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Interannual Variation of Benthic Macroinvertebrate Communities at Long-Term Monitoring Sites Impacted by Human Activities: Implications for Bioassessment

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Abstract: Bioassessment assumes that ecological conditions remain stable in the absence of environmental changes. Evidence suggests this assumption may hold for reference streams, but knowledge gaps remain for impacted streams. Our study quantified interannual variation of benthic macroinvertebrate communities, monitored for at least 14 years in eight impacted streams in the Upper Thames River watershed in Ontario, Canada. Benthic communities exhibited moderate interannual variation in relative abundance of EPT (Ephemeroptera, Plecoptera and Trichoptera) and Chironomidae taxa. Year-to-year changes were reflected in lower community persistence than that observed in studies of reference streams. In contrast, tolerance-based metrics showed minimal interannual variation, suggesting compositional changes were because of taxonomic substitutions, in which one tolerant taxon replaced another. Analyses indicated limited directionality in temporal variation for most bioassessment metrics. An exception was taxa richness, which increased at most sites, possibly because of changes in subsampling. However, no associations between calculated bioassessment metrics and measured environmental factors (stream flow and water chemistry) or sampling procedures were observed. We conclude interannual variation in ecological conditions can be substantial and may not be associated with deterministic factors routinely measured in stream assessments. We recommend increased sampling frequency and traits-based assessment as options for limiting effects of interannual variation on assessment results.

Keywords: benthic macroinvertebrates; bioassessment; community composition; impacted streams; interannual variation; long-term monitoring; stream flow; water chemistry

1. Introduction

Biomonitoring and bioassessment are the collection and analysis of biological data to determine the biological condition of an ecosystem. Bioassessment programs are particularly common for stream ecosystems where a variety of ecological communities, such as, fish, diatoms, or macrophytes, described using either multivariate or metric-based approaches, are used to assess ecological conditions [1]. However, by far the most commonly used indicators in stream bioassessments are benthic macroinvertebrate communities [2,3].

Benthic macroinvertebrate communities have been regularly observed to be sensitive to a variety of stressors (e.g., nutrients, pesticides, and metals) associated with human activities [4–6]. Moreover, it has been noted that benthic macroinvertebrate communities in streams exposed to human activity are compositionally distinct from nearby reference streams [7]. However, it has also been noted that the status of macroinvertebrate communities varies through time at a stream [8,9]. Understanding the extent and source of interannual variability in benthic macroinvertebrate communities is essential given the assumption in bioassessment that long-term changes in community composition are more driven by deterministic factors associated with environmental conditions (e.g., flow, water chemistry, habitat availability, and heterogeneity) than by stochastic factors (e.g., dispersal). Under this premise, community composition should remain relatively stable from year to year in the absence of major environmental perturbations. However, there is only a small number of studies that have tested this hypothesis [10–12], primarily because of the limited availability of high-quality datasets collected using consistent protocols over long-term periods, especially time series greater than 10 years.

Two parameters are often employed to characterize temporal variability in the structure of the macroinvertebrate community: Community persistence and compositional stability. Community persistence accounts for the consistency of taxa presence/absence over time [13–15]. It is expected that macroinvertebrate communities in sites with constant environmental conditions would show a higher persistence than sites with more variable conditions [11,16–18]. Compositional stability describes the similarity of taxa abundance over time [18–20]. Similar to persistence, stability is expected to be greater through time at sites with more constant environmental conditions [11,16–18]. Most studies on reference sites have reported that benthic community composition has high persistence and stability over time (e.g., [10,11,16] but see [12]), and some have linked the existing variation to either large-scale environmental patterns [17] or small scale disturbances [11,19]. Fewer studies have taken place in streams located in highly modified anthropogenic landscapes and results from such studies are more equivocal or based on shorter time series [18,20].

The goal of our study was to quantify interannual variation in the composition of benthic invertebrate communities collected in impacted streams that have been subject to annual monitoring for at least 14 years. We addressed this goal by 1) quantifying community persistence and stability for each site, and; 2) measuring and comparing interannual variation in six bioassessment metrics describing community composition and tolerance. A secondary goal of our study was to assess if interannual variation in the bioassessment metrics could be attributed to catchment factors (i.e., land cover and presence of dams), as well as environmental disturbances associated with preceding hydrological events and annual averages of water quality parameters or changes in sampling procedures. We used the results of our analyses to draw inferences regarding implications of interannual variability for the biomonitoring of streams using benthic macroinvertebrates.

2. Materials and Methods

Our study was conducted in the Upper Thames River Basin (UTRB) in southwestern Ontario, Canada (Figure 1). The UTRB's climate is temperate in nature and the regional surface geology is a mixture of tills, sand, and clays deposited during the last glaciation. The 3420 km^2 basin is dominated by anthropogenic land cover types, with intensive agricultural and urban land use comprising 76% and 8% of the watershed, respectively [21]. Approximately 472,000 people reside in the watershed [21]. Channelization and burial have impacted 64% of the water courses in the UTRB [21]. Moreover, streams in the watershed are exposed to numerous non-point sources, increasing nutrient and sediment loads to the river system. Mean annual flows (from 2011 to 2015) in tributary and main-stem subwatersheds range from 2.8 $m^3 s^{-1}$ to 13.6 $m^3 s^{-1}$, respectively [21]. From the 28 subwatersheds that constitute the UTRB, 8 subwatersheds were chosen for our study due to the availability of long-term datasets generated from co-located monitoring of ecological and environmental conditions (Figure 1c).

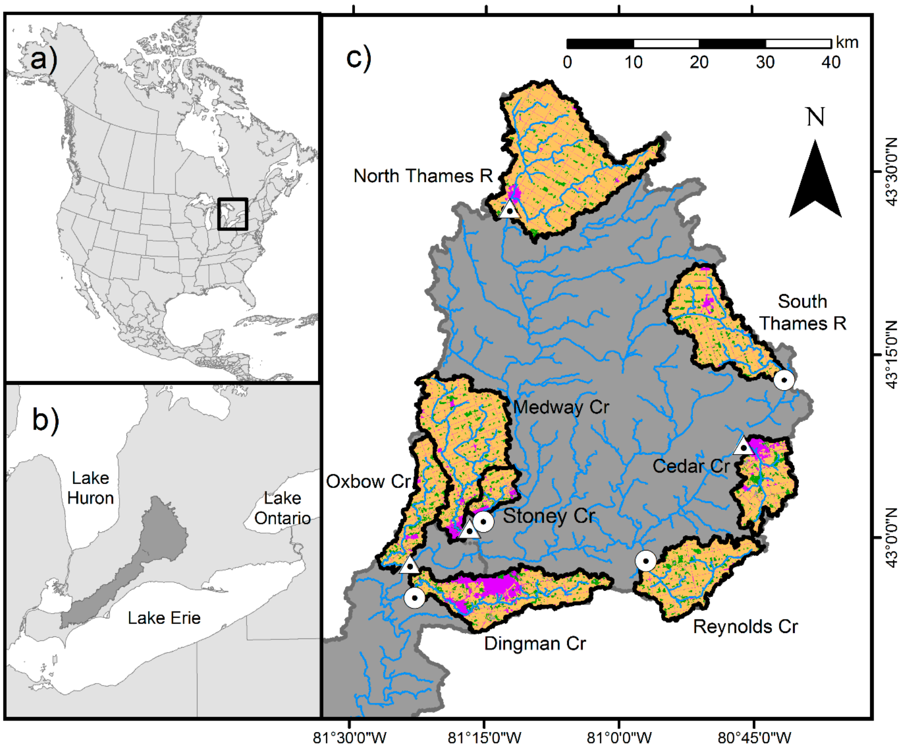

Figure 1. Position of study area in the Great Lakes region of North America (**a**) and location of Thames River watershed in southern Ontario (**b**). Location and land cover (agriculture [orange]; forest [green]; urban [purple]) of eight impacted streams and their associated catchments (black outlines) within the Upper Thames River Basin (**c**). Streams influenced by dams have site points as triangles. Streams without dams have sites represented by circles.

