

Project Report
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In support of the
Research Proposal

**Examination of bacterial levels in water and sediment for the development of
refined monitoring protocols for inland recreational waters**

by

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Examination of bacterial levels in water and sediment for the development of refined monitoring protocols for inland recreational waters

Focus Category

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- Logan Martin Lake Protection Association volunteer monitors, Wayne Wilcox and Margaret Mize, for assisting in field sampling at Lake Logan Martin.

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Identification and Statement of the Major Regional Water Problem

The health and well-being of Alabama's citizens relative to recreational water usage depends on credible and timely monitoring of public swimming areas and other recreational waters to assess these areas for contamination with pathogens and other pollutants. *Escherichia coli* bacteria are commonly used as indicator organisms for the presence of fecal contamination and its associated pathogens in inland waters, while *Enterococci* are used in marine waters (USEPA, 2012). The State (Alabama Department of Environmental Management) routinely monitors swim areas along Alabama's coast (the Coastal Alabama Beach Monitoring Program involves the routine collection of water samples from 25 high use and/or potentially high risk public recreational sites from Perdido Bay to Dauphin Island, for details see <http://adem.alabama.gov/programs/coastal>). Inland swimming and recreational-use areas are not routinely monitored by the State. With increasing pressures on these inland waters from urban development, industrial needs, agricultural needs and others, there is increasing risk to the public health from contaminated waters (Natural Resources Defense Council, 2013).

The Alabama Water Watch Program (AWW), based at Auburn University, has been training and certifying volunteer citizen monitors in Bacteriological Monitoring since 1996, and attained EPA approval on its bacteriological monitoring protocols in 1999. AWW monitors have been monitoring waters for *E. coli* contamination using AWW's Coliscan Easygel method, and have compiled over 14,300 data records from over 2,000 sample sites throughout the state. Recent citizen monitoring efforts at public swimming areas have suggested significant differences in

E. coli concentrations measured at the same site at different times of the day. Side-by-side monitoring by citizen monitors, agency personnel and private laboratory personnel have also yielded differences. Recent research supports these citizen monitoring results. Research results and citizen data throw into question the adequacy of monitoring public swim areas only once a day. And if sampled once a day, what time of day would be most protective of public health. They also throw into question the source or sources of *E. coli* – emanating from the gut of warm-blooded animals, or also emanating from sources living out in the environment, such as in beach/lake/stream sediments.

Related Research

Recent research supports observed AWW citizen monitoring results indicating significant diurnal differences in bacterial contamination at public swimming areas that appear to be emanating from bacterial reservoirs harbored in sand/sediments underlying these areas.

Jamieson et al. (2005) found that the association of microorganisms with sediment particles is one of the primary complicating factors in assessing microbial fate in aquatic systems. They employed an experimental procedure, involving the use of a tracer-bacteria, to simulate the transport and deposition of bacteria-laden bed sediments in a small alluvial stream during steady flow conditions. The experimental data and a mathematical model were used to determine dispersion coefficients, deposition rates, and partitioning coefficients for sediment-associated bacteria in two natural streams.

Garzio-Hadzick, et al. (2010) found that in agricultural watersheds that were studied, substantial numbers of *E. coli* may reach surface waters, and subsequently be deposited into sediments, along with fecal material in runoff from land-applied manures, grazing lands, and/or wildlife excreta; and *E. coli* survived in sediments much longer than in the overlaying water.

Piorkowski et al. (2013) found that *E. coli* concentrations in streambed sediments were significantly different among monitoring sites during baseflow; significant interactive effects occurred among monitoring sites and morphological features following stormflow; and *E. coli* can persist in streambed sediments and influence water quality monitoring programs through their resuspension into overlying waters.

Ikonen et al. (2013) found significant differences in *E. coli* concentrations measured at the same site at different times of the day; and that *E. coli* levels in the water directly correlated with activity in the water, UV absorbance and turbidity.

Statement of the Results, Benefits, and Information

Expected project results include the following:

- Evaluation and quantitative estimates of bacterial contamination (*E. coli*, *Salmonella*) at swimming/public use areas at various times of the day throughout the recreational season (April-September) at two major reservoirs in Alabama, lakes Martin and Logan Martin.
- Evaluation and quantitative estimates of bacterial contamination (*E. coli*, *Salmonella*) in the sediment of these swimming/public use areas at various times of the day throughout the recreational season.

- Evaluation of coincident water column sampling by AWW volunteer monitors and state agency personnel.
- Examination of relationships among project parameters including water column bacterial concentrations, sediment bacterial concentrations, time of day and human activity levels.

Expected project benefits and information include the following:

- Development of recommendations for swim beach/recreation area monitoring protocols that are most protective of human health will be developed.
- Dissemination of project results, conclusions and recommendations to AWW volunteer monitors throughout the state and to state agencies involved in monitoring public waters.
- Improved monitoring of public swimming and recreational-use areas in inland waters based on the results and recommendations of this project.

Nature, Scope, and Objectives of the Research

AWW bacteriological monitoring data have been used by municipalities, county agencies, universities, private companies and state agencies for detecting, sourcing and solving bacterial contamination in inland surface waters. Recent citizen monitoring efforts at public swimming areas have suggested significant differences in *E. coli* concentrations measured at the same site at different times of the day. Side-by-side monitoring by citizen monitors, agency personnel and private laboratory personnel have also yielded difference results. Recent research supports these citizen monitoring results (see Related Research).

The research proposed in this project is aimed at determining the temporal and spatial distribution of bacterial contamination at public swimming areas in three recreational-use areas at lakes Logan Martin and Martin, as well as developing recommendations that best protect the public health for swim-area monitoring protocols for inland waters.

The objectives of this project are to:

1. sample multiple public swimming/recreational-use areas for bacterial contamination on two major reservoirs, one in the Tallapoosa Basin (Lake Martin) and one in the Coosa Basin (Lake Logan Martin) throughout the outdoor recreational season;
2. sample these same sites multiple times during the same day (morning, midday-afternoon) to evaluate temporal differences within the same day to examine the relationship between sediment *E. coli* counts and the surface water *E. coli* counts;
3. sample swim beach sediments to test for the presence of *E. coli*, and to test for other fecal bacteria (*Salmonella*);
4. conduct additional side-by-side AWW volunteer monitor bacteriological testing and agency testing, and;
5. test for sourcing of *E. coli* using selective antibiotic disks on bacterial media cultures.

Methods, Procedures, and Facilities

I. Conducting Bacteriological Sampling at Public Swimming Areas

A. AU Coliscan Easygel Sampling

The Coliscan Easygel method employed by the AWW Program was used for water monitoring of *E. coli* (Alabama Water Watch, 2012). Sampling sites were at public swimming/recreation areas on lakes Martin and Logan Martin, sites that have a history of AWW citizen/state agency monitoring (Table 1; Figure 1). Two samples were taken at each sampled beach – one on the left side and one on the right side of the beach.

Table 1. Sample sites at public swimming areas at Lake Logan Martin (Site 1 at Lakeside Park at Cropwell and Site 2 at Camp Cosby near St Ives, AL) and at Lake Martin (Site 3 at Wind Creek State Park near Alexander City, AL).

AWW Site	Lake	AWW Monitoring Group	Site Location	County	Lat (N)	Lon (W)
07001021	Lake Martin	Lake Watch of Lake Martin	Wind Creek State Park swim area	Tallapoosa	32.8564	85.9251
05012028	Logan Martin	Logan Martin Lake Protection Association	Camp Cosby (YMCA camp)	Talladega	33.4425	86.2782
05012046	Logan Martin	Logan Martin Lake Protection Association	Pell City Lakeside Park swim area	Saint Clair	33.5460	86.2689

Water samples were collected using a sterile pipette to collect one ml of water that will be discharged into a *Coliscan Easygel* media bottle. Sampling was done in triplicate at each site per the AWW EPA-approved QA plan. Samples were placed in a cooler with ice to prevent bacterial replication until samples could be plated. Plating was done after transport of samples back to the AWW laboratory if this could be accomplished within three hours of sample collection. Otherwise, samples were plated in the field and incubated in a portable incubator so that they were plated within a three-hour holding time. On a given sample date, each site was sampled two times – morning and midday/afternoon.

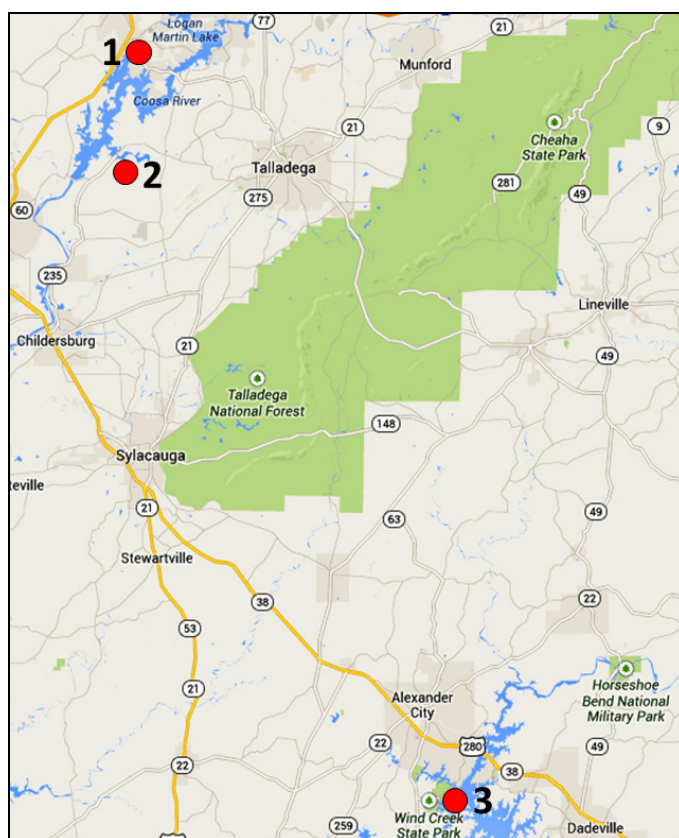


Figure 1. Sample sites on lakes Logan Martin and Martin.

