2014

Cyanobacteria harmful algal blooms in South Louisiana estuaries : a synthesis of field research, management implications, and outreach

Emily Anne Smith
Louisiana State University and Agricultural and Mechanical College

CYANOBACTERIA HARMFUL ALGAL BLOOMS IN SOUTH LOUISIANA ESTUARIES: A SYNTHESIS OF FIELD RESEARCH, MANAGEMENT IMPLICATIONS, AND OUTREACH

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Oceanography and Coastal Sciences

by
Emily Anne Smith
B.S., University of Southern Mississippi, 2002
M.Ed., University of Tennessee at Chattanooga, 2010
May 2014

ACKNOWLEDGEMENTS

I want to thank my advisor Sibel Bargu, for being patient, understanding and a confidant; for calling me, “Soon to be Dr. Emily;” for laughing and listening; and most importantly pushing me to be the best I could be. I also want to thank the rest of my committee: Pam Blanchard, for understanding my education point of view and planting trees; John White, for asking me every time he saw me, “How’s the writing?”; Ralph Portier, for parlaying with me whenever I needed it; Chunyan Li, for making me remember my physical oceanography; and Brenda Tubaña, for being an outside perspective during this whole process.

I honestly could have not even started on this journey if it weren’t for the support of my family. When I didn’t know what to do with my life, I was reminded that I always wanted my PhD and that I should go out and get it—Thanks, Jim! Thanks to my mom and dad for always believing in my ability to achieve this dream, so they can now call me Doctor Daughter.

And friends…wow, there are so many that have been a part of this process. To Jenny Lentz, who started out as an advisor to the process and became a dear friend; Koray Ozhan, who started this journey with me and helped in sample processing for two of my chapters; Beth LaBone, who is quirky and quiet and one of the best people you can have on your trivia team; Elizabeth Robinson— for providing an ear to let me vent and provide counseling; all the rest—Kari, Gio, Tiffany, Lauren, Lilly, Greg, Ashley, and Sarah: I couldn’t have made it here without you; Jillybean—my best friend for almost half of my life, for always being on my side no matter what the situation was; Robert Ray at Louisiana Sea Grant for his artistic contribution in my educational brochure; and all of

the people who helped in sample collection and processing: Chase Weidert, Anthony Nguyen, and Nathan Nguyen.

To my research partner, Eric Roy—two summers of going out on the lake to sample. Our first year, I didn't know if we would ever not be tired. Our drives made me laugh, but we became friends to the point that quiet was comfortable. We worked in unison, ate on the boat, and gave each other looks when our captains would do strange things. Your help on my final research chapter was invaluable and I will never forget your time you gave up to help me with it.

This work was supported in part by the Louisiana Sea Grant College Program with funds from the National Oceanic and Atmospheric Administration Office of Sea Grant, Department of Commerce, under grant no. NA10OAR4170077, project no. E/MUR-01 (Undergraduate Research Opportunities Program); a National Science Foundation grant (Grant no. EAR-1139997); and the National Oceanic and Atmospheric Administration, Louisiana Sea Grant College Program under NOAA Grant award NA10OAR4170077. E.A.S was supported by a research fellowship from The Department of Oceanography and Coastal Sciences at Louisiana State University and LSU's Graduate School.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Estuaries are biologically productive and important habitats for several fisheries. However, human intervention has separated many estuaries from their needed freshwater source and the commonly used solution is to use diversions to regulate the flow. This episodic increase in nutrients into estuaries has sometimes led to the formation of freshwater cyanobacteria HABs (CyanoHABs). The goal of this dissertation was to look at a field research study of phytoplankton bloom dynamics; management implications for cyanobacteria entering estuaries; and an outreach effort in relation to residents knowledge about cyanobacteria and algae. The first study compared the phytoplankton bloom dynamics, specifically CyanoHABs, in Lake Pontchartrain, Louisiana (LA) during a diversion opening year and a non-opening year. While variations in freshwater flow were found to be important to determine which phytoplankton group dominates the system, species diversity within a group likely was regulated by the water source. During the large flow year (21.9 km^3) in 2011, chlorophytes and diatoms were the dominant groups. In 2012, with a much lower flow of 0.3 km^3, again chlorophytes and diatoms were dominant in the spring, but both years cyanobacteria numbers significantly increased in the late summer. The second study was conducted in a different estuary of LA (Breton Sound) to determine the salt tolerance of toxic cyanobacteria; the ability of oysters to feed on toxic cyanobacteria; and their ability to retain those toxins in their viscera. The study found that the cyanotoxin, microcystin (MC) was present, where the native oysters inhabit (<10 g kg^{-1}). When the oysters were exposed to toxic *Anabaena* sp. cells, they fed on these cells and retained MCs. This led to a concern for the public health. The last study surveyed fishermen about their knowledge of algae and HABs. This baseline data was used to create an educational brochure which was distributed to the marinas around

Lake Pontchartrain and Lac Des Allemandes. There was also a follow-up survey to determine the effectiveness of the educational brochure. Many of the people surveyed had a basic understanding of algae, but 60% were not familiar with harmful algal blooms.

CHAPTER 1: INTRODUCTION

The fascination of coastal living for humans extends back for millennia and evidence of human habitation along a coast dates back to at least 125 thousand years ago. This possible move from the interior of Africa is theorized to be due to increased arid conditions further inland thus prompting the community to look for other water sources (Walter et al. 2000). Early civilizations understood the need to take care of their water source in order to continue to be able to use those resources. “Where there is water there are fish. If we care for the water, the fish will take care of us.” This ecological understanding developed out of necessity and was passed on to future generations through such proverbs (Kurien 1998). This may be an over simplistic point of view now, but the simplicity speaks volumes.

Beyond initial reasons that humans migrated to the coasts, they continued to live there because of a constant food supply and later because of ease of transport and shipping of goods (Smith 1863; Gillis 2012). Human habitation of these coastal areas continued to grow once transportation of goods and services became the way to connect with other areas of the world (Gillis 2012). As the human population expanded, more areas along the coastline were utilized by humans for habitation and agricultural use, which decreased the amount of habitat for nature. Initially this was not seen as a problem, but years of manipulating the coastal system to fit human needs, has greatly damaged our environment including degradation to marshes, mangroves, and reef communities (Valiela et al. 2001; Brown et al. 2006). In an effort to preserve our coastal areas, many scientists and policy makers have started working on ways to reverse the damage caused (Barbier et al. 2008). The need for preservation of these coastal

communities is strong because they have continued to be where a large portion of the world's population lives, and now contains approximately one third of the world's population (Small and Nicholls 2003; Gillis 2012). Population along the coasts is only expected to continue to increase in numbers with an estimated more than 2 billion people by 2025 (Vörösmarty et al. 2000).

All along the coast are wetlands and estuaries that serve as a transition zone between the fresh and salt water. An estuary is a body of water that has an open connection with the ocean, but is also influenced by a fresh water source. These areas are geologically young, filters for nutrients and pollutants, biologically productive, and important habitats for a wide variety of organisms, where many species of fish and crustaceans are dependent for either all or part of their life (De Sylva et al. 1962; Chubb and Potter 1984, 1986; Schindler and Vallentyne 2008; Dando 2011). Estuaries are ever changing environments that can be difficult for organisms to adapt, thus lead to high diversity and shifts in the biological communities. Typically, estuaries receive fresh water, nutrients, and sediments from periodic flooding of the river source that is near, together with other smaller tributaries (Paerl and Justić 2011). Depending upon the size of the river and the amount of watershed, the estuarine areas that are fed by these rivers can be very dynamic and productive (Hopkinson and Vallino 1995). However, in recent times this access to the river has been cut off due to human intervention. Levee systems were built to contain the rivers in order to prevent flooding and have greatly changed the hydrology of estuaries and surrounding areas. One effort to restore fresh water, nutrients, and sediments to estuaries was the creation of diversions from the river (Rozas et al. 2005; Lane et al. 2007; Schindler and Vallentyne 2008). Diversions are constructed

differently depending on the main purpose that the ecosystem needs. Some were designed for flood prevention (Roy et al. 2013), or designed to direct freshwater and nutrients into wetlands (Martin 2002), while others have been designed for land creation (Lane et al. 1999) or irrigation for agriculture (Kingsford 2000). Diversion use has been successful in many instances when judged by what purpose they were built.

Human habitation and use together with industrial development along these land-water interfaces have contributed to the decline of water quality in estuaries, mainly due to excess nutrient and sewage inputs from land sources (Kennish 2002). Around the world, rivers are contributing to higher nutrient concentrations in their surrounding water bodies and leading to sudden increases in primary production (Richardson 1997). River flow can also play a role in water flushing rates and residence times which can also greatly affect the planktonic communities (Pinckney et al. 1998; Paerl and Justić 2011). Primary production in marine and in many estuarine systems is regulated by phytoplankton diversity and biomass (Paerl and Justić 2011). Phytoplankton plays a very important role in providing carbon and energy flow to these systems (Richardson 1997). Their growth is vital to supporting food webs and they determine the overall water quality of that system (Paerl 1988; Paerl and Justić 2011). Seasonally, phytoplankton biomass increases as a natural phenomenon, but the frequency and higher biomass accumulation over the past several decades is due to eutrophication, and can sometimes lead to negative outcomes such as hypoxia or harmful algal blooms (HABs) (Cloern 1999; Harding et al. 2002; Jochens et al. 2010).

When a large bloom of phytoplankton occurs it can be considered harmful. Some species of phytoplankton are able to produce toxins that can kill fish and shellfish or

bioaccumulate in higher trophic level organisms and possibly be a threat to humans through ingestion (Landsberg 2002). In coastal areas, wave actions may also aerosolize the toxins that could then be inhaled by humans or animals (Backer and McGillicuddy 2006). Some HAB species cause physical harm through spine structures that can cause irritation to fish gills and eventually cause suffocation. HABs can be also harmful simply when there is a high biomass accumulation that can lead to environmental damages, such as shading of submerged plant life, hypoxia, or anoxia (Rabalais et al. 1996; Rabalais et al. 2002; Glibert et al. 2005).

HABs have been expanding in frequency, duration, and location around the world. The increase in frequency of HAB detection could be from two possible reasons. One is new technologies that can detect these events more accurately and the availability of these technologies around the world (Glibert et al. 2005 and references therein). The other reason is the increase in eutrophication from run-off and hydrologic manipulations, such as diversions, that have severely impacted our coastal communities. It has been reported that the amount of nutrients that are entering into our coastal systems is giving rise to HABs in estuarine systems (Paerl et al. 2001). Increasing fertilizer use in agriculture, particularly in the past 30 years, is attributed to greater amounts of nutrients into the watersheds of rivers (Carmichael 2008; Gilpin et al. 2004). In freshwater and estuarine systems, cyanobacteria are the major harmful group that can proliferate when conditions are optimal for growth and cause cyanobacteria harmful algal blooms (CyanoHABs) (Downing et al. 2001; Glibert et al. 2005; Boyer 2008). CyanoHABs are also becoming more prevalent in coastal areas due to an increase in agricultural run-off, groundwater inputs, and weather perturbations (Paerl et al. 2001). Some species are salt-

tolerant (Tonk et al. 2007; Lehman et al. 2008) which allows them to be present in both saltwater and freshwater habitats (Miller et al. 2010). CyanoHABs can cause many of the same problems as marine HAB species, but can also have more of a direct connection with humans due to the areas in which they reside. In some areas of the world, CyanoHABs have become a persistent problem throughout the year. This continual duration of CyanoHABs has caused problems particularly when the freshwater systems are the main drinking source for many cities (Paerl et al. 2011). Cyanobacteria can produce a variety of toxins including microcystins, anatoxins, and saxitoxins. These toxins have a wide range of mechanisms that can affect aquatic organisms and humans, either through direct contact or from ingesting the cells containing the toxin. In many mammals, fish, mollusks, and zooplankton, these toxins can cause death or developmental and behavioral abnormalities (Råbergh et al. 1991; Oberemm et al. 1999; Miller et al. 2010), while in humans the effects can be a simple skin irritation or more severely gastrointestinal problems or even death (Codd 1995; Codd et al. 2005; Metcalf and Codd 2009).

Louisiana is a unique setting that includes many estuaries and wetlands that are essential to highly productive seafood grounds as well as to the humans that live next to these water bodies. The Mississippi (MS) River feeds the coastal system and has a watershed of 41% of the continuous United States (Rabalais et al. 1996). This very large watershed also includes extensive agricultural lands and the subsequent fertilizer run-off. The amount of excess nutrients that are then delivered downstream have caused many problems for the northern Gulf of Mexico coastal system, including increased phytoplankton blooms that have led to a large hypoxic zone (Rabalais et al. 2002;

Rabalais et al. 2010). The estuaries in Louisiana have been greatly modified by levee systems and diversions, as previously mentioned. Some of the diversions are operated year round to regulate the salinity of the estuary. For example, the Caernarvon diversion in Breton Sound Estuary was built to help regulate salinity for oyster production and has been successful at this (Chatry et al. 1983; Snedden et al. 2007). There are even additional benefits from this diversion that include sediment deposition which helps build up this wetland (Wheelock 2003). Davis Pond is a diversion that feeds the Barataria Estuary and is also built for salinity regulation against salt water intrusion from the Gulf of Mexico (Das et al. 2012). A different type of diversion that is operated year round is the Atchafalaya River diversion, which diverts approximately 30% of the MS River. This diversion was built to prevent the MS River from completely leaving its current course as part of the natural deltaic switching that rivers periodically engage (Roberts 1998). Two other diversions that were built only for flood prevention of downriver cities are the Bonnet Carré Spillway (BCS), in South Louisiana that opens into Lake Pontchartrain, a large oligohaline estuary, and the Morganza Spillway, also in southern Louisiana to the northwest of Baton Rouge opens into a wetlands area. However, even when these many diversions are used appropriately for their intended purpose, increased nutrient input from river discharge also promoted higher than usual phytoplankton biomass and occurrences of CyanoHABs (Turner et al. 2004; Czubakowski 2010; Garcia et al. 2010; Bargu et al. 2011). The presence of toxin cyanobacteria and the associated toxins in several Louisiana estuaries has already been documented including blue crab contamination in Lac Des Allemandes (Garcia et al. 2010); high levels of cyanobacteria

abundance in Breton Sound (Czubakowski 2010); and CyanoHABs in Lake Pontchartrain following BCS openings (Turner et al. 2002; Bargu et al. 2011; Roy et al. 2013).

Any field of science is not single-dimensional; therefore the state of the coastal systems needs to be approached in a multi-dimensional way. The following chapters look at the increase of CyanoHABs in estuaries from a pure research approach, a management approach, and an educational outreach approach. Studying the problem of CyanoHABs in LA estuaries using these different approaches gives scientists and managers a better understanding of how interconnected the issue of diversions, seafood contamination, and human health are. This is especially important considering the Louisiana State Master Plan includes more diversions to be built along the MS River. This study investigates how our changing environment is affecting the phytoplankton community dynamics and subsequently how toxic species of cyanobacteria may be contaminating seafood. It also examines the knowledge level of humans living in near these estuaries on CyanoHABs and algae in general and the potential impact on their lives.

As mentioned previously, river water discharge events can potentially translate into enhanced primary production, phytoplankton community shifts, and potential harmful/toxic bloom formation. Lake Pontchartrain is a very large estuary and has multiple inputs for freshwater from tributaries and river run-off. In the past, when the spillway was opened it has led to toxic cyanobacteria blooms including species of *Microcystis* and *Anabaena,* following the closing of the spillway (Turner et al. 2002; Bargu et al. 2011; Roy et al. 2013). Therefore, there was great concern that another CyanoHAB would form with the most recent opening of the BCS in 2011. In the first research chapter (Chapter 2), phytoplankton bloom dynamics in conjunction with the

opening of the 2011 BCS are examined in Lake Pontchartrain, LA. In addition, further research was needed on a more regular basis with spaced spatial–temporal scales during non-diversion years to determine if there are any direct impacts of river water nutrient input on the development of phytoplankton blooms, specifically toxic cyanobacteria, in the estuary. Therefore, our study looks at both the spillway opening year that is regulated by river flow and a non-spillway opening year, when main flow comes from tributaries, in order to understand how the phytoplankton community may respond over time and space.

In the second research chapter (Chapter 3), another South Louisiana estuary, Breton Sound, was chosen for the study specifically concerning oyster contamination from cyanotoxins. The oyster fishery provides millions of dollars towards Louisiana revenue and is supported by a large number of both private and state oyster beds that may be adversely affected by the presence of CyanoHAB toxins. This estuary receives nutrient-rich MS River water via the Caernarvon diversion structure at the northernmost point of the estuary, and the Gulf of Mexico at the southern end supplying salt water. This creates a salinity range that is excellent for oyster growth, due to the fact that oysters grow best in salinities of 10-30 g kg^{-1}. A recent study by Czubakowski (2010) has shown that the phytoplankton community in Breton Sound estuary was dominated by cyanobacteria for early and late summer months and the cyanotoxin, microcystin, was detected throughout the estuary with highest concentrations found to be in downstream waters entering the Breton Sound coastal waters. Therefore, the main goals of this research were to determine whether toxic cyanobacteria can grow successfully in more saline coastal stations of Louisiana estuaries, and whether they have the potential to

contaminate oysters in that area. Laboratory experiments were conducted to determine if oysters would feed on these toxin producing species and to determine if they would retain those toxins.

In the final research chapter (Chapter 4), local fishermen were interviewed around Lake Pontchartrain and Lac Des Allemandes to gather baseline data about their knowledge of algae and HABs. Humans that live in communities along coastal systems have a personal connection with the water and they need to understand how fast the environment in which they live is changing. Scientists need to find more effective ways to disseminate information accurately to the public on topics such as CyanoHABs, and the potential problems they can cause, but also ways that people can be involved in protecting their environment by understanding what causes these CyanoHABs. In order to increase awareness of potential contamination from CyanoHABs, the information needs to have correct terminology and explanations to help with the public's understanding of scientific concepts. For example, red tide was the generally accepted term for any phytoplankton that produced toxins, but now harmful algal bloom is the more widely accepted terminology (Glibert et al. 2005). This shift helps people to understand that not all harmful algal blooms cause red coloration and not all toxic blooms are colored, but that it is a broader and more diverse problem. Fisheries are very important in Louisiana not only as a local food source but also economically for the nation. Louisiana is one of the largest exporters for seafood in the United States (Banks 2010). This is also socially important because many residents of Louisiana are dependent upon the seafood industry for their income. Therefore, the need to have these fishermen understand more about how their product can be contaminated is crucial for their

immediate health and anyone who may purchase seafood from them. The surveys in this study also included a visual aspect to test whether fishermen could visually recognize a HAB or CyanoHAB because a large component of fishing is visual, meaning fishermen need to be able to recognize when a water condition is not favorable for fish. This initial information gathered was then used to create an educational brochure that was distributed to the same areas as the initial surveys. Follow-up interviews were also conducted to find out if the educational brochures were effective in helping people understand the role that algae plays in the environment and the difference between beneficial algae and HABs. There have been outreach efforts in other parts of the country concerning HABs, but no concerted effort in Louisiana for CyanoHABs. This is a very big concern given how much seafood and interactions the residents of Louisiana have with these estuaries on a daily basis.

References

Anderson, D., P. Glibert, and J. Burkholder. 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. *Estuaries* 25: 704-726.

Backer, L., and D. McGillicuddy. 2006. Harmful algal blooms. *Oceanography* 19: 94.

Banks, P. 2010. Oyster Stock Assessment Report of the public oyster areas in Louisiana. *In* L. W. a. Fisheries [ed.].

Barbier, E. B., E. W. Koch, B. R. Silliman, S. D. Hacker, E. Wolanski, J. Primavera, E. F. Granek, et al. 2008. Coastal Ecosystem-Based Management with Nonlinear Ecological Functions and Values. *Science* 319: 321-323.

Bargu, S., J. White, C. Li, J. Czubakowski and R. Fulweiler 2011. Effects of freshwater input on nutrient loading, phytoplankton biomass, and cyanotoxin production in an oligohaline estuarine lake. Hydrobiologia 661: 377-389.

Boyer, G. 2008. Cyanobacterial Toxins in New York and the Lower Great Lakes Ecosystems. *In* H. K. Hudnell [ed.], Cyanobacterial Harmful Algal Blooms: State

of the Science and Research Needs, vol. 619, Advances in Experimental Medicine and Biology, 153-165. Springer New York.

Brown, C., E. Corcoran, P. Herkenrath, and J. E. Thonell. 2006. Marine and Coastal Ecosystems and Human Well-Being; A Synthesis Report Based on the Findings of the Millennium Ecosystem Assessment. United Nations Environment Programme, Nairobi.

Carmichael, W. 2008. A world overview—One-hundred-twenty-seven years of research on toxic cyanobacteria—Where do we go from here? In Cyanobacterial harmful algal blooms: State of the science and research needs, 105-125: Springer.

Carmichael, W. 2001. Health Effects of Toxin-Producing Cyanobacteria: 'The CyanoHABs'. *Human & Ecological Risk Assessment* 7: 1393.

Chatry, M., R. J. Dugas, and K. A. Easley. 1983. Optimum salinity regime for oyster production on Louisiana's state seed grounds. *Contributions in Marine Science* 26: 81-94.

Chorus, I., and J. Bartram. 1999. Toxic cyanobacteria in water a guide to their public health consequences, monitoring, and management. E & FN Spon, London; New York.

Chubb, C. F. and I. C. Potter 1984. The reproductive biology and estuarine movements of the gizzard shad, Nematalosa vlaminghi (Munro). *Journal of Fish Biology* 25: 527-543.

Chubb, C. F. and I. C. Potter 1986. Age, growth and condition of the Perth herring, Nematolosa vlaminghi (Munro) (Dorosomatinae), in the Swan Estuary, south-western Australia. *Marine and Freshwater Research* 37: 105-112.

Cloern, J. E. 1999. The relative importance of light and nutrient limitation of phytoplankton growth: a simple index of coastal ecosystem sensitivity to nutrient enrichment. *Aquatic Ecology* 33: 3-15.

Codd, G. A. 1995. Cyanobacterial toxins: Occurrence, properties and biological significance. *Water Science and Technology* 32: 149-156. doi: 10.1016/0273-1223(95)00692-3

Codd, G. A., J. Lindsay, F. M. Young, L. F. Morrison and J. S. Metcalf 2005. Harmful cyanobacteria: From mass moralities to management measures. In Harmful cyanobacteria, eds. J. Huisman, H.C.P. Matthijs and P.M. Visser, 1-23. Netherlands: Springer.

Czubakowski, J. 2010. Estuarine phytoplankton response to annual and manipulated river inputs. Master’s Thesis Baton Rouge, La.: Louisiana State University.

Dando, P. R. 2011. Site fidelity, homing and spawning migrations of flounder Platichthys flesus in the Tamar estuary, South West England. *Marine Ecology-Progress Series* 430: 183-196. doi: 10.3354/meps09116

Das, A., D. Justic, M. Inoue, A. Hoda, H. Huang and D. Park 2012. Impacts of Mississippi River diversions on salinity gradients in a deltaic Louisiana estuary: Ecological and management implications. *Estuarine, Coastal and Shelf Science* 111: 17-26. doi: http://dx.doi.org/10.1016/j.ecss.2012.06.005

De Sylva, D., C. Shuster and F. Kalber 1962. Fishes and ecological conditions in the shore zone of the Delaware River estuary: with notes on other species collected in deeper water. In Fishes and ecological conditions in the shore zone of the Delaware River estuary: with notes on other species collected in deeper water, 164. Newark: Dept. of Biological Sciences, University of Delaware.

Downing, J. A., S. B. Watson, and E. McCauley. 2001. Predicting Cyanobacteria dominance in lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 1905-1908.

