Wakulla Spring Dark Water: Causes and Sources
Upper Lake Lafayette and Lake Jackson Dye Studies

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Wakulla Springs Alliance
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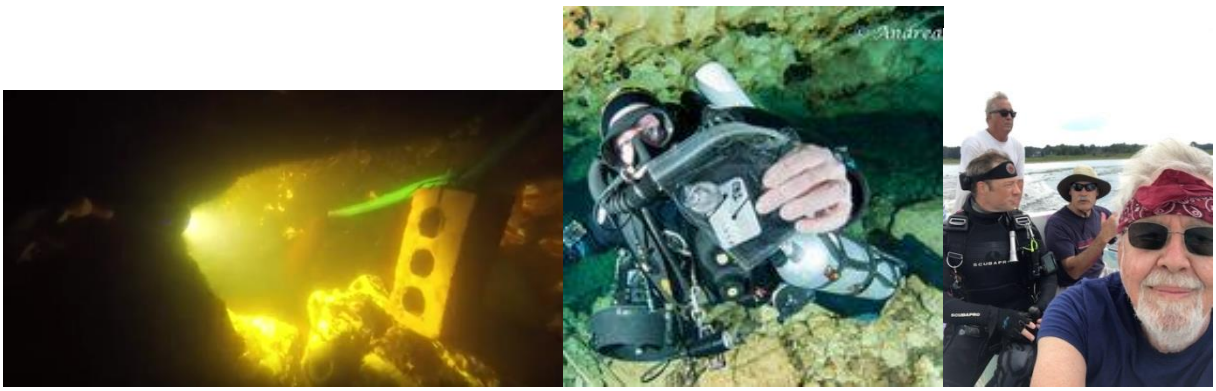
This project was conducted for the Wakulla Springs Alliance by McGlynn Laboratories, Inc. (MLI) with financial assistance provided by the Fish and Wildlife Foundation of Florida, Inc. through the Protect Florida Springs Tag Grant Program, project PFS #1617-08.

Cal Jamison assisted with injecting dye into Porter Hole Sink at Lake Jackson and oversaw designing and implementing the charcoal pack monitoring initiative. Diver Andreas Hegberg assisted with injecting dye into Porter Hole Sink and with placing and retrieving charcoal packs at several sites. Divers Michael Barnette, Travis Kersting, and Brandon McWilliams assisted with deploying and retrieving other charcoal packs. Sherry Carpenter assisted with the Porter Hole Sink dye injection. Brendan McGlynn assisted with injecting dye into Fallschase Sink at Upper Lake Lafayette. Thanks also to the following property owners for providing access to sinks and springs on or through their properties: Bob Brown, Don Fortner, the Harveys, Norma Skaggs, Mitch and Barbara Spears, Teddy Tollett, and Wakulla Springs State Park.

Upper Lake Lafayette, Fallschase sink, 01/19/17

100 lbs of approximately 20% liquid rhodamine WT dye from Abbey Color Inc. was pumped from the shore with a 300 GPH, 50 psi, 4500 RPM, 12 volt, Pacific Hydrostar utility pump through tubing lowered 30 feet down into the orifice of the sinkhole.

GPS coordinates for Fallschase Sink in Upper Lake Lafayette: latitude: 30.455352 and longitude: -84.202655.


Lake Jackson, Porter Hole sink, 09/19/17

100 lbs of approximately 20% of liquid rhodamine WT dye from Abbey Color Inc. was pumped from a 17 foot Boston Whaler with a 300 GPH, 50 psi 4500 RPM, 12 volt, Pacific Hydrostar utility pump. A professional diver, Andreas Hagberg, swam the tubing down about 22 feet to Porter Hole Sink in Lake Jackson. A video of this is on YouTube: https://www.youtube.com/watch?v=JmNWs_I9-oU&feature=youtu.be.

GPS coordinates for Porter Hole Sink in Lake Jackson: latitude: 30.524662 and longitude: -84.322059.

Type of Dye Used

All of the fluorescent dyes used in the Wakulla Springs drainage basin are very different and distinctive. They are distinguished from one another by their signature emission and excitation wavelength. The fluorescent properties allow for detection at parts per billion (ppb) concentrations. We 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 in 2004. The other dye studies run by the FGS used Fluorescein, which has different excitation and emission spectra.

