A ONE HEALTH APPROACH TO DETERMINING ADVERSE EFFECTS OF CONCENTRATED ANIMAL FEEDING OPERATION (CAFO) FARMING ON SURROUNDING WATER QUALITY IN SAMPSON COUNTY, NC

A Thesis By ABIGAIL LEIGH HRABOSKY

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Abstract

A ONE HEALTH APPROACH TO DETERMINING ADVERSE EFFECTS OF CONCENTRATED ANIMAL FEEDING OPERATION (CAFO) FARMING ON SURROUNDING WATER QUALITY IN SAMPSON COUNTY, NC

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Sampson County, NC contains the second more CAFOs in the United States. CAFO waste is commonly stored in open-air basins comprised of fecal matter, urine, and elemental contaminants. Surrounding water sources are at risk of contamination due to both the runoff from these lagoons, and the use of their contents as 'organic' fertilizer. Additionally, periodic flooding due to Atlantic hurricanes has been shown to contaminate surface waters. Hog feces and urine are stores in open-air basins called lagoons. Although rich in nutrients, hog lagoons also contain elemental, anion, microbial, and nutrient wastes. The concentration of these contaminants was analyzed in both household and stream water using ion chromatography (IC), inductively coupled plasma – optical emission spectrometry (ICP-OES), and 3M Petrifilm E. coli tests. Additionally, the quality of surface water near hog and poultry CAFOs was analyzed with the use of benthic macroinvertebrate biomonitoring using several indices of biodiversity and aquatic health. The One Health approach considers both human and environmental health concerns. We hypothesized that would be correlated with proximity to CAFOs and density of CAFOs. Statistical analyses showed no significant correlations between CAFO proximity and density and water quality.

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Dedication

This thesis is dedicated to my father, Michael Joseph Hrabosky Jr., who passed away during my first semester of graduate school. His encouragement to never give up in pursuit of my dreams led me through graduate school and will continue to motivate me through the rest of my life.

Table of Content

Abstract
Acknowledgements
Dedication
List of Tables
List of Figures
Foreword
Introduction
Purpose of Study
Materials and Methods
2021 Spring & Fall Collections
2022 Spring Collection
Inductively Coupled Plasma – Optical Emission Spectrometry
Ion Chromatography
3M Petrifilm [™] Escherichia coli (<i>E. coli</i>) Testing
Benthic Macroinvertebrate Biomonitoring11
Statistical Analyses
Surface Water Analyses 13
Results and Discussion
Correlation Analyses
Linear Regression

S	urface Water Analyses
[iscussion
C	onclusions
Referen	es19
Tables &	Figures
Vita	

List of Tables

Table 1. Surface water collection sites sorted by name, collection site, distance to nearest hogfarm (kilometers), and classification of collections by time period (F21 = Fall 2021, S22 = Spring2022). (page 29)

Table 2. Surface water collection site field parameters. Measurements were not taken for Crane Creek and Little Coharie Creek; Crane Creek had negligible flow, and Little Coharrie had a large beaver dam blocking all flow from upstream of the collection site, so calculating discharge was not possible. (page 30)

 Table 3. ICP-OES elements and emission wavelengths. (page 31)

Table 4. ICP-OES analytes and their respective NC & EPA benchmark standard values. (page 32)

 Table 5. Ion chromatography analytes and their respective NC & USEPA benchmark standard

 values. (page 33)

Table 6. Fall 2021 surface water samples *E. Coli* and total coliform analyses using 3M PetrifimTM *E. coli* testing measured in 1mL. Values are converted to total number of colonies per 100 mL to effectively compare to standard NC benchmark levels. (page 34) **Table 7.** Spearman's correlation matrix values, including p-values to determine significance and Spearman's Rho values to analyze co-occurrence between values. Any significant p-values are indicated in bold and assigned a Spearman's Rho to further classify them. (page 35)

Table 8. Linear regression model fit measures. Each contaminant of concern is accompanied by an AIC value to determine the best predictor, an adjusted R² value to determine the strength of the model fit explanation, an intercept estimate to represent the baseline contaminant level when the predictor = 0, and a predictor estimate to represent the rate at which the intercept increases or decreases. The best fit models are characterized between 1-3, where 1 = distance to closest CAFO, 2 = density of CAFOs within a 10km² radius, and 3 = both distance to closest CAFO + density of CAFOs within a 10km² radius. (page 36)

Table 9. Results of benthic macroinvertebrate monitoring analyses for each field site. (page 37)

Table 10. Comparison of stream water EPT bioclassification data between DEQ published data(NCDEQ 2019) and 2021-2022 Appalachian State University collected data. (page 38)

List of Figures

Figure 1. CAFOs in eastern North Carolina, with emphasis on Sampson and Duplin Counties. Red indicates hog farms, and violet indicates poultry farms (Graddy et al., 2020). (page 27)

Figure 2. Collection sites in Sampson County, NC. Red house icons indicate residential sites and are stacked to protect privacy- each house icon may indicate multiple collection sites. Blue water icons indicate surface water collection sites, with a single point being a single site. (page 27)

Figure 3. Correlation matrix visually displaying any significant relationships between variables analyzed using the Spearman's correlation. Each relationship is assigned a correlation value between -1 to +1, where 1 is perfectly, positively correlated, 0 indicates no correlation, and -1 indicates a perfect negative correlation. Each correlation value is also assigned an asterisk classification, where a higher number of asterisks indicates a stronger correlation. (page 28)

Foreword

This thesis manuscript will be submitted to the journal *EcoHealth*. The thesis has been formatted according to the style guide for this journal for minimal revisions before publication.

Introduction

The term 'One Health' was introduced in the early 2000s, when it was used to study the effect of severe acute respiratory disease (SARS) on humans, animals, and the economy (Mackenzie & Jeggo, 2019). A One Health approach combines human, animal, and environmental health concerns when addressing an ecological issue (Middleton et al., 2014). The One Health approach frames human and animal health as interdependent and bound to the health of the ecosystems in which they exist (Bonilla-Aldana et al., 2020). In this research, we use the One Health approach to understand how CAFOs produce stressors to human health, and how those stressors ultimately impact the surrounding ecosystem.

