

## FINAL Report

# **Vertebrate Community Response to Regenerative Stream Conveyance (RSC) Restoration as a Resource Trade-Off**

Short Title: Vertebrate Community Trajectory in RSCs

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## **Abstract**

This study assessed the trade-offs inherent in stream restorations using the Regenerative Stream Conveyance (RSC) or restored stream-wetland technique. Specifically, it quantifies the aquatic vertebrate community trajectories that should be expected from RSC restorations implemented in lowland Coastal Plain streams with nutrient-rich waters. We defined the reference conditions (minimally-disturbed) for Coastal Plain aquatic vertebrate communities in both single-thread streams and stream-wetland complexes, using a literature review and data from Maryland Biological Stream Survey (MBSS). We sampled and analyzed the aquatic vertebrate communities (fish and herpetofauna) in 11 streams that have been converted to RSCs, along with 24 comparable references of three types. In general, RSC fish communities were more similar to low-quality single streams than to high-quality single streams or stream wetland complexes. Specifically, fish diversity in RSCs was lower than in high-quality sites and decreased with higher conductivity and lower dissolved oxygen. Sensitive fish species found in high-quality references (e.g., creek chubsucker, fallfish, madtoms, lampreys) were absent from RSCs and low-quality sites. Fish indices of biotic integrity (IBIs) were also lower in RSCs than in high-quality sites, but may be higher than in low-quality sites. While RSCs recreated the physical conditions typical of high-order stream wetland complexes in low-order reaches, they did not attain the levels of dissolved oxygen, conductivity, and flow found in high-quality sites. Herpetofauna diversity showed few patterns, except for higher frog abundance in RSCs than references. Overall, vertebrate uplift in RSCs appears to be constrained by continuing poor water quality. Variability among the 11 RSCs likely resulted from differences in water quality, canopy shading and temperature, stream width and depth, as well as restoration design and landscape setting. These results should help practitioners and regulators develop realistic expectations of biotic resource changes that occur when defined-channel stream systems are transformed into less-defined stream wetland complexes in urban-suburban settings.

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# **1. Introduction**

This study provides a baseline for assessing the trade-offs inherent in stream restorations using the Regenerative Stream Conveyance (RSC) or restored stream-wetland technique. Specifically, it helps quantify the aquatic vertebrate community changes that should be expected as part of the ecological change resulting from RSC restorations implemented in lowland Coastal Plain streams with nutrient-rich waters. First, we defined the reference conditions (disturbed and minimally-disturbed) for Coastal Plain aquatic vertebrate communities in both streams and stream-wetland complexes, using a literature review and existing Maryland DNR's Maryland Biological Stream Survey (MBSS) data. Second, we sampled and analyzed the aquatic vertebrate communities in streams that have been converted to RSCs and in appropriate references. Basic water quality, stream metabolism, habitat, and canopy cover measurements were also recorded. The results of this study should help practitioners and regulators more appropriately quantify the biotic resource changes that occur when defined-channel stream systems with riparian canopy cover are transformed into less-defined stream-wetland complexes, and ultimately consider those changes in the context of nutrient reduction benefits expected from restoration.

## **1.1 Key Restoration Questions**

This project addresses “resource trade-offs in different types of restoration projects.” Specifically, we focus on the aquatic vertebrate community changes that should be expected as part of the ecological change resulting from RSC or stream-wetland restoration. It is well-known that RSCs and similar designs generally transform defined-channel stream systems into less-defined stream-wetland complexes with a sequence of weirs, pools, and even small dams, with the intent of producing nutrient and sediment reduction benefits. To fairly quantify resource trade-offs, and ultimately apply a common currency, requires an accurate understanding of the biotic assemblages present in these two different reference ecosystems types. To date, the fish, amphibians, and reptiles that comprise the aquatic vertebrate community have been much less studied than benthic macroinvertebrates. Without an understanding of aquatic vertebrates in these systems, conclusions on trade-offs will be incomplete, especially because the recovery of macroinvertebrate and aquatic vertebrate communities in RSCs may follow different trajectories.

Our study includes two components:

- (1) Definition of reference conditions for lowland Coastal Plain aquatic vertebrate communities in both streams and stream-wetland complexes, using a literature review and existing MBSS data
- (2) Contemporaneous sampling and analysis of aquatic vertebrate communities in streams converted to RSCs, along with appropriate references for minimally-disturbed streams, minimally-disturbed stream-wetland complexes, and degraded streams

## 2. Literature Review and Conceptual Model

### 2.1 Definitions of Regeneration Stream Conveyance and Three References

Regenerative stream conveyances (RSCs) typically transform degraded, single-channel, lower-order streams into stream-wetland complexes designed to provide more opportunity for sediment retention and nutrient removal (Maryland DNR 2019). RSCs result in channel widening and partial impoundments that slow flow rates, typically reduce shading, create periodic anoxia, and increase diel dissolved oxygen variation and ecosystem gross primary production (GPP). Photographs of two sampled RSCs (Wilelinor and North Cypress Branch), taken immediately post-construction and after the RSCs have matured (17 and 10 years) are shown below (Figure 1).



*Figure 1. Photographs of two Regenerative Stream Conveyances (RSCs), taken immediately post-construction and after the RSCs have matured (17 and 10 years). Source: Anne Arundel County (Wilelinor 2003 and North Cypress Branch 2010), Tetra Tech (Wilelinor 2020 and North Cypress Branch 2020)*

We envision that RSC vertebrate communities may continue to mirror the communities in the degraded streams that were restored or may transform into communities more similar to either minimally-disturbed versions of these degraded streams or minimally-disturbed stream-wetland



complexes that better match the physical reconfiguration of the RSC construction. Typical photos of each of the following references are shown with comparison to an RSC (Figure 2).

- LSS – Low-quality, Single Streams as degraded, pre-restoration streams
- HSS – High-quality, Single Streams as minimally-disturbed streams
- HSW – High-quality, Stream-Wetlands as minimally-disturbed stream-wetland complexes
- RSC – Regenerative Stream Conveyances

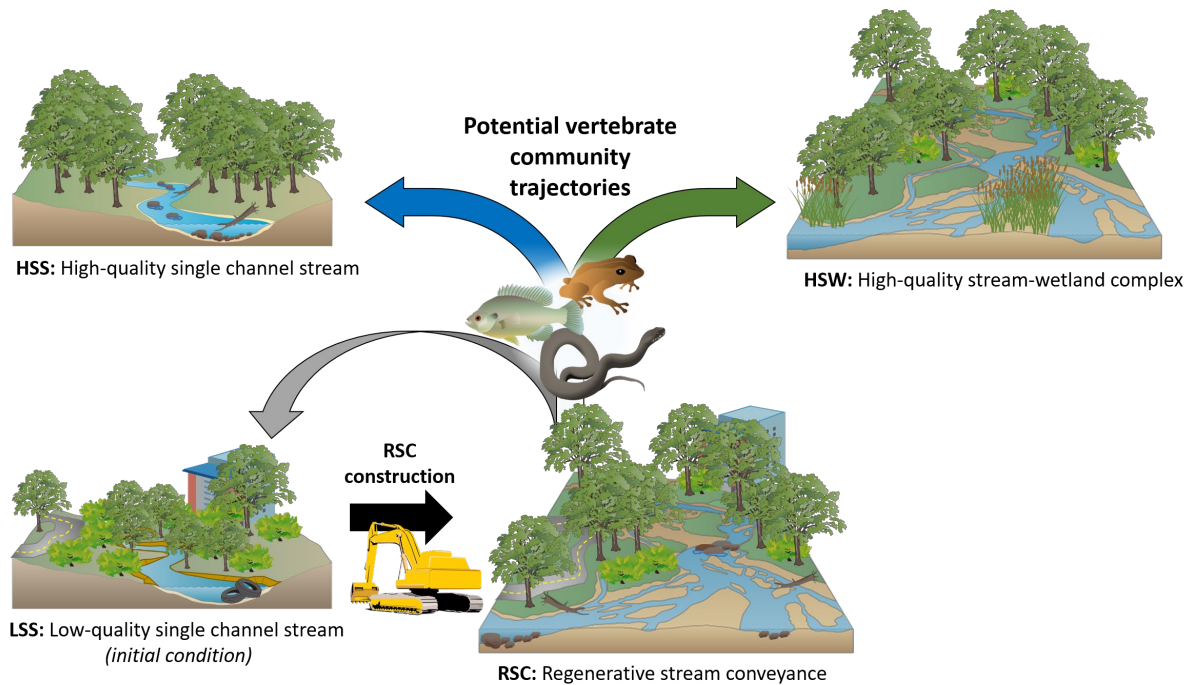


*Figure 2. Typical photos of three references shown with comparison to a Regenerative Stream Conveyance (RSC) in the lower right: High-quality, Single Streams (HSS) as minimally-disturbed stream (upper left); High-quality, Stream-Wetlands (HSW) as minimally-disturbed stream-wetland complex (upper right); and Low-quality, Single Streams (LSS) as degraded, pre-restoration streams (lower left). Source: Tetra Tech*

## **2.2 Conceptual Model Predictions from Existing Data**

Regenerative stream conveyance and restored stream-wetlands are being employed more frequently as a stream restoration technique to reduce nutrient and sediment pollution loading to downstream waters, especially at the tidal boundary. Implementation of RSCs in lowland channels typically involve transforming degraded, low-quality single-channel streams (LSS) into stream-wetland

complexes with less-defined channels that consist of a series of weirs and partial impoundments (Maryland DNR 2019; Figure 3). When degraded, these channels are often incised and disconnected from their floodplain, have flashy discharge profiles, and have relatively low in-stream primary productivity (i.e., production/respiration or P/R ratio) owing to riparian shading and turbulence. Following RSC implementation, channel widening slows flow rates and typically reduces shading, characteristics that can increase in-stream primary productivity considerably in nutrient-rich waters and lead to excessive biological oxygen demand, higher water temperatures, periodic anoxia, and increased concentrations of soluble reactive phosphorus (Filoso 2020). Such changes in the physical, chemical, and biotic conditions following RSC implementation should have a profound influence on the restored stream's habitats and biotic communities.



**Figure 3.** Graphical comparison of habitat-related differences associated with regenerative stream conveyance (RSC) construction relative to the putative initial condition (LSS: low-quality single channel stream), and reference conditions for three potential vertebrate community trajectories (HSW: high-quality stream-wetland complex; HSS: high-quality single channel stream; LSS).

Accurate evaluations of RSC-associated resource trade-offs between nutrient processing and biotic habitat suitability can only be achieved if the constituent biotic assemblages are quantified in a manner that allows for consideration of (1) community composition pre- and post-RSC implementation, (2) expected community composition based on habitat characteristics of post-RSC reaches, and (3) recovery trajectories that provide insight into the temporal scope of recovery by different community components (i.e., vertebrates and invertebrates). Despite structural similarities in habitat conditions between RSCs and naturally occurring high-quality stream-wetland complexes (HSW), it is possible that the aquatic, semi-aquatic, and riparian vertebrate communities in RSCs could ultimately resemble communities in single-channel streams. These single-channel alternative trajectories include communities reflective of the initial low-quality habitat conditions (low-quality single-channel stream, LSS), present prior to the RSC, or communities that resemble higher quality single-channel streams (high-quality single-channel streams, HSS) that are less impacted by human activity (Figure 3). The uncertainty surrounding the effect of RSCs on vertebrate communities reflects the lack of previous studies, as well as the potentially interacting influences of local environmental

conditions, catchment characteristics, and proximal vertebrate communities upstream or downstream of the RSC. For example, while RSC construction alters habitat conditions at the reach-scale, watershed land use characteristics, as well as stream conditions upstream and downstream, typically remain unchanged.

In order to develop a typology of these three potential recovery conditions based on diagnostic habitat and vertebrate community features, we analyzed existing stream data from the MBSS, held discussions with state and county personnel, and conducted a literature review. The MBSS database was queried to identify a suite of sampled sites that could be representative of LSS, HSS, and HSW conditions, by first isolating sites from the Coastal Plain province on the western shore of Chesapeake Bay. We elected to focus on the western shore Coastal Plain, because all of the RSC sites to be sampled in this study are found on the western shore. From this reduced dataset, we identified the pool of HSW sites as those classified as having extensively braided channel habitat and fish Index of Biotic Integrity (FIBI) scores in the Good range ( $\geq 4.0$  on a scale of 1 to 5) (Southerland et al. 2007). HSS and LSS sites were identified as those having no braiding present and FIBI scores  $\geq 4.0$  and  $\leq 2.0$ , respectively. This initial process yielded initial site pools of  $n = 46$  HSW sites,  $n = 43$  HSS sites, and  $n = 46$  LSS sites, sampled between 2000 and 2018. These site pools were further constrained by

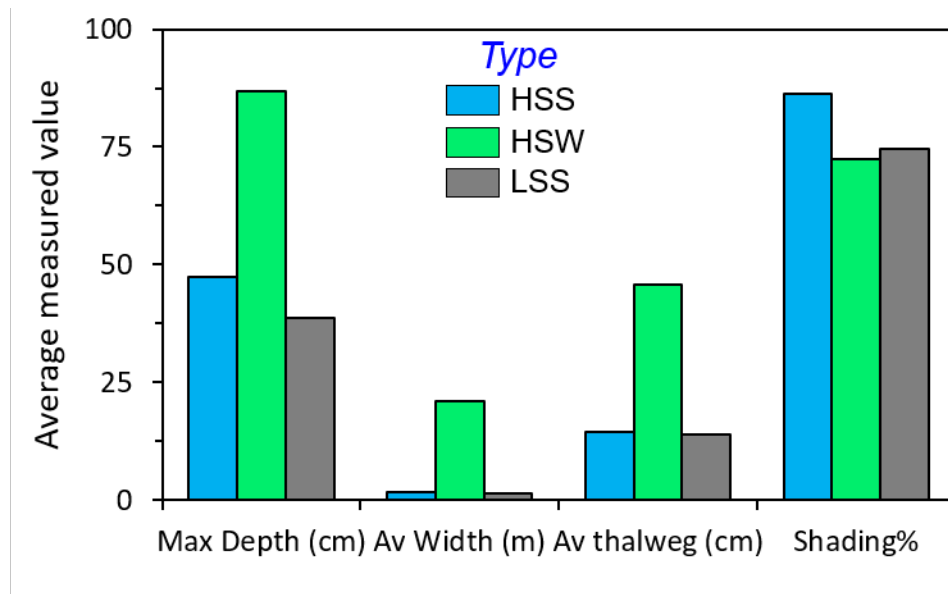


Figure 4. Average value of 4 habitat variables: maximum depth (*Max Depth*), average stream width (*Av width*), average thalweg depth (*Av thalweg*), and % shading of reach (*Shading%*) reported for each reference type (HSS: high-quality single channel stream; HSW: high-quality stream-wetland complex; LSS: low-quality single channel stream)

(1) selecting HSS and LSS sites with smaller watersheds ( $< 600$  acres) to align with the smaller catchment size of most RSC sites (HSW sites have naturally larger catchments); (2) excluding sentinel or current special project sampling sites to avoid excessive sampling of those sensitive locations; and (3) reviewing site photographs to ensure actual reach conditions were consistent with our target reference conditions. MBSS data from the final site pool of  $n = 8$  HSW,  $n = 10$  HSS, and  $n = 28$  LSS were used to generate a conceptual model of RSC habitat conditions and vertebrate community recovery trajectories. Final field site locations also included several Anne Arundel County sampling sites of an appropriate condition and size, selected to supplement the sites available (see Section 5 - Site Selection, below).



Conceptual model development focused on a qualitative analysis of the existing MBSS data using a combination of univariate graphical analysis and preliminary multivariate analysis targeted to identify habitat and community differences in fish and herpetofauna (amphibians and reptiles) among reference types (if present). Clear patterns in habitat conditions were apparent when comparing variables associated with stream depth and width across reference types (Figure 4). On average, the selected HSW sites had maximum depth values approximately 45% deeper than HSS and 55% deeper than LSS. Average thalweg depth was also deeper in HSWs than either HSS or LSS, by approximately 70%. Finally, HSW averaged ~95% wider than HSS and LSS. These patterns are explained by the physical characteristics of the systems as well as the higher average stream order of the HSW, which ranged from 1 to 4 with an average stream order = 2.75 (on 1:100,000-scale map). Conversely, all HSS and LSS sites had stream orders = 1. While these differences in stream order are sizeable, and may explain some of the patterns seen, HSW naturally occur on higher-order streams. This pattern underscores the novelty of the RSC approach, in that RSCs may recreate conditions typical of high-order streams in reaches that are low-order. We do not have incident light or stream metabolism measurements from the MBSS dataset, but there are estimates of overhead shading of stream reaches, as a percentage of the reach shaded. There are not large differences in the amount of shading by reference type, with slightly more shading occurring at the HSS sites than the LSS or HSW sites (Figure 4).

Similar to the habitat patterns among reference types, the composition and diversity of the fish and herpetofauna communities differed among reference types. Overall, HSW sites consistently had higher species richness among both vertebrate groups (Figure 5 and Figure 6). There was a qualitative pattern of HSS having intermediate species richness and LSS having the lowest species richness. There was also a consistent pattern of elevated variability in measured species richness in LSS for both vertebrate groups, as indicated by the coefficient of variation (CV). Because only presence-absence herpetofauna data were available for all MBSS sites, we were not able to calculate additional diversity indices; however, we were able to calculate additional indices for the fish community (Figure 5). These additional indices (Shannon-Wiener index of diversity  $H'$ , Margalef's  $d$ ) both showed the same pattern, with HSW reaches showing the highest  $H'$  and  $d$  values, HSS showing intermediate values of both indices, and LSS having the lowest values. Both HSW and HSS showed relatively low levels of fish diversity index variability (CVs < 0.5) while LSS variability was > 1.0 for all fish diversity indices.

Community composition data for fish and herpetofauna were analyzed (Similarity percentage profiles [SIMPER], Primer v7) by comparing the contribution of individual taxa within each reference type to determine which species occurred most frequently and contributed the most to community similarity among sites of each type. The SIMPER analysis quantifies the species-level contribution to among-site similarities, based on Bray-Curtis similarity matrix calculated among sites (Clarke 1999).

Analysis of fish community data showed that >90% of the LSS fish community similarity among sites was explained by three species: American eel (*Anguilla rostrata*), eastern mudminnow (*Umbra pygmaea*), and pumpkinseed (*Lepomis gibbosus*; Table 1). Both American eel and eastern mudminnow were among the most frequently encountered species (and explained the most among-site similarity in community composition) in all three reference types. These two species are native to the region, are habitat generalists, and are tolerant of degraded water quality and habitat conditions. For example, eastern mudminnow can survive in waters with pH = 3.0, at oxygen concentrations of 2 ppm or lower, and are capable of aestivation in stream muds during dry periods and air-breathing to supplement low dissolved oxygen conditions (Scott and Hall 1997, Kuehne and Olden 2014). In a previous study of Maryland Coastal Plain streams, Scott and Hall (1997) found that American eel were common in both disturbed and undisturbed stream reaches. Three additional species, least brook lamprey (*Lampetra aepyptera*) and two cyprinids (blacknose dace *Rhinichthys atratulus*, creek



chubsucker *Erimyzon oblongus*), were frequently encountered at HSS sites. A total of 18 species (including American eel and eastern mudminnow) were required to explain > 90% of the among-site variability in the HSW type, underscoring the greater diversity and the among-site variability in community composition in HSW sites relative to HSS and LSS sites (Figure 5, Table 1). Many of the species that were commonly observed at HSW sites are tolerant of a range of environmental conditions, including high temperatures, relatively low dissolved oxygen, and low pH (e.g., eastern mosquitofish *Gambusia holbrooki*, golden shiner *Notemigonus crysoleucas*, bluespotted sunfish *Enneacanthus gloriosus*; Scott and Hall 1997, Nordlie 2006, Stone et al. 2016), suggesting that conditions in HSW sites likely select for diverse yet tolerant species communities.

