Evaluation of biotic responses to urban stream restoration: a comparison of the sampling methods of chironomid pupal exuviae vs. benthic invertebrates

Susan Gresens[1] and Dennis Genito[2]

[1]*Department of Biological Sciences, Towson University, 8000 York Road, Towson, Maryland 21252-0001, USA.* sgresens@towson.edu *Corresponding author.*
[2]*Watershed Restoration Section, Baltimore County Department of Environmental Protection and Sustainability, 111 W. Chesapeake Ave., Towson, Maryland 21204, USA.* dgenito@baltimorecountymd.gov

Abstract

Stream restoration in the Mid-Atlantic USA is intended to reduce delivery of nutrients and sediment to Chesapeake Bay. However, the macroinvertebrate communities of urban streams have shown little improvement following restoration. To detect biological responses to restoration of Cloisters Branch, two sampling methods and two analytical approaches were compared. The restoration minimized disturbance of the existing forest buffer and wetlands to the extent possible during construction. Concurrent sampling using benthic D-net and collection of floating chironomid pupal exuviae (CPEX) occurred once pre- (2013) and twice post-restoration (2023 and 2024) at five stations on Cloisters Branch. Additional CPEX sampling of Cloisters Branch and three non-restored control streams, for two years pre- and post-restoration, in a Before-After-Control-Impact design allowed us to distinguish a restoration response from other sources of interannual variation. Multimetric analysis of D-net samples indicated declines of biotic integrity post-restoration. In contrast, multivariate analyses of D-net and CPEX samples showed significant taxonomic responses to restoration. Lower conductivity in Cloisters Branch facilitated a strong increase in CPEX diversity and abundance of Chironominae. Post-restoration increase in stability and abundance of epilithic algae suggests that restoration improved physical habitat stability. Temporal and spatial variation in conductivity showed that road salt deicers were a greater biotic stressor than nutrients.

Introduction

Human activity within the watershed degrades the physical, chemical and biological condition of streams, especially in urban areas with a high degree of impervious land cover. The 'urban stream syndrome' (Walsh et al. 2005) summarized a pattern of increased stormflow, stream bank erosion, sediment deposition and loading of nutrients and contaminants in streams subject to urban runoff. Stream channel degradation and removal of riparian vegetation have resulted in loss of aquatic biodiversity even at low levels of watershed impervious area (IA) (Booth and Jackson 1997, Gresens et al. 2007, Cuffney et al. 2010; Wang et al. 2012, Bohus et al. 2023). Physical restoration of streams is increasingly used to decrease export of nitrogen and sediment and reduce flooding (Doll et al. 2020). Stream restoration is a preferred best management practice in urban streams of the Chesapeake Bay watershed, USA (Wood et al. 2021). Restoration has been shown to increase instream and riparian denitrification rates (Kaushal et al. 2008) and to reduce nitrogen export (Filoso and Palmer 2011). Restoration projects have also stabilized stream banks and promoted riparian forest regrowth (Christenson et al. 2024), and improved floodplain soils (Galella et al. 2024). However, despite large financial investments there has been limited evidence of improvement in benthic macroinvertebrate communities following restoration. A survey of European rivers subject to floodplain restoration found significant improvement in habitat diversity across the floodplain, at sites sampled a long time (between 5-13 years) after restoration (Jähnig et al. 2010). In contrast, macroinvertebrate diversity did not respond consistently to improved habitat within the wetted channel. Studies comparing restored and unrestored urban streams with forested reference sites found no effect of restoration on multi-metric indices and diversity of urban macroinvertebrate assemblages (Violin et al. 2011, Stranko et al. 2012). Multimetric benthic indices of biotic integrity (B-IBI) are widely used to assess stream condition and inform management decisions (Kerans and Karr 1994). Yet the reliance of BIBI scores on taxa sensitive to a low degree of urbanization, e.g., Ephemeroptera, Plecoptera and Trichoptera (EPT) (Purcell et al. 2009, Coles et al, 2012) may reduce their response to change in tolerant benthic communities.

Two approaches are used in the design of stream restorations: natural channel design and floodplain reconnection. Natural channel design applies structures composed of stone and wood to areas of high erosion stress, with varying degrees of bank and floodplain grading and stream channel realignment. In floodplain reconnection, eroded stream banks are graded down and/or the stream bed is raised to promote frequent overbank flows and increased stream and floodplain interaction. Restoration designs typically combine these approaches due to the constraints imposed by human development, e.g., sewer lines, overhead utilities, roads, housing, and commercial land use, which constrict the riparian zone. In some cases, existing stream buffers are partially removed to accommodate the channel realignment and bank grading that are needed to complete the work.

Cloisters Branch, a stream in suburban Baltimore County, MD, USA, was exceptional for retaining a riparian zone with mature forest and wetlands. The stream was restored in 2018 to increase interaction of the stream channel, groundwater and floodplain, and thus increase stream baseflow and hydrologic retention, thereby promoting nutrient removal and improving instream physical habitat. By design, the existing riparian forest and wetlands were minimally disturbed and access to the site was through utility rights-of-way and other previously disturbed areas. In this design, the channel bed was raised, cobble riffles were installed, and pools were reshaped and deepened (Biohabitats 2016).

To determine whether this physical habitat manipulation would result in a consistent positive biotic response to stream restoration, we compared two different methods for sampling macroinvertebrates pre- and post-restoration. The first method used a D-net to sample the entire macrozoobenthic community following standard protocol (Harbold et al. 2024). The second method was the collection of floating chironomid pupal exuviae (CPEX) to measure the emergence of adult Chironomidae from the stream (Wilson and Bright 1973). As sensitive EPT taxa are eliminated by urban stressors, chironomid larvae increase in relative abundance (Gresens et al. 2007, Hopkins et al. 2022). However, chironomid genera and species vary in their tolerance of urban stressors such as salinity, sediment-bound metals and pesticides (Wright et al. 1996, Ruse et al. 2000, Gresens 2010), and may provide insight into the nature of environmental stress. We hypothesized that in urban streams dominated by chironomid larvae, CPEX would be sufficient to detect biotic responses to stream restoration without the need to sample the entire benthic macroinvertebrate community. To validate the results of our CPEX survey, it was necessary to make a quantitative comparison of CPEX data with data from the standard benthic sampling method.

Our first goal was to identify a biotic response to restoration distinct from other sources of environmental variation. A CPEX survey was conducted on Cloisters Branch and three nearby streams within the local watershed for 2 years pre-restoration and 2 years post-restoration. This survey followed a multiple Before-After-Control-Impact design (MBACI) (Steward-Oaten et al. 1986), Martin et al. 2012) in which the three additional streams functioned as controls for the restoration of Cloisters Br.

Our second goal was to compare the results of CPEX sampling with those from the standard benthic monitoring method. To assess sampling efficiency, indices of taxa richness, diversity and relative abundance of shared chironomid genera were compared. We also compared the results obtained from benthic indices of biotic integrity (B-IBI) scores, vs, multivariate analysis of data from each sampling method.

Our third goal was to document the ecological regime that these taxa must tolerate. Environmental variables measured in Cloisters Branch and the control streams include seasonal changes in water chemistry and nitrogen, as well as riparian canopy cover and attached algal abundance.

Methods

Study Sites and Survey Design

Cloisters Branch and the three additional study streams are first-order tributaries of Towson Run, located in Towson, Maryland, USA, in the Eastern Piedmont physiographic province (Fig. 1).

The study streams run through the Towson University campus, where the surrounding watershed is dominated by institutional and high-density residential land uses. Cloisters Branch, subject to restoration, emerges from a stormwater outfall and was divided into upper and lower reaches by the confluence with the outfall of another "buried" stream. The catchment area of upper Cloisters Branch was 95.8 ha, with 52% impervious area (IA) and 15% forest cover. The total catchment area of the restored section of Cloisters Branch, including both reaches, was176.1 ha, with 53.7% IA and 11% forest cover (U.S. Geological Survey, 2019). Prior to restoration, Cloisters Branch was characterized by an incised channel with eroded meanders and excess fine sediment, but it had retained a mature, forested riparian zone and as-

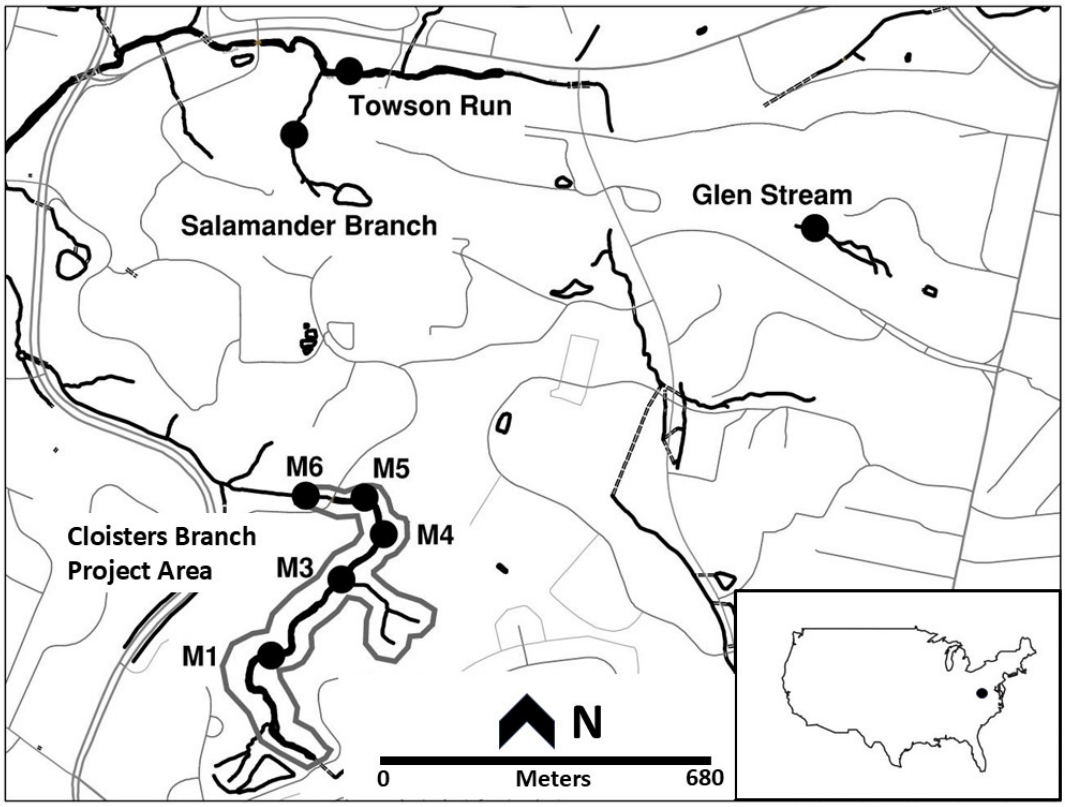

Figure 1. Study location in central Maryland. The Cloisters Branch restoration area is outlined and sample sites on restored and unrestored control streams are marked with dots. Streams are indicated with bold lines and roads with fine lines.

sociated wetlands. Specific enhancements to the stream included installation of 64 riffle-pool or cascade-pool sequences to improve bedform and sediment transport, avoidance of 40 large, streamside trees, and horizontal realignment of approximately 213 m of stream to improve planform. The riffles and cascades were placed to either raise (ca. 274 m of stream channel) or lower (ca. 165 m) the stream bed to establish overbank flow and floodplain reconnection. Tree removal was avoided to the maximum extent possible, but where trees were cleared 2,039 new trees and 3,008 shrubs were planted.

