



- I would like to share with you today information about the apparent water color of the spring and its causes drawn from a presentation I made in November 2020 based on research conducted by Sean McGlynn that was financed by several Protect Florida Springs tag grants that I secured and managed for WSA [\[click\]](#)



- When I moved to Tallahassee in 1991 you could see all the way to the bottom of the spring from a boat or the top of the dive tower through aqua blue water “as clear as gin” and “sapphire blue,” as one British travel writer once described it
- **[CLICK]** Historically, the water turned tea-colored after major rain events.
- Typically the spring would clear after a few weeks, unless we had prolonged periods of rain.
- But now the spring stays dark most of the time
- The periods of time when the water is brown have increased in duration and frequency
- **[click to next slide]** In addition, during times when the water has historically cleared, i.e. when you could see all the way to the bottom, the water is now green, with varying degrees of intensity

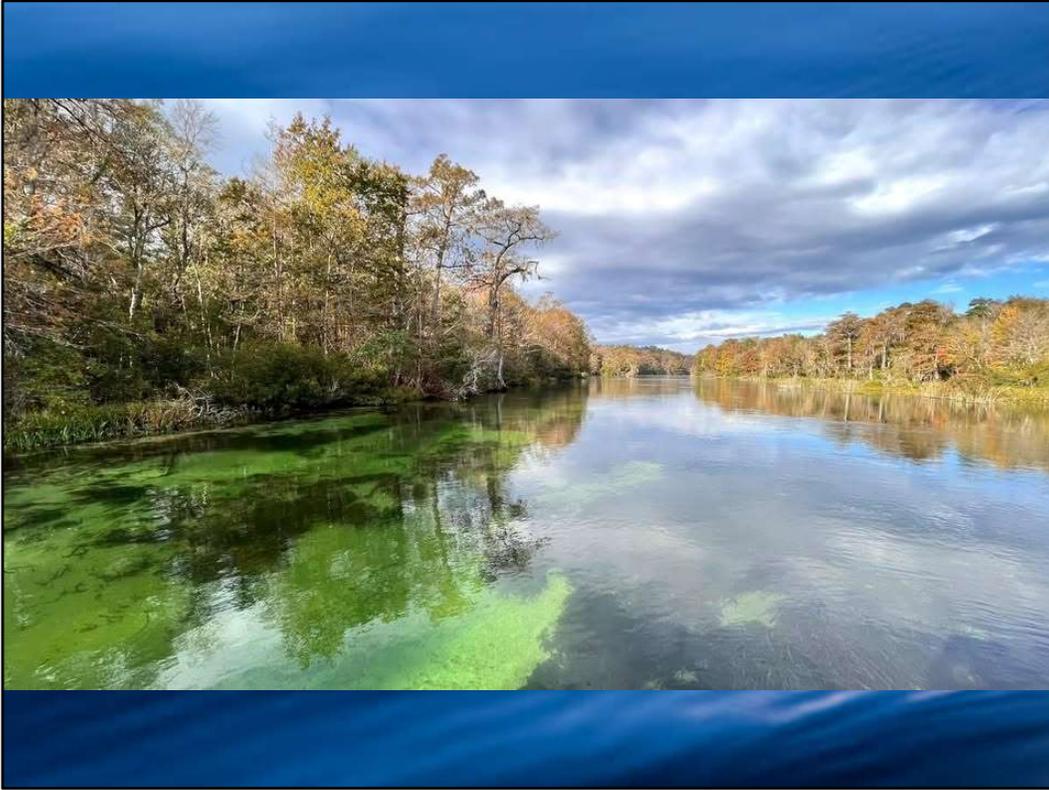


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- The change is starkly shown in these two photos of the spring bowl taken from the cave entrance





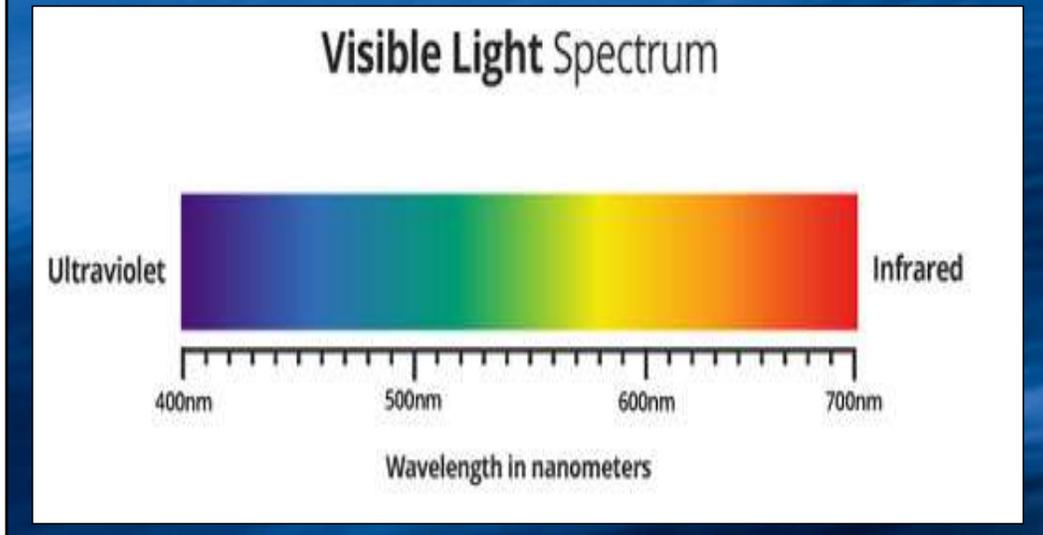
- The green color is sometimes even apparent in shallow water downstream as seen in this photo taken by wildlife survey volunteer Nico Wienders in November 2022

Causes of Apparent Color

- Colored Dissolved Organic Matter (CDOM): measured as “true color” (tannins)
- Chlorophyll a
- Pheophytin a

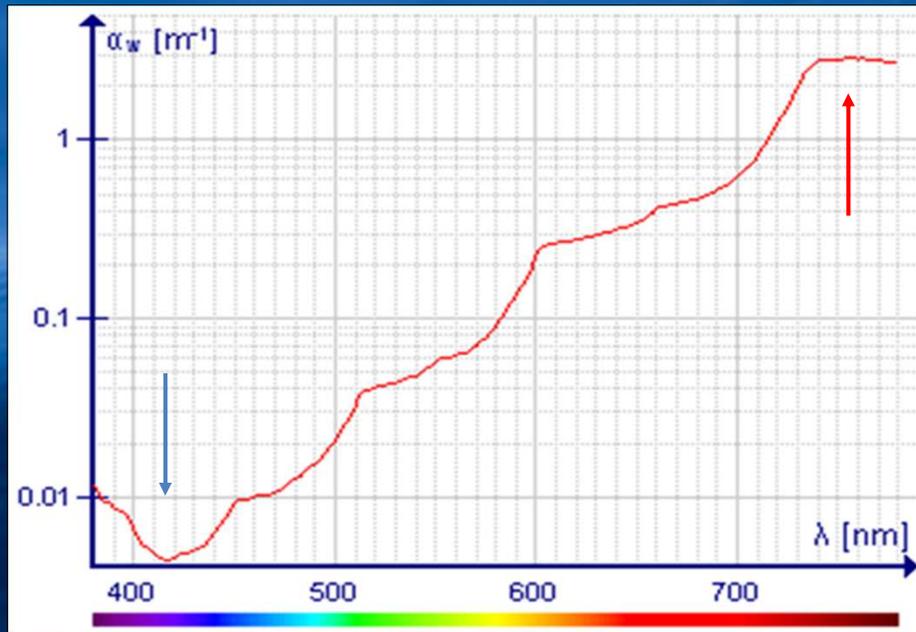
- **[click]** Three light-absorbing substances are present in the spring:
 - **[click]** Colored dissolved organic matter referred to as CDOM of which tannins are the predominant form in freshwater springs in Florida
 - **[click]** Chlorophyll a, the predominant form of chlorophyll in algae and vascular plants
 - **[click]** Pheophytin a, which is a degraded form of chlorophyll a that can occur when algal cells are damaged or die
- Analysis of the data collected by Sean McGlynn between December 2015, and January 2020, demonstrates that both CDOM and chlorophyll a have statistically significant negative impacts on visibility depth
- I am going to primarily focus on CDOM and chlorophyll a because the pheophytin results were anomalous (quantile regression indicated that pheophytin had a statistically significant positive impact when visibility depth was high – just the reverse of what is expected)
- **Reference:** Suspended particulates tend to be very low in most freshwater springs;
 - the common metric, turbidity, however, captures more than suspended inorganic particles
 - turbidity is an aggregate measure that reflects the presence of algae cells as well as colored dissolved organic matter
 - Therefore it is not very helpful in differentiating the individual causes of dark water conditions

Visibility Depth: 0% PAR Depth Limit



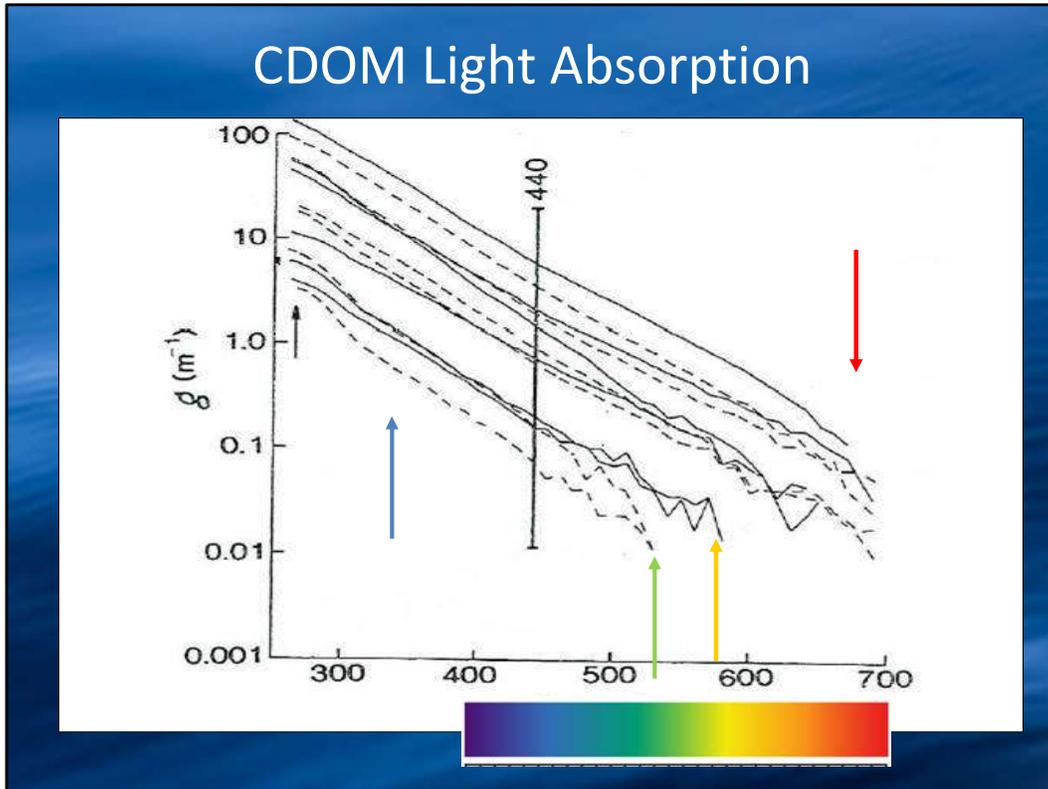
- Dr. McGlynn defined visibility depth as the depth at which all photosynthetically active radiation or PAR is extinguished by absorption or the 0% PAR depth limit
- The PAR spectrum is equivalent to the spectrum that humans can see, what we call "visible light" with wavelengths ranging from 400 to 700 nanometers

