EXHIBIT "H"

# Investigation on Fecal Indicator Bacteria, Estero River, 2019-2021


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Florida Gulf Coast University


*April 7, 2021*

# FGCU Estero River study

# July 2019 –

# March 2021

**Florida Gulf Coast University**

**The Water School**

**Estero River Bacteria-Nutrient Source Identification Project**
**Contract EC 2019-29**

**Final Report to Village of Estero**

**March 15, 2021**

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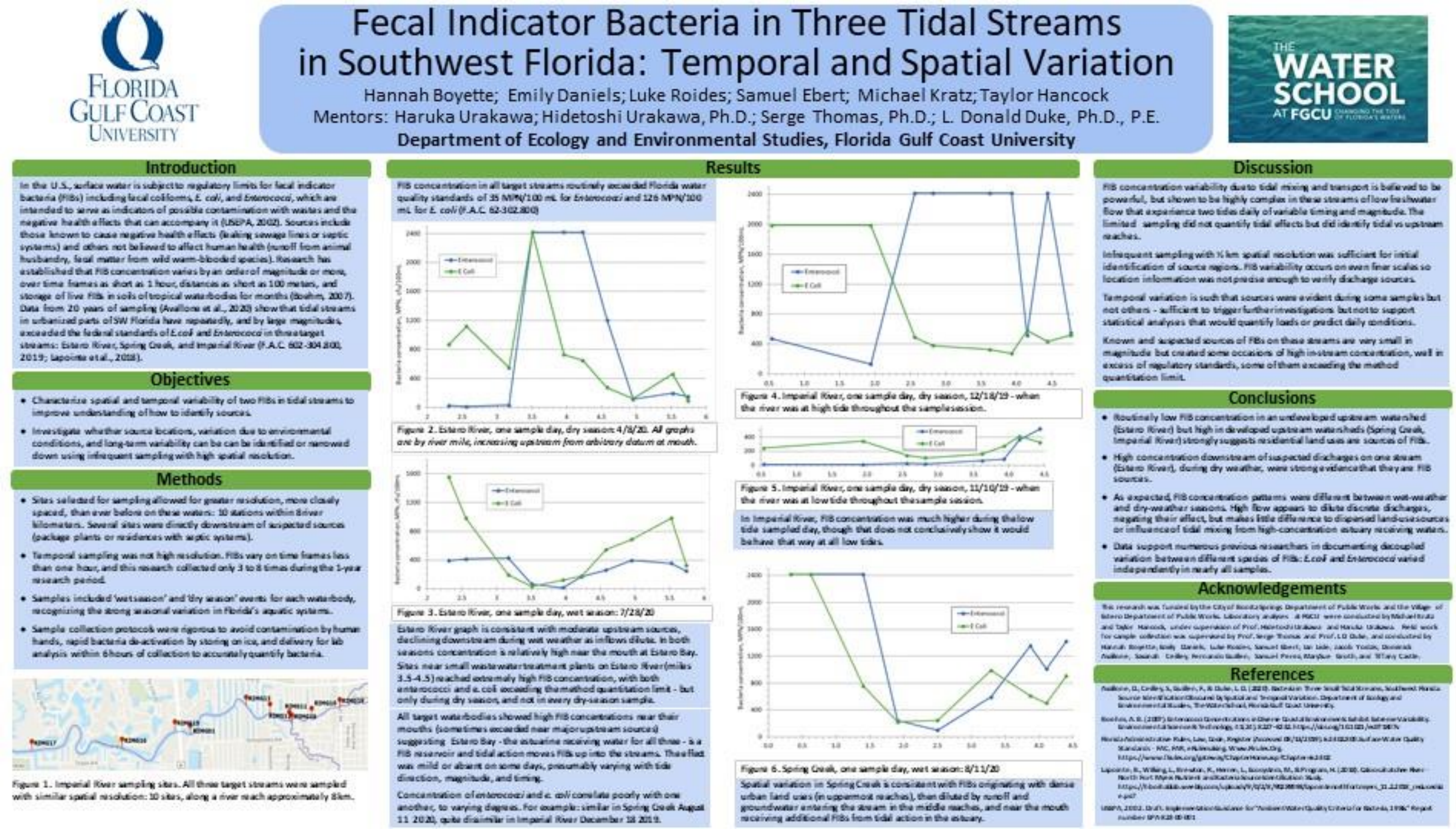
# Student participation

## Poster presented at AWRA Annual Conference, Nov 2021



# Fecal indicator bacteria: FIB

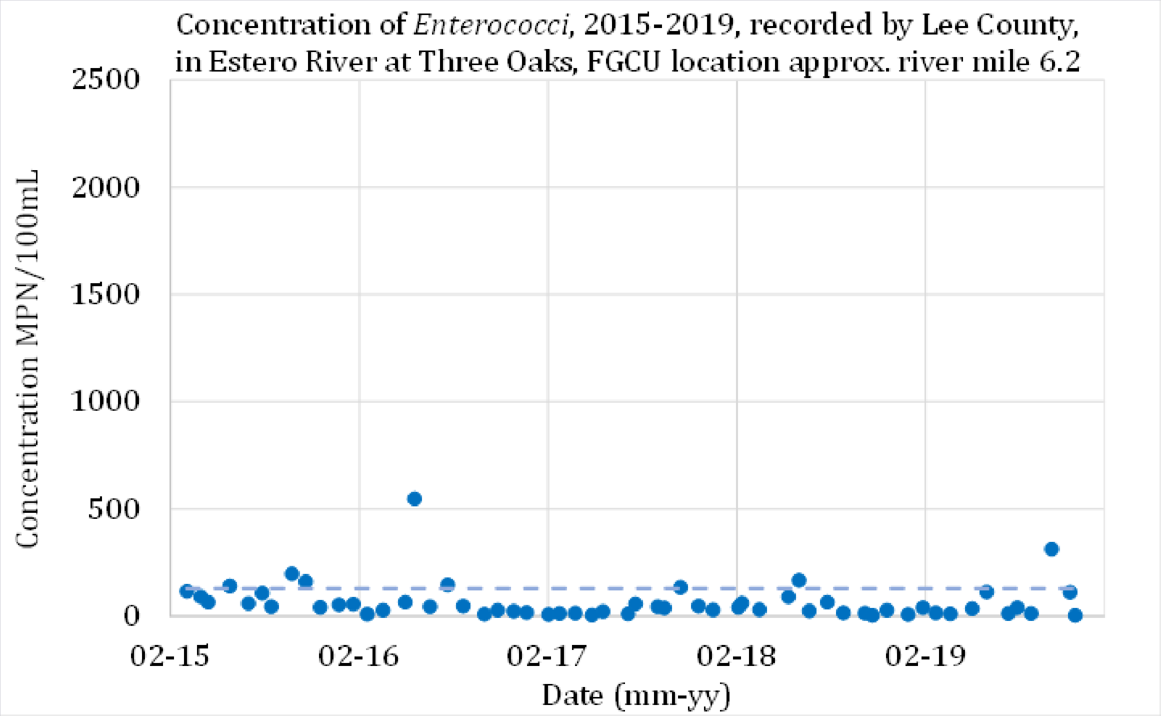
- Designated by USEPA and FL DEP for water quality standards, two species:
  - *Enterococcus*
  - *E. coli*
- Surrogates for species that can cause human health effects
- Relatively easy to measure – while potentially harmful species are very difficult to measure
- Highly variable in the environment, by time and place

