RESEARCH ARTICLE

Assessing Biological Condition of Restored Streams in an Agriculturally Impaired Chesapeake Bay Sub-Watershed

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ABSTRACT

Intensive agricultural practices can be detrimental to aquatic biological conditions; however, restoration such as removing livestock and creating in-stream habitat can strengthen biotic communities. Smith Creek, a sub-watershed of the Chesapeake Bay, was designated a showcase watershed in 2010 by the United States Department of Agriculture to demonstrate the efficacy of widespread restoration. Our study sought to follow up on these efforts by assessing the individual and combined impacts of restoration, habitat, and land cover on aquatic macroinvertebrate communities in Smith Creek. We predicted that longer-restored sites, sites with less surrounding pasture, and streams with larger substrate size would have the highest stream condition. At 14 properties that were restored between zero and 20 years ago, we conducted kick-net surveys for macroinvertebrates and measured in-stream habitat characteristics. We calculated the Chesapeake Basin-wide Index of Biotic Integrity (Chessie B-IBI) and the Virginia Stream Condition Index (VSCI) to indicate biological condition and calculated land cover within 10 and 100 meters (m) of the stream on the property. Time since restoration was a significant predictor of both indices. The Chessie B-IBI was best predicted by time since restoration and substrate size together. Our results suggest that the restoration initiative may help improve stream conditions given enough time, but additional efforts are needed to establish causal effects of restoration.

Keywords: in-stream habitat, land cover, livestock, macroinvertebrates, stream condition

Restoration Recap

- In areas with a history of stream restoration, monitoring multiple restoration sites with varied times since restoration may be an effective way to examine post-restoration responses.
- Follow-up assessments should not only consider biological stream condition metrics, but also stream habitat conditions, as these will be important for determining the quality of communities that can survive.
- Conducting active versus passive restoration on streams in agricultural areas did not significantly impact stream condition in our study, but this is an interesting area for further investigation.

Land cover surrounding streams can impact stream condition, or the biological condition of flowing water habitats. Agricultural land cover is a common pressure on aquatic health, generating stressors such as nonpoint source pollution from pesticides, fertilizers, and organic matter (Karuppiah and Gupta 1996). Fertilizer runoff into streams, particularly nitrogen (N) and phosphorous (P), can elevate nutrient levels and cause long-term shifts in aquatic community structure (Inamdar et al. 2001, Barmentlo et al. 2019). Pesticides have been shown to significantly stress macroinvertebrates, which are indicators of stream biological condition (Hall et al. 1999, Anderson et al. 2006, McClure et al. 2020). Livestock grazing in and around streams frequently causes physical damage and introduces excess nutrients and microbes from excrement; these may combine to substantially decrease macroinvertebrate species richness (Inamdar et al. 2001, Horak et al.

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2019). Mitigating these pressures through riparian restoration must be considered to maintain both local stream condition and that of the surrounding watershed.

Riparian restoration can be implemented using several methods, broadly categorized as passive or active restoration. Restoration practitioners initiate passive restoration by simply removing the stressor or impairment that is causing a decline in stream condition (Kauffman et al. 1997). In agricultural areas—such as the pastoral landscapes prevalent in the Chesapeake Bay watershed—this is typically accomplished by fencing livestock out of streams (livestock exclusion). This can prevent physical damage to the streambed and direct fecal inputs to the water, while allowing in-stream and riparian habitats to improve (Ranganath et al. 2009). For the purposes of our study, we refer to passive restoration only as sites with cattle excluded from the stream. One drawback of passive restoration is the potential need for landowners to provide separate water sources and maintain the fencing; however, these costs may be supported by local and state agencies.

Alternatively, restoration practitioners can implement active restoration methods in situations where passive restoration is unlikely to satisfy restoration goals within the desired timeline. Creating in-stream habitat, such as adding gravel and log vanes to create riffles and pools, can improve macroinvertebrate diversity, an indicator of stream condition, by providing varied food sources, egg-laying surfaces, and diverse microhabitats (Selego et al. 2012). Anchoring trees and bales of woody debris to stream banks can stabilize them, thus reducing erosion, sediment deposition, and N and P input to streams (Meals 2001). However, streambank stabilization is labor intensive, expensive, and can typically only be conducted on short stretches of stream. Riparian buffer zones of at least 15 m around the impacted stream can also be established by actively planting trees; although riparian buffer zones can also be established by passively allowing regeneration to occur, our study considers riparian buffers to be active restoration. Additionally, buffers of at least 30 m and potentially up to 100 m are needed to support healthy macroinvertebrate communities (Moraes et al. 2014, Sweeney and Newbold 2014). The planted trees can stabilize the streambank and create shade, thus decreasing water temperature, while also reducing N and P loads entering the stream over time (Inamdar et al. 2001, Albertson et al. 2018). Along a given agricultural stream, many different restoration approaches may be utilized, which presents an opportunity for assessing single versus combined effects of restoration practices. However, in one review of restoration monitoring practices, Rubin et al. (2017) found that 62% of studies only monitored water quality for one year post-restoration, while only 4% sampled five years post-restoration, indicating a gap in monitoring efforts.

Assessing water quality following restoration is essential, and aquatic macroinvertebrates can serve as useful indicators of stream condition. Aquatic macroinvertebrates reflect stream conditions beyond those present while sampling due to their multi-year aquatic larval stages, sensitivity to local habitat differences, and sensitivity to changes in water conditions (Berkman et al. 1986, Bonada et al. 2006). Many macroinvertebrate species also respond differently to stressors, so researchers can understand the impairment that a stream is facing based on which organisms are present (Feld and Hering 2007). In particular, the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) are intolerant of pollutants, so their presence indicates quality habitat and biotic conditions (Merritt et al. 2008). As such, collecting aquatic macroinvertebrates can be effective for monitoring the impacts of restoration by assessing the proportion of pollution-tolerant to pollution-sensitive species.

