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Evaluating macroinvertebrate communities at the nexus of freestone and limestone streams

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Abstract

The Yellow Breeches, a tributary of the Susquehanna River, is a freestone stream flowing 49 miles through limestone-dominated valleys. The character of the stream changes as limestone streams join it at several points, altering the bedrock, formation and water source. Stream chemistry and macroinvertebrate communities consequently change in conjunction with the physical and chemical transformations. As cornerstones of the food chain and ecosystem, shifts in these populations can have widespread effects on the stream community as a whole. It is essential to determine factors promoting community changes to be able to accurately determine the conservation measures that can be safely taken without changing the overall ecosystem structure. Therefore, this project strives to assess whether there is significant difference between macroinvertebrate communities in the two streams as they join and if one exists, to identify the chemical and physical parameters contributing to that shift. To accomplish this, visual assessments, nutrient analysis, and macroinvertebrate sampling were performed at eleven sites within thirty meters of the mixing site. Preliminary data reveals significant difference in macroinvertebrate communities in the limestone and freestone influenced sites in some keystone species, as well as significant differences nearly all chemical parameters and only one physical parameter, substrate composition. Additional testing will be performed at this site as well as two additional sites to further specify the cause of the change in community structure and composition.

Introduction

Benthic macroinvertebrates are animals without backbones, dwelling primarily in rocks, sediments, debris, and aquatic plants. This group encompasses a wide variety of organisms, including crayfish, mollusks, and aquatic worms and insects. Macroinvertebrates have a nearly ubiquitous distribution in aquatic habitats due to a large array of tolerance levels and habitat requirements. They occupy a wide variety of trophic levels, some acting as predators while others as processors of detritus and organic material. They are an essential part of the food chain by feeding on algae and bacteria while simultaneously being consumed by fish and larger organisms. As intermediates in the food chain, their absence would disrupt the natural flow of energy and nutrients (Maryland Department of Natural Resources 2004).

Alterations in the community structure of macroinvertebrates could drastically change the overall aquatic ecosystem; however, there is a dearth of information on exactly which ecological factors significantly impact macroinvertebrate communities. Several studies (Smith and Wood 2002; Jackson, Gibbins, and Soulsby 2007; Russell 2010) have demonstrated that perhaps the most critical factor determining macroinvertebrate communities is velocity. The existence of dams significantly reduces the organisms present in orders intolerant of flow change, such as Plecoptera and Ephemeroptera; tolerant orders, such as Diptera and Oligochates, however, show no significant change upstream or downstream of dams (Jackson, Gibbins, and Soulsby 2007). Smith and Wood (2002) focused on temporary springs and found that no physical or chemical data affected community structure except for velocity.

In permanent streams, there is evidence to suggest the diversity is more likely a product of the interaction of multiple environmental determinants, particularly chemical factors, substrate, depth, and algae. High levels of nutrient loads from land runoff or pollution can eliminate or greatly reduce numbers of more sensitive species and allow tolerant species of Oligochaetes and Chironomids to dominate (Walsh et al 2005). Even in cases where chemical differences are natural, population shifts can occur because macroinvertebrates are adapted to specific chemical conditions. Additionally, substrate can have a significant effect on the ability of some organisms to survive. Some macroinvertebrates require large particles and interstitial space for predator protection, attachment sites for feeding, and increased oxygen exchange (Roy et. al., 2003), whereas others build protective cases and require either course or fine gravel-sand conditions (Neuswanger 2010). Finally, algae or vegetative growth can provide a microclimate sheltered from flow and abundant with organic material for food and case construction. This benefits several organisms, but too much can eliminate species that require bed surfaces free of algae (Jackson, Gibbins, and Soulsby 2007).

