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Estuarine phytoplankton response to annual and manipulated river inputs

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ESTUARINE PHYTOPLANKTON RESPONSE TO ANNUAL AND MANIPULATED RIVER INPUTS

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ABSTRACT

River water entering estuaries affects the physical and chemical environment at irregular intervals creating a highly dynamic aquatic habitat. Phytoplankton are important primary producers in estuaries that respond quickly to their changing environment. Since 1991, Breton Sound estuary in southeast Louisiana has been directly influenced by Mississippi River water through the Caernarvon Freshwater Diversion structure. Over a 12 month period, the phytoplankton response was examined, in terms of biomass, abundance, community composition and potential phycotoxin production to seasonal changes in river input into the estuary. Within this 12 month period, a short pulse study was also carried out to examine the immediate response of phytoplankton to pulsed river input. Chlorophyll *a* (chl *a*) measurements estimated phytoplankton biomass and light microscopy identified phytoplankton abundance and community composition. Phycotoxins were measured using ultra-sensitive ELISA. During the seasonal study, chl *a* and cell abundance concentrations exhibited an inverse relationship with river input rates. Mean chl *a* concentrations were 79.1$\pm$38.7 and 55.2$\pm$48.5 µg chl *a* l^{-1} for low and high river input, respectively. Phytoplankton cell abundance concentrations averaged $7.5x10^5 \pm 6.7x10^5$ cells l^{-1} and $1.2x10^5 \pm 2.5x10^5$ cells l^{-1} during low and high river input, respectively. The community was dominated by cyanobacteria for most of the year, corresponding to higher temperatures. For the rest of the year, cyanobacteria decreased while chlorophytes and centric diatoms increased to approximately equal contributions. Chlorophytes dominated during the entire pulse study, however, cyanobacteria increased during high river input. Over both studies, the phytoplankton community composition was most commonly moderated by salinity and nutrient availability. Salinity, temperature and nutrient availability primarily influenced phytoplankton biomass and abundance during the seasonal study. The distance from the diversion and phosphate availability were the most important factors influencing phytoplankton biomass and abundance during the pulse study. Microcystins (MCs) were detected throughout the seasonal and pulse studies, ranged from below detection to 2.92 µg MCs l^{-1} and were

highest during low river input and toward the outer estuary. The detection of MCs in Breton Sound estuary illustrates a potential risk to human health and economically important estuarine food webs.

CHAPTER 1

INTRODUCTION

Estuaries are important coastal environments subject to natural and anthropogenic changes on scales of days to tens of thousands of years. These coastal systems occur where the confluence of fresh and marine environments create a salinity gradient from the inner to outer estuary. Estuaries of the Gulf of Mexico, which are typically shallow systems with easily mixed water columns, are further characterized by low tidal energy. In the summer months, high solar radiation and increased water temperatures can create strong stratification. Spring is a time of high river flow due to increased precipitation and snowmelt. This nutrient rich freshwater pulsed into estuaries affects turbidity, water temperature, salinity, and nutrient concentrations and ratios at irregular intervals, creating a highly dynamic habitat (Day et al. 1983, Lane et al. 1999). Estuaries are highly productive ecosystems containing numerous primary producers that contribute to secondary production (Nielson et al. 2004). Phytoplankton can be especially important basal resource to higher trophic levels in some estuaries and their growth and abundance can determine the potential productivity of the entire ecosystem (Wissel and Fry 2005). Phytoplankton are unicellular, pelagic primary producers with communities composed of numerous species with a broad range of physiological responses to their environment (Pinckney 1999). Due to short generation times, phytoplankton can respond quickly to changes in their aquatic environment and changes in phytoplankton may indicate when significant physical, chemical or biological changes occur in the water column (Hotzel and Croome 1999).

Nutrients are a required resource for phytoplankton. Nutrient availability is essential for phytoplankton growth and reproduction (Harris 1996, Sparthis et al. 2007). Therefore, when nutrients are available in the water column at non-limiting concentrations and all other environmental conditions are favorable, phytoplankton growth is not restricted and changes in the community composition can

occur. Subsequently, alterations in nutrient availability and stoichiometery can affect the biomass and community composition (Garcia-Soto et al. 1990, Justić et al. 1995, Piehler et al. 2004, Buyukates and Roelke 2005). The sources of these nutrients in coastal ecosystems are both natural and anthropogenic, including benthic resuspension, upwelling, increased nutrient loading within the watershed from increased fertilizer use, surface runoff, precipitation, and nutrient loaded river water.

Rivers are an important source of nutrients to coastal and estuarine systems, with the concentration of nutrients in river water often directly related to land use activities within their watershed (Hecky and Kilham 1988, Cloern 1996, Rabalais 2002a and references therein). In recent decades, the amount of nutrients entering estuaries has increased significantly around the world from land use activities associated with increases in agriculture, industry, and population growth (Justić et al. 1995, Nixon 1995, Smith et al 1999). This increase has resulted in increased phytoplankton growth, increased hypoxia, and shifts in the phytoplankton community that lead to harmful algal blooms (HABs) in many coastal and estuarine systems (Justić et al. 1995, Glibert et al. 2005, Rabalais et al. 2002b). River nutrient delivery to the coastal zone is considered a direct route of eutrophication into these areas (Rabalais et al 2002a and references therein).

The Mississippi River and its watershed containing large amounts of land use associated with agricultural fields for either crop production and/or pasturing livestock is a prime example of how changes in river nutrients impacting the coastal zone. The Mississippi River, which contains high concentrations of essential nutrients, such as nitrogen (N) and phosphorus (P), required for phytoplankton growth and algal bloom formation, achieves peak flow during the spring due to snowmelt and surface runoff into its tributaries (Turner et al. 1994, Snedden et al. 2007, Hyfield et al. 2008). As a result, phytoplankton blooms occur in late spring through early fall in the receiving estuaries from increased nutrient delivery, with seasonal community composition shifts commonly observed (Murrell et al. 2007, Thronson et al. 2008, Costa et al. 2009).

Near the mouth of the Mississippi River, a deltaic plain forms one of the most extensive coastal wetland landscapes in North America created by the river switching course over the past 6000 years (Coleman et al. 1998). Several coastal basins have been created from the river flooding its extensive floodplain and forming large associated wetlands. In recent years, these river pulsed events have been limited by construction of levees that were completed in the mid-1900's to protect increased industry and population in Louisiana's estuarine and coastal system from flooding. Levees prevent natural flooding of sediment rich waters into the wetlands.

In the twentieth century, 25% of Louisiana coastal wetlands were lost, partially due to the prevention of sediment delivery to deltaic coasts (Day et al. 2009). Water control structures have been installed to reestablish the connection between the river and estuaries. River diversion structures, a specific type of water control structure, are designed to control introductions of sediment and nutrient rich river water into specific coastal basins to reduce salinity intrusions and promote wetland productivity (Delaune et al. 2008). Two large diversion structures, the Caernarvon Diversion structure and the Davis Pond Diversion structure, have been operating in southeastern Louisiana for the past two decades controlling the input of nutrient loaded freshwater into Breton Sound estuary and Barataria Bay estuary, respectively.

The biomass and community composition of phytoplankton in Breton Sound and Barataria Bay estuaries are dependent on changes in temperature and by factors that are highly influenced by river input controlled by the diversions; nutrient availability, salinity, and water mixing. Water column mixing and salinity are also influenced by seasonal wind and weather patterns (Swenson et al. 2006, Snedden et al. 2007). These forces act to resuspend sediments, increasing nutrient availability for phytoplankton growth, but also reducing light availability. Fluctuations in chlorophyll *a* (chl *a*) concentrations in portions of the estuary adjacent to the diversion structure are strongly related to river input throughout the year (Day et al. 2009, Reynolds 2006, Jöhnk et al. 2008). Studies have also shown

that certain phytoplankton groups proliferate, specifically cyanobacteria, during summer months when water temperatures increase due to additional sunlight and nutrients decrease from plant and algal uptake (Jöhnk et al. 2008, Paerl and Huisman 2008). Potentially toxic cyanobacterial genera, such as *Anabaena*, *Microcystis*, and *Cylindrospermopsis*, have been frequently observed in Louisiana estuaries (Dortch et al. 1999, Dortch et al. 2001, Rabalais 2005, Garcia et al. 2010). The phycotoxins they produce can contaminate the food web when ingested by primary consumers. Cyanobacterial phycotoxins have already been detected in water and blue crab tissues from Barataria Bay estuary during the summer (Garcia et al. 2010). In Breton Sound estuary, cyanobacteria have been observed (Day et al. 2009), but specific toxic genera have not been noted, nor have toxin measurements been performed.

Phycotoxins produced by phytoplankton may serve single or multiple purposes for the cell. There are a number of hypotheses set forth to explain why different phycotoxins are produced including; grazer deterrent, infochemicals, metal detoxification, and allelopathy (Van Dolah 2000, Sukenik et al. 2002, Jang et al. 2003, Pohnert 2008, Poulson et al. 2009). Cellular toxin production can be affected by changes in environmental conditions such as light availability, temperature, and nutrient availability and ratios (Huisman and Hulot 2005, Vézie et al. 2002). However, not all species produce toxins in all conditions or locations. The toxin-producing species present in an ecosystem determine the type and nature of phycotoxins that could potentially be detected. Some groups of phytoplankton contain several species that are capable of producing toxin, while other groups contain species that are capable of producing several toxins. Changes in nutrient concentrations and ratios can cause shifts in phytoplankton community assemblages that could favor groups containing more toxin producing species over other groups (Sivonen and Jones 1999).

The three most prevalent phycotoxins produced by cyanobacteria are microcystins (MCs), anatoxins, and cylindrospermopsin. Of these, MCs are the most commonly detected and well-studied phycotoxins in fresh and brackish waters (Sivonen 1996, Chorus and Bartram 1999, Sivonen and Jones

1999). MCs are a group of hepatotoxic (affecting the liver) cyclic peptides produced by several species, including (but not limited to) *Microcystis* and *Anabaena*, and have been detected in blue crab and catfish tissues from the upper Barataria estuary (Garcia et al. 2010). Numerous studies have also reported detecting MCs in other aquatic organisms commonly found in estuaries (i.e. fish, crustaceans, benthic invertebrates and shellfish, Ibelings and Havens 2007 and references therein, Galván et al. unpublished data). MC production is influenced by various physical and chemical variables (Sivonen and Jones 1999 and references therein). Tonk et al. (2007) observed that salinity fluctuations in brackish water, such as estuarine systems, favored MC-producing species over other freshwater phytoplankton species, but also resulted in increased dissolved MC concentrations measured in waters with a salinity greater than 10‰ (Tonk et al. 2007).

Louisiana estuaries act as important nurseries and habitat for economically important species of fish, crustaceans and shellfish. The largest amount of menhaden, oyster, and blue crab in the nation, as well as a large portion of commercially harvested shrimp, originates from these estuaries and these fisheries are a large part of the state's economy. The productivity of these fisheries is based on the energy provided by lower trophic levels. The phytoplankton response in estuaries impacted by diversion structures needs closer examination. Specifically, determining how altered river input influences shifts in community composition and potential phycotoxin production is of great interest (Dortch et al. 1999, Turner and Streever 2002, Lane et al. 2007, Day et al. 2009). This is especially important considering that numerous phycotoxin producing species have been observed in Louisiana waters (Dortch et al. 1999, Dortch et al. 2001, Rabalais 2005). The production of phycotoxins not only affects fisheries, but also places a potential risk on human health as Louisiana's fish and shellfish are consumed by millions of people. While the concentration of toxins previously measured in Louisiana is not alarming, observations of sub-lethal chronic exposure of MCs suggest that they are tumor promoters and possible carcinogens (Falconer et al. 1999, Ito et al. 1997, Grosse et al. 2006). This emphasizes the need for more

monitoring of the distribution and concentrations of such toxins in relation to important fisheries species.

Each year when river levels on the Mississippi River rise due to increased precipitation and snowmelt, diversion structures are used to alleviate flooding pressure and mimic seasonal flooding to adjacent estuaries. River input rates into the estuary are increased, which affects the environmental conditions. These conditions typically occur during the cooler portions of the year (November to April). Salinity decreases in the upper estuary while TSS increases in the immediate vicinity to the diversion structure (Lane et al. 2007). Because the river water is loaded with N and P, these resources become more available within the estuary during times of high river input (Lane et al. 1999, 2004, Hyfield et al. 2008). Water residence times also decrease with high river input while turbulence and water column mixing increase (Hyfield et al. 2008, Day et al. 2009). However, when river levels decrease, usually during warmer months (May to October), these parameters exhibit a reverse response. Salinities increase, TSS decreases and nutrient availability decreases as it is removed or assimilated into the estuarine ecosystem, water residence times increase and turbulence and mixing decrease (Lane et al. 1999, 2004, Hyfield et al. 2008, Day et al. 2009). Aside from changes in river input rates, seasonal changes in temperatures also occur. Temperatures are typically lower during high river input, which occurs during the cooler part of the year, and increase during warmer months when river input is low. Research on the effects of these changing variables for specific phytoplankton groups and individual species, especially harmful algal species, throughout the year within Louisiana estuaries has yet to be fully investigated. Because freshwater diversion structures can be controlled to mimic natural seasonal flow patterns into the estuary, they can also be used to create experimental conditions to determine how the phytoplankton community would respond to maximum flow rates of river input or significantly different input patterns than are typically observed.

In Breton Sound estuary, the Caernarvon freshwater diversion structure is the most influential factor on the physical and chemical environment within the estuary. Extended periods, from weeks to months, of either high or low river input are expected to promote different community assemblages based on the environmental tolerances of the species present in this estuary. Large pulses of nutrient rich river water entering the estuary, which is generally considered nutrient limited, could cause increases in phytoplankton biomass. However, high river input through the structure would decrease residence time of open water areas, large channels, and bayous and could advect phytoplankton toward the open bay and higher salinities. This changing habitat will likely favor certain groups of phytoplankton over others at different times of year and under different river input rates (Day et al. 2009).

During this study, the diversion structure in Breton Sound estuary was used as a manipulation tool to determine the response of the phytoplankton community and potential phycotoxin production to different river input rates and the associated changes they affect on the environmental parameters. This research examines the response of the phytoplankton community, in terms of biomass, cell abundance and community composition, to varying environmental parameters (i.e., nutrients, salinity and mixing) based on the rate of river input entering the estuary. Based on preliminary observations of MC-producing genera, sometimes in bloom conditions, within Louisiana estuaries, MCs are also expected in Breton Sound estuary in response to changing environmental conditions, including nutrient availability, temperature and salinity. Additionally, it was predicted that concentrations of MCs would reflect changes in cyanobacterial cell abundances within the estuary. This examination of the phytoplankton community will identify the toxic cyanobacteria genera and phycotoxins present and the conditions that potentially favor their production in Breton Sound estuary.