Concentrated animal feeding operations (CAFOs) are large-scale agricultural farms that specialize in raising animals for human consumption. CAFOs externalize environmental, social, and public health issues with the goal of maximum animal protein output and profit (Cassuto, 2010; Hribar, 2010). CAFO waste comes in direct contact with the surrounding water supply via runoff, flooding, leaching, or other forms of water movement (Hribar, 2010). Although the Clean Water Act regulates the water contaminants from CAFOs, it does not explicitly define CAFOs, thereby allowing for the creation of numerous enforcement loopholes (Centner, 2011). Additionally, CAFOs are disproportionally located in eastern North Carolina as opposed to the rest of the state of North Carolina (Wing et al., 2000), and an estimated 6 x 10⁶ hogs live in Sampson County alone (USDA 2017, Fig. 1). Additionally, roughly 7 x 10⁶ turkeys were produced in Sampson County in 2020, ranking number 1 in all of North Carolina (NC Department of Agriculture and Consumer Services, 2021).

[Insert Figure 1 here]

The high density of CAFOs in Sampson County—inhabited by marginalized populations is an environmental injustice. Environmental injustice is described as the disproportionate burden of pollution on people of Color and poverty (Bryant, 1995; Bullard & Borgmann, 1993). The environmental justice movement was initially sparked in Warren County, NC in 1982, when a small, majority African American community was designated as a toxic waste dump site. This decision caused protests, and an estimated 500 protestors in the community were arrested. These protests kickstarted the environmental justice movement in eastern NC by raising awareness of the injustices that the communities were experiencing daily and initiating groups and studies to fight for environmental equality (Office of Legacy Management). Sampson County, NC is home to a larger percentage (33.6%) of Black, Hispanic, and impoverished residents as opposed to the rest of the state of North Carolina (29.9%) (NCDEQ, 2021; U.S. Census Bureau, 2021). Additionally, according to the NCDEQ, Sampson County ranks 77th (of 100 counties total) in the state for health outcomes, such as lifespan and self-reported health status. In 2019, Sampson County was named a Tier 1 county, meaning that the county has a population of fewer than 50,000 residents and a poverty rate > 19% (NCDEQ, 2021). We collaborated with the Environmental Justice Community Action Network (EJCAN, 2019), a nonprofit founded in 2020 by Sampson County resident and attorney, Sherri White-Williamson, to empower communities with the technical, scientific, legal, educational, and funding resources needed to address environmental problems (EJCAN, 2022).

Poultry and hog farming may significantly impact water quality at broad spatial scales. The environmental and human health impacts include elevated nutrient, metal, anion, and *E. coli* exposures. The application of animal wastes to croplands may result in the movement of

associated nutrients and contaminants to receiving waters through runoff (Burkholder et al., 2017). Changes in the water chemistry of surrounding surface and drinking water can cause detrimental effects to both aquatic biota and human health via well water contamination (Villanueva et al., 2014). CAFOs can impact human health and have been linked to elevated incidences of bronchitis, mucus membrane irritation, asthma-like symptoms, and organic toxic dust syndrome (Donham et al., 2007). Once CAFO waste reaches local water sources including surface streams and adjoining aquifers, infections of the eyes, skin, ears, nose, and throat may result (Thorne, 2007). Additionally, CAFOs can emit toxic gases and vapors containing ammonia, carbon dioxide, and other toxic chemicals, which can result in respiratory complications in humans as well as exacerbate global climate change (Heederik et al., 2007). The overuse of antibiotics and toxic chemicals by CAFOs can negatively impact the surrounding environment by reducing landscape diversity and native species and causing eutrophication in streams (Centner, 2003). Additionally, the heavy use of antibiotics in CAFOs has raised concerns about their role in the ongoing evolution of microbial antibiotic-resistant bacterial strains (West et al., 2011).

Hog lagoons are open-air basins comprised of fecal matter, urine, wash water, and rain (Arfken et al., 2015). Surrounding water sources are at risk of contamination due to both the runoff from these lagoons, and the use of their contents as 'organic' fertilizer. This fertilizer is then sprayed onto surrounding crops and may then leach or runoff into water sources. Depending how long the lagoon wastes sit before either leaching or being used as fertilizer, several chemical process can take place that alter the original animal waste into more harmful substances, like nitrates or microbial pollutants (Arfken et al., 2015; Whitall et al., 2003). The presence of these chemicals in surrounding water sources may degrade stream water quality

and impact aquatic life, as well as impacting the human populations relying on those water sources. Known impacts of CAFOs on nature and human health include eutrophication, foul odors/tastes of waters, fish kills, low oxygen levels, and the threat of pathogenic bacterial infections (West et al., 2011). Hog lagoons have the potential to produce elemental, anion, microbial, and nutrient wastes, including, but not limited to, toxic metals, nitrates, phosphates, chloride, sulfates, and fecal coliform (Krapac et al., 2002; Sobsey & Hill, 2008); the discharges from CAFO water treatment lagoons may lead to adverse human health and environmental impacts.

Aquatic scientists monitor water quality using benthic macroinvertebrates to assess stream health. Bioindicators are living organisms that are used to monitor an ecosystem's health, usually aquatic (Rosenberg & Resh, 1993). Freshwater macroinvertebrates are arguably one of the best choices for bioindication for several reasons- they are easy to collect, relatively inexpensive to sample, they are abundant (representing the second largest group of organisms in aquatic ecosystems), and taxonomically rich (Allan, 1995; Dodson, 2001). Monitoring benthic macroinvertebrate populations can be used to assess the overall health of that ecosystem based on different sensitivity characteristics possessed by the species. Benthic macroinvertebrates are assigned different tolerance values based on how sensitive or tolerant they are to changing water chemistry and harsh living conditions. Sampling aquatic insects, considering their tolerance value, and calculating an Index of Biologic Integrity (IBI) can indicate the water quality present. The IBI value can determine if the stream's water quality is considered excellent, good, good-fair, fair, or poor (dependent on ecoregion of the stream). The

simplicity of the IBI makes it easy to understand, interpret, and apply, as well as making it a widely accessible tool (Van Dolah et al., 1999).

The EPT percentage (summation of Ephemeroptera, Plecoptera, and Trichoptera species richness) can indicate the health of a stream ecosystem (DeWalt & Webb, 1998). The EPT index uses the relative abundance of each insect (all of which have a low tolerance to pollution) (Hamid & Md Rawi, 2017).

