

Isolation of Fecal Coliform Bacteria from the American Alligator (*Alligator mississippiensis*) in South Carolina

Michelle A. Johnston^{1*}, Dwayne E. Porter¹, Geoffrey I. Scott², Walter E. Rhodes³, Laura F. Webster²

¹University of South Carolina – Environmental Health Sciences, ²National Oceanic and Atmospheric Administration Center for Coastal Environmental Health and Biomolecular Research, ³South Carolina Department of Natural Resources, *Arnold School Graduate Research Fellow



Introduction to Bacterial Sources

Many of South Carolina's waterbodies do not meet the Clean Water Act "fishable and swimmable" goal due to elevated numbers of fecal coliform bacteria, which belong to the enteric family of bacteria. Various sources may contribute pathogenic bacteria to waters in the coastal environment including humans, pets, livestock, and wildlife. It is generally accepted that the main source of fecal coliform bacteria (especially *E. coli*) is unique to warm-blooded animals. Although alligators are commonly found throughout the coastal zone of South Carolina, there is little information known regarding whether or not alligators contribute to fecal contamination in waterbodies. Due to abundant numbers of alligators living in natural waterbodies and stormwater retention ponds as a result of coastal development, fecal bacterial output into the environment may be significant.

Hypothesis and Research Objectives

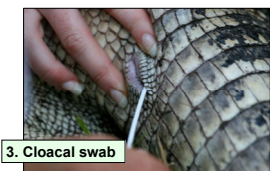
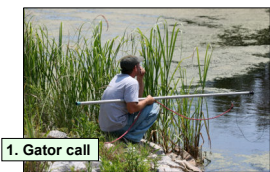
This study tested the null hypothesis that alligators do not harbor enteric bacteria, and therefore, are not a source of fecal coliform bacterial contamination in waterbodies in the South Carolina coastal zone. The objectives were to:

- Discover the predominant fecal coliform species in alligator feces,
- Assess human health risks associated with alligator fecal coliforms,
- Determine if alligator fecal coliform bacteria could contribute to water quality degradation.

Study Area and Field Sampling Techniques

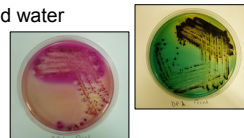


- Obtained cloacal and fecal samples from 31 American alligators residing in various waterbodies on the property of Palmetto Bluff, in Bluffton, SC.
- Collected water samples from 7 study area ponds containing alligators.
- Cloacal swab sampling process is outlined below:



Microbiological Laboratory Techniques

- Cloacal and fecal samples, as well as filtered water samples, were streaked for isolation.
- Isolates were identified using the API 20 E Identification System for Enterobacteriaceae.
- The mFC agar method was used to enumerate fecal coliforms by Colony Forming Unit (CFU) estimates.
- Bacteria from alligator fecal samples were enumerated by multiple dilution Most Probable Number (MPN) using APHA standard techniques.



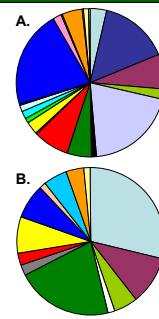
Sampled Alligator Locations and Fecal Coliforms

Waterbody	Alligators sampled (cloacal samples)	CFU/100 mL
1 – Rice Field Pond	16	153*
2 – Golf Course Pond	2	176*
3 – Golf Course Pond	3	2600*†
4 – Golf Course Pond	3	1
5 – Stormwater Pond	3	120*
6 – Stormwater Pond	3	<1
7 – Stormwater Pond	1	2

* exceeds allowable standards for shellfish harvesting (>14 CFU/100 mL)
† exceeds allowable standards for contact recreational water use (>200 CFU/100 mL)

Results: Bacteria Identification and Enumeration

- 21 species of enteric bacteria were identified from the fecal and cloacal isolates (A).
- 13 species of enteric bacteria were identified from the water samples (B).
- 10 bacteria species were common between the alligator and water isolates.
- One pond exceeded the allowable amount of fecal coliform bacteria for contact recreational waters and 4 exceeded the shellfish standards.
- The MPN for one gram of alligator fecal coliform bacteria was 8.0×10^9 .



