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Impact of a Cattle Crossing on Water Quality Along a Tributary of the Muddy Fork of the Illinois River, Northwest Arkansas

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Abstract

Cattle are known to have an effect on water quality in various bodies of water. Studying how cattle impact water quality along various streams and tributaries is important to understanding how certain water parameters may be affected at the individual farm level. It is known that unrestricted access to a cattle crossing has been shown to increase the occurrence of downstream pollutants such as *E. coli*, ammonium, total kjeldahl nitrogen, total suspended solids, total phosphorus, and turbidity. However, many studies focus on large-scale operations and neglect the individual farm level. In this study, samples were collected twice for each parameter studied. An upstream, crossing, and downstream sampling site were established in order to evaluate water quality across the reach of the studied tributary of the Muddy Fork of the Illinois River in Northwest Arkansas. Results were obtained out in the field or within a lab, but exact instructions were followed for both collections. It was discovered that cattle had an impact on water quality downstream from the cattle crossing on the individual farm level. However, many parameters were shown to exhibit poor quality from the upstream collection area. Specifically, *E. coli* increased at and downstream from the cattle crossing. Dissolved oxygen and biochemical oxygen demand also increased downstream from the cattle crossing. Results suggested that best management practices, such as off-stream watering points, should be implemented to reduce cattle occurrence in riparian zones to improve in-stream water quality.

Introduction

Background and Need

Within the last twenty years, the world human population has grown by 1.6 billion. (United Nations, 2019). With increased population comes growing concern for environmental impacts. Increases in population have resulted in an increase in the number of pollutants being released into the environment and overall environmental degradation. Meat consumption, particularly in the most developed countries, has led to increased livestock production. The production of livestock results in degradation to the environment through decreases in surface water quality and increased greenhouse gas emissions such as methane. In as little as 50-100 years, it is estimated that cattle will contribute approximately 2% to total global warming (Johnson & Johnson, 1995). Water quality degradation is a problem, particularly on cattle farms that contain at least one stream crossing. Unrestricted access to the crossing has been shown to increase the occurrence of downstream pollutants such as *E. coli*, ammonium, total kjeldahl nitrogen (TKN), total suspended solids (TSS), total phosphorus (TP), and turbidity (Vidon, Campbell, & Gray, 2008).

According to the World Health Organization (2019), contaminated water is linked to transmission of deadly diseases such as cholera, dysentery, diarrhea, hepatitis A, polio, and typhoid. The importance of understanding the potential for polluted waters to exist in an area and the ability to limit human exposure to that water is key to minimizing infection and death from waterborne illnesses worldwide.

Not only can pollutants affect water quality, but pollutants can also impact the health of adjacent riparian buffers as well as soil quality. Riparian buffers are found to trap phosphorus and other sediments released from cattle production facilities (Georgakakos, Morris, & Walter,

2018). Maintaining the health of riparian buffers is key to maintaining the high quality of waterways surrounding cattle facilities. Identifying consequences from cattle crossings and how they play a role in the ecosystem is vital to ensure a future in which there is limited exposure to pollutants and other harmful substances.

Problem Statement

Common nutrient pollutants, such as nitrogen and phosphorus, as well as pathogenic organisms, continue to be problematic in waterways (World Health Organization, 2019). At cattle crossings, cattle can release a number of contaminants into the water such as nitrogen, phosphorus, coliform bacteria, especially *E. coli*, and ammonia (Vidon, Campbell, & Gray, 2008). The riparian areas adjacent to waterways are degraded through compaction and removal of vegetation, often leading to sedimentation and increased turbidity due to soil erosion. Research into the impacts of cattle crossings and potential best management practices have not been well studied at the individual farm level.

Purpose of the Study

The purpose of this study was to measure common physical (turbidity and temperature), chemical (dissolved oxygen, biochemical oxygen demand, pH, nitrogen, and phosphorus), and biological (coliform and *E. coli* bacteria) water quality parameters across two dates at three locations within a reach of a tributary of the Muddy Fork of the Illinois River in Northwest Arkansas (See Figures 1, 2, and 3 in Appendix 1). By sampling upstream of the crossing, at the crossing, and downstream of the crossing on multiple sampling dates, the potential impact of the cattle crossing on the water quality was quantified. Results from studies like these may allow for better cattle management decisions and practices regarding placement and potential impacts of water crossings on in-stream water quality.

Research Objective

The following research objective guided this study:

- To compare water quality parameters upstream and downstream to those same parameters located at a cattle-crossing area within a small stream across time

Hypothesis

The null hypothesis was that there would be no difference in water quality parameters between sampling locations. The alternative hypothesis was that water quality parameters would differ at each location with the area of the cattle crossing exhibiting the poorest water quality overall as cattle are shown to have impacts on these parameters.

Literature Review

In the past 20 years, human population has grown substantially (United Nations, 2019). World meat consumption has grown by 0.34 kg/capita/year from 2000 through 2019 (Whitton et. al, 2021). The total number of inventoried cattle in the United States during 2020 reached 93.8 million head (USDA ERS, 2021). In cattle production, farmers may set up a crossing point to move cattle across a river or stream. The crossing allows cattle to move from one pasture to the next to continue grazing as well as providing water for drinking and cooling. Cattle are shown to impact water sources through non-point source pollution, and only a limited number of solutions have been proposed.