Although the factors influencing macroinvertebrate communities have been studied at several specific sites, such as temporary streams, dam sites, and polluted streams, little has been done at sites where different stream types intersect. Similar to the aforementioned sites, conditions change because the chemical and physical parameters in these streams are drastically different. This study in particular examines the nexus of a limestone and freestone stream. Conditions in these streams differ primarily in formation, underlying bedrock, and water source. Limestone streams originate from underground water sources, such as springs, so they form rapidly and fluctuate little with rainfall (Yellow

Breeches Conservation Plan 2005). A true limestone stream has a year-round temperature of 40-65°, an alkalinity above 140mg/L, and a maximum drainage area of twenty square miles. Conversely, a freestone stream grows slowly from a small trickle to a large river, gathering water from land runoff and rainfall. Consequently, there are wide fluctuations in both flow levels and temperature. Although many freestone streams have naturally higher diversity, they are in general less productive than limestone streams, with little algae, slightly acidic pH, and a plethora of gravel. Limestone streams are naturally fertile because of their high alkalinity and are also protected from acid precipitation because of their natural production of carbonate and carbon dioxide. (Yellow Breeches Conservation Plan 2005).

Studies have cited natural differences in macroinvertebrate communities between limestone and freestone streams. Macroinvertebrate populations in limestone streams are usually abundant and dominated by a few taxa such as Ephemeralla, Amphipoda, Isopoda, and Chironomidae, but naturally lack stonefly taxa; aquatic insects dominate in freestone streams. It is thought that macroinvertebrate assemblages differ because of environmental variations and the ecological requirements of each species rather than competition or interspecific interactions (Glazier and Gooch 1987). Despite some literature on the differences in community structure in limestone and freestone streams and additional information on some of the parameters influencing macroinvertebrates, there has been no clear connection between which of the changing parameters in the two streams is the cause of potential community differences.

This study attempts to answer that question by first determining if there are significant differences in communities between limestone and freestone streams where they join, and if there is, to determine the chemical and physical factors involved in that community shift. The site selected to examine this is where Trout Run, a limestone stream 7.2 miles long, joins the Yellow Breeches at Messiah College, Grantham, Pennsylvania. Yellow Breeches originates in Michaux State Forest and flows 49 miles until it empties into the Susquehanna River (Figure 1). The upper, western portions flow through freestone areas, whereas the lower, eastern areas are limestone influenced due to limestone bedrock and several limestone tributaries. Despite the limestone influence, there are still significant chemical and physical difference between the Yellow Breeches and Trout Run, making it an ideal site for this study. I believe that there will be a significant difference in the composition of the communities, and that substrate, velocity, and chemical parameters will be the primary determinants of that change.

Materials and Methods

Surveying occurred in 11 sites spanning a small section of the Yellow Breeches, a freestone stream, where it intersects with Trout Run, a limestone stream, at Messiah College in Grantham, Pennsylvania (Figure 2). Samples at control sites were taken in each stream; the remaining nine sites were in close proximity to the confluence of the two streams. Sites were selected in the Yellow Breeches 10m, 20m, and 30m downstream of where Trout Run enters (Figure 3). Each of those distances was divided into three zones based on conductivity resembling the Yellow Breeches, a mixing zone, and Trout Run.

Three components of stream health were assessed. A physical assessment of the stream was performed at each site. Habitat parameters measured include: velocity, depth, sediment deposition, and conductivity. Velocity and depth were assessed utilizing a General Oceanics flow meter and conductivity was measured with a Cole-Parmer conductivity meter. Substrate composition was estimated in terms of percent present of boulder, cobble, gravel, sand, and silt. Water samples were taken from each site and analyzed within 24- 48 hours of collection. Levels measured include: nitrate, chloride, alkalinity, calcium hardness and hardness. Testing was done primarily through buret titrations, except for nitrate which was measured using a spectrometer. Techniques were performed according to the Water Analysis Handbook written by the purchasing company HACH. Finally, macroinvertebrates were collected utilizing standardized traveling kick samples. Procedures were performed according to the Rapid Bioassessment Protocol dictated by the EPA. Jabs or kicks from several different locations within each site were taken to compose one homologous sample. The macroinvertebrate samples were preserved in enough 95% ethanol to cover the sample. All macroinvertebrates were separated from the surrounding substrate utilizing forceps and were classified to the genus level utilizing taxonomic keys and a dissecting microscope. Several indices were tested: Shannon's diversity index, species richness, Hilsenhoff's Biotic Index, and EPT, which tests richness within the 3 most sensitive orders, Ephemeroptera (mayflies), Plecoptera (stoneflies), and Tricoptera (caddisflies). ANOVA (one way, unstacked, 95% confidences) tests were also executed to test for significant differences in chemical and physical data in the three zones. Finally, correlation tests were run to assess correlations between the physical, chemical, and macroinvertebrate data.

