EA Engineering, Science, and Technology, Inc., PBC

FINAL REPORT ON TEMPORAL TRENDS OF IRON IN STREAMS AND EFFECTS TO STREAM COMMUNITIES

Prepared for:

The Chesapeake Bay Trust
108 Severn Avenue
Annapolis, Maryland 21403

Prepared by:

EA Engineering, Science, and Technology, Inc., PBC
225 Schilling Circle, Suite 400
Hunt Valley, Maryland 21031

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Introduction

Stream restoration projects are undertaken to mitigate concerns associated with urban stream syndrome, such as flash-flooding, increased nutrient loading and overall reduced ecosystem function (Walsh et al. 2005). Another important rationale for restoration is to increase connectivity in systems that have disturbed hydrology (Stromberg et al. 2007). In Maryland, stream restorations are undertaken for all the aforementioned reasons, but a predominant theme is to reduce sediment loading and nutrient transport to the Chesapeake Bay. Despite nearly all stream restoration activity within Maryland sharing this common goal, there is not a singular restoration approach to reduce nutrient and sediment loadings to the Bay (Berg et al. 2013). One approach gaining more traction is a restoration technique that involves significant construction and environmental modification of the stream or conveyance to 'restore to natural conditions'; this restoration design has many terms, Regenerative Streamwater Conveyance, Regenerative Stormwater Conveyance, Step Pool Storm Water Conveyance, Coastal Plain Outfall, etc. A common theme of this design is to modify the hydrology and other physical characteristics of the stream to create a tiered pool system of sand seepages using large boulders (frequently sandstone) in between the pools to provide stability and reduce heavy flash flooding of the system following storms (Flores, et al. 2012). Successful implementation of the Regenerative Streamwater Conveyance (RSC), is how we will refer to it from hereon, will filter surface water flow to shallow groundwater for pollutants by reducing flowrates through diversion of water to pools and the newly reconnected floodplain. Sediments and nutrients are also expected to be retained within the RSC and therefore, reducing overall loading downstream and into the Chesapeake Bay.

Another observation on streams that have been restored following RSC design is the presence of iron flocculate (Williams et al. 2016). Constructed wetlands and RSCs are designed to promote conditions necessary for denitrification by creating reducing conditions (lowering oxygen; Beebe et al. 2013, Beebe et al. 2015). These reducing conditions can favor the generation of iron flocculate. The appearance of iron in streams can be aesthetically concerning, especially following an expensive restoration design and implementation. Iron chemistry is complex; and is driven by biotic and abiotic factors (Willams et al. 2016; Martin 2003). Iron flocculate and particulate iron observed in streams are iron oxides (FeOH) and Fe^{3+} where conditions favor lower low oxygen and higher pH; importantly, iron may also be present in surface water in the aqueous form (Fe^{2+}) under conditions with moderate oxygenation and lower pH (Martin et al. 2003). Water depth and degradable organic carbon (e.g. mulch, leaf litter) are also abiotic factors that are manipulated during RSC construction that influence iron chemistry. In addition to concerns regarding the aesthetics of iron flocculate in streams, there is also the potential that iron in streams may impact the biological community (Kotalik et al. 2019).

The associated effects of iron to the biotic community in streams are not fully characterized; direct toxicity may occur when iron is in the dissolved (aqueous) form and direct + indirect effects may result from iron in the solid form (flocculate/particulate). Within the macroinvertebrate community, Mayflies (Ephemeroptera; Leptophlebiidae) appear to be most sensitive to dissolved iron (Linton et al. 2007). Colptera (Psephindae) was found to have intermediate sensitivity and Diptera (Tipulidae) showing the greatest tolerance to dissolved iron (Linton et al. 2007). A field study by Rasmussen and Lindegaard (1998) found Ephemeroptera and Plecoptera taxa were absent from river sites with elevated dissolved iron while Tubificidae, Chironomidae and Tupulidae taxa were present. Iron in the solid form (particulate or flocculate) is not expected to result in direct toxicity; however, it may disturb respiration in gilled organisms (Dalzell and MacFarlane 1999). Iron (solid) may also decrease habitat availability for invertebrates through deposition (Rasmussin and Lindegaard 1988; Vuori 1995).

The presence of iron in streams following RSC construction can be attributed to the design materials used (ironstone boulders; Williams et al. 2016) but is also likely a result of dissolved iron in groundwater infiltration, disturbed soils at lower depths and the combination of the RSC conditions (reducing) and added organic material that result in observable iron flocculate. Here we address several objectives related to iron in streams in Anne Arundel County (AAC), MD. Our first objective was to better understand the occurrence and concentrations of Fe in restored and non-restored streams. In essence, we sought to characterize Fe in AAC streams with an eye toward whether streams were restored or not. Secondly, we sought to determine whether observed Fe in AAC streams could lead to adverse effects on aquatic animal species.

This report addresses the following objectives:

Objectives 2&3. Characterizing Iron and its impact on biological communities in Anne Arundel County, MD streams including those with Regenerative Streamwater Conveyance (RSC) Systems

Hypotheses:

(1) Temporal shifts in water chemistry and temperature influence measurable iron concentrations in streams.
(2) Biodiversity of the stream community will decrease when iron in streams is higher.
(3) Survival of caged macroinvertebrates will be lower in streams with high iron compared to reference streams.

Methods

Site Selection and Water Chemistry

Stream sites were selected in Anne Arundel County (AAC), MD to maintain a consistent underlying geological foundation consistent with the Coastal Plain. Additionally, many streams in AAC have observable levels of iron (Fig. 1). Collectively, we have obtained water quality and iron concentration data at 38 different sites across 24 different streams in AAC; 19 of the sites have been repeatedly sampled more than five times and 8 sites have been repeatedly sampled 12 times or more. Our goal was to better understand Fe and stream water quality across a wide range of streams while also obtaining more temporal resolution for a smaller subset of streams. Streams can vary considerably with regard to impervious surface inputs, riparian zone condition, substrate, restoration activity, and a wide variety of other variables. Representative sites that included streams restored via step-pool design with and without iron flocculate present and streams without restoration serving as reference locations (Table 1). Examples of streams that have had a Step-pool restoration were Cowhide Branch and Cattail Creek which had restoration projects completed in 2018 and 2019, respectively. Dividing Creek restoration was completed in 2016; Church creek at Wilelinor and Gingerville Creek were completed in 2014. Example non-restored streams include the headwaters of the Magothy River, Severn Run at the KOA campsite, and Mill Creek.


Figure. 1. Image of Jabez Branch (DNR land) showing iron in the stream. This is a non-restored site.

There were challenges in visually identifying step-pool restoration sites that did not have iron flocculate present. As previously noted, iron flocculate tends to be a characteristic of this restoration technique (Williams et al. 2016, Cope undated presentation). Through reconnection of the floodplain during step-pool construction, the infusion of groundwater into the reducing conditions of the pools likely results in iron precipitating into flocculate. Additionally, the use of ironstone to stabilize pools provides another iron source into the restored system (Williams et al. 2016). However, it is important to note that many streams within AAC that have not been restored have evidence of iron flocculate either currently or historically as seen by iron stained substrate (e.g. Fig 1, Appendix [high iron site]).

