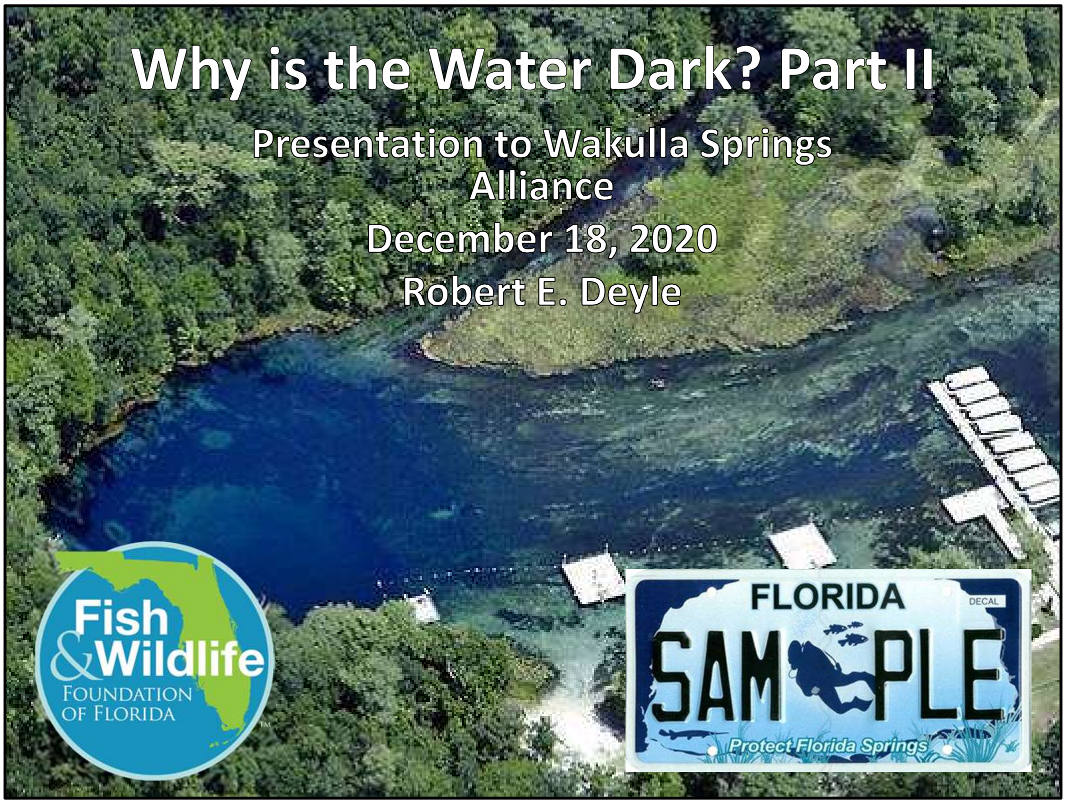
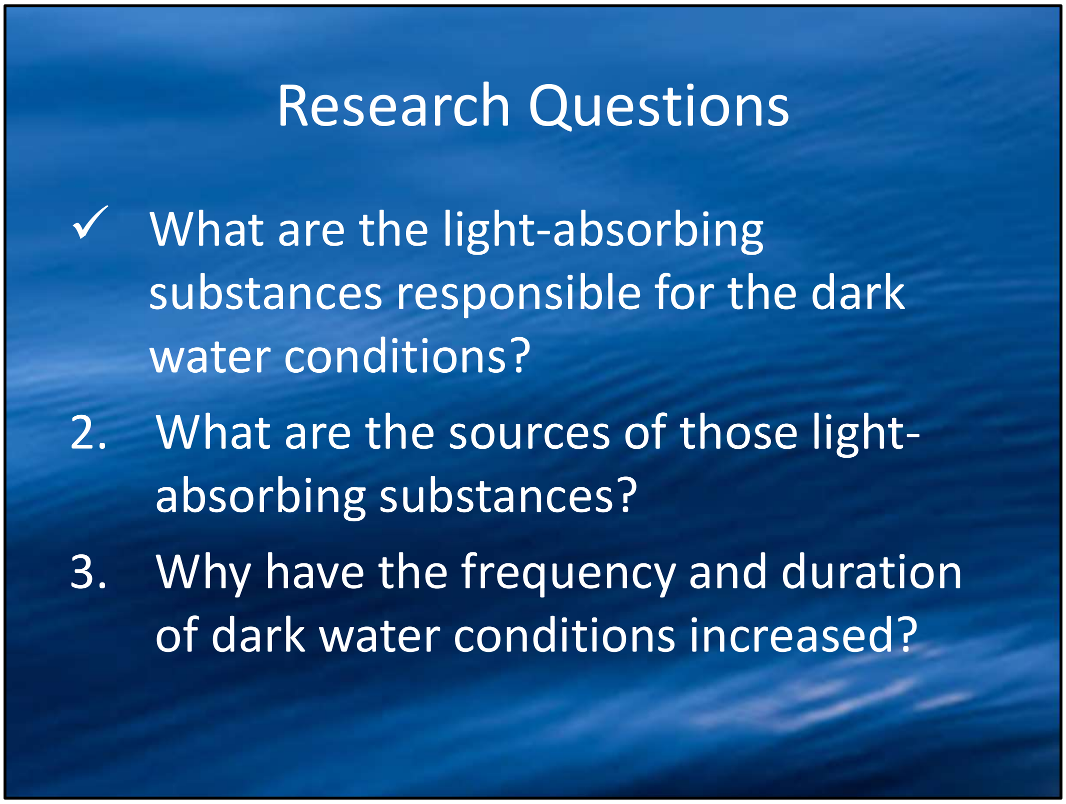
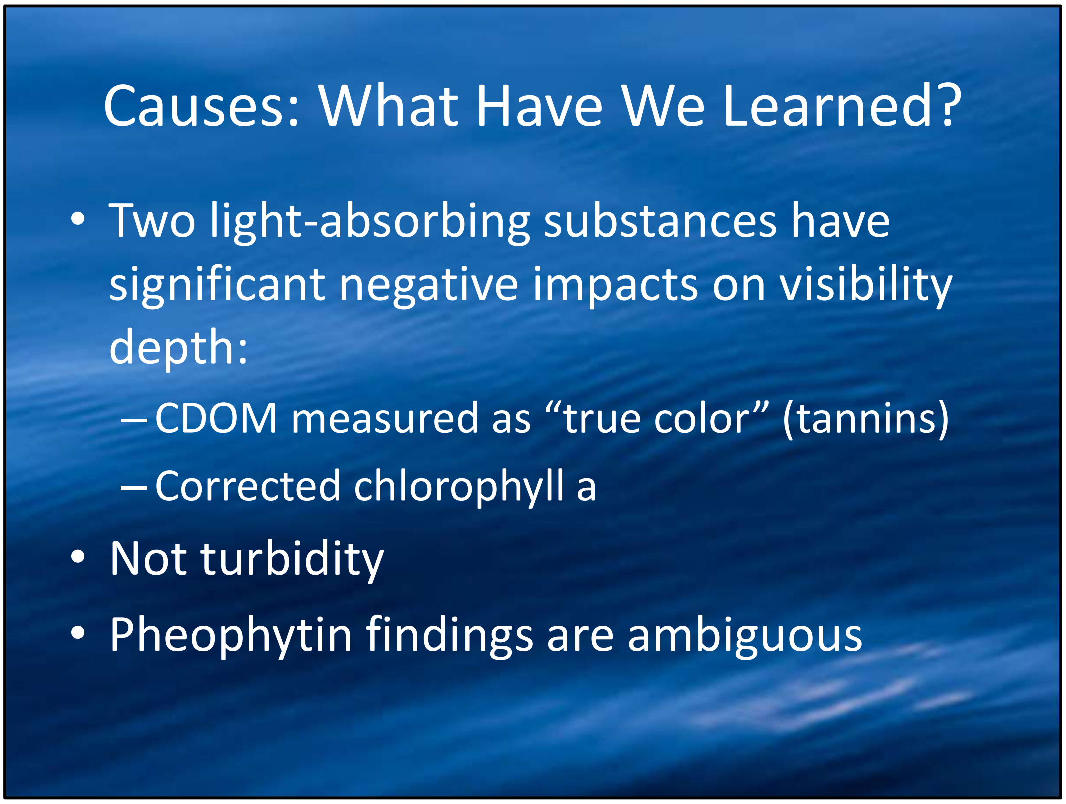


• This is Part II of a series of three presentations sharing what I believe we now know about why the water in the spring is "dark" so much more often than in the past,
• I will be drawing on research conducted by the US Geological Survey and other hydrogeologists and a recently completed 4.5-year WSA project funded with three Protect Florida Springs tag grants from the Fish and Wildlife Foundation of Florida **[click]**.
• McGlynn Laboratories Inc. collected and analyzed the weekly water quality and light data that are the backbone of the study, as well as conducting the dye studies and water quality analyses of karst lakes and sinking streams that are sources of the light-absorbing substances responsible for the dark water conditions in the spring

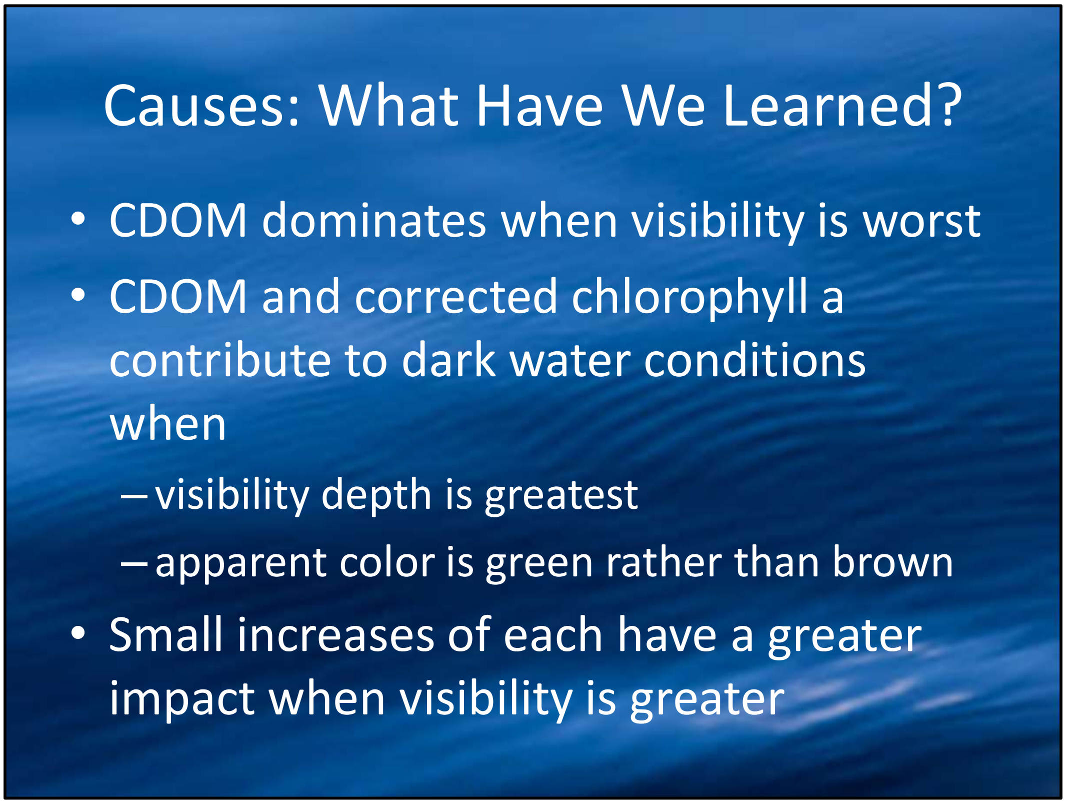


- I’ve split this into three presentations, each addressing a separate research question
- Having established in November that CDOM measured as tannins and chlorophyll a measured as corrected chlorophyll a are the primary causes of reductions in spring visibility
- We will move on today to the second research question: What are the sources of those light-absorbing substances?
- I plan to address the third question in January.
- We’ll start with a quick synopsis of the findings regarding the first question **[next slide]**

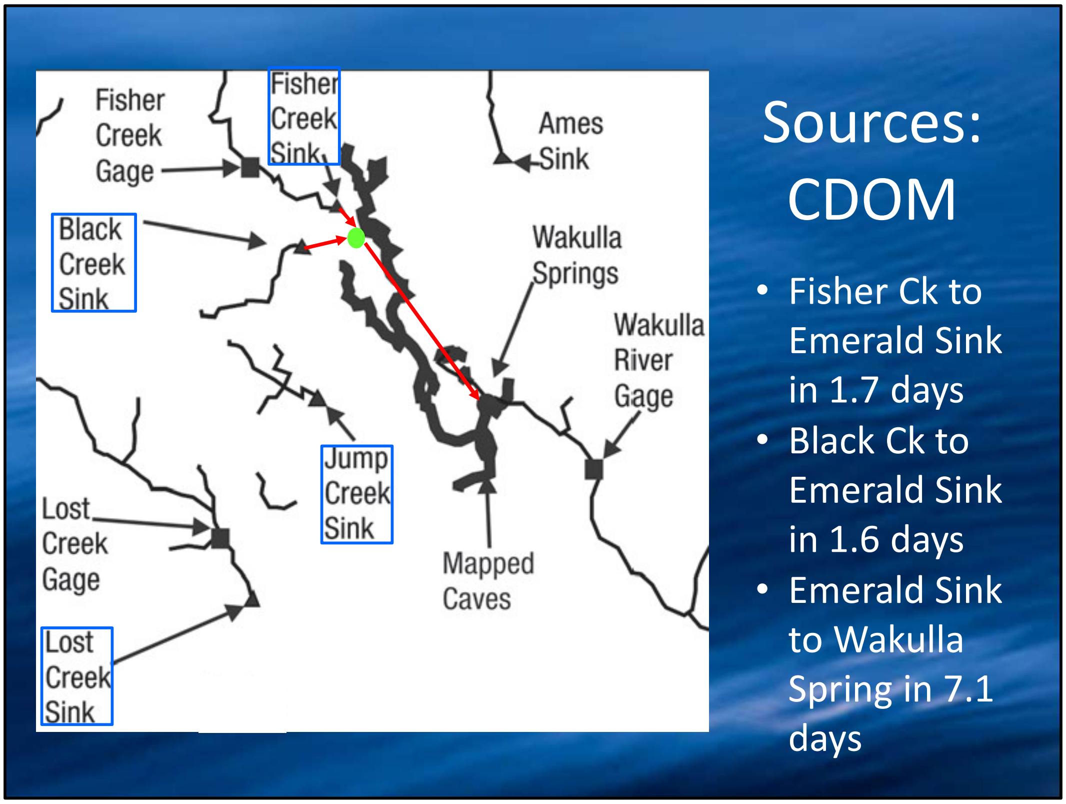


Statistical analysis reveals that

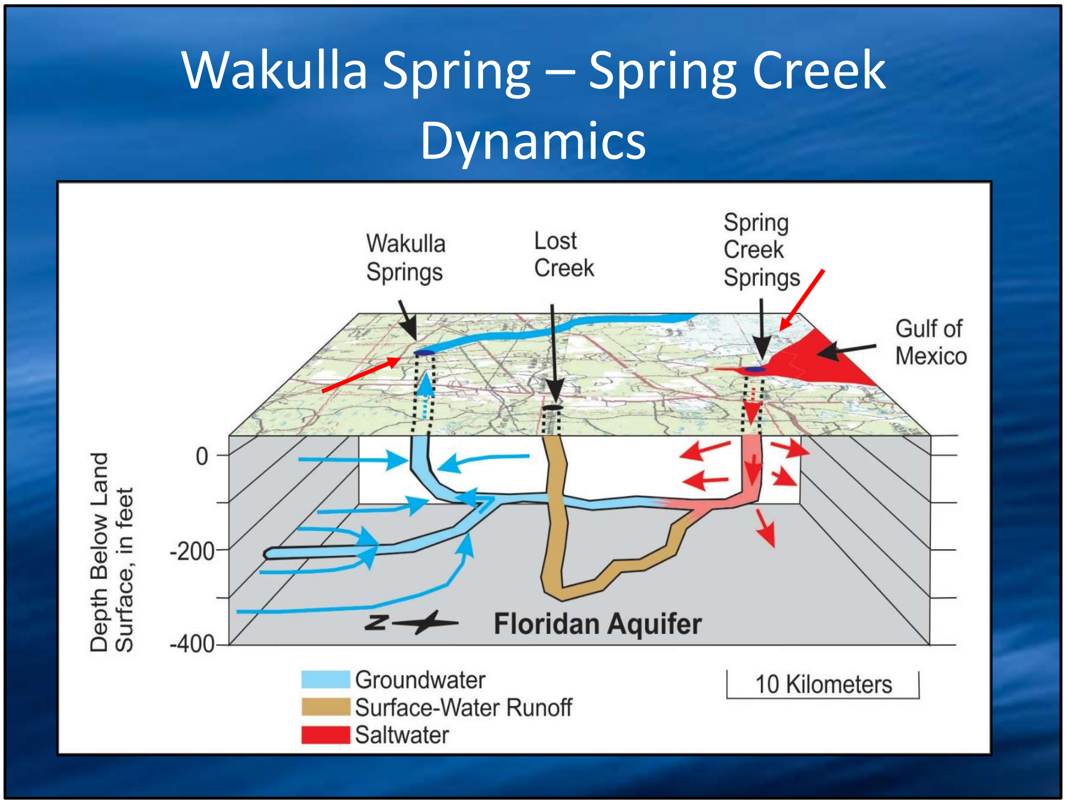
- **[click]** Two color-absorbing substances have significant negative impacts on visibility depth:
    - **[click]** CDOM measured as true color and
    - **[click]** chlorophyll a measured as corrected chlorophyll a
- **[click]** Turbidity has no significant effect
- **[click]** Pheophytin findings are ambiguous.



