WATERFOWL INFLUENCE ON FECAL INDICATOR BACTERIA IN CENTRAL FLORIDA FRESHWATER LAKES

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ABSTRACT

Bacteria are important natural components of virtually every environment, including water systems. While many are beneficial to the ecosystem in which they are found, some can be indicators of pathogens that can endanger human health. Fecal coliform bacteria such as *Escherichia coli* are bacterial indicators that can originate from many of the same sources as pathogenic bacteria and serve as a sign that pathogens may be present. These bacterial counts can be influenced by many different well-studied environmental factors, including pH, temperature, and nutrient availability. In addition to these factors, mammalian and waterfowl presence can influence coliform abundance. While this area of research has been examined before, conflicting conclusions have been reached as to whether or not waterfowl abundance positively correlates with coliform bacteria abundance. Levels of *E. coli* as well as *Enterococcus*, a genus of non-coliform bacterial organisms that are also found in high concentrations in feces, were measured by membrane filtration of water samples collected from six freshwater lakes around Lakeland, FL and were isolated from fresh fecal samples that were simultaneously collected from waterfowl species present at the lakes. Results suggest a correlation between the abundance of *E. coli* and the presence of waterfowl.

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KEYWORDS

- waterfowl
- fecal indicator
- freshwater
- *E. coli*
- *Enterococcus*

INTRODUCTION

Bacteria are natural components of virtually every environment and many play important roles in the ecosystems of which they are found. For example, the human microbiome is imperative for metabolic processes that occur in the human digestive tract and is responsible for variations in metabolic phenotypes in humans (21). Human health would drastically change if these bacteria did not exist as human symbionts (16). Further, bacteria play a great part in the natural world. Nitrogen fixation and nitrification, for instance, are processes by which bacteria fix atmospheric nitrogen from the air and convert it into a usable form for other organisms. Both of these

processes are parts of the nitrogen cycle, which is a key process for almost all living organisms (17). In freshwater and marine systems, bacterial roles vary from primary producers to decomposers to pawns in the carbon cycle (14). Additionally, bacteria can produce oxygen (5, 12, 31), recycle many primary elements of living systems (6, 28, 29), and detoxify systems (3, 11), among other processes.

While bacteria occupy many important and beneficial niches across ecosystems, the presence of certain types of bacteria can indicate environmental contamination that can raise concerns for public health, such as coliform bacteria in aquatic environments (20, 33). Coliform bacteria can be naturally occurring in the environment; however, they can also be found within the digestive tracts and therefore from there the feces of warm-blooded animals, such as mammals and birds (1, 23). Because coliform bacteria can be found in the digestive tracts and from there the feces of these organisms, their presence in aquatic habitats can be indicative of fecal contamination. This is a human health concern because many pathogens survive under similar conditions as these fecal coliforms. Thus, high levels of coliform bacteria may indicate high levels of human pathogens. The hypothesis that the presence of fecal coliforms in water suggests the presence of harmful bacteria was first introduced in 1892 and is still used as a basis for water quality standards today (15, 33).

Standards for measuring fecal coliform levels in water can vary, but the most commonly measured bacterial indicators can be split into three groups: total coliforms (TC), fecal coliforms (FC), and *Enterococcus* organisms (EC). However, because some coliform bacteria are naturally occurring in the environment, TC counts can be inaccurate and may not be the best indicators of fecal contamination (26). *Escherichia coli* counts, which is a subset of the FC count, and the *Enterococcus* EC counts are becoming the most preferred methods, primarily because *E. coli* strains and *Enterococcus spp.* have not yet been shown to be naturally occurring, while some other fecal coliforms can have questionable origins (26).

Many factors can impact coliform abundance in aquatic environments, including pH, nutrient availability, temperature, and anthropogenic pollution, as well as mammalian and avian abundances (8, 22, 30, 36). Waterfowl excrete a large amount of fecal matter, and their feces have commonly been found to harbor pathogens (4, 9, 13, 25). Microorganisms that are excreted in the feces of ducks have been linked to human health hazards such as *Salmonella*, pathogenic bacteria that reside in the digestive tracts of animals and can lead to gastrointestinal disruptions in humans (32). Moreover, a parasite known as *Echinostoma revolotum* harbored in waterfowl feces has been linked to a condition known as "swimmers' itch", an infection that appears as a skin rash, but is actually an allergic reaction to the parasite (24). Ecologically, increases in duck fecal concentrations have affected shellfish beds in the past and have been shown to influence algal blooms, which can have multiple negative impacts on aquatic systems (7).