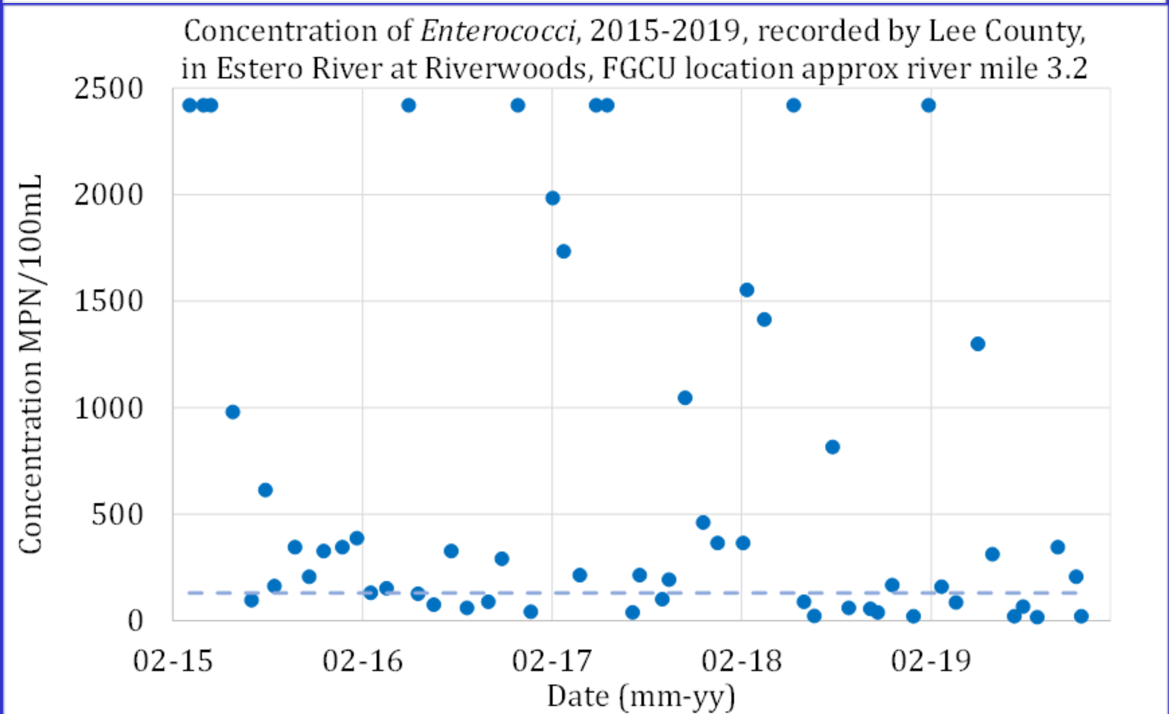
# Key findings

- High FIB numbers in upstream locations are evidence that human activities produce and/or enhance sources
- High FIB variability, temporally and spatially, confirms sources are episodic, short-term, varied in action/inputs: <u>in aggregate, sources are substantial</u>
- Variability within and between sampling events, affected by many factors, masks specific sources
- Frequency of high-FIB events, with presence of sucralose and microbe species found in humans, confirms presence of human waste AND other sources
- Groundwater does not appear to convey waste in soils
- Small, steady surface flows of groundwater may convey human waste into the Estero River

# Outline: Evidence for Findings

- Historic data from Lee County monitoring
- Estero River “length of river” samples
- Groundwater sampled near river
- Surface stormwater backup, Sherrill Lane, one morning
- Sucralose, genetic sequencing: additional evidence
- Summary of key findings / weight of evidence



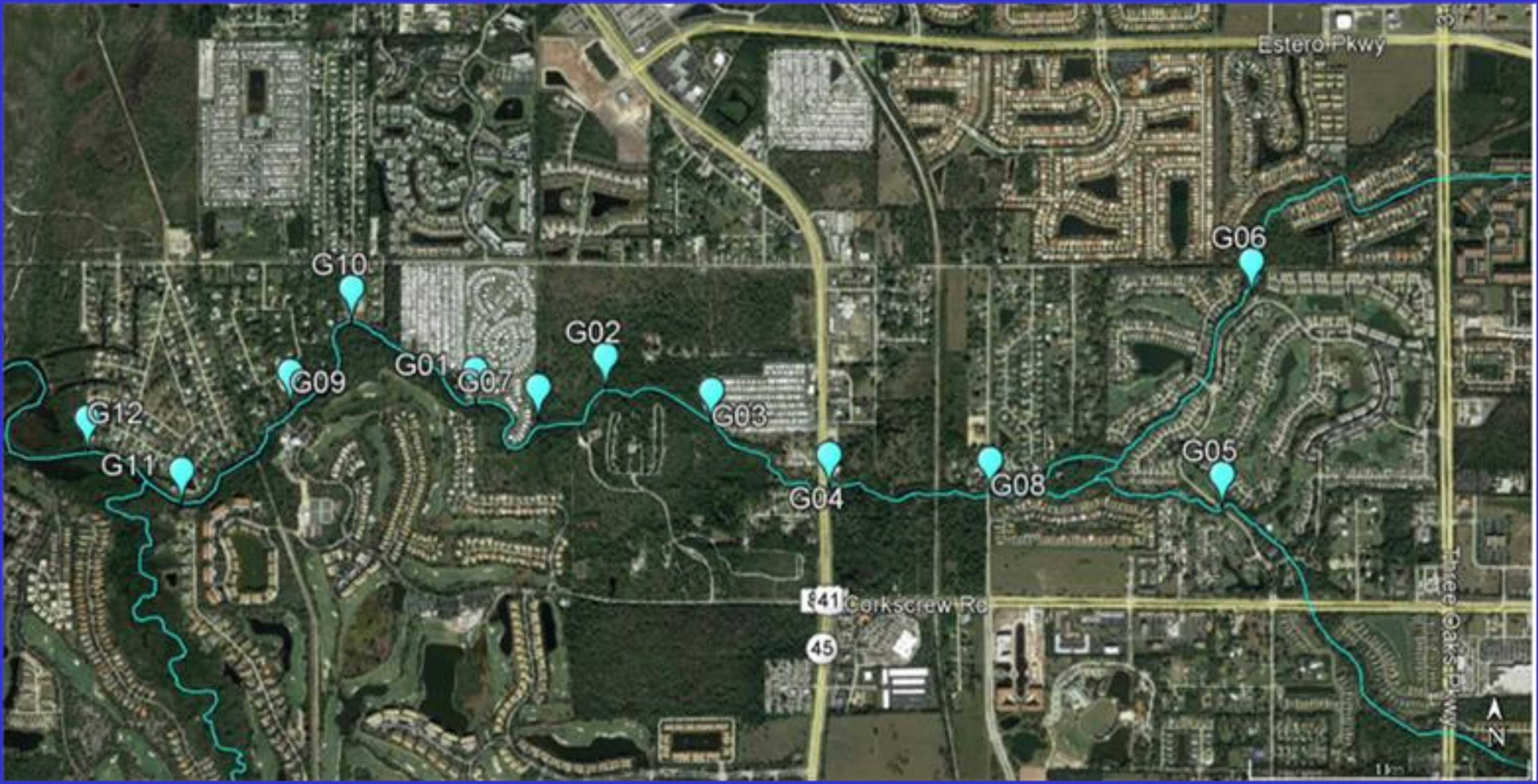

# Frequency of high-MPN (high abundance) points: greater downstream than upstream

*Enterococcus,* Three Oaks (upstream – top)

and Riverwoods (downstream – bottom)

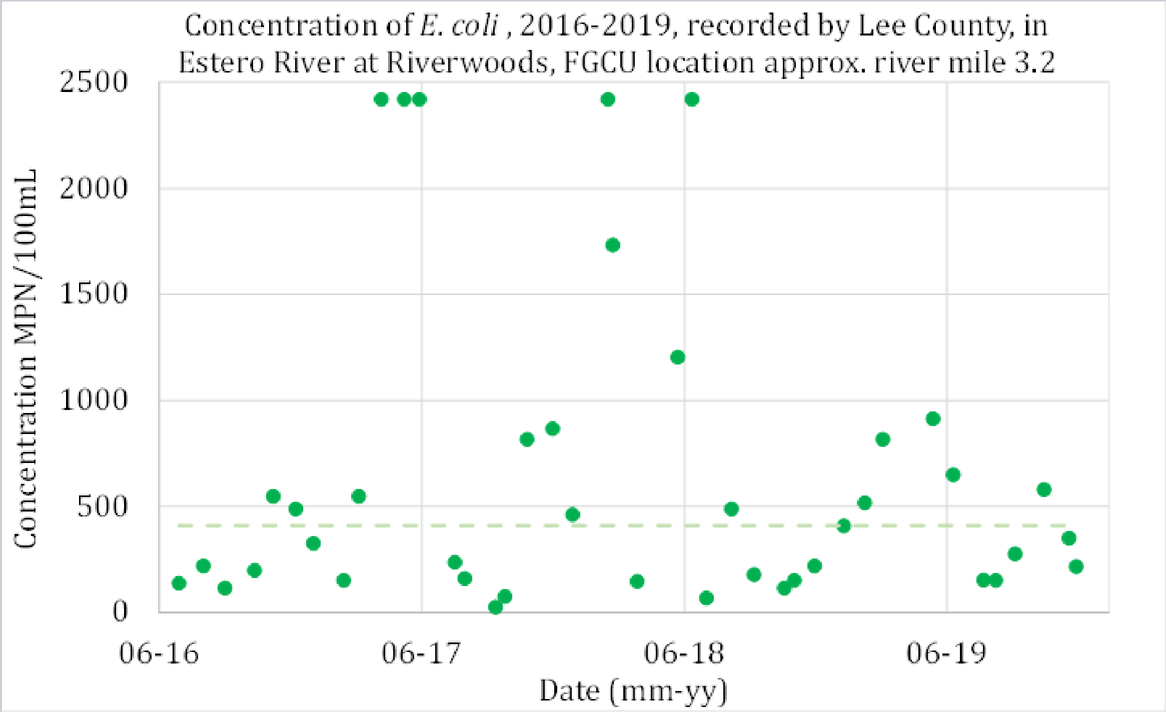
Lee County Natural Resources, March 2015 – January 2020

