DEP PROJECT: AT011

FINAL REPORT

Project 1: Water quality variability in karst aquifers and implications on spring monitoring

Project 2: Identifying source waters to priority springs using isotopes of sulfate, geochemical modelling, and data analysis.

Prepared by Patricia Spellman, Sunhye Kim, and Mahnoor Kamal for the Florida Department of Environmental Protection

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Executive Summary

The Florida Department of Environmental Protection (FDEP) funded two separate, but related projects that begin to outline the implications of spatial and temporal water quality variability on water management decisions in the Upper Floridan Aquifer (UFA). The UFA is the primary source of drinking water for most Floridians and has characteristics that pose unique challenges to water resource management. These challenges arise because the UFA is a complex, heterogeneous karst aquifer whereby groundwater is transported quickly through large, extensive conduits while simultaneously transported more slowly through fissures and intergranular porosity of the limestone matrix. Further nuanced flow dynamics include local flow paths created by conduits that direct groundwater from the matrix toward a cave passage. These characteristic features of the UFA generate highly variable flow and solute transport dynamics which need to be comprehensively understood for effective water resource management.

Over the last several decades, changes in land use have corresponded to changes in water quality recorded at springs across Florida with the primary attention focused on nitrates. The overall negative impact of anthropogenic land use alterations to spring water quality prompted more rigorous monitoring of spring water chemistry. This included regular water quality sampling as well as long term, continuous monitoring of physiochemical parameters including pH, dissolved oxygen (DO), specific conductance, and temperature along with nitrate at selected Florida springs. Though most attention focused on increasing nitrates, analyses of major ions and specific conductance from the monitoring initiative showed increases in specific conductance along with coincident increases in major groundwater ions such as Na^+, K^+, Mg^{2+}, SO_4^{2-} and Cl^-. Because natural groundwater chemistry is primarily controlled by limestone (Ca^{2+}, HCO_3^-) weathering with minor mineral inclusions, these changes have disrupted natural major ion chemistry and are likely because of anthropogenic practices. These anthropogenic practices include pumping from agriculture, which could locally increase the flow contributions from the Lower Floridan Aquifer (LFA) via fractures, increase in older, matrix storage flow to conduits, or from surface derived application of fertilizers and soil amendments; and potentially a combination of these factors.

The expansiveness of conduits in the UFA means they will likely intersect different land uses which may cause substantial differences in water chemistry across conduits within a hydraulically connected cave system. Thus, the changes in water chemistry observed at springs from anthropogenic factors, represents a convoluted signal and the extent of this convolution remains relatively unexplored. The differences in water quality between conduits may impact the interpretation of spring basin processes and even spring basin management success. For example, dilution at the spring vent after multiple converging passages may show a target threshold concentration of a solute has been reached, however individual passages may still drain areas that are disrupting major ion chemistry and overall water quality.

Because it is still unclear whether nitrates are the primary cause of spring ecosystem degradation which has fueled algae proliferation, holistically understanding the spatial and temporal causes of all water quality changes are necessary for both practical and research applications. The two funded projects from the FDEP thus focused on starting to comprehensively address the spatial and temporal variability observed in water quality across selected regions of the UFA.

Project 1 focused on quantifying and qualifying the suspected *spatial variability* in water chemistry by conducting water quality surveys in caves by measuring major physiochemical water parameters and took discrete measurements in a subset of those cave systems. The project also aimed to inform the location of optimal water quality sampling for objectives such as assessing aquifer and spring health. Project 2 aimed to quantify the effects of *temporal variability* in water chemistry by quantifying the contributions of different potential water sources using isotopes of water and sulfate, geochemical analyses, and statistical models.

Project 1 results highlighted the extent of the spatial variability in water quality across a single cave system, and qualified some of the causes which impact interpretation at monitored spring basins. Water quality surveys performed using a YSI EXO^2, that took continuous measurements of pH, dissolved oxygen (DO), specific conductance, and temperature, showed high variability at individual conduits (Table 1.1) whereby DO was the most substantially different and a non-unique water quality indicator. Changes in pH and specific conductance were also observed at converging conduit passages, illustrating how different natural processes such as recharge mechanisms guided by drainage depths in the aquifer influence the chemistry that emerges at springs (see, for example, Figure 1.7). We showed how small conduits can mix quickly with water (Jug Hole) or larger conduits could coalesce in a passageway to alter the final water chemistry at the spring (Peacock, Madison Blue Springs). Further, discrete water quality measurements including nutrients, dissolved organic carbon, and DO, showed high variability (Figure 1.10) demonstrating how different land use drainage may potentially impact water chemistry across the entire cave system and convolute spring water quality signals. These observations helped guide discussions in Project 2.

The preliminary analysis from Project 2 strongly indicated that water quality changes in major ion chemistry and specific conductance are primarily from agriculture. Because the groundwater chemistry in the UFA is primarily controlled by limestone weathering, there exists a threshold specific conductance that is theoretically achieved, and many springs are approaching this value suggesting other factors aside from increased matrix storage are contributing to increases in specific conductance. The remaining contributions would be deeper, more saline LFA waters or surface derived anthropogenic land use practices. We used sulfur and oxygen isotopes from sulfate, a common major ion in groundwater, to determine the contributions from these two sources. The results of the sulfur isotope analysis showed, for example, that at Fanning Spring, there is little indication of LFA waters impacting spring water chemistry (Figure 2.18), however it was one of the most impaired springs regarding major ion chemistry and resulting changes in specific conductance (Figure 2.20). In contrast, sites such as Peacock Springs need additional interpretation of water quality for a definitive conclusion. Further, unique isotopic signatures (for our dataset) from a farm/livestock operation outside Manatee Spring matched closely to the sulfur isotopes emerging from Manatee Spring, indicating that the farm is strongly influencing the water chemistry at Manatee Spring. Our analyses are ongoing, however, as we complete the mixing models and further work beyond the project scope needed for greater certainty in the conclusions.

Our work further highlights the uniqueness of individual springs and their water chemistry, hydraulic responses, and extent which emphasizes individualized water management strategies should be applied to Florida springs.

Project funding

We completed two projects for the Florida Department of Environmental Protection (FDEP). The project finances were aggregated, and compensation for both projects is $139,594.44.

The budget for **Project 1: Water quality variability in karst aquifers and implications on spring monitoring** project was $72,513.

The budget for **Project 2: Identifying source waters to priority springs using isotopes of sulfate, geochemical modelling, and data analysis** project was $67,081.

Task No.	Budget Category	Budget Amount
1	Salaries	$8,381.00
	Fringe	$1,415.55
	Supplies/Miscellaneous	$2,547.00
	Indirect	$1,618.00
	Total for Task:	**$13,961.55**
2	Salaries	$25,142.00
	Fringe	$4,246.49
	Supplies/Miscellaneous	$7,640.00
	Indirect	$4,850.10
	Total for Task	**$41,878.59**
3	Salaries	$25,143.00
	Fringe	$4,246.65
	Supplies/Miscellaneous	$7,640.00
	Indirect	$4,849.00
	Total for Task	**$41,878.65**
4	Salaries	$25,143.00
	Fringe	$4,246.65
	Supplies/Miscellaneous	$7,638.00
	Indirect	$4,848.00
	Total for Task	**$41,875.65**

Figure A.1: Breakdown of costs for each quarter from the combined project funding.

Project 1: Water quality variability in karst aquifers and implications on spring monitoring
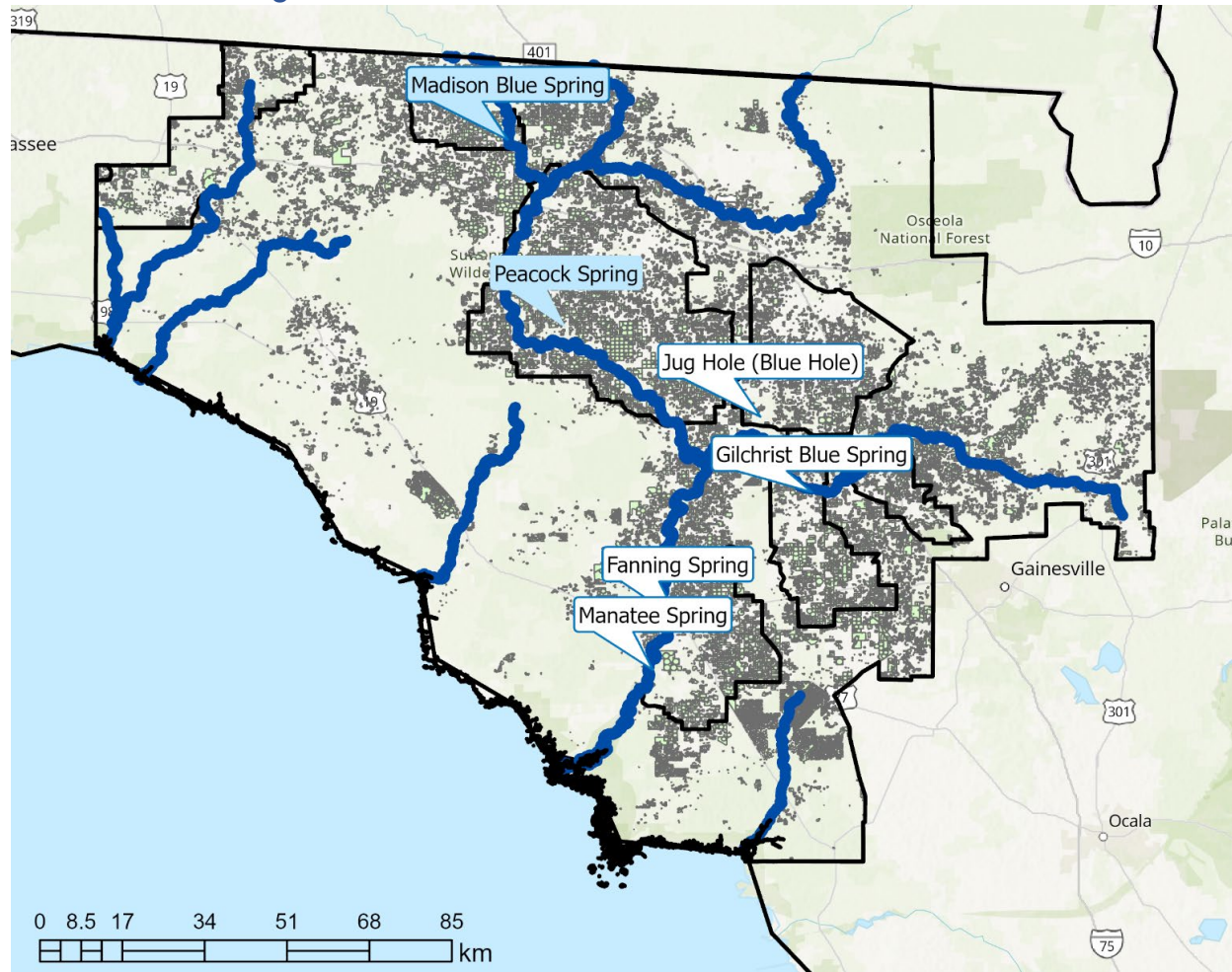
Review and background

Figure 1.1: Sites of priority focus for sensor comparisons. We tried to target springs within the priority focus areas (outlined black). Sites with blue background are also where discrete water quality samples (major ions, nitrate) were taken at multiple cave passages. The green shaded areas show the extent of agricultural lands (FSAID database, 2019).

Review

Phreatic (saturated) conduits within the Upper Floridan Aquifer (UFA) are extensive, transmitting a large portion of groundwater through the FAS very rapidly. Their contested (geologist conversations) inception and evolution have resulted in complex morphologies that can branch out expansively draining large areas that eventually coalesce to a discharge point (i.e., spring). The network of conduits that creates these complex phreatic cave systems create localized flow paths that direct water toward main conduit passages (Spellman, 2012). Though most water resource managers develop regional potentiometric surface maps for karst aquifers, the localized flow paths are important but often overlooked due to data density required (and often lacking) including cave maps and water level data to identify them.

These local flowpaths become significant, however, when they drain different land uses and can transmit contaminated or different water quality signals to a spring. Because of local

flowpaths and discrete features (i.e. sinkholes, karst windows) that can rapidly recharge the aquifer, water quality such as nutrients, specific conductance, temperature, and dissolved oxygen in caves may vary across passages. For example, discrete recharge points (sinkholes, fractures/fissures) and relatively quick diffuse recharge can impact the water chemistry along each passageway. The changes can be even more drastic when different land uses are close to a cave passage. Further, because different passages may contribute different volumes due to hydrologic changes, the changes may be transient. Water temperature changes, visibility changes, and water stratification have been observed by cave divers along passages within a single, defined cave system. Further, extreme water quality changes have also been observed at passages that run directly underneath active farming operations (see Future Work). Thus, the spring where these flowpaths converge produces a convoluted signal derived from each contributing passage which can affect water chemistry interpretation and ultimately water quality management. However, to date, no quantification of water quality variability across multiple systems has been systematically documented.

Once these convoluted waters emerge from the spring vent, the water quality can further change, particularly dissolved oxygen, due to aerated spring runs and vegetation. If the intent is to monitor groundwater, changes that occur may affect water quality trend analysis and interpretation of processes occurring in the aquifer if the sensor is not in a representative location. For example, a recent study used dissolved oxygen sampled at spring vents to interpret average potential for biogeochemical reactions (Spellman et al., 2022). However, assumptions about dissolved oxygen thresholds that would indicate potential denitrification may have been in error.

The overarching goal of this project was to document water quality variability within cave systems draining to priority springs and assess the efficacy of the water quality sensor placement used to address water quality concerns across the groundwater contributing area. We completed our project within the restrictions of the diving allowed (i.e. no rebreathers or decompression) but was still optimized to bring attention to the variability observed within a single cave system and highlight individualized treatment some springs may need when undergoing restoration or land use management.

We chose a variety of accessible, safe, cave systems for water quality surveys that represented 1) simple morphologies (Manatee Spring, Jug Hole), 2) anastomosing, extensive cave morphologies draining different land uses (Peacock Springs, Madison Blue Spring), and 3) shallow cave systems that have multiple discrete features from the surface (Lafayette Blue Spring). We surveyed water quality using a YSI EXO^2 water quality sonde that continuously measured pH, temperature (T), dissolved oxygen (DO), and specific conductance (SpC). We did this in limited parts of the cave typically near the entrance, but ensured we covered areas within range where known cave water quality changed or was suspected. In some cave systems, we took water quality samples at discrete locations which included nitrate, dissolved organic carbon, and major groundwater ions (Ca^{2+}, K^+, Mg^{2+}, SO_4^{2-}, HCO_3^-, Cl^-, Na^+).

We also aimed to quantify the changes in water quality that occurred between water emerging at the spring vent and location of a monitoring sensor to monitor general water quality. We did this for SRWMD and FDEP monitored priority springs. We assessed the differences in continuously monitored water quality between sensor location and the spring vent at accessible priority springs that were monitored and had sensors located a significant distance from the vent

(Manatee Springs, Fanning Spring, Gilchrist Blue Spring) or were in potentially stagnant waters (Jug Hole, Peacock Springs).

This data also provide background information for more individualized priority springs in Florida. They can be used to launch new investigations targeting specific concerns for springs that require restoration or management. We note we did not investigate the full scope of variability and had only proposed to achieve what was possible within the timeline available.

Project 1 goals

1. Address the reliability and efficacy of monitoring karst groundwater at springs when monitoring point is far away from spring vent or suspected to be impacted by processes that could affect water quality.
2. Quantify the degree of variability of major ions (metals, anions) and other water quality parameters at selected locations to understand how variable water chemistry might be within the cave system which informs water quality interpretation and resource decisions.

Hypotheses:

1. <u>Ho</u>: The monitoring point selected at most springs reliably captures groundwater emerging from the spring vent without bias.
   <u>Ha</u>: Water quality monitored at a location outside the spring vent is not reliable.
2. <u>Ho</u>: Minimal variability in water quality exists in cave passages draining the UFA
   <u>Ha</u>: High variability in surface sourced constituents and water quality across a cave system will exist likely due to drainage of different regions of the landscape and different recharge mechanisms

Data availability and description

All YSI EXO^2 sonde data are found in **/Project1/Data** folder and all NELAC lab water quality analyses are in /**NELAC Lab Water Quality Results** folder.

Highlighted results

1. Both null hypotheses were rejected. However, some sites had minimal differences in water quality at the vent compared to the monitoring location.
2. All cave systems surveyed showed water quality changes from an intersecting cave passage.
3. Dissolved oxygen (DO) was the most variable parameter whereby some ranges of DO *within* the cave system varied over 2 mg/L.
4. DO was also the least representative parameter monitored at locations in the spring run, as almost all springs showed changes in DO between the vent and the monitoring location.
5. Peacock Springs monitoring location was the poorest location selected for monitoring, as water seems to stagnate near the sensor. Temperatures showed over 0.5 C degree difference. The DO was also significantly higher than water emerging from the vent.
6. Peacock springs had the highest variability in water quality in nutrients, DO, dissolved organic carbon (DOC), and some major ions.

Detailed results

Cave system water quality variability

Cave surveys with a YSI EXO^2 were completed **in** the following caves (Figure 1.1):

1. Jug Hole (Ichetucknee Blue Hole)
2. Manatee Spring
3. Madison Blue Spring
4. Peacock Springs

From the cave surveys completed, we compiled the **<u>range</u>** in major water quality parameters (Table 1.1) measured on the EXO^2 including dissolved oxygen (DO), pH, specific conductance (SpC), and temperature.

Spring	DO (mg/L)	SpC (µS/cm)	T (C)	pH
Jug Hole	1.21 (13.7%)	15.5	0.067	0.27
Manatee Spring	0.36 (4.7%)	6.6	0.21	0.05
Madison Blue Spring	0.98 (11.1%)	18.2	0.57	0.21
Peacock Spring*	2.95 (30%)	8	0.1	0.01*

Table 1.1: Water quality parameters within the cave system which include the vent. **Bolded** sites indicate where additional water quality sampling of nitrate, major ions, and dissolved organic carbon (DOC) were obtained. * for Peacock indicates values are only for the main P1/P2 cave system. P3 was not used in this data summary. We however reported variation in P3 within this report.

Jug Hole

Jug Hole is located on the Ichetucknee River and is the second monitoring point of spring discharge that supports the Ichetucknee River. The cave system is small, as the primary drainage and capture area for Jug Hole is under unconfined, semi-confined and confined conditions (Figure 1.2), however much of the recharge area is under semi-confined and confined conditions. Most of the catchment area is defined as clayey sand. Temporal variability in nitrate and water quality are low at Jug Hole possibly owing to more restrictive land use management and low density of row crops (Figure 1.2) along with degree of confinement.

There was a noticeable shift in DO, pH, and SpC at a source tunnel that is likely a recharge point to the cave system. There is no identifiable location on the land surface (from LiDAR data) as to where this recharge point is, but it is likely a discrete feature such as a sinkhole, enlarged fracture, or karst window. Because the feeder is small, it likely mixes and is diluted by all the water emerging from the diamond sands restriction (see Figure 1.4 – End of sensor data).
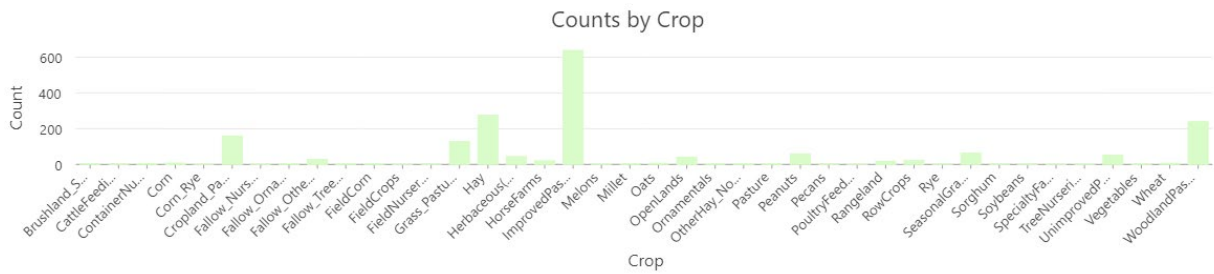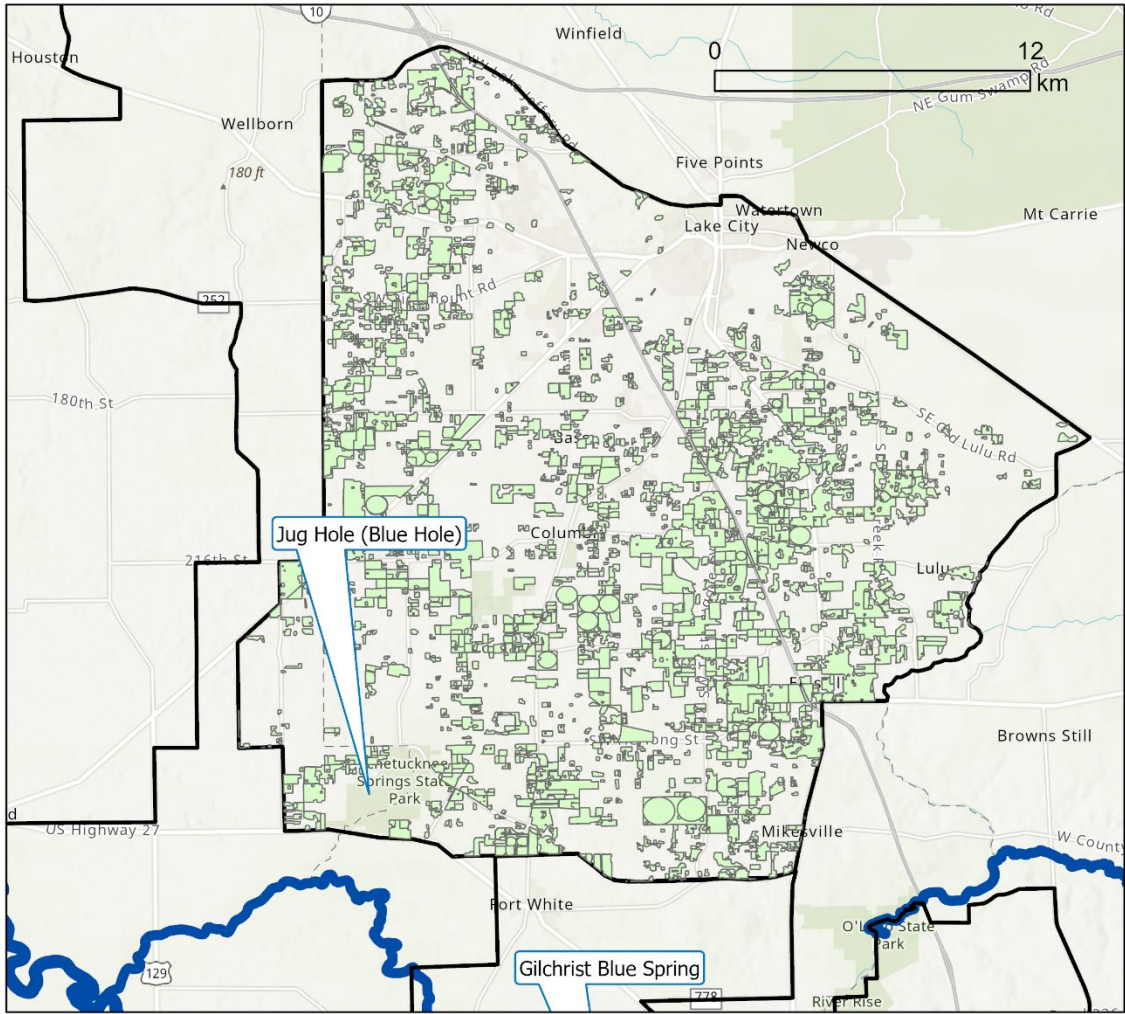
Figure 1.2: The 'springshed' area for the whole of the Ichetucknee River within Jug Hole near the southern part. The bar graph breaks down the agriculture in the region, showing a dominant land use of pasture. Only limited row crops are grown.
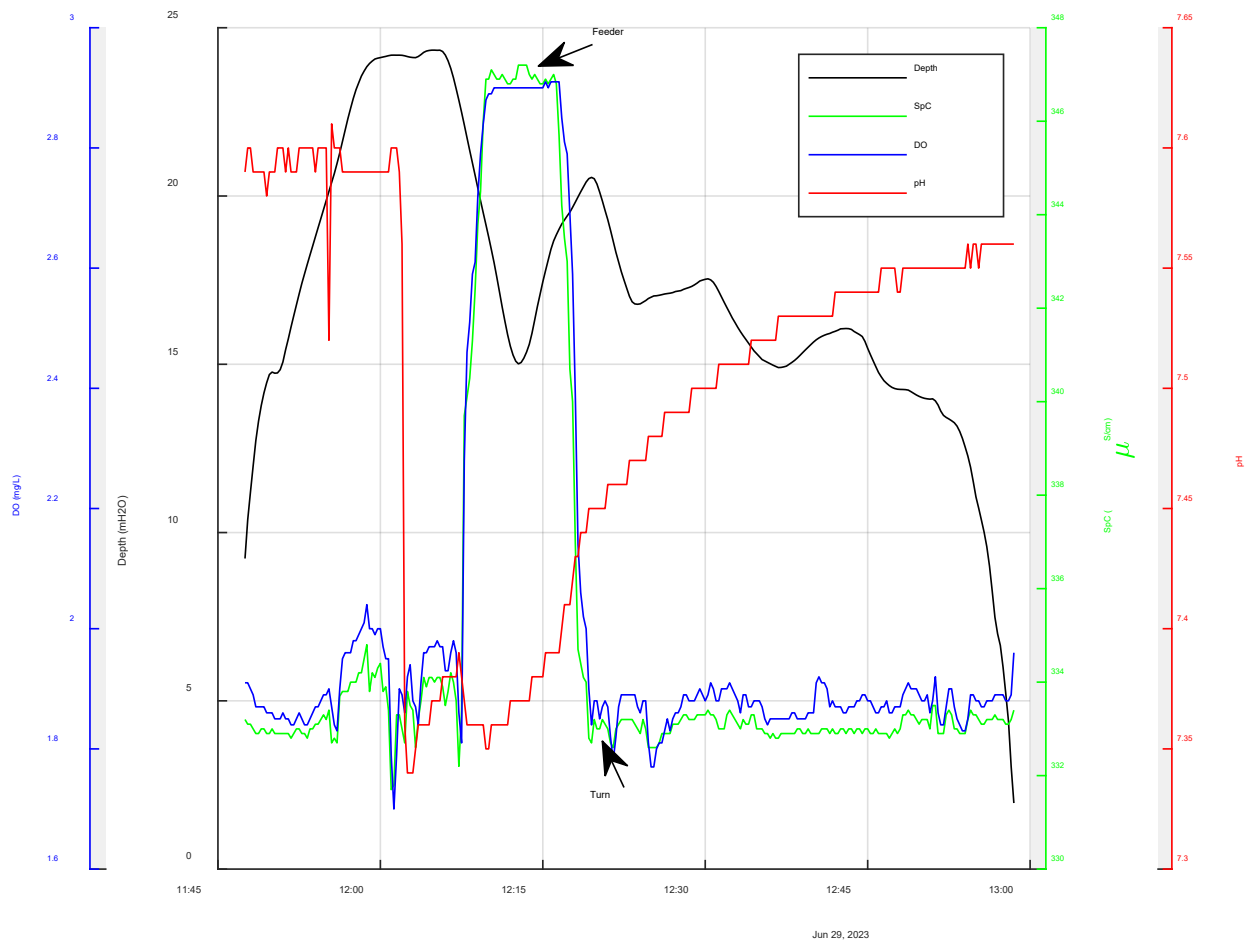
Figure 1.3: Water quality profile of Jug Hole. The spike is where the feeder discharges different water (See Figure 1.4 - Feeder). During the EXO² profile, there was some sample collection for University of Florida (UF). The dive plan was to reach the end of the passage and then on the exit, take the samples. Therefore, the profile is longer after reaching the feeder passage.
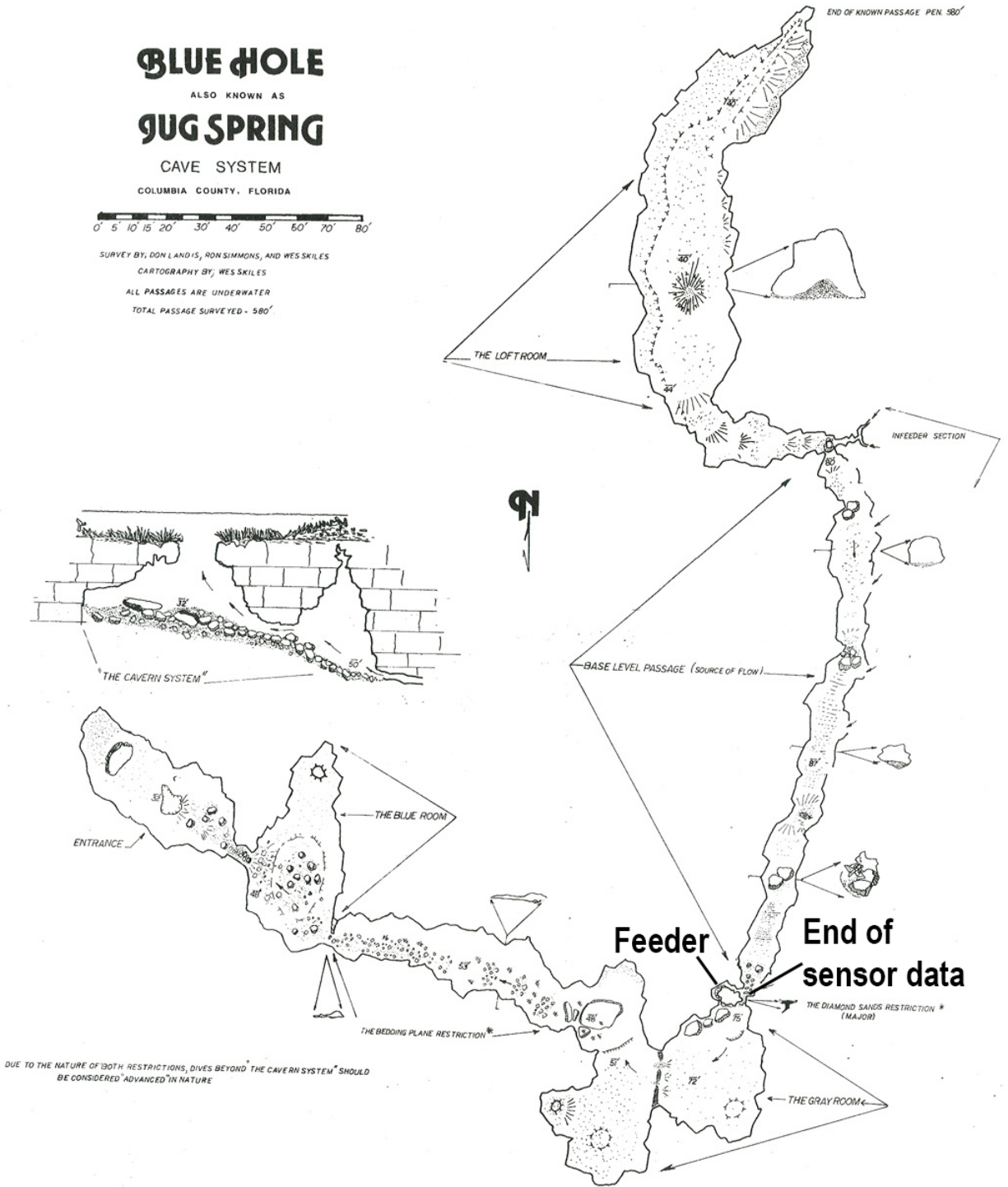
Figure 1.4: Map of Jug Hole (source: https://www.cavediving.com/where/jughole/). The locations of the end of the sensor data are shown. The beginning of the dive was at the _Entrance_ (on map).

