

Introduction:

Pasture and animal grazing constitutes the largest type of land use in the contiguous United States, taking up one-third of the total acreage (Merrill and Leatherby 2018). Domestic grazers are frequently kept in the valley bottoms adjacent to streams for easy access to water, but this makes it easy for fecal runoff to enter waterways (Meehan and Platts 1978). Various studies allude to strong negative effects of grazers in relation to stream health, with grazing reshaping stream communities through interactive effects on nutrient loadings, bank stability, channel morphology, substratum size composition and riparian vegetation (Kauffman, Krueger and Vavra 1983, Quinn et al. 1992, Trimble 1994, Harding et al. 1998, Belsky, Matzke and Uselman 1999, Clary 1999). While there are known linkages between streams and grazers, negative effects from grazers are hard to directly quantify compared to other contaminants due to the diffuse nature of agricultural pollution and the diversity of nutrients involved (Schepers and Francis 1982). As a result, further research is needed on the interrelationships between livestock grazing and streams with attention to physical and chemical aspects, and to the development of tests that can measure the effects of animal waste and effectiveness of various management practices (Meehan and Platts 1978, Robbins et al. 1972, Scrimgeour and Kendall 2003). Likewise, it is important to understand runoff characteristics in order to fully assess and understand their impacts (Kato 2009).

Pollution from livestock has been shown to be a major contributor to eutrophication across the globe (Kato 2009). Eutrophication is the presence of an abundance of nutrients that allows algae to bloom and clog waterways with excessive organic matter, depleting oxygen, and killing other aquatic life (Kato 2009). Globally, freshwater ecosystems are rapidly losing their biodiversity because of human activity, and eutrophication is a major contributing factor to these losses (Dahl et al. 2004). Studies have shown that agriculture is the largest contributor of nitrogen and phosphorus to large bodies of water around the world, and these nutrients are agriculture's primary contribution to eutrophication (Stanner and Bordeau 1995, Kato 2009). Numerous studies have shown that livestock excrement contains a significant amount of nitrogen and phosphorus and that watersheds with livestock tend to have significantly higher mean concentrations of nutrients like nitrogen and phosphorus than watersheds without livestock (Azevedo and Stout 1974, Meehan and Platts 1978, Schepers and Francis 1982, Nader et al. 1998, Kato 2009). These nutrients are more likely to be moved with rainfall induced runoff, increasing the potential for them to be associated with surface pollution (Frey et al. 2015). Rainfall can also cause exposed stream beds to erode more which can release fecal material located along the shoreline (Khan et al. 2013, Frey et al. 2015). Livestock tend to graze by small lower order streams, but the contamination of these waterways can still have a significant effect on the composition of the larger, higher order streams that they flow into (Alexander et al. 2007). One study found that first water headwaters contribute to approximately 65% of the nitrogen flux in second order streams and this value only slightly declines to 40% for streams that are fourth order or higher indicating that the effect of livestock runoff on nutrient concentrations of small streams is an important factor in the eutrophication of larger bodies of water (Alexander et al. 2007).

Macroinvertebrates are commonly used to assess the stream health, in part because they can provide a more complete picture of the long term health and environmental stresses of

a stream than any other metrics that can only be measured in a single visit (Burton and Gerritsen 2003, Sanchez-Montoya et al. 2010). Some European agencies have been using benthic macroinvertebrates in bioassessment programs for many decades, and they have become popular in citizen science initiatives across the United States (Dahl et al. 2004, Chessman and McEvoy 1998, Reynoldson and Metcalfe-Smith 1992). The general scientific consensus is that community structure of macroinvertebrate populations is a valuable water quality indicator for monitoring and assessing aquatic systems (Chessman and McEvoy 1998, Reynoldson and Metcalfe-Smith 1992). It has been displayed that livestock runoff can alter the abundance and composition of benthic invertebrate communities, mostly through modifying resource availability (Lenat 1984, Townsend et al. 1997, Delong and Brusven 1998, Weigel et al. 2000, Scrimgeour and Kendall 2003). It is unclear how long invertebrate community recovery from livestock grazing pressure takes, but it could take years to decades with lasting impacts on stream health (Scrimgeour and Kendall 2003).

In our review of the literature, there was minimal past research concerning specific equine fecal runoff, although there were correlations between equine fecal runoff and a lower abundance of macroinvertebrates at sites downstream from fecal runoff compared to upstream (Jeffrey et al. 2015). Research is currently limited in regards to the differences between bovine and equine fecal runoff. This study seeks to characterize bovine and equine fecal runoff and answer questions about what differences may exist between them.