CHAPTER 2

MATERIAL & METHODS

Study Site: Breton Sound Estuary

Breton Sound estuary was chosen to examine seasonal and pulse changes in phytoplankton biomass, total phytoplankton cell abundances, and shifts in phytoplankton community composition in response to changes in environmental parameters determined by diverted river input. Breton Sound estuary is located southeast of New Orleans, Louisiana and is composed of approximately 1100 km^2 of fresh and brackish wetlands with several large lakes throughout the upper estuary and opens up into Breton Sound coastal waters (Fig. 2.1). Breton Sound estuary receives Mississippi River water via the Caernarvon freshwater diversion structure at the northernmost point of the estuary (open arrow, Fig. 2.1). The diversion structure is one of the largest in southern Louisiana and discharges freshwater into the estuary at a maximum rate of approximately 226 m^3s^{-1}, but has averaged approximately 54 m^3s^{-1} since 2001 (http://waterdata.usgs.gov). The discharge rate is regulated according to river height to simulate natural seasonal flow trends, but also to manage the salinity in portions of the estuary. Intermittent pulsing up to the maximum flow from December to June occurs to benefit oyster production and sediment delivery to the estuary. The operation of the diversion structure during the study period was consistent with previous years of diversion operation based on the discharge volume per year.

The salinity gradient is influenced by river water input, as well as seasonal wind and weather patterns (Swenson et al. 2006, Snedden et al. 2007). River water diverted into Breton Sound estuary is the major source of nutrients and suspended sediments, all other sources are negligible, including atmospheric and groundwater input (Hyfield et al. 2008). The rate of river input (discharge, cfs^{-1}) through the Caernarvon Diversion structure was accessed from the USGS National Water Information System: Web Interface (Site #: 295124089542100, Hydrologic Unit #: 08090203,

http://waterdata.usgs.gov) and converted to the appropriate metric units. Daily water temperature data was also retrieved from the USGS National Water Information System: Web Interface (http://waterdata.usgs.gov) at three stations (Fig. 2.1), one each from the inner (Reggio Canal near Will's Point, LA), middle (Crooked Bayou near Delacroix, LA) and outer estuary (Black Bay near Point-a-la-Heche, LA). The three stations were averaged to achieve the daily mean water temperature for the estuary for each day samples were collected during the seasonal and pulse studies. Mean daily air temperatures were retrieved from the National Weather Service station at the New Orleans International Airport in Kenner, LA (http://www.weather.gov/data/obhistory/KMSY.html).

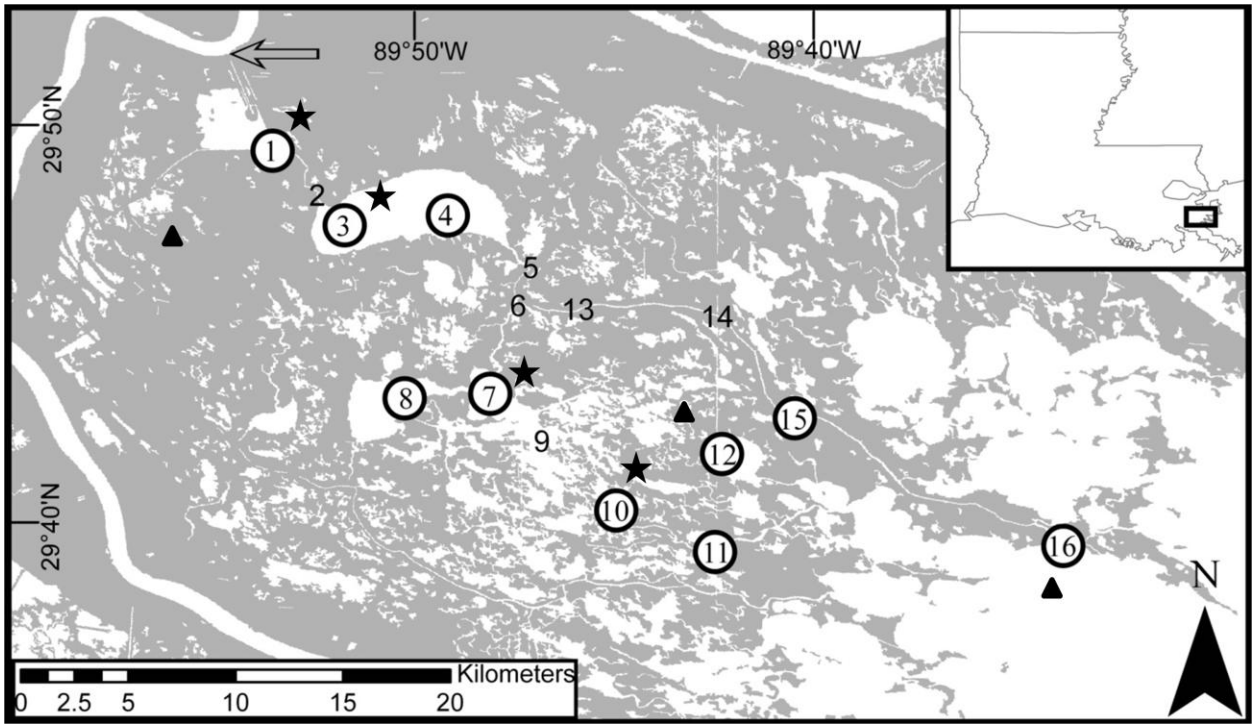

Figure 2.1. Breton Sound estuary, southeast Louisiana. Numbers indicate stations along the sample transect. Circled numbers signify stations sampled for this research. Stations without a star were sampled for the seasonal portion of this research. A star denotes additional stations sampled for the manipulation portion of this research. The open black arrow indicates the diversion structure on the Mississippi River. Closed triangles represent USGS sites that were used to determine the daily average water temperature in the estuary during the study period.

Methods

Two studies were conducted to accomplish the stated objectives. A seasonal study over the course of 12 months from September 2007 to August 2008 to explore the influence of seasonal

variations in river input on the phytoplankton community. And a pulse study to specifically investigate the immediate influence of pulsed river input on the phytoplankton community by manipulating the river input rates at the diversion structure. The purpose of the pulse study during the seasonal study was also to separate out the affects of seasonal influences from the influences of river input.

Field Sampling

For the seasonal study, surface water samples were collected approximately once every month between September 2007 and August 2008 at 6 stations along a transect composed of 16 stations that were located from near the diversion structure at the top of the estuary to the mouth of the navigation channel at the lower end of the estuary (Table 2.1, Fig 2.1). The distance of each station from the diversion structure by flow path was determined by laying a string on a current map of Breton Sound estuary between the diversion and each station following the most direct water route. The distance was then compared to the map scale. The 6 stations used in this study were selected to represent the gradient of environmental conditions from the inner to outer estuary. Sampling days occurred at various river input rates ranging from 0 to 180 $m^3 s^{-1}$. The collected water samples were kept dark and cold while being transported to the laboratory within a few hours for processing. At each station, salinity was collected using a hand held YSI. Additional water samples were also collected to characterize the physicochemical conditions of the study site.

For the pulsed study, surface water samples were collected twice during both low and high river input conditions between March 17, 2008 and April 3, 2008 from 10 stations (Table 2.1, Fig. 2.1) along a transect composed of 16 stations that were located from near the diversion structure at the top of the estuary to the mouth of the navigation channel at the lower end of the estuary. Six of the 10 stations (4, 8, 11, 12, 15, and 16) were chosen for sampling along the salinity gradient, while the remaining four stations (1, 3, 7, and 10) were chosen to increase sample representation in the upper estuary and create a

more representative sampling regime of the entire estuary. Water samples were collected from each station on the third and tenth day after the river input rate was decreased to capture the response of the phytoplankton community at the beginning and toward the end of the low river input period (Table 2.2, Fig. 2.2). Subsequently, water samples were collected on the third and ninth day after the river input rate was increased to determine the changes in the phytoplankton community from the beginning to the end of the high river input period. The water samples were stored and transported identical to the monthly samples. Salinity was also collected with a hand held YSI and additional water collected to determine the physicochemical characteristics for each station during the period of river input pulse.

In late March through early April 2008, the river input rate was manipulated to allow for sampling to determine the phytoplankton response to pulsed river input (Table 2.2). On March 14, 2008, the discharge rates were lowered from 224 m^3s^{-1} to approximately 23 m^3s^{-1} and remained at this rate through March 24, 2008. The discharge rate was then increased to greater than 200 m^3s^{-1} on March 25, 2008 and held at this rate for the remainder of the short term study period.

Table 2.1. Coordinates for stations sampled in Breton Sound estuary and the distance of each from the diversion structure by flow.

Station	Latitude	Longitude	Distance from Diversion (km)
1	29.8225°N	-89.8929°W	4.4
3	29.7913°N	-89.8628°W	9.7
4	29.7954°N	-89.8197°W	14.9
7	29.7209°N	-89.8020°W	23.3
8	29.7190°N	-89.8378°W	27.4
10	29.6721°N	-89.7494°W	30.6
11	29.6548°N	-89.7081°W	35.8
12	29.6957°N	-89.7052°W	35.8
15	29.7107°N	-89.6748°W	32.6
16	29.6573°N	-89.5626°W	44.7

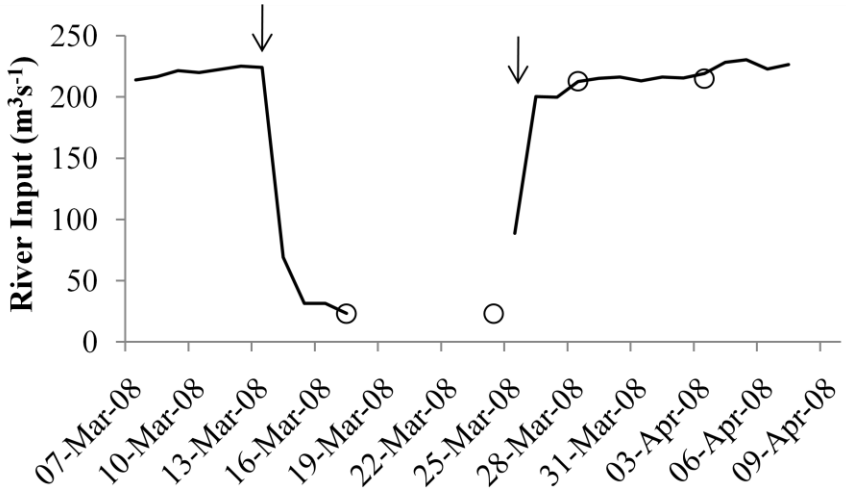

Figure 2.2. River input rate during the pulse study. Arrows indicating when river input rate was adjusted. Open circles indicate sampling days. Missing data due to instrument malfunction.

Table 2.2. Sampling of Breton Sound estuary and manipulation of river input conditions at the Caernarvon Freshwater Diversion structure during the pulse study period.

Date	**Event**
March 14, 2008	Discharge rate was decreased from 224 m^3s^{-1} to approximately 23 m^3s^{-1}
March 17, 2008	1st Low river input sampling
March 24, 2008	2nd Low river input sampling
March 25, 2008	Discharge rate was increased to >200 m^3s^{-1}
March 28, 2008	1st High river input sampling
April 3, 2008	2nd High river input sampling

Laboratory

Sample Processing

The water surface samples from each station were processed immediately on delivery to the laboratory. A portion of the water samples were preserved using 50% gluteraldehyde to yield a final concentration of 2% gluteraldehyde and then stored at 4°C until analyzed to determine the community composition. For particulate (within the cell) and dissolved (extra-cellular) phycotoxin measurements, additional portions of the water were vacuum filtered through GF/F filters. From 15 to 500 ml of water or as much as could pass through up to three GF/F filters was filtered for each replicate of particulate toxin. For each replicate, the filters were stored in a 2 ml microcentrifuge tube at -20°C until analyzed.

A portion of the filtrate from each station was collected and stored in a 50 ml plastic centrifuge tube at -20°C until analyzed for dissolved toxin.

The environmental parameters (temperature, salinity, TSS, DIN, P and distance from the diversion structure by flow) for both the seasonal and manipulated study periods and the seasonal chlorophyll *a* (chl *a*) were analyzed by a collaborating lab as part of a monthly monitoring program. These parameters were examined to determine if they influenced the phytoplankton biomass, community composition or harmful phytoplankton abundance specifically. Chl *a*, which was used as a proxy for phytoplankton biomass, was measured for the seasonal study according to EPA Method 445 (USEPA 1997a) utilizing acidification. Chl *a* for the pulse study was also measured using this method, however, sonication was used in place of the tissue grinder. The environmental parameters measured include nitrogen (N), phosphorus (P), silica (Si), and total suspended solids (TSS). Multiple forms of N were measured, including ammonia (NH_4) according to EPA Method 350.1 (USEPA 1993a) and nitrate plus nitrite (NO_2+NO_3) according to EPA Method 353.2 (USEPA 1993b). Phosphate was measured as phosphate according to EPA Method 365.1 (USEPA 1993c). Silica was measured according to EPA Method 366.0 (USEPA 1997b). Additionally, due to the introduction of turbid riverine waters into the estuary and the general shallow depth of the estuary, TSS was also measured as an indicator of mixing using APHA Method 2540D (APHA 1999).

Cell Enumeration and Community Composition Analysis

A gridded Sedgwick-Rafter slide (Wildco) was used to examine two 1 ml replicates of water from each gluteraldehyde preserved archive sample at 400X on an Axio Observer - A1 inverted microscope (Zeiss). In preparation for examination, each archive sample was uniformly mixed by inversion of the sample container for approximately 1 min before the 1 ml subsample was loaded onto a Sedgwick-Rafter slide. The sample was then allowed to settle for 30-45 min before examination began.

Solitary cells and colonies/chains whose cells were easily differentiated were enumerated as individual cells. When colonies were too dense to count individual cells, the number of colonies was counted and up to 10 were photographed using AxioVisionLE V4.6.1.0 camera software (Zeiss). The surface area of each photographed colony was measured with the camera software to achieve an average surface area per colony. This was done for colonies of the cyanobacteria genus *Aphanothece*, *Eucapsis*, *Microcystis*, and *Merismopedia*. The average surface area per colony was multiplied by the number of colonies counted to estimate colonial surface area per liter. Subsequently, to convert colonial surface area per liter to cells per liter, up to 100 colonies of each genus where individual cells could be counted were measured for surface area and individual cells were enumerated to create a linear equation of cells per surface area for each genus. The equation for each genus was then used to calculate cells per liter from the total surface area per liter. These cell numbers were added to each specific genus' cell abundance so they could be included in the total cyanobacterial cell abundance per liter. Additionally, the length of filaments of the cyanobacteria genus *Planktolynbya* were measured and divided by the average cell length to achieve cells per liter, which were then added to the total cyanobacteria cell abundance per liter.