Flourescent Dye	Excitation (nm)	Emission (nm)	Color
Rhodamine WT	530	555	Orange-Red
Fluorescein (FITC)	490	525	Green

Sampling and Analysis

In situ sample readings were collected within the field with Hydrolab MS5 multiparameter water quality sondes equipped with rhodamine WT submersible fluorimeter sensors on a compact and lightweight multiprobe designed for either profiling or unattended monitoring. The rhodamine WT sensor is a modified Turner Designs Cyclops-7 submersible fluorimeter with sensitivities as low as 0.04 µg/L (ppb) and capable of detecting concentrations as high as 10 µg/L (ppb). It is designed to fit into 2" wells. Dye concentrations were measured continuously every 10 minutes.

The sondes were swapped out at weekly intervals with fully charged calibrated sondes and their data downloaded to a PC.

Sensors were placed in the field about a month before the expected arrival of the dye and discrete grab samples were measured for background fluorescence weekly for another two months prior to deploying the sensors in the field. All of our background levels were below detection limit for rhodamine WT. The grab 2 liter water samples were collected according to Florida Department of Environmental Protection (FDEP) protocol and analyzed at our National Environmental Laboratory Accreditation Program (NELAP) certified environmental laboratory. The sides of the vials were labeled with the project name, sample ID, sample date and time with a black permanent felt tip pen. The vials were placed in the dark and refrigerated immediately after collection, and maintained under refrigeration until shipment.

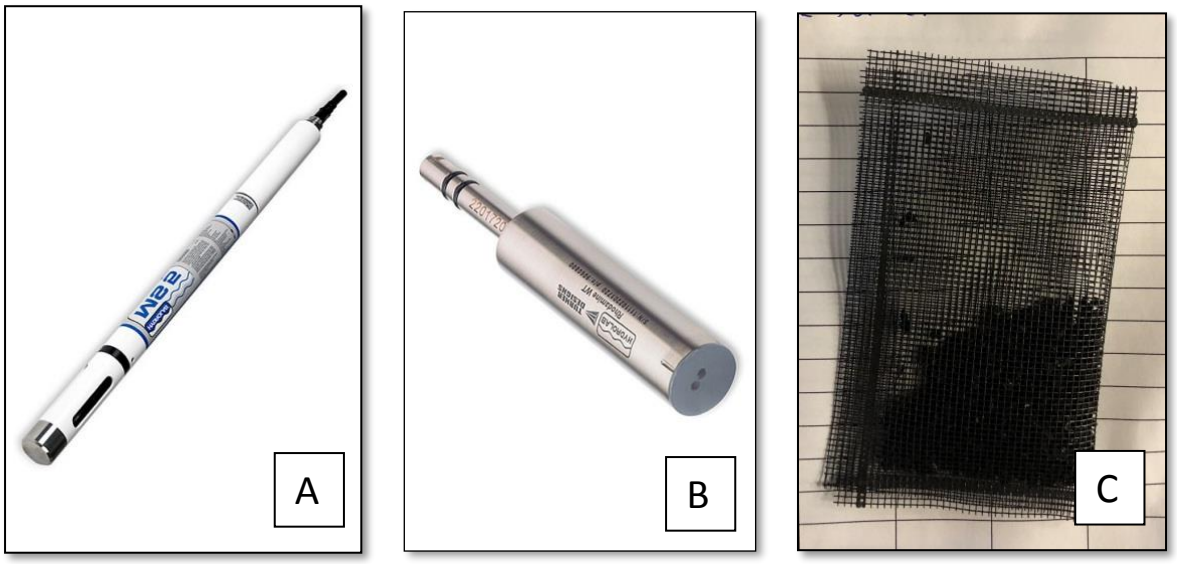
Our Lake Jackson dye trace study also included charcoal samplers (also called activated carbon or charcoal packets) placed at the Wakulla Spring and Sally Ward Spring boils and at 13 other sites. We used packs to check for background levels at 4 of the sites: Emerald Sink, Indian Spring, Harvey's Clear Lake, and Harvey's Aphasta Pit. At the Sally Ward Spring we deployed a second charcoal pack after detecting dye for the first time at the Wakulla Spring boil.