We hypothesize that fecal runoff from livestock pastures causes depressed stream health in waterways that are close to grazing animals. This study will specifically address 3 questions related to fecal pollution from livestock pastures: (i) Do livestock inputs affect water quality parameters, including conductivity, pH, and DO, and do these effects depend on whether the inputs are from horses or cows or on the amount of recent rainfall? (ii) Do livestock inputs affect the abundance, diversity, evenness, or richness of stream invertebrates, and do these effects depend on whether the inputs are from horses or cows? (iii) Do water quality parameters have a direct effect on abundance, diversity, evenness, or richness of stream invertebrates?

Methods:

Site Determination

This study was conducted between September and November 2019 at 10 streams, located within 20 miles of Blacksburg, Virginia. Five of the streams were located near bovine pastures and five were located near equine pastures. A list of potential sites was generated through a Google Maps satellite view search of streams that had areas that looked like they could be pastures for cows or horses nearby. Potential sites were then selected by assessing potential accessibility to sites upstream and downstream from the identified fecal runoff input. Finally, sites were visited in person to ensure feasibility prior to the start of the study. Each stream had a site A that was upstream from major fecal runoff inputs, and a site B that was downstream from fecal runoff inputs.

Sites

Stream 1, Tom's Creek, was selected because of its proximity to the Walnut Springs Horse Stable, and was denoted as Tom's Creek 1 to differentiate from the other Tom's Creek site. Stream 2, Stroubles Creek, was selected because of its proximity to the Virginia Tech teaching farms at the Smithfield Horse Center. Stream 3, Cedar Run, was located two miles northeast of Blacksburg near a small cow pasture. Stream 4, Indian Run, was also northeast of Blacksburg near a small horse pasture. Stream 5, Spruce Run, was located northwest of Blacksburg and was close to a horse pasture. Stream 6, also Toms Creek (denoted as Tom's Creek 2), was located at a separate location close to a horse pasture. Stream 7, Slate Branch, was south of Blacksburg near Christiansburg, and was located near a cow pasture. Stream 8, Crab Creek, was located near Christiansburg and was close to a cow pasture. Stream 9, Smith Creek, was located directly south of Blacksburg across route 81, and was close to a cow pasture. Stream 10, Elliott Creek, was also located across route 81 and was close to a cow pasture. The median distance between upstream and downstream sites for an input was 708.51 meters with a maximum distance of 6260.35 meters at Slate Branch, and a minimum distance of 514.96 meters at Tom's Creek 1.

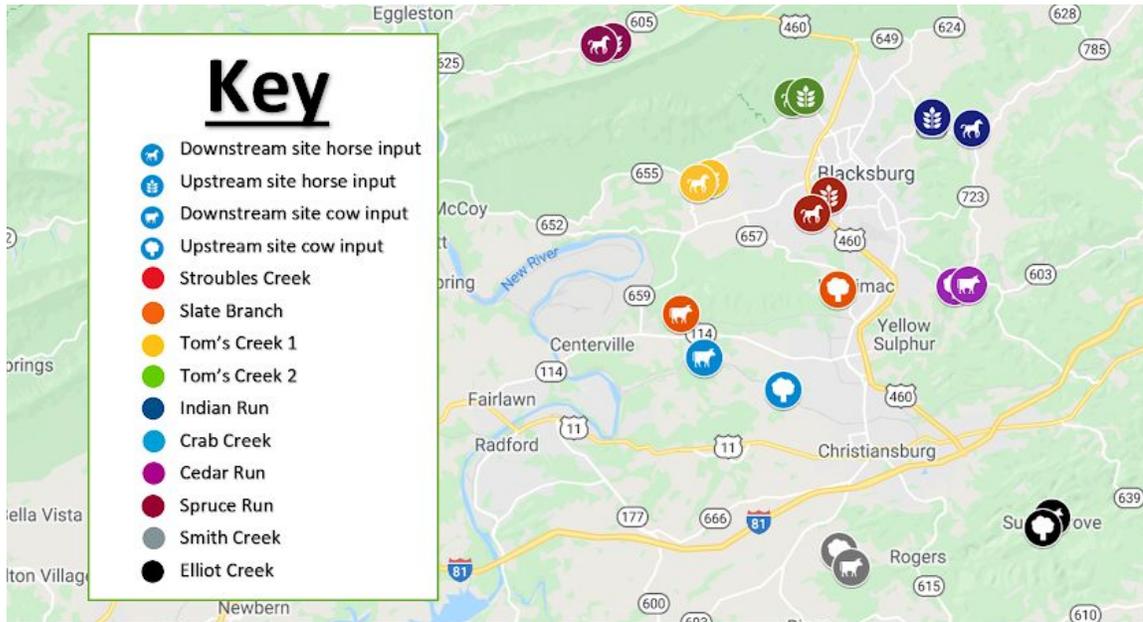


Figure 1: Map of study sites

Study Approach

This study took an observational approach by measuring water quality parameters including dissolved oxygen, pH, conductivity in streams, and frequency of macroinvertebrates in several orders within streams. Rainfall levels near streams were also measured. Water quality was measured with probes at each site, sampling on two separate occasions at each upstream and downstream site. Macroinvertebrate collection and identification was conducted with a surber sampler and each site visit consisted of two separate samples. All data collection at each site was conducted between the hours of 10:00 AM-2:00 PM to minimize natural changes and variance in recorded measurements. The study sites selected to visit on a given sampling day and the order in which sites were visited was randomized using a random number generator.