Site Name	Latitude	Longitude	Step Pool Restoration	Visual Flocculate	Iron Category	Biotic Sampling
Ref_Stocketts Run	38.87358	-76.66405	-	Yes	Low	-
Ref_Rock Branch	38.87129	-76.66612	-	-	Low	Yes
Ref_Magothy River	39.0704	-76.6504	-	-	Low	Yes
Ref_SevernRun_DNR	39.07751844	-76.64034066	-	Yes	Low	-
Low Flocc_CowhideBranch_down	39.086986	-76.548846	Yes	Yes	Med	Yes
Low Flocc_CattailCreek	38.990835	-76.525908	Yes	-	Med	Yes
Ref_SevernRun_KOA	39.050597	-76.515798	-	Yes	Med	-
High Flocc_GingerCreek	38.968187	-76.76555683	Yes	Yes	High	-
HighFlocc_DividingCreek_down	38.968128	-76.76555687	Yes	Yes	High	Yes
HealthDept_BroadCreek_MidPool	38.979818	-76.563973	Yes	Yes	High	-
High Flocc_ChurchCreek	39.1154874	-76.5582277	Yes	Yes	High	Yes
- indicates Absence of Restoration, Flocculate or Biotic Sampling						

Table 1. Example selected stream sites within Anne Arundel County, MD and the associated features related to this project. Note, that more streams than those listed here were sampled.

Stream sampling was initiated in February 2020 and occurred on a monthly or semi-monthly basis. Note, there were some challenges in completing routine stream sampling as noted in the *Challenges that have impacted the project* section. Streams sampled changed somewhat through time based on data and knowledge obtained in the process of conducting this research. Water samples were obtained at each sampling event and frozen for future iron analyses. At the time of sampling, standard water quality measurements were obtained using a YSI (Cole-Parmer® Vernon Hills, IL) including dissolved oxygen, conductivity, and pH. In February, 2021, we started collecting oxidative-reductive potential (ORP) and also collected filtered water samples for analyzing dissolved iron. Unfiltered water samples, when analyzed for iron, provide a measure of total iron as some particulate iron is likely suspended in the water column.

Iron was measured in filtered and unfiltered water samples using atomic absorption spectrophotometry at Towson University.

Biota Sampling

Macroinvertebrate sampling was conducted on 14 July 2020 at the six sites selected to represent Low, Medium and High iron concentrations (as noted in Table 1). Sampling protocols followed US EPA Rapid Bioassessment guidelines and focus was on riffles and runs. In addition, we obtained data from the Maryland Department of Natural Resources (DNR) and AAC from 16 stream sites in AAC that had a range of observable iron flocculate. For these 16 sites, water was sampled in July 2021 for water quality and iron analysis for use in comparing benthic Index of Biotic Integrity (IBI) scores across total iron.

Fish sampling occurred over the course of three days in August and September, 2020 following MBSS guidelines (Table 2). Each stream was sampled twice along a 75-m transect using one or two backpack electroshockers as determined by stream width.

Date	**Location**	**Stream Name**	**Sample Method**
8/11/20	MAG	Magothy River	Backpack Electroshocking
8/11/20	CATT	Cattail Creek	Backpack Electroshocking
8/12/20	CHBR	Cowhide Branch	Backpack Electroshocking
8/12/20	RKBR	Rock Branch	Backpack Electroshocking
9/2/20	WLCR	Church Creek	Backpack Electroshocking
9/2/20	DVCR	Dividing Creek	Backpack Electroshocking

Table 2. Stream sampling schedule for fish communities

Fish have been identified and biodiversity indices and the index of biotic integrity (IBI) have been calculated. Fish IBI methods were prescribed by the DNR and Roth et al. 2004. Fish IBI scores were also obtained from DNR for 5 additional stream sites in AAC and with observed range of iron flocculate. As above, these sites were sampled in July 2021 for iron and water quality analysis.

Invertebrate Field Enclosure Methods

Enclosures and methods were modeled after DeNicola and Stapleton (2002); where they performed an *in-situ* macroinvertebrate experiment (~5 days) in an Acid Mine Drainage (AMD) impacted stream. At minimum, we have selected seven stream sites for the *in situ* experiment: one reference site (Magothy River), one site with a newer RSC (low flocculation- Cattail Creek), one site with an older RSC (high flocculation- Dividing Creek at ACC), and four non-restored sites (low flocculation- unnamed tributary to Patuxent at Davidsonville Park; mid-level flocculation- a northern tributary to Flat Creek and Jabez Run at KOA; high flocculation- Mill Creek at AACC).

The enclosures consist of one 6-inch-long PVC pipe (3-inch diameter) with squares of 500µm mesh zip tied to each end, with the downstream end sealed with silicon. The screening pipe is zip-tied to two bricks to provide weight to reduce the chances of enclosure migration downstream (Fig. 2). Before the enclosure was zip-tied, closed substrate and macroinvertebrates are added and the experimental unit was placed in-stream.

Figure 2. *in situ* experimental design

In late May 2021, 240 macroinvertebrates (caddisflies) were sampled from the Magothy River site over two days. Per day, macroinvertebrates were held in 1L Nalgene bottles that were filled with water from the Magothy River and kept in coolers with a small layer of ice to maintain cool temperatures that helped to conserve dissolved oxygen during transport. Ten live macroinvertebrates were placed in each enclosure with a standardized amount of substrate, rocks and leaves, from the Magothy River (inset of Fig. 3). Three enclosures were left in each stream for 7 days (Fig. 3). Total and ferrous iron were measured in the field with Hach IR-18B and IR-18C kits; respectively, at both the point of set up and removal. At these time points total and ferrous iron were also measured, as were temperature, dissolved oxygen (DO), conductivity, pH, and Oxidation Reduction Potential (ORP) using a YSI Professional Plus.

Figure 3. *Deployment of the enclosures; inset image is substrate inside enclosures for resources and enrichment.*

After 7 days the enclosures were removed, and caddisflies/casings were counted and observed for survivorship. Macroinvertebrates were fixed in 95% ethanol in the field and later preserved in 70% ethanol in the lab. Caddisflies will be identified to genus where possible and official counts will be conducted outside of this project period.

<u>Statistical Analyses</u>

The R software environment was used for all statistical analyses and to generate plots in this report (R Core Team 2019). Regression analysis was used to explore the relationship between water quality parameters and total and ferrous Fe in AAC streams. Regression was also used to explore the relationship between the mean and standard deviation of total Fe. The 'relaimpo' package in R was used to assess the relative importance of DO, pH, conductivity and ORP on total and ferrous iron. The 'relaimpo' package provides estimates of regressors in linear models along with bootstrap confidence intervals.

To determine alpha-diversity, Shannon diversity index and evenness were analyzed in the R software environment (R Core Team 2019) using the Vegan Package (Jari Oksanen et al. 2020); these were then analyzed with respect to Fe using linear regression. To determine the effects of iron on fish communities, a regression analysis (linear model) was performed using the Shannon index and mean iron concentrations of the streams. For FIBI and BIBI data that was provided by DNR, linear or exponential regression was used to assess impacts of iron on these metrics. When considering results of regression analyses, the p-value corresponds to a test of the slope of the regression and R^2 provides an estimate of model fit.

