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## **Influence of Nutrient Contamination on Macroinvertebrate Communities in Two Tidal Freshwater Creeks**

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## Introduction

Freshwater ecosystems are some of the most biodiverse ecosystems in the world. Freshwater ecosystems are also facing a rapid losses of this biodiversity. One human driven cause of these losses is nutrient runoff from high fertilizer use and increased impervious surfaces. Macroinvertebrates have long been used as indicators of overall stream health because they are relatively easy to survey, and different species of macroinvertebrates have different sensitivities to pollution. While the research is clear that in areas of high pollution, macroinvertebrate biodiversity is lowered, further research is needed to examine the relationship between macroinvertebrate biodiversity and nutrient overloading. Additionally, much less is known about the effects of nutrients in tidal freshwater streams compared to high gradient rocky bottom streams.

This study has 3 objectives: a) to examine the differences in macroinvertebrate diversity between upstream sample sites and downstream sample sites b) to examine the differences in macroinvertebrate community diversity between Accokeek Creek and Aquia Creek and c) to investigate the relationship between nutrient concentration and macroinvertebrate community assemblage. Because we expect nutrient levels to be higher downstream, we hypothesize that upstream sites will have higher macroinvertebrate biodiversity compared to downstream sites. We also hypothesize that biodiversity will be higher in Accokeek Creek than Aquia Creek due to the higher predicted levels of nutrients in Aquia Creek.

## Study Sites

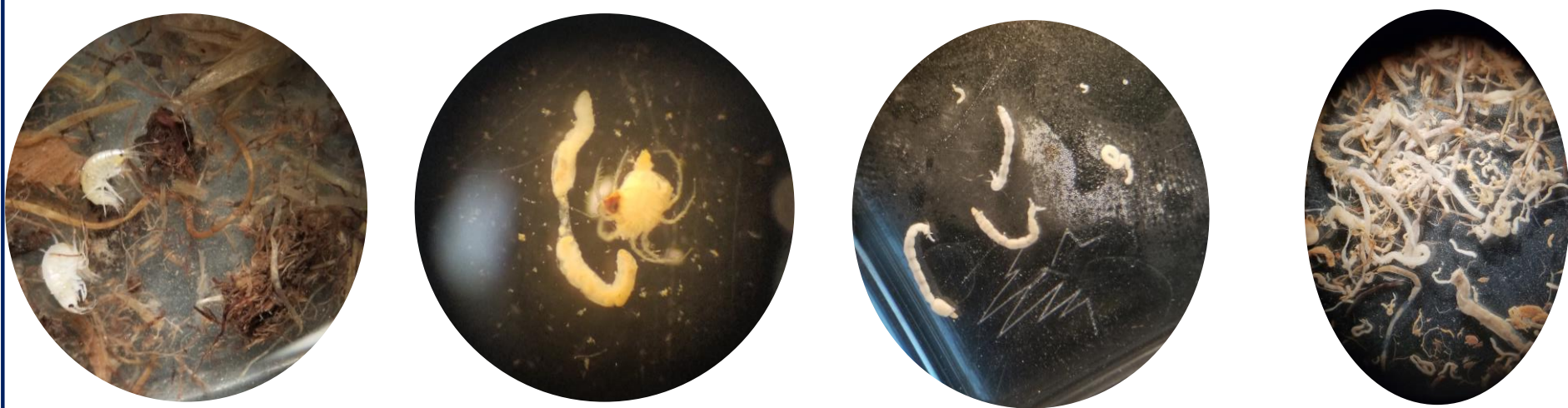
Accokeek Creek and Aquia Creek are two tidal, soft bottom, freshwater streams found within Stafford County, VA.

### Accokeek Creek

Accokeek Creek is found partly within the Crow's Nest Natural Preserve Area which is nearly 3,000 acres of state-owned land. Because of this, upstream sites are completely free of human development. Downstream, development is still low with several houses found along the northern bank of the stream. Sampling was done at six Accokeek Creek sites on May 25<sup>th</sup>, 2021, and June 1<sup>st</sup>, 2021.