Non-Point Source Pollution

As ranchers strive to meet the demand for meat worldwide, one can see an increase in various pollutants present within nearby water sources (O'Callaghan et. al, 2019). Many of the contaminants originate directly from the cattle crossings located on farmland waterbodies

(Vidon, Campbell, & Gray, 2008). Multiple access points for cattle directly correlated to an increase in total kjeldahl nitrogen, total phosphorus, suspended sediments, *E. coli*, chloride, cation concentrations, atrazine, and silica, during the summer and fall months in the midwest (Vidon, Campbell, & Gray, 2008) indicating that cattle directly influence water quality when given free access to on-site waterbodies. Although the study by Vidon, Campbell, & Gray (2008) was conducted with no limitations as to where the cattle crossed, there was still an impact on water quality when crossings were monitored. Concentrations of *E. coli,* total nitrogen, and suspended solids numerically decreased when cattle crossed singularly rather than as a herd, but these pollutants were present for a longer time (Davies-Colley et al., 2004). In the study by Davies-Colley et al. (2004), cattle crossed only at a single point, but the results were similar to the study by Vidon, Campbell, & Gary (2008), indicating that limiting where the cattle cross may not have a significant impact on decreasing water pollutants.

Although cattle may be a primary cause for contaminated waterbodies, it is important to note that they are not always the sole cause. Graves et al. (2007) discovered that microbes from a specific Virginia watershed stream site were directly tied to deer, geese, and other various waterfowl, along with cattle which supports the importance of identifying where the contaminants originated. Not only does cattle access to water points impact the overall water quality, but cattle access impacts aquatic life, particularly amphibian life (Scmutzer et al., 2008). The presence of cattle in local farm ponds was correlated with decreasing overall species richness and diversity of larval amphibians (Scmutzer et al., 2008).

Although cattle mainly tend to pollute water through direct contact when crossing, drinking, or cooling, there are multiple studies showing how cattle can have an indirect impact on local waterbodies through means other than crossings. For example, one study identified how

grazing cattle decreased the water quality of a bedrock aquifer down-gradient from a pasture in Canada (Levison & Novakowski, 2009). Grazing led to an increase of nitrate and bacteria within the aquifer, demonstrating that cattle can have an indirect effect on the water quality from feeding up gradient of a hidden groundwater source. A second example was provided in a study conducted in the Sierra Nevada Mountains in California, where greater algal biomass and an increased presence of *E. coli* were discovered off-site from cattle grazing lands, which tended to compromise local water quality (Derlet et al., 2012).

Although living cattle play an important role in water pollution, deceased cattle can have a similar impact. Leachate collected from dead cattle carcass burial pits was shown to have increased levels of total Kjeldahl nitrogen, phosphorus, total organic carbon, and chemical oxygen near Mead, Nebraska (Yuan, Snow, & Bartelt-Hunt, 2013). Leachate collected from dead cattle carcass burial pits foreshadows the importance of proper environmental treatment of animals before and after death.

Reducing Water Pollution

Methods have been proposed to help slow the contamination of local water sources. Off-stream watering points (OSWPs) were determined to reduce cattle frequency in riparian buffer zones (Malan et al., 2018). Removing the cattle from the riparian zone with relocation into pastureland was proven to have significant benefit to water quality. Another proposed best management practice (BMP) was to decrease the elevation at which cattle were grazing. Preventing nonpoint pollution in high-elevation meadows was achieved by relocating cattle to lower-elevation areas (Derlet et al., 2009). Solutions to the problem tend to be limited, as removing cattle seems to be one of the only viable options, which poses an inconvenience for the farmer. One other method that may be viable would be the use of water troughs. Using cattle

troughs may allow cattle to reduce time in the riparian zone. However, water trough sediments contaminated with feces from cattle excreting *E. coli* O157 can serve as a long-term reservoir of this organism on farms, as well as a source of infection for cattle (LeJeune, Besser, & Hancock, 2001). Tighter regulations have shown to affect economic output. The Dairy Rule and Dairy Easement Program of 1994 based out of Okeechobee County, Florida were shown to decrease dairy sales by $47.6 million and reduce 465 full-time jobs (Morse, 1996). Future research could help determine a practical solution that would benefit both the environment and the farmer.

Water pollution continues to be a problem in cattle farming operations, even for small rural operations. Cattle can have direct and indirect impacts on water quality, and it is important to address both issues in future research. Solutions to the problem have been limited, but the importance of developing best management practices remains high.

Methodology

Research Design

This project utilized a quantitative quasi-experimental research design that was used to measure water quality parameters and quantify coliform bacteria, especially *E. coli,* present at three locations within a tributary located on a cattle and dairy farm. A quasi-experimental design allows the researcher to assign research conditions in an arranged manner to estimate the effect of a treatment (Millsap & Maydeu-Olivares, 2009). Quasi-experimental designs allow for partial control over procedures and methods. In this case, three sampling locations were purposefully selected for sampling so there was no randomization.

Site Characteristics

This study was conducted on a small cattle and dairy farm located in northwest Arkansas. The farm is 88.59 acres based on the Lincoln, AR Geographic Information System (GIS) map (see Figure 4 in Appendix 1). The land consisted of multiple grazeable pastures suitable for a small herd of cattle, separated by a flowing water body. The land on the east side of the crossing consisted of steep slopes covered by trees (see Figure 5 in Appendix 1). This landscape makes crossing the stream difficult except for the one identified location. The tributary of Muddy Fork is located within the Illinois River watershed. The stream is separated by agricultural and forested areas which is indicative of gravel mantled stream beds (Shepherd et al., 2011).