The question asking how waterfowl abundance and fecal coliform bacteria abundance are related is not a new one. A previous study conducted by Standridge et

al., suggested that fecal coliform counts were high during their study time frame due to increased duck abundances and increased waterfowl fecal matter (32). However, in a similar study by Brierley *et al.* there was no direct correlation between waterfowl abundance and fecal coliform counts observed (7). This pattern of conflicting results can be seen across many other similar studies, leaving the question as to whether or not waterfowl abundance plays a significant role in fecal contamination levels unanswered (18). The goal of this study was to investigate this question and relationship by testing the hypothesis that the bacterial water quality of freshwater lakes in Central Florida will vary across a gradient of waterfowl abundance. From a big picture perspective, the study also addressed what varying water quality results could mean for overall ecosystem and human health.

MATERIALS AND METHODS

SAMPLING METHODS

Five lakes in Lakeland, Florida were sampled: Lake Hollingsworth, Lake Morton, Lake Mirror, Lake Belulah, and Lake Hunter. These lakes were selected based on their close proximity and locations surrounding Florida Southern College. At each lake, water was collected from ten different widely distributed and randomly selected accessible sites on the same day using sterilized beakers. The GPS coordinates, pH, and temperature of each sample site was measured and recorded, as well as plant abundance, anthropogenic pollution, and human trafficking that may influence water quality and therefore bacterial abundance. Acreage for each lake was determined from the Polk County Water Atlas (http://www.polk.wateratlas.usf.edu/). The waterfowl present at each lake was quantified and identified using the waterfowl identification guide from Ducks Unlimited (http://www.ducks.org/hunting/waterfowl-id/). Fresh fecal samples were collected from as many waterfowl as possible using sterile swabs and aseptic techniques. Fecal samples were collected from the ground, plated by continuous streaking on Eosin Methyl Blue (EMB) agar plates (RemelTM) and m *Enterococcus* Agar Plates (Fisher Scientific), and incubated overnight at 35°C.

ISOLATION AND IDENTIFICATION

Bacteria from water samples were filtered by membrane filtration using 0.2 µm membranes (Pall Corporation). For all *Enterococcus* isolations, 100 mL of sampled water was vacuum filtered. For potential *E. coli* isolations, 10 mL of sampled water was filtered. All 0.2 µm filters used for isolating *Enterococcus* bacteria were placed on *Enterococcus* media plates, while all filters used for isolating *E. coli* were placed on EMB plates. All of the plates were placed in a 35°Celsius incubator for 24–48 hours.

After incubation, the plates were removed and the colony forming units (CFU) count for each sample site was measured. CFU counts were determined based solely off of specific fecal coliform presence, meaning that for EMB plate

isolates, only those bacteria that were observed by color change to be fecal coliforms (metallic green) were quantified, and for *Enterococcus* plates, all of the bacteria were quantified. For each lake, colonies that appeared unique were isolated to obtain pure cultures of all isolated bacteria. Isolated colonies from the EMB plates were confirmed to be *E. coli* by 20E Analytical Profile Index (API) strips (bioMérieux). Strips were inoculated with fresh overnight cultures following the manufacturer's instructions and were incubated at 35°C for 16–24 hours. The profile index codes were obtained following the manufacturer's guidelines and APIweb was used to determine identifications from the codes. All of the fecal coliform bacteria cultured from the fresh fecal samples were also isolated and quadrant streaked to obtain pure cultures for use in comparative experiments against the lake isolates.