### Aquia Creek

Aquia Creek has higher levels of development including a marina, residential development, and a railroad that crosses over the creek. The differing nature of the creeks will allow us to compare differences in macroinvertebrate composition between areas of higher and lower development. Sampling was done at three Aquia Creek sites on June 8<sup>th</sup>, 2021, and one Aquia Creek site on June 16<sup>th</sup>, 2021.



Scuds (Family Gammaridae) Worm (Subclass Oligochaeta) and Water Mite (Family Hydrachnida) Midges (Family Chironomidae) A variety of worms (family Oligochaeta) and midges (subclass Chironomidae)

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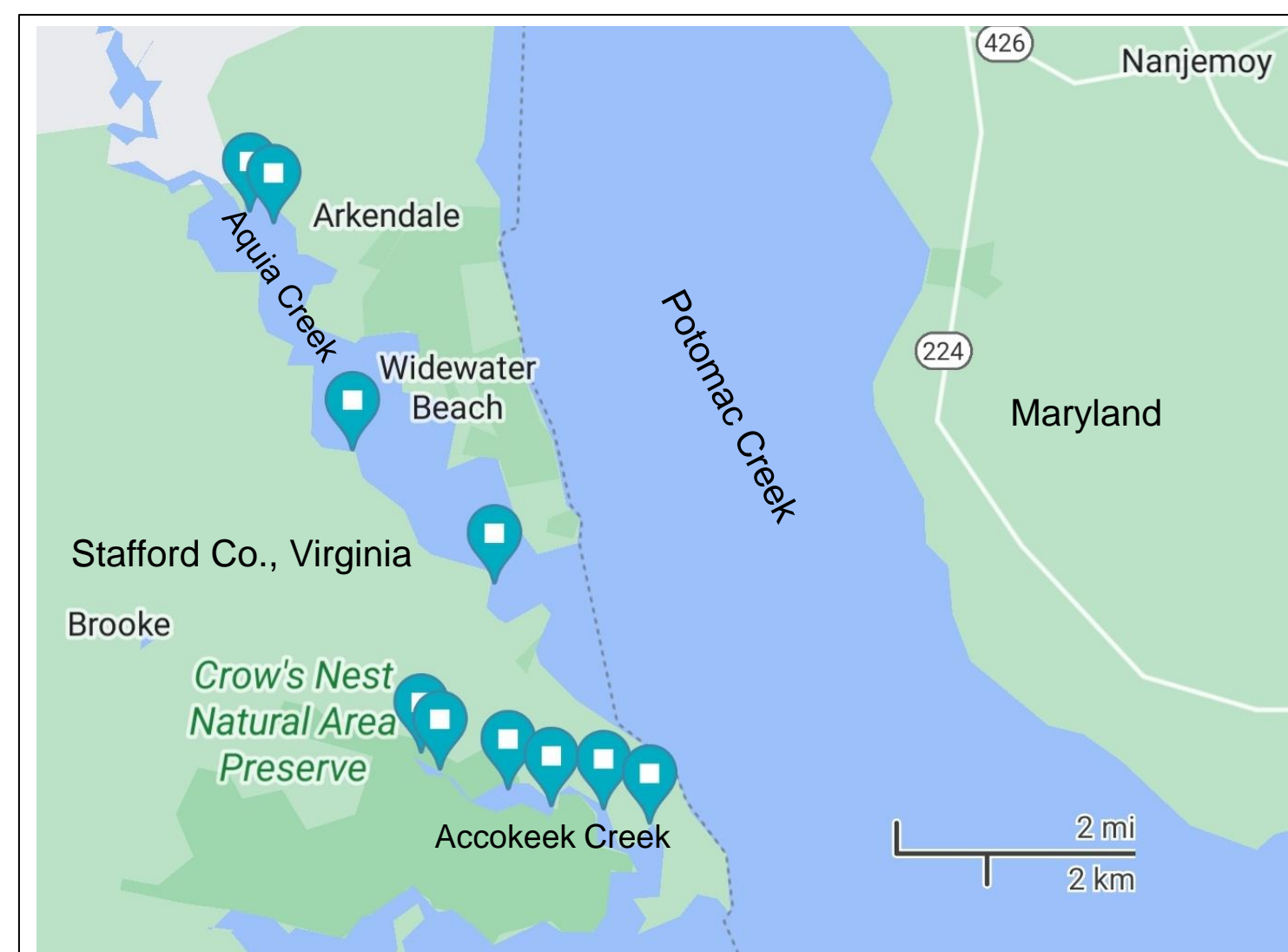


Figure 1. Map of sites found at Accokeek Creek and Aquia Creek in Stafford County, Virginia. Each teal marker represents one site where three macroinvertebrate samples were collected in late Spring 2021.

