Wakulla Spring Dark Water: Causes and Sources Phase I Final Report

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Photo: Bob Thompson

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Executive Summary

Wakulla Spring's water quality and its optical properties are the focus of this study. Wakulla Spring is the deepest and largest single-vent spring in Florida and perhaps in the world. As a result of its colossal size and complex hydrogeology, mysteries are numerous and answers are somewhat illusive. 'Dark' water is one of the causes of the decline of the Wakulla Spring and River natural ecosystem. 'Dark' water has virtually eliminated the park's most popular attraction, glass bottom boat tours. 'Dark' water also impacts the submerged aquatic vegetation and the biological productivity of the Wakulla spring and river ecosystem. The Wakulla Springs Alliance (WSA) undertook this project in an effort to determine the causes and sources of the more prolonged "dark water" conditions experienced at Wakulla Spring.

Approach

Glass bottom boat tour data offer a proxy for the frequency of dark water conditions in the spring because tours are only conducted when visibility is at least 75 feet. Those data demonstrate that dark water episodes at Wakulla Spring have been increasing over the last 30 years with tour frequencies (percentage of days per year tours are provided) dropping from 42% between 1987 and 1996 to 5% between 2007 and 2014 and less than 1% of the year from 2014 to 2016.

Tannins, the predominant type of colored dissolved organic matter (CDOM) in freshwater springs, have long been recognized as the principal cause of dark water conditions in Wakulla Spring. Under classic "dark water" conditions, the water appears reddish-brown from the tannins which absorb light predominantly at the short blue and green wavelengths while transmitting the longer-wavelength yellows and reds. High tannin levels have historically followed periods of prolonged rainfall and resulting discharges of three sinking streams north of the spring (Black, Fisher, and Jump Creeks) into the Upper Floridan Aquifer. After those flows subsided, the spring would "clear," returning to a pale blue color with the spring bottom completely visible.

In recent decades, the spring has reverted to a greenish apparent color after the tannins subside. That observation led us to hypothesize that chlorophylls may be contributing to the dark water conditions of the spring. Hydrogeologic research also has demonstrated that beginning in the mid-2000s, discharges into the aquifer from a fourth sinking stream, Lost Creek, which typically flow south to the Spring Creek spring complex on the shores of the Gulf of Mexico, sometimes flow north to Wakulla Spring bringing additional tannin loadings.

We therefore designed this study to track measures of tannins and chlorophylls over time and assess their trends against each other as well as rainfall and the flows of the sinking streams. We also tracked levels of turbidity in the spring, a composite measure of light absorbance and reflectance by particulate matter and dissolved color.

We analyzed daily and weekly samples collected between 8/25/15 and 9/29/16 from the Wakulla Spring boat dock and the spring boil for tannins, measured as true color, and chlorophylls, measured as corrected chlorophyll a and phaeophytin, a degraded form of chlorophyll. We also analyzed daily grab samples for specific conductance and nitrates. We conducted weekly spectral radiometric (spec rad) measurements in the field at the spring boil of transmittance of photosynthetically active radiation (PAR) light and its absorbance at each wavelength of the visible light spectrum (400-700 nanometers). This enabled us to calculate the 0% PAR depth limit, i.e. the depth at which no light is available for plant growth. We also measured Secchi disk visibility, a proxy for PAR depth limit.

In addition, we conducted light/dark event sampling on eight occasions: four “light” events during periods when Wakulla Spring visibility was relatively high and four “dark” events when visibility was low and the apparent water color was reddish-brown. From those measurements we constructed “optical fingerprints” of the spring boil and the major karst feature sources of inflow, i.e. the sinking lakes and sinks (Bradford Brook Chain of Lakes, Lake Iamonia, Lake Jackson, Upper Lake Lafayette, Lake Miccosukee, Lake Munson, and Cheryl Sink) and the sinking streams (Black Creek, Fisher Creek, Jump Creek, Lost Creek, and Mill Creek). For these events we analyzed samples for true color, corrected chlorophyll a, and phaeophytin from the Wakulla Spring boil and each of the karst sources. We also conducted light absorbance scans of filtered water from the karst sources and PAR transmittance analyses at the spring boil. We calculated loadings of tannins, corrected chlorophyll a, and phaeophytin to the ground water from the sinking streams and lakes to assess the likely relative importance of these different sources to the dark water conditions at the spring.

<u>Hydrology of the Wakulla Spring System</u>

Wakulla Spring is a complex dynamic hydrogeologic system. Based on the work of Davis and Verdi (2014) and Dyer (2015), it is likely that the driving forces behind the flow regime of the spring include (a) base flow from the Upper Floridan Aquifer within its very large springshed estimated at 1,600 to 2,900 square miles, (b) precipitation within the near springshed area, (c) flow from the intermittent streams that lie to the north, accounted for here by the discharges of Fisher and Black Creeks into their respective sinks, and (d) periodic discharges of Lost Creek into its sink when Spring Creek is not flowing and those discharges flow north to Wakulla Spring.

The flow regime of the Spring Creek springs complex is likely driven by (a) rainfall within its near springshed, and (b) discharges by Lost Creek into its sink under those conditions when its discharges flow south. The flow dynamics we observed at Spring Creek were consistent with the description presented by Davis and Verdi (2014). They explain the periodic cessation and sometimes negative discharge of the Spring Creek springs as the result of prolonged periods of little or no rainfall. Under these circumstances, the hydraulic head differential between the Spring Creek water table and sea level is too little to maintain flow from the Spring Creek springs.

We used discharge data from the USGS gauges at Lost Creek, Fisher Creek, Black Creek, Wakulla River at Shadeville Road bridge, and Spring Creek. Because of prolonged gaps and inaccuracies in the USGS Spring Creek discharge data, we constructed a polynomial model to use salinity/specific conductance as a proxy.

- Spring Creek had highly variable flow, sometimes ceasing altogether. Its average daily flow during the study period was 446 cubic feet per second (cfs).
- Wakulla Spring averaged almost twice as much flow, 875 cfs.
- The two sinking streams to the north flowed intermittently with modest average daily flows: Black Creek (18 cfs) and Fisher Creek (43 cfs). Lost Creek, at 158 cfs, is the dominant sinking stream, flowing almost all of the time except after very prolonged periods of little or no rainfall.
- The sinking lakes flow constantly into the aquifer within the Wakulla springshed at rates defined by seepage and sinkhole characteristics unique to each waterbody (McGlynn and Deyle, 2016).

Discharges by the three sinking streams are directly associated with rainfall within their watersheds. We used rainfall data from the NOAA gauge at Tallahassee International Airport because of gaps in data from the Wakulla Spring gauge maintained by the Northwest Florida Water Management District.

<u>Photosynthetically Active Radiation (PAR)</u>

Plants need almost the full spectrum of visible light to photosynthesize. This so-called photosynthetically active radiation (PAR) encompasses the spectral range of solar radiation from 400 to 700 nanometers (nm) which is the same as the light range visible to humans.

We defined the 0% PAR depth limit, calculated from an extinction coefficient using the spec rad data, as the depth at which PAR falls to 0% transmittance. This quantifies the depth at which all photosynthetic productivity ceases. During the period of study, the PAR depth limit at the spring boil averaged 12 feet, ranging from as little as 4 feet to as deep as 32 feet. Thus, at times, the light necessary for aquatic plant growth within the spring bowl, with depths as great as 80-90 feet, virtually disappears.

The compensation point for a plant is the depth at which it receives just enough PAR to produce by photosynthesis the amount of sugar needed to offset the amount consumed by respiration. This is 10% PAR for the two dominant submerged aquatic grass species in the spring bowl and Upper Wakulla River, American eelgrass (*Vallisneria americana*) and springtape (*Sagittaria kurziana*). The average depth of the 10% PAR compensation point during the period of study is about 10 feet, suggesting that survival of the native aquatic grasses may be limited at greater depths.

Turbidity

Turbidity is an aggregate measure that reflects the presence of algae cells measured as corrected chlorophyll a and phaeophytin as well as tannins measured as true color, in addition to other CDOM and suspended particulates. It is typically very low in spring outflow in Florida and is not likely to be a very significant factor in freshwater spring light regimes.

Turbidity data recorded at the Northwest Florida Water Management District's gauge on the boat dock during the study period revealed average levels lower than the long-term (1970-2008) average for the spring boil, despite the fact that limited synchronous data from the boat dock and boil indicate that levels are 23 to 32% higher at the dock. The study period average at the boat dock also was lower than the long-term average mean of 10 other Florida springs for the period 1996-2008 but comparable to the average mean of six other springs analyzed between 2004 and 2007. While both the tannins and other CDOM as well as the algae cells that contain the chlorophyll and phaeophytin detected in the water contribute to turbidity, we found only a weak statistical correlation between turbidity at the boat dock and our measure of pheophytin and no significant correlation to corrected chlorophyll a or true color. On the other hand, turbidity levels were correlated with the combined flows of Black and Fisher Creeks suggesting that some other turbidity constituents are entering the spring.

Tannins

Our analyses of true color in samples from the spring boil and the boat dock indicate that Wakulla Spring is more tannic than many other major springs in Florida. It also appears that tannin levels at the spring have increased over the long term since 1966. Four true color peaks occurred at Wakulla Spring during the study period, each shortly after peaks in the flows of the three sinking streams. Three of the peaks were associated with times when Spring Creek flow had ceased. In these cases, tannins from Lost Creek presumably contributed to the observed peaks at the spring. Regression analyses yielded a significant correlation between true color levels at Wakulla Spring and the combined flows of Black and Fisher Creeks, but not with the occasional flows of Lost Creek when Spring Creek was not flowing. The latter is not surprising because of its intermittent nature.

Chlorophyll

Our water quality analyses of samples from the spring boil and the boat dock confirm the presence of chlorophyll a in the spring, including its degraded form, phaeophytin. Few historical data are available for corrected chlorophyll a levels at the Wakulla Spring boil. Those we could identify suggest that chlorophyll a may not have increased in the spring since 2006, the earliest year for which data are available. However, chlorophyll a levels measured at Wakulla Spring are considerably higher than those measured at a number of other major springs in Florida between 1988 and 2008. Historical phaeophytin data for the Wakulla Spring boil also are limited. Those few available data similarly indicate no significant increase in phaeophytin levels at the spring boil since 2006 and possibly a decrease. Our average is comparable, however, to those measured at 11 other springs in Florida.

Total chlorophyll (corrected chlorophyll a + phaeophytin) levels do not vary directly with either rainfall or sinking stream inflows into the aquifer. This is not surprising, as we suspect that the principal source of chlorophylls measured at the spring boil is the base flow of the aquifer fed by discharges from the sinkholes of one or more of the karst lakes in Leon County that experience frequent algae blooms: Lakes Iamonia, Jackson, Lafayette, and/or Munson.

Apparent Causes of Dark Water Conditions at Wakulla Spring

Graphic analysis of turbidity and 0% PAR depth limit trends reveal an inverse relationship that is statistically significant at the 95% level for a simple linear regression model. A similar statistically significant inverse relationship occurs between true color and 0% PAR depth limit over the study period prior to June 30, 2015, but the relationship shifts thereafter and is no longer significant. Corrected chlorophyll a and phaeophytin exhibit inconsistent dynamic relationships with 0% PAR depth limit that are not statistically significant.

A multiple regression model to assess the combined linear effects of turbidity, true color, chlorophyll a, and phaeophytin on PAR depth limit offers a simplified approximation of the complex and dynamic combined effects of these four factors treating them as independent variables. While the model is significant at the 95% level, results suggest that light absorbance is not a simple linear function of turbidity and the concentrations of tannins and/or chlorophylls and that more sophisticated statistical analysis may be appropriate with a larger data set.

Our light/dark event spec rad analyses show that during “light events” when visibility depth is greatest and the water has an apparent green color, both the shortest wavelengths (< 480 nm) and the longer wavelengths (>570 nm) were being absorbed, with a distinct absorbance dip at 664 nm that is characteristic of chlorophyll a, and with greatest transmittance in the middle (about 480-570 nm, i.e. greens and yellows). These “optical fingerprints” are indicative of the presence of tannins and other CDOM as well as chlorophyll a and phaeophytin which have absorption peaks at about 410-430 nm and

660-675 nm. They are similar to those for the karst lakes with low tannin levels and substantial algae populations.

The entire spectrum is shifted to the right by the tannic condition in the "dark" events with the peak intensity moving from about 520 nm in the light events to about 620 nm in the dark events. In some dark events there is some overlap into the greens (500-560 nm), because of the presence of chlorophyll as well as tannins and other CDOM.

True color and chlorophyll level data for the light/dark events reveal higher levels of true color during the four dark events than three of the four light events as well as higher total chlorophyll a (corrected chlorophyll a + phaeophytin) levels during the dark events than any of the light events. Corrected chlorophyll a was detected in three of the four dark events. During two of those events it was 8 to 13 times greater than levels measured during the two light events when it was detected. Phaeophytin also was detected during three of the four dark events at levels 2 to 7 times greater than levels recorded during the two light events when it was present. Each of the light events had some low level of one or more of the three color sources: tannins, corrected chlorophyll a, or phaeophytin.

Absorbance scans of filtered water samples for the visible spectrum (400-700 nm) revealed greatest total light absorbance within the tannic waterbodies during both light and dark events: Cascade Lake and the sinking streams - Black, Fisher, Jump, Lost, and Mill Creeks. The lowest absorbance readings were recorded for Wakulla Spring and Sally Ward Spring. The lakes vary considerably with the more eutrophic lakes (Miccosukee, Iamonia, and Munson) having higher mean total absorbance (the area under the absorbance curve) than the less enriched systems (Jackson and Lafayette).

Measures of true color in the major sinking streams and karst lakes of the near-springshed taken within a week or less of those at the spring boil reveal that the sinking streams contribute the greatest tannin loadings to the Upper Floridan Aquifer during all four dark events and two of the light events. Lake loadings were greater sources during the other two light events. Both components of total chlorophyll a loads, corrected chlorophyll a and phaeophytin, are predominantly from the karst lakes in all events, but the creeks are discharging some total chlorophyll a to the aquifer during most of the events.

Spring Creek spring flows were negative during three of the four dark events when tannins probably flowed into Wakulla Spring directly from Lost Creek. However, tannin levels were greatest during dark event E8 when Spring Creek flows were nearly as great as they were during light event E7 when the PAR depth limit was greatest.

Phases II and III of this project have been designed to demonstrate that chlorophyll a and phaeophytin found in the spring boil originate from the sinking lakes. These subsequent studies analyze samples collected from the caverns and conduits that flow into the spring, conduct dye tests of Lakes Jackson, Upper Lake Lafayette, and Lake Iamonia, and attempt to link the chlorophyll a and phaeophytin in the lakes and the spring using algal taxonomy and analysis of environmental DNA sequenced from cyanobacteria (blue-green algae) and eukaryotic algae.[1]

Summary of Findings and Conclusions

- Both tannins, measured as true color, and other color dissolved material (CDOM) as well as chlorophyll a, measured as corrected chlorophyll a and chlorophyll's degradation product phaeophytin, contribute to reduced visibility in the spring boil much of the time.
- Chlorophyll a contributes to dark water conditions during both "brown dark water" conditions when tannins lend a predominantly reddish-brown color to the water and during "green dark water" conditions when the water appears greenish-brown and visibility is greater but still much lower than historic conditions when the water was a clear pale blue in color and the bottom was visible from the surface.
- Chlorophyll a loadings to the Upper Floridan Aquifer in the near-springshed of Wakulla Spring are predominantly from the karst lakes with sinkholes that discharge to the aquifer.
- As has been historically the case, tannins are the predominant cause of the brown dark water conditions that prevail after periods of prolonged rainfall when the intermittent sinking streams north of the spring are discharging to the aquifer through their swallets. Tannins also are present at low levels during some "light" events when visibility is greater.
- We did observe conditions consistent with the understanding that at times when the Spring Creek springs cease flowing, tannin loads from Lost Creek can be diverted north to Wakulla Spring.
- Simple linear statistical analyses are insufficient for unravelling the complex dynamics of the dark water conditions at Wakulla Spring. A larger data set is needed to support more sophisticated analyses.
- Additional analyses of water quality in the caverns and conduits are needed to demonstrate that chlorophylls detected in the spring boil are carried into the spring in the ground water.
- Dye tests are required to establish hydrogeologic connections between Wakulla Spring and the major karst lakes that have not been previously tested, i.e. Lakes Jackson, Upper Lake Lafayette, and Lake Iamonia.
- Other research strategies are needed to link chlorophyll and phaeophytin detected in the spring boil to specific karst features that discharge to the Upper Floridan Aquifer.

[1] Unlike the cyanobacteria, eukaryotic algae have a nucleus that contains their DNA and perform photosynthesis in separate membrane-bound organelles called chloroplasts.

1. Introduction

It's all about the water at Wakulla Spring, particularly the stuff dissolved in it, and in this project, it's the optical properties that are of primary concern. Wakulla Spring is the largest single vent spring in North America with caverns 350 feet in the earth. It is now also the largest based on flow, due to the demise of many larger springs, mostly due to our thirsty population. As a result of its colossal size, mysteries are numerous and answers are somewhat illusive.

The Wakulla Spring and River aquatic ecosystems have experienced several severe insults over the past quarter century including excessive inflows of nitrogen from urban and rural domestic wastewater; invasion by the exotic aquatic plant, Hydrilla; multiple years of herbicide treatment to control the Hydrilla; and an increase in the frequency and duration of dark water conditions that have impaired visibility in the spring.

This project examines the unique light absorption properties of the water of Wakulla Spring and of the karst features which recharge the spring in an effort to understand the sources and causes of the dark water conditions and to identify practical management strategies to mitigate the problem.

1.1 Consequences of Dark Water Conditions

'Dark' water is one of the causes of the demise of the Wakulla Spring and River natural ecosystem. It has also virtually eliminated the Park's most popular attraction, glass bottom boat tours.

Dark water conditions reduce the amount of light available to the submerged aquatic plants that comprise the base of the aquatic food web in freshwater spring and river ecosystems such as Wakulla Spring. More prolonged and frequent intermittent dark water conditions reduce plant growth resulting in less food and cover for manatee and the invertebrates and fish that support the entire spring and upper river ecosystem. The spring and upper river are losing this aquatic plant community, mainly the grass beds of *Vallisneria americana* and *Sagittaria kurziana*, that once thrived in the spring run all the way to its confluence with the St. Marks River (figures 1.1, and 1.2). Quarterly surveys of submerged aquatic vegetation (SAV) in the spring and upper river conducted by park staff and volunteers since 2013 have documented that extensive areas are now dominated by bare sediment and algal mats (figure 1.3).

There also has been a drastic decline in the number of days when the state park is able to offer glass-bottom boat tours. Data collected by state park staff for the last 30 years, separated into decades (figure 1.4), indicate that the water at Wakulla Spring was clear enough for the glass-bottom boats to run on average 42% of the time between 1987 and 1996. The next decade, 1997-2006, dropped to 24%, and in the last 10 years the glass-bottom boats ran on average only 5% of the time. No tours were offered in 2010, during the first 11 months of 2014, or during all of 2015. Surprisingly, conditions improved at the park for the Memorial Day weekend in 2016. Glass-bottom boats plied the spring again for five days (figure 1.5).

While dark water conditions have been observed in the spring since at least the 1930s due to tannins associated with heavy rains, since the mid-1990s the spring has experienced more frequent and more prolonged periods of dark water. It is likely that these periods of “brown dark water” have increased due to changes in hydro-geologic conditions that have increased flows into Wakulla Spring from the Spring Creek springshed, and in particular Lost Creek, which lies to the south (Davis and Verdi, 2014; Dyer, 2015; Kincaid, 2011), but no analyses have been completed to determine if this is the case. In the intervals between brown dark water conditions, when the spring and river have historically run clear, nearly continuous "green dark water” conditions of unknown nature and origin have occurred (Howard T. Odum Florida Springs Institute, 2014, p. 52).

1.2 Project Objectives

The Wakulla Springs Alliance (WSA) undertook this project in an effort to determine the causes and sources of the more prolonged "dark water" conditions experienced at Wakulla Spring and to identify practical management strategies that might be developed to mitigate the problem. WSA contracted with McGlynn Laboratories, Inc. (MLI) to conduct water quality analyses to define the light absorption properties of pigments in the water of Wakulla Spring and in water from karst features which recharge the spring with surface waters with differing optical properties and to document the hydrogeological attributes of the Wakulla springshed that may also contribute to those conditions. Karst features analyzed include several large “sink-hole lakes” (Lake Cascade, Lake Iamonia, Lake Miccosukee, Lake Jackson, Upper Lake Lafayette, and Lake Munson) and "disappearing" streams that flow into the aquifer through swallet sinkholes (Black Creek, Fisher Creek, Jump Creek, Lost Creek, and Mill Creek) as well some sinkholes (Cheryl Sink). Samples were collected at these sinking water bodies for eight light/dark events followed by sampling at Wakulla Spring and Spring Creek in an effort to "fingerprint" the color at the spring and match it to one or more sources.

Figure 1.1: Aerial photo of Wakulla Spring and the Upper Wakulla River, 1967, depicting extensive coverage by native submerged aquatic plants.

Figure 1.2: Submerged aquatic vegetation in 1960 comprising luxurious flats of aquatic grass meadows.

Figure 1.3: Submerged aquatic vegetation monitoring, Wakulla Springs State Park: bare sediment/sand (02/24/15); algal mats (05/25/16).

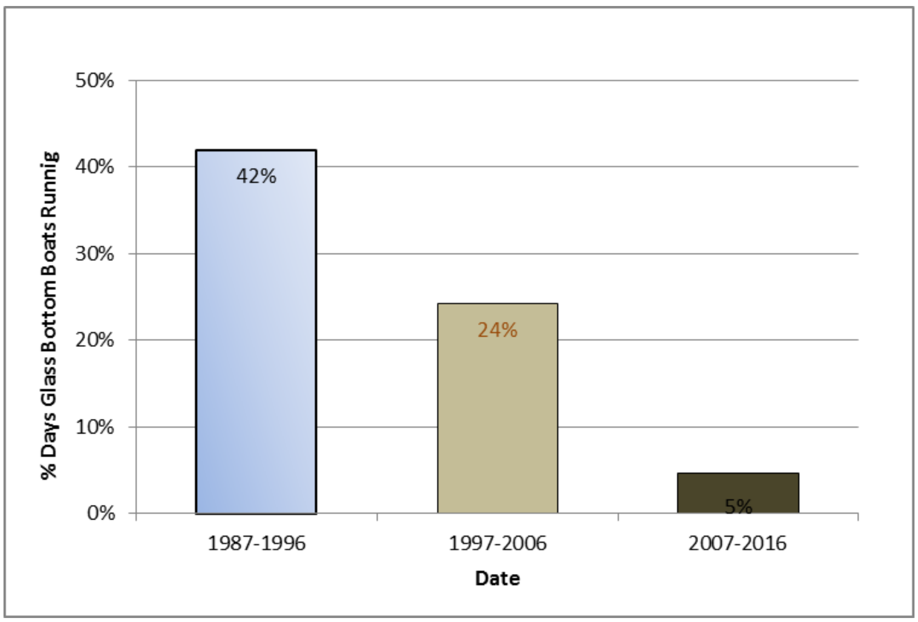

Figure 1.4: Percent of days per year glass-bottom boat tours conducted at Wakulla Spring over three decades.

Figure 1.5: Glass-bottom boat tour, 06/12/16.

2. Methods

2.1 General Sampling and Analysis

McGlynn Laboratories Inc. performed all analytical work and participated in all sampling except for the collecting of the daily samples which was independently performed by park staff. The sample analyses were certified according to The NELAC Institute (TNI). Sampling followed Florida Department of Environmental Protection, Standard Operating Procedures 01-001, revised 2014 (FDEP, 2014): FC 1000-Field Decontamination; FD 1000-Documentation; FM 1000-Field Mobilization; FQ 1000-Quality Control; FS 1000-General Sampling; FS 2000-General Water Sampling; FS 2100-Surface Water Sampling; FT 1000-Field Testing General; FT 1100-Field pH; FT 1200-Field Specific Conductance; Specific laboratory quality assurance (QA) objectives for chlorophylls, true color, and nitrate-nitrite are listed in Table 2.1

Sample analyses followed APHA (2005). True color samples were run in a quartz cuvette with a 10 cm path length to increase accuracy per SM 2120C on filtered samples (glass fiber filters, 0.45 um), pH adjusted (standard pH) at 465 nm. Chlorophyll a and phaeophytin were analyzed according to SM 1032000H. Nitrate was analyzed following SM 4500-NO3-E. Specific conductance/salinity was measured according to EPA method 120.1.

For most broad-spectrum light sources, including natural sunlight, artificial, or mixed sources, plants use light in the wavelength range from about 400 to 700 nm to drive photosynthesis. We measured PAR light transmittance in the field under natural conditions without filtration or pH adjustment of the water using two devices on each sampling date: (1) a LICOR LI-188B Integrating Quantum Radiometer (figure 2.1) and (2) an Ocean Optics USB2000 portable submersible spectrometer (figure 2.2). We also measured visibility in the field with a Secchi disk (figure 2.3).

We recorded PAR at 0.5-meter intervals to a depth of 5 meters using an underwater fiber optic cable fitted with a cosine collector with an LI-192 Underwater Quantum Sensor and the LICOR LI-188B radiometer which measures and integrates PAR directly. We used the Ocean Optics USB2000 to measure the underwater light field at 0.3 nm intervals of from 200nm to 1000 nm following SM 2120D (APHA, 2005). We calculated PAR for the 400 to 700 nm range from these values and normalized by dividing each integrated transmittance reading by the transmittance of incident light at the surface to generate a percent PAR. We calculated an integrated PAR extinction coefficient across the 400 to 700 nm spectrum[2] and used that to estimate the depth at which PAR falls to 0%

[2] Normalized absorbances from each depth were integrated to determine the absorbance area under each curve (10 areas). The extinction coefficient was calculated in accordance with the Beer Lambert Law regressing the 10 integrated absorbance values against depth yielding acceptable results when the F-test was significant at the 95 percent level or better. The measurement at 0.5 meter was sometimes eliminated due to non-linear conditions resulting from scatter due to turbulence, air bubbles, or reflectance from under the surface. The regression models were then used to calculate the 0% transmittance depth. As a quality

transmittance, i.e. the depth limit of visible light. This measure provides a basis for assessing the suitability of light conditions for glass-bottom boat tours which operate when visibility is at least 75 feet as well as the depth at which all photosynthetic activity ceases.

It should be noted that light intensity measurements in water will be affected by other factors besides the presence of substances that absorb light: sun angle, surface ripples, cloud cover, and bottom reflectance. These will affect Secchi disk readings, but we normalized our light absorbance readings against light absorption in air at the start and end of each measurement sequence to minimize the effects of these factors on the individual readings. We also minimized the effect of changing cloud conditions by taking readings during clear-sky intervals or under heavy overcast. Variable wind levels and associated ripples may have contributed to some differences in light intensity from one sample depth to the next during any given sampling.

We measured Secchi disk visibility according to FLORIDA LAKEWATCH methodology (Hoyer et al., 2016). We used the standard Secchi disk modified for fresh water applications equipped with a linear open reel fiberglass engineer's tape measure and weights attached to the bottom of the disc. The disc was suspended from the tape and lowered slowly into the water until no longer visible. The depth in feet at which the disk is no longer visible is recorded as a measure of the transparency of the water. Secchi readings can also be used to calculate an approximate extinction coefficient, but we did not do so as the spectrometer-derived values are far more accurate.

2.2 Daily Sampling from Wakulla Spring

Park staff collected samples off the boat dock for analysis by MLI of specific conductance, true color, and nitrates. These samples were taken according to FDEP (2014) sampling protocol in 50 ml polypropylene centrifuge tubes and stored in a secure refrigerator before being picked up every two to three days for analysis.

Daily grab samples were collected on 319 dates: 10/16/15; 10/17/15; 10/19/15; 10/21/15; 10/22/15; 10/23/15; 10/24/15; 10/25/15; 10/26/15; 10/29/15; 10/30/15; 10/31/15; 11/1/15; 11/2/15; 11/3/15; 11/5/15; 11/6/15; 11/7/15; 11/7/15; 11/8/15; 11/9/15; 11/10/15; 11/11/15; 11/12/15; 11/13/15; 11/14/15; 11/16/1; 11/17/15; 11/17/15; 11/18/15; 11/20/15; 11/21/15; 11/22/15 11/24/15; 11/25/15; 11/26/15; 11/27/15; 11/28/15; 11/29/15; 11/30/15; 12/1/15; 12/2/15; 12/3/15; 12/4/15; 12/5/15; 12/6/15; 12/7/15; 12/8/15; 12/9/15; 12/11/15; 12/12/15; 12/13/15; 12/14/15; 12/15/15; 12/18/15; 12/20/15; 12/21/15; 12/23/15; 12/25/15; 12/26/15; 12/27/15; 12/28/15; 12/29/15; 12/30/15; 12/31/15; 1/1/16; 1/3/16; 1/4/16; 1/5/16; 1/6/16; 1/8/16; 1/10/16; 1/11/16; 1/12/16; 1/13/16; 1/15/16; 1/16/16; 1/17/16; 1/20/16; 1/21/16; 1/23/16; 1/25/16; 1/26/16; 1/27/16; 1/30/16; 1/31/16; 2/2/16; 2/3/16; 2/4/16; 2/5/16; 2/6/16; 2/7/16; 2/8/16; 2/9/16;

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assurance protocol, we also calculated weekly 0% PAR depth limits by regressing the LICOR absorbance values.

2/12/16; 2/13/16; 2/14/16; 2/15/16; 2/16/16; 2/17/16; 2/20/16; 2/21/16; 2/22/16; 2/23/16; 2/24/16; 2/26/16; 2/27/16; 2/28/16; 2/29/16; 3/1/16; 3/2/16; 3/4/16; 3/5/16; 3/7/16; 3/8/16; 3/10/16; 3/11/16; 3/12/16; 3/13/16; 3/14/16; 3/15/16; 3/20/16; 3/21/16; 3/23/16; 3/24/16; 3/25/16; 3/26/16; 3/27/16; 3/28/1; 3/30/16; 3/31/16; 4/2/16; 4/3/16; 4/4/16; 4/5/16; 4/6/16; 4/7/16; 4/9/16; 4/10/16; 4/12/16; 4/14/16; 4/15/16; 4/16/16; 4/17/16; 4/19/16; 4/20/16; 4/22/16; 4/23/16; 4/24/16; 4/25/16; 4/27/16; 4/28/16; 4/29/16; 4/30/16; 5/1/16; 5/2/16; 5/3/16; 5/5/16; 5/6/16; 5/11/16; 5/12/16; 5/13/16; 5/14/16; 5/15/16; 5/16/16; 5/18/16; 5/19/16; 5/22/16; 5/23/16; 5/27/16; 5/28/16; 5/29/16; 5/30/16; 5/31/16; 6/1/16; 6/7/16; 6/8/16; 6/9/16; 6/11/16; 6/13/16; 6/14/16; 6/15/16; 6/16/16; 6/17/16; 6/18/16; 6/19/16; 6/20/16; 6/24/16; 6/25/16; 6/26/16; 6/27/16; 6/28/16; 6/29/16; 7/1/16; 7/3/16; 7/4/16; 7/10/16; 7/12/16; 7/14/16; 7/16/16; 7/17/16; 7/18/16; 7/19/16; 7/20/16; 7/21/16; 7/24/16; 7/23/16; 7/25/16; 7/26/16; 7/27/16; 7/28/16; 7/30/16; 7/31/16; 8/2/16; 8/6/16; 8/7/16; 8/8/16; 8/9/16; 8/10/16; 8/11/16; 8/12/16; 8/13/16; 8/14/16; 8/15/16; 8/15/16; 8/17/16; 8/19/16; 8/20/16; 8/21/16; 8/22/16; 8/23/16; 8/24/16; 9/1/16; 9/6/16; 9/9/16; 9/11/16; 9/14/16; 9/16/16; 9/17/16; 9/18/16; 9/20/16; 9/21/16; 9/22/16; 9/25/16; 9/28/16 and 9/29/16.

2.3 Weekly Sampling from Wakulla Spring

We collected weekly grab samples from Wakulla Spring for analysis of chlorophylls, true color, specific conductance, and nitrate. Grab samples were collected in 1.8-liter bottles and stored on ice until analysis at the laboratory according to the sampling and analytical protocols and certifications documented above. Simultaneous scans of light transmittance in the water column were also taken, in situ, as detailed above. Secchi disk readings were added to the sampling regime on 06/30/16.

Initially samples were taken off the end of the boat dock. Then on 12/24/15 sampling from a boat at the spring boil was added in an effort to obtain data purely reflective of the ground water being discharged into the spring. Samples were taken from both the dock and the boil until 06/30/16 and exclusively from the boil thereafter. Regression analyses show limited correlation between the sample values obtained from the dock versus the boil for the 11 occasions when we sampled both stations. For true color, dock data explain 32% of the observed variation in boil data significant at only the 90% level. Corrected chlorophyll a data from the dock are a better fit explaining 43% of the observed variation at the boil at the 95% level of significance. However, the relationship between dock and boil phaeophytin data is not statistically significant. We therefore differentiate between data from the two stations in our findings.

Weekly grab samples were collected on 52 dates: 08/25/15; 09/03/15; 09/09/15; 09/25/15; 10/03/15; 10/15/15; 10/21/15; 10/29/15; 11/06/15; 11/12/15; 11/20/15; 11/27/15; 12/10/15; 12/16/15; 12/24/15; 01/07/16; 01/14/16; 1/18/16; 01/22/16; 01/28/16; 2/5/16; 02/11/16; 02/18/16; 02/24/16; 03/03/16; 03/10/16; 03/22/16; 03/28/16; 04/02/16; 04/05/16; 04/07/16; 04/13/16; 04/21/16; 04/28/16; 05/05/16; 05/12/16; 05/19/16; 05/26/16; 06/02/16; 06/10/16; 06/15/16, 06/23/16; 06/30/16; 07/07/16; 07/14/16; 07/21/16; 07/28/16; 08/04/16; 08/11/16; 08/12/16; 08/18/16; and 08/29/16.

2.4 Light and Dark Event Sampling

Light and dark event sampling was conducted during periods when the water at Wakulla Spring was relatively clear and obviously dark to construct “optical fingerprints” of the springhead and the major karst feature sources of inflow: Bradford Brook Chain of Lakes, Lake Iamonia, Lake Jackson, Upper Lake Lafayette, Lake Miccosukee, Lake Munson, Black Creek, Cheryl Sink, Fisher Creek, Jump Creek, Lost Creek and Mill Creek (figures 2.4 and 2.5). Grab samples were collected in 1.8-liter bottles and stored on ice for laboratory analyses of chlorophylls, true color, and specific conductance following the sampling and analytical protocols and certifications described above. Color absorbance scans of filtered samples were run at 400-700 nm) at approximately 1-nm intervals, using the Ocean Optics spectrometer. We used a 10-cm cuvette rather than the typical 1-cm to maximize absorbance as light passed through the sample. Because these scans are filtered, they measure only dissolved color (CDOM) most of which is attributable to tannins. Chlorophylls, predominantly within discrete algae cells, are removed by filtering.

Sampling was conducted for four light events: Light #1: 10/09/15 - 10/26/15; Light #5: 05/12/16 - 05/26/16; Light #6: 06/11/16 - 06/20/16; and Light #7: 08/06/16 - 08/13/16, and four dark events: Dark #2: 12/04/15 - 12/10/15; Dark #3:01/04/16 - 01/14/16; Dark #4: 02/15/16 - 02/18/16; and Dark #8: 08/27/16 - 09/04/16.

Table 2.1: Laboratory Quality Assurance Objectives (Method, MDL, PQL)

PARAMETER	METHOD	MDL	PQL	Units
Chlorophylls	SM-10200-H	0.178	0.890	ug/L
Color (True)	SM-2120-C	0.19	0.95	CoPt
Nitrate + Nitrite	SM-4500-NO3-E	0.005	0.048	mg/L

MDL = method detection limit; PQL = practical quantitation limit.

Figure 2.1: LICOR integrating quantum radiometer.

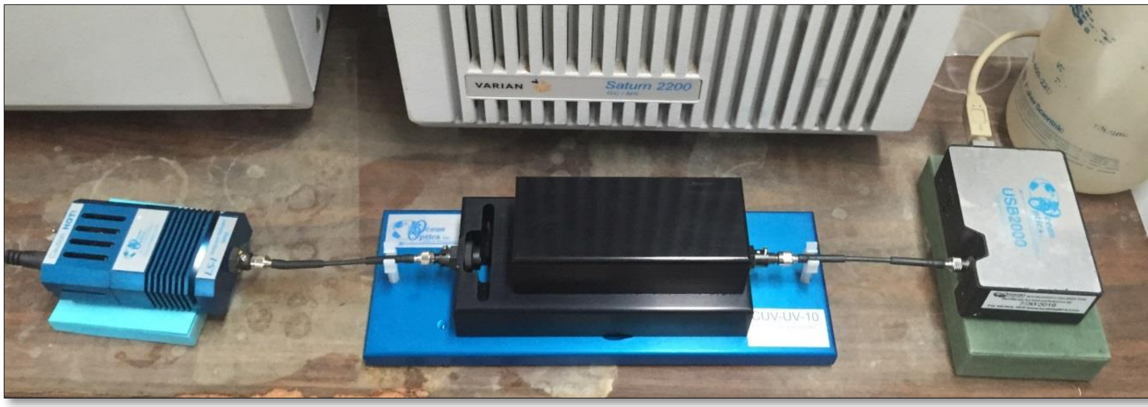

Figure 2.2: Ocean Optics USB2000 portable spectrometer.


Figure 2.3: Secchi disk.

Figure 2.4: Light/dark event sampling in the Wakulla springshed: karst lake sinks denoted by a yellow sun; sinking creeks denoted by a blue snow flake; major discharge sites denoted by a green star.