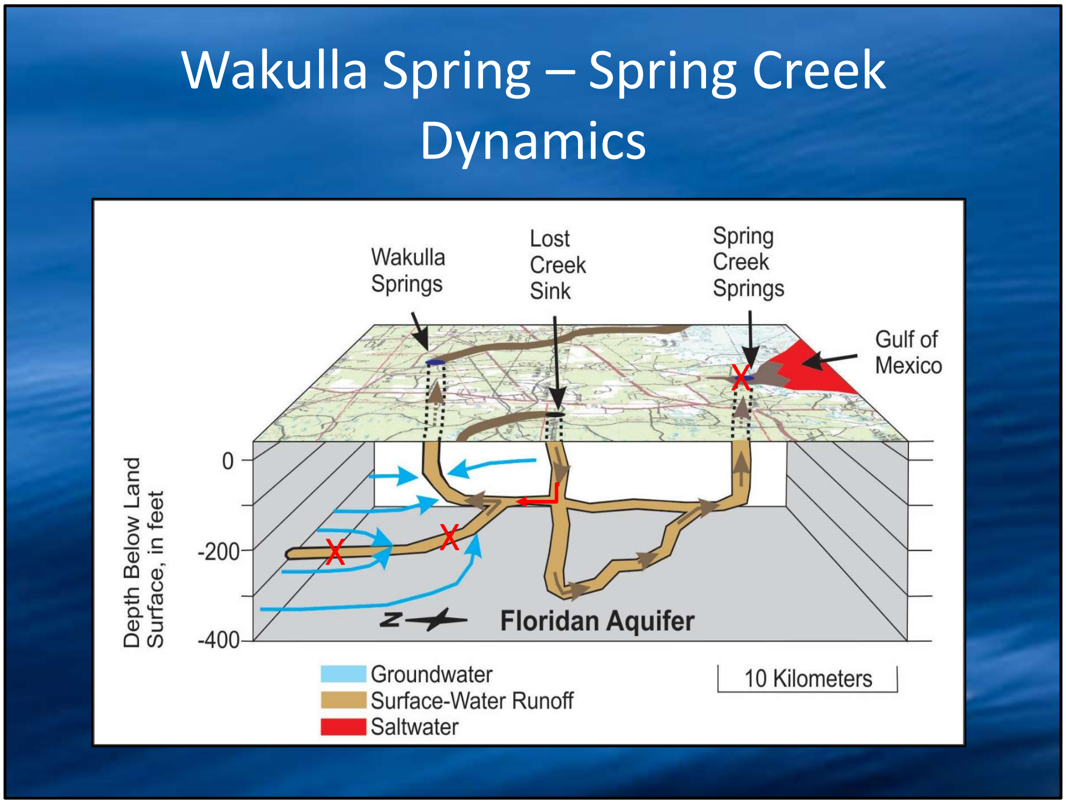
- **[click]** CDOM dominates when water is darkest and visibility is worst
- **[click]** CDOM and corrected chlorophyll a contribute to dark water conditions when
    - visibility depth is greatest and
    - the apparent color is green rather than brown
- **[click]** Small increases of each have a greater impact when visibility is greater
- So, let's see what we know about the sources of CDOM and chlorophyll that affect spring visibility



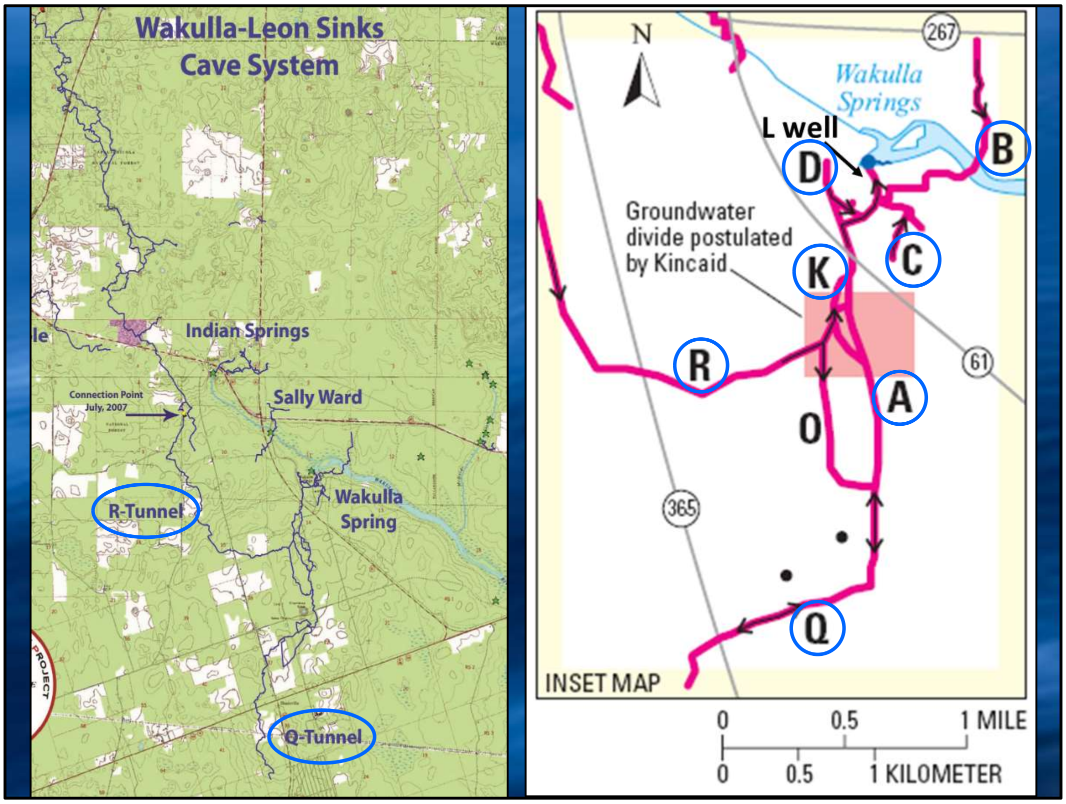
- In 1992, in one of the first meetings of the Florida Department of Natural Resources Wakulla Springs Water Quality Working Group, U.S.G.S. geologist Hal Davis hypothesized that the principal sources of tannins causing dark water conditions at the spring are the sinking streams that originate within the Apalachicola National Forest:
    - **[click]** namely Black, Fisher, Jump, and Lost Creeks discharging to the aquifer through their respective sinkholes or swallets.
- Dye tests conducted by Todd Kincaid of Geohydros in early 2000s demonstrated hydrogeologic connections between both Black Creek and Fisher Creek and Wakulla Spring
    - **[click]** Fisher Creek to Emerald Sink (2002): in about 1.7 days
    - **[click]** Black Creek to Emerald Sink (2003): in about 1.6 days
    - **[click]** Emerald Sink to Wakulla Spring (2004): in about 7.1 days



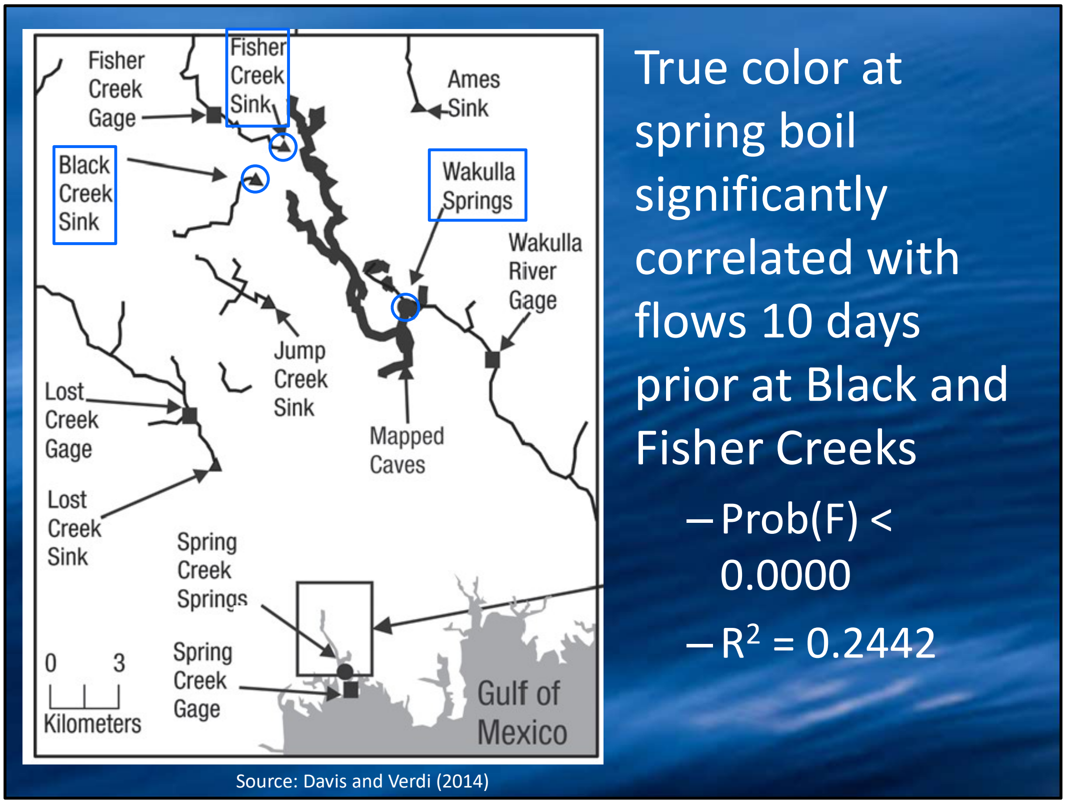
- As we’ve seen on several occasions, most recently in James Sutton’s presentation on Wakulla Spring-Spring Creek Dynamics in September,
  - Hal Davis and David Verdi described what happens when the Spring Creek spring group stops flowing due to saltwater intrusion associated with low rainfall conditions
    - **[click]** Saltwater flows into the Spring Creek caverns forming a high-density "plug" that resists outflow into the Gulf
    - **[click]** Water levels begin to rise at Wakulla Spring as some of the conduits south of the spring reverse flow



- Davis and Verdi also illustrated the scenario when, after sufficient rainfall
  - the sinking streams, including Lost Creek, begin to flow again as shown in this figure
- However, there is a transition period when the saltwater plug at Spring Creek still holds back the southward flow **[click]**
- Scott Dyer's 2015 master's thesis documented flow between the Lost Creek swallet and Wakulla Spring under these conditions
  - Dye tests he completed in 2008 and 2009 revealed that discharge into the Lost Creek swallet arrived at Wakulla Spring in 45-47 days
  - Thus while the Spring Creek plug still holds, tannic water from Lost Creek can flow north to Wakulla adding to the dark water load **[click]**
- It is likely that a transitional scenario also sometimes occurs when rainfall is not sufficient to unplug Spring Creek during which
  - tannic flows from the Leon Sinks sinking streams cease **[click]**
  - but Lost Creek continues to flow and its tannic discharge continues to enter Wakulla Spring,
    - albeit at lower concentrations,
    - perhaps contributing to the apparent green dark water conditions at the spring



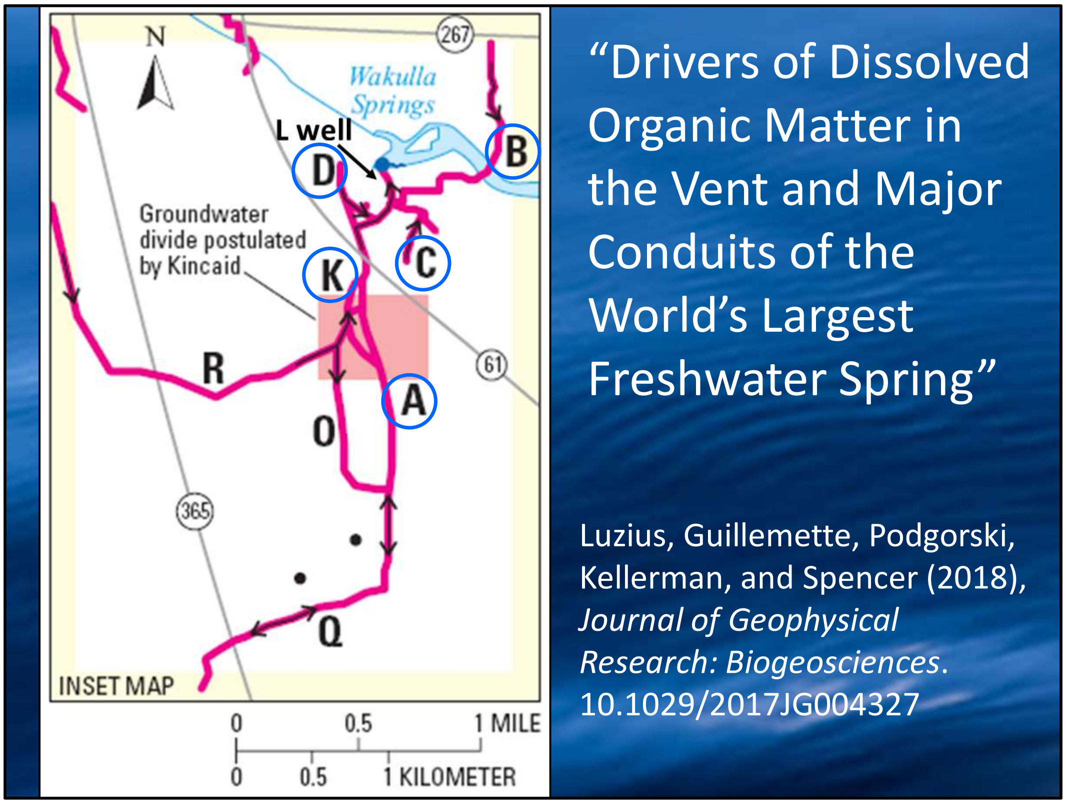
- Weekly sampling conducted by Dr. McGlynn between August 2016 and August 2017 established that
  - Both CDOM (tannins) and chlorophyll (corrected chlorophyll a and pheophytin) are delivered to the spring in ground water flowing through all the major caves that converge upstream of the spring vent
- The K cave **[click]** receives flow from the R tunnel which connects to the Leon Sinks sinking streams and caves to the north **[click]**
- The A cave **[click]** receives flow from the Q tunnel which flows north to the spring **[click]**
  - This would likely be discharge from Lost Creek and small sinks discharging into the conduit system to the south
- The D, C, and B caves are fed by smaller conduits and matrix flow



• The interconnection between the sinking streams and Wakulla Spring is further demonstrated by testing the relationship between CDOM/tannin levels at the spring with flows of the creeks

- Combined creek flows explain 24% of observed variation in true color at spring boil (R-squared = 0.2442)

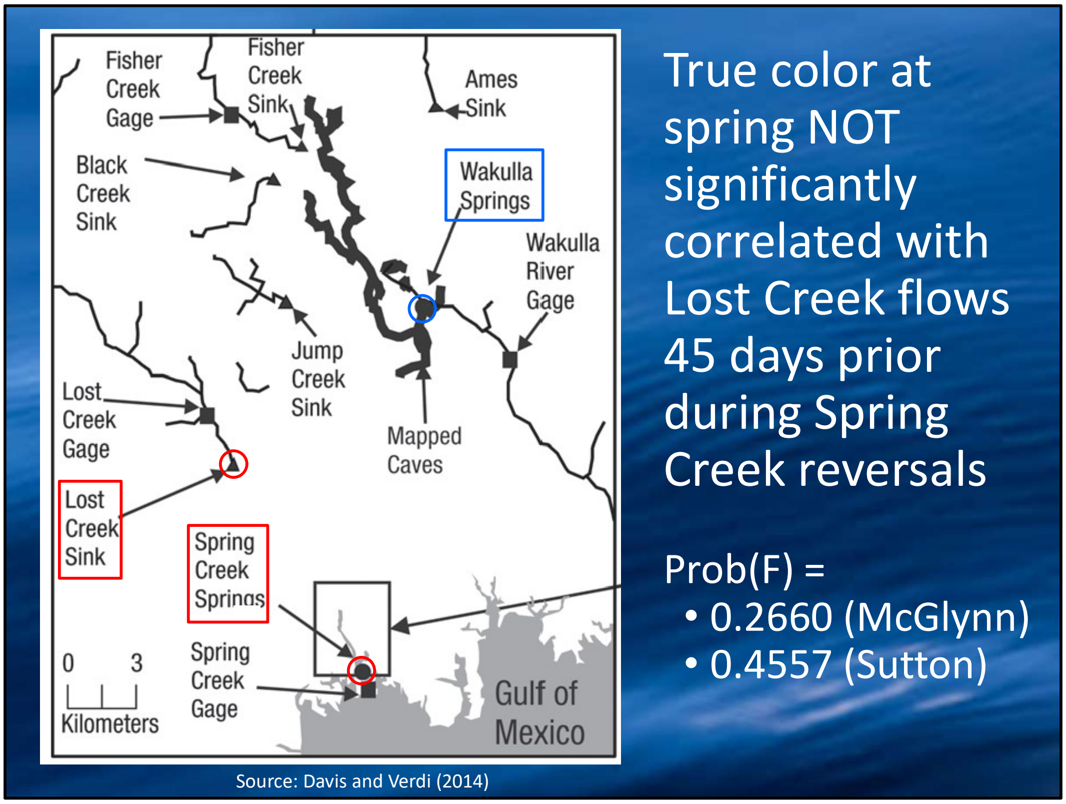
•



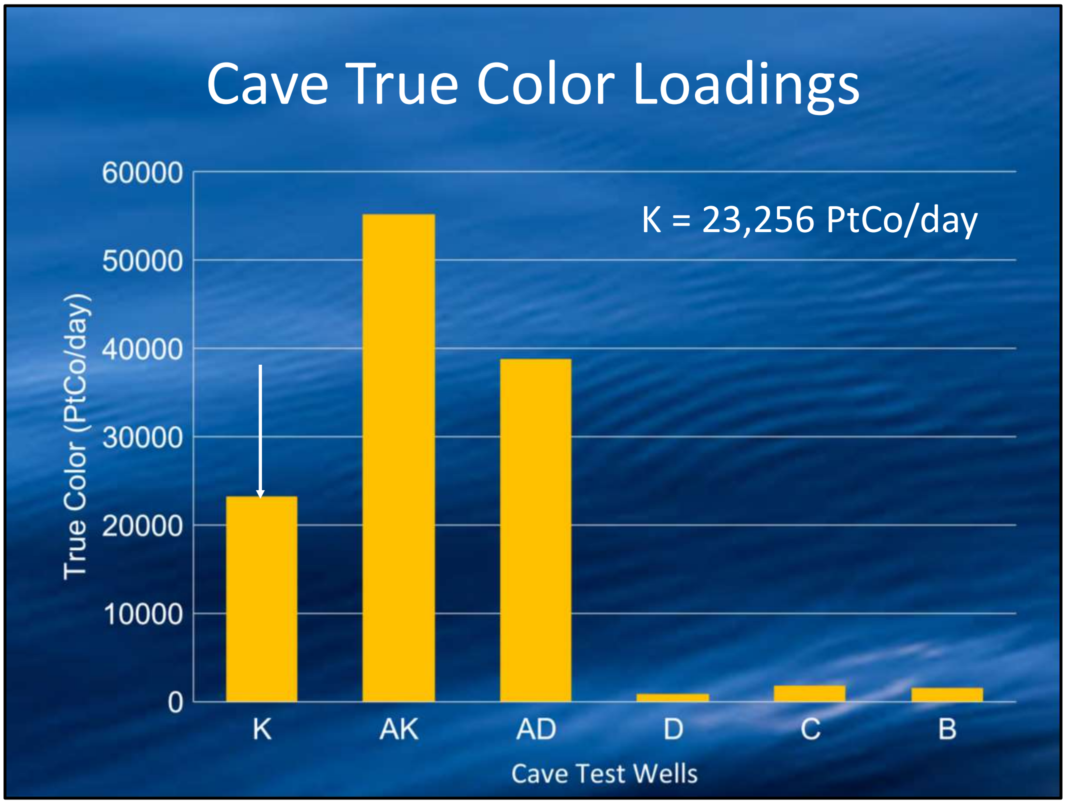
• These findings are reinforced by a study conducted by FSU researchers at the National High Magnetic Field Lab who analyzed dissolved organic matter (DOM) from the cave test wells and the spring vent

• They consistently found that the light absorbing properties of DOM from the A and K caves **[click]** was more similar to that observed at the vent than that observed in samples from D, C, and B caves **[click]** (Luzius et al., 2018, pp. 2778-2784).

