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Phytoplankton Abundance and Species Composition in Relation to Environmental Paramenters in Coastal Mississippi Waters

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PHYTOPLANKTON ABUNDANCE AND SPECIES COMPOSITION IN RELATION TO ENVIRONMENTAL PARAMENTERS IN COASTAL MISSISSIPPI WATERS

by

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ABSTRACT

PHYTOPLANKTON ABUNDANCE AND SPECIES COMPOSITION IN RELATION TO ENVIRONMENTAL PARAMENTERS IN COASTAL MISSISSIPPI WATERS

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Phytoplankton pigments from Coastal Mississippi waters were measured to determine the spatial and temporal distributions and composition of phytoplankton communities. Concentration of phytoplankton pigments were analyzed using High Performance Liquid Chromatography (HPLC) and the compositional changes in phytoplankton communities were determined with CHEMTAX 1.95. Surface water was collected for two years (September 2007-November 2009) at three sampling sites on a monthly basis. The stations were located at the Bay of St. Louis (station 1), the Mississippi Sound (station 4) and the Mississippi Bight (station 8), following a salinity gradient. A time series of the observations documented the variability of different taxonomic groups and phytoplankton abundance in Mississippi waters. Phytoplankton abundance and species group composition were related to environmental variables. Phytoplankton abundance did vary within stations and seasons being greater in coastal waters during the summer months. Diatoms were the major group at stations 1 and 4 where there was no major seasonal trend. At station 8 there was a clear seasonal trend where diatoms predominated in the winter, prymnesiophytes increased in spring, and cyanobacteria and diatoms predominated during the summer.

DEDICATION

This study is dedicated to my family, especially my mom, Maria Teresa García. Her encouragement, hard work, and love of science and nature inspired me to successfully finish this research.

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There are many students, staff and faculty members who helped me to successfully finish this thesis. I would especially like to thank Dr. Donald G. Redalje for guiding me through this research, providing my funding and allowing me to participate in a series of projects that have given me the experience I could not have acquired anywhere else. Also, thanks to Merrit Tuel and Sumit Chakraborty for teaching me how to use the HPLC and to NGI for funding this project.

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TABLE OF CONTENTS

LIST OF TABLES

Table

LIST OF ILLUSTRATIONS

Figure

LIST OF ABBREVIATIONS

B: Bottom depth

CCA: Canonical Correspondence Analysis

CDOM: Colored Dissolved Organic Carbon

chl *a*: Chlorophyll *a*

DCA: Detrended Correspondence Analysis

DIN: Dissolved Inorganic Nitrogen

DO: Dissolved Oxygen

Fig.: Figure

Figs: Figures

FNU: Formazin Nephelometric Units

HPLC: High Performance Analysis

M: Middle depth

N: Nitrogen

P: Phosphorus

RDA: Canonical redundancy analysis

S: Surface

Sal: Salinity

station: Station

WD: Wind Direction

WS: Wind Speed

CHAPTER I

INTRODUCTION

The major roles of phytoplankton may be summarized as being the basis of the trophic food web in marine environments, plus mediating flows of energy and conversions of matter. They govern biogeochemical cycles in the ocean (Miller 2004). Nevertheless, their importance does not only lie in their quantity. The global impact of marine phytoplankton depends on the taxonomic composition of algal assemblages (Kudela 2008). For example, different algal groups are specific food sources because of size selective grazing by zooplankton (Riegman et al. 1993). They can be useful as indicator organisms in polluted habitats and can serve as markers of long-term environmental changes (Gameiro et al. 2007; Kristiansen 2009). However, "the specifics of why a particular species blooms at a given time and place remain elusive" (Adolf et al. 2008, p. 119).

To understand the mechanisms controlling phytoplankton assemblages and primary production, it is necessary to understand the relationships of environmental factors to algae throughout the ocean. The ambient variables that influence phytoplankton growth and composition include nutrient concentration (Sommer 1993), temperature (Murrel and Lores 2004), salinity (Marshall et al. 2006) and light availability (Defew et al. 2004). Nutrients may limit algae growth; temperature regulates metabolic reactions; salinity affects osmoregulation; light controls photosynthesis; and the lack of any set of these factors may result in an overall negative effect.

Fundamental characteristics of ocean biology and chemistry vary in space and time with physical and chemical conditions that determine the richness of the ocean

(Cullen et al. 2007). Environmental change, such as turbulence and stoichiometry of the physical environment, like the N:P ratios, have a strong influence on survival of phytoplankton species (Cullen et al. 2007). But more than just survival, these variations may result in phytoplankton succession that characterizes an area that may be used to model and predict marine communities (Margalef 1978; Cullen et al. 2007).

Background

Good areas to study the relationship of surrounding variables and microalgae would be along short transects where environmental factors change significantly. These conditions are easily found in coastal regions where freshwater systems, such as rivers, meet with seawater and sharp gradients in physical and chemical parameters occur.

To examine differences along a transect, The University of Southern Mississippi (USM) and the Northern Gulf Institute (NGI) have conducted a study of different constituents along a transect in the northern Gulf of Mexico in an ongoing monitoring and assessment project. The key environments along the gradient are a small estuary, the Bay of St. Louis, the Mississippi Sound and an estuary and shelf waters outside the barrier islands. During monthly NGI cruises, physical and chemical factors, such as temperature, turbidity, conductivity, nutrient concentration and salinity at the stations located along this transect were measured.

The water depth of the station outside the barrier islands makes it reasonable to sample and analyze variables at three different depths, therefore supplying a third dimension to this study, providing insight as to how the mixing process of low salinity water encountering sea water throughout the water column interacts with primary producers and affects their growth.

Because surrounding factors not only influence phytoplankton quantities but also community structure (Gameiro et al. 2007), this study centered on how phytoplankton concentration and community composition varied along the coastal transition zones in relation to diverse environmental conditions. In order to address variation in community composition and determine the taxonomic groups present, different pigments were quantified using High Performance Liquid Chromatography (HPLC) and analyzed with CHEMTAX 1.95 (Mackey et al. 1997) to estimate phytoplankton community composition.

Given that seasonal variations also affect phytoplankton density and community structure (Murrel and Lores 2004), a time series of the observations that documented seasonal variability of different taxonomic groups was conducted. This time series continues previous surveys done in the Bay of St. Louis, so what has happened in this estuary during the last 10 years will be useful for baselines and trends to assess effects on climate change.

The northern Gulf waters are known as the "Fertile Fisheries Crescent" where estuarine and marine habitats are important due to the area's remarkable productive fisheries (MMNS 2005, p. 264). So far, there are many studies regarding temperate estuaries throughout the East coast of the United States, but very few include warm-temperate estuaries like those present in coastal Mississippi waters (Holtermann 2001). Currently, Holtermann (2001) is the only published study that includes phytoplankton species composition in the Bay of St. Louis, and no similar study has been performed in the Mississippi Sound or beyond the barrier islands. This study provided such information. The hypothesized relationships between the different phytoplankton groups

with the physical, chemical and temporal variables were established based on the study in the Bay of St. Louis made by Holltermann (2001).

Study Area

Bay of St. Louis

The Bay of St. Louis is a small, protected, low energy subtidal estuary enclosed on three sides by land. The bottom sediment consists of muddy sand that supports a diverse group of polychaetes, mollusks, insects and crustaceans (Phelps 1999; MMNS 2005). It is a well mixed system that receives fresh water from the Wolf and the Jourdan Rivers. The Bay's salinity levels range from 5 to 14 (Holtermann 2001) and constantly change, depending on the ebb and flow of the tides, the weather and the season (MMNS 2005). Urban exploitation, channel modification and incompatible water quality are the major threats to this estuarine bay (MMNS 2005).

Mississippi Sound

The Mississippi Sound is an estuary bounded by the coast of Mississippi to the north; Lake Borgne, Louisiana, to the west; Mobile Bay, Alabama, to the east; and a series of barrier islands including Ship, Horn, Petit Bois Islands, Dauphin and Cat Island to the south (Moncreiff 2007). The general current is westward and it induces sand movement along the shorelines. Salinity levels are polyhaline with the lowest values close to the mainland (MMNS 2005).

The Mississippi Sound exchanges salt water with the Gulf through the barrier island passes. From the north, the watersheds drain the mainland bringing freshwater. These watershed systems include the Pearl River, the Jourdan River, the Wolf River, the Tchoutacabouffa River, the Pascagoula River and the Mobile Bay basin that encompasses

seven river systems including the Mobile, Middle, Tensaw, Raft, Apalachee, Blakeley, Dog and Fish Rivers (Moncreiff 2007).

Land use varies in the eight watersheds feeding the Mississippi Sound, from agriculture and forestry for the paper and lumber industries to residential and casino development, plastics, and chemicals industries. Commercial shipping, shipbuilding, phosphate fertilizer, and electric power generating complexes have resulted in higher nutrient, sediment and contaminant loads that have caused a loss of water quality (Moncreiff 2007).

Mississippi Bight

The Mississippi Bight is south of the Barrier Islands and extends southward along the shelf. The Bight has marine habitats with salinity levels that may exceed 30, but seasonal current shifts bring fresh water from the Mississippi river to the Mississippi Bight (MMNS 2005). Multiple connections between the Northern Gulf of Mexico and the Mississippi Sound through a number of passes between the barrier islands, allow an interaction between the estuarine Sound and the Gulf of Mexico waters (Vinogradova et al. 2005). The irregular coastline and the islands result in a distinctive circulation pattern that adds spatial variability to water mixing (Vinogradova et al. 2005). Outside the Barrier Islands there is a system of reefs that support commercial and recreational fisheries, as well as spawning areas, though they are being threatened by excessive fishing, chemical spills and overboard discharge (MMNS 2005).

Objective

In this study, concentrations of phytoplankton pigments were quantified using High Performance Liquid Chromatography (HPLC), and it was determined if their concentrations changed in space and time in coastal Mississippi waters.

Hypotheses

H1. Phytoplankton abundance and species composition will vary spatially between the Bay of St. Louis, the Mississippi Sound and Shelf waters outside the barrier Islands.

- Diatoms will predominate in inshore waters with higher concentration of nutrients.
- Dinoflagellates and prymnesiophytes will prevail in offshore waters with lower nutrient concentration and higher salinities.
- Phytoplankton pigment concentration will vary with increasing salinity.

H2. The temporal variability of phytoplankton abundance and composition is related to environmental factors, such as weather and nutrient load in the study area.

- Cyanobacteria and chlorophytes will increase towards the summer.
- Diatoms and Cryptophytes will increase in the winter.
- Phytoplankton pigment concentration and composition will vary in response to wind speed and wind direction due to nutrient increase, storms and rainfall.

CHAPTER II

MATERIALS AND METHODS

Field Sampling

NGI cruises included 8 stations where casts were deployed. Water samples for pigments were collected at three of these stations: NGI 1 at the Bay of St. Louis, NGI 4 at the Mississippi Sound and NGI 8 at the Mississippi Bight (Fig. 1).

Monthly collections were performed between 8:00AM and 1:00PM at the three stations when weather conditions allowed sampling from the RV Lemoyne. There was surface sampling at stations 1, 4 and 8. At station (station) 8, samples were also collected at mid depth (9 m) and the bottom depth (19 m) for a total of 134 pigment samples (Table 2.1). Seawater was collected in 2 L bottles previously rinsed with nanopure water and rinsed in the field with sample water, then placed in coolers while being transported to the laboratory.

Fig. 2.1. Sampling stations. Station 1 was at the Bay of St. Louis, station 4 was at the pass between St. Bernard Parrish LA and Cat Island but it is considered to be Mississippi Sound and station 8 at the Mississippi Bight.

Sample Preparation

The same day of collection, samples were filtered onto 47mm GF/F filters at a vacuum of not more than 0.5 atm to avoid damaging cells. The filters were patted dry with Kimwipes®, placed in cryotubes, and frozen in dewars with liquid nitrogen at -196ºC until extraction (modified from Jeffrey et al. 1997). Samples were protected from light to avoid photoxidation.

	NGI Station	1		2		4		8					
	Depth	Surface		Surface		Surface		Surface		Middle		Bottom	
	Replicate	A	B	A	B	A	B	A	B	A	B	A	B
Dates	17-Sep-07												
	11-Oct-07												
	13-Nov-07												
	Dic-07												
	Jan-10												
	2-Feb-08												
	12-Mar-08												
	17-Apr-08												
	20-May-08												
	17-Jun-08												
	15-Jul-08												
	18-Aug-08												
	Sep-10												
	22-Oct-08												
	20-Nov-08												
	8-Dec-10												
	29-Jan-09												
	Feb-10												
	12-Mar-09												
	16-Apr-09												
	28-May-09												
	23-Jun-09												
	Jul-09												
	9-Aug-09												
	9-Sep-09												
	Oct-09												
	11-Nov-09												

Fig. 2.2. Samples collected and analyzed with HPLC. During September 2007 sampling sites had not been established definitely and station 2 was included once. Before February 2008 a middle depth had not been defined for station 8, so they are missing from the first 3 months. Dates with no data correspond to days with bad weather and sampling was not possible on the R/V Lemoyne.

Cryotubes containing the filters, and with the caps removed, along with an ice pack, were placed into a dark glass cylinder that was connected to a LABCONCO 6 Liter Console Freeze Dry System (Lyophilizer). The filters were dried at <0.05 mlliBar

vacuum and <-51ºC for 18 hours. This was to extract all the water from the filters. After the filters were dried, the cryotubes were capped and placed back in the dewars until extraction.

Filters were taken from the cryotubes and placed in 7 mL scintillation vials with 3 mL of 90% acetone. Then, they were placed on a Fisher Scientific Vortex mixer for 1 min. Next, the vials were put in a freezer at -17 ºC for 24 hours. After that, the filters were vortexed for 1 min. The 90% acetone extracted pigments were then filtered through a 0.2 μm Teflon syringe filter and filtered into a 2 mL microcentrifuge tube.

Sample Analysis

Prior to injection, a 1:1 mixture of sample extract was prepared with 350 μL sample mixed with 350 μL of 0.5 M ammonium acetate ion pairing solution that had been previously adjusted to pH 7.2 with ammonium hydroxide. The mixture was immediately injected into a 500μL injection loop, allowing the excess sample/ion pairing agent mixture to overflow into a collection vial. The loop contents were then injected onto an Alltech Alltima High Purity C-18 spherical silica, monomerically bonded, end capped, 100, 120, 190Å pore size analytical column. The sample was then analyzed using a Waters 600 Controller and Pump HPLC and the absorption spectra chromatograms were acquired using a Waters 2996 Photodiode Array Detector.

Reagents

Solvents used in the HPLC were degassed with He to prevent the formation of air bubbles in the column. Before use, acetone and acetonitrile were filtered with a 0.2μm pore size Millipore Nylon membrane. Methanol was filtered through a 0.2μm pore size Micron Osmonics Membrane.

A nonlinear gradient was used for pigment separation with the following solvents:

- A: 80% Methanol, 20% Ammonium Acetate 0.5M brought to pH 7.2 with drops of ammonium hydroxide.
- B: Acetonitrile (100%), 0.01% of BHT.
- C: 100% Acetone.
- D: 100% Nanopure Water.

Pigment Analysis

Pigments were identified by comparing their retention times and absorption spectra to those of pure standards. The equation to calculate pigment concentration is from Jeffrey et al. (1997):

1) $Cp = (A_p)(W_{is})(F_p)/(A_{is})(V)$

Where: *Cp* is the concentration of pigment in seawater, A_p is the Peak area of pigment from seawater (μV.s), W_{is} is the weight of mass of internal standard, F_p is the response factor of pigment with respect to the internal standard, A_{is} is the peak area of the internal standard (μV.s) and *V* is the volume of seawater filtered.

Diversity Index

According to Noble *et al*. (2003) and Estrada *et al*. (2004), pigment concentration can be used to calculate the richness of biological elements in a community. The diversity present at the three stations was determined using Shannon Diversity Index:

2) $H = -\sum_{i=1}^{s} (P_i * \ln P_i)$, where

H is the Shannon Diversity Index, P_i is the fraction of the entire population made up of pigment i, S is the number of pigments encountered and $\sum$ is the sum from pigment 1 to pigments S.

Initial Pigment Ratio

The computer program CHEMTAX 1.95 (Mackey et al. 1997) was used to establish the relative abundance of a phytoplankton group as a fraction of chlorophyll *a* (chl *a*). The absence, existence and combination of pigments in the samples were used to choose the groups present that had to be included in the initial pigment ratios. The groups whose pigments were not found were not included. The initial pigment ratios matrix for calculating algal class abundances were assembled from Schluter et al. (2000), Lewitus et al. (2005) and Laza-Martinez et al. (2007) (Table 2.1). Because the version of CHEMTAX 1.95 required more than one matrix of initial ratios, the second set of ratios were obtained from Schlüter et al. (2000), Lewitus et al. (2005), Laza-Martinez et al. (2007), and Pinckney et al. 2009 (Table 2.2) to best fit the results. The final pigment ratio matrixes from CHEMTAX 1.95 are in Appendix A, Tables A.2.a to A.2.c.

Table 2.1. First Initial pigment ratio.

	chl c 1+2	per	but	fuc	neo	hex	pra	vio	diad	all	lut	zea	chb	chl_a
Diat	0.326	0.000	0.000	0.779	0.000	0.000	0.000	0.000	0.317	0.000	0.000	0.000	0.000	1.000
Cyan	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.245	0.000	1.000
Chlor	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.031	0.000	0.000	0.320	0.040	0.277	1.000
Dino	0.219	0.533	0.000	0.000	0.000	0.000	0.000	0.000	0.232	0.000	0.000	0.000	0.000	1.000
Euglen	0.000	0.000	0.000	0.000	0.077	0.000	0.000	0.000	0.086	0.000	0.022	0.020	0.828	1.000
Prym	0.137	0.000	0.007	0.304	0.000	0.270	0.000	0.000	0.113	0.000	0.000	0.000	0.000	1.000
Crypto	0.221	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.405	0.000	0.000	0.000	1.000
Pras	0.000	0.000	0.000	0.000	0.096	0.000	0.229	0.069	0.000	0.000	0.067	0.061	0.605	1.000
Chryso	0.127	0.000	0.933	0.625	0.000	0.000	0.000	0.000	0.438	0.000	0.000	0.000	0.000	1.000
Eustig	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.221	0.000	0.000	0.000	0.015	0.000	1.000

Table 2.2. Second Initial pigment ratio.

	chl c 1+2	per	but	fuc	neo	hex	pra	vio	diad	all	lut	zea	chb	chl_a
Diat	0.3010	0.000	0.000	0.810	0.000	0.000	0.000	0.000	0.318	0.000	0.000	0.000	0.000	1.000
Cyan	0.0000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.324	0.000	1.000
Chlor	0.0000	0.000	0.000	0.000	0.049	0.000	0.000	0.040	0.000	0.000	0.174	0.018	0.282	1.000
Dino	0.2280	0.545	0.000	0.000	0.000	0.000	0.000	0.000	0.245	0.000	0.0000	0.000	0.000	1.000
Euglen	0.0000	0.000	0.000	0.000	0.071	0.000	0.000	0.007	0.203	0.000	0.000	0.053	0.236	1.000
Prym	0.137	0.000	0.007	0.304	0.000	0.270	0.000	0.000	0.113	0.000	0.000	0.000	0.000	1.000
Crypto	0.1600	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.354	0.000	0.000	0.000	1.000
Pras	0.000	0.000	0.000	0.000	0.096	0.000	0.229	0.069	0.000	0.000	0.067	0.061	0.605	1.000
Chryso	0.1270	0.000	0.933	0.625	0.000	0.000	0.000	0.000	0.438	0.051	0.000	0.000	0.000	1.000
Eustig	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.221	0.000	0.000	0.000	0.015	0.000	1.000

Environmental Variables

Environmental variables were obtained from *in situ* casts deployed during sampling. Variables, such as colored dissolved organic matter (CDOM) and chl *a* fluorescence, were obtained from a Wetlabs ECO FL3 optics package. Turbidity was acquired from an *InSitu* Instruments 9500 sensor. Dissolved oxygen and salinity were obtained from a Seabird Electronics SB43 sensor. Both instruments were field calibrated periodically with Winkler titration and Autosal Guildline 8400 respectively. Temperature, conductivity, and density (σ_t) were obtained from the SBE 49 CTD sensor. Nutrient samples were filtered and frozen at -17ºC until analysis with an Astoria Pacific A2 Nutrient Analyzer in the laboratories of The University of Southern Mississippi at the Stennis Space Center.

Values for tides, wind direction, wind speed, precipitation and river flow were obtained from stations throughout coastal Mississippi (Fig. 2.3). Environmental stations and environmental variable stations are presented on Table 2.3.

Precipitation

Rainfall data were compiled as the sum over 3 days prior to sampling (Gameiro et al. 2007). Precipitation values were only obtained for the Bay of St. Louis area since the weather station was at Waveland, MS.

Fig. 2.3. Sampling stations and environmental parameter locations. See Table for icons.

Table 2.3. Station description.

Variable	Icon	Location	Agency	Website	Station/ Buoy	Latitude	Longitude	Dates
Pigments, Nutrients, Salinity, Temperature, Turbidity, DCOM, DO		Bay	USM		NGI1	30.3095 N	89.3064 W	Most
		Bight	USM		NGI4	30.1979 N	89.1820 W	Most
		Sound	USM		NGI8	30.0425 N	88.6473 W	Most
Atm Pressure, Wave height, Wind Speed and Direction		Bight	USM	http://www.cengoos.org/archive.html	42067	30.043N	88.647W	M,J,J A,S/09
			NDBC	http://www.ndbc.noaa.gov/historical_data.shtml	42007	30.090N	88.769W	Most
		Bay	NOS	http://www.ndbc.noaa.gov/historical_data.shtml	WYCM6	30.326 N	89.326 W	A/08-N/09
Precipitation		Waveland	NCDC LAND SURFACE COOP COOP-A USHCN A	a.gov/IPS/coop/coop.html?_page=2&state=MS&foreign=false&stationID=229426&_targe	229426	30°18'0.00"N	89°23'0.00"W	Most
River Gage		Jourdan River	USGS	http://waterwatch.usgs.gov/?m=real&r=ms	2481660	30°23'14"N	89°26'29" W	Most
		Wolf River	USGS	http://waterwatch.usgs.gov/?m=real&r=ms	2481510	30°29'01"N	89°26'28" W	Most
		Baight Sound	USCarolina	http://tbone.biol.sc.edu/tide/worldmap.html	Chaneleur I.	30.0500° N	88.8667° W	All
					Cat I.	30°13'59.88"N	89°10'0.12"W	All
Tides		Bay	NOAA	http://tidesandcurrents.noaa.gov/data_menu.shtml?stn=8747437%20Bay%20Waveland%20Yacht%20Club,%20MS&type=Tide+Data	MS 8747437	30°19.5'N	89° 19.5' W	All

Tides

Verified data for tides were only obtained for the Bay of St. Louis (NGI 1). The values for the Sound (NGI 4) and the Bight (NGI 8) were estimated using the Xtide (Pentcheff 2010) program, which calculates the mean sea level (MSL) for a given date and location. The software uses recorded tide and current observations to tabulate and reduce them to a set of factors that can be used to model tide values.

To use the tidal values, the tidal phase angle was estimated. This can be calculated for any time during the cycle by standardizing the measure of location along the tidal curve regardless of the tidal period or amplitude (Dustan and Pinckney 1989), using the following equation:

3) Tidal Phase Angle=t*360/T

Where: t is the time in minutes between low slack time and sampling time, T is the difference between low slack times.

River Gage

River gage height data were used to determine averaged over 3 days before sampling because rainfall events three days prior to sampling have been reported to be the most influential in elevated river flows (Rowe 2008).

Wind Speed and Direction

According to Wilkerson et al. (2006), wind events as short as one day, or similar duration to phytoplankton generation times, are long enough to be favorable for vertical mixing of nutrients that may support subsequent blooms. Therefore wind speeds and directions from two days prior to sampling were averaged using the following equations (M. Gonsalves, pers. comm.):

$$4)\ Rad = (90-WD)*¶/180$$

$$5)\ W_x = \text{two day Ave } [WS*\cos(Rad)]$$

$$6)\ W_y = \text{two day Ave } [WS*\sin(Rad)]$$

$$7)\ \text{Average Wind Speed} = \sqrt{\{(W_x)^2+(W_y)^2\}}$$

$$8)\ \text{Average Wind Direction} = 90+\{180*\ [\text{Arctan}(W_y/W_x)]/\ ¶\}$$

Where WD is wind direction and WS is wind speed.

Data Analysis

The spatial and temporal variation of phytoplankton groups, pigments and environmental variables had less than 2000 values; thus, a Shapiro Wilkinson Test in (SPSS) was used to test if the numbers were distributed normally. Since the data were not distributed normally, statistical analyses were performed using nonparametric statistics.

Dendrograms, Kruskal-Wallis test, and Spearman Correlation were performed using MATLAB 2010b. Box plots were created using SPSS. Dendrograms were made to cluster phytoplankton groups and variables. The function used to measure distance was standardized euclidean distance; thus, the distance was divided by the standard deviation of each variable. The linkage used was complete (i.e., the furthest distance between the samples in each cluster).

Sample temperature values had a gap at 24ºC. For this reason a cluster analysis was performed to check which samples were above or below this gap. Samples with the warmer temperatures were mainly between May and October (Appendix C, Fig. C.7), thus providing a basis to cluster data into hot or cold time of year.

The Kruskal-Wallis ranking test was performed to examine differences between groups of variables. The first variable was station, and it grouped the surface values from stations 1, 4 and 8. At station 8, we also measured variables at different depths, so another Kruskal-Wallis test was performed to determine if the different depths had significant differences. Variables were also grouped according to the month, season and hot or cold time of year.

Variability in temporal and spatial distributions of pigments and phytoplankton groups was also analyzed in relation to abiotic factors. This was done using Spearman's rank correlation analysis. Each pair of variable was correlated to determine if they were associated. Gage height and precipitation data were only correlated for values in the Bay of St. Louis (station 1).

Ordination

Ordination approaches like detrended correspondence analysis and redundancy analysis were used to relate assemblages, composition and environmental data directly using the program CANOCO 4.5 (Braak and Šmilauer 2002). Because the assemblage composition and environmental data was skewed, a transformation was required to perform more ordination tests. A log transform, which is the most common solution, was challenging because of the presence of zeros in the species data set. An alternate solution was adding 1 to the log transformed data. Because concentration values were so small, in most cases below 0.1 μg L^{-1}, calculating ln(n+1) would definitely change and distort the estimates (C. F. Rakocinski, per. comm.). In addition, according to Palmer (2011), logarithmic transforms are not recommended because they give different values depending on the mass units used, for example whether they are grams or kilograms. Since species abundances have asymmetric distributions and organisms tend to have exponential growth when conditions are favorable, taking the fourth root was used to reduce asymmetry of the distribution before performing further ordination techniques (Legendre and Birks 2010). There was no need to worry about normality since the tests of significance in canonical redundancy analysis (RDA) were done with Monte Carlo permutation tests (Legendre and Birks 2010). Data were standardized in all ordination procedures; thus, the selection of units in environmental measurements did not play a role (Lepš and Šmilauer 2003).

The purpose of ordination is to find axes of greatest variability in the community and to visualize the similarity structure for samples and species (Lepš and Šmilauer 2003). The aim of constrained ordination is to find the variability in species composition

that can be explained by measured environmental variables (Lepš and Šmilauer 2003). The Monte Carlo permutation statistical test was used to relate the general null hypothesis, stating the independence of species data with respect to the values of the explanatory variables (Lepš and Šmilauer 2003). The Monte Carlo test reshuffles the environmental rows data available and keeps the species rows intact (Lepš and Šmilauer 2003). For each data set permuted, a constrained ordination is calculated using an F-statistic (Lepš and Šmilauer 2003). The chances that any combination of the data is the same as the true data set implies that the null hypothesis is true (Lepš and Šmilauer 2003).

To determine if the species response along environmental gradients was linear or unimodal detrended correspondence analysis (DCA) was used (Legendre and Birks 2010). This analysis minimized the arch effect and edge effect, so the lengths of the new ordination "axes were given by the range of object scores and were expressed in standard deviation units" (Legendre and Birks 2010, p. 15). Because the gradient length was narrow (SD<2.5), a linear approach like RDA was used (Legendre and Birks 2010).

CHAPTER III

RESULTS

In this study, samples were collected from three stations that encompassed a range of salinities from the Bay of St. Louis (an estuary) to outer coastal waters of the Gulf of Mexico. The samples were analyzed for pigments with HPLC and these pigments were used to determine pigment taxonomic groups based on CHEMTAX analysis. These results were then used to test the main hypotheses:

- Phytoplankton abundance and species composition will vary spatially between the Bay of St. Louis, the Mississippi Sound and Shelf waters outside the barrier Islands.
- The temporal variability of phytoplankton abundance and composition is related to environmental factors, such as weather and nutrient load in the study area.

Because the hypotheses link biological factors to spatial and temporal variability among the properties measured, the distribution of pigments and phytoplankton group data has been presented, and environmental data was described to explore relationships between pigment-based taxonomy and environmental factors. As stated in the methods, the differences found between temporal and spatial data has been analyzed statistically with the Krukal-Wallis test. Once these results have been presented, it is also possible, using ordination, to relate phytoplankton composition within an environmental context.

Pigments

Pigment concentrations were determined by analysis with HPLC. The most important and noticeable pigment analyzed was chl *a*. The bar graph on Fig. 3.1,

illustrates that the highest single value was in the Mississippi Sound on August of 2009. There was a significant ($p<0.01$) difference in chl *a* from surface values between stations and seasons (Table 3.1). The highest chl *a* values among all seasons and at all the stations were observed in the summer (Fig. 3.2). There was no significant difference in chl *a* values from station 8 between depths or seasons (Table 3.2).

