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Quantifying the Relationship Between Pond Size and Water **Quality**

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Quantifying the Relationship Between Pond Size and Water Quality

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Abstract

The water quality of ponds, streams, and groundwater is at risk due to agricultural and urban development. Implementation of ponds near developmental areas can act as catchment sites to reduce further water pollution (Bichsel et al., 2015). However, maintenance of pond water quality is necessary for continued water supply to livestock and general recreational use. The present study aimed to determine the relationship between pond size (surface area range from 142 to 5336 $m²$) and water quality. Analysis of water quality parameters, including dissolved oxygen, nitrate, phosphate, and chlorophyll-a, were conducted during the summer of 2021 and winter of 2022. Attention was also given to site characteristics such as buffer zone presence and whether the pond was a lotic or lentic system. The results of summer and winter samples were aggregated in Excel™. T-tests and ANOVA tests were used to determine the statistical significance for within and between pond variation. A p-value > 0.05 was statistically insignificant, whereas a p-value < 0.05 was statistically significant. Both medium sized ponds, Davis B and Fields, resulted in statistically significant within pond seasonal variation. Davis B had seasonal variation for pheophytin-a with a p-value of 0.017. Fields had statistically significant within pond seasonal variation for chlorophyll-a, with a p-value of 0.001, and pheophytin-a, with a p-value 0.002. All ANOVA tests indicated that there was no statistical significance between pond size and any of the parameters tested (p-value > 0.05). The lack of statistical significance regarding each parameter's relation to pond size suggests that the pond with the largest volume can act as the greatest catchment site without corresponding water quality degradation. However, an increased sampling size during each season may result in more statistically significant results in the future.

Introduction

Background and Need

The extent of agricultural and urban development within the United States poses a threat for water resources. Of the 2.3 billion acres in the United States alone, 17% is cropland, 29% is pasture, and 3% is urban (USDA, 2012). Due to the extent of agricultural and urban development in the United States, land management is necessary to protect water resources for current and future generations. The natural state, Arkansas, offers a prime model for the United States as a whole. Arkansas's 34 million acres contains 20.93% cropland, 15.76% pasture, and 5.53 % urban development (USDA, 2017). Overall, the ratio of urbanized land to agricultural land in Arkansas is relatively analogous to the overall country. Therefore, Arkansas land should be considered in relation to water resource management, notably, that of pond systems.

A pond can be defined as a freshwater system that holds water for at least four months of the year and ranges in size from 1 $m²$ to 2 hectares (Bichsel et al., 2015). Ponds provide a unique ecosystem due to the small size and shallowness of the water body, play an important role in global cycles, and cover more total area than lakes (Boix et al., 2012). Typically, ponds are eutrophic or hypereutrophic in nature resulting in an increase of vegetative growth (Fairchild et al., 2004). Whether man-made or naturally occurring, ponds provide a multitude of environmental services such as functioning as catchment sites, supplying water for agriculture and livestock, acting as erosion protection, and contributing space for recreational activities (Bichsel et al., 2015). The water quality of ponds is directly related to nearby land use and can be characterized on the basis of physical, biological, and chemical properties.

Many pond features are indicative of water quality and nearby land use. Among the physical aspect of ponds, water body size contributes to the pond's ability to perform ecosystem services and maintain water quality. The way in which a pond receives water, either through

stream inlet, ditch inlet, pipe inlet, or no inflow plays a major role in pond health (Bichsel et al., 2015). Additionally, berms (raised bank) and buffer strips are important management practices used to protect pond ecosystems. The biodiversity of groups such as Amphibia, Odonata, and general micro and macroinvertebrates depend heavily on pond water quality (Oertli et al., 2000). Indicators of water quality such as dissolved oxygen, pH, and chlorophyll are especially important when viewing overall pond health (Bichsel et al., 2015). Generally, pond characteristics are strongly influenced by the surrounding agricultural and urban areas. While limited, earlier research on ponds creates a foundation that aids further studies.

Previously, there have been a handful of important studies examining pond attributes. Oertli et al. (2000) concluded that the prevalent assumption regarding ponds that the larger the water body, the greater biodiversity should be present, is false. Rather all pond sizes should be promoted, at least in terms of biodiversity. Bichsel et al. (2015) deduced that the presence of inflow, heavy precipitation, and management practices led to increased water quality within pond environments. Oertli et al. (2000) and Bichsel et al. (2015) have proposed a list of problems associated with determining pond water quality.