Once pure cultures of all of the lake and fecal samples were obtained, antibiotic profiles were obtained for each pure culture bacterial isolate to determine if any lake isolates matched any strains of bacteria isolated from waterfowl at the same lake. To obtain antibiotic profiles, a single colony of each isolate was resuspended in a solution of 0.9% saline to an OD600 value between 0.05 and 0.1. From this suspension, 100 µL was spread onto a Mueller–Hinton plate. Antibiotic disks (Carolina Biological Supply Company) were dispensed onto the plate. The following disks were used: Chloramphenicol (30 mcg), Erythromycin (15 mcg), Gentamycin (10 mcg), Kanamycin (30 mcg), Neomycin (30 mcg), Novobiocin (30 mcg), Penicillin G (10 units), Streptomycin (10 mcg), and Tetracycline (30 mcg). The plates were incubated at 35°C for 24 hours. The zones of inhibition for each antibiotic were measured, and isolates were classified as sensitive, intermediate, or resistant to each antibiotic using Antibiotic Disk Diffusion Interpretation Guide (27). Any isolates that showed a potential positive match was used for biochemical analysis and comparisons.

The 20E Analytical Profile Index (API) strips (bioMérieux) were used to perform biochemical tests on the waterfowl fecal samples that were determined to be potential matches to the lake isolates based on identical results from the antibiotic susceptibility testing. The strips were inoculated from fresh cultures following the manufacturer's guidelines and were incubated at 35°C for 16–24 hours. A known sample of *E. coli* K12 (ATCC 10798) was cultured on a 20E API test strip as a positive control. The profile index codes were obtained following the manufacturer's guidelines and APIweb was used to determine identifications from the codes.

RESULTS

The Environmental Protection Agency (EPA) defines the bacteriological water quality criteria for Lake Class waters,bodies of water that can be used for domestic, industrial, and agricultural water supply; stock watering; seafood rearing; wildlife habitat; ceremonial use; primary contact recreation; and commerce or navigational use, as water that should not exceed an Enterococci density of 107 colony forming units (CFU) per 100 mL in any single water sample or water that should not exceed an Enterococci density

geometric mean of 33 CFU/100 mL, where the mean was calculated with at least 5 samples over a period of 30 days (34). The *E. coli* standards for this same water class state that no single water sample should exceed 409 CFU/100 mL or that the geometric mean should not exceed 126 CFU/100 mL (34).

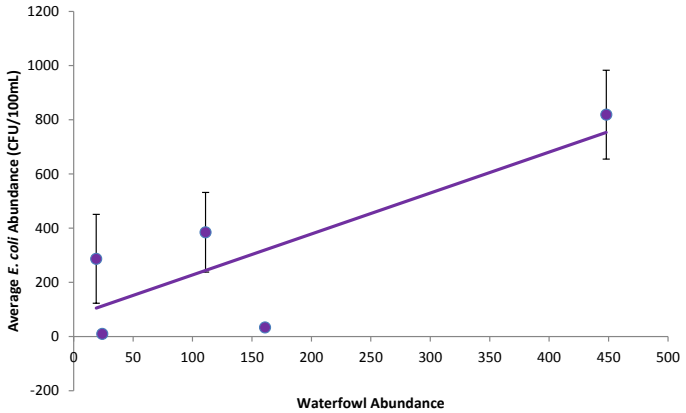
A summary of the abundance of waterfowl relative to abundance of *E. coli* and *Enterococcus* is shown in Table 1. The waterfowl abundance was 2.7-fold greater at Lake Morton than the next most abundant site, Lake Mirror. Abundance of *E. coli* was also the greatest at Lake Morton while abundance of *Enterococcus* was greatest at Lake Mirror. Lake Hunter showed the lowest level of waterfowl abundance. The lowest levels of *E. coli* and *Enterococcus* were found at Lake Belulah and Lake Hollingsworth, respectively.

Table 1 – Average Abundance of Bacteria and Waterfowl at Each Lake
The table shows the average abundance of bacteria and the abundance of waterfowl at the five sampled lakes. Abundance values for both *E. coli* and *Enterococcus* represent the mean ± standard error of n=10 samples taken from unique locations on the same day around each lake. *E. coli* and *Enterococcus* were isolated by membrane filtration. The number of samples exceeding the standards was determined by comparing the *E. coli* and *Enterococcus* CFU counts from each individual sample to the EPA standards of 409 CFU/100 mL and 107 CFU/100 ml for *E. coli* and *Enterococcus*, respectively.