Water Light Absorption Spectrum



- Pure water absorbs light most strongly at the higher wavelengths in the red range **[click]**
- Thus it's the shorter blue light wavelengths that are most fully transmitted, hence clean water looks blue **[click]**

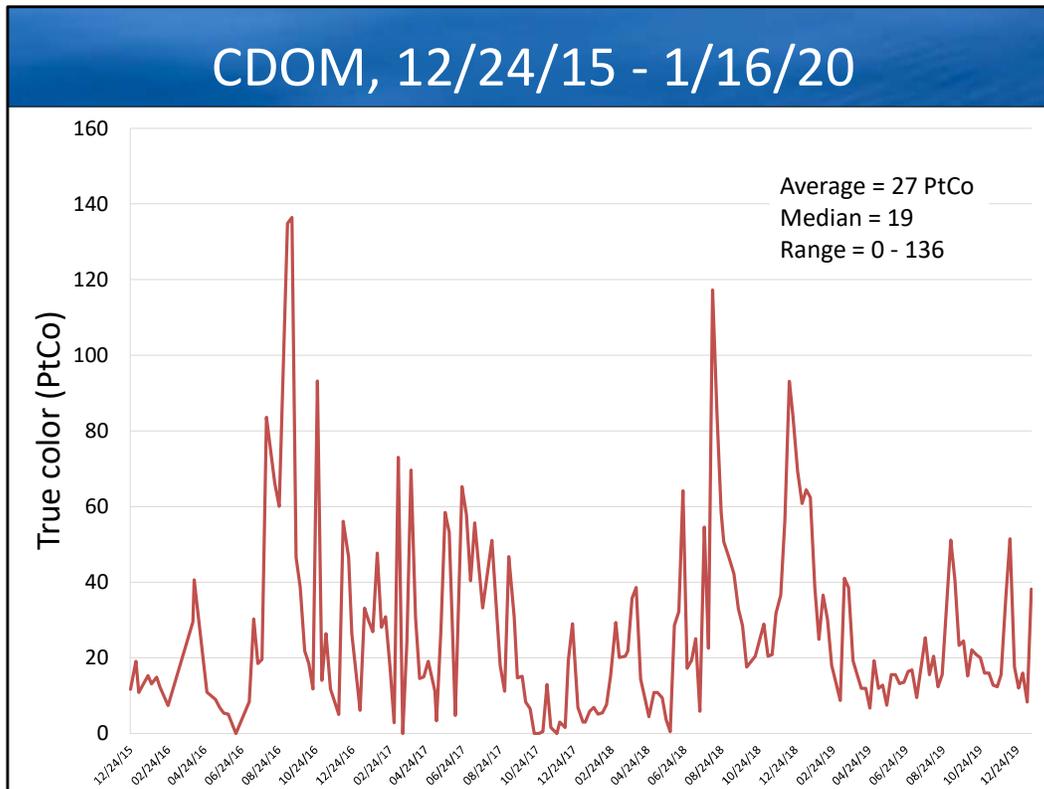
CDOM Light Absorption



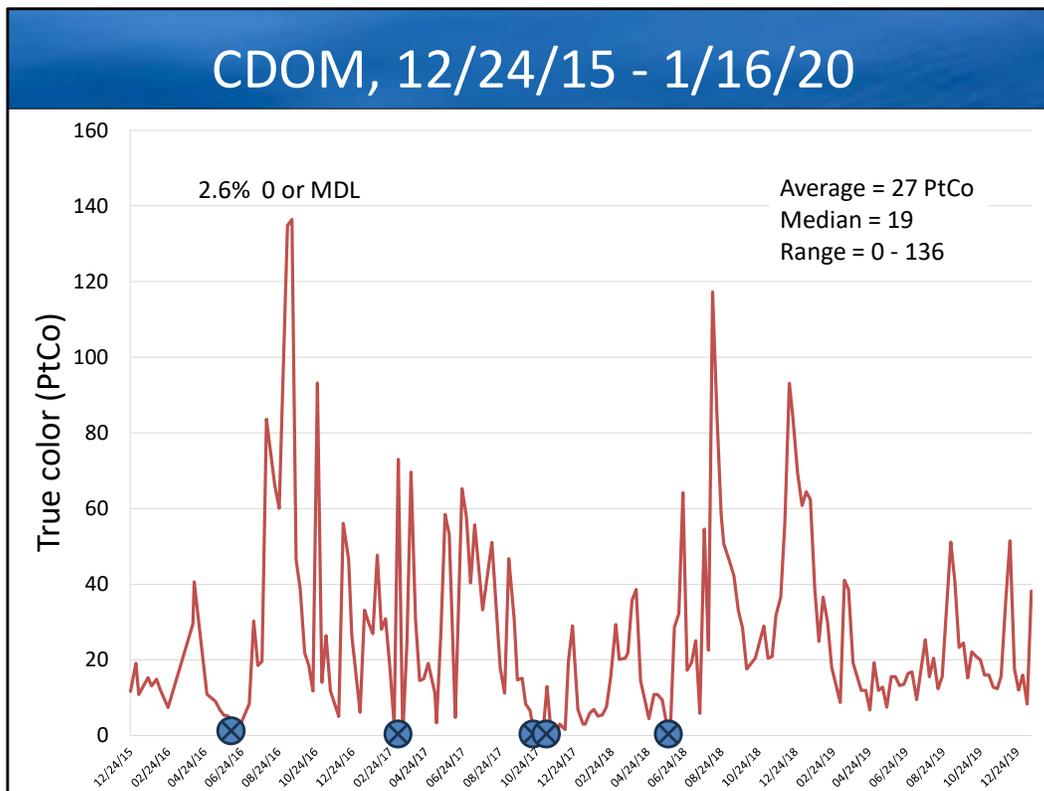
- This slide depicts light absorption curves for water bodies with different concentrations of colored dissolved organic matter, or CDOM
- When CDOM is present, it absorbs predominantly the shorter blue as well as ultra-violet wavelengths less than 400 nm which are outside the visible range **[click]**
- This shifts the dominant light transmission back to the right **[click]**
- Higher concentrations of CDOM absorb enough of the blue light to make the water appear reddish brown
- At lower concentrations, less blue light is absorbed and the transmitted wavelengths shift to the left
- Intermediate concentrations can yield a yellow to orange color **[click]**
- Low concentrations can produce an apparent green color **[click]**
- This is nicely illustrated in the next slide



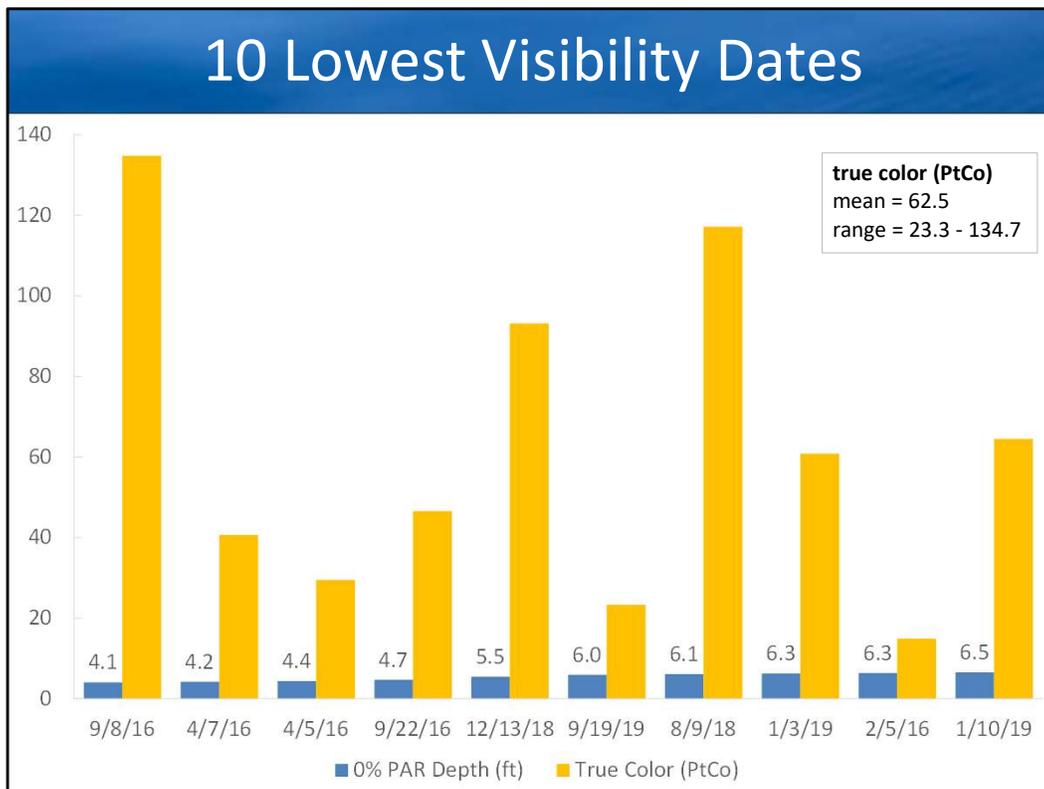
- This National Geographic photo depicts highly tannic water from the Santa Fe river mixing with the outflow from the Ginnie Spring run
 - You can see the color shifts with decreasing concentrations of CDOM in the area above the diver



- Let's take a look now at the individual light-absorbing substances
- CDOM measured as true color (tannins) over the 4.5-year study period, from Dec 24, 2015 to Jan 16, 2020 averaged 27 Platinum-Cobalt units
 - With a median of 19
 - And a range of 0 to 136
- **[click]** True color was zero or less than the method detection limit (0.19 PtCo) for only 5 of the 189 weekly samples (2.6%)