Nitrogen and phosphorus can be direct indicators of livestock pollution. In the scope of our study it would have been unfeasible to measure these variables, as we lacked the ability to precisely measure aqueous nitrogen and phosphorus concentrations. A good alternative is measuring the conductivity of the water, which measures the capacity of the water to conduct electricity, and has been shown to be an effective method of estimating the dissolved ionic species in streams (Wenner and Ruhlman 2003). Among streams with similar geology, conductivity is an effective way to measure pollutants (such as nitrogen and phosphorus), with one study showing that conductivity was the parameter that best differentiated minimally impacted streams from highly impacted streams (Wenner and Ruhlman 2003). In this previous study, conductivity was used to assess the significance of urban pollution in streams and did so effectively, proving its efficacy as a measure of pollution (Wenner and Ruhlman 2003).

Variables

Invertebrates were sampled on two separate occasions per upstream and downstream site with a surber sampler in riffle stream conditions. The invertebrate counts recorded for each upstream or downstream site were a summation of the total invertebrates collected across two samples collected at each visit. All stream bed material within the square frame of the surber sampler was disturbed for 20 seconds, and then the contents of the surber sampler was emptied into a white tray. Invertebrates were then sorted into an ice cube tray, and counts of individuals within each class and order were tallied. Individuals were primarily classified to the order level, however with some specimens only class level classification was possible, and individuals of the order Odonata was classified further into the two suborders of Anisoptera and Zygoptera. The orders that were identified were Ephemeroptera, Amphipoda, Diptera, Coleoptera, Tricladida, Decapoda, Plecoptera, Megaloptera, and Trichoptera. The organisms that could only be identified to class were in the classes Oligochaeta, Bivalvia, and Gastropoda.

The invertebrate communities at the sites were then evaluated for abundance, richness, evenness, and diversity. Abundance was evaluated by taking the total number of invertebrates per species in each sample. Richness, evenness, and diversity were calculated in R Studio using the vegan package. Diversity was calculated with Shannon's diversity index and evenness was calculated using Pielou's evenness, a method which compares the true diversity value with the maximum possible diversity value. Finally, richness was calculated by taking the total number of taxa identified at each site.

The probes that were used when sampling were the Sper Scientific Water Quality Meter (model 850081) which measured pH, the Sper Scientific Dissolved Oxygen Meter Kit (model 850081DOK) which measured dissolved oxygen, and an ATC Conductivity Probe (model 850081C/TD) which recorded conductivity. Rainfall levels were taken from the Montgomery County, VA WeatherSTEM dating mining tools. Cumulative rainfall data was recorded for 48 hours prior to each data collection date using the station "Virginia Tech," output format "Table," interval "day," and operation "maxima."

Data Analysis

All statistical analyses were carried out in R Studio version 3.6.1 using the packages `vegan`, `lme4`, `plyr`, `dplyr`, `ggplot`, and `ggplot2`.

To test whether there were differences in invertebrate diversity, abundance, richness, or evenness upstream and downstream of livestock inputs, we used generalized linear mixed models. These models tested whether site (upstream vs. downstream), input (horse vs cow), or the interaction of input and site, had an effect on these response variables. Separate models were run for each response variable. All models also included sample period nested within stream ID as random effects. For DO, pH, or conductivity, we used identical models but also included 48 hour rainfall and all possible three and two-way interactions with site and input as predictors. Rainfall was taken into account as a possible predictor variable for the water quality measures but not for the macroinvertebrate indices. This is because macroinvertebrate indices paint a more long-term picture of stream health and would be unlikely to be affected by a single large rain event whereas water quality parameters show the more immediate effects of fecal runoff.

In models that were run with the `lmer` function, we tested for the significance of fixed effects by comparing the model in question with a simplified models with single terms deleted through likelihood ratio tests. The likelihood ratio tests provided a chi-squared test statistic and p-value. When site had a significant effect on the response variables, the average difference between upstream and downstream sites in our data was taken to provide a ballpark on the level of biological significance of the finding by subtracting the downstream values for a site from the upstream values. Based on significant interactions involving input (see results), we also wanted to determine significant effects on only cow or horse data. To conduct this testing, two new data sets were created in R that were filtered for either H (horse) or C (cow) for input. These data sets were then used as the data for a model that included site (upstream vs. downstream) as the predictor variable with conductivity, pH, DO, evenness, richness, abundance, and diversity being used as response variables with a separate analysis for each response variable. All of these models included stream identify and sample period as nested random effects.