The Before-After-Control-Impact design for detection of a planned perturbation (Green 1979, Stewart-Oaten et al.1986, Underwood 1994) accounts for biological variation in time and space that could mask detection of an impact. In our study, *M*BACI refers to pre- and post-restoration sampling of *m*ultiple control streams in addition to Cloisters Branch, and to our *m*ultivariate analysis of invertebrate data. Cloisters Branch was the restored "impact" stream. Following Baltimore County protocols, five sampling stations were defined: M1, M3, M4 on the upper reach and M5 and M6 on the lower reach of Cloisters Branch (Fig. 1) to assess change in the benthic invertebrate community from before to after restoration. The purpose of additional control streams was to represent change over time in chironomid assemblages of urban streams comparable to Cloisters Branch, but which did not undergo physical habitat restoration during the period of study. We expected that floods, snowmelt events and droughts would impact the biota of local streams similarly, which could mask a restoration response in Cloisters Branch. We did not believe it would be possible for restoration to return Cloisters Branch to the condition of a pristine forest stream. Our more realistic expectation was that physical restoration of the channel would lead to increased diversity of relatively tolerant invertebrates, in response to improved microhabitat diversity and greater hydrologic stability.

Three nearby streams were the MBACI controls for restoration: Towson Run, Salamander Branch, and Glen Stream. The Towson Run watershed defined by our sampling site had a catchment area of 266.8 ha, with 56.3% IA and 9% forest cover (U.S. Geological Survey 2019). Salamander Branch was the smallest stream, with a catchment area of 10.4

ha; it drained parking lots and its deeply incised channel ran downhill through a forested area to join Towson Run downstream of our sampling reach. Glen Stream had a catchment area of 22.8 ha, with 56% IA and 15% forest cover, as it emerged from a stormwater outfall and flowed through the University campus arboretum in a deeply incised channel shaded by buildings and trees.

Sampling Methods

CPEX and D-net sampling occurred within a mid-April to mid-May sampling window, when streams were at baseflow, at least 2 days since the previous rainfall. Although the Cloisters Branch restoration occurred in 2018, post-restoration sampling was intentionally delayed to allow the stream to recover from physical disturbance. Samples for the CPEX-MBACI study were collected for two years pre-restoration (2013, 2014) and two years post restoration (2023, 2024) at the 5 sites on Cloisters Branch and at one site on each of the three control streams. This yielded 8 CPEX samples collected on the same day per date (although a couple of Cloisters Branch stations were not sampled in May 2013). Samples for comparison of D-net and CPEX methods were collected simultaneously at the five Cloisters Branch stations for one year pre- (2013) and two years post-restoration (2023, 2024) due to budget limitations. The efficiency of collection and processing of CPEX samples made the MBACI survey feasible, as it was originally envisioned as an undergraduate research project for Towson University students.

Benthic macroinvertebrates were sampled, subsampled, and identified following Maryland Biological Stream Survey (MBSS) methods (Harbold et al. 2024, Resource Assessment Service 2024) at five 75 m long stations within the reach of Cloisters Branch subject to restoration (Fig. 1). Within each 75 m station, benthic macroinvertebrates were collected using a 500 μm mesh D-net (30 cm width) to sample 930 cm^2 of the best available habitat. Material was preserved in the field using 95% ethanol and returned to the laboratory for sub-sampling and identification. D-net samples were sub-sampled by removing all individuals from randomly selected grids in a standardized, gridded tray until a total of at least 120 organisms were removed from each sample. Macroinvertebrates were identified to genus or the lowest possible taxon.

Chironomid pupal exuviae were collected using a shallow pan to skim exuviae from the water surface where floating material accumulated. Exuviae were concentrated on a 63 μm sieve and preserved in 70% ethanol. Sampling effort was standardized by collecting for a 10-minute period at each station, although at times of extreme abundance of exuviae collection lasted 5 minutes. Exuviae were sorted from debris and counted at 12x magnification (Gresens 2010). Abundance of exuviae was standardized to a 10-minute sample; this was estimated for 5-minute samples by doubling their abundance. In some cases, 10-minute samples yielded multiple thousands of exuviae so it was necessary to subsample, given the time required for exuviae identification. Random subsamples were obtained by suspending a sample in a larger, known volume of water, stirring well and rapidly pouring off half to a third of the volume. The volume of the subsample was also measured so that the abundance of the entire sample could be estimated mathematically. Subsamples produced at least 400 exuviae and usually more, which proved sufficient for taxonomic analysis. Using a stereomicroscope, exuviae were identified to genus using keys by Wiederholm (1986) and Ferrington and Berg (2019). *Corynoneura* and *Thienemanniella* were combined as Tribe Corynoneurini, whereas congeneric species of *Micropsectra*, *Paratanytarsus* and *Tanytarsus*, easily distinguished at 12x, were counted as separate taxa.

Our sampling method resembles that of Wilson and Bright (1973) who sampled exuviae in rivers using mesh nets supported by floats to collect surface drift for known periods of time. Our sampling method and survey design are similar to that of Ruse (2025) who used the Chironomid Pupal Exuviae Technique to sample farm ponds in an MBACI design. These studies concluded that the relative abundance of chironomid genera in such samples are a consistent measure of assemblage composition. Our CPEX method is based on that of Ferrington et al. (1991), who compared CPEX samples with D-net samples of chironomid larvae, and showed that CPEX samples were more efficient in detecting chironomid species richness than D-net samples, and that the processing and identification of CPEX took one third of the time that was needed for D-net samples.

Water quality data were measured in the field at baseflow using a YSI Pro 2030 meter, including specific conductivity, dissolved oxygen as percent saturation, pH, and water temperature. These measurements were taken at monthly intervals from October 2012 to November 2013, and intermittently thereafter, in conjunction with CPEX and periphyton sampling. A limited number of measurements were made at the two outfalls on Cloisters Branch.

To indirectly assess potential limitation of attached algal production and resource supply for the biota, we conducted seasonal sampling of attached algal abundance, total nitrogen (TN), total carbon (TC) concentration and % canopy cover at all eight reaches. Water samples for nutrient analysis were frozen until analysis on a Shimadzu TOC-L total organic carbon analyzer with a TMN-L total nitrogen measuring unit. Total phosphorus (TP) data were only available from samples before, during, and after one snowmelt/flood event pre-restoration. Unfiltered water samples were subject to persulfate digestion, followed by analysis of orthophosphate by the ascorbic acid-molybdenum blue method (Wetzel and Likens 1991).

Attached algal abundance was quantified as algal thickness score (ATS) following Stevenson and Bahls (1999). Using a plexiglass-bottom viewing bucket inscribed with 5 sampling dots, the thickness of epilithic algal mats was scored into one of six categories based on modal length of algal filaments. At least 100 sample points were measured at random points along a zig-zag path across the stream reach. Each thickness category was assigned a numeric weight (from 0.5 -5) such that an ATS score is the weighted average of the number of points in each length class. Riparian canopy cover before and after restoration was estimated using a spherical densiometer.

Data Analysis

The Maryland Benthic Index of Biotic Integrity (BIBI) (Southerland et al. 2005) was calculated using Excel, as were other basic data manipulations. Wilcoxon signed rank tests were used in paired comparisons of pre-post changes in BIBI, percent Chironomidae and Simpsons diversity at the sites (n = 5) on Cloisters Branch. Kruskal-Wallis tests were used to assess differences in conductivity, TC and TN between Cloisters Branch and control streams.

Simpsons diversity values and multivariate analyses were conducted using PC-ORD v. 7 software (McCune and Mefford 2016). Rarefaction analysis (Colwell et al. 2012) was used to estimate taxa richness for CPEX samples randomly resampled to a smaller sample size. Rarefaction results were obtained using iNEXT software (Hsieh et al. 2013). The Chao 1 estimate of intrinsic taxa richness of CPEX assemblages uses the observed number of singletons (number of taxa with only one individual counted) and doubletons (taxa with only 2 individuals) to calculate the number of taxa that should have been observed if sampling were "complete", i.e., no taxa were missed due to random sampling of the community (Chao 1984). Chao 1 estimates and their 95% confidence intervals were obtained using SpadeR software (Chao et al. 2015).

Both D-net and CPEX sampling methods are semi-quantitative: they employ standard effort in the field: 20 D-net jabs or 10-minute collection of CPEX. The total abundance of organisms in CPEX samples varied greatly, whereas D-net samples subject to fixed-size subsampling. Therefore, all data were input as relative abundance (taxon abundance as a proportion of the sample total) in analyses of taxonomic composition. Multivariate response variables were used, assuming that some taxa would respond to the environment in similar ways so that an assemblage-based analysis should be more sensitive to the effects of restoration.