Problem Statement

Presently, there is a gap in the research regarding agricultural and urban pond water quality related to the preferred water body size for specific land use locations in the United States, and specifically within Arkansas. Pond conservation value is just as important as that of other surface water bodies, yet the destruction of ponds is occurring at rapid rates that outweigh their creation (Thornhill et al., 2017). Therefore, pond water quality, design, and management are of great concern in agricultural and urban areas.

Purpose of the Study

The current study aimed to further identify and test pond physical, biological, and chemical characteristics through correlational and comparative research on pond water quality and size. Five ponds located on both agricultural (livestock production) and urban sites, and ranging in size (small, medium, and large), were examined during the summer of 2021 and winter of 2022.

Research Objectives

The following research objectives guided this study:

- 1. Determine the relationship between pond size and water quality in agricultural and urban areas within Northwest Arkansas.
- 2. Examine the physical, biological, and chemical factors of each pond seasonally.
- 3. Deduce which pond size (small, medium, large) maintains high water quality when implemented on agricultural or urban landscapes.

Literature Review

Despite the amount of research that has been conducted on the topic of ponds, there are few studies on the specific relationship between pond size and water quality in agricultural and urban areas. Rather, previous studies have focused on general concepts concerning ponds. Prior to the current study, research was focused on determinants of pond health, the relation between pond water quality and use, and the effects of pond size on biodiversity and catchment abilities.

Pond Characteristics Overview

Research on general pond ecosystem characteristics has often been paired with the development of methods to determine pond health and biodiversity. An overview of the pond ecosystem was created by Bronmark and Hansson (2002) in which pond health was found to be mainly affected by anthropogenic disturbances such as the introduction of exotic species, increased nutrient loads, and overall contamination. Of these anthropogenic disturbances, nearby land use has been identified as a major factor affecting conservation value and biodiversity of ponds (Thornhill et al., 2017). Aside from human-related impacts upon ponds, another notable feature of pond health is the presence of insects or lack thereof. The population of insects is directly related to the measurement of pond invertebrates using intra-patch habitat quality and inter-patch geometry (Jeffries, 2005). Earlier research on pond features, especially those pertaining to the health of these water bodies, provides a foundation for further investigation on pond ecosystems. Exploration of pond traits and nearby habitats can further be applied to the study of water quality.

Pond Water Quality and Related Use

Water quality and the intended use of ponds have been repeatedly tied together through investigative research within the agricultural sector. Examination of the impacts of nearby land use on stream water quality was performed by Maillard et al. (2008). Land-use type was recognized as a contributor to elevated turbidity levels and increased nitrogen, phosphorus, and fecal coliform levels within stream systems. The construction of ponds in areas possessing negative impacts on surface waters such as streams due to nearby land use could provide relief by acting as catchment sites. Aside from the ability of ponds to protect separate water bodies and land health, they can provide habitats for diverse organisms. Pond management is directly related to the assortment of aquatic organisms (Lemmens et al., 2013). Management practices such as buffer strips can contribute to enhanced levels of biodiversity. The correlation between pond water quality and how the water body is used was noted by Bichsel et al. (2015) in which water health standards for pH, dissolved oxygen, total chlorophyll, and conductivity were established.

In addition to the important parameters discussed by Bichsel et al. (2015), pond catchment intake, buffer zone presence, and zooplankton and macrophyte populations are also key to determining pond water quality (Joniak et al., 2017). In general, there is an important relationship between pond water quality and the surrounding land use type which can further be understood by the investigation of pond size.

Examination of Pond Size

Over the past twenty five years, the link between pond size and species diversity has been extensively researched. Oertli et al. (2000) examined the relationship between pond size and species diversity and concluded that the principle that "larger areas support more species" was not always accurate. Additionally, the correlation between pond size and depth with water quality reveals that many features, such as phosphorus, nitrogen, phytoplankton biomass, and light penetration, are both positively and negatively related (Fairchild et al., 2004). Pond size should also be considered for catchment abilities. The ability of a pond to function as a catchment site is directly related to pond size (Novikmec et al., 2016). An understanding of the importance of pond size is lacking and remains an open area of inquiry for further research.