Results

Characterization of Iron in AAC Streams

Total iron concentrations (Fe_{tot}) and Ferrous iron concentrations (Fe^{2+}) in multiple AAC streams were measured starting in February 2020 and continuing through July 2021. Total iron concentrations measured support observations at stream sites; for example, if the stream sites have noticeable iron flocculate then the streams had higher total iron concentrations compared to streams with less or no observed iron flocculate. Of the streams represented in Figure 4, those with higher iron (Cowhide Branch and others to the right) have RSCs. Not surprisingly, total iron concentrations vary more when the mean total iron in streams is higher (Figure 5); however, overall variability in total iron per stream is low as all coefficients of variation (standard deviation /mean) were less than 1.0 except for Broad Creek (Health Dept).

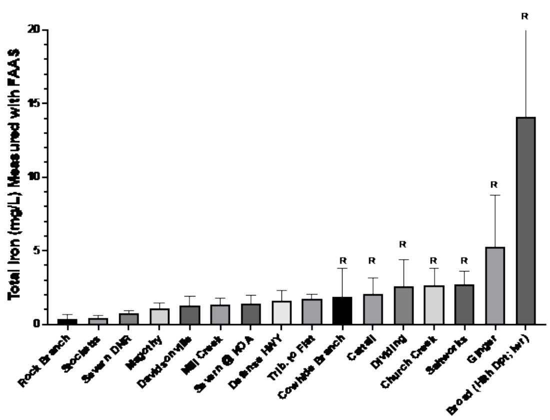

Figure. 4. Mean Fe_{tot} concentration ± standard deviation as measured with Flame Atomic Absorption Spectrophotometry

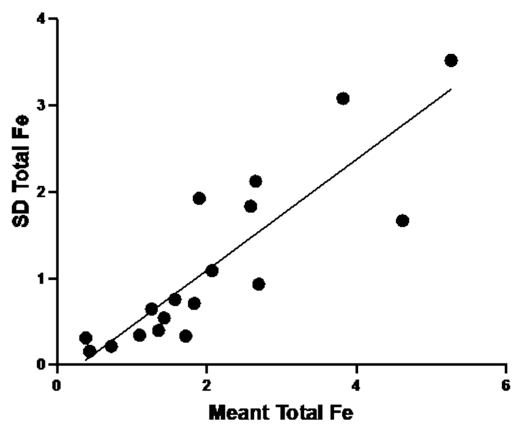

Figure. 5. Standard deviation of total Fe as related to mean total Fe (FAAS). Excludes Broad Creek at the Health Dept.

Ferrous iron (Fe^{2+}) concentrations for most of the same streams in Fig. 4 are shown in Fig. 6. Note that ferrous iron concentrations closely follow trends in total iron across streams and, in fact, represent close to half of the total measured iron (proportion of total iron that was Fe^{2+} was 0.51 with a standard deviation of 0.19). There was also a strong, positive relationship between the mean total Fe and Fe^{2+} in streams (Fig. 7).

Iron chemistry is strongly dependent on other water quality and chemical parameters. Dissolved oxygen, pH and temperature can all impact iron chemistry (Kotalik et al. 2019). Across all sampled streams at all time points, pH did not vary considerably with an overall mean of 7.06 and a standard deviation of 0.47. The overall mean concentration of dissolved oxygen was 6.66 mg/L with a standard deviation of 2.90. Conductivity, is an indirect measure of inorganic dissolved solids in surface water and, in general, is highly correlated to urbanization. The average conductivity in streams was 303 µs/cm with a standard deviation of 229 µs/cm. As anticipated, conductivity varied considerably because inputs to streams from surrounding areas vary considerably and is heavily influenced by the amount of impervious surfaces. The maximum conductivity measured was 2621 measured at Cowhide Branch in February 2021. Temperature varied seasonally with an overall average of 16 °C and a standard deviation of 5.99 °C.

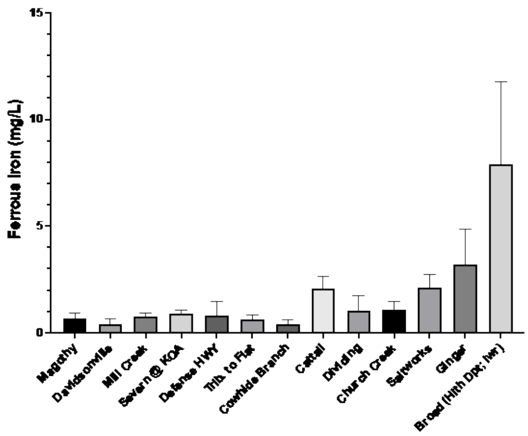

Figure. 6. Mean Ferrous Fe^{2+} concentration ± standard deviation as measured with Flame Atomic Absorption Spectrophotometry

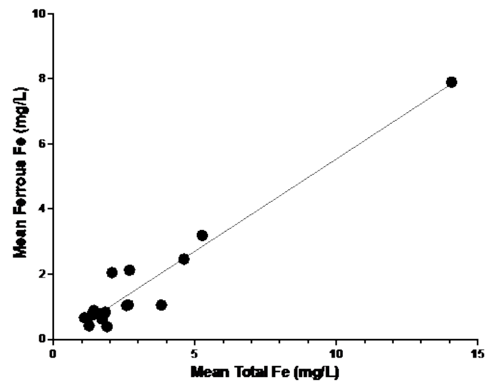

Figure. 7. Relationship between Mean total Fe and Ferrous Fe^{2+} for streams sampled 5 times or more. $R^2 = 0.94$.

We explored the relationship between the abovementioned water quality parameters and total iron which again, is highly correlated to ferrous iron and likely representative of what would be iron flocculate levels. As such, we can view these analyses as the contribution of factors related to the overall iron in the sampled AAC streams. For this analysis, we used data obtained from streams sampled from February through July 2021. These stream samples represent the most consistent, high quality samples collected and span three seasons. Additionally, for these samples we also measured oxidative-reductive potential (ORP), which is also directly related to dissolved oxygen but also other ions that influence the redox conditions in the stream.