Non-metric multidimensional scaling (NMDS) ordinations were used to describe variation in taxonomic composition among samples. Stress values, a diagnostic, were calculated according to the Kruskal stress formula 1 and expressed as a percentage: stress values in the range of 10 to 15 are considered satisfactory for interpretation (McCune and Mefford 2016). To interpret the ordination axes in terms of taxa, correlations between axis score and the relative abundance of each taxon were calculated for each data point. Correlations were reported for taxa most influential to the ordination (i.e., correlations arbitrarily greater than 0.4 in absolute value). Changes in relative abundance of individual taxa were also inspected for comparison with changes in assemblage composition.

Changes in taxonomic composition were analyzed with Multi-response Permutation Procedures, MRPP (Mielke and Berry 2001), based on a matrix of relative Sørensen distance between samples. If samples within each group (e.g., Cloisters *Pre* vs. *Post* and Control *Pre* vs. *Post*) are more similar to one another (but not to samples from other groups) they will each have a small *within-group distance*. Conversely, if samples are highly variable, *within-group* distances will be larger, and the groups are unlikely to have distinctive taxonomic composition. The test statistic, *Delta*, the weighted mean *within-group* distance, is expected to be large under the null hypothesis where taxonomic composition of samples is random within and among groups. If groups do differ significantly in composition, the observed *Delta* will be small. The probability of *Delta* under the null hypothesis is determined through random permutations of samples among groups. Given that *p*-values from MRPP tests are often very low, it is easier to use the effect size, *A,* to examine the degree of difference between

groups. If all assemblages in a group are identical, $A = 1$, whereas $A = 0$ for random variation among all groups. Distinctiveness of sample composition within groups was also shown graphically using NMDS ordinations.

Kruskal-Wallis tests were used to assess differences in conductivity, TC and TN between Cloisters Branch and control streams. Separate tests were conducted on data from pre-restoration and post-restoration periods separately.

Results

Standard indices of biotic condition

Standard measures of stream biotic condition failed to show a consistent response of Cloisters Branch macrobenthos to restoration. Pre-restoration (2013) BIBI values rated reaches M1, M4 and M6 as "poor" while M3 and M5 rated "very poor". Post-restoration BIBI scores indicated declines in stream condition: in 2023, M4 and M6 remained "poor", but in 2024 all Cloisters Branch reaches had declined to the "very poor" category (Fig. 2c). Percent Chironomidae, one of the metrics contributing to the BIBI score, increased strongly post-restoration at M1and M4 but showed lesser, inconsistent responses at the remaining reaches. Dominance of chironomid larvae was highest in 2024 (Fig. 2b). Reach M5 maintained high percent chironomids both pre- and post-restoration. The five Cloisters sites showed no significant pre-post restoration change in either BIBI scores or percent Chironomidae (Wilcoxon signed ranks test, n = 5, $p > 0.05$). Diversity of macrobenthos, measured by Simpson's Index (Fig. 2a), was relatively high at all reaches, ranging from 0.75 to 0.91 out of a maximum of 1.0. The most consistent increases in diversity were observed post-restoration. Pre-post increases in diversity were only significant (Wilcoxon signed ranks test $p = 0.05$) for 2023 comparisons, but not for 2024.

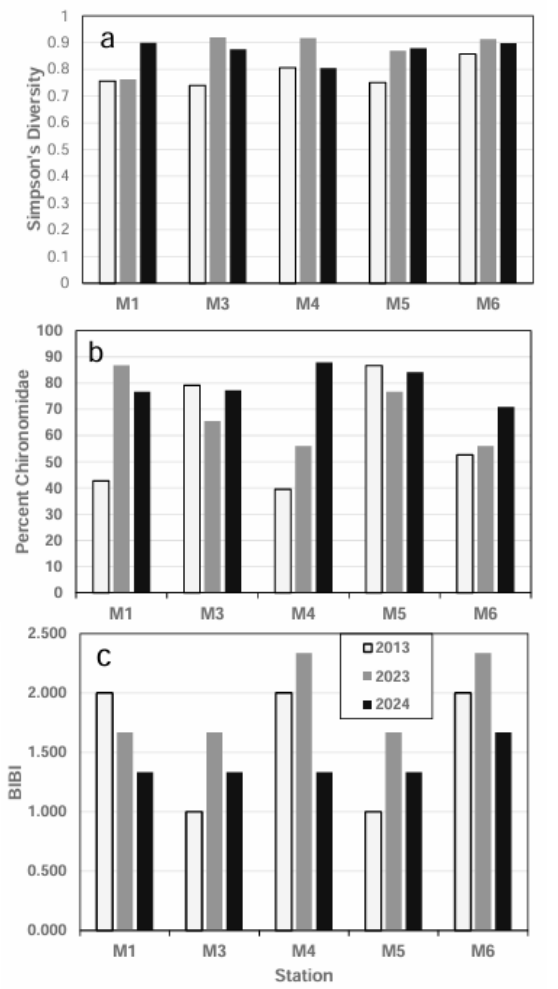

Figure 2. Cloisters Branch D-net sample indices pre-restoration (2013) and post-restoration (2023 and 2024). Reaches from upstream to downstream are listed from left to right: a) Simpsons diversity index, b) Relative abundance of chironomid larvae, c) Benthic Index of biological integrity (BIBI). Diversity increased significantly ($p = 0.05$) from 2013 to 2023, but not from 2013 to 2024. No significant pre-post differences were observed for the other variables (Wilcoxon signed ranks test, n = 5).

D-net samples and benthic response to restoration

Taxonomic composition clearly distinguished pre-restoration vs. post-restoration D-net samples. Three groups of samples corresponding to year: 2013, 2023 and 2024, were subject to a MRPP analysis which found a significant difference in assemblages between years ($p = 0.00019$, $A = 0.1594$). Ordination (Fig. 3) showed pre-restoration D-net samples with distinctly higher scores on axis 2 than for post-restoration (NMDS, $p = 0.008$ and final Stress = 13.4). Post-restoration, axis 2 scores for M5 and M6, lower Cloisters Br., were noticeably higher than for upper Cloisters Branch (M1, M3, M4) in both 2023 and 2024. Ordination distances were strongly associated ($R^2 = 0.772$) with distances in the original dataset, although axis 2 accounted for a smaller amount of biotic variation ($R^2 = 0.162$).

The following taxa were associated with pre-restoration, based on strong positive correlations with axis 2: *Baetis*, Elmidae, Ceratopogonidae, Simuliidae, *Tipula*, Amphipoda and Turbellaria (Table 3). Pre-restoration samples were also associated with several orthoclad chironomids: *Chaetocladius*, *Cricotopus, Hydrobaenus*, *Limnophyes*, and *Orthocladius*. Post-restoration taxa, with negative correlations to axis 2 (Table 3), included Hydropsychidae, Zygoptera, and chironomids of sub-

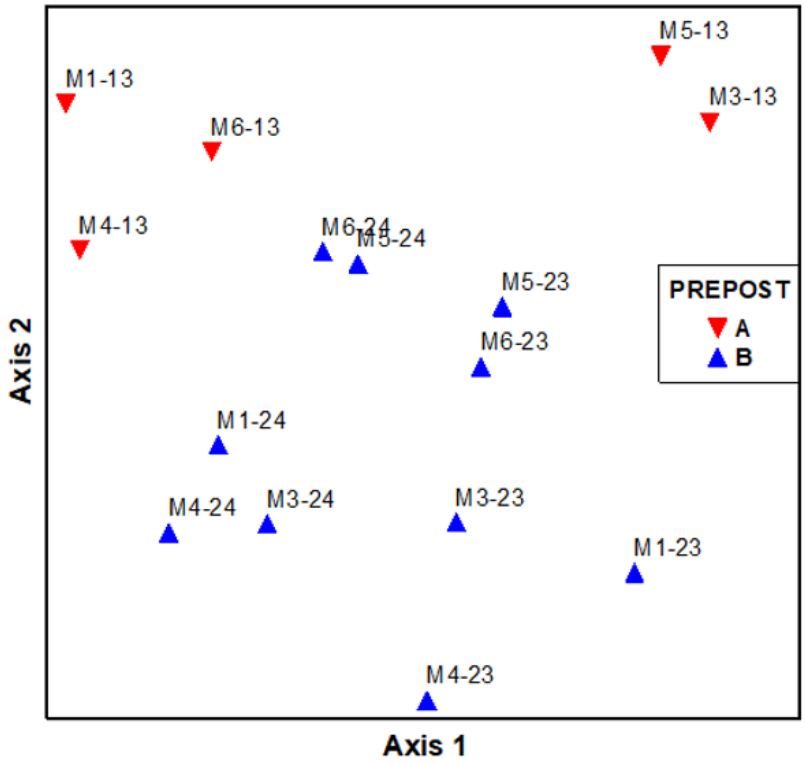

Figure 3. NMDS ordination of Cloisters Branch macroinvertebrate assemblages in D-net samples ($p = 0.008$). Legend: pre-restoration (2013) samples = "A" and post-restoration (2023, 2024) samples = "B". Benthic samples differed significantly between years (MRPP, $p < 0.0001$).

families Chironominae and Tanypodinae.

Comparison of sampling methods

D-net and CPEX methods were compared in terms of sample size, number of organisms per sample unit (N), and number of taxa detected (S) at Cloisters Branch (Table 1). Benthic richness ranged from 14 - 20 genera pre-restoration and increased to 18 - 26 post-restoration. Reaches M3, M5 and M6 showed consistent increases in the number of taxa observed. Post-restoration taxa richness of CPEX samples was more often slightly greater than richness of all benthic taxa combined. Chironomid richness based on CPEX, i.e., adult emergence, was almost always greater than larval chironomid richness in benthic samples but this is biased by sample size. The number of individual exuviae per CPEX sample varied by two orders of magnitude, whereas D-net samples were subsampled to a fixed goal of 120 organisms. Less common taxa are more likely to be included in samples with more individuals; therefore, rarefaction was used to estimate CPEX-r richness per random subsamples of 120 exuviae. Taxa richness of rarefied CPEX-r was almost always substantially lower than total benthic richness (Table 1)

Taxonomic composition of paired D-net vs. CPEX samples were on average 61% different when Sørensen distance was calculated with relative abundance (Fig. 4). Sampling method had less effect on presence-absence data, with only 39% average dissimilarity. This shows that both methods detect many of the common taxa. However, the D-net method under-sampled small-bodied larvae such as *Corynoneura* and *Thienemaniella* which were rarely recorded in D-net samples, although their combined abundance in paired CPEX samples was often 50 to over 200 times greater.