Manatee Spring

Manatee Springs had minimal variability but is one of the most extensive (by distance from spring vent) in the SRWMD and is one of the least geomorphologically complex. Manatee Spring contains a large conduit that has minimal larger, intersecting and drains a large, entirely unconfined recharge area of the UFA. There was still some variability captured, likely around the cold-water tunnel (see Figure 1.6). However, water quality data collected and reported from SRWMD has shown variation in nitrate ~2 mg/L increase in the back section (Blue Water Tunnel) of the cave compared to what is sampled at the spring vent. Further, suspected differences in water chemistry occur at the Milk Tunnel where visibility also changes, but flow in Manatee Springs precludes much exploration without scooters, rebreathers, and decompression obligations.
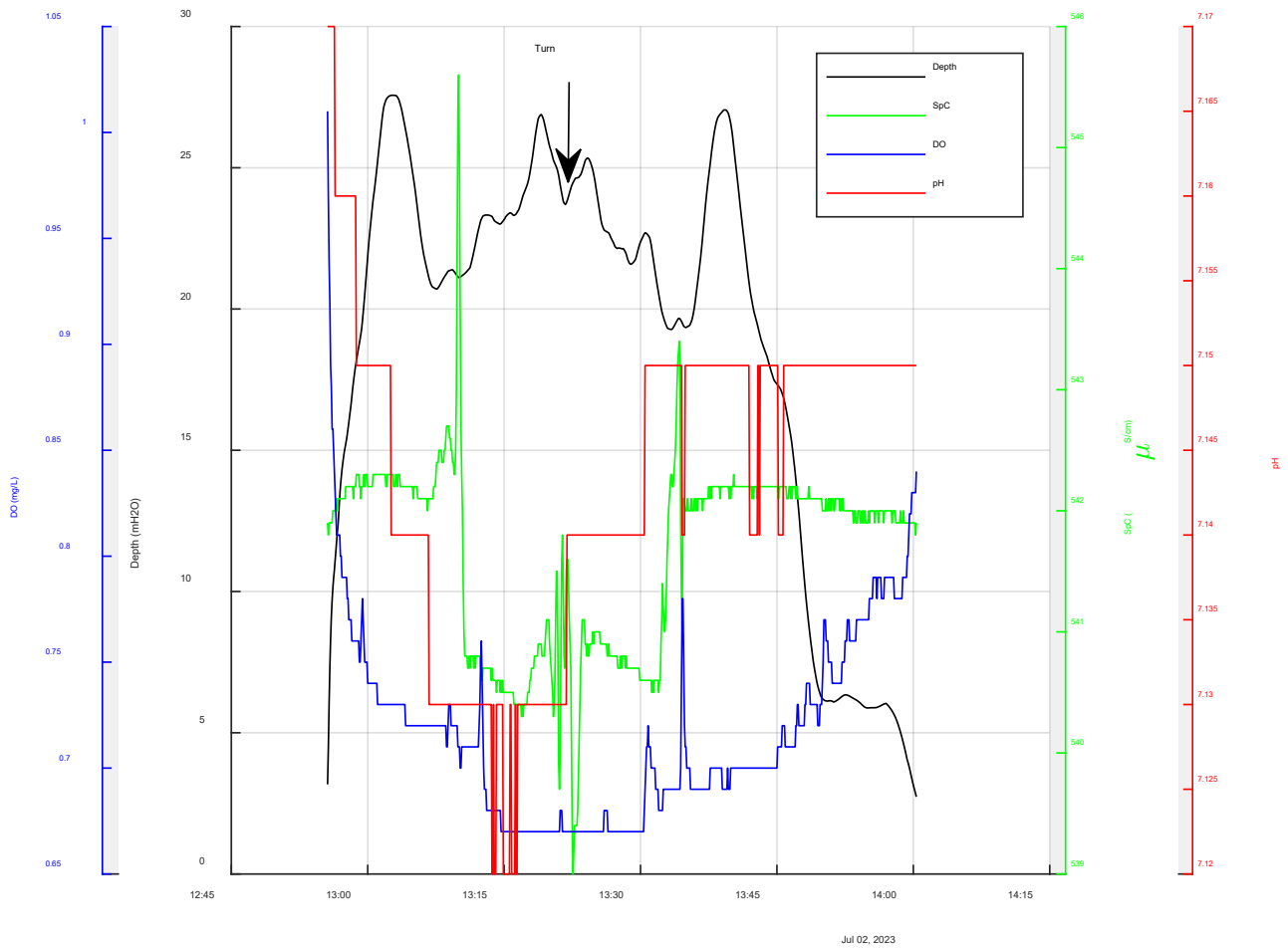

Figure 1.5: Manatee Spring dive timeseries. Turn indicates where divers turned around. Assume depth of __10m is entering the cave system__.
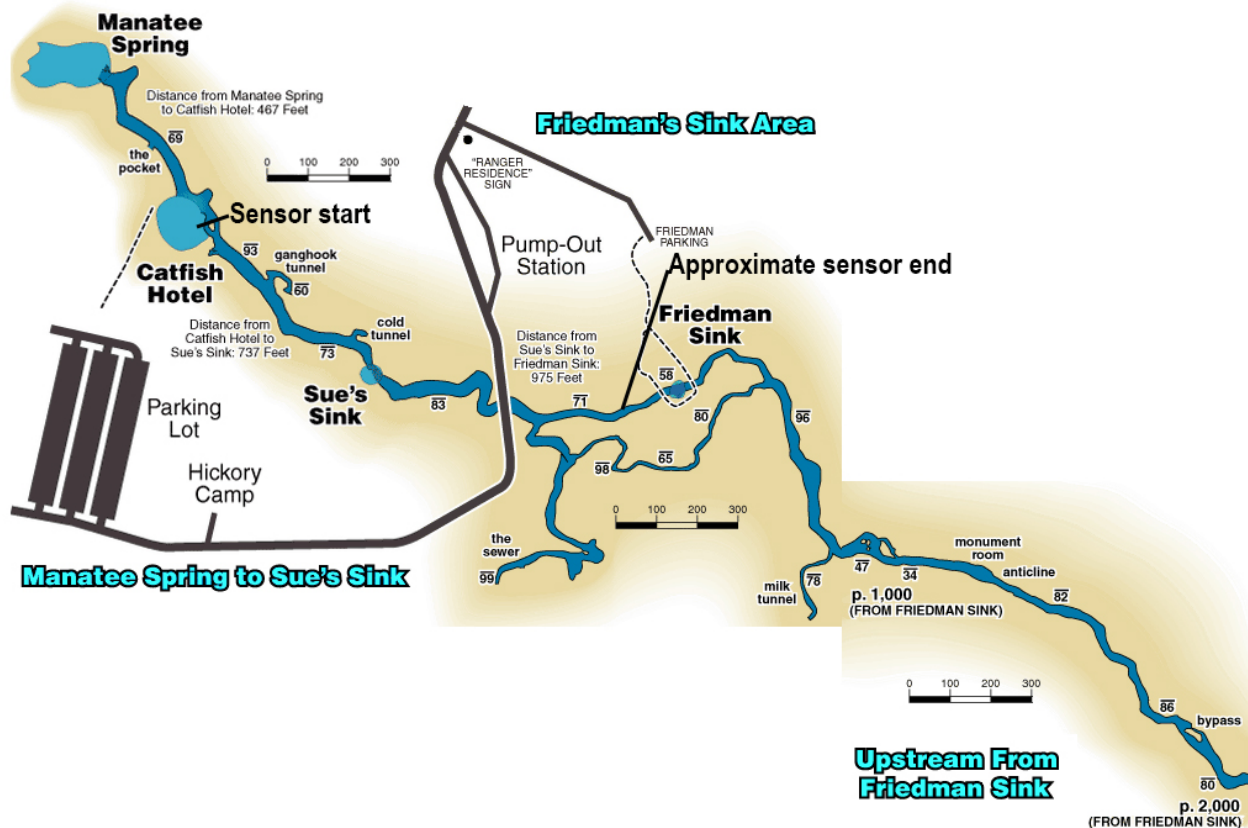
Figure 1.6: Manatee Spring cave map. No georeferenced cave map was available (though it exists) to USF at the time. Map courtesy of https://www.cavediving.com/where/manatee/

Peacock Springs

The variability in water quality both as continuously monitored water quality and discrete water sampling was most extreme at Peacock Springs. The anastomosing complexity of Peacock Springs cave system and the potential interconnectivity of the spring system with other cave systems along the Middle Suwannee River (MSR) (Greenhalgh et al, 2014) suggest that there are highly variable recharge points and land uses being drained. The variability in NO_x-N and DO together illustrates this point (Figures 1.9 and 1.10). Further, river water is known to stagnate in certain parts of the cave after a reversal. The flow dynamics in Peacock Spring are very complex, and we only captured a small part of the expected variability.

The survey of Peacock Springs water quality variability (pH, DO, T, SpC), shows some differences between the seasons as well. The route was the same for both surveys and began at Orange Grove and ended at the P1 vent (Figure 1.14). The water quality surveys were conducted at the same time as the discrete water sampling. The differences in pH and SpC across the same transect changed from each season with pH ranges increasing in the spring, while SpC ranges decreased in the spring. The surveys also highlight how the mixing waters change the chemistry that emerges at P1. For example, the DO is highest near the entry point at Orange Grove (even after descent beyond the cavern zone), a substantial decrease is observed throughout the dive toward site 2. On the transect back to P1, the DO is lower than the entry point but higher than the lowest value observed. Similar effects are observed on pH and SpC.

Peacock Springs is likely connected to multiple other cave systems and sinks in the region including Cow Spring, Running Spring, Bonnet sink and possibly Telford. Dye tracing and flow meter installations from Tom Greenhalgh showed that the middle Suwannee River (MSR) area is largely connected (Greenhalgh et al, 2014). Currently, discussions are underway to dye trace Peacock Springs proper and attempt to connect some of these cave systems and karst surface expressions.

Major ion chemistry did not change substantially between passages, albeit some changes occurred, however this just suggests that the waters are draining the Ocala limestone. The passage with lower DO is suspected to not have a direct recharge source from a surface feature unlike the other cave passages. That possibility also explains the lower NO_x-N concentrations, which may be buffered by diffuse rather than discrete recharge mechanisms, but also because of different flowpaths intersecting the cave passage. We do suspect a deeper flowpath in Peacock, but current restrictions make that difficult to sample (see Project 2). The passage, however, that had a high DO value was shown to have a recharge signal from specific conductivity sensors installed in that section of cave from 2008-2010 (possibly 2011) (Jason Gulley, personal communication).
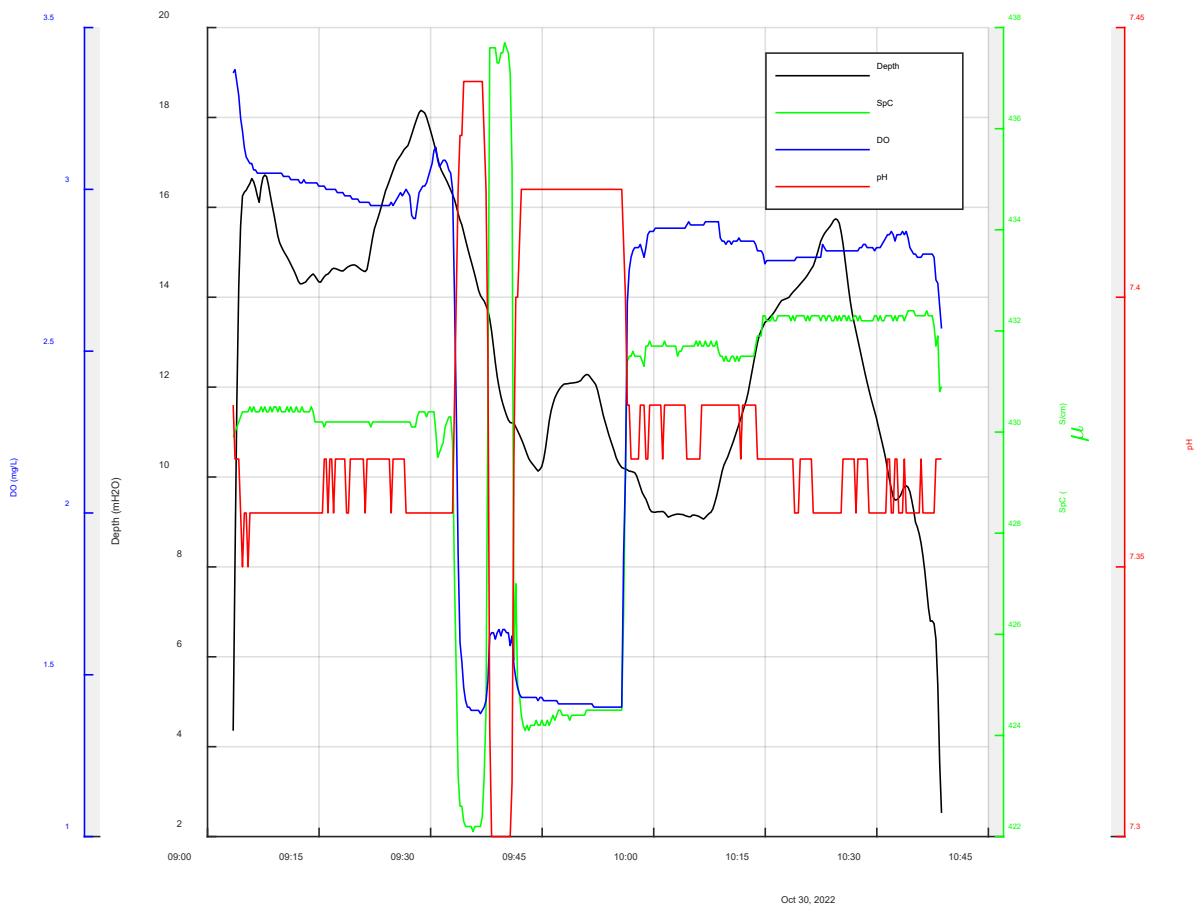

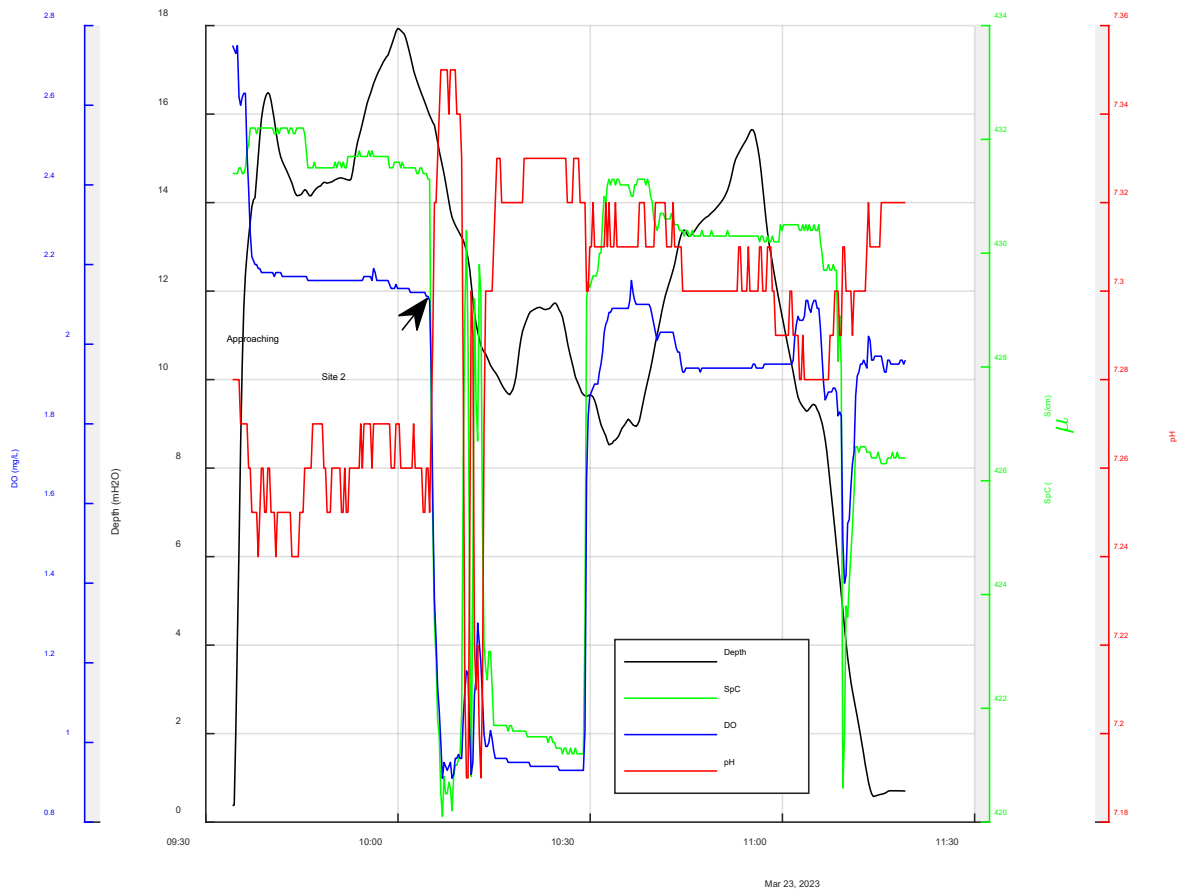Figure 1.7: Fall 2022 cave profile of Peacock Spring.

Figure 1.8: Spring 2023 profile of Peacock Spring. Similar relative water chemistry differences through the Distance Tunnel and around the Orange Grove area as from Fall 2022. Additionally, other conduits along the transect appear to be contributing more flow and thus altering DO and SpC more substantially than from Fall, 2022.
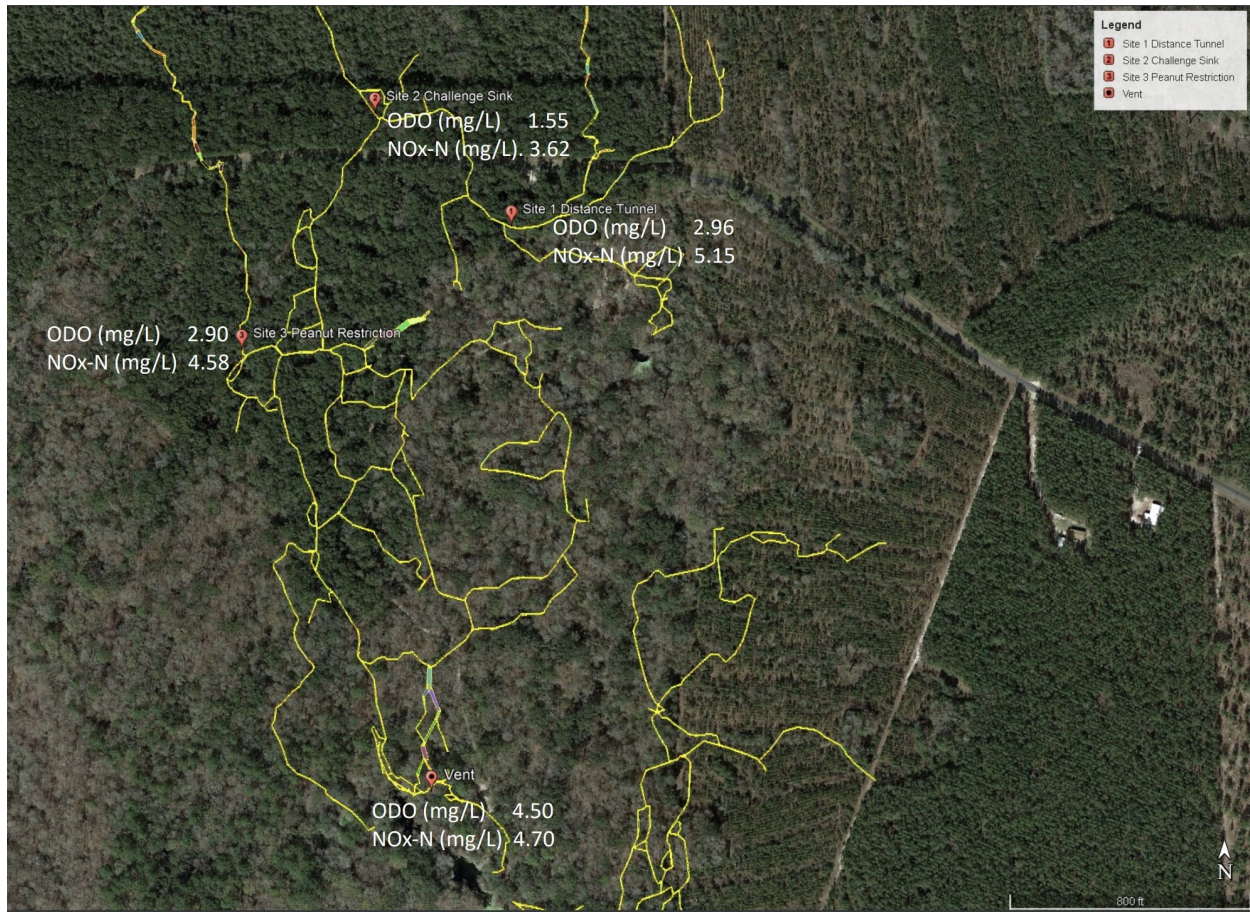
Figure 1.9. Measured variability of Nitrogen of Nitrate+Nitrite as NOₓ-N in Peacock springs, FL during November 2022. Cave water samples taken at 4 locations, including the vent at Peacock Spring (P1) indicate high variability between adjacent cave passages. For example, Site 2 and Site 1 are less than 1000 feet apart and have almost 2 mg/L difference in NOₓ-N. It should be clarified that Site 2 is not the actual sink, but upstream of Challenge Sink. Values represent average DO around sample point (20 points).
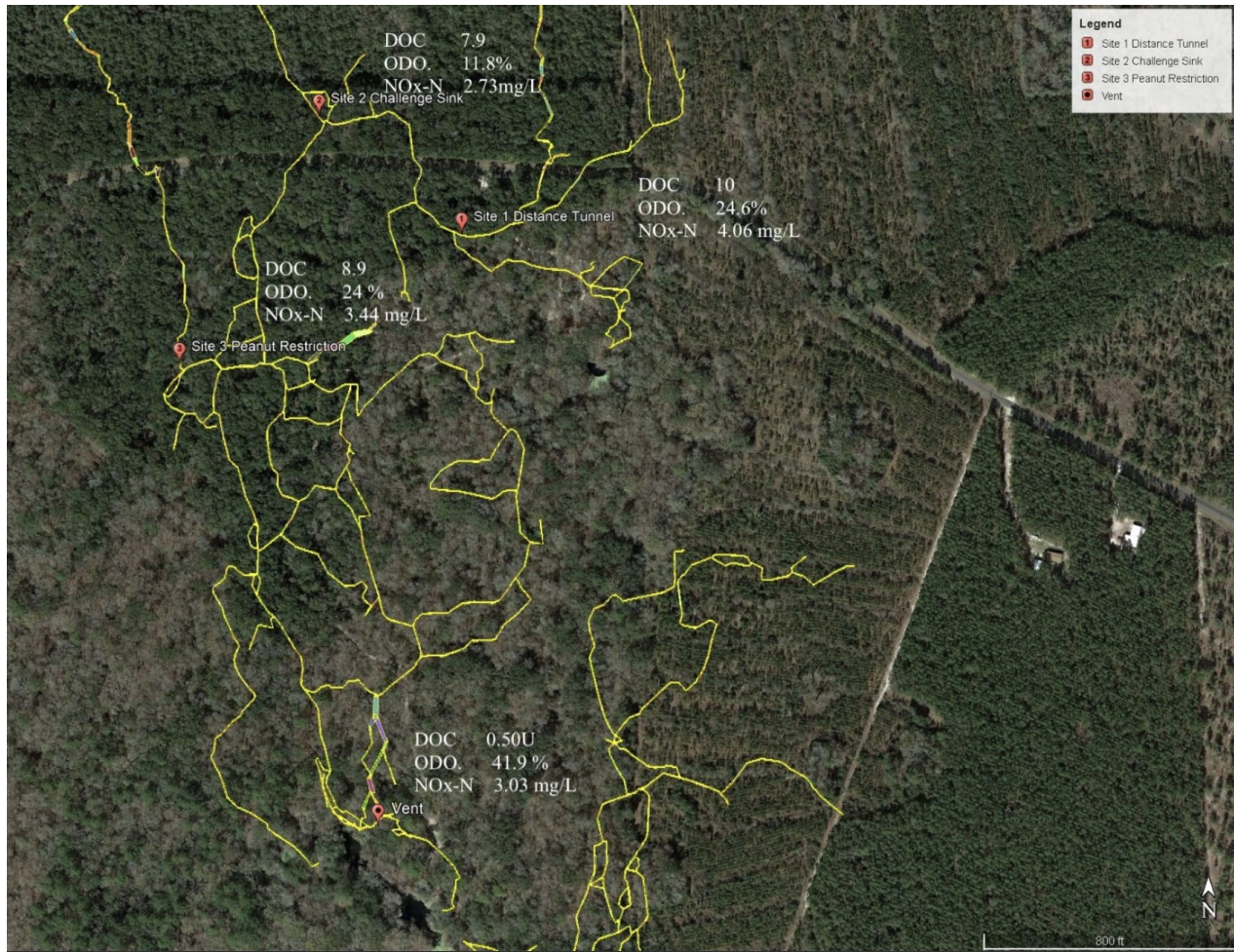
Figure 1.10. Measured variability of Nitrogen of Nitrate+Nitrite as NO_x-N in Peacock springs, FL during April 2023. Cave water samples taken at 4 locations, including the vent at Peacock Spring (P1) indicate high variability between adjacent cave passages. For example, Site 2 and Site 1 are less than 1000 feet apart and have almost 2 mg/L difference in NO_x-N. It should be clarified that Site 2 is not the actual sink, but upstream of Challenge Sink. DOC is in mg/L. U indicates undetectable below 0.5 mg/L.