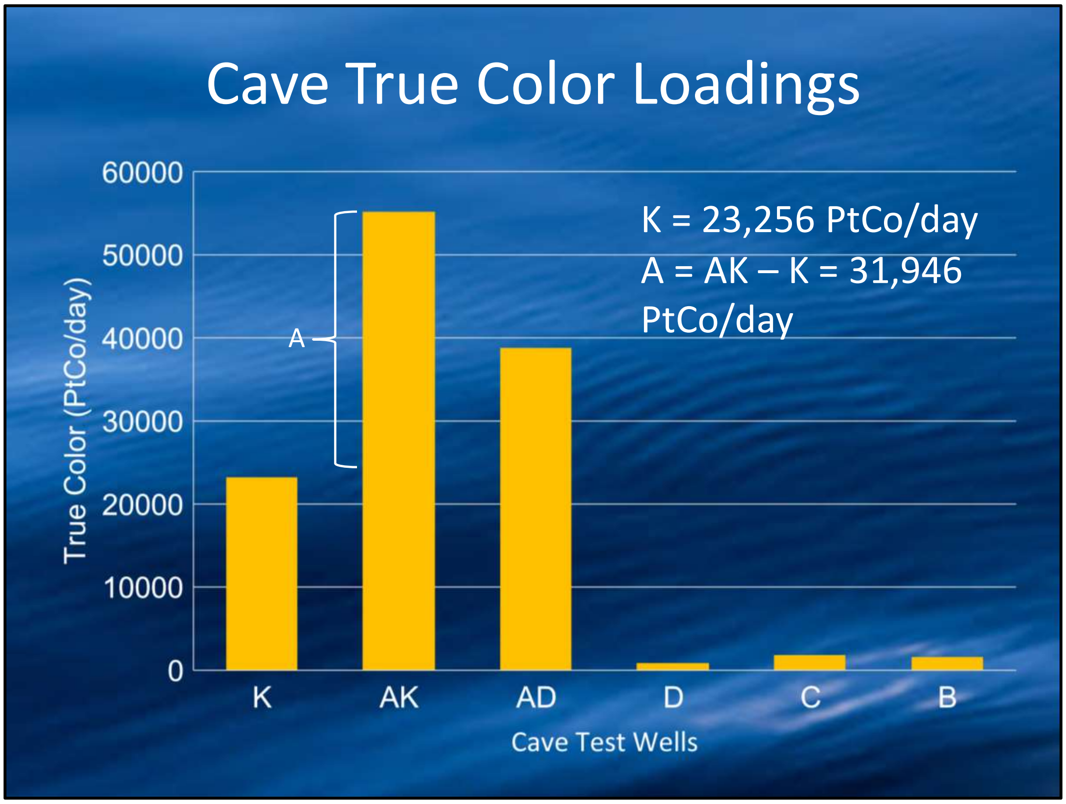
• They also found that DOM levels peaked with higher flows following prolonged dry spells (p. 2787)



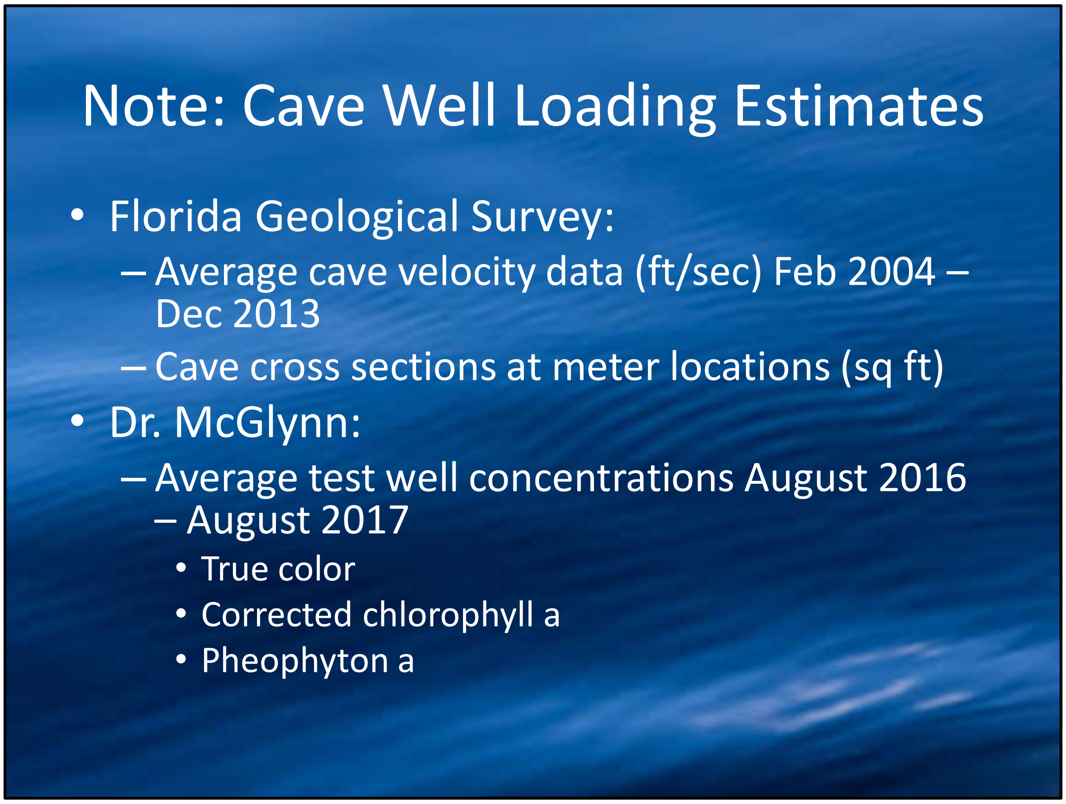
- I attempted to test possible impacts of Lost Creek swallet discharges on spring color by correlating spring boil CDOM levels with those of Lost Creek 45 days prior (based on Dyer's dye tests) at times when Spring Creek springs group appeared to have stopped or reversed.
  - Dr. McGlynn estimated reversals based on a model of flow and salinity measured as specific conductivity.
  - NWFWMD hydrologist James Sutton estimated reversals using the method devised by Davis and Verdi (2014) to calculate head differentials between Wakulla Spring and Spring Creek based on flow and salinity data
- Neither regression model was statistically significant **[click]**:
  - McGlynn estimation: prob(F) = 0.2600
  - James Sutton estimation: prob(F) = 0.4557



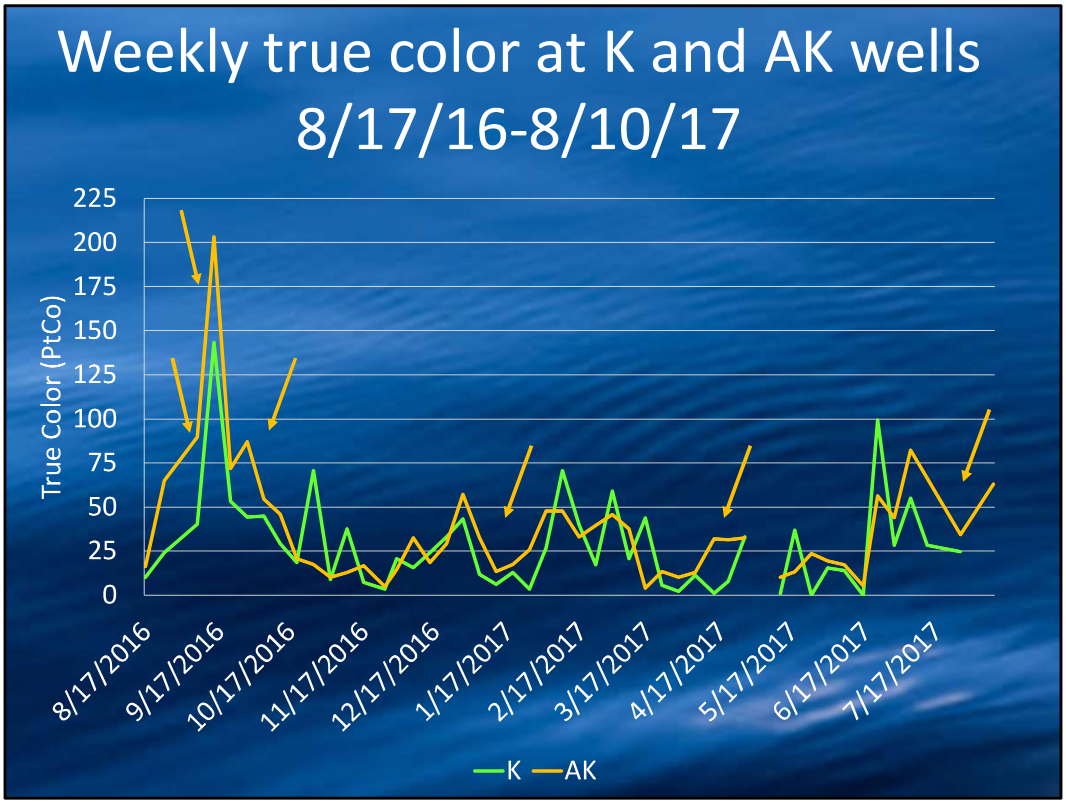
- While this may suggest that Lost Creek CDOM inflows are inconsequential, loading calculations for CDOM measured as true color at the K and AK wells indicate the A cave carries more CDOM than the K cave:
    - **[click]** K = 23,256 PtCo/day
    - **[click to next slide]**



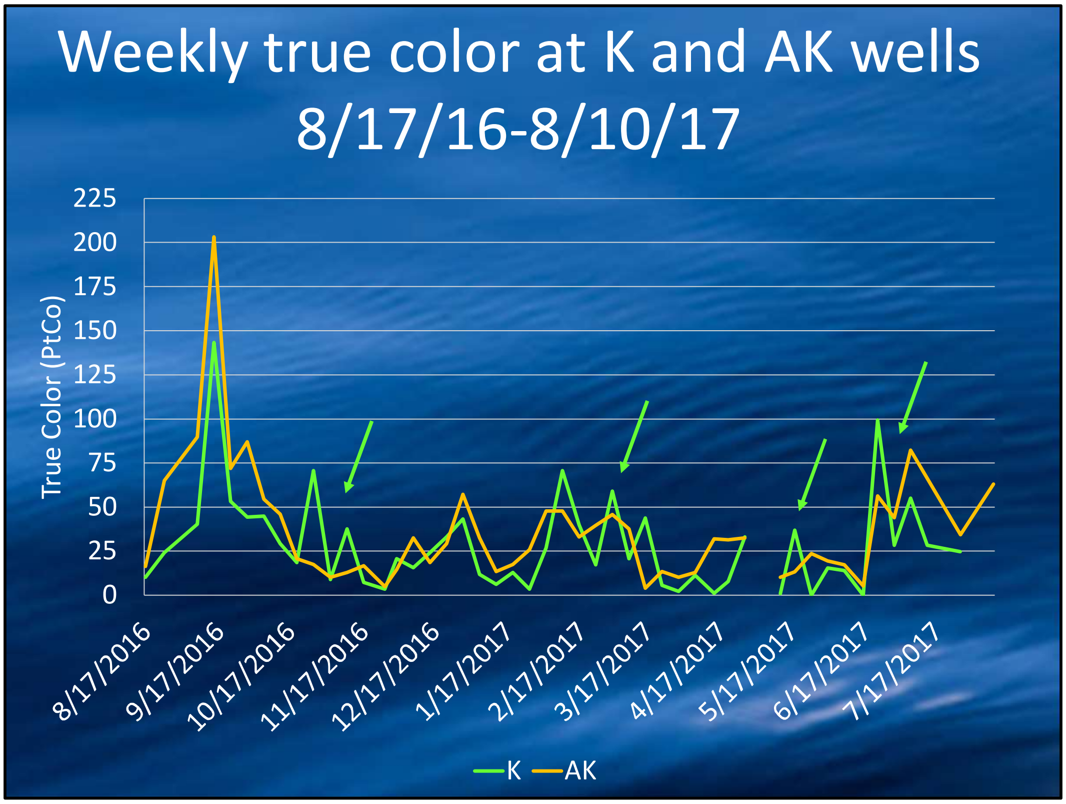
- A = AK – K = 31,946 PtCo/day
- Dr. McGlynn's data also showed that **mean CDOM levels** measured as true color were higher at the AK well than at the K well
    - This finding is consistent with the Mag Lab study of DOM
- Evidently the dynamics associated with tannic flows from Lost Creek and the matrix south of Wakulla Spring are considerably more complex than can be captured by simple linear models.



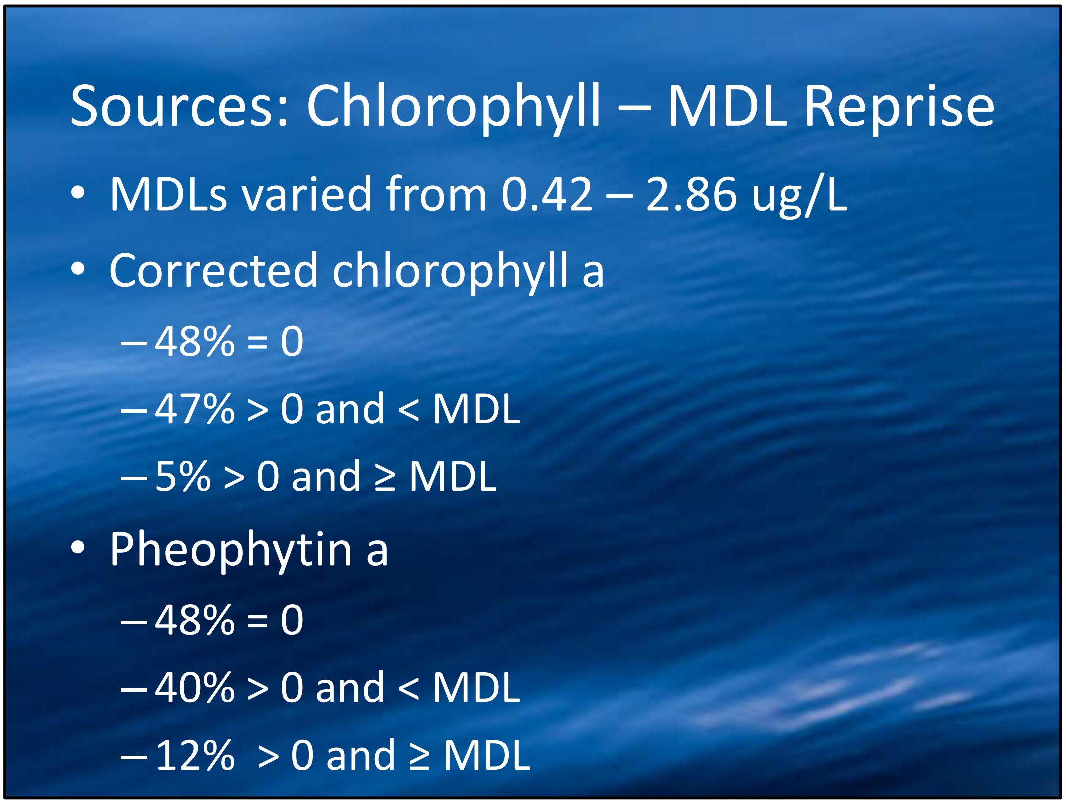
- I estimated the cave well loadings for true color, corrected chlorophyll a, and pheophytin a based on
  - Florida Geological Survey cave velocity data from Feb 2004 – Dec 2013 and cross sections to calculate average flow in cu ft/sec
  - Dr. McGlynn’s water quality data for August 2016 – August 2107



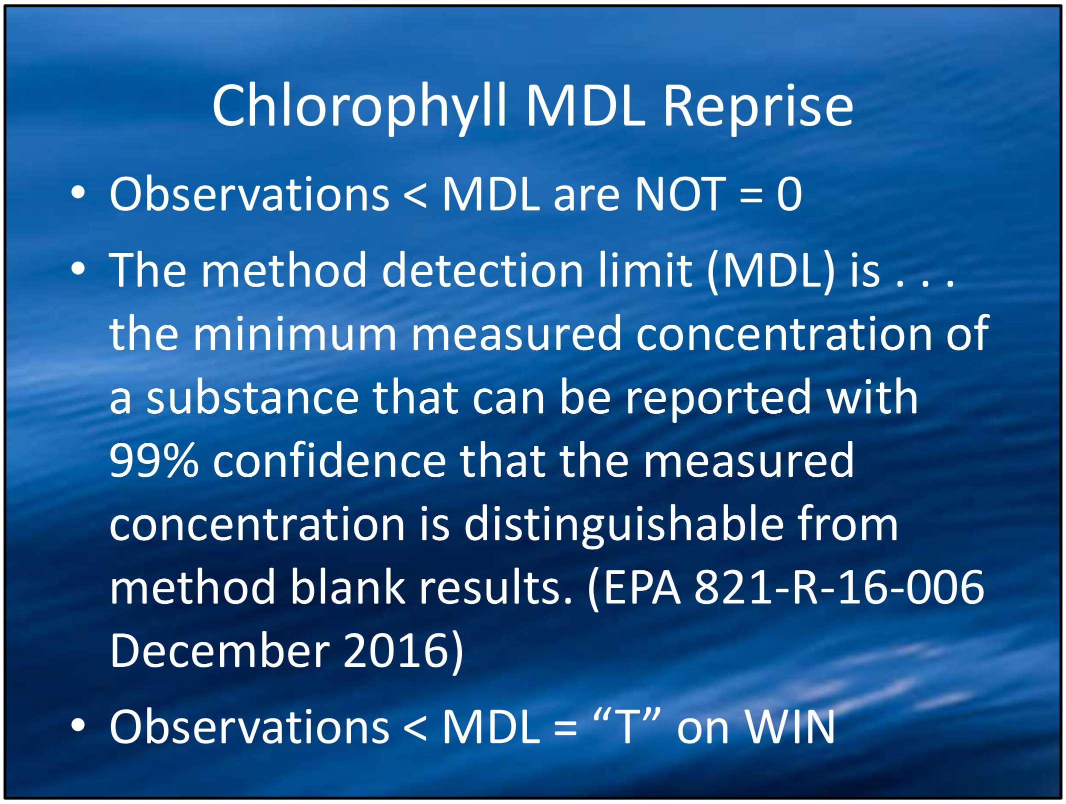
- Comparison of true color levels at the K and AK wells also suggests CDOM contributions from Lost Creek
- **[click]** When color levels are greater at the AK well than at the K well
    - tannic inflows from Lost Creek via the A cave are likely supplementing those in the K cave from the sinking streams to the north.
- **[click for next slide]**



- When color levels are higher at the K well than at the AK well
  - it is likely that there is little if any tannic inflow from Lost Creek via the A cave
  - and CDOM levels are due primarily to inflows from the karst limestone matrix to the R tunnel and the K cave
- Let’s take a look now at what we know about sources of the chlorophyll detected at the spring

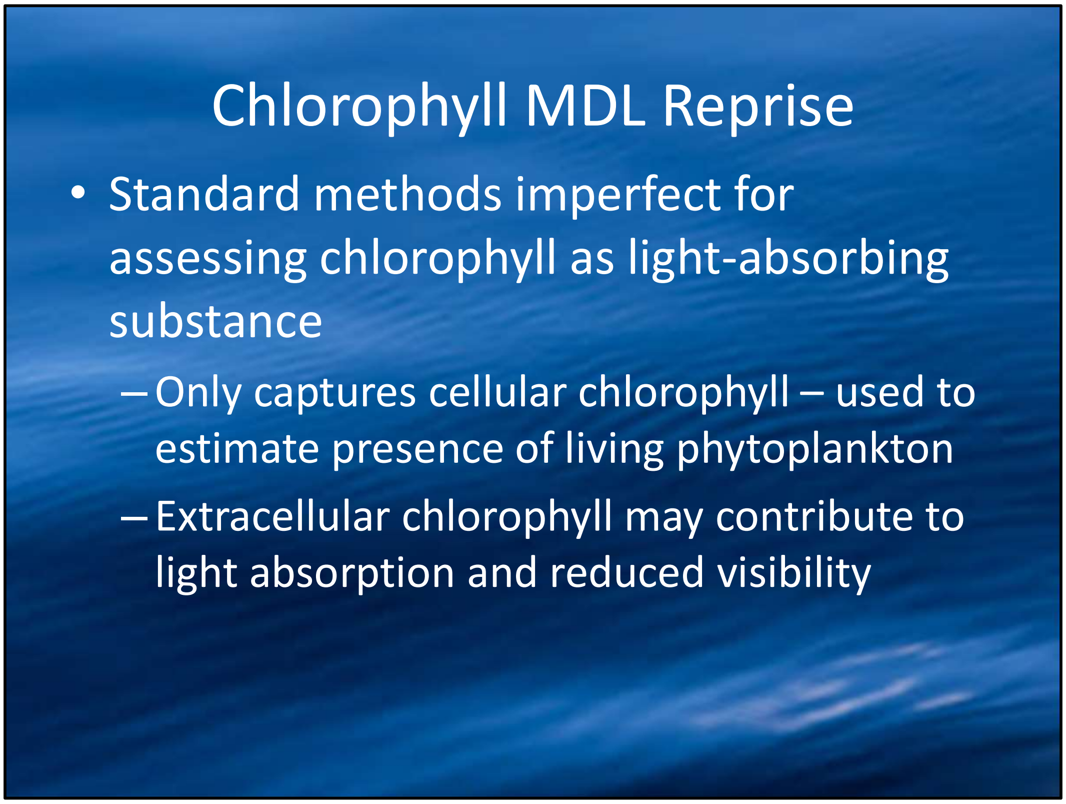


- Last month, Mark Heidecker correctly pointed out that many of the corrected chlorophyll a and pheophytin a observations recorded by Dr. McGlynn were less than his reported method detection limit or MDL
- I looked up Dr. McGlynn's reported MDLs on FDEP's online Watershed Information Network (WIN) database, previously called STORET
- MDLs for these two forms of chlorophyll ranged from 0.42 to 2.86 micrograms per liter **[click]**
- Reviewing the 189 observations for each of these factors reveals that

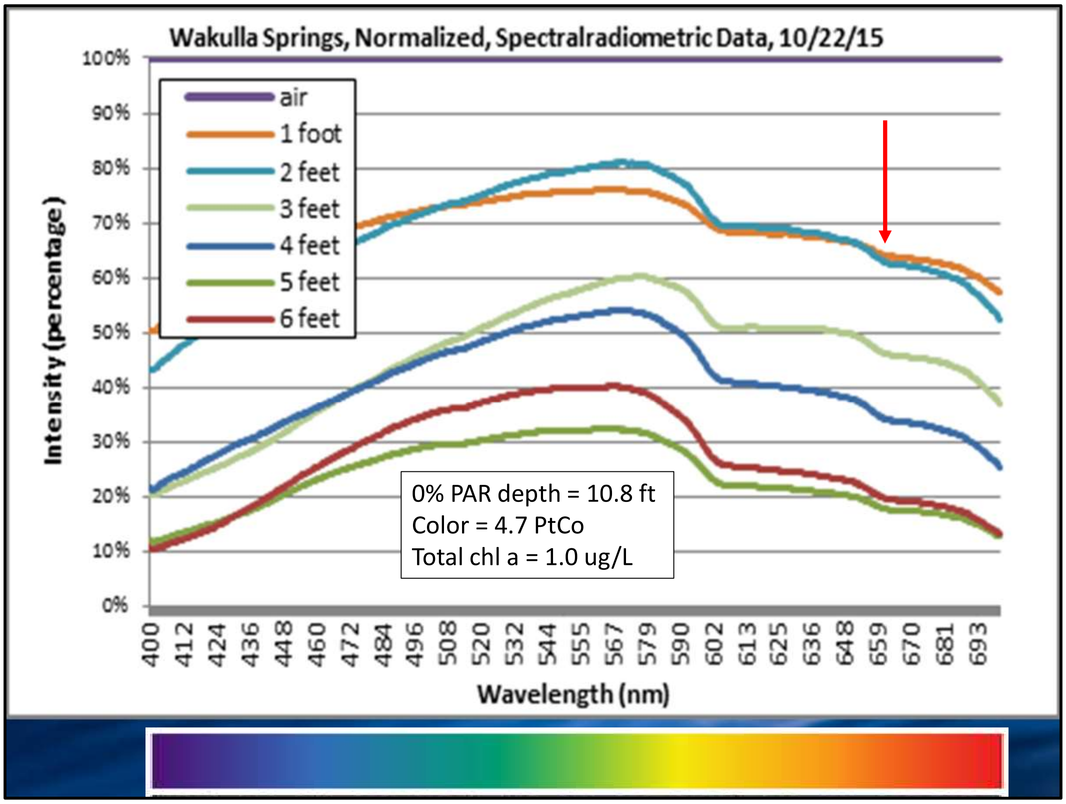


- It’s important to recognize, however, that observations < MDL are not = 0
- **[click]** EPA defines the MDL as

