Evaluating a Regenerative Stormwater Conveyance Stream Restoration and its Effects on Water Quality and Benthic Macroinvertebrates: A Case Study at Muddy Creek

May 2023

Department of Natural Resources
Resource Assessment Service

DNR 12-042623-352

Maryland

Wes Moore, Governor

Josh Kurtz, Secretary

MARYLAND DEPARTMENT OF NATURAL RESOURCES

Resource Assessment Service
Monitoring and Non-Tidal Assessment
580 Taylor Ave, C-2
Annapolis, Maryland 21401
410-260-8610 Phone
410-260-8620 Fax
dnr.maryland.gov

Prepared by Kyle Hodgson, Lindsay Powers, and Scott Stranko

Additional Telephone Contact Information:
Toll free in Maryland: 877-620-8DNR ext. 8540 OR
Individual unit/program toll-free number
Out of state call: 410-260-8540
Text Telephone (TTY) users call via the Maryland Relay

ACKNOWLEDGEMENTS

The Maryland Department of Natural Resources would like to thank several contributors from the Smithsonian Environmental Research Center, including: Tom Jordan, Joshua Thompson, Cynthia Gilmour, and Carey Pelc. Additionally, the Maryland Department of Natural Resources would like to thank the Cheseapeake and Atlantic Coastal Bays Trust Fund for funding this project.

Suggested citation: Resource Assessment Service. 2023. Evaluating a Regenerative Stormwater Conveyance Stream Restoration and its Effects on Water Quality and Benthic Macroinvertebrates. Maryland Department of Natural Resources. 580 Taylor Avenue, Annapolis, Maryland 21401. DNR 12-042623-352.

Table of Contents

ABSTRACT

Regenerative stormwater conveyances (RSCs) have gained popularity in Maryland and neighboring states as a relatively novel stream restoration designed to improve nutrient and sediment reduction and enhance floodplain connectivity, though few peer-reviewed studies addressing their performance exist. An RSC was installed along 452 linear stream meters on North Branch Muddy Creek on the Smithsonian Environmental Research Center (SERC) property in Edgewater, Maryland to reconnect the stream to its floodplain to increase water storage and sediment deposition, increase nutrient and sediment processing, and provide biological uplift. Biological and water chemistry monitoring were conducted pre- and post-restoration, and dissolved oxygen and temperature were monitored post-restoration above the RSC, and at the downstream end of the restored reach. Dissolved oxygen concentrations and saturation levels were frequently significantly lower at the downstream monitoring station compared to the upstream station in the same month, and DO concentrations more frequently fell below the 5.0 mg/L Maryland Use Class I water quality standard violation threshold at the downstream station. Stream temperatures were significantly higher at the downstream station compared to the upstream station in more than half of the months studied. Significant decreases in Benthic Index of Biotic Integrity (BIBI) scores, Number of Taxa, Shannon-Wiener Index, and Percent Predators, and significant increases in the Percent Chironomidae and Percent Collectors were observed after restoration. Benthic macroinvertebrate communities also experienced a marked shift in composition to the dominance of tolerant taxa. Although water chemistry data previously reported by SERC showed significant retention of some nutrients within the reach, dissolved oxygen, water temperature, and benthic macroinvertebrate communities appear to have worsened after restoration.

INTRODUCTION

Channel incision and the subsequent disconnection of the stream from its floodplain can impair stream ecosystem function by increasing hydrological flashiness, degrading physical habitat, and increasing downstream transport of sediment and nutrients (Shields et al. 2009). These excessive amounts of nutrients and sediments ultimately reach downstream estuaries, contributing to eutrophication and habitat loss (Boesch et al. 2001). Stream restoration techniques and stormwater best management practices (BMPs) are increasingly implemented with the expectation that they will restore hydrochemical regimes and improve water quality, habitat, and biodiversity (Williams et al. 2016). Across the U.S., more than 9 billion dollars was spent between 1990 and 2003 on a variety of stream restoration projects, including channel reconfigurations, in-stream habitat improvements, and water quality management (Bernhardt et al. 2005). Restorations aimed at water quality improvements have grown in popularity in the mid-Atlantic region, including the Chesapeake Bay watershed's coastal plain area, which must meet required Total Maximum Daily Loads (TMDLs) to reduce pollution in the Bay (Thompson et al. 2018).

Because only modest improvements with regard to water quality and sediment transport commonly occur with traditional stream restoration techniques and stormwater BMP installations (Selvakumar et al. 2010, Filoso and Palmer 2011, Palmer et al. 2014), environmental engineers have more recently attempted to improve nutrient and sediment reduction efficiencies by creating novel stream restoration designs such as regenerative stormwater conveyances (RSCs; Williams et al. 2016). Incorporating a combination of techniques, RSC construction fills in the channel with a mixture of sand, gravel, and woodchips, and creates a series of pools and riffles. In addition, this technique improves connectivity with the floodplain using large rock weirs and perpendicular berms across the channel (Brown et al. 2010).

Among Maryland and its neighboring states, RSCs have gained popularity since they are designed to increase stream residence time, reduce erosion impacts, and enhance floodplain connectivity (Palmer et al. 2014). Despite RSCs having been implemented throughout Maryland and neighboring areas for over a decade, limited information is available concerning their nutrient and sediment reduction capabilities and sustainability (Filoso and Palmer 2011, Palmer et al. 2014). It has been argued that RSCs improve water quality (Berg and Underwood 2007, Bowen 2012, Brown et al. 2010) but peer-reviewed studies addressing their performance are sparse. In addition, ecological effects associated with RSCs have rarely been evaluated (Williams et al. 2016).

In 2013, an RSC stream restoration project was proposed for the North Branch Muddy Creek (henceforth Muddy Creek) located on the 2,650-acre property of the Smithsonian Environmental Research Center (SERC) in Edgewater, Maryland. The primary objectives of this restoration project were to reconnect Muddy Creek to its floodplain to increase water storage and sediment deposition, increase nutrient and sediment processing, and provide biological uplift. These objectives were addressed with the installation of an RSC along 452 linear stream meters on Muddy Creek at an estimated cost of 1,077,585 dollars with funding provided by the Chesapeake and Atlantic Coastal Bays Trust Fund. The RSC construction at Muddy Creek was carried out between December 2015 and February 2016 by Underwood and Associates, LLC.

The long history of water sampling on SERC property combined with monitoring before and after restoration make this project an ideal research opportunity to test the efficacy of RSC projects at reducing constituent loads in Maryland coastal plain streams, and to monitor ecological responses of stream biota.

One component of this study that focused on the restoration's potential effects on water chemistry was conducted and previously reported by SERC. Thompson et al. 2018 described analyses and findings related to loads and flow-weighted mean concentrations of the following parameters upstream and downstream of the restoration reach: total nitrogen, total phosphorus, total organic carbon, pH, nitrate plus nitrite (combined total), total ammonium, and total phosphate. Additional information about this part of the study can be found in their publication.

This report describes results from two years of pre-restoration monitoring (2014 – 2015) and six years of post-restoration monitoring (2016 – 2021) of benthic macroinvertebrate communities within the restoration, and six years of post-restoration monitoring (2016 – 2021) of dissolved oxygen and temperature above and below the restored reach.

METHODS

Muddy Creek restoration monitoring was a collaborative effort involving the Smithsonian Environmental Research Center (SERC) and the Maryland Department of Natural Resources (MDNR). SERC collected hydrologic and physiochemical data upstream and downstream of the restoration reach between March 2015 and April 2019. MDNR was tasked with documenting impacts on biological communities before and after restoration between March 2014 and March 2021.

Dissolved Oxygen and Temperature

Continuous Dissolved Oxygen (DO) loggers (Onset HOBO U26-001) were deployed upstream of the restoration reach and at its farthest point downstream between February 2016 and April

2019 by SERC staff (Figure 1). The loggers were programmed to record DO and temperature readings at 10-minute intervals. Between June 2019 and July 2021, Maryland DNR redeployed continuous DO loggers using the same methodology and type of equipment as SERC, with the exception that DO and temperature readings were changed to hourly intervals. For comparability between SERC and DNR data, mean daily measurements were calculated from raw data, and data were excluded from the months of December, January, and February. Temperature data prior to March 2018 were comparatively sparse and were excluded from analyses.

Continuous DO logger data were assessed in several ways for quality assurance. Air temperatures from the on-site weather station were compared to water temperatures recorded by DO loggers. Records with matching air and water temperatures were removed from the dataset due to suspected logger dewatering. Records corresponding to days that received one or more inches of precipitation were also removed due to suspected logger burial. In Microsoft Excel, DO was graphed by month to view potential changes in daily patterns. Records that corresponded to days preceded by precipitation and did not exhibit normal diel temperature fluctuations were removed due to suspected logger burial (Figure 2). Data were only presented and analyzed if a full 24-hour period of consecutive raw data was captured.

Dissolved oxygen is a measure of the amount of oxygen dissolved in the water as a function of variables such as water temperature, atmospheric pressure, physical aeration, and chemical/biological oxygen demand. Low DO concentrations may indicate pollution due to heterotrophic oxygen consumption and may stress aquatic organisms (MDE 2014). Since the Code of Maryland Regulations (COMAR) criterion for Use I waters states that the DO concentration may not be less than 5.0 mg/L at any time (COMAR 2014), the number of days in which mean daily DO concentrations fell below this threshold was investigated, by month, at both monitoring stations.

Additionally, mean daily temperature, mean daily DO concentration, and elevation were used to calculate DO saturation by following procedures described in the BSID process (MDE 2014). DO saturation values were only calculated on datasets containing temperature (i.e., 2018 – 2021). Since statistically significant relationships between biology and low DO saturation (below 40% in the Coastal Plain) were documented by MDE 2014, the number of days below 40% DO saturation at each station was also investigated to determine potential biological impairment.

To test for statistical differences in DO and temperature measurements between stations, a series of Welch's t-tests using mean daily values were performed by month. DO and temperature data were not collected prior to restoration activities; therefore all statistical comparisons were performed between upstream and downstream stations in the post-restoration period.

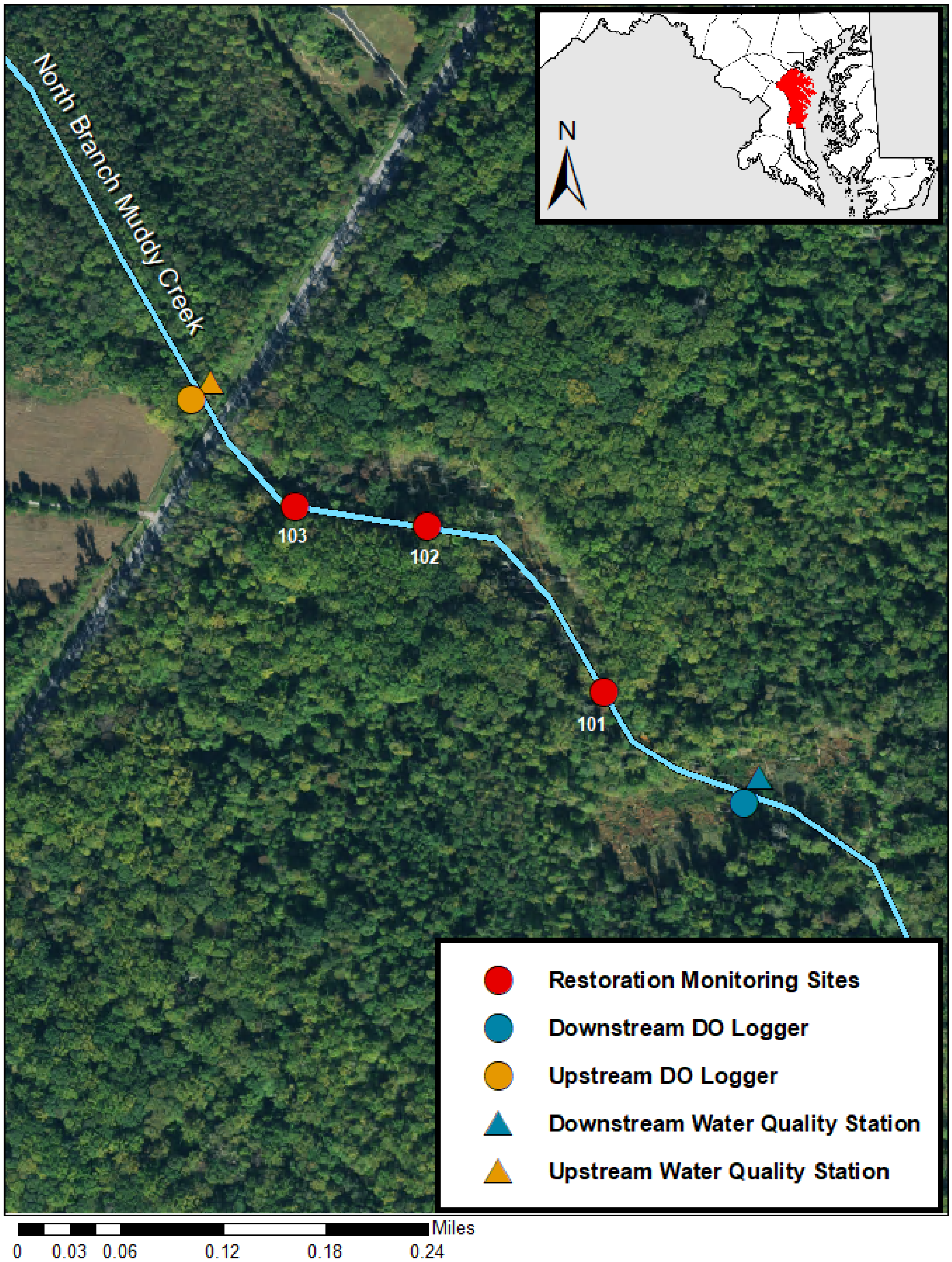


Figure 1. Map of Maryland DNR biological monitoring sites in the restoration reach of Muddy Creek (red circles), locations of upstream and downstream dissolved oxygen loggers (circles), and locations of SERC's fixed water quality monitoring stations (triangles).