Furthermore we evaluated whether water quality measurements had an effect on macroinvertebrate community indices. For example, we used a model where conductivity was the predictor variable and evenness was the response variable. Additional similar models were used with the same predictor variable, and diversity and abundance being the response variable.

Results

Livestock input's effect on water quality parameters

Conductivity

There was a marginally significant three-way interaction between site, 48 hour rainfall, and input's effect on conductivity ($\chi^2=3.34$, $p=.068$; Fig 2), with a significant two-way interaction between 48 hour rainfall and input ($\chi^2=4.67$, $p=.031$) but no significant two-way interactions

between 48 hour rainfall and site ($p=.94$, $\chi^2=.33$) or site and input ($\chi^2=.77$, $p=.38$). There was a significant single effect of site on conductivity ($\chi^2=4.64$, $p=.031$, Fig. 3) and 48 hour rainfall ($\chi^2=5.47$,

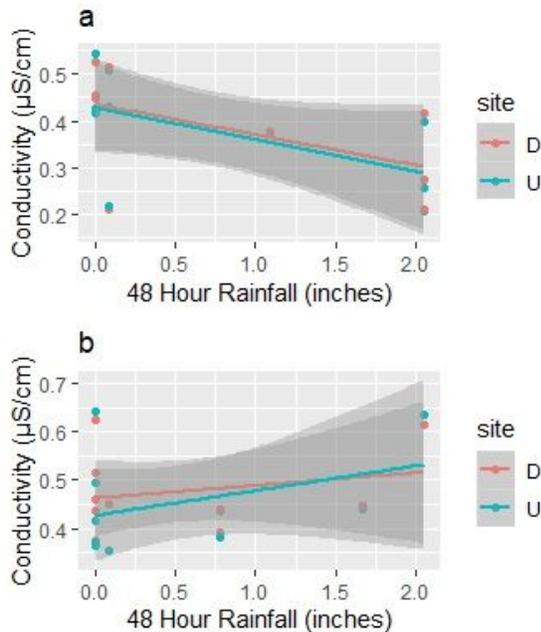


Figure 2: The effect of 48 hour rainfall on conductivity divided by site (U=upstream, D=downstream) for (a) all horse input streams and (b) all cow input streams.

$p=.019$) but there was no significant single effect of input ($\chi^2=1.48$, $p=.22$). There was no significant interaction of 48 hour rainfall and site on the cow only data set ($\chi^2=2.58$, $p=.11$) and there were no significant single effects of 48 hour rainfall ($\chi^2=1.29$, $p=.25$) or site ($\chi^2=3.17$, $p=.075$). There was no significant interaction between 48 hour rainfall and site on the horse only data set ($\chi^2=.39$, $p=.53$) or significant single effects of site ($\chi^2=2.77$, $p=.096$) but there was a significant single effect of 48 hour rainfall ($\chi^2=5.81$, $p=.016$).

DO

For DO, there was no significant three-way interaction between site, input, and 48 hour rainfall ($\chi^2=1.072$, $p=.30$) or any significant two-way interactions between site and 48 hour rainfall ($p=.84$, $\chi^2=.36$), site and input ($\chi^2=.01$, $p=.92$), or 48 hour rainfall and input ($\chi^2=1.19$, $p=.27$). There was a marginally significant single effect of input on DO ($\chi^2=3.33$, $p=.068$; Fig. 4) but no significant single effects of either 48 hour rainfall ($\chi^2<.001$, $p=.99$) or site ($\chi^2=.065$, $p=.79$). Within the cow only data set, there was no significant interaction between 48 hour rainfall and site ($\chi^2=.039$, $p=.84$) or significant single effects of 48 hour rainfall ($\chi^2=.74$, $p=.39$) or site ($\chi^2<.001$, $p=.98$). For the horse only data set, there was no significant interaction between

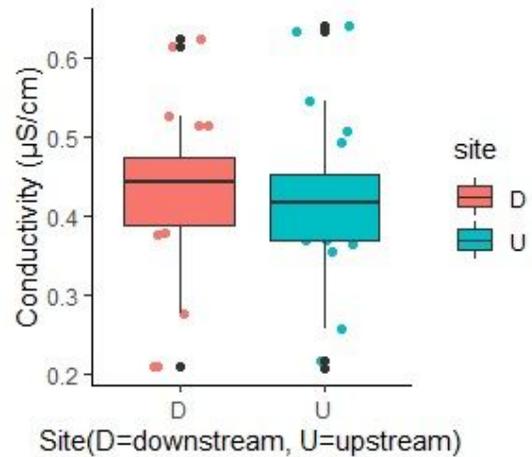


Figure 3: The effect of site (upstream or downstream) on conductivity.

site and 48 hour rainfall ($\chi^2=2.33$, $p=.13$) or significant single effect of site ($\chi^2=.13$, $p=.72$) or 48 hour rainfall ($\chi^2=.47$, $p=.49$).