B. Enumeration of *E. coli* and *Salmonella*

To correlate the AWW Coliscan Easygel method with FDA microbial enumeration methods, 50 ml of water sample or 25 g of sediment sample was collected using sterile disposable long-handled dippers at each sampling site each time (USFDA 2013). The samples were kept on ice and delivered to Wang's microbiology lab located on Auburn campus. Each water sample was vortexed for 2 min before six milliliters of water was picked and plated on to two 3M™ Petrifilm™ *E.coli*/Coliform count plate, two MacConkey agar and two XLT4 agar (1ml for each plate). After plating, 25 ml of the same water sample got enriched with 225 ml of lactose broth. Those enriched samples were plated only if on colony was found from the Petrifilm or the MacConkey or the XLT4 plate. For sediment samples, 100 ml of autoclaved MilliQ water was added to each 25 g sediment sample, and the samples were homogenized for 2 min before plating. The same plating procedure was followed as described above for water samples. Plates were incubated at 37°C for 24 hours before enumeration. Once suspected pathogenic *E. coli* and *Salmonella* isolates were found, they were sent to Iowa State University for serotyping.

C. Antibiotic Resistant Analyses

To understand the potential antibiotic resistant properties the isolates may have, one hundred microliters of each confirmed *E. coli* or *Salmonella* isolate was plated onto Mueller Hinton plates. The plates were divided into four quarters and appropriate antibiotic disks were placed on the surface of each quarter (Table 2). Plates were then incubated at 37°C for 18 hours and the resistant zone was measured.

Table 2. Antibiotic disks used in the antibiotic resistant tests.

Class	Antibiotics	Disk concentration
Penicillin	Amoxicillin-clavulanic acid	20µg
Penicillin	Ampicillin	10 µg
Cephalosporin	Ceftriaxone	30µg
Cephalosporin	Cephalothin	30 µg
Phenicol	Chloramphenicol	30 µg
Quinolone & Fluoroquinolones	Ciprofloxacin	5 µg
Cephalosporin	Cefoxitin	30 µg
Cephalosporin	Ceftiofur	30 µg
Aminoglycoside	Gentamicin	10 µg
Quinolone & Fluoroquinolones	Naladixic acid	30 µg
Sulfonamide	Sulfamethoxazole/Trimethoprim	23.75/1.25 µg
Tetracycline	Tetracycline	30 µg

D. AWW Citizen Monitoring

AWW-certified citizen monitors conducted concurrent monitoring during the project. AWW Program personnel oriented citizen monitors in the project goals, protocols and QA/QC plan prior to field data collection. Lake Watch of Lake Martin and Logan Martin Lake Protection Association volunteer monitors that had been trained and certified in AWW's Bacteriological Monitoring protocols sampled alongside AU researchers. It was not possible to coordinate with Alabama Department of Environmental Management personnel to conduct coincident sampling

at select sites on the same date at the same time, but coincident sampling was coordinated with the City of Pell City personnel when possible.

II. Analysis of Bacterial Sampling Results

Concentrations of *E. coli* measured in the water column was interpreted relative to human health by comparison with USEPA and Alabama Department of Environmental Management water quality criteria (USEPA 2012; ADEM 2012). Water column and sediment bacteria (*E. coli*, *Salmonella*) data was compiled and analyzed for significant differences relative to time of day, testing procedure, and level of human/animal activity using SAS/STAT Software (Schlotzhauer and Little 1991). Bacteria data was analyzed to examine relationships/correlations among water column bacterial concentrations, sediment bacterial concentrations, time of day and human activity levels.

III. Facilities

Project bacteriological analyses were conducted in the Alabama Water Watch Watershed Stewardship Laboratory in the Center for Advanced Science, Innovation and Commerce located in the AU Research Park, and Dr. Wang's laboratory in the Animal Sciences Building on the main AU campus.

Results

Salmonella Monitoring:

Twenty-five g of each sediment sample was weighed and 25 ml of each surface water sample was measured out. Samples were enriched in 225 ml 1× Lactose broth for 24 hours and the enriched broth was streaked onto XLT4 agar and incubated at 37°C for an additional 24 hours. Suspect *Salmonella* colonies were checked the next day. *Salmonella* tests were done for all of the 24 samples collected on May 1st 2014. However, no suspect *Salmonella* colony was seen on XLT4 plates. *Salmonella* was not found in the water or the sediment samples, therefore the research team decided to discontinue the *Salmonella* testing starting from the second sampling time (June 4th 2014).

One phenomenon that is worth mentioning is that on June 4th 2014, a Canada goose fecal sample was collected from Wind Creek. This sample was enriched and plated, suspect *Salmonella* colonies were seen on the selective agar from this particular sample. According to the literature, wild animals can carry *Salmonella* at a level of approximately 10% (Silva-Hidalgo et al., 2013), thus retrieving *Salmonella* from goose feces was not unusual. However, *Salmonella* was not detected in the water. Our hypothesis is that wild animals can have *Salmonella* in their feces; however, if *Salmonella* is present in low concentrations, it will not survive long in the environment (water) and will not pose a human health risk after it is outside of the animal's body. Of note was the extremely high count of *E. coli* bacteria in the fecal sample, indicating that Canada geese can be a significant contributor to *E. coli* contamination of surface waters.

Escherichia coli Monitoring:

A. Standard Methods Monitoring – Temporal Comparison

A total of six sampling trips were made from May 2014 to October 2014. Three sampling sites were chosen at the beginning, one was dropped after August due to issues with access. Lake Martin and Lake Logan Martin are the two lakes from which data will be presented in this report. As shown in Figure 2 (A, below), season (in other words, temperature, rain fall, etc.) played significant role in *E. coli* concentrations in surface water. The *E. coli* concentrations of Lake Martin in the afternoons were not significantly different from the morning samples (T-test, $P > 0.05$). For Lake Logan Martin, from the month of May to August, the concentrations of *E. coli* in the afternoon samples were significantly higher than the morning samples. In September and October, the morning samples contained higher concentrations of *E. coli* than the afternoon samples. However, if all of the *E. coli* samples from all six sampling months are analyzed collectively, there is no significant difference between morning and afternoon *E. coli* concentrations (T-test, $P > 0.05$). The occasional differences seen in *E. coli* counts in Lake Logan Martin between morning and afternoon samples might be caused by disturbance of sediments from swimming and other beach activities.

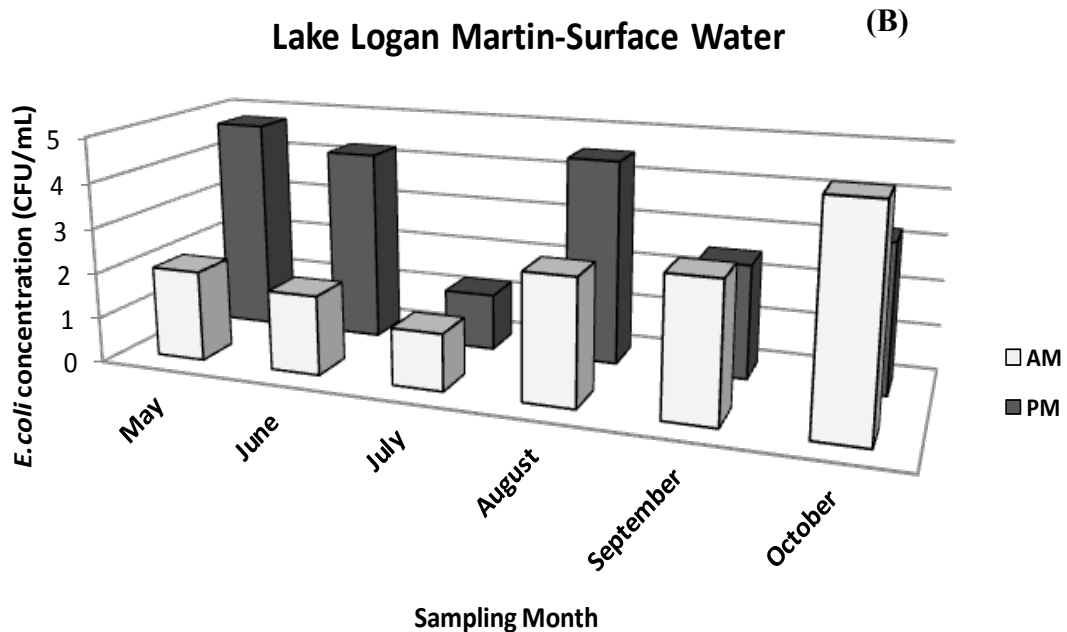
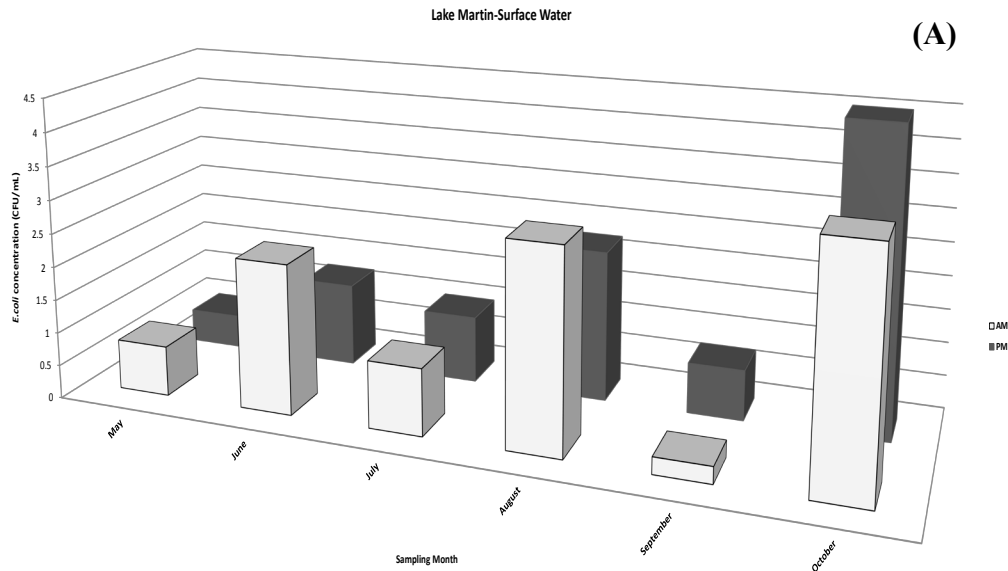


