## HIGH DENSITY LAGRANGIAN SAMPLING FOR PATHOGEN SOURCE IDENTIFICATION

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Abstract. In compliance to the Clean Water Act, each US state compiles a list of water bodies not meeting regulatory criteria. The most common impairment in US water bodies is elevated pathogens measured by fecal indicator bacteria (FIB). Reasons for this prevalence probably include the true magnitude of pathogen contamination, monitoring bias from human health concern, inaccuracy of FIB monitoring compared to other parameters, and difficulty estimating background condition. In practice, identification and citation of impairment is extensive, while development of plans that identify the source with certainty and implement highprobability remediation lags behind. The difficulty in confidently identifying sources of impairment is an impediment to the protection of water bodies and increases the cost of remediation due to the need for casting a wider net of lower probability solutions. With a high proportion of resources directed to pathogen contamination, it is important to confidently identify sources. Increased confidence will improve efficacy of remediation and ability to secure funding. To achieve these objectives, we designed a study method to investigate Rocky Creek, a pathogen impaired stream in Augusta, GA. This method applied a Lagrangian FIB sampling approach to reduce confounding variability and a high sampling density to identify high contribution watershed areas. We then layered typical pathogen sources (e.g. septic, pet waste, sewer, wildlife) and alternative sources (e.g. sediment, instream growth) along with their GIS data over the FIB data. In this way, we were able to target remediation efforts on the convergence of sources and regions and thereby decrease the scale of remediation efforts.

**Introduction.** In regulatory compliance to the Clean Water Act, each US state is required to compile a list of water bodies that are not meeting regulatory criteria for their designated use. Monitoring efforts take place in order to accomplish this directive. These efforts identify those water bodies that are not meeting their designated use, cite the monitored parameter that is out of compliance, and

develop a plan that both identifies the source of the impairment and proposes remediation steps for that source of impairment. In practice, the identification of impaired water bodies and the designation of a particular impairment has proceeded in large numbers, while the development of plans that identify, with any certainty, the exact source of the impairment have lagged behind. Remediation steps that can produce measureable improvements have lagged even further behind. There is probably improvement to be made at all levels of this process but the relative difficulty in accurately identifying and locating sources of impairment is a major impediment to the protection of our water bodies. A lack of source specificity increases the cost of remediation due to the need to cast a wider net of lower probability solutions in hope of achieving some measureable improvement.

The most common impairment in US water bodies is elevated concentrations of pathogens measured by fecal indicator bacteria (FIB). The reasons for the prevalence of this impairment are probably multidimensional, including not only the true magnitude of pathogen contamination, but also monitoring bias for pathogens before other parameters based on real or perceived human health concerns, relative inaccuracy of FIB monitoring compared to other parameters such as pH or sediment, and difficulty in developing estimates for background condition. Due to the high proportion of time any money applied to pathogen contamination relative to other potential water quality impairments, it is critically important to do as much as possible to correctly identify the background conditions, hone the list of water bodies to those truly in need of remediation, and improve our ability to confidently identify and locate the specific sources of elevated FIB. This confidence and specificity will then improve the efficacy of remediation and the ability to appropriate funding from tight municipal budgets. In an effort to achieve these objectives, we designed a new study method to investigate Rocky Creek, a pathogen impaired stream in Augusta, GA. While there are several pathogen impaired streams in Augusta,

GA, Rocky was the focus of this study due to historic data which suggests it consistently has the highest concentrations.

**Methods.** The study method applied a Lagrangian approach, based on a dye tracer, to the sampling schedule in order to eliminate some of the variation that often plagues source identification efforts with FIB data. We also employed a high spatial sampling density in order to target specific (< 6 km<sup>2</sup>) regions of the 60 km<sup>2</sup> watershed that are major contributors to elevated pathogen concentration. Eight tributaries were identified and delineated in addition to the mainstem and its adjoining drainage area.