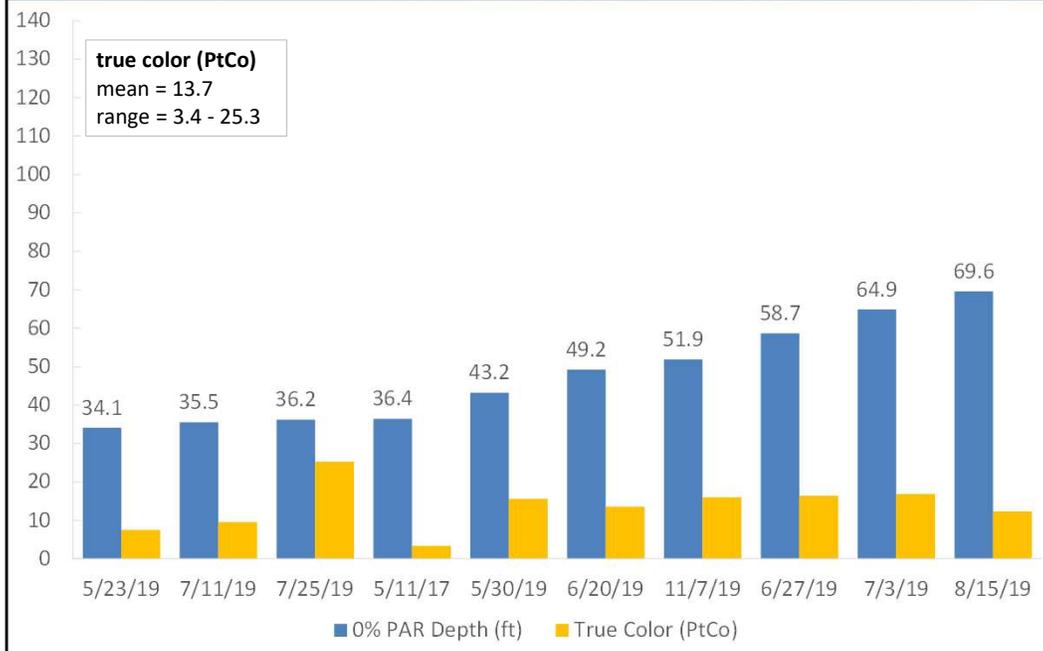


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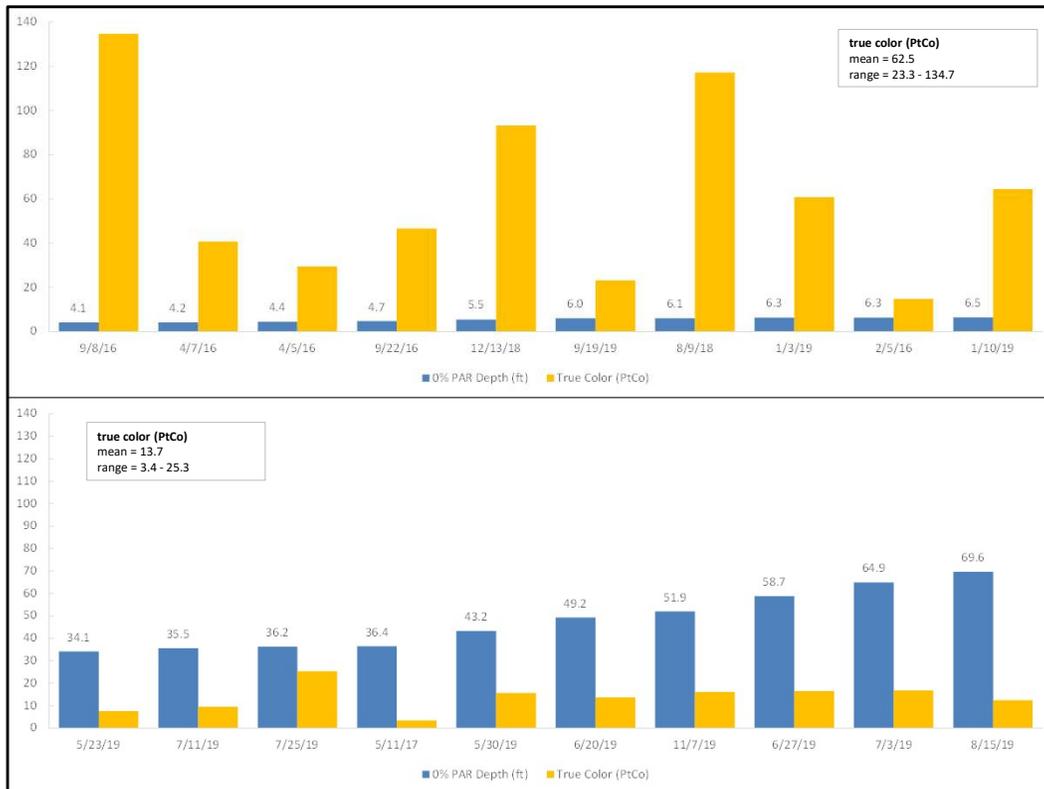


- If we examine the 10 dates when visibility was lowest during the study period, what we might call “dark water events,” we encounter visibility depths (blue bars) ranging from 4.1 feet on the left to 6.5 feet on the right
- True color values (yellow-orange bars) range from 23 to 135 PtCo but do not appear to be directly correlated with the variation in visibility

10 Highest Visibility Dates

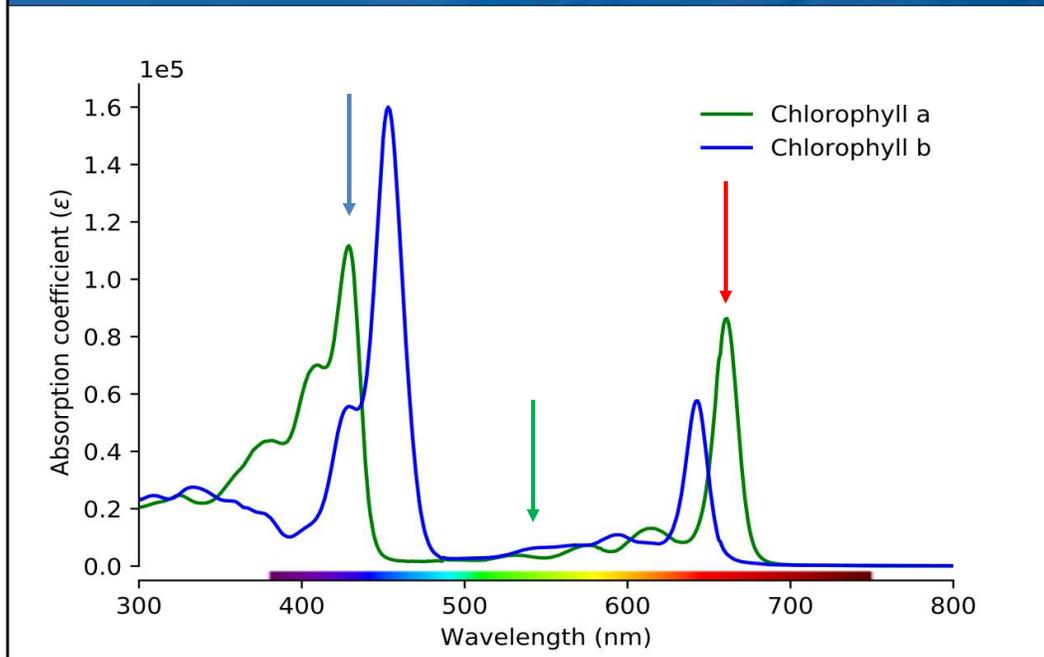


- If we examine the 10 dates when visibility was highest, what we might call “light water events” we encounter visibility depths ranging from 34 feet on the left to nearly 70 feet on the right
- True color values range from 3 to 55 PtCo but again do not appear to be directly correlated with visibility levels
- Note however, that CDOM is present on each of these dates when the perceived color of the water would have been green or greenish brown