Figure 2. Concentrations of *E. coli* in surface water samples collected from Lake Martin (graph A) and Lake Logan Martin (graph B). Two sampling times were conducted for each trip, one in the morning when human activities were minimal and one in the afternoon when most activities occurred.

B. Standard Methods – Comparison of *E. coli* Concentrations in Water versus Sediment
 As shown in Figure 3 (below), regardless of the sampling month or the lake, *E. coli* concentrations were always significantly higher in the sediments samples (T-test, $P < 0.05$). This phenomenon may help to explain the *E. coli* surface water concentration differences happening

between morning and afternoon samples. Swimming and other activities can resuspend some of the *E. coli* cells from the sediment into the surface water.

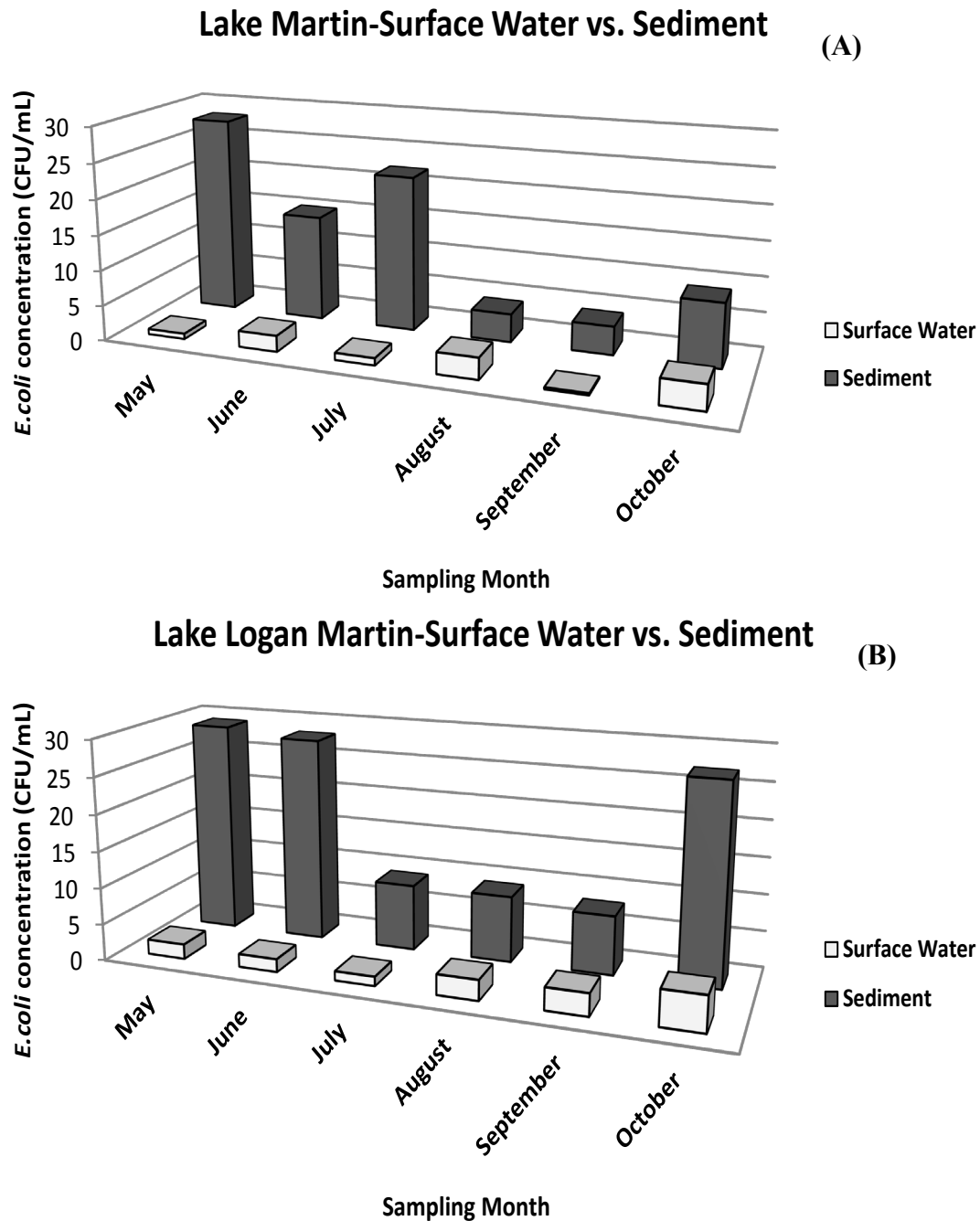


Figure 3. Concentrations of *E. coli* in surface water vs. sediment from Lake Martin (graph A) and Lake Logan Martin (graph B) (sediments were sampled during morning sampling only).

C. AWW Methods Results of AU Personnel versus AWW Volunteers

AWW staffers (AU Water Resources Center personnel) sampled side-by-side AWW volunteer monitors of Lake Watch of Lake Martin at the Wind Creek sample site and AWW volunteer monitors of Logan Martin Lake Protection Association at the Lake Side and Camp Cosby sites. All volunteer monitors were certified by AWW for bacteriological monitoring. Both AU personnel and AWW volunteer monitors followed AWW's EPA-approved bacteriological monitoring protocol, using Coliscan Easygel media and petri dishes.

Results are shown below (Figure 4). Side-by-side results were analyzed using T-tests. In all cases at all sites, AU and AWW volunteer monitor results of *E. coli* levels in surface waters at the three swim areas were not significantly different samples (T-test, $P > 0.05$), except for a single instance at Camp Cosby (on 7/8/2014: AU *E. coli* count per petri dish = 0,0,0,0,0,0; AWW *E. coli* count per petri dish = 0,1,0,1,1,1). Thus, out of 16 side-by-side sample events, AU and AWW results of *E. coli* levels were not significantly different 15 of 16 times, or 94% of the time.