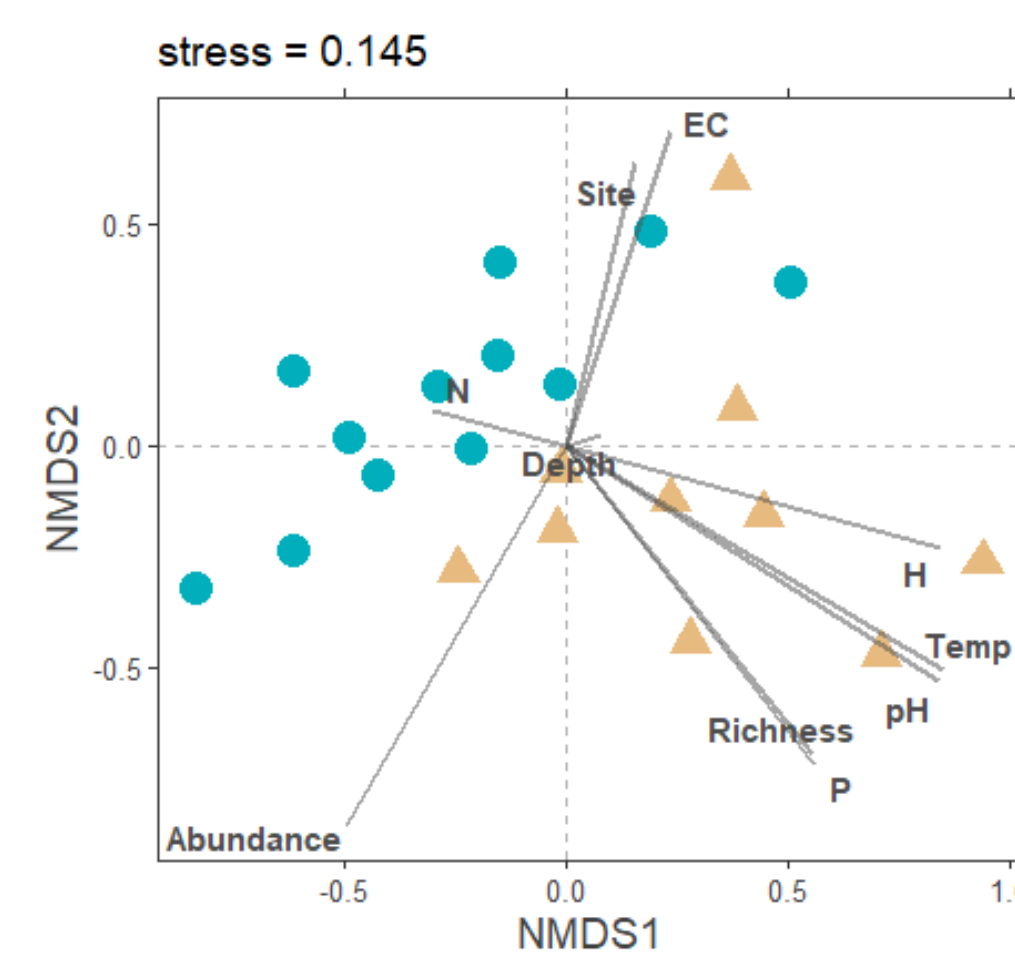


Figure 3. Non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis dissimilarities of macroinvertebrate taxa composition of 22 sites. Sites are color-coded by Stream. Arrows indicate parameters associated with species composition at each site. Arrow length is proportional to the degree of correlation between the parameters and the ordination. Orthophosphate level ( $p=0.004$ ), Shannon Diversity ( $p=0.001$ ), richness ( $p=0.001$ ), abundance ( $p=0.001$ ), temperature ( $p=0.001$ ), pH ( $p=0.001$ ), and electroconductivity ( $p=0.016$ ) are the best predictors.

