Biomonitor

Steuben County Lakes Council
317 South Wayne Street,
Suite 2A
Angola, IN 46703

STEUBEN COUNTY ENVIRONMENTAL DNA TESTING

INTRODUCTION

On July 6, 2022, 31 wet weather samples were collected in and around Steuben County to determine the source of elevated *Escherichia coli (E. coli)* concentrations at several locations throughout Steuben County. This was the third such sampling event, previous samples were collected in October 2020 and June 2021. At the request of the Steuben County Lakes Council, thirteen new sites were added for the 2022 sampling event. The monitoring sites are summarized in Table 1 and maps are included in Appendix A. These samples were analyzed for *E. coli* and the presence of human, swine, bovine, poultry, sheep, and dog DNA.

Site Number	Latitude	Longitude	Water Body	Years Sampled
3	41.729226	-84.824110	Clear Lake	2020
4	41.768574	-85.020190	Lake George Watershed	2020, 2021
5	41.776168	-85.001989	Lake George Watershed	2020
6	41.771334	-85.004431	Lake George Watershed	2020, 2021
7	41.655504	-85.019579	Carpenter Creek	2020, 2021
8	41.656863	-85.023053	Carpenter Creek	2020, 2021
9	41.660794	-85.028259	Carpenter Creek	2020, 2021
10	41.667092	-85.036215	Carpenter Creek	2020, 2021
11	41.668156	-85.036002	Carpenter Creek/Crooked Lake	2020, 2021
12	41.671376	-85.026319	Palfreyman Ditch	2020, 2021
13	41.664479	-85.018234	Palfreyman Ditch	2020, 2021
14	41.664136	-85.018111	Palfreyman Ditch	2020, 2021
15	41.664331	-85.017397	Palfreyman Ditch	2020, 2021
16	41.658881	-85.004190	Palfreyman Ditch	2020, 2021
17	41.719156	-85.033741	Snow Lake	2021
18	41.542063	-85.194894	McClish Lake	2021
19	41.546779	-85.191998	McClish Lake	2021
21	41.618222	-85.156835	Lake Arrowhead	2021
22	41.727454	-85.024531	Snow Lake	New
23	41.736383	-85.027020	Snow Lake	New
24	41.735771	-85.036618	Snow Lake	New
25	41.729612	-84.847775	Clear Lake	New
26	41.651418	-85.174436	Pigeon River	New
27	41.741473	-85.171456	Fawn River	New

Site Number	Latitude	Longitude	Water Body	New
28	41.558906	-84.835616	Fish Creek	New
29	41.631997	-84.937648	Pigeon River	New
30	41.625871	-84.930258	Jack Ditch	New
31	41.757408	-85.078012	Lake Pleasant	New
32	41.53942	-84.957821	Ball Lake	New
33	41.624472	-85.026793	Fox Lake	New
34	41.680974	-85.019476	Lake James	New

Table 1: July 6, 2022 Sampling Locations

RESULTS

E. coli samples were filtered and incubated in the field by Biomonitor to stay within the short holding time for bacterial samples. A field sheet showing sample collection data is in Appendix B. The complete laboratory report can be found in Appendix C. Elevated *E. coli* levels were detected at many of the sampling locations. A summary of the *E. Coli* results is displayed in Table 2 below.

The samples were also analyzed for human, dog, bovine, swine, poultry, and sheep DNA. The complete lab report can be found in Appendix D. A summary of the eDNA results is shown in Table 3 below. The filtered volume of sample is included in Table 3. Although this value has been normalized in the eDNA results, it is useful to consider the overall turbidity of the water body, since samples are filtered until the membrane fouls. Figure 1 shows a graph with eDNA results plotted alongside *E. coli* results.

Site Number	Water Body	Description	Estimated Flow	E Coli CFU/100 mL
3	Clear Lake	Across 114 Channel Drive	Med	2420
4	Lake George Watershed	Flint Road Boat ramp	Med	664
5	Lake George Watershed	Angola Road	High	182
6	Lake George Watershed	Kope Kon Road	High	524
7	Carpenter Creek	CR 155 W	Med	420
8	Carpenter Creek	CR 100 N	Med	2420
9	Carpenter Creek	CR 200 W	High	672
10	Carpenter Creek	Beach Road	High	2420
11	Carpenter Creek/Crooked Lake	Beach	NA	2
12	Palfreyman Ditch	CR 200 W	High	100
13	Palfreyman Ditch	Downstream Northwood	Med	816
14	Palfreyman Ditch	Northwood	Med	214
15	Palfreyman Ditch	Upstream Northwood	Med	1538
16	Palfreyman Ditch	Wohlert	Med	86
17	Snow Lake	Pokagon Treatment Plant	Med	0
18	McClish Lake	Discharge on west end	Low	276

Site Number	Water Body	Description	Estimated Flow	E Coli CFU/100 mL
19	McClish Lake	Inlet on east end	Low	2420
21	Lake Arrowhead	Ditch entering	High	2420
22	Snow Lake	Bridge on 175 West	Med	214
23	Snow Lake	Crooked Creek by the Hang Out	Med	829
24	Snow Lake	North Snow Bay near trailer park	None	4
25	Clear Lake	Tributary to Clear Lake	High	280
26	Pigeon River	At IND 327	High	470
27	Fawn River	At IND 327 near the fish hatchery	High	896
28	Fish Creek	At IND 427	High	2420
29	Pigeon River	At US 20 east of Angola	High	1210
30	Jack Ditch	Off HWY 1	Med	2420
31	Lake Pleasant	East end of lake	Low	546
32	Ball Lake	Ditch entering lake from east	High	2420
33	Fox Lake	At swimming beach	NA	54
34	Lake James	Discharge of Croxton Ditch	Med	890

Table 2: *E. Coli* Results Summary

**Conditional formatting shows green highlights for E. coli below the Indiana Recreational Limit of 235 MPN/100 mL and red highlights above 1000 MPN/100 mL*

***2420 CFU/100 mL is the upper detection limit of the test*

Site Number	Water Body	Filtered Volume (mL)	eDNA (Copies/100 mL)			
			Dog	Human	Poultry	Sheep
3	Clear Lake	60	31	1246	0	0
4	Lake George Watershed	20	0	1034	0	0
5	Lake George Watershed	180	0	725	0	0
6	Lake George Watershed	120	0	702	0	0
7	Carpenter Creek	30	0	655	0	0
8	Carpenter Creek	60	0	759	0	0
9	Carpenter Creek	60	0	692	6	0
10	Carpenter Creek	60	0	868	0	0
11	Carpenter Creek/Crooked Lake	180	0	78	0	0
12	Palfreyman Ditch	60	0	1360	0	0
13	Palfreyman Ditch	30	0	854	11	0
14	Palfreyman Ditch	120	0	364	0	0
15	Palfreyman Ditch	60	0	607	0	0
16	Palfreyman Ditch	60	0	338	0	0
17	Snow Lake	300	12	7367	32	0

Site Number	Water Body	Filtered Volume (mL)	eDNA (Copies/100 mL)			
			Dog	Human	Poultry	Sheep
18	McClish Lake	300	0	182	0	0
19	McClish Lake	65	0	242	0	0
21	Lake Arrowhead	80	0	0	0	0
22	Snow Lake	120	0	1063	0	0
23	Snow Lake	180	0	371	7	0
24	Snow Lake	180	0	128	0	0
25	Clear Lake	35	0	1303	0	0
26	Pigeon River	240	0	358	0	0
27	Fawn River	120	0	469	0	0
28	Fish Creek	15	11	955	0	8
29	Pigeon River	60	0	1139	0	0
30	Jack Ditch	30	0	381	0	0
31	Lake Pleasant	100	0	511	0	0
32	Ball Lake	30	0	324	0	1
33	Fox Lake	120	0	2126	0	0
34	Lake James	180	0	466	0	0