The Chesapeake Bay is the largest estuary in the United States and the third largest in the world, with a surface area of over 11,000 km^2 and a watershed area of greater than 166,000 km^2 (Figure 1). The Chesapeake Bay and its surrounding watershed support approximately 18 million people and 3,600 plant and animal species, provide ecological utility, and sustain threatened and endangered species (Morimoto et al. 2003, Phillips and McGee 2016, USFWS 2022). Economically, the Chesapeake Bay is valued at approximately $100 billion USD annually, due primarily to fishing and recreation industries (Phillips and McGee 2016). However, the Chesapeake Bay has a decades-long history of pollution throughout its watershed, making it an ideal region in which to study restoration (Chesapeake Bay Foundation 2020).

Our purpose was to a) assess water quality using biotic indices across a gradient of restoration ages in an agriculturally impaired sub-watershed of the Chesapeake Bay (the Smith Creek watershed), and b) identify factors that influence biotic stream condition in order to help inform future restoration projects. We hypothesized that the type of restoration conducted (active versus passive), the length of time since restoration efforts occurred (ranging from zero to 20 years), land cover around the stream, and the in-stream habitat would be important factors for macroinvertebrate communities based on the Virginia Stream Condition Index (VSCI) and the Chesapeake Bay Basin-wide Index for Biotic Integrity (Chessie B-IBI) (Table 2). We predicted that water quality would be greater in actively restored sites than passively restored sites. We also predicted that older restoration sites would have higher stream condition scores. Lastly, we predicted that improved habitat quality (e.g., larger substrate, less bank erosion) would predict higher stream condition scores.

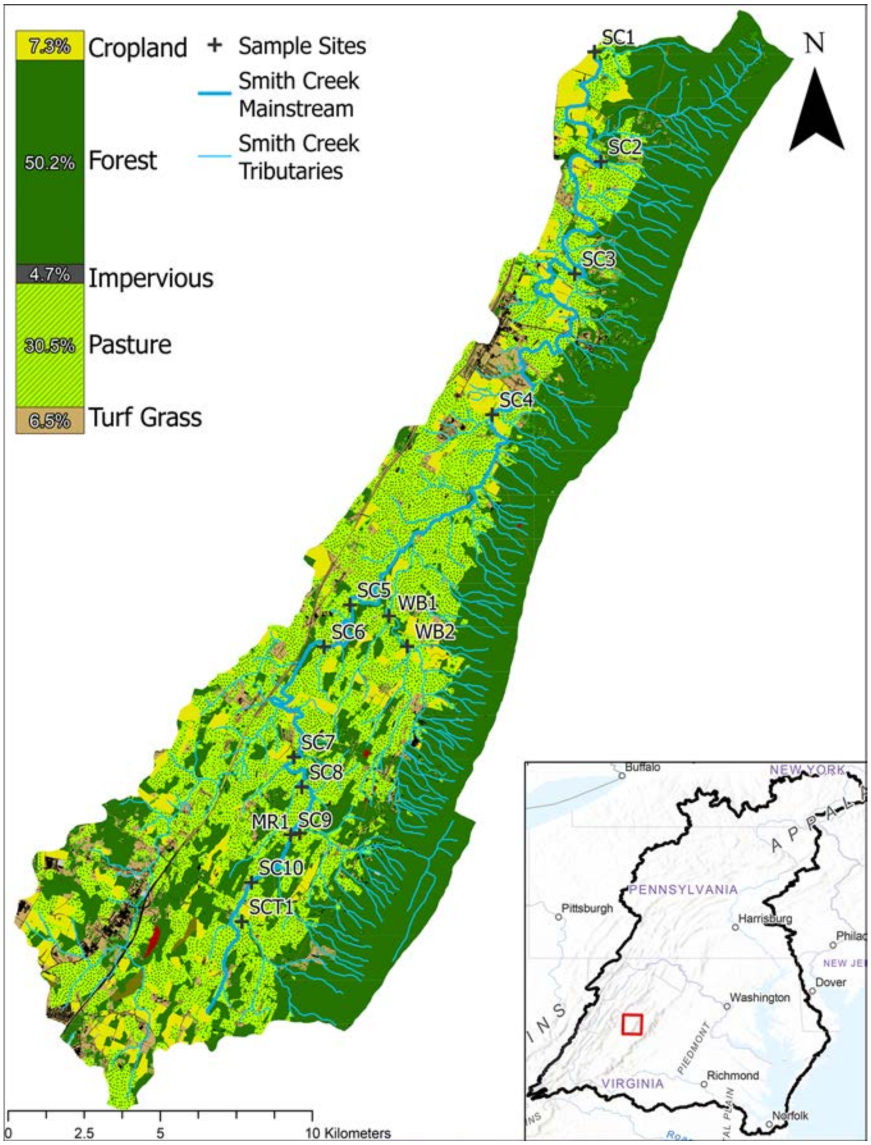

Figure 1. Land cover in the Smith Creek watershed with sampling sites. The inset map shows the entire Chesapeake Bay watershed with the location of Smith Creek indicated by the square. Land cover types representing greater than 4% of the watershed are shown in the stacked bar chart. SC=Smith Creek mainstem, WB=War Branch, MR=Mountain Run, SCT=unnamed tributary to Smith Creek. Base map credits: data.pa.gov, Esri, HERE, Garmin, FAO, NOAA, USGS, EPA.

Methods

Study Area

The Smith Creek watershed, located in the Shenandoah Valley in Virginia, USA, is a 272 km² sub-watershed of the Chesapeake Bay with 457.1 km of streams and served as the focal area for this study (Figure 1). The primary land cover in the Smith Creek watershed is agricultural, particularly cattle pasture and poultry farms, although restoration has been conducted over the past 20 years (~38% agriculture; Figure 1). In 2010, the Smith Creek watershed was designated as a United States Department of Agriculture (USDA) Showcase Watershed, part of an initiative aiming to demonstrate that widespread stream restoration can reduce agricultural pollutants in watersheds that feed into the Chesapeake Bay (Chesapeake Bay Foundation 2010). Over the ten years since this designation, substantial riparian restoration has occurred through extensive collaboration between landowners, local agencies, and other organizations. Despite these efforts, Smith Creek and most of its headwater streams are still classified as impaired (VA DEQ 2020).