Sampling

Water was collected at three locations along the stream. For the purpose of this study, they were Upstream, Crossing, and Downstream (See Figure 6 in Appendix 1). The Upstream sample was located approximately 645 m upstream from the Crossing and was representative of the water flowing onto the property (See Figure 7 in Appendix 1). The Crossing was located in an area of the stream where the herd routinely crossed to graze on the south side of the farm. (See Figures 8 and 9 in Appendix 1). The Downstream sample was situated approximately 804 m from the Upstream location and approximately 159 m from the Crossing (See Figure 10 in Appendix 1). Samples were collected first at the Upstream location, then the Crossing location, and lastly the Downstream location. Samples were collected from the midstream by lowering a sample bottle into the water, flushing it out and then collecting the sample by turning the bottle sideways until all air bubbles were removed. The bottle was then sealed prior to removal from the water. In-situ measurements (temperature and turbidity) were taken from approximately the same location within the stream.

Sampling occurred on August 20th, 2021 (summer) and February 19th, 2022 (winter). For the first collection date, the daily high air temperature was 31.7°C with a low of 22.2°C (National Oceanic and Atmospheric Administration, 2022). The air temperature at the time of collection was 25°C. Two days prior to sampling, 0.61cm of precipitation fell. One day prior, there was no reported precipitation (NOAA, 2022). On the second sampling and collection date, the daily high temperature was 17.2°C with a low of -4.4°C. Three days prior to sampling, 2.87cm of precipitation fell. Two days prior to sampling, 2.13cm of precipitation fell. There was no reported precipitation one day prior to sampling (NOAA, 2022). Sampling was also conducted on March 13th, 2022. For the March 2022 date, the temperature high was 17.7°C with a low of 3.3°C. Three days prior to sampling, 5.6 cm of precipitation fell. One day prior, there was no reported precipitation (NOAA, 2022). All weather data were reported from the Fayetteville Drake Field weather station and were found on the NOAA website.

Data Collected

Water Temperature. Water temperature was measured in-situ using a LaMotte thermometer-Code 1066 (LaMotte, Chestertown, MD). The thermometer was placed in the stream by the stream edge, allowed to equilibrate, and then read in degrees Celcius.

pH. The pH was determined in-situ using a Hach Pocket Pro pH pen calibrated using Hach Singlet pH buffer solutions of 4.0, 7.0, and 10.0 (Hach, Loveland, Colorado).

Turbidity. Turbidity was measured using an EISCO 40-inch Transparent Turbidity Tube with Secchi Disk. The tube was filled with flowing water from the creek. The tube was then placed on a rock or other solid surface so the spring-activated stopper in the bottom of the tube could be depressed to slowly let water out of the tube. The spring was depressed until, while looking straight down into the bottom of the tube (through the water), the Secchi disk was

visible. The depth of the water remaining in the tube was then recorded in centimeters with the larger the number recorded meaning the less turbid the water. One sample collection was performed at each of the three testing locations.

Nitrogen – Nitrate and Ammonia. Nitrate was measured using the HACH Low Range Nitrate Test Kit. The 0-10 mg/L test procedure was followed. Demineralized water was filled into the color viewing tube to the mark. The tube was stoppered and shaken vigorously. The tube was then emptied, and the same procedure was repeated. The plastic dropper was rinsed with the sample and then filled to the 0.5 mL mark. The contents of the dropper were added to the rinsed color viewing tube. The color viewing tube was then filled to the mark with demineralized water. Using clippers, one NitraVer 6 Nitrate Reagent Powder Pillow was opened and added to the tube sample to be tested. The tube was then stoppered and shaken for three minutes. After shaking, the sample stood undisturbed for 30 seconds. The prepared sample was then poured into a second color viewing tube. Clippers were then used to open one NitraVer 3 Nitrite Reagent Powder Pillow and the contents of the pillow were added to the tube sample. The tube was stoppered and shaken for 30 seconds. This tube was then set aside for at least 10 minutes, but no more than 20 minutes. The prepared sample tube was inserted into the right top opening of the color comparator. The color viewing tube was filled to the mark with original water sample and placed in the left top opening of the comparator. The comparator was held up to a light source, such as the sky or a window, and the color disc was rotated to obtain a color match. The amount of nitrogen was recorded based on the color match, if any. That number was then multiplied by 10 to obtain the mg/L (or ppm) of nitrate nitrogen present in the sample. This test was performed once at each sampling location and was recorded in ppm.

Ammonia nitrogen was measured using the HACH Ammonia Nitrogen Test Kit (NI-SA 2428700). The standard test procedure was followed. Two glass 18 mm sample tubes (Item # 173006) were rinsed with water to be tested and then filled to the 5 mL mark with the water sample. Using clippers, one Ammonia Salicylate Reagent Powder Pillow was opened and added to the sample tube. The tube was then capped and shaken until all the powder was dissolved. The sample was set aside for three minutes. The contents of one Ammonia Cyanurate Reagent Powder Pillow were added to the tube containing the salicylate-treated sample. The tube was recapped and shaken until all powder was dissolved. The tube was set aside for 15 minutes to allow for color development. The outside of both 18 mm tubes were cleaned with a dry cloth. The color-developed sample was placed into the right-hand opening of the top of the color comparator (Item # 173200). The non-reagent tube was inserted into the left-hand opening of the color comparator. The comparator was held up to a light source, such as the sky or a window, and the color disc (ammonia nitrogen, salicylate, 0-2.0 mg/L - Item # 9261300) was rotated to obtain a color match. The amount of ammonia nitrogen was then recorded in mg/L (or ppm) based on the color match, if any. This test was performed once at each sampling location and was recorded in ppm.