The eight streams included in our study all exhibit perennial flow, with drainage areas ranging from 37 to 315 km^2, and had been monitored for at least 14 years between 1997 and 2016 (Table 1). Selected streams had larger amounts of either agricultural and urban land cover (i.e., greater than 76% and 8%, respectively) compared to land use in the UTRB as a whole [21]. Specifically, Medway, North Thames, Oxbow, Reynolds, and South Thames had more than 80% agriculture in the catchment, whereas Cedar, Dingman, and Stoney Creek catchments had more than 10% urban land cover throughout the study period. Based on available land cover information, land cover was stable for the duration of the study period in nearly all catchments [21–24]. The only exception was Stoney Creek, which experienced an approximately 6% increase in urban cover prior to 2006. Moreover, four of the streams had small dams located upstream of the sampling site that are operated largely to maintain recreational ponds.

Table 1. Sampling location and landscape characteristics (drainage area, land cover, dam presence) for eight streams located in the Upper Thames River Basin of southern Ontario, Canada. Subwatershed characteristics drawn from Maaskant and Quinlan (2007). North Thames River includes both Whirl and North Mitchell subwatersheds.

Stream	Latitude	Longitude	Drainage Area (km^2)	Urban (%)	Agriculture (%)	Forest (%)	Dam Present
Cedar Creek	43.122	−80.7515	87.75	10.0	70.6	3.8	yes
Dingman Creek	42.934	−81.3513	148.59	15.5	64.0	8.7	no
Medway Creek	42.966	−81.4180	85.66	3.8	82.4	6.6	yes
North Thames River	43.450	−81.2068	315.4	1.3	90.4	3.1	yes
Oxbow Creek	43.014	−81.2804	203.19	1.8	85.3	5.0	yes
Reynolds Creek	42.982	−80.9546	145.14	2.4	79.6	9.6	no
South Thames River	43.215	−80.6919	148.85	0.6	84.8	5.7	no
Stoney Creek	43.022	−81.2534	37.33	14.4	62.8	7.2	no

2.1. Data Collection

Benthic macroinvertebrate communities were sampled annually from 1997 to 2016, except for Stoney Creek where monitoring of benthic macroinvertebrates began in 2003 (Table 2). Samples were collected in the same reach where the hydrological and water quality monitoring took place. Upper Thames River Conservation Authority staff collected the benthic samples in late May or early June of each year. Samples were collected using a three-minute travelling kick with a D-frame net of 500-µm mesh. Sampling resulted in the collection of a single sample of invertebrates from the habitats present in the sampling reach. Samples were fixed for a minimum of 48 hours in 10% formalin solution prior to being preserved in 70% ethanol. Collected samples were subsampled in the lab using a gridded pan and a fixed count-subsampling process. Invertebrates were subsampled from randomly selected grid cells until the set minimum count had been attained.

Table 2. Years for which monitoring data of benthic macroinvertebrates, water chemistry, and stream flow were available for eight streams in the Upper Thames River Basin of southern Ontario, Canada. Number in parentheses indicates total number of years sampled.

Stream	Benthic	Water Chemistry	Stream Flow
Cedar Creek	1997 to 2016 (20)	2004 to 2014 (11)	1997 to 2016 (20)
Dingman Creek	1998 to 2016 (19)	2004 to 2014 (11)	1997 to 2015 (19)
Medway Creek	1997 to 2016 (20)	1997 to 2014 (11)	1997 to 2015 (19)
North Thames River	1997 to 2016 (20)	2004 to 2014 (11)	1997 to 2015 (19)
Oxbow Creek	1998 to 2016 (19)	2004 to 2014 (11)	2003 to 2015 (14)
Reynolds Creek	1998 to 2016 (19)	2004 to 2014 (11)	2003 to 2016 (14)
South Thames River	1997 to 2016 (20)	2004 to 2014 (11)	1997 to 2015 (19)
Stoney Creek	2003 to 2016 (14)	2004 to 2014 (11)	2003 to 2015 (14)

The minimum count used for subsampling increased from 100 to 300 individuals over the 20-year sampling period. Prior to 2000, benthic samples were subsampled to 100 individuals. In contrast, the number of organisms subsampled was increased first to 200 and subsequently to 300 through the early 2000s. The required fixed minimum count (i.e., subsampling protocol) was included as a predictor variable in analyses to assess the potential impact of changes to subsampling procedures on temporal variability in the monitored benthic communities.

Taxa were identified to the family level in order to avoid rarities of isolated species between sampling streams, resulting in a total of 61 unique families being collected across all streams over the study period. The resultant relative abundances for each stream for each year were used to calculate six bioassessment metrics describing aspects of community composition (Berger–Parker dominance (% dominant), family richness (richness), percentage of Chironomidae (% Chironomidae), percentage of Emphemeroptera, Plecoptera, and Trichoptera (% EPT)), community tolerance to flow and organic pollution (Canadian Ecological Flow Index (CEFI), and Hilsenhoff Family Biotic Index (FBI); Table 3; [25–28]).

Table 3. List and description of bioassessment metrics as well as hydrologic and water quality parameters computed from monitoring data collected over at least 14 years from eight streams of the Upper Thames River Basin, southern Ontario, Canada.

	Parameter	Description	Reference
Bioassessment Metrics	Berger–Parker dominance index	Measure of taxa dominance based on the proportional abundance of the most abundant taxon	[22]
	Canadian Ecological Flow Index (CEFI)	Measure to assess ecological responses to hydrological alterations	[23]
	Hilsenhoff Family Biotic Index (FBI)	Index based on the tolerance of family taxa to organic pollution	[24]
	Taxa richness	Total number of taxa in a sample	[25]
	% of Chironomidae	Percentage of Chironomidae taxa in the sample	[25]
	% of EPT families	Percentage of Ephemeroptera, Plecoptera, and Trichoptera taxa in the sample	[25]
Water Quality	Conductivity	Mean annual conductivity of stream water (μS cm^{-1})	
	Total Phosphorus (TP)	Mean annual concentration of Total Phosphorus (mg P/l)	
	Nitrate	Mean annual concentration of Nitrate (mg N/l)	
Hydrology	Magnitude of spring freshet	Maximum flow rate (m^3 s^{-1}) measured during the spring freshet	[26]
	Date of spring freshet	Hydrologic date the maximum flow rate during the spring freshet was measured	[26]
	Magnitude of peak flow in ice-influenced period	Maximum flow rate (m^3 s^{-1}) measured during the ice-influenced period	[26]
	Date of peak flow in ice-influenced period	Hydrologic date the maximum flow rate during the ice-influenced period was measured	[26]
	Magnitude of peak flow in open-water period	Maximum flow rate (m^3 s^{-1}) measured during the open-water period	[26]
	Date of peak flow in open-water period	Hydrologic date the maximum flow rate during the open-water period was measured	[26]

Community stability and persistence were determined by calculating measures of similarity between samples in consecutive years (e.g., 2003–2004) (sensu [11,18]). Bray–Curtis dissimilarity was calculated from relative abundance data for every two consecutive years at each stream to generate a measure of between-years compositional stability. Similarly, Jaccard's similarity was used to determine the degree of community persistence for each stream based on the presence and absence of taxa between consecutive years.

