Finding Sources of Fecal Coliform Bacteria in Stormwater Runoff: The Importance of Nonfecal Origins

David Tomasko, Emily Keenan, Jessica Hudson, Cheryl Propst, Matt Logan, and Joanne Vernon

The Sunshine Lake/Sunrise Waterway system, located in Charlotte County, previously experienced extensive and persistent algal blooms, accompanied by noxious odors and deep organic-rich soils. This condition resulted in Charlotte County implementing a lakewide dredging project to remove the algal material from the lake and waterway system. Two reports, a preliminary assessment (Atkins, 2012), and a more-detailed water quality management plan (Atkins and ESA, 2014) determined that the algal bloom in the lake was associated with a growth form that assimilated nutrients into a mass of material associated with the bottom of the lake and waterway, dominated by cyanobacteria, which is also known as blue-green algae.

As part of the development of the water quality management plan, a six-month monitoring program was implemented to quantify the nutrient concentrations of stormwater runoff and groundwater seepage, so that external nutrient loads could be quantified. The results from that effort indicated that the nutrient loads required for the algal bloom in the lake and the waterway were due in large part to excessive concentrations of phosphorus in stormwater runoff; however, nitrogen concentrations were not similarly elevated. The algal mat that was removed by dredging was comprised of various species of cyanobacteria, many of which have been shown to be able to "fix" nitrogen from the atmosphere (Tomasko et al., 2009).

David Tomasko is principal associate and Emily Keenan is an environmental scientist at Environmental Science Associates in Tampa. Jessica Hudson is a scientist in Tampa and Cheryl Propst is a senior scientist in Jacksonville at Atkins North America. Matt Logan is projects manager and Joanne Vernon is a county engineer with Charlotte County in Punta Gorda.

The lack of similarly and consistently elevated levels of nitrogen in stormwater runoff (compared to phosphorus) suggested that sewage and/or fertilizer were likely not the source(s) of phosphorus, as stormwater runoff that is influenced by sewage overflows and/or excessive fertilizer application is typically characterized by high levels of both phosphorous and nitrogen. Instead, it was concluded that the high levels of phosphorus in stormwater runoff were associated with the influence of the Peace River Formation of the Hawthorn Group, otherwise known as the Bone Valley Formation (Atkins and ESA, 2015). This geological feature, which extends into Charlotte County, is characterized by its elevated phosphorus content.

High levels of fecal coliform bacteria (>1,000,000 colony-forming units [cfu] per 100 ml) were often found in the same stormwater runoff samples with low concentrations of nitrogen. When considered as a whole, these findings suggested that the high levels of fecal coliform bacteria were not associated with sewage, since a sewage source would also be expected to have high concentrations of nitrogen. To further test the hypothesis that sewage was not the source of the high levels of fecal coliform bacteria in stormwater runoff, molecular source identification efforts were initiated, using polymerase chain reaction (PCR) techniques to de-

Table 1. Bucket assignment and quantity of material added per treatment.

Designation	Treatment	Weight of material added (grams)
A	Dog waste	58
B	Control	NA
C	Soil	56
D	Dog waste	46
E	Control	NA
F	Dog waste	50
G	Soil	56
H	Soil	56
I	Grass clippings	100
J	Grass clippings	100
K	Grass clippings	100
L	Control	NA

tect the presence of human-related deoxyribonucleic acid (DNA) sequences from *Baceroidetes* sp. and Enterococci sp. bacteria, a technique similar to those previously applied in the Miami River (Florida Department of Environmental Protection [FDEP], 2006) and in Collier County's Clam Bay (Atkins, 2012).

After sampling three stormwater discharge locations twice, results from the PCR study did not find evidence of humans as a significant source of the high levels of fecal coliform bacteria. After conducting a thorough examination of the stormwater conveyance system, a number of other potential sources of bacteria were considered, including wildlife, pet wastes, soils, and rotting vegetation.

In response to the findings of high levels of bacteria in stormwater runoff, and the finding that human fecal material did not appear to be the source of those bacteria, a follow-up manipulative experiment was conducted to determine not only what source(s) were not responsible for the high levels of bacteria found, but also what source(s) *were* likely responsible.