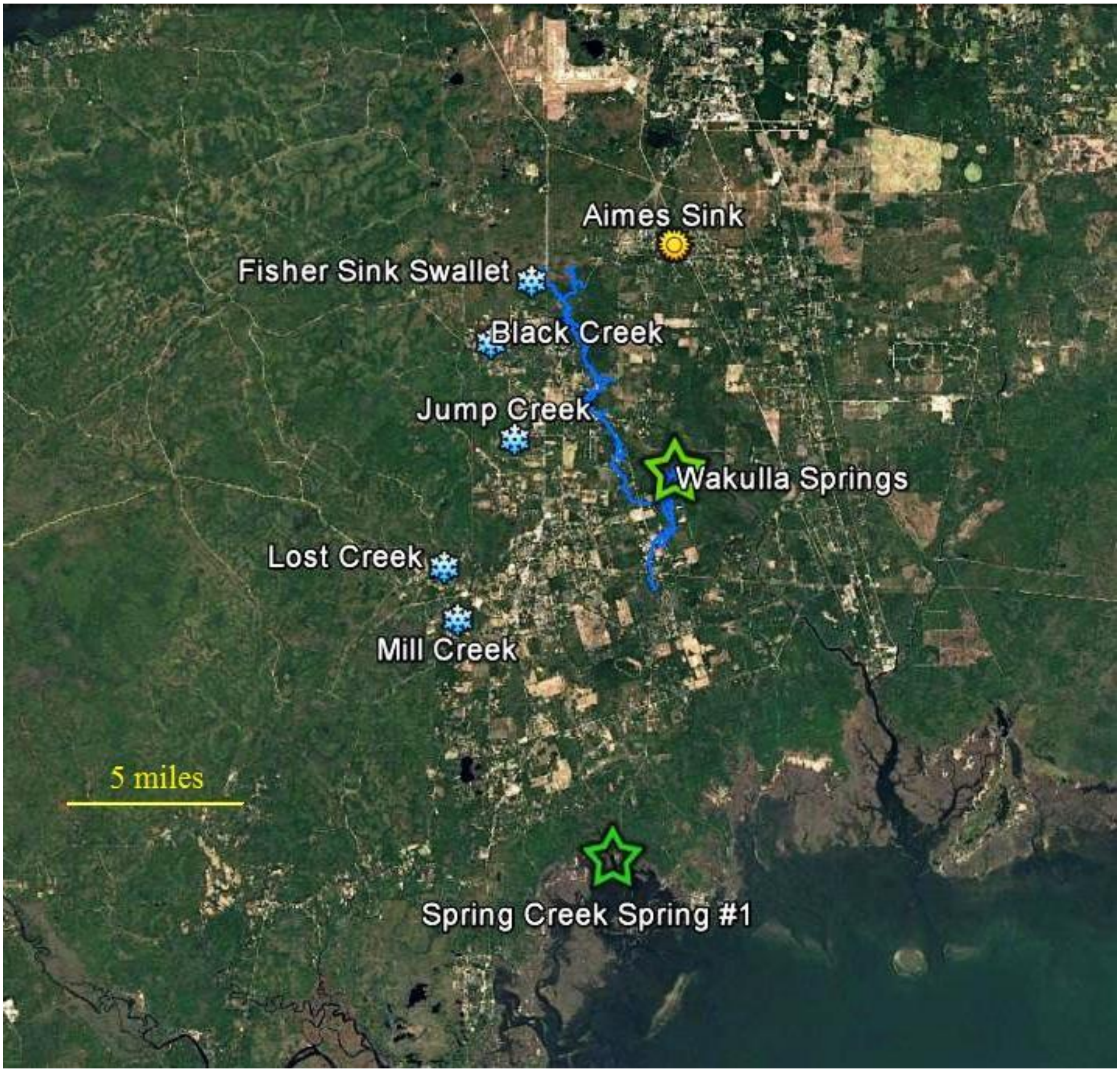

Figure 2.5: Close up of sinking creek area of the southern portion of the Wakulla springshed where light/dark event sampling performed: karst lake sinks denoted by a yellow sun; sinking creeks denoted by a blue snow flake; major discharge sites denoted by a green star.

3. Rainfall in the Coupled Springsheds: Wakulla and Spring Creek

We began the study using rainfall data from the Wakulla Spring boat dock and Tallahassee International Airport in this study (figure 3.1). However, the Wakulla Spring data record (figure 3.2) ceased on 07/08/16 when the gauge at the boat dock, which is owned by the Northwest Florida Water Management District (NWFWMD), went down. It was subsequently retrofitted by the USGS which is operating the gauge for the district. The National Oceanic and Atmospheric Administration (NOAA) Tallahassee Airport data (figure 3.3) are complete but may not be an entirely accurate representation of rainfall closer to Wakulla Spring or Spring Creek. As shown in figure 3.4, the Wakulla Spring and Tallahassee rainfall patterns are similar, but rainfall amounts differed, sometimes substantially, on some occasions. Because of the data gap in the Wakulla Spring gauge data, we have used the Tallahassee data in these analyses.

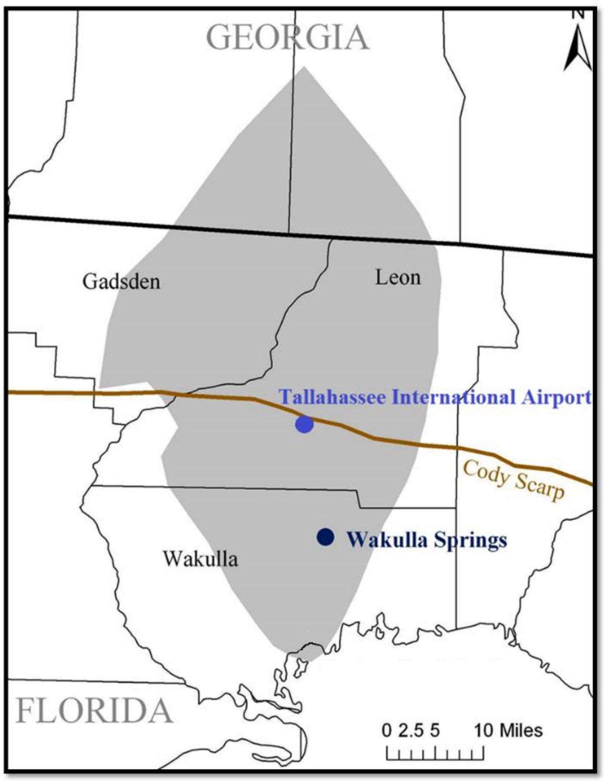

Figure 3.1: Wakulla springshed and weather stations used in this report: Tallahassee International Airport and Wakulla Spring Boat Dock.

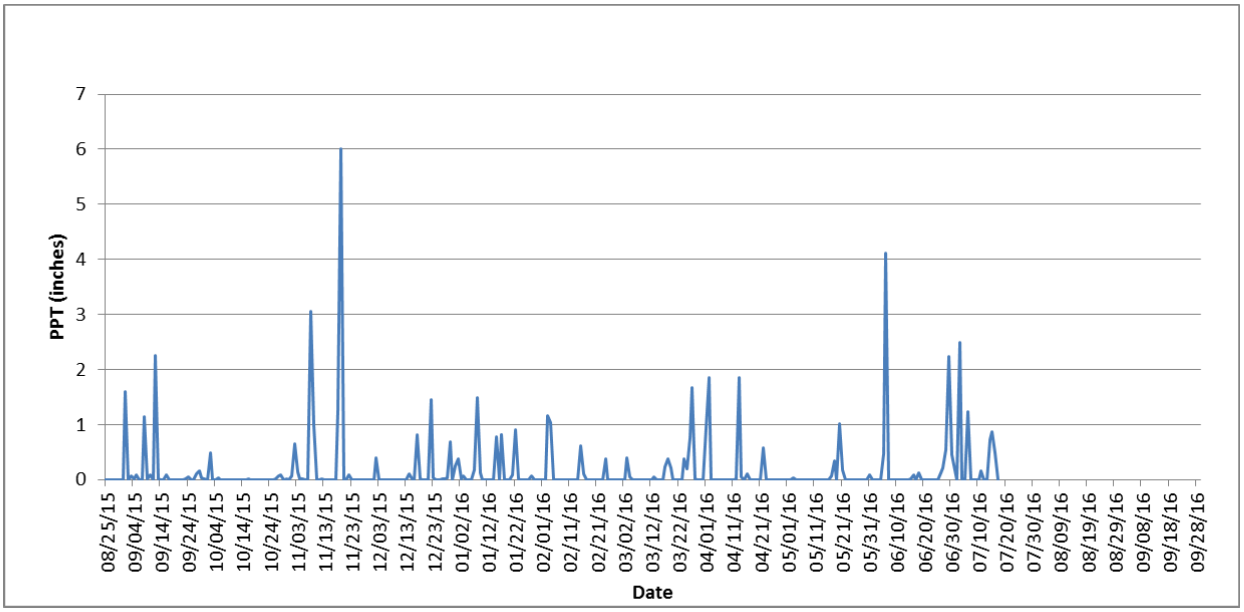

Figure 3.2: Wakulla Spring daily rainfall data from the NWFWMD gauge at the boat dock near the Wakulla Spring boil.

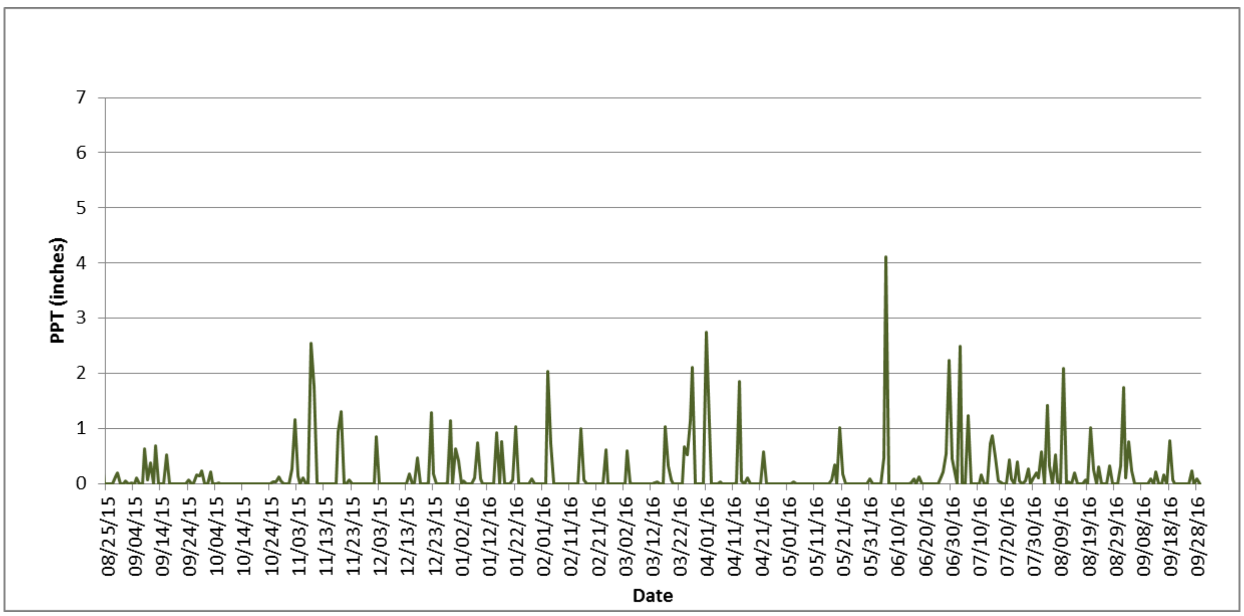

Figure 3.3: Tallahassee daily rainfall data from the NOAA gauge at the Tallahassee International Airport.

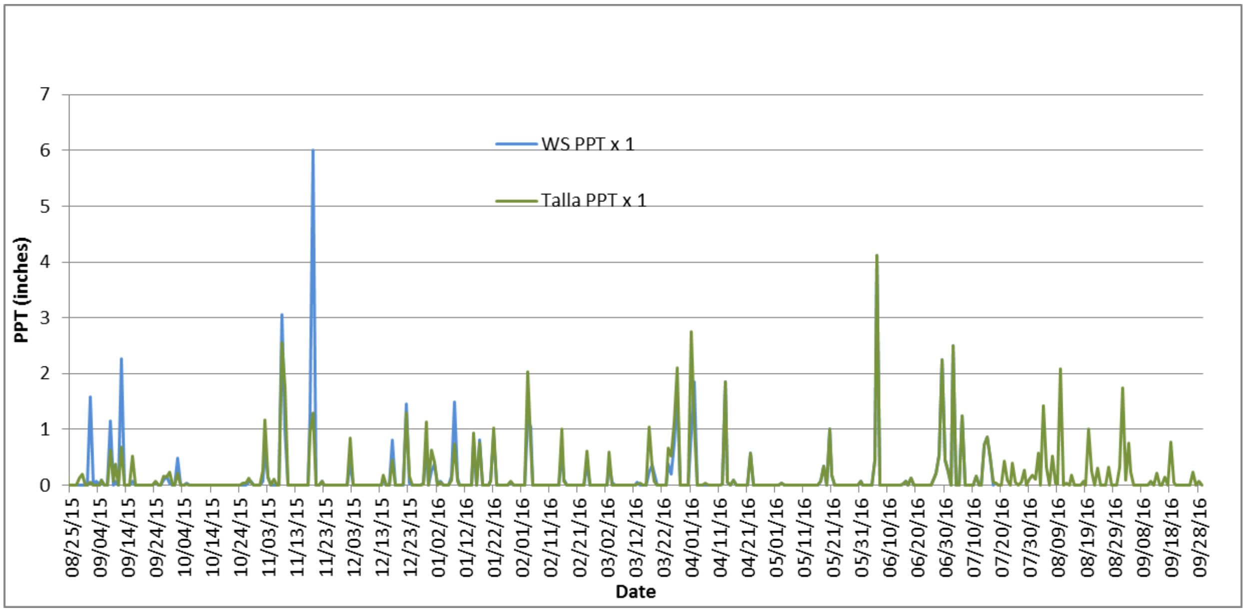

Figure 3.4 Tallahassee International Airport and Wakulla Spring boat dock rainfall.

4. Discharge in the Coupled Springsheds: Wakulla and Spring Creek

We used discharge data from the USGS gauges at Lost Creek, Fisher Creek, Black Creek, Wakulla River, and Spring Creek (figure 4.1). The gauges were working and there were fewer data gaps than with the weather data, except, there were some other problems with the Spring Creek data.

4.1 Spring Creek Discharge

The Spring Creek springs complex comprises 14 springs within Spring Creek and beyond its mouth in the Gulf of Mexico. According to Ron Knapp, the Field Office Chief for the USGS in Tallahassee, the flow meter at Spring Creek, (USGS 02327031 SPRING CREEK NEAR SPRING CREEK, FL), is located out in the estuary, approximately 0.6 mile downstream from Spring Creek vent #1 and in the vicinity of vents 8, 10, and 11, but totally missing flows from vents 12 and 13. It was placed to catch most of the flows from the multiple vents, however, it misses some and is to the side of the channel. It was designed to monitor the flow at that spot, not the flow of the entire Spring Creek system. In addition, the flow meter seems to have been moved by a dragging anchor, into a rather difficult rocky area, not optimal for flow measurements. Furthermore, the salinity guage is at the top of the water column and the flow meter is at the bottom. Spring Creek is a marine estuary and is heavily impacted by the tides and is often stratified with respect to salinity and temperature. The less dense freshwater flows out of the spring vents and into the salty estuary, usually flowing out over the surface of the water column. The denser salt water either lies listlessly in the lower reaches of the water column, not flowing, or flows with the tides, in and out.

During this study period, the Spring Creek vents ceased flowing several times. The flow seemed to be uncoupled from the tides in the area, which vary about 3 feet, a water level change much greater than sea level rise which has been cited as the cause of a sufficiently reduced head differential to cause a cessation of flow at Spring Creek (figures 4.2 and 4.3). There is also inexplicable variability in the observed flow rate of the vents, which seem to cut in and out independently of one another. Vortexes have been visible in the past at the Spring Creek vents (figure 4.4). The late Lee Nell Spears, a lifelong resident at Spring Creek, said that several times he heard the vortex whistling like a freight train. One instance was during Hurricane Dennis, which had an 11 foot storm surge, the height of which is prominently marked on a telephone pole near the dock. While our polynomial model (see below) predicted some negative flows during the study period, we never saw a vortex at a Spring Creek vent during this study.

Because of the problems with the Spring Creek gauge data, we calculated flow using the theory behind the "Fraction of Salinity Model' of Thomann and Mueller (1987). We estimated both linear and polynomial regression models of the Spring Creek estuary based on over 1,102 daily average readings of salinity and discharge from the USGS gauge from 06/25/07 to 07/01/10 for model development. We used the polynomial regression, which provided the better fit with a coefficient of determination (R^2) of 72.54% significant at the 99.9999% level (F-test probability = 3.3847E-172) (Figure 4.5).

Spring Creek discharge, for the study period, based on these USGS salinity readings (Figure 4.6), showed considerable flow variation. We had an initial five months of negative and/or zero discharge with one exception in mid-November 2015, followed by seven months of substantial, but intermittent discharge. During the study period, discharge ranged from -150 to 1,674 cubic feet per second (cfs) with an average of 446 cfs.

4.2 Wakulla Spring Discharge

While the NWFWMD maintains a flow meter in the spring vent, that meter was down during much of the study. We used the USGS guage (USGS 02327000 WAKULLA SPRING NR CRAWFORDVILLE, FL) on the Shadeville Road Bridge (county route 365), which is at the southern boundery of the park approximately 3 miles downstream of the vent (figure 4.7). As a result, the discharge values include outflow from the spring vent as well as inflow to the river from the Sally Ward Spring run and several smaller springs along the upper three miles of the river. Those additional volumes are trivial. During the study period, the discharge was highly variable ranging from 528 to 1,650 cfs with an average of 875 cfs.

4.3 Sinking Stream Discharges

We used flow data from three USGS gauges to measure sinking stream discharges into their respective swallets within the Wakulla springshed: Lost Creek (USGS 02327033 LOST CREEK AT ARRAN FLA), Black Creek (USGS 02326995 BLACK CREEK

NEAR HILLIARDVILLE, FL), and Fisher Creek (USGS 02326993 FISHER CREEK NEAR HILLIARDVILLE,FL). None of these guages had associated rainfall data during our study period.

Lost Creek flowed intermittently during the study period with discharges ranging from 0.37 to 1,690 cfs and an average of 158 cfs (figure 4.8). Lost Creek is the dominant sinking stream in the springshed, but its discharge is believed to flow predominantly to Spring Creek (Davis and Verdi, 2014; Dyer, 2015). Lost Creek Sink is located several miles southwest of Wakulla Spring (figure 4.1).

Black Creek and Fisher Creek also discharged intermittently during the study period (figures 4.9 and 4.10) with flows ranging from 0.01 to 250 cfs at Black Creek and from 0.03 to 443 cfs at Fisher Creek. Black and Fisher Creeks contribute significantly less flow than Lost Creek averaging 18 cfs and 43 cfs respectively (figure 4.11). However, the discharges of these two streams into their respective sinks are assumed to flow exclusively to Wakulla Spring. As a result, they are the major sources of tannin and other CDOM color in the spring when Lost Creek's discharge flows south to Spring Creek (Davis and Verdi, 2014).

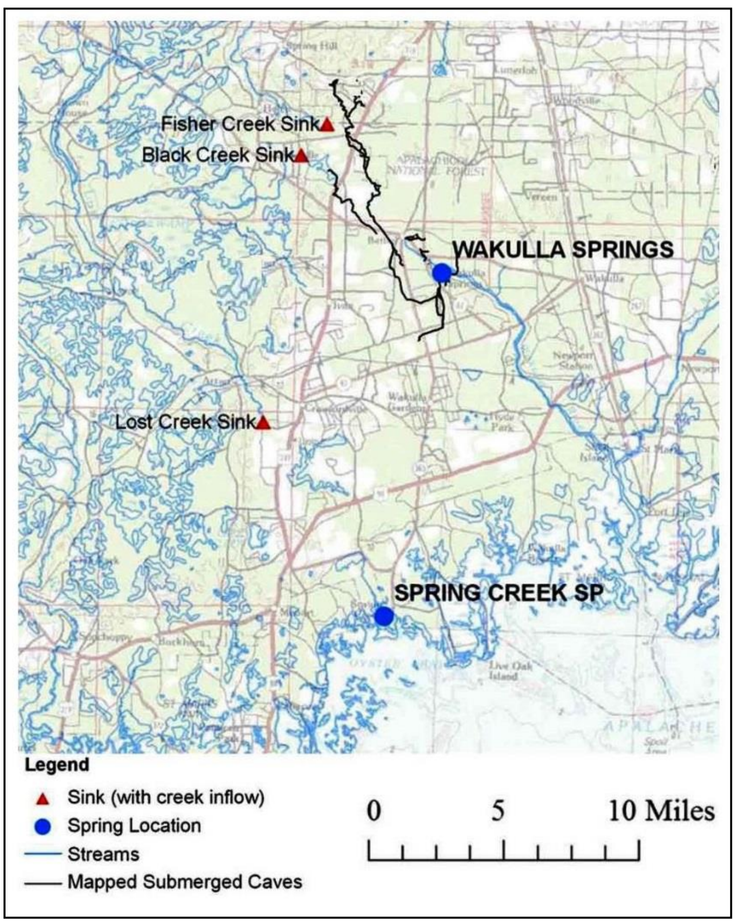

Figure 4.1: Map showing the locations of springs and sinking streams for which discharges were analyzed in this project.

Figure 4.2: Spring Creek flow (08/08/16).

Figure 4.3: Spring Creek: No flow and no vortex (10/28/16).

Figure 4.4: Vortexes at Spring Creek

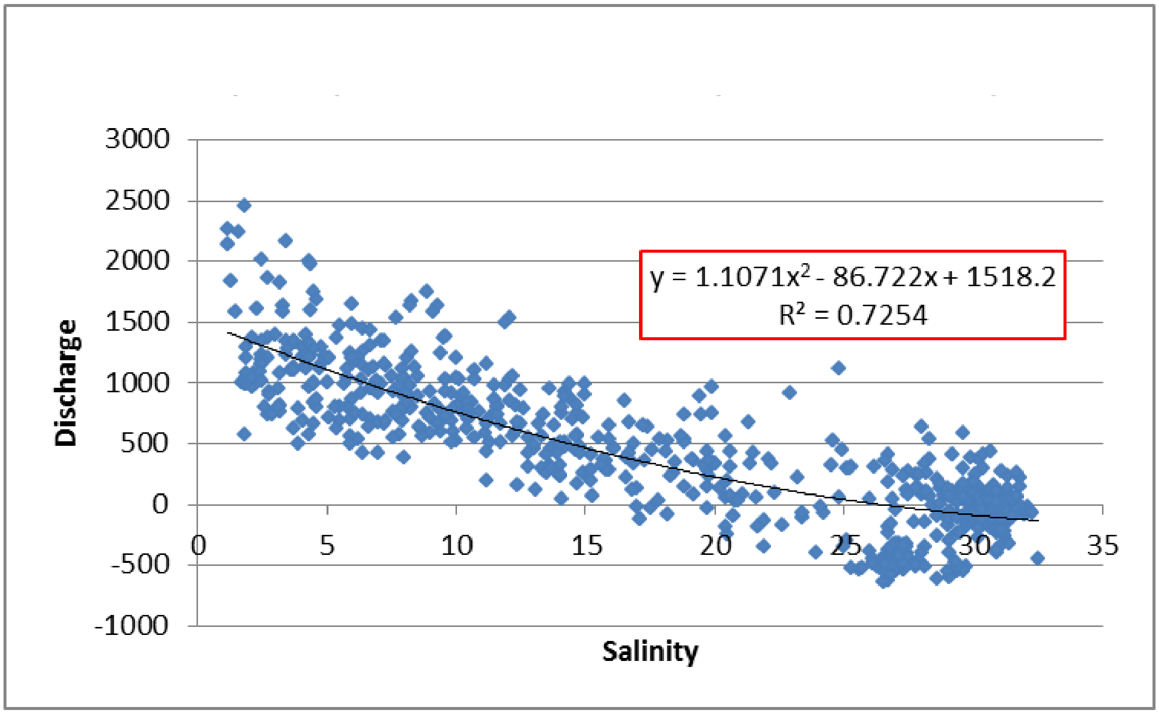

Figure 4.5: Polynomial regression model of Spring Creek flow and salinity data from USGS guage 02327031, 06/25/07 to 07/01/10.

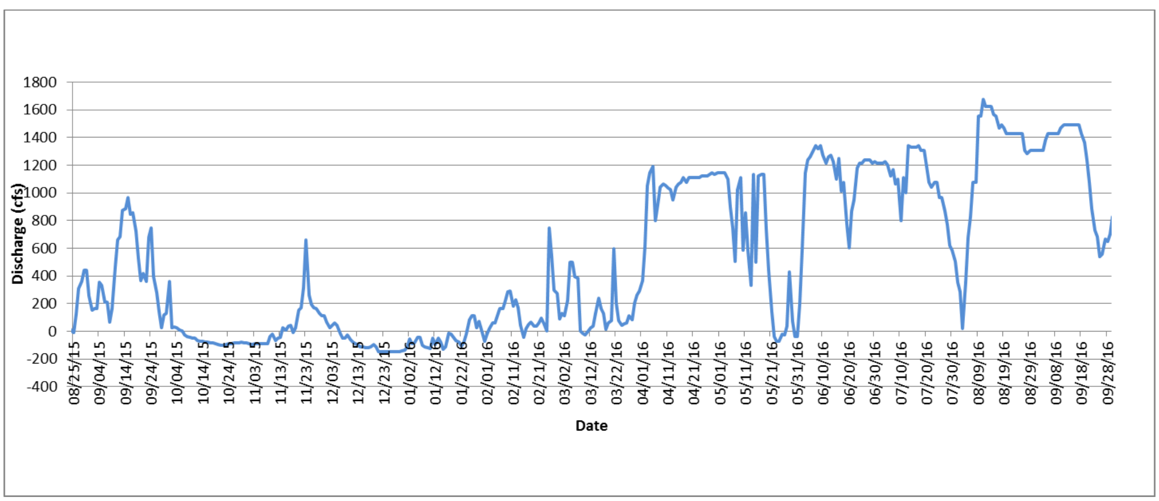

Figure 4.6: Spring Creek discharge, for the study period, as modeled with polynomial regression.

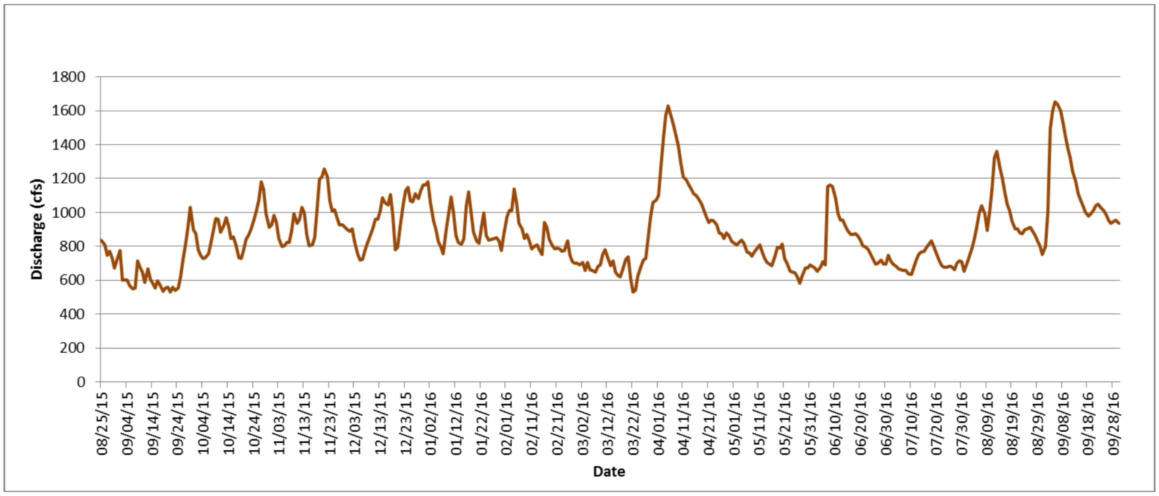

Figure 4.7: Wakulla Spring discharge for the study period, as measured at the Shadeville Road Bridge, USGS gauge 02327000.

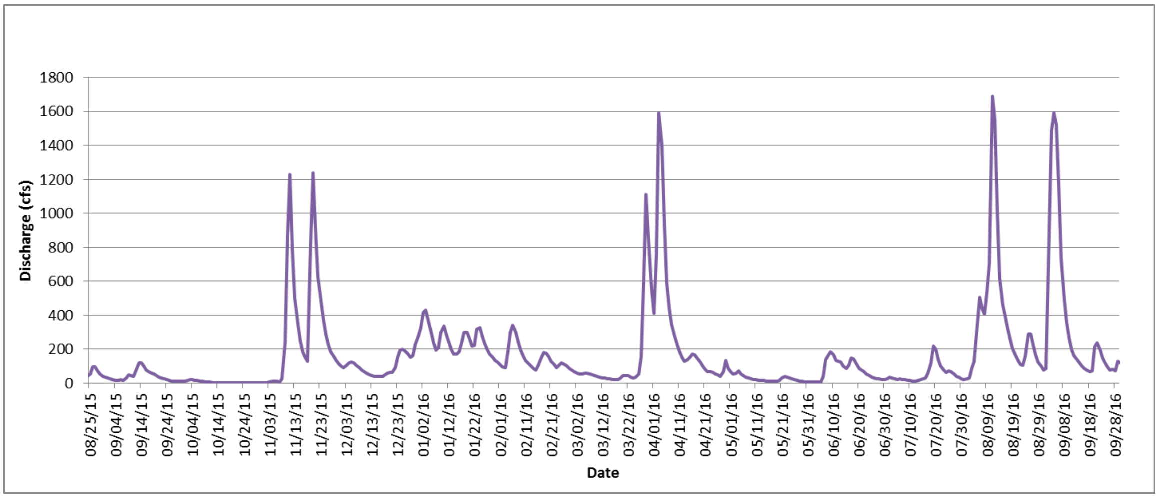

Figure 4.8: Lost Creek discharge for the study period, USGS gauge 02327033.

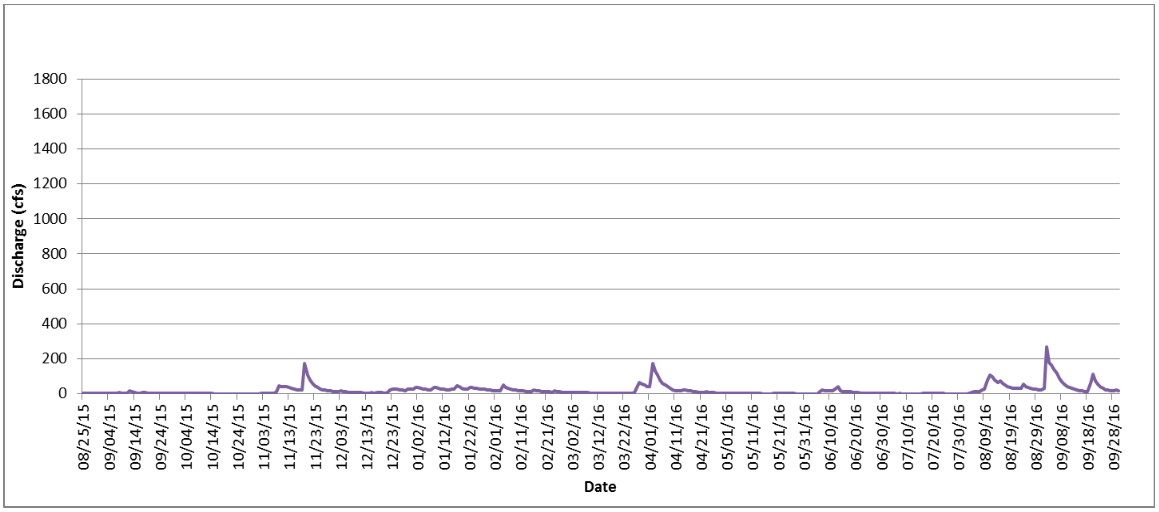

Figure 4.9: Black Creek discharge for the study period, USGS gauge 02326995.

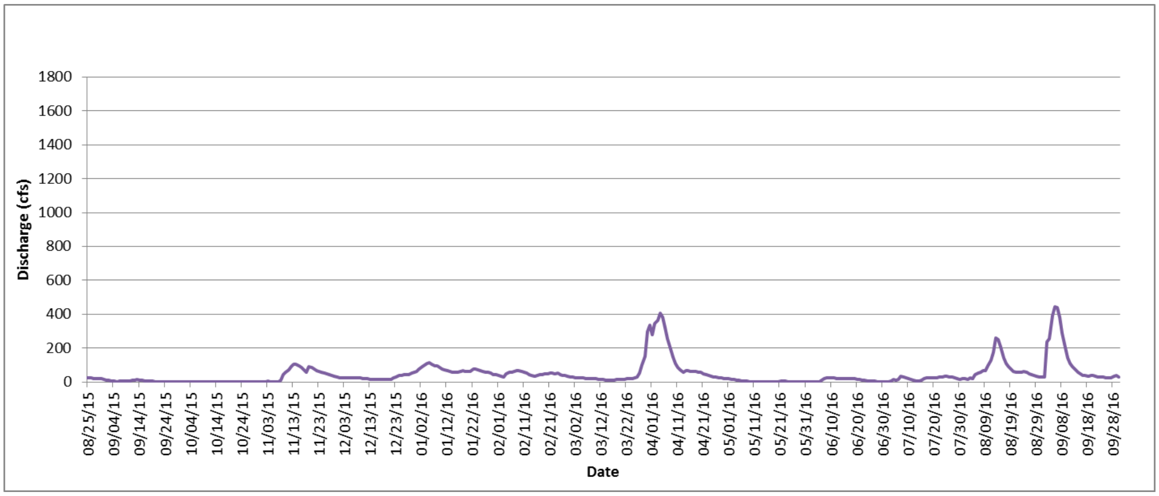

Figure 4.10: Fisher Creek discharge for the study period, USGS gauge 02326993.

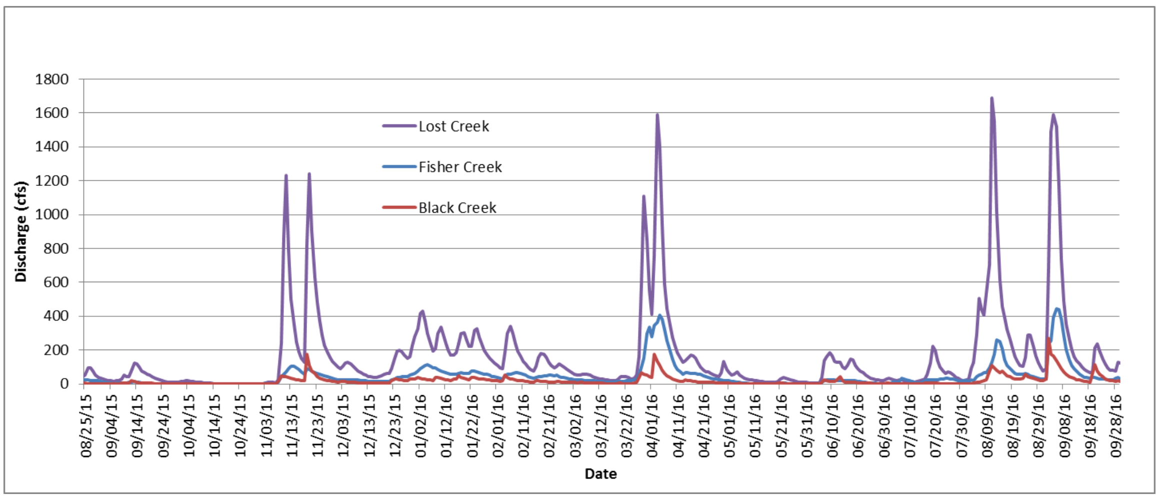

Figure 4.11: Comparison of sinking stream discharges for the study period: Lost, Black, and Fisher Creeks.

5. Flow Dynamics in the Coupled Springsheds: Wakulla and Spring Creek

Based on the work of Davis and Verdi (2014) and Dyer (2015), it is likely that the driving forces behind the flow regime of Wakulla Spring include (a) rainfall within the near springshed area, represented in this study by rainfall data from the Tallahassee Airport, (b) flow from the intermittent streams that lie to north, accounted for here by the discharges of Fisher and Black Creeks into their respective sinks, and (c) periodic discharges of Lost Creek into its sink when Spring Creek is not flowing and those discharges flow north to Wakulla Spring. Discharges by the three sinking streams generally respond directly to rainfall events within their watersheds except after prolonged periods of little or no rainfall. The flow regime of the Spring Creek springs is likely driven by (a) rainfall within its near springshed, represented in this study by Tallahassee Airport data, and (b) discharges from Lost Creek into its sink under those conditions when its discharges flow south to Spring Creek. We begin by examining the Spring Creek springs flow dynamics.

5.1 Spring Creek Springs Flow Dynamics

Davis and Verdi (2014) explain the periodic cessation and sometimes negative discharge of the Spring Creek springs as the result of prolonged periods of little or no rainfall coupled with higher mean sea level. Under these circumstances, the hydraulic head differential between the Spring Creek water table and sea level is sometimes too little to maintain flow from the Spring Creek springs. As noted above, the Spring Creek springs ceased flowing at some points of time during the study and per our model results, may have reversed at others (see figure 4.6).

The flow dynamics we observed during this study are consistent with those explanations. As shown in figure 5.1, regional rainfall and associated discharges from Lost Creek into its sink correspond well with discharges from the Spring Creek springs. The Spring Creek springs discharge peaks at nodes A, B, D, and E follow shortly after rain events that also are associated with peak discharges from Lost Creek. The restoration of flow at Spring Creek at node C coincides with a rainfall event measured at the Tallahassee Airport for which there is a considerably smaller increase in discharge from Lost Creek.

5.2 Wakulla Spring Flow Dynamics

Wakulla Spring's discharge reflects base flow from the Upper Floridan Aquifer within its very large springshed estimated at 1,600 to 2,900 square miles (Howard T. Odum Florida Springs Institute, 2014) and periodic pulses from the intermittently flowing sinking streams.

Looking at the effects of rainfall on Wakulla Spring flows, minor rainfall events of one inch or less have little apparent effect on spring discharge, but larger rainfall events are associated with discharge peaks (figure 5.2). Each of the major discharge peaks (nodes B-E) is associated with preceding rainfall patterns. Prior to the peak discharge at node B, rainfall shows little obvious relationship to discharge peaks. In particular two large rainfall events recorded at the Tallahassee Airport in November 2015 precede a very minor discharge peak at node A. The discharge peak at C follows closely after a major rainfall event measured at the Tallahassee Airport. Peaks at nodes B, D, and E each appear to be associated with the cumulative effects of several rain events.

As shown in figure 5.3, the impacts of the sinking streams on spring discharge, while sometimes pronounced, are generally short-lived. The dominant determinant of spring discharge is the base flow from the aquifer. With the exception of spring discharge peak C, the major peaks are associated with increases in flow from all three of the sinking streams. However, we cannot ascertain easily from these figures if Lost Creek discharges are flowing north to Wakulla Spring or flowing south to Spring Creek.