Lake	Hollingsworth	Morton	Mirror	Hunter	Belulah
Waterfowl Abundance	111	448	161	19	24
Average *E. coli* Abundance (CFU/100 mL)	385 ± 147	819 ± 164	34.0 ± 7.92	287 ± 164	10.0 ± 5.16
E. coli Individual Samples Exceeding Standards	3	9	0	2	0
Average *Enterococcus* Abundance (CFU/100 mL)	3.00 ± 0.760	14.8 ±6.83	36.9 ± 6.93	14.2 ± 5.56	16.2 ± 8.94
Enterococcus Individual Samples Exceeding Standards	0	0	0	0	0

The abundance of *E. coli* from three lakes, Lake Hollingsworth, Lake Morton, and Lake Hunter exceeded the geometric mean standard of 126 CFU/100 mL, while 14 individual samples (three from Lake Hollingsworth, nine from Lake Morton, and two from Lake Hunter) exceeded the single sample standard of 409 CFU/100 mL. The abundance of *Enterococcus* from one lake, Lake Mirror, exceeded the accepted EPA geometric mean standard of 33 CFU/100 mL while the other four lakes fell within in the acceptable range. No single *Enterococcus* sample exceeded 107 CFU/100 mL. Figure 1 shows the abundance of *E. coli* (Panel A) and *Enterococcus* (Panel B) vs. the waterfowl abundance for each lake. There is a strong positive correlation between *E. coli* and waterfowl abundance with a Pearson's correlation coefficient of 0.809. The abundance of *Enterococcus* and waterfowl showed no real correlation with a coefficient of 0.067. In order to determine whether the presence of *E. coli* might affect levels of *Enterococcus* and vice versa, their abundances were compared for each lake. The relationship between the average *E. coli* abundance and the average *Enterococcus* abundance per lake is shown in Figure 2.

A.



B.

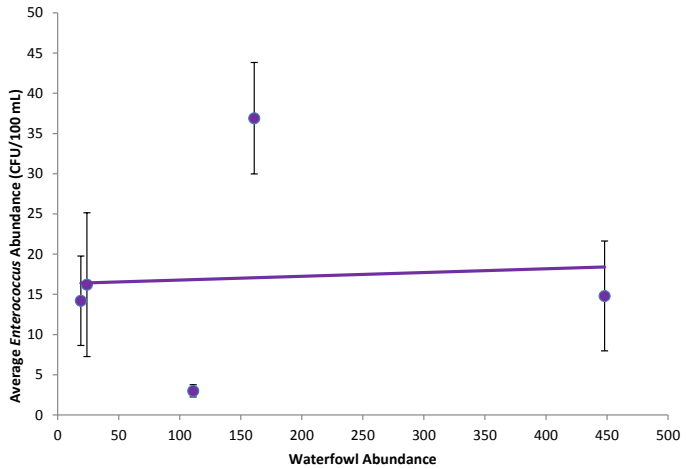
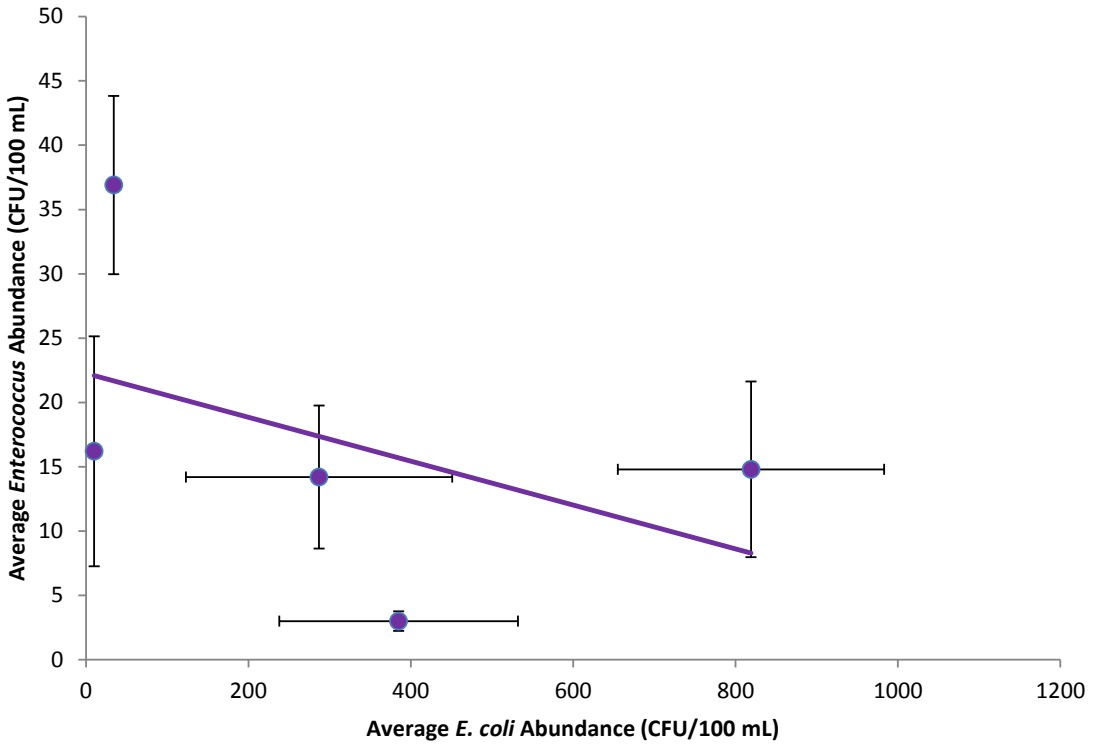


