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COMPARISON OF VNIR DERIVATIVE AND VISIBLE FLUORESCENCE SPECTROSCOPY METHODS FOR PIGMENT ESTIMATION IN AN ESTUARINE ECOSYSTEM: OLD WOMAN CREEK, HURON, OHIO

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by

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SUMMARY

This study provides a useful comparison between traditional fluorometric methods of testing for algal contamination and a newer analytical technique that has been developed for assessing water quality. This new technique, referred to as visible/near-infrared (VNIR) derivative spectroscopy, uses multivariate statistics to rapidly identify and quantify the distribution of phytoplankton in aquatic systems. Unlike traditional methods, VNIR derivative spectroscopy does not require chemical reagents can thus be considered easier, quicker, and more cost-effective to use. Samples are filtered onto a 47 mm, 0.4 µm glass-fiber filter (GF/F), dried, and measured using a VNIR spectrophotometer. This results in a hyper-spectral reflectance recording for each sample (400 – 2500 nm). Statistics then provide a means by which to separate out important pigment classes. Reflectance data may also be converted to chlorophyll *a* concentrations using wavelength index numbers, allowing for independent techniques of measurement to be compared.

In this study, an *in-situ* Hach hydrolab sensor and a Trilogy laboratory fluorometer were used to obtain direct chlorophyll *a* concentrations. A correlation value of 0.90 was found between these two methods. Comparison of reflectance data with Hach and Trilogy measurements also produced good results, with correlation values of 0.82 and 0.64, respectively.

This study took place over the summer of 2011 in a dynamic estuary on the southern central shore of Lake Erie. A dramatic change occurred halfway through the study period when water passage into Lake Erie was prohibited due to sediment accumulation at the mouth bar. Multivariate statistics of reflectance data suggest there was a shift in which in-water constituents were responsible for determining the optical variability of the estuary when this change took place. Clay, chlorophyceae (green algae) and bacillariophyceae (diatoms) were found to be the most important in-water constituents during the full range of the study, while cyanobacteria (blue-green algae) and fucoxanthin (an accessory pigment) played a lesser role. Cryptophyta was not important when the mouth bar was open but became more prominent with the closing of the mouth bar later in the summer.

CHAPTER 1: INTRODUCTION

1.1 General Statement

Nishanthi Wijekoon (2007) measured the total amount of chlorins in Old Woman Creek (OWC) in Huron, Ohio, using a combination of remote sensing techniques, field observations, and filtering water samples. While her results provided valuable information to management decisions at the OWC State Nature Preserve and National Estuarine Research Reserve, the drying method she used to identify plant pigments based on reflectance spectra from filtered water samples may not have allowed for the differentiation of chlorophyll *a* from its degradation products. To build upon her work, this study examined the impact of drying samples to provide a means of testing for the presence of degradation products and to rapidly identify the taxa of alga present. This was done by comparing the dry, visible/near-infrared (VNIR) reflectance spectroscopy method used by Wijekoon (2007) and wet, visible-range fluorometery. Multivariate statistical methods were used to separate out the relative importance of different classes of alga. In addition, a three-month time series was constructed to observe changes in algal populations in a dynamic estuarine ecosystem. The detailed experiments developed here document the applicability and extend the utility the methods first introduced by Wijekoon (2007).

1.2 Objectives

This method-based study had four main objectives:

1. Determine the best method(s) by which to process and treat filtered water samples in order to minimize degradation of chlorophyll *a*.
2. Determine the rate of degradation of chlorophyll *a* into its principal degradation products.
3. Compare VNIR derivative spectroscopy and visible-range fluorometery in the identification and quantification of chlorophyll *a*.
4. Create a time series of variations in pigment classes during a three-month study of the Old Woman Creek estuary.

1.3 Purpose of Study

Being able to rapidly and effectively assess and monitor the composition, growth, and distribution of phytoplankton has become of increasing importance to researchers, government agencies, public officials, and water managers due to the deleterious effects of eutrophication and harmful algal blooms (HABs) in many aquatic environments (Sellnar, Doucette, and Kirkpatrick, 2003; Herdendorf, Klarer, and Herdendorf, 2006; Heisler et al., 2008). Algal blooms have become of particular interest in Lake Erie (especially the western basin), where their presence can even be observed by satellite (see Figure 1).

Figure 1. Harmful algal bloom (HAB) in Lake Erie.

This image was obtained on October 9, 2011, from the Moderate Resolution Imaging Spectroradiometer (MODIS) on the Aqua satellite. (Earth Observatory, NASA, http://earthobservatory.nasa.gov/IOTD/view.php?id=76115)

HABs can produce toxins that may lead to skin rashes, allergic reactions, neurotoxicity, or liver damage in humans, but even non-toxic blooms can be problematic, by reducing oxygen levels or adding unwanted taste, odor, or color to the water (Ohio Sea Grant Fact Sheet 91, 2011). Because many people rely on freshwater ecosystems for drinking water—approximately 11 million on Lake Erie alone (EPA, 2011)—it has become critical for researchers to accurately predict where and when these blooms may occur. In addition, being able to monitor these blooms and quickly identify the taxa of

phytoplankton present can provide managers enough time and information to take proper action. This will require making improvements on the current techniques.

As will be described in greater detail in Chapter 2.2, the current techniques for measuring plant pigments are all lacking in some area. While sensors and laboratory testing can provide good data, their methods can sometimes be complicated, expensive, or time-consuming, and it becomes difficult to monitor large aquatic environments, such as the Great Lakes. Remote sensing techniques have been shown to be a powerful tool in assessing wide spatial areas, but often require ground-truthing for calibration (Aminot and Rey, 2002). Single-point collection and filtering methods are used in the traditional spectrophotometric and fluorometric approach of chlorophyll analysis (APHA, 1999), but both require pigment extraction, which is a time-costly procedure that requires the use of chemical reagents and effectively destroys the filter in the process. While no one technique can be described as "perfect," this study attempted to compare methods—the traditional fluorometric approach and a modified spectrophotometric approach (called "VNIR derivative spectroscopy")—to demonstrate how filtered water samples can be analyzed without the need for pigment extraction. While there is still a time component to this method (samples must be dried first in order to be analyzed; see Chapter 3.5), it does not require chemical reagents and the filters remain intact. In addition, the use of multivariate statistics can be used to identify the taxa of phytoplankton that exist (based on spectral signatures; see Chapter 4.8). When used in conjunction with these other methods for chlorophyll measurements, VNIR derivative spectroscopy becomes a powerful method for pigment analysis.

CHAPTER 2: BACKGROUND

2.1 Geographic Setting

The Old Woman Creek (OWC) freshwater estuary is located 5 km east of Huron, Ohio, along the south-central shoreline of Lake Erie (Cornell and Klarer, 2008) (see Figure 2). The creek has a watershed area of approximately 79 km^2, and the primary use of the surrounding land is agriculture (Klarer and Millie, 1992). In addition to supplying the lake with nutrients, OWC provides an important ecological habitat and breeding grounds for birds, fish, plants, and other forms of wildlife (Herdendorf, Klarer, and Herdendorf, 2006). In 1980, the site was designated as part of the National Oceanic and Atmospheric Administration (NOAA) National Estuarine Research Reserve (NERR) System (Herdendorf, Klarer, and Herdendorf, 2006). Interestingly, of the 27 coastal reserves currently in the NERR System, OWC is the only one located in the Great Lakes biogeographic region (Wijekoon, 2007). This has made this location an appealing site for freshwater studies and lake research. Since 1980, OWC has been the focus of more than 200 research and monitoring projects, which have encompassed a wide range of academic disciplines, such as ecology, biology, geology, hydrology, and archeology (Herdendorf, Klarer, and Herdendorf, 2006).

There are three distinct segments of the OWC estuary: (1) a lagoon north of US Route 6, (2) a large central basin with a star-shaped island in the middle (Star Island), and (3) a smaller southern basin (see Figure 3). Water depths in the estuary vary by location

and are prone to fluctuation, but most parts are less than 0.5 m deep, with depths reaching up to 3.6 m in the inlet channel (Herdendorf, Klarer, and Herdendorf, 2006).

As part of the NERR System-Wide Monitoring Program (SWMP), water quality data loggers were established at specific points along the OWC estuary. These stations are identifiable by two-letter code names. Site "WM" (wetland mouth) was established in 1995 and is located in the lower estuary, just before the creek enters into Lake Erie (Cornell and Klarer, 2008). Sites "OL" (overlook), located slightly upstream of WM, and "BR" (near Berlin Road), located in the upper estuary, were established in 2001 (Cornell and Klarer, 2008). There is also a site "SU", located near US Route 2, and "DR", located beneath the bridge on Darrow Road (see Figure 3). These data loggers record water quality data, including pH, temperature, and dissolved oxygen, at 15 minute intervals (Cornell and Klarer, 2008). Due to the availability of *in-situ* data and the proximity to the mouth bar (allowing for better correlations with Lake Erie), the WM site (shown on Figure 3) was chosen for all sampling and measurements collected in this study. A single sampling site was possible, as Wijekoon (2007) identified spatial variability in the estuary based on surface reflectors. (Future work, however, may consider using the methods described in this study to examine spatial variability and test reproducibility across various regions.) Wijekoon's study included fifteen sampling sites, with thirteen sites located in the central basin (including the WM site), one in the northern lagoon, and one in the southern basin.

An interesting and dynamic feature of the OWC estuary is the barrier beach that sometimes forms at the mouth bar (Figure 2) due to wave action from Lake Erie. When

this happens, the waters of the creek are essentially isolated from Lake Erie (Cornell and Klarer, 2008). The build-up of sediment at the mouth opening is particularly common in the summer or fall, when there are periods of low flow or low rainfall (McCarthy et al., 2007). High water levels or moderate to heavy rainfall, however, can break open the barrier and allow mixing between Old Woman Creek and Lake Erie (Klarer and Millie, 1994). As such, this estuary has been identified as a storm-driven system (Cornell and Klarer, 2008). Annually, the mouth bar is closed about 40% of the time, but there is a high annual variability (Cornell and Klarer, 2008). When OWC is not barred across, water retention in the estuary is less than a day (Herdendorf, Klarer, and Herdendorf, 2006).

As part of this study, a photograph of the mouth bar was taken every eight days, beginning June 15 and continuing until September 11, 2011. This allowed for a visual, time-lapse representation of the mouth bar progression. Photographs were captured from the western margin of the bar mouth, directly north of the WM site, and can be found in Appendix C. The mouth bar was open during the early summer and began to pinch off in mid-July. The mouth bar officially closed around July 21, 2011, and remained closed for the remainder of the study (see Appendix C).

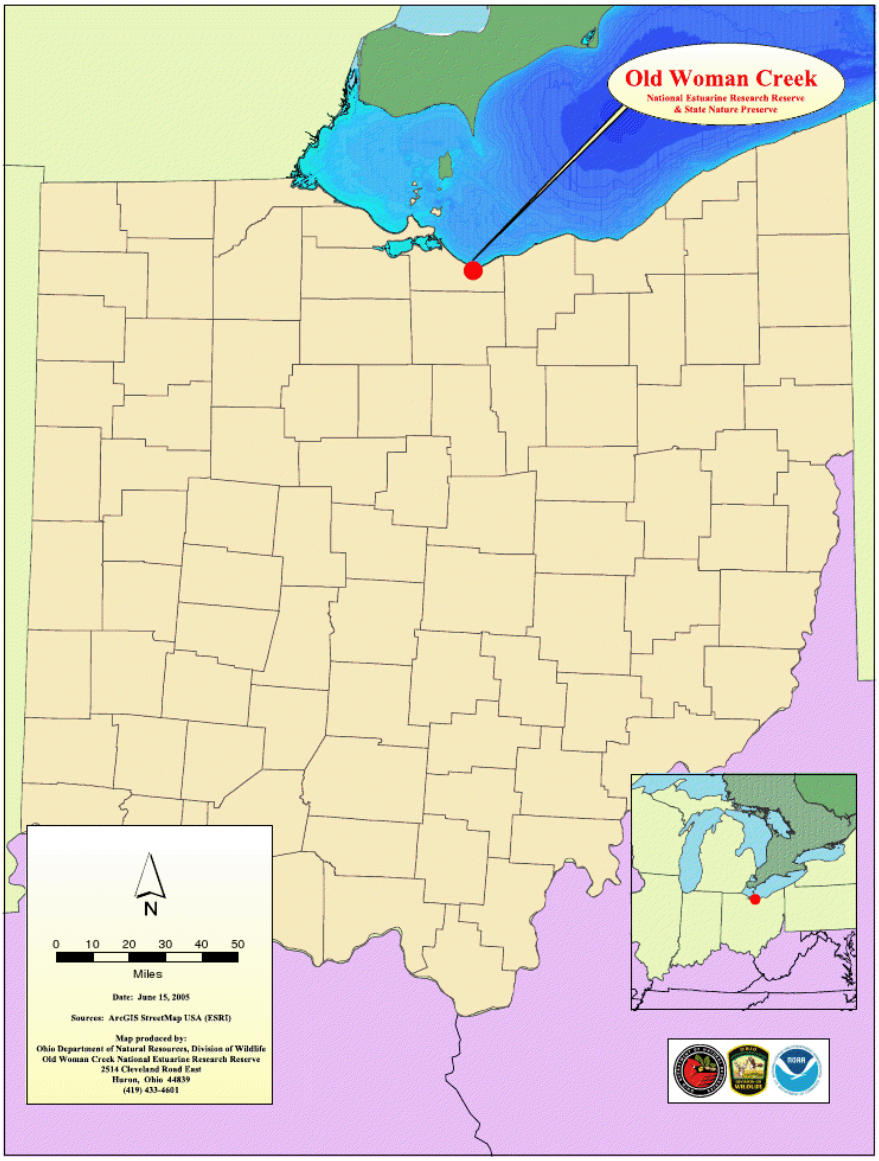

Figure 2. Location of Old Woman Creek in Huron, Ohio.

(Image provided by the OWC management staff, 2011)

Figure 3. The Old Woman Creek Estuary.

(Modified from Wijekoon, 2007)

2.2 Measuring Plant Pigments

The three materials that determine the optical properties (e.g. color, transparency) of natural bodies of water are: (1) phytoplankton (algae), (2) non-algal suspended solids (e.g. clays), and (3) humic substance (colored dissolved organic matter, or CDOM) (Menken, Brezonik, and Bauer, 2005). Observing and quantifying concentrations of phytoplankton biomass directly can be difficult because of its microscopic size and high populations in some ecosystems. By measuring the concentration of photosynthetic pigments in the water, however, we can obtain a proxy that will allow us to estimate the total phytoplankton biomass (American Public Health Association (APHA), 1999). There are many plant pigments that can be examined, but chlorophyll *a* is the most commonly used (APHA, 1999). Other pigments that might be considered are chlorophyll *b*, chlorophyll *c*, xanthophylls, phycobilins, carotenes, and the degradation products of chlorophyll *a*: chlorophyllide *a*, pheophorbide *a*, and pheophytin *a* (APHA, 1999; Carpenter, Elser, and Elser, 1986). Each of these pigments has its own diagnostic spectral signature, with maxima and minima at specific wavelengths, which can be extracted and used for identification purposes. Comparing pigments to concentrations can be done using spectral indices (such as those determined by Wolfe et al., 2006), as will be described in Chapter 4.4.

The APHA "Standard Methods for the Examination of Water and Wastewater" (1999) discusses the procedures for three methods of determining chlorophyll *a* in phytoplankton. These methods are described as: (1) the spectrophotometric approach, (2) the fluorometric approach, and (3) high-performance liquid chromatography (HPLC).

This study considered comparing all three of these techniques; however, plant pigment analysis through HPLC is an involved process that requires specialized training, equipment, and reagents that were not available, and as such has been excluded.

The spectrophotometric approach implemented in this study was the same used by Wijekoon (2007). It varies from the traditional spectrophotometric approach described in the APHA (1999) protocol in that pigments do not have to be extracted. Instead, sample water is filtered through a glass-fiber filter (GF/F) and the collected biomass is dried and then measured spectrophotometrically with the filter still intact. Detailed information on this procedure, along with instrument specifications and the drying technique, are described in Chapters 3.5 and 3.6. The traditional fluorometric approach is described in Chapter 3.7.

One problem with these optical methods is they can severely under- or overestimate the concentrations of chlorophyll *a*. This happens, in part, because of overlapping signals in the adsorption and fluorescence bands of chlorophyll *a*, its degradation products, and other accessory pigments (APHA, 1999; Aminot and Rey, 2002). In Case I waters—that is, where chlorophyll *a* is the dominant color-producing agent (CPA) (Ali, Witter, & Ortiz, 2012)—this is less of a concern. In Case II waters, however, where there are multiple CPAs, this can be exceptionally problematic, as the presence of one pigment may ‘mask’ or cover-up the presence of another. For example, Ortiz et al. (2013) discovered high concentrations of phycocyanin, an accessory pigment to chlorophyll *a*, in Lake Erie will mask the peak for chlorophyll *a* at 440 nm, as well as partially mask and cause an apparent shift in the chlorophyll *a* peak at 660 nm (see Figure

4A). These noticeable changes with higher concentrations of phycocyanin resulted because of the asymmetry in the derivative spectra shown in Figure 4B (Ortiz et al., 2013).

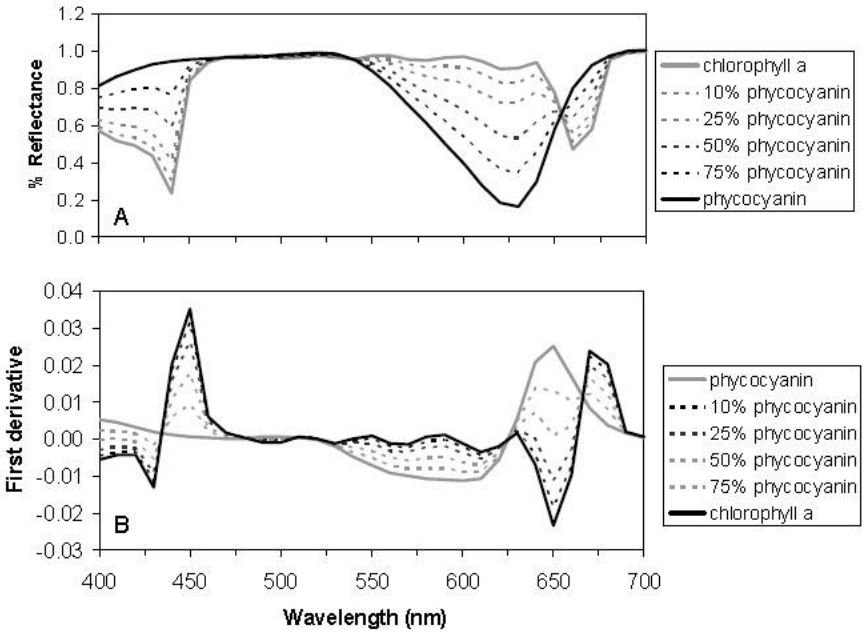

Figure 4. Reflectance spectra for chlorophyll *a* and phycocyanin.

(Ortiz et al., 2013)

There are other complications in these methods as well. One might argue the drying of filters (see Chapter 3.5) may actually facilitate chlorophyll *a* degradation, resulting in inaccurate spectral readings. In this case, the resultant spectra would be expected to have signatures indicative of pheophytin *a*, pheophorbide *a*, and/or

chlorophyllide *a*, the three principal degradation products of chlorophyll *a*. This study examined the drying technique used by Wijekoon (2007) in order to determine what impact, if any, these degradation products might have in the determination of chlorophyll *a* concentrations.

Before continuing, it is important to describe chlorophyll *a* and to explain how the breakdown process works. Chlorophyll *a* is a specific form of chlorophyll (along with chlorophylls *b* and *c*) that exists in all green plants and constitutes ~1-2% of the dry weight of phytoplankton (APHA, 1999). The molecular structure of chlorophyll *a* contains a central magnesium atom and a long phytol tail made up of hydrocarbons. This leads to a pathway by which chlorophyll *a* can break down. If the magnesium atom is removed, pheophytin *a* is formed (Carpenter et al., 1986); if the phytol tail is removed, chlorophyllide *a* is formed (Hendry, Houghton, and Brown, 1987). Removal of both magnesium and the phytol tail produces pheophorbide *a* (Hendry, Houghton, and Brown, 1987). This 'forked' model was first proposed by Hendry et al. (1987), but recent studies have argued against it. Eckardt (2009), for example, has suggested a more linear pattern of degradation, in which the first step is the removal of the central magnesium atom in chlorophyll *a*, followed by the loss of the phytol tail, which is catalyzed by a pheophytinase enzyme. In this model, chlorophyllide *a* is not considered an intermediate step of breakdown at all. While much is known about chlorophyll, more research may be needed to fully understand the inherent complexities of the breakdown process.

CHAPTER 3: METHODOLOGY

3.1 Methods for Water Collection

In the summer of 2011, thirteen sampling trips were scheduled for Old Woman Creek (OWC). These sampling trips began June 7 and continued every eight days until September 11, 2011. During each sampling trip, water samples were collected near the mouth of the estuary, at the WM site (Figure 3). Samples were collected from the surface, an intermediate depth, and near bottom. Additional samples (surface only) from the same site were collected by David Klarer, beginning June 19 and continuing until September 7, 2011, at an eight-day interval. As such, there is a four-day frequency between surface samples and an eight-day frequency between samples at depth. All water samples collected by David Klarer were kept bottled and in a fridge at the OWC field station and processed during the next scheduled sampling trip, four days later.

Multiple experiments relating to sampling and filtration methods were performed throughout this study. As a result, the amount of water collected each day and the method of processing each sample varied. The tables in Appendix A provide a detailed description of each sample, from collection to filtering to analysis. In addition, the phrase "*standard sampling day*" has been defined below. This describes the standard protocol that was followed for each sampling trip. Any changes to this procedure have been recorded for individual samples in the "Notes" column in Appendix A.

Standard sampling day (the collection of water samples followed these exact procedures, unless otherwise noted):

All water samples were collected at the WM site of Old Woman Creek, located at 41°22'57 N, -82°30'54 W (Figure 3). All water samples were collected between 10:00 AM and 1:00 PM, with the exceptions of July 9 and July 17, on which samples were collected in the late afternoon, and September 11, on which samples were collected in the early morning. On the days when David Klarer collected samples, the time was approximately 11:00 AM. All bottles used for water collection were opaque, 1-liter plastic containers, which were cleaned between sampling trips. All water samples were collected via row boat, with the exceptions of June 7 and all David Klarer collection days, where samples were collected on shore by using an extended pole arm with an open bottle attached to one end. This device only allowed for surface samples to be collected. The loading and unloading dock for the row boat used was directly north of the WM site; as such, paddling to the WM site was always done upstream. When using oars near the collection site, precaution was taken to minimize surface disturbance. All surface samples collected from the row boat were taken by first holding an open bottle parallel to the surface of the water and then submerging the bottle slowly. Once filled, these bottles were sealed with a screw-cap, labeled, and stored in a dark cooler until they could be taken back to the lab. Water samples were also collected at depth. Before collecting samples at depth, a meter stick was used to calculate the depth of the estuary at the WM site. Depth values changed daily at this site, so this process had to be repeated for each sampling trip. Once measured, the depth value was divided by three, and additional

samples were collected from depths equal to one-third and two-thirds of the distance to the bottom (see Chapter 3.2 for further explanation). Samples were not collected from the very bottom, as doing so may have released sediments into suspension and interfered with results. All samples at depth were collected with a standard Van Dorn sampler. This device is a narrow plastic tube attached to a cord that allows it to be lowered through the column of water. In order for water to be allowed to pass through the tube, there are clamps on either side that can be pinned back prior to deploying the device. Once the sampler reaches the desired depth, a weight messenger is sent down along the cord, striking a mechanism that triggers the clamps to shut and seals in the water at that depth. The device is then brought back up and its contents emptied into a container. Colored electric tape was used to mark off the depths on the cord from which to collect samples. The tape had to be re-adjusted each time to account for fluctuations in depth. On most sampling days, two bottles of water were collected from each of the three depths (surface, one-third distance to bottom, and two-thirds distance to bottom), for a total of six 1-liter bottles. More water samples, however, were collected on certain days (refer to Appendix A for a complete list). Once collected, water samples were brought back to the Old Woman Creek field station and filtered with the help of David Klarer. The only exception to this was on June 7, when water samples were carried back to Kent State University, stored in a dark cooler, and filtered over the following two days.

3.2 Methods for Measuring Depth

Due to the dynamics of Old Woman Creek described in Chapter 2.1, the water depth in the estuary was not consistent throughout the summer. During each sampling trip (with the exception of June 7), a wooden meter stick was used to determine the depth of the estuary at the sampling site. This value ("bottom depth") was then used to calculate which depths from which to collect samples, according to the following formula (given in depth from the surface):

Water collection depth #1 = surface

Water collection depth #2 = (bottom depth)*0.33

Water collection depth #3 = (bottom depth)*0.66

It should be noted the sampling site was very close to shore (within one meter) and the bed directly below the boat was steep and rocky. This made it difficult to obtain consistent depth measurements from day to day. A simple adjustment of the meter stick could have had a dramatic impact on the recorded depth—perhaps as high as ±10 cm. To minimize this, an attempt was made to maintain the position of the boat at all times, with the bow of the boat touching land, secured to a nearby branch, and the stern tied to the white pipe casing used for the WM monitoring sonde (see Chapter 3.3). This positioning also helped to minimize movement of the boat when sampling, as the pipe casing acted as a wedge to prevent the boat from drifting downstream. Depth measurements (and all other measurements; see Chapter 3.3) were taken off the port side of the boat. The WM

monitoring sonde was on the starboard side, so there was some lateral distance (roughly an arms-length) between the water being collected and measured with the on-board instruments and those measurements being recorded by the WM monitoring sonde.

All depth measurements have been recorded in Appendix B.3.

3.3 Methods for Measuring Water Properties

Water property data were obtained through a combination of field measurements and *in-situ* measurements. *In-situ* measurements were recorded by the WM site monitoring sonde, one of four water quality data loggers located in the Old Woman Creek estuary as part of the NERR System-Wide Monitoring Program (Cornell and Klarer, 2008). The WM monitoring sonde measured water temperature, specific conductivity, salinity, depth, pH, turbidity, and dissolved oxygen at 15 minute intervals. All of the sensors for this sonde were ~30 cm from the bottom of the estuary, with the exception of the depth sensor, which was ~40 cm from the bottom. The automated data logger was located ~20 cm from the bottom.

Field data were obtained by using (a) a standard, 20-cm diameter Secchi disk with alternating black and white quadrants, (b) a Hach hydrolab multiparameter sonde, and (c) a Turner® Designs Cyclops-7 colored dissolved organic matter (CDOM) sensor. Secchi depth measures were recorded by gradually lowering the device off the shaded side of the boat and measuring the distance on the rope from which the disk completely disappeared in the water column. An average of three measurements was used as the Secchi depth reading for each sampling trip. These data can be found in Appendix B.3.

The Hach hydrolab multiparameter sonde measured a full suite of limnological properties, such as pH, dissolved oxygen, and temperature, similar to that of the WM monitoring sonde. The Turner® Designs Cyclops-7 sensor measured colored dissolved organic matter, but was not capable of recording depth. In order to obtain a depth profile, it was necessary to manually align the cables of the Cyclops-7 CDOM and the Hach hydrolab, such that the sensors were parallel, and lower both instruments through the water column simultaneously. This allowed the sensors to record measurements at the same depth. Recordings were taken every 10 cm. Later, data from the CDOM data logger were matched up to the depth profiles from the Hach hydrolab using a time offset. CDOM data are missing for July 25, 2011, because the battery died in mid-recording.

Water samples and Cyclops-7 CDOM/Hach hydrolab measurements were collected in proximity of the WM site monitoring sonde and correlation was performed by using sonde data from the nearest 15-minute interval to the time when sampling took place. Because the sensors for the data logger were located 30 cm from the bottom of the lake, 30 cm was subtracted from the calculated depth of the lake at the WM site each day in order to determine the row of data from the depth profile to use for correlation. For example, on June 15, 2011, the total depth of the lake at the WM site was determined to be 85 cm, so instrumental data collected at 55 cm (5.5 m) was used. Because instrumental data were collected only at 10 cm intervals, data were sometimes averaged or weighted.

Correlations between the Hach hydrolab instrument and WM site monitoring sonde were calculated for the five matching parameters: temperature, specific conductivity, pH, turbidity, and dissolved oxygen. All of these parameters showed a

strong correlation (Figure 5), except for dissolved oxygen, in which no correlation was found. The membrane on the Hach instrument was most likely not working correctly at the time this study was conducted.

All water property data have been recorded in the tables in Appendix B.

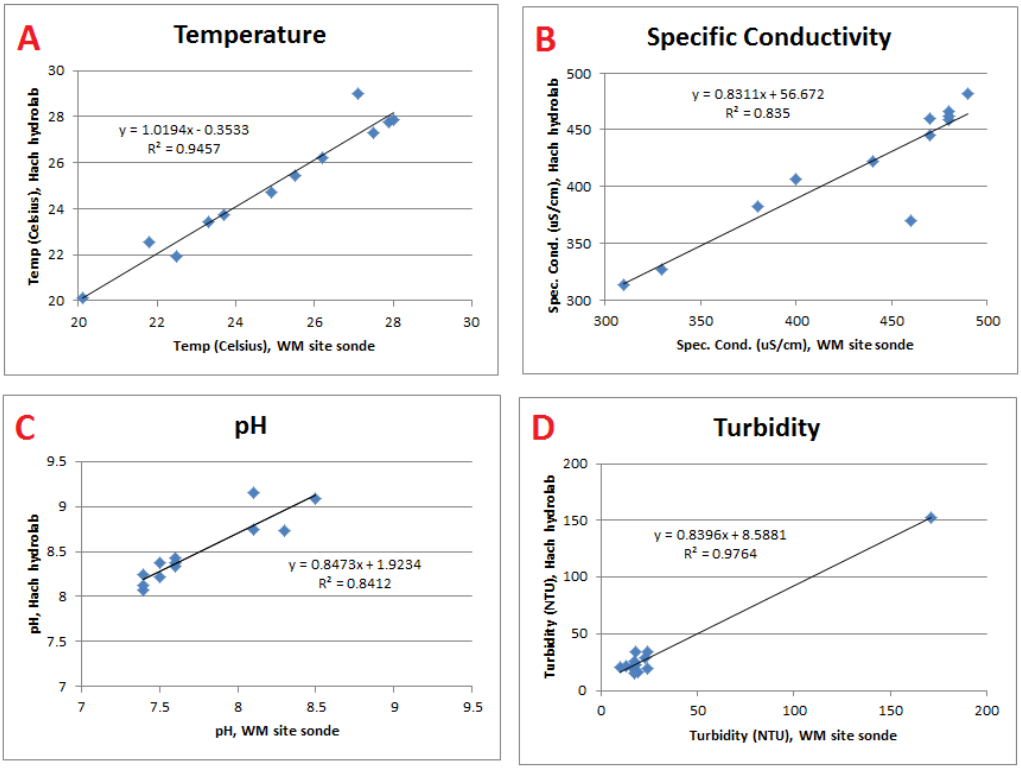

Figure 5. Correlation of Hach hydrolab and WM site sonde.

Strong correlations between the Hach hydrolab multiparameter sonde and WM site in situ monitoring sonde were calculated for temperature (A), specific conductivity (B), pH (C), and turbidity (D). Dissolved oxygen values are not shown, as no significant correlation was found (r2 = 0.01).

3.4 Methods for Filtering

The standard method of filtering water samples in this study was through the use of a 60-psi vacuum pump at the field station at Old Woman Creek. The apparatus allowed for up to four samples to be filtered at a time; however, no more than two samples were ever run simultaneously, and those two were almost always replicates. Bottles were shaken prior to filtering to homogenize the water and a graduated cylinder was used to measure out the desired volume of water. All filters used in this study were 47 mm, 0.4 µm glass-fiber filters (GF/F) and were handled with metal forceps (to prevent contamination of organics from skin) with a non-pointed tip (to prevent tearing). GF/Fs used for hyperspectral, VNIR derivative spectroscopy (henceforth referred to as "ASD filters" or "ASD analysis"; see Chapter 3.6) were weighed three times: empty (pre-weight), wet (immediately before being placed in the oven), and dry (immediately after being removed from the oven). All weighing was done using an analytical balance with a precision of ±0.1 mg. Once filtering was done, wet GF/Fs were wrapped in aluminum foil. During transport, GF/Fs were protected from loss of material through contact with the aluminum foil by placing a small, doughnut-shaped PVC ring on top of the filter, outside the mass of collected particulate matter, and carefully wrapping the aluminum foil over top of the disc. No problems with smearing ever occurred. GF/Fs intended for visible-range fluorescence analysis (henceforth referred to as "Trilogy filters" or "Trilogy analysis"; see Chapter 3.7) were carefully folded shut, with the particulate matter on the inside. Unlike ASD filters, it was not necessary to preserve the structure of Trilogy filters because they were ultimately ground up and macerated in Mg-acetone solution (as

described by the standard method of pigment extraction in Chapter 3.7). All GF/Fs (both ASD and Trilogy) were wrapped in small square packets of aluminum foil. This not only helped to prevent contamination from outside sources, but also allowed for the GF/Fs to be labeled, which was done using a black marker on the outside of the packet prior to wrapping the filter pad. Wrapped GF/Fs were placed in plastic bags and transported in a cooler with ice to Kent State University, where they were stored in a freezer until ready to be processed. The time in transit was about an hour.