Overall, the historical approach to research involving ponds focused on broad ideas and concepts. Past studies often examined basic pond components, the correlation between pond water quality and use, and general investigation of pond size. Nonetheless, these extensive results can be combined to create a foundation for the current study on pond water quality in relation to pond size within agricultural and urban landscapes.

Methodology

The current study compared pond size with water quality in urban and agricultural settings using a quantitative research design. The study centered on five ponds located in

Northwest Arkansas over the course of one year. Pond were distinguished as small, medium, or large. Pond size $\lt 750$ m² was small, pond size between 750 and 2000 m² was medium, and pond size > 2000 m² was large. Of the five ponds, two were small and titled Davis A and Austin, two were medium and labeled Davis B and Fields, and one was large, named Thompson. Seasonal data collection was performed to gain insight into the physical, biological, and chemical characteristics of each pond. Factors indicative of trophic productivity were measured including organic and inorganic phosphate concentrations, ammonia, initial and final dissolved oxygen (DO), nitrate, pH, temperature, chlorophyll-a, pheophytin-a, and coliform bacteria concentrations to establish pond water quality. The following sections detail the research design, rigor, pond selection rationale, site characteristics, and describes sampling procedures and data collection.

Research Design

A quasi-experimental paradigm was selected by the researcher for the purpose of this study. The quasi-experimental research design type was chosen as the study did not involve manipulation or prevent confounding variables and the chosen ponds were not randomly assigned (BCcampus, n.d.). Since the current research study investigated water quality parameters in agricultural ponds and presented a relationship with pond size, comparative or correlational research was necessary. Comparative design involves the description of similarities and differences, explanations of these factors, and can create opportunities for prediction (Esser & Vliegenthart, 2017). This study pertains to descriptive research objectives and how variance occurs between cases, making this research primarily focused on descriptive comparison (Esser & Vliegenthart, 2017).

Rigor

An array of potential threats to validity and reliability existed within this study that needed to be met with the appropriate preventative steps. Internally, validity could have suffered from changes in instrumentation, observers, and scoring. Externally, validity was heavily subject to environmental changes such as weather and time of day. Steps were taken to lessen the effects of these validity threats largely by the use of critical appraisal checklists (study protocol) to create a set map of how water sampling procedures occurred or could change (Claydon, 2015). This included using set standards for buffer strip presence, maintaining a consistent observer and water sampling methods as well as instrumentation which would be calibrated each testing session.

Site Characteristics

Water body size and nearby land use type were considered when determining which ponds would provide useful information for the current study. Additionally, minor consideration was given to properties that would allow for further comparison such as whether the water body was lotic (flowing water-system) or lentic (less flowing/stagnant), bermed, and if a buffer zone was present. Ultimately, five ponds were chosen (Figure 1) of which two were categorized as small (Davis A and Austin), two as medium (Davis B and Fields), and one large (Thompson). Table 1 provides details pertaining to each pond including GPS coordinates, size designation, surface area, and surrounding land use as determined from aerial photography.

The small sized ponds, Davis A (Figures 2 and 3) and Austin (Figures 4 and 5), were located on land dedicated to the rearing of livestock. Davis A was frequented by horses, whereas Austin was positioned on a dairy farm with chickens, geese, and ducks also present. Davis A was measured to have a maximum depth of 160.02 cm (Figure 6), while Austin, the shallowest of all

the ponds was measured to have a maximum depth of 69.85 cm (Figure 7). The north side of Davis A was exposed to sunlight for longer intervals than the south side where mature trees surrounded the perimeter. During summer months, the north shoreline supported thick vegetative growth. Algae growth in Davis A remained present throughout the summer and winter. Algal growth in Austin, however, remained on the water surface in the summer but was minimal during the winter. Austin, established as lotic, had a spring-fed water inlet (Figure 8) as well as an outfall to a small stream. Measurements determined approximately 10,800 gallons of springfed water per day was flowing through a PVC pipe into the pond. The rate of flow at the outfall was not measured. The land surface had minimal vegetative growth during the summer and no ground cover during the winter. Additionally, the pasture in which Austin was situated was open to young calves during the winter, thus destroying the vegetative cover observed in the summer. The opposing lotic and lentic variables of Davis A and Austin allowed comparison between flowing and non-flowing pond systems.