To determine the abundance and composition of the phytoplankton community for each replicate, up to 20-1 mm^2 grids were examined and cells/colonies were enumerated and categorized into the following major groups: centric diatoms, pennate diatoms, cyanobacteria, chlorophytes, dinoflagellates, and flagellates. All of these groups are taxonomic, with the exception of flagellates. The flagellate group is a functional group composed of small (<5µm) autotrophic, heterotrophic and mixotrophic plankton. Potentially harmful or toxin producing phytoplankton were also identified to at least genus level and their abundances recorded for each replicate. The cells and colonies observed were used to calculate the cells per liter for each replicate.

Phycotoxin Measurements

Preliminary examinations of the phytoplankton community of Breton Sound estuary indicated that cyanobacteria species producing the phycotoxin microcystins (MCs) were the most frequently observed group in the estuary and MCs would be the most likely toxin detected. Therefore, to measure both particulate and dissolved MC, the recently developed Enzyme-Linked Immunosorbant Assay (ELISA) was utilized. ELISA is a competitive binding assay that is highly sensitive for MCs with a detection limit of 0.10 µg l^{-1} based on the most common variant, MC-LR, and its congeners.

To measure particulate MC, an extraction process was determined to allow for the detection of the phycotoxin with the purchased MC-ADDA ELISA kits (Abraxis, LLC, USA). A spike and recovery experiment following the extraction protocol produced extraction efficiencies of 93% for particulate MC. This involved adding MC-LR standard (Abraxis, LLC) directly to a clean GF/F filter to accomplish a final concentration of 7.5 µg l^{-1} in the extraction volume and then extracting as described below. For the particulate samples, the glass fiber filters from each replicate were allowed to reach room temperature and then placed in a 15 ml round bottom glass centrifuge tubes with 5 ml of a 50% methanol, 1% acetic acid extraction solution. Glass vials and pipettes were used as much as possible to reduce the risk of MC being lost by absorption to plastic as been found to occur by Codd and Bell (1996) and others (Metcalf et al. 2000, Hyenstrand et al. 2001a, Hyenstrand et al. 2001b). The filters and solution were then vigorously swirled using a vortex (Fisher Scientific) for 1 min and sonicated using a Misonix Sonicator 3000, equipped with a microtip, for 2 min at 30-40W to ensure that the cells on the glass fiber filter were ruptured to release MC into the extraction solution. This solution was then centrifuged for 10 min at 3000 RPM with a relative centrifugal force of 1399 g in an IEC Centra CL2 centrifuge (Thermo Electron Corp.). The supernatant was collected and passed through a 0.20 µm syringe filter (Corning) with a surfactant free cellulose acetate (SFCA) membrane filter into a 7 ml scintillation vial. An additional 5 ml of extract solution was added to the already homogenized filter in

each 15 ml centrifuge tube and the process of vortexing, sonicating and centrifuging was repeated. The resulting supernatent was filtered and collected into the same scintillation vial used to collect the previous supernatant volume for a total extraction solution volume of up to 7 ml. Samples for the measurement of dissolved toxins were directly passed through a 0.20 µm syringe filter with a SFCA membrane filter and collected in a 7 ml scintillation vial. All the processed samples were temporarily stored at -4°C until needed for analysis.

All samples were analyzed following the protocol included in the ELISA kit (Abraxis, LLC). The samples were analyzed in duplicate at a dilution of 1:10 to eliminate possible false positives from the methanol extraction solution (Beattie et al. 1998 and Metcalf et al. 2000). Additionally, if samples analyzed were found to be greater than the range of the assay, they were diluted to 1:100 and reanalyzed. The absorbance data for each sample was collected using a micro-plate spectrophotometer set at a wavelength of 450 nm.

Statistical Analyses

The seasons were determined using mean daily air temperature measurements and the daily discharge values over the entire study period accessed from the USGS National Water Information System: Web Interface (http://waterdata.usgs.gov). The warm season was determined to include the period from approximately September to the end of October 2007 and from the beginning of May thru August 2008 that was characterized by consistently warmer temperatures and low river input, while the cool season included November 2007 thru April 2008 and high river input. Student's t tests using the transformed (LogX+1) environmental parameter data were performed to determine if there were significant differences between seasons, for the seasonal study, or between river input conditions, for the short term study. If any assumptions were not met, a Mann-Whitney Rank Sum test was utilized. Analysis of variances (ANOVAs) were performed on the transformed (LogX+1) chl *a*, total and group

cell abundances, and particulate and dissolved MC data to determine if there was a significant difference in these biological responses between the seasons or with distance from the diversion structure, for the seasonal study, and between river input conditions, for the short term pulse study. Where significant differences were found between treatments, Tukey tests were use for pairwise comparison. Additionally, when data failed any assumptions, a Kruskal-Wallis ANOVA on ranks test was utilized and where statistically significant differences were found, Dunn's Method was used to further analyze the pair wise comparisons; in such cases these methods are specified. To determine the influence of environmental parameters on chl *a* and total and group cell abundances in both the seasonal and pulse studies, multiple linear regression (MLR) of the transformed (LogX+1) data was also performed. The independent parameters included temperature, salinity, DIN, P, TSS and distance from the diversion structure.

PRIMER (v6, PRIMER-E Ltd) is a statistical program used frequently for analyzing communities and environmental parameters. The Bio-Env procedure in PRIMER was used to determine which environmental parameters best explained the biological patterns of the phytoplankton community composition among the samples under different seasons and river input conditions. This procedure selects the subset of environmental parameters that gives the maximum rank correlation (Spearman, ρ_s) between the resemblance matrixes of the transformed group abundance and transformed and normalized environmental parameters as outlined in Clark and Ainsworth (1993). A further permutation analysis of the Bio-Env procedure was used to determine if the correlation coefficient achieved was significantly different from the coefficients achieved by randomly permutating the samples relative to each other and running the Bio-Env procedure 99 additional times.

CHAPTER 3

RESULTS

Seasonal Study

Environmental Parameters

River input through the Caernarvon freshwater diversion structure ranged from zero to 242 m^3s^{-1} during the study period from September 2007 to August 2008 (Fig. 3.1). River input rates were significantly higher during the high river input period (102 m^3s^{-1}), compared to the low river input period (20.1 m^3s^{-1}, Mann-Whitney Rank Sum test, $P<0.001$). Breton Sound estuary contains fresh to brackish water with salinities ranging from 0.1 to 18.9‰ and increasing from the inner to outer estuary (Fig. 3.2a). The variation in salinity values also increased from the inner to outer estuary. TSS concentrations were variable throughout the sampling period and appear to increase with increasing distance from the diversion structure (Fig. 3.2b). Neither salinity nor TSS concentrations were significantly different between high and low river input. Water and air temperatures followed a seasonal pattern; however, air temperatures were more variable than water temperatures (Fig. 3.3).

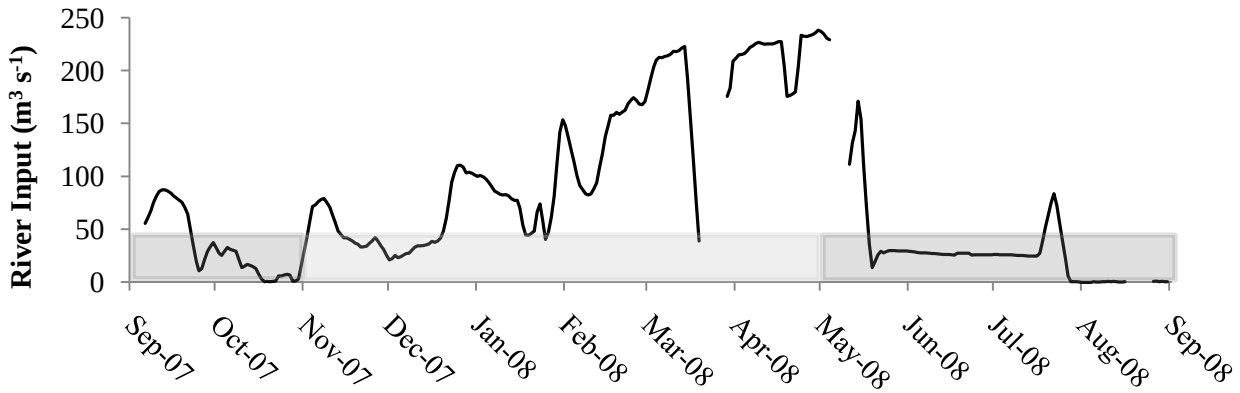

Figure 3.1. The five day averaged river input rate for the Caernarvon freshwater diversion structure from September 2007 to August 2008. The light and dark shaded areas indicate the high and low river input periods, respectively. Missing data due to instrument malfunction.

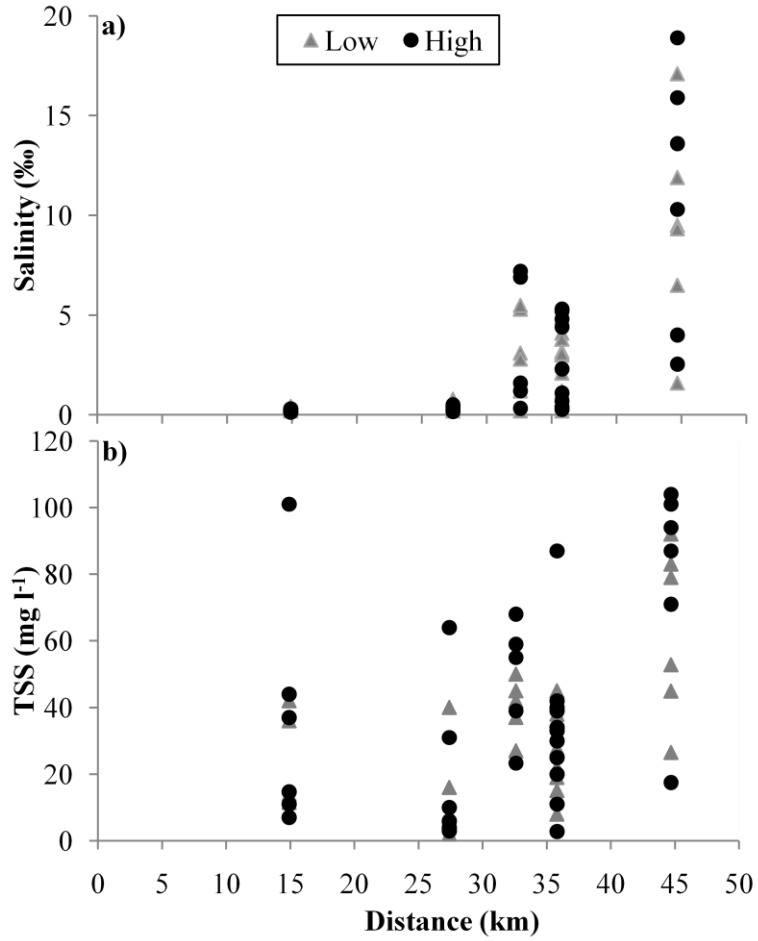

Figure 3.2. Salinity (a) and TSS (b) values measured in Breton Sound estuary with increasing distance from the diversion structure by flow for September 2007 to August 2008. Triangles and closed circles indicate sample values from the low and high river input periods, respectively.

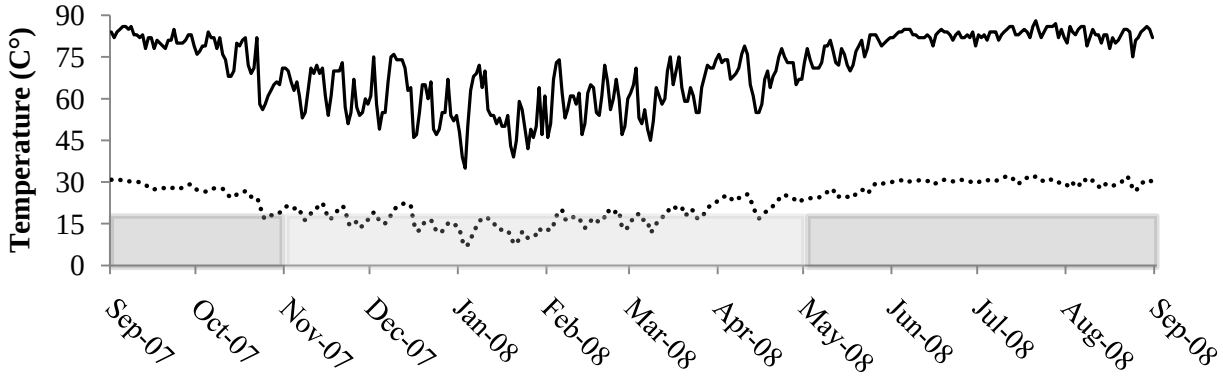

Figure 3.3. Daily mean water (dotted line) and air (solid line) temperature values for Breton Sound estuary for September 2007 through August 2008. The light and dark shaded areas indicate the high and low river input periods, respectively.

During the study period, DIN concentrations averaged 25.5 ± 36.9 µM and ranged from 1.0 to 158.6 µM with the highest values occurring toward the inner estuary (Fig.3.4a). DIN concentrations were significantly higher in the high river input compared to the low river input ($P<0.001$, Mann-Whitney Rank Sum test). Phosphate concentrations ranged from 0.3 to 3.8 µM, with an average of 1.4 ± 0.8 µM during the study period (Fig. 3.4b). Significant differences in P concentrations between high and low river input were not found. Since DIN concentrations decreased with distance from the diversion structure while P concentrations remained fairly consistent, the DIN:P ratio also decreased toward the outer estuary, more strongly during low river input (Fig. 3.4c). DIN:P ratio values ranged from 0.5 to 53.2 during the seasonal study period and were significantly higher during high river input compared to low river input ($P<0.001$, Mann-Whitney Rank Sum test). However, decreasing DIN concentrations toward the outer estuary during high river input along with variable, but consistent P concentrations across the estuary caused large variation the high river input DIN:P ratio values. This resulted in an average of 17.1 ± 17.1 over the whole estuary during the seasonal study period; a ratio value just above the Redfield ratio of N:P of 16 (Redfield 1958).