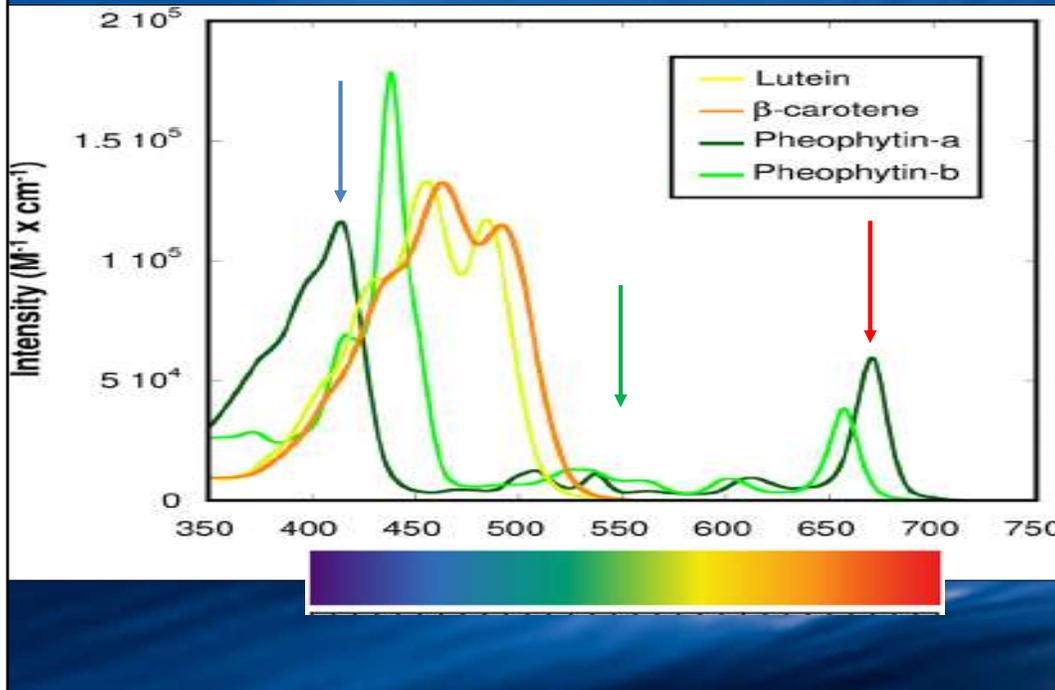
- Here are the two graphs compared

Chlorophyll a Light Absorption



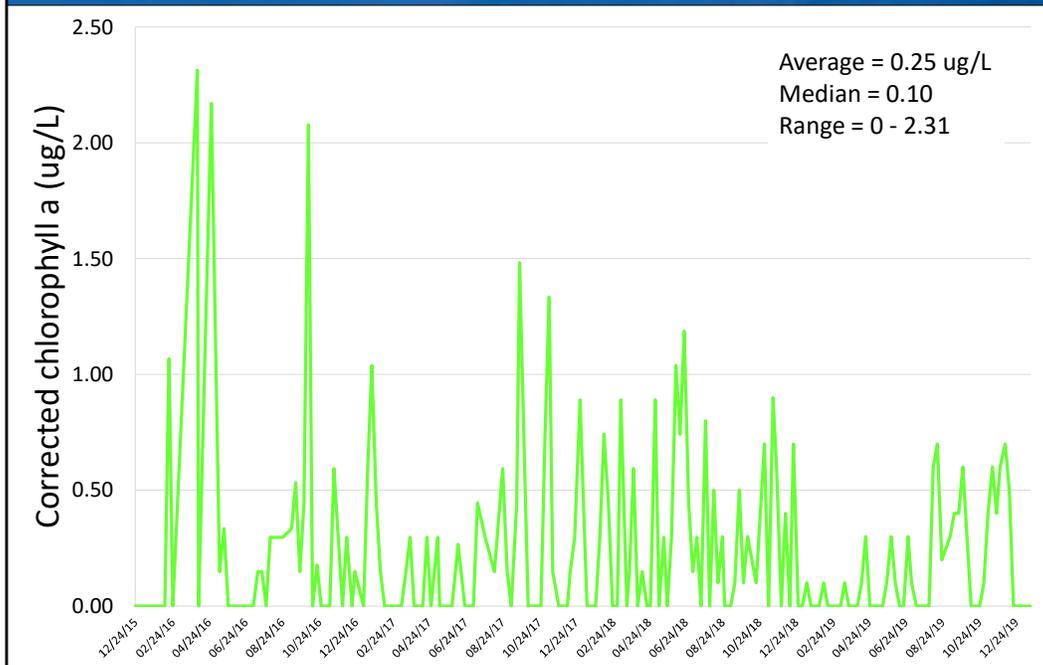
- The chlorophyll a absorption spectrum (green line) is bi-nodal with one peak in the blue range at 430 nm **[click]** and a second in the red range at 665 nm **[click]**
- If we just introduce chlorophyll a to clean water the transmission peak shifts to the middle of the visible light spectrum and the apparent color is green **[click]**
- **Reference:** Chlorophyll *b* is not typically measured in water quality monitoring because chlorophyll *a* is the primary pigment found in all types of algae and cyanobacteria and is a much more effective, universal indicator of total algal biomass and overall water body trophic status.

Pheophytin a Light Absorption



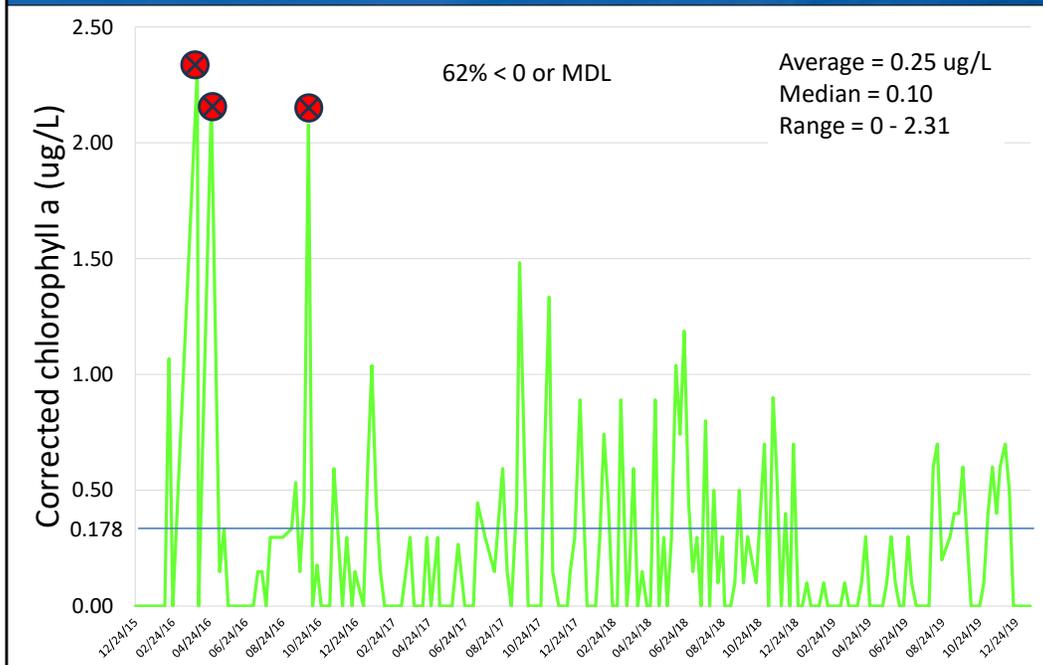
- Pheophytin a also is commonly measured as a water quality parameter.
- As I mentioned earlier, it is a degraded form of chlorophyll a that can occur when algal cells are damaged or die
- It has a light absorption spectrum (black line) similar to that of chlorophyll a with slightly different peaks: a blue range peak at 420 nm [\[click\]](#) and a red-range peak at 654 nm [\[click\]](#)
- Again yielding an apparent color that is green [\[click\]](#)

Chlorophyll a, 12/24/15 - 1/16/20



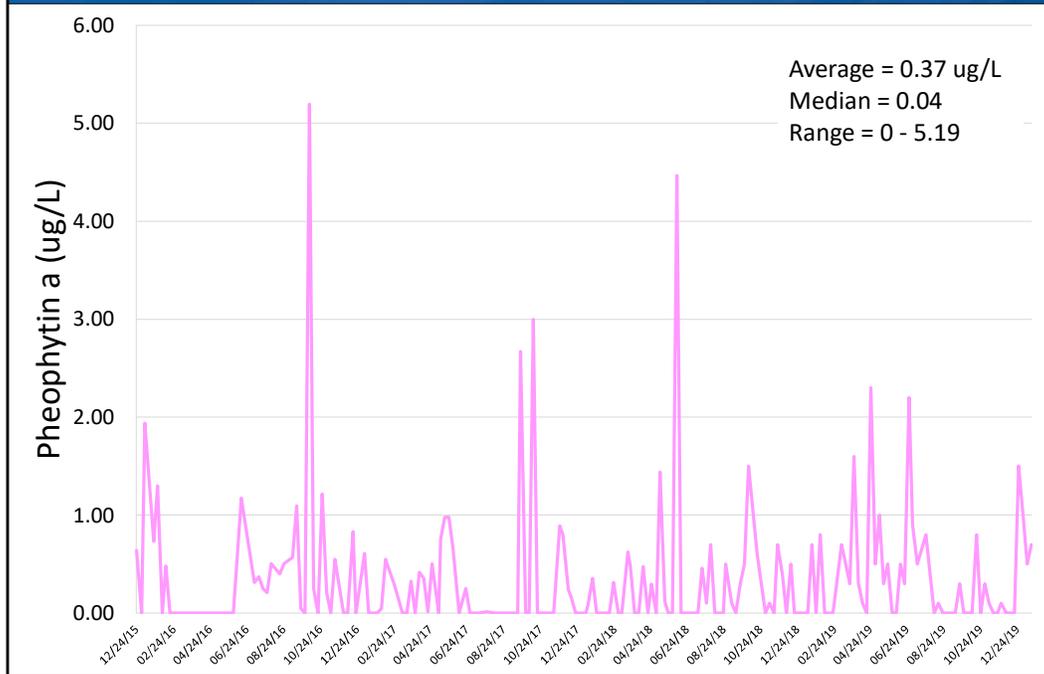
- This is a graph of chlorophyll a concentrations, measured as corrected chlorophyll a
 - They range from 0 to 2.31
 - These are not high levels
 - Water bodies with chlorophyll a levels less than or equal to 2 micrograms per liter are considered oligotrophic,
 - i. e. water bodies that have low concentrations of nutrients, low rates of productivity and generally low biomass, such as alpine lakes, are termed oligotrophic
(<https://nationallakesassessment.epa.gov/webreport/#chlorophyll-a>)
 - The average concentration of 0.25 ug/L was nearly 10 times less than that
- **[click]** Only three of the 189 observations in the database exceeded 2 ug/L
- Corrected chlorophyll a was zero or less than the method detection limit (0.178 ug/L) for 62% of the 189 weekly samples

Chlorophyll a, 12/24/15 - 1/16/20



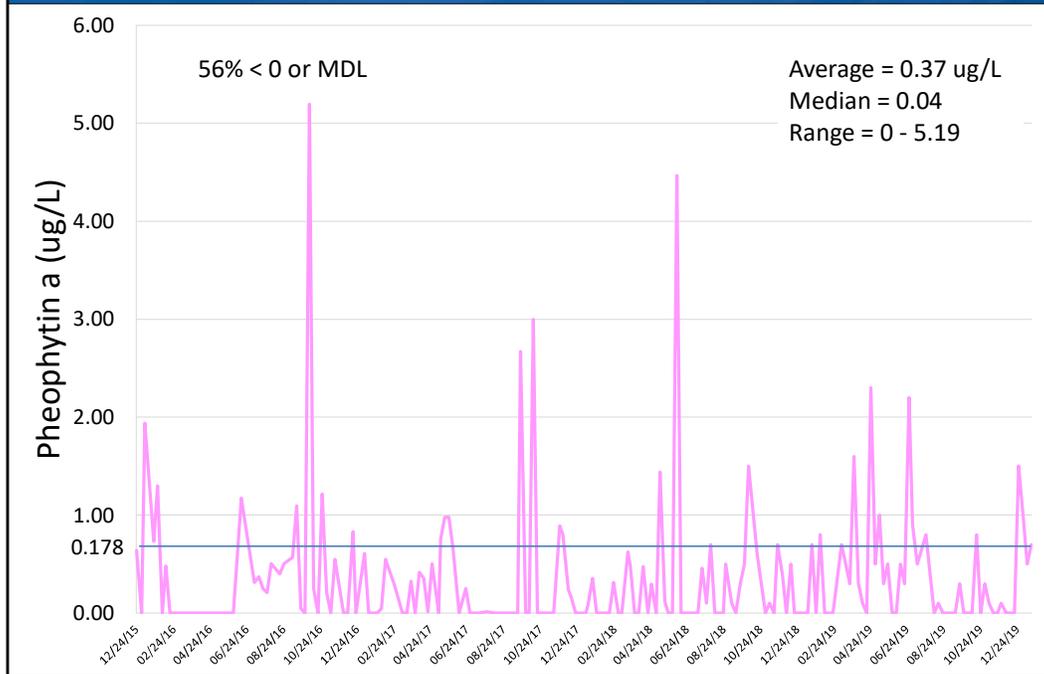
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Pheophytin a, 12/24/15 - 1/16/20