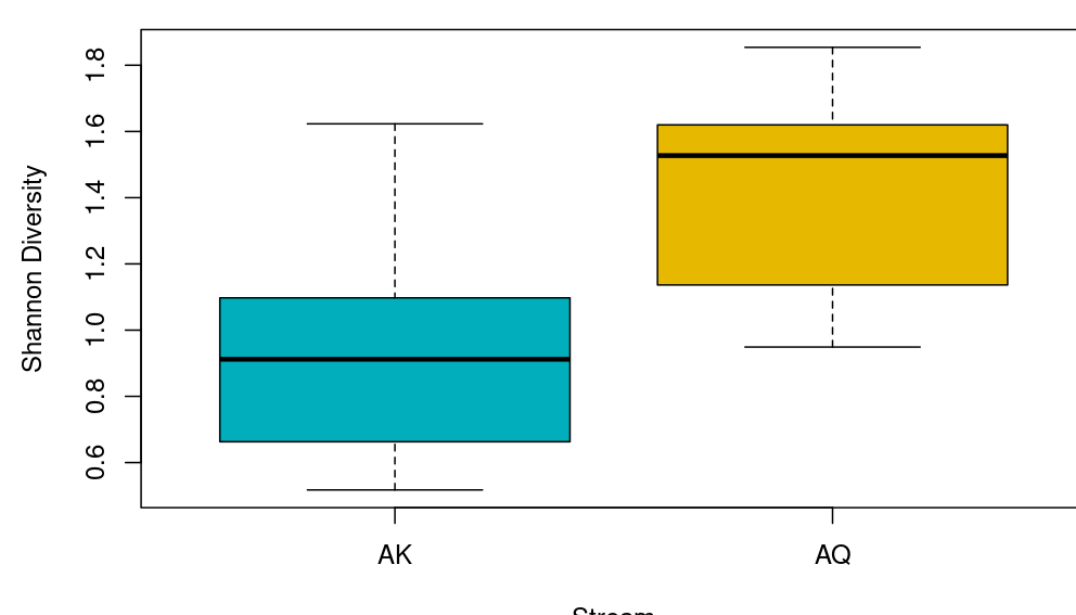


Figure 4. Box plots comparing macroinvertebrate Shannon Diversity between Accokeek Creek (n=12) and Aquia Creek (n=10) ( $t(20) = -3.4$ ,  $p=0.003$ ).