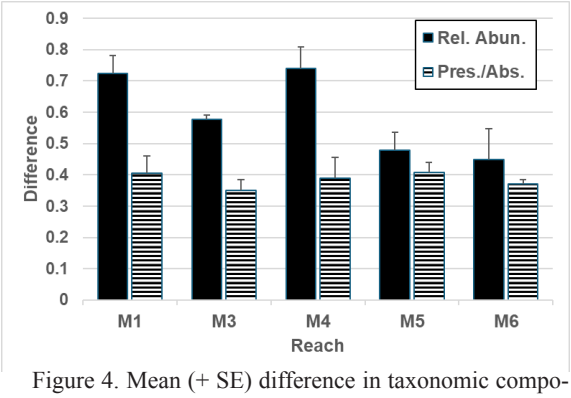

Figure 4. Mean (+ SE) difference in taxonomic composition of D-net vs. CPEX samples collected concurrently at the same sites on Cloisters Branch. Difference was based on either relative abundance or presence/absence of taxa.

Table 1. Comparison of taxa richness (S) in paired benthic D-net and CPEX (exuviae) samples from Cloisters Branch pre- and post-restoration. Sample size (N), rarefaction of CPEX sample size to 120 organisms (CPEX-r). Larval chironomids only in D-net samples (B. chiro). Taxa richness is either observed or estimated, depending on method. Confidence interval for rarefied richness estimate (95% CI).

	Pre-restoration 2013				Post-restoration 2023				Post-restoration 2024			
Reach	N	Method	S	95% CI	N	Method	S	95% CI	N	Method	S	95% CI
M1	428	CPEX	14		133	CPEX	15		924	CPEX	25	
	120	CPEX-r	10.7	9.6-11.7	120	CPEX-r	14.5	11.7-17.3	120	CPEX-r	15.0	14.0-16.1
	124	Benthic	19		128	Benthic	18		124	Benthic	22	
		B. chiro	14			B. chiro	9			B. chiro	13	
M3					230	CPEX	26		708	CPEX	22	
					120	CPEX-r	21.3	18.7-23.9	120	CPEX-r	13.8	12.7-15.0
	91	Benthic	14		119	Benthic	24		126	Benthic	22	
		B. chiro	8			B. chiro	15			B. chiro	13	
M4	1020	CPEX	14		168	CPEX	23		322	CPEX	20	
	147	CPEX-r	9.5	8.7-10.3	120	CPEX-r	20.9	18.4-23.5	120	CPEX-r	14.6	12.7-16.4
	147	Benthic	20		136	Benthic	20		123	Benthic	21	
		B. chiro	14			B. chiro	8			B. chiro	14	
M5	317	CPEX	11		421	CPEX	29		838	CPEX	24	
	120	CPEX-r	8.5	7.0-10.0	120	CPEX-r	19	16.8-21.1	120	CPEX-r	13.9	13.0-14.9
	98	Benthic	14		137	Benthic	20		137	Benthic	20	
		B. chiro	6			B. chiro	13			B. chiro	12	
M6					1188	CPEX	28		283	CPEX	17	
					120	CPEX-r	17.8	16.9-18.6	120	CPEX-r	12.8	10.9-14.8
	114	Benthic	17		134	Benthic	26		117	Benthic	21	
		B. chiro	13			B. chiro	16			B. chiro	11	

MBACI and chironomid response to restoration

Chironomid emergence, i.e., CPEX sample size, varied by three orders of magnitude across Cloisters Branch and the three Control streams (Table 2). Emergence sampled in 2014 was low across all four study streams. Despite high interannual variation in sample size, observed taxa richness at all Cloisters Branch reaches was consistently higher post-restoration than pre-restoration, with no overlap in values (Table 2). Chao 1 richness estimates also showed this pattern of increased richness post-restoration while accounting for sample size bias. Simpson's diversity, more robust to sample size variation, showed non-overlapping increase in values post-restoration at all Cloisters Branch reaches. Towson Run had higher values of diversity in 2023 and 2024. Although richness values were the highest in 2023, in 2024 richness was lower and within the range of 2013-14 richness values. Fewer samples were available from Salamander Branch and Glen Branch and sample sizes were low. In Salamander Branch both diversity and richness were higher in 2023-24 than in 2014. Interannual variation in Glen Stream was so great that the range of diversity and richness values overlapped between time periods. An opportunistic CPEX sample, GLEN 2024b, was collected two weeks after GLEN 2024a, during regular algal sampling, because emergence was obviously much higher than seen there before (no peak emergence was noted at the other sample sites on this date). This illustrates the impact of temporal variation in sample size and number of sampling dates on detecting trends in richness. Richness and diversity may be less sensitive as indicators of a response to restoration.

Chironomid emergence along Cloisters Branch underwent significant change in taxonomic composition from pre- to post restoration periods. The test of a restoration effect requires that CPEX samples from Cloisters Branch show consistent change in composition from pre- to post restoration, while the control streams (sampled concurrently) show very different changes in composition over time, unrelated to the restoration of Cloisters Branch. A highly significant difference in composition of

Table 2. Emergence of chironomid pupal exuviae from Cloisters Branch and three reference streams during pre- and post-restoration periods. Abundance in 10-minute samples (N), observed taxa richness (Obs S), estimated richness (Chao1) and its associated 95% CI, and Simpsons diversity (SimDiv). *GLEN 2024b was collected 2 weeks after GLEN 2024a when a peak of emergence was observed during algal sampling. This demonstrates that emergence can exceed that measured at the limited number of prior sampling dates.

Reach-Year	N	Obs S	Chao1	Chao 95% CI	SimDiv
M1 2013	428	14	14.0	14.0-16.6	0.667
M1 2014	22	4	5.0	4.1-16.7	0.517
M1 2023	133	15	18.3	15.5-36.9	0.744
M1 2024	924	25	32.5	26.3-67.5	0.786
M3 2023	230	26	28.0	25.5-42.9	0.876
M3 2024	708	22	24.5	22.4-39.0	0.779
M4 2013	1020	14	14	14-16.0	0.381
M4 2014	28	5	7.9	5.4-28.6	0.367
M4 2023	168	23	24.0	22.3-35.9	0.906
M4 2024	322	20	22.0	20.3-33.9	0.780
M5 2013	317	11	11.2	11.0-15.8	0.566
M5 2014	56	4	4	4-6.1	0.198
M5 2023	420	28	32.2	28-54.5	0.893
M5 2024	838	24	33.3	25.9-68.9	0.833
M6 2014	208	9	14.0	9.8-41.0	0.121
M6 2023	1188	28	28	28-30.7	0.887
M6 2024	283	17	24.5	18.3-59.4	0.840
TOWS 2013	6820	14	13	13-13.3	0.578
TOWS 2014	415	8	8	8-9.8	0.549
TOWS 2023	320	16	18.0	15.4-37.9	0.681
TOWS 2024	2637	10	11	10.1-24.0	0.630
SALA 2014	38	3	3	3-4.5	0.342
SALA 2023	15	5	5	5-7.2	0.692
SALA 2024	230	10	10.3	10.0-15.9	0.507
GLEN 2014	24	3	3	3-3.5	0.497
GLEN 2023	4	4	--	--	0.75
GLEN 2024a	57	4	4	4-5.0	0.336
GLEN 2024b*	629	14	14.3	14.0-20.0	0.589

pre- versus post-restoration assemblages (MRPP, $p < 0.0001$, A=0.3235) was seen in Cloisters Branch. In contrast, "pre and post" control stream assemblages were highly variable and did not differ significantly (MRPP, $p = 0.6608$, A -0.0256). An ordination plot (NMDS, 2 dimensions, $p = 0.004$, stress = 12.7) shows pre-restoration intermingling of both Cloisters Branch and Control stream samples (Fig. 5). Post-restoration, Cloisters Branch samples were separated from the Control stream points, with lower values on axis 1, and moderate to high values on axis 2 (Fig. 5). Almost half of the 42 total chironomid taxa had a strong negative correlation with axis 1, thus were associated with Cloisters Branch post-restoration (Table 4).

Post-restoration CPEX richness increased in Cloisters Branch, as 22 new taxa were gained and only 1 taxon lost, for a total of 40 taxa in 2023-24. Changes in the abundance of chironomid taxa further explain the response of CPEX assemblages visualized in Fig. 4. Sixteen Chironomini taxa and eight Tanypodinae genera that were largely absent pre-restoration were well-represented in abundance data post-restoration. The dominant Orthocladinae taxa: Corynoneurini spp., *Eukiefferiella coerulescens* (Kieffer) and *Cricotopus* spp. (Table

Table 3. Ordination of benthic macroinvertebrate D-net samples from Cloisters Branch. Pearson correlation of sample scores on ordination axes with taxon relative abundance. Only taxa with r > 0.30 on an axis are reported. Taxa with negative correlation on axis 2 are associated with post-restoration samples.

Family (subfamily)	Genus	*r* axis 1	*r* axis 2
Baetidae	*Baetis*	-0.399	0.356
Hydropsychidae	*Cheumatopsyche*	0.157	-0.586
	Hydropsyche	0.231	-0.517
Philopotamidae	*Chimarra*	0.060	-0.556
Elmidae	*Stenelmis*	0.457	0.312
Lampyridae	Lampyridae larvae	-0.393	0.134
Ceratopogonidae	*Culicoides*	0.390	0.406
Chironomidae (Chironominae)	*Dicrotendipes*	-0.040	-0.543
	Micropsectra	-0.582	0.343
	Microtendipes	-0.005	-0.607
	Polypedilum	-0.336	-0.289
	Paratanytarsus	-0.354	-0.332
	Rheotanytarsus	-0.406	-0.003
Chironomidae (Diamesinae)	*Diamesa*	0.850	-0.003
Chironomidae (Orthocladiinae)	*Chaetocladius*	-0.375	0.413
	Corynoneura	-0.454	0.145
	Cricotopus	-0.278	0.463
	Eukiefferiella	-0.786	0.261
	Hydrobaenus	0.457	0.312
	Limnophyes	-0.469	0.503
	Orthocladius	0.734	0.499
	Paracricotopus	0.240	-0.419
	Parametriocnemus	0.261	-0.503
	Rheocricotopus	-0.590	0.347
	Thienemanniella	-0.626	-0.027
	Tvetenia	-0.580	-0.408
Chironomidae (Tanypodinae)	*Ablabesmyia*	0.129	-0.681
	Thienemannimyia gr.	0.160	-0.509
Simuliidae	*Simulium*	-0.629	0.373
Tipulidae	*Tipula*	0.519	0.343
Calopterygidae	*Calopteryx*	0.164	-0.642
Coenagrionidae	*Argia*	0.225	-0.835
	Enallagma	0.139	-0.476
Crangonyctidae	*Crangonyx*	-0.539	0.111
Ancylidae	“limpet”	0.076	-0.501
Turbellaria	“flatworm”	0.620	0.507

5), had disproportionately large increases in total abundance. Nevertheless, four taxa decreased in abundance, even as CPEX sample sizes trended larger post-restoration (Table 2); *Orthocladius obumbratus* (Johannsen), formerly a co-dominant, underwent a 73% decrease compared to its pre-restoration abundance in Cloisters Branch.