Figure 1 – Average Bacteria Abundance vs. Waterfowl Abundance per Lake

The figure shows the average abundance of E. coli (Panel A) and *Enterococcus* (Panel B) vs. waterfowl abundance for each lake. Average abundance values were calculated from membrane filtration of ten samples obtained from each lake. Error bars represent the standard error of the mean. The trendline shows the best-fit linear line of regression. The Pearson's Correlation Coefficient for this data is 0.809 (E. coli) and 0.067 (*Enterococcus*).

Figure 2 – Average *Enterococcus* Abundance per Lake vs. Average *E. coli* Abundance per Lake
The figure shows the average abundance of *Enterococcus* vs the average abundance of E. coli for each lake. Average abundance values were calculated from membrane filtration of ten samples obtained from each lake. Error bars represent the standard error of the mean. The trendline shows the best-fit linear line of regression. The Pearson's Correlation Coefficient for this data is -0.455.



There is a negative correlation between the presences of these two bacterial varieties with a correlation coefficient of -0.455. Since other factors besides waterfowl are believed to affect the abundance of fecal indicator bacteria the effects of pH and temperature on abundance of E. coli and *Enterococcus* were examined. There is no apparent correlation between pH levels and either *E. coli* or *Enterococcus* with correlation coefficients of -0.0121 and -0.221 (data not shown). Neither E. coli nor *Enterococcus* showed a correlation with temperature, producing coefficients of -0.104 and -0.0875, respectively (data not shown).

The five lakes sampled in this study vary somewhat in size. To determine if there is any relationship between the size of the lake and the abundance of *E. coli* or *Enterococcus*, the abundance of organisms from each lake was compared to the size of the lake. Figure 3 shows the results of this comparison. The relationship between *E. coli* abundance and lake size resulted in a correlation coefficient of 0.191, suggesting a slight positive correlation may exist. However, the abundance of *Enterococcus* and lake

