically significant, they often explained only a fraction of the observed variability in metric responses (R^2 <0.34). Furthermore, there was evidence of lack of fit of the models, as indicated by the relatively high variability around predicted values when compared to the measurement error (variability between replicate samples). These results imply that diagnosis power of individual site condition assessments is low. However, the scaled deviation scores incorporate an evaluation of uncertainty in site condition diagnoses, given by the variability estimates (i.e., residuals) around each predicted metric reference value. This makes them suitable for use in a 'probabilistic' risk assessment framework – where a large deviation score indicates a higher probability (risk) that an observed metric value is outside of reference condition (i.e., impacted). By further manipulating anthropogenic activity levels in the developed model equations, it also becomes possible to test for alternative management scenarios and estimate associated risks. These features of the scaled metric deviation scores make them a useful tool in watershed management decisions.

RÉSUMÉ

Des données d'échantillonnage d'invertébrés et des prédicteurs à l'échelle du bassin versant ont été utilisées pour estimer des modèles de régressions multiples afin de déterminer le statut écologique des ruisseaux situés dans l'est de l'Ontario. En utilisant ces équations nous avons prédit de façon rétrospective les conditions de référence pour chacune de nos métriques à chaque site échantillonné, en l'absence d'activités anthropogéniques. Les valeurs observées de nos métriques ont été scorées basé sur leur déviation des valeurs attendues en état de référence. Ces scores ont ensuite été normalisés par un estimé de la variabilité naturelle mesurée dans la région étudiée (i.e., la distribution des résidus) et observée pour chacune de nos métriques. Les scores normalisés ont été utilisés pour construire un index sommaire incorporant de multiples métriques rendues maintenant compatibles. Malgré le fait que nos modèles simplifiés étaient hautement statistiquement significatifs, ils expliquaient généralement seulement une fraction de la variabilité observée dans la réponse des métriques aux prédicteurs (R^2 <0.34). De plus, il y avait évidence d'un manque d'ajustement des modèles indiqué par la haute variabilité relative autour des valeurs prédites comparé à l'erreur de mesure (variabilité entre les réplicats). Ces résultats impliquent que la valeur diagnostique pour un site en particulier n'est pas très élevée. Cependant, les scores normalisés des déviations incorporent une évaluation de l'incertitude par rapport à ces diagnostics de condition des sites, définie par nos estimés de la variabilité (i.e., les résidus) autour des valeurs de référence prédites pour chacune de nos métriques. Ceci permet l'évaluation des risques par probabilité – où de grandes déviations dans les scores indiquent une plus grande probabilité (risque) qu'une valeur observée pour une métrique quelconque soit hors de l'état de référence (i.e., site

impacté). Ces équations permettent de comparer des stratégies alternatives de gestion des bassins versants et d'estimer les risques associés avec de tels scénarios. Ces caractéristiques des scores de déviations pour les métriques en font un outil utile au processus décisionnel des gestionnaires de bassins versants.

INTRODUCTION

Regulatory agencies around the world rely on the monitoring of benthic macroinvertebrate assemblages as indicators of stream ecological status (e.g., Canada, USA, Europe, Australia; McElligott 2006, Buss et al. 2015). This biologically based method, or bioassessment, is appealing because these indicators are ubiquitous, well-known, inexpensive and quick to sample, and sensitive to a range of factors (natural and anthropogenic) influencing streams (Malmqvist 2002, McElligott 2006, Jones et al. 2007). Most biomonitoring programs in Canada are regional efforts (McElligott 2006, Buss et al. 2015). In Ontario, different local organisations (e.g., Conservation Authorities) and governmental agencies are mandated with stream monitoring to assess current ecological status and to take action towards maintaining stream health. In order to make evidence-based decisions about which stream sites to prioritize in terms of management action, regulators rely on scientific expertise (using best available science) to guide them and support them in their decision-making process. These 'decision-support tools', often in the form of statistical predictive models, should enable planners to assess current status based on the collected biomonitoring information, and to explore the projected impacts of alternative management scenarios.

The determination of reference conditions is essential to all bioassessment approaches (Reynoldson et al. 1997, Soranno et al. 2011, Buss et al. 2015). Reference conditions represent the biological attributes expected at a group of ecologically similar sites that are unaffected by human activity. In order to assess site ecological status, the reference condition serves as a benchmark against which sites of unknown status are assessed. The benchmark thus provides the necessary context for interpreting measured biomonitoring information gathered at stream sites and to subsequently evaluate the degree of stream site ecological impairment.

Many of the different existing methods involve the classification of a group of minimally impacted sites as being in reference condition (Reynoldson et al. 1997, Stoddard et al. 2006, Hawkins et al. 2010b, Soranno et al. 2011, Buss et al. 2015). However, reference conditions vary naturally across space and it would be inappropriate to have a single reference condition for sites sampled across large, often physically and biologically variable, geographical regions (Seelbach et al. 2002, Stoddard et al. 2008, Soranno et al. 2011, Buss et al. 2015). This natural variability must be accounted for in order to properly evaluate stream ecological status.

One approach to account for this variability is to use groups of reference sites within relatively homogeneous regions (i.e., the 'Reference Condition Approach'; Reynoldson et al. 1997, Hawkins et al. 2010b, Soranno et al. 2011). The range of measured biological characteristics observed across these sites is then used as a benchmark to which sites of unknown condition ('test' sites) are compared to in order to assess their status. The assumption made under this classification approach is that reference sites and 'test' sites have equivalent natural background conditions in the absence of human activity and thus should have similar biological features in the absence of human-induced impact.

An alternative approach for determining reference conditions is to develop site-specific biological expectations obtained from statistical modeling, a method also known as 'hindcasting' (Seelbach et al., 2002, Dodds and Oakes 2004, Baker et al. 2005, Kilgour and Stanfield, 2006, Soranno et al. 2008, 2011, Angradi et al. 2009, Hawkins et al. 2010b, Herlihy et al. 2013). Statistical models relating both natural and human activity predictors to site-specific indicators of stream health can be developed for particular regions. Using these models, site-specific expectations of reference conditions can be estimated by setting human stressor predictors to zero and extrapolating ‘true’ undisturbed condition for each individual site. Similar to the previously mentioned classification methods, this approach also accounts for natural variability among streams by incorporating these variables as predictors in modelling. Therefore, this method eliminates the need for the *a-priori* categorization of sites as being in reference (i.e., ‘least-disturbed’) condition, removing associated sources of bias in bioassessment.

The main objectives of the current study are to characterize the current ecological condition of streams in eastern Ontario as described by their benthic macroinvertebrate assemblages and to develop predictive models to describe the effects of future development. We first develop multiple linear regression models using catchment-scale characteristics, both non-anthropogenic and anthropogenic. These equations are then used to model site-specific reference conditions for a set of benthic macroinvertebrate assemblage descriptors routinely used in bioassessments. Next, we build a summary metric (i.e., a multimetric index; Klemm et al. 2003, Maloney and Feminella 2006, Stoddard et al. 2008) with which we summarize the ecological status and evaluate associated risk for streams across the region of study, using scaled deviations from hindcasted expected reference conditions. Finally, we describe the methodology for making projections of expected effects of land use changes on stream ecological status by further manipulating human activity levels in the generated equations. This will enable decision-makers to test alternate environmental management scenarios.

METHODS

Study area

Our study focused on the eastern region of Ontario for which multiple regional biomonitoring datasets have been combined. This was made possible through a collaboration with the Stream Monitoring and Research Team in Eastern Region (SMARTER), a multi-sector team composed of University researchers, non-government organisations, government organisations (Ministry of Natural Resources, Ministry of the Environment, and the City of Ottawa) and Conservation Authorities which are watershed-based organizations (Figure 1).

Within the region, there are varying degrees of urban and agricultural land use as well as varying types of natural landscape features, unaffected by human influence. Regions of sedimentary bedrock formations dominate the major urban centers, Ottawa and Kingston. The middle portion of the study area is characterized by highly variable metamorphic rock formations, and a mixture of agricultural land use types and forested regions.

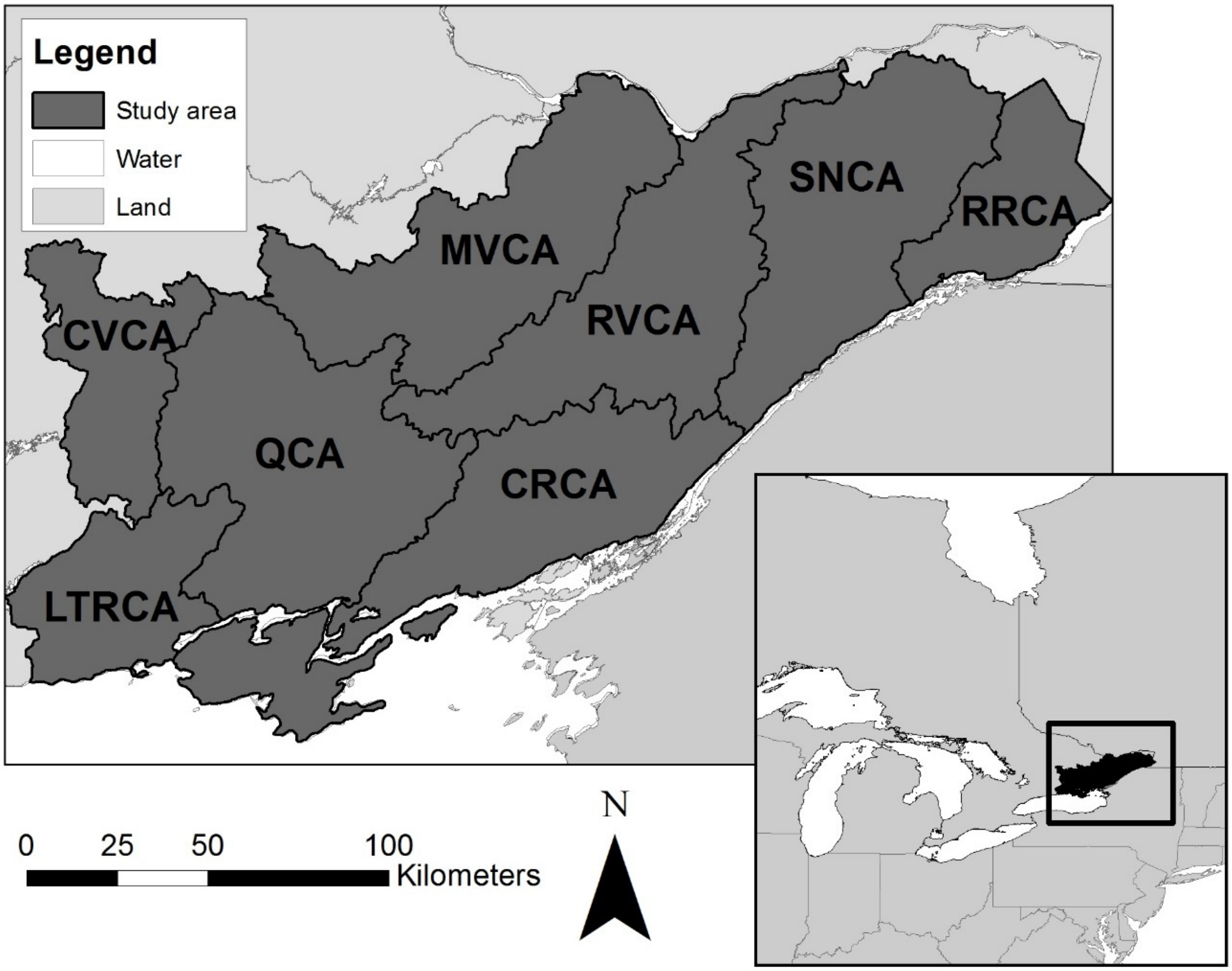


Figure 1: Study region and Conservation Areas in eastern Ontario: CVCA=Crowe Valley Conservation Area; LTRCA=Lower Trent Region Conservation Area; QCA= Quinte Conservation Area; MVCA= Mississippi Conservation Area; RVCA= Rideau Valley Conservation Area; CRCA= Cataraqui Region Conservation Area; SNCA= South Nation Conservation Area; RRCA= Raisin Region Conservation Area.

Biological data

Sampling protocol

Biomonitoring data covering the period between 2003 and 2013 were obtained from SMARTER participants and consisted in a sample-by-taxa benthos data matrix, with a total of 2110 records (i.e., 258 sites, 706 site visits, with generally 3 samples each). Benthic macroinvertebrate sampling was carried out by several agencies according to the Ontario Benthos Biomonitoring Network protocol (OBBN; Jones et al 2007). Sampling was performed following the Travelling-Kick-and-Sweep-Transect Method in wadeable streams found across the study region, generally at locations that were easily accessible from a nearby road (i.e., upstream of a stream and road-crossing). A stream site consisted of a stream segment encompassing two riffles and one pool, typically contained within a single stream meander wavelength (see Appendix I for more details on the sampling protocol).

Data aggregation and taxonomic resolution

Because the taxonomic level of invertebrate identification was inconsistent across records, we discarded some of the data of lower quality (i.e., broad taxonomic classification) and used 160 Operational Taxonomic Units (OTUs; Appendix II) in an attempt to maximize the proportion of data kept while minimizing potential biases. The chosen 160 OTUs mostly consisted of family level taxonomic units. Ambiguities in taxonomy arose when a given taxon had been identified to multiple different taxonomic levels across records. For example, Heptageniidae mayflies were identified to the order level *Ephemeroptera* in some records and to the family level Heptageniidae in others. Such inconsistencies can bias certain metrics such as taxonomic richness, and lead to inappropriate cross-record comparisons. To reduce such potential biases, we eliminated records where 50% or more of the organisms had only been identified to a broad taxonomic level (above family level). Through this process, 16% of records were discarded resulting in a total of 1743 remaining records (i.e., 236 sites, 601 site visits). Removal of broadly identified records caused some sites to have incomplete sample sets (i.e., less than the standard 3). As a result, 215 sites out of the 236 had intact sample sets. Lastly, of the remaining 1743 records, organisms that were identified to a finer taxonomic resolution (e.g., to genus level) were aggregated to fit designated OTU categories.

Sub-sampling effort varied across records and we attempted to correct for potential biases that could have been created by this variability. Most records consisted of 100-individual sub-sample counts (Appendix I), but some had considerably higher counts (up to 3005). Taxa richness increases as sub-sample count increases (Doberstein et al. 2000, Cao and Hawkins 2005) therefore, sub-sample counts were standardized across records by rarefaction (random resampling without replacement to a count of 100 individuals; Heck et al. 1975, Van Sickle 2011).

Metrics describing macroinvertebrate assemblages

Biological metrics that quantified various aspects of macroinvertebrate assemblage structure were calculated for each record in the dataset. A suite of 21 metrics were calculated (Table 1). Chosen metrics covered sensitivities to different human stressors and reflected metrics meaningful to the sampling agencies. The Hilsenhoff Biotic Index (HBI) was calculated using tolerance values obtained from Hilsenhoff (1987) and Bode et al. (2002) (Appendix II). Diversity metrics were calculated using the diversity command in the R vegan package (Oksanen et al. 2013). The ratio of Observed-over-Expected (O/E) number of taxa, derived from the River InVertebrate Prediction and Classification System (RIVPACS) was also calculated for each record following the method outlined in Van Sickle (2008) and using Van Sickle's (2011) R scripts available online (http://www.epa.gov/wed/pages/models/rivpacs/rivpacs.htm). For the purpose of calculating the O/E metric only, reference sites (following a 'least-disturbed' definition; Stoddard et al. 2006, Hawkins et al. 2010b) were characterized as those that were below the 40th percentile for each of the following catchment-scale stressors: % intensive agriculture, % urban, road density. Rare OTUs were removed to improve the RIVPACS-model performance (i.e., taxa occurring in less than 5% of reference calibration sites). Taxa with capture probabilities below 10% were excluded from the models to further improve their performance (Ostermiller and Hawkins 2004, Van Sickle et al. 2007, Hawkins et al. 2010a). An O/E site score that was close to 1 indicated closeness to reference conditions while an O/E score close to 0 indicated site impairment. Preliminary analyses revealed that benthos composition varied little geographically. Therefore, we used a null model, where all reference sites belonged to the same regional class (Van Sickle et al. 2005, Hawkins et al. 2010a) to compute O/E. Lastly, we calculated multivariate community composition indices, such as described by principal component analysis (PCA) and principal coordinate analysis (PCoA) axis scores. The axis scores were calculated from a Bray-Curtis distance matrix. Biological data were log-transformed and a Lingoes correction was applied to the PCoA distance matrix values to insure positive eigenvalues (Lingoes 1971, Legendre and Legendre 1998, Bocard et al. 2011). All analyses were conducted using R (version 3.0.3; R Development Core Team, http://www.r-project.org/).

Table 1: Definition and predicted responses to anthropogenic stress of the considered macroinvertebrate metrics.

Metric (abbreviation)	Definition	Predicted response to stress[1]
Diversity		
Shannon-Wiener's Diversity Index	Taxonomic diversity of assemblage	Decrease
Simpson's Diversity Index	Taxonomic diversity of assemblage	Decrease
Pielou's Evenness Index	Assemblage's evenness	Decrease
Richness		
Taxonomic richness	Total number of taxa	Decrease
EPT richness	Total number of Ephemeroptera (E), Plecoptera (P), and Trichoptera (T) taxa	Decrease
O/E	Index comparing observed (O) taxa to those expected (E) at reference sites	Decrease

Tolerance		
Hilsenhoff Biotic Index (HBI)	Organic pollution tolerance index	Increase
Composition		
% EPT	Proportion of individuals that are Ephemeroptera (E), Plecoptera (P), and Trichoptera (T)	Decrease
% chironimidae	Proportion of sampled individuals that are midges	Increase
% diptera	Proportion of sampled individuals that are true flies	Increase
% amphipoda	Proportion of sampled individuals that are scuds	Increase
% isopoda	Proportion of sampled individuals that are sowbugs	Increase
% worms	Proportion of sampled individuals that are aquatic worms (e.g., Oligochaeta)	Variable
% molluscs	Proportion of sampled individuals that are molluscs (e.g., snails)	Variable
% CIGH[2]	Proportion of sampled individuals that are Coroxidae (C), Isopoda (I), Gastropoda (G), or Hirudinae (H)	Increase
% insect	Proportion of sampled individuals that are insects	Decrease
% non-insect	Proportion of sampled individuals that are not insects	Increase
PCA axis 1 scores	Multivariate community composition index	Variable
PCA axis 2 scores	Multivariate community composition index	Variable
PCoA axis 1 scores	Multivariate community composition index	Variable
PCoA axis 2 scores	Multivariate community composition index	Variable

[1] Each metric's predicted response to anthropogenic stress was based on published literature (Hilsenhoff 1987, Klemm et al. 2003, Maloney and Feminella 2006, SNCA 2014).
[2] % CIGH (proportion of individuals that are water boatmen, sowbugs, snails, or leaches in a sample) is a metric sensitive to impacts associated with agricultural land use (SNCA 2014).

Quantifying variability

Random nested models were fitted for each metric to quantify the variance accounted for at each of the sampling scales. Sites, site visits, and replicate samples were treated as random nested factors. These components of variance provided a relative measure of variability at each of these scales (variance partitioning), allowing for cross-metric comparisons.

Since only riffles were sampled twice per site according to the OBBN protocol, variance due to replicates (or within site variability) could be quantified only for the riffle habitat.

Determination of catchment characteristics

Site catchments were delineated from a digital elevation model (DEM; OMNR 2006) with a 10-m resolution using ArcMap 10.0 (ESRI, Redlands, California, USA) and ArcHydro extension's batch watershed delineation tool. Neighbouring sites, less than 40-m apart, were identified and considered as replicates; the same catchment was used for such sites.