Table 3: eDNA Results Summary

**Conditional formatting shows green highlights for no eDNA detected and red highlights above 1000 copies/100 mL*

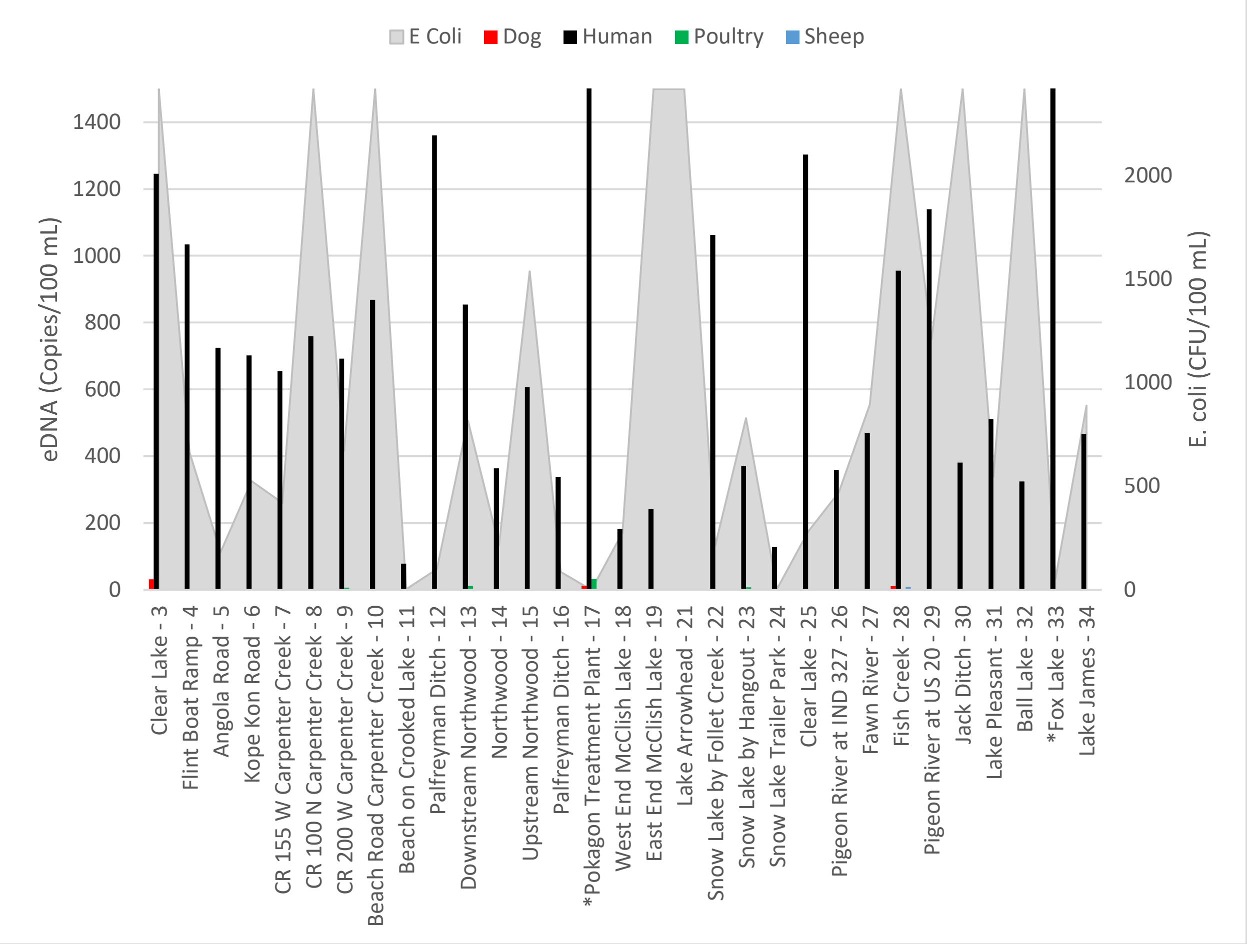


Figure 1: E. Coli and eDNA results

Human eDNA values for sites 17 and 33 were beyond the scale of the chart and are listed in Table 3

CONCLUSIONS

As in previous years, *E. coli* was detected at high concentrations at many locations throughout the sampling area. However, Human DNA was detected at levels much higher than seen in previous sampling events. Tables 4 and 5 compare *E. coli* and Human eDNA levels from the past three sampling events.

Site Number	Water Body	E. Coli (CFU/100 mL)		
		Jul-22	Jun-21	Oct-20
3	Clear Lake	*2420	NT	*2420
4	Lake George Watershed	664	185	*2419.6
5	Lake George Watershed	182	NT	28
6	Lake George Watershed	524	82	178
7	Carpenter Creek	420	1046	184
8	Carpenter Creek	*2420	687	727

Site Number	Water Body	E. Coli (CFU/100 mL)		
		Jul-22	Jun-21	Oct-20
9	Carpenter Creek	672	770	579
10	Carpenter Creek	*2420	249	1300
11	Carpenter Creek/Crooked Lake	2	25	4
12	Palfreyman Ditch	100	64	*2419.6
13	Palfreyman Ditch	816	*2420	*2419.6
14	Palfreyman Ditch	214	*2419.6	NT
15	Palfreyman Ditch	1538	1553	*2419.6
16	Palfreyman Ditch	86	1553	*2419.6
17	Snow Lake	0	2	NT
18	McClish Lake	276	308	NT
19	McClish Lake	*2420	64	NT
21	Lake Arrowhead	*2420	866	NT
22	Snow Lake	214	NT	NT
23	Snow Lake	829	NT	NT
24	Snow Lake	4	NT	NT
25	Clear Lake	280	NT	NT
26	Pigeon River	470	NT	NT
27	Fawn River	896	NT	NT
28	Fish Creek	*2420	NT	NT
29	Pigeon River	1210	NT	NT
30	Jack Ditch	*2420	NT	NT
31	Lake Pleasant	546	NT	NT
32	Ball Lake	*2420	NT	NT
33	Fox Lake	54	NT	NT
34	Lake James	890	NT	NT

Table 4: E. Coli results from October 2020, June 2021, and July 2022

**The upper detection limit of the m-coli blue E. coli test is 2420 CFU/100 mL*

***Conditional formatting shows green highlights for E. coli below the Indiana Recreational Limit of 235 MPN/100 mL and red highlights above 1000 MPN/100 mL*

****NT=Not Tested*

Site Number	Water Body	Human eDNA (Copies/100 mL)		
		Jul-22	Jun-21	Oct-20
3	Clear Lake	1246	NT	0
4	Lake George Watershed	1034	71	1
5	Lake George Watershed	725	NT	4
6	Lake George Watershed	702	44	31
7	Carpenter Creek	655	233	0

Site Number	Water Body	Human eDNA (Copies/100 mL)		
		Jul-22	Jun-21	Oct-20
8	Carpenter Creek	759	89	5
9	Carpenter Creek	692	258	0
10	Carpenter Creek	868	110	18
11	Carpenter Creek/Crooked Lake	78	36	33
12	Palfreyman Ditch	1360	149	0
13	Palfreyman Ditch	854	264	60
14	Palfreyman Ditch	364	90	NT
15	Palfreyman Ditch	607	117	144
16	Palfreyman Ditch	338	193	40
17	Snow Lake	7367	1631	NT
18	McClish Lake	182	78	NT
19	McClish Lake	242	230	NT
21	Lake Arrowhead	0	28	NT
22	Snow Lake	1063	NT	NT
23	Snow Lake	371	NT	NT
24	Snow Lake	128	NT	NT
25	Clear Lake	1303	NT	NT
26	Pigeon River	358	NT	NT
27	Fawn River	469	NT	NT
28	Fish Creek	955	NT	NT
29	Pigeon River	1139	NT	NT
30	Jack Ditch	381	NT	NT
31	Lake Pleasant	511	NT	NT
32	Ball Lake	324	NT	NT
33	Fox Lake	2126	NT	NT
34	Lake James	466	NT	NT

Table 5: eDNA results from October 2020, June 2021, and July 2022

**Conditional formatting shows green highlights for no eDNA detected and red highlights above 1000 copies/100 mL*

***NT = Not Tested*

There are some key differences in sampling conditions between these three events that could explain the discrepancies. The rain event that occurred on July 5th through the morning of July 6th was much larger than the rain events from previous years. This led to a change in water level which was greater than previously seen. Figures 2, 3, and 4 below show the United States Geological Survey (USGS) stream gage data for Pigeon Creek near Angola for the weeks surrounding the 2020, 2021, and 2022 sampling events. Additionally, the 2022 samples were collected immediately after the weekend of the 4th of July, which could lead to increased recreation throughout Steuben County. This is in contrast with the 2020 samples which were collected in the fall and the 2021 which were collected towards the beginning of the recreation season.

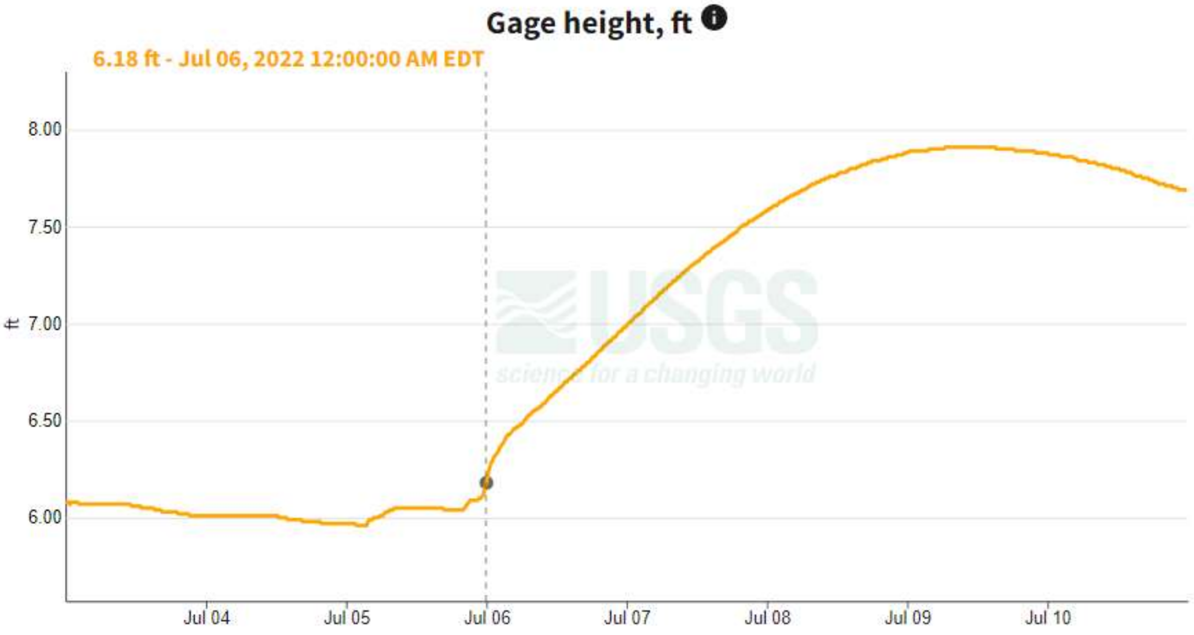


Figure 2: USGS Stream Gage Data for Pigeon Creek from July 3, 2022 to July 10, 2022

**Orange color shows provisional data*

***Samples collected beginning at 7:39 AM on July 6, 2022*

Figure 3: USGS Stream Gage Data for Pigeon Creek from June 7, 2021 to June 14, 2021