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| <input type="checkbox"/> <i>Aeromonas hydrophila</i> group 1 | <input type="checkbox"/> <i>Enterobacter sakazakii</i> | <input type="checkbox"/> <i>Plesiomonas shigelloides</i> |
| <input type="checkbox"/> <i>Aeromonas hydrophila</i> group 2 | <input type="checkbox"/> <i>Escherichia coli</i> 1 | <input type="checkbox"/> <i>Proteus mirabilis</i> |
| <input type="checkbox"/> <i>Citrobacter braakii</i> | <input type="checkbox"/> <i>Hafnia alvei</i> 1 | <input type="checkbox"/> <i>Providencia alcalifaciens/rustigianii</i> |
| <input type="checkbox"/> <i>Citrobacter freundii</i> | <input type="checkbox"/> <i>Klebsiella pneumoniae</i> | <input type="checkbox"/> <i>Providencia rettgeri</i> |
| <input type="checkbox"/> <i>Citrobacter youngiae</i> | <input type="checkbox"/> <i>Morganella morganii</i> | <input type="checkbox"/> <i>Salmonella choleraesuis</i> ssp <i>arizonae</i> |
| <input type="checkbox"/> <i>Edwardsiella tarda</i> | <input type="checkbox"/> <i>Pantoea</i> spp 2 | <input type="checkbox"/> <i>Serratia marcescens</i> |
| <input type="checkbox"/> <i>Enterobacter aerogenes</i> | <input type="checkbox"/> <i>Pantoea</i> spp 3 | <input type="checkbox"/> <i>Vibrio cholerae</i> |
| <input type="checkbox"/> <i>Enterobacter cloacae</i> | <input type="checkbox"/> <i>Pasteurella pneumotropica</i> | <input type="checkbox"/> <i>Vibrio fluvialis</i> |

Fecal Coliform Density from Animal Sources

Species	Fecal Coliform (Density/g feces)	Source
Alligator	8.0×10^9	Current study
Duck	3.3×10^7	Harwood et al., 1999
Dog	2.3×10^7	Schueler and Holland, 2000
Human	1.3×10^7	Schueler and Holland, 2000
Turtle	1.6×10^6	Schueler and Holland, 2000
Cow	2.3×10^5	Schueler and Holland, 2000

Conclusions

- American alligators are sources of enteric bacteria.
- Evidence suggests alligators can contribute to water quality degradation, however, further research using bacterial source tracking is recommended to better confirm the observations.
- The fact that cold-blooded animals excrete fecal coliform bacteria is significant because alligators live in habitats where these bacteria may impact humans.
- Some of the bacteria isolated (i.e. *E. coli* and *Salmonella*) are public health concerns and should be further investigated.
- The ability to distinguish between human and animal sources of fecal contamination is an important public health assessment tool as fecal contamination from human sources may pose different risks than bacteria from animal sources.
- From a water quality perspective, the ability to narrow the source of fecal contamination among the many potential sources will facilitate more tailored and cost effective pollution abatement efforts, such as proper BMPs and measuring risk.

References

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- Harwood, V. J., J. Butler, D. Parrish, and V. Wagner. 1999. Isolation of Fecal Coliform Bacteria from the Diamondback Terrapin (*Malaclemys terrapin centrata*). Applied and Environmental Microbiology 65:865-870.
- Schueler, T. R., and H. K. Holland. 2000. Microbes in Urban Watersheds: Concentrations, Sources, and Pathways, p. 74-84, Watershed Protection Techniques. Center for Watershed Protection, Ellicott City, MD.

Contact Info: **Michelle A. Johnston**, Environmental Health Sciences, Public Health Research Center, University of South Carolina, 921 Assembly Street, Columbia, South Carolina
Email: michelle@inlet.geol.sc.edu