Land cover information was obtained from the Agri-Environment Service Branch (Agriculture and Agri-food Canada 2011) and the Southern Ontario Land Resource Information System version 1.2 (SOLRIS; OMNR 2010b). The two land use layers were superimposed and merged as one – to which a road layer was subsequently added (OMNR 2010a) – providing the best land use category separation. This was accomplished by giving priority to certain categories (Appendix III) over others depending on which land cover layer had the best differentiation for given land use types. For example, the AAFC layer was given priority over the SOLRIS layer for agricultural land use types as SOLRIS' land cover layer aggregated all agriculture types as the single broad category of 'cropland' whereas the AAFC layer distinguished among cropland types. The information on the newly made land use layer provided most recent information on agricultural and urban extent and differentiation of category types.

We aggregated the 40 resulting specific land use classes into 4 broad categories of land use: % urban, % developed, % agriculture, and % intensive agriculture (Appendix III). Proportions for each broad land use category, and a land disturbance index, were estimated for each catchment (Table 2). The Land Disturbance Index (LDI; Stanfield and Kilgour 2012) was calculated – where each land use category in a catchment was ranked by expert judgement according to the severity of their expected effects on biota and weighted by area. Ultimately, this provided a single metric of the overall land cover in a catchment that reflected all classes of natural, agricultural and urban lands.

Topographical, geological, and physiographical characteristics known to be associated with properties of invertebrate assemblages were calculated for each catchment. These natural landscape features – now referred to as 'non-anthropogenic' variables – were assumed to be unmodified or unaffected by human activities. Topographical aspects of catchments were measured as the mean, standard deviation, and range in elevation within a catchment. These elevation measures along with catchment size were calculated from the DEM (Table 2; OMNR 2006). The extent of geological and physiographical variables were calculated for each site's catchment as a proportion (Table 2).

Model development

Sites were randomly allocated to a calibration (80%) and validation (20%) set, resulting in 190 calibration sites and 46 validation sites. The calibration set was used in model development, whereas the validation dataset served to test how well models predicted on new sets of sites (model evaluation).

Only records corresponding to the most recent site visit were used to screen metrics and select independent variables to reduce variability due to mismatches with the land use layer, and ensure equal weighing of sites in the regression models as the number of site visits was not consistent across sites.

Riffle and pool samples were analysed separately to control for spatial variation in metric values introduced by major differences in habitat types. The distribution of macroinvertebrates has the potential to be influenced by habitat types through their varying sensitivities to associated differences in physical conditions (e.g., current velocities, food types/availabilities, dissolved oxygen-levels, substrate compositions) and anthropogenic impacts (Kerans et al. 1992, Malmqvist 2002, Winemiller et al. 2010). Given this, we accounted for the effects of within-site habitat variability on macroinvertebrate assemblage composition to avoid introducing noise in the model analyses.

Catchment characteristics were used to estimate multiple linear regression models predicting each metric. Saturated (full) models using all possible independent variables were first fitted for all of the response metrics for exploratory purposes. The partial (i.e., marginal) effect of each group of variables (Table 2) on each of the calculated metrics was quantified. At this stage, the focus was on quantifying the variability in each metric that could be explained by each of these variable groups once the effect of all other variables under consideration has been taken in account.

Table 2: List of predictors used in modelling, grouped in three major categories: non-anthropogenic, physiographic, and anthropogenic. Units and source of the layers used to derive the predictors are provided in brackets.

Non-anthropogenic	**Physiographic** (MNDM 2007)	**Anthropogenic** (OMNR 2010a, 2010b, AAFC 2011)
Location	% Bare Rock Ridges	% Urban[2]
Catchment area (m^2)	% Beaches	% Developed[3]
Longitude	% Clay plains	Road density (km/km^2)
Latitude	% Drumlins	% Agriculture[4]
Mean elevation (m)	% Eskers	% Intensive agriculture[5]
Elevation range[1] (m)	% Kame moraines	LDI rating[6]
Elevation SD (m)	% Limestone plains	
	% Peat and muck	
Geology (MNDM 2003)	% Sand plains	
% Igneous	% Shallow till	
% Metamorphic	% Spillways	
% Sedimentary	% Till moraines	
	% Till plains and drumlins	

[1] Elevation range is measured as the difference in elevation between the highest and the lowest point within a site's catchment.

[2] Described by both pervious and impervious built-up areas such as roads, commercial, industrial, and residential land use types.

[3] Includes both urban and agricultural land use types.

[4] Includes both intensive and non-intensive agricultural land use types.

[5] Described by monoculture agricultural land use types characteristic of pesticide, tilling, and heavy fertilizer use.

[6] Land disturbance Index (Stanfield and Kilgour 2012).

When necessary to meet normality or homoscedasticity assumptions of linear models, dependent and independent variables were log-transformed. Similarly, catchment area and road density were $\log_{10}$ transformed to improve linearity.

Full models were then scrutinized for signs of overfitting and metrics exhibiting clear signs of model overfitting were excluded from further analyses. Only metrics which had comparable R^2 and residual mean square (RMS) values for both the calibration and validation datasets were conserved. Further model evaluation involved comparing residual variance against that of a null model – the simple mean – for both the calibration and validation datasets. Metrics for which the variance of the residuals (observed minus predicted using the model built from the calibration data) of the validation set was larger than variance of the metric, indicated non-responsiveness of the metric to model parameters. For such cases, the metric was not conserved for further analyses.

Simplified models

To simplify models predicting each metric, we removed redundant variables keeping those that were most easily calculated. A PCA was performed separately on each group of variables. Subsets of variables having similar loadings on the first 2 axes were then identified as being mostly redundant. For each subset, one of the variables was selected and the others removed from the model.

Stepwise deletion method was then applied to each metric model to remove non-significant terms, using the step function in R. In order to increase statistical power, calibration and validation datasets were aggregated for the simplification of the regressions. To quantify the reduction in explanatory power of these simplified models, where redundant and uninformative predictors had been removed, the R^2 values were compared to that of the full models including the complete set of predictors in Table 2. The full models indicated the maximum R^2 value that could be reached with the full set of independent variables. The simplified models were as close as possible to this maximum attainable R^2 value without compromising simplicity – and without substantially compromising the predictive ability of the model.

Hindcasting reference conditions

Simplified models for each metric were used to hindcast metric values in the absence of human activities in the catchment, while controlling for the confounding influences of natural gradients. Expected macroinvertebrate metric values in the absence of human disturbance were estimated for each site, accounting for other non-anthropogenic variables found to explain a significant fraction of the variability in the metric (i.e., non-human variables retained in the simplified models).

Each metric at each site was then scored as a function of its deviation from the hindcasted metric reference value. Retained metrics were scored following Blocksom (2003) and Minns et al. (1994). Scoring transformed metrics to a standard scale ranging from 0 (i.e., site was in bad condition) to 100 (i.e., site was in good condition). Metrics that decreased with stress were scored as follow:

$$\frac{(Deviation\ from\ expected - Min) \times 100}{(Max - Min)} \tag{1}$$

Where *Deviation from expected* referred to the extent a given metric's observed value deviated from its expected value in the absence of human activity, while correcting for the effects of natural background conditions. *Min* is the 5^{th} percentile of the residuals (observed minus predicted by the simplified model) and *Max* is the 95^{th} percentile of residuals. Metrics that increased with stress were scored as follow:

$$\frac{(Deviation\ from\ expected - Max) \times 100}{(Min - Max)} \tag{2}$$

Scores were then trimmed to 0 or 100.

Multimetric index (MMI) and estimation of probabilities

To summarize current site condition as a single measure, uncorrelated metrics were combined into an index. A correlation based PCA was performed to identify which metrics were redundant. Among the strongly correlated metrics (high loading on PCs 1 or 2, and Pearson's $r > |0.5|$), those which were easiest to calculate were preferred. Scored metrics, selected separately for riffle and pool habitats, were then averaged into the MMI_{RRP} – the multimetric index combining riffle (R) and pool (P) information into a single index value for a site.

To estimate the probability that an observed MMI_{RRP} value could be observed by chance in a site not affected by human activities in the catchment, we used the empirical distribution of the residuals of a regression relating the MMI_{RRP} to anthropogenic variables. We assumed these residuals represented a fair measure of variability around the predicted reference value for sites unaffected by human activities. The probability of being in reference condition was estimated as a one-tailed (left) test. This procedure assumes that the distribution of residuals was unaffected by the level of human activities in the catchment. For the purpose of this study, the threshold for site impairment was set at $\alpha=0.10$.

RESULTS

Quantifying variability

There was considerable temporal (within a site, among visits) and spatial (within a site visit, between riffles) variability beyond what existed among sites (Table 3). Variance component analysis for riffle habitats revealed a large temporal variability representing on average 34% (range: 26% - 48%) of the total variance. Within site variability between replicate riffles accounted on average for 35% (range: 22% - 48%) of the total variance. Lastly, variability due to site differences accounted for on average 31% (range: 9% - 51%) of the total variability. In general, metric variance among sites was less than among sampling dates within a site or replicate measurements within a site on a given date.

Pool habitat data revealed similar patterns. Temporal variability among site visits for pool habitats was large compared to that existing among sites (Table 4). Given the nested design of the variance component analysis, the temporal variability encompassed both the temporal and the 'in-stream site' spatial variability – causing relative variance due to visits for the pool habitat metrics to have much larger values compared to that same measure for the riffle sample metrics (Table 3) ranging from 39% to 83%.

Table 3: Results of the variance components analysis for riffle habitat data. Relative (%) variability at different scales and associated absolute variance (***s^2***) estimates are presented in the table's body. Bolded text denotes the scale at which most of the variation is explained. N= 1131

Metrics	**Sites**		**Visits**		**Replicates**	
	%	***s^2***	**%**	***s^2***	**%**	***s^2***
% EPT	**39**	165.77	35	145.45	26	109.73
EPT richness	**39**	1.95	29	1.45	32	1.59
HBI	18	0.15	**48**	0.38	33	0.27
Log % isopoda	**42**	0.71	29	0.49	28	0.47
Log % CIGH	**43**	0.66	26	0.41	31	0.47
Taxonomic richness	10	1.22	**42**	4.87	**48**	5.61
Shannon-Wiener's	9	0.016	**44**	0.074	**47**	0.078
PCA axis 1 scores	**48**	1.20	30	0.75	22	0.55
PCoA axis 1 scores	**51**	0.014	27	0.0072	22	0.0060
O/E	13	0.0043	37	0.013	**51**	0.018
MMI	**54**	149.86	25	129.24	21	100.94

Table 4: Results of the variance components analysis for pool habitat data. Relative (%) variability at different scales and associated absolute variance (*s^2*) estimates are presented in the table's body. Bolded text denotes the scale at which most of the variation is explained. N= 612 (MMI_{RRP} N=527).

Metrics	Sites		Visits	
	%	s^2	%	s^2
Log % EPT	44	0.60	**56**	0.75
EPT richness	43	2.00	**57**	2.71
Log % amphipoda	**52**	0.84	48	0.76
Log % isopoda	44	0.67	**56**	0.87
% CIGH	40	92.52	**60**	139.94
% insect	**60**	359.98	40	240.40
% non-insect	**61**	365.71	39	234.35
Taxonomic richness	17	1.85	**83**	9.23
PCA axis 1 scores	37	0.64	**63**	1.08
PCA axis 2 scores	49	0.98	**51**	1.03
PCoA axis 1 scores	40	0.0078	**60**	0.012
PCoA axis 2 scores	**59**	0.017	41	0.012
O/E	22	0.0080	**78**	0.029
MMI	**55**	175.31	45	146.19
MMI_{RRP}	**53**	146.97	47	130.06

Exploratory full models

Physiographic, non-anthropogenic, and anthropogenic activity variables together could account only for a relatively small fraction of the variance of individual metrics (Appendix IV, Table 9). Full models accounted for between 11% and 45% of the variance in metrics (average=24%) from riffle habitats. Similarly, full models accounted between 17% and 43% of the variance in metrics (average= 24%) from pool habitats.

In general, non-anthropogenic variables accounted for the largest proportion of explained variance, followed by physiographic and human activity variables (Appendix IV, Table 9). The average proportion of the variance explained by the partial effects of each variable groups to the full models (average of (partial R^2/full model R^2)) for riffles were, in order of importance: 24% for the non-anthropogenic group, 21% for the physiographic group, and 14% for the anthropogenic activity group (with the remaining 41% of explained variability left ambiguously attributed to the collinearities of the 3 groups of variables). Similar patterns were detected for the pool samples with 26% for the non-anthropogenic variable group, 20% for physiographic variables, and 12% for the anthropogenic activity variables. Overall, it was observed that the anthropogenic variable group had a smaller impact on metric values compared to the other measured variable groups.

The slight improvement in overall model R^2 (explanatory power) attributable to physiographic variables was not substantial enough to justify the added model complexity, and for these reasons, the physiographic variables were excluded from further analyses. Although the physiographic variable group occasionally had a higher contribution to the full model R^2 values relative to the anthropogenic variable group, the large number of correlated variables contained within the physiographic group (i.e., 13) increased considerably collinearity and overfitting problems.

Overfitting assessment

There was strong evidence of overfitting of the full models for approximately half of the metrics, and these were removed from further consideration. Ten out of 21 metrics for riffle samples and 13 out of 21 metrics for pool samples demonstrated no clear evidence of model overfitting and were conserved for model simplification (Appendix III). Model overfitting was most often exhibited by negative R^2 values for the validation datasets.

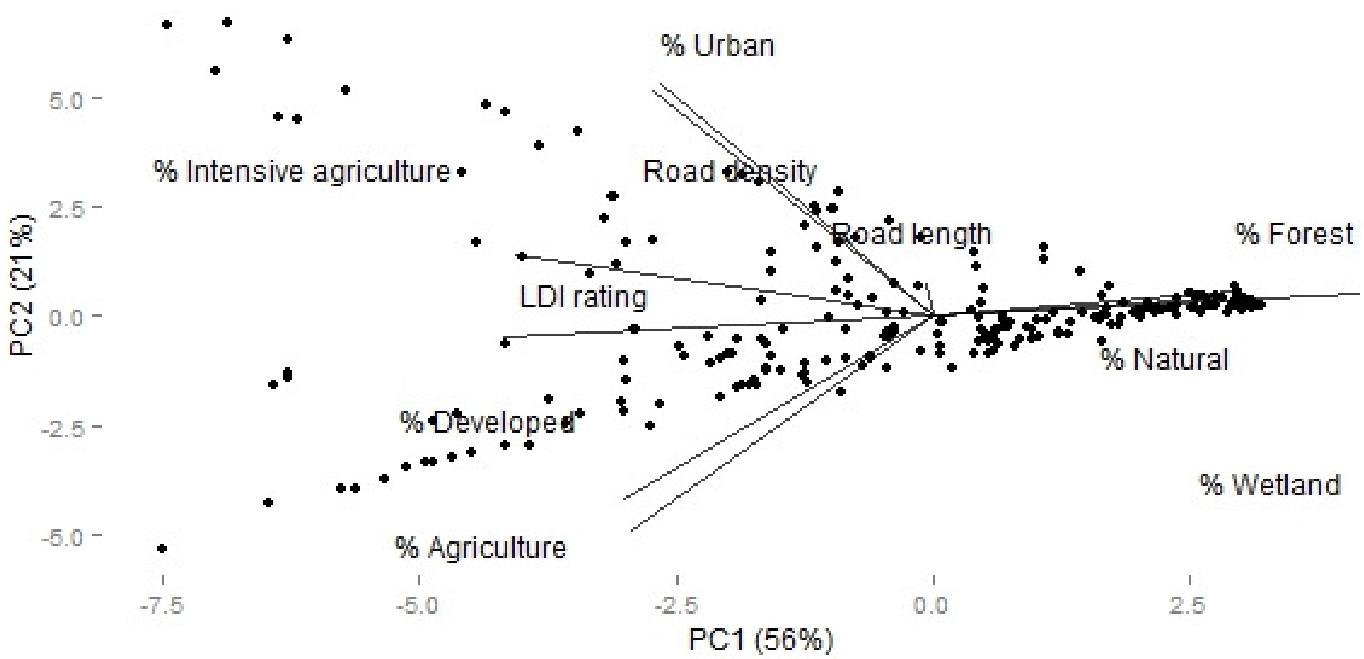
Simplified models

There was considerable correlation and redundancy among the anthropogenic activity variables. The first 2 principal components accounted for 77% of the variance in the data

(Figure 2a; Appendix V, Table 14). Final variables within the anthropogenic variable group retained for the simplified models were: % developed (high loading on axis 1), % intensive agriculture (high loading on axis 2), and $\log_{10}$ of road density (high loading on axis 2).

There was also high correlation among non-anthropogenic variables and the first 2 components of the PCA accounted for 72% of their combined variability (Figure 2b, Appendix V, Table 14). Based on highest loadings, these uncorrelated variables were kept for further modeling: % sedimentary (high loading on axis 1) and $\log_{10}$ of catchment area (high loading on axis 2).

a



b

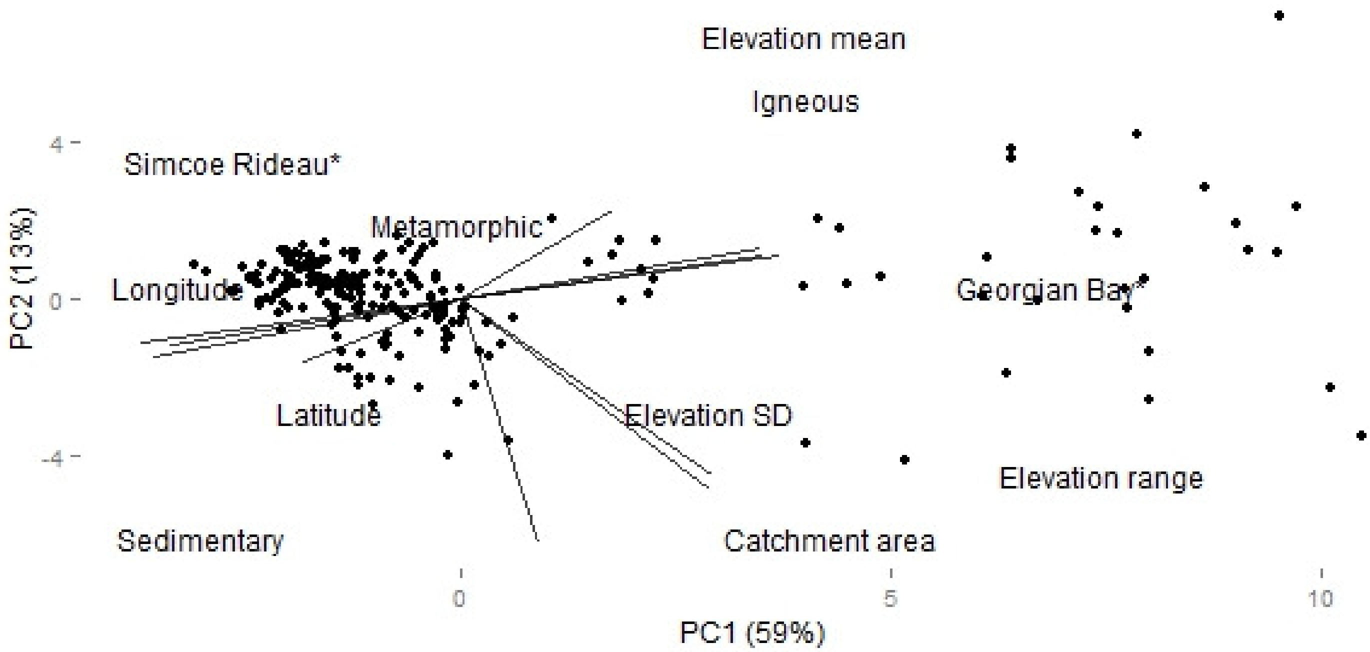


Figure 2: PCA biplots of sites based on (a) anthropogenic variables, and (b) non-anthropogenic variables where the asterisks (*) mark ecoregions. The loadings for each variable group are indicated by vectors. Symbols denote the stream sampling sites within our study region. N=236.