Davis B (Figures 9 and 10) and Fields (Figures 11 and 12), both categorized as medium and lentic, were situated within a donkey and cattle pasture, respectively. Davis B was measured to have a maximum depth of 142.24 cm (Figure 13), while Fields was measured to have a maximum depth of 195.58 cm (Figure 14). Davis B was bermed and had a vegetative buffer zone composed of tall grasses present throughout the summer and on the east side throughout the winter. Submerged moss and other submerged vegetative growth was supported during the summer and winter near the bank of Davis B. Bermed, lentic, and surrounded by trees, Fields had significant amounts of algal growth throughout the summer and winter. The similarities and differences of Davis B and Fields provide insight into the unique characteristics of the medium sized ponds used for this study.

Lastly, Thompson (Figures 15 and 16), with a maximum measured depth of 231.14 cm (Figure 17) was the largest and only residential pond used in this study. Located in an area with surface drainage issues, Thompson was lotic with a stream inlet secluded by a tree grouping at the east side of the pond. A small pier on the west side of the pond was indicative of the pond's use for recreational activities. On the south side of the pond there was a rocked outfall allowing water to overflow the pond and run into a ditch during periods of water level rise. During winter sampling, the pond was approximately three feet higher than during summer sampling, concealing the bermed bank. Thompson, compared to the other four ponds, provided the largest water body with no animal production.

Sampling

During each sampling session, travel west of Fayetteville was necessary to access the ponds to collect field observations and related water quality tests of the five ponds. The same two researchers performed the water quality tests and observations for the sake of consistency. Upon arrival to each location, weather conditions were noted, and physical, biological, and chemical examinations/tests were conducted. The two sampling sessions were conducted on August $11th$ and $12th$, 2021 and on February 19th, 2022. Microbial sampling was conducted on March 13th, 2022.

Summer water quality tests were performed on August $11th$ and $12th$, 2021. Midday August $11th$ was 33.89 $^{\circ}$ C and 35 $^{\circ}$ C on August $12th$. During summer sampling, a kayak was used to collect water samples near the pond center. Dissolved oxygen, temperature, pH, turbidity, nitrate, ammonia, and phosphate were tested in the field during the summer sampling sessions. Summer chlorophyll-a and pheophytin-a samples were filtered in the field and processed in the lab. The final sampling session occurred during the winter on February 19th, 2022, following

1.97 inches (per Drake Field data) of precipitation two days prior to sampling. Winter water samples were collected toward the pond bank and processed similar to the summer samples. Dissolved oxygen, temperature, pH, and turbidity were determined in the field. Nitrate, ammonia, and phosphate were processed in the lab ten days following the sampling date due to inclement weather that prevented laboratory access. Chlorophyll-a and pheophytin-a were filtered and processed in the lab. During both summer and winter sampling, 7-day dissolved oxygen was analyzed in the lab to determine biochemical oxygen demand.

Data Collection

The water quality parameters evaluated were temperature, pH, turbidity, nitrate, ammonia, organic and inorganic phosphate, initial and 7-day dissolved oxygen, biochemical oxygen demand, chlorophyll-a, and pheophytin-a. The Hach Stream Survey Kit (#2712000) was used to test for pH, dissolved oxygen, phosphate, ammonia, nitrate, and nitrite if applicable. Turbidity was determined using a transparent turbidity tube with a Secchi disk. Aside from minor changes, chlorophyll-a and pheophytin-a were measured using the procedure established by Maris (2019).

Water Temperature. Water temperature was measured in-situ using a LaMotte thermometer - Code 1066 (LaMotte, Chestertown, MD). The thermometer was lowered into the water body, allowed to equilibrate and then was read in degrees Celsius.

pH. The water 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) each sampling session. The pH pen was lowered into the water sample, allowed to equilibrate, and was then read.

Turbidity. Turbidity was measured using an EISCO 40-inch Transparent Turbidity Tube with Secchi Disk. The tube was filled with a sample collected in a bucket. The tube was 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 cm.

Nitrogen.

Nitrate. 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. 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. 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 was added to the tube containing the salicylatetreated 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 lefthand 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 (0-4 mg/L PO₄) test procedure was followed. 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 also 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 precipitate formed in the sample, indicating oxygen was present. The bottle was placed on a flat surface to allow the precipitate 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 precipitate 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)*.* BOD 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° 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.