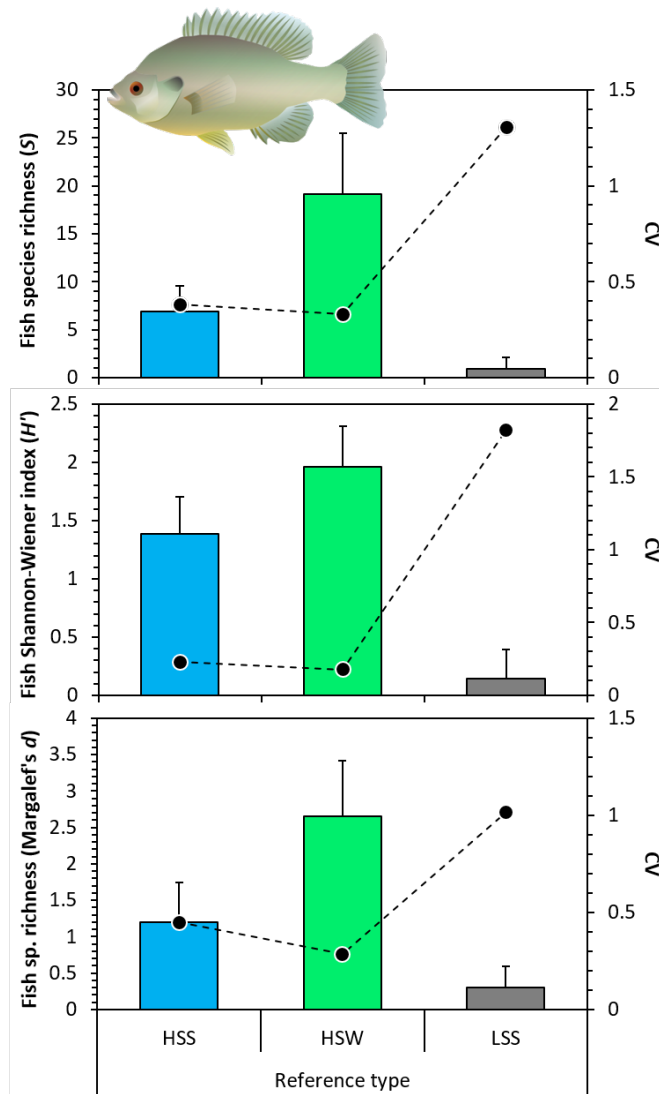


Figure 5. Fish community univariate biodiversity indices (bars, primary y-axis) calculated for each reference type (HSS: high-quality single channel stream; HSW: high-quality stream-wetland complex; LSS: low-quality single channel stream) and associated measures of variability (error bars  $\pm 1$  SD [primary y-axis]; coefficient of variation [CV; filled symbols, dashed line; secondary y-axis]). Indices calculated using total species catches per standardized sampling event.

Table 1. Similarity percentage profiles for each reference condition (LSS: low-quality single channel stream; HSS: high-quality single channel stream; HSW: high-quality stream-wetland complex) showing diagnostic fish % frequency of occurrence across sites in each reference condition (% Occur.), the average among-site community similarity contributed by each species (Av.Sim), and the contribution of each species to the total among-site community similarity (% Cont.).

Type	Species	% Occur.	Av.Sim	% Cont.
LSS	AMERICAN EEL	21	2.6	44.55
	EASTERN MUDMINNOW	21	2.31	39.68
	PUMPKINSEED	11	0.45	7.68
HSS	AMERICAN EEL	100	15.41	27.92
	EASTERN MUDMINNOW	100	15.41	27.92
	LEAST BROOK LAMPREY	80	9.79	17.74
	BLACKNOSE DACE	70	6.36	11.53
	CREEK CHUBSUCKER	50	2.87	5.2
HSW	AMERICAN EEL	100	5.49	10.25
	EASTERN MUDMINNOW	100	5.49	10.25
	PUMPKINSEED	88	3.99	7.45
	BLUEGILL	88	3.65	6.83
	FALLFISH	88	3.65	6.83
	REDFIN PICKEREL	88	3.65	6.83
	TESSELLATED DARTER	88	3.65	6.83
	CREEK CHUBSUCKER	75	2.7	5.04
	LARGEMOUTH BASS	75	2.7	5.04
	LEAST BROOK LAMPREY	63	2.13	3.98
	EASTERN MOSQUITOFISH	63	1.98	3.7
	TADPOLE MADTOM	63	1.84	3.43
	GOLDEN SHINER	63	1.76	3.29
	CHAIN PICKEREL	63	1.7	3.18
	BLUESPOTTED SUNFISH	50	1.37	2.55
	WARMOUTH	50	1.08	2.01
	MARGINED MADTOM	50	1.06	1.98
	PIRATE PERCH	50	1.06	1.98

Analysis of the herpetofauna data showed a similar pattern as fish with > 90% of the among-site similarity of LSS sites explained by two species, the northern green frog (*Lithobates clamitans*) and American bullfrog (*Lithobates catesbeianus*; Table 2). Among HSS and HSW sites, five and six species, respectively, explained >90% of the within-type similarity. The larger number of species needed to describe similarity patterns in the high-quality reference types agrees with the pattern of higher overall species richness observed in these types (Figure 6). Northern green frog was among the mostly frequently encountered herpetofauna taxa across all reference types. American bullfrogs were frequently encountered at LSS and HSW reference types, but were not commonly encountered at the

HSS sites. Several turtle and frog species, and two salamanders (northern two-lined salamander *Eurycea bislineata*, northern red salamander *Pseudotriton ruber*) were individually diagnostic of HSS or HSW sites but not both.

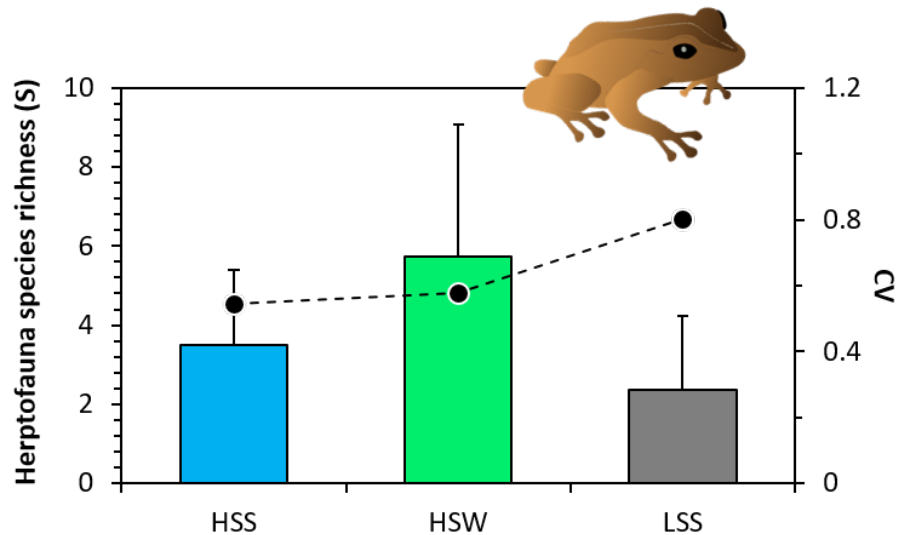


Figure 6. Herpetofauna community species richness (bars, primary y-axis) calculated for each reference type (HSS: high-quality single channel stream; HSW: high-quality stream-wetland complex; LSS: low-quality single channel stream) and associated measures of variability (error bars  $\pm 1$  SD [primary y-axis]; coefficient of variation [CV; filled symbols, dashed line; secondary y-axis]). Index calculated species presence/absence per standardized sampling event.

Table 2. Similarity percentage profiles for each reference condition (LSS: low-quality single channel stream; HSS: high-quality single channel stream; HSW: high-quality stream-wetland complex) showing diagnostic herpetofauna % frequency of occurrence across sites in each reference condition (% Occur.), the average among-site community similarity contributed by each species (Av.Sim), and the contribution of each species to the total among-site community similarity (% Cont.).

Type	Species	% Occur.	Av.Sim	% Cont.
LSS	NORTHERN GREEN FROG	75	24.39	80.78
	AMERICAN BULLFROG	29	3.25	10.77
HSS	NORTHERN TWO-LINED SALAMANDER	60	9.59	41.76
	NORTHERN GREEN FROG	60	7.67	33.41
	PICKEREL FROG	40	2.68	11.69
	EASTERN BOX TURTLE	20	0.63	2.77
	NORTHERN RED SALAMANDER	20	0.63	2.77
HSW	NORTHERN GREEN FROG	75	8.1	26.8
	AMERICAN BULLFROG	75	8.1	26.8
	SOUTHERN LEOPARD FROG	50	5.02	16.63
	EASTERN SNAPPING TURTLE	38	2.38	7.89
	EASTERN CRICKET FROG	38	2.01	6.64
	STINKPOT TURTLE	38	1.65	5.45

Based on our analysis of existing MBSS data and review of the literature, we have developed a conceptual model for the three stream reference types identified as possible endpoints of RSC vertebrate community trajectories (LSS, HSS, HSW; Figure 7). Stream channel increases in stream width and depth are expected at RSC sites, similar to HSW reference sites, owing to the purposefully engineered characteristics of RSCs (Maryland DNR 2019). These habitat conditions and the expectation of high, in-situ primary production associated with high nutrient loads, suggest RSC vertebrate communities should recover toward HSW reference conditions. In terms of biotic community structure, and relative to putative initial conditions (i.e., LSS), species turnover and species recruitment is expected to lead to higher species richness and diversity. Actual species community composition will depend on the geographic pool of available species, but it is likely that herpetofauna and fish species tolerant of slow moving, warm waters and potentially oxygen-limiting conditions are among those likely to occur in RSCs.

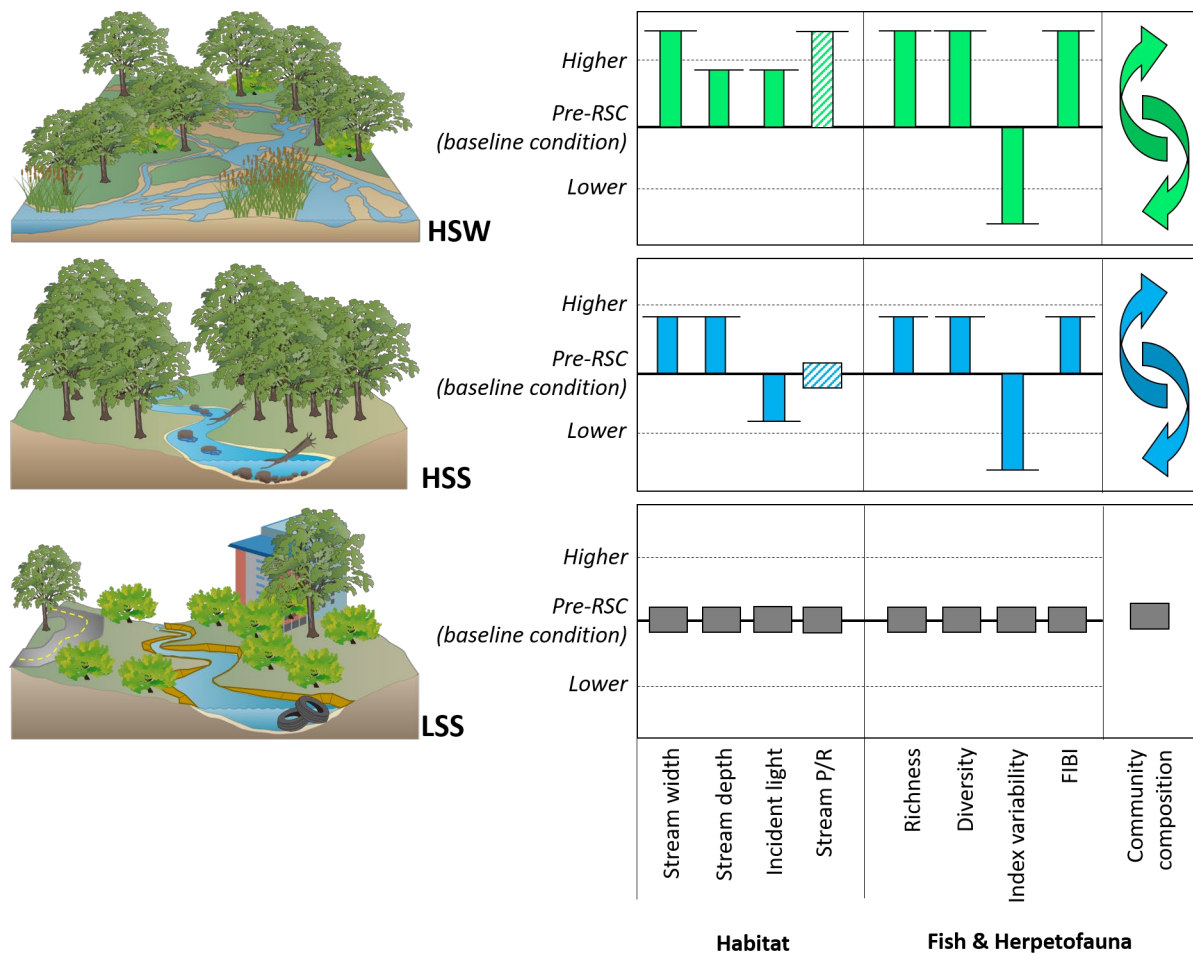


Figure 7. Key habitat and biotic community variables showing direction and magnitude of differences associated with each reference condition (top - HSW: high-quality stream-wetland complex; middle - HSS: high-quality single channel stream; bottom - LSS: low-quality single channel stream).

\*Hypothesized patterns depicted with patterned fill; community composition change represented by cycling arrows.



## 2.3 Stream Metabolism in Regenerative Stream Conveyances

Biological assemblages and water quality are commonly used for assessing stream health, but there is increased interest among the freshwater research community in incorporating measures of stream function to strengthen stream-health assessments (Munn et al. 2020). Stream metabolism is an integrative whole-ecosystem estimate of the carbon fixed and respired in a stream, which is used as a functional indicator of the overall health of stream ecosystems (Young et al. 2008). Low-order streams are typically heterotrophic, i.e. they consume more carbon through ecosystem respiration than they produce ( $\text{Production} < \text{Respiration}$ ,  $P < R$ ) (Dodds 2006), and most of the carbon in the system is allochthonous, meaning that it comes from outside sources such as the upstream watershed and riparian vegetation. By determining whether production in the stream is higher than respiration or vice-versa on a diurnal cycle, it is possible to assess if the stream is moving away from a more natural state. This information provides helpful insights into the trophic status and nutrient cycling in streams (Munn et al., 2020).

A combination of different environmental variables such as nutrients, light, and discharge rates control stream metabolism (Roberts et al. 2007). Under natural conditions, gross primary production (GPP) is usually limited by light, nutrients, grazing pressure, or a combination of these environmental variables. When human activities such as land-use changes or stream restoration affect these variables, they can shift stream metabolism away from a more natural state, with consequences to aquatic life (as these changes affect the availability of dissolved oxygen and carbon).

For example, in agricultural streams, GPP is typically elevated due to higher nutrient inputs and increased solar radiation from the loss of riparian canopy, causing changes in ecosystems structure and function (Munn et al. 2020). In urban streams, GPP is usually reduced by frequent flow disturbances, which scour benthic algal biomass, increase turbidity, and reduce light penetration, thereby reducing photosynthesis (O'Donnell and Hotchkiss 2020).

For this study, we hypothesize that degraded urban streams will have low rates of GPP in comparison to high-quality, single-channel streams because of the characteristic problems. When converted into RSCs, we predict that GPP rates will increase because of a combination of nutrient-rich waters, more light incidence from widened channels, and less scouring from high flows. Furthermore, because increases in GPP rates in streams are usually associated with the growth of algal biomass (Dodds 2007), we also predict that RSCs will have more autochthonous organic matter and, consequently, higher ecosystem respiration rates. Increased GPP and ecosystem respiration (ER) rates influence DO concentrations, which can result in DO levels becoming limiting to specific taxa, particularly at night (Munn et al. 2020).

In a comprehensive analyses of productivity in stream ecosystems based on data from over 200 streams, Finlay (2011) has shown that human actions have increased primary production in streams to a greater degree than heterotrophic respiration, shifting streams toward autochthony (i.e., organic matter production within the stream channel) and away from allochthonous sources of organic matter, which have long been known to fuel stream food webs (Tank et al. 2010). The increase in autotrophic-based production is considered a major factor causing shifts in biodiversity, trophic dynamics, and nutrient cycling in streams worldwide (Findlay 2001).

### 3. Methods

#### 3.1 Hypotheses

This study will test the following hypotheses about expected characteristics of aquatic vertebrate assemblages occurring in RSCs, compared with three different reference conditions.

Hypothesis 1 – RSC assemblages are the same as degraded, pre-restoration streams

Hypothesis 2 – RSC assemblages are the same as minimally-disturbed streams

Hypothesis 3 – RSC assemblages are the same as minimally-disturbed stream-wetland complexes

The expected results of our hypothesis testing will be significant differences in diversity and composition metrics of the fish and herpetofauna (amphibian and reptile) assemblages among all, some, or none of the aquatic system types described above. Covariates such as size of catchment draining to each site and percent of forest in the catchment were considered in site selection. Supporting information such as, basic water quality conditions, habitat conditions, and light incidence, will be included as explanatory variables.

#### 3.2 Site Selection

Based on our literature review and conceptual model described above, we worked closely with the Maryland Department of Natural Resources and Anne Arundel County to identify 10+ sites (8 target sites and 2+ alternatives) representative of each of our four stream classes:

- RSC – Regenerative Stream Conveyance restorations
- LSS – Low-quality, Single Streams as degraded, pre-restoration streams
- HSS – High-quality, Single Streams as minimally-disturbed streams
- HSW – High-quality, Stream-Wetlands as minimally-disturbed stream-wetland complexes

Using data from sites previously sampled by Maryland DNR's Maryland Biological Stream Survey (MBSS) and the Anne Arundel County Bureau of Watershed Protection and Restoration, we identified candidate sites with the desired stream quality and stream form for each class as described below. The final selection of sites among the candidates also involved review of site photos and field reconnaissance. Only sites with landowner permissions and no sampleability problems were sampled. A total of 35 sites with a range of catchment sizes were sampled as follows:

- 11 RSC *Regenerative Stream Conveyances* = 30–4550 acres
- 8 LSS *Low-quality Single Streams* = 134–669 acres
- 8 HSS *High-quality Single Streams* = 453–665 acres
- 8 HSW *High-quality Stream Wetlands* = 552–52,936 acres

#### RSC Sites

The number of RSC restorations that have been completed (and are at least 2 years post-construction to avoid immediate construction impacts) is limited and they primarily occur in the Coastal Plain. We

worked with Anne Arundel County and Arundel Rivers Federation to identify RSC sites that represent the most similar cohort of Coastal Plain RSC restorations. Nonetheless, these sites include varying design approaches (within the RSC type) and times since construction. The catchment areas draining to each RSC ranged from 30 to 4550 acres. Additional design information and photographs of each RSC site are included in the Appendix.

The RSC sites located on Church Creek have sampling data by Arundel Rivers Federation from previous years (RSC-9, RSC-11, RSC-12; 2015-2019), but all were sampled contemporaneously for this project. Note that RSC-4 Dividing Creek and RSC-10 were electrofished by Anne Arundel County in 2020, and RSC-9, RSC-11, and RSC-12 were electrofished by Arundel Rivers Federation in 2020, so we used those fish data (so as not to further disturb the fish community) but completed all other sampling at these sites.

<b>ID</b>	<b>Site Name</b>	<b>Age (years)</b>	<b>Catchment (acres)</b>	<b>Lat</b>	<b>Long</b>
RSC-1	Bacon Ridge	2	1757	39.000337	-76.613901
RSC-2	N Branch Cypress Creek	10	461	39.075049	-76.539927
RSC-3	Crofton Tributary	9	211	38.999339	-76.697001
RSC-4	Dividing Creek	4	220	39.050682	-76.515928
RSC-5	Howard's Branch	17	237	39.020913	-76.549196
RSC-6	Cabin Branch Saltworks Creek	7	121	38.994141	-76.548351
RSC-8	Wilelinor	16	262	38.965987	-76.541020
RSC-9	Church Creek at Allen Apartments	3	30	38.974970	-76.534451
RSC-10	Cowhide Branch to Weems Creek	7	4550	38.990208	-76.525618
RSC-11	Church Creek at Bywater	5	67	38.968582	-76.532140
RSC-12	Church Creek at Annapolis Harbour	6	151	38.975297	-76.541749

### **LSS Sites**

The 8 LSS sites were selected to represent degraded Coastal Plain streams that could reasonably be restored as RSCs. Only sites that were confirmed as degraded with FIBIs of less than or equal to 2 (on the 1-5 scale) were considered.

Four of the following sites were provided by Anne Arundel County as being candidates or similar to candidates for restoring as RSCs through their program. Each is part of their countywide, probability-based monitoring program and has been previously sampled. The catchment areas for these 4 sites range from 134 to 407 acres.

The other four LSS sites were selected from MBSS sites that have also been previously sampled. The selected MBSS sites also scored 2 or less on the FIBI, had similar catchment areas, and were located in the general vicinity of Anne Arundel County. The catchment areas for these four sites range from 177 to 667 acres.

<b>ID</b>	<b>Site Name</b>	<b>Catchment (acres)</b>	<b>Lat</b>	<b>Long</b>
LSS-2	BALT-101-R-2009 UT Marley Creek	177	39.120233	-76.628285
LSS-3	SEVE-110-R-2008 Hockley Branch	259	39.014638	-76.54949
LSS-4	PAXU-118-R-2004 UT14 Patuxent River	304	39.073650	-76.815770

LSS-6	Mill Creek at Dividing Creek	247	39.051321	-76.508081
LSS-7	05-R3M-05-18 Marley Creek	407	39.126638	-76.624002
LSS-8	08-R3M-02-18 Lower Magothy	270	39.033363	-76.446650
LSS-10	01-L2M-01-18 Piney Run	134	39.161420	-76.758123
LSS-13	SEVE-106-R-2017 UT Severn Run	669	39.113234	-76.667384

### HSS Sites

The HSS sites were selected to represent minimally-disturbed Coastal Plain streams with a distinct single channel. This represents one potential trajectory for RSC restorations. Only sites that were confirmed as high-quality with FIBIs of greater than or equal to 4 (good on the 1-5 scale) were considered. None of the sites in the Anne Arundel County countywide monitoring program had FIBIs greater or equal to 4, so none were included.