Engström-Öst, J., S. Repka, and M. Mikkonen. 2011. Interactions between plankton and cyanobacterium Anabaena with focus on salinity, growth and toxin production. *Harmful Algae* 10: 530-535.

Garcia, A. C., S. Bargu, P. Dash, N. N. Rabalais, M. Sutor, W. Morrison, and N. D. Walker. 2010. Evaluating the potential risk of microcystins to blue crab (Callinectes sapidus) fisheries and human health in a eutrophic estuary. *Harmful Algae* 9: 134-143.

Gillis, J. R. 2012. The Human Shore. The University of Chicago Press, Chicago.

Gilpin, LC, Keith Davidson and E Roberts 2004. The influence of changes in nitrogen: silicon ratios on diatom growth dynamics. *Journal of Sea Research* 51: 21-35.

Glibert, P. M., D. M. Anderson, P. Gentien, E. Graneli, and K. G. Sellner. 2005. The global, complex phenomena of harmful algal blooms.

Harding Jr, L. W., M. E. Mallonee, and E. S. Perry. 2002. Toward a Predictive Understanding of Primary Productivity in a Temperate, Partially Stratified Estuary. *Estuarine, Coastal and Shelf Science* 55: 437-463.

Hopkinson, C., and J. Vallino. 1995. The relationships among man's activities in watersheds and estuaries: A model of runoff effects on patterns of estuarine community metabolism. *Estuaries* 18: 598-621.

Hupp, C., C. Demas, D. Kroes, R. Day, and T. Doyle. 2008. Recent sedimentation patterns within the central Atchafalaya Basin, Louisiana. *Wetlands* 28: 125-140.

Ibelings, B. W., and I. Chorus. 2007. Accumulation of cyanobacterial toxins in freshwater “seafood” and its consequences for public health: A review. *Environmental Pollution* 150: 177-192.

Jochens, A. E., T. C. Malone, R. P. Stumpf, B. M. Hickey, M. Carter, R. Morrison, J. Dyble, et al. 2010. Integrated Ocean Observing System in Support of Forecasting Harmful Algal Blooms. *Marine Technology Society Journal* 44: 99-121.

Kennish, M. J. 2002. Environmental threats and environmental future of estuaries. *Environmental Conservation* 29: 78-107.

Kingsford, R. T. 2000. Ecological impacts of dams, water diversions and river management on floodplain wetlands in Australia. *Austral Ecology* 25: 109-127.

Kurien, J. 1998. Traditional Ecological Knowledge and Ecosystem Sustainability: New meaning to Asian coastal proverbs. *Ecological Applications* 8: S2-S5.

Landsberg, J. H. 2002. The Effects of Harmful Algal Blooms on Aquatic Organisms. *Reviews in Fisheries Science* 10: 113-390.

Lane, R., J. Day, B. Marx, E. Reyes, E. Hyfield, and J. N. Day. 2007. The effects of riverine discharge on temperature, salinity, suspended sediment and chlorophyll a in a Mississippi delta estuary measured using a flow-through system. *Estuarine Coastal & Shelf Science* 74: 145-154.

Lane, R., J. Day, and B. Thibodeaux. 1999. Water quality analysis of a freshwater diversion at Caernarvon, Louisiana. *Estuaries and Coasts* 22: 327-336.

Lehman, P., G. Boyer, M. Satchwell and S. Waller 2008. The influence of environmental conditions on the seasonal variation of <i>Microcystis cell density and microcystins concentration in San Francisco Estuary. *Hydrobiologia* 600: 187-204. doi: 10.1007/s10750-007-9231-x

Martin, J. F. 2002. Emergy valuation of diversions of river water to marshes in the Mississippi River Delta. *Ecological Engineering* 18: 265-286.

Metcalf, J. S. and G. A. Codd 2009. Cyanobacteria, neurotoxins and water resources: Are there implications for human neurodegenerative disease? *Amyotrophic Lateral Sclerosis* 10: 74-78. doi: 10.3109/17482960903272942

Miller, M. A., R. M. Kudela, A. Mekebri, D. Crane, S. C. Oates, M. T. Tinker, M. Staedler, W. A. Miller, S. Toy-Choutka, C. Dominik, D. Hardin, G. Langlois, M. Murray, K. Ward and D. A. Jessup 2010. Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters. *PLoS ONE* 5: 1-11. doi: 10.1371/journal.pone.0012576

Oberemm, A., J. Becker, G. A. Codd and C. Steinberg 1999. Effects of cyanobacterial toxins and aqueous crude extracts of cyanobacteria on the development of fish

and amphibians. *Environmental Toxicology* 14: 77-88. doi: 10.1002/(SICI)1522-7278(199902)14:1<77::AID-TOX11>3.0.CO;2-F

Paerl, H. 1988. Nuisance Phytoplankton Blooms in Coastal, Estuarine, and Inland Waters. *Limnology and Oceanography* 33: 823-847.

Paerl, H., R. Fulton, P. Moisander, and J. Dyble. 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *The Scientific World Journal* 1: 76-113.

Paerl, H., and D. Justić. 2011. 6.03 Primary Producers: Phytoplankton Ecology and Trophic Dynamics in Coastal Waters.

Paerl, H., H. Xu, M. J. McCarthy, G. Zhu, B. Qin, Y. Li and W. S. Gardner 2011. Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): The need for a dual nutrient (N & P) management strategy. *Water Research* 45: 1973-1983. doi: 10.1016/j.watres.2010.09.018

Pinckney, J. L., H. W. Paerl, M. B. Harrington, and K. E. Howe. 1998. Annual cycles of phytoplankton community-structure and bloom dynamics in the Neuse River Estuary, North Carolina. *Marine Biology* 131: 371-381.

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

Rabalais, N., R. E. Turner, D. Justić, Q. Dortch, W. Wiseman, and B. Sen Gupta. 1996. Nutrient changes in the Mississippi River and system responses on the adjacent continental shelf. *Estuaries* 19: 386-407.

Rabalais, N., R. Diaz, L. A. Levin, R. E. Turner, D. Gilbert, and J. Zhang. 2010. Dynamics and distribution of natural and human-caused hypoxia. *Biogeosciences* 7: 585-619

Rabalais, N., R. E. Turner, Q. Dortch, D. Justic, V. Bierman, Jr., and W. Wiseman, Jr. 2002. Nutrient-enhanced productivity in the northern Gulf of Mexico: past, present and future. *Hydrobiologia* 475-476: 39-63.

Richardson, K. 1997. Harmful or Exceptional Phytoplankton Blooms in the Marine Ecosystem. *In* J. H. S. B. a. A. J. Southward [ed.], *Advances in Marine Biology*, 31, 301-385. Academic Press.

Roberts, H. H. 1998. Delta switching: early responses to the Atchafalaya River diversion. *Journal of Coastal Research*: 882-899.

Roy, E., J. White, E. A. Smith, S. Bargu, and C. Li. 2013. Estuarine Ecosystem Response to Three Large-Scale Mississippi River Flood Diversion Events. *Science of the Total Environment*: 374-387.

Rozas, L. P., T. J. Minello, I. Munuera-Fernández, B. Fry, and B. Wissel. 2005. Macrofaunal distributions and habitat change following winter–spring releases of freshwater into the Breton Sound estuary, Louisiana (USA). *Estuarine, Coastal and Shelf Science* 65: 319-336.

Råbergh, C. M. I., G. Bylund, and J. E. Eriksson. 1991. Histopathological effects of microcystin-LR, a cyclic peptide toxin from the cyanobacterium (blue-green alga) Microcystis aeruginosa on common carp (Cyprinus carpio L.). *Aquatic Toxicology* 20: 131-145.

Schindler, D. W., and J. R. Vallentyne. 2008. The Algal Bowl; Overfertilization of the World's Freshwaters and Estuaries. Houghton Boston Printers, Canada.

Small, C., and R. J. Nicholls. 2003. A Global Analysis of Human Settlement in Coastal Zones. *Journal of Coastal Research* 19: 584-599.

Smith, A. 1863. An Inquiry into the Nature and Causes of the Wealth of Nations.

Snedden, G. A., J. E. Cable, C. Swarzenski, and E. Swenson. 2007. Sediment discharge into a subsiding Louisiana deltaic estuary through a Mississippi River diversion. *Estuarine, Coastal and Shelf Science* 71: 181-193.

Srivastava, A. K., P. Bhargava, R. Thapar, and L. C. Rai. 2008. Salinity-induced physiological and proteomic changes in Anabaena doliolum. *Environmental and Experimental Botany* 64: 49-57.

Tonk, L., K. Bosch, P. M. Visser, and J. Huisman. 2007. Salt tolerance of the harmful cyanobacterium Microcystis aeruginosa. *Inter-Research*.

Turner, R. E., Q. Dortch, D. Justic and E. M. Swenson 2002. Nitrogen loading into an urban estuary: Lake Pontchartrain (Louisiana, U.S.A.). *Hydrobiologia* 487: 137-152. doi: 10.1023/A:1022994210268

Valiela, I., J. L. Bowen, and J. K. York. 2001. Mangrove Forests: One of the World's Threatened Major Tropical Environments. *BioScience* 51: 807-815.

Vörösmarty, C. J., P. Green, J. Salisbury, and R. B. Lammers. 2000. Global water resources: vulnerability from climate change and population growth. *Science* 289: 284.

Walter, R. C., R. T. Buffler, J. H. Bruggemann, M. M. M. Guillaume, S. M. Berhe, B. Negassi, Y. Libsekal, et al. 2000. Early human occupation of the Red Sea coast of Eritrea during the last interglacial. *Nature* 405: 65-69.

Wheelock, K. 2003. Pulsed river flooding effects on sediment deposition in Breton Sound estuary, Louisiana, University of Rhode Island.

CHAPTER 2: PHYTOPLANKTON COMMUNITY DYNAMICS IN AN OLIGOHALINE ESTUARY COMPARING EPISODIC AND CONTINUOUS FRESHWATER INPUT INFLUENCES

Abstract

Hydrologic variability in estuaries due to storm events, drought, and human manipulation of water systems can greatly influence both nutrient availability and phytoplankton populations. This is especially true for some shallow estuaries where residence time is altered substantially. In Louisiana, freshwater diversions are used to achieve multiple objectives including flood control. The Bonnet Carré Spillway is a flood management structure used to protect the city of New Orleans by diverting Mississippi River water into the Pontchartrain Estuary. Nutrient loading associated with these events consistently produces relatively high phytoplankton biomass. However, the formation of toxic cyanobacteria blooms is variable. In this study, we build upon previous research to increase understanding of biological response in Lake Pontchartrain under different hydrologic forcing. We compared the phytoplankton community dynamics during the latest diversion year (2011) and a non-diversion year (2012). In 2011, the majority of the phytoplankton abundance in the spring was attributable to chlorophytes and centric diatoms and then shifted to a majority of cyanobacteria in the summer. However, the biomass was mainly attributable to dinoflagellates throughout the entire year with cyanobacteria only contributing 3%. In 2012, there was a similar trend of chlorophytes, pennate diatoms and centric diatoms in the spring and then an increase in cyanobacteria in the summer. This year the overall biomass was much lower than 2011, but again dinoflagellates were the major contributor to biomass throughout the entire sampling period. Although cyanobacteria numbers have increased significantly in

the summer time, we did not observe a toxic bloom in either 2011 or 2012. Factors related to hydrologic variability, including flushing in 2011 (21.9 km^3 input from the opening of the spillway) and dry conditions in 2012 (0.4 km^3 input from tributaries), together with changes in nutrient availability likely influenced these observations.

Introduction

Estuaries are highly productive coastal areas, which are affected both by marine (tides) and freshwater inputs (tributaries, rivers, run-off, and intrusions) (Kennish 2002 and references therein). The freshwater inputs can be both continuous and episodic, depending upon the source. While continuous inputs are generally from small tributaries that serve the immediate drainage basin, episodic inputs are usually in the form of extreme run-off or from a specific freshwater source (i.e. a river) that can vary greatly in the amount depending on initial spring flood discharge and overall precipitation (Rabalais et al. 1996). Precipitation patterns have increased in variability between extreme drought and extreme flooding due to global warming (IPCC 2007; Paerl and Huisman 2008). Resulting changes in freshwater input can in turn modify the water column nutrient levels, flushing rates, residence times, and overall volume of an estuary (Vilhena et al. 2010).

In some estuaries, the main river freshwater source has been cut off through hydrologic human manipulations. Natural watersheds have been altered for flood prevention by building levees or other manmade structures to keep the river on a specific course. Diversions are used as a remediation technique to help restore natural flow to these estuaries. Each diversion is built with a specific remediation concept and can be

used for regulation of freshwater, sediment or nutrient inputs (Lane et al. 1999; Rozas et al. 2005; Piazza and La Peyre 2007; Schindler and Vallentyne 2008). Estuaries and wetlands not receiving the annual natural amounts of freshwater but rather exposed episodically have been experiencing sudden changes in salinity, nutrient loadings, and sediment loads, which in turn affect the biological communities (Kennish 2002).

Phytoplankton are considered to be one of the major primary producers in estuaries and they are particularly susceptible to community shifts corresponding to changes in their environment. Systems that experience episodic river runoff due to precipitation (directly or from increased upstream precipitation warranting the need to open diversions downstream), the shift can be unpredictable (Beaugrand et al. 2000). Water flushing rates, changes in temperature, salinity, turbidity, nutrient and light availability, as well as selective grazing, competition, and allelopathy are all associated parameters that cause shifts in phytoplankton communities (Paerl 1988; Cloern 1999; Quinlan and Phlips 2007). Specifically nutrient pulsing associated with these episodic high freshwater discharge events can result in large blooms of certain phytoplankton groups (Heisler et al. 2008; Cook et al. 2010). Different phytoplankton groups are known to require different optimal conditions and can respond differently to alterations of their environment. Correspondingly, estuaries are generally dominated by diatoms and chlorophytes in the spring with a shift to cyanobacteria in the summer (e.g. Pinckney et al. 1998; Rocha et al. 2002; Bargu et al. 2011). Cyanobacteria dominance in early summer months is usually stimulated by warmer temperatures, a more stable water column, and a nutrient enrichment event (increased run-off or episodic pulse). During a storm event, resuspension of nutrients from sediments can occur and stimulate primary

production as well (Paerl 1988). When nutrients get too low to support large phytoplankton biomass in later summer months, they can continue to be the major phytoplankton group, especially some cyanobacteria species that are able to fix atmospheric nitrogen (Bouvy et al. 1999; Paerl et al. 2001; Paerl and Huisman 2008). Elevated biomass due to periodic nutrient enhancement in freshwater and estuarine systems can be of concern when toxin-producing species, like some species of cyanobacteria, are the dominant species. Toxic cyanobacteria blooms can significantly cause deterioration in water quality and their toxins may have negative health impacts on higher trophic levels through direct contact or ingestion.

Louisiana is a very unique setting for estuaries in that all of the estuaries receive their freshwater inflow from the Mississippi (MS) River that has many man-made manipulations (levees and diversions) for flood prevention. The two main Louisiana estuaries that receive MS River water are Breton Sound and Barataria Basin. Another estuary basin that was historically connected to the river is the Pontchartrain basin. The MS River has a drainage basin of 41% of America, including a large amount of agriculture run-off increasing the nutrient loading substantially (Rabalais et al. 1996; Dortch et al. 1999; Rabalais et al. 2010; Roy et al. 2013), and annually delivers 1.57×10^{7} tons $year^{-1}$ nitrogen to the Gulf of Mexico (Duan et al. 2014). This large amount of nutrients has been shown to cause regional problems such as hypoxia and a rise of harmful algal blooms (HABs) (Rabalais et al. 1996). There are previous studies showing the presence of cyanobacteria HABs (CyanoHABs) in Louisiana estuaries as a potential contamination source (Mize and Demchek 2009; Czubakowski 2010; Bargu et al. 2011). Two cyanobacteria genera of concern in Louisiana estuaries are certain species of

Anabaena and *Microcystis* (Dortch et al. 1999; Garcia et al. 2010; Czubakowski 2010; Bargu et al. 2011). Breton Sound Estuary has experienced high cyanobacteria numbers (up to $3.7x10^5$ cells L^{-1}) during the summer and fall months (Czubakowski 2010); and Lac Des Allemandes in Barataria Estuary has also had blooms of *Microcystis* sp. (up to 2.4×10^6 colonies L^{-1}) and *Anabaena* sp. (up to 5×10^6 cells L^{-1}) in spring and summer months, respectively (Garcia et al. 2010). Differences in the timing of occurrence between these two cyanobacteria species are largely driven by nitrogen biogeochemistry. *Microcystis* spp. depend on bioavailable nitrogen and they are typically observed in Louisiana estuaries in early summer when run-off or an episodic event has provided a nitrogen source to the water column. *Anabaena* spp. are able to fix atmospheric nitrogen under nitrogen limitation, and become the dominant species after the nitrogen is depleted in later summer months (White et al. 2009; Czubakowski 2010; Garcia et al. 2010; Bargu et al. 2011). The toxins that certain species of *Microcystis* and *Anabaena* can potentially produce are anatoxins, microcystins, and saxitoxins (Sivonen and Jones 1999). These toxins can cause neuro- and hepatotoxicity when the cells are ingested (Ibelings and Chorus 2007), and they can also cause dermal irritations from recreational use and contaminate drinking water sources (Chorus and Bartram 1999; Paerl et al. 2011).

Lake Pontchartrain is a large, oligotrophic estuary in South Louisiana that receives both continuous and episodic freshwater inputs. Run-off from New Orleans and fresh water inputs from several northern tributaries provide a continuous source of freshwater with seasonal variability (Wu and Xu 2007; Pinckney et al. 2009). Occasionally, episodic freshwater inflows occur in the form of Bonnet Carré Spillway (BCS) openings. The BCS is used to divert a large amount of Mississippi River water

into Lake Pontchartrain when the spring flood stage endangers the city of New Orleans, LA. High cell numbers of toxic cyanobacteria and their associated toxins have been observed with variability in time and space following past BCS openings (Dortch and Achee 1998, Turner et al. 2002; White et al. 2009; Bargu et al. 2011). The impact of a CyanoHAB in this estuary can be great considering it is used extensively for recreation and fishing, both commercial and sport. The Lake Pontchartrain phytoplankton community has historically only been studied when a BCS diversion event or a weather event, such as a hurricane, occurs. Understanding the phytoplankton community dynamics during years with relatively low and continuous freshwater input is necessary to fully understand the effects of diversion-related perturbations.

The BCS was most recently opened between May 9 and June 20, 2011 and the diverted high flow of river water influencing the majority of the estuary (Roy et al. 2013). High nutrient loading was expected to promote primary production, phytoplankton community shifts, and potentially toxic cyanobacterial blooms. During this event in 2011, we monitored water quality, environmental parameters, and corresponding changes in the phytoplankton abundance, biomass, and community under the influence of river water. The following year in 2012, a non-spillway or “normal” year, we monitored the lake’s environmental conditions similar to 2011, and compared the findings on phytoplankton responses to their changing environment in 2011 to 2012. The objectives of our study were to: (1) determine the effects of the episodic large freshwater influx associated with the 2011 BCS opening on the Lake Pontchartrain phytoplankton community and assess controls on toxic cyanobacteria blooms during these episodic events, and (2) determine the baseline information on the dynamics of the natural phytoplankton community in a

non-diversion year with relatively low and continuous freshwater input from tributaries (2012).

Materials and Methods

Study Site

Lake Pontchartrain has a surface area of 1637 km^2 and is a 6 km^3, shallow (mean depth = 3.7 m), and an oligohaline (salinity = 2-9 psu) estuary with in southeast Louisiana receiving water input from the fresher Lake Maurepas, several northern tributaries, and urban run-off from New Orleans (Figure 2.1) (Turner et al. 2004; Hastings 2009). Water exiting Lake Pontchartrain eventually enters the Gulf of Mexico following passage through Lake Borgne.

Bonnet Carré Spillway and northern tributary water discharge data

Discharge data from the BCS in 2011 was provided by the US Army Corps of Engineers. Daily discharge data for five northern tributaries were obtained from the USGS, and corrected to represent the entire watershed using methods outlined in Roblin (2008). The five tributaries were the Amite River near Denham Springs (USGS Station 7378500); the Tangipahoa River at Robert (USGS Station 7375500); the Tchefuncte River near Folsom (USGS Station 7375000); the Tickfaw River at Holden (USGS Station 7376000); and the Natalbany River at Baptist (USGS Station 7376500).

2.3.3 Sample Collection

The sampling regime involved multiple components. First, a 3-station, 30-km transect extending northeastwardly from the BCS inflow to the Lake Pontchartrain causeway bridge was utilized to monitor water quality and phytoplankton community

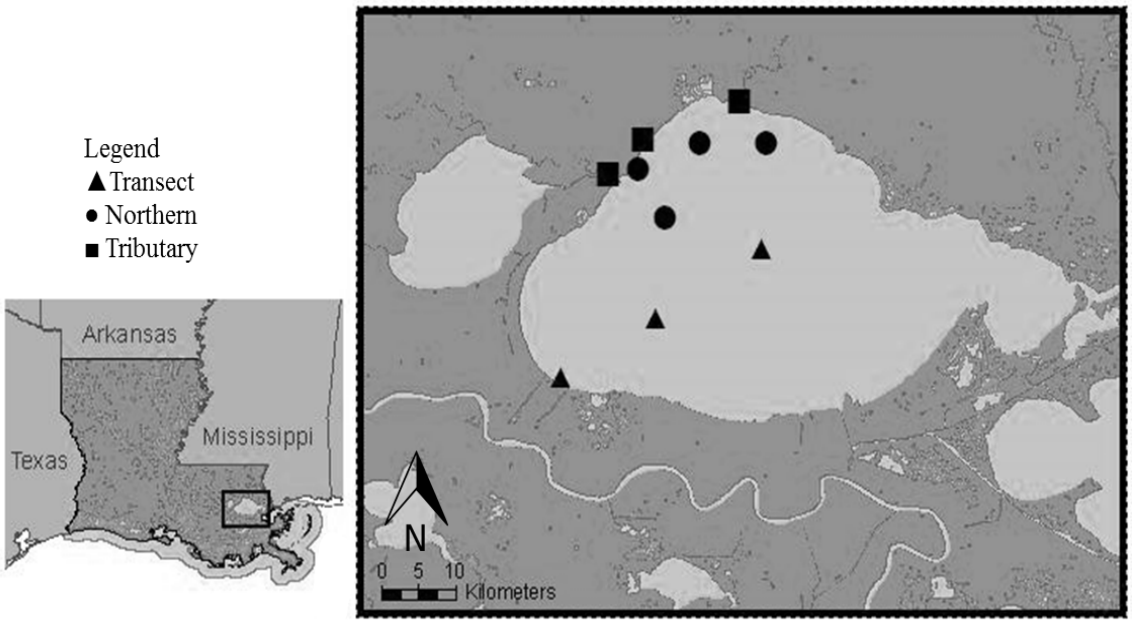


Figure 2.1. Map of the sampling stations for 2011 and 2012 in Lake Pontchartrain. Transect (▲) and northern (●) stations were sampled in 2011 and 2012, and tributary (■) stations were only added for 2012.

dynamics before, during, and after the 2011 BCS opening event (Figure 2.1). This transect was again monitored during March-August in 2012. During the 2011 BCS opening, two of the three stations were located directly within the river water plume, while the other station was within the area where diverted river water interacted more with estuarine water. Secondly, additional stations nearer to the northern shore were sampled in 2011 and 2012 (Figure 2.1) to: (1) expand the spatial coverage of the study and (2) assess the question of whether or not tributary-dominated northern waters host a potential seed population of toxic cyanobacteria. Finally, additional stations within three northern tributaries (Manchec Pass, Tangipahoa River, and Tchefuncte River) were added in 2012 (Figure 2.1). The sampling period for both years was divided into different time periods of early spring (March and April), late spring (May and early June, which corresponds to the opening of the spillway in 2011), early summer (late June and July,

post-spillway opening of 2011) and late summer (August and September). This was done so that comparisons could be made between the two sampling years.