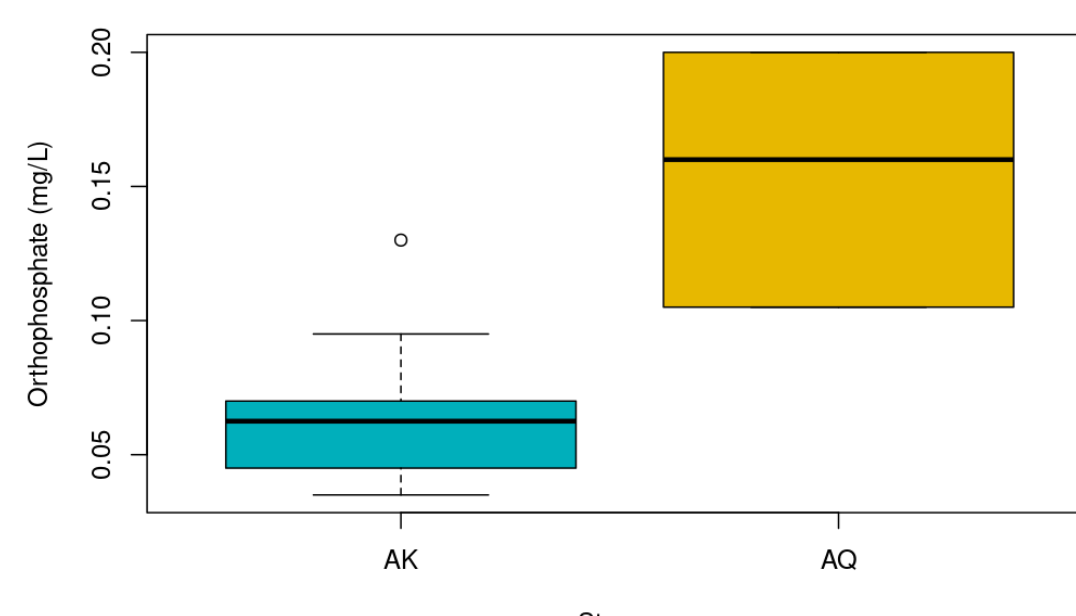


Figure 5. Orthophosphate levels by Accokeek Creek and Aquia Creek. This figure shows box plots of the stream-related variation in orthophosphate levels. Orthophosphate levels are significantly higher in Aquia Creek (n=10) than in Accokeek Creek (n=12) ( $t(20) = -6.3$ ,  $p=0.000004$ ).

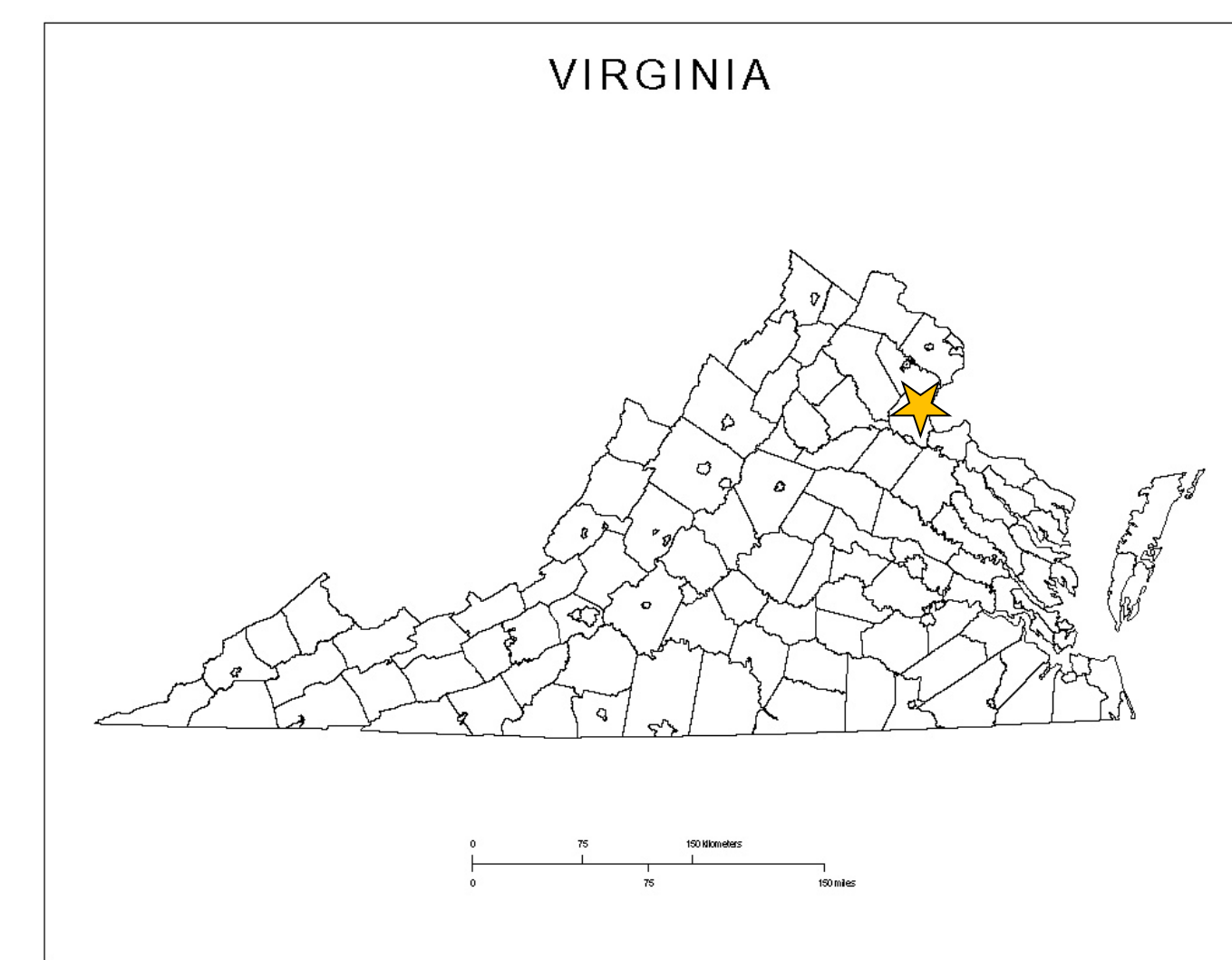


Figure 2. Map showing the locations of Accokeek Creek and Aquia Creek in Stafford County, Virginia. Location indicated by star.