The eight HSS sites were selected from MBSS sites that have also been previously sampled and scored greater than or equal to 4 on the FIBI. The sites are in the Coastal Plain of southern Maryland closest to Anne Arundel County. The catchment areas for these eight sites range from 453 to 665 acres.

ID	Site Name	Catchment (acres)	Lat	Long
HSS-6	ZEKI-106-R-2001 UT Zekiah Swamp	453	38.504810	-76.882492
HSS-9	PAXU-101-R-2018 Newstop Branch	609	39.997852	-76.785007
HSS-10	SOUT-101-R-2016 Saint George Barber Marsh	658	38.969878	-76.609398
HSS-12	STMA-103-R-2009 UT St Mary's River	600	38.281611	-76.517430
HSS-17	MATT-104-R-2007 UT5 Mattawoman Creek	594	38.582277	-77.133896
HSS-18	WICO-113-R-2004 Chaptico Run	650	38.366110	-76.780417
HSS-20	PRMT-118-R-2002 Reeder Run	544	38.517763	-77.200080
HSS-21	STMA-113-R-2000 UT Bacon Ridge Branch	665	38.198024	-76.418188

### HSW Sites

The HSW sites were selected to represent minimally-disturbed Coastal Plain stream-wetland complexes with braided channels and Rosgen DA5 or E5 classifications. This represents the expected trajectory for RSC restorations. Seven sites had fish IBIs of greater than or equal to 4 (good on the 1-5 scale); one of the Anne Arundel County sites from their countywide monitoring program had a fish IBI of 3.67.

Six HSW sites were selected from MBSS sites that have also been previously sampled and scored greater than or equal to 4 on the FIBI. The sites are in the Coastal Plain of southern Maryland closest to Anne Arundel County. The catchment areas for these six sites range from 552 to 52,936 acres. The HSW catchment sizes are much greater than for the other classes, but are characteristic of the stream-wetland complexes that RSCs seek to mimic. The two of the Anne Arundel County sites were classified as the Rosgen DA5 or E5 stream forms indicative of the target for RSCs and had catchment areas of 552 and 4135 acres. Note that HSW-30 was electrofished by Anne Arundel County in 2020, so we used those fish data (so as not to disturb the fish community) but completed all other sampling.



ID	Site Name	Catchment (acres)	Lat	Long
HSW-1	PRMT-151-X-2006 UT28 Potomac River	588	38.473930	-77.255737
HSW-2	MATT-210-R-2014 Piney Branch	5258	38.654961	-76.981788
HSW-3	ZEKI-301-X-2016 Zekiah Swamp Run	8526	38.610080	-76.831709
HSW-6	ZEKI-312-R-2001 Zekiah Swamp Run	52,936	38.492852	-76.927364
HSW-8	04-L2M-03-19 Bacon Ridge Branch	4135	39.009877	-76.621008
HSW-17	PTOB-201-X-2017 Port Tobacco Creek	7060	38.557011	-77.017023
HSW-18	ZEKI-202-A-2012 Zekiah Swamp Run	2897	38.630406	-76.806337
HSW-30	04-L2M-03-19 Sawmill Creek	552	39.164025	-76.640088

### 3.3 Field Sampling

Our data collection methods followed the procedures of the MBSS (Stranko et al. 2019), with which both restoration proponents and regulators are familiar. These methods provide comparability with previously recorded data collected by MBSS and Anne Arundel County (which uses MBSS methods). Specifically, we used MBSS methods for fish electrofishing, herpetofauna (amphibian and reptile) timed searches, and habitat assessment, with refinements for water quality (dissolved oxygen, temperature, nutrients) and stream metabolism (described below).

We sampled each site between August 1 and September 30, which was the minimal period within the MBSS Summer Index Period that all 35 sites could be sampled. This shortened period limited seasonal variation among sample dates and the dates of sampling each type of stream was spread over the entire sampling period. Additional measurements for stream metabolism were conducted on a subset of sites in May 2021.

#### 3.3.1 Fish

Double-pass electrofishing of 75-m stream sites using block nets was conducted at each site. MBSS electrofishing occurs only during the Summer Index Period (June-September). This time period was chosen to characterize fish communities during the low flow period. Sampling during this period is also advantageous because spawning effects are minimized, temperatures are conducive to wading and water contact, and capture efficiency using electrofishing is typically best when streams are relatively low and warm. In the case of stream-wetland complex sites that access the floodplain, sampling was done during lower flow periods when fish are generally constrained to the sampleable segment.

The sampleability of each site was assessed prior to fish sampling, in accordance with MBSS sampling protocols (Stranko et al. 2017). If depth or velocity at a site was too high following a rain event, sampling at that site was deferred to a later date, after water levels had returned to a lower level, so that sampling could be conducted safely and with good visibility. Because the 2020 sampling occurred during a rainy period, weather conditions were monitored daily and adjustments to the sampling schedule were made accordingly. Smaller sites often returned to a low enough water level more quickly, while larger sites sometimes required additional days to return to a sampleable level.

#### 3.3.2 Amphibians and Reptiles (Herpetofauna)

Amphibians and reptiles have been shown to be good indicators of site conditions at typical MBSS streams (Southerland et al. 2004). However, it should be noted that stream salamanders are less

diverse and abundant in Coastal Plain streams, so the stream salamander sampling protocol was not used. We anticipated that frogs, turtles, and snakes will be the most abundant amphibians and reptiles in the RSCs and reference stream-wetland complexes. Therefore, we implemented a custom MBSS sampling method that draws on amphibian and reptile methods used across different MBSS rounds. Specifically, we searched for 60 minutes using two meandering transects on each side of the stream to cover the 10 meters from stream bank into the riparian area, as follows:

1. 60 minutes is the total for both sides and searching was generally 30 minutes on each side
2. Two transects were completed on each side, with the first transect meandering upstream to include the stream bank and adjacent 5 m and the second transect meandering downstream between 5-10 m from the stream
3. Transects were conducted at the beginning of the site set up, along with block net installation and water quality sampling, so as to sample amphibians and reptiles before they were disturbed
4. Transect searching used a pace to cover the best habitat within 60 minutes total, turning accessible cover objects (and returning them to their original position)
5. If there was a lot of stream braiding, the middle habitat was also searched, but within the 60-minute time limit
6. Any amphibians and reptiles captured during electrofishing were recorded, but noted as separate from the transects to limit double counting
7. Frog calls were included, but noted as species identified by calls
8. Visual identification was as adult or larvae, but size was not recorded
9. Where necessary and possible, individuals were captured for identification (by hand or using dip nets) and photographed, but no specimens were preserved

### **3.3.3 Habitat**

We conducted physical habitat assessments using MBSS methods (Stranko et al. 2019), which are intended to represent the habitat conditions available to the organisms living in the streams and the extent to which certain anthropogenic factors may be affecting the stream. MBSS habitat assessment protocols are based on a combination of metrics modified and adapted from USEPA's Rapid Bioassessment Protocols (RBP) and Ohio EPA's Qualitative Habitat Evaluation Index (QHEI). Although EPA's RBP habitat assessment protocols differentiate between riffle-run and pool-glide stream types, all metrics selected for the MBSS are scored at all MBSS sample sites to allow direct comparisons across physiographic regions and summaries of conditions on a statewide basis. As all the project sites are in the Coastal Plain, metric values can be easily compared.

### **3.3.4 Water Quality and Stream Metabolism**

Dissolved oxygen (DO) and water temperature were measured continuously in 5-minute intervals for 2 to 3-week periods in 19 of the study streams. Measurements were done in different times (late summer and early fall of 2020, and in the spring of 2021) for separate groups of streams which included RSCs and reference streams (LSS, HSS, HSW). The main goal was to assess how DO concentrations and metabolism in RSCs compared to DO and metabolism in reference streams.

Stream metabolism is a functional metric of ecosystem activity used to assess stream health and estimate the relative contribution of allochthonous and autochthonous carbon sources to the stream food web. Diel trends in DO have been used to measure whole-system metabolism since Odum (1956) introduced the method. Stream ecosystem metabolism (in units of oxygen,  $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ) is estimated from the daily variation in the production and consumption of oxygen by stream organisms, following a simple model described in Bernhardt et al. (2018):

$$\frac{dDO}{dt} = \frac{GPP + ER}{z} + K(DO_{sat} - DO)$$

where  $dDO/dt$  is the change in dissolved oxygen concentration through time, GPP is the rate of photosynthetic  $O_2$  production, ER is the rate of oxygen consumption through both autotrophic and heterotrophic respiration, and  $K(DO_{sat} - DO)$  is the net exchange of oxygen between the water column and the overlying air governed by a per unit time gas exchange rate  $K$  (Grace and Imberger 2006). By convention, any process that lowers DO concentration in the water takes on a negative value so that ER is always a negative number. Whenever autotrophs are present, GPP increases with light. Typically, GPP results in peak DO during the day.

We hypothesized that minimally disturbed streams with riparian canopy shading are net heterotrophic, with the allochthonous contribution being predominant compared to the autochthonous production. We also hypothesize that GPP and ER is greater in minimally disturbed stream-wetland complexes than in minimally disturbed single channel streams because they receive higher solar irradiance (influencing GPP) and higher temperature (influencing ER). Finally, we expect GPP and ER to be higher in RSCs as a consequence of both greater light incidence, higher temperatures from the decreased shading effect of channel widening, and sometimes riparian deforestation and increased nutrient loadings.

### Field Sampling

Field campaigns were carried out in August, September, and early October of 2020, and again in May of 2021, to cover the period of maximum primary production in streams in the region. Stream metabolism was calculated using only data recorded on days with clear skies and no rain event within the antecedent 24-hour period.

#### 3.3.4.1 Stream Metabolism

Stream metabolism was estimated using the open-water exchange method, which measures natural diel changes in  $O_2$  concentrations downstream of a stream reach to determine primary production (PP) and respiration (R) rates along the reach. The open water exchange method integrates metabolism over a large spatial scale, and thus, to minimize uncertainty in P and R estimates, requires accurate measurements of  $O_2$  concentrations and additional inputs of environmental/lotic variables such as water temperature and reaeration coefficient.

Diel DO and water temperature were measured in the study streams using temperature and DO optical sensors (Onset-HOBO, Onset Corporation, Bourne, MA) installed in the water column at a site of low disturbance. Metabolism was measured during base flow conditions in intervals of 5 minutes for continuous periods of 2 to 3 weeks.

The reaeration coefficient was estimated using the surface renewal method described in Grace and Imberger (2006), which is based on stream hydrological parameters including mean depth and flow velocity.

The reaeration coefficient at 20°C,  $KO_2$  (20°C) in  $d^{-1}$  is given by:

$$KO_2 (20^\circ C) = 50.8 \times (v^{0.67}) \times (D^{-0.85})$$

where

$v$  = velocity ( $\text{cm s}^{-1}$ ),  $D$  = depth (cm)

The value of  $\text{KO}_2$  in  $\text{d}^{-1}$  can be calculated for any temperature (in  $^{\circ}\text{C}$ ) using an equation, also provided by Grace and Imberger (2006).

#### **3.3.4.2 Water Quality and Other Environmental Variables**

An important step in using stream metabolism for understanding stream health is the identification of environmental factors that control metabolism. Therefore, in addition to determining metabolism for the 19 study streams monitored for DO and temperature, we also assessed environmental factors that could help explain stream metabolism and to what degree metabolism were related to fish metrics in restored versus reference streams. To explore which environmental factors control stream metabolism at each site, we measured stream discharge, drainage area, level of imperviousness, extent of canopy shading over the stream channel and water quality represented by conductivity and nutrient concentrations.

The physical and chemical characteristics of the selected stream segments were monitored during baseflow conditions at the time of installation of DO and temperature loggers in the study streams. Discharge was estimated from flow velocity measurements taken with an electronic flow meter (Marsh-McBirney Flo-Mate; Marsh-McBirney, Frederick, Maryland) using the cross-sectional area method. Specific conductance, temperature and pH were measured in stream flow using a field probe (Manta+35, Eureka Water Probes, Austin, TX), while water samples for analyses of nitrate, ammonium, soluble reactive P (SRP) and total suspended solids (TSS) were collected manually and filtered in the field through pre-combusted glass-fiber filters (Whatman GF, 0.45- $\mu\text{m}$  pore size) for analyses of nutrients. Water samples for determination of suspended solids were filtered in the lab through pre-weighted glass-fiber filters.

The concentration of nitrate in the streams was determined in the laboratory at the Chesapeake Biological Lab using a Dionex IC 1000 ion chromatograph, according to the EPA 300.0 Method for Inorganic Anions Ion Chromatography. Total nitrogen (TN) and total phosphorus (TP) concentrations were determined on unfiltered stream water samples using the persulfate digestion method. Conductivity was measured in situ and in the lab with a handheld YSI Conductivity/Temperature meter.

The area drained by each stream to the point of DO data collection and the percent imperviousness in the area were determined using the U.S. Geological Survey StreamStats program (<http://streamstats.usgs.gov>). The percentage canopy cover in the stream reaches was assessed with a spherical densitometer.

### **3.4 Statistical Methods**

#### **3.4.1 Vertebrate Community Analysis**

Fish and herpetofauna community assemblage data were analyzed using parametric univariate and non-parametric multivariate approaches. Univariate indices of fish community structure calculated for each site include the fish index of biotic integrity (IBI). Calculation of individual site fish IBI scores followed the current MBSS protocol (Stranko et al. 2019) and is dependent on the species composition, number of individuals captured, ecoregional stratum, catchment area, area of stream sampled, and total fish biomass. Univariate community metrics calculated for both fish and herpetofauna included species richness (Richness), species richness per unit area sampled



(Richness<sub>Area</sub>), and an index of biodiversity (Shannon-Wiener diversity index,  $H'$ ). Richness is the number of individual species-level taxa encountered at each stream and Richness<sub>Area</sub> is the Richness divided by the stream area sampled (reach length  $\times$  average wetted width). The Shannon-Wiener index is calculated as

$$H' = -\sum_{i=1}^R p_i \ln p_i$$

where

$R$  is species Richness,  $p_i$  is the proportion of species  $i$  relative to the total number of species, which is then multiplied by the natural log of  $p_i$ .

Additional aggregate variables that were considered included total number of individuals and total biomass (across species), and number of individuals and biomass per unit area sampled (i.e., individuals  $m^{-2}$  and  $g\ m^{-2}$ , respectively).

Differences in univariate community assemblage metrics across stream types were analyzed for fish and herpetofauna using ANOVA (i.e., HSS, HSW, RSC, LSS). Post-hoc pairwise comparisons were conducted using Tukey protected tests. Multiple linear regression was used to model the relationship between biodiversity indices (response variable) and three environmental variables (conditions) that included dissolved oxygen concentration, water temperature, and specific conductivity. To evaluate evidence for changes in vertebrate community composition over time in RSCs, each RSC was assigned to one of three age classes based on the amount of time elapsed since construction: 1.9-4.3 years ( $n=3$ ; see RSC site Table, Section 3.2), 5.3-7.4 years ( $n=4$ ), and 8.9-16.2 years ( $n=4$ ). Vertebrate abundance, biomass, Richness, Richness<sub>Area</sub>, and  $H'$  were analyzed using ANOVA to test for differences in assemblage composition over time. Parametric assumptions of all ANOVA and regression models were examined visually (residual normality) and statistically using Levene's test (homoscedasticity).

A Bray-Curtis dissimilarity matrix was calculated separately for the fish and herpetofauna datasets to support multivariate analyses. Fish and herpetofauna abundances were square-root transformed to down-weight the contribution of the most abundant species prior to calculation of dissimilarity matrices. Sites with zero observations were removed from the analysis prior to matrix calculation. Non-metric multidimensional scaling (nMDS;  $N = 999$  permutations) was used to visually assess similarity across sites by stream types for each vertebrate group and these patterns were analyzed quantitatively using Analysis of Similarity (ANOSIM; Clarke 1999). The contribution of individual species to within stream type similarity and between stream type dissimilarities were assessed using SIMPER.

### 3.4.2 Water Quality and Habitat Analysis

Water quality measured at the time of sampling was compared across stream types using ANOVA. Dissolved oxygen concentration, water temperature, and specific conductivity were compared individually across stream types and post-hoc pairwise comparisons were conducted using Tukey protected tests. Additional habitat variables were graphed by stream type to support visual analysis and habitat-context for observed biotic patterns.

## 4. Results

The analyses below were used to test the following hypotheses about expected characteristics of aquatic vertebrate assemblages occurring in RSCs, compared with three different reference conditions. The results of the 2020 sampling were also compared to the predictions of the conceptual model described above.

Hypothesis 1 – RSC assemblages are the same as degraded, pre-restoration streams

Hypothesis 2 – RSC assemblages are the same as minimally-disturbed streams

Hypothesis 3 – RSC assemblages are the same as minimally-disturbed stream-wetland complexes

Specifically, we evaluated the field sampling results for the RSCs and reference types by comparing differences in diversity and composition metrics of fish and herpetofauna assemblages. We also evaluated results among stream types for habitat and basic water quality that may be affecting the vertebrate assemblage results.

### 4.1 Fish Results

#### 4.1.1 Fish IBIs

The fish Index of Biotic Integrity (IBI) developed by the MBSS is a well-known and accepted indicator of stream condition in Maryland. A comparison of fish IBIs calculated for RSCs and the three references showed that there were significant differences among the stream types (ANOVA, *F-statistic*<sub>3,31</sub> (*df* = num., den.),  $p < 0.0001$ ). HSS and HSW reference sites were consistently above the threshold of impairment (fish IBI = 3) and averaged  $3.7 \pm 0.6$  (SD) and  $3.9 \pm 0.3$ , respectively. RSCs and LSS sites had significantly lower fish IBIs with averages of  $2.3 \pm 1.0$  and  $1.9 \pm 0.7$ , respectively. Two RSC sites had fish IBIs exceeding 3. Tukey's pairwise comparisons showed HSS and HSW sites were higher than RSC and LSS sites ( $p$ -adjusted  $\leq 0.001$ ; Figure 8).

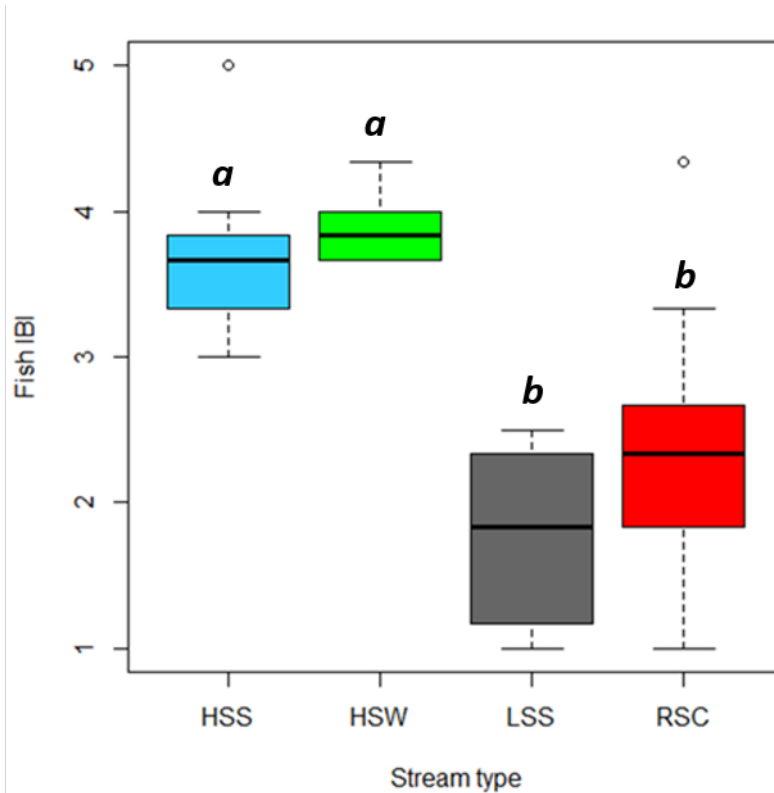


Figure 8. Boxplot of fish index of biotic integrity (IBI) scores by stream type. Different letters above groups indicate significant differences based on Tukey's post-hoc HSD.

#### 4.1.2 Fish Diversity and Composition

Fish assemblages in RSCs were more similar to the LSS reference streams than either the HSS or HSW reference streams (Figure 9). Total biomass and abundance of fish was typically high at RSC sites relative to reference stream types, but RSC sites ranked lower than HSS and HSW sites when biomass and abundance was scaled to area sampled. Species richness per unit area sampled was lowest at RSC sites, followed by LSS sites, then by HSW and HSS sites.