Biological Response

Phytoplankton biomass within the estuary was estimated by chl *a* concentrations during the 12 month study period (chl *a* measurements were missed for October 2007). Chl *a* concentrations varied from 5.5 to 242.5 µg chl *a* l^{-1} from September 2007 to August 2008 with an annual estuary wide average of 66.2 ± 45.5 µg chl *a* l^{-1} (Fig. 3.5a). During the seasonal study period, the highest chl *a* concentration (242.5 µg chl *a* l^{-1}) occurred in November 2007. Chl *a* concentrations were significantly higher during low river input (79.1±38.7 µg chl *a* l^{-1}) compared to high river input (55.2±48.5 µg chl *a* l^{-1}, $P=0.005$, Mann-Whitney Rank Sum test, Fig. 3.5a). Additionally, monthly average chl *a* concentrations were inversely correlated with river input rates over the entire seasonal study period (Fig. 3.6).

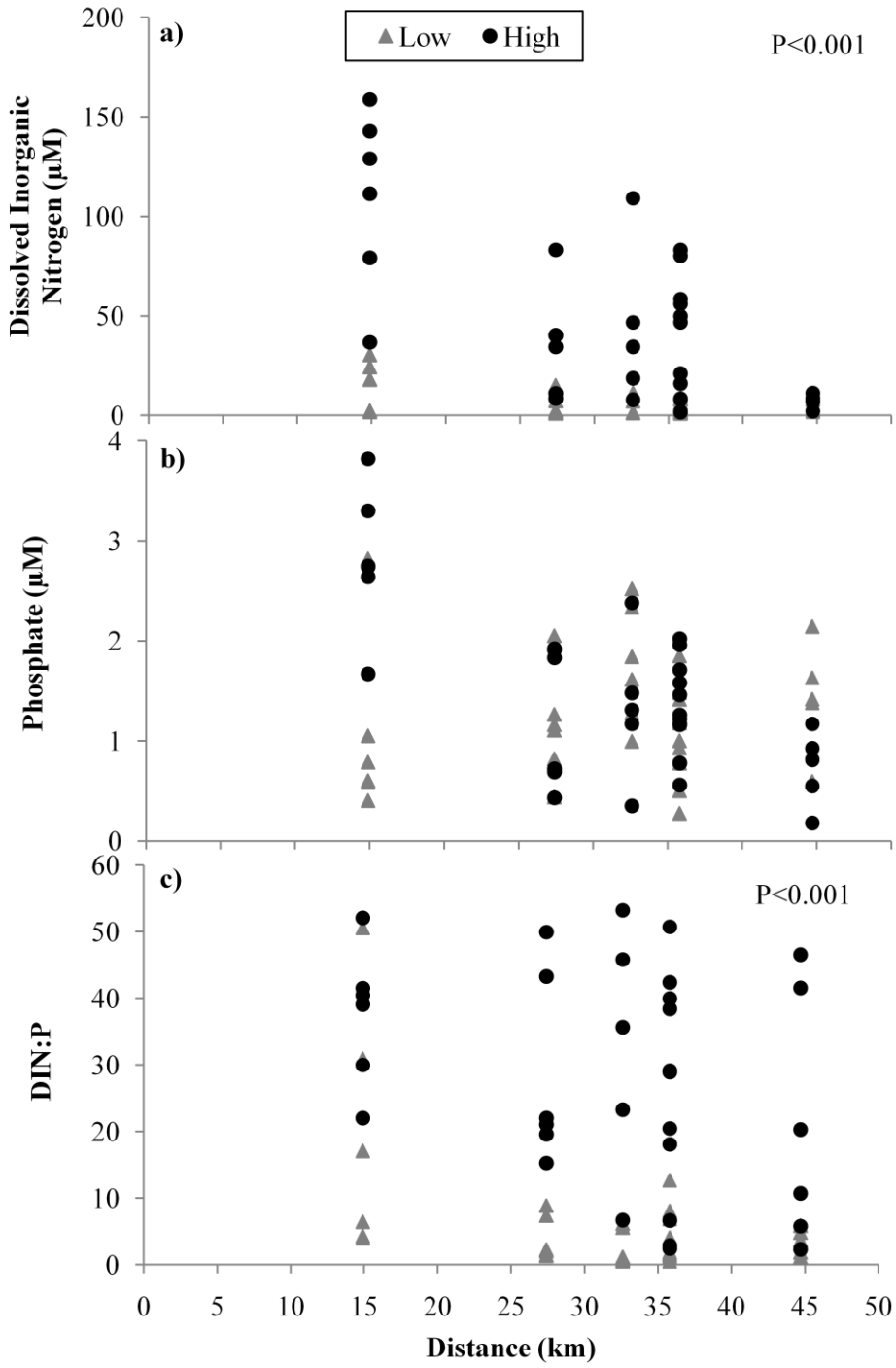

Figure 3.4. DIN (a), P (b) and DIN:P ratios (c) for each sample collected from September 2007 to August 2008 in relation to the distance from the diversion structure by flow in Breton Sound estuary. The triangles and closed circles represent samples collected from the low and high river input periods, respectively.

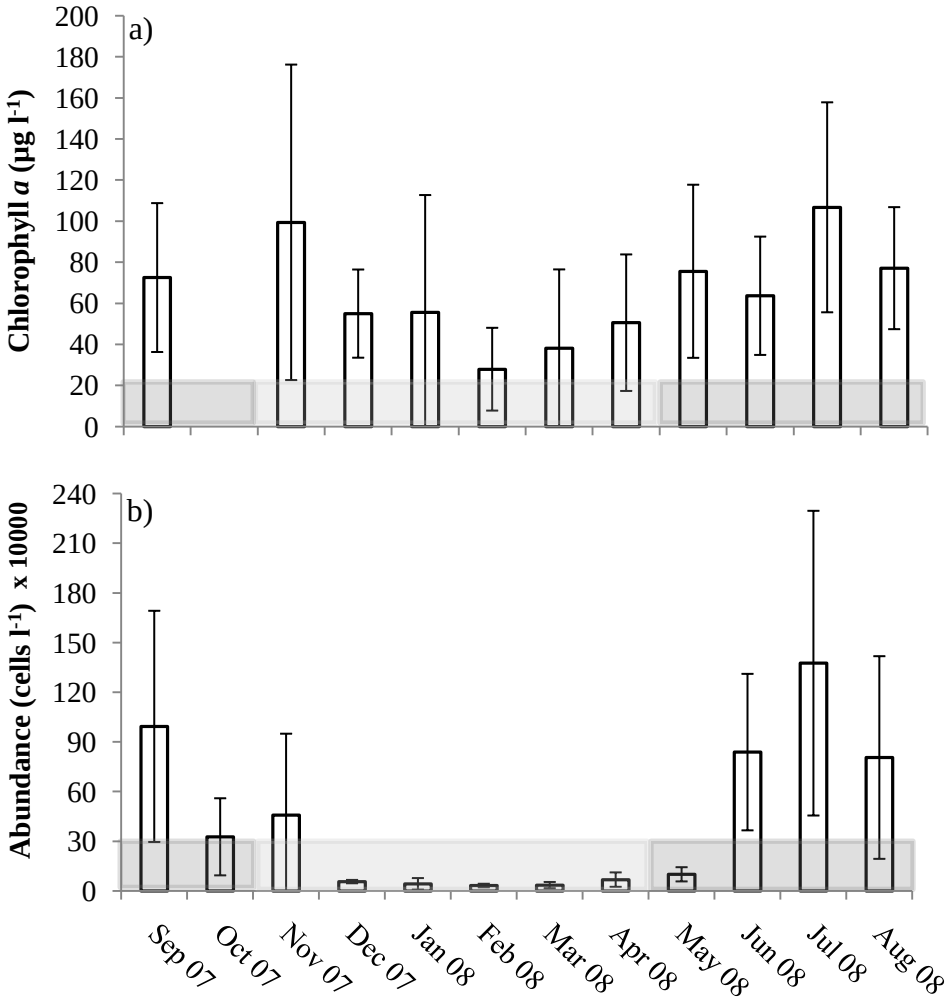

Figure 3.5. Mean monthly chlorophyll *a* concentrations (a) and total phytoplankton cell abundance concentrations (b) for Breton Sound estuary during the seasonal study period from September 2007 to August 2008. The light and dark shaded areas indicate the high and low river input periods, respectively. Error bars represent one standard deviation.

Among the stations sampled, the annual mean chl *a* concentrations were only significantly different between station 11 (89.1 $\pm$ 38.3 µg chl *a* l^{-1}) toward the outer estuary and station 4 (46.0 $\pm$ 45.9 µg chl *a* l^{-1}, P=0.015) in the inner estuary (Fig. 3.7), all other station annual mean chl *a* concentrations fell between these values, except station 12 (Fig. 3.7). Station 12 toward the outer estuary had the lowest mean chl *a* concentration of all stations (45.5 $\pm$ 38.2 µg chl *a* l^{-1}), but was not significantly different due to variation among the samples for each station.

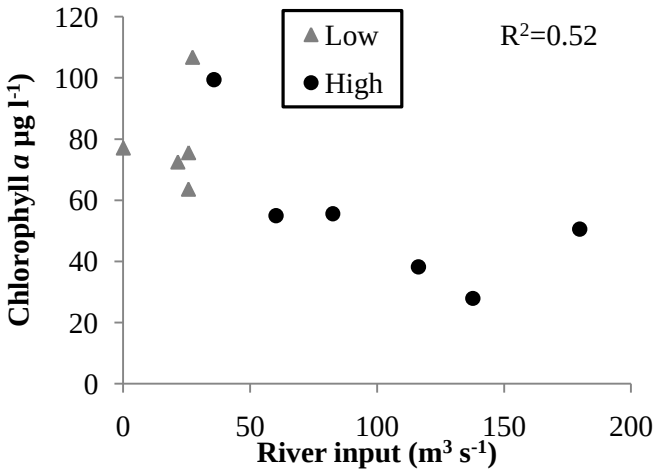

Figure 3.6. Relationship between river input rate and mean monthly chlorophyll *a* concentrations for September 2007 to August 2008. R^2=0.52, n=11. The triangles and closed circles represent samples collected from the low and high river input periods, respectively.

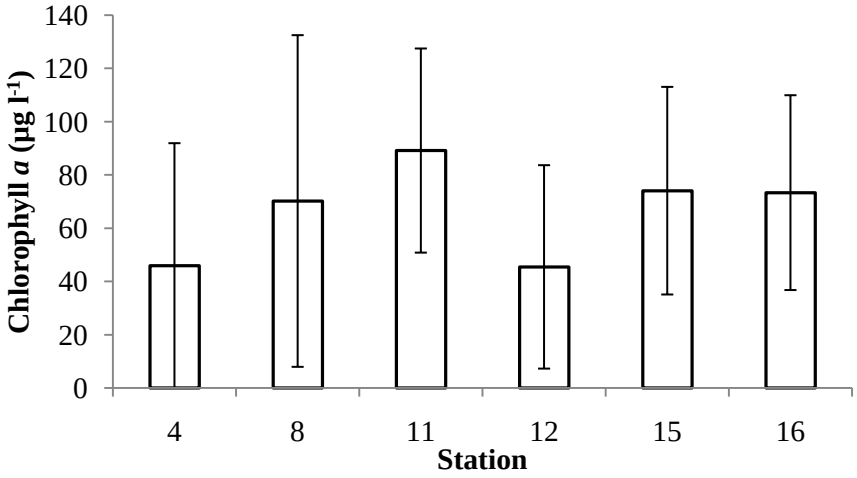

Figure 3.7. Mean chlorophyll *a* concentrations with error bars representing one standard deviation for each station sampled in Breton Sound estuary from September 2007 to August 2008.

Phytoplankton cell abundance concentrations ranged from $1.6x10^4$ to $2.7x10^6$ cells l^{-1} with an average concentration of $4.3x10^5 \pm 6.1x10^5$ cells l^{-1} and generally followed the same temporal trends as chl *a* concentrations, but with less variability (Fig. 3.5b). Overall, significantly higher mean phytoplankton cell abundance concentrations were observed in the months with low river input ($7.5x10^5 \pm 6.7x10^5$ cells l^{-1}) compared to the months with high river input ($1.2x10^5 \pm 2.5x10^5$ cells l^{-1}, $P<0.001$, Fig. 3.5b, 3.8). Low river input was also associated with higher temperatures. Due to variation among the samples for each station, there were no significant differences in the phytoplankton cell

abundance concentrations between stations (Fig. 3.9). Both chl *a* concentrations and total cell abundances had an inverse pattern in relation to the rate of river water coming into the estuary; concentrations and abundances decreased when river input was high and increased when river input was low (Fig. 3.1, 3.5).

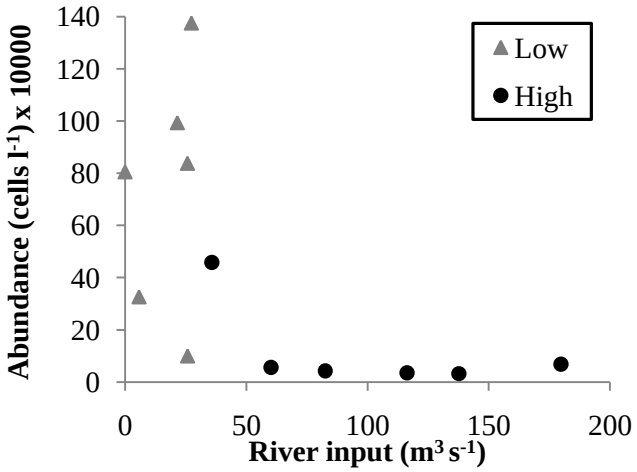

Figure 3.8. Relationship between river input rate and mean monthly phytoplankton cell abundance concentrations from September 2007 to August 2008. The triangles and closed circles represent samples collected from the low and high river input periods, respectively.

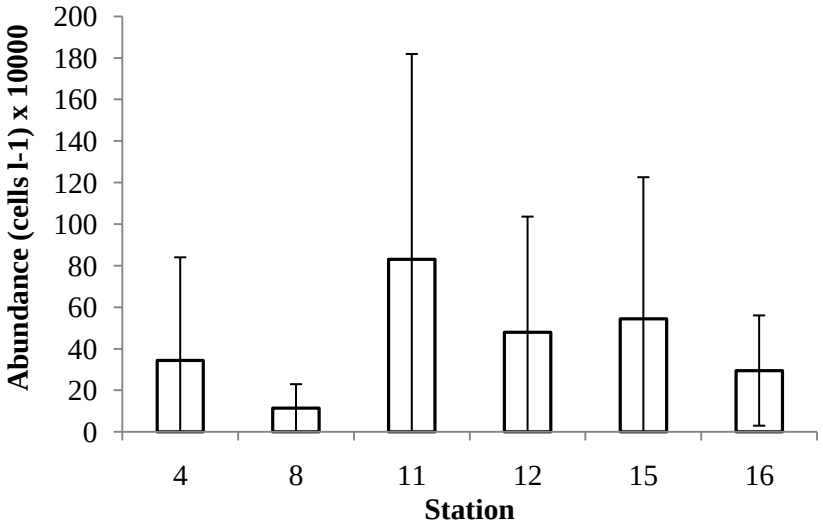

Figure 3.9. Mean phytoplankton cell abundance with error bars representing one standard deviation for each station sampled in Breton Sound estuary from September 2007 to August 2008.