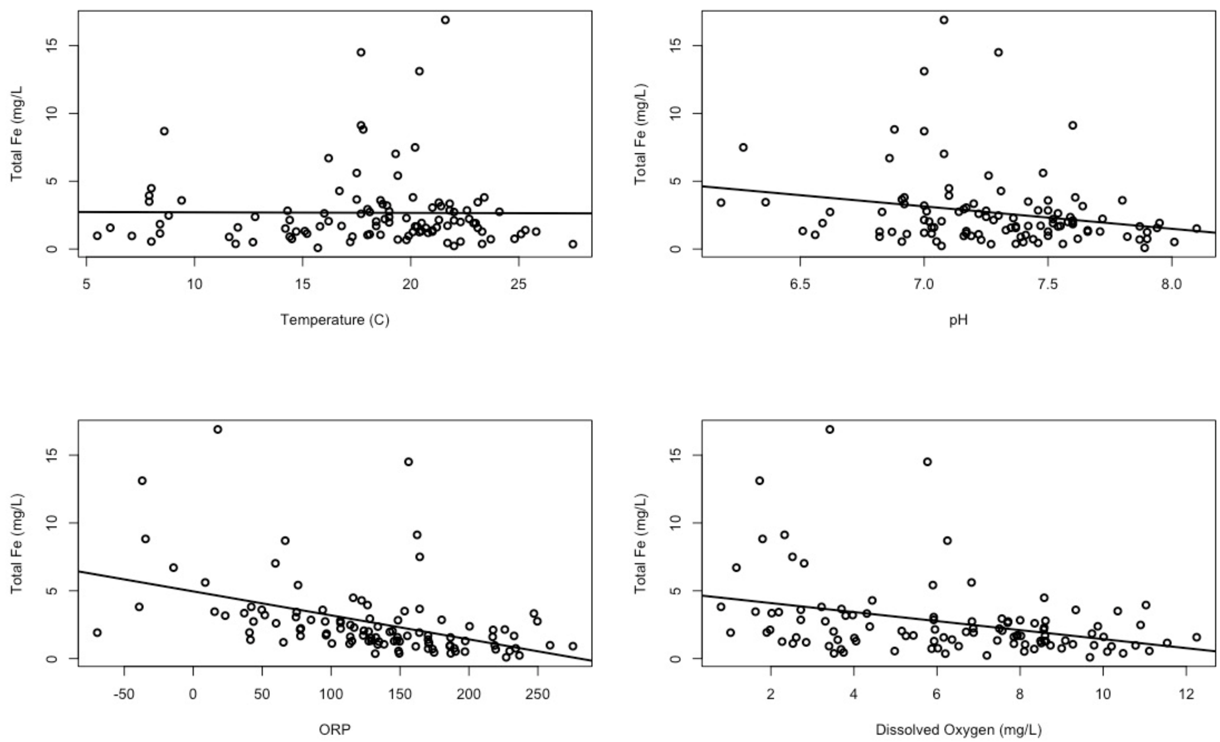

Figure. 8. Relationship between Total Fe measured in AAC streams and temperature, pH, ORP, and dissolved oxygen. R^2 values were all low.

Analyzed individually via linear regression (Fig. 8) shows that pH, ORP, and DO are negatively related to total Fe. That is, as pH, ORP, and DO increase, total Fe tends to decrease. That said, in all cases, the data are considerably variable and all R^2 values were less than 0.2 indicating a relatively poor fit of the data to the model. Another method implemented in the R software environment designed to determine the relative importance of different parameters (Figure 9) to a response variable yielded insights similar to an inspection of Figure 8.

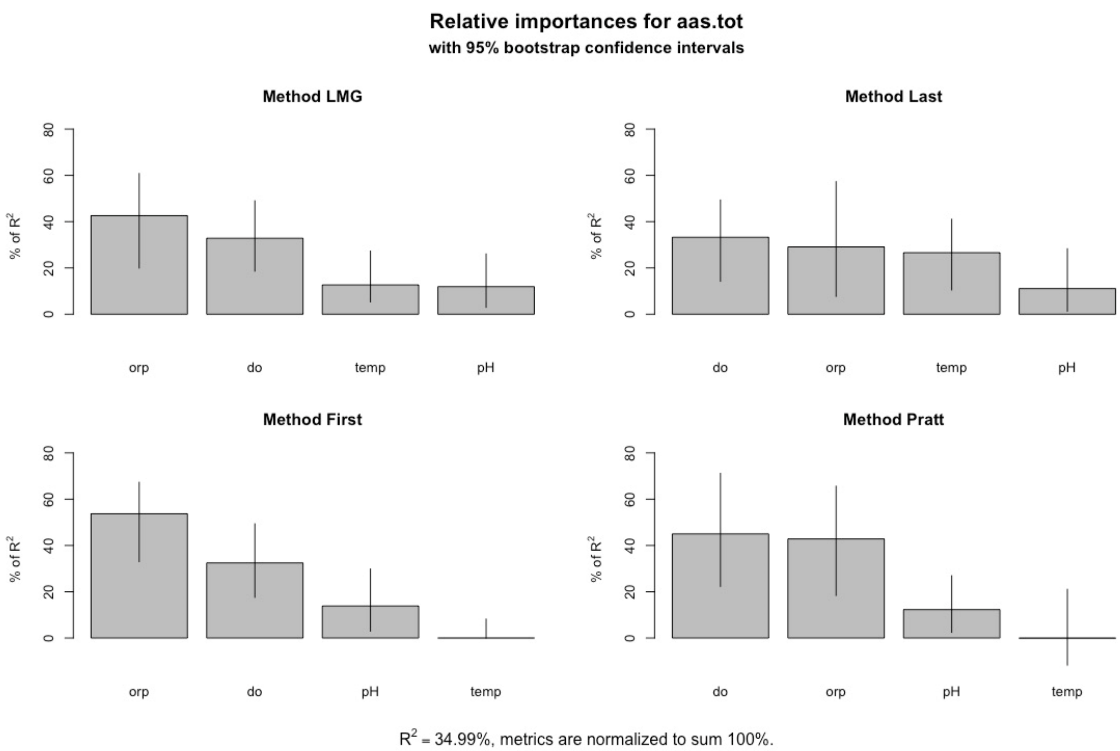

Figure. 9. Relative importance was calculated for total Fe for the four parameters measured when stream samples were obtained. Dissolved oxygen and oxidative-reductive potential are most the most important factors with regard to the concentration of total Fe.

Figure 9 shows that dissolved oxygen and ORP are relatively more important for determining total iron in AAC streams compared to temperature and pH. These results likely reflect that there was not much variability in pH across streams and that temperature was not a key factor in total iron concentrations. These data provide some insight into water quality parameters that contribute to total iron in streams although, because there was considerable variability in the data, other factors we did not measure likely play more important roles in determining iron in streams. We hypothesize as to what these other factors may be in the discussion below.

<u>Assessment of In-Situ Effects of Fe on Stream Biota</u>

<u>Macroinvertebrates</u>

An assessment of the benthic community was conducted utilizing one benthic sample from each location, with macroinvertebrates being processed at EA's environmental toxicology laboratory. The specimens of most taxonomic groups were identified to the most practical taxon, in most cases genus, depending on maturity and condition. Cattail Creek and Magothy River had the greatest invertebrate abundance, resulting in 7,642 and 4,990 collected individuals, respectively. Comparatively, Rock Branch and Dividing Creek had the lowest abundance of 24 and 219 individuals, respectively. Despite low invertebrate abundance Rock Branch had comparatively high local diversity and evenness (likewise for Cowhide Branch) considering the other sampling sites (Fig 10). The most dominant taxa within sites is the chironomid, which ultimately decreases the diversity and evenness of invertebrates within the Magothy River and Cattail Creek, where chironomids comprise > 75% of individuals identified. There was no significant effect of total Fe on Shannon diversity ($p = 0.19$; $R^2 = 0.24$) nor was there a significant effect of total Fe on evenness ($p = 0.14$; $R^2 = 0.33$).