Phosphate. Total orthophosphate was measured using the HACH Total Phosphate Test Kit (Hach, PO-23 – 225001). The medium range test procedure was followed 0-4 mg/L PO_4. The sampled water was filled into the square 29 mL bottle (Item # 232706) to the 20 mL mark. One PhosVer® 3 Reagent Powder Pillow was added to the bottle and then swirled to mix. The bottle was placed on a flat surface for at least two, but no more than 10 minutes for blue color development. One glass 18 mm color viewing tube was then filled to the lowest mark with the prepared sample. This tube was then inserted into the right top opening of the color comparator

(Item # 173200). The other 18 mm glass tube was filled to the lowest mark with untreated sample. This tube was then inserted into the left top opening of the color comparator. The comparator was then held up to a light source, such as the sky or a window, and viewed through the front opening. The disc (phosphate 0-40 mg/L, Item # 9262100) was then rotated to obtain a color match. The reading was then divided by 10 to obtain the mg/L (or ppm) of orthophosphate. This test was preformed once at each sampling location and was recorded in ppm.

Orthophosphate was measured using AquaCheck Water Quality Phosphate Test Strips (Hach, Item # 2757150). The strip was submerged in sample water for one minute. The strip was then removed and set aside for three minutes to allow for color development. The color of the reagent pad of the strip was then compared to the reagent pad color chart on the bottle. This test was performed once at each sampling location as a pre-test validation tool and was recorded in ppm.

Dissolved Oxygen. Dissolved Oxygen was measured using the HACH Dissolved Oxygen Test Kit (OX-2P). The high range test procedure was followed. The water sample was collected in a glass 60 mL BOD bottle (Item # 190902) by first rinsing the bottle with the water to be sampled and then placing the bottle entirely under the water for 2-3 minutes. The bottle was inclined, and the stopper was inserted when no bubbles were evident in the sample. The stopper was then removed, and the contents of the Dissolved Oxygen 1 Reagent Powder Pillow was added, followed by the Dissolved Oxygen 2 Reagent Powder Pillow. The stopper was then inserted without trapping air bubbles in the sample. The bottle was inverted several times until the powders were dissolved. A brownish-orange flocculant precipitate formed in the sample, indicating oxygen was present. The bottle was placed on a flat surface to allow the flocculant to settle to half the bottle volume. The bottle was then inverted again to mix. The bottle was placed

on a flat surface to allow the flocculant to settle to half the bottle volume. The stopper was removed and the contents of one Dissolved Oxygen 3 Reagent Powder Pillow was added to the sample. The stopper was once again inserted, and the bottle was inverted several times to allow the flocculant to dissolve. The sample then turned yellow if oxygen was present. The sample was poured into the 5.83 mL plastic tube (Item # 43800) and the contents of the tube were poured into the square mixing bottle (Item # 43906). Sodium Thiosulfate Standard Solution (0.0109 N) drops were added to the square mixing bottle, swirling the sample after every drop. Drops were added until the sample became colorless. The number of drops used indicated the amount of dissolved oxygen in mg/L. This test was run twice at each sampling location and was recorded as the average of the two tests in mg/L.

Biochemical Oxygen Demand (BOD). Biochemical Oxygen Demand was measured by collecting a second water sample in the glass 60 mL BOD bottle. The bottle was wrapped in aluminum foil and samples were stored in an incubator in the laboratory at 25^0 C for seven days. At the end of seven days, the samples were removed from the incubator and the dissolved oxygen content was measured using the procedure outlined above. The BOD was calculated by taking the initial dissolved oxygen content and subtracting from it the 7-day dissolved oxygen content. The result was reported as the BOD in mg/L.

Nitrite, Hardness, Chlorine, and Alkalinity – Test Strips. Nitrite, Hardness, Chlorine, and Alkalinity were measured using a 6 in 1 Tetra aquarium test strip. The strip was submerged in sample water for one minute. The strip was then removed and set aside for three minutes to allow for color development. The reagent pads on the strip were then compared to the reagent pad color chart on the bottle for freshwater samples. This test was performed once at each sampling location and was recorded in ppm as a reference.

Microbial Analysis. Total coliform bacteria and *E. coli* was measured using the ColiQuick™ Environmental procedure (Environmental Bio-Detection Products, Inc., Ontario, Canada). Sample water was collected using a sterile bottle. Using a multi-channel pipette, 200µL of water sample was dispensed into each of the 96 wells in the well microplate. Using the same lid, the microplate was covered and inserted into the sample bag provided. Upon returning to the laboratory, the plates were placed in an incubator for 24 hours at 35ºC. The plates were then removed from the incubator and the number of blue/green wells were counted. The wells were then examined with a 360Nm UV light. Wells that were both blue and fluorescent under the UV light were counted to determine the total number of *E. coli*. The counted numbers were then converted to a Most Probable Number (MPN) using the chart provided. The MPN chart converts the cell counts into the most probably number of colony forming units (CFU) per 100 mL of water.