Enumeration of the phytoplankton community revealed that cyanobacteria was the most abundant phytoplankton group and dominated the community in terms of abundance for a majority of the year (Table 3.1, Fig. 3.10). Subsequently, cyanobacterial abundances followed a similar temporal

abundance pattern as the total phytoplankton cell abundances. Cyanobacteria generally consisted of very small cells (<5 µm) either solitary or in colonies. When cyanobacteria were not the most abundant group, from January to May 2008, chlorophytes were dominant with moderate contributions by both cyanobacteria and centric diatoms. Relatively small contributions were made by flagellates, pennate diatoms and dinoflagellates.

Table 3.1. The mean and range of phytoplankton group cell abundances in Breton Sound estuary from September 2007 to August 2008.

Group	**Mean (cells l^{-1})**	**St Dev (cells l^{-1})**	**Range (cell l^{-1})**
Cyanobacteria	$3.7x10^5$	$6.0x10^5$	$1.9x10^3$-$2.7x10^6$
Chlorophytes	$2.7x10^4$	$1.7x10^4$	$7.1x10^3$-$8.7x10^4$
Centric Diatoms	$6.9x10^3$	$1.3x10^4$	$3.7x10^2$-$7.9x10^4$
Flagellates	$2.2x10^3$	$1.7x10^3$	0.0-$8.4x10^3$
Pennate Diatoms	$1.3x10^3$	$2.6x10^3$	0.0-$1.9x10^4$
Dinoflagellates	$1.1x10^2$	$3.8x10^2$	0.0-$3.1x10^3$

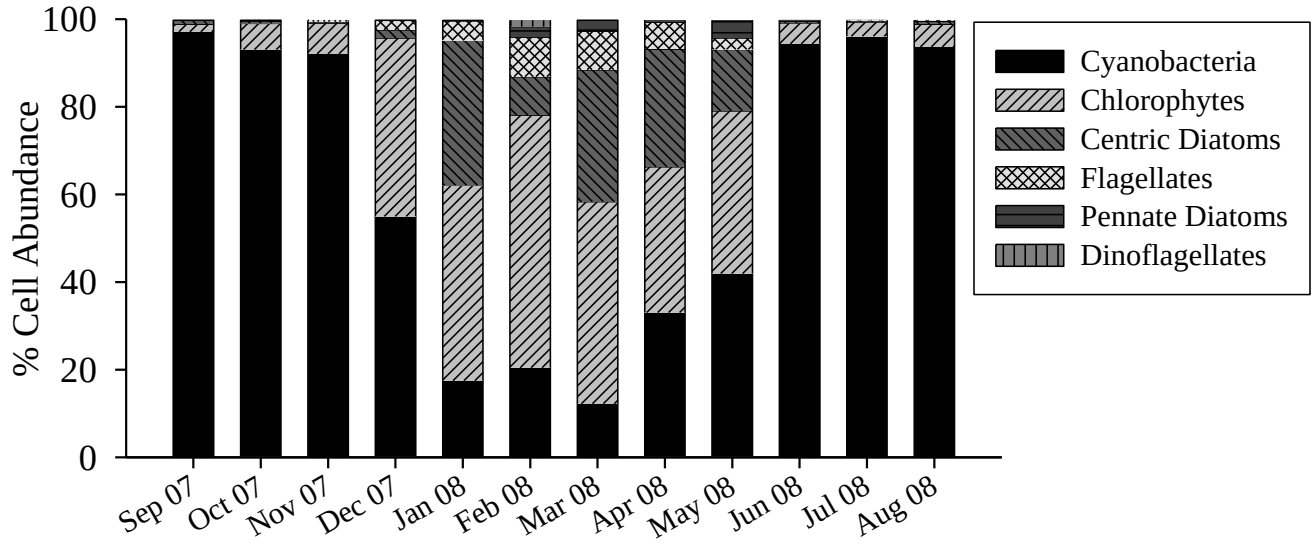

Figure 3.10. Percent abundance of individual phytoplankton groups for each month from September 2007 to August 2008 during the seasonal study.

Statistical analysis of the chl *a* concentrations and total cell abundances with the environmental parameters, including MLR and the BIO-ENV procedure, illustrated significant relationships (Table 3.2). MLR indicated that annual chl *a* concentrations were more strongly and significantly correlated

with salinity and DIN, but were also significantly correlated with concentrations of TSS and PO_4. However, the BIO-ENV procedure of the environmental parameters with chl *a* concentrations over the entire study period indicated that DIN concentrations and distance from the diversion by flow best explained the pattern in chl *a* concentrations. When breaking the seasonal study into high and low river input periods, different relationships emerge for each period. The MLR of chl *a* concentrations during low river input indicated a positive correlation with TSS concentrations and a negative correlation with DIN concentrations. The corresponding Bio-Env procedure presents a different set of variables that predict the pattern chl *a* concentrations during low river input as temperature, DIN and distance from the diversion structure. Alternatively, for high river input, chl *a* concentrations were correlated with salinity, P and TSS, while the Bio-Env indicated that DIN, P and distance from the diversion structure were the best predictors of high river input chl *a* concentrations.

Annual total cell abundance concentrations were significantly correlated with salinity, DIN and distance from the diversion structure (negative, P=0.003). However, the Bio-Env procedure of the environmental parameters and total abundance concentrations indicated that temperature and DIN explained the patterns in total cell abundance concentrations. When analyzing the total cell abundance concentrations by river input rates, different relationships are observed. During low river input, the MLR indicated that cell abundances were positively correlated with temperature, salinity and DIN, while the Bio-Env indicated that only temperature was the best predictor of total cell abundances during low river input. Alternatively, high river input MLR indicated that salinity had the strongest correlation with total cell abundance concentrations, and the Bio-Env did not present any significant relationships.

Further BIO-ENV analysis of the community composition with the environmental variables significantly indicated that a combination of temperature, salinity and DIN concentrations of samples best explained the annual patterns in the community composition (Table 3.2). However, during low river

input, Bio-Env indicated salinity, DIN and distance from the diversion structure were the best predictors of the community and during high river input, salinity and temperature were the best predictors.

Table 3.2. MLR and Bio-Env procedure relationships between the environmental parameters and chl *a*, phytoplankton cell abundance concentrations and the phytoplankton community composition during the seasonal study. Low river input includes samples from September to October 2007 and May to August 2008. High river input includes samples from November 2007 to April 2008.

	MLR				Bio-Env		
	Parameter	P-value	R^2	n	Parameter	Spearman, ρ_s	Significance Level
Chlorophyll *a*							
Annual	Salinity (+)	0.005	0.43	65	DIN	0.287	1%
	DIN (-)	0.004			Distance		
	TSS (-)	0.049					
	P (-)	0.048					
Low River	TSS (+)	0.029	0.34	30	Temp	0.247	10%
Input	DIN (-)	0.035			DIN		
					Distance		
High River	Salinity (+)	0.01	0.48	35	DIN	0.203	6%
Input	P (-)	0.016			P		
	TSS (-)	0.002			Distance		
Abundance							
Annual	Salinity (+)	<0.001	0.70	70	Temp	0.375	1%
	DIN (+)	<0.001			DIN		
Low River	Temp (+)	0.022	0.68	35	Temp	0.231	3%
Input	Salinity (+)	<0.001					
	DIN (+)	<0.001					
High River	Salinity (+)	0.002	0.34	35			
Input							
Community							
Annual					Temp	0.285	1%
					Salinity		
					DIN		
Low River					Salinity	0.256	1%
Input					DIN		
					Distance		
High River					Salinity	0.219	4%
Input					Temp		

Toxic Phytoplankton Abundance and Distribution

Nine toxin producing genera were observed within Breton Sound estuary from September 2007 to August 2008, comprising 5 genera of cyanobacteria and 4 genera of dinoflagellates (Table 3.3). Toxin

producing cyanobacteria genera were at least an order of magnitude more abundant than toxin producing dinoflagellate genera throughout the seasonal study period. The cyanobacteria genera included *Anabaena, Anabeanopsis, Microcystis, Raphidiopsis,* and *Cylindrospermopsis*, in order of most to least abundant, which were most frequently observed toward the outer portion of the estuary (stations 11, 12, 15 and 16) during the low river input. *Anabaena* reached the highest abundance of all toxic phytoplankton observed, $8.9x10^4$ cells l^{-1}, during this period. The dinoflagellate genera included *Prorocentrum, Gymnodinium, Peridinium*, and *Heterocapsa,* from most to least abundant, which were ubiquitously observed throughout the estuary during the entire study period. These genera are capable of producing numerous phycotoxins and harmful compounds that are summarized in Table 3.3.

Phycotoxin Production

Species of *Anabaena, Anabaenopsis* and *Microcystis* capable of producing MCs were observed throughout the estuary. Subsequently, particulate and dissolved MCs were detected throughout the estuary during all months except March and April 2008 (Fig. 3.11a). Particulate MCs (PTox) concentrations ranged from below detection (0.10 µg l^{-1}) to 2.92 µg l^{-1} and were consistently higher than dissolved MCs (DTox), which ranged from below detection to 0.26 µg l^{-1}. The highest concentration of MCs were measured in the particulate phase at station 16 in the outer estuary during Oct 2007 (2.92 µg l^{-1}, Fig. 3.11a, b). Variations in concentrations of MCs followed the same temporal trends as the estuary wide mean cyanobacterial cell abundances during the seasonal study period (Fig. 3.11a, 3.12).

Pulse Study

Environmental Parameters

During the pulse study period (March 14 to April 3, 2008), salinity ranged from 0.1 to 8.7‰ within the estuary and averaged 1.0 $\pm$ 2.0 and 0.6 $\pm$ 1.0‰ during low and high river input, respectively (Fig.

3.13a). This difference was not significant, however, salinity increased with distance from the diversion structure toward the outer estuary during both the low and high river input conditions. TSS concentrations were variable throughout the estuary, ranging from 5 to 190 mg l^{-1} (Fig. 3.13b). The TSS concentrations during low and high river input were 56 $\pm$ 46 and 30 $\pm$ 23 mg l^{-1}, respectively, which was a significant difference (P=0.01) between river input rates. The highest TSS concentrations were observed at the stations closest to the source of river input in the inner estuary (<15 km from diversion structure). Water temperatures ranged from 16.7 to 24.8, increasing from the beginning to the end of the pulse experiment (Fig. 3.14).

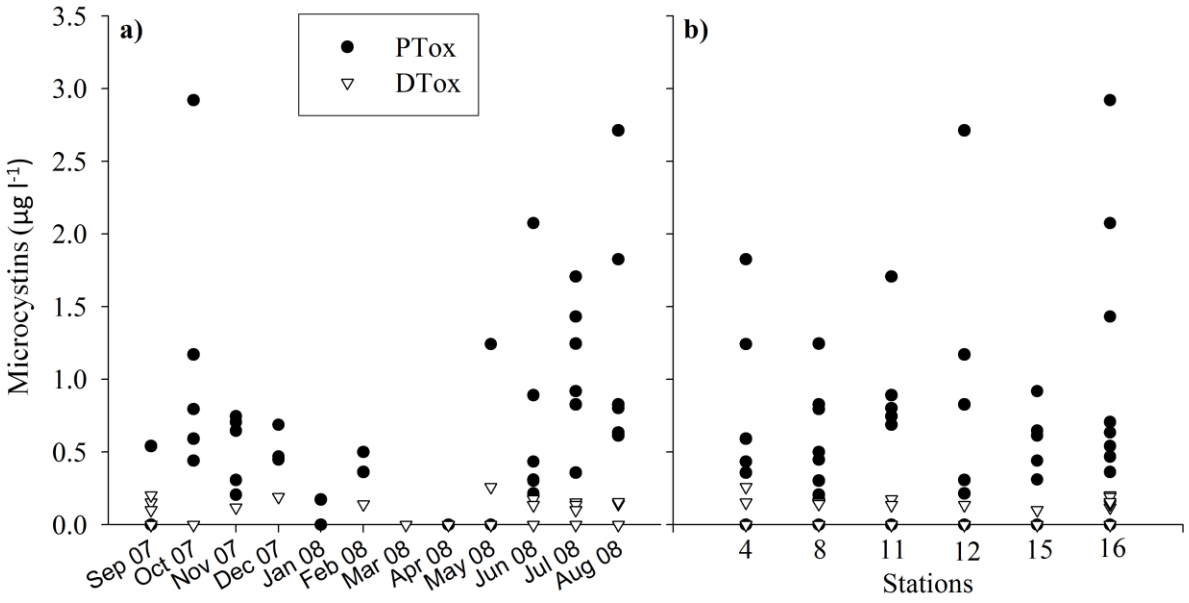

Figure 3.11. Concentrations of particulate (closed circles) and dissolved (open inverted triangles) microcystins temporally (a) and spatially (b) in samples collected during the seasonal study from September 2007 to August 2008 in Breton Sound estuary, Louisiana.

The DIN concentrations averaged 51.6 $\pm$ 39.3 µM across the estuary under low river input rates and 42.1 $\pm$ 36.3 µM during high river input rates, which were not significantly different (Fig. 3.15a). Estuary wide phosphate concentrations were also not significantly different between low and high river input rates, with averages of 1.3 $\pm$ 0.9 µM and 0.9 $\pm$ 0.7 µM, respectively, and ranged from below detection to 2.9 µM (Fig. 3.15b). DIN and P concentrations tended to decrease with increasing distance

from the diversion during this experiment, therefore, DIN:P ratios appeared to have the same trend (Fig. 3.15c). DIN:P ratio values ranged from 0 to 204 across the estuary, with an average of 34 ± 21 during low river input and 58 ± 57 during high river input, which was not significantly different.