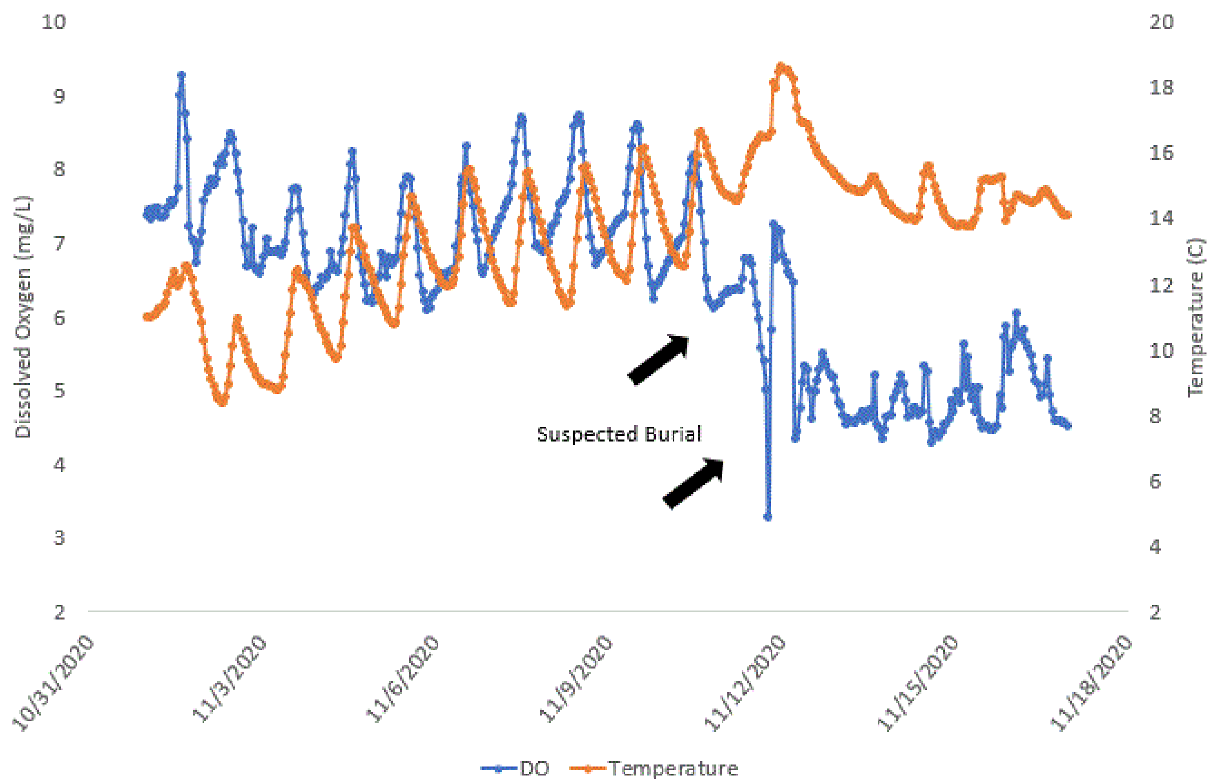


Figure 2. An example of a break in the cyclical patterns of data collected by a dissolved oxygen logger. The suspected burial arrows indicate a day preceded by precipitation. These data were removed from the analysis.

Benthic Macroinvertebrate Monitoring

The 452-meter restored section of Muddy Creek is located on SERC property immediately downstream of Muddy Creek Road (Rt. 468) and upstream of a SERC water quality monitoring station (Figure 1). In 2014, MDNR established nine biological monitoring sites to assess the impacts of the restoration on stream biota, particularly benthic macroinvertebrates (Figure 3). Three sites (101, 102, 103) are located within the restoration reach, where average land use is primarily forested (50%), with agriculture (28%) and urban (20%) land cover also present (Table 1). Three upstream control sites (104, 105, 106) are directly upstream of the restoration reach, and their land use is similar to that of the restoration reach (Table 1). The upstream control reach resembles a first-order flowing stream typical of headwater coastal plain streams in Maryland. An additional three biological monitoring sites (108, 109, 110) were also surveyed as adjacent control comparisons. Sites 108 and 109 are within Bluejay Branch, approximately 600 meters from the restoration reach and generally characterized as a wide, swampy stream with no discernable channel and minimal flow. Site 110 is within Mill Swamp Run, which has the largest drainage area among all biological monitoring sites, and is characterized as a second-order flowing stream typical of coastal plain streams in Maryland (Table 1).

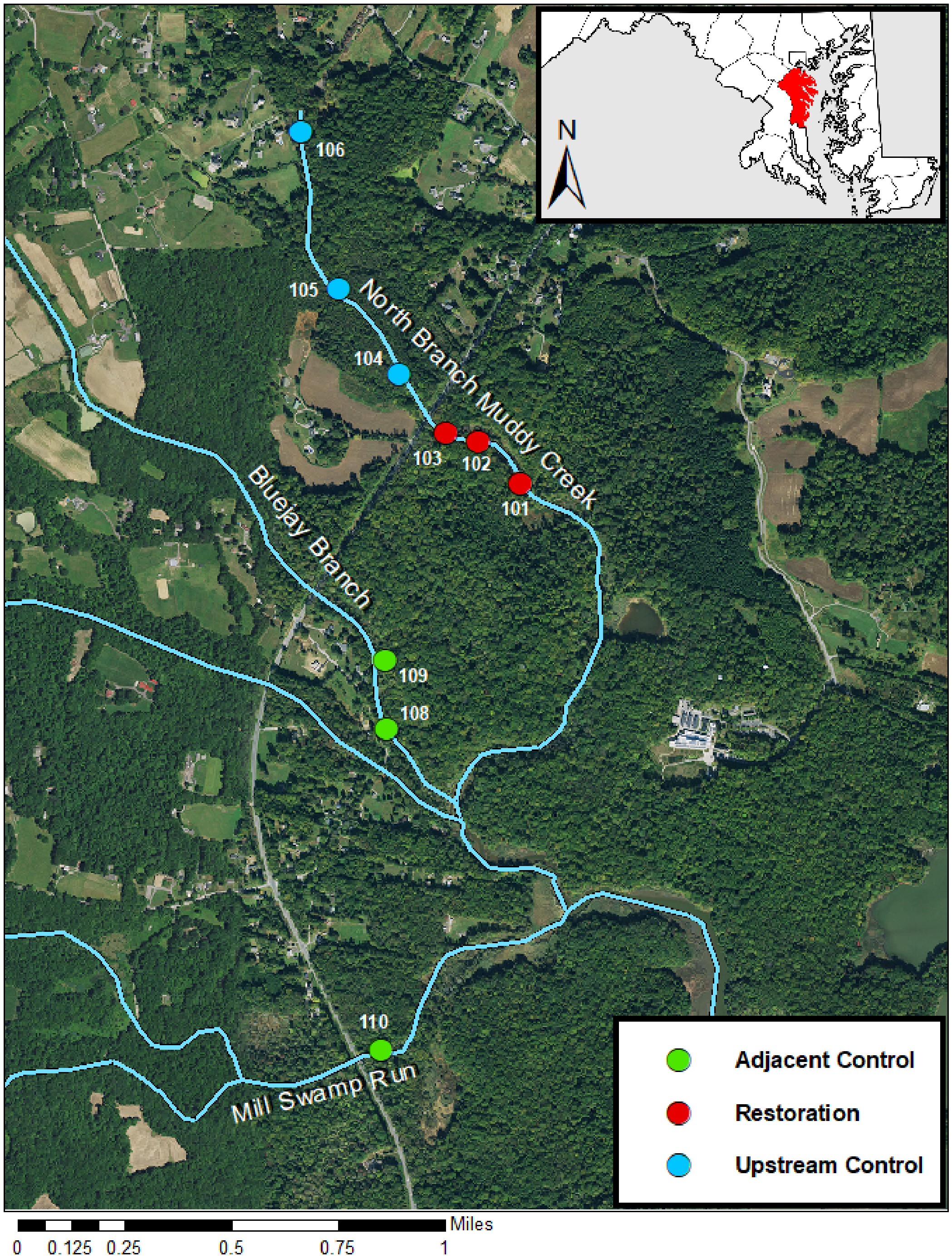


Figure 3. Map of Maryland Department of Natural Resources biological monitoring sites in the Muddy Creek Watershed.

Table 1. Catchment drainage area of Muddy Creek, Bluejay Branch, and Mill Swamp Run biological monitoring sites.

	Catchment Drainage Area (acres)
Muddy Creek Restoration Sites	
101	362
102	346
103	331
Muddy Creek Upstream Control Sites	
104	294
105	207
106	80
Bluejay Branch Adjacent Control Sites	
108	450
109	406
Mill Swamp Run Adjacent Control Sites	
110	2596

Table 2. Average watershed land use for Muddy Creek restoration and control biological monitoring sites. Land use metrics were calculated using the 2011 National Land Cover Database.

	Land Cover Type (%)		
Watershed	*Urban*	*Agriculture*	*Forest*
Muddy Creek Restoration Sites	20	28	50
Muddy Creek Upstream Control Sites	20	27	51
Bluejay Branch Adjacent Control Sites	13	28	50
Mill Swamp Run Adjacent Control Site	9	28	53

Maryland Biological Stream Survey (MBSS) methods (Stranko et al. 2014) were used to sample benthic macroinvertebrates at each biological monitoring site. All sites were sampled annually between 2014 and 2021, with the exception of site 110 which was added in 2016 (Figure 3). Benthic Index of Biotic Integrity (BIBI) scores were calculated at each site sampled for benthic macroinvertebrates (Southerland et al. 2005). BIBIs are calculated based on metrics that are indicative of stream health, as evidenced by impacts on the biotic community. Results are combined into a scaled IBI score, ranging from 1.0 to 5.0, with an applied narrative ranking ranging from Very Poor to Good (Table 3). Pre- and post-restoration benthic macroinvertebrate samples from the restoration reach, in conjunction with upstream and adjacent control sites, were used to determine if the restoration reach is changing in a manner that is consistent with restoration objectives.

Table 3. MBSS IBI scoring and narrative ranking.

IBI Score	Narrative Ranking
4.0 – 5.0	Good
3.0 – 3.9	Fair
2.0 – 2.9	Poor
1.0 – 1.9	Very Poor

In addition to investigating BIBI score responses to restoration, several functional feeding group, diversity, habit, and phylogenetic metrics were compared between the pre- (2014-2015) and post-restoration (2016-2021) periods, and compared against trends observed from both adjacent and upstream control groups. Select metrics that have predictable responses to stress were tested for statistical significance using a one-way Analysis of Variance (ANOVA) and a post-hoc Tukey's test (Table 4). In addition, non-metric multidimensional scaling (NMDS), a multivariate graphing technique using a Bray-Curtis distance measure in two-dimensional ordination space (McCune et al. 2002), was used to investigate how benthic communities compare between pre- and post-restoration periods and to determine community responses to restoration. To further investigate which taxa are strong drivers of clustering effects in two-dimensional ordination space, an Indicator Species Analysis (ISA) was used to determine taxon weighting using Indicator Values and associated Monte Carlo p-values (McCune et al. 2002).

A supporting cluster dendrogram was produced alongside NMDS graphics, which indicated all 2020 samples were closest in community composition regardless of sampling location. All 2020 samples were collected in late April, whereas sampling was conducted in early to mid-March during the other years of this study. Therefore, 2020 samples were removed from both NMDS and ISA analyses to avoid seasonal bias in community composition comparisons.

Table 4. Definitions of Coastal Plain benthic macroinvertebrate metrics tested and expected response to stress. Descriptions and Predicted Responses adapted from Southerland et al. 2005.

Metric	Description	Predicted Response
BIBI	Benthic Index of Biotic Integrity score	Decrease
Number of Taxa	Measures the total count of taxa within each sample	Decrease
Shannon-Wiener Index	A measure of general richness and composition (diversity and evenness)	Decrease
Number of Grids	Number of grids sorted to reach target number of organisms during processing	Increase
Percent Intolerant Urban	Percent of sample considered intolerant to urbanization (tolerance values 0-3)	Decrease
Percent EPT	Percentage of taxa in the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)	Decrease
Percent Chironomidae	Percent midge larvae and pupae	Increase
Percent Collectors	Percent of sample that feed on detrital deposits or loose surface films	Increase
Percent Predators	Percent predator individuals	Decrease
Percent Filterers	Percent of sample that feeds on suspended detritus	Decrease
Percent Clingers	Percent of sample primarily adapted for inhabiting flowing water, as in riffles	Decrease
Percent Burrowers	Percent of sample that primarily burrows in sandy substrate	Increase
Percent Sprawlers	Percent of sample that primarily lives on top of plant or sediment substrates	Increase

Fish and Physical Habitat Monitoring

Maryland Biological Stream Survey (MBSS) methods (Stranko et al. 2014) were used to sample fish communities and physical habitat during the summer index period at each biological monitoring site. All sites were sampled annually in the pre-restoration period (2014 - 2015) except site 110 which was added in 2016.

Since fish community assessments in the post-restoration period detected only one species at site 101, fish and physical habitat monitoring was discontinued as of 2017. The limited post-restoration data, collected only at site 101 within the restoration reach, made it difficult to determine any possible effects of the restoration on fish communities and physical habitat.

RESULTS

Dissolved Oxygen

Analysis of mean daily dissolved oxygen (DO) concentrations showed a pattern of significant differences between the downstream and upstream stations. For the majority of the study period from 2016 to 2021 in which adequate data were available from both stations (30 out of 34 months), dissolved oxygen concentrations were significantly lower at the downstream station compared to the upstream station in the same month. DO concentrations were significantly higher at the downstream station in March 2019, and the two stations were not significantly different for three out of the 34 months (March 2018, August 2019, and September 2020). Only one data point was collected at the upstream station in September 2018, which did not allow for statistical analysis. Data were not collected at one or both stations for 19 months from 2016 to 2021 (Table 7).

In three of the six years of the study period (2016, 2017, and 2021), significantly lower mean daily DO concentrations occurred at the downstream station compared to the upstream station in all months with adequate data collected from both stations, with similarly high degrees of significance based on Welch's t-tests. Daily mean DO concentrations from 2016 were significantly lower at the downstream station in March ($p < 0.001$), April ($p < 0.001$), May ($p < 0.001$), June ($p < 0.001$), July ($p < 0.001$), and August ($p < 0.001$). In 2017, lower DO concentrations were observed at the downstream station in March ($p < 0.001$), April ($p < 0.001$), May ($p < 0.001$), and June ($p = 0.001$). All five months in which data were collected in 2021 showed lower DO concentrations at the downstream station, including March ($p = 0.001$), April ($p < 0.001$), May ($p < 0.001$), June ($p < 0.001$), and July ($p < 0.001$; Table 7).