Figure 5.4, which displays Wakulla Spring discharge with rainfall plus discharges from Lost Creek and Spring Creek, offers some insight. This shows that Spring Creek was not flowing prior to Wakulla Spring discharge peak A, so Lost Creek flows should have been a major contributor to flow at Wakulla. However, the impact is relatively modest and short-lived, perhaps because of the relatively low rainfall prior to the rainfall event that the triggered peak A. Spring Creek discharge is near zero prior to Wakulla Spring discharge peaks B and D, suggesting that Lost Creek flows played a major role in those events. However, Spring Creek was flowing at a high level shortly thereafter and throughout the time of Wakulla Spring discharge peak E, indicating that Lost Creek flow would likely not have contributed to that discharge event at Wakulla Spring.

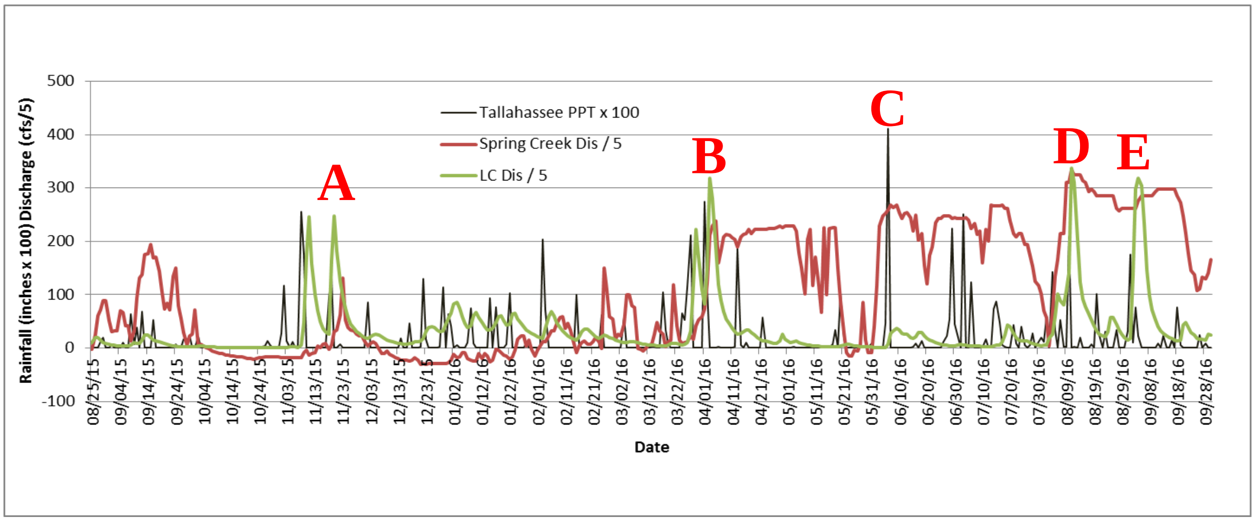

Figure 5.1: Spring Creek and Lost Creek discharges with rainfall.

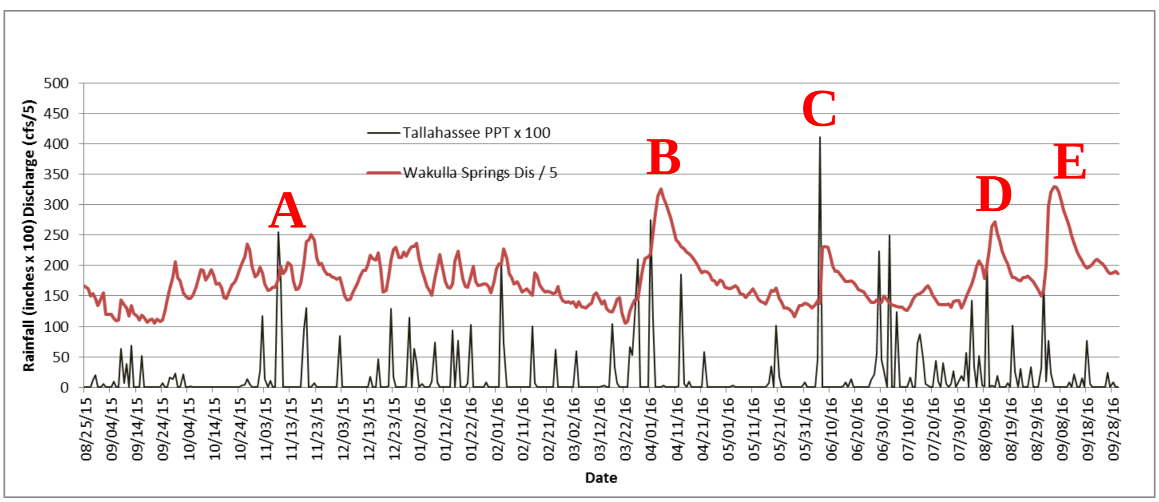

Figure 5.2: Wakulla Spring discharge with rainfall.

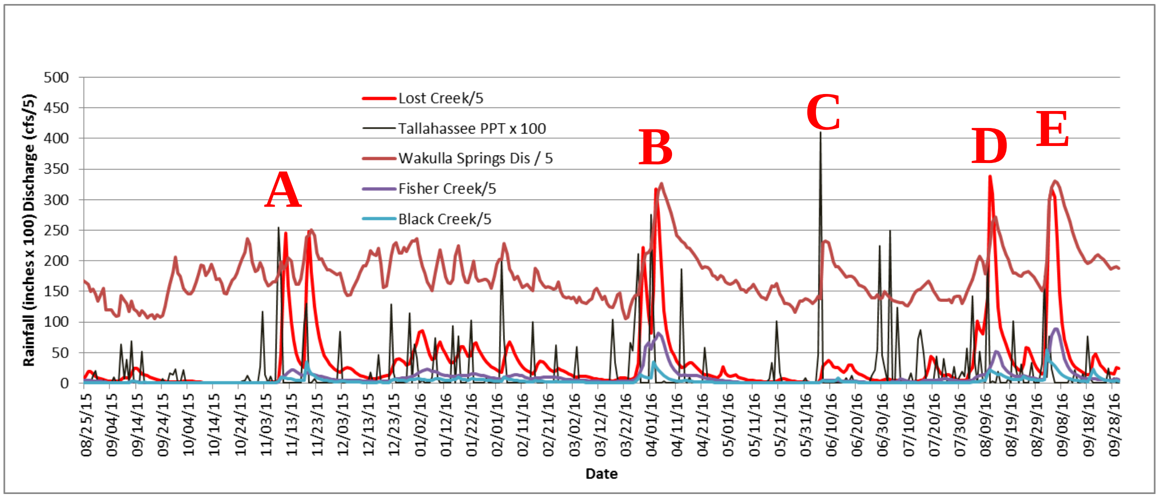

Figure 5.3: Wakulla Spring and sinking stream discharges with rainfall.

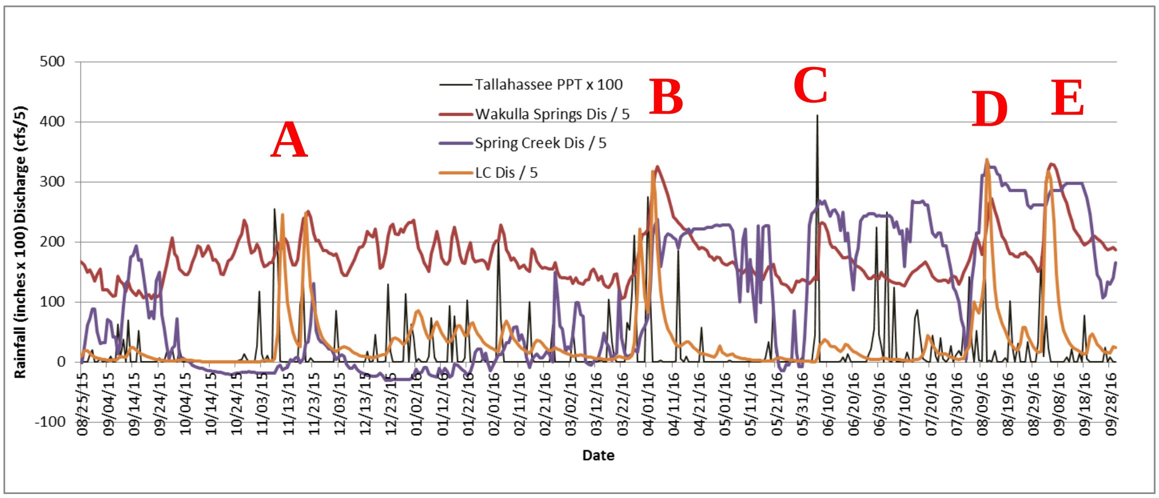

Figure 5.4: Wakulla Spring, Lost Creek, and Spring Creek discharges with rainfall.

6. Variation in Water Quality at Wakulla Spring

This chapter presents data for five water quality parameters: chlorophylls, true color, turbidity, specific conductance, and nitrates.

6.1 Chlorophylls

Chlorophyll levels are considered to be proportional to the algae content in aquatic systems and are used in trophic state analysis. We initially measured them weekly from samples collected at the boat dock then transitioned to sampling by boat from the boil which we did exclusively after 7/7/16. The methods and sampling techniques are detailed in Chapter 2. Chlorophyll measures used in this analysis include (a) corrected chlorophyll a (chlorophyll a corrected for phaeophytin), (b) phaeophytin, and (c) “total chlorophyll a” (sum of corrected chlorophyll a plus phaeophytin).

Chlorophyll a is the most common photosynthetically active pigment in plants and algae. When the porphyrin ring of the chlorophyll molecule is degraded, usually by the loss of its magnesium ion, this heterocyclic macrocycle organic compound falls apart and is analyzed as phaeophytin. Measuring phaeophytin separately and subtracting it from the total chlorophyll value provides a robust measure of the chlorophyll a in viable plant cells. This is the “corrected chlorophyll a” parameter used by the Florida Department of Environmental Protection in their Total Maximum Daily Load (TMDL) program (FDEP, 2011).

Because of the high rate of discharge of the spring (400 million gallons per day), we hypothesize that chlorophyll detected in the boil originates elsewhere and flows into the spring from the aquifer. The most likely sources are one or more of the large sinking lakes north of Wakulla Spring that discharge algae-laden water into the aquifer via sinkholes (McGlynn and Deyle, 2016).

Figures 6.1.1 – 6.1.3 present weekly corrected chlorophyll a and phaeophytin levels during the study period plus their sum. Colored data points indicate the sampling locations (blue at the boat dock, red at the spring boil).

Corrected chlorophyll a (figure 6.1.1) ranged from 0.00 to 2.94 ug/L with an average of 0.40 and a median of 0.10 (values below the method detection limit (MDL) of 0.178 ug/l coded as zero).[3] Thirty-seven percent of the observations were below the MDL. The average of 0.40 ug/L is less than one third that reported by the Howard T. Odum Florida Springs Institute (2014, p. 48) from the EPA STORET database for 12 samples collected between April 10 and November 28, 2006, i.e. 1.63. However, if we include only values greater than the FDEP STORET MDL of 0.55 ug/L, the average for the current study is 1.66 ug/L (see table 6.1.1). This value is higher than the averages for samples reported in the FDEP STORET data base for the boat dock and tram sampling stations for time periods extending to 2017 from 2013 and 2014 respectively. The average from our study applying the FDEP MDL also is twice the average reported for two samples collected from the boil during the Wetland Solutions Inc. survey in April 2009, i.e. 0.83 ug/L (2010b, p. G-4).

Corrected chlorophyll a levels at Wakulla Spring are far lower than those of the karst lakes in the springshed, which averaged over 20 ug/L during the study with algae bloom peaks of more than 300 ug/L. However, when compared to other springs in Florida, the average values recorded at the spring boil are similar or considerably higher. Wetland Solutions (2010b, pp. G-1 – G-4) reported an average of 0.99 ug/L for samples from the boils of ten other springs included in its 2008-2009 survey: DeLeon, Homosassa, Jackson Blue, Madison Blue, Manatee, Ponce de Leon, Rainbow, Silver, Silver Glen, and Weeki Wachee. The Wakulla Spring boil average during our study also is considerably higher

[3] Excludes outlying value of 8.5 ug/L measured on 8/25/15 which appears to be anomalous. Where duplicate values occur, dock reading increased by 0.05 to offset on figure 6.1.1.

than the average of 1.11 ug/L for historical data from six of the springs reported by Wetland Solutions (2010b): DeLeon Springs, Ichetucknee, Manatee, Rainbow, Silver, and Weeki Wachee. However, it is comparable to the average of 1.6 ug/L reported by Walsh et al. (2009) for six springs within the St. Johns River Water Management District (SJRWMD) sampled between 2004 and 2007: Alexander, De Leon, Gemini, Green, Silver Glen, and Wekiwa (65% of the samples were below the reported MDL of 0.1 ug/L).

Looking at maximum corrected chlorophyll a levels, we recorded five samples of 2.0 ug/L or greater at Wakulla with a maximum of 2.94. Only one of the ten springs sampled by Wetland Solutions in 2008-2009 yielded a spring boil corrected chlorophyll a level greater than 1.10 ug/L: Rainbow Spring at 2.10. Maxima for the historical data reported by Wetland Solutions ranged from 0.48 to 7.10 with four springs recording maxima greater than 2.0. The maximum reported by Walsh et al. was 5.7 ug/L from DeLeon Spring.[4]

Phaeophytin (figure 6.1.2) ranged 0.00 to 2.15 ug/L with an average of 0.32 and a median of 0.05 (values below the MDL of 0.178 ug/l coded as zero).[5] Forty-eight percent of the observations were below the MDL. Limiting the values to those greater than the FDEP STORET MDL of 0.40 ug/L, the average for 20 observations for the current study is 0.90 ug/L. As shown in table 6.1.2, our study average exceeds those for the limited number of observations reported in the FDEP STORET data base at the boat dock (N=3) and the tram (N=5) that exceeded the FDEP MDL.

While Wetland Solutions (2010) did not report phaeophytin values for its 2008-2009 study of Florida Springs, Walsh et al. (2009) report an average of 0.7 ug/L and a median of 0.3 for eight samples from six springs within the SJRWMD between 2004 and 2007 (61% of the samples were below the reported MDL of 0.1 ug/L): Alexander, De Leon, Gemini, Green, Silver Glen, and Wekiwa. The Wakulla Spring boil average of 0.90 during our study is considerably lower than the 3.68 ug/L average reported by the Springs Institute for 12 samples collected from the Wakulla Spring boil in 2006 and the 3.55 ug/L average for historical data from seven of the springs reported by Wetland Solutions (2010b): DeLeon, Ichetucknee, Manatee, Rainbow, Silver, Silver Glen, and Weeki Wachee. The 2006 Wakulla average is driven by a maximum value of 23 ug/l. The Wetland Solutions average of seven springs is driven by a very high maximum value from Rainbow Spring of 100 ug/l and an average of 19.60 ug/L in six samples collected in 2006. The average of the other six springs reported by Wetland Solutions is more nearly the same as Wakulla during our study: 0.87 ug/L.

Figure 6.1.3 presents the sum of corrected chlorophyll a and phaeophytin for the study period. As figure 6.1.4 reveals, rainfall, as measured at the Tallahassee Airport, has no apparent effect on total chlorophyll a (linear regression model is not statistically

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[4] Excluding samples from the Lake Apopka spring which discharges on the bottom of that highly eutrophic lake and a single sample from Bugg Spring, of 24.8 ug/L collected 300 feet downstream from the vent.

[5] Excludes outlying value of 9.2 ug/L measured on 1/7/16 which appears to be anomalous. Where duplicate values occur, dock reading increased by 0.05 to offset on figure 6.1.2.

significant). Neither does the inflow to the aquifer from the combined flows of Black Creek and Jump Creek 10 days prior to total chlorophyll measurements at the spring boil (figure 6.1.5).[6] or the inflows from Lost Creek 45 days prior when its flow is diverted north to Wakulla Spring when the Spring Creek springs are not flowing (figure 6.1.6).[7] These findings suggest that chlorophyll levels at the spring boil are not directly influenced by precipitation within the springshed or by sinking stream inflows to the aquifer.

6.2 True Color

We measured true color light absorbance at 465 nm from samples filtered to remove suspended matter. This is a wavelength absorbed by the tannin pigments that impart the reddish-brown to yellowish apparent color in the sinking streams that discharge through swallets into the Upper Floridan Aquifer in areas that flow into Wakulla Spring: Black Creek, Fisher Creek, Jump Creek, and Lost Creek (Kulakowski, 2010).[8] We initially measured true color weekly from samples collected at the boat dock then transitioned to sampling by boat from the boil which we did exclusively after 7/7/16. The methods and sampling techniques are detailed in Chapter 2. True color values over 40 PtCo (Platinum-Cobalt) units are considered to be "black, dark, or tannic."

Wakulla Spring is more tannic than many other major springs in Florida, and it appears that tannin levels have increased over the long term. As shown in figure 6.2.1 and table 6.2.1, weekly true color varied from 4.7 to 136.4 PtCo units during the study period with an average of 22.7 and a median of 12.6 PtCo units (for all samples greater than the FDEP STORET MDL of 2.5 PtCo units).

Table 6.2.1 reveals that the study period mean is somewhat higher than the means for STORET data sampled from the boil between 2009 and 2014 and from the boat dock between 2013 and 2015. The study period median of 12.6 PtCo is substantially greater than the STORET medians. Combining the STORET boil data with those from our study reveals a trivial increasing trend with an R^2 of 0.03 significant at only the 90% level (F-test p-value = 0.098). However, our study average of 22.75 is substantially higher than the STORET 40-year average of 4.2 (1966-2006) reported by the Florida Springs Institute (2014). It also is much higher than both the near-term (2008-2009) and long-term (1946-2008) averages reported by Wetland Solutions for 10 other springs in the state: De Leon Springs, Homosassa Springs, Ichetucknee Springs, Jackson Blue Spring, Madison Blue Spring, Manatee Springs, Rainbow Springs, Silver Springs, Silver Glen Springs, and Weeki Wachee Springs.

[6] Dye studies demonstrated a 9- to 10-day travel time from Fisher and Black Creek sinks to Wakulla Spring (http://www.geohydros.com/FGS/Tracing/).
[7] Dye studies demonstrate a 45- to 47-day travel time from the Lost Creek sink to Wakulla Spring (http://www.geohydros.com/FGS/Tracing/; Dyer (2015)).
[8] Tannins are the predominant type of colored dissolved organic matter or CDOM in freshwater springs (USGS, 1995). Other CDOM components, e.g. fulvic and humic acids, absorb light most strongly in the ultra violet range at short wavelengths outside the visible spectrum (Bricaud et al., 1981; Ghabbour and Davies, 2009; Kumada, 1955).

Four peaks occurred during the study period. Figure 6.2.2 depicts weekly true color with flows from the two sinking streams to the north of Wakulla Spring for which flow data are available, Black Creek and Fisher Creek, measured 10 days prior to reflect the travel time from their sinks to Wakulla Spring established by dye studies. Three of the true color peaks occur within a few days of peaks 10 days prior in the flows of these two sinking streams into their swallets: A, B, and D. Linear regression analysis of the relationship yields a model with a coefficient of determination (R^2) of 0.32 that is significant at better than the 99.999% level (F-test p-value = 9.06×10^{-6}). In other words, the combined flows of the two sinking streams measured 10 days prior explain 32 percent of the observed variation in tannin levels at the spring measured as true color.

Figure 6.2.3 presents true color with flows at Lost Creek and Spring Creek 45 days prior. No substantial increases in true color appear to be associated with Lost Creek flows 45 day prior when Spring Creek was nearly not flowing. Including these Lost Creek flows 45 days prior as a second variable with the summed flows of Black Creek and Fisher Creek 10 days prior does not improve the regression model for true color. Although the model remains significant (99.99% level), the adjusted R^2 value remains the same at 0.30.[9]

As shown in figure 6.2.4 during much of the study period (10/21/15 - 6/10/16), true color and total chlorophyll a exhibit a generally inverse relationship consistent with the patterns in figures 6.1.5, 6.1.6, 6.2.2, and 6.2.3, i.e. higher stream flows carry more tannins and other CDOM into the spring while diluting chlorophyll carried in the base flow of the aquifer. However, the relationship is not statistically significant. During the first month of the study (9/3/15 – 10/15/15) and during the latter few months of the sampling period (6/15/16 – 9/29/16) the two parameters seem to run in parallel, but again the relationships are not statistically significant.

6.3 Turbidity

"Turbidity is caused by the presence of suspended and dissolved matter, such as clay, silt, finely divided organic matter, plankton and other microscopic organisms, organic acids, and dyes" (Anderson, 2005, p. TBY-3). Thus, turbidity is an aggregate measure that will reflect the presence of algae cells measured as corrected chlorophyll a and phaeophytin as well as tannins measured as true color, in addition to other CDOM and suspended particulates. Turbidity is typically very low in spring outflow in Florida (Wetland Solutions Inc., 2010a) and is not likely to be a very significant factor in freshwater spring light regimes. We did not collect turbidity data during our study, relying instead on data recorded at the Northwest Florida Water Management District gauge on the boat dock.[10] Those data, as well as historical data, confirm that turbidity levels are low at Wakulla

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[9] The adjusted R^2 statistic accounts for the number of independent variables in a regression model. It only increases if the additional independent variable improves the predictive power of the model more than would be expected by chance. (http://blog.minitab.com/blog/adventures-in-statistics-2/multiple-regession-analysis-use-adjusted-r-squared-and-predicted-r-squared-to-include-the-correct-number-of-variables).

[10] No spring boil turbidity data are currently available from STORET for the period 1/1/97 to 2/6/19 or from FDEP's successor WIN data base.

Spring. Figure 6.3.1 presents weekly boat dock turbidity data, measured as nephelometric turbidity units (NTU), recorded during this study comprising 58 observations between 9/3/15 and 9/29/16. The mean for these values is 0.21 NTU with a median of 0.16 and a range of 0.06 to 1.01.

As shown in table 6.3.1, these values are lower than the long-term STORET database average of 0.44 NTU from 1970 to May 2008 reported for the spring boil by the Howard T. Odum Florida Springs Institute (2014, p. 46). Our maximum also is smaller than that reported by the Springs Institute: 1.01 versus 6.20 NTU. Levels measured at Wakulla Spring on two dates in April 2009 by Wetlands Solutions, Inc. (2010b, p. G-4) averaged 0.27 NTU near the boil and 0.34 at the boat dock suggesting that boat dock data may be measuring turbidity produced within the spring bowl as well as that present in the vent discharge at the boil.

The 0.21 NTU average turbidity level at the Wakulla boat dock during the study period also is lower than the 0.27 NTU average for the 10 other springs in Florida (De Leon, Homosassa, Ichetucknee, Jackson Blue, Madison Blue, Manatee, Rainbow, Silver, Silver Glen, and Weeki Wachee) surveyed by Wetland Solutions (2014b) in 2008 and 2009 and substantially lower than the mean average of 0.54 reported by Wetland Solutions (2014b) for those springs between 1966 and 2008. However, it is nearly the same as the mean of 0.20 reported for six springs by Walsh et al. (2009).

Algae cells comprise some portion of turbidity. However, as shown in figures 6.3.2 and 6.3.3 there is not a strong correlation between our measures of corrected chlorophyll a or phaeophytin and turbidity. A simple regression model of turbidity and corrected chlorophyll a is not significant at the 90% or better level. The model for phaeophytin and turbidity is significant at the 95% level (F-test p-value = 0.0489), but the R^2 value is only 0.07. A multiple regression model including both corrected chlorophyll a and phaeophytin is not significant at the 90% level or better.

Tannins and other CDOM also may contribute to turbidity measurements, however, while there are some parallels evident in figure 6.3.4 at some times during the study period, a regression model of our true color measurements with the boat dock turbidity data is not significant at the 90% level or better. We do, however, find a correlation between turbidity at the boat dock and discharges from the two sinking streams north of Wakulla Spring, Black and Fisher Creeks. Figure 6.3.5 shows turbidity with the summed flows of these creeks 10 days prior. A regression model of this relationship has an R^2 of 0.22 that is significant at the 99.9% level. Adding Lost Creeks flows 45 days prior, at times when Spring Creek flows are less than or equal to zero, to the summed flows of Black and Fisher Creeks 10 days prior, yields a significant model, but with a lower R^2 and adjusted R^2. This indicates that addition of the Lost Creek flow data does not enhance the explanatory power of the regression model. Similar effects result from including the Lost Creek flow data as a separate independent variable. It appears, nonetheless, that some portion of the turbidity measured at the boat dock is comprised of particles other than algae cells and/or CDOM other than that measured as true color.

6.4 Specific Conductance

Specific conductance serves as a proxy for the concentration of ions in solution by measuring the ability of a solution to conduct electricity. We measured it from weekly samples prior to 10/15/15 supplemented by daily samples thereafter collected off the boat dock at Wakulla Spring State Park. The methods and sampling techniques are detailed in chapter 2. Typically, in karst systems the calcium carbonate ions that comprise the lime rock come into equilibrium and dissolve into the ground water yielding specific conductance levels that range from 300 to 400 micro Siemens per meter (uS/m). Rainwater has almost no specific conductance, and the area lakes and sinking streams have typically less than 50 uS/m. Salt water, on the other hand, exhibits about 40,000 uS/m.

As shown in figure 6.4.1, specific conductance samples from Wakulla Spring ranged from about 75 to 400 uS/m during the study period with an average of 298 uS/m. Values at the lower end of the range are not typical of those found in most karst systems. These dips in specific conductance values may reflect substantial fresh water intrusion into the aquifer, perhaps from periodic inflow from the sinking streams. However, as shown in figure 6.4.1, no correlation is evident between specific conductance at Wakulla Spring and the discharge from the dominant sinking stream, Lost Creek, during those times when Spring Creek has stopped flowing or flow is very low and Lost Creek discharges to the aquifer are assumed to be flowing north to Wakulla Spring.

6.5 Nitrate

Background levels of nitrate, a form of oxidized nitrogen (NO_3), are typically quite low in natural karst systems, originating primarily from atmospheric deposition on the land surface and runoff from soils into sinking streams. Nitrates are mobile in soils, having a low capacity for adsorption. Human sources predominate in the Wakulla springshed. Septic tank loading replaced wastewater treatment plant loading as the primary nitrate source after retrofit at the City of Tallahassee’s Thomas P. Smith Water Reclamation Facility in 2012 to meet advanced wastewater treatment standards (FDEP, 2015). We measured nitrate from weekly samples prior to 10/15/15 supplemented by daily samples thereafter collected off the boat dock at Wakulla Springs State Park. The methods and sampling techniques are detailed in chapter 2.

As shown in figure 6.5.1, concentrations varied considerably with a range of 0.2 to 0.7 mg/L during the study period and an average of 0.373 mg/L. The Total Maximum Daily Load (TMDL) target set by FDEP is a monthly average of 0.35 mg/L (Gilbert, 2012). As also shown in figure 6.5.1 there is no relationship between northerly flows of Lost Creek (when Spring Creek is very low or not flowing) and nitrate levels.

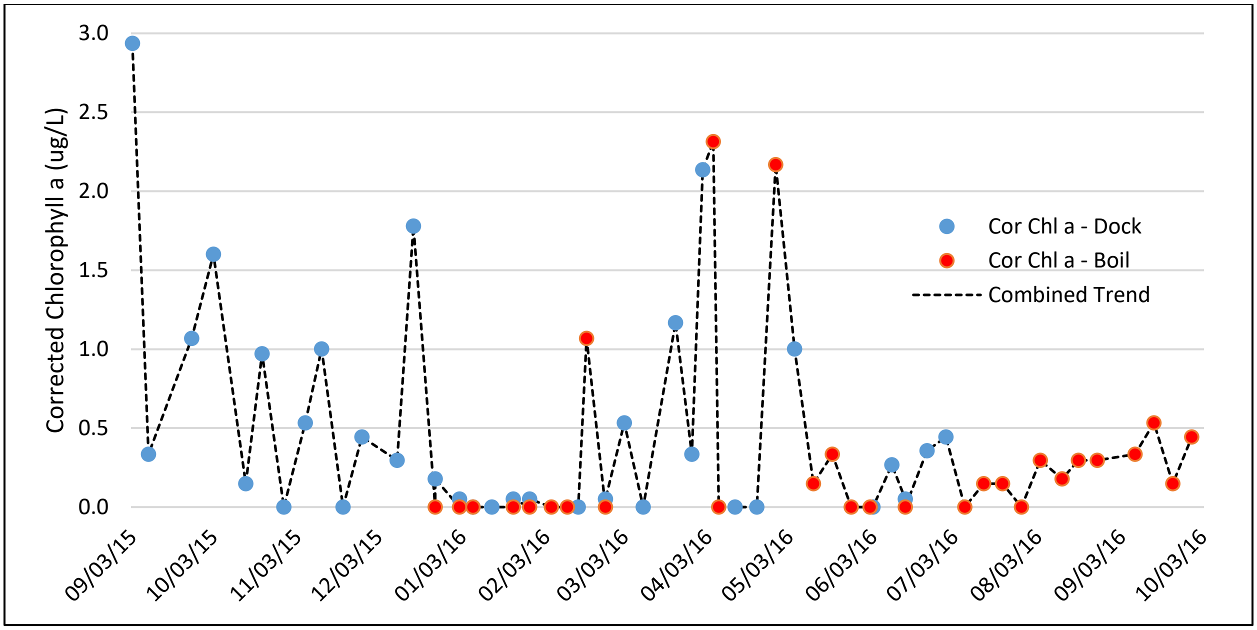


Figure 6.1.1: Weekly levels of corrected chlorophyll a at the boat dock and the spring boil, 09/03/15 – 09/29/16. Where duplicate values occur, dock reading increased by 0.05 ug/L.

Source	Sample Station	Dates	Sample Size	Range	Average	Median
Current Study	Wakulla boil and boat dock	09/03/15 – 09/29/16	13	0.97 – 2.94	1.66	1.60
FDEP STORET	Wakulla boat dock (#44061)	06/10/14 - 04/26/17	7	0.55 – 1.50	0.90	0.77
FDEP STORET	Wakulla tram (#44059)	10/30/13 – 7/26/17	10	0.55 – 2.10	0.98	0.90
EPA STORET (Springs Institue, 2014)	Wakulla boil	4/10/06 – 11/28/06	12	1.00 - 5.30	1.63	n/a
Wetland Solutions (2010b)	Wakulla boil	4/13/09 - 4/16/09	2	0.55 – 1.10	0.83	n/a
	10 springs at boil/vent	2008-2009	20	0.55 - 2.10	0.99	1.10
		1999-2008	177	n/a	1.11	n/a
Walsh et al. (2009)	Six springs at or near boil/vent	2004 - 2007	5	0.2 – 5.7	1.6	0.8

Table 6.1.1: Correcetd chlorophyll a concentrations (ug/L) for the study compared to other Wakulla Spring and River data and other springs (MDL = 0.55 ug/L).

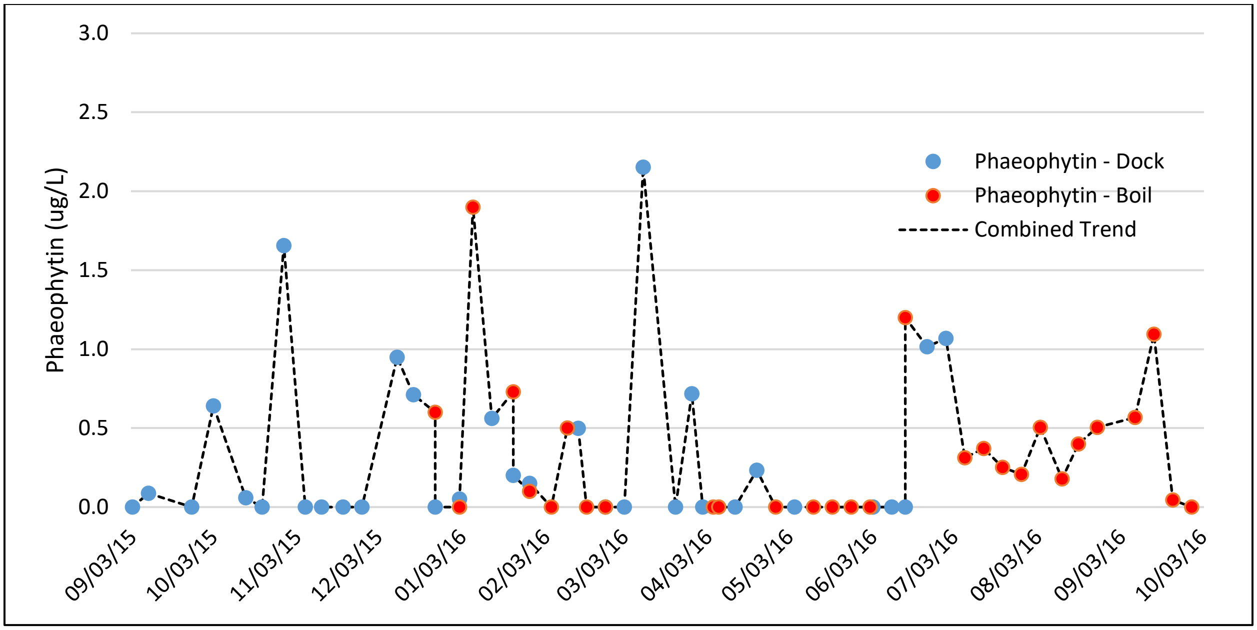


Figure 6.1.2: Weekly levels of phaeophytin at the boat dock and the spring boil, 09/03/15 – 09/29/16. Where duplicate values occur, dock reading increased by 0.05 ug/L.

Source	Sample Station	Dates	Sample Size	Range	Average	Median
Current Study	Wakulla boil and boat dock	09/03/15 – 09/29/16	20	0.40 – 2.15	0.90	0.72
FDEP STORET	Wakulla boat dock (#44061)	06/10/14 - 04/26/17	3	0.40 – 0.43	0.41	0.41
FDEP STORET	Wakulla tram (#44059)	10/30/13 – 7/26/17	5	0.45 – 1.50	0.69	0.53
EPA STORET (Springs Institue, 2014)	Wakulla boil	4/10/06 – 11/28/06	12	1.00 – 23.00	3.68	n/a
Wetland Solutions (2010b)	10 springs at boil/vent	1988-2008	202	n/a	3.55	n/a
	Nine springs at boil/vent (excluding Rainbow Springs)	1988-2008	196	n/a	0.87	n/a
Walsh et al. (2009)	Six springs at or near boil or vent	2004 - 2007	8	0.1 – 1.8	0.7	0.3

Table 6.1.2: Phaeophytin concentrations (ug/L) for the study compared to other Wakulla Spring and River data and other springs (MDL = 0.40 ug/L).

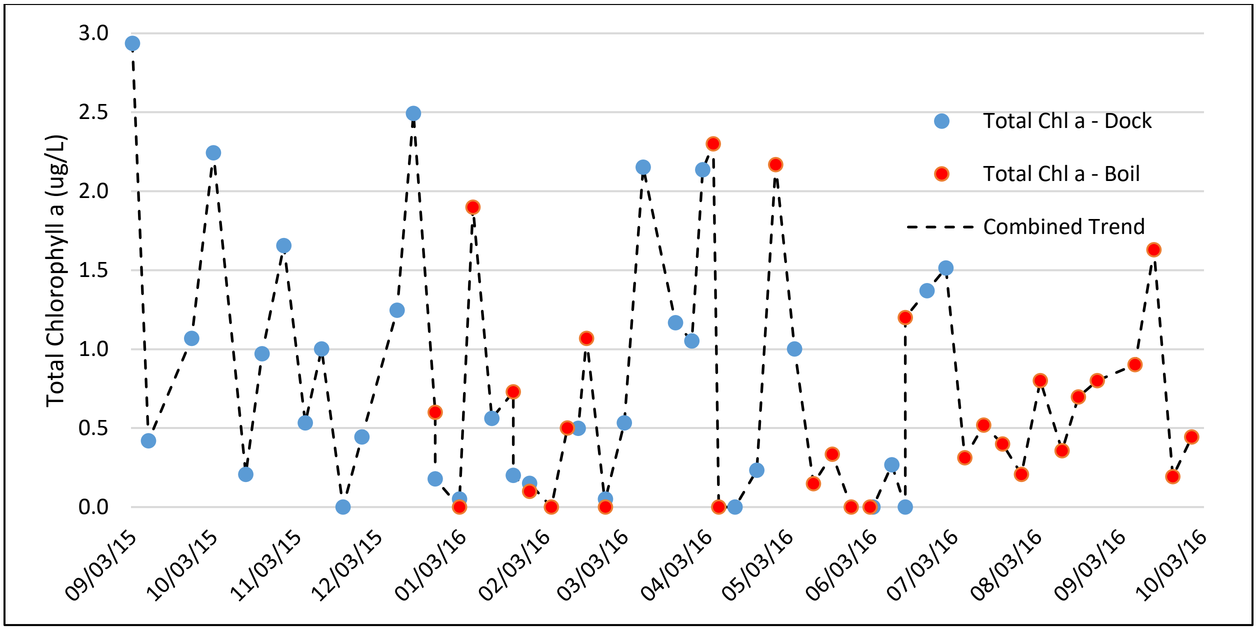


Figure 6.1.3: Weekly levels of total chlorophyll a (corrected chlorophyll a + phaeophytin) at the boat dock and the spring boil, 09/03/15 – 09/29/16. Where duplicate values occur, dock reading increased by 0.05 ug/L.

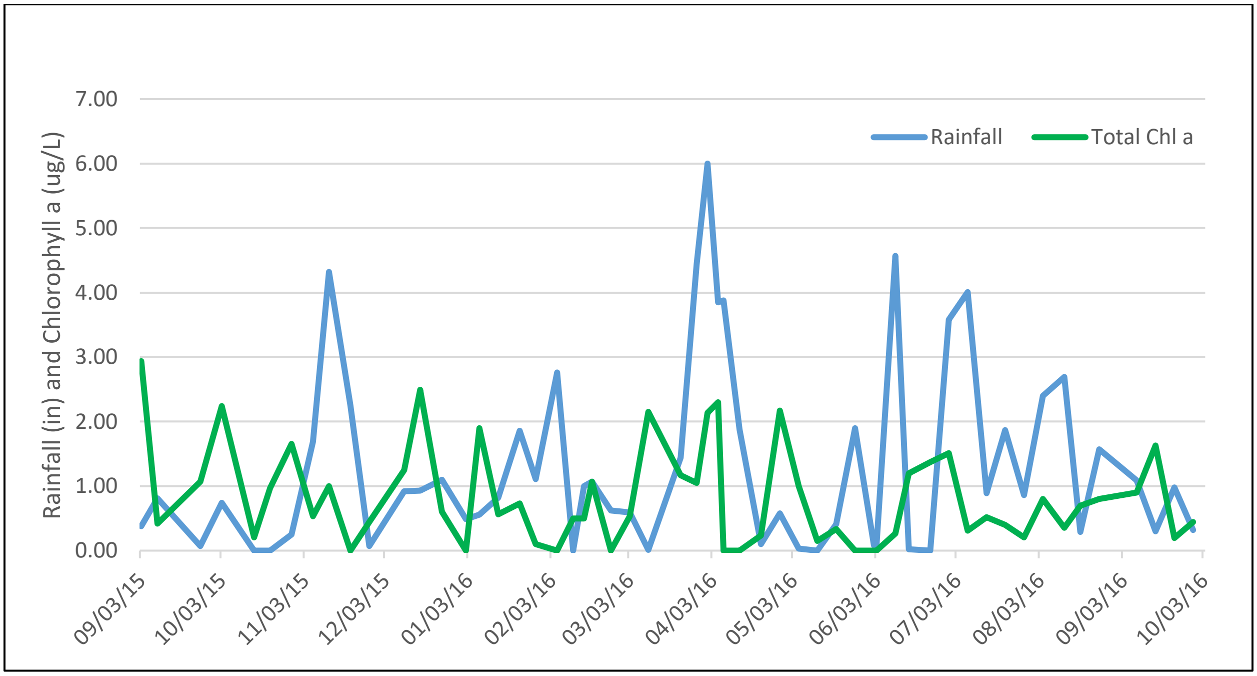


Figure 6.1.4: Weekly total chlorophyll a and rainfall at Tallahassee Airport, 09/03/15 – 09/29/16.

