To swim or not to swim; are *Escherichia coli* and coliform abundances related to distance from shore, pH, and dissolved oxygen in recreational lakes?

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Sharlo Bayless

Advisor: Sara Benevente

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ABSTRACT

Fecal contamination assessments, often measured by indicator bacteria such as *Escherichia coli* and coliform, are required by the Environmental Protection Agency to evaluate water quality. While much is known about plants and animals affecting fecal contamination, the influence of pH and dissolved oxygen (DO) is less frequently studied. This study looked at 4 lakes with differing morphologies—Tenderfoot, Roach, Morris, and Bay—to determine if fecal contamination was lower in recreational lakes and varied with pH, DO, and distance from the shore. I took 100mL water samples from two distances on each lake on 5 sampling events and incubated 1mL replicates on Petrifilms at 37°C for 24 hours. The results showed that 1) the samples closer to the shoreline had higher coliform abundance, 2) Roach had significantly more coliform than other lakes and *E. coli* presence was significantly higher in Bay Lake, and 3) coliform abundance had a positive relationship with pH, but not DO. The results suggest that *E. coli* and coliform levels were probably influenced by lake morphology, such as discharge patterns and shoreline development. Furthermore, bacterial tolerance to slightly basic environments could explain coliform abundance in lake samples with higher pH levels. Future research can look at isolating fecal coliform from total coliform as a more accurate indicator of fecal contamination and measure it in conjunction with *E. coli* to further assess recreational safety.

INTRODUCTION

When assessing the water quality of lakes, a common method is to look at the fecal contamination, which often indicates the potential for other illness-causing pathogens. *Escherichia coli (E. coli)* and fecal coliform, which reside in the guts of birds and mammals, are

used as indicator bacteria of the presence of feces (Bower et al. 2005). Fecal contamination occurs in close proximity to water treatment plants or agricultural areas where it can travel through runoff water, making its way into streams and lakes (Ahmed et al. 2015). As such, monitoring fecal levels can prevent a number of illnesses from the contamination itself, as well as suggest the presence of similar pathogens.

The US Environmental Protection Agency (EPA) requires regular assessments of the water quality of lakes, commonly performed by local health officials and natural resource departments (Lam and Surbeck 2011). To prevent infections and illnesses, the EPA establishes water quality standards that lakes must satisfy to be considered acceptable for swimmers. The Estimated Illness Rate associated with this standard is 32 health incidents (including vomiting, diarrhea, stomachaches, nausea, and fevers) per 1000 recreational swimmers, or 3.2% (U.S. EPA 2012). In recent years, the EPA has suggested multiple methods to collect data, such as testing for *E. coli* and coliform as indicator bacteria. Health departments do this by counting the number of colony-forming units (CFUs) in a 100mL sample, which are bacterial colonies that can be distinguished by the naked eye. Coliforms were first used as indicator bacteria for fecal contamination in the 1960s, and the limit of total coliform in a 100mL sample was 2400 CFUs (Griffin et. al 2000). In the late 1970s, the Environmental Protection Agency began to monitor *E. coli* levels in place of coliform as a more efficient method for freshwater testing. The cutoff for *E. coli* is 100 CFUs per 100mL sample (U.S. EPA 2012).

While animals clearly contribute to fecal contamination and plants impact contamination levels by providing habitats, it is unclear if abiotic factors such as pH, dissolved oxygen (DO), and distance from the shore are related to fecal contamination in lakes. Bacteria reproduce most successfully at a near-neutral pH of around 7.4 (Zhu 2007), and bodies of water with high pH

levels increase bacterial susceptibility to harmful ultraviolet rays from the sun, often resulting in lower bacterial concentrations (Curtis et al. 1992). On the other hand, bodies of water with lower levels of pH, such as bogs, do not support the wildlife or proper nutrients for warm-blooded animals, and therefore, these locations likely have lower fecal contamination (Connor 1953). Additionally, indicator bacteria reproduce better in oxygen-rich conditions for the purposes of cellular functions such as protein synthesis (Sandoval-Basurto et al. 2004). High oxygen levels also increase algae growth, which blocks harmful UV rays and supports bacteria populations (Curtis et al. 1991). Also, shorelines are known to pose as sinks for general bacteria, with heavier presences of contaminants from animals and humans (Parkera et al. 2010). Therefore, it would be interesting to see if there are also higher levels of fecal contamination in areas close to shores.