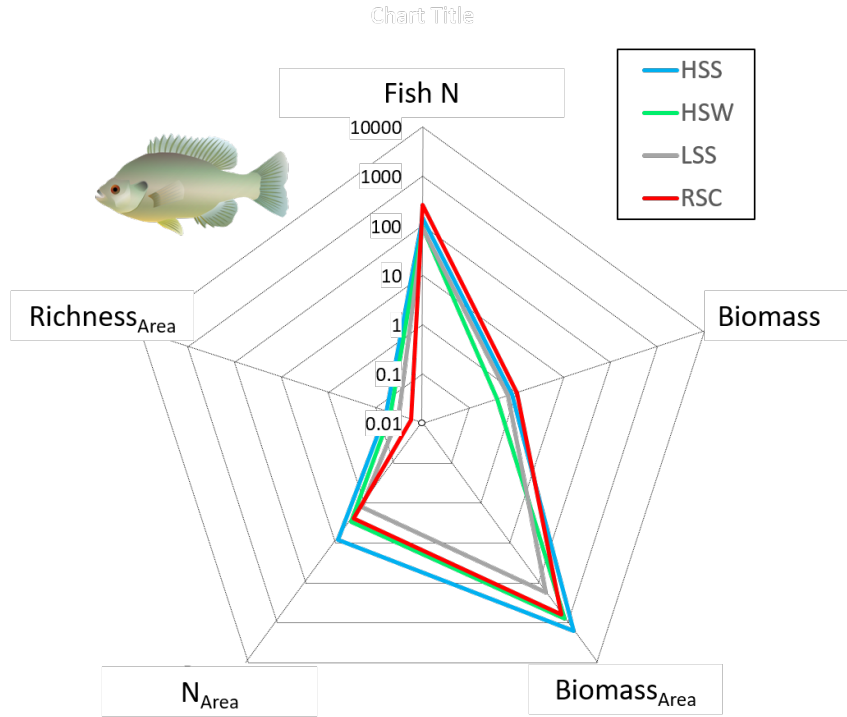


Figure 9. Radar diagram showing mean values for general fish assemblage indices measured at each stream type. Indices include total abundance (N), total biomass (Biomass), total biomass per unit area sampled (Biomass<sub>Area</sub>, g m<sup>-2</sup>), total abundance per unit area sampled (N<sub>Area</sub>, ind m<sup>-2</sup>), and species Richness per unit area sampled (Richness<sub>Area</sub>, spp. m<sup>-2</sup>).

A one-way ANOVA was performed to test all three fish biodiversity indices: Richness, Richness<sub>Area</sub>, and  $H'$ . All tests showed significant differences among stream types (Table 3). Tukey's post-hoc tests showed that fish biodiversity differed among stream types with RSCs having lower Richness than HSS streams, and lower Richness<sub>Area</sub> and  $H'$  than both high-quality (HSS and HSW) reference streams (Figure 10). There were no differences between RSC and the low quality LSS reference streams for any biodiversity metrics ( $p > 0.05$ ).

Table 3. ANOVA table for fish biodiversity metrics, including species richness (Richness), species richness per unit area (spp. m<sup>-2</sup>; Richness<sub>Area</sub>), and the Shannon-Wiener biodiversity index ( $H'$ ). ANOVA components (in addition to  $F$ -statistic and associated  $p$ -value) include degrees of freedom ( $df$ ), sum of squares (SS), and mean squares (MS).

Index	Component	$df$	SS	MS	$F$	$p$
Richness	Model	3	360.4	120.2	8.17	0.0004
	Residuals	31	455.7	14.7		
Richness <sub>Area</sub>	Model	3	0.009	0.003	7.106	0.0009
	Residuals	31	0.013	0.0004		
$H'$	Model	3	12.94	4.31	15.48	<0.0001
	Residuals	31	8.64	0.28		

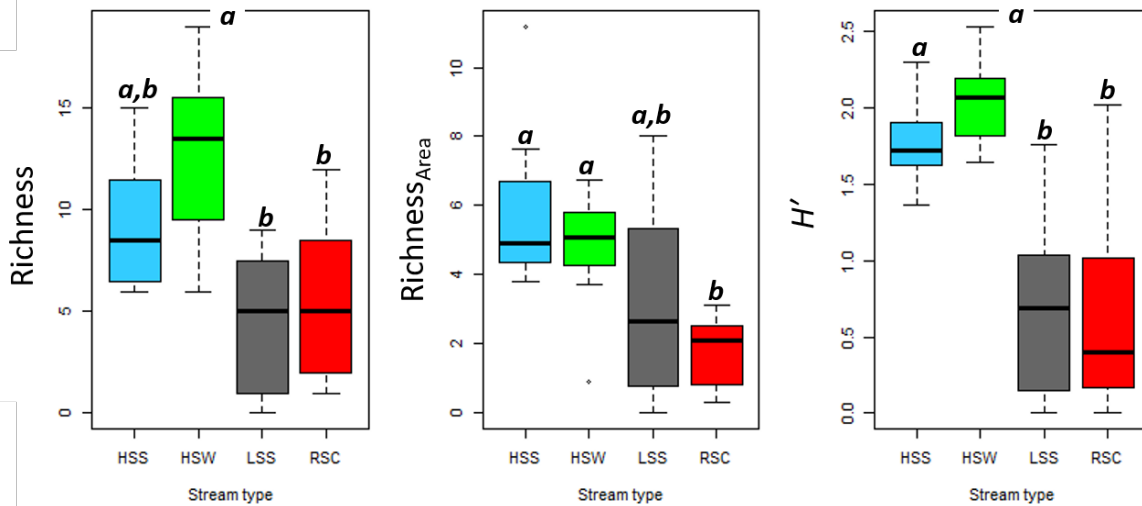


Figure 10. Boxplot of fish biodiversity indices by stream type. Different letters above groups indicate significant differences based on Tukey's post-hoc HSD.

Species richness and the within-stream type variability (coefficient of variability, CV) measured during this study were compared with the MBSS data used to develop the conceptual model (Figure 11). Both means and CV show good agreement in terms of consistent rank-order of reference streams between the two datasets. While RSC data were only available for the current study, RSC fish Richness mean and variability was similar to LSS reference streams.

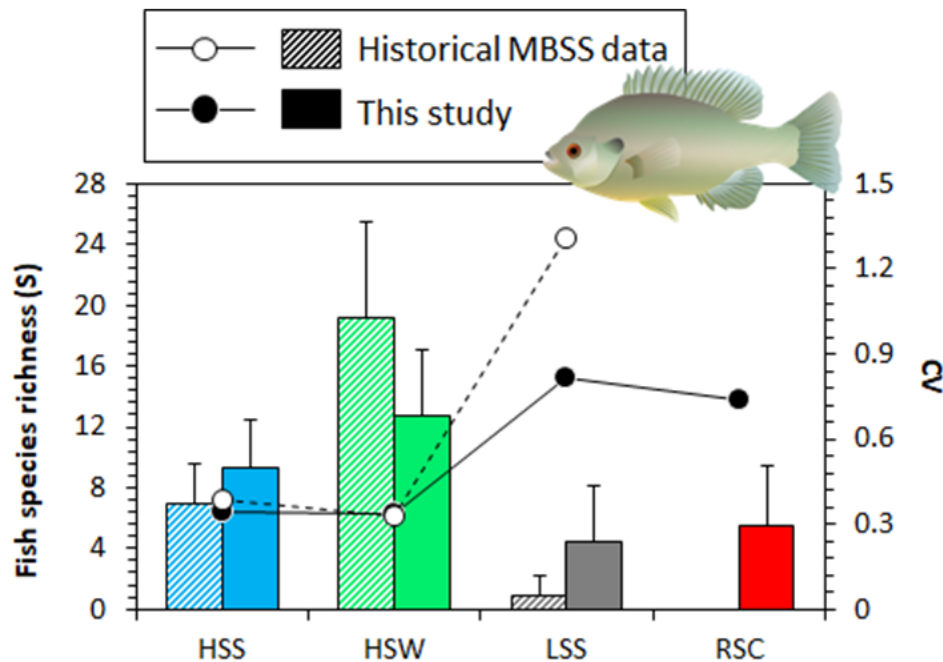


Figure 11. Comparison of fish species Richness (primary y-axis) measured during the current study (solid shaded bars) and that calculated during development of the conceptual model for reference stream types (diagonal filled bars). Error bars = 1 standard deviation. Coefficient of variation (CV) for the current study (filled symbols) and the historical MBSS data (empty symbols) is shown (secondary y-axis).

Fish multivariate assemblage composition showed a pattern across stream types that was similar to the pattern identified for fish biodiversity (Figure 12). The stress for the 2-dimensional plot was high ( $> 0.2$ ), indicating that two dimensions are likely insufficient to represent the relationship among sites in multivariate space and that representative ordination requires additional axes (Figure 12). Stream type described significant patterns in fish assemblage composition (1-way ANOSIM, Global  $R = 0.20$ ,  $p = 0.003$ ). Permutation-based pairwise comparisons (Table 4) showed no statistical difference between RSC and LSS assemblage composition ( $p = 0.06$ ) or HSW and HSS assemblage composition ( $p = 0.19$ ). Both RSC and LSS assemblages were significantly different from HSW and HSS assemblages ( $p \leq 0.02$ ).

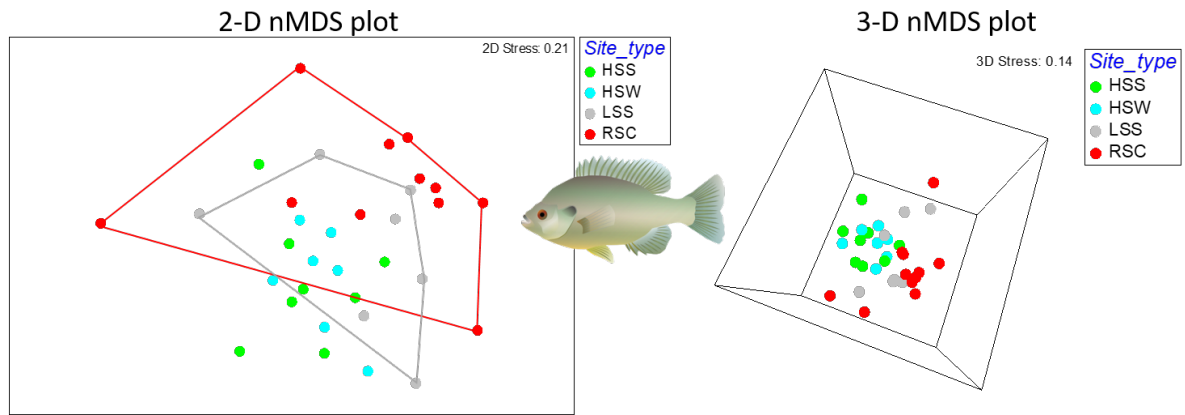


Figure 12. Non-metric multidimensional scaling plot of fish community data across stream types in 2-dimensions (left) and 3-dimensions (right). Convex hulls (solid lines) show the location of RSC (red) and LSS (grey) sites in 2-dimensional nMDS space.

Table 4. Permutation-based pairwise comparison tests of multivariate fish assemblage composition between stream types based on a Bray-Curtis dissimilarity matrix.

Groups	R	P	Possible permutations	Number $\geq$ observed
HSS vs HSW	0.07	0.19	6,435	186
<b>HSS vs LSS</b>	<b>0.25</b>	<b>0.02</b>	<b>3,003</b>	<b>20</b>
<b>HSS vs RSC</b>	<b>0.35</b>	<b>0.002</b>	<b>75,582</b>	<b>1</b>
<b>HSW vs LSS</b>	<b>0.36</b>	<b>0.006</b>	<b>3,003</b>	<b>5</b>
<b>HSW vs RSC</b>	<b>0.26</b>	<b>0.002</b>	<b>75,582</b>	<b>1</b>
LSS vs RSC	-0.04	0.06	12,376	621

\*global test significant (see text), pairwise comparisons significant have been bolded in table

A SIMPER analysis of the species responsible for similarity and dissimilarity of fish assemblage composition within and between stream types identified several species that occurred across all stream types and others that were unique to one or two types (Table 5). American eel (*Anguilla rostrata*) and eastern mudminnow (*Umbra pygmaea*) were common to all stream types, including RSCs and reference streams. No species uniquely contributed substantially to RSC assemblage similarity, but a number of species were shared among HSS and HSW stream assemblages, including



creek chubsucker (*Erimyzon oblongus*), fallfish (*Semotilus corporalis*), least brook lamprey (*Lampetra aepyptera*), and tessellated darter (*Etheostoma olmstedii*), among others.

*Table 5. Fish species responsible for similarity within stream types identified using SIMPER. Cells are species average square-root abundance for each stream type and, in parentheses, is the percentage that each species contributes to the average Bray-Curtis similarity of sites within each stream type. Only species contributing 90% of the cumulative similarity within sites are shown.*

<b>Species</b>	<b>HSS</b>	<b>HSW</b>	<b>LSS</b>	<b>RSC</b>
American eel	2.91 (7.04)	2.99 (7.56)	2.04 (3.43)	1.56 (8.23)
Banded killifish				
Blacknose dace	3.35 (6.90)		2.04 (1.47)	
Brown bullhead				
Creek chubsucker	2.13 (0.81)	1.21 (1.69)		
Eastern mosquitofish		2.43 (4.47)	3.71 (5.39)	5.78 (8.95)
Eastern mudminnow	2.36 (4.48)	2.88 (4.57)	1.97 (3.36)	1.01 (4.00)
Fallfish	1.27 (1.05)	0.72 (0.73)		
Golden shiner				
Green sunfish		1.66 (1.31)		
Largemouth bass		0.73 (1.04)		
Least brook lamprey	2.37 (3.73)	1.60 (2.06)		
Margined madtom		0.79 (0.81)		
Pumpkinseed	1.79 (1.59)	1.21 (0.81)		
Tessellated darter	1.88 (2.90)	3.12 (6.46)		

Figure 13 shows the square-root transformed average species abundance for pairs of stream types that most contribute to Bray-Curtis dissimilarity of sites between each type pair identified as significantly different in ANOSIM (Table 5, above). Fish assemblages differed between high-quality (HSS and HSW) and both low-quality (LSS) and RSC streams. Several species were diagnostic of these differences, particularly sensitive species that were absent from RSCs and LSS sites (e.g., creek chubsucker, fallfish, madtoms, lampreys).

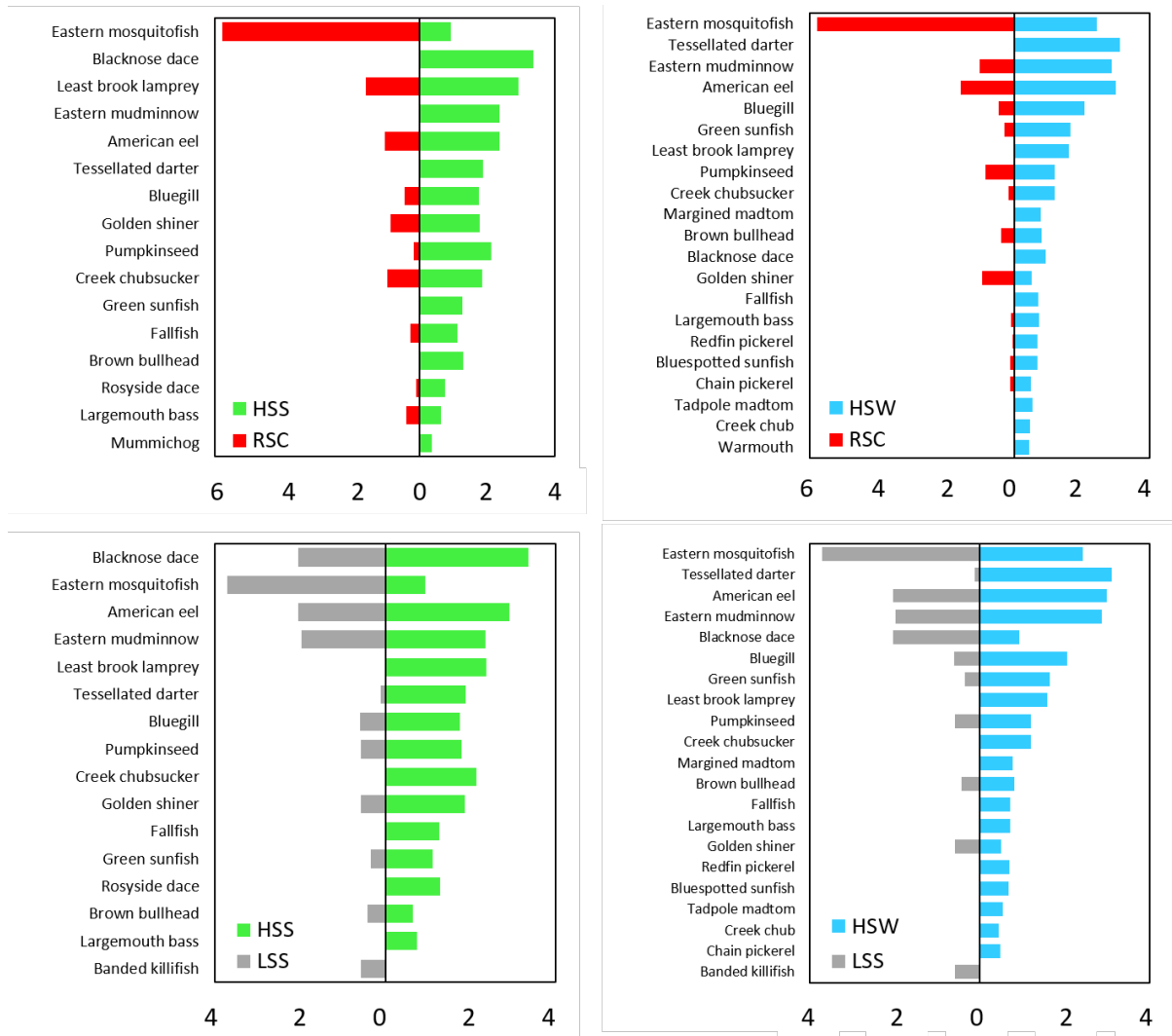


Figure 13. Species average square-root transformed abundance for pairs of stream type (upper: HSS vs RSC, HSW vs RSC; lower: HSS vs LSS, HSW vs LSS) for species that contribute to ~ 90% of the average Bray-Curtis dissimilarity of sites between each type pair. Figures are only shown for pairs of stream types identified as significantly different in ANOSIM (above).

#### 4.1.3 Fish Environment Modeling

Multiple linear regressions of fish biodiversity metrics were significantly related to water quality conditions (Table 6). Specific conductivity was a significant, negative predictor for both fish Richness and  $H'$ ; whereas DO was a positive predictor of fish  $H'$  (Table 7, Figure 14). There was equivocal evidence of a weak, positive relationship between DO and fish Richness<sub>Area</sub>. Water temperature was not significant in any of the regression models.

Table 6. Model results from multiple linear regression models relating fish biodiversity to environmental conditions. Fish indices include species richness (Richness), species richness per unit area ( $spp. m^{-2}$ ,  $Richness_{Area}$ ), and the Shannon-Wiener biodiversity index ( $H'$ ). Multiple regression components (in addition to  $F$ -statistic and associated  $p$ -value) include residual standard error (RSE), adjusted- $R^2$  ( $adj-R^2$ ), and numerator and denominator degrees of freedom ( $df_{num}$  and  $df_{den}$ , respectively).

Index	RSE	$adj-R^2$	$F$	$df_{num.}$	$df_{den.}$	$p$
Richness	4.42	0.19	3.59	3	31	0.03
$Richness_{Area}$	2.37	0.15	2.99	3	31	0.046
$H'$	0.66	0.31	6.01	3	31	0.002

Table 7. Model coefficients (including estimate and standard error [SE]) from multiple linear regression models relating fish biodiversity to environmental conditions. Predictor variables with  $p < 0.05$  are bolded, predictor variables with  $0.05 < p < 0.10$  are italicized.