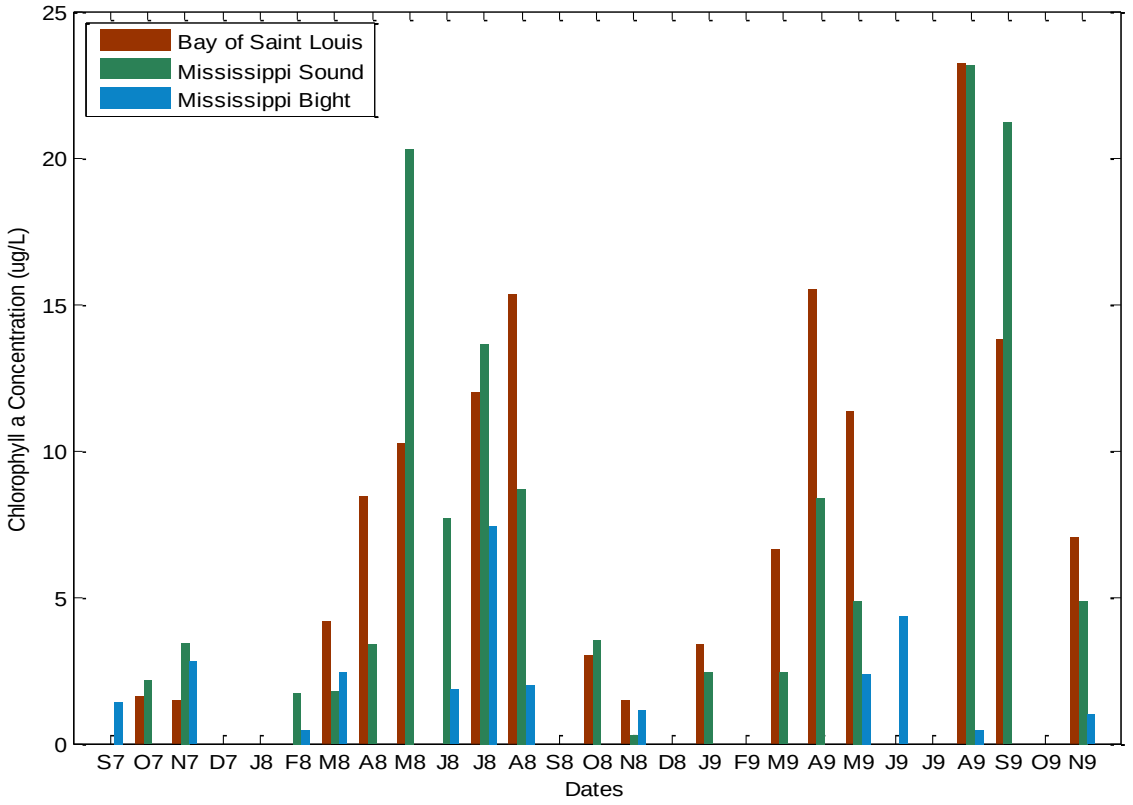


Fig. 3.1. Bar graph of chl *a* concentration at station 1, Bay of St. Louis; station 4 MS Sound; station 8 Surface MS Bight during sampling dates.

The highest median values for chl *a* concentrations were found at stations 1 and 4. Station 8 at 9m depth had the lowest chl *a* concentration. The gaps between pigments are days where there was no sampling due to poor weather conditions. Station 8 at 9m was included in the sampling after the research had started, hence the low number of samples.

There was a linear correlation between chl *a* from HPLC analysis and from the extracted chl *a* fluorometric method (Welschmeyer 1994). A Model II linear regression of HPLC with Welschmeyer (1994) chl *a* values gave a slope (b_{II}) of 1.03 and an intercept (a_{II}) value of 0.5. At 95% level of confidence, the confidence interval for the slope is (0.912, 1.014) (Appendix A, Table A2). The fact that the composition of both models gave a slope close to one assures that both methods gave similar results (Appendix A Fig. A.1).

The pigments determined with HPLC (Appendix A, Figs. A.2a to A.2e) also include pigments that were not used for the initial ratio required by CHEMTAX and degradation products like Phaeophytin a, Phaeophorbide a and b. All samples had similar amounts of pigments with the exception of the sample taken during November 2008 at station 4. For that sample, the concentration of Fucoxanthin and chlorophyll c_{1+2} were even greater than chl *a*.

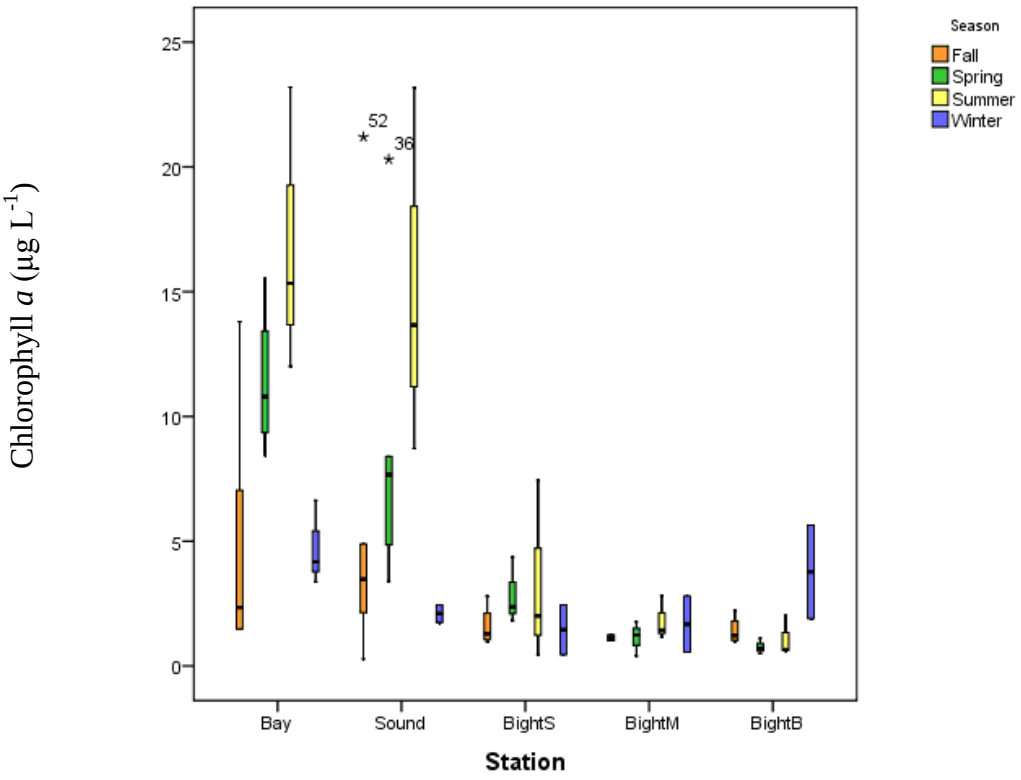


Fig. 3.2. Box plot of chl *a* (µg L^{-1}). The Bay is station 1, the Sound is station 4, Bight S is station 8 Surface, Bight M is station 8 (9m) and Bight B is station 8 at 19m. The central line in the SPSS box plots (Figs. 3.6. and 3.9) represent the median. The color box represents the Interquartile Range of the sample, the top of the box represents the 75th percentile, the bottom of the box represents the 25th percentile. The fact that the 50th percentile or median is not in the middle means the data is skewed. The top and bottom lines represent the largest and smallest values, within 1.5 times the interquartile range. The symbols in the graphs represent outliers that only happen if the data does not have a normal distribution and are beyond 1.5 the interquartile range.

Kruskal-Wallis Test

A Kruskal-Wallis Test was performed to determine if there was a significant difference between the variables for station, depth or time of the year. Thus, Table 3.1 illustrates surface water differences among variables at stations and Table 3.2 illustrates differences between the depths at station 8. Only those variables that had a complete data set, and that were referenced in the hypothesis are included.

Table 3.1. Kruskal-Wallis Test results for surface samples at stations 1, 4 and 8 (n=46). High significant values ($p<0.01$) are denoted with ** and significant values of ($p<0.05$) are denoted with *.

	station	Month	Season	Hot-Cold
chl. *a*	0.0047**	0.0544	0.0018**	0.0147*
Diatoms	0.0004**	0.0653	0.0269*	0.1529
Cyanobacteria	0.3055	0.0007**	0.0000**	0.0001**
Chlorophytes	0.0012**	0.2426	0.1237	0.1831
Dinoflagellates	0.4198	0.4957	0.3827	0.3670
euglenophytes	0.0134*	0.2543	0.2854	0.0423*
eustigmatophpytes	0.0618	0.4171	0.2854	0.8613
prymnesiophytes	0.2201	0.2277	0.3492	0.8185
cryptophytes	0.0001**	0.4144	0.2174	0.2613
prasinophytes	0.0188*	0.0991	0.5867	0.0143*
chrysophytes	0.7260	0.5057	0.7291	0.0560
temperature	0.5909	0.0000**	0.0000**	0.0000**
salinity	0.0000**	0.0589	0.0415*	0.7919
DIN	0.5061	0.4357	0.1696	0.5381
SiO_3	0.0017**	0.5482	0.5023	0.2912
PO_4	0.1128	0.3356	0.2988	0.4816
Shannon Diversity	0.3490	0.3091	0.6078	0.3444

Chlorophyll *a*, diatoms, chlorophytes, euglenophytes, cryptophytes, and prasinophytes varied significantly ($p<0.05$) in surface waters between stations 1, 4 and 8. Cyanobacteria was the only group that changed sinificantly ($p<0.01$) between seasons in surface waters (Table 3.1)

Prymnesiophytes, cryptophytes and chrysophytes changed significantly ($p<0.01$) between depths (Table 3.2). Diatoms, cyanobacteria, chlorophytes, and euglenophytes changed between seasons at station 8.

Table 3.2. Kruskal-Wallis Test results for samples from surface, middle and bottom depths at station 8 (n=34). High significant values ($p<0.01$) are denoted with ** and significant values of ($p<0.05$) are denoted with *.

	Depth	Month	Season	Hot-Cold
chl. *a*	0.4712	0.2210	0.6953	0.2210
diatoms	0.6268	0.0060**	0.0204*	0.0060**
cyanobacteria	0.6855	0.0005**	0.0000**	0.0005**
chlorophytes	0.1044	0.0177*	0.0066**	0.0177*
dinoflagellates	0.1856	0.0203*	0.1169	0.0203*
euglenophytes	0.0957	0.0072**	0.0006**	0.0072**
eustigmatophpytes	0.6996	0.1984	0.3970	0.1984
prymnesiophytes	0.0435*	0.2060	0.8266	0.2060
cryptophytes	0.0489*	0.1907	0.1908	0.1907
prasinophytes	0.3502	0.3136	0.1194	0.3136
chrysophytes	0.0167*	0.4218	0.5917	0.4218
temperature	0.5465	0.0015**	0.0001**	0.0015**
salinity	0.0016**	0.5197	0.5437	0.5197
DIN	0.0001**	0.9035	0.6731	0.9035
SiO_3	0.4197	0.0052**	0.1988	0.0052**
PO_4	0.0868	0.1218	0.0180*	0.1218
Shannon Diversity	0.0689	0.0307*	0.0045**	0.0307*

Phytoplankton Groups

Phytoplankton taxonomic composition was determined with CHEMTAX 1.95 (Mackey et al. 1997), and the relative contribution of observed taxa are in Appendix D (Table D.1). Figures 3.3 to 3.7 illustrate the relative abundance of each phytoplankton group as a fraction of the total chl *a*. Diatoms predominated at stations 1 and 4. Cyanobacteria and diatoms predominated at station 8. Chlorophytes were present at stations 1 and 4, but almost absent at station 8, while prymnesiophytes and chrysophytes increased toward open waters. At station 4 during June 2008, dinoflagellates increased their abundance dramatically. In July 2008, it was chlorophytes that increased at station 4. In figures D.1a to D.1j of Appendix D, it is also possible to see that peak contributions for each group were different for every station and date.

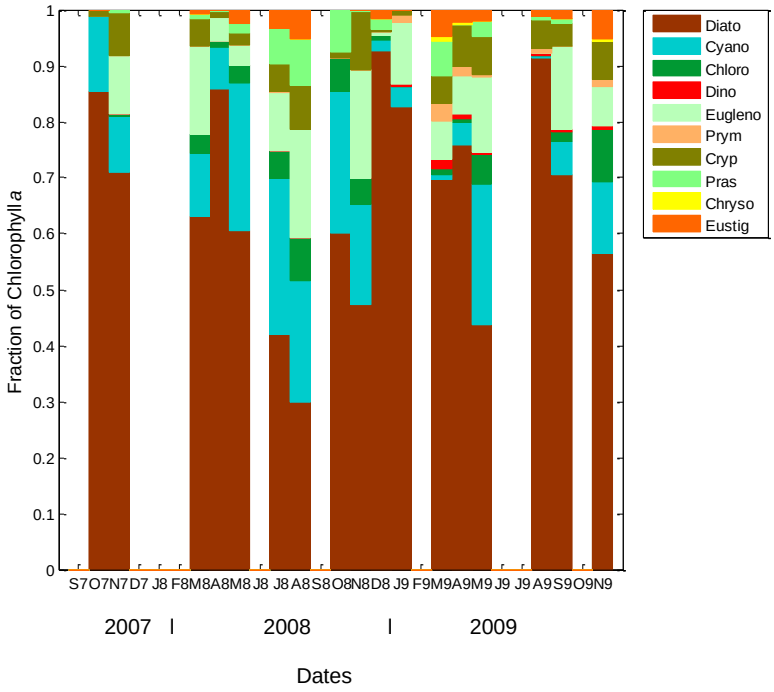


Fig. 3.3. Fraction of chl *a* present on each sampling day at station 1 (Bay of St. Louis).

The percentage of a particular group of the total chl *a* concentration was used to look at seasonal changes (Appendix B, Table B.2). Phytoplankton group percentages were similar at station 1 and station 4, but different for station 8 (Figs. 3.3 to 3.5). Diatoms went from a median of 66% of the chl *a* in the summer to 56% in the winter at station 1, while their median concentration went from 78% in the winter to 44% in the summer at station 8.

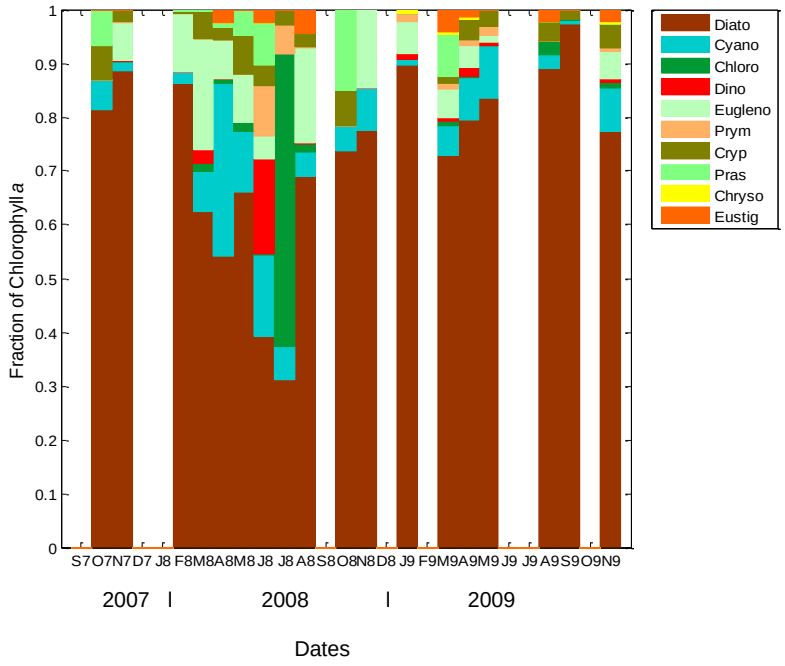


Fig. 3.4. Fraction of chl *a* present on each sampling day at station 4 (Mississippi Sound).

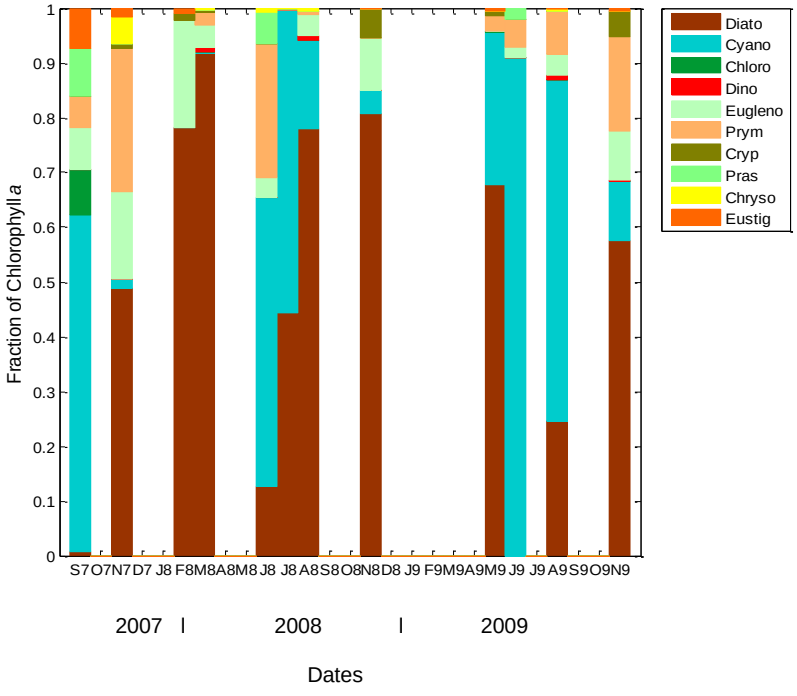


Fig. 3.5. Fraction of chl *a* present on each sampling day at station 8 Surface (Mississippi Bight).

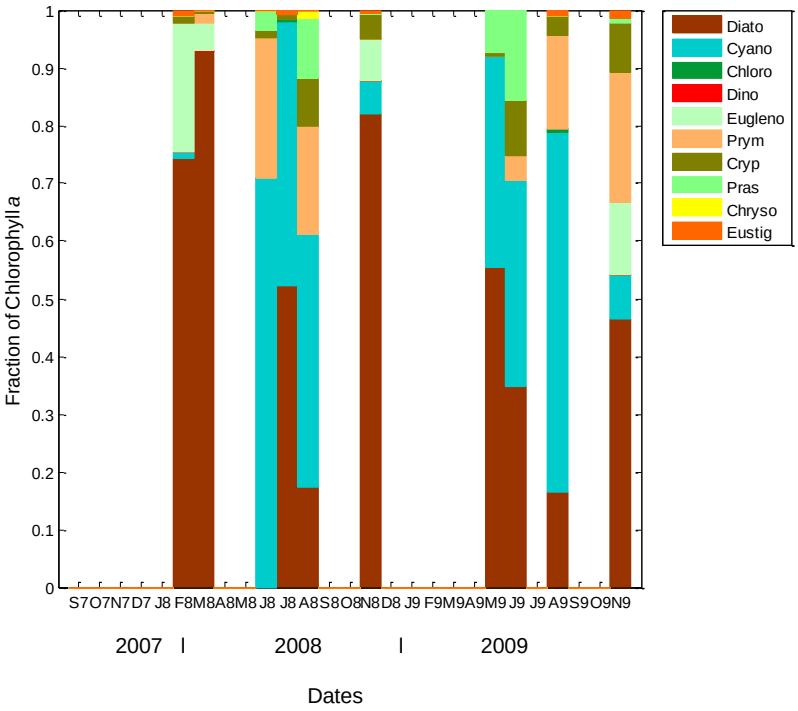


Fig. 3.6. Fraction of chl *a* present on each sampling day at station 8 (Mississippi Bight) Middle (9m).

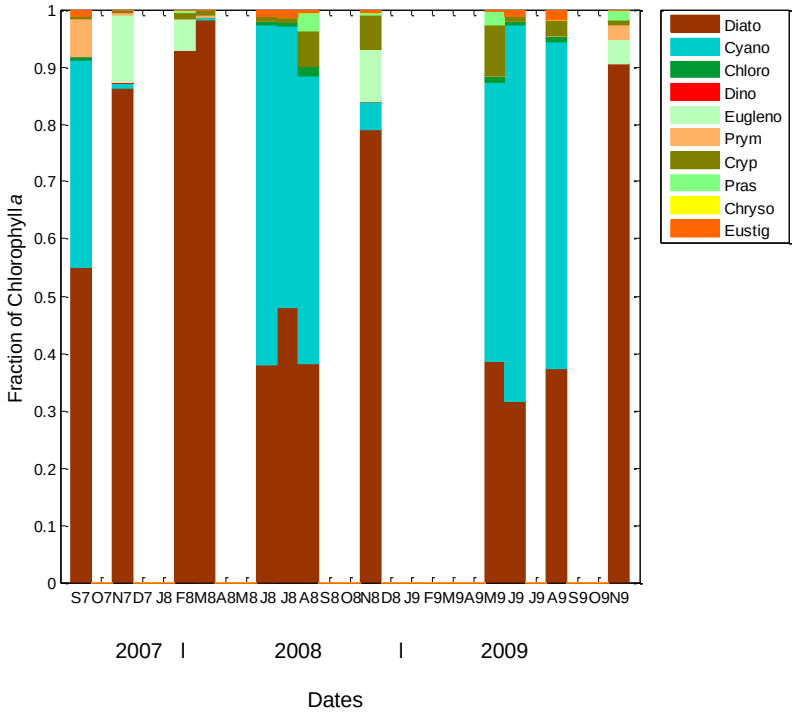


Fig. 3.7. Fraction of chl *a* present on each sampling day at station 8 (Mississippi Bight) Bottom (19m).

Cyanobacteria started at 2% in the winter and during the summer increased to 13% at station 1 while at station 8 they went from 0% in the winter to 55% in the summer. Prymnesiophytes were between 0% and 0.6% throughout the year at station 1 while at station 8 they increased from 0% in the summer to 17% median in the spring. The changes in other algae groups ratios were not as great as these described above.

The quantity of each phytoplankton group has been described, but it is also important to look at each phytoplankton group individually in relation to the stations (spatial variability) and the seasons (temporal variability). Diatom abundance was different significantly between stations ($p<0.01$), and between seasons ($p<0.05$) in surface waters (Tables 3.1 and 3.2). Diatoms were more important in the summer (Fig. 3.8) at station 1 and station 4. There was no significant difference between diatom abundance at different depths at station 8. The highest biomass was at station 1 and station 4 in August and September 2009 (Fig. B.1a).

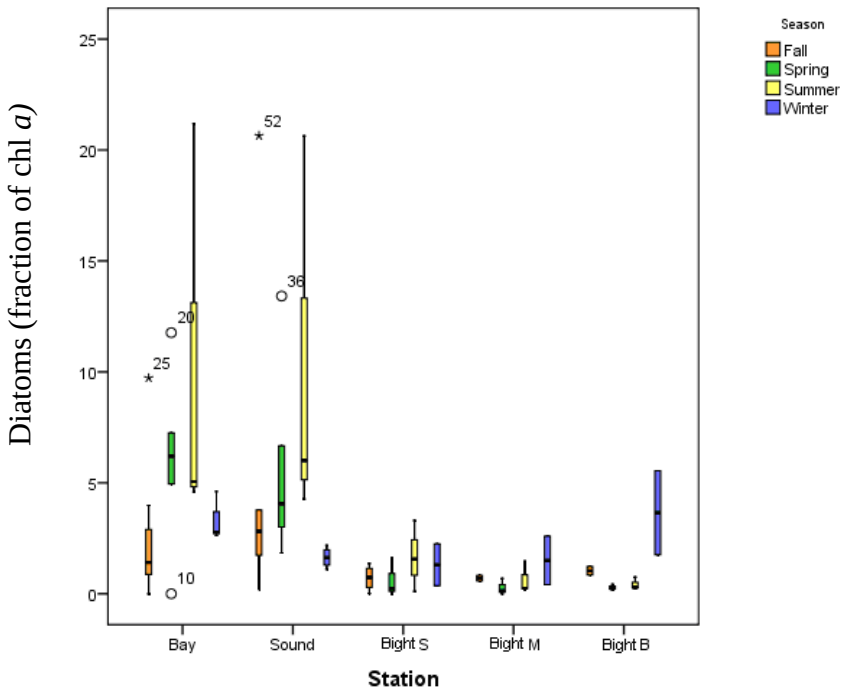


Fig. 3.8. Box plot of diatom fraction of chl *a* (µg L^{-1}) in each station per season. The Bay is station 1, the Sound is station 4, Bight S is station 8 Surface, Bight M is station 8 (9m) and Bight B is station 8 19m. Values beyond 1.5 times the interquartile range (outliers) are represented as º, values beyond 3 times the interquartile range (extremes) are represented as *. The numbers next to these symbols represent sample numbers.

Cyanobacteria did not vary significantly between stations. In surface waters, cyanobacteria changed significantly ($p<0.01$) between seasons (Table 3.1), but not between depths for station 8. The highest values for cyanobacteria were at stations 1 and 8 during the summer (Fig. B.1b).

Chlorophyte values were different significantly ($p<0.01$) between stations, but not between seasons (Table 3.1). The highest values for chlorophytes were in the summer (Fig. 3.10), specifically July 2008 at station 4 (Fig. B.1c).

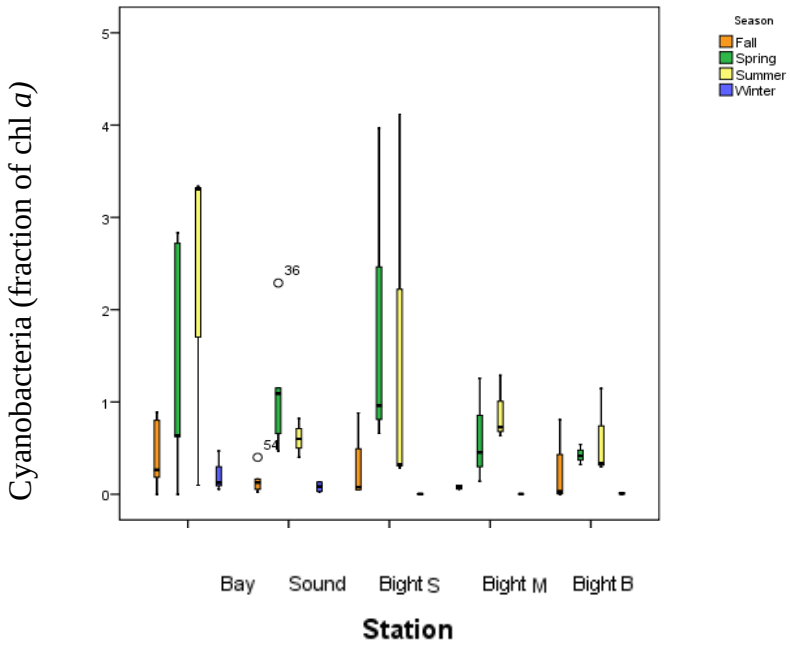


Fig. 3.9. Box plot of cyanobacteria fraction of chl *a* (µg L^{-1}) per station each season. The Bay is station 1, the Sound is station 4, Bight S is station 8 Surface, Bight M is station 8 (9m) and Bight B is station 8 (19m).

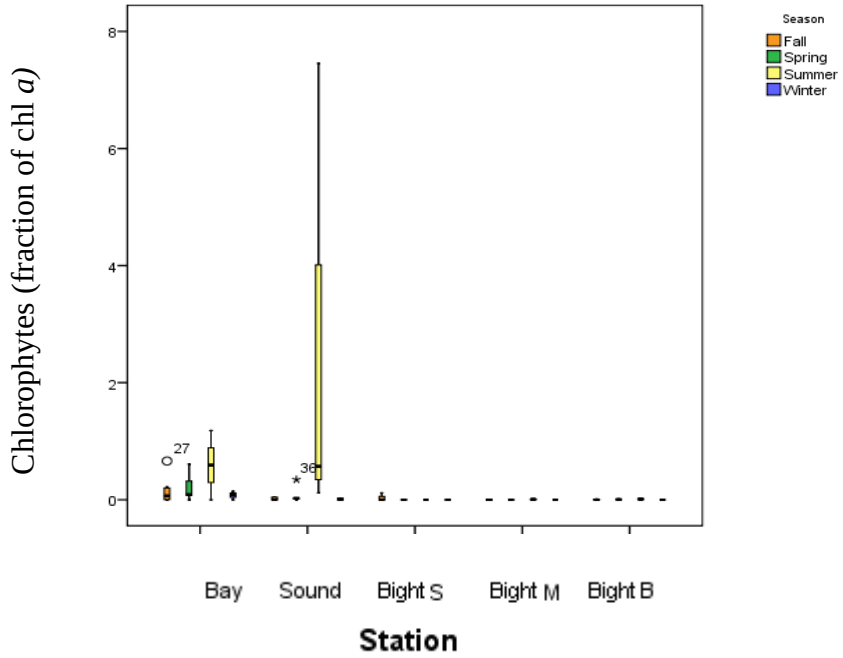


Fig. 3.10. Box plot of chlorophyte fraction of chl *a* (µg L^{-1}) per station each season. The Bay is station 1, the Sound is station 4, Bight S is station8 Surface, Bight M is station 8 (9m) and Bight B is station 8 (19m).

Dinoflagellate density did not change significantly between stations, depths or seasons (Tables 3.1 and 3.2). There was one event when there was a higher contribution of dinoflagellates at station 4 (Fig. 3.11, Appendix B Fig. B1.c) in July of 2008.

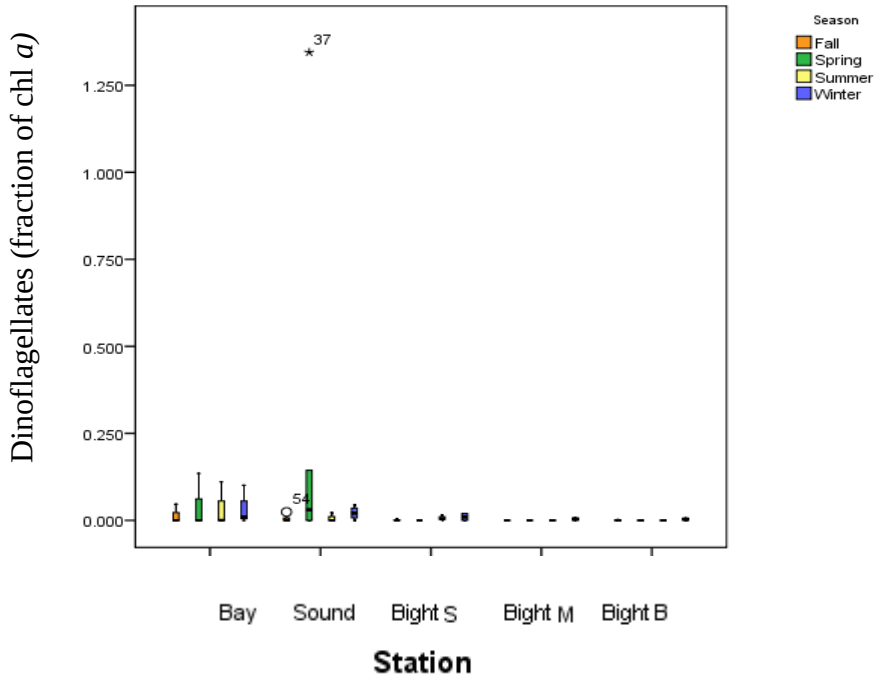


Fig. 3.11. Box plot of dinoflagellate fraction of chl *a* (µg L^{-1}) per station each season. The Bay is station 1, the Sound is station 4, Bight S is station 8 Surface, Bight M is station 8 (9m) and Bight B is station 8 (19m).