The simplified models were highly statistically significant as indicated by their small *p*-values for retained model terms. All regression models revealed the influence of catchment area on metrics for the riffle (Table 5) and pool habitats (Table 6). Generally, an increase in this variable resulted in an increase in metric values that decreased with human activity (e.g., % EPT). The most consistently retained anthropogenic variable was % developed for riffles and pools.

Predictive power of simplified models was similar to that of full models. R^2 values were comparable to the R^2 values for equivalent full models. The simplified models generally explained a modest amount of variation in the metric responses with the highest attained R^2 value for riffles <0.28 (Table 5) and for pools <0.34 (Table 5). The simplified models for riffle samples did not cause a substantial loss in model explanatory power, with a maximum decrease of 4% in R^2 value. The simplified model for pool samples resulted in slightly larger decrease in R^2 values, with a maximum reduction of 10% in R^2 value.

There was evidence of lack of fit of the models. Variability around predicted values was large compared to measurement error, and the models accounted for only a fraction of the variance attributable to sites. The simplified models' residual variances (RMS; Table 5) were consistently at least double that of the variances between riffle replicates (Table 3), and full model R^2 values (Table 5; Table 6) were typically much lower (average: riffle= 21%; pool= 28%) than the proportion of variance attributable to sites in the variance component analysis (average: riffle= 33%; pool= 44%; Table 3; Table 4).

Table 5: Importance of simplified model parameters on each conserved metric (as the response variable) for riffle habitats only. Direction of response (+/-), intercepts, coefficients (standard error) and *p*-values are displayed in the body of the table. R^2 and RMS of the simplified and full models are included as a measure of statistical 'goodness of fit' for each model type. Calibration and validation datasets were combined for these models. N= 236

Response variable/metric	Simplified regression model			R^2		RMS	
	Parameter	Coefficient (SE)	Coefficient *p*-value	Simplified	Full	Simplified	Full
% EPT	Intercept	-45.83 (9.05)					
	Road density	-7.40 (2.30)	10^{-4}				
	% Intensive agriculture	-0.19 (0.052)	10^{-5}	0.16	0.17	382.4	365.5
	Catchment area	9.74 (1.25)	10^{-15}				
EPT richness	Intercept	-5.58 (1.04)					
	Road density	-0.76 (0.31)	0.0223				
	% Developed	-0.023 (0.0047)	10^{-7}				
	Catchment area	1.43 (0.14)	10^{-17}	0.28	0.29	4.60	4.01
	Elevation SD	-0.064 (0.022)	10^{-4}				
HBI	Intercept	7.86 (0.42)					
	% Developed	0.0039 (0.0015)	10^{-4}	0.06	0.07	0.77	0.76
	Catchment area	-0.24 (0.057)	10^{-6}				
Log % isopoda	Intercept	33.35 (12.75)					
	% Intensive agriculture	-0.013 (0.0042)	10^{-4}				
	% Developed	0.019 (0.0031)	10^{-10}				
	Catchment area	-0.17 (0.081)	0.0319	0.16	0.17	1.41	1.27
	Latitude	-0.69 (0.28)	0.0139				
	Elevation mean	-0.0043 (0.0015)	10^{-4}				

Log % CIGH	Intercept	59.1 (12.34)					
	% Developed	0.014 (0.0025)	10^{-9}				
	Catchment area	-0.22 (0.079)	10^{-4}	0.20	0.21	1.35	1.21
	Latitude	-1.23 (0.27)	10^{-7}				
	Elevation mean	-0.0072 (0.0015)	10^{-7}				
Taxonomic richness	Intercept	2.61 (1.82)					
	% Intensive Agriculture	0.047 (0.11)	10^{-6}				
	% Developed	-0.060 (0.0084)	10^{-13}				
	Catchment area	1.82 (0.22)	10^{-16}	0.25	0.25	10.33	10.05
	Elevation mean	-0.013 (0.0044)	10^{-4}				
	Elevation SD	-0.11 (0.039)	10^{-4}				
Shannon-Wiener's Diversity Index	Intercept	1.32 (0.20)					
	% Intensive agriculture	0.0045 (0.0013)	10^{-5}				
	% Developed	-0.0057 (0.00097)	10^{-10}	0.17	0.17	0.14	0.14
	Catchment area	0.12 (0.025)	10^{-7}				
	Elevation mean	-0.0030 (0.00045)	10^{-12}				
PCA axis 1 scores	Intercept	4.04 (0.72)					
	% Developed	0.013 (0.0025)	10^{-8}	0.14	0.15	2.21	2.12
	Catchment area	-0.60 (0.097)	10^{-10}				
PCoA axis 1 scores	Intercept	0.52 (0.075)					
	% Developed	0.0012 (0.00026)	10^{-7}	0.16	0.16	0.024	0.023
	Catchment area	-0.074 (0.010)	10^{-14}				
O/E	Intercept	0.098 (0.087)					
	% Intensive agriculture	0.0016 (0.00063)	10^{-4}				
	% Developed	-0.0029 (0.00041)	10^{-13}	0.22	0.26	0.032	0.031
	Catchment area	0.096 (0.012)	10^{-15}				
	Elevation SD	-0.0084 (0.0018)	10^{-7}				
MMI	Intercept	42.86 (1.45)					
	Road density	-5.47 (2.58)	0.0345	0.11	0.21	321.8	299.0
	% Developed	-0.19 (0.037)	10^{-8}				

Table 6: Importance of simplified model parameters on each conserved metric (as the response variable) for pool habitats only. Direction of response (+/-), intercepts, coefficients (standard error) and *p*-values are displayed in the body of the table. R^2 and RMS of the simplified and full models are included as a measure of statistical 'goodness of fit' for each model type. Calibration and validation datasets were combined for these models. N= 236

Response variable/metric	Simplified regression model			R^2		RMS	
	Parameter	Coefficient (SE)	Coefficient *p*-value	Simplified	Full	Simplified	Full
Log % EPT	Intercept	-2.88 (0.66)					
	Road density	-0.47 (0.22)	0.0300				
	% Developed	-0.0076 (0.0032)	0.0175	0.27	0.34	1.25	1.19
	Catchment area	0.745 (0.091)	10^{-16}				
EPT richness	Intercept	-5.47 (1.21)					
	% Developed	-0.026 (0.0051)	10^{-8}				
	Catchment area	1.31 (0.16)	10^{-15}	0.27	0.36	4.06	3.86
	Igneous	-0.011 (0.0045)	0.0216				
Log % amphipoda	Intercept	-2.70 (38.27)					
	% Intensive agriculture	-0.014 (0.0067)	0.0327				
	% Developed	0.023 (0.0042)	0.0248				
	Longitude	-0.93 (0.44)	0.0343				
	Latitude	-1.44 (0.36)	10^{-6}	0.13	0.23	1.51	1.42
	Igneous	0.0076 (0.0036)	0.0353				
	Elevation mean	-0.018 (0.0043)	10^{-6}				
Log % isopoda	Intercept	-58.83 (35.34)					
	% Intensive agriculture	-0.014 (0.0062)	0.0237				
	% Developed	0.023 (0.0042)	10^{-9}				
	Longitude	-1.39 (0.40)	10^{-4}	0.20	0.27	1.30	1.24
	Latitude	-0.98 (0.33)	10^{-6}				
	Elevation mean	-0.014 (0.0038)	0.0219				
% CIGH	Intercept	861.47 (198.84)					
	% Developed	0.17 (0.046)	10^{-5}				
	Catchment area	-3.32 (1.26)	10^{-4}	0.13	0.20	233.8	227.6
	Latitude	-18.31	10^{-6}				
	Elevation mean	-0.062	0.0219				
% insect	Intercept	-1145 (295.5)					
	% Intensive agriculture	0.32 (0.12)	10^{-4}				
	% Developed	-0.49 (0.084)	10^{-9}				
	Catchment area	5.25 (1.87)	10^{-4}	0.23	0.30	510.9	498.3
	Latitude	26.18 (6.51)	10^{-6}				
	Elevation mean	0.089 (0.040)	0.0261				

% non-insect	Intercept	1168.50 (294.57)					
	% Intensive agriculture	-0.32 (0.12)	10^{-4}				
	% Developed	0.49 (0.083)	10^{-9}				
	Catchment area	-5.21 (1.86)	10^{-4}	0.23	0.31	507.9	496.4
	Latitude	-24.50 (6.49)	10^{-5}				
	Elevation mean	-0.092 (0.040)	0.0214				
Taxonomic richness	Intercept	0.077 (2.01)					
	% Developed	-0.020 (0.0085)	0.0163				
	Catchment area	1.66 (0.27)	10^{-10}	0.17	0.23	11.12	10.74
	Igneous	-0.029 (0.0075)	10^{-5}				
PCA axis 1 scores	Intercept	-27.35 (14.49)					
	% Developed	0.0087 (0.0029)	10^{-4}				
	Catchment area	-0.54 (0.094)	10^{-9}	0.19	0.20	1.35	1.30
	Latitude	0.70 (0.32)	0.0300				
PCA axis 2 scores	Intercept	50.65 (39.00)					
	% Intensive agriculture	0.013 (0.0068)	0.0500				
	% Developed	-0.028 (0.0046)	10^{-10}				
	Longitude	1.39 (0.44)	10^{-4}	0.27	0.34	1.55	1.47
	Latitude	1.20 (0.37)	10^{-4}				
	Elevation mean	0.019 (0.0044)	10^{-6}				
	Elevation SD	-0.050 (0.020)	0.0107				
PCoA axis 1 scores	Intercept	-3.10 (1.44)					
	Catchment area	-0.081 (0.010)	10^{-15}	0.22	0.27	0.01617	0.01564
	Latitude	0.10 (0.032)	10^{-4}				
PCoA axis 2 scores	Intercept	-5.26 (4.34)					
	% Intensive agriculture	-0.0015 (0.00075)	0.0412				
	% Developed	0.0035 (0.00051)	10^{-12}				
	Longitude	-0.16 (0.050)	10^{-4}	0.34	0.41	0.01917	0.01812
	Latitude	-0.14 (0.042)	10^{-5}				
	Elevation mean	-0.0025 (0.00049)	10^{-8}				
	Elevation SD	0.0054 (0.0022)	0.0134				
O/E	Intercept	-0.023 (0.12)					
	% Developed	-0.0016 (0.00049)	10^{-4}				
	Catchment area	0.099 (0.016)	10^{-10}	0.19	0.22	0.03703	0.03650
	Igneous	-0.0016 (0.00043)	10^{-5}				
MMI	Intercept	52.68(1.84)					
	% Intensive agriculture	0.26 (0.082)	10^{-4}				
	% Developed	-0.36 (0.051)	10^{-12}	0.16	0.26	250.3	232.5
	Igneous	-0.090 (0.036)	0.0117				
MMI_{RRP}	Intercept	43.89 (1.88)					
	Road density	-7.07 (3.50)	0.0446	0.14	0.28	230.4	209.9
	% Developed	-0.16 (0.049)	10^{-5}				

Multimetric Indices

There was considerable variation in the scaled metric scores observed across sampled sites. For the majority of scaled metrics, the spread contained the full possible range of values from 0 to 100, regardless of habitat type (Appendix VI).

There was high redundancy observed among metrics (Figure 3; Appendix V, Table 15), and 5 out of the 15 metrics (riffle and pool habitats) were conserved for building the MMI_{RRP}. As would be expected, the highest correlation was observed between the mutually exclusive % insect and % non-insect metrics ($r = -0.99$) at pool habitats, followed by a strong correlation between PCA axis 1 scores and PCoA axis 2 scores ($r = 0.97$) at riffle habitats. Consequently, the MMI_{RRP} combined the following uncorrelated metrics incorporating both riffle (R) and pool (P) habitats: % EPT (R and P), HBI (R), % amphipoda (P), % isopoda (R and P), and taxonomic richness (R and P). The lack of correlation with any non-anthropogenic variables was a result of combining metric residuals into an index – for which the confounding effects of non-anthropogenic variables had already been removed.

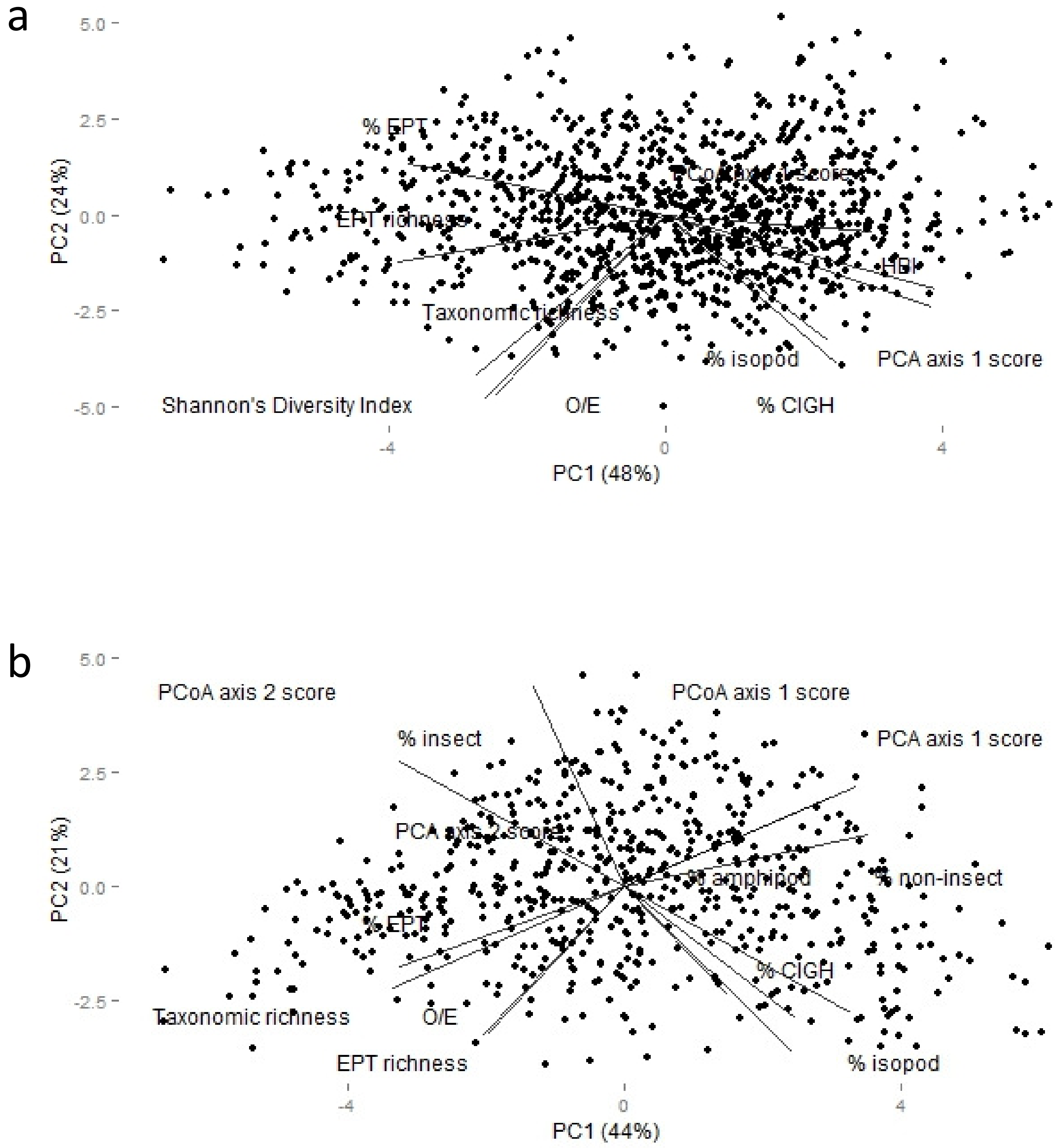


Figure 3: PCA biplots of sites based on (a) scored metric at riffle habitats N=1131, and (b) scored metrics at pool habitats N=612. The loadings for each variable group are indicated by vectors. Symbols denote the stream sampling sites within our study region.

The MMI_{RRP} declined with increasing road density and % developed as:

$$\mathrm{MMI}_{\mathrm{RRP}} = 43.89 - 7.07\ (\log_{10} road\ density) - 0.16\ (\%\ developed) \qquad (3)$$

The predictive power of the simplified model was reduced compared to the full model equivalent and, similar to patterns observed for individual metrics, the models accounted for a small portion of the variance attributable to sites. The full model – containing physiographic (P), Non-Anthropogenic (NA), and Anthropogenic (A) variable groups – explained 28% (R^2= 0.28; RMS=209.9; N=215; Table 6) of the variability in the MMI_{RRP}, while the simplified model explained 14% (R^2= 0.14; RMS=230.4; N=215; Table 6), cutting the model explanatory power by half for the final metric. These values, when compared to the relative and absolute variance attributable to sites (53%; MSE=146.97; N=527; Table 4), indicated lack of precision of the MMI_{RRP} model (Equation 3).

Assessment of site status

Residual variability around the MMI_{RRP} model (Equation 3) represent both sampling variability and lack of fit of the model due to misspecification and unconsidered explanatory variables. This variability is substantial (observed MMI_{RRP} scores could deviate by as much as 46.73 units from predicted values; Appendix VII; Figure 4), and has to be considered in assessing stream site status. One method of doing so requires the use of the distribution of residuals to determine thresholds for classification of sites into condition categories. MMI_{RRP} deviation scores can then be used to determine whether a site should be categorized as being in reference or out of reference condition, based on the established threshold. We used a type I error rate (alpha) of 10% for classifying a site as being out of reference, and an error rate of 25% for classifying a site as being in reference condition. According to these criteria, a total of

85 sites were assigned as likely in reference condition ('pass'), while 102 sites were classified as unlikely in reference condition ('fail') at a -16.6 critical value determined at α=0.10. The remaining 28 sites within the study area were categorized as being inconclusive (Figure 5; Table 7).