Results

In examining physical and chemical data between edge, mix, and outer sites (in which edge sites resemble limestone stream conditions and outer sites represent freestone conditions), there was a significant difference in conductivity (p=0.021), alkalinity (p=0.023), hardness (p=0.001), calcium hardness (p=0.024), and chloride (p=0.011) (Figures 4-8). All five of these parameters decreased from the edge to the outer sites. Although there was no significant difference in depth and velocity, both of these values increased from edge to outer sites (Table 1). Control sites mimicked these trends.

Substrate was analyzed in terms of percent present of cobble (2.5”-10”), gravel (0.1”-2.5”), sand (gritty), and silt (fine) at each site. The percentage of cobble and gravel increased in the outer sites whereas the percentage of sand and silt increased in the edge sites (Table 1). In examining each type of substrate separately, only cobble (p=0.028) was significantly different between edge, mix, and outer sites (Figure 9).

When comparing physical and chemical parameters to indices, there were significant correlations. Diversity was negatively correlated with conductivity (p=0.05) and alkalinity (p=0.044), but positively correlated with depth (p=0.03). EPT was negatively correlated with conductivity (p=0.022), alkalinity (p=0.022), hardness (p=0.015), calcium hardness (p=0.039), chloride (p=0.043), and sand (p=0.004), and positively correlated with depth (p=0.002) and velocity (p=0.006). Richness and HBI were not significantly correlated with any chemical values; richness was positively correlated with depth (p=0.005) and velocity (p=0.016) and negatively correlated with sand (p=0.016). HBI was negatively correlated with cobble (p=0.003) (Table 2).

In the eleven sites surveyed, 33 genera were represented in the 2330 individuals collected. Several dominant genera were significantly correlated with specific chemical and physical parameters. *Gammarus* was positively correlated with conductivity (p=0.032) and all chemical parameters but nitrate, whereas *Ephemerella* and *Brachycentrus* were negatively correlated with conductivity (p=0.019 and p=0.006 respectively) and all chemical parameters but nitrate. *Gammarus* was negatively correlated with depth (p=0.007); Ephemerella was positively correlated with depth (p=0.014) and cobble (p=0.004); Brachycentrus was positively correlated with depth (p=0.001), velocity (p=0.042), and cobble (p=0.048), but negatively correlated with sand (p=0.037) (Table 2). Finally, although there were significant differences in specific genera, there were also changes in feeding groups. There is not enough data to test for statistical significance, but in general, the percentage of filter/collectors and scrapes increased from the edge to the outer sites, whereas the percentage of collector/gatherers decreased slightly (Table 3).

Discussion

In examining the physical and chemical data, we can determine characteristics of the limestone and freestone influenced sites. There are significant differences in the chemical and physical parameters of the outer and edge sites as well as between the two control sites. In general, the edge sites resembling Trout Run had higher conductivity, alkalinity, hardness, calcium hardness, and chloride. The substrate was more sandy and silty. Conversely, the outer sites resembling Yellow Breeches had lower chemical values, higher velocities, were deeper, and had a cobble and gravel based substrate. Out of those parameters, only alkalinity, hardness, calcium hardness, chloride, and cobble were statistically significant. Since depth and velocity were not significant, they can be eliminated as factors affecting the changes in invertebrate communities. Depth and velocity are physical factors more closely associated to size and flow of a stream, which vary regardless of the particular type of stream. The physical and chemical parameters that the literature cites as differing from limestone to freestone streams also differed in our study; therefore, we can conclude that the edge and outer sites represent significantly different habitats, and the mixing zone is a convergence of those two habitats.