Within the Smith Creek watershed, several non-biotic assessments have occurred. Although some agencies such as the VA DEQ have collected macroinvertebrate data on Smith Creek historically, these have been collected along roadsides rather than on farms and are not compatible with our study. Previous studies have found that the total N values still indicate significant inputs from sewage, manure, or fertilizer, that around 75% of the sediment in Smith Creek comes from streambank erosion throughout the watershed, and that riparian planting success has been diminished by deer browse (Hyer et al. 2016, Gellis and Gorman Sanisaca 2018, Peters et al. 2021). These studies are important for understanding the nuances of stream restoration in Smith Creek and our study hopes to introduce a biological component to the body of literature.

Table 1. Properties sampled, the length of time they have been restored, the distance along the stream they are located, the types of restoration implemented, the length of the stream on the property, and the area of the watershed around the site. Site position category is noted in parentheses after the river kilometer: Low = lower, Mid = Middle, Upp = Upper. SC=Smith Creek mainstem, WB=War Branch, MR=Mountain Run, SCT=unnamed tributary to Smith Creek.

Site	Time Restored (Years)	River Kilometer	Type of Restoration	Passive/Active	Restored Length (km)	Watershed Area (km²)
SC1	12	0.06 (Low)	Cattle exclusion	Passive	1.09	272.05
SC2	10	5.70 (Low)	Cattle exclusion + mature buffer	Active	0.65	242.74
SC3	10	13.11 (Low)	Cattle exclusion + bank stabilization	Active	1.2	225.73
SC4	8	24.85 (Low)	Cattle exclusion + mature buffer	Active	1.3	197.58
WB1	20	34.67 (Mid)	Cattle exclusion	Passive	0.38	28.3
SC5	19	35.48 (Mid)	Cattle exclusion	Passive	1.03	125.19
WB2	3	36.42 (Mid)	Cattle exclusion	Passive	0.21	23.61
SC6	15	38.29 (Mid)	Partial exclusion	N/A	1.18	120.66
SC7	20	45.50 (Upp)	Cattle exclusion + mature buffer + bank stabilization	Active	2.35	52.24
SC8	16	47.48 (Upp)	Cattle exclusion + mature buffer	Active	1.4	46.58
SC9	5	49.83 (Upp)	Cattle exclusion	Passive	0.41	24.39
MR1	8	49.96 (Upp)	Cattle exclusion + mature buffer	Active	1.01	18.54
SC10	0	52.27 (Upp)	No restoration	N/A	0.75	16.7
SCT1	7	53.58 (Upp)	Cattle exclusion	Passive	0.76	10.4

Site Selection

Fourteen privately-owned farms with perennial streams were sampled (Figure 1) representing a variety of restoration practices and restoration ages (Table 1). River km served as a metric to control for site position within the watershed and was calculated as the river distance from the sampling site to the mouth of Smith Creek. Sites were grouped into position categories for use as a random effect (Upper: >45km, Middle: 34–45 km, Lower: <34 km, Table 1). For tributaries, the river km at which the tributary met the main stem was added to the distance along the tributary at which the site was located.

Restoration types included full or partial cattle exclusion (passive restoration, cows not allowed access to stream), mature buffer (active restoration, trees were >15 cm DBH at the time of the surveys), and bank stabilization (active restoration, bank erosion reduced using large boulders). Of sites that had mature buffers, these were either planted over a decade ago as seedlings and grown to maturity or had naturally regenerated over approximately the same time period. Partial exclusion refers to a property that removed cattle from most of the stream but had one point of access for drinking water in the middle of the property. We compared passive restoration (cattle exclusion only) to active, which included cattle exclusion plus any additional restoration conducted.

We split the length of the restored stream on each property into three equal sections using ArcGIS (v.10.8.1 Esri, Redlands, CA) and sampled the downstream-most riffle in each section. We used georeferenced PDF maps in Avenza Maps (Toronto, Ontario) to navigate to each stream section. We used a Trimble Geo 7x GPS (Trimble, Sunnyvale, CA) to collect coordinates (WGS 1984) from the center of each riffle. We sampled each property once between May 17 and July 2 2021, with two or three streams sampled per week depending on landowner availability. We noted Julian day sampled, total precipitation for day of, 24 hours prior, and 48 hours prior to sampling (obtained from PRISM) as potential confounding variables for water quality. We sampled all riffles from each property on the same day to avoid introducing additional confounding variables.

Benthic Macroinvertebrate Sampling

We collected benthic macroinvertebrate samples following the Environmental Protection Agency (EPA) Rapid Bioassessment Protocol single habitat methodology, which was among the methods used to develop the Virginia Stream Condition Index (Barbour et al. 1999, Burton and Gerritsen 2003). At each riffle, we collected benthic macroinvertebrates using a 1 m^2 kick-net with 500 µm mesh size, placed at the downstream end of the riffle. Each kick-net sample lasted two minutes: one minute scrubbing rocks within a 1 m^2 area upstream of the net, and one minute shuffling around the substrate within that same area. Using forceps, we then collected invertebrates from the net until no more were visible, then washed leaf packs and branches with stream water and picked small invertebrates that passed through the net onto a white tarp under the net. We stored all specimens from each site in 70% ethanol.

We stored the invertebrates collected at each riffle separately by sample for identification and counting. We

Table 2. Description of stream health indices and predictor variables that were used. EPT=Ephemeroptera, Trichoptera, Plecoptera, D_{50}= 50th percentile substrate size, D_{90}= 90th percentile substrate size, P10=within 10 m of the stream on the property, P100=within 100 m of the stream on the property, W10=within 10 m of the stream throughout the upstream watershed, W100=within 100 m of the stream throughout the upstream watershed.