- **[click]** The curators of FDEP’s WIN database permit uploading values less than the MDL; they are labelled as “T”
- While observations < MDL are not used for regulatory purposes they may still be still valuable for describing an ecosystem,

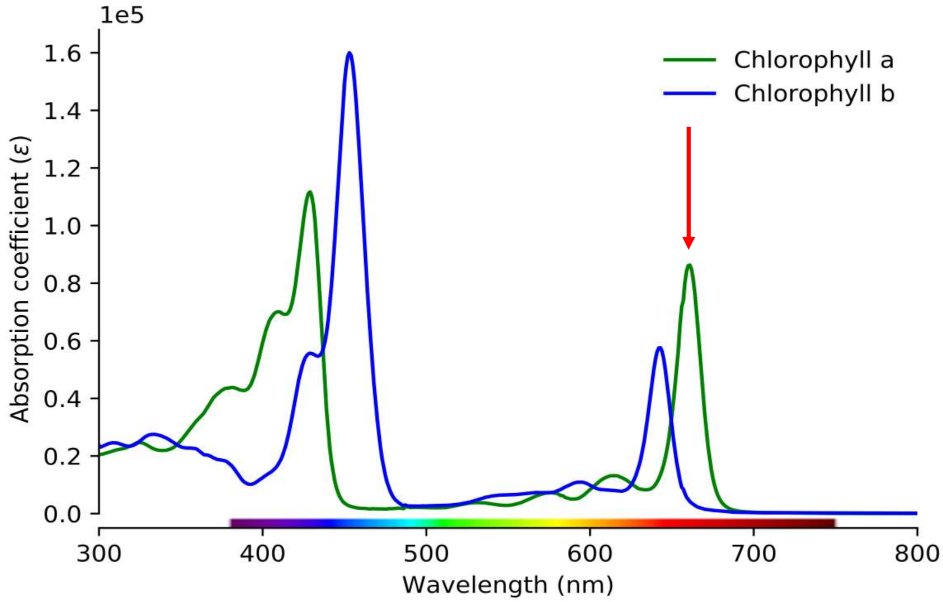


- I believe that taking account of observations < MDL is of value in this particular study
- It’s important to recognize that the



- The eight light and dark event normalized spectrographs created by Dr. McGlynn provide further evidence of the presence of chlorophyll and its effect on visibility
- As we saw last month, each one displays an absorbance dip at approximately 665 nm **[click]**
    - Here the percent absorbance at 665 nm at one-foot depth is approximately 35% (inverse of intensity)
- 665 nm corresponds with the red-range peak of the chlorophyll a absorbance spectrum **[click to next slide]**

Chlorophyll a Light Absorption



Light/Dark Event Chlorophyll Absorbances

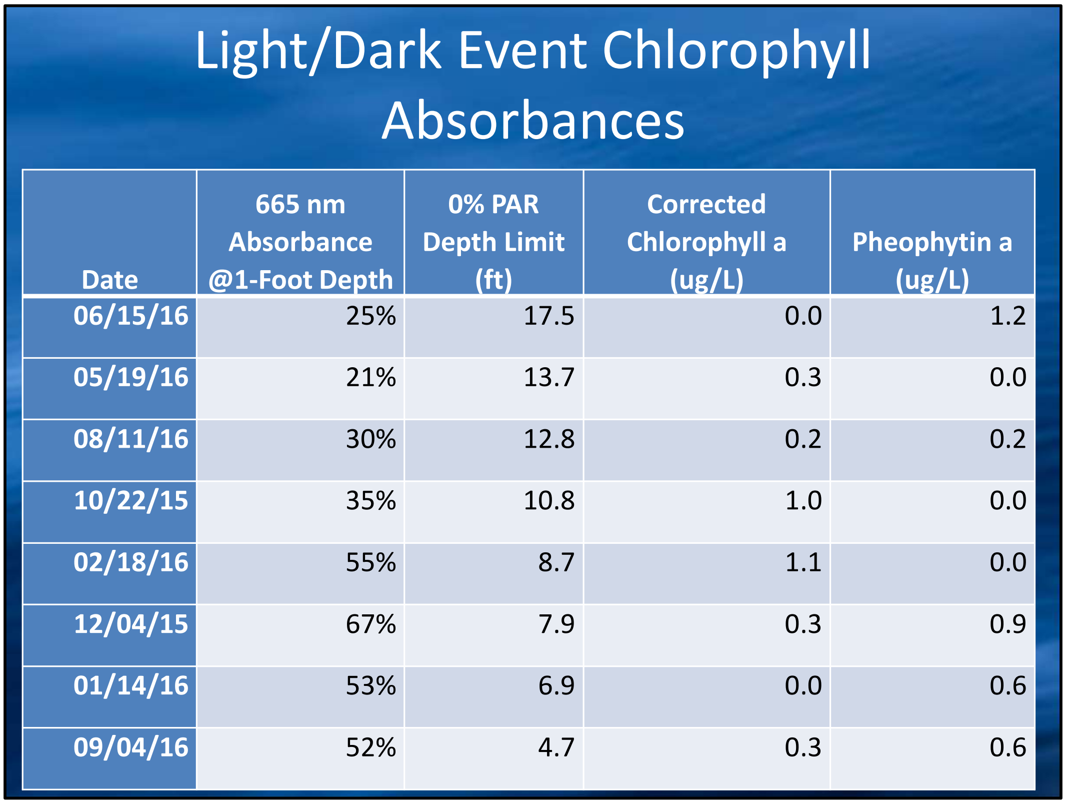
Date	665 nm Absorbance @1-Foot Depth			
06/15/16	25%			
05/19/16	21%			
08/11/16	30%			
10/22/15	35%			
02/18/16	55%			
12/04/15	67%			
01/14/16	53%			
09/04/16	52%			

- This table displays
  - the percent absorbance at the 665 nm chlorophyll peak
  - The corresponding 0% PAR depth limit on that date
  - And the levels of corrected chlorophyll a and pheophytin a measured
- Note that some amount of one or the other form of chlorophyll was recorded for each date

Light/Dark Event Chlorophyll Absorbances

Date	665 nm Absorbance @1-Foot Depth	0% PAR Depth Limit (ft)		
06/15/16	25%	17.5		
05/19/16	21%	13.7		
08/11/16	30%	12.8		
10/22/15	35%	10.8		
02/18/16	55%	8.7		
12/04/15	67%	7.9		
01/14/16	53%	6.9		
09/04/16	52%	4.7		

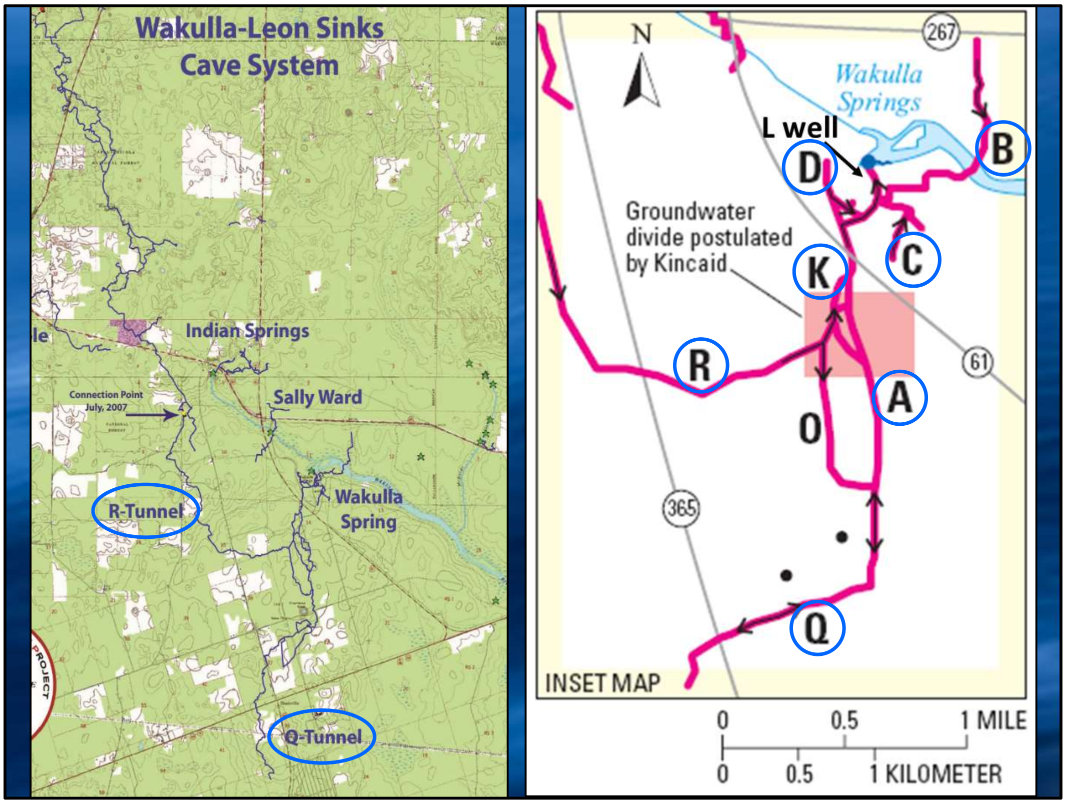
- This table displays
    - the percent absorbance at the 665 nm chlorophyll peak
    - The corresponding 0% PAR depth limit on that date
    - And the levels of corrected chlorophyll a and pheophytin a measured
- Note that some amount of one or the other form of chlorophyll was recorded for each date



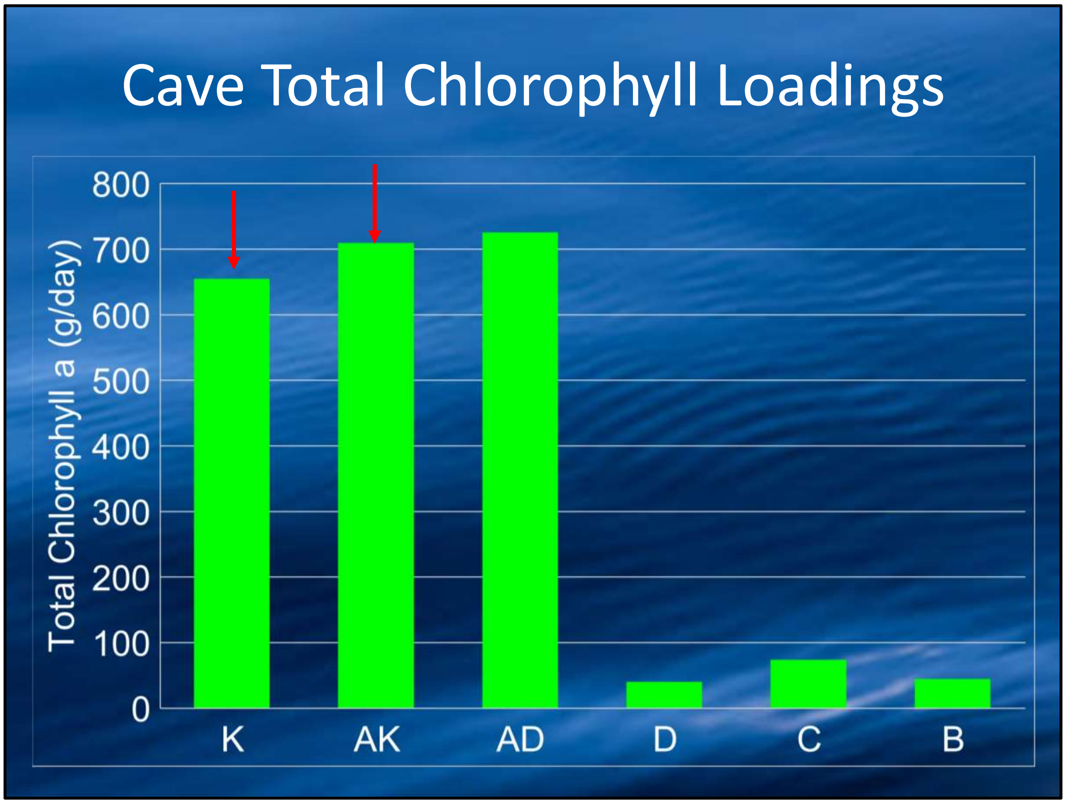
Light/Dark Event Chlorophyll Absorbances

Date	665 nm Absorbance @1-Foot Depth	0% PAR Depth Limit (ft)	Corrected Chlorophyll a (ug/L)	Pheophytin a (ug/L)
06/15/16	25%	17.5	0.0	1.2
05/19/16	21%	13.7	0.3	0.0
08/11/16	30%	12.8	0.2	0.2
10/22/15	35%	10.8	1.0	0.0
02/18/16	55%	8.7	1.1	0.0
12/04/15	67%	7.9	0.3	0.9
01/14/16	53%	6.9	0.0	0.6
09/04/16	52%	4.7	0.3	0.6

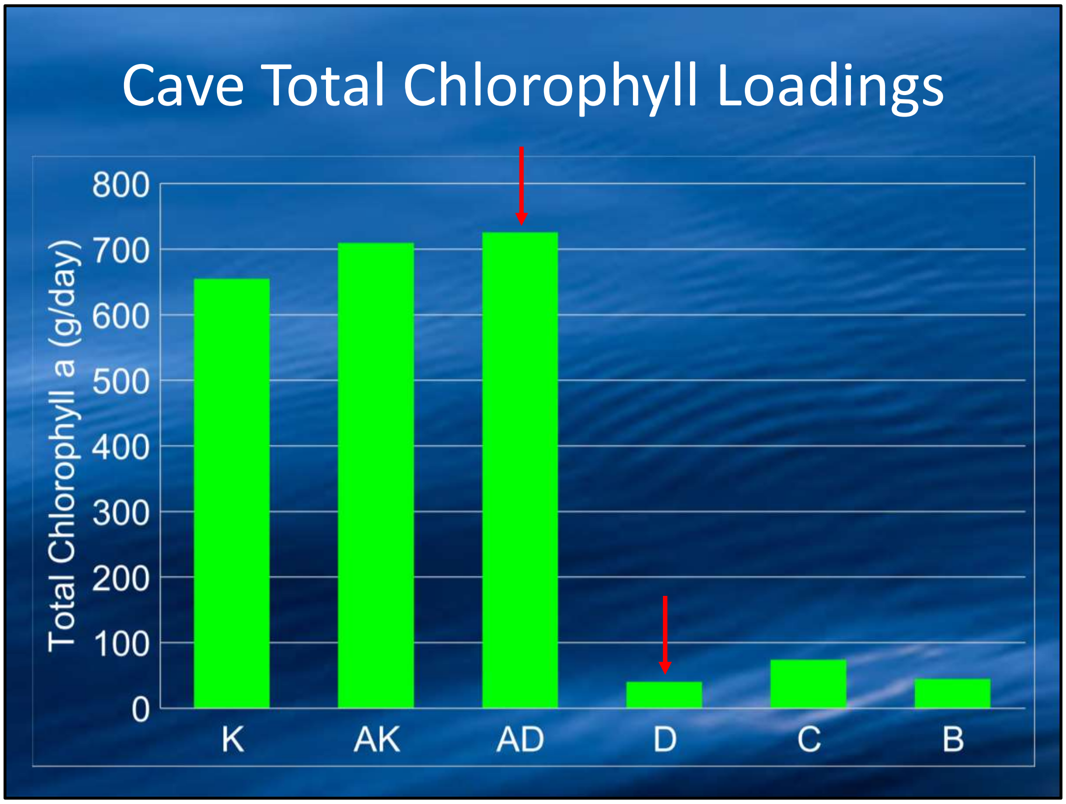
- This table displays
  - the percent absorbance at the 665 nm chlorophyll peak
  - **[click]** The corresponding 0% PAR depth limit on that date
  - And **[click]** the levels of corrected chlorophyll a and pheophytin a measured
- Note that some amount of one or the other form of chlorophyll was recorded for each date



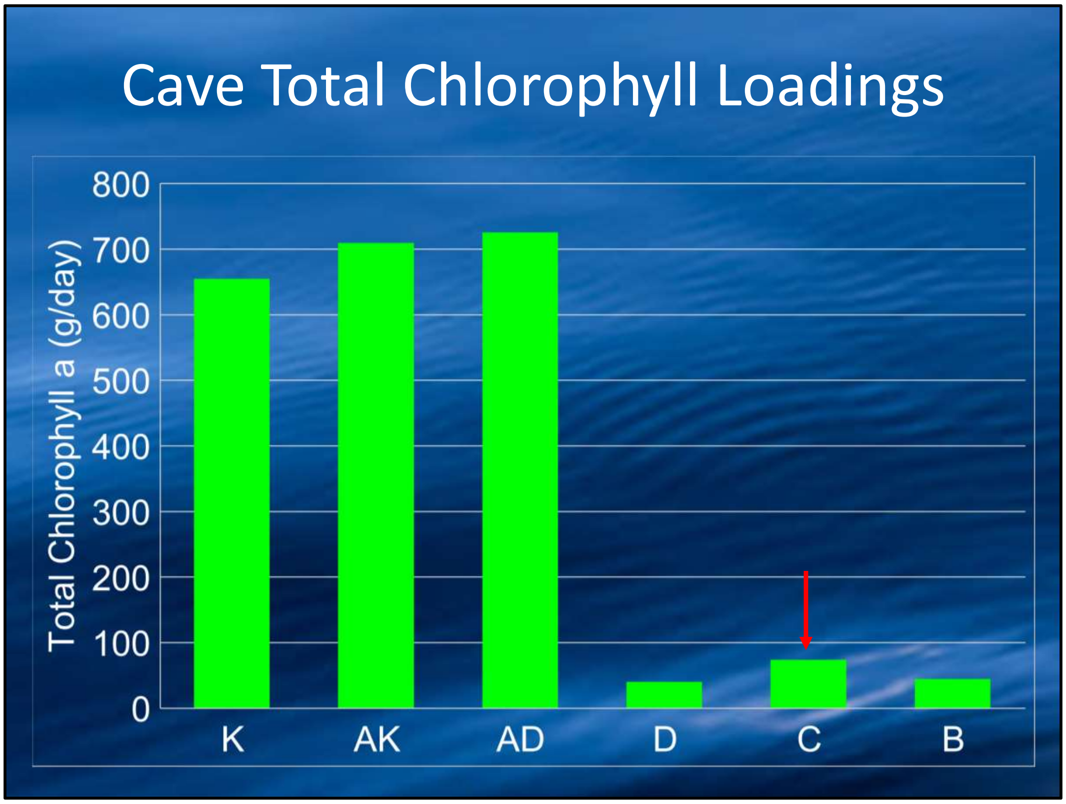
- So where is the chlorophyll coming from?
- As noted above, Dr. McGlynn detected both forms of chlorophyll at each cave well
- This suggests that chlorophyll is arriving both via the R and Q tunnels that feed the K and A caves as well as via smaller conduits and matrix flow that feed the B, C, and D caves