Index	Coefficients	Estimate	SE	$t$	$p$
Richness	(Intercept)	11.60	7.67	1.51	0.14
	DO	0.22	0.42	0.52	0.61
	Temp	-0.09	0.29	-0.30	0.77
	<b>Cond</b>	<b>-0.018</b>	<b>0.006</b>	<b>-2.79</b>	<b>0.009</b>
Area richness	(Intercept)	3.17	4.11	0.77	0.45
	<i>DO</i>	<i>0.44</i>	<i>0.23</i>	<i>1.93</i>	<i>0.06</i>
	Temp	-0.09	0.16	-0.57	0.58
	Cond	-0.003	0.003	-0.94	0.36
Shannon-Wiener diversity	(Intercept)	0.90	1.15	0.78	0.44
	<b>DO</b>	<b>0.13</b>	<b>0.06</b>	<b>2.09</b>	<b>0.05</b>
	Temp	-0.001	0.04	-0.03	0.98
	<b>Cond</b>	<b>-0.003</b>	<b>0.0009</b>	<b>-2.77</b>	<b>0.009</b>

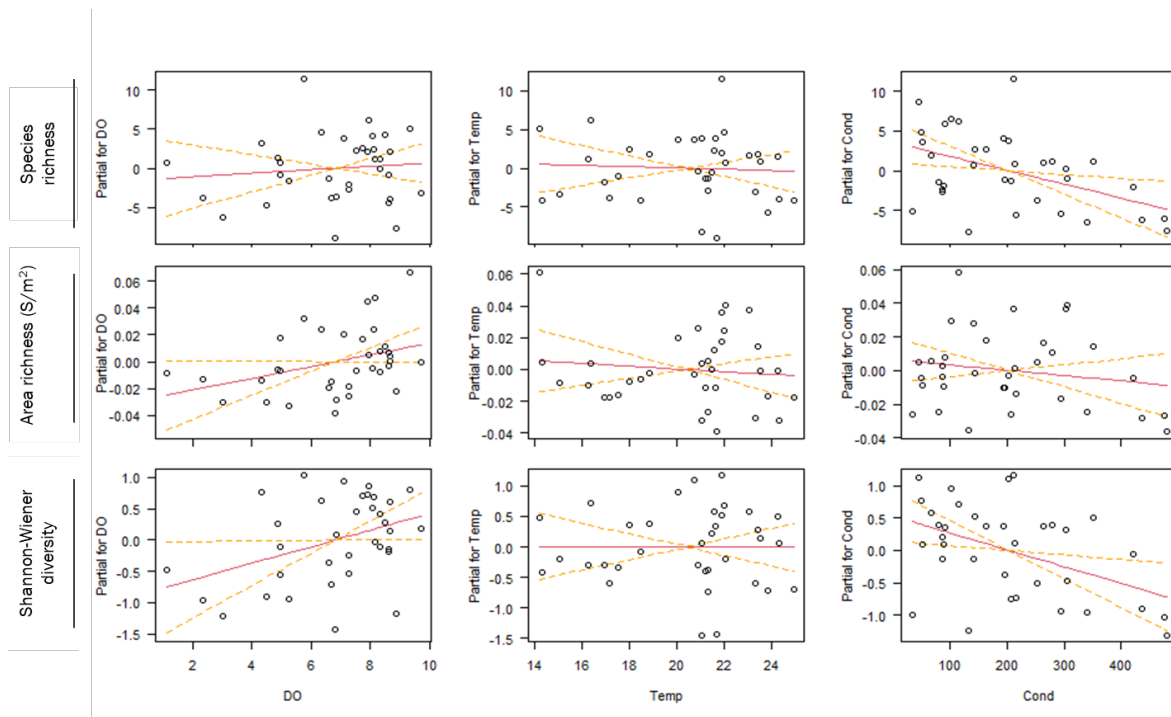


Figure 14. Partial residual plot matrix from multiple linear regression models relating fish biodiversity to environmental conditions. Plot matrix is designed as follows: upper row – Richness, middle row – Richness<sub>Area</sub>, bottom row – Shannon-Wiener diversity; first column – dissolved oxygen concentration (DO), second column – water temperature (Temp), third column – specific conductivity (Cond). Red lines = fitted partial regression, dashed yellow lines = 95% confidence intervals.

#### 4.1.4 Fish Assemblage Changes with Time Since Construction

The following five measures of fish assemblages in RSCs were compared across the three most consistent bins of time since construction (Recovery at Age 1=1.9-3.4 years, Age 2=5.3-7.4, and Age 3=8.9-16.2) to quantify how fish assemblages are changing with maturation of RSC stream restoration (Figure 16).

- Fish abundance
- Fish biomass
- Species richness
- Area richness
- Shannon-Wiener diversity

Analysis of variance found no relationship between fish assemblage indices and the amount of time elapsed since RSC construction ( $p > 0.05$ ). Despite the absence of statistically significant patterns, there was a qualitative suggestion of an increase in mean total abundance through time; however, variance also increased with time elapsed since construction (Figure 15). There was no obvious evidence of a recovery trajectory for RSCs based on the examined fish assemblage indices.

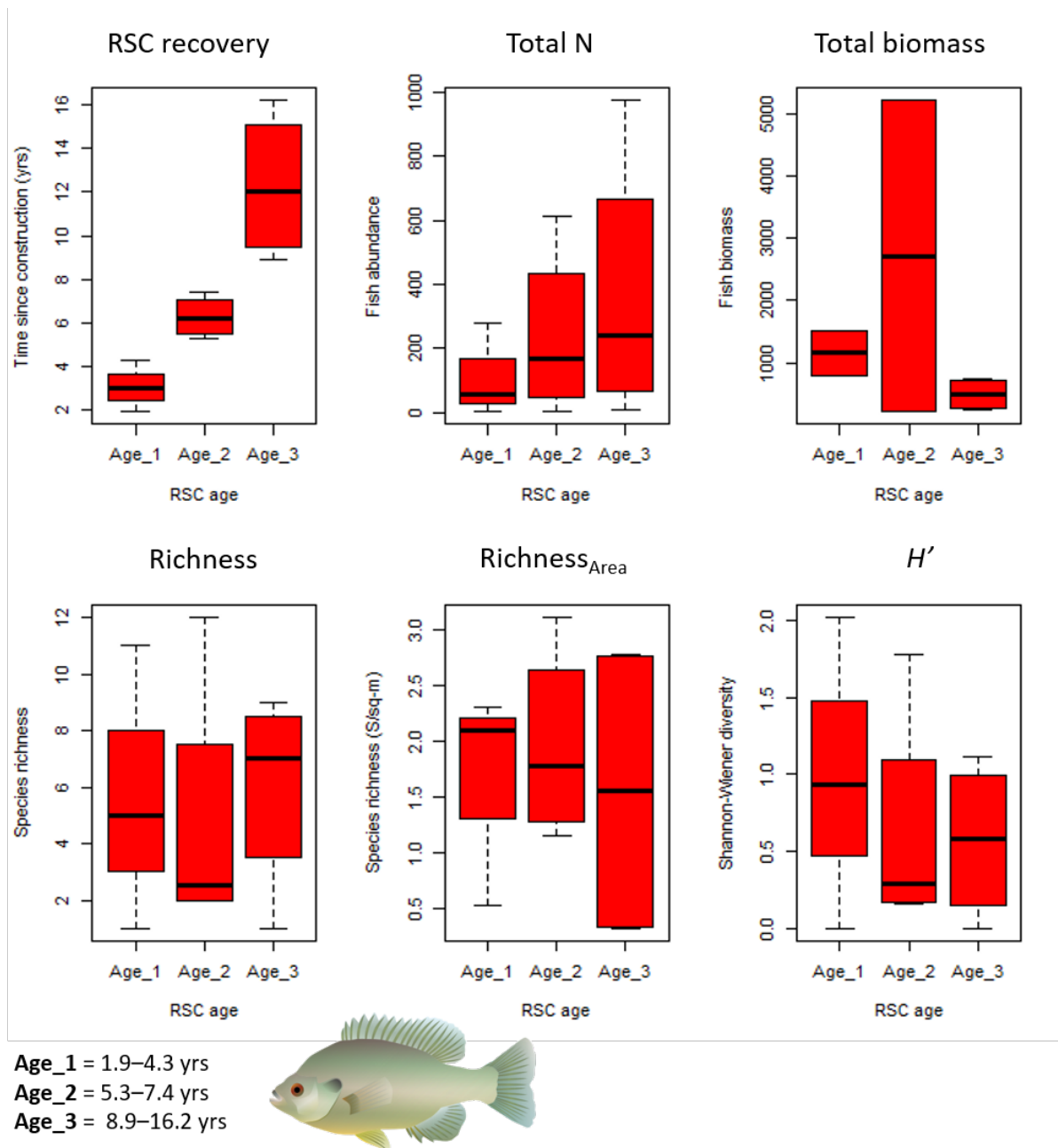


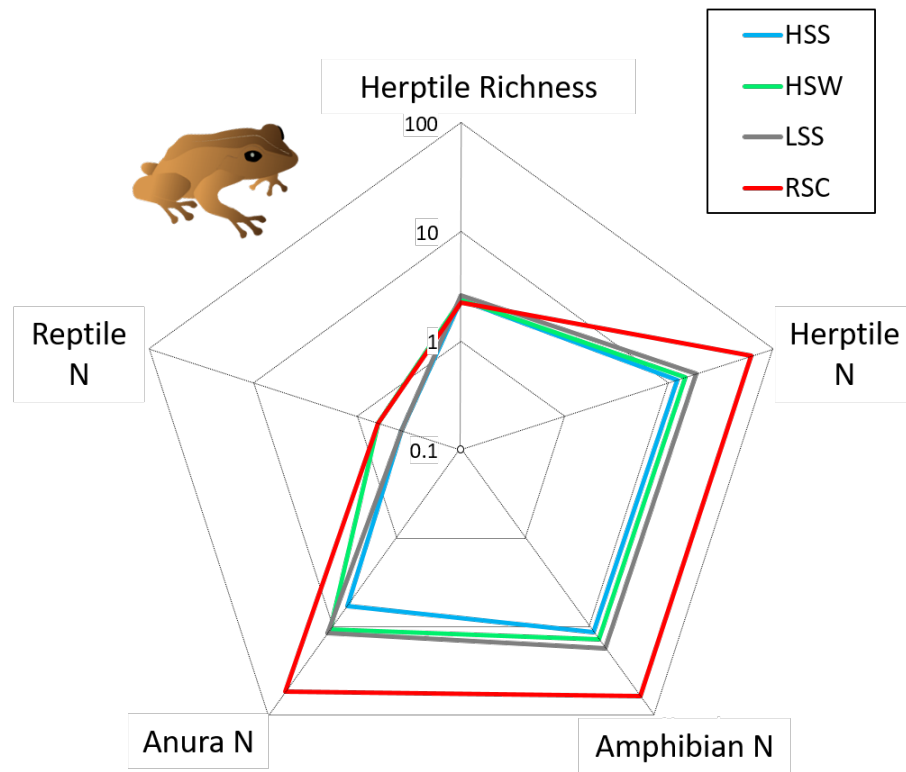
Figure 15. Age of RSC construction projects and fish assemblage indices measured at RSCs grouped by amount of time elapsed since construction. Indices include total fish abundance ( $N$ ), total fish biomass, species richness, species richness per unit area sampled ( $\text{Richness}_{\text{Area}}$ ,  $\text{spp. m}^{-2}$ ), and Shannon-Wiener diversity index ( $H'$ ).

## 4.2 Amphibians and Reptiles (Herpetofauna)

### 4.2.1 Herpetofauna Diversity and Composition

Herpetofauna assemblages in RSCs were typically more abundant (total abundance) than reference stream types (Figure 16). This was particularly true of the Anurans (frogs and toads), which resulted

in the greater abundance estimates for higher taxonomic groupings (i.e., amphibians, herptiles). Total abundance of reptiles was also high at RSC sites relative to LSS and HSS reference streams, but was similar to reptile abundance at HSW sites. Total abundance of reptiles but were not more similar to the LSS reference streams than either the HSS or HSW reference streams. Overall taxonomic richness of herpetofauna was low-to-intermediate at RSC sites relative to reference streams.



*Figure 16. Radar diagram showing mean values for general herpetofauna assemblage indices measured at each stream type. Indices include total taxonomic richness, total herptile abundance (N), total amphibian abundance, total anuran abundance, and total reptile abundance.*

Patterns in abundance were further explored with ANOVA to test for differences among stream types for abundance-based indices for reptiles, amphibians, and for a single species taxon (bullfrogs; Table 8). A significant difference across stream types was identified for amphibians with RSCs having a higher abundance than HSS reference streams (Figure 17). There was no evidence of differences in reptile or bullfrog abundance across stream types.



Table 8. ANOVA table for herpetofauna abundance indices, including total abundance of amphibians, reptiles, and bullfrogs. ANOVA components (in addition to  $F$ -statistic and associated  $p$ -value) include degrees of freedom ( $df$ ), sum of squares ( $SS$ ), and mean squares ( $MS$ ).

Index	Component	$df$	$SS$	$MS$	$F$	$p$
Amphibian	Model	3	9,113	3037.5	3.43	0.03
	Residuals	31	27,425	884.7		
Reptile	Model	3	0.333	0.11	0.14	0.93
	Residuals	31	24.35	0.79		
Bullfrog	Model	3	3.71	1.24	0.14	0.93
	Residuals	31	267.26	8.62		

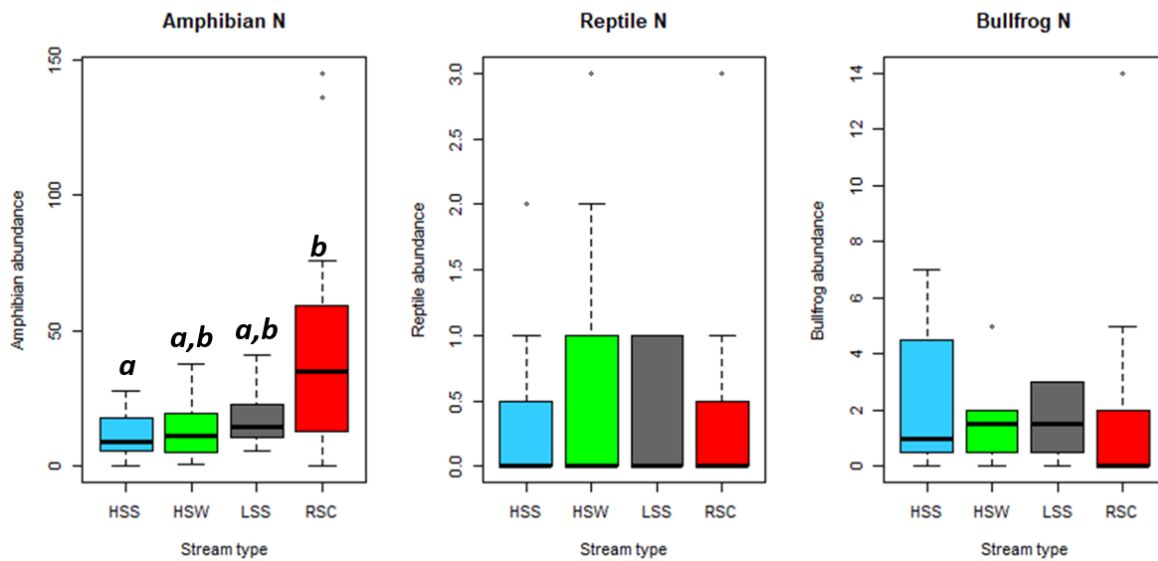


Figure 17. Boxplot of herpetofauna abundance indices by stream type. Different letters above groups indicate significant differences based on Tukey's post-hoc HSD.

A one-way ANOVA was performed to test all three herpetofauna biodiversity indices: Richness, Richness<sub>Area</sub>, and  $H'$ . Significant differences in herpetofauna Richness<sub>Area</sub> was observed among stream types (Table 9). Tukey's post-hoc tests showed that RSCs had the lowest median Richness<sub>Area</sub> among stream types although the only pairwise comparison that was significant at  $p < 0.05$  was between RSC and LSS streams (Figure 18). There was no relationship identified between stream type and either the Richness or Richness<sub>Area</sub> herpetofauna biodiversity metrics ( $p > 0.05$ ).

Table 9. ANOVA table for herpetofauna biodiversity metrics, including taxonomic richness (Richness), taxonomic richness per unit area (taxa  $m^{-2}$ , Richness<sub>Area</sub>), and the Shannon-Wiener biodiversity index ( $H'$ ). ANOVA components (in addition to  $F$ -statistic and associated  $p$ -value) include degrees of freedom ( $df$ ), sum of squares ( $SS$ ), and mean squares ( $MS$ ).

Index	Component	$df$	$SS$	$MS$	$F$	$p$
Richness	Model	3	3.42	1.14	0.60	0.62
	Residuals	31	59.26	1.91		
Richness <sub>Area</sub>	Model	3	10.57	3.52	3.87	0.018
	Residuals	31	28.2	0.91		
$H'$	Model	3	0.80	0.27	1.04	0.39
	Residuals	28	7.17	0.26		

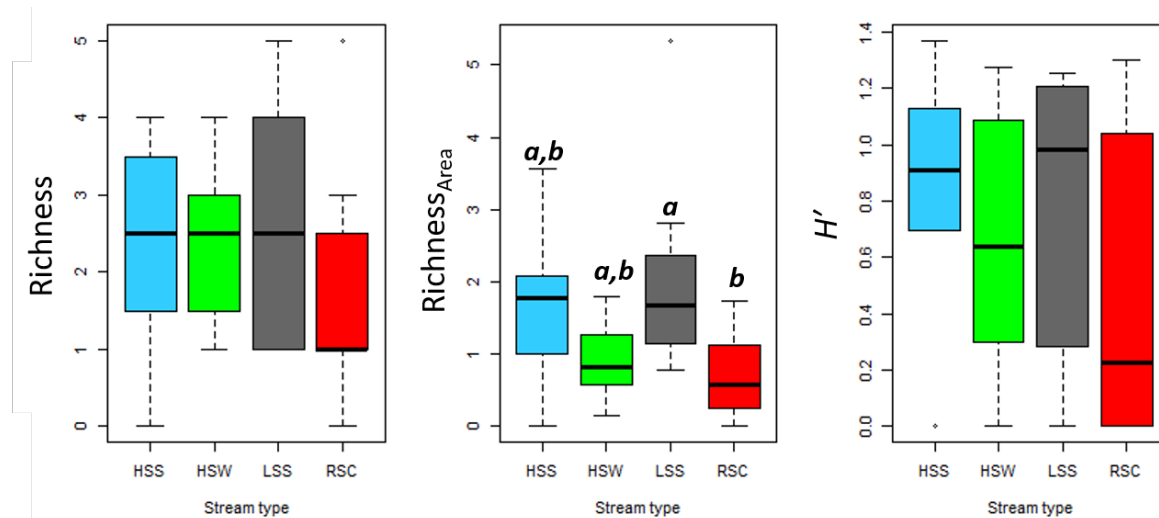


Figure 18. Boxplot of herpetofauna biodiversity indices by stream type. Different letters above groups indicate significant differences based on Tukey's post-hoc HSD.

Herpetofauna taxonomic Richness and the within-stream type variability (coefficient of variability, CV) measured during this study were compared with the MBSS data used to develop the conceptual model (Figure 19). In contrast to the historical MBSS data used to develop the conceptual model for this project, 2020 sampling mean herpetofauna taxonomic richness was relatively invariant across stream types during the current study. Similarly, variability in Richness (as quantified using CV) did not show close agreement between the historical and current datasets. While RSC data were only available for the current study, mean RSC herpetofauna Richness was qualitatively lower while variability was somewhat higher than that of reference stream types.

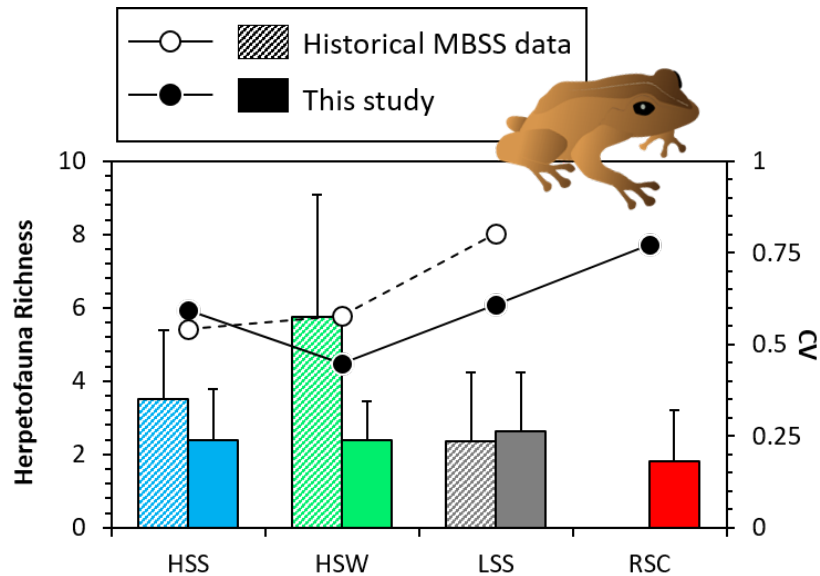


Figure 19. Comparison of herpetofauna taxonomic Richness (primary y-axis) measured during the current study (solid shaded bars) and that calculated during development of the conceptual model for reference stream types (diagonal filled bars). Error bars = 1 standard deviation. Coefficient of variation (CV) for the current study (filled symbols) and the historical MBSS data (empty symbols) is shown (secondary y-axis).

Herpetofauna multivariate assemblage composition showed no discernible pattern across stream types (Figure 20). The stress for the 2-dimensional plot was sufficiently low ( $= 0.12$ ) to suggest that it was a robust representation of the dissimilarity among sites in multivariate space. One-way ANOSIM corroborated the visual assessment, finding no evidence of a statistical difference in multivariate herpetofauna assemblage composition among stream types (Global  $R = -0.02$ ,  $p = 0.64$ ).