Index name	Description	Variables included
Chesapeake Bay B-IBI (Smith et al. 2017)	Index developed for the Southern Great Valley region of the Chesapeake Bay watershed to standardize reporting of stream scores	% Gastropoda and Oligochaeta, % Clinger, % Collector, % Ephemeroptera except Baetidae, % EPT except Hydropsychidae, % EPT richness except tolerant families, % Filterer, % Heptageniidae, % Moderately tolerant, % Trichoptera except Hydropsychidae, % Pisciforma, % Predators, % Pterygota, % Scraper, % Sprawler, % Tolerant, richness of collectors, richness of filterers, richness of Trichoptera
Virginia Stream Condition Index (Burton and Gerritsen 2003)	Index developed to evaluate stream health in Virginia, includes reference streams for the region	EPT index, total taxa, % Ephemeroptera, % Plecoptera and Trichoptera (except Hydropsychidae), % Chironomidae, % top 2 dominant taxa, the Hilsenhoff biotic index (HBI), and the % scrapers
Predictors	Restoration	Time since restoration, restored length, restoration type
	Habitat	Canopy cover, bank height, bank angle, D50, D90, %fines, algal density
	Land cover	Road density 10P, 100P, 10W, 100W, %cropland 10P, 100P, 10W, 100W, %pasture 10P, 100P, 10W, 100W, %forest 10P, 100P, 10W, 100W

identified all individuals in the lab under a dissecting microscope to the family level, where possible, using a regional key based on Merritt et al. (2008). We discarded specimens degraded beyond the point of identification (<0.01% of total). Following identification, we pooled the data from all three riffles per site to calculate stream condition indices, per the EPA Rapid Bioassessment Protocol. Morisita Horn similarity indices show the variation in macroinvertebrate communities within each site (Chao et al. 2016, Figure S1).

We used macroinvertebrate community compositions to calculate two stream condition indices, the Chesapeake Bay Basin-wide Index of Biotic Integrity (Chessie B-IBI, Smith et al. 2017) and the Virginia Stream Condition Index (VSCI, Burton and Gerritsen 2003). Both are comprised of multiple metrics and are standardized to produce scores between 0–100 (Table 2). Of the two indices, the Chessie B-IBI was created more recently, for a more specific region (subsections of the Chesapeake Bay watershed), and is more complex, including 21 metrics focusing heavily on functional feeding groups (FFGs) (Smith et al. 2017, Table 2). The VSCI has been in use for two decades and focuses on fewer metrics, primarily EPT taxa and pollution tolerance (Burton and Gerritsen 2003, Table 2). As both indices are indicative of different aspects to stream condition, it is useful to consider both.

For both the Chessie B-IBI and the VSCI, we subsampled data to 100 random individuals per sample, aggregated to site, and calculated stream condition scores averaged from 500 iterations in RStudio (Doberstein et al. 2000, Burton and Gerritsen 2003, Smith et al. 2017).

Habitat Variables

We conducted habitat classification on the same day as the macroinvertebrates were sampled. We visually classified channel-reach morphology according to categories described by Montgomery and Buffington (1997) and measured the slope of the riffle in percent using a clinometer. We conducted a modified Wolman's pebble count using the pace-and-point method to select particles throughout the riffle and measured the intermediate axis of the particle using a gravelometer (US SAH-97TM Particle Size Analyzer) (Wolman 1954). We repeated this throughout the riffle until 100 particles within the wetted channel were measured. We calculated the median substrate size (D_{50}; the particle size at which 50% of substrate pieces were smaller), the 90th percentile of substrate sizes (D_{90}; the particle size at which 90% of substrate pieces were smaller), and the percent fines (pfine; percent of particles < 4 mm) for each sample. We conducted point counts of the presence or absence of filamentous algal mats along the same paths as the pebble counts and calculated algal density (% presence).

We measured the bank height from the base (where the stream bank meets the stream bed) to the top of the bank, as close to the water as possible and measured bank angle using a clinometer set on a short rod laid flush to the bank (US EPA 2009). We collected both measurements at the upstream, center, and downstream section of the riffle on the left and right banks and averaged them for each riffle. We measured canopy cover at each riffle, aligned east-west and held level, using hemispherical photography with a fish-eye lens attachment (Criacr) on a Samsung Galaxy S10 12-megapixel smartphone. To account for glare and shadows, we enhanced and normalized images, converted to black-and-white pixels in RStudio using the caiman package, and averaged the scores for overall site canopy coverage (Bianchi et al. 2017, Diaz 2018).

Land Cover Variables

We calculated land cover (percent cropland, pasture, forest) using a high resolution (1 m) land cover dataset (VGIN 2021). We combined forest (patches of trees covering greater than one acre) and tree (patches of trees covering less than one acre) in analyses and hereafter refer to the variable as forest. To calculate road density (km roads/km^2), we divided total road length (US Census Bureau 2021) by the total area of interest. We calculated road density and percent forest, cropland, and pasture in 10 m and 100 m buffers around the stream on either only the property (P) or throughout the upstream watershed (W). Although they are not independent of each other, when combined they serve as a complete measure of what was draining into the site. To address this potential error, we assessed the relationship between watershed position, based on river km, and stream condition scores using linear regressions. We clipped the variables P10 (10 m on either side of the stream) and P100 (100 m on either side of the stream) to the boundaries of the landowner's property, while we clipped W10 and W100 to the upstream watershed feeding into the sample point. Riparian land cover has been shown to be an effective predictor of macroinvertebrate indices, so our study focused on riparian-scale rather than catchment (e.g., Sponseller et al. 2001, Moore and Palmer 2005, Schiff and Benoit 2007).

Statistical Analyses

We conducted all statistical analyses in an RStudio programming environment (R, Boston, MA) and used the packages data.table (Dowle and Srinivasan 2019), ggplot2 (Wickham 2016), kit (Jacob 2021), tidyr (Wickham 2020), vegan (Oksanen et al. 2020), and nlme (Pinheiro et al. 2022). All predictor variables are described in Table 2. We used an alpha of 0.05 to denote statistical significance. We conducted Shapiro Wilk normality tests on all variables and transformed non-normally distributed variables (log10, natural log, or square). Because we could not transform algal density, road density 10P, road density 100P, cropland 10P, cropland 10W, and cropland 100P, we removed these variables from analyses. We also tested for multicollinearity between variables using variance inflation factors, which led us to remove elevation, pasture 10W and 100W, forest 10W and 100W, and road density 100W from our models (Naimi et al. 2014).

To compare stream condition and habitat characteristics between sites that implemented passive restoration only and those that implemented any active restoration, we used two-tailed t-tests and Wilcoxon rank sum tests (for normally and non-normally distributed data, respectively). We excluded sites SC6 (only partial cattle exclusion) and SC10 (no restoration) from these analyses.

We conducted linear mixed effects models between the two stream condition indices and the measured predictor variables, with site category as a random effect, and restoration type (Table 2). We ran models in a forward stepwise fashion, based on the predictors identified as important by the linear mixed models with one fixed effect. Due to our study's small sample size, we did not construct any models using more than three variables at one time. We ranked all models by the change in Akaike's Information Criterion scores adjusted for small sample sizes (ΔAIC).