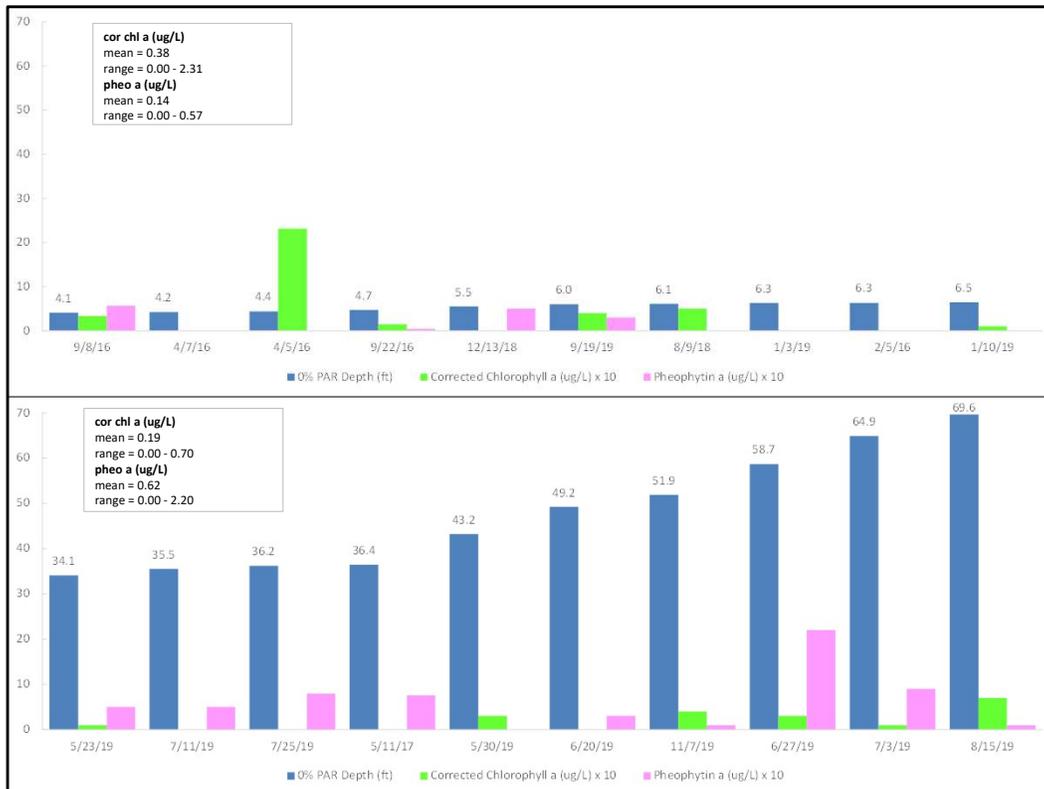


- Pheophytin a averaged 0.37 ug/L over the 4.5-year study period
 - With a median of 0.04
 - And a range of 0 to 5.19
- **[click]** Pheophytin a was zero or less than the method detection limit (0.178 ug/L) for 56% of the 189 weekly samples

Pheophytin a, 12/24/15 - 1/16/20

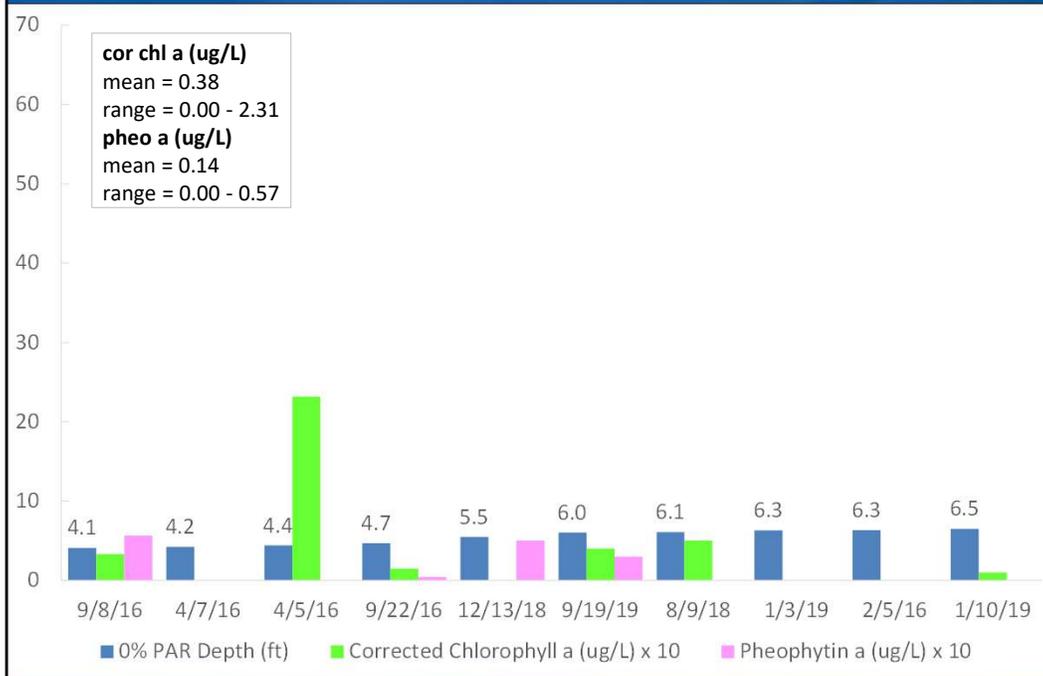


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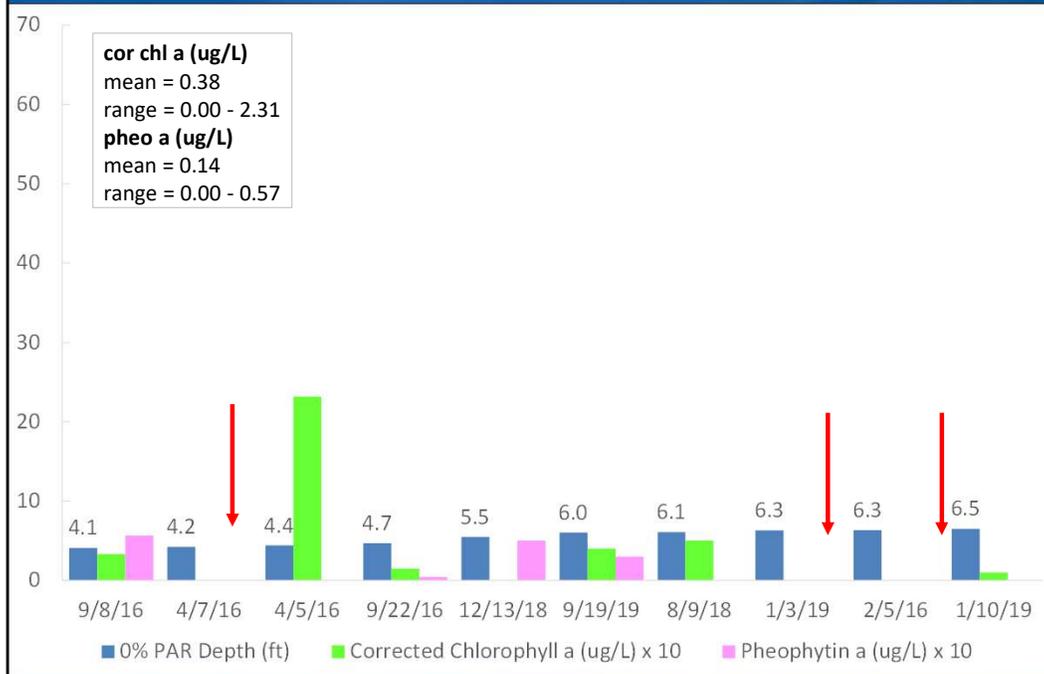
- Looking at the 10 dates when visibility was lowest during the study period: **[hidden enlarged slide follows]**
 - corrected chlorophyll a values range from 0.00 to 2.31 ug/L but show no evidence of being directly correlated with the shift in visibility
 - neither does pheophytin a which ranges from 0.00 to 0.057 ug/L
 - On three dark event dates **[click]**, neither form of chlorophyll a is present above the method detection limit of 0.178 ug/L
- Examining the 10 dates when visibility was highest during the study period: **[hidden enlarged slide follows]**
 - corrected chlorophyll a values range from 0.00 to 0.70 ug/L but show no evidence of being directly correlated with the shift in visibility
 - neither does pheophytin a which ranges from 0.00 to 2.20 ug/L
 - One or the other form of chlorophyll a is present above the method detection limit of 0.178 ug/L on each light event date

10 Lowest Visibility Dates

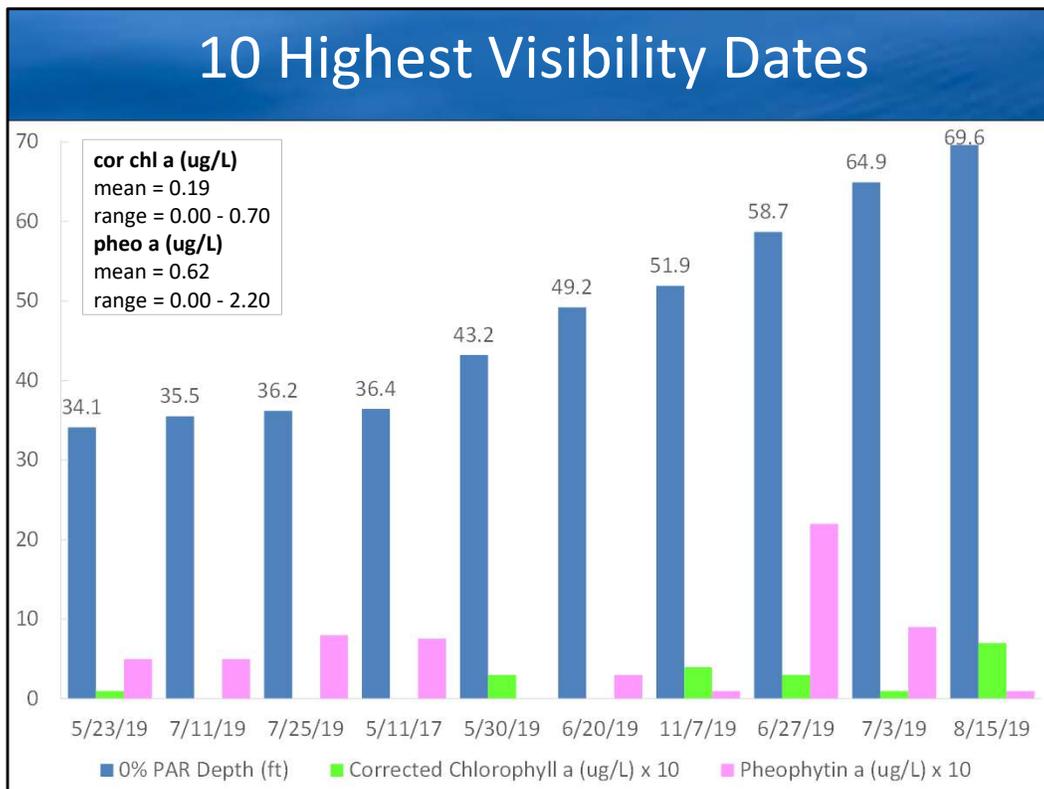


- Looking at the 10 dates when visibility was lowest during the study period:
 - corrected chlorophyll a values (green) range from 0.00 to 2.31 ug/L but show no evidence of being directly correlated with the variation in visibility
 - neither does pheophytin a (pink) which ranges from 0.00 to 0.57 ug/L
- On three dark event dates **[click]**, neither form of chlorophyll a is present above the method detection limit of 0.178 ug/L