Euglenophytes varied their numbers significantly ($p<0.05$) between stations in surface waters (Table 3.1). Their values did not vary significantly between depths nor seasons, but did vary significantly ($p<0.05$) between hot and cold times of year. The highest values where closer to the coast in warmer months (Fig. 3.12).

Prymnesiophyte relative abundance was not different significantly between stations or seasons, but was different significantly ($p<0.05$) between depths (Tables 3.1 and 3.2). Prymnesiophyte values were high at stations 4 and 8 in spring, summer and fall

(Fig. 3.13). The highest relative abundance of prymnesyophytes was at station 4 in June and July 2008 (Fig. B.1f).

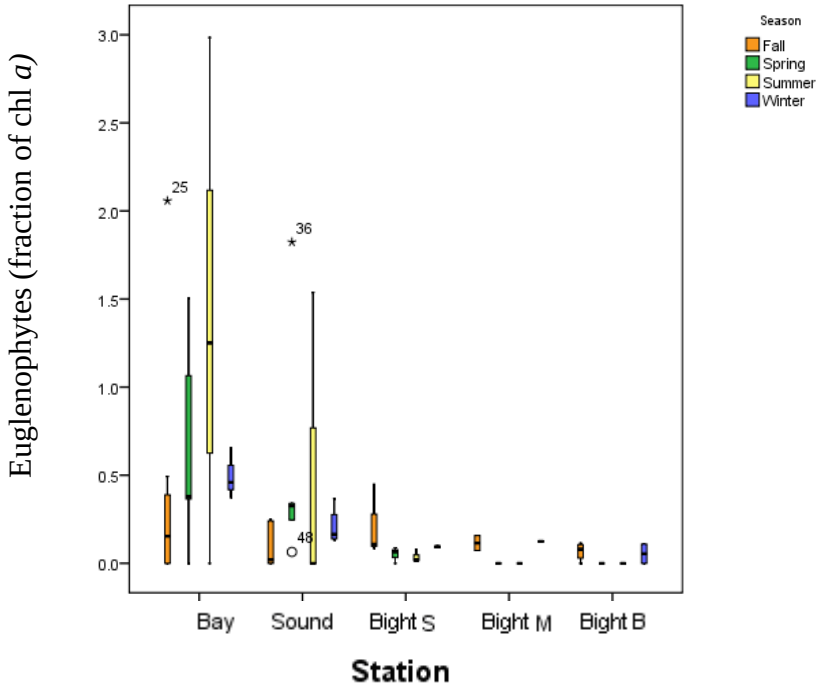


Fig. 3.12. Box plot of euglenophyte fraction of chl *a* (µg L^{-1}) per station each season. The Bay is station 1, the Sound is station 4, Bight S is station 8 Surface, Bight M is station 8 (9m) and Bight B is station 8 (19m).

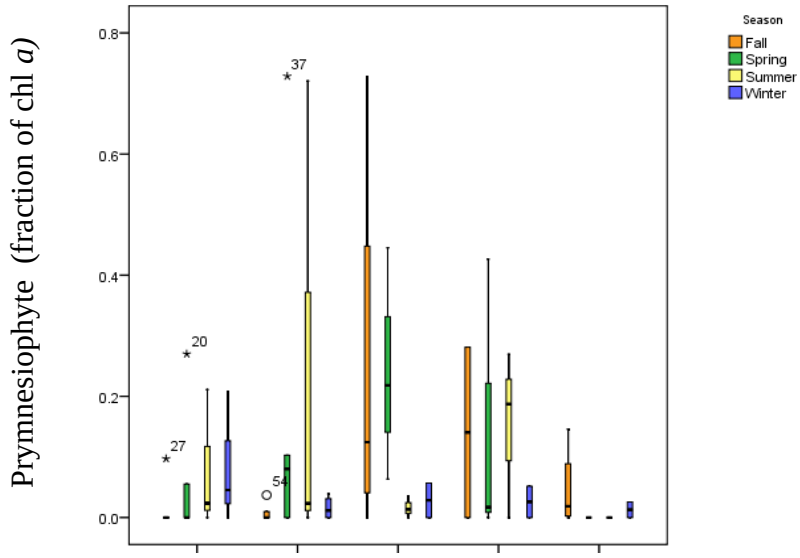


Fig. 3.13. Box plot of prymnesiophyte fraction of chl *a* (µg L^{-1}) per station each season. The Bay is station 1, the Sound is station 4, Bight S is station8 Surface, Bight M is station 8 (9m) and Bight B is station 8 (19m).

Cryptophyte values were different significantly ($p<0.01$) between stations, and depths at station 8 ($p<0.05$) (Tables 3.1 and 3.2), but there was no significant difference between seasons (Fig. 3.14). The highest relative abundance of cryptophytes was at station 1 during the summer.

Prasinophyte relative abundance was different significantly ($p<0.05$) between the three stations (Table 3.1 and 3.2), but not different between depths or seasons. However, prasinophyte concentration was different significantly ($p<0.05$) between hot and cold times of year. The highest contribution was during the summer at station 1 (Fig. 3.15).

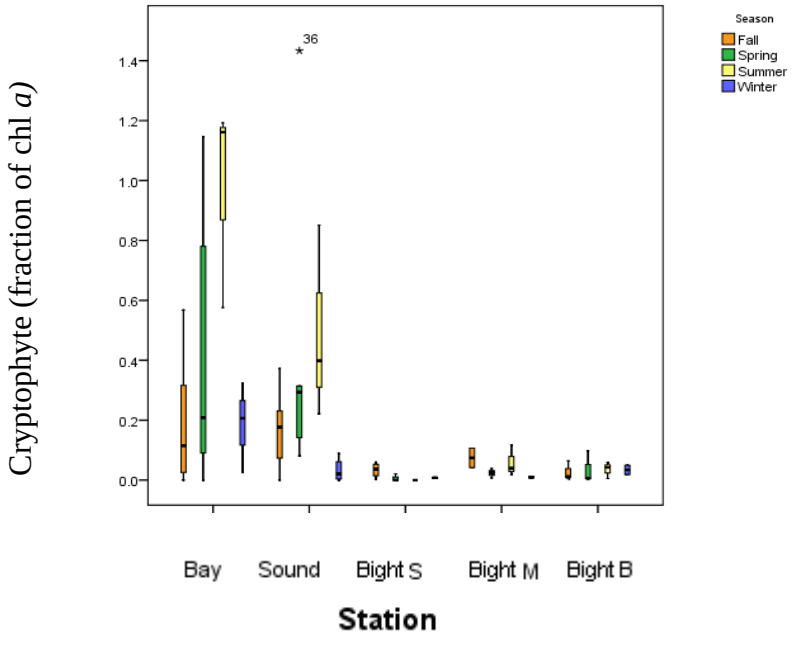


Fig. 3.14. Box plot of cryptophyte fraction of chl *a* (µgL-1) per station each season. The Bay is station 1, the Sound is station 4, Bight S is station Surface, Bight M is station 8 (9m) and Bight B is station 8 (19m).

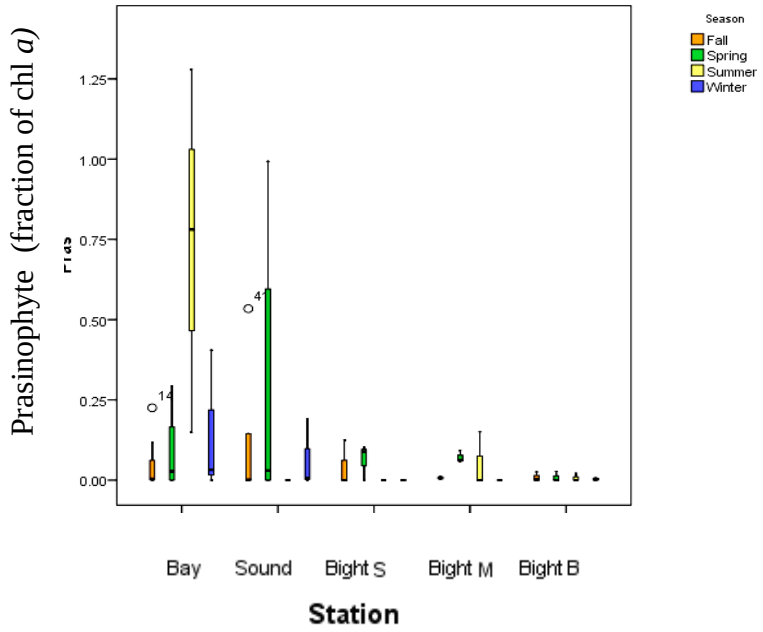


Fig. 3.15. Box plot of prasinophyte fraction of chl *a* (µg L^{-1}) per station each season. The Bay is station 1, the Sound is station 4, Bight S is station8 Surface, Bight M is station 8 (9m) and Bight B is station 8 (19m).

Chrysophyte relative abundance did not vary significantly between stations or seasons, though the concentration changed significantly ($p<0.05$) between depths at station 8. The highest concentration was at station 8 surface waters during the fall (Fig. 3.16). Chrysophytes had the lowest concentration among all phytoplankton groups studied.

Eustigmatophpyte abundance was not different between stations, seasons or depth (Tables 3.1 and 3.2). Nevertheless, their values ranged higher at station 1 during summer months (Fig. 3.17).

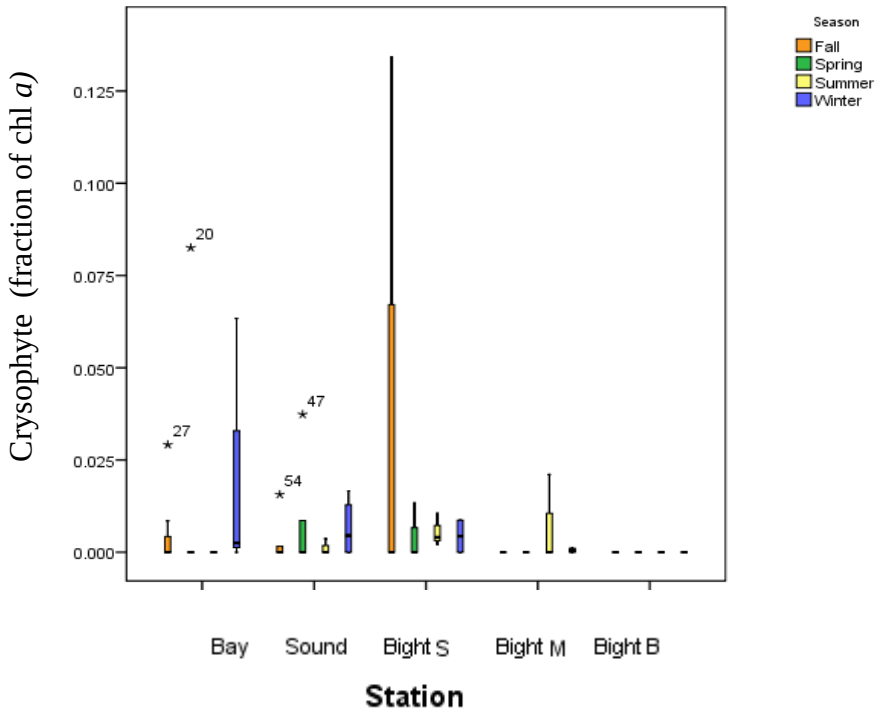


Fig. 3.16. Box plot of chrysophyte fraction of chl *a* (µg L^{-1}) per station each season. The Bay is station 1, the Sound is station 4, Bight S is station 8 Surface, Bight M is station 8 (9m) and Bight B is station 8 (19m).

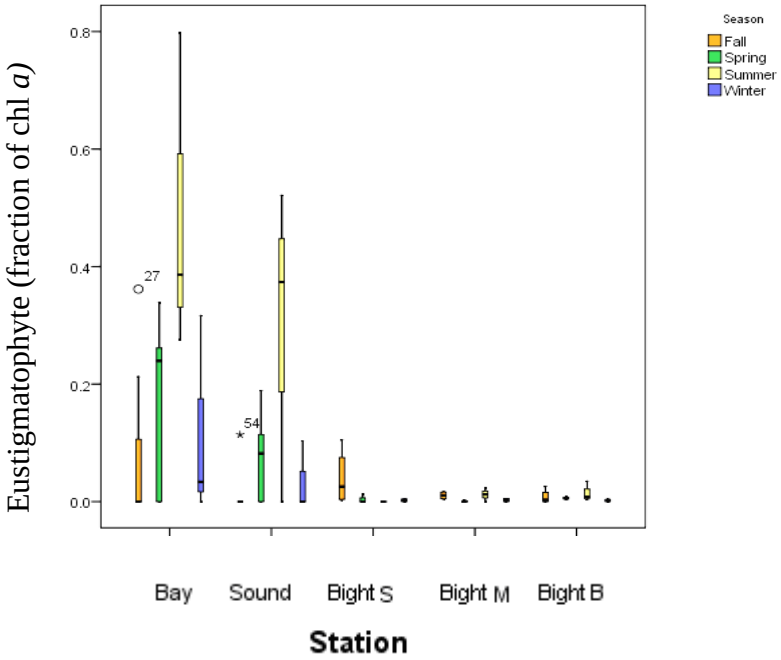


Fig. 3.17. Box plot of eustigmatophpyte fraction of chl *a* (µg L^{-1}) per station each season. The Bay is station 1, the Sound is station 4, Bight S is station 8 Surface, Bight M is station 8 (9m) and Bight B is station 8 (19m).

Spatial and Temporal Variability

The first hypothesis relates phytoplankton groups and their abundance to their spatial surroundings in coastal Mississippi waters, and salinity was the variable selected to assess that gradient between the three stations. Salinity was higher significantly ($p<0.01$) further from the coast and higher significantly ($p<0.01$) in deeper waters (Figs. 3.5 and 3.8). Salinity changed throughout the year, being significantly ($p<0.05$) lower in spring and higher in the fall for surface values (Table 3.1). Station 8 values did not change significantly throughout the year (Table 3.2).

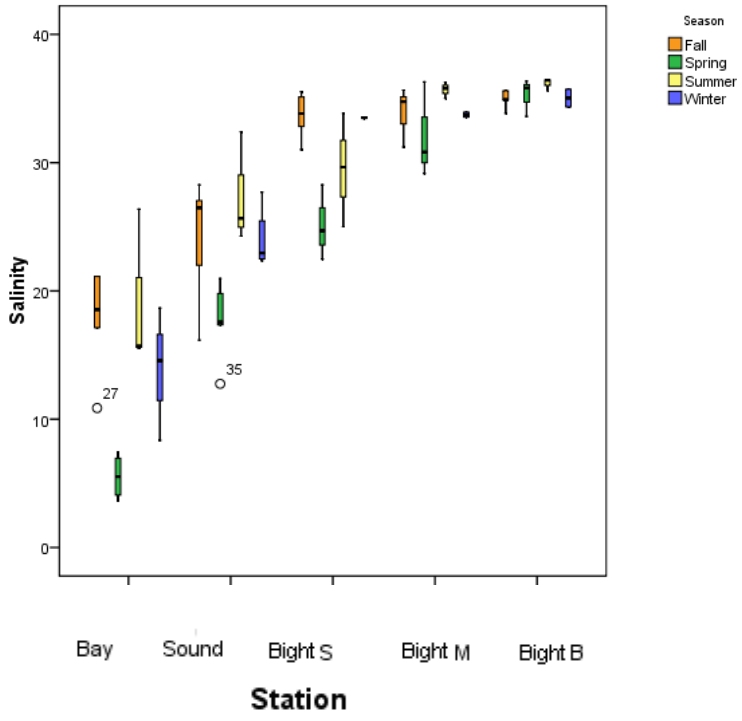


Fig. 3.18. Box plot of Salinity. The Bay is station 1, the Sound is station 4, Bight S is station 8 Surface, Bight M is station 8 (9m) and Bight B is station 8 (19m). Values beyond 1.5 times the interquartile range (outliers) are represented as º, values beyond 3 times the interqauartile range (extremes) are represented as *. The numbers next to these symbols represent sample numbers.

Temporal Variability

The second main hypothesis relates phytoplankton groups and their abundance to temporal changes. The environmental variables that were influenced mainly by daily weather conditions did not show a clear trend during the sampling period. Gage height trends differ greatly between the Wolf River and the Jourdan River (Appendix C Fig. C.1). Precipitation trends were also not similar between 2008 and 2009 (Fig. C.3). Monthly average precipitation for Waveland (Fig. C.2) did not show a clear pattern, so it is not easy to establish which were the wet and dry seasons during sample collection. Monthly El Niño Southern Oscillation (ENSO) Index values (Fig. C.4) were used to look for possible associations with climate. ENSO values were positive indicating La Niña conditions for values >1.0 until April 2009 when they turned negative with values <1.0, representing El Niño conditions, but this clear pattern was not linked with the ambient values for environmental characteristics already mentioned.

Winds averaged for two days prior to sampling did not show a clear seasonal trend, at least during the sampling days. Northerly and Southerly winds of 3 m s^{-1} were the most common, with the highest speed occurring during the sampling of September 2007 (Fig. C.5). Tidal phase graphs did not show any tendency with salinity or chl *a* to increase during Flood or Ebb (Fig. C.6), or in relation to other variables.

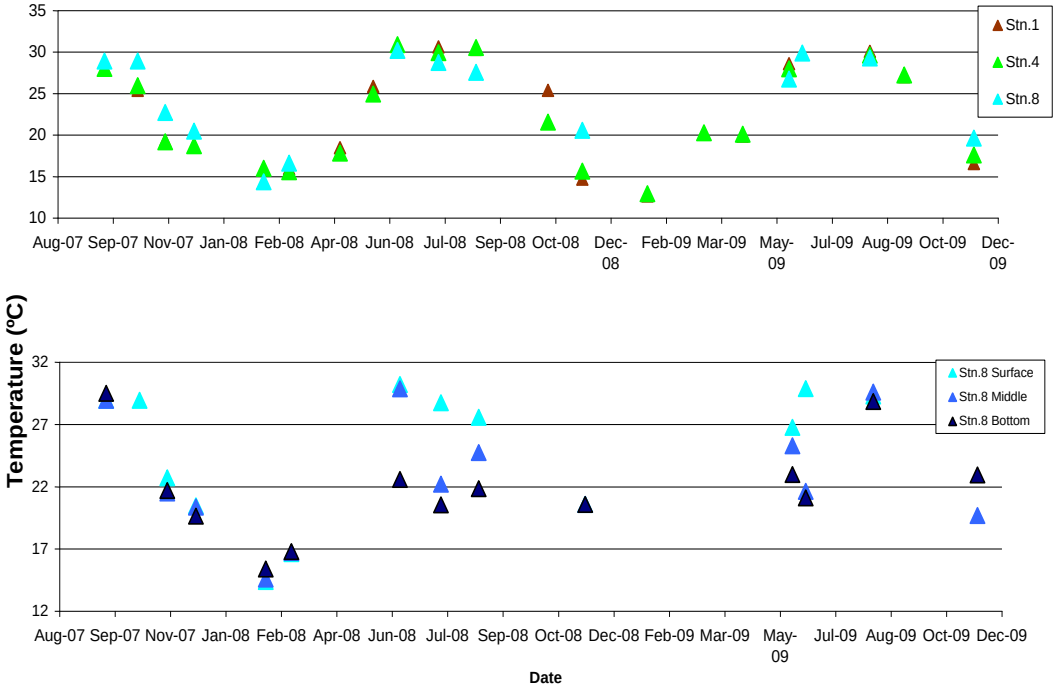


Fig. 3.19. Temporal variation of Temperature, showing the dates of sampling. On top the three stations and at the bottom the 3 depths at station 8 Surface, Middle (9m) and Bottom (19m).

Temperature was an environmental variable that did show a seasonal trend in coastal Mississippi waters. Temperature values increased significantly ($p<0.01$) during the summer and decreased during winter in surface waters. Middle and bottom temperatures did not follow the same trend as surface values at station 8, but their values were also different significantly ($p<0.01$) each month and season. During the summer of 2008 bottom values did not increase (Fig. 3.19). Temperature values between stations and depths did not vary significantly (Tables 3.1. and 3.2) throughout the year.

Nutrients

Nutrient concentrations were influenced by environmental variables over time and space in the study region (Justić et al. 2005) and were referenced in the hypotheses as a major force that may determine the abundance and composition of phytoplankton groups.

Among all nutrients measured, silicate was the only nutrient that changed significantly ($p<0.01$) between stations (Table 3.1). Values did not change much between depths; only silicate values had a significant ($p<0.01$) differences between months and hot and cold times of the year (Table 3.1). Silicate values were higher ($p<0.01$) significantly in the bight during winter months (Fig. 3.20).

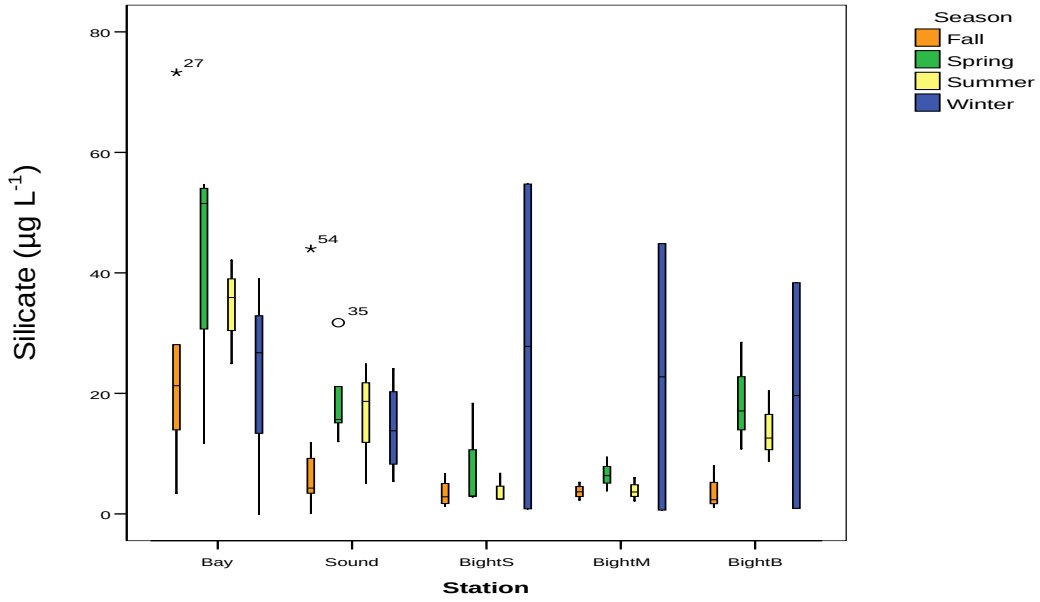


Fig. 3.20. Box plot of silicate concentration (µg L^{-1}) in each station per season. The Bay is station 1, the Sound is station 4, Bight S is station 8 surface, Bight M is station 8 at (9m) and Bight B is station 8 at 19m. Values beyond 1.5 times the interquartile range (outliers) are represented as º, values beyond 3 times the interauartile range (extremes) are represented as *. The numbers next to these symbols represent sample numbers. silicate values at the Bight in the winter had a leptikutic distribution hense no visible maximum or minimum values.

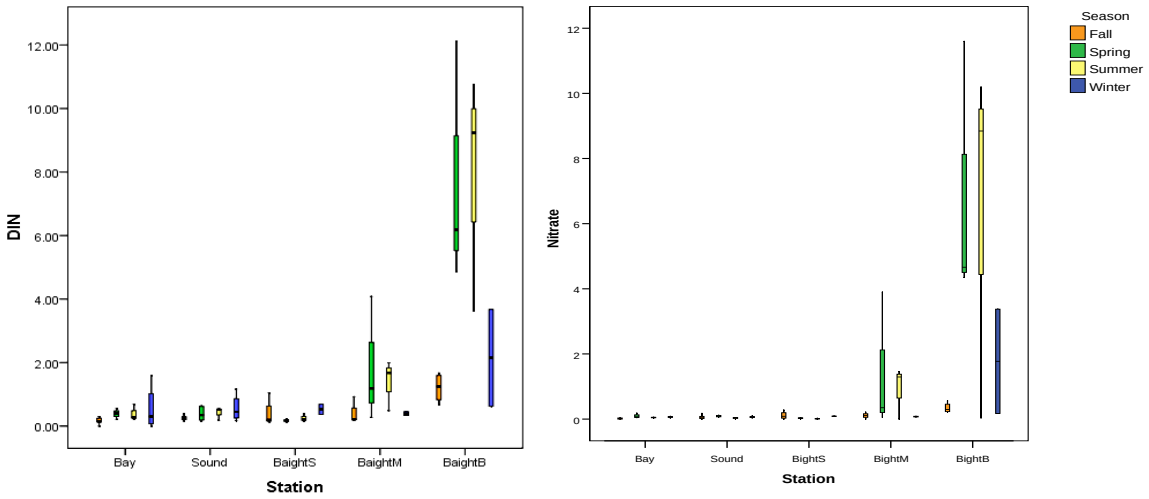
Nitrate (Fig. 3.21.b) had the highest values at 19m at station 8, mainly during spring and summer. Nitrite (Fig. 3.21.d) had the lowest values for all nutrients. Its highest values were at station 8 during the fall. Ammonium (Fig. 3.21.c) had very low concentrations at all stations, but concentrations were more homogeneous than other

nutrients, peaking in the summer at 19m depth at station 8. Dissolved inorganic nitrogen (DIN) (Fig.3.21.b) was obtained from adding nitrate, nitrite and ammonium values with nitrate as the major contributor. There was no significant difference in DIN values between stations or seasons, but there was a significant ($p<0.01$) difference between depths (Table 3.2) with the highest concentration at 19m at station 8.

There was no significant difference in phosphate concentration between stations or between depths (Tables 3.1. and 3.2). There was a significant ($p<0.05$) difference between seasons at station 8, with summer having the highest values (Fig. 3.22). Phosphate values decreased slightly with time since the sampling program was initiated (Appendix C Fig. C.). The correlation between phosphate and date, seen in Appendix D Table D.1 indicates that throughout the sampling period there was a slight decrease in phosphate concentration in the three sampling sites.

All nutrients were expected to be higher at station 1, and these were the results found for surface values. But if we include nutrient values from 9m and 19m at station 8, deep samples had higher nutrient concentrations with the exception of phosphate.

a. b.



c. d.

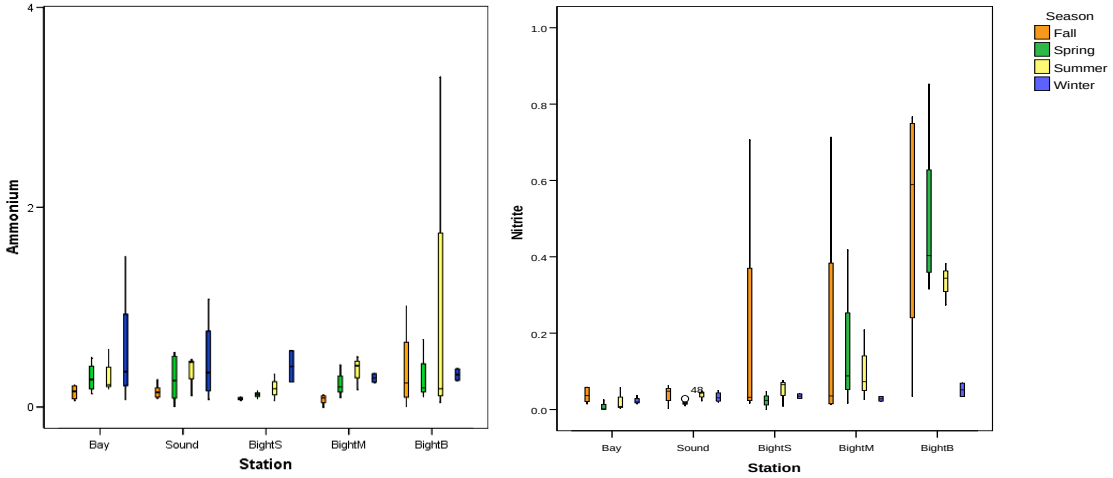


Fig. 3.21. Box plots of a. Dissolved Inorganic nitrogen (DIN), b. nitrate, c. Ammonium and d. Nitrite concentration (µg L^{-1}) in each station per season. The Bay is station 1, the Sound is station 4, Bight S is station 8 Surface, Bight M is station 8 (9m) and Bight B is station 8 at 19m depth.

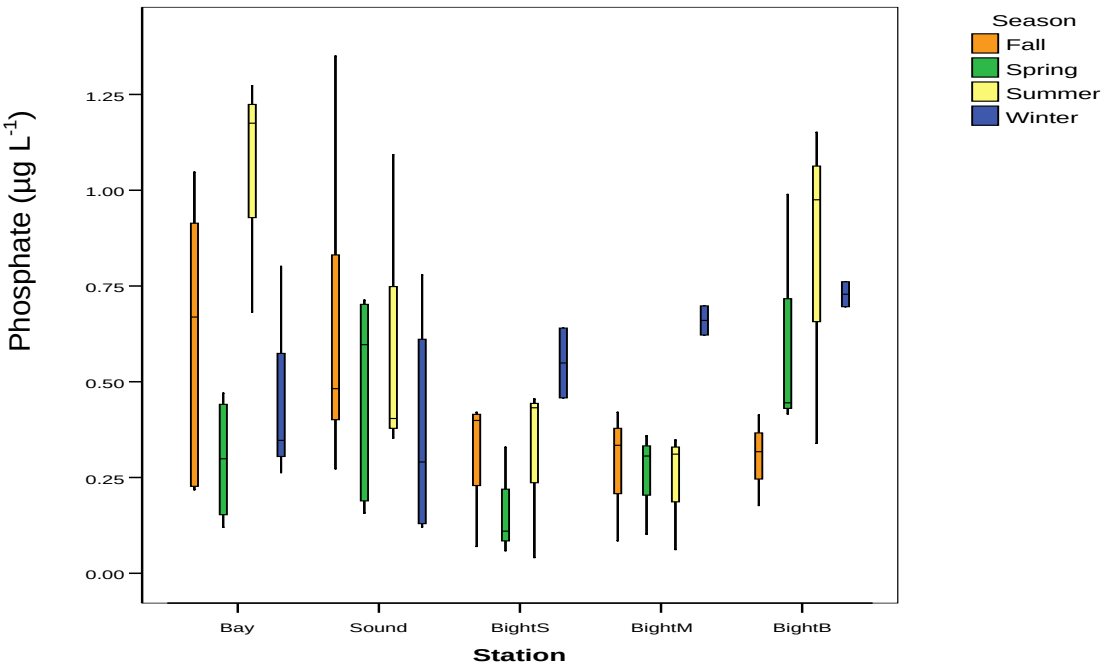


Fig. 3.22. Box plot of phosphate concentration (µg L^{-1}) in each station per season. The Bay is station 1, the Sound is station 4, Bight S is station 8 Surface, Bight M is station 8 (9m) and Bight B is station 8 (19m).