The charcoal samplers are packets of fiberglass screening partially filled with 4.25 grams of activated 20 mesh size coconut charcoal. Our charcoal samplers are about 4 inches long by 2 inches wide. Prewashed charcoal packs were placed in the field to be exposed to as much water as possible. Packs were attached with plastic cable or baling wires in the flow. The frequency of sampler collection and replacement varied during this study. Collections at one week intervals were common, but longer and shorter frequencies occurred and are recorded in Table 1. Collected samplers were rinsed with distilled, demineralized, tracer free reagent water to remove dirt and accumulated organic material. The packs were shaken to remove excess water. Next, the packs were placed in a plastic Ziploc or Whirl-pak bag. Only pens that have black ink were used as colored inks may contain fluorescent dyes. The notations included station name or number and the date and time of collection.

At the laboratory, we used a saturated solution of alcohol, water, aqueous ammonia and sodium hydroxide as our elution solution. This is a mixture of 5% aqua ammonia and 95% isopropyl alcohol solution and saturated with sodium hydroxide pellets. The isopropyl alcohol solution is 70% alcohol and 30% water. The aqua ammonia solution is 29% ammonia. The sodium hydroxide is added until a super-saturated layer is visible in the bottom of the container. This eluting solution will elute fluorescein, eosine, rhodamine WT, and sulforhodamine B dyes..

The charcoal pack was emptied into a 50 ml centrifuge tube. The centrifuge tube was filled with eluting solution. The sample beaker was capped. The sample was allowed to stand for 60 minutes under gentle agitation. Then the liquid was decanted off the charcoal into an appropriately labeled beaker with the laboratory identification number. After decanting, a small amount of the elutant will remain in the initial sample beaker. Samples were kept refrigerated until analyzed with the rhodamine WT submersible fluorimeter from the Hydrolab MS5 multiparameter water quality sonde which functions reproducibly and reliably under different

ambient light conditions. The fluorimeter was emersed in the eluant and read in the same manner as in the field.



A) Hydrolab MS5 multiparameter water quality sonde equipped with
B) Rhodamine WT modified Turner Designs Cyclops-7 submersible fluorimeter.
C) Charcoial pack

Quality Assurance / Quality Control

We run 6 standards made from one commercial standard and a second source standard from another standard source. Standard number 3 is used as a continuing calibration standard every 10 samples. The standard curve must have a coefficient of correlation greater than 99.5 percent. The recoveries of all standards must be above 90%. The concentrations of the standards must also bracket the range of concentrations of the elution samples. Laboratory blanks are run before and after each sample set. Duplicates are run every 10 samples and matrix spike duplicates are run one per sample set and must have a recovery of +/- 90%. All materials used in sampling and analysis work are routinely analyzed for the presence of any compounds that might create fluorescence peaks in or near the acceptable wavelength ranges for any of the tracer dyes.

Criteria used for quantifying rhodamine WT dye in elutants from charcoal samplers:

1. There must be at least one fluorescence peak in the sample in the range of 565.2 to 571.8 nm.
2. The dye concentration associated with the rhodamine WT peak must be at least 3 times the detection limit. The detection limit in elutant samples is 0.170 ppb, thus this dye concentration limit equals 0.510 ppb.
3. The dye concentration must be greater than the lab blank test and at least 10 times greater than any other concentration reflective of background at the sampling station in question.
4. The shape of the fluorescence peak must be typical of rhodamine WT. In addition, there must be no other factors which suggest that the fluorescence peak may not be dye from the groundwater tracing work under investigation.

Criteria used to quantify rhodamine WT dye in water samples:

1. In most cases, the associated charcoal samplers for the station should also contain rhodamine WT dye in accordance with the criteria listed above. These criteria may be waived if no charcoal sampler exists.
2. There must be no factors which suggest that the fluorescence peak may not be rhodamine WT dye from the tracing work under investigation. The fluorescence peak should generally be in the excitation (530 nm) and emission (555 nm).
3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. Our rhodamine WT detection limit in water samples is 0.015 ppb, thus this dye concentration limit is 0.045 ppb.
4. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Findings

Results from the January 2017 dye study of Upper Lake Lafayette (ULL) and the September 2017 study of Lake Jackson are presented in Table 1. For the ULL study we deployed a single sonde at the Wakulla Spring boil. For the Lake Jackson study, we also deployed a sonde at the Sally Ward Spring boil as well as charcoal packs at both the Wakulla Spring and Sally Ward Spring boils and at 13 additional sites (see Figures 1 and 2).

Sondes placed at Wakulla Spring first detected dye from both the ULL and Lake Jackson dye injections after 35 days. The sonde placed at the Sally Ward Spring for the Lake Jackson dye study first detected dye after 31 days.