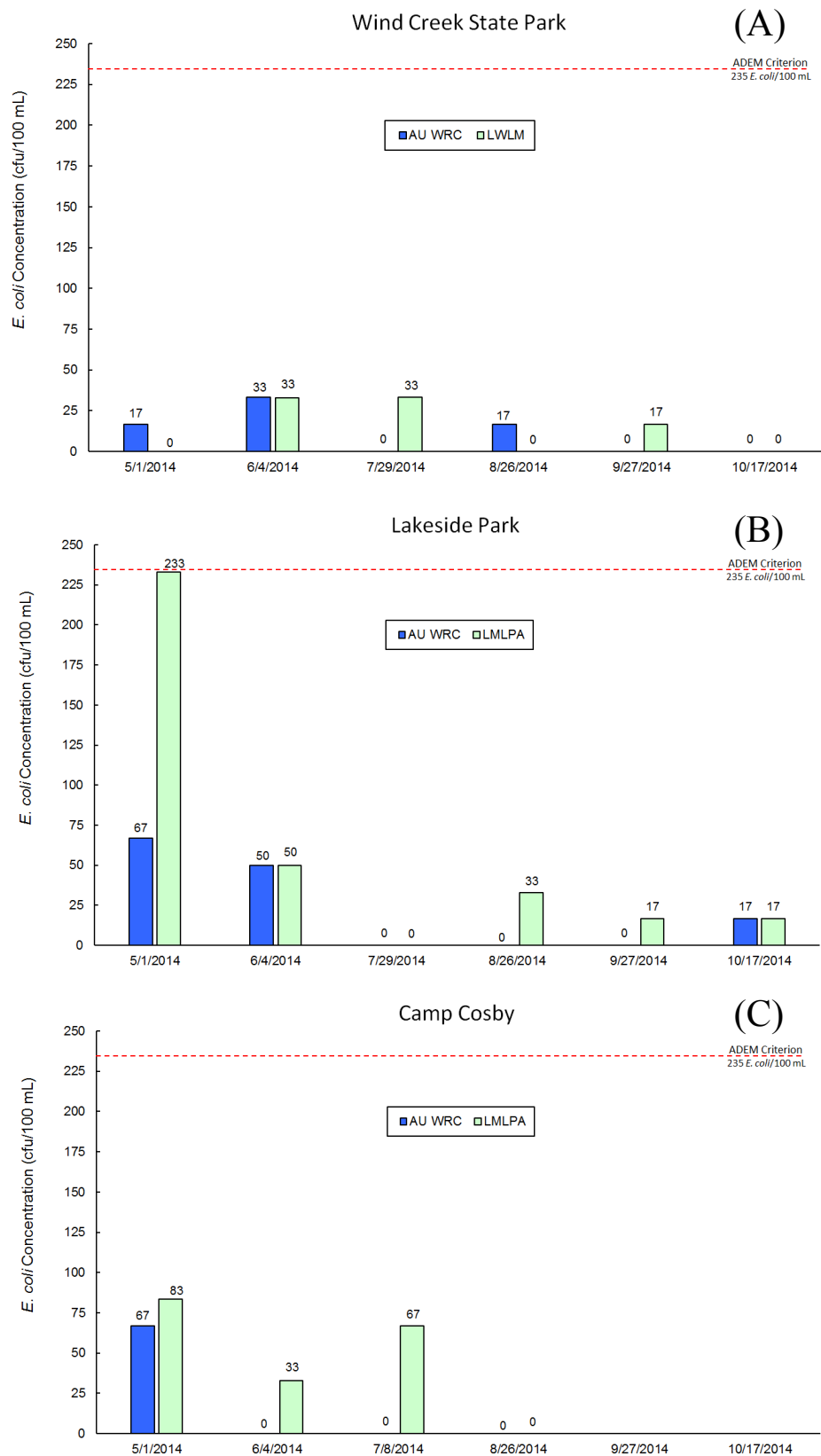


Figure 4. Concentrations of *E. coli* in surface water from Lake Martin (graph A) and Lake Logan Martin (graphs B, C) measured by AU personnel (blue bars) and AWW volunteer monitors (green bars).

D. Comparison of AU, AWW Volunteer and Pell City Results:

The City of Pell City sampled side-by-side the AU personnel and LMLPA volunteer monitors on three different sample dates in May, June and July. The City conducts routine bacteriological monitoring on a weekly basis at the Lakeside Park swimming area in collaboration with Logan Martin Lake Protection Association. Water samples are delivered to a private laboratory for analyses. Results are presented below (Figure 5).

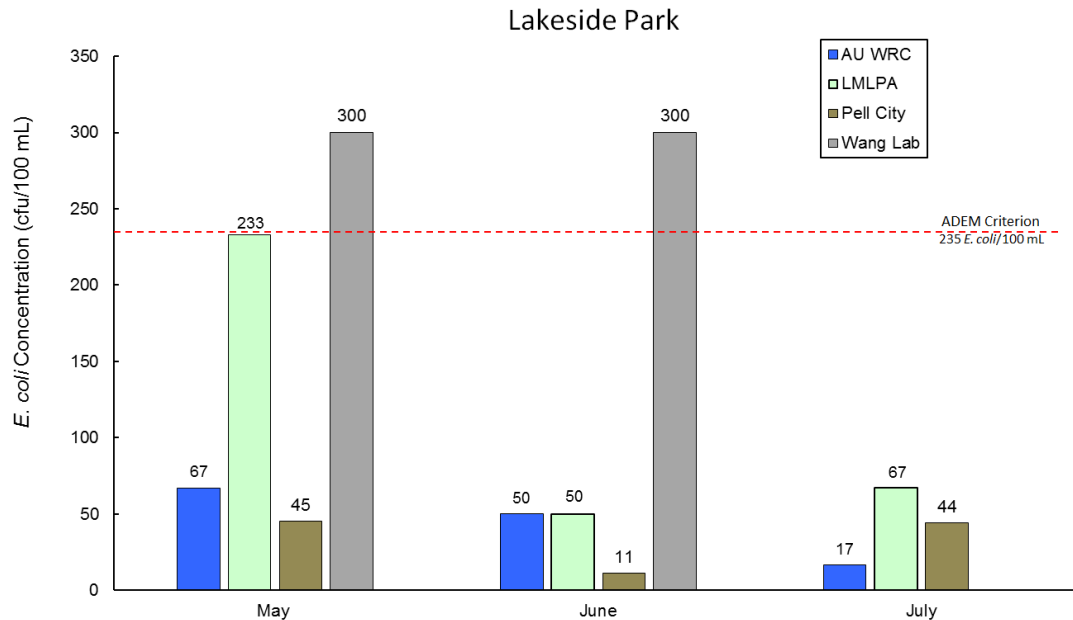


Figure 5. Concentrations of *E. coli* in surface water from Lake Logan Martin (graph B) measured by AU personnel (blue bars), Logan Martin Lake Protection Association volunteer monitors (green bars), Pell City (brown bars) and Wang lab (grey bars).

Results indicate that:

1. Even with side-by-side sampling, bacterial concentrations are not evenly distributed in the sampled environment (lake water), in other words, there is significant variability among samples even on the same date and time.
2. In general, lakeside plating yielded lower *E. coli* concentrations.
3. Agreement was best between AU Water Resources sampling and City of Pell City sampling (mean of three events was 44.5 cfu/100 ml and 33.3 cfu/100 ml, respectively).

E. Correlation between *E. coli* Concentration and Number of Waterfowl Present:

Researchers conducted visual observations and counts of the number of waterfowl present at each sampling location each date-and-time the location was sampled. Enumeration of waterfowl along with *E. coli* concentrations (mean of left-beach and right-beach sample taken by AU Water Resources personnel) are presented below (Table 3.)

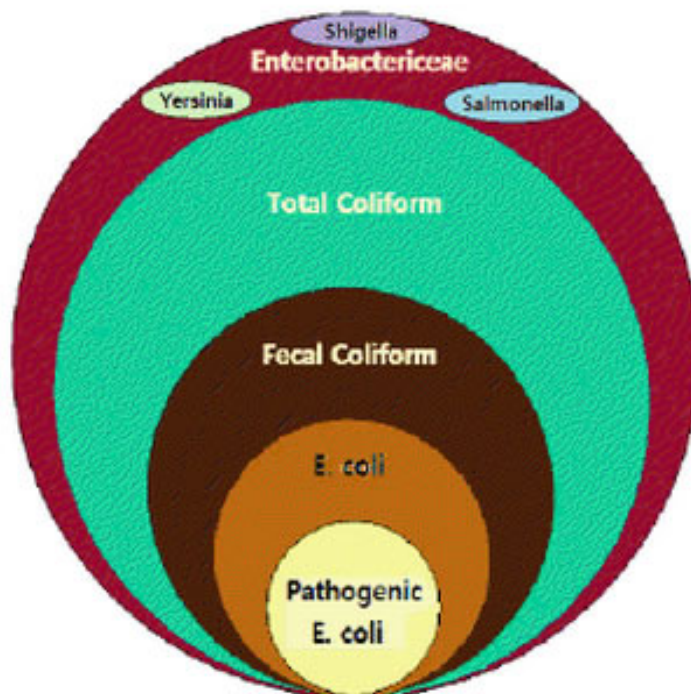
Table 3. Number of waterfowl present at sample sites and associated *E. coli* concentration in the surface water.

Location	Date	Time	N	<i>E.coli</i>	Fowl
Camp Cosby	05/01/14	After	2	150.0	0
Camp Cosby	05/01/14	Morn	2	66.5	0
Camp Cosby	06/04/14	After	2	0.0	0
Camp Cosby	06/04/14	Morn	2	16.5	0
Camp Cosby	07/08/14	After	2	0.0	0
Camp Cosby	07/08/14	Morn	2	0.0	0
Camp Cosby	08/26/14	After	2	0.0	0
Camp Cosby	08/26/14	Morn	2	0.0	0
Lakeside	05/01/14	After	2	100.0	0
Lakeside	05/01/14	Morn	2	66.5	0
Lakeside	06/04/14	After	2	50.0	10
Lakeside	06/04/14	Morn	2	50.0	2
Lakeside	07/29/14	After	2	16.5	0
Lakeside	07/29/14	Morn	2	0.0	0
Lakeside	08/26/14	After	2	67.0	0
Lakeside	08/26/14	Morn	2	0.0	0
Lakeside	09/27/14	After	2	0.0	0
Lakeside	09/27/14	Morn	2	0.0	3
Lakeside	10/17/14	After	2	16.5	0
Lakeside	10/17/14	Morn	2	16.5	0
Wind Creek	05/01/14	After	2	16.5	18
Wind Creek	05/01/14	Morn	2	0.0	.
Wind Creek	06/04/14	After	2	33.0	40
Wind Creek	06/04/14	Morn	2	100.0	30
Wind Creek	07/29/14	After	2	0.0	0
Wind Creek	07/29/14	Morn	2	0.0	0
Wind Creek	08/26/14	After	2	16.5	0
Wind Creek	08/26/14	Morn	2	16.5	0
Wind Creek	09/27/14	After	2	0.0	0
Wind Creek	09/27/14	Morn	2	0.0	3
Wind Creek	10/17/14	After	2	0.0	0
Wind Creek	10/17/14	Morn	2	0.0	0

Correlation analyses revealed that there was no significant correlation between *E. coli* concentrations in the surface water and number of waterfowl observed at a sample site ($P > 0.05$).