Results

Benthic Macroinvertebrate Communities

In total, we identified 28,875 macroinvertebrates of 61 unique families from the 42 riffles at 14 sites. The most abundant taxon was Elmidae (riffle beetle; n=7,453, 25.8% of total), of which 4,984 (66.9%) were larvae and 2,469 (33.1%) were adults, followed by Hydropsychidae (net-spinning caddisfly; n=4,984, 17.3%), Prosobranchia (gilled snail; n=3,884, 13.5% of total), Ephemerellidae (spiny crawler mayfly; n=3,444, 11.9% of total), and Chironomidae (nonbiting midge; n=2,137, 7.4% of total) (Figure 2). We only found pollution-tolerant invertebrates such as Simuliidae, Turbellaria, and Oligochaeta in passively restored sites (Figure 2). The most prevalent FFG across all sites were scrapers, followed by gatherers, filterers, and predators (Table S1). Site SC9 had the lowest Chessie B-IBI score (50.2; poor) and the highest Chessie B-IBI score (73.4; good) occurred at SC4 (Table 3). Site WB2 had the lowest VSCI score (55.3; impaired), while SC8 had the highest VSCI score (73.1; least impaired; Table 3).

Habitat Characteristics

All stream bed morphologies were classified as pool-riffle. Riffle-scale channel slopes ranged from 1–5%. The remaining habitat characteristics are summarized in Table 3. Median particle sizes ranged from 16–32 mm, all within the pebble size class, while 90th percentile sizes ranged from 45–128 mm (spanning pebble and cobble size classes) but had high variation within each site. The percent of particles less than 4 mm was generally small, ranging from 0–4 %. Algal density was generally low across sites, ranging from 0–35.7%. Bank heights were variable, with the lowest average height 0.32 m and the highest at 1.64 m. Bank angles were fairly steep (35.9%–74.2%) but were not undercut on average. All but one site (SC3) had at least 50% canopy cover over the stream and percent cover ranged from 46%–76%.

Land Cover Characteristics

Each of four land cover variables (road density, percent cropland, percent pasture, and percent forest) was calculated within 10- and 100-meter (m) buffers around the

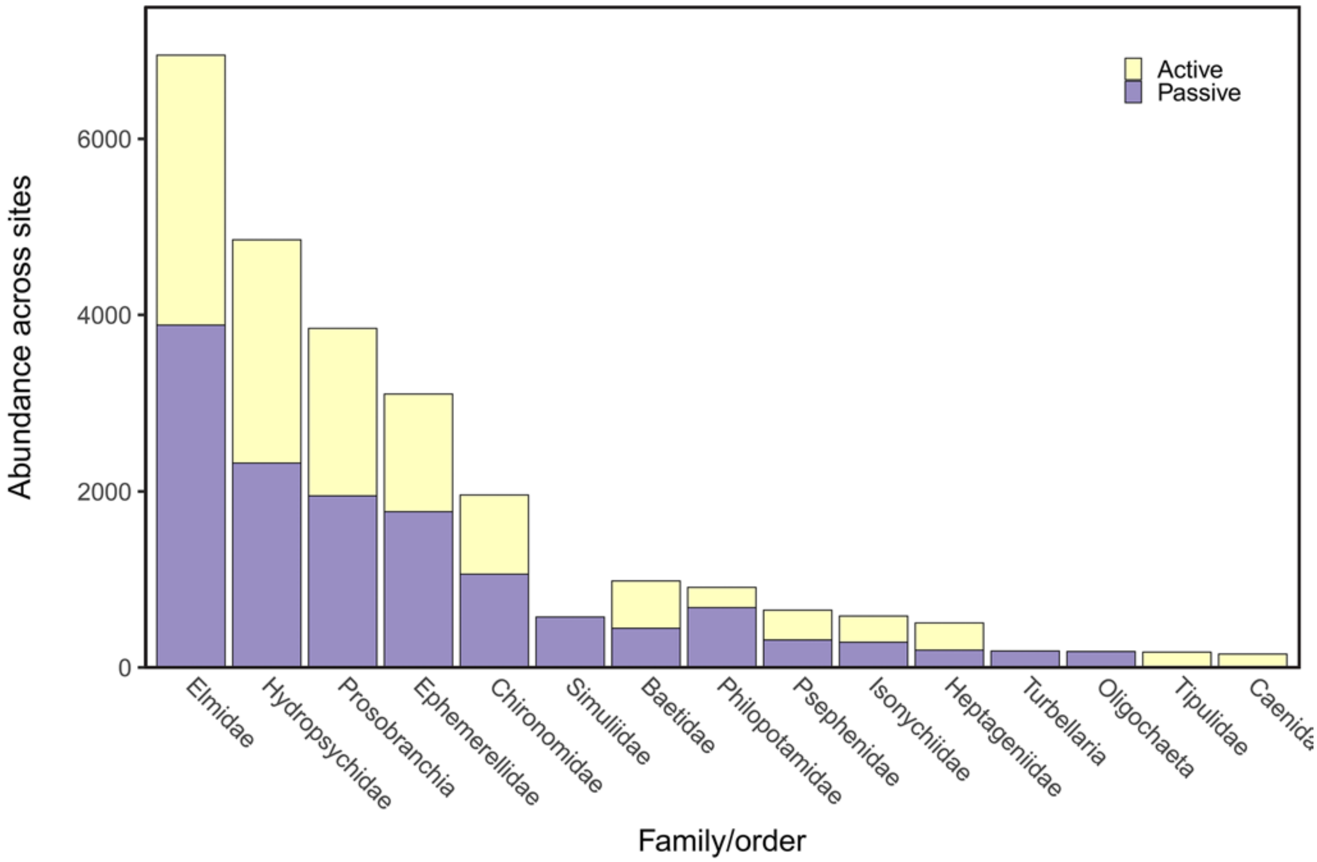

Figure 2. Total counts from all samples combined of the top fifteen most abundant families or orders across all sites. The top bar (yellow) indicates abundance in actively restored sites, while the bottom (purple) indicates abundance in passively restored sites.