Experimental Design

The experimental design tested the ability of different materials found in the storm drain conveyance system to produce fecal coliform bacteria. Prior to the initiation of the study, Charlotte County Public Works identified a secure facility to perform the experiment, which was conducted in an abandoned trailer without electricity, which resulted in temperatures more similar to those expected within the stormwater conveyance pipes.

During field review of the storm drains, areas were found where exposed soils were likely to be eroded down into the stormwater conveyance system. This soil was collected for later use in the experimental phase of this project. A yard with newly mowed (that morning) grass was found, and with the homeowner's approval, fresh grass clippings were collected as well for the experiment. Due to the uncertainty of locating fresh dog droppings, the fecal material used for this experiment was provided by one of the researcher's dog, collected the morning of the start of the experiment.

The replicated incubation experiment consisted of 12 buckets (5 gal each) with four treatments (soil, fresh pet waste, fresh grass clippings, and controls). Each bucket contained 4 gal of water collected from the lake and three buckets were randomly assigned to each of the four treatments. For all buckets, excluding the controls, the material (soil, pet waste, and grass clippings) was weighed prior to adding the substance to each bucket (Table 1). A decision was made to double the amount of grass clippings added to the buckets, compared to the amount of soil or dog fecal material used. This decision was based on a presumption that grass clippings would have a low bacterial abundance associated with them, and therefore, a larger amount might be needed to produce any measurable counts.

Each bucket was then stirred gently to fully distribute any added materials and randomly placed in one room within the empty trailer.

After adding the materials to be tested to each bucket (with no material added to controls) a water sample was taken from each bucket on the day that the experiment was started. Additional samples were taken two, four, eight, 16, and 30 days after the start of the experiment. Samples were collected from each bucket using standard operating procedures, as outlined by FDEP, placed on ice, and provided to a private laboratory certified by an Environmental Laboratory Advisory Committee for standard analysis of fecal coliform bacteria concentrations.

Table 2. Results of incubation experiment. Values shown are means of three replicates per treatment in units of colony-forming units of fecal coliform bacteria per 100 mL of water.

	Day 0	Day 2	Day 4	Day 8	Day 16	Day 30
Controls	47	2,860	647	13	10	10
Dog feces	123	100,000	77,000	4,900	7,633	20
Soil	127	90	137	10	10	10
Grass clippings	103,333	2,000,000	19,366,667	1,033,333	678,333	55,667

Results

Results from the experiment are listed in Table 2, and graphically displayed in Figure 1. The results in Figure 1 are displayed on a log-10 scale, a common technique for results from bacterial abundance assessments.

On day zero, the controls, dog feces, and soil treatments had average fecal coliform bacteria abundances lower than 200 cfu/100 ml. In contrast, the treatment with grass clippings started off on day zero with more than 100,000 cfu/100 ml. At day two, fecal coliform bacteria in the dog feces treatment increased to a value similar to that seen at day zero in the grass clippings treatment, which had itself increased to 2,000,000 cfu/100 ml. By day four, the dog feces treatment had decreased from 100,000 to 77,000 cfu/100 ml of fecal coliform bacteria, while the grass clippings treatment had increased further to nearly 20,000,000 cfu/100 ml. By day eight, fecal coliform bacteria concentrations in both the controls and the soil treatments had decreased to 10 cfu/100 ml, where they stayed until day 30.

By day 16, the fecal coliform bacteria amount in the grass clippings treatment averaged 678,333 cfu/100 ml, while the dog feces treatment values averaged 7,633 cfu/100 ml. By the end of the experiment, the dog fecal treatment averaged 20 cfu/100 ml of fecal coliform bacteria, while the grass clippings treatment averaged 55,667 cfu/100 ml.

These findings suggest that the decomposition of grass clippings in warm and anoxic conditions, such as those used in this experiment, can give rise to an abundance of bacteria that test positive as fecal coliform, and that these bacteria are not likely due to potential contamination with pet waste. Instead, it appears that the test for fecal coliform bacteria is not specific enough to detect that grass clippings are likely decomposed in part by bacteria that also grow under the conditions used to test for fecal coliform bacteria.