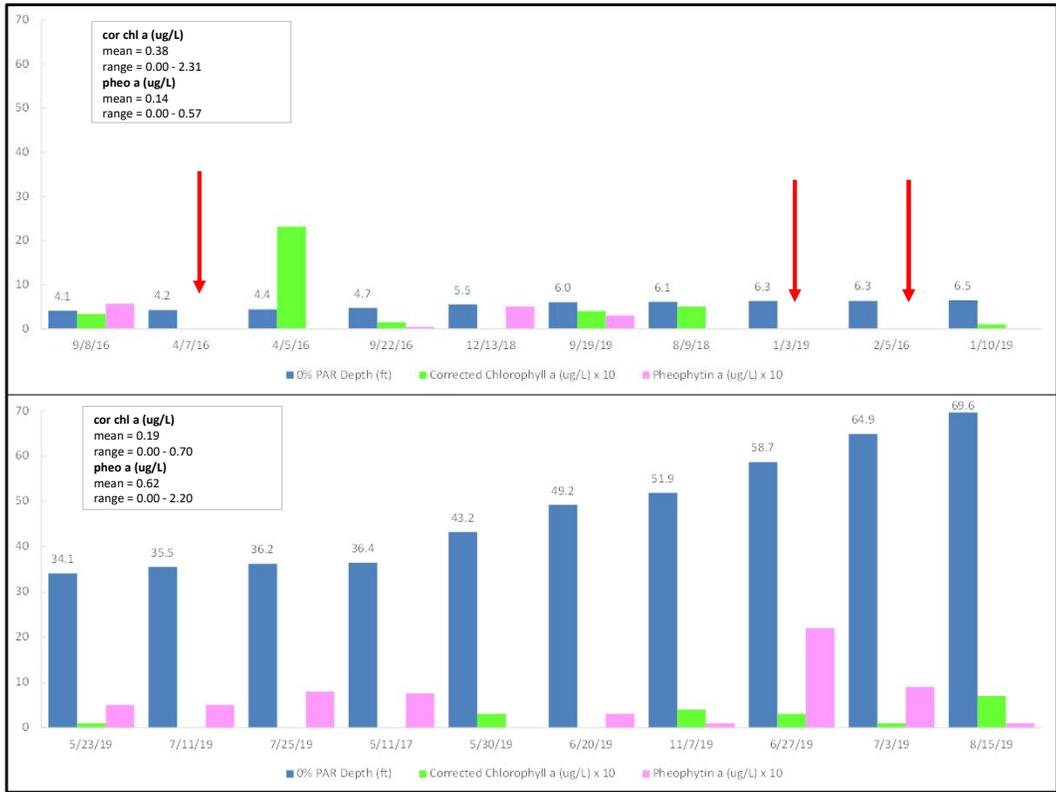
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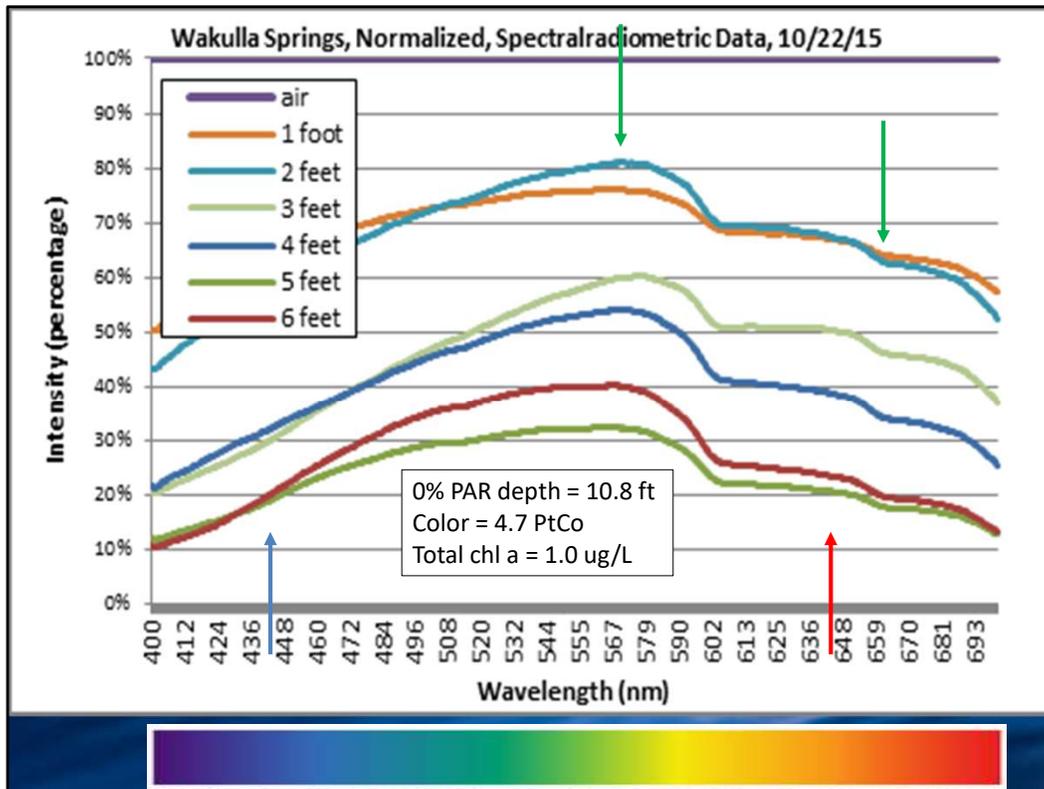


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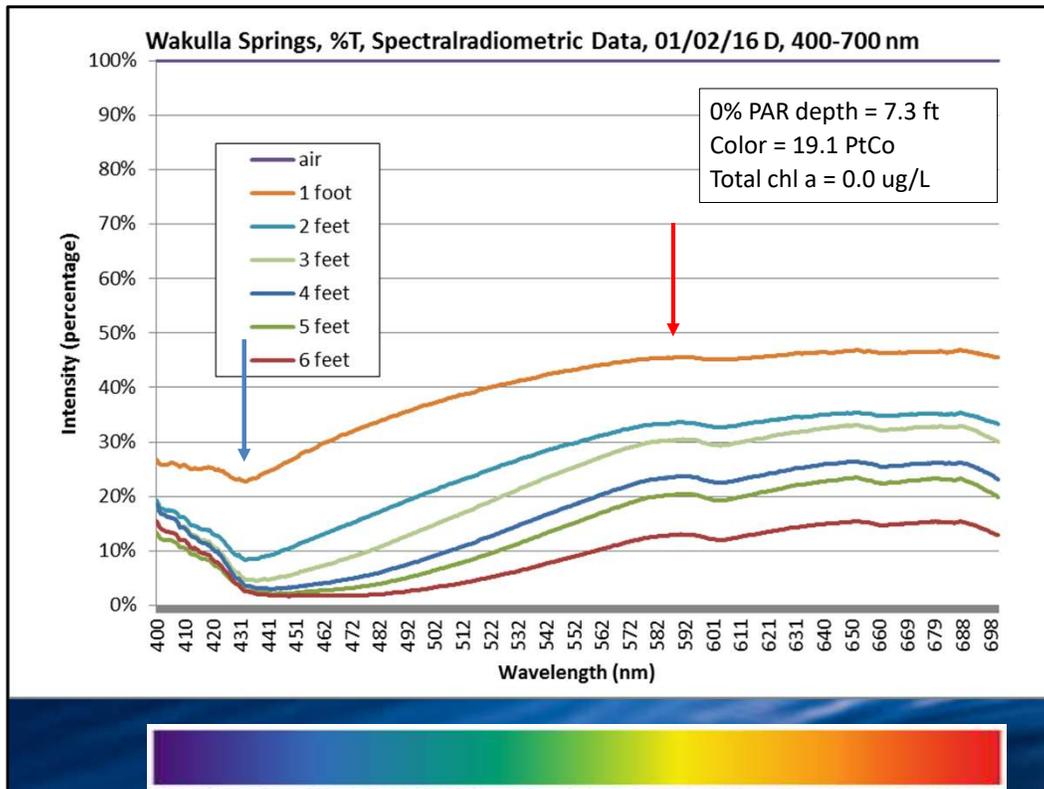


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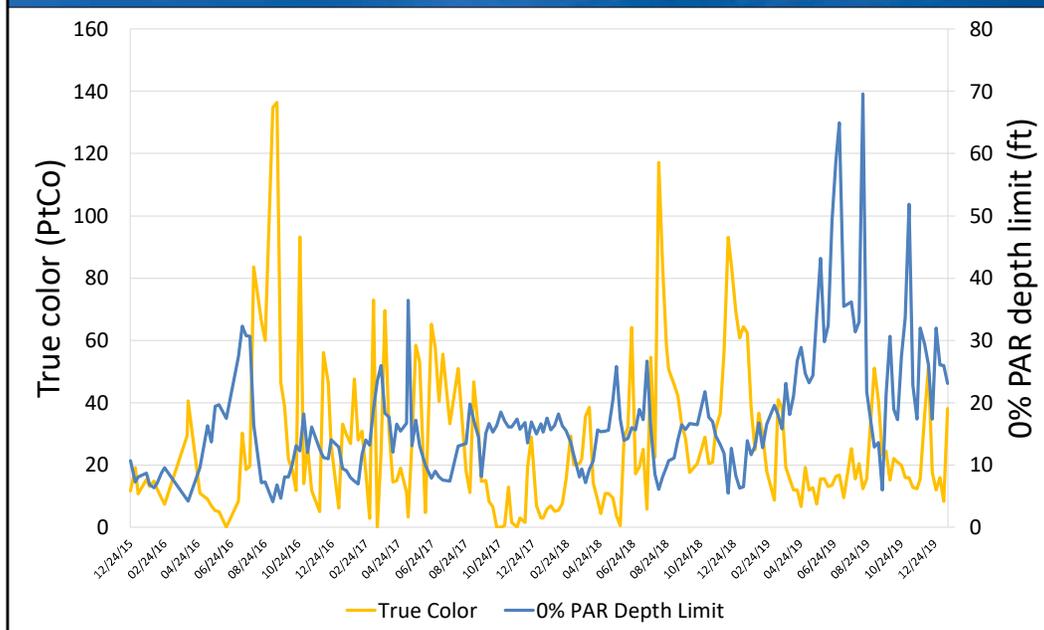


- As we've just seen, chlorophyll a and pheophytin a are present in the spring boil, and, therefore must be contributing to some degree to the absorption of PAR or visible light and reduced visibility, along with CDOM measured as true color.
- This is demonstrated by graphs of visible light transmission created by Dr. McGlynn from spectral radiometric readings taken at the spring boil at one-foot intervals
- This normalized graph compares the percent light transmission at each depth at each wavelength with transmission measured in the air just above the water surface – the purple line at the top, i.e. 100 percent
 - Visibility depth was 10.8 ft
 - Color was 4.7 PtCo
 - Total chlorophyll a (sum of corrected chlorophyll a + pheophytin a) was 1.0 ug/L
- As is to be expected, transmission decreases with depth: as light passes through more water, more light is absorbed by the water itself and any light-absorbing substances that are present
- This specrad graph illustrates the typical profile of a so-called "light event" when visibility depth is greater and the apparent color is green rather than the reddish brown we associate with high CDOM or tannin levels
- Both the shortest wavelengths (< 470 nm) **[click]** and the longer wavelengths (>590 nm) **[click]** were being absorbed
- Greatest transmittance is in the middle at about 500-590 nm, i.e. greens and yellows at 75-80% **[click]**
- Each curve displays a dip in transmittance at about 665 nm which corresponds to the chlorophyll a longer wavelength absorbance peak **[click]**