We collected more data than planned for the proposal at Peacock Springs because 1) a student wanted to prioritize Peacock Spring for her M.S. thesis and 2) it was the most accessible during the time of the project due to unforeseen reversals at some targeted springs. We document some of it in here to provide a more comprehensive picture of Peacock Springs water quality variability.

We collected additional pH, DO, T, and SpC readings with the YSI EXO[2] at 4 locations in the Peacock Springs cave system (Figure 1.12) and collocated our YSI with the SRWMD installed YSI at the Peacock Spring basin during our April 2022 trip. The first location was the vent where we sent the YSI down on a line to record at P1 and recorded the entry way until the bottom. The Peanut and Pothole measurements were taken for 1 hour along each passageway and the sensor placed at either side of the junction leading to the different cave sections. The P3 measurement was done by a KUR diver who was diving P3 and offered to take the sensor on their dive and leave the sensor on the sign roughly 50-100 ft into P3 until they finished their dive (>1 hr). The results of the DO, SpC, and T readings are shown in Table 1.3. The additional data shows how

water changes as it emerges from the vent, and then flows over the land and siphons into the P3 cave.
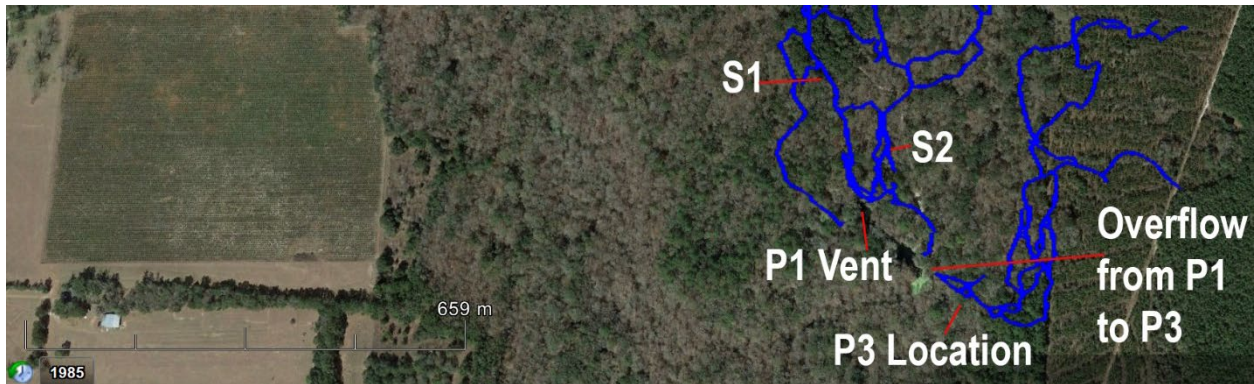

Figure 1.12: Locations of additional YSI EXO² sampling from Peacock Springs. The entrance and immediate cave passage following entry into Peacock Springs are in the window.

	Basin Sensor	Vent	Cave S1	Cave S2	P3 (siphon)
DO mg/L	3.65	1.96	2.63	1.55	2.95
DO %	41.80	21.6	30.0	18.0	33.8
SpC (µS/cm)	447	444	448	440	444
T	22.1	21.8	21.9	21.8	21.9

Table 1.3: Water quality parameters at the basin sensor, and 2 passages along Peacock Springs when entering P1 (Peanut, Pothole) and another measurement taken at the siphon (P3). Data obtained March, 2022. During this period, our pH meter was broken on the YSI EXO². This was done before project was funded.
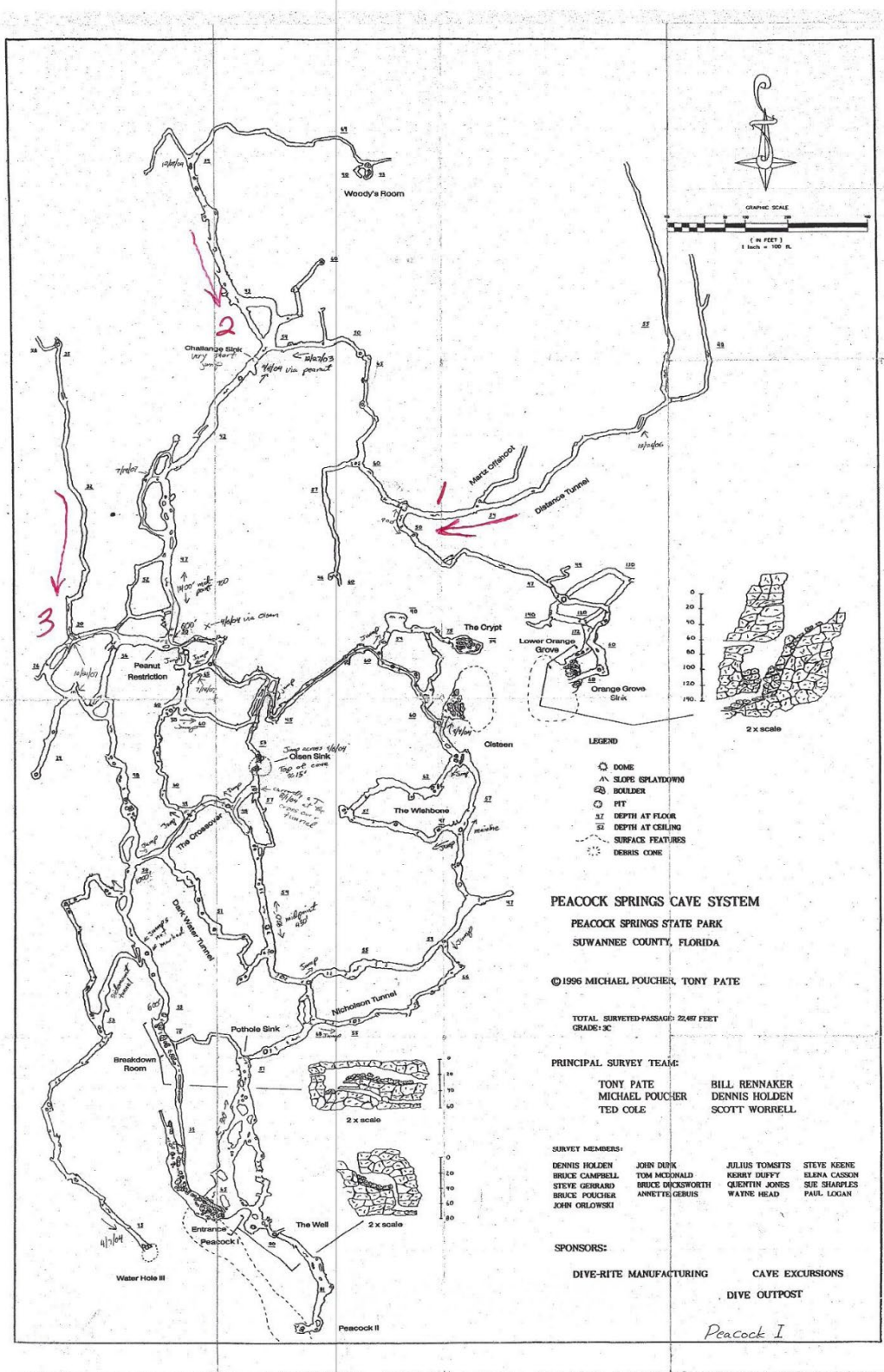
Figure 1.14: Peacock Springs cave system map with locations identified. The numbers represent the sampling locations and their respective names. Peacock Springs cave map provided by Mike Stine.

Madison Blue

Water chemistry profiles taken with the YSI EXO[2] obtained from Madison Blue Spring are shown in figure 1.14 and key water quality parameters from the discrete sampling are shown in Figure 1.15. The water chemistry survey in Figure 1.14 shows an increase in SpC, drop in DO, and drop in pH. This is potentially from a deeper water source that is not as readily recharged (lower DO) and more equilibrated (higher SpC) to matrix rock. It could also be draining upgradient water (Figure 1.16) from above the known and mapped location of Madison Blue Spring cave proper, but this is speculation. The changes in water quality across the cave system also impact the signal at the vent much like in Peacock Springs. The Madison Blue Spring water quality survey began at Martz sink and ended at Madison Blue vent. The DO and pH are lower and SpC is higher at the vent than at the entry point at Martz. The values change after intersection of the Cross-under tunnel with the main cave passage.

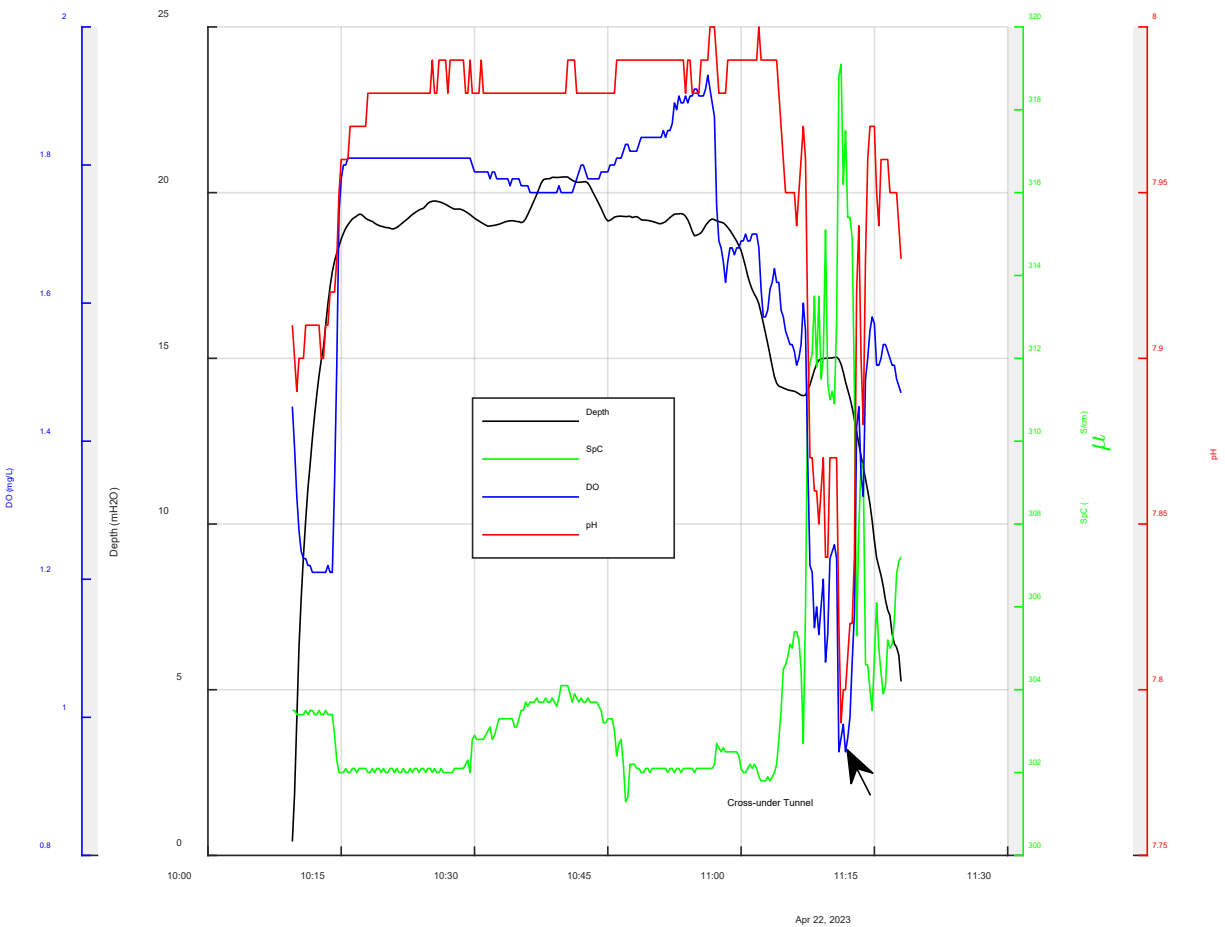Figure 1.14: Madison Blue cave profile. Cross-under tunnel identified (Figure 1.15) where major water chemistry shift occurs. pH decreases >0.2, SpC increases almost 20 µS/cm and DO decreases by over 1 mg/L as you enter the tunnel. Volatility in parameters also indicates water from additional intersecting passages. See video: https://www.youtube.com/watch?v=tb_nJCk9808 for shifts in water clarity observations.

Figure 1.15: Available water chemistry from NELAC lab during water sampling. Additional chemistry in Supplemental B. It should be noted a more detailed map exists, but is currently unavailable to USF.

Major ion and NO_x-N from Madison Blue Spring indicate that water chemistry is not as variable as it is in Peacock Springs. In fact, they are *surprisingly* similar, even at the Cross-under tunnel, and major ion chemistry was also almost identical. The similarity in major ion carbonate chemistry is not surprising, especially when compared to Peacock Spring. We strongly suspect sample data taken from the Cross-under tunnel was not representative and a duplicate sample from another site was analyzed. Possible issues could be either 1) Sampling at the site was from the wrong water sample for NO_x-N or 2) a duplicate run of NO_x-N for a different site was performed and reported as Cross-under (a separate sample bottle is used). Changes in DO, T, SpC, and pH along with persistent diver observations suggest a different water source (Figure 1.14). We are going to contact the NELAC lab about potentially clearing any problems up on their end and then sample the same sites again to re-do the water chemistry.

Unfortunately, continuous reversals made accessing Madison Blue Spring difficult during the spring and early summer 2023. We wanted to capture water that would be representative of aquifer conditions along the different cave passages, but the storms kept affecting the condition of the cave system and subsequently, our sampling schedule. This season was particularly bad for some reason.

It should be noted it is possible Madison Blue Spring is impacted by frequent river water intrusion. The cave system at M2 Blue north of Madison Blue Spring (Figure 1.16) is connected into Fannels Funnel which is known to siphon river water from the Withlacoochee River rather regularly (Spellman, 2012). The potential connectivity of M2 Blue to Madison Blue Spring could

have implications for increased river water intrusion if head gradients are persistently reduced near the river due to climate changes or pumping. However, because the connection has not been made, and efforts are still underway to connect these two cave systems, we cannot say for certain if that would be a possibility. The passageway connection also intersects the regional flow gradients toward the Withlacoochee River, indicating the sources to Madison Blue Spring may be more extensive than can be understood from current cave maps.

Figure 1.16. Colocation of Fannels (sometimes Fennels) Funnel, M2 Blue and Madison Blue Spring. Photo sourced from Karst Underwater Research (KUR) found at https://sites.google.com/view/karstunderwater/projects/m2-blue.

Monitoring point comparisons

Table 1.4 details the differences between the vent and the sensor at the monitoring point by % change. The data are all collected with the same calibrated EXO^2 for standardization. We

show images at each location of the spring monitoring point location and vent with the DO reported. All other water quality parameters are found in the original EXO^2 data provided.

Special notes

   Fanning Spring monitoring point sensor data showed very different readings in DO at the vent compared to the sensor placed at the gaging station. We point out we went to Little Fanning Spring to get some data from that as a comparison. We found that Little Fanning Spring had DO more comparable to measurements at the sensor which was slightly higher (~2.52 mg/L). The measurements at Little Fanning Spring mostly showed that the water chemistry was similar, with SpC almost identical to the vent SpC as well as the temperature. Additional major ion chemistry and nutrients collected at Little Fanning Spring also showed almost identical water quality (See Supplemental B).

   Surprisingly, Gilchrist Blue was not as bad as suspected. We anticipated some differences due to the long spring run, but the sensor recorded values similar to what was recorded at the vent with limited increase in DO.

Spring	% Change DO (mg/L)	% Change SpC (µS/cm)	%Change T (C)	%Change pH	Date
Jug Hole	14%	<0.1%	<0.1%	<0.1%	June 2023
Manatee Spring	56%	1%	<0.1%	<0.1%	June 2023
Peacock Spring*	86%	<0.1%	1%	<0.1%	April 2022
Fanning Spring	50%	<0.1%	<0.1%	<0.1%†	April 2022
Gilchrist Blue	5%	2%	<0.1%	<0.1%	June 2023
Madison Blue Spring*	2%	9%	2%	<0.1%	April 2023

Table 1.4: Percent difference between vent recorded values and sensor location. The * at Madison Blue and Peacock indicate the most extreme differences were reported although multiple sampling times occurred. † Used an external pH (calibrated) meter on water obtained from spring. All values are positive increases of the parameter at the sensor location unless noted with a negative sign.
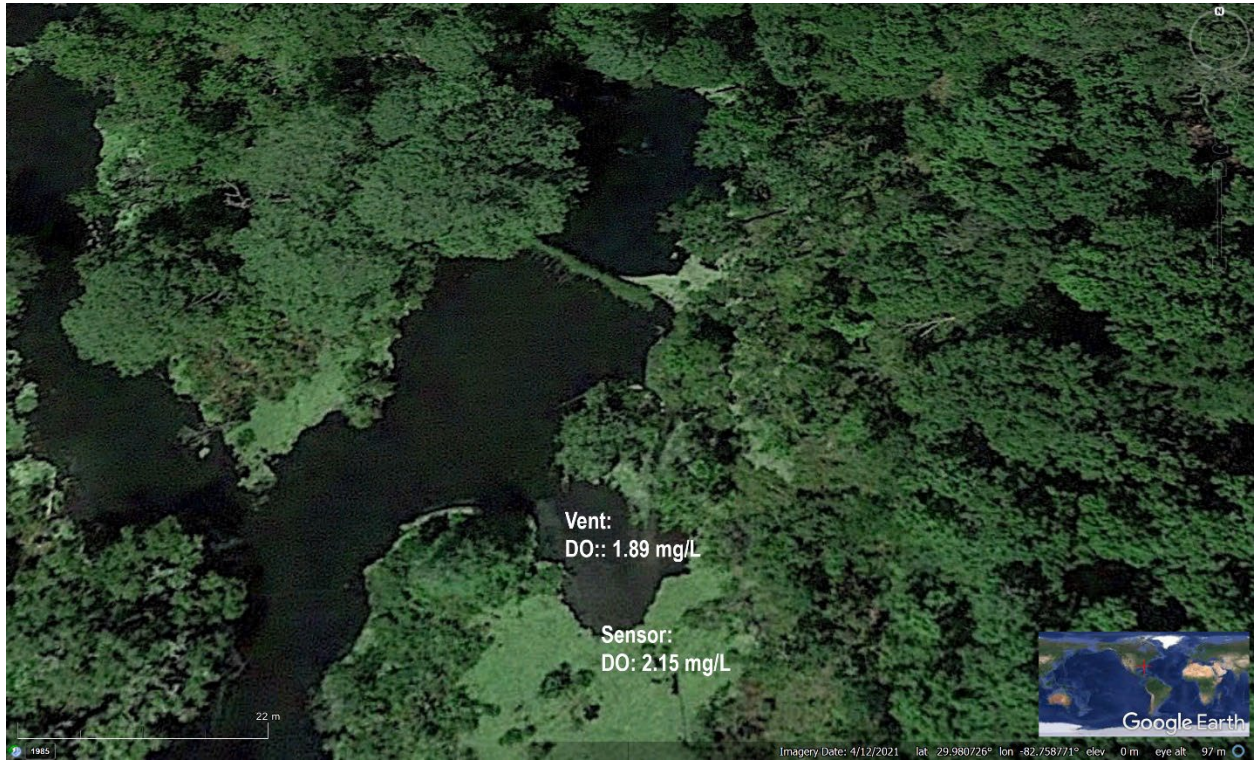
Jug Hole

Figure 1.17: Jug Hole sensor vs vent DO values

Manatee Spring

Figure 1.18: Manatee Spring sensor vs vent DO values
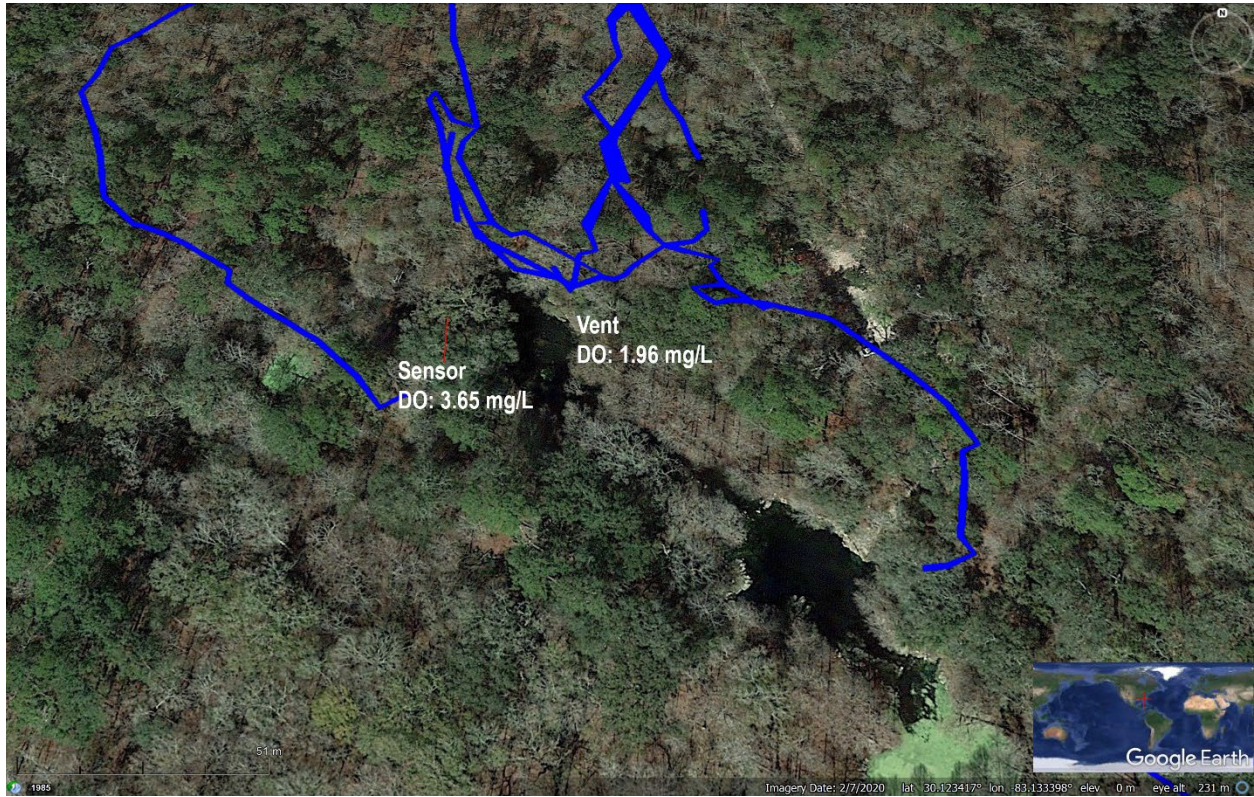
Peacock Spring

Figure 1.19: Peacock Spring (P1) sensor vs vent DO values. Cave map (Blue lines) superimposed on land surface map

Figure 1.20: Fanning Spring and Little Fanning Spring DO comparisons to sensor DO values

Gilchrist Blue Spring

Figure 1.21: Gilchrist Blue Spring sensor vs vent DO values

Madison Blue Springs

We took sensor comparisons of Madison Blue Spring during high and low water conditions and report the lower water conditions (Figure 1.22). During high conditions, data comparisons between the sensor and the vent were not very different.

We also placed a sensor long-term inside the cave at the sign to monitor how water quality changes occur over a longer period (>3 months). We managed to capture a reversal and recovery and showed the water quality differences reported in the cave and sensor (Figures 1.23-1.25). We only got to Madison Blue Spring for this experiment, as we could not afford to lose our EXO^2 for long periods of time due to our DO sensor on our YSI ProDSS not working properly.
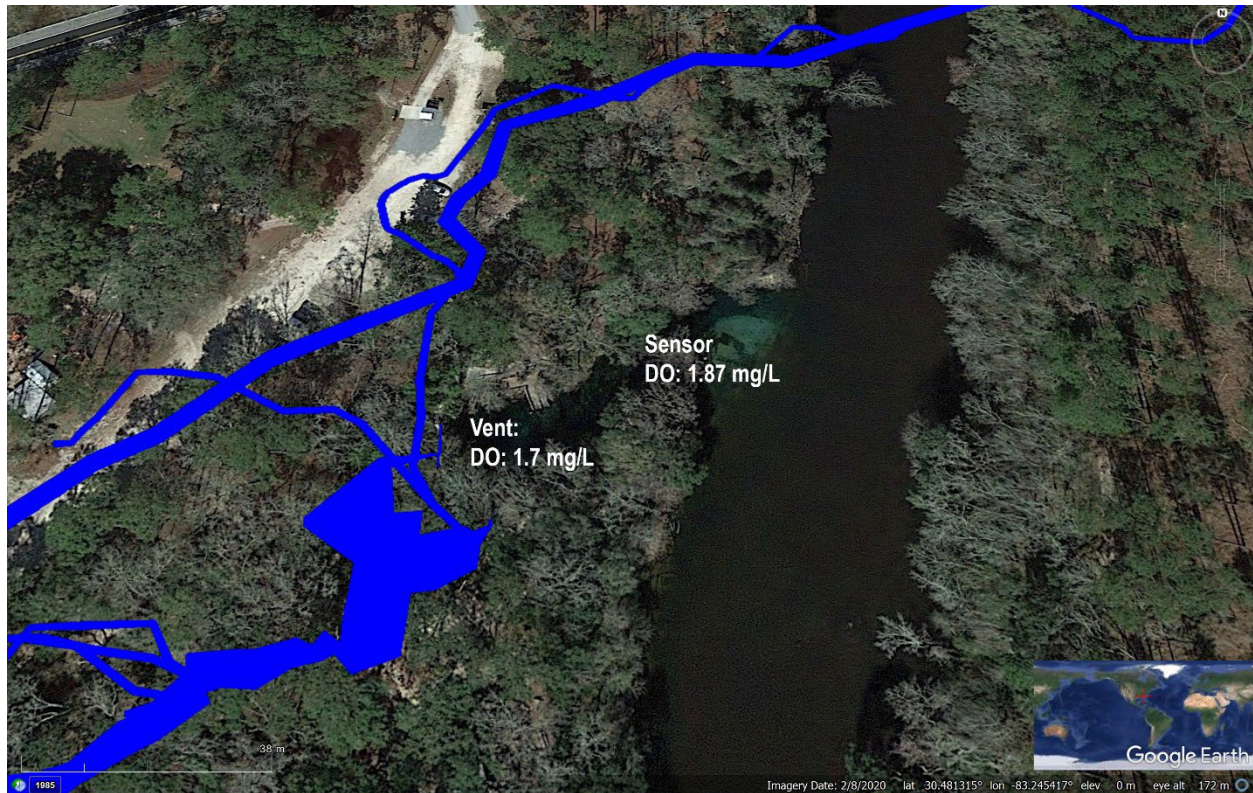

Figure 1.22: Madison Blue Spring sensor vs vent DO values. Cave map (Blue lines) superimposed on land surface map
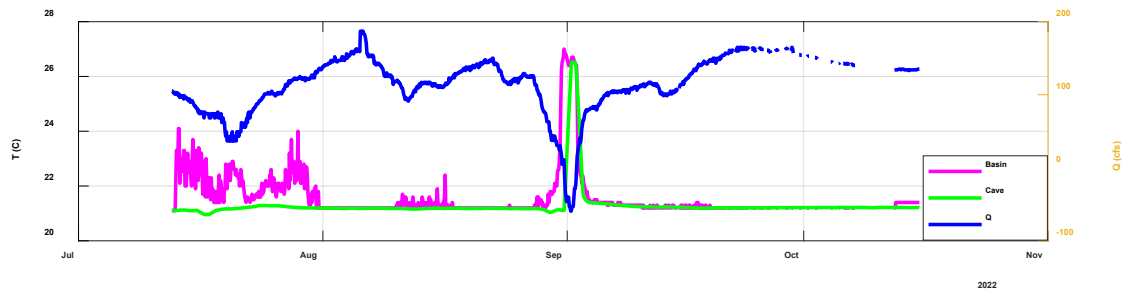
Figure 1.23:

TOP: Absolute time series of temperature comparing the values in the Basin (DEP monitoring site), in the Cave, and the reported discharge (Q) from the USGS. Different axes are used for temperature (left) and discharge (right).