The analysis of nutrient related to stations and seasons was not just limited to their concentrations, but also to their ratios. Ratio analysis has proven important because their values many times determine if a nutrient is limiting to phytoplankton groups in a study area despite of their concentration (Rocha et al. 2002). Elemental ratios given in Fig. 3.23 and Fig. C.10, with red lines representing Redfield ratios (1958), highlight that in the study area phosphate values were high (Fig. 3.23a.), especially near shore. Figure 3.23a. and c. illustrate that DIN values were low and that silicate values Fig. 3.23b. and c. were high.

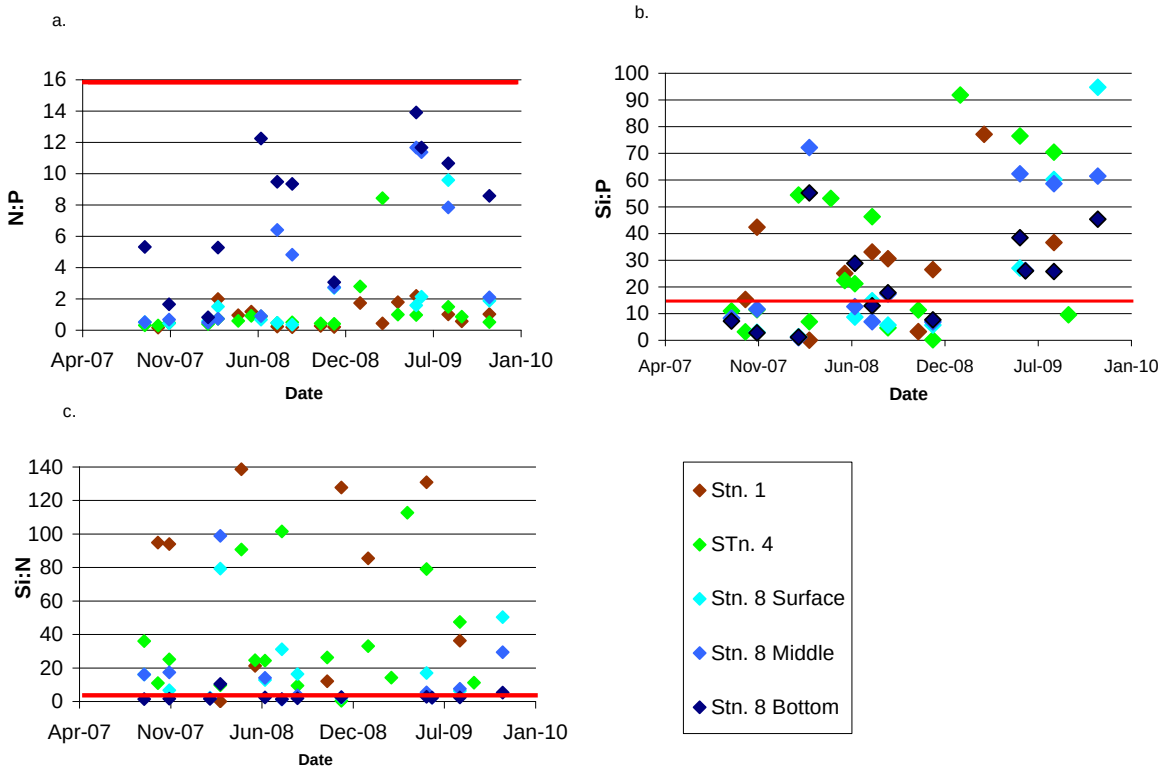


Fig. 3.23. Nutrient Molar ratios. a. N:P, b. Si:P and c. Si:N. The red horizontal line corresponds to the Redfield ratio Si:N:P (15:16:1).

Statistical Analyses

Correlations

A Spearman Rank Correlation Test was performed to determine significant correlations among the data (Appendix D Table D.1 and D.2). Ratios between nutrients were also included. The Spearman Rank Correlation test showed that environmental variables, such as precipitation, wind direction, wind speed, Jourdan River gage height and Wolf River gage height, did not correlate significantly ($p<0.01$) with biological variables during sampling days.

Chlorophyll *a* had a positive correlation with diatoms, and phaeophytin *a*, a chlorophyll breakdown product from grazing. Chlorophyll *a* was also negatively

correlated with salinity, density and nitrite. Salinity had a positive correlation with density and a negative correlation with CDOM. Most phytoplankton groups had a significant ($p<0.01$) positive correlation with other groups with the exception of cyanobacteria, prymnsesiophytes, chrysophytes and prasinophytes.

Ordination

Ordination analyses were used to look for those factors that contributed more variability to the dataset. There were two ordination methods which were used to examine at the relationships between environmental and biological variables while constraining biological variables to environmental factors, i.e., canonical correspondence analysis and redundancy analysis.

Redundancy analysis. There were two options for the type of ordination method to be used: a model of linear species response, and an unimodal species response (weighed averaging ordination method). Each one tests the species response with respect to the environmental variables. The method chosen was determined on the basis of gradient length in detrended correspondence analysis (DCA), which estimates the heterogeneity of the community composition (Legendre and Birks 2010). An example of phytoplankton group response can be viewed in the dissolved oxygen gradient graph Appendix E (Fig. E.1). The gradient in this case was not long enough, and the response to dissolved oxygen by most phytoplankton groups was linear. Because the lengths of gradients were <2 (Table 3.3), the best approach was to use a linearly constrained method such as RDA.

Table 3.3. Summary of DCA for the biological (10 phytoplankton groups) and environmental data derived from 68 samples from coastal Mississippi waters.

Axes	1	2	3	4	Total

					inertia
Eigenvalues :	0.138	0.078	0.052	0.028	0.472
Lengths of gradient:	1.564	1.737	1.228	1.077	
Cumulative percentage variance of species data:	29.2	45.6	56.5	62.4	
Sum of all eigenvalues					0.472

Species-environmental correlations (Table E.1) ranged from 0.868 to 0.494; this suggested that the measured environmental variables are those responsible for species composition variation (Lepš and Šmilauer 2003). Species-environmental correlations were high for the four axes. The cumulative percentage of species variance totaled 45.3% and the cumulative percentage of species-environmental relation totaled 93.8% for the first 4 axes. The first 2 axes were used to generate the ordination diagram.

A Monte Carlo test (Table 3.4) was used to prove the performed significance. The test on the first axis and the test on all axes were highly significant (P=0.006 with 499 permutations), so the null hypothesis that phytoplankton groups were independent from the explanatory variables was rejected.

Because there was a close correlation between environmental variables and species composition (Table 3.5), forward selection was used to build a simpler model with enough, but fewer, explanatory variables that would explain the species composition pattern (Lepš and Šmilauer 2003).

Table 3.4. Summary of the Monte Carlo test with 499 permutations.

Summary of Monte Carlo Test
Test of significance of first canonical axis: eigenvalue = 0.282

	F-ratio =	20.843
	P-value =	0.0060
Test of significance of all canonical axes : Trace	=	0.483
	F-ratio =	3.532
	P-value =	0.0060

From the marginal effects (Table 3.5), the most important factor for species composition was salinity followed by CDOM and silicate (SiO_3). These variables were correlated because they may be dependent on freshwater outflows. After salinity was selected, the conditional effect of CDOM decreased dramatically. That is why other variables qualify for the final model with a probability of 0.05 thresholds. The variables that can be included in the model are salinity, temperature, turbidity, phosphate, silicate and DIN. Some of them may have had low marginal effects, but they were independent of other variables, and because they probably affected the species composition, they added an explanatory power to the previously selected variables (Lepš and Šmilauer 2003). The final selection was in fact "a sufficient set of predictors and further addition of variables do not significantly improve the fit" (Lepš and Šmilauer 2003, p. 190).

Table 3.5. Marginal and Conditional Effects from the forward selection.

	Marginal Effects			Conditional Effects			
Variable	Var. N	Lambda 1	Variable	Var. N	Lambda A	P	F
Sal	3	0.2	Sal	3	0.2	0.014	16.28
CDOM	5	0.13	Temperature	2	0.09	0.02	8.15
SiO_3	12	0.11	Turbidity	6	0.03	0.036	3
Temperature	2	0.08	PO_4	7	0.03	0.024	3.23
Depth	1	0.08	SiO_3	12	0.03	0.008	3.25
Turbidity	6	0.08	DIN	11	0.03	0.032	3.1
DO	4	0.06	CDOM	5	0.02	0.066	1.98
DIN	11	0.05	NO_2	10	0.01	0.322	1.16
NO_2	10	0.05	WD	14	0.01	0.352	1.14
NO_3	9	0.05	WS	13	0.02	0.346	1.11
PO_4	7	0.03	NH_4	8	0	0.652	0.69
WD	14	0.02	DO	4	0.01	0.698	0.66
WS	13	0.02	Tides	15	0	0.962	0.24
Tides	15	0.02	Depth	1	0	0.978	0.25
NH_4	8	0.01					

Ordination diagrams (Fig. 3.24 to Fig. 3.31) were used to illustrate the relationships from RDA already presented. Arrows represent species. If the angle between two species is close to a right angle, these species are predicted to have low or no correlation (Lepš and Šmilauer 2003), for example euglenophytes and cyanobacteria. Arrows pointing in the same direction correspond to species that are predicted to have a positive correlation, as was the case for eustigmatophpytes and chlorophytes (Fig. 3.24). Species with arrows pointing in opposite directions would have a negative correlation (that is not the case in this data set). The maximum and minimum values for the plots are 1 and -1. This is because variables were standardized before the ordination analyses.

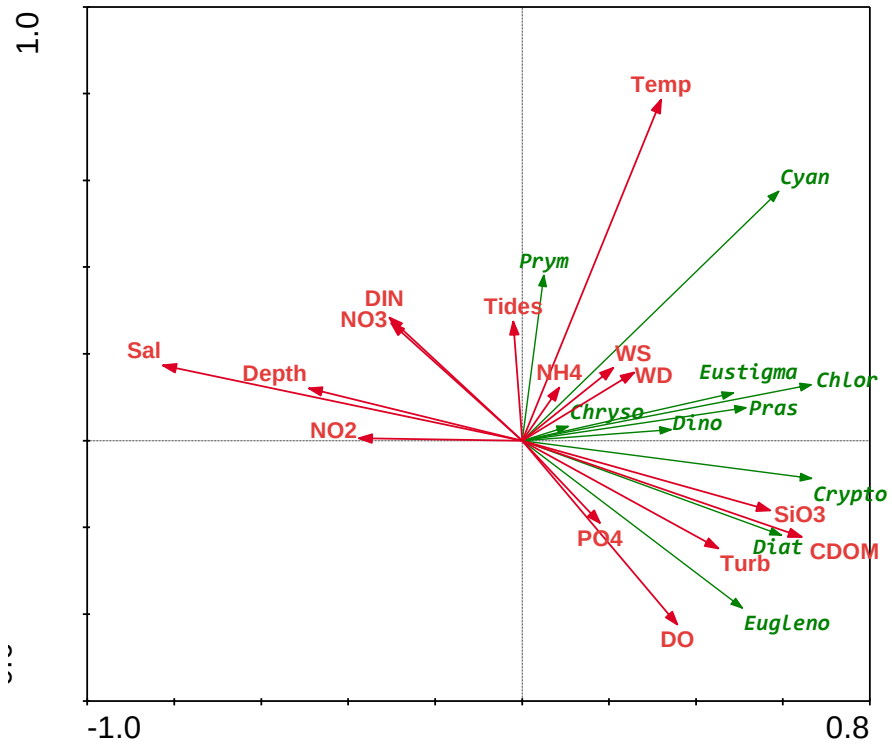


Fig. 3.24. Ordination diagram of environmental variables (red arrows) with constrained phytoplankton groups (green arrows) from RDA results.

The same approximation can be applied to environmental variable arrows and species arrows. If the arrow directions are the same, it is predicted that the variables are correlated positively (Lepš and Šmilauer 2003; Fig.3.24). For example, diatoms increased when silicate increased or that euglenophytes tended to increase with turbidity. Variables like Wolf River gage, Jourdan River gage and precipitation were not included in this ordination because their values were measured only for station 1.

Attribute Loess plots (Figs. 3.25 to 3.30) are also ordination diagrams, but they show isolines of environmental variable gradients, in each case only one environmental variable. For the Loess plots, the data from all stations and depths was included. For

example, in Fig. 3.25 most phytoplankton groups increased with decreased salinity, whereas prymnesiophytes increased when salinity remained at 26.

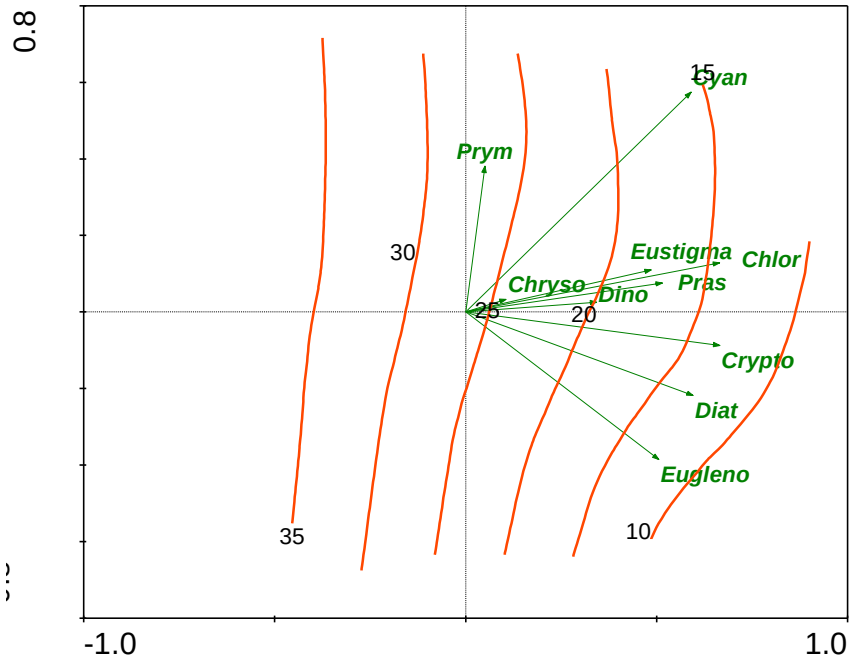


Fig. 3.25. Attribute Loess plot of Salinity combined with phytoplankton group.

Temperature had a different effect on several groups. Diatoms grew between 22ºC and 24ºC. Euglenophytes increased when temperatures decreased slightly over time, while most phytoplankton groups grew with slightly increased temperatures (Fig. 3.26). Cyanobacteria and prymnesiophytes definitely increased with increasing temperatures within the ranges measured in coastal Mississippi waters.

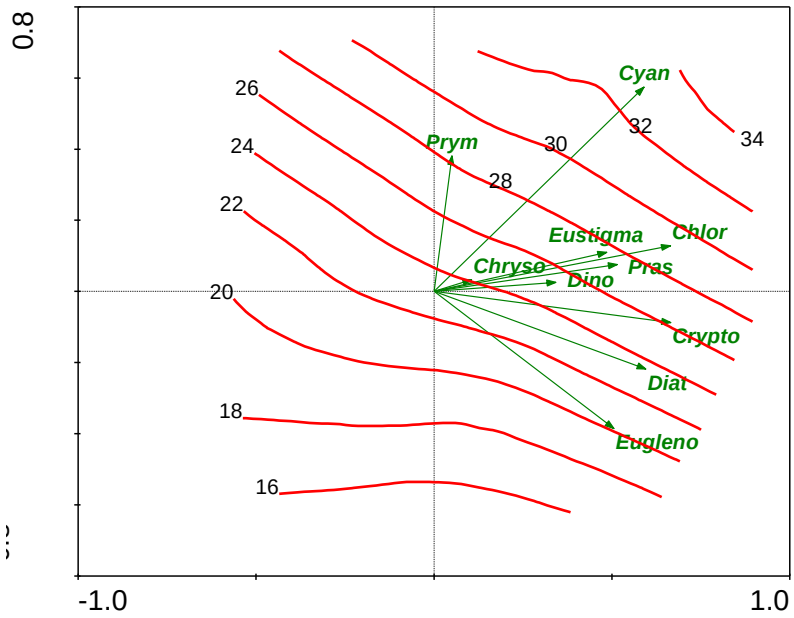


Fig. 3.26. Attribute Loess plot of Temperature combined with phytoplankton group.

Turbidity also increased with the presence of most phytoplankton groups, with the exception of prymnesiophytes which decreased with turbidity. Cyanobacteria relative abundance tended to be within 3 and 3.5 Formazin Nephelometric Units (Fig. 3.27).

Nutrients had a different effect on algal density. Euglenophytes, diatoms and cryptophytes increased with phosphate concentration. Prymnesiophytes decreased with phosphate concentration, while most algal groups were associated with increasing phosphate concentrations (Fig. 3.28). Most algal group densities increased with silicate (Fig. 3.29), but diatoms were correlated with it most. The prymnesiophyte fraction of chl *a* did not increase with silicate. Most phytoplankton groups were associated with a low concentration of DIN (Fig. 3.30).

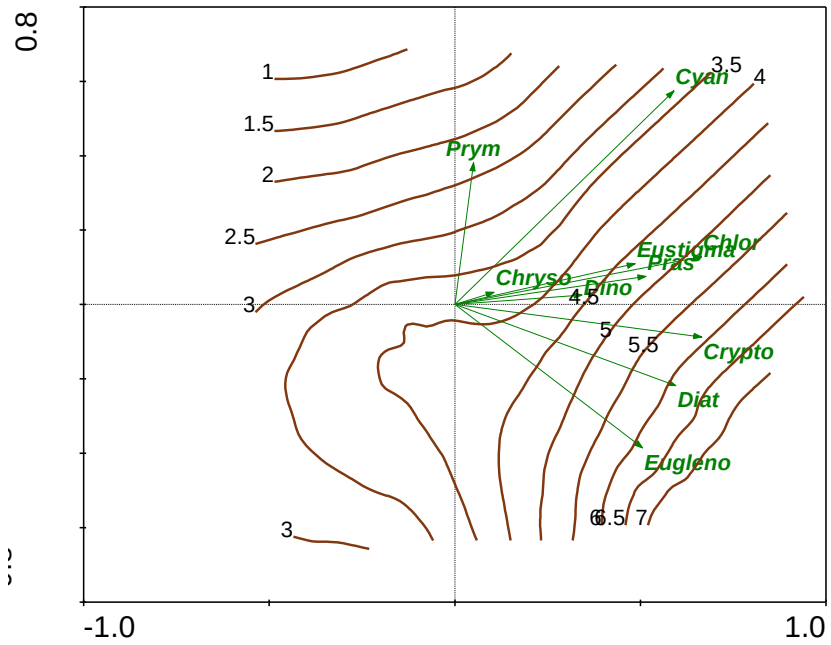


Fig. 3.27. Attribute Loess plot of Turbidity combined with phytoplankton group.

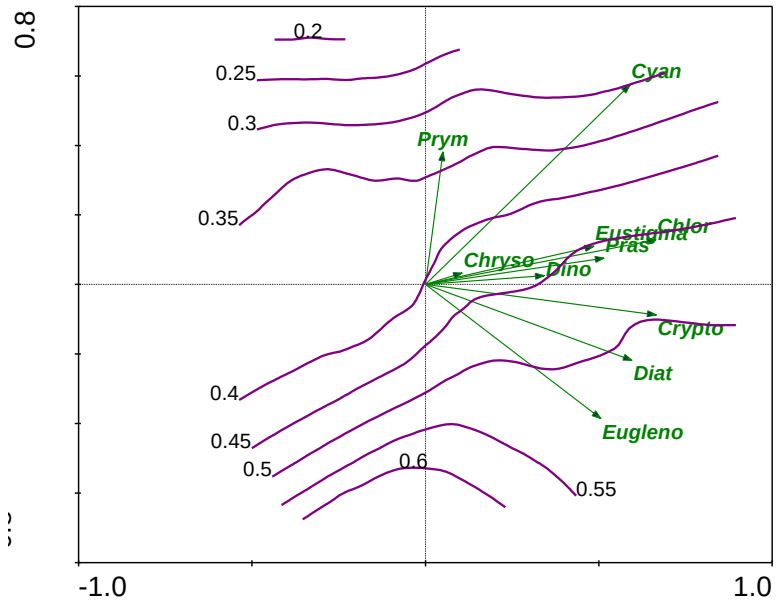


Fig. 3.28. Attribute Loess plot of phosphate combined with phytoplankton group.

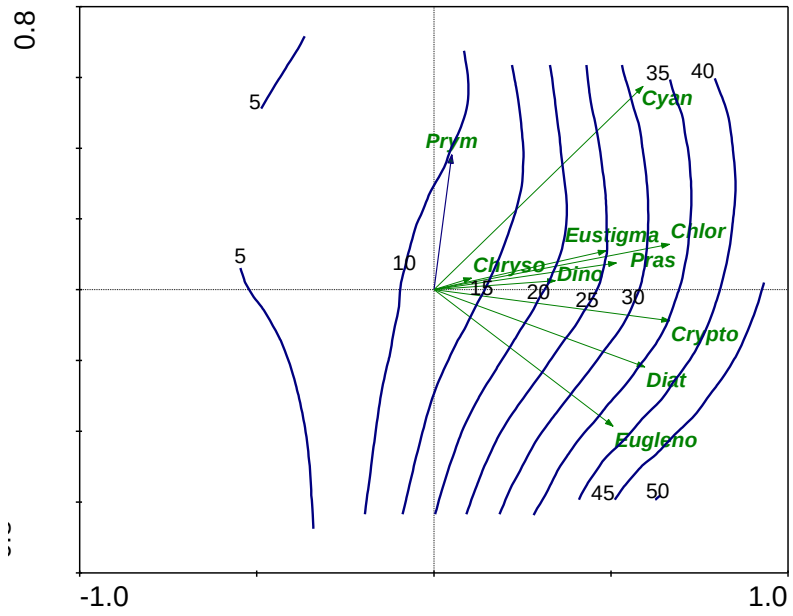


Fig. 3.29. Attribute Loess plot of silicate combined with phytoplankton group.

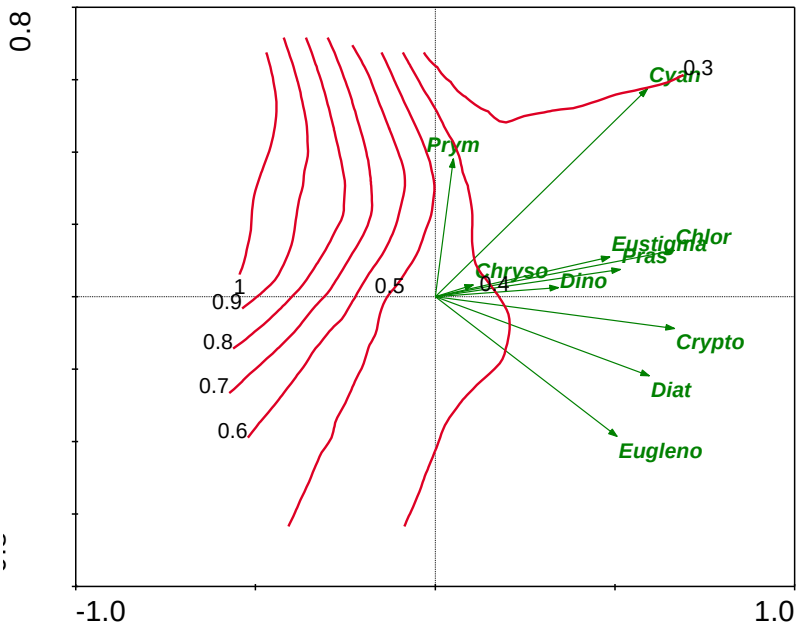


Fig. 3.30. Attribute Loess plot of DIN combined with phytoplankton group.

As observed in the majority of attribute plots, most algal groups were related closely, except cyanobacteria and prymnesiophytes, possibly meaning that in the study area they had similar requirements. It is also clear that environmental variables had differing effects in prymnsiophytes and euglenophytes.

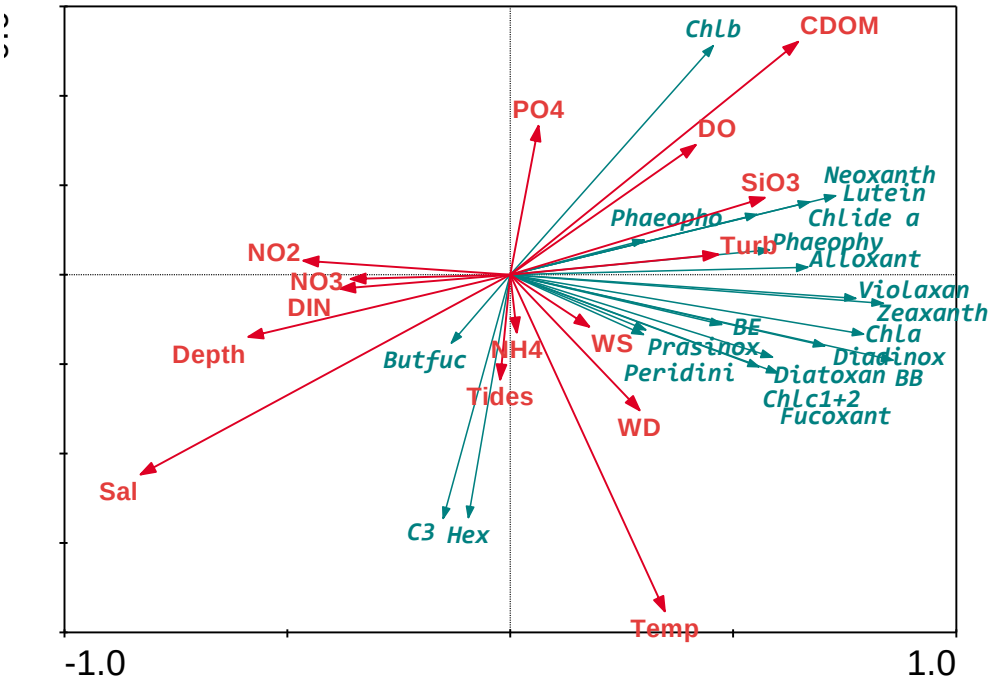


Fig. 3.31. Ordination diagram of environmental variables (red arrows) with pigments, constrained variable (blue arrows) from RDA results.

Ordination was also used to explain the pigment composition in relation to environmental variables. Redundancy analysis (Table E.2) performed for all pigments indicated that environmental variables were related to the observed pigment distribution. The pigments, 19'butanoylofucoxanthin, 19'hexanoylofucoxanthin and chlorophyll c 3 (Fig. 3.31), depart from the other pigments. It is important to note that these pigments are found in prymnesiophytes and chrysophytes. The concentration of chl *a* increased with silicate but decreased with salinity (Fig. 3.32).

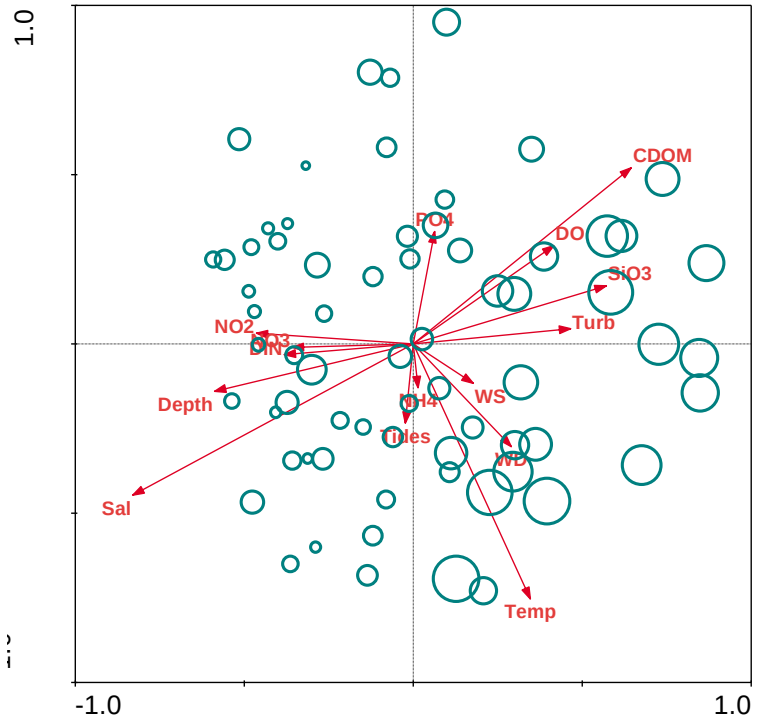


Fig. 3.32. Sample-environmental variables biplot with symbol size corresponding to chl *a* concentration, red arrows represent environmental variables.

Diversity

The Shannon Diversity Index was calculated to determine if there was a change in species diversity throughout the sampling program or in relation to the environmental variables (Fig. E.3). There was no significant difference in diversity (Table 3.1) between stations or between depths (Fig. 3.33). Although, at station 8, there was a significant difference in diversity with depth (Table 3.2).

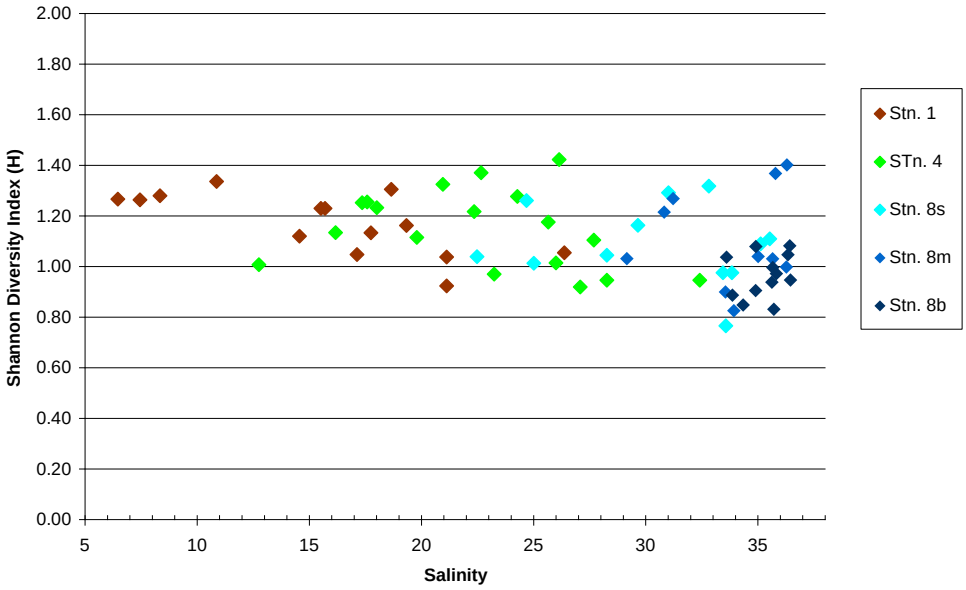


Fig. 3.33. Shannon diversity index vs. salinity at the three stations and three depths of station 8.