In addition to an IBI and % EPT, both a Simpson's biodiversity index and a Shannon Wiener index can be calculated. The Simpson's biodiversity index is based on the relative abundance of species, and it models the probability that two randomly selected individuals will be from the same category (He & Hu, 2005; McLaughlin et al., 2016). With this metric, each species' contribution is determined by the probability that it will appear in a random sample of the population (Smith & Grassle, 1977). The Shannon-Wiener index is a combined measurement of both the number of species in a community, or richness, and the relative frequency of those species, or the equitability (Di Bitetti, 2000). The use of these metrics, in combination with the IBI and % EPT, helped to determine if the range of species in a single site is objectively variated.

Purpose of Study

The objectives of this project were to quantitatively analyze the combined effect that hog and poultry CAFOs have on health risks that residents of Sampson County and organisms inhabiting the surface waters are experiencing as a result of poor water quality and to raise awareness of the environmental injustices that residents of Sampson County experience daily. By employing the One Health approach, the combination of both drinking and surface water

results can be associated to make broader conclusions of the impacts of CAFOs to watershed health. The hypothesis that areas with poor drinking water quality will also be reflected in localized poor surface water quality will be tested.

Materials and Methods

This study combined ion chromatography (IC), inductively coupled plasma-optical emission spectroscopy (ICP-OES), 3M Petrifilm[™] *E. Coli* testing with a macroinvertebrate sampling approach to encompass the overall quality of nearby drinking and surface waters due to continuous agricultural pollution from hog lagoons and spray fertilizer. The concentration of these chemicals were IC, ICP-OES, and 3M Petrifilm[™] *E. coli* tests. Additionally, the quality of surface water sources surrounding hog lagoons were analyzed with the use of macroinvertebrate biomonitoring, where the presence (or absence) of sensitive macroinvertebrates qualitatively (and quantitatively, using both the NC biotic index, % EPT, and a biodiversity index) assessed the water quality. Comparison of this project's findings to EPA benchmarks helped to indicate any water sources that were adversely affected by the amount and density of hog land poultry operations surrounding them.

Collection sites for both drinking and surface water samples are mapped (Fig. 2). Residential drinking water sites may be stacked to protect resident confidentiality and are meant to give relative locations of drinking water sampling sites.

[Insert figure 2]

2021 Spring & Fall Collections

Surface water samples were collected from 11-12 September 2021. All surface water sampling was completed in two days and included five sites (Fig. 2, Table 1). Stream sites were chosen based on presence (or absence) of CAFOs near the water source; one site (Little Coharie) was picked based on the lack of hog lagoons close to them to serve as reference sites. Surface water samples were collected in acid-washed 250mL Nalgene bottles and kept on ice until they were brought back to the lab. 137 drinking water samples were taken from resident's homes between March – October 2021. Drinking water sample collections were led by the EJCAN collaborative research group and were collected by running the resident's sink for 60 seconds, then collecting a 250mL room temperature water sample. Samples were kept on ice until they were brought back to the lab. Samples for ion chromatography are poured into 50mL centrifuge tubes and placed in the freezer until analysis. Samples for ICP-OES were acidified with approximately 2mL concentration nitric acid and placed in the fridge until analysis. 2022 Spring Collection

Drinking water samples were collected by the same protocol as spring/fall 2021 samples; the EJCAN collaborative research group led the residential collections.

Surface water samples were collected in the field. Benthic macroinvertebrates were collected using the US EPA qual-4 method, using a kick seine, D-frame dip net and sieve. Macroinvertebrates were removed from sieves and preserved in ethanol on site. Stream discharge was calculated for streams with measurable flow (Table 2). Each site was analyzed for approximately one hour time frames. Benthic macroinvertebrates collected in the field were placed in glass jars with 80% ethanol (EtOH) and taken back to the lab for identification.

[Insert table 1]

[Insert table 2]

Inductively coupled plasma – optical emission spectrometry

Drinking water and stream water samples were acid digested using a modified EPA Method 3015A (USEPA, 2018). 40mL of each water sample and 10-mL of nitric acid were added to a MARSXpress digestion vessel. Digestion vessels were placed in the Microwave Assisted Reaction System (MARS) and ramped to 170°C for 10 minutes and held at 170°C for 10 more minutes. Water samples were allowed to cool, filtered into a 50-mL volumetric flask, and placed in a 50-mL centrifuge tube. Nitric acid and deionized (DI) water were digested for quality control using the same technique as the samples.

A Varian 710ES inductively coupled plasma-optical emission spectrometer (ICP-OES) with a MirraMist Teflon nebulizer and glass cyclonic spray chamber was used to analyze the digested samples. A 2-ppm yttrium (Y) internal standard was added via the sample introduction system. Emission intensities were recorded in duplicate; emission wavelengths used in this study are noted (Table 3). The plasma power was 1.00 kW, the argon flow rate was 15.0 L/min with an auxiliary flow of 1.50 L/min. The nebulizer pressure was 300 kPa. A Teledyne ASX-560 autosampler was used.

[Insert table 3]

The concentration range of standards used to create calibration curves was 0.01 – 20.0 mg/L. For quality control during the run, a laboratory fortified blank (LFB, 0.2 ppm), a 1% nitric acid blank, a matrix spike (1 ppm added to sample), and a sample duplicate was analyzed every 10 samples.

Results from the ICP-OES were corrected by multiplying the concentration by 1.2 to account for the dilution factor; final concentrations are in parts per million (ppm or mg/L). Detection limits were determined once per year, or anytime a major change was made to the method, for example operator, preventative maintenance visit, etc. Metals analyzed by ICP-OES were compared to their EPA accepted standards (Table 4).

[Insert table 4]

Ion chromatography

A Dionex ICS-3000 ion chromatograph with a Dionex AS autosampler and Dionex IonPac[™] AS11-HC column (4x250mm) and conductivity detection were used. The Dionex suppressor was set to 91 mA. The flow rate was 1.4 mL/min (isocratic), and the eluent was 21.00 mM KOH. If the eluent concentration changed, the suppressor current was adjusted accordingly. The concentration range of standards used to create calibration curves was 0.1 -10.0 mg/L.

For this study, ion chromatography was used to detect several analytes (Table 5).