MIDDLE: The PERCENT DIFFERENCE in temperature between the basin and the cave. The difference is taken by subtracting the values recorded at the cave from the values recorded at the basin and dividing by values in the cave.

BOTTOM: A relationship between the difference in temperature at both locations and discharge
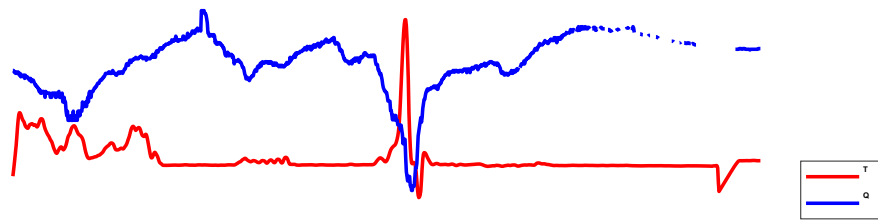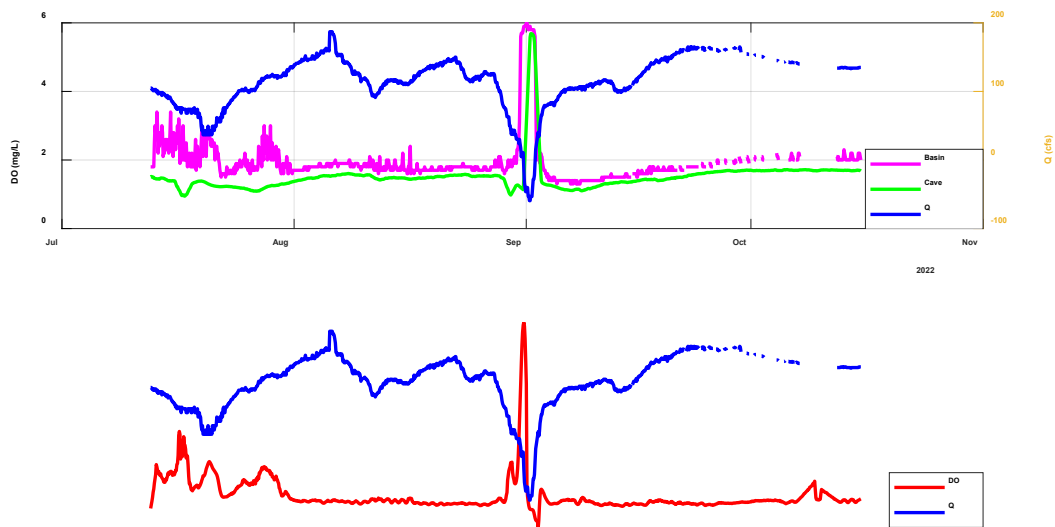
Figure 1.24:

TOP: *Absolute time series of dissolved oxygen (DO) comparing the values in the Basin (DEP monitoring site), in the Cave, and the reported discharge (Q) from the USGS. Different axes are used for DO (left) and discharge (right).*

MIDDLE: *The PERCENT DIFFERENCE in DO between the basin and the cave. The difference is taken by subtracting the values recorded at the cave from the values recorded at the basin and dividing by values in the cave.*

BOTTOM: *A relationship between the <u>difference</u> in DO between locations and discharge*

Figure 1.25:

TOP: Absolute time series of specific conductance (SpC) comparing the values in the Basin (DEP monitoring site), in the Cave, and the reported discharge (Q) from the USGS. Different axes are used for SpC (left) and discharge (right).

MIDDLE: The PERCENT DIFFERENCE in SpC between the basin and the cave. The difference is taken by subtracting the values recorded at the cave from the values recorded at the basin and dividing by values in the cave.

BOTTOM: A relationship between the difference in SpC between the locations and discharge
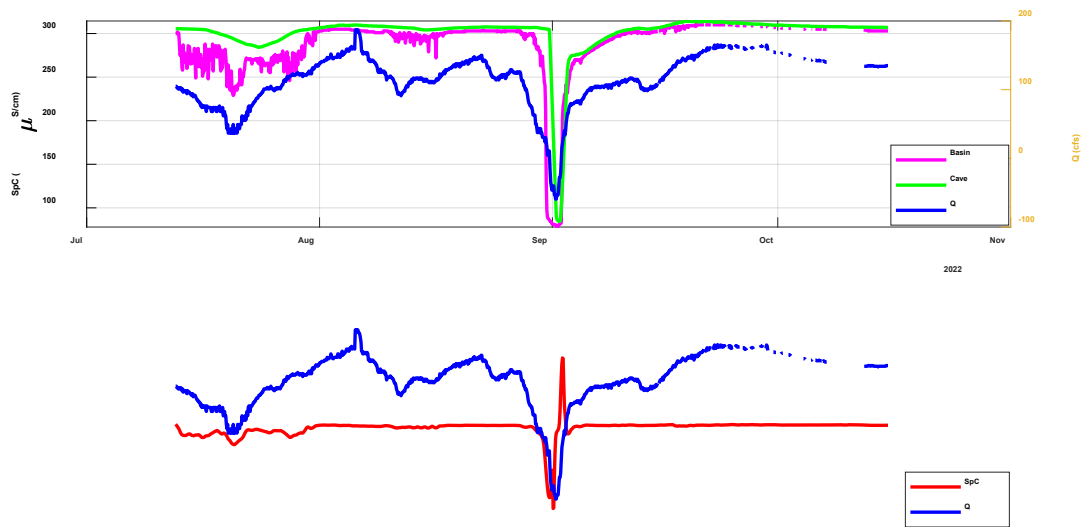
Data from the long-term deployment of the sensor in Madison Blue Spring cave system showed there was a bias between spring monitoring location and the sensor in the cave system. The most extreme differences were observed in DO and T, but less so for SpC, which was expected based on the other results. The most extreme differences occurred during a reversal (Sep, 2022), and smaller differences occurred during the end of the rainy season (October, 2022). The average difference in DO was around ~0.3 mg/L with a maximum difference of 1.8 mg/L (after a pseudo-reversal in July). There appeared to be an increase in the difference between the spring basin value and cave sensor DO prior to removal which coincided with entering the dry season.

It is hard to tell if there is a discharge threshold with which we may expect to see some differences in the water quality, or if lack of calibration during deployment may have been causing some drift in the data. Although there did not appear to be any substantial drift in the water quality data, so likely not a calibration issue.

It was also challenging to create a relationship between the differences in the sensor readings (basin and cave) and discharge. Though the data was initially screened for the major reversal and the pseudo-reversal (river water appeared to mostly impact the sensor), the relationship was still poor. The relationship using polynomials improved when re-integrating the pseudo reversal (Figures 1.23-1.25), but that is not an appropriate technique. It became clear that it would be difficult to determine when any bias correction could be applied at Madison Blue as any data truncated between 60-120 cfs, which represents average conditions, contained no significant relationship. The lack of being able to create a relationship for bias correction at a site where only small differences were observed, indicated it is probably more challenging where more substantial differences in water chemistry are known to occur.

We had chosen Madison Blue Spring because discussions with SRWMD personnel and observations indicated that the spring run likely aerated water across the rocky shoals prior to reaching the sensor location. We suspected higher differences in DO at normal flow conditions (non-reversals) but were surprised at the similarities. However, we will likely try again when flows are even lower (which we somehow missed this year) and see if there is much of a difference during extremely low flows.

Discussion

We knew accessing some of the caves would be challenging and was dependent upon weather and safety conditions within the cave systems and we had planned for that by collecting additional data or replacing some springs with others. Unfortunately, one spring we were unable to achieve our survey and water quality sampling goals was in Lafayette Blue Spring due to consistently poor conditions for diving. There is only one access point that is not on private property and the visibility was apparently not suitable in that entry point for safe navigation. Because I had never been in that cave system before, I was relying on experienced cave divers who had navigated Lafayette Blue Spring previously. Lafayette Blue Spring is a shallow cave relative to other cave systems we selected and has multiple sinkholes along the cave which certainly impact the water quality in the cave, and the shallowness likely is why it is also so large in size (cavern width). This can also make it dangerous to access based on current access points available to us. The lack of data is disappointing since nitrate concentrations at Lafayette Blue Spring are some of the most rapidly increasing among the springs in the SRWMD as of 2010 (Figure 1.26).
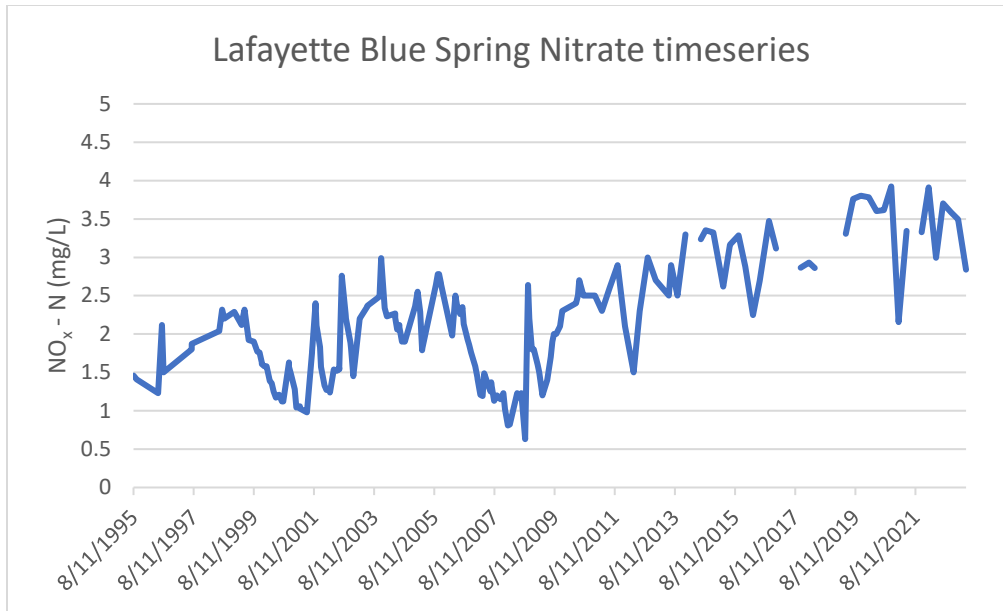
Figure 1.26: Lafayette Blue Spring nitrate concentrations through time. Data acquired from Suwannee River Water Management District.

Implications on monitoring

The implications for monitoring karst aquifer dynamics at a single discharge point (i.e. spring) are dependent upon the intent of the monitoring as the spring vent is a convolution of different sources. As observed in the water quality surveys, variability in the conduits contributed to changes in the overall water chemistry emerging at the vent. The extent of the changes is dependent upon the discharge from each contributing passageway. It is also dependent on the consistency to which each passage contributes water. Parameters such as pH can vary, even seasonally, which was slightly surprising. Monitoring long term trends in some of the more stable parameters such as pH and SpC is probably reasonable, but still comes with individualized interpretation at each spring.

Parameters such as SpC can be difficult to assess regarding continued monitoring and interpretation. We observed changes in SpC along conduit passages, but the change was usually small due to different passages still draining primarily limestone (Krawczyk and Ford, 2005). More extreme changes may be observed at passages we were unable to get to for this project. However, increases in SpC across many springs (see Project 2) suggest that something has changed, which includes possibilities such as discrete fracture contributions from LFA waters, changes in length of contributing flowpaths due to hydrologic shifts in the aquifer (e.g., drainage divide changes), or long-term influx from surface contaminants. This makes it important to continue monitoring SpC.

High variability in DO within the cave system, coupled with changes in DO between the spring vent and most monitoring locations, suggest that DO is not entirely reliable for monitoring changes that are extrapolated to widespread recharge basin changes. If a sinkhole opens up (see Gilchrist Blue recent collapse) or land use practices change (see Project 2), the amount of recharge and water quality of recharge would change and be reflected at the monitoring point. However, this would be from a point/isolated source, and could erroneously be attributed to changes in a basin process. For example, the two different water sources in Madison Blue Spring

and Peacock Springs illustrate that the mixing caused a difference in DO that emerged as a converged signal at the vent. Different passages may contribute different amounts due to nonuniform recharge across the basin and the hydraulic state of the aquifer. The relative contributions of each cave passage may also change, potentially seasonally (see Peacock Springs), and therefore would introduce an uncertain cause of variability in DO. Some biogeochemical reactions within the cave system can also reduce DO values under locally reducing conditions. The high variability in DO across cave systems indicates it would be challenging to determine what that measured DO ultimately represents.

The data from Peacock Springs spring vent comparison to continuous monitoring location is the most dramatic illustration that choosing the monitoring point for water quality is critical, with advice to do a comparison of the water quality sensor at the vent along with potential monitoring sites. That is, if the intention is to try and capture overall trends aggregated over the recharge area. All water quality parameters were somewhat biased compared to vent water quality and of course, this varied at different locations.

The same interpretation made for DO within cave systems can be made for DOC and NO_x-N concentrations. The high variability in NO_x-N along with no distinct relationship between NO_x-N and DOC or NO_x-N and DO (albeit small sample size), indicate that NO_x-N could be varying due to different land use drainage, however without more detailed chemistry to rule out biogeochemical processes, this is only hypothesized. However, for monitoring overall changes in the entirety of the basin recharge area, sampling NO_x-N will still provide a useful monitoring tool at the vent to establish metrics at the spring. However, caution should be used for monitoring surface derived contaminants and reactive species and making general inferences from data collected only at the spring vent.

Fanning and Manatee Springs have sensors that are located significant distances away from the spring vent which only seemed to affect the DO measured. The sensor at Fanning Spring is a combination of both Fanning and Little Fanning Spring discharge. The sensor frequently reports a 'reversal', but in many cases, this is just some river water moderately intruding into the spring run while Fanning Spring still discharges, and the basin water near the vent is clear. Little Fanning Spring and Fanning Spring have similar major ion chemistries, however the shoals at Little Fanning Spring aerate the water impacting the DO downstream. Manatee Spring also has a sensor a fair bit away from the spring vent and DO has been known to increase downgradient (farther along the spring run) under some monitoring timeframes. Therefore, it is clear that water quality sampling should be performed as close to the vent as possible for monitoring basin management success. In some cave systems, monitoring wells that are within the conduit system provides more reliable indicators of groundwater changes. However, capturing the variability in the cave system remains challenging using current water sampling protocol.

Future work

We would like to continue the same type sampling at different springs as well as ones we missed for this project and keep building the database of water quality variability and potentially use these techniques for conduit tracing to source contamination. Springs can drain a range of different cave morphologies, each with potentially unique management needs. Understanding the flow and water quality dynamics more holistically can improve individual restoration and water quality management. We are also trying to complement these analyses with more dye tracing to connect cave systems, sinks, and karst windows and understand the extent of contributing areas to caves and their discharge points.

We would also like to sample cave systems that have known cave passage directly underneath active farms. Cave diver observations have noted visible changes to water quality (brown, murky water) underneath many of these farms where the caves occur (Lineeater/ Falmouth, Weeki Wachee to name a few cave divers have mentioned), and sampling for water quality, particularly nutrients, upstream, below, and at discrete points downstream from the farm can help identify and possibly constrain nitrate transformation from the source (Figure 1.27 is Lineeater example).

Figure 1.27: Lineeater cave system whose conduit passage runs directly underneath an active farm. From Cropscape, I identified corn/peanut rotation (Only 2017-2022).

Completion of tasks

Cave survey		
Site	*Completed?*	*Notes*
Jug Hole	Yes	Completed: June 2023 - Replaced Convict (collapse)
Manatee Spring	Yes	Completed: June 2023
Peacock Springs	Yes	Completed: March 2023
Madison Blue Spring	Yes	Completed: March 2023
Cave water chemistry		
Peacock spring	Yes	Completed: March 2023
Madison Blue Spring	Yes (50%)	We were unable to get back to access it as more frequent than typical reversals and scheduling made it difficult
Sensor comparisons		
Gilchrist Blue	Yes	Completed: June 2023
Fanning Spring	Yes	Completed: June 2022 (multiple times)
Manatee Spring	Yes	Completed: June 2023 (multiple times)
Jug Hole	Yes	Completed: June 2023
Madison Blue Spring	Yes	Completed: March 2023 (multiple times)

Table 1.5: Project tasks and notes about completion.

Project 2: Identifying source waters to priority springs using isotopes of sulfate, geochemical modelling, and data analysis.

Review

The goals of Project 2 were to collect $\delta^{34}S$ and $\delta^{18}O$ isotopes of sulfate (SO_4^{2-}) along with major ion chemistry, nutrients (NO_3^-), and $\delta^{18}O$ and δ^2H isotopes of water to develop a comprehensive understanding of shallow vs deep water sources on spring water quality changes. The deep-water source is defined as the Lower Floridan Aquifer (LFA) which has a distinctive water chemistry due to gypsum beds and dolomite that were deposited during the Eocene (Scott et al., 1990). Because $\delta^{34}S$ and $\delta^{18}O$ of SO_4^{2-} from the LFA would have different characteristics to that of shallow derived, Upper Floridan Aquifer (UFA) SO_4^{2-}, it would be a useful indicator for mixing models and geochemical interpretation (Sacks and Tihansky, 1996). Changing water chemistry observed as increases in key major ions and specific conductance at springs draining the UFA (Spellman, 2022) has given thought to 1) the potential of increasing LFA water contributions via existing fractures (Moore et al., 2009) due to pumping or 2) anthropogenic sources. Our project sought to collect initial data to constrain LFA contributions at priority springs where increases in specific conductance and major ions have been observed alongside nutrient enrichment.

The motivation for this project was multifold and part of a more comprehensive project to understand the hydrology, solute mobility, and geometry (as porosity space) of the Floridan Aquifer System (FAS) in the context of eogenetic (Florea and Vacher, 2004) karst systems in general. Directly, the results of the project are an indicator of the contribution of LFA vs shallower water sources to key priority springs that could be sampled and completed in the timeframe of the project. However, quantifying fractions of these two contrasting end members, or eliminating the LFA altogether, allows us to narrow assumptions and begin to parse out other information about the FAS which has implications for water resource management that are described hereafter.

First, when the contributions of LFA waters can be quantified, major ions that are associated with agriculture but not UFA minerals (e.g., K^+, Mg^{2+}, SO_4^{2-}) can potentially be used as tracers to track surface source contaminants and changes to land use and/or agricultural practices. For example, if we eliminate the LFA as a contributing or minimal source, we can use solutes that are associated with different agricultural and livestock practices (i.e, peanuts require potassium/magnesium, cattle bedding and feed contains agricultural gypsum, etc.) along with land use maps and DEM data to constrain the potential major source contaminants to springs. Coupled with methods from Project 1 and/or (potentially) DNA analysis, conduit tracing can also be used to identify pollution sources more precisely. Trends in ions would also now be used to determine average diffuse intergranular contributions over time from shallow storage (Figure 2.1), which helps us more holistically understand the general geometry of the aquifer and its function.
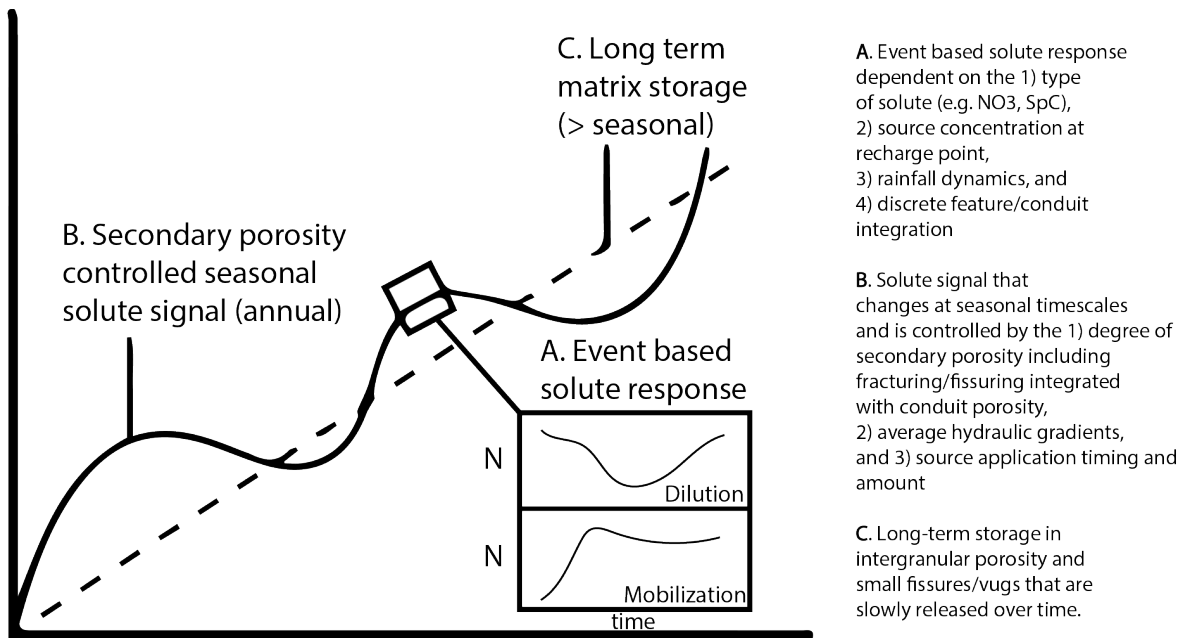
C. Long term matrix storage (> seasonal)

B. Secondary porosity controlled seasonal solute signal (annual)

A. Event based solute response

N — Dilution

N — Mobilization

time

A. Event based solute response dependent on the 1) type of solute (e.g. NO3, SpC), 2) source concentration at recharge point, 3) rainfall dynamics, and 4) discrete feature/conduit integration

B. Solute signal that changes at seasonal timescales and is controlled by the 1) degree of secondary porosity including fracturing/fissuring integrated with conduit porosity, 2) average hydraulic gradients, and 3) source application timing and amount

C. Long-term storage in intergranular porosity and small fissures/vugs that are slowly released over time.

*Figure 2.1: Solute dynamics in the FAS based on high temporal resolution observations on $NO_2 + NO_3$ as **N** across selected springs in the SRWMD. Adapted from Spellman et al., 2022.*

Second, the redox state of the aquifer could be locally controlled by different source waters. LFA waters are lower in DO with respect to UFA waters (albeit this is variable) and mixing of LFA waters could affect the redox state at springs. From Project 1, we showed that contributions from a small section in Peacock Springs could change the average DO concentrations that emerge at the spring vent. For example, if LFA water contributions are negligible, then changes in redox state would be regulated by surface controls including land use changes and practices. It is known that biochemical oxygen demand (BOD) can increase from land uses that contain animal wastes, septic tanks, and spray fields (FDEP Springs Literature Report, 2007) (see Manatee Spring discussion – this report). Over time, that would locally impact the overall groundwater quality in the aquifer affecting biogeochemical reactions (e.g., denitrification). Understanding the primary source helps target specific remediation efforts.

Third, a proxy for dissolved ions, specific conductance, has been increasing over the last few decades at priority springs (Spellman SRWMD Report, 2022) and the exact implications of the increases on spring ecosystem health are unclear. These increases have been attributed to older, more equilibrated natural water which could include UFA matrix water or LFA water that is more saline from evaporites and proximity to saltwater, depending on location. For simplicity, we bulk any water types from depth as LFA waters in this report.

UFA matrix water is likely more equilibrated with respect to carbonate minerals compared to newly recharged water, but there would be a threshold reached based on local geology. In a previous study, geochemical modelling using PHREEQc (USGS) coupled with field data were used to determine the relationship (linear) between measured $CaCO_3$ (mg/L) (i.e. limestone) and the expected specific conductance in pure limestone (Krawczyk and Ford, 2006). The study determined a maximum upper limit of specific conductance from natural limestone weathering to be 600 µS/cm. Using the relationships developed in Krawczyk and Ford, 2006 and reported $CaCO_3$ values from springs selected for analysis as inputs, we calculated a local *theoretical*

threshold of specific conductance for each spring where we may start to expect contamination/sources beyond natural UFA limestone mineral weathering (Figure 2.2).
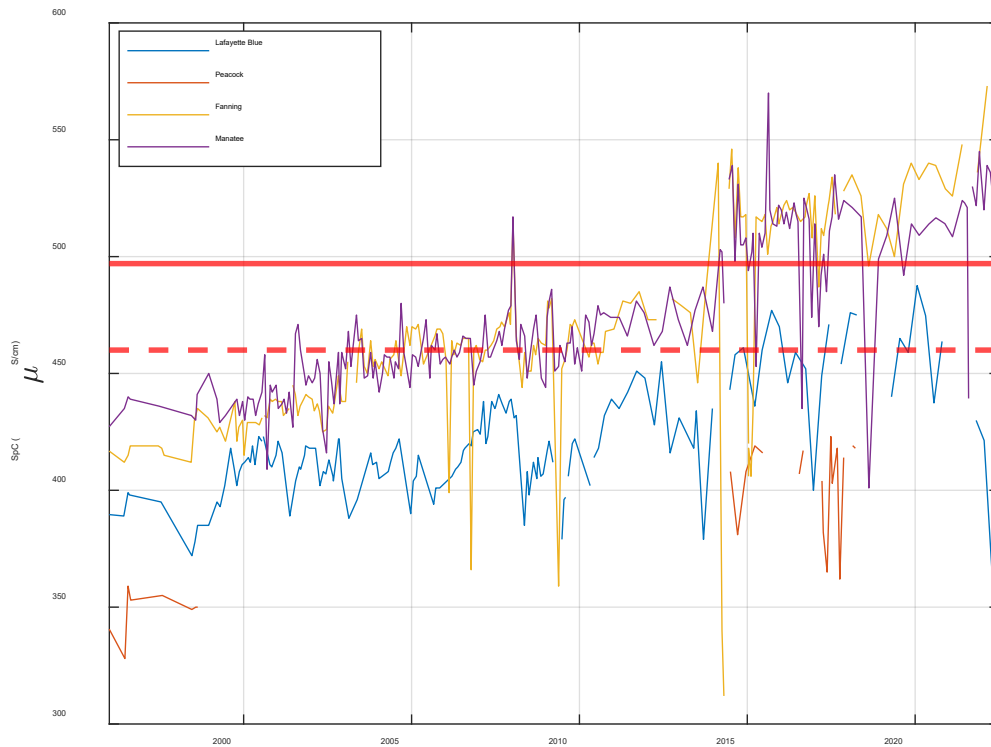

Figure 2.2: Specific conductance (SpC) for the 4 springs studied for this project and equilibrium thresholds. The dashed red line indicates the <u>maximum</u> SpC that would be expected based on the maximum hardness values (as CaCO₃) observed after combining the data at each spring in the legend. The upper solid line is the threshold where an additional source of increased SpC waters or pollution would need to occur for those SpC's to be achieved based on the maximum total CaCO₃ observed from all springs in the SRWMD (249 mg/L as CaCO₃). <u>Though the relationship used was developed using geochemical modelling and validated using 1949 natural samples, we suggest more rigorous local relationships should be considered for a UFA specific threshold and should be considered in interpretation of the above graph.</u> The UFA has some variability which may alter theoretical limits.

Figure 2.2 shows that some springs are above the derived, *theoretical* local threshold (>496 µS/cm) and approaching the defined limestone threshold of 600 µS/cm. These observations suggest specific conductance has reached levels where contamination or a high specific conductance source of water is likely required to produce the values observed, warranting investigation. The increase in specific conductance is also correlated to increases in major ions related to both agriculture and LFA waters, but not with UFA waters (see Review and Discussion).