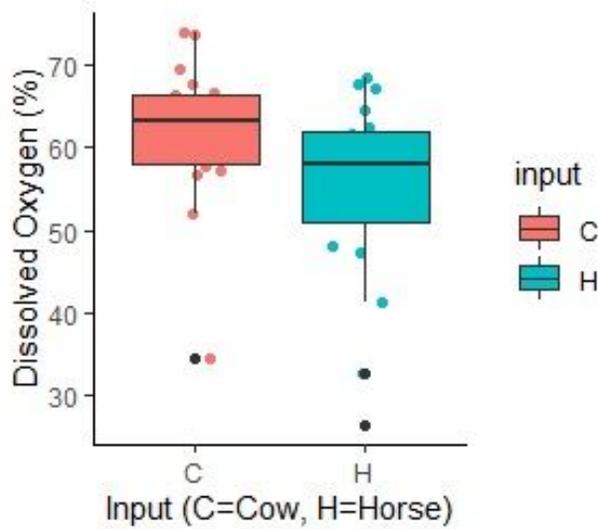


Figure 4: The effect of input (cow or horse) on dissolved Oxygen (DO) percentage observed in streams.

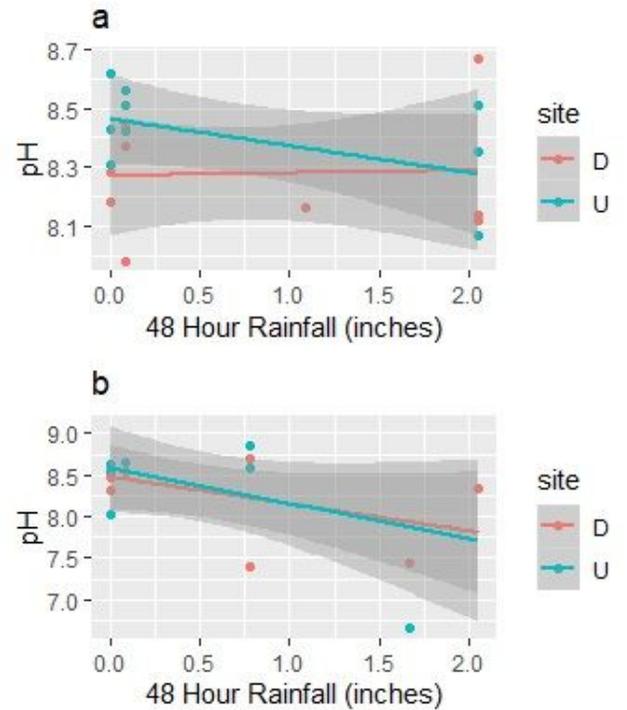


Figure 5: The effect of 48 hour rainfall on pH Observed in streams divided by site (U=upstream, D=downstream) for (a) all horse input streams and (b) all cow input streams.

pH

There was no significant three-way interaction between site, input, and 48 hour rainfall for pH ($\chi^2=.20$, $p=.65$) or significant two-way interactions between site and 48 hour rainfall

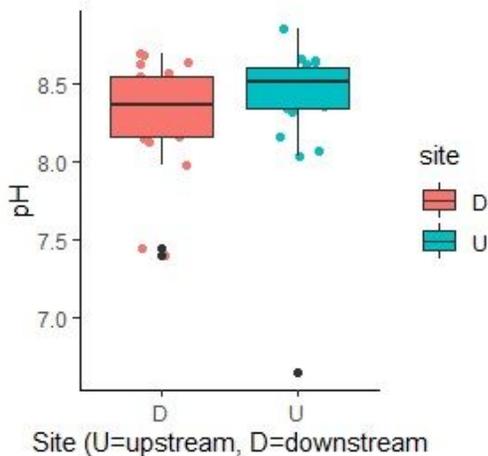


Figure 6: The effect of site (upstream or downstream) on pH observed in streams.

($p=.96$, $\chi^2=.33$) or site and input ($\chi^2=.14$, $p=.71$). There was a marginally significant two-way interaction between input and 48 hour rainfall ($\chi^2=3.39$, $p=.065$; Fig. 5). There were no significant single effects of site ($\chi^2=1.95$, $p=.16$; Fig. 6), 48 hour rainfall ($\chi^2=3.23$, $p=.072$) or input ($\chi^2=.029$, $p=.87$) on pH. In the cow only data, there was no significant interaction of 48 hour rainfall and site ($\chi^2=.049$, $p=.82$) or single effect of site ($\chi^2=.47$, $p=.49$), but there was a significant single effect of 48 hour rainfall ($\chi^2=3.66$, $p=.056$). In the horse only data, there was no significant interaction between 48 hour rainfall and site ($\chi^2=2.022$, $p=.16$) or single effects of 48 hour rainfall ($\chi^2=.49$, $p=.49$) or site ($\chi^2=2.87$, $p=.090$).