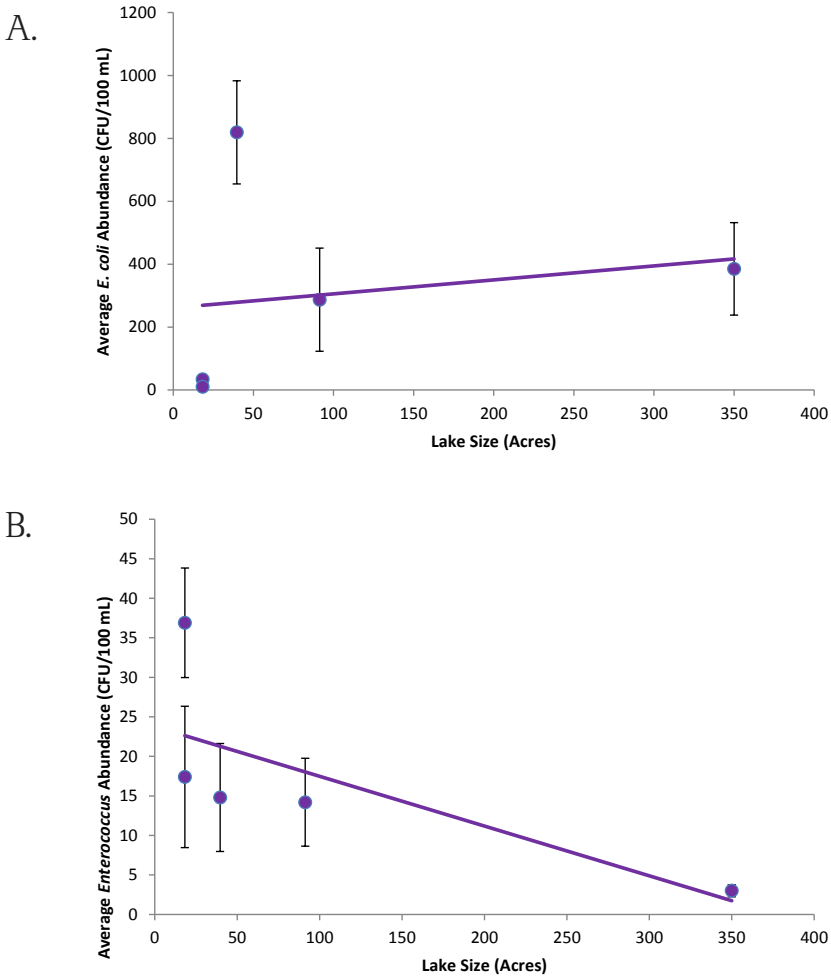


Figure 3 – Average Bacteria Abundance per Lake vs. Lake Size
The figure shows the average abundance of *E. coli* (Panel A) and *Enterococcus* (Panel B) as a function of lake size for each lake. Average abundance values were calculated from membrane filtration of ten samples obtained from each lake. Error bars represent the standard error of the mean. The trendline shows the best-fit linear line of regression. The Pearson's Correlation Coefficient for this data is 0.191 (E. coli) and -0.722 (*Enterococcus*).

size produced a strong negative correlation coefficient of -0.722.

Preliminary source tracking through the use of antibiotic susceptibility screening resulted in eight positive matches where the antibiotic susceptibility pattern of the E. coli sample from the lake matched the pattern from a fecal coliform sample isolated from waterfowl feces at the same lake (data not shown). After testing patterns of Biochemical characterizations, one of these matches showed identical Biochemical characterization using 20E API strips (data not shown).

DISCUSSION

The question as to whether or not the abundance of waterfowl impacts the bacterial water quality of lakes is one that has been asked previously, leading to controversial results. This study aimed to provide insight into this topic in order to determine how the relationship between waterfowl and bacteria can impact the ecosystem as a whole and human health. Results suggest that as the abundance of waterfowl present at a lake increases, the average *E. coli* abundance increases, with a positive correlation coefficient of 0.809, as shown in Figure 1. While this coefficient is below the critical value for significance at the 95% confidence level, it is significant at the 90% confidence level and shows a strong positive trend worthy of future research with a larger sample size. This data lends support to the hypothesis that an increase in waterfowl abundance at a lake does increase the *E. coli* abundance and thus decreases the bacterial water quality and increases the potential human health risks.

However, there was no definite correlation observed between waterfowl abundance and *Enterococcus* abundance (Figure 1), while there was a negative correlation (Pearson's correlation coefficient being –0.455) between the *Enterococci* densities and *E. coli* densities at each lake (Figure 2). While this correlation is not particularly strong, combined with the absence of a correlation between the waterfowl and the *Enterococcus* spp., it suggests that there may be some type of competitive interaction occurring between the *E. coli* and *Enterococcus* organisms and/or the surrounding bacterial community that may be complicating the ecosystem dynamics observed in this study. A strong negative correlation (correlation coefficient of –0.722) was observed between lake size and *Enterococcus* organisms (Figure 3) while a very slight positive correlation was observed between *E. coli* and lake size (correlation coefficient 0.191). This further suggests there may be an interaction occurring between the two bacterial groups that is impacting their abundances. No relationship was found between pH and bacterial abundance, nor between temperature and bacterial abundance, so these factors do not appear to complicate the relationship.