DO concentrations from the remaining three years of the study period (2018, 2019, and 2020) indicated mean daily DO concentrations were significantly lower at the downstream station compared to the upstream station during most months with adequate data for analysis. In 2018, significantly lower downstream daily means occurred in seven out of eight months with adequate data: April ($p = 0.001$), May ($p < 0.001$), June ($p < 0.001$), July ($p < 0.001$), August ($p < 0.001$), October ($p < 0.001$), and November ($p < 0.001$). In 2020, significantly lower downstream daily means occurred in four out of five months with adequate data: June ($p < 0.001$), July ($p < 0.001$), October ($p < 0.001$), and November ($p = 0.005$). While 2019 generally followed the same pattern with lower downstream mean daily concentrations in April ($p = 0.004$), June ($p < 0.001$), July ($p < 0.001$), and November ($p < 0.001$), it contained the only point at which daily DO concentration means were significantly higher at the downstream station compared to the upstream station in a given month (March, $p = 0.021$; Table 7).

The upstream station was below the 5.0 mg/L water quality violation threshold (COMAR 2014) as a monthly mean once during the 36-month study period (3.48 mg/L in August 2019). The downstream station's monthly mean fell below this threshold in 20 out of 36 months.
The downstream station frequently experienced a greater number of days in which dissolved oxygen levels fell below the 5.0 mg/L water quality violation threshold compared to the upstream station (Figure 4), though it is important to note that data were not consistently collected on the same days or for the same number of days at both stations each month. In 2016, mean daily DO concentrations at the downstream station fell below the 5.0 mg/L threshold on 87.2% of days sampled from April to August. In 2018, DO concentrations below 5.0 mg/L were recorded on 28 days each in June and August at the downstream station, while the upstream station did not fall below 5.0 mg/L on any day during those same months. The downstream station daily means remained below 5.0 mg/L for all 31 days of July 2019, and for 29 of 30 days in June of 2021 (Table 7).

Table 7. Mean daily DO concentrations (mg/L) with standard deviations (SD) and number of days below 5.0 mg/L by month from 2016 to 2021. DO concentrations at the downstream site (below restoration activities) compared to the upstream site are noted as higher or lower where significant, based on a Welch's t-test, with a corresponding p-value. NS indicates no significant difference.

	UPSTREAM			DOWNSTREAM				
	Mean	SD	Days below 5.0 mg/L	Mean	SD	Days below 5.0 mg/L	Change	p-value
2016								
March	10.27	0.63	0	7.16	1.65	4	lower	<0.001
April	9.63	0.74	0	4.14	2.11	18	lower	<0.001
May	9.15	0.92	0	3.78	1.59	18	lower	<0.001
June	6.62	1.11	3	0.58	0.86	23	lower	<0.001
July	5.91	1.49	6	1.00	1.30	24	lower	<0.001
August	6.29	1.46	2	0.15	0.38	19	lower	<0.001
September	—	—	—	—	—	—	—	—
October	—	—	—	—	—	—	—	—
November	8.96	1.09	0	—	—	—	—	—
2017								
March	11.00	0.82	0	9.39	1.26	0	lower	<0.001
April	8.83	0.62	0	4.87	2.17	16	lower	<0.001
May	6.35	2.54	8	3.64	1.84	20	lower	<0.001
June	5.75	2.00	2	1.14	1.02	6	lower	0.001
July	—	—	—	—	—	—	—	—
August	—	—	—	—	—	—	—	—
September	—	—	—	—	—	—	—	—
October	—	—	—	—	—	—	—	—
November	—	—	—	7.23	2.53	4	—	—

2018								
March	11.26	0.58	0	11.21	0.67	0	NS	—
April	9.18	0.87	0	7.85	2.10	4	lower	0.001
May	8.16	0.40	0	5.50	0.83	10	lower	<0.001
June	7.41	0.64	0	2.20	1.84	28	lower	<0.001
July	7.39	0.32	0	4.52	1.86	17	lower	<0.001
August	6.81	0.55	0	3.36	1.49	28	lower	<0.001
September	7.44	—	0	6.43	1.07	4	—	—
October	8.99	1.06	0	6.69	1.34	5	lower	<0.001
November	10.11	0.67	0	8.08	1.36	1	lower	<0.001
2019								
March	11.05	0.60	0	11.53	0.77	0	higher	0.021
April	10.39	0.97	0	9.18	1.44	0	lower	0.004
May	—	—	—	—	—	—	—	—
June	7.92	0.45	0	4.84	1.23	11	lower	<0.001
July	6.58	1.42	3	1.87	1.46	31	lower	<0.001
August	3.48	1.33	8	2.70	1.62	12	NS	—
September	—	—	—	—	—	—	—	—
October	—	—	—	—	—	—	—	—
November	7.71	0.77	0	4.17	1.85	16	lower	<0.001
2020								
March	—	—	—	—	—	—	—	—
April	—	—	—	—	—	—	—	—
May	—	—	—	—	—	—	—	—
June	8.09	0.50	0	0.77	0.69	19	lower	<0.001
July	7.11	0.25	0	0.86	1.41	13	lower	<0.001
August	—	—	—	—	—	—	—	—
September	6.82	0.00	0	5.72	1.01	0	NS	—
October	6.88	1.33	4	5.56	1.05	9	lower	<0.001
November	8.14	0.80	0	7.55	0.90	0	lower	0.005
2021								
March	10.93	0.80	0	9.96	1.12	0	lower	0.001
April	9.79	0.78	0	8.23	1.12	0	lower	<0.001
May	8.75	0.72	0	4.30	1.84	16	lower	<0.001
June	6.63	0.95	2	1.54	1.72	29	lower	<0.001
July	5.15	1.46	10	1.05	1.50	24	lower	<0.001
August	—	—	—	—	—	—	—	—
September	—	—	—	—	—	—	—	—
October	—	—	—	—	—	—	—	—
November	—	—	—	—	—	—	—	—

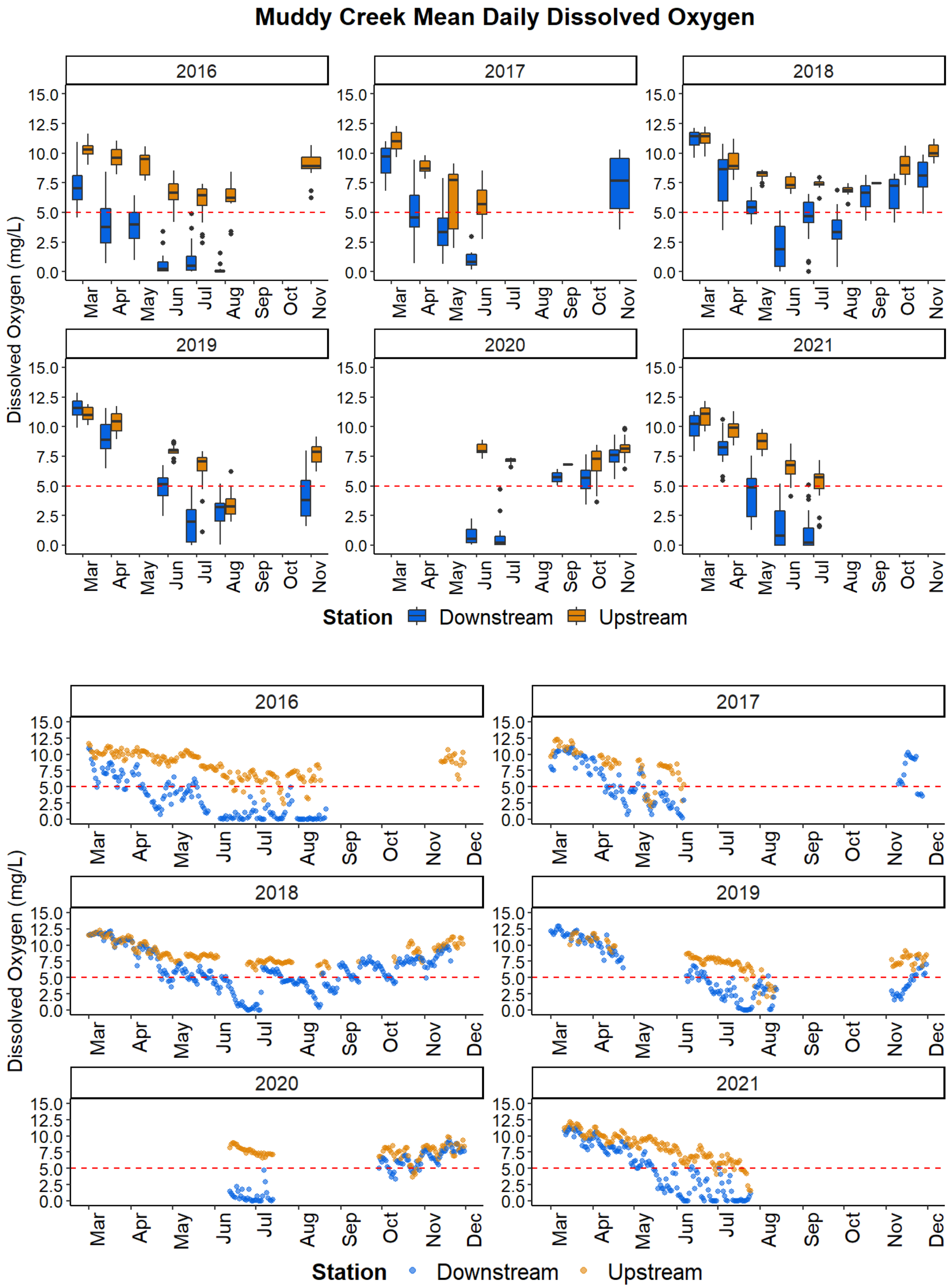


Figure 4. Mean daily dissolved oxygen concentrations (mg/L) from 2016 to 2021 at the upstream and downstream stations at Muddy Creek. Red dotted lines represent the COMAR water quality threshold of 5.0 mg/L.

Additionally, dissolved oxygen saturation was markedly different between the upstream and downstream stations. From 2018 to 2021, the mean daily DO saturation levels at the downstream station were significantly lower compared to the upstream station in 19 out of 23 months with adequate temperature and DO data for statistical analysis (Table 8).

In 2018, the downstream station's mean daily DO saturation was significantly below the upstream station in six out of seven total months with adequate data: April ($p = 0.001$), May ($p < 0.001$), June ($p < 0.001$), July ($p < 0.001$), October ($p < 0.001$), and November ($p < 0.001$). DO saturation was not significantly different between the stations in March 2018, while the amount of data collected in August and September 2018 was insufficient for DO saturation calculations. In 2019, downstream mean daily DO saturation was significantly lower in April ($p = 0.002$), June ($p < 0.001$), July ($p < 0.001$), and November ($p < 0.001$), and was not significantly different from the upstream station in March or August. In 2020, mean daily DO saturation at the downstream station was significantly lower in June ($p < 0.001$), July ($p < 0.001$), October ($p < 0.001$), and November ($p < 0.001$), with no significant difference detected in September. In 2021, mean daily DO saturation levels were significantly lower downstream compared to the upstream station in all five months when data was collected: March ($p < 0.001$), April ($p < 0.001$), May ($p < 0.001$), June ($p < 0.001$), and July ($p < 0.001$; Table 8).

The mean daily DO saturation at the upstream station generally did not fall below the BSID threshold of 40%, with the exception of July 2019 (one day), October 2020 (one day), July 2021 (three days), and August 2019 (six days). The downstream station, however, experienced several months with a high number of days below the threshold. In 2018, the mean DO saturation at the downstream station fell below 40% on 22 days in June and 15 days in August. In 2019, the downstream station was below the threshold for 25 days in July and 16 days in November. In 2020, the downstream station's mean DO saturation fell below 40% during 19 days in June and 12 days in July 2020. In 2021, three out of five months with adequate data included a high number of days with mean DO saturation under 40% at the downstream station: 11 days in May, 25 days in June, and 22 days in July (Table 8).

Both the upstream and downstream stations experienced zero days or one day below the BSID threshold during the two earliest months of data collection (March and April) in 2018, 2019, and 2021 (no data were collected in March or April of 2020). The downstream station experienced its highest number of days below the threshold in either June or July during all four years, while the number of days under 40% remained comparatively low at the upstream station and did not exceed 3 days in those same months (Table 8, Figure 5).

Table 8. Mean daily dissolved oxygen saturation and number of days DO saturation fell below the 40% BSID threshold for the Coastal Plain region from 2018 to 2021. DO saturation at the downstream site (below restoration activities) compared to the upstream site is noted as higher or lower where significant, based on a Welch's t-test, with a corresponding p-value. NS indicates no significant difference.