Table 3. Summary of stream condition index scores and mean in-stream habitat characteristics at each site, plus or minus the standard deviation. %fine was aggregated across sites and thus does not have a standard deviation listed. Sites are ordered from downstream to upstream.

Site	Chessie B-IBI	VSCI	D_{50} (mm)	D_{90} (mm)	%fine (%)	Algal Density (%)	Bank Height (m)	Bank Angle (o)	Canopy Cover (%)
SC1	60.1	66.9	35.33 ± 16.74	115.33 ± 21.94	2.00	6.3 ± 11.0	1.64 ± 0.34	41.33 ± 9.93	57.7 ± 8.0
SC2	70.5	72.4	33.20 ± 11.25	102.67 ± 21.94	4.00	26.3 ± 17.6	0.90 ± 0.22	49.28 ± 6.32	60.9 ± 7.0
SC3	52.3	69.0	18.20 ± 3.81	72.67 ± 47.92	1.00	9.3 ± 8.5	1.10 ± 0.23	43.50 ± 5.57	45.1 ± 16.0
SC4	73.4	70.1	22.60 ± 0.00	106.67 ± 36.95	1.00	26.0 ± 11.4	1.17 ± 0.53	51.33 ± 18.94	76.5 ± 6.0
WB1	66.0	73.1	22.60 ± 0.00	87.67 ± 41.55	0.67	9.7 ± 8.5	0.67 ± 0.39	59.06 ± 10.71	52.9 ± 24.0
SC5	66.3	69.7	33.20 ± 11.25	83.47 ± 14.73	1.00	35.7 ± 12.5	0.64 ± 0.14	35.94 ± 15.66	74.0 ± 9.0
WB2	52.0	55.3	33.20 ± 11.25	90.00 ± 0.00	2.33	1.3 ± 0.6	0.52 ± 0.20	56.56 ± 10.48	71.3 ± 7.0
SC6	71.0	70.9	31.00 ± 14.53	82.20 ± 13.51	1.00	11.3 ± 5.5	1.30 ± 0.65	60.11 ± 12.70	65.2 ± 24.0
SC7	72.9	72.1	18.20 ± 3.81	115.33 ± 21.94	0.33	0.7 ± 0.6	0.49 ± 0.24	38.67 ± 13.52	58.7 ± 18.0
SC8	69.1	73.5	36.33 ± 7.51	102.67 ± 21.94	2.33	1.0 ± 1.7	0.96 ± 0.12	74.17 ± 15.26	58.6 ± 25.0
SC9	50.2	64.7	16.00 ± 0.00	55.67 ± 30.44	0.00	0.0 ± 0.0	0.68 ± 0.03	60.83 ± 13.98	57.1 ± 25.0
MR1	61.2	68.4	36.40 ± 23.90	107.53 ± 35.45	0.67	0.3 ± 0.6	0.49 ± 0.21	44.00 ± 8.43	52.4 ± 20.0
SC10	60.7	66.5	21.87 ± 10.52	106.67 ± 36.95	1.50	2.3 ± 2.5	0.40 ± 0.22	67.61 ± 10.01	50.3 ± 13.0
SCT1	62.4	68.7	26.67 ± 9.24	115.33 ± 21.94	3.00	0.0 ± 0.0	0.32 ± 0.10	59.61 ± 19.19	52.4 ± 26.0

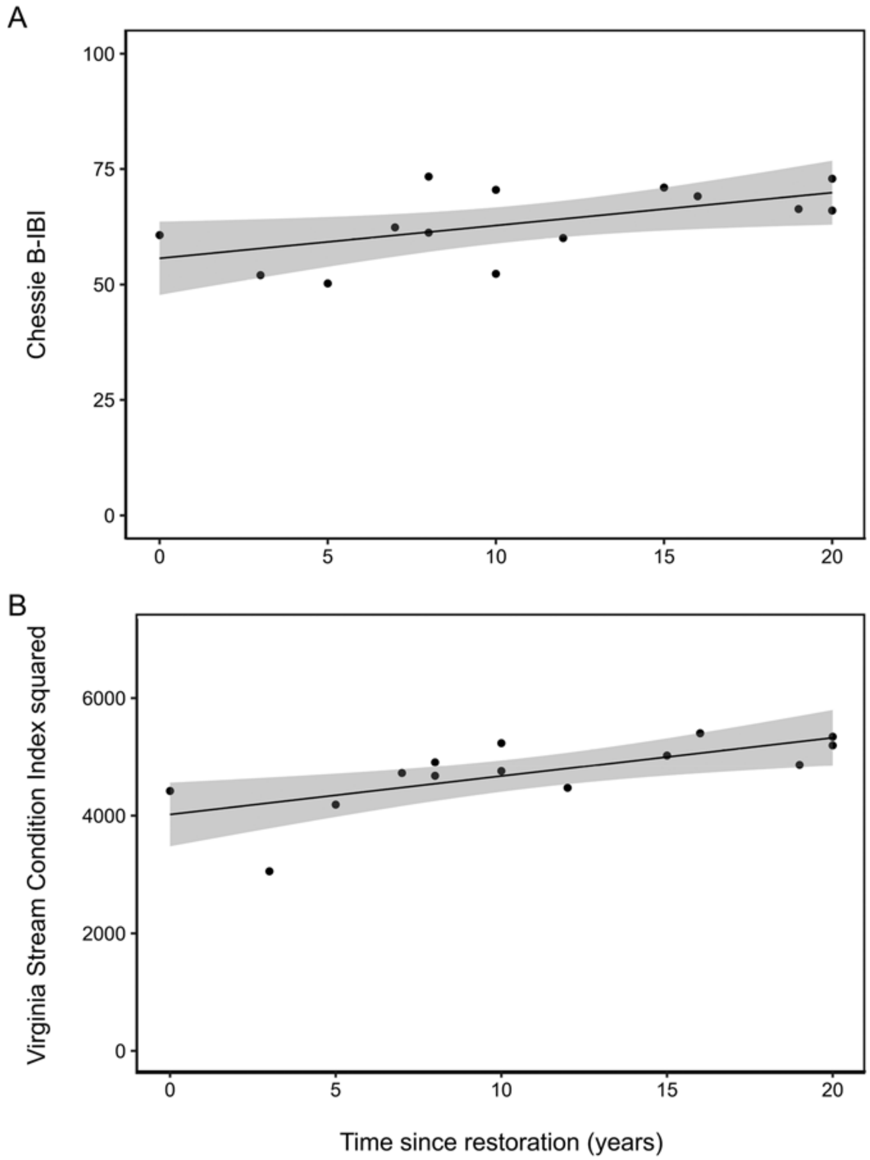

Figure 3. A) The Chessie B-IBI was significantly predicted by time since restoration (t=2.446, p=0.035), as was B) the Virginia Stream Condition Index (t=4.156, p=0.002). A 95% confidence interval is indicated by the shaded gray area.

