TEMPORAL AND SPATIAL GRADIENTS IN PHYTOPLANKTON PIGMENTS IN THE TAR-PAMLICO RIVER ESTUARY

by

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ABSTRACT

Phytoplankton form the base of most aquatic food webs, and their biomass and the composition of their communities directly impact upper trophic levels. Phytoplankton chlorophyll a concentrations in aquatic environments have been long used to estimate phytoplankton biomass, and the concentrations of accessory chlorophylls and carotenoids used to diagnose the taxa composing the phytoplankton community of an area. In this study, the photopigments present in water samples collected at eleven sites from October to December 2016 along the Tar-Pamlico River estuary were measured using high performance liquid chromatography (HPLC). From the concentrations of these pigments, phytoplankton biomass and community composition was estimated along the spatial gradient of the freshwater end of the Tar-Pamlico River estuary to the mouth of the Tar-Pamlico River. Chlorophyll a concentrations were found to be significantly lower in upstream stations than downstream stations. Concentrations of accessory pigments varied between stations and dates sampled, but no consistent trends were observed over time or space. Changes in pigment concentrations were compared to environmental characteristics such as water temperature, salinity, and dissolved nutrient content to determine the magnitude of their influence on the phytoplankton community. No strong correlations were observed between any combination of these factors and pigment concentrations of samples.

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INTRODUCTION

Phytoplankton function as the primary producers for aquatic food webs, directly and indirectly influencing the energy and biomass available in aquatic systems. However, the way and the extent to which phytoplankton production affects the food web are dependent on the phytoplankton community structure (Cloern 2001). Certain phytoplankton taxa are preferentially grazed on by zooplankton, suspension feeders, and deposit feeders, while the ungrazed phytoplankton taxa provides an input of energy for the aquatic microbial community (Baird and Ulanowicz 1989).

The structure of phytoplankton communities has far broader impacts than simple trophic interactions, though. Phytoplankton photosynthesis is partially responsible for the dissolved oxygen concentration in the water, which is required for fish and invertebrate survival (Los and Wisjman 2007). Under particular environmental conditions, several taxa of phytoplankton reproduce at a rate much faster than the consumption by their grazers, causing blooms. Microbial respiration from the decomposition of ungrazed phytoplankton blooms can cause anoxic bottom waters, choking out benthic communities (Cloern 2001; Kemp et al. 2005). The increase in turbidity caused by phytoplankton blooms on the water surface can also limit seagrasses access to light, eventually killing them (Greening and Janicki 2006). Additionally, certain phytoplankton taxa produce toxins that under high cell concentrations, like blooms, are responsible for fish kills (Glibert et al. 2005). The number of phytoplankton blooms and subsequent anoxic conditions has increased lately worldwide and are responsible for more than 245,000 square kilometers of coastal waters being effectively devoid of life, affecting more than 400 aquatic systems (Diaz and Rosenburg 2008).

A diverse, changing phytoplankton community structure seems counterintuitive, according to ecological theory. Phytoplankton have an apparently simple habitat consisting of little physical structure, and only two nutrients, nitrogen and phosphorus, usually limit their growth (Ryther et al. 1971). How then can the structure of a phytoplankton community be so variable? The answer lies in the complex physical processes that create a dynamic, rather than simple, habitat and drive the availability of resources for phytoplankton (Hutchinson 1961). Spatially and temporally variable environmental conditions such as salinity, temperature, flow rates, and nutrient and light availability all directly affect phytoplankton community structure (Harding 1994). In estuaries, the interfaces between rivers and the ocean, strong gradients are present in all of these environmental factors (Harding 1994). Their interaction result in variable conditions for phytoplankton growth both spatially and temporally across estuaries (Harding 1994).

Phytoplankton growth is primarily limited by nitrogen in marine systems and phosphorus in freshwater systems, but estuaries represent an unique environment where these two systems meet (Ryther et al. 1971). In the Chesapeake Bay, the largest estuary in the United States, nutrient limitation varies seasonally, with phosphorus often limiting growth in the spring, and nitrogen during the summer (Malone et al. 1996). In contrast, estuaries in North Carolina are typically nitrogen limited, with phosphorus occasionally co-limiting phytoplankton growth (Mallin 1994).

The overall availability of both of these nutrients is dictated by the flow rate and discharge to the estuary, as riverine discharge is the primary input of nutrients into estuarine systems (Hall et al. 2013). However, increased river flow simultaneously

provides nutrients to the phytoplankton community while flushing phytoplankton cells to the sea (Hall et al. 2013). Consequently, maximum estuarine phytoplankton biomass has been found at moderate discharge rates, and the flushing time of estuaries have been shown to be an indicator of phytoplankton community composition, as different rates of flushing give certain taxa advantages over others (Hall et al. 2013). The rainfall responsible for estuarine discharge rates is associated with a variety of other factors that can also cause changes to the phytoplankton community structure, though, such as wind mixing of the water column, cloud cover, and wind and wave driven resuspension of benthic sediments (Cloern and Dufford 2005). These factors complicate the relationship between the phytoplankton community and estuarine discharge, making it nearly impossible to predict the phytoplankton community structure based on any sole variable. It is important that the effect of discharge rates on the phytoplankton community be understood, though, as predictions of increased storm frequency and subsequently increased discharge are a part of climate change (Goldenberg et al. 2001; Webster et al. 2005).

Since taxa of phytoplankton respond differently to these conditions of nutrient availability, light availability, temperature, salinity, estuarine discharge, etc., the environment plays a huge role in phytoplankton community structure. Increased nutrient availability leads to increased phytoplankton biomass, which has subsequently been linked to alterations to phytoplankton community structure, species evenness, and species richness (Pickney et al. 1999; Tsirtsis and Karydis 1998).

Differing growth rates and abilities to take up nutrients results in certain taxa such as fast-growing, negatively buoyant diatoms flourishing in nutritious, well-mixed

waters; and slower-growing, motile dinoflagellates thriving when nutrients are less concentrated and the water column more stratified (Smayda 1997). Salinity also impacts the structure of phytoplankton communities, with some species being more tolerant than others to highly saline waters (Quinlan and Philips 2007).

The environmental conditions across North Carolina's estuaries lend themselves to certain pervasive trends in their phytoplankton communities. Diatoms tend to dominate North Carolina's sounds, and up into the saline portion of its river estuaries (Mallin 1994). Studies in the New River Estuary have found them to reach peak abundance in the spring and summer, rarely reaching the biomass of a full bloom (Pickney et al. 1998). Dinoflagellates and cryptomonads most frequently bloom in the late winter to early spring, primarily following high-preciptation winters that enhance nitrogen loading to the estuaries (Mallin 1994; Pickney et al. 1999). Dinoflagellates may also bloom in the fall when calm, sunny periods lead to stratification of the water column (Hall et al. 2008).

Human development along watersheds is vastly changing the composition and function of estuarine phytoplankton communities, though (Vitousek et al. 1997). Over half of the human population lives in the coastal zone, and our impact on estuarine systems is increasing as watersheds become more and more developed (Vitousek et al. 1997). The concentrations of estuarine nitrogen and phosphorus responsible for seasonal changes in phytoplankton communities are 6-50 and 18-180 times greater, respectively, than they were before human development (Conley 2000). The combustion of fossil fuels, runoff of agricultural and commercial fertilizers, and industry waste all contribute to anthropogenic nutrient loading of estuaries (Galloway 2008). This nutrient loading led

to a dramatic increase in phytoplankton biomass and harmful algal bloom frequency in the Mid-Atlantic from 1999 to 2007; an increase that is predicted to continue to worsen (Bricker et al. 2008). Land clearing around estuaries also impacts the phytoplankton community by changing the estuarine discharge rates that are so intimately linked to the phytoplankton community's structure (Cooper and Brush 1993). Analysis of sediments in the Chesapeake Bay showed a drastic increase in anoxic conditions, eutrophication, and sedimentation rates following the initial land clearing by European settlers (Cooper and Brush 1993).

The phytoplankton community's sensitivity to environmental conditions, as well as the effects it has on aquatic systems, make the community's structure and overall abundance a good indicator of a system's health (Paerl et al. 2010). The National Oceanic and Atmospheric Administration and the U.S. Clean Water Act currently list phytoplankton community assessment as an indicator of ecosystems changes in estuaries (Garmendia et al. 2013; Bricker et al. 2008). However, to use the phytoplankton community as an indicator of environmental change, baseline conditions must be established for how an average phytoplankton community is structured (Domingues et al. 2008). Establishing this baseline of community structure is an urgent task, as intense, rapid development of watersheds are quickly making it more difficult to gather accurate reference conditions (Domingues et al. 2008).

Traditionally, light microscopy has been used to assess phytoplankton communities. This technique is time consuming, though and accurate description of certain phytoplankton taxa requires significant experience (Pan et al. 2011). A quicker, easier method is necessary to monitor a community rapidly changing in response to

anthropogenic nutrient inputs and altered river flows. Analysis of photopigments has become the standard method for quickly assessing the phytoplankton community (Pickney et al. 1998). Like terrestrial plants, nearly all phytoplankton use Chlorophyll *a* (Chl *a*) to capture the sun's energy, therefore concentrations of Chl *a* can be used to estimate phytoplankton biomass (Behrenfield and Falowski 1997). Additionally, phytoplankton use a variety of accessory photopigments to capture wavelengths of light outside of the range of Chl *a* (Gieskies and Kraay 1986). Many of these accessory pigments are particular to specific taxa, and consequently certain photopigments are diagnostic of the functional groups that compose a phytoplankton community (Pickney et al. 1998; Mackey et al. 1996).

Phytoplankton pigments can be measured in a variety of ways. For example, satellite remote sensing of ocean color can be used to study large-scale spatial distributions in Chl *a* concentrations, and consequently variations in phytoplankton biomass (McClain 2009). Satellites technology is limited by its ability to distinguish between individual accessory photopigments, though, and thus ocean color alone is not descriptive of the structure of phytoplankton communities (McClain 2009). A better assessment of the structure of phytoplankton communities can be done using the chemical technique high-performance liquid chromatography (HPLC) (Gieskies and Kraay 1986). HPLC can be used to separate, identify, and quantify the concentrations of pigments present in water samples (Mackety et al. 1996; Pickney et al. 1998).

The goal of the study was to use HPLC to quantify the spatial and temporal variability in phytoplankton biomass (as a measure of Chl *a*) and community composition (as a measure of other pigments) in North Carolina's Tar-Pamlico River estuary over the

fall of 2015, and attempt to relate environmental conditions these factors' variability. In doing this, we addressed the following questions: 1) Does the phytoplankton community vary spatially across the Tar-Pamlico River estuary? 2) Does the community vary temporally across the fall season? 3) What phytoplankton taxa are primarily responsible for this variability? 4) What, if any, environmental conditions seem to be driving this variability? Similar studies in North Carolina's New and Neuse River estuaries have found phytoplankton biomass to be largely related to nutrient loading, and community structure dependent on other factors, specifically temperature and river flow (Hall et al. 2013; Peirls et al. 2012).