Results & Discussion

Data were aggregated in an Excel spreadsheet. Due to the limited replication in sampling within time and across time, statistical analyses were not conducted. Therefore, data will be discussed based on numerical differences and practical implications rather than detailed statistical analyses. Some descriptive statistics, such as averages, will be presented.

Physical Characteristics

Results for turbidity and water temperature, the two physical characteristics measured, are presented in Table 1 (See Appendix). The stream was flowing at or near base at the time of the 8/20/21 sampling. As a result, the water was clear and free of any appreciable sedimentation. The Secchi disk was visible in all sampling locations to the maximum depth of the Eisco turbidity tube, 95cm of water. On February 19, 2022, increased amounts of sedimentation were

observed due to a significant rainfall event that occurred prior to sampling. The stream was flowing rapidly and was higher than base flow. Turbidity measurements averaged 47.3 cm on 2/19/22 with the upstream location representing the most turbid location and the downstream sample representing the least turbid sample. These findings were expected given the precipitation event that preceded. The upstream location was more of a pool feature while the water at the crossing and the downstream location were riffles flowing rapidly.

Water temperature was consistent throughout the reach of the water body sampled on each sampling date, averaging 21.5°C, 8.8°C, and 16.6°C on 8/20/21, 2/19/22, and 3/13/22, respectively.

Chemical Characteristics

Chemical parameters evaluated included nitrate, ammonia, orthophosphate, dissolved oxygen, biochemical oxygen demand, and pH. Data for nitrate, ammonia, and orthophosphate are shown in Table 2. Nitrate sampling was variable across sampling dates as well as between sampling locations. On 8/20/21, the Upstream sampling location was 7.4 ppm of nitrate while the sample tested at the crossing was 0 ppm. It is unlikely that there was no nitrate present in the water sample. The Hach stream test kit used for testing in the field is not as sophisticated or sensitive as lab-based procedures. Therefore, nitrate levels were likely below the limits of detection by the field test. The downstream sample resulted in 0.5 ppm nitrate. The average across the reach sampled was 2.6 ppm nitrate. On 3/13/22, the Upstream and Downstream samples both measured 1.5 ppm while no nitrate was detected at the Crossing. The lower levels detected on 3/13/22 could be the result of increased water flow. The flow in August 2021 was base flow, so less flushing and movement of water. The contamination level to produce

deleterious effects in humans, freshwater invertebrates, fishes, and amphibians is a nitrate concentration of 10 ppm or mg/L (Camargo, Alonso, & Salamanca, 2005). All samples tested were below this limit, indicating nitrate was not negatively contributing to the nutrient load of the stream.

No ammonia levels were detected at any locations on either of the sampling dates using the Hach stream kit. Again, this does not mean there was no ammonia present, simply that the level was below the detection limit of the field test.

The content of orthophosphate across the sampling reach varied between and within sampling dates. On 8/20/21, orthophosphate was measured at 2 ppm across all sampling locations. On 3/13/22, orthophosphate levels increased 2 ppm at the Upstream location and 4 ppm at the Downstream location was remained at 2 ppm at the Crossing. Averaged across time, orthophosphate levels were 3.0, 2.0, and 5.0 for the Upstream, Crossing, and Downstream locations, respectively. Nutrients such as orthophosphate added from urine and fecal material over the winter may end up in water sources such as ground water and run-off water (Smith et al., 2011). Increased levels of orthophosphate at the Downstream site may be indicative of cattle spending increased time at the Crossing sampling location compared to the Upstream location.

Dissolved oxygen content varied both within sampling dates and between sampling dates (Tabl2 3). Data were included for the 2/19/22 sampling date since measurements were taken for dissolved oxygen in the field even though the nitrogen and phosphorus tests were not conducted on the samples. Dissolved oxygen levels were higher on 8/20/21, averaging 12 mg/L across sampling locations. The Downstream sample had the highest dissolved oxygen content at 13.5 mg/L. Little numerical difference was noted between the 2/19/22 and 3/13/22 sampling dates. Dissolved oxygen content ranged from 8.44 to 9.16 mg/L. Averaged across sampling dates, the

mean dissolved oxygen levels were 9.30, 9.91, and 10.37 for the Upstream, Crossing, and Downstream locations, respectively.

Sufficient levels of dissolved oxygen are required to support aquatic life (Murphy, 2006). Flowing streams typically have higher dissolved oxygen levels than stagnant water bodies. Aquatic organisms use oxygen for respiration. According to multiple sources, healthy water has dissolved oxygen levels between 6 and 8 mg/L, though 5 mg/L can support most aquatic life. Therefore, all dissolved oxygen levels measured in this study are indicative of a healthy stream as the lowest measurement was 8.44 mg/L.

The concentration of dissolved oxygen is related to water temperature. Cold water can hold more dissolved oxygen than warmer water. Typically, in early spring the water temperature is lower, so the dissolved oxygen level is higher. The findings of this study were the opposite. The warmer water (August 2021) had higher dissolved oxygen levels. This was likely due to the flowing nature of the stream even in the summer as oxygen enters the water through diffusion on the surface as well as through rapidly moving water such as the case for most parts of the stream in this study. Therefore, the flow likely had a larger impact on dissolved oxygen than the water temperature.