This study looked at abiotic factors in four selected lakes in Vilas County, Wisconsin, USA to determine if there are optimal abiotic conditions for E. *coli* growth and survival across various lake morphologies. I hypothesized that (1) *E. coli* and coliform concentrations are higher by the shore than at chest-deep water, (2) *E. coli* and coliform levels are lowest in lakes commonly used for recreational swimming, and (3) *E. coli* and coliform concentrations are greatest with neutral pH and high DO levels.

MATERIALS AND METHODS

Study Site:

I selected four recreational lakes based on their distance from each other and variations in morphology: Tenderfoot, Roach, Morris, and Bay. These lakes are located in the University of Notre Dame Environmental Research Center (UNDERC) property in Vilas County, Wisconsin,

USA. Tenderfoot is a drainage lake that receives water from many smaller lakes on property and empties into Tenderfoot Creek. The shoreline development index (D_L), which indicates how much the perimeter deviates from a perfect circle, is 1.91 (U.S. Geological Survey 1981). Roach is a seepage lake without any discharge outlets, known for its highly transparent water, and also has a D_L of 1.91 (Johnson 1997). It is the only lake on property that is regularly used for recreational swimming. Morris Lake is much darker in color and is also part of a flowing water system. It is very round with a D_L of 1.19 (Huftalen and Larvey 1991). Bay Lake is highly transparent, and receives water from Tuesday Lake, which it discharges into a series of other lakes. It deviates greatly from a circle, having a D_L of 2.64 (U.S. Forest Service 1997; S.R. Carpenter, unpublished).

Sample Collection and Techniques:

In each study lake, I designated one "Near" and one "Far" sampling distance. The "Near" samples were collected from the shore of the lake closest to the dock, and the "Far" samples were collected from chest-deep water. Sample collection occurred in the morning on the following dates: June 2, June 15, June 17, July 2, and July 7. I collected 100mL of water from each location. I also recorded pH and DO at each sampling location using a pH electrode (Oakton, Orangeburg, NY) and DO probe (YSI, Yellow Springs, OH). In the laboratory, I added 1mL from each sample to a Petrifilm *E. coli* count plate (3M, Saint Paul, MN), repeating each sample in triplicates. One milliliter of distilled water served as an uncontaminated control. The Petrifilms incubated at 37°C for 24 hours. Following incubation, I counted *E. coli* and coliform CFUs on the plates. The number of CFUs per 1mL sample was then scaled up to reveal the *E.*

coli and coliform concentrations in the 100 mL water samples for comparison with EPA standards.

Statistical Analysis:

I transformed the data by taking the natural logarithm of total coliform counts and the square of pH values, and verified that the coliform, pH, and DO data were normally distributed with Shapiro-Wilk tests. I used a t-test to compare the coliform abundance between all "Near" and "Far" distances. I also used a one-way ANOVA and TukeyHSD to compare the total coliform between all lakes and distances. Additionally, I used a chi-square test to compare the *E. coli* presence between lakes. I performed linear regressions to look at the relationship between pH and coliform levels and between DO and coliform levels.

RESULTS

The "Near" distances had significantly higher coliform counts ($M = 89.89$, $SD = 61.98$) than "Far" distances ($M = 55.2$, $SD = 35.19$) among all lakes ($p = 0.000618$, $F = 11.1$, $t(95) = 52.88$) in 1mL samples (Fig. 1). Furthermore, there were significant differences in coliform abundances between distances and lakes in 1mL samples ($F(7, 89) = 4.261$): the "Near" location in Roach was significantly higher in coliform abundance than the "Far" distances in Bay, Morris, and Roach ($p = 0.0004$, 0.0162, and 0.009 respectively) and the "Near" distance in Morris Lake ($p = 0.038$). The "Near" distance at Bay was also significantly higher in coliform abundance than the "Far" distance at Bay Lake ($p = 0.022$) (Fig. 2). See Table 1 for average coliform abundances per lake and distance.