**Samples collected beginning at 9:26 AM on June 10, 2022*

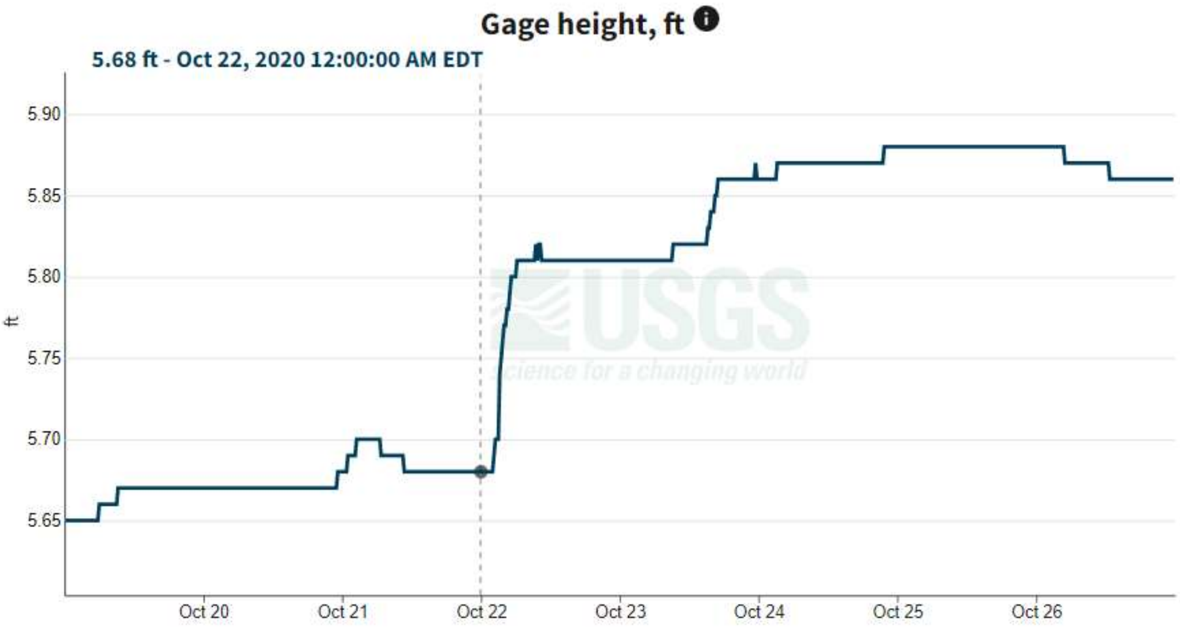


Figure 4: USGS Stream Gage Data for Pigeon Creek from October 19, 2020 to October 26, 2020
**Samples collected beginning at 10:15 AM on October 22, 2020*

Although values for human DNA in the low thousands seem incredibly high when compared to previous sampling efforts, it is worth noting that this data is not unusual when compared to other source tracking data collected. Figure 5 below shows a compilation of all the source tracking data analyzed by JonahVentures in the past year. Density refers to the relative likelihood to encounter host DNA at a given level across samples. Humans are far and away the most common source of environmental DNA and values a little over 1000 copies/100 mL are the most common.

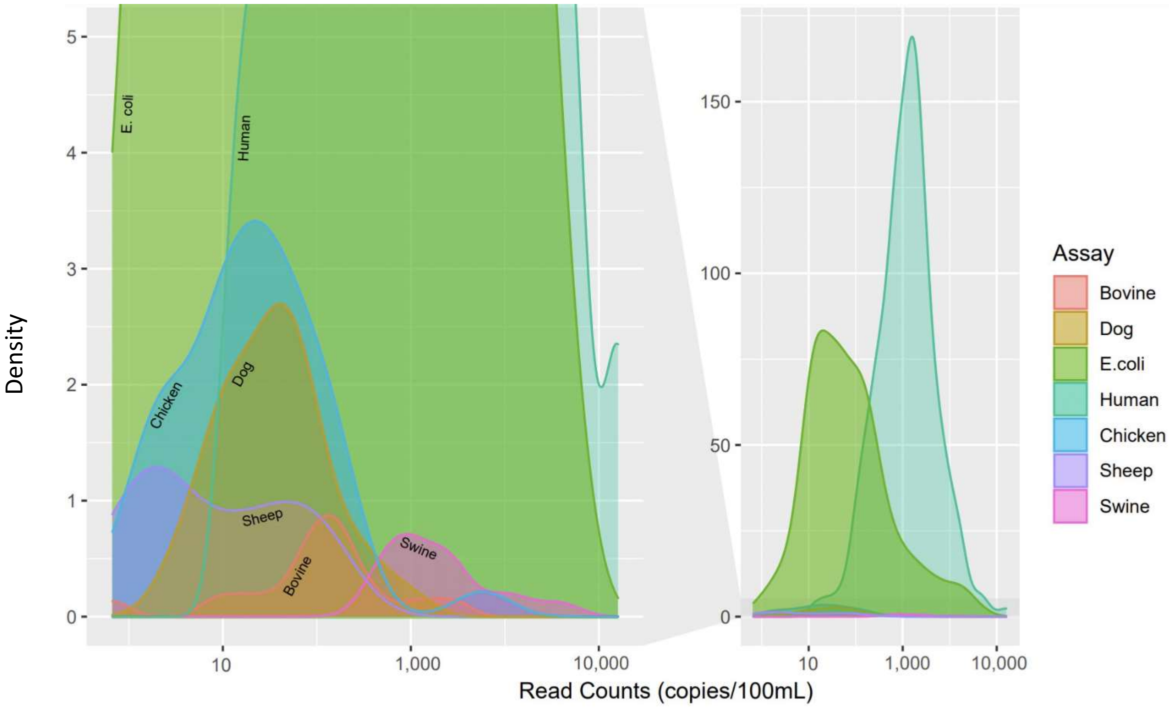


Figure 5: Graph generated by JonahVentures displaying all E. coli source tracking data analyzed by their laboratory in the past year

There are a few outliers that require further explanation and some interesting findings to discuss. Once again, the highest levels of eDNA were found at site 17. Sample 17 was collected from the effluent of the wastewater treatment plant at Pokagon State Park. The treatment process removes *E. coli* and other fecal coliforms from the water, but DNA from human cells remain. The presence of poultry DNA can be explained by the diets of the people discharging their sewage to the plant. One potential explanation for sample 17 having an increased level of human DNA in 2022 compared to 2021 could be the increased use the park saw over the holiday weekend.

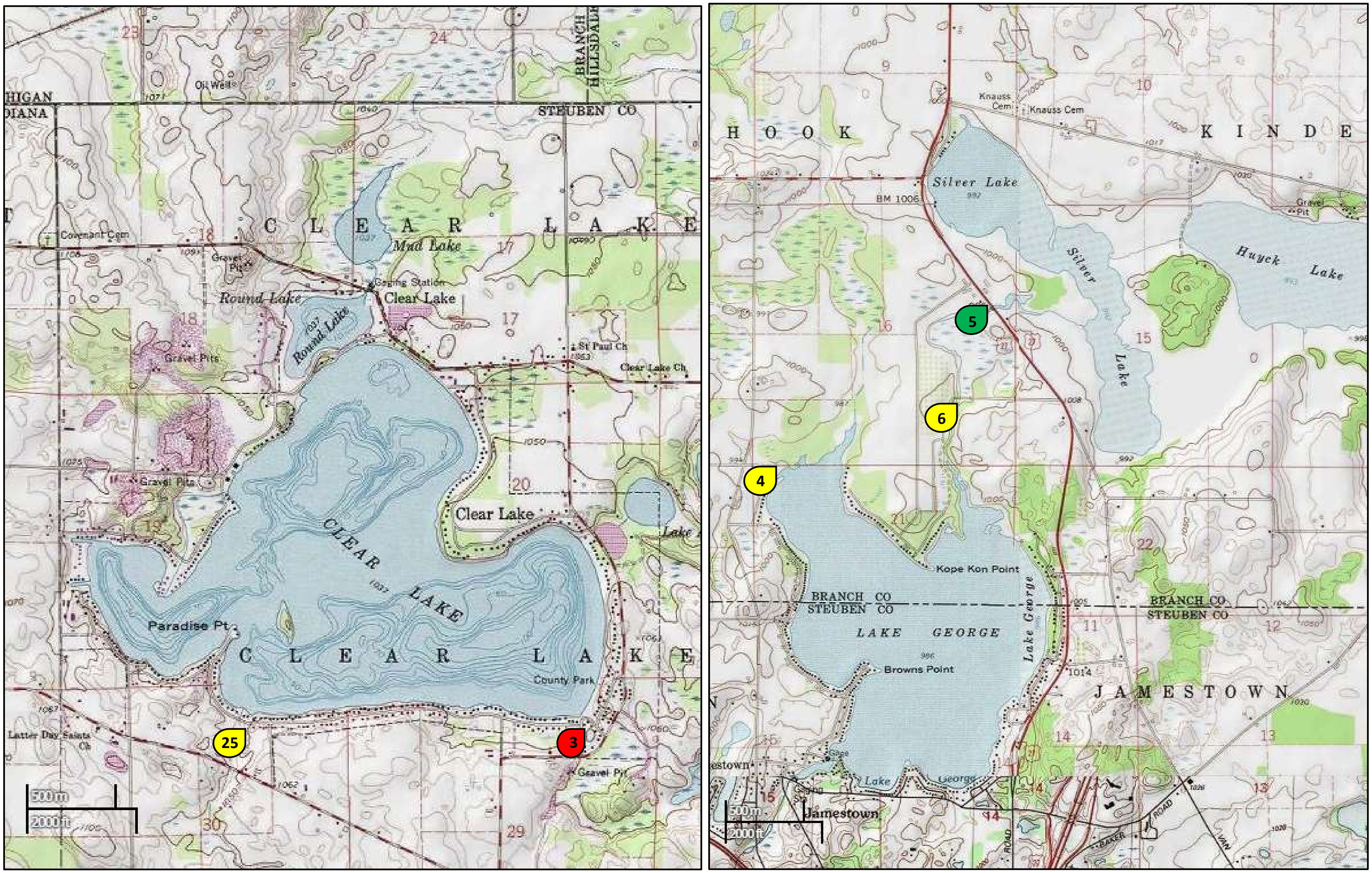
Site 33 is another interesting case. This sample was collected from the swimming beach at Fox Lake. At the time of sample collection, several people were swimming at the beach. Although the sample was collected towards the end of the pier away from the swimmers, levels of human DNA were much higher than those observed at other locations. In contrast, levels of *E. coli* were within safe recreation levels. This provides evidence that humans may be leaving more DNA in the water when swimming than previously thought.