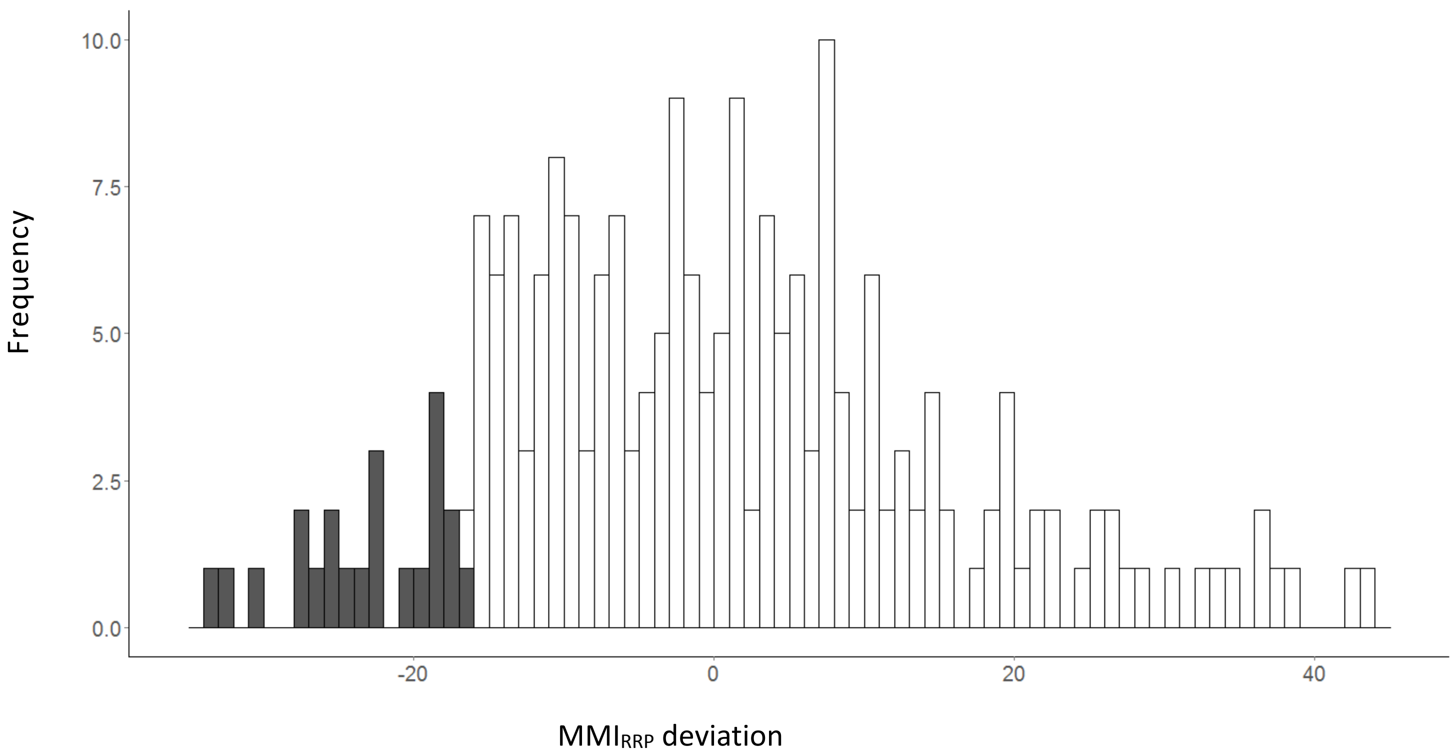


Figure 4: Frequency distribution of residuals of the model relating multimetric index score (MMI_{RRP}) to human activity variables used to determine thresholds to classify sites. The portion in dark grey represent deviations that would have a site considered to be out of reference condition, based on a 10% threshold (α=0.10) critical value of -16.6. N = 215

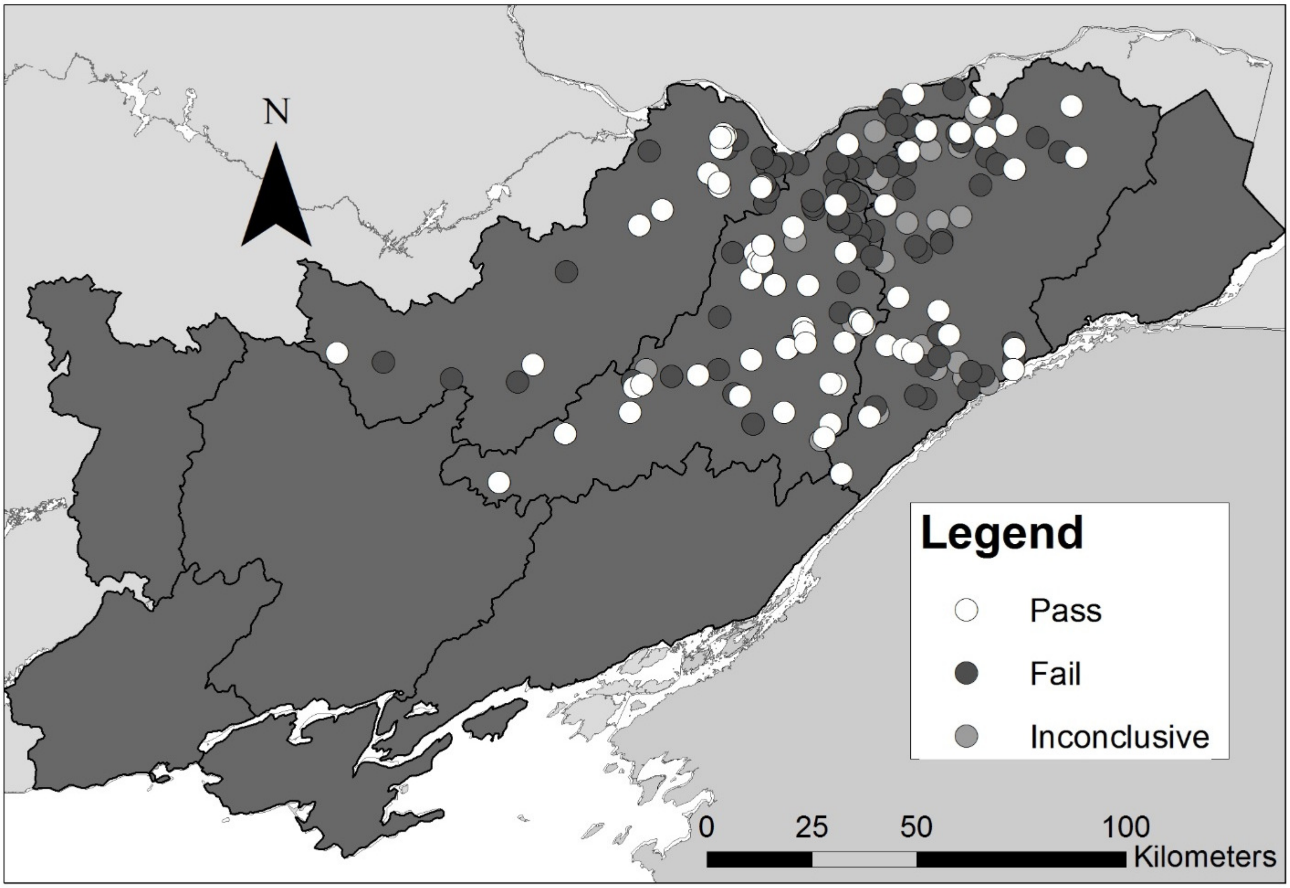


Figure 5: Map showing site ecological status within the study region (N= 215). Different circle colours indicate site condition: in white are the sites that were deemed in reference condition (p≥0.25), in dark grey are the sites that were deemed outside of reference condition (p≤ 0.10), and sites in light grey are those that were classified as inconclusive.

Table 7: Sampled stream site status by Conservation Area within the study region (N= 215). Sites in the 'Pass' column are classified as being in reference condition (p≥0.25), while sites in the 'Fail' column were classified as being outside of reference condition (p≤ 0.10). Remaining sites were inconclusive.

Conservation Area	**Pass**	**Fail**	**Inconclusive**	**TOTAL**
South Nation	24	31	17	72
Rideau Valley	44	52	8	104
Mississippi Valley	17	19	3	39
TOTAL	85	102	28	215

DISCUSSION

Features associated with current site condition

Catchment area was usually the most significant explanatory variable in our models predicting macroinvertebrate metrics and illustrates well the importance of accounting for possible confounding effects of non-anthropogenic catchment-scale predictors on biological indicators. Catchment/stream size has been recognized as being an important environmental determinant of biotic assemblages (Vinson and Hawkins 1998, Hawkins et al. 2010a, Waite et al. 2010, Ligeiro et al. 2013). As was observed in our study, catchment area tends to have a positive influence on stream health indicators, especially for richness and diversity based metrics (Table 5; Table 6). An underlying species-area relationship could explain this phenomenon; where larger catchments, and consequently larger streams, result in higher taxonomic richness/diversity partly related to increased flow and greater habitat heterogeneity (Vinson and Hawkins 1998, Van Sickle et al. 2004). This highlights the need to account for natural, non-anthropogenic factors in modeling efforts – with the objective of 'separating' these effects on the assemblage descriptors to evaluate the effects of human disturbance, independent of these.

The proportion of developed land in the catchment was the anthropogenic variable most often retained in the models. The combination of agriculture and urban land use types performed better than each land use variable individually and generally had a negative influence on the invertebrate descriptors (Table 5; Table 6). Both agriculture and urban disturbances can have direct and indirect effects on macroinvertebrate assemblages that are difficult to parse out (Stepenuck et al. 2002, Van Sickle et al. 2004, Riseng et al. 2011). However, some studies find that stream biological assemblages can tolerate higher levels of agriculture (>30-50%; Allan 2004, Riseng et al. 2010) than urban land use (measured through impervious surface cover = 10-20%; Paul and Meyer 2001, Stepenuck et al. 2002) in a catchment before becoming impaired (Paul and Meyer 2001, Stepenuck et al. 2002, Allan 2004, Moore and Palmer 2005, Riseng et al. 2010) Numerous studies have documented declines in biological assemblage metrics as the extent of urban (Paul and Meyer 2001, Stepenuck et al. 2002, Roy et al. 2003, Walters et al. 2009, Riseng et al. 2010, Bellucci et al. 2013, Wallace et al. 2013) and agricultural land use types increases within catchments (Allan 2004, Blann et al. 2009, Riseng et al. 2010, 2011). Negative biological responses to these impacts have been linked to associated changes in hydrological regimes, alterations in habitat structure (e.g., changes in channel geomorphology, and a reduction in streambed heterogeneity), increases in bank erosion and sedimentation, temperature rises, and higher nutrient and/or pollutant inputs through stormwater runoff.

The use of multimetric indices (MMIs) over individual metrics as a way to summarize biological aspects of a site into a single measure continues to be widespread in bioassessment (Hawkins et al. 2010b, Ruaro and Gubiani 2013). More recent advances on this approach have shown that removing biologically redundant information (i.e., correlated metrics) to build a MMI improves their performance in terms of precision, bias, and sensitivity/responsiveness to human-induced impacts (Van Sickle 2010, Chen et al. 2014). Therefore, for optimal MMI performance, redundancy among metrics should be reduced. Correlation was high among individual metrics (Figure 3; Appendix V, Table 15) reflecting the biological redundancy of certain metrics as they are either based on similar subsets of taxa or represent slight variations of each other (e.g., % isopod vs % CIGH; O/E vs taxonomic richness) and therefore likely respond to similar stressors (Stoddard et al. 2008). Furthermore, the MMI_{RRP} – as a single measure to summarize overall site ecological condition – provides an objective and practical management tool for decision-makers. The task of describing the condition of individual sites and their relative condition compared to other sites within a given region is simplified, rather than using >10 individual metrics, most of which, essentially measure similar site characteristics.

The combined multimetric index (MMI_{RRP}), correlated negatively to road density and to development in catchments (Equation 3). Similar to individual metrics, our summary index was mostly responsive to an overall measure of disturbance in the catchment. The inclusion of road density as a predictor in the final model supports the suggestion that urban land use is directly (or indirectly through increased road density) more detrimental than agricultural development in our sites (Paul and Meyer 2001, Stepenuck et al. 2002, Allan 2004, Moore and Palmer 2005, Riseng et al. 2010). Road density is higher in urban (e.g., residential, commercial, and industrial urban land uses are associated with greater road density) than agricultural landscapes, and can be used as an effective surrogate measure for quantifying the effects of urbanization on stream biota (Alberti et al. 2007, Bailey et al. 2007, Wallace et al. 2013).

Urban and agricultural land use generally follow natural gradients, therefore these environmental features are intertwined with gradients of human disturbance (Allan 2004, Van Sickle et al. 2004, King et al. 2005, Stoddard et al. 2008, Waite et al. 2010). This collinearity limits our ability to unambiguously partition explained variability by our models to covarying variables. Indeed, the sum of the partial R^2 values (anthropogenic (A) + non-anthropogenic

(NA)) was on average about only 60% of the R^2 values of the full models (Table 7; Table 8). The difference cannot be uniquely attributed to neither the anthropogenic nor the non-anthropogenic variables – a portion of the total variance explained is shared among these variable groups. Consequently, some degree of confounding between 'non-anthropogenic' and 'anthropogenic' effects appears inevitable when attempting to develop regression models from catchment-scale predictors.

Explanatory power of the models for assemblage metrics

Although the correlations between the modeled catchment-scale predictors and assemblage metrics were highly significant, our simplified models accounted for only a modest proportion of the observed variability. Given the proportion of the total variability attributable to among-site differences (riffles<54%, pools<61%; Table 3; Table 4) that could be attained by a 'perfect' model, there remains a portion of that among-site variability not captured by our full (riffles R^2<0.29, pools R^2<0.41; Table 5, Table 6) or simplified models (riffles R^2<0.28, pools R^2<0.34; Table 5, Table 6). Recent large-scale studies relating macroinvertebrate indicators to catchment-scale predictors through predictive modeling rarely report strong relationships, with R^2 values typically ranging between 0.2 and 0.7 (Alberti et al. 2007, Bailey et al. 2007, Goetz and Fiske 2008, Walters et al. 2009, Riseng et al. 2010, Waite et al. 2010, Bellucci et al. 2013). Our obtained simplified model R^2 values are within the expected range, albeit on the lower end of it. For the most part, lack of model fit could be attributed to unaccounted temporal and spatial sources of variability such as would be expected from modeling across larger and thus more variable regions. We elaborate below on possible causes for low precision of our models and potential ways to improve on them.

The absence of strong disturbance and natural gradients within our study region has the potential to limit the R^2 achievable by models. When compared with more densely populated areas, our study region may not have severely impacted sites, therefore limiting the disturbance gradient that can be attained. For example, streams in the greater Toronto area, in southern Ontario, are considered highly degraded and studies conducted in this densely populated region have been able to measure greater adverse effects of urbanization on fish and invertebrate assemblage metrics (Stanfield 2012, Wallace et al. 2013). Lack of relatively obvious anthropogenic disturbance within our region could result in natural habitat (catchment-scale and local-scale) variability to obscure any subtle human-induced impacts on stream health indicators (i.e., high signal:noise ratio; Waite et al. 2010, Ligeiro et al. 2013).

Part of the variability unexplained by our model could reflect the combined impact of catchment and local-scale variables, such as riparian and in-stream characteristics (Walters et al. 2009, Riseng et al. 2010, Winemiller et al. 2010, Waite et al. 2010, Ligeiro et al. 2013). Several variables are known to affect invertebrates but could not be examined due to the unavailability of data for most sites. Stream current velocity and streambed composition (Vinson and Hawkins 1998, Roy et al. 2003, Van Sickle et al. 2004, Walters et al. 2009, Ligeiro et al. 2013) or stream slope (Walters et al. 2009, Waite et al. 2010, Bellucci et al. 2013) as a surrogate variable encompassing a series of habitat qualities (e.g., stream velocity and streambed composition) may be the most important missing variables. Additional stressors to be modeled could include point-source pollution, such as sewage treatment discharges (Kratzer et al. 2006).

The proximity of land use to the stream sampling location could have an effect on the predicted response of the macroinvertebrate assemblages to its impacts. It is possible that natural forested land cover could buffer the streams from detrimental effects linked to human activity occurring further away in the catchment (King et al. 2005, Alberti et al. 2007, Goetz and Fiske 2008, Riseng et al. 2011, Stanfield and Kilgour 2012). A number of studies have explored the influence of landscape configuration on predicting macroinvertebrate metrics through the use of distance weights or other configuration descriptors, where patches of particular land use types are allocated greater importance when they occur closer to the stream sampling site (King et al. 2005, Alberti et al. 2007, Goetz and Fiske 2008). Considering the spatial arrangement of developed areas within the catchments could account for land use effects on stream ecological condition that otherwise would be missed by using aggregate measures of land use (e.g., percentage in whole catchment). Moreover, addressing the influence of development patterns within catchments can reduce unexplained variability and improve predictions (Alberti et al. 2007, Goetz and Fiske 2008) of macroinvertebrate assemblage metrics.

Site status diagnostic value

The combination of high variability of measurement with low precision of our models implies that measurements taken at a single visit have low diagnostic value. Samples taken in close spatial and temporal proximity should, in theory, resemble each other. The degree of similarity between such replicate samples could therefore offer a measure of repeatability/precision (Stribling et al. 2008, Ogren and Huckins 2014). This precision measure can subsequently be used as a quantification of measurement error (i.e., variability introduced by field sampling/sample processing) associated with the entire dataset from which our models are built. A high degree of similarity between replicate samples would be indicative of low measurement error linked to the dataset and high precision of metric model predictions. Higher precision and lower measurement error would mean higher confidence that detected differences between sites are real, and would therefore increase diagnostic value of site assessments (Stribling et al. 2008, Zuellig et al. 2012, Ogren and Huckins 2014). In the case of our developed models, replicate samples taken at a site on the same day were very variable, as observed through the MSE estimates and the proportion of variance at the replicate scale (Table 3; Table 4). Therefore, our models account for only a fraction of the variability and the 'true' metric values in the absence of human disturbance cannot be estimated precisely for any individual stream site.

In itself, uncertainty in predictions does not render models inadequate for use in the decision-making process (Harris et al. 2003, Stribling et al. 2008) As long as quantifications of the uncertainties and the risk associated with individual site condition diagnosis are communicated along with model predictions (Appendix VII), effective decisions – based on the best available scientific information – can be made in terms of watershed management.

Scenario testing and risk of fail

The regression relating the MMI_{RRP} to anthropogenic variables (% developed and road density; Equation 3) can be used to describe expected changes under different development scenario. Using the regression model for MMI_{RRP}, predicted change can be estimated for a range of % developed (0-100%) and road density (0-10 km/km^2) combinations. For example, at 50% developed and with a road density of 1.5km/km^2, there is a predicted decrease in MMI_{RRP}

by 9.4 units from expected reference value. This estimate represents the 'mean' expected deviation from reference condition under this development scenario.