Water samples were analyzed *in situ* for temperature, salinity, dissolved oxygen (DO), Secchi depth, and pH using a handheld YSI (Model 556). Whole water samples were collected in acid washed and cleaned polyethylene two or three 1L bottles at 10 cm below the surface to avoid surface scum (if any was observed) and placed on ice to be returned to the laboratory for analysis of chlorophyll *a* (chl *a*), microscopy, nitrate, ammonium, silica, soluble reactive phosphorus (SRP), and total suspended solids (TSS).

Nutrient Analysis

In the laboratory, 30 mL of each water sample was vacuum-filtered through 0.45µm membrane filters and then analyzed for nutrients. All of the following analyses were performed on a Seal Analytical (Mequon, Wisconsin) AQ2+ discrete analyzer using standard colorimetric methods. Multiple forms of N were measured, including ammonia (NH_4) according to EPA Method 350.1 (USEPA 1993) and nitrate plus nitrite (NO_2+NO_3) according to EPA Method 353.2 (USEPA 1993). SRP was measured according to EPA Method 365.1 (USEPA 1993). Silica concentrations were measured on filtered subsamples using an O.I. Analytical Flow Solutions IV Autoanalyzer (Method 4500-SiO_2). Detection limits for NO_x-N is 0.016 mg N L^{-1}, for NH_4-N is 0.022 mg N L^{-1}, for SRP is 0.003 mg P L^{-1}, and for Si is 0.071 µmoles L^{-1}. Any amount found below this limit is indicated by BD (below detection).

Suspended solids in the water column influence light availability to primary producers. TSS was also measured by filtering a measured volume of water through a

pre-ashed glass fiber filter (GFF Gelman), dried at 105°C, and weighed. TSS data was only collected for the transect stations in 2012.

Chlorophyll *a* (chl *a*) and Microscopy Analyses

Chl *a* was measured in triplicate for all stations as a measure of phytoplankton biomass using a Turner fluorometer (Model 10-AU) following the protocol from Parsons et al. (1984). Subsamples (50 mL) were vacuum-filtered onto glass-fiber filters (GF/F) (Whatman). The filters were placed in 15 mL Corning centrifuge test tubes, covered with foil and kept in -20°C until analyzed. In order to analyze the samples, filters were allowed to come to room temperature and then 10 mL of 90% acetone was added to the test tube, vortexed for 1 minute and returned to -20°C for 24 hours. After the 24 hour period, samples were brought back to room temperature, centrifuged for 10 minutes (3000 rpm), then the supernatant was suctioned off and placed in a glass test tube and fluorescence was measured before and after acidification with 10% hydrochloric acid.

Additional whole water subsamples were preserved with 2% Lugol's solution and kept in a dark room at room temperature. A gridded Sedgwick-Rafter slide (Wildco) was used to examine duplicates of water from each Lugol's preserved archive sample on an inverted microscope (Axiovert 135, Zeiss). In preparation for examination, each archive sample was uniformly mixed by inversion of the sample container for approximately thirty seconds before the one ml subsample was loaded onto a Sedgwick-Rafter slide. The sample was then allowed to settle for 30-45 min before examination began. Cells were enumerated by individual or colony/chains. These samples were used to determine the phytoplankton community structure by enumerating cells into taxa of centric diatoms, pennate diatoms, chlorophytes, cyanobacteria, cryptophytes, and dinoflagellates. In order

to determine abundance, the entire slide or a minimum of 150 individuals from the most dominant taxa were counted. These abundances were then averaged with their replicate to determine cells L^{-1} for each station and date.

Biomass was also calculated for each taxa. Several of the most common species per taxa were measured and biomass was calculated using published formulas for geometric models of phytoplankton (Sun and Liu 2003). The average size for each taxa was then multiplied by the cell counts to obtain a representative total biomass.

Toxin Extraction and Analyses

Water samples were analyzed for both particulate and dissolved forms of the cyanobacterial toxin microcystin (MC). The samples were filtered through GF/F circles (Whatman) until clogged. The filtrates were collected for dissolved toxins, while the filters were kept for analysis of the particulate toxins. Both samples were kept in -20°C until processed.

Enzyme-linked immunosorbent assay (ELISA) kit (Abraxis LLC) was used to measure the MC equivalents in the two collected subsamples following the protocol in Garcia et al. (2010). ELISA is a competitive binding assay that is highly sensitive for MCs with a detection limit of 0.15 μg L^{-1} based on the most common variant, MC-LR, and its congeners. The cross-congener reactivity is high and has high sensitivity (Fischer et al. 2001). Dissolved toxin samples were allowed to come to room temperature, passed through a 0.2 μm syringe filter (Corning), and then analyzed according to the protocol in the kit. The particulate sample filters were allowed to come to room temperature, placed in glass test tubes, and then 5 mL of 50% methanol plus 1% acetic acid was added to each tube. Each sample was then vortexed (1 minute) to displace cells from the filter,

sonicated (2 minutes, 30-40 W) in an ice bath using a sonicator probe, and centrifuged (10 minutes, 3000 rpm). The supernatant was removed and filtered through a 0.2 µm syringe filter (Corning). The remaining pellet was re-suspended using 5 ml of the above extraction solution before repeating the process and finally pooling the filtered supernatant. Samples were then analyzed following the protocol included in the kit, with each sample run in duplicate and at several dilutions in order to reduce interference from matrix effects (impact of physical components of the sample on measurement of the analyte). Absorbance was read at 450 nm using a micro-plate spectrophotometer.

Statistical Analysis

The environmental parameters were compared using a Student's t-test assuming unequal variances with $\alpha \leq 0.05$. Each time period was compared linearly and then the total spring and total summer data were compared in the same manner to identify significant shifts.

All environmental data and biological abundance data were analyzed using PRIMER 6. The phytoplankton abundance data was converted using the fourth root to reduce the importance of very abundant taxa and take into account the less common taxa. A similarity matrix was then calculated using Bray Curtis (BC). The BC matrix was then used to create a multi-dimensional scaling plot (MDS) to observe the patterns in the different taxa over the time periods and locations.

In order to compare the patterns in the biological abundance data to the environmental data, the analysis called BEST ("best" match of sample patterns between environmental and biological data) was performed in order to determine which environmental data was significantly affecting the biological taxa groups. BEST was run

with 999 permutations with a significance levels calculated for each year and each location (tributary, northern and transect).

Results

2011 Bonnet Carré Spillway Event Year

All environmental parameters were previously published in Roy et al. (2013) for 2011 through the beginning of August. This paper uses seasonal averages for 2011 (Table 2.1) for the transect stations, but also includes northern stations from 2011 that were previously unpublished. All data for 2012 (Table 2.2) is original to this work.

The Mississippi River freshwater input from the Bonnet Carré Spillway had a total discharge of 21.9 km^3 (approximately 330% of the estuary's volume). The total amount of discharge from the northern tributaries was 0.4 km^3 (Roy et al. 2013). The highest peak in flow occurred in early September (see A & D in Figure 2.2).

All environmental data for 2011 is summarized in Table 2.1. Water temperature significantly increased at the transect stations from late spring (25.1 ± 1.0°C) to early summer (30.6 ± 1.0°C) ($p < 0.001$), and then declined slightly to 28.9 ± 1.8°C in the late summer. In response to the opening of the BCS during the late spring, salinity decreased from 3.8 ± 0.5 psu to 1.4 ± 1.5 psu and continued to decrease into early summer as diversion water continued to influence the estuary (0.6 ± 0.3 psu), and then significantly increased ($p=0.05$) in the late summer to 1.7 ± 1.3 psu. DO levels remained fairly constant throughout the spring and summer with the largest range of 6.5-14.0 mg L^{-1} during the early summer period immediately following BCS closure. In the late spring to early summer, pH increased significantly (6.7 ± 1.4 to 7.5 ± 0.4; $p< 0.05$), but then did

not change greatly in late summer (7.9 ± 0.5). Nitrate levels decreased significantly from late spring to early summer (0.6 ± 0.5 to 0.2 ± 0.3 mg N^{-1} L^{-1}; $p<0.05$) and continued to decrease into the late summer (0.01 ± 0.02 mg N^{-1} L^{-1}). The average N:P molecular ratio also decreased drastically from the spring to the summer, going from 34:1 to 0.4:1, respectively, indicating that N was the limiting nutrient for phytoplankton growth only later in the summer. This large decrease in N availability also affected the Si:N ratios decreasing from 1:0.9 to 1:0.02. Ammonium was only present during the spillway opening (0.02 ± 0.02 mg N^{-1} L^{-1}) and immediately after the closing (0.02 ± 0.02 mg N^{-1} L^{-1}), before completely being depleted in summer. SRP followed a different trend from N, and was usually present in the system (BD-0.08 mg P L^{-1}) increasing significantly in the late summer (0.06 ± 0.02 mg P^{-1} L^{-1}; $p<0.05$). There was a significant decrease in silica from late spring to early summer (0.3-3.4 mg Si^{-1} L^{-1}; $p=0.01$), but no significant change into late summer (1.7 ± 1.0 mg Si^{-1} L^{-1}). As expected, TSS was very high in the late spring (corresponding to the opening of the BCS) (28.9 ± 34.04 mg L^{-1}) and decreased significantly after the BCS closed in early summer (8.6 ± 10.7 mg L^{-1}; $p\leq0.05$). Secchi depth ranged from 0.2-2.7 m with lowest depth at the station nearest the entry point of the BCS into Lake Pontchartrain. Total Chl *a* values (range of 2.5-35.2 µg L^{-1}) reached the maximum in early summer after the spillway was closed and then a significant decrease to 6.6 ± 2.2 µg L^{-1} ($p<0.01$) was observed in the late summer.

Data was not collected for the northern stations in late spring. The early to late summer time periods had similar trends to the transect stations during the same time period (Table 2.1). Temperature, salinity and DO did not change significantly. The pH did decrease significantly ($p<0.01$) from early to late summer (8.0 ± 0.2 to 7.8 ± 0.2).

Table 2.1. 2011 Water quality parameters for Lake Pontchartrain, Louisiana. Any concentrations that are below the detection limit for a nutrient are indicated by BD and any samples not collected are indicated by N/A.

	Transect Stations			Northern Stations		
	Late Spring	Early Summer	Late Summer	Late Spring	Early Summer	Late Summer
Water quality measure	*Average ± SD (Range)*	*Average ± SD (Range)*	*Average ± SD (Range)*		*Average ± SD (Range)*	*Average ± SD (Range)*
temperature (°C)	25.1 ± 1.0 (20.2-29.5)	30.6 ± 1.0 (28.9-32.3)	28.9 ± 1.8 (25.7-31.4)	N/A	30.3 ± 0.9 (29.5-31.6)	28.5 ± 2.0 (25.5-30.6)
salinity (psu)	1.4 ± 1.5 (0.1-4.4)	0.6 ± 0.3 (0.2-1.2)	1.7 ± 1.3 (0.6-4.8)	N/A	0.5 ± 0.2 (0.3-0.8)	1.3 ± 1.0 (0.4-3.0)
dissolved O_2 (mg L^{-1})	8.4 ± 1.1 (6.9-10.7)	8.6 ± 2.3 (6.5-14.0)	8.1 ± 1 (7-9.8)	N/A	7.3 ± 0.5 (7-8.2)	7.6 ± 0.6 (7.1-8.8)
pH (pH units)	6.7 ± 1.4 (4.9-8.3)	7.5 ± 0.4 (6.8-8.3)	7.9 ± 0.5 (7.1-8.7)	N/A	8.0 ± 0.2 (7.8-8.2)	7.8 ± 0.2 (7.3-8.1)
nitrate (mg N L^{-1})	0.6 ± 0.5 (0-1.4)	0.2 ± 0.3 (BD-1.3)	0.01 ± 0.02 (BD-0.04)	N/A	0.01 ± 0.01 (0-0.02)	0.005 ± 0.01 (BD-0.04)
ammonium (mg N L^{-1})	0.02 ± 0.02 (BD-0.04)	0.01 ± 0.02 (BD-0.07)	BD	N/A	BD	BD
SRP (mg P L^{-1})	0.04 ± 0.02 (BD-0.07)	0.04 ± 0.03 (BD-0.08)	0.06 ± 0.02 (0.02-0.08)	N/A	0.02 ± 0.03 (BD-0.07)	0.06 ± 0.01 (0.03-0.07)
silica (mg Si L^{-1})	2.8 ± 0.4 (2.2-3.4)	1.8 ± 1.3 (0.3-3.4)	1.7 ± 1.0 (0.3-2.7)	N/A	3.3 ± 0.2 (3.0-3.6)	3.1 ± 0.2 (2.8-3.2)
TSS (mg L^{-1})	28.9 ± 34.04 (2-124)	8.6 ± 10.7 (BD-46.9)	4.7 ± 4 (BD-11.5)	N/A	4.4 ± 2.5 (BD-7.8)	5.5 ± 3.7 (1.3-13.0)
Chl *a* (µg L^{-1})	10.7 ± 9.7 (2.5-33.3)	16.9 ± 9.9 (4.2-35.2)	6.6 ± 2.2 (3.5-11.4)	N/A	12.9 ± 7.2 (4.4-27.6)	7.3 ± 1.8 (4.2-9.9)
N:P	34:1	12:1	0.4:1	N/A	1.1:1	0.2:1
Si:N	1:0.9	1:0.5	1:0.02	N/A	1:0.01	1:0.007

Nitrate concentrations in the northern stations were lower than the transect stations for the entire summer, never greater than 0.04 mg N^{-1} L^{-1}, while ammonium was never present at detectable concentrations in this part of the estuary. SRP increased significantly ($p<0.01$) throughout the summer, reaching 0.07 mg P L^{-1}. There was not a significant change in the early to late summer for silica and had a range of 2.7-3.6 mg L^{-1}. The N:P ratio was very low in early summer (1.1:1) and decreased even more in late summer (0.2:1). Correspondingly the Si:N ratio was very low as well going from 1:0.01 to 1:0.007. Because samples were not taken in the late spring, there was not very high TSS (range of 0-13.0 mg L^{-1}), and Secchi depth also had less variability with a range of 0.8-2.0 m. Chl *a* levels ranged from 4.2 to 27.6 µg L^{-1} but with only an average of 8.9 ± 5.8 µg L^{-1} and there was not significant change over the summer.

The phytoplankton community response was different both spatially and temporally due to the varying environmental conditions throughout the sampling period (see B & E in Figure 2.2). During the late spring, while the spillway was still open, within the transect stations, the community consisted of several different freshwater taxa, with chlorophytes, such as desmids, *Scenedesmus,* and *Klebsormidium,* and centric diatoms, mainly *Melosira,* accounting for the largest cell abundances (8.0×10^5 cells $L^{-1}$and 5.0×10^5 cells L^{-1}, respectively) (see B in Figure 2.2). However, the cell biomass indicated that dinoflagellates were responsible for the largest amount, (unfortunately the samples were preserved with Lugol's and did not allow for accurate species identification) with chlorophytes and centric diatoms contributing the second largest amount (see C in Figure 2.2). In the early summer, following the BCS closure, chlorophytes became the most abundant group (1.5×10^6 cells L^{-1}), but dinoflagellates

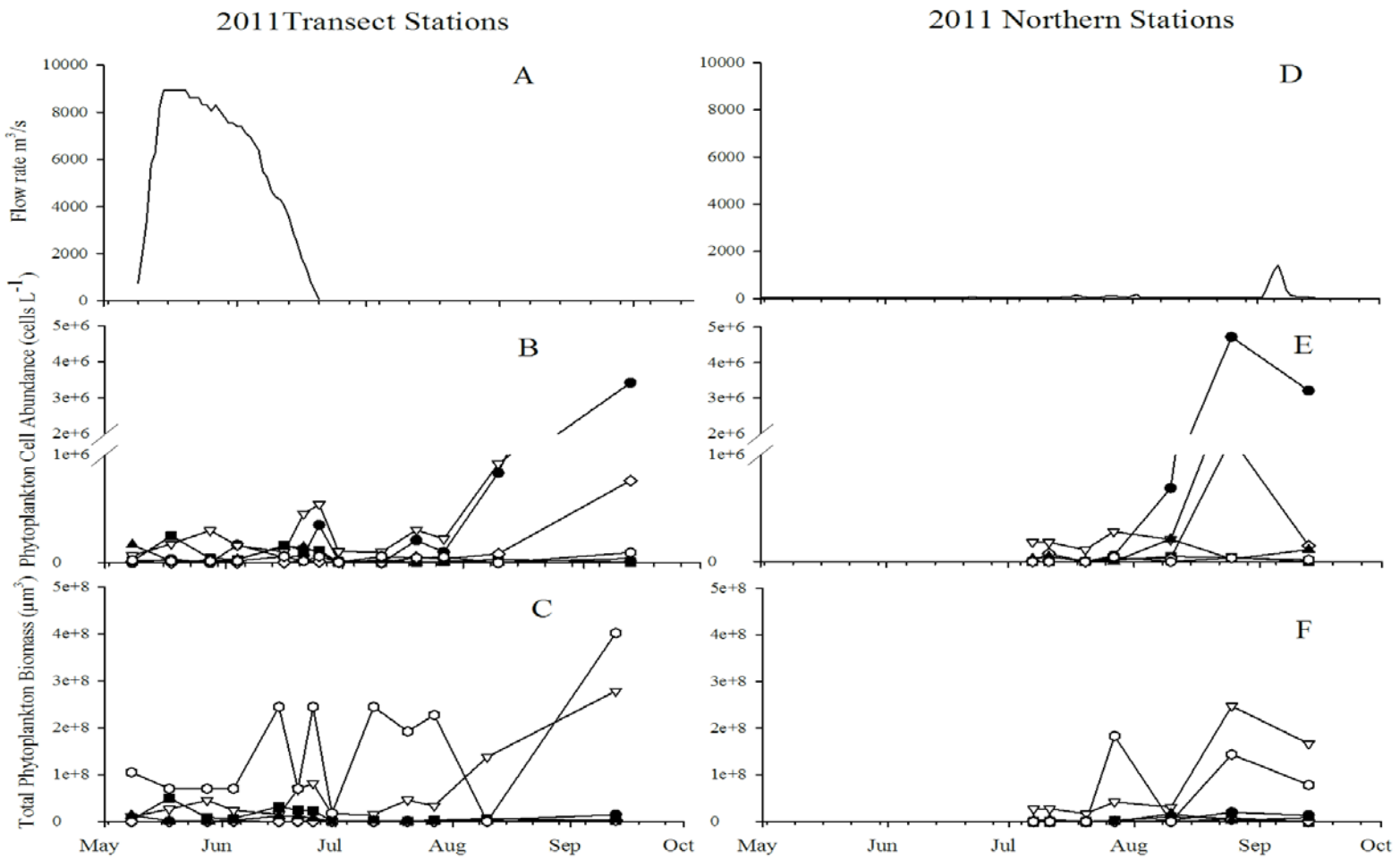


Figure 2.2. 2011 flow rate for the Bonnet Carré Spillway (A) and flow rate from the northern tributaries (D). The cell abundances and biomass for the transect stations influenced by the spillway (B and C). The cell abundances and biomass of the northern stations influenced by the northern tributaries (E and F). Legend: cyanobacteria (●), chlorophytes ( ), centric diatoms (■), cryptophytes (◊), pennate diatoms (▲), and dinoflagellates (○).

were still the largest contributor to cell biomass with chlorophytes remaining the second largest contributor. In the late summer cyanobacteria became the largest group in terms of cell abundance, mainly in single cell or small groups of *Microcystis* with a few chains of *Anabaena,* (range of 1.0×10^5 to 3.4×10^6 cells L^{-1}), but despite these large abundances they only made up 3% of the total cell biomass, while chlorophytes and dinoflagellates were still the main contributors to the biomass.

The phytoplankton cell abundances in the northern area of the estuary went from relatively low in the early summer (6.3×10^5 cells L^{-1}) to much higher cell abundances in late summer (1.4×10^7 cells L^{-1}) (see E in Figure 2.2). Chlorophytes were the most abundant group in the early summer, but cyanobacteria, mainly *Anabaena* sp., had a large increase (range of 6.9×10^5 to 4.7×10^6 cells L^{-1}), in the late summer with chlorophytes and cryptophytes being the second most abundant. Again cyanobacteria was not the main contributor to biomass (4.8% of total biomass) despite high abundance. The main biomass contributor throughout the summer was chlorophytes, and dinoflagellates were the second largest contributor in late summer (see F in Figure 2.2).

The MDS plot was utilized for phytoplankton community similarities. The more similar a specific sampling (one station at one time period) is to another sampling is represented by proximity. For the transect stations, there was high variability demonstrated by several overlapping groups with 60% similarity and only a few that exhibited 80% similarity within those groups (see A in Figure 2.3). There was not a clear shift over time of the phytoplankton community indicated by different time periods being represented in many different similarity groups, which corresponded to the observed mixed population of phytoplankton. MDS bubble plots were initially run for all

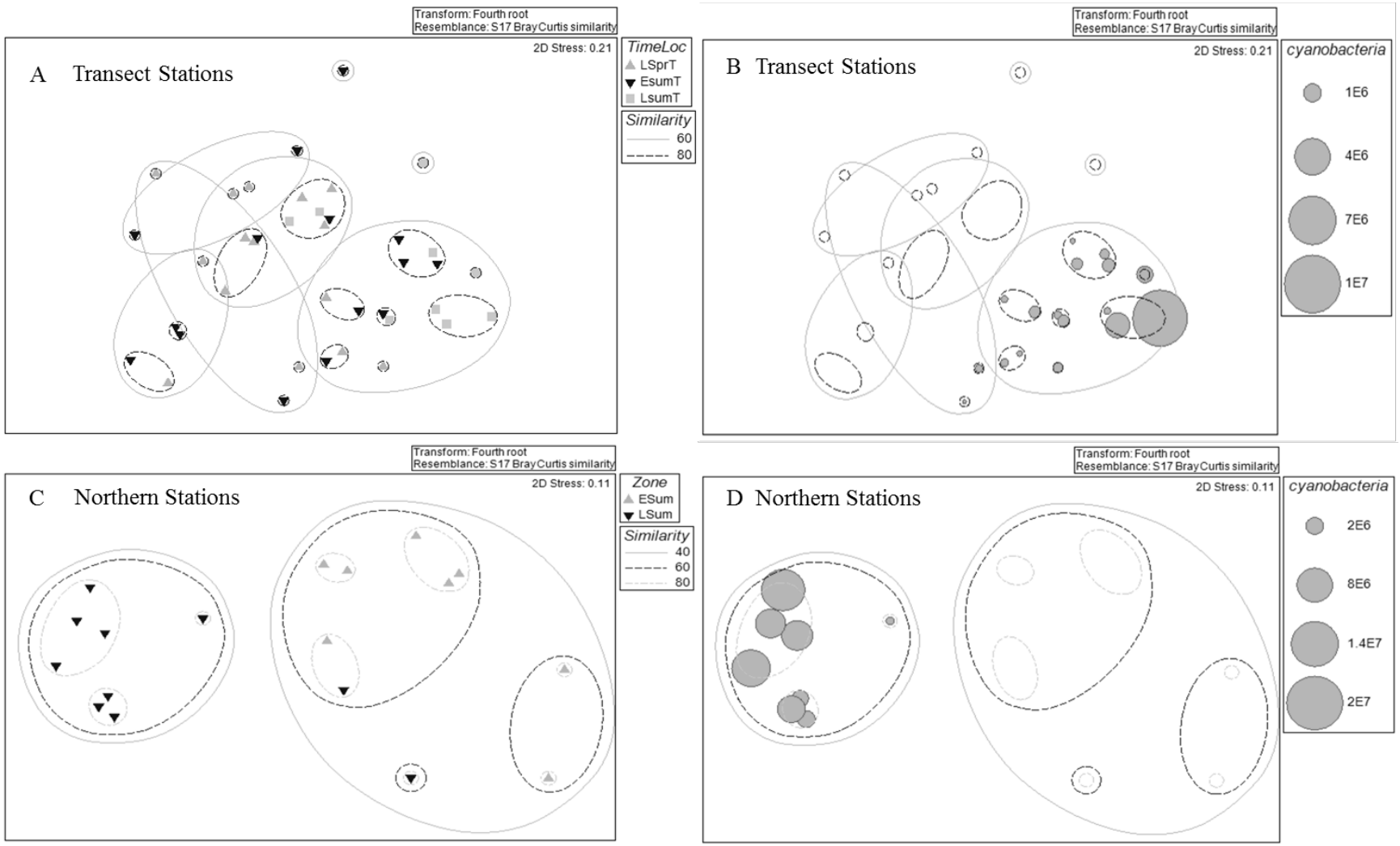


Figure 2.3. 2011 MDS plots of phytoplankton community over time and location. Transect stations (A), with cyanobacteria abundance (B); northern stations (C), with cyanobacteria abundance (D). Each plot has the different time periods of late spring (LSpr), early summer (ESum) and late summer (LSum).

phytoplankton groups at all time periods for all stations but did not show a specific pattern. However, the MDS bubble plot for cyanobacteria concentrations showed a distinct pattern and the majority was contained within one 60% similarity group. That group included stations from all time periods, but had the majority of early and late summer groups (see B in Figure 2.3). The MDS for the northern stations showed a very clear separation for the majority of stations with a shift in the phytoplankton community occurring between early and late summer (see C in Figure 2.3). This shift can mainly be attributed to cyanobacteria only being present in the late summer as indicated in D in Figure 2.3.