Figure 1. Tributaries and sampling locations

This led to the selection of 12 sampling sites in the watershed (Fig. 1) in order to isolate particular contributing areas either directly by measurement or by subtraction. Sampling and analysis included E. coli by most probable number, flow measurements, temperature and conductivity measurements, dye tracer quantification, nitrogen series, and total reactive phosphorus. E. coli were quantified both in the water column and in pore space of the underlying sediment by taking sediment cores and extracting the pore water for analysis. Flow measurements were made with a Marsh McBirney velocimeter. Rhodamine WT was used as the dye tracer and was measured in the stream, along with temperature and conductivity, with a YSI multiparamter data sonde. Nutrients were quantified by standard methods on a SEAL AQ2+ Automated Discrete Analyzer. E. coli quantification was with the IDEXX Colilert-18 method. We then applied both typically held pathogen source assumptions (e.g. failing septic, pet waste, sewer leakage, wildlife) and alternative source possibilities (e.g. sediment, detritus, in-stream growth) along with GIS data on the location of these potential sources. This

2

GIS data was then layered over the high spatial density Lagrangian sampling data. In this way, we were able to target remediation efforts on the convergence and correlation of potential sources and specific regions and thereby significantly decrease the scale of remediation efforts.

**Results and Discussion.** During the scheduled study period, what was forecast to be a very light rain turned into something more significant and somewhat hampered sampling as the study progressed. However, much information was still gained from the study. Instead of the originally planned 12 sampling locations, 8 were actually measured as increased flow and contribution from rain prohibited sampling for the 4 most downstream sampling sites. For the sites that were measured, hydrology data revealed some potential impact of the rain event (Fig. 2). It is likely that the rain event was contributing some additional flow possibly beginning at Wheeless Road and almost certainly by Jennings Road.



Figure 2. Stream flow and precipitation

Dye tracer recovery indicated almost complete transport of the tracer material through the first two sites below the release point at North Leg Road (Fig. 3). Error bars reflect the difference between the known volume of dye added and the additional amount estimated at the next downstream location. However, only about 30% of the originally released mass was measured at Jennings Road, likely due to retention in the wetland area located between Wheeless and Jennings Roads. The response was sufficient at all measured sites to determine the peak of the tracer curve, and hence the Lagrangian sampling time for the water column concentrations. Peak concentrations ranged from 300 ug/l at Milledgeville Road to 30 ug/l at Jennings Road.



Figure 3. Dye tracer recovery remained steady until Jennings Road

Nutrient concentrations were relatively low in the upstream portions of the watershed above Milledgeville Road (Fig. 4), with the exception of a high NH<sub>3</sub> value at Noland Connector, which may be associated with the wetland just upstream of this site and the relative decrease in nitrification and denitrification in the wetland during winter months. Nutrient concentrations increased after Milledgeville Road, with an apparent spike of NO<sub>3</sub> entering at least partially from Trib 4, and decreasing somewhat as it moves into the mainstem and travels further downstream. Total reactive phosphorus concentrations were relatively low throughout the sampled region with the possible exception of the Noland Connector sampling site.



Figure 4. Nutrient concentrations were dominated by NO<sub>3</sub>

Temperature and specific conductivity data were also taken during the study at North Leg, Milledgeville, Wheeless, and Jennings Roads. Values in Table 1 were given as averages over the measured duration of the dye tracer curve. A notable change in temperature between Milledgeville and Wheeless Roads may have indicated the contribution of warmer surface runoff from impermeable surfaces, while the decrease at Jennings Road may have indicated temperature buffering in the wetland. Increases in conductivity at Milledgeville Road and again at Wheeless Road could have been due mobilization of stream bed material, to surface runoff, or to contributing tributaries with different geology or various other sources of conductivity.

**Table 1. Temperature and Conductivity Values** 

Sampling Site	Temperature (°C)	Specific Conductivity (uS/cm)
North Leg	12.4	43
Milledgeville	12.4	57
Wheeless	13.1	68
Jennings	12.9	66

E. coli was the central focus of the study and there was much information provided from the data collected (Fig. 5). First, it was noteworthy that in all of the sites upstream of Jennings Road, the sediment concentrations exceeded the surface water, indicating that the sediment may have either served as a repository for bacteria that was at a higher concentration previously, as an incubator for bacterial growth, or both.