Chlorophyll-a/Pheophytin-a. The current study followed methodology established by Maris (2019) with few deviations to measure pond chlorophyll-a and pheophytin-a. Due to availability and easier filtration, 934-AH (1.5 micrometer pore size) filters were used during the

filtration process rather than the GF/F filters. Additionally, samples were discrete rather than composite wherein the number of samples taken per pond depended on pond size such that Davis A, Davis B, Fields, Austin, and Thompson had three, four, five, four, and seven samples collected, respectively. The discrete samples provided variability throughout pond locations. Samples were taken near the pond center and pond edges to gain a comprehensive view of chlorophyll-a levels throughout each water body. One hundred twenty-five mL of pond water was filtered for each discrete sample, unless the levels of algae were too dense to filter the full 125 mL. If 125 mL was unable to be filtered completely, a lower amount was filtered as noted.

Total Coliforms and *Escherichia coli.* The Coli-Quick laboratory procedure (ebpi, Burlington, Ont. Canada) was used to detect viable total coliforms and *e. coli*. A 96-well plate utilizing the nutrient indicators X-Gal and MUG was used. Two hundred microliters of water sample was placed into each of the 96 wells using a multichannel pipette. Plates were incubated at 35^o C for 24 hours. After incubation, the plates were removed from the incubator and placed on a white surface where the number of blue/ green colored wells was counted. That number was recorded. The plate was moved to a black surface and a 360 nm UV light was shown on the plate. The number of blue/ green wells that fluoresced were counted and recorded. Both numbers recorded were compared to Most Probable Number (MPN) chart, converting the cell counts to the most probable number of colony forming units (CFU) per 100 mL of water. The number of MPN of the number of wells that turned blue/ green were total coliform bacteria. The number of those that fluoresced were *E. coli*. Data were only collected on one date, March 13, 2022, to compare the coliform bacteria between ponds to evaluate the impact of livestock as an adjacent land use.

Data Analysis

Water quality measurements were aggregated in an Excel™ spreadsheet organized by sampling date, pond location, and parameter analyzed. The Excel^{TM} data analysis add-in was used to run multiple t-tests to calculate the statistical significance between the means of two groups. Analysis of Variance (ANOVA) tests were also run to determine the statistical significance between the means of three or more groups. Within pond t-tests were used to determine the significance of seasonality on chlorophyll-a and pheophytin-a. Between pond ANOVA tests were used on initial dissolved oxygen, biochemical oxygen demand, pH, turbidity, nitrate, ammonia, organic and inorganic phosphate, chlorophyll-a, and pheophytin-a to determine if pond size impacted water quality parameters. Following the t-tests and ANOVA tests, a pvalue > 0.05 meant the difference between groups was not statistically significant using a 95% confidence level, whereas a p-value < 0.05 was statistically significant. A paired two sample for means t-test was used for within pond seasonal variation, and either a Student's t-test or Welch's t-test was used for comparisons between the two small ponds and between the two medium ponds. The variance of each pond was calculated using the summer and winter water quality sample results which were then used to determine whether Student's or Welch's t-test was used. Upon comparing two sample groups, if the ratio of the larger sample variance to the smaller sample variance was greater than three, Welch's t-test was used. After establishing whether Student's or Welch's t-test was appropriate for the particular analysis, the t-test was used to determine if the two small ponds (Davis A and Austin) were statistically different from one another and if the two medium ponds (Davis B and Fields) where statistically different from one another. If the p-value was > 0.05 , the difference between the small or medium ponds was statistically insignificant and therefore grouped together resulting in three categories (small,

medium, large). ANOVA tests were then used to compare each water quality parameter between the small, medium, and large ponds.

Results and Discussion

Sampling Adjustments

Pond water characteristics, such as water level and algae concentrations, were variable throughout sampling dates and required slight adjustments to sampling procedures. Despite aiming for chlorophyll-a sample filtrations of 125 mL, extensive pond algae in the medium sized pond, Fields, during the summer prevented total discrete sample filtration. As a result, 90 mL of the first discrete sample was filtered and only 50 mL of the remaining four samples were filtered from Fields during summer sampling. During winter sampling, a rainfall event occurred two days prior to sampling and resulted in water level rise for four of the five ponds.