[Insert table 5]

For each IC run, two lab fortified blanks (LFB, 0.752 and 3.0 mg/L) and a matrix spike (MS) were run every 10 samples; a percent recovery was calculated for each LFB and MS sample. Once analyzed, the peak area of each sample was used to calculate the concentration of each anion in the sample and its uncertainty (Eq. 1 & 2).

$$C = \frac{|(A-b)|}{m}$$
 Equation 1

where C = Concentration of analyte in sample (mg/L), A = Peak area, b = intercept, and m = slope, and

$$S_{\chi} = \frac{\binom{s_{y}}{m}\sqrt{\binom{1}{k} + \binom{1}{n} + (A-y)^{2}}}{(m^{2})(\Sigma(x_{i}-mean\ x)^{2})}$$
 Equation 2

Where $S_x =$ uncertainty, $S_y =$ vertical deviation in y, m = slope, k = number of replicates, n = number of responses in the calibration curve, A = average peak area of the sample, y = average peak area of the standards, and x = concentration of an individual standard. The percent recovery was calculated for all QC and MS samples and monitored in control charts. 3M PetrifilmTM Escherichia coli (E. coli) Testing

Surface water samples were also tested for *E. coli* using 3M Petrifilm[™] *E. coli* testing. *E. coli*, an indicator bacterium, is the most common cause of bacterial infections, sepsis, and surface water impairment in the United States (Harmel et al., 2010; Johnson & Russo, 2002). The possibility of surface waters being contaminated with *E. coli* is a direct threat to the health of any living organism around it. 3M Petrifilm[™] *E. coli* testing takes advantage of the blue precipitate associated with the beta glucuronidase that most *E. coli* produces (3M Petrifilm[™] Interpretation Guide). Other coliform colonies produce red gas bubbles; the combination of red and blue bubbles within the same quadrant of the Petrifilm[™] plate indicates presence of total coliform in the sample, and the presence of blue colonies with gas indicates presence of *E. coli* in the sample. Surface water samples that were collected in September 2021 were tested for *E. coli* and total coliform (Table 6). Drinking water samples were tested for *E. coli* and total coliform by collaborators at University of North Carolina Chapel Hill.

[Insert table 6]

Benthic Macroinvertebrate Biomonitoring

The IBI equation is calculated (Eq. 3),

$$IBI = \frac{\Sigma T_i n_i}{N}$$
 Equation 3

where T_i is the tolerance value of a specific organism, n_i is the number of a specific organism (where 1-2 organisms is counted as 1, 3-9 as 3, and >10 as 10), and N is the total number of organisms sampled. The BI value is used to categorize stream's water quality as excellent, good, good-fair, fair, or poor, and is dependent on each stream's ecoregion.

In addition to an IBI, a % EPT was also calculated. The % EPT equation is calculated (Eq. 4),

% Abundance =
$$\frac{Total EPT Taxa}{Total Taxa Found} \times 100$$
 Equation 4

where EPT can be defined as E = Ephemeroptera (mayflies), P = Plecoptera (stoneflies), and T = Trichoptera (caddisflies). The sensitivity of these organisms to pollution is considered when calculating a % EPT to analyze water quality; the higher the % EPT value, the better the water quality.

Simpson's biodiversity index quantifies the overall biodiversity of the surface water habitats by considering the benthic macroinvertebrate collections (Eq. 5),

$$D = \sum \frac{n_i(n_i-1)}{N(N-1)}$$
 Equation 5

where *D* is the Simpson's biodiversity value, n_i is the number of organisms that belong to species *i*, and N is the total number of organisms. The value for *D* ranges from 0-1 for each species, with a higher value for *D* correlating to lower diversity. Finally, the Shannon-Wiener index quantifies the biodiversity in a surface water habitat while considering the ecosystem's richness and relative frequency of species (Eq. 6).

$$H = -\sum p_i \ln (p_i)$$
 Equation 6

where H is the Shannon-Wiener index value, generally ranging from 1.5 - 3.5, and p_i is the relative frequency of species *i* in the community.

Statistical Analysis

Several statistical analyses were performed in Jamovi version 2.2.5 to find different relationships between variables. First, all variables were tested for normality to ensure the correct tests were run. A correlation matrix was done to understand the dataset and results better visually and statistically. The Akaike information criterion (AIC) score was used for any significant relationships to determine which relationships would be the best fit for the linear model. Any significant (p < 0.05) correlations were noted in bold and assigned a Spearman's Rho value (also known as Spearman's rank-order correlation coefficient) to better understand the strength of the significant relationship between the variables (Table 7). Generally, a higher positive rank-order coefficient means there is higher co-occurrence between the variables.

[Insert table 7]

Next, any significant correlations were plotted into a correlation matrix (Fig. 3) to visually understand and compare the relationships.

[Insert figure 3]

A linear regression model was done for each contaminant, using distance and density as covariates, to find any correlation between either distance to closest CAFO or density of CAFOs within a 10km² radius and contaminant level. A model fit measures table was produced for the

best fit model of each contaminant, displaying the adjusted R² value, AIC value, p-values, and estimate values for both the intercept and each predictor. The software assigns values to determine which model (distance to closest CAFO, density of CAFOs within a 10km radius, or a combination of both) is the best predictor for each contaminant. Contaminants with significant correlations included chloride, sulfate, iron, and manganese (Table 8). For statistical purposes, any contaminant concentration that was below the detection limit (< LOD) was reported as ½ the value of the detection limit for that contaminant (Venturini et al., 2015).

[Insert table 8]

Surface Water Analyses

Benthic macroinvertebrates were identified in the lab to the lowest taxonomic unit possible, several parameters were analyzed for each surface water site (Table 9).

[Insert table 9]

Overall productivity can be defined as the total taxa collected. Family and genus richness alike are the total number of different family and genus groups determined during the identification process. Percent EPT (% EPT) is scored on a percentage scale from 0-100, with a higher percentage indicating a higher presence of EPT taxa, which are generally pollution-sensitive, thus indicating a healthier ecosystem. The percentage is then assigned a qualitative measure to classify the stream as wither poor, fair, good-fair, good, or excellent.

The North Carolina Index of Biologic Integrity (NCIBI) Index is a weighted average of tolerance values of all taxa found in a collection site. Following equation 3, the NCIBI value will generally be calculated to be between 0-10; the value is then "scored" between 1.0-5.0 (depending on ecoregion), and a bioclassification can be assigned.

The Shannon Wiener index is scored on a scale generally from 1.5-3.5, with a higher score explaining more diversity. Associated with the Shannon Wiener index is the evenness, which describes the overall distribution of taxa in an ecosystem. This value ranges from 0-1, with a higher value indicating a more evenly distributed ecosystem. Like evenness, Simpson's biodiversity index is scored on a scale of 0-1, with a higher value indicating more biodiversity.

Results and Discussion

Correlation Analyses

Table 7 shows the correlation matrix results.