Enterobacteriaceae Monitoring:

The family Enterobacteriaceae encompasses approximately 20 genera, including *E. coli* and all members of the coliform group; as well as foodborne pathogens *Salmonella*, *Shigella*, and *Yersinia*. The family was originally proposed as an indicator alternative to the coliform group because testing for the entire family would be more inclusive for the pathogenic bacteria. The Enterobacteriaceae may be superior to coliforms as indicators of sanitation GMPs because they have collectively greater resistance to the environment than the coliforms. This group is more widely used as indicators in Europe than in the United States (see www.mybiolumix.com/the-debate-coliforms-fecal-coliforms-and-enterobacteriaceae-as-indicator-organisms).



The protocol of preparing, plating, and enumerating Enterobacteriaceae is the same with *E. coli*, except 3M Petrifilm™ Enterobacteriaceae plates were used instead of 3M Petrifilm™ *E. coli* plates.

As shown in Figure 6(A) below, no significant difference was seen between the morning and afternoon samples collected in Lake Martin (T-test, $P > 0.05$). For Lake Logan Martin (graph B), the afternoon samples contained significantly higher numbers of Enterobacteriaceae than the morning samples for May and August, while the morning samples contained slightly higher numbers of Enterobacteriaceae than the afternoon samples for September and October.

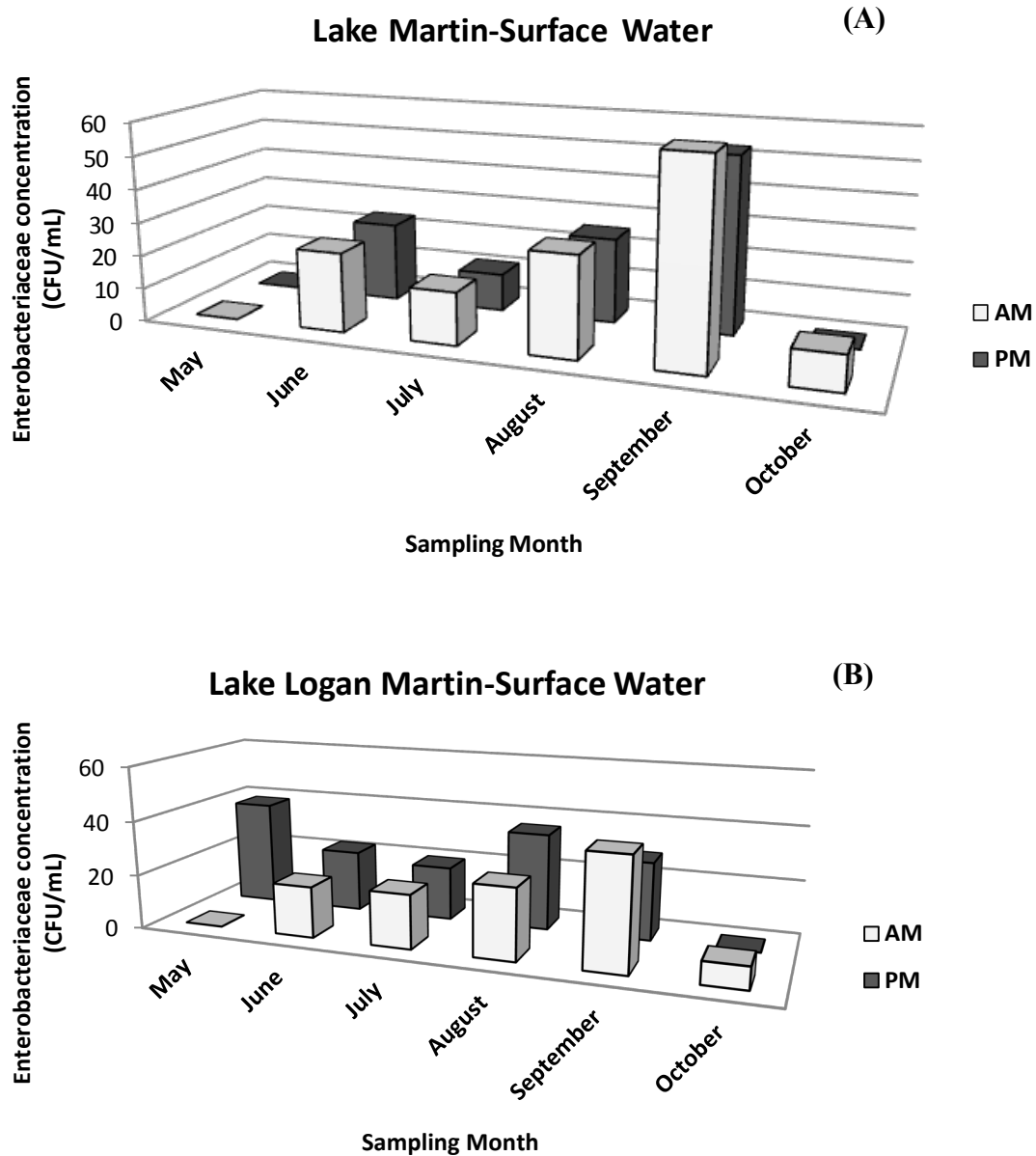


Figure 6. Concentrations of Enterobacteriaceae in surface water samples collected from Lake Martin (graph A) and Lake Logan Martin (graph B) in the morning vs. in the afternoon.

As shown in Figure 7 below, regardless of the sampling month or the lake, concentrations of Enterobacteriaceae were significantly higher in the sediments compared to the surface water (T-test, $P < 0.05$). In addition, the differences between surface water vs. sediments in Lake Martin were greater compared to the differences existed between surface water vs. sediment in Lake Logan Martin. In Lake Martin we saw several geese, and some also at Logan Martin, and collected one goose fecal sample that tested positive for *Salmonella*. Enterobacteriaceae can

indicate the potential presence of *Salmonella* and *Shigella*, thus, higher Enterobacteriaceae may correlate to a higher probability of *Salmonella* in the water, though we did not detect any *Salmonella* in water samples that we tested.

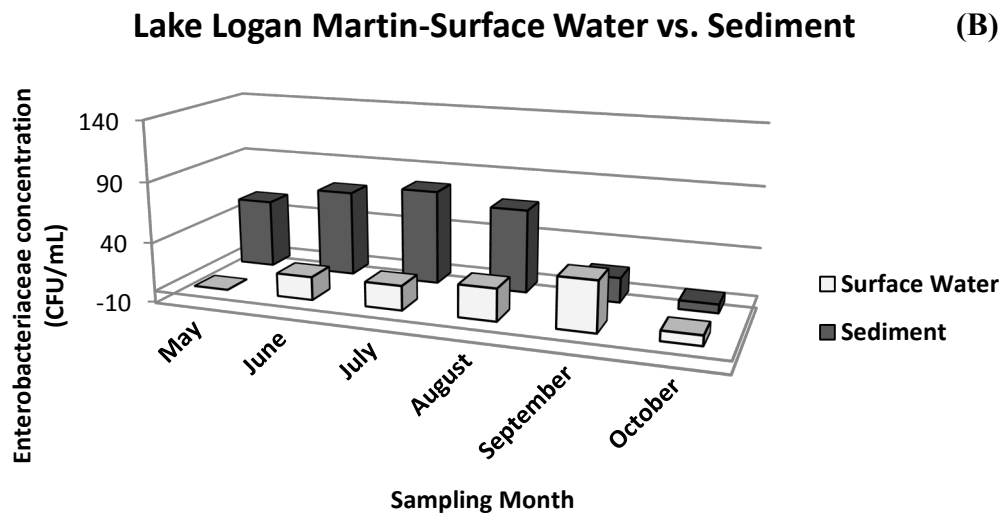
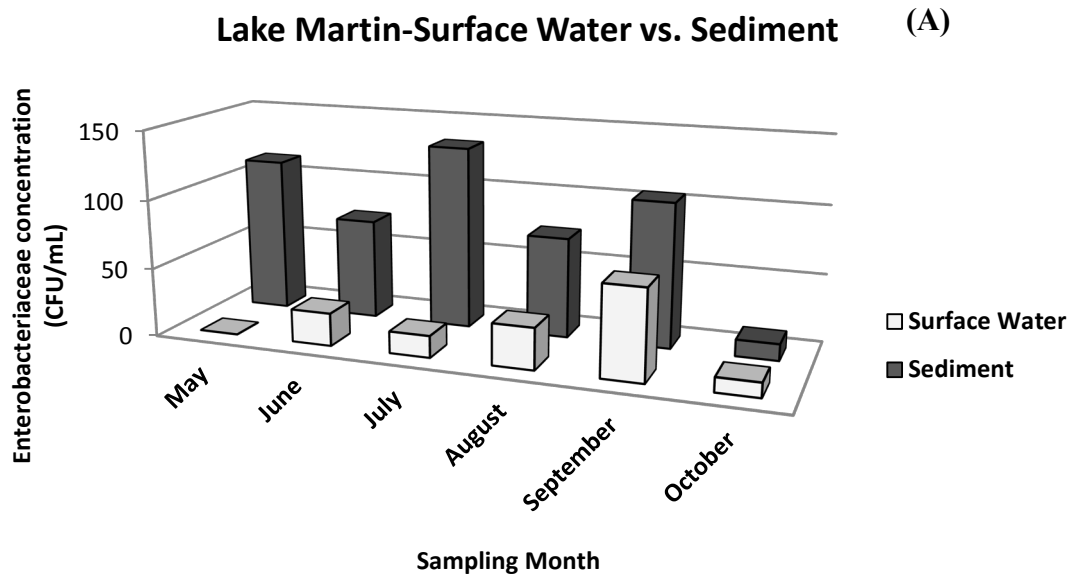


Figure 7. Comparison in Enterobacteriaceae concentrations between surface water and sediments collected from Lake Martin (graph A) and Lake Logan Martin (graph B).