	UPSTREAM		DOWNSTREAM			
	Mean DO Saturation (%)	Days below 40%	Mean DO Saturation (%)	Days below 40%	Change	p-value
2018						
Mar	91.36	0	90.15	0	NS	—
Apr	83.30	0	71.56	1	lower	0.001
May	85.71	0	58.70	1	lower	<0.001
Jun	88.14	0	24.25	22	lower	<0.001
Jul	85.08	0	52.55	5	lower	<0.001
Aug	77.22	0	40.08	15	—	—
Sep	85.06	0	74.42	0	—	—
Oct	87.15	0	66.75	0	lower	<0.001
Nov	86.96	0	74.03	0	lower	<0.001
2019						
Mar	96.26	0	95.84	0	NS	—
Apr	95.86	0	88.22	0	lower	0.002
May	—	—	—	—	—	—
Jun	87.19	0	55.56	4	lower	<0.001
Jul	77.06	1	22.58	25	lower	<0.001
Aug	41.03	6	31.52	7	NS	—
Sep	—	—	—	—	—	—
Oct	—	—	—	—	—	—
Nov	63.20	0	34.84	16	lower	<0.001
2020						
Mar	—	—	—	—	—	—
Apr	—	—	—	—	—	—
May	—	—	—	—	—	—
Jun	89.78	0	8.72	19	lower	<0.001
Jul	83.36	0	10.37	12	lower	<0.001
Aug	—	—	—	—	—	—
Sep	73.07	0	61.75	0	NS	—
Oct	69.08	1	55.83	2	lower	<0.001
Nov	75.26	0	68.96	0	lower	<0.001
2021						
Mar	96.30	0	88.91	0	lower	<0.001
Apr	91.53	0	78.43	0	lower	<0.001
May	87.09	0	43.76	11	lower	<0.001
Jun	72.53	0	17.17	25	lower	<0.001
Jul	59.93	3	12.16	22	lower	<0.001
Aug	—	—	—	—	—	—
Sep	—	—	—	—	—	—
Oct	—	—	—	—	—	—
Nov	—	—	—	—	—	—

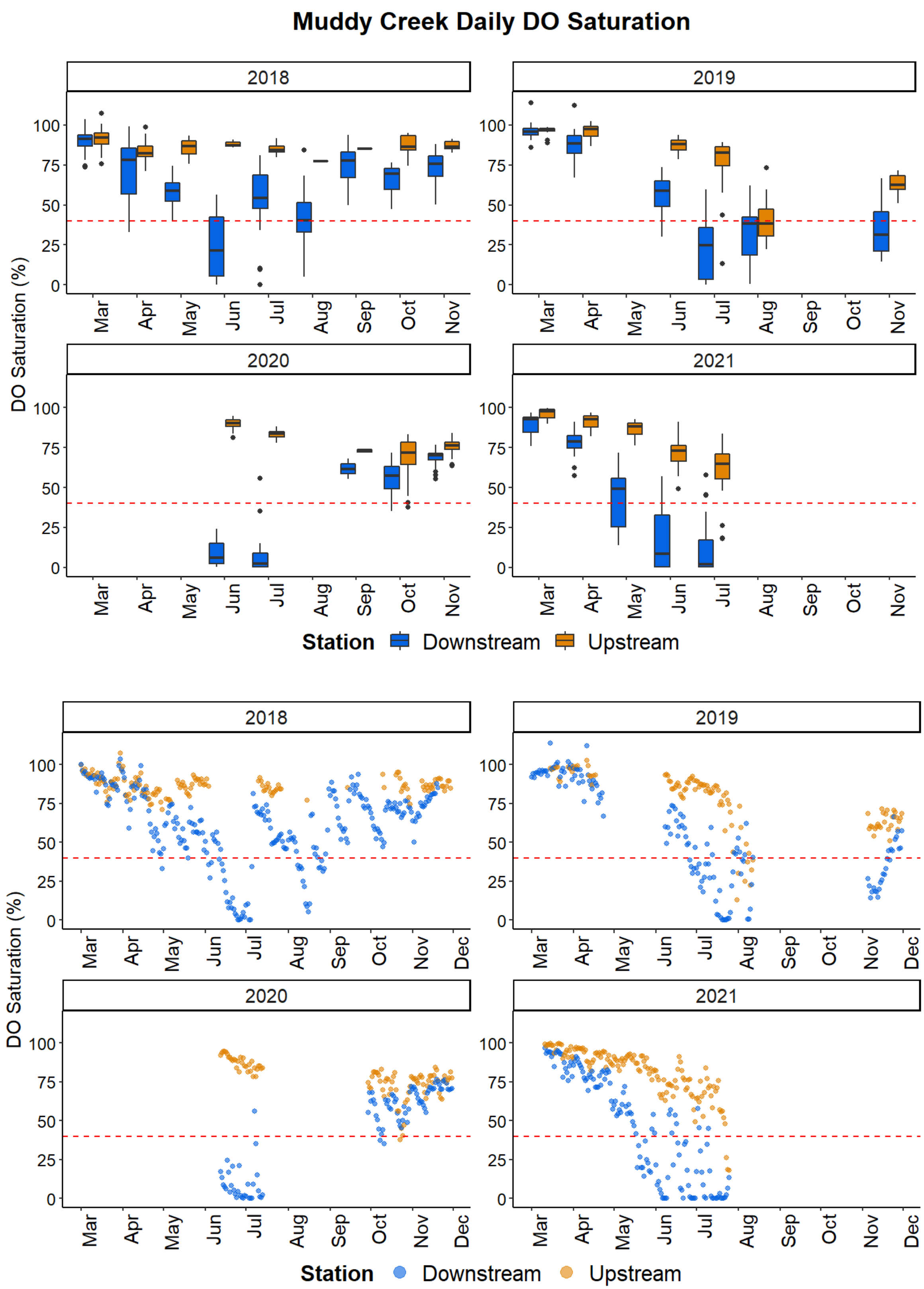


Figure 5. Mean daily dissolved oxygen saturation calculated from mean daily dissolved oxygen concentrations (mg/L) and temperature at the upstream and downstream stations. Red dotted lines represent the 40% BSID threshold for DO saturation in the Coastal Plain.

Temperature

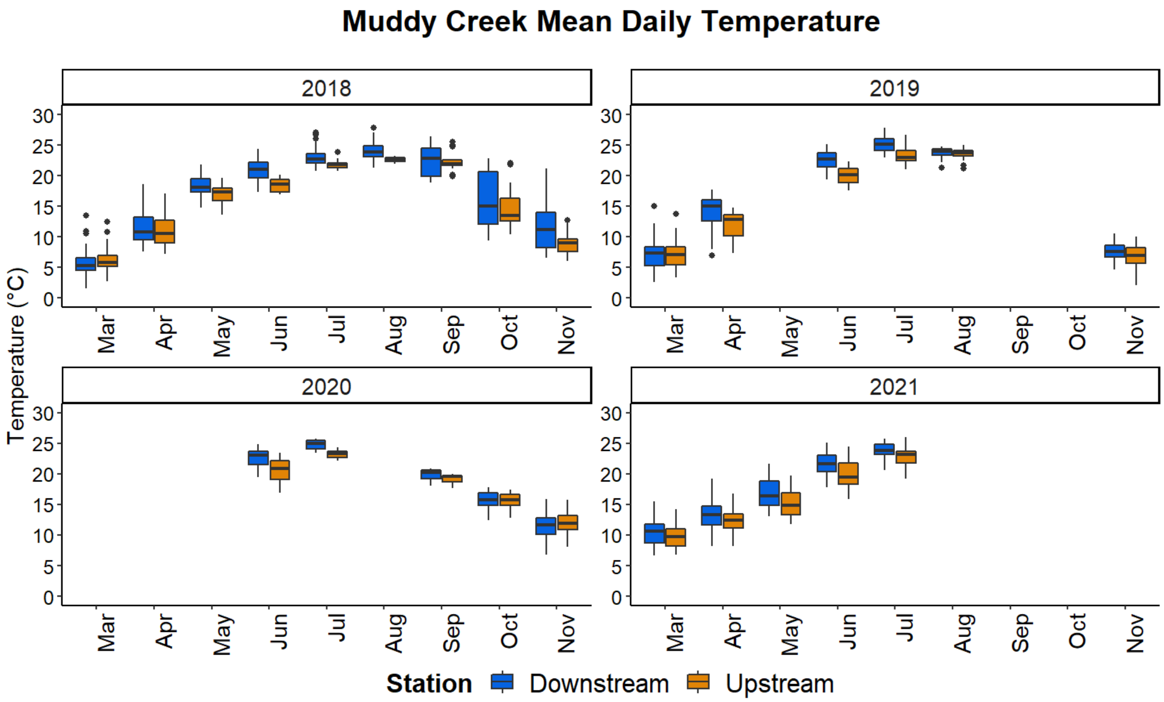
Analysis of temperatures recorded above and below the restoration project also indicated differing upstream and downstream conditions from 2018 to 2021, particularly during the summer months. Daily mean temperatures were significantly higher at the downstream station compared to the upstream station in 15 out of 25 months in which data were collected, with 10 months showing no significant difference among the daily means at the two stations based on Welch's t-tests.

In 2018, when data were collected during nine months from March to November, daily mean temperatures at the downstream station were significantly higher compared to the upstream station in five months: May ($p = 0.002$), June ($p < 0.001$), July ($p < 0.001$), August ($p < 0.001$), and November ($p = 0.002$). In 2019, daily mean temperatures at the downstream station were significantly higher compared to the upstream station in four out of six months: April ($p = 0.030$), June ($p < 0.001$), July ($p < 0.001$), and November ($p = 0.036$). In 2020, there were two out of five months with adequate data – June ($p < 0.001$) and July ($p < 0.001$) – when mean temperatures were higher at the downstream station compared to the upstream station. In 2021, mean temperatures at the two stations were not significantly different in March, but downstream mean temperatures were significantly higher than upstream mean temperatures for the subsequent four months: April ($p = 0.045$), May ($p = 0.003$), June ($p < 0.001$), and July ($p = 0.013$; Table 9).

Though data collection spanned varying months in each year of the study period, temperature differences were most pronounced during the summer months (Figure 6). Daily mean temperatures at the downstream station were consistently significantly higher in June and July of all four years compared to daily mean temperatures at the upstream station. This trend occurred with a high degree of significance in each year of this study.

Table 9. Temperature means with standard deviations (SD) by month from 2018 to 2021. Temperature readings at the downstream site (below restoration activities) compared to the upstream site are noted as higher or lower where significant, based on a Welch's t-test, with a corresponding p-value. NS indicates no significant difference.

	UPSTREAM		DOWNSTREAM			
	Mean	SD	Mean	SD	Change	p-value
2018						
March	6.34	1.99	6.02	2.42	NS	—
April	11.08	2.35	11.53	2.65	NS	—
May	17.21	1.37	18.44	1.78	higher	0.002
June	18.50	1.13	20.98	1.97	higher	<0.001
July	21.85	0.72	23.21	1.72	higher	<0.001
August	22.62	0.44	24.10	1.55	higher	<0.001
September	22.39	1.75	22.61	2.53	NS	—
October	14.77	3.25	16.02	4.52	NS	—
November	8.81	1.89	11.88	4.01	higher	0.002
2019						
March	7.32	2.54	7.44	2.95	NS	—
April	11.91	2.59	13.89	2.95	higher	0.030
May	—	—	—	—	—	—
June	20.03	1.47	22.45	1.73	higher	<0.001
July	23.38	1.39	25.22	1.39	higher	<0.001
August	23.51	1.19	23.64	1.08	NS	—
September	—	—	—	—	—	—
October	—	—	—	—	—	—
November	6.82	1.96	7.73	1.57	higher	0.036
2020						
March	—	—	—	—	—	—
April	—	—	—	—	—	—
May	—	—	—	—	—	—
June	20.57	1.86	22.62	1.51	higher	<0.001
July	23.23	0.66	24.77	0.82	higher	<0.001
August	—	—	—	—	—	—
September	19.08	1.22	19.76	1.46	NS	—
October	15.64	1.28	15.71	1.42	NS	—
November	11.96	1.90	11.50	2.13	NS	—
2021						
March	9.86	2.02	10.51	2.28	NS	—
April	12.40	2.06	13.45	2.61	higher	0.045
May	15.26	2.34	17.01	2.45	higher	0.003
June	19.93	2.13	21.71	1.81	higher	<0.001
July	22.80	1.60	23.77	1.41	higher	0.013
August	—	—	—	—	—	—
September	—	—	—	—	—	—
October	—	—	—	—	—	—
November	—	—	—	—	—	—



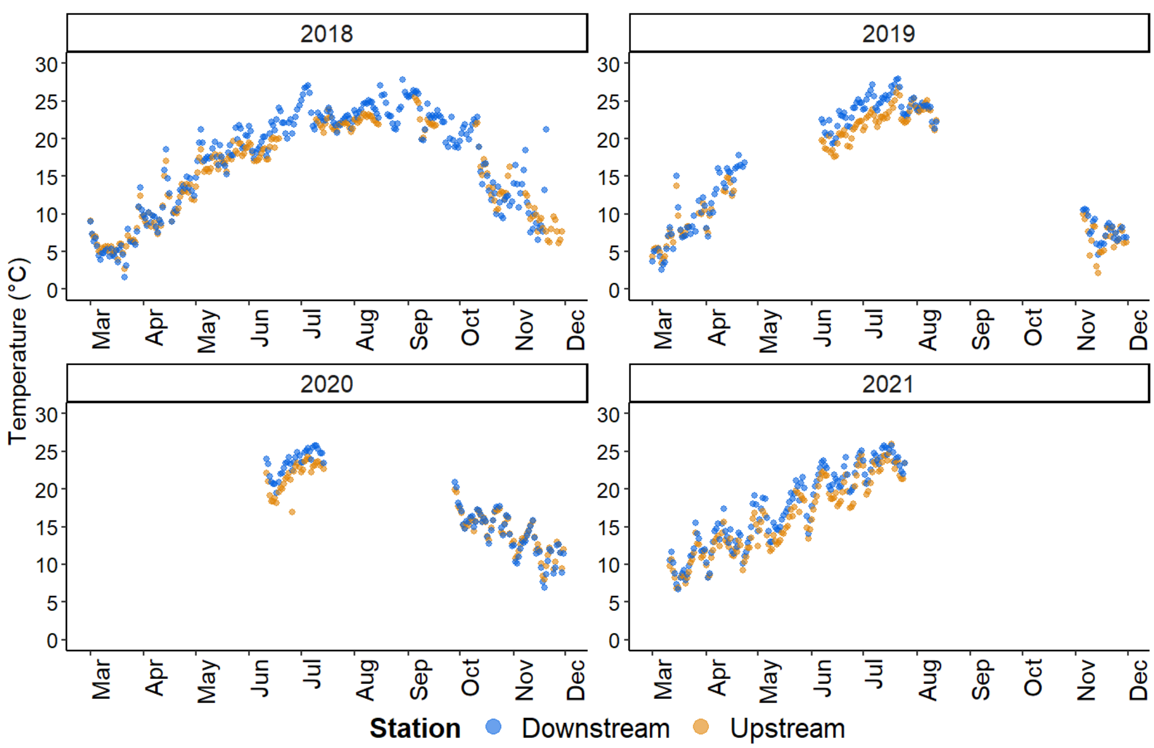


Figure 6. Mean daily temperature by month from 2018 to 2021 at the upstream and downstream stations at Muddy Creek.