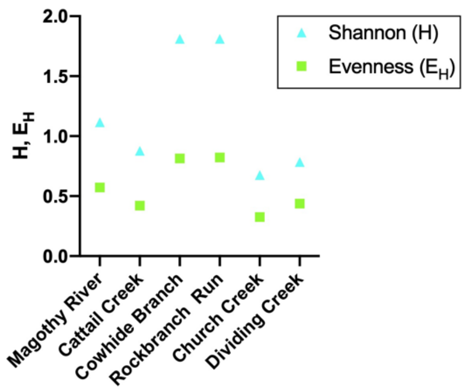

Figure 10. Local Diversity of Macroinvertebrate Communities

Discerning effects of specific stressors on streams in the environment is extremely challenging given the multitude of factors that can cause effects on stream biota. In an effort to better evaluate effects of Fe on stream macroinvertebrates, we obtained stream assessment data from DNR that included benthic indexes of biotic integrity (BIBI) scores for 16 stream sites in AAC from 2019 and 2020. We subsequently collected water samples in June and July of 2021 and measured Fe at the exact sites corresponding to the BIBI scores. Because the iron and benthos data were not collected concurrently, caution is warranted in interpreting these results. Nonetheless, there is evidence that Fe as well as the biological community are somewhat stable over a short span of years. We explored the relationship between total and ferrous iron and BIBI scores using regression analysis. The strongest model evaluated was an exponential regression model for ferrous iron; other models that included total iron yielded slightly worse fit statistics and did not yield any significant results. The exponential regression of all BIBI scores (16 sites) yielded a statistically significant effect of ferrous iron on BIBI scores (Fig. 11.A.; $p = 0.040$) although the model fit was poor ($R^2 = 0.23$). The relationship shown in Fig. 11.A. appears to be driven strongly by a single data point, the site with a Fe^{2+} concentration of about 14 mg/L. This is an extremely high iron concentration. We re-ran the analysis excluding the highest Fe^{2+} site and the regression was no longer significant ($p = 0.14$) and the fit of the model worsened ($R^2 = 0.11$). As well, there is no obviously apparent pattern of BIBI scores for Fe2+ concentrations below 4 mg/L. To place this concentration in context, we only observed Fe^{2+} concentrations greater than 4 mg/L at one site: Broad Creek (at the Health Department) at the tail end of a series of step pools where the stream was little more than a trickle.

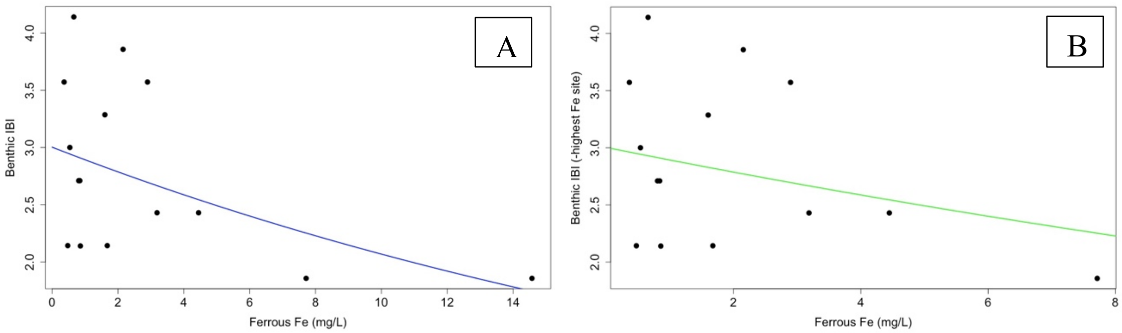

Figure 11. Exponential regression analyses to evaluate the relationship between Fe^{2+} and BIBI scores for stream data obtained from DNR. Figure 11.A yielded a significant effect but removing the highest iron site resulted in a non-significant relationship. In both cases, R^2 values were very low (see text for details).

As mentioned, it is very challenging to discern effects of specific stressors on biological entities in the field given the multitude of factors that influence streams. We sought to further address potential effects of Fe on stream invertebrates by conducting an exposure and effects experiment in a subset of AAC streams. This approach has been used to also assess impacts of acid mine drainage on stream invertebrates (DeNicola and Stapleton 2002). There were no discernable effects of iron or the different stream conditions and water quality parameters on caddisflies that were added to stream enclosures (Fig. 12). Results of the logistic regression showed no significant effect of total Fe on survival of caddisflies ($p = 0.42$) nor Fe2+ on caddisfly survival ($p = 0.96$). These results corroborate those of the mesocosm in which caddisflies were not sensitive to Fe in those systems. Importantly, the average survival of caddisflies in the enclosures in the Magothy River, which was the control, was 0.83 with overall survival on the order of 0.70. These data suggest that the field experiment was robust as acceptability criteria for laboratory studies often require 80% survival or better in control treatments.

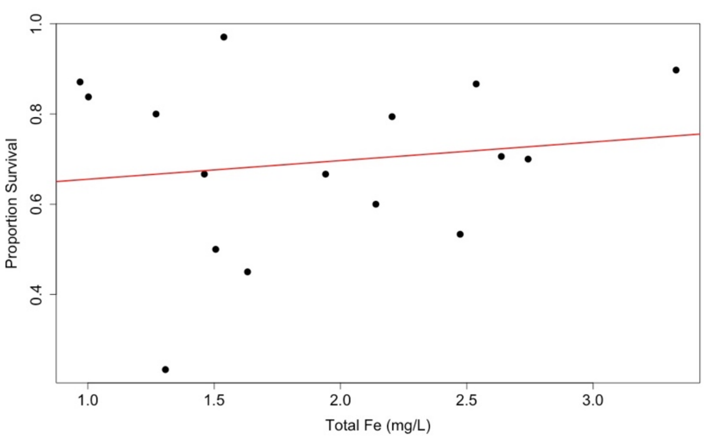

Figure 12. Relationship between Proportion Survival of caddisflies in enclosures and Total Fe measured in streams. Logistic regression was used to statistically analyze these data, but a linear regression is plotted for ease of interpretation. See text for details.

Fish Community

Biodiversity indices were calculated on the fish communities of the streams sampled to provide insight into the health of the fish community structure. Local diversity was higher in both the Magothy River and Cattail Creek (Figure 13). Furthermore, evenness of the communities was slightly greater in these waterbodies compared to the others sampled. The other sites had relatively similar local diversity and evenness of the fish communities, although Church Creek had notably less evenness compared to other sites because 87% of the fish sampled were Eastern Mosquitofish (*Gambusia affinis*).

Shannon diversity was regressed against total iron (Fe_{tot}) to provide insight into potential effects of iron on the local diversity of the fish community (Fig. 14). While there is a trend of reduced Shannon diversity with increasing iron concentrations, this was not statistically significant.

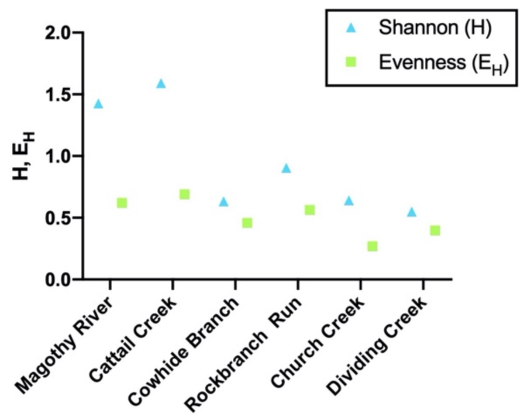

Figure. 13. Local Diversity of Fish Communities

In addition to local diversity, compositional turnover (or beta-diversity) was also investigated. While these data are not statistically robust in the current form, there is an obvious visual trend in fish community grouping with the assigned iron category (Figure 15). Again, it is important to note the preliminary nature of the compositional turnover model. More data are needed to make a stronger statement on potential impacts of iron to beta diversity of the fish community. Data collection in conjunction with AAC or MD DNR annual sampling would be a great way to fold in iron analysis and increase the robustness of this dataset to provide a clearer picture. This is would a be a cost and energy effective approach to supplementing these preliminary findings.