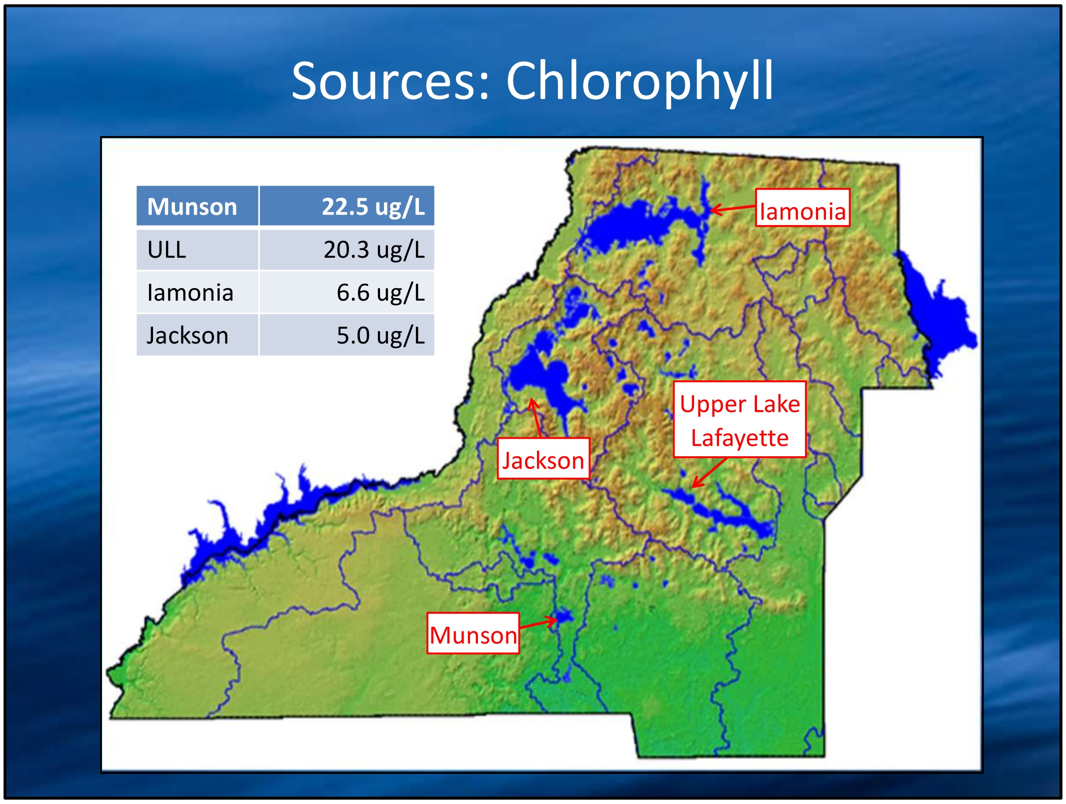
- As with color, the greatest loadings of total chlorophyll a (corrected chlorophyll a + pheophytin a) are from the K and A caves **[click]**
- But in this case, the predominant source is the K cave which receives inflow from the R tunnel



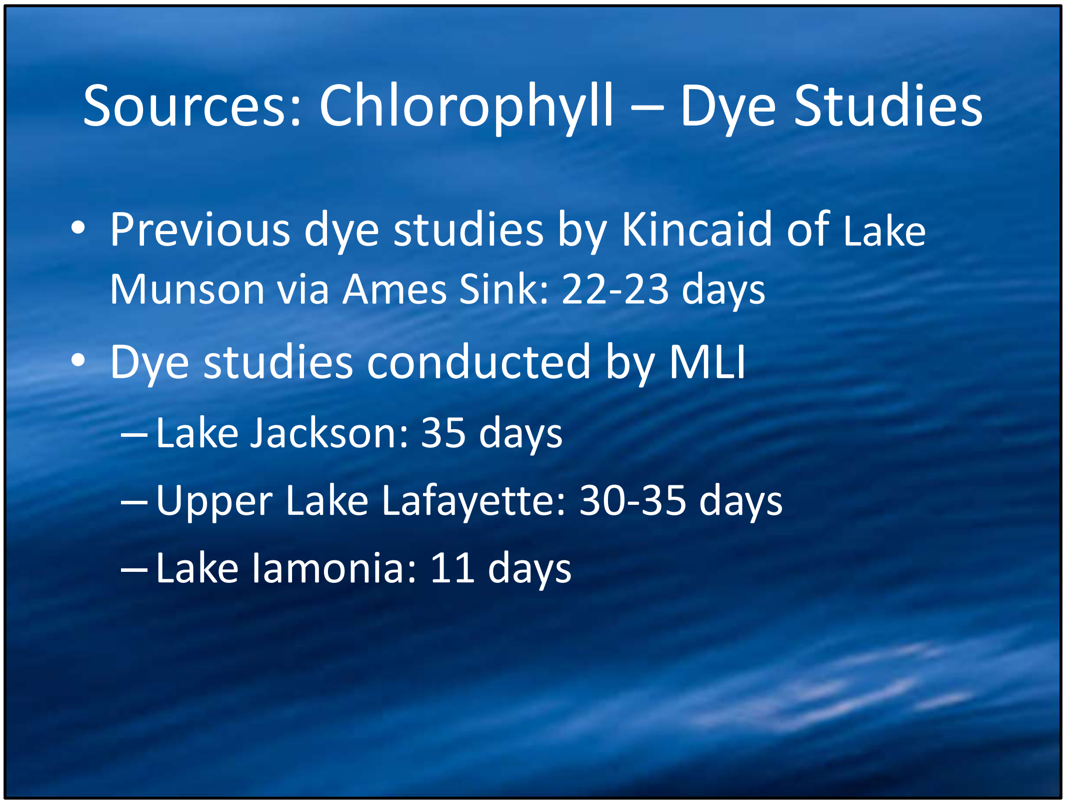
- The higher loadings at the AD well reflect input from the D cave **[click]**



- And, loadings from the C cave are greater than those from D or B

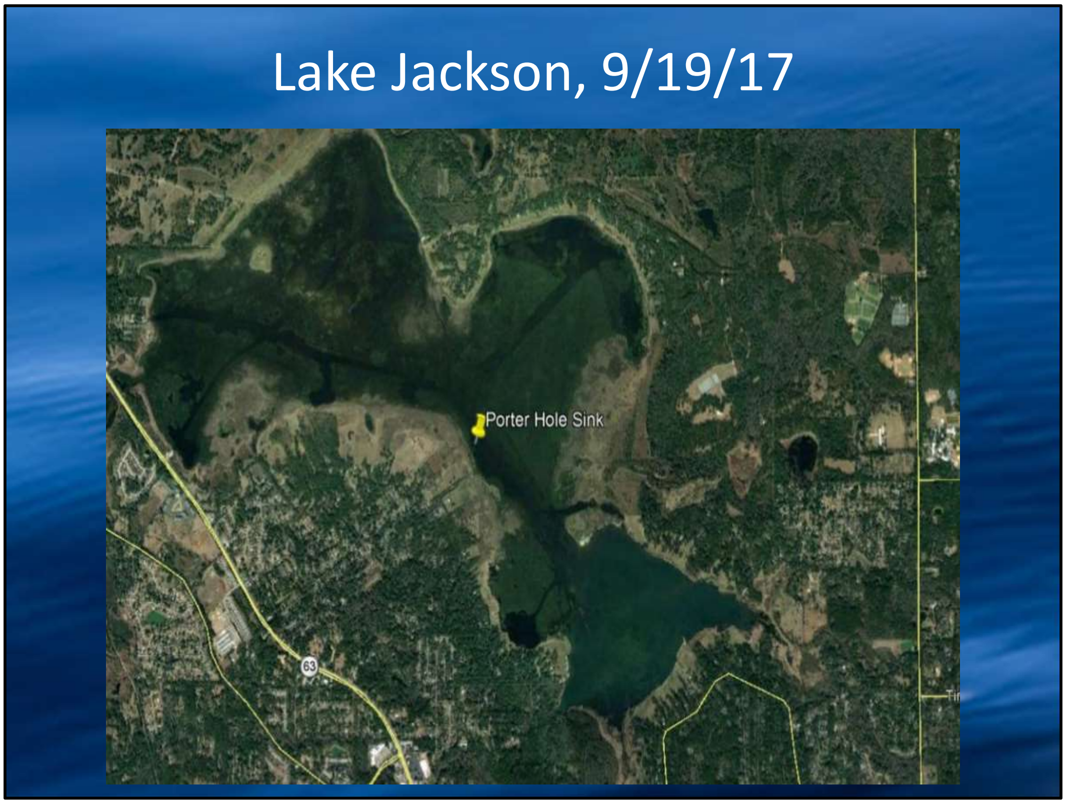


- The prime suspect sources are the large karst lakes north of the spring, each of which has one or more sink holes that drain to the aquifer:
    - **[click]** Lake Iamonia
    - **[click]** Upper Lake Lafayette
    - **[click]** Lake Jackson
    - **[click]** Lake Munson
- **[click]** Lake Munson and Upper Lake Lafayette have recorded the highest levels of chlorophyll a, averaging 22.5 and 20.3 µg/L over the most recent 12 to 13-year records reported by the Leon County Public Works Division
- Lakes Jackson and Iamonia have considerably lower concentrations, averaging 5.0 and 6.6 µg/L respectively over comparable time periods

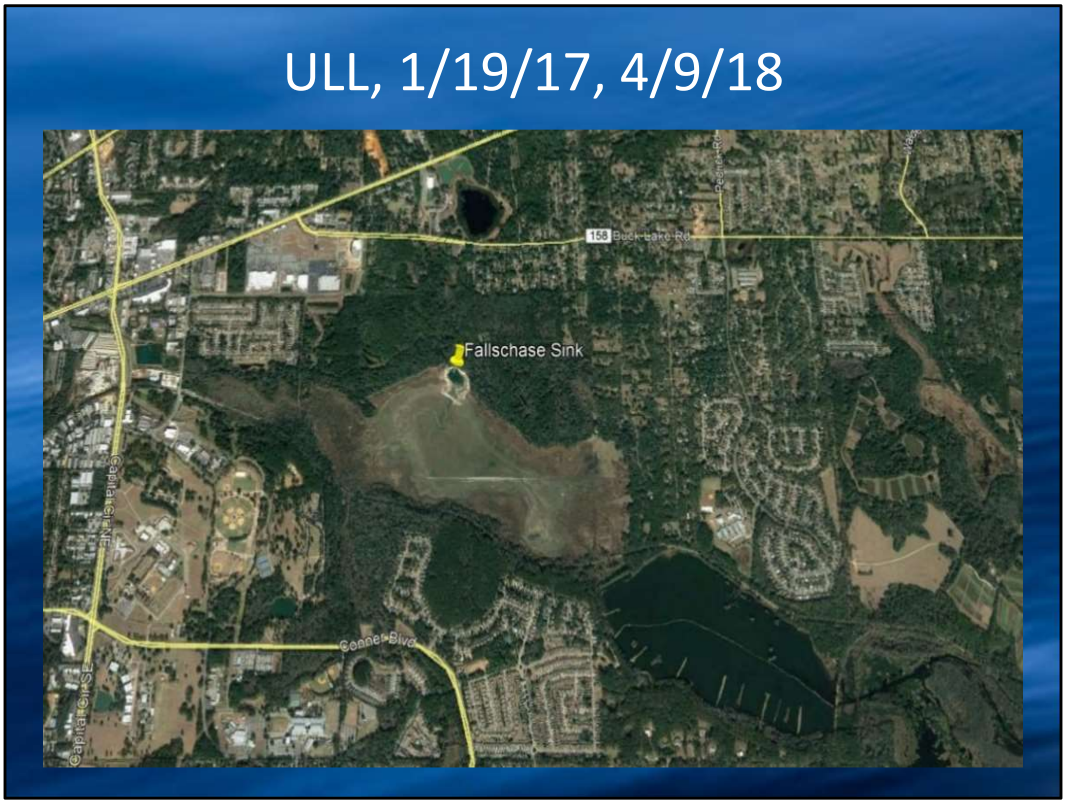


- Previous dye studies completed by Todd Kincaid documented connections to Wakulla Spring from Lake Munson via Ames Sink in 22 to 23 days
- **[click]** Dye studies conducted by McGlynn Laboratories Inc have, for the first time, shown connections between Wakulla Spring and the other large karst lakes in the springshed:
    - **[click]** Lake Jackson in 35 days
    - **[click]** Upper lake Lafayette in 30-35 days
    - **[click]** Lake Iamonia in 11 days
- The following slides show the dates and locations of the dye injections

- Lake Munson via Ames Sink located 3.75 miles south at the terminus of Munson Slough

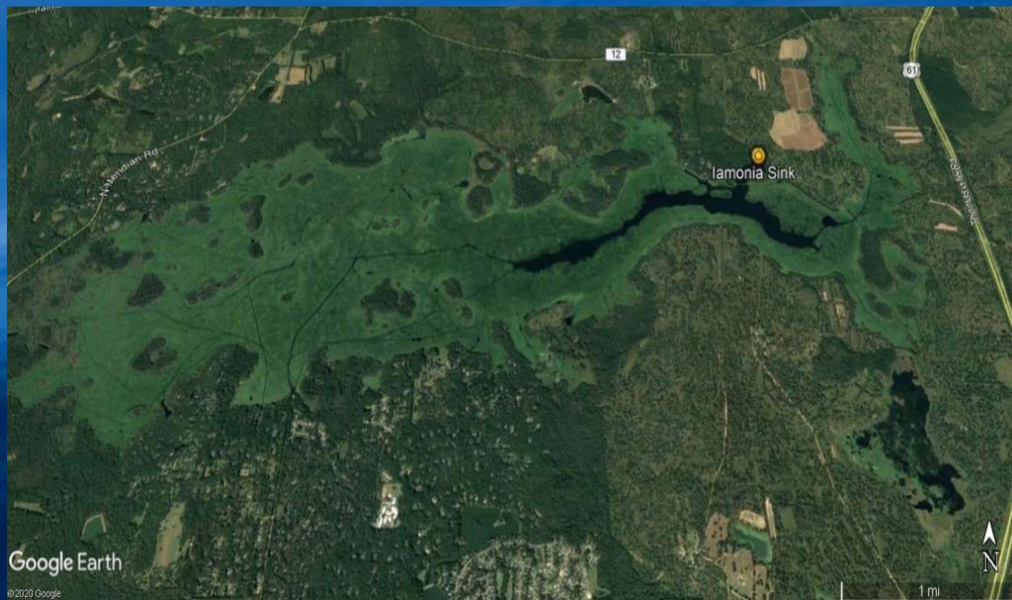


- Lake Jackson at Porter Hole Sink

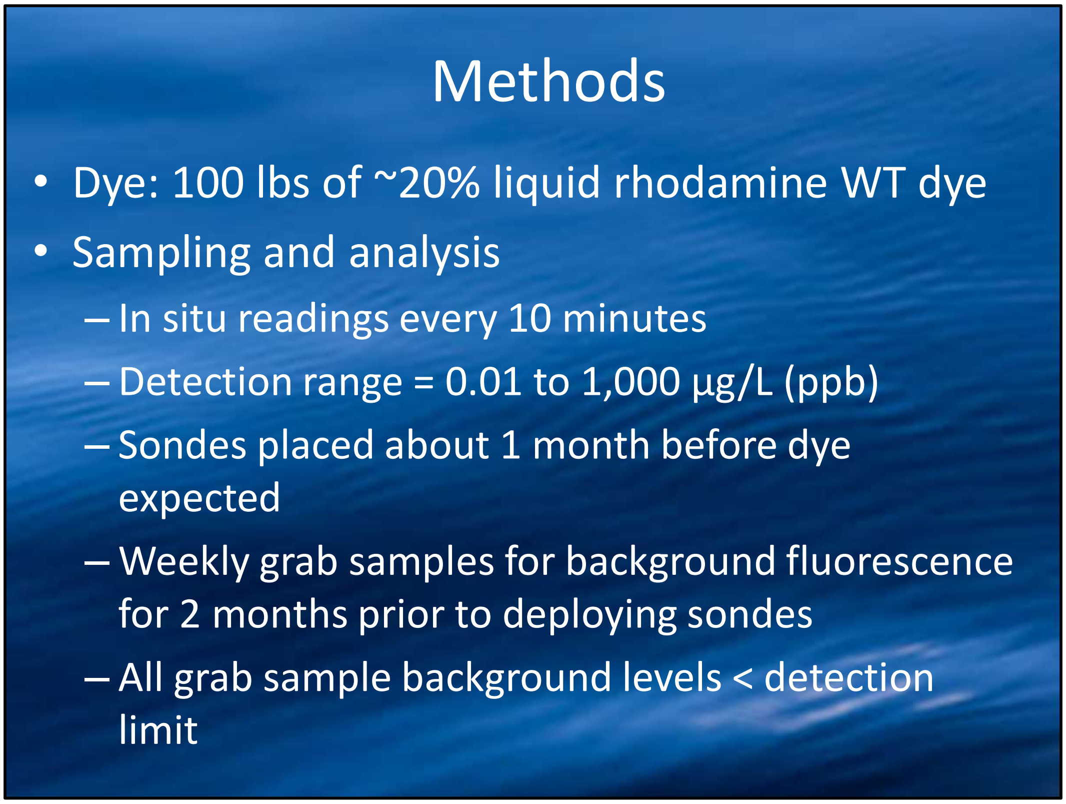


- Two at Upper Lake Lafayette, injecting dye at the Fallschase Sink

Iamonia, 7/16/18



- And one during Phase III at Lake Iamonia



- **[click]** MLI chose a dye, rhodamine WT, that is never used by the Florida Geological Survey (FGS) and has only been used by MLI in the Wakulla Springshed, most recently prior to this project in 2004.
    - For each dye study MLI injected 100 lbs of approximately 20% liquid rhodamine WT dye
- Sampling and analysis:
    - **[click]** In situ sample readings were taken every 10 minutes with water quality sondes equipped with submersible rhodamine fluorimeter sensors
        - **[click]** with sensitivities as low as 0.01 µg/L (ppb) and capable of detecting concentrations as high as 1,000 µg/L (ppb).
    - The fluorimeters in the sondes were swapped out at weekly intervals with fully charged and calibrated devices and their data downloaded to a laptop computer.
    - **[click]** Sondes were placed in the field about a month before the expected arrival of the dye
    - **[click]** Grab samples were collected weekly for another two months prior to deploying the sondes in the field and analyzed for background fluorescence with the rhodamine fluorimeter.