Benthic Macroinvertebrate Results

Average pre-restoration Benthic Index of Biotic Integrity (BIBI) scores fell within the Fair narrative ranking, whereas average post-restoration BIBI scores remained in the Poor narrative ranking throughout the post-restoration monitoring period (2016-2021; Figure 7). Average BIBI scores in the post-restoration period showed a significant decrease ($p = 0.011$) from pre-restoration scores (Figure 7, Table 12). Metrics that exhibited significant decreases in the post-restoration period compared to the pre-restoration period include: Number of Taxa ($p = 0.047$), Shannon-Wiener Index ($p = 0.0039$), and Percent Predators ($p < 0.001$; Figure 8, Table 12). The Number of Grids picked to reach an acceptable number of organisms for the BIBI also significantly decreased ($p < 0.001$; Figure 8C, Table 12), which indicates that the density of benthic macroinvertebrates increased in the post-restoration period. Metrics that exhibited significant increases in the post-restoration period compared to the pre-restoration period include: Percent Chironomidae ($p < 0.001$) and Percent Collectors ($p = 0.010$, Figure 8, Table 12). Metrics that did not significantly change between pre- and post-restoration periods include: Percent Intolerant, Percent EPT, Percent Filterers, Percent Clingers, Percent Burrowers, and Percent Sprawlers (Figure 8, Table 12).

Of the four metrics that exhibited significant decreases in the post-restoration period compared to the pre-restoration period (BIBI score, Number of Taxa, Shannon-Wiener Index, and Percent Predators; Table 12), site 103, in particular, does not appear to be following this trend entirely. Site 103 had similar pre- and post-restoration BIBI scores (except in 2016; Figure 7A), Number of Taxa (Figure 8A), and Shannon-Wiener Index scores (Figure 8B), and a lower percentage of predators in the post-restoration period (Figure 8H). Of the two metrics that exhibited significant increases in the post-restoration period compared to the pre-restoration period (Percent Chironomidae and Percent Collectors), site 103 remained higher for both of these metrics in the post-restoration period, but is less dominated by Chironomidae and collectors compared to sites 101 and 102 (Figure 8F, Figure 8G). Additionally, site 103 had higher post-restoration medians of Percent Intolerant Urban and Percent EPT compared to sites 101 and 102 (Figure 8D, Figure 8E).

Non-metric multidimensional scaling (NMDS) graphics (Figure 9) suggest that there was significant benthic macroinvertebrate community similarity between all upstream control and pre-restoration samples. In contrast, no significant community similarity between pre-restoration and post-restoration samples is evident. The post-restoration samples in 2016, 2017, and 2018 shared no community overlap with adjacent control samples, with one exception of site 103 in 2017 falling within the convex hull of the adjacent control group. In 2019 and 2021, nearly all post-restoration samples fell within the convex hull of the adjacent control group, and clustered near its centroid. This finding suggests that the post-restoration community structure has shifted over time to resemble the adjacent control community, and is becoming less similar to the upstream control community.

Indicator Species Analysis (ISA) results suggest that the pre-restoration community was dominated by Diptera taxa within Families Chironomidae (tolerant midges; three genera), Tabanidae (intolerant horse flies; one genus), Tipulidae (crane flies; three genera), and Ceratopogonidae (biting midges, one taxon). These samples were also dominated by one Coleoptera taxon, one Amphipoda taxon, one Ephemeroptera taxon, and one Bivalvia taxon. In contrast, the post-restoration community is dominated by two tolerant midge taxa and two tolerant snail taxa, and the adjacent control is dominated by three tolerant midge taxa and one Amphipoda taxon (Table 13).

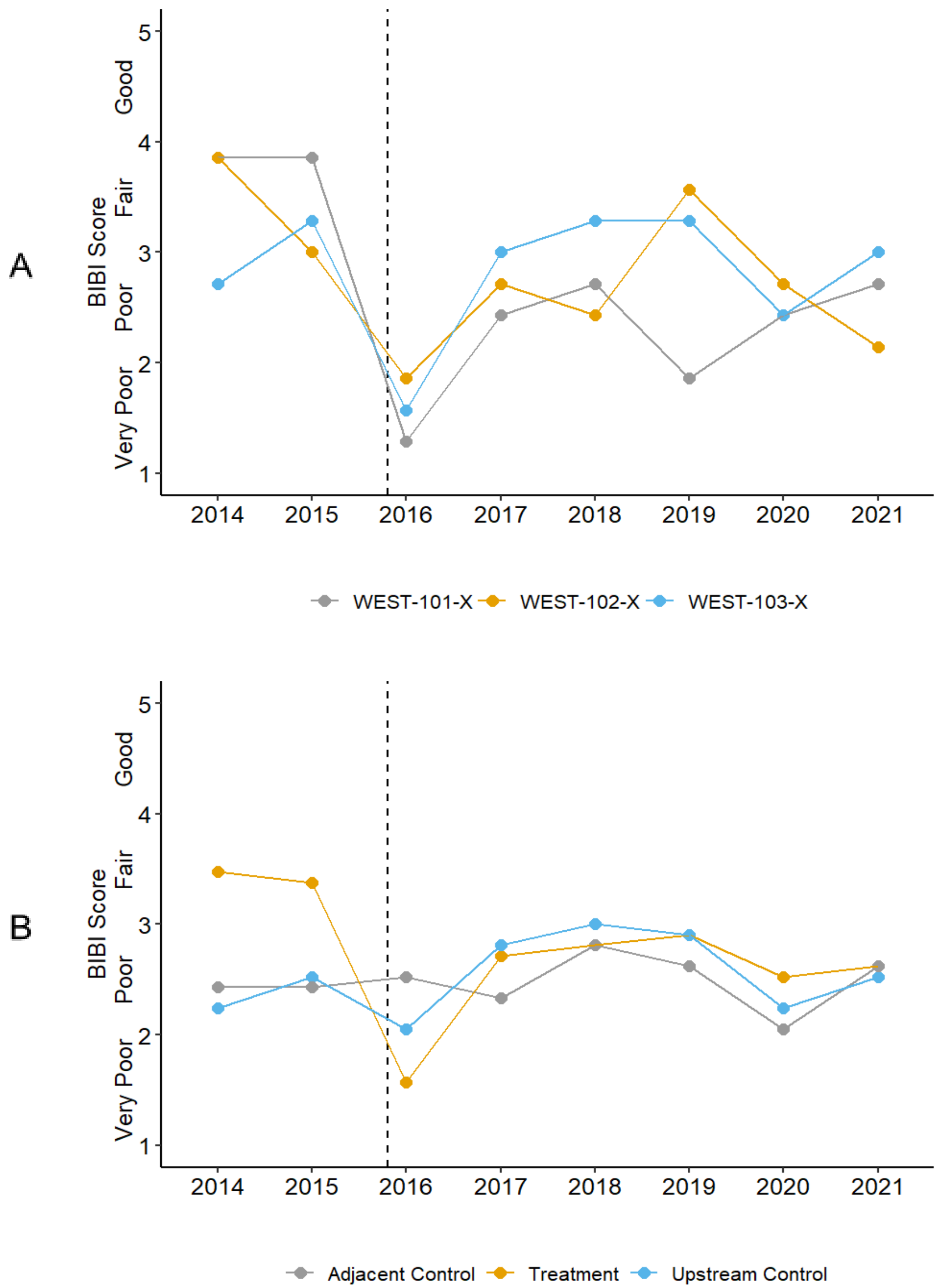


Figure 7. Raw Benthic Index of Biotic Integrity (BIBI) scores within the restoration reach (A), and average BIBI scores among site types by sampling year (B). Dotted line delineates pre- (2014-2015) and post-restoration (2016-2021) sampling.

Table 12. Summary statistics of select diversity, density, sensitive groupings, tolerant groupings, functional feeding group and habit metrics, as well as Benthic Index of Biotic Integrity (BIBI) scores. Pre-restoration sites include sites 101, 102, and 103 collected in 2014 and 2015. Post-restoration sites include sites 101, 102, and 103 collected between 2016 - 2021. Upstream control sites include sites 104, 105, and 106 collected between 2014 - 2021. Adjacent control sites include sites 108 and 109 collected between 2014 - 2021 and site 110 collected between 2016-2021. * = pre-restoration mean significantly higher than post-restoration mean; ** = pre-restoration mean significantly lower than post-restoration mean. S-W Index = Shannon-Weiner Diversity Index, St Deviation = standard deviation.

		Pre-Restoration	Post-Restoration	Upstream Control	Adjacent Control
BIBI	Mean	3.43*	2.52	2.54	2.48
	St Deviation	0.50	0.61	0.69	0.56
	Range	2.71 - 3.86	1.28 - 3.57	1.28 - 3.86	1.57 - 3.86
	Tukey Letter	A	B	B	B
	ANOVA p-value	0.011	—	—	—
Number of Taxa	Mean	23.17*	16.78	18.71	17.86
	St Deviation	4.36	4.6	5.42	4.26
	Range	19 - 29	5 - 23	10 - 30	11 - 26
	Tukey Letter	A	B	AB	AB
	ANOVA p-value	0.047	—	—	—
S-W Index	Mean	2.72*	2.17	2.22	2.13
	St Deviation	0.30	0.35	0.41	0.27
	Range	2.32 - 3.11	1.68 - 2.74	1.44 - 2.79	1.43 - 2.52
	Tukey Letter	A	B	B	B
	ANOVA p-value	0.004	—	—	—
Number of Grids	Mean	12.03*	8.67	12.43	6.36
	St Deviation	6.65	11.34	11.16	5.08
	Range	6 - 21	2 - 47	2 - 50	1 - 17
	Tukey Letter	A	B	A	C
	ANOVA p-value	<0.001	—	—	—
Percent Intolerant	Mean	43.82	28.09	42.02	25.51
	St Deviation	23.74	21.9	26.91	23.07
	Range	13.27 - 74.28	0.8 - 71.09	0.73 - 88.33	1.36 - 84.91
	Tukey Letter	A	A	A	A
	ANOVA p-value	0.072	—	—	—
Percent EPT	Mean	13.66	6.97	11.85	9.37
	St Deviation	7.96	8.26	9.78	11.93
	Range	3.54 - 26.09	0 - 25.22	0 - 38.79	0 - 44.12
	Tukey Letter	A	A	A	A
	ANOVA p-value	0.352	—	—	—
Percent Chironomidae	Mean	26.57**	59.98	33.79	55.07
	St Deviation	11.38	25.12	21.68	26.3
	Range	11.50 - 38.94	7.59 - 89.76	4.17 - 82.48	3.25 - 84.91
	Tukey Letter	B	A	B	A
	ANOVA p-value	<0.001	—	—	—

Percent Collectors	Mean	23.39**	54.3	42	47.15
	St Deviation	9.08	18.33	24.25	16.42
	Range	13.27 - 39.09	18.54 - 77.95	8.47 - 92.70	17.07 - 78.30
	Tukey Letter	B	A	AB	AB
	ANOVA p-value	0.010	—	—	—
Percent Predators	Mean	22.47*	6.15	9.26	8.5
	St Deviation	12.41	8.38	6.36	6.99
	Range	6.96 - 38.94	0 - 37.09	0 - 25	0 - 26.47
	Tukey Letter	A	B	B	B
	ANOVA p-value	<0.001	—	—	—
Percent Filterers	Mean	19.61	17.38	12.73	10.46
	St Deviation	10.88	15.52	16.89	12.99
	Range	6.36 - 33.63	1.32 - 67.19	0 - 67.80	0.8 - 46.22
	Tukey Letter	A	A	A	A
	ANOVA p-value	0.376	—	—	—
Percent Clingers	Mean	30.34	27.91	27.25	25.65
	St Deviation	15.55	17.8	20.76	21.3
	Range	10.61 - 48.67	1.98 - 71.09	4.13 - 79.66	3.25 - 81.08
	Tukey Letter	A	A	A	A
	ANOVA p-value	0.958	—	—	—
Percent Burrowers	Mean	21.34	19.2	13.61	25.87
	St Deviation	10.88	12.72	11.05	15.42
	Range	7.62 - 38.94	0 - 45	1.67 - 44.14	4.07 - 48.72
	Tukey Letter	AB	AB	B	A
	ANOVA p-value	0.021	—	—	—
Percent Sprawlers	Mean	44.16	58.33	48.97	64.25
	St Deviation	8.84	17.62	18.45	16.28
	Range	32.38 - 57.27	22.82 - 77.39	11.57 - 83.56	29.73 - 93.10
	Tukey Letter	B	AB	B	A
	ANOVA p-value	0.009	—	—	—

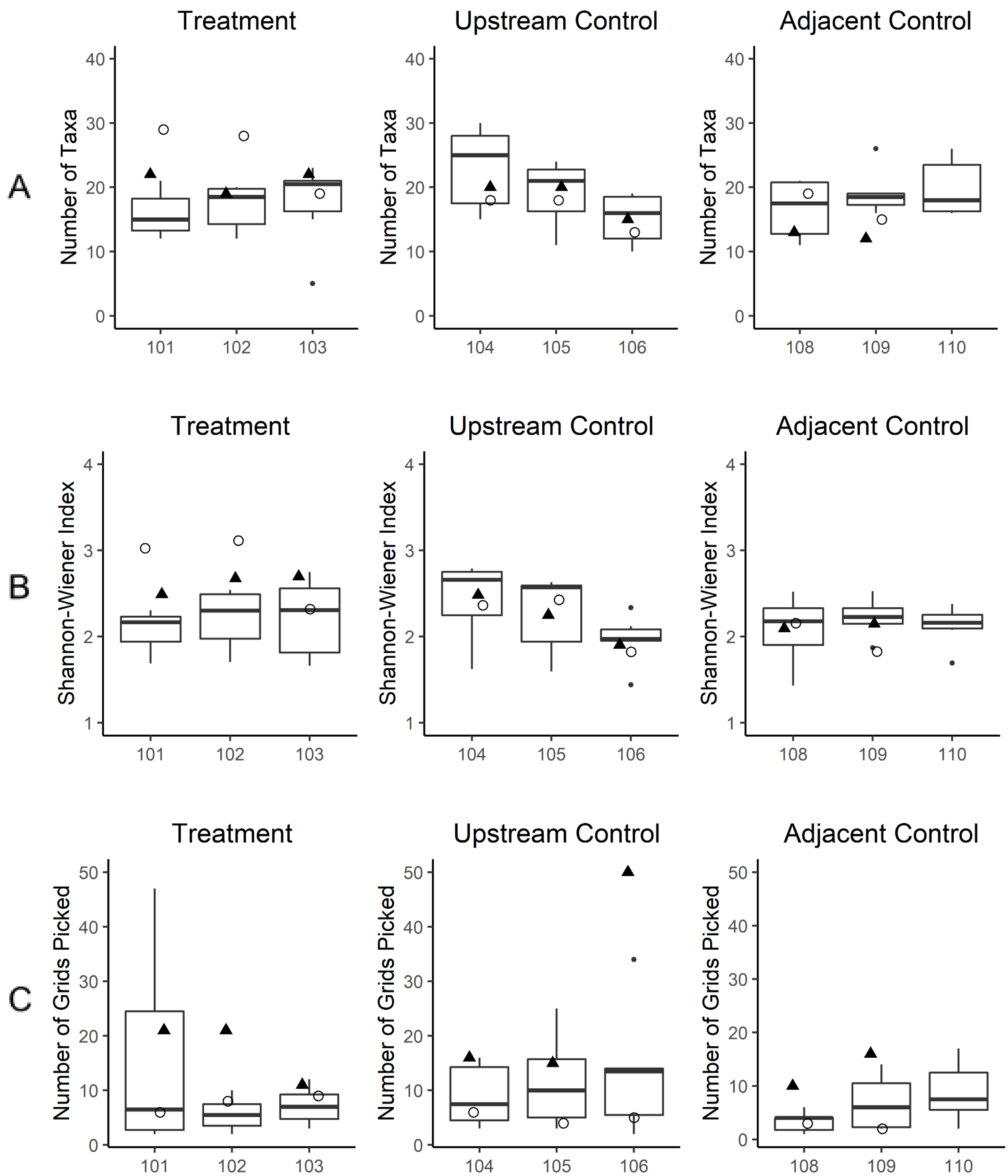


Figure 8. Observed taxa richness (A), diversity (B), number of grids picked, estimated density (C), proportions of intolerant groups (D and E) and proportions of tolerant groups (F). Communities are also grouped by functional feeding groups (G - I) and by habit (J - L). Open circles and closed triangles represent values from pre-restoration years, 2014 and 2015 respectively. Boxplots summarizing the median (horizontal black bar), 0.25/0.75 quantiles (bottom and top of box respectively), 0.025/0.975 quantiles (vertical black line extent), and outliers (smaller black points) for post-restoration years (2016 - 2021) are also included. Site names are included on the x-axis.