Macroinvertebrate community composition changed as well. *Gammarus* (order Amphipoda) was found in conditions reflecting limestone stream influences, while *Brachycentrus* (order Trichoptera) and *Ephemerella* (order Ephemeroptera) favored conditions akin to freestone streams. This is consistent with the literature; aquatic insects are less abundant in limestone streams and *Gammarus* thrive in limestone conditions. This may be due to tolerance or sensitivity to higher chemical levels in limestone streams or to the switch from sand dominated substrate in Trout Run influenced sites to cobble and gravel dominated substrate in sites reflecting the Yellow Breeches. Additionally, feeding group percentages changed, with filter/collectors, shredders, and scrapers favoring freestone stream conditions. Although there is no significance that can be drawn from the feeding groups(due to lack of data), it does reflect that both individual genera and the overall composition of the community change from freestone to limestone conditions.

Indices also displayed several correlations. In general, diversity, richness, and EPT increased with conditions indicative of freestone streams. This would seem to indicate that Yellow Breeches is a healthier stream, but it would be erroneous to drawn this conclusion.

Indices are not adapted for limestone streams, which naturally have fewer aquatic insects (which are in the orders measured by EPT). Although the indices do show significant differences in data and provide a reference point in displaying the differences between outer and edge sites, they cannot be utilized to draw any concrete conclusions.

More data is necessary to further specify the causes of the macroinvertebrate community changes. Depth and velocity are eliminated as significant factors contributing to that shift, but it is unclear which remaining factors are responsible: chemical parameters, conductivity, or substrate. Although literature cites velocity as the most significant factor contributing to community changes, it is clear from this data that community changes can happen outside of significant velocity changes. Therefore, one of the parameters characteristic of freestone or limestone streams is responsible for the community shift. Additional data will be taken at this site as well as at two other sites to compare results when physical and chemical parameters change. One site, where Dogwood Run enters the Yellow Breeches, should have no chemical differences because two freestone streams are converging, but physical factors may differ. Additionally, at Coover Park in Dillsburg, PA, a sandstone stream enters a limestone stream. Significant chemical values will most likely be found there. Between the different sites, this preliminary data may be supported and hopefully factors contributing to the change may be specified. Data will be useful in assessing consequences of stream alterations and in evaluating parameters necessary for maintaining the current composition of macroinvertebrate communities in both freestone and limestone streams.

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Table 1: Physical, chemical, and substrate results (substrate in percentage present) for edge, mix, and outer sites as well as for control sites. Edge sites represent Trout Run influence and outer sites demonstrate Yellow Breeches influence

	Edge	Mix	Outer	TR	YB
Physical					
Conductivity (uS/cm)	581.66667	395	246.6667	575	235
Depth (cm)	25.5	34	42.83333	29	44
Velocity (cm/s)	57.733333	75.55	83.13333	37.15	93.9
Chemical					
Alkalinity (mg/L)	203.66667	138.3333	93.66667	203	88
Hardness (mg/L)	256.33333	179.3333	116.3333	269	123
Ca Hardness (mg/L)	203.66667	131.3333	75	209	85
Chloride (mg/L)	36.083333	25.16667	16.75	32.5	18.75
Nitrate (mg/L)	3.9333333	3.933333	2.116667	5.55	2.15
Substrate					
Cobble	3.3333	5.833333	20.83333	5	20
Gravel	43.333333	65	60.83333	40	65
Sand	39.166667	28.33333	18.33333	35	15
Silt	14.166667	0	0	20	0

Table 2: Results of the Pearson correlation test comparing physical and chemical data to indices and dominant macroinvertebrate genera. Values shown for significant correlation ($p<0.05$).