Another set of interesting results are the three samples around Northwood. Those are sites 13, 14, and 15. This is the first year where the small ditch exiting Northwood and flowing into Palfreyman Ditch (site 14) had flowing water. In 2020 site 14 was dry and no sample was collected. In 2021, there was no flowing water but a sample was collected from a puddle in the ditch. This year, Biomonitor's field staff noted that the water exiting Northwood was flowing and was visibly clear. Included in Appendix E are two photos which show Biomonitor's staff in the field. Although not the primary focus of the photos, the clear water exiting Northwood can be seen in one photo and Palfreyman Ditch can be seen in the other photo. These visual observations of water quality were supported by the data. *E. coli* levels upstream of Northwood (site 15) were highest with an *E. coli* reading of 1538 CFU/ 100 mL. The water exiting Northwood had surprisingly low levels of *E. coli,* below the limit for safe recreation. Downstream of Northwood (site 13) the two streams had mixed leading to an *E. coli* result of 816 CFU/100 mL. In all three of these samples human DNA was the primary source of *E. coli* detected. Improperly maintained septic tanks continue to be a potential source of *E. coli* but this most recent sampling event rules out the Northwood subdivision as a key contributor to that problem.

Finally, it is clear from these results and from previous sampling evidence that the overwhelming source of the *E. coli* problem in Steuben County is human waste. This has also been seen in other sampling efforts throughout the country as seen in Figure 5. Although humans are leaving behind DNA when doing normal recreation activities, the lack of DNA evidence pointing to any other source of *E. coli* leads to the conclusion that human waste is the major problem. Unfortunately, the exact source of this human waste is still unknown. Possibilities include unsafe waste practices during recreational activities such as camping or improperly maintained septic systems.

APPENDIX A – July 2022 Sampling Maps
APPENDIX B – July 2022 Field Sheet
APPENDIX C – July 2022 E. coli Lab Report
APPENDIX D – July 2022 eDNA Lab Report
APPENDIX E – July 2022 Photos

APPENDIX A – July 2022 Sampling Maps



Steuben County eDNA Sampling Locations with *Escherichia coli* Results

Legend:

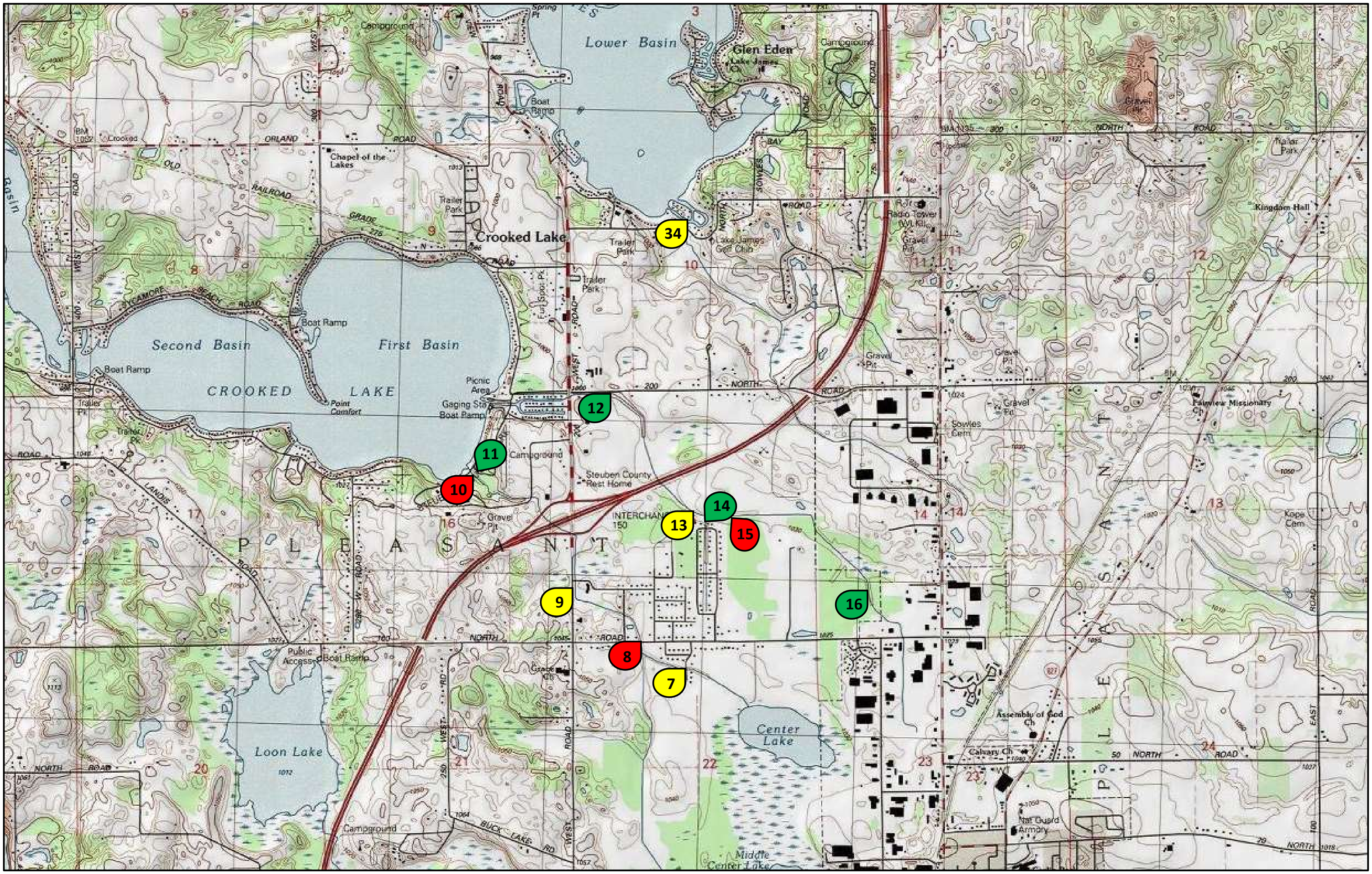
17 Site ID

July 2022 *E. Coli* results:

🟩	0 - 234 MPN/100 mL
🟨	235 - 999 MPN/100 mL
🟥	1000 – 2420 MPN/100 mL

BASEMAP:
USGS Topographic Maps 2000-2022

Biomonitor



Steuben County eDNA Sampling Locations with *Escherichia coli* Results

Legend:

(17) Site ID

July 2022 *E. Coli* results:

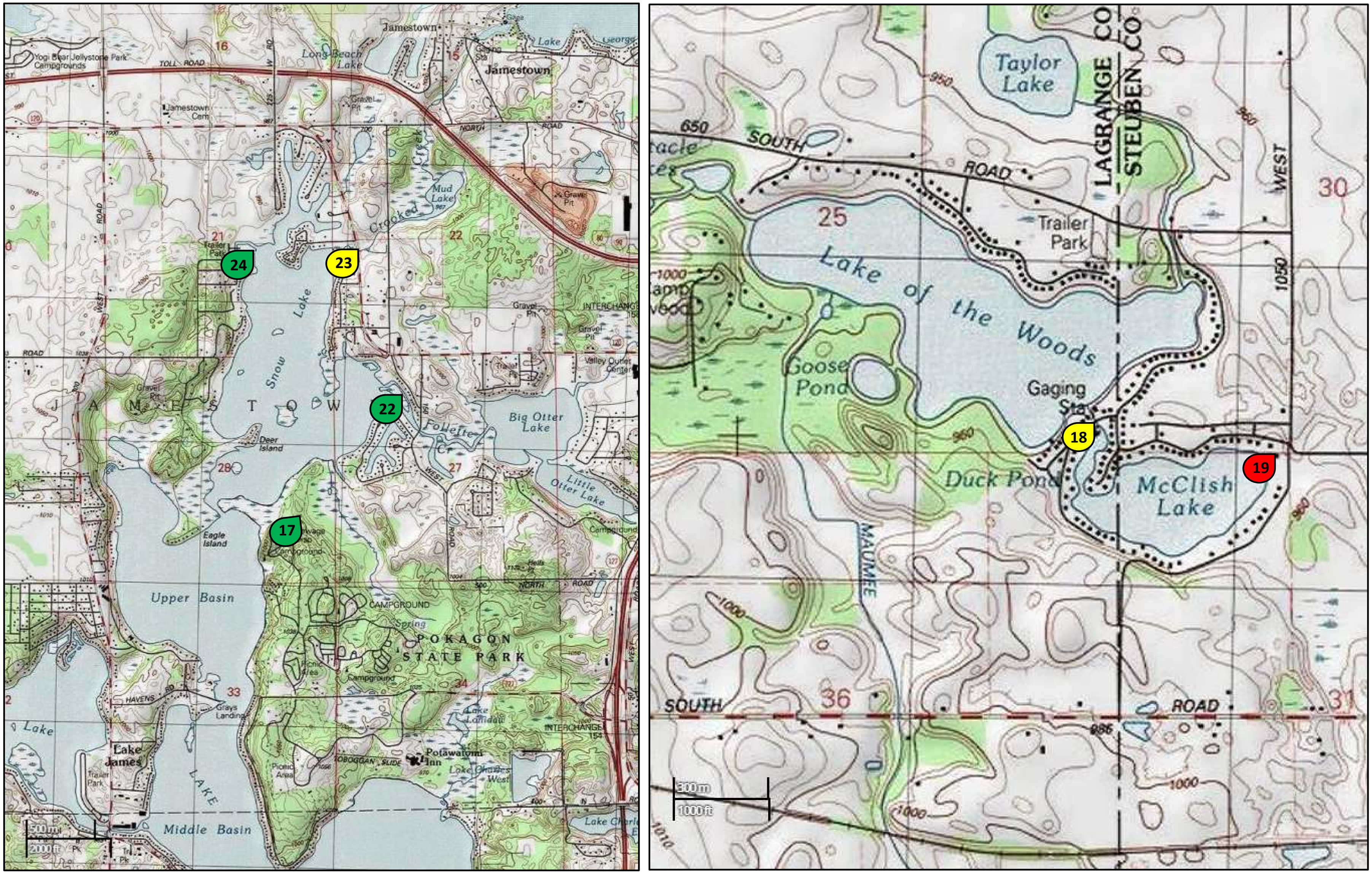
🟩 0 - 234 MPN/100 mL

🟨 235 - 999 MPN/100 mL

🟥 1000 – 2420 MPN/100 mL

BASEMAP:
USGS Topographic Maps 2000-2022

Biomonitor



Steuben County eDNA Sampling Locations with *Escherichia coli* Results

Legend:

17 Site ID

July 2022 *E. Coli* results:

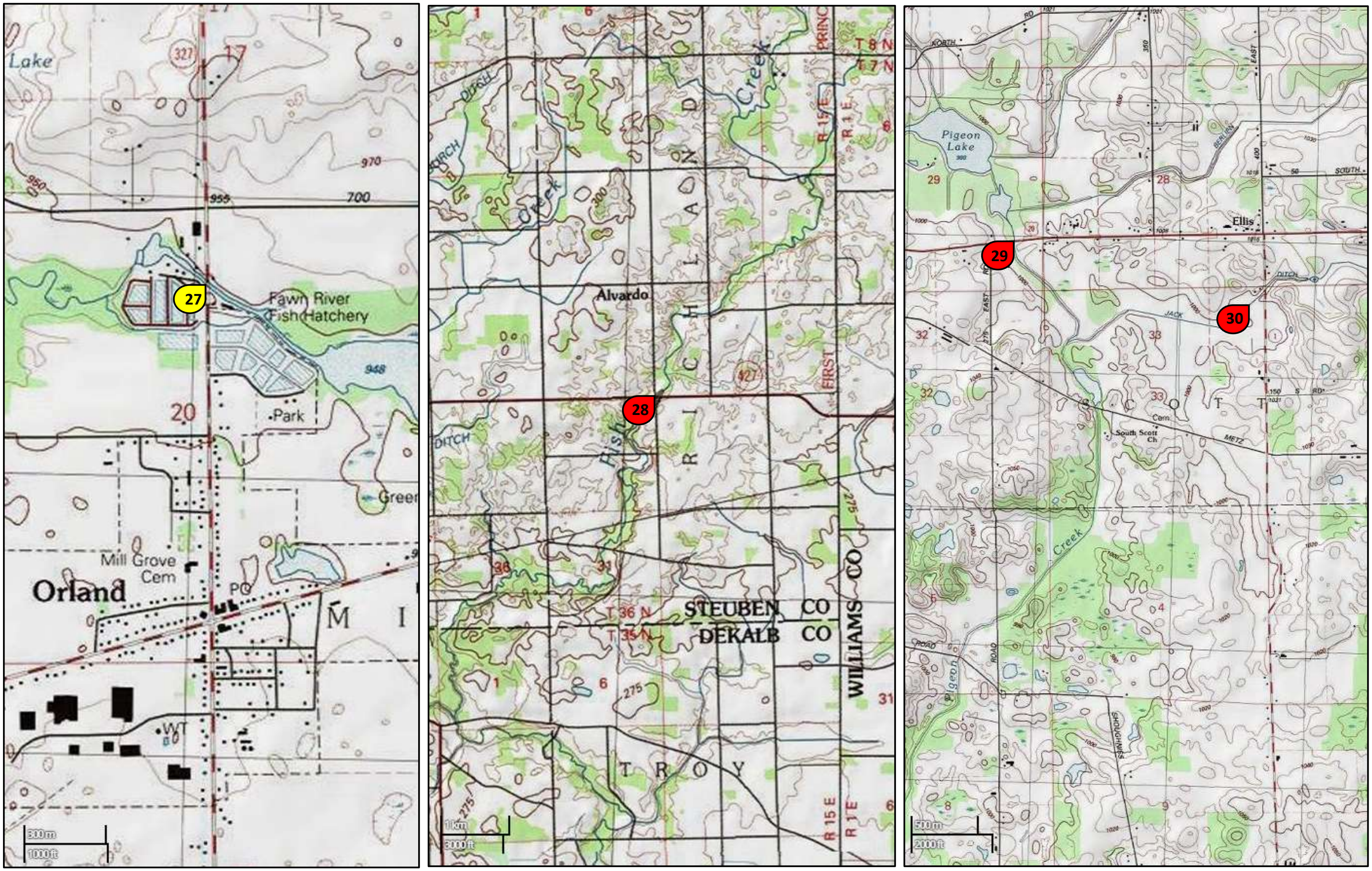
🟩 0 - 234 MPN/100 mL

🟨 235 - 999 MPN/100 mL

🟥 1000 – 2420 MPN/100 mL

BASEMAP:
USGS Topographic Maps 2000-2022

Biomonitor



Steuben County eDNA Sampling Locations with *Escherichia coli* Results

Legend:

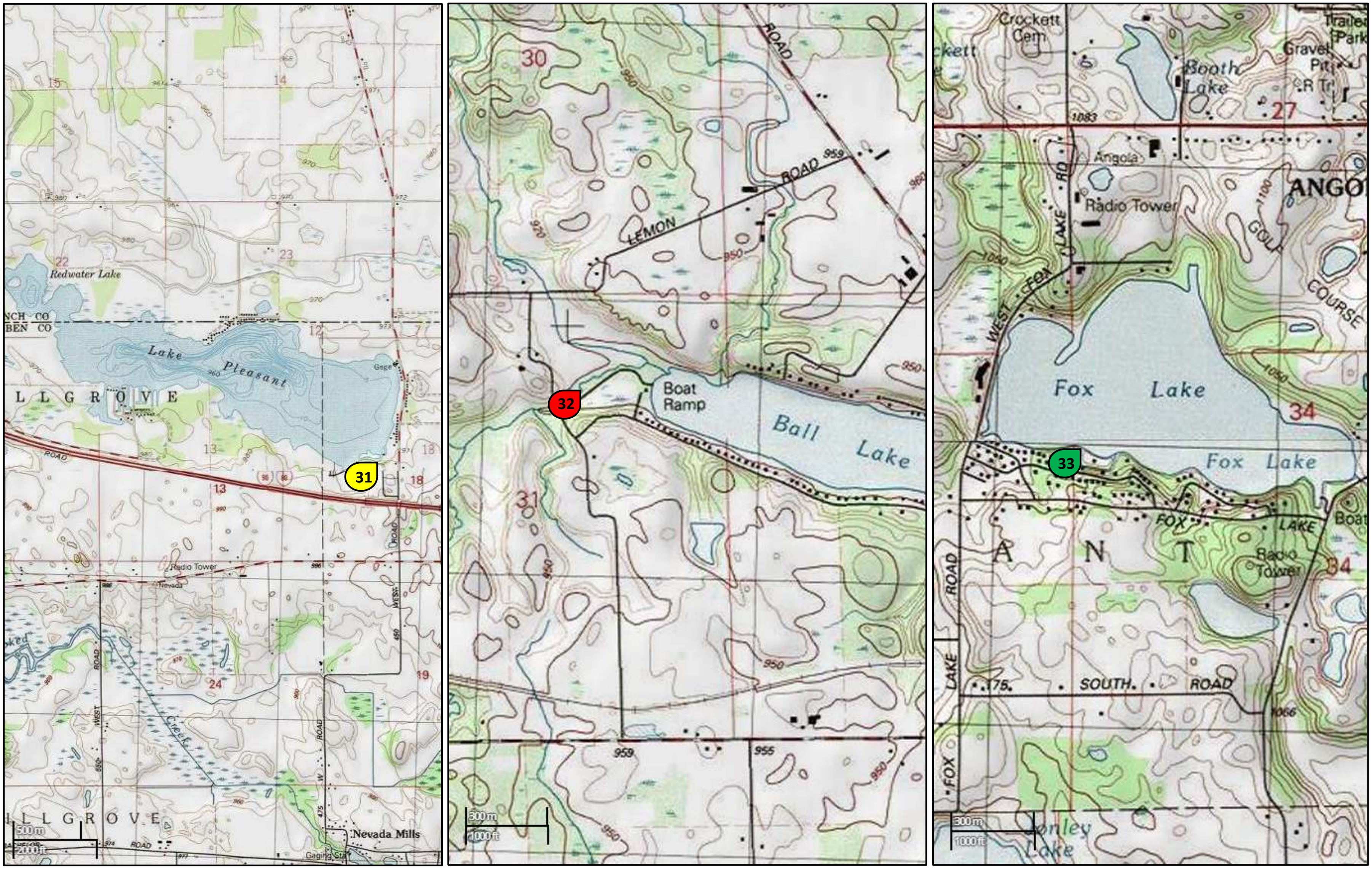
(17) Site ID

July 2022 *E. Coli* results:

- 🟢 0 - 234 MPN/100 mL
- 🟡 235 - 999 MPN/100 mL
- 🔴 1000 – 2420 MPN/100 mL

BASEMAP:
USGS Topographic Maps 2000-2022

Biomonitor



Steuben County eDNA Sampling Locations with *Escherichia coli* Results

Legend:

17 Site ID

July 2022 *E. Coli* results:

🟩 0 - 234 MPN/100 mL

🟨 235 - 999 MPN/100 mL

🟥 1000 – 2420 MPN/100 mL

BASEMAP:
USGS Topographic Maps 2000-2022

Biomonitor

APPENDIX B – July 2022 Field Sheet

*132A6C6M is 18

Site Number	Time Sampled	Sample Code	Filtered Volume	Estimated Flow
19	7:39	132A6C6M	65mL	Low +
18	7:51	LJ04YK07	300 mL	Low
21	8:16	NDC8ZFR3	80mL	High
26	8:29+1	03D2IU2H	240 mL	High
27	8:42	4T0NDX0F	120 mL	High
31	8:58	933H245V	100mL	Low
4	9:15	LCAM4GW1	20 mL	Med.
6	9:40	I377HCH1	120 mL	High
5	9:27	HJB6BRJW	180 mL	M/High
25	10:03	09AYDUIU	35 mL	High
3	10:18	DOQJ9XYI	60 mL	Med.
22	10:41	524FZAD0	120 mL	Med
23	10:51	Z11ZE71Q	180 mL	Med
24	11:06	5BHCJT4D	180 mL	Low/None
17	11:38	4WXGH4RB	300 mL	~~Low~~ Med/Avg.
34	12:01	KECWOOVW	180 mL	Med.
12	12:10	L25BIHW8	60 mL	High
10	12:28	MZBSJ8FK	60mL	High
11	12:25	IOIQ48Z6	180mL	NA
9	12:39	HDY2L8AP	60 mL	High
8	12:45	E1WSN6P8	60mL	Med.
7	12:52	WA0K9DN8	30 mL	Med.
16	1:01	VWDBTTXT	60 mL	Med.
15	1:11	IIWXRY93	60 mL	Med
14	1:15	C8Y9B3I6	120 mL	Med
13	1:20	8QTBXR92	60 mL	Med
33	2:09	Z9XNXYAN	120 mL	NA
29	2:29	18YXTAK4	60mL	High
30	2:54	QGZZGE11	30mL	Med.
28	3:08	SHVGGMEJ	15 mL	High
32	3:30	ZG8GZOZW	30 mL	High

sampled on bridge next to 150W

@bridge on Hwy 1

L.

APPENDIX C – July 2022 E. coli Lab Report

Simultaneous Detection and Enumeration of Total Coliforms and *Escherichia coli* using m-ColiBlue24 Membrane Filtration

July 7, 2022

Steuben County Lakes Council *E. coli* Source Tracking:

The following table includes results from 32 total samples. This includes 31 site samples and a lab blank (LB). Samples collected and cultures inoculated by Biomonitor laboratory personnel on July 7, 2022.

SAMPLE ID	*E. coli* CFU/100ml
Site – 3	>2420
Site – 4	664
Site – 5	182
Site – 6	524
Site – 7	420
Site – 8	>2420
Site – 9	672
Site – 10	>2420
Site – 11	2
Site – 12	100
Site – 13	816
Site – 14	214
Site – 15	1538
Site – 16	86
Site – 17	0
Site – 18	276
Site – 19	>2420
Site – 21	>2420
Site – 22	214
Site – 23	829
Site – 24	4
Site – 25	280
Site – 26	470
Site – 27	896
Site – 28	>2420
Site – 29	1210
Site – 30	>2420
Site – 31	546
Site – 32	>2420
Site – 33	54
Site – 34	890
LB	0

APPENDIX D – July 2022 eDNA Lab Report

JONAH VENTURES
KNOWLEDGE IN SEQUENCE

July 29 2022

For more information go to www.jonahventures.com

info@jonahventures.com

Report prepared for arizona@biomonitor.com

BatchId = JVB1772

Number of samples analyzed = 31

Average number of copies detected

This table shows the average number of copies detected for each target organism in each sample. Values represent the average number of copies / 100 mL when sample volume was provided. When sample volumes are not known, the values indicate the estimated number of copies in the sample.

SampleId	Bovine01	Dog01	E.coli01	Human01	Poultry01	Sheep01	Swine01
03D2IU2H	0	0	107	358	0	0	0
132A6C6M	0	0	7	182	0	0	0
18YXTAK4	0	0	137	1139	0	0	0
4T0NDXOF	0	0	150	469	0	0	0
4WXGH4RB	0	12	40	7367	32	0	0
524F2AD0	0	0	27	1063	0	0	0
5BHCJT4D	0	0	3	128	0	0	0
8QTBXR92	0	0	79	854	11	0	0
933H245V	0	0	198	511	0	0	0
C8Y9B3I6	0	0	90	364	0	0	0
DOQJ9XYI	0	31	206	1246	0	0	0
E1WSN6P8	0	0	496	759	0	0	0
HDY2L8AP	0	0	501	692	6	0	0
HJB6BRJW	0	0	12	725	0	0	0
I0IQ48Z6	0	0	123	78	0	0	0
I377HCH1	0	0	63	702	0	0	0
IIWXRY93	0	0	184	607	0	0	0
KECWOOVW	0	0	67	466	0	0	0
L25BIHW8	0	0	279	1360	0	0	0
LCAM4GW1	0	0	157	1034	0	0	0
LJ04YK07	0	0	166	242	0	0	0
M2BSJ8FK	0	0	97	868	0	0	0
NDC8ZFR3	0	0	103	0	0	0	0
O9AYDU1U	0	0	125	1303	0	0	0
QG2ZGE11	0	0	157	381	0	0	0
SHV6GMEJ	0	11	1283	955	0	8	0
VWDBTTXT	0	0	68	338	0	0	0
WA0K9DN8	0	0	342	655	0	0	0
Z11ZE71Q	0	0	47	371	7	0	0
Z9XNXYAN	0	0	24	2126	0	0	0
ZG8GZOZK	0	0	46	324	0	1	0

Percent of replicates above detection limit

This table provides data on what percentage of the replicates that were run were above the detection limit. The detection limit is as high as the lowest positive on the calibration curve, but can be up to an order of magnitude lower. For example, a calibration curve might generate a positive at 100 copies and no positive for 10 copies, but the actual detection limit would be 11 copies. See the next section for the range of copy numbers estimated for each assay.

SampleId	Bovine01	Dog01	E.coli01	Human01	Poultry01	Sheep01	Swine01
03D2IU2H	0	0.0	100.0	66.7	0.0	0.0	0
132A6C6M	0	0.0	100.0	33.3	0.0	33.3	0
18YXTAK4	0	0.0	100.0	66.7	0.0	0.0	0
4T0NDXOF	0	0.0	100.0	66.7	0.0	0.0	0
4WXGH4RB	0	33.3	100.0	100.0	66.7	0.0	0
524F2AD0	0	0.0	100.0	100.0	0.0	0.0	0
5BHCJT4D	0	0.0	33.3	33.3	0.0	0.0	0
8QTBXR92	0	0.0	100.0	66.7	66.7	0.0	0
933H245V	0	0.0	100.0	66.7	0.0	0.0	0
C8Y9B3I6	0	0.0	100.0	66.7	0.0	0.0	0
DOQJ9XYI	0	33.3	100.0	66.7	0.0	0.0	0
E1WSN6P8	0	0.0	100.0	66.7	0.0	0.0	0
HDY2L8AP	0	0.0	100.0	66.7	33.3	0.0	0
HJB6BRJW	0	0.0	66.7	66.7	0.0	0.0	0
I0IQ48Z6	0	0.0	100.0	33.3	0.0	0.0	0
I377HCH1	0	0.0	100.0	66.7	0.0	0.0	0
IIWXRY93	0	0.0	100.0	66.7	0.0	0.0	0
KECWOOVW	0	0.0	100.0	33.3	0.0	0.0	0
L25BIHW8	0	0.0	100.0	66.7	0.0	0.0	0
LCAM4GW1	0	0.0	100.0	33.3	0.0	0.0	0
LJ04YK07	0	0.0	100.0	66.7	0.0	0.0	0
M2BSJ8FK	0	0.0	100.0	66.7	33.3	0.0	0
NDC8ZFR3	0	0.0	100.0	0.0	0.0	0.0	0
O9AYDU1U	0	0.0	100.0	100.0	0.0	0.0	0
QG2ZGE11	0	0.0	100.0	66.7	0.0	0.0	0
SHV6GMEJ	0	33.3	100.0	66.7	0.0	66.7	0
VWDBTTXT	0	0.0	100.0	66.7	0.0	0.0	0
WA0K9DN8	0	0.0	100.0	100.0	0.0	0.0	0
Z11ZE71Q	0	0.0	100.0	66.7	33.3	0.0	0
Z9XNXYAN	0	0.0	100.0	100.0	0.0	0.0	0
ZG8GZOZK	0	0.0	100.0	100.0	0.0	66.7	0

Detailed results

The following table provides the estimated copy number for individual technical replicates for each qPCR assay. Missing values indicate failed reactions or outliers that were removed from the analysis.