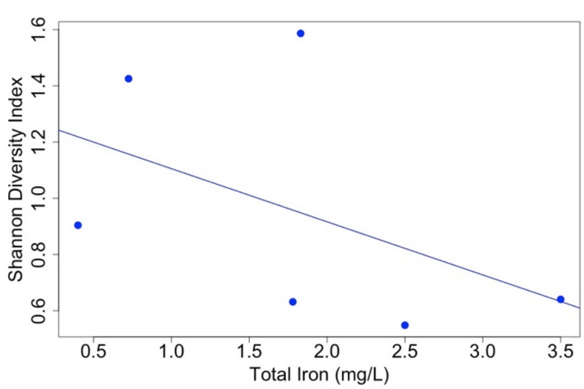

Figure. 14. Regression of Shannon Index of fish communities and mean total iron concentrations of sampling sites.

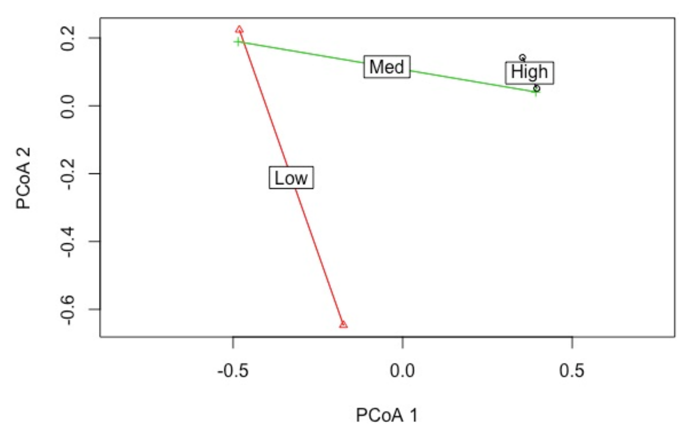

Figure. 15. Principle coordinates analysis of compositional turnover of fish communities grouped by low, medium and high iron concentrations.

As Maryland Department of Natural Resource and other regulators typically favor commonly used indexes for grading stream health, we calculated the Fish Index of Biotic Integrity (F-IBI). Interestingly, Cattail Creek has the lowest F-IBI score of 2.25 (Figure 16). The divergence in this score versus local diversity indices is due to the F-IBI weighting metrics, whereas local diversity does not account for differences in species sensitivity or feeding preferences. While Cattail Creek does have comparatively high diversity of the fish present, there are low numbers of benthic feeders, intolerant species and overall low biomass of species. It is worth noting that this location had restoration completed in 2019 and is therefore also the "youngest" of the RSC systems.

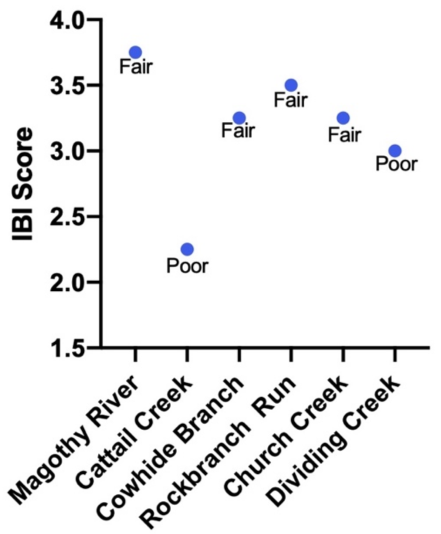

Figure. 16. Fish Index of Biotic Integrity (F-IBI) for streams sampled.

To better inform our understanding of the effects of iron in AAC stream on the fish community, we were successful in collaborating with Anne Arundel County and MD Department of Natural Resources (DNR) to incorporate additional fish community data that they have collected. As in the analysis of DNR BIBI data, we obtained Fish Indexes of Biological Integrity (FIBI) scores for five stream sites in addition to the sites we sampled (e.g., Fig. 16), however, one of the sites was undergoing restoration when we were sampling water for iron analysis. As such, the exploration of effects of stream iron on FIBI scores was based on 10 stream sites. As with the BIBI scores, these data should be interpreted with caution because of the temporal mismatch between when DNR FIBI scores were obtained (2019 and 2020) and when we obtained stream chemistry data (summer 2021). A linear regression exploring the effects of total Fe on FIBI scores showed no significant relationship ($p = 0.55$), however, the relationship was significant for Fe^{2+} ($p = 0.028$; Figure 17). Importantly, the fit of the model was poor ($R^2 = 0.41$), although it is better than the corresponding model for BIBI scores. There were three sites from the DNR data in Mill Creek with identical FIBI scores (1.67). Although these might be considered different sites there is some element of pseudoreplication by treating these all as independent. We re-analyzed the data combining the three Mill Creek sites into a single composite Mill Creek site by using the arithmetic average. Although the relationship is similar (Fig. 17.B.) there was not a statistically significant effect ($p = 0.076$) and model fit worsened ($R^2 = 0.34$). Nonetheless, these data are suggestive that adverse effects to fish associated with Fe^{2+} might occur in some AAC streams that have relatively higher iron than others.

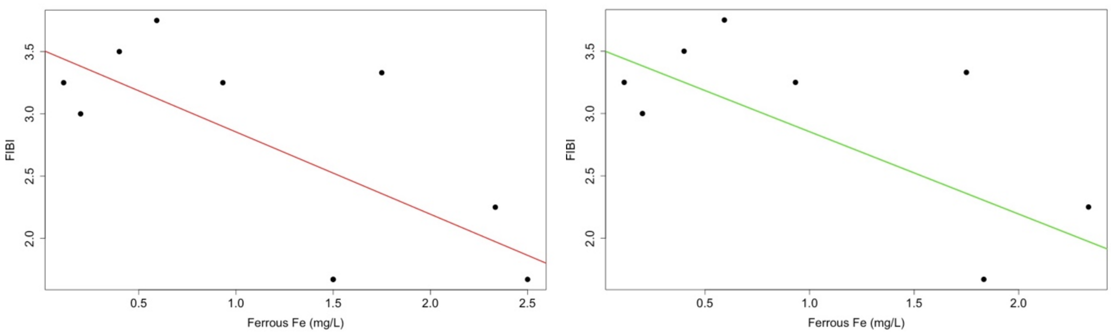

Figure. 17. FIBI as a function of Fe2+ concentrations for (A) the full dataset as provided by DNR and (B) combining Mill Creek samples into a single composite. See text for details.