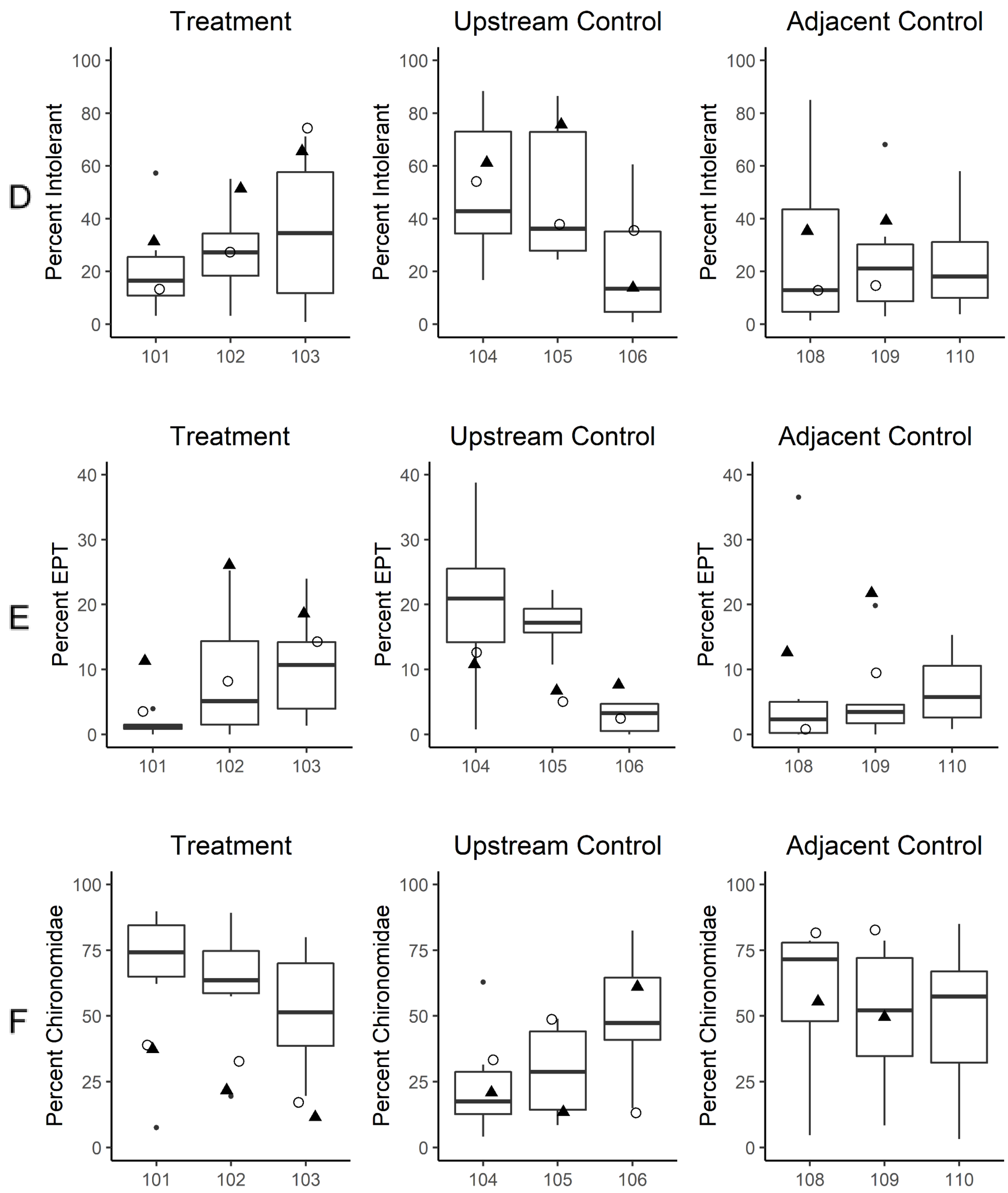


Figure 8 (continued). Observed taxa richness (A), diversity (B), number of grids picked, estimated density (C), proportions of intolerant groups (D and E) and proportions of tolerant groups (F). Communities are also grouped by functional feeding groups (G - I) and by habit (J - L). Open circles and closed triangles represent values from pre-restoration years, 2014 and 2015 respectively. Boxplots summarizing the median (horizontal black bar), 0.25/0.75 quantiles (bottom and top of box respectively), 0.025/0.975 quantiles (vertical black line extent), and outliers (smaller black points) for post-restoration years (2016 - 2021) are also included.

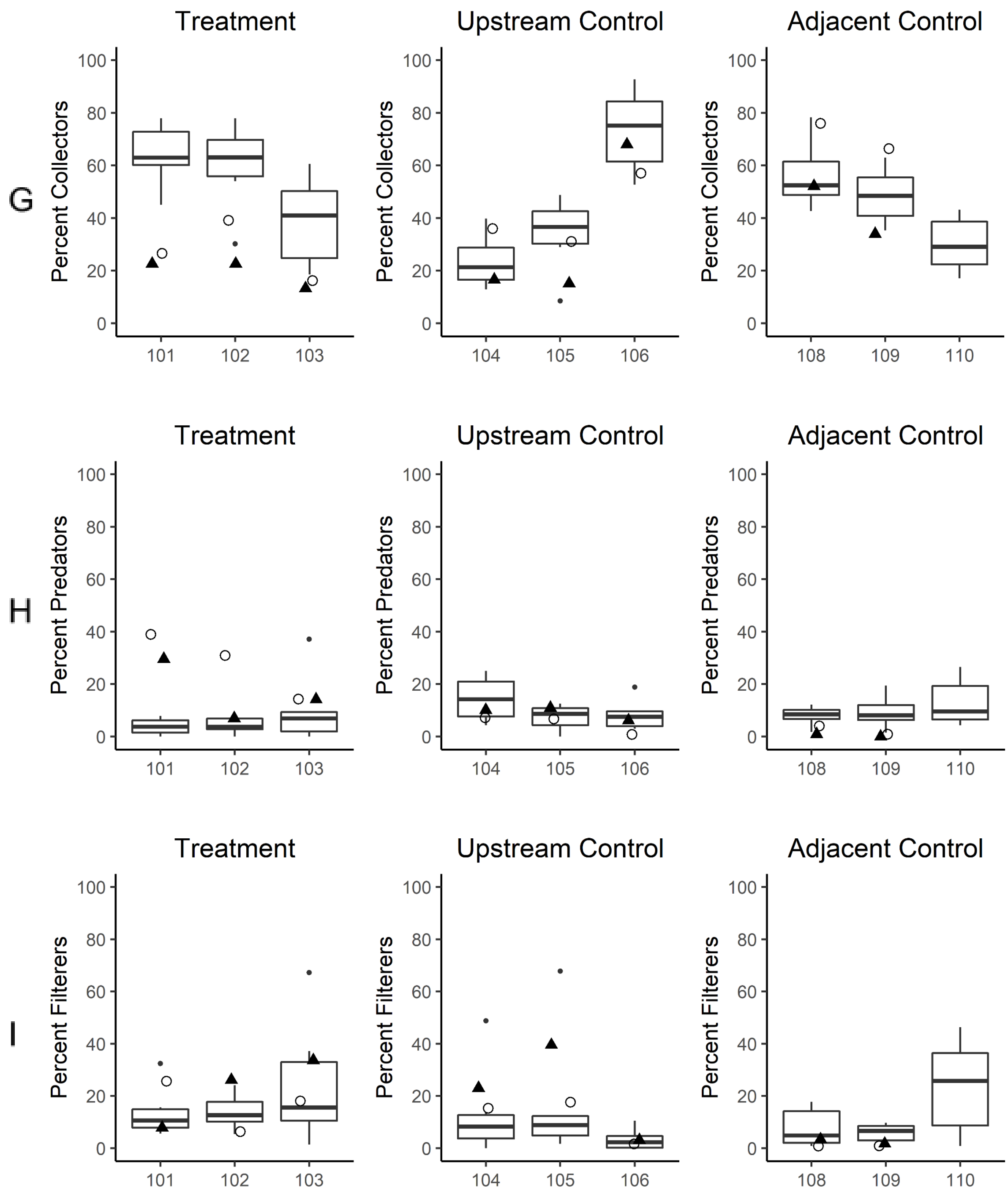


Figure 8 (continued). Observed taxa richness (A), diversity (B), number of grids picked, estimated density (C), proportions of intolerant groups (D and E) and proportions of tolerant groups (F). Communities are also grouped by functional feeding groups (G - I) and by habit (J - L). Open circles and closed triangles represent values from pre-restoration years, 2014 and 2015 respectively. Boxplots summarizing the median (horizontal black bar), 0.25/0.75 quantiles (bottom and top of box respectively), 0.025/0.975 quantiles (vertical black line extent), and outliers (smaller black points) for post-restoration years (2016 - 2021) are also included.

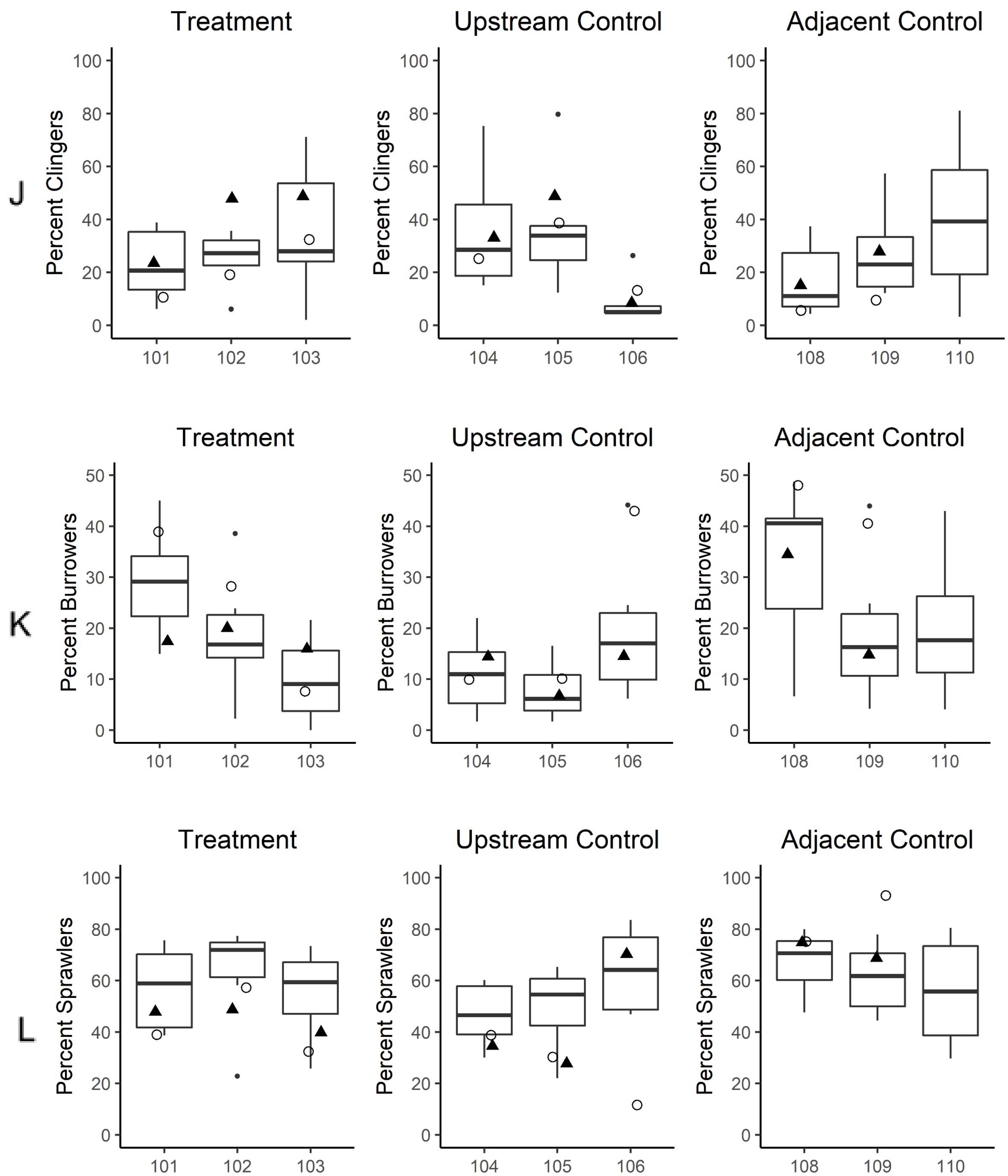


Figure 8 (continued). Observed taxa richness (A), diversity (B), number of grids picked, estimated density (C), proportions of intolerant groups (D and E) and proportions of tolerant groups (F). Communities are also grouped by functional feeding groups (G - I) and by habit (J - L). Open circles and closed triangles represent values from pre-restoration years, 2014 and 2015 respectively. Boxplots summarizing the median (horizontal black bar), 0.25/0.75 quantiles (bottom and top of box respectively), 0.025/0.975 quantiles (vertical black line extent), and outliers (smaller black points) for post-restoration years (2016 - 2021) are also included.