MATERIALS AND METHODS

Study Site

Originating from the fourth largest drainage area in North Carolina, the Tar-Pamlico River drains a 15923 km^2 river basin, a mixture of agriculture (29%), wetlands (23%) and forested areas (27%) (Keith 2014; NCDWQ 2014). The river terminates at the 2685 km^2 Tar-Pamlico River estuary, which flows into the second largest estuarine system in the United States, the Pamlico Sound (NCDWQ 2014; NCDENR 1991). In 1989, frequent algal bloom-related fish kills caused the Tar-Pamlico River Basin to be declared a Nutrient Sensitive Water (NSW), resulting in the implementation of a nutrient management strategy to reduce the total nitrogen in the system by 30%, and maintain total phosphorus concentrations at a baseline level (NCDWQ 2014). Organic nitrogen input continues to rise in the likely nitrogen limited estuary, though, resulting in large areas of the estuary frequently exceeding the state's 40 $\mu g\ L^{-1}$ chl *a* concentration limit for NSWs (Piehler et al. 2004; Keith 2014). Runoff from agriculture, confined animal feeding operations (CAFOs), and highly erodible soils are the likely sources of this excess nitrogen input (Keith 2014). The Tar-Pamlico estuary also has a history of major hurricanes and tropical storms increasing the nitrogen and phosphorus loads in the system, due to the flooding and increased runoff associated with these events (Paerl et al. 2001).

Environmental Conditions

Sampling was conducted at eleven sites ranging from freshwater to mesohaline in the Tar-Pamilco River estuary on October 12th, October 29th, November 15th, and December 13^{th,} 2015 (Figure 1). Vertical profiles of salinity, temperature, and dissolved oxygen were measured at each station using a YSI Pro2030

DO/Conductivity/Temperature meter (YSI, Yellow Springs, OH). Surface water collected from each station was analyzed for nutrient content. Dissolved Kjeldahl nitrogen (DKN), nitrate (NO_3^-+ NO_2^-, referred to as NO_3), ammonium (NH_4^+), and orthophosphate (PO_4^{3-}) concentrations were determined colorometrically using a SmartChem 200 discreet analyzer (Westco Scientific Instruments Inc. 2008). Total dissolved phosphate (TDP) concentrations were determined chemically by persulfate digestion (Ameel et al. 1993). Due to inclement weather, sites 1 and 1A were not sampled on October 12th or December 13th. Due to physical constraints, temperature and salinity were not measured at site S.B. Also, due to equipment malfunction only nutrient data was collected on December 13th.

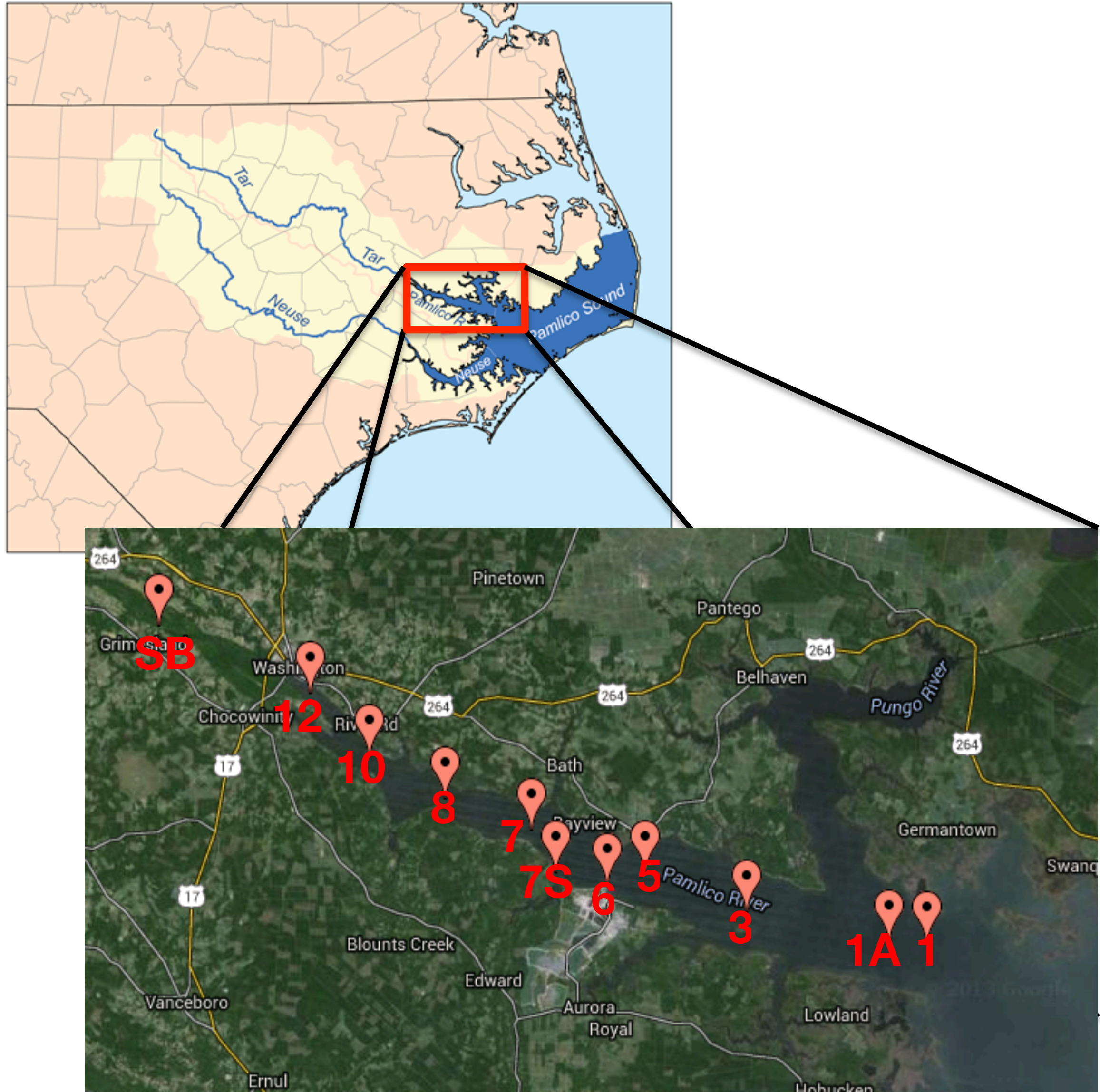

Figure 1. Map of study site on the Tar-Pamlico estuary. Sampling was conducted at eleven sites (labeled by pins) ranging from freshwater to mesohaline on October 12th, October 29th, November 15th, and December 13th, 2015.

Sample Collection and Photopigment Extraction

Sampling was conducted at eleven sites ranging from freshwater to mesohaline in the Tar-Pamilco River on October 12th, October 29th, November 15th, and December 13th, 2015 (Figure 1). Surface water was collected at each of these stations for pigment analysis. Due to inclement weather, sites 1 and 1A were not sampled on October 12th or December 13th, and due to time constraints site S.B. was not sampled on October 29th. To prevent photopigment degradation, water samples were stored in dark bottles, on ice during transport to the laboratory. Aliquots of water (200-450 mL) from each station were filtered onto Whatman GF/F glass fiber filters under vacuum in reduced light conditions, and immediately frozen at -20° C. Within 48 hours, these filters were then transferred to -80° C. Frozen filters were sonicated in 100% HPLC grade acetone (3 mL), pigments extracted in -20° C for 20-24 hours, and centrifuged. The supernatant containing the extracted photopigments was filtered into amber glass autosampler vials, which reduced light degradation of pigments during transport to the HPLC system.

Phytoplankton Photopigment Analysis

200 µL of pigment extracted from each sample was injected into the Shimadzu HPLC system (HPLC; system controller model CBM-20A; solvent delivery module LC-20AB) coupled with a UV/Vis photodiode array (PDA) spectrophotometric detector (PDA; Shimadzu model SPD-M20A; 200-800 nm; deuterium and tungsten lamps), using a non-linear 2-solvent gradient adapted from Van Heukelem et al. (1994). This non-linear, binary gradient was composed of Solvent A [80% methanol:20% ammonium acetate] and Solvent B [80% methanol:20% acetone]. The following series of C18 reverse-phase columns, adapted from Pickney et al., were used to separate the pigment extract into individual pigments: one Varian Dynamax Microsorb guard column (0.46 x

1.5cm; 3µm packing), followed by one monomeric reverse-phase C18 column (Varian Microsorb-MV; 0.46 x 10cm; 3µm packing, 100 Angstoms), followed by two polymeric reverse-phase C18 columns (Vydac 201TP5; 0.46 x 25cm, 5 µm packing) (1999). Separated pigments were passed though the PDA, and the absorbance of the extract from 380-700 nm was measured.

Shimadzu's EZStart software was used to collect and analyze HPLC data. Pigments were identified by retention times of peaks in the column, shape and signature of absorbance spectra, and visual matching of unknown peaks to those of known standards. Known quantities of pure pigment standards (peridinin, fucoxanthin, alloxanthin, chlorophyll b, chlorophyll a, and pheophytin a) were processed by HPLC to obtain these pigments' response factors. These response factors were multiplied by the peak area of each pigment's chromatogram to obtain the concentration of each pigment in the water sample (in µg L^{-1}).

Data Analysis

Concentrations of pigments across stations were plotted for each month, and the resulting line graphs visually interpreted to determine when each pigment was most abundant. The total pigment composition was also plotted across stations for each month, with each accessory pigment represented as its concentration's percentage of the chlorophyll a concentration. These graphs were visually interpreted to determine how the phytoplankton community changed across stations.

All statistical tests were performed using PRIMER 6 & PERMANOVA+ software. A Bray-Curtis dissimilarity matrix was used to generate the dissimilarities between photopigment compositions of samples. These dissimilarities between samples were ranked, and these rankings used in multi-dimensional scaling (MDS) analysis.

Cluster analysis was used to determine the levels of similarity and dissimilarity present in the MDS. An analysis of similarities (ANOSIM) was used to determine the strength and significance of differences in pigment concentrations between stations and between dates. To determine which pigment(s) had the greatest contribution to these differences, similarity percentage (SIMPER) analysis was used. A BIOENV test was then used to determine which, if any, environmental conditions correlated to the similarities observed between stations' pigment concentrations.

Pigment extrapolation to algal taxa

Observed pigments were matched with the algal taxa they represent based on previous work on phytoplankton photopigments (Table 1). Chlorophyll a is found in nearly all photosynthetic algae, and is thus representative of phytoplankton biomass. Pheophytin a does not correspond with a particular algal taxa, but rather is a product of the degradation of chlorophyll a, and is representative of decomposing phytoplankton and phytoplankton detritus (Jeffrey et al. 2005). Pigment concentrations were not extrapolated to represent exact abundances of particular phytoplankton taxa, but were rather used to suggest the presence of these taxa when discussing results.

Table 1. Phytoplankton taxa represented by pigments measured (Adapted from Paerl et al. 2003; Jeffery et al. 2005). Filled squares indicate the pigment is used by the taxa.

	Chlorophyll a	Chlorophyll b	Alloxanthin	Fuxoanthin	Zeaxanthin	Peridinin
Chlorophytes	■	■				
Chrysophytes	■			■	■	
Cryptophytes	■		■			
Cyanobacteria	■				■	
Diatoms	■			■		
Dinoflagellates	■					■
Prymnesiophytes	■			■		
Prasinophytes	■	■				
Raphidophytes	■			■	■	

RESULTS

Spatial Distribution of Pigments

Descriptive Analysis

Chlorophyll a concentrations were lowest at the most upstream stations, S.B. and 12, ranging from 0.27-1.29 μg L-1 at these two sites. On October 29, November 15, and December 13, chlorophyll a concentrations sharply rose moving downstream from stations S.B. and 12, peaking at stations 10 (Nov 15, 11.47 μg L-1), 8 (Oct 29, 16.72 μg L-1), and 7S (Dec 13, 16.74 μg L-1) (Figure 2). Following this peak, chlorophyll a concentrations sharply decreased at the next station downstream, then gradually increased until site 1A, when concentrations began to decrease again (Figure 2). On October 12, which had the lowest chlorophyll a concentrations of any date sampled, the concentrations gradually increased moving upstream to downstream.