Within Pond t-Test

Fields and Davis B, both medium sized ponds, resulted in the only statistically significant seasonal variation for either chlorophyll-a or pheophytin-a concentrations (Table 2). Fields had the only p-value less than 0.05 for the chlorophyll-a parameter, indicating a statistically significant difference between chlorophyll-a concentrations during summer and winter. Both Fields and Davis B had p-values less than 0.05 for the pheophytin-a parameter, resulting in statistically significant variations of pheophytin-a during the summer and winter. All other within-pond chlorophyll-a and pheophytin-a p-values were greater than 0.05 and therefore statistically insignificant for the remaining four ponds.

Pond Size Grouping

Davis A and Austin were grouped together, and Davis B and Fields were grouped together and categorized as small and medium, respectively. Since within pond t-tests indicated

some seasonal variation for chlorophyll-a and pheophytin-a, separate seasonal groupings were used for these parameters. Between pond t-tests were used for the remaining parameters to determine whether statistically significant results existed between two ponds of the same size. Mean results for the small, medium, and large pond groupings for all parameters are provided in Tables 3 and 4. Davis A and Austin resulted in p-values greater than 0.05 for all water quality parameters, therefore the difference between both small ponds was statistically insignificant. Water quality parameters compared between Davis B and Fields also resulted in p-values greater than 0.05, therefore the difference between the two medium sized ponds were statistically insignificant. According to the previously mentioned results, Davis A and Austin were categorized together as small and Davis B and Fields were grouped together as medium.

The large p-values associated with the small ponds may indicate a lack of statistical significance between these particular lotic (Austin) and lentic (Davis A) systems. Furthermore, the large p-values associated with the medium ponds suggests a lack of statistical significance pertaining to the impact of a buffer zone (Davis B) and no buffer zone (Fields) on water quality. However, the smaller, yet statistically insignificant, p-value associated with turbidity between Davis B and Fields indicates a possible correlation between buffer zones and turbidity for future research. Figure 18 depicts how Fields (no buffer) was more turbid in the summer and winter than Davis B (buffer). The difference between turbidity in a buffer and no buffer pond system suggests the prevention of increased sediment loads due to runoff in pond systems with a buffer zone. Nevertheless, further investigation of lotic and lentic systems and buffer zone presence could yield statistically significant results.

Between Pond ANOVA

The p-values obtained through ANOVA tests between small, medium, and large ponds all yielded values > 0.05, and therefore are not statistically significant (Tables 5 and 6). However, the pond water quality parameter p-values between pond sizes can indicate whether further investigation of the parameter would yield more statistically significant results. Although none of the water quality parameters are statistically significant with pond size, turbidity shows the greatest probability of a correlation. A p-value of 0.178 for this parameter suggests that further investigation may yield a statistically significant correlation.

The smaller, yet statistically insignificant, p-values of turbidity indicate the parameter's potential importance in relation to pond size and warranted further investigation between turbidity and other pond water quality parameters. Turbidity, initial dissolved oxygen, and biochemical oxygen demand appeared to be the most correlated. Figure 19 depicts the biochemical oxygen demand and initial dissolved oxygen for small, medium, and large ponds. The large pond had the lowest biochemical oxygen demand and largest initial dissolved oxygen during the summer and winter. Figure 20 represents the turbidity of each pond size, of which the largest pond has the least turbid water. Comparison of Figure 19 and Figure 20 indicate that less turbidity allows greater dissolved oxygen concentrations. Additionally, increased turbidity leads to less light and therefore reduced photosynthesis which creates less dissolved oxygen production (USDA, 2011).

All ponds in the present study, with the exception of Austin, receive the vast majority of water from rainfall and runoff. Pond water volume is directly related to catchment area and ponds with larger volumes provide larger catchment abilities (USDA, 1997). Since the water quality parameters for each pond were not statistically significant between pond sizes, large

ponds have the most water input while maintaining similar water quality as small and medium ponds. Therefore, larger ponds may be able to tolerate greater inputs of pollution from runoff than small ponds without associated water quality degradation.