BEST analysis for the transect stations demonstrated that only Secchi depth affected changes in the biological community (Rho 0.10, p = 80.1%). BEST analysis for the northern stations indicated that TSS affected the phytoplankton community (Rho 0.29, p=15.9%). Both of these environmental parameters are the indicators of a highly mixed system. High turbidity and probable light limitation seem to be the major factors that greatly impacted the phytoplankton community in 2011.

2012 Non- Bonnet Carré spillway event year

The 2012 study period was relatively dry with low run-off from the northern tributaries and no episodic BCS discharge event. The discharge amount from the northern tributaries only ranged from 44.7 – 4168.1 $m^3 s^{-1}$ (see A in Figure 2.4). Environmental parameters for 2012 are summarized in Table 2.2.

Surface water temperature at transect stations increased significantly from the spring (24.1 ± 2.1°C) to the summer (range 27.9-31.5°C) with the highest average in the early summer of 31.5°C ($p<0.001$). Salinity throughout the entire sampling period was

between 2.7 and 4.0 psu, with a significant increase from early to late summer (3.0 ± 0.1 to 3.5 ± 0.4; $p<0.05$). DO and pH decreased significantly from spring to summer (8.4 ± 0.2 mg L^{-1} to 7.1 ± 0.3 mg L^{-1}; $p<0.001$ and 7.9 ± 0.2 to 7.1 ± 0.3; $p<0.001$, respectively). Nitrate was present at low levels in the early spring (0.01 ± 0.02 mg N^{-1} L^{-1}), but was below the detection limit for the rest of the sampling period. Therefore, the average N:P and Si:N ratios were only calculated for early spring (1:0.4 and 1:0.004, respectively). Ammonium had the opposite trend from nitrate and was only present in the system during the late summer with very low concentrations at a range of BD-0.02 mg N^{-1} L^{-1}. Despite variability in dissolved inorganic N availability and speciation, SRP was usually present in the system at a range of BD-0.08 mg P L^{-1}. There was a significant increase at silica levels from early summer to late summer (11.5 ± 0.02 to 12.2 ± 0.05 mg Si^{-1} L^{-1}, respectively) ($p=0.03$), and when comparing averaged spring totals to average summer totals, there was a significant increase in the summer ($p<0.01$). Mean TSS concentration for the entire sampling period was much lower than it was for 2011, however, the range of 2.6-18.5 mg L^{-1} was similar to levels that were observed in late summer of 2011. Secchi depth also had a smaller range of only 0.7-2.0 m. Chl *a* concentrations (range of 1.8-12.4 µg L^{-1}) for the entire sampling period were relatively low in comparison to the previous year, with the highest concentrations occurring during the early spring.

The northern stations experienced significant increases in water temperatures throughout the entire spring and summer periods (range of 20.4-31.6°C) ($p<0.001$) with the highest mean temperature also occurring in the early summer time period. The salinity range was larger and more variable than the transect stations (0.9-7.4 psu), but there was not any significant changes over the different time periods. DO had an overall

Table 2.2. 2012 Water quality parameters for Lake Pontchartrain, Louisiana. Any concentrations that are below the detection limit for a nutrient are indicated by BD. Nutrient ratios that could not be calculated are indicated by NC.

	Transect Stations			
	Early Spring	Late Spring	Early Summer	Late Summer
Water quality measure	*Average* ± *SD* *(Range)*	*Average* ± *SD* *(Range)*	*Average* ± *SD* *(Range)*	*Average* ± *SD* *(Range)*
temperature (°C)	22.8 ± 1.1 (21.1-24.6)	26.7 ± 0.5 (26.2-27.3	30.7 ± 0.5 (30.3-31.5)	29.4 ± 1.2 (27.9-31.1)
salinity (psu)	3.1 ± 0.4 (2.7-4.0)	3.1 ± 0.3 (2.9-3.5)	3.0 ± 0.1 (2.8-3.1)	3.5 ± 0.4 (3.0-4.0)
dissolved O_2 (mg L^{-1})	8.8 ± 0.7 (7.4-9.5)	8.4 ± 0.2 (8.1-8.6)	n/a	7.1 ± 0.3 (6.8-7.5)
pH (pH units)	7.6 ± 0.2 (7.2-7.8)	8.0 ± 0.1 (7.9-8.2)	7.2 ± 0.05 (7.1-7.3)	7.0 ± 0.4 (6.3-7.4)
nitrate (mg N L^{-1})	0.01 ± 0.02 (BD-0.04)	BD	BD	BD
ammonium (mg N L^{-1})	BD	BD	BD	0.004 ± 0.008 (BD-0.02)
SRP (mg P L^{-1})	0.05 ± 0.02 (0.03-0.08)	0.03 ± 0.01 (0.02-0.05)	0.05 ± 0.02 (0.03-0.08)	0.03 ± 0.02 (BD-0.06)
silica (mg Si L^{-1})	10.1 ± 0.7 (8.8-10.9)	10.4 ± 0.5 (9.8-11.0)	11.5 ± 0.2 (11.3-11.7)	12.2 ±0.5 (11.3-12.8)
TSS (mg L^{-1})	5.2 ± 1.8 (3.0-8.0)	6.9 ± 0.4 (6.6-7.6)	4.7 ± 0.8 (3.7-5.6)	9.8 ± 5.1 (2.6-18.5)
Chl *a* (µg L^{-1})	5.7 ± 3.3 (3.1-12.4)	3.4 ± 1.2 (1.8-4.8)	4.2 ± 1.8 (2.9-6.8)	4.3 ± 0.5 (3.6-5.2)
N:P	0.4:1	NC	NC	0.03:1
Si:N	1:0.004	NC	NC	NC
	Northern Stations			
temperature (°C)	26.4 ± 1.1 (25.0-28.2)	22.3 ± 1.3 (20.4-24.1)	31.1 ± 0.5 (30.3-31.6)	29.5 ± 1.2 (28.0-31.0)
salinity (psu)	2.3 ± 0.7 (1.4-3.5)	2.9 ± 2 (1.1-7.4)	2.3 ± 0.3 (1.8-2.9)	1.9 ± 0.6 (0.9-3.1)
dissolved O_2 (mg L^{-1})	7.6 ± 0.4 (6.9-8.1)	8.4 ± 0.5 (7.2-8.9)	n/a	7.4 ± 0.4 (6.6-7.8)
pH (pH units)	7.6 ± 0.6 (6.7-.82)	7.7 ±0.07 (7.6-7.8)	7.1 ± 0.2 (6.7-7.3)	7.4 ± 0.1 (7.2-7.6)
nitrate (mg N L^{-1})	BD	0.008 ± 0.01 (0-0.03)	BD	BD
ammonium (mg N L^{-1})	BD	BD	BD	BD

(Table 2.2 continued) Northern Stations

Water quality measure	Early Spring *Average ± SD (Range)*	Late Spring *Average ± SD (Range)*	Early Summer *Average ± SD (Range)*	Late Summer *Average ± SD (Range)*
SRP (mg P L^{-1})	0.09 ± 0.08 (0.04-0.3)	0.04 ± 0.01 (0.02-0.07)	0.04 ± 0.01 (0.04-0.05)	0.05 ± 0.004 (0.04-0.06)
silica (mg Si L^{-1})	10.5 ± 0.5 (9.6-11.0)	11.0 ± 0.7 (9.9-12.2)	11.3 ± 0.5 (10.7-12.0)	11.3 ± 0.9 (10.2-13.1)
TSS (mg L^{-1})	n/a	n/a	n/a	n/a
Chl *a* (µg L^{-1})	5.3 ± 1.8 (3.1-7.5)	6.9 ± 1.9 (4.5-9.6)	3.0 ± 0.3 (2.6-3.4)	4.8 ± 1.3 (2.8-6.7)
N:P	NC	0.4:1	NC	NC
N:Si	NC	0.03:1	NC	NC
	Tributaries			
temperature (°C)	21.9 ± 1.1 (20.5-23.8)	26.1 ± 1.1 (24.1-27.8)	31.6 ± 1.0 (30.9-32.9)	29.9 ± 1.8 (27.2-32.4)
salinity (psu)	0.7 ± 0.6 (0.04-1.1)	0.8 ± 0.6 (0.1-1.6)	0.8 ± 0.4 (0.3-1.4)	0.9 ± 1.1 (0.06-3.1)
dissolved O_2 (mg L^{-1})	7.1 ± 1.4 (4.6-8.3)	5.4 ± 1.8 (2.2-7.3)	n/a	6.7 ± 1.1 (4.5-7.8)
pH (pH units)	7.3 ± 0.2 (7.0-7.5)	7.4 ± 0.6 (6.6-8.1)	6.8 ± 0.2 (6.5-7.1)	7.0 ± 0.4 (6.5-7.5)
nitrate (mg N L^{-1})	0.07 ± 0.04 (0.02-0.14)	0.05 ± 0.05 (BD-0.1)	BD	0.05 ± 0.06 (BD-0.1)
ammonium (mg N L^{-1})	0.03 ± 0.02 (BD-0.08)	0.02 ± 0.03 (BD-0.1)	BD	0.03 ± 0.05 (BD-0.1)
SRP (mg P L^{-1})	0.05 ± 0.02 (0.03-0.09)	0.1 ± 0.04 (0.02-0.2)	0.07 ± 0.02 (0.05-0.09)	0.07 ± 0.03 (0.04-0.1)
silica (mg Si L^{-1})	13.1 ± 3.7 (9.1-20.4)	13.0 ± 4.6 (9.4-22.8)	13.4 ± 4.1 (9.9-19.1)	14.1 ± 3.8 (10.4-21.8)
TSS (mg L^{-1})	n/a	n/a	n/a	n/a
Chl *a* (µg L^{-1})	7.1 ± 4.1 (3.1-15.6)	8.3 ± 2.4 (4.5-11.7)	26.0 ± 15.7 (9.9-47.4)	18.6 ± 18.2 (6.2-57.7)
N:P	4:1	2:1	NC	3:1
N:Si	0.03:1	0.02:1	NC	0.02:1

range of 6.6-8.9 mg L^{-1}, significantly increased from early to late spring ($p \leq 0.01$), and then significantly decreased during the summer ($p < 0.01$). The pH range of 6.7-8.2 was a similar trend to the transect stations of a significant decrease from the spring to the summer ($p < 0.05$). Nitrate was only present in the late spring in low concentrations of

0.008 ± 0.01 mg N^{-1} L^{-1} thus giving only 0.4:1 N:P ratio, and ammonium was never detected during the sampling period. The N:Si ratio was slightly higher than the transect stations at 0.03:1 during the late spring. SRP was always present in this part of the estuary (range of 0.02-0.3 mg P L^{-1}), similar to the transect stations. A significant increase in silica concentrations occurred between the early and late spring (9.6-12.2 mg Si^{-1} L^{-1}) ($p=0.03$), and then stabilized to an average of 11.3 ± 0.7 mg Si^{-1} L^{-1} in the summer. TSS data was not collected for these stations. Secchi depth had a similar range to the transect stations going from 0.4-2.0 m. Chl *a* concentrations were low (2.6-9.6 µg L^{-1}), similar to the transect stations, but exhibited a significant decrease from spring to summer ($p<0.01$).

The northern tributaries had the same temporal pattern of surface water temperatures (range of 20.5-32.9°C), which significantly increased throughout the spring and summer ($p<0.001$) with the highest average again in the early summer. Salinity was much lower in the tributaries (0.64 ± 0.58 psu) as it would be expected due to freshwater runoff and no direct link to a salt water source. DO and pH were stable with averages of 6.2 ± 1.6 mg L^{-1} and 7.2 ± 0.5, respectively, throughout the sampling period with no significant changes. Nitrate and ammonium were present at all time periods except the early summer with ranges of BD-0.14 mg N^{-1} L^{-1} and BD-0.1 mg N^{-1} L^{-1}, respectively. N:P ratios varied through the sampling period starting at 3:1 and decreasing slightly through the late summer to 2:1. SRP was available continuously with a range of 0.02-0.2 mg P L^{-1}. There was not a significant shift in silica levels during any of the time periods for the tributary data, but did have a larger range of 9.1-21.8 mg Si L^{-1}. N:Si ratios also remained stable at an average of 0.02:1 for the entire sampling period. Secchi depth in the tributaries had a smaller range of only 0.4-1.3 m. Chl *a* levels were generally low but

an increase from the early spring to the late summer (7.1 ± 4.1 to 18.6 ± 18.2 μg L^{-1}) was observed.

The 2012 phytoplankton community in the transect stations showed a different pattern from the transect stations sampled during the 2011 spillway event year (see B &C in Figure 2.4). The early spring had much higher cell abundances (0-3.2x10^6 cells L^{-1}) than later in the year with centric diatoms, such as *Thalassionema*, and pennate diatoms, such as *Fragilaria*, being the dominant contributors (see B in Figure 2.4). Pennate diatoms and chlorophytes were responsible for the majority of the biomass during early spring (see C in Figure 2.4). There was then a decrease in cell abundances through the rest of the sampling period with chlorophytes always being present and cyanobacteria, including species of *Microcystis* and *Anabaena*, becoming more abundant later in the summer. The biomass was mostly attributable to chlorophytes and dinoflagellates equally for the rest of the sampling period, except for late summer when dinoflagellates became the major contributor.

The northern stations experienced a shift in cell abundances from a mixed population, including cryptophytes, chlorophytes and pennate diatoms, in early spring to an increase in chlorophyte and cyanobacteria abundances later in the summer (see D in Figure 2.4). The rise in abundance of cyanobacteria did not add much to the overall biomass, which was mostly attributable to chlorophytes and dinoflagellates (see E in Figure 2.4).

The tributaries started with a very mixed population in the early spring of pennate diatoms, cryptophytes, centric diatoms, chlorophytes and cyanobacteria (see F in Figure 2.4). The biomass was also made up of a mixed population of dinoflagellates, pennate

diatoms, centric diatoms and chlorophytes (see G in Figure 2.4). However, in the late pring there was an increase in chlorophyte abundance with smaller numbers of cyanobacteria. The summer brought more diversity to the cell abundances with cryptophytes becoming more abundant in addition to the chlorophytes and cyanobacteria. The late summer period was characterized by the same diversity but a decrease in overall abundance. The biomass for the rest of the sampling period, despite high abundance diversity, was mainly composed of dinoflagellates and chlorophytes.

The MDS plot for the 2012 transect stations again had several 60% similarity groups, but there was not any overlap indicating very different compositions throughout the sampling (see A in Figure 2.5). The cyanobacteria populations were mostly in one 60% similarity group; however this group included stations from all time periods. This indicates that when cyanobacteria is present, the other groups of phytoplankton are in similar proportions no matter what time of the year this occurs. There was on outlier group from the late summer that did not include any cyanobacteria (see B in Figure 2.5). MDS plot for the northern stations indicated that the overall community was very similar throughout the sampling time period by the majority of stations contained within an 80% similarity group (see C in Figure 2.5). The cyanobacteria population also followed a similar pattern suggesting that a population of cyanobacteria persists throughout the sampling period in this part of the estuary (see D in Figure 2.5). The MDS plot for the tributary stations also had a very similar pattern to the northern stations with most being within the 80% similarity and all but one being within 60% (see E in Figure 2.5). Again, this shows not a large shift in the composition of the phytoplankton community over

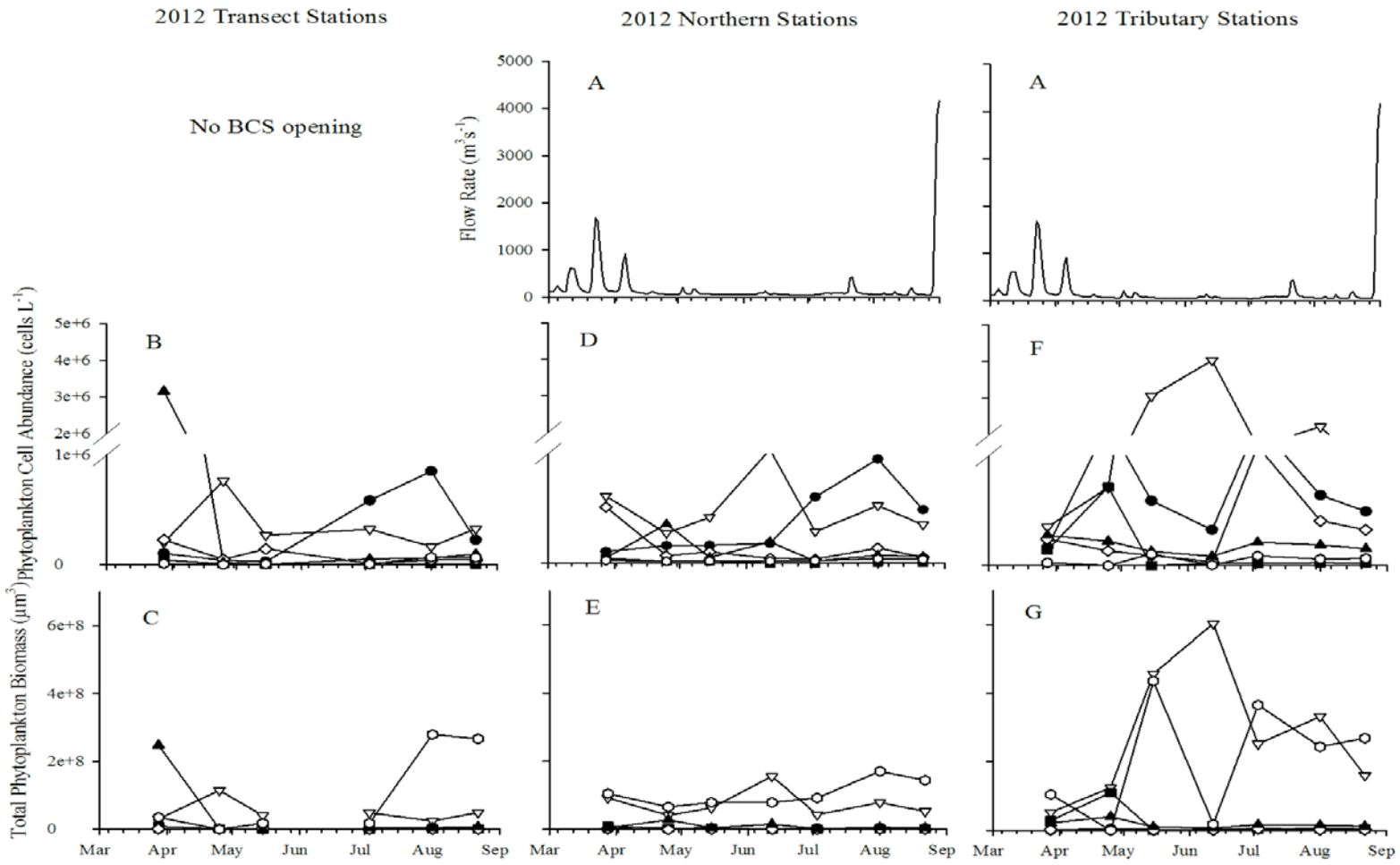


Figure 2.4. 2012 flow rate of the northern tributaries (A) and the phytoplankton cell abundance data from transect stations (B), northern stations (D) and tributary stations (F). The corresponding phytoplankton biomass for the transect stations (C), northern stations (E), and the tributary stations (G). Legend: cyanobacteria (●), chlorophytes (▽), centric diatoms (■), cryptophytes (◊), pennate diatoms (▲), and dinoflagellates (○).