The highest number of *E. coli* colonies per 100mL sample was 100 (M = 33.3125, SD = 35.62). Additionally, the chi square test revealed that the *E. coli* presence was significantly higher in Bay Lake than the other lakes ($p = 0.0039$, $df = 3$, $\chi^2 = 13.353$) (Table 2).

When I analyzed the pH and DO data, I ignored the "Near" and "Far" differences, looking only at patterns between entire lakes. The linear regressions revealed that a positive significant relationship existed between pH and coliform ($p = 0.0034$, $t(39) = 3.121$, $R^2 = 0.204$) but not between DO and coliform ($p = 0.225$, $t(39) = -1.234$, $R^2 = 0.16878$) (Fig. 3 and 4).

DISCUSSION

In the overall coliform averages, the significantly higher count in "Near" samples implies that more bacteria contamination occurs in shallow shoreline areas. This is possibly due to the runoff that makes its way onto shores, which produces high microbial counts (Bergstein-Ben and Koppel 1992). Additionally, mammals and especially birds defecate close to the shores—often because the littoral zone is rich in food sources for birds—causing a buildup of feces there (Pieczyńska 1986). Research also suggests that aquatic plants and algae, which are especially present in eutrophic lakes, and plastic debris serve as sinks for indicator bacteria. These are often concentrated closest to shores (Quilliam et al. 2014). Furthermore, storms cause runoff levels to increase, which bring pollutants, such as fecal contamination, to shore areas and drastically elevate coliform counts (Parkera et al. 2010).

The "Near" distance in Roach Lake has significantly higher coliform counts than the "Far" distances in Bay and Morris. This could be due to the fact that Roach Lake does not have

any discharge outlets, as the other three lakes do (Fig. 5). Water flow mobilizes sinks of high *E. coli* and coliform concentration to further contaminate bodies of water downstream (Quilliam et al. 2014). For example, a previous study used artificial floods to replicate discharge outlets to flush water rapidly downstream. Researchers observed an exponential decrease in *E. coli* concentration after successive floods, suggesting that a never-ending series of floods would remove every single *E. coli* bacterium from the sampled area (Muirhead et al. 2004). In this context, Roach Lake does not have an outlet to remove bacterial indicators, and events such as heavy rain bring microorganisms to water shores, rather than expel them to downstream bodies of water.

It is curious that Bay Lake was the only lake where *E. coli* was found. Although a number of factors may contribute to this finding, it is likely that the shoreline development (D_L) of Bay Lake contributes to this idea. Bay Lake has a much greater perimeter relative to its area than the other lakes, deviating greatly from a perfect circle. Pollution levels tend to be higher along densely developed shorelines, as more of the perimeter is available as habitats for birds and mammals, and additionally provides many opportunities for fecal contamination from runoff water (Moses et al. 2011).

It is also interesting that coliform counts have a positive relationship with pH but not dissolved oxygen. One reason may be that the internal environment of coliform is slightly basic at a pH of about 7.4-7.8 (Beuria et al. 2006). Additionally, studies have shown that both coliform and *E. coli* are more readily cultured at a pH of 7.9 - 8.2 than at a neutral level (Martin et al. 1982; Grandjean et al. 2005). Coliform is capable of adapting to changes in pH that naturally occur due to cellular mechanisms for ensuring pH homeostasis (Bearson et al. 1997),

but are more affected by extreme pH values lower than 4 or above 11 (Foster et al. 2000). Therefore, while coliform can survive in environments with slight pH deviations, mammals are more likely to be found in slightly basic than slightly acidic environments, increasing fecal contamination in those areas (Connor 1953). It is more difficult to find relationships with dissolved oxygen, in part because bacteria can function in anaerobic conditions. Additionally, while bacteria survive most readily at high DO levels, excess bacteria and high levels of biological waste use up DO and actually cause a decrease in its levels (Oram 2014).