No peridinin was found in any water samples. The concentrations of other accessory pigments varied between stations. No consistent trends were seen in any pigment's concentrations between stations, except a decline in all pigments at sites 12 and S.B. (Figure 2). The highest fucoxanthin concentrations occurred at stations 3 and 5 on Nov 15 (2.50, 1.95 μg L-1). The highest alloxanthin concentration occurred at station 7S on Dec 13 (2.16 μg L-1). The highest zeaxanthin concentrations occurred at stations 8 and 10 on Oct 29 (2.38, 1.85 μg L-1). Chlorphyll b's highest concentrations occurred at stations 7 and 8 on Dec 13 (1.21, 1.34 μg L-1) Pheophytin a concentrations were highly variable (Figure 2). No pheophytin a was found in any samples taken on Dec 13. The highest concentrations of pheophytin a were at stations 8 and 10 on Oct 29 (0.99, 1.04 μg L-1)

Figure 3 shows changes across stations in the concentrations of each accessory pigment in relationship to chlorophyll a concentrations. The photopigment 'community' changed significantly between sites, but no trends were consistent across the months sampled. Fucoxanthin, alloxanthin, and zeaxanthin were usually the most abundant accessory pigments at each station (Figure 3). Which pigment was the most abundant varied greatly between stations, however none of these variations were consistent across the months sampled. Pheophytin a was the most abundant pigment at stations 10, 12 and S.B. on November 15, and chlorophyll b was the most abundant at stations 7 and 8 on December 13 (Figure 3).

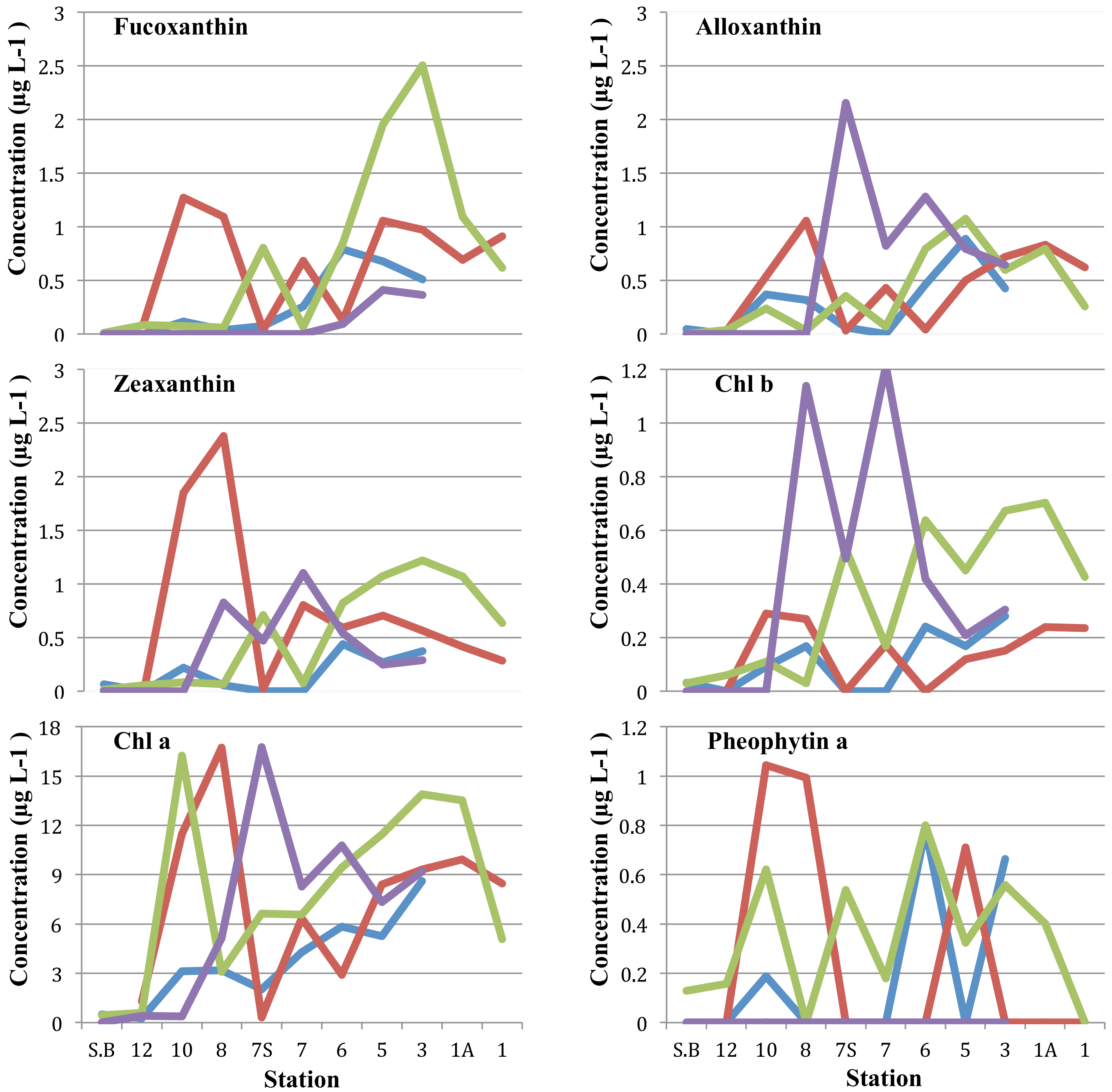

Figure 2. Changes in pigment concentrations across stations. Colored lines indicate sampling dates; labels on graphs indicate pigments shown. No consistent trends were seen in any pigment's concentrations between stations, except a decline in all pigments at sites 12 and S.B. Stations 1 and 1A were not sampled on October 12th or December 13th, and station S.B. was not sampled on October 29th.

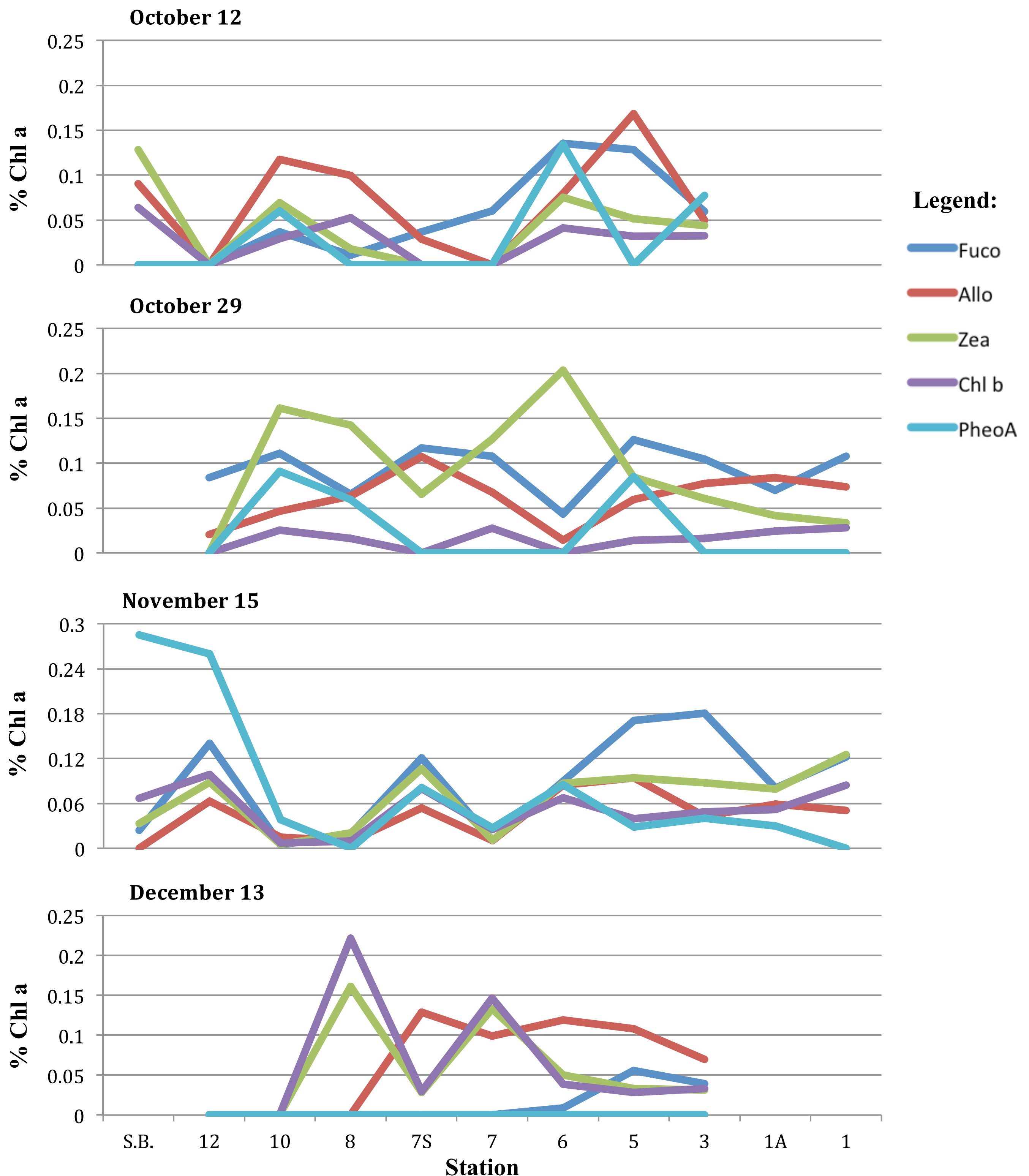

Figure 3. Changes in pigment compositions across stations. Pigments are shown as their percentage of the chlorophyll a concentration at each station. Colored lines indicate pigments; labels on graphs indicate dates sampled. Stations 1 and 1A were not sampled on October 12th or December 13th, and station S.B. was not sampled on October 29th. The photopigment 'community' changed significantly between sites, but no trends were consistent across the months sampled.

Statistical Analysis

Multi-dimensional scaling (MDS) of the dissimilarities between pigment samples showed certain clusters of sites did appear more and less similar then others. Specifically, most samples collected at stations 1-7S (downstream stations) showed 60% similarity to one another, and most samples collected at stations 12 and S.B. (upstream stations) showed 60% similarity to one another. There was an 80% dissimilarity between these two groups (Figure 4).

An analysis of similarities (ANOSIM) of the pigment compositions of the stations determined strong, significant differences were present between the pigment composition of station 12 and the pigment compositions of stations 3, 5, 6, 7, and 8 (R-values 1, 0.99, 0.938, 0.958, 0.781; p=0.029) (Table 2). Strong, nearly significant differences were also seen between the pigment composition of station 12 and those of stations 1 and 1A (R-values 0.964, 1; p=0.067); and that of station S.B. and those of stations 3, 5, 6, 7, and 8 (R-values 1, 1, 1, 1, 0.857; p=0.067) (Table 2). Strong differences were seen between the pigment compositions of station S.B. and stations 1 and 1A, but these differences were insignificant, likely due to a small samples size at these stations (Table 2). No strong differences (R>0.75) were seen between any other stations.

SIMPER analysis determined differences in chlorophyll a concentrations accounted for 73-82% of the dissimilarities between the pigment compositions of station 12 and stations 3, 5, 6, 7, and 8 (Table 3). A visual representation of these differences can be seen in Figure 5. Station S.B. also had noticeably lower chlorophyll a concentrations than stations 1-8 (Figure 5).