Towson Run assemblages experienced greater turnover: 9 taxa were gained and 5 lost for a total of 18 taxa present in 2023-24. The pattern of

Table 4. Ordination of Chironomidae. CPEX from Cloisters Branch and three control streams. Kendall *tau* correlation of sample scores on ordination axes with taxon relative abundance. Only taxa with r > 0.30 on an axis are reported. Taxa with negative correlation on axis 1 are associated with Cloisters Branch post-restoration.

Subfamily	Genus / Tribe /species	*tau*, axis1	*tau*, axis 2
Tanypodinae	*Ablabesmyia*	-0.635	0.166
	Conchapelopia	-0.341	-0.034
	Zavrelimyia	-0.071	-0.336
Diamesinae	*Potthastia*	-0.394	0.118
	Sympotthastia	-0.279	0.335
Orthocladiinae	Corynoneurini	-0.450	-0.139
	Cricotopus	-0.358	-0.239
	Eukiefferiella	-0.173	-0.520
	Nanocladius	-0.334	0.250
	O. Eudactylocladius	-0.203	-0.305
	Paracricotopus	-0.459	-0.140
	Parakiefferiella	-0.492	-0.180
	Parametriocnemus	-0.319	-0.153
	Rheocricotopus	-0.411	0.120
	Tvetenia	-0.596	-0.105
Chironominae	*Dicrotendipes*	-0.676	0.102
	Microtendipes	-0.541	-0.193
	Micropsectra polita	-0.321	0.014
	Paratendipes	-0.487	0.040
	Phaenopsectra	-0.616	0.123
	Polypedilum	-0.474	0.102
	Stictochironomus	-0.304	-0.315
	Paratanytarsus sp.1	-0.570	-0.144
	Rheotanytarsus	-0.400	0.111
	Tanytarsus signatus gr.	-0.534	0.103
	Tanytarsus cf. sepp	-0.404	0.333

change in the dominant taxa of Towson Run was opposite that in Cloisters Branch. *Orthocladius* increased, whereas Corynoneurini, *Cricotopus*, and *Eukiefferiella coerulescens* all decreased in abundance (Table 5). These four taxa, plus *Limnophyes*, were also the dominant taxa in Salamander Branch and Glen Stream, where their emergence was low but highly variable.

Conductivity

The upstream reach of Cloisters Branch (M1-M4) averaged 30%-40% lower conductivity compared to the downstream reach (M5, M6) due to the confluence of a buried tributary with mean baseflow conductivity of 1357 μScm^{-1} (n = 10) (Fig. 6). The control streams had much higher values of mean conductivity than Cloisters Branch. Kruskal-Wallis tests indicated significant differences in conductivity across the five reaches (upper and lower Cloisters Branch, and the three control streams) in both the pre-restoration ($\alpha < 0.001$) and post-restoration ($\alpha << 0.001$) time periods. Conductivity was highest in Salamander Branch and Glen Stream, which had the smallest catchments which were dominated by parking lots. There was a trend across all streams for lower mean conductivity in 2023-24 than in 2013-14.

Baseflow conductivity was significantly related to assemblage composition. Ordination of Cloisters Branch D-net benthos showed conductivity strongly correlated with sample scores on axis 2 ($r = 0.741$), but not on axis1 ($r = 0.169$). Pre-restoration D-net samples had the highest axis 2 scores whereas the lowest post-restoration scores were for upper Cloisters Branch sites (Fig. 3). Scores from the CPEX ordination were also related to conduc-

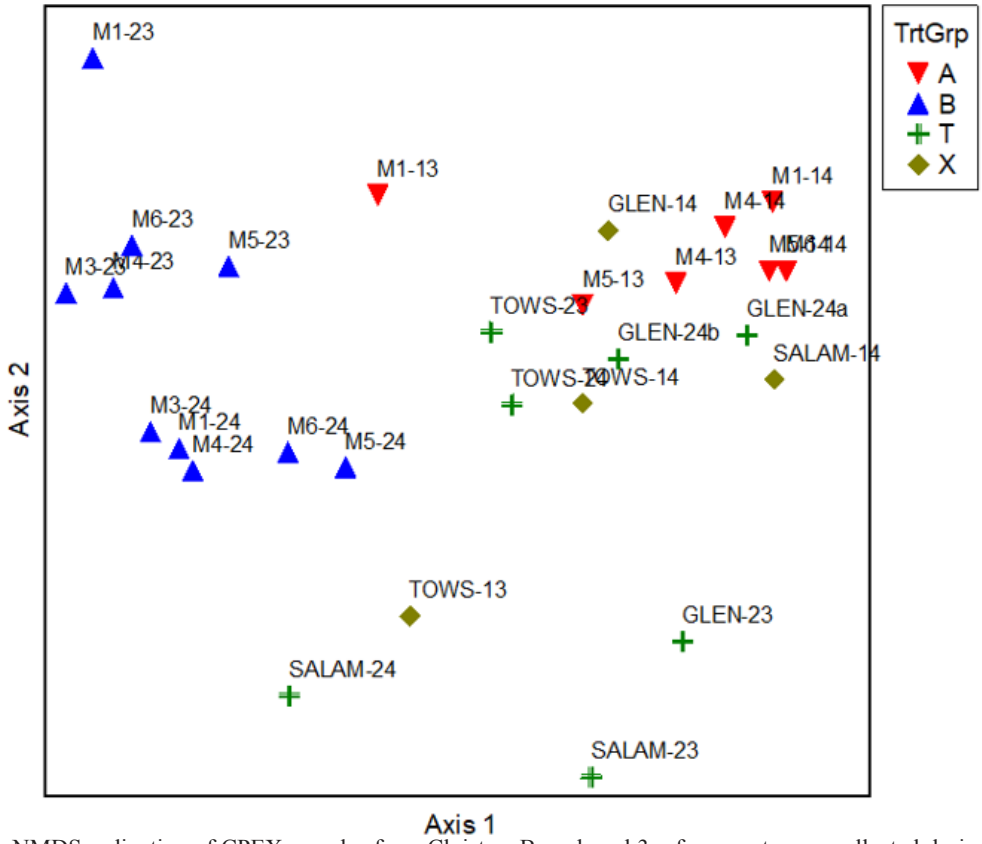

Figure 5. NMDS ordination of CPEX samples from Cloisters Branch and 3 reference streams collected during April/May, $p = 0.004$. Legend: "A" = Cloisters Branch pre-restoration, "B" = Cloisters Branch post-restoration, "X" = control streams during pre- period, "T" = control streams during post- period. Points labelled by reach and year. Pre-post restoration samples were significantly different only for Cloisters Branch (MRPP, $p < 0.0001$).

Table 5. The 12 most abundant CPEX taxa and their change in temporal abundance from 2013-2014 (Pre) to 2023-2024 (post) periods in Cloisters Branch (CLST) vs. Towson Run (TOWS). UP = increase, DN = decrease, np = not present, 0 = no consistent change.

		Pre-post change	
Taxon	Total N	CLST	TOWS
Cricotopus spp.	6745	UP	DN
Orthocladius obumbratus	4315	DN	UP
Eukiefferiella coerulescens	2472	UP	DN
Corynoneurini genera	1860	UP	DN
Tvetenia sp.	684	UP	0
Dicrotendipes sp.	626	UP	UP
Tanytarsus signatus gr. sp.	304	UP	np
Polypedilum spp.	243	UP	DN
Paratanytarsus sp.	210	UP	np
Limnophyes sp.	145	DN	DN
Micropsectra nigripila	89	DN	DN
Paracricotopus sp.	69	UP	np
Diamesa sp.	69	0	UP

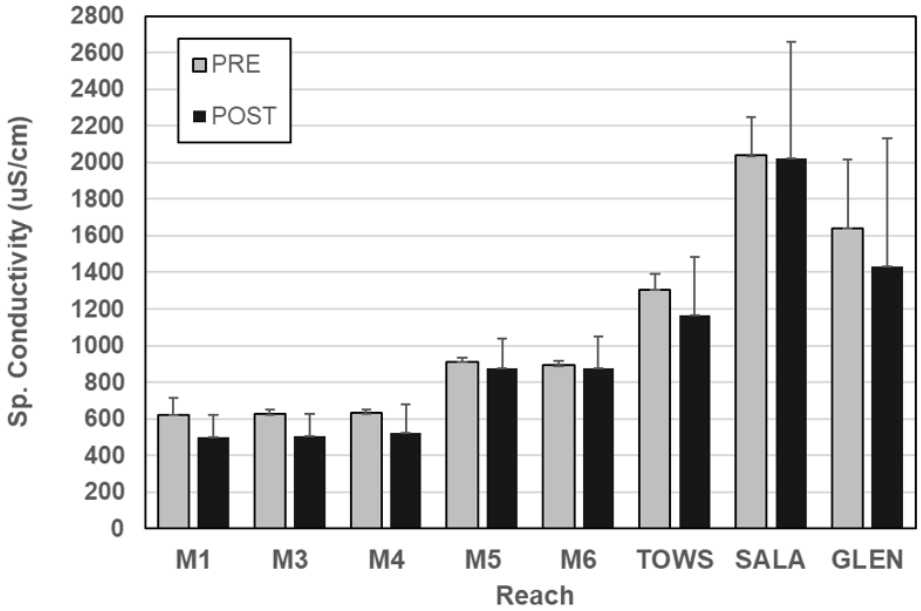

Figure 6. Means (+ standard deviation) of baseflow specific conductivity (μScm^{-1}) measured concurrently in Cloisters Branch (M1-M6), and control streams Towson Run (TOWS), Salamander Branch (SALA) and Glen Stream (GLEN) during pre-restoration (2013, 2014) and post-restoration (2023, 2024) periods.

tivity: sample scores on axis 1 had a positive correlation with conductivity (r = 0.404) whereas scores on axis 2 had a negative correlation with conductivity (r = - 0.538). Thus, Cloisters Branch CPEX samples lie in the upper half of Fig. 5, shifting left on axis 1 post-restoration, while control streams lie towards the right side of the diagram.