The regional geology and land use implicates LFA mixing, agriculture, or both are likely responsible for the increases in specific conductance. However, because the UFA has undergone a wide range of hydraulic head fluctuations over the last few decades (Figure 2.3) without any detectable changes to rate of increase in specific conductance across springs (Spellman, 2022, and example spring in Figure 2.4), coupled with data from Project 1 where discrete inputs of different specific conductivity water into even small cave system mixes quickly (Jug Hole), there would have to be large scale inputs of LFA water or agriculture may play a larger role than previously assumed.
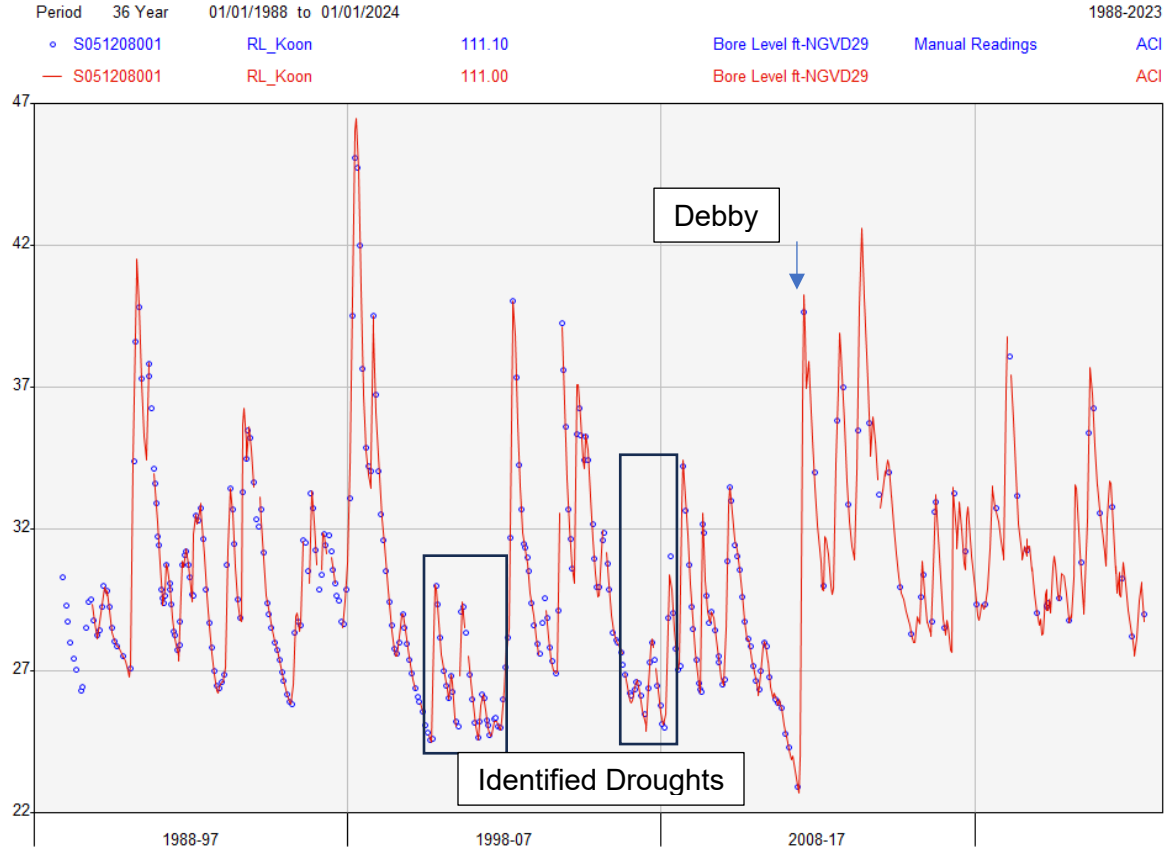
Figure 2.3: Water level changes in a continuously monitored well in Lafayette County near Lafayette Blue Spring. The well had one of the longest records within this region. Note mean groundwater level change after Debby. The data were verified to be real with the SRWMD after the change was detected.

Our project seeks to collect data that will help quantify the LFA fractions at selected priority springs using the collected field data, models, and geochemical analyses. We selected a subset of springs that are primarily unconfined with differences in *suspected* source contributions including mostly shallow (Lafayette Blue Spring, Fanning Spring) and the highest potential for deeper water mixing (Manatee Spring and Peacock Springs). We do note other springs have a larger variety of potential different sources, including the LFA, due to size, depth, proximity to the coast, and differences in regional hydrostratigraphy (e.g., Wakulla Springs, Weeki Wachee, Silver Springs, etc). We focus on priority springs in the agriculturally dense Suwannee River Basin. Selecting sites that are unconfined was purposeful because confinement can add hydrogeological complexities, which for brevity, are discussed at request. For review and reference, Figures 2.5-2.6 show the hydrostratigraphy in the study region. Figures 2.7-2.10 show the sampling locations and key landscape characteristics discussed later in the report.
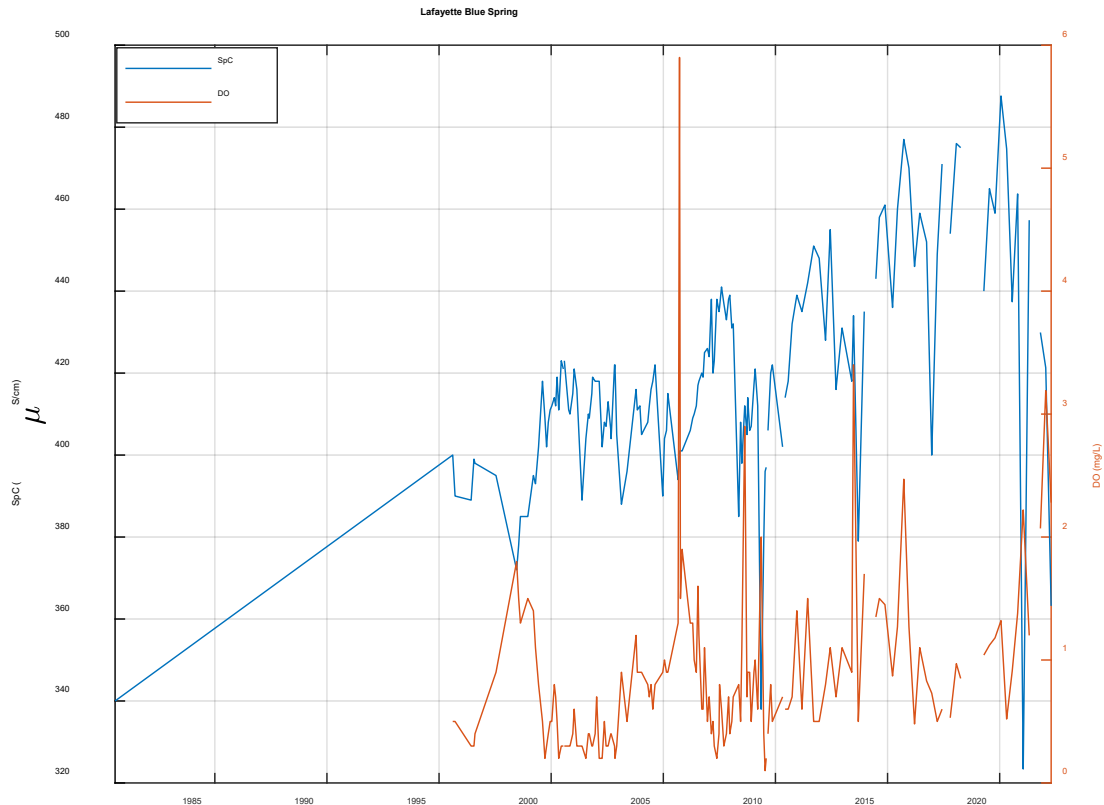
Figure 2.4: Grab sample DO and SpC from Lafayette Blue Spring which shows one of the largest increases in SpC across the SRWMD. Lafayette Blue spring was used because the sensor is located in an offset sink not far from the vent and the morphology of Lafayette Blue Spring is relatively simple. Note low DO during the major drought of 2000-2002 and levelling off of both DO and SpC, and subsequent acceleration of SpC increase around 2010. Uncertain if data prior to 1990s aligns with current sampling protocol.

Hypotheses

1. <u>Ho</u>: The LFA is the primary contributor to major ion and specific conductivity changes in the UFA springs
<u>Ha</u>: The LFA is <u>not</u> the primary contributor to major ion and specific conductivity changes in UFA springs.

Our work does not contain all the pieces needed to understand water chemistry changes and the consequences of them at priority springs, but rather seeks to isolate and understand a single cause and what the relative implications of eliminating or confirming and quantifying that cause may be at different springs. The project funded was the initial step for collecting data and analyzing the potential to understand these processes. The results herein should lead to further research as more needs to be done to understand changes in water quality and quantity at springs in northern Florida.
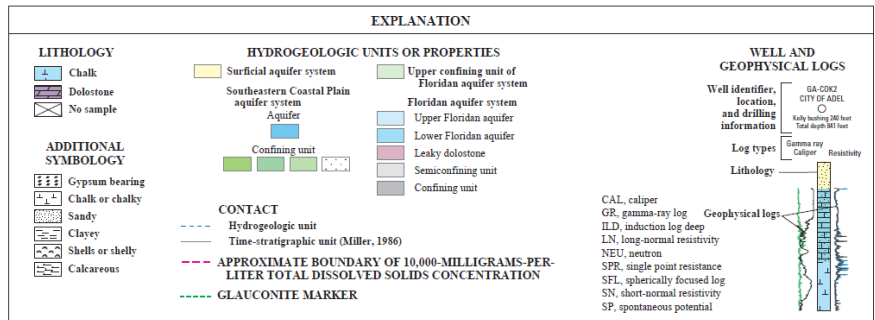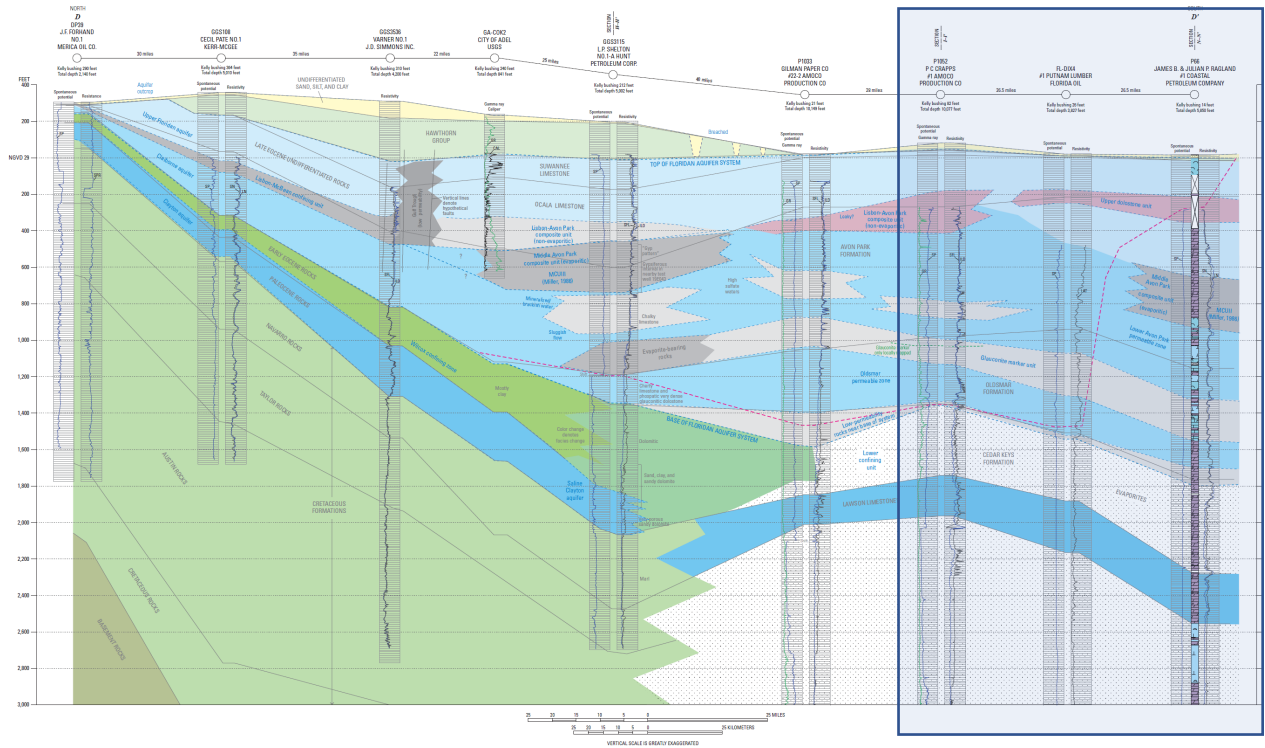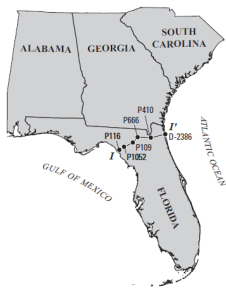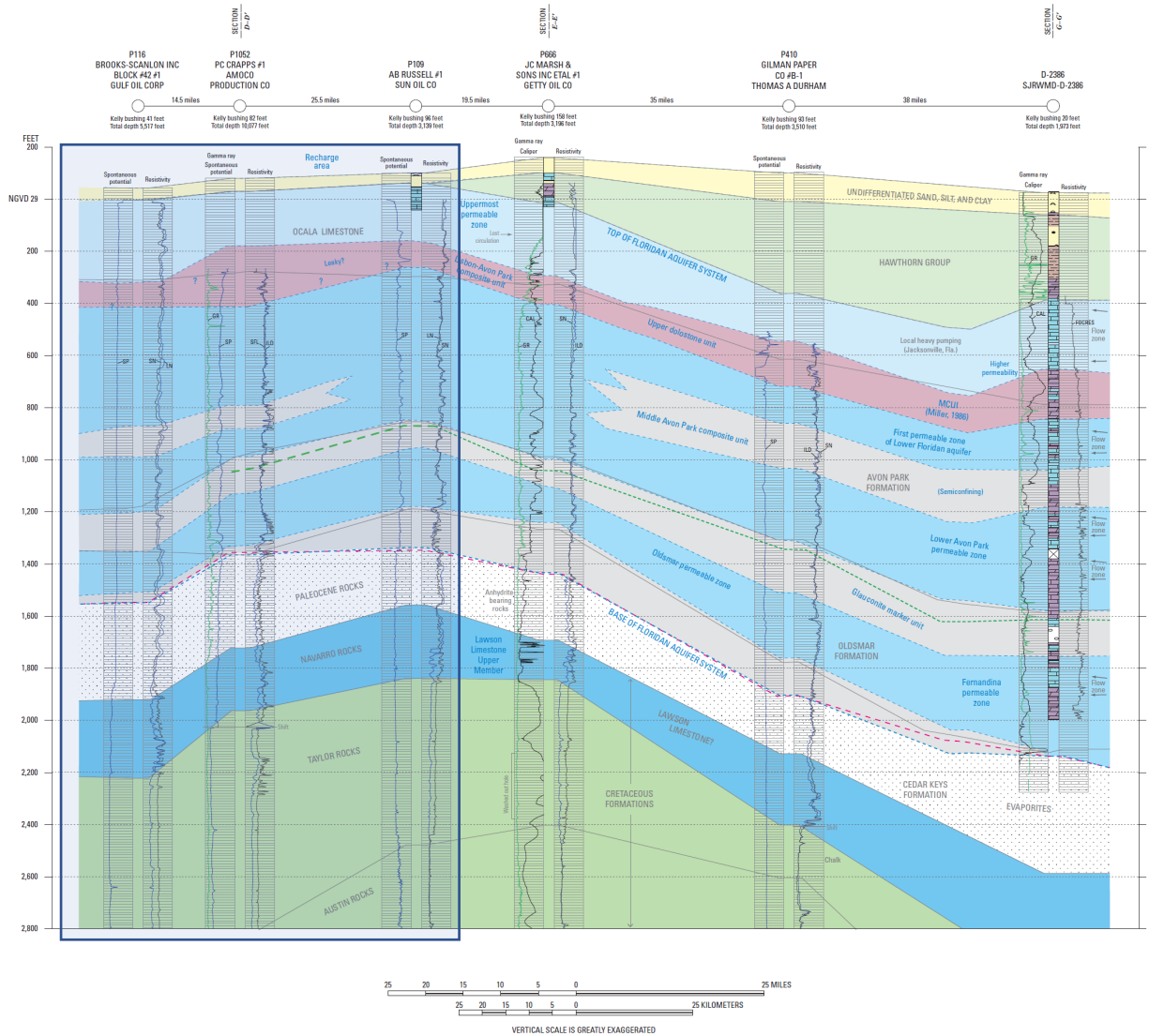
Figure 2.5: North-South cross section through sampling region. The blue box indicates hydrostratigraphy in region where samples were obtained designated lower Suwannee River - **LSR**

Figure 2.6: East-West Cross Section through sampling region. The blue box indicates hydrostratigraphy in region where samples were obtained designated middle Suwannee River - **MSR**
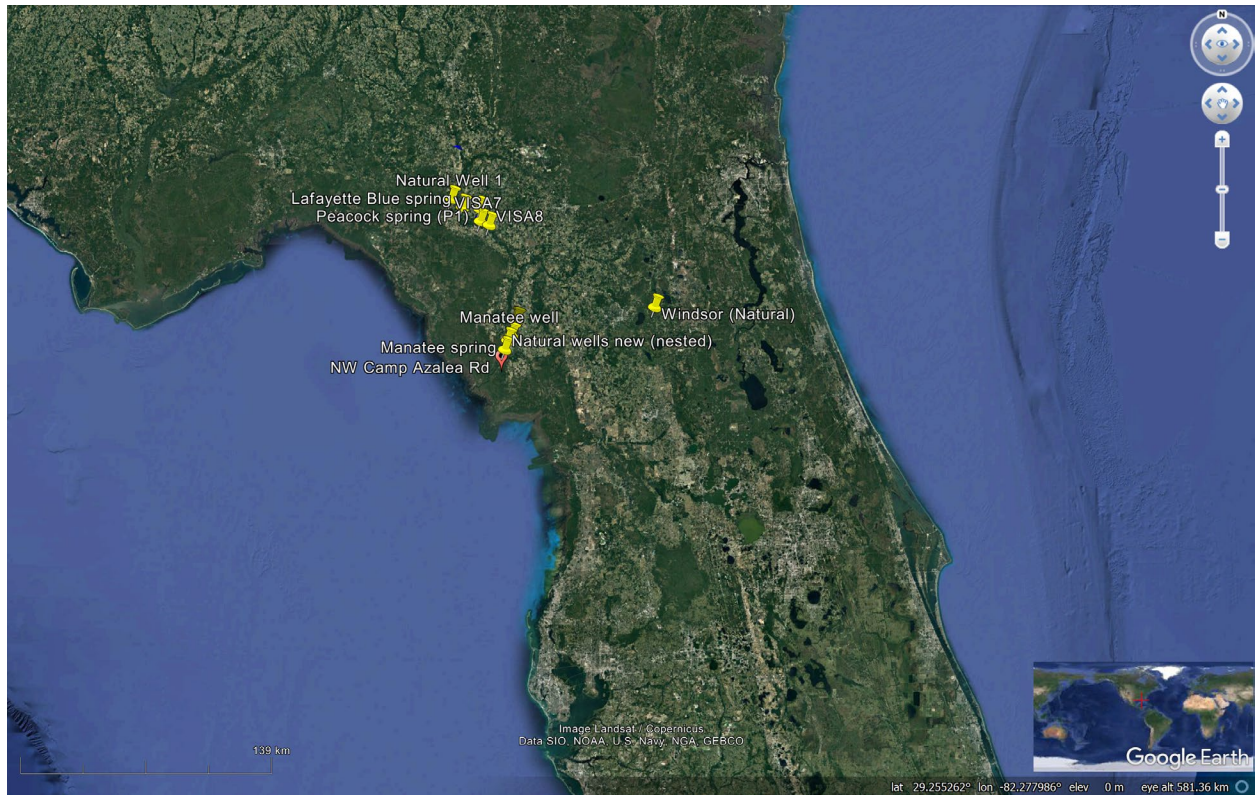
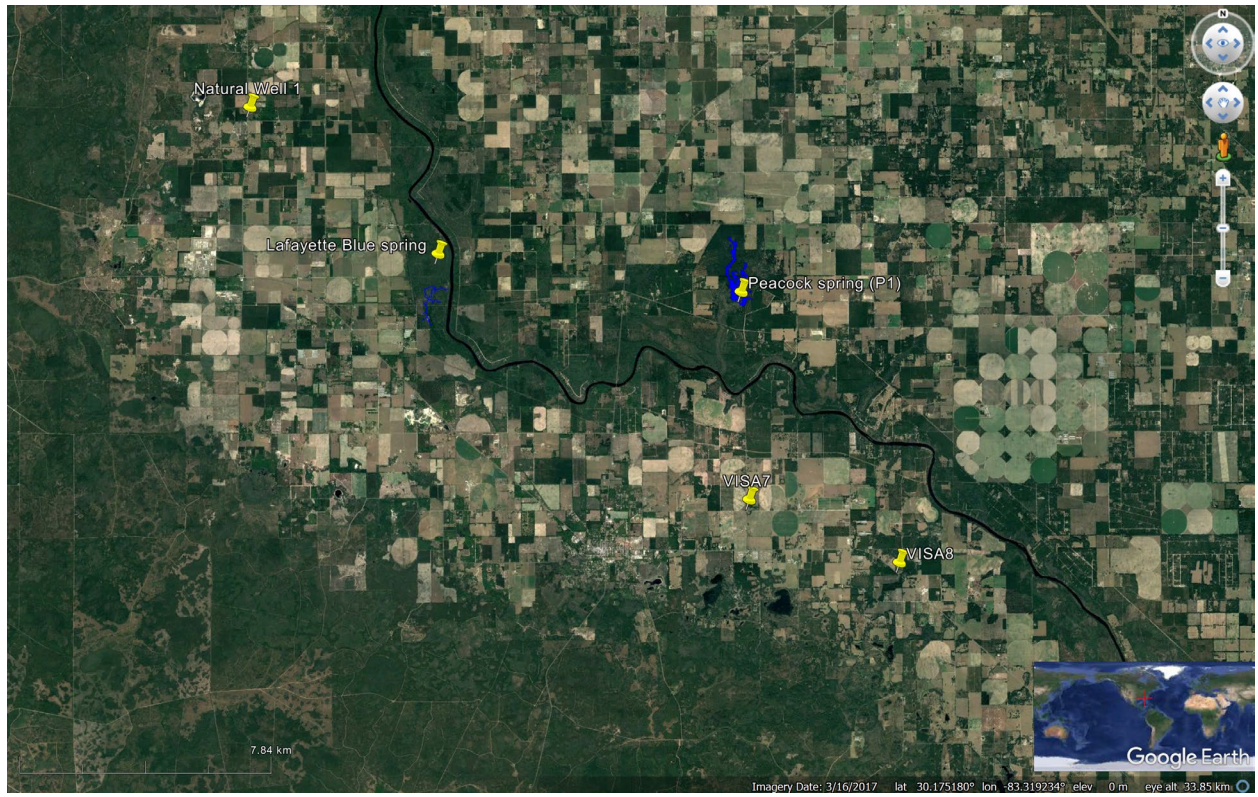*Figure 2.7: Sampling locations at zoomed out view*

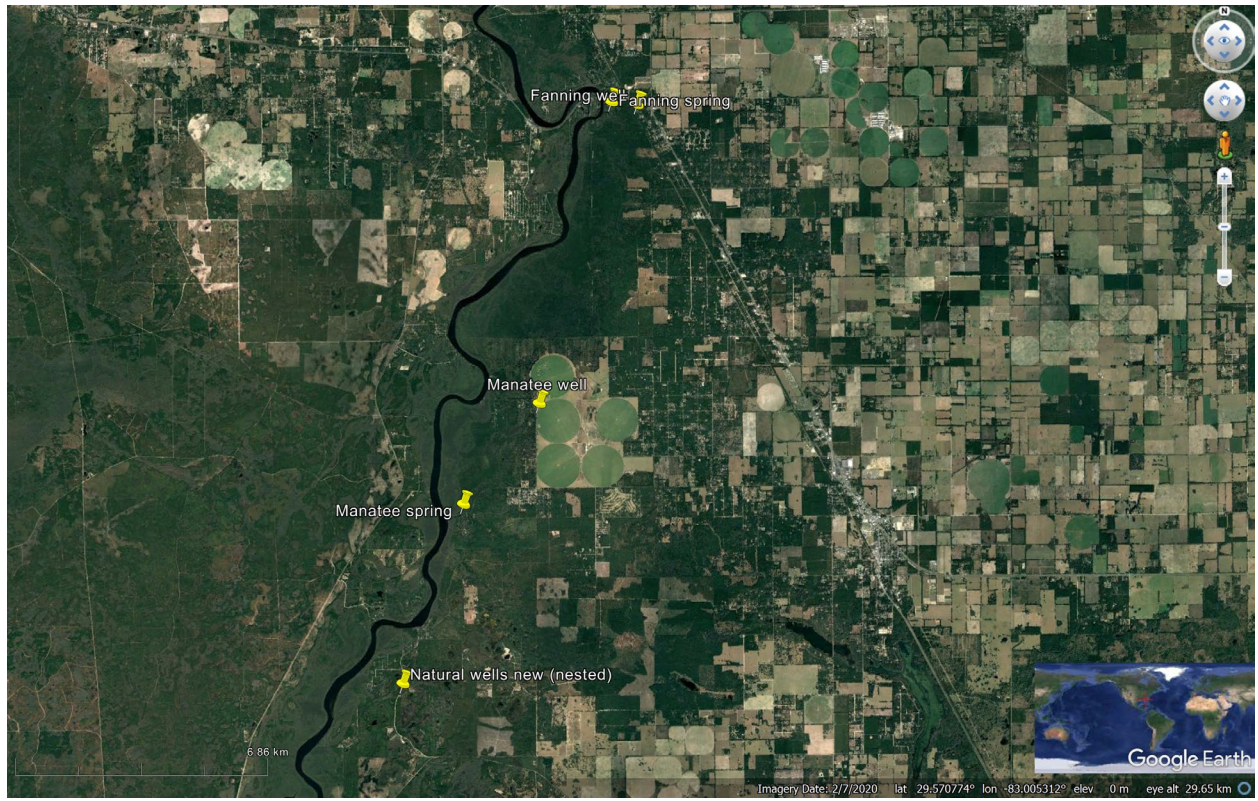*Figure 2.8: Middle Suwannee River (MSR) region of sampling*

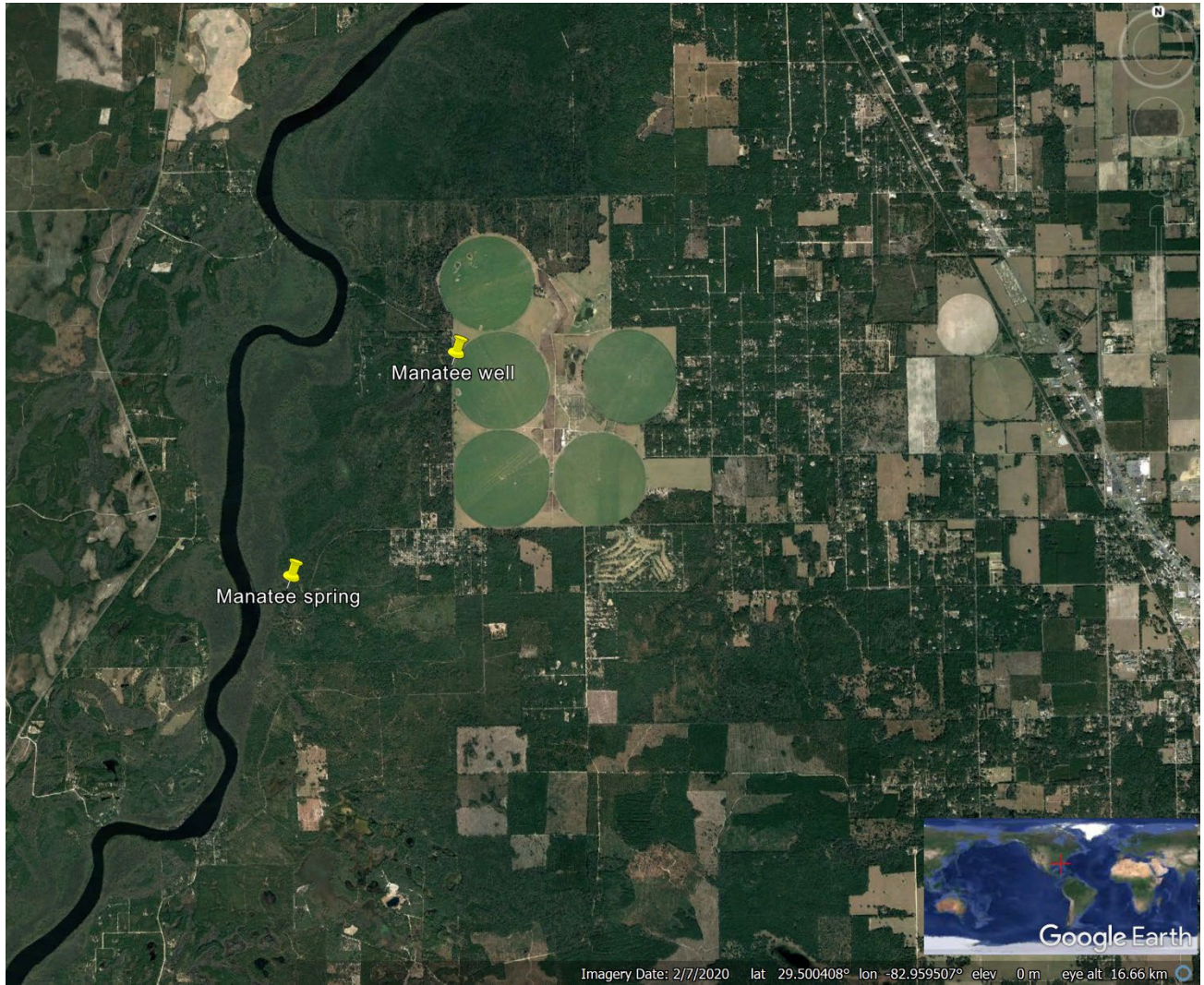*Figure 2.9: Lower Suwannee River (LSR) region of sampling*

Figure 2.10: Close up location of Manatee Spring and the farm and cattle operation that is likely impacting Manatee Spring water quality.