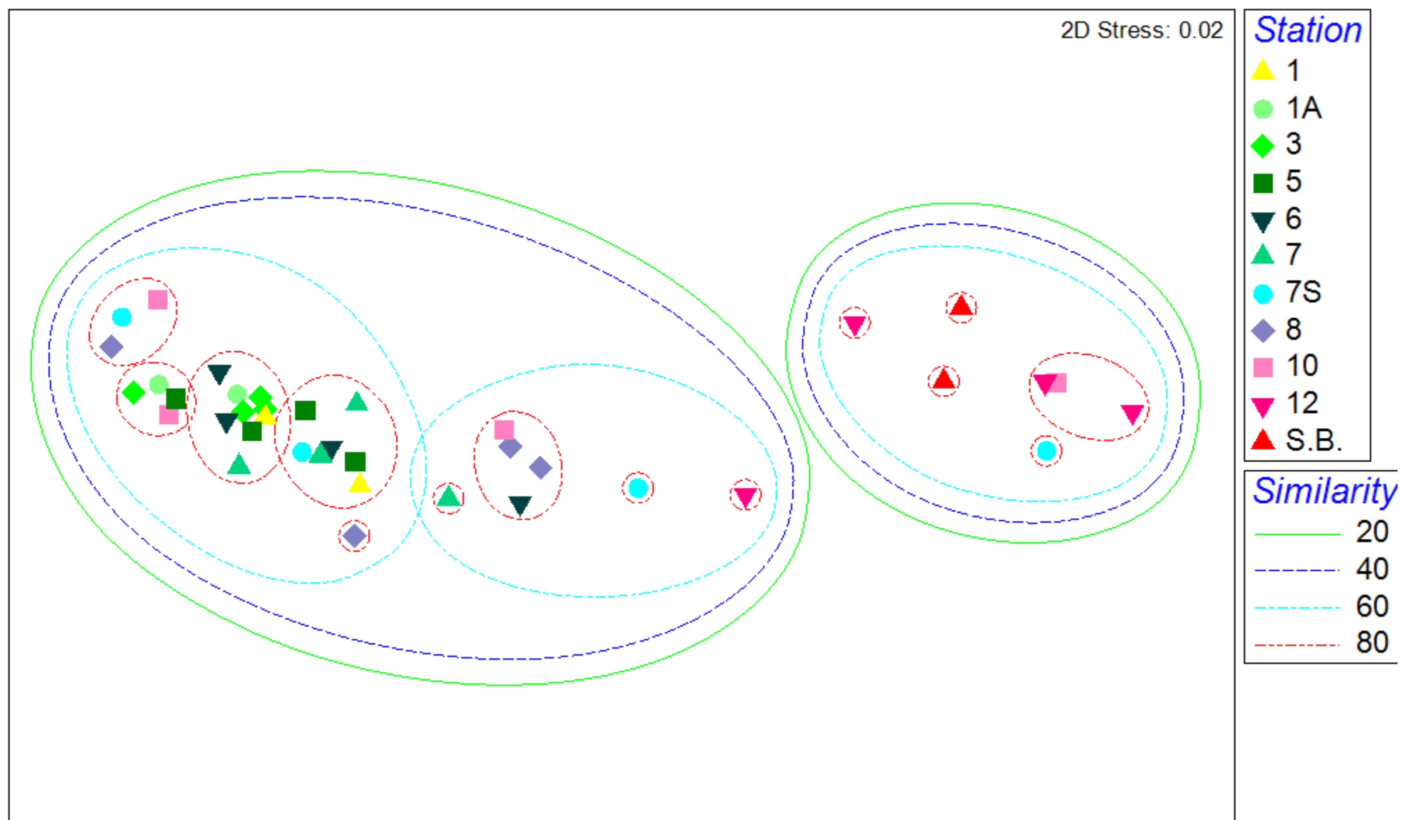

Figure 4. Multi-dimensional scaling (MDS) of pigment data, labeled by station number. A Bray-Curtis dissimilarity matrix was used to compare photopigment compositions of samples. From this, the similarities between samples were ranked, and these rankings used in the above MDS analysis. In MDS, the stress value represents how well the analysis fits the data. The above chart is a sound representation of the similarity and dissimilarity between samples (stress<0.1). Samples are labeled by the station they were collected from. Solid, dashed, and dotted lines cluster samples by levels of similarity.

Table 2. Analysis of similarities (ANOSIM) of pigment concentrations between stations. R-statistic ranging from 1 to -1 indicates dissimilarity between samples. Values approaching 1 indicate strong dissimilarity between samples. Negative R-statistic indicates dissimilarities in pigments within stations are greater than the dissimilarity between stations. P-values indicate level of significance. Stations highlighted in yellow have strong, significant differences between photopigment compositions ($R>0.75$, $p<0.05$). Stations highlighted in orange have strong, nearly significant differences between photopigment compositions ($R>0.75$, $p>0.05$).

Stations	***R-Stat***	*% Sig.*	***p-value***
3, 5	**-0.115**	74.3	**0.743**
3, 6	**-0.063**	74.3	**0.743**
3, 7	**0.25**	8.6	**0.086**
3, 7S	**0.177**	8.6	**0.086**
3, 8	**0.354**	14.3	**0.143**
3, 10	**0.125**	22.9	**0.229**
3, 12	**1**	2.9	**0.029**
3, S.B.	**1**	6.7	**0.067**
3, 1	**0.214**	40	**0.4**
3, 1A	**-0.25**	93.3	**0.933**
5, 6	**-0.167**	94.3	**0.943**
5, 7	**-0.042**	57.1	**0.571**
5, 7S	**0.063**	34.3	**0.343**
5, 8	**0.271**	11.4	**0.114**
5, 10	**0.104**	20	**0.2**
5, 12	**0.99**	2.9	**0.029**
5, S.B.	**1**	6.7	**0.067**
5, 1	**-0.25**	86.7	**0.867**
5, 1A	**-0.25**	93.3	**0.933**
6, 7	**0.01**	42.9	**0.429**
6, 7S	**-0.042**	48.6	**0.486**
6, 8	**-0.052**	42.9	**0.429**
6, 10	**-0.104**	77.1	**0.771**
6, 12	**0.938**	2.9	**0.029**
6, S.B.	**1**	6.7	**0.067**
6, 1	**-0.357**	93.3	**0.933**
6, 1A	**-0.25**	86.7	**0.867**
7, 7S	**0.063**	25.7	**0.257**
7, 8	**0.021**	34.3	**0.343**
7, 10	**0.167**	14.3	**0.143**
7, 12	**0.958**	2.9	**0.029**
7, S.B.	**1**	6.7	**0.067**
7, 1	**-0.357**	100	**1**
7, 1A	**0.286**	20	**0.2**
7S, 8	**-0.104**	71.4	**0.714**
7S, 10	**-0.24**	94.3	**0.943**
7S, 12	**0.219**	11.4	**0.114**
7S, S.B.	**0.036**	46.7	**0.467**
7S, 1	**-0.321**	100	**1**
7S, 1A	**-0.214**	80	**0.8**
8, 10	**-0.073**	68.6	**0.686**
8, 12	**0.781**	2.9	**0.029**
8, S.B.	**0.857**	6.7	**0.067**
8, 1	**-0.25**	86.7	**0.867**
8, 1A	**0.036**	40	**0.4**
10, 12	**0.302**	14.3	**0.143**
10, S.B.	**0.107**	40	**0.4**
10, 1	**-0.286**	86.7	**0.867**
10, 1A	**-0.321**	100	**1**
12, S.B.	**-0.286**	80	**0.8**
12, 1	**0.964**	6.7	**0.067**
12, 1A	**1**	6.7	**0.067**
S.B., 1	**1**	33.3	**0.333**
S.B., 1A	**1**	33.3	**0.333**
1, 1A	**0.25**	33.3	**0.333**

Table 3. Similarity percentage (SIMPER) analysis of stations with significantly different pigment compositions (Table 2). Chlorophyll a (Chl a) concentrations contributed the most to the differences in pigment compositions between these stations (73-82%).

Stations 3 & 12
Average dissimilarity = 88.91

	Group 3	*Group 12*				
Species	*Av.Abund*	*Av.Abund*	*Av.Diss*	*Diss/SD*	*Contrib%*	*Cum.%*
Chl a	**10.25**	**0.64**	**69.55**	**10.89**	**78.22**	**78.22**
Fuco	1.09	0.05	6.58	1.72	7.4	85.63
Allo	0.6	0.02	4.37	3.46	4.91	90.54

Stations 5 & 12
Average dissimilarity = 86.63

	Group 5	*Group 12*				
Species	*Av.Abund*	*Av.Abund*	*Av.Diss*	*Diss/SD*	*Contrib%*	*Cum.%*
Chl a	**8.11**	**0.64**	**63.42**	**8.36**	**73.21**	**73.21**
Fuco	1.02	0.05	7.75	2.73	8.95	82.16
Allo	0.81	0.02	7.25	2.72	8.37	90.53

Stations 6 & 12
Average dissimilarity = 83.04

	Group 6	*Group 12*				
Species	*Av.Abund*	*Av.Abund*	*Av.Diss*	*Diss/SD*	*Contrib%*	*Cum.%*
Chl a	**7.23**	**0.64**	**61.05**	**5.17**	**73.51**	**73.51**
Zea	0.6	0.01	6.83	1.72	8.22	81.74
Allo	0.64	0.02	5.02	1.59	6.04	87.78
Fuco	0.46	0.05	3.99	1.23	4.81	92.59

Stations 7 & 12
Average dissimilarity = 82.56

	Group 7	*Group 12*				
Species	*Av.Abund*	*Av.Abund*	*Av.Diss*	*Diss/SD*	*Contrib%*	*Cum.%*
Chl a	**6.38**	**0.64**	**67.65**	**6.22**	**81.93**	**81.93**
Zea	0.5	0.01	4.64	1.08	5.62	87.56
Chl b	0.39	0.01	3.45	0.88	4.17	91.73

Stations 8 & 12
Average dissimilarity = 78.66

	Group 8	*Group 12*				
Species	*Av.Abund*	*Av.Abund*	*Av.Diss*	*Diss/SD*	*Contrib%*	*Cum.%*
Chl a	**7.04**	**0.64**	**61.66**	**4.87**	**78.39**	**78.39**
Zea	0.83	0.01	5.71	1.2	7.26	85.65
Chl b	0.4	0.01	4.89	0.85	6.22	91.87

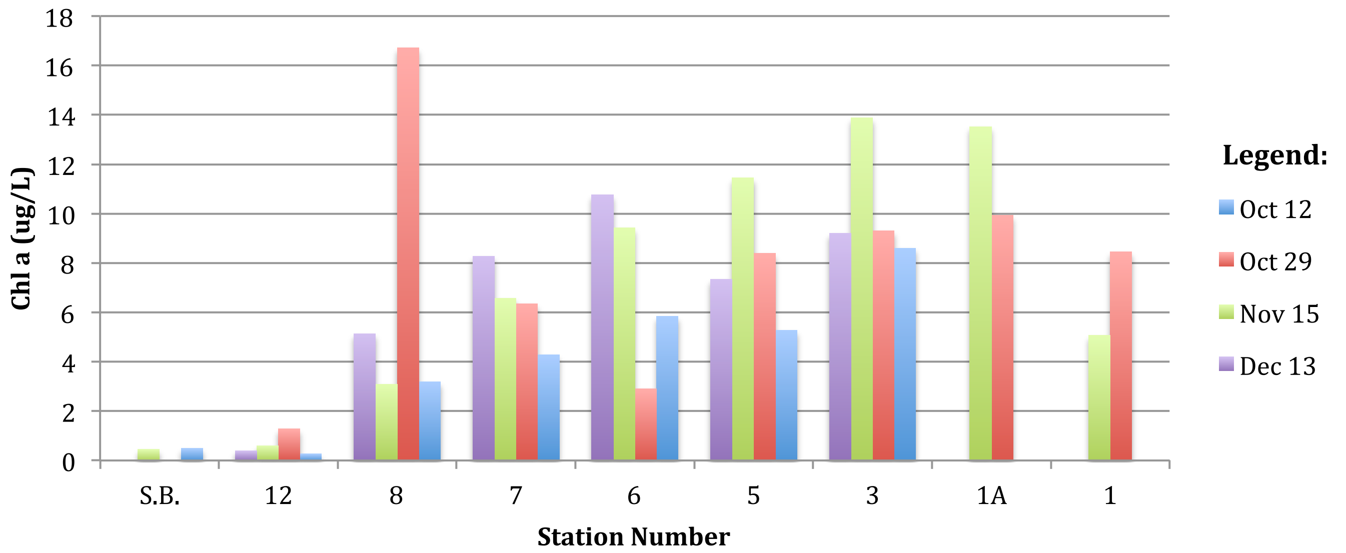

Figure 5. Chlorophyll a concentrations across stations with strong differences between pigment compositions. Bar color indicates date samples were taken. Stations S.B. and station 12 had noticeably lower chlorophyll a concentrations than stations 1-8.