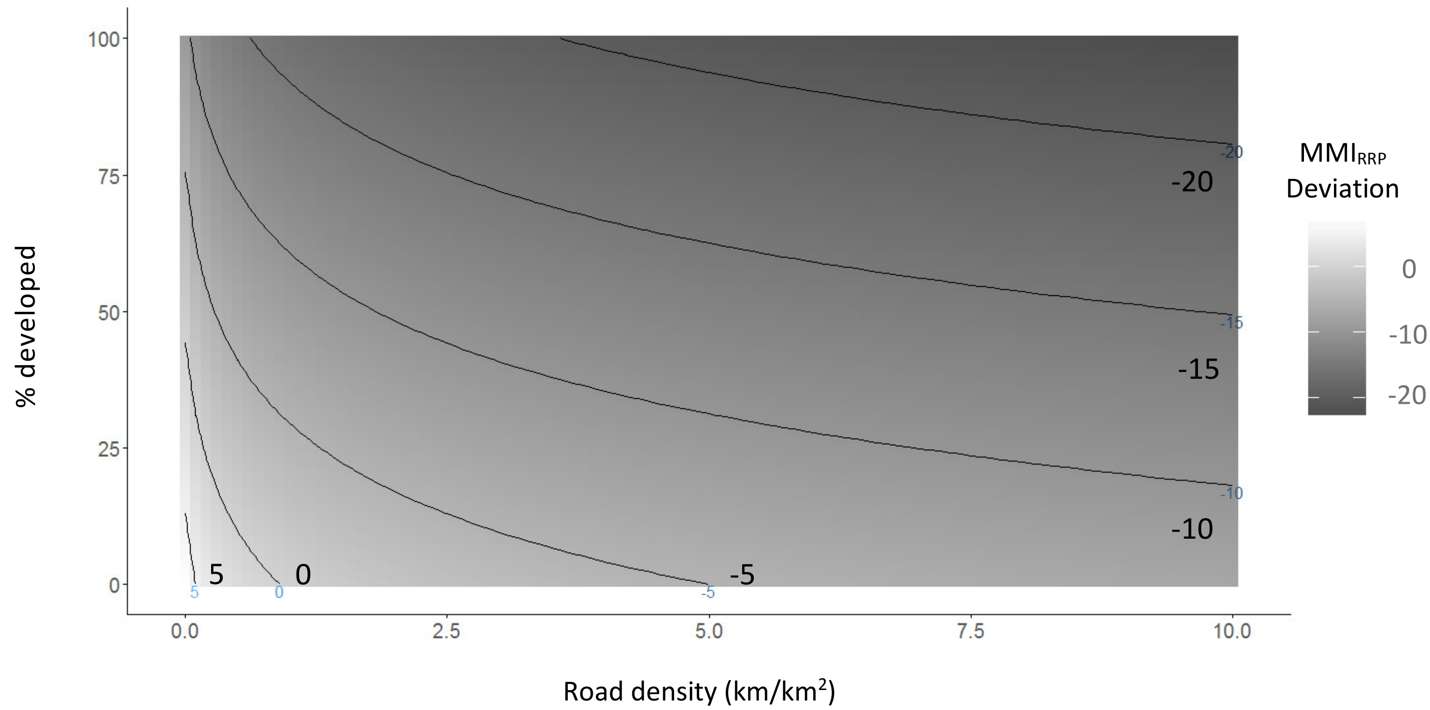
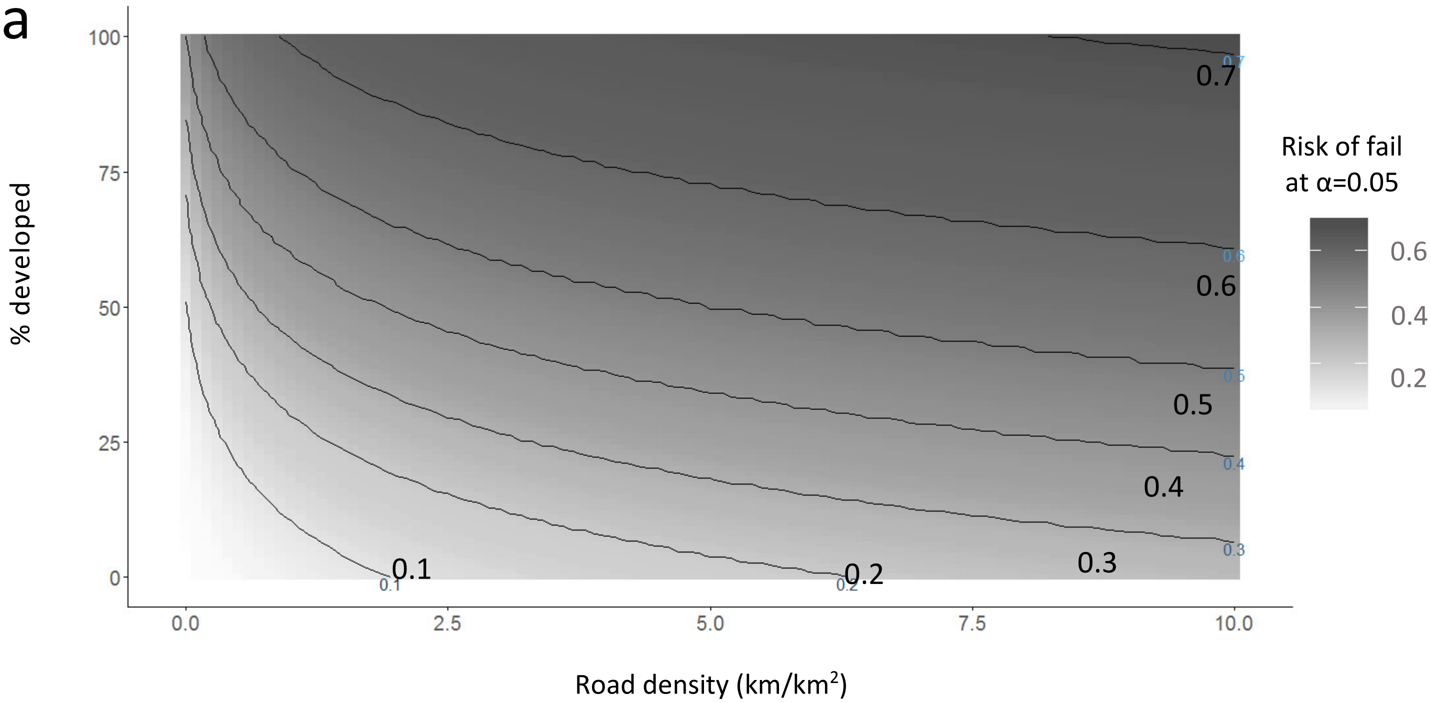


Figure 6: Contour plot illustrating various combinations of land use change scenarios and how these changes are 'predicted' to affect the MMI_{RRP} (as predicted deviations from expected value at reference condition). N= 215

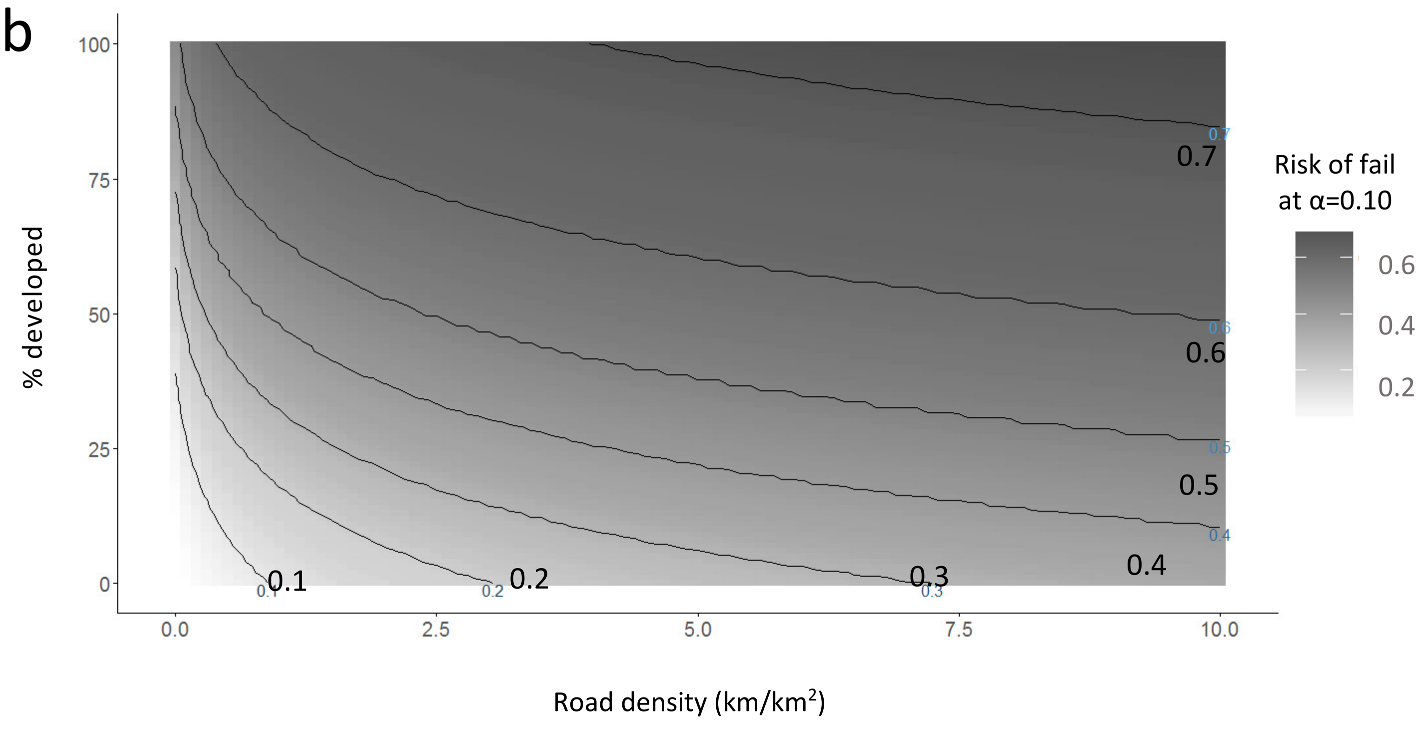
The risk that a future assessment would classify a site as not being in reference condition can be estimated, taking into account unexplained variability in the models and sampling error. Using deviation from expected and the residual distribution of the regression model (due to sampling error and lack of fit of the model), the probability that an assessment would conclude that a site is out of reference condition under each development scenario (Figure 6) was estimated. For the purpose of this exercise, the threshold for site impairment was set at α=0.10 (Figure 7b).

a



% developed
Road density (km/km²)
Risk of fail
at α=0.05
0.7
0.6
0.5
0.4
0.3
0.2
0.1

b



% developed
Road density (km/km²)
Risk of fail
at α=0.10
0.7
0.6
0.5
0.4
0.3
0.2
0.1

C

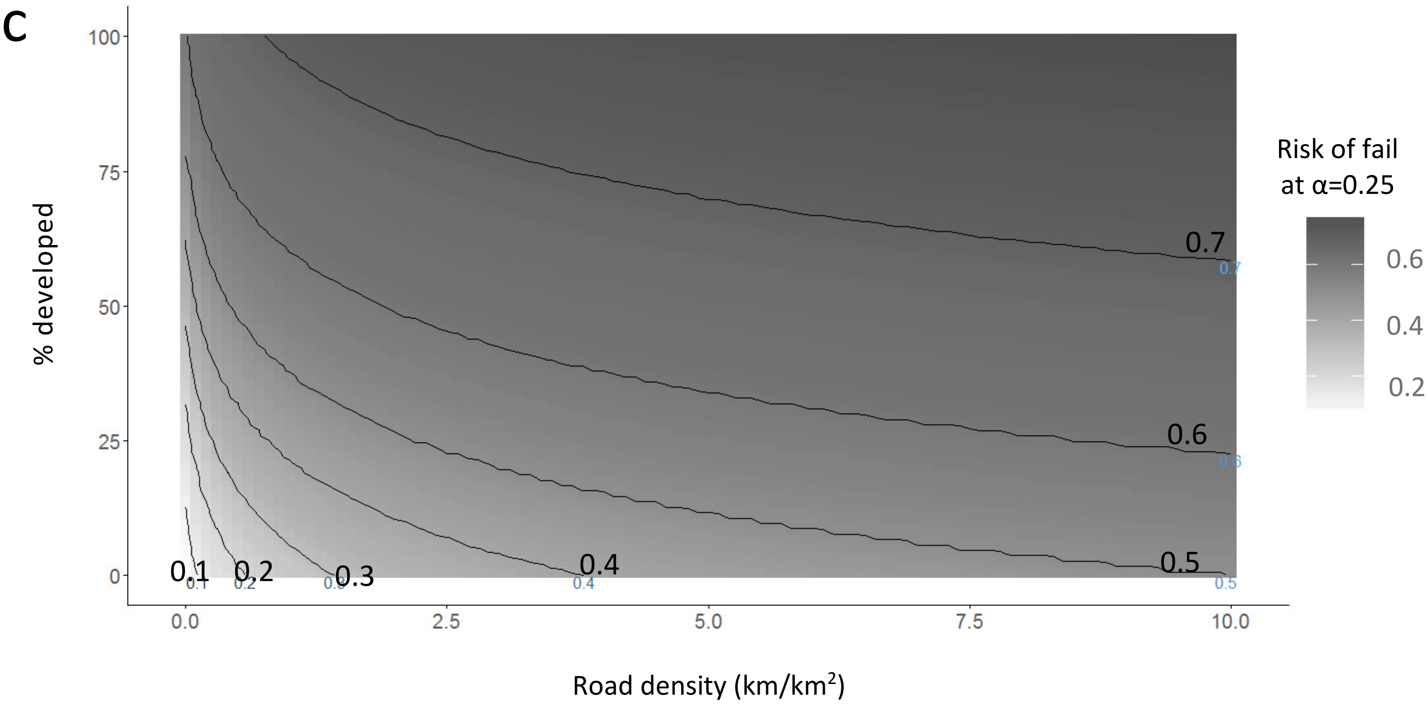


Figure 7: Development scenarios, using the MMI_{RRP}, and associated risk of fail under different thresholds: (a) alpha=0.05, (b) alpha= 0.10, and (c) alpha = 0.25. N = 215

The uncertainty around the predicted 'mean' change related to development can be quantified by the residuals, using them to generate probabilities to subsequently quantify the risk (at α=0.10; Figure 7b) that a site is out of reference condition. The -16.6 critical/cut off value identified at the 10% threshold (Figure 4), when applied to this specific development scenario results in a 35.3% probability of detecting an adverse effect on the MMI_{RRP} from the changes in development (Figure 8). That is, there is a 35.3% probability that a single assessment of a site located in a catchment with a road density of 1.5 km/km^2 and where 50% of the drainage area has been developed will be identified as out of reference condition/impacted (at this particular impact threshold of α=0.10).

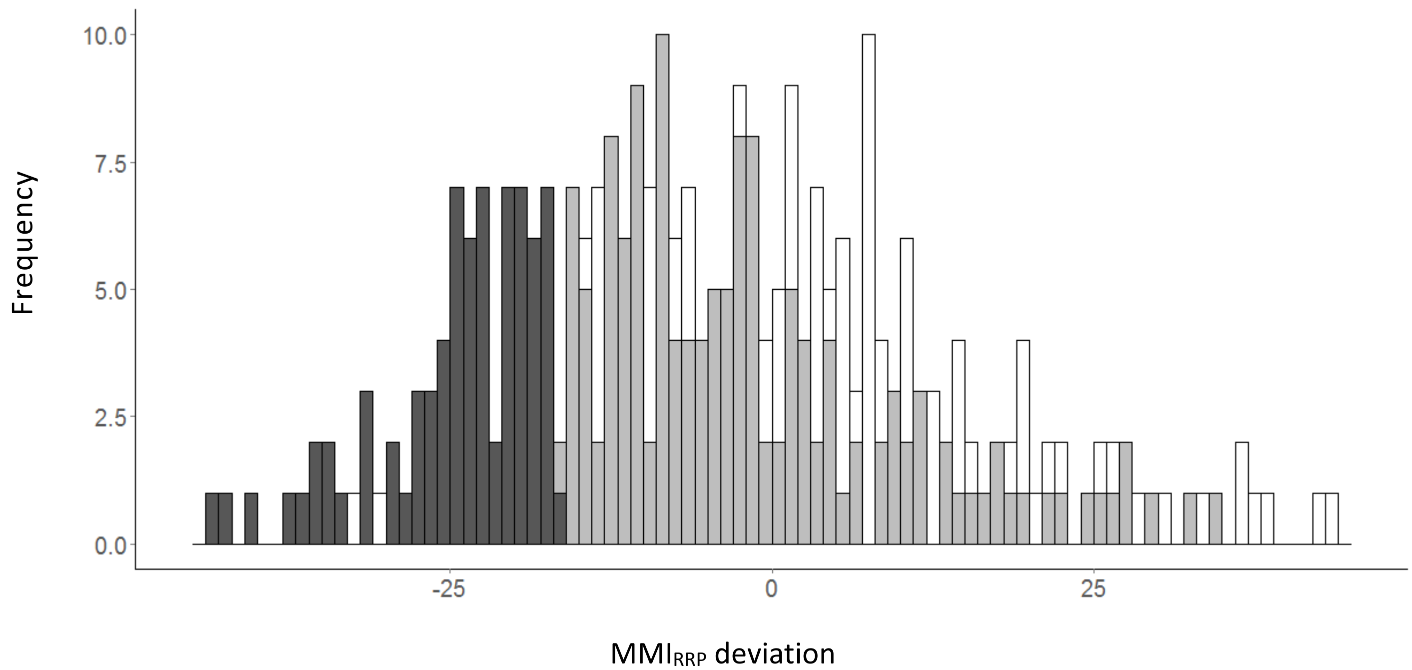


Figure 8: Estimating the probability (risk of 'fail') that a single assessment, using the MMI_{RRP}, would lead to the conclusion that a site is out of reference condition if its catchment's land cover is altered to 50% developed and road density is 1.5km/km^2 (α=0.10) resulting in a predicted mean decline of the MMI_{RRP} by 9.4 units. The white histogram represents the distribution of residuals at reference condition (observed - predicted at 0-level disturbance) while the light grey histogram (a portion is in dark grey) represents the expected distribution of individual observations of the MMI_{RRP} at a site following this development scenario. Approximately 35% of the observations (dark grey portion of the light grey histogram) would be below the threshold used to declare a site out of reference condition.

Implications for stream management

Caution is necessary when interpreting the effects of each retained predictor on the measured stream health indicators. In the model simplification phase, we removed correlated human disturbance variables, keeping the ones that were most easily calculated. Therefore, variables that were retained in our models are simply 'associated' with the assemblage descriptors; causality cannot be implied from our models. The identified anthropogenic stressors in this study can be considered as 'proxies' for various processes that can impact streams in eastern Ontario. Therefore, a reduction in the stressors selected by individual metric and/or MMI_{RRP} models may not be sufficient in addressing the mechanisms affecting stream health within the study region.

Variability associated with site assessments could be reduced by improving sampling methods and collecting more information. Estimates could increase in precision with a larger number of collected replicate samples (in pools and riffles) at each site; uncertainty in the estimates (due to sampling variability) would decrease with an increase in the number of replicates (Kerans et al. 1992, Li et al. 2001, Stribling et al. 2008, Ogren and Huckins 2014). Following seasonal and annual temporal trends in assemblage metrics would avoid erroneously modeling temporal variation as spatial variance; if unaccounted for, this natural temporal fluctuation could potentially lead to incorrect site condition conclusions (Reece et al. 2001, Ogren and Huckins 2014, Chen et al. 2014). Lastly, increasing subsample counts (>300 individuals; Doberstein et al. 2000, Cao and Hawkins 2005) and counting to finer taxonomic levels (e.g., species- or genus-level instead of family-level; Schmidt-Kloiber and Nijboer 2004, Jones 2008, Ogren and Huckins 2014) would insure more accurate/representative assemblage metric values and could lead to the development of metrics requiring detailed taxonomic information (e.g., richness, diversity, tolerance, trait and/or multivariate based metrics) – resulting in higher sensitivity to detecting the potentially subtle impacts within our study region. These proposed modifications could all contribute to increasing measurement precision and offer the potential to develop more precise models, insuring higher confidence in modeled predictions.

The confidence in individual site status assessments could be increased by further examination of residual distributions. Our current method assumes that the residuals to the

MMI_{RRP} regression model (Equation 3) represent a reasonable measure of the range in expected values across all levels of human activity in the catchments. Acknowledging potential shifts in the shape of the residual distribution in response to changes in types and extent of human activities would provide improved estimation of risk. Therefore, to improve confidence in probabilistic risk estimations, we recommend further examination of residual distributions under different disturbance levels.

Arbitrarily setting thresholds (alpha) to interpret whether site deviation scores indicate departure from reference condition is inevitable, often involving a trade-off between different risks (type I vs type II errors) and the costs associated with these risks (Oris and Bailer 2003, Reece et al. 2001, Mudge et al. 2012). Although lowering the alpha (e.g., $\alpha = 0.05$; Figure 7a) would generate a lower number of sites as being out of reference condition, there is more risk associated with misclassifying a site that is actually impaired (type II error). On the other hand, raising the alpha (e.g., $\alpha = 0.25$; Figure 7c) would make site assessments more sensitive to detecting site impairment (increased power), while increasing the rate of false positives (sites in reference condition declared out of reference condition). Higher alpha (type I error) may be desirable for early warnings for site impairment, highlighting streams that warrant further investigation. In the context of large uncertainties inherent in aquatic ecosystems and associated with our developed models, it may be appropriate to follow such a more 'precautionary approach' (Carpenter et al. 1999).

CONCLUSION

With the developed statistical catchment-scale equations to estimate site-specific reference conditions, it was possible to characterize current ecological status of sites within our study region. Further manipulation of these equations enables the exploration of alternative remedial or development scenarios. Although our models have their limitations, related to reducible and irreducible sources of uncertainties, we advocate for the use of our models in the decision-making process within a watershed management context. Essential to this process is the inclusion of explicit estimates of uncertainty in the site assessment procedure in order to communicate relative risks of impairment. Here, we incorporate estimations of risk that a site is 'out of reference condition' through the probabilities associated with model predictions. These tools allows decision-makers to make informed decisions based on scientific evidence. Furthermore, our models lend themselves to updates as additional data becomes available allowing for continued refinement of the model to reduce uncertainties around model predictions and subsequent site condition assessments. As knowledge improves and predictions become more certain, management actions can be altered and updated to improve the chances of obtaining the desired results

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APPENDIX I- Ontario Benthos Biomonitoring Network (OBBN) stream sampling protocol

Stream macroinvertebrate sample collection:

Site information was obtained from members of the SMARTER network, following the OBBN sampling protocol for wadeable streams (Jones et al. 2007). Each site consisted of three macroinvertebrate samples each – at two riffles and one pool habitat (i.e., fast/shallow vs. slow/deep habitats) – contained within one meander wavelength (typically 14-20 times the stream width) (Figure 9).