Dye Study Details

	Lake Jackson	Upper Lake Lafayette 1	Upper Lake Lafayette 2	Lake Iamonia
Dye injection sink	Porter Hole	Fallschase	Fallschase	Iamonia
Date of dye injection	9/19/17	1/19/17	4/9/18	7/16/18
Wakulla Spring				
Date in				
Initial detection date				
Transit time				
Maximum concentration (ppb)				
Second pulse detection date				
Transit time				
Maximum concentration (ppb)				

Dye Study Details

	Lake Jackson	Upper Lake Lafayette 1	Upper Lake Lafayette 2	Lake Iamonia
Dye injection sink	Porter Hole	Fallschase	Fallschase	Iamonia
Date of dye injection	9/19/17	1/19/17	4/9/18	7/16/18
Wakulla Spring				
Date in	9/21/17			
Initial detection date				
Transit time				
Maximum concentration (ppb)				
Second pulse detection date				
Transit time				
Maximum concentration (ppb)				

Dye Study Details

	Lake Jackson	Upper Lake Lafayette 1	Upper Lake Lafayette 2	Lake Iamonia
Dye injection sink	Porter Hole	Fallschase	Fallschase	Iamonia
Date of dye injection	9/19/17	1/19/17	4/9/18	7/16/18
Wakulla Spring				
Date in	9/21/17			
Initial detection date	10/24/17			
Transit time				
Maximum concentration (ppb)				
Second pulse detection date				
Transit time				
Maximum concentration (ppb)				

Dye Study Details

	Lake Jackson	Upper Lake Lafayette 1	Upper Lake Lafayette 2	Lake Iamonia
Dye injection sink	Porter Hole	Fallschase	Fallschase	Iamonia
Date of dye injection	9/19/17	1/19/17	4/9/18	7/16/18
Wakulla Spring				
Date in	9/21/17			
Initial detection date	10/24/17			
Transit time	35 days			
Maximum concentration (ppb)				
Second pulse detection date				
Transit time				
Maximum concentration (ppb)				

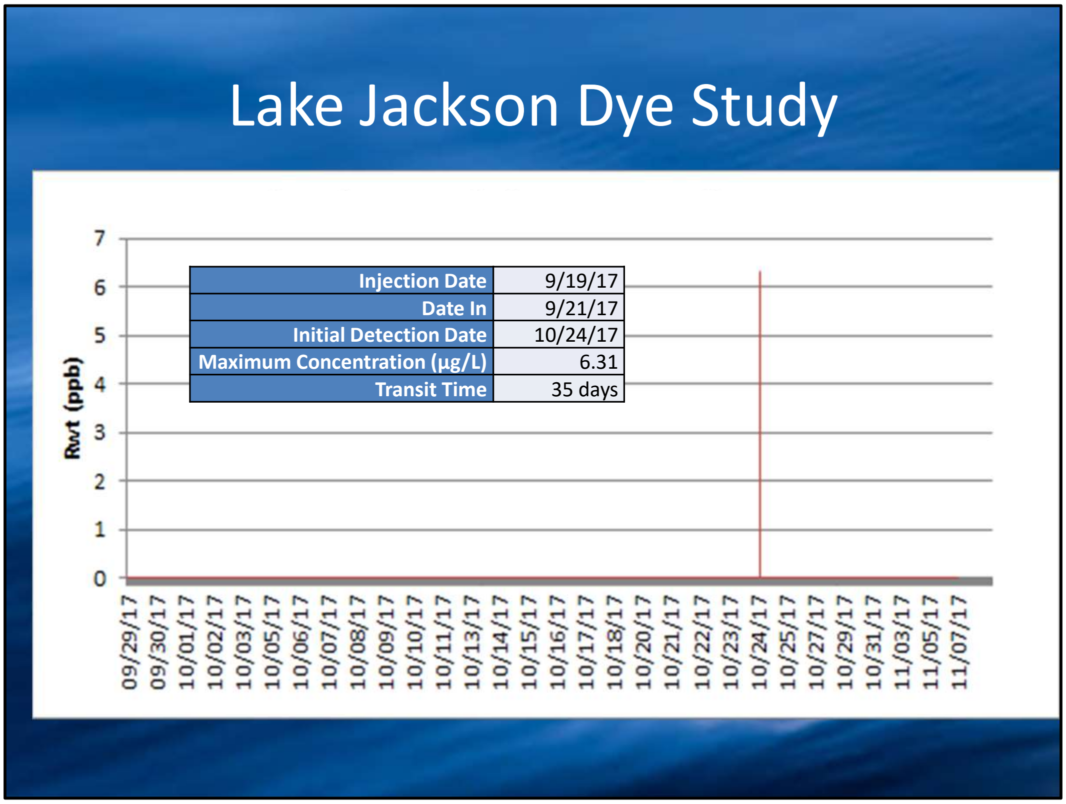
Dye Study Details

	Lake Jackson	Upper Lake Lafayette 1	Upper Lake Lafayette 2	Lake Iamonia
Dye injection sink	Porter Hole	Fallschase	Fallschase	Iamonia
Date of dye injection	9/19/17	1/19/17	4/9/18	7/16/18
Wakulla Spring				
Date in	9/21/17			
Initial detection date	10/24/17			
Transit time	35 days			
Maximum concentration (ppb)	6.31			
Second pulse detection date				
Transit time				
Maximum concentration (ppb)				

Dye Study Details

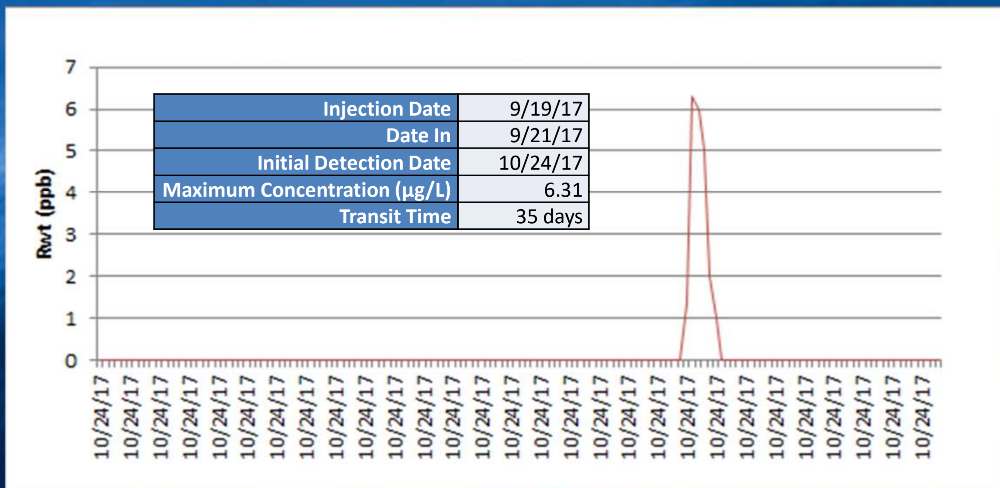
	Lake Jackson	Upper Lake Lafayette 1	Upper Lake Lafayette 2	Lake Iamonia
Dye injection sink	Porter Hole	Fallschase	Fallschase	Iamonia
Date of dye injection	9/19/17	1/19/17	4/9/18	7/16/18
Wakulla Spring				
Date in	9/21/17			
Initial detection date	10/24/17			
Transit time	35 days			
Maximum concentration (ppb)	6.31			
Second pulse detection date	n/a			
Transit time				
Maximum concentration (ppb)				

- This table presents the details:
  - **[click]** Injection location and date
  - **[click]** Date the sonde fluorimeter meter was deployed at Wakulla Spring
  - **[click]** Initial detection date
  - **[click]** Transit time
  - **[click]** Maximum concentration of the rhodamine dye detected at the spring
  - **[click]** And if applicable the detection date, transit time, and maximum concentration of a second dye pulse



- The Lake Jackson study generated a single pulse after 35 days spread over about 60 minutes **[click]**

Lake Jackson Dye Study

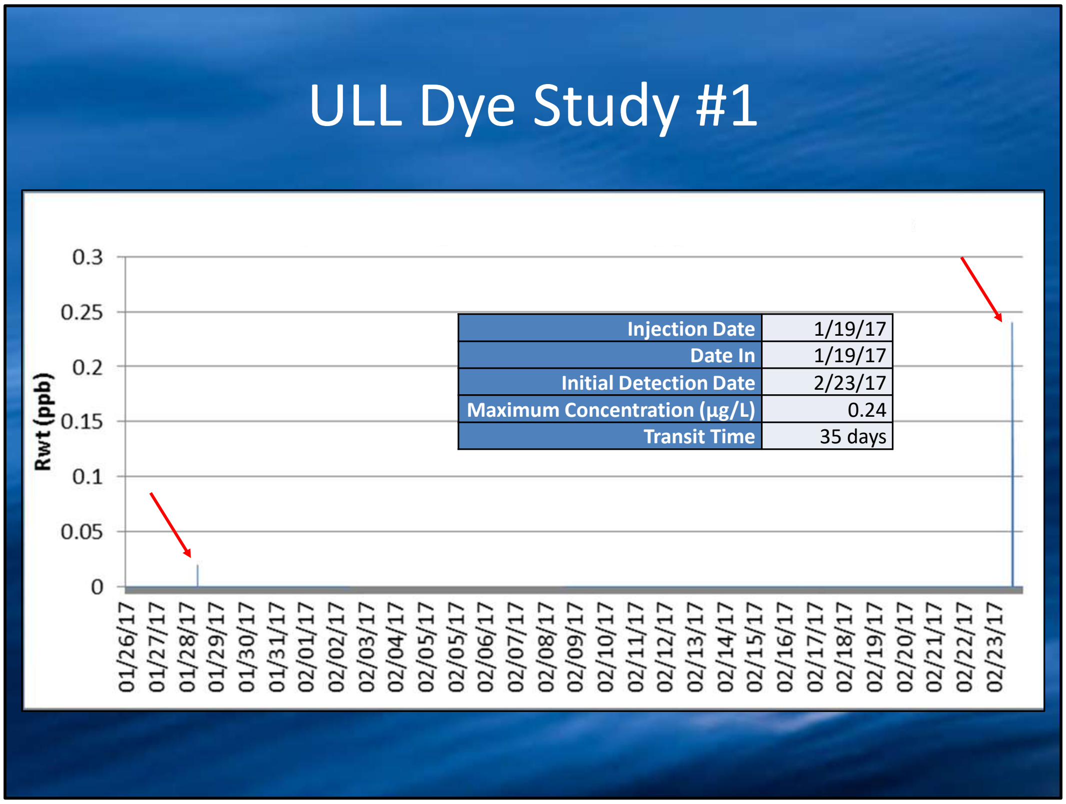


Injection Date	9/19/17
Date In	9/21/17
Initial Detection Date	10/24/17
Maximum Concentration (µg/L)	6.31
Transit Time	35 days

Dye Study Details

	Lake Jackson	Upper Lake Lafayette 1	Upper Lake Lafayette 2	Lake Iamonia
Dye injection sink	Porter Hole	Fallschase		
Date of dye injection	9/19/17	1/19/17		
Wakulla Spring				
Date in	9/21/17	1/19/17		
Initial detection date	10/24/17	2/23/17		
Transit time	35 days	35 days		
Maximum concentration (ppb)	6.31	0.24		
Second pulse detection date	n/a	n/a		
Transit time				
Maximum concentration (ppb)				

- The first Upper Lake Lafayette study was conducted on January 19, 2017
- A single pulse was detected 35 days later
- The dye concentration was quite low, 0.24 ppb

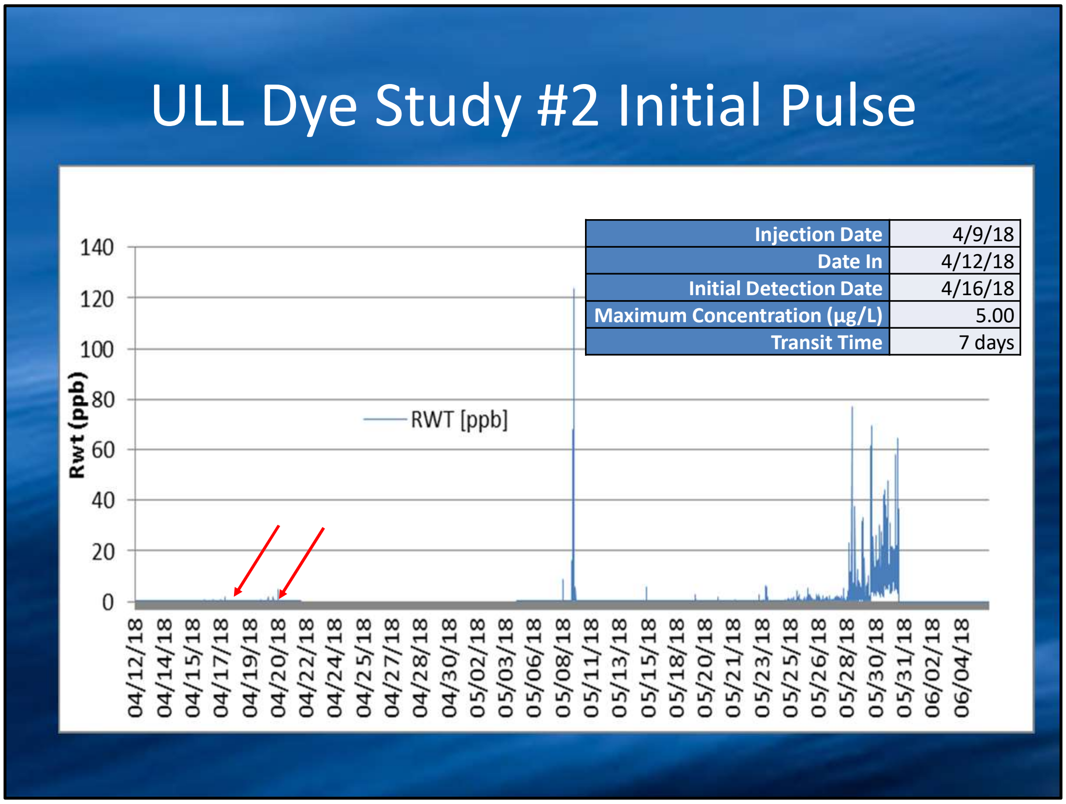


- **[click]** An earlier pulse on 1/28/17 was barely above detection limit of 0.01 ug/L

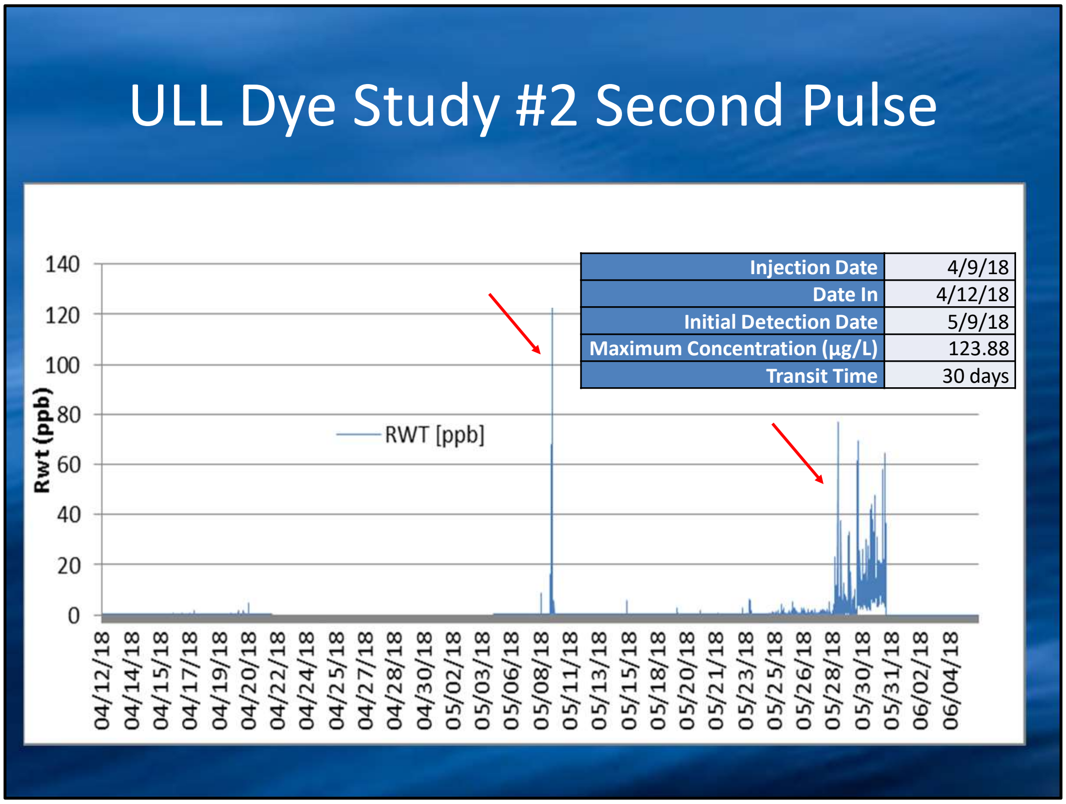
Dye Study Details

	Lake Jackson	Upper Lake Lafayette 1	Upper Lake Lafayette 2	Lake Iamonia
Dye injection sink	Porter Hole	Fallschase	Fallschase	
Date of dye injection	9/19/17	1/19/17	4/9/18	
Wakulla Spring				
Date in	9/21/17	1/19/17	4/12/18	
Initial detection date	10/24/17	2/23/17	4/16/18	
Transit time	35 days	35 days	7 days	
Maximum concentration (ppb)	6.31	0.24	5.00	
Second pulse detection date	n/a	n/a	5/9/18	
Transit time			30 days	
Maximum concentration (ppb)			123.88	

- In part because of the low concentration detected in the first ULL dye study, MLI conducted a second one on April 9, 2018
- That study was complicated by a peculiar situation



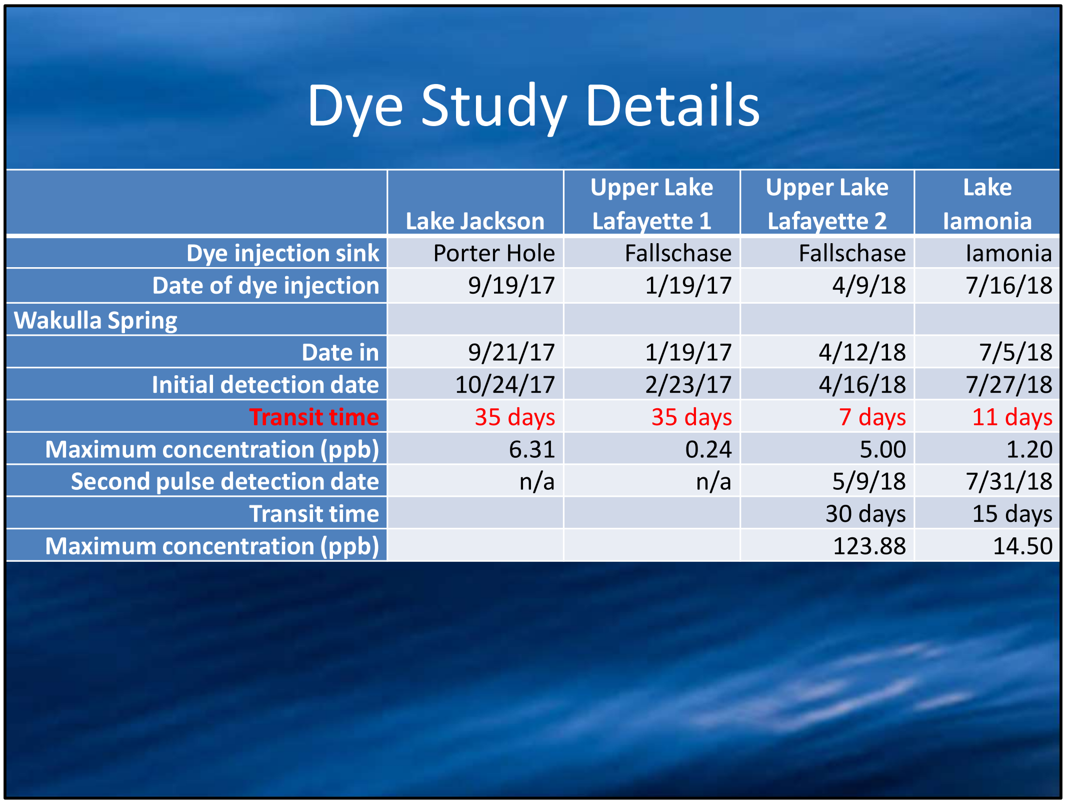
- In the second ULL dye study, the Wakulla sonde fluorimeter recorded multiple dye peaks:
  - an initial pulse (0.59 µg/L) appeared on 4/16/18 **[click]**, only 7 days after injection
  - and peaked at about 5.0 µg/L after 11 days on 4/20/18 **[click]**
  - **CLICK for next slide**



- **[CLICK]** A second larger pulse that peaked at 123.88 µg/L followed on 5/9/18 after 30 days
- **[CLICK]** followed by a cluster of intermediate level spikes on 5/28/18-5/31/18.