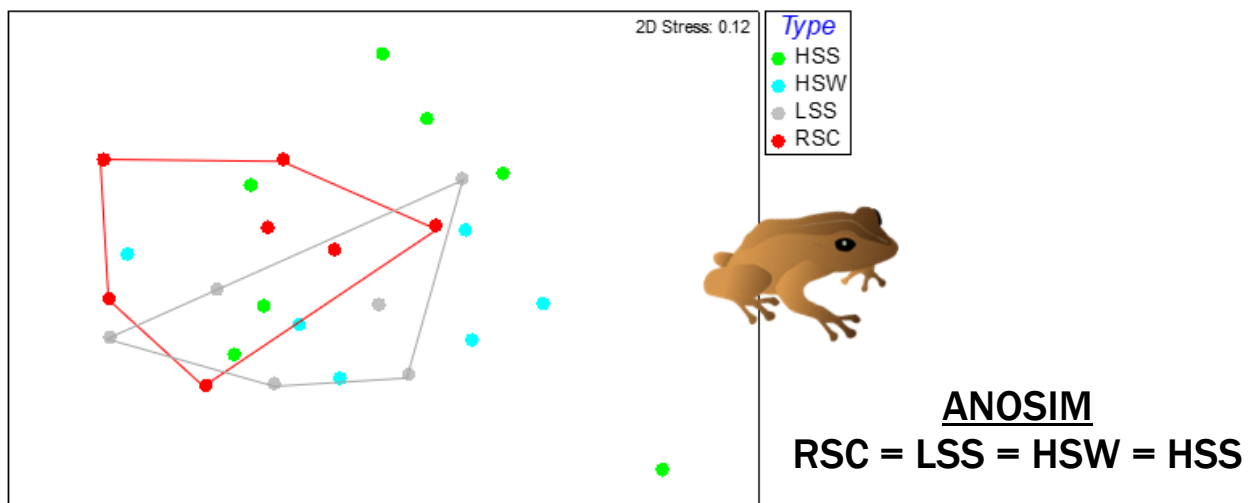


Figure 20. Non-metric multidimensional scaling plot of herpetofauna community assemblage data across stream types in 2-dimensions. Convex hulls (solid lines) show the location of RSC (red) and LSS (gray) sites in 2-dimensional nMDS space. Inset results from analysis of similarity (ANOSIM) summarize absence of significant differences between stream type differences.

Northern green frog was among the mostly frequently encountered herpetofauna taxon across all reference types. American bullfrogs were frequently encountered at LSS and HSW reference types, but was not commonly encountered at the HSS sites. Several turtle and frog species, and two salamanders (northern two-lined salamander *Eurycea bislineata*, northern red salamander *Pseudotriton ruber*) were individually diagnostic of HSS or HSW sites but not both.

#### 4.2.2 Herpetofauna environment modeling

Multiple linear regressions of herpetofauna biodiversity metrics in response to water quality conditions did not identify significant biodiversity-environment relationships (Table 10 and Table 11). Multiple linear regression was also used to assess relationships between herpetofauna abundance indices and water quality conditions. The model relating amphibian abundance to water quality was significant with an adjusted- $R^2$  of 0.53 ( $F = 13.73$ ,  $p = 0.0001$ ). Within the model, DO was negatively related to amphibian abundance (Table 12, Figure 21). There was no relationship observed between amphibian abundance and water temperature or specific conductivity across the range measured. Neither of the other abundance index models (reptile abundance, bullfrog abundance) was significantly related to water quality conditions.

Table 10. Model results from multiple linear regression models relating herpetofauna biodiversity to environmental conditions. Herpetofauna indices include taxonomic richness (Richness), taxonomic richness per unit area (taxa  $m^{-2}$ ,  $Richness_{Area}$ ), and the Shannon-Wiener biodiversity index ( $H'$ ). Multiple regression components (in addition to  $F$ -statistic and associated  $p$ -value) include residual standard error (RSE), adjusted- $R^2$  (adj- $R^2$ ), and numerator and denominator degrees of freedom ( $df_{num}$  and  $df_{den}$ , respectively).

Index	RSE	adj- $R^2$	$F$	$df_{num}$	$df_{den}$	$p$
Richness	1.36	<0.01	1.01	3	31	0.40
$Richness_{Area}$	1.08	-0.02	0.72	3	31	0.54
$H'$	0.49	0.08	1.85	3	28	0.16

Table 11. Model results from multiple linear regression models relating herpetofauna abundance indices to environmental conditions. Multiple regression components (in addition to  $F$ -statistic and associated  $p$ -value) include residual standard error (RSE), adjusted- $R^2$  (adj- $R^2$ ), and numerator and denominator degrees of freedom ( $df_{num}$  and  $df_{den}$ , respectively).

Abundance index	RSE	adj- $R^2$	$F$	$df_{num}$	$df_{den}$	$p$
<b>Amphibian</b>	<b>22.5</b>	<b>0.53</b>	<b>13.73</b>	<b>3</b>	<b>31</b>	<b>0.00001</b>
Reptile	0.84	0.01	1.10	3	31	0.36
Bullfrog	2.88	-0.04	0.57	3	31	0.64

Table 12. Model coefficients (including estimate and standard error [SE]) from multiple linear regression models relating amphibian abundance to environmental conditions. Predictor variables with  $p < 0.05$  are bolded.

Abundance index	Coefficients	Estimate	SE	<i>t</i>	<i>p</i>
Amphibian	<b>(Intercept)</b>	<b>162.04</b>	<b>39.03</b>	<b>4.15</b>	<b>0.0002</b>
	<b>DO</b>	<b>-13.41</b>	<b>2.15</b>	<b>-6.25</b>	<b>&lt;0.00001</b>
	Temp	-1.90	1.48	-1.28	0.21
	Cond	-0.03	0.03	-0.97	0.34

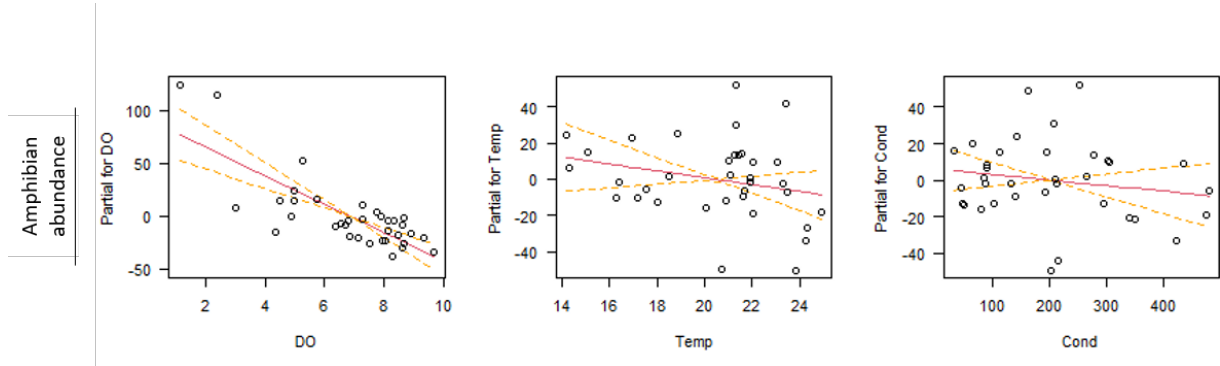


Figure 21. Partial residual plots from multiple linear regression models relating amphibian abundance to environmental conditions. Plot panels show partial residuals for three predictors: first column – dissolved oxygen concentration (DO), second column – water temperature (Temp), third column – specific conductivity (Cond). Red lines = fitted partial regression, dashed yellow lines = 95% confidence intervals.

#### 4.2.3 Herpetofauna Assemblage Changes with Time Since Construction

The following five measures of herpetofauna assemblages in RSCs were compared across three bins of time (Recovery at Age 1=1.9-3.4 years, Age 2=5.3-7.4, and Age 3=8.9-16.2) since construction to quantify how herpetofauna assemblages are changing with maturation of RSC stream restoration.

- Amphibian abundance
- Herpetofauna abundance
- Herpetofauna species richness
- Herpetofauna area richness
- Herpetofauna Shannon-Wiener diversity

Analysis of variance found no relationship between herpetofauna abundance or biodiversity indices and the amount of time elapsed since RSC construction ( $p > 0.05$ ). Despite the absence of statistically significant patterns, there were several qualitative patterns that suggest an increase in amphibian abundance, Richness, and  $H'$  over time. None of these variables were statistically different across RSC recovery intervals, but the consistency of the pattern is suggestive of an overall trend related to the age of RSC sites (Figure 22).

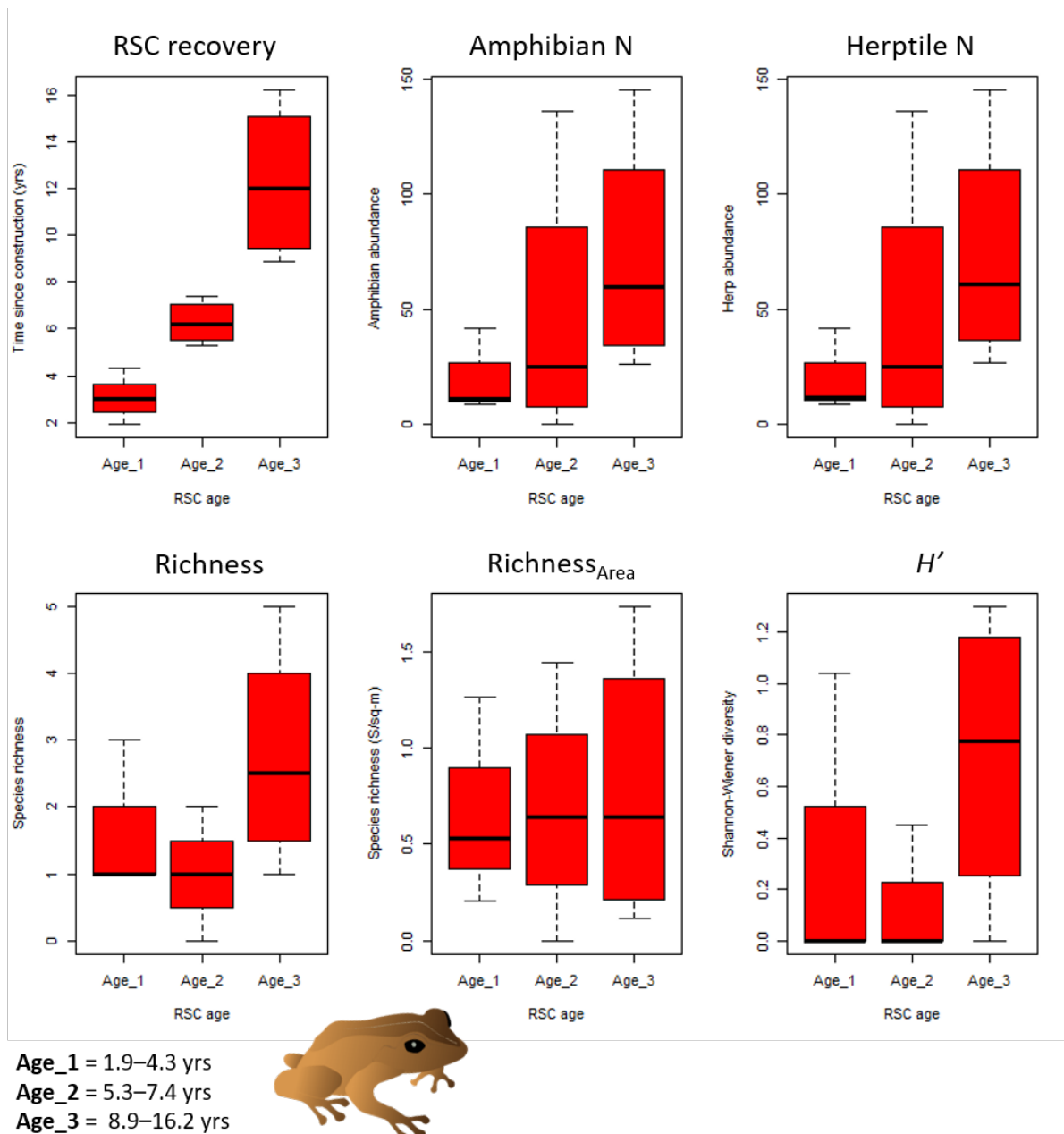


Figure 22. Age of RSC construction projects and herpetofauna assemblage indices measured at RSCs grouped by amount of time elapsed since construction. Indices include total amphibian abundance (N), total herpetofauna abundance (Herptile N), taxonomic richness, taxonomic richness per unit area sampled ( $Richness_{Area}$ , taxa  $m^{-2}$ ), and Shannon-Wiener diversity index ( $H'$ ).

To further explore patterns of amphibian abundance over time at RSC sites, a locally weighted (loess) regression was used to fit a smoothed curve to amphibian total abundance as a function of time elapsed since RSC construction (Figure 23). Amphibian abundance shows an increase at approximately 9 years post-construction, as evidenced by the lower 95% confidence interval becoming positive at that point. The number of RSC sites with post-construction periods  $> 10$  years is low ( $n = 2$ ) and the 95% confidence intervals increase in size to include 0 at the oldest range of the modeled RSC projects.



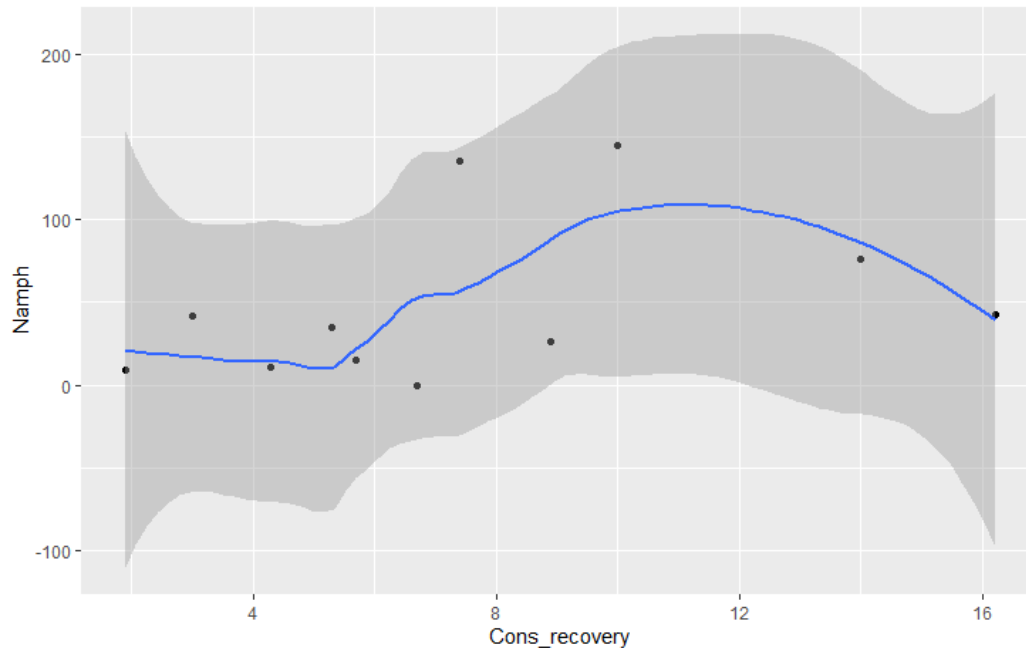


Figure 23. Loess smooth plot of amphibian total abundance in RSCs versus years of recovery post construction. Blue line – fitted loess curve, shaded grey area – 95% confidence interval.

### 4.3 Habitat

Figures 24a and 24b summarize the values measured at the RSCs and three reference stream types for the following 12 habitat elements:

- Maximum depth of the stream channel
- Shading (densiometer reading) over the stream
- Thalweg depth of the stream channel
- Width of the stream channel
- Current velocity of stream flow
- Woody debris in the stream
- Buffer width along the stream
- Cobble in the stream bed
- Gravel in the stream bed
- Sand in the stream bed
- Silt in the stream bed
- Rootwads in the stream channel

For most of these habitat elements, the stream types are not statistically different. Noteworthy differences are as follows:

- Maximum depth (likely pools) and stream width are greater in HSWs and RSCs, as is expected for stream wetland complexes
- Current velocity is slower for RSCs than HSWs and other stream types
- High-quality stream types, HSSs and HSWs, have maximum buffers (50m or greater) while the LSSs and RSCs have variable buffer widths

- Stream bed materials are similar among stream types, except for the absence of cobbles in the high-quality HSS and HSW

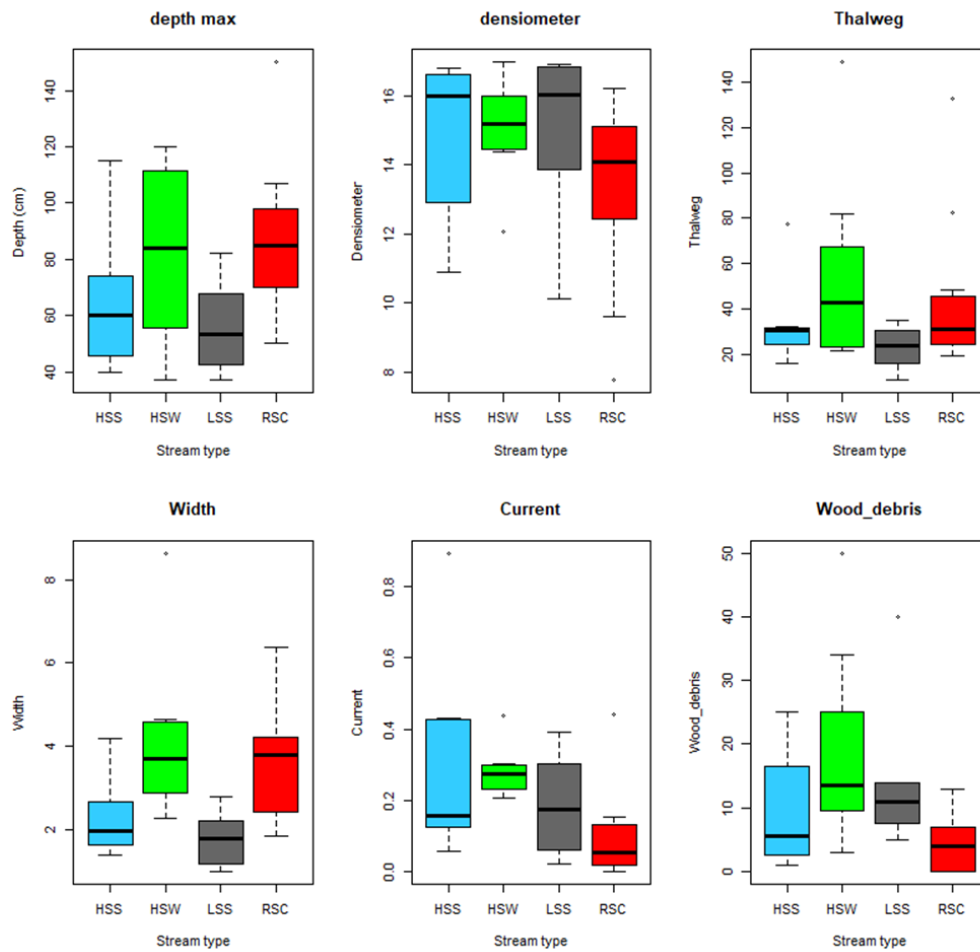


Figure 24a. Boxplots of habitat elements by stream type. Different letters above groups indicate significant differences based on Tukey's post-hoc HSD.

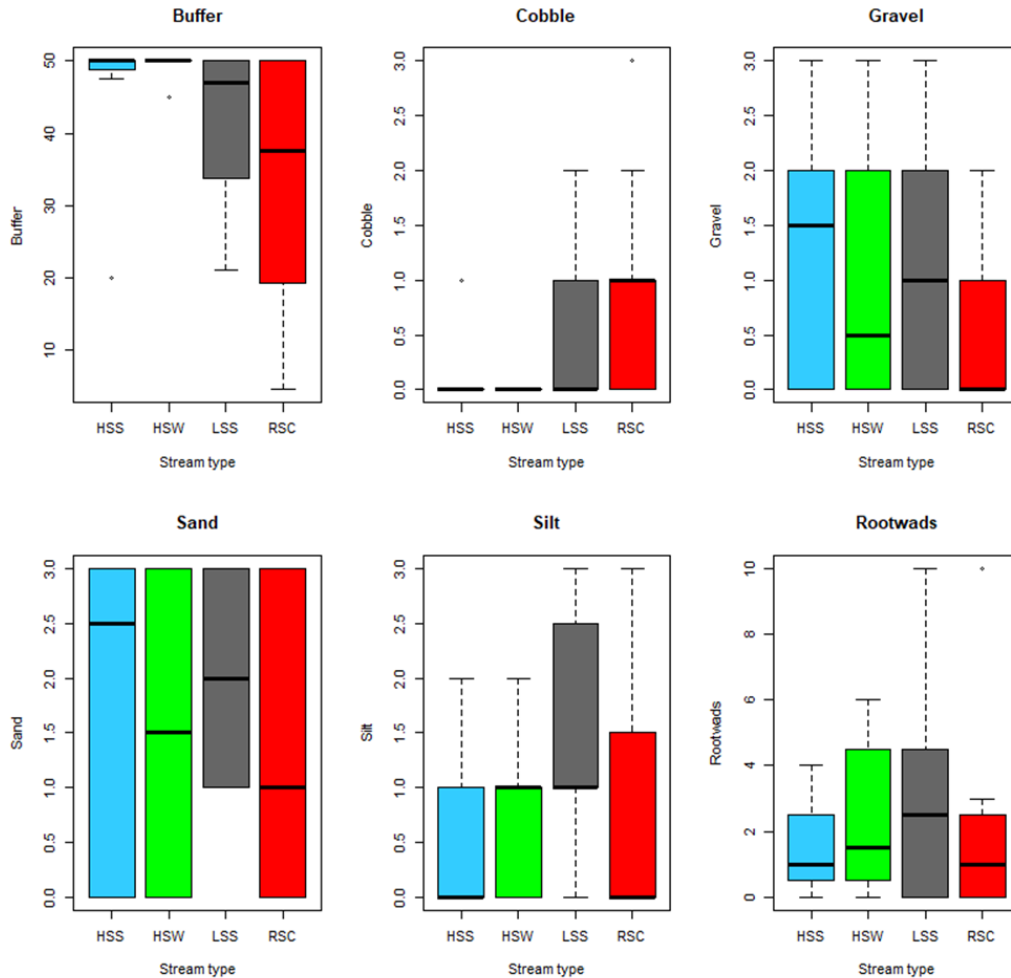


Figure 24b. Boxplots of habitat elements by stream type. Different letters above groups indicate significant differences based on Tukey's post-hoc HSD.