Results of the incubation experiment are somewhat compromised by the fact that the buckets with grass clippings had roughly twice as much material added to them (100 grams wet weight) as the buckets with either soils or dog feces; however, the finding that day-zero samples of grass clippings had approximately 1,000 times as much bacteria as the buckets with soil or dog feces suggest that there are large amounts of bacteria that test positive as fecal coliform associated with grass clippings. Samples incubated with dog feces showed very high levels of fecal coliform bacteria at days two, four, eight, and 16, but values decreased substantially from day 16 to day 30. At all dates, however, there were more bacteria in the samples incubated with grass clippings than those with dog feces. Samples incubated with soil did not appear to be a significant source of fecal coliform bacteria.

These results help to explain how the stormwater conveyance system discharging into the lake had such high levels of fecal coliform bacteria, even though more sophisticated molecular techniques did not find evidence of human sewage as the likely source of such high values.

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Potential Sources of Fecal Coliform Bacteria

For over a century, either total coliform bacteria or fecal coliform bacteria have been used as indicators of the presence of various pathogens, whether bacterial, viral, or associated with parasites. Their use as indicators of pathogens was valuable, as they have been used to set standards and determine the likelihood of fecal contamination and associated human health risk in surface waters; however, the use of indicator organisms to warn of fecal contamination and associated risks to human health is confounded by many variables, including differences among the life cycles, environmental requirements, overall abundance, and likelihood of being detected, compared to actual pathogens (Harwood et al., 2013). Furthermore, total and fecal coliform bacteria have been shown to persist and grow in terrestrial soils, aquatic soils, and aquatic vegetation (LaLiberte and Grimes, 1982; Fujioka et al., 1999; Solo-Gabriele et al., 2000; Desmarais et al., 2000; Byappanahalli et al., 2000; Topp et al., 2003; Whitman et al., 2003; Anderson et al., 2005; Ishii et al., 2000; Ksoll et al., 2007; Badgley et al., 2011; Harwood et al., 2013).

The fecal coliform group is comprised of thermotolerant bacteria, such as *Escherichia coli* and *Klebsiella pneumonae*, and is used to indicate the presence of fecal contamination from warm-blooded animals (Bitton, 2005); however, *K. pneumoniae* may be present in the environment from a variety of nonfecal sources, such as pulp and paper mill wastes (Knittel, 1975; Caplenas et al., 1984) and fresh vegetables (Duncan and Razzell, 1972). Similarly, *E. coli* can persist and grow in soil, resulting in *E. coli* levels that are artificially elevated above those expected from fecal contamination alone (LaLiberte and Grimes, 1982). The warm, humid conditions and the availability of organic matter typical of tropical and subtropical environments may play an important role in *E. coli* persistence and growth in these regions (Rochelle-Newall et al., 2015; Desmarais et al., 2002; Solo-Gabriele et al., 2000; Fujioka et al., 1999).

In addition to terrestrial and aquatic soils, aquatic vegetation has also been shown to harbor and support the growth of *E. coli* and enterococci bacteria, which can persist and grow on decomposing seaweeds, such as the green algae *Cladophora glomeratai* (Byappanahalli et al., 2003; Whitman et al., 2003). The presence and persistence of fecal coliform bacteria and *E. coli* was also associated with algal communities growing on hard substrates along the shoreline of Lake Superior (Ksoll et al., 2007). Although some of the fecal indicator bacteria growing on the algal films along Lake Superior could be associated with impacts from wildlife, many of the strains of *E. coli* found appeared to be unique to the algal film alone, rather than being due to fecal material from wildlife (let alone fecal contamination from humans).