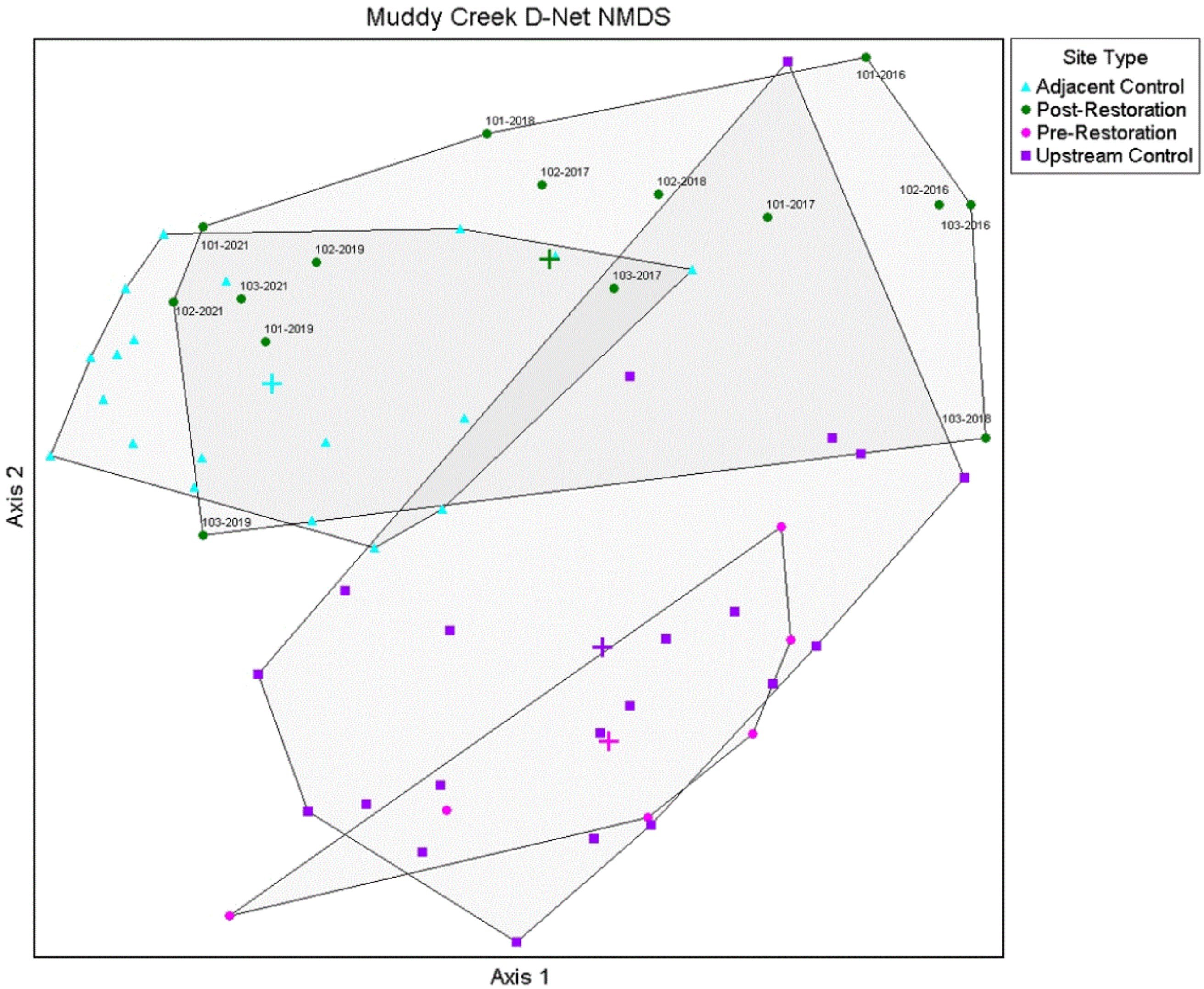


Figure 9. Non-metric multidimensional scaling (NMDS) graphic displaying all D-net benthic samples shown as triangles, circles, or squares after applying a square root transformation to raw data. Upstream control samples are shown as purple squares, adjacent control samples are shown blue triangles, pre-restoration samples are shown as pink circles, and post-restoration samples are shown as green circles. Each site type is enclosed via convex hulls. Individual post-restoration samples are labeled by site number followed by year. All 2020 samples were excluded from this analysis. Stress = 0.140.

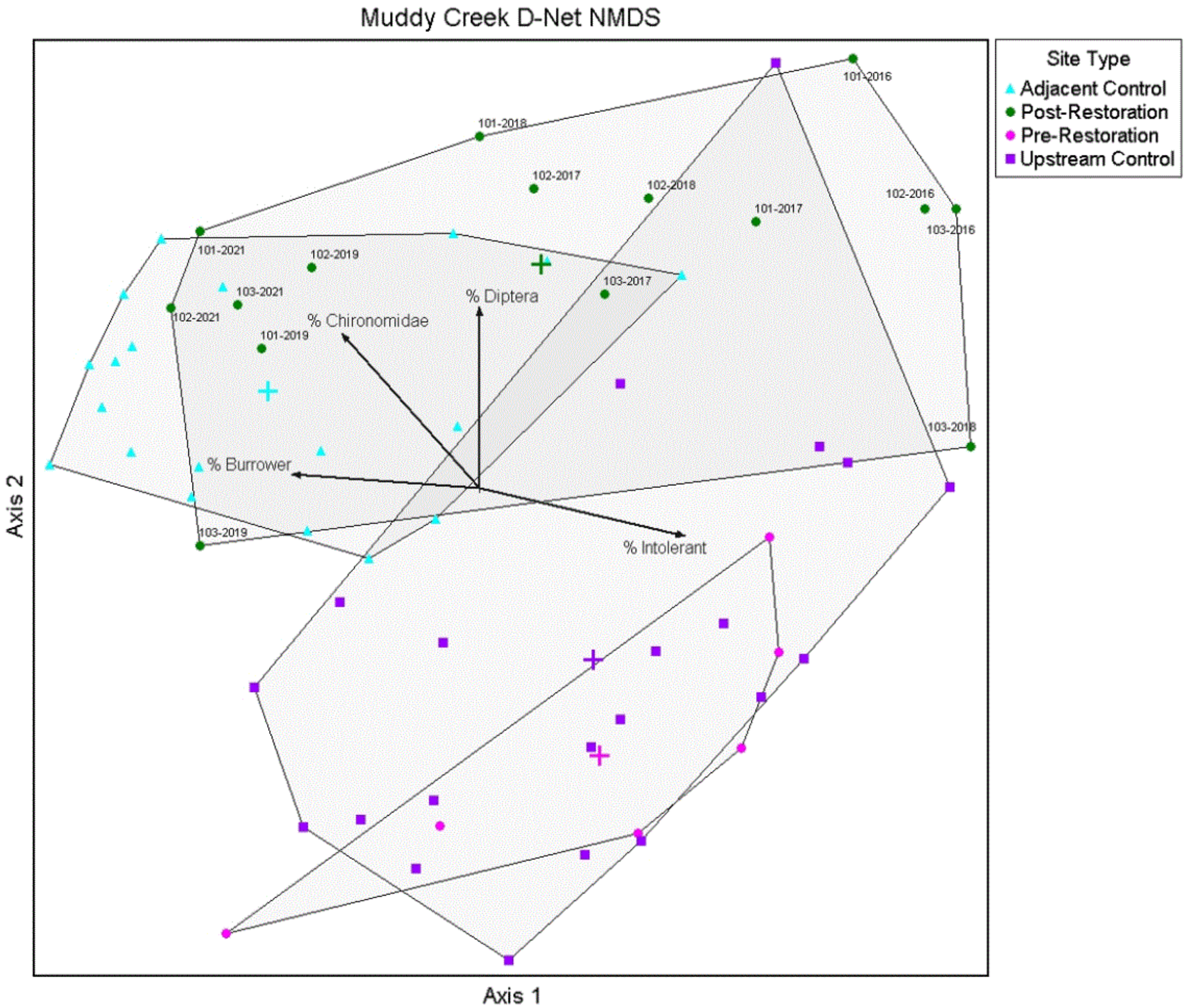


Figure 9 (continued). Non-metric multidimensional scaling (NMDS) graphic displaying all D-net benthic samples shown as triangles, circles, or squares after applying a square root transformation to raw data. Black lines with corresponding arrows indicate covariate overlays with an R-squared value of 0.400 or greater. Upstream control samples are shown as purple squares, adjacent control samples are shown blue triangles, pre-restoration samples are shown as pink circles, and post-restoration samples are shown as green circles. Each site type is enclosed via convex hulls. Individual post-restoration samples are labeled by site number followed by year. All 2020 samples were excluded from this analysis. Stress = 0.140.

Table 13. Indicator Species Analysis grouped by site types: Pre-restoration sites include sites 101, 102, and 103 collected in 2014 and 2015. Post-restoration sites include sites 101, 102, and 103 collected between 2016 - 2021. Upstream control sites include sites 104, 105, and 106 collected between 2014 - 2021. Adjacent control sites include sites 108, 109, and 110 collected between 2014 - 2021. All 2020 samples were excluded from this analysis. Taxa listed are those with a p-value of <0.05 using a Monte Carlo test of significance. FFG = Functional Feeding Group, sp = sprawler, bu = burrower, sw = swimmer, cn = clinger, cb = climber, TV = Tolerance Value, IV = Indicator Species Analysis Indicator Value.

	Taxon	Order	Family	FFG	Habit	TV	IV	p-value
Upstream Control	Synurella	Amphipoda	Crangonyctidae	–	–	0.4	45.1	0.0140
Pre-Restoration	Ceratopogonidae	Diptera	Ceratopogonidae	Predator	sp, bu	3.6	40.3	0.0118
	Chrysops	Diptera	Tabanidae	Predator	sp, bu	2.9	27.4	0.0458
	Erioptera	Diptera	Tipulidae	Collector	bu	4.8	24.2	0.0458
	Neoporus	Coleoptera	Dytiscidae	Predator	sw, cb	5.0	41.3	0.0038
	Paraphaenocladius	Diptera	Chironomidae	Collector	sp	4.0	34.3	0.0106
	Phaenopsectra	Diptera	Chironomidae	Collector	cn	8.7	24.8	0.0354
	Pisidiidae	Veneroida	Pisidiidae	Filterer	bu	6.5	39.9	0.0166
	Pseudolimnophila	Diptera	Tipulidae	Predator	bu	2.8	42.6	0.0010
	Siphlonurus	Ephemeroptera	Siphlonuridae	Collector	sw, cb	7.0	39.2	0.0040
	Stygobromus	Amphipoda	Crangonyctidae	Collector	–	4.0	36.9	0.0070
	Tipula	Diptera	Tipulidae	Shredder	bu	6.7	50.5	0.0034
	Zavrelimyia	Diptera	Chironomidae	Predator	sp	5.3	40.2	0.0344
Post-Restoration	Cricotopus	Diptera	Chironomidae	Shredder	cn, bu	9.6	20.1	0.0356
	Diplocladius	Diptera	Chironomidae	Collector	sp	5.9	56.8	0.0070
	Menetus	Basommatophora	Planorbidae	Scraper	cb	7.6	20.1	0.0336
	Pseudosuccinea	Basommatophora	Lymnaeidae	Collector	cb	6.3	20.1	0.0356
Adjacent Control	Gammarus	Amphipoda	Gammaridae	Shredder	sp	6.7	21.1	0.0462
	Hydrobaenus	Diptera	Chironomidae	Scraper	sp	7.2	62.8	0.0004
	Mesocricotopus	Diptera	Chironomidae	–	–	6.6	57.9	0.0006
	Orthocladius	Diptera	Chironomidae	Collector	sp, bu	9.2	52.3	0.0476

DISCUSSION

The implementation of the RSC restoration at Muddy Creek has dramatically altered the hydrology, channel geomorphology, and its adjacent riparian buffer between pre- and post-restoration conditions. A clearly well-defined stream channel, with high channel incision and little bedform diversity defined pre-restoration conditions, and its geomorphic, hydraulic, and hydrologic conditions were all deemed non-functional by United States Fish and Wildlife Service's pre-restoration assessments (Starr and Cullen 2016).

Post-restoration conditions differed between the top half and bottom half of the 452-meter restoration area; however, both sections contain several rocky riffles, clear floodplain access, and adjacent vernal pools. The top half of the restoration area resembles a restored stream with a narrower average width (where biological site 103 resides), whereas the bottom half of the restoration reach more closely resembles a stream-wetland complex with more backwater lentic habitat and greater variability of stream widths (where biological sites 102 and 101 reside).

The floodplain connectivity that was accomplished with RSC implementation is expected to dissipate the energy from storms, which may decrease their power to erode and scour stream banks, resulting in less downstream sediment and nutrient export. Greater retention times within the restoration are expected to increase in-stream nutrient uptake. Detaining surface runoff within the floodplain may decrease nutrient export through the processes of filtration and denitrification.

Thompson et al. 2018 reported that SERC's water chemistry data collected upstream and downstream of the Muddy Creek restoration reach indicated a net export of ortho-phosphate, TP, NH4, and TSS, and a net retention of NO3 and TN in pre-restoration conditions. Post-restoration water chemistry data showed a significant reduction in ortho-phosphate and TP loads, a marginally significant reduction in NH4 and TN, and a statistically insignificant reduction in NO3 and TSS using a Randomized Intervention Analysis (RIA) at the reach scale. TSS concentrations seem to suggest retention at a much higher percentage post-restoration, but was not confirmed via RIA because of high variability from storm flow events.

It was suggested by Thompson et al. 2018 that a beaver pond downstream of the restoration – which has significantly reduced total nitrogen, total phosphorus, and TSS loads in the past (Correll et al. 2000) – could help explain why significant load and FWMC reductions were observed at the restoration's outlet but not on a larger scale in the watershed.