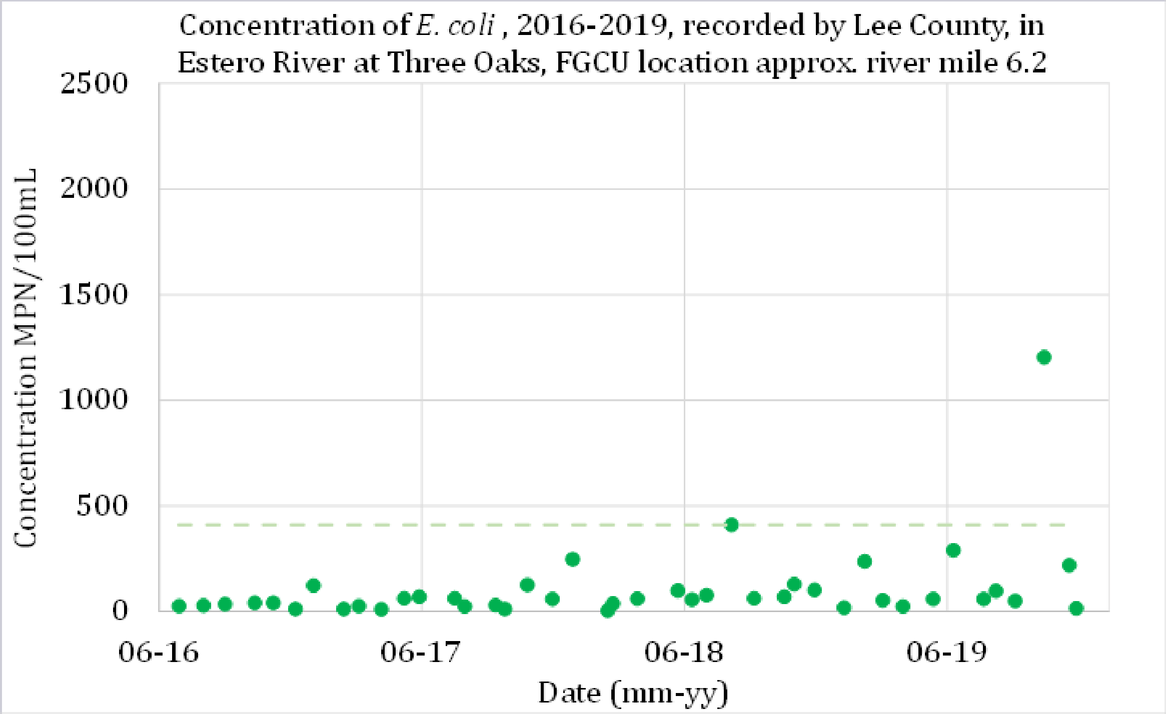
Dashed line is TPTV, "ten percent threshold value," numeric target not to be exceeded by more than 10% of samples, at 130 MPN/100mL for *Enterococci*




Ten sites on Estero River used for length-of-river sampling

- Lee County "Riverwoods" site is G10
- Lee County "Three Oaks" is upstream (right) of G06




# E. coli also greater downstream than upstream

*E. coli,* Three Oaks (upstream – top)

and Riverwoods (downstream – bottom)

Lee County Natural Resources, June 2016-January 2020

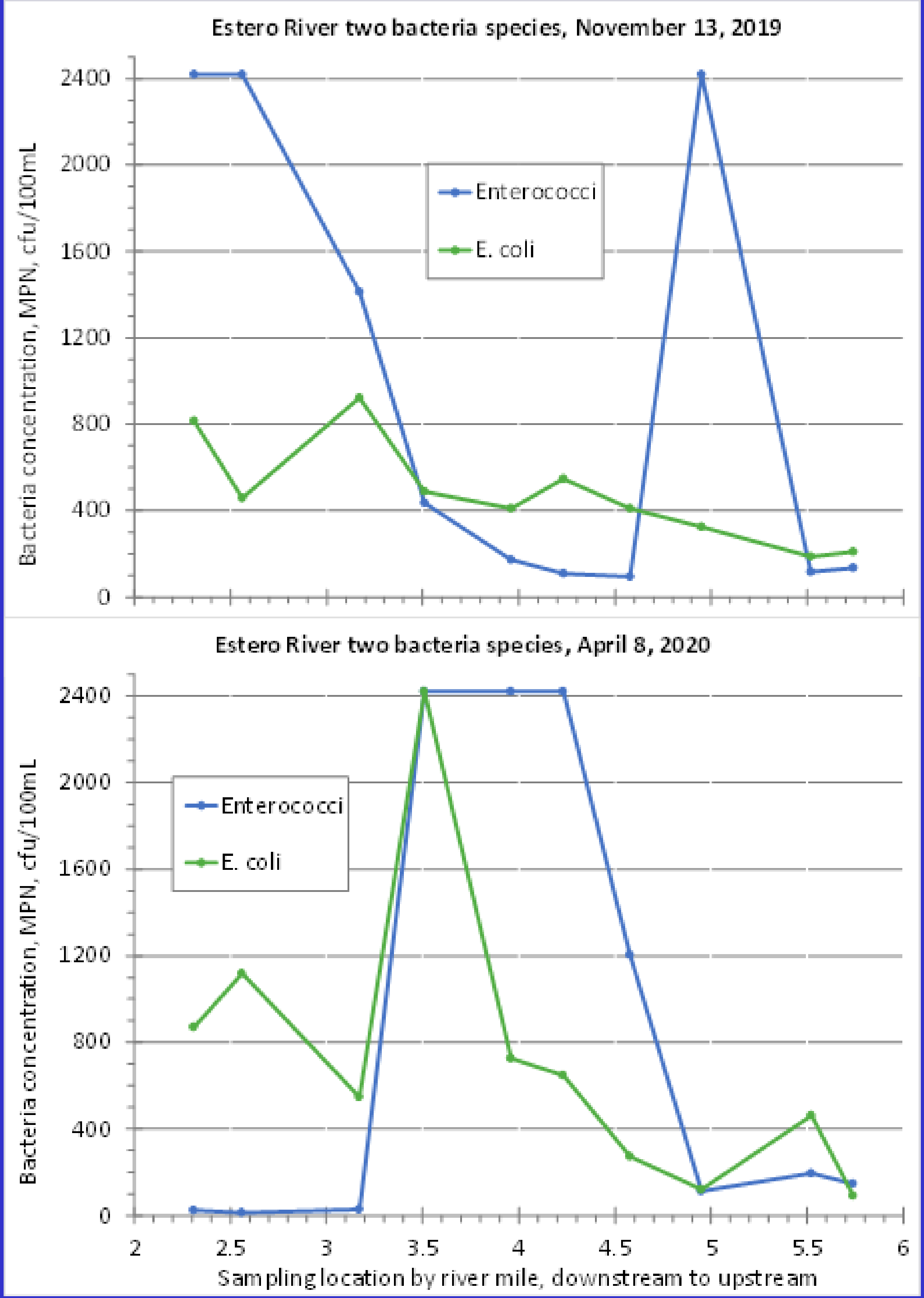
Dashed line is TPTV, “ten percent threshold value,” numeric target not to be exceeded by more than 10% of samples, at 410 MPN/100mL for *E. coli.*

# Summary of 5 years historical data, 2 sites

| | *Enterococci* | | | | *E. coli* | | |
|---|---|---|---|---|---|---|---|
| | 3 Oaks | Rt 41 | Riverwoods | | 3 Oaks | Rt 41 | Riverwoods |
| n | 58 | 58 | 58 | | 42 | 42 | 42 |
| Median | 40 | 345 | 214 | | 59 | 236 | 350 |
| TPV = 90$^{th}$ %ile | 146 | 1,203 | 2,420 | | 238 | 770 | 2,420 |
| | | | | | | | |
| TPTV | 130 | 130 | 130 | | 410 | 410 | 410 |
| | | | | | | | |
| Exceed-ences | 8 | 28 | 39 | | 2 | 12 | 20 |
| >1000 MPN | 0 | 7 | 15 | | 0 | 2 | 8 |
| > 2420 MPN | 0 | 2 | 9 | | 0 | 1 | 6 |