Nutrients

Most samples for TC and TN were collected in conjunction with attached algal thickness measurements. All sites showed substantial seasonal variation in TC: the highest values occurred from late July to November, as fallen leaves accumulated in-stream. Kruskal-Wallis tests showed that TC concentrations at Cloisters Branch sites were significantly lower than among the control streams ($0.005 < \alpha < 0.01$) pre-restoration (Fig. 7a). Although TC was lower in the control streams than in Cloisters Branch post-restoration, this was not a significant difference ($0.95 < \alpha < 0.975$).

Although mean TN concentration trended higher in Towson Run during both study periods, there were no significant differences in TN between the Cloisters Branch sites and the control streams during pre-restoration (Kruskal-Wallis test, $0.95 < \alpha < 0.99$). Although mean TN decreased post-restoration by 39-63% at upper Cloisters Branch sites and by 32% at lower Cloisters Branch, TN in Cloisters Branch was not significantly different from that in the control streams ($0.75 < \alpha < 0.90$). TN was lower in both Towson Run and Glen Stream post-restoration, therefore there was no evidence that stream restoration decreased baseflow nitrogen in Cloisters Branch.

It was not possible to incorporate total phosphorus (TP) analyses into the stream restoration study. Limited water column TP data were only available for 2013. Mean baseflow TP (n = 2) was lower in upper Cloisters Branch (9.6 μg L^{-1}) than in lower Cloisters Branch (12.5 μg L^{-1}) which was comparable to the control streams (12.8 – 18.2 μg L^{-1}). Stormflow TP was sampled at Cloisters Branch M5 on 3 days during a major snowstorm in 2013: baseflow TP pre-storm (10.0 μg L^{-1}) rose to 161.1 μg L^{-1} at the start of snowmelt, declined to 16.4 μg L^{-1} at peak runoff and returned to 11.7 μg L^{-1} at baseflow on day 4. In 2013, sewage input to the storm drain headwaters of Cloisters Branch was a measurable source of phosphorus: TP in the effluent averaged 29 μg L^{-1} (S.D. = 10.2, n = 2).

Periphyton, pH and dissolved oxygen

Epilithic algae, dominated by diatoms, were conspicuous in Cloisters Branch both before and after restoration. Riparian canopy cover was largely preserved during the restoration project in 2018, with less than 10% decrease in canopy cover at all stations except M3 where canopy was reduced by 16% (Table 6). Towson Run had the lowest canopy cover and Salamander Branch and Glen Stream had the highest canopy cover. Algal sampling dates were spread across seasons to encompass seasonal variation in average algal thickness scores (ATS)

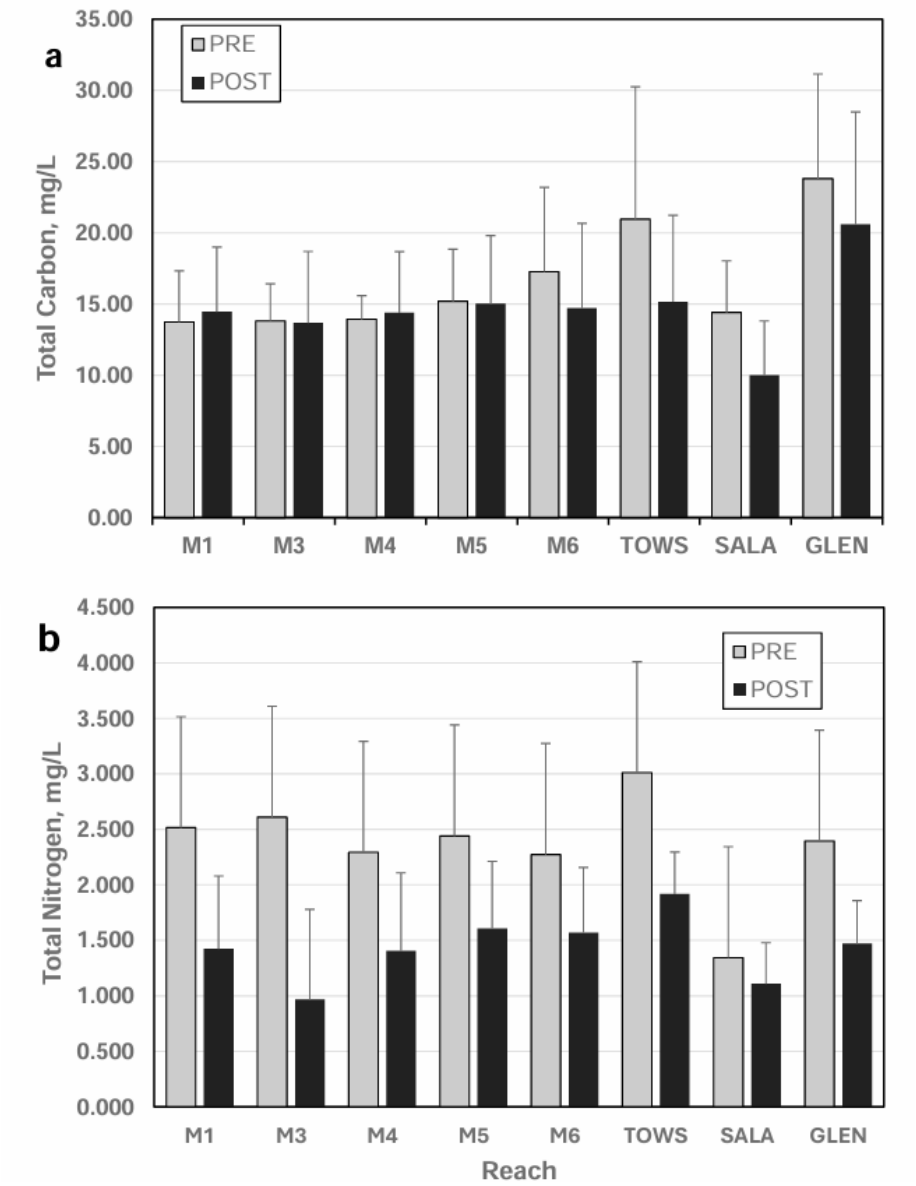

Figure 7. Mean concentrations, mg L^{-1} (+ standard deviation) of nutrients in stream water at baseflow, sampled seasonally pre- and post-restoration in Cloisters Branch (M1-M6) and control streams Towson Run, Salamander Branch and Glen Stream. 7a) Total dissolved carbon (organic + inorganic), 7b) Total nitrogen concentration.

(Table 6). Light limitation of algae was evidenced by spring algal blooms (February – early April, before leaf-out of trees) with ATS scores ranging from 3 to 3.8 (i.e., modal algal filament lengths from 2 mm to 2 cm) with patches of macroalgae, i.e., *Melosira* and filamentous green algae, reaching 3 – 5 centimeters in length. Increased shading of the stream channels by the tree canopy from May to September, restricted algal scores to between 2 and 2.4 for most streams, corresponding to a fuzzy layer of epilithon between 0.5 and 1 mm thick. However, algal biomass was rarely evenly distributed across the stream bed, with patches of longer algal filaments.

Restoration of Cloisters Branch was intended to increase stream channel stability, suggesting that epilithic algal biomass benefitted from decreased stormflow disturbance and more stable substrates. Along Cloisters Branch, mean ATS increased post restoration at all sites, except M3 where there was little change. Increases in ATS did not correspond to changes in canopy cover at sites (Table 6). As mean ATS increased, the variability of ATS decreased (as indicated by the coefficient of variation) (Table 6). The exception was at site M6, where both mean ATS and its CV increased sharply at this least-shaded reach. Post restoration, crayfish and minnows were observed in the deeper pools that were constructed at M3 and M6 where

Table 6. Environmental variables related to algal metabolism pre- and post-restoration. Mean, coefficient of variation (CV) and number of observations (n) for: dissolved oxygen as percent saturation (%DO); pH, algal thickness score (ATS) and percent riparian canopy cover (%can). Minimum %DO observation (DO lowest). Attached algal thickness categories: 1 = thin, visible color; 2 = 0.5-1 mm; 3 =1-5 mm; 4 = 5-20 mm; 5 > 20 mm.

	M1		M3		M4		M5		M6		TOWS		SALA		GLEN	
	pre	post	pre	post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
%DO																
mean	89.8	94.4	98.5	97.0	98.0	90.5	86.3	95.3	103.7	95.2	111	115	92.9	97.4	84.4	84.2
CV	0.19	0.17	0.28	0.13	0.10	0.19	0.29	0.08	0.06	0.11	0.11	0.15	0.08	0.06	0.23	0.37
lowest	56	79	86	81	83	70	80	86	99	80	93	96	82	90	55	58
n	10	10	11	10	10	10	11	10	5	10	11	10	9	10	9	10
pH																
mean	7.4	7.1	7.2	7.0	7.4	7.1	7.4	7.1	7.7	7.3	8.0	8.0	7.7	7.5	7.1	6.8
CV	0.02	0.02	0.05	0.03	0.03	0.03	0.02	0.02	0.03	0.02	0.03	0.05	0.02	0.04	0.03	0.02
n	10	11	8	10	10	11	10	11	6	11	10	11	9	10	10	9
ATS																
mean	2.00	2.39	2.38	2.31	2.17	2.29	1.94	2.28	1.44	2.55	2.56	2.18	1.14	1.23	2.06	2.16
CV	0.54	0.23	0.41	0.31	0.34	0.07	0.44	0.27	0.16	0.25	0.31	0.30	0.69	0.41	0.50	0.50
n	8	4	8	4	6	4	8	4	6	4	8	4	7	4	7	4
%can																
	90	82	93	78	79	73	88	88	69	63	78	80	95	96	93	95

their grazing may have affected algal and invertebrate abundance (Bengsten et al. 2008).