Charcoal packs placed at the Wakulla Spring and Sally Ward Spring boils confirmed dye detection by the sondes placed at those locations. Positive charcoal pack dye test results were observed at Indian Spring and Meeting House Sink to the north of Wakulla Spring; at the McBride Slough bridge, downstream from the McBride Slough springs, located east of the Wakulla Spring boil; at No Name Spring which discharges to the Wakulla River approximately 2.9 miles south of the boil; and in the river at the south boundary of the park at the Shadeville Road bridge. Charcoal pack concentrations should be interpreted with caution as they reflect the adsorption of dye over time.

Table 1. Upper Lake Lafayette and Lake Jackson Dye Trace Studies

	Upper Lake Lafayette	**Lake Jackson**
Dye Injection Site	Fallschase sink	Porter Hole sink
Date of Dye Injection	1/19/17	9/19/17
Dye Type	Rhodamine WT liquid	Rhodamine WT liquid
Dye Concentration	~20%	~20%
Dye Amount	100 lbs	100 lbs
Wakulla Spring - sonde		
Date In	1/19/17	9/21/17
Initial Detection Date	2/23/17	10/24/17
Maximum Concentration (µg/L)	0.24	6.31
Transit Time	35 days	35 days
Wakulla Spring – charcoal pack*	n/a	
Date In		10/5/17
Date Out		10/27/17
Concentration (µg/L)		**18.0**
Wakulla River Shadeville Road Bridge – charcoal pack*	n/a	
Date In		10/04/17
Date Out		11/07/17
Concentration (µg/L)		**18.5**
Sally Ward Spring - sonde	n/a	
Date In		10/04/17
Initial Detection Date		10/20/17
Maximum Concentration (µg/L)		3.6
Transit Time		31 days
Sally Ward Spring - charcoal pack 1*	n/a	
Date In		10/04/17
Date Out		10/24/17
Concentration (µg/L)		**23.0**
Sally Ward Spring - charcoal pack 2*	n/a	
Date In		10/24/17
Date Out		11/07/17
Concentration (µg/L)		**30.3**
Freedom Sink – charcoal pack*	n/a	
Date In		10/24/17
Date Out		11/07/17
Concentration (µg/L)		10.8
Spiral Garden Sink – charcoal pack*	n/a	
Date In		10/24/17
Date Out		11/07/17
Concentration (µg/L)		7.5

	Upper Lake Lafayette	Lake Jackson
Emerald Sink – charcoal pack 1*	n/a	
Date In		10/10/17
Date Out		10/15/17
Concentration (µg/L)		5.9
Emerald Sink – charcoal pack 2*	n/a	
Date In		10/15/17
Date Out		11/12/17
Concentration (µg/L)		4.2
Whiskey Sink – charcoal pack*	n/a	
Date In		10/11/17
Date Out		11/07/17
Concentration (µg/L)		3.2
Meeting House Sink – charcoal pack 1*		
Date In		10/15/17
Date Out		11/12/17
Concentration (µg/L)		12.4
Indian Spring - charcoal pack 1*	n/a	
Date In		10/10/17
Date Out		10/15/17
Concentration (µg/L)		4.9
Indian Spring - charcoal pack 2*	n/a	
Date In		10/15/17
Date Out		11/12/17
Concentration (µg/L)		**20.8**
McBride Slough @ park boundary – charcoal pack*	n/a	
Date In		10/11/17
Date Out		11/07/17
Concentration (µg/L)		**26.9**
No Name Spring – charcoal pack*	n/a	
Date In		10/24/17
Date Out		11/07/17
Concentration (µg/L)		**30.2**
Harvey's Clear Lake – charcoal pack 1*	n/a	
Date In		10/09/17
Date Out		10/16/17
Concentration (µg/L)		4.8
Harvey's Clear Lake – charcoal pack 2*	n/a	
Date In		10/16/17
Date Out		11/13/17
Concentration (µg/L)		3.2

	Upper Lake Lafayette	**Lake Jackson**
Harvey's Aphasta Pit – charcoal pack 1*	n/a	
Date In		10/09/17
Date Out		10/16/17
Concentration (µg/L)		6.1
Harvey's Aphasta Pit – charcoal pack 2*		
Date In		10/16/17
Date Out		11/13/17
Concentration (µg/L)		7.8
Spring Creek @ Spears' dock – charcoal pack*	n/a	
Date In		10/04/17
Date Out		11/07/17
Concentration (µg/L)		6.5
Lab blank - charcoal pack		
Concentration (µg/L)		3.5

* Significant levels for charcoal packs indicated in bold.