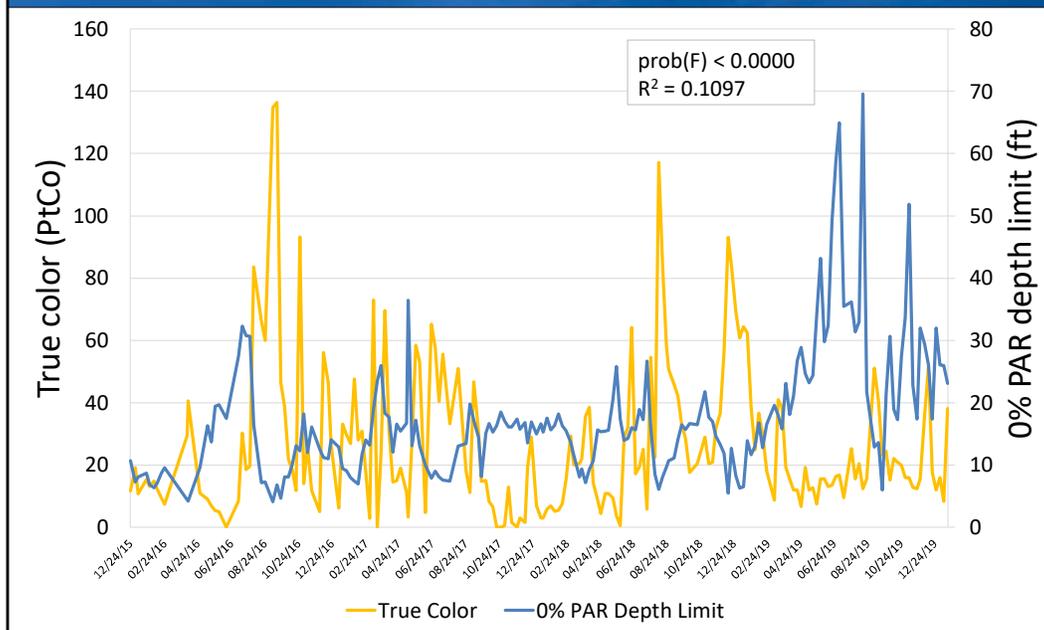
- This specrad graph illustrates the typical profile of a so-called “dark event” when visibility depth is less and the apparent color is reddish brown associated with high CDOM levels
- The shortest wavelengths (< 470 nm) were strongly absorbed as is expected with high levels of CDOM [\[click\]](#)
- While the longer wavelengths were being more fully transmitted at about 45% at one-foot depth [\[click\]](#)

CDOM with Visibility Depth, 12/24/15 - 1/16/20



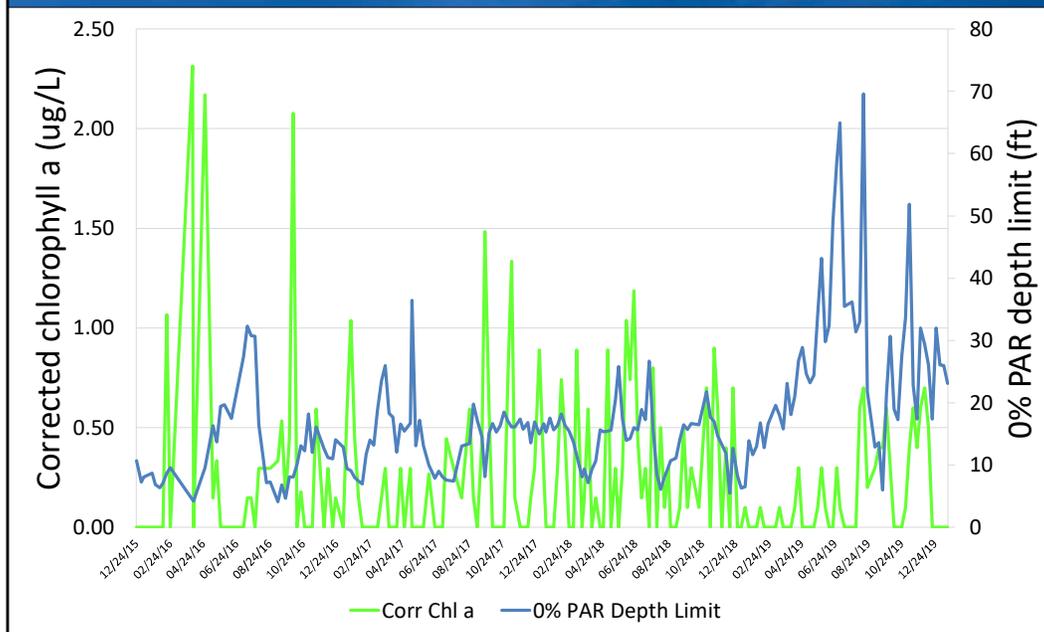
- I want to wrap up by looking at statistical tests of the effects of CDOM and chlorophyll on visibility
- When we look at the full data set we see evidence of an inverse relationship between CDOM measured as true color (yellow line) and visibility (blue line), i.e. higher levels of CDOM are associated with reduced visibility
- **[click]** Ordinary least squares regression confirms this apparent relationship with a model that is statistically significant at better than 99.99%
 - However, true color only explains about 11% of the observed variation in visibility depth (R-squared = 0.1097)

CDOM with Visibility Depth, 12/24/15 - 1/16/20



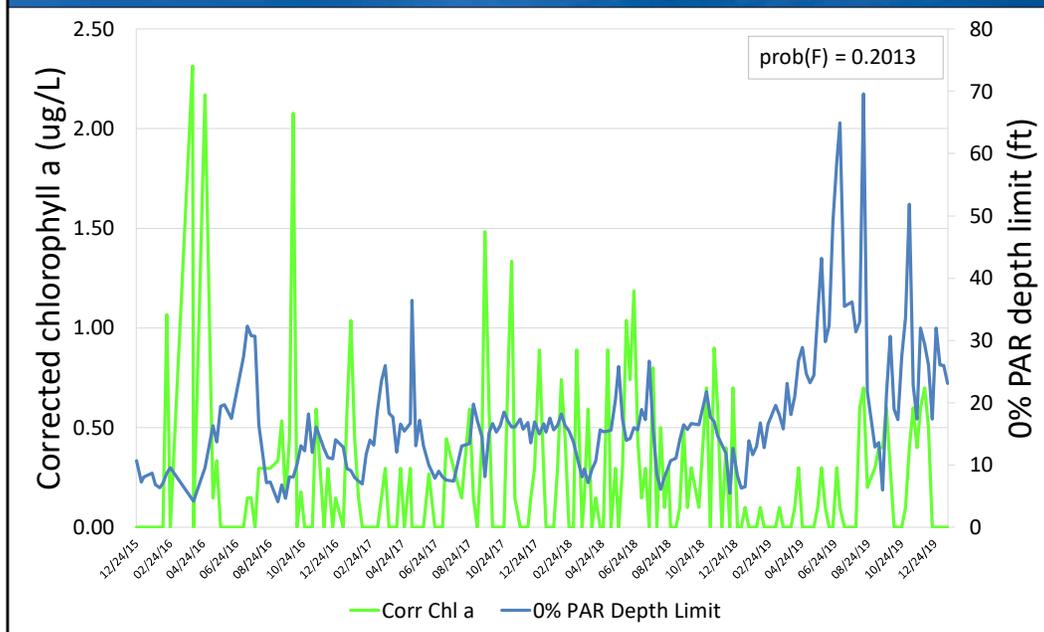
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Chlorophyll a with Visibility Depth, 12/24/15 - 1/16/20



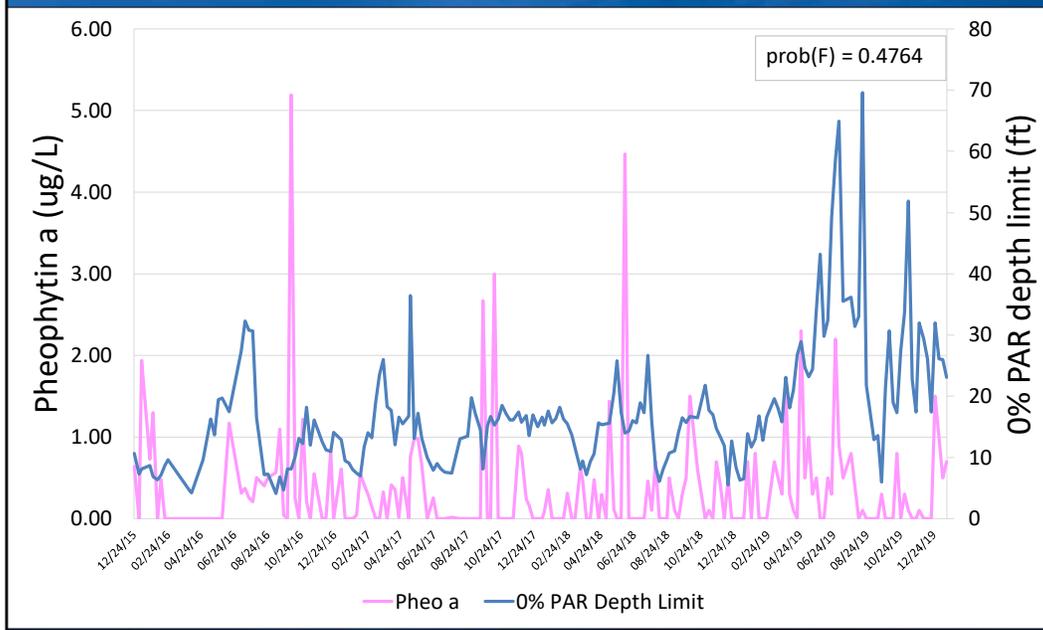
- When we plot chlorophyll a measured as corrected chlorophyll a with visibility, we see no evidence of a consistent relationship
- **[click]** An ordinary least squares regression model is not statistically significant at the 90% level or better, so the R-squared value is irrelevant