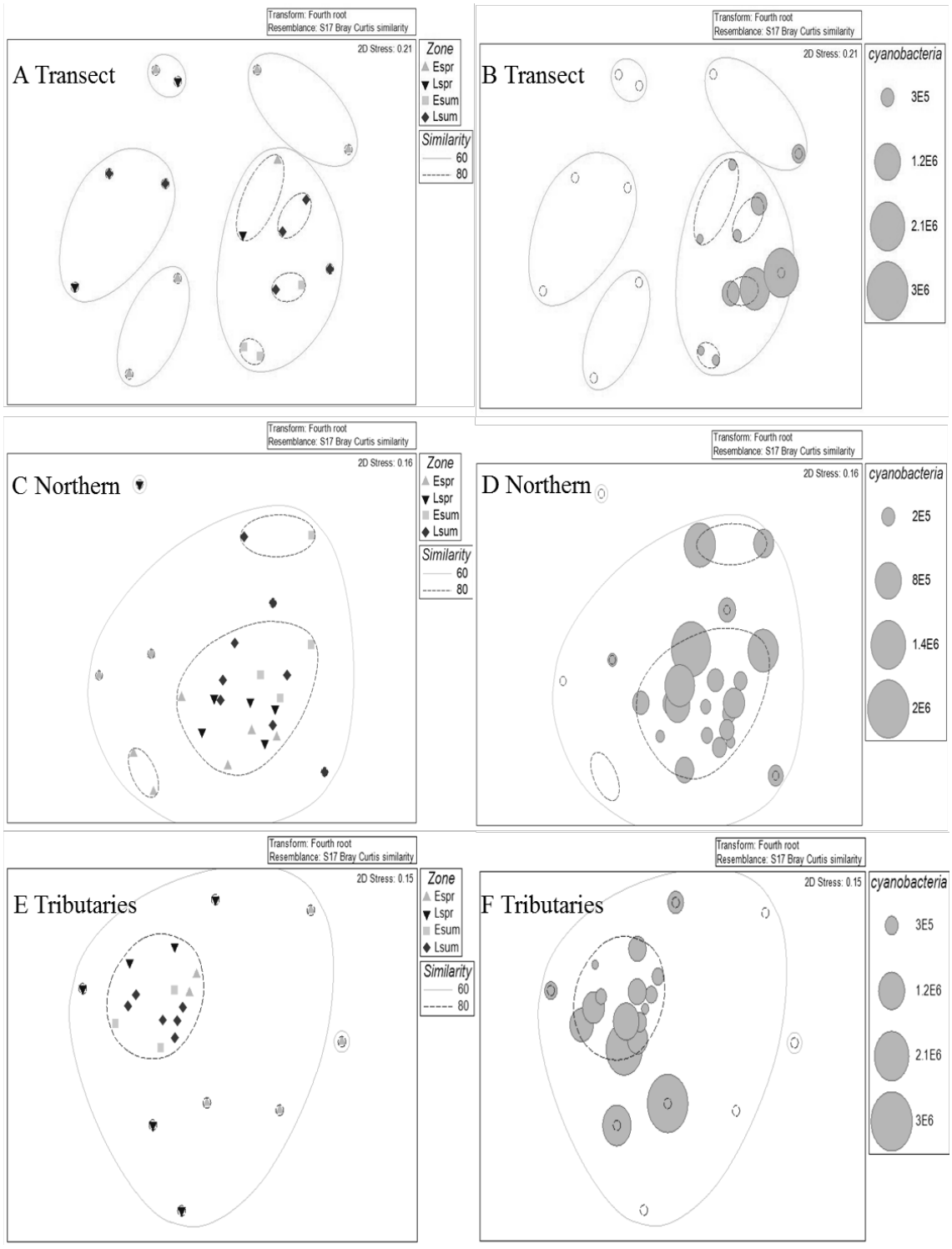


Figure 2.5. 2012 MDS plots of phytoplankton community over time and location. Transect stations (A) with cyanobacteria abundance (B); northern stations (C) with cyanobacteria abundance (D); and tributary stations (E) with cyanobacteria abundance (F).

time. The cyanobacteria population was similarly configured again indicating a persistent community of cyanobacteria throughout the year in the tributaries (see F in Figure 2.5).

BEST analysis for the transect stations indicated that silica, water temperature, and ammonium were all affecting the changes in phytoplankton community composition, but not highly significantly (Rho 0.29, p=37.8%). While the northern phytoplankton community was significantly affected by Secchi depth, SRP, and daily discharge (Rho 0.60, p=0.2%), the tributary phytoplankton community was affected by silica, water temperature, Secchi depth, salinity, and wind speed (Rho=0.53, p=5.7%).

Potentially Toxic Cyanobacteria Presence and Microcystin Levels

Potentially toxic cyanobacteria species of *Microcystis* sp. and *Anabaena* sp. were present in both years, but in different temporal and spatial distributions. In 2011, *Microcystis* sp. was present throughout the sampling period, with the highest abundance in both transect and northern stations occurring in late summer [3.4×10^6 (55% of total abundance) and 2.2×10^6 cells L^{-1} (70% of total abundance), respectively]. *Anabaena* sp. was only detected during the late summer in the northern stations. In 2012, *Microcystis* sp. and *Anabaena* sp. were both found throughout the sampling period. *Microcystis* sp. was initially found in the early spring in the transect stations at abundances of 1.4×10^5 cells L^{-1} and was continually found in all stations throughout the rest of the sampling period. *Anabaena* sp. was first present in low numbers only in the tributaries in late spring, before being abundantly found in both transect and northern stations in the early and late summer.

Particulate toxins were not detected either year, but dissolved toxins were present at varying concentrations for each year. In 2011, dissolved toxins were detected along

the transect stations just below the World Health Organizations (WHO) limit of 1.0 µg MC L^{-1} until late summer when the toxin was detected just above the limit (Table 2.3). In the northern stations, dissolved toxins were detected initially at low levels (0.4 ± 0.5 µg MC equivalent L^{-1}) and then increased greatly in the late summer (3.4 ± 1.5 µg MC equivalent L^{-1}) corresponding to an increase in cyanobacteria cell numbers.

In 2012, dissolved toxins had the highest concentration in early spring (range of BD-4.4 µg MC equivalent L^{-1}), but that did not corresponded to the highest concentration of cyanobacteria abundance. The levels then decreased to below the WHO limit by late spring (range of 0-1.0 µg MC equivalent L^{-1}), and by the late summer, dissolved toxins for all stations were below the detection limit, even though cyanobacteria cell numbers were higher compared to other times sampled.

Discussion

Estuaries are already complex environments, and since there has not been any study conducted to look at Lake Pontchartrain phytoplankton dynamics during a non-event year, whether it was a BCS opening or a weather related event, understanding the mechanisms causing changes in phytoplankton biomass and diversity in response to changes in their environment was challenging due to the lack of background data. The aim of this two-year study was to look at the phytoplankton bloom dynamics related to the high river input associated with a BCS opening year and a non-BCS opening year or "normal" year, where all freshwater comes from the tributaries and run-off. These two study years also proved to be vastly different in many regards from the previous "event" or "normal" years, which caused further complications to make comparative

Table 2.3. Dissolved toxins found in the water column and corresponding cyanobacteria cell counts during the 2011 and 2012 sampling period of Lake Pontchartrain.

		2011		2012	
Time period	Stations	Dissolved toxin (µg MC equivalent L^{-1})	Cyanobacteria cell counts (cells L^{-1})	Dissolved toxin (µg MC equivalent L^{-1})	Cyano. cell counts (cells L^{-1})
		Average ± SD (range)	*Average ± SD*	*Average ± SD (range)*	*Average ± SD*
Early Spring	Transect	Not collected	Not collected	1.5 ± 1.5 (0-3.5)	2.9 ± 4.7x10^5
Late Spring	"	0.9 ± 0.2 (0.6-1.2)	2.1 ± 2.6x10^5	0.3 ± 0.5 (0-1.0)	1.0 ± 1.5x10^5
E. Summer	"	0.5 ± 0.3 (0.2-0.9)	5.4 ± 5.6 x10^5	0.5 ± 0.4 (0-0.9)	2.4 ± 2.9 x10^6
L. Summer	"	1.3 ± 0.3 (1.0-1.7)	6.1 ± 6.0 x10^6	BDL	2.2 ± 3.1 x10^6
Early Spring	Northern	Not collected	Not collected	1.9 ± 1.8 (0-4.4)	5.6 ± 6.0 x10^5
Late Spring	"	Not collected	Not collected	0.4 ± 0.4 (0-0.9)	7.1 ± 4.8 x10^5
E. Summer	"	0.4 ± 0.5 (0.8-1.1)	5.9 ± 10 x10^4	0.3 ± 0.3 (0-0.6)	2.5 ± 1.2 x10^6
L. Summer	"	3.4 ± 1.5 (1.3-5.5)	12 ± 7.0 x10^6	BDL	3.0 ± 2.2 x10^6
Early Spring	Tributary	Not collected	Not collected	1.9 ± 1.7 (0-4.1)	2.4 ± 4.4 x10^6
Late Spring	"	"	"	0.1 ± 0.3 (0-0.9)	1.9 ± 2.0 x10^6
E. Summer	"	"	"	BDL	5.4 ±3.1 x10^6
L. Summer	"	"	"	BDL	2.4 ± 1.6 x10^6

BDL = Below Detection Limit (0.15 µg MC equivalent L^{-1})

interpretations. One year was had the vast amount of high flood volume (500 year flood) from the MS River which drains 41% of the continental United States compared to the regional drainage basin of only a small portion of two states.

During 2011, Lake Pontchartrain had the highest volume of water come through the BCS in its history and was opened much later in the year than previous openings. Additionally, water remained turbid after the BCS closing during the summer due to frequent storm activities and in particular, Tropical Storm Lee in early September. On the other hand, 2012 was a drought year providing an unusually low freshwater flow from the northern tributaries. As previously mentioned, the amount of freshwater and the source of the water can change phytoplankton resident times and flushing rates and can affect the overall community structure (Paerl et al. 2013). During 2011 BCS opening, phytoplankton biomass was expected to be low at the river source where flow rate was very high, but gradual increase farther away from the river source was also expected. However, the existing estuarine water was completely flushed out (Roy et al. 2013), which reduced water resident times preventing high accumulation of phytoplankton biomass at any time period. Prolonged turbidity (represented by high TSS and low Secchi depth) also generated a non-stable water column and likely light limitation for an extended time period. Phytoplankton diversity remained high, with no observed single species dominating the system even when the Chl *a* levels were somewhat high. Then in 2012, very low freshwater flow from the northern tributaries resulted in a low turbid environment and therefore phytoplankton composition and biomass were minimally influenced by the flow rate.

In addition to the differences in freshwater flow that determined the level of turbidity and light limitation in the estuary, nutrient dynamics were also quite different between the two sampled years. In 2011, N was available in high amounts during the BCS opening, but declined quickly, likely due to uptake by phytoplankton or direct

transport to the Gulf of Mexico, leading to overall nitrogen limitation in the system (Roy et al. 2013). Lake Pontchartrain was not generally P limited either year possibly due to resuspension of P from the sediments, which may have been increased due to the above mentioned storm activities or as was shown in Roy et al. (2012). Gilpin et al. (2004) suggest that when Si:N ratios are less than 1:2 that N becomes the limiting factor and when it is greater than 1:2 then Si is limiting. With this rational, Si was also not the limiting for diatoms in spring months for both years as evidenced by a large abundance of centric diatoms in 2011 and a large abundance of pennate and centric diatoms in 2012. Even water was much more stable in 2012 compared to 2011, due to overall nutrient limitation, biomass remained low with no single species dominance. For both years, the spring had a mixed population of several groups including diatoms, chlorophytes, and dinoflagellates but then had a noticeable shift to cyanobacteria in the late summer.

CyanoHABs are a growing concern worldwide in freshwater and estuarine systems. The specific concern about a CyanoHAB forming in Lake Pontchartrain stems from previous BCS opening observations. During 1997 BCS opening, Chl *a* reached a maximum of 855 µg L^{-1} and the CyanoHAB consisted of a mixed population of *Microcystis* spp. and *Anabaena* spp. during summer months (Turner et al. 2004). During 2008 BCS opening, the maximum Chl *a* was much lower only reaching 58 µg L^{-1}, but was similar with an observable CyanoHAB consisting of *Microcystis* sp. in early summer followed by *Anabaena* sp. in the late summer time with associated toxin production (Bargu et al. 2011). The most recent 2011 BCS opening yielded similar Chl *a* levels to the 2008 opening with a maximum Chl *a* of 45µg L^{-1}, but with higher phytoplankton diversity. Both species of these potentially toxic cyanobacteria were observed, with

Microcystis sp. only observed at low levels in the estuary throughout the summer, and *Anabaena* sp. observed more frequently late in the summer near the north shore. However, both species only contributed less than 0.6% to the total biomass for the entire sampling period. The late closing date of the BCS followed by a large storm made Lake Pontchartrain water very turbid creating unfavorable conditions for cyanobacteria to bloom in the summer time. Even though non-nitrogen fixing *Microcystis* spp. was present early in the summer when N:P ratio was still high, their bloom did not occur possibly due to the high turbidity and flushing of the system as previously mentioned. Since *Anabaena* spp. are able to fix atmospheric nitrogen, they would have been expected to increase following the N depletion, but low flow rates from the northern tributaries may have impeded *Anabaena* sp. from being pushed out into the main part of the estuary with the assumption that the *Anabaena* sp. seed population is found in these tributaries. This assumption was initiated by observation of higher *Anabaena* numbers and detection of dissolved toxins in only the northern part of the estuary following the BCS opening.

In 2012, both *Microcystis* and *Anabaena* were present in the estuary, *Anabaena* was only initially observed within the tributaries and then moved out into the main part of the estuary later in the summer, supporting the idea that *Anabaena* spp. is likely originating from the northern tributaries. *Microcystis* was continually part of the natural phytoplankton community throughout the estuary at low levels, but likely limited by overall low N in the system. Further research is needed for more closely spaced spatial–temporal scales on non-diversion years to determine if there is any direct impact of river water input verses tributary water input for the development of toxic cyanobacterial blooms in the estuary.

The amount of carbon availability in an aquatic system is dependent upon the total phytoplankton and plant biomass. However, phytoplankton species, with rapid turnover rates, size and toxicity can regulate grazing and corresponding carbon transport efficiency. Larger diatoms and dinoflagellates are found to be important carbon transporters for the Lake Pontchartrain system during spring when nutrients are sufficient enough to support their biomass. The fate of the carbon in the water column during this time will be tightly linked to the timing of their bloom, which would determine how efficiently they can be grazed by zooplankton. Biomass that is not grazed will eventually sink and can then support benthic communities. There is great need to further explore these thoughts to see how the timing of the river input would influence the fate of the carbon in this system for BCS opening years. In both studied years, potentially toxin producing cyanobacteria became more abundant in the summer months. Cyanotoxins are known to be a grazer deterrent (Codd 1995) so their presence can lead to more carbon, from ungrazed biomass, sinking to the benthic community along with the associated toxins. In 2011 and 2012, there was not any particulate toxins detected, however in past years of BCS openings, toxins were detected and the overall transfer of toxins to higher trophic organisms was not studied. We do need to know the mechanisms underlying the initiation and accumulation of these species, especially the toxic ones to be able to determine the fate of carbon and toxins in the food web and fisheries in this estuary.

Lake Pontchartrain has many dimensions of space, mixing, inflow rates, weather events and potential diversion openings, which lead it to be difficult to predict how the phytoplankton community will react. However, there were distinct patterns of hydrological parameters, nutrient concentrations, and phytoplankton diversity/biomass

observed in Lake Pontchartrain, both spatially and temporally within each year, and between the two years. While seasonal variations are found to be important to determine which phytoplankton group dominates the system, species diversity within a group seems to be regulated by the water source. Nutrient inputs from rivers and tributaries, and possible from sediment nutrient resuspension can all play important role in controlling phytoplankton community structure and their biomass, and consequently the fate of carbon in the system. Given the shallow and well-mixed nature of the system, benthic flux of deposited nutrients, especially phosphorus can be readily accessible to the water column and further support nitrogen-fixing cyanobacteria in summer months. Since this estuary is extensively used for fishing and recreation, continuous monitoring needs to take place to observe changes in phytoplankton bloom dynamics and the presence of these toxic species because of the great effect they can have on the overall quality of this ecosystem and it's fisheries productivity.

References

Bargu, S., J. White, C. Li, J. Czubakowski, and R. Fulweiler. 2011. Effects of freshwater input on nutrient loading, phytoplankton biomass, and cyanotoxin production in an oligohaline estuarine lake. *Hydrobiologia* 661:377-389.

Beaugrand, G., F. Ibanez, and P. C. Reid. 2000. Spatial, seasonal and long-term fluctuations of plankton in relation to hydroclimatic features in the English Channel, Celtic Sea and Bay of Biscay. *Marine Ecology Progress Series* 200:93-102.

Boney, A.D. 1976. Phytoplankton. Great Britain: The Camelot Press Ltd, Southhampton.

Bouvy, M., R. Molica, S. De Oliveira, M. Marinho, and B. Beker. 1999. Dynamics of a toxic cyanobacterial bloom (Cylindrospermopsis raciborskii) in a shallow reservoir in the semi-arid region of northeast Brazil. *Aquatic Microbial Ecology* 20:285-297.

Chorus, I., and J. Bartram. 1999, Toxic cyanobacteria in water a guide to their public health consequences, monitoring, and management. London; New York, E & FN Spon.

Cloern, J. E. 1999. The relative importance of light and nutrient limitation of phytoplankton growth: a simple index of coastal ecosystem sensitivity to nutrient enrichment. *Aquatic Ecology* 33:3-15.

Codd, G. A. 1995. Cyanobacterial toxins: Occurrence, properties and biological significance. *Water Science and Technology* 32: 149-156.

Cook, P. L., D. P. Holland, and A. R. Longmore. 2010. Effect of a flood event on the dynamics of phytoplankton and biogeochemistry in a large temperate Australian lagoon. *Limnology and Oceanography* 55:1123.

Czubakowski, J. 2010. Estuarine phytoplankton response to annual and manipulated river inputs. M.S. Thesis, Baton Rouge: Louisiana State University.

Dortch, Q. & S. Achee. 1998. Lake Pontchartrain 1997 algal bloom: identification, toxicity, and similar occurrences elsewhere in Louisiana coastal waters. In Malek-Wiley, R. R. (ed.), Clean enough? Lake Pontchartrain Basin Foundation, New Orleans: 19–20.

Dortch, Q., M. L. Parsons, N. N. Rabalais, and R. E. Turner. 1999. What is the threat of harmful algal blooms in Louisiana coastal waters? *in* L. P. Rozas, J. A. Nyman, C. E. Proffitt, N. N. Rabalais, D. J. Reed, and R. E. Turner, eds., The Symposium Recent Research in Coastal Louisiana: Natural System Function and Response to Human Influence. LA, Louisiana Sea Grant College Program.

Duan, S., M.A. Allison, T.S. Bianchi, B.A. McKee, A.M. Shiller, L. Guo, and B.E. Rosenheim. 2014. Biogeochemical dynamics at Major River-Coastal Interfaces: Linkages with Global Change. Cambridge University Press, New York.

Fischer, W. J., I. Garthwaite, C. O. Miles, K. M. Ross, J. B. Aggen, A. R. Chamberlin, N. R. Towers, and D. R. Dietrich. 2001. Congener-Independent Immunoassay for Microcystins and Nodularins: *Environmental Science & Technology*, v. 35, p. 4849-4856.

Garcia, A. C., S. Bargu, P. Dash, N. N. Rabalais, M. Sutor, W. Morrison, and N. D. Walker. 2010. Evaluating the potential risk of microcystins to blue crab (*Callinectes sapidus*) fisheries and human health in a eutrophic estuary. *Harmful Algae* 9:134-143.

Gilpin, L., K. Davidson, and E. Roberts. 2004. The influence of changes in nitrogen: silicon ratios on diatom growth dynamics. *Journal of Sea Research* 51: 21-35.

Hastings, R. W. 2009. The Lakes of Pontchartrain: Their History and Environments: Jackson, MS, University Press of Mississippi, 319 p.

Heisler, J., P. M. Glibert, J. M. Burkholder, D. M. Anderson, W. Cochlan, W. C. Dennison, Q. Dortch et al. 2008. Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae* 8:3-13.

Ibelings, B. W., and I. Chorus. 2007. Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health: A review. *Environmental Pollution* 150:177-192.

IPCC. 2007. Working Group III Report. Climate Change 2007*in* U. N. E. Programme, ed. New York.

Kennish, M. J. 2002. Environmental threats and environmental future of estuaries. *Environmental Conservation* 29:78-107.

Lane, R., J. Day, and B. Thibodeaux. 1999. Water quality analysis of a freshwater diversion at Caernarvon, Louisiana. *Estuaries and Coasts* 22:327-336.

Mize, S. and D.K. Demcheck. 2009. Water quality and phytoplankton communities in Lake Pontchartrain during and after the Bonnet Carré Spillway opening, April to October 2008, in Louisiana, USA. *Geo-Marine Letters* 29: 431-440.

Paerl, H. 1988. Nuisance Phytoplankton Blooms in Coastal, Estuarine, and Inland Waters. *Limnology and Oceanography* 33:823-847.

Paerl, H., R. Fulton, P. Moisander, and J. Dyble. 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *The Scientific World Journal* 1:76-113.

Paerl, H., N.S. Hall, B.L. Peierls, K.L. Rossignol, and A.R. Joyner (2013). Hydrologic Variability and Its Control of Phytoplankton Community Structure and Function in Two Shallow, Coastal, Lagoonal Ecosystems: The Neuse and New River Estuaries, North Carolina, USA. *Estuaries and Coasts* pp 1-15.

Paerl, H., and J. Huisman. 2008. Blooms Like It Hot. *Science* 320:57-58.

Paerl, H., H. Xu, M. J. McCarthy, G. Zhu, B. Qin, Y. Li, and W. S. Gardner. 2011. Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): The need for a dual nutrient (N & P) management strategy. *Water Research* 45:1973-1983.

Parsons, T., Maita, Y., Lali, C. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford.

Piazza, B.P. and M.K. La Peyre. 2007. Restoration of the annual flood pulse in Breton Sound, Louisiana, USA: habitat change and nekton community response. *Aquatic Biology* 1: 109-119.

Pinckney, J.L., H.W. Paerl, M.B. Harrington, and K.E. Howe. 1998. Annual cycles of phytoplankton community-structure and bloom dynamics in the Neuse River Estuary, North Carolina. *Marine Biology* 131:371-381.

Pinckney, J.L., J.L. Wee, A. Hou, and N. Walker. 2009. Phytoplankton community structure responses to urban effluent inputs following Hurricanes Katrina and Rita. *Marine Ecology Progress Series* 387: 137-146.

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

Rabalais, N., R. J. Diaz, L. A. Levin, R. E. Turner, D. Gilbert, and J. Zhang. 2010. Dynamics and distribution of natural and human-caused hypoxia. *Biogeosciences* 7:585-619.

Rabalais, N., R. E. Turner, D. Justić, Q. Dortch, W. Wiseman, and B. Sen Gupta. 1996. Nutrient changes in the Mississippi River and system responses on the adjacent continental shelf. *Estuaries* 19:386-407.

Roblin, R. 2008. Water quality modeling of freshwater diversions in the Pontchartrain Estuary. M.S. Thesis, New Orleans: University of New Orleans.

Rocha, C., H.M. Galvão, and A.B. Barbosa. 2002. Role of transient silicon limitation in the development of cyanobacteria blooms in the Guadiana estuary, south-western Iberia. *Marine Ecology Press Series* 228: 35-45.

Roy, E., N. T. Nguyen, S. Bargu, and J. R. White. 2012. Internal loading of phosphorus from sediments of Lake Pontchartrain (Louisiana, USA) with implications for eutrophication. *Hydrobiologia* 684: 69-82.

Roy, E., J. White, E. A. Smith, S. Bargu, and C. Li. 2013. Estuarine Ecosystem Response to Three Large-Scale Mississippi River Flood Diversion Events. *Science of the Total Environment*:374-387.

Rozas, L. P., T. J. Minello, I. Munuera-Fernández, B. Fry, and B. Wissel. 2005. Macrofaunal distributions and habitat change following winter–spring releases of freshwater into the Breton Sound estuary, Louisiana (USA). *Estuarine, Coastal and Shelf Science* 65:319-336.

Schindler, D. W., and J. R. Vallentyne. 2008, The Algal Bowl; Overfertilization of the World's Freshwaters and Estuaries. Canada, Houghton Boston Printers.

Sivonen, K., and G. Jones. 1999. Cyanobacterial toxins *in* I. Chorus, and J. Bartram, eds. Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. Great Britain, St. Edmundsbury Press.

Sun, J., and D. Liu. 2003. Geometric models for calculating cell biovolume and surface area for phytoplankton: *Journal of Plankton Research*, v. 25, p. 1331-1346.

Turner, R. E., Q. Dortch, D. Justic, and E. M. Swenson. 2002. Nitrogen loading into an urban estuary: Lake Pontchartrain (Louisiana, U.S.A.). *Hydrobiologia* 487:137-152.

Turner, R. E., Q. Dortch, and N. N. Rabalais. 2004. Inorganic nitrogen transformations at high loading rates in an oligohaline estuary. *Biogeochemistry* 68:411-422.

USEPA. 1993. Methods of chemical analysis of water and wastes, *in* USEPA, ed., Cincinnati, Environmental Monitoring Support Laboratory.

Vilhena, L. C., I. Hillmer, and J. Imberger. 2010. The role of climate change in the occurrence of algal blooms: Lake Burragorang, Australia. *Limnol. Oceanogr* 55:1188-1200.