Nitrate, Ammonia, Organic & Inorganic Phosphate

Table 7 provides the results of summer and winter pond water temperature, pH, nitrate, ammonia, organic phosphate, and inorganic phosphate values. Nitrate levels were absent or relatively minimal in each pond, excluding the summer sample of Austin which had a nitrate level of 15 mg/L, a value that has a slight possibility of causing harm to livestock (USDA, 2011). Winter ammonia concentrations for Davis A, Davis B, and Fields, and the summer ammonia concentration for Austin were all above 0.01 mg/L. These levels of ammonia in ponds are generally thought to be a result of human input (USDA, 2011). Lastly, the organic and inorganic phosphate concentrations were often above 0.03 mg/L, the maximum phosphate level capable of limiting excessive algae/vegetative pond growth (USDA, 2011). The large pond, Thompson, had organic and inorganic phosphate levels of zero, suggested that there was plant uptake occurring often and preventing the growth of algae throughout the pond.

Coliform Bacteria

A positive total coliform test was recorded at each pond (Figure 21). The number of colony forming units per 100 mL of water ranged from 200 CFU in the Thompson pond (largest pond) to 1693 CFU in Davis A and Austin (small ponds) and Fields (medium pond). The *E. coli* ranged from 0 in Davis A and B and Thompson to 1,038 CFU in Austin. The Austin pond was small and also had ducks and geese in addition to the livestock access. The Austin pond was impacted by animal waste contamination. The spring water flowing into the Austin pond at approximately 10,800 gallons per day was also tested for comparison. The spring water had only 43 CFU total

coliform and 19 CFU *E. coli*. Primary contact standards in most states are <200 CFU of *E. coli*/100 mL water. The Austin pond, then, was the only water body that exceeded primary contact standards.

Conclusion

The lack of statistical significance between pond sizes is indicative of the ability for large pond systems to provide greater catchment abilities while maintaining similar water quality to that of small and medium ponds. Therefore, the results of the current study support the "larger is better" assumption. However, multiple limitations should be considered concerning the current study and future research.

Limitations

Limitations in methodology, funding, and scheduling became apparent throughout the current study. The Hach Stream Survey Kit possessed detection limits and was prone to human error. Particularly, the use of the color comparator was impacted by lighting and the user's ability to accurately compare the sample color with the comparator. Additionally, COVID-19 implications and seasonal weather conditions pushed sampling dates back, impeded analysis of samples, and prevented additional seasonal sampling. Generally, the final evaluation on pond size contribution to water quality was impacted by confounding variables. While the current study aimed to attribute water quality to pond size, parameter results could have been impacted by differences in nearby land use (urban, pasture, or cropland), management practices, source of water input, etc. Therefore, differences in water quality between ponds cannot be conclusively linked to pond size. Despite these limitations, the outcomes of the current study can act as preliminary results for further research concerning ponds.

Practical Implications

The current study provides a basis for future research. Enlargement of the sampling population could limit the impact of confounding variables and present a stronger correlation between pond size and water quality. Additionally, if replicated, the current study could benefit from slight alterations including increased water sampling frequency, additional sampling dates within each season, and incorporation of nearby soil samples to determine the source of nitrogen and phosphorous additions. Furthermore, the comparatively high CFU results for Austin can be used in follow-up research to determine the source of *E.coli* inputs into the pond. Lastly, the reduction of turbidity in ponds with buffer zones in the present study supports previous research that suggests buffers prevent water contamination.

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Appendix 1: Site Characteristics

Figure 1

Pond Locations in Relation to One Another

Note. Map from Google Earth (2022) indicating the pond locations used in the current study. Davis A and Austin, Davis B and Fields, and Thompson were distinguished as small, medium, and large respectively.

Table 1

Pond ID, GPS Coordinates, Size Designation, Surface Area, and Surrounding Land Use of the

Individual Ponds Used in the Current Study

Figure 2

Google Earth (2022) Image of the Smallest Pond Titled 'Davis A'

Photograph of Davis A on August 11th, 2021

Figure 4

Google Earth (2022) Image of the Pond Titled 'Austin'

Austin Photographs

Note. Left: Photograph of Austin on August 12th, 2021 (Summer Sampling). Right: Photograph of Austin on February 19th, 2022 (Winter Sampling).

Figure 6

Bathymetric Profile of Davis A

Bathymetric Profile of Austin

Figure 8

Austin Spring Inlet (August 12th, 2021)

Google Earth (2022) Image of the Pond Titled 'Davis B'

Figure 10

Davis B Photographs

Note. Left: Photograph of Davis B on August $11th$, 2021 (Summer Sampling). Right: Photograph of Davis B on February 19th, 2022 (Winter Sampling).