In Towson Run and Glen Stream algal variability changed little from pre- to post restoration and substrates often appeared scoured after rain events. In Towson Run, thick turfs of water moss and *Cladophora* were restricted to large rocks and sheltered areas, with adnate biofilm covering smaller substrata in riffles. Glen Stream had the largest algal variation due to low ATS values (ca. 1.5) on most sampling dates, except during spring blooms when ATS more than doubled and filamentous algae choked the channel.

Seasonal variation in biofilm metabolism was evident in values of dissolved oxygen (percent saturation) and pH measured during midday. Mean %DO for Cloisters Branch varied between 90-100%, (Table 6); oxygen supersaturation was measured during spring algal blooms and lowest %DO was measured in October and November when stream channels were full of fallen leaves. Towson Run had both the highest mean DO $\geq$ 111% and pH = 8.0, followed by Cloisters Branch M6, pre-restoration; these reaches also had the least riparian canopy cover. Glen Stream had the lowest mean DO, only 84%, and lowest pH, 6.8, as well as the greatest range in %DO: from below 60% and as low as 30% in October and November, to supersaturation during spring algal blooms. In contrast to the marked decline in ATS variability along Cloisters Branch post-restoration, there was no evidence of similar trends in the CVs for mean %DO or for pH. (Table 6). A final perturbation to DO and nutrients in Cloisters Branch was discovered by students in summer 2013, who repeatedly sampled the pool below the stormwater outfall that is the head of Cloisters Branch and measured DO below 3.5% and observed raw sewage. The source of the illicit sewerage connection was identified and corrected by Baltimore County in subsequent months.

Discussion

Comparison of sampling methods

The D-net and CPEX methods sample different assemblages, benthic macroinvertebrates vs. emergent chironomids, respectively. However, both methods yield data as abundance per sample unit. CPEX abundance varied greatly, as illustrated by CPEX samples from GLEN 2024a and 2024b taken one week apart. Chironomid species vary in peak emergence from late winter to summer (Gresens and Ferrington 2010), so that the April-May sampling window included a broad array of chironomid taxa. A total of 43 chironomid taxa were detected by CPEX sampling vs. 32 taxa detected in concurrent D-net samples. Eleven genera observed only by CPEX were either singletons or were present in just one sample (except for *Paratendipes*).

Small-bodied chironomid species, e.g., *Corynoneura* and *Thienemanniella*, were greatly underrepresented in D-net samples, likely due to the large mesh-size (500 µm). Ferrington et al. (1991) also found that CPEX sampling was more efficient in detecting chironomid taxa than benthic samples of chironomid larvae. CPEX samples also integrate emergence across all upstream microhabitats, so sampling takes less time, and less material is collected and preserved compared to a D-net sample. Identification of genera (and even species) of pupal exuviae is much more efficient than the laborious process of identifying larval chironomids. Although D-net samples are needed to include the diversity of other orders and phyla, the CPEX method is valuable for efficient sampling of urban streams dominated by chironomids.

Sample size bias

The small size of D-net subsamples, 120 organisms, appears to have underestimated benthic diversity and limited the ability to detect larger non-chironomid taxa. Observed CPEX richness was often greater than D-net richness, in paired samples post-restoration (Table 1) partly because CPEX sample sizes were always larger than fixed-count D-net samples. When sampling bias was removed by rarefaction of CPEX-r richness to 120 individuals, estimated CPEX-r richness was less than that of the entire macrobenthos. However, CPEX-r richness was usually still greater than the observed number of larval chironomid taxa in D-net samples, especially when % chironomid larvae was high (e.g., 2024). These sample size effects suggest that a fixed sample size of 120 organisms underestimated the richness of the benthic macroinvertebrate community, as proposed by Cao et al. (1998). Large-bodied taxa such as odonate nymphs and trichopteran larvae were sometimes observed in bulk D-net samples, but large, low-density organisms had little chance of inclusion in fixed subsamples of 120 when chironomid larvae were abundant. Rare taxa often make up a significant portion of benthic communities (Resh et al. 2005, Arscott et al. 2006), and many uncommon taxa present in Cloisters Branch in 2023 were not detected again in 2024. We cannot know whether these taxa were missed during sampling or laboratory subsampling, or whether they were excluded by environmental factors. Organisms of a larger individual body mass and lower population density are less likely to be counted. We recommend a stratified method of sub-sampling like that of Walsh et al. (2023) where large-bodied organisms are counted in a rapid visual scan of the entire sample with a subsampling fraction of 1.0, and the remaining organisms are subsampled as usual. A benthic sub-sample size of 300 individuals was used by Walsh et al. (2023) and was also considered adequate for CPEX samples (Bouchard and Ferrington 2011). An inadequate sample size will limit the ability to detect a signal in taxonomic composition.

Response of invertebrates to restoration

The MBACI sampling design and comparison of sampling methods allowed us to detect changes in the chironomid assemblage of Cloisters Branch which we attribute to physical habitat restoration, despite interannual fluctuation in water chemistry. Changes in the wider macroinvertebrate community were likely due, in part, to restoration, although limited sampling could not confirm this.

The Cloisters Branch restoration created a sequence of clearly defined riffles and pools. The channel was altered to increase groundwater and stormwater interaction with the floodplain and adjacent wetlands, and the bank-full channel width was increased. Evidence of improved channel-floodplain connection was provided by stormflow flotsam stranded in the floodplain after heavy rainfall post restoration. We infer that these very visible changes in macro-habitat improved the stability and variety of microhabitats for invertebrates, which led to the consistent increases in taxa richness for Cloisters Branch, but not for the control streams. The most notable taxonomic changes post-restoration were the increased diversity of invertebrate feeding modes and microhabitat preferences. The conspicuously large rocks in restored riffles improved habitat for filter feeders: Hydropsychidae and Philopotamidae (Trichoptera) and *Rheotanytarsus* (Chironomidae). Several predatory genera of Zygoptera and Tanypodinae (Chironomidae) appeared post-restoration and should have benefited from increases in chironomid prey. The appearance of odonates (*Argia*, *Calopteryx*, *Enallagma*) in 2023 was likely a response to improvement of their preferred habitat in submerged rootwads and large woody debris. Mature streamside trees were preserved as part of the restoration, and natural treefall over the five years since restoration has noticeably augmented large woody debris in the channel. Higher baseflow post-restoration should have increased availability of such habitat for odonates.

Subfamily Chironominae increased in diversity as 9 new genera appeared and 6 genera became more common; of these, *Dicrotendipes*, *Polypedilum* and *Paratanytarus* increased greatly in abundance post-restoration. Nine of these genera prefer

depositional habitats (Ferrington and Berg 2019) which we interpret as response to the expansion of pool and run habitats in Cloisters Branch. Seven new genera of subfamily Orthocladiinae associated with erosional habitats (Ferrington and Berg 2019) appeared, and *Cricotopus*, Corynoneurini spp. *Tvetenia* and *Eukiefferiella coerulescens* became dominant taxa post-restoration. Attached algae provided ample food resources for chironomid larvae, particularly for *Cricotopus* spp. (dominated by *C. triannulatus*) which are associated with attached algae (LeSage and Harrison 1980). The marked increase of small-bodied genera *Corynoneura* and *Thienemanniella* (Corynoneurini), which inhabit crevices and interstitial spaces between stones (Anderson et al. 2013), may reflect decreased embeddedness and improved stability of the channel post-restoration.

Ironically, the pre-restoration dominant, *Orthocladius obumbratus*, declined 72% in Cloisters Branch, while it became 1.5 times more abundant in Towson Run post restoration. We hypothesize that this reflects a preference of *O. obumbratus* larvae for coarser substrates. Field notes from Cloisters Branch pre-restoration mention "lots of sand movement and big dunes" in the channel. In contrast, silt and fine detritus accumulated in pools and runs in Cloisters Branch post restoration. Towson Run contained little fine interstitial sediment, ranking it in the lowest third of 25 Baltimore area streams. Stormflow scour in Towson Run maintained coarse substrate in riffles: 17% sand, 49% gravel/pebbles and 27% cobble (Gresens 2010). This is comparable to a riffle in Juday Creek, an Indiana trout stream, with 25% sand, 65% gravel and 10% cobble. *Orthocladius obumbratus* was common in Juday Creek (Berg and Hellenthal 1992a) and contributed 9.6% of chironomid secondary production (Berg and Hellenthal 1992b). Larvae of *O. obumbratus* may be better able to survive stormflow disturbance by living deeper within gravel/pebble substrates compared to *Cricotopus* larvae, which appear to prefer surficial substrates supporting diatoms and filamentous algae in sunlit reaches (LeSage and Harrison 1980). Of the 15 *Cricotopus* spp. in Salem Creek, *C. triannulatus* and *C. bicinctus* were the most successful in exploiting depositional areas that accumulated thin layers of diatoms and detritus (LeSage and Harrison 1980). The increase of *C. triannulatus* and *C. bicinctus* in Cloisters Branch may reflect their ability to utilize the increase in diatoms and detritus better than *O. obumbratus*, given a more stable flow regime.

Conductivity and chironomids

Salinity limited which genera could survive in Cloisters Branch and the control streams. Genera abundant pre-restoration had values of XC_{95}, i.e., the maximum conductivity that a genus is predicted to tolerate (Cormier 2017), from 938 to 1518 µS cm^{-1}, which exceeded mean baseflow conductivity in Cloisters Branch, but which approximated mean conductivity for Towson Run. (Fig. 5). This suggests that peak salinity during snowmelt may limit the distribution of these genera, as predicted by the results of Moore et al. (2020). Grab samples from the beginning of a major snowmelt event in 2013 measured over 3500 µS cm^{-1} in Cloisters Branch and Towson Run and 15,210 µS cm^{-1} in Salamander Branch. Salinity appears to be responsible for the very low abundance and diversity of chironomids in Salamander Branch and Glen Stream, where mean baseflow conductivity exceeded the XC_{95} for all taxa. Might hyporheic groundwater of lower salinity offer temporary refugia during high salinity runoff events? In Cloisters Branch, XC_{95} values of taxa that newly appeared or increased in abundance post-restoration ranged from 817 to 1730 µS cm^{-1}, overlapping the range for pre-restoration taxa.