Linear Regression

Table 8 shows the linear regression results.

Surface Water Analyses

While each surface water sample possessed less than five total *E. coli* colonies/1mL, the presence of any number of colonies representative of possible *E. coli* poses a threat to the community surrounding the water source.

It is important to note that productivity and richness are not directly correlated- a site can have a high productivity and low richness, or vise versa.

A high Shannon Wiener value and a low Simpson's biodiversity index value (shown by Six Runs and Rowan) indicates a single species is more dominant than any other taxa in that specific ecosystem; for Six Runs, genera *Cheumatopsyche* and *Stenonema* dominated. Rowan showed a high number of *Elimidae* and *Chironomidae*.

Six Runs Creek showed the highest overall productivity, family and genus richness, % EPT, Shannon-Wiener index, and evenness of of the five sites. Despite being only 0.40 km from

the closest hog farm and having 5 hog farms in a 10 km² radius upstream, Six Runs Creek was concluded the healthiest of the surface sites samples. This can most likely be attributed to the higher flow observed when compared to the other sites.

When collecting benthic macroinvertebrates in spring 2022, it was noted that there was a beaver dam sitting upstream of the collection site that could most likely be responsible for the poor quality of the stream due to the obstruction of flow.

Overall, the surface sites showed minimal adverse impacts due to hog lagoon presence. Several sensitive taxa were collected, including *Acroneuria evoluta*, *Isoperla holochlora*, and *Pycnopsyche sp.* Some of the most pollution-tolerant taxa collected included members of the *Libellulidae* family, *Callibaetis sp.*, and members of the *Coenagrionidae* family. Due to the variability associated with surface sites, it is difficult to confidently link lack of sensitive taxa and major impacts, like low flow or diversity, to hog farm presence. Flooding, beaver dams, human impacts, and flow are all factors that could contribute to differences in overall health between surface sites.

The data collected, specifically the EPT bioclassifications, can be compared to previous DEQ data collected for several, but not all, of the sites (Table 10).

[Insert table 10]

It can be observed that Little Coharie Creek has declined in quality, likely due to the beaver dam that was observed upstream at the time of collection. Additionally, Six Runs and Crane Creek have both increased in water quality since the NCDEQ last sampled.

Discussion

The purpose of the correlation matrix is to try to find any strong relationships between significant variables. Figure 3 visually depicts the correlations that the statistical software found significant; the correlations are categorized on a scale of -1 to 1, where -1 is a perfect negative relationship and 1 is a perfect positive relationship. Each number is then denoted with a range of 0-3 asterisks, where 0 indicates a weak significance and "***" indicates a strong significance. The contaminants that were considered significant enough to be included in the correlation matrix were chloride, sulfate, iron, and manganese; all the contaminants had a strong positive correlation with density of CAFOs in a 10km² radius, meaning that as density increased, contaminant level increased. Distance to closest CAFO as an independent variable was not significant enough to be included in the correlation matrix. Strong intra-elemental correlations presented between sulfate & chloride and iron & manganese; these could likely be explained by lead pipe corrosion (for sulfate & chloride ratios) or both the catchment erosion and the dissolution of iron and/or manganese-containing sediments and minerals (for iron & manganese ratios) as opposed to CAFO contamination (Edwards & Triantafyllidou, 2007; Zaw & Chiswell, 1999).

The linear regression analysis aims to give better understanding for relationships. Each element is analyzed separately for the most accurate results. Each contaminant is analyzed against three models (distance to closest CAFO (1), density of CAFOs in a 10km² radius (2), and distance to closest CAFO + density of CAFOs in a 10km² radius (3)) to find the best fit using the AIC values. The adjusted R² value for each contaminant is used to tell what percentage of the variance in that specific contaminant can be described by the given model. The intercept

estimate value is the level that the contaminant should be when the regression line crosses the y-axis. Finally, the estimate value for each predictor explains the change in that value as the position on the x-axis increases by one unit.

The surface water analyses show that there are high numbers of sensitive taxa, meaning that the surface waters are healthier than expected. If there is any contamination to surface waters due to CAFO farming, it is likely negligible or diffused before causing any true harm to the ecosystem.

Conclusions

Holistically, the results of the benthic macroinvertebrate and drinking water analyses show that there is no immediate threat to ecosystem health as a result of CAFOs in Sampson County, NC with the use of our methods specifically. ~19% of drinking water samples tested above benchmark values for at least one anion, and ~40% of drinking water samples tested above the threshold levels for at least one metal. Overall, distance to closest hog farm shows almost no significant contaminant levels when measuring anions or metals. While there are some correlations between density of hog farms in a 10km² radius and a handful of contaminants, they are weak and can most likely be better explained by geological features, such as rock formation or chemical makeup. Benthic macroinvertebrate monitoring results showed several sensitive taxa present in surface waters across Sampson County, including *Acroneuria evoluta, Isoperla holochlora,* and *Pycnopsyche sp.* The presence of these taxa indicates that there is minimal, if any, impact to surface waters as a result of CAFOs. The lack of impacted freshwater ecosystems in Sampson County can be attributed to dilution or other forms of chemical uptake by other organisms (Chen & Barko, 1988).

It is important to recognize the environmental justice conclusions, as well. While the statistical and biological analyses showed little to no significant data, the residents of Sampson County can gain a new level of security in knowing that there is no immediate threat to their health and well-being according to our methods. Additionally, further research in the field can use this method as a baseline for the development of new methods and ideas to advance the overall understanding of the effects that CAFOs have on not only water, but all aspects of ecosystem and human health. Future research should focus on the possibility of modeling CAFO runoff in a more controlled environment to see any adverse effects without the potential interference of other variables. Additionally, research focusing more on the air quality, or effects of CAFO waste being used as fertilizer in the context of air quality, could be very valuable. Finally, modeling a study with a focus on the analysis of molecular biomarkers as indicators of water quality could expand the understanding that we have on how these contaminants may interact with the surrounding watershed.

References

- Allan JD (1995) Stream Ecology: Structure and Function of Running Waters, London: Chapman & Hall.
- Arfken A, Song B, Mallin M (2015) Assessing hog lagoon waste contamination in the Cape Fear Watershed using Bacteroidetes 16S rRNA gene pyrosequencing. Applied Microbiology & Biotechnology 99(17):7283–7293
- Di Bitetti MS (2000) The Distribution of Grooming Among Female Primates: Testing Hypotheses With the Shannon-Wiener Diversity Index, Behaviour 137(11):1517-1540.