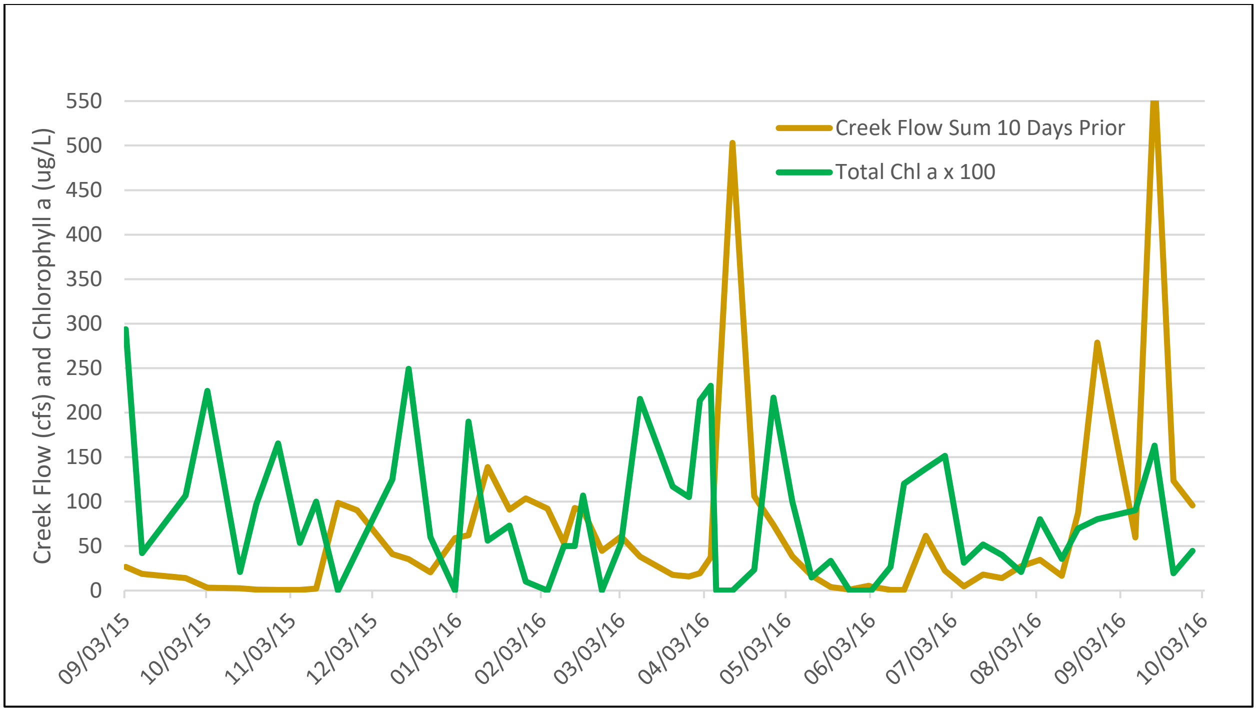


Figure 6.1.5: Weekly total chlorophyll a with summed flows of Black and Fisher Creeks 10 days prior, 09/03/15 – 09/29/16.

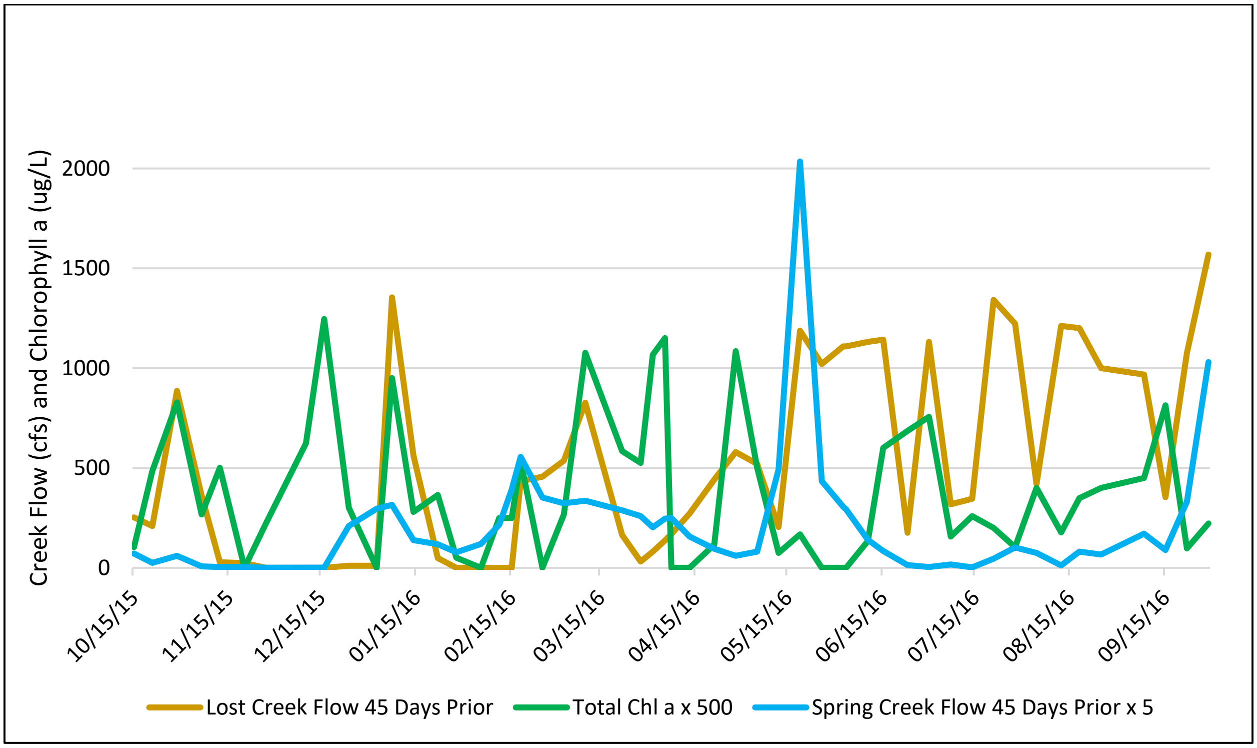


Figure 6.1.6: Weekly total chlorophyll a with Lost and Spring Creek flows 45 days prior, 10/15/15 – 9/29/16.

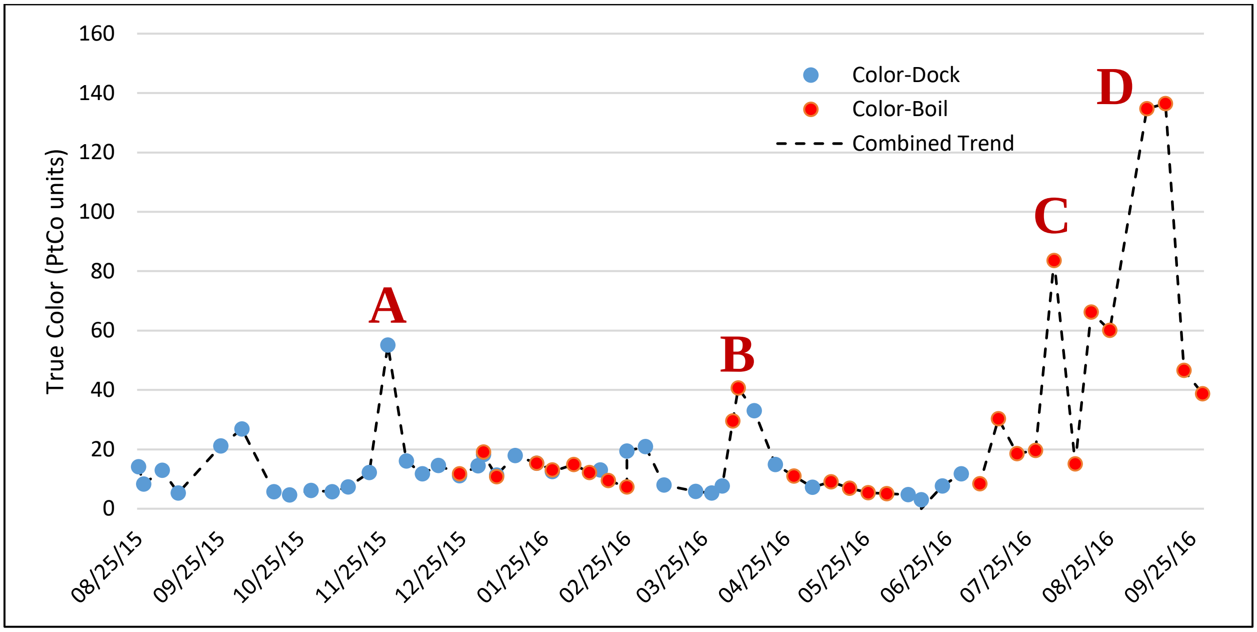


Figure 6.2.1: Weekly levels of true color at the boat dock and the spring boil, 09/03/15 – 09/29/16. Where duplicate values occur, dock reading increased by 0.05 ug/L.

Source	Sample Station	Dates	Sample Size	Range	Average	Median
Current Study	Wakulla boil and boat dock	09/03/15 – 09/29/16	54	4.7 – 136.4	22.7	12.6
FDEP STORET	Wakulla boil (#9695)	09/23/09 - 12/2/14	26	0.0 - 82.0	18.7	5.0
FDEP STORET	Wakulla boat dock (#44061)	10/31/13 – 8/18/15	23	23.0 - 56/0	16.5	9.3
EPA STORET (Springs Institue, 2014)	Wakulla boil	5/19/66 – 12/5/06	108	0.0 – 40.0	4.2	n/a
Wetland Solutions (2010b)	Wakulla boil	4/13/09 – 4/16/09	2	50.0 – 70.0	60.0	n/a
	10 springs at boil/vent	2008-2009	20	2.5 – 5.0	3.1	2.5
		1946-2008	3,104	0.0 – 160.0	3.4	n/a

Table 6.2.1: True color (PtCo units) for the study compared to other Wakulla Spring data and other springs (MDL = 2.5 PtCo units).

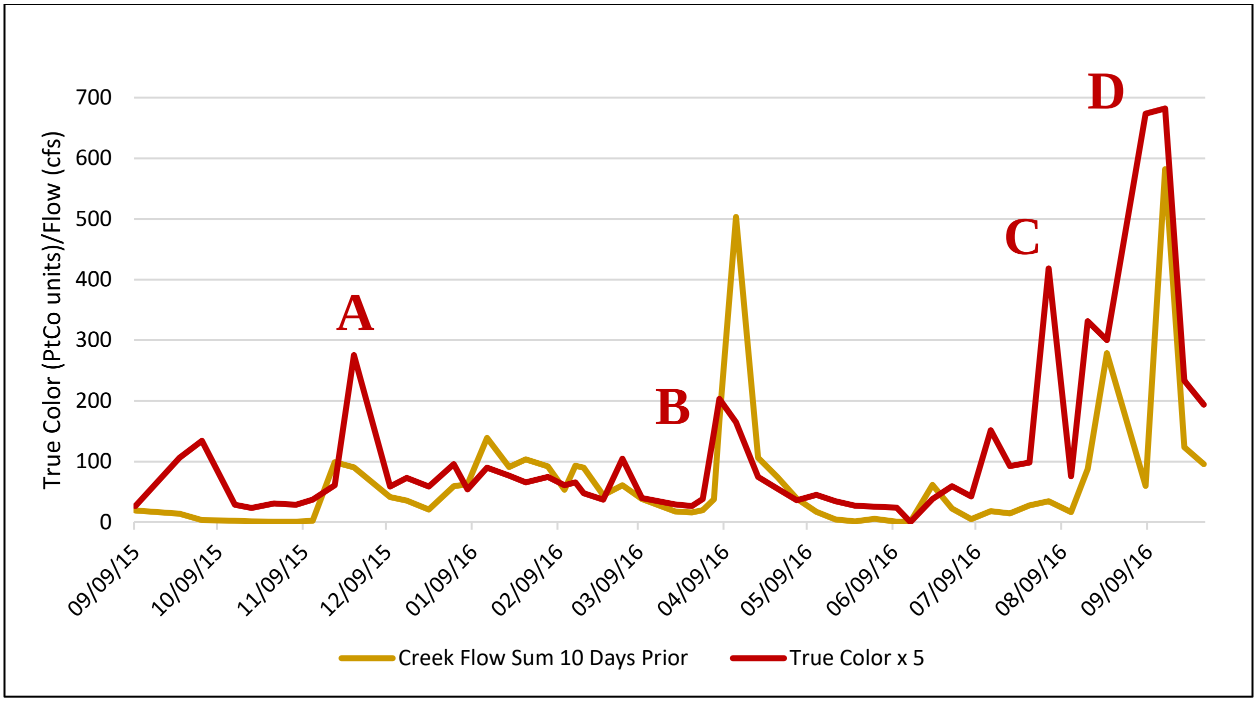


Figure 6.2.2: Weekly true color with summed flows of Black and Fisher Creeks 10 days prior, 09/09/15 – 09/29/16.

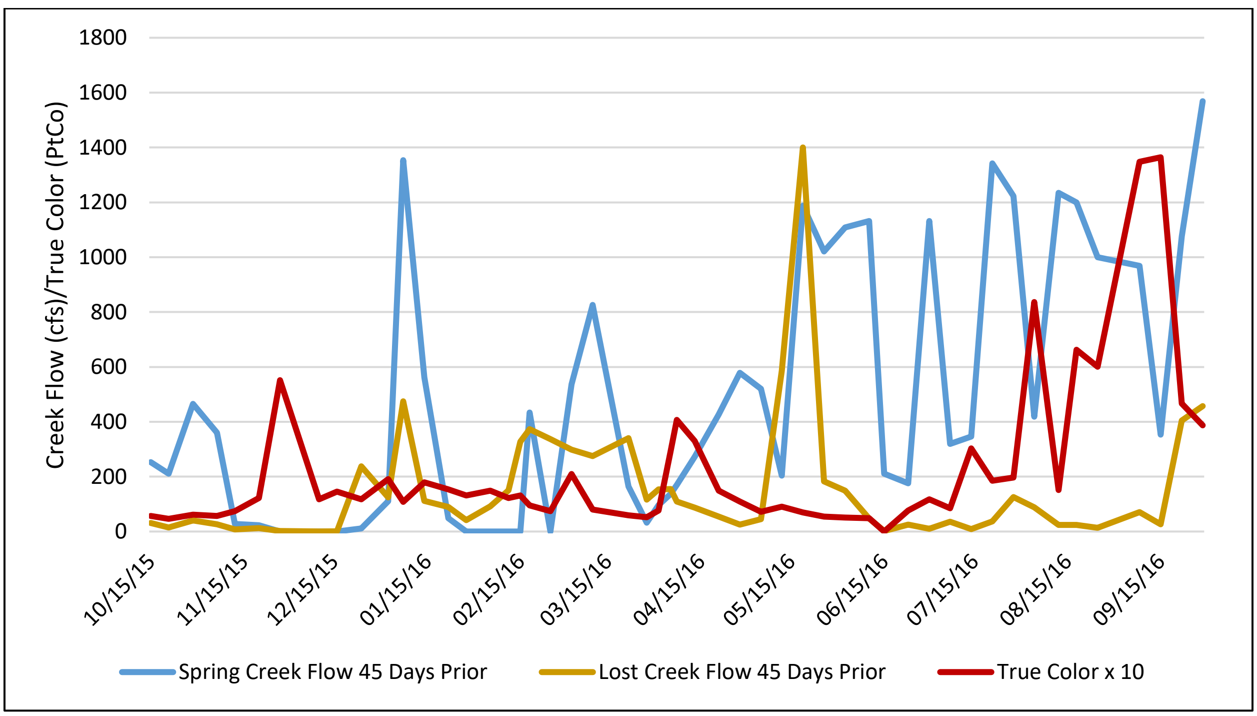


Figure 6.2.3: Weekly true color with discharges of Lost Creek and Spring Creek 45 days prior, 10/15/15 – 09/29/16.

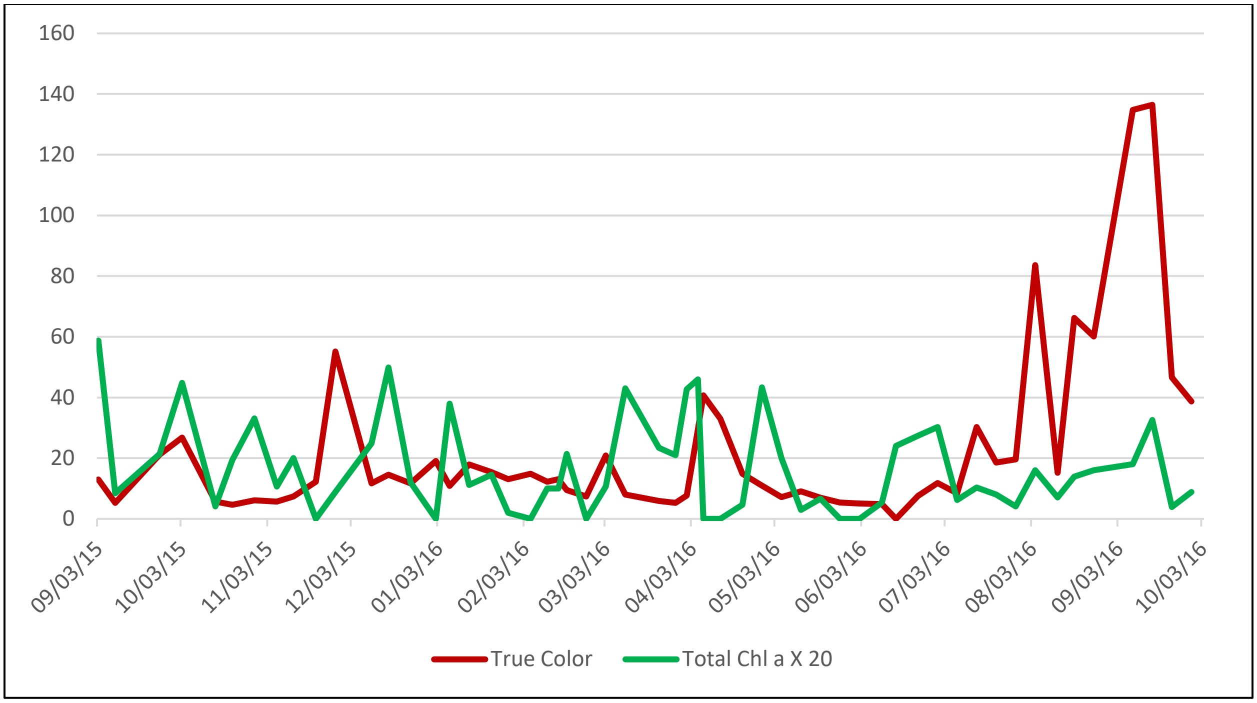


Figure 6.2.4: Weekly true color and total chlorophyll a, 09/03/15 – 09/29/16.

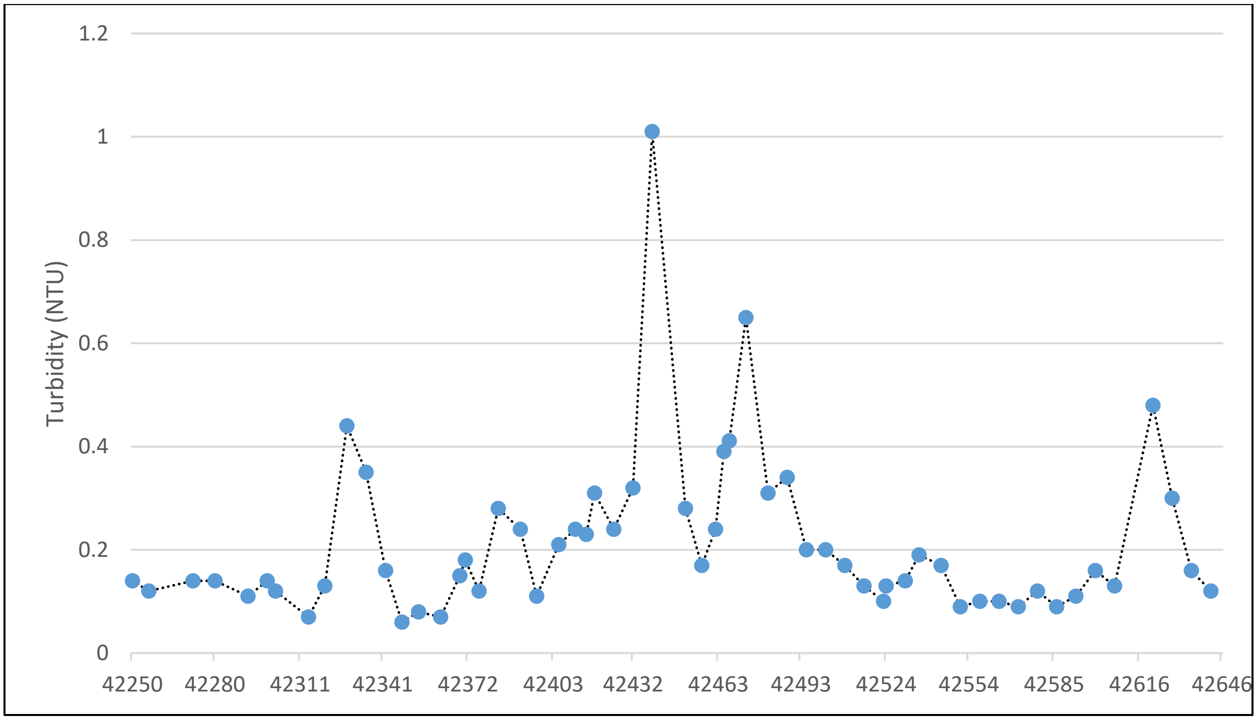


Figure 6.3.1: Weekly turbidity at the boat dock, 09/03/15 – 09/29/16.

Source	Sample Station	Dates	Sample Size	Range		Average	Median
NWFWMD	Wakulla boat dock (#44061)	09/03/15 – 09/29/16	58	0.06 – 1.01		0.21	0.16
EPA STORET (Springs Institue, 2014)	Wakulla boil	1970-2008	132	0.00 – 6.20		0.44	n/a
Wetland Solutions (2010b)	Wakulla boil	4/13/09 - 4/16/09	2	0.22 - 0.31		0.27	n/a
	Wakulla boat dock	4/13/09 - 4/16/09	2	0.29 – 0.38		0.34	n/a
	Multiple springs at boil/vent	2008-2009	20	0.02 - 0.9		0.27	0.12
		1966-2008	2,318	n/a		0.54	n/a
Walsh et al. (2009)	Multiple springs at or near boil/vent	2004-2007	13	0.0 – 0.6		0.20	0.10

Table 6.3.1: Turbidity for the study compared to other Wakulla Spring and River data and other springs (MDL = 0.10 NTU).

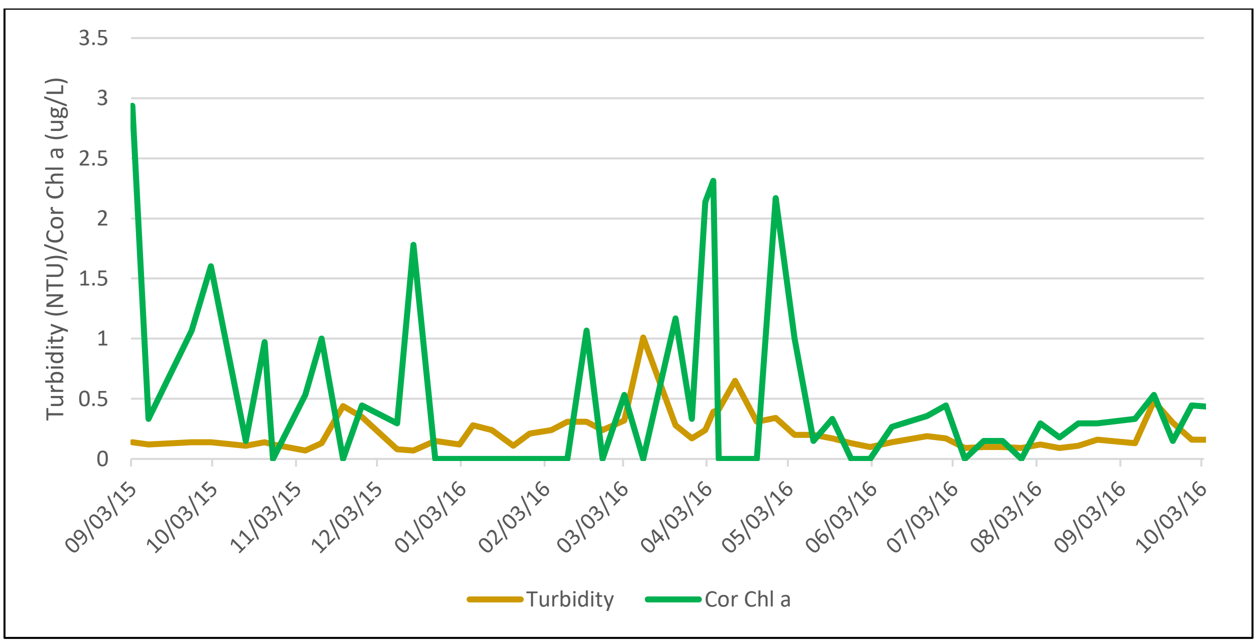


Figure 6.3.2: Weekly turbidity and corrected chlorophyll a, 09/03/15 – 09/29/16.

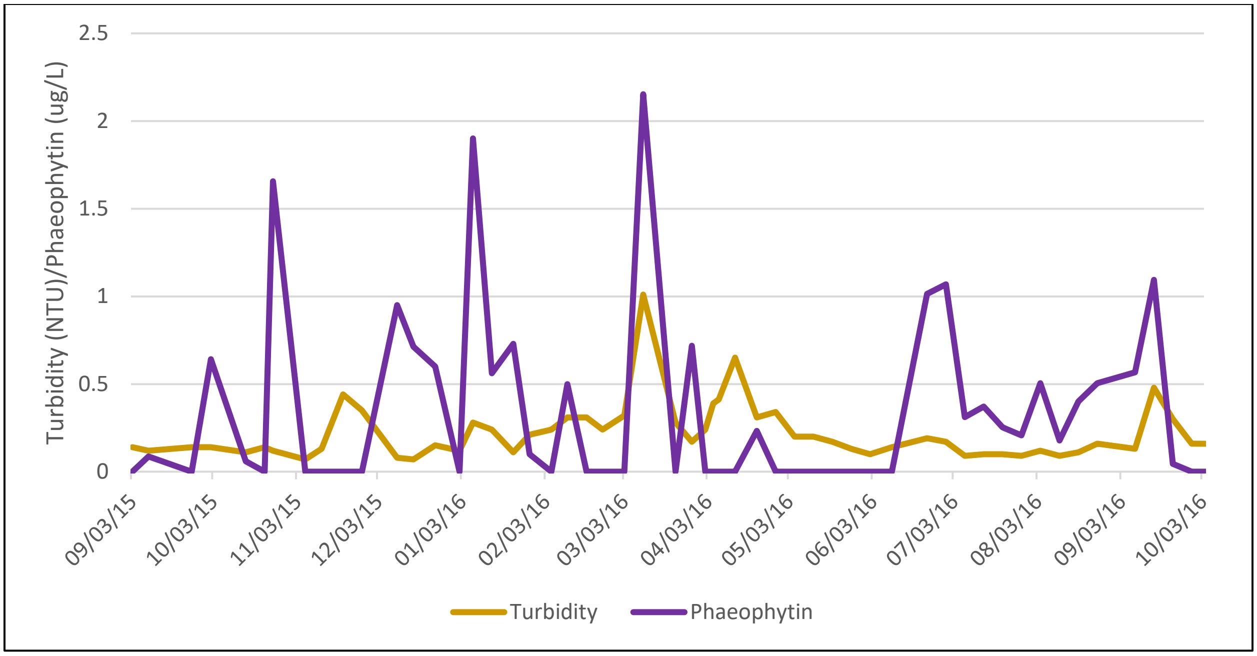


Figure 6.3.3: Weekly turbidity and phaeophytin, 09/03/15 – 09/29/16.

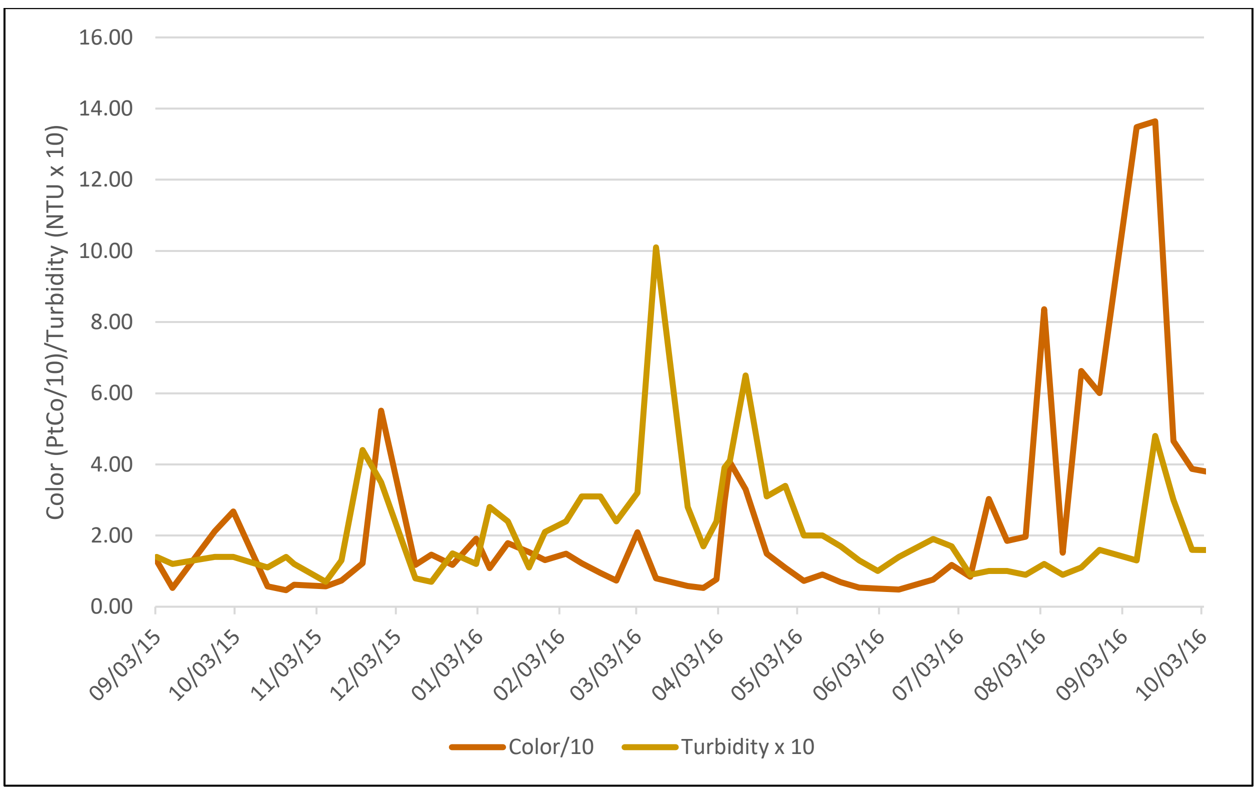


Figure 6.3.4: Weekly turbidity and true color, 09/03/15 – 09/29/16.

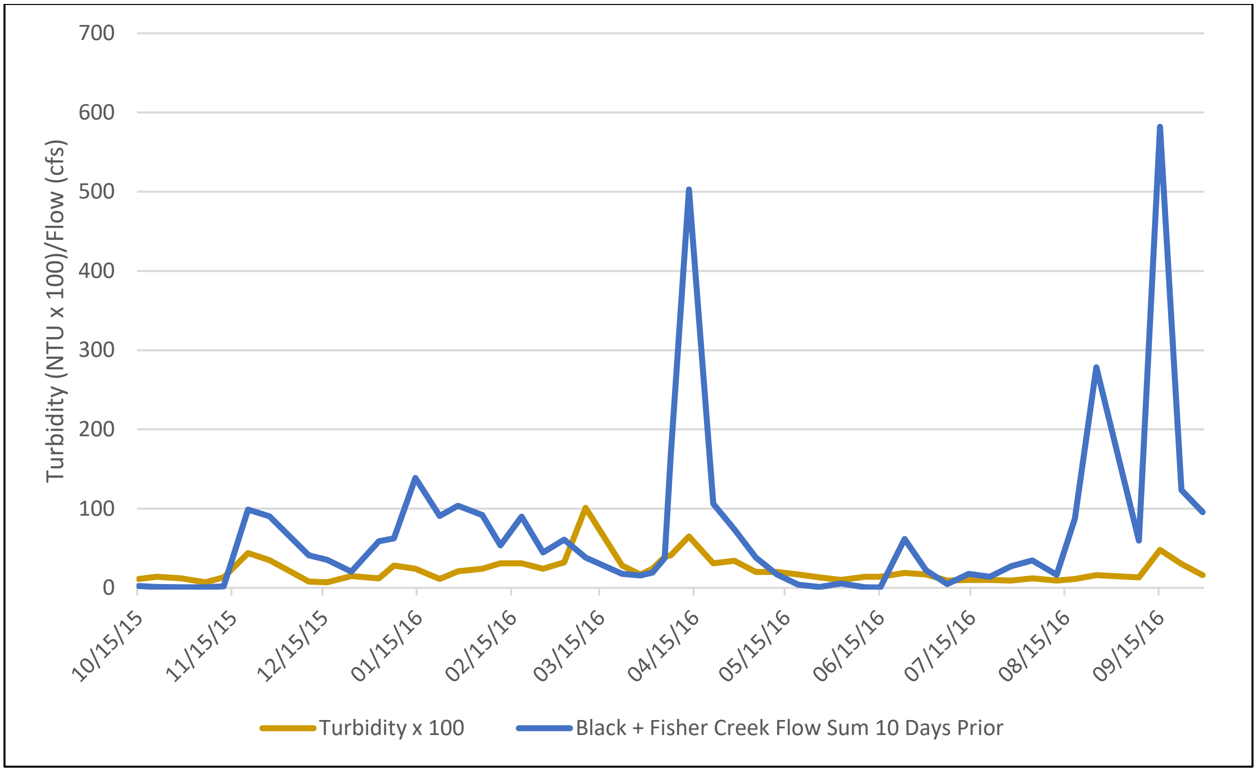


Figure 6.3.5: Weekly turbidity with summed flows of Black and Fisher Creeks 10 days prior, 10/15/15 – 9/29/16.

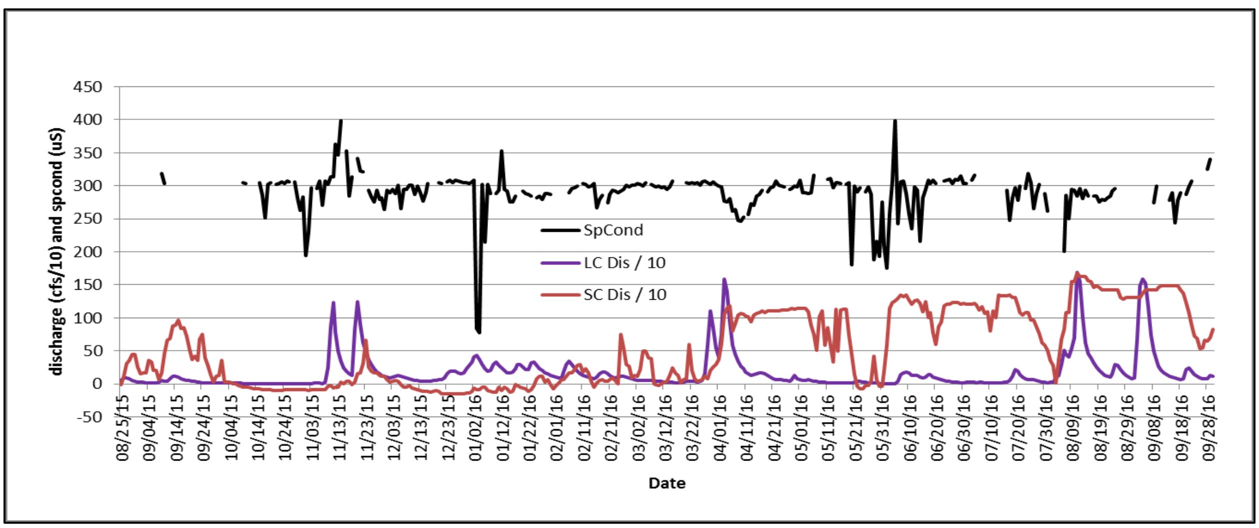


Figure 6.4.1: Specific conductance with discharges of Lost Creek and Spring Creek.