- The rapid transit of the first pulse was likely the result of an unusual situation.
- On April 11, 2018, two days after dye injection at Fallschase Sink, a sinkhole opened in nearby Buck Lake and drained about 48 acre-feet of water into the aquifer.
- **CLICK for next slide**

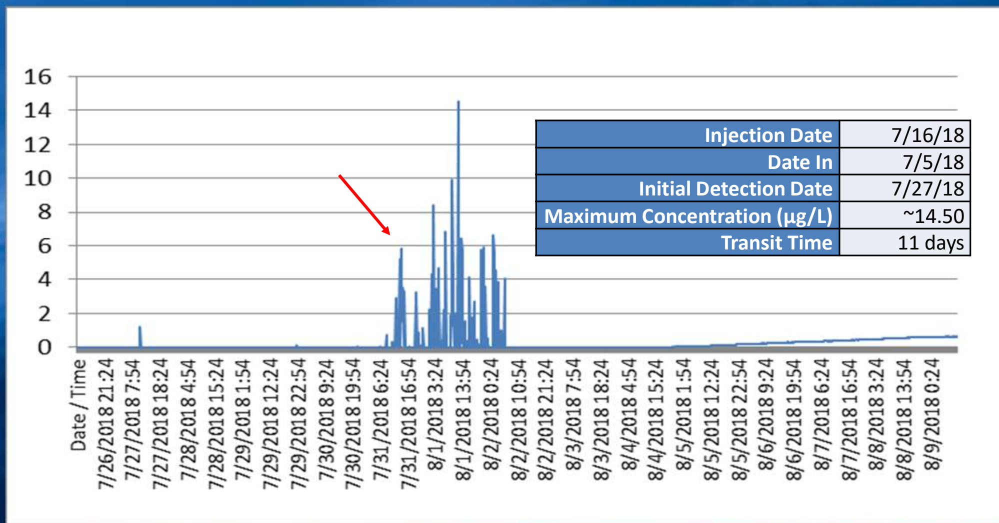
- Dye that had been injected into the aquifer was pushed back into ULL the next day raising its water level about three feet.
- The lake subsequently cleared 12 days later (4/23/18).
- Dr. McGlynn hypothesized that the Buck Lake discharge may have quickly pushed a small amount of the dye south toward Wakulla Spring at the same time that it pushed most of the dye back into ULL



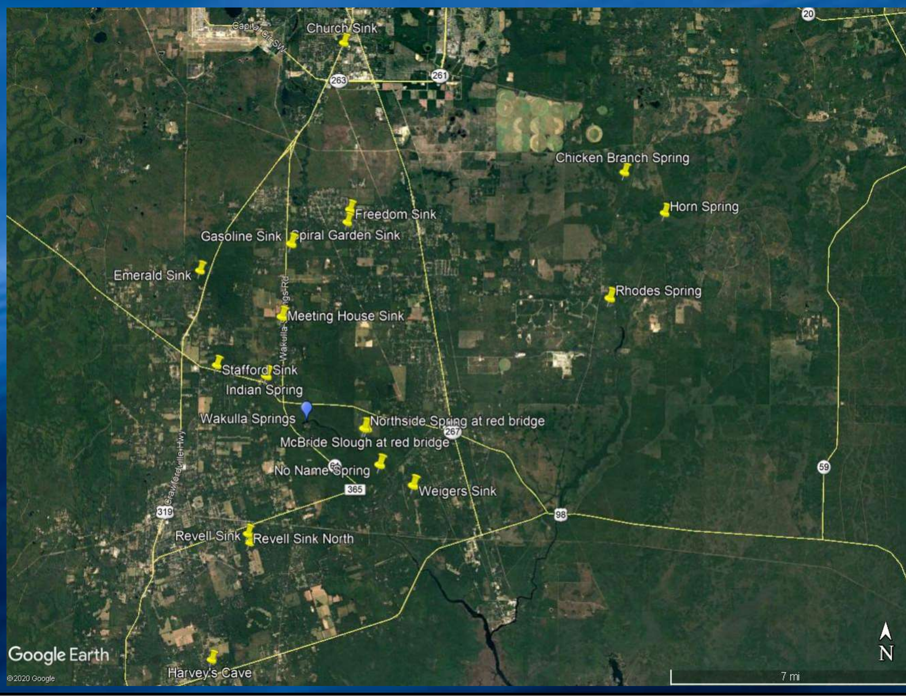
	Lake Jackson	Upper Lake Lafayette 1	Upper Lake Lafayette 2	Lake Iamonia
Dye injection sink	Porter Hole	Fallschase	Fallschase	Iamonia
Date of dye injection	9/19/17	1/19/17	4/9/18	7/16/18
Wakulla Spring				
Date in	9/21/17	1/19/17	4/12/18	7/5/18
Initial detection date	10/24/17	2/23/17	4/16/18	7/27/18
Transit time	35 days	35 days	7 days	11 days
Maximum concentration (ppb)	6.31	0.24	5.00	1.20
Second pulse detection date	n/a	n/a	5/9/18	7/31/18
Transit time			30 days	15 days
Maximum concentration (ppb)			123.88	14.50

- MLI initiated the Lake Iamonia dye study on 7/16/18
- Dye was initially detected at Wakulla Spring only 11 days later suggesting there may be a substantial conduit that moves Iamonia discharge quickly south
- Major spike arrived 4 days later, on July 31 **[click to next slide]**

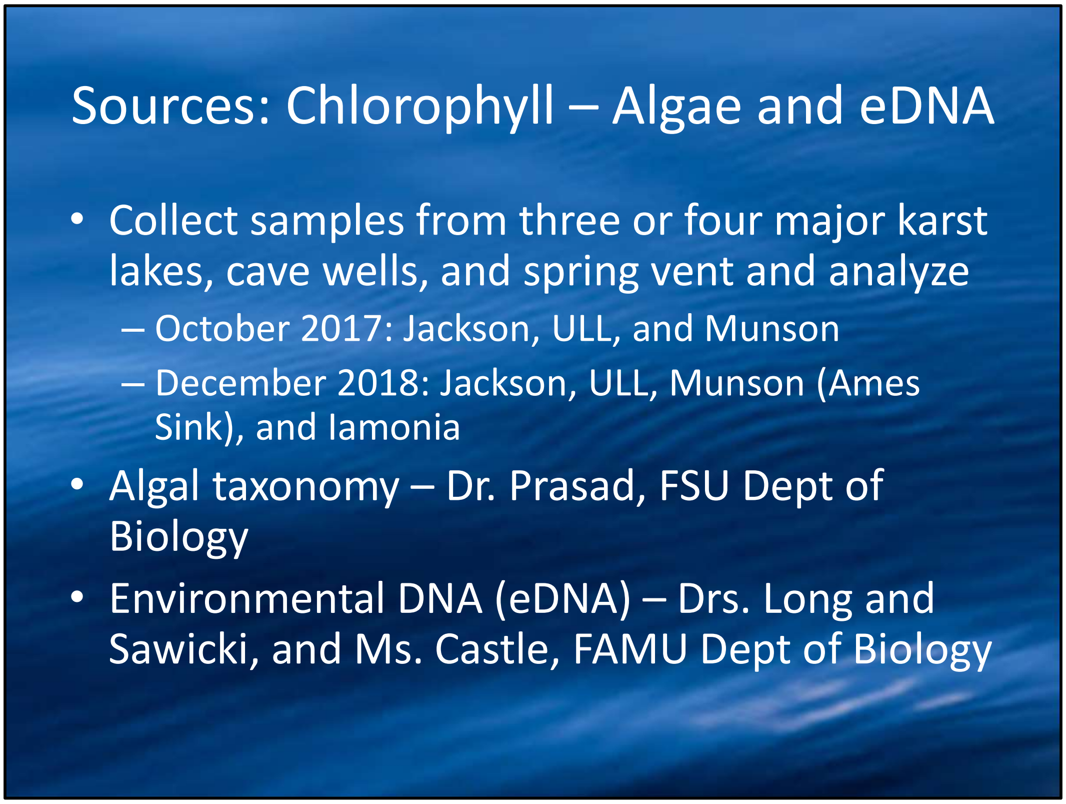
Lake Iamonia Dye Study



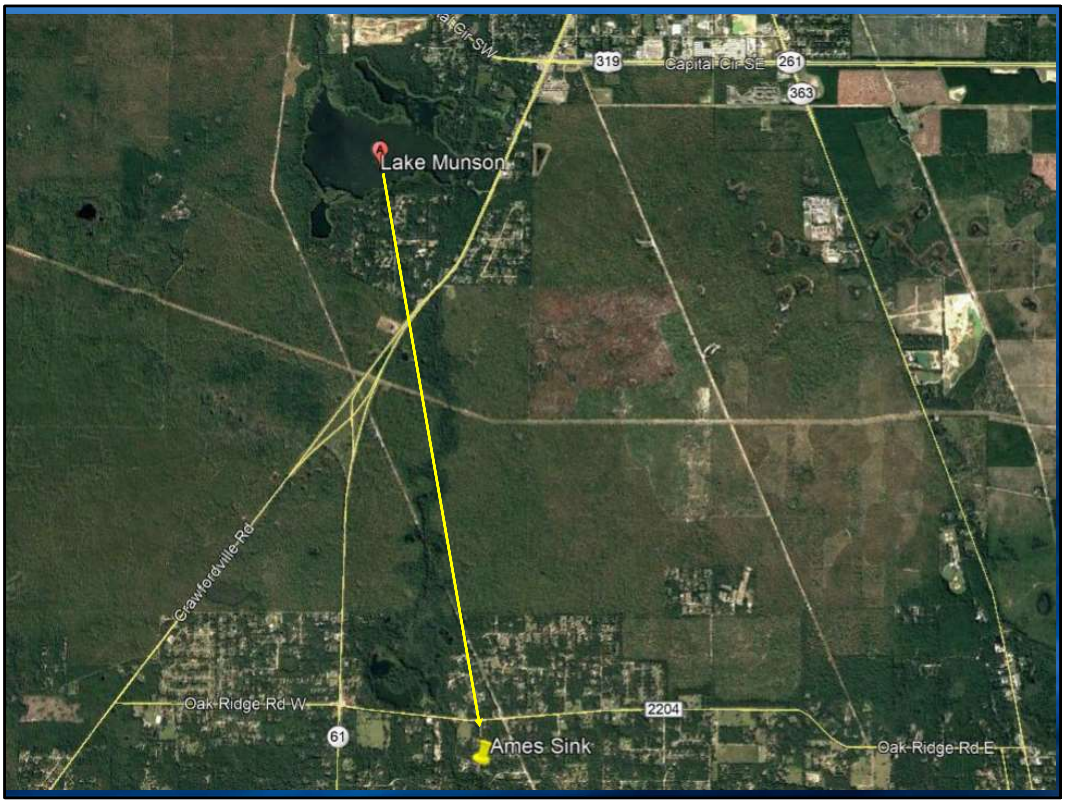
Charcoal Pack Sample Sites



- **Add description; explain my method for calculating pre/post ratio**
- **Use zoomed-in copies and circles to identify sites with non-zero net increases for each study**
- **Use table 4.2 for reference – don't need to show here**



- We also undertook two approaches to look for tracers that might help identify the sources of the chlorophyll
- **[click]** One-liter samples were taken from the karst lakes for algae identification and environmental DNA analysis
    - **[click]** October 2017: Jackson, ULL, and Munson
    - **[click]** December 2018: Jackson, ULL, Munson (Ames Sink), and Iamonia
- One-liter samples also collected from L well and spring boil as well as the K, D, C, and B cave wells for eDNA analysis
- The algae sample was only taken from L well because a grab sample from spring boil would be too dilute
    - In 2017 the L well sample of 160 gallons was pumped through a 64-micron mesh plankton net to produce a sufficiently concentrated sample, then filtered in the lab
    - In 2018 the L well sample was to be pumped through a finer 25-micro plankton net to try to capture smaller algae;
        - that didn’t happen, and the resulting sample yielded no algae
    - Hence, I am only presenting 2017 results
- 2018 sample timing was keyed to findings from dye studies to enhance likelihood that algae/eDNA collected from lakes was representative of what reached the spring by the time it was sampled
- **[click]** Dr. Akshinthala Prasad of the Florida State University Department of Biology performed algal taxonomic analysis
- **[click]**

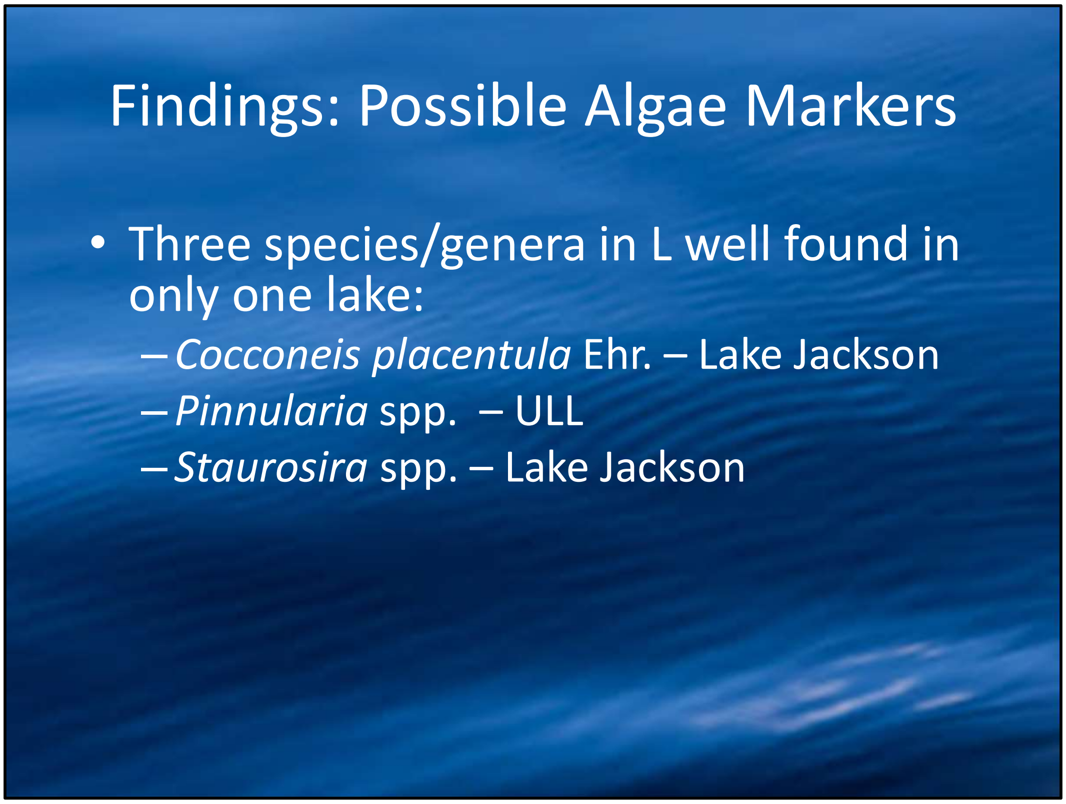


- In 2018, MLI moved the Lake Munson sample site to Ames Sink **[click]** where the outflow from the lake enters the aquifer.
    - As a result, the eDNA from that sample reflects algae not only in Lake Munson but also in Munson Slough between the lake and the sink and a smaller waterbody, Sellar's Pond, that is situated in between

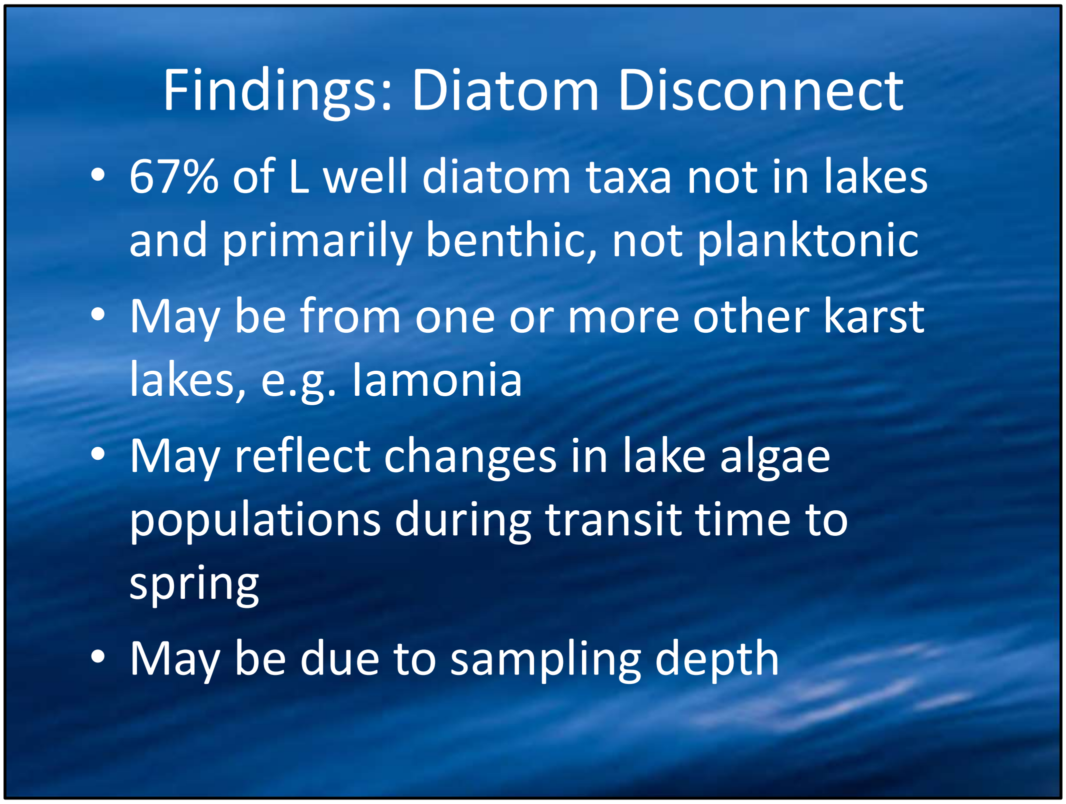
Findings: Dominant Algae Classes

- Lake Jackson:
  - golden brown algae (38%)
  - green algae (19%)
  - diatoms (19%)
  - cryptophytes (13%)
- Upper Lake Lafayette:
  - green algae (73%)
  - diatoms (11%)
  - raphidophytes (8%)
- Lake Munson:
  - diatoms (71%),
  - cryptophytes (12%),
  - green algae (7%)
- L well:
  - diatoms (100%)

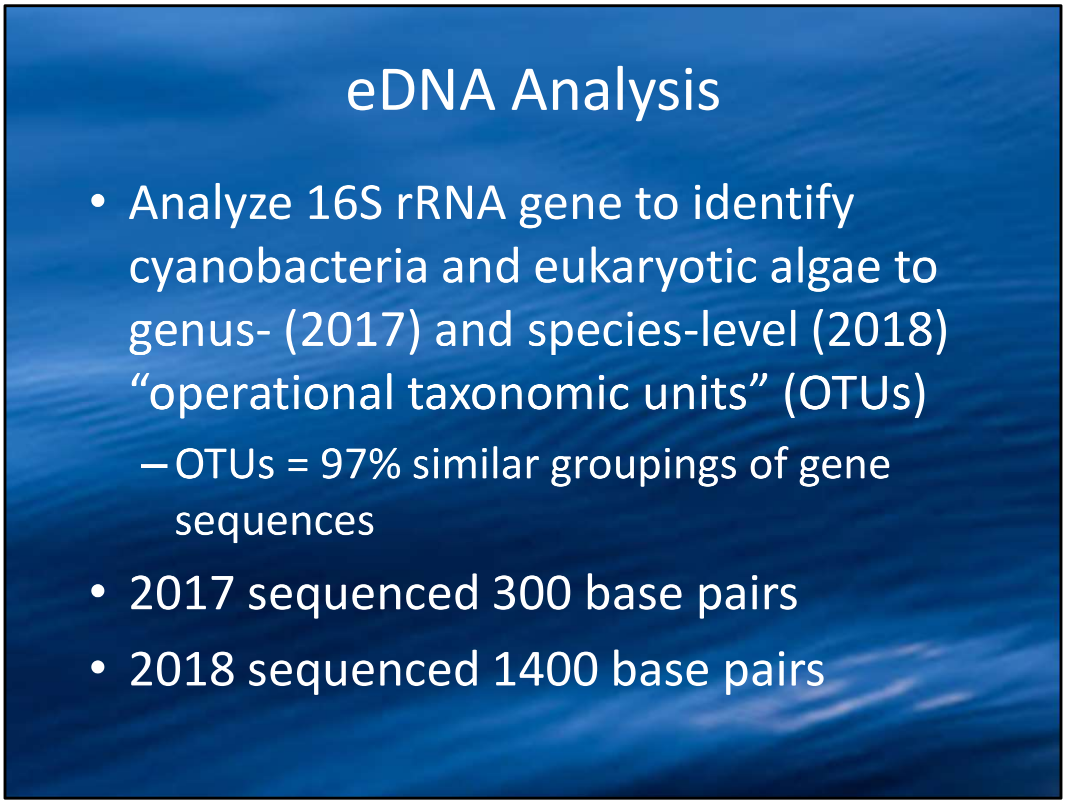
- The mix of dominant algae classes in 2017 was quite different among the three lakes
- 100% diatom sample from L well probably reflects
  - Relatively large plankton net mesh size of 64 microns; Dr. Prasad recommended using 10-25 micron the next time
  - More robust cells – silicaceous shells less likely to deteriorate during passage through cavern system even if cells die



- Finding species/genera unique to two of the lakes suggests that both Lake Jackson and ULL are contributing to the chlorophyll present in the groundwater in the L well
- With no algal species or genus unique to Lake Munson we can’t draw any even tentative conclusions about its contributions

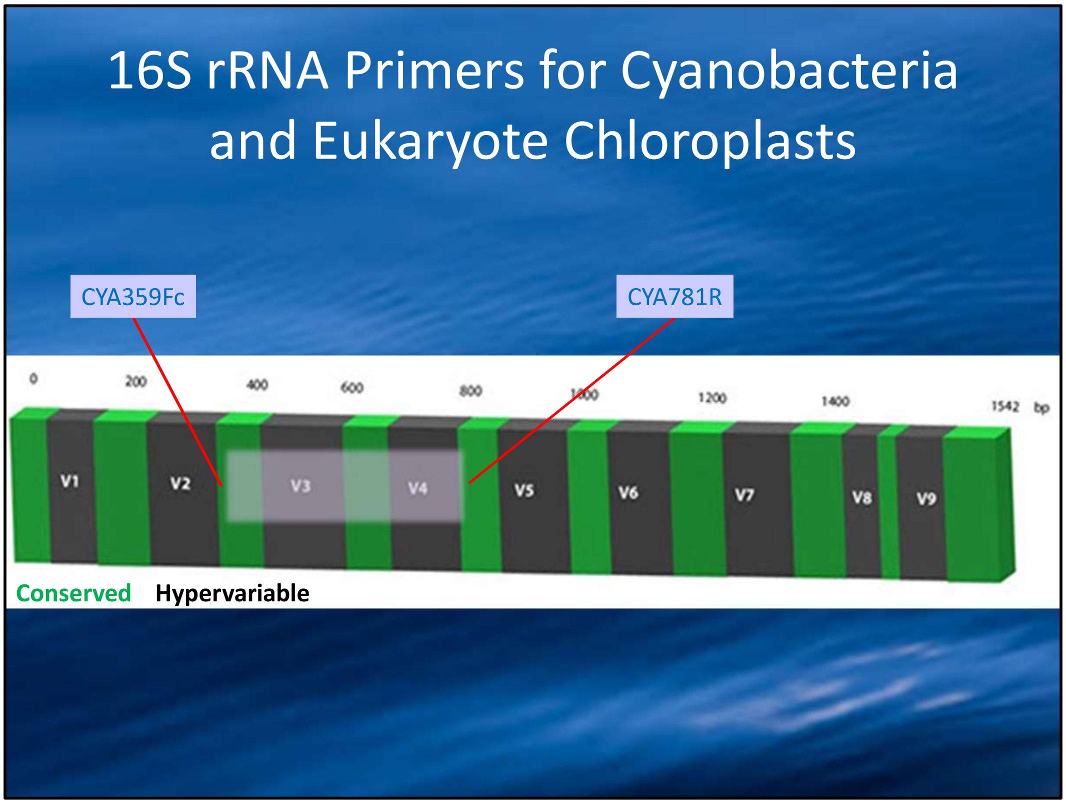


- There was, however, another confounding finding
  - **[click]**
  - Dr. Prasad reported that the L well diatom taxa were primarily benthic – species found on sediment and rock substrates on the bottom of a lake
    - while the [m]ajority of algal taxa in the lake samples were strictly planktonic – species that live suspended in the water
  - These discrepancies may reflect one or more factors:
    - **[click]** The other diatom taxa at the spring may have originated from other karst water bodies in the springshed such as Lake Iamonia, the Killearn Chain of Lakes, the Bradford Brook Chain of Lakes
    - **[click]** Changes in lake algae populations that occurred in the 30-some days during which the lake discharges travelled to Wakulla Spring
    - **[click]** Or the fact that we collected our samples from the surface waters of the lakes while in both Lake Jackson and Upper Lake Lafayette, the principal discharge to ground water is through sinkholes in the bottoms of the lakes.
      - The grab samples would likely have had a higher proportion of planktonic species while the water discharged through the sinkholes may have had higher proportions of benthic species.