SampleId	Rep 1	Rep 2	Rep 3
Bovine01			
03D2IU2H	0	0	0
132A6C6M	0	0	0
18YXTAK4	0	0	0
4T0NDXOF	0	0	0
4WXGH4RB	0	0	0
524F2AD0	0	0	0
5BHCJT4D	0	0	0
8QTBXR92	0	0	0
933H245V	0	0	0
C8Y9B3I6	0	0	0
DOQJ9XYI	0	0	0
E1WSN6P8	0	0	0
HDY2L8AP	0	0	0
HJB6BRJW	0	0	0
I0IQ48Z6	0	0	0
I377HCH1	0	0	0
IIWXRY93	0	0	0
KECWOOVW	0	0	0
L25BIHW8	0	0	0
LCAM4GW1	0	0	0
LJ04YK07	0	0	0
M2BSJ8FK	0	0	0
NDC8ZFR3	0	0	0
O9AYDU1U	0	0	0
QG2ZGE11	0	0	0
SHV6GMEJ	0	0	0
VWDBTTXT	0	0	0
WA0K9DN8	0	0	0
Z11ZE71Q	0	0	0
Z9XNXYAN	0	0	0
ZG8GZOZK	0	0	0
Dog01			
03D2IU2H	0	0	0
132A6C6M	0	0	0
18YXTAK4	0	0	0
4T0NDXOF	0	0	0
4WXGH4RB	35	0	0
524F2AD0	0	0	0
5BHCJT4D	0	0	0
8QTBXR92	0	0	0
933H245V	0	0	0
C8Y9B3I6	0	0	0
DOQJ9XYI	94	0	0
E1WSN6P8	0	0	0

HDY2L8AP	0	0	0
HJB6BRJW	0	0	0
I0IQ48Z6	0	0	0
I377HCH1	0	0	0
IIWXRY93	0	0	0
KECWOOVW	0	0	0
L25BIHW8	0	0	0
LCAM4GW1	0	0	0
LJ04YK07	0	0	0
M2BSJ8FK	0	0	0
NDC8ZFR3	0	0	0
O9AYDU1U	0	0	0
QG2ZGE11	0	0	0
SHV6GMEJ	34	0	0
VWDBTTXT	0	0	0
WA0K9DN8	0	0	0
Z11ZE71Q	0	0	0
Z9XNXYAN	0	0	0
ZG8GZOZK	0	0	0
E.coli01			
03D2IU2H	80	115	125
132A6C6M	3	8	9
18YXTAK4	141	125	144
4T0NDXOF	175	126	150
4WXGH4RB	39	35	47
524F2AD0	29	38	14
5BHCJT4D	0	0	10
8QTBXR92	69	80	87
933H245V	195	179	220
C8Y9B3I6	62	83	126
DOQJ9XYI	253	158	206
E1WSN6P8	487	511	490
HDY2L8AP	500	459	543
HJB6BRJW	14	0	22
I0IQ48Z6	152	85	132
I377HCH1	52	63	73
IIWXRY93	193	194	166
KECWOOVW	35	63	104
L25BIHW8	349	253	236
LCAM4GW1	164	138	170
LJ04YK07	182	134	183
M2BSJ8FK	58	120	114
NDC8ZFR3	120	53	135
O9AYDU1U	133	155	86
QG2ZGE11	106	209	156
SHV6GMEJ	1306	1141	1401
VWDBTTXT	58	86	59
WA0K9DN8	292	365	368
Z11ZE71Q	82	39	19
Z9XNXYAN	4	21	48
ZG8GZOZK	28	41	68
Human01			

03D2IU2H	0	665	410
132A6C6M	545	0	0
18YXTAK4	0	1533	1883
4T0NDXOF	1014	0	393
4WXGH4RB	6458	8258	7384
524F2AD0	430	2017	742
5BHCJT4D	0	0	384
8QTBXR92	1220	0	1343
933H245V	0	686	846
C8Y9B3I6	710	0	381
DOQJ9XYI	2190	0	1548
E1WSN6P8	0	829	1449
HDY2L8AP	383	0	1694
HJB6BRJW	1469	707	0
I0IQ48Z6	234	0	0
I377HCH1	1126	980	0
IIWXRY93	447	1373	0
KECWOOVW	1398	0	0
L25BIHW8	1129	0	2952
LCAM4GW1	0	0	3103
LJ04YK07	365	361	0
M2BSJ8FK	0	1823	781
NDC8ZFR3	0	0	0
O9AYDU1U	1218	1418	1274
QG2ZGE11	0	387	756
SHV6GMEJ	0	1998	867
VWDBTTXT	0	364	651
WA0K9DN8	697	746	523
Z11ZE71Q	886	226	0
Z9XNXYAN	1664	2653	2061
ZG8GZOZK	617	351	5
Poultry01			
03D2IU2H	0	0	0
132A6C6M	0	0	0
18YXTAK4	0	0	0
4T0NDXOF	0	0	0
4WXGH4RB	0	49	48
524F2AD0	0	0	0
5BHCJT4D	0	0	0
8QTBXR92	0	22	11
933H245V	0	0	0
C8Y9B3I6	0	0	0
DOQJ9XYI	0	0	0
E1WSN6P8	0	0	0
HDY2L8AP	0	17	0
HJB6BRJW	0	0	0
I0IQ48Z6	0	0	0
I377HCH1	0	0	0
IIWXRY93	0	0	0
KECWOOVW	0	0	0
L25BIHW8	0	0	0
LCAM4GW1	0	0	0
LJ04YK07	0	0	0

M2BSJ8FK	0	0	1
NDC8ZFR3	0	0	0
O9AYDU1U	0	0	0
QG2ZGE11	0	0	0
SHV6GMEJ	0	0	0
VWDBTTXT	0	0	0
WA0K9DN8	0	0	0
Z11ZE71Q	0	22	0
Z9XNXYAN	0	0	0
ZG8GZOZK	0	0	0
Sheep01			
03D2IU2H	0	0	0
132A6C6M	0	0	1
18YXTAK4	0	0	0
4T0NDXOF	0	0	0
4WXGH4RB	0	0	0
524F2AD0	0	0	0
5BHCJT4D	0	0	0
8QTBXR92	0	0	0
933H245V	0	0	0
C8Y9B3I6	0	0	0
DOQJ9XYI	0	0	0
E1WSN6P8	0	0	0
HDY2L8AP	0	0	0
HJB6BRJW	0	0	0
I0IQ48Z6	0	0	0
I377HCH1	0	0	0
IIWXRY93	0	0	0
KECWOOVW	0	0	0
L25BIHW8	0	0	0
LCAM4GW1	0	0	0
LJ04YK07	0	0	0
M2BSJ8FK	0	0	0
NDC8ZFR3	0	0	0
O9AYDU1U	0	0	0
QG2ZGE11	0	0	0
SHV6GMEJ	0	15	9
VWDBTTXT	0	0	0
WA0K9DN8	0	0	0
Z11ZE71Q	0	0	0
Z9XNXYAN	0	0	0
ZG8GZOZK	0	1	1
Swine01			
03D2IU2H	0	0	0
132A6C6M	0	0	0
18YXTAK4	0	0	0
4T0NDXOF	0	0	0
4WXGH4RB	0	0	0
524F2AD0	0	0	0
5BHCJT4D	0	0	0
8QTBXR92	0	0	0
933H245V	0	0	0

C8Y9B3I6	0	0	0
DOQJ9XYI	0	0	0
E1WSN6P8	0	0	0
HDY2L8AP	0	0	0
HJB6BRJW	0	0	0
I0IQ48Z6	0	0	0
I377HCH1	0	0	0
IIWXRY93	0	0	0
KECWOOVW	0	0	0
L25BIHW8	0	0	0
LCAM4GW1	0	0	0
LJ04YK07	0	0	0
M2BSJ8FK	0	0	0
NDC8ZFR3	0	0	0
O9AYDU1U	0	0	0
QG2ZGE11	0	0	0
SHV6GMEJ	0	0	0
VWDBTTXT	0	0	0
WA0K9DN8	0	0	0
Z11ZE71Q	0	0	0
Z9XNXYAN	0	0	0
ZG8GZOZK	0	0	0

Sample metadata

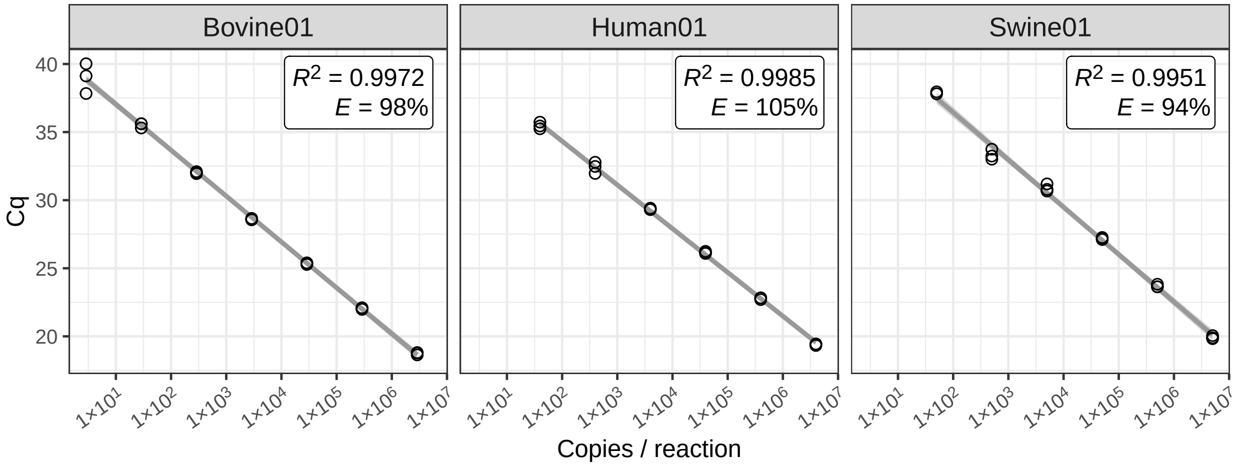
SampleId
03D2IU2H
132A6C6M
18YXTAK4
4T0NDXOF
4WXGH4RB
524F2AD0
5BHCJT4D
8QTBXR92
933H245V
C8Y9B3I6
DOQJ9XYI
E1WSN6P8
HDY2L8AP
HJB6BRJW
I0IQ48Z6
I377HCH1
IIWXRY93
KECWOOVW
L25BIHW8
LCAM4GW1
LJ04YK07
M2BSJ8FK
NDC8ZFR3
O9AYDU1U
QG2ZGE11
SHV6GMEJ
VWDBTTXT
WA0K9DN8
Z11ZE71Q
Z9XNXYAN
ZG8GZOZK

Methods and calibration curves

The following pages provide details of the methods used for each qPCR assay and the associated standard curves. Each assay in each run is associated with a calibration curve based typically on a series of 7, 10-fold dilutions of a standard with a known concentration. The calibration curves show the relationship between the $\log_{10}$-transformed standard concentration and the number of PCR cycles at which the detection threshold was reached (Cq). A linear regression is applied to this relationship and the r2 intercept and slope extracted for further analyses.