	Conductivity	Alkalinity	Hardness	Ca Hardness	Chloride	Depth	Velocity	Cobble	Sand
Indices									
Diversity	-0.601	-0.614	*	*	*	0.81	*	*	*
EPT	-0.677	-0.677	-0.705	-0.627	-0.618	0.817	0.767	*	-0.79
Richness	*	*	*	*	*	0.772	0.7	*	-0.7
HBI	*	*	*	*	*	*	*	-0.798	*
Genera									
Gammarus	0.646	0.64	0.656	0.67	0.709	-0.756	*	*	*
Ephemerella	-0.69	-0.684	-0.691	-0.725	-0.626	0.713	*	0.79	*
Brachycentrus	-0.768	-0.764	-0.764	-0.743	-0.684	0.848	0.62	0.606	-0.63

Table 3: Percentage of feeding groups present in each zone and at control sites.

Feeding Groups	Edge	Mix	Outer	TR	YB
%Collector/Gatherer	93.3572711	88.21839	84.28144	33.8345865	86.7133
%Filter/Collector	2.6929982	3.448276	7.185629	11.2781955	2.0979
%Scraper	2.87253142	6.034483	6.287425	38.7218045	10.4895
%Predator	0.53859964	0.574713	0.598802	1.12781955	0.6993
%Shredder	0	0.431034	0.299401	0	0