Livestock input's effects on macroinvertebrate community indices

Evenness

There was no significant two-way interaction between site and input ($\chi^2=1.28$, $p=.26$) but there were significant single effects of input ($\chi^2=4.50$, $p=.034$; Fig. 8) and site ($\chi^2=5.10$, $p=.024$) on evenness. There was a significant effect of site on the horse only data set ($\chi^2=7.58$, $p=.0059$) but not on the cow only data set ($\chi^2=.90$, $p=.34$).

Abundance

There was no significant two-way interaction between site and input ($z=-.33$, $p=.74$) or significant single effects of site ($z=-.49$, $p=.62$) or input ($z=1.42$, $p=.16$) on abundance. There was no significant single effect of site found in the cow only data set ($z=-.49$, $p=.62$) or the horse only data set ($z=-1.31$, $p=.19$).

Diversity

There was no significant two-way interaction between site and input ($\chi^2=.39$, $p=.53$) or significant single effects of site ($\chi^2=1.99$, $p=.16$; Fig. 7) or input ($\chi^2=1.81$, $p=.18$) on diversity.

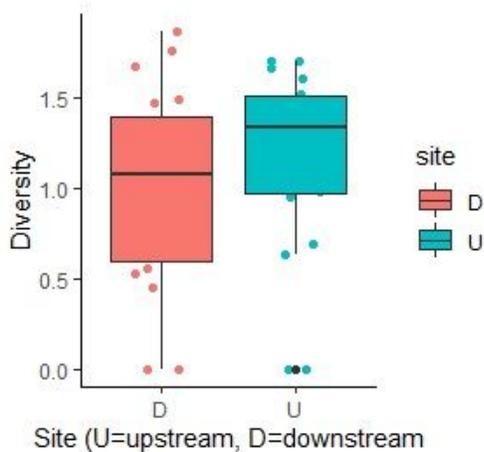


Figure 7: The effect of site (upstream or downstream) on diversity observed in

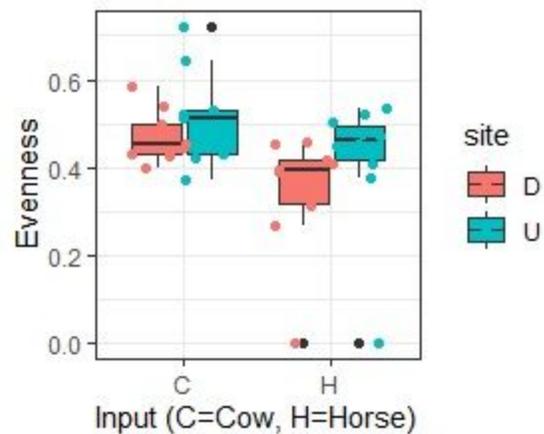


Figure 8: The effect of input (cow or horse) on evenness observed in streams.

stream.

There was no significant single effect of site found in the cow only data set ($\chi^2=.26$, $p=.61$) or the horse only data set ($\chi^2=2.78$, $p=.095$).

Richness

There was no significant two-way interaction between site and input ($\chi^2=0$, $p=1$) or significant single effects of site ($\chi^2=.37$, $p=.54$) or input ($\chi^2=2.12$, $p=.15$) on richness. There was no significant single effect of site found in the cow only data set ($\chi^2=.16$, $p=.69$) or the horse only data set ($\chi^2=.22$, $p=.64$).

Water quality measures' effect on macroinvertebrate community indices

Conductivity

Conductivity had a marginally significant effect on evenness observed ($\chi^2=3.78$, $p=.052$; Fig. 9) but did not have a significant effect on richness ($\chi^2=.039$, $p=.84$), diversity ($\chi^2=.095$, $p=.76$), or abundance ($z=-.56$, $p=.58$).

DO

DO had a significant effect on abundance ($z=2.582$, $p=.0098$; Fig. 10) but did not have a significant effect on diversity ($\chi^2=.036$, $p=.85$), richness ($\chi^2=.089$, $p=.77$), or evenness ($\chi^2=.26$, $p=.61$).