stream both on the property (P) and in the watershed (W) and are summarized in Table S2. Road density in P10 and P100 was low across all sites (0–0.004 km roads/km² and 0–0.012 km roads/km², respectively), while road density in W10 and W100 was greatest at the downstream sites (0.002–0.003 km roads/km² and 0.003–0.005 km roads/km², respectively). Within P10 and P100, SC4 had the greatest amount of cropland (0–1.3%, 0–34.5%, respectively), and cropland in W10 and W100 decreased moving downstream (0–2.6%, 0.1–4.4%, respectively). Pasture in P10 and P100 were greatest at site WB2 (0.2–64.3%, 2.1–74.8%, respectively), and pasture in W10 and W100 were lowest at site MR1 (0–2.6%, 0.1–4.4%, respectively). Pasture was primarily used for cattle, while cropland was primarily corn and soy. Conversely, percent forest cover in W10 and W100 were highest at site MR1 (53.3–85.3%, 48.2–82.6%, respectively), but the median forest cover in P10 and P100 at MR1 were only slightly above the overall average (12.4–97.1%, 1.7–68.9%).

Stream Condition and Habitat by Restoration Type

Stream condition, as indicated by the Chessie B-IBI and the VSCI, between sites with passive restoration and sites with active restoration also did not vary, although the actively restored sites had marginally higher scores (B-IBI: t=1.365, *p*=0.206; VSCI: KW=1.4615, *p*=0.8334; Figure S2A, S2B). Bank height was significantly lower at streams that used passive restoration compared to those that implemented active restoration: 0.57 m on average compared to 0.89 m (t=2.393, *p*=0.043; Figure S2C). No other variables were significantly predicted by restoration type and restoration type was not statistically significant in any mixed effects models.

Table 4. Significant model outputs according to AICc scores. Time=time since restoration, D_{90}=Dth percentile substrate size, length=length of restored area. All models included stream position as a random effect.

Index	Predictors	*p*	t	ΔAIC
Chessie B-IBI	Time (+) + D_{90} (+)	0.007; 0.0098	3.507; 3.263	0
	Time (+)	0.035	2.446	4.444
VSCI	Time (+)	0.002	4.156	0

Stream Condition and Environmental Variable Relationships

Both the Chessie B-IBI and the VSCI were significantly and positively predicted by time since restoration (t=2.446, p=0.035; Figure 3A; t=4.156, p=0.002; Figure 3B). No other single linear relationships were statistically significant. Neither the VSCI or Chessie B-IBI were significantly predicted by sampling day of year, river km, watershed area, or precipitation (p>0.05). The variance inflation factors for variables in each model were low (<1.2) and no multicollinearity was detected. When multiple predictor variables were included, the Chessie B-IBI was best predicted by increasing time since restoration and D_{90} (time: t=3.507, p=0.007; D_{90}: t=3.263, p=0.009; Table 4).

Discussion

During a single sampling season, our study surveyed sites restored over a wide range of time to discern the relationship between restoration time and water quality, indicated by two stream condition indices. As we predicted, the Chessie B-IBI and VSCI scores significantly increased as time since restoration increased, suggesting that there is hope for long-term restoration success in agricultural watersheds. Rather than sampling over several years, we used a space-for-time substitution. With this approach, we attempted to fill a sampling gap in which many long-term monitoring efforts of water quality occur less than a decade after the restoration was conducted and therefore may miss long-term recoveries of biotic communities (Muotka et al. 2002 [4–8 years], Buchanan et al. 2013 [8 years], Rubin et al. 2017, dos Reis Oliviera et al. 2020). Some exceptions include work by Pilotto et al. (2019) and Leps et al. (2016), who assessed stream condition (using multi-habitat sampling of fish and macroinvertebrates) in sites restored 1 to 26 years ago. Contrary to our results, they did not find a positive relationship between restoration age and stream condition, so our findings provide important new insights to the question of restoration age and water quality.

The presence of large cobbles and small boulders was an important predictor for models with the Chessie B-IBI. Previous research shows that adding in-stream habitat (e.g., log vanes, wood, gravel) during restoration improves biotic diversity, creates egg-laying habitat, and provides channel stability (Lester et al. 2007, Lancaster et al. 2010, Reich et al. 2011, Hussain and Pandit 2012, Selego et al. 2012). Although altering the stream channel and creating habitat create more disturbance initially, biotic communities have been shown to recover quickly (Selego et al. 2012). When considering adding habitat as an active restoration measure, it is important to note that adding large substrate is only effective where the stream historically had such large substrate present. Additional experimental research is needed to confirm causal relationships, as we did not detect a strong signal in our study.

When assessing overall site-level benthic communities, the implementation of any type of active restoration (e.g., cattle exclusion and mature buffer) compared to only excluding cattle (passive restoration) did not statistically affect stream condition, although there was a nonsignificant trend of higher scores on actively restored sites. This may have been due in part to the variety of active restoration methods conducted (e.g., bank stabilization, tree buffers of varied ages and compositions) compared to only one passive restoration method (cattle exclusion). Another potential explanation for this relationship is that sites with riparian buffers, the most common active restoration method, typically had small buffer widths as the minimum required under the Conservation Reserve Enhancement Program (CREP) in Virginia is only ~10 m. However, Moraes et al. (2014) indicated that wider buffers (>15 m) were essential for healthy biotic communities, while Sweeney and Newbold (2014) suggest that at least 30 m of buffer are needed. We did not measure riparian buffer areas in our study, but it would be a useful variable to include in future studies.