DOI: https://doi.org/10.1163/156853900502709 [Online January 1, 2000]

Bonilla-Aldana DK, Dhama K, Rodriguez-Morales AJ (2020) Revisiting the one health approach in the context of COVID-19: a look into the ecology of this emerging disease. Advances in Animal and Veterinary Science. 8(3):234-237

Bryant B (1995) Environmental Justice: Issues, Policies. Solutions. Washington: Island.

Bullard RD, Borgmann A (2007). Environmental justice for all. Available:

https://www.uky.edu/~tmute2/GEI-Web/password-protect/GEI-readings/Bullard-

Environmental%20justice%20for%20all.pdf [accessed October 2022]

Burkholder J, Libra B, Weyer P, Heathcote S, Kolpin D, Thorne PS, Wichman M (2007) Impacts
of waste from concentrated animal feeding operations on water quality.
 Environmental Health Perspectives 115(2):308-312
 Cassuto DN (2010) The CAFO Hothouse: Climate Change, Industrial Agriculture and the

Law. Animals & Society Institute Policy Paper SSRN:

https://ssrn.com/abstract=1646484 [accessed March 2022]

Census of Agriculture County Profile: Sampson County North Carolina (2017) USDA. Available: https://www.nass.usda.gov/Publications/AgCensus/2017/Online_Resources/County_P

rofiles/North_Carolina/cp37163.pdf [accessed 1 March 2022]

- Centner TJ (2011) Addressing water contamination from concentrated animal feeding operations. Land Use Policy 28(4):706-711
- Centner TJ (2003) Regulating concentrated animal feeding operations to enhance the environment. Environmental Science & Policy 6(5):433-440
 - Chen RL, Barko JW (1988) Effects of freshwater macrophytes on sediment chemistry. Journal of Freshwater Ecology 4(3):279-289
- Coates JD, Cole KA, Michaelidou U, Patrick J, McInerney MJ, Achenbach LA (2005) Biological control of hog waste odor through stimulated microbial Fe (III) reduction. Applied and Environmental Microbiology 71(8):4728-4735
- DeWalt RE, Webb DW (1998) Summer ephemeroptera, Plecoptera, and trichopteran (EPT) species richness and hilsenhoff biotic index at eight stream segments in the lower Illinois river basin. INHS Center for Biodiversity
- Dodson SI (2001) Zooplankton communities of restored depressional wetlands in Wisconsin USA. Wetlands 21:292–300

- Donham KJ, Wing S, Osterberg D, Flora JL, Hodne C, Thu KM, Thorne PS (2007) Community health and socioeconomic issues surrounding concentrated animal feeding operations. Environmental Health Perspectives 115(2):317-320
- Edwards M, Triantafyllidou S (2007) Chloride-to-sulfate mass ratio and lead leaching to water. Journal-American Water Works Association 99(7):96-109

EJCAN (2019) Available: <u>http://ejcan.org/</u> [accessed March 2022]

Fritz JS (1987) Ion Chromatography. American Chemical Society, Instrumentation 59(4)

Graddy S, Simon E, Rundquist S (2020) UPDATE: Exposing Fields of Filth: Factory Farms

Disproportionately Threaten Black, Latino, and Native American North Carolinians.

Waterkeeper Alliance & Environmental Working Group. Available:

https://www.ewg.org/interactive-maps/2020-fields-of-filth/ [accessed March 2022]

Ab Hamid S, Md Rawi CS (2017) Application of Aquatic Insects (Ephemeroptera,

Plecoptera And Trichoptera) In Water Quality Assessment of Malaysian

Headwater. Tropical Life Sciences Research 28(2):143–162

Harmel RD, Karthikeyan R, Gentry T, Srinivasan R (2010) Effects of agricultural management, land use, and watershed scale on E. coli concentrations in runoff and streamflow.

Transactions of the ASABE 53(6):1833-1841

He F, Hu XS (2005) Hubbell's fundamental biodiversity parameter and the Simpson diversity index. Ecology Letters 8(4):386-390

- Heederik D, Sigsgaard T, Thorne PS, Kline JN, Avery R, Bønløkke JH, et al. (2007) Health effects of airborne exposures from concentrated animal feeding operations. Environmental Health Perspectives 115(2):298-302
- Hribar C (2010) Understanding concentrated animal feeding operations and their impact on communities. Available: https://stacks.cdc.gov/view/cdc/59792 [accessed March 2022]

Johnson JR, Russo TA (2002) Extraintestinal pathogenic Escherichia coli: "the other bad E coli". Journal of Laboratory and Clinical Medicine 139(3):155-162

- Krapac IG, Dey WS, Roy WR, Smyth CA, Storment E, Sargent SL, Steele JD (2002) Impacts of swine manure pits on groundwater quality. Environmental Pollution 120(2):475-492
- Mackenzie JS, Jeggo M (2019) The One Health approach—Why is it so important? Tropical Medicine and Infectious Disease 4(2):88
- McLaughlin JE, McLaughlin GW, McLaughlin JS, White CY (2016) Using Simpson's diversity index to examine multidimensional models of diversity in health professions education. International Journal of Medical Education 7:1–5
- Middleton D, Pallister J, Klein R, Feng YR, Haining J, Arkinstall R, Frazer L, et al. (2014) Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. Emerging Infectious Diseases 20(3):p.372

North Carolina Department of Agriculture (2021) North Carolina Agricultural Statistics. Available:

https://www.nass.usda.gov/Statistics_by_State/North_Carolina/Publications/Annual_S tatistical_Bulletin/AgStat/NCAgStatBook.pdf [accessed October 2022]

North Carolina Department of Environmental Quality (2019) Benthic Monitoring Data Web

Mapping Application. Available: https://data

ncdenr.opendata.arcgis.com/apps/ncdenr::benthic-monitoring-data/explore [accessed

October 2022]

North Carolina Department of Environmental Quality (January 6, 2021) Environmental Justice

Report. Available:

https://files.nc.gov/ncdeq/Air%20Quality/permits/align-energy/Align-Energy-

EJReport.pdf [accessed March 2022]

North Carolina Department of Environmental Quality (2019) Environmental Justice Report: Enviva Pellets Sampson. Available: <u>https://deq.nc.gov/media/13865/download</u>

[accessed March 2022]