Water chemistry data were obtained from the Ontario Provincial Water Quality Monitoring Network (PWQMN) database [29]. Water quality samples were generally collected monthly under ambient flow conditions during the open water period for each year from 2004 to 2014 (Table 2). Medway Creek had a longer dataset extending from 1997 to 2014. Data from 2006 were missing from Reynolds Creek. Of the water quality parameters collected by the PWQM program, only conductivity, nitrate, and total phosphorus were collected with sufficient regularity (at least 5 times per year) to calculate annual averages at all sites (Table 3). Moreover, the 5 to 7 samples available in most years for most streams were considered insufficient to generate meaningful annual averages of water temperature for the sampled streams. Annual averages of conductivity, nitrate, and total phosphorus were calculated from all water samples available for the year preceding each benthic sampling event.

Hydrologic data were extracted for the eight study streams from the HYDAT database using HEC-DSSVue v2.0.1 [30]. Hydrological variables were calculated based on the Canadian Hydrological Indicators of Change (CHIC; [31,32]). Hydrologic parameters were calculated for two periods prior to each year's macroinvertebrate sampling: (i) The ice-influenced period, from 1 December of the year preceding sampling to the last day ice was present in the catchment; (ii) the open water period, from the last day of ice to the macroinvertebrate sampling date of each year. Ice-influenced flow variables described the spring freshet and the peak flow event of the ice-influenced period as well as the date these events occurred on (Table 3). Open water variables described the magnitude and date of the peak flow event occurring between spring freshet and benthic sampling. Hydrologic variables were selected based on evidence that these hydrologic events are ecologically relevant [31,32]. Flow magnitudes are presented as discharge (m^3 s^{-1}) and flow parameters represented by dates are given as numerical values representing the hydrologic day of the year, where day 1 was 1 October and day 365 was 30 September of the following year (sensu [31]).

2.2. Data Analysis

A two-factor permutational analysis of variance (PERMANOVA; [33]) was used to assess differences between the biological communities in terms of community composition (i.e., presence/absence and relative abundance) using the raw taxonomic data from the annual monitoring samples for all streams and years. The PERMANOVA considered year (20 years, 1997–2016) and stream (eight streams). The analysis was done using the vegan package [34] using 999 permutations using R version 2.12.0 [35]. This analysis was conducted to determine if streams should be analyzed as a group or individually to best detect interannual variation.

Community persistence and stability were assessed in a descriptive fashion by calculating descriptive statistics (i.e., mean, median, quartiles), as well as the coefficient of variation (CV), for each stream. Similarly, interannual variation in the bioassessment metrics was determined through calculation and analysis of the CV for each metric for each individual stream. Among the metrics, differences in the amount of interannual variability were established by comparing the CVs of the six bioassessment metrics across all streams. These comparisons were conducted using the non-parametric Kruskal–Wallis test, as the distributions of the metric CVs did not meet assumptions of normality for parametric tests. The significance level for the Kruskal–Wallis test was set at $\alpha = 0.05$. Analyses were conducted using Systat 13.

A non-parametric regional Mann–Kendall test [36,37] was used to assess the presence of monotonic trends in the biological, environmental, and sampling procedures' time series. Trend analyses were conducted for each individual biological metric, environmental parameters (i.e., water chemistry and hydrological variables), and sampling procedures (sampling date and subsampling effort) for each individual stream to detect within-stream variation using the longest dataset available for each parameter. For the purposes of this study, we defined a "trend" as a long-term unidirectional change (either increasing or decreasing) in the mean as indicated by the Mann–Kendall analyses. To account for the large number of tests being run, trends were considered as statistically significant if $p < 0.01$. Theil–Sen's slope was also calculated as part of the analysis, indicating the linear rate of change (calculated as the median of all slopes), where negative values indicated decreasing trends and positive values indicated increasing trends. The analyses were completed using the rkt package [38] in R version 2.12.0 [35].

Effects of land cover type and the presence of dams on variation in each individual bioassessment metric was assessed by conducting separate Kruskal–Wallis tests ($\alpha = 0.05$) in Systat 13 using land cover and presence of dams as the factors and the coefficient of variation of the individual sites as the dependent variable. The land cover groups were based on the criteria of whether urban land use equaled or exceeded 10%. Thus, three streams (Cedar Creek, Dingman Creek, and Stoney Creek) were assigned to the urban group and the remaining five were assigned to the agricultural group (Medway Creek, North Thames River, Oxbow Creek, Reynolds Creek, and South Thames River).

Partial least squares (PLS) regressions [39] were used to assess the relationships between the calculated bioassessment metrics and the three groups of annually monitored environmental parameters (i.e., water quality parameters, ice-influenced and open-water variables) for each individual stream. Sampling date and minimum subsampling effort, measured as the number of organisms counted, were also included in the PLS to account for potential effects of variation in the sampling protocol. PLS is a well-tested approach used to identify relationships among dependent and independent variables in time series analysis (e.g., [40,41]). PLS is also robust for assessing whether variables show significant temporal trends [42]. This analysis also enables the description of multiple ecological response variables simultaneously, while avoiding multicollinearity issues among environmental indices [43,44].

PLS regressions were completed on each individual stream using the bioassessment metrics as response variables, and environmental and sampling variables as predictors. Years included in the analysis were restricted to ones with a complete set of response and predictor variables. Consequently, models tested relationships for 11 years of complete data, excluding Reynolds Creek, which had only 10 years of data in the PLS regression (Table 2). PLS model performance is expressed in terms of the cross-validated explained variances of the environmental variables (r^2x), cross-validated explained variances of the biological community (r^2y), and predictive ability of the model (Q^2y). PLS models were considered significant when $Q^2y > 0.097$ and to have a good predictive capacity when $r^2y > 0.5$, (sensu [45]). A 10-fold cross-validation method, iterated 999 times, was used to select the number of significant components through the calculation of the Q^2y. PLS analyses were computed using the pls package [46] using R version 2.12.0 [35].

3. Results

According to the two-factor PERMANOVA comparing the taxonomic composition of benthic invertebrates, stream/site location (factor: Stream) explained the largest portion of the variance observed (partial $R^2 = 0.32$ and $R^2 = 0.38$, for presence/absence and relative abundance, respectively). In contrast, the portion of overall variance explained by interannual variability (factor: Year) was small (partial $R^2 = 0.06$ and $R^2 = 0.01$ for presence/absence and relative abundance, respectively). The factors stream and year interacted significantly for the relative abundance of taxa (partial $R^2 = 0.04$), but not presence/absence. As the benthic communities differed significantly by stream location, it was determined that further temporal analyses should be conducted for each stream individually to remove confounding spatial effects.