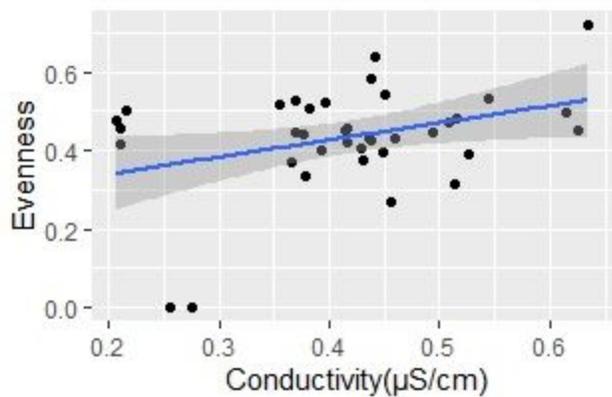


Figure 9: The effect of conductivity ($\mu\text{S/cm}$) on evenness

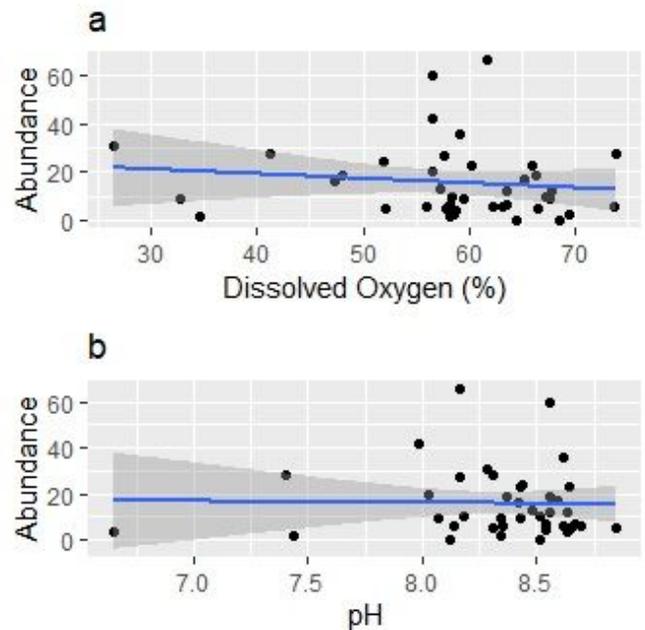


Figure 10: The effect of (a) dissolved oxygen (%)

observed in streams.

and **(b)** pH on abundance observed in streams.

pH

There was a marginally significant effect of pH on abundance ($z=-1.86$, $p=.062$; Fig. 10) but no significant effects of pH on diversity ($\chi^2=2.11$, $p=.15$), richness ($\chi^2=1.55$, $p=.21$), or evenness ($\chi^2=.11$, $p=.74$).

Conductivity had a significant effect on evenness across all sites ($p=.05$) displaying that when the conductivity increases so does the evenness.

Discussion

Equine and bovine fecal runoff has been shown to have negative effects on the health of nearby waterways (Meehan and Platts 1978, Airakensen et al. 2007). Livestock runoff demonstrated to have high nutrient concentrations, especially with nitrogen and phosphorus accumulation (Meehan and Platts 1978, Kistemann et al. 2002, Kato et al. 2009). Conductivity was used as a measure of the approximate concentrations of nutrients in the water, which was shown to be an effective method by Thomas et al. (2012). Our results generally displayed that there are relationships between bovine and equine fecal runoff, water quality parameters, and macroinvertebrate indices. These effects were further shown to be different based on the bovine and equine input, as well as the rainfall amount (Fig. 2, Fig. 8). In addition, we found that both livestock inputs and water quality parameters affect certain stream invertebrate indices with certain indices affected more by one type of input than another.

In this study, we showed that the average relative difference between upstream and downstream conductivity levels was -0.0437 , displaying that downstream sites tended to have a higher conductivity when compared to upstream sites (Fig. 3). Sites downstream of fecal runoff displayed higher conductivity, which is consistent with past studies displaying higher levels of nutrients downstream from nutrient runoff (Azevedo and Stout 1974, Meehan and Platts 1978, Schepers and Francis 1982, Nader et al. 1998, Kato 2009). Furthermore, we were able to see a correlation between 48 hour rainfall and conductivity, with conductivity increasing for bovine sites as rainfall increased, and conductivity decreasing for equine sites as rainfall increased (Fig. 2). This result may be due to a variety of factors, one of which being the different stocking densities of cattle and horses. In the pastures acting as fecal runoff sources in our study, cattle pastures tended to hold many more individuals than horse pastures. The higher density of individuals and feces in cow pastures may have made so that the rain washed more fecal material into stream and increased water quality parameters associated with fecal pollution while the lower density of fecal material in equine pastures may have made so rain simply had a dilutatory effect on stream pollution metrics.

Overall, these results suggest that fecal runoff from livestock pastures near streams studied carried nutrients that influence the conductivity and concentrations of the nutrients present in the streams.