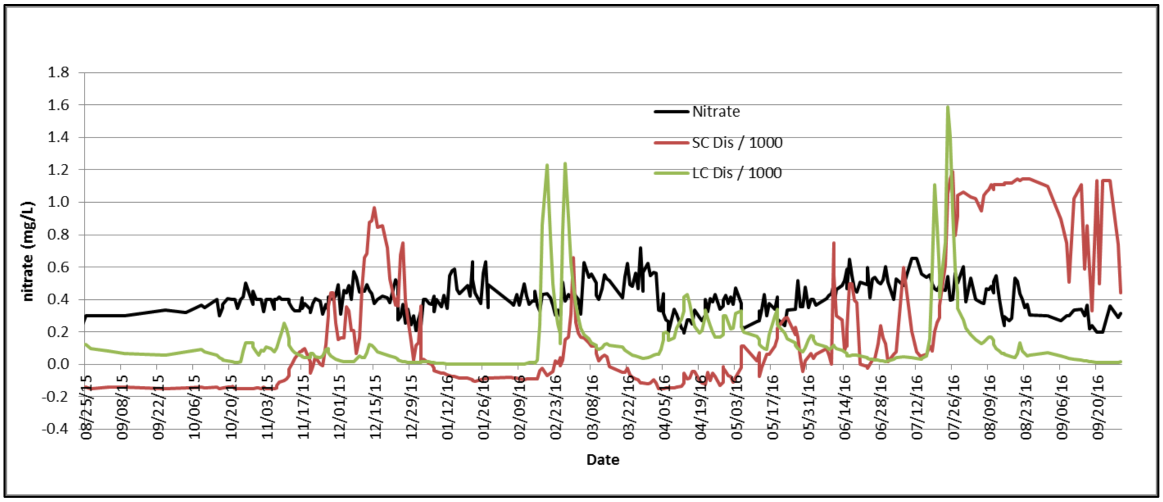


Figure 6.5.1: Nitrate concentrations with discharges of Lost Creek and Spring Creek.

7. Variation in Photosynthetically Active Radiation at Wakulla Spring

7.1 Optical Properties of Wakulla Spring and its Springshed

Light is one of the most important parameters determining the diversity and abundance of aquatic biological communities. The underwater light field is a critical factor for primary production: plants need almost the full spectrum of visible light to photosynthesize. This so-called Photosynthetically Active Radiation (PAR) encompasses the spectral range (wave band) of solar radiation from 400 to 700 nanometers (nm) which is the same as the light range visible to humans (figure 7.1.1).

Light attenuates with depth in water because water molecules absorb light radiation. Other substances cause further attenuation including dissolved substances which absorb light over different wavelengths and suspended particles, measured as turbidity, which can absorb, reflect, and scatter incoming solar radiation. This aspect of our study focuses primarily on two dissolved light-absorbing substances: tannins and chlorophyll a, measured as corrected chlorophyll a and its degradation product, phaeophytin. We also examine the effects of turbidity. Tannins absorb light at shorter wavelengths extending from UV light to blue visible light up to about 475 nm.[11] Both chlorophyll a and pheophytin absorb in two regions of the visible light spectrum. The shorter wavelengths of their absorbance spectra overlap with that of tannins. Chlorophyll a exhibits absorbance peaks at about 430 and 665 nm (see figure 7.1.2), while pheophytin a absorbs most strongly at about 410 nm and to a lesser extent at about 675 nm (see figure 7.1.3). Turbidity measurements account for both suspended matter and dissolved substances and thus overlap with measures of color and chlorophylls.

[11] See Bricaud et al. (1981).

We examined the light spectrum transmitted through the water column at Wakulla Spring using spectral radiometric (spec rad) analysis of unfiltered water in the field to define "optical fingerprints" of light conditions at the spring on a weekly basis as well as for eight events that exhibited "dark" and "light" water conditions. "Dark" conditions comprise those when visibility is low, and the apparent color is reddish-brown associated with significant amounts of tannins in the water. "Light" conditions are those when visibility is high, and tannins are not noticeably present. Historically, the water in the spring was a clear pale blue during "light" conditions. In recent decades, the spring has had a greenish apparent color under "light" conditions.

We also developed optical fingerprints for some of the karst lakes and streams that discharge water into the Upper Floridan Aquifer within the Wakulla springshed by conducting color absorbance scans of filtered samples with the Ocean Optics spectrometer. Absorbance is measured on a logarithmic scale in absorbance units (Au): 1.0 Au is approximately equal to 10% transmittance; 2.0 Au is approximately equal to 1% transmittance. Here we present several examples.

We begin with optical fingerprints of the water in Wakulla Spring for a "light" event on 07/07/16 (Secchi 63 feet) and a "dark" event on 09/15/16 (Secchi 5.5 feet). The former was about one month after the highest visibility water conditions in three years, with the glass bottom boats running for a short time beginning Memorial Day weekend. The latter was during the darkest period of the project. In just two months Secchi disc visibility decreased by almost 50 feet; a dramatic decrease in visibility.

Figures 7.1.4 and 7.1.6 present raw spectral radiometric light measurements for the two different events at Wakulla Spring. Figures 7.1.5 and 7.1.7 are the same optical fingerprints but show the percentage of incident light transmission at each depth and wavelength. Light readings were normalized by dividing them by the incident (air) light intensity, which eliminates atmospheric optical effects, like the absorption bands of nitrogen and oxygen, and yields the percent of light transmitted at each depth.

Under the "light event" conditions (figures 7.1.4 and 7.1.5), both the shortest wavelengths (< 480 nm) and the longer wavelengths (>570 nm) were being absorbed, with the greatest transmittance in the middle (about 480-570 nm, i.e. greens and yellows) and a distinct absorbance dip at 664 nm that is characteristic of chlorophyll a. So, the apparent water color would have been greenish. The entire spectrum is shifted to the right by the tannic condition in the dark events (figures 7.1.6 and 7.1.7) with the peak intensity moving from about 520 nm in the light event (figure 7.1.4) to about 620 nm in the dark event (figure 7.1.6). The tannins in the water absorb highly in the blues and greens, the short wavelengths of the PAR. The longer wavelength yellows and reds (580-700 nm) are typically transmitted giving the tannic water its characteristic brown or reddish-brown apparent color. Here there is some overlap into the greens (500-560 nm), possibly because of the presence of chlorophyll as well as tannins and . Notice that the light intensity falls quickly with depth under the dark event condition (figure 7.1.6) registering just over 1000 counts at 1 foot versus nearly 2500 counts at 1 foot during the light event (figure 7.1.4).

We defined the PAR depth limit, calculated from an extinction coefficient using the spectral radiometric data, as the depth at which PAR falls to 0% transmittance. This quantifies the depth at which all photosynthetic productivity ceases. The 0% PAR depth limit for the study averaged 12 feet ranging from a low of 4 to a high of 32. The 12-foot average is shallower than the vent ledge (21 feet) where American eelgrass (*Vallisneria americana*) once grew and shallower than several of the deepest stands that were present in December 2016 (see figure 7.1.8). Thus, prolonged dark water conditions are likely to lead to further declines in the aquatic grasses that dominate the submerged aquatic plant community of Wakulla Spring with accompanying loss of primary productivity and habitat.

Punch Bowl Sink offers an example of a dark water "karst window" that is directly connected to the aquifer and has very high levels of tannins and other CDOM from open conduits connected to the underground flow from Lost Creek.[12] Figure 7.1.9 shows that on 11/07/15 the water was very dark: transmittance at 1 foot was only 500 counts which is half the intensity experienced during the 09/15/16 dark water event at Wakulla Spring (figure 7.1.6). The color peak was at approximately 680 nm, further to the right into the longer red wavelengths than in both the 07/07/16 light event at Wakulla Spring (figures 7.1.4 and 7.15), and the 09/15/16 dark event at the spring (figures 7.1.6 and 7.1.7). This is likely because tannin levels are not as high in the spring discharge as they are in Punch Bowl Sink and there is likely little chlorophyll in Punch Bowl Sink. The percent transmission curves are also further to the right in the Punch Bowl Sink dark event (figure 7.1.10) than in the Wakulla Spring 09/15/16 dark event (figure 7.1.7).

The optical characteristics in a sinkhole lake, the Lafayette (Fallschase) Sink in Upper Lake Lafayette, during its almost continuous algae bloom, are graphically depicted in figures 7.1.11 and 7.1.12. This sink drains approximately 30% of the urban area within the City of Tallahassee. The Lafayette Sink in Upper Lake Lafayette is directly connected to the aquifer. Its water is not very tannic but rather green with chlorophyll from high numbers of microalgae in the water column. Here the spectrum is shifted to the left compared to Punch Bowl Sink, into the shorter green wavelengths, peaking at about 550 nm (figures 7.1.11 and 7.1.12), similar to the pattern observed during the 09/15/16 light event at Wakulla Spring (figures 7.1.4 and 7.1.5). In the raw spec rad data (figure 7.1.11), transmittance is low at both ends of the spectrum, in the violet-blue shorter wavelengths and in the yellow-red longer wavelengths. In figure 7.1.12, transmittance is low below 500 nm and there is a distinct dip at about 664 nm. These patterns are consistent with the typical absorbance patterns of chlorophyll a (figure 7.1.2) and phaeophytin (figure 7.1.3).

A somewhat similar pattern is apparent at Wakulla Spring for the light event on 07/07/16 with a decreasing transmittance above 580 nm as well as evidence of light absorption in the low 400s (figures 7.1.4 and 7.1.5). The transmittance spectrum for the 11/20/15 light event at Wakulla Spring bears an even greater resemblance to the 11/04/15 green event at Upper Lake Lafayette (figures 7.1.13 and 7.1.14). This shows high absorbance in the low 400 nm and high 600 nm ranges characteristic of chlorophyll a and phaeophytin, again

[12] Confirmed by dye studies (Cal Jamison, personal communication).

with a distinct dip at 664 nm.

Figure 7.1.15 presents a series of absorbance scans, as opposed to transmittance, measured in the laboratory from filtered water from various waterbodies in the Wakulla springshed. This is another version of the optical fingerprint of the water, but it is limited to CDOM, principally from tannins. Chlorophylls and phaeophytin, which are associated with cellular material removed by filtering, are not detected by this method. The two tannic waterbodies shown here, Lost Creek and Fisher Creek, have very similar absorbance scans. They absorb strongly in the short wavelengths, the blues and greens, and transmit the longer reds and yellows. Therefore, they appear brown. The clearer waterbodies (Wakulla Spring and Sally Ward) do not absorb much at any wavelength. They have very little color with strongest absorbance at 400 nm and below, which likely reflects low levels of tannins and other CDOM. Spring Creek,[13] which often receives significant inflow from the Lost Creek sink, exhibits an intermediate scan.

7.2 Variation of PAR at Wakulla Spring

In this section we examine the effects of turbidity as well as the two light-absorbing dissolved substances that may result in shallow PAR extinction depths: (a) chlorophyll and/or phaeophytin and (b) tannins (measured as true color). The 0% PAR depth limit was calculated weekly, based on in situ measurements taken with two devices: a LICOR photometric cell and an Ocean Optics underwater, integrating spectrometer (OOS). The methods, calculations, and sampling techniques are detailed in chapter 2. Figure 7.2.1 presents these data for the study period between 8/25/15 and 9/29/16. As shown in Table 7.2.1, LICOR and OOS values were comparable for the study period with nearly identical ranges, averages, and geometric means.

It is obvious from the graph depicted in figure 7.2.1 that we had two clear events towards the end of the project, in late-May and mid-July 2016. It was clear enough on May 30 to run the glass bottom boats for the first time since 2014. The 0% PAR depth limit exceeded 30 feet on July 10. Then in mid-September the 0% PAR depth limit decreased to less than 5 feet.

The depth at which a plant receives just enough PAR to produce by photosynthesis the amount of sugar needed to offset the amount consumed by respiration is called the compensation point. No net plant growth occurs at or below the depth of the compensation point. The compensation points for the two dominant submerged aquatic grass species in the spring bowl and Upper Wakulla River, *Vallisneria americana* and *Sagittaria kurziana*, are estimated at 10% PAR based on studies of other spring-fed rivers in Florida (Hoyer et al., 2004). Figure 7.2.2 displays the 10% PAR depth limit for the study period. With a 10% PAR compensation point, these plants may not be able to grow as deeply as a plant like *Hydrilla* which is reported to grow in lower light environments down to about 1% PAR (Steward, 1991). *Hydrilla* adapts to lower light conditions in part by elongation of its stems toward the surface. *V. americana* can adapt to low light

[13] We collected Spring Creek water samples from the boat dock downstream of the main boil.

intensity by extending its leaf length, but its capacity to do so diminishes substantially at less than 8% PAR (French and Moore, 2003). The shorter-leafed *S. kurziana*, however, is not able to adapt in this manner.

Figure 7.2.3 displays weekly boat dock turbidity levels with the 0% PAR depth limit for the same dates as measured with the Ocean Optics spectrometer (OOS). The chart suggests the expected inverse relationship between turbidity and PAR depth limit. A simple linear regression model of the relationship is statistically significant at the 95% level (F-test = 0.6.70; p-value = 0.0124) with turbidity explaining about 11% of the observed variation in the 0% PAR depth limit: the coefficient of determination (R^2) equals 0.11.

Figure 7.2.4 reveals a generally negative relationship between the 0% PAR depth limit and true color as well, however the PAR depth limit does not directly reflect true color levels in all instances, particularly after June 30, 2015. True color explains only 6% of the observed variation in the 0% PAR depth limit over the full study period ($R^2 = 0.06$). While the effect is small, it is statistically significant at the 90% level (regression F-test = 3.49; p-value = 0.0674). Regression analysis for the period 9/3/15 – 6/30/15 reveals a much stronger statistical relationship with an R^2 of 0.36 that is significant at the 99.99+ level (F-test = 23.61; p-value = 0.0000).

A graph of 0% PAR depth limit with total chlorophyll a, i.e. corrected chlorophyll a plus phaeophytin (figure 7.2.5), exhibits an inconsistent dynamic relationship. At times the variations in PAR depth limit and chlorophyll appear independent. At others a positive correlation appears. After May 5, 2016, the relationship is inverse as would be expected, i.e. lower levels of total chlorophyll a are associated with greater PAR depth limit. Figures 7.2.6 and 7.2.7, which break out corrected chlorophyll a and phaeophytin, exhibit similar inconsistencies. It is only during the exceptionally clear episodes towards the end of the project, circa May 30 and July 10, 2016, that increased PAR depth limit is associated with low concentrations of corrected chlorophyll a and phaeophytin in the spring. It is not surprising, therefore, that when corrected chlorophyll a and phaeophytin are plotted against PAR and simple regressions calculated, the R^2 values are very small (0.02 for corrected chlorophyll a and 0.002 for phaeophytin) and neither relationship is statistically significant at the 90% level or better.

A multiple regression model to assess the combined linear effects of true color, chlorophyll a, and phaeophytin on PAR depth limit offers a simplified approximation of the complex and dynamic combined effects of these three factors treating them as independent variables. Such a model has very weak explanatory power with an R^2 of only 0.08 and is not statistically significant at the 95% level or better.

Adding turbidity yields a model that is statistically significant at the 95% level (F-test = 2.89; p-value = 0.0318). However, as noted above, the turbidity measure captures tannins and chlorophylls, and our data show a statistically significant correlation between turbidity and phaeophytin. Thus, this simple multivariate analysis is complicated by some multicollinearity. While the R^2 is 0.19, the adjusted R^2, which accounts for the number of independent variables in the regression model, is only 0.12. Only the turbidity coefficient is significant (95% level), but that is not surprising given its likely multicollinearity with the other independent variables. These results suggest that light absorbance is not a simple linear function of turbidity and the concentrations of tannins or chlorophylls and that more sophisticated statistical analysis may be appropriate.

Figure 7.2.8 demonstrates that PAR depth is moderately affected by flow of the two tannic creeks to the north of Wakulla Spring, Black and Fisher Creeks exhibiting a generally inverse relationship between the 0% PAR depth limit at the spring and the summed flows of Black and Fisher Creeks 10 days prior. Regression analysis reveals that this association is significant at the 99.5% level and explains 14% of the observed variation in 0% PAR depth limit ($R^2 = 0.14$).

As shown in figure 7.2.9, our data provide no compelling evidence of an effect of Lost Creek discharges to the aquifer 45 days prior on the 0% PAR depth limit at Wakulla when Spring Creek flows are minimal or cease altogether. Lost Creek flow peak A, which begins while Spring Creek is almost not flowing, is associated with a subsequent decrease in the PAR depth limit. The dips in PAR depth limit following or during Lost Creek flow peaks B, C, and E, however, occur when Spring Creek is flowing. During the prolonged period of highest PAR depth limit at D, Spring Creek flows vary substantially while Spring Creek flows are low. No doubt the travel times from the Lost Creek sink to Wakulla Spring vary considerably so the 45-day time, based on dye studies, may not be representative of conditions during this study.

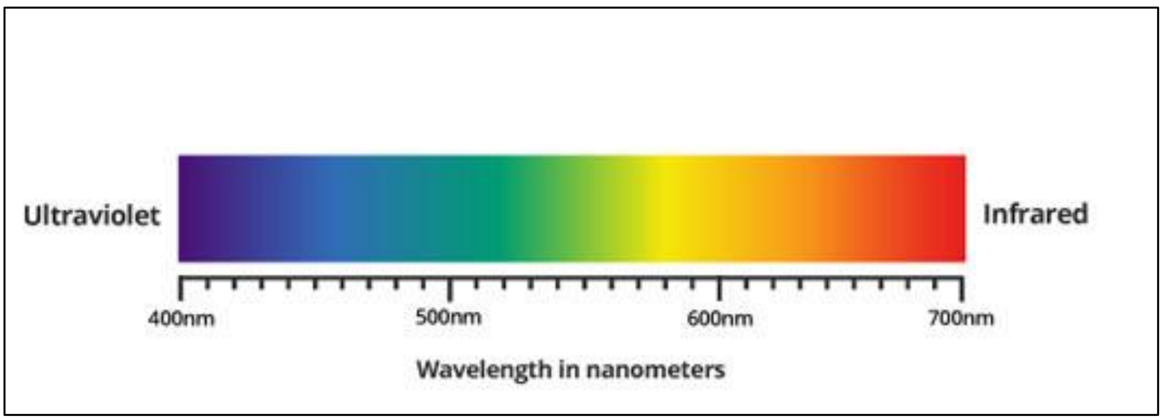


Figure 7.1.1: Visible light spectrum (www.mylighttherapy.com/common/images/spectrum_pic.jpg).

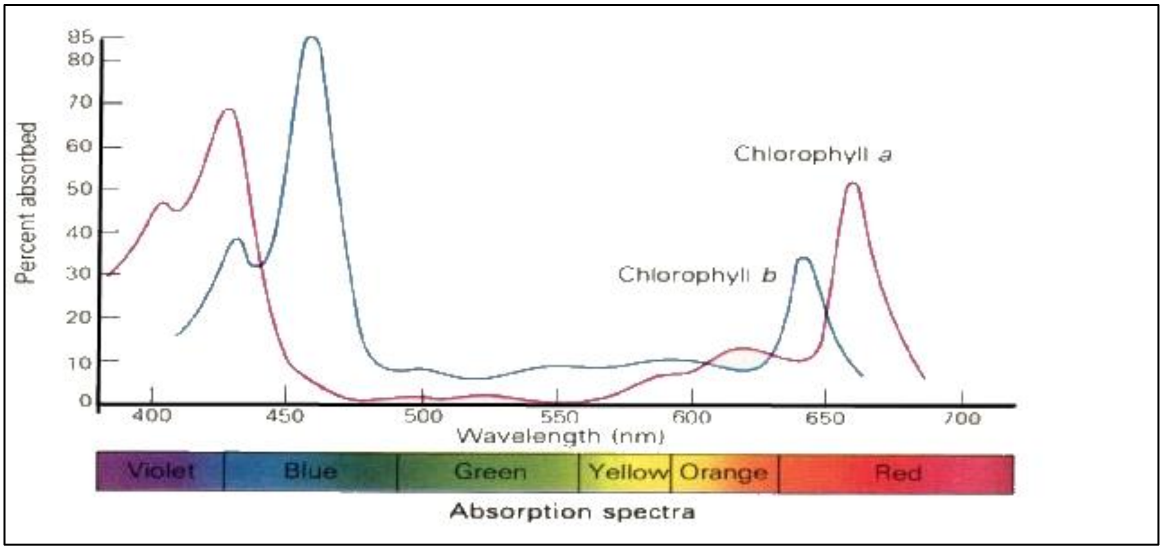


Figure 7.1.2 Light absorbance spectra of chlorophyll a and b (www.austincc.edu/biocr/1406/labm/ex7/prelab_7_4.htm).

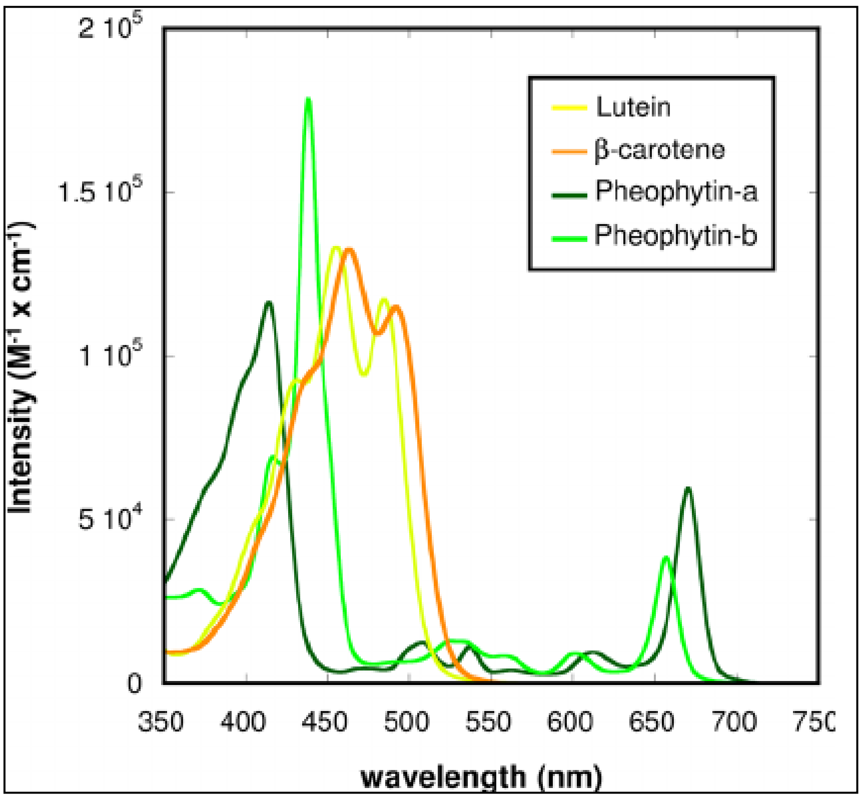


Figure 7.1.3 Light absorbance spectra of phaeophytin a and b (https://www.researchgate.net/ figure/265213606_fig1_Figure-1-UV-vis-molar-extinction-spectra-of-lutein-b-carotene).

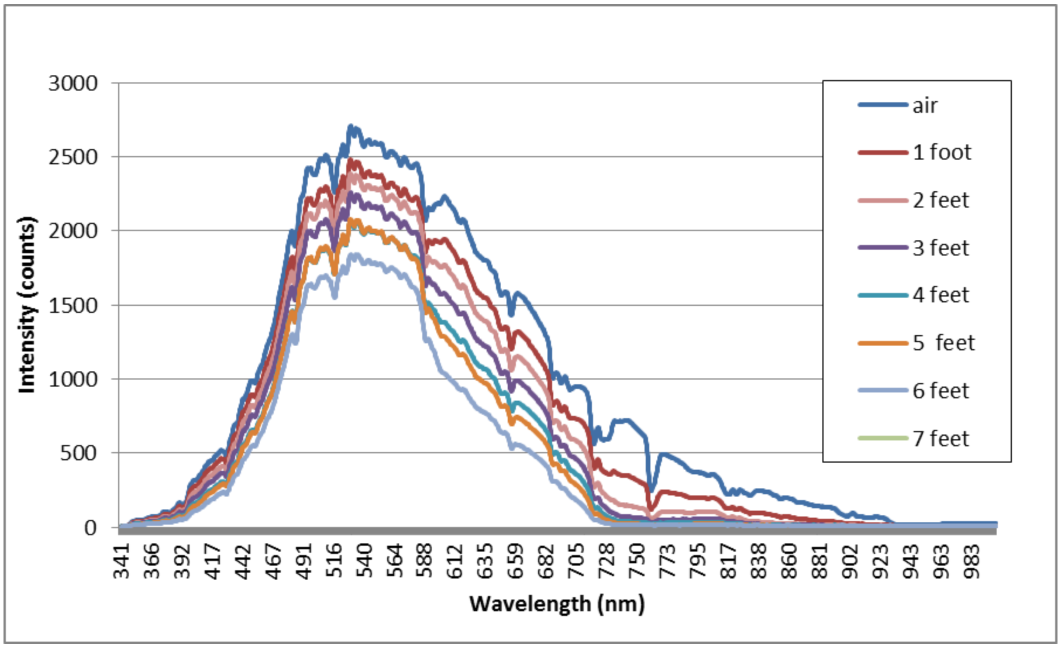


Figure 7.1.4 'Light event' spectral radiometric transmittance through the water column at Wakulla Spring, 07/07/16.

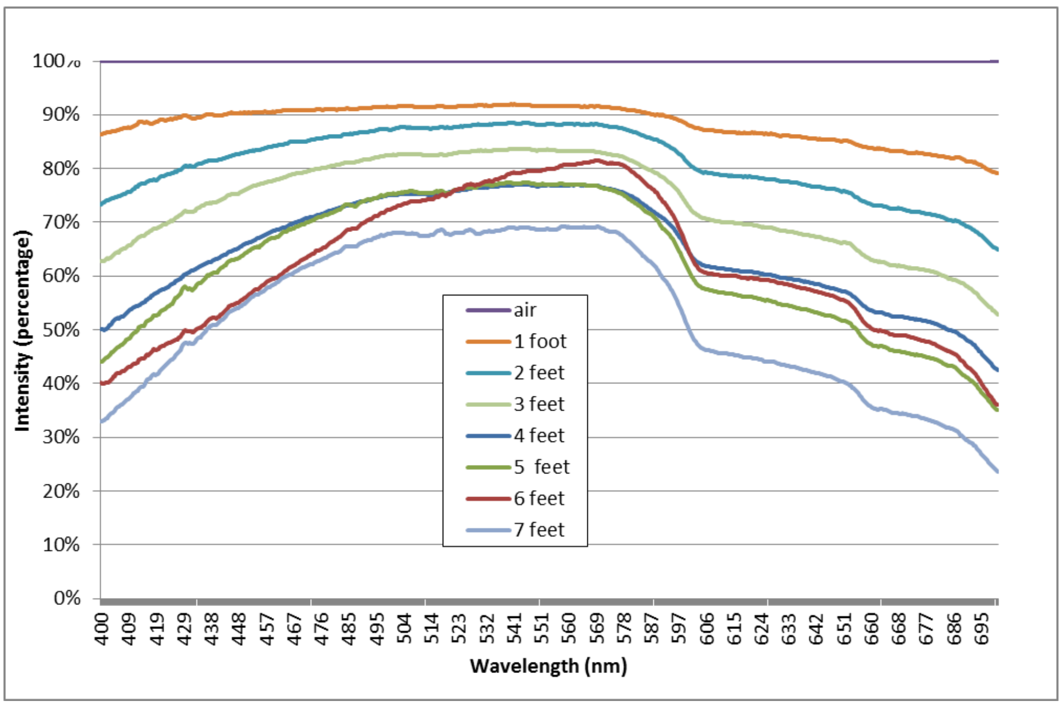


Figure 7.1.5 'Light event' spectral radiometric percent transmittance through the water column at Wakulla Spring, 07/07/16.

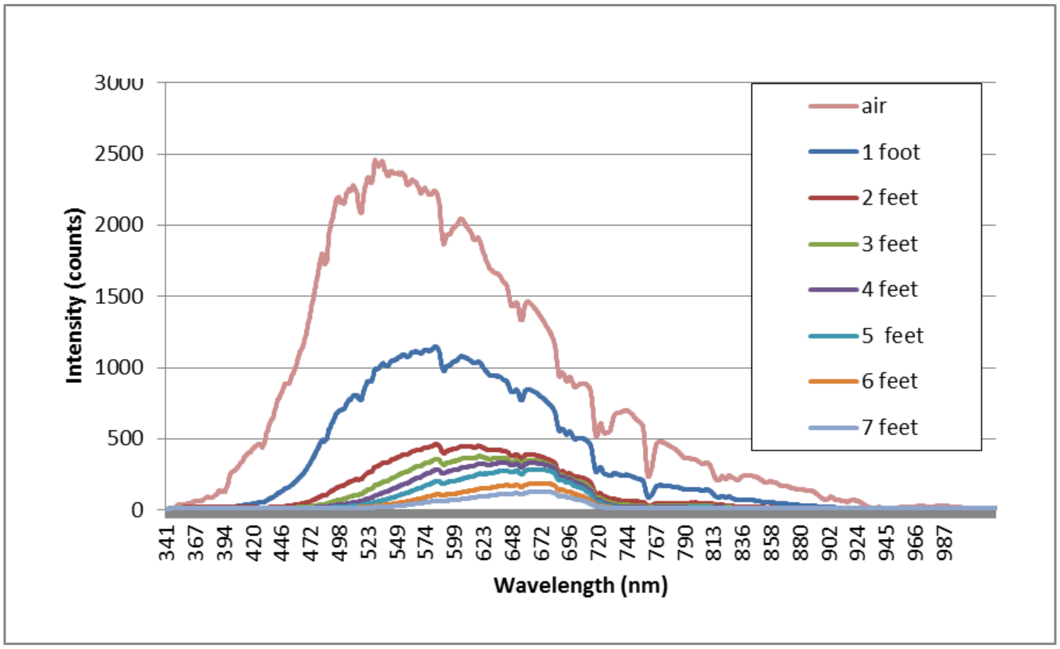


Figure 7.1.6: 'Dark event' spectral radiometric transmittance through the water column at Wakulla Spring, 09/15/16.

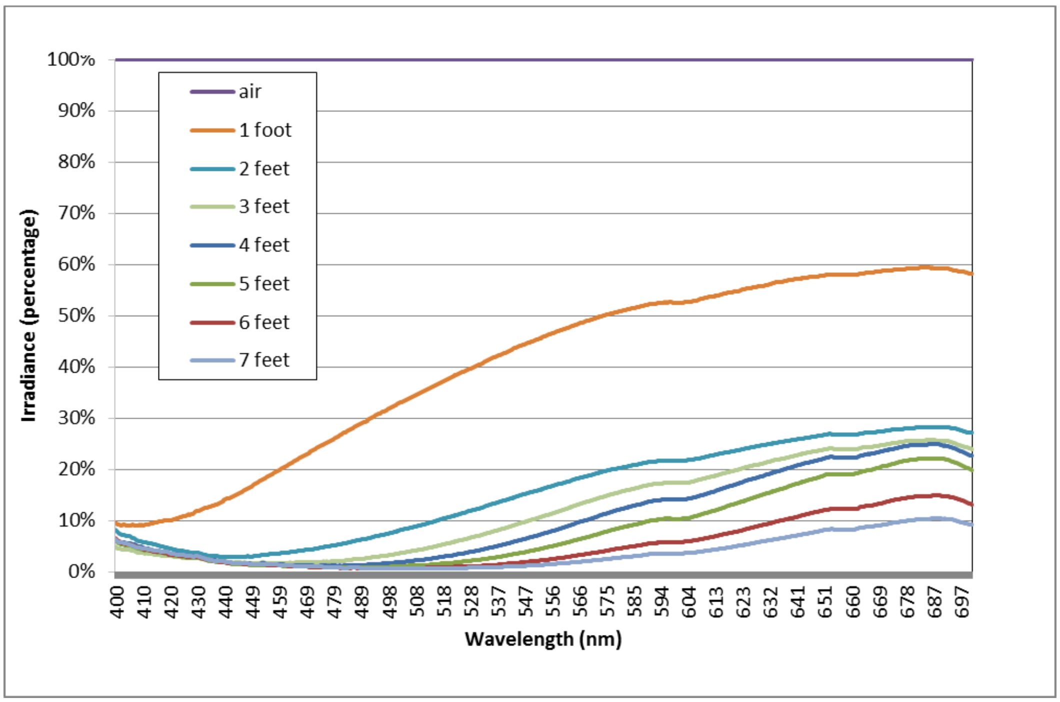


Figure 7.1.7: 'Dark event' percent transmittance through the water column at Wakulla Spring, 09/15/16.

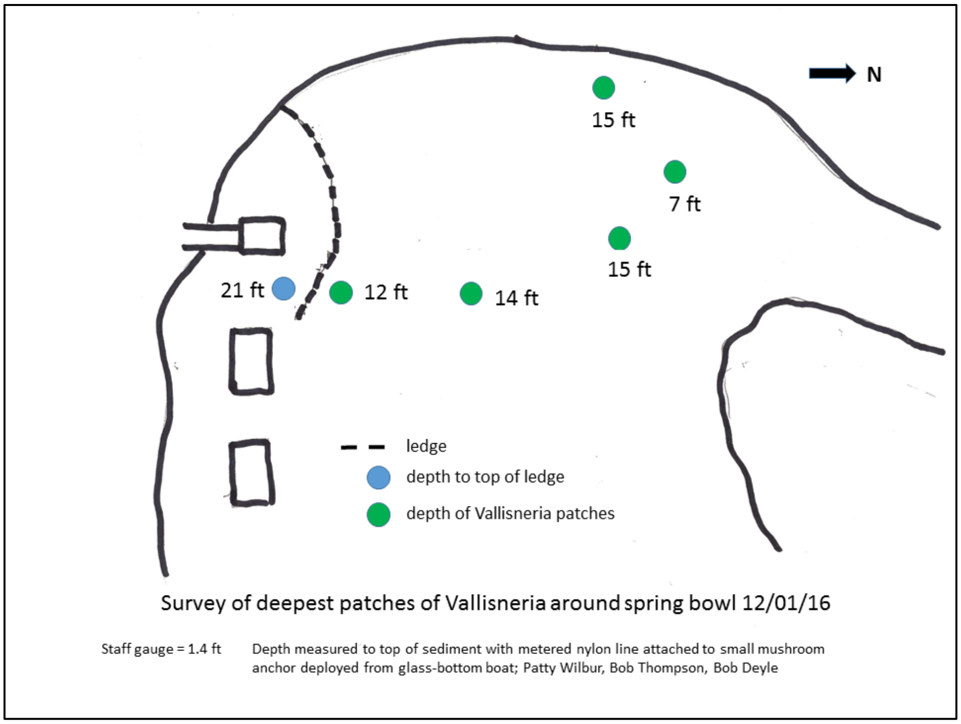


Figure 7.1.8: Survey of deepest patches of *Vallisneria americana* around spring bowl, 12/01/16 (Deyle, 2017).

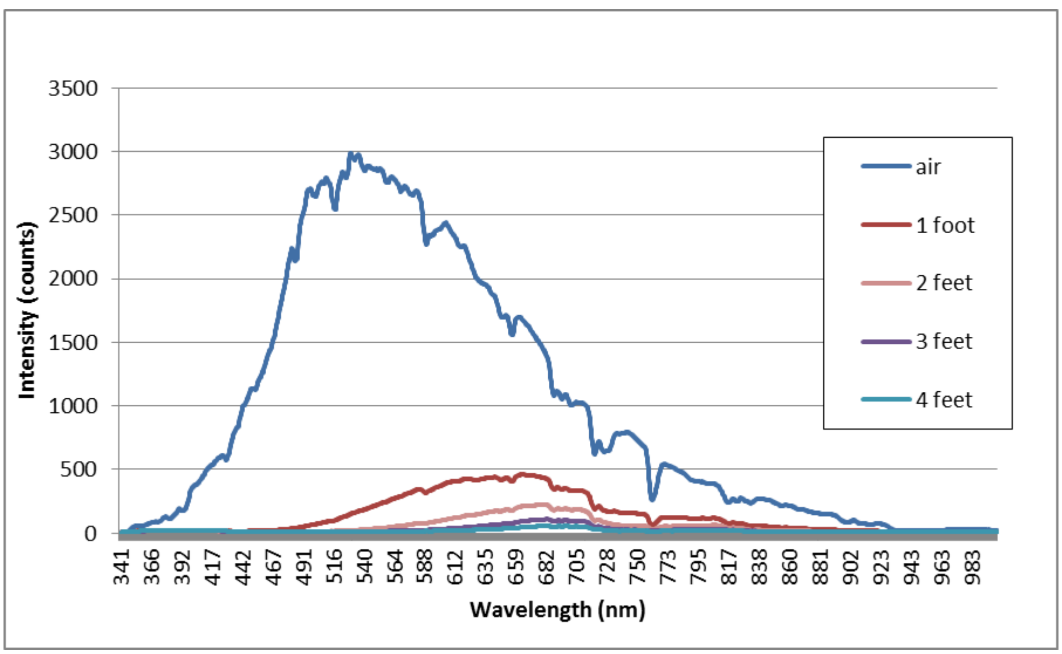


Figure 7.1.9: Spectral radiometric transmittance through the water column at Punch Bowl Sink in Wakulla County, 11/07/15.

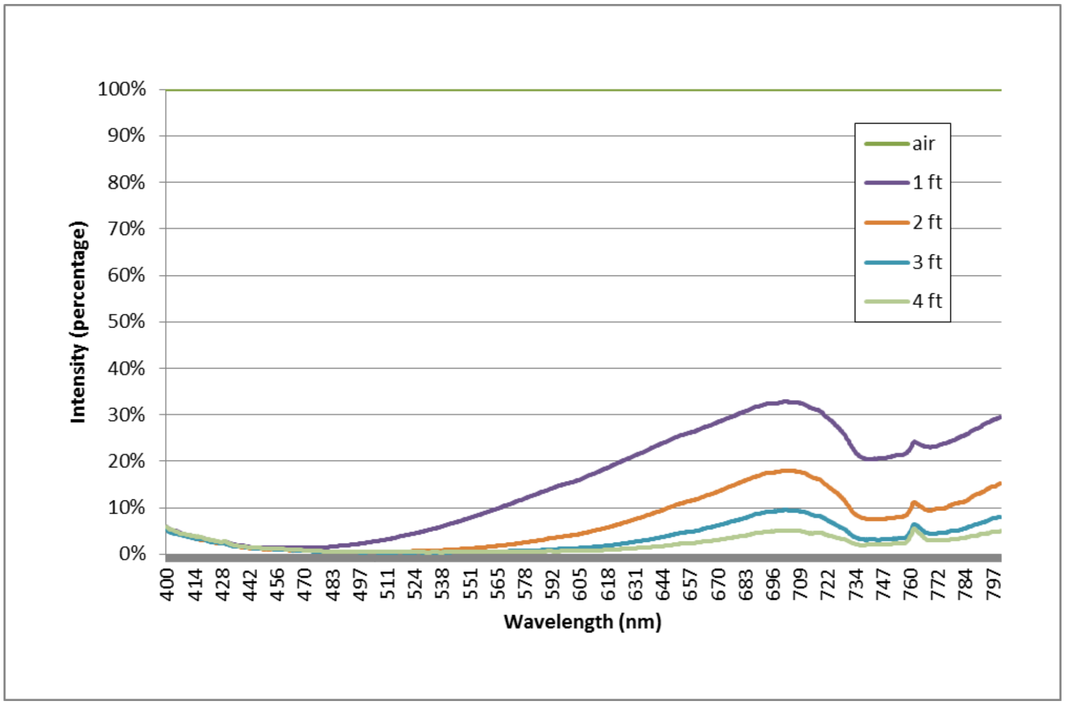


Figure 7.1.10: Spectral radiometric percent transmittance through the water column at Punch Bowl Sink, 11/07/15.