The description above was the standard method of filtering used throughout the study; variations or changes to these methods will be discussed below.

The first change was the amount of water filtered per GF/F. For Trilogy GF/Fs, only 100 mL of water was used, as fluorometry is considered to be more sensitive than spectrophotometry and, thus, less water is needed to obtain an accurate reading (APHA, 1999). Larger volumes of water, however, were required for ASD analysis. In the early stages of the study (June 7 and June 15), 500 mL of water was used for all GF/Fs intended for ASD analysis. These GF/Fs became heavily clogged after about 300 mL. Further filtering was slowed dramatically and led to overworking of the vacuum pump. Ultimately, it was decided to decrease the filtration amount to 250 mL for all samples intended for ASD analysis. This volume was found to provide good quality readings, without the risk of further damage to the vacuum pump. As a result of this change in the methodology, the samples collected on June 7 and June 15 were disregarded, as it would have been difficult to compare samples with different filtration amounts. (Regardless, an attempt was made on June 23 to examine the effects of filtering multiple levels of water,

but this may have to be the work of future studies. See Chapter 4.1: Multiple Levels Experiment for more information.)

The second change to the standard method of filtering was when the filtering actually took place (relative to when the water was collected). All samples were filtered within an hour after collection, with the exception of the samples collected on June 7 and all the samples collected by Dr. David Klarer. The samples collected on June 7 were filtered over the course of two days (standard preservation techniques were still applied during this time). The samples collected by David Klarer were always filtered during the next scheduled OWC trip, which took place four days later (e.g. the Klarer bottles "D5" and "D6" were collected on July 5 and were filtered on July 9; see Appendix A).

The third change was the type of apparatus used for filtering. The 60-psi vacuum pump was used almost exclusively for this study. On July 1, however, an aspirator was used to filter two 250 mL surface samples (replicates). These took an exceptionally long time to filter (~31 minutes) and the aspirator was not used again. Similarly, all samples collected on June 7 were filtered using a hand pump. A hand pump was again used on August 10 to filter replicate 100 mL samples. The intention of using these different devices was (a) to find the most effective means of filtering samples, and (b) to determine if a change in apparatus would lead to a change in the resulting concentrations of biomass (see Chapter 4.1: Filtering Apparatus Experiment). It was determined that the vacuum pump provided the most rapid means of filtration (usually less than five minutes for a 250 mL sample), but future studies may consider looking into the effectiveness of this device compared to a standard hand pump.

All changes from the standard method of filtering, as described in the first paragraph of this section, have been recorded in Appendix A. In general, only samples that were filtered with a vacuum pump and had filtration amounts of 100 mL (for Trilogy analysis) or 250 mL (for ASD analysis) were considered in the quantitative data analysis sections in Chapter 4. All other samples were disregarded or only used as a means of determining the best method of filtration.

3.5 Methods for Drying (ASD samples only)

Filtered samples intended for ASD analysis were first required to be dry, as the presence of water molecules on the filter pad may have interfered with hyperspectral readings. As such, these filters were brought back to the Kent State lab, weighed, and placed in a drying oven at 60°C for 24 hours. This was sufficient time for the water molecules to evaporate. This procedure was almost always done immediately upon returning from Old Woman Creek from a sampling trip; in the case of the Drying Time Series Analyses (Chapter 4.1), however, it was necessary to wait one or more days before drying certain samples. In this case, the samples were preserved by keeping them wrapped in packets of aluminum foil and storing them in a dark freezer.

After 24 hours in the oven, the filters were removed, weighed again, and processed by the methods described in Chapter 3.6. Figure 6 shows a typical result of these filters after drying.

It was not necessary to dry samples intended for Trilogy analysis (see Chapter 3.7).

Figure 6. Glass-fiber filters (GF/Fs) after oven-drying.

Six glass-fiber filters (GF/Fs) after the filtration of 500 mL of sample water from the sampling site at Old Woman Creek. These samples were collected and filtered on June 15, 2011. Filters 27 and 28 were collected from the surface, 29 and 30 at an intermediate depth, and 31 and 32 near bottom. This photograph was taken after the samples had been oven-dried for 24 hours.

3.6 Methods for Measuring Reflectance (ASD analysis)

Reflectance spectra of dry filtered samples were recorded using an Analytical Spectral Device (ASD) LabSpec® Pro Full-Resolution ultraviolet/visible/near-infrared (UV/VIS/NIR) spectrophotometer (Figure 7) with a high intensity contact probe. This instrument was capable of measuring between 250-2500 nm, with a 2-nm resolution in

the UV-VIS range and 4-10 nm in the NIR range. For this study, only data from 400-2500 nm was used, as significant noise was generated below 400 nm.

Figure 7. ASD Device LabSpec® Pro FR UV/VIS/NIR spectrophotometer.

Three steps were involved in the preparation of using the ASD spectrophotometer. First, samples were dried (refer to Chapter 3.5 for the oven-drying procedure) to prevent interference from water molecules during spectral analysis. Drying time was about 24 hours. Second, the spectrophotometer was allowed at least 30 minutes to warm up. This was done shortly before the 24 hour drying period was complete, allowing sufficient time for the filters to cool before removing them from the oven. Third, an empty glass-fiber filter (GF/F) was used as a blank. For this study, the spectrophotometer was re-calibrated

(using a blank ceramic panel) each day the instrument was used, but only the reflectance values of the two blanks measured on the first day were averaged for use in blank-correction.

Once all of these steps were completed, a dry filtered sample was placed on a clean white ceramic panel on a stand directly below the contact probe of the spectrophotometer. A knob on the side of the stand allowed for the platform to be raised and lowered. The platform was raised until the GF/F touched the sensor and was centered beneath the probe. Hyperspectral readings were collected on a laptop with the ASD software. Reflectance values were given in graphical format, and data were saved to the hard drive as an ASD binary data file. Once a recording was taken, the platform was lowered and the GF/F was removed, wrapped back up in aluminum foil, and put away. This process was repeated for each sample.

At the conclusion of the study, ASD files on the laptop were converted to ASCII, which allowed the spectral data to be exported into Excel® for easier quantitative analysis. Samples were separated out by experiment due to the large volume of data generated.

Once in Excel®, the data were blank-corrected. This was done by dividing each reflectance value by the average of two blank GF/Fs, measured on the first day of sampling. Next, offset adjustments had to be performed. This was done because there were three detectors within the instrument and each detector only measured a specific part of the spectrum. Offset adjustments were calculated at 987 nm and 1766 nm. To do this, each reflectance value from 987-2500 nm was multiplied by the quotient of the

reflectance value at 986 nm divided by the reflectance value at 987 nm. This set the reflectance values at 986 nm and 987 nm equal. The same technique was applied at 1766 nm, where each reflectance value from 1766-2500 nm was multiplied by the quotient of the reflectance value at 1765 nm divided by the reflectance value at 1766 nm (in addition to being multiplied by the quotient of the reflectance value at 986 nm divided by the reflectance value at 987 nm). This set the reflectance values at 1765 nm and 1766 nm equal. This procedure allowed for a single, full hyperspectral reading from 200-2500 nm.

After blank-correction and offset adjustments were made, the data were band-averaged to a width of 10 nm. This provided a useful hyperspectral recording from 400-2500 nm. Data below 400 nm were discarded due to noise. Values were then multiplied by 100 to give percentage reflectance values.

3.7 Methods for Measuring Fluorescence (Trilogy analysis)

Visible-range fluorescence spectroscopy was accomplished using a Trilogy® Laboratory Fluorometer and was conducted according to the guidelines of the American Public Health Association's (APHA), "Standard Methods for Examination of Water and Wastewater" (1999). The following is an abbreviated version of this technique. The standard APHA (1999) method was modified slightly to accommodate the particular Trilogy® fluorometer used in this study. (This method is almost identical to the EPA's Method 445.0, "*In Vitro* Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Algae by Fluorescence" (1997), except that a 90% Mg-acetone solution is used instead of a 90% acetone solution with distilled water.) Mg-acetone solution was

chosen because this is standard method used by researchers in the Aquatic Ecology lab of the Department of Biological Sciences of Kent State University, which was the facility used for this study.

Part I (pigment extraction):

1. Filter 100 mL of water onto a glass-fiber filter (47 mm, 0.4 µm).
2. Fold filter in half, such that the collected particulate material is on the inside, wrap in aluminum foil, and store on ice or at 4°C in the dark until ready to process (no more than three weeks).
3. Slide filter into a 15-mL graduated, screw-cap centrifuge tube.
4. Add 10 mL of 90% Mg-Acetone solution (90% aqueous acetone, 10% saturated magnesium carbonate).
5. Macerate filter with a glass rod to create extraction slurry.
6. Cap tube, wrap in aluminum foil, and store in a fridge at 5°C overnight (24 hours).

Part II (fluorometery):

1. Insert correct module lamp (Chl-a acid) in fluorometer while fluorometer is off.
2. Turn fluorometer on and allow 10 minutes to warm up.
3. Centrifuge samples at 4000 revolutions per minute (RPM) for 10 minutes.

4. Pipette 4 mL of 90% Mg-Acetone into a clean cuvette to use as blank. (Chlorophyll *a* value should read 0.0 ug/L.)
5. Pipette 4 mL of sample into a clean cuvette, wipe with a Kimwipe, and place in fluorometer. Close lid.
6. Record first reading (Fb = fluorescence before acidification).
7. Remove cuvette from fluorometer and acidify with 125 µL 0.1 N HCl. Mix solution thoroughly by holding from top of cuvette and thumping its base several times.
8. Wait 90 seconds. (If needed, wipe cuvette with a Kimwipe to remove condensation.)
9. Place cuvette back into fluorometer, close lid, and record second reading (Fa = fluorescence after acidification).
10. Repeat Steps 6-10 for all samples.

Subdued lighting is important for all stages of this procedure. When not in use, samples were wrapped completely in aluminum foil. GF/Fs were only handled with metal forceps to prevent contamination of organics from skin.

For all samples in this study, the fluorometer was set to automatically calculate the concentrations of chlorophyll *a* and pheophytin *a* based on the values of "Fa" and "Fb" that were determined by the instrument. Chlorophyll *a* and pheophytin *a* concentrations have been recorded in Appendix A. Some of these samples, such as those collected on June 15, have been discarded due to incorrect procedures (indicated in the

"Notes" column). Other samples are suspect, due to chlorophyll *a* and pheophytin *a* values seeming inverted (e.g. filters #60-62 in Appendix A.4, in which the concentrations of pheophytin *a* are notably higher than chlorophyll *a*). As these potentially erroneous values were noticed at the time of recording, they have also been discarded from analysis.

3.8 Methods for Preservation

Standard preservation methods, as described in APHA (1999), were used throughout this study. All water that was collected was stored in clean, opaque, 1-liter plastic bottles, and kept cold and in the dark until ready to be processed. Filtered samples were kept wrapped in aluminum foil and in a cooler or on ice at all times. GF/Fs were handled with metal forceps only. No filtered samples were subjected to extended periods of light, with the exception of the Air-drying Experiment (see Chapter 4.1). All frozen samples were processed within at least three weeks of being collected, with the exception of the Drying Time Series Analyzes (see Chapter 4.1).

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Experiment Summary

This section provides a brief description of each experiment performed in this study. Experiments were assigned as being either qualitative or quantitative. The purpose of qualitative experiments was to determine the best means by which to process and treat samples (Objective #1); these were excluded from any statistical analysis but may be of use in further studies, when more data can be provided. Quantitative experiments, however, were intended to provide statistical insights and were thus used in the data analysis sections found later in Chapter 4.

Experiments considered to be qualitative were: (1) the Magnesium Carbonate (MgCO3) Experiment, (2) the Filtering Apparatus Experiment, and (3) the Multiple Levels experiment. The details for each of these experiments have been described below. Quantitative experiments include: (1 and 2) the two Drying Time Series, (3) the Air-drying Experiment, (4) the Surface Time Series, and (5) the Depth Time Series. These have also been described below.

Qualitative experiments:

- **Magnesium Carbonate (MgCO3) Experiment (June 7, 2011; ASD samples only)**. For this study, it was sometimes necessary to preserve water samples for up to four days at a time (see Chapter 3.1). One technique that can be utilized to help prevent rapid degradation of chlorophyll *a* is adding a small amount of powdered magnesium carbonate (MgCO3) to the sample (UNESCO, 1966). To test this, replicate samples from the Drying Time Series #1 (June 7) were filtered with a few drops of 10 mL of powdered MgCO3 solution (see Appendix A.1). This was done to see if more chlorophyll *a* was retained in the samples filtered with magnesium carbonate than those that were left untreated. The white residue left behind by the solution, however, interfered with hyperspectral readings (see Figure 8), even when only small amounts were used. In addition, Lium and Shoaf (2007) measured chlorophyll concentrations with and without the MgCO3 treatment and found no difference in either the retention of algae on the filter or in the stability of chlorophyll, and Aminot and Rey (2002) found magnesium carbonate actually adsorbs the degradation pigments, making it more of a hindrance than a help. Due to the collective effort of these findings, the use of the magnesium carbonate as a preservation agent was discontinued for the remainder of the study.

Figure 8. GF/Fs with and without MgCO3 treatment.

Water samples were collected on June 7, 2011, and filtered onto these two GF/Fs two days later. The sample on the right was mixed with 10 mL of powdered magnesium carbonate (MgCO3) prior to filtering. The sample on the left was untreated. This photograph was taken after the samples had been oven-dried. As shown, there was a significant color variation between the samples, which made it difficult to get an accurate hyperspectral reading in the visible part of the spectrum.

- **Filtering Apparatus Experiment**. As described in Chapter 3.4, there were three devices by which water was filtered through a GF/F: a 60-psi vacuum pump, a hand pump, and an aspirator. The purpose of testing these different apparatuses was to see if there was potential for material to become lost or destroyed during the filtering process. Due to the potential

for high variability between replicates in this study (due to incomplete mixing, etc.), obtaining a quantitative result from this experiment would be difficult without the collection of more data. Qualitatively, however, the vacuum pump was determined to be the most effective. As noted in Appendix A.4, it took ~31 minutes to filter 250 mL of water with the aspirator. Replicates filtered with the vacuum pump took less than a minute. Given time constraints (at least eight 250 mL samples had to be filtered per sampling trip) and the potential for chlorophyll *a* to degrade in the presence of light for an extended period of time, all aspirator samples were disregarded. The hand pump was discontinued for similar reasons, as samples collected (on June 7) took two days to completely filter. (It should be noted, however, that the volume of water being filtered at that time was 500 mL, and a hand pump might be more practical with lesser volumes of water.) More work would need to be done to provide a quantitative comparison between filtering apparatuses, but this experiment at least confirmed the most efficient device to filter samples was being used.

- **Multiple Levels Experiment (June 23, 2011; ASD samples only)**. Another issue that was previously discussed in Chapter 3.4 was determining how much water should be filtered per GF/F. Attempting to filter too much water caused the GF/Fs to become clogged and impeded further filtering, but enough material needed to be collected to obtain a

useful hyperspectral reading. It was also important to be consistent so samples could be readily compared. The Multiple Levels Experiment examined the results of filtering 50, 100, 150, 200, and 250 mL of water. This was done on each of the two bottles at each of the three depths collected on June 23 (for a total of six times). Ultimately, however, this experiment was disregarded, as it was deemed easier to simply remove the samples that had been filtered with 500 mL (i.e. those collected on June 7 and June 15) than to find a way to extrapolate to 500 mL from the results of this experiment. An additional sampling trip (September 11, 2011) was scheduled to provide three months' worth of data.

Quantitative experiments:

- **Drying Time Series #1 (June 7, 2011; ASD samples only)**. This experiment examined the results of drying GF/Fs on increasing numbers of days after initial collection. For this experiment, all water samples were collected on June 7, 2011, and were homogenized in an 18-liter carboy prior to filtering. Filtering was done with 500 mL of water per GF/F. Samples were then preserved until a pre-determined number of days had passed (see Appendix A.1). Then, they were dried and a spectral reading was taken. The purpose of this experiment was to determine how long it would take for the samples to begin to show a significant loss of chlorophyll.

- **Drying Time Series #2 (July 17, 2011; ASD samples only)**. This experiment was a replication of the drying time series on June 7. In this experiment, however, 250 mL of water per GF/F was used to coincide with the volume used for both the Surface Time Series and the Depth Time Series Analyses. (Refer to Appendix A.6 for oven-drying dates.)
- **Air-drying Experiment (September 11, 2011; ASD samples only)**. This experiment compared standard oven-drying (see Chapter 3.6) and air-drying near an open window for 24 hours. The purpose of this experiment was to see if air-drying would lead to a significant loss of chlorophyll *a* due to radiation from sunlight.
- **Surface Time Series**. This experiment examined surface samples from the WM site (Figure 3) at a four-day interval. Two samples were taken every four days, beginning June 23 and ending September 11, 2011, for roughly a three-month time series. Water samples collected before June 23 were excluded from this experiment (see Chapter 3.4). All GF/Fs intended for ASD analysis were filtered with 250 mL of water (100 mL for Trilogy analysis).
- **Depth Time Series**. This experiment examined samples at three depths from the WM site (Figure 3) at an eight-day interval. Two samples were taken at three depths (surface, intermediate, and near bottom; see Chapter 3.2) every eight days, beginning June 23 and ending September 11, 2011, for roughly a three-month time series. Water samples collected before

June 23 were excluded from this experiment (see Chapter 3.4). All GF/Fs intended for ASD analysis were filtered with 250 mL of water (100 mL for Trilogy analysis).

4.2 Determining the Rate of Degradation of Chlorophyll a

Objective #2 was to determine the rate of degradation of chlorophyll *a* into its principal degradation products: pheophytin *a*, pheophorbide *a*, and/or chlorophyllide *a* (see Chapter 2.2). This was necessary to determine how quickly frozen ASD samples would need to be processed in order to obtain accurate hyperspectral data. For traditional spectrophotometric and fluorometric analysis, three to four weeks has been suggested as the maximum holding time for frozen samples (Arar and Collins, 1997; Aminot and Rey, 2002). After this time, samples may begin to show a significant loss of chlorophyll *a*. To test this for the modified ASD analysis used in this study, two drying time series were conducted (one on June 7 and one on July 17; see Chapter 4.1: Drying Time Series #1 and #2). These experiments involved the typical procedure for water collection and filtering, but ASD samples were kept frozen for a pre-determined number of days before analyzing them. The experiment conducted on June 7, 2011, used 500 mL of water as the filtration amount. The experiment conducted on July 17, 2011, used 250 mL of water as the filtration amount. Water was homogenized in a large carboy so that all samples in either experiment could be considered replicates.

The results for the experiment on June 7, 2011, are shown in Figure 9A (full hyperspectral range from 400-2500 nm) and Figure 9B (only the visible range, 400-700

nm, shown). It should be noted all sample water in this experiment was collected on June 7, but it took two days to filter all samples. Thus, it is possible some chlorophyll was lost to grazer activity. As shown in the legend in Figure 9, one filtered sample was dried and analyzed on the day of collection ("Day 0"), two days later ("Day 2"), three days later ("Day 3"), four days later ("Day 4"), and so on, up to 21 days later ("Day 21"). Even after three weeks, no significant difference in chlorophyll *a* was noticed (Figure 9B). Had there been a difference, there would be a notably higher degree of variability in the red part of the spectrum (particularly 660-690 nm, where chlorophyll *a* peaks). No such variability exists. The high variability around 400 nm is a product of noise. There is also some variability between the "Day 0" sample and the other samples in the blue part of the spectrum; this was caused by not filtering all the samples on the day of collection. As such, this experiment was performed again on July 17, 2011 (Figure 10). In this case, all water was filtered on the day of collection (July 17) and samples were kept frozen for up to 36 days. Again, all samples show a high degree of similarity at every wavelength, and the high variability between "Day 0" and other samples that was observed in the June 7 experiment (Figure 9B) is not observed here (Figure 10B). Some noise still exists around 400 nm.

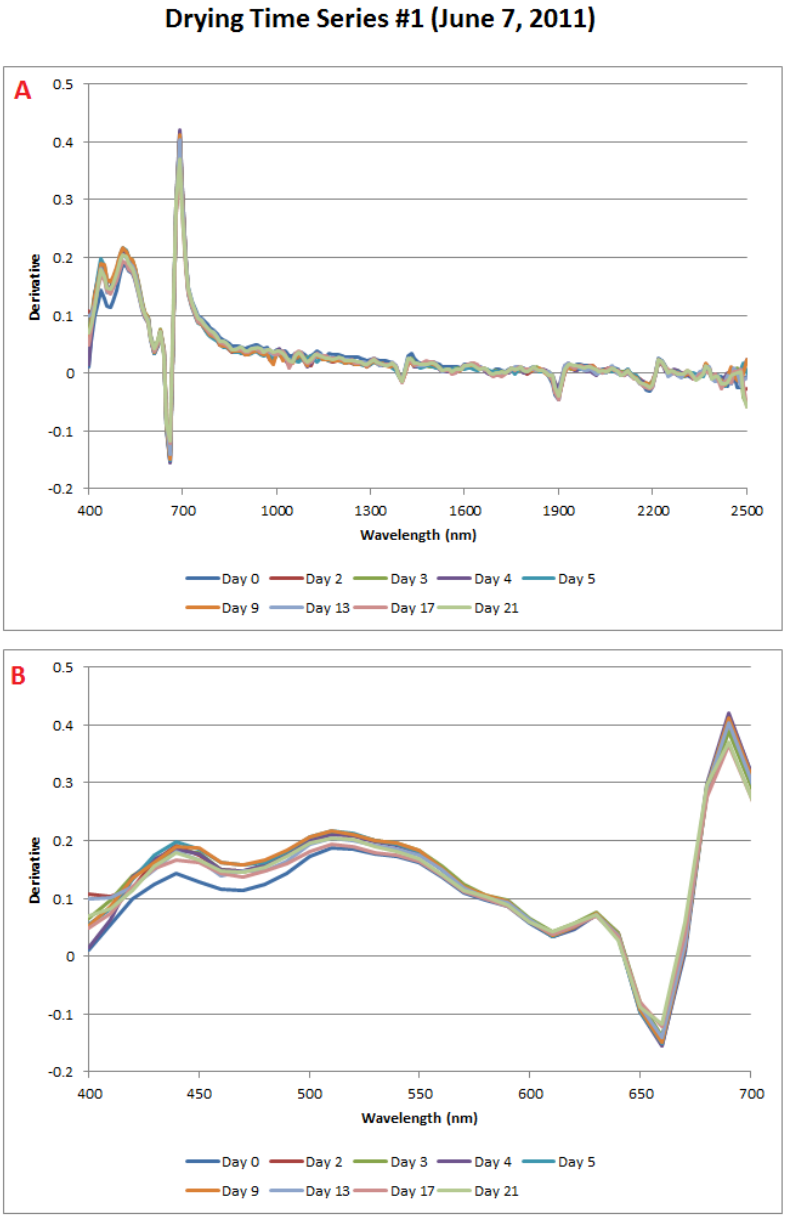

Figure 9. Drying time series on June 7, 2011.

VNIR derivative results from the first drying time series, with a hyperspectral range of (A) 400-2500 nm, and (B) 400-700 nm. Filter samples were kept frozen until a certain number of days after collection (shown in legend).

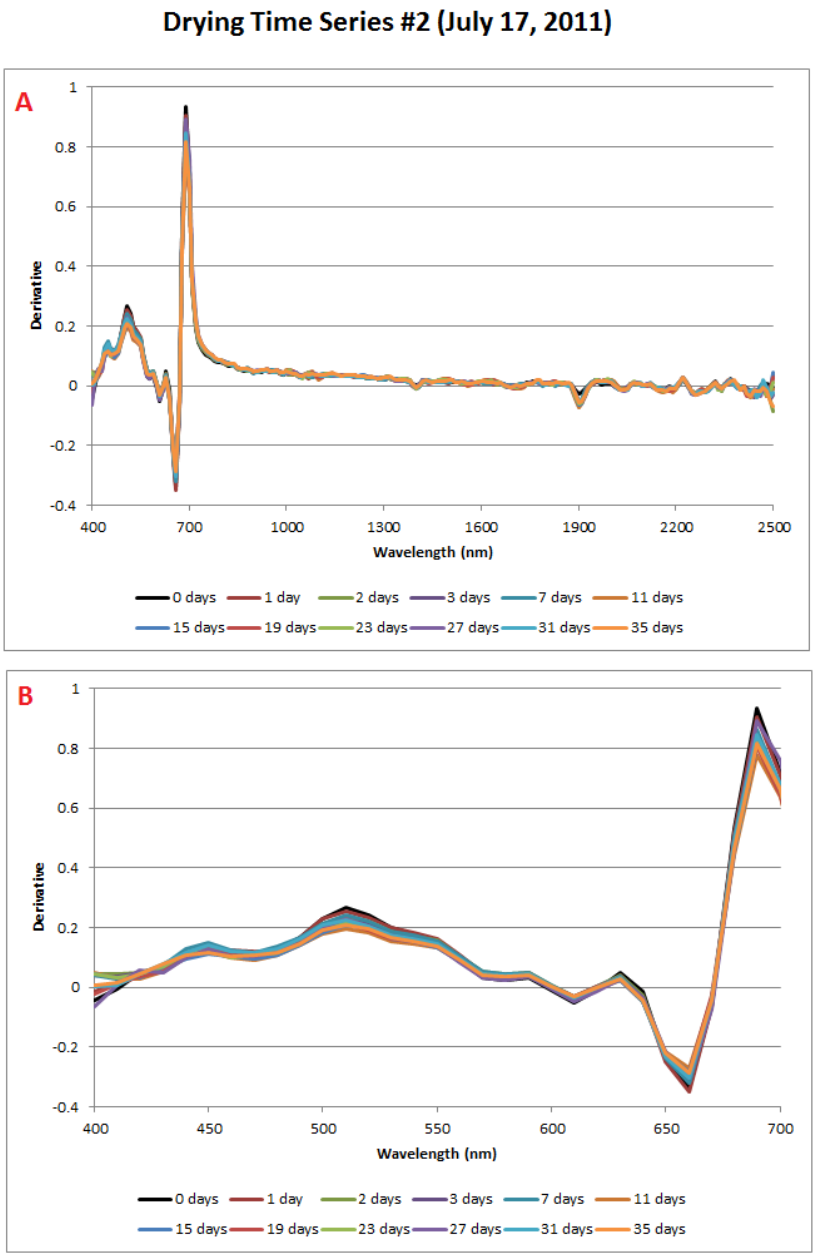

Figure 10. Drying time series on July 17, 2011.

VNIR derivative results from the first drying time series, with a hyperspectral range of (A) 400-2500 nm, and (B) 400-700 nm. Filter samples were kept frozen until a certain number of days after collection (shown in legend).

In addition, major inflection points from the July 17 drying time series experiment were plotted individually in Figure 11. Major inflection points were based off the plots in Figure 10A and include 420 nm, 440 nm, 470 nm, 510 nm, 550 nm, 570 nm, 590 nm, 610 nm, 630 nm, 660 nm, and 690 nm (see Figure 11A-K). The y-axis in these graphs was kept constant (derivative 0-1.0) to make for easier comparisons (except in the cases of 610 and 660 nm, where values drop below 0). Almost all of the major inflection points remain constant from the initial collection date, even up to 36 days after. The four inflection points with the greatest variability are 440 nm, 510 nm, 660 nm, and 690 nm. The first two (Figure 11B and Figure 11D), however, only show a notable change about a week after collection (7 days) and then return to their initial value. Interestingly, the variability at 660 nm (Figure 11J), which may correspond to chlorophyll *a*, increases after only a week of being frozen (although this is a trough in the full signature and actually corresponds to a slight decrease in strength). Regardless, this is not a significant difference. Variability at 690 nm (Figure 11K) is highest; however, there is no linear trend to the data, so it cannot be said a significant amount of chlorophyll *a* has been lost, even after 36 days. Had significant loss of chlorophyll *a* been lost during this experiment, there would be a noticeable decrease at 690 nm (one of the prominent peaks for chlorophyll *a*) and a subsequent increase around 410 or 420 nm (prominent peaks for the principal degradation products of chlorophyll *a*).

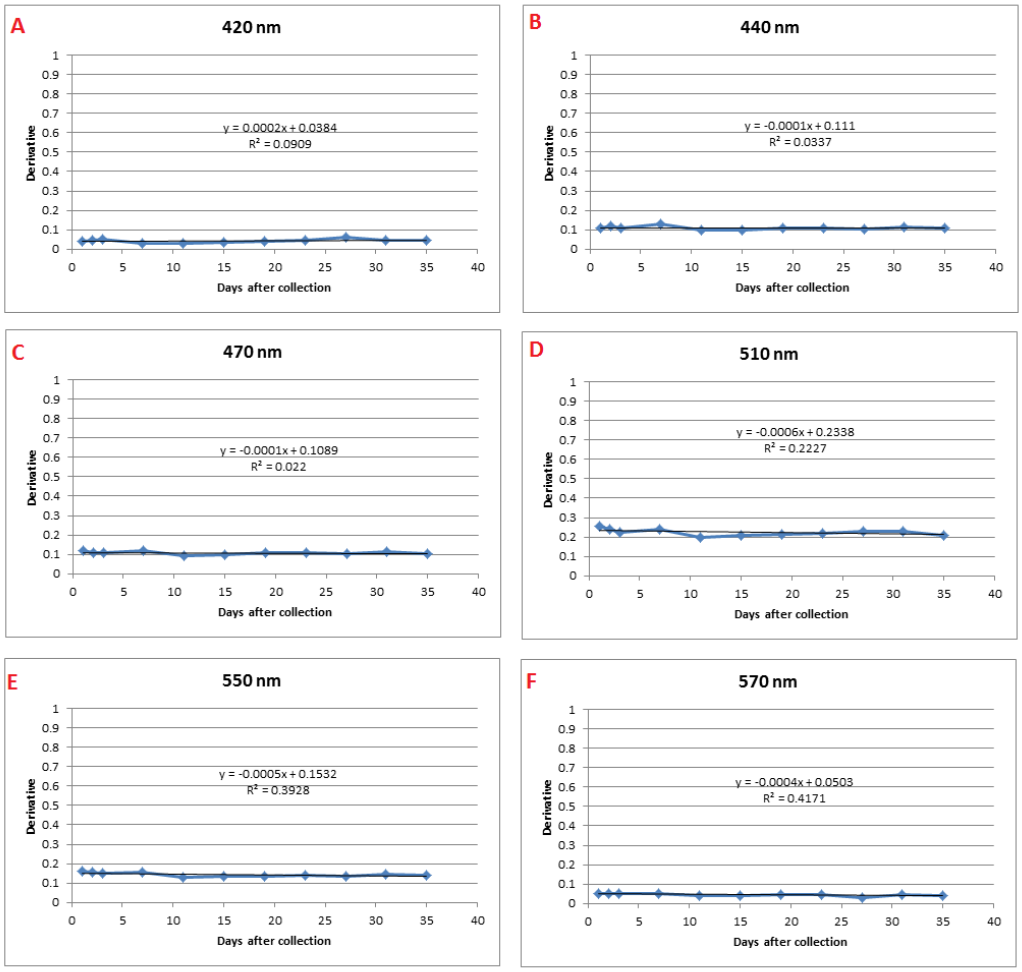

Figure 11. Major inflection points for drying time series on July 17, 2011.

Major inflection points for plant pigments (based on drying time series results) in the visible range of the spectrum (400-700 nm) have been plotted. All samples were collected and filtered on July 17 ("Day 0") and were kept frozen until their pre-determined day of analysis. The final sample in the experiment was kept frozen for 36 days.