Total coliform was the original indicator of fecal contamination, but its presence can indicate a diverse range of bacteria, from microorganisms in polluted water to naturally occurring bacteria. As such, it is still used in assessing drinking water (U.S. EPA 2001), since total coliform is a better indicator of overall environmental bacteria, rather than bacteria of any particular origin (Paruch and Maehlum 2012). The Petrifilms specifically tested for total coliform and *E. coli*, and because it indicated a large presence of total coliform, the next step is to use a more specific assay to determine the presence of *fecal* coliform. Fecal coliform is a variant of coliform that is thermotolerant, and plates of colonies are incubated at a higher temperature of 44◦C to kill the coliform from other sources (Bartram and Rees 2000). Additionally, since fecal coliform and *E. coli* are found together in animal gastrointestinal systems, we would most likely see high levels of fecal coliform in Bay Lake (U.S. EPA 2001).

E. coli was only found in Bay Lake, with an estimated maximum of 100 colonies per 100mL sample. As it just barely satisfies the cutoff of 100 colonies in a 30-day period, Bay Lake is still safe for swimming, according to EPA guidelines on *E. coli* abundance. Total coliform levels exceeded the EPA maximum in all lakes, making none of the lakes safe for recreational

swimming according to EPA guidelines on total coliform. However, it is necessary to carry out further tests on the *fecal* coliform abundance in order to make a more definite claim about the fecal contamination. With this information, we will have a better understanding of abiotic factors in lakes and how they influence microbial populations and fecal contamination, which will play a role in protecting the health of swimmers and recreational users.

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TABLES

Table 1. Average coliform per lake and distance.

Lake/Distance	Average Coliform per 1mL	Standard Deviation
Tenderfoot "Near"	83.80	50.25
Tenderfoot "Far"	73.69	45.54
Roach "Near"	141.56	77.96
Roach "Far"	51.08	31.69
Morris "Near"	52.08	25.84
Morris "Far"	48.85	29.06
Bay "Near"	62.03	62.03
Bay "Far"	27.87	27.87

Table 2. Chi square test on *E. coli* presence between lakes. Bay lake had a significantly higher presence of *E. coli* than Tenderfoot, Roach, and Morris Lakes ($p = 0.0039$, $df = 3$, $\chi^2 = 13.353$).

Lake	Events with *E. coli* Present	Events with *E. coli* absent
Tenderfoot	0	5
Roach	0	5
Morris	0	5
Bay	4	1