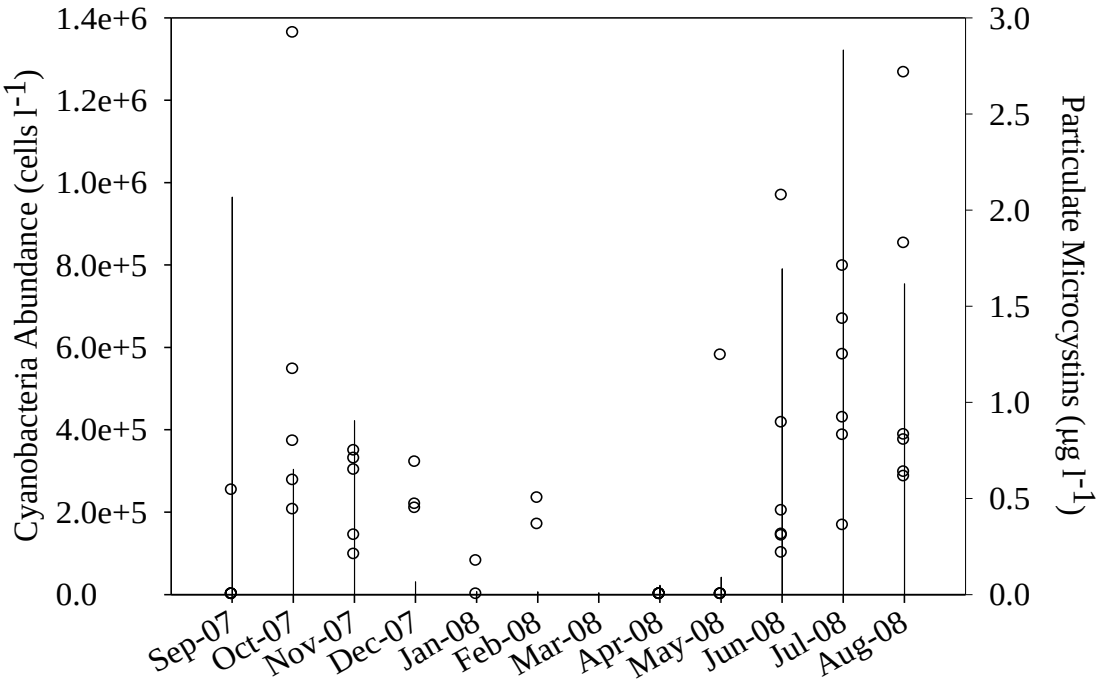

Figure 3.12. Average cyanobacteria cell abundances (vertical bars) from September 2007 to August 2008 with particulate microcystin concentrations (open circles) measured for each month in Breton Sound estuary during the seasonal study period.

Biological Response

Chl *a* concentrations observed in the estuary over the entire pulse study period, during both low and high river input, ranged from 2.5 to 44.8 µg l^{-1} (Fig. 3.16). Higher chl *a* concentrations were observed during low river input, averaging 15.2 ± 12.6µg l^{-1} with the highest chl *a* concentration (44.8 µg l^{-1}) measured toward the outer estuary at station 11. During high river input, chl *a* concentrations averaged 8.9 ± 5.3 µg l^{-1}. Although the average chl *a* concentration decreased from the low to high river input rates, this change was not significant. Total phytoplankton cell abundance concentrations varied from $1.4x10^4$ to $1.3x10^5$ cells l^{-1} throughout the sampling period. The average abundance during low and high

river input was $4.2x10^4 \pm 2.2x10^4$ and $4.4x10^4 \pm 3.0x10^4$ cells l^{-1} and this difference was also not significant (Fig. 3.17).

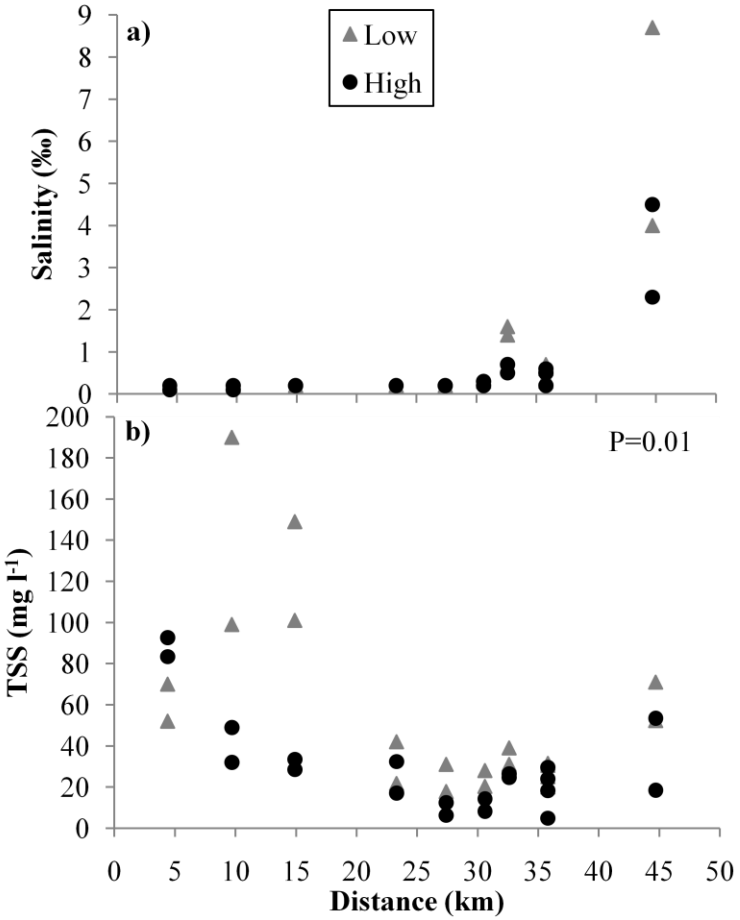

Figure 3.13. Salinity (a) and TSS concentrations (b) for samples from low and high river input during the pulse study from March 17 to April 3, 2008. Triangles and closed circles indicate low and high river input, respectively.

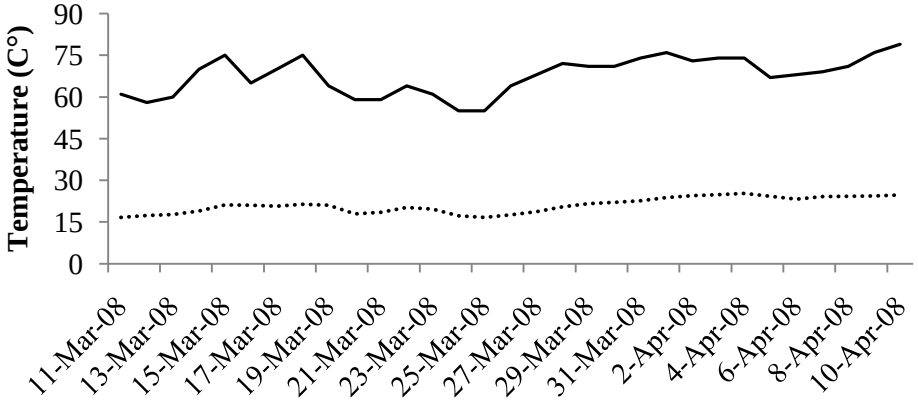

Figure 3.14. Daily mean air (solid line) and water (dotted line) temperature during the pulse study in Breton Sound estuary.

Table 3.3. Toxin producing harmful algal genera observed in Breton Sound estuary during the seasonal and pulse study periods, September 2007 to August 2008.

Genera	Range (Cells L^{-1})	Station(s)	Potential Toxin	Reference
Cyanobacteria				
Anabaena	$3.0x10^2$-$8.9x10^4$	4,8,10,11,12,15	Anatoxin-a	Devlin et al. 1977
			Anatoxin-a(s)	Mahmood et al. 1986, Matsunaga et al. 1989
			Microcystins	Harada et al. 1991
			Saxitoxin	Negri et al. 1995
			Cylindrospermopsin	Schembri et al. 2001
Anabaenopsis	$4.0x10^2$-$1.1x10^4$	8,11,12,15	Microcystins	Lanaras et al. 1994
Cylindrospermopsis	$3.3x10^2$-$8.3x10^2$	11,12,15	Cylindrospermopsin	Ohtani et al. 1992
			Deoxycylindrospermopsin	Norris et al. 1999
			Saxitoxin	Lagos et al. 1999, Molica et al. 2002
Microcystis	$2.7x10^2$-$9.6x10^3$	8,11,12,15,16	Microcystins	Carmichael et al. 1988
Raphidiopsis	0-$7.0x10^3$	1,3,4,7,8,10,11,12,15,16	Anatoxin-a	Namikoshi et al. 1996
			Cylindrospermopsin	Li et al. 2001
			Deoxycylindrospermopsin	Li et al. 2001
Dinoflagellate				
Gymnodinium	$0.3x10^2$-$2.0x10^2$	1,4,8,11,12,15,16	Hemolytic Compounds	Paster et al. 1969
			Aerosolized Endotoxins	Devassy et al. 1991
			Gymnodimine	Seki et al. 1995
Heterocapsa	$0.3x10^2$-$3.0x10^2$	11,12,16	Hemolytic Compounds	Oda et al. 2001
Peridinium	$0.3x10^2$-$2.0x10^2$	4,8,11,15,16	Glenodinine	Hashimoto et al. 1968
			Algacidal Compounds	Wu et al. 1998
Prorocentrum	$0.3x10^2$-$2.6x10^3$	1,3,4,8,10,11,12,15,16	Hemolytic Compounds	Nakajima et al. 1981
			Okadaic Acid	Murakami et al. 1982
			Dinophysistoxin	Yasumoto et al. 1987
			Prorocentrolid	Torigoe et al. 1988
			Venerupin	Grzebyk et al. 1997

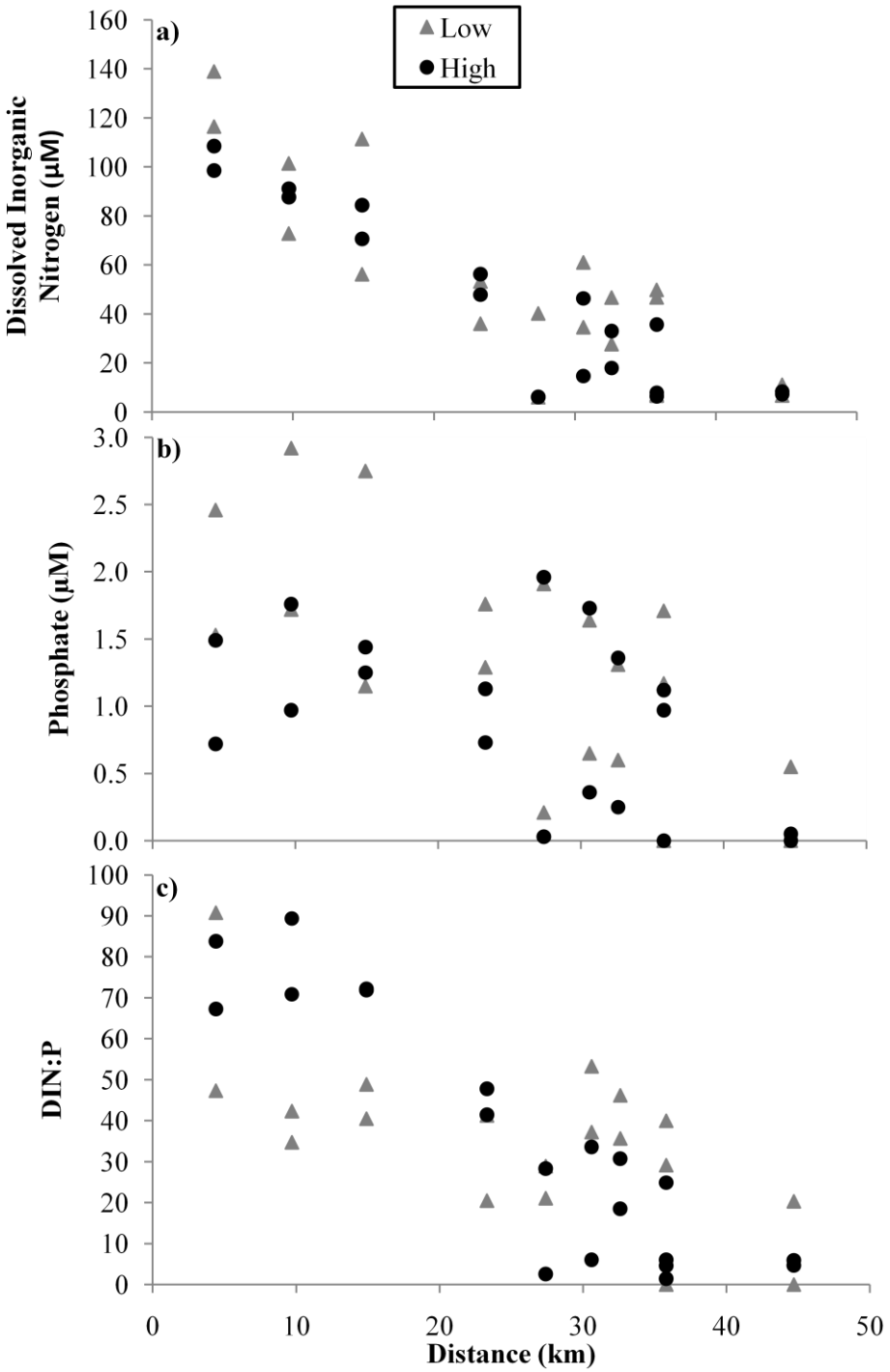

Figure 3.15. DIN (a), P (b) and DIN:P ratio (c) for each sample collected during the pulse study in relation to distance from the diversion structure by flow in Breton Sound estuary. Triangles and closed circles indicate samples collected during low and high river input, respectively.

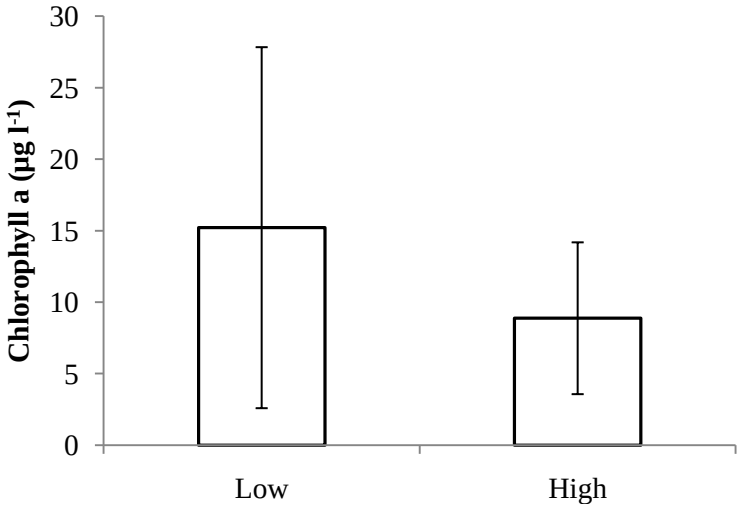

Figure 3.16. Mean chl *a* concentrations during low and high river input of the pulse study from March 14 to April 3, 2008 in Breton Sound estuary.