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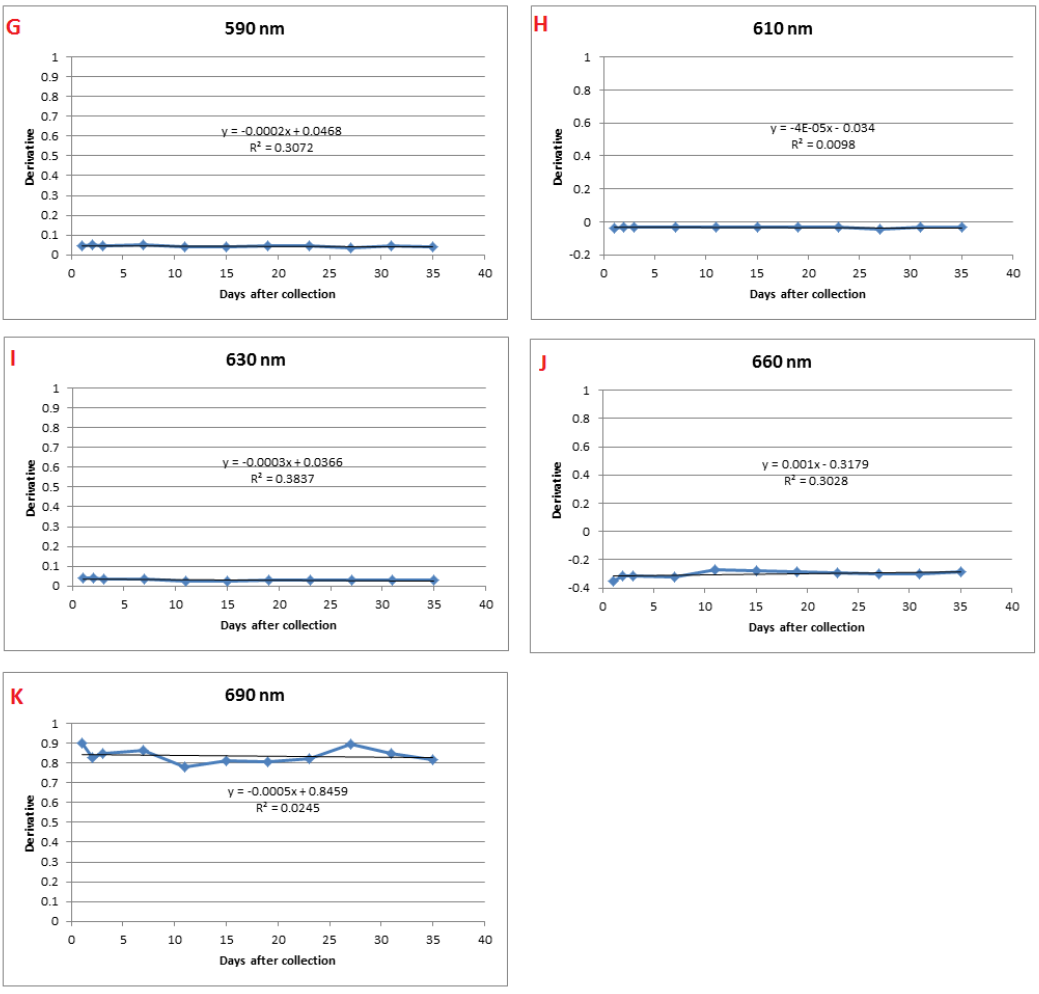

(continued from previous page)

The results of these experiments confirm filtered samples can be kept frozen for at least three weeks for ASD analysis. This did not come as a surprise, as the only differences between the modified 'dry' spectrophotometric method used in this study and the 'wet' spectrophotometric method used by the APHA (1999) are how samples are analyzed and not their storage conditions. It was, however, useful to confirm this and to see if hyperspectral signatures remain constant even at higher wavelengths (700-2500 nm), which they do (Figure 9A and Figure 10A).

The drying experiments could also have been used to see if a significant loss of chlorophyll *a* could be observed in the samples collected by David Klarer (see Chapter 3.1), which were not processed until four days after collection. Unfortunately, a comparison could not be made because Klarer water samples were kept unfiltered for the four day time period, whereas samples in these experiments were immediately filtered and frozen. Unfiltered samples can be treated with 10% isopropyl alcohol, which essentially kills grazers in the water to prevent an underestimation of chlorophyll *a*. Furthermore, Aminot and Rey (2002) suggest filtering samples immediately after collection (within an hour), but any time within twenty-four hours of collection may be acceptable. Should an experiment like this be performed again, it would be a good idea to consider fixing samples (with 10% isopropyl alcohol) or filtering water immediately after collection and storing all samples in a freezer until they can be retrieved by the researcher performing the experiment. Regardless, as was suggested by Arar and Collins (1997) and confirmed in these two drying experiments, frozen filters can last for at least up to three or four weeks without showing a significant difference in hyperspectral readings.

4.3 Results of Air-drying Experiment

As an additional element to determine Objective #1 (choosing the best methods by which to process and treat samples), samples collected on September 11, 2011, were allowed to dry one of two ways. Six samples (#232-237; see Appendix A.13) were dried using the standard oven-drying (see Chapter 3.5). Replicate samples (#248-253; see Appendix A.13) were placed on sheets of aluminum foil, unwrapped, and left near an open window, facing west, for 24 hours. Weather conditions during this time were sunny and 18°C (65°F), with a brief period of rain showers. Air-drying samples were far enough away from the window that they did not get wet. VNIR derivative data from this experiment are represented graphically in Figure 12. The one notable anomaly in the data (Sample #251) was caused by a strand of hair that was found on the filter after the drying period was over. This hair was not removed from the filter before measurement, as there was risk of ruining the sample further. Suspect readings from this sample were found at 1400, 1480, 1900, and 1970 nm, but the visible range (400-700 nm) appeared unaffected. Contamination of this type was never problem while oven-drying.

Averaged data from the drying treatments in the visible range (Figure 12) show a strong overlay, suggesting a high degree of similarity. Upper and lower 95% confidence intervals for each treatment have also been plotted. The confidence band is narrower for the oven-dried (red lines) samples, indicating a higher degree of certainty the true mean lies somewhere within these parameters. It is possible the air-dried (blue lines) samples show a greater variance at certain wavelengths because of chlorophyll *a* degradation in the presence of sunlight. The wide confidence band at ~400 nm for the air-dried samples

(and, to a lesser extent, the oven-dried samples) is a product of noise. This is common at lower wavelengths. A close-up look at the red end of the spectrum, where chlorophyll *a* peaks (Figure 13B), again shows no statistical difference between oven-drying and air-drying.

Figure 14 shows another way to represent the data. Here, values for the oven-dried samples were subtracted from their counterpart air-dried samples. The resulting values were then summed and an average was calculated at each wavelength (the dark blue "x-bar" line in Figure 14). The lighter blue lines represent the upper and lower 2-sigma values. The y-axis (derivative) was kept the same as what is shown in Figure 13A, for easy comparison. As can be seen, there is almost no variability from zero, except at some of the higher wavelengths (the wider spread between the 2-sigma values around 400 nm is a product of noise). Even so, the variability at these wavelengths (650 and 660 nm) is not significant when compared to the full range of data in Figure 13A.

These results suggest filtered samples can be air-dried without losing a significant amount of chlorophyll *a*; however, more research is needed be done to confirm this. Even if this method is used, care should be taken to ensure samples are not interfered with while air-drying. Hair, dust, and other falling or moving particles may lead to contamination. Contamination could also potentially be avoided by air-drying in front of a closed window, or by keeping samples partially wrapped in aluminum foil. Future studies may consider air-drying samples in a multitude of ways (e.g. sunlight versus a well-lit room versus a darkened room) to determine the best method of treatment.

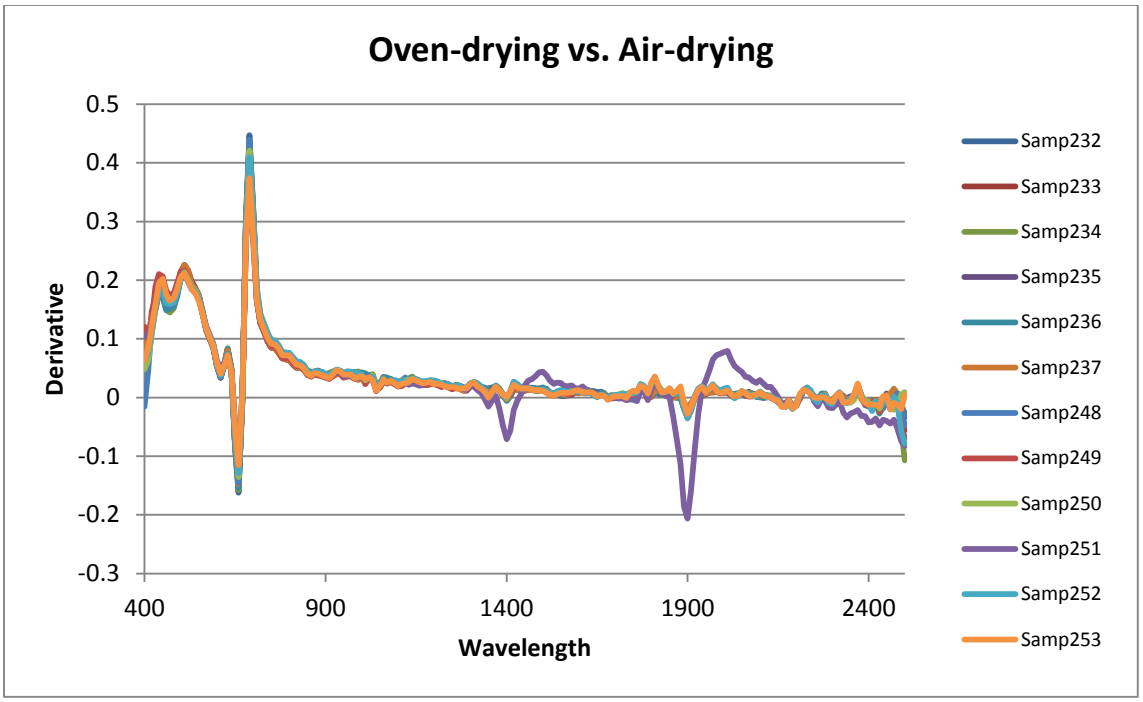

Figure 12. Individual VNIR derivative spectra for air-drying experiment.

Samples were collected at three depths: surface (232, 233, 248, and 249), intermediate (234, 235, 250, and 251), and near bottom (237, 238, 252, and 253). Samples #232-237 were dried using the standard oven-drying technique. Samples #248-253 were air-dried near an open window for 24 hours. Notable peaks in the visible range occur at 400, 440, 470, 510, 530, 630, 660, and 690 nm. Irregular peaks of Sample #251 (purple line) at higher wavelengths were caused by a strand of hair caught on the filter.

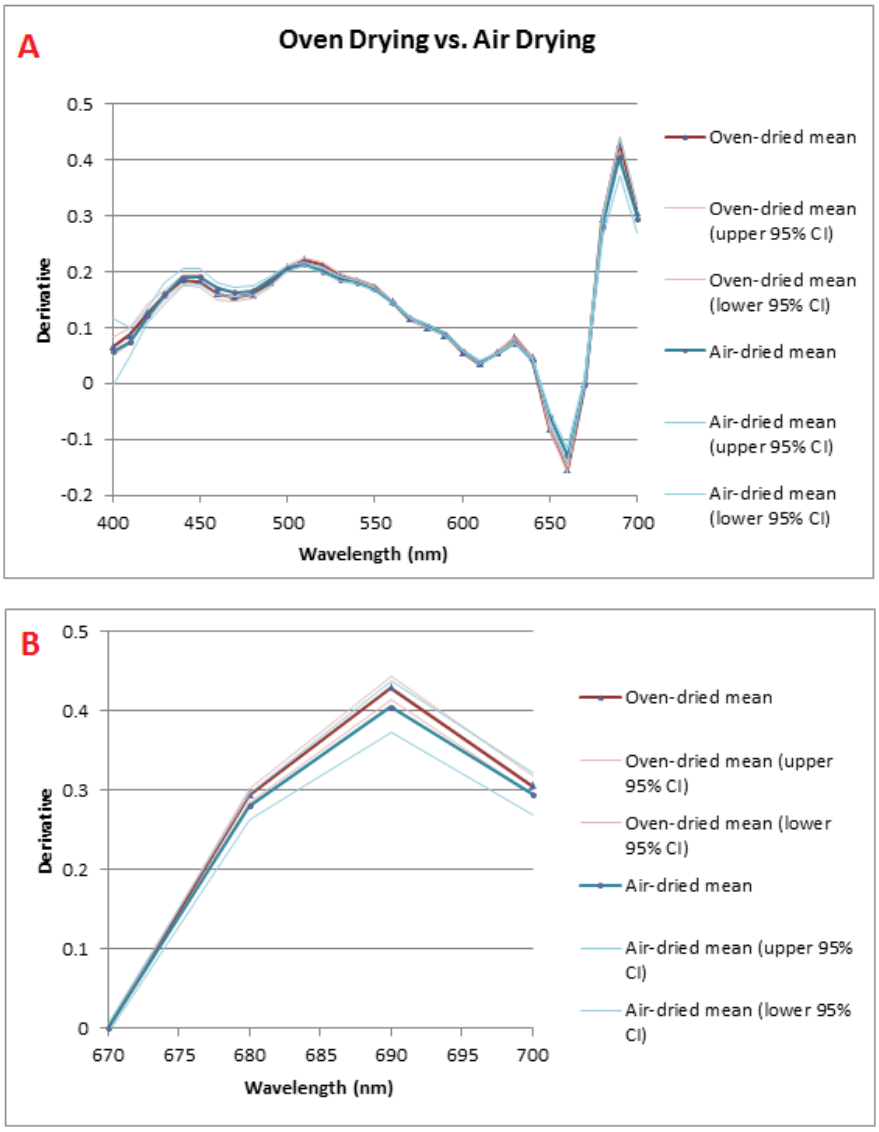

Figure 13. Combined VNIR derivative spectra for air-drying experiment.

Averaged VNIR derivative spectra in the visible spectrum have been plotted for oven-dried samples (red line; n=6) and air-dried samples (blue line; n=5) from (A) 400-700 nm, and (B) 670-700 nm. Sample #251 (air-dried) was excluded from this analysis due to contamination. No significant difference was found between air-drying and oven-drying.

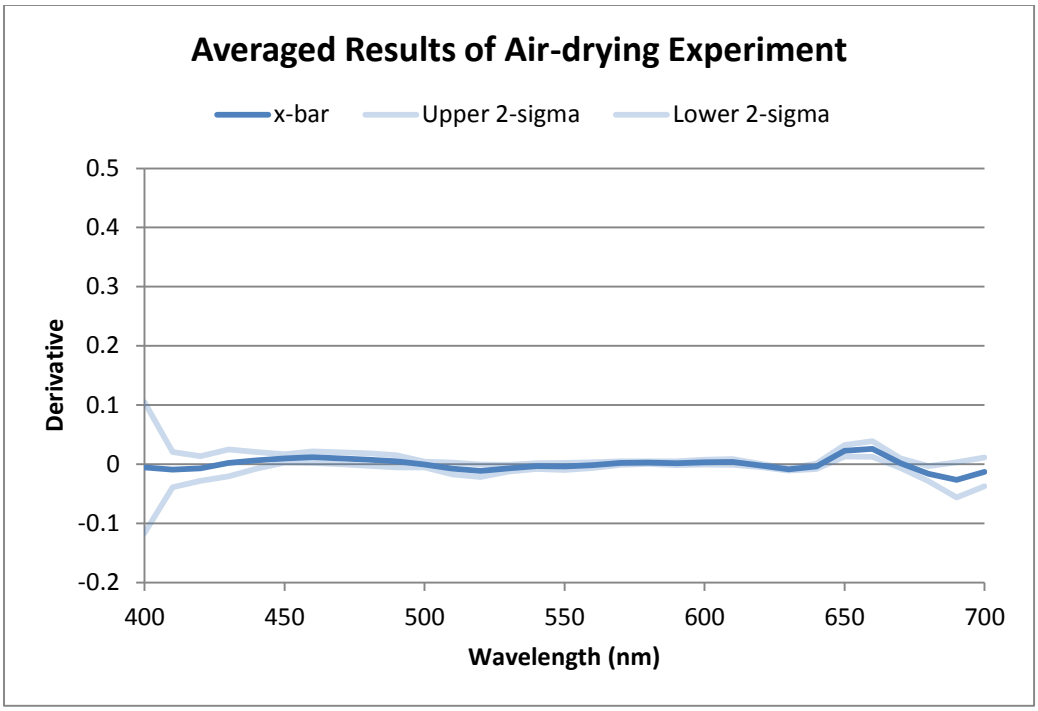

Figure 14. Averaged data from air-drying experiment.

Values for oven-dried samples were subtracted from air-dried samples, summed, and an average calculated at each wavelength (blue line "x-bar"). Upper and lower 2-sigma values have also been plotted. Noise detectable at 400 nm.

4.4 Comparison of Methods of Chlorophyll a Measurements

As discussed previously, there are numerous methods by which to measure chlorophyll *a*. In this study, concentrations of chlorophyll *a* were determined by both a Hach hydrolab sensor (see Chapter 3.3) and a Trilogy laboratory fluorometer (see Chapter 3.7). Reflectance spectra from filtered samples also contain data that can be readily compared to direct concentration measurements by using various spectral indices (Wolfe et al., 2006). These index numbers, henceforth referred to as Wolfe indices, can be determined by manipulating spectral data in some way; for example, dividing the reflectance value at 700 nm by the reflectance value at 675 nm calculating the reflectance derivative at 690 nm. There are seven such indices identified by Wolfe et al. (2006). This section provides a statistical comparison between these three unique methods of measuring plant pigments.

The easiest two methods to compare are the *in-situ* Hach and the Trilogy lab fluorometer, as both of these instruments measured direct concentrations of chlorophyll *a*. There are eleven days during the study period (separated by eight-day intervals) on which data was collected from both measurements. Figure 15 shows a comparison of surficial Hach measurements and concentrations of chlorophyll *a* as measured by the Trilogy lab fluorometer (n=2). A very strong correlation of $r^2 = 0.90$ was found between these methods (Figure 16).

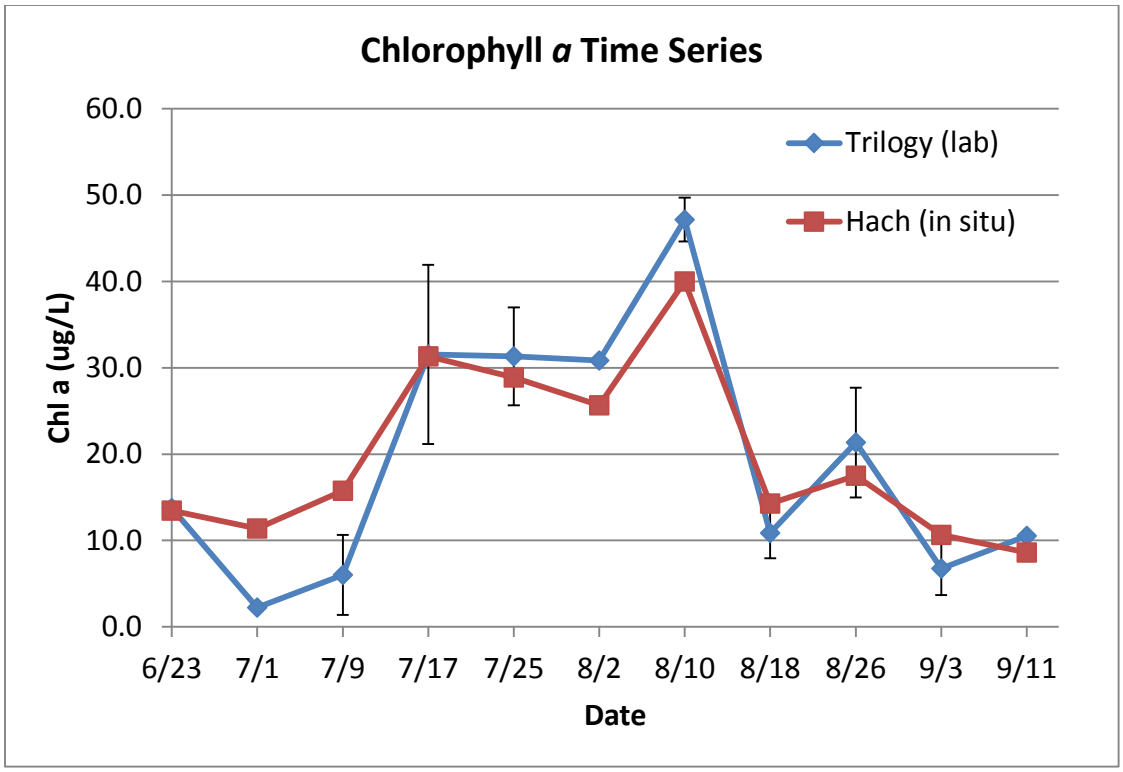

Figure 15. Direct chlorophyll a concentration measurements.

Direct chlorophyll *a* concentrations were calculated every eight days by a Trilogy laboratory fluorometer (blue) and a Hach hydrolab sensor (red). Error bars using standard error (n=2) have been plotted for laboratory data. Only surface samples have been recorded.

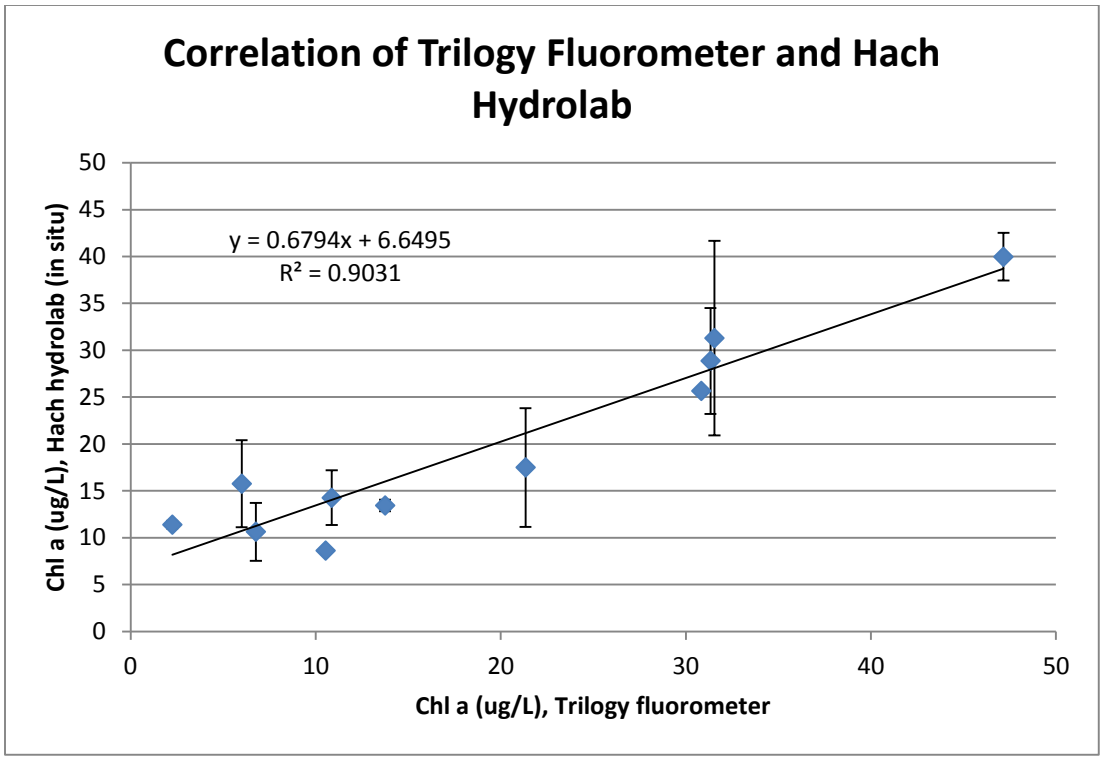

Figure 16. Trilogy lab fluorometer and Hach correlation.

Chlorophyll *a* was measured using both a Trilogy lab fluorometer and Hach hydrolab sensor and Trilogy fluorometer on eleven separate occasions. Data for the Trilogy lab have been averaged and error bars using standard error (n=2) are shown.

Comparing direct concentrations of chlorophyll *a* to reflectance data requires the use of one of the Wolfe indices. The Wolfe index of R700/675 (reflectance value at 700 nm divided by reflectance value at 675 nm) was used, as it gave the best correlation value: r^2=0.82 (Figure 17). These data were not averaged and all depths and days have been included to show the full extent of data. In addition, at least two of the other Wolfe indices showed strong correlations (R650/675 with r^2=0.77 and R675-750 with r^2=0.66).

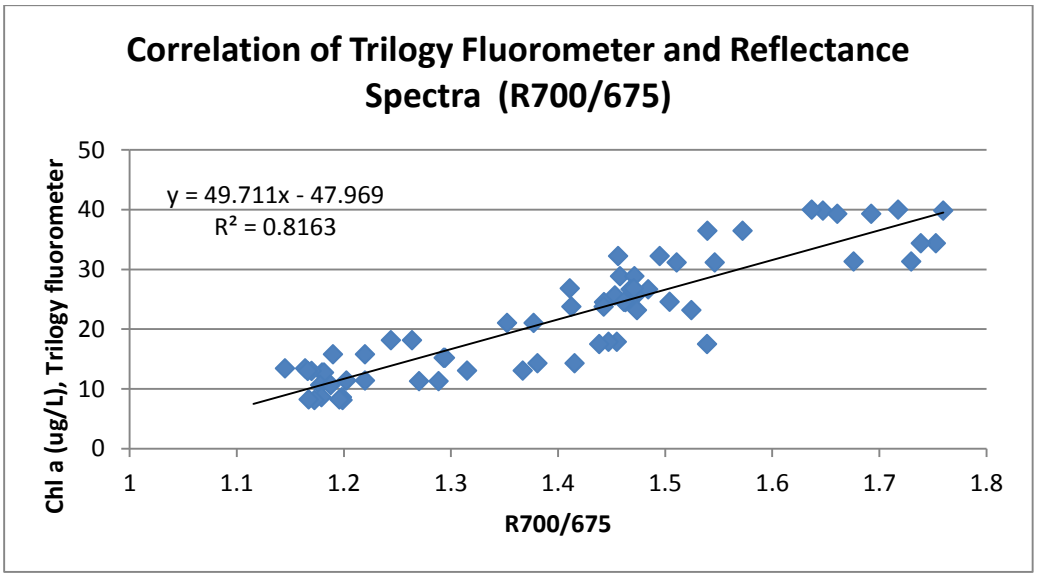

Figure 17. Hydrolab and reflectance spectra correlation.

Chlorophyll *a* concentrations were measured directly using a Hach hydrolab sensor. A reflectance spectra index was calculated by taking the value at 700 nm and dividing it by the value at 675 nm (R700/675) for each spectrum.

The Wolfe index of R700/675 was used again to compare Trilogy lab data to reflectance measurements. Using the full extent of data provides a correlation value of r^2=0.64 (Figure 18). The correlation value was found to be the same if only samples collected every eight days were considered.

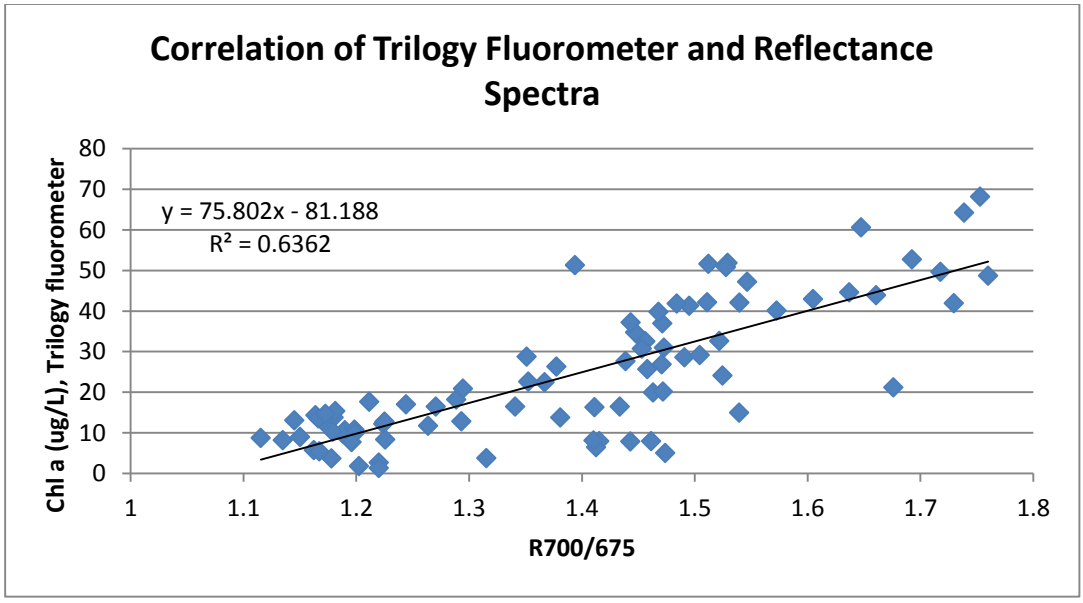

Figure 18. Fluorometer and reflectance spectra correlation.

Chlorophyll *a* concentrations were measured directly using a Trilogy fluorometer. A reflectance spectra index was calculated by taking the value at 700 nm and dividing it by the value at 675 nm (R700/675) for each spectrum.

Lastly, Wolfe indices can be converted to chlorophyll *a* estimations by using the trendline equation from Figure 18 and substituting in R700/675 values for "x". Doing so allows for a single graph to be made to compare all three methods (Figure 19). All three methods show a high degree of similarity.

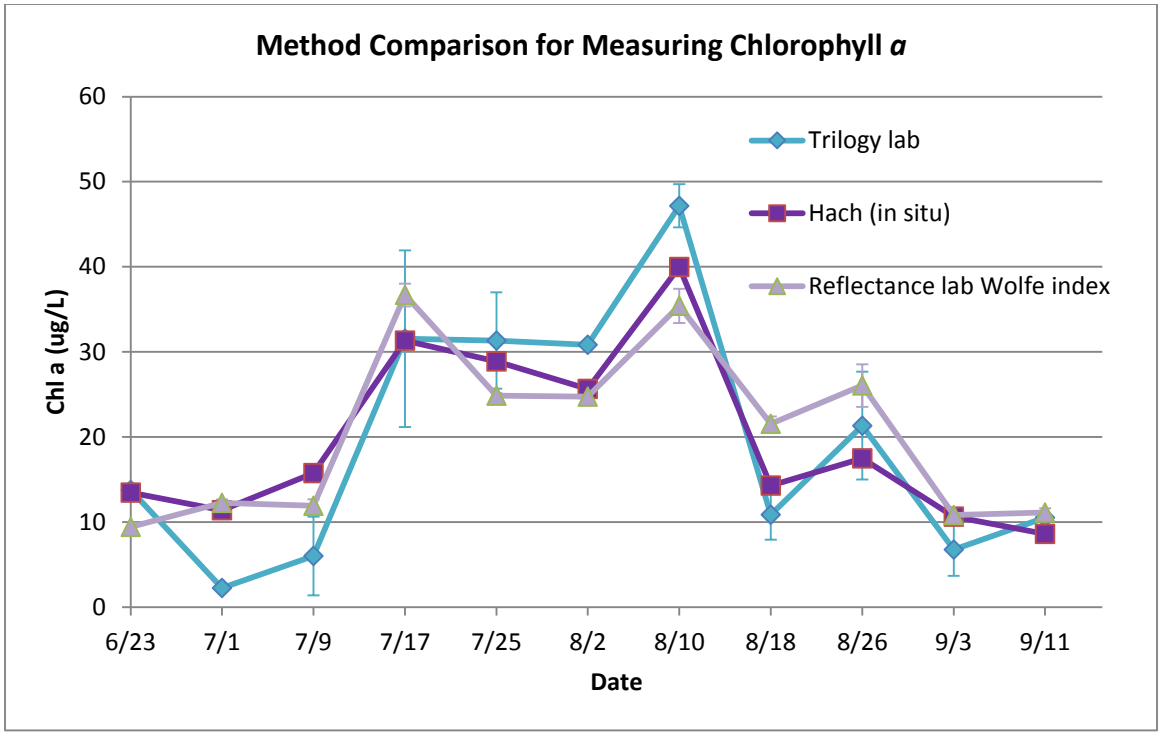

Figure 19. Comparison of all three methods of measuring chlorophyll a.

Chlorophyll *a* concentrations were measured directly using an *in-situ* Hach hydrolab sensor and a Trilogy lab fluorometer. The Wolfe indices calculated in Figure 18 were converted to chlorophyll *a* concentrations (ug/L) to allow them to be plotted on the same graph. Error bars have been included for the Trilogy lab and converted Wolfe indices (both n=2). Only surface samples are shown.