3.1. Interannual Variability of the Biological Assemblage

Between-year differences in community persistence and stability were similar among the eight study streams over the course of the study period (Figure 2). Mean community persistence, represented by the Jaccard similarity index, varied from a maximum of 62% at the South Thames River to a minimum of 49% at the North Thames River. Mean persistence across all streams was about 56%. Mean community stability as represented by Bray–Curtis dissimilarity indicated that the North Thames River had, on average, the greatest between-year changes in composition, with a mean dissimilarity of 45%, whereas Medway Creek exhibited the smallest mean dissimilarity (27%). The mean stability across all streams was near 36%. Furthermore, there was only moderate variation of persistence and stability over the course of the study period, with CVs varying between 0.2 and 0.4 for most streams. An exception was Cedar Creek, which had a CV of 0.6 for stability. However, the overall variance was not completely representative of the range of values exhibited by several of the streams. For example, at Medway Creek, the Jaccard similarity exceeded 90% between two consecutive years, whereas at the North Thames River similarity was found to be below 20% between two consecutive years. Maximum and minimum Bray–Curtis dissimilarity values also deviated by about 40% from the mean value for the North Thames River. Cedar Creek also exhibited a maximum dissimilarity value nearly 50% greater than the stream's mean.

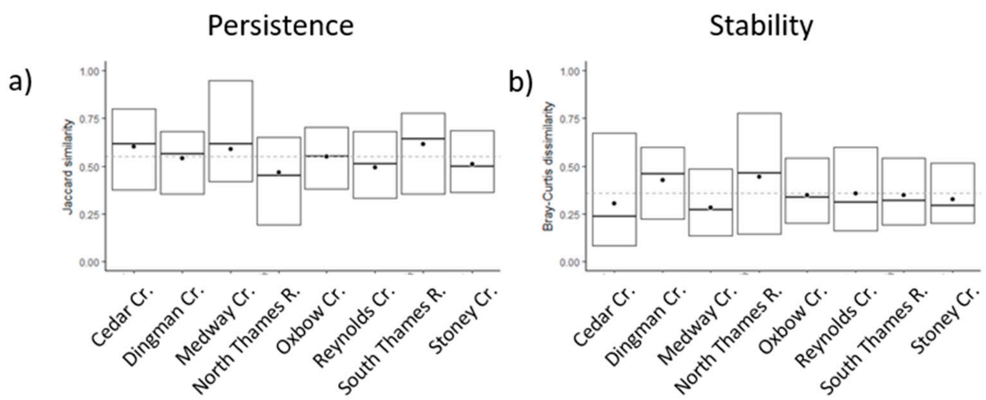

Figure 2. Boxplots of persistence (**a**) and stability (**b**) of benthic macroinvertebrate communities for eight rivers in the Upper Thames River Basin (southern Ontario, Canada) monitored for at least 14 years. Bars represent range of values, solid black lines represent median values, and black circles represent means. Gray dashed line indicates mean of all streams combined.

Regional Mann–Kendall tests on community persistence and stability revealed eight significant monotonic trends ($p < 0.01$; Table 4). Three streams exhibited trends for community persistence, with Cedar Creek exhibiting a decreasing trend and the South Thames River and Stoney Creek showing increasing trends. The South Thames River and Stoney Creek also exhibited a decreasing trend for community stability, as did Medway Creek and Reynolds Creek. Dingman Creek showed an increasing trend for community stability. However, all trends in community persistence and stability had low slopes (<0.025).

Benthic macroinvertebrate assemblages exhibited an average richness of 11 to 21 families across the eight sampled streams. Oxbow Creek and the South Thames River had the greatest richness (Figure 3). Assemblages at most streams were dominated by EPT taxa (mean range = 10.6–64.6%) and Chironomids (18.5–45.8%). Moreover, the mean Berger–Parker dominance was similar to % Chrionomidae at most sites. Exceptions were Cedar Creek and Dingman Creek, suggesting that these two streams were dominated by taxa other than EPT and Chironomids. Mean CEFI scores ranged from 0.27 to 0.35. Six of the eight streams had mean FBI scores between 5.51 and 7.5, indicating that these communities scored fair to fairly-poor in terms of exposure to organic enrichment. The remaining two streams, Oxbow Creek and the South Thames River, had mean FBI scores within the upper end of the good category (4.51 to 5.50).

Table 4. Significant trends detected by regional Mann–Kendall tests for community persistence and stability, as well as six bioassessment metrics measured in eight different streams in the Upper Thames River Basin (southern Ontario, Canada). RKT-slope derived from the significant (p-value < 0.01) Mann–Kendall tests. K-score in brackets.

Biological Parameter	Cedar Cr	Dingman Cr	Medway Cr	North Thames R	Oxbow Cr	Reynolds Cr	South Thames R	Stoney Cr
Community Persistence	−0.009 (−56)						0.014 (80)	0.023 (47)
Community Stability		0.012 (50)	−0.008 (−53)			−0.014 (−67)	−0.013 (−59)	−0.023 (−38)
Taxa richness	0.273 (83)		0.444 (102)		0.500 (50)	0.333 (54)	0.631 (74)	
% of EPT families								
% of Chironomidae						−0.005 (−56)		
Berger–Parker dominance index								
Hilsenhoff Family Biotic Index							−0.004 (−56)	
Canadian Ecological Flow Index								