#### 4.4 Water Quality and Stream Metabolism

##### 4.4.1 Dissolved Oxygen and Temperature

Median DO concentrations during the long-term monitoring periods ranged between about 4 mg/L to 10 mg/L, with median concentrations around 7 mg/L (Figure 25). Concentrations were consistently lower in RSC sites than in other sites, including low-quality single channels (LSS). Except for Wilelinor (RSC-8), which is the second oldest RSC in the study, DO concentrations in RSCs frequently reached levels below 2 mg/L. The DO concentrations recorded in early fall were generally higher than those recorded in late summer, but hypoxic and anoxic episodes in RSC sites persisted.

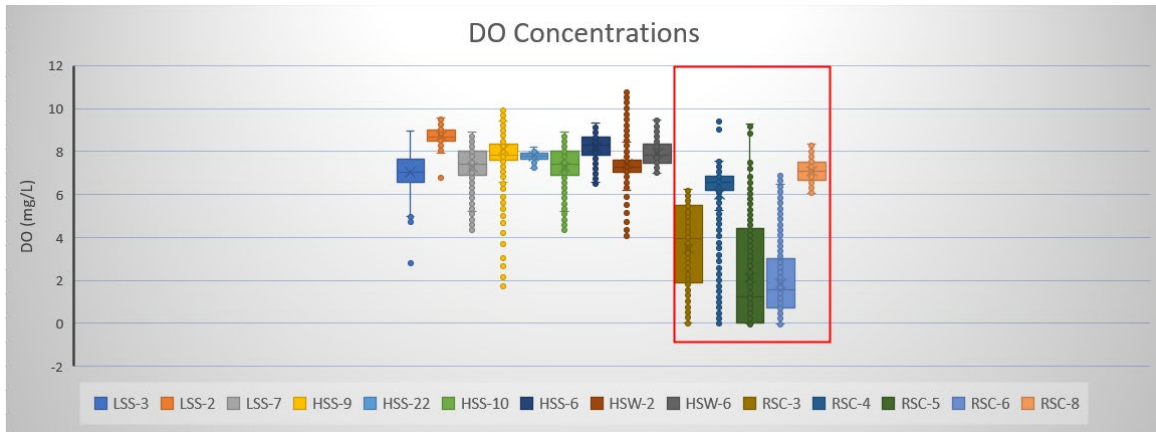


Figure 25. Long-term dissolved oxygen concentrations in streams monitored for 2 to 3 weeks in late summer and early fall 2020. RSC-4, RSC-5 and RSC-6 were monitored in late summer, and RSC-3 and RSC-8 were monitored in early fall.

The long-term median temperatures in the study streams varied between about 15°C and 24°C, with higher values in late summer and lower values in early fall. Therefore, differences in temperature among stream types were more evident from instantaneous measurements obtained during the fish sampling campaigns. Figure 26 shows that median temperatures in the different stream types ranged from about 14°C to 25°C, and that HSS, HSW, and RSC streams had the highest temperature variability. LSS had the lowest variability but also the highest median temperature.

Temperature of surface water influences the concentration of dissolved oxygen by controlling the solubility of oxygen. Therefore, as temperature increases, the solubility of DO decreases, meaning that the same volume of water holds less DO when it is warm. This helps explain why HSS streams, which had the lowest medium temperatures, also had the highest DO concentrations while RSCs had the lowest (Figure 26). Slower flow velocities probably also contributed to the lower DO concentrations observed in RSCs, since reaeration becomes limited (Dodds 2006).

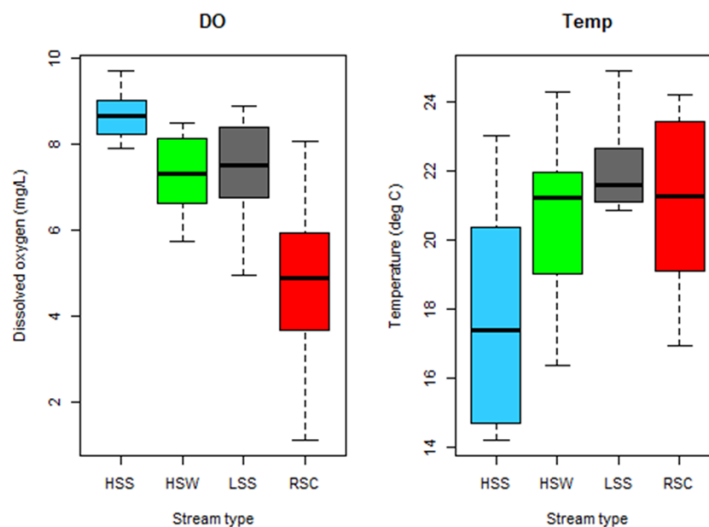


Figure 26. Instantaneous dissolved oxygen concentrations and temperature measured immediately prior to fish sampling in the streams monitored in late summer and early fall 2020.

#### 4.4.2 Stream Metabolism

The daily rates of Gross Primary Production (GPP), Ecosystem Respiration (ER) and Net Ecosystem Production (NEP) in the 19 streams were calculated using the long-term DO and temperature data measured in RSCs and references streams in late summer/early fall of 2020, or spring of 2021. Average values are shown in Table 13.

Average GPP rates ranged from 0.13 to 2.43 mg O<sub>2</sub> m<sup>-2</sup> d<sup>-1</sup> across sites, with the lowest rates observed in reference sites and the highest in RSCs. Ecosystem respiration rates were considerably higher than GPP rates in all streams, resulting in negative NEP values. Negative NEP means that the system is consuming more carbon than producing, i.e., all streams are heterotrophic.

*Table 13. Daily Rates of Gross Primary Production (GPP), Ecosystem Respiration (ER), and Net Ecosystem Production (NEP) per Unit Area (g O<sub>2</sub> m<sup>2</sup> d<sup>-1</sup>).*

<b>Streams</b>	<b>GPP</b> (mg O <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup> )	<b>ER</b> (mg O <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup> )	<b>NEP</b> (mg O <sub>2</sub> m <sup>-2</sup> d <sup>-1</sup> )	<b>Monitoring period</b>
LSS-2	0.32	-1.63	-1.31	late summer 2020
LSS-7	0.24	-2.19	-1.96	early fall 2020
LSS-3	0.13	-2.92	-2.78	early fall 2020
HSS-9	0.22	-2.51	-2.29	early fall 2020
HSS-10	0.31	-9.16	-8.85	early fall 2020
HSW-2	0.55	-1.76	-1.21	late summer 2020
HSS-22	0.18	-9.15	-8.97	late summer 2020
HSW-6	0.23	-3.25	-3.02	early fall 2020
HSW-18	0.31	-12.78	-12.47	early fall 2020
RSC-8	0.39	-4.92	-4.54	early fall 2020
RSC-3	0.19	-6.41	-6.22	early fall 2020
RSC-5	0.81	-13.70	-12.90	late summer 2020
RSC-4	0.30	-3.42	-3.12	late summer 2020
RSC-6	1.04	-17.0	-15.95	late summer 2020
RSC-2	2.43	-38.2	-35.8	spring 2021
LSS-8	0.60	-9.86	-9.26	spring 2021
HSW-3	0.49	-8.22	-7.73	spring 2021
RSC-5b	1.71	-27.1	-25.3	spring 2021
HSS-6	0.14	-2.83	-2.69	spring 2021

GPP rates were consistently higher in RSCs than in degraded and reference streams (Figure 27), supporting our hypothesis that the conversion of degraded streams into RSCs increases primary production. The exception was RSC-3, which had GPP rates similar to those observed in HSSs and LSSs. It is important to mention that RSC-3 is the only restored stream that was not converted into a stream-wetland complex.

All GPP rates were lower in the fall than in the other monitoring periods; hence, differences between RSCs and the other stream types were smaller during this monitoring period. Yet, excluding RSC-3, rates in RSCs were still significantly higher than in the other stream types.

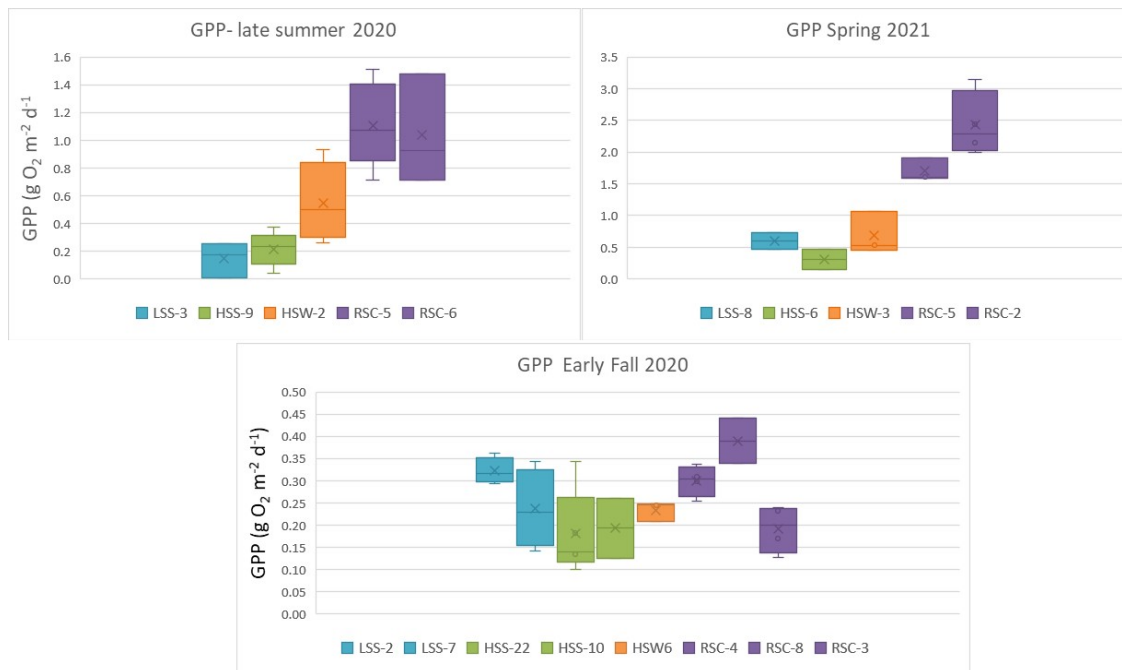


Figure 27. Gross primary production (GPP) rates calculated for the streams monitored for DO and temperature for long time periods between late summer 2020 and spring 2021.

GPP rates varied among RSCs as well, with averages ranging from 0.19 to 2.4 g O<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>. However, despite the relatively elevated GPP rates, ecosystem respiration rates in the RSCs were substantially higher (Figure 28), indicating that these systems still consume more carbon than produce, as it is typical of low-order streams, which are known for being hot spots for organic matter processing and retention (Roberts et al. 2007). It is also possible that grazing by fish is keeping GPP rates in RSCs low relative to that of ecosystem respiration (Dodds 2006). However, the frequent hypoxia and anoxia observed in several RSCs suggest that, where GPP rates are substantially elevated in comparison to reference streams (HSSs and HSWs), growth of primary producers may be causing excessive growth of heterotrophic micro-organisms such as bacteria, which increases metabolic activity in the stream water and lead to a depletion of dissolved oxygen.

Many natural streams are net heterotrophic, so we propose that trophic state be divided into autotrophic and heterotrophic state. This division allows consideration of the influence of external carbon sources as well as nutrients such as nitrogen and phosphorus. Empirical results suggest that phosphorus and nitrogen are the most important nutrients regulating autotrophic state in flowing waters and that benthic algal biomass is positively correlated to gross primary production in streams. Reference (minimally influenced by human activities) nutrient concentrations and correlations of nutrients with algal biomass are used to characterize reference distributions of stream autotrophic state. Only when reference nutrient concentrations are in the upper one third of those expected in the United States, is maximum benthic chlorophyll projected to exceed 100 mg m<sup>-2</sup> (a concentration commonly used to indicate nuisance levels) >30% of the time. Average reference nutrient concentrations lead to sestonic chlorophyll concentrations above those considered typical of eutrophic lakes (>8 mg m<sup>-3</sup>) less than half the time. Preliminary analysis suggests that the autotrophic state is variable in small pristine streams, because it is influenced by canopy cover (light), but the heterotrophic state is less variable because it can be based on allochthonous or autochthonous production. Nitrogen and phosphorus enrichment can influence both the heterotrophic and autotrophic state, and these effects could cascade to animal communities. Stoichiometry should be considered



because carbon, nitrogen, and phosphorus are all involved in trophic state. The proposed definition of trophic state offers a starting conceptual framework for such considerations.

Decomposition of primary producers and macrophytes also consumes oxygen, which can increase the severity of DO depletion in these RSCs, especially if stream flow velocity is reduced by vascular vegetation and restoration structures.

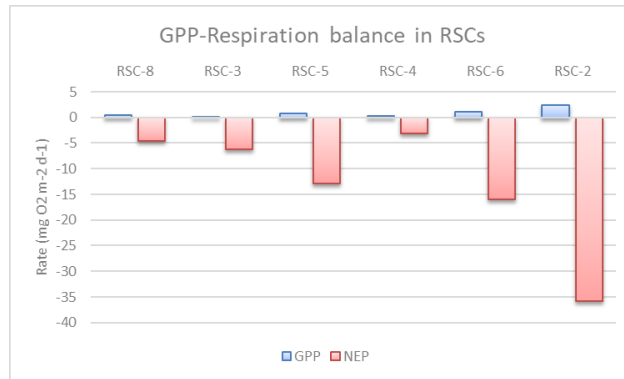


Figure 28. Average ratios of gross primary production and ecosystem respiration in the different RSCs.

#### 4.4.3 Nutrients, Conductivity and Other Environmental Variables

An important step in using stream metabolism for understanding stream health is the identification of environmental factors that control metabolism. Therefore, in addition to determining metabolism for the 19 study streams, we assessed environmental factors that could help explain stream metabolism and to what degree metabolism were related to fish metrics in restored versus reference streams. To explore which environmental factors control stream metabolism at each site, we measured drainage area, level of imperviousness, and water quality represented by conductivity and nutrient concentrations (Table 14). Nitrogen was determined in the forms of nitrate and total nitrogen.

Table 14. Study streams monitored for DO and temperature and measured environmental variables.

Streams	Drainage Area (km <sup>2</sup> )	Imperviousness (%)	TN (mg/L)	Nitrate (mg/L)	TP (mg/L)	Conductivity $\mu$ S	Shading (%)
LSS-2	0.39	50.9	2.01	0.82	NA	374	90
LSS-7	1.58	34.5	1.51	0.62	NA	401	95
LSS-3	1.89	18.4	1.12	0.46	NA	305	70
LSS-8	0.43	34.9	0.75	0.29	NA	141	30
HSS-9	2.51	25.5	0.73	0.30	NA	296	100
HSS-10	1.22	12.1	1.10	0.45	NA	302	75
HSS-22	2.25	28.9	1.71	0.70	NA	346	80
HSS-6	0.53	4.9	0.26	0.11	NA	86.6	95
HSW-2	20.8	20	0.32	0.14	NA	102	85
HSW-6	209	9.07	0.24	0.10	NA	80.8	100

HSW-18	2.31	5.79	0.18	0.08	NA	55.3	60
RSC-8	0.65	33.2	0.91	0.43	0.035	214	90
RSC-3	0.85	39.1	0.82	0.31	0.027	216	85
RSC-5	0.36	12.3	0.49	0.22	0.051	184	65
RSC-4	0.88	35.4	1.17	0.44	0.015	241	80
RSC-6	0.49	55	0.52	0.22	0.038	227	35
RSC-2	1.4	42	0.64	0.14	0.048	NA	15

Among all the environmental factors listed in Table 14, the only one that had a significant relationship with stream metabolism represented by net ecosystem production (NEP) was the level of canopy shading on the stream channel (Figure 29). The less shading, the more negative was NEP, suggesting that light incidence was a key factor controlling metabolism in the study streams. A more negative NEP means a lower ratio of production to respiration. In fact, GPP rates are negatively correlated with level of shading in the streams.

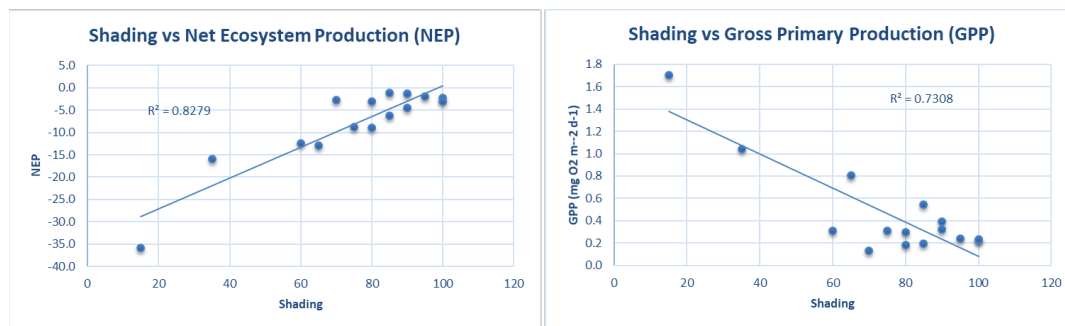


Figure 29. Relationships between canopy shading and net ecosystem production (NEP) and GPP rates in the study streams monitored for DO.

When we analyzed the influence of environmental factors on the metabolism of RSCs only, other interesting patterns emerged. For instance, nitrate is positively correlated with NEP while TP is negatively correlated (Figure 30), suggesting that nitrate enhances ecosystem respiration while TP enhances GPP.

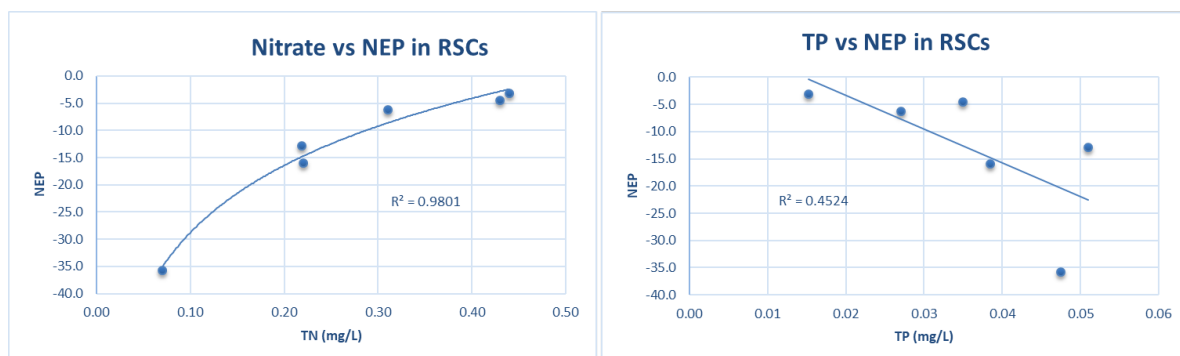
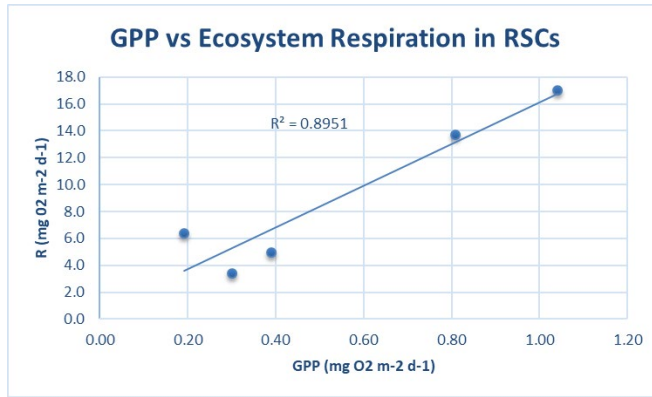


Figure 30. Relationships between nutrient concentrations and net ecosystem production (NEP) in the RSCs.

The rates of gross primary production (GPP) in the RSCs is positively correlated with rates of ecosystem respiration (R) (Figure 31), which indicates that RSCs with relatively high GPP rates also have high respiration rates and, therefore, are more likely to have anoxia or hypoxia episodes.



*Figure 31. Relationships between gross primary production (GPP) and net ecosystem production (NEP) in the RSCs.*

The net ecosystem production (NEP), which is the sum of respiration and GPP, is a fundamental indicator of whether a system is a net producer or consumer of carbon. In the RSCs, nutrient concentrations, especially nitrate, seem to be key factors influencing NEP.