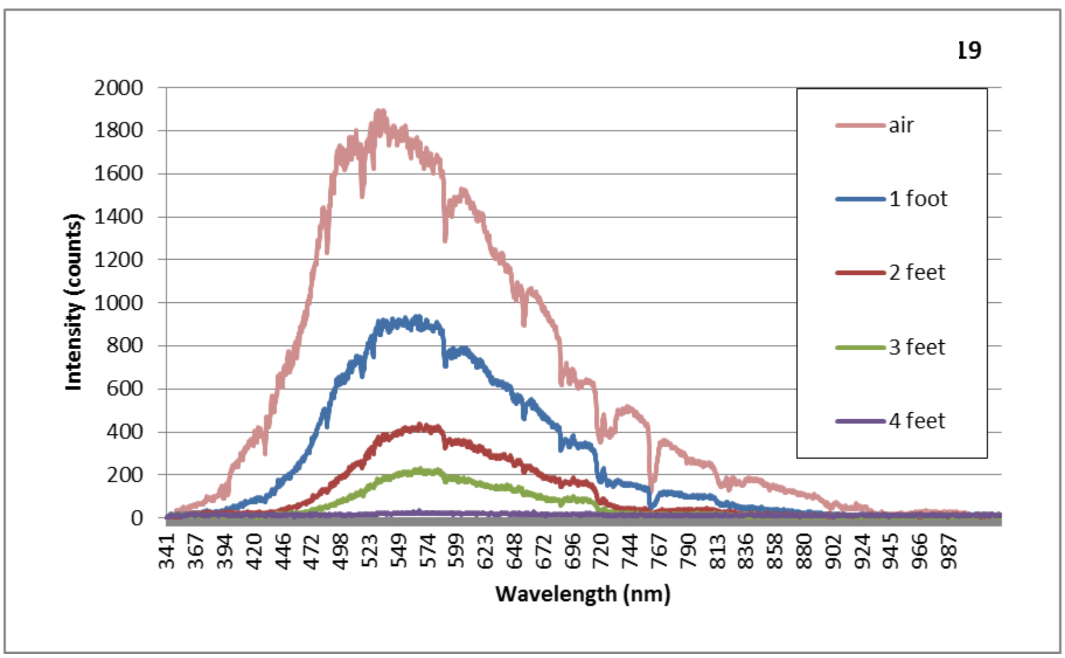


Figure 7.1.11: Spectral radiometric transmittance through the water column in Lafayette Sink in Upper Lake Lafayette, 11/04/15.

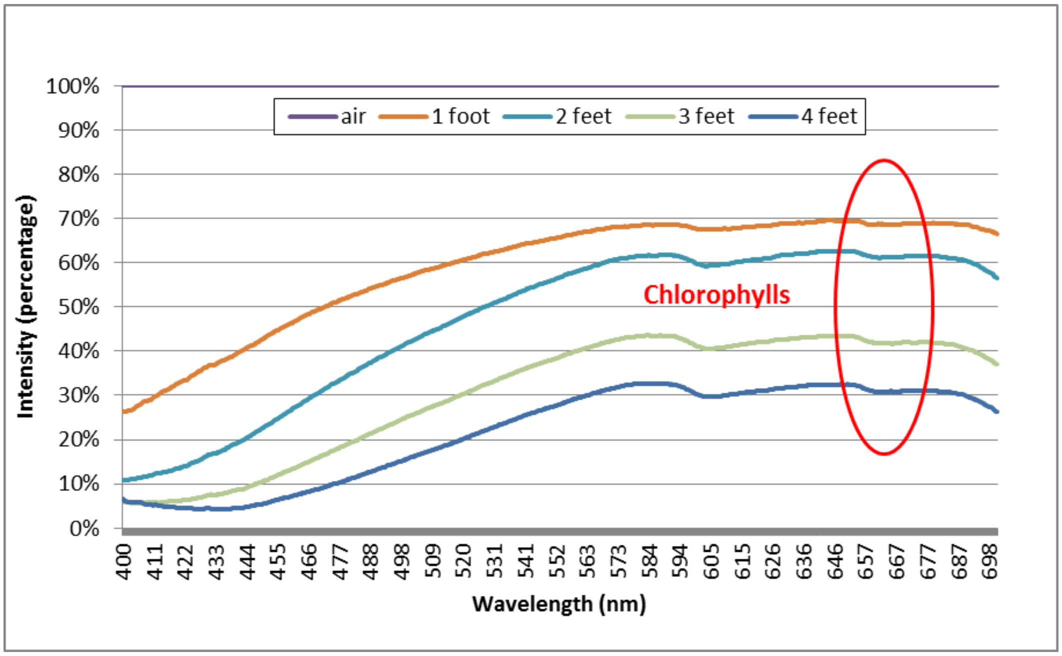


Figure 7.1.12: Spectral radiometric percent transmittance through the water column in Lafayette Sink in Upper Lake Lafayette, 11/04/15.

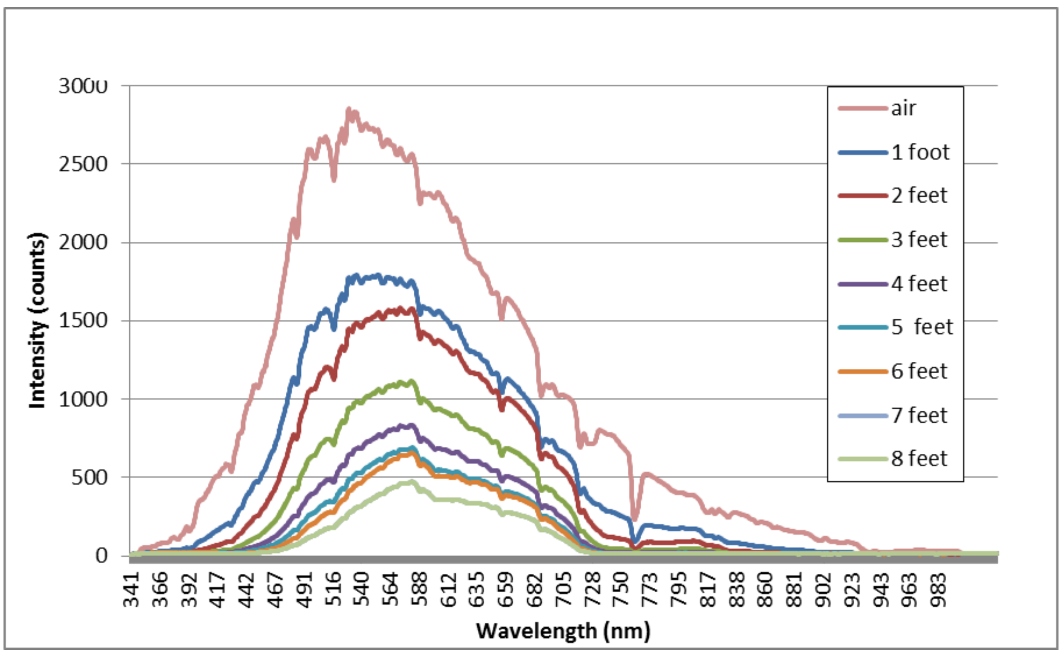


Figure 7.1.13: 'Light event' spectral radiometric transmittance through the water column at Wakulla Spring, 11/20/15.

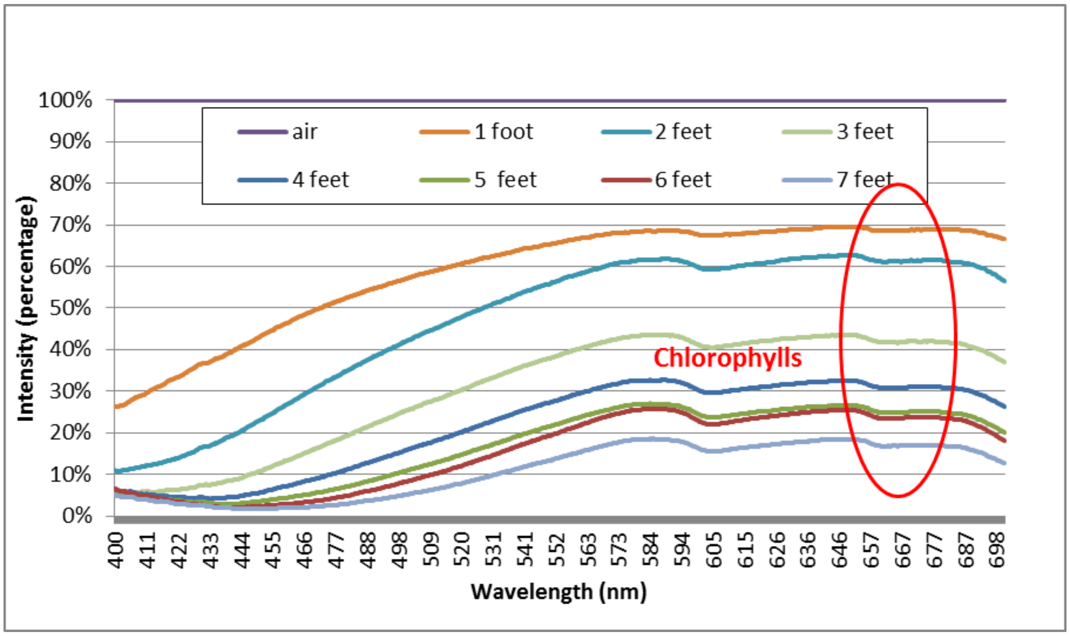


Figure 7.1.14: 'Light Event' percent transmittance through the water column at Wakulla Spring, 11/20/15.

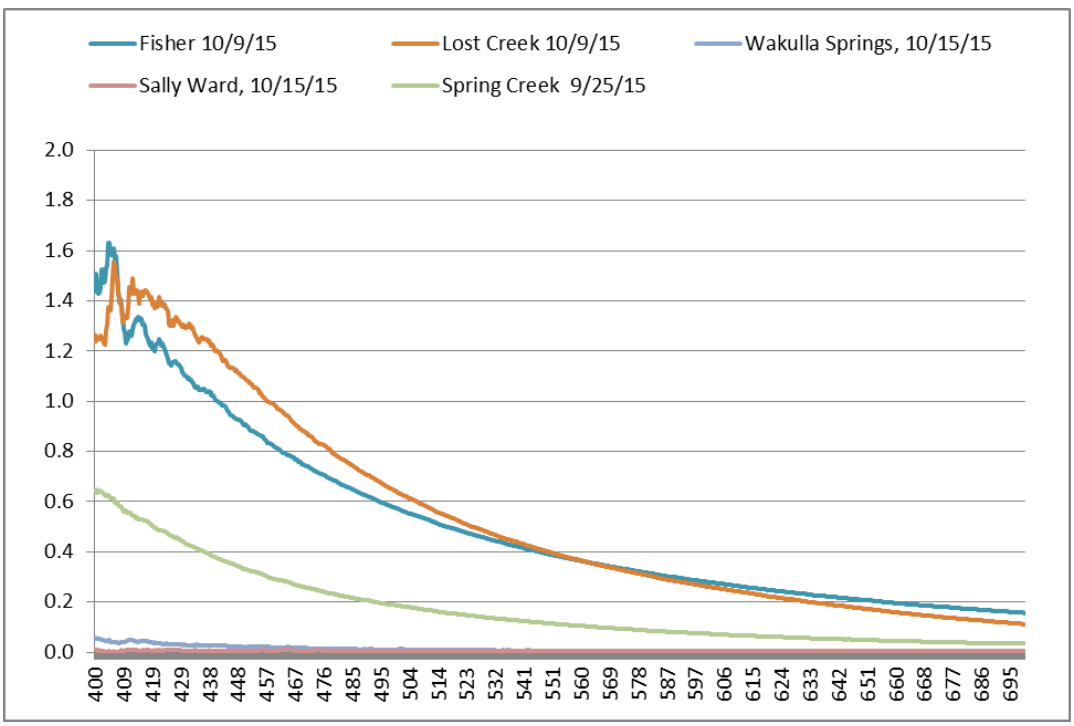


Figure 7.1.15: Absorbance scans of two sinking streams that impact Wakulla Spring – Fisher Creek and Lost Creek - as well as Spring Creek, Sally Ward Spring, and Wakulla Spring measured as optical density units (OD).

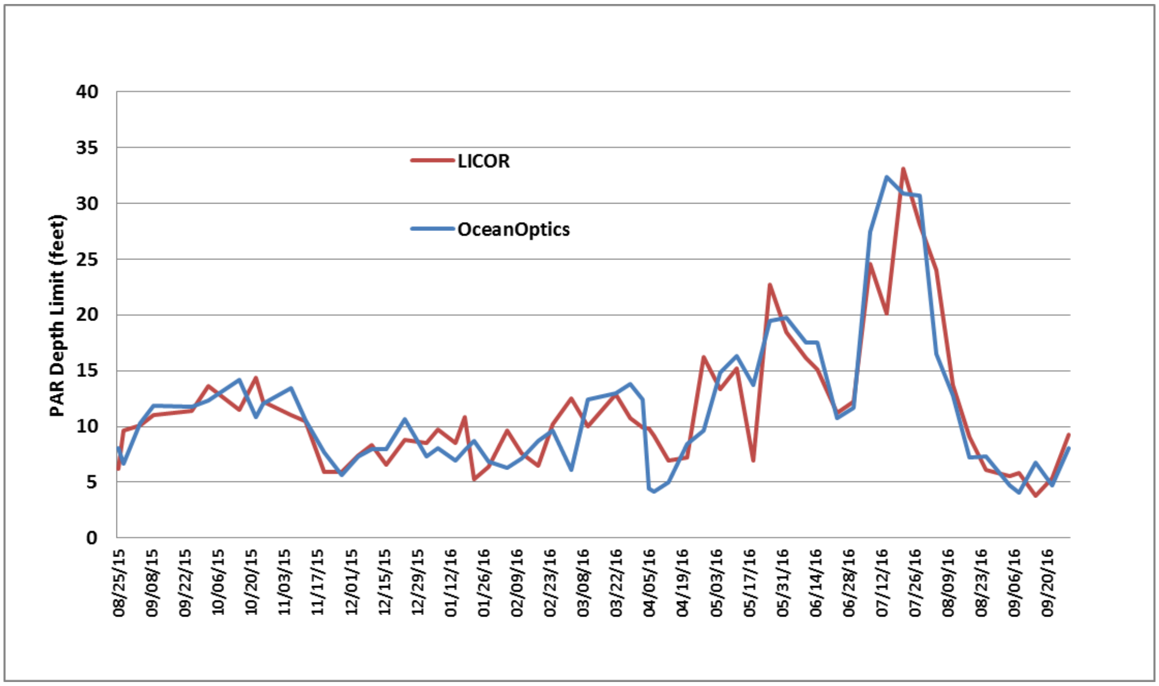


Figure 7.2.1: 0% photosynthetically active radiation (PAR) depth limit during the study period as measured by LICOR photometric cell and Ocean Optics spectrometer (OOS).

PAR Depth Limit Summary Statistics (feet)		
	LICOR	OOS
Count Sort	94.0	59.0
Minimum	3.8	4.1
Maximum	33.1	32.3
Median	10.8	9.6
Average	11.9	11.6
Geometric Mean	11.0	10.0

Table 7.2.1: 0% PAR depth limit summary statistics for the two measurement devices: LICOR photometric cell and Ocean Optics spectrometer (OOS), 08/25/15 - 09/29/16.

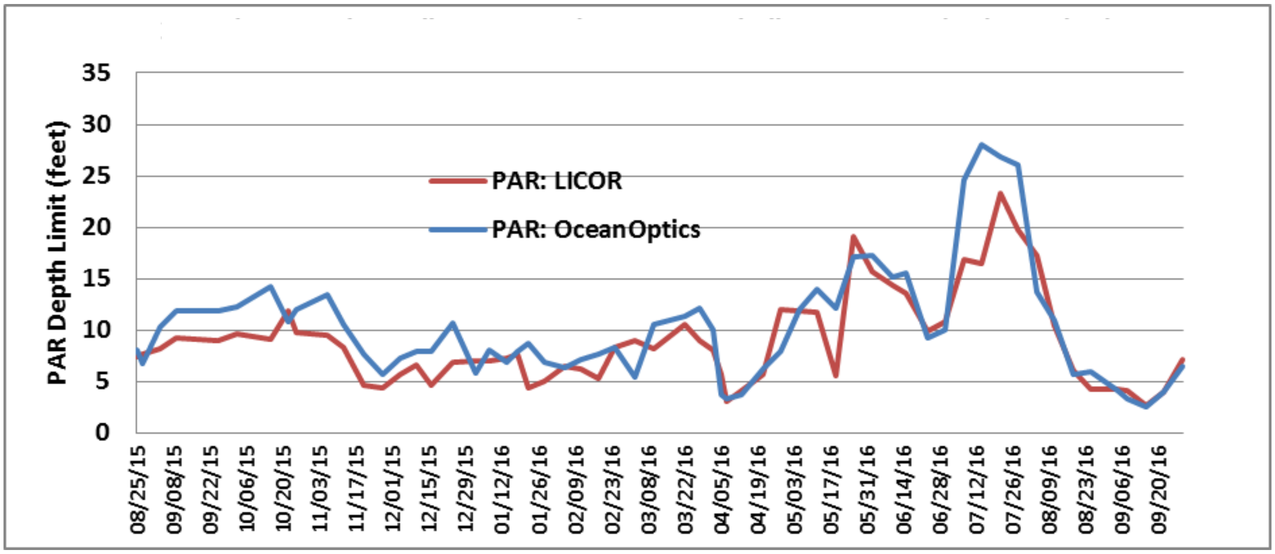


Figure 7.2.2: Compensation point depth limit (10% PAR) during the study period as measured by LICOR photometric cell and Ocean Optics spectrometer (OOS).

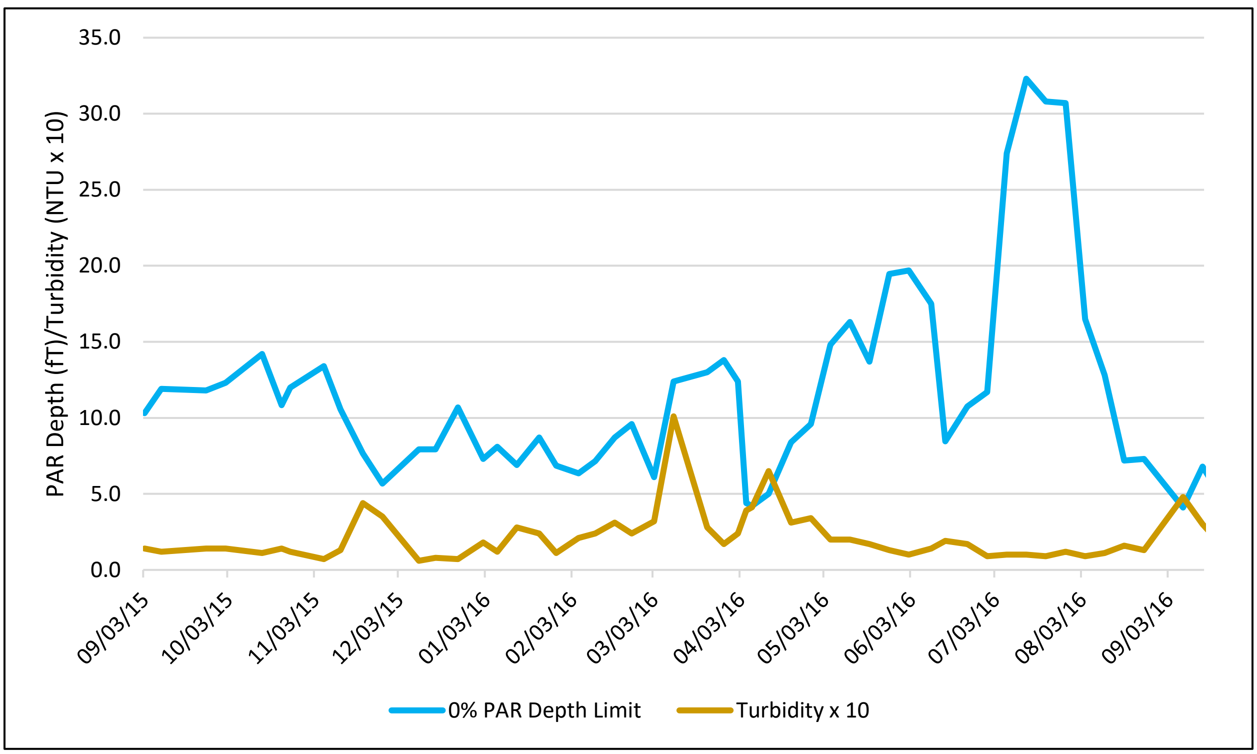


Figure 7.2.3: Weekly 0% PAR depth limit (OOS) versus turbidity at the boat dock, 9/3/15 – 9/29/16.

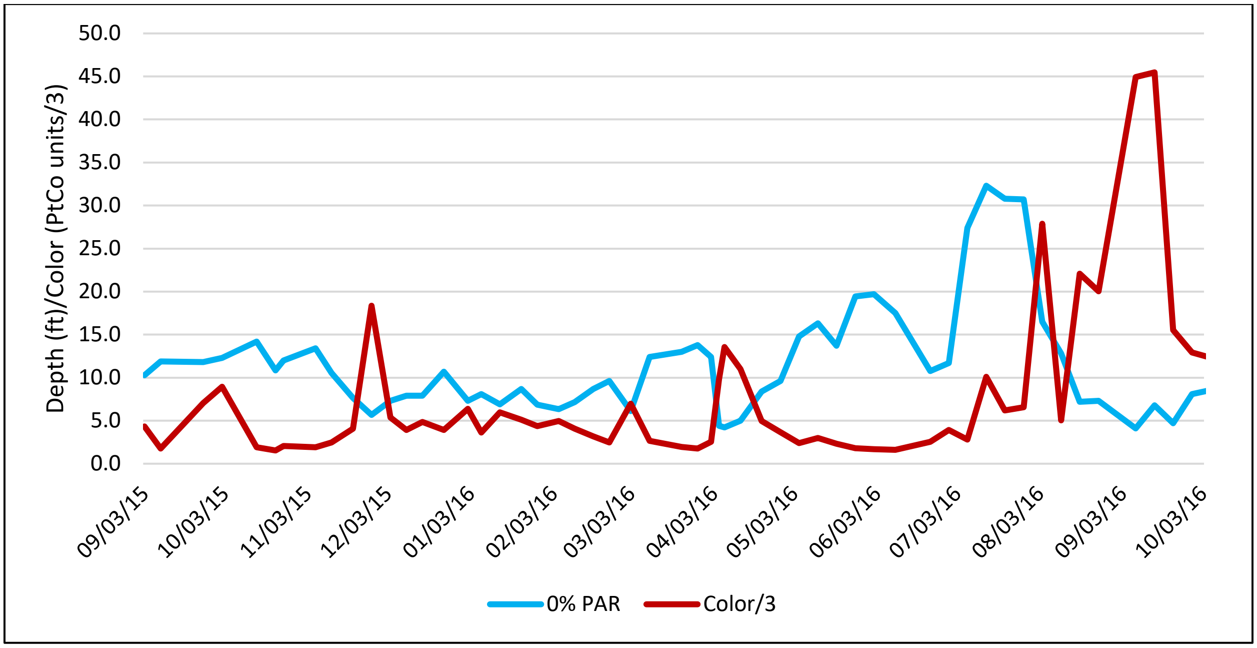


Figure 7.2.4: Weekly 0% PAR depth limit (OOS) versus true color, 9/3/15 – 9/29/16.

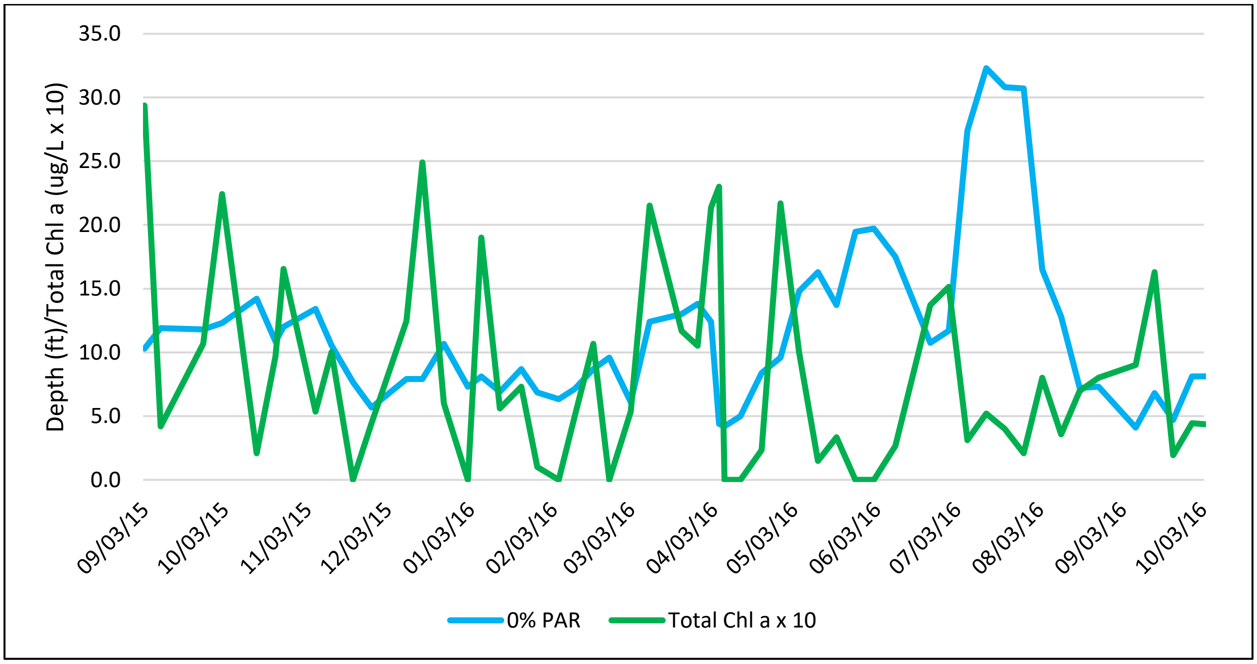


Figure 7.2.5: Weekly 0% PAR depth limit (OOS) versus total chlorophyll a, 9/3/15 - 9/29/16.

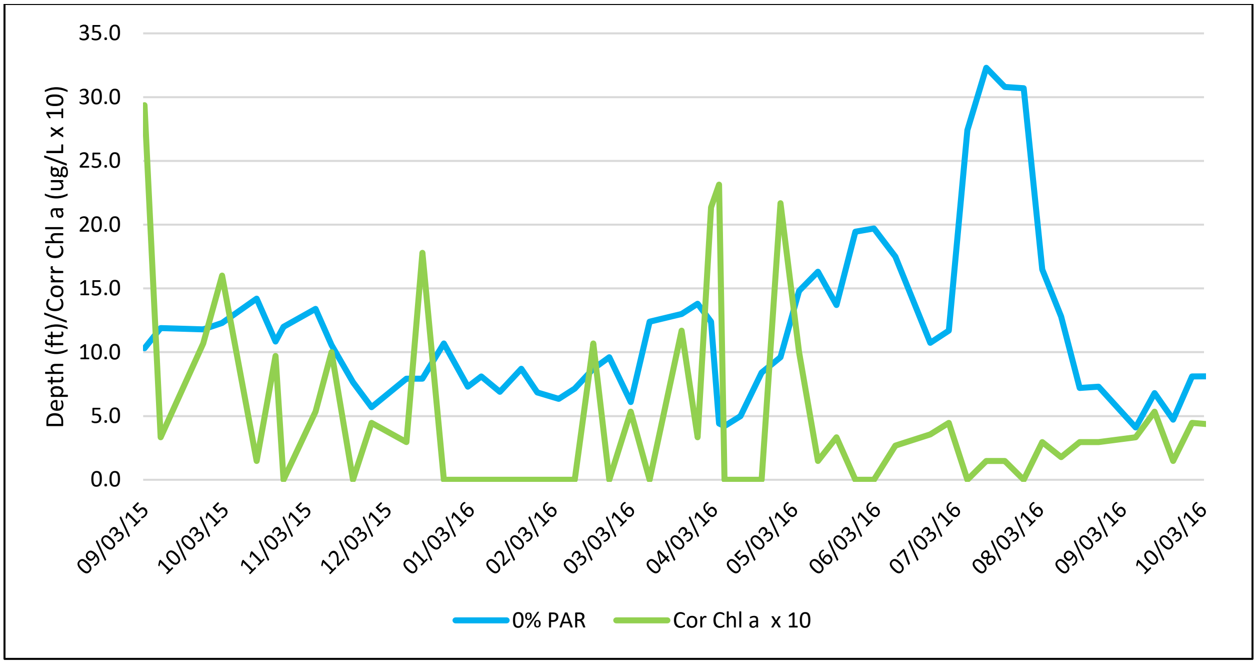


Figure 7.2.6: Weekly 0% PAR depth limit (OOS) versus corrected chlorophyll a, 9/3/15 – 9/29/16.

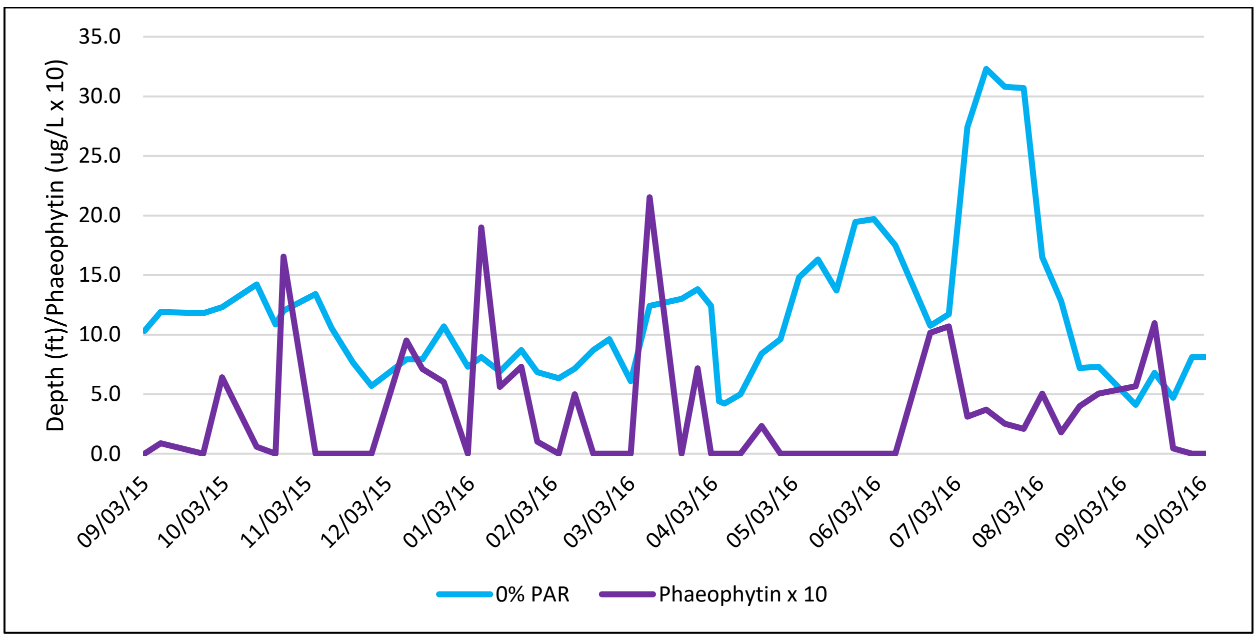


Figure 7.2.7: Weekly 0% PAR depth limit (OOS) versus phaeophytin, 9/3/15 – 9/29/16.

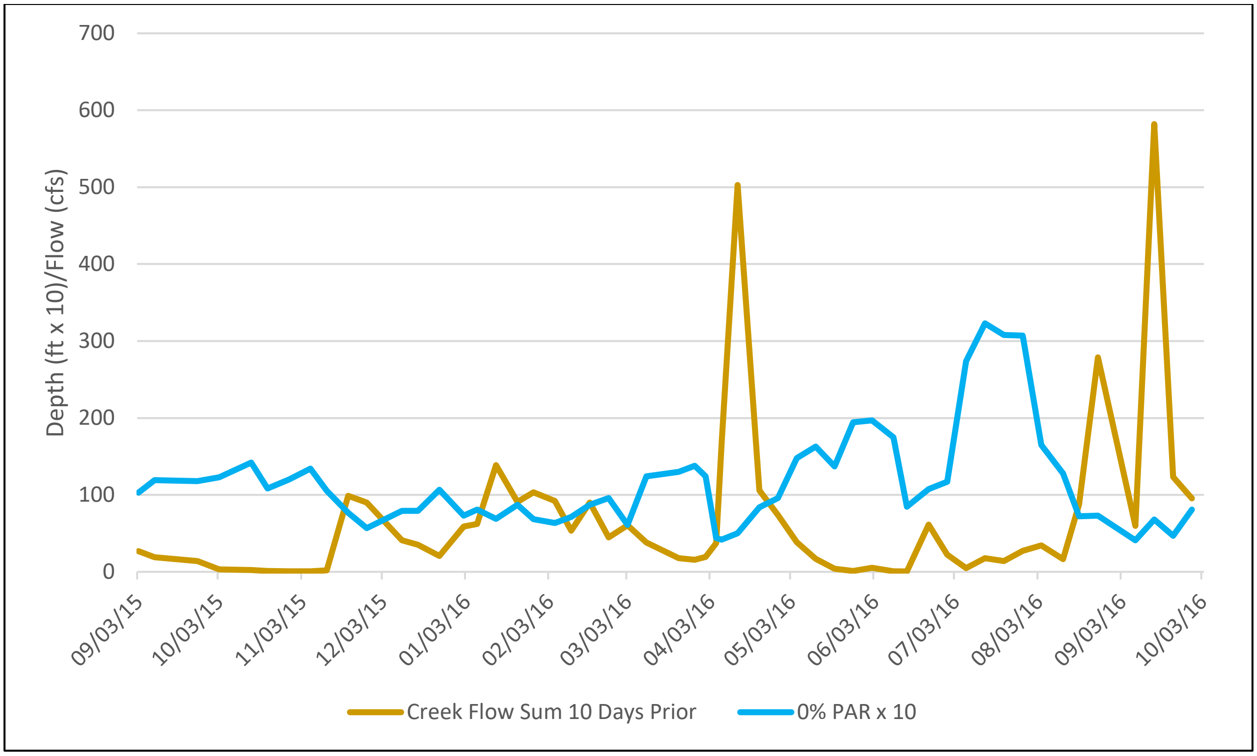


Figure 7.2.8: Weekly 0% PAR depth limit with summed flows of Black and Fisher Creeks 10 days prior, 09/03/15 – 09/29/16.

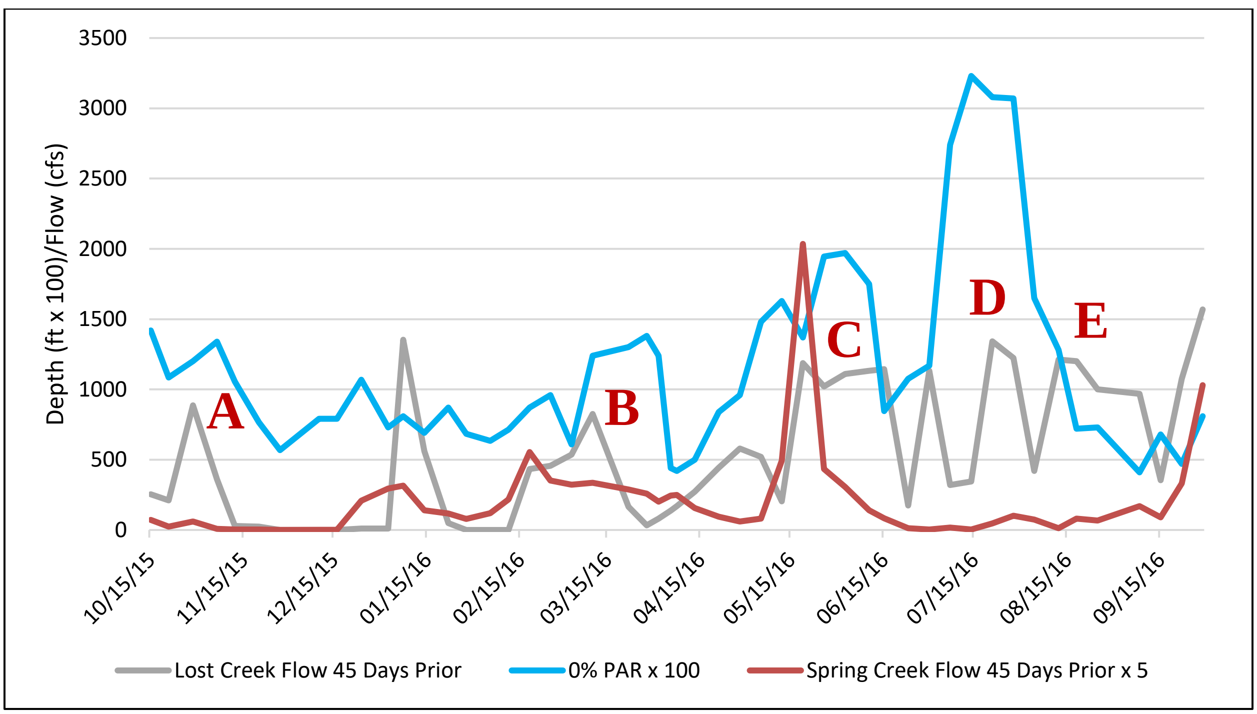


Figure 7.2.9: Weekly 0% PAR Depth Limit with discharges of Lost Creek and Spring Creek 45 days prior, 10/15/15 – 09/29/16.

8. Wakulla Springshed Light and Dark Events

As reported above, dramatic decreases in glass bottom boat tours reflect an increase in "dark water" episodes at Wakulla Spring over the last 30 years. Long-term informal observations by park staff characterize both brown and green dark water conditions. Brown dark water conditions have been attributed to the influx of tannins and other CDOM discharged into the groundwater that flows into the spring from several sinking streams that discharge into sinkholes (swallets), primarily Black Creek, Fisher Creek, and Lost Creek. We have shown in the preceding chapter, however, that measurements of true color do not correlate with visibility at Wakulla Spring measured as the 0% PAR depth limit over the full period of study, although they are significantly correlated over a portion of that time. Similar simple linear regressions with corrected chlorophyll a and phaeophytin are also insignificant. However, turbidity measured at the boat dock is a significant predictor of 0% PAR depth limit. A multiple regression model combining all four variables is significant suggesting a complex relationship determining spring visibility.