Specific conductivity was a potentially confounding factor that could obscure a biotic response to restoration. Baseflow conductivity of Cloisters Branch was lower than in the control streams, but higher than 227 µS cm^{-1}, the level of baseflow conductivity at which the 5% most salt-sensitive invertebrate genera of the northern Piedmont ecoregion of Maryland are predicted to disappear (Cormier et al 2018). The large interannual variation in CPEX sample size reflected the annual total amount of road deicing material applied by Baltimore County (DEPS 2024). Low CPEX abundance in 2014 coincided with the highest recorded rate of deicer application, but not with average baseflow conductivity in Cloisters Branch, which was 11% lower in 2014 than in 2013, although conductivity in Towson Run was 22% higher in 2014. Winter 2014 also saw 39" of snowfall compared to 8" in 2013 and 0 and 9.8" in 2023 and 2024, respectively. Application of deicing salts for 2013 and 2014 combined was 9.5 times greater than deicer use in 2023 and 2024. Without snow, 2023 saw the lowest deicer application recorded, which then rebounded in 2024, when mean conductivity increased by over 12% in Cloisters Branch, and up to 40% in Glen Stream. Baseflow conductivity values underestimate the exposure of invertebrates to acute toxicity during high salinity snowmelt and runoff events. Moore et al. (2020) used high-fre-

quency records of specific conductivity to estimate corresponding chloride concentrations for urban areas of the eastern US, which in turn showed that urban streams can be expected to exceed the EPA criteria for acute and chronic toxicity to chloride concentration many times per year in watersheds with over 10% IA.

This range in annual deicer use was reflected in both benthic and CPEX assemblage structure, as conductivity was correlated with the results of both biotic ordinations. Fortunately, two years of CPEX samples both pre- and post-restoration permitted us to distinguish a road-salt response from a restoration response. Confounding was a bigger issue for the D-net data from Cloisters Branch, with two years of sampling post-restoration but only one year pre-restoration, and no sampling of a control stream. These inter-annual changes in D-net benthos cannot be attributed to restoration without reference to a control stream and additional years of sampling.

Comparison of stressors

A major goal of stream restoration is reduction of nutrient export. Nutrients might indirectly stress invertebrates through spring algal blooms. Periphyton growth in all study streams was limited by light, except during periphyton blooms in early spring. In Cloisters Branch, algal blooms were dominated by the filamentous diatom *Melosira* and by green algae, *Oedogonium* and *Cladophora,* which are all associated with poor water quality (Potapova 2009, Stevenson et al. 2012). Very high algal biomass during blooms in low-flow periods can create stressful conditions of dissolved oxygen and pH, as documented in an enriched Ozark stream with extensive growth of green filamentous algae (Stevenson et al. 2012). During the day, photosynthesis raised water column pH to 9.0, a level injurious to aquatic life, and raised dissolved oxygen to supersaturation, while microbial respiration at night often decreased oxygen to stressful levels during low flow. We observed similar phenomena: moderate to high algal biomass increased water column pH up to 8.0, especially in Towson Run and at Cloisters Branch M6, where canopy cover was lowest. The range of pH we measured in the water column suggests that locally stressfully high pH and diurnal oxygen fluctuations occurred within large patches of attached algae in our study streams.

Many methods have been used to set nutrient criteria for guidance in avoiding or remediating excessive enrichment (Morgan and Kline 2011). Nutrient criteria for biologically protective levels of TN and TP in Maryland streams were developed by Morgan et al. (2007) using MBSS data and multimetric BIBI scores. With best quality streams scoring "5", a BIBI score of 3 was the breakpoint between streams judged fair vs. poor, based on their macroinvertebrate assemblage. Nutrient data were used to estimate a corresponding BIBI value using quantile regression. The nutrient criteria that predicted BIBI = 3 were 1.3 mg L^{-1} for TN and 0.043 mg L^{-1} for TP. Pre-restoration, mean TN in Cloisters Branch was from 2.3-2.6 mg L^{-1}, almost twice as high as the Biological TN criterion, and TN surpassed 3 mg L^{-1} in Towson Run (Fig. 6b). Post-restoration, upper Cloisters Branch had mean TN from 1.0 to 1.4 mg L^{-1}, close to the TN criterion, which was surpassed in the control streams except for Salamander Branch. Our limited TP data with mean TP of 0.0096 mg L^{-1} and 0.0125 mg L^{-1} in upper and lower Cloisters Branch, respectively, and mean TP from 0.014 mg L^{-1} to 0.018 mg L^{-1} in the control streams, rank all our streams well below the Biological TP criterion. Similar methods were used to estimate a conductivity criterion of 247 µS cm^{-1} (Morgan et al. 2007), close to 227 µS cm^{-1}, the level of conductivity estimated by Cormier et al. (2018) to be protective of salt-sensitive invertebrates. Mean baseflow conductivity, pre-restoration, of upper Cloisters Branch. (626 µS/cm) and lower Cloisters Branch (901 µS cm^{-1}) and the control streams (1689 µS cm^{-1}) greatly exceeded these criteria both pre- and post-restoration. Our field-measured BIBI values were well below those predicted using the method of Morgan et al. (2007) for TN and TP but were closer to their conductivity values. We conclude that salinity was more limiting than nitrogen to the invertebrate communities of the four study streams.

Restoration monitoring design and effectiveness

The ability to detect a biological response to restoration of urban streams depends on a robust survey design. The BACI design with multiple years of sampling pre- and post-intervention is the most effective method for detecting an effect within a given restoration, although labor-intensive. Lacking prior sampling, an "extensive post treatment" (EPT) design involving matched pairs of restored vs. control streams monitored for multiple years post-restoration can inform restoration effectiveness across a greater number of paired streams (Roni et al. 2018). Unexpected assaults on urban water quality, e.g., sewage leaks and dumping of chemical waste in storm drains (as observed in this study) can occur without the knowledge of stream monitors. Such high temporal variability in urban streams emphasizes the importance of multiple years of pre- and post-restoration sampling.

The use of BACI sampling designs is increasing to meet the demand for rigorous evaluation of a wide variety of stream restoration projects. Studies which failed to detect biotic response to restoration compared restored streams to large groups of unrestored streams classified by land-use (Stranko et al. 2012), or involved restorations compromised by continued flow of stressors from upstream catchments (Violin et al. 2011, Walsh et al. 2023). These studies concluded that restoration efforts should focus on streams in less urbanized catchments. Although the Cloisters Branch watershed has over 50% IA, positive responses of invertebrate diversity and functional taxonomic groups to restoration were detected. Application of BACI designs in other studies of hydro-morphological stream restoration in rural watersheds with good water quality detected macroinvertebrate improvement (England et al. 2021, Omoniyi et al. 2022), but not in a suburban restored stream exposed to multiple anthropogenic stressors (Omoniyi et al. 2022).

Choice of response variables affects the ability to avoid Type II error and to detect a restoration response. Initial analysis of the Cloisters Branch D-net data used multi-metric BIBI scores and related metrics of tolerant (e.g., % Chironomidae) and sensitive taxa (virtually absent) to conclude that biotic condition continued to decline post-restoration. In contrast, multivariate analyses of taxonomic composition were sensitive to changes of invertebrate assemblages due to restoration of Cloisters Branch as well as to variation in conductivity. Taxonomic analysis is also valuable to explain the nature of a restoration response, and even the lack of a response, in urbanized streams (Walsh et al. 2023).

Addressing chemical water quality is essential to any ecological study of urban streams. In climates where deicing salts are applied to roadways in winter, chloride content of stream water has risen over time (Wallace and Biastoch 2016) despite stormwater management (Hopkins et al. 2022). Stream flow and groundwater deliver road salts to drinking water reservoirs, such as those in the Baltimore area where chloride concentrations have doubled since the 1980s (Baltimore County Council on Environmental Quality 2024). Reduction of deicer usage is necessary not only to protect drinking water sources and aquatic life, but also to minimize corrosion of concrete structures. Urgency to protect public drinking water wells in Canada and Finland motivated reductions in deicer application from 25% to 35%, respectively (Salminen et al. 2011, Rudolph et al. 2023.

Conclusions

In a stream dominated by chironomid larvae, CPEX sampling was sufficient to detect a significant biotic response to stream restoration, without analysis of the entire macroinvertebrate community. CPEX samples integrate chironomid emergence from all upstream microhabitats, produce a smaller volume of preserved material than D-net samples, and exuviae are more time-efficient to identify to genus (and often to species) compared to identification of chironomid larvae. CPEX sampling was more efficient than D-net sampling in detecting chironomid species, especially small-bodied genera producing abundant exuviae. Use of a MBACI survey design involving Cloisters Branch and three unrestored control streams was essential to distinguish the restoration response from substantial inter-annual variation in water chemistry and invertebrate assemblage composition in all streams. The efficiency of CPEX sampling made it feasible to collect 2 years of both pre- and post-restoration data from all four streams.

The restoration of Cloisters Branch led to a 50% increase in chironomid taxa richness, marked by increases in the number of Chironominae and Tanypodinae genera. Emergence of *Dicrotendipes*, *Polypedilum* spp. and *Paratanytarsus* spp. increased greatly post-restoration. Several Orthocladiinae taxa become more abundant post-restoration: *Cricotopus* spp. (especially *C. triannulatus*), *Eukiefferiella coerulescens*, *Tvetenia*, and Corynoneurini spp. This response by chironomids tracked increased abundance and stability of epilithic algae. This suggests that the stability of the stream substrate improved, a goal of the Cloisters Branch restoration. In contrast, there was little change in either algal variability or abundance in unrestored Towson Run, where *Orthocladius obumbratus* emergence increased greatly, even as it decreased sharply in Cloisters Branch. Almost all taxa which become common in Cloisters Branch decreased in emergence from Towson Run or were never found there or in other control streams. Conductivity was more limiting than food and habitat stability to the distribution of chironomids in these urban streams.

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