The results from this study suggest that fecal coliform bacteria may be similarly associated with the decomposition of grass clippings, at least in the warm, anoxic environments likely found in the storm drain system flowing into the lake and waterway. The findings, as well as anecdotal observations, suggest that grass clippings can decompose rather quickly in Florida, at least in the summertime. Decomposition of grass clippings likely involves some combination of either fungi or bacteria. If the rapid decomposition of grass clippings is achieved in part by bacteria, then it is possible that at least some of the bacteria involved also test positive as fecal coliform bacteria. Strynchuk and Royal (2003) showed an immediate rise in biochemical oxygen demand (BOD) in the water column during incubation of mixed oak leaves (*Quercus* sp.) and St. Augustine yard grass (*Stenolaphrum secundalum*) and a slightly slower rise in BOD during incubation of St. Augustine grass clippings in Florida, indicating the rapid initiation of decomposition of such materials. Similarly, Kopp and Guillard (2004) found rapid decomposition of turf grass clippings and release of nitrogen during a study conducted in the more temperate environment of the state of Connecticut.

Currently, it is unknown if bacteria associated with the decomposition of grass clippings would also test positive for either *E. coli* or enterococci, both of which have been proposed as criteria that are expected to be more precisely associated with fecal contamination from humans, or at least mammals. This data gap should be addressed, so that the various regulatory programs in Florida and elsewhere focus on the most likely sources of bacteria in impaired waters, which could require public education related to the proper disposal of grass clippings, as opposed to the typical conclusion that elevated bacteria are likely to require septic tank retrofits, and/or upgrades to sewer collection and treatment systems.

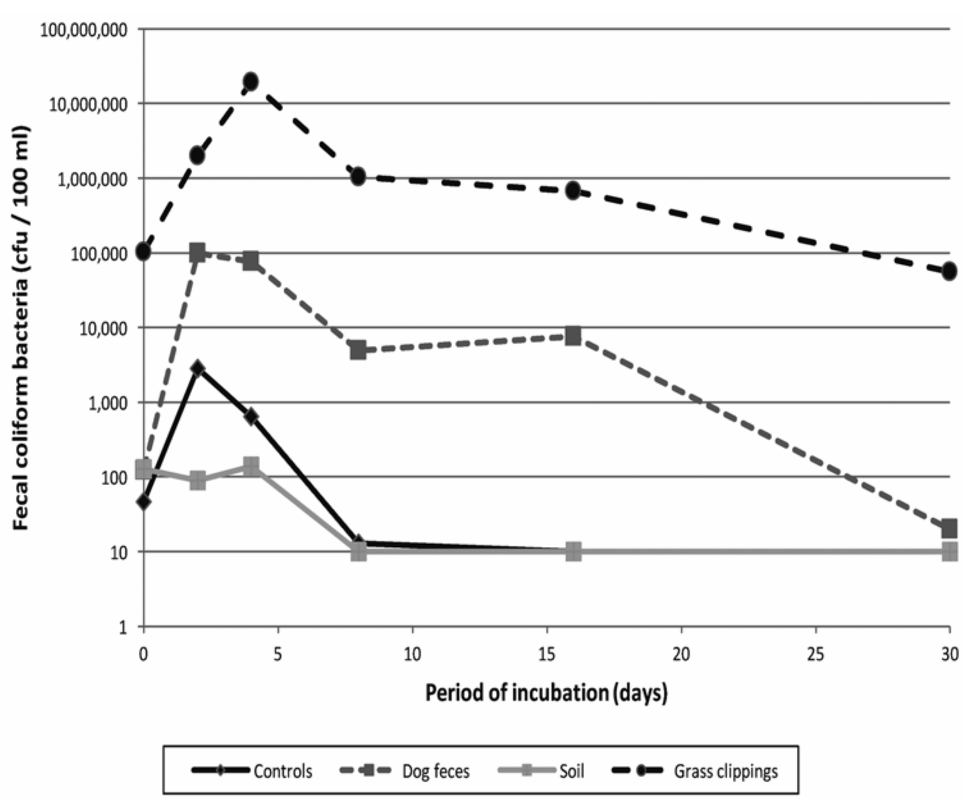

Figure 1. Results of incubation experiment. Values shown are log-10-based means of three replicates per treatment in colony-forming units of fecal coliform bacteria per 100 mL of water.

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