This chapter extends that analysis by presenting detailed findings from synoptic surveys of Wakulla Spring and its major karst feature sources associated with eight "events" when the water at Wakulla Spring had no obvious tannins or other CDOM present and visibility was relatively good (light event) and when the water appeared very dark and tannic (dark event) (table 8.1.1). Sampling was conducted to characterize concentrations of true color, corrected chlorophyll a, and phaeophytin and light absorption of the spring and the major karst feature sources of inflow to the Upper Floridan Aquifer within the Wakulla near springshed: Bradford Brook Chain of Lakes (Lake Cascade), Lake Iamonia, Lake Jackson (Porter Hole Sink), Upper Lake Lafayette (Lafayette Sink), Lake Miccosukee, Lake Munson (Ames Sink), Black Creek, Cheryl Sink, Fisher Creek, Jump Creek, Lost Creek and Mill Creek. Using the concentrations of these pigments in the waterbodies we also calculated loading estimates for these sinking streams, karst lakes, and the flow from Wakulla Spring.

8.1 Light Absorbance at Wakulla Spring During Light and Dark Events

Looking exclusively at our samples of light and dark events, we find a mixed story indicating that all three dark water agents are present at various levels, often reinforcing each other in reducing visibility. Figure 8.1.1 differentiates between the two sources of dissolved color: tannins (true color) and total chlorophyll a (corrected chlorophyll a + phaeophytin) depicted as loadings. This reveals that the four dark events are characterized by substantially higher loadings of tannins and/or chlorophyll a while the light events have lower levels of both (units are not comparable). Note, however, that some tannins are present in all four light events.

Table 8.1.2 further distinguishes between the two forms of chlorophyll a – corrected chlorophyll a (total chlorophyll a minus phaeophytin) and phaeophytin. Tannin loads are higher for dark events than light events (average true color of 26 units versus 6 units per day). Corrected chlorophyll a loads are much higher for two of the dark events (E2 and E3) than the light events but absent and very low for two others (E8 and E4). Nevertheless, average loadings are much higher for the dark events (69 versus 7 g/day). Phaeophytin loads are high during three of the dark events but absent in one (E3) with an average of 44 g/day versus 7 g/day for light events. Figure 8.1.2 depicts these patterns scaling the values to fit.

8.2 Optical Fingerprints of Karst Water Bodies and Wakulla Spring

Absorbance scan "optical fingerprints" of filtered water samples for the visible spectrum (400-700 nm) revealed greatest total light absorbance (total area under the curve) within the tannic waterbodies during both light and dark events (see table 8.2.1): Lake Cascade and the sinking streams - Black, Fisher, Jump, Lost, and Mill Creeks (absorbance values of 40 or greater are indicative of tannic water). The lowest absorbance values were recorded for Wakulla Spring and Sally Ward Spring. The lakes vary considerably with the more eutrophic lakes (Miccosukee, Iamonia, and Munson) having higher mean total absorbance than the less enriched systems (Jackson and Lafayette). Cheryl Sink varies tremendously ranging from 1 to 329 total absorbance units. The absorbance of Spring Creek also varies considerably, attaining its two highest total absorbance values during two of the lights events (E7 and E5), but also not flowing during two of the events (light event E6 and dark event E2). The absorbance patterns (see figures 8.2.1.3, 8.2.2.3, etc.) are consistent between the light and dark events.

High resolution spectral radiometric transmittance "optical fingerprints" were taken for Wakulla Spring during each of the eight light/dark events (sections 8.2.1-8.2.8). The light event fingerprints are consistent with the example presented in section 7.1: the normalized light intensity graphs show that both the shortest wavelengths (< 470 nm) and the longer wavelengths (>590 nm) were being absorbed, with the greatest transmittance in the middle (about 500-590 nm, i.e. greens and yellows). Transmittance peaks range from 75 to 88 percent at a depth of one foot below the surface. Each displays a dip in transmittance at about 664 nm which is characteristic of the presence of chlorophyll a.

The dark event fingerprints show the typical shift to the right of the visible spectrum resulting from substantial absorbance of the short wavelengths by tannins and other CDOM as well as chlorophyll a and phaeophytin. Greatest transmittance occurs between 580 and 690 nm, with peaks ranging from 33 to 58 percent. Maximum absorbance is in the range of 432 to 436 nm in three of the four dark events. In dark event E2 (see figure 8.2.2.2), which has the lowest visible light transmittance, peak absorbance is at about 450 nm and the transmittance curve is more nearly flat, consistent with the high levels of corrected chlorophyll a and phaeophytin (see figure 8.1.2) which absorb at both the short and long wavelengths (see figures 7.1.10 and 7.1.11).

We also calculated loadings of true color, corrected chlorophyll a, phaeophytin, and total chlorophyll a (corrected chlorophyll a + phaeophytin) for each of the events for the combined sinking streams (creek), the combined lakes (lake), and the Wakulla Spring (WS) discharge (see tables 8.2.1.2, 8.2.2.2, etc.). The loading values are based on the average of measurements of concentrations and flow taken during the event time window. The creek and lake loads comprise seepage and sinkhole/swallet discharges to the Upper Floridan Aquifer from those karst features. Flows in sinking streams were taken directly from USGS gauges. Flows for sinking lakes were determined by modeling evapotranspiration and local precipitation and stream flows where applicable to determine seepage or water loss to the aquifer, then standard attenuation factors were applied to the loadings to account for any treatment the water may sustain in its course into the aquifer. For details on these calculations please see McGlynn and Deyle (2016).

These data reveal that the sinking streams also are contributing some chlorophyll and phaeophytin to the aquifer. In section 8.3 we present bar charts of each event for each water quality parameter. These enable a visual assessment of which sources of those water quality parameter loadings are the most important under different light and dark event conditions. Estimated tannin loadings to Wakulla Spring are predominantly from the sinking streams in both the light and dark events, however, the Lost Creek discharges to the aquifer very likely did not flow towards Wakulla Spring during three or four of the events when Spring Creek flows were less than or equal to zero. Corrected chlorophyll a loads in the lakes exceed that of Wakulla Spring in six events. Creek loads are lower than Wakulla Spring in six of the eight events. Phaeophytin loading values are considerably different from those of corrected chlorophyll a. The lake loadings exceed the Wakulla Spring level seven times, while creek loads are less than the spring in six of the eight events, as is the case for corrected chlorophyll a.

Event Number	Light/Dark Status	Sample Dates
1	E1, Light	10/09/15 - 10/26/15
2	E2, Dark	12/04/15 - 12/10/15
3	E3, Dark	01/04/16 - 01/14/16
4	E4, Dark	02/15/16 - 02/18/16
5	E5, Light	05/12/16 - 05/26/16
6	E6, Light	06/11/16 - 06/20/16
7	E7, Light	08/06/16 - 08/13/16
8	E8, Dark	08/27/16 - 09/04/16

Table 8.1.1: Light and dark events.

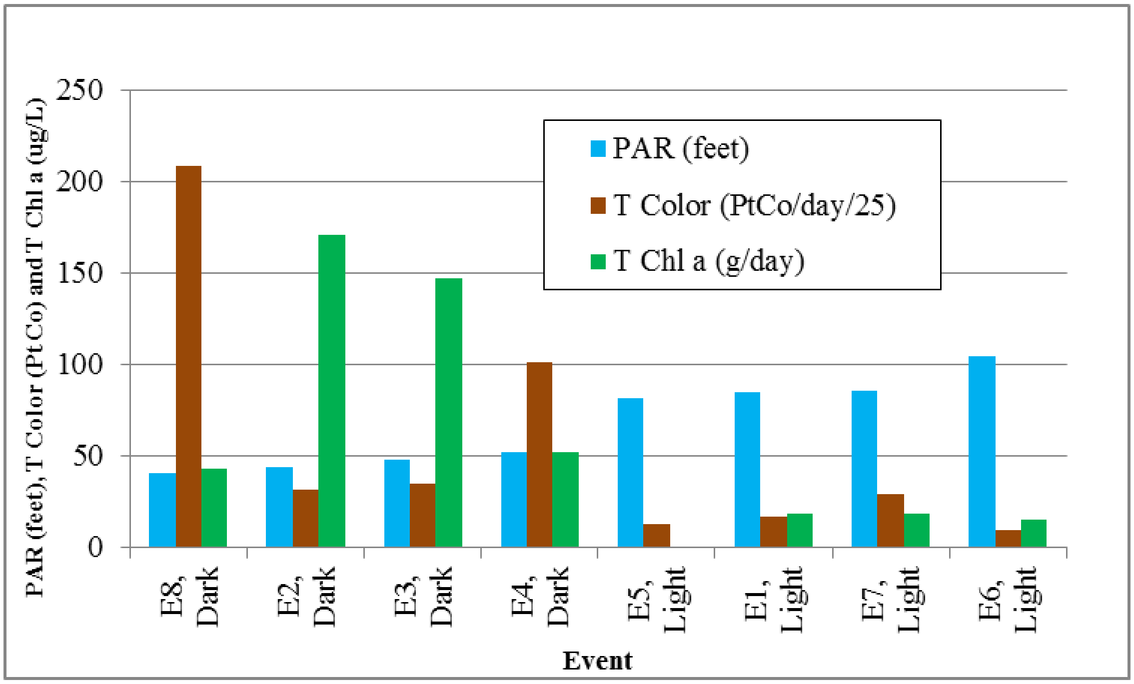


Figure 8.1.1: Summary of Wakulla Spring tannin (true color) and total chlorophyll a loadings during light and dark events arranged according to increasing 0% PAR depth limit.

Event	0% PAR Depth Limit	True Color Load (PtCo units/day)	Phaeophytin Load (g/day)	Corrected Chlorophyll a (g/day)	Total Chlorophyll a (g/day)	Mean Total Creek Flow (cfs)	Mean Spring Creek Flow (cfs)
Dark E8	6.8	5210	43	0	43	503	1358
Dark E2	7.3	803	48	123	171	98	<0
Dark E3	8.1	875	0	147	147	255	<0
Dark E4	8.7	2535	45	7	52	128	23
Light E5	13.7	333	0	0	0	23	465
Light E1	14.2	425	7	11	18	0	<0
Light E7	14.3	746	19	0	19	855	1373
Light E6	17.5	236	0	16	16	120	1080

Table 8.1.2: Summary of Wakulla Spring boil light/dark conditions, loadings, and springshed mean flow regimes during light and dark events, arranged by increasing 0% PAR depth limit.

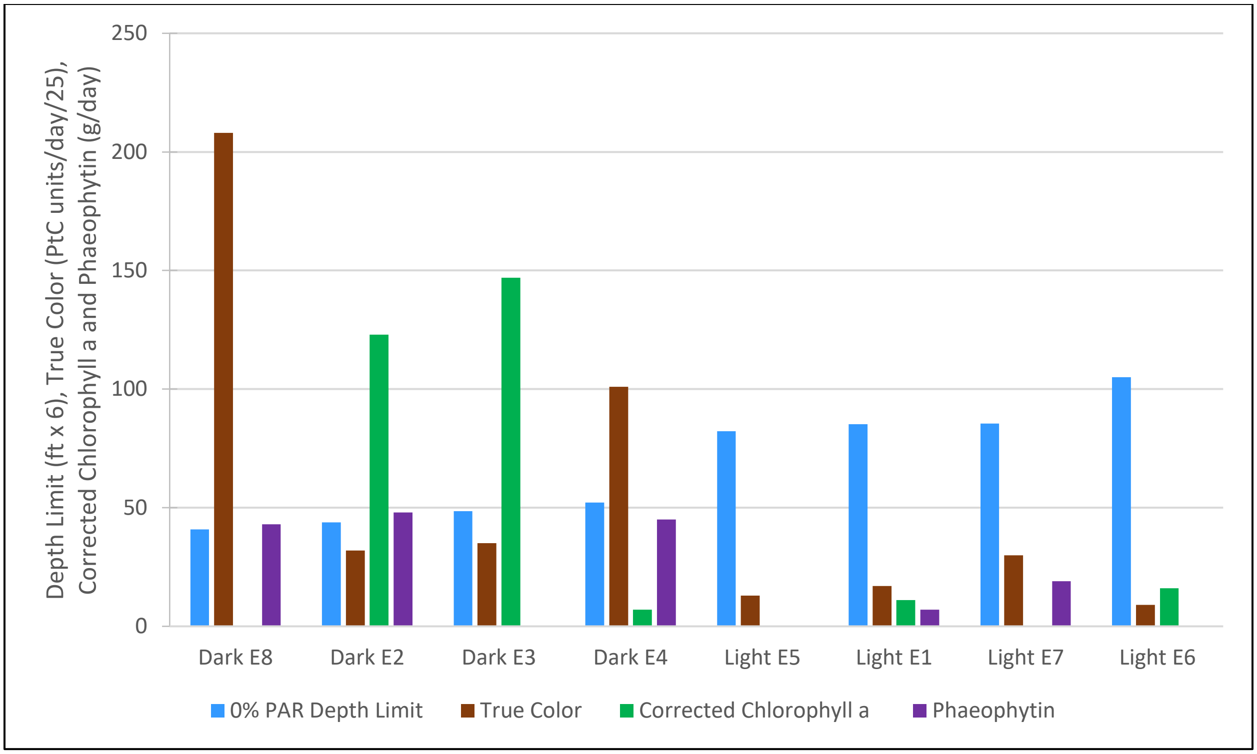


Figure 8.1.2: Loadings of true color, corrected chlorophyll a, and phaeophytin during light and dark events arranged by increasing 0% PAR depth limit.

Water Body	Light Events				Dark Events				Mean
	E7 08/06/16-08/13/16	E6 06/11/16-06/20/16	E1 10/9/15 - 10/26/15	E5 05/12/16-05/26/16	E4 02/15/16-02/18/16	E3 01/04/16-01/14/16	E2 12/04/15-12/10/15	E8 08/27/16-09/20/16	
Jump Creek	441	n/a	n/a	n/a	n/a	544	407	668	515
Lost Creek	679	454	463	460	374	545	440	693	514
Black Creek	604	464	470	n/a	268	523	481	737	507
Mill Creek	430	416	355	477	327	417	468	588	435
Fisher Creek	471	284	436	n/a	268	527	302	503	399
Spring Creek Spgs	535	n/a	148	427	241	270	n/a	322	324
Lake Cascade	267	367	318	139	252	308	321	431	300
Lake Miccosukee	140	123	114	126	60	150	134	71	115
Cheryl Sink	329	1	11	6	107	211	n/a	n/a	111
Lake Iamonia	100	98	82	101	71	140	105	89	98
Lake Munson	n/a	94	175	11	79	73	89	123	92
Lake Jackson	47	69	38	38	39	209	45	29	64
Lake Lafayette	n/a	46	43	113	26	35	55	n/a	53
Wakulla Spring	66	-5	9	10	24	47	23	64	30
Sally Ward Spring	n/a	n/a	5	8	3	n/a	n/a	68	21

Table 8.2.1: Light absorbance totals for Wakulla Spring and major karst sources .

8.2.1 Event 1 (Light): 10/9/15-10/26/15

Sinking streams not flowing; Spring Creek negative flow.

Event # 1 was considered a clear or 'light event' for Wakulla Spring, but not clear enough for glass bottom boats. Spring Creek and the sinking streams or creeks were not flowing. So there was minimal tannic loading. Color was at its lowest, almost for the entire project, at about 5 PtCo units, virtually non-existent, so why was there poor visibility?

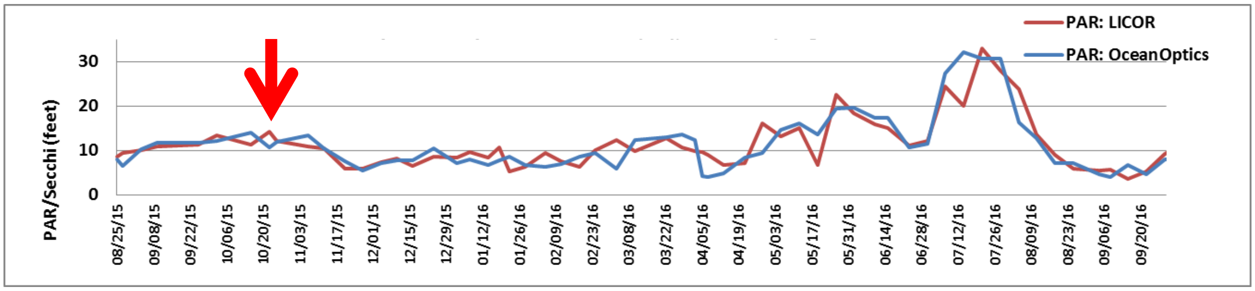
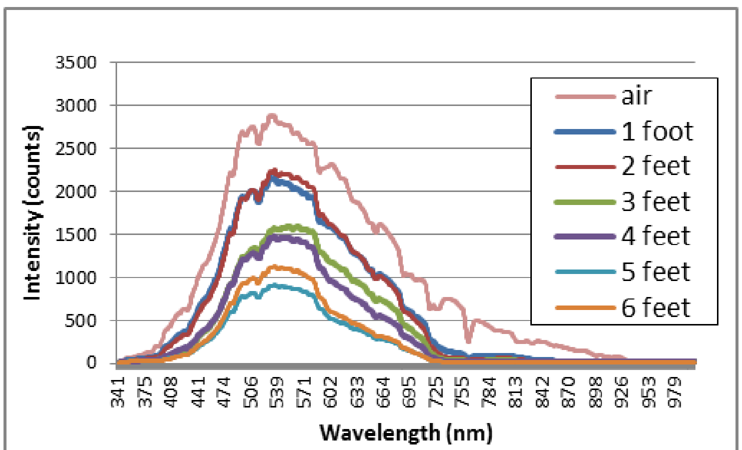


Figure 8.2.1.1: 0% PAR depth limit as measured 10/22/15 during Event 1 (light), 10/9/15-10/26/15.



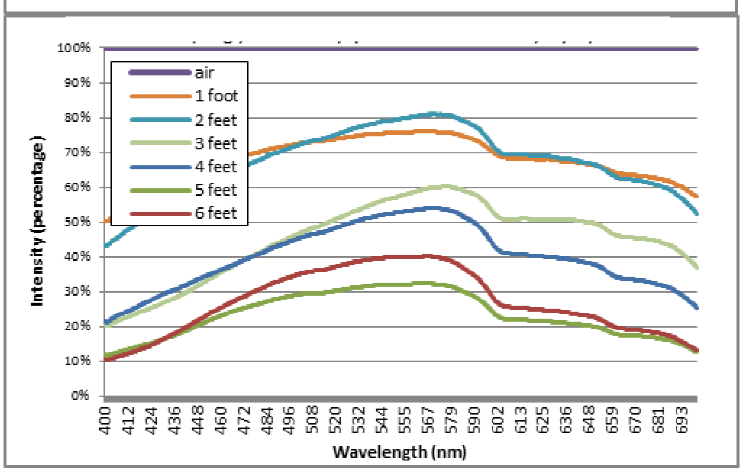


Figure 8.2.1.2: Event 1 (light) incident and normalized spectral radiometric transmittance through the water column at Wakulla Spring, 10/22/15.

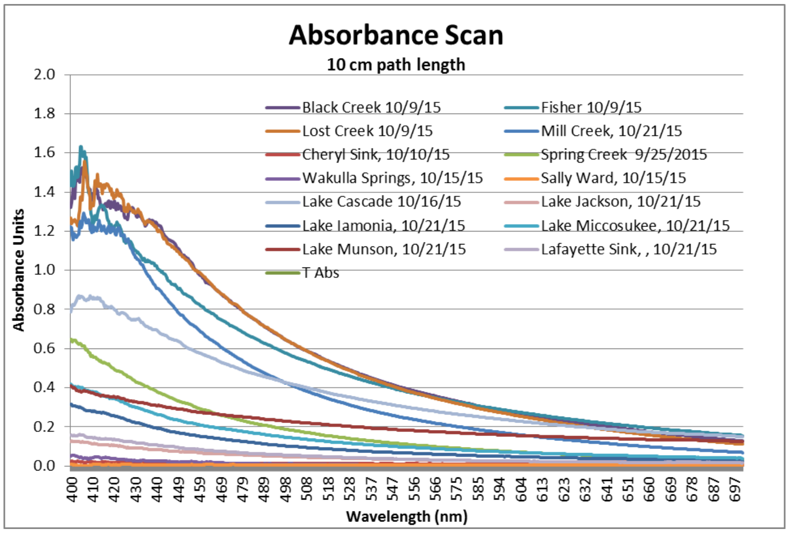


Figure 8.2.1.3: Event 1 (light) absorbance scans of Wakulla Spring and major karst sources, 10/9/15-10/26/15.

Waterbody	T Abs
Sally Ward Spring, 10/15/15	5
Wakulla Springs, 10/15/15	9
Cheryl Sink, 10/10/15	11
Lake Jackson, 10/21/15	38
Lafayette Sink, , 10/21/15	44
Lake Iamonia, 10/21/15	82
Lake Miccosukee, 10/21/15	114
Spring Creek, 9/25/2015	135
Lake Munson, 10/21/15	176
Lake Cascade, 10/16/15	318
Mill Creek, 10/21/15	335
Fisher Creek, 10/9/15	437
Lost Creek, 10/9/15	463
Black Creek, 10/9/15	470

Table 8.2.1.1: Event 1 (light) sum of PAR absorbance by Wakulla Spring and major karst sources, 10/9/15-10/26/15.

Light #1: 10/9/15 - 10/26/15					
Loads	PtCo/day	g/day	g/day	g/day	cfs
Watertype	**Color**	**Phaeophyti**	**Cor Chl a**	**T Chloro a**	**Discharge**
Lost Creek Load	14	0	0	2	2
Fisher Creek Load	5	0	0	0	0
Black Creek Load	18	0	0	1	1
Spring Creek Load	95	0	16	80	80
Sum Lake Load	259	8	78	87	81
Wakulla Spring Load	425	7	11	18	862

Table 8.2.1.2: Water quality parameter loadings during Event 1 (light).

8.2.2 Event 2 (Dark): 12/04/15-12/10/15
Sinking streams flowing; Spring Creek negative flow.

Event #2 had the second darkest color reading of all the events. All the sinking streams were flowing. Spring Creek had negative flow.

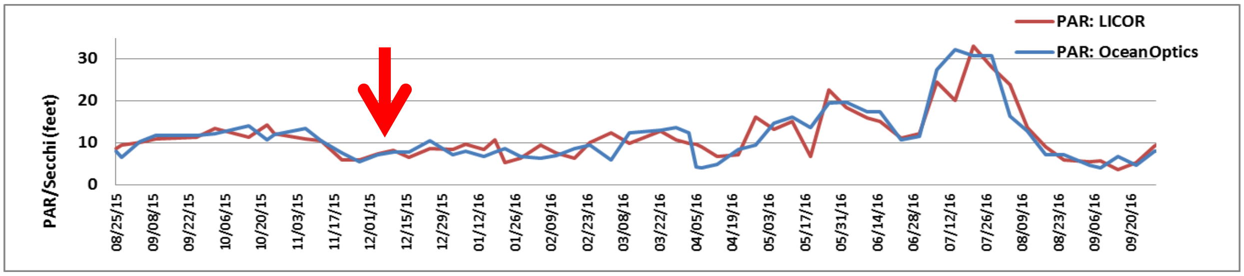


Figure 8.2.2.1: 0% PAR depth limit as measured 12/4/15 during Event 2 (dark), 12/04/15-12/10/15.

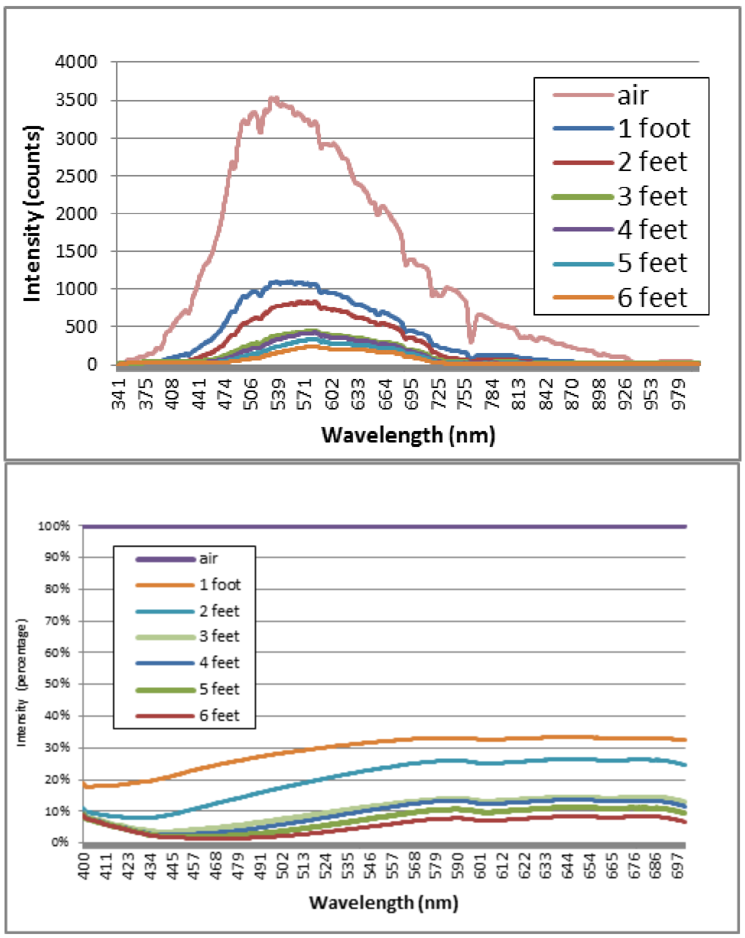


Figure 8.2.2.2: Event 2 (dark) incident and normalized spectral radiometric transmittance through the water column at Wakulla Spring, 12/04/15.

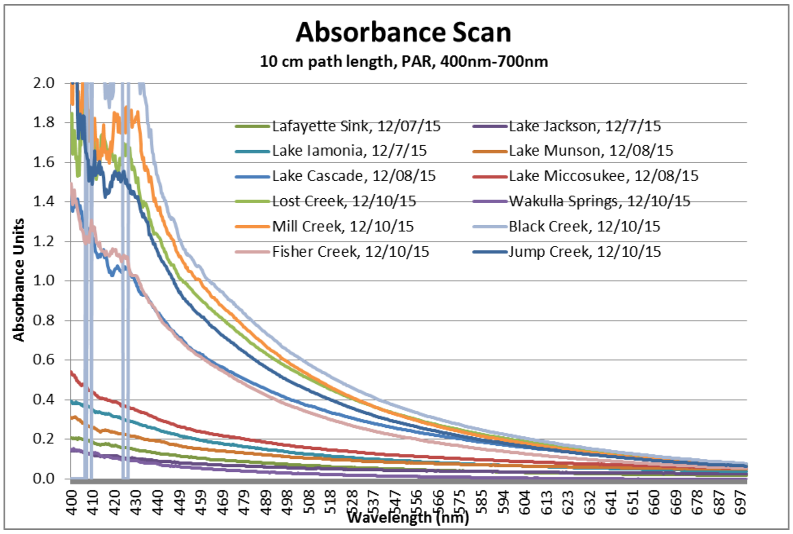


Figure 8.2.2.3: Event 2 (dark) absorbance scans of Wakulla Spring and major karst sources, 12/04/15-12/10/15.

Waterbody	T Abs
Wakulla Springs, 12/10/15	23
Lake Jackson, 12/7/15	61
Lafayette Sink, 12/07/15	55
Lake Munson, 12/08/15	90
Lake Iamonia, 12/7/15	105
Lake Miccosukee, 12/08/15	134
Fisher Creek, 12/10/15	303
Lake Cascade, 12/08/15	321
Jump Creek, 12/10/15	407
Lost Creek, 12/10/15	441
Mill Creek, 12/10/15	468
Black Creek, 12/10/15	482

Table 8.2.2.1: Event 2 (dark) sum of PAR absorbance for Wakulla Spring and major karst sources, 12/04/15-12/10/15.

Dark #2: 12/4/15 - 12/10/15					
Loads	PtCo/day	g/day	g/day	g/day	cfs
Watertype	**Color**	**Phaeophyti**	**Cor Chl a**	**TChloro a**	**Discharge**
Lost Creek Load	1138	0	8	100	100
Fisher Creek Load	187	1	0	24	24
Black Creek Load	133	0	0	10	10
Spring Creek Load	29	2	0	14	14
Sum Lake Load	253	17	24	41	81
Wakulla Spring Load	803	48	123	171	792

Table 8.2.2.2: Water quality parameter loadings during Event 2 (dark).

8.2.3 Event 3 (Dark): 01/04/16 – 01/14/16

Sinking streams flowing; Spring Creek negative flow.

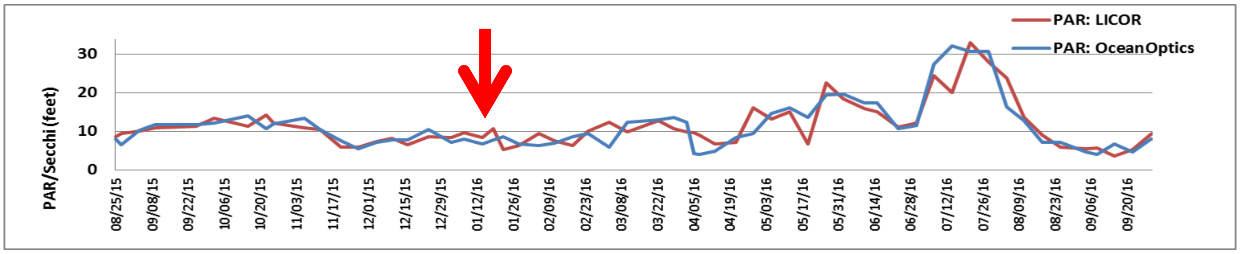


Figure 8.2.3.1: 0% PAR depth limit as measured 1/14/16 during Event 3 (dark), 01/04/16 – 01/14/16.

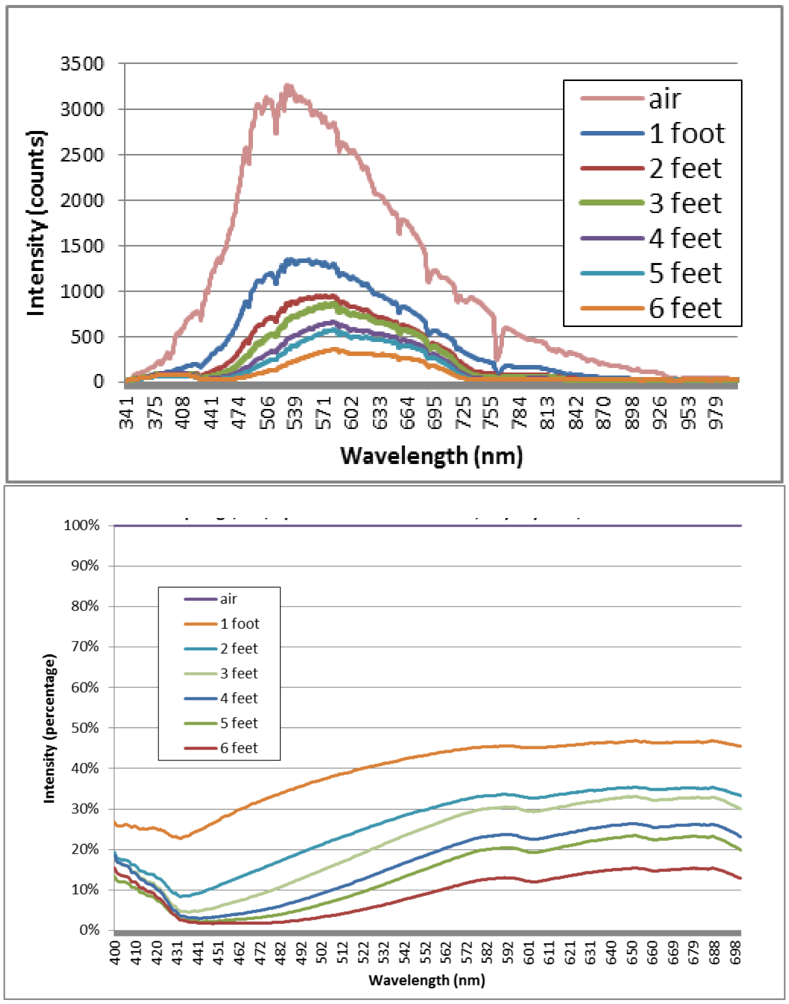


Figure 8.2.3.2: Event 3 (dark) incident and normalized spectral radiometric transmittance through the water column at Wakulla Spring, 01/14/16.

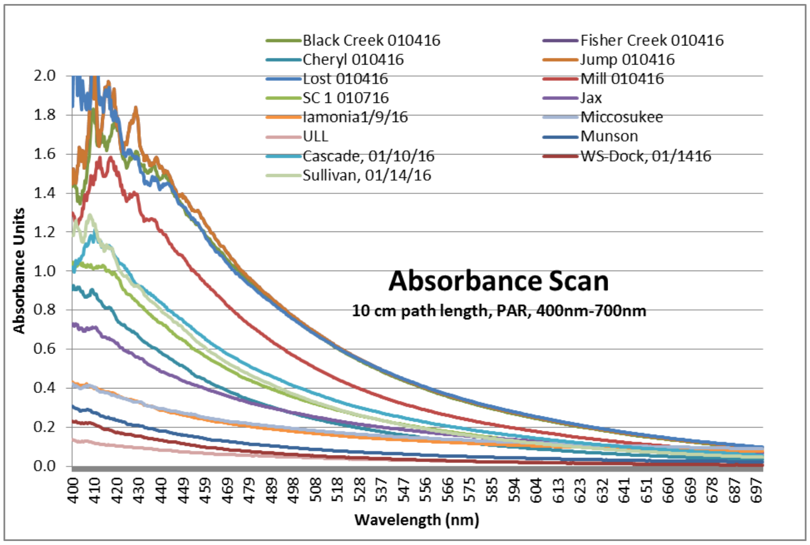


Figure 8.2.3.3: Event 3 (dark) absorbance scans of Wakulla Spring and major karst sources, 01/04/16 – 01/14/16.

Waterbody	T Abs
Upper Lake Lafayette, 01/09/16	35
Wakulla Springs-Dock, 01/14/16	47
Lake Munson, 01/09/16	73
Lake Iamonia, 01/09/16	140
Lake Miccosukee, 01/09/16	150
Lake Jackson, 01/09/16	209
Cheryl Sink, 01/04/16	211
Spring Creek #1, 01/07/16	270
Sullivan Sink, 01/14/16	285
Lake Cascade, 01/10/16	308
Mill Creek, 01/04/16	417
Black Creek 01/04/16	523
Fisher Creek 01/04/16	527
Jump Creek, 01/04/16	544
Lost Creek, 01/04/16	545

Table 8.2.3.1: Event 3 (dark) sum of PAR absorbance for Wakulla Spring and major karst sources, 01/04/16 – 01/14/16.

Dark #3:1/4/16 - 1/14/16					
Loads	PtCo/day	g/day	g/day	g/day	cfs
Watertype	**Color**	**Phaeophyti**	**Cor Chl a**	**T Chloro a**	**Discharge**
Lost Creek Load	3758	0	36	258	258
Fisher Creek Load	836	0	8	85	85
Black Creek Load	413	0	0	27	27
Spring Creek Load	366	2	2	82	82
Sum Lake Load	234	14	29	43	81
Wakulla Spring Load	875	0	147	147	896

Table 8.2.3.2: Water quality parameter loadings during Event 3 (dark)

8.2.4 Event 4 (Dark): 02/15/16 – 02/18/16

Sinking streams and Spring Creek flowing and Spring Creek flow at 23 cfs was just barely positive.

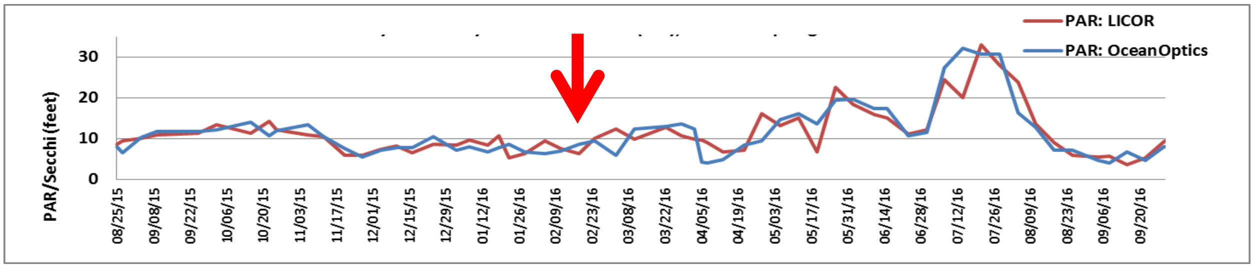


Figure 8.2.4.1: 0% PAR depth limit as measured 2/18/16 during Event 4 (dark), 02/15/16 – 02/18/16.

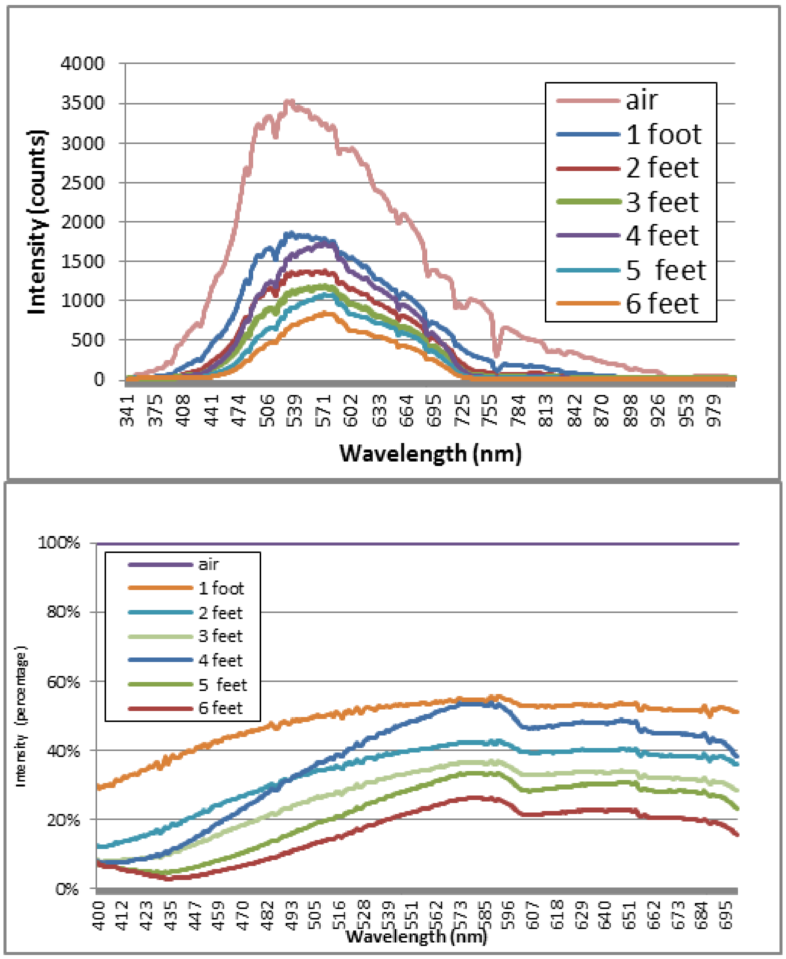


Figure 8.2.4.2: Event 4 (dark) incident and normalized spectral radiometric transmittance through the water column at Wakulla Spring, raw and normalized data, 02/18/16.