- RunId = An internal identifier for the standard curve(s) used to calculate copy numbers in the submitted samples. Assays that share a RunId are multiplexed (i.e., multiple targets amplified in a single reaction).
- R^2 = The coefficient of determination, or goodness of fit for the linear relationship (should be > 0.98).
- (E) = The reaction efficiency, or how close to a doubling of product was achieved with each PCR cycle. For a 10-fold dilution, 100% efficiency is for ~3.3 cycles per 10-fold dilution. A range of values is acceptable here, but we try to keep efficiency between 85% - 110%.

RunID: JVQ0172



Human Forward primer: 5’ CAGCAGCCATTCAAGCAATCC 3

Human Reverse primer: 5’ GGTGGAGACCTAATTGGGCTGATTAG 3

Human Probe: 5’ /5Cy5/TATCGGCGA/TAO/TATCGGTTTCATCCTCG/3IAbRQSp/ 3

Bovine Forward primer: 5’ CAGCAGCCCTACAAGACCTGT 3

Bovine Reverse primer: 5’ GAGGCCAAATTGGGCGGATTAT 3

Bovine Probe: 5’ /5HEX/CATCGGCGACATTGGTTTCATTTTAG/BHQ1/ 3

Swine Forward primer: 5’ ACAGCTGCACTACAAGCAATGC 3

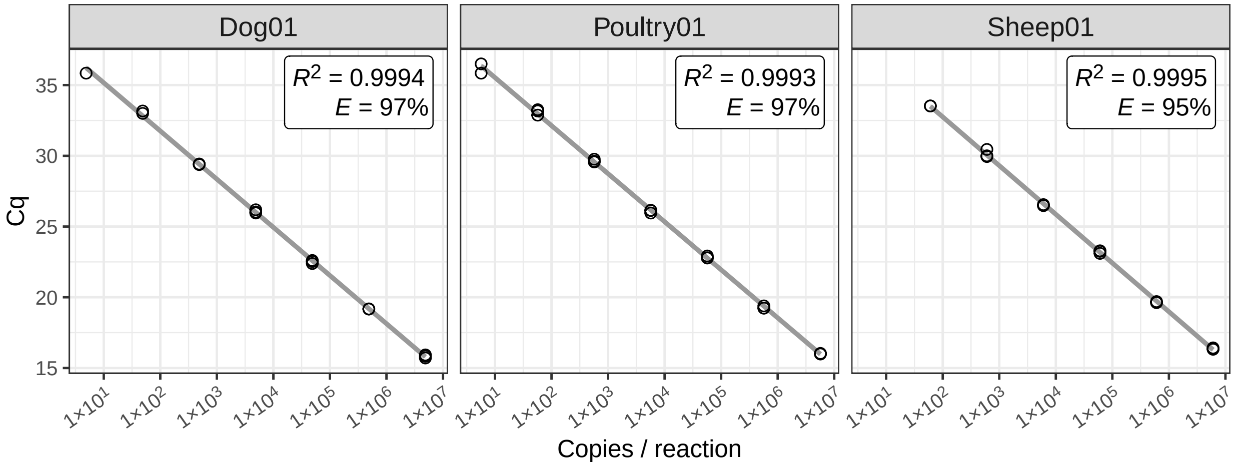
Swine Reverse primer: 5’ GGATGTAGTCCGAATTGAGCTGATTAT 3

Swine Probe: 5’ /56-FAM/CATCGGAGA/ZEN/CATTGGATTTGTCCTAT/3IABkFQ/ 3

Primer/probe reference: Cadwell et al., 200

Amplicons from the NADH dehydrogenase, subunit 5 (ND5) gene were amplified via qPCR from genomic DNA samples using the ND5 FWD and ND5 REV primers, and ND5 Probes corresponding to human, bovine, and swine. A standard curve was generated for each run to correspond to targeted regions of each of the ND5 genes. qPCR reactions contain 4.0 uL of QuantaBio PerfeCTa Multiplex qPCR ToughMix Low ROX (Catalog Number 89497-290), 500 nM of each primer, 300 nM of each probe, 4.0 uL of gDNA (or 1.33 uL of each gBlock for standard curve wells), and 2.4 uL of Nuclease-free H2O for a total reaction volume of 20 uL. qPCR amplification was carried out on the QuantStudio 5 qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95 C; followed by 50 cycles of 15 seconds at 95 C and 1 minute at 60 C.

RunID: JVQ0173



Dog Forward primer: 5’ CACATCTAAGCAACGCAGCATAA 3’

Dog Reverse primer: 5’ AGATCGGCGACTAAAAGTCAGAA 3’

Dog Probe: 5’ /5HEX/TCCGGCCCC/ZEN/TTAGCCAATGCC/3IABkFQ/ 3’

Poultry Forward primer: 5’ CGTYATCACAAACCTATTCTCAGCAAT 3’

Poultry Reverse primer: 5’ TTGGGTTGTCGACTGAAAATCC 3’

Poultry Probe: 5’ /5Cy5/CCCTACATY/TAO/GGACAMACCCTAGTAGAGTGAGCC/3IAbRQSp/ 3’

Sheep Forward primer: 5’ GCAATACACTATACACCTGACACAACAA 3’

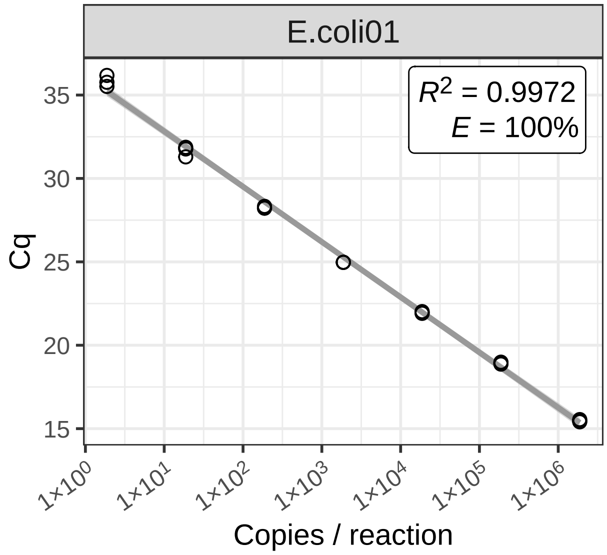
Sheep Reverse primer: 5’ CAGATAAAAAATATTGATGCCCCGTTTG 3’

Sheep Probe: 5’ /56-FAM/CTCCTCTGT/ZEN/AACCCACATTTGCCGAGA/3IABkFQ/ 3’

Primer/probe reference: Dancer et al., 2014

Amplicons from the Cytochrome b (Cytb) gene were amplified via qPCR from genomic DNA samples using the Cytb FWD and Cytb REV primers, and Cytb Probes corresponding to dog, poultry, and sheep. A standard curve was generated for each run to correspond to targeted regions of each of the Cytb genes. qPCR reactions contain 4.0 uL of QuantaBio PerfeCTa Multiplex qPCR ToughMix Low ROX (Catalog Number 89497-290), 500 nM of each primer, 300 nM of each probe, 4.0 uL of gDNA (or 1.33 uL of each gBlock for standard curve wells), and 2.4 uL of Nuclease-free H2O for a total reaction volume of 20 uL. qPCR amplification was carried out on the Agilent AriaMx qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95 C; followed by 50 cycles of 15 seconds at 95 C and 1 minute at 60 C.

RunID: JVQ0174



Forward primer: 5' CAATGGTGATGTCAGCGTT 3

Reverse primer: 5' ACACTCTGTCCGGCTTTTG 3

Probe: 5' /56-FAM/TTGCAACTG/ZEN/GACAAGGCACCAGC/3IABkFQ/ 3

Primer/probe reference: Srinivasan et al., 201

An amplicon from the uidA gene was amplified via qPCR from genomic DNA samples using E. coli FWD and REV primers and probe. A standard curve was generated for each run to correspond to targeted region of the E. coli, uidA gene. Each qPCR reaction is run in triplicate and contains 8.0 uL of QuantaBio PerfeCTa qPCR ToughMix Low ROX (Catalog Number 97065-966), 500 nM of each primer, 300 nM of probe, 4.0 uL of gDNA, and 4.8 uL of Nuclease-free H2O for a total reaction volume of 20 uL. qPCR amplification was carried out on the QuantStudio 5 qPCR instrument with the following thermal profile conditions: 1 cycle of initial denaturation for 5 minutes at 95 C; followed by 50 cycles of 15 seconds at 95 C and 1 minute at 60 C.

APPENDIX E – July 2022 Photos

Photo 1: Arizona Fox of Biomonitor preparing to collect an eDNA sample from site 14, water shown is ditch exiting Northwood prior to flowing into Palfreyman Ditch

Photo 2: Michael Britton of Biomonitor observing vegetation along Palfreyman Ditch