Temporal Distribution of Pigments

Descriptive Analysis

Chlorophyll a concentrations were relatively stable across October 29, November 15, and December 13 (avg. 7.51, 7.90, 6.47 µg L-1). October 12, had much lower chlorophyll a concentrations than the three later dates (avg. 3.68 µg L-1) (Figure 6b).

Fucoxanthin and zeaxanthin concentrations were higher on October 29 and November 15 than October 12 and December 13 (Fucoxanthin: avg. 0.70,0.74 vs. 0.27,0.10 µg L-1 ; Zeaxanthin: avg. 0.76,0.53 vs. 0.16,0.39 µg L-1) (Figure 6a, 6b). Fucoxanthin was absent from stations 12 and S.B. on October 12, and from stations 7-S.B. on December 13th. Zeaxanthin was absent from stations 7, 7S, and 12 on October 12; station 12 on October 29; and stations 10-S.B. on December 13.

Alloxanthin and chlorophyll b concentrations generally increased across the dates sampled, from averages of 0.29 and 0.11 µg L-1 , respectively, on October 12, to averages of 0.63 and 0.42 µg L-1 on December 13 (Figure 6a, 6b). Alloxanthin was absent from stations 7 and 12 on October 12, station S.B. on November 15, and stations 8-S.B. on December 13. Chlorophyll b was absent from stations 7, 7S, and 12 on October 12; stations 6, 7S, and 12 on October 29; and stations 10-S.B. on December 13.

Pheophytin a was only consistently observed across stations on November 15, though it did peak at stations 3, 6, and 10 on October 12 and stations 5, 8, and 10 on October 29. December 13 was the only date where no pheophytin a was observed.

Statistical Analysis

Sample collection dates did not appear to be related to clusters formed by MDS of the dissimilarities between pigment samples (Figure 7). ANOSIM analysis of pigment samples by date showed no differences between the pigment compositions of any dates ($R<0.1$) (Table 4).

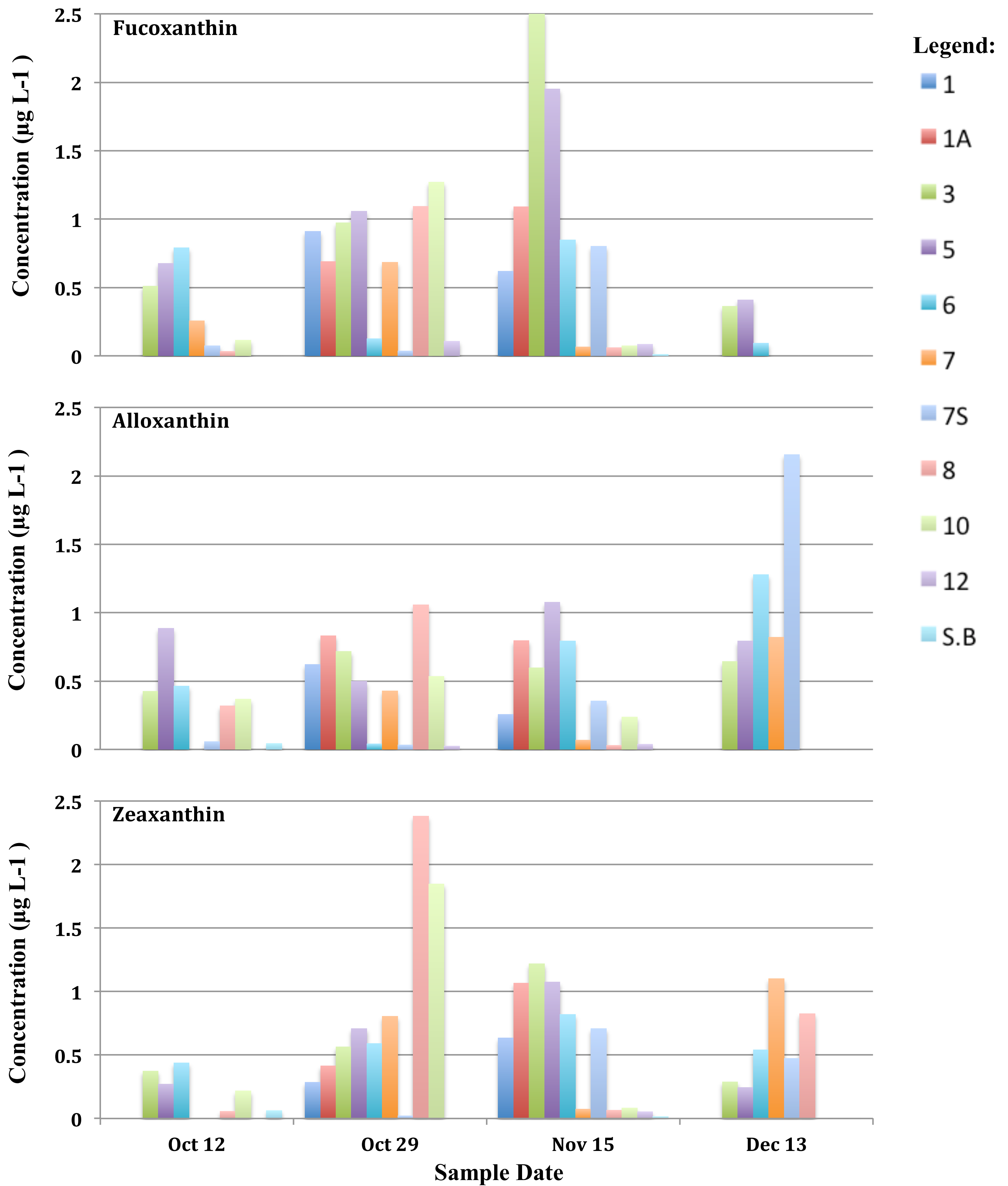

Figure 6a. Changes in pigment compositions between sampling dates. Bar colors represents sample stations. Fucoxanthin and zeaxanthin concentrations were highest on October 29 and November 15 (0.70, 0.74 µg L-1). Alloxanthin concentrations generally increased across dates sampled.

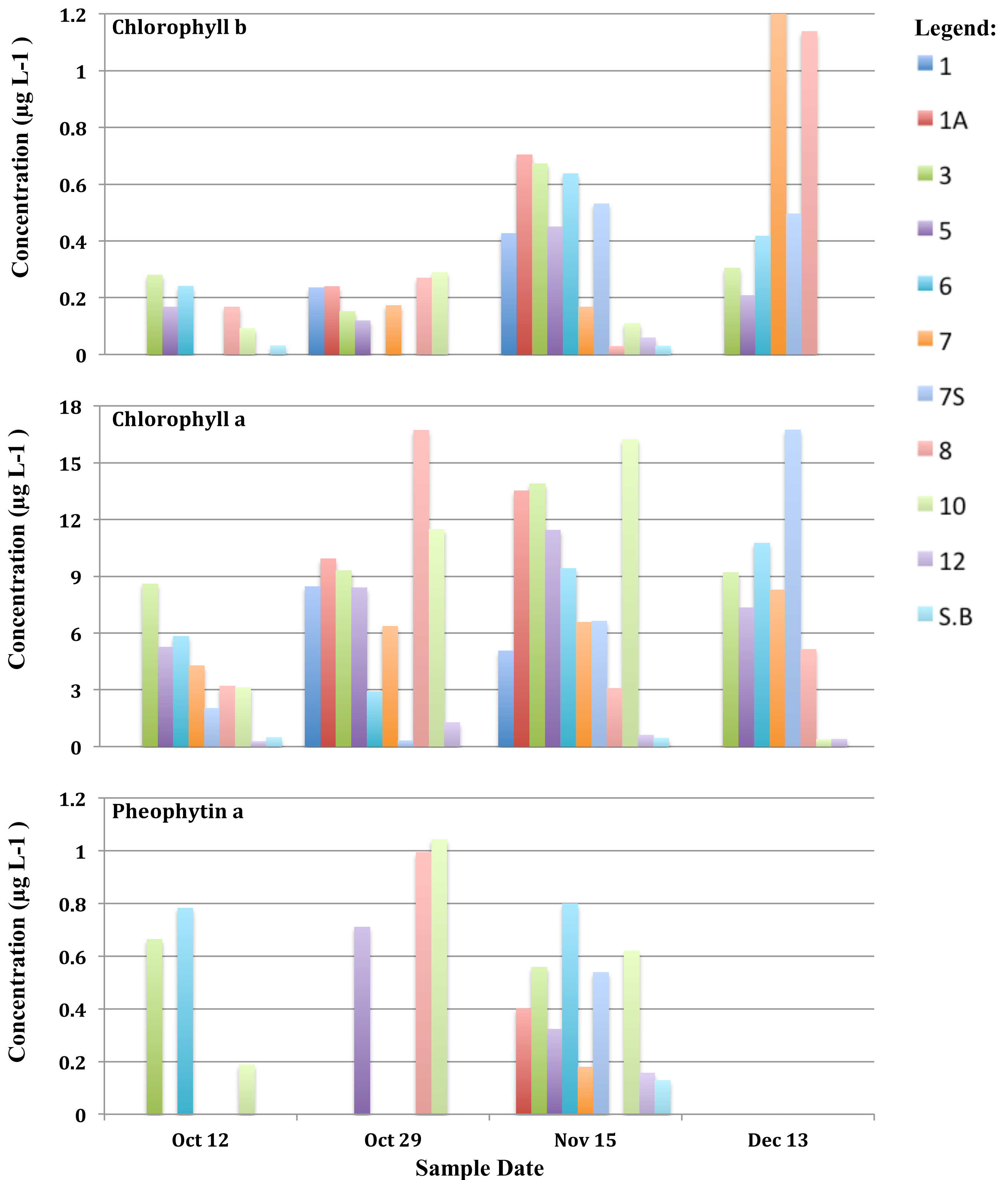

Figure 6b. Changes in pigment compositions between sampling dates. Bar colors represents sample stations. Chlorophyll b concentrations generally increased across dates sampled. Chlorophyll a concentrations were relatively stable across October 29, November 15, and December 13. October 12 had much lower chlorophyll a concentrations. Pheophytin a was only consistently observed across stations on November 15, and was not found on December 13.