Lee County Natural Resources, March 2015 – January 2020
TPV: ten percent value, i.e. 90%ile of data at a location
TPTV: ten percent threshold value, regulatory numeric target for ten percent value at a location
TPTV = 130 MPN/100mL for *Enterococci,* 410 MPN/100mL for *E. coli*

# Surface water sampling, Estero River

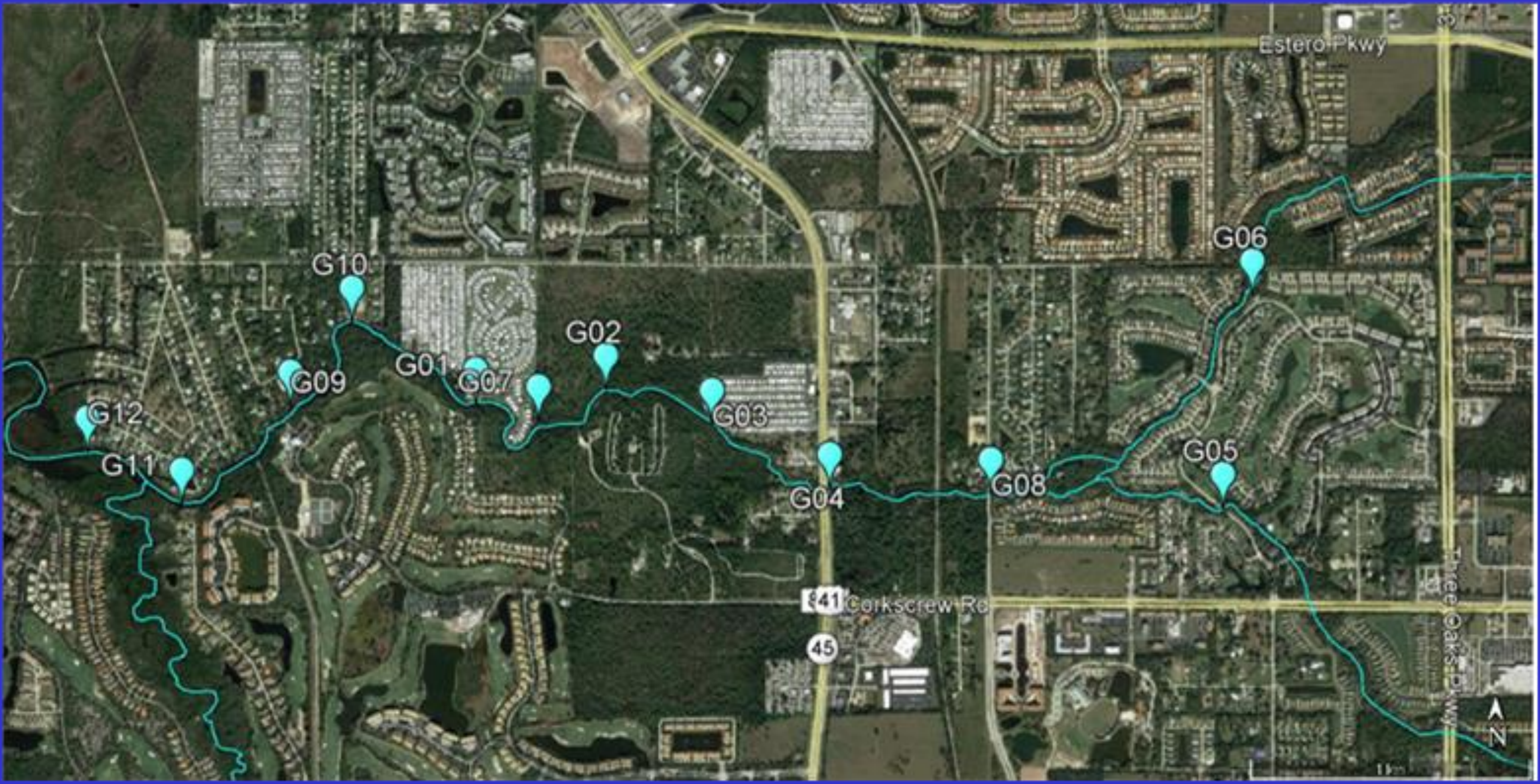

Two dry-season "length of river" sampling days on the Estero River:

11/13/19,

4/8/20

Estero Bay Village is about mile 3.5

Sunny Groves is about mile 4.2

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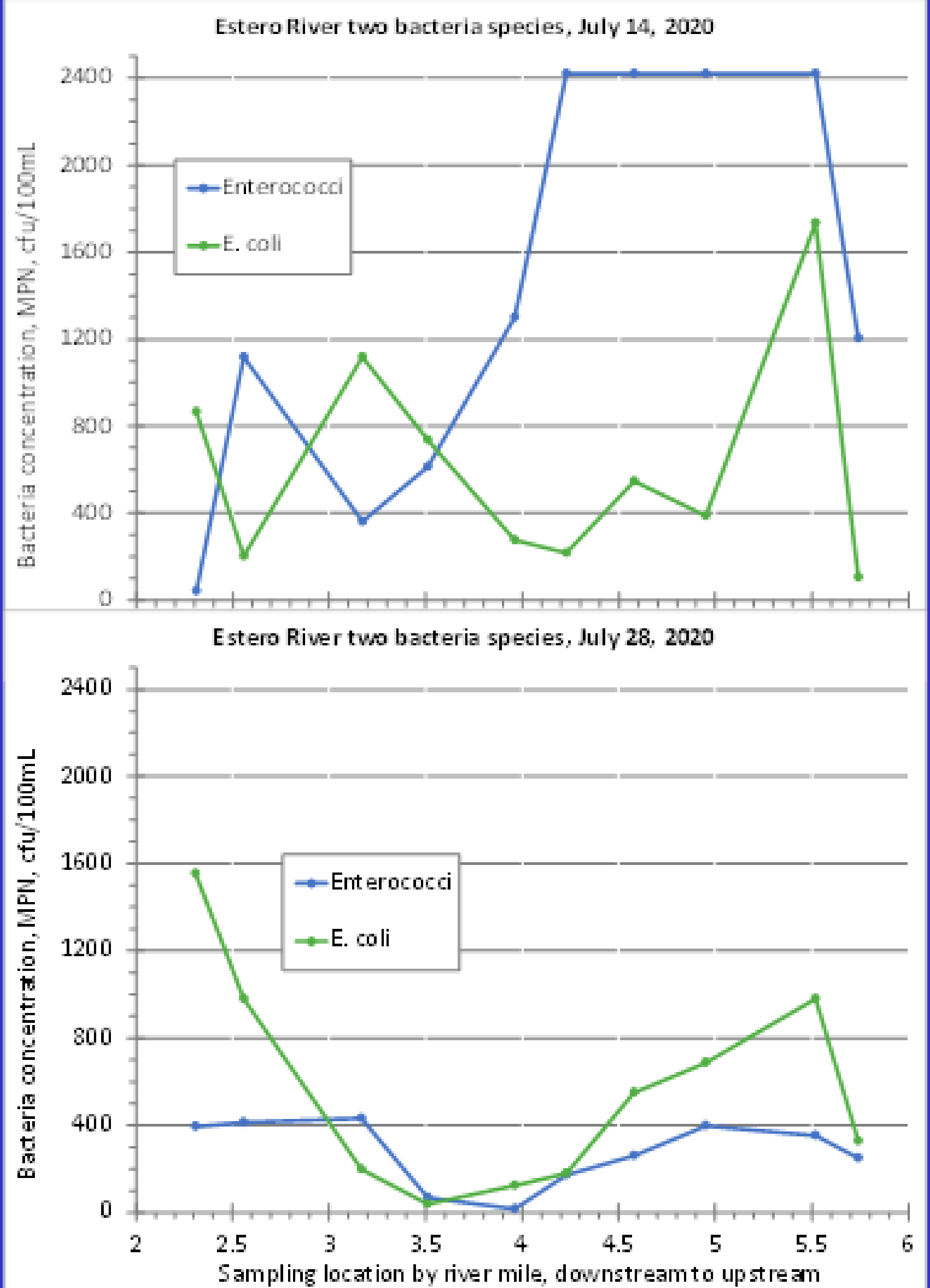



Two wet-season "length of river" sampling days on the Estero River:

7/14/20,

7/28/20

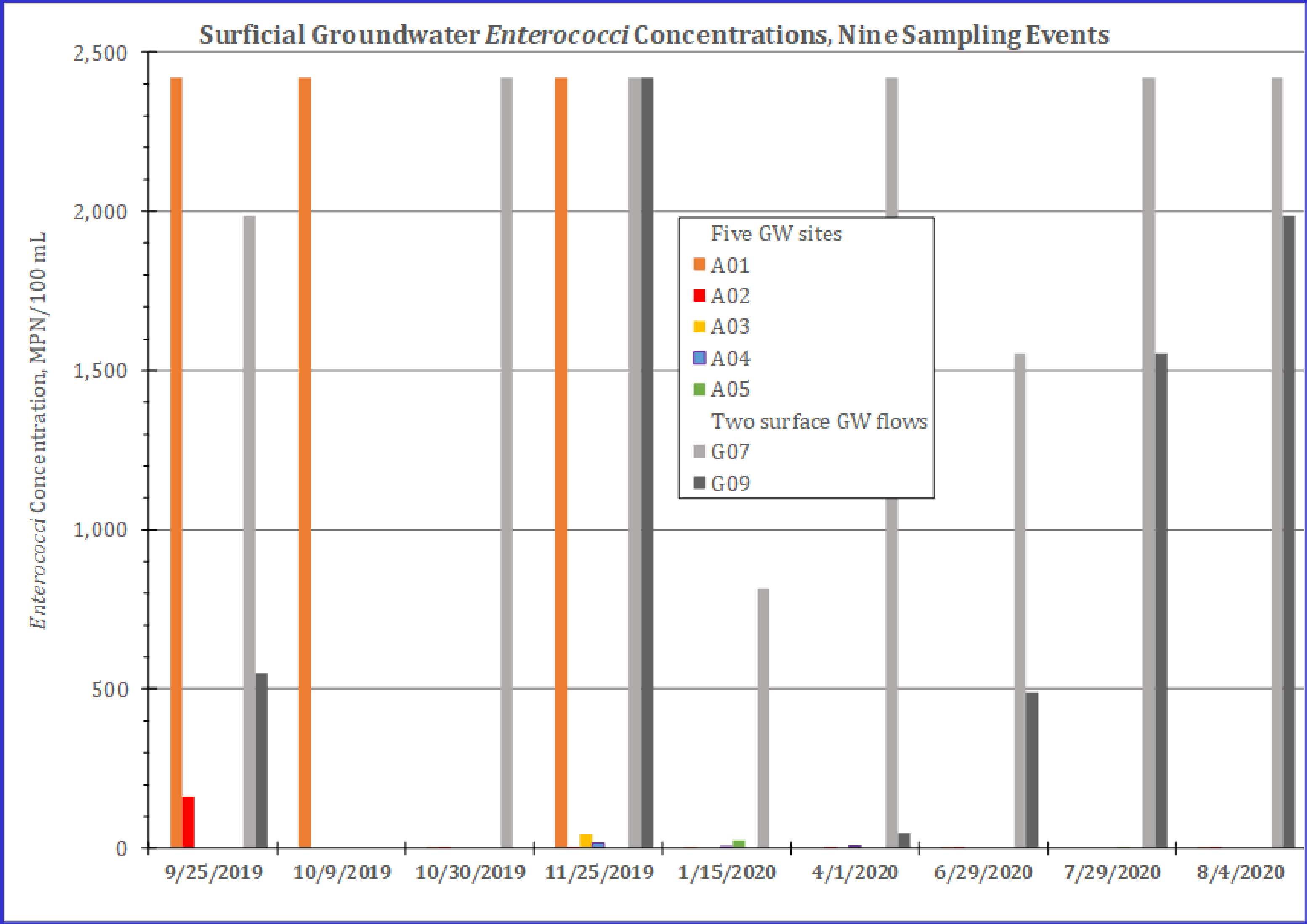
Estero Bay Village is about mile 3.5

Sunny Groves is about mile 4.2

# Groundwater sampling near Estero River

# Groundwater samples

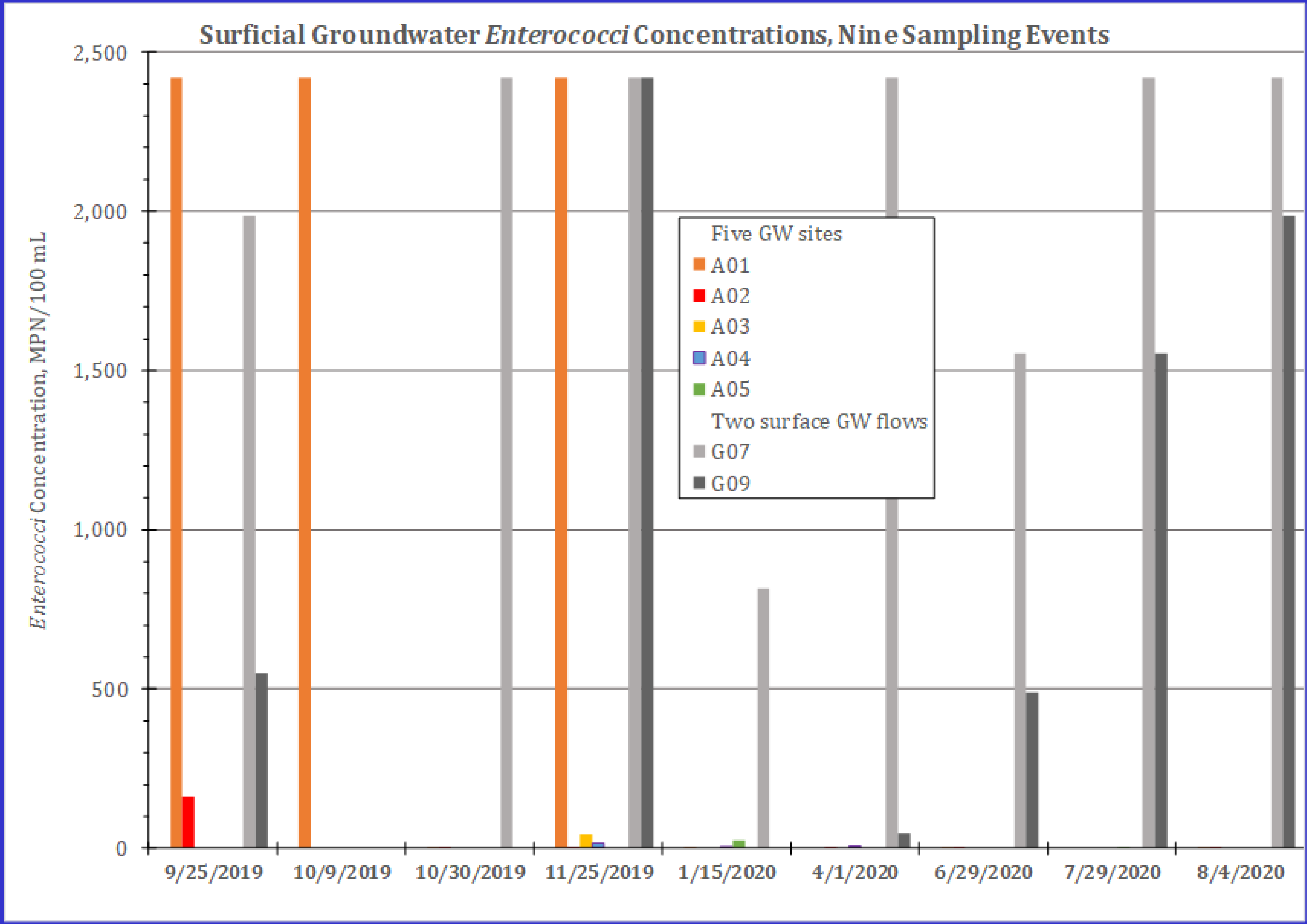
Ground-water at 5 piezo-meters and 2 surface flows




A01 through A05: near-surface groundwater (piezometers) – almost no *Enterococci* except A01 (believed erroneous)

Small surface drainages conveying groundwater – the "ditches"

Ground-water at 5 piezo-meters and 2 surface flows




G07, Estero Bay Village "ditch"; G09, Charing Cross "ditch:" convey groundwater year-round, routinely high *Enterococci*

# Sample, stormwater ponding, Sherrill Lane north of Broadway, September 4, 2020: Very high FIB, other factors not surprising

| Constituent | Measurement |
|---|---|
| enterococci | 2420 MPN/100 mL |
| *E. coli* | 2420 MPN/100 mL |
| Dissolved oxygen concentration | 7.33 mg/L |
| Dissolved oxygen proportion of saturation | 93.5% |
| pH | 7.58 |
| Electric conductivity | 255 µS/cm |
| Turbidity | 24.1 NTU |