At each site, macroinvertebrate samples were collected using the Travelling-Kick-and-Sweep-Transect method (Figure 9). Macroinvertebrate samples were collected using a 500-µm mesh net positioned directly above the streambed, facing downstream from the sample collector (which insured the stream current carried any dislodged macroinvertebrate into the net). Beginning at either the right/left side stream bank, the sample collector kicked up the substrate and swept the net back and forth along a transect through the water column until the next stream bank was reached. Standardized sampling efforts were obtained by kicking and sweeping 10-m in 3 minutes, adjusting the amount of time spent kicking/sweeping according to the stream width. The collected benthos samples (net contents) were sieved and transferred to an appropriate container, rinsing into the net any large debris before removing them (as to collect any attached benthos). These steps were repeated along the 3 sample transects (1 transect per sample), always moving against the current in order to avoid disturbing unsampled transects. Benthos sample were either kept cool and stored live (to be processed within 48-hrs) or preserved in alcohol.

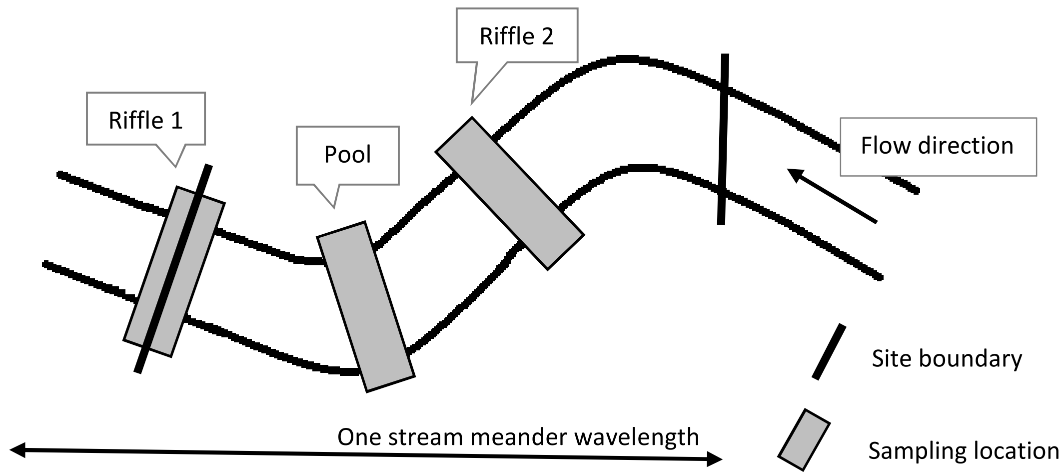

Figure 9: Conceptual diagram of a theoretical site location sampled using the Travelling-Kick-and-Sweep-Transect Method for wadeable streams. Above diagram is modified from Jones et al. (2007).

Macroinvertebrate sample processing:

Once in a lab setting, sub-samples were typically obtained following the Marchant Box method. This method involved evenly spreading a given sample (riffle 1, pool, or riffle 2) in a box equipped with a water-tight lid and divided into 100 equally sized cells (the cells have dividing walls). The even spread was accomplished by emptying a given sample in the box, adding water (to just below the height of the dividing cell walls), closing the box lid, inverting the box and lightly mixing the sample before setting it upright. Cells were randomly selected and each cell's content were transferred to a petri dish for microscope taxonomic identification (usually to family level) of picked macroinvertebrates individuals. Cells were sequentially removed and picked until at least 100-individuals were found for each sample (thus consisting of 100-individual fixed count sub-sample).

APPENDIX II- Benthic macroinvertebrate Operational Taxonomic Units

Table 8: Operational Taxonomic Units (OTUs) selected for macroinvertebrate metric calculation, along with the taxonomic level of the designated OTUs. Tolerance values are those used to calculate the Hilsenhoff Biotic Index (HBI; Bode et al., 2002; Hilsenhoff, 1987) – dashes indicate information gaps for those taxa.

OTUs	Taxonomic level	Tolerance values	Broader taxonomic level
Aeshnidae	Family	3	Anisoptera
Ameletidae	Family	0	Ephemeroptera
Ancylidae	Family	6	Gastropoda
Arrenuridae	Family	-	Acari
Asellidae	Family	8	Isopoda
Athericidae	Family	4	Misc. Dipterans
Baetidae	Family	6	Ephemeroptera
Bdellidae	Family	-	Acari
Belostomatidae	Family	-	Hemiptera
Beraeidae	Family	-	Trichoptera
Bithyniidae	Family	8	Gastropoda
Brachycentridae	Family	2	Trichoptera
Caenidae	Family	6	Ephemeroptera
Calopterygidae	Family	6	Zygoptera
Cambaridae	Family	6	Decapoda
Capniidae	Family	3	Plecoptera
Carabidae	Family	4	Coleoptera
Ceratopogonidae	Family	6	Ceratopogonidae
Chaoboridae	Family	8	Misc. Dipterans
Chironomidae	Family	8	Chironomidae
Chloroperlidae	Family	1	Plecoptera
Chrysomelidae	Family	4	Coleoptera
Coenagrionidae	Family	8	Zygoptera
Corbiculidae	Family	6	Bivalvia
Cordulegastridae	Family	3	Anisoptera
Corduliidae	Family	5	Anisoptera
Corixidae	Family	5	Hemiptera
Corophiidae	Family	-	Amphipoda
Corydalidae	Family	0	Megaloptera
Crambidae	Family	-	Lepidoptera
Crangonidae	Family	-	Decapoda
Crangonyctidae	Family	6	Amphipoda
Culicidae	Family	8	Culicidae
Curculionidae	Family	5	Coleoptera
Dipseudopsidae	Family	5	Trichoptera
Dixidae	Family	1	Misc. Dipterans
Dolichopodidae	Family	4	Misc. Dipterans
Dreissenidae	Family	8	Bivalvia

Dryopidae	Family	5	Coleoptera
Dytiscidae	Family	5	Coleoptera
Elmidae	Family	5	Coleoptera
Empididae	Family	6	Misc. Dipterans
Ephemerellidae	Family	2	Ephemeroptera
Ephemeridae	Family	4	Ephemeroptera
Ephydridae	Family	6	Misc. Dipterans
Gammaridae	Family	4	Amphipoda
Gerridae	Family	-	Hemiptera
Glossosomatidae	Family	1	Trichoptera
Gomphidae	Family	4	Anisoptera
Gyrinidae	Family	4	Coleoptera
Halacaridae	Family	-	Acari
Haliplidae	Family	5	Coleoptera
Helicopsychidae	Family	3	Trichoptera
Heptageniidae	Family	3	Ephemeroptera
Hyalellidae	Family	8	Amphipoda
Hyalidae	Family	-	Amphipoda
Hydrachnidae	Family	-	Acari
Hydridae	Family	5	Acari
Hydrobiidae	Family	8	Gastropoda
Hydrochidae	Family	-	Coleoptera
Hydrodromidae	Family	-	Acari
Hydrophilidae	Family	5	Coleoptera
Hydropsychidae	Family	5	Trichoptera
Hydroptilidae	Family	6	Trichoptera
Hydrozeitidae	Family	-	Acari
Hydryphantidae	Family	-	Acari
Hygrobatidae	Family	-	Acari
Isonychiidae	Family	2	Ephemeroptera
Isotomidae	Family	5	Springtails
Lebertiidae	Family	-	Acari
Lepidostomatidae	Family	1	Trichoptera
Leptoceridae	Family	4	Trichoptera
Leptohyphidae	Family	4	Ephemeroptera
Leptophlebiidae	Family	4	Ephemeroptera
Lestidae	Family	6	Zygoptera
Leuctridae	Family	0	Plecoptera
Libellulidae	Family	2	Anisoptera
Limnephilidae	Family	4	Trichoptera
Limnesiidae	Family	-	Acari
Limnocharidae	Family	-	Acari
Lymnaeidae	Family	6	Gastropoda
Malaconthricidae	Family	-	Acari
Mideopsidae	Family	-	Acari

Molannidae	Family	6	Trichoptera
Muscidae	Family	6	Misc. Dipterans
Nemouridae	Family	2	Plecoptera
Nepidae	Family	-	Hemiptera
Noctuidae	Family	-	Lepidoptera
Notonectidae	Family	-	Hemiptera
Odontoceridae	Family	0	Trichoptera
Oligoneuriidae	Family	-	Ephemeroptera
Oxidae	Family	-	Acari
Perlidae	Family	3	Plecoptera
Perlodidae	Family	2	Plecoptera
Philopotamidae	Family	4	Trichoptera
Phryganeidae	Family	4	Trichoptera
Physidae	Family	8	Gastropoda
Pionidae	Family	-	Acari
Plagiostomidae	Family	6	Turbellaria
Planariidae	Family	6	Turbellaria
Planorbidae	Family	6	Gastropoda
Pleidae	Family	-	Hemiptera
Pleuroceridae	Family	6	Gastropoda
Poduridae	Family	-	Springtails
Polycentropodidae	Family	6	Trichoptera
Pomatiopsidae	Family	8	Gastropoda
Potamanthidae	Family	4	Ephemeroptera
Psephenidae	Family	4	Coleoptera
Psychodidae	Family	10	Misc. Dipterans
Psychomyiidae	Family	2	Trichoptera
Ptychopteridae	Family	9	Misc. Dipterans
Pyralidae	Family	5	Lepidoptera
Rhyacophilidae	Family	0	Trichoptera
Sciomyzidae	Family	-	Misc. Dipterans
Scirtidae	Family	5	Coleoptera
Sialidae	Family	4	Megaloptera
Simuliidae	Family	6	Simuliidae
Siphlonuridae	Family	4	Ephemeroptera
Sisyridae	Family	5	Spongeflies
Sminthuridae	Family	-	Springtails
Sperchonidae	Family	-	Acari
Sphaeriidae	Family	6	Bivalvia
Stratiomyidae	Family	7	Misc. Dipterans
Syrphidae	Family	-	Misc. Dipterans
Tabanidae	Family	5	Tabanidae
Taeniopterygidae	Family	2	Plecoptera
Tipulidae	Family	4	Tipulidae
Torrenticolidae	Family	-	Acari

Trypochthonidae	Family	-	Acari
Uenoidae	Family	3	Trichoptera
Unionicolidae	Family	-	Acari
Unionidae	Family	6	Bivalvia
Unknown_Acari	Subclass	-	Acari
Unknown_Amphipoda	Order	7	Amphipoda
Unknown_Anisoptera	Suborder	5	Anisoptera
Unknown_Bivalvia	Class	6	Bivalvia
Unknown_Clitellata	Class	8	Clitellata
Unknown_Coleoptera	Order	5	Coleoptera
Unknown_Decapoda	Order	6	Decapoda
Unknown_Dipterans	Order	7	Misc. Dipterans
Unknown_Ephemeroptera	Order	3	Ephemeroptera
Unknown_Gastropoda	Class	7	Gastropoda
Unknown_Hemiptera	Order	5	Hemiptera
Unknown_Hirudinea	Subclass	8	Clitellata
Unknown_Horsehair_worms	Phylum	-	Horsehair worms
Unknown_Isopoda	Order	8	Isopoda
Unknown_Lepidoptera	Order	5	Lepidoptera
Unknown_Megaloptera	Order	2	Megaloptera
Unknown_Nematoda	Phylum	5	Nematoda
Unknown_Oligochaeta	Subclass	8	Clitellata
Unknown_Plecoptera	Order	1	Plecoptera
Unknown_Ribbon_worms	Phylum	6	Ribbon worms
Unknown_Springtails	Subclass	5	Springtails
Unknown_Trichoptera	Order	3	Trichoptera
Unknown_Turbellaria	Class	6	Turbellaria
Unknown_Zygoptera	Suborder	7	Zygoptera
Valvatidae	Family	8	Gastropoda
Veliidae	Family	-	Hemiptera
Veneridae	Family	-	Bivalvia
Viviparidae	Family	6	Gastropoda

APPENDIX III- Land cover categories and final broad groupings

Table 9: Land cover categories from SOLRIS and AAFC layers to be merged along with associated priority (top layer vs bottom layer) and final broad groupings used in analyses. Dashes in the grouping column indicate that the given land use category was completely covered (and thus eliminated) by categories from the prioritized layer (top).

Land use category	Source layer	Category priority	Broad grouping
Alvar	SOLRIS	Top	Natural
Open sand barren and dune	SOLRIS	Top	Natural
Treed sand barren dune	SOLRIS	Top	-
Water*	AAFC, SOLRIS	SOLRIS	Humid
Tallgrass woodland	SOLRIS	Top	Natural
Tallgrass savannah**	SOLRIS	-	-
Forest	SOLRIS	Top	Forest
Exposed land	AAFC	Bottom	Developed
Developed	AAFC	Bottom	Urban
Plantation-tree cultivated	SOLRIS	Top	Forest
Hedge rows	SOLRIS	Top	Natural
Transportation	SOLRIS	Top	Urban
Extraction	SOLRIS	Top	Developed
Built-up area pervious	SOLRIS	Top	Urban
Built-up area impervious	SOLRIS	Top	Urban
Shrubland	AAFC	Bottom	Natural
Forested swamp	SOLRIS	Top	Humid
Fen	SOLRIS	Top	Humid
Bog	SOLRIS	Top	Humid
Marsh	SOLRIS	Top	Humid
Wetland	AAFC	Bottom	Humid
Grassland	AAFC	Top	Natural
hay/pasture	AAFC	Top	Agriculture
Pasture and abandoned fields	SOLRIS	Bottom	-
Mine tailings, quarries, and bedrock outcrop	SOLRIS	Top	Developed
Too wet to be seeded	AAFC	Bottom	Humid
Fallow	AAFC	Top	Agriculture
Cereals	AAFC	Top	Intensive Agriculture
Corn	AAFC	Top	Intensive Agriculture
Canola/rapeseed	AAFC	Top	Intensive Agriculture
Soybeans	AAFC	Top	Intensive Agriculture
Peas	AAFC	Top	Agriculture
Beans	AAFC	Top	Agriculture
Vegetables	AAFC	Top	Agriculture
Fruits	AAFC	Top	Agriculture

Herbs	AAFC	Top	Agriculture
Nursery	AAFC	Top	Agriculture
Buckwheat	AAFC	Top	Agriculture
Other crops	AAFC	Top	Agriculture
Cropland	SOLRIS	Bottom	-
Coniferous trees*	AAFC, SOLRIS	SOLRIS	Forest
Deciduous trees*	AAFC, SOLRIS	SOLRIS	Forest
Mixed trees*	AAFC, SOLRIS	SOLRIS	Forest
Open tallgrass prairies	SOLRIS	Top	Natural
Swamp	SOLRIS	Top	Humid
Undifferentiated	SOLRIS	Bottom	-

*Category contained in both layers. Category from designated layer in the 'category priority' column was selected to be the top layer.

**Category not contained within our study region, but one of the SOLRIS layer land use categories

APPENDIX IV- Full model information for riffle and pool habitats

Table 10: Exploratory full model R^2 values for riffle habitats. Models include either Physiographic (P), Non-Anthropogenic (NA), or Anthropogenic activity variables (A) of table 2. Partial R^2 for P, NA, and A variables, along with R^2 of the full models (includes all variable groups) are also presented. Data were separated in calibration (N= 190) and (validation) (N=46). Metrics retained for further analyses are indicated by an asterisk (*).

Metric	R^2 P only	R^2 partial P	R^2 NA only	R^2 partial NA	R^2 A only	R^2 partial A	R^2 full model (P+NA+A)
% EPT*	0.10 (0.10)	0.04 (0.03)	0.13 (0.19)	0.09 (0.11)	0.04 (0.10)	0.02 (-0.03)	0.20 (0.19)
EPT richness*	0.20 (0.26)	0.10 (0.06)	0.19 (0.28)	0.10 (0.08)	0.12 (0.18)	0.03 (0.02)	0.35 (0.38)
HBI*	0.07 (0.04)	0.04 (-0.02)	0.06 (0.07)	0.04 (0.01)	0.01 (0.09)	0.01 (0.05)	0.11 (0.06)
% chironomidae	0.20 (-0.09)	0.05 (-0.03)	0.18 (-0.19)	0.04 (-0.20)	0.13 (-0.40)	0.05 (-0.39)	0.29 (-0.59)
% diptera	0.18 (-0.08)	0.04 (0.04)	0.18 (-0.20)	0.04 (-0.12)	0.10 (-0.33)	0.03 (-0.30)	0.26 (-0.45)
Log % amphipoda	0.13 (-0.009)	0.09 (0.086)	0.12 (-0.07)	0.08 (-0.024)	0.03 (0.009)	0.01 (0.086)	0.22 (-0.004)
Log % isopoda*	0.19 (0.19)	0.07 (0.22)	0.13 (-0.001)	0.03 (-0.08)	0.14 (0.11)	0.05 (0.07)	0.27 (0.20)
Log % worms	0.10 (0.12)	0.04 (-0.02)	0.15 (0.13)	0.07 (0)	0.11 (0.05)	0.03 (-0.11)	0.21 (0.03)
Log % mollusc	0.08 (-0.07)	0.03 (-0.09)	0.06 (-0.03)	0.02 (-0.14)	0.12 (-0.14)	0.07 (-0.17)	0.17 (-0.29)
Log % CIGH*	0.22 (0.30)	0.06 (0.18)	0.16 (0.11)	0.04 (-0.07)	0.15 (0.12)	0.02 (0)	0.29 (0.26)
% insect	0.24 (0.23)	0.05 (0.23)	0.18 (-0.01)	0.04 (-0.18)	0.21 (0.02)	0.05 (-0.06)	0.33 (0.06)
% non-Insect	0.23 (0.19)	0.05 (0.22)	0.18 (-0.009)	0.06 (-0.16)	0.20 (0.02)	0.05 (-0.04)	0.33 (0.06)
Taxonomic richness*	0.11 (0.10)	0.03 (0.04)	0.15 (0.25)	0.08 (0.15)	0.07 (0.17)	0.05 (0.05)	0.25 (0.38)

Shannon-wiener's*	0.11 (-0.05)	0.02 (-0.02)	0.13 (0.07)	0.04 (0.07)	0.03 (0.02)	0.02 (0)	0.18 (0.10)
Simpson's	0.13 (-0.07)	0.02 (-0.02)	0.13 (-0.04)	0.02 (0.02)	0.14 (-0.08)	0.01 (-0.07)	0.15 (-0.06)
Pielou's evenness	0.12 (-0.12)	0.03 (-0.02)	0.11 (-0.16)	0.02 (-0.07)	0.03 (-0.13)	0 (-0.08)	0.15 (-0.24)
PCA axis 1 scores*	0.09 (0.15)	0.04 (0.02)	0.09 (0.19)	0.05 (0.05)	0.05 (0.14)	0.01 (0.01)	0.15 (0.22)
PCA axis 2 scores	0.27 (0.13)	0.06 (0.07)	0.22 (-0.18)	0.05 (-0.38)	0.21 (-0.02)	0.04 (-0.02)	0.37 (-0.26)
PCoA axis 1 scores*	0.09 (0.12)	0.03 (0.02)	0.11 (0.20)	0.06 (0.09)	0.04 (0.13)	0.01 (0)	0.16 (0.23)
PCoA axis 2 scores	0.33 (0.21)	0.06 (-0.42)	0.29 (-0.14)	0.05 (-0.42)	0.28 (-0.05)	0.04 (0.02)	0.45 (-0.25)
O/E*	0.11 (0.14)	0.03 (0.01)	0.13 (0.22)	0.06 (0.10)	0.08 (0.23)	0.05 (0.06)	0.23 (0.36)
MMI[1]*	0.09 (0.19)	0.06 (0.07)	0.04 (0.10)	0.01 (0.02)	0.09 (0.19)	0.03 (0.04)	0.15 (0.26)

[1] MMI combines the following metrics: % EPT, HBI, log % isopoda, and taxonomic richness.