FIGURES

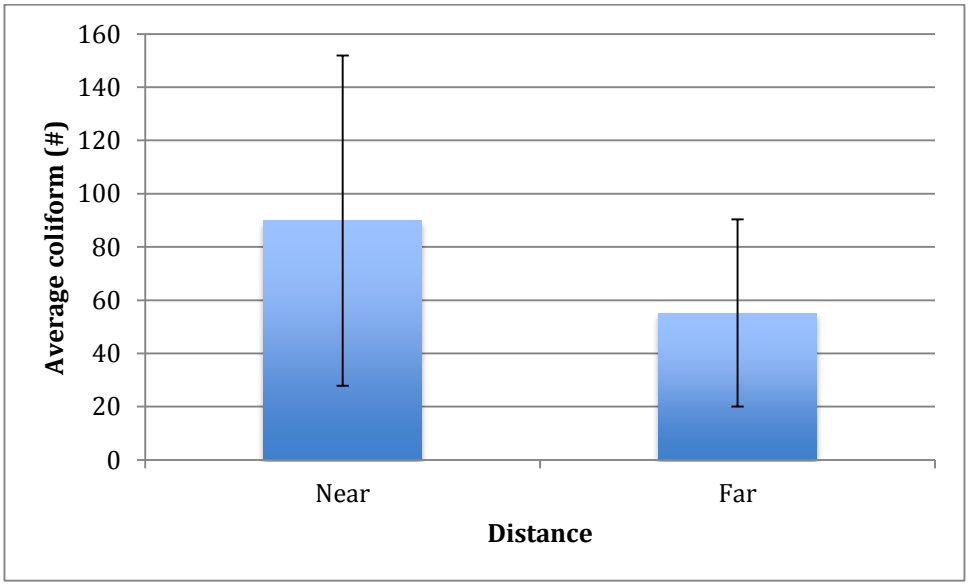

Fig. 1 Comparing average coliform between "Near" and "Far" distances. The average coliform count of all "Near" distances (M = 89.89, SD = 61.98) was statistically greater than the average of all "Far" distances (M = 55.2, SD = 35.19), in 1mL samples ($p = 0.000618$, $F = 11.1$, $t(95) = 52.88$).

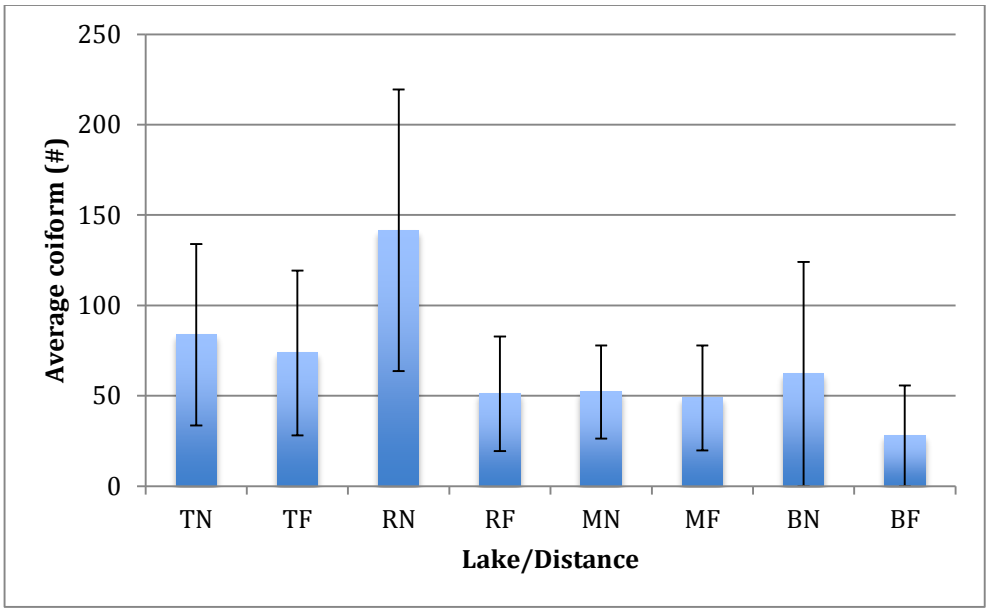

Fig. 2 Average coliform across all locations. There were significant differences between the distances and lakes [F(7, 89) = 4.261] in 1mL samples: the "Near" distance at Bay Lake had a significantly higher coliform abundance than "Far" at Bay Lake ($p = 0.022$). The "Near" distance at Roach had a significantly higher abundance than the "Far" distances at Bay Lake ($p < 0.001$), Morris Lake ($p = 0.016$), and Roach Lake ($p = 0.009$). The "Near" location at Roach also had significantly more coliform than the "Near" distance at Morris Lake ($p = 0.038$).