# Sucralose

- Sucralose is a chemical tracer: originates in the environment almost exclusively with human waste
- Can be measured in ng/L, $10^{-12}$ (parts per trillion)

Table 10.1. Sucralose concentration, MPN and other environmental factors.

| Date | FGCU ID | Sample type | Sucralose (ng/L) | Temp (C) | DO (mg/L) | DO (%) | pH | Electric cond (µS/cm) | Turbidity (NTU) | ORP (mV) | enterococci (MPN/100 ml) | E. coli (MPN/100 ml) | Ratio (ent/ecoli) | TIN (ug-N/L) | NH4+ (ug-N/L) | NOx (ug-N/L) | SRP (ug-P/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8/19/2019 | G04 | Surface | 285.56 | 29.2 | 3.5 | 45.1 | 7.5 | 430 | 2.7 | 491.7 | 96 | 75 | 1.28 | 197 | 138 | 60 | 16 |
| 9/18/2019 | G04 | Surface | 358.83 | 29.3 | 3.2 | 42.2 | 7.5 | 626 | 2.9 | 442.8 | 1986 | 411 | 4.83 | 224 | 75 | 149 | 11 |
| 8/19/2019 | G07 | Ditch | 17418.41 | 28.3 | 2.1 | 26.5 | 7.3 | 454 | 2.3 | 81.3 | 78 | 172 | 0.45 | 1270 | 1257 | 13 | 22 |
| 10/30/2019 | G07 | Ditch | 35568.04 | 26.2 | 2.1 | 25.8 | 7.1 | 532 | 1.1 | 163.7 | 2420 | 2420 | 1 | 1018 | 512 | 506 | 1690 |
| 11/25/2019 | G07 | Ditch | 42710.33 | 22.0 | 4.9 | 55.0 | 7.8 | 495 | 0.0 | 495.0 | 2420 | 236 | 10.26 | 517 | 104 | 413 | 1711 |
| 1/15/2020 | G07 | Ditch | 36647.11 | 24.1 | 4.8 | 51.7 | 7.7 | 449 | 1.0 | 313.7 | 816 | 45 | 18.14 | 2117 | 950 | 1167 | 1989 |
| 9/25/2019 | A01 | Groundwater | 0 | 28.1 | 1.8 | 23.4 | 6.3 | 652 | 45.1 | -71.1 | 2420 | 0 | 2420 | 5206 | 5157 | 49 | 1773 |
| 9/25/2019 | A02 | Groundwater | 17310.35 | 27.5 | 4.2 | 52.4 | 6.9 | 535 | 3.6 | -22.7 | 164 | 0 | 164 | 890 | 852 | 38 | 1511 |
| 9/25/2019 | A03 | Groundwater | 20287.92 | 27.2 | 2.2 | 27.1 | 6.8 | 517 | 6.0 | -92.4 | 0 | 7 | 0.14 | 1164 | 1120 | 44 | 1589 |
| 10/30/2019 | A01 | Groundwater | 0 | 27.8 | 3.4 | 42.0 | 6.4 | 6 | 2.7 | 30.6 | 2 | 0 | 2 | 4517 | 3823 | 694 | 932 |
| 10/30/2019 | A02 | Groundwater | 22310.33 | 27.6 | 2.4 | 30.2 | 6.9 | 680 | 1.0 | 78.9 | 2 | 1 | 2 | 579 | 486 | 93 | 1387 |
| 10/30/2019 | A03 | Groundwater | 19675.39 | 28.9 | 2.7 | 34.3 | 7.0 | 446 | 14.0 | 21.4 | 0 | 0 | 0 | 1705 | 1545 | 161 | 1678 |
| 11/25/2019 | A01 | Groundwater | 0 | 24.2 | 3.3 | 38.4 | 7.0 | 434 | 5.6 | 169.3 | 2420 | 0 | 2419.6 | 3179 | 2745 | 434 | 143 |
| 11/25/2019 | A02 | Groundwater | 32891.69 | 28.7 | 4.3 | 49.0 | 7.2 | 585 | | -48.4 | 4 | 0 | 4.1 | 1710 | 225 | 1484 | 1272 |
| 11/25/2019 | A03 | Groundwater | 27708.41 | 25.1 | 2.2 | 26.0 | 7.1 | 542 | 10.3 | -20.5 | 42 | 0 | 42.2 | 2951 | 2211 | 740 | 1885 |
| 11/25/2019 | A04 | Groundwater | 940.87 | 21.1 | 3.9 | 45.4 | 7.1 | 3517 | 6.2 | -97.8 | 17 | 112 | 0.15 | 620 | 313 | 308 | 117 |
| 11/25/2019 | A05 | Groundwater | 953.16 | 23.0 | 3.4 | 40.3 | 7.0 | 6502 | 4.7 | | 0 | 1 | 0 | 2083 | 668 | 1415 | 52 |
| 1/15/2020 | A01 | Groundwater | 0 | 23.9 | 3.7 | 43.1 | 6.6 | 443 | 2.2 | 51.7 | 2 | 0 | 2 | 1614 | 356 | 1258 | 2013 |
| 1/15/2020 | A02 | Groundwater | 31287.88 | 22.4 | 2.2 | 25.8 | 7.0 | 445 | 2.2 | -114.4 | 0 | 1 | 0 | 387 | 303 | 84 | 1991 |
| 1/15/2020 | A03 | Groundwater | 30540.15 | 24.7 | 1.6 | 19.3 | 6.9 | 560 | 10.0 | -105.0 | 0 | 0 | 0 | 3332 | 2214 | 1118 | 1780 |
| 1/15/2020 | A04 | Groundwater | 2568.72 | 21.7 | 3.9 | 43.2 | 7.0 | 874 | 3.7 | 29.3 | 2 | 0 | 2 | 647 | 84 | 564 | 109 |
| 1/15/2020 | A05 | Groundwater | 649.48 | 25.1 | 3.0 | 31.2 | 6.9 | 7592 | 1.5 | -198.4 | 25 | 0 | 24.9 | 1579 | 552 | 1027 | 164 |

# Sucralose

- High concentration in A02 and A03: Estero Bay Village About 15 - 35 μg/L, or 15 - 35 x $10^{-9}$, parts per billion
- Expected to be high in groundwater near human use
- Not present at A01, A04, or A05 in soils (35 – 45 ng/L, i.e. 35 x $10^{-12}$) – little human waste in those locations
- Tested 'ditch' at G07 also: surprised to find high concentrations (35 – 45 μg/L)
- Unusual in surface water – evidence that flow in the 'ditches' is mostly groundwater recently reaching surface

# DNA sequencing

- A biological tracer: Compare DNA in samples to a database, and identify the species of microbes present in samples of Estero River water
- Captures only those species of microbes present in sufficiently high numbers to be detected in the lab – i.e. presence means something; absence means nothing
- Species of microbes in some cases might correspond to the gut biomes of some species of warm-blooded animals
- When certain species of microbes are present, it might suggest a higher than random probability that waste from certain warm-blooded animals are present

# DNA sequencing: Species of microbes that the literature identifies as "markers" that were present in Estero River samples, 2019-2020

| Specific genetic marker | Source |
|---|---|
| *Bacteroides barnesiae* | Chickens, other birds |
| *Bacteroides fragilis* | Human |
| *Bacteroides intestinalis* | Human |
| *Bacteroides massiliensis* | Human |
| *Bacteroides* sp. | Human, mammal, bird |
| *Barnesiella* sp. | Human |
| *Dysgonomonas gadei* | Human |
| *Dysgonomonas* sp. | Animal |
| *Dysgonomonas termitidis* | Termites, other insects |
| *Paludibacter* sp. | Human, cattle, other mammals |
| *Parabacteroides chinchillae* | Rodents, esp. chinchilla (*Chinchilla lanigera*) |
| *Prevotella* sp. | Human |
| *Alistipes* sp. | Human |
| *Rikenella* sp. | Chicken, Japanese quail, other birds |

# DNA sequencing

- Human waste is present in Estero River
- Waste from other species is present in Estero River – birds, rodents, other mammals