Analyte	Hold time	Preservative	Filter (micron)	Analysis/Method	Storage	Field Quality Control
Cl⁻ **SO₄²⁻**	28 days	None	None	NELAC Lab[1] EPA Method 300.1	Transport on ice, store <6°C	Field blank (5% of time)
Alkalinity	14 days	None	None	NELAC Lab[1] & Manual Titration EPA Method 310.1[2]	Transport on ice, store <4°C	
$NO_3^-+NO_2$	28 days	H_2SO_4	None	NELAC Lab[1] EPA Method 300.1	Transport on ice, store <6°C	
K⁺ **Ca²⁺** **Mg²⁺** **Na⁺**	6 months 6 months 6 months 6 months	HNO_3	None	NELAC Lab[1] EPA Method 200.7	Transport on ice, store <6°C	Field blank (5% of time)
Sulfide (H_2S)	Immediate sampling		None	HACH DR/2400/2800 Method of Methylene Blue[3]	Field tested if necessary	Field blank (100% of time)
Dissolved Organic Carbon (DOC)	28 days	HCl	0.45	NELAC Lab[1] SM 5310B	Transport on ice, store <6°C	Field blank (5% of time)
$\delta^{18}O$ (water) δ^2H (water)	28 days	None	0.22	Picarro L2130-i Cavity Ring-Down Spectroscopy at USF	Transport on ice, store <6°C	Field blank (5% of time)
Sulfur/Oxygen Isotope of SO_4^{2-}	28 days[4]	HCl	0.45	Costech Elemental Analyzer (EA) ECS 4010 at USF	Transport on ice, store <6°C	Field blank (5% of time) and duplicate samples

*Table 2.1: Details regarding samples obtained for Project 2. **Bolded values** indicate major ions defined in this study.*

We selected shallow wells (total depth <70' bgs) that were representative of natural (unimpaired by agriculture or urban settings) and anthropogenic conditions primarily from agriculture. The shallow condition was imposed to reduce any LFA water influence due to the substantial flow depth of the UFA in the region (~500 ft) studied and therefore wells would capture the first 20-50 ft of the water table. We ensured selected wells had a substantial amount of legacy data (>20 data points) to make this determination. For deep well selection, we expanded our wells to represent both Avon Park and Oldsmar formation waters. We had access to all borehole data from the DEP (https://ca.dep.state.fl.us/mapdirect/?focus=fgsgeologicwell) and water quality data provided by the SRWMD for our final selection. The well characteristics are defined in Table 2.2 and more information was provided in the preliminary proposal. We also show the relationship between SO_4^{2-} and NO_3^- and K^+ and NO_3^- from legacy spring data (for context) from all springs in the SRWMD averaged over several years which guided some of our methods.

Previous investigations into shallow water and depth transformations of major ions showed that depths up to 500 ft (') did not show substantial differences in water chemistry (Katz, 1992) across the SRWMD. We performed the same analysis to define how water quality changes with depth using newer, more recent data as well as a larger dataset. We found that with the newer dataset available, the only parameters that increased with depth were Cl^- and SO_4^{2-} and that was only after 500' bgs within the SRWMD. Those analyses were for another study, but are available upon request.

Well Name	Well ID	Average SO_4^{2-} (mg/L)	Region	Depth (ft)
VISA 7	S051209001	22.07	MSR	62
VISA 8	S051214008	14.11	MSR	52
Manatee Well	S111324036	412.97	LSR	28
Fanning Well	S101429020	29.31	LSR	32
Lafayette Blue Well	S031035001	3.50	MSR	47
Camp Azalea 1	S121302010	0.25	LSR	30
Camp Azalea 2	S121302011	2.5	LSR	40
Avon Park 1	A-0997	56	LSR	1350
Avon Park 2	902208	1400	SWFWMD	1200
Oldsmar 1	949451	2550	SWFWMD	2234
Oldsmar 2	949452	2475	MSR	1550

Table 2.2: Well ID's and average conditions for anthropogenic constituents. See Legacy Data Folder for original water quality data.

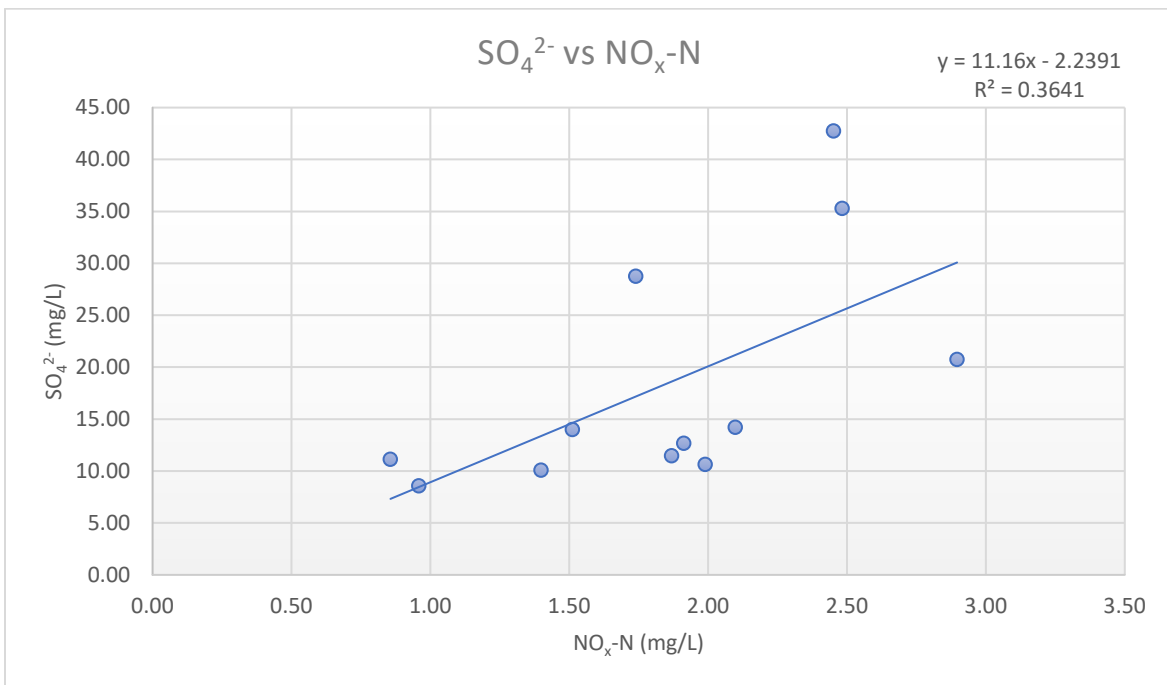

Figure 2.11: Legacy NO_x-N vs SO_4^{2-} data for springs in Suwannee River Basin. Values of K, NOx-N and SO_4^{2-} are averaged after year 2000. Differences in management practices and potential source waters would affect the relationship.

K vs NO$_x$-N

$y = 0.6715x - 0.3961$
$R^2 = 0.7058$

Figure 2.12: Legacy NO$_x$-N vs SO4 data for springs in Suwannee River Basin. Values of K, NO$_x$-N and SO$_4^{2-}$ are averaged after year 2000. Differences in management practices and potential source waters would affect the relationship.
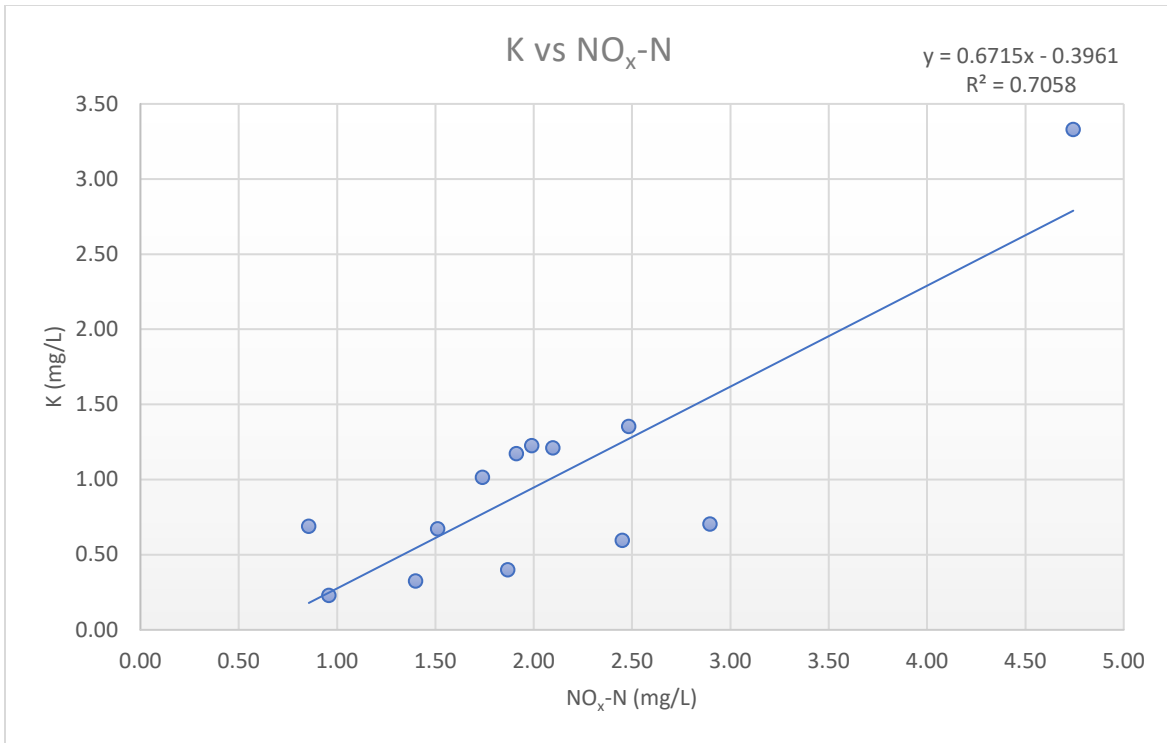
Parameter		Unimpaired		Impaired shallow wells					
		Shallow Lafayette Blue Well		Moderately Impaired VISA 7			Significantly Impaired Manatee Well		
		n=(After 2000)		n=33			n=55		
Parameter		Mean	Std	Mean	Std	% Change	Mean	Std	%Change
pH		7.05	0.48	7.20	0.42	2%	7.05	0.26	0%
Temperature	C	21.82	0.35	22.35	0.40	2%	23.04	0.58	6%
DO	mg/L	0.82	0.69	1.41	1.10	73%	1.42	1.24	73%
Ca	mg/L	60.45	24.75	50.95	4.65	-16%	199.46	22.10	**230%**
Mg	mg/L	22.12	9.69	13.07	1.58	-41%	28.52	5.36	29%
K	mg/L	0.35	0.28	1.04	0.14	**198%**	12.89	1.11	**3579%**
Na	mg/L	3.42	0.65	3.19	0.40	-7%	11.70	2.78	**242%**
Cl	mg/L	6.10	1.45	5.08	0.81	-17%	31.20	6.82	**411%**
NO3	mg/L	0.08	0.11	1.57	1.21	**1923%**	17.63	4.94	**22645%**
SO4	mg/L	3.12	1.27	14.11	2.96	**352%**	410.96	168.03	**13072%**
HCO3	mg/L	421.05	27.54	311.35	29.19	-26%	298.03	17.29	-29%
Ca/Mg	mg/L	2.73	0.15	3.90	0.36	43%	6.99	0.77	156%
SpC (Field)	µS/cm	412.61	21.80	342.19	21.29	-17%	1157.82	115.87	181%

Table 2.3: Water quality changes from a selected natural well in a recharge area, compared to a moderately impaired (NO$_x$-N <2 mg/L) and significantly impaired (NO$_x$-N >10mg/L) well.

Background

Sulfur ($\delta^{34}S$) and oxygen ($\delta^{18}O$) isotopes of sulfate (SO_4^{2-}) were selected as the primary isotope signature because of the unique $\delta^{34}S$ isotope signature from LFA vs shallower, surface derived sources (Sacks and Tihansky, 1996). The $\delta^{34}S$ of SO_4^{2-} is much more enriched (higher values) in the LFA (> 19.4 ‰) as compared to surface derived sources such as soils, rainwater, and atmospheric deposition which are more depleted (lower values), and thus the end members would be dissimilar enough to identify deep vs shallow water mixing. The oxic conditions in the UFA (Katz, 1992), limited dissolved organic carbon (DOC) (Flint et al., 2022), and small differences in SO_4^{2-} in matrix and nonmatrix porosity (Yang et al., 2023) suggest SO_4^{2-} and its isotopes would be a relatively conservative tracer (Sim et al., 2023). Additionally, the methods used which produce most fertilizers with SO_4^{2-} (https://www.epa.gov/system/files/documents/2023-03/Sulfuric%20Acid%20Supply%20Chain%20Profile.pdf) generates a highly depleted $\delta^{34}S$ signal further adding to the usefulness for shallow source identification and potential to identify unique sources of impairment in agricultural areas. $\delta^{34}S$ had previously been used to detect and interpret the evolution of natural source waters in the UFA (Rye, 1981, Sacks and Tihansky, 1996, Sacks, 1996). The $\delta^{34}S$ were used because, as described, significant differences occur between LFA waters and surface derived waters which led to previous analyses of comparative contributions of deep vs shallow flow fields to discrete wells in the FAS (Sacks, 1996).

The $\delta^{18}O$ and δ^2H of water would provide additional end member constraints. The shallow groundwater isotopes are predicted to have uniquely different water signatures from older water (Sacks 1996, Girard et al., 1997). We also acquired local isotopes of rainwater from the water isotope database (Bowen and Revenaugh, 2003) which are averaged and reported monthly. We then select a distinctive water isotope to be used as the additional isotope for a 3-end member mixing model that includes $\delta^{34}S$ of SO_4^{2-}.

We also wanted to determine if there may be a seasonal signal which would strengthen our alternative hypothesis. Seasonality in NO_x-N was previously observed at several springs in the SRWMD (Spellman et al., 2022). Further, we assumed a seasonal signal would be plausible after doing comparisons of impaired vs unimpaired wells and analyzing variation in parameters with the available data. For example, Table 2.3 shows the major ion and water chemistry values of a moderately impaired well (VISA 7) a significantly impaired well (Manatee Well) and an unimpaired well (Lafayette Blue Well) (Figures 2.7-10). As observed, the standard deviation in SO_4^{2-} was the highest for all parameters and increased with NO_x-N. The Manatee Well represented a strong, agricultural end member due to being shallow and immediately downgradient (hydraulic gradient) from a cattle and farming operation. VISA 7 is downgradient from a farm but shows moderate impairment as quantified by NO_x-N concentrations (< 2 mg/L). Reinforced by the understanding that SO_4^{2-} is shown to behave conservatively in aerobic environments (Sim et al., 2023) the data indicated a change would be detected from seasonal application of fertilizers/soil amendments.

Factors affecting $\delta^{34}S$ and $\delta^{18}O$ isotopes of SO_4^{2-}

The primary control on SO_4^{2-} reduction is the existence and metabolic activity of sulfate reducing bacteria (SRB) and it is the most stable form of sulfur under aerobic conditions (Aravena and Mayer, 2009, Miao et al., 2011). SRB proliferation is controlled by the existence of available organic carbon, whereby the preferred carbon sources are organic acids. Therefore, SO_4^{2-} reduction is limited by the amount of organic carbon as well as the redox state and concentration

of nutrients and trace metals higher up on the redox chain than SO_4^{2-} ($NO_3^- \rightarrow Mn^x \rightarrow Fe^x \rightarrow SO_4^{2-}$) (Jurgens et al., 2009). Thus, appreciable NO_3^- concentrations in the waters would imply limited SO_4^{2-} reduction.

When conditions do facilitate SO_4^{2-} reduction, the resulting impact on $\delta^{34}S$ enrichment is fractionation of up to +12 ‰ of sulfur. However, the magnitude of enrichment will be dependent upon the concentration and ratio of both SO_4^{2-} to sulfides (H_2S, HS). Because the enrichment would take place in reducing environments with limited or lack of oxygen, the primary location of this would likely be the LFA, where, on average, DO is more likely to be below the low oxygen defined threshold of 0.5 mg/L than in the recharged UFA and NO_3^- concentrations are also low. Therefore, this adds to the utility of $\delta^{34}S$ in the source water analysis.

Pyrite or iron sulfide oxidation and oxidation of organic matter depletes the $\delta^{34}S$, whereby typical $\delta^{34}S$ of these processes results in depleted values on average of -8‰. Lack of substantial iron minerals in the UFA and LFA (Sprinkle, 1989) indicates that is unlikely to be a major control. However, local oxidation of organic sulfur derived from the surface may occur.

Geology affecting major ion chemistry in the UFA and LFA

The hydrogeology and degree of confinement control most of the natural major ion chemistry of the FAS. The primary groundwater ions are Ca^{2+} and HCO_3^- (bicarbonate) in the UFA as limestone/calcite ($CaCO_3$). Gypsum ($H_2O * CaSO_4$) and dolomite ($Ca(Mg)HCO_3)_x$) are found in the primarily limestone LFA, including within the Avon Park and Oldsmar (LFA) formations. These minerals would contribute additional SO_4^{2-}, Mg^{2+} and Ca^{2+}. Significant sources of K^+, Na^+, and Cl^- are not known to exist (Katz et al., 1995b), as the aquifer is thick and sources of Na^+, K^+, and Cl^- such as clays are not present in large quantities (Williams and Kuniansky, 2016) and seawater is at substantial depths (Katz et al., 2004). For a more thorough review of factors influencing natural geochemistry, see Sprinkle, 1989 and Katz, 1992.

Data availability and description

Folder of water sample analyses are provided from the NELAC lab (Advanced Environmental) in /**NELAC Lab Water Quality Results** and the USF isotope lab results with data reporting in the folder **Project 2/Isotope Data**. Well and spring samples were coordinated with the Suwannee River Water Management District (SRWMD) and the St. Johns River Water Management District (SJRWMD) and some legacy data are used from their databases. We do not provide the legacy data that is available on the website.

Initial quality control of sulfur ($\delta^{34}S$) and oxygen ($\delta^{18}O$) isotopes of sulfate (SO_4^{2-}).

We performed experiments (PI funded) to ground truth the hydrogen peroxide (H_2O_2) sample treatment to determine if the H_2O_2 treatment and heating time of water samples fractionated the $\delta^{18}O$ when heated. The USGS sulfur isotope analysis protocol suggests treating all samples with 6% H_2O_2 to remove organic sulfur that may be present (Carmody et al., 1996). Then the sample is heated to 90°C for 45 minutes to degas any SO_2 produced via oxidation of organic sulfur with H_2O_2. The USGS protocol, however, was only for $\delta^{34}S$, without obtaining any $\delta^{18}O$ from SO_4^{2-} using the recommended sample treatment. After reading about possible fractionation of $\delta^{18}O$ when water is heated, we questioned how much the reactive H_2O_2 may affect the $\delta^{18}O$ values when heating the sample. We experimented with heating times and H_2O_2 concentration. We used a standard with known $\delta^{18}O$ value (8.59‰ +/-0.26) and compared the $\delta^{18}O$ treatment under the conditions in Table 2.4.

Sample ID	Heating time	H_2O_2 concentration
1	45 minutes	0 H_2O_2
2	45 minutes	6% H_2O_2
3	45 minutes	18% H_2O_2
4	45 minutes	30% H_2O_2
5	45 minutes	6% H_2O_2
6	90 minutes	6% H_2O_2
7	135 minutes	6% H_2O_2
8	0 minutes	0 H_2O_2
9	0 minutes	6% H_2O_2

Table 2.4: Experimental runs for testing the impact of the USGS method on $\delta^{18}O$ fractionation.

The results of the incremental increase in H_2O_2 under standard heating conditions (ID's 1-4) showed that up to 6% (in our experiment) did not affect the $\delta^{18}O$ isotopes within the expected precision of the instrument. The addition of 6% H_2O_2 with variable heating times also showed an increase in the time induced fractionation. The heating experiment was performed as multiple heating times were reported in the literature.
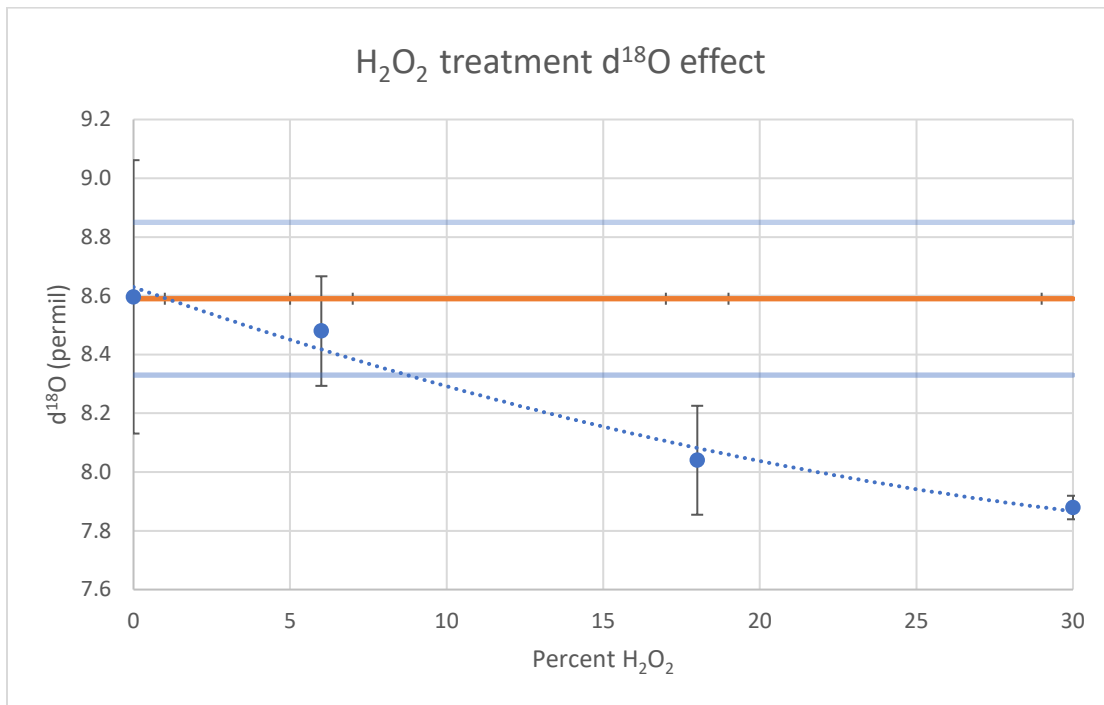

Figure 2.13: The results of the H_2O_2 experiment for items 1-4 from Table 2.4. The samples were all heated to 90C for 45 minutes. Orange line is the $\delta^{18}O$ standard value (8.59 +/- 0.26). Light blue lines show the max and min for standard bounds.
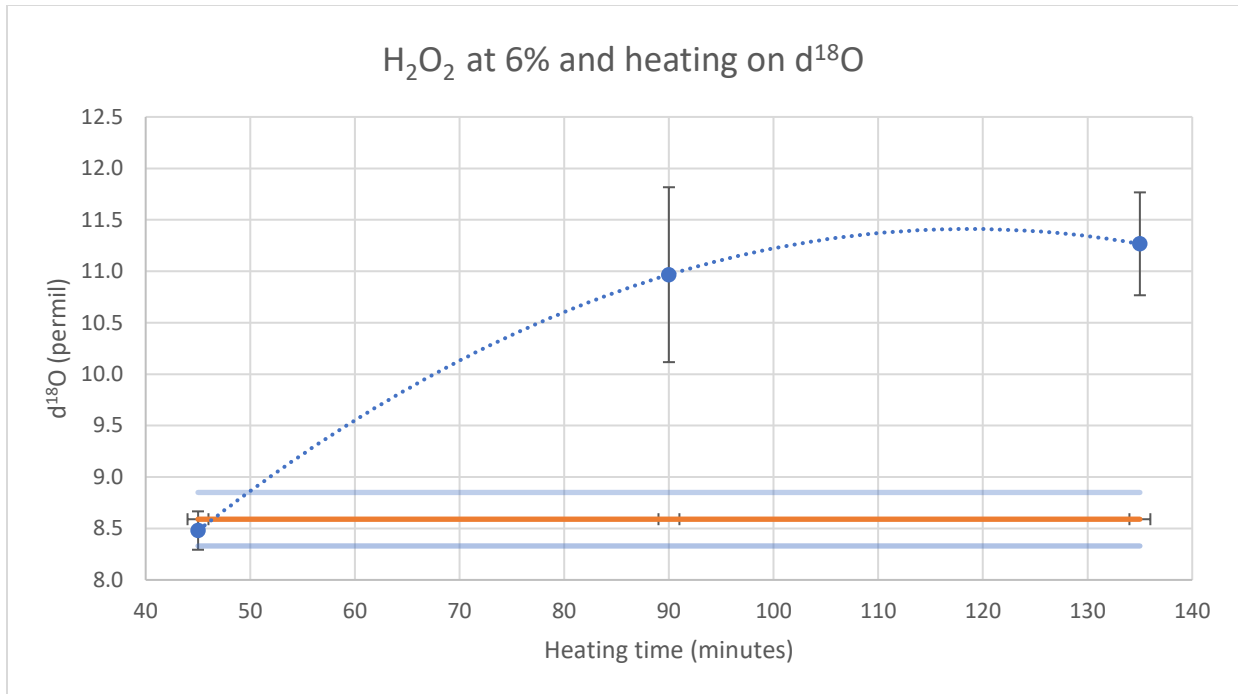
Figure 2.14: The results of treating the sample with 6% H_2O_2 and changing the heating time representing items 5-7 in Table 2.4. Orange line is the $d^{18}O$ standard value (8.59 +/- 0.26). Light blue lines show the max and min for standard bounds.

Therefore, we treated our samples with no more than 6% H_2O_2 and heated them according to USGS protocol (45 minutes) without any concern on the effect on our $\delta^{18}O$ values. Further, we realized that if samples were high in organic carbon, that additional treatment using H_2O_2 would lead to fractionation of $\delta^{18}O$, so that method is not recommended.

We used research grade anion-exchange resin with the properties outlined in USGS report (Carmody et al., 1992). The anion-exchange resin was to be used when SO_4^{2-} values were low, approximately < 0.5 mg/L (as per reported protocol in proposal), or sulfide was detected and the ratio of SO_4^{2-} to sulfide was 40:1. The method is described in detail in Carmody et al., 1992. We however performed the entire process on a test sample using deionized water to determine if there was any sulfur residue on the resin. We precipitated very small mass (.01 mg) but detected sulfur signal with $\delta^{34}S$ of 9.3 ‰. We then pre-treated the resin (effectively rinsed) via the recommended methods and the signal disappeared. Thus, we pre-treated all resin before passing any samples through. We also ran an experiment where we extracted precipitates from a sample (VISA 8) with a pre-treated and not pre-treated resin to compare the results. There was no significant difference in the value of $\delta^{34}S$ outside the error of the instrument indicating that any residue must have been a trace amount. If there was sulfide (H_2S) in the sample, and the ratio of 40 to 1 SO_4^{2-} to sulfide, we also passed the sample through the pre-treated anion-exchange resin (Carmody et al, 1992).