North Carolina Department of Environmental Quality (2016) Standard Operating Procedure for

the Collection and Analysis of Benthic Macroinvertebrates. Division of Water

Resources, Water Sciences Section. Biological Assessment Branch, Version 5.0

Office of Legacy Management. Environmental Justice Report. Available:

https://www.energy.gov/lm/services/environmental-

justice/environmentaljusticehistory [accessed October 2022]

Olesik JW (1991) Elemental analysis using ICP-OES and ICP/MS. Analytical Chemistry 63(1):

12A-21A

Rosenberg DM, Resh VH (1993) Freshwater Biomonitoring and Benthic

Macroinvertebrates. New York: Chapman and Hall

Sandstrom MW, Lewis JA (2009) Instructions for Field Use of Spike Solutions for

Organic-Analyte Samples. Processing of Water Samples (5.3)2

Smith W, Grassle JF (1977) Sampling Properties of a Family of Diversity Measures. Biometrics

33(2):283-292

Sobsey MD, Hill VR (2008) Hog Waste Treatment to Control Microbial Contamination. Water Resources Research Institute of the University of North Carolina. Available:

https://repository.lib.ncsu.edu/bitstream/handle/1840.4/4110/NC-WRRI

380.pdf?sequence=1 [accessed March 2022]

Thorne PS (2007) Environmental health impacts of concentrated animal feeding operations: anticipating hazards—searching for solutions. Environmental Health Perspectives 115(2):296-297

- United States Environmental Protection Agency (2018) Chapter 3 of the SW-846 Compendium: Inorganic Analytes. pp. 1-22.
- United States Environmental Protection Agency (2022) Drinking Water Regulations and Contaminants. Available: https://www.epa.gov/sdwa/drinking-water-regulations-andcontaminants [accessed March 2022]

United States Environmental Protection Agency (2014) North Carolina Criteria Water Quality Survey. Available: https://www.epa.gov/sites/default/files/2014-12/documents/nc criteriawqs.pdf [accessed March 2022] United States Census Bureau (2021) QuickFacts. Available:

https://www.census.gov/quickfacts/NC [accessed October 2021]

University of New Hampshire. EPT Index. Available:

http://cfb.unh.edu/StreamKey/html/biotic_indicators/indices/EPT.html [accessed

March 2022]

- Van Dolah RF, Hyland JL, Holland AF, Rosen JS, Snoots TR (1999) A benthic index of biological integrity for assessing habitat quality in estuaries of the southeastern USA. Marine Environmental Research 48(4-5):269-283
- Venturini N, Bicego MC, Taniguchi S, Sasaki ST, Garcia-Rodriquez F, Brugnoli E, Munix P (2015) A multi-molecular marker assessment of organic pollution in shore sediments from the Rio de la Plata Estuary, SW Atlantic. Marine Pollution Bulletin 91(2015):461 475
- Villanueva CM, Kogevinas M, Cordier S, Templeton MR, Vermeulen R, Nuckols JR, et al. (2014) Assessing exposure and health consequences of chemicals in drinking water: current state of knowledge and research needs. Environmental Health Perspectives 122(3):213-221
- West BM, Liggit P, Clemans DL, Francoeur SN (2011) Antibiotic resistance, genetransfer, and water quality patterns observed in waterways near CAFO farms and wastewater treatment facilities. Water, Air, & Soil Pollution 217(1):473-489
- Whitall D, Hendrickson B, Paerl H (2003) Importance of atmospherically deposited nitrogen to the annual nitrogen budget of the Neuse River estuary, North Carolina. Environment International 29(2-3):393-399

Williams, M. NC DWR Benthos Sample Results. NC Department of Environmental Quality.

Available: https://data

ncdenr.opendata.arcgis.com/datasets/713e6200142d4c88850cc35ac207b157_0/explo

e?location=34.869346%2C-78.488829%2C11.45 [accessed September 2022]

Wing S, Cole D, Grant G (2000) Environmental injustice in North Carolina's hog

industry. Environmental health perspectives 108(3):225-231

Zaw M, Chiswell B (1999) Iron and manganese dynamics in lake water. Water Research

33(8):1900-1910

3M Petrifilm[™] Interpretation guide (2017) Available:

https://multimedia.3m.com/mws/media/2362460/petrifilm-ecoli

coliforminterpretation-guide.pdf [accessed March 2022]

Tables/Figures



Figure 1. CAFOs in eastern North Carolina, with emphasis on Sampson and Duplin Counties. Red indicates hog farms, and violet indicates poultry farms (Graddy et al 2020).



Figure 2. Collection sites in Sampson County, NC. Red house icons indicate residential sites and are stacked to protect privacy- each house icon may indicate multiple collection sites. Blue water icons indicate surface water collection sites, with a single point being a single site.



Figure 3. Correlation matrix visually displaying any significant relationships between variables analyzed using the Spearman's correlation. Each relationship is assigned a correlation value between -1 to +1, where 1 is perfectly, positively correlated, 0 indicates no correlation, and -1 indicates a perfect negative correlation. Each correlation value is also assigned an asterisk classification, where a higher number of asterisks indicates a stronger correlation.

Table 1. Surface water collection sites sorted by name, collection site, distance to nearest hog farm (kilometers),
and classification of collections by time period (F21 = Fall 2021, S22 = Spring 2022).

Site name	Collection Site Coordinates	Distance to nearest hog farm (km)	Density of hog farms upstream (10 km ² radius)	Water collection	Aquatic Macroinvertebrate Collections
Six Runs Creek	N 34055.326, W 078012.802	0.40	5	F21, S22	S22
Crane Creek	N 34091.173 W 078026.328	0.22	2	F21, S22	S22
Buckhorn Creek	N 34□53.579, W 078□18.266	1.22	7	F21, S22	S22
Rowan Branch	N 34□58.006, W 078□14.778	0.60	1	F21, S22	S22
Little Coharie Creek	N 35011.940, W 078028.525	2.46	0	F21, S22	S22

Table 2. Surface water collection site field parameters. Measurements were not taken for Crane Creek and Little Coharrie Creek; Crane Creek had negligible flow, and Little Coharrie had a large beaver dam blocking all flow from upstream of the collection site, so calculating discharge was not possible.

Site Name	Width (ft)	Average Depth (ft)	Flow (ft/s)	Discharge (ft ³ /sec)
Six Runs Creek	47.24	1.08	1.5	76.53
Crane Creek	Not measured	Not measured Not measured		Not measured
Buckhorn Creek	7.87	0.65	0.77	3.92
Rowan Branch	12	0.53	1.2	7.68
Little Coharie Creek	Not measured	Not measured	Not measured	Not measured

 Table 3. ICP-OES elements and emission wavelengths.