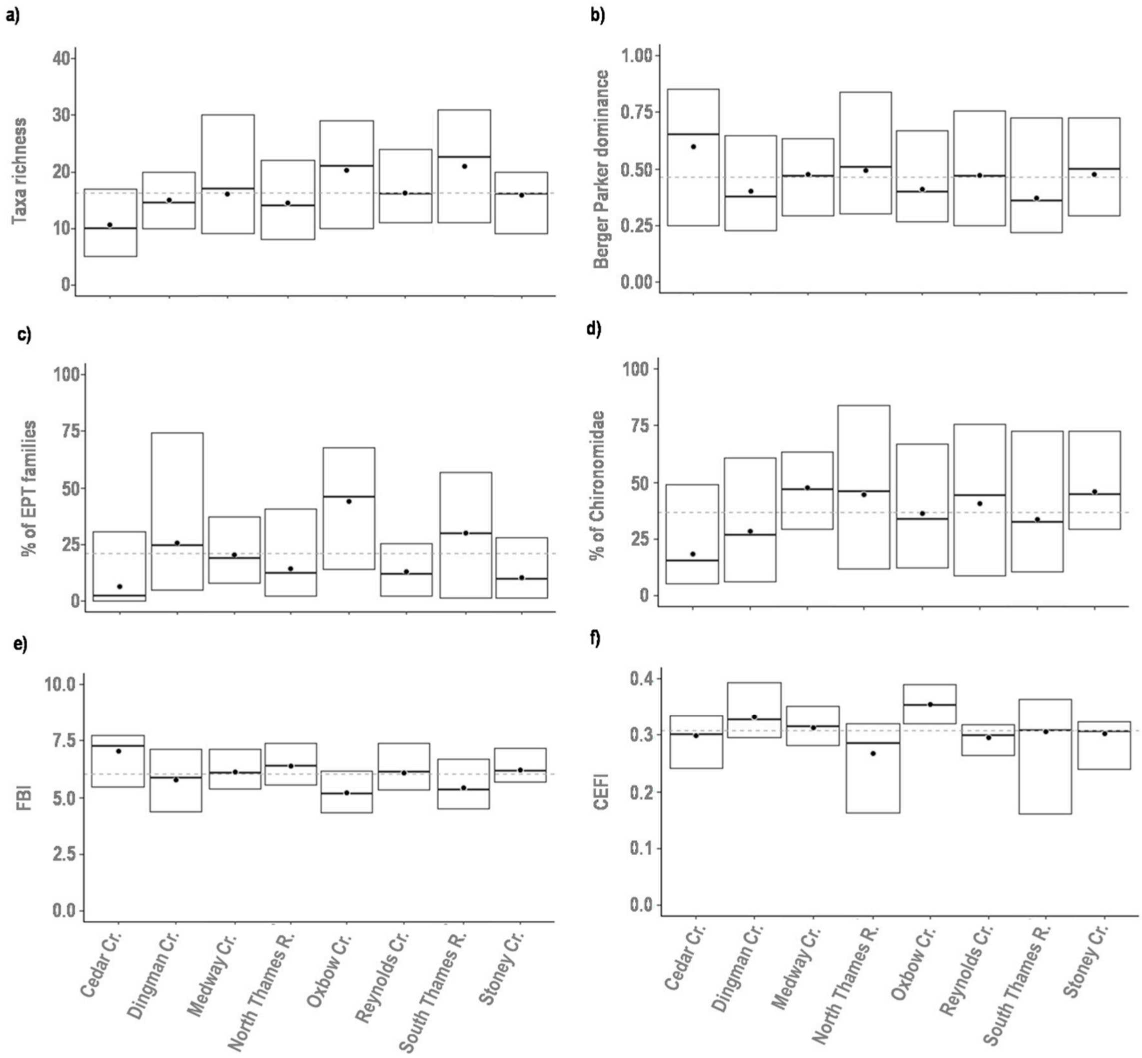

Figure 3. Boxplots of six bioassessment metrics (taxa richness (**a**), Berger Parker dominance (**b**), % of Empheroptera, Plecoptera and Trichoptera (EPT) families (**c**), % of Chironomidae (**d**), Family Biotic Index (FBI) (**e**), and Canadian Ecological Flow Index (CEFI) (**f**)) summarizing benthic macroinvertebrate communities for eight rivers in the Upper Thames River Basin (southern Ontario, Canada) monitored for at least 14 years. Bars represent range of values, solid black lines represent median values, and black circles represent means. Gray dashed line indicates mean of all streams combined.

Results from the Kruskal–Wallis test comparing the CVs of the six bioassessment metrics indicated that there were differences among the metrics (KW = 41.25, $p < 0.001$). Indeed, pairwise comparisons showed that across all sites the tolerance metrics with mean CVs of 0.086 and 0.096 for CEFI and FBI, respectively, were less variable than the four composition-based metrics (Figure 4). Comparisons also revealed that %EPT (mean CV = 0.69) was more variable than all metrics except %Chironomidae (mean CV = 0.41). The average amount of variation did not differ between %Chironomidae, taxa richness (mean CV = 0.24), and Berger dominance (mean CV = 0.29).

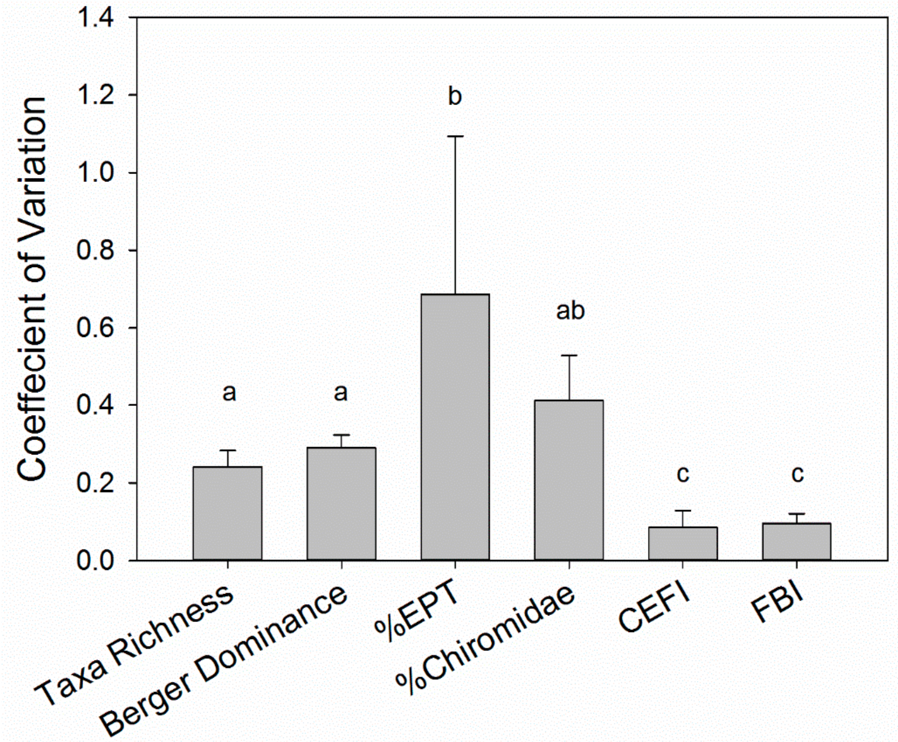

Figure 4. Comparison of mean coefficients of variation (bars) for bioassessment metrics calculated for eight streams sampled at least 14 years. Error bars indicate one standard deviation. Letters indicate pairwise differences ($p < 0.05$) among metrics in terms of the amount of interannual variation as indicated by the coefficients of variation such that metrics with the same letters did not differ.

3.2. Assessment of Environmental Variability

Analysis of annual means of the water quality parameters indicated water quality varied substantially among streams (Figure 5). Mean annual conductivity was greatest at Dingman Creek where conductivity values were, on average, greater than 300 $\mu S\ cm^{-1}$ larger than the minimum mean annual average observed at Oxbow Creek. CVs of mean annual conductivity were largest at the North Thames River and smallest at Cedar Creek. Overall, conductivity was less variable over time within streams (all CVs <0.20) than concentrations of nitrate and total phosphorus (all CVs >0.20). The largest mean annual average nitrate concentrations were measured at the North Thames River and exceeded 8.5 mg/L. In contrast, Stoney Creek exhibited the smallest mean annual average nitrate concentration at 2.3 mg/L. Mean annual nitrate concentration at Stoney Creek also exhibited substantial year-to-year variation (CV >0.5). Maximum and minimum mean annual nitrate concentrations were often 2- to 3-fold different from the mean annual average. Moreover, at the North and South Thames Rivers, the maximums exceeded 12 mg/L. Mean annual TP exhibited comparable within-stream variability to nitrate (CVs within 0.2). However, there was no strong correlation between the mean annual averages of nitrate and TP ($r_s = 0.62$, $p = 0.10$). Mean annual average TP concentrations were also more similar among streams than nitrate, as all streams had average TP concentrations within 0.07 mg/L.