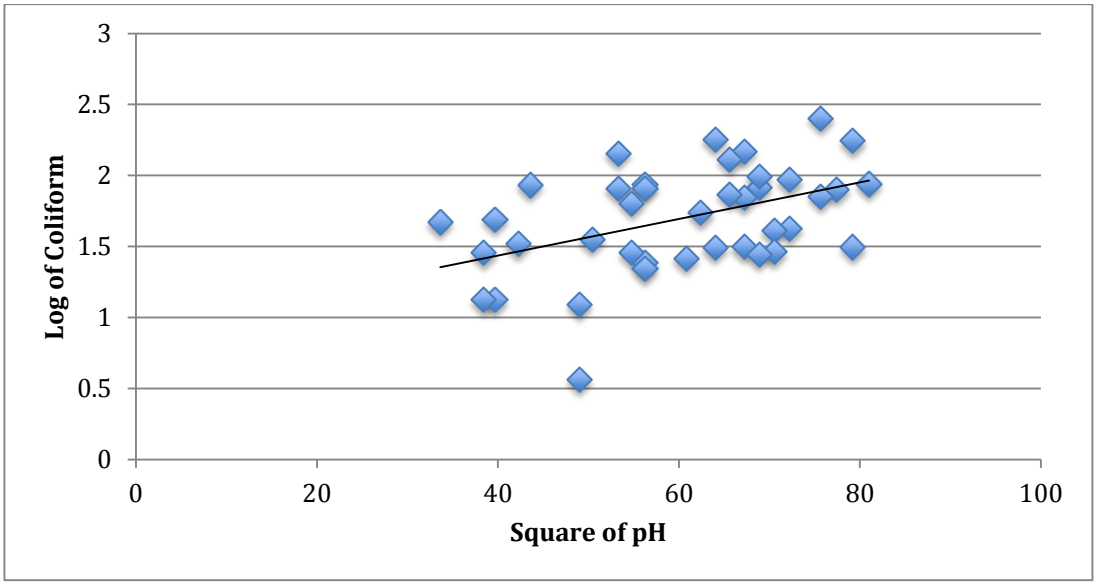

Fig. 3 Average coliform against pH. A significant positive relationship exists between the total coliform and pH ($p = 0.0034$, $t(39) = 3.121$, $R^2 = 0.204$).

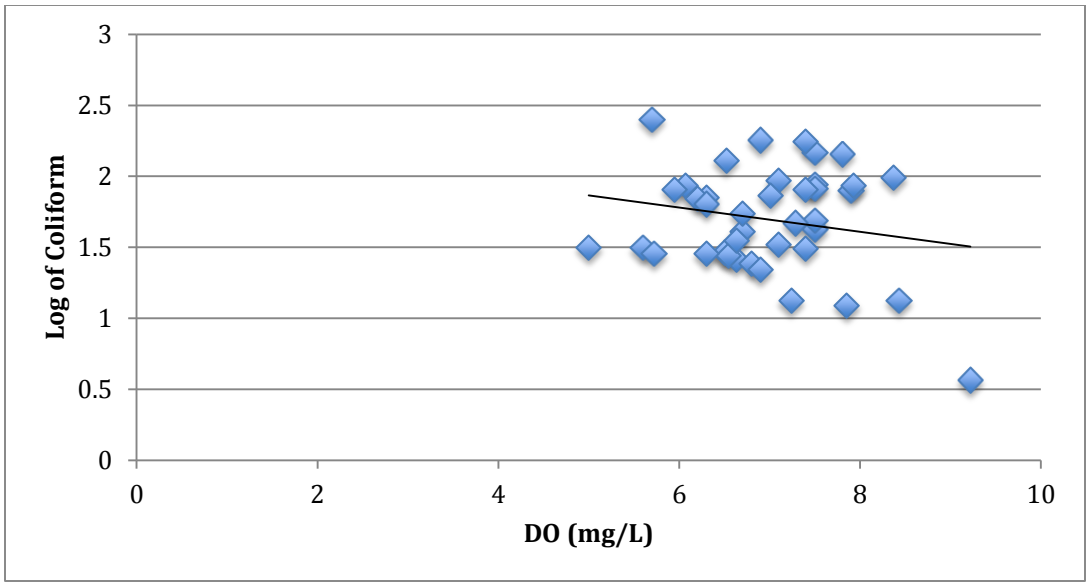

Fig. 4 Coliform against dissolved oxygen. There is no significant linear relationship between coliform abundance and DO ($p = 0.225$, $t(39) = -1.234$, $R^2 = 0.16878$).