Conclusions

Based on temporal stream water sampling, streams are relatively consistent with regard to iron concentrations. That is, streams with low iron tend to have low iron concentrations throughout the year and vice versa for streams with high iron. That said, we have observed that iron concentrations vary more as iron concentrations increase but that, again, the overall variability is low as evidenced by coefficients of variation that are generally less than 1.0. Importantly, we have also observed that the highest iron concentrations have been measured in streams with RSCs. The most likely potential explanation for this is that the RSCs facilitate reconnection of the stream to the surrounding habitat and groundwater which likely serves to move iron from surrounding soils to the stream. We have observed in a number of cases, the obvious presence of iron in surrounding seeps and soils that lends supports to this point. Moreover, when we consider the lack of strong relationships between water quality parameters and Fe concentrations in streams, we hypothesize that the key determinant of Fe in streams is Fe in the surrounding soil. As a good example of this, see Fig. 18 that shows a small tributary or seep to Broad Creek at Defense Highway, one of our stream sites. This clearly shows high Fe concentrations in the tributary leading to the main stream. Fe concentrations in the main stream; however, are relatively low. The highest Fe concentrations in streams were observed at the tail-end of one RSC, Broad Creek (Health Dept.). In this case, this is a very steep "stream" that functions more as intermittent

Figure 18. Seep entering Broad Creek at Defense Highway, Anne Arundel County, MD

catchments to help in dissipating the energy of the water during storm events. Where we measured high Fe concentrations was little more than a trickle emerging from the last step pool.

When we consider whether adverse effects to the biological stream community are occurring as a result of Fe, there is little evidence that Fe is causing adverse effects over and above what likely results from considerable impervious surface leading to urban stream syndrome. That said, when we explored the relationship between ferrous Fe and BIBI scores, there was a statistically significant relationship although there was considerable variability in the dataset. Moreover, when an extremely high Fe site was removed from the analysis, the relationship was no longer significant. Our own stream invertebrate sampling as well as a field experiment using caddisflies in an enclosure did not suggest strong, adverse effects to the invertebrate communities observed nor in the caddisflies within enclosures. This is not to say effects of Fe are not possible. It may be that Fe is more toxic to other species (maybe amphipods using results from the mesocosm exposure) but these are not common in AAC streams that we assessed.

Currently, there are trends that suggest iron may impact the fish community assemblages. An analysis of the data we collected and the associated Shannon Diversity Index showed a significant negative relationship as Fe increased. Interestingly, when we analyzed FIBI scores as a function of ferrous Fe concentrations, we saw a significant negative effect of increasing Fe concentrations. That said, similar to the invertebrate data, when we removed a high Fe stream the relationship was no longer significant ($p = 0.076$). Nonetheless, it appears possible that dissolved Fe may adversely effect fish which may occur through interactions with the gill. Field data is inherently variable making it a challenge to see the "signal through the noise". Additional studies focused on fish may be warranted to better understand whether Fe is really causing adverse effects to these species.

Challenges that have impacted the project

In addition to the previously mentioned challenges of the pandemic coinciding with this project kick start, there were some other challenges imposed by nature. For example, Church Creek near Wilielinor experienced catastrophic failure following a severe rain even in early spring of 2021. This prevented routine sampling of this site as the RSC was destroyed. Obviously, occasional rain events and or weather related delays occurred during planned routine sampling events; however, these were recoverable.

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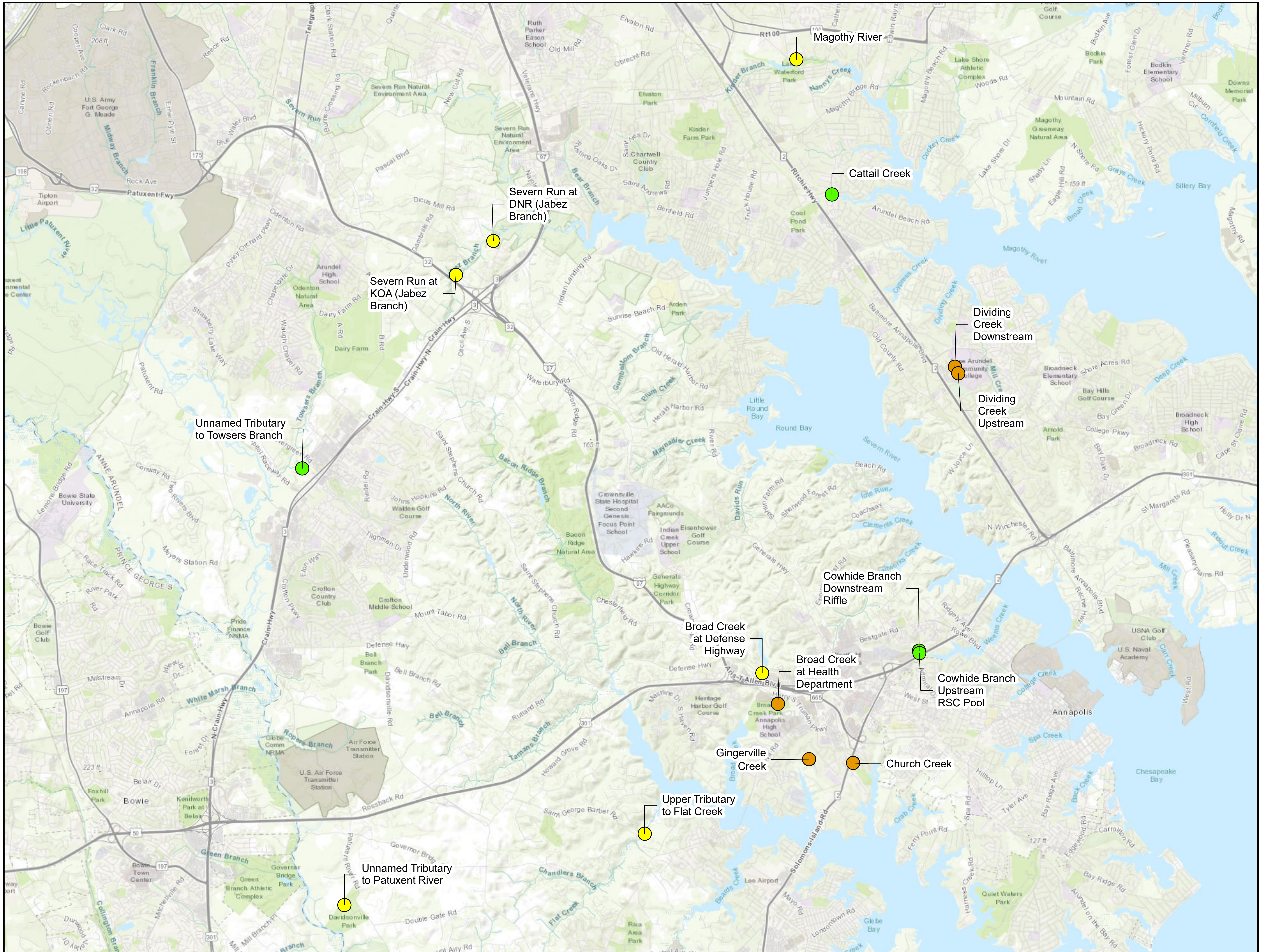
Appendix