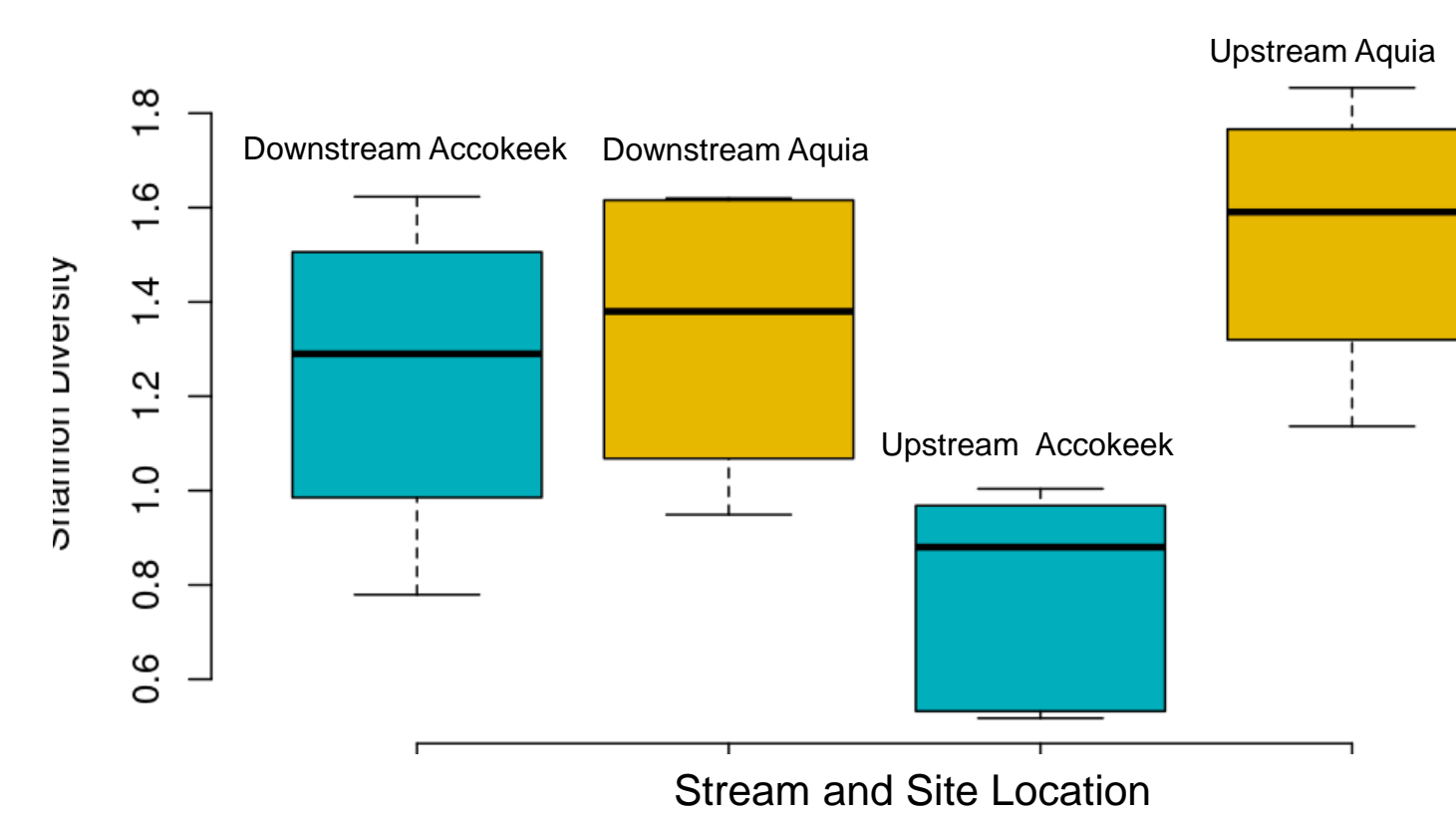


Figure 6. Box plots comparing Macroinvertebrate Shannon Diversity between sites (Accokeek and Aquia) and site location (upstream and downstream). There was a statistically significant interaction between the effects of stream and site location [two-way ANOVA  $F(1, 18)=7.03$ ,  $p = 0.01624$ ]. Upstream Accokeek sites had a mean Shannon Diversity that was significantly lower than both upstream Aquia sites ( $p=0.0019$ , Tukey's HSD post hoc tests) and downstream Aquia sites ( $p=0.01624$ ).