Table 11: Residual mean square (RMS) values for exploratory full models (Physiographic (P) + Non-Anthropogenic (NA) + Anthropogenic activity variables (A) of Table 2) vs a null model (the variance of the metric) – for riffle habitats. Data are separated in calibration (N=190) and validation (N=46) sets. Metrics retained for further analyses are indicated by an asterisk (*).

Metric	Null model RMS		Full model RMS	
	Calibration	**Validation**	**Calibration**	**Validation**
% EPT*	425.0	571.9	358.0	465.9
EPT richness*	5.27	10.61	3.58	6.52
HBI*	0.85	0.71	0.79	0.66
% chironomidae	471.9	404.1	352.6	640.8
% diptera	547.1	463.3	422.4	670.3
Log % amphipoda	1.697	1.691	1.398	1.697
Log % isopoda*	1.693	1.437	1.305	1.152
Log % worms	1.305	1.611	1.082	1.570
Log % mollusc	1.212	1.269	1.054	1.637
Log % CIGH*	1.672	1.605	1.246	1.192

% insect	650.7	516.9	455.9	484.8
% non-insect	666.9	537.8	471.8	505.4
Taxonomic richness*	13.5	14.2	10.6	8.78
Shannon-wiener's*	0.1688	0.1569	0.1447	0.1406
Simpson's	0.0193	0.0147	0.0171	0.0156
Pielou's evenness	0.0148	0.0118	0.0132	0.0147
PCA axis 1 scores*	2.26	3.74	2.03	2.91
PCA axis 2 scores	2.26	1.78	1.50	2.25
PCoA axis 1 scores*	0.0253	0.0409	0.0224	0.0316
PCoA axis 2 scores	0.028	0.020	0.016	0.025
O/E*	0.0405	0.0423	0.0329	0.0270
MMI[1]*	337.6	473.5	300.3	349.1

[1] MMI includes: % EPT, HBI, log % isopoda, taxonomic richness

Table 12: Exploratory full model R^2 values for pool habitats. Models include either Physiographic (P), Non-Anthropogenic (NA), or Anthropogenic activity variables (A) of table 2. Partial R^2 for P, NA, and A variables, along with R^2 of the full models (includes all variable groups) are also presented. Data were separated in calibration (N= 190) and (validation) (N=46). Metrics retained for further analyses are indicated by an asterisk (*).

Metric	**R^2 P only**	**R^2 partial P**	**R^2 NA only**	**R^2 partial NA**	**R^2 A only**	**R^2 partial A**	**R^2 full model (P+NA+A)**
Log % EPT*	0.15	0.07	0.21	0.13	0.09	0.03	0.33
	(0.06)	(0.26)	(0.16)	(0.20)	(0.16)	(0.01)	(0.27)
EPT richness*	0.18	0.09	0.22	0.12	0.09	0.01	0.35
	(0.15)	(0.01)	(0.31)	(0.20)	(0.12)	(0)	(0.34)
HBI	0.09	0.06	0.09	0.06	0.017	0.01	0.17
	(-0.08)	(0.0012)	(0.09)	(0.1912)	(-0.13)	(-0.079)	(0.0012)
% chironomidae	0.20	0.03	0.24	0.07	0.15	0.04	0.32
	(-0.04)	(0.001)	(0.03)	(0.19)	(-0.12)	(-0.081)	(0.019)
% diptera	0.20	0.03	0.23	0.08	0.14	0.04	0.32
	(-0.04)	(0.02)	(0.03)	(0.20)	(-0.20)	(-0.13)	(-0.019)
Log % amphipoda*	0.13	0.08	0.14	0.10	0.022	0.01	0.23
	(0.12)	(0.15)	(0.017)	(-0.03)	(0.027)	(0.08)	(0.17)
Log % isopoda*	0.13	0.05	0.13	0.06	0.13	0.06	0.26
	(0.15)	(0.03)	(0.18)	(0.05)	(0.17)	(0.05)	(0.27)
% worms	0.16	0.06	0.13	0.05	0.16	0.07	0.29
	(0.11)	(0.02)	(0.03)	(-0.067)	(-0.11)	(-0.13)	(-0.06)

Log % mollusc	0.09 (-0.02)	0.06 (-0.09)	0.07 (-0.03)	0.04 (-0.07)	0.07 (-0.07)	0.05 (-0.086)	0.17 (-0.13)
% CIGH*	0.16 (0.22)	0.03 (0.05)	0.18 (0.19)	0.07 (0.05)	0.11 (0.14)	0.03 (0.04)	0.26 (0.28)
% insect*	0.20 (0.18)	0.06 (0.03)	0.15 (0.18)	0.04 (0.11)	0.17 (0.14)	0.05 (0.01)	0.30 (0.29)
% non-insect*	0.20 (0.22)	0.05 (0.03)	0.15 (0.22)	0.03 (0.10)	0.17 (0.20)	0.05 (0.02)	0.29 (0.34)
Taxonomic richness*	0.16 (-0.08)	0.06 (-0.07)	0.16 (0.16)	0.06 (0.17)	0.013 (0.03)	0.01 (0.01)	0.24 (0.11)
Shannon-wiener's	0.19 (-0.18)	0.05 (-0.05)	0.19 (0.05)	0.05 (0.20)	0.02 (-0.10)	0.01 (-0.026)	0.25 (-0.02)
Simpson's	0.22 (-0.23)	0.05 (-0.08)	0.23 (-0.007)	0.05 (0.20)	0.05 (-0.17)	0.01 (-0.07)	0.28 (-0.12)
Pielou's evenness	0.22 (-0.10)	0.04 (0)	0.22 (0)	0.04 (0.15)	0.06 (-0.13)	0.01 (-0.05)	0.27 (-0.03)
PCA axis 1 scores*	0.10 (0.18)	0.05 (0.03)	0.13 (0.29)	0.07 (0.13)	0.06 (0.15)	0 (0.01)	0.20 (0.32)
PCA axis 2 scores*	0.21 (0.19)	0.04 (0.13)	0.19 (0.05)	0.06 (0.03)	0.22 (0.07)	0.06 (-0.01)	0.35 (0.20)
PCoA axis 1 scores*	0.07 (0.10)	0.04 (0)	0.18 (0.30)	0.14 (0.22)	0.02 (0.09)	0.11 (-0.01)	0.23 (0.30)
PCoA axis 2 scores*	0.27 (0.23)	0.05 (0.09)	0.26 (0.08)	0.06 (0.04)	0.29 (0.15)	0.07 (-0.01)	0.43 (0.24)
O/E*	0.15 (-0.06)	0.05 (-0.07)	0.15 (0.19)	0.06 (0.18)	0.02 (0.04)	0.01 (0)	0.23 (0.13)
MMI[1]*	0.16 (0.19)	0.08 (0.11)	0.04 (0.02)	0.01 (-0.03)	0.11 (0.20)	0.05 (0.07)	0.24 (0.23)
MMI_{RRP}*	0.12 (0.22)	0.09 (0.12)	0.04 (0.09)	0.01 (0.01)	0.12 (0.17)	0.06 (0.03)	0.21 (0.28)

[1] MMI includes log % EPT, log % amphipoda, log % isopoda, and taxonomic richness

Table 13: Residual mean square (RMS) values for exploratory full models (Physiographic (P) + Non-Anthropogenic (NA) + Anthropogenic activity variables (A) of Table 2) vs a null model (the variance of the metric) – for pool habitats. Data are separated in calibration (N=190) and validation (N=46) sets. Metrics retained for further analyses are indicated by an asterisk (*).

Metric	Null model RMS		Full model RMS	
	Calibration	**Validation**	**Calibration**	**Validation**
Log % EPT*	1.607	2.152	1.178	1.575
EPT richness*	4.817	8.745	3.401	5.814
HBI	0.812	0.665	0.731	0.664
% chironomidae	609.8	612.3	452.5	600.8
% diptera	632.6	585.7	468.6	597.1
Log % amphipoda*	1.711	1.694	1.435	1.409
Log % isopoda*	1.642	1.381	1.327	1.001
% worms	103.26	288.76	79.94	305.79
Log % mollusc	1.184	1.136	1.072	1.280
% CIGH*	1.715	1.729	1.379	1.243
% insect*	632.7	748.4	483.8	534.1
% non-insect*	636.7	732.3	489.0	482.6
Taxonomic richness*	12.41	17.60	10.27	15.64
Shannon-wiener's	0.198	0.211	0.163	0.215
Simpson's	0.028	0.024	0.022	0.026
Pielou's evenness	0.021	0.020	0.016	0.021
PCA axis 1 scores*	1.453	2.470	1.260	1.674
PCA axis 2 scores*	2.134	1.825	1.499	1.466
PCoA axis 1 scores*	0.0187	0.0286	0.0156	0.0201
PCoA axis 2 scores*	0.0289	0.0279	0.0179	0.0213
O/E*	0.0423	0.0584	0.0353	0.0508
MMI[1]*	270.81	399.78	222.5	308.8
MMI_{RRP}*	234.2	390.2	206.7	280.6

[1] MMI includes log % EPT, log % amphipoda, log % isopoda, and taxonomic richness

APPENDIX V- Principal Component Analysis details

Table 14: Anthropogenic and non-anthropogenic variable loadings on the first two components axes. In bold are the selected variables for use in the simplified models. N= 1743

Anthropogenic	Component 1	Component 2	Non-anthropogenic	Component 1	Component 2
Road length			*Longitude (X)*	-0.336	-0.116
Log$_{10}$ Road density	-0.272	0.515	*Latitude (Y)*	-0.182	-0.161
% Urban	-0.266	0.533	***Log$_{10}$ Catchment Area***		-0.626
% Wetland	0.278		*% Sedimentary*	-0.357	-0.151
% Intensive Agriculture	-0.301	-0.421	***% Igneous***	0.348	0.128
% Forest	0.294		*% Metamorphic*	0.178	0.228
% Agriculture	-0.294	-0.488	*Georgian Bay ecoregion*	0.370	0.109
% Natural	0.417		*Simcoe Rideau ecoregion*	-0.370	-0.109
% Developed	-0.418		*Elevation Range*	0.290	-0.489
LDI rating	-0.407	0.142	*Elevation Mean*	0.351	0.114
			Elevation Std	0.292	-0.449
Cumulative percentage of variance explained	56%	77%	Cumulative percentage of variance explained	59%	72%

Table 15: Metric variable loadings on the first two components axes. In bold are the uncorrelated metrics selected for the MMI_{RRP}. N= 1743

Riffle metric scores	Component 1	Component 2	Pool metric scores	Component 1	Component 2
% EPT	-0.372	0.132	***Log % EPT***	-0.325	-0.174
EPT richness	-0.389	-0.121	*EPT richness*	-0.335	-0.224
HBI	0.300		***Log % amphipoda***	0.151	-0.236
Log % isopoda	0.234	-0.327	***Log % isopoda***	0.243	-0.361
Log % CIGH	0.248	-0.387	*% CIGH*	0.249	-0.288
Taxonomic richness	-0.262	-0.475	*% insect*	-0.324	0.274
Shannon-Wiener's	-0.274	-0.414	*% non-insect*	0.327	-0.279
PCA axis 1 scores	0.385	-0.239	***Taxonomic richness***	-0.195	-0.321
PCoA axis 1 scores	0.389	-0.190	*PCA axis 1 scores*	0.353	0.113
O/E	-0.247	-0.468	*PCA axis 2 scores*	-0.131	0.438
			PCoA axis 1 scores	0.337	0.217
			PCoA axis 2 scores	0.301	0.197
			O/E	-0.204	-0.322
Cumulative percentage of variance explained	48%	77%	Cumulative percentage of variance explained	44%	65%

APPENDIX VI- Cross-site variation in observed scaled metric scores

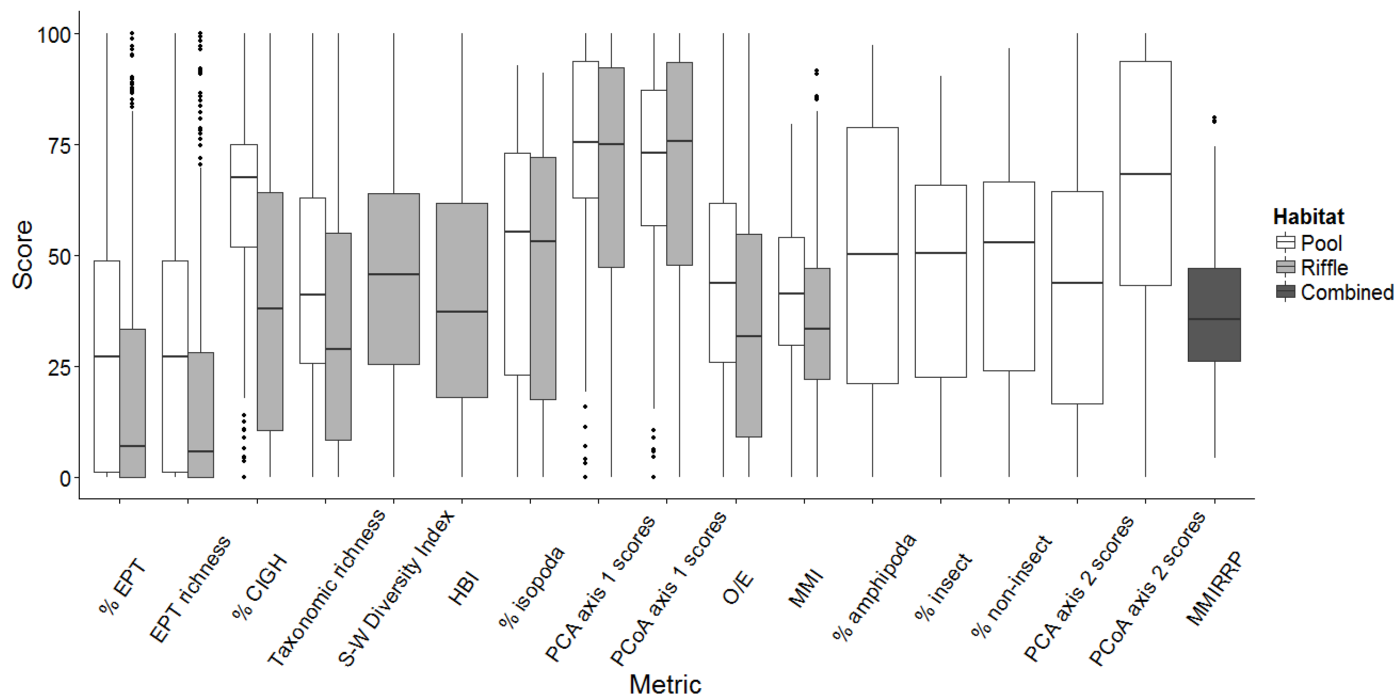

Figure 10: Box-and-whisker plots of the scaled scores of metrics selected for use in the simplified models – shown data includes information from last site visits only. The range of observed values for each scaled metric is separated by habitat (riffle vs. pool). Due to different metrics being selected for simplified models (based on habitat), there are a few cases for which there is only metric information for one of the habitat types (riffle vs. pool). The MMI_{RRP} combines both riffle and pool samples

APPENDIX VII- Stream site-specific MMI_{RRP} information

Table 16: MMI_{RRP} information for each stream site in the study region (N=215), sorted by Conservation Area (CA). Included are the observed and expected (modeled at 0-level of human activity) values as well as calculated scaled deviation scores and associated probabilities of being in reference condition (*p*-values).

Site code	Stream name	Longitude	Latitude	CA	MMI_{RRP} observed	MMI_{RRP} expected at 0 human activity	Score	*p*-value
235	Gull Creek	-76.866373	44.873861	MVCA	32.07	42.82	-18.81	0.07
56	Casey Creek	-76.043491	45.378311	MVCA	29.25	45.48	-21.63	0.06
153	Casey Creek	-76.048424	45.378425	MVCA	39.68	45.01	-11.20	0.24
193	Tributary of Carp River	-76.065116	45.298779	MVCA	51.86	43.73	0.98	0.55
176	Feedmill Creek	-75.935542	45.297391	MVCA	44.39	32.47	-6.49	0.36
171	Poole Creek	-75.91551	45.287208	MVCA	26.40	32.76	-24.48	0.04
97	Poole Creek	-75.914643	45.290028	MVCA	26.24	32.60	-24.64	0.04
189	Corkery Creek	-76.098797	45.324124	MVCA	66.73	39.96	15.86	0.87
93	Shirleys Brook	-75.923089	45.352325	MVCA	14.47	36.59	-36.41	0.00
120	Feedmill Creek	-75.937678	45.29486	MVCA	42.26	32.68	-8.62	0.32
176	Feedmill Creek	-75.935542	45.297391	MVCA	39.02	32.47	-11.85	0.21
101	Unnamed Tributary	-77.073779	44.906346	MVCA	30.70	44.77	-20.18	0.07
152	Casey Creek	-76.046489	45.406062	MVCA	47.42	39.80	-3.46	0.42
45	Casey Creek	-76.05941	45.379149	MVCA	42.92	45.87	-7.95	0.33
58	Casey Creek	-76.054245	45.410563	MVCA	44.01	42.87	-6.87	0.36
29	Shirleys Brook	-75.921811	45.340706	MVCA	33.94	45.23	-16.94	0.10
124	Unnamed Tributary	-76.667642	44.870146	MVCA	32.12	47.78	-18.76	0.07
104	Unnamed Tributary	-76.306902	45.210457	MVCA	44.89	43.21	-5.98	0.39
234	Gordon's Creek	-76.621025	44.908086	MVCA	44.70	46.20	-6.17	0.38
191	Casey Creek	-76.012432	45.396872	MVCA	30.05	44.04	-20.83	0.06
252	Tributary of Carp River	-76.063318	45.296062	MVCA	45.03	44.40	-5.85	0.39
97	Poole Creek	-75.914643	45.290028	MVCA	36.45	32.60	-14.43	0.15
113	Poole Creek	-75.925918	45.262103	MVCA	8.85	34.67	-42.02	0.00
209	Tributary of Mississippi River	-76.280144	45.37058	MVCA	25.79	36.24	-25.08	0.04
113	Poole Creek	-75.925918	45.262103	MVCA	24.79	34.67	-26.09	0.03