White, J. R., H. W. Fulweiler, C. Y. Li, S. Bargu, N. D. Walker, H. H. Twilley, and S. E. Green. 2009. Mississippi River Flood of 2008: Observations of a Large Freshwater Diversion on Physical, Chemical, and Biological Characteristics of a Shallow Estuarine Lake. *Environmental Science & Technology* 43:5599-5604.

Wu, K., and Y. J. Xu. 2007. Long-term freshwater inflow and sediment discharge into Lake Pontchartrain in Louisiana, USA. *Hydrological Series Journal* 52: 166-180.

CHAPTER 3: POTENTIAL FOR OYSTER CONTAMINATION FROM FRESHWATER CYANOBACTERIAL TOXINS IN COASTAL LOUISIANA

Abstract

Documented occurrences and severity of toxin-producing cyanobacteria blooms are increasing in estuarine environments throughout the world leading to negative impacts on the environmental health and creating a human health risk through shellfish consumption. In Louisiana (LA), when freshwater diversions are opened into estuaries, toxin producing freshwater cyanobacteria can be flushed into more brackish coastal waters increasing the possibility of exposure of toxins to oyster beds. Toxic *Anabaena* sp. was experimentally grown in surface waters collected from Breton Sound Estuary, LA, at a range of salinities (0-10 g kg^{-1}). Oysters were then collected from that field site and allowed to feed on toxic *Anabaena* over a three-hour period. The results indicate that toxic *Anabaena* can sustain or even increase biomass at the salinities (5-10 g kg^{-1}) typical of oyster beds in coastal Louisiana. The oysters collected from the field were found to already contain microcystin in their tissue (8.11 µg MC equivalent kg^{-1}). Additionally, we found the oysters fed on the toxic *Anabaena* and their tissue was found to contain the microcystin (7.23 ± 6.76 µg MC equivalent kg^{-1}). These findings suggest that the original intent of the diversions to create more productive systems may also potentially foster a human health problem given that LA is a leading provider of oysters to the United States.

Introduction

Estuaries are some of the most biologically productive areas on earth, and a critical environment where many species of fish and crustaceans are dependent for either

all or part of their life cycle (De Sylva et al. 1962; Chubb and Potter 1984, 1986; Dando 2011). These complex systems are also subject to great variations in hydrology, tidal interactions, salinity, sedimentation and geomorphology, supporting this diverse biota. In the past, estuaries relied heavily on the natural periodic flooding of the rivers to supply sediments, nutrients, and freshwater to maintain the ecosystem. Over the years, estuaries have also become separated from the river flooding due to hydrologic manipulations by humans. Additionally, increased human habitation and industrial development bordering estuarine systems have led to the degradation of estuarine water quality.

In an effort to restore freshwater flow to estuaries, surface water diversions have been adopted recently to introduce fresh water into coastal basins estuaries along with associated nutrients and sediment to prompt coastal restoration (Rozas et al. 2005; Lane et al. 2007; Schindler and Vallentyne 2008). Diversions are managed based on the ecosystem they serve, where some are designed to prevent flooding to downriver communities (Roy et al. 2013), while others are managed to direct water and nutrients into marshes, while other have been designed for land creation (Lane et al. 1999). Addition of nutrients and freshwater are needed in estuarine systems to maintain productivity; however, a large nutrient pulse from such diversions can quickly increase primary production causing associated problems, such as hypoxia, harmful algal blooms (HABs), and excessive plant growth (Kennish 2002; Bricker et al. 2008; Bargu et al. 2011).

Prolonged phytoplankton blooms can cause many problems such as foul odors, fish kills, food web alterations, and toxicity (Paerl et al. 2001). In freshwater and oligohaline systems, the main group of phytoplankton linked to toxicity is cyanobacteria,

also called blue-green algae (Paerl 1988). Cyanobacterial toxins (cyanotoxins), including microcystins, anatoxins, and saxitoxins, can present several health hazards for aquatic organisms and humans through direct contact or through their diet. Many studies have shown that these toxins can cause death or developmental and behavioral abnormalities in mammals, fish, mollusks, and zooplankton (Råbergh et al. 1991; Oberemm et al. 1999; Miller et al. 2010). Symptoms caused by these toxins in humans can range from skin irritation and gastrointestinal problems to death (Codd 1995; Codd et al. 2005; Metcalf and Codd 2009). Although some species of toxin producing cyanobacteria are shown to be salt-tolerant (Moisander et al. 2002; Tonk et al. 2007; Lehman et al. 2008); and present in both saltwater and freshwater habitats (Miller et al. 2010), consistent monitoring efforts are generally limited to freshwater systems.

In the northern Gulf of Mexico (GOM), marine HAB toxins have been the subject of regional interest for many decades, while the presence of toxin producing cyanobacteria in estuaries have recently received increased attention. Toxin producing cyanobacteria have been shown to occupy the Louisiana (LA) estuaries with species of *Microcystis* and *Anabaena* (Czubakowski 2010). It is now known that when the water flow is high in the upper part of these estuaries due to opening of the river diversions, toxin producing cyanobacteria can reach the coastal parts of the estuaries, where high toxin intake into the filter-feeding oysters can occur (Czubakowski 2010). However, there has been very limited local research into understanding estuarine cyanobacteria bloom dynamics and their ability to contaminate brackish water organisms.

Louisiana's coastline on the Gulf of Mexico (GOM) has large numbers of both private and state oyster beds that can be potentially adversely affected by the presence of

both marine and freshwater toxins. Louisiana has the largest production of eastern oysters (*Crassostrea virginica*) in the United States (Banks 2012). One important oyster seed ground, Breton Sound, LA, which is influenced by nutrient-rich freshwater coming from the Mississippi River via Caernarvon diversion, has more than 10 % bottom coverage of oyster beds. The Caernarvon diversion was originally built in 1981 to mimic periodic flooding of the Mississippi River into the adjacent coastal wetlands to lower the salinity of the estuary and to stimulate oyster production. The diversion is operated on a year to year basis to maintain target salinities in various portions of the estuary (Chatry et al. 1983). Phytoplankton growth can be stimulated by regular additions of nutrients from diversion openings that vary depending on rainfall patterns, river stage and wind driven tides (Lane et al. 1999; VanZomeren et al. 2013).

Breton Sound Estuary offers an opportunity to observe whether toxin producing cyanobacteria are capable of growing in higher saline waters of the Estuary and if the native oyster population is consuming the cells and concentrating the toxins in their tissue. Therefore, preliminary experiments were designed to address following questions: (1) What is the range of survival of freshwater-adapted toxin producing cyanobacteria in the coastal waters of the Breton Sound Estuary?; (2) Is associated toxin production affected by such salinity changes?; and (3) Can local oysters consume toxin producing cyanobacteria and to what level do they contain the microcystins (MCs)?

Materials and Methods

Cyanobacteria cultures and maintenance

The freshwater cyanobacterium used in the experiments was a toxic strain of *Anabaena* sp. that was obtained from Provasoli-Guillard National Center for Culture of

Marine Phytoplankton (NCMA 2066). Freshwater (0 g kg^{-1}) was collected during a non-bloom period from the Breton Sound Estuary, LA, and natural phytoplankton were removed using 0.2 µm pleated capsule filter (Pall). Nutrient rich stock media, Alga-Grow (Carolina Biological) was added and then sterilization of the media was accomplished by autoclaving. The semi-continuous stock cultures of toxic *Anabaena* sp. were maintained in 250 mL Erlenmeyer flasks containing approximately 150 mL of freshwater media and kept in a light and temperature controlled incubator at 25 ± 1 C° on a 12-h light/12-h dark cycle.

Toxic *Anabaena* sp. Growth Experiments at Varying Salinities

For the first experiment, 150-ml cultures of toxic *Anabaena* sp. at 0, 5, 10, 15 and 20 g kg^{-1} salinities (n = 3 for each) were maintained in a controlled incubator (25°C on a 12-h light/12-h dark cycle) for 9 days. Every 24 hours, starting from t = 0 day, subsamples were removed from each flask for Chlorophyll *a* (Chl *a*) measurements.

Chl *a* levels were used as the indicator of cyanobacteria biomass. Subsamples were filtered onto 25 mm GF/F (Whatman) circles and the filters were placed in 15-ml centrifuge tubes, covered with aluminum foil and kept frozen until the analyses. Filters were then extracted for 24 h in 90% aqueous acetone at –20 °C, and fluorescence was measured before and after acidification with hydrochloric acid using a Turner fluorometer (Model 10-AU) (Parsons et al. 1984).

For the second experiment, the first experiment was repeated at narrower salinity range of 0, 5, 6,7,8,9, and10 g kg^{-1} to be able to define the tolerance threshold better. Zero g kg^{-1} was used as the control. The highest salinity used was 10 g kg^{-1} since the previous experiment showed that *Anabaena* sp. did not show significant growth in any of the

cultures with salinities above 9 g kg^{-1} and because oysters are found in salinities of 5 to 35 g kg^{-1}, but prefer 10-30 g kg^{-1} (Strom and Thompson 2000). Sampling and processing were the same as in the first experiment, except microscopy examinations were conducted throughout the experiment and at the end of this experiment, subsamples were also taken for the cyanotoxin, microcystin (MC), measurements. The whole stock culture was also measured at the beginning of the experiment for a baseline of MC levels. The growth rate (μ) for cyanobacterial growth was calculated using the modified formula

$$\mu = (\ln N_2 - \ln N_1)/t_2 - t_1$$

where N_1 and N_2 are Chl *a* at the end and beginning of time (*t*) during the exponential growth phase (Fogg and Thake 1987).

Enzyme-linked immunosorbent assay (ELISA) kit (Abraxis LLC) was used to measure MC equivalents in water subsamples following the protocol in Garcia et al. (2010). ELISA is a competitive binding assay that is highly sensitive for MCs with a detection limit of 0.15 μg L^{-1} based on the most common variant, MC-LR, and its congeners. The cross-congener reactivity is high and has high sensitivity (Fischer et al. 2001). Particulate samples were collected by filtering the water through GF/F circles until clogged. The filtrate was also collected for the dissolved toxins. All the processed samples were temporarily stored at -20°C until needed for analysis. Filters were allowed to come to room temperature and placed in glass test tubes, and then 5mL of 50% methanol plus 1% acetic acid was added to each tube. Each sample was then vortexed (1 minute) to displace cells from the filter, sonicated (2 minutes, 30-40 W) in an ice bath using a sonicator probe, and centrifuged (10 minutes, 3000 rpm). The supernatant was removed and filtered through a 0.2 μm syringe filter (Corning). The remaining pellet was

re-suspended using 5 ml of extraction solution before repeating the process and finally pooling the filtered supernatant. The dissolved toxins were only filtered through a 0.2 µm syringe filter (Corning) before analyzing. Samples were then analyzed following the protocol included in the kit, with each sample run in duplicate and at several dilutions in order to reduce interference from matrix effects (impact of physical components of the sample on measurement of the analyte). Absorbance was read at 450 nm using a micro-plate spectrophotometer.

Three Hour Time Course Experiment with Eastern Oysters (*Crassostrea virginica*) Grazing on a Low Toxicity Strain of *Anabaena* sp.

Eastern oysters, *Crassostrea virginica*, were collected in May 2010 from a historical transect where Station 16 (previously named as station 11 in the literature) is the most Gulfward station in Breton Sound Estuary, LA (29.659772°N, 89.564792°W) (Lane et al. 2007). At the time of oyster collection, water samples were also taken where the oysters were collected. Background Chl *a* and MC analyses (water and oysters) were done with the collected field samples to obtain information of the water conditions at the time of the oyster collection before the grazing experiments started. Chl *a* analyses and MC analyses were conducted the same way as described above.

The oysters were brought back to the lab located in Baton Rouge, Louisiana, within five hours of collection, and then were scrubbed with wire brushes to remove any barnacles or other debris and placed in 10 gal aquariums. Oysters were held in 10 gal aquariums with continuous flow of ambient water (24°C) and bubbled air for 24 hours to acclimate. The 24 hours of acclimation also served as the starvation period.

Toxic *Anabaena* sp. was added to each clear polycarbonate containers (Tundra Specialties) (n = 9) with 2 L of sterilized media using filtered estuarine water collected

during non-bloom period. The salinity was adjusted to10 g kg^{-1} based on what the typical salinity is for lower part of the estuaries in LA in the spring (Turner 1985). Each container held one oyster. The containers were air bubbled to ensure even distribution of *Anabaena*. Animals were allowed to feed on *Anabeana* with an initial concentration of $2x10^4$ cells mL^{-1} (2.32 µg Chl *a* L^{-1}) for 3 h. Other field studies have indicated *Anabaena* sp. cell concentrations at a high concentration of $1.5x10^4$ cells mL^{-1} (Tsujimura and Okubo 2003). Water aliquots of 10-mL were drawn from each container at t=0 and every 20 minute interval for the 3 h, and immediately filtered onto 25 mm GF/F circles to determine the cyanobacteria removal due to grazing. Filters were then extracted for 24 h in 90% aqueous acetone and analyzed for Chl *a* as described above. Each oyster was removed individually at the end of 3 h and frozen for further viscera content and toxin analyses. Water samples were measured for toxin levels at time zero and at the end of 3h.

Three sets of controls were also used in the oyster grazing experiment (Table 3.1). They were all exposed to the same set-up as the grazing experimental containers. Subsamples were similarly taken every 20 min to measure Chl *a* for changes in phytoplankton biomass over 3 h. Both ingestion and clearance rates were calculated from changes in chlorophyll *a* (Chl *a*) concentrations in the feeding containers relative to changes in Chl *a* concentrations in control bottles using Frost's (1972) equations.

Water toxin levels were analyzed as described above. The potential toxin in oyster viscera was extracted by homogenizing the viscera in a beaker until it was smooth using a Biohomogenizer with a 1.4 cm generator (Biospec). Four grams (of one or more oysters when needed) of the homogenate was weighed on a Mettler balance, as the Biosense ELISA protocol specifies. Homogenates were then placed in a clean 50 mL centrifuge

Table 3.1 Control treatments for the oyster grazing experiment. Describes all treatments with methods and reasoning.

Control Treatments for Oyster Grazing Experiment			
Treatment	Algae present?	Method	Purpose
1-bleached oyster	No	Scrubbed oyster with wire brush and 10% bleach solution; rinsed with fresh water	Remove any epibionts that may also feed on algae
2-shucked and bleached shell	Yes	Shucked and scrubbed shell with 10% bleach solution	Account for any settling of the algae onto the shell
3-algae only	Yes	Algae only in container	Account for any growth or death of cells over the time of the experiment

tube and 75% MeOH was added in a 1:4 weight to volume ratio, 1 part viscera weight to 4 parts MeOH. After this step, the homogenates were vortexed, sonicated, filtered and analyzed in the same manner as the water samples except they were only extracted once. Microcystin extraction efficiencies were tested for oyster tissue by using spike and recovery methods on oysters done similarly to the methods in Garcia et al. (2011). Microcystin LR Standard, 0.75 µg/L (Abraxis kits) was injected into homogenized viscera from those oysters, extracted at 50% MeOH, 75% MeOH and 100% MeOH. The recovery efficiencies were 55%, 66% and 37% respectively. Control samples not injected with the standard were also processed the same way and were below the detection limit.

Statistical Analysis

All statistical analyses were initially run with multi-variate ANOVA. The matrices were not invertible, so final analysis was done using pairwise comparisons or difference of least mean squares.

Results

Toxic Cyanobacteria Growth Experiments at Varying Salinities

The initial *Anabaena* sp. growth experiment at varying salinities (0, 5, 10, 15, and 20 g kg^{-1}) only had positive growth in 0 and 5 g kg^{-1} treatments. Ten g kg^{-1} treatment maintained a variable biomass, while 15 g kg^{-1} treatment showed a decline in biomass starting on day 5, and 20 g kg^{-1} treatment had no biomass by day 6 (data not shown). The 10-day growth experiment at the narrower salinities (5-10 g kg^{-1}) showed that *Anabaena* can positively grow in almost all treatments, except 9 and 10 g kg^{-1}, where the biomass did not change significantly over time ($p<0.0001$, $\alpha=0.05$) (Figure 3.1). There was not a significant correlation between salinity and Chl *a* concentrations per cell ($p<0.001$, $\alpha=0.05$). However, there was a significant difference between terminal Chl *a* at 0, 5 and 6 g kg^{-1} compared to the terminal Chl *a* at 9 and 10 g kg^{-1} treatments ($p<0.005$). The growth rate (μ) for the control (0 g kg^{-1}) was 0.34 µg Chl *a* day^{-1}. In the experimental treatments (5-10 g kg^{-1}), 6 g kg^{-1} had the highest growth rate of 0.61 µg Chl *a* day^{-1}, but 5 g kg^{-1} treatment had the largest biomass (461.8 µg Chl *a* L^{-1}) at the end of day 10. At 7 and 8 g kg^{-1}, there was a longer lag phase in growth where biomass did not start to increase until 7 and 9 days, respectively. Particulate toxins were below the level of detection (BD) of 0.15 µg MC equivalent L^{-1} in all of the salinities (Table 3.2). However, dissolved toxin levels were not only detectable at all salinities tested, but also showed an increase from 0-8 days with a range of 0.40 -1.25 µg MC equivalent L^{-1} (Table 3.2), with no observed cell lysis.

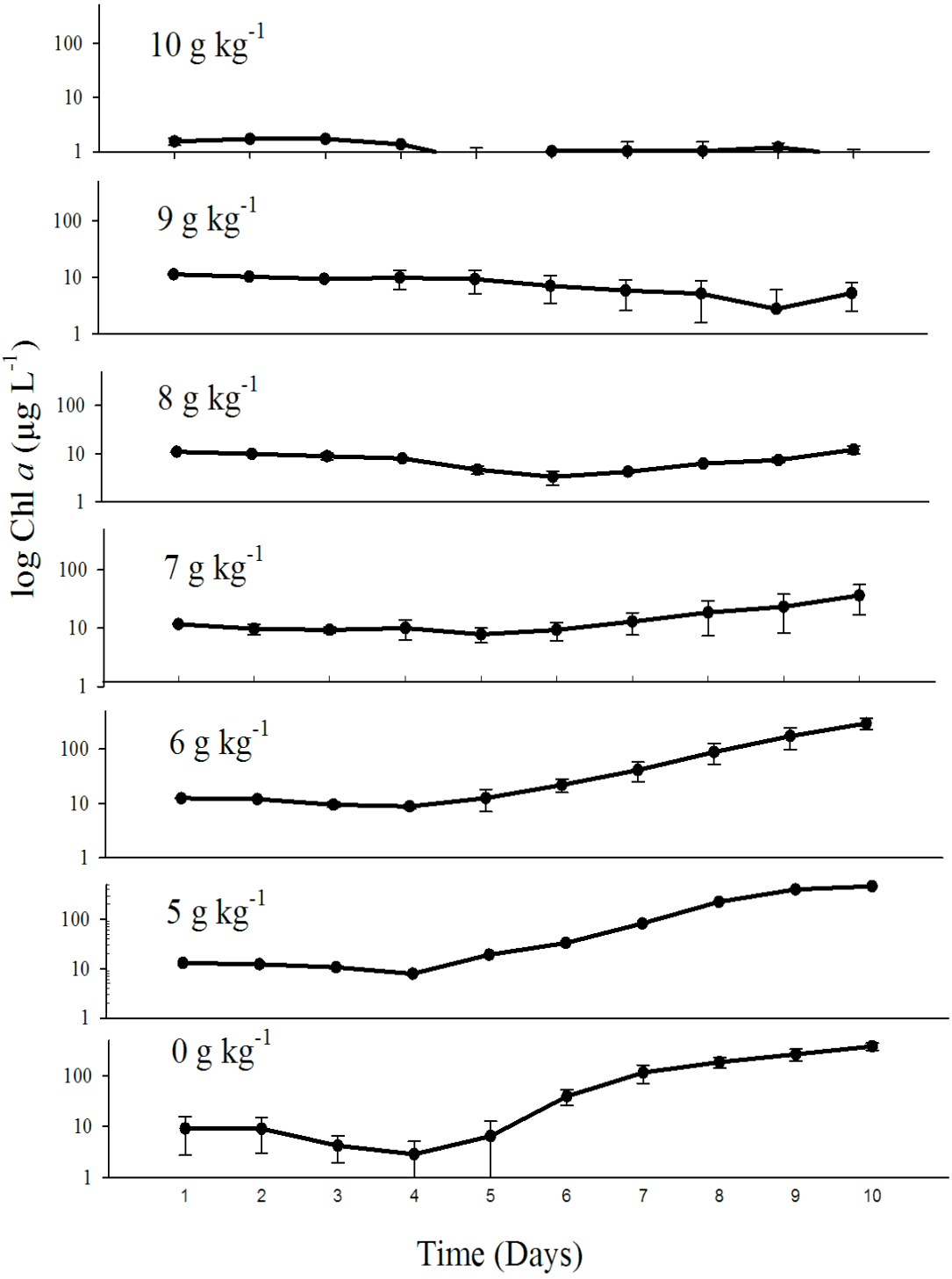


Figure 3.1. Changes in Chl *a* concentrations (µg L^{-1}) in log scale of toxic *Anabaena* sp. cultures at different salinities over a 10 day time span.

Table 3.2. The comparison of dissolved and particulate toxin levels for increasing salinity treatments over a 10-day growth period. The dissolved MC equivalent increased until 8 g kg^{-1}, and then decreased slightly at 10 g kg^{-1}.

Time	Treatment group (salinity g kg^{-1})	Dissolved toxin level (µg MC equivalent L^{-1})	Particulate toxin level (µg MC equivalent L^{-1})
Day 0	Stock culture	0.66	BDL
Day 10	0	0.66	BDL
"	5	0.51	BDL
"	6	0.72	BDL
"	8	1.25	BDL
"	10	0.40	BDL

BDL = Below Detection Limit (0.15 µg MC equivalent L^{-1})

Three Hour Time Course Experiment with Eastern Oysters (*Crassostrea virginica*) Grazing on a Low Toxicity Strain of *Anabaena* sp.