The results here demonstrate these various methods of plant pigment estimations (two direct and one indirect) are readily comparable. In Chapter 4.8, an additional way to compare direct concentrations of chlorophyll *a* to reflectance spectra has been identified using component scores from multivariate statistics. The procedure for these statistical methods will be explained in the next section.

4.5 Principal Component Analysis (PCA) Explained

The next sections use principal component analysis (PCA) as a means of analyzing and interpreting data. Before continuing, it is necessary to provide some background information on this particular multivariate procedure.

Morel and Prieur (1977) classified marine waters based on their optical properties. "Case I" waters are those in which chlorophyll is the dominant color-producing agent (CPA), while "Case II" waters are more complex and have other constituents (e.g. dissolved organic matter, suspended sediments) that are the dominant CPAs (Morel and Prieur, 1977). Another way to visualize this would be to consider Case I and Case II waters as end-members on a spectrum, where all natural waters (marine and freshwater) fall somewhere in between. Case II waters present a challenge to researchers because the optical properties of the water are governed by more than one component (Ortiz et al., 2013), and a technique must be utilized to separate out these components. This can be done by developing remote sensing algorithms specific for Case II waters, such as those by Witter et al. (2009), or by using a multivariate statistical technique, such as Principle Component Analysis (PCA). For this study, PCA was used. The sampling trips conducted

in this study, however, were purposely scheduled to coincide with Landsat 5/7 overpasses. Future researchers may consider comparing the hyperspectral readings assembled in this study to existing remote sensing data, which may provide useful information in the development of modified algorithms for assessing the optical properties of the Case II water at OWC.

Principal Component Analysis (PCA) is a multivariate technique that "involves the transformation of a number of possibly correlated variables into a smaller number of uncorrelated indices (eigenvectors)" (Ali, Witter, and Ortiz, 2012). Principle components (PCs) calculated from PCA are essentially eigenvectors of a variance-covariance (or correlation) matrix (Davis, 1986). PCs can be used to determine important information regarding variability in the data.

In this study, varimax-rotated PCA (VPCA) was performed on both sensor data (see Chapter 4.6) and reflectance spectra (see Chapter 4.8). VPCA, where the axes of the matrix are rotated and kept perpendicular to each other, is often used for this type of analysis because it "allows sufficient orthogonality between derived component axes to [spectrally distinguish…] several in-water constituents" (Ali, Witter, and Ortiz, 2012). When analyzing the results of PCA, the first PC explains the greatest amount of variance in the data (usually given as both an eigenvalue and percentage), the second PC explains the next greatest amount of variance, and so on. As a general rule of thumb, PCs with eigenvalues less than 1 are often disregarded (Ali, Witter, and Ortiz, 2012), but may be included if they can be shown to provide valuable information (as was the case for the limnological parameter set discussed in Chapter 4.5).

All PCA techniques were performed using the Statistical Program for Social Sciences (SPSS®).

4.6 Analysis of Water Parameters

As explained in Chapter 3.3, sensor data were collected from the field site used in this study (see Figure 3). Sensor data consisted of both a monitoring sonde (updated every 15 minutes) and an *in situ* Hach hydrolab multiparameter sonde (with measurements made every eight days). As was shown in Figure 5, strong correlations (r^2 = 0.83 or better) between these sondes were found for all common parameters (temperature, specific conductivity, pH, and turbidity) except dissolved oxygen. (In the case of dissolved oxygen, the membrane on the Hach hydrolab DO sensor was faulty at the time of collection.)

The following six time series (Figure 20 through Figure 25) were made using monitoring sonde data only. Sensor data were collected at 15 minute intervals, beginning midnight on the first day of field work (June 7, 2011) and ending at 11:59 PM on the last day of collection (September 11, 2011). Data were averaged hourly (blue line in each graph) and given a 12 hour, low pass filter (red line in each graph) to illustrate trends more clearly.

The most interesting thing to note is this is a dynamic ecosystem, with a mouth bar that is continually opening and closing (as was explained in Chapter 2.1 and can be seen in Appendix C). For this study, the mouth bar was open when sampling first began (June 7, 2011) and started to close in July. The official "closing" date was July 21, 2011,

which was halfway between sampling days. After that, the mouth bar remained closed throughout August and September. Essentially, this means free flow was allowed through the system during June and halfway through July and then the flow was stopped for the rest of the study period. As such, many, if not all, of the limnological parameters show changes in their variability before and after July 21, 2011.

Water temperature (Figure 20), for example, shows a steady increase until the official close date of the mouth bar; after this, it begins a steady decrease. This is not altogether unusual, however, as the water temperature in Old Woman Creek often reaches its maximum in late July (Herdendorf, Klarer, and Herdendorf, 2006).

Specific conductivity (Figure 21) shows a dramatic change when the mouth bar closed. Prior to closure, specific conductivity showed a high amount of variability; afterwards, however, it showed a slow and steady increase with only an occasion drop that quickly returned to normal values. Depth (Figure 22) shows a similar trend. These values were influenced by precipitation, with steady (but increasing) values after the mouth bar had closed, when the system was not capable of flushing itself out. Conversely, dissolved oxygen (Figure 25) and pH (Figure 23) show high variability both before and after the mouth bar closed, but the average values are much lower in both cases after the mouth bar has closed. This was impacted by changes in the growth and populations of phytoplankton due to the dynamic shift in the ecosystem. Turbidity (Figure 24) is the only aquatic parameter that does not seem to be influenced by the status of the mouth bar.

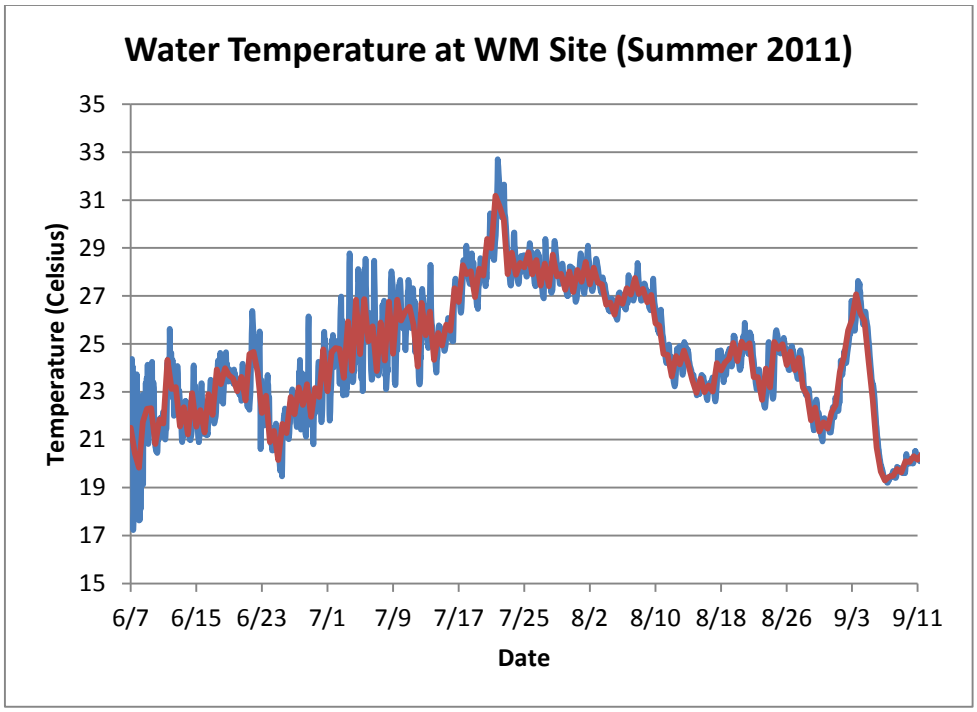

Figure 20. Water temperature time series, from monitoring sensor data.

This graph shows changes in water temperature across the duration of the study period. Data has been averaged hourly (blue line) and given a 12 hour, low pass filter (red line). The mouth bar was open during the beginning of the study (June 7) and remained open until July 21. The mouth bar remained closed throughout the rest of the study.

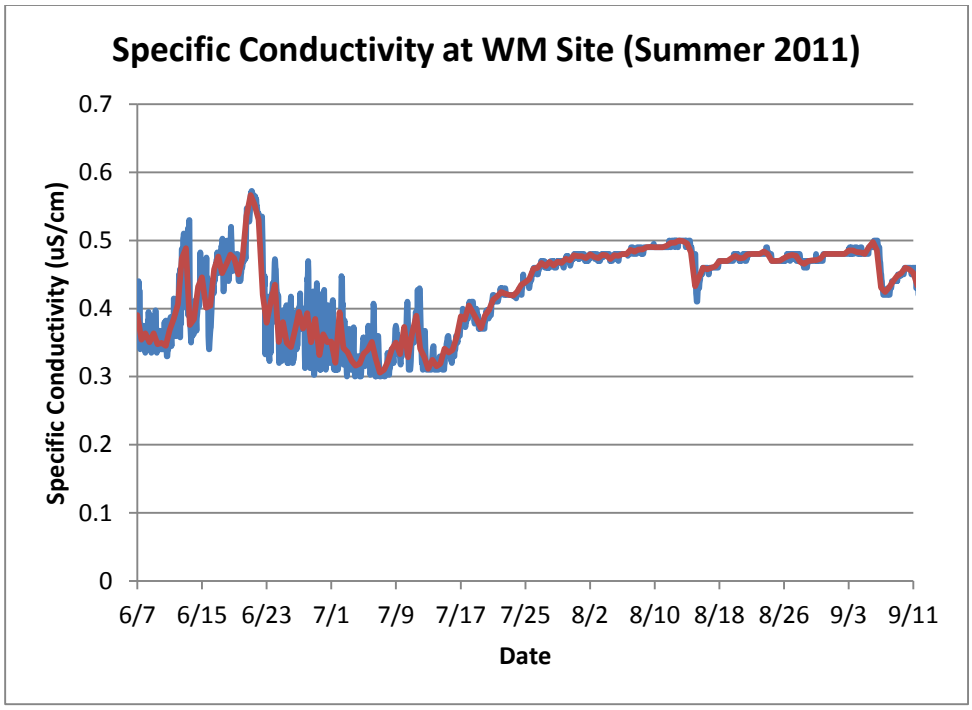

Figure 21. Specific conductivity time series, from monitoring sensor data.

This graph shows changes in specific conductivity across the duration of the study period. Data has been averaged hourly (blue line) and given a 12 hour, low pass filter (red line). The mouth bar was open during the beginning of the study (June 7) and remained open until July 21. The mouth bar remained closed throughout the rest of the study.

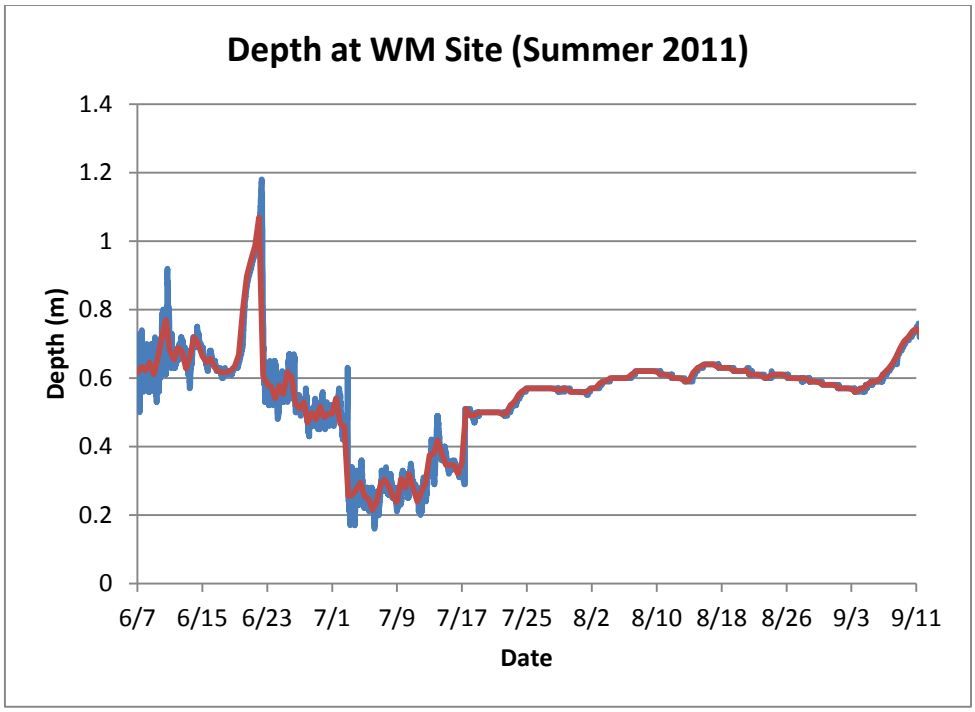

Figure 22. Depth time series, from monitoring sensor data.

This graph shows changes in depth across the duration of the study period. Data has been averaged hourly (blue line) and given a 12 hour, low pass filter (red line). The mouth bar was open during the beginning of the study (June 7) and remained open until July 21. The mouth bar remained closed throughout the rest of the study. (NOTE: the depth axis is inverted. Higher values = deeper water.)

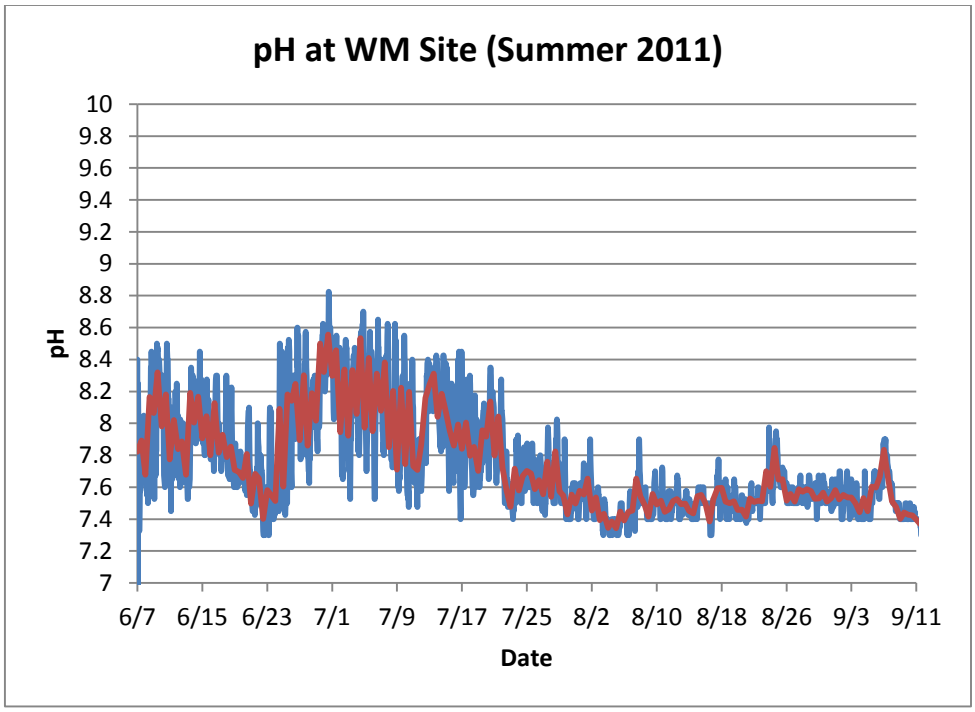

Figure 23. pH time series, from monitoring sensor data.

This graph shows changes in pH across the duration of the study period. Data has been averaged hourly (blue line) and given a 12 hour, low pass filter (red line). The mouth bar was open during the beginning of the study (June 7) and remained open until July 21. The mouth bar remained closed throughout the rest of the study.

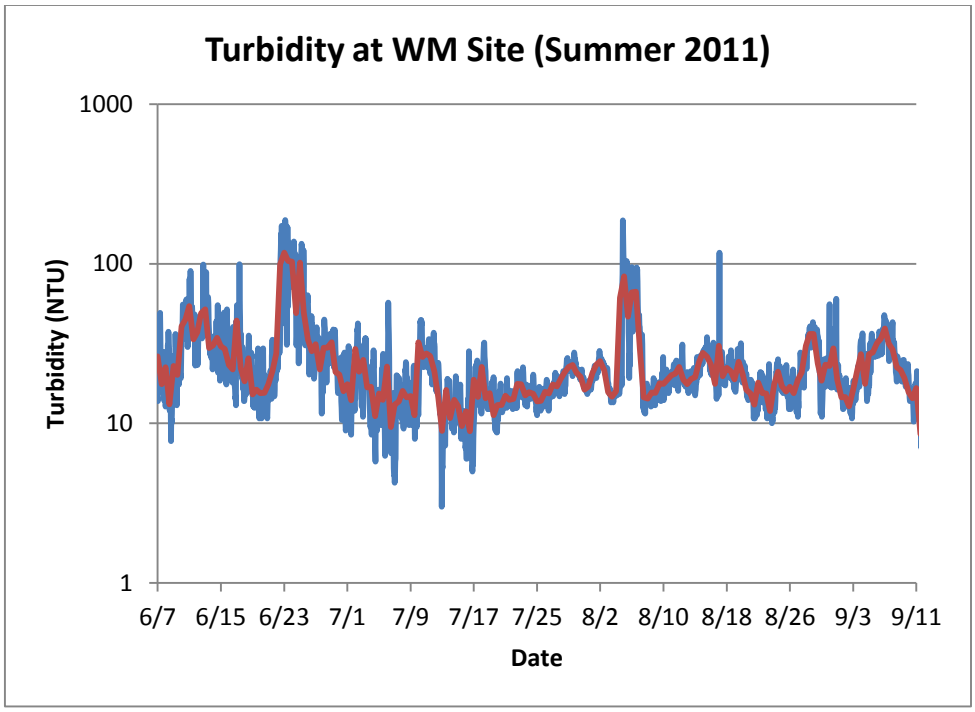

Figure 24. Turbidity time series, from monitoring sensor data.

This graph shows changes in turbidity across the duration of the study period. Data has been averaged hourly (blue line) and given a 12 hour, low pass filter (red line). The mouth bar was open during the beginning of the study (June 7) and remained open until July 21. The mouth bar remained closed throughout the rest of the study.

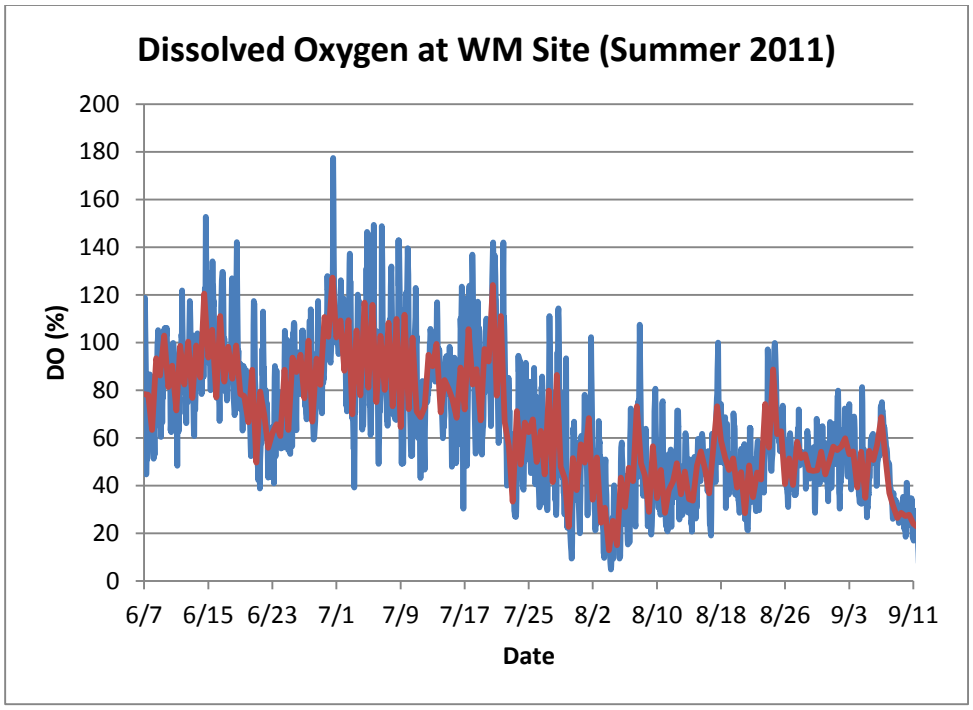

Figure 25. Dissolved oxygen time series, from monitoring sensor data.

This graph shows changes in dissolved oxygen across the duration of the study period. Data has been averaged hourly (blue line) and given a 12 hour, low pass filter (red line). The mouth bar was open during the beginning of the study (June 7) and remained open until July 21. The mouth bar remained closed throughout the rest of the study.

To examine this further, varimax-rotated PCA (VCPA) was performed on the six limnological parameters (temperature, specific conductivity, depth, pH, turbidity, and DO). The result produced four components (see Table 1). Only two of these components had an eigenvalue >1, but it was decided to include the other two, as these four components combined explain over 95% of the data.

Table 1. VPCA Results for Six Aquatic Parameters

Total Variance Explained				
Component	Initial Eigenvalues	Rotation Sums of Squared Loadings	% of Variance	Cumulative %
1	2.85	2.15	35.85	35.85
2	1.32	1.50	25.01	60.86
3	0.90	1.09	18.19	79.05
4	0.69	1.01	16.83	95.87

Component 1 explains 35.85% of the variance (Table 1) and is strongly influenced by pH and DO, with an inverse relationship with specific conductivity (Table 2). This is related to influence by biological activity. During photosynthesis, algae remove carbon dioxide from the system and increase the pH of the water. Photosynthesis also results in an increase in DO in the water. The inverse relationship with specific conductivity is a little more puzzling. It might be explained, however, by nutrients being removed from the system during intense periods of algal growth, higher production in fresher water due to phytoplankton preference, or the correlation of fresher water with

increased runoff and greater nutrient supply. The variability of Component 1 through time (Figure 26) is similar to what was observed with pH (Figure 23) and DO (Figure 26), with steady values before and after the mouth bar closed but notably lower values after the closure.

Component 2 explains 25.01% of the variance (Table 1) and is strongly influenced by specific conductivity and depth (Table 3 and Figure 27). This is related to runoff, as the input of nutrients and water into the system would cause these values to increase. (In the next section, we will compare these results to meteorological data.)

Component 3 explains 18.19% of the variance (Table 1) and is strongly influenced by temperature and evaporative forces (Table 4). Values are highest during the peak of the summer and the closing of the mouth bar (Figure 28).

Component 4 explains 16.83% of the variance (Table 5) and is strongly influenced by turbidity (Table 5 and Figure 29), which is related to precipitation and runoff. As such, in the next section, we will compare each of these components to meteorological data.

Table 2. VPCA Results for Component 1

Parameter	Component 1 values
Temperature	0.01
Specific Conductivity	-0.57
Depth	-0.14
pH	0.93
Turbidity	-0.06
DO	0.97

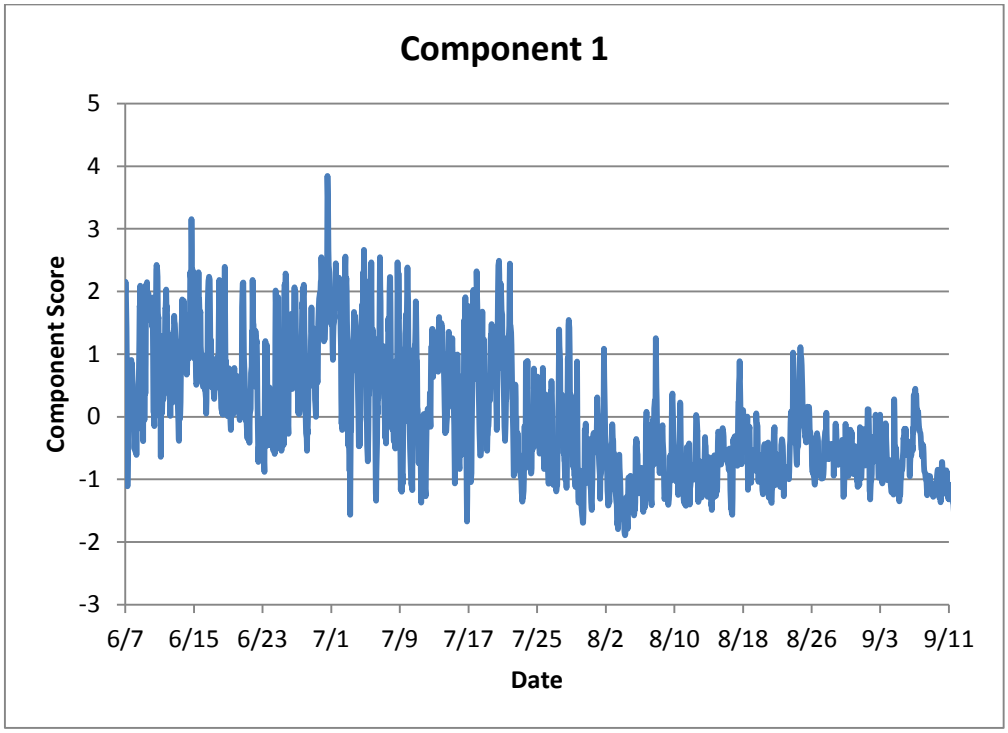

Figure 26. VPCA results for component 1 (time series).

Table 3. VPCA Results for Component 2

Parameter	Component 2 values
Temperature	-0.11
Specific Conductivity	0.74
Depth	0.92
pH	-0.29
Turbidity	0.04
DO	-0.11

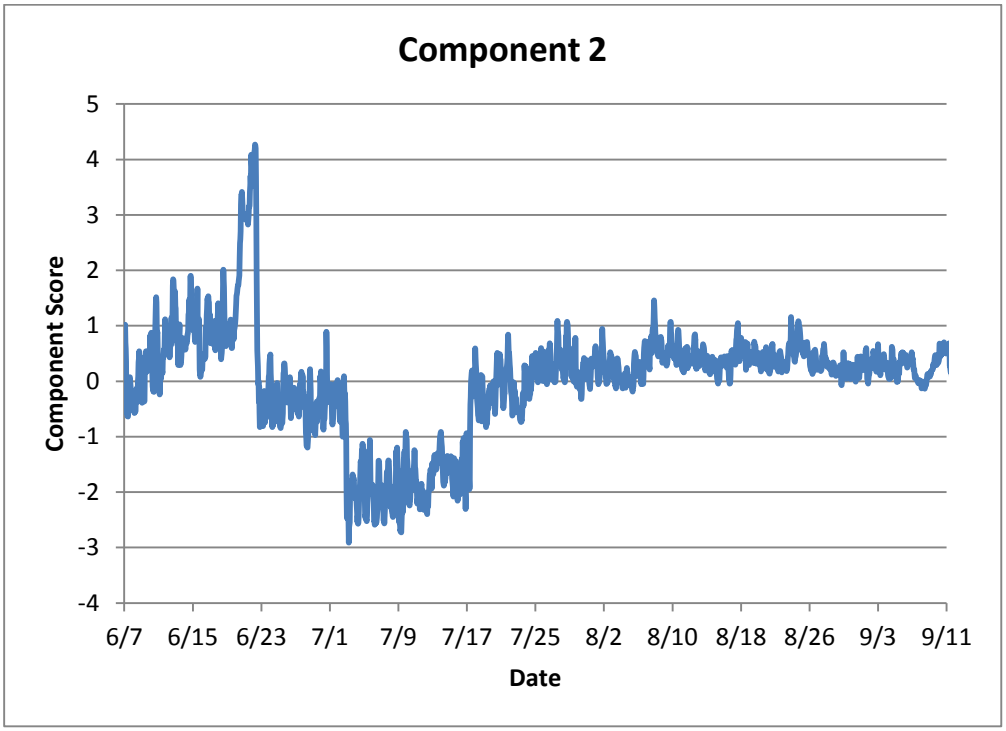

Figure 27. VPCA results for component 2 (time series).

Table 4. VPCA Results for Component 3

Parameter	Component 3 values
Temperature	0.97
Specific Conductivity	0.21
Depth	-0.27
pH	-0.05
Turbidity	-0.12
DO	0.09

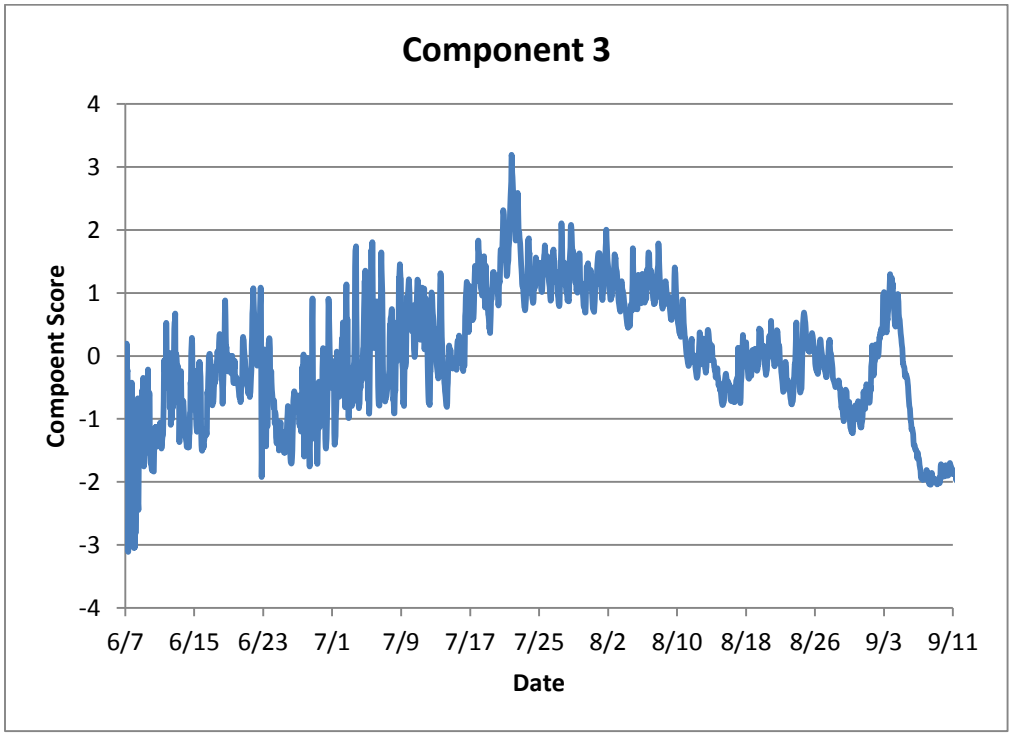

Figure 28. VPCA results for component 3 (time series).

Table 5. VPCA Results for Component 4

Parameter	Component 4 values
Temperature	-0.13
Specific Conductivity	0.01
Depth	0.05
pH	-0.11
Turbidity	0.99
DO	-0.00

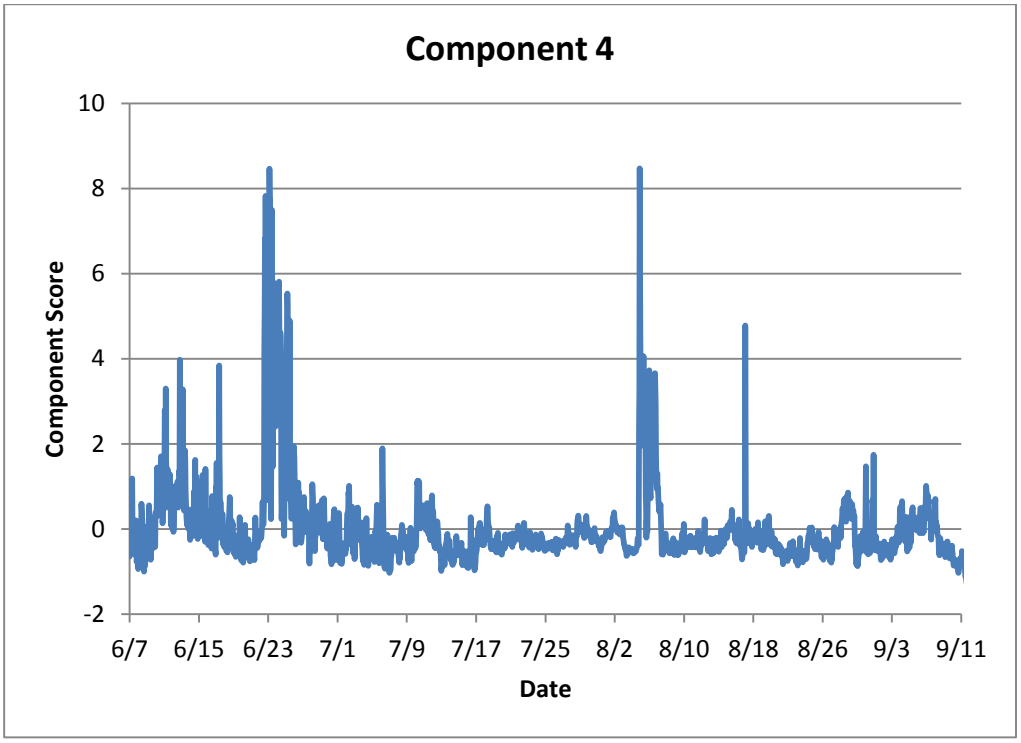

Figure 29. VPCA results for component 4 (time series).