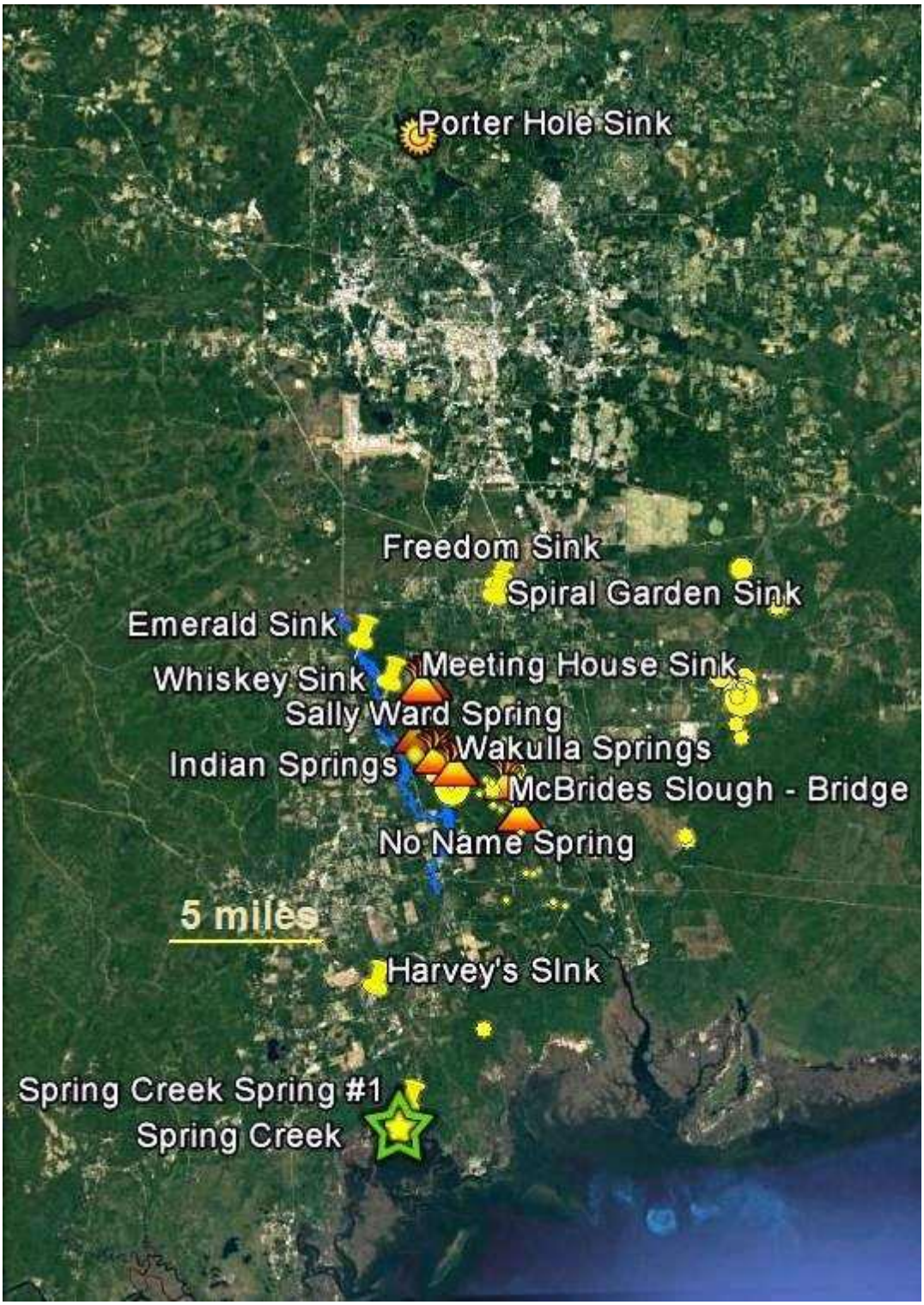


Figure 1: Route of dye trace of Lake Jackson (Porter Hole Sink) to Wakulla Springs. Porter Hole Sink is marked with a star burst. Positive dye results are indicated by a Hawaiian volcano, negative or questionable results are indicated by a thumb tack, mapped caves are indicated by a blue line, and springs are indicated by a yellow circle.