- The environmental DNA studies analyzed sequences of the 16S rRNA (ribosome ribonucleic acid) gene to identify cyanobacteria and eukaryotic algae (have a nucleus) operational taxonomic units (OTUs)
  - OTUs are groupings of gene sequences that are 97% similar
  - They are not identical to taxonomic identification to species or genus
- DNA sequencing was performed for 300 base pairs in 2017 allowing for specification of genus-level OTUs
- For 2018 we paid for analysis of a longer DNA sequence of 1400 base pairs to attempt to identify species-level OTUs
  - Ideally to achieve greater differentiation among the lakes

NOTE: A base pair is two chemical bases bonded to one another forming a "rung of the DNA ladder.“ (https://www.genome.gov/genetics-glossary/Base-Pair)



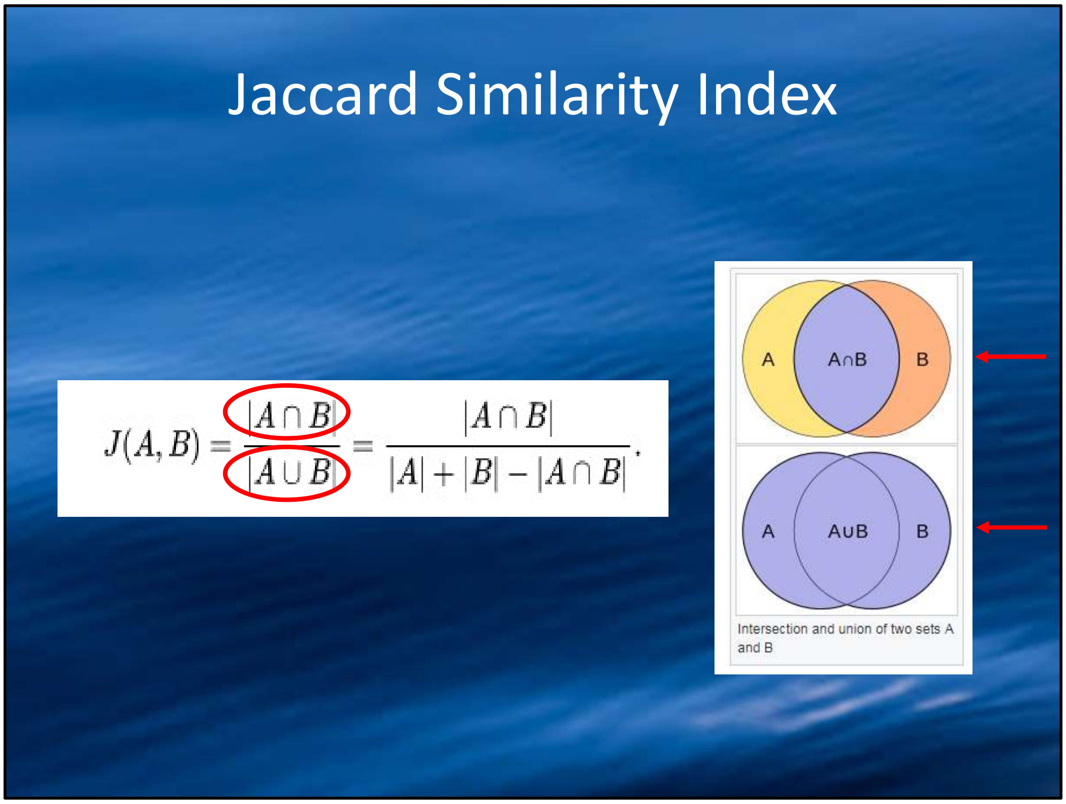
- 16sRNA gene is present in all organisms
  - It is the primer* organisms use to produce ribosomal RNA which is essential to cell function.
  - Deleterious mutations lead to cell death.
    - Therefore "conserved" regions of the genomic sequence are very stable and can be targeted in all species
    - While "variable regions" allow for differentiation among genera and species
- There are nine hypervariable (black) regions found in the 16s rRNA gene, and each of these regions is flanked by a highly conserved (green) region
  - The two regions of 300 base pairs used for the initial study are shown here
  - They are targeted for identifying cyanobacteria and eukaryote chloroplasts, the organelles that use chlorophyll to accomplish photosynthesis
- I'm going to focus on the findings from the 2018 eDNA study because I think those are likely to be more accurate
  - First because we keyed the sample timing to what we learned form the dye studies about travel time from the individual lakes to Wakulla Spring
  - Second because analysis of the longer gene sequences should provide greater precision in identifying species-level OTUs

* A primer is a short strand of RNA or DNA (generally about 18-22 bases) that serves as a starting point for DNA synthesis. It is required for DNA replication because the enzymes that catalyze this process, DNA polymerases, can only add new nucleotides to an existing strand of DNA." (https://en.wikipedia.org/wiki/Primer_(molecular_biology))

Findings: eDNA Cyanobacteria

- Cyanobacteria far more prevalent in lakes than in L well and spring
- Cyanobacteria of three lakes more similar to each other than to those at spring
- Four most prevalent cyanobacteria genera at spring boil are each present in two or more lakes
  - *Leptolyngbya*
  - *Cyanobacterium*
  - *Synechococcus*
  - *Prochlorococcus*

- Differences between cyanobacteria population of spring and those of the lakes likely due to high susceptibility of bacterial rDNA to degradation during transit through cave system



- Dr Long and his colleagues calculated Jaccard similarity indices for each of the lakes, each of the cave wells, and the spring boil
    - The Jaccard index is the ratio of the overlap between samples A and B **[click]** to the sum of the two samples **[click]**

Species-Level Eukaryote Algae OTUs: Lakes and Spring

	ULL	Lake Jackson	Lake Munson/ Ames Sink	Lake Iamonia	Spring Boil
ULL	1	0.583	0.714	0.593	0.533
Lake Jackson	0.583	1	0.619	0.640	0.517
Lake Munson	0.714	0.619	1	0.625	0.500
Lake Iamonia	0.593	0.640	0.625	1	0.441
Spring Boil	0.533	0.517	0.500	0.441	1

- There is substantial similarity among the four lakes and the spring boil based on Jaccard index for species-level eukaryote algal OTUs **[click]**
  - ULL - 0.533
  - Lake Jackson - 0.517
  - Lake Munson - 0.500
  - Lake Iamonia – 0.441
- These suggest that each is contributing to the chlorophyll load at the spring
  - Lake Iamonia less so than the other three

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- Note also that the lakes are more similar to each other than to the spring boil
- ULL is most similar to Lake Munson/Ames Sink and vice versa **[click]**
- Lake Jackson is most similar to Lake Iamonia and vice versa **[click]**

Species-Level Eukaryote Algae OTUs: Lakes and Cave Wells

	K Well	D Well	C Well	B Well
ULL	0.474	0.486	0.567	0.475
Lake Jackson	0.385	0.351	0.500	0.425
Lake Munson/Ames Sink	0.444	0.500	0.483	0.375
Lake Iamonia	0.439	0.375	0.429	0.476

- Jaccard similarity coefficients also may offer some clues as to the routes by which chlorophyll arrives at the spring from the individual lakes
- ULL and Lake Jackson are most similar to the C well **[click]**
- Lake Munson is most similar to the K well **[click]**
- Lake Iamonia is most similar to the B well **[click]**

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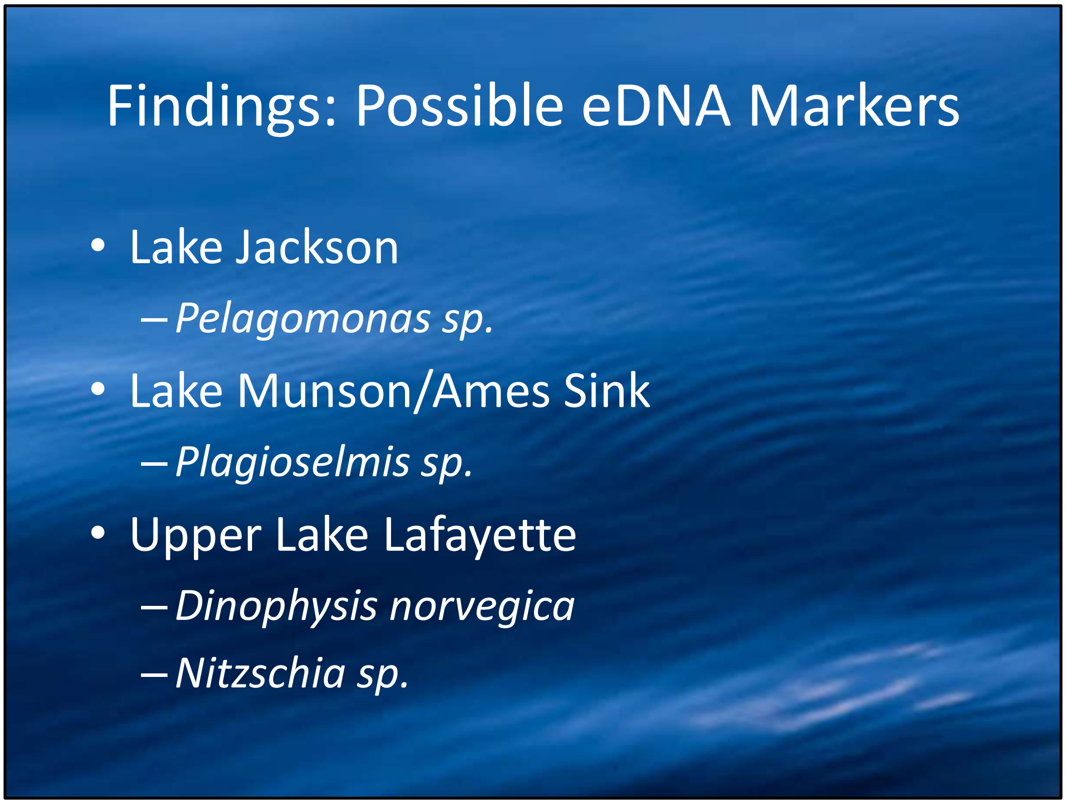
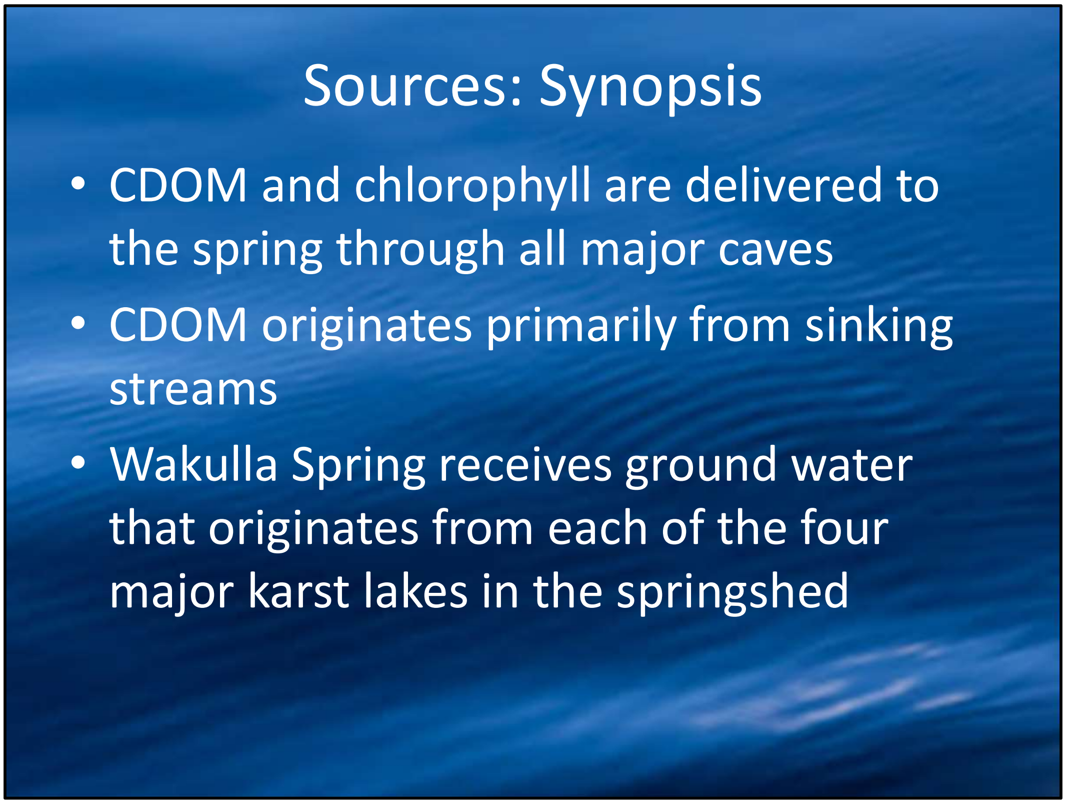
Heat Maps

Species	L Well	Spring Boil	Lake Jackson	Lake Munson/ Ames Sink	Upper Lake Lafayette	Lake Iamonia
Ankylochrysis lutea	0	3.5	2.8	0	0	28.3
Antithamnionella spirographidis	259.1	244.1	7.7	0.6	4.7	3.7
Asterionella ralfsii	68.2	89.2	144.4	54.9	286.3	2528.3
Chrysochromulina sp.	6.3	0	0.7	0.1	0.1	79.5
Chrysosphaera sp.	114.5	102.4	1389.7	406	297.4	507.9
Corethron pennatum	2.3	3.4	0.5	0	4.6	0.7
Coscinodiscus radiatus	105.1	77.9	119	27.3	16.1	41.4
Cryptoglena pigra	2	0	4.7	0.4	23.3	1.2
Cryptomonas pyrenoidifera	183.3	200.2	3433.7	961.3	2861.4	2187.5
Cyanoptyche gloeocystis	29.9	33.6	7.6	0	0	0.4

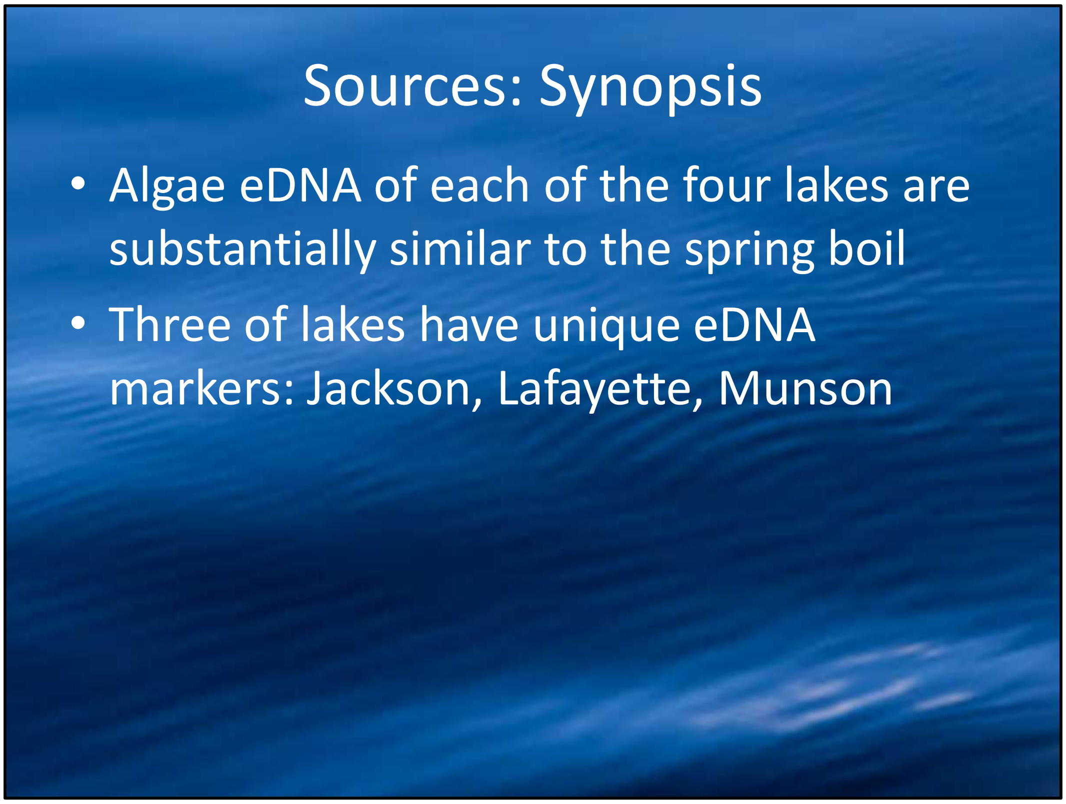
- Dr. Long and his colleagues also constructed “heat maps” that display the prevalence of each OTU per 10,000 OTUs at each site
- This is a sample of the first few rows of the 2018 heat map for eukaryote algae
- Prevalence increases from less than 10 per 10,000 OTUs (grey) to pale green and then through various shades of orange



- We can treat OTUs found at prevalances greater than 10 per 10,000 at the spring boil and in single lakes as markers that suggest those lakes are contributors to the chlorophyll load at Wakulla Spring
- **[click]** Species-level heat maps constructed in 2018 for eukaryote algae revealed that three of the lakes included one or more eukaryote algae species-level OTUs defined for the spring boil sample that were not found in any of the other lakes.
    - Lake Jackson – 1 genus
    - Munson/Ames Sink – 1 genus
    - Upper Lake Lafayette – 1 species and 1 genus
- Note, however, that three of these were not differentiated below genus level
- None unique to Lake Iamonia were found at the spring boil.



- I’d like to wrap this up with a summary of what I think we now know about the sources of CDOM and chlorophyll responsible for reduced visibility at Wakulla Spring
- **[click]** CDOM and chlorophyll are delivered to the spring through all major caves
- **[click]** CDOM originates primarily from sinking streams
- **[click]** Wakulla Spring receives ground water that originates from each of the four major karst lakes in the springshed



- **[click]** Algae eDNA of each of the four lakes are substantially similar to the spring boil
- **[click]** Three of lakes have unique eDNA markers: Jackson, Lafayette, Munson
- I plan to wrap this up next month with an exploration of possible causal explanations for why the frequency and duration of dark water conditions have increased at Wakulla Spring