Chlorophyll a with Visibility Depth, 12/24/15 - 1/16/20



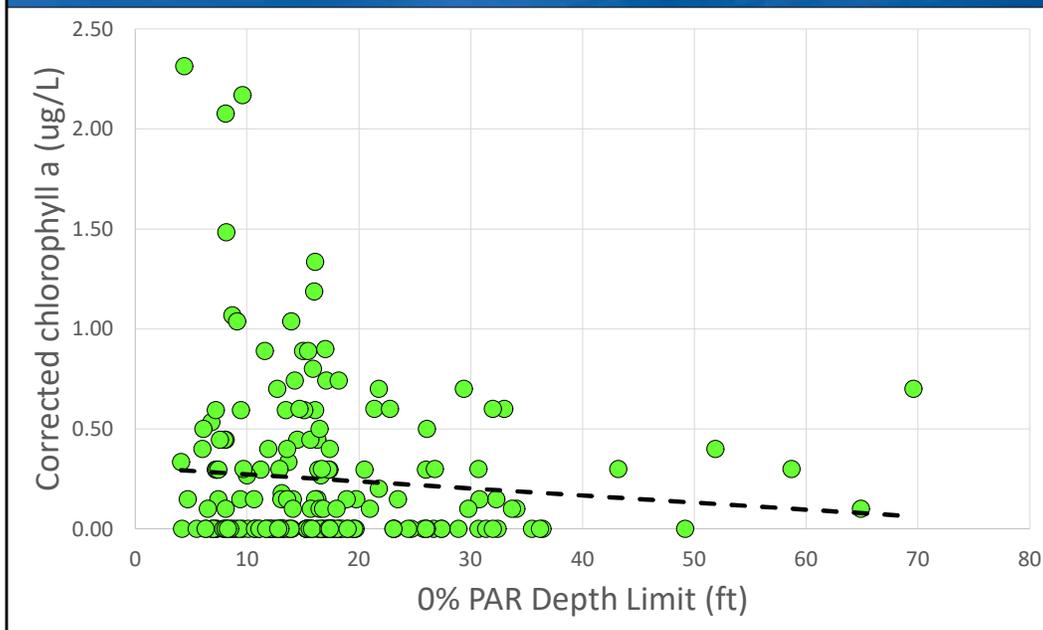
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Pheophytin a with Visibility Depth, 12/24/15 - 1/16/20



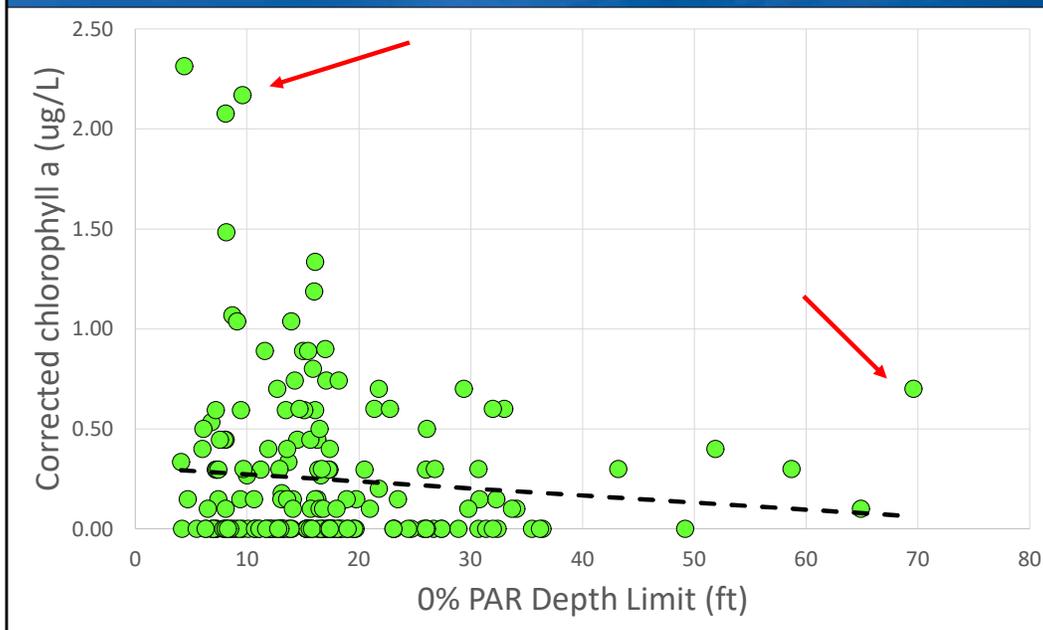
- We get similar results when we plot pheophytin a with visibility: there is no apparent statistically significant linear relationship

Scatter Plot of Chlorophyll a and Visibility, 12/24/15 – 01/16/20



- However, if we look at the scatterplot for corrected chlorophyll a, we see that ordinary least squares or OLS regression is not the most effective way to analyze possible relationships between these two variables and visibility depth
- Values are not linearly distributed, i.e. they are clustered at the lower levels of visibility, and there are a number of outlying values **[click]**
- My statistical consultant, who is my son Dr. Ethan Deyle, a quantitative ecologist at Tufts University, recommended quantile regression as a more suitable approach
 - This is an approach that is more appropriate when the independent variable, i.e.. chlorophyll a concentration, does not have proportional impacts at all levels of the dependent variable, i.e. visibility depth
- Rather than estimating the mean expected value of visibility depth for each corrected chlorophyll a observation, quantile regression estimates the expected value for a given quantile or percentile of the dependent variable observations
 - For example, one can apply quantile regression to estimate the expected value of the 50th percentile of visibility depth, i.e. the median, or any other percentile of interest
- I engaged Dr. Deyle to conduct quantile regression for CDOM measured as true color as well as corrected chlorophyll a and pheophytin a. The results are presented in the next slides

Scatter Plot of Chlorophyll a and Visibility, 12/24/15 – 01/16/20



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Quantile and OLS Regression Results

| Light Absorbing Substance | Visibility Depth Model | Coefficient* | t Statistic** | Prob(t)*** |
|---------------------------|-----------------------------|--------------|---------------|------------|
| CDOM (true color) | OLS | -0.1414 | -4.80 | < 0.0000 |
| | 25 th percentile | -0.1120 | -7.28 | < 0.0000 |
| | 50 th percentile | -0.0968 | -4.42 | < 0.0000 |
| | 75 th percentile | -0.1240 | -3.07 | 0.0025 |
| | 90 th percentile | -0.2190 | -9.89 | < 0.0000 |
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- The coefficient is the slope of the regression line for a given independent variable.
 - If the coefficient is negative there is an inverse relationship between the independent variable and the dependent variable.
 - Therefore, in these models, a negative coefficient indicates that the higher the level of the light-absorbing substance, the shallower the visibility depth.
 - So in the OLS model, a one unit increase in true color results in a 0.14 ft decrease in visibility depth **[click]**
- The t statistic tests the hypothesis that the actual value of the coefficient is zero.
 - The higher the t statistic value, the lower the probability that the actual value is zero.
- The prob(t) value is the probability that the actual value of the coefficient is zero.
 - The lower the prob(t) value, the greater the probability that the coefficient is NOT equal to zero, in other words that the apparent relationship is real
 - Values highlighted in green are significant at the 95 percent level or higher, i.e. prob(t) is less than or equal to 0.0500.
 - Values highlighted in yellow (none shown here) are significant at the 90 percent level or higher, i.e. prob(t) is less than or equal to 0.1000.
- Here we see the results for CDOM measured as true color
 - The coefficient is significant for the OLS regression as we saw earlier
 - The coefficients also are significant for all four quantiles tested
- Notice that the 90th percentile coefficient is almost twice as large as that for the 75th percentile **[click]**
 - That tells us that when visibility depth is greater than 90% of the observations, a one unit increase in CDOM measured as true color has nearly twice the impact that it has when visibility depth is greater than 75% of the other observations

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| | 75 th percentile | -0.1240 | -3.07 | 0.0025 |
| | 90 th percentile | -0.2190 | -9.89 | <0.0000 |
| Corrected chlorophyll a | OLS | -2.4713 | -1.28 | 0.2013 |
| | 25 th percentile | -2.4000 | -1.36 | 0.1740 |
| | 50 th percentile | -3.1300 | -3.50 | 0.0006 |
| | 75 th percentile | -3.7000 | -1.20 | 0.2320 |
| | 90 th percentile | -10.3000 | -1.77 | 0.0783 |

- Looking at corrected chlorophyll a, we see again that the OLS model is not statistically significant, i.e. the prob(F) value is 0.2013 **[click]**
- The 50th percentile model, i.e. the median, is significant at the 99.94% level **[click]**
- And the 90th percentile model is significant at the 92.17% level **[click]**
- Comparing the coefficients for the 50th and 90th percentiles we see that visibility depth at the 90th percentile **[click]** is more than 3 times as sensitive to a one ug/L increase in corrected chlorophyll a than at the 50th percentile **[click]**
 - i.e. at the 90th percentile of visibility depth, a 1 ug/L increase in corrected chlorophyll a will lead to a 10.3-foot decrease in visibility

Quantile and OLS Regression Results

| Light Absorbing Substance | Visibility Depth Model | Coefficient* | t Statistic** | Prob(t)*** |
|---------------------------|-----------------------------|--------------|---------------|------------|
| CDOM (true color) | OLS | -0.1414 | -4.80 | < 0.0000 |
| | 25 th percentile | -0.1120 | -7.28 | < 0.0000 |
| | 50 th percentile | -0.0968 | -4.42 | <0.0000 |
| | 75 th percentile | -0.1240 | -3.07 | 0.0025 |
| | 90 th percentile | -0.2190 | -9.89 | <0.0000 |
| Corrected chlorophyll a | OLS | -2.4713 | -1.28 | 0.2013 |
| | 25 th percentile | -2.4000 | -1.36 | 0.1740 |
| | 50 th percentile | -3.1300 | -3.50 | 0.0006 |
| | 75 th percentile | -3.7000 | -1.20 | 0.2320 |
| | 90 th percentile | -10.3000 | -1.77 | 0.0783 |
| Pheophytin a | OLS | 0.7809 | 0.71 | 0.4764 |
| | 25 th percentile | -0.5890 | -1.10 | 0.2720 |
| | 50 th percentile | -0.4220 | -0.46 | 0.6260 |
| | 75 th percentile | 4.2000 | 1.12 | 0.2630 |
| | 90 th percentile | 11.0000 | 1.77 | 0.0791 |

- Looking at pheophytin a, only the 90th percentile model is significant at the 90% level or better
- BUT note, the 90th percentile coefficient is positive
- That means that a 1 ug/L increase in pheophytin is associated with an 11-foot INCREASE in visibility
- I cannot think of a plausible explanation for this result

Summary

- Apparent green water color can be caused by both CDOM (tannins) and chlorophyll
- Both CDOM and chlorophyll a measured as corrected chlorophyll a contribute to reductions in spring visibility
- The spring is especially sensitive to these substances when visibility is higher

- To summarize:
- Apparent green water color can be caused by both CDOM (tannins) and chlorophyll
- Both CDOM and chlorophyll a measured as corrected chlorophyll do contribute to reductions in spring visibility, even though chlorophyll levels are very low
- The spring is especially sensitive to these substances when visibility is higher

Other Research Questions

1. What are the sources of those light-absorbing substances?
2. Why have the frequency and duration of dark water conditions increased?

- The study addresses two other research questions
- I could resurrect those presentations if there is interest in hearing them again five years later.