In the 3-h grazing experiment, Chl *a* concentrations in grazing containers (n=9) decreased significantly over time ($p<0.05$, $\alpha=0.05$, Tukey Test), with initial concentrations of 2.28 ± 0.32 µg Chl *a* L^{-1} decreasing to 0.27 ± 0.12 µg Chl *a* L^{-1} indicating 89% reduction of total biomass in the water over 3 hrs (Figure 3.2). There was also significant difference in the Chl *a* concentrations for the grazing treatment compared to all of the controls ($p<0.001$, $\alpha=0.05$) (Figure 3.2). Control 1 (bleached shell with oyster + *Anabaena* sp.) showed only a small decrease in biomass from t=0 to t=180 min of 2.62 ± 0.16 to 2.44 ± 0.03 µg Chl *a* L^{-1} indicating possible stress from the bleaching process, making the oysters unable to feed in that short period of time. Biomass in control 2 (bleached shell without oyster + *Anabaena* sp.) stayed the same over time with beginning average Chl *a* concentration of 2.62 ± 0.16 µg Chl *a* L^{-1} and ending with 2.60 ± 0.15 µg Chl *a* L^{-1}. Control 3 (*Anabaena* sp. only) had rather a small decrease in biomass,

possibly due to salinity stress, over time with net population growth (μ) of -0.09 ± 0.03 µg Chl *a* L^{-1} hour $^{-1}$ (Figure 3.2). In the

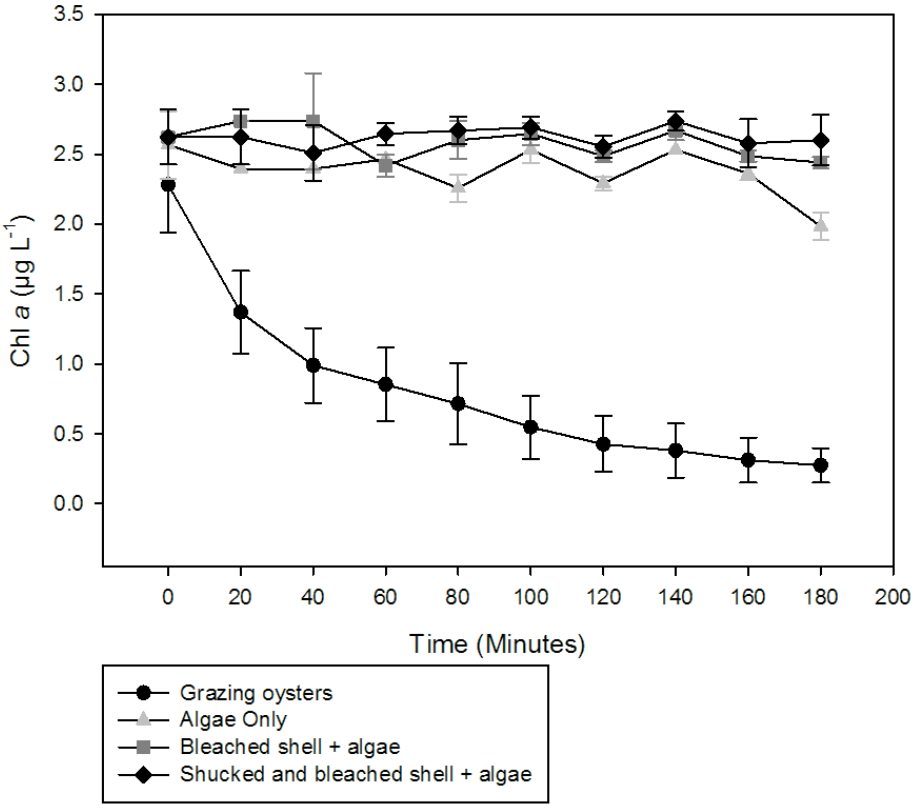


Figure 3.2. Changes in Chl *a* concentrations (µg L^{-1}) in experimental and control containers during the oyster grazing experiment on toxic *Anabaena* sp. maintained that rate until the end of the experiment. Pseudofeces were not observed in any of the oysters.

grazing containers, the average Chl *a* ingestion rate for the oysters while they were actively feeding was 0.05 ± 0.02 µg Chl *a* oyster^{-1} min $^{-1}$. In the first 20 minutes, the majority of the oysters were actively feeding with a higher filtration rate of 45.87 ± 24.44 mL oyster $^{-1}$ min^{-1}. From 20-180 minutes, the oysters had a decrease in filtration rate (20.5 ± 2.75 mL oyster $^{-1}$ min^{-1}). The toxin levels measured in samples related to the grazing experiments showed an increase up to 8 g kg^{-1} (Table 3.3). The water where the

oysters were originally collected had initial particulate toxins present (0.04 µg MC equivalent L^{-1}), but dissolved toxins were below the detection limit. All oysters collected

Table 3.3. Toxin concentrations of both water and tissue samples collected from Breton Sound Estuary and from the grazing experiment. Tissue in the grazing experiment showed an increase over time with exposure to the toxin-producing algae.

	Dissolved toxin level (µg MC equivalent L^{-1})	Particulate toxin level (µg MC equivalent L^{-1})	Tissue toxin level (µg MC equivalent kg^{-1})
Breton Sound Estuary	BDL	.04	8.11
Grazing Experiment oyster t=0 min.	0.52	BDL	2.42[a]
Grazing Experiment oyster t=180 min.	0.91 ± 0.44	BDL	7.23 ± 6.76
Bleached shell oyster + algae	0.93 ± 0.39	BDL	BDL
Bleached shell with no oyster + algae	0.34 ± 0.02	BDL	N/A
Algae only t=0 min.	0.52	BDL	N/A
Algae only t=180 min.	0.31 ± 0.13	BDL	N/A

BDL = Below Detection Limit
[a] only had one extra oyster to be able to run toxins on

from Breton Sound Estuary also likely contained toxins in their viscera, one that we measured reaching to 8.11µg MC equivalent kg^{-1}. The viscera toxin sample taken from t=0, after the 24 h acclimation, had a lower MC equivalent concentration of 2.42 µg kg^{-1}, indicating potential toxin depuration over 24-h time starvation period. The stomach contents were analyzed using microscopy, but no cells were visible in any of the tissue samples. The initial concentrations of particulate MCs in the water of the experimental

containers were below the detection limit of 0.15 µg L^{-1} with dissolved MC equivalent reaching to 0.52 µg L^{-1}. Particulate toxin levels at the end of the experiment was still below the detection limit but the dissolved MC equivalent increased to 0.91 ± 0.44 µg L^{-1}. The oyster viscera that were measured for MC equivalent after 3 hours of exposure had concentrations of 7.23µg kg^{-1}± 6.75 µg kg^{-1}.

Discussion

The Caernarvon diversion was built to allow nutrients and fresh water into the Breton Sound Estuary, but has opened the possibility of fresh-water adapted phytoplankton being flushed into the more saline parts of the system as well. The flushing of the system can bring cyanobacteria into a lower saline range of less than 10 g kg^{-1}; a range our study showed can be tolerated by toxin producing *Anabaena* sp. The experiment showed that even though toxic *Anabaena sp.* growth was lower over the increasing salinity treatments; up to 8 g kg^{-1}, they still demonstrated a positive growth during the 10-day period, indicating potential for cyanobacteria to become abundant within these more saline waters. Other studies showed similar results with some freshwater cyanobacteria species not only tolerating an increase in salinity (2 to >30 psu) but continuing to grow (Otsuka et al. 1999; Paerl et al. 2001; Moisander et al. 2002; Tonk et al. 2007). Toxin producing cyanobacteria, such as *Microcystis* sp. and *Anabaena* sp., are commonly found in Louisiana's freshwater and estuarine systems with varying salinities, especially during warmer months at low turbidity (Czubakowski 2010; Garcia et al. 2010; Bargu et al. 2011; Roy et al. 2013). This ability by toxin producing freshwater cyanobacteria to grow in higher salinities can be very important in coastal

regions, in particular in southern Louisiana estuaries, where the salinity can range from 0-30 g kg^{-1} depending on rainfall, winds and storms.

Not only did our experiment demonstrate growth of the toxin producing cyanobacteria at higher salinities, but it also demonstrated that *Anabaena* sp. produced the cyanotoxin MCs under a range of salinities and in the case of the higher salinities, produced more extracellular (dissolved) toxins similar to findings in Czubakowski (2010), indicating potential salinity stress on these organisms. When the required conditions are present, cyanotoxin production mainly occurs intracellularly (particulate) and food web contamination may occur through the digestion of the toxic prey (Ibelings and Chorus 2007; Karjalainen et al. 2007). However when the cells are stressed due to environmental changes such as nutrient limitation or salinity shifts, then these toxins can be released out of the cell into the water column (Chorus and Bartram 1999) creating a vector for threats to marine and human health from direct uptake of the dissolved toxins or recreational exposure. One noteworthy result was higher increase in extracelluar toxin production in treatments containing oysters than the *Anabaena* only treatment. The benefit of releasing the toxin outside of the cells to the organism is not clear. They can possibly increase extracellular toxin production as a defense mechanism to reduce their predation. More research is needed on the examination of toxin production when cyanobacteria encounters environmental stressors, like low nutrients or shifting salinity, compared to more immediate stress factors, like grazing.

The coastal areas of the Breton Sound Estuary provide habitats for many filter feeding organisms that are economically important to LA, especially oysters, and they are potentially at risk of exposure to cyanotoxins. The oyster samples collected in this study

from the lower part of the Breton Sound Estuary naturally contained cyanotoxins indicating that these toxins are not only present in LA's estuaries but also are being accumulated by shellfish. Our grazing experiment further supports the active consumption of toxic cyanobacteria by oysters. At the end of our experiment, the amount of the toxin found in the viscera of the oysters was higher than expected based on the low amount of particulate toxin found in the *Anabaena* sp. cells. This result is likely due to previous toxin exposure of the oyster in the environment before collection. Several studies have shown effective bioaccumulation of cyanotoxins (both particulate and dissolved) occurred in fresh and saltwater bivalves (Negri and Jones 1995; Amorim and Vasconcelos 1999; White et al. 2006; Ibelings and Chorus 2007), but variability between species, uptake and retention mechanism, and relationship between bioconcentration and environmental parameters were found to be high. There is very limited knowledge on cyanotoxin exposures to oysters in general, and none for the eastern oysters, *Crassostrea virginica.* Our toxin measurements were limited due to opportunistic sampling and in order to have enough oysters left for the study, only 2 oysters were initially sacrificed. The toxin concentrations that were found in the local species initially compared to the end of the experimental phase were highly variable, indicating that the experimental oysters may have had higher initial toxin levels. Our study can only suggest a trend in oysters' accumulation of the toxic species, but need further study on their feeding efficiencies in reference to these toxins. Further research efforts are needed to understand the complexity of both ingestion and toxin retention of cyanotoxins in this economically valuable oyster species.

Like in any aquatic system with land-sea interfaces, Breton Sound Estuary is also exposed to other natural and anthropogenic derived stressors to organisms. Our study only emphasized one potential stressor of toxin producing cyanobacteria to the oyster community in Louisiana. There are numerous other stressors such as pathogenic *Vibrio*, salinity changes, low dissolved oxygen and turbidity that can also affect the oysters' health in estuarine systems (Lenihan et al. 1999; Breitburg and Riedel 2005). The overall synergistic effects of these stressors are also not known and need further attention.

Fisheries in Louisiana are largely dependent upon estuarine productivity. Despite evidence that toxin producing cyanobacteria can be present in and tolerant to estuarine and partially marine conditions, there is currently limited monitoring for detection of MCs in shellfish in America and none for LA (Miller et al. 2010). Considering that Louisiana has the largest landings (34% for USA) of eastern oysters (*Crassostrea virginica*) in the United States (Banks 2010), we need to have a better understanding for the variation in diversion flow and cyanobacterial bloom dynamics. In addition, there needs to be a better monitoring program (both water and shellfish) in place to understand how often and at what level oyster communities are exposed to these cyanotoxins in order to evaluate both oysters and consequent human health risks. Diversion management should also consider cyanobacterial blooms as part of their decision to regulate when diversions are opened in order to decrease the risk to these estuarine systems from potentially toxic cyanobacteria.

References

Amorim, Á. and V.T. Vasconcelos. 1999. Dynamics of microcystins in the mussel Mytilus galloprovincialis. *Toxicon* 37, 1041-1052.

Banks, P. 2012. Oyster Stock Assessment Report of the public oyster areas in Louisiana. In: Fisheries, L.W.a. (18).

Bargu, S., J.R. White, C.Y. Li, J. Czubakowski and R.W. Fulweiler. 2011. Effects of freshwater input on nutrient loading, phytoplankton biomass, and cyanotoxin production in an oligohaline estuarine lake. *Hydrobiologia* 661:377-389.

Breitburg, D.L. and G.F. Riedel. 2005. Marine Conservation Biology: The Science of Maintaining the Sea's Biodiversity. Island Press, Washington D.C.

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

Chatry, M., R.J. Dugas, and K.A. Easley. 1983. Optimum salinity regime for oyster production on Louisiana's state seed grounds. *Contributions in Marine Science* 26, 81-94.

Chorus, I. and J. Bartram. 1999. Toxic cyanobacteria in water a guide to their public health consequences, monitoring, and management. E & FN Spon, London; New York.

Chubb, C.F. and I.C. Potter. 1984. The reproductive biology and estuarine movements of the gizzard shad, Nematalosa vlaminghi (Munro). *Journal of Fish Biology* 25, 527-543.

Chubb, C.F. and I.C. Potter. 1986. Age, growth and condition of the Perth herring, Nematolosa vlaminghi (Munro) (Dorosomatinae), in the Swan Estuary, south-western Australia. *Marine and Freshwater Research* 37, 105-112.

Codd, G.A. 1995. Cyanobacterial toxins: Occurrence, properties and biological significance. *Water Science and Technology* 32, 149-156.

Codd, G.A., J. Lindsay, F.M. Young, L.F. Morrison, and J.S. Metcalf. 2005. Harmful cyanobacteria: From mass moralities to management measures. In: Huisman, J., Matthijs, H.C.P., Visser, P.M. (Eds.), *Harmful cyanobacteria*. Springer, Netherlands, pp. 1-23.

Czubakowski, J. 2010. Estuarine phytoplankton response to annual and manipulated river inputs. Louisiana State University, Baton Rouge, La.

Dando, P.R. 2011. Site fidelity, homing and spawning migrations of flounder Platichthys flesus in the Tamar estuary, South West England. *Marine Ecology-Progress Series* 430, 183-196.

De Sylva, D., C. Shuster, and F. Kalber. 1962. Fishes and ecological conditions in the shore zone of the Delaware River estuary: with notes on other species collected

in deeper water. Dept. of Biological Sciences, University of Delaware, Newark, p. 164.

Fischer, W.J., I. Garthwaite, C.O. Miles, K.M. Ross, J.B. Aggen, A.R. Chamberlin, N.R. Towers, and D.R. Dietrich. 2001. Congener-Independent Immunoassay for Microcystins and Nodularins. *Environmental Science & Technology* 35, 4849-4856.

Fogg, G. and B. Thake. 1987. Algal Cultures and Phytoplankton Ecology. The University of Wisconsin Press, Madison, Wisconsin.

Frost, B.W. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnology and Oceanography* 17 (6) 805-815.

Ibelings, B.W. and I. Chorus. 2007. Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health: A review. *Environmental Pollution* 150, 177-192.

Ibelings, B. and K. Havens. 2008. Cyanobacterial toxins: a qualitative meta–analysis of concentrations, dosage and effects in freshwater, estuarine and marine biota. In: Hudnell, H.K. (Ed.), Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Springer New York, pp. 675-732.

Karjalainen, M., J. Engstrom-Ost, S. Korpinen, H. Peltonen, J.P. Paakkonen, S. Ronkkonen, S. Suikkanen, and M. Viitasalo.2007. Ecosystem consequences of cyanobacteria in the northern Baltic Sea. *Ambio* 36, 195-202.

Kennish, M.J. 2002. Environmental threats and environmental future of estuaries. *Environmental Conservation* 29, 78-107.

Lane, R., J. Day, B.D. Marx, E. Reyes, E. Hyfield, and J. Day.2007. The effects of riverine discharge on temperature, salinity, suspended sediment and chlorophyll a in a Mississippi delta estuary measured using a flow-through system. *Estuarine Coastal & Shelf Science* 74, 145-154.

Lane, R., J. Day, and B. Thibodeaux. 1999. Water quality analysis of a freshwater diversion at Caernarvon, Louisiana. *Estuaries and Coasts* 22, 327-336.

Lehman, P., G. Boyer, M. Satchwell, and S. Waller. 2008. The influence of environmental conditions on the seasonal variation of *Microcystis* cell density and microcystins concentration in San Francisco Estuary. *Hydrobiologia* 600, 187-204.

Lenihan, H.S., F. Micheli, S.W. Shelton, and C.H. Peterson. 1999. The Influence of Multiple Environmental Stressors on Susceptibility to Parasites: An Experimental Determination with Oysters. *Limnology and Oceanography* 44, 910-924.

Metcalf, J.S. and G.A. Codd. 2009. Cyanobacteria, neurotoxins and water resources: Are there implications for human neurodegenerative disease? *Amyotrophic Lateral Sclerosis* 10, 74-78.

Miller, M.A., R.M. Kudela, A. Mekebri, D. Crane, S.C. Oates, M.T. Tinker, M. Staedler, W.A. Miller, S. Toy-Choutka, C. Dominik, D. Hardin, G. Langlois, M. Murray, K. Ward, and D.A. Jessup. 2010. Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters. *PLoS ONE* 5, 1-11.

Moisander, P.H., E. McClinton III, and H.W. Paerl. 2002 Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. *Microbial Ecology* 43:432-442.

Negri, A.P. and G.J. Jones. 1995. Bioaccumulation of paralytic shellfish poisoning (PSP) toxins from the cyanobacterium Anabaena circinalis by the freshwater mussel Alathyria condola. *Toxicon* 33, 667-678.

Oberemm, A., J. Becker, G.A. Codd, and C. Steinberg. 1999. Effects of cyanobacterial toxins and aqueous crude extracts of cyanobacteria on the development of fish and amphibians. *Environmental Toxicology* 14, 77-88.

Otsuka, S., S. Suda, R. Li, M. Watanabe, H. Oyaizu, S. Matsumoto, and A.M.M. Watanabe. 1999. Characterization of morphospecies and strains of the genus Microcystis (Cyanobacteria) for a reconsideration of species classification. *Phycological Research* 47, 189-197.

Paerl, H. 1988. Nuisance Phytoplankton Blooms in Coastal, Estuarine, and Inland Waters. *Limnology and Oceanography* 33, 823-847.

Paerl, H., R. Fulton, P. Moisander, and J. Dyble. 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *The Scientific World Journal* 1, 76-113.

Paerl, H. and J. Huisman. 2008. Blooms Like It Hot. *Science* 320, 57-58.

Parsons, T., Y. Maita, and C. Lali. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford.

Rozas, L.P., T.J. Minello, I. Munuera-Fernández, B. Fry, and B.Wissel. 2005. Macrofaunal distributions and habitat change following winter–spring releases of freshwater into the Breton Sound estuary, Louisiana (USA). *Estuarine, Coastal and Shelf Science* 65, 319-336.

Råbergh, C.M.I., G. Bylund, and J.E. Eriksson. 1991. Histopathological effects of microcystin-LR, a cyclic peptide toxin from the cyanobacterium (blue-green alga) *Microcystis aeruginosa* on common carp (*Cyprinus carpio*). *Aquatic Toxicology* 20, 131-145.

Roy, E.D., J.R. White, E.A. Smith, S. Bargu, and C. Li. 2013. Estuarine Ecosystem Response to Three Large-scale Mississippi River Flood Diversion Events. *Science of the Total Environment* 458:374-387.

Schindler, D.W. and J.R. Vallentyne. 2008. The Algal Bowl; Overfertilization of the World's Freshwaters and Estuaries. Houghton Boston Printers, Canada.

Strom, D. and M. Thompson. 2000. Biological Implications of Stable Salinity Gradients within the Context of the St. Lucie Estuary, Florida. In: Protection, F.D.o.E., Program, S.D.W.Q. (Eds.).

Tonk, L., K. Bosch, P.M. Visser, and J. Huisman. 2007. Salt tolerance of the harmful cyanobacterium Microcystis aeruginosa. Inter-Research.

Tsujimura, S. and T. Okubo. 2003. Development of Anabaena blooms in a small reservoir with dense sediment akinete population, with special reference to temperature and irradiance. *Journal of Plankton Research* 25, 1059-1067.

Turner, H. 1985. Parasites of eastern oysters from subtidal reefs in a Louisiana estuary with a note on their use as indicators of water quality. *Estuaries and Coasts* 8, 323-325.

USGS. 2012. Water resources data for the United States, Water Year 2012: U.S. Geological Survey Water. Data Report: WDR,US-2012, site 295124089542100

VanZomeren, C., J.R. White and R.D. DeLaune. 2013. Ammonification and denitrification rates in coastal Louisiana bayou sediment and marsh soil: Implications for Mississippi river diversion management. *Ecological Engineering* 54:77-81.

White, S.H., L.J. Duivenvoorden, L.D. Fabbro, and G.K. Eaglesham. 2006. Influence of intracellular toxin concentrations on cylindrospermopsin bioaccumulation in a freshwater gastropod (*Melanoides tuberculata*). *Toxicon* 47, 497-509.

CHAPTER 4: EDUCATION AND PUBLIC OUTREACH CONCERNING FRESHWATER HARMFUL ALGAL BLOOMS IN SOUTHERN LOUISIANA

Abstract

Scientific Literacy in America is very low compared to other developed countries. The public has a poor understanding of basic scientific principles (28% are considered scientifically literate) and outreach efforts to address this problem are limited. When looking at specific issues such as harmful algal blooms in freshwater systems there is hardly any active outreach programs. The basis for this study was to help the public understand what harmful algal blooms are and what visual indicators exist to warn of their presence. Initial surveys were conducted to find out what fishermen knew about algae and harmful algal blooms. The participants (age 11-70) had heard of algae (100%), but very few had heard of a harmful algal bloom (40%) and when the participants were pressed on the subject, few could define algae. An educational brochure was created from the baseline of data collected from the public and was distributed to the same fishing areas. A follow-up interview of these areas showed that the brochures were being taken by people, but finding those actual people proved difficult. Once brochures were viewed, many people were able to indicate something new they learned about algae such as algae produces oxygen. Scientists need to take a more active role in conveying their research to the public in order to increase scientific knowledge.

Introduction

Scientific Literacy

A common theme of our society today is that education in the United States is not sufficient compared to other developed countries around the world. Areas of education

that the United States consistently ranks low in are math and science. Within the education world, there are terms used to identify the specific areas that are deficient. "Scientific literacy" is a term that has been used to try and raise education standards around the country (Brown et al. 2005) and can have a different definition depending upon which agency is using the term. For example, The National Science Foundation defines scientific literacy as "the knowledge and understanding of scientific concepts and processes required for personal decision making, participation in civic and cultural affairs, and economic productivity" (NSES 1996). This allows for many interpretations and uses of scientific literacy. A more general definition is what the public should know and understand about science in order to live respectfully in the natural world (DeBoer 2000).

There has been a common theme of education to improve scientific literacy in America and other countries for decades. In the past, the largest push for more effective science education in America was during the Cold War era Space race (Laugksch 2000). In the years since, support for scientific literacy has waxed and waned dependent upon how much trust was put into the scientific community. In the 1980's when the United States thought that they were going to suffer from economic loss based on not having enough inventors, there was another surge to support scientific literacy (Prewitt 1983). A recent survey to determine how scientifically literate Americans are was conducted by the California Academy of Sciences, using the NSF's definition, and found that 4 out of 5 adults do not even know basic scientific principles (2009). Couple this with the fact that Americans are more distrusting of scientists, and you have a lot of scientific knowledge

being sheltered within the purely scientific community thereby contributing to the decline of scientific literacy in America (Mooney and Kirshenbaum 2009).

Harmful Algal Bloom Outreach

Harmful Algal Blooms (HABs) are increasing in frequency and habitats. Initially HABs were observed mainly in marine systems, but in more recent years, their presence has been increasing in freshwater systems as well. This can be of concern to humans due to their higher chance of interacting with a freshwater or estuarine system verses a marine system. To help protect the public, national plans have been established to help understand HABs (Bauer 2006). There are also several regional efforts to help monitor HABs, but despite the increase of events, few of these areas actively engage in public outreach, such as Oregon, California, Vermont, and Florida, that have active programs to alert the public about these events (Lewitus et al. 2003; Stone and Bress 2007; Nierenberg et al. 2011).

Literature exists in large amounts about the types of effects that HABs have on human health, economic loss and damage to the environment (Chorus and Bartram 1999; Ibelings and Chorus 2007; Heisler et al. 2008). However, these studies are all written by scientists for scientists in scientific journals; and they are not being passed easily on to the public. The few regional efforts to provide information about HABs to the public (e.g., educational information, warning signs on beaches, etc.) have limited assessment to determine effectiveness (Fleming et al. 2007; Nierenberg et al. 2010; Nierenberg et al. 2011). Of these regional efforts, Florida has been particularly active using state funds to support centers for HAB education (Kuhar et al. 2009).