# Key findings

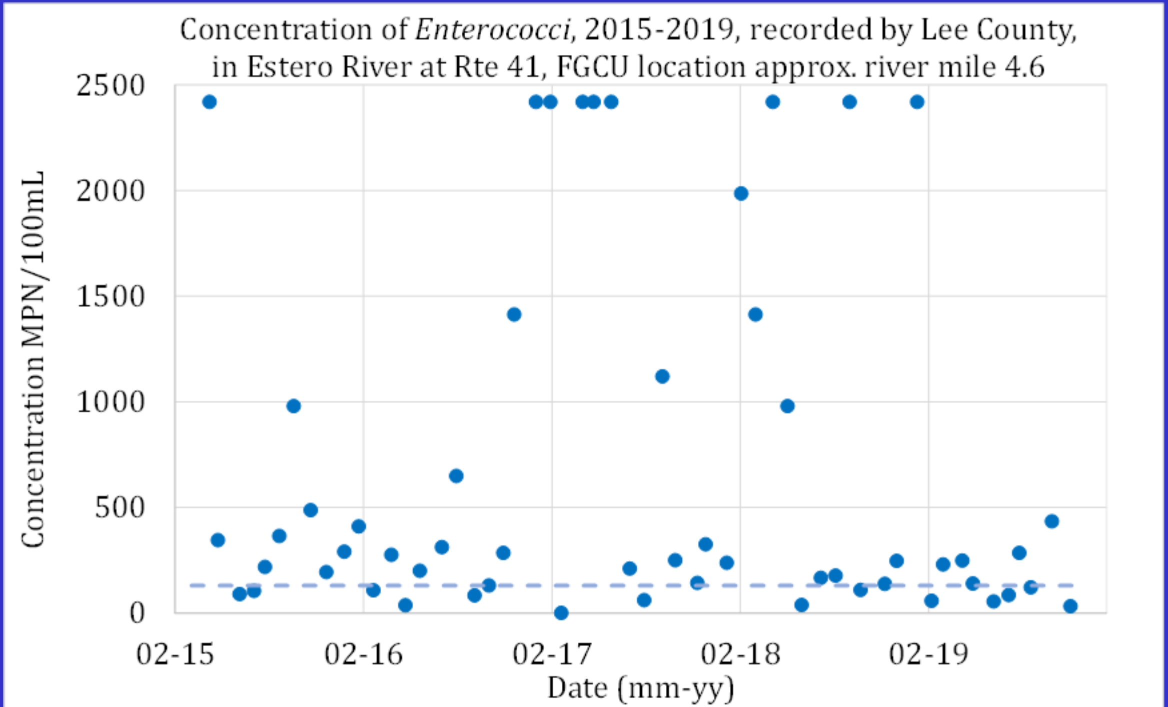
- High FIB numbers in upstream locations are evidence that human activities produce and/or enhance sources
- High FIB variability, temporally and spatially, confirms sources are episodic, short-term, varied in action/inputs: <u>in aggregate, sources are substantial</u>
- Variability within and between sampling events, affected by many factors, masks specific sources
- Frequency of high-FIB events, with presence of sucralose and microbe species found in humans, confirms presence of human waste AND other sources
- Groundwater does not appear to convey waste in soils
- Small, steady surface flows of groundwater may convey human waste into the Estero River

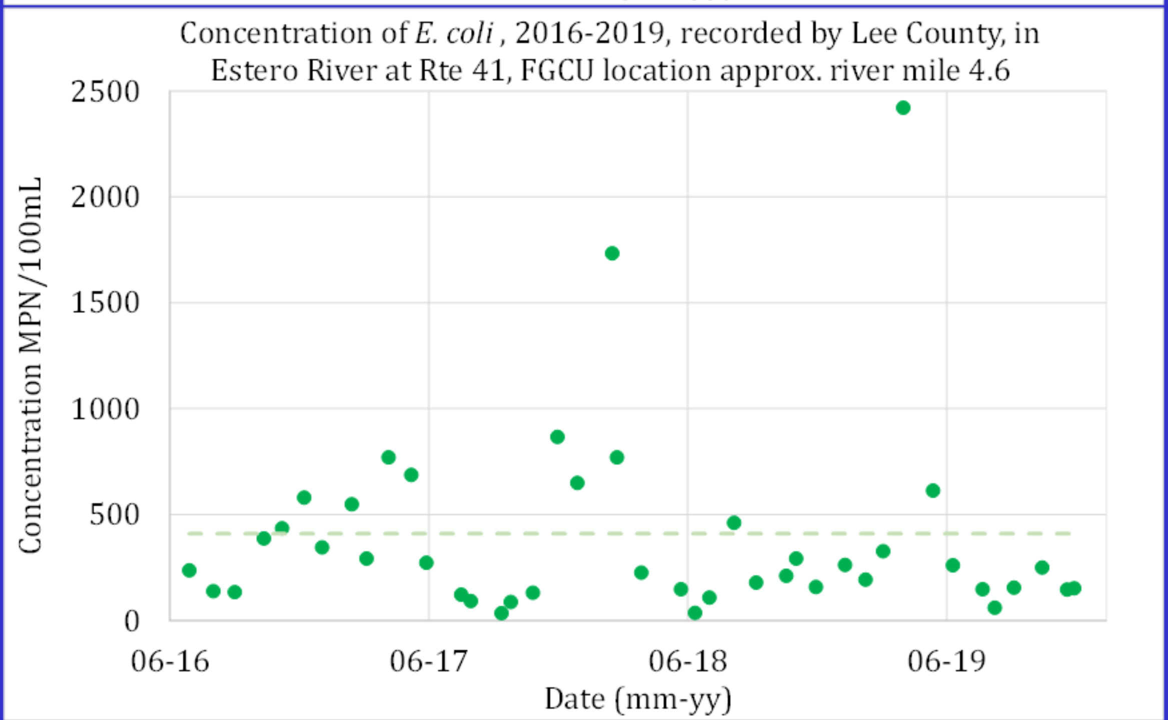
# Key findings: advances in knowledge

- High variability masks sources: not able to pinpoint without intensive studies. Typical in US waters, perhaps more so in S FL tidal waters
- Seasonal variability (wet-weather vs dry-weather) is not so powerful as to be visible among other factors: tides, source variability, precipitation/mobilization, human activities, animal activities, sediment disturbances, etc.
- Variation affected by factors not discernible here: tides, precipitation patterns, lawn activities, sediment disturbances – including very small scale
- Presence of FIB in some soils and in riverbed sediments suggests sediments may be proximate sources for some sampling events, so may confound ability to identify originating sources
- The two regulatory FIB (*Enterococcus, E. coli*) do not track one another – need to monitor both to verify condition of Estero River

# Questions

# Additional slides follow – in case of questions




# Intermediate location:

# Estero River at Rt 41 bridge

# *Enterococci* (top) and *E. coli* (bottom)

Lee County Natural Resources, June 2016-January 2020

Dashed line is TPTV, "ten percent threshold value," numeric target not to be exceeded by more than 10% of samples,