Past studies have also showed changes in the pH and dissolved oxygen levels of waterways near livestock pastures (Meehan and Platts 1978, Kistemann 2002). Dissolved oxygen levels have been shown to be higher at upstream sites than downstream sites (Meehan and Platts 1978). This can be partially attributed to increased algae growth due to greater

nutrients from fecal runoff which uses a lot of oxygen from the water and depletes the dissolved oxygen supply (Kato 2009). In this study, we did not see any significant difference between the dissolved oxygen levels in upstream and downstream sites. Our failure to find significant differences for these metrics was likely due to our small sample size and type of sample stream type, a ripple. The precision of the meters used may also have contributed to the insignificance of the data upstream and downstream due to tool inaccuracies. Although we were unable to find a difference between dissolved oxygen and site, there was a moderately significant difference between the input, horse or cow, and dissolved oxygen, displaying that horse input lowered the dissolved oxygen more than cow input (Fig. 4). In addition, potential of hydrogen (pH) has demonstrated to be lower in relation to higher runoff loads as a result of increased concentrations of alkaline nutrients which are more acidic (Kistemann 2002). As a result, it was expected to see lower pH values at the downstream sites than upstream sites. Although our pH data was insignificant, there still appears to be a trend between sites, displaying that the downstream sites tended to contain a lower pH (Fig. 6). Furthermore, we were able to see a marginally significant effect between 48 hour rainfall and bovine input, displaying that with increased rainfall, there was a decrease in the pH most likely attributed to rainfall nutrient runoff release (Fig. 5).

Macroinvertebrate community structure is a useful indicator of overall stream health (Gaufin and Tarzwell 1952, Li et al. 2001). There was little information in the literature directly relating macroinvertebrate assessment to livestock fecal runoff, but generally results suggested that a variety of characteristics of macroinvertebrate community structure are altered by intensity and proximity to pollution (excess nitrogen and phosphorous), including abundance and diversity (Li et al. 2001, Jeffrey et al. 2015). We found no significant difference between upstream and downstream sites for both richness and abundance. A possible cause of the lack of significance was the differences in the variety of habitats that the sites were in and the small sample size. Wood et al. (2005) found that headwater streams with intermittent flow showed a smaller number of taxa and individuals than expected. Many streams in this study were headwater streams, and as a result the upstream sites were frequently close to the start of a stream with resulting intermittent flow patterns. Due to these intermittent flow patterns at upstream sites, we would predict to see lower abundance and richness than what would otherwise be expected (Wood et al. 2005). These streams, likely influenced the trends in relative difference between upstream and downstream sites causing the lack of significance.

Although there was a lack of significance with abundance and richness, we saw a trend towards higher diversity at upstream sites compared with downstream sites (Fig. 7). This is consistent with past studies that have shown diversity of macroinvertebrates being lower at more polluted sites (Jeffrey et al. 2015). With an increased sample size and more sites, this trend could be shown as more significant.

We did see a significant relative difference in the evenness of macroinvertebrates between upstream and downstream sites. Our results showed that upstream sites had greater species evenness than downstream sites in the same stream (Fig. 8). This is consistent with past research displaying greater macroinvertebrate evenness in sites with less nutrient pollution (Gaufin and Tarzwell 1952). Concurrently, we were also able to see a difference in the effect between bovine and equine input and site, on evenness, displaying that evenness was lowered

based on horse input (Fig. 8). This is a unique finding as there is not much literature comparing the effects of cows and horses on streams and alludes to horses having a greater effect on stream macroinvertebrates communities comparatively to cows.

Furthermore, our results showed that there were significant relationships between water quality parameters and macroinvertebrate indices, showing that conductivity was a significant predictor of macroinvertebrate evenness, with higher conductivity relating to higher species evenness (Fig. 9). This finding was atypical because higher conductivity has been historically associated with higher pollution levels (Thomas et al. 2012). This abnormality may have been due to large differences in the conductivity of different streams, and the fact that streams with a high overall conductivity at both the upstream and downstream sites also demonstrated a high overall evenness at both the upstream and downstream sites. We also displayed that both pH and dissolved oxygen affected abundance, with both higher pH and dissolved oxygen slightly reducing the abundance of macroinvertebrates (Fig. 10).

Consistently throughout this study we were able to see trends between fecal runoff at upstream and downstream sites, to certain water quality parameters and macroinvertebrate indices. Future studies should focus on identifying a larger number of study sites to increase statistical power for identifying trends in dissolved oxygen concentrations and pH, and should stay away from using headwaters with intermittent flow for macroinvertebrate sampling due to effects intermittent flow has on macroinvertebrate community composition. Overall, the demonstrated difference in conductivity at upstream and downstream sites suggests that fecal runoff does have a direct effect on the nutrient composition of waterways near livestock pastures. Differences in the macroinvertebrate community evenness and diversity suggest that fecal runoff negatively impacts macroinvertebrate communities, which are a good indicator of impact on overall stream health. This study also displayed that horse and cow fecal runoff have separate effects on macroinvertebrate evenness and as a result, suggests that they have independent effects on stream health. Finally, this study displays the need for further research into stream health and pollution, specifically to demonstrate the distinct polluting effects of different grazing animals, which will help determine best management practices and resource allocation to help preserve stream health.

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