CHAPTER IV

DISCUSSION

The results of this study have demonstrated that there is a strong spatial variation in phytoplankton relative abundance and group composition. In locations closer to the shore, there was greater seasonal variability in phytoplankton abundance; whereas, for the offshore areas there was a greater seasonal trend in species composition. Diatoms dominated coastal areas, while cyanobacteria, prymnesiophytes and diatoms were abundant in offshore waters.

Hypothesis I

Phytoplankton Abundance and Species Composition will Vary Spatially Between the Bay of St. Louis, the Mississippi Sound, and Shelf Waters outside the Barrier Islands

Phytoplankton abundance determined by chl *a* concentration did vary because there was a significant difference in chl *a* concentration between the three stations sampled. From the stations surveyed, station 8 had the lowest chl *a* concentration. Wysocki et al. (2006) and Quian et al. (2003) also found that chl *a* concentration in shelf waters of the Gulf of Mexico was higher in coastal regions and that chl *a* was related to freshwater outflows or attributed to salinity.

Group density at each station varied depending on the species group. The diatom, chlorophyte, euglenophyte, cryptophyte and prasinophyte relative abundance changed significantly between stations. These groups had higher concentrations at station 1 and station 4. Prymnesiophyte and chrysophyte fraction of chl *a* increased at station 8.

Diatoms Will Predominate in Inshore Waters With Higher Concentration of Nutrients

This research found that diatoms did predominate in coastal waters with high nutrient concentrations. However, not all nutrient concentrations were significantly higher in inshore waters. DIN increased with salinity (Appendix D, Table D.1); thus, lower N concentrations were found closer to the coast. What this implies is that phytoplankton groups are efficiently utilizing DIN to the point that they are depleting it (Fig. 3.30). Phosphate was higher, but not significantly; only silicate was significantly higher at station 1 (Table 3.1). Quian et al. (2003) established that throughout the Gulf of Mexico, diatoms were the main source of chl *a* in inner shelf waters. Lohrenz et al. (2003) found similar results in Chesapeake Bay, where diatoms were the most abundant relative to chl *a* in waters with lower salinities; it was also considered that higher temperatures contributed to diatom abundance in that study area. Another study, also in Chesapeake Bay, attributed diatom prevalence to excess nutrient concentration (Marshall et al. 2006).

Dinoflagellates and Prymnesiophytes Will Prevail in Offshore Waters With Lower Nutrient Concentration and Higher Salinities

Nutrient concentrations were not significantly (Table 3.1) lower in offshore waters except for surface silicate. Dinoflagellates did not vary significantly between stations, although they were more important at station 4, which was characterized as having intermediate nutrient and salinity values. Even though no particular conditions were shown to be a direct cause for dinoflagellate abundance, Springer et al. (2005) found that dinoflagellate density in the Neuse Estuary were linked to ammonium values close to 4µM. However, coastal Mississippi waters had lower ammonium concentrations

($\leq 2\mu M$) (Fig. 3.21.c), so ammonium concentration was not the likely reason for the absence of dinoflagellates. Moreover, dinoflagellates in the Gulf of Mexico may be estuarine, neritic or oceanic (Steidinger et al. 2009), so there was no basis to support their higher concentrations at higher salinities in the study area.

Low densities of dinoflagellates in the area were also noticed by Quian et al. (2003), but it is important to note that Adolf et al. (2008) found dinoflagellate blooms of *Karlodinium veneficum* in eutrophic estuaries, and their abundance was correlated with cryptophyte abundance. Mixotrophic organisms like *Karlodinium veneficum* may have autotrophic or heterotrophic nutritional strategies. Adolf et al. (2008) study implied that mixotrophic dinoflagellates were using cryptophytes as prey, and neither of these groups was found with high abundances, in this survey.

Prymnesiophyte contribution to the fraction of chl *a* was higher at station 8. At this station, salinities were much higher, and there were no significant differences in nutrient concentration, with the exception of silicate that was lower in relation to the other stations. This result was expected, as prymnesiophytes do not require nutrient concentrations as high as diatoms or chryptophytes (Schlüter 1998). Quian et al. (2003) reported that in the Gulf of Mexico, this group was associated with high salinities. Bontempi and Lyons (1998) also published a shift in phytoplankton composition as salinity increased from a diatom-dominated community at low salinities to a coccolithophorid-dominated community at high salinities on the Texas-Louisiana Shelf.

Chrysophyceae were more abundant at station 8, but this group was not included in the hypotheses. The main reason for this was because inadequate sampling provided little information on this group for the Gulf of Mexico (Nicholls and Wujek 2003).

Chrysophytes form stomatocysts (rest stage) that allow them to survive through adverse conditions and undergo passive transport by winds; they may also use a heterotrophic mode of nutrition, and have a rapid growth rate, attributions which enable them to have a broad distribution (Nicholls and Wujek 2003). However, as chlorophytes, this taxon is a good competitor for phosphorus, due to symbiotic associations with bacteria when phosphorus is limiting (Nicholls and Wujek 2003; Kristiansen 2009). But low phosphate conditions do not characterize coastal Mississippi waters, possibly explaining the low relative abundance density of chrysophytes in the area (Fig. C.10)

Phytoplankton Pigment Concentration Will Vary with Increasing Salinity

There was a significant difference ($p<0.01$) in chl *a* concentration between stations and a significant negative correlation ($p<0.01$, $r=-0.762$) between chl *a* and salinity. Spearman Rank Correlation (Appendix D, Table D.2) also demonstrated that most pigments decreased with salinity with the exception of 19'butanoylfucoxanthin, chlorophyll c3 and 19'hexanoylofucoxanthin (Fig. 3.31). This negative correlation was expected since these were diagnostic pigments for chrysophytes and prymnesiophytes, the groups that had higher biomass outside the barrier islands.

Phytoplankton and depth. While differences between depths were not addressed in the hypotheses, some remarks may be stated about depth at station 8. There was no significant difference in chl *a* between depths, and from the nutrients, only DIN increased significantly ($p<0.01$) at 19m. From the ten groups studied only chrysophytes and prymnsiophytes varied significantly with depth (Table 3.2). The decrease in prymnesiophytes and chrysophytes may be related to low light levels or higher nutrient concentrations than those needed by these groups.

As mentioned before, algal abundance had a pronounced negative correlation with nitrite (Table D.1). The negative correlation of nitrite with chl *a* may be explained by the fact that the nitrite maximum results from a series of mechanisms, including excretion of nitrite from light-limited phytoplankton (Olson 1981; Lomas and Lipschultz 2006), which coincided with higher concentrations in deeper waters (Fig. 3.21.d).

Redundancy analysis. Redundancy analysis was used to determine if phytoplankton composition was explained by environmental factors. The results from the RDA suggested that phytoplankton group composition was dependent on a combination of environmental variables (Table 3.3), mainly salinity, temperature, phosphate, silicate, and DIN plus turbidity (Table 3.4).

Turbidity was not included in the hypotheses, but it varied in relation to phytoplankton composition patterns. It is probable that algal group composition was related to light penetration, and its effects on different phytoplankton groups. There was evidence of other reasons behind this association. Euglenophytes were positively correlated with CDOM (Table D.1) and increased with turbidity, which is not a surprise because they are also photoauxotrophs that require vitamins or amino acids from their surroundings (Rosowski 2003). Therefore, euglenophytes are found in high CDOM areas due to their vitamin B (cyanocobalamin and thiamine) requirement. Vitamin B is available from bacteria that utilize organic matter in nutrient rich environments (Rosowski 2003). Some euglenoids are facultative heterotrophs and may thrive in waters enriched (polluted) with organic matter (Rosowski 2003). Turbidity also plays a role when organic aggregates provide attachments for micrograzers and bacteria, which function as patches of enhanced nutrient recycling that maintain phytoplankton growth

(Estrada and Berdalet 1997). If there was a link between turbidity and organic compounds required by auxotrophic and heterotrophic phytoplankton, it might also support that turbidity was included among the variables that explain phytoplankton composition.

Hypothesis II

The Temporal Variability of Phytoplankton Abundance and Composition is Related to Environmental Factors Such as Weather and Nutrient Load in the Study Area

Chlorophyll *a* changed with seasons, with concentrations that increased from spring to summer. Similar results have been observed for the Neuse River Estuary (Valdes-Weaver et al. 2006) and the Northern Inlet Estuary in South Carolina (Lewitus 1998). Since there were time lags between seasonal values and the related increase in chl *a*, and not all groups increased with higher temperatures. The Spearman test did not find a significant correlation between temperature and chl *a*. In Appendix C, Fig. C.9, it was possible to see that temperature increases were followed by chl *a* increace, with very large error bars, hence their lack of correlation but their similar variation with seasons.

Wysocki et al. (2006) found a relationship between chl *a*, nutrients and the discharge of major rivers, such as the Mississippi and the Atchafalaya, in the Gulf of Mexico. Unlike the research from Wysocki et al. (2006), in this study the gages of two small rivers, Jourdan and Wolf Rivers, were used. The discharges of these small rivers did not correlate with the variables during the sampling period (Appendix Fig. C.1 and Table D.1) and thus were very unlikely to control the nutrient load in the study area. Quian et al. (2003) proposed that in the northern Gulf of Mexico, an efficient utilization of nutrients by phytoplankton was the reason for the lack of correlation. Even more,

Wysocki et al. (2006) explained that a de-coupling between nutrients and biomass due to spatial variability in the uptake and recycling of nutrients was found in the Mississippi River Plume, providing additional reasons why there was no correlation between flow, nutrients and chl *a* that might apply to this data.

Microtidal estuaries (tides<2m range) are dominated by wind and wave effects and maximum turbidity by river floods (Monbet 1992), characteristics that describe the Bay of St. Louis. However, as noted by the same author, the effects of vertical mixing on phytoplankton populations are not direct, but mediated by light fluctuations. In relation to the association between biomass and nutrient load in the sampling area, Noble et al. (2003) explained that biomass response to nutrient increases in two estuaries in South Carolina, were highly dependent on salinity, tidal mixing, river drainage and optical properties of the water column that affected light exposure of phytoplankton. Thus, the combined effects of wind speed, wind direction, precipitation, tides, Wolf River gage, and Jourdan River gage plus light attenuation and time lag response may be the reasons why phytoplankton abundance was not correlated with each of these variables in coastal Mississippi waters (Appendix A, Table A.1).

Seasonal succession. Dissolved inorganic nitrogen values did not change significantly with seasons. Phosphate and silicate did have a significant temporal difference ($p<0.05$) for surface waters of station 8. This observed nutrient increase during winter is supported by the fact that during winter months there was no stratification (Appendix C, Fig. C8). The consequences of no stratification result in changes of nutrient concentration and species succession (Estrada and Berdalet 1997; Smayda and Reynolds 2001; Smayda and Reynolds 2003; Cullen et al. 2007).

According to Margalef's Mandala (1978), diatoms are non-motile organisms that thrive in turbulent, nutrient rich waters. In these conditions, diatom cells are resuspended due to turbulence. Dinoflagellates, which are motile because of their flagella, can change their position in stratified waters (Margalef 1978). This way they can acquire nutrients from deep layers. Meanwhile coccolithophorids (prymnesiophytes) occupy intermediate positions (Margalef 1978). The seasonal succession observed in coastal Mississippi waters may be attributed to this framework where diatoms thrive in nutrient rich estuarine areas and silicate rich waters during winter (Fig. 3.20). Margalef's Mandala (1978) predicts the presence of prymnesiophytes in intermediate turbulent and low nutrient waters, which may be the case for coastal Mississippi waters. It was also likely that turbulent conditions of the study area were harmful to dinoflagellates (Estrada and Berdalet 1997).

In relation to the analysis of seasonal phytoplankton succession, it is important to take into account time lags (Sommer 1993). Even though in this study there was evidence of a seasonal succession of algal groups, phytoplankton species composition rarely change due to optimal resource ratios as they do in chemostat experiments because of mortality, physical boundaries and conditions that change from week to week (Sommer, 1993). If light, temperature and nutrient changes are not the same every year, we may not expect the same timing for species abundance and succession, even on consecutive years.

Cyanobacteria and Chlorophytes Will Increase toward the Summer

Cyanobacteria and chlorophytes increased in the summer (Appendix B Table B.1 and Figs. 3.9 and 3.10). The difference between seasons was significant for cyanobacteria

(Table 3.2), and a positive correlation with temperature was also found for cyanobacteria (Table A.1, Fig. 3.26).

Some differences in cyanobacteria and chlorophyte density could be explained by variations in nutrient concentration. Cyanobacteria increased in the summer after diatoms used excess silicate during winter and spring (Rocha et al. 2002). Rocha et al. (2002) also concluded that a chlorophyte bloom would be diminished if N:P ratios were lower than 16 (Fig. 3.23.a) and suggested that chlorophytes may have outcompeted other taxa for P but not for N. This is the case for most of the sampling area, where an excess of P and not N may be the reason why chlorophytes are not predominant.

Quian et al. (2003) also found a high abundance of prokaryotes in the Gulf of Mexico. Prokaryotes were distributed in patches, while chlorophytes were found in low numbers, similar to what was found at the NGI stations in coastal Mississippi waters. The lower densities of chlorophytes were also related to the fact that true starch is their principal photosynthetic product, and it is preferred by juvenile fish (Zemke-White and Clements 1999). Coastal Mississippi waters are nursery grounds for juvenile fish (Christmas 1973), which are more likely to selectively graze on chlorophytes than other algal groups in the area.

There were a series of studies that provide information that may help to explain the evident presence of cyanobacteria in coastal Mississippi waters in the summer. A study done in the Neuse River Estuary-Pamlico found that cyanobacteria were most abundant during summer months, but also suggested that when river flow rates were reduced and water residence times were longer, cyanobacteria algae concentrations increased (Valdes-Waver et al. 2006). A similar succession was documented in the

Bermuda Atlantic time series study, where a prymnesiophyte spring bloom was followed by a cyanobaceria early summer bloom (Steinberg et al. 2001). Research in the Baltic Sea provided similar N:P ratios as our sampling site. The sources of P were from anthropogenic sources and from anoxic sediments that decreased N:P ratios (Vahtera et al. 2007). Once the N was exhausted in the summer months, nitrogen fixing cyanobacteria gained competitive advantage and produced blooms (Vahtera et al. 2007). The conditions in coastal Mississippi waters may not be exactly the same, but unicellular and diazotrophic cyanobacterium have been detected in the Mississippi Sound (Ren 2010). These organisms were more abundant in the summer, likely controlled by temperature and low N:P ratios (Ren 2010).

Diatoms and Cryptophytes Will Increase in the Winter

Diatom relative abundance was only greater in the winter at station 8 (Fig. 3.8; Table 3.2). Adolf et al. (2006) found that diatoms dominated in Chesapeake Bay, accounting for 70% of phytoplankton groups in winter, while in the summer, there was a mixed assemblage of diatoms, dinoflagellates, cryptophytes and cyanobacteria. The results of that study suggest that the community responded to environmental forcing associated with variability of river flow and nutrient loading. In Chesapeake Bay and at station 8 of this study, it was clear that winter conditions supported an increase in diatoms during winter.

Diatoms and cyanobacteria increased seasonally in Galveston Bay because of an increase in N+P load, but diatoms decreased due to grazing while cyanobacteria were not grazed upon (Ornolfsdottir et al. 2004). The reason for this was that the nutritional value and filamentous characteristics of cyanobacteria like *Trichodesmium*, made them less

attractive for zooplankton (Rocha et al. 2002). Cyanobacteria are not likely used as a food source, as they often have toxins (Porter and Orcutt 1980). Thus, grazing may explain the decrease of diatoms and increase of cyanobacteria at station 8 during the summer.

Diatom density was much higher in the summer at station 1 and station 4. This may imply that at these stations, light is what limits their growth, while in the bight (station 8), we can see a better algal group succession, which may be due to a change in nutrient concentration.

Chryptophytes, along with diatoms, were more closely associated with river flow and nutrient concentration of the winter season in the Bay of St. Louis (Holtermann 2001). Hence, the inclusion of this group in the hypothesis. However, in this study that also included offshore waters, no association was found. Because chryptophytes are extremely delicate and rupture with fixatives or high temperatures, few studies have extensive information on their ecology (Kugrens and Clay 2003); thus, no further assumptions were made with respect to their distribution.

The group that unexpectedly increased during winter at station 8 were the euglenophytes, which went from a median 3% to 4% of chl *a*. Euglenophyte connection to organic matter and fresh outflows (Rosowski 2003) may explain the increase in their abundance during winter months during low light intensities when they can take advantage of organic matter.

Phytoplankton Pigment Concentration and Composition Will Vary in Response to Wind Speed, Wind Direction, Nutrient Increase, Storms and Rainfall

Phytoplankton pigment concentration did not have a correlation with wind speed, wind direction, tides, gage height or rainfall (Table A.2). Chlorophyll *a* and most

pigments had a slightly positive correlation with silicate, which meant that most of the pigment contribution was due to groups like diatoms that flourished in high silicate concentrations. Pigment composition evaluated with RDA demonstrated that it was strongly related to salinity, silicate and temperature (Fig. 3.31 and Appendix E, Table E.2).

The Shannon Diversity Index was performed because it takes into account the number of species and the evenness of species in a sample, giving relative abundances of different groups. The Shannon Diversity Index determined from pigments (Noble et al. 2003) gave values between 1.6 and 2.8 while Estrada et al. (2004) found values between 0.5 and 2.5. Estrada et al. (2004) also found that there were no differences in diversity values regardless of whether the index was calculated from microscopic counts, flow cytometry or genetic fingerprinting. This supported the approach used in this study. Shannon Diversity Index values measured in Mississippi coastal waters ranged between 0.7 and 1.6. No significant differences were observed between stations or depth (Table 3.1 and 3.2). The observed range of the Shannon Diversity Index was about half the range found by Noble et al. (2003) and Estrada et al. (2004). From this, it may be inferred that the phytoplankton community for this study period was characterized by having a relatively low diversity. Because the number of phytoplankton groups was relatively stable and the samples evenness was similar, the index did not change much. This does not mean that algal groups were not influenced by external variables; it is just that specific groups at different times were affected without affecting evenness or that more than one factor was responsible for the pigment concentration (Noble et al. 2003). That is the case for diatoms and cyanobacteria at station 8. Cyanobacteria concentration

increased at station 8 almost replacing the diatoms at other stations, such that the number of groups and the evenness was almost the same and that most groups in small numbers were being dominated by a major group. At station 8, diversity did change with seasons, suggesting that the seasonal change in community composition was so strong in the summer that the majority of phytoplankton did not belong to one group, but was more evenly distributed among several groups.

Suggestions for further research would include studies of nutrient uptake by major groups, sediment iron oxides, selective grazing by zooplankton, pigment ratios from local strains, photosynthetically available radiation studies, CO_2 relationships with phytoplankton assemblage, euglenophyte and eustigmatophyte ecology in coastal Mississippi waters, and a one week daily sampling per season to evaluate closely tidal effects.

Conclusions

There was a significant difference in phytoplankton abundance and species composition along the salinity gradient from the Bay of St. Louis to the coastal Mississippi Bight. As expected, chl *a* was higher in low salinity areas. Diatoms dominated station 1 throughout the sampling period. Prasinopytes and chlorophytes were present in stations 1 and 4, but almost absent from station 8. Prymnesiophytes had higher abundances in surface waters with lower phosphate concentrations. Even though cryptophytes and dinoflagellates were present, their relative abundances were lower than expected. There was a seasonal succession in phytoplankton groups at station 8. Diatoms predominated in shelf waters outside the barrier islands during winter while in the summer cyanobacteria and prymnesiophytes increased their fraction of the total chl *a*.

Variation in community composition were related to salinity, temperature, turbidity, nutrient ratios and nutrient concentrations in coastal Mississippi waters. Shannon Diversity Index did not change significantly throughout the study area and sampling period.

APPENDIX A

PIGMENT CONCENTRATION

Table A.1. Pigment concentration (μg L^{-1}). Station 1 surface waters, station 4 Surface waters, station 8 surface, station 8 Middle (9m) and station 8 bottom (19m).

Station	Date	Chloro-phyll c3	Chloro-philide a	Chl. c1+2	Peri-dinin	19'Butan oyloxyfu coxanthin	Fuco-xanthin
1	Oct-07	0.0000	0.0000	0.1647	0.0000	0.0000	0.5749
1	Nov-07	0.0000	0.0000	0.1275	0.0000	0.0000	0.2391
1	Mar-08	0.0000	0.1319	0.3418	0.0000	0.0000	0.8191
1	Apr-08	0.0000	0.2081	0.5553	0.0000	0.0000	2.1171
1	May-08	0.0000	0.3267	0.3805	0.0000	0.0000	1.9507
1	Jul-08	0.0000	0.0000	0.6540	0.0000	0.0000	2.1879
1	Aug-08	0.0000	0.0000	0.4938	0.0000	0.0000	2.3364
1	Oct-08	0.0000	0.0000	0.2060	0.0000	0.0000	0.6155
1	Nov-08	0.0000	0.0811	0.0356	0.0000	0.0000	0.1244
1	Jan-09	0.0000	0.0000	0.3535	0.0000	0.0000	0.9293
1	Mar-09	0.0000	0.0635	0.6873	0.0000	0.0000	1.9417
1	Apr-09	0.0000	0.2543	1.2136	0.0000	0.0000	3.1393
1	May-09	0.0000	0.0908	0.8086	0.0202	0.0000	1.7026
1	Aug-09	0.0000	0.8397	3.6908	0.0382	0.0000	9.2090
1	Sep-09	0.0000	0.1619	0.0503	0.0869	0.0000	5.1480
1	Nov-09	0.0000	0.1913	0.4402	0.0000	0.0000	1.1250
4	Oct-07	0.0000	0.0000	0.2786	0.0000	0.0000	0.7591
4	Nov-07	0.0000	0.0000	0.4092	0.0000	0.0000	1.0809
4	Feb-08	0.0000	0.0000	0.1872	0.0000	0.0000	0.5525
4	Mar-08	0.0000	0.0000	0.2448	0.0314	0.0000	0.2656
4	Apr-08	0.0000	0.0000	0.1063	0.0000	0.0000	0.3323
4	May-08	0.0591	0.4950	1.8566	0.0000	0.0000	5.6007
4	Jun-08	0.1076	0.0000	0.6617	0.6789	0.0000	1.3667
4	Jul-08	0.0659	0.0000	1.2182	0.0000	0.0000	2.7301
4	Aug-08	0.0000	0.2978	0.9593	0.0000	0.0000	2.6747
4	Oct-08	0.0000	0.0000	0.4172	0.0000	0.0000	1.1431
4	Nov-08	0.0000	0.6323	0.0881	0.0000	0.0000	0.5194
4	Jan-09	0.0000	0.0000	0.2468	0.0000	0.0000	0.8553
4	Mar-09	0.0390	0.0238	0.2697	0.0000	0.0000	0.8071
4	Apr-09	0.1054	0.1033	0.9106	0.0381	0.0000	2.0236
4	May-09	0.0000	0.0472	0.7320	0.0000	0.0000	1.6103

Table A.1. (continued).

Station	Date	Chloro-phyll c3	Chloro-philide a	Chl. c1+2	Peri-dinin	19'Butan oyloxyfu coxanthin	Fuco-xanthin
4	Aug-09	0.0686	0.1266	2.4616	0.0000	0.0000	9.8909
4	Sep-09	0.1640	0.0000	3.4738	0.0000	0.0000	11.4693
4	Nov-09	0.0513	0.0522	0.4571	0.0000	0.0000	1.4455
8 Surface	Sep-07	0.0373	0.0000	0.0999	0.0000	0.0000	0.1576
8 Surface	Nov-07	0.1507	0.0000	0.2067	0.0000	0.1250	0.1742
8 Surface	Feb-08	0.0000	0.0000	0.0434	0.0000	0.0000	0.1286
8 Surface	Mar-08	0.1593	0.0000	0.2908	0.0000	0.0000	1.0897
8 Surface	Jun-08	0.0349	0.0000	0.2013	0.0000	0.0187	0.4354
8 Surface	Jul-08	0.0985	0.0000	0.9091	0.0000	0.0000	2.8581
8 Surface	Aug-08	0.0000	0.0736	0.3783	0.0000	0.0000	1.0247
8 Surface	Nov-08	0.0152	0.0000	0.1087	0.0000	0.0000	0.3707
8 Surface	May-09	0.0796	0.0356	0.4392	0.0000	0.0000	1.2278
8 Surface	Jun-09	0.0425	0.0000	0.2042	0.0000	0.0000	0.6206
8 Surface	Aug-09	0.0000	0.0000	0.0504	0.0000	0.0000	0.0679
8 Surface	Nov-09	0.0249	0.0000	0.1285	0.0000	0.0000	0.2944
8 Middle	Feb-08	0.0000	0.0000	0.0548	0.0000	0.0000	0.1592
8 Middle	Mar-08	0.0742	0.0000	0.2642	0.0000	0.0000	1.0696
8 Middle	Jun-08	0.0833	0.0000	0.1285	0.0054	0.0000	0.2643
8 Middle	Jul-08	0.0912	0.0074	0.4564	0.0000	0.0000	1.3950
8 Middle	Aug-08	0.1698	0.0000	0.2270	0.0000	0.0455	0.6127
8 Middle	Nov-08	0.0000	0.0000	0.0985	0.0000	0.0000	0.3520
8 Middle	May-09	0.0672	0.0000	0.2145	0.0000	0.0000	0.5261
8 Middle	Jun-09	0.0133	0.0000	0.0852	0.0000	0.0000	0.2966
8 Middle	Aug-09	0.0790	0.0000	0.1499	0.0000	0.0000	0.4334
8 Middle	Nov-09	0.0880	0.0000	0.1566	0.0000	0.0000	0.4155
8 Bottom	Sep-07	0.0447	0.0000	0.2978	0.0000	0.0000	1.0445
8 Bottom	Nov-07	0.0000	0.0000	0.0866	0.0000	0.0000	0.3199
8 Bottom	Feb-08	0.0266	0.0000	0.2010	0.0000	0.0000	0.8251
8 Bottom	Mar-08	0.3089	0.0000	0.6019	0.0000	0.0000	2.8123
8 Bottom	Jun-08	0.0000	0.0000	0.0915	0.0000	0.0000	0.3437
8 Bottom	Jul-08	0.0276	0.0000	0.0892	0.0000	0.0000	0.3524
8 Bottom	Aug-08	0.0000	0.0000	0.0937	0.0000	0.0000	0.3246
8 Bottom	Nov-08	0.0000	0.0000	0.1093	0.0000	0.0000	0.4181
8 Bottom	May-09	0.0279	0.0000	0.1403	0.0000	0.0000	0.4615
8 Bottom	Jun-09	0.0000	0.0000	0.0538	0.0000	0.0000	0.2185
8 Bottom	Aug-09	0.0751	0.0000	0.2517	0.0000	0.0000	0.9359
8 Bottom	Nov-09	0.0787	0.0000	0.1632	0.0000	0.0000	0.6494

Table A.1. (continued).

Station	Date	Neo-	19'Hexano	Prasino	Viola-	Phaeoph

		xanthin	yloxyfuco xanthin	xanthin	xanthin	orbide a
1	Oct-07	0.0000	0.0000	0.0000	0.0000	0.0085
1	Nov-07	0.0000	0.0000	0.0000	0.0000	0.0624
1	Mar-08	0.0681	0.0000	0.0000	0.0209	0.1862
1	Apr-08	0.0000	0.0000	0.0000	0.0133	0.2629
1	May-08	0.0349	0.0000	0.0000	0.0806	0.5810
1	Jul-08	0.1964	0.0000	0.1179	0.2004	0.0000
1	Aug-08	0.2884	0.0000	0.1435	0.3501	0.0000
1	Oct-08	0.0000	0.0000	0.0000	0.0000	0.0405
1	Nov-08	0.0142	0.0000	0.0000	0.0000	0.0252
1	Jan-09	0.0325	0.0000	0.0000	0.0000	0.0559
1	Mar-09	0.1429	0.0000	0.1398	0.1684	0.0000
1	Apr-09	0.1158	0.0000	0.0000	0.1358	0.3791
1	May-09	0.1173	0.0000	0.0000	0.1293	0.2153
1	Aug-09	0.1790	0.0000	0.1174	0.1611	0.2815
1	Sep-09	0.0504	0.0000	0.0000	0.0915	0.0000
1	Nov-09	0.1323	0.0000	0.0000	0.1284	0.0572
4	Oct-07	0.0000	0.0000	0.0000	0.0000	0.0000
4	Nov-07	0.0000	0.0000	0.0000	0.0000	0.0000
4	Feb-08	0.0113	0.0000	0.0000	0.0000	0.1600
4	Mar-08	0.0158	0.0000	0.0000	0.0000	0.0663
4	Apr-08	0.0150	0.0000	0.0000	0.0096	0.0312
4	May-08	0.2118	0.0000	0.0000	0.0521	0.3412
4	Jun-08	0.0000	0.1823	0.0000	0.0631	0.0000
4	Jul-08	0.0000	0.2013	0.0000	0.0810	0.8597
4	Aug-08	0.0842	0.0000	0.0000	0.0996	0.0000
4	Oct-08	0.0190	0.0000	0.0000	0.0000	0.0557
4	Nov-08	0.0000	0.0000	0.0000	0.0000	0.0000
4	Jan-09	0.0000	0.0000	0.0000	0.0000	0.0000
4	Mar-09	0.0637	0.0000	0.0477	0.0503	0.0585
4	Apr-09	0.0955	0.0000	0.0000	0.0423	0.2241
4	May-09	0.0000	0.0127	0.0000	0.0000	0.0586
4	Aug-09	0.1311	0.0000	0.0464	0.0742	0.0197
4	Sep-09	0.0351	0.0000	0.0000	0.0000	0.5398
4	Nov-09	0.0493	0.0000	0.0000	0.0385	0.0799
8 Surface	Sep-07	0.0821	0.0307	0.0000	0.0468	0.0152
8 Surface	Nov-07	0.0000	0.1723	0.0000	0.0000	0.0000
8 Surface	Feb-08	0.0000	0.0000	0.0000	0.0000	0.0000

Table A.1. (continued).