Figure 5. E. coli concentrations were greater in sediment than in the water column until Jennings Road

This relationship between water column and sediment bacteria was particularly true for Trib 2, North Leg Road, and Trib 4 where sediment concentrations were 2.5-7.0 times that of the overlying water column. This may have indicated a pulsed sourcing of bacteria at these locations, potentially based on rainfall but with other possibilities such as septic overuse. Also of note were the particularly high concentrations found in Trib 2, and at Wheeless Road. These concentrations were well above generally accepted background levels, indicating probable sources of contamination somewhere in Trib 2 above the sampling site, and somewhere between Milledgeville Road and Wheeless Road other than Trib 4. When discharge was considered along with concentrations, the bacterial loading can be determined (Fig. 6). This representation illuminates the most significant contributing areas to the overall bacterial contamination and to the concentrations measured at the portion of the stream currently listed as impaired. From this perspective, the dominant contribution to the stream was the region between Milledgeville and Wheeless Roads, excluding Trib 4. This leaves Trib 5 and the mainstem portion between the two roads as probable sources. It is important to note, however, that the contribution of surface runoff due to the rain event was probably beginning somewhere in this region as well and could have confounded these results to some degree, particularly in the dominance of the contribution from this particular region compared to the contribution of other regions.



Figure 6. The bacterial load in the stream is dominated by an addition between Milledgeville and Wheeless, other than Trib 4

Consideration of this spatial result along with the subwatershed parameters in Table 2 revealed further insights. First, bacterial concentration across all subwatersheds showed probable positive correlation to the number of active septic sites in that subwatershed. Probable positive correlations with estimated bacterial load across all subwatershed also occurred including feet of sewer pipe adjacent to the stream and contributing watershed area. Wildlife refuge area seemed to correlate negatively to the estimated bacterial loading.

Table 2. Comparison of subwatershed parameters with bacterial load contributions indicates some correlation

Watershed	Subwatershed	Contributing	Contributing	Vacant	Flood	Parcels	Parcel	No	Septic	Mean	Mean	Sewer Pipe <100ft	Critical Sewer	Occupied Septic	Critical Septic	Wildlife	Wildlife	Est. E.	Est E. Coli
		Area	Area	(%)	Zone	(#)	Density	Wastewater	(%)	Residential	Parcel Size	from Stream	Pipe Density	System <100ft	Density	Refuge	Refuge	Coli Load	(mg/l)
		(km^2)	(%)		(%)		(#/km^2)	(%)		Value (\$)	(acres)	(feet)	(ft/km^2)	from Stream (#)	(#/km^2)	(Acres)	(%)	(cfu/s)	
Rocky	Upper Main	3.2	7%	37%	7%	418	131	17%	28%	41000	1.13	5511	1722	3	0.9	232	29%	41000	300
Rocky	Trib1	2.9	6%	16%	14%	421	145	3%	36%	98000	0.90	4872	1680	1	0.3	211	29%	0	0
Rocky	Trib2	2.4	5%	16%	0%	382	159	7%	17%	40000	0.92	3327	1386	3	1.3	162	27%	65000	900
Rocky	Trib3	4.0	8%	12%	9%	647	162	14%	24%	44000	0.99	6384	1596	0	0.0	149	15%	214000	600
Rocky	Trib4	1.0	2%	11%	0%	261	261	1%	22%	48000	0.42	1916	1916	16	16.0	44	18%	15000	200
Rocky	Trib5	4.2	9%	8%	7%	656	156	6%	23%	79000	0.84	10330	2460	23	5.5	115	11%	1500000	2800
Rocky	Trib6	3.8	8%	10%	0%	1276	336	5%	2%	44000	0.47	8915	2346	0	0.0	31	3%	0	0
Rocky	Lower Main	13.4	27%	12%	18%	3407	254	13%	16%	33000	0.65	25695	1918	11	0.8	467	14%	*	*
Rocky	Trib7	3.1	6%	27%	5%	744	240	9%	3%	15000	0.76	1755	566	0	0.0	141	18%	*	*
Rocky	Trib8	10.8	22%	6%	8%	3948	366	6%	1%	45000	0.50	24424	2261	3	0.3	121	5%	*	*
*Weather co	anditions prohibi	tod compling h	obond Ionnir	arc Road															