Analyte	Emission Wavelength (nm)
Aluminum (Al)	396.152
Arsenic (As)	188.980
Boron (B)	249.772
Barium (Ba)	233.527
Calcium (Ca)	422.673
Cadmium (Cd)	214.439
Chromium (Cr)	267.716
Copper (Cu)	327.395
Iron (Fe)	238.204
Magnesium (Mg)	285.213
Manganese (Mn)	257.610
Molybdenum (Mo)	202.032
Nickel (Ni)	231.604
Lead (Pb)	220.353
Selenium (Se)	196.026
Strontium (Sr)	407.771
Zinc (Zn)	213.857

Analyte	Surface Water benchmark (mg/L)	Drinking Water benchmark (mg/L)
Aluminum (Al)	6.5	0.05-0.02
Arsenic (As)	0.01	0.01
Boron (B)	No data	No data
Barium (Ba)	200	200
Calcium (Ca)	No data	No data
Cadmium (Cd)	0.002	0.005
Chromium (Cr)	0.05	0.01
Copper (Cu)	0.007	1.0
Iron (Fe)	1.0	0.3
Magnesium (Mg)	No data	No data
Manganese (Mn)	0.2	0.05
Molybdenum (Mo)	2.0	2.0
Nickel (Ni)	0.088	0.025
Lead (Pb)	0.025	0.015
Selenium (Se)	0.005	0.05
Strontium (Sr)	40	40
Zinc (Zn)	0.05	5.0

 Table 4. ICP-OES analytes and their respective NC & EPA benchmark standard values.

Analyte	Benchmark (mg/L)
Chloride (Cl ⁻)	230
Nitrate (NO ₃ -, measured as N)	10
Fluoride (F ⁻)	2.0
Sulfate (SO ₄ ²⁻)	250
Bromide (Br-)	No data
Phosphate (PO ₄ ³⁻)	0.05

Table 5. Ion chromatography analytes and their respective NC & USEPA benchmark standard values.

Table 6. Fall 2021 surface water samples *E. Coli* and total coliform analyses using 3M Petrifim™ *E. coli* testing measured in 1mL. Values are converted to total number of colonies per 100 mL to effectively compare to standard NC benchmark levels.

Sample Name	Total Coliform (number of colonies/ 1mL)	Total Coliform (number of colonies/ 100mL)	<i>E. coli</i> (number of colonies/ 1mL)	<i>E. coli</i> (number of colonies/ 100mL)
Six Runs Creek	5	500	4	400
Crane Creek	7	700	0	0
Buckhorn Creek	6	600	1	100
Rowan Branch	2	200	0	0
Little Coharie Creek	9	900	1	100

Table 7. Spearman's correlation matrix values, including p-values to determine significance and Spearman's Rho values to analyze co-occurrence between values. Any significant p-values are indicated in bold and assigned a Spearman's Rho to further classify them.

Contaminant	Distance p-value	Density p-value	Spearman's Rho
Chloride	0.310	0.010	0.237
Nitrate	0.910	0.254	
Fluoride	0.261	0.109	
Sulfate	0.265	0.002	0.283
Bromide	0.633	0.114	
Phosphate	0.997	0.357	
Aluminum	0.090	0.466	
Barium	0.513	0.491	
Calcium	0.757	0.650	
Cadmium	0.073	0.110	
Chromium	0.996	0.868	
Copper	0.523	0.584	
Iron	0.135	0.033	0.199
Magnesium	0.026	0.213	-0.208
Manganese	0.849	<0.001	0.367
Nickel	0.128	0.643	
Lead	0.289	0.425	
Strontium	0.142	0.571	
Zinc	0.076	0.438	

Table 8. Linear regression model fit measures. Each contaminant of concern is accompanied by an AIC value to determine the best predictor, an adjusted R^2 value to determine the strength of the model fit explanation, an intercept estimate to represent the baseline contaminant level when the predictor = 0, and a predictor estimate to represent the rate at which the intercept increases or decreases. The best fit models are characterized between 13, where 1 = distance to closest CAFO, 2 = density of CAFOs within a $10km^2$ radius, and 3 = both distance to closest CAFO + density of CAFOs within a $10km^2$ radius.

Contaminant	Best fit model	Model AIC	Adjusted R ²	Intercept Estimate	Predictor Estimate
Chloride	1	878	-0.008	4.246	-0.293
Sulfate	2	741	0.039	1.133	0.537
Iron	2	369	0.062	-0.095	0.156
Manganese	2, 3	-442, -442	0.060, 0.073	0.010, 0.019	0.005, 0.004

	Six Runs Creek	Rowan Branch	Little Coharie	Crane Creek	Buckhorn Creek
			Creek		
Productivity	168	150	118	109	144
Family Richness	24	12	18	17	13
Genus Richness	27	14	19	18	14
% EPT	65.5%	13.3%	10.2%	22.0%	52.1%
EPT Classification	Excellent	Good-fair	Fair	Good	Excellent
NCIBI Score	4.21	4.49	4.37	4.50	5.86
NCIBI	Excellent	Excellent	Excellent	Excellent	Good
Classification					
Shannon-	2.92	1.91	2.08	2.43	2.25
Wiener Index					
Evenness	0.89	0.72	0.71	0.84	0.85
Simpson's	0.06	0.23	0.19	0.10	0.12
Biodiversity Index					

Table 9. Results of benthic macroinvertebrate monitoring analyses for each field site.

 Table 10. Comparison of stream water EPT bioclassification data between DEQ published data (NCDEQ 2019) and

 2021-2022 Appalachian State University collected data.

	EPT Classification		
Site	NCDEQ	Appalachian State	
Little Coharie	Good	Fair	
Six Runs Creek	Fair	Excellent	
Crane Creek	Good-Fair	Good	

Abigail Leigh Hrabosky was born in Greensboro, North Carolina, to Michael Hrabosky Jr. and Denise McGee. She graduated from Northwest Guilford High School in 2016. The following winter, she entered Appalachian State University to study Chemistry, and in December 2020 she was awarded the Bachelor of Science degree. In the fall of 2021, she began a Master of Science study in Biology at Appalachian State University. The M.S. was awarded in December 2022. In January 2023, Hrabosky began work in the department of Chemistry and Fermentation Sciences at Appalachian State University.