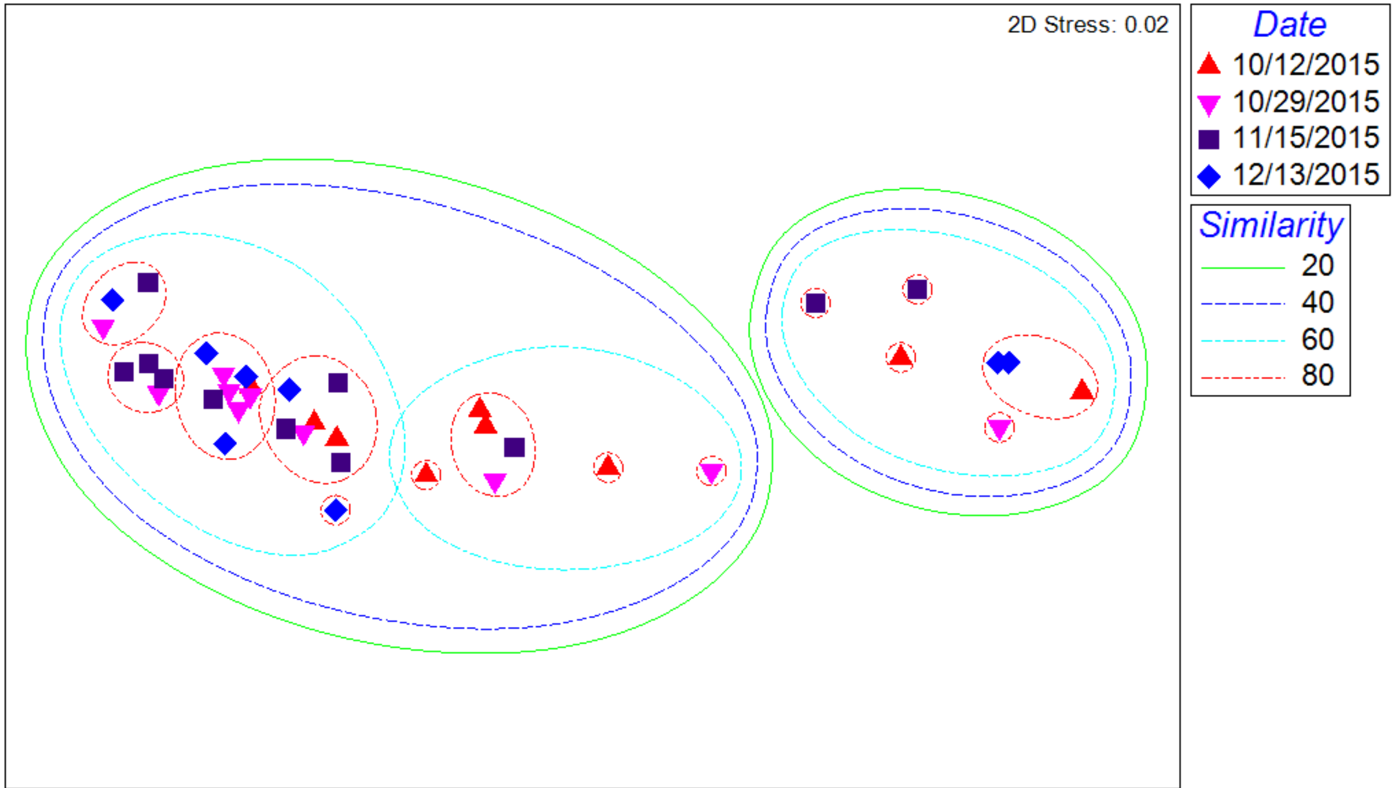

Figure 7. Multi-dimensional scaling (MDS) of pigment data, labeled by date sampled. A Bray-Curtis dissimilarity matrix was used to compare photopigment compositions of samples. From this, the similarities between samples were ranked, and these rankings used in the above MDS analysis. In MDS, the stress value represents how well the analysis fits the data. The above chart is a sound representation of the similarity and dissimilarity between samples (stress<0.1). Samples are labeled by the dates they were collected. Solid, dashed, and dotted lines cluster samples by levels of similarity.

Table 4. Analysis of similarities (ANOSIM) of pigment concentrations between dates. R-statistic ranging from 1 to -1 indicates dissimilarity between samples. Values approaching 1 indicate strong dissimilarity between samples. Negative R-statistic indicates dissimilarities in pigments within stations are greater than the dissimilarity between stations. P-values indicate level of significance. No strong, nor significant differences were observed between any dates sampled.

Sample Dates	***R-Stat***	*% Sig.*	***p-value***
10/12/2015, 10/29/2015	**0.068**	14.8	**0.148**
10/12/2015, 11/15/2015	**0.064**	11.9	**0.119**
10/12/2015, 12/13/2015	**0.064**	16.9	**0.169**
10/29/2015, 11/15/2015	**-0.045**	78.3	**0.783**
10/29/2015, 12/13/2015	**-0.003**	36.9	**0.369**
11/15/2015, 12/13/2015	**0.008**	33.7	**0.337**

Environmental Condition Changes

Descriptive Analysis

Nutrient concentrations differed between stations. Nitrate, orthophosphate, and total dissolved phosphate concentrations generally decreased moving downstream, whereas trends in ammonia and dissolved Kjedahl nitrogen concentrations differed from month to month (Table 5). Ammonia, orthophosphate, and total dissolved phosphate concentrations were especially high at station six on October 12, November 15, and December 13.

Surface water temperature and dissolved oxygen remained mostly steady moving downstream. Surface water was considerably cooler on November 15 (15.6 C) than it was on October 12 (19.9 C) or October 29.(19.3 C) (Figure 8). The salinity of surface water generally increased moving downstream, and was highest on October 29 (Figure 8).

On October 29, November 15, and December 13, temperature, salinity, and dissolved oxygen generally differed little across the water column. On October 12, however, water temperature and salinity increased with depth, indicating there was likely a salt wedge in the estuary (Figure 9). This difference between surface and bottom waters was especially great upstream of station three, with the exception of the shallowest station, station six (depth: 1.5m). Hypoxic bottom waters (D.O.< 3.0ppm) were also present upstream of station seven (Figure 9).

Table 5. Nutrient concentrations of water samples. Higher concentrations of nutrients are represented by darker colors. Nutrients concentrations varied between sites and months, with few consistent trends. Nitrate (NO3+NO2) and total dissolved phosphate (TDP) concentrations generally decreased moving downstream. TDP and orthophosphate (PO4) concentrations were especially high at station six.

	NH4 (µM)				PO4 (µM)			
	Oct 12	Oct 29	Nov 15	Dec 13	Oct 12	Oct 29	Nov 15	Dec 13
S.B.	2.940	3.009	0.969	2.100	1.286	2.054	1.076	1.006
12	6.048	8.431	2.093	3.239	1.153	0.516	1.230	0.908
10	14.224	2.639	4.247	2.589	1.310	0.436	1.355	0.971
8	8.887	3.294	3.115	1.487	1.412	0.443	1.069	0.918
7	8.146	5.392	2.735	0.851	1.317	0.464	1.090	0.859
7S	11.177	2.014	2.618	0.851	1.317	0.702	0.859	0.540
6	12.743	8.578	32.401	8.525	2.866	0.979	11.038	7.498
5	9.673	6.117	0.851	0.851	1.163	0.750	0.358	0.337
3	4.513	1.544	0.851	0.851	0.996	0.365	0.372	0.302
1A		4.883	1.501			0.412	0.365	
1		3.742	1.027			0.344	0.418	

	NO3+NO2 (µM)				TPD (µM)			
	Oct 12	Oct 29	Nov 15	Dec 13	Oct 12	Oct 29	Nov 15	Dec 13
S.B.	23.949	69.132	11.198	40.143	2.983	3.889	3.285	2.878
12	14.823	24.552	12.765	31.300	2.541	2.077	3.347	2.694
10	9.516	2.194	14.305	27.475	2.152	1.171	3.254	2.348
8	11.789	0.396	11.919	31.609	2.414	1.156	2.018	2.317
7	8.600	0.002	9.763	25.603	2.114	1.051	1.694	2.402
7S	8.833	0.400	13.936	17.912	2.002	1.455	1.555	1.094
6	10.103	0.615	17.176	19.864	3.313	1.658	10.499	7.915
5	7.394	0.089	3.117	13.846	1.837	1.366	0.883	1.287
3	5.984	0.002	2.095	4.497	1.650	0.931	1.006	0.771
1A		0.255	1.448			0.999	0.976	
1		0.002	0.006			0.916	0.960	

	DKN (µM)			
	Oct 12	Oct 29	Nov 15	Dec 13
S.B.	43.691	20.547	56.788	29.635
12	56.961	62.321	56.216	33.496
10	68.763	82.436	56.089	31.008
8	63.433	81.814	34.263	30.193
7	58.566	90.612	27.249	33.797
7S	72.135	82.382	40.938	33.539
6	80.429	92.453	65.815	41.669
5	82.767	94.024	26.189	32.317
3	82.332	89.205	58.144	39.545
1A		102.308	78.508	
1		100.359	60.835	

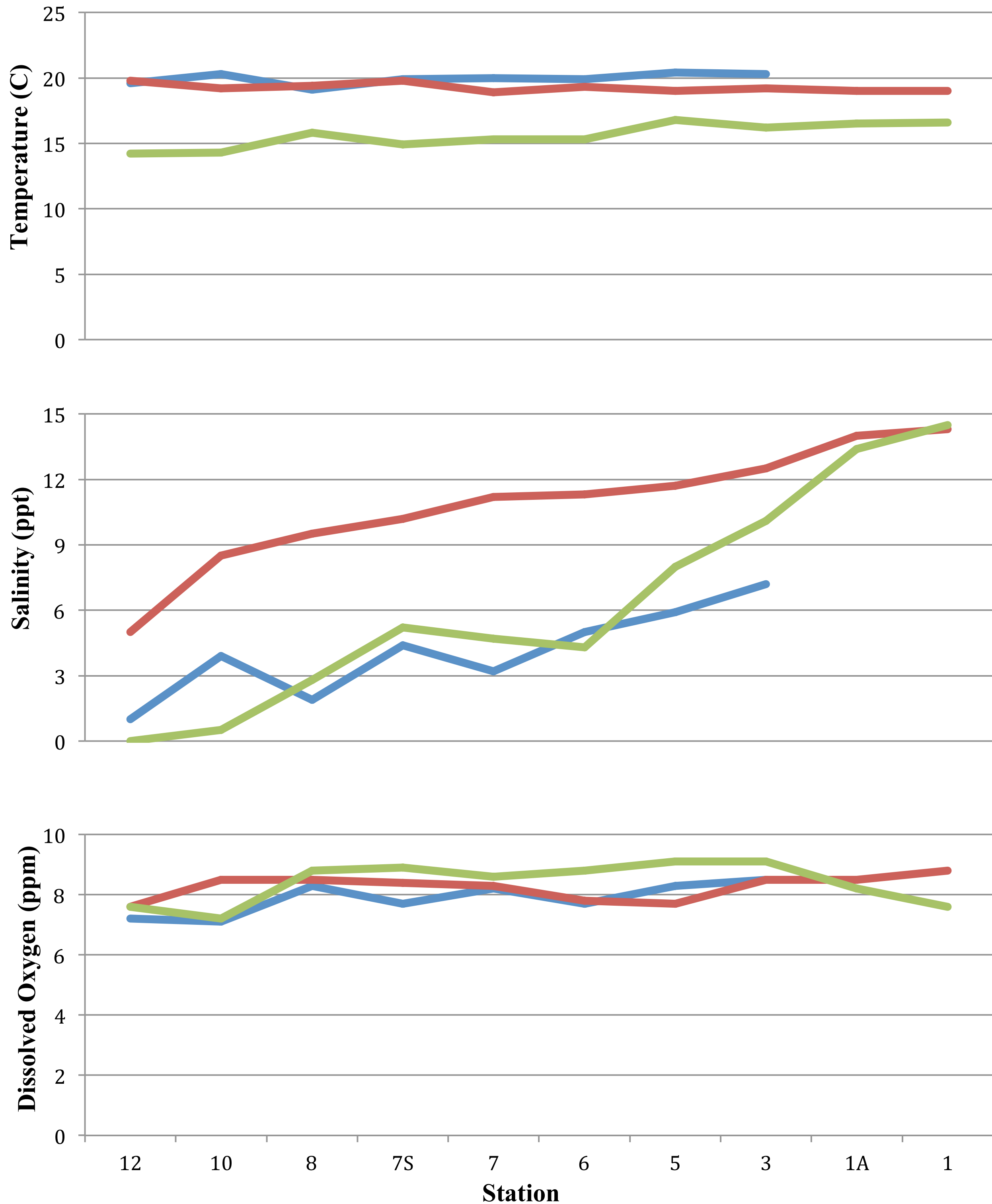

Figure 8. Changes in surface water temperature, salinity, and dissolved oxygen (D.O.) across stations and dates sampled. Colored lines represent months sampled. Temperature remained steady across stations, but decreased between months sampled. Salinity increased moving downstream, and was highest on October 29. D.O. remained steady across stations and dates sampled.