The 7-day dissolved oxygen content was measured after the water samples were incubated at 25°C for seven days. The 7-day dissolved oxygen was subtracted from the initial concentration to calculate the biochemical oxygen demand (BOD). Data are shown in Table 3 for the three sampling dates. The highest BOD was measured on 8/20/21 at the Crossing and Downstream locations, 8.1 and 7.6 mg/L, respectively. Averaged across sampling dates, the mean BOD levels were 4.03, 5.59, and 4.90 mg/L, for the Upstream, Crossing, and Downstream locations, respectively. BOD measures the oxygen consumed by microorganisms in decomposing organic

matter. With a greater BOD, oxygen is depleted quicker in the system, causing less oxygen to be available to higher aquatic life forms (EPA, 2012). Unpolluted stream systems typically have BOD values below 1 mg/L. Moderately polluted vary between 2 and 8 mg/L. The higher the BOD, the more organic matter there is in the stream. Sources of organics include stormwater runoff from agricultural lands, feedlots, and failing septic systems (EPA, 2012). The findings of this study indicate there is some organic matter present as average values for BOD ranged from 4.03 to 5.59 ppm, the highest of which was in the Crossing location where the cows cool off in the water, drink water, and certainly defecate in the water.

The pH at the sampling sites remained relatively similar both within and across sampling dates. Measurements taken across sampling dates averaged 8.3, 8.57, and 8.43 for the Upstream, Crossing, and Downstream locations, respectively (Table 3). A pH of 8.3 is slightly alkaline. Drinking water, for reference, generally ranges from 7 to 8.5. The majority of aquatic life prefer a pH range of 6.5-9.0. The optimum pH levels for streams ranges from 6.0-8.5, for crabs, snails and mussels ranges from 7.3-10, and for fish ranges from 6.5-9.0 (Fondriest Environmental, Inc., 2013). Therefore, the pH measured in this study does not indicate any concerns for water quality.

Biological Characteristics

Microbial analysis varied both across and within sampling dates Table 4). Total coliform bacteria were measured on 2/19/22 and 3/13/22. The data samples collected on 2/19/22 were collected following significant precipitation that resulted in a rapidly flowing stream with water levels well above base flow. The total coliform measurements were 388, 307, and 939 CFU for the Upstream, Crossing, and Downstream locations, respectively. On 3/13/22, once the water had receded to levels that more approximated base flow, the measurements were 619, 1038, and 587 CFU at the upstream, crossing, and downstream locations. *Escherichia coli* concentrations on

3/13/22 ranged from 255 to 559 CFU across sampling locations while on 3/13/22 they ranged from 28 to 188 CFU. On 2/19/22 the Downstream location had the greatest amount of *E. coli* while the Crossing location had the least. This could be due to the rapidly flowing water within this section of the reach flushing the *E. coli* downstream. On 3/13/22 the largest *E.coli* count was measured at the Crossing location. E. coli is an indication of fecal contamination by animals. Therefore, measurements at the Crossing location would be expected to have greater counts than elsewhere.

The environmental protection agency (EPA) recommends less than 200 CFU per 100 mL of water for primary human contact. Therefore, primary human contact would not have been recommended on 2/19/22 but would have been acceptable on 3/13/22. High percentages of *E. coli* isolates were identified as originating from wildlife such as geese and deer in New York (Somarelli et al., 2007). In a farm setting such as was used for this study, isolating the *E. coli* to simply the cattle crossing the stream is not possible. Other non-point sources of fecal contamination could contribute to the large levels of measured *E. coli* observed.

Conclusions

Multiple factors contributed to the limitations of this study. The Covid-19 pandemic created restrictive guidelines through the university, making it more difficult to conduct on-site sampling and to use the lab to process samples. Limited testing supplies as well as time constraints led to a decrease in sampling dates and replication during sampling. Time was also an issue, as schedule conflicts were common throughout the duration of the study. Additionally, the University of Arkansas was shut down for seven days during the spring 2022 semester due to inclement weather, which caused cancellations of important meetings as well.

The best method to fight the increased water pollution due to cattle crossings is the removal of the cattle. However, this option is not feasible as cattle are required to create a livelihood for the farmer. Off-stream watering points (within the pastures) may draw cattle out of the reach of the water body, resulting in less time present at the Crossing location. Off-stream watering points could aid in reduction of total coliform and *E. coli* in the stream.

To better understand water quality due to cattle crossings, more research may need to be conducted. Increasing the sampling frequency would allow for more information to be collected, resulting in an increased sample size. Collecting samples at multiple locations along the entire stretch of the tributary may allow for a better representation of the data than sampling within the boundaries of one small farm. Combining these two methods may allow for significance testing to confirm results to a more accurate degree.

Laboratory analysis of samples with more robust methods of detection, particularly for nitrate, ammonia, and orthophosphate would also improve the quality and reliability of the measurements. Field tests are intended to provide a rough estimate of parameters tested, but do not provide research-grade results.

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Appendix 1 – Figures and Tables

Figure 1

Google Maps Muddy Fork Tributary aerial view, 2022.

Figure 2

Aerial view of stream from Google Maps with highlight, 2022.