171	Poole Creek	-75.91551	45.287208	MVCA	26.51	32.76	-24.36	0.04
232	Waddle Creek	-76.524689	45.108237	MVCA	23.09	45.27	-27.79	0.02
149	Unknown	-77.213516	44.923065	MVCA	48.01	46.85	-2.87	0.46
189	Corkery Creek	-76.098797	45.324124	MVCA	50.48	39.96	-0.39	0.53
188	Trip of Carp	-76.064992	45.304823	MVCA	58.60	43.02	7.72	0.74
184	Casey Creek	-76.052506	45.401474	MVCA	46.99	42.78	-3.89	0.42
135	Feedmill Creek	-75.926699	45.303328	MVCA	18.54	31.46	-32.34	0.01
188	Trip of Carp	-76.064992	45.304823	MVCA	68.49	43.02	17.62	0.87
121	Watts Creek	-75.875277	45.348264	MVCA	14.76	26.02	-36.12	0.00
122	Watts Creek	-75.897697	45.340829	MVCA	28.57	27.39	-22.31	0.05
123	Shirleys Brook	-75.93435	45.358893	MVCA	21.14	35.51	-29.74	0.01
135	Feedmill Creek	-75.926699	45.303328	MVCA	25.80	31.46	-25.08	0.04
125	Casey Creek	-76.064126	45.402336	MVCA	50.15	41.80	-0.73	0.52
108	Unnamed Tributary	-76.237157	45.245175	MVCA	51.26	40.60	0.38	0.54
140	RVCARIDE	-75.693767	45.029348	RVCA	17.13	30.55	-33.74	0.00
49	RVCAKEMP	-75.720758	44.790102	RVCA	79.97	43.87	29.09	0.95
96	Sawmill Creek	-75.664595	45.365414	RVCA	12.83	27.58	-38.04	0.00
115	Unnamed	-75.632832	45.189684	RVCA	18.08	44.31	-32.80	0.00
238	Bilberry Creek	-75.534255	45.485982	RVCA	13.21	21.63	-37.67	0.00
190	Brassils Creek	-75.805651	44.996573	RVCA	51.63	44.39	0.76	0.55
35	Nichols Creek	-75.892351	45.087501	RVCA	43.66	45.03	-7.21	0.35
185	Sawmill Creek	-75.629404	45.339828	RVCA	18.45	33.93	-32.43	0.01
94	Stillwater Creek	-75.826433	45.34704	RVCA	30.86	32.45	-20.01	0.07
77	Hobbs Drain	-75.931994	45.17144	RVCA	80.07	37.68	29.20	0.95
75	Hunt Club Creek	-75.67432	45.338624	RVCA	14.57	23.98	-36.31	0.00
84	Sawmill Creek	-75.675951	45.389806	RVCA	35.08	26.17	-15.80	0.11
154	Stevens Creek	-75.790931	45.086466	RVCA	52.75	47.01	1.87	0.60
238	Bilberry Creek	-75.534255	45.485982	RVCA	11.46	21.63	-39.41	0.00
92	Voyageur Creek	-75.549061	45.466552	RVCA	6.59	22.52	-44.28	0.00
182	Unnamed	-75.635302	45.198897	RVCA	19.03	46.22	-31.85	0.01
174	Mosquito Creek	-75.670573	45.284696	RVCA	32.02	33.33	-18.86	0.07
90	Mosquito Creek	-75.645578	45.269265	RVCA	25.32	34.40	-25.55	0.03
159	Kings Creek	-75.964642	45.100299	RVCA	74.04	37.53	23.16	0.92
211	O'Keefe Drain	-75.775756	45.250558	RVCA	13.46	28.26	-37.41	0.00
73	O'Keefe Drain	-75.779128	45.256138	RVCA	20.68	28.65	-30.19	0.01
213	O'Keefe Drain	-75.781744	45.268443	RVCA	11.28	28.39	-39.59	0.00
69	Jock River	-76.022399	45.155234	RVCA	14.07	39.60	-36.81	0.00
79	Becketts	-75.352471	45.509334	RVCA	29.85	32.21	-21.02	0.06

	Creek							
83	Black Rapids Creek	-75.710573	45.317945	RVCA	14.56	24.93	-36.32	0.00
160	Jock River	-75.954348	45.156221	RVCA	41.01	39.79	-9.87	0.29
81	Greens Creek	-75.592964	45.418688	RVCA	37.69	29.76	-13.19	0.20
350	Stevens Creek	-75.67851	45.157796	RVCA	47.59	36.68	-3.29	0.43
350	Stevens Creek	-75.67851	45.157796	RVCA	27.25	36.68	-23.62	0.05
297	Dowdall Drain	-75.943756	45.136937	RVCA	40.33	31.12	-10.55	0.26
332	North Branch	-75.705074	44.874415	RVCA	62.26	43.15	11.38	0.80
309	Grants Creek	-76.324727	44.809819	RVCA	43.87	42.53	-7.01	0.35
351	Tay River	-76.313454	44.862244	RVCA	71.76	43.78	20.88	0.90
23	Otter Creek	-76.012583	44.852516	RVCA	19.04	39.01	-31.84	0.01
28	Jebbs Creek	-76.201256	44.88776	RVCA	12.42	40.29	-38.46	0.00
28	Jebbs Creek	-76.201256	44.88776	RVCA	19.94	40.29	-30.94	0.01
32	Rideau Creek	-75.854076	44.950775	RVCA	58.68	44.34	7.81	0.74
32	Rideau Creek	-75.854076	44.950775	RVCA	32.65	44.34	-18.22	0.08
33	Kemptville Creek	-75.738586	44.760193	RVCA	64.78	39.64	13.90	0.84
34	Barbers Creek	-75.860517	44.813859	RVCA	42.28	42.98	-8.60	0.32
36	Black Creek	-76.059458	44.90341	RVCA	27.57	40.33	-23.31	0.05
37	Brassils Creek	-75.801196	44.985071	RVCA	54.92	44.20	4.04	0.64
38	Jock River	-75.832172	45.18218	RVCA	37.54	40.97	-13.34	0.19
41	Cranberry Creek	-75.670412	45.093979	RVCA	33.61	42.70	-17.27	0.09
42	Mud Creek	-75.70111	45.224364	RVCA	24.75	30.77	-26.12	0.03
44	Hutton Creek	-75.99324	44.846711	RVCA	47.55	40.72	-3.33	0.43
60	Barnes Creek	-75.62036	45.002757	RVCA	42.35	39.08	-8.53	0.32
60	Barnes Creek	-75.62036	45.002757	RVCA	42.98	39.08	-7.90	0.33
65	Fish Creek	-76.716879	44.653152	RVCA	66.69	44.12	15.81	0.86
65	Fish Creek	-76.716879	44.653152	RVCA	65.37	44.12	14.50	0.85
65	Fish Creek	-76.716879	44.653152	RVCA	64.11	44.12	13.23	0.83
102	Jock River	-75.932085	45.135236	RVCA	41.77	39.39	-9.10	0.30
155	RVCARIDE	-75.846522	44.954065	RVCA	25.09	43.16	-25.79	0.03
118	Barnes Creek	-75.632887	45.013313	RVCA	42.44	37.87	-8.44	0.32
118	Barnes Creek	-75.632887	45.013313	RVCA	45.01	37.87	-5.87	0.39
118	Barnes Creek	-75.632887	45.013313	RVCA	26.29	37.87	-24.58	0.04
134	Nichols Creek	-75.892351	45.087501	RVCA	53.24	45.03	2.36	0.60
148	Mud Creek	-75.749234	44.753266	RVCA	37.83	39.91	-13.04	0.20
150	Barnes Creek	-75.63563	45.017467	RVCA	22.00	37.79	-28.88	0.01
150	Barnes Creek	-75.63563	45.017467	RVCA	22.75	37.79	-28.13	0.01
150	Barnes Creek	-75.63563	45.017467	RVCA	33.95	37.79	-16.93	0.10
161	Barnes Creek	-75.625721	45.006941	RVCA	28.13	38.66	-22.75	0.05
161	Barnes Creek	-75.625721	45.006941	RVCA	46.37	38.66	-4.51	0.41
161	Barnes Creek	-75.625721	45.006941	RVCA	50.98	38.66	0.10	0.53
194	Black Rapids	-75.714378	45.31627	RVCA	21.67	24.47	-29.20	0.01

	Creek							
196	Tay River	-76.519796	44.759831	RVCA	59.89	44.06	9.01	0.77
196	Tay River	-76.519796	44.759831	RVCA	63.41	44.06	12.54	0.83
196	Tay River	-76.519796	44.759831	RVCA	54.30	44.06	3.42	0.62
197	Blueberry Creek	-76.278084	44.901279	RVCA	34.79	39.63	-16.09	0.11
200	Rudsdale Creek	-76.318745	44.876496	RVCA	18.40	40.93	-32.48	0.01
200	Rudsdale Creek	-76.318745	44.876496	RVCA	10.13	40.93	-40.74	0.00
200	Rudsdale Creek	-76.318745	44.876496	RVCA	8.18	40.93	-42.70	0.00
203	Kings Creek	-75.932467	45.119044	RVCA	32.06	42.03	-18.82	0.07
204	Sawmill Creek	-75.675951	45.389806	RVCA	48.69	26.17	-2.18	0.48
206	Hobbs Drain	-75.928535	45.171558	RVCA	71.75	37.84	20.87	0.90
207	Flowing Creek	-75.838501	45.210481	RVCA	45.51	35.08	-5.37	0.40
215	Rosedate Creek	-75.961197	44.925586	RVCA	46.53	43.50	-4.34	0.41
35	Nichols Creek	-75.892351	45.087501	RVCA	31.77	45.03	-19.11	0.07
217	Mosquito Creek	-75.676483	45.287076	RVCA	33.75	32.64	-17.12	0.09
220	Irish Creek	-75.953536	44.787192	RVCA	33.26	39.77	-17.62	0.09
220	Irish Creek	-75.953536	44.787192	RVCA	31.57	39.77	-19.31	0.07
221	Jock River	-76.058248	45.016426	RVCA	25.84	41.17	-25.04	0.04
40	Unnamed Creek	-75.663941	45.218942	RVCA	14.54	38.33	-36.34	0.00
222	Jock River	-75.71032	45.259948	RVCA	68.05	37.24	17.17	0.87
43	Unnamed	-75.639236	45.17145	RVCA	17.83	36.32	-33.04	0.00
223	Dales Creek	-75.796944	44.963238	RVCA	48.72	42.87	-2.15	0.48
231	Tay River	-76.122038	44.891722	RVCA	70.23	35.79	19.35	0.88
231	Tay River	-76.122038	44.891722	RVCA	31.71	35.79	-19.16	0.07
231	Tay River	-76.122038	44.891722	RVCA	74.39	35.79	23.51	0.92
244	Grants Creek	-76.291949	44.870117	RVCA	39.35	40.90	-11.53	0.23
244	Grants Creek	-76.291949	44.870117	RVCA	42.17	40.90	-8.71	0.32
244	Grants Creek	-76.291949	44.870117	RVCA	39.98	40.90	-10.90	0.24
245	Kemptville Creek	-75.655354	45.007721	RVCA	35.43	40.88	-15.45	0.13
173	Nepean Creek	-75.705006	45.350559	RVCA	29.44	23.99	-21.44	0.06
247	Kemptville Creek	-75.679043	44.965091	RVCA	46.95	41.18	-3.92	0.42
98	Barrhaven Creek	-75.706008	45.290682	RVCA	19.87	22.84	-31.01	0.01
174	Mosquito Creek	-75.670573	45.284696	RVCA	30.43	33.33	-20.45	0.06
71	Mud Creek	-75.701188	45.229738	RVCA	31.36	30.76	-19.51	0.07
210	Mud Creek	-75.701188	45.229738	RVCA	17.12	30.76	-33.76	0.00

216	Cardinal Creek	-75.476422	45.499172	RVCA	48.87	28.06	-2.01	0.48
82	Mud Creek	-75.526379	45.433074	RVCA	11.35	28.49	-39.53	0.00
107	Hobbs Drain	-75.929321	45.171552	RVCA	66.57	37.82	15.70	0.86
117	Doyle Creek	-75.598394	45.150415	RVCA	5.37	20.66	-45.51	0.00
GRVCA44|SI39|	RVCAKEMP	-75.721542	44.876597	RVCA	80.95	48.91	30.07	0.95
67	North Indian Creek	-75.236714	45.47236	SNCA	28.13	44.98	-22.74	0.05
170	Middle Castor River	-75.493443	45.221855	SNCA	38.10	36.51	-12.78	0.21
78	Middle Castor River	-75.593286	45.205279	SNCA	31.19	38.87	-19.69	0.07
183	South Castor River	-75.449788	45.160466	SNCA	13.49	28.28	-37.39	0.00
169	Bear Brook Creek	-75.530391	45.352404	SNCA	32.00	34.32	-18.88	0.07
224	Shields Creek	-75.559677	45.259555	SNCA	46.76	28.20	-4.12	0.41
76	South Indian Creek	-75.250304	45.365867	SNCA	23.72	36.80	-27.16	0.02
91	McKinnons Creek	-75.43524	45.419441	SNCA	51.58	30.26	0.71	0.55
80	South Castor River	-75.399002	45.226354	SNCA	38.54	31.17	-12.34	0.21
119	Bear Brook	-75.491062	45.428001	SNCA	30.72	33.83	-20.16	0.07
167	Findlay Creek	-75.584359	45.31681	SNCA	36.22	34.19	-14.66	0.15
89	Buckles Municipal Drain	-75.564091	45.138296	SNCA	39.30	31.97	-11.58	0.22
187	North Castor River	-75.466639	45.17174	SNCA	4.15	38.11	-46.73	0.00
72	North Castor River	-75.502136	45.294997	SNCA	27.83	32.75	-23.05	0.05
127	Cheney Drain	-75.387537	45.187557	SNCA	5.53	30.17	-45.35	0.00
GSNC667|SI413|	Wade Road 01	-75.329243	45.236767	SNCA	37.88	30.62	-12.99	0.20
GSNC669|SI414|	Bearbrook	-75.095622	45.407277	SNCA	31.16	40.36	-19.72	0.07
GSNC671|SI416|	NULL	-75.23641	45.470494	SNCA	36.73	44.57	-14.15	0.16
GSNC675|SI420|	NULL	-75.218957	45.349743	SNCA	33.62	44.77	-17.26	0.09
GSNC676|SI421|	Louis Lafleur M.D.	-75.236714	45.47236	SNCA	34.51	44.98	-16.36	0.10
GSNC679|SI424|	Wolf Creek	-75.165707	45.339131	SNCA	33.61	46.47	-17.27	0.09
GSNC680|SI425|	Saddlemire MD	-75.244247	44.877516	SNCA	35.28	35.26	-15.60	0.12
GSNC682|SI427|	Bear Brook	-75.501424	45.350806	SNCA	27.24	34.22	-23.64	0.05
GSNC687|SI432|	Bear Brook	-75.254456	45.408524	SNCA	46.98	32.96	-3.90	0.42
GSNC690|SI435|	Bear Brook	-75.334556	45.387026	SNCA	39.24	34.69	-11.64	0.22
GSNC691|SI436|	Bear Brook	-75.323855	45.396643	SNCA	30.17	33.47	-20.71	0.06

GSNC692|SI437|	Bear Brook	-75.332202	45.417465	SNCA	46.85	28.54	-4.03	0.42
GSNC694|SI438|	Bear Brook	-75.341309	45.413857	SNCA	14.13	32.50	-36.75	0.00
GSNC695|SI439|	Bear Brook	-75.500306	45.345626	SNCA	33.67	36.70	-17.21	0.09
GSNC696|SI440|	Upper Bearbrook	-75.488437	45.37549	SNCA	43.35	35.89	-7.53	0.35
GSNC697|SI441|	North Indian Creek Tributary	-75.272275	45.472456	SNCA	48.58	36.97	-2.30	0.48
GSNC698|SI442|	North Indian Creek Tributary	-75.28576	45.458311	SNCA	35.81	28.00	-15.07	0.13
GSNC699|SI443|	Wolf Creek	-75.165707	45.339131	SNCA	45.83	46.47	-5.04	0.40
GSNC700|SI444|	Mer Bleue	-75.422916	45.379906	SNCA	38.20	35.04	-12.68	0.21
GSNC701|SI445|	Mer Bleue	-75.43614	45.420856	SNCA	50.24	30.29	-0.64	0.53
GSNC702|SI446|	North Indian Creek	-75.190894	45.433425	SNCA	49.70	36.57	-1.18	0.51
GSNC705|SI449|	South Nation River	-75.501757	44.950853	SNCA	41.22	34.44	-9.65	0.29
GSNC706|SI450|	South Nation River	-75.552695	44.958825	SNCA	55.14	41.30	4.26	0.64
GSNC707|SI452|	South Nation River	-75.501757	44.950853	SNCA	52.25	34.44	1.37	0.59
GSNC713|SI457|	South Nation River	-75.585342	44.82684	SNCA	30.46	41.24	-20.41	0.06
GSNC714|SI458|	NULL	-75.395836	45.034546	SNCA	41.78	37.03	-9.09	0.30
GSNC717|SI461|	South Nation River	-75.684792	44.681553	SNCA	54.45	40.19	3.57	0.63
GSNC718|SI462|	Indian Creek	-75.603597	44.806433	SNCA	45.92	41.49	-4.96	0.40
GSNC723|SI467|	Glen Becker	-75.259108	44.895017	SNCA	27.02	31.94	-23.85	0.04
GSNC724|SI468|	Doron	-75.303221	44.866236	SNCA	29.63	35.77	-21.24	0.06
GSNC725|SI469|	Hoasic Creek	-75.167067	44.93079	SNCA	30.39	38.31	-20.48	0.06
GSNC726|SI470|	Hoasic Creek	-75.165572	44.953394	SNCA	43.83	40.30	-7.04	0.35
GSNC727|SI471|	Ault Drain	-75.317544	44.890868	SNCA	35.20	32.07	-15.68	0.12
GSNC728|SI472|	Ault Drain	-75.336965	44.924194	SNCA	37.13	31.16	-13.75	0.18
GSNC729|SI473|	Sandy Creek	-75.446448	44.958212	SNCA	38.15	39.01	-12.73	0.21
GSNC730|SI474|	Sandy Creek	-75.431585	44.915507	SNCA	32.76	31.87	-18.12	0.08
GSNC731|SI475|	South Branch	-75.434436	44.845362	SNCA	25.57	38.99	-25.31	0.04
GSNC732|SI476|	Ault Drain	-75.297189	44.903902	SNCA	16.35	30.48	-34.53	0.00
GSNC733|SI477|	Ault Drain	-75.395391	44.984055	SNCA	16.07	29.87	-34.81	0.00
GSNC735|SI479|	Wylie Creek	-75.51788	45.063147	SNCA	58.84	34.64	7.96	0.75
GSNC736|SI480|	Head Water	-75.577865	44.814672	SNCA	38.09	40.62	-12.79	0.21
GSNC737|SI481|	Black Creek	-74.993181	45.474585	SNCA	41.46	36.11	-9.41	0.30
GSNC738|SI482|	Fraser Drain	-74.978453	45.364065	SNCA	74.17	30.59	23.29	0.92
GSNC743|SI488|	Bear Brook	-75.27038	45.304203	SNCA	30.19	29.99	-20.68	0.06
GSNC744|SI489|	South Branch of South	-75.463574	44.851942	SNCA	33.95	42.73	-16.93	0.10

	Nation River							
GSNC748|SI492|	South Branch of South Nation River	-75.36255	44.982464	SNCA	48.13	35.65	-2.75	0.46
GSNC749|SI493|	South Branch of South Nation River	-75.39354	44.93602	SNCA	25.88	37.51	-25.00	0.04
GSNC750|SI494|	South Branch of South Nation	-75.4026	44.909398	SNCA	37.62	39.46	-13.26	0.20
GSNC752|SI496|	Moose Creek	-75.028285	45.376501	SNCA	30.49	41.21	-20.38	0.06
GSNC756|SI499|	South Nation River	-75.474449	44.942902	SNCA	57.25	42.45	6.38	0.69
GSNC757|SI500|	Hoasic Creek	-75.167938	44.965593	SNCA	21.65	40.45	-29.23	0.01
GSNC758|SI501|	Hoasic Creek	-75.169278	44.908353	SNCA	50.28	38.09	-0.60	0.53
GSNC759|SI502|	Shield's Creek	-75.560572	45.259354	SNCA	65.56	28.20	14.68	0.85
GSNC761|SI503|	Shield's Creek	-75.559202	45.261113	SNCA	54.94	28.06	4.06	0.64
GSNC762|SI504|	Findlay Creek	-75.552129	45.342687	SNCA	26.15	34.03	-24.72	0.04
GSNC763|SI505|	Hoasic Creek	-75.165728	44.951239	SNCA	37.42	39.74	-13.45	0.18
GSNC764|SI506|	Lough Municipal Drain	-75.386967	45.180838	SNCA	22.91	30.47	-27.96	0.01