Sampling Technique Comparison:

Due to the differences we saw between the EasyGel method results and the Petrifilm method results, we proposed that sampling technique differences could be one of the reasons that caused discrepancies in test results. To test this hypothesis, parallel comparisons were conducted. The student collected two 1-ml surface water samples from the lake and directly plated on Petrifilm plates on site and incubated them in the lab. The student also collected water samples ~50 ml or ~50 g of sediments and conducted the preparation and plating after she came back to the lab. Results from the Alabama Water Watch method, referred to as ‘Lakeside’ plating, while the *Standard Methods* method, referred to as ‘In-lab’ plating are presented in Figure 8 below.

As shown in Figure 8, regardless of the sampling month, sampling time (AM vs. PM), or sampling site, the in-lab plating yielded relatively higher counts compared to lakeside plating. One explanation is that in-lab plating deals with larger sample sizes and the vortex step gave a more even distribution of the *E. coli* in that 1 ml plated sample. Another possible explanation is that the in-lab plating allowed possible reproduction of bacterial cells in transit because of the longer holding time of media before plating. Nonetheless, based on statistical analysis, no significant difference was detected between these two different sampling methods with regards to *E. coli* counts obtained ($P > 0.05$).

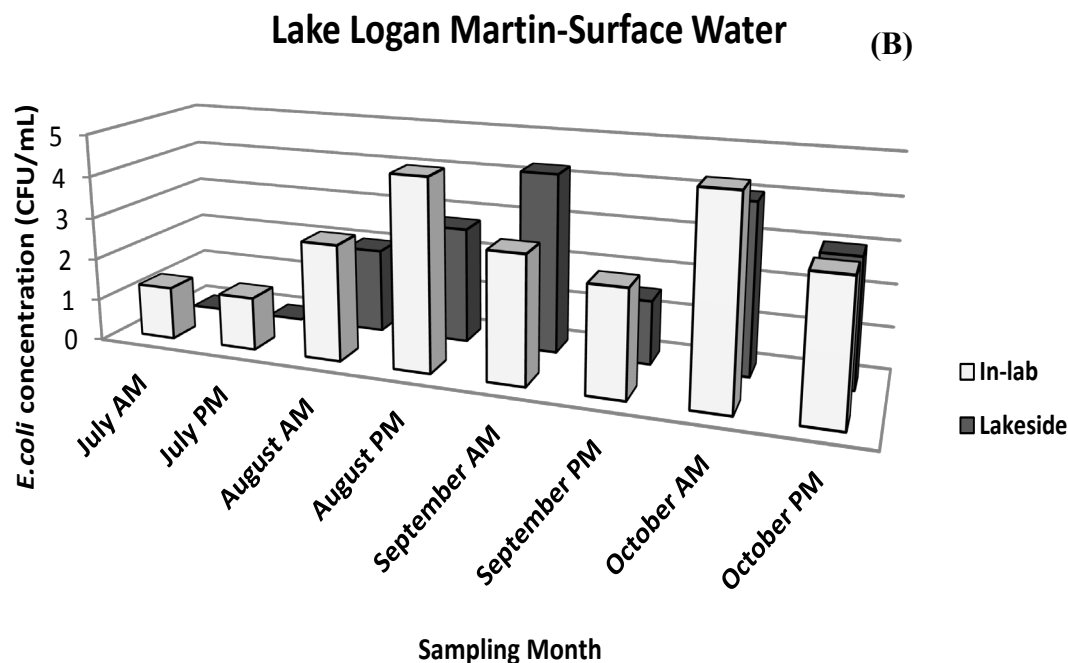
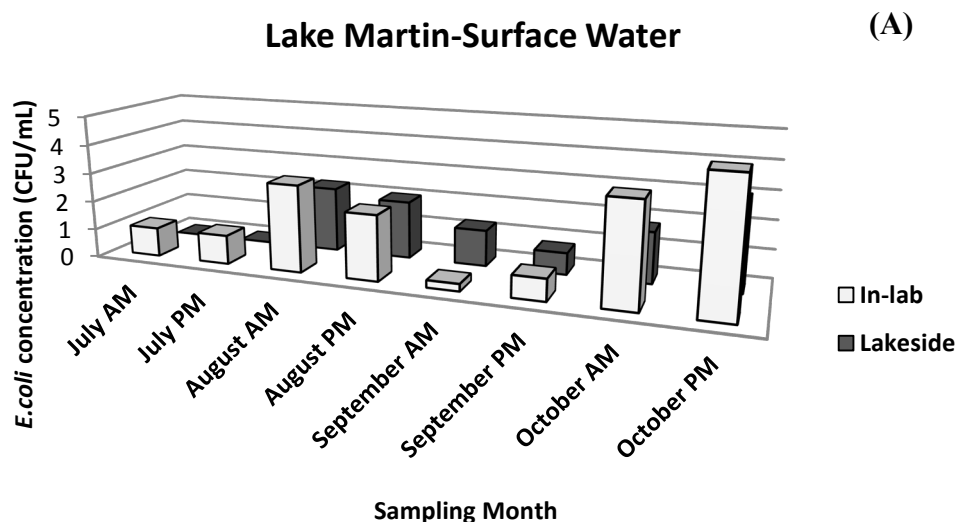


Figure 8. Comparison of lakeside plating vs. in-lab plating (*E. coli* concentrations).

As shown in Figure 9 below, with higher Enterobacteriaceae concentrations (relative to *E. coli* concentrations), it is more evident that the in-lab plating gave higher counts than the lakeside plating method. Results of the statistical analysis indicated that the two sampling methods for Enterobacteriaceae yielded significantly different counts ($P < 0.05$).

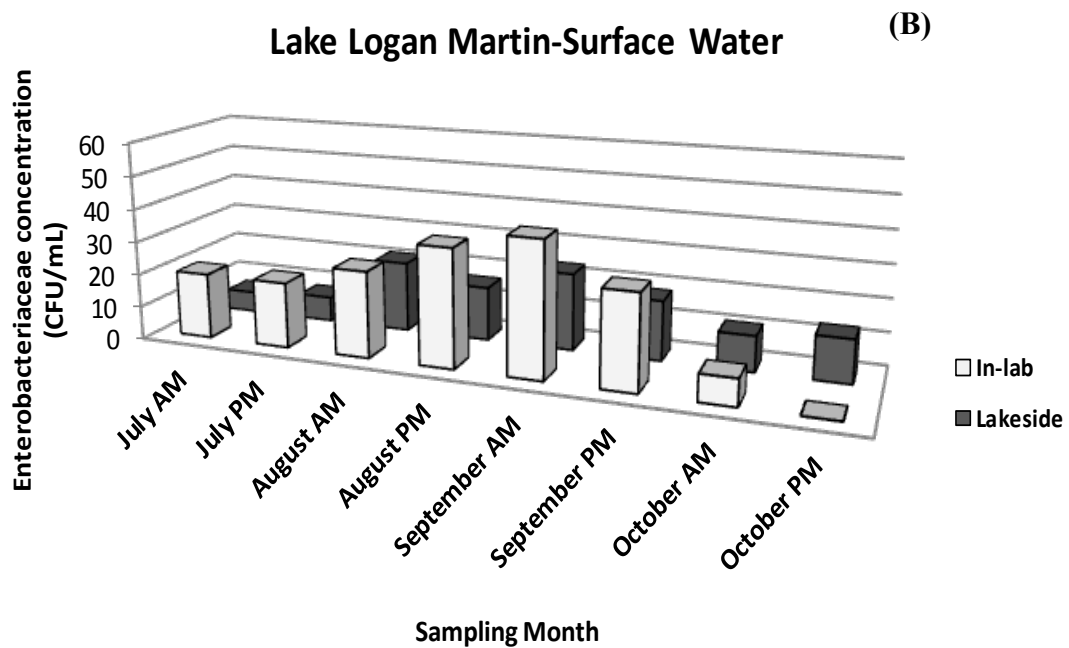
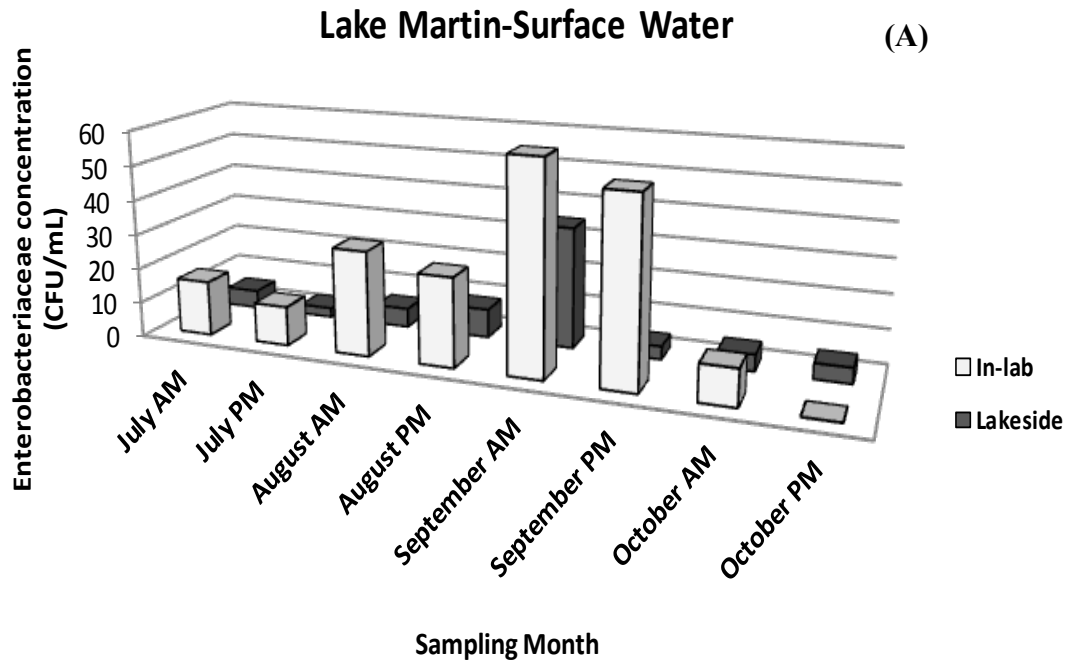