Appendix A

Sampling Locations and Photolog



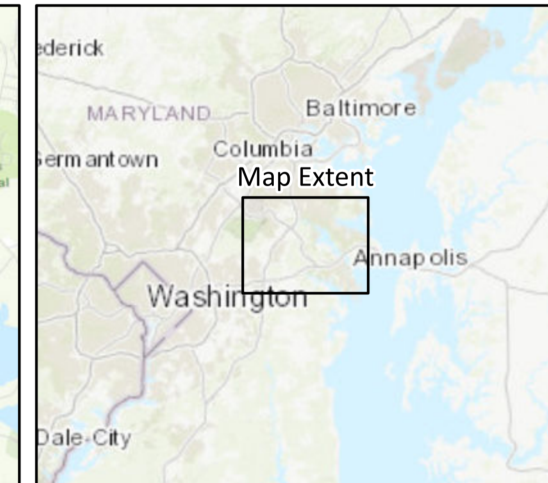


Figure 1-1
Floc Categories
Chesapeake Bay Trust Iron in Restored Streams
Chesapeake Bay, Maryland

Low Iron Sites

Figure 1. Stocketts Run, Natural Site

Figure 2. Rock Branch, Natural Site

Figure 3. Magothy Run, Natural Site

Figure 4. Severn Run DNR, Natural Site

Medium Iron Sites

Figure 5. Cowhide Branch, RSC site

Figure 6. Cattail Creek, RSC site

Figure 7. Severn Run KOA, Natural Site

High Iron Sites

Figure 8. Ginger Creek, RSC Site

Figure 9. Dividing Creek, RSC Site

Figure 10. Broad Creek (Health Department), RSC Site

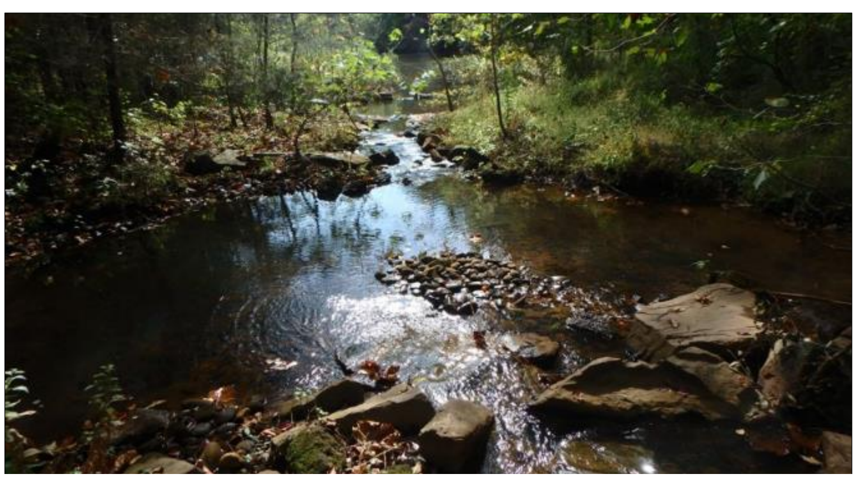


Figure 11. Church Creek, RSC Site

Appendix B
Raw Data Macroinvertebrate Field Sampling

RESULTS

Taxa	Magothy				Wilelinor (Church Creek)				Dividing Creek			
	Run[a]		Riffle[a]		Run		Riffle		Run		Riffle	
	Total (#)	Percent (%)	Total (#)	Percent (%)	Total (#)	Percent (%)	Total (#)	Percent (%)	Total (#)	Percent (%)	Total (#)	Percent (%)
Oligochaeta (Worm)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	6	2.8
Chironomidae (Midge)	2736	84.2	744	42.8	499	87.9	213	80.7	1	100.0	169	77.5
Tipulidae (Crane Fly)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Similiidae (Black Fly)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	7	3.2
Diptera pupae	114	3.5	96	5.5	0	0.0	0	0.0	0	0.0	0	0.0
Gammarus (Amphipod)	0	0.0	0	0.0	23	4.0	40	15.2	0	0.0	0	0.0
Zygotpera (Dragonfly)	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0	3	1.4
Anisoptera (Damselfly)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Physella (Snail)	0	0.0	36	2.1	5	0.9	1	0.4	0	0.0	0	0.0
Planorbella (Snail)	232	7.1	72	4.1	2	0.4	0	0.0	0	0.0	2	0.9
Pisidiidae (Bivalve)	120	3.7	228	13.1	0	0.0	1	0.4	0	0.0	0	0.0
Hydropsychidae (Caddisfly)	48	1.5	180	10.3	1	0.2	7	2.7	0	0.0	31	14.2
Hirudinea (Leach)	0	0.0	0	0.0	36	6.3	0	0.0	0	0.0	0	0.0
Colepoptera (Beetle)	0	0.0	384	22.1	0	0.0	0	0.0	0	0.0	0	0.0
Isopoda	0	0.0	0	0.0	0	0.0	2	0.8	0	0.0	0	0.0
TOTAL	3250		1740		568		264		1		218	
TAXA RICHNESS	5		7		7		6		1		6	
EPT TAXA RICHNESS	1		1		1		1		0		1	

(a) Sample was sub-sorted at 1/6.

RESULTS

Taxa	Cattail Creek				Cowhide				Rock Branch			
	Run		Riffle[(a)]		Riffle		Pool		Run		Riffle	
	Total (#)	Percent (%)	Total (#)	Percent (%)	Total (#)	Percent (%)	Total (#)	Percent (%)	Total (#)	Percent (%)	Total (#)	Percent (%)
Oligochaeta (Worm)	0	0.0	204	2.7	1	1.0	30	7.4	1	11.1	1	7.1
Chironomidae (Midge)	39	67.2	5472	72.2	26	25.5	120	29.4	3	33.3	7	50.0
Tipulidae (Crane Fly)	0	0.0	24	0.3	0	0.0	0	0.0	0	0.0	3	21.4
Similiidae (Black Fly)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Diptera pupae	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	7.1
Gammarus (Amphipod)	6	10.3	48	0.6	19	18.6	68	16.7	1	11.1	0	0.0
Zygotpera (Dragonfly)	0	0.0	0	0.0	0	0.0	3	0.7	0	0.0	0	0.0
Anisoptera (Damselfly)	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	1	7.1
Physella (Snail)	0	0.0	0	0.0	9	8.8	10	2.5	0	0.0	0	0.0
Planorbella (Snail)	0	0.0	24	0.3	1	1.0	81	19.9	0	0.0	0	0.0
Pisidiidae (Bivalve)	0	0.0	0	0.0	0	0.0	11	2.7	2	22.2	0	0.0
Hydropsychidae (Caddisfly)	9	15.5	1404	18.5	38	37.3	2	0.5	1	11.1	1	7.1
Hirudinea (Leach)	4	6.9	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Colepoptera (Beetle)	0	0.0	408	5.4	0	0.0	0	0.0	0	0.0	0	0.0
Isopoda	0	0.0	0	0.0	8	7.8	82	20.1	1	11.1	0	0.0
TOTAL	58		7584		102		408		9		14	
TAXA RICHNESS	4		7		7		10		6		6	
EPT TAXA RICHNESS	1		1		1		1		1		1	

(a) Sample was sub-sorted at 1/12.