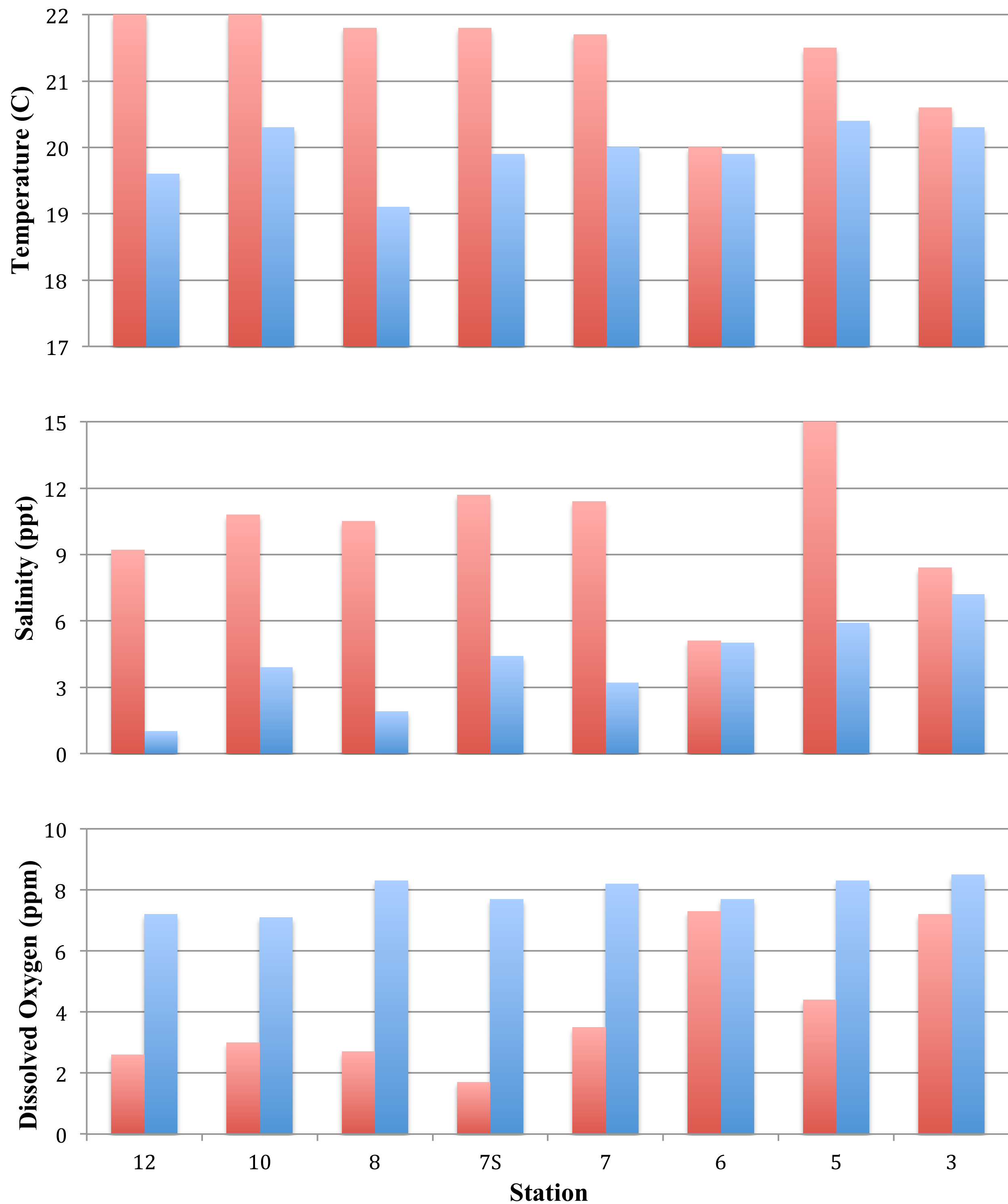

Figure 9. Changes in surface (blue bar) and bottom (red bar) water temperature, salinity, and dissolved oxygen (D.O.) across stations on October 12. Temperature and salinity differences between surface and bottom water indicates stratification of the water column, and likely a salt wedge in the estuary. This was not observed at station six, which was the shallowest station (1.5m). Hypoxic bottom waters were also present further upstream in the estuary.

Legend:
Surface
Bottom

Statistical Relationship to Pigment Changes

BIOENV analysis showed no strong correlation between the pigment compositions of samples and the chemical or physical water quality of the stations they were sampled from (Tables 6 & 7). Because temperature, salinity, and dissolved oxygen were not measured on December 13, these factors were analyzed separately from water nutrients, which were collected across all dates and stations except S.B. Of the nutrients measured for, total dissolved phosphate had the strongest relationship with pigment concentrations, but the correlation between these factors was weak (p_w=0.224). Overall a combination of depth, bottom temperature, difference in surface and bottom temperature, and surface dissolved oxygen had the strongest correlation with pigment concentrations, though this correlation was also relatively weak (p_w=0.293). Because these correlations were weak, they were not tested for statistical significance.

Table 6. BIOENV analysis of the relationship between the pigment compositions of samples and the water chemistry of the stations where they were sampled. Factors analyzed are shown. Total dissolved phosphorus (TDP) had the highest correlation to pigment concentrations (p_w=0.224). All correlations observed were relatively weak (p_w<0.225).

BIOENV Results, Spearman correlation			
# Variables	Variables	Spearman Correlation (p_w)	Variables
1	5	0.224	1 NH4
2	4,5	0.213	2 NO3+NO2
1	2	0.190	3 DKN
2	2,5	0.186	4 PO4
2	2,4	0.182	5 TDP
3	2,4,5	0.180	
3	1,2,5	0.157	
2	1,2	0.156	
4	1,2,4,5	0.156	
3	1,2,4	0.154	

Table 7. BIOENV analysis of the relationship between the pigment compositions of samples and the physical water quality of the stations where they were sampled. Factors analyzed are shown. A combination of station depth, bottom temperature, difference in temperature, and surface dissolved oxygen (D.O.) had the highest correlation to pigment concentrations (p_w=0.293). All correlations observed were relatively weak ($p_w<0.3$).

BIOENV Results, Spearman correlation			
# Variables	Variables	Spearman Correlation (p_w)	Variables
4	1,3,4,8	0.293	1 Depth
3	1,3,8	0.287	2 Surface temp
4	1,2,4,8	0.285	3 Bottom temp
3	1,2,8	0.279	4 Temp difference
5	1,2,3,4,8	0.266	5 Surface salinity
5	1,3,4,8,10	0.265	6 Bottom salinity
3	2,4,8	0.263	7 Salinity difference
4	1,2,3,8	0.262	8 Surface D.O.
2	8,9	0.262	9 Bottom D.O.
4	1,3,8,10	0.262	10 D.O. Difference

DISCUSSION

The goal of this study was to use quantify the spatial and temporal variability in phytoplankton biomass (as a measure of Chl *a*) and community composition (as a measure of other pigments) in North Carolina's Tar-Pamlico River estuary over the fall of 2015 using HPLC. I also attempted to relate environmental conditions these factors' variability.

Our results indicate that there is spatial variation in the concentrations of photopigments, specifically chlorophyll a. A decrease in phytoplankton biomass at the most upstream stations, S.B. and 12, was consistently observed across the four dates sampled. Phytoplankton biomass also peaked at a different mid-estuary station on each sampling date, indicating a local chlorophyll maximum may occur between stations 7S and 10. Often times, areas of estuaries consistently have high levels of chlorophyll a, and studies have found such areas to exist in the nearby Neuse River Estuary (Kimmel et al. 2015).

The concentrations of accessory pigments varied greatly across stations, but no changes in concentrations occurred consistently across the sample dates. The most notable result was a complete lack of peridinin in water samples, indicating either complete absence or extremely low abundances of dinoflagellates in the estuary on the dates sampled. Dinoflagellates tend to bloom in the late winter or early spring, so it is not completely surprising that they were not seen (Mallin 1994; Pickney et al. 1999). Fucoxanthin, alloxanthin, and zeaxanthin were the most abundant accessory pigments overall, but which was the most abundant at a particular station varied greatly. This suggests that crysophytes, cryptomonads, cyanobacteria, diatoms, prymnesiophytes, and raphidophytes were the most abundant phytoplankton across samples, but the actual

abundance of these taxa were impossible to quantify from the data collected. All pigments were completely absent from at least one sample over the study, evidencing that the success of phytoplankton taxa is patchy across the estuary.

Temporal variation in the phytoplankton community was also observed, though it was also largely random. The average phytoplankton biomass across stations was similar on the last three dates sampled, but was considerably lower on October 12. This is possibly due to the weather, as it was raining on this day and phytoplankton may have been lower in the water column.

Though pigment concentrations at individual sites varied greatly from month to month, the average concentrations of pigments across the estuary may evidence that particular taxa of phytoplankton were more abundant at certain times of the study. Fucoxanthin and zeaxanthin concentrations were highest on October 29 and November 15, indicating that chrysophtyes, cyanobacteria, diatoms, prymnesiophytes, and radiophytes may have been more successful on these dates. Alloxanthin and chlorophyll b concentrations increased across the dates sampled, indicating that chlorophytes, cryptophytes, and prasinophytes may be more successful in the estuary in the late winter. No statistical differences were seen in pigment concentrations across dates, though, so this is just speculation. Diatoms, which dominate the phytoplankton community in many of North Carolina's sounds and estuaries, peak in abundance in the spring and summer, so a repeat of this study from March-August would likely show a large increase in fucoxanthin (Mallin 1994; Pickney et al. 1998). Interestingly, December 13 was the only sampling date where no pheophytin a was observed. As mentioned, phytoplankton biomass on this date was similar to that on October 29 and November 15, but absence of

pheophytin a indicates that very little of this biomass was degrading. This could possibly be due to an increase in zooplankton grazing or a decrease in the flushing time of the estuary on December 13, both of which would result in phytoplankton biomass being removed from the estuary before it could degrade. Further experimentation would be needed to substantiate these hypotheses.