Hydrologic variables exhibited substantial interannual variability in the date of spring freshet and peak flows (Figure 6). On average, spring freshet occurred on the 86th day (max = 88, min = 85) of each hydrologic year for all eight study streams. However, maximum annual values were as late as the 115th day for most streams. However, Oxbow, Reynolds, and Stoney Creeks did not exhibit these late spring freshet dates and exhibited reduced interannual variation in the initiation of spring freshet. In contrast, the date of peak water level during the ice-influenced period exhibited comparable ranges of dates for all eight streams. The average date of the peak water level in the ice-influenced period was more variable among streams, with the earliest dates observed at Reynolds and Stoney Creek

(105th and 101st days respectively) and the latest at Oxbow Creek (131st day). In contrast, the date of maximum flow within the open water period was less variable for all streams, except Stoney Creek. Furthermore, the range of dates of maximum in the open water period was greater for all streams than those observed for the ice-influenced period, with most streams having a range of more than 80 days. Flow magnitudes at spring freshet, as well as peak flows in the ice-influenced and open water periods, showed similar patterns in terms of within-stream interannual variability. The North Thames River exhibited the largest range of peak flows at 50, 200, and 150 $m^3 s^{-1}$ for spring freshet, ice-influenced, and open water periods, respectively. In contrast, Cedar and Stoney Creeks exhibited comparatively small ranges of interannual variation in peak flow. Differences in the mean peak flow at spring freshet were less than 5 $m^3 s^{-1}$ for all streams and with the exception of the North Thames River had for peak flows below 30 $m^3 s^{-1}$ in the ice-influenced and open water periods.

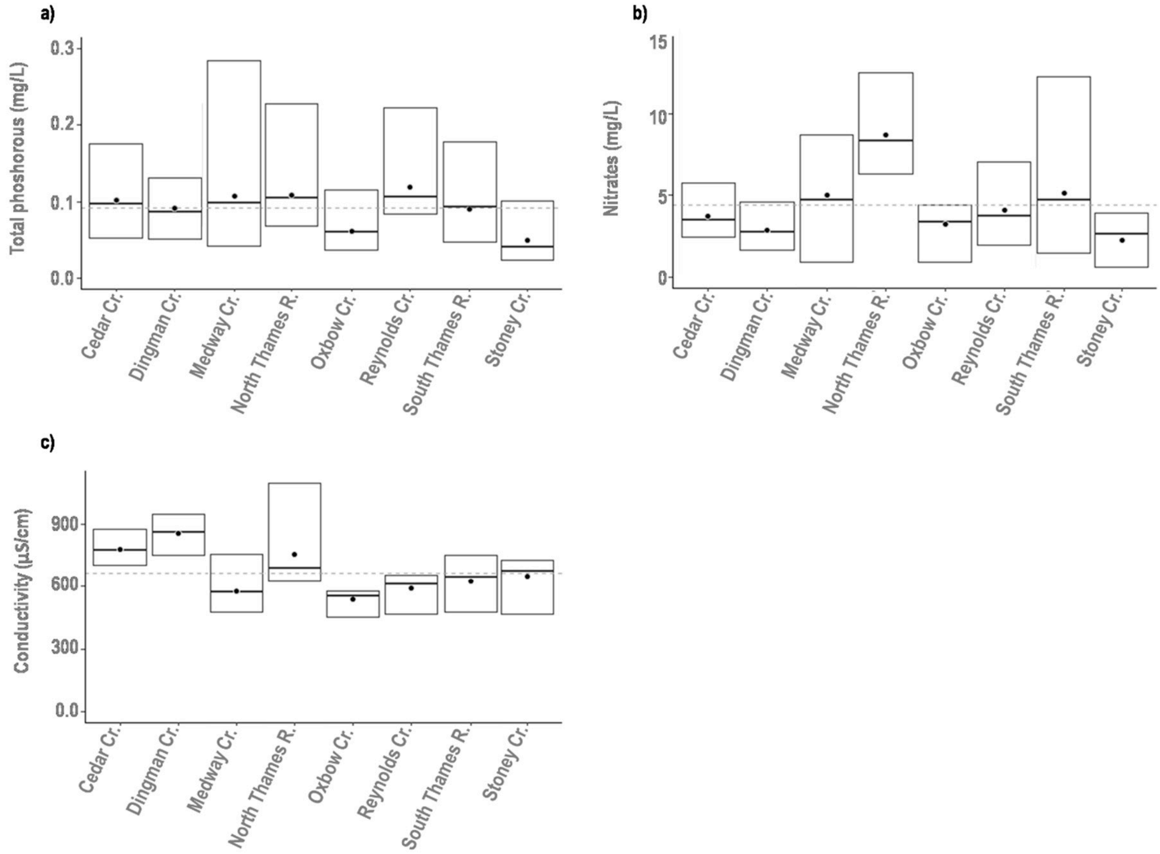

Figure 5. Box plots summarizing mean annual values of total phosphorus (**a**), nitrate (**b**) and conductivity (**c**) sampled five to seven times annually in eight rivers in the Upper Thames River Basin (southern Ontario, Canada) monitored for at least 10 years. Bars represent range of values, solid black lines represent median values, and black circles represent means. Gray dashed line indicates the mean of all streams combined.

The mean sampling date was within 5 days of the 240th day for all streams except Oxbow Creek, for which the mean sampling day was day 250. Oxbow Creek was the most variable in terms of sampling date, with a CV of 0.13. This variation was primarily due to two outliers where sampling took place on days 318 and 348. Reynolds Creek was also sampled later in the year in one instance over the course of the study period. The remaining six streams had standard deviations of less than 3 weeks and all sampling took place between days 215 and 275. The subsampling count was highly variable at most streams over the course of the study period (Figure 5), with standard deviations exceeding 100 individuals for all streams but Stoney Creek. The mean number of individuals subsampled was between 260 and 320 for all streams. Furthermore, subsample counts had minimums near 100 for all streams, reflecting the change in the subsample count target over the course of the study period.