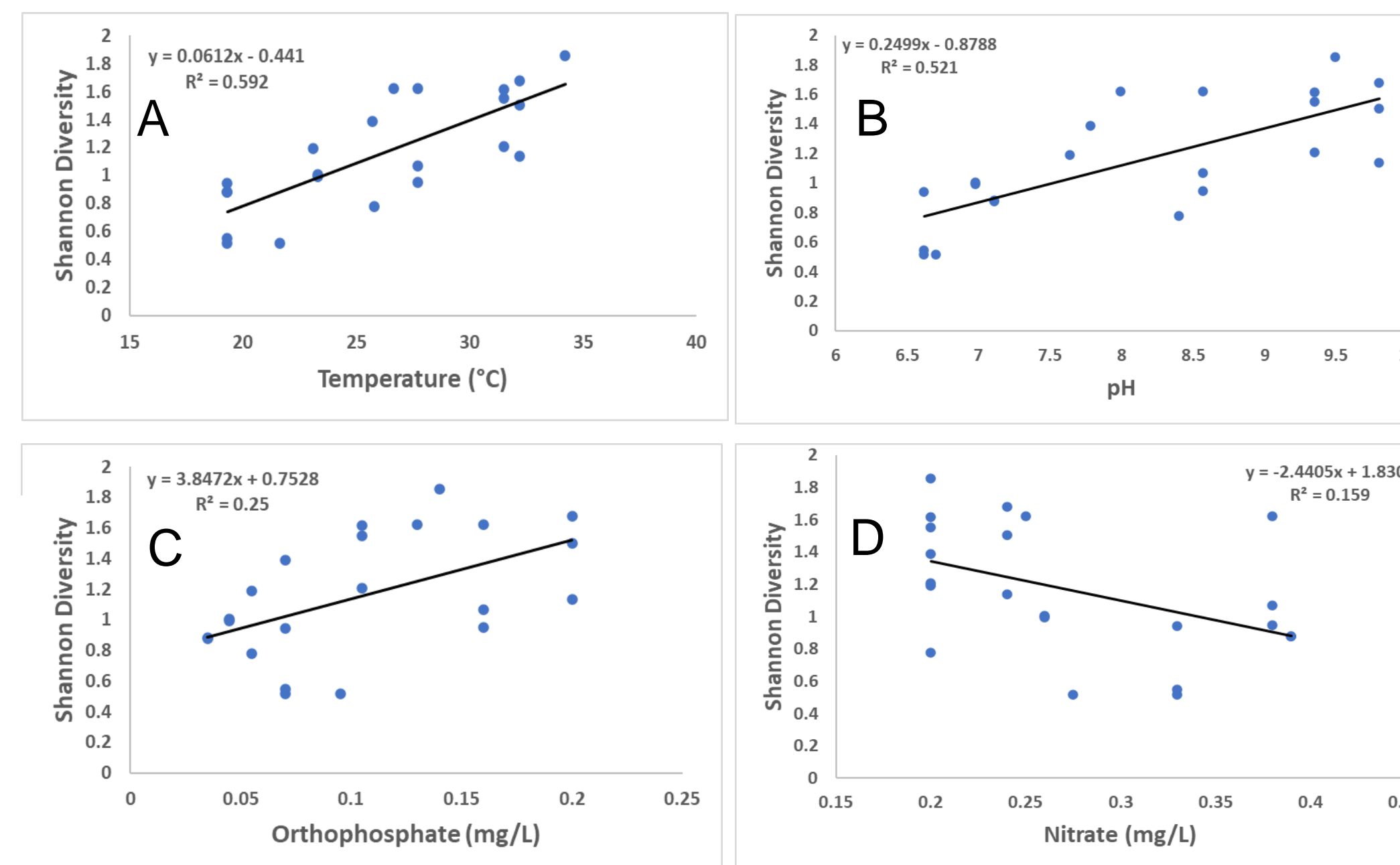


Figure 7. Linear regressions depicting variables that predict Shannon Diversity of macroinvertebrate communities. Temperature (A) [ $R^2=0.592$ ,  $p=0.0000174$ ], pH (B) [ $R^2=0.521$ ,  $p=0.0000897$ ], orthophosphate (C) [ $R^2=0.25$ ,  $p=0.0104$ ] levels, and nitrate levels (D) [ $R^2=0.159$ ,  $p=0.0372$ ] each significantly predict the Shannon Diversity of macroinvertebrate communities.

## Methods

### Surface Water Sampling

- Water samples were taken at each site.
  - Nitrate levels measured with the Hach TNT 835 Nitrate test kit (Loveland, CO).
  - Orthophosphate levels were measured with the Hach Reactive Phosphorus PhosVer 3 (Ascorbic Acid) test kits (Loveland, CO).

### Field Procedures

- Macroinvertebrate surveys were conducted at six Accokeek Creek sites on May 25, 2021, and June 1<sup>st</sup>, 2021, and at four Aquia Creek sites on June 8<sup>th</sup>, 2021. Three samples were collected at each site for a total of 36 samples. The two uppermost sites and two lowermost sites from Accokeek were used for analysis.
- Scoops of sediment with an approximate volume of 25.13 in<sup>3</sup> were collected from the bottom of the creeks, preserved in 95 percent ethanol, and refrigerated until lab work began.
- Water temperature, pH, electroconductivity, and dissolved oxygen (ppm and percent saturation) were measured for each site using a Hanna Instruments multiparameter meter model # HI98194 (Woonsocket, RI). Depth was measured for each of the three samples taken per site.

### Lab Procedures

- Using a randomized grid system, macroinvertebrates were removed from the sample, identified to the family level, and placed in vials of 70 percent ethanol.

## Results

- Of the 32 samples to be analyzed, 22 (12 Accokeek and 10 Aquia) have been processed at this time and were used for the following statistical analyses.