Though trends were apparent in physical and chemical water quality across stations and dates sampled, no combination of the factors measured showed a strong correlation to differences in pigment concentrations between samples. Because of instrument malfunction on December 13, water salinity, temperature, and dissolved oxygen concentrations had to be analyzed separately from nutrient concentrations, though. As a result, certain combinations of environmental factors could not be tested.

Future studies should also sample the pigment concentrations of both surface and bottom waters. Though YSI measurements evidenced the water column was well-mixed on the last three dates sampled, on October 12 a definite halocline was present. A salt wedge seemed to extend from stations 5 to S.B. that stratified the water column, and it is likely that the phytoplankton communities differed above and below this salt wedge. In addition to being more saline, bottom waters were hypoxic at the four most upstream stations on October 12. Hypoxic bottom waters are frequently associated with microbial decomposition of algal blooms, therefore it is possible a bloom occurred at some point prior to the first sampling.

Overall, this study provides only a snapshot, or rather four snapshots, of the phytoplankton community in the Tar-Pamlico River estuary. Phytoplankton can grow rapidly in response to changing environmental conditions, and it is possible that the

sampling regime of this study missed changes in the phytoplankton community. To paint a more detailed picture of community changes, more intensive sampling is recommended for future studies. The stations sampled were also irregularly spaced along both the length and width of the estuary. To more accurately determine how the phytoplankton community changes spatially across the estuary, future sampling stations should be selected more systematically. Lastly, river flow and estuarine discharge were not measured in this experiment. Prior studies in nearby estuaries have shown phytoplankton biomass and community structure to be largely dependent on these factors, as they are responsible for nutrient loading into estuaries and flushing of phytoplankton from estuaries (Hall et al. 2013; Peirls et al. 2012). In the future, these factors should be measured, and their relation to the Tar-Pamlico River estuary phytoplankton community assessed.

REFERENCES CITED

Ameel, J.J., R.P. Axler, and C.J. Owen. 1993. Persulfate digestion for determination of total nitrogen and phosphorus in low-nutrient waters. American Environmental Laboratory.

Baird, D., and R.E. Ulanowicz. 1989. The seasonal dynamics of the Chesapeake Bay ecosystem. Ecological Monographs 59(4): 329-364.

Behrenfield, M.J., and P.G. Falowski. 1997. Photosynthetic rates derived from satellite-based chlorophyll concentration. Limnology and Oceanography 42(1): 1-20.

Bricker, S.B., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2008. Effects of nutrient enrichment in the nation's estuaries: A decade of change. Harmful Algae 8: 21-32.

Cloern, J.E. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series 210: 223-253.

Cloern, J.E., and R. Dufford. 2005. Phytoplankton community ecology: Principles applied in San Francisco Bay. Marine Ecology Progress Series 285: 11-28.

Conley, D.J. 2000. Biogeochemical nutrient cycles and nutrient management strategies. Hydrobiologia 410: 87-96.

Cooper, S.R., and G.S. Brush. 1993. A 2,500-year history of anoxia and eutrophication in Chesapeake Bay. Estuaries 16: 617-626.

Diaz, R.J., and R. Rosenburg. 2008. Spreading dead zones and consequences for marine ecosystems. Science 321(5891): 926-929.

Domingues, R.B., A. Barbosa, and H. Galvao. 2008. Constraints on the use of phytoplankton as a biological quality element within the Water Framework Directive in Portuguese waters. Marine Pollution Bulletin 56(8): 1389-1395.

Galloway, J.N., A.R. Townsend, J.W. Erisman, M. Bekunda, Z. Cai, J.R. Freney, L.A. Martinelli, S.P. Seitzinger, and M.A. Sutton. 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. Science 320(5878): 889-892.

Garmendia, M., Á. Borja, J. Franco, and M. Revilla. 2013. Phytoplankton composition indicators for the assessment of eutrophication in marine waters: Present state and challenges within the European directives. Marine Pollution Bulletin 66: 7-16.

Gieskies, W.W., and G.W. Kraay. 1986. Floristic and physiological differences between the shallow and the deep nanophytoplankton community in the euphotic zone of the open tropical Atlantic revealed by HPLC analysis of pigments. Marine Biology 91: 567-576.

Gilbert, P.M., D.M. Anderson, P. Gentien, E. Graneli, and K.G. Sellner. 2005. The global, complex phenomena of harmful algal blooms. Oceanography 18(2): 130-141.

Goldenberg, S.B., C.W. Landsea, A.M. Mestas-Nunez, and W.M. Gray. 2001. The recent increase in Atlantic hurricane activity: Causes and implications. Science 293: 474-479.

Greening, H., and A. Janicki. 2006. Toward reversal of eutrophic conditions in a subtropical estuary: Water quality and seagrass response to nitrogen loading reduction in Tampa Bay, Florida, USA. Environmental Management 38(2): 163-178.

Hall, N.S., W. Litaker, E. Fensin, J.E. Adolf, H.A. Bowers, A.R. Place, and H.W. Paerl. 2008. Environmental factors contributing to the development and demise of a

toxic dinoflagellate (*Karlodinium veneficum*) bloom in a shallow, eutrophic, lagoonal estuary. Estuaries and Coasts 31: 402-418.

Hall, N.S., H.W. Paerl, B.L. Peierls, A.C. Whipple, K.L. Rossignol. 2013. Effects of climatic variability on phytoplankton community structure and bloom development in the eutrophic, microtidal, New River Estuary, North Carolina, USA. Estuarine Coastal and Shelf Science 117: 70-82.

Harding, L.W. 1994. Long-term trends in the distribution of phytoplankton in Chesapeake Bay: Roles of light, nutrients and streamflow. Marine Ecology Progress Series 104: 267-291.

Hutchinson, G.E. 1961. The paradox of the plankton. The American Naturalist 95(882): 137-145.

Jeffrey, S.W., R.F.C. Mantoura, and S.W. Wright. 2005. Phytoplankton pigments in oceanography: guidelines to modern methods. Madrid, Spain: UNESCO

Keith, D.J. 2014. Satellite remote sensing of chlorophyll *a* in support of nutrient management in the Neuse and Tar-Pamlico River (North Carolina) estuaries. Remote Sensing of Environment 153: 61-78.

Kemp, W.M., W.R. Boynton, J.E. Adolf, D.F. Boesch, W.C. Boicourt, G. Brush, J.C. Cornwell, T.R. Fisher, P.M. Glibert, J.D. Hagy, L.W. Harding, E.D. Houde, D.G. Kimmel, W.D. Miller, R.I.E. Newell, M.R. Roman, E.M. Smith, and J.C. Stevenson. 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. Marine Ecology Progress Series 303: 1-29.

Kimmel D.G., B.D. McGlaughon, J. Leonard, H.W. Paerl, J.C. Taylor, E.K. Cira, and M.S. Wetz. 2015. Mesozooplankton abundance in relation to the chlorophyll maximum in the Neuse River Estuary, North Carolina, USA: implications for trophic dynamics. Estuarine, Coastal, and Shelf Science 157:59-69.

Los F.J., and J.W.M. Wijsman. 2007. Application of a validated primary production model (BLOOM) as a screening tool for marine, coastal and transitional waters. Journal of Marine Systems 64: 201-215.

McClain, C.R. 2009. A decade of satellite ocean color observations. Annual Review of Marine Science 1: 19–42.

Mackey, M.D., D.J. Mackey, H.W. Higgins, and S.W. Wright. 1996. CHEMTAX—a program for estimating class abundance from chemical markers: Application to HPLC measurements of phytoplankton. Marine Ecology Progress Series 144: 265-283.

Mallin, M.A. 1994. Phytoplankton ecology of North Carolina estuaries. Estuaries 17(3): 561-574.

Malone, T.C., D.J. Conley, T.R. Fisher, P.M. Glibert, L.W. Harding, and K.G. Sellner. 1996. Scales of nutrient-limited phytoplankton productivity in Chesapeake Bay. Estuaries 19(2): 371-385.

NCDENR. 1991. Executive summary of the status and trends report of the Albemarle-Pamlico Estuarine Study. Raleigh, NC: North Carolina Department of Environment and Natural Resources

NCDWQ. 2014. Tar-Pamlico River Basinwide water quality management plan. Raleigh, NC: North Carolina Division of Water Quality, North Carolina Department of Environment and Natural Resources

Paerl, H.W., J.D. Bales, L.W. Ausley, C.P. Buzzelli, L.B. Crowder, L.A. Eby, J.M. Fear, M. Go, B.L. Peierls, T.L. Richardson, and J.S. Ramus. 2001. Ecosystem impacts of three sequential hurricanes (Dennis, Floyd, and Irene) on the United States' largest lagoonal estuary, Pamlico Sound, NC. Proceedings of the National Academy of Sciences (USA) 98: 5655-5660.

Paerl, H.W., K. Rossignol, S.N. Hall, B.L. Peierls, and M.S. Wetz. 2010. Phytoplankton community indicators of short- and long-term ecological change in the anthropogenically and climatically impacted Neuse River Estuary, North Carolina, USA. Estuaries and Coasts 33: 485-497.

Pan, X., A. Mannino, H.G. Marshall, K.C. Filippino, and M.R. Mulholland. 2011. Remote sensing of phytoplankton community composition along the northeast coast of the United States. Remote Sensing of Environment 115: 3731-3747.

Peierls, B.L., N.S. Hall, and H.W. Paerl. 2012. Non-monotonic responses of phytoplankton biomass accumulation to hydrologic variability: a comparison of two coastal plain North Carolina estuaries. Estuaries and Coasts 35(6): 1376-1392.

Piehler, M.F., L.J. Twomey, N.S. Hall, and H.W. Paerl. 2004. Impacts of inorganic nutrient enrichment on phytoplankton community structure and function in Pamlico Sound, NC, USA. Estuarine, Coastal and Shelf Science 61: 197-209.

Pinckney, J.L., H.W. Paerl, M.B. Harrington, and K.E. Howe. 1998. Annual cycles of phytoplankton community structure and bloom dynamics in the Neuse River Estuary, NC (USA). Marine Biology 131: 371-382.

Pinckney, J.L., H.W. Paerl, and M.B. Harrington. 1999. Responses of the phytoplankton community growth rate to nutrient pulses in variable estuarine environments. Journal of Phycology 35: 1455-1463.

Quinlan, E.L., and E.J. Philips. 2007. Phytoplankton assemblages across the marine to low-salinity transition zone in a blackwater dominated estuary. Journal of Phytoplankton Research 29(5): 401-416.

Ryther, J.H., and W.M. Dunstan. 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science 171(3975): 1008-1013.

Smayda, T.J. 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton. Limnology and Oceanography 42(5): 1137-1153.

Tsirtsis, G., and M. Karydis. 1998. Evaluation of phytoplankton community indices for detecting eutrophic trends in the marine environment. Environmental Monitoring and Assessment 50: 255-269.

Van Heukelem, L., A.J. Lewitus, T.M. Katan, and N.E. Craft. 1994. Improved separations of phytoplankton pigments using temperature-controlled high performance liquid chromatography. Marine Ecology Progress Series 114: 303-313.

Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, and D.G. Tilman. 1997. Technical report: Human alteration of

the global nitrogen cycle: Sources and consequences. Ecological Applications 7(3): 737-750.

Webster, P.J., G.J. Holland, J.A. Curry, and H.R.Chang. 2005. Changes in tropical cyclone number, duration, and intensity in a warming environment. Science 309: 1844-1846.

Westco Scientific Instruments, Inc. 2008. SmartChem 200 Method 375-100E-1.