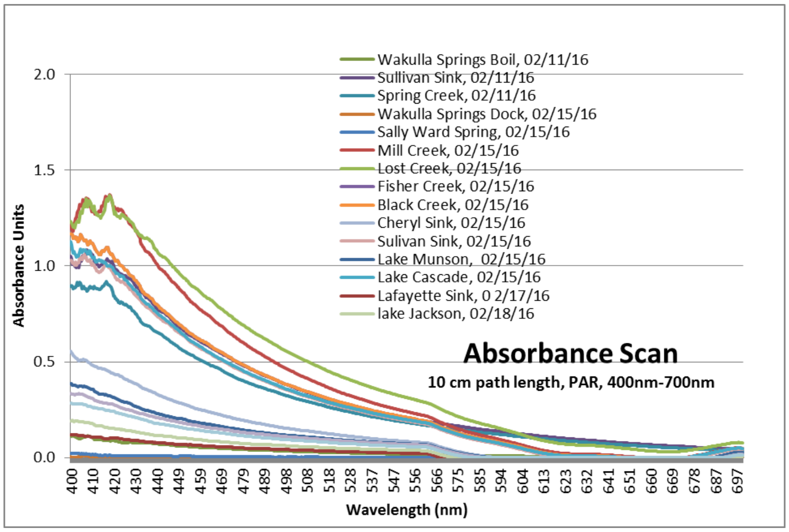


Figure 8.2.4.3: Event 4 (dark) absorbance scans of Wakulla Spring and major karst sources, 02/15/16 – 02/18/16.

Waterbody	T Abs
Wakulla Springs Dock, 02/15/16	0
Sally Ward Spring, 02/15/16	3
Wakulla Springs Boil, 02/11/16	24
Lafayette Sink, 0 2/17/16	26
lake Jackson, 02/18/16	39
Lake Micc, 02/18/16	60
Lake Iamonia, 02/18/16	71
Lake Munson, 02/15/16	79
Cheryl Sink, 02/15/16	107
Spring Creek, 02/11/16	241
Sulivan Sink, 02/15/16	245
Lake Cascade, 02/15/16	252
Fisher Creek, 02/15/16	268
Black Creek, 02/15/16	268
Sullivan Sink, 02/11/16	282
Mill Creek, 02/15/16	327
Lost Creek, 02/15/16	374

Table 8.2.4.1: Event 4 (dark) sum of PAR absorbance for Wakulla Spring and major karst sources, 02/15/16 – 02/18/16.

Dark #4: 2/15/16 - 2/18/16					
Loads	PtCo/day	g/day	g/day	g/day	cfs
Watertype	**Color**	**Phaeophyti**	**Cor Chl a**	**TChloro a**	**Discharge**
Lost Creek Load	1270	22	0	129	129
Fisher Creek Load	440	0	0	40	40
Black Creek Load	200	0	0	16	16
Spring Creek Load	26	1	0	23	23
Sum Lake Load	233	87	14	101	81
Wakulla Spring Load	2535	45	7	52	862

Table 8.2.4.2: Water quality parameter loadings during Event 4 (dark)

8.2.5 Event 5 (Light): 05/12/16 – 05/26/16
Sinking streams and Spring Creek flowing at low levels.

Creek flows totaled only 19 cfs, nearly an order of magnitude less than during all other events except Event 1 (light). Spring Creek flow of only 498 cfs was 600 to 900 cfs less than other events for which it had positive flow.

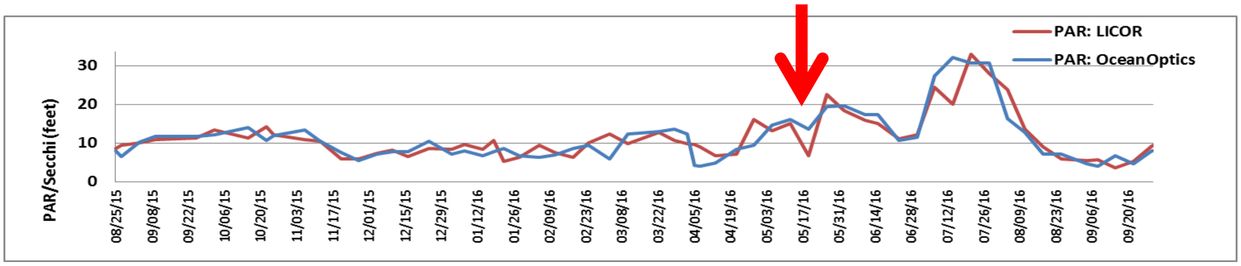


Figure 8.2.5.1: 0% PAR depth limit as measured 5/19/16 during Event 5 (light), 05/12/16 – 05/26/16.

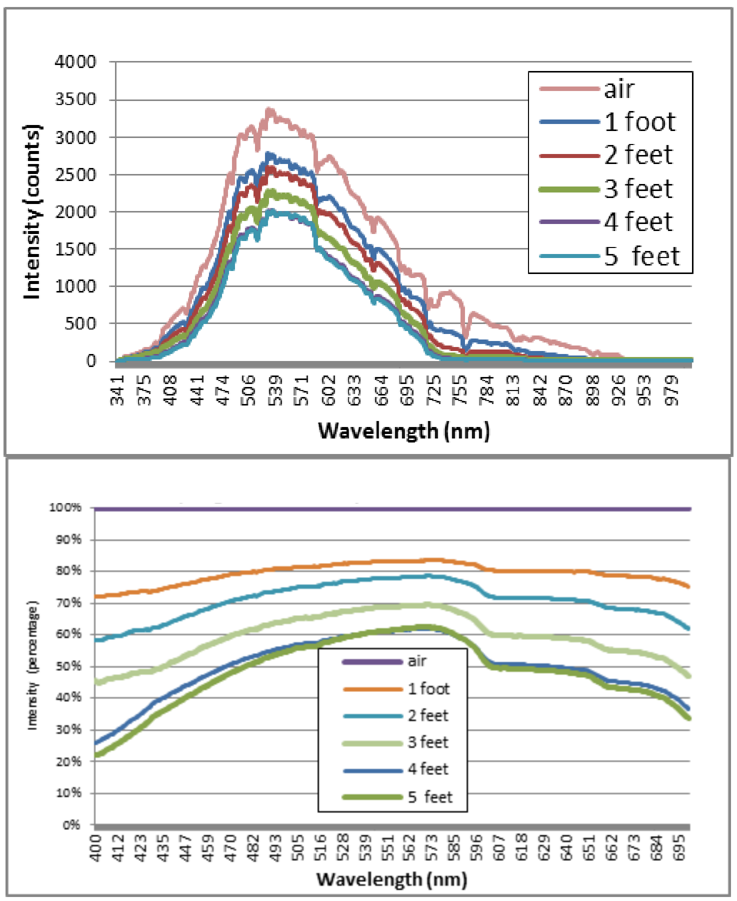


Figure 8.2.5.2: Event 5 (light) incident and normalized spectral radiometric transmittance through the water column at Wakulla Spring, raw and normalized data, 05/19/16.

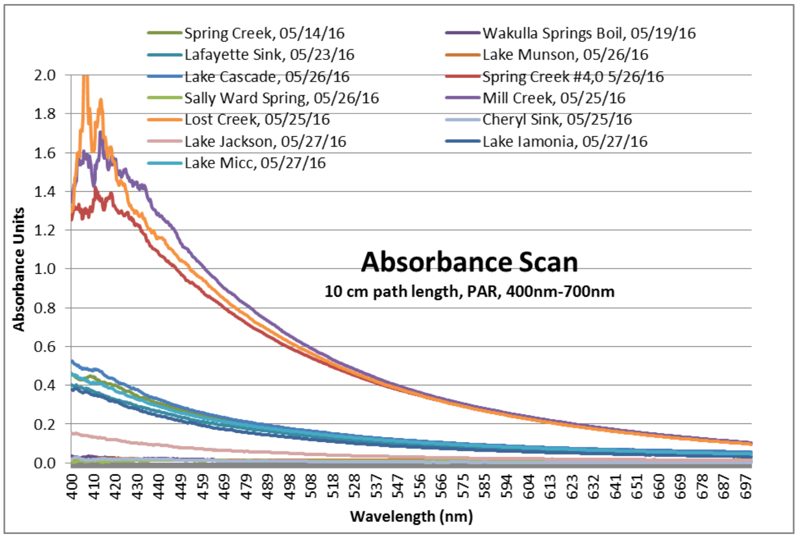


Figure 8.2.5.3: Event 5 (light) absorbance scans of Wakulla Spring and major karst sources, 05/12/16 – 05/26/16.

Waterbody	T Abs
Cheryl Sink, 05/25/16	6
Sally Ward Spring, 05/26/16	8
Wakulla Springs Boil, 05/19/16	10
Lake Munson, 05/26/16	11
Lake Jackson, 05/27/16	38
Lake Iamonia, 05/27/16	101
Lafayette Sink, 05/23/16	113
Spring Creek, 05/14/16	122
Lake Miccosukee, 05/27/16	126
Lake Cascade, 05/26/16	139
Spring Creek #4, 05/26/16	427
Lost Creek, 05/25/16	460
Mill Creek, 05/25/16	477

Table 8.2.5.1: Event 5 (light) sum of PAR absorbance for Wakulla Spring and major karst sources, 05/12/16 – 05/26/16.

Light #5: 5/12/16 - 5/26/16					
Loads	PtCo/day	g/day	g/day	g/day	cfs
Watertype	**Color**	**Phaeophyti**	**Cor Chl a**	**TChloro a**	**Discharge**
Lost Creek Load	52	0	4	19	19
Fisher Creek Load	27	1	0	2	2
Black Creek Load	20	3	0	2	2
Spring Creek Load	0	0	0	468	468
Sum Lake Load	338	194	312	506	81
Wakulla Spring Load	333	0	0	0	714

Table 8.2.5.2: Water quality parameter loadings during Event 5 (light).

8.2.6 Event 6 (Light): 06/11/16 – 06/20/16

Sinking streams and Spring Creek flowing.

This event experienced good PAR depth limit at 17.5 feet. Chlorophylls and phaeophytin were zero and true color was also low.

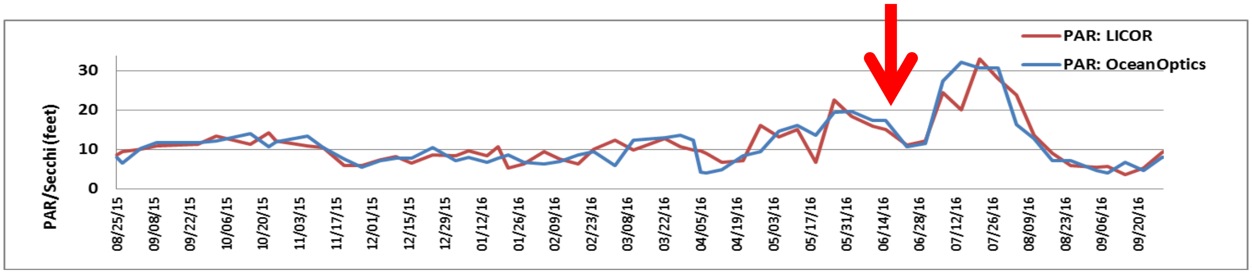


Figure 8.2.6.1: 0% PAR depth limit as measured 6/15/16 during Event 6 (light), 06/11/16 – 06/20/16.

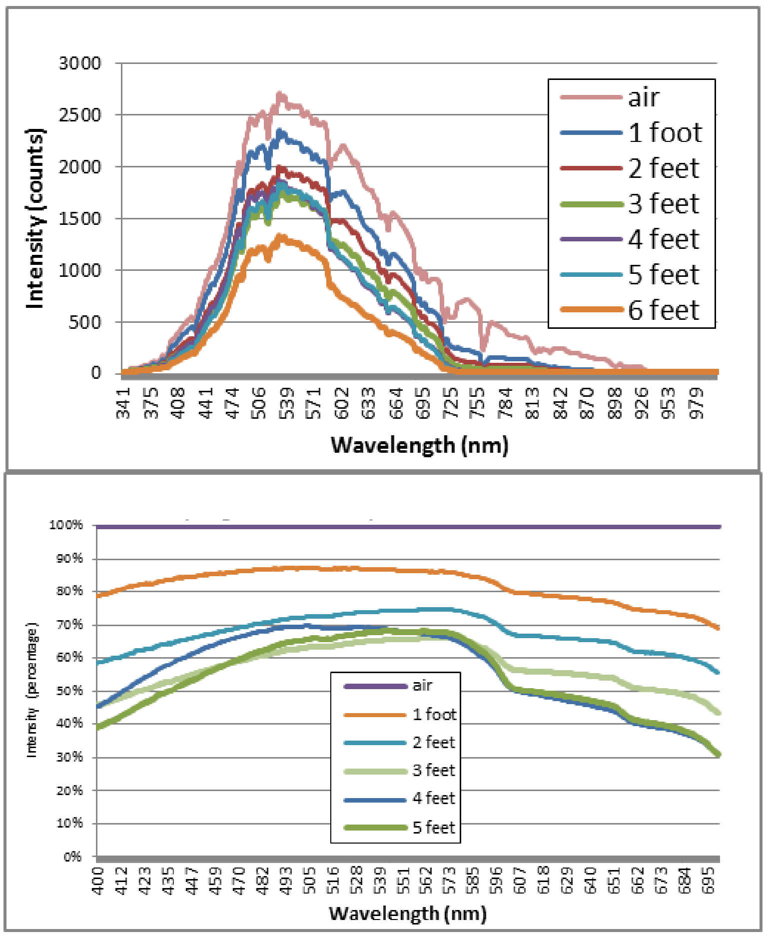


Figure 8.2.6.2: Event 6 (light) incident and normalized spectral radiometric transmittance through the water column at Wakulla Spring, 06/15/16.

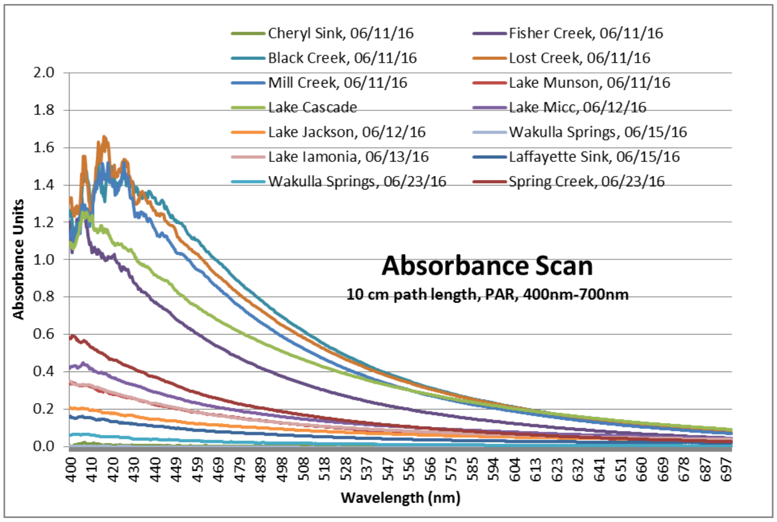


Figure 8.2.6.3: Event 6 (light) absorbance scans of Wakulla Spring and major karst sources, 06/11/16 – 06/20/16.

Waterbody	T Abs
Wakulla Springs, 06/15/16	0
Cheryl Sink, 06/11/16	1
Wakulla Springs, 06/23/16	14
Laffayette Sink, 06/15/16	46
Lake Jackson, 06/12/16	69
Lake Munson, 06/11/16	94
Lake Iamonia, 06/13/16	98
Lake Micc, 06/12/16	123
Fisher Creek, 06/11/16	284
Lake Cascade, 06/11/16	367
Mill Creek, 06/11/16	416
Lost Creek, 06/11/16	454
Black Creek, 06/11/16	464

Table 8.2.6.1: Event 6 (light) sum of PAR absorbance for Wakulla Spring and major karst sources, 06/11/16 – 06/20/16.

Light #6: 6/11/16 - 6/20/16					
Loads	PtCo/day	g/day	g/day	g/day	cfs
Watertype	**Color**	**Phaeophyti**	**Cor Chl a**	**TChloro a**	**Discharge**
Lost Creek Load	1415	12	7	117	117
Fisher Creek Load	144	10	1	19	19
Black Creek Load	223	1	1	16	16
Spring Creek Load	11761	28	104	1078	1078
Sum Lake Load	322	32	152	183	81
Wakulla Spring Load	236	0	16	16	902

Table 8.2.6.2: Water quality parameter loadings during Event 6 (light).

8.2.7 Event 7 (Light): 08/06/16 – 08/13/16

Sinking streams and Spring Creek flowing.

Event #7 included a period when, for the first time the spring was sufficiently clear for the glass bottom boats to operate. True color was comparable to two dark events (#3 and 4), but chlorophyll and phaeophytin concentrations were mixed.

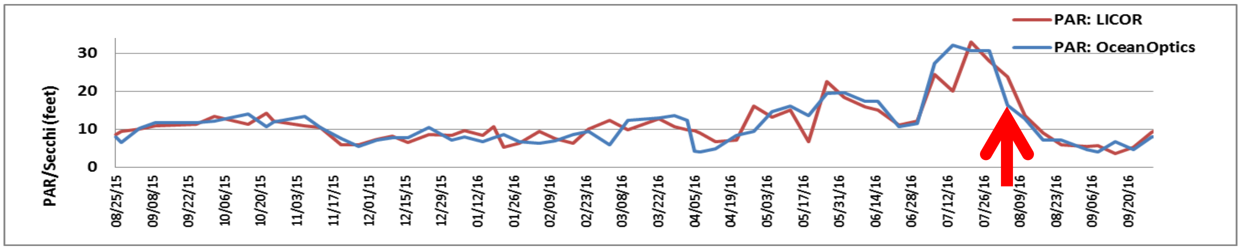


Figure 8.2.7.1: 0% PAR depth limit as measured 8/11/16 during Event 7 (light), 08/06/16 – 08/13/16.

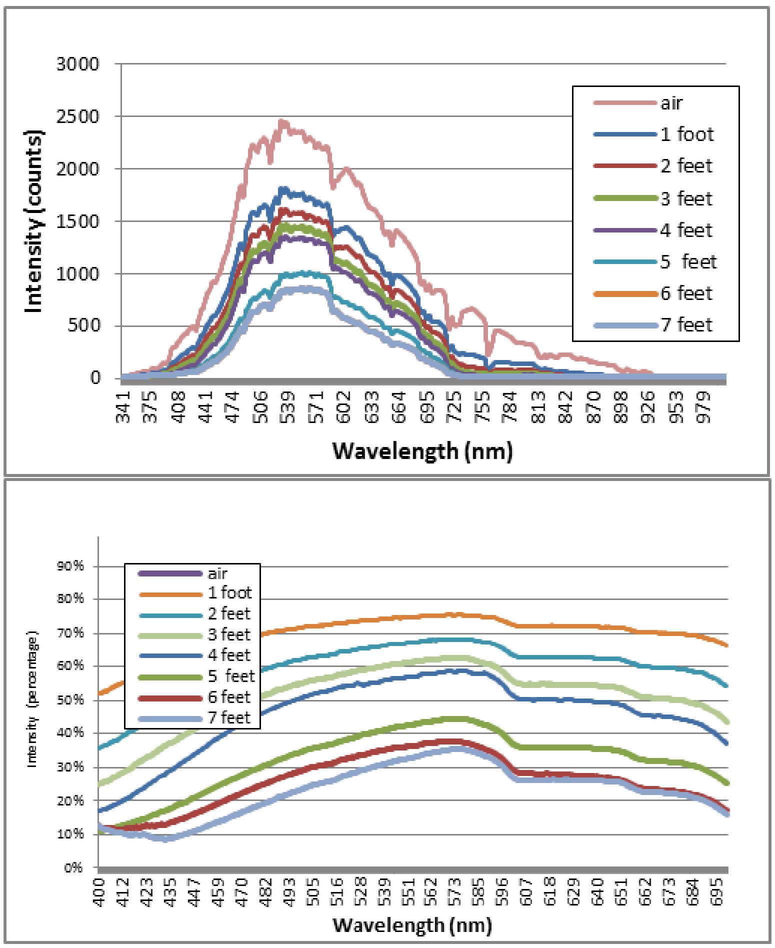


Figure 8.2.7.2: Event 7 (light) incident and normalized spectral radiometric transmittance through the water column at Wakulla Spring, 8/11/16.

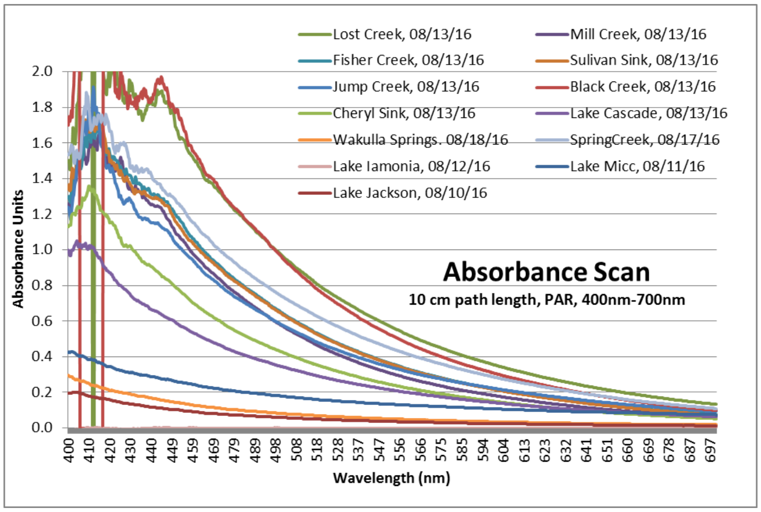


Figure 8.2.7.3: Event 7 (light) absorbance scans of Wakulla Spring and major karst sources, 08/06/16 – 08/13/16.

Waterbody	T Abs
Lake Jackson, 08/10/16	47
Wakulla Spring,. 08/18/16	66
Lake Iamonia, 08/12/16	100
Lake Miccosukee, 08/11/16	140
Lake Cascade, 08/13/16	267
Cheryl Sink, 08/13/16	329
Mill Creek, 08/13/16	430
Jump Creek, 08/13/16	441
Sulivan Sink, 08/13/16	466
Fisher Creek, 08/13/16	471
Spring Creek, 08/17/16	535
Black Creek, 08/13/16	604
Lost Creek, 08/13/16	679

Table 8.2.7.1: Event 7 (light) sum of PAR absorbance for Wakulla Spring and major karst sources, 08/06/16 – 08/13/16.

Light #7: 8/6/16 - 8/13/16					
Loads	PtCo/day	g/day	g/day	g/day	cfs
Watertype	**Color**	**Phaeophyti**	**Cor Chl a**	**TChloro a**	**Discharge**
Lost Creek Load	17366	88	0	261	261
Fisher Creek Load	556	0	0	12	12
Black Creek Load	2448	0	0	35	35
Spring Creek Load	1123	15	0	858	858
Sum Lake Load	1164	151	1018	1170	81
Wakulla Spring Load	746	19	0	19	1093

Table 8.2.7.2: Water quality parameter loadings during Event 7 (light).

8.2.8 Event 8 (Dark): 08/27/16– 09/04/16
Sinking streams and Spring Creek flowing.

Event #8 is the ultimate dark event with very high tannins (true color = 100) and moderately high concentrations of chlorophylls and phaeophytin.

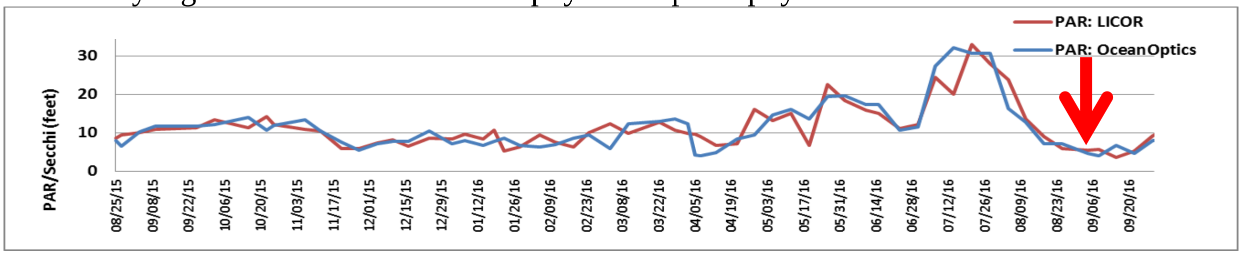


Figure 8.2.8.1: 0% PAR depth limit as measured 9/4/16 during Event 8 (dark), 08/25/16–09/20/16.

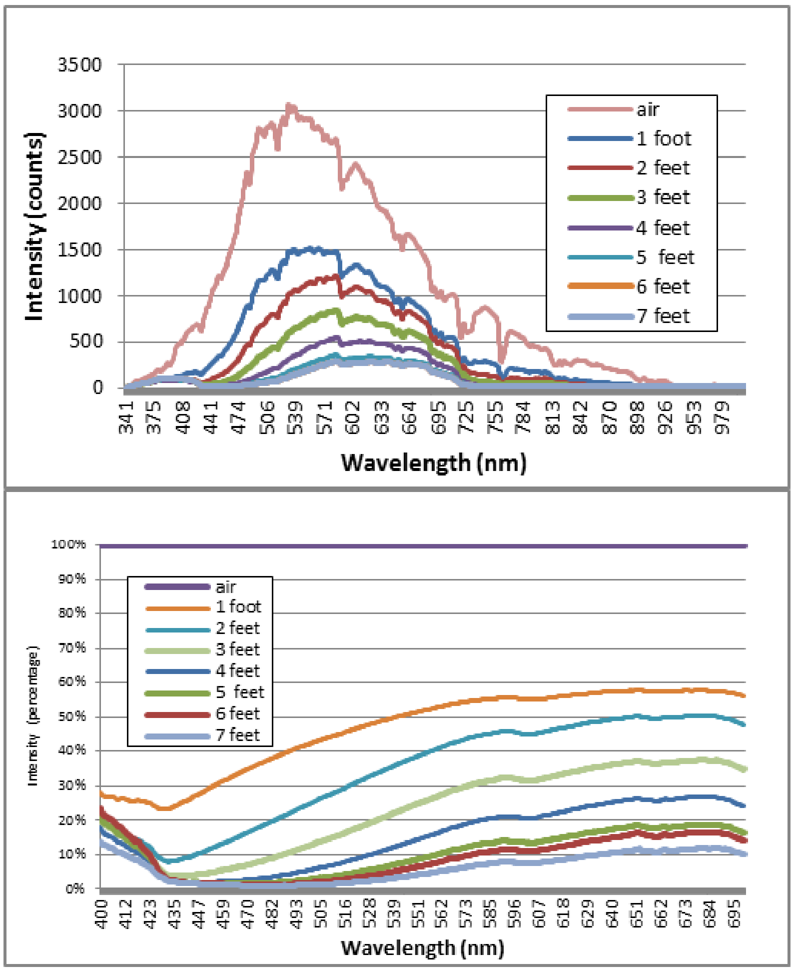


Figure 8.2.8.2: Event 8 (dark) incident and normalized spectral radiometric transmittance through the water column at Wakulla Spring, 09/04/16.

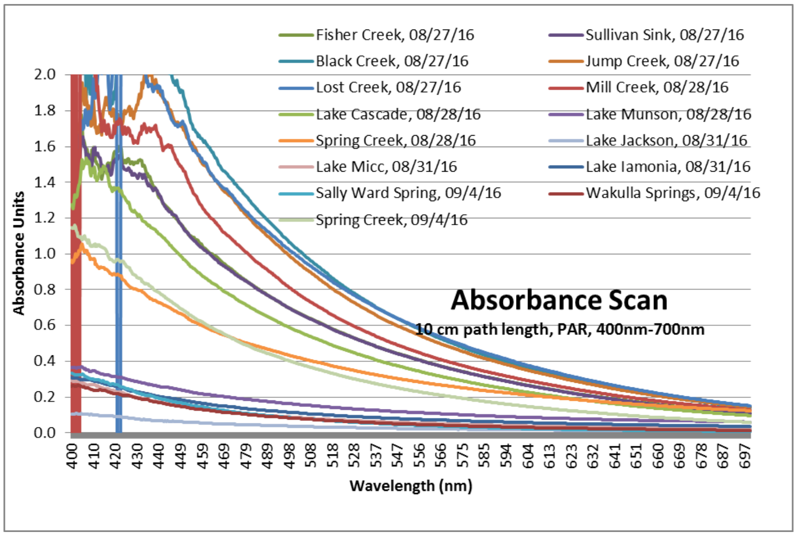


Figure 8.2.8.3: Event 8 (dark) absorbance scans of Wakulla Spring and major karst sources, 08/27/16– 09/04/16.

Waterbody	T Abs
Lake Jackson, 08/31/16	29
Wakulla Springs, 09/4/16	64
Sally Ward Spring, 09/4/16	68
Lake Miccosukee, 08/31/16	71
Lake Iamonia, 08/31/16	89
Lake Munson, 08/28/16	123
Spring Creek, 09/4/16	297
Spring Creek, 08/28/16	322
Lake Cascade, 08/28/16	431
Sullivan Sink, 08/27/16	497
Fisher Creek, 08/27/16	503
Mill Creek, 08/28/16	588
Jump Creek, 08/27/16	668
Lost Creek, 08/27/16	693
Black Creek, 08/27/16	737

Table 8.2.8.1: Event 8 (dark) sum of PAR absorbance for Wakulla Spring and major karst sources, 08/27/16-09/04/16.

Dark #8: 8/27/16 - 9/04/16					
Loads	PtCo/day	g/day	g/day	g/day	cfs
Watertype	**Color**	**Phaeophyti**	**Cor Chl a**	**TChloro a**	**Discharge**
Lost Creek Load	34718	35	0	504	504
Fisher Creek Load	5801	6	0	121	121
Black Creek Load	6270	12	0	86	86
Spring Creek Load	0	0	0	1311	1311
Sum Lake Load	630	177	156	333	81
Wakulla Spring Load	5210	43	0	43	1004

Table 8.2.8.2: Water quality parameter loadings during Event 8 (dark).

8.3 Sources of Water Quality Parameter Loadings to Wakulla Spring

This section presents bar charts water quality parameter loadings for each event offering an easy visual assessment of the principal sources of the different water quality parameters during light and dark events. Loads are divided into sinking streams (creek load), sinking lakes (lake load), and Wakulla Spring (WS load). The creek and lake loadings are from seepage and sinkhole/swallet discharges to the Upper Floridan Aquifer. The Wakulla Spring loadings are the discharge from the spring to the river. The creek loadings include those from Lost Creek. Sometimes its loading goes to Wakulla Spring and sometimes it does not depend on whether flow at Spring Creek is positive.

8.3.1 True Color Loading

True color loading is a measure of tannin levels in the water. Loadings to Wakulla Spring are predominantly from the sinking streams. During five events the total creek load exceeds the Wakulla Spring load, but Lost Creek flows very likely did not flow towards Wakulla Spring during events E1, E2, E3, and possibly E4 when Spring Creek flows were less than or equal to zero (see table 8.1.2).

Figure 8.3.1.1: True color loadings.

8.3.2 Corrected Chlorophyll a Loadings

We assume that chlorophyll originates in the lakes in the Wakulla springshed because the high flow at the spring vent cannot sustain a standing crop of phytoplankton. Corrected chlorophyll a loads in the lakes exceed that of Wakulla Spring in six events. Creek loads are lower than Wakulla Spring in six of the eight events.

Figure 8.3.2.1: Corrected chlorophyll a loadings.

8.3.3 Phaeophytin Loadings

While we also assume that phaeophytin loadings ultimately originate from the lakes, their levels behave considerably differently from corrected chlorophyll a. The lake loadings exceed the Wakulla Spring level seven times, while creek loads are less than the spring in six of the eight events as is the case for corrected chlorophyll a.

Figure 8.3.3.1: Phaeophytin loadings.

8.3.4 Total Chlorophyll a Loadings

This is the sum of phaeophytin and corrected chlorophyll a. The patterns parallel those for the two components.

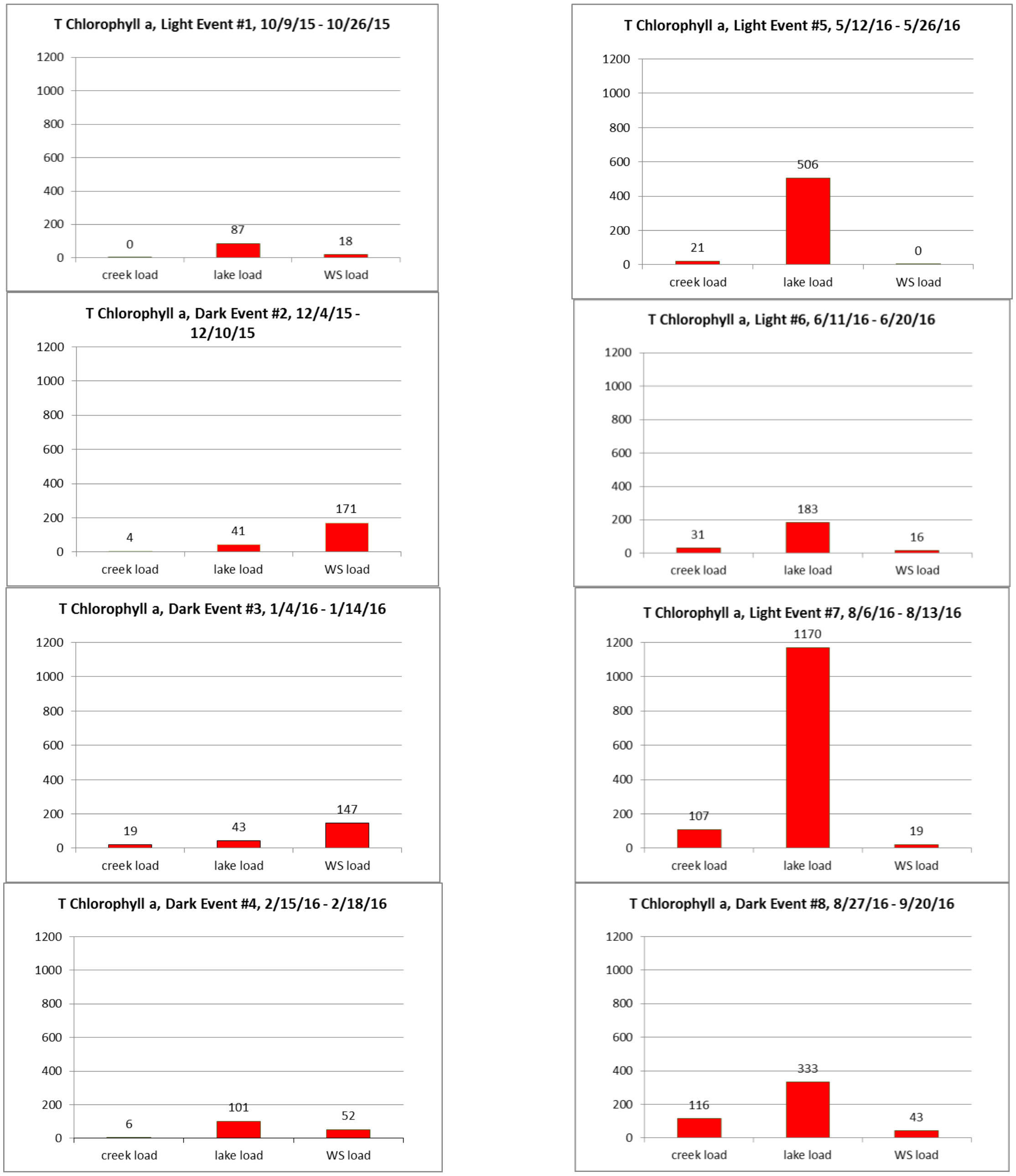


Figure 8.3.4: Total chlorophyll a loadings.

9. Summary of Findings and Conclusions

- Both tannins, measured as true color, and other color dissolved material (CDOM) as well as chlorophyll a, measured as corrected chlorophyll a and chlorophyll's degradation product phaeophytin, contribute to reduced visibility in the spring boil much of the time.
- Chlorophyll a contributes to dark water conditions during both "brown dark water" conditions when tannins lend a predominantly reddish-brown color to the water and during "green dark water" conditions when the water appears greenish-brown and visibility is greater but still much lower than historic conditions when the water was a clear pale blue in color and the bottom was visible from the surface.
- Chlorophyll a loadings to the Upper Floridan Aquifer in the near-springshed of Wakulla Spring are predominantly from the karst lakes with sinkholes that discharge to the aquifer.
- As has been historically the case, tannins are the predominant cause of the brown dark water conditions that prevail after periods of prolonged rainfall when the intermittent sinking streams north of the spring are discharging to the aquifer through their swallets. Tannins also are present at low levels during some "light" events when visibility is greater.
- We did observe conditions consistent with the understanding that at times when the Spring Creek springs cease flowing, tannin loads from Lost Creek can be diverted north to Wakulla Spring.
- Simple linear statistical analyses are insufficient for unravelling the complex dynamics of the dark water conditions at Wakulla Spring. A larger data set is needed to support more sophisticated analyses.
- Additional analyses of water quality in the caverns and conduits are needed to demonstrate that chlorophylls detected in the spring boil are carried into the spring in the ground water.
- Dye tests are required to establish hydrogeologic connections between Wakulla Spring and the major karst lakes that have not been previously tested, i.e. Lakes Jackson, Upper Lake Lafayette, and Lake Iamonia.
- Other research strategies are needed to link chlorophyll and phaeophytin detected in the spring boil to specific karst features that discharge to the Upper Floridan Aquifer.

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