We ensured the isotopes of SO_4^{2-} were obtained, treated, and precipitates extracted in a timely manner after the samples were acquired (<1 week). The precipitates are chemically stable and are always kept in tightly sealed centrifuge tubes in the lab.
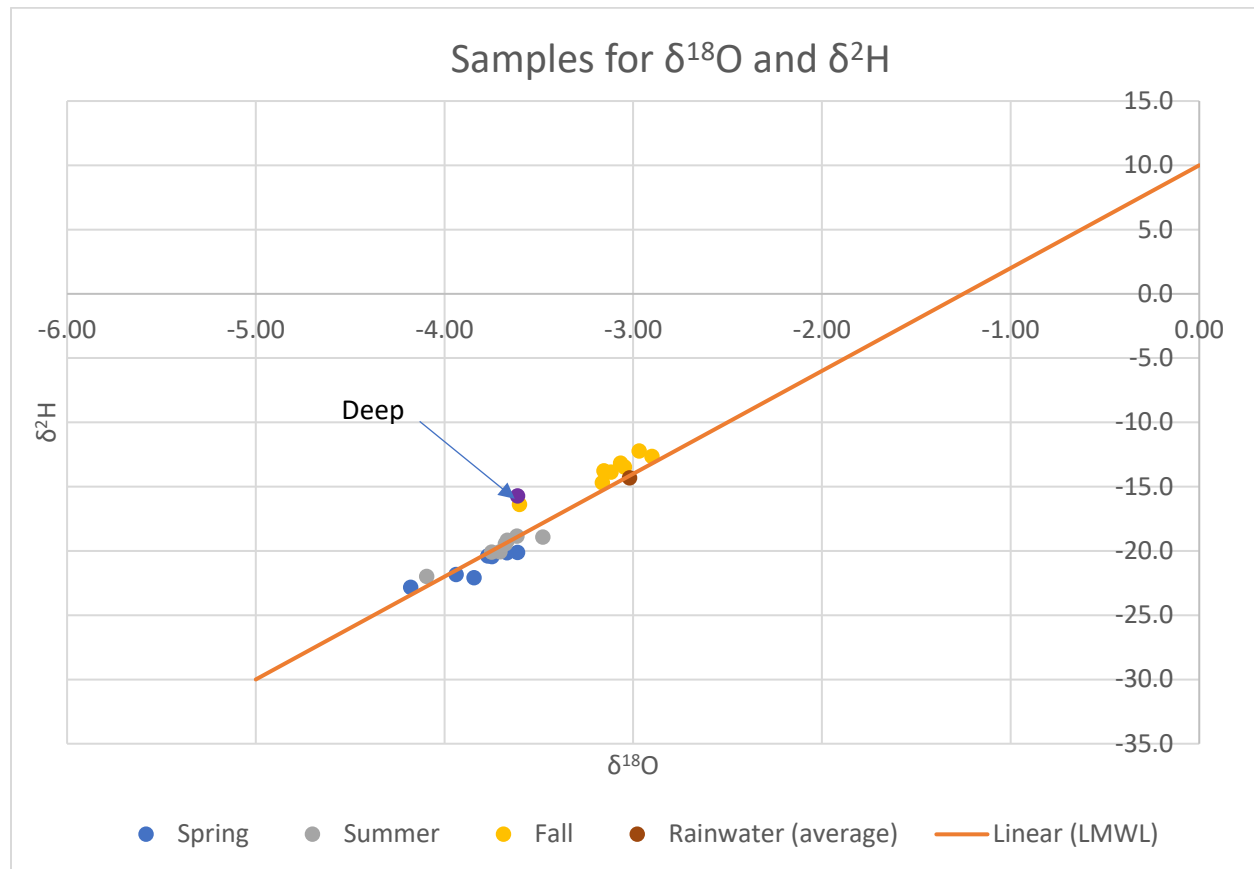
Detailed results
Water isotopes

Figure 2.15: Water isotopes for current analyses that have been concluded. The fall well and spring waters appear to be matched closest to rainwater compiled from the Rainwater Isotope database (https://wateriso.utah.edu/waterisotopes/index.html). The LMWL is the local meteoric water line which was regionally derived from Sacks,1996. The deep wells showed a substantial distinction in both hydrogen and oxygen isotopes of water giving us more confidence for our mixing models.

We collected $\delta^{18}O$ and δ^2H from water as another classification mechanism for isotope mixing (Figure 2.15). Older, deeper water is typically depleted in $\delta^{18}O$ and δ^2H relative to shallower sources, and thus we assumed this could provide another end member for newly recharged vs older water sources. Indeed, the δ^2H of the LFA sampled had a unique signature compared to the shallower water sources.

We are also investigating the yellow point (Peacock Spring) next to the arrow to determine if that is correct. To do this, we will be analyzing raw data from Picarro water isotope analyzer. We have not worked closely with this isotope data as it is primarily to be used as an end member for mixing and not used for interpretation of any project goals.

$\delta^{34}S$ and $\delta^{18}O$ isotopes of SO_4^{2-}

The following graphs plot the sulfur ($\delta^{34}S$) and oxygen ($\delta^{18}O$) isotopes of sulfate (SO_4^{2-}) against each other as a *preliminary analysis* to understand sources of water. The shaded boxes are the ranges of different sources identified by our isotope results and supplemented by literature sources (Table 2.5). The data in some cases is preliminary until we more thoroughly screen some

data and acquire a final suite of analyses which are expected in August 2023. Natural end members are from wells that have no nitrate pollution based on legacy data and are in regions where upgradient flowpaths do not intersect agricultural operations.

Type	Isotope sources
Fertilizer	• Kim et al., 2023. Analyzed 3 different common, local fertilizers.
Seawater*	• Literature (known)
Gypsum (Feed)	• Kim et al., 2023. Used common gypsum provided to cattle. It is also commonly used as a soil amendment that increases the pH (Ben Franklin).
Lower Floridan Aquifer	• Literature for deep LFA - Sacks 1996 (δ^{34}S). • Literature for d^{18}O of SO$_4^{2-}$ through time (Seal et al., 2000) • 2 sample analyses forthcoming Kim et al., 2023. *Expect δ^{18}O range to change.* • New well located for Kim et al., 2023. No sample yet.
Natural	• Kim et al., 2023. • New data collected and will be analyzed in July 2023. *Expect this range to expand*.
Rainwater*	• Literature (known in FL from Katz, 1995b)
Manure/Sewage*	• Literature (Cravotta, 1995, Otero et al., 2007, Shin et al., 2015, Karpierz and Slosarczyk, 2022)

*Table 2.5: Sources of ranges in figures. Some values from previous literature are used as they would be relatively static. Data analyzed by **this project is labeled Kim et al., 2023** (student).* Indicates no data collection planned for these analyses due to known values being reported.*

Notes on sources of isotope data

Our δ^{34}S and δ^{18}O isotopes for fertilizer fell within the boundaries established from other studies done in the US (Szynkiewicz et al., 2015) albeit our fertilizer results were more restricted than other published data. Other sources have reported values of δ^{34}S within the ranges of -2.1 to 13.6‰, while δ^{18}O was reported between 8.3-16.9‰. Because the source of sulfur affects the isotopic composition of δ^{34}S, regional variation exists, and limited data existed for the agriculturally dense northern Florida region. Our source was from two companies, one of which is Nutrien® and known as one of the primary distributors in Florida and the region (SRWMD correspondence). Mayo Fertilizer® is another major distribution center in Mayo, FL (MSR region), and we analyzed one of their fertilizer blends.

Values for manure and septic were similar in ranges for δ^{34}S according to previous literature sources, but only δ^{18}O could be found for manure. Because it is difficult to get the local isotopes from these sources (however, still possible), we decided that the literature would be sufficient, and the ranges were similar enough and could be grouped as a single anthropogenic source labeled as 'Manure' on the graphs (Figures 2.17-2.18). The additional justification for the aggregation was the lack of δ^{18}O able to be found for septic.

Because we used shallow, natural end members of water as a source, we neglect showing soil derived SO$_4^{2-}$ and atmospheric deposition. The natural end member likely has some more variability, and we have additional water samples (Camp Azalea) to constrain that a little better. Previous literature sources have shown that soil derived δ^{34}S and δ^{18}O are between -5 to 9‰ and 0.5 to 5‰ respectively (Cravotta, 1997, Otero et al., 2007, Shin et al., 2015, Karpierz and

Slosarczyk, 2022). These data fall well outside the range of the results in our dataset and would have zoomed out the graph more than we assumed necessary.

The seawater and LFA end members were obtained from literature sources. The seawater values are known and fall within the $\delta^{34}S$ and $\delta^{18}O$ of $SO_4{}^{2-}$ from gypsum deposits. We included seawater as most naturally derived $SO_4{}^{2-}$ from depth in the FAS is from gypsum and saltwater (Rightmire et al., 1974). Because gypsum deposits occur in closed marine basins, the isotopic signatures of gypsum are enriched in $\delta^{34}S$ and $\delta^{18}O$. Literature values of $\delta^{18}O$ from changes in $\delta^{18}O$ of marine gypsum have showed up to 25‰ in the Cretaceous, however the most recent reported values are under 15‰ (Claypool et al., 1980) and data collected from evaporites during the depositional timeframe of LFA gypsum has an upper bound of ~14.5‰ (Seal et al., 2000). We use this upper boundary until our LFA data is analyzed (Figure 2.16).
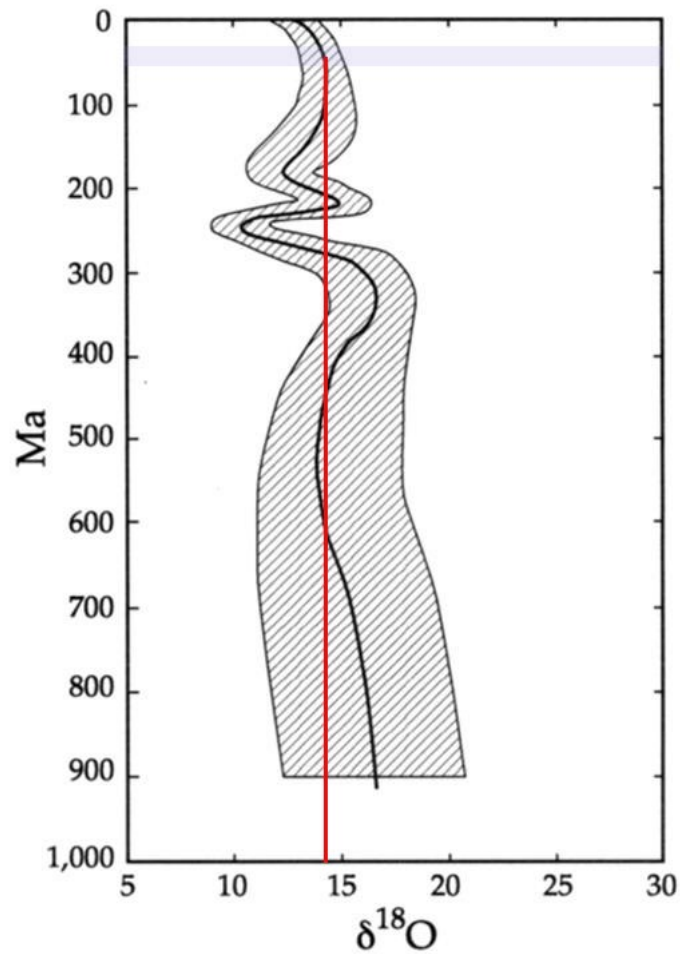

Figure 2.16: Figure obtained from Seal et al., 2000. Red line is added to illustrate value. Highlighted zone is roughly the Eocene epoch. Maximum value based on interpolation is ~14.5‰.

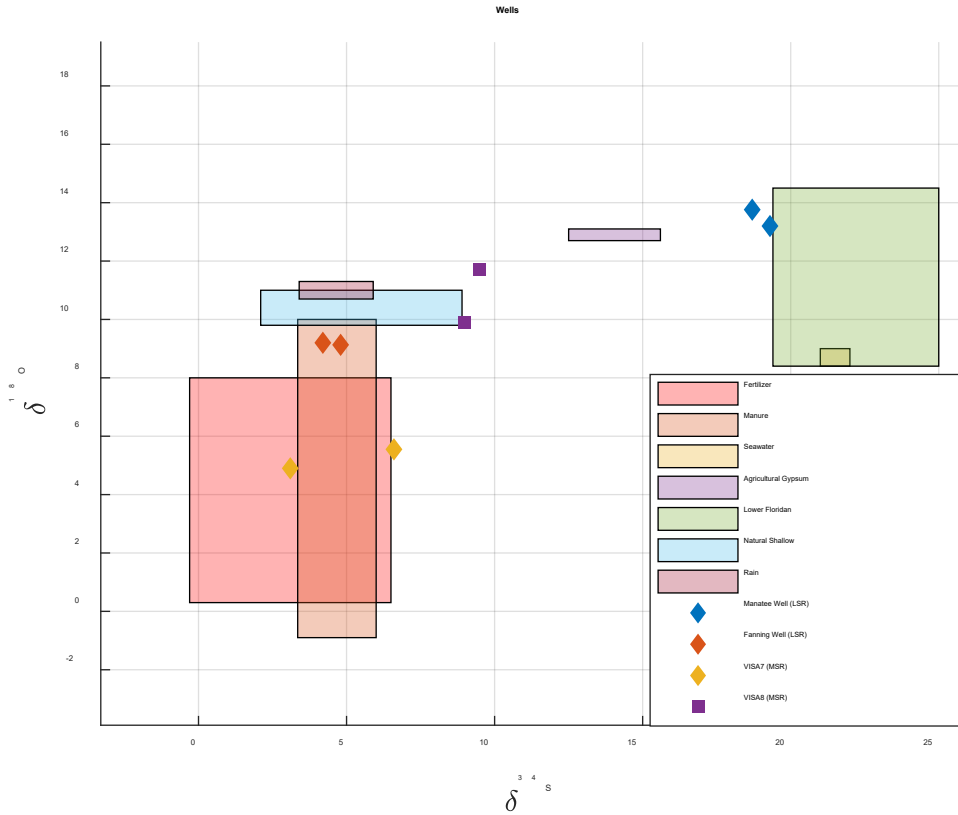*Figure 2.17: Sulfur (δ³⁴S) and oxygen (δ¹⁸O) isotopes of sulfate (SO₄²⁻) plotted for the **wells** sampled in the lower Suwannee River (LSR) region (Figure 2.9) and Middle Suwannee River (MSR) region (Figure 2.8). The legend does not hide any samples.*

*Figure 2.18: Sulfur (δ³⁴S) and oxygen (δ¹⁸O) isotopes of sulfate (SO₄²⁻) plotted for the **springs** sampled in the lower Suwannee River (LSR) region (Figure 2.9) and Middle Suwannee River (MSR) region (Figure 2.8). The legend does not hide any samples.*
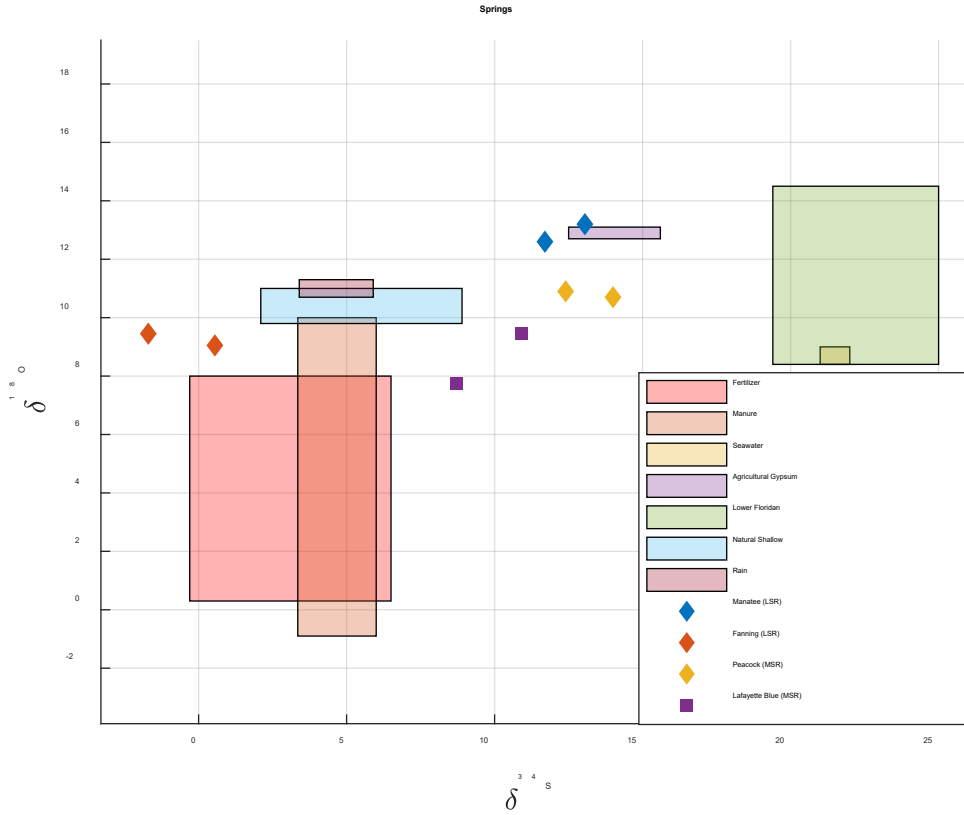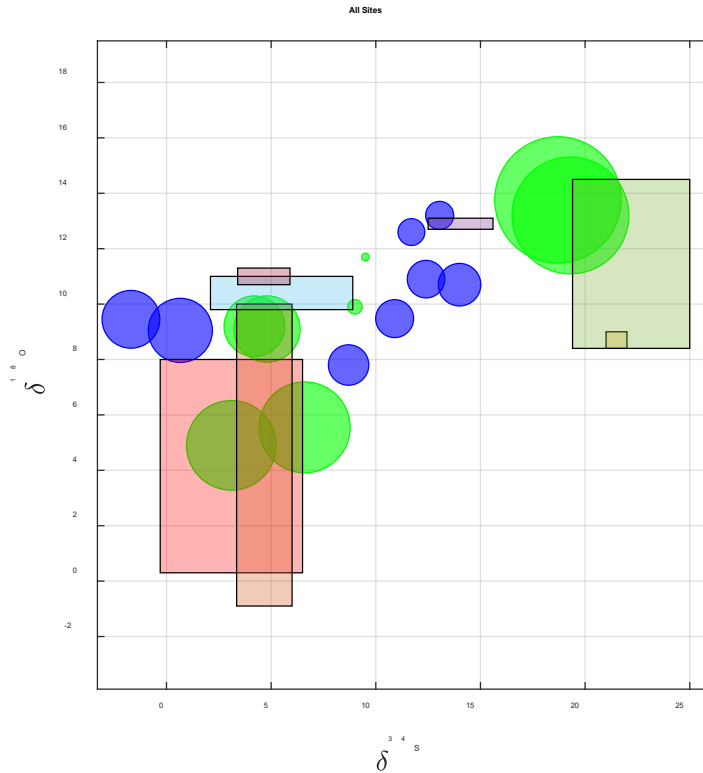
Figure 2.19 Relationship between nitrate and δ³⁴S and δ¹⁸O isotope values for all samples (springs and wells).

Additional observations from the dataset collected include the relationship between nitrate and $\delta^{34}S$ and $\delta^{18}O$ (Figure 2.19). It was observed that sites with the higher nitrate concentrations on average showed more depleted $\delta^{34}S$. The only outlier was Manatee Well, which showed enriched $\delta^{34}S$ and had the highest NO_x-N concentrations of all the sites. It is also observed there is a definite shift in $\delta^{34}S$ and $\delta^{18}O$ whereby there is a change from the groundwater system to the springs where they were sampled.

Discussion of $\delta^{34}S$ and $\delta^{18}O$ results (Preliminary)

Hereafter we briefly discuss some of the preliminary results with the caveat that the analyses are not complete, most importantly, our mixing models. We only discuss the results within the context of the data available and highlight in Future Work some of the remaining analyses that complement the results of the dataset we acquired.

Lower Suwannee River (LSR) discussion

The LSR region was both the most distinct for interpretation (Fanning Spring) and the most interesting regarding Manatee Spring dynamics.

Fanning Spring showed substantially depleted $\delta^{34}S$ compared to all other sites. Fanning Spring has also showed some of the more significant continuous increases in major ion concentrations apart from NO_x-N (Figure 2.20) and has some of the highest NO_x-N spring values on average (~ 9 mg/L). The significantly isotopically low $\delta^{34}S$ and high oxygen content of the springs and wells suggest that the water quality has been affected by both natural sources and anthropogenic sources, but likely minimal LFA mixing.
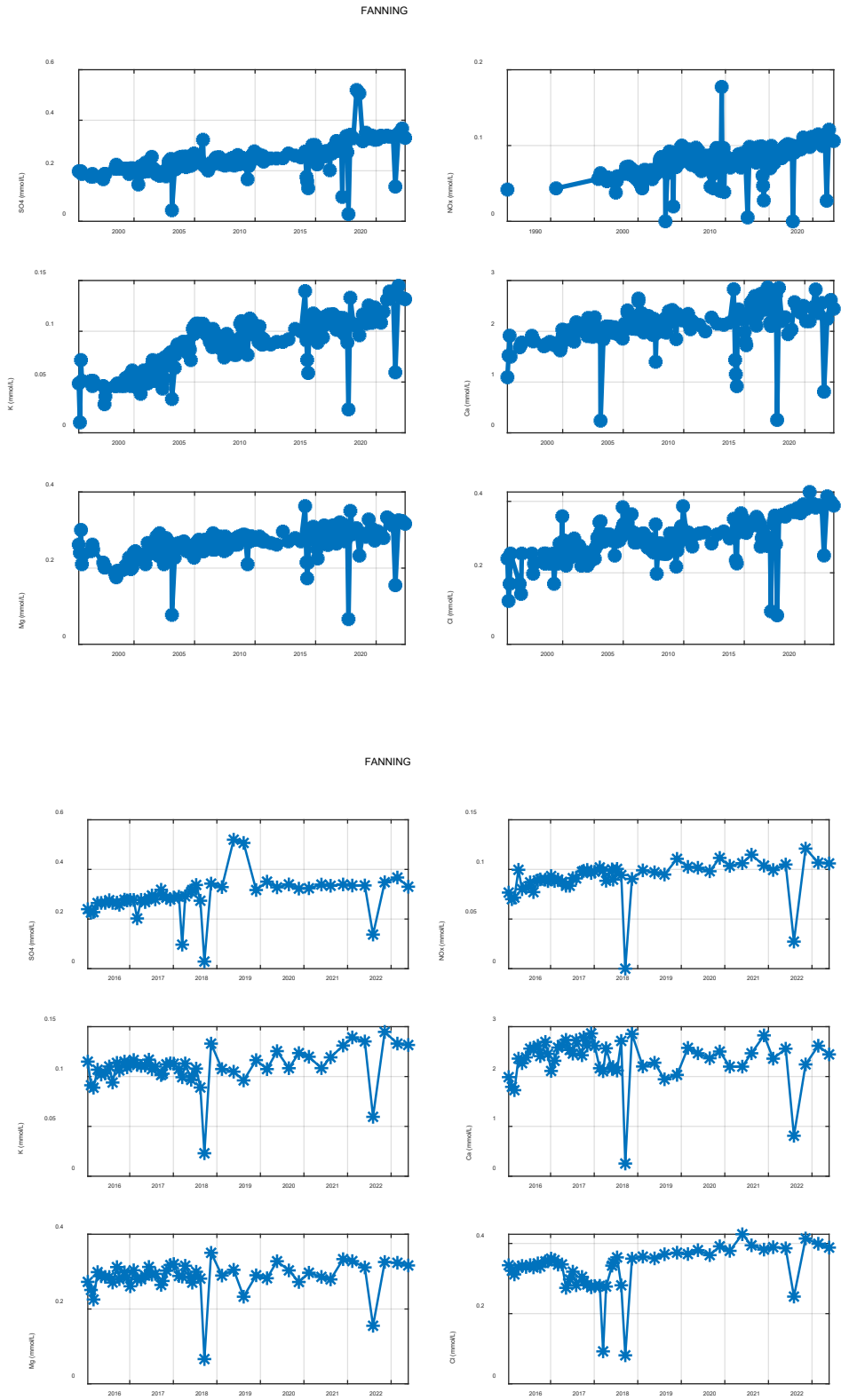
*Figure 2.20: Fanning Spring selected major ions through time (Top: Whole record; Bottom: Recent record (2015+). Note y-axis is in **mmol/L**. A shift in method of analysis is conducted prior to 2000 (analysis from dissolved to total ions) and therefore we also truncate the record to be comparable to Peacock Springs.*

The Fanning Well plots in a similar corner of the $\delta^{34}S$ and $\delta^{18}O$ isotope plot as Fanning Spring, however a noticeable transformation of $\delta^{34}S$ to the spring occurs. The final spring values suggest flowpaths may be converging, mixing, influencing the final values emerging at the spring. Another possibility could be oxidation of organic sulfur or pyrite that would deplete the $\delta^{34}S$ (Sacks, 1996), however the persistently negligible total iron ($Fe^{3+}+Fe^{2+}$) concentrations at Fanning Spring except during reversals are indicative that oxidation of pyrite or iron sulfides are not an appreciable cause of the change observed in $\delta^{34}S$ (Figure 2.21). Therefore, it is possible to be from more depleted sources such as organic sulfur that could be introduced via surface sources, fertilizers, or mixture of waters with depleted $\delta^{34}S$.

At this stage in Fanning Spring analysis, we can rule out any substantial LFA waters mixing with the cave system. However, mixing and flowpath models will still be performed as a confirmation.
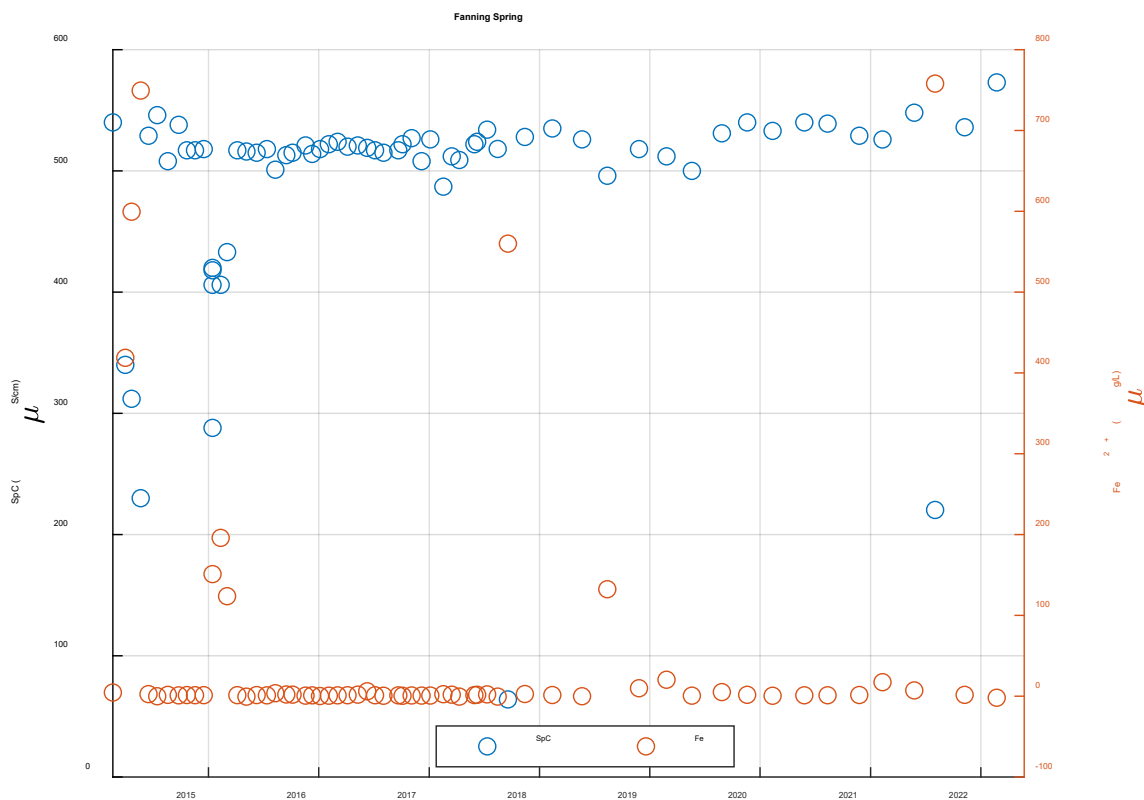
Figure 2.21: The data showing the relationship between iron and specific conductance, where decreases in specific conductance indicate river water at the sampling location. All increases in iron are associated with these decreases in SpC. Average Fe (without reversals) 1.49 μg/L.