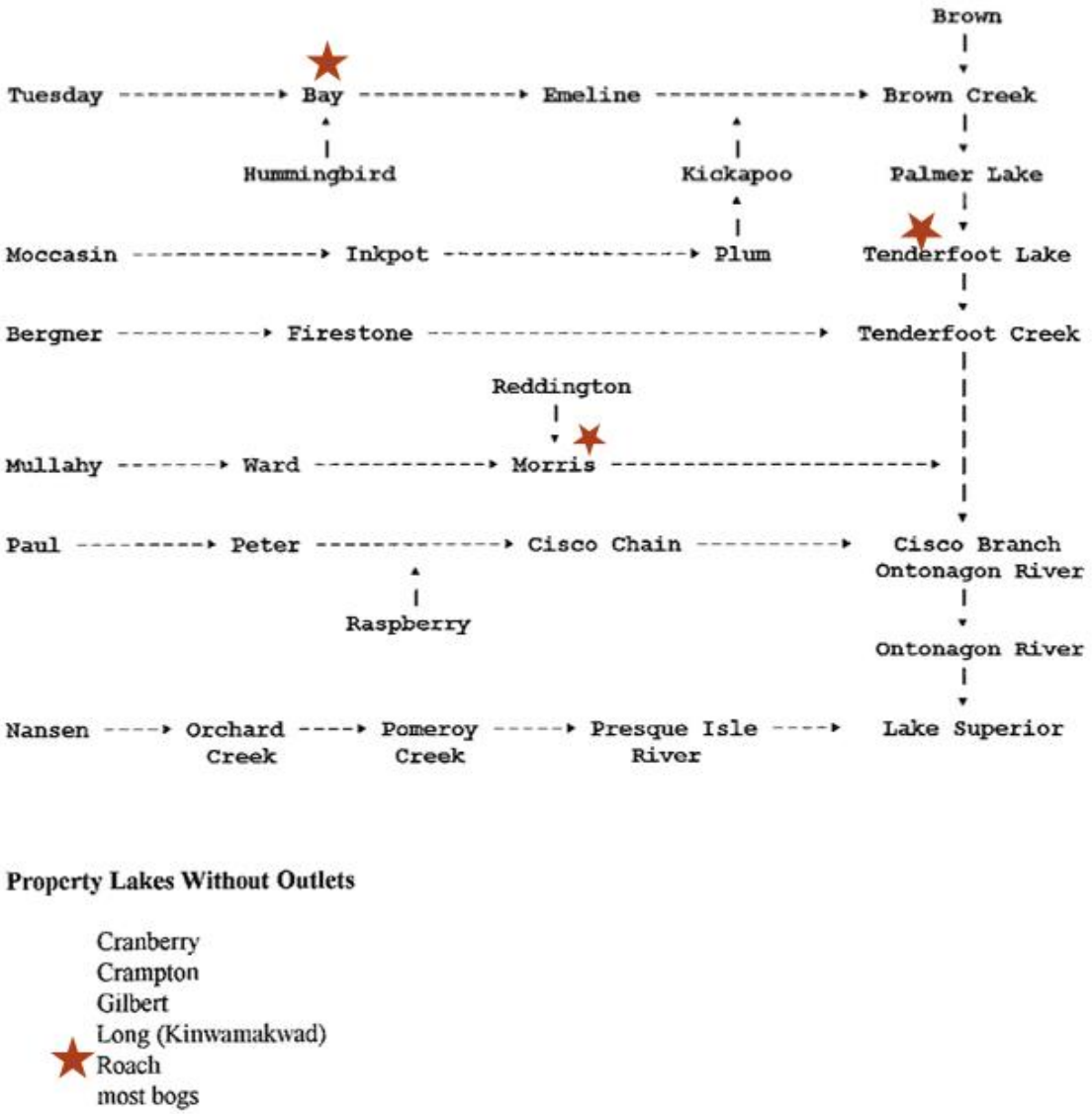

Fig. 5 Drainage Pattern of Property Lakes. Bay, Morris, and Tenderfoot are part of flowing water systems. Roach, on the other hand, does not have any outlets.