## 5. Variation in Regenerative Stream Conveyances

While every effort was made to find RSCs with comparable restoration designs and landscape settings, the 11 RSCs sampled did vary in design, setting, and age (see the Appendix for details on the design of each RSC). These differences may have contributed to the variation in vertebrate trajectories described above. While the differences found among stream types are significant, examining the results of individual RSCs may shed light on which factors contribute to the overall trajectories found.

Table 15 shows the fish and herpetofauna richness, abundance, biomass, and FIBI results for each of the 11 RSCs. Table 16 lists the numbers of each fish species found in each RSC. Using the well-established FIBI as an indicator of fish community health, we see that RSC 10 (Cowhide Branch to Weems Creek) has a Good FIBI rating, RSC 1 (Bacon Ridge) has a Fair IBI, 6 other RSCs have Poor ratings, and 3 RSCs have Very Poor ratings. RSC 10 has the most taxonomically diverse assemblage with a number of high trophic level predators (pickerel) and several functional groups. It also has species that suggest connectivity with estuarine habitats (eel, killifish, stickleback, silverside, mummichog). Note that all of these species live in freshwater, but are salt tolerant. RSC 1 is the next most diverse fish assemblage, but has a different species composition, including a large number of centrarchids (bluegill, bluespotted sunfish, green sunfish, pumpkinseed), very few mid-trophic level consumers (bullhead, eel), and only one higher trophic level predator (single redbfin pickerel). Note that RSC 1 was the largest site sampled, both in width and depth, but had little flow and therefore a more lentic character, which is consistent with the presence of centrarchids.

*Table 15. Biota sampling data from RSC sites. The following abbreviations are used in the table: taxonomic richness (S), abundance (N), biomass (B), fish index of biotic integrity (FIBI). Fish area metrics are calculated based on wetted stream area sampled. FIBI calculated using Maryland Biological Stream Survey methodology. Data are unavailable for blank cells.*

RSC site	Herpetofauna		Amphibians			Reptiles	Fish fauna			Fish area metrics			FIBI
	S	Total N	Total N	Anuran N	Larval N	N	S	N	B	S m <sup>-2</sup>	N m <sup>-2</sup>	B m <sup>-2</sup>	
1	1	9	9	8	0	0	11	57	796	0.023	0.12	1.66	3.33
2	1	145	145	143	99	0	9	974	709	0.028	2.99	2.17	2.67
3	3	27	26	22	15	1	1	10	266	0.003	0.03	0.87	1.00
4*	3	12	11	8	0	1	5	278	1509	0.021	1.17	6.34	2.67
5	2	76	76	66	58	0	6	362	244	0.004	0.21	0.14	2.33
6	2	136	136	130	0	0	3	254	208	0.022	1.83	1.5	2.33
8	5	46	43	17	0	3	8	122	743	0.028	0.42	2.57	2.33
9†	1	42	42	40	0	0	1	5		0.005	0.03		1.00
10*	0	0	0	0	0	0	12	614	5208	0.031	1.59	13.48	4.33
11†	1	35	35	35	0	0	1	9		0.007	0.06		1.67
12†	1	15	15	15	0	0	1	86		0.006	0.50		2.00

\* Fish data collected by Anne Arundel County in July (RSC 10) and August (RSC 4) 2020

† Fish data collected by Arundel Rivers Federation in July 2020 (RSC 9, RSC 11, RSC 12)

Table 16. Species counts associated with each RSC site. Species richness and total abundance associated with each RSC site are summarized at the bottom of the table.

Species	RSC site ID										
	1	2	3	4	5	6	8	9	10	11	12
American eel	1	2	0	3	3	2	13	0	49	9	0
Banded killifish	0	0	0	0	0	0	0	0	78	0	0
Black crappie	0	0	0	0	0	0	0	0	1	0	0
Bluegill	9	25	0	0	0	0	12	0	0	0	0
Bluespotted sunfish	7	0	0	0	0	0	0	0	0	0	0
Brown bullhead	1	10	0	0	5	0	0	0	14	0	0
Chain pickerel	0	0	0	0	0	0	0	0	9	0	0
Creek chubsucker	15	0	0	0	0	0	0	0	0	0	0
Eastern mosquitofish	3	716	0	170	341	246	84	0	183	0	86
Eastern mudminnow	4	0	0	86	1	6	3	5	0	0	0
Fourspine stickleback	0	0	0	0	0	0	0	0	3	0	0
Golden shiner	2	166	0	15	11	0	0	0	9	0	0
Goldfish	0	0	0	4	0	0	0	0	0	0	0
Green sunfish	2	0	10	0	0	0	6	0	0	0	0
Inland silverside	0	0	0	0	0	0	0	0	41	0	0
Largemouth bass	0	2	0	0	0	0	1	0	0	0	0
Mummichog	0	48	0	0	0	0	0	0	13	0	0
Northern snakehead	0	2	0	0	0	0	0	0	0	0	0
Pumpkinseed	12	3	0	0	1	0	2	0	201	0	0
Rainwater killifish	0	0	0	0	0	0	0	0	13	0	0
Redfin pickerel	1	0	0	0	0	0	0	0	0	0	0
White perch	0	0	0	0	0	0	1	0	0	0	0
<i>Species richness (S)</i>	11	9	1	5	6	3	8	1	12	1	1
<i>Abundance (N)</i>	57	974	10	278	362	254	122	5	614	9	86

Table 17 lists the values for 11 physical, environmental, and metabolic parameters measured at each RSC. Figure 32 is a Principal Components Analysis (PCA) plot of the 11 RSC sites based on normalized canopy shading (Densio), depth (Thalweg), Width, dissolved oxygen (DO), Temperature, and Conductivity values. The PCA shows clusters of (1) larger RSCs (1 and 10), (2) RSCs with more tree canopy and higher temperature (2, 3, 8, 6, and 4), and (3) RSCs with higher conductivity and DO (9, 12, and 11). Some of these differences may reflect variation in restoration design, such as more of less pools and faster or slower flow, with implications for fish communities.

Table 17. Environmental and habitat data from RSC sites. The following abbreviations are used in the table: catchment area – **Area** (acres), average densitometer reading – **Densio**, average thalweg – **Thalweg** (cm), average stream width – **Width** (m), current speed – **Current** (m/sec), dissolved oxygen concentration – **DO** (mg/L), water temperature – **Temp** (C), specific conductance – **Cond** (μs/cm), gross primary production – **GPP** (mg/O<sub>2</sub>/m<sup>2</sup>/d), ecosystem respiration – **ER** (mg/O<sub>2</sub>/m<sup>2</sup>/d), net ecosystem production – **NEP** (mg/O<sub>2</sub>/m<sup>2</sup>/d). Data are unavailable for blank cells.

RSC site	Physical					Environmental			Metabolic		
	Area	Densio	Thalweg	Width	Current	DO	Temp	Cond	GPP	ER	NEP
1	478.1	9.6	132.5	6.4	<0.01	4.33	20.7	202.8			
2	326.3	14.1	82.8	4.4	0.04	1.11	23.4	163.4	2.11	-34.05	-31.97
3	305.6	15.8	24.3	4.1	0.03	3.008	23.8	215.6	-0.13	-2.26	-2.39
4	238.1	14.5	30.3	3.2	0.02	4.89	24.2	422.1	-0.02	0.73	0.71
5	1684.7	12.1	42.0	3.8	0.07	5.26	21.3	207.5	0.49	-9.55	-9.07
6	138.8	12.9	19.3	1.9	0.02	2.36	21.3	253.3	0.72	-12.85	-12.12
8	288.8	16.2	25.0	3.9	0.15	4.95	23.5	214.0	0.07	-0.77	-0.71
9	189.4	12.8	43.3	2.5		4.51	17.2	340.4			
10	386.3	7.8	48.3	5.2	0.44	8.06	21.6	193.6			
11	142.7	15.8	21.0	1.9	0.13	7.27	17.0	436.5			
12	172.3	14.3	31.0	2.3	0.07	6.58	17.5	475.1			

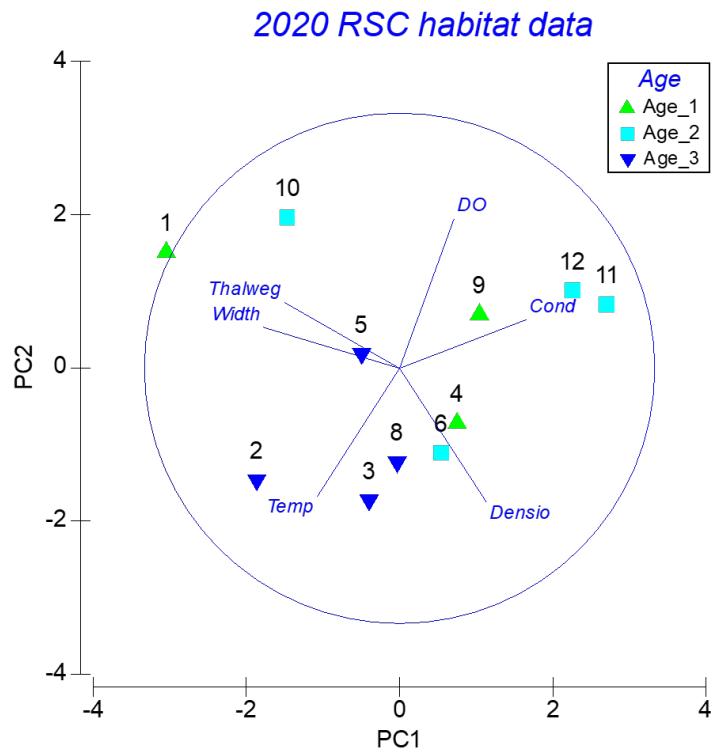


Figure 32. Principal Components Analysis plot of RSC sites based on normalized *Densio*, *Thalweg*, *Width*, *DO*, *Temp*, and *Cond* values. RSC's identified by site number and coded by RSC age (represents time since completion: Age\_1 = 1.9-4.3 yrs, Age\_2 = 5.3-7.4 yrs, Age\_3 = 8.9-16.2 yrs). Labelled vectors show loading of environmental variables on the first 2 principal components (% variance explained – PC1 = 49.3%, PC2 = 27.9%). See Table 16 for variable definitions.

Figures 33 and 34 show abundance of fish and herpetofauna, respectively, versus ecosystem respiration (ER) and gross primary production (GPP) at the 6 RSCs sampled for stream metabolism. Based on our estimates of net ecosystem production, the metabolism of some RSCs was further away from a more natural state than others. The best potential explanatory factor were canopy shading, followed by nutrient concentrations. The RSC with metabolism deviating the most from that of natural streams was RSC 2 (N Branch Cypress Creek), which has strong wetland features. In contrast, the RSC deviating the least was also the oldest—RSC 8 (Wilelinor). RSC 3 (Crofton Tributary) was also similar to natural streams, probably because of canopy shading and healthy riparian forest. RSC 2 had the highest abundance of fish and amphibians (with RSC 6 also high for amphibians) among the RSCs sampled for stream metabolism.

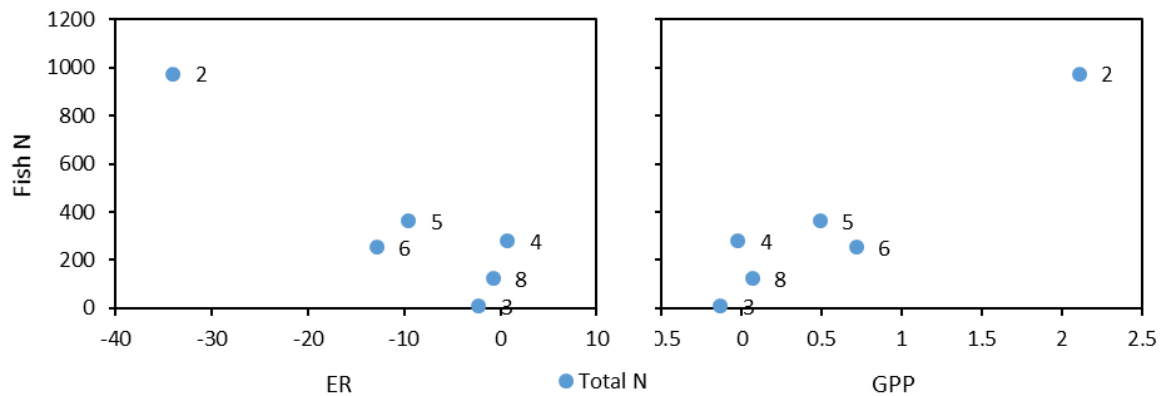


Figure 33. Fish abundance plotted against ecosystem respiration (ER) and gross primary production (GPP) measured at RSC sites 2, 3, 4, 5, 6 and 8 (RSC site numbers provided as data labels).

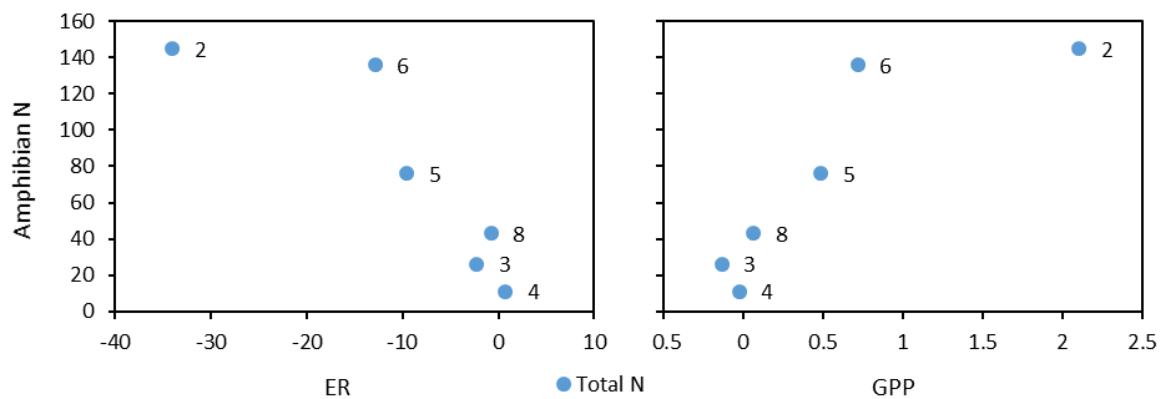


Figure 34. Amphibian abundance plotted against ecosystem respiration (ER) and gross primary production (GPP) measured at RSC sites 2, 3, 4, 5, 6 and 8 (RSC site numbers provided as data labels).

## 6. Conclusions

The conceptual model derived from existing MBSS sample data provided quantitative expectations for three reasonable trajectories of vertebrate communities after restoration as Regenerative Stream Conveyances (RSCs). Comparisons of fish and herpetofauna sampling in 11 RSCs and 24 reference sites in August-September of 2020 provided support for our three hypotheses as follows:

Hypothesis 1 – RSC assemblages are the same as degraded, pre-restoration streams - SUPPORTED

- RSC fish IBIs, biodiversity indices (Richness, Richness<sub>Area</sub>, H'), and multivariate species compositions were not significantly different between RSC sites and Low-quality Single Streams (LSS) sites, i.e., the reference for degraded, pre-restoration streams
- RSC herpetofauna indicators were generally not significantly different from LSS sites, with the exception of Richness<sub>Area</sub> being lower than for LSS sites

Hypothesis 2 – RSC assemblages are the same as minimally-disturbed streams – NOT SUPPORTED

- RSC fish IBIs, biodiversity indices (Richness, Richness<sub>Area</sub>, H'), and multivariate species compositions were nearly always significantly different between RSC sites and High-quality Single Streams (HSS) sites, i.e., the reference for minimally-disturbed single streams
- RSC herpetofauna indicators were generally not significantly different from HSS sites, with the exception of Amphibian N (abundance) being higher than for HSS sites

Hypothesis 3 – RSC assemblages are the same as minimally-disturbed stream-wetland complexes – NOT SUPPORTED

- RSC fish IBIs, biodiversity indices (Richness, Richness<sub>Area</sub>, H'), and multivariate species compositions were nearly always significantly different between RSC sites and High-quality Stream Wetlands (HSW) sites, i.e., the reference for minimally-disturbed stream-wetland complexes
- RSC herpetofauna indicators were not significantly different from HSW sites

The primary details of our analysis of the vertebrate communities are summarized below:

- **Fish Index of Biotic Integrity (IBI)** was significantly lower in RSC and LSS sites than in HSS and HSW sites, with RSC sites non-significantly higher than LSS sites
- **Fish diversity** differed among stream types with RSC sites having lower richness (Richness), lower richness per unit area sampled (Richness<sub>Area</sub>), and lower Shannon diversity (H'), than the HSS and HSW sites
- **Fish richness** was negatively related to conductivity and fish diversity was positively related to DO and negatively related to conductivity
- **Fish assemblages** differed between HSS/HSW and LSS/RSC sites, several species were diagnostic of these differences—particularly sensitive species that were absent from RSC and LSS sites (creek chubsucker, fallfish, madtoms, lampreys)
- **Herpetofauna diversity** showed few patterns, although richness per unit area was lower in RSC than LSS sites



- **Abundance of amphibians** was highest in RSC sites and lowest in HSS sites (significant pairwise difference)
- **Abundance of frogs** was higher in RSC sites than in LSS or high-quality HSS and HSW sites
- **Abundance of amphibians** was negatively related to DO in the streams

Overall, fish and herpetofauna communities in RSC sites are similar to their presumptive pre-construction state, represented by low-quality LSS sites, and are not similar to either the high-quality HSS or HSW sites. Potential reasons for this lack of trajectory toward higher quality states include the following (Figure 35):

- While RSC sites recreated physical stream-wetland structure (such as width and depth) typical of high-order streams in reaches that are low-order, high-quality reference (HSS and HSW) levels of DO, conductivity, and flow levels were not attained
- Therefore, vertebrate uplift in RSC sites appear constrained by continuing poor water quality
- The only vertebrate community element to increase in RSC sites was frog abundance, which is consistent with the fact that frogs can survive slow-velocity, low-DO aquatic systems because of their semi-aquatic life cycle
- Both less shading (more light) and smaller buffer widths in RSC sites compared to reference sites, likely fuel in-stream primary productivity, which leads to greater diel DO swings

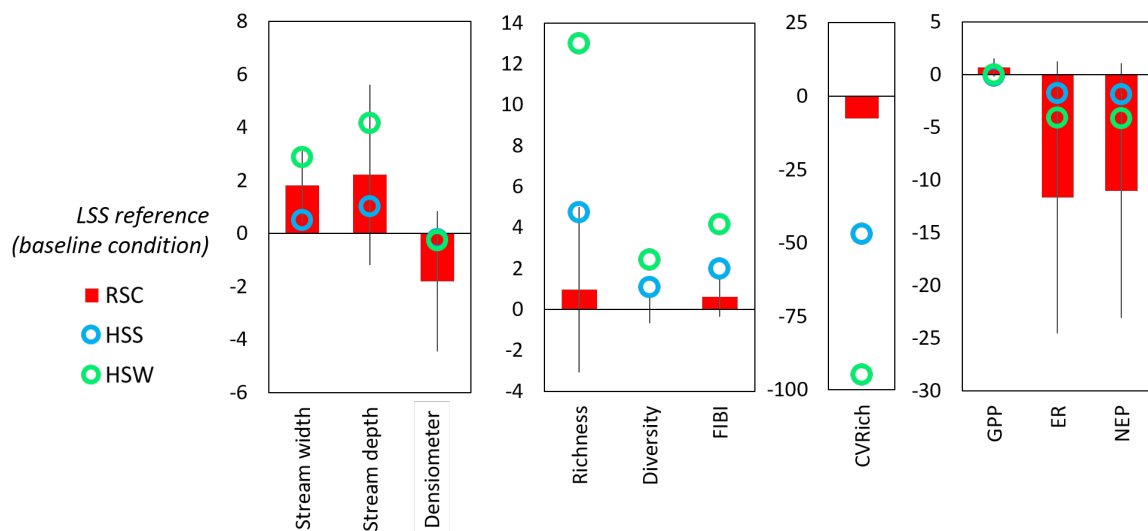


Figure 35. Trajectory of Regenerative Stream Conveyance (RSC) sites (red bars) compared to the baseline of presumed pre-restoration, low-quality single streams (LSS) (zero line), with relative values of high-quality (HSS and HSW) sites (circles) also shown for (1) environmental variables (stream width, stream depth, densimeter), (2) fish (richness, diversity, IBI), (3) variance in fish richness (CVRich), and (4) stream metabolism (GPP, ER, NEP). Note that decreased densimeter values indicate increased incident light.

While fish and herpetofauna communities in RSCs as a group did not approach the high-quality references, HSS and HSW, it is possible they improved on the pre-existing degraded conditions. It is impossible to quantify this change since pre-restoration data were not available. It is likely that pre-restoration conditions were as bad or worse than our LSS reference or, possibly, low-quality stream-wetland complexes that existed before restoration. Vertebrate communities may also improve as RSCs mature and riparian vegetation returns, but this is not strongly indicated in our results.

Although we are hopeful that RSC designs (and stream restoration in general) will evolve to better approach vertebrate communities found in high-quality references, the constraints of human perception of ecological states may continue to limit restoration toward the natural state. Prober et al. (2019) posits that “ecological renovation” may be a better term for stream manipulation than restoration.

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