Preliminary source tracking of fecal coliforms occurred in this study. A combination of antibiotic-susceptibility testing and 20E API strips was used to match samples isolated from water and fecal samples with the same patterns for both tests. One bacteria isolated from Lake Belulah was both positively identified as *E. coli* and matched the antibiotic susceptibility pattern and 20E API strip biochemical test results of an *E. coli* sample isolated from the feces of a White Ibis at the same lake. Sequencing of the 16s rRNA and multiple other housekeeping genes would be necessary to confirm these two isolates are the same and identify the White Ibis as the source of the fecal coliform isolated from the lake. While one positively source-tracked sample would not be considered significant, it would show that deposition of feces from waterfowl does contribute to the microbial community of

freshwater lakes.

It is important to note that not all bacteria were able to be tracked to a source for varying reasons. First, some of the bacteria did not survive in the lab until the end of the extensive study, and because of this, they could not be tracked to a source. Additionally, some bacteria were not able to be matched back to an organism because the number of fecal samples gathered was limited. It was not possible to obtain fecal samples from every waterfowl, nor from every other potential source such as dogs, cats, livestock, and humans. Future research will increase the number of samples collected and will examine the effects of plant abundance, anthropogenic pollution, and human traffic may have on waterfowl abundance.

While the relationship between the bacteria and other factors at each lake was the primary focus of this study, the impacts that these relationships may have on the ecosystem and human health must be acknowledged. The bacterial water quality standards for Lake Class waters have been previously defined by the EPA, and the results of this study were compared to those standards. Only at one lake, Lake Mirror, was the average *Enterococcus* density greater than the acceptable average, as displayed in Table 1. Moreover, none of the CFU counts for each individual site was greater than the acceptable value for *Enterococcus* organisms. The *E. coli* densities, on the other hand, need to be addressed. The average *E. coli* abundance samples from three of the five lakes exceeded the EPA geometric mean standards, and 14 of the 50 individual samples (28%) exceeded the individual sample standards. These potentially high levels of *E. coli* pose a threat to human health, given that such high levels of these coliforms may indicate high levels of pathogens.

Though a positive correlation between *E. coli* densities and waterfowl abundance was observed, it is possible that other factors may be contributing to these unusually high levels of bacteria, such as nutrient availability, competition between bacteria, vegetation presence and abundance, and anthropogenic pollution, such as the addition of fertilizer and oil run-off. In particular, if antibiotics have somehow entered these waters due to anthropogenic interactions and fecal contamination, any opportunistic bacteria or pathogens present in these waters may pose an even greater risk (10). A study conducted by Costanzo, Murby, and Bates has shown that an increase in antibiotics in waterways can lead to bacterial resistance, contributing to the current worldwide antibiotic resistance crisis (10, 35). Regardless of additional factors that may be influencing the bacterial abundance, these *E. coli* densities should not be ignored. These lakes are historically known to attract tourists and are often used for human recreational activities, such as kayaking, boating, and water skiing.

On an ecological scale, bacteria occupy many niches, both harmful and beneficial. An increase in any number of microorganisms in an aquatic system may impact the bacterial ecosystem as a whole, changing the dynamic between organisms, as well as altering the biogeochemical cycles. For instance, increasing the nutrient availability in an aquatic ecosystem can lead to increased numbers of phytoplankton blooms, which can alter an ecosystem by depleting the oxygen and nutrients present in the water,

as well as decreasing the amount of sunlight to submerged vegetation, thus leading to a change in the entire ecosystem (2). Increasing the bacterial abundance in an aquatic ecosystem may alter the entire dynamic, especially if these bacteria are introduced to the environment *via* contamination, thus essentially acting as invasive species, these introduced *E. coli* and *Enterococcus* organisms must adapt and occupy niches that were previously occupied by the naturally occurring bacteria (19).

Overall, the bacterial water quality of the lakes sampled in this study was shown to be unacceptable based on the EPA Lake Water standards when looking at *E. coli* densities. The ecosystem and human health risks that are potentially posed due to these values should raise concerns both for environmental health and public health officials. Additionally, the water sampling protocol used in this study did not account for the presence of stressed or injured Enterobacteriaceae that might not grow when plated directly onto EMB agar, suggesting the actual densities of *E. coli* could be higher than those reported here. Moreover, the relationships observed between the bacteria and the factors measured in this study suggest that there may be an interaction component between *E. coli* and *Enterococcus* spp. that needs to be taken into account when bacterial water quality assessments are being made, and this interaction should be investigated further for confirmation.

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