Figure 9. Concentrations of Enterobacteriaceae using different plating methods (Enterobacteriaceae).

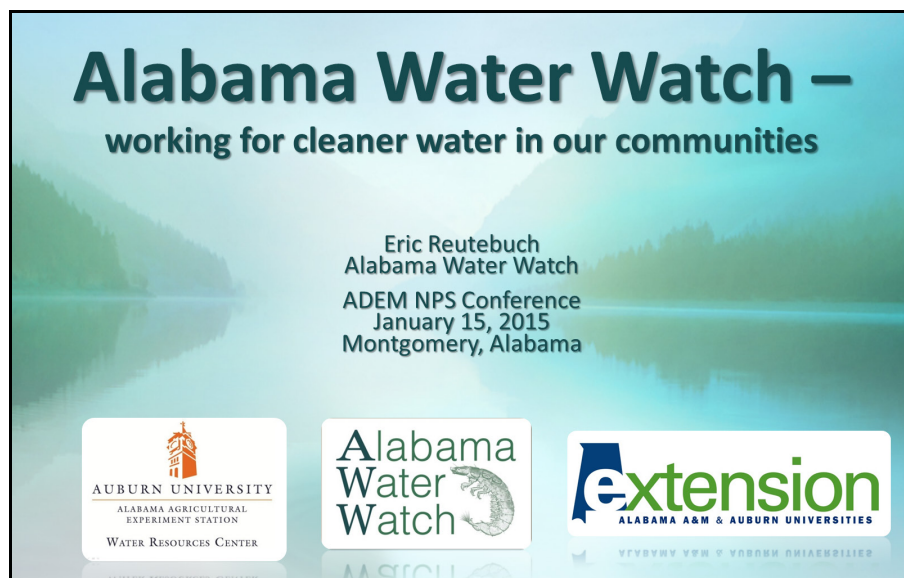
Outreach

Project results were communicated to Wind Creek State Park officials at a meeting at the park, and in an article published by Tallapoosa Publishing Company in the January 2015 issue of Lake Magazine (see Appendix).

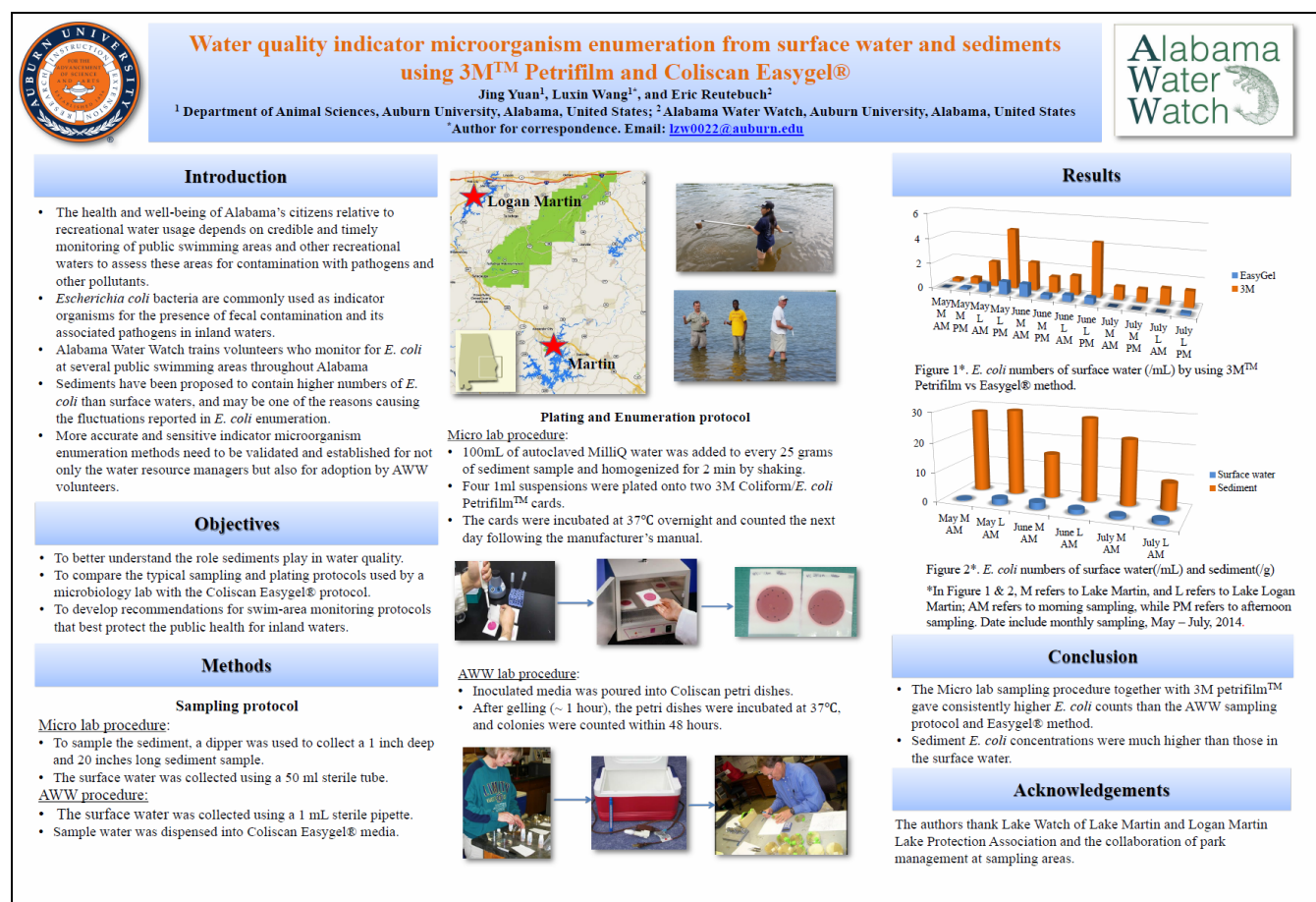
Project results (as of 7/2014) were communicated to the Logan Martin Lake Protection Association during a presentation at the Pell City Civic Center on July 17, 2014 (see below).



Project results from the Logan Martin sample site at Lakeside Park were presented at the ADEM Nonpoint Source Conference on January 15, 2015 in Montgomery, AL (see slides below).



Project results were also communicated in a poster presentation at the 2014 Alabama Water Resources Conference on September 4, 2014 in Orange Beach, AL (see poster below).



Conclusions

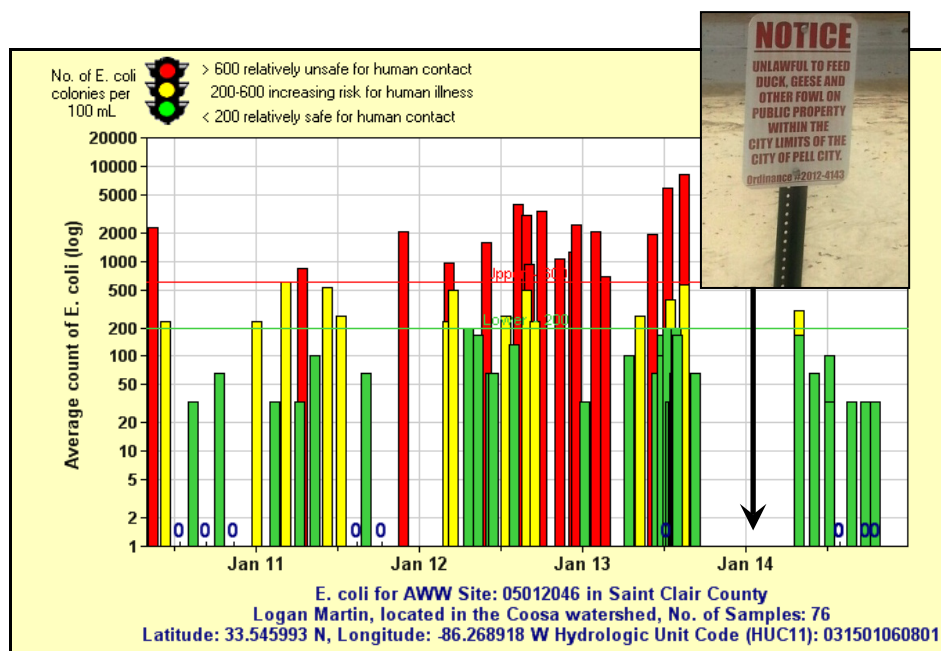
- E. coli* concentrations were sometimes significantly different when sampling the surface water at different times of the day.
- Enterobacteriaceae is present in lake water in much higher numbers than *E. coli* and has a potential to be used for lake water monitoring as it includes more pathogens like *Salmonella* and *Shigella* than coliform and *E. coli* tests.
- Both *E. coli* and Enterobacteriaceae were present in significantly higher numbers in the sediments than the surface water ($P < 0.05$).
- Although there was no significant correlation between the number of waterfowl observed at each beach and the concentration of *E. coli* present in the water ($P > 0.05$), Canada goose feces was found to test positive for *Salmonella* and high levels of *E. coli*.
- Differences were observed in the AWW *E. coli* 'lakeside' sampling and plating method, which routinely yielded lower counts, compared to the FDA microbial enumeration method (plating back in the lab); although the differences were not significant ($P > 0.05$).