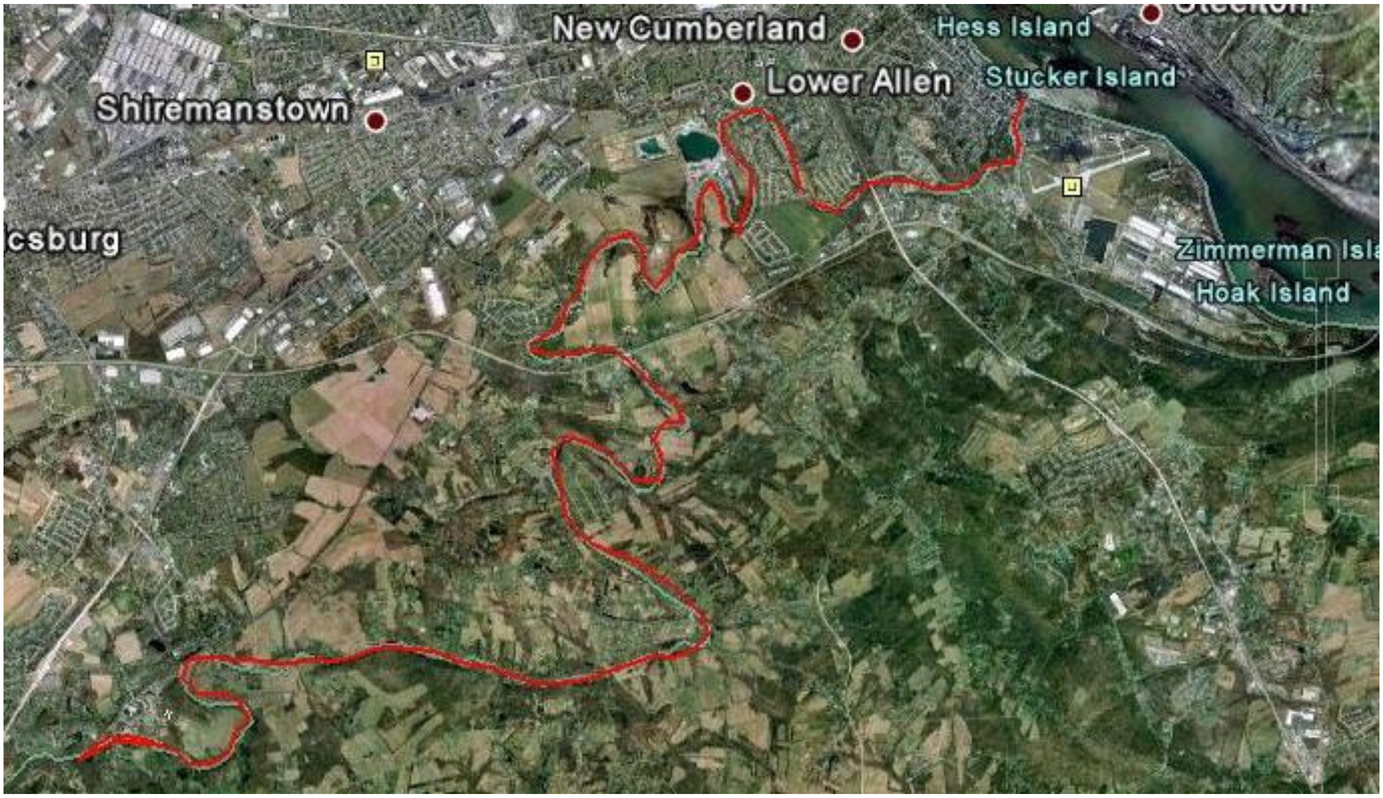

Figure 1: Aerial view of the Yellow Breeches. It empties into the Susquehanna River near New Cumberland.

Figure 2: Aerial view of where Trout Run enters the Yellow Breeches at Messiah College, Grantham, Pennsylvania, United States.