Station	Date	Neo-xanthin	19'Hexano yloxyfuco	Prasino xanthin	Viola-xanthin	Phaeoph orbide a

		xanthin				
8 Surface	Mar-08	0.0000	0.0066	0.0000	0.0000	0.0000
8 Surface	Jun-08	0.0000	0.1382	0.0000	0.0069	0.0000
8 Surface	Jul-08	0.0000	0.0000	0.0000	0.0000	0.1143
8 Surface	Aug-08	0.0000	0.0000	0.0000	0.0000	0.0904
8 Surface	Nov-08	0.0000	0.0000	0.0000	0.0000	0.0583
8 Surface	May-09	0.0000	0.0203	0.0000	0.0000	0.0147
8 Surface	Jun-09	0.0000	0.1061	0.0000	0.0000	0.0000
8 Surface	Aug-09	0.0000	0.0097	0.0000	0.0000	0.0000
8 Surface	Nov-09	0.0000	0.0477	0.0000	0.0030	0.0214
8 Middle	Feb-08	0.0000	0.0000	0.0000	0.0000	0.0000
8 Middle	Mar-08	0.0000	0.0103	0.0000	0.0000	0.0119
8 Middle	Jun-08	0.0000	0.1386	0.0000	0.0074	0.0271
8 Middle	Jul-08	0.0000	0.0000	0.0000	0.0000	0.1147
8 Middle	Aug-08	0.0000	0.0930	0.0000	0.0000	0.0375
8 Middle	Nov-08	0.0000	0.0000	0.0000	0.0000	0.0176
8 Middle	May-09	0.0000	0.0000	0.0000	0.0000	0.0000
8 Middle	Jun-09	0.0000	0.0097	0.0000	0.0000	0.0313
8 Middle	Aug-09	0.0000	0.0644	0.0000	0.0000	0.0125
8 Middle	Nov-09	0.0000	0.0969	0.0000	0.0000	0.0091
8 Bottom	Sep-07	0.0000	0.0419	0.0000	0.0000	0.0000
8 Bottom	Nov-07	0.0000	0.0000	0.0000	0.0000	0.0000
8 Bottom	Feb-08	0.0000	0.0000	0.0000	0.0000	0.0173
8 Bottom	Mar-08	0.0000	0.0000	0.0000	0.0000	0.0000
8 Bottom	Jun-08	0.0000	0.0000	0.0000	0.0000	0.0174
8 Bottom	Jul-08	0.0000	0.0000	0.0000	0.0000	0.0098
8 Bottom	Aug-08	0.0000	0.0000	0.0000	0.0000	0.0775
8 Bottom	Nov-08	0.0000	0.0000	0.0000	0.0000	0.0000
8 Bottom	May-09	0.0000	0.0000	0.0000	0.0000	0.0111
8 Bottom	Jun-09	0.0000	0.0000	0.0000	0.0000	0.0365
8 Bottom	Aug-09	0.0000	0.0000	0.0000	0.0000	0.0701
8 Bottom	Nov-09	0.0000	0.0151	0.0078	0.0000	0.0277

Table A.1. (continued).

Station	Date	Diadino xanthin	Allo xanthin	Diato xanthin	Lutein	Zea xanthin	Chl. b
1	Oct-07	0.0363	0.0000	0.0000	0.0000	0.1904	0.0000
1	Nov-07	0.0000	0.0432	0.0000	0.0051	0.1997	0.2558
1	Mar-08	0.0606	0.0794	0.0000	0.0700	0.6958	1.0821
1	Apr-08	0.0892	0.0228	0.0000	0.0440	0.9094	0.7634
1	May-08	0.4139	0.0721	0.0654	0.0361	2.2109	0.3492
1	Jul-08	0.5532	0.2078	0.0476	0.1235	3.1699	1.1510
1	Aug-08	0.7773	0.4413	0.0000	0.1884	3.0994	2.2333
1	Oct-08	0.0256	0.0000	0.0000	0.0082	0.6151	0.2944
1	Nov-08	0.0152	0.0609	0.0000	0.0258	0.3232	0.3912
1	Jan-09	0.1303	0.0000	0.0000	0.0154	0.2323	0.6394
1	Mar-09	0.5388	0.1614	0.0431	0.1080	0.2680	1.0478
1	Apr-09	0.8470	0.5126	0.1445	0.0893	1.0639	1.5402
1	May-09	0.6280	0.2668	0.0583	0.0922	2.6546	0.9061
1	Aug-09	1.0605	0.3424	0.0833	0.0796	0.7063	0.0000
1	Sep-09	1.1660	0.2929	0.0913	0.0374	0.9103	0.8059
1	Nov-09	0.2989	0.2119	0.0155	0.2525	1.3084	0.8445
4	Oct-07	0.0761	0.0395	0.0000	0.0000	0.1462	0.1038
4	Nov-07	0.1339	0.0288	0.0000	0.0000	0.1000	0.3684
4	Feb-08	0.0309	0.0000	0.0000	0.0000	0.0526	0.2825
4	Mar-08	0.0273	0.0277	0.0000	0.0164	0.1647	0.5264
4	Apr-08	0.0182	0.0257	0.0000	0.0076	1.1906	0.3158
4	May-08	1.0691	0.4420	0.1171	0.0639	2.9294	1.1212
4	Jun-08	0.5592	0.0858	0.0352	0.0278	1.4225	0.4892
4	Jul-08	0.3133	0.1307	0.0900	1.5816	1.2543	0.4103
4	Aug-08	0.7882	0.0712	0.1131	0.0319	0.6134	0.3648
4	Oct-08	0.0347	0.0602	0.1704	0.0000	0.2070	0.3688
4	Nov-08	0.0000	0.0000	0.0000	0.0000	0.0924	0.1650
4	Jan-09	0.1707	0.0000	0.0000	0.0000	0.0712	0.2128
4	Mar-09	0.1255	0.0170	0.0153	0.0278	0.2444	0.3315
4	Apr-09	0.4484	0.1287	0.0293	0.0211	0.9118	0.4228
4	May-09	0.3912	0.0451	0.0669	0.0000	0.6117	0.0000
4	Aug-09	0.9709	0.2415	0.0825	0.0203	0.6816	0.0000
4	Sep-09	1.2626	0.1002	0.1238	0.0000	0.1851	0.0000
4	Nov-09	0.2265	0.0971	0.0127	0.0291	0.5785	0.3580
8 Surface	Sep-07	0.0225	0.0000	0.0000	0.0228	0.3235	0.1844
8 Surface	Nov-07	0.1259	0.0000	0.0096	0.0000	0.0547	0.3715

Table A.1.

(continued).

Station	Date	Diadino xanthin	Allo xanthin	Diato xanthin	Lutein	Zea xanthin	Chl. b
8 Surface	Feb-08	0.0000	0.0000	0.0000	0.0000	0.0000	0.0936
8 Surface	Mar-08	0.1215	0.0000	0.0045	0.0000	0.0137	0.1260
8 Surface	Jun-08	0.0986	0.0000	0.0072	0.0000	0.3792	0.1167
8 Surface	Jul-08	0.3386	0.0000	0.0000	0.0000	1.2478	0.0000
8 Surface	Aug-08	0.1914	0.0000	0.0373	0.0000	0.1704	0.0000
8 Surface	Nov-08	0.0165	0.0225	0.0000	0.0000	0.0685	0.1186
8 Surface	May-09	0.1279	0.0045	0.0143	0.0000	0.0846	0.0000
8 Surface	Jun-09	0.0786	0.0132	0.0000	0.0000	2.1173	0.1452
8 Surface	Aug-09	0.0289	0.0000	0.0000	0.0000	0.1188	0.0000
8 Surface	Nov-09	0.0436	0.0176	0.0000	0.0000	0.1475	0.0992
8 Middle	Feb-08	0.0000	0.0000	0.0000	0.0000	0.0104	0.1315
8 Middle	Mar-08	0.1007	0.0000	0.0000	0.0000	0.0042	0.1477
8 Middle	Jun-08	0.0200	0.0095	0.0000	0.0000	0.3814	0.0535
8 Middle	Jul-08	0.0799	0.0000	0.0000	0.0000	0.1838	0.0000
8 Middle	Aug-08	0.0135	0.0431	0.0000	0.0000	0.0971	0.1412
8 Middle	Nov-08	0.0048	0.0142	0.0000	0.0000	0.0792	0.0835
8 Middle	May-09	0.0431	0.0000	0.0000	0.0000	0.0725	0.0754
8 Middle	Jun-09	0.0063	0.0184	0.0000	0.0000	0.0642	0.0717
8 Middle	Aug-09	0.0000	0.0111	0.0000	0.0000	0.1178	0.0000
8 Middle	Nov-09	0.0000	0.0397	0.0000	0.0000	0.1336	0.1813
8 Bottom	Sep-07	0.0920	0.0000	0.0000	0.0000	0.0502	0.0000
8 Bottom	Nov-07	0.0317	0.0000	0.0000	0.0000	0.0139	0.1247
8 Bottom	Feb-08	0.0170	0.0000	0.0045	0.0000	0.0000	0.1331
8 Bottom	Mar-08	0.1956	0.0127	0.0072	0.0000	0.0307	0.0000
8 Bottom	Jun-08	0.0041	0.0000	0.0000	0.0000	0.0573	0.0000
8 Bottom	Jul-08	0.0093	0.0000	0.0000	0.0000	0.0294	0.0000
8 Bottom	Aug-08	0.0000	0.0132	0.0000	0.0000	0.0242	0.0210
8 Bottom	Nov-08	0.0000	0.0244	0.0000	0.0000	0.0772	0.1161
8 Bottom	May-09	0.0143	0.0332	0.0000	0.0000	0.0624	0.0260
8 Bottom	Jun-09	0.0000	0.0000	0.0000	0.0000	0.0486	0.0000
8 Bottom	Aug-09	0.0000	0.0136	0.0000	0.0000	0.0889	0.0000
8 Bottom	Nov-09	0.0212	0.0000	0.0459	0.0000	0.0000	0.0951

Table A.1. (continued).

Station	Date	Chl. *a*	Phaeo-phytin a	βε Carothene	ββ Carothene
1	Oct-07	1.6486	0.0553	0.0000	0.0642
1	Nov-07	1.4804	0.0451	0.0000	0.0484
1	Mar-08	4.1765	0.1083	0.0000	0.1497
1	Apr-08	8.4440	0.6813	0.0000	0.3229
1	May-08	10.2513	0.3660	0.0000	0.7490
1	Jul-08	12.0032	0.1446	0.0127	0.7531
1	Aug-08	15.3342	0.2662	0.0000	0.7441
1	Oct-08	3.0240	0.0680	0.0000	0.1232
1	Nov-08	1.4665	0.0000	0.0000	0.0455
1	Jan-09	3.3705	0.0879	0.0000	0.0840
1	Mar-09	6.6314	0.1148	0.0000	0.2099
1	Apr-09	15.5202	0.4320	0.0598	0.6544
1	May-09	11.3315	0.1646	0.0000	0.7832
1	Aug-09	23.1959	0.3389	0.0398	0.3334
1	Sep-09	13.7888	0.1978	0.0418	0.4923
1	Nov-09	7.0395	0.1163	0.0210	0.3650
4	Oct-07	2.1375	0.0524	0.0000	0.0648
4	Nov-07	3.4060	0.0402	0.0000	0.0397
4	Feb-08	1.7173	0.0579	0.0000	0.0273
4	Mar-08	1.7738	0.0518	0.0000	0.0336
4	Apr-08	3.3965	0.0714	0.0000	0.2110
4	May-08	20.3015	0.3537	0.0590	1.0511
4	Jun-08	7.6705	0.0931	0.0000	0.4587
4	Jul-08	13.6655	0.1486	0.0000	1.0302
4	Aug-08	8.7083	0.1085	0.0000	0.2828
4	Oct-08	3.5381	0.0824	0.0000	0.1052
4	Nov-08	0.2747	0.1624	0.0000	0.0000
4	Jan-09	2.4373	0.0628	0.0000	0.0510
4	Mar-09	2.4412	0.0354	0.0000	0.1087
4	Apr-09	8.3897	0.1673	0.0093	0.3968
4	May-09	4.8523	0.0473	0.0000	0.2666
4	Aug-09	23.1785	0.7276	0.0299	0.6031
4	Sep-09	21.1995	0.2566	0.0092	0.4707
4	Nov-09	4.8819	0.0655	0.0078	0.2041
8 Surface	Sep-07	1.4305	0.0238	0.0000	0.0856
8 Surface	Nov-07	2.7941	0.0273	0.0000	0.0348

Table A.1. (continued).

Station	Date	Chl. *a*	Phaeo-phytin a	βε Carothene	ββ Carothene
8 Surface	Feb-08	0.4598	0.0000	0.0000	0.0040
8 Surface	Mar-08	2.4438	0.0387	0.0000	0.0431
8 Surface	Jun-08	1.8186	0.0133	0.0000	0.1060
8 Surface	Jul-08	7.4423	0.1023	0.0000	0.2317
8 Surface	Aug-08	2.0067	0.0288	0.0000	0.0939
8 Surface	Nov-08	1.1400	0.0281	0.0000	0.0255
8 Surface	May-09	2.3663	0.0218	0.0000	0.0953
8 Surface	Jun-09	4.3611	0.0400	0.0000	0.5211
8 Surface	Aug-09	0.4588	0.0000	0.0000	0.0237
8 Surface	Nov-09	0.9761	0.0066	0.0000	0.0455
8 Middle	Feb-08	0.5510	0.0000	0.0000	0.0034
8 Middle	Mar-08	2.7953	0.0518	0.0000	0.0471
8 Middle	Jun-08	1.7678	0.0227	0.0000	0.1470
8 Middle	Jul-08	2.8121	0.0504	0.0000	0.0492
8 Middle	Aug-08	1.4420	0.0367	0.0056	0.0371
8 Middle	Nov-08	1.0097	0.0352	0.0000	0.0310
8 Middle	May-09	1.2424	0.0000	0.0000	0.0384
8 Middle	Jun-09	0.3994	0.0303	0.0000	0.0205
8 Middle	Aug-09	1.1660	0.0000	0.0000	0.0331
8 Middle	Nov-09	1.2490	0.0086	0.0000	0.0492
8 Bottom	Sep-07	2.2268	0.0277	0.0000	0.0491
8 Bottom	Nov-07	0.9805	0.0198	0.0000	0.0184
8 Bottom	Feb-08	1.8995	0.0133	0.0000	0.0294
8 Bottom	Mar-08	5.6446	0.1168	0.0000	0.1028
8 Bottom	Jun-08	0.7026	0.0560	0.0000	0.0291
8 Bottom	Jul-08	0.6047	0.0228	0.0000	0.0124
8 Bottom	Aug-08	0.6638	0.0518	0.0000	0.0166
8 Bottom	Nov-08	1.0787	0.0362	0.0000	0.0281
8 Bottom	May-09	1.1076	0.0336	0.0000	0.0361
8 Bottom	Jun-09	0.4918	0.0678	0.0000	0.0164
8 Bottom	Aug-09	2.0100	0.0554	0.0000	0.0517
8 Bottom	Nov-09	1.3701	0.0339	0.0000	0.0326

Table A.2. Excel Summary output.

Regression Statistics	
Multiple R	0.994662593
R Square	0.989353675
Adjusted R Square	0.98872742
Standard Error	0.848100974
Observations	19

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1136.307509	1136.30751	1579.795	3.26294E-18
Residual	17	12.22767944	0.71927526		
Total	18	1148.535188			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.44654529	0.263240079	-1.69634234	0.108054	-1.001933306	0.108842721
X Variable 1	0.963478247	0.024240498	39.7466359	3.26E-18	0.912335268	1.014621227

Table A.2a. Final Pigment ratio from Chemtax 1.95, station 1, Bay of Saint Louis.

Station 1

	chc1+2	per	but	fuc	neo	hex	pra	vio	diad	all	lut	zea	chb	chl_a
Diat	0.0295	0.0000	0.0000	0.1252	0.0000	0.0000	0.0000	0.0000	0.0218	0.0000	0.0000	0.0000	0.0000	0.8235
Cyan	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.5546	0.0000	0.4454
Chlor	0.0000	0.0000	0.0000	0.0000	0.0246	0.0000	0.0000	0.0181	0.0000	0.0000	0.1871	0.0234	0.1620	0.5848
Dino	0.1102	0.2687	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1170	0.0000	0.0000	0.0000	0.0000	0.5042
Eugleno	0.0000	0.0000	0.0000	0.0000	0.0312	0.0000	0.0000	0.0000	0.0348	0.0000	0.0089	0.0081	0.5124	0.4047
Prym	0.0748	0.0000	0.0038	0.1660	0.0000	0.1475	0.0000	0.0000	0.0617	0.0000	0.0000	0.0000	0.0000	0.5461
Crypto	0.1359	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2491	0.0000	0.0000	0.0000	0.6150
Pras	0.0000	0.0000	0.0000	0.0000	0.0451	0.0000	0.1077	0.0324	0.0000	0.0000	0.0315	0.0287	0.2844	0.4701
ChrysoB	0.0407	0.0000	0.2988	0.2001	0.0000	0.0000	0.0000	0.0000	0.1402	0.0000	0.0000	0.0000	0.0000	0.3202
Eustigma	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1788	0.0000	0.0000	0.0000	0.0121	0.0000	0.8091

Table A.2b. Final Pigment ratio from Chemtax 1.95, station 4, Mississippi Sound.

Station 4

	chc1+2	per	but	fuc	neo	hex	pra	vio	diad	all	lut	zea	chb	chl_a
Diat	0.0580	0.0000	0.0000	0.2197	0.0000	0.0000	0.0000	0.0000	0.0218	0.0000	0.0000	0.0000	0.0000	0.7005
Cyan	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.5546	0.0000	0.4454
Chlor	0.0000	0.0000	0.0000	0.0000	0.0246	0.0000	0.0000	0.0181	0.0000	0.0000	0.1871	0.0234	0.1620	0.5848
Dino	0.1102	0.2687	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1170	0.0000	0.0000	0.0000	0.0000	0.5042
Eugleno	0.0000	0.0000	0.0000	0.0000	0.0303	0.0000	0.0000	0.0000	0.0338	0.0000	0.0087	0.0079	0.5259	0.3934
Prym	0.0748	0.0000	0.0038	0.1660	0.0000	0.1475	0.0000	0.0000	0.0617	0.0000	0.0000	0.0000	0.0000	0.5461
Crypto	0.1359	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2491	0.0000	0.0000	0.0000	0.6150
Pras	0.0000	0.0000	0.0000	0.0000	0.0451	0.0000	0.1077	0.0324	0.0000	0.0000	0.0315	0.0287	0.2844	0.4701
ChrysoB	0.0407	0.0000	0.2988	0.2001	0.0000	0.0000	0.0000	0.0000	0.1402	0.0000	0.0000	0.0000	0.0000	0.3202
Eustigma	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1788	0.0000	0.0000	0.0000	0.0121	0.0000	0.8091

Table A.2c. Final Pigment ratio from Chemtax 1.95, station 8, Mississippi Bight.

Station. 8 Surface, Middle and Bottom

	chc1+2	per	but	fuc	neo	hex	pra	vio	diad	all	lut	zea	chb	chl_a
Diat	0.0272	0.0000	0.0000	0.2274	0.0000	0.0000	0.0000	0.0000	0.0218	0.0000	0.0000	0.0000	0.0000	0.7236
Cyan	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.5546	0.0000	0.4454
Chlor	0.0000	0.0000	0.0000	0.0000	0.0246	0.0000	0.0000	0.0181	0.0000	0.0000	0.1871	0.0234	0.1620	0.5848
Dino	0.1102	0.2687	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1170	0.0000	0.0000	0.0000	0.0000	0.5042
Eugleno	0.0000	0.0000	0.0000	0.0000	0.0362	0.0000	0.0000	0.0000	0.0155	0.0000	0.0103	0.0094	0.4589	0.4697
Prym	0.0748	0.0000	0.0038	0.1660	0.0000	0.1475	0.0000	0.0000	0.0617	0.0000	0.0000	0.0000	0.0000	0.5461
Crypto	0.1359	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.2491	0.0000	0.0000	0.0000	0.6150
Pras	0.0000	0.0000	0.0000	0.0000	0.0451	0.0000	0.1077	0.0324	0.0000	0.0000	0.0315	0.0287	0.2844	0.4701
ChrysoB	0.0407	0.0000	0.2988	0.2001	0.0000	0.0000	0.0000	0.0000	0.1402	0.0000	0.0000	0.0000	0.0000	0.3202
Eustigma	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1788	0.0000	0.0000	0.0000	0.0121	0.0000	0.8091

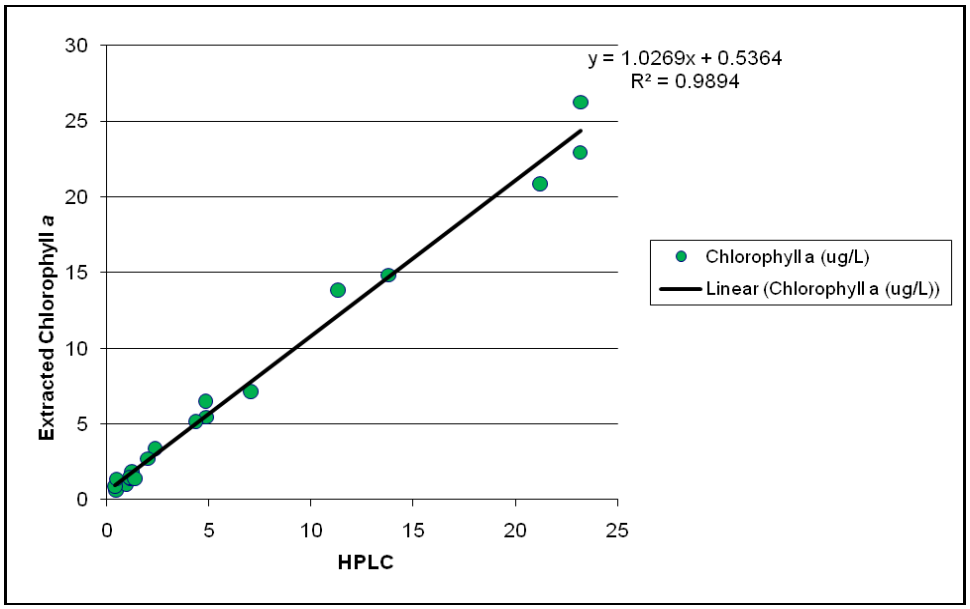


Fig. A.1. Extracted Chlorophyll a vs HPLC with the coefficient of determination between extracted Chlorophyll a from the Welschmeyer method and Chlorophyll a from HPLC.

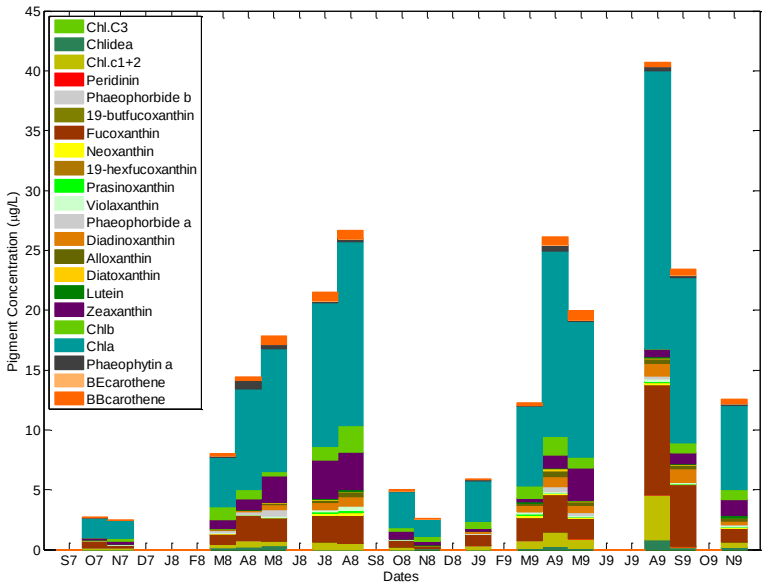


Fig. A.2a. Pigment concentration (µg L^{-1}) present in Station 1.

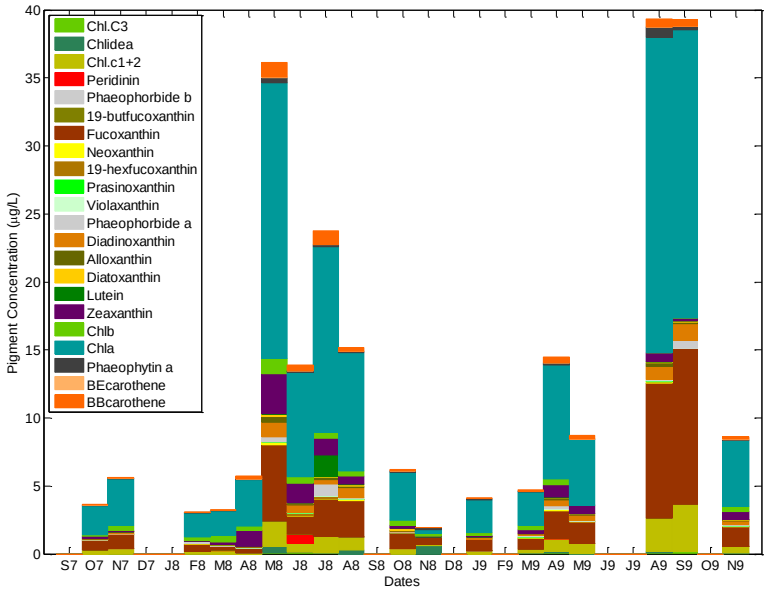


Fig. A.2b. Pigment concentration (µg L^{-1}) present in Station 4.

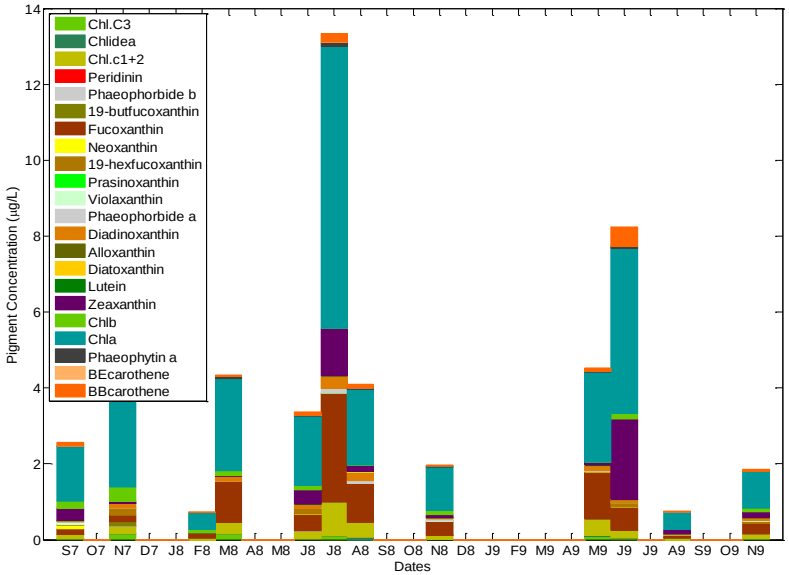


Fig. A.2c. Pigment concentration (µg L^{-1}) present in Station 8 Surface.

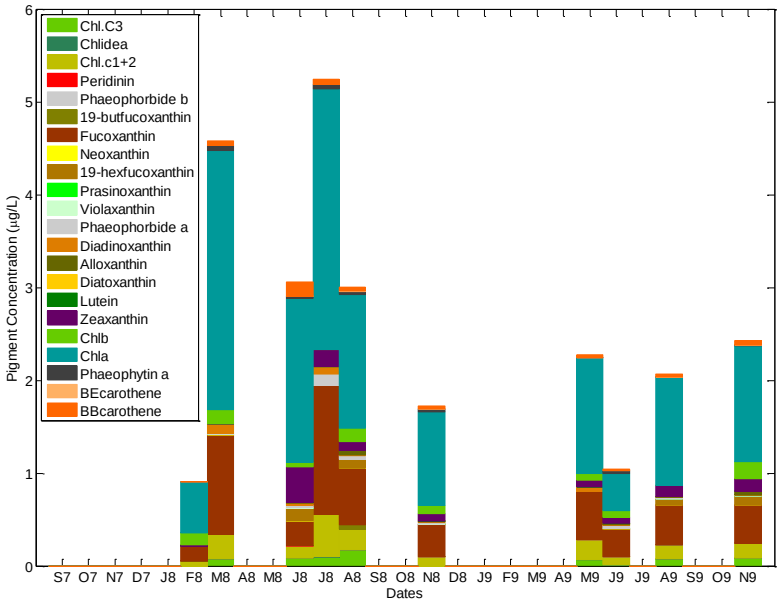


Fig. A.2d. Pigment concentration (µg L^{-1}) present in Station 8 at the Middle (7m).

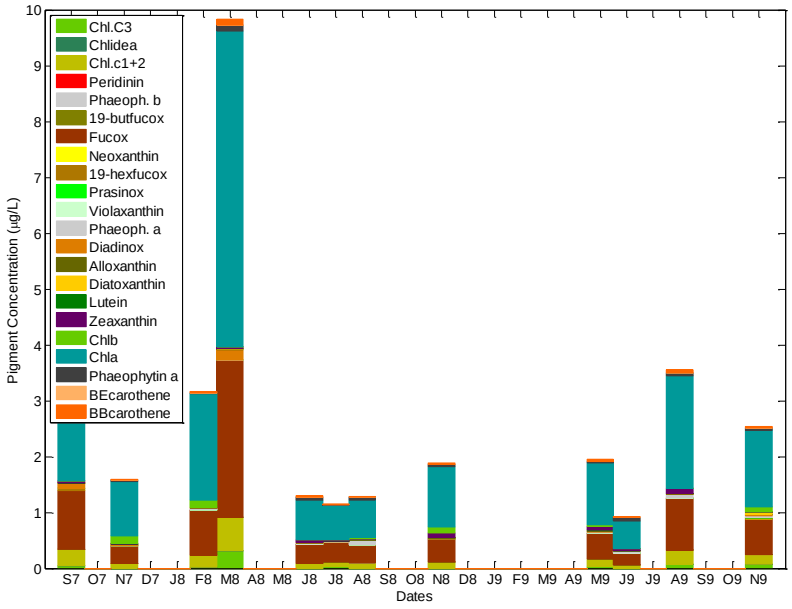


Fig. A.2e. Pigment concentration (µg L^{-1}) present in Station 8 at the Bottom (19m).

APPENDIX B

PHYTOPLANKCTON GROUP FRACTION OF CHLOROPHYLL A

Table B.1. Phytoplankton group fraction of chl *a* (µg L^{-1}). Station 1 surface waters, station 4 Surface waters, station 8 surface, station 8 (7m) and station 8 (19m).