Differences in the two methods were significant for Enterobacteriaceae enumeration ($P < 0.05$). Possible reasons for these differences may be:

- Replication of Enterobacteriaceae may occur during the transport back to the lab (FDA microbial enumeration method) because of longer holding time or due to the fluctuations of the internal temperature of the holding cooler,
- Increased probability of sampling Enterobacteriaceae from the environment using the standard method procedure because of the much larger sample volume used (50 ml versus 1 ml).

Recommendations

- Because of uneven distribution of bacteria in the aquatic environment, replicate sampling (preferably triplicate sampling) is recommended for bacteriological monitoring of surface waters.
- Results from Enterobacteriaceae sampling suggests that this group of bacteria (which includes Enterococcus) may be a more sensitive bacterial indicator for fecal contamination of surface waters. Further study is recommended.
- Since Canada goose feces contains both *Salmonella* and high levels of *E. coli*, avoid feeding water fowl at public swimming areas. It is recommended that signage be posted (see image below) and local ordinances be passed to restrict the feeding of water fowl at public swimming areas, as was done at the Lakeside Park swimming beach. The impact was very dramatic in curtailing *E. coli* contamination at the beach – only on one instance was the concentration of *E. coli* above 235/100 ml (on 5/1/2014 the concentration was 300 *E. coli*/100 ml), see graph below.



Source: <https://fp.auburn.edu/icaae/ddBacHistory.aspx?dg=1&GroupName=LoganMartinLakeProtectionAssociation&AwwSiteCode=05012046&ChartID=6>

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APPENDIX: Letters of Support from Collaborating AWW Water Monitoring Groups and Publication about the Project

- Letter of Support from Logan Martin Lake Protection Association
- Letter of Support from Lake Watch of Lake Martin
- Lake Magazine article (January 2015 issue)

December 17, 2013

Dr. Luxin Wang
Department of Animal Sciences
210 Upchurch Hall
Auburn University, AL 36849

Dear Dr. Wang:

I am pleased to offer this letter of support for your program's Alabama Water Resources Research Institute (WRRRI) proposal. Working with local nonprofit organizations to identify less expensive but meaningful methodologies that will assist in identifying pollutants of concern for water quality and aid in effective implementation of practices to improve water quality is a critical step in the long-term protection and restoration of Alabama waters.

Logan Martin Lake Protection Association has partnered with Alabama Water Watch on many occasions to implement outreach and research programs. Each program has been a success due to the highly qualified, technical professionals that lead your programs and the trust that has been built between Alabama Water Watch and stakeholders throughout the State of Alabama.

I have no doubt future funding of this WRRRI project will result in improved techniques for assessing and monitoring water quality in Alabama. Importantly, results from this project will serve as a template for other groups around the state and region seeking to improve water quality.

Please contact me at (205) 525-5309 or mike.riley@lmlpa.org with any questions.

Sincerely,



Mike Riley
President
Logan Martin Lake Protection Association

Cc: AWW



December 18, 2013

Dr. Luxin Wang
Department of Animal Sciences
210 Upchurch Hall
Auburn University, AL 36849

Dear Dr. Wang:

I am pleased to offer this letter of support for your proposal to conduct in-depth bacteriological monitoring at the Wind Creek State Park swimming beach in 2014. I understand this research would also include the use of volunteer monitors, including those associated with Lake Watch. As presented, this testing would monitor for *E. coli* and Salmonella, both in the water column and in the sand/sediment below the water at early morning and at mid-day/afternoon throughout the recreational season in 2014, then look at relationships between the sediment levels of bacteria and levels in the water column. Test results would allow for the development of recommendations for bacterial monitoring of inland swimming areas that are most protective of public health. These recommendations would then be disseminated to AWW volunteer monitors throughout Alabama and to state agency personnel who are involved in water quality monitoring.

Lake Watch of Lake Martin is delighted by this proposal and we are eager to participate. Recent concerns regarding *E. coli* and salmonella have been discussed by our monitors and we have been contacted regarding testing for bacteriological contaminants by local agencies. This proposal would help us develop better testing protocols and improve our volunteer water monitoring program. We fully support the project.

Please contact me at 256-825-2624 or khbraund@mac.com with any questions.

Sincerely,

A handwritten signature in black ink that reads "Kathryn H. Braund". The signature is written in a cursive, flowing style.

Dr. Kathryn H. Braund, President
Lake Watch of Lake Martin



AU graduate student Jing Yuan takes a sediment sample at Wind Creek State Park's beach



AWW Director Eric Reutebuch tests samples for E. coli bacteria

Lake Martin added to swim guide site

Extensive Auburn University study affords 100 percent rating for Wind Creek State Park Beach

STORY BY BETSY ILER

PHOTOS & GRAPH COURTESY OF AU WATER RESOURCES CENTER

Lake Martin's Wind Creek State Park Beach last month joined theswimguide.org website with a 100 percent rating as one of a handful of clean freshwater swimming beaches in the interior of Alabama. The post came after several months of vigorous chemical and bacterial water sample tests conducted through the Auburn University Alabama Water Watch (AWW) office in the AU Water Resources Center and local Lake Watch volunteers.

AWW Director Eric Reutebuch
20 LAKE

posted the results of the six-month testing program in mid-December, noting that bacterial levels remained well below harmful levels throughout the testing.

"Every time we sampled, the levels were well below the limit for E. coli," Reutebuch noted. "It was always well within the safe zone for human contact."

Levels under 200 E. coli colonies per 100 milliliters are deemed safe by AWW, with Alabama's Department of Environmental Management criteria at 235 E. coli

colonies per 100 milliliters of lake water.

The samples taken from Lake Martin were never higher than 33 colonies per 100 milliliters, Reutebuch said.

The study was funded by the U.S. Geological Society through the Water Resources Research Institute. Research was conducted by Auburn University microbiologist Dr. Luxin Wang and her graduate student Jing Yuan, along with Lake Watch Lake Martin volunteer water quality monitors. Samples were taken from

JANUARY 2015

the site two times on one day each month from May through October, and test results can be found online at www.alabamawaterwatch.org.

"Click on water data; then, summary data and bacteria and site histories," Reutebuch said.

"One of the biggest things that came out of the study was getting Lake Martin onto theswimguide site," he added. "The guide lists swimming beaches from all over the U.S., Canada and Mexico. You can click on any site on the map and get a report of the cleanliness of that beach. There's an app for it, too, so you can check it on your smart-phone."

In addition to taking water samples in the morning and afternoon, the testing team took sediment samples in the morning on testing days, as recent research has indicated there could be *E. coli* concentrations below the water, Reutebuch said. The teams dipped up the sediment on the lake floor after taking their morning samples; then, they returned to the site in the afternoon to take additional samples.

"In general, the sediment tested did have higher levels, but again

in Lake Martin it was a non-issue. Levels were still substantially lower than limits," Reutebuch reported.

One other lake in Alabama was part of the study; Logan-Martin north of Lake Martin. While some water quality issues were identified there – and preventative actions taken as a direct result – Lake Martin's bacterial counts were never a cause of concern over the course of the testing program, Reutebuch said.

Another objective of the local testing program was to check and validate the procedures and test results of Lake Watch Lake Martin, said Ann Campbell, chair of water quality monitoring at Lake Watch.

"We took samples beside the Auburn testers, and we consistently had the same results," Campbell said.

The Lake Watch program this year added six volunteer monitors and nine sites to their program. The non-profit watchdog organization now includes 18 volunteers who take monthly water samples at 23 sites around Lake Martin, including the state park beach.

Lake Watch will continue to test

the beach area and will post test results on theswimguide.org site, which also posts information of interest to visitors to the area.

Lake Watch Education Chair Dick Bronson said students in the gifted program at Alexander City's Radney Elementary School also have been sampling and testing water at the state park beach.

"This swim guide site has given them a practical application for something they have been doing in the classroom for three years. It is meaningful and useful as a public service," Bronson said.

Lake Watch hopes to add the D.A.R.E. Park beach to the program in the future, he added.