4.7 Analysis of Meteorological Data

In addition to monitoring sondes, meteorological data were collected at Old Woman Creek from a weather station not far (within a mile) from the field site used in this study. As with the monitoring sonde, weather data were collected every 15 minutes. In the case of temperature, these data were averaged hourly and given a 12 hour, low pass filter (blue and red lines, respectively; see Figure 30). Precipitation data were also collected (see Figure 31).

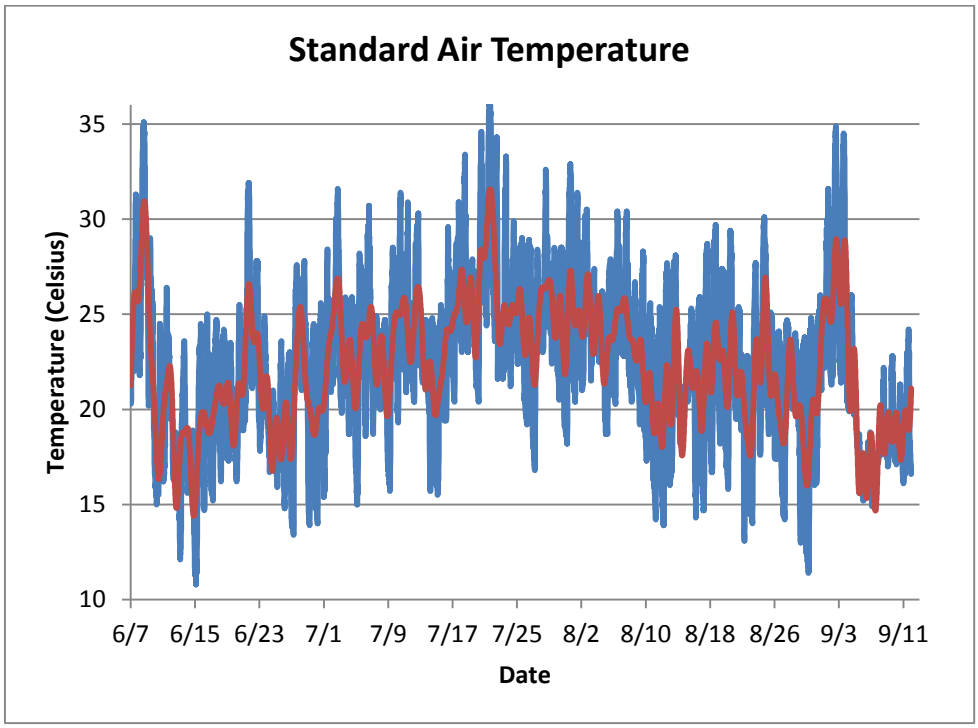

Figure 30. Air temperature at OWC field site.

Data has been averaged hourly (blue line) and given a 12 hour, low pass filter (red line).

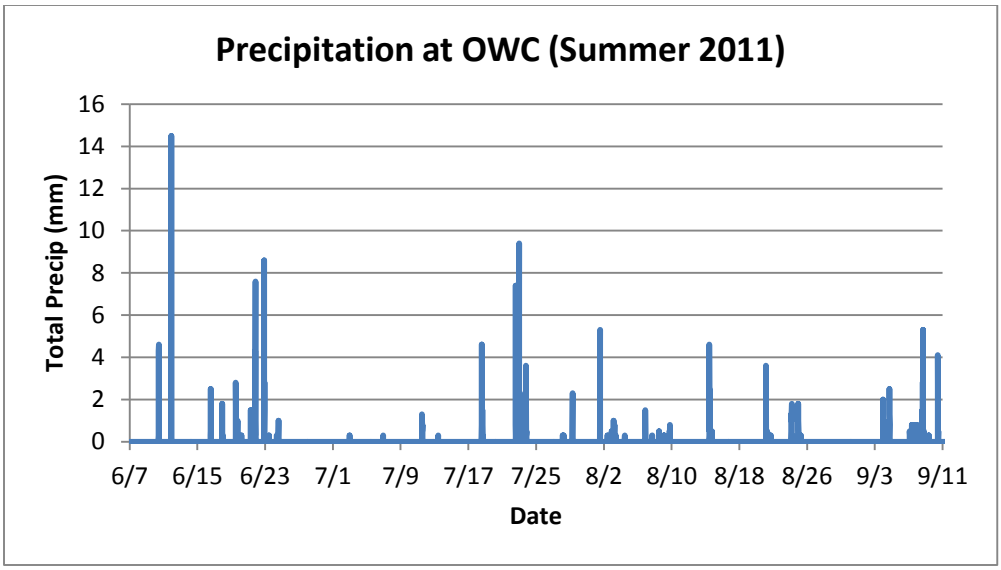

Figure 31. Precipitation values at OWC field site.

The standard air temperature pattern shows both a diurnal and seasonal frequency, which looks very similar to the VPCA results for Component 3 (Figure 28) in the previous section. In fact, if we overlay these plots (see Figure 32), we can see they are almost identical. There is also reason to suspect Components 2 and 4 are closely related to storm events (as was explained in Chapter 4.5). As such, each of the component time series was matched up against precipitation values. No correlation was found for Component 1 (Figure 33), which makes sense, as Component 1 was strongly influenced by biological activity and the closing of the mouth bar. Component 2, however, shows a strong correlation to precipitation data (Figure 34), although the system appears to stabilize once the mouth bar closed off (around July 21). This suggests Component 2 is more closely related to storm events when the mouth bar is open and is unrelated when

flow to Lake Erie is blocked. Component 3 has already been shown to be related to temperature (Figure 32); as such, no apparent correlation to precipitation seems to exist (Figure 35). Component 4, however, does seem to be influenced by precipitation (Figure 36), which further suggests it is most related to turbidity.

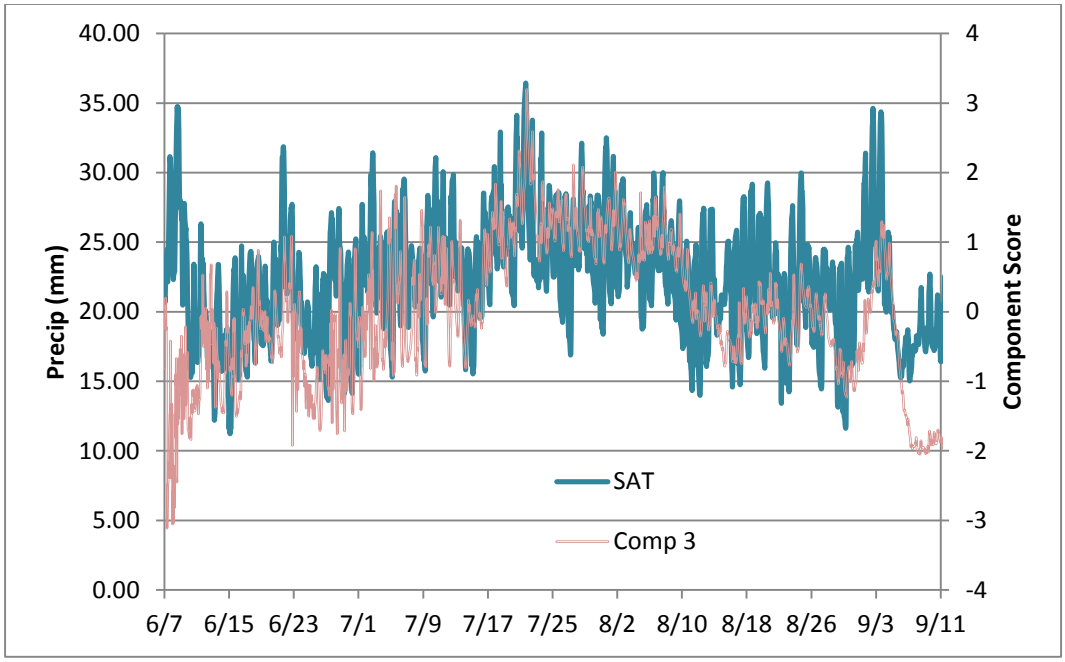

Figure 32. Component 3 (water parameter VPCA) and air temperature.

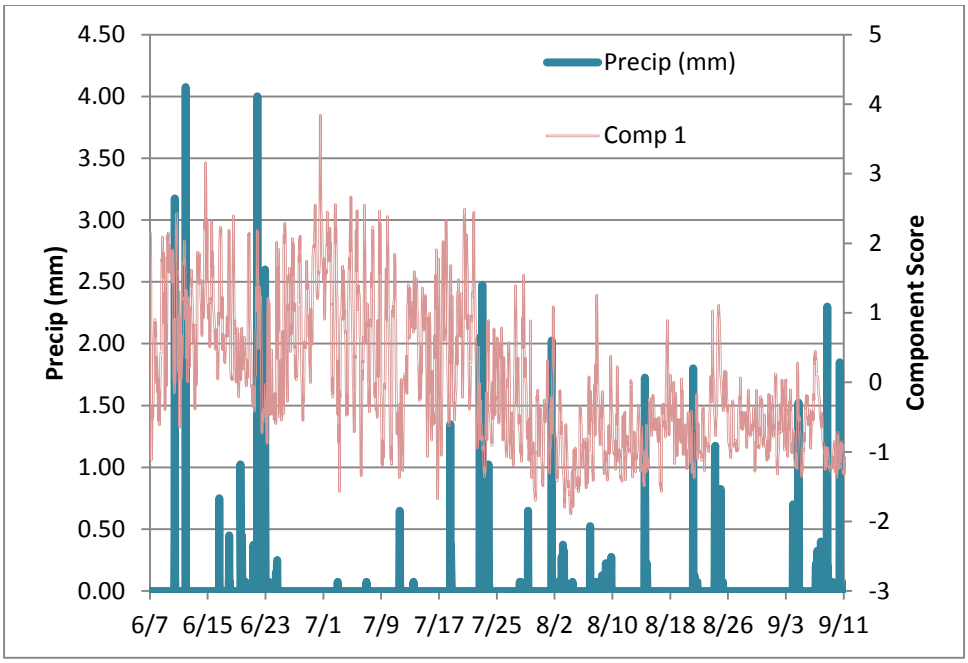

Figure 33. Component 1 (water parameter VPCA) and precipitation.

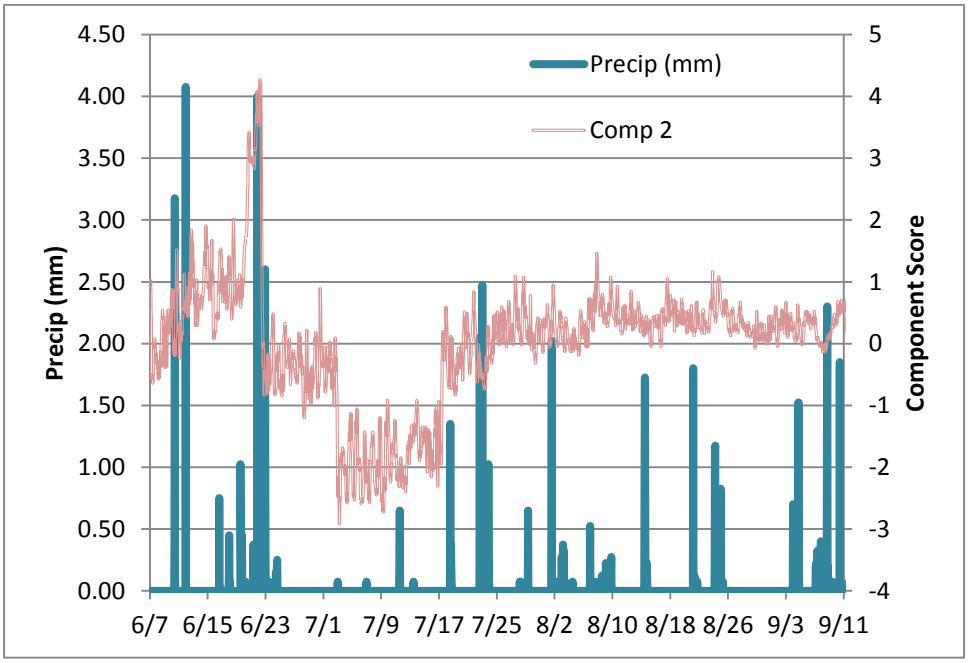

Figure 34. Component 2 (water parameter VPCA) and precipitation.

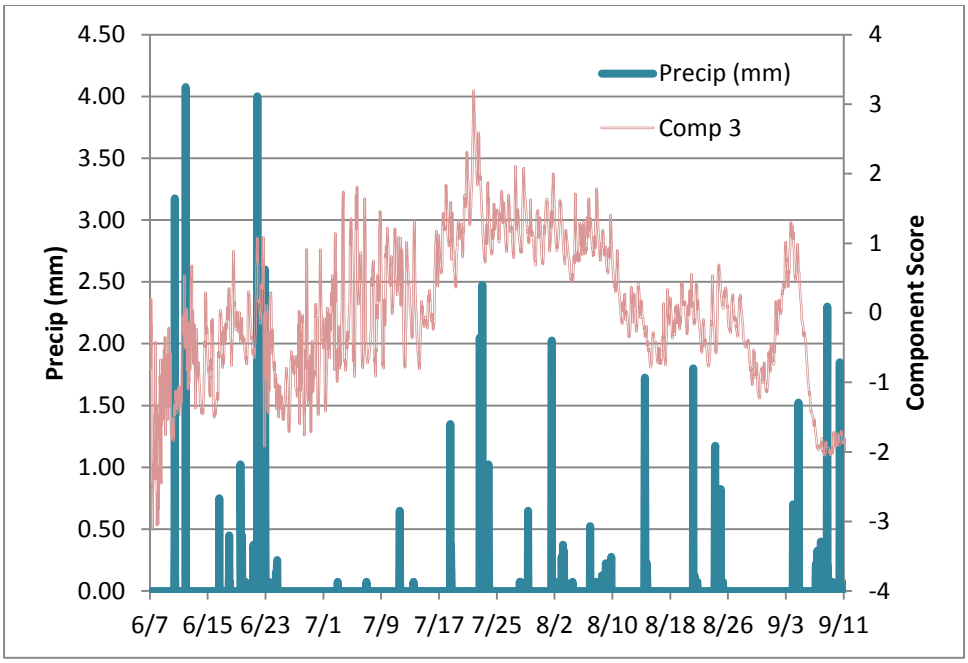

Figure 35. Component 3 (water parameter VPCA) and precipitation.

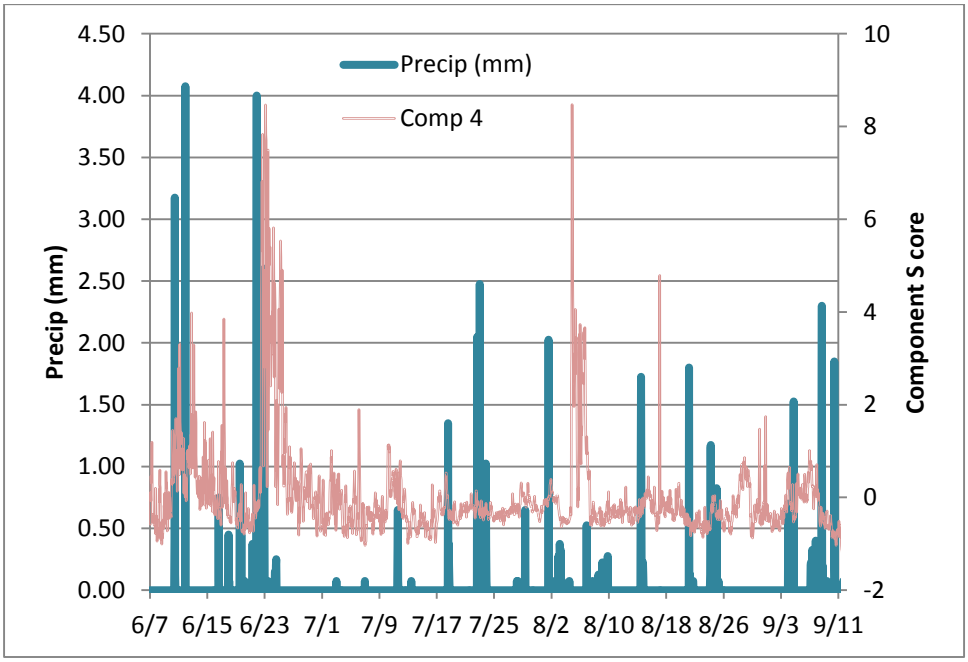

Figure 36. Component 4 (water parameter VPCA) and precipitation.

4.8 Identifying Plant Pigments Using PCA

Varimax-rotated principal component analysis (VPCA) was performed on reflectance spectra. Before conducting PCA, data were first blank-corrected, offset adjusted, and band-averaged to a width of 10 nm, as described in Chapter 3.6. The first-derivative of the reflectance spectra was calculated to minimize background noise (Ali, Witter, and Ortiz, 2012). Next, the first-derivative reflectance spectra were transformed, where *m* rows were comprised of time and *n* columns were comprised of spectral bands (in 10 nm increments). Only the visible part of the spectrum (400-700 nm) was considered. This allowed for a total of 31 bands to be analyzed.

Samples from the Surface Time Series and Depth Time Series (see Chapter 4.1) were combined into a single data matrix. Samples from the Surface Time Series occurred at a frequency of every four days (two per day, or n=2) from June 23 to September 11, 2011. Samples from the Depth Time Series occurred at a frequency of every eight days (six per day, or n=2 at three depths) from June 23 to September 11, 2011. A total of 86 samples over the three-month study were analyzed. Descriptive statistics, including the mean values and standard deviations for each of the 31 bands, have been included in Table 6. Four components with eigenvalues greater than 1 were extracted from the dataset, and these components explain 95.1% of the variability in the data (Table 7). (A PCA run that excluded the samples collected by David Klarer at an eight-day frequency produced almost identical components, with four similar components explaining approximately 95.6% of the optical variability in the dataset. There was concern these

samples might have been compromised due to remaining unfiltered for four days, but no problems seemed to occur during the PCA analysis.)

These components can now be matched up to existing pigment and mineral reflectance spectra to determine the most important in-water constituents governing the optical variability at one specific site at Old Woman Creek during the period of sampling. A library of pigment spectra was created using Moberg et al., 2002; Toepel et al., 2005; and Chazottes et al., 2006. Before using the library, however, it was decided to subset the data to see if one or more components were more important during specific times of the study period. This can happen due to changes in phytoplankton populations, which can be influenced by a number of different factors. Because the opening and closing of the mouth bar is an important physical process that governs much of the variability in Old Woman Creek, it was decided to perform a PCA analysis using only samples when the mouth bar was *open* (June 23 – July 17, 2011) and an additional PCA when the mouth bar was *closed* (July 21 – September 11, 2011). This allowed for an analysis of the estuary under two very different conditions—both physically and ecologically.

Table 6. Descriptive Statistics for Full Time Series

June 23 – Sept 11, 2011

	Mean	Std. Deviation	N
drdl400	0.019	0.041	86
drdl410	0.042	0.034	86
drdl420	0.076	0.037	86
drdl430	0.105	0.042	86
drdl440	0.146	0.048	86
drdl450	0.150	0.041	86
drdl460	0.133	0.035	86
drdl470	0.128	0.033	86
drdl480	0.133	0.033	86
drdl490	0.166	0.039	86
drdl500	0.214	0.042	86
drdl510	0.233	0.042	86
drdl520	0.214	0.032	86
drdl530	0.188	0.022	86
drdl540	0.177	0.019	86
drdl550	0.159	0.017	86
drdl560	0.117	0.018	86
drdl570	0.074	0.023	86
drdl580	0.066	0.021	86
drdl590	0.064	0.014	86
drdl600	0.021	0.022	86
drdl610	-0.008	0.031	86
drdl620	0.028	0.027	86
drdl630	0.068	0.016	86
drdl640	0.009	0.027	86
drdl650	-0.194	0.084	86
drdl660	-0.261	0.096	86
drdl670	0.005	0.088	86
drdl680	0.459	0.144	86
drdl690	0.658	0.198	86
drdl700	0.459	0.178	86

Table 7. VPCA Results for Full Time Series

June 23 – Sept 11, 2011

	Initial Eigenvalues			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	12.026	38.792	38.792	10.025	32.340	32.340
2	11.147	35.958	74.751	7.136	23.019	55.359
3	3.764	12.142	86.893	7.012	22.618	77.977
4	2.533	8.171	95.064	5.297	17.087	95.064
5	.460	1.483	96.547			
6	.312	1.006	97.553			
7	.230	.742	98.295			
8	.140	.452	98.747			
9	.128	.414	99.161			
10	.071	.230	99.391			
11	.042	.134	99.525			
12	.035	.114	99.639			
13	.025	.082	99.721			
14	.018	.057	99.778			
15	.015	.050	99.828			
16	.011	.036	99.863			
17	.009	.030	99.894			
18	.008	.026	99.920			
19	.006	.019	99.938			
20	.004	.014	99.952			
21	.004	.012	99.964			
22	.002	.008	99.972			
23	.002	.007	99.979			
24	.002	.006	99.984			
25	.001	.005	99.989			
26	.001	.003	99.992			
27	.001	.003	99.996			
28	.001	.002	99.998			
29	.000	.001	99.999			
30	.000	.001	100.000			
31	.000	.000	100.000			

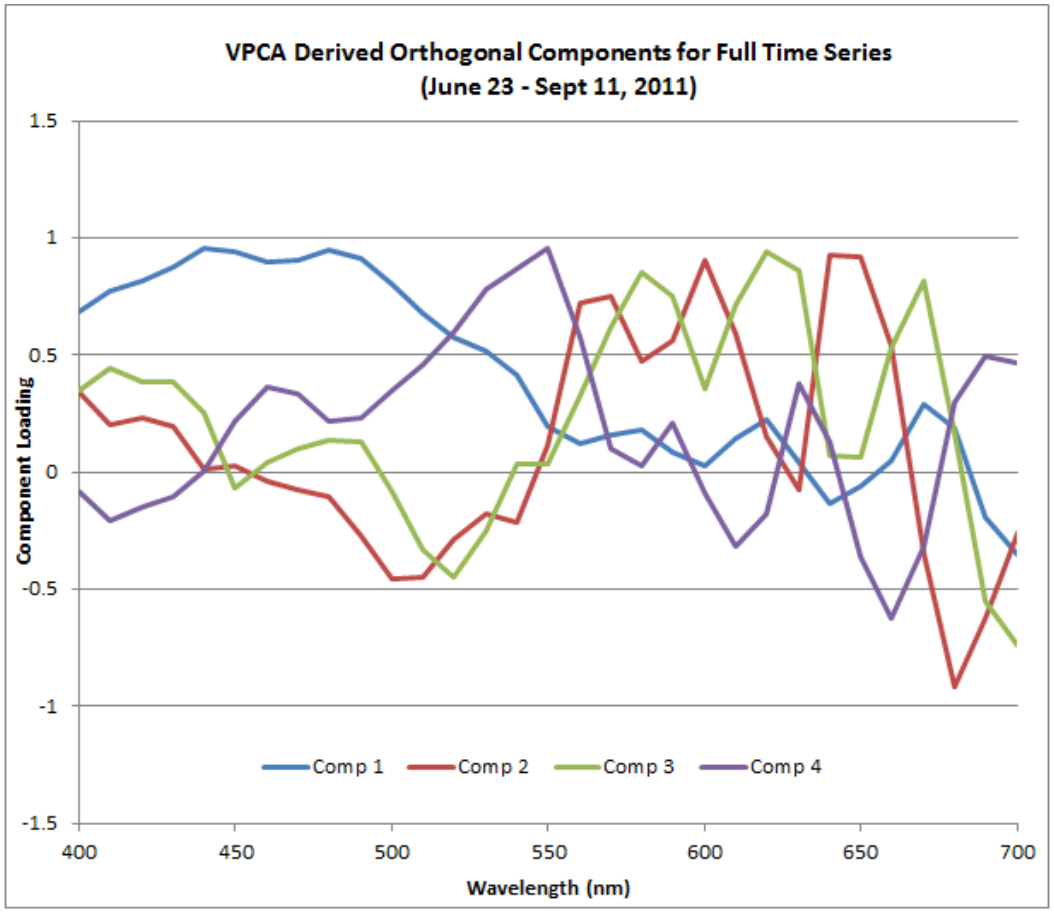

Figure 37. VPCA results for full time series.

Varimax-rotated principal component analysis on the full range of spectral data produced four components that account for approximately 95% of the optical variability in the OWC dataset.

PCA was performed on all samples collected before the closing of the mouth bar on July 21, 2011. Descriptive statistics for all 24 reflectance spectra have been provided in Table 8. Three components with eigenvalues greater than 1 were extracted, with a total variance explained of 97.8% (Table 9). Each of these components was compared to known pigments and minerals in the spectral library and the best matches were assigned to each component (Figure 39). The first component explains almost 64% of the optical variability during the closed mouth bar season and is heavily influenced by the red and green region of the spectrum (560-670 nm). The first component has a strong correlation (r^2=0.81) and appears to correspond to a mixture of two phytoplankton groups: chlorophyceae and bacillariophyceae. Both of these are important algal groups in OWC, as a past study by Klarer and Millie (1994) found ranges of bacillariophytes between 22-99% and chlorophytes between 1-32%. The second component explains almost 29% of the optical variability and is heavily influenced by the blue and green region of the spectrum (420-500 nm). No good correlations were found with known plant pigment spectra; strong correlations, however, were found with clay minerals. The second component has been identified as corresponding to a 50/50 mixture of chlorite and muscovite (r^2=0.86), although good correlations from illite and amphibole have also been identified. The third component explains an additional 5% of the optical variability of the estuary during the early summer and is heavily influenced in the green region of the spectrum (500-550 nm) with additional peaks in the red region. The third component corresponds to a mixture of two phytoplankton groups: cyanobacteria and fucoxanthin (r^2=0.64).

Of these three components, the first component should be the most easily comparable to direct concentrations of chlorophyll *a* (which is Objective #3 and was discussed in detail in Chapter 4.4). Figure 40 illustrates how the component scores of the first component can be compared to direct concentration measurements (in this case, sensor data from the Hach hydrolab) to produce a good correlation (r^2=0.65). Much weaker correlations are found if the component scores from the second component or third component are used (r^2=0.21 and r^2=0.05, respectively). Unfortunately, there was not a good correlation when comparing the component scores from the first component to fluorometer data. This could perhaps be due to the small sample set during this time period. If the first data point is excluded (June 23), however, the remaining three points give a correlation of r^2=0.92. Because there were not many full sample dates before the mouth bar closed (only four), we will have to rely on closed mouth bar data to draw correlations using component scores. There were seven full sampling trips during that time period.

Table 8. Descriptive Statistics for Open Mouth Bar

June 23 – July 21, 2011

	Mean	Std. Deviation	N
drdl400	0.017	0.033	24
drdl410	0.040	0.028	24
drdl420	0.074	0.030	24
drdl430	0.101	0.042	24
drdl440	0.143	0.058	24
drdl450	0.149	0.061	24
drdl460	0.122	0.052	24
drdl470	0.117	0.046	24
drdl480	0.127	0.041	24
drdl490	0.160	0.048	24
drdl500	0.210	0.062	24
drdl510	0.232	0.068	24
drdl520	0.213	0.053	24
drdl530	0.181	0.033	24
drdl540	0.164	0.024	24
drdl550	0.146	0.017	24
drdl560	0.105	0.014	24
drdl570	0.062	0.026	24
drdl580	0.051	0.025	24
drdl590	0.053	0.017	24
drdl600	0.019	0.026	24
drdl610	-0.015	0.038	24
drdl620	0.011	0.025	24
drdl630	0.050	0.009	24
drdl640	0.008	0.027	24
drdl650	-0.157	0.089	24
drdl660	-0.240	0.121	24
drdl670	-0.031	0.042	24
drdl680	0.385	0.130	24
drdl690	0.632	0.257	24
drdl700	0.473	0.204	24

Table 9. VPCA Results for Open Mouth Bar

June 23 – July 21, 2011

Component	Initial Eigenvalues			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	19.822	63.940	63.940	13.921	44.905	44.905
2	8.959	28.900	92.841	9.340	30.128	75.033
3	1.536	4.954	97.795	7.056	22.762	97.795
4	.297	0.958	98.752			
5	.139	.447	99.199			
6	.131	.421	99.621			
7	.055	.177	99.797			
8	.022	.072	99.869			
9	.019	.060	99.929			
10	.007	.022	99.951			
11	.004	.014	99.965			
12	.003	.011	99.976			
13	.002	.007	99.982			
14	.002	.005	99.987			
15	.001	.005	99.992			
16	.001	.002	99.994			
17	.001	.002	99.996			
18	.000	.001	99.998			
19	.000	.001	99.999			
20	.000	.001	99.999			
21	.000	.001	100.000			

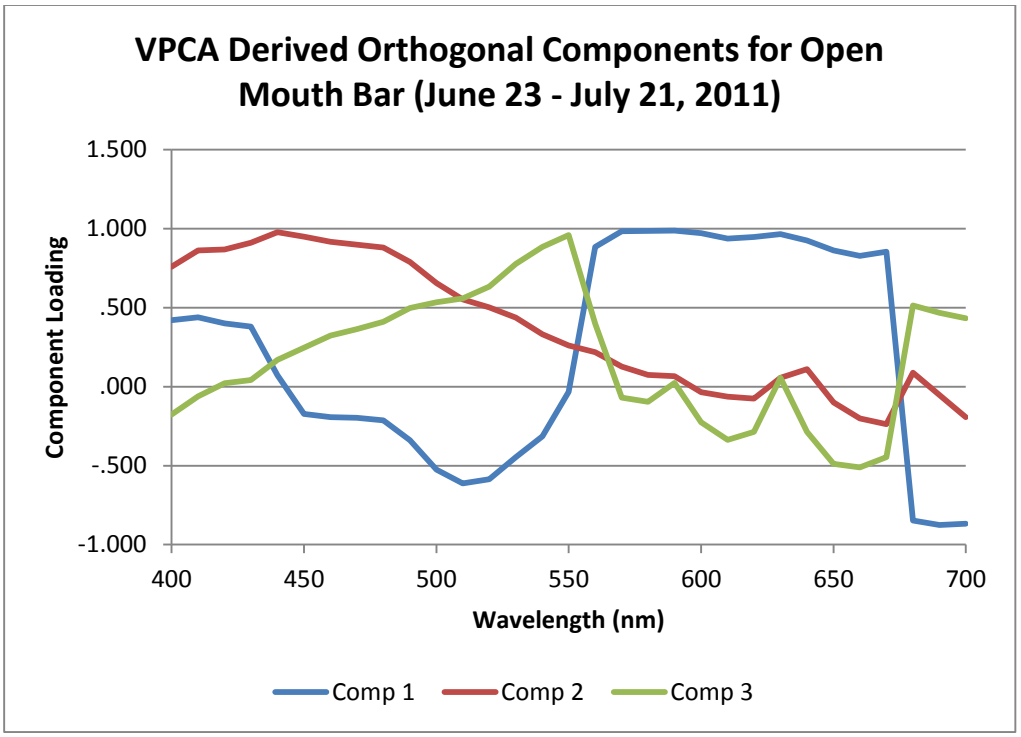

Figure 38. VPCA results for open mouth bar.

Varimax-rotated principal component analysis on spectral data produced three components that account for approximately 98% of the optical variability in the OWC dataset during the period when the mouth bar was open.