Our study found minimal impacts of land cover on in-stream habitat and macroinvertebrate communities. Although we attempted to compare land cover at four different scales, none were significant individually or combined with other predictors. This was unexpected, as previous studies have documented variable impacts of land cover on numerous metrics of water quality; Donatich et al. (2020) suggest that reach-scale variables, such as land cover in the riparian corridor, are important for improving biological function in the stream. Potentially, other factors that we had not considered affected the models and could have been accounted for by sampling above and below restoration sites. For example, several restored sites in this study had upstream neighbors that still allowed cattle in the stream which may have contributed to these contradictory results, reducing the efficacy of purely local effects. Potentially, the inclusion of physico-chemical data, such as nitrate, phosphate, and dissolved oxygen levels, could explain some of these trends in future studies (Sponseller et al. 2001, Le Gall et al. 2021).

Our study used both the Chessie B-IBI and VSCI, multimetric indices, to assess stream biological condition. The Chessie B-IBI tended to be predicted by in-stream habitat, such as substrate size and canopy cover, along with time since restoration. The VSCI was best predicted by various combinations of land cover characteristics, although time since restoration was also often the most important predictor. This difference may be due to the inclusion of more FFGs in the Chessie B-IBI, compared to only one FFG (scrapers) in the VSCI. At our study sites, scrapers were the most prevalent FFG due to the high abundance of Elmidae and Prosobranchia. Sites with higher proportions of these taxa tended to have higher VSCI scores as a result, although there was no statistically significant relationship between percent scraper and VSCI score. This marginal relationship could have influenced the unexpected land cover trends we identified, or they could have been driven by our relatively small sample size. However, in-stream habitat and water quality are also driven by catchment-level factors, so the relationships here are more complex than our study can explain (Feld and Hering 2007, Merritt et al. 2008). Assessing stream condition using multiple indices can be useful to researchers for understanding a more complete picture of stream condition, which can then be shared with land managers.

Streams are complex and dynamic; identifying a single factor to ensure high water quality is not possible. However, our study isolated some variables that may help predict biotic stream condition by examining a uniquely wide span of time since restoration. Streams that historically had large rocky substrate prior to disturbance may benefit from the reintroduction of large cobble, which our study found to be an important predictor for biotic stream condition by one index. Most importantly, our findings suggest that older restoration projects in agricultural areas typically have higher water quality and biotic stream condition, although precise driving factors are still unclear.

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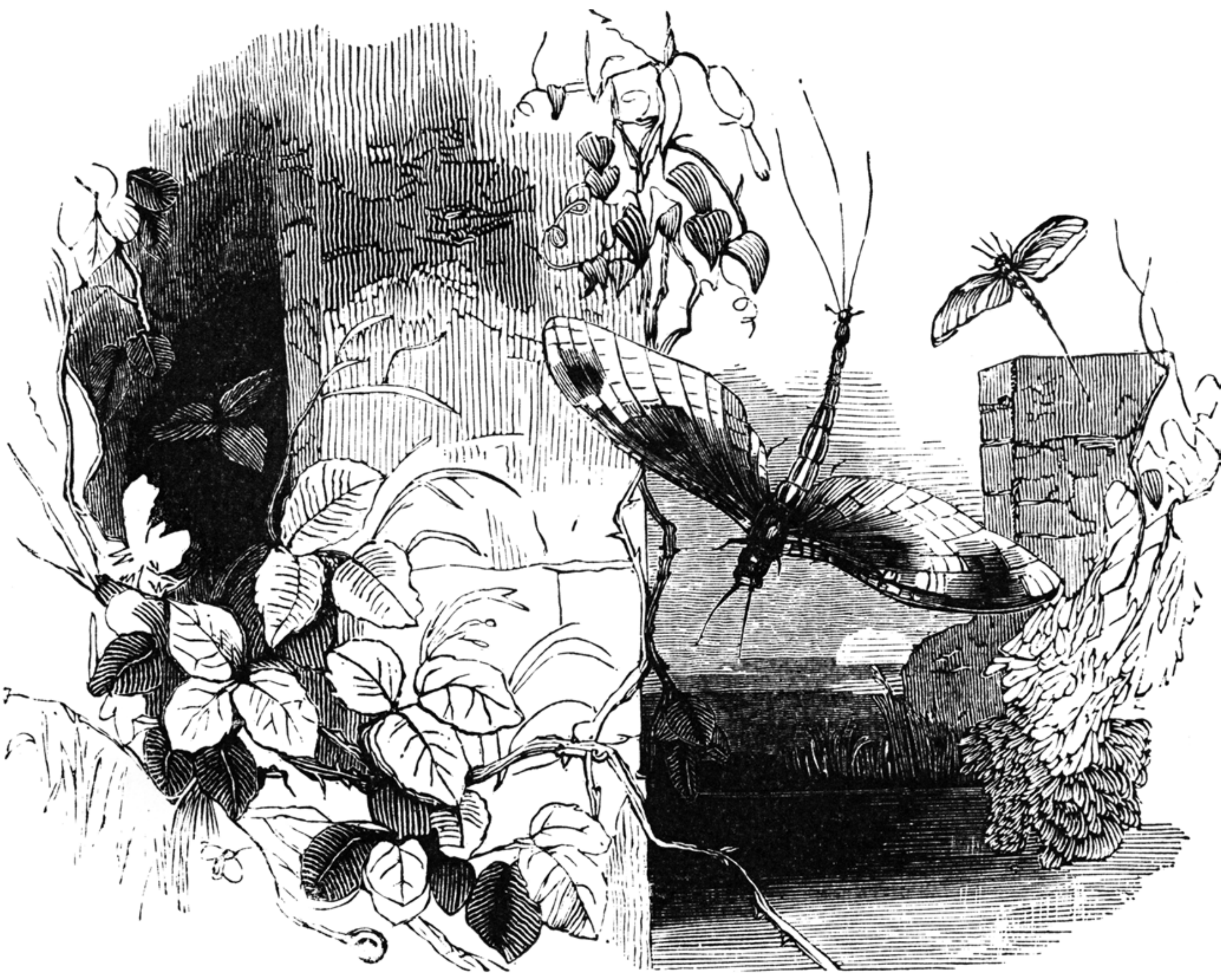

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