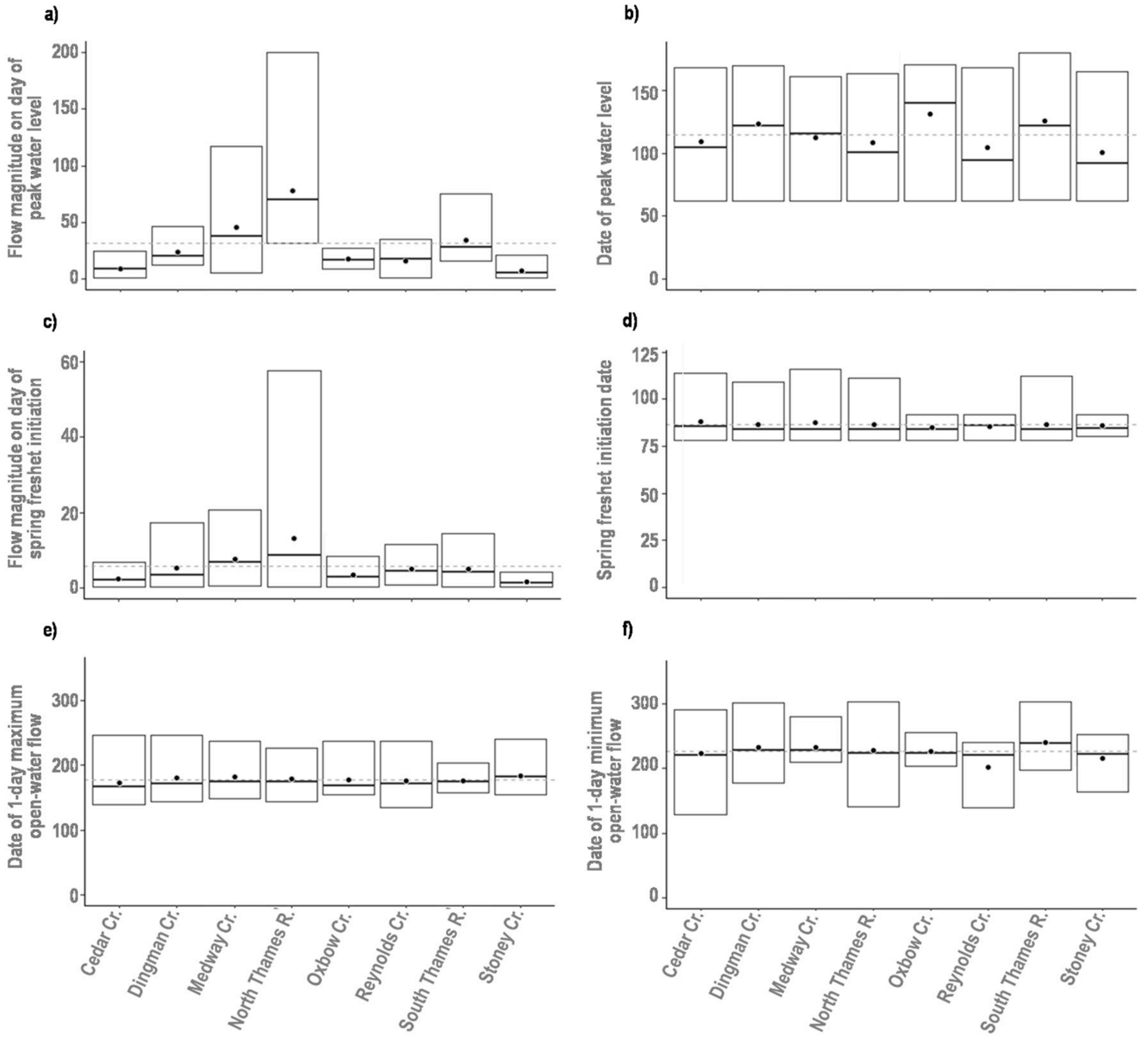

Figure 6. Box plots summarizing hydrologic measures (flow magnitude on day of peak water level (**a**), date of peak water level (**b**), flow magnitude on day of spring freshet initiation (**c**), spring freshet initiation date (**d**), date of 1-day maximum open-water flow (**e**), and date of 1-day minimum open-water flow (**f**)) for eight rivers in the Upper Thames River Basin (southern Ontario, Canada) monitored for at least 14 years. Bars represent range of values, solid black lines represent median values, and black circles represent means. Gray dashed line indicates the mean of all streams combined.

Mann–Kendall tests revealed five statistically significant ($p < 0.01$) trends for water quality. Decreasing trends were observed for nitrate at the Dingman (RKT-slope = −0.16; K-score = −27) and Medway Creek (RKT-slope = −0.25; K-score = −54), whereas decreasing trends for TP were detected at Cedar (RKT-slope = −0.006; K-score = −29), Medway (RKT-slope = −0.007; K-score = −85), and Stoney (RKT-slope = −0.007; K-score = −37) Creeks. The slopes for TP were substantially lower (two orders of magnitude) than for nitrate. No trends were observed for any of the hydrologic variables, but monotonic trends were observed for both sampling variables at all streams. Sampling date showed decreasing trends for all sites, indicating progressively earlier sampling over the course of the study period (Cedar [RKT-slope = −2.62; K-score = −129]; Dingman [RKT-slope = −1.73; K-score = −79]; Medway [RKT-slope = −1.33; K-score = −90], North Thames [RKT-slope = −2.63; K-score = −132], Oxbow [RKT-slope = −2.46; K-score = −110], Reynolds [RKT-slope = −3.00; K-score = −132]; South Thames [RKT-slope = −2.32; K-score = −125]; Stoney [RKT-slope = −1.80; K-score = −49]). In contrast, subsample count increased over the course of the study, reflecting the systematic shift in subsampling protocol from a 100- to 200- and then 300-count subsample target (Cedar [RKT-slope = 14.1; K-score = 116], Dingman [RKT-slope = 7.1; K-score = 62], Medway [RKT-slope = 10.8; K-score = 94], North Thames [RKT-slope = 10.6; K-score = 103], Oxbow [RKT-slope = 18.0; K-score = 59], Reynolds [RKT-slope = 13.0; K-score = 94], South Thames [RKT-slope = 33.6; K-score = 114], Stoney [RKT-slope = 9.54; K-score = 43]).

3.3. Environment to Biota Associations

Comparison of CVs of each bioassessment metric between streams designated as urban and agricultural indicated that none of the metrics were more variable in one land use type ($p > 0.05$). However, the Kruskal–Wallis test on CVs for %EPT did generate a p-value of 0.053. Similarly, the Kruskal–Wallis tests revealed that CVs did not differ between streams with and without dams for any of the metrics ($p > 0.05$), although taxa richness and CEFI had p-values near the statistical threshold ($p = 0.083$).

PLS regressions associating the calculated bioassessment metrics with the environmental and sampling variables (i.e., water quality parameters, ice-influenced variables, open-water variables, and sampling descriptors) for each of the eight streams indicated that 12% to 27% and 12% to 47% of the variance was captured in the dependent and predictive variables, respectively. However, cross-validated Q^2Y values for all streams were below 0.097, indicating temporal variation in the bioassessment metrics could not be predicted by interannual variation in environmental and sampling variables. Based on this finding, we increased the length of record for each stream by up to eight years for each stream by removing the water chemistry variables from the analyses. However, cross-validated Q^2Y values for all streams were still below the 0.097 threshold.

4. Discussion

Several publications have focused on understanding temporal biological patterns in reference (unimpacted) sites (e.g., [10–12]), but few have assessed temporal variation in invertebrate community structure in streams impaired by anthropogenic activities. Our long-term study of eight impacted streams in southern Ontario revealed substantial interannual variation in benthic macroinvertebrate community composition. However, our assessment also indicates that the observed variation lacks directionality and is unrelated to measured hydrological and water chemistry conditions. Moreover, it does not appear that changes in sampling procedures have affected biomonitoring endpoints. These findings have implications for effectively using benthic macroinvertebrates as indicators of stream conditions.

4.1. Temporal Variations in Benthic Macroinvertebrate Communities

Analysis of the community persistence in the eight study streams indicates substantial year-to-year variation in community composition. Indeed, persistence indicated that on average there was a change of more than 45% of the species across all of the sampled streams. In contrast, past studies assessing reference sites have generally found high levels of community persistence (e.g., [10,11,47]), although the calculation methods used in these past studies are not always consistent with ours. For example, in a study of 26 streams, Scarsbrook [11] observed an average dissimilarity of less than 0.3 and no individual stream exceeded 40% as an average rate of taxonomic turnover. In contrast, our findings are more consistent with those of Collier [18], who also measured persistence to frequently be less than 60% for 49 streams in New Zealand that were exposed to a gradient of human land use. Moreover, Collier [18] a observed similarly large variation in year-to-year persistence within individual streams to that observed in our studies. Our findings thus contribute to a growing body of literature that collectively indicate that the suite of taxa making up the community in impacted streams may exhibit less persistence than is observed at reference sites.