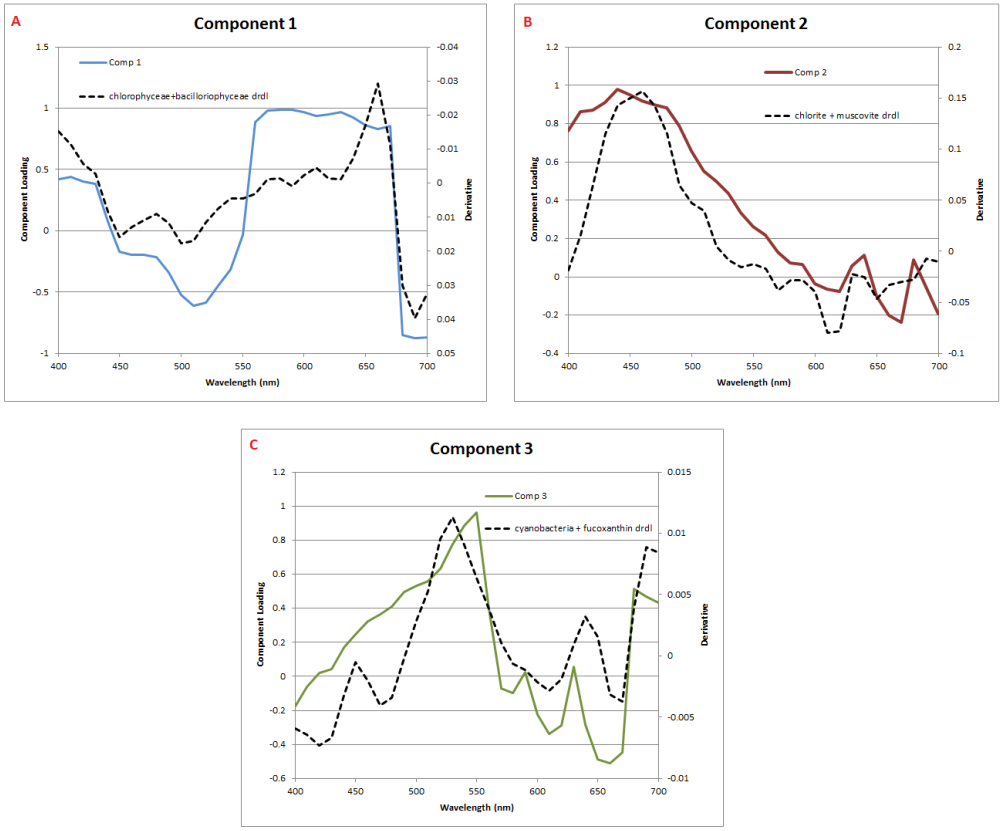

Figure 39. PCA component loadings reference derivative spectra.

Component loadings for PCA are compared to selected reference derivative spectra during the open mouth bar season (June 23 – July 21, 2011). The first component (A) relates to chlorophyceae and bacillariophyceae; the second component (B) relates to a mixture of clay minerals (e.g. chlorite and muscovite); the third component (C) relates to cyanobacteria and fucoxanthin.

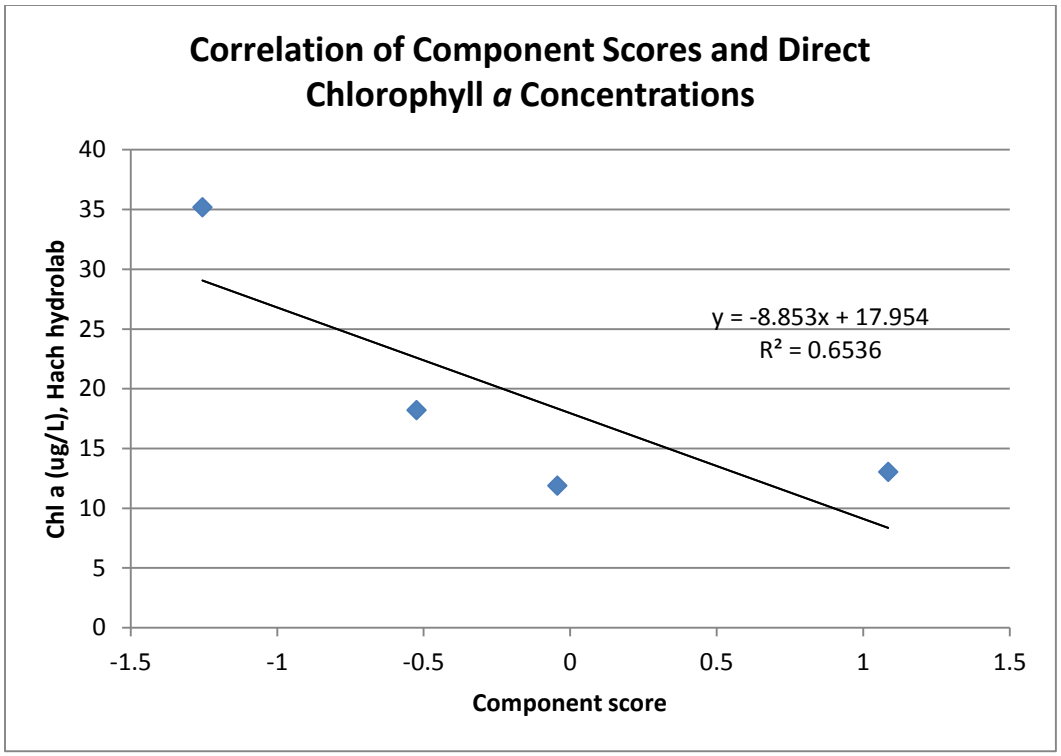

Figure 40. Component score and Hach hydrolab correlation.

Chlorophyll *a* was measured using a Hach hydrolab sensor. Here, averaged hydrolab data are compared to averaged PCA component scores derived from the first component in the open mouth bar study (June 23 – July 21, 2011).

A separate PCA was performed during the period of the summer when the mouth bar was closed (July 21 – September 11, 2011). Descriptive statistics for all 56 reflectance spectra have been provided in Table 10. As before, three components with eigenvalues greater than 1 were extracted. These components, however, were different from the ones produced during the open mouth bar period, and they explained 92.1% of the optical variability in the dataset. The relative importance of these components was also found to be different than the open mouth bar components, with the first component explaining about half (49.7%) of the variance, the second component explaining 23.2%, and the third component explaining 19.2% of the variability in the dataset (Table 11). This suggests a change in the importance of the CPAs that could perhaps be attributed to the prevention of flow into Lake Erie (although seasonal variability might be important as well).

As before, these components were compared to known spectra for common pigments and minerals (see Figure 42). The first component is somewhat similar to the first component for the open mouth bar period, but only explains about half (49.6%) of the optical variability when the mouth bar was closed. The first component corresponds to a mixture of chlorophyceae (green algae) and cyanobacteria (r^2=0.65), although bacillariophyta may be included as well. The second component explains 23.2% of the optical variability and, again, corresponds to a mixture of clay (and, in this case, iron oxide) minerals. A 50/50 mixture of illite and goethite provided a correlation value of r^2=0.78. The third component explains 19.2% of the optical variability and appears to

correspond to additional phytoplankton assemblages; a mixture of chlorophyta and cryptophyta provided a correlation value of r^2=0.64.

Despite having a longer dataset for the closed mouth bar study, there seems to be a greater variability among the in-water constituents, making it more difficult to find a suitable reflectance spectra from the known pigment/mineral library. Additionally, much less of the dataset is explained by a single component when the mouth bar is closed. When the mouth bar was open, almost 64% of the dataset was explained by the first component with the second component explaining an additional 29% of the variance. Thus, two components during the open mouth bar period explained more of the variance than three components during the closed mouth bar period. Additionally, while it is difficult to determine the exact mineral assemblages based on their spectra, there does seem to be a change in phytoplankton populations when the mouth bar is closed. Cryptophyta, for example, does seem to be a dominant algal group during June or early July, but becomes more important towards the autumn season. This is similar to what was found by Klarer and Millie (1994).

Table 10. Descriptive Statistics for Closed Mouth Bar

July 21 – Sept 11, 2011

	Mean	Std. Deviation	N
drdl400	0.021	0.045	56
drdl410	0.042	0.037	56
drdl420	0.075	0.039	56
drdl430	0.105	0.042	56
drdl440	0.146	0.043	56
drdl450	0.150	0.030	56
drdl460	0.137	0.025	56
drdl470	0.133	0.025	56
drdl480	0.136	0.030	56
drdl490	0.169	0.036	56
drdl500	0.216	0.034	56
drdl510	0.233	0.028	56
drdl520	0.215	0.021	56
drdl530	0.191	0.016	56
drdl540	0.184	0.014	56
drdl550	0.165	0.013	56
drdl560	0.121	0.017	56
drdl570	0.078	0.019	56
drdl580	0.071	0.016	56
drdl590	0.069	0.009	56
drdl600	0.020	0.020	56
drdl610	-0.007	0.026	56
drdl620	0.036	0.025	56
drdl630	0.077	0.010	56
drdl640	0.008	0.027	56
drdl650	-0.217	0.073	56
drdl660	-0.276	0.080	56
drdl670	0.023	0.101	56
drdl680	0.504	0.132	56
drdl690	0.682	0.161	56
drdl700	0.459	0.168	56

Table 11. VPCA Results for Closed Mouth Bar

July 21 – September 11, 2011

	Initial Eigenvalues			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	15.40436	49.69148	49.69148	14.03487	45.27379	45.27379
2	7.199185	23.22318	72.91466	7.438769	23.99603	69.26982
3	5.952353	19.20114	92.1158	7.082256	22.84599	92.1158
4	0.786246	2.536277	94.65208			
5	0.608322	1.962331	96.61441			
6	0.306882	0.989943	97.60435			
7	0.226567	0.730861	98.33521			
8	0.162709	0.524868	98.86008			
9	0.106348	0.343059	99.20314			
10	0.057762	0.18633	99.38947			
11	0.045398	0.146446	99.53592			
12	0.030697	0.099022	99.63494			
13	0.026361	0.085036	99.71997			
14	0.019251	0.062101	99.78207			
15	0.014409	0.046482	99.82856			
16	0.011165	0.036017	99.86457			
17	0.009629	0.031062	99.89564			
18	0.006708	0.021639	99.91727			
19	0.006182	0.019943	99.93722			
20	0.003328	0.010737	99.94795			
21	0.002965	0.009564	99.95752			
22	0.002811	0.009069	99.96659			
23	0.00211	0.006806	99.97339			
24	0.00186	0.005999	99.97939			
25	0.001714	0.005529	99.98492			
26	0.001652	0.00533	99.99025			
27	0.001362	0.004393	99.99464			
28	0.001042	0.00336	99.998			
29	0.00031	0.000999	99.999			
30	0.000207	0.000669	99.99967			
31	0.000102	0.000328	100			

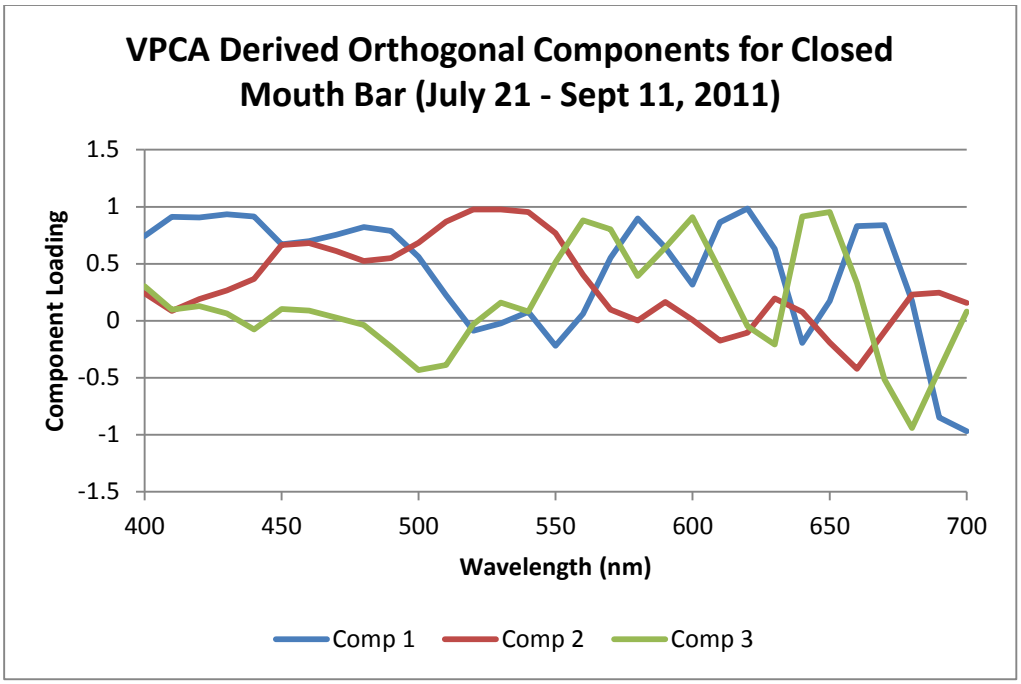

Figure 41. VPCA results for closed mouth bar.

Varimax-rotated principal component analysis on spectral data produced three components that account for approximately 92.1% of the optical variability in the OWC dataset during the period when the mouth bar was closed.

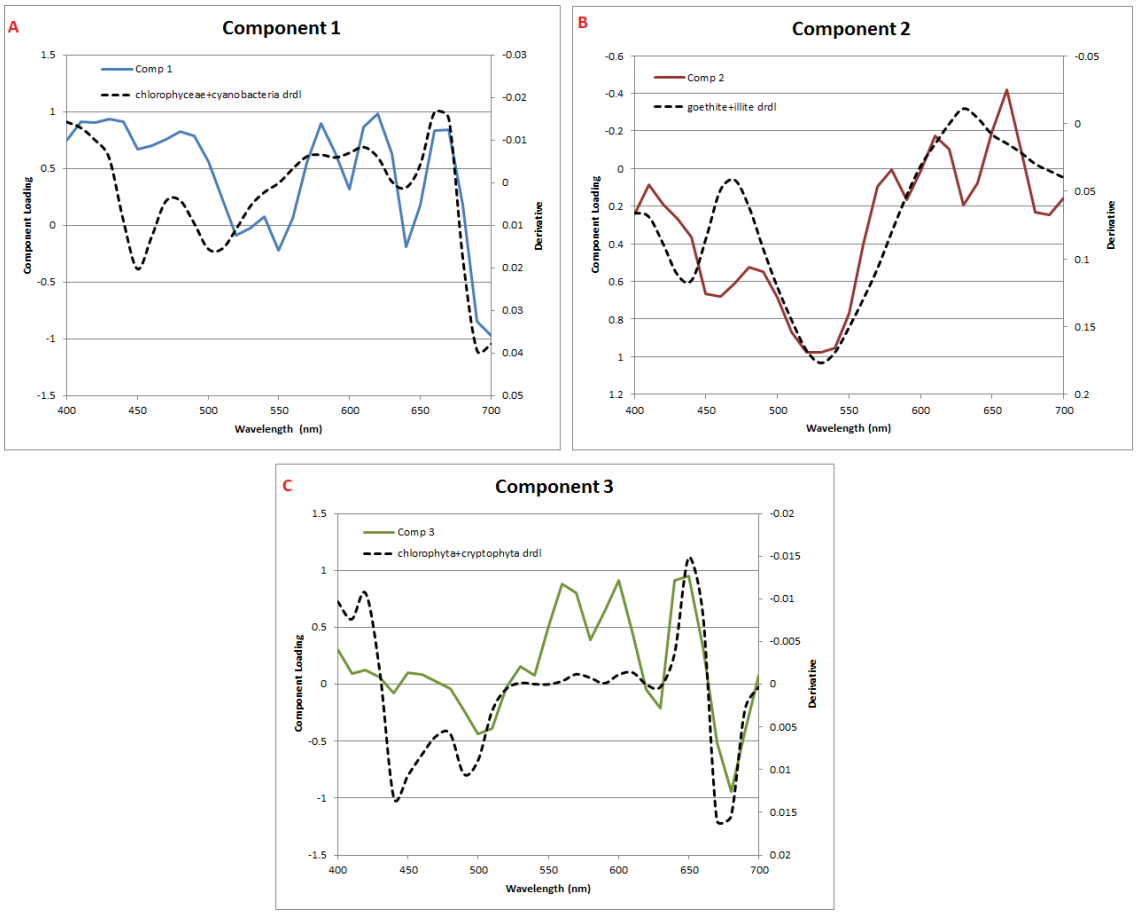

Figure 42. PCA component loadings and reference derivative spectra.

Component loadings for PCA are compared to selected reference derivative spectra during the closed mouth bar season (July 21 – September 11, 2011). The first component (A) relates to chlorophyceae and cyanobacteria; the second component (B) relates to a mixture of clay and iron oxide minerals (e.g. illite and goethite); the third component (C) relates to chlorophyta and cryptophyta.

Lastly, we can compare the component scores from the reflectance data to the direct concentration measurements. Doing so over the entire study period was not possible, as there were changes in the importance of components during periods when the mouth bar was open versus when it was closed. Averaging data for sampling periods when the mouth bar was closed, however, provides seven days (data points) from which data can be compared, and is thus be more useful than the four days during the open mouth bar period. Doing so provides excellent results. A correlation value of r^2=0.74 was found between the averaged component scores for the first component of the closed mouth bar study and averaged data from the Trilogy lab fluorometer (Figure 43). The correlation between component scores for the first component and averaged *in-situ* Hach data was r^2=0.92 (Figure 43). There does appear to be one outlier in the Trilogy dataset. If averaged Trilogy data on August 18 is excluded from the dataset, the correlation value of Trilogy measurements and component scores increases from 0.74 to 0.94, which makes sense. The two laboratory measurements were performed on the same parcel of water and can thus be expected to provide a stronger correlation than either of the laboratory measurements with *in-situ* data.

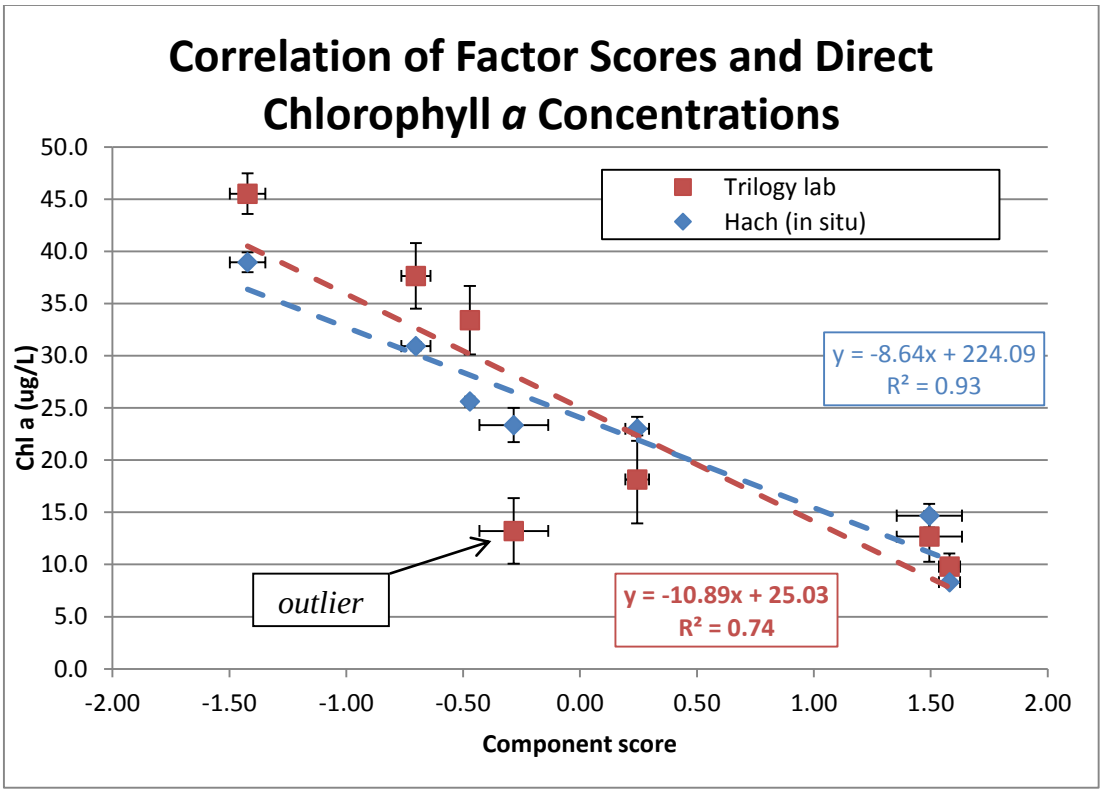

Figure 43. Component score and chlorophyll *a* concentrations.

Chlorophyll *a* concentrations were measured using an *in-situ* Hach hydrolab sensor and a Trilogy fluorometer. Here, averaged Hach data (blue) and averaged Trilogy data (red) are compared to averaged PCA component scores derived from the first component in the closed mouth bar study (July 21 – September 11, 2011). Hach chlorophyll *a* values were calculated by averaging the full profile of measurements taken on each day (see Appendix B.1). Hach error bars were calculated using standard error (standard deviation divided by the square root of *n*, where *n* equals the number of measurements recorded each day). Trilogy chlorophyll *a* measurements were calculated by averaging all daily measurements (two samples at three samples, or *n*=6), and component scores were calculated in a similar fashion (two samples at three depths or *n*=6). Error bars for these measurements were calculated using standard error (standard deviation divided by the square root of *n*, where *n*=6). The correlation value for the Trilogy lab (red) increases from r^2=0.74 to r^2=0.94 when the outlier is excluded, due to instrument error.

4.9 Full Time Series Analysis

The last thing that was done in this study was a PCA on the entire dataset. This included reflectance data, fluorometer data, sensor data, and meteorological data. Due to the issue with some water samples being filtered on the day of collection and others remaining unfiltered for four days, only the data from the 8-day frequency have been included here (see Chapter 3.1). Next, it was decided to use the PCA results for the entire dataset (Figure 37) rather than a subset. This allowed for a comparison between reflectance data at depth, as it was expected there might be variability among phytoplankton populations with depth as well as time. For the most part, however, spectra were similar at all depths (see Figure 44). The one exception to this was component four, in which surface reflectance is somewhat higher in the green part of the spectrum (500-560 nm). This signature is identical to the third component in the open bar mouth study (Figure 39), which was assigned to cyanobacteria and fucoxanthin. Because most of the components were similar at depth, all reflectance data have been averaged. These four components (and variations of their difference; e.g. component 2 minus component 1, competent 2 minus component 3, etc.) were than correlated to every existing dataset, which included sensor data from the Hach hydrolab, sensor data from the WM site monitoring sonde, meteorological data from the OWC weather station, CDOM, and Trilogy fluorometer data. Due to the large volume of data generated, a complete table of these correlations has been provided in Appendix D. Values statistically significant at the 1% level (greater than 0.74) have been shaded pink. Values statistically significant at the 5% level (greater than (0.60) have been shaded green.

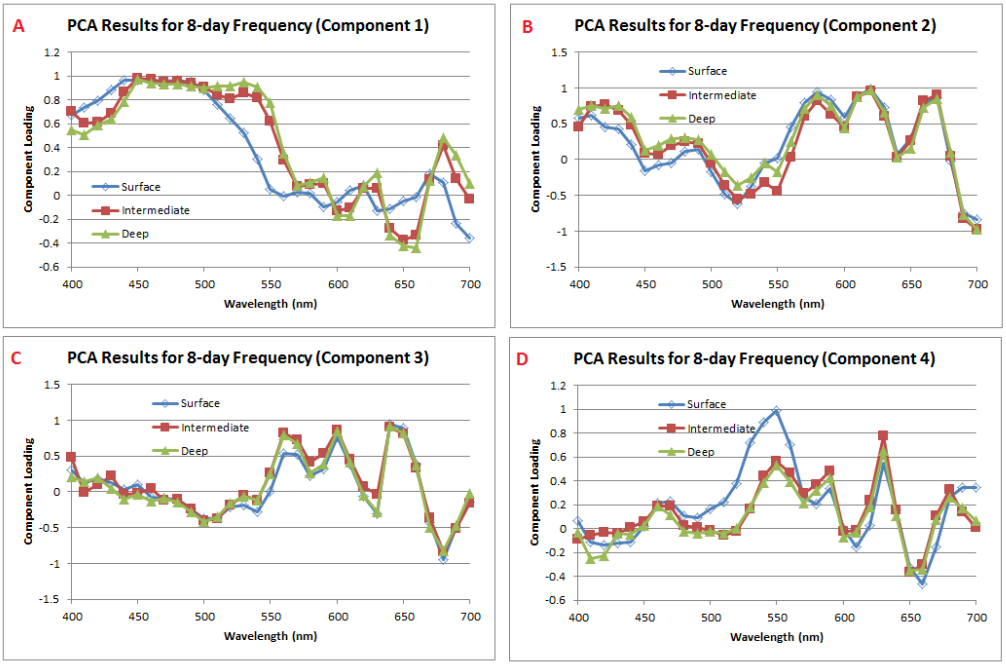

Figure 44. PCA results for samples at various depths.

PCA was performed on all samples during the 8-day frequency sampling. Samples were collected at three depths. Spectral comparisons have been made for each of the components at each depth.

Before this can be done, however, it is useful to analyze the PCA results for the reflectance spectra over the full time series. This was done previously in Figure 37, with some basic statistics and total variance provided in Table 6 and Table 7, respectively. Now, however, we will consider each of the four components and attempt to identify the components using known reference derivative spectra, as was done with the subset data.

The first component explains about 38.8% of the optical variability across the entire three-month study at OWC (see Table 7) and is best attributed to clays (illite has a correlation value of r^2=0.88; see Figure 45) or other minerals. This would suggest sediment—not phytoplankton—is the dominant factor controlling the optical variability at OWC; at least, in the time period that was considered. In both of the cases in which the data were separated based on the mouth bar status, clays were the second dominant factor in explaining the variance. Interestingly, the first component shows good correlation values (significant at the 5% level) for both surface chlorophyll *a* measurements (fluorometric) and surface phycocyanin (Appendix D).

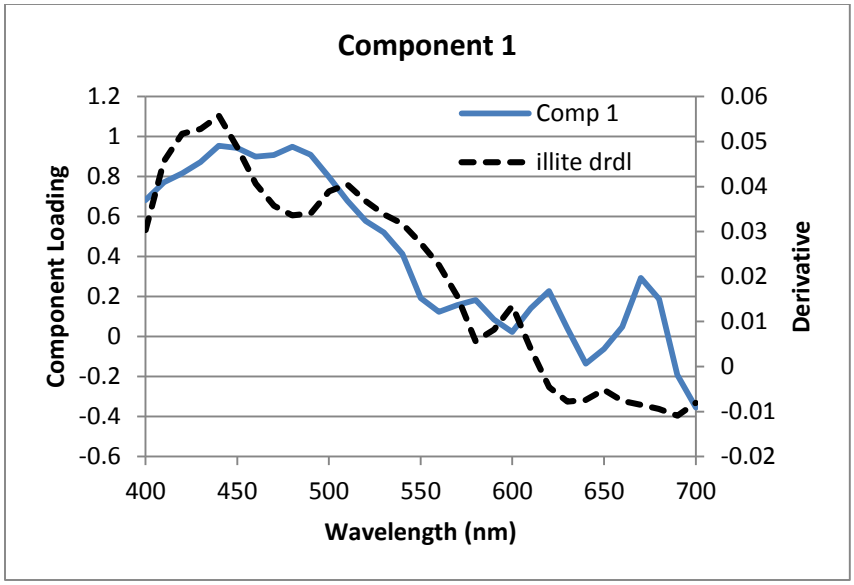

Figure 45. Component 1: competent loading and reference spectrum.

The second component explains almost 36% of the optical variability in the dataset (and is thus almost as important as the first component) and is similar to the first component in the subset data, corresponding to a mixture of chlorophyceae and bacillariophyceae (r^2=0.70; see Figure 46). Based on the data in Appendix D, strong correlations exist between component 2 and chlorophyll *a* (intermediate depth), pheo-pigments (intermediate depth), and most of the limnological parameters, including temperature, pH, DO, and depth, as well as two components from the monitoring sonde data: component 1 (which was attributed to biological activity) and component 3 (which was attributed to temperature; see Chapter 4.6). Thus, it makes sense that the second component here is associated with the dominant algal groups found in the estuary.

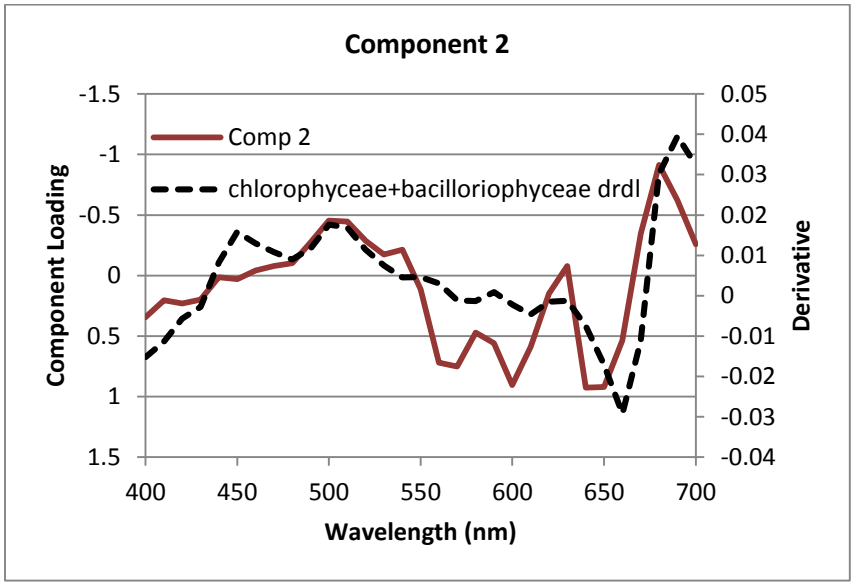

Figure 46. Component 2: competent loading and reference spectrum.

The third component explains approximately 12.1% of the optical variability during the three-month study and can be attributed to cyanobacteria (r^2=0.78; see Figure 47), although it is not well-correlated to any of the other datasets in this study (besides reflectance spectra) (Appendix D). This further suggests cyanobacteria are of less importance to the optical variability in the estuary when compared to the more dominant groups of green algae.

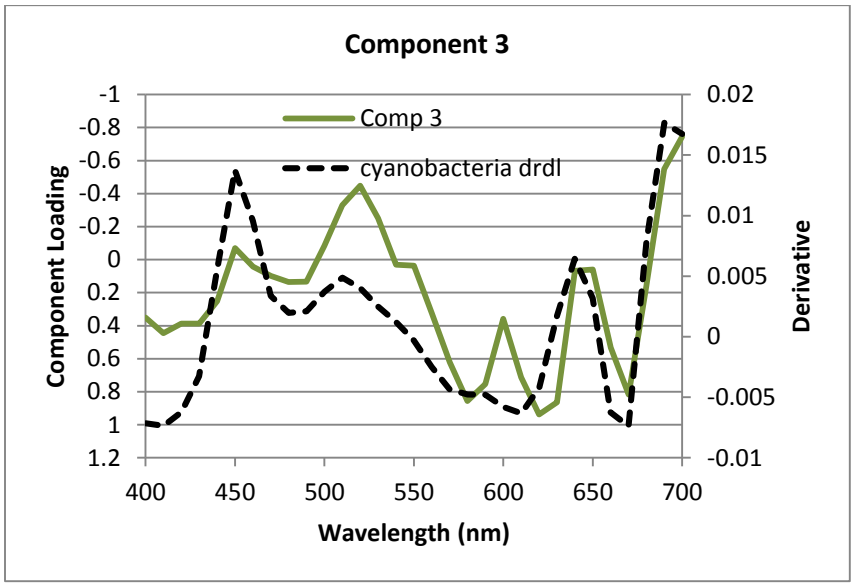

Figure 47. Component 3: competent loading and reference spectrum.

The fourth component explains 8.2% of the variance and appears to be associated with a mixture of cyanobacteria and fucoxanthin (Figure 48). It has a good correlation with oxygen reduction potential (ORP) at all three measured depths and specific conductivity (Appendix D). More correlations are found, however, when the difference between components 3 and 4 are calculated (Appendix D, last column), which makes sense, as component 3 and 4 appear to share a similar algal group (cyanobacteria).

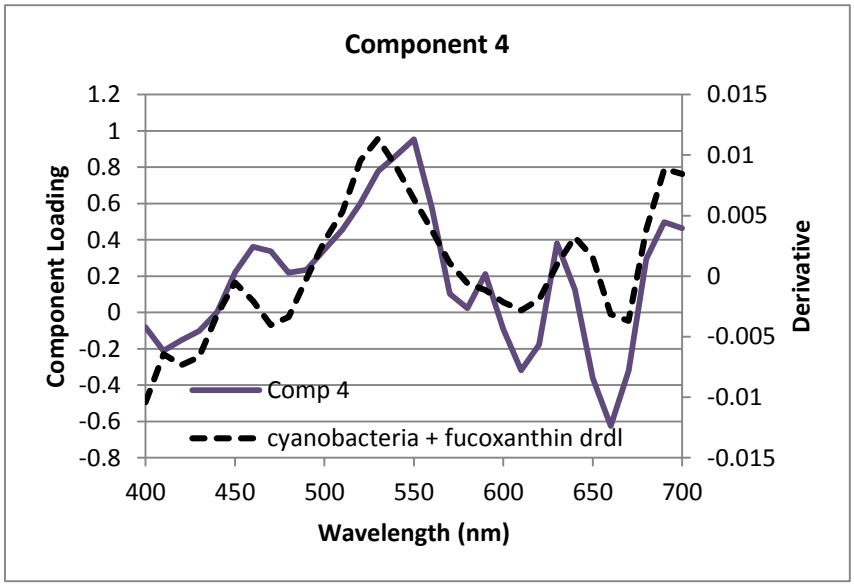

Figure 48. Component 4: competent loading and reference spectrum.