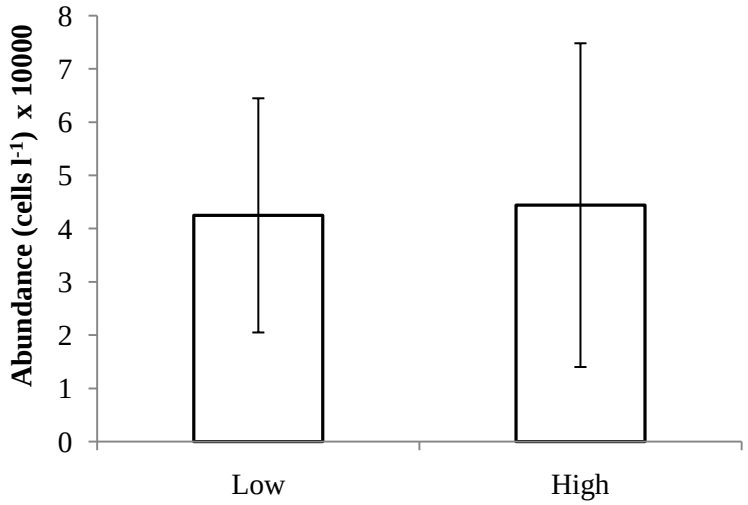

Figure 3.17. Mean phytoplankton cell abundance concentrations during low and high river input of the pulse study from March 14 to April 3, 2008 in Breton Sound estuary.

In general, the phytoplankton community was dominated by small chlorophytes and cyanobacteria cells based on average cells per liter among all the samples examined (Table 3.4). These cells were approximately <5 µm in size. Larger chlorophytes, centric and pennate diatoms, and flagellates composed the remainder of the community, while dinoflagellates provided little contribution to the community composition based on cell abundance (Table 3.4, Fig. 3.18). Station 16, the outermost station

sampled in the lower portion of the estuary, had the highest total cell numbers recorded, which occurred during high river input conditions with cyanobacteria as the dominant phytoplankton group.

Table 3.4. Mean phytoplankton group cell abundances over the whole pulse study, but also under the low and high river input conditions in Breton Sound estuary during this study.

	Mean Abundance (cells l^{-1})			
Group	**Study Period**	**±St Dev**	**Low Input**	**High Input**
Chlorophytes	$2.0x10^4$	$\pm 1.1x10^4$	$2.2x10^4$	$1.8x10^4$
Cyanobacteria	$1.1x10^4$	$\pm 2.0x10^4$	$5.4x10^3$	$1.7x10^4$
Centric Diatoms	$8.0x10^3$	$\pm 7.9x10^3$	$1.0x10^4$	$6.0x10^3$
Flagellates	$3.3x10^3$	$\pm 2.1x10^3$	$4.1x10^3$	$2.3x10^3$
Pennate Diatoms	$5.0x10^2$	$\pm 4.1x10^2$	$6.3x10^2$	$3.7x10^2$
Dinoflagellates	$0.5x10^2$	$\pm 0.8x10^2$	$0.8x10^2$	$0.2x10^2$

Statistical analysis using MLR analysis and the Bio-Env procedure for the chl *a* and total phytoplankton cell abundance concentrations showed few significant relationships with any of the environmental parameters during the pulse study period (Table 3.5). During both low and high river input, chl *a* concentrations lacked significant correlation with any environmental parameter according to MLR. The Bio-Env procedure, however, indicated that distance was the best parameter to explain the patterns in chl *a* concentrations during high river input conditions. Subsequently, MLR analysis showed that total phytoplankton cell abundance concentrations were negatively correlated with temperature values and positively correlated with TSS concentrations during low river input conditions, but was not significantly correlated with any environmental parameter during the high river input conditions. The corresponding Bio-Env procedures indicated that patterns in abundance concentrations were best predicted by temperature, TSS and P concentrations under low river input conditions and by salinity, DIN, P and distance under high river input conditions.

During low river input conditions, chlorophytes dominated the phytoplankton community, with the highest cell abundance at station 3 in the upper estuary ($5.4x10^4$ cells l^{-1}, Fig. 3.18, Table 3.4). The second most abundant phytoplankton group, cyanobacteria, was present at a consistent abundance

throughout the estuary ($1.9x10^3$ -$1.0x10^4$ cells l^{-1}). Centric diatoms were moderately abundant throughout the estuary ($8.3x10^2$-$3.4x10^4$ cells l^{-1}), with the highest cell abundance also occurring at station 12 toward the outer estuary (Fig. 3.18). The abundance of centric diatoms was greater in the outer estuary farther from the diversion structure (11, 12, 15, and 16) compared to the inner stations closer to the structure (1, 3, and 4), but increased in abundance at the outer stations during low river input (Fig. 3.18). Other phytoplankton groups, pennate diatoms, flagellates and dinoflagellates, did not significantly contribute to the community during these conditions. The Bio-Env procedure indicated that P concentrations were the strongest environmental parameter influencing the patterns in the composition of the community during low river input conditions.

During the high river input conditions, chlorophyte cell numbers decreased. Cyanobacteria were consistently present throughout the estuary ($2.2x10^3$-$1.3x10^4$ cells l^{-1}) with the exception of higher abundances at station 8 mid estuary and station 16 in the outer estuary (Fig. 3.18). Early in high river input period, cyanobacteria cell abundance reached $1.0x10^5$ cells l^{-1} at station 16 in the outer estuary, the highest cell abundance for a single phytoplankton group for the entire study period. Cyanobacteria was the only group to achieve its highest cell abundance during high river input. Similar to chlorophytes, centric diatoms also decreased during these conditions. Centric diatoms continued to be more abundant at the outer stations. Pennate diatoms, flagellates and dinoflagellates made negligible contributions to the community during this time. The Bio-Env procedure indicated that P concentrations and distance from the diversion structure where the best predictors of the patterns in the community composition during high river input conditions.

Toxic Phytoplankton Abundance and Distribution

Seven genera containing harmful algal bloom species were observed in low to moderate abundances throughout the estuary during the pulse study period, including the cyanobacteria *Anabaena,*

Microcystis, and *Raphidiopsis* and the dinoflagellate *Prorocentrum*. These genera were present throughout the estuary, but at relatively low cell abundances, being observed more frequently during low river input. Most of the genera were found only during low river input at one or two stations, however, *Raphidiopsis* and *Prorocentrum* were observed throughout the estuary in low abundances during both low and high river input conditions, up to $7.0x10^3$ and $2.0x10^2$ cells l^{-1}, respectively. Two MC-producing genera, *Microcystis* and *Anabaena*, were observed in the estuary during the pulse study period. However, each of these genera were only identified in one sample in the outer estuary under low river input ($1.2x10^3$ cells l^{-1} at station 10 and $4.8x10^2$ cells l^{-1} at station 15, respectively).

Phycotoxin Production

Even though MC-producing genera were observed only twice during the pulse study period, MCs concentrations were detected in both the particulate and dissolved fractions of the water column at seven of the ten sampling stations during this time. In the samples analyzed, the average concentration of MCs was 0.40 µg l^{-1} in the particulate fraction and below detection in the dissolved fraction. High concentrations of particulate MCs were observed toward the end of low river input to early in the high river input conditions at stations in the outer estuary (Fig. 3.19). However, the highest concentration of particulate MCs (2.17 µg l^{-1}) occurred at station 16 (44.7 km from the diversion structure) under high river input conditions. In general, higher concentrations of dissolved MCs were observed in the outer estuary during high river input conditions, with the highest concentration of dissolved MCs (0.17 µg l^{-1}) occurring at station 12 (35.8 km from the diversion structure).

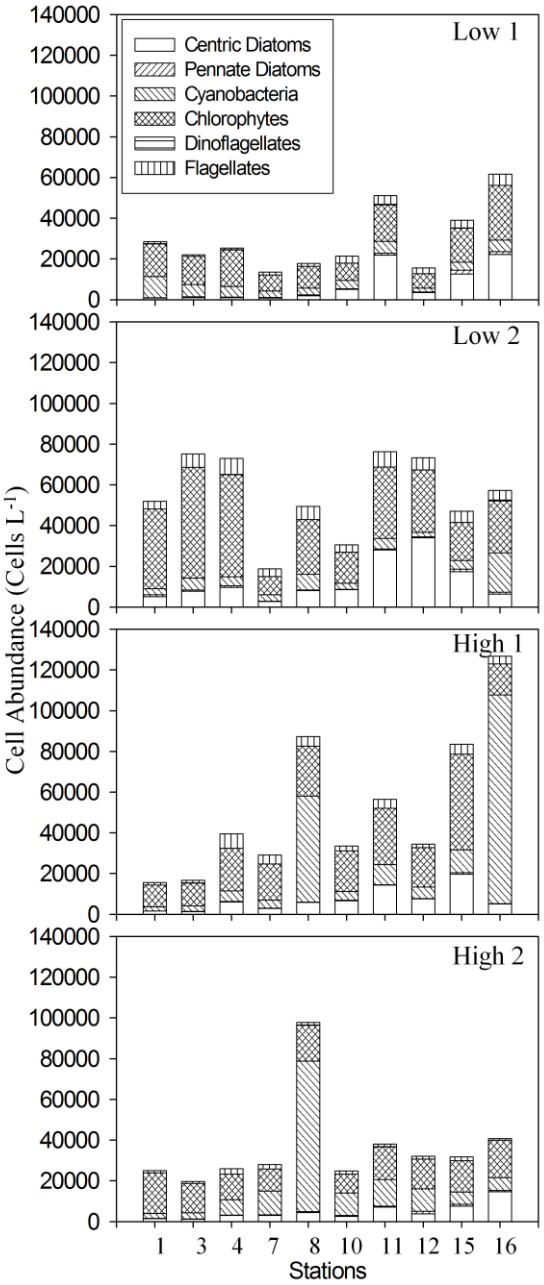

Figure 3.18. Abundance of phytoplankton groups in Breton Sound estuary over the four sampling dates during the pulse study from March 14 to April 3, 2008. Low 1 and 2 represents the phytoplankton community on the third and tenth days, respectively, after low river input began. High 1 and 2 represents the phytoplankton community on the third and ninth days, respectively, after high river input began.

Table 3.5. MLR and Bio-Env procedure relationships between the environmental parameters and chl *a*, phytoplankton cell abundance concentrations and the phytoplankton community composition during the pulse study. Low river input includes samples from March 17 and 24, 2008. High river input includes samples from March 28 and April 3, 2008.

	MLR				Bio-Env		
	Parameter	P-value	R^2	n	Parameter	Spearman, ρ_s	Significance Level
Chlorophyll *a*							
Low River Input							
High River Input					Distance	0.567	1%
Abundance							
Low	Temp (-)	0.045	0.62	20	Temp	0.267	4%
	TSS (+)	0.05			TSS		
					P		
High					Salinity	0.443	2%
					DIN		
					P		
					Distance		
Community							
Low					P	0.332	3%
High					P	0.366	1%
					Distance		

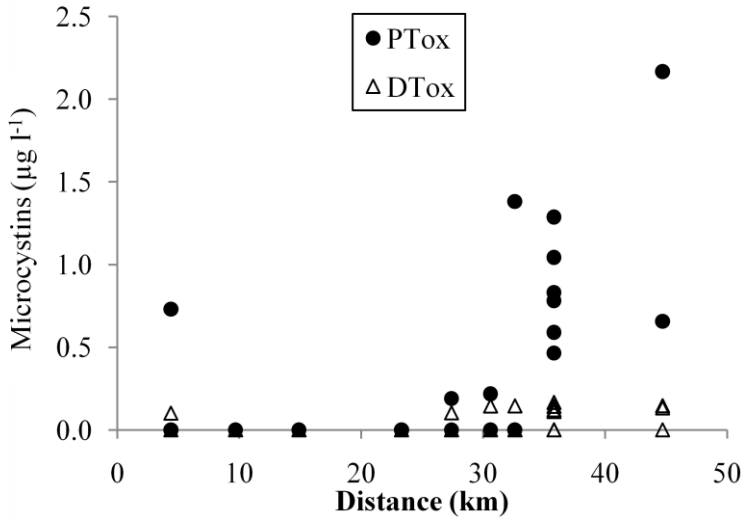

Figure 3.19. Concentrations of particulate and dissolved microcystins (MCs) at each station in Breton Sound estuary during the pulse study. The closed circles and open triangles represent concentrations of particulate and dissolved MCs, respectively.

CHAPTER 4

DISCUSSION AND CONCLUSIONS

The Breton Sound estuarine system is unique in that its main source of river water is provided through a manually controlled diversion structure. This source is also one of the main providers of freshwater and the main provider of total nitrogen (TN) and phosphorus (P) to the estuary (Lane et al. 1999, Hyfield et al. 2008). Although this structure is managed to mimic seasonal river input and flooding, changes in the river input rate can generally be made at any time, depending on the height of the water in the river. The ability to determine the response of the phytoplankton community, harmful algal species and phycotoxin production in relation to the pulse of river input rates is beneficial for both scientific and economic interests.

The phytoplankton community of Breton Sound estuary was examined under both typical seasonal and sudden pulses in river input rate. Changes in phytoplankton biomass, total cell abundances, community composition and phycotoxin production were observed over a 12 month period and during a short pulse event. Phytoplankton shifted in response to seasonal changes, but also responded quickly, in a matter of days, to changes in environmental conditions (i.e. nutrients, salinity and turbulence) under manipulated river input rates as they were advected toward the outer estuary. Variation of river input rates created a highly dynamic and potentially turbulent environment that favored specific groups of phytoplankton resulting in shifts in community composition. Additionally, specific toxic dinoflagellate and cyanobacteria genera were observed, including cyanobacteria species that produce microcystins (MCs). The toxic cyanobacteria genera were observed most frequently during the warmer months when temperatures were high and nutrient availability was decreased.

Seasonal Study

Phytoplankton responses, in terms of biomass, abundance, community composition and phycotoxin production to changes in river input were first examined on a seasonal time scale. River input into Breton Sound estuary through the Caernarvon Freshwater Diversion structure follows typical seasonal patterns (Swenson et al. 2006). Increases in river input mimics high water levels associated with snowmelt and spring precipitation runoff into the Mississippi River. During the summer and early fall, river water levels drop, decreasing the amount of river input delivered to coastal estuaries. This means that Breton Sound estuary has two time periods throughout the year that are influenced by different river input rates, a period with low river input that usually occurs during warmer months, and a period with high river input that usually occurs during the cooler months of a given year. This river input along with seasonal influences (temperature and light availability), is responsible for the condition of the estuarine environment. Over the entire seasonal study period, the salinity gradient created by incoming river water keeps the inner to mid estuary relatively fresh. Mean station salinity values do not exceed 2‰ until distances greater than approximately 30 km from the diversion structure. Before the diversion structure was installed, higher salinity values were found closer to the diversion structure (Chatry and Chew 1985, Meffert and Good 1996). Total suspended solid (TSS) concentrations increased toward the outer estuary potentially as a result of wind resuspension, which was previously suggested by Lane et al. (2007) for this estuary. During the seasonal study, stations were not close enough to the diversion structure to demonstrate typical high TSS values from sediment laden river waters entering the estuary that were previously observed by Lane et al. (1999, 2007) and Snedden et al. (2007).