Manatee Spring represents a more uncertain dataset, and our research is extending the data analysis to comprehensively understand the causes of water quality shifts at Manatee Spring. The major ion chemistry and isotope data are outliers from other sampled springs across the SRWMD. The $\delta^{34}S$ for Manatee Spring and Manatee Well plot similarly and along what would be assumed to be LFA or approaching LFA water mixing. But we discovered it is also near the agricultural gypsum that is potentially used for cattle nutrition and soil pH adjustment that we

analyzed (https://www.usg.com/content/usgcom/en/products/industrial/agricultural-gypsum-products/calcium-sulfate-feed-grade-gypsum.html). The farm is a facility that has cattle, and agricultural gypsum may be used both to increase soil pH of persistently farmed fields and provide cattle nutritional supplements which is possibly done on the grazing fields. We may not have the representative sample in our dataset that would be used at the farm and thus, it is still speculation without confirmed evidence of management practices on the farm. The potential for increases in LFA mixing from this farm is still possible and we continue to investigate using geochemical analyses.

It is noted that although no sulfide was detected via smell, the DO concentrations at Manatee Well were surprisingly low and from legacy data, have dipped to 0.42 mg/L. Upon further investigation of the DO levels at Manatee Spring, we did observe a significant changepoint in DO after 2016 and the DO appeared to be declining since 2015 (Figure 2.23). We are considering these data in our continued work on Manatee Spring. The change in DO which occurs relatively abruptly, could be from changes in management practices on nearby farming operations, a shift in source waters, or some other factor occurring nearby that is unexplained.
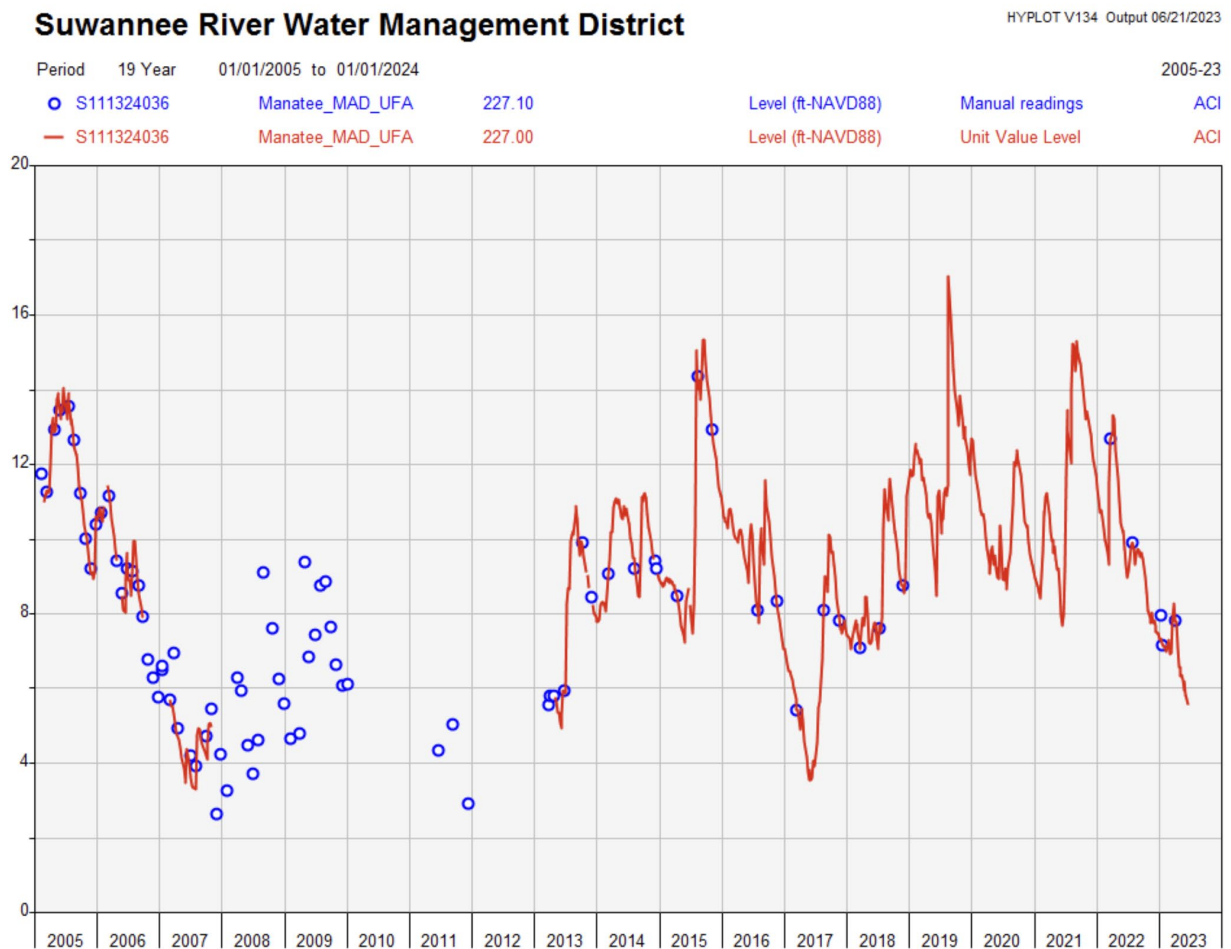

Figure 2.22: Water levels at Manatee Well from 2005-2023. Courtesy of SRWMD website.
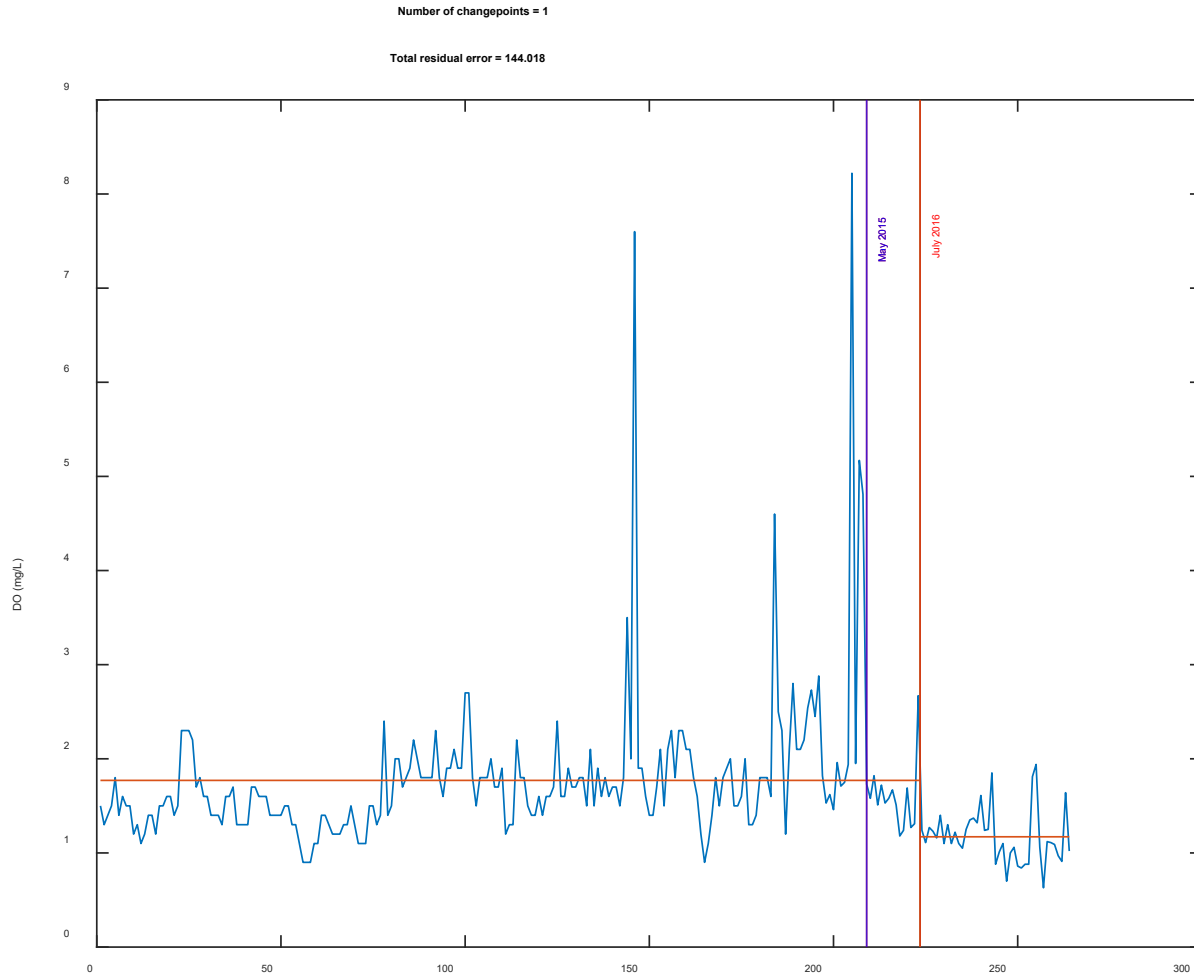
Figure 2.23: DO at Manatee Springs. Detection of statistical change point in the mean observed July 2016, and the mean values are noted as horizontal lines. The purple vertical line indicates a noticeable change in DO starting May 2015. No rigorous investigation of the cause has been done. Steady decline indicates it is unlikely a change in sampling location. .

The Manatee Well also had SpC > 1000 µS/cm on average. The well is shallow (depth < 40' bgs), and it has been shown that SpC > 600 µS/cm, foreign ions are contributing to pollution in carbonate aquifers (Krawcyzk and Ford, 2005). Additionally, high NO_x-N (> 30 mg/L) and K^+ (> 12 mg/L) concentrations corroborate a pollution narrative at this well. The similar, and somewhat unique isotopic value of $\delta^{34}S$ (based on data collected from the other sites) in both Manatee Spring and Manatee Well (and the farm impacting it) strongly suggests the two locations are hydraulically linked as these isotope signals are not expected from the UFA in this region (Figure 2.5). We expect to use major ion covariance structure (see Statistical model) and ion ratios in addition to isotope data to confirm these results.

Middle Suwannee River (MSR) discussion

The MSR region showed more enriched $\delta^{34}S$ at the springs, overall, as opposed to LSR region. Further, it is observed that the MSR wells seem to be depleted in $\delta^{34}S$ compared to the springs sampled. This could be from LFA waters emerging closer to the discharge point, microbial

reduction via locally reducing waters, agricultural gypsum sources, a combination of these or additional factors not considered.

Peacock Springs showed slight enrichment with respect to $\delta^{34}S$. The trends in major ions are not as prevalent as they are for other springs (Figure 2.24 and see LSR comparisons). Aside from positive significant trends in NO_x-N and Cl, most major ions except for a few spikes which may be related to seasonality, are relatively stable in the recent data including SO_4^{2-}. We know from the variable water chemistry in Peacock that it can contain high DOC in some passages along with lower DO (see Project 1), which may indicate possible SRB mediated reduction, LFA contributions, or just deeper flowpaths that are lower in DO due to lack of recharge. We did potentially suspect this may have deeper water sources, but the extent and depth of that is unknown.
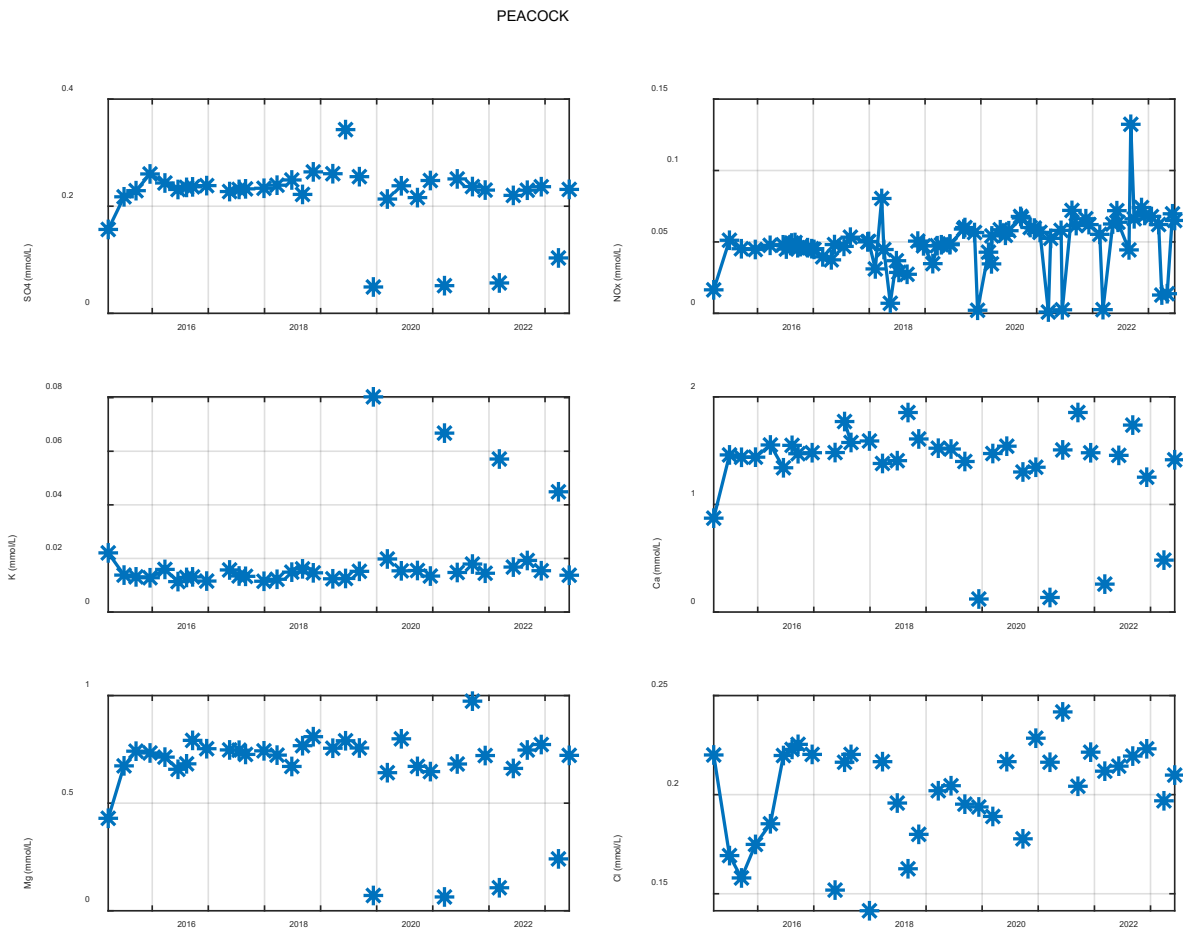

Figure 2.24: Major ions for Peacock Springs. Data are truncated to more contemporary values as early values appeared to represent a shift in the method of analysis (Changed from dissolved to Total).

We hold off on much interpretation on Lafayette Blue Spring as issues arose with $\delta^{34}S$ precipitate analysis. The sampling location of Lafayette Blue Spring was next to the sensor in a sink that had some appreciable algae growth. Our filters were consistently clogged and the color of one of our precipitates was tinged brown and orange which was unlike what we expected, and what we saw in all our other samples. It could be organics or a metal, but we are still looking into it because we are unsure if it may impact our overall $\delta^{34}S$ and $\delta^{18}O$ results. We therefore want to investigate and more thoroughly review the data prior to interpretation.

Summary

The current data shows that each spring is complex, shows no one-size fits all approach and thus should be treated individually. For example, Fanning Spring shows the greatest changes in specific conductance, nitrates, major ions, and likely minimal LFA water mixing, suggesting the water quality changes observed are from surface practices as agriculture or livestock/animal operations. The changes in water quality observed have been some of the most remarkable in the region, fueling suspicion it was primarily shallow. Fanning Spring thus represents the most interpretable signal and may be a potential end member for water quality changes from anthropogenic alterations. If LFA waters are contributing to Fanning Spring, they are overwhelmed by the surface signal and would likely not contribute to the changes in other major ions as extensively as observed. In contrast, Manatee Spring may be significantly impacted by a single farm near the cave system while Peacock Springs may have some deeper upwelling that needs to be quantified. All springs, however, require further analyses which we will undertake within the next few months for a PhD students dissertation. Further, recent work has suggested that passive seawater intrusion is occurring at some of Florida's key springs, even in the extreme interior of the state (Copeland et al., 2019). Our work suggests this concept may require further scrutiny.

Discussion on data limitations and impact on current interpretation

We sought an ambitious project that was tasked to a PhD student for their dissertation and had some additional steps and setbacks we didn't foresee. First, there were the possible impacts of heating and H_2O_2 on oxygen isotope fractionation using the steps taken to extract $\delta^{34}S$ from the water samples. We read about the possibility of H_2O_2 enhancing fractionation only after project submittal. Thus, we needed to confirm our results for $\delta^{18}O$ of SO_4^{2-} would be acceptable. Therefore, some time was dedicated to running experiments (out of Spellman startup) and ensuring we had reliable $\delta^{18}O$ of SO_4^{2-}. Second, we purchased research grade resin to ensure that we had no residual by products on our resin. However, when we analyzed the elution to be safe, we discovered that it did have a sulfur signal. This required us to pre-treat the resin (USGS method) which added to my students learning and actionable time. Third, instrument problems and IRMS turnover (form analyzing $\delta^{18}O$ of SO_4^{2-} to $\delta^{34}S$ of SO_4^{2-} and vice versa) delayed analyses which is also discussed hereafter.

We had some results from the $\delta^{34}S$ and $\delta^{18}O$ data that required further scrutiny. We have performed reruns for samples in which data points were highly dissimilar to each other (PI funded). The points that are suspect are described in **Project 2/Isotope Data** folder and the Excel sheet provided to identify those points and the reason for any flags. We are going to perform reruns of specific samples, particularly the LSR samples, by sending them out to the Arizona isotope lab for confirmation of the values. Additionally, the IRMS may not be functional due to a recent flood in the lab, and therefore, the delays may be greater than we anticipated.

We had no issues with the δ^2H and $\delta^{18}O$ of water for our sample runs except for a single point that looked suspicious from Peacock Springs. We only had one (possible) issue with the NELAC lab on another project (Project 1).

We ran into no sulfide issues in our sampling except in the deeper wells in the Oldsmar Formation. Sulfide has not been abundantly observed in the region to our knowledge in shallow wells and again, due to primarily oxic UFA, is unlikely where we sampled. We suspect the sulfide would be restricted to hotspots of bacterial reduction where organic carbon has been discretely introduced to the aquifer creating reduced conditions, much like what is expected for denitrification in the FAS (Heffernan et al., 2012, Henson, et al., 2019). When we did detect sulfide, we ensured the molar ratio was 40:1 (SO_4^{2-}:sulfide) and used anion-exchange resin for extraction of precipitates.

Because of some delays with sample runs, extra time my student spent learning the Isotope Ratio Mass Spectrometer (IRMS) and Picarro (δ^2H and $\delta^{18}O$) isotopes, additional experiments and methods learning, the mixing models were not completed. Further, detection of potential poor quality data further delayed any analysis because of reruns and further delays in results. There was some instrument malfunction on the IRMS which caused a back log for running samples until it could be fixed. Then, turnover time for each isotope ($\delta^{34}S$ and $\delta^{18}O$) was longer than anticipated prior to writing the proposal. For example, the gases and analyses performed need instrument adjustments prior to analyzing a different isotope. The mixing models along with additional NETPATH and PHREEQc (geochemical models) are to be incorporated into the PhD dissertation (see Future Work). It should be noted the NETPATH models are not part of this proposal.

We have included a deeper Oldsmar formation end member to expand the isotope graph boundaries. We also anticipate additional widening of the natural end member which was constrained using samples from newly installed nested wells (Camp Azalea) at shallow depths. The expected widening is based on previous $\delta^{34}S$ analyses in the UFA (Sacks and Tihansky, 1996).

Future work

Some of the more immediate work includes:

- Mixing models using the isotope data to determine the contributions of LFA vs. shallow sources. We will likely do an ensemble of scenarios that considers combinations or aggregates of isotope data as well as individual values.
- Molar ratio analysis of different ions, the correlation of the enrichment/changes in those ions to land use, spring discharge, and other factors to further constrain all possible causes of enrichment and water quality changes. It also includes in-depth analysis of collected and legacy geochemical data to further constrain other processes occurring (ion-exchange, dedolomitization, gypsum dissolution, etc).
- Mixing fronts in PHREEQc can be constructed that identify the evolution of water under different mixing fractions (e.g.. natural UFA equilibrated water with LFA water). Deviations from these lines for certain major ions would indicate additions from an anthropogenic source. These mixing scenarios can provide us with constraints on some of our data observations. We will also develop local thresholds.
- Geochemical evolution models in NETPATH to understand the potential sources of variability in our isotope data (see Sacks and Tihansky, 1996).

Quantifying the fraction of water arriving from deep LFA sources vs. shallower UFA at springs allows us to start using major water chemistry constituents that are agricultural and nonreactive or have limited reactivity (K^+, Ca/Mg, SO_4^{2-}) as additional constraints for determining the spring water fractions of diffuse, fracture/fissure components and rapidly recharged water via large discrete features (sinkholes). These tracers may also be used to identify or narrow down potential source areas of pollution as well.

We will focus significant effort on the LSR region in the future. We have started discussions of a vadose trace with Florida Geological Survey in the Manatee Springs basin area. We want to learn how quickly water moves through the vadose zone under diffuse, fracture dominated recharge (i.e., non-sinkhole). We hope to move forward with this project in the next few years.

Our work has established a baseline of isotope data of $\delta^{34}S$ and $\delta^{18}O$ of $SO_4^{2-}$and constraining local, $\delta^{34}S$ and $\delta^{18}O$ ranges expected for the SRWMD for use in future comparisons of changes in spring water quality. Our work can be used as a foundational support for adding additional isotopes for analysis of more targeted and precise anthropogenic impacts studies. We hope to improve on our local isotope graph with additional data collection and the new results for my students PhD dissertation. We will also add error bounds once we have a better constraint on the error we are observing in our samples. We also performed experiments and put together a robust methods paper on treating $\delta^{34}S$ and $\delta^{18}O$ together for analysis and will write these up in a data paper.

Statistical model with extended discussion on water quality

The isotope data will be used along with a statistical geochemical model we developed to provide broader context for water quality interpretation. The development of the model is described in the supplemented paper (Spellman 2023-WRR). We should note that the paper is almost ready to be sent for peer review but has been through all authors (colleagues with backgrounds in Geochemistry, Physics, Geophysics) and the model has been validated. We document some key points of the model needed for interpretation hereafter.

The statistical model is based on analyzing the covariance of major ions and ratios in agriculturally impaired water vs natural waters in shallow wells and mathematically defining that covariance to use in assessing impairment of spring waters. The description of each water source is documented in *METHODS* (paper) and the results showed distinctive covariance of major ions for each water source. The water sources were based on groundwater legacy data from wells that were sampled by the SRWMD. We chose wells that spanned across the entirety of the SRWMD as much as possible. We also used some LFA quality data for the natural end member. We used principal component analysis (PCA) to quantify the covariance signatures and used the properties of PCA subspace to define a dimensionless impairment metric denoted as **IM** (see METHODS section PCA).

The **IM** showed that Manatee and Fanning Springs, among the LSR springs plotted similarly with respect to agricultural impairment and that impairment from agriculture was observed in major ions at most springs in the SRWMD. The impairment metric was then independently related to the nitrate concentrations at each spring, and the relationship became a strong log-linear relationship (Figure 2.25) further verifying that major covariance structure may be substantially altered by agriculture. We intend to modify and use this metric with the data we collected for this project. Further information can be found in the text.
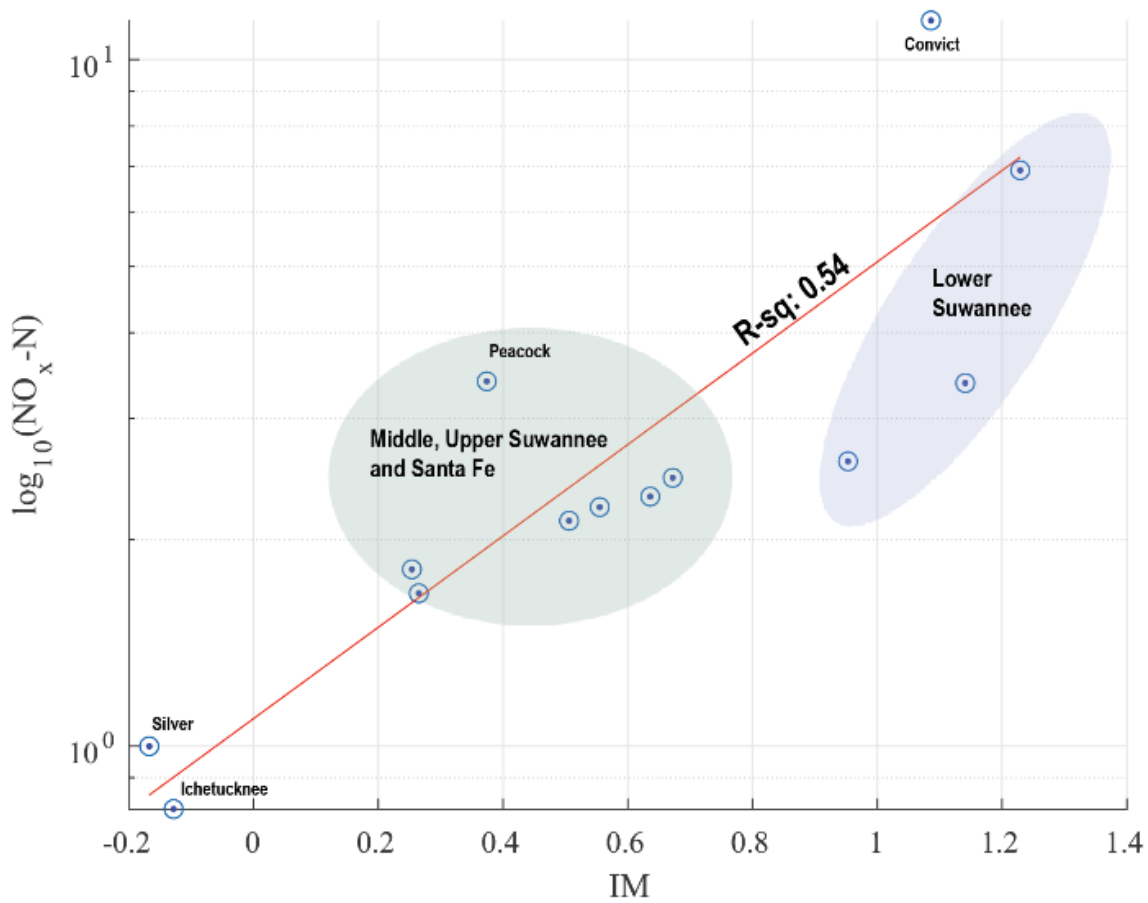
Figure 2.25: Impairment metric (IM) vs logarithm of NO_x-N concentrations at the springs. Peacock and Convict springs are labelled due to their removal and reanalysis. Ichetucknee and Silver Springs are also identified due to their confinement, management, and relative unimpairment. A distinctive clustering was shown by regions along the Suwannee and Santa Fe rivers. Madison Blue is on the Withlacoochee River but is grouped into the upper Suwannee River region. R^2 line shown is for the least-squares regression for <u>all springs</u> and is 0.54. When Peacock was removed the R^2 was 0.71. When both Peacock and Convict (probably impacted by septic)are removed, the R^2 is 0.79.

Completion of tasks

Water chemistry samples		
Sampling Activity	*Completed*	*Notes*
Summer (2022)	Yes	Completed: July 2022
Fall (2022)	Yes	Completed: November 2022
Spring (2023)	Yes	Completed: March 2023
Major ion chemistry, nitrate, DOC		
Summer (2022)	Yes	Completed: August 2022
Fall (2022)	Yes	Completed: November 2022
Spring (2023)	Yes	Completed: April 2022
$\delta^{34}S$ and $\delta^{18}O$ of SO_4^{2-}		
Summer (2022)	Yes	Completed: April 2023
Fall (2022)	Yes	Completed: April 2023
Spring (2023)	Yes ($\delta^{34}S$)/No ($\delta^{18}O$)	--
$\delta^{18}O$ and δ^2H of water		
Summer (2022)	Yes	Completed: August 2022
Fall (2022)	Yes	Completed: October 2022
Spring (2023)	Yes	Completed: March 2023
Modeling and analyses		
Statistical model	Yes	Completed: January 2023 - Included as separate journal paper
Mixing models	No	Time constraints, instrument issues, and data scrutiny caused this to be delayed

Table 2.6: Tasks identified from proposal. Completion marked and notes on the task are provided.

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