The average degree of constancy in abundance, as indicated by community stability, was greater in our study than persistence and was more comparable with observations of past studies at reference sites [10,11]. Moreover, the average mean dissimilarity that we observed for all streams combined was near 35%, which is about 10% greater than that observed by Collier [18] in impacted streams. The increased stability, relative to persistence, is consistent with our finding of high levels of dominance and suggests that the more abundant taxa remain in the communities from year to year whereas the taxonomic turnover indicated by the low amount of persistence is due to substitutions of less abundant taxa. Such a trend is not surprising in impacted streams as anthropogenic exposure is known to increase the abundance of major groups of tolerant taxa (e.g., Chironomidae), while reducing taxa richness through the loss of less abundant sensitive taxa [48].

At the outset of the study, we were concerned by the possibility that observations of community persistence and stability may be confounded with changes in subsampling protocols over the 20 years of monitoring. In particular, we expected that increased subsampling would affect the capture rate of taxa, as less abundant taxa have been shown to be biased against when subsample sizes are small [49,50]. However, despite observing trends of increasing taxonomic richness in biomonitoring samples for five of the eight streams, the change in subsampling does not appear to have had a substantial effect on persistence or stability in these streams as no consistent, strong monotonic trends were observed. Thus, we have confidence that the changes in subsampling protocol did not greatly affect our estimates of stability and persistence.

Interannual variation in the common bioassessment metrics depended on the nature of the metric. The two tolerance-based metrics, CEFI and FBI, showed very little variation among years for all eight study streams. The composition-based metrics (taxa richness, dominance, %EPT, % Chironomid) showed more substantial variation (2.5 to 6 times more based on CVs) among years for all streams. As previously indicated, our findings suggest measures of taxa richness were impacted by changes in subsampling protocols, with a trend towards increased richness in the latter years of monitoring coincident with the shift towards larger subsample counts. However, this change in subsampling does not appear to have influenced the relative abundance of EPT families and Chironomids, as these metrics did not exhibit similarly strong monotonic trends. Moreover, there is no evidence from our analyses that the shift in sampling time to earlier in the spring impacted either the compositional- or the tolerance-based metrics. Thus, the contrasting responses between the compositional and tolerance metrics suggest that the interannual variation in the benthic invertebrate community may largely be the result of species substitutions, where one tolerant taxon is replaced by another.

Among-catchment differences in land use type and the presence of dams were not associated with among-stream differences in variability in the bioassessment metrics. A lack of influence of differences in land use on temporal variability in benthic communities is consistent with the findings of Collier [18]. However, our finding that the presence of dams has no impact on temporal variation contrasts other studies showing interannual variations are associated with the stability of substrate and flow conditions [11,19], the effects of which could be dampened by the presence of dams. Overall, our findings indicate that interannual variations in benthic community composition in our eight study streams are not driven by the measured catchment scale factors, however, a broader study with a greater number of streams should be undertaken to ensure that our findings are not a result of our small sample size (n = 8).

We did not find interannual variations in the measured hydrologic and water quality parameters to be predictors of the observed changes in the bioassessment metrics. The lack of a relationship between the measured environmental parameters and community composition is not consistent with past studies, which have found changes in flow conditions and water chemistry to impact temporal variation in benthic invertebrate communities (e.g., [19,20,47]). A possible explanation for the difference between our study and past studies is that the widespread and long-term exposure the study streams have had to anthropogenic disturbance has removed all sensitive taxa from the regional species pool and thus the measured parameters did not impart significant stress on these remaining tolerant taxa. Indeed, there is a growing number of studies demonstrating that communities in anthropogenic landscapes are increasingly homogenized and less sensitive to variation in environmental conditions [51–53]. This explanation is supported by the dominance of typically tolerant Chrionomid taxa and the high FBI scores. These community characteristics suggest that the observed taxa substitutions may be the result of small-scale factors, such as resource availability, habitat heterogeneity, and community interactions (e.g., competition and predation); other larger scale factors not measured in this study; or more stochastic factors related to dispersal and variation in sampling efficiency.

An additional explanation for the lack of association between the measured environmental parameters and the bioassessment metrics is that environmental conditions were not adequately captured at the time scale the communities are responding to. Mismatch of temporal scales between environmental and biological sampling is a constant concern in biomonitoring as snapshot biological data, such as that used in our study, are undertaken at an annual time scale based on the premise that benthic insect communities are an integrated reflection of stream conditions over the course of a year. However, it is possible that the hydrologic and water quality parameters used in our study did not accurately reflect the dominant conditions in the streams that drove the benthic community composition. This explanation is likely to be particularly pertinent to the water chemistry data available for the sampled streams as the monitoring programs were unlikely to capture extreme events, particularly those in winter, which may have had a disproportionate effect on contaminant and nutrient loadings. Thus, while the data we had access to is typical of, or even exceeds, what is often available for large scale monitoring programs, we recommend that future studies aim to include water quality sampling regimes that are better able to capture seasonally and event-based variations in stream conditions.

4.2. Implications for Biomonitoring Using Benthic Macroinvertebrates

The findings of our study have implications for effectively applying benthic macroinvertebrate communities to the biomonitoring of stream ecosystems. First, our study indicates that interannual variations in bioassessment metrics derived from taxonomic composition can be significant and may not be linked to the tested environmental drivers. Thus, the observed make-up of the community and subsequent conclusions about stream conditions from a single snapshot sampling of streams may be insufficient. For example, we found that that the %EPT and %Chironomid metrics, metrics that respectively are often positively and negatively associated with stream condition, varied by as much as 60% among years within a single stream. Clearly, such dramatic shifts in composition could influence the outcome of status assessments based on these commonly used metrics. Given that many stream assessment programs infrequently resample test sites, our results suggest that compositional metrics have a high probability of generating type I and type II errors regarding the status of a stream. Continued application of metrics describing taxonomic composition should thus be assessed with the potential effect of temporal variability in mind.

A second implication of our findings presents a possible solution to the aforementioned issue of potential assessment errors related to substantive variation in composition-based metrics. Indeed, our assessment of the two tolerance-based metrics, CEFI and FBI, suggests that these metrics describing ecological traits may be less sensitive to interannual variations in community composition and provide a more consistent estimate of the stream condition through time. This finding supports calls for increased adoption of descriptors of community function using trait-based approaches (e.g., [54]), based on the principle that compared taxonomic measures traits are more strongly linked to habitat-filtering effects (sensu [55]) and will be insensitive to taxonomic replacements where functionally redundant taxa are substituted as a result of stochastic events. However, our ability to draw strong conclusions around this idea were limited by the fact that the monitoring data we had access to for this study were only identified to the family level, making it difficult to generate high-quality trait assignments. Indeed, the widespread use of family-level identification by many benthic biomonitoring programs (e.g., CABIN; [56], AUSRIVAS; [57]) continues to inhibit the testing and adoption of traits in many jurisdictions as does the availability of high-quality trait information for many species and genera, although the latter is incrementally being addressed (e.g., [58,59]). Thus, we would recommend that future studies, where longer-term datasets have been collected using genus- or species-level identifications, explore the degree of interannual variation in trait-based compositions to address this knowledge gap.

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