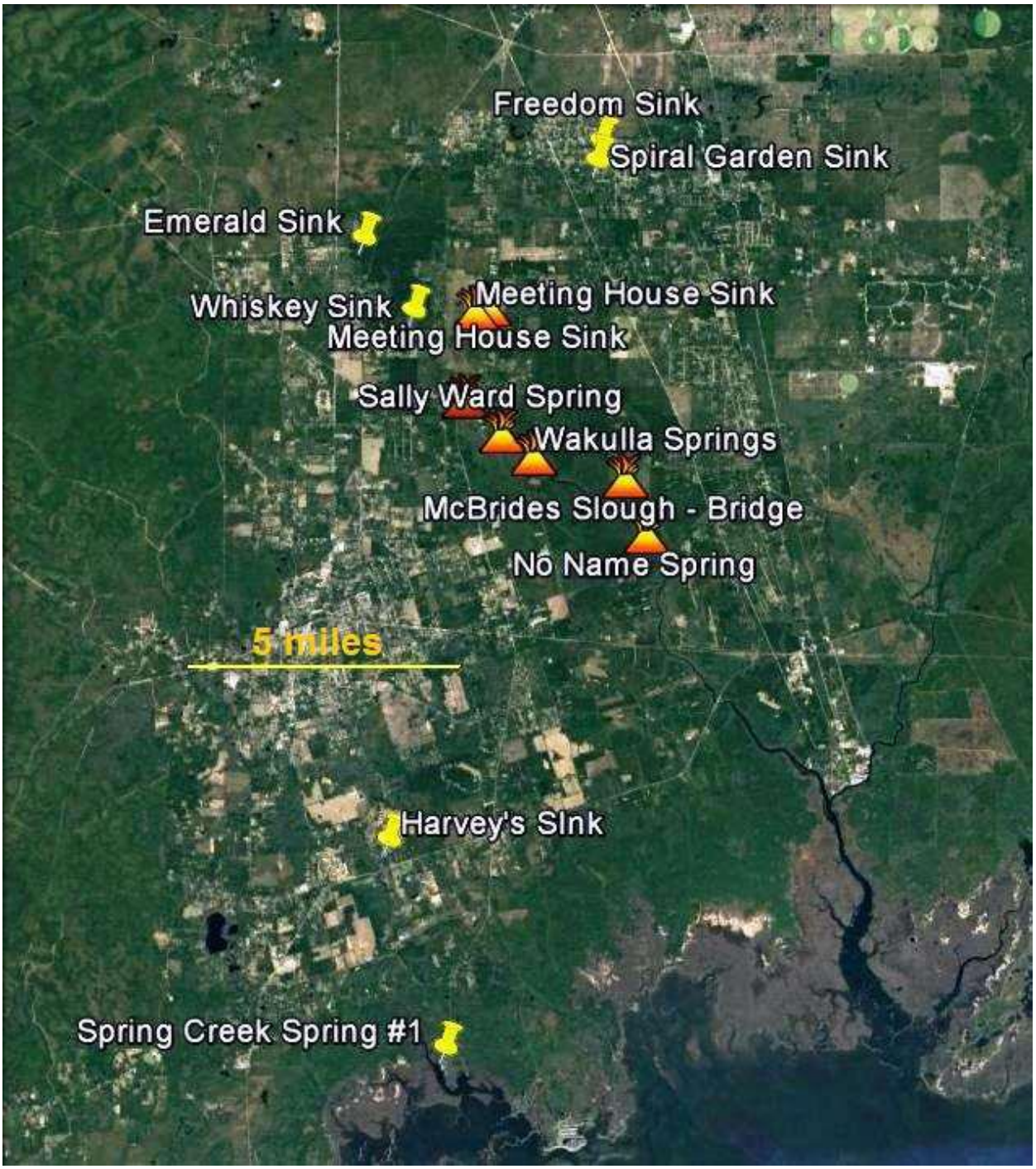


Figure 2: Close-up of the charcoal pack results from the dye trace of Lake Jackson (Porter Hole Sink) to Wakulla Springs. Positive dye results are indicated by a Hawaiian volcano, negative or questionable results indicated by a thumb tack.