CHAPTER 5: CONCLUSION

This was a method-based study with four principal objectives. These objectives were to (1) determine the best way to process samples, (2) determine the rate of degradation of chlorophyll *a*, (3) compare VNIR derivative spectroscopy and visible-range fluorometry, and (4) create a time series of variations in pigment classes during a three-month study of OWC.

Objective #1 (determining the best method(s) by which to process and treat filtered water samples in order to minimize degradation of chlorophyll *a*) was handled in trial-and-error fashion by testing out various methods of filtering, preserving, and drying samples. It was determined (a) 250 mL is the ideal filtration amount for ASD analysis in an estuarine ecosystem such as OWC, (b) samples should *not* be treated with MgCO3 as a preservation agent (as it interferes with spectral readings, and (c) both oven-drying and air-drying provided good results, although oven-drying is probably safer. In addition, water should be filtered on the day of collection. If that is not possible, the water should be treated with 10% isopropyl acetone to prevent the loss of chlorophyll.

Objective #2 (determining the rate of degradation of chlorophyll *a* into its principal degradation products) was investigated by performing a drying time series analysis. This analysis was performed twice (once when filtering 500 mL of water and once with 250 mL). In both cases, samples did not show a significant loss of chlorophyll after 3-4 weeks, as long as proper preservation techniques were used. This confirmed

what was found in the literature and allows for samples to be collected and filtered on one day and processed much later, if needed.

Objective #3 (comparing VNIR derivative spectroscopy and visible-range fluorometery in the identification and quantification of chlorophyll *a*) was done in multiple ways, using fluorometry, independent sensor data, reflectance indices, and averaged component scores from principal component analysis. Despite the high variability in samples, strong correlations were found between all methods of chlorophyll *a* measurements. The results of these first three objectives demonstrate filtered samples can be dried without losing a significant amount of chlorophyll *a*, and VNIR derivative spectroscopy does much more than simply supplement direct concentration measurements; it provides key insights into the composition, variability, and distribution of pigment classes. This is evidenced in the numerous time series analyses that were performed to address Objective #4 (creating a time series of variations in pigment classes). Through the use of VNIR derivative spectroscopy, the important in-water constituents were determined during a three-month study of Old Woman Creek, while using a method of analysis that is much more rapid and less destructive than traditional techniques for measuring pigments. When coupled with these traditional methods, however, VNIR derivative spectroscopy becomes a powerful tool that can provide some very interesting and useful data that can be used to rapidly identify and quantify phytoplankton distributions in aquatic systems.

APPENDIX A – FILTER DATA

The following tables are a record of the filter data used in this study. Below is a guide for reading these tables.

- Date: The date on which the water was collected.
- Filter #: The number of the filter. Some samples also used letters to differentiate between samples from the same bottle (e.g. on June 23), and some filters were completely excluded from this study (e.g. #152-157).
- From bottle #: The number of the bottle which was filtered. Bottles were filtered multiple times, so they required separate numbering from filters. Bottle numbers that are preceded by a "D" (e.g. D1, D2, etc.) were collected by David Klarer, four days before or after each sampling trip. On June 7, "H" refers to a homogenous mixture of 18-liters of water. Listed below each table is also a record of the depth each bottle was collected from. (Refer to Chapter 3.2 for the calculations of these depths.)
- Filt'd (mL): The amount of water filtered.
- MgCO3 sol'n used (mL): The amount of magnesium carbonate powder added to the water, prior to filtering. Only used on June 7.
- Wt empty (mg): Empty weight of filter. (ASD samples only.)
- Wt wet (mg): Wet weight of filter. Taken immediately before being placed into oven. (ASD samples only.)
- Wt dry (mg): Dry weight of filter. Taken immediately after being removed from oven. (ASD samples only.)
- Total (mg): amount of material collected (dry weight minus empty weight). (ASD samples only.)
- In oven: The date on which the filter was put into the oven. ASD samples only.
- Chl a (ug/L): The concentration of chlorophyll *a*. (Trilogy samples only.)
- Pheo (ug/L): The concentration of pheophytin *a*. (Trilogy samples only.)
- Notes: Any additional information. Generally, this refers to a change from the standard method of filtration (such as a different apparatus) or suspect samples from which erroneous data may have resulted.

A.1 Filter data from June 7, 2011

Date	Filter #	From bottle #	Filt'd (mL)	MgCO 3 Sol'n (mL)	Wt empty (mg)	Wt wet (mg)	Wt dry (mg)	Total (mg)	In oven	Chl a (ug/L)	Pheo (ug/L)	Notes
6/7	1	1	500	none	129.2	1230.0	137.7	8.50	6/7	N/A	N/A	Hand pump
6/7	2	2	500	none	127.3	1341.2	135.9	8.60	6/7	N/A	N/A	Hand pump
6/7	3	3	500	none	130.2	1258.7	139.3	9.10	6/7	N/A	N/A	Hand pump
6/7	4	4	100	none	N/A	N/A	N/A	N/A	N/A	12.20	0.87	Hand pump
6/7	5	5	100	none	N/A	N/A	N/A	N/A	N/A	9.01	0.37	Hand pump
6/7	6	6	100	none	N/A	N/A	N/A	N/A	N/A	6.77	1.22	Hand pump
6/7	7	H	500	none	129.5	955.7	133.1	3.60	6/9	N/A	N/A	Hand pump
6/7	8	H	500	none	128.9	1180.9	136.7	7.80	6/10	N/A	N/A	Hand pump
6/7	9	H	500	none	129.3	1049.6	136.7	7.40	6/11	N/A	N/A	Hand pump
6/7	10	H	500	none	127.9	969.1	140.8	12.90	6/12	N/A	N/A	Hand pump
6/7	11	H	500	none	127.9	949.4	135.1	7.20	6/16	N/A	N/A	Hand pump
6/7	12	H	500	none	130.2	970.3	139.4	9.20	6/20	N/A	N/A	Hand pump
6/7	13	H	500	few drops	135.7	867.8	143.6	7.90	6/16	N/A	N/A	Hand pump
6/7	14	H	500	10	128.2	1065.3	144.0	15.80	6/9	N/A	N/A	Hand pump
6/7	15	H	500	10	128.0	1246.0	205.6	77.60	6/10	N/A	N/A	Hand pump
6/7	16	H	500	10	128.8	1443.3	235.2	106.40	6/11	N/A	N/A	Hand pump
6/7	17	H	500	10	129.4	1410.9	231.7	102.30	6/12	N/A	N/A	Hand pump
6/7	18	H	500	10	128.0	1428.1	243.4	115.40	6/16	N/A	N/A	Hand pump
6/7	19	H	500	5 (at end)	130.0	1442.5	178.7	48.70	6/7	N/A	N/A	Hand pump

6/7	20	H	500	few drops (at end)	131.8	1445.5	148.0	16.20	6/7	N/A	N/A	Hand pump
6/7	21	H	500	few drops (at end)	127.2	1283.4	144.9	17.70	6/7	N/A	N/A	Hand pump
6/7	22	H	500	10	128.2	1404.6	274.3	146.10	6/20	N/A	N/A	Hand pump
6/7	23	H	500	none	134.2	941.5	144.3	10.10	6/24	N/A	N/A	Hand pump
6/7	24	H	500	none	134.8	909.0	142.7	7.90	6/28	N/A	N/A	Hand pump
6/7	25	H	500	10	134.7	1415.9	257.5	122.80	6/24	N/A	N/A	Hand pump
6/7	26	H	500	10	131.7	1350.2	248.4	116.70	6/28	N/A	N/A	Hand pump

Bottles collected from depth 1 (surface): All

Trilogy measurements were made June 25, 2011

A.2 Filter data from June 15, 2011

Date	Filter #	From bottle #	Filt'd (mL)	Wt empty (mg)	Wt wet (mg)	Wt dry (mg)	Total (mg)	In oven	Chl a (ug/L)	Pheo (ug/L)	Notes
6/15	27	27	500	133.5	607.5	147.5	14.00	6/15	N/A	N/A	
6/15	28	28	500	133.4	579.0	147.2	13.80	6/15	N/A	N/A	
6/15	29	29	500	133.9	1116.5	147.5	13.60	6/15	N/A	N/A	
6/15	30	30	500	135.0	720.7	149.6	14.60	6/15	N/A	N/A	
6/15	31	31	500	134.2	783.8	149.0	14.80	6/15	N/A	N/A	
6/15	32	32	500	133.5	644.2	149.7	16.20	6/15	N/A	N/A	
6/15	33	27	100	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Incorrect procedure
6/15	34	28	100	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Incorrect procedure
6/15	35	29	100	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Incorrect procedure
6/15	36	29	85	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Incorrect procedure
6/15	37	31	100	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Incorrect procedure
6/15	38	31	100	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Incorrect procedure

Bottles collected from depth 1 (surface): 27, 28

Bottles collected from depth 2 (via Van Dorn): 29, 30

Bottles collected from depth 3 (via Van Dorn): 31, 32

Trilogy measurements were made on June 25, 2011

A.3 Filter data from June 23, 2011

Date	Filter #	From bottle #	Filt'd (mL)	Wt empty (mg)	Wt wet (mg)	Wt dry (mg)	Total (mg)	In oven	Chl a (ug/L)	Pheo (ug/L)	Notes
6/19	51	D1	500	134.1	858.4	144.7	10.60	6/23	N/A	N/A	
6/19	52	D1	100	N/A	N/A	N/A	N/A	N/A	29.51	12.83	
6/19	53	D2	500	135.2	726.0	144.8	9.60	6/23	N/A	N/A	
6/19	54	D2	100	N/A	N/A	N/A	N/A	N/A	34.77	12.70	
6/23	39a	39	50	135.1	797.9	138.5	3.40	6/23	N/A	N/A	
6/23	39b	39	100	135.0	877.5	141.8	6.80	6/23	N/A	N/A	
6/23	39c	39	150	134.4	880.5	144.4	10.00	6/23	N/A	N/A	
6/23	39d	39	200	134.1	911.9	147.8	13.70	6/23	N/A	N/A	
6/23	39e	39	250	132.7	1093.3	149.5	16.80	6/23	N/A	N/A	
6/23	40a	40	50	135.2	686.8	137.9	2.70	6/23	N/A	N/A	
6/23	40b	40	100	134.4	871.7	140.8	6.40	6/23	N/A	N/A	
6/23	40c	40	150	134.4	901.0	144.6	10.20	6/23	N/A	N/A	
6/23	40d	40	200	134.7	881.7	147.7	13.00	6/23	N/A	N/A	
6/23	40e	40	250	133.5	951.6	150.5	17.00	6/23	N/A	N/A	
6/23	41a	41	50	134.7	722.5	137.9	3.20	6/23	N/A	N/A	
6/23	41b	41	100	134.5	888.0	141.0	6.50	6/23	N/A	N/A	
6/23	41c	41	150	136.3	900.8	146.1	9.80	6/23	N/A	N/A	
6/23	41d	41	200	134.5	875.8	147.6	13.10	6/23	N/A	N/A	
6/23	41e	41	250	134.5	908.2	149.5	15.00	6/23	N/A	N/A	
6/23	42a	42	50	133.8	778.9	136.8	3.00	6/23	N/A	N/A	
6/23	42b	42	100	134.6	904.5	141.1	6.50	6/23	N/A	N/A	
6/23	42c	42	150	135.9	918.2	145.6	9.70	6/23	N/A	N/A	
6/23	42d	42	200	133.0	922.0	146.1	13.10	6/23	N/A	N/A	
6/23	42e	42	250	133.2	733.4	151.0	17.80	6/23	N/A	N/A	
6/23	43a	43	50	134.2	881.5	138.3	4.10	6/23	N/A	N/A	
6/23	43b	43	100	136.2	889.8	143.8	7.60	6/23	N/A	N/A	
6/23	43c	43	150	134.0	884.2	145.3	11.30	6/23	N/A	N/A	
6/23	43d	43	200	134.9	913.4	148.8	13.90	6/23	N/A	N/A	
6/23	43e	43	250	134.4	878.3	151.0	16.60	6/23	N/A	N/A	
6/23	44a	44	50	134.6	862.3	138.2	3.60	6/23	N/A	N/A	
6/23	44b	44	100	135.0	865.0	143.4	8.40	6/23	N/A	N/A	
6/23	44c	44	150	134.2	939.4	145.3	11.10	6/23	N/A	N/A	
6/23	44d	44	200	134.0	912.7	148.7	14.70	6/23	N/A	N/A	

6/23	44e	44	250	132.7	918.0	150.8	18.10	6/23	N/A	N/A	
6/23	45	39	100	N/A	N/A	N/A	N/A	N/A	13.12	4.77	
6/23	46	40	100	N/A	N/A	N/A	N/A	N/A	14.37	4.26	
6/23	47	41	100	N/A	N/A	N/A	N/A	N/A	13.83	4.63	
6/23	48	42	100	N/A	N/A	N/A	N/A	N/A	15.40	4.75	
6/23	49	43	100	N/A	N/A	N/A	N/A	N/A	12.95	4.09	
6/23	50	44	100	N/A	N/A	N/A	N/A	N/A	13.55	5.75	

Bottles collected from depth 1 (surface): D1, D2, 39, 40

Bottles collected from depth 2 (via Van Dorn): 41, 42

Bottles collected from depth 3 (via Van Dorn): 43, 44

Trilogy measurements were made on June 25, 2011

A.4 Filter data from July 1, 2011

Date	Filter #	From bottle #	Filt'd (mL)	Wt empty (mg)	Wt wet (mg)	Wt dry (mg)	Total (mg)	In oven	Chl a (ug/L)	Pheo (ug/L)	Notes
6/27	67	D3	250	134.1	793.4	140.5	6.40	7/1	N/A	N/A	
6/27	68	D3	100	N/A	N/A	N/A	N/A	N/A	17.68	4.45	
6/27	69	D4	250	133.5	902.9	140.9	7.40	7/1	N/A	N/A	
6/27	70	D4	100	N/A	N/A	N/A	N/A	N/A	12.19	9.57	
7/1	55	55	250	134.3	1003.9	138.0	3.70	7/1	N/A	N/A	Aspirator (31 min)
7/1	56	56	250	135.0	986.2	137.1	2.10	7/1	N/A	N/A	Aspirator (31 min)
7/1	57	57	250	135.0	727.2	139.9	4.90	7/1	N/A	N/A	Vacuum (36 sec)
7/1	58	58	250	135.2	814.3	140.1	4.90	7/1	N/A	N/A	Vacuum (36 sec)
7/1	59	59	250	133.7	755.5	148.0	14.30	7/1	N/A	N/A	Vacuum (36 sec)
7/1	60	60	250	135.6	888.7	142.3	6.70	7/1	N/A	N/A	Vacuum pump (36 sec)
7/1	61	55	100	N/A	N/A	N/A	N/A	N/A	2.66	19.19	Chl a/Pheo inverted?
7/1	62	56	100	N/A	N/A	N/A	N/A	N/A	1.83	14.67	Chl a/Pheo inverted?
7/1	63	57	100	N/A	N/A	N/A	N/A	N/A	18.22	2.87	
7/1	64	58	100	N/A	N/A	N/A	N/A	N/A	16.48	3.70	
7/1	65	59	100	N/A	N/A	N/A	N/A	N/A	3.81	24.23	Chl a/Pheo inverted?
7/1	66	60	100	N/A	N/A	N/A	N/A	N/A	22.47	3.87	
7/1	71	55	500	133.0	860.4	141.8	8.80	7/1	N/A	N/A	
7/1	72	57	500	135.4	909.4	145.9	10.50	7/1	N/A	N/A	
7/1	73	59	500	135.3	975.4	143.7	8.40	7/1	N/A	N/A	
7/1	74	D4	500	133.2	837.6	147.7	14.50	7/1	N/A	N/A	
7/1	75	56	250	131.3	711.5	134.7	3.40	7/1	N/A	N/A	Vacuum (36 sec)

Bottles collected from depth 1 (surface): D3, D4, 55, 56

Bottles collected from depth 2 (via Van Dorn): 57, 58

Bottles collected from depth 3 (via Van Dorn): 59, 60

Trilogy measurements were made on July 12, 2011

A.5 Filter data from July 9, 2011

Date	Filter #	From bottle #	Filt'd (mL)	Wt empty (mg)	Wt wet (mg)	Wt dry (mg)	Total (mg)	In oven	Chl a (ug/L)	Pheo (ug/L)	Notes
7/5	88	D5	250	127.3	972.4	135.7	8.40	7/9	N/A	N/A	
7/5	89	D5	100	N/A	N/A	N/A	N/A	N/A	42.94	3.63	
7/5	90	D6	250	134.2	863.8	142.6	8.40	7/9	N/A	N/A	
7/5	91	D6	100	N/A	N/A	N/A	N/A	N/A	51.88	3.03	
7/9	76	76	250	134.7	674.1	137.4	2.70	7/9	N/A	N/A	
7/9	77	77	250	136	762.2	138.6	2.60	7/9	N/A	N/A	
7/9	78	78	250	131.8	756.2	137.0	5.20	7/9	N/A	N/A	
7/9	79	79	250	132.9	834.1	138.1	5.20	7/9	N/A	N/A	
7/9	80	80	250	133.3	732.8	138.5	5.20	7/9	N/A	N/A	
7/9	81	81	250	133.6	829.2	138.8	5.20	7/9	N/A	N/A	
7/9	82	76	100	N/A	N/A	N/A	N/A	N/A	1.37	8.78	Chl a/Pheo values inverted?
7/9	83	77	100	N/A	N/A	N/A	N/A	N/A	10.63	0.91	
7/9	84	78	100	N/A	N/A	N/A	N/A	N/A	32.65	4.86	
7/9	85	79	100	N/A	N/A	N/A	N/A	N/A	34.74	2.98	
7/9	86	80	100	N/A	N/A	N/A	N/A	N/A	26.36	2.66	
7/9	87	81	100	N/A	N/A	N/A	N/A	N/A	22.61	3.02	
7/9	92	77	500	135.2	707.4	140.2	5.00	7/9	N/A	N/A	
7/9	93	79	500	134.3	882.5	144.3	10.00	7/9	N/A	N/A	

Bottles collected from depth 1 (surface): D5, D6, 76, 77

Bottles collected from depth 2 (via Van Dorn): 78, 79

Bottles collected from depth 3 (via Van Dorn): 80, 81

Trilogy measurements were made on July 12, 2011

A.6 Filter data from July 17, 2011

Date	Filter #	From bottle #	Filt'd (mL)	Wt empty (mg)	Wt wet (mg)	Wt dry (mg)	Total (mg)	In oven	Chl a (ug/L)	Pheo (ug/L)	Notes
7/13	117	D7	250	130.0	790.9	133.9	3.90	7/17	N/A	N/A	
7/13	118	D7	100	N/A	N/A	N/A	N/A	N/A	8.17	2.57	
7/13	119	D8	250	129.1	860.0	133.2	4.10	7/17	N/A	N/A	
7/13	120	D8	100	N/A	N/A	N/A	N/A	N/A	8.75	2.81	
7/17	94	94	250	128.4	816.2	134.6	6.20	7/17	N/A	N/A	
7/17	95	95	250	130.1	924.5	137.9	7.80	7/17	N/A	N/A	
7/17	96	96	250	129.7	855.5	137.6	7.90	7/17	N/A	N/A	
7/17	97	97	250	130.0	870.1	137.1	7.10	7/17	N/A	N/A	
7/17	98	98	250	129.1	821.7	136.8	7.70	7/17	N/A	N/A	
7/17	99	99	250	129.8	862.8	138.2	8.40	7/17	N/A	N/A	
7/17	100	94	100	N/A	N/A	N/A	N/A	N/A	21.17	3.09	
7/17	101	95	100	N/A	N/A	N/A	N/A	N/A	41.92	4.94	
7/17	102	96	100	N/A	N/A	N/A	N/A	N/A	68.19	7.45	
7/17	103	97	100	N/A	N/A	N/A	N/A	N/A	64.26	6.30	
7/17	104	98	100	N/A	N/A	N/A	N/A	N/A	48.69	5.93	
7/17	105	99	100	N/A	N/A	N/A	N/A	N/A	60.65	9.69	
7/17	106	106	250	128.9	876.2	135.2	6.30	7/18	N/A	N/A	
7/17	107	107	250	128.6	913.5	136.5	7.90	7/19	N/A	N/A	
7/17	108	108	250	127.6	812.9	134.4	6.80	7/20	N/A	N/A	
7/17	109	109	250	129.0	866.9	135.7	6.70	7/24	N/A	N/A	
7/17	110	110	250	129.4	797.2	137.2	7.80	7/28	N/A	N/A	
7/17	111	111	250	128.7	752.3	137.0	8.30	8/1	N/A	N/A	
7/17	112	112	250	129.5	888.0	136.9	7.40	8/5	N/A	N/A	
7/17	113	113	250	129.1	765.0	137.1	8.00	8/9	N/A	N/A	
7/17	114	114	250	127.9	408.2	135.4	7.50	8/13	N/A	N/A	
7/17	115	115	250	128.4	597.8	135.4	7.00	8/17	N/A	N/A	
7/17	116	116	250	129.4	647.0	136.8	7.40	8/21	N/A	N/A	

Bottles collected from depth 1 (surface): D7, D8, 94, 95, 106-116

Bottles collected from depth 2 (via Van Dorn): 96, 97

Bottles collected from depth 3 (via Van Dorn): 98, 99

Trilogy measurements were made on July 26, 2011

A.7 Filter data from July 25, 2011

Date	Filter #	From bottle #	Filt'd (mL)	Wt empty (mg)	Wt wet (mg)	Wt dry (mg)	Total (mg)	In oven	Chl a (ug/L)	Pheo (ug/L)	Notes
7/21	133	D9	250	128.5	938.5	134.5	6.00	7/25	N/A	N/A	
7/21	134	D9	100	N/A	N/A	N/A	N/A	N/A	50.88	4.99	
7/21	135	D10	250	128.6	926.3	135.2	6.60	7/25	N/A	N/A	
7/21	136	D10	100	N/A	N/A	N/A	N/A	N/A	51.67	5.12	
7/21	137	D9	100	N/A	N/A	N/A	N/A	N/A	51.73	5.93	
7/21	138	D9	100	N/A	N/A	N/A	N/A	N/A	42.14	5.90	
7/21	139	D9	100	N/A	N/A	N/A	N/A	N/A	32.44	4.21	
7/25	121	121	250	128.8	879.3	133.6	4.80	7/25	N/A	N/A	
7/25	122	122	250	128.8	892.5	134.2	5.40	7/25	N/A	N/A	
7/25	123	123	250	128.9	910.5	133.1	4.20	7/25	N/A	N/A	
7/25	124	124	250	134.3	841.4	139.2	4.90	7/25	N/A	N/A	
7/25	125	125	250	127.8	786.1	132.8	5.00	7/25	N/A	N/A	
7/25	126	126	250	130.1	904.2	136.5	6.40	7/25	N/A	N/A	
7/25	127	121	100	N/A	N/A	N/A	N/A	N/A	25.67	8.17	
7/25	128	122	100	N/A	N/A	N/A	N/A	N/A	36.99	9.39	
7/25	129	123	100	N/A	N/A	N/A	N/A	N/A	41.33	9.12	
7/25	130	124	100	N/A	N/A	N/A	N/A	N/A	32.59	7.50	
7/25	131	125	100	N/A	N/A	N/A	N/A	N/A	42.16	9.69	
7/25	132	126	100	N/A	N/A	N/A	N/A	N/A	47.20	7.81	

Bottles collected from depth 1 (surface): D9, D10, 121, 122

Bottles collected from depth 2 (via Van Dorn): 123, 124

Bottles collected from depth 3 (via Van Dorn): 125, 126

Trilogy measurements were made on July 26, 2011

A.8 Filter data from August 2, 2011

Date	Filter #	From bottle #	Filt'd (mL)	Wt empty (mg)	Wt wet (mg)	Wt dry (mg)	Total (mg)	In oven	Chl a (ug/L)	Pheo (ug/L)	Notes
7/29	158	D11	250	127.0	919.0	132.8	5.80	8/2	N/A	N/A	
7/29	159	D11	100	N/A	N/A	N/A	N/A	N/A	16.51	3.29	
7/29	160	D12	250	130.0	883.5	135.5	5.50	8/2	N/A	N/A	
7/29	161	D12	100	N/A	N/A	N/A	N/A	N/A	28.80	9.04	
8/2	140	140	250	127.7	932.0	132.1	4.40	8/2	N/A	N/A	
8/2	141	141	250	128.5	867.2	133.3	4.80	8/2	N/A	N/A	
8/2	142	142	250	128.1	814.3	132.6	4.50	8/2	N/A	N/A	
8/2	143	143	250	129.1	828.6	134.0	4.90	8/2	N/A	N/A	
8/2	144	144	250	127.5	920.0	132.3	4.80	8/2	N/A	N/A	
8/2	145	145	250	126.6	834.7	132.2	5.60	8/2	N/A	N/A	
8/2	146	140	100	N/A	N/A	N/A	N/A	N/A	30.96	0.49	
8/2	147	141	100	N/A	N/A	N/A	N/A	N/A	30.71	2.66	
8/2	148	142	100	N/A	N/A	N/A	N/A	N/A	41.83	4.01	
8/2	149	143	100	N/A	N/A	N/A	N/A	N/A	39.84	2.71	
8/2	150	144	100	N/A	N/A	N/A	N/A	N/A	37.23	4.44	
8/2	151	145	100	N/A	N/A	N/A	N/A	N/A	19.91	3.52	

Bottles collected from depth 1 (surface): D11, D12, 140, 141

Bottles collected from depth 2 (via Van Dorn): 142, 143

Bottles collected from depth 3 (via Van Dorn): 144, 145

Trilogy measurements were made on August 11, 2011

A.9 Filter data from August 10, 2011

Date	Filter #	From bottle #	Filt'd (mL)	Wt empty (mg)	Wt wet (mg)	Wt dry (mg)	Total (mg)	In oven	Chl a (ug/L)	Pheo (ug/L)	Notes
8/6	174	D13	250	134.5	851.9	140.0	5.50	8/10	N/A	N/A	
8/6	175	D13	100	N/A	N/A	N/A	N/A	N/A	32.64	2.19	
8/6	176	D14	250	132.4	899.0	137.7	5.30	8/10	N/A	N/A	
8/6	177	D14	100	N/A	N/A	N/A	N/A	N/A	20.20	2.08	
8/6	181	D13	100	N/A	N/A	N/A	N/A	N/A	30.01	1.60	Hand pump
8/10	162	162	250	135.1	865.2	141.0	5.90	8/10	N/A	N/A	
8/10	163	163	250	134.8	831.7	141.4	6.60	8/10	N/A	N/A	
8/10	164	164	250	134.7	892.0	141.8	7.10	8/10	N/A	N/A	
8/10	165	165	250	135.3	867.3	141.8	6.50	8/10	N/A	N/A	
8/10	166	166	250	134.5	915.6	141.5	7.00	8/10	N/A	N/A	
8/10	167	167	250	132.4	907.6	140.8	8.40	8/10	N/A	N/A	
8/10	168	162	100	N/A	N/A	N/A	N/A	N/A	49.70	2.43	
8/10	169	163	100	N/A	N/A	N/A	N/A	N/A	44.63	3.17	
8/10	170	164	100	N/A	N/A	N/A	N/A	N/A	52.75	3.64	
8/10	171	165	100	N/A	N/A	N/A	N/A	N/A	43.93	1.78	
8/10	172	166	100	N/A	N/A	N/A	N/A	N/A	42.12	1.51	
8/10	173	167	100	N/A	N/A	N/A	N/A	N/A	40.12	1.87	
8/10	178	162	100	N/A	N/A	N/A	N/A	N/A	37.96	2.20	Hand pump
8/10	179	164	100	N/A	N/A	N/A	N/A	N/A	49.00	3.92	Hand pump
8/10	180	166	100	N/A	N/A	N/A	N/A	N/A	57.33	5.51	Hand pump

Bottles collected from depth 1 (surface): D13, D14, 162, 163

Bottles collected from depth 2 (via Van Dorn): 164, 165

Bottles collected from depth 3 (via Van Dorn): 166, 167

Trilogy measurements were made on August 11, 2011

A.10 Filter data from August 18, 2011

Date	Filter #	From bottle #	Filt'd (mL)	Wt empty (mg)	Wt wet (mg)	Wt dry (mg)	Total (mg)	In oven	Chl a (ug/L)	Pheo (ug/L)	Notes
8/14	194	D15	250	133.5	933.6	139.6	6.10	8/18	N/A	N/A	
8/14	195	D15	100	N/A	N/A	N/A	N/A	N/A	28.65	8.54	
8/14	196	D16	250	132.2	825.7	137.5	5.30	8/18	N/A	N/A	
8/14	197	D16	100	N/A	N/A	N/A	N/A	N/A	51.31	27.27	
8/18	182	182	250	132.3	852.2	136.4	4.10	8/18	N/A	N/A	
8/18	183	182	250	133.0	867.3	137.2	4.20	8/18	N/A	N/A	
8/18	184	182	250	133.2	898.6	138.9	5.70	8/18	N/A	N/A	
8/18	185	182	250	134.4	874.5	140.0	5.60	8/18	N/A	N/A	
8/18	186	182	250	132.2	905.3	138.5	6.30	8/18	N/A	N/A	
8/18	187	182	250	133.6	873.6	139.8	6.20	8/18	N/A	N/A	
8/18	188	182	100	N/A	N/A	N/A	N/A	N/A	7.94	4.09	
8/18	189	183	100	N/A	N/A	N/A	N/A	N/A	13.78	5.17	
8/18	190	184	100	N/A	N/A	N/A	N/A	N/A	7.87	3.00	
8/18	191	185	100	N/A	N/A	N/A	N/A	N/A	6.49	3.30	
8/18	192	186	100	N/A	N/A	N/A	N/A	N/A	26.88	6.57	
8/18	193	187	100	N/A	N/A	N/A	N/A	N/A	16.33	5.94	

Bottles collected from depth 1 (surface): D15, D16, 182, 183

Bottles collected from depth 2 (via Van Dorn): 184, 185

Bottles collected from depth 3 (via Van Dorn): 186, 187

Trilogy measurements were made on September 2, 2011

A.11 Filter data from August 26, 2011

Date	Filter #	From bottle #	Filt'd (mL)	Wt empty (mg)	Wt wet (mg)	Wt dry (mg)	Total (mg)	In oven	Chl a (ug/L)	Pheo (ug/L)	Notes
8/22	210	D17	250	136.6	950.3	141.2	4.60	8/26	N/A	N/A	
8/22	211	D17	100	N/A	N/A	N/A	N/A	N/A	16.51	3.35	
8/22	212	D18	250	135.6	882.8	139.9	4.30	8/26	N/A	N/A	
8/22	213	D18	100	N/A	N/A	N/A	N/A	N/A	8.10	1.85	
8/26	198	198	250	133.9	905.4	138.8	4.90	8/26	N/A	N/A	
8/26	199	199	250	133.8	872.3	139.6	5.80	8/26	N/A	N/A	
8/26	200	200	250	136.3	871.9	143.0	6.70	8/26	N/A	N/A	
8/26	201	201	250	136.0	921.8	140.2	4.20	8/26	N/A	N/A	
8/26	202	202	250	136.6	887.0	142.1	5.50	8/26	N/A	N/A	
8/26	203	203	250	136.8	920.7	143.0	6.20	8/26	N/A	N/A	
8/26	204	198	100	N/A	N/A	N/A	N/A	N/A	14.99	3.37	
8/26	205	199	100	N/A	N/A	N/A	N/A	N/A	27.68	6.08	
8/26	206	200	100	N/A	N/A	N/A	N/A	N/A	29.16	7.46	
8/26	207	201	100	N/A	N/A	N/A	N/A	N/A	7.93	2.03	
8/26	208	202	100	N/A	N/A	N/A	N/A	N/A	24.15	6.12	
8/26	209	203	100	N/A	N/A	N/A	N/A	N/A	5.01	1.50	