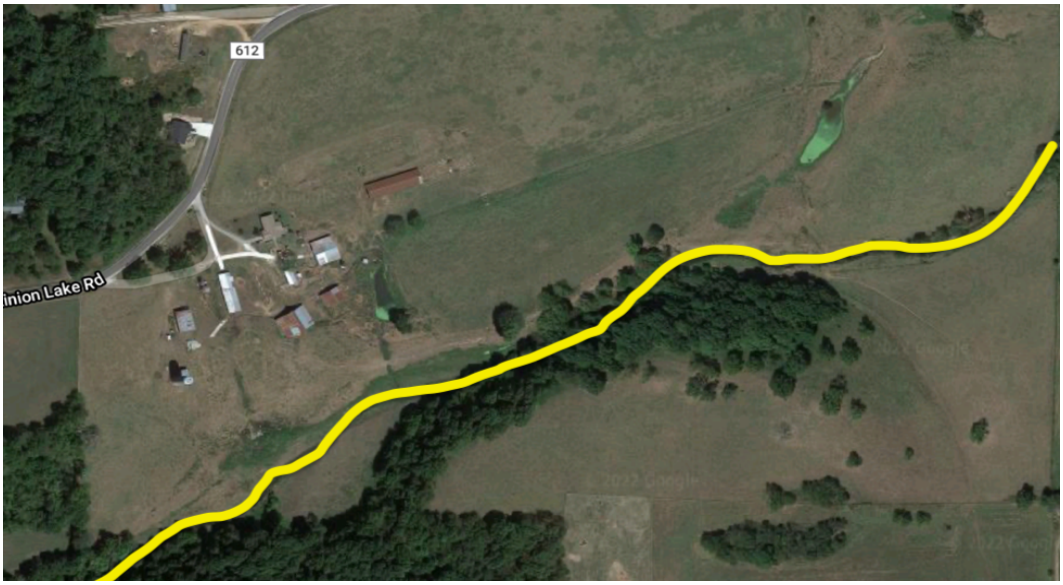

Figure 3

Highlighted aerial view of the Muddy Fork of the Illinois River, 2022. Retrieved from https://arkansaswater.org/29-watershed/136-illinois-watershed-11110103

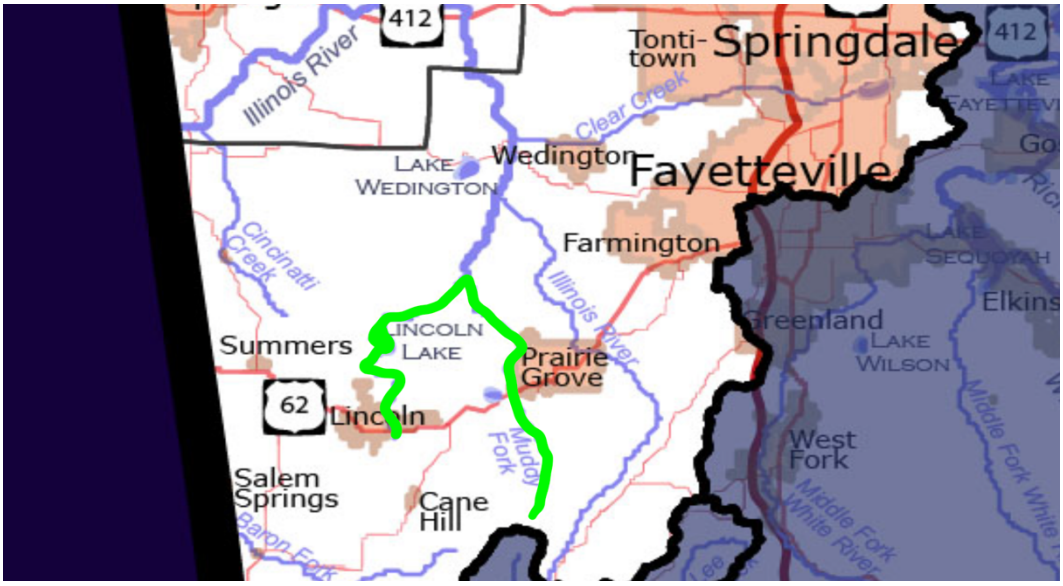

Figure 4

Lincoln, AR Parcel Map 2022. This image shows the boundaries of the property line for the sample collection site. Retrieved from ArcGIS web application