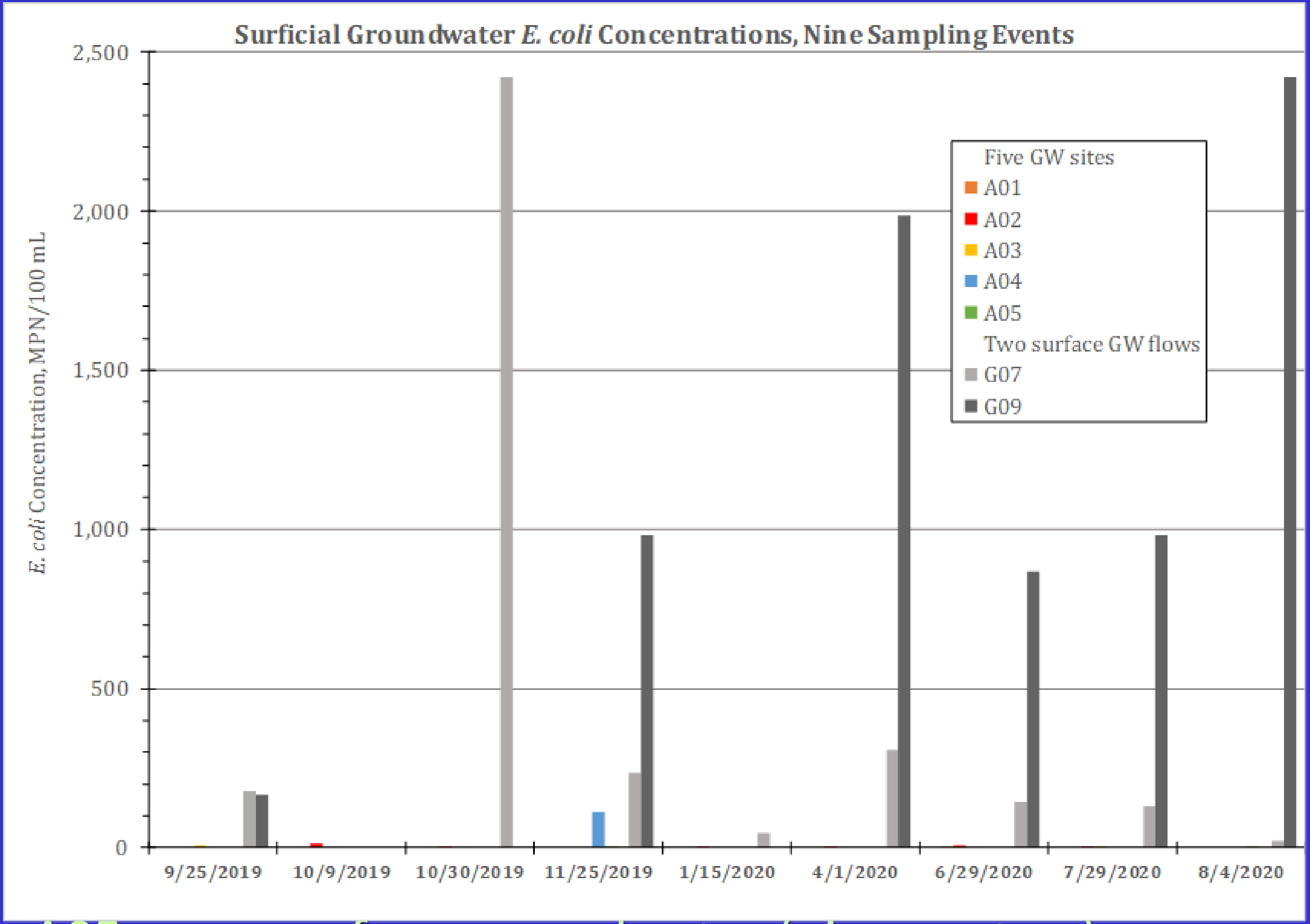
130 MPN/100mL *Enterococcus*

and 410 MPN/100mL *E. coli*

| Site name | River mile | FGCU unique identifier |
|---|---|---|
| Armada Ct below canal | 2.31 | G12 |
| Estero Ct above tributary | 2.56 | G11 |
| At boat launch near Broadway (Lee County 47A-4GR) | 3.17 | G10 |
| Below Tahiti | 3.51 | G01 |
| At Koreshan boat launch | 3.96 | G02 |
| At Sunny Grove | 4.23 | G03 |
| Below Rt 41 bridge (Lee County 47A-15GR) | 4.58 | G04 |
| At Sandy Lane bridge | 4.95 | G08 |
| S Branch – Country Ck Dr bridge | 5.52 | G05 |
| N Branch – Country Ck nr Candlewood Hollow | 5.74 | G06 |

| Site name | River mile | FGCU unique identifier |
| --- | --- | --- |
| Estero Bay Village near retaining wall | 3.70 | A01 |
| Estero Bay Village near “ditch” | 3.78 | A02 |
| Estero Bay Village near lagoon | 3.70 | A03 |
| Koreshan near boat launch | 3.96 | A04 |
| Charing Cross | 2.90 | A05 |
| Estero Bay Village “ditch” | 3.78 | G07 |
| Charing Cross “ditch” | 2.90 | G09 |

Ground-water at 5 piezo-meters and 2 surface flows




A01 through A05: near-surface groundwater (piezometers) – almost no *E. coli*

G07, Estero Bay Village "ditch", and G09, Charing Cross "ditch:" convey groundwater year-round, routinely high in *E. coli*

1. Data support numerous previous researchers in documenting decoupled variation between different species of FIB: *E.coli* and *Enterococci* varied in ways that did not correspond to one another in nearly all samples. This finding supports the conventional wisdom that no one species is an ideal indicator of potential presence of bacteria originating with human waste. Our data suggested that both bacterial species had multiple sources, which likely vary both temporally and spatially. As both FIB species are present to varying extent in humans and in other organisms, and any group of humans or other species will have both of these, and other organisms, present in their wastes in ways that vary between individuals, and between groups, over time both within the digestive track and in environmental systems affected by the wastes of warm-blooded species.

2. Data on FIB in the waters of the Estero River varied spatially and temporally. In almost no cases – wet or dry seasons, or in any run-of-the-river sample – was the MPN either high (above 1000 MPN/mL) or low (below 200 MPN/mL) in all locations sampled. The data thus show that spatial variability within the stream at a given time is greater than variability between times. That finding indicates that high MPN counts can be triggered by highly local and short-term events, and it is not clear if those events endure for hours, days, or weeks or whether they may have dissipated within hours after the sample was collected.

3. FIB concentration variability due to tidal mixing and transport is believed to be powerful, but known to be highly complex in a southwest Florida water such as Estero River with low freshwater flow that experiences semi-diurnal tides (two tides daily, on most days) of variable timing and magnitude. Two wet weather samples, and one dry weather sample, appear to show higher concentrations in the downstream portion (approximately river miles 2 through 3) where we would expect tidal action to produce conditions of resuspension of deposited sediment, or of tides 'piling up' freshwater discharges in a way that might concentrate suspended sediments, or both. That portion of the river was sampled only three times during wet weather and twice during dry weather. The results suggest that one or the other of those mechanisms, or both, might contribute to high FIB concentration under some conditions but not all. It is not possible to attribute those results to either high or low tide, or incoming or outgoing tide, because tidal conditions changed over the course of every 4-hour sampling event. Future research might further investigate that mechanism.

# Key findings

1. Routinely low FIB concentration in the upstream portion of the watershed strongly suggests there is little or no source from wild warm-blooded non-human animals in that undeveloped area. Increased (though highly variable) FIB concentration as the Estero River moves downstream through residential land uses indicates that either human activities, or animals coexisting with human activities, are the sources of FIB in the waterbody. As a point of comparison, data from two waterbodies studied by FGCU during this same time period in a nearby municipality (Spring Creek, Imperial River) showed that FIB concentrations were higher, though moderate, under most conditions in the upstream portions of the watershed, which have substantially higher development density than the Estero River reaches above river mile 6. Those two other waterbodies showed routinely increasing FIB concentrations as the streams moved downstream through developed residential areas. Those observations together with the Estero River data strongly suggest that dense residential land use corresponds to areas where bacteria enter the river.
2. The effect of several suspected source activities (small wastewater treatment facilities, septic systems, residential lawns used by pets extending directly to river's edge, and others) could not be reliably differentiated from other land uses, as there were no locations where persistent high concentrations were co-located with any of the suspected sources. The findings are consistent with all those sources, and more, contributing to the periodically very-high FIB concentrations on the Estero River.
3. It was expected that FIB concentration patterns would be different between wet-weather and dry-weather seasons. Instead, concentration patterns varied substantially among sampling events in each season, and no discernible pattern shows more variability between seasons than within seasons. The high variability of FIB concentration in the environment, and the high variability of source activities, outweighs any differences that may be produced by high or low in-stream flow diluting discrete discharges, or source-mobilizing action of precipitation events, in the samples collected for this study. Those effects may be present, but they do not influence the concentration at a given site or a given time to a discernible extent.

# Key findings

1. Although tested numbers of samples were small, our data showed river bed sediment, river bank soil, ditch water and road standing water harboured a large numbers of FIB and demonstrated that these could be potential sources of FIB input to the Estero River. Those sediments are not believed to be the point of origin of those FIB – they receive FIB from biological sources such as fecal matter originating with human wastewater or other warm-blooded animals – but short-term disturbance of river sediments, riverbank soils, or soil from the watershed mobilized by heavy precipitation – can theoretically trigger local and temporal high FIB events, and could be the proximal source of FIB measured in any one water sample.
2. Groundwater, in the areas studied, does not appear to convey large quantities of FIB to the Estero River, even though it does receive some human wastewater. That human wastewater appears to have any FIB satisfactorily attenuated by biological and physical activity in the soils before it reaches the river, and it is not likely that direct groundwater flows into the river are a major source of high MPN counts of FIB.
3. However, surface flows of discharging groundwater that has “short-circuit” the preferred underground path do appear to convey FIB to the Estero River. Surface flows in the “ditches” does not receive the same attenuation as groundwater; rather, the soil beneath the ditches appears to provide a stable environment for FIB, so that flowing water can re-suspend FIB and convey them to the river. It is not clear how large these contributions may be, or how many neighborhoods are drained by this kind of small surface discharge, but it could potentially be a significant source of FIB to the Estero River.

# Image of a field sheet