Bottles collected from depth 1 (surface): D17, D18, 198, 199

Bottles collected from depth 2 (via Van Dorn): 200, 201

Bottles collected from depth 3 (via Van Dorn): 202, 203

Trilogy measurements were made on September 2, 2011

A.12 Filter data from September 3, 2011

Date	Filter #	From bottle #	Filt'd (mL)	Wt empty (mg)	Wt wet (mg)	Wt dry (mg)	Total (mg)	In oven	Chl a (ug/L)	Pheo (ug/L)	Notes
8/30	226	D19	250	134.5	1045.7	137.5	3.00	9/3	N/A	N/A	May be contaminated
8/30	227	D19	100	N/A	N/A	N/A	N/A	N/A	12.73	4.88	
8/30	228	D20	250	135.4	939.1	138.2	2.80	9/3	N/A	N/A	
8/30	229	D20	100	N/A	N/A	N/A	N/A	N/A	8.38	4.26	
8/30	230	D20	100	N/A	N/A	N/A	N/A	N/A	10.10	4.77	
8/30	231	D20	100	N/A	N/A	N/A	N/A	N/A	10.26	4.25	
9/3	214	214	250	134.4	963.7	137.9	3.50	9/3	N/A	N/A	Soap on filter?
9/3	215	215	250	135.2	948.8	137.7	2.50	9/3	N/A	N/A	
9/3	216	216	250	133.7	912.8	137.5	3.80	9/3	N/A	N/A	
9/3	217	217	250	134.0	947.0	137.4	3.40	9/3	N/A	N/A	
9/3	218	218	250	134.1	951.4	138.8	4.70	9/3	N/A	N/A	
9/3	219	219	250	136.0	955.5	140.0	4.00	9/3	N/A	N/A	
9/3	220	214	100	N/A	N/A	N/A	N/A	N/A	3.68	1.33	
9/3	221	215	100	N/A	N/A	N/A	N/A	N/A	9.86	3.47	
9/3	222	216	100	N/A	N/A	N/A	N/A	N/A	11.78	2.63	
9/3	223	217	100	N/A	N/A	N/A	N/A	N/A	17.01	3.89	
9/3	224	218	100	N/A	N/A	N/A	N/A	N/A	12.83	2.57	
9/3	225	219	100	N/A	N/A	N/A	N/A	N/A	20.87	4.90	

Bottles collected from depth 1 (surface): D19, D20, 214, 215

Bottles collected from depth 2 (via Van Dorn): 216, 217

Bottles collected from depth 3 (via Van Dorn): 218, 219

Trilogy measurements were made on September 13, 2011

A.13 Filter data from September 11, 2011

Date	Filter #	From bottle #	Filt'd (mL)	Wt empty (mg)	Wt wet (mg)	Wt dry (mg)	Total (mg)	In oven	Chl a (ug/L)	Pheo (ug/L)	Notes
9/7	244	D21	250	136.3	864.7	142.8	6.50	9/11	N/A	N/A	
9/7	245	D21	100	N/A	N/A	N/A	N/A	N/A	5.76	2.36	
9/7	246	D22	250	135.9	871.6	142.1	6.20	9/11	N/A	N/A	
9/7	247	D22	100	N/A	N/A	N/A	N/A	N/A	8.95	2.43	
9/11	232	232	250	135.9	883.4	139.1	3.20	9/11	N/A	N/A	
9/11	233	233	250	136.0	873.4	139.2	3.20	9/11	N/A	N/A	
9/11	234	234	250	135.5	878.8	139.3	3.80	9/11	N/A	N/A	
9/11	235	235	250	135.3	843.8	138.8	3.50	9/11	N/A	N/A	
9/11	236	236	250	135.4	871.5	140.0	4.60	9/11	N/A	N/A	
9/11	237	237	250	136.2	857.0	139.9	3.70	9/11	N/A	N/A	
9/11	238	232	100	N/A	N/A	N/A	N/A	N/A	10.76	3.45	
9/11	239	233	100	N/A	N/A	N/A	N/A	N/A	10.32	3.38	
9/11	240	234	100	N/A	N/A	N/A	N/A	N/A	9.96	3.75	
9/11	241	235	100	N/A	N/A	N/A	N/A	N/A	14.63	-6.10	Bad sample
9/11	242	236	100	N/A	N/A	N/A	N/A	N/A	7.71	2.88	
9/11	243	237	100	N/A	N/A	N/A	N/A	N/A	5.40	2.31	
9/11	248	232	250	137.4	913.7	140.4	3.00	Sept 11	N/A	N/A	
9/11	249	233	250	136.0	872.6	139.1	3.10	Sept 11	N/A	N/A	
9/11	250	234	250	134.8	884.1	138.2	3.40	Sept 11	N/A	N/A	
9/11	251	235	250	136.1	880.9	150.4	14.30	Sept 11	N/A	N/A	Hair on filter?
9/11	252	236	250	134.4	854.8	138.1	3.70	Sept 11	N/A	N/A	
9/11	253	237	250	136.2	846.4	140.2	4.00	Sept 11	N/A	N/A	

Bottles collected from depth 1 (surface): D21, D22, 232, 233

Bottles collected from depth 2 (via Van Dorn): 234, 235

Bottles collected from depth 3 (via Van Dorn): 236, 237

Trilogy measurements were made on September 13, 2011

APPENDIX B – WATER PROPERTY DATA

B.1 Hydrolab/CDOM data

Date	Depth (m)	Temp (C)	SpCond (uS/cm)	TDS (g/L)	ORP (mV)	pH	DO % (Sat)	DO (mg/L)	TurbSC (NTUs)	PCY (cell/mL)	CHL (ug/L)	CDOM (mV)	CDOM (ppm)
6/15	0.1	21.93	425.4	0.2722	405	8.68	26.9	1.96	27.2	5191	25.53	1040	8.01
6/15	0.2	21.94	428.7	0.2744	406	8.67	27.1	1.97	27.3	5436	24.09	1037	7.98
6/15	0.3	21.96	431.9	0.2764	406	8.7	26.6	1.94	26.9	5512	25.67	1045	8.05
6/15	0.4	21.91	435.3	0.2786	406	8.7	26.6	1.93	29.7	5552	30.54	1052	8.12
6/15	0.5	21.92	441.8	0.2827	406	8.73	26.1	1.9	28.5	5545	30.48	1072	8.29
6/15	0.6	21.91	445.3	0.285	406	8.73	25.3	1.85	28.9	5568	33.34	1067	8.25
6/15	0.7	21.92	440.4	0.2819	407	8.71	25	1.82	32.3	5148	35.46	1070	8.28
6/15	0.8	21.81	443.1	0.2836	407	8.7	24.3	1.78	26.2	5489	36.93	1065	8.23
6/23	0.1	22.68	403	0.2579	348	8.1	21.9	1.57	165.5	5904	13.45	1038	7.99
6/23	0.2	22.6	405	0.2592	345	8.09	22	1.58	164.1	5208	12.87	1043	8.04
6/23	0.3	22.61	406.7	0.2603	343	8.1	21.6	1.55	163.1	5524	12.71	1039	8
6/23	0.4	22.6	403.8	0.2584	337	8.09	20.9	1.5	155.7	5350	12.65	1050	8.1
6/23	0.5	22.57	406	0.2598	336	8.07	20.8	1.49	152.5	4826	12.16	1059	8.18
6/23	0.6	22.58	404.8	0.2591	335	8.07	20.2	1.45	162	5225	12.99	1054	8.13
6/23	0.7	22.59	403.1	0.258	334	8.07	20.2	1.45	170.9	5810	13.92	1013	7.77
6/23	0.8	22.6	405.9	0.2598	333	8.07	19.8	1.42	170.7	6153	15.83	1056	8.15
7/1	0.1	26.08	373.2	0.2388	360	8.98	28.9	1.95	30.2	3522	N/A	659	4.64
7/1	0.2	25.83	366.4	0.2345	360	8.99	28.6	1.93	28.2	3618	11.4	653	4.59
7/1	0.3	25.6	365.3	0.2338	360	8.98	28.3	1.93	27.8	3460	11.26	654	4.59
7/1	0.4	24.55	348.1	0.2228	360	9.03	27.5	1.91	25.8	3408	10.15	524	3.44
7/1	0.5	23.43	312.7	0.2001	359	9.09	26.9	1.9	20.8	3158	9.6	426	2.58
7/1	0.6	22.53	304.8	0.1951	359	9.09	26.3	1.89	24.3	3288	13.04	382	2.19
7/1	0.7	22.69	308.7	0.1976	359	9.09	25.9	1.86	27.7	3619	12.2	394	2.29
7/9	0.1	29.15	332.3	0.2127	341	9.19	28.2	1.8	20	3656	15.76	398	2.33
7/9	0.2	29.2	329.5	0.2109	343	9.14	26.1	1.66	20.2	4038	15.34	394	2.29
7/9	0.3	29	326.9	0.2092	345	9.15	26.7	1.71	21.3	4027	17.85	388	2.24
7/9	0.4	28.25	325.7	0.2085	346	9.13	25.9	1.68	22.2	4480	18.2	385	2.21
7/9	0.5	27.82	325	0.208	347	9.13	25.1	1.64	22.8	4512	20.38	382	2.19
7/9	0.6	27.76	316.6	0.2026	348	9.1	24.3	1.59	24	4877	21.04	381	2.18
7/9	0.7	25.85	312.5	0.2	355	8.93	23.6	1.6	37.4	4545	19.59	370	2.08
7/9	0.8	25.81	312.5	0.2	356	8.92	23	1.56	36.8	4235	20.64	392	2.28
7/9	0.9	25.81	311.8	0.1996	356	8.92	22.6	1.53	35.8	4186	21.75	383	2.2
7/17	0.1	28.61	382.6	0.2449	351	8.96	23.4	1.51	33.3	9906	31.31	901	6.78
7/17	0.2	28.11	381.1	0.2439	354	8.86	23.2	1.51	33.3	9530	31.29	894	6.72

7/17	0.3	27.85	381.3	0.244	356	8.82	22.8	1.49	32.5	9288	34.39	906	6.82
7/17	0.4	27.8	382.1	0.2445	358	8.82	22.5	1.47	32	10097	37.73	912	6.88
7/17	0.5	27.74	382.6	0.2449	360	8.75	22.2	1.45	34.1	9266	38.01	927	7.01
7/17	0.6	27.73	383.1	0.2452	362	8.72	22.1	1.45	37.3	9589	39.85	928	7.02
7/17	0.7	27.68	384.3	0.246	363	8.69	21.9	1.43	36.1	8532	42.97	944	7.16
7/17	0.8	27.58	384.6	0.2461	365	8.64	21.8	1.43	35.1	8111	46.74	944	7.16
7/25	0.1	28.08	423	0.2707	397	8.49	18.5	1.21	20.5	7835	28.86	1289	10.21
7/25	0.2	28	422.6	0.2704	398	8.48	18.6	1.21	20.9	7675	31.23	1291	10.23
7/25	0.3	27.97	422.5	0.2704	398	8.47	18.1	1.18	22.1	8394	32.25	1291	10.23
7/25	0.4	27.91	421.9	0.27	398	8.45	17.7	1.16	21.5	7718	30.87	1293	10.25
7/25	0.5	27.88	421.7	0.2699	398	8.44	17.6	1.14	20.2	8178	31.22	N/A	N/A
7/25	0.6	27.84	421.6	0.2698	398	8.43	17.4	1.13	20.7	7287	31.17	N/A	N/A
7/25	0.7	27.8	421.8	0.2699	398	8.41	17.2	1.12	21.5	6917	30.87	N/A	N/A
8/2	0.1	28	465.9	0.2982	383	8.34	17.2	1.12	17.7	8413	25.65	1356	10.81
8/2	0.2	27.6	466.2	0.2983	384	8.27	15.4	1.01	19.1	7698	25.43	1394	11.14
8/2	0.3	27.54	466.4	0.2983	384	8.26	14.8	0.97	18.3	6846	26.67	1407	11.26
8/2	0.4	27.49	466.2	0.2983	384	8.25	14.5	0.95	18	6167	26.06	1431	11.47
8/2	0.5	27.38	466.2	0.2984	384	8.25	14.3	0.94	18.6	6453	25.64	1418	11.35
8/2	0.6	27.32	466.2	0.2984	383	8.24	13.9	0.92	19.7	6305	24.49	1408	11.27
8/2	0.7	27.29	466.3	0.2984	381	8.24	13.3	0.87	19.9	5125	25.35	1410	11.28
8/10	0.1	25.72	482.5	0.3088	402	8.28	15.2	1.03	24.2	8428	39.98	875	6.55
8/10	0.2	25.77	481.7	0.3083	403	8.29	14.5	0.98	23.9	8276	41.62	879	6.59
8/10	0.3	25.71	482.3	0.3087	403	8.27	13.8	0.94	26.8	6706	39.3	941	7.13
8/10	0.4	25.62	482.5	0.3088	403	8.23	13	0.88	33	7116	37.95	956	7.27
8/10	0.5	25.53	481.9	0.3084	402	8.22	12.5	0.85	34.5	6125	35.35	979	7.47
8/10	0.6	25.45	481.7	0.3083	400	8.22	12.2	0.82	34.8	6710	36.46	1009	7.74
8/10	0.7	25.43	482.1	0.3085	398	8.22	11.7	0.8	35.5	5899	42.01	947	7.19
8/18	0.1	25.12	460.3	0.2946	419	8.48	17.9	1.23	25.4	4204	14.27	1073	8.3
8/18	0.2	24.79	459.6	0.2942	420	8.44	17.7	1.22	25.3	6568	22.5	985	7.52
8/18	0.3	24.24	460.1	0.2945	420	8.43	17.8	1.24	24.9	6989	23.74	1181	9.26
8/18	0.4	23.93	460.1	0.2945	421	8.41	17.7	1.24	25.1	6801	24.25	939	7.12
8/18	0.5	23.79	460.4	0.2947	421	8.39	17.5	1.23	25.4	6550	25.2	983	7.51
8/18	0.6	23.72	460.3	0.2948	422	8.38	17.2	1.21	25.5	7312	26.81	890	6.68
8/18	0.7	23.7	460.6	0.2948	422	8.36	17.1	1.2	25.8	6684	26.8	1201	9.43
8/26	0.1	25.03	458.4	0.2933	400	8.39	31.7	2.18	21	8082	17.49	1652	13.43
8/26	0.2	25.01	458	0.2931	400	8.39	30.5	2.1	21.6	8131	21.95	1631	13.24
8/26	0.3	24.91	458.5	0.2934	400	8.37	30.3	2.09	22.3	8402	24.57	1637	13.29
8/26	0.4	24.82	458.4	0.2934	400	8.36	29.9	2.06	23	7650	25.81	1697	13.82
8/26	0.5	24.79	458.4	0.2934	400	8.36	28.6	1.97	23.2	7390	26.34	1734	14.15
8/26	0.6	24.74	458.4	0.2934	400	8.33	28.9	1.99	23.1	6429	23.2	1740	14.2

8/26	0.7	24.7	458.6	0.2935	390	8.32	31.8	2.2	150.8	7831	21.69	1814	14.86
9/3	0.1	28.19	464.7	0.2974	398	8.43	40.6	2.63	17.3	3757	10.63	1250	9.87
9/3	0.2	27.48	465.2	0.2977	399	8.36	38.4	2.52	17.7	3540	12.58	1264	9.99
9/3	0.3	26.79	462.9	0.2963	400	8.38	42.6	2.83	18.7	6514	18.12	1247	9.84
9/3	0.4	26.34	462.7	0.2961	399	8.39	44.1	2.96	18.6	5025	N/A	1267	10.02
9/3	0.5	26.23	461.8	0.2955	399	8.38	45.6	3.07	17.4	4372	17.05	1275	10.09
9/3	0.6	26.2	462.1	0.2957	399	8.37	40.5	2.72	16.8	3964	15.18	1271	10.05
9/3	0.7	26.06	461.9	0.2956	399	8.36	40.7	2.74	16.6	3835	14.51	1283	10.16
9/11	0.1	20.11	369.6	0.2366	398	8.13	52.6	3.97	13.7	2007	8.62	1148	8.97
9/11	0.2	20.11	369.1	0.2362	398	8.12	56.5	4.26	13.7	2267	8.34	1149	8.97
9/11	0.3	20.11	369.8	0.2366	398	8.12	63.6	4.8	13.9	1935	8.12	1148	8.97
9/11	0.4	20.12	369.6	0.2366	397	8.12	65.2	4.92	14.1	2120	7.86	1148	8.97
9/11	0.5	20.12	369.6	0.2366	396	8.12	57.7	4.35	13.9	2063	8.09	1149	8.97
9/11	0.6	20.12	369.2	0.2363	393	8.12	59.8	4.51	13.8	2111	8.24	1143	8.92
9/11	0.7	20.12	369.6	0.2366	392	8.12	55.9	4.22	14.4	1915	8.53	1139	8.89
9/11	0.8	20.12	369.6	0.2366	390	8.12	60.4	4.56	15.5	2200	8.69	1137	8.87

B.2 OWC sonde data

Date*	Depth (m)	Temp (C)	SpCond	pH	DO (%)	DO (mg/l)	Turb
6/15	0.64	22.5	470	8.3	131.4	11.4	23
6/23	0.54	21.8	400	7.4	49.3	4.3	171
7/1	0.52	23.3	310	8.5	108.2	9.2	10
7/9	0.32	27.1	330	8.1	107	8.5	13
7/17	0.51	27.9	380	8.1	106.8	8.4	18
7/25	0.57	28	440	7.6	50.2	3.9	16
8/2	0.57	27.5	480	7.4	25.9	2.1	24
8/10	0.61	25.5	490	7.5	34	2.8	24
8/18	0.63	23.7	470	7.6	58.5	5	17
8/26	0.6	24.9	480	7.6	61.4	5.1	18
9/3	0.56	26.2	480	7.5	51.8	4.2	19
9/11	0.76	20.1	460	7.4	24	2.2	17

* This sonde was located at the WM site and made a recording every 15 minutes. All recordings were time-stamped. The recordings shown here were extracted from the nearest 15-minute interval to the time when field measurements were collected (see Appendix B.1).

B.3 Depth recordings

Date	Bottom depth (cm)	Water collection depth #1 (cm)	Water collection depth #2 (cm)	Water collection depth #3 (cm)
June 15	85	0	28.1	56.1
June 23	90	0	29.7	59.4
July 1	85	0	28.1	56.1
July 9	104	0	34.3	68.6
July 17	96	0	31.7	63.4
July 25	96	0	31.7	63.4
Aug 2	100	0	33.0	66
Aug 10	100	0	33.0	66
Aug 18	91	0	30.0	60.1
Aug 26	94	0	31.0	62.0
Sept 3	99	0	32.7	65.3
Sept 11	109	0	36.9	72.0

Date	Secchi depth (cm)
June 15	40
June 23	20
July 1	34
July 9	50
July 17	36
July 25	45
Aug 2	46
Aug 10	40
Aug 18	38
Aug 26	46
Sept 3	46
Sept 11	55

APPENDIX C – MOUTH BAR PROGRESSION

June 15, 2011

June 23, 2011

July 1, 2011

July 9, 2011

July 17, 2011

July 25, 2011

August 10, 2011

August 18, 2011

August 26, 2011

September 3, 2011

September 11, 2011

APPENDIX D – PCA REFLECTANCE SPECTRA CORRELATIONS

Four components were determined using varimax-rotated principal component analysis (VPCA) on the full range of spectral data. Shown here are the correlations of each of these reflectance components (1-4, as well as variations of the differences of components) to all datasets. “TRILOGY” refers to Trilogy fluorometer data, “TURNER CDOM” refers to CDOM data, “HYDROLAB” refers to Hach hydrolab data, “MET DATA” refers to meteorological data collected from the OWC weather station, “WM SONDE” refers to monitoring sonde data. Also included are the derivatives of the 31 bands in the visible spectrum (400-700 nm, at 10 nm increments). Correlation values statistically significant at the 1% level are those greater than 0.74 and have been shaded pink. Correlation values statistically significant at the 5% level are those greater than 0.60 and have been shaded green.

COMPONENT	1	2	3	4	1-2	1-3	1-4	2-3	2-4	3-4
surface depth TRILOGY chl a (ug/L)	-0.69	-0.32	-0.25	0.34	-0.24	-0.30	-0.73	-0.06	-0.47	-0.41
intermediate depth TRILOGY chl a (ug/L)	-0.29	-0.77	-0.17	0.04	0.37	-0.08	-0.23	-0.44	-0.59	-0.15
deep depth TRILOGY chl a (ug/L)	-0.31	-0.51	-0.13	-0.12	0.16	-0.12	-0.15	-0.28	-0.30	-0.02
surface depth TRILOGY pheophytin and phaeophorbid (ug/L)	0.21	-0.29	0.24	-0.17	0.36	-0.02	0.27	-0.38	-0.10	0.29
intermediate depth TRILOGY pheophytin and phaeophorbid (ug/L)	-0.37	-0.72	-0.16	0.26	0.27	-0.15	-0.45	-0.42	-0.71	-0.29
deep depth TRILOGY pheophytin and phaeophorbid (ug/L)	0.09	-0.37	0.23	-0.12	0.34	-0.10	0.15	-0.43	-0.20	0.25
surface depth TURNER CDOM (ppm)	-0.29	0.59	-0.46	0.16	-0.64	0.12	-0.32	0.75	0.33	-0.44
intermediate depth TURNER CDOM (ppm)	-0.33	0.58	-0.44	0.24	-0.66	0.08	-0.41	0.73	0.27	-0.49
deep depth TURNER CDOM (ppm)	-0.35	0.54	-0.50	0.13	-0.64	0.11	-0.34	0.74	0.31	-0.45
surface depth HYDROLAB chl a (ug/L)	-0.58	-0.58	-0.21	0.34	0.02	-0.26	-0.65	-0.28	-0.65	-0.38
intermediate depth HYDROLAB chl a (ug/L)	-0.53	-0.51	-0.41	0.43	0.01	-0.07	-0.68	-0.09	-0.67	-0.60
deep depth HYDROLAB chl a (ug/L)	-0.49	-0.65	-0.37	0.33	0.14	-0.08	-0.58	-0.22	-0.71	-0.50
surface depth HYDROLAB PCY (cell/mL)	-0.68	-0.45	-0.36	0.03	-0.14	-0.21	-0.51	-0.07	-0.35	-0.28
intermediate depth HYDROLAB PCY (cell/mL)	-0.51	-0.28	-0.52	0.16	-0.15	0.01	-0.47	0.16	-0.31	-0.48
deep depth HYDROLAB PCY (cell/mL)	-0.59	-0.54	-0.32	0.16	-0.01	-0.19	-0.53	-0.18	-0.51	-0.34
surface depth HYDROLAB temp (Celsius)	0.24	-0.68	-0.24	0.21	0.67	0.33	0.03	-0.33	-0.65	-0.32
intermediate depth HYDROLAB temp (Celsius)	0.20	-0.74	-0.22	0.19	0.68	0.29	0.01	-0.39	-0.68	-0.29

COMPONENT	1	2	3	4	1-2	1-3	1-4	2-3	2-4	3-4
deep depth HYDROLAB temp (Celsius)	0.03	-0.62	-0.29	0.23	0.48	0.22	-0.14	-0.25	-0.62	-0.37
surface depth HYDROLAB Cond (uS/c)	-0.45	0.32	-0.39	0.57	-0.55	-0.04	-0.72	0.51	-0.14	-0.67
intermediate depth HYDROLAB Cond (uS/c)	-0.47	0.34	-0.37	0.55	-0.58	-0.07	-0.72	0.50	-0.12	-0.65
deep depth HYDROLAB (uS/c)	-0.50	0.37	-0.39	0.52	-0.62	-0.08	-0.72	0.54	-0.08	-0.64
surface depth HYDROLAB TDS (gal/L)	-0.45	0.32	-0.39	0.57	-0.55	-0.04	-0.72	0.51	-0.14	-0.67
intermediate depth HYDROLAB TDS (gal/L)	-0.47	0.34	-0.37	0.55	-0.58	-0.07	-0.72	0.51	-0.12	-0.65
deep depth HYDROLAB TDS (gal/L)	-0.50	0.37	-0.39	0.52	-0.62	-0.08	-0.72	0.54	-0.08	-0.64
surface depth HYDROLAB ORP (mV)	-0.12	0.51	-0.39	0.72	-0.46	0.18	-0.58	0.64	-0.10	-0.77
intermediate depth HYDROLAB ORP (mV)	-0.08	0.45	-0.43	0.74	-0.39	0.24	-0.57	0.63	-0.16	-0.82
deep depth HYDROLAB ORP (mV)	-0.04	0.35	-0.49	0.76	-0.29	0.31	-0.55	0.59	-0.25	-0.87
surface depth HYDROLAB pH	0.49	-0.79	-0.08	-0.19	0.93	0.40	0.49	-0.52	-0.46	0.07
intermediate depth HYDROLAB pH	0.51	-0.77	-0.06	-0.20	0.92	0.39	0.51	-0.52	-0.43	0.10
deep depth HYDROLAB pH	0.55	-0.72	-0.01	-0.21	0.91	0.39	0.54	-0.52	-0.39	0.13
surface depth HYDROLAB DO (mg/L)	0.56	0.60	-0.10	-0.31	-0.05	0.45	0.61	0.51	0.65	0.14
intermediate depth HYDROLAB DO (mg/L)	0.52	0.61	-0.05	-0.25	-0.09	0.39	0.55	0.48	0.62	0.14
deep depth HYDROLAB DO (mg/L)	0.54	0.60	-0.05	-0.25	-0.07	0.40	0.56	0.48	0.62	0.13
surface depth HYDROLAB Turb(NTU)	-0.51	0.22	0.54	-0.56	-0.52	-0.73	0.02	-0.22	0.54	0.77
intermediate depth HYDROLAB Turb(NTU)	-0.51	0.22	0.54	-0.55	-0.52	-0.73	0.01	-0.21	0.53	0.77

COMPONENT	1	2	3	4	1-2	1-3	1-4	2-3	2-4	3-4
deep depth HYDROLAB Turb(NTU)	-0.55	0.19	0.52	-0.55	-0.52	-0.74	-0.02	-0.22	0.50	0.75
MET DATA comp1	0.39	-0.67	0.00	-0.46	0.76	0.26	0.60	-0.49	-0.19	0.32
MET DATA comp 2	-0.25	0.38	-0.56	0.47	-0.45	0.22	-0.50	0.66	-0.04	-0.73
MET DATA comp 3	-0.11	-0.53	-0.29	0.17	0.32	0.13	-0.19	-0.18	-0.50	-0.33
MET DATA comp 4	-0.51	0.26	0.52	-0.52	-0.55	-0.71	-0.01	-0.17	0.54	0.73
MET DATA standard air temp (Celsius)	0.42	-0.35	-0.21	0.32	0.55	0.43	0.08	-0.12	-0.48	-0.37
WM SONDE temp (Celsius)	0.08	-0.66	-0.33	0.27	0.54	0.28	-0.13	-0.25	-0.67	-0.42
WM SONDE spec cond (uS/c)	-0.27	0.45	-0.49	0.64	-0.52	0.16	-0.63	0.67	-0.09	-0.79
WM SONDE salinity	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WM SONDE depth (m)	-0.42	0.70	-0.10	0.30	-0.81	-0.22	-0.51	0.58	0.32	-0.28
WM SONDE pH	0.36	-0.71	0.05	-0.50	0.78	0.21	0.60	-0.56	-0.19	0.38
WM SONDE turbidity (NTUs)	-0.51	0.28	0.56	-0.51	-0.56	-0.74	-0.02	-0.18	0.55	0.75
WM SONDE DO (%)	0.31	-0.62	-0.03	-0.54	0.67	0.23	0.59	-0.43	-0.10	0.35
WM SONDE Comp 1	0.26	-0.63	0.02	-0.57	0.65	0.17	0.58	-0.48	-0.09	0.40
WM SONDE Comp 2	-0.33	0.44	-0.53	0.44	-0.56	0.14	-0.54	0.69	0.03	-0.69
WM SONDE Comp 3	0.01	-0.61	-0.35	0.22	0.46	0.25	-0.15	-0.21	-0.61	-0.40
WM SONDE Comp 4	-0.50	0.19	0.54	-0.54	-0.49	-0.72	0.01	-0.24	0.50	0.76
drdl400	0.69	0.51	0.24	0.01	0.10	0.31	0.50	0.21	0.38	0.17
drdl410	0.79	0.54	0.12	-0.16	0.15	0.46	0.68	0.31	0.51	0.19
drdl420	0.87	0.36	0.22	-0.13	0.33	0.45	0.72	0.12	0.36	0.25
drdl430	0.91	0.35	0.11	-0.12	0.37	0.54	0.74	0.18	0.34	0.16
drdl440	0.96	0.14	0.00	-0.07	0.56	0.65	0.74	0.10	0.16	0.05
drdl450	0.92	-0.20	0.05	0.13	0.78	0.60	0.57	-0.18	-0.23	-0.06
drdl460	0.90	-0.07	-0.13	0.28	0.67	0.71	0.45	0.04	-0.24	-0.29

COMPONENT	1	2	3	4	1-2	1-3	1-4	2-3	2-4	3-4
drdl470	0.90	-0.03	-0.13	0.29	0.65	0.71	0.45	0.07	-0.22	-0.29
drdl480	0.96	0.10	-0.14	0.14	0.60	0.76	0.60	0.17	-0.02	-0.20
drdl490	0.94	0.11	-0.30	0.09	0.58	0.85	0.62	0.29	0.02	-0.27
drdl500	0.84	-0.17	-0.45	0.19	0.71	0.89	0.47	0.19	-0.26	-0.46
drdl510	0.68	-0.46	-0.43	0.30	0.81	0.77	0.28	-0.03	-0.54	-0.52
drdl520	0.55	-0.55	-0.28	0.47	0.79	0.57	0.07	-0.21	-0.72	-0.52
drdl530	0.43	-0.25	-0.27	0.73	0.49	0.48	-0.19	0.00	-0.68	-0.69
drdl540	0.24	0.10	-0.36	0.83	0.09	0.42	-0.39	0.33	-0.48	-0.83
drdl550	-0.01	0.19	-0.07	0.93	-0.14	0.05	-0.65	0.19	-0.48	-0.69
drdl560	0.01	0.53	0.47	0.64	-0.39	-0.33	-0.43	0.06	-0.03	-0.09
drdl570	0.10	0.79	0.51	0.16	-0.52	-0.29	-0.04	0.22	0.48	0.26
drdl580	0.11	0.95	0.24	0.07	-0.62	-0.09	0.03	0.52	0.66	0.12
drdl590	-0.03	0.87	0.32	0.26	-0.66	-0.24	-0.20	0.41	0.48	0.06
drdl600	0.02	0.54	0.78	-0.02	-0.39	-0.53	0.03	-0.14	0.42	0.58
drdl610	0.16	0.83	0.43	-0.26	-0.50	-0.19	0.29	0.31	0.79	0.49
drdl620	0.20	0.96	-0.01	-0.13	-0.58	0.14	0.23	0.71	0.81	0.09
drdl630	-0.10	0.84	-0.30	0.40	-0.69	0.14	-0.34	0.82	0.36	-0.49
drdl640	-0.14	0.09	0.89	0.22	-0.17	-0.72	-0.25	-0.55	-0.08	0.50
drdl650	0.01	0.19	0.89	-0.31	-0.13	-0.61	0.22	-0.48	0.35	0.86
drdl660	0.11	0.65	0.45	-0.57	-0.41	-0.24	0.47	0.16	0.86	0.71
drdl670	0.29	0.81	-0.38	-0.33	-0.40	0.46	0.43	0.85	0.82	-0.05
drdl680	0.10	0.05	-0.96	0.20	0.03	0.73	-0.07	0.70	-0.10	-0.83
drdl690	-0.31	-0.64	-0.53	0.42	0.25	0.16	-0.52	-0.09	-0.76	-0.67
drdl700	-0.44	-0.75	-0.18	0.44	0.25	-0.17	-0.62	-0.42	-0.85	-0.44
reflectance component 1		0.03	-0.05	-0.01	0.67	0.72	0.73	0.06	0.03	-0.03

COMPONENT	1	2	3	4	1-2	1-3	1-4	2-3	2-4	3-4
reflectance component 2			0.02	0.00	-0.72	0.01	0.02	0.72	0.74	0.01
reflectance component 3				-0.01	-0.04	-0.73	-0.03	-0.68	0.02	0.73
reflectance component 4					-0.01	0.00	-0.69	0.01	-0.67	-0.69
Surface Ref 1-2						0.49	0.49	-0.49	-0.53	-0.03
Surface Ref 1-3							0.52	0.51	0.01	-0.53
Surface Ref 1-4								0.03	0.48	0.45
surface Ref 2-3									0.53	-0.50
surface Ref 2-4										0.47
Surface ref 3-4										

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