The river input was also the source of biologically available inorganic nutrients, as observed in previous studies of the estuary (Lane et al 1999, Hyfield et al. 2008). Dissolved inorganic nitrogen (DIN) was highest toward the inner estuary and when river input was high. As suggested in several other studies (e.g. Lane et al. 1999, Reddy and Patrick 1984), DIN might become limiting toward the outer

estuary due to denitrification, burial, biological uptake and dilution. During low river input, decreased DIN values with fairly consistent P concentrations toward the outer estuary resulted in decreased DIN:P ratio values from the inner to outer estuary. Lane et al. (2004) previously observed DIN:P ratios to decrease to 26 toward the outer estuary, however, this study revealed ratios as low as 17 in the outer estuary, even closer to the Redfield ratio (Redfield 1958). Justić et al. (1995) have hypothesized that this trend toward more balanced nutrient ratios in river water entering estuarine systems may be relieving nutrient limitations for phytoplankton and allowing the increase of harmful algal species.

Chl *a* concentrations and phytoplankton cell abundance concentrations were influenced by seasonal and river input conditions. Both chl *a* and abundance concentrations were higher during low river input, however, variability was high among stations during the study period. Other sources of variation in chl *a* concentrations include cell size and chl *a* per cell. Larger phytoplankton can contain more chl *a* than small phytoplankton. Additonally, environmental factors such as light availability may influence how much chl *a* is produced by a cell (Steemann Nielson and Jörgenson 1968, Beardall and Morris 1976, Falkowski and Owens 1980). When light levels are high, such as in the warmer months on the Gulf coast, phytoplankton cells need less chl *a* to capture sufficient energy. Alternatively, when light levels decrease, phytoplankton cells produce more chl *a* to capture light energy. Numerous physical, chemical and biological processes act on phytoplankton at different magnitudes of spatial and temporal scales, which may explain the patchiness of phytoplankton (Platt and Denman 1980 Glibert et al. 2005). Temperature moderates phytoplankton cellular processes, increasing or decreasing growth, photosynthesis and reproduction as temperatures change (Reynolds 1984). Decreases in phytoplankton biomass and abundance during high river input is most likely due to the seasonal effects of decreased temperature, decreased light availability and increased turbulence, since nutrient availability increases during high river input, alleviating nutrient limitation for phytoplankton. The difference in magnitude of the chl *a* response versus cell abundance concentration response and the nonlinear correlation between

cell abundance concentrations and the river input rate suggests that dilution is not the only factor influencing decreases in chl *a* or abundances.

The phytoplankton community shifted from cyanobacteria dominating during the majority of the year to chlorophytes becoming dominant during the high river input. The higher temperatures along with water column stability and likely increase in water residence time during low river input may have supported cyanobacteria, as was observed in other studies of phytoplankton communities (e.g. Reynolds 1984, Elliot et al. 2006, Jöhnk et al. 2008, Paerl and Huisman 2008). Huisman et al. (2004) observed a similar shift from cyanobacteria to chlorophytes in the phytoplankton community of Lake Niuewe in the Netherlands in response to increases in turbulence and water column mixing. They hypothesized that this shift occurred because cyanobacteria are buoyant phytoplankton, including *Microcystis*, that can generally only become established during stable water column conditions (Walsby 1994, Kononen et al. 1996, Walsby et al. 1997). Stronger turbulence during high river input favors sinking phytoplankton, such as chlorophytes, by increasing their duration within the photic zone (Harris and Baxter 1996, Visser et al. 1996). Furthermore, the availability of DIN within the estuary and between the seasons created more favorable conditions for cyanobacteria. Cyanobacteria's high affinity for N and P, (Smith 1983, Klemer and Konopka 1989), as well as the ability of certain species to fix nitrogen and/or store P (Thompson et al. 1994, Dignum et al. 2005), enable them to outcompete other groups during low river input and toward the outer estuary where nutrient concentrations decreased. Since cyanobacteria generally have relatively low growth rates (Canale and Vogel 1974, Robarts and Zohary 1987), increased temperatures during low river input might have allowed increased growth rates.

Statistical analysis of the biological responses using multiple linear regression (MLR) and the Bio-Env procedure revealed significant relationships with the change in the environmental parameters. Chl *a* appeared to be mostly influenced by DIN and P concentrations throughout the seasonal study period, while phytoplankton cell abundance concentrations and community composition were influenced

by salinity and temperature variations, as well as DIN concentrations. When nutrients were available in the water column at non-limiting concentrations and all other environmental conditions were favorable, phytoplankton growth was not restricted and shifts in the dominant phytoplankton in the community occurred. Subsequently, altered nutrient availability and stoichiometry might also have majorly impacted phytoplankton biomass and community composition, as seen in several previous studies (e.g. Garcia-Soto et al. 1990, Justić et al. 1995, Piehler et al. 2004, Buyukates and Roelke 2005). Decreased nutrient concentrations as chl *a* and abundance increased suggested biological assimilation occurred within the estuary, as well as other natural nutrient cycling processes. Salinity restricts species of phytoplankton to specific ranges within the environment due to their physiological tolerances, thereby influencing abundance concentrations in different areas of the estuary and causing shifts in the community composition. Since Breton Sound estuary is mostly fresh to slightly brackish and the phytoplankton genera observed generally have low salinity tolerances. However, some cyanobacteria genera, such as *Microcystis*, can tolerate salinities up to 14‰, which may enable them to increase in times of higher salinity during low river input. Additionally, increased temperature and light availability would potentially allow increased cellular activity and growth rates reflected by increased phytoplankton cell abundance concentrations during low river input.

The environmental conditions during low river input, characterized by low turbulence, high temperatures and decreased nutrients, supported cyanobacteria, including harmful algal species such as *Microcystis* and *Anabaena*. Furthermore, levels of detected MCs followed cyanobacteria abundance patterns spatially and temporally as was predicted. In a culture study, Orr and Jones (1998) found that the production of MCs was proportional to cellular growth rates of *Microcystis aeruginosa*. Subsequently, optimum conditions for specific species favor the highest toxin production (Sivonen and Jones 1999). Cyanobacteria's ability to produce toxins has been found to be genetically determined

based on strain (Kurmayer and Christiansen 2009), the conditions within Breton Sound estuary support toxic strains of cyanobacteria and the production of MCs.

Pulse Study

In the pulse study, the diversion structure in Breton Sound estuary was used as a manipulation tool to determine the immediate response of the phytoplankton community and potential phycotoxin production to a short river pulse event. The environmental parameters followed similar trends during the pulse study as were observed during the seasonal study with salinity, TSS and nutrients most influenced by river input. Salinity values for the pulse study were similar to the seasonal study values with little variation and a lack of noticeable increase within approximately 30 km of the diversion structure. Inclusion of sampling stations closer to the diversion structure revealed high TSS concentrations near the diversion structure, likely from the introduction of sediment laden river water, as expected and previously observed to occur in the estuary by Lane et al. (1999, 2007) and Snedden et al. (2007). TSS concentrations were also slightly higher toward the outer estuary from wind resuspension as observed during high river input. DIN concentrations and DIN:P ratio values also decreased toward the outer estuary, as in the seasonal study. Decreased P concentrations from the diversion structure toward the outer estuary may have been caused by high P loading from typical spring river input rates, but was further increased by manipulating the diversion structure to maximum river input rates during the pulse study.

The duration of the sampling periods during low and high river input of the pulse experiment (approximately 2 weeks) may not have been long enough to capture a significant change in chl *a* concentrations and phytoplankton cell abundance concentrations. An absence of a significant decrease in phytoplankton cell abundance concentrations during the pulse study also suggests dilution may not be a major factor. However, the phytoplankton community composition shifted in response to changes in

river input rates and environmental conditions during this study. Chlorophytes were the dominant phytoplankton group throughout the pulse study period, as was also observed during the cooler months of the seasonal study. However, in the pulse study, cyanobacteria increased from the low to the high river input periods and achieved their highest cell abundance concentrations during high river input conditions. It is possible that this occurred because cyanobacteria might have been able to increase in backwater regions during low river input, increased residence times and decreased turbulence. The resumption of high river input potentially advected these seed populations of cyanobacteria toward the outer estuary explaining the increase in cyanobacteria during high river input. The affinity of cyanobacteria for nutrients at low concentrations (Smith 1983, Klemer and Konopka 1989) and their ability to tolerate higher salinities than other freshwater phytoplankton (Tonk et al. 2007) potentially enabled them to survive the conditions of the outer estuary. Alternatively, the delayed increase in cyanobacteria might have resulted from their slow growth rates during this cooler period.

MLR and the Bio-Env procedure indicated that P concentrations appeared to be an environmental factor influencing the phytoplankton cell abundance concentrations and community composition under both low and high river input conditions during the pulse study. Because high river input delivered abundant N to the estuary during the high river input, the system became P limited based on frequent DIN:P ratio values above 16 and as high as 204.3 observed at this time. Similar seasonal alterations in nutrient limitation have also been observed in estuaries throughout the world (Murrell et al. 2007). Furthermore, the Bio-Env procedure suggested that the distance from the diversion structure was an important factor during high river input on a pulse scale for all biological responses. This illustrates that the river input rate is influential in determining the environmental conditions from the inner to outer estuary on a short time scale.

As in the seasonal study, harmful cyanobacteria were observed and MCs were detected during the pulse study. MCs also followed the same trend as cyanobacterial abundances, which were higher

during low river input and toward the outer estuary. However, dissolved toxins detected in higher salinities of the outer estuary during the pulse study could be the result of toxins leaked from cyanobacteria cells into the water column in response to salinity stress, as shown to occur in a recent study by Tonk et al. (2007).

The results of the present research, for the first time, confirmed the presence of MCs in Breton Sound estuary. However, for numerous samples in which MCs were detected, there was a lack of corresponding MC-producing genera observed or enumerated. The potential reasons for the lack of MC-producing species observed in samples that had a detectable amount of MCs could be that an insufficient amount of sample was enumerated to observe large colonies of these species at low concentrations, the presence of other MC-producing species were not observed or identified, or physical disruption caused colonial MCs-producing genera, such as *Microcystis*, to break apart. The latter is more likely since the majority of cyanobacteria cells counted were singular coccoid cells around 2 µm in diameter. These cells may have been from colonies that were disrupted by mixing in the natural environment or homogenization of the sample water after collection for processing and examination on a Sedgewick Rafter slide. These small, singular cells could potentially be the source of the detected particulate and dissolved MC concentrations when MCs-producing species were not positively identified. Since MCs-producing genera were only counted by colonies, cell abundances of these toxic genera are most likely an underestimate. Counting colonies is a common practice for certain genera, such as *Microcystis*, and positive identification of singular cells from these colonies in natural phytoplankton assemblages is difficult, especially with the techniques utilized in this study.

Conclusions

In order to understand how a system functions, we must first be aware of how it responds to the influences placed upon it, whether man-made or natural. In the Breton Sound estuary, biological

responses to environmental changes are driven by nutrient availability and seasonal changes in salinity and water temperatures. During pulse changes in river input rates, P availability, as well as the distance from the diversion structure were important. The phytoplankton community composition shifted from cyanobacteria for a majority of the year to chlorophytes in response to changes in environmental conditions during high river input. Overall, the phytoplankton community of Breton Sound estuary appears to be moderated by temperature during high river input and nutrient availability during low river input. Grazing impacts on the phytoplankton abundance and community composition could also be significant in an estuarine environment (Bledsoe and Phlips 2000), such as Breton Sound estuary and should be further investigated. A study by Buyukates and Roelke (2005) suggested that increases in zooplankton under pulsed conditions may be preventing biomass accumulation in estuaries influenced by pulsed river input. Huisman et al. (2004) also observed phytoplankton community composition shifts in response to interactions between turbulence and light availability in a similar system, suggesting that further research including light availability measurements may provide additional information. Including more environmental variables and utilizing more frequent and dense spatial sampling could also help determine additional influences on the phytoplankton community and potentially aid in model validation.

Phycotoxins, including MCs, have now been detected in water samples, as well as primary and secondary consumers (chironomids, clams, blue crab, gulf menhaden and catfish), in two estuaries and coastal Louisiana (Garcia et al. 2010, Del Rio et al. 2010, Galvan et al. unpublished data), illustrating the need for continued monitoring and research to discover the underlying factors that control toxin production. The production of phycotoxins in the estuary and MCs detected during this research are not only a risk to fisheries, but also to human health as Louisiana's fish and shellfish industries feed millions of people. These MCs are toxic chemicals and several studies of sub-lethal chronic exposure of mammals to MCs suggest they can be tumor promoters and possible carcinogens (Falconer et al. 1994,

Ito et al. 1997, Grosse et al. 2006). Higher trophic levels, including humans, could possibly be exposed to MCs through multiple routes of exposure because these compounds are very stable in water and heat tolerant (Wannemacher 1989). Finding phycotoxins in the estuary illustrates the potential for harmful effects on consumers and the entire food web, as well as the need to understand what underlying conditions may increase the potential for toxin production.

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VITA

Jessica Czubakowski was born in 1984 to Jerry and Lori Czubakowski and grew up in the small typical Midwestern town of Stanley, Wisconsin, where she enjoyed spending time outdoors, reading and spending time with family. She spent her summers at Girl Scout camp and traveling with her family. This led to her love of traveling, the outdoors and her interest in the natural sciences. Upon finishing high school, Jessica began her college career at Carroll College in Waukesha, Wisconsin, in marine biology. As she learned more and her interests evolved, she transferred to the University of Wisconsin in La Crosse, Wisconsin. During her years at UW-La Crosse, she participated in research for over two years under Dr. Jasmine Saros studying nutrient limitation of phytoplankton in prairie salt lakes. She finished her bachelor's degree in biology with an aquatic science concentration and a chemistry minor. From La Crosse, Jessica journeyed to Louisiana State University in Baton Rouge, Louisiana, in pursuit of a master's degree advised by Dr. Sibel Bargu Ates, a phytoplankton ecologist researching harmful algal blooms. She is now a candidate for a Master of Science degree from LSU in August 2010. Jessica currently resides in Baton Rouge and still loves traveling, the outdoors, reading and the natural sciences.