Google Earth (2022) Image of the Pond Titled 'Fields'

Figure 12

Fields Photographs

Note. Left: Photograph of Fields on August 11th, 2021 (Summer Sampling). Right: Photograph of Fields on February 19th, 2022 (Winter Sampling).

Bathymetric Profile of Davis B

Figure 14

Bathymetric Profile of Fields

Google Earth (2022) Image of the Pond Titled 'Thompson'

Figure 16

Photographs of Thompson on August 12th, 2021

Bathymetric Profile of Thompson

Appendix 2: Results

Table 2

Within Pond Seasonal Variation for Chlorophyll-a and Pheophytin-a

Note. Results were obtained through paired two sample t-tests for repeated measurements taken within each pond on two sampling dates.

*****Indicates significant p-value at alpha = 0.05

Table 3

Means of Parameters without Seasonal Variation for Small, Medium, and Large Ponds

Table 4

	Pond Size Means			
Sampling Season & Parameter	Small	Medium	Large	
Summer				
Chlorophyll-a	10.95	263.36	6.43	
Pheophytin-a	6.63	-76.36	1.82	
Winter				
Chlorophyll-a	19.38	45.75	7.02	
Pheophytin-a	2.65	-7.68	1.30	

Means of Parameters with Seasonal Variation for Small, Medium, and Large Ponds

Table 5

Between Pond Variation for Water Quality Parameters Without Seasonal Variation

Parameter	p-value	Variance	Standard Deviation
DO initial (mg/L)	0.518	2.627	1.621
BOD (mg/L)	0.884	7.317	2.705
pH	0.394	0.952	0.976
Turbidity (cm)	0.178	580.6943	24.098
Nitrate (mg/L)	0.498	22.019	4.692
Ammonia (mg/L)	0.604	0.046	0.214
Organic Phosphate (mg/L)	0.463	1.003	1.001
Inorganic Phosphate (mg/L)	0.145	13.511	3.676

Note. Results were acquired through the use of single factor Analysis of Variance tests for all

parameters tested across sampling dates by pond size (small, medium, large).

Table 6

Sampling Season &			
Parameter	p-value	Variance	Standard Deviation
Summer			
Chlorophyll-a	0.613	50039.16	223.694
Pheophytin-a	0.619	5226.89	72.297
Winter			
Chlorophyll-a	0.727	1114.562	33.385
Pheophytin-a	0.655	85.72	9.259

Between Pond Variation for Water Quality Parameters With Seasonal Variation

Note. Results were acquired using a single factor Analysis of Variance test for parameters with seasonal variation by pond size (small, medium, large).

Figure 18

Turbidity in a Buffer and Non-Buffer Pond System During Summer 2021 and Winter 2022

Note. Turbidity (reported in cm of H₂O through which the Secchi disk can be seen) of the two medium sized ponds, Fields (no buffer) and Davis B (buffer) during the Summer of 2021 and Winter of 2022.

Dissolved Oxygen and Biochemical Oxygen Demand Means of Small, Medium, and Large Ponds

Figure 20

Turbidity Means of Small, Medium, and Large Ponds

Table 7

Pond &					Organic	Inorganic
Sampling	Temperature		Nitrate	Ammonia	Phosphate	Phosphate
Date	$({}^\circ\mathrm{C})$	pH	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Davis A						
8/11/2021	27.5	8.6	$\overline{0}$	0		4
2/19/2022	9.5	9	$\boldsymbol{0}$	0.4	2	8
Davis B						
8/11/2021	29.9	7.6	θ	θ	0.2	4
2/19/2022	10	8.8	0.05	0.2	2	θ
Fields						
8/11/2021	33.5	10.5	$\overline{0}$	θ	0.2	0
2/19/2022	12	8.4	0.75	0.6	2	$\overline{2}$
Austin						
8/12/2021	27	7.8	15	0.25	$\overline{2}$	θ
2/19/2022	10	8.2		θ	Ω	10
Thompson						
8/12/2021	32	6.8	θ	Ω	Ω	Ω
2/19/2022	11.5	8.4	0.05			0

Measured Parameters for Each Pond by Sampling Date

Figure 21

Total coliform bacteria and Escherichia coli bacteria per pond

Note. Total coliform sampled following summer and winter samples on March 13th, 2022.