- A total of 1,874 macroinvertebrates across 25 taxa were identified with the most common taxa being Oligochaeta (n=954), Chironomidae (n=314), and Hydrobiidae (n=191).
- Shannon Diversity was significantly higher in Aquia Creek (1.4184±0.0959 mean±SE) than in Accokeek Creek (0.9387±0.099),  $t(20) = -3.4$ ,  $p=0.003$  (see Figure 4).
- Orthophosphate levels are significantly higher in Aquia Creek (0.1535mg/L±0.012) than in Accokeek Creek (0.0646mg/L±0.008,  $t(20) = -6.3$ ,  $p=0.000004$ . (See Figure 5).

## Discussion

- Our hypotheses predicting that upstream sites and Accokeek Creek would have higher levels of biodiversity were both proven to be incorrect. Rather, Aquia Creek was found to have statistically higher macroinvertebrate biodiversity, and no significant difference in biodiversity was found between upstream sites and downstream sites.
- Orthophosphate levels were found to be positive predictors of Shannon Diversity while nitrate levels were negative predictors. Of the habitat data collected, only pH and temperature were found to positively predict Shannon diversity.
- The EPA has set the recommended nitrate level at 10 mg/L and the recommended orthophosphate level at 0.1 mg/L. Nitrate levels were well below the recommended level in both Accokeek Creek and Aquia Creek. Orthophosphate levels in Accokeek Creek were below the recommended level, but in Aquia Creek, orthophosphates levels were found to be above the recommended level. Aquia Creek, which had significantly higher orthophosphate levels than Accokeek Creek,
- The positive relationship between orthophosphates and macroinvertebrate diversity is likely explained by an increase in resource availability caused by macrophyte growth.
- Past literature supports the findings that pH is a positive predictor of macroinvertebrate diversity as a low pH may weaken exoskeletons and shells of macroinvertebrates.

## Acknowledgements

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