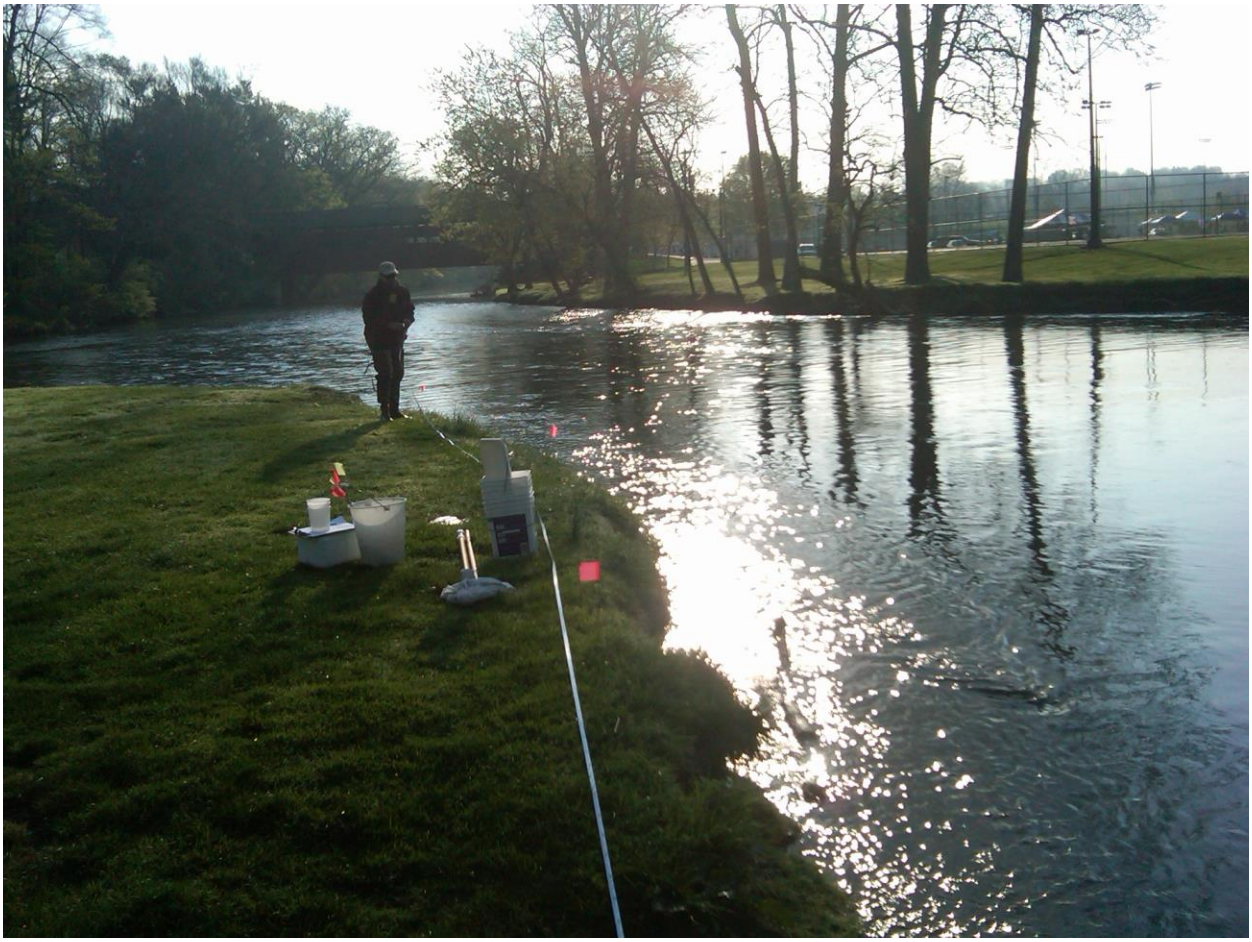

Figure 3: Flags marking sites 10m, 20m, and 30m downstream of where the streams intersect.

```
                                Individual 95% CIs For Mean Based on
                              Pooled StDev
Level       N    Mean  StDev  --+---------+---------+---------+-------
Cond Edge   2  581.67   2.36                        (--------*-------)
Cond Mix    2  395.00  96.64            (-------*--------)
Cond Outer  2  246.67   9.43  (-------*--------)
                              --+---------+---------+---------+-------
                              150       300       450       600
```

Figure 4: Results of ANOVA (one way, unstacked, 95% confidence) displaying significance for conductivity (p=0.021) between edge, mix, and outer sites.

```
                             Individual 95% CIs For Mean Based on
                             Pooled StDev
Level      N    Mean  StDev   +---------+---------+---------+---------
Alk Edge   2  203.67   2.36                          (--------*-------)
Alk Mix    2  138.33  32.53            (--------*-------)
Alk Outer  2   93.67   0.47   (--------*-------)
                              +---------+---------+---------+---------
                             50      100       150       200
```

Figure 5: Results of ANOVA (one way, unstacked, 95% confidence) displaying significance for alkalinity (p=0.023) between edge, mix, and outer sites.

```
                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level       N    Mean  StDev   -+---------+---------+---------+--------
Hard Edge   2  256.33   9.90                               (---*---)
Hard Mix    2  200.33   9.90                    (---*---)
Hard Outer  2  116.33   6.13   (---*---)
                               -+---------+---------+---------+--------
                              100       150       200       250
```

Figure 6: Results of ANOVA (one way, unstacked, 95% confidence) displaying significance for hardness (p=0.001) between edge, mix, and outer sites.

```
                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level     N    Mean  StDev  ------+---------+---------+---------+---
Ca Edge   2  203.67   3.30                         (-------*-------)
Ca Mix    2  138.00  38.66              (-------*-------)
Ca Outer  2   75.00   0.47  (--------*-------)
                            ------+---------+---------+---------+---
                                 60       120       180       240
```

Figure 7: Results of ANOVA (one way, unstacked, 95% confidence) displaying significance for calcium hardness (p=0.024) between edge, mix, and outer sites.

```
                           Individual 95% CIs For Mean Based on
                            Pooled StDev
Level     N    Mean  StDev  ------+---------+---------+---------+---
Cl Edge   2  36.083  2.003                           (------*------)
Cl Mix    2  25.167  3.771            (------*-------)
Cl Outer  2  16.750  1.061  (------*------)
                            ------+---------+---------+---------+---
```

Figure 8: Results of ANOVA (one way, unstacked, 95% confidence) displaying significance for chloride (p=0.011) between edge, mix, and outer sites.

```
                            Individual 95% CIs For Mean Based on
                             Pooled StDev
Level      N    Mean  StDev  ----+---------+---------+---------+-----
Cob Edge   2   3.333  0.000  (------*-------)
Cob Mix    2   5.833  1.179    (-------*-------)
Cob Outer  2  20.833  5.893                    (-------*-------)
                             ----+---------+---------+---------+-----
                                 0        10        20        30
```

Figure 9: Results of ANOVA (one way, unstacked, 95% confidence) displaying significance for cobble (p=0.028) between edge, mix, and outer sites.