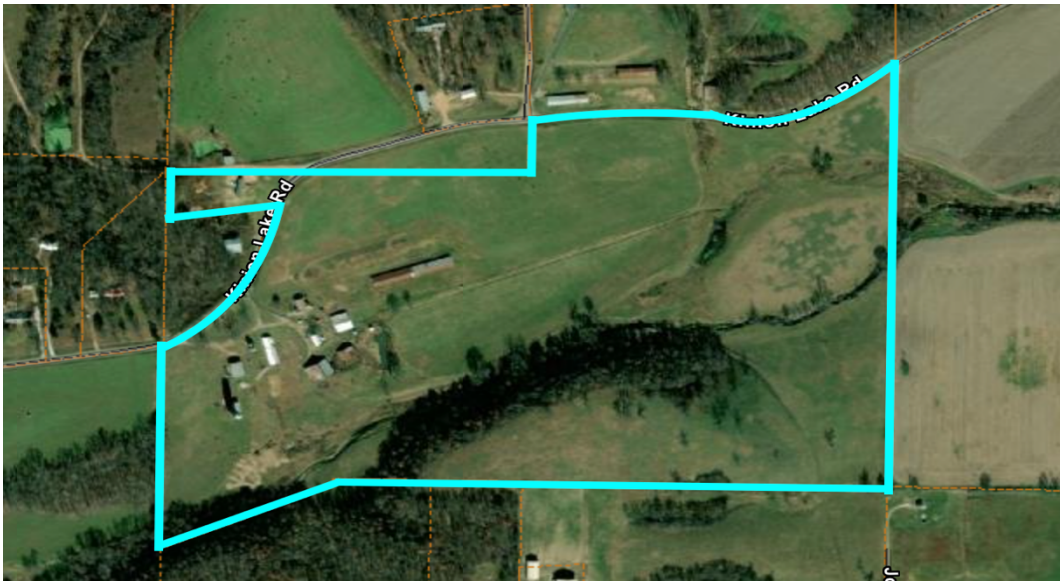

Figure 5

On-site view of steep slope at Crossing sample location, 2022.

Figure 6

Aerial identifications of sampling sites along the Muddy Fork tributary of the Illinois River, 2022.3

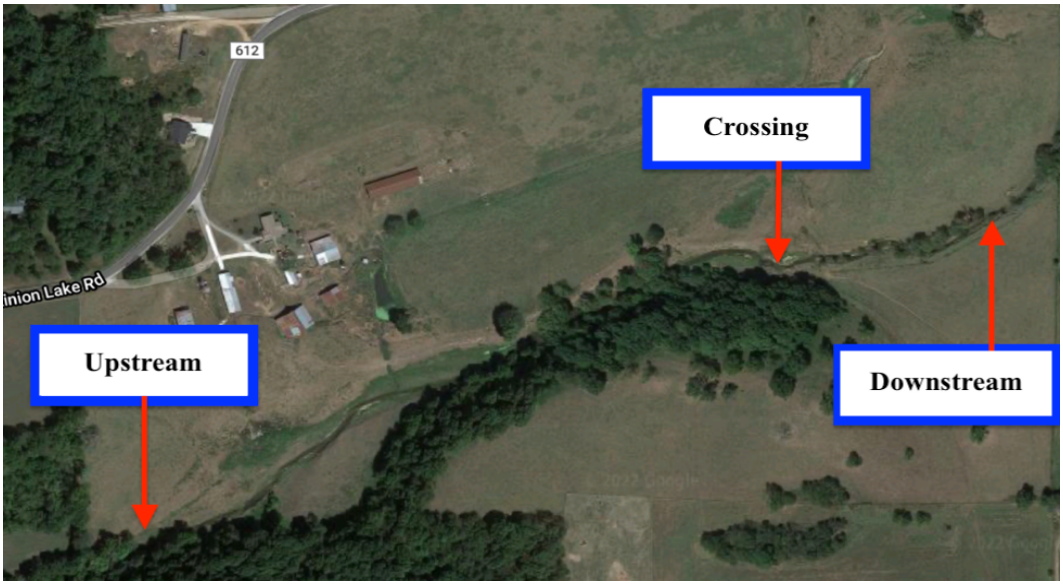

Figure 7

On-site view of the Upstream collection site as well as the water entering onto the property.

Figure 8

Cattle crossing the stream at the Crossing sample site, August 20th, 2022.

Figure 9

On-Site view of the Crossing collection site, 2022.

Figure 10

On-site view of the Downstream collection site, 2022.

Table 1

Results of Physical Parameters

Collection Site	Turbidity (cm of water)		Temperature (^{O} C)		
	8/20/21	2/19/22	8/20/21	2/19/22	3/13/22
Upstream	>95	43.5	22	9.51	16.6
Crossing	>95	45.5	20	8.0	16.7
Downstream	>95	53	22.5	9.0	16.4

Table 2

Results of Chemical Parameters – Part 1

Collection Site	Nitrate (ppm)		Ammonia (ppm)		Orthophosphate (ppm)	
	8/20/21*	3/13/22	8/20/21	3/13/22	8/20/21	3/13/22
Upstream	7.4	1.5	0**	0	2	4
Crossing	0	0	0	0	2	2
Downstream	0.5	1.5	0	0	2	8

* No data for sampling date 2/19/22 due to sample collection immediately prior to a university closure for inclement weather. Samples could not be processed in a timely manner.
** Actual values are likely not zero but were below the limits of detection by the field method used to measure the parameters.

Table 3

Results of Chemical Parameters – Part 2

Collection Site	Dissolved Oxygen (mg/L)			Biochemical Oxygen Demand (mg/L)			pH		
	8/20/21	2/19/22	3/13/22	8/20/21	2/16/22	3/13/22	8/20/21	2/19/22	3/13/22
Upstream	11	8.47	8.44	5.3	3.41	3.38	8.1	8.5	8.3
Crossing	11.5	9.16	9.06	8.1	4.12	4.56	8.8	8.7	8.2
Downstream	13.5	9.16	10.37	7.6	3.72	3.37	8.3	8.8	8.2

Table 4

Results of Biological Parameters

Collection Site	Total Coliform (CFU)		*E. coli* (CFU)	
	2/19/22*	3/13/22	2/19/22	3/13/22
Upstream	388	619	298	28
Crossing	307	1038	255	188
Downstream	939	587	559	132

* Significant precipitation preceded water collection; Stream was flowing rapidly with water levels higher than base flow.