Station	Date	Diat	Cyano	Chlor	Dino	Eugleno
1	Oct-07	1.4095	0.2193	0.0000	0.0000	0.0000
1	Nov-07	1.0503	0.1491	0.0059	0.0000	0.1535
1	Mar-08	2.6380	0.4691	0.1443	0.0000	0.6526
1	Apr-08	7.2491	0.6227	0.0889	0.0000	0.3649
1	May-08	6.1982	2.7199	0.3192	0.0000	0.3782
1	Jul-08	5.0561	3.3372	0.5911	0.0005	1.2512
1	Aug-08	4.5934	3.3065	1.1812	0.0000	2.9837
1	Oct-08	1.8190	0.7651	0.1820	0.0000	0.0000
1	Nov-08	0.6931	0.2645	0.0668	0.0000	0.2851
1	Jan-09	2.7836	0.1263	0.0000	0.0103	0.3753
1	Mar-09	4.6142	0.0561	0.0831	0.1010	0.4596
1	Apr-09	11.7680	0.6390	0.0768	0.1347	1.0646
1	May-09	4.9553	2.8341	0.6079	0.0615	1.5051
1	Aug-09	21.1893	0.0983	0.0000	0.1106	0.0000
1	Sep-09	9.7228	0.8371	0.2211	0.0448	2.0577
1	Nov-09	3.9830	0.8870	0.6622	0.0464	0.4930
4	Oct-07	1.7396	0.1165	0.0000	0.0000	0.0000
4	Nov-07	3.0207	0.0536	0.0000	0.0068	0.2400
4	Feb-08	1.4828	0.0362	0.0000	0.0000	0.1858
4	Mar-08	1.1095	0.1289	0.0282	0.0443	0.3670
4	Apr-08	1.8407	1.0911	0.0254	0.0000	0.2465
4	May-08	13.4185	2.2888	0.3445	0.0000	1.8234
4	Jun-08	3.0103	1.1514	0.0322	1.3447	0.3256
4	Jul-08	4.2725	0.8209	7.4531	0.0000	0.0000
4	Aug-08	6.0084	0.4013	0.1172	0.0217	1.5372
4	Oct-08	2.6085	0.1647	0.0000	0.0000	0.0000
4	Nov-08	0.2128	0.0221	0.0000	0.0000	0.0398
4	Jan-09	2.1850	0.0265	0.0000	0.0262	0.1439
4	Mar-09	1.7803	0.1355	0.0177	0.0149	0.1334
4	Apr-09	6.6644	0.6575	0.0155	0.1436	0.3400
4	May-09	4.0558	0.4701	0.0000	0.0308	0.0645
4	Aug-09	20.6345	0.6002	0.5717	0.0000	0.0000
4	Sep-09	20.6431	0.1376	0.0450	0.0000	0.0000

Table B.1. (continued).

Station	Date	Diatoms	Cyanobac	Chloro	Dino	Eugleno
4	Nov-09	3.7732	0.4003	0.0527	0.0242	0.2479
8 Surface	Sep-07	0.0113	0.8800	0.1167	0.0000	0.1102
8 Surface	Nov-07	1.3643	0.0469	0.0000	0.0000	0.4479
8 Surface	Feb-08	0.3599	0.0000	0.0000	0.0000	0.0895
8 Surface	Mar-08	2.2446	0.0063	0.0000	0.0194	0.0983
8 Surface	Jun-08	0.2298	0.9614	0.0000	0.0000	0.0656
8 Surface	Jul-08	3.3045	4.1175	0.0000	0.0012	0.0151
8 Surface	Aug-08	1.5627	0.3265	0.0000	0.0163	0.0774
8 Surface	Nov-08	0.9204	0.0495	0.0000	0.0000	0.1076
8 Surface	May-09	1.6034	0.6586	0.0068	0.0000	0.0000
8 Surface	Jun-09	0.0000	3.9658	0.0000	0.0000	0.0888
8 Surface	Aug-09	0.1130	0.2853	0.0000	0.0045	0.0178
8 Surface	Nov-09	0.5614	0.1056	0.0000	0.0034	0.0866
8 Middle	Feb-08	0.4094	0.0062	0.0000	0.0000	0.1230
8 Middle	Mar-08	2.5960	0.0002	0.0000	0.0073	0.1269
8 Middle	Jun-08	0.0000	1.2561	0.0000	0.0000	0.0000
8 Middle	Jul-08	1.4693	1.2880	0.0128	0.0000	0.0000
8 Middle	Aug-08	0.2506	0.6327	0.0000	0.0000	0.0000
8 Middle	Nov-08	0.8292	0.0576	0.0000	0.0000	0.0728
8 Middle	May-09	0.6887	0.4540	0.0000	0.0000	0.0000
8 Middle	Jun-09	0.1393	0.1422	0.0000	0.0000	0.0000
8 Middle	Aug-09	0.1933	0.7268	0.0068	0.0000	0.0000
8 Middle	Nov-09	0.5803	0.0964	0.0000	0.0000	0.1576
8 Bottom	Sep-07	1.2244	0.8066	0.0132	0.0000	0.0000
8 Bottom	Nov-07	0.8468	0.0083	0.0000	0.0021	0.1134
8 Bottom	Feb-08	1.7624	0.0000	0.0000	0.0000	0.1087
8 Bottom	Mar-08	5.5442	0.0186	0.0000	0.0065	0.0000
8 Bottom	Jun-08	0.2669	0.4166	0.0046	0.0000	0.0000
8 Bottom	Jul-08	0.2896	0.2977	0.0043	0.0000	0.0000
8 Bottom	Aug-08	0.2531	0.3336	0.0108	0.0000	0.0000
8 Bottom	Nov-08	0.8524	0.0540	0.0000	0.0000	0.0987
8 Bottom	May-09	0.4287	0.5381	0.0127	0.0000	0.0000
8 Bottom	Jun-09	0.1554	0.3231	0.0032	0.0000	0.0000
8 Bottom	Aug-09	0.7509	1.1473	0.0183	0.0000	0.0000
8 Bottom	Nov-09	1.2399	0.0000	0.0000	0.0000	0.0601

Table B.1. (continued).

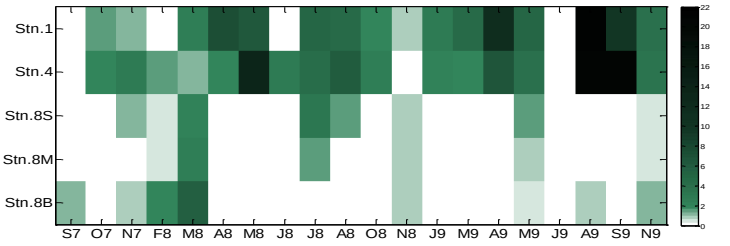
Station	Date	Prym	Crypto	Pras	Chryso	Eustigma
1	Oct-07	0.0006	0.0189	0.0004	0.0000	0.0000
1	Nov-07	0.0000	0.1150	0.0068	0.0000	0.0000
1	Mar-08	0.0000	0.2065	0.0323	0.0000	0.0336
1	Apr-08	0.0000	0.0906	0.0278	0.0000	0.0000
1	May-08	0.0000	0.2084	0.1657	0.0000	0.2616
1	Jul-08	0.0236	0.5763	0.7813	0.0000	0.3861
1	Aug-08	0.0000	1.1922	1.2793	0.0000	0.7979
1	Oct-08	0.0000	0.0326	0.2253	0.0000	0.0000
1	Nov-08	0.0000	0.1525	0.0043	0.0000	0.0003
1	Jan-09	0.0455	0.0269	0.0000	0.0025	0.0000
1	Mar-09	0.2077	0.3244	0.4053	0.0634	0.3166
1	Apr-09	0.2700	1.1460	0.0000	0.0825	0.3385
1	May-09	0.0552	0.7802	0.2927	0.0000	0.2395
1	Aug-09	0.2113	1.1615	0.1493	0.0000	0.2755
1	Sep-09	0.0000	0.5675	0.1170	0.0085	0.2123
1	Nov-09	0.0972	0.4800	0.0000	0.0292	0.3617
4	Oct-07	0.0000	0.1365	0.1449	0.0000	0.0000
4	Nov-07	0.0099	0.0734	0.0000	0.0016	0.0000
4	Feb-08	0.0000	0.0072	0.0052	0.0000	0.0000
4	Mar-08	0.0000	0.0885	0.0073	0.0000	0.0000
4	Apr-08	0.0000	0.0810	0.0297	0.0000	0.0821
4	May-08	0.0000	1.4333	0.9930	0.0000	0.0000
4	Jun-08	0.7284	0.2930	0.5957	0.0000	0.1891
4	Jul-08	0.7205	0.3985	0.0000	0.0000	0.0000
4	Aug-08	0.0233	0.2218	0.0000	0.0037	0.3737
4	Oct-08	0.0000	0.2308	0.5341	0.0000	0.0000
4	Nov-08	0.0000	0.0000	0.0000	0.0000	0.0000
4	Jan-09	0.0391	0.0000	0.0000	0.0166	0.0000
4	Mar-09	0.0232	0.0343	0.1894	0.0090	0.1033
4	Apr-09	0.1029	0.3142	0.0000	0.0373	0.1142
4	May-09	0.0802	0.1424	0.0000	0.0086	0.0000
4	Aug-09	0.0000	0.8510	0.0000	0.0000	0.5211
4	Sep-09	0.0000	0.3724	0.0013	0.0000	0.0000
4	Nov-09	0.0371	0.2170	0.0000	0.0156	0.1138
8 Surface	Sep-07	0.0812	0.0017	0.1246	0.0000	0.1048
8 Surface	Nov-07	0.7282	0.0272	0.0000	0.1342	0.0454
8 Surface	Feb-08	0.0000	0.0059	0.0000	0.0000	0.0045

Table B.1. (continued).

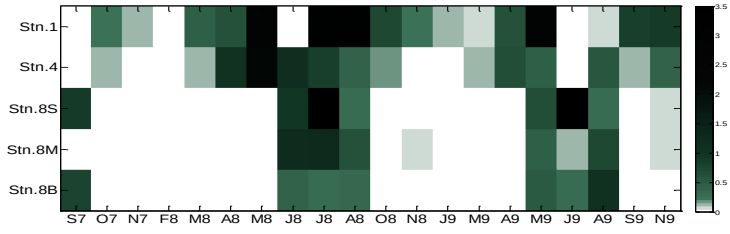
Station	Date	Prym	Crypto	Pras	Chryso	Eustigma
8 Surface	Mar-08	0.0569	0.0095	0.0000	0.0087	0.0000
8 Surface	Jun-08	0.4450	0.0000	0.1035	0.0134	0.0000
8 Surface	Jul-08	0.0000	0.0000	0.0000	0.0041	0.0000
8 Surface	Aug-08	0.0133	0.0000	0.0000	0.0105	0.0000
8 Surface	Nov-08	0.0000	0.0602	0.0000	0.0000	0.0022
8 Surface	May-09	0.0635	0.0210	0.0000	0.0000	0.0131
8 Surface	Jun-09	0.2181	0.0000	0.0884	0.0000	0.0000
8 Surface	Aug-09	0.0360	0.0000	0.0000	0.0022	0.0000
8 Surface	Nov-09	0.1676	0.0457	0.0000	0.0000	0.0058
8 Middle	Feb-08	0.0000	0.0071	0.0000	0.0000	0.0053
8 Middle	Mar-08	0.0516	0.0123	0.0000	0.0010	0.0000
8 Middle	Jun-08	0.4264	0.0247	0.0577	0.0000	0.0029
8 Middle	Jul-08	0.0000	0.0182	0.0000	0.0000	0.0238
8 Middle	Aug-08	0.2694	0.1178	0.1503	0.0211	0.0000
8 Middle	Nov-08	0.0000	0.0420	0.0035	0.0000	0.0047
8 Middle	May-09	0.0000	0.0074	0.0924	0.0000	0.0000
8 Middle	Jun-09	0.0170	0.0385	0.0625	0.0000	0.0000
8 Middle	Aug-09	0.1873	0.0396	0.0000	0.0000	0.0123
8 Middle	Nov-09	0.2811	0.1066	0.0107	0.0000	0.0164
8 Bottom	Sep-07	0.1456	0.0112	0.0000	0.0000	0.0258
8 Bottom	Nov-07	0.0051	0.0049	0.0000	0.0000	0.0000
8 Bottom	Feb-08	0.0000	0.0182	0.0064	0.0000	0.0039
8 Bottom	Mar-08	0.0258	0.0495	0.0000	0.0000	0.0000
8 Bottom	Jun-08	0.0000	0.0061	0.0000	0.0000	0.0085
8 Bottom	Jul-08	0.0000	0.0051	0.0000	0.0000	0.0081
8 Bottom	Aug-08	0.0000	0.0422	0.0210	0.0000	0.0032
8 Bottom	Nov-08	0.0000	0.0641	0.0039	0.0000	0.0057
8 Bottom	May-09	0.0000	0.0979	0.0272	0.0000	0.0031
8 Bottom	Jun-09	0.0000	0.0041	0.0000	0.0000	0.0059
8 Bottom	Aug-09	0.0000	0.0589	0.0000	0.0000	0.0346
8 Bottom	Nov-09	0.0322	0.0124	0.0254	0.0000	0.0000

Table B.2. Phytoplankton median group percentages for each station and season.

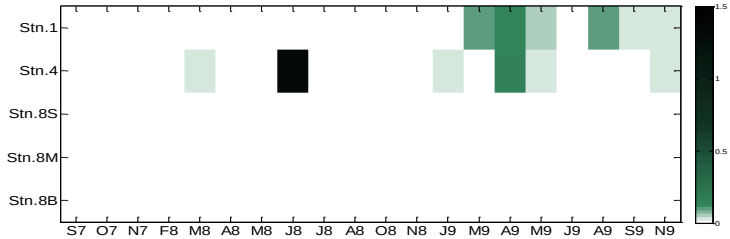
	Season	Diat	Cyan	Chlor	Dino	Eugleno	Prym	Crypto	Pras	Chryso	Eustigma
Station 1	Fall	60.1534	13.2989	6.0196	0.0000	7.0036	0.0000	6.8180	0.4623	0.0000	0.0000
	Spring	68.1434	16.1925	2.0834	0.2716	5.5907	0.2434	4.4591	0.9730	0.0000	2.1474
	Summer	56.3176	13.8169	3.2639	0.1646	12.6732	0.0984	4.9041	3.6787	0.0000	2.3781
	Winter	66.3726	2.2964	0.6265	0.1525	9.0325	0.6755	2.8460	0.3864	0.0374	0.4019
Station 4	Fall	77.2898	4.6562	0.0000	0.1998	5.0781	0.2673	4.4454	0.0000	0.0421	0.0000
	Spring	66.0962	11.2740	0.4200	0.6340	4.2450	1.2269	3.7455	0.8741	0.0000	1.3610
	Summer	79.0104	3.5991	1.9061	0.0000	0.0000	0.1337	2.7315	0.0000	0.0000	1.1242
	Winter	72.9266	2.1108	0.0000	0.6112	5.9046	0.0000	0.4217	0.3017	0.0000	0.0000
Station 8 Surface	Fall	57.5156	10.8170	0.0000	0.3478	8.8723	17.1675	0.9726	0.0000	0.5223	0.5945
	Spring	12.6362	52.8667	0.0000	0.0000	2.0352	5.0009	0.0000	2.0281	0.0000	0.0000
	Summer	44.4015	55.3257	0.0000	0.8132	3.8586	0.6641	0.0000	0.0000	0.4880	0.0000
	Winter	78.2737	0.0000	0.0000	0.0000	4.0244	0.0000	0.3907	0.0000	0.0000	0.0000
Station 8 Middle	Fall	31.9203	25.7979	0.0000	0.0000	6.3075	20.5942	8.3516	5.6395	0.7305	0.6585
	Spring	34.8674	36.5430	0.0000	0.0000	0.0000	4.2562	1.3962	7.4334	0.0000	0.0000
	Summer	17.3815	45.8032	0.4540	0.0000	0.0000	16.0613	3.3952	0.0000	0.0000	0.8479
	Winter	74.2877	0.0060	0.0000	0.0000	4.5397	0.0000	0.4406	0.0000	0.0000	0.0000
Station 8 Bottom	Fall	86.3578	0.8495	0.0000	0.0000	4.3881	0.5169	0.9086	1.8507	0.0000	0.0000
	Spring	37.9819	59.2840	0.6552	0.0000	0.0000	0.0000	0.8659	0.0000	0.0000	1.2051
	Summer	38.1332	50.2505	0.9083	0.0000	0.0000	0.0000	2.9320	0.0000	0.0000	1.3319
	Winter	92.7789	0.0000	0.0000	0.0000	0.0000	0.0000	0.8772	0.0000	0.0000	0.0000



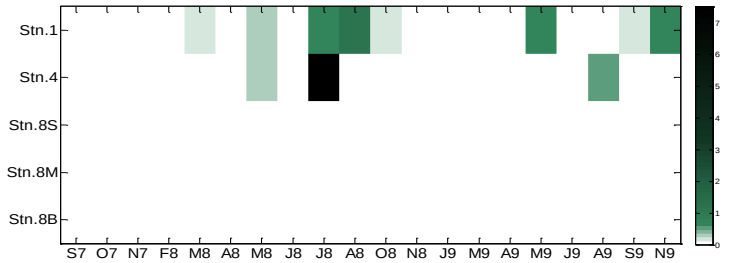
a. Diatoms



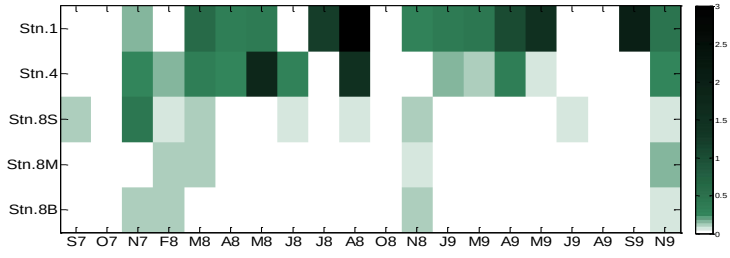
b.Cyanobacteria



c.Dinoflagellate

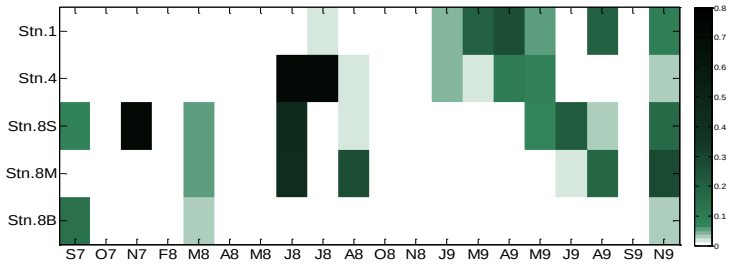


d.Chlorophyte

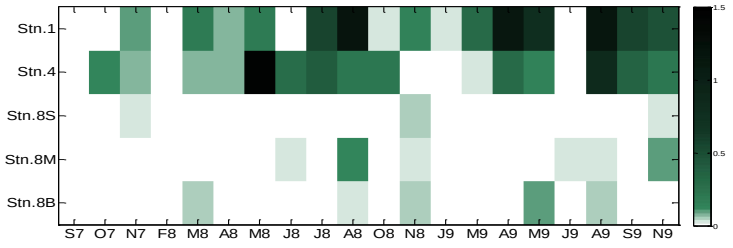


e.Euglenophyte

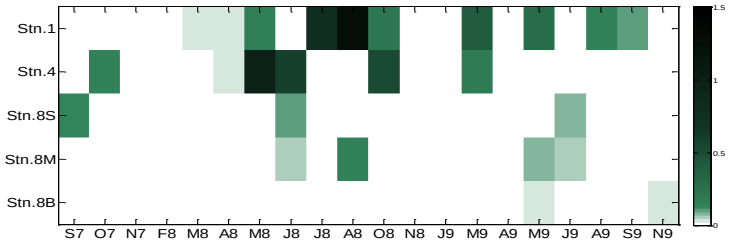
Fig. B.1a. to Fig. B.1e. Phytoplankton group fraction of chl *a* (μgL^{-1}) on each station and each sampling day.



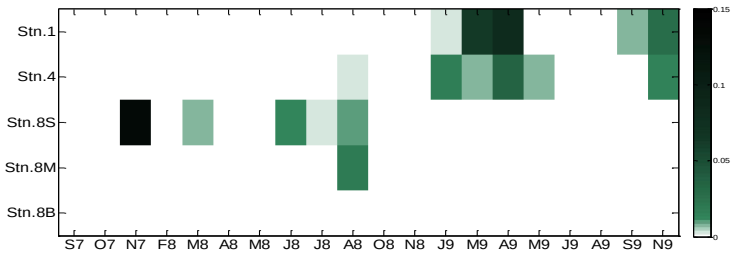
f.Prymnesiophyte



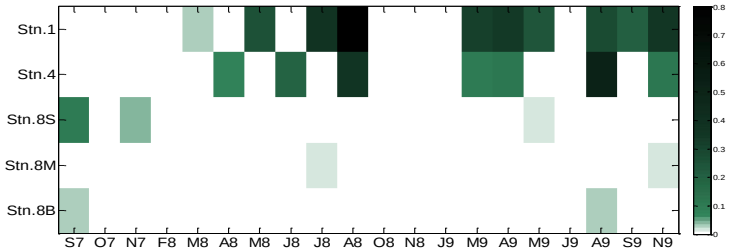
g.Cryptophyte



h.Prasinophyte



i.Chrysophyte



j.Eustigmatophyte

Fig. B.1f. to Fig. B.1j. Phytoplankton group fraction of chl *a* (μg L^{-1}) on each station and each sampling day.

APPENDIX C

ENVIRONMENTAL VARIABLES

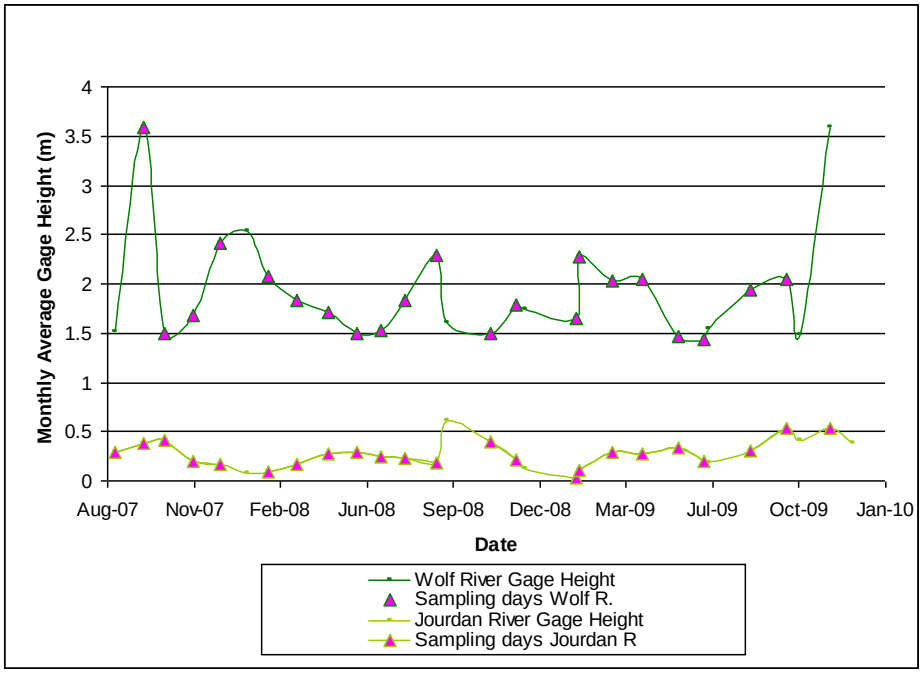


Fig. C.1. Monthly average gage height for the Wolf River and the Jourdan River. Pink triangles indicate sampling days. Refer to sampling stations and environmental parameters stations map (Fig. 2.2). Graph created with data obtained from http://waterwatch.usgs.gov/?m=real&r=ms, and http://waterwatch.usgs.gov/?m=real&r=ms.

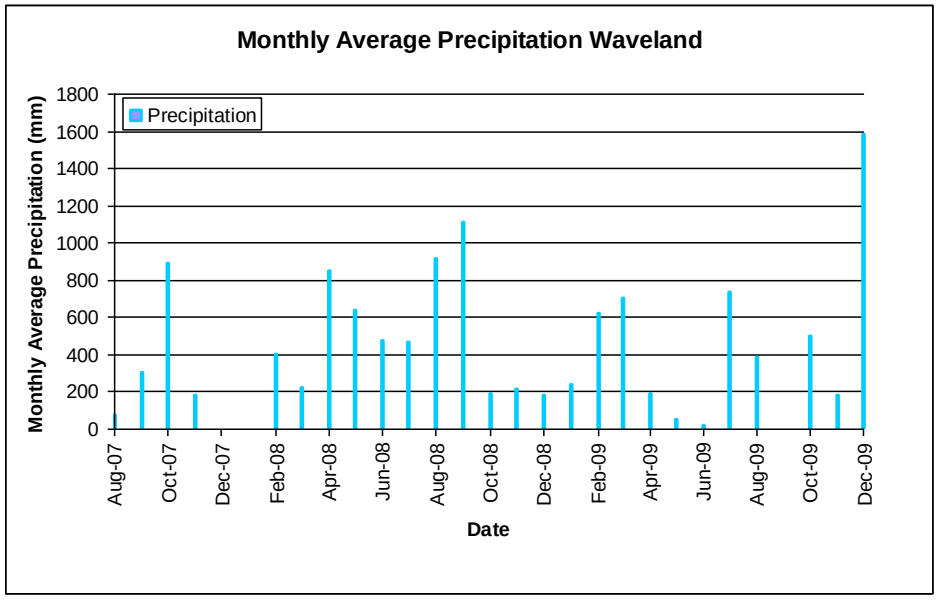


Fig. C.2. Monthly precipitation averages in Waveland, MS. http://www7.ncdc.noaa.gov/IPS/coop/coop.html?_page=2&state=MS&foreign=false&stationID=229426&_target3=Next+%3E

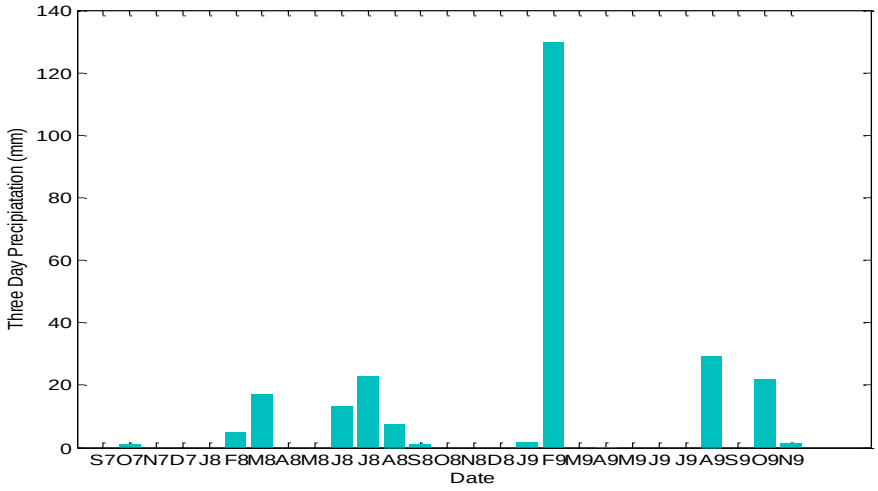


Fig. C.3. Precipitation during sampling dates in Waveland, MS. Each value is equal to the addition of three precipitation days prior to sampling. Refer to sampling stations and environmental parameters stations map (Fig. 2). Graph created with values obtained from http://www7.ncdc.noaa.gov/IPS/coop/coop.html?_page=2&state=MS&foreign=false&stationID=229426&_target3=Next+%3E.

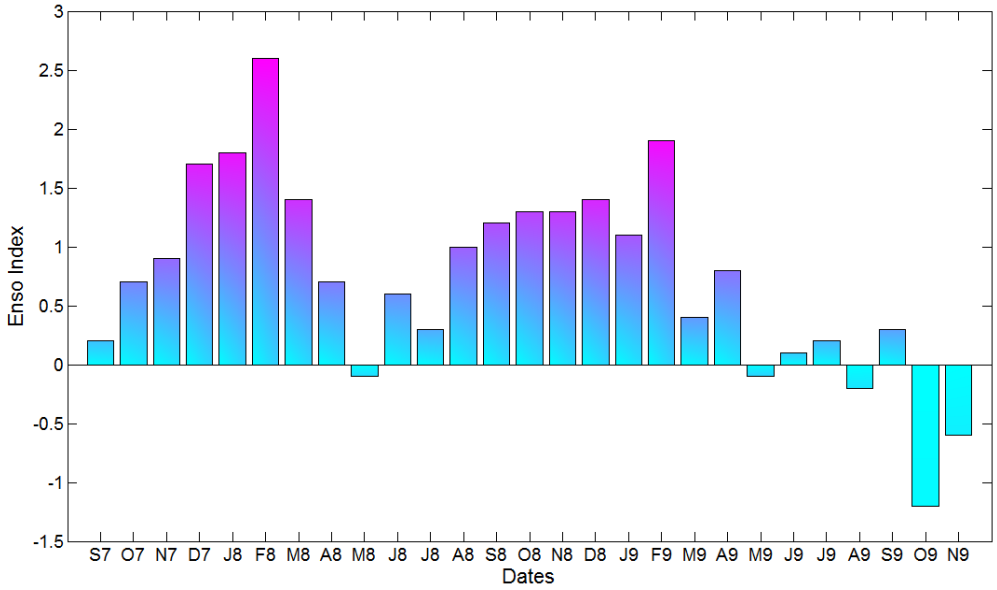


Fig. C.4. Monthly El Niño Southern Oscillation (ENSO) Index. Values obtained from ftp://ftp.cpc.ncep.noaa.gov/wd52dg/data/indices/soi.

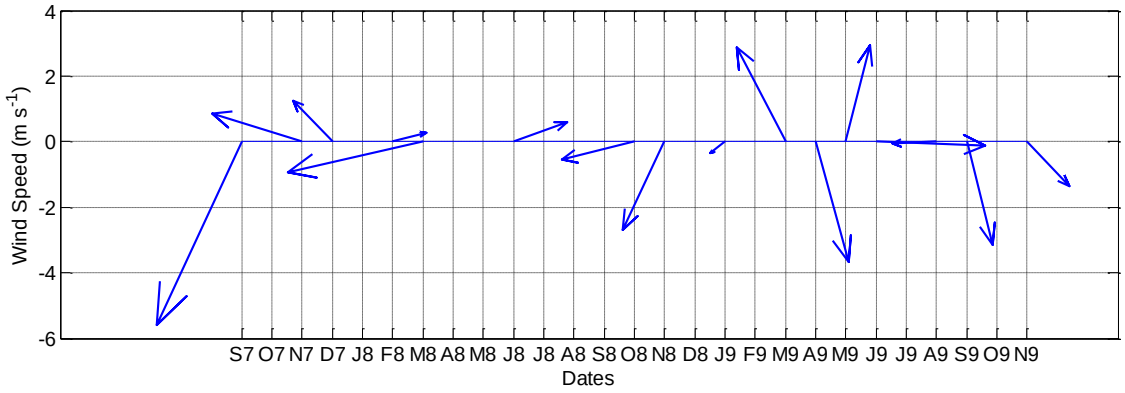


Fig. C.5. Wind Speed and Direction for the three sampling stations. True North is 90º and the length of the vectors is the average speed m s-1. Refer to sampling stations and environmental parameters stations map (Fig. 2).