Dissolved oxygen and temperature

Data collected at two fixed monitoring stations strongly indicate restoration activities may have impacted dissolved oxygen at the station located at the downstream end of the restoration reach. Compared to the upstream station above the restoration reach, DO conditions were often distinctly lower at the downstream station based on multiple factors, including mean daily DO concentrations, number of days with DO concentrations below 5.0 mg/L, and DO saturation levels.

One likely contributor to lower DO concentrations at the downstream station are large blooms of iron-oxidizing bacteria, which have been observed along with high concentrations of iron flocculent in the restoration reach (Figure 10). These bacteria can substantially contribute to the iron oxidation process (Emerson et al. 2010), have a high oxygen demand (Fleming et al. 2014), and block sunlight necessary for algal growth (Hayer et al. 2013), which can further decrease dissolved oxygen. *Leptothrix*, the same type of iron-oxidizing bacteria observed in the restoration reach at Muddy Creek, has been shown to reduce algae within its blooms, possibly as a result of the iron oxide deposits it helps produce creating toxic conditions, smothering the algae, or binding to important nutrients like phosphorus (Sheldon and Wellnitz 1998).

Iron flocculation can occur naturally. Iron that is introduced to a stream via restoration construction materials (e.g., as a constituent of introduced substrates) can also contribute to the proliferation of iron flocculation. Additionally, proliferation of iron flocculation can be facilitated by the movement of adjacent soils and consequent increase in iron they contribute to a stream (Williams et al. 2016). RSCs in the mid-Atlantic region are commonly built with ironstone (Fanelli et al. 2019), which was used in the form of boulders and sand in weirs within some Maryland RSCs (Williams et al. 2016). Lab experiments conducted by Williams et al. 2016 suggested that construction materials at RSC sites, as well as riparian soils, contributed to high iron concentrations in stream water and groundwater. Chemical oxygen demand, and possibly biological oxygen demand depending on other factors, could be affected in downstream waters by the dissolved iron sourced from these materials (Fanelli et al. 2019).

Figure 10. Iron flocculent observed in the restoration reach of Muddy Creek in Fall 2020.

Decreased DO concentrations downstream could also be tied to increased dissolved organic carbon (DOC) from decomposing wood chips and trees in the restoration reach. RSC sites containing high concentrations of DOC from organic matter as well as high iron concentrations can help create the conditions that allow iron-oxidizing bacteria to produce large and dense amounts of iron flocculate (Williams et al. 2016). Duan et al. 2019 found that two streams with regenerative stormwater conveyance projects in Anne Arundel County experienced lower DO saturation levels compared to unrestored control sites, resulting in part from bacterial oxygen consumption during the decomposition of wood chips and accumulated leaf litter. Additional factors such as reduced streamflow velocity in the restoration areas may have possibly contributed as well.

Other monitored RSC systems in Maryland have demonstrated similar effects on dissolved oxygen. Three urban streams with RSCs constructed in their watersheds had lower dissolved oxygen concentrations than four forested streams as well as four urban streams located in what were considered degraded watersheds that had not undergone restoration. The average of the lowest minimum DO concentrations was below 5.0 mg/L in each of the three restored urban streams, but only at two of the four degraded urban streams; averages of the forested streams' very lowest concentrations never fell below the threshold (Fanelli et al. 2019).

Significantly higher daily mean temperatures at the downstream station compared to the upstream station in 15 out of 25 months studied could also stem from the RSC at Muddy Creek, and may also help contribute to the lower DO concentrations downstream. Ten months of the monitoring period showed no significant differences between temperatures at the upstream and downstream stations, but clear patterns of warmer temperatures were observed at the downstream station during summer months.

Other studies have shown no significant effects or inconclusive and possibly positive effects on stream temperature by RSC systems. Fanelli et al. 2019 found that, compared to the degraded urban streams, the restored urban streams experienced smaller temperature surges resulting from rainfall and runoff. Maximum daily summer temperatures, however, were not clearly different between the two groups of urban streams, and Fanelli et al. 2019 concluded that significant differences in DO saturation between the restored and degraded urban streams were likely not caused by water temperature. Duan et al. 2019 also did not see a pattern of significantly different temperatures in the RSC sites compared to the unrestored streams.

Benthic Macroinvertebrates

Benthic macroinvertebrate communities appear to have changed between the pre- and post-restoration periods, with significant declines in BIBI scores, taxa richness, Shannon-Wiener diversity scores, and significant increases in percentages of Chironomidae and collectors.

Despite the restoration's success in improving physical habitat heterogeneity for benthic macroinvertebrate colonization, particularly within the constructed rocky riffles, biological improvement measured by BIBI scores, taxa richness, and Shannon-Wiener Index scores was not observed in the post-restoration period. In fact, it appears that declines in biological communities are evident within Muddy Creek after six years of post-restoration monitoring. Documented declines in BIBI scores associated with RSC restored streams have also been discussed by Hilderbrand et al. 2019, despite a few case studies of marginal biological improvement. Additionally, Palmer et al. 2010 found that in 78 restoration projects examined, only two demonstrated increases in taxonomic diversity and that diversity was more likely explained by surrounding land use and water quality. Similarly, Zerega et al. 2021 found that benthic macroinvertebrate communities sometimes did not appear to benefit from the restoration approaches implemented, including flow modification and improved habitat heterogeneity. Such hydromorphological measures could be limited in their ability to help benthic macroinvertebrates if either water quality issues or larger issues in the watershed continue to affect the stream (Zerega et al. 2021).

Despite documented decreases in some nutrient concentrations at Muddy Creek, the biological community appears to be poorer in the post-restoration period with higher percentages of collectors and Chironomidae, which are both silt- and sand-tolerant organisms and early colonizers to disturbance (Selvakumar et al. 2010). This shift in community composition to the dominance of tolerant organisms may be a result of persistent iron flocculent blooms present within post-restoration conditions, particularly observed in the downstream half of the restoration reach (where sites 102 and 101 reside). Iron precipitation is known to reduce benthic macroinvertebrate diversity (Vuori 1995), and, in some cases, results in dissolved oxygen crashes through significant algae reductions or oxygen demands (Sheldon and Wellnitz 1998, Hayer et al. 2013, Fleming et al. 2014). This may explain why site 103 was least impacted, from a biological perspective, compared to sites 102 and 101.

Iron precipitation can physically change stream habitat by covering substrate such as rocks and leaves, and filling interstitial spaces between substrate particles, making them difficult for some benthic invertebrate species to live on or feed from (McKnight and Feder 1984, Wellnitz et al. 1994, Hayer et al. 2013, Cadmus et al. 2018). Williams et al. 2016 concluded that, at certain times of the year, iron flocculate in RSC streams accumulated to such a problematic level that benthic macroinvertebrates might be unable to use the covered substrate. In addition, blooms of iron-oxidizing bacteria can play a role in decreasing macroinvertebrate diversity as they cover substrate, act as a low-quality or even harmful food source, and accumulate on some species' gills (Wellnitz et al. 1994). The ability of iron precipitation to cover substrate, increase turbidity, and absorb nutrients can also reduce algal growth in streams (Sheldon and Wellnitz 1998, Hayer et al. 2013). A decline in algae can in turn contribute to decreased abundance of macroinvertebrates that consume algae (Hayer et al. 2013). As a food source themselves, macroinvertebrates can pass iron precipitates in their bodies to larger consumers in the ecosystem (Vuori 1995).

The multi-pronged monitoring approach at Muddy Creek showed varying possible responses within the stream ecosystem to the implementation of the RSC. Based on SERC's research, the restoration significantly increased the retention of some nutrients within the reach, though these effects were not observed across all parameters analyzed and did not appear to significantly contribute to nutrient retention within the entire watershed (Thompson et al. 2018). However, the MDNR results from this study showed evidence of potentially harmful effects of the RSC on dissolved oxygen, temperature, and benthic macroinvertebrate communities.

Literature Cited

Berg, J., Underwood, K., 2007. Constructed weirs and pools for safe, non-erosive conveyance of stormwater runoff: an example of regenerative design. Ecological Society of America Annual Meeting.

Bernhardt, E.S., Palmer, M.A., Allan, J., Alexander, G., Barnas, K., Brooks, S., Carr, J., Clayton, S., Dahm, C., Follstad-Shah, J., 2005. Synthesizing US river restoration efforts. Science 308 (5722), 636–637.

Boesch, D.F., R.B Russell and R.E. Magnien. 2001. Chesapeake Bay eutrophication: Scientific understanding, ecosystem restoration, and challenges for agriculture. Journal of Environmental Quality 30:303-320.

Bowen, R., 2012. Regenerative Step Pool Conveyance (SPSC) Design Guidelines. Anne Arundel County Government Department of Public Works Bureau of Engineering.

Brown, T., Berg, J., Underwood, K., 2010. Regenerative stormwater conveyance: an innovative approach to meet a range of stormwater management and ecological goals. In: World Environmental and Water Resources Congress.American Society of Civil Engineers, pp. 3399–3413..

Cadmus, P., Brinkman, S. F., & May, M. K., 2018. Chronic toxicity of ferric iron for North American aquatic organisms: Derivation of a chronic water quality criterion using single species and mesocosm data. Archives of environmental contamination and toxicology, 74(4), 605-615.

COMAR (Code of Maryland Regulations). 2014. 26.08.02.02 http://www.dsd.state.md.us/comar/getfile.aspx?file=26.08.02.02.htm.

Correll, D.L., Jordan, T.E., Weller, D.E., 2000. Beaver pond biogeochemical effects in the Maryland Coastal Plain. Biogeochemistry 49 (3), 217–239.

Duan, S., Mayer, P. M., Kaushal, S. S., Wessel, B. M., & Johnson, T., 2019. Regenerative stormwater conveyance (RSC) for reducing nutrients in urban stormwater runoff depends upon carbon quantity and quality. Science of The Total Environment, 652, 134-146.

Emerson, D., Fleming, E. J., & McBeth, J. M., 2010. Iron-oxidizing bacteria: an environmental and genomic perspective. Annual Review of Microbiology, 64(1), 561-583.

Fanelli, R. M., Prestegaard, K. L., & Palmer, M. A., 2019. Urban legacies: Aquatic stressors and low aquatic biodiversity persist despite implementation of regenerative stormwater conveyance systems. Freshwater Science, 38(4), 818-833.

Filoso S, Palmer MA. 2011. Assessing stream restoration effectiveness at reducing nitrogen export to downstream waters. Ecol. Appl. 21(6):1989–2006.

Fleming, E. J., Cetinić, I., Chan, C. S., Whitney King, D., & Emerson, D., 2014. Ecological succession among iron-oxidizing bacteria. The ISME journal, 8(4), 804-815.

Hayer, C. A., Holcomb, B. M., & Chipps, S. R., 2013. Associations between iron concentration and productivity in montane streams of the Black Hills, South Dakota. The Prairie Naturalist 45:68–76.

Hilderbrand, R. H., Acord, J., Nuttle, T. J., & Ewing, R., 2019. Quantifying the ecological uplift and effectiveness of differing stream restoration approaches in Maryland. *Final Report to Chesapeake Bay Trust*.

McCune, B., J. B. Grace & D. L. Urban 2002. Analysis of Ecological Communities, Vol 28. MjM Software Design, Gleneden Beach.

McKnight, D. M., & Feder, G. L., 1984. The ecological effect of acid conditions and precipitation of hydrous metal oxides in a Rocky Mountain stream. Hydrobiologia, 119, 129-138.

MDE (Maryland Department of the Environment). 2014. Maryland Biological Stressor Identification Process. Baltimore, MD: Maryland Department of the Environment.

Palmer, M. A., Menninger, H. L., & Bernhardt, E., 2010. River restoration, habitat heterogeneity and biodiversity: a failure of theory or practice?. Freshwater Biology, *55*, 205-222.

Palmer, M.A., Hondula, K.L., & Koch, B.J., 2014. Ecological restoration of streams and rivers: shifting strategies and shifting goals. Annual Review if Ecology, Evolution, and Systematics. 45, 247-272. https://doi.org/10.1146/annurev-ecolsys-120213-091935.

Selvakumar, A., O'Connor, T.P., & Struck, S.D., 2010. Role of stream restoration on improving benthic macroinvertebrates and in-stream water quality in an urban watershed: case study. J. Environ. Eng. 136(1):127–139.

Sheldon, S. P., & Wellnitz, T. A., 1998. Do bacteria mediate algal colonization in iron-enriched streams?. Oikos, 83(1), 85-92.

Shields Jr, F.D., Lizotte Jr, R.E., Knight, S.S., Cooper, C.M., & Wilcox, D., 2009. The stream channel incision syndrome and water quality. Ecological Engineering doi:10.1016/j.ecoleng.2009.09.014

Southerland, M.T., G.M. Rogers, M.J. Kline, R.P. Morgan II, D.M. Boward, P.F. Kazyak, R.J. Klauda, and S.A. Stranko. 2005. Improving biological indicators to better assess the condition of streams. Ecological Indicators 7: 751-767.

Starr, R.R. and Cullen, K., 2016. North branch Muddy Creek stream restoration: pre-restoration function-based geomorphic monitoring report. U.S. Fish and Wildlife Service, Chesapeake Bay Field Office.

Stranko, S., Boward, D., Kilian, J., Becker, A., Ashton, M., Southerland, M., Franks, B., Harbold, W., & Cessna, J. 2014. Maryland Biological Stream Survey: Round Four Field Sampling Manual. Maryland Department of Natural Resources, Annapolis, Maryland.

Thompson, J., Pelc, C.E., Brogan, W.R., & Jordan, T.E., 2018. The multiscale effects of stream restoration on water quality. Ecological Engineering 124: 7-18.

Vuori, K. M., 1995 Direct and indirect effects of iron on river ecosystems. Ann. Zool. Fennici, 32(3), 7-329.

Wellnitz, T. A., Grief, K. A., & Sheldon, S. P., 1994. Response of macroinvertebrates to blooms of iron-depositing bacteria. Hydrobiologia, 281(1), 1-17.

Williams, M. R., Wessel, B. M., & Filoso, S., 2016. Sources of iron (Fe) and factors regulating the development of flocculate from Fe-oxidizing bacteria in regenerative streamwater conveyance structures. Ecological Engineering, 95, 723-737.

Zerega, A., Simões, N. E., & Feio, M. J., 2021. How to Improve the Biological Quality of Urban Streams? Reviewing the Effect of Hydromorphological Alterations and Rehabilitation Measures on Benthic Invertebrates. Water, 13(15), 2087.