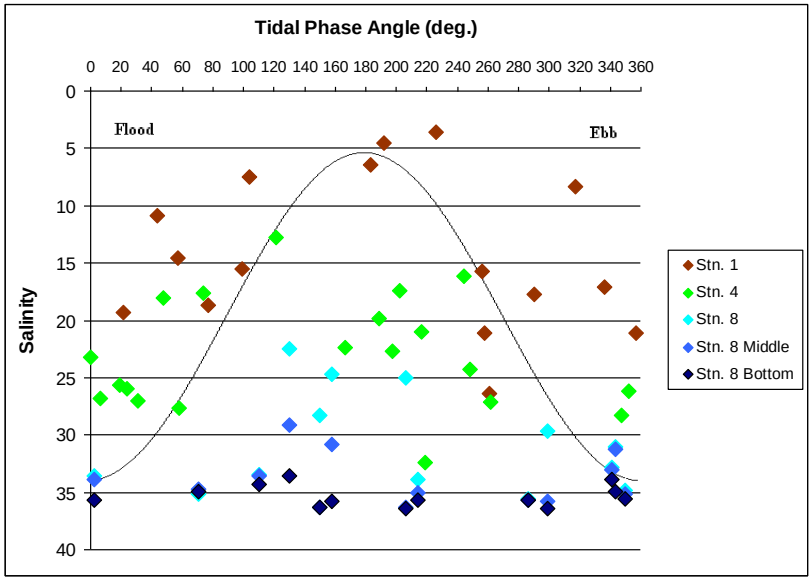


Fig. C.6. Salinity vs. Tidal Phase Angle scatter plot.

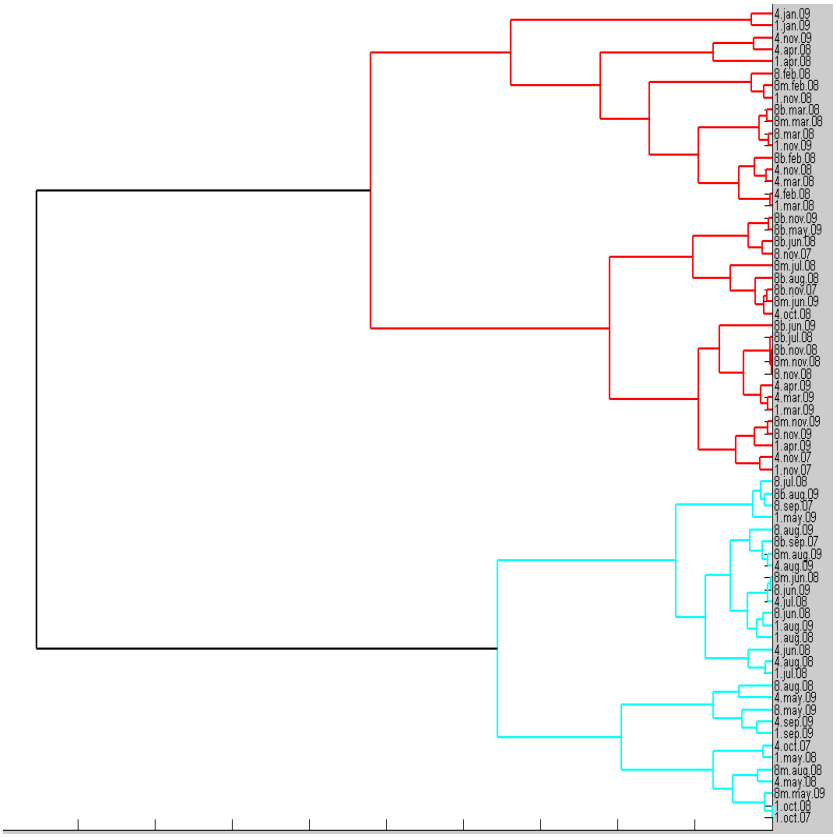


Fig. C.7. Dendrogram clustering samples into two groups, dates above 24ºC and below 24ºC.

Fig. C.8. Density profiles from Station 8.

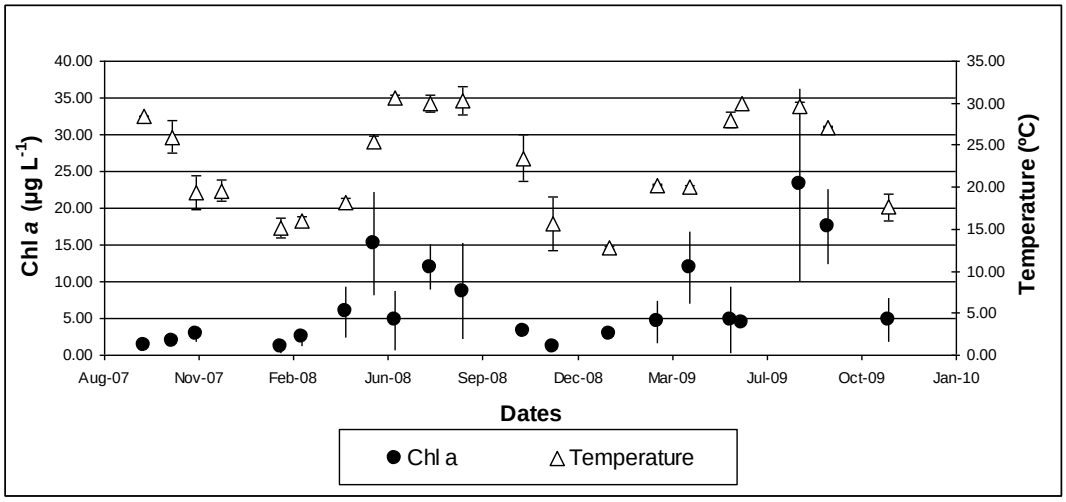


Fig. C.9. Scatter plot of chl a (average surface stations) and Temperature (average surface stations) vs. Date. Error bars are the standard deviations.

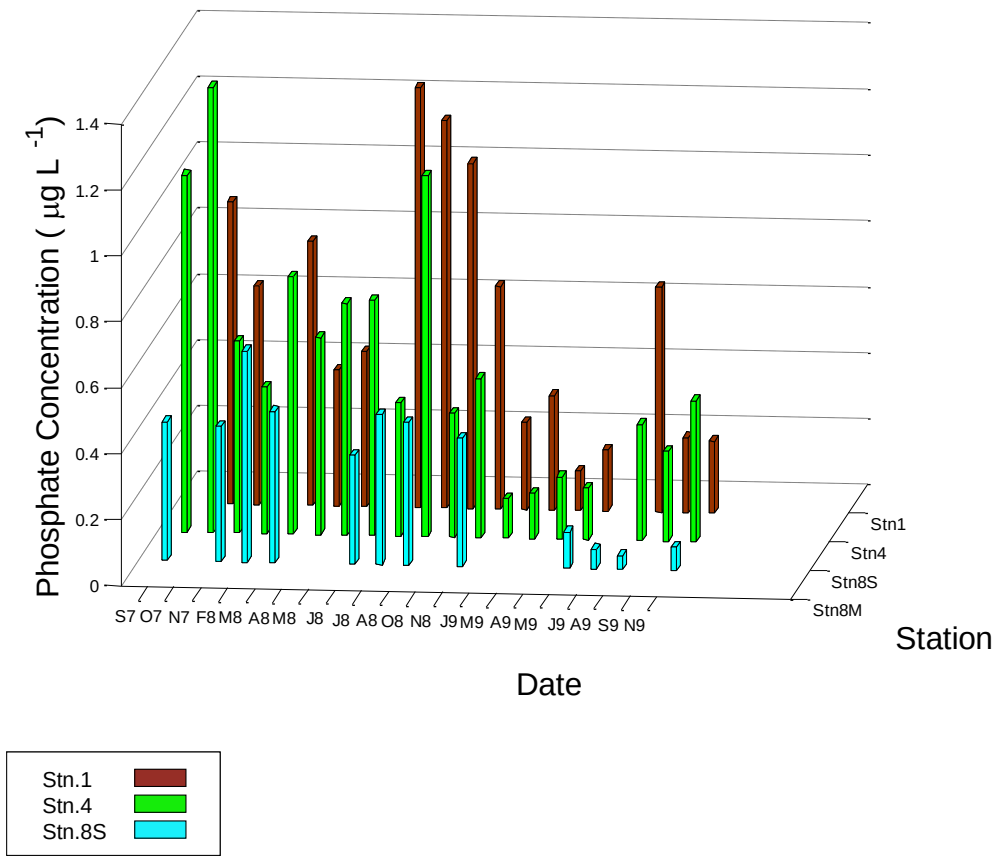
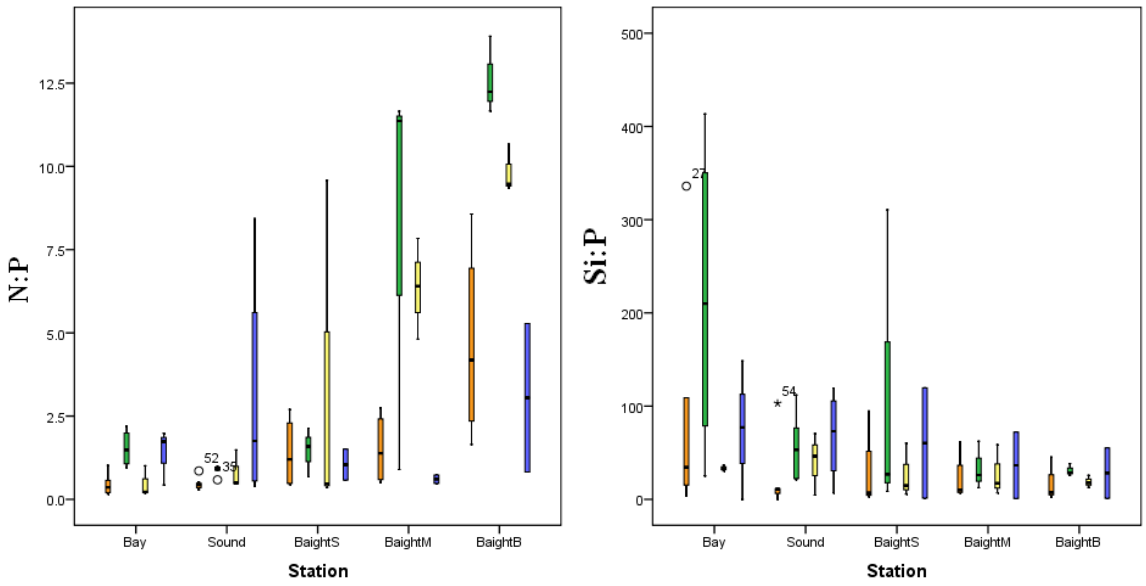


Fig. C.10. Bar graph with Phosphate concentration vs. Date.

a.

b.



c.

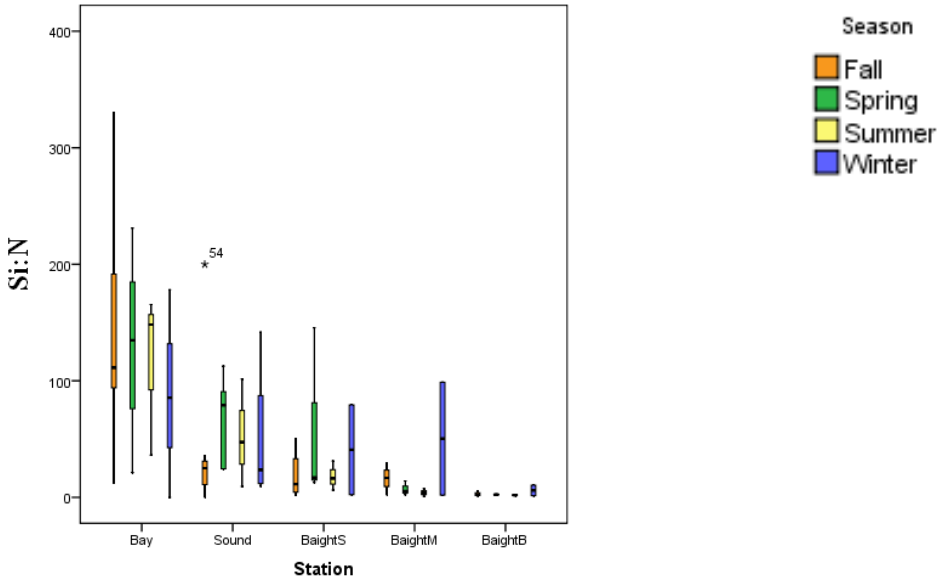


Fig. C.11. Box plots of a. Si:N ratio b. Si:P and c. Si:N in each station per season. The Bay is station 1, the Sound is station 4, Bight S is station 8 Surface, Bight M is station 8 (7m) and Bight B is station 8 at 19m depth.

APPENDIX D

CORRELATION

Table D.1. Two tailed Spearman's Rank Correlation for the phytoplankton group dataset. Only highly significant correlation coefficients (n=68, $p \leq 0.01$) are presented.

	Diat	Cyan	Chlor	Dino	Euglen	Eustig	Prym	Crypt	Pras
Diatoms	1.000								
Cyan	--	1.000							
Chlor	0.558	0.578	1.000						
Dino	0.593	--	--	1.000					
Euglen	0.592	--	0.518	0.514	1.000				
Eustig	--	--	0.611	0.380	0.534	1.000			
Prym	--	--	--	0.444	--	--	1.000		
Crypt	0.677	--	0.618	0.372	0.547	0.427	--	1.000	
Pras	--	--	--	--	--	--	--	0.370	1.000
Chrys	0.356	--	--	0.709	--	--	0.413	--	--
Chl. *a*	0.876	0.595	0.630	0.533	0.570	--	--	0.636	--
Phaeoph. a	0.740	0.395	0.573	0.423	0.442	--	--	0.558	--
Shannon Div.	--	--	0.359	--	0.367	--	0.504	0.437	--
Temp.	--	0.669	--	--	--	--	--	--	0.398
Salinity	-0.717	-0.423	-0.543	-0.446	-0.664	--	--	-0.587	--
Density	-0.676	-0.533	-0.561	-0.445	-0.622	--	--	-0.561	--
DO	0.418	--	--	--	0.503	--	--	--	--
CDOM	0.640	0.350	0.490	0.442	0.582	--	--	0.463	--
Phosphate	--	--	--	--	--	--	-0.508	--	--
Silicate	0.506	--	0.541	0.455	0.442	--	--	0.552	--
Nitrate	--	--	--	--	-0.390	--	--	--	--
Ammonium	--	--	--	--	--	--	--	--	--
Nitrite	-0.476	-0.618	-0.488	-0.442	-0.585	-0.369	--	--	--
DIN	--	--	--	--	--	--	--	--	--
ENSO	--	-0.431	--	--	--	--	--	--	--
Tides	--	--	--	--	--	--	--	--	--
Date	--	--	--	0.365	--	--	--	--	--
Wolf R.	--	--	--	--	--	--	--	--	--
Wind Direction	--	--	--	--	--	--	--	0.219	--

Table. D.1. (continued).

	Chryso	Chl. *a*	Phaeo phytin	Shannon	Tº	Salinity	Density	DO
Chryso	1.000							
Chl. *A*	0.361	1.000						
Phaeoph. a	--	0.737	1.000					
Shannon	--	--	--	1.000				
Temp	--	--	--	--	1.000			
Salinity	--	-0.762	-0.615	-0.360	--	1.000		
Density	--	-0.777	-0.608	-0.421	--	0.943	1.000	
DO	--	0.350	--	--	--	-0.644	-0.545	1.000
CDOM	--	0.659	0.589	--	--	-0.922	-0.915	0.667
PO_4	--	--	--	--	--	--	--	--
Silicate	--	0.545	0.603	--	--	-0.572	-0.601	--
Nitrate	--	-0.398	--	--	--	0.586	0.506	-0.442
NH_4	--	--	--	--	--	--	--	--
Nitrite	--	-0.703	-0.499	-0.373	--	0.683	0.689	-0.401
DIN	--	--	--	--	--	0.532	0.463	-0.418
N:P	--	-0.360	--	--	--	0.523	0.464	-0.528
O:N	--	--	--	--	--	-0.562	-0.481	0.483
ENSO	--	--	--	--	--	--	--	--
Tides	--	--	--	--	--	--	--	--
WS	--	--	--	--	--	--	--	--
WD	--	--	--	--	--	--	--	--
Precipitation	--	--	--	--	--	--	--	--

	Jourdan R.	Wolf R.	WS	WD	Precipitation
Jourdan R.	1.000				
Wolf R.	--	1.000			
WS	--	--	1.000		
WD	-0.450	-0.152	0.550	1.000	
Precipitation	-0.308	0.288	0.139	--	1.000

Table. D.1. (continued).

	PO_4	SiO_3	NO_3	NH_4	NO_2	DIN	N:P	O:N	ENSO
PO_4	1.000								
Silicate	--	1.000							
Nitrate	--	--	1.000						
NH_4	--	--	--	1.000					
Nitrite	--	-0.432	0.435	--	1.000				
DIN	--	--	0.839	0.438	0.450	1.000			
N:P	-0.422	--	0.686	--	0.393	0.697	1.000		
O:N	--	--	-0.839	-0.432	-0.440	-0.992	-0.703	1.000	
ENSO	0.419	--	--	--	--	--	--	--	1.000
Tides	--	--	--	--	--	--	--	--	--
Date	-0.638	--	--	--	--	--	--	--	-0.447
CDOM	--	0.597	-0.547	--	-0.635	-0.486	-0.498	0.522	--

Table D.2. Two tailed Spearman's Rank Correlation for the pigment dataset. Only highly significant correlation coefficients (n=68, $p \leq 0.01$) are presented.

	Alloxanthin	BB	BE	Butfuc	C3	Chl b	Chl c 1+2
BB	0.644	1.000					
BE	0.557	0.409	1.000				
Butfucoxanthin	--	--	--	1.000			
C3	--	--	--	--	1.000		
Chl *a*	0.638	0.948	0.447	--	--		
Chl b	0.714	0.618	0.481	--	--	1.000	
Chl c1+2	0.466	0.763	--	--	--	0.382	1.000
ChlideA	0.457	0.439	0.428	--	--	0.413	0.387
Diadinoxanthin	0.535	0.868	0.433	--	--	0.505	0.813
Diatoxanthin	0.568	0.634	0.442	--	--	0.446	0.617
Lutein	0.712	0.737	0.466	--	--	0.857	0.494
Neoxanthin	0.655	0.619	0.548	--	--	0.766	0.413
Peridinin	--	0.348	--	--	--	--	--
Phaeophorbidea	--	--	--	--	--	--	--
Phaeophytina	0.552	0.664	0.422	--	--	0.573	0.584
Prasinoxanthin	--	--	--	--	--	--	--
Violaxanthin	0.693	0.758	0.470	--	--	0.743	0.500
Zeaxanthin	0.643	0.913	0.397	--	--	0.674	0.595
Density	-0.594	-0.827	--	--	--	-0.664	-0.640
Salinity	-0.598	-0.780	-0.364	--	--	-0.743	-0.529
Temperature	--	0.459	--	--	--	--	--
DO	--	--	--	--	--	0.575	--
CDOM	0.480	0.673	--	--	--	0.618	0.507
PO_4	--	--	--	--	--	--	--
SiO_3	0.570	0.548	0.402	--	--	0.503	0.361
NO_3	--	-0.468	--	--	--	-0.411	--
NH_4	--	--	--	--	--	--	--
NO_2	-0.422	-0.778	-0.358	--	--	-0.590	-0.478
DIN	--	-0.432	--	--	--	--	--
NP	--	-0.373	--	--	--	-0.412	--
ON	--	0.421	--	--	--	0.363	--
WD	--	--	0.409	--	--	--	--
Wolf	0.523	--	--	--	--	--	--
Jourdan	0.046	--	--	--	--	--	--
Precipitation	0.327	--	--	--	--	--	--

Table D.2. (continued).

	Chlide a	Diadino xanthin	Diato xanthin	Lutein	Neo xanthin	Peridinin
Diadinoxanthin	0.511	1.000				
Diatoxanthin	0.554	0.707	1.000			
Lutein	0.521	0.648	0.516	1.000		
Neoxanthin	0.528	0.615	0.539	0.805	1.000	
Peridinin	--	--	--	--	--	1.000
Phaeophorbidea	0.364	--	--	--	--	--
Phaeophytina	0.481	0.620	0.473	0.655	0.543	--
Prasinoxanthin	--	--	--	0.349	0.414	--
Violaxanthin	0.472	0.705	0.578	0.867	0.778	0.373
Zeaxanthin	0.437	0.714	0.448	0.753	0.630	--
Density	-0.497	-0.710	-0.472	-0.738	-0.617	--
Salinity	-0.517	-0.660	-0.490	-0.742	-0.667	--
Temperature	--	0.407	--	--	--	--
DO	0.450	--	--	0.489	0.450	--
CDOM	0.532	0.582	0.353	0.665	0.560	--
PO_4	--	--	--	--	--	--
SiO_3	--	0.453	--	0.646	0.526	--
NO_3	--	-0.348	--	--	--	--
NH_4	--	--	--	--	--	--
NO_2	-0.365	-0.634	-0.360	-0.639	-0.593	-0.357
DIN	--	--	--	--	--	--
NP	--	--	--	-0.380	--	--
ON	--	--	--	--	--	--
Day	--	--	--	--	--	--
WS	--	--	--	--	--	--
WD	--	--	--	--	--	--
Wolf	--	--	--	--	--	--
Jourdan	--	--	--	--	--	--
Precipitation	--	--	--	--	--	--

Table D.2. (continued).

	Phaeophytin a	Prasinoxanthin	Violaxanthin	Zeaxanthin
Phaeophytin a	1.000			
Prasinoxanthin	--	1.000		
Violaxanthin	0.577	0.390	1.000	
Zeaxanthin	0.629	--	0.740	1.000
Density	-0.608	--	-0.664	-0.840
Salinity	-0.615	--	-0.632	-0.793
Temperature	--	--	--	0.426
DO	--	--	--	0.372
CDOM	0.589	--	0.522	0.704
PO_4	--	--	--	--
SiO_3	0.603	--	0.520	0.499
NO_3	--	--	--	-0.527
NH_4	--	--	--	--
NO_2	-0.499	--	-0.686	-0.813
DIN	--	--	--	-0.489
NP	--	--	--	-0.475
ON	--	--	--	0.492
Day	--	--	--	--
WS	--	--	--	--
WD	--	--	--	--
Wolf	--	--	--	--
Jourdan	--	--	--	--
Precipitation	--	--	--	--
Phaeophytina	--	--	--	--
Prasinoxanthin	--	--	--	--

APPENDIX E

ORDINATION

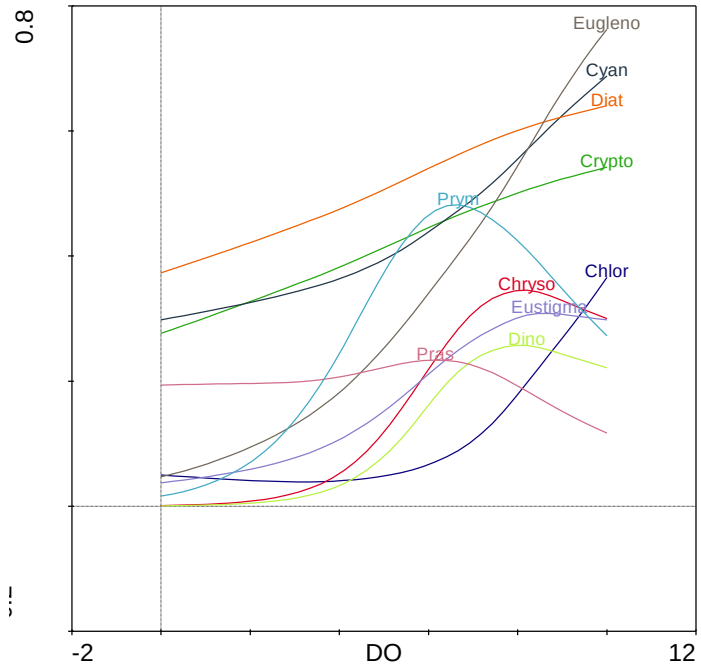


Fig. E.1. Species response curve to Dissolved Oxygen gradient.

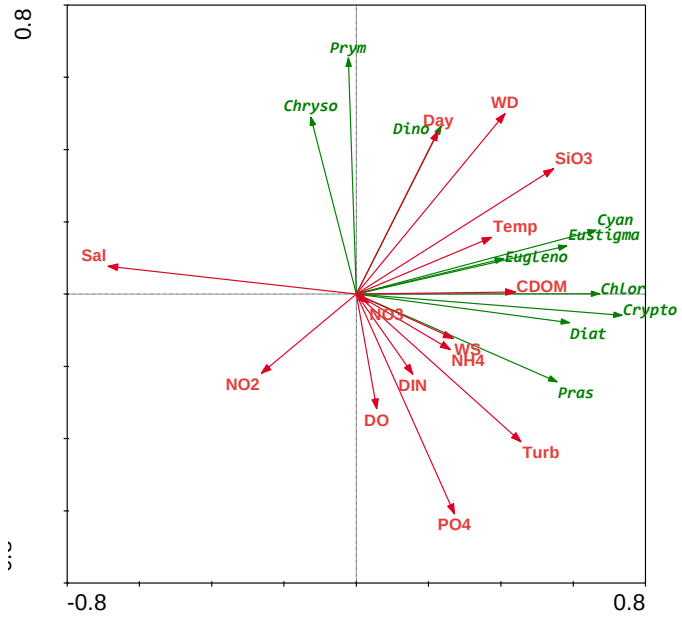


Fig. E.2. Ordination diagram of environmental variables (red arrows) with constrained phytoplankton groups (green arrows) from RDA results with only surface data included.

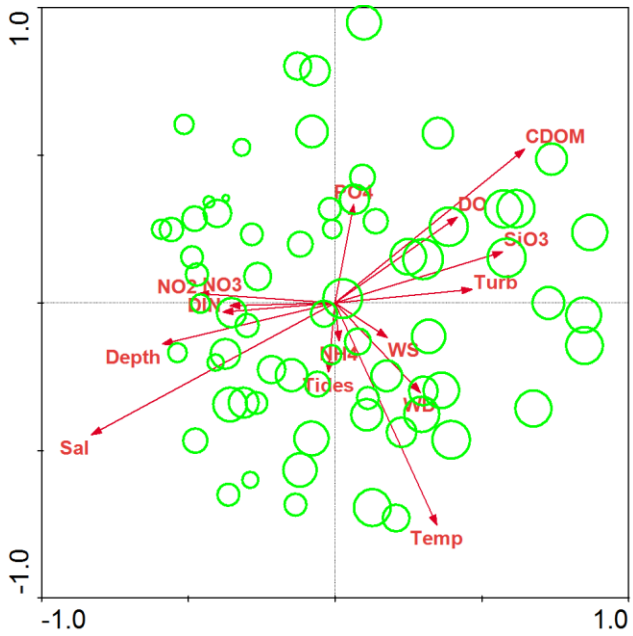


Fig. E.3. Sample-environmental variables biplot with symbol size corresponding to Shannon Diversity Index, red arrows represent environmental variables.

Table E.1. Summary of the canonical redundancy analysis (RDA) for the biological (10 phytoplankton groups) and environmental data derived from 68 samples from coastal Mississippi waters. All four eigenvalues reported in this table are canonical and correspond to exes that are constrained by the environmental variables. Eigenvalues which are equivalent to measures of importance of RDA, axes vary from 0 to 1. The highest values were found in the first two axes which indicate that these axes explained most of the variance of phytoplankton groups with respect to the measured environmental variables. The sum of the first two RDA axes (0.282 + 0.085) explain 36.7% of variability, and the sum of all canonical eigenvalues explain 48.3% of total phytoplankton variability (Lepš and Šmilauer 2003).

Axes	1	2	3	4	Total variance
Eigenvalues :	0.282	0.085	0.064	0.022	1
Species-environment correlations :	0.868	0.791	0.685	0.494	
Cumulative percentage variance					
of species data :	28.2	36.7	43.1	45.3	
of species-environment relation:	58.5	76.1	89.3	93.8	
Sum of all eigenvalues					1
Sum of all canonical eigenvalues					0.483

Table E.2. Summary of the canonical redundancy analysis (RDA) for the pigments measured and environmental data derived from 68 samples from coastal Mississippi waters. All four eigenvalues reported in this table are canonical and correspond to exes that are constrained by the environmental variables. At the bottom summary of Monte Carlo test with 499 permutations.

Axes	1	2	3	4	Total variance
Eigenvalues :	0.381	0.067	0.031	0.019	1
Species-environment correlations :	0.868	0.777	0.674	0.718	
Cumulative percentage variance					
of species data :	38.1	44.8	48	49.9	
of species-environment relation:	70.9	83.4	89.3	92.9	
Sum of all eigenvalues					1
Sum of all canonical eigenvalues					0.537

Summary of Monte Carlo test	
Test of significance of first canonical axis: eigenvalue =	0.381
F-ratio =	32.604
P-value =	0.0060
Test of significance of all canonical axes : Trace =	0.537
F-ratio =	4.394
P-value =	0.0060

Table E.3. Marginal and Conditional Effects from the forward selection in environmental variables related to pigment concentration.

Marginal Effects			Conditional Effects				
Variable	Var.N	Lambda1	Variable	Var.N	LambdaA	P	F
Sal	3	0.28	Sal	3	0.28	0.01	25.26
CDOM	5	0.18	Temperature	2	0.09	0.02	9.64
Depth	1	0.14	SiO_3	12	0.03	0.02	3.52
SiO_3	12	0.13	Turbidity	6	0.02	0.108	2.17
Turbidity	6	0.1	CDOM	5	0.02	0.058	1.98
Temperature	2	0.09	DIN	11	0.02	0.26	1.81
NO_2	10	0.09	WD	14	0.01	0.03	1.97
DO	4	0.08	PO_4	7	0.02	0.112	1.79
DIN	11	0.06	WS	13	0.02	0.064	1.92
NO_3	9	0.06	NO_2	10	0	0.418	1
WD	14	0.04	DO	4	0.01	0.668	0.72
WS	13	0.02	Depth	1	0.01	0.738	0.66
PO_4	7	0.02	NH_4	8	0	0.764	0.63
Tides	15	0.01	Tides	15	0.01	0.64	0.61
NH_4	8	0.01					

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