Some of the studies in Florida have looked at the effectiveness of HAB outreach materials. One study looked at improving a HAB hotline that citizens could call. The improvement allowed callers to talk to a specialist if they wanted and this option showed a 68% increase in satisfaction. However, this increase was based on only of people who called a HAB hotline, which were mainly older non-Hispanic white females (Fleming et al. 2007). Another study looked at the risk assessment of red tide compared to the perception of red tide, mainly focusing on if negative effects from red tide would change the activities of both tourists and Florida residents. The main finding from this study was that the more familiar people were with the topic of red tide, the better they were able to asses risk given different scenarios (Kuhar et al. 2009). A third study solely looked at what the general public knows about Florida red tide. The comparison focused on tourists and residents and their knowledge of general human health questions, red tide information, and if they knew where to get more information about red tide (i.e., were they aware of the current outreach efforts?) Perceptions of both tourists and residents were not accurate about what is safe to eat during a red tide. This fear of consumption of any seafood could potentially cause a negative economic impact on the region and associated industries, specifically fisheries (Nierenberg et al. 2010). Two of the studies encouraged additional outreach material to be evaluated after it is distributed in order to be more effective to communicating to the public.

Cyanobacteria in Louisiana

Cyanobacteria harmful algal blooms (CyanoHABs) are increasing in both frequency and geographic extent in which they are found (Paerl et al. 2001; Huisman and Hulot 2005; Heisler et al. 2008; Jöhnk et al. 2008). A CyanoHAB can be harmful to

humans and other animals mainly due to toxin production. Alternatively, it can result in surface scums that can block sunlight to lower water column (Codd 1995). The common pathways for human exposure of cyanotoxins are through recreational use of waters, consumption of drinking water, or contaminated seafood (Carmichael 2001; Codd et al. 2005). The effects of the toxins vary from rashes and other skin irritations in association with exposure during recreational use (Pilotto et al. 2004) to gastrointestinal distress as a result of consumption of seafood (Chorus and Bartram 1999) or respiratory problems (Heise 1949). Different countries have had to monitor their fresh water drinking supplies when a CyanoHAB occurs to insure that the public does not get exposed to cyanotoxins through their drinking water (Qin et al. 2010)

In Louisiana, CyanoHABs (specifically toxin-producing species) have been widely detected in several of the estuaries and their associated toxins have been found in seafood (White et al. 2009; Garcia et al. 2010; Bargu et al. 2011; Roy et al. 2013; Smith et al. in review). Despite evidence of cyanobacterial presence, there has not been any funded outreach in association with HABs or CyanoHABs attempted in the state. Information needs to be provided to both the public and policy makers about the possible sources of contamination. The increase of occurrence of CyanoHABs and the need for more effective education for the public is vital. A study to find out baseline knowledge about algae and harmful algal blooms was then conducted.

Methods

The purpose of this project was to conduct initial surveys with fishermen to determine baseline knowledge about algae in general as well as harmful algal blooms.

An educational brochure was then produced based on that information, and distributed to areas that fishermen frequent. Follow-up surveys were then conducted to determine if the educational brochure improved the baseline knowledge of fishermen concerning algae and harmful algal blooms.

An initial survey was created with the intent of assessing how fishermen perceived the function of algae in their environment and if they knew that it could potentially be a problem. The survey also checked for scientific literacy of terms associated with water, and included a visual research component where participants looked at different pictures of water conditions. IRB exemption status was applied for on 3/7/12 and obtained on 4/3/12 (Appendix 1). The interviews then were conducted at several locations around Lakes Pontchartrain and Des Allemandes, LA during the summer of 2012. These two areas were chosen due to cyanobacteria bloom presence and toxin production previously detected, in addition to having active fisheries (Garcia et al. 2010). There were a total of seven sites visited: at Lake Des Allemandes, the local crab house; around Lake Pontchartrain: Frenier Landing, Madisonville pier, Rigolets Marina, Spanish Trail Road, Sunset Point, and Williams Boulevard pier (Figure 4.1).

Initial Surveys

The initial surveys were constructed in a similar manner to surveys in other studies by using a Likert scale, yes/no and forced answer questions (Fleming et al. 2007; Kuhar et al. 2009) and included open-ended questions as well in order to allow each participant to explain his/her answers. This was done to probe for deeper understanding of people's knowledge and not allow them to choose a given answer.

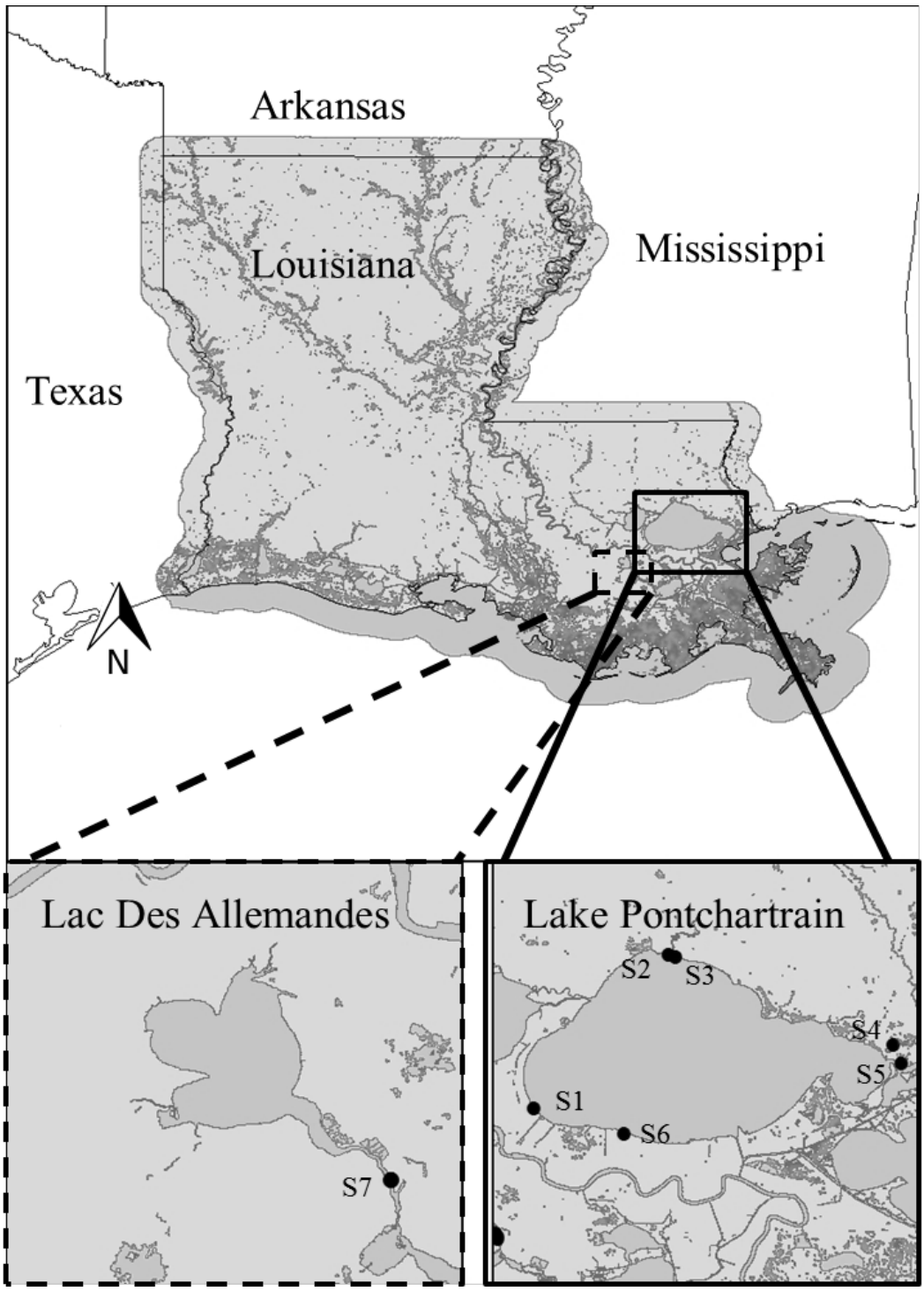


Figure 4.1. Map of the interview locations around Lake Pontchartrain and Lake Des Allemandes. Site 1 (S1) is Frenier Landing in LaPlace; S2 is the Madisonville Pier; S3 is Sunset Point; S4 is Spanish Trail Road; S5 is the Rigolets Marina; S6 is Williams Blvd. pier; and S7 is the crab house at Lake Des Allemandes.

Initial surveys were conducted at selected public fishing piers and marinas surrounding Lakes Pontchartrain and Des Allemandes. The researcher asked individuals coming off of boats or fishing from the pier if they would like to participate in a study as part of a research project. If the person chose to participate, the researcher asked questions related to their fishing habits (i.e., number of years fishing and frequency of fishing) and their knowledge of harmful algal blooms (Table 4.1 and Appendix 2). The researcher made notes of the participant's responses to the questions on a response form. Questions were also asked in regards to several different pictures of water conditions. For the visual component participants were allowed to look at all of the pictures at once, for as long as they wanted (Figure 4.2). At the end of the survey, the participant read

Table 4.1. Initial Survey Questions

1. Type of fisherman: Commercial or Sports
2. Experience of fishing (years)
3. How often do you go fishing? Once a year, once a month, twice a month, once a week, more than once a week
4. Do you: eat what you catch? Sell what you catch? Share with friends or family?
5. Have you heard of algae (Can you please describe what you know about it?)
6. Have you ever caught a "bad fish" or a fish that you would not eat? Yes or no
    a. If so, why did you think it was "bad"? taste, visual, other
7. Have you ever gone to a doctor/hospital from eating bad fish or shellfish? Yes or no
    a. Did you catch this fish or buy it?
8. Where do you get your information (on fishing conditions) about the lake?
9. Have you ever seen dead fish in the lake?
    a. Did you quit fishing that day?
    b. Did you contact anyone about the dead fish? Who?
10. Is all algae "bad"? Why or why not?
11. Are you familiar with the term harmful algal bloom? What does it mean?
12. Have you seen any of the types of water conditions before?
13. Which of these pictures would you not go fishing?

A.

B.

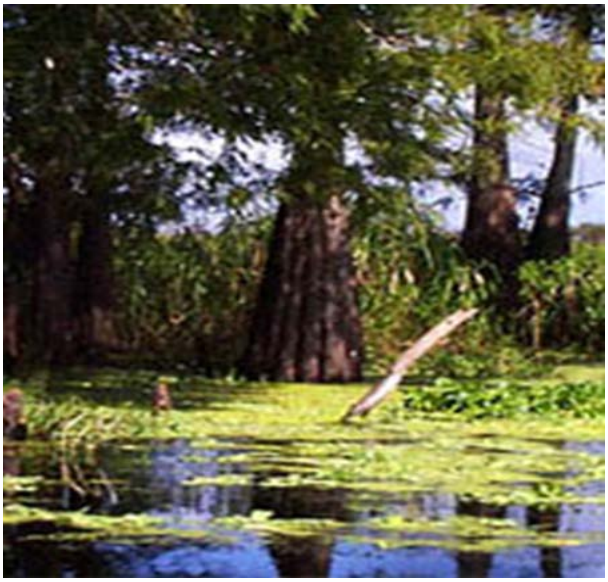
C.

D.

E.

F.

G.



H.

Figure 4.2. Pictures that participants were shown to answer questions about different water conditions. A—control picture of Lake Pontchartrain (courtesy of Eric Roy); B—control picture of duckweed (Emily Smith); C—picture of red tide (courtesy of Pinellas County, FL); D—brown tide (courtesy of Jeff Tittel); E—red tide (courtesy of Peter Lindeman); F—brown tide scum (courtesy of Marion Beck); G—control picture of duckweed in shallow water (courtesy of Graphic Mac); and H—cyanobacteria bloom in shallow waters (courtesy of Blue Water Satellite)

over the survey notes for accuracy, made any necessary corrections and then initialed and dated the survey notes. The results of the surveys were analyzed to determine the fishermen's existing level of knowledge of harmful algal blooms and any misconceptions that might need to be addressed. The participant was then allowed to ask any questions to the researcher in regards to the project.

Statistical Analysis for Initial Surveys

The number of years a person has been fishing may not be equivalent with total experience, so total fishing experience was determined by multiplying the frequency of fishing by the number of years they have been fishing. The total fishing experience numbers were placed in rank order and then plotted on a best fit line (R^2 =0.87) (Figure

4.3). The best fit line had such a high R^2 value; it was then used to divide the experience into three categories: novice, intermediate, and expert. Data that fell below zero for the calculated y-axis were identified as novices, while those with data below the best fit line but above zero were classified as intermediate. Those located above the best fit line were identified as experts. These three groups were then able to be used to run pair-wise comparisons for all of the questions from the survey to determine statistical differences, if any.

Sometimes a participant would give more than one answer in the open-ended questions, therefore each answer was counted separately when comparing and grouping the responses. All statistics and percentages regarding the open-ended questions were based on total responses and not the total number of participants.

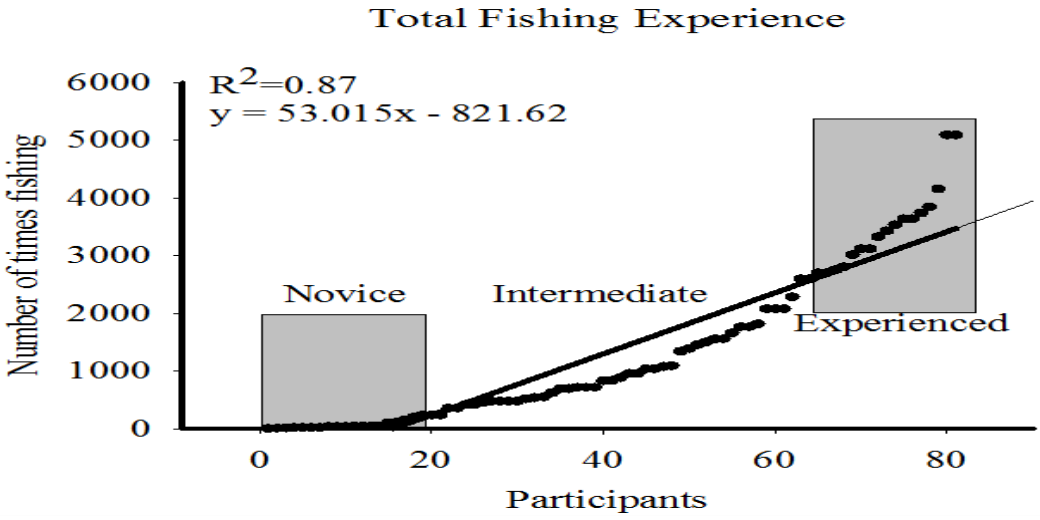


Figure 4.3. Total fishing experience calculated by number of times a year each person went fishing multiplied by the number of years they have been fishing. The groups were then broken down using a best fit line to determine if they were a novice, intermediate or experienced fisherman.

Creation of Educational Brochure

The results of the initial survey were then used to create an educational brochure that was distributed to the same marinas that were surveyed around Lakes Pontchartrain and Des Allemandes. Based on the responses to the questions specifically about algae and the visual based questions a brochure was created. It was designed to address areas of misunderstanding (i.e. algae removes oxygen from water), and included pictures to help distinguish between commonly seen duckweed and harmful algae. The help of an artist from Louisiana Sea Grant College Program was sought for his input on design and color scheme. The brochures were left for two weeks to a month before follow-up interviews were conducted.

Follow-up Interviews

After the brochures had been distributed for the aforementioned time period, the researcher returned to the same piers and marinas and interviewed fishermen. The intent was to discover the effectiveness of the document and to determine if the document had served to increase the public's knowledge about harmful algal blooms. The same demographics questions were asked along with the follow-up questions (Table 4.2 and Appendix 3). If the participant had not seen the brochure previously, then he/she was allowed to look it over quickly in order to answer the questions. At the end of the interview, the interviewee read over the interview notes for accuracy, made any necessary corrections, then initialed and dated the document. The results of this interview were analyzed in a similar method to the initial interviews to determine if the educational brochure had the desired impact of increasing knowledge about harmful algal blooms.

Table 4.2. Follow-up survey questions. All demographic information was collected exactly the same as the initial survey.

1. Have you seen this public service document?
2. Was the information easy to understand?
3. Did you know about harmful algal blooms before reading it?
4. What did you learn from the document?

Results

Initial Surveys

The number of participants for the initial surveys was 81 with 89% being male and 11% female (Table 4.3). The age range was 11-70 years old with an average age of 44. Sports fishermen made up 85.2% of the population while commercial were only 14.8%. The range of years of fishing was 1-70 years with the average of 31 years and a mode of 40 years. The total fishing experience ranged from 5 - 5096 times. These total times then were broken down into the three categories of experience with, 16 novice fishermen, 45 intermediate fishermen and 20 experienced fishermen. What fishermen did with the fish they caught allowed the participant to have more than one answer so the percentages that were calculated were based off of the total number of participants rather than the total number of responses. The majority of the fishermen ate what they caught (98%) and shared with friends or family (72%). Only a few sold their catch (16%) or practiced catch and release methods (7%). The type of fish caught ranged greatly and included speckled trout, drum, redfish, blue crab, shrimp, catfish, white perch, flounder, bass, and brim.

When asked if they had ever caught a fish they would not eat, novice fishermen had 50% that had caught one. The main reason (6 of the 8 people) given was that it was

Table 4.3. Demographics of initial and follow-up surveys. *The last question was able to have more than one choice as an answer so the total number of responses was recorded, but percentages were still based on total number of participants.

		Initial surveys *N=81*	Follow-up surveys *N=32*
		Total (%)	Total (%)
Gender	Male	72 (89)	30 (94)
	Female	9 (11)	2 (6)
Age	10-20	7 (9)	1 (2)
	21-30	10 (12)	9 (28)
	31-40	15 (18)	8 (25)
	41-50	19 (24)	4 (13)
	51-60	18 (22)	6 (19)
	61-70	12 (15)	4 (13)
Type of Fisherman	Recreational	69 (85)	24 (75)
	Commercial	12 (15)	8 (25)
Fishing Experience (years x trips per year)	Novice	16 (20)	3 (9)
	Intermediate	45 (56)	23 (72)
	Experienced	20 (24)	6 (19)
Frequency of fishing	Once a year	8 (10)	2 (6)
	Once a month	20 (25)	9 (28)
	Twice a month	15 (18)	6 (19)
	Once a week	16 (20)	4 (13)
	Multiple times per week	22 (27)	11 (34)
What do you do with fish that you catch?*	Eat	79 (98)	32 (100)
	Share (with friends and family)	58 (72)	12 (38)
	Sell	13 (16)	10 (31)
	Catch and Release	6 (7)	0

not a type they would eat, while the other 2 people said there was a visual reason. Of the intermediate fishermen, 51% said they had caught a fish they would not eat, but their reasons varied with 12 of the 23 participants attributing it to a visual reason which ranged from sores to worms. The other 11 participants attributed this to the fact that the type of

fish is not one that they stereotypically eat. In the experienced group, 75% of the participants had caught a fish they would not eat. Six of the 15 participants attributed it to type, while the remaining 9 attributed it to visual reasons and one also said the taste was bad. The entire surveyed group answered affirmatively when asked if they had ever heard of algae. However, not all knew how to describe it when asked for a definition. All three experience levels most commonly described it as green/grassy/slime/gunk (23.5%) and did know it is associated with water (21.6%). However, the majority of the participants (60.8%) did not know what a harmful algal bloom was. Experienced and intermediate fishermen had a higher rate of at least some knowledge of HABs with 48% and 45% respectively, compared to only 13% of novice prior knowledge. The definitions varied from not knowing what a HAB is to it meaning that it kills fish and red tide (Table 4.4).

The visual component of this project yielded some interesting results. There was one control picture without anything visible in the water and then two additional control pictures that each had duckweed present. For the plain water condition, 27.2% recognized the first picture as something they had seen before (see A in Figure 4.2), and 85.2% of all participants would go fishing in those conditions. Of the other two controls with duckweed present (see B & G in Figure 4.2), 38.3% and 51.9% recognized these as conditions they had seen before and the majority would go fishing in those conditions (80.2% and 71.6%, respectively). The other pictures ranged from 14.8%--38.3% that they had seen that type of water condition before and from 19.8%--85.2% would not fish in a certain type of water (Table 4.5). However, some participants would fish in any condition (4.9%).

Table 4.4. Answers to open-ended questions for initial survey. Some questions had different number of responses from the number of participants. These questions have a total response number at the top of each question. **indicates a scientific term used by the participant.

		Novice (16)		Intermediate (45)		Experienced (20)	
		Total (%)		Total (%)		Total (%)	
Question		Yes	No	Yes	No	Yes	No
Have you heard of algae?		16 (100)	0 (0)	45 (100)	0 (0)	20 (100)	0 (0)
Can you describe it?	TOTAL RESPONSES	33		87		35	
	don't know/care	1 (6)		1 (2)		0	
	plankton**	0 (0)		1 (2)		1 (3)	
	uses sunlight	1 (6)		1 (2)		1 (3)	
	associated with spillway	0 (0)		2 (4)		1 (3)	
	organism /microscopic**	2 (13)		1 (2)		1 (3)	
	removes oxygen from water/low oxygen	0 (0)		4 (9)		2 (6)	
	considered bad/not good/damage/dirty	1 (6)		8(18)		0 (0)	
	associated with pools/fish tanks/water	9 (56)		17 (38)		7 (20)	
	considered good/needed/natural	1 (6)		4 (9)		1 (3)	
	plant/seaweed/weed/fungus/bacteria/ fish food	3 (19)		12 (27)		7 (20)	
	grows/blooms	5 (31)		19 (42)		5 (14)	
	Green stuff/gunk/grassy/slime	10 (63)		17 (38)		9 (26)	

(Table 4.4 continued)

		Total (%)		Total (%)		Total (%)	
		Yes	No	Yes	No	Yes	No
Is all algae "bad"?		2 (12)	14 (88)	5 (11)	40 (89)	2 (10)	18 (90)
Why or why not?							
	don't know		1 (6)		3 (7)		1 (5)
	natural/needed/beneficial		10 (63)		33 (73)		16 (80)
	takes oxygen out of the water/causes harm/negative response	2 (12)		5 (11)	4 (9)	2 (10)	
	No reason given		3 (19)				1 (5)
What is a harmful algal bloom?							
	don't know	14 (88)		26 (58)		10 (50)	
	kills fish/lowers oxygen	1 (6)		8 (18)		5 (25)	
	health risk	0		0		1 (5)	
	red tide	0		4 (9)		3 (15)	
	related to spillway	0		1 (2)		1 (5)	
	too much algae	1 (6)		6 (13)		0	

Table 4.5. Percent of participants that would not go fishing in the shown water condition. Pictures A, B and G were control pictures; C-red tide, D- brown tide, E-red tide, F-brown tide, and H-cyanobacteria bloom.

	Would not fish in water condition shown.
A	14.8%
B	19.8%
C	70.4%
D	44.4%
E	85.2%
F	69.1%
G	28.4%
H	39.5%
Would fish in any shown condition	4.9%

Follow-up Surveys

There were a total of 32 participants in the follow-up surveys with 30 (94%) being male (Table 4.3). The age range was 16-67 with an average age of 40 and a mode of 23, 34, and 54. The majority of fishermen were recreational (75%) verses commercial. The experience levels were divided according to the same scale as the initial surveys and there were 3 experts, 23 intermediates and 6 novices. The majority of fishermen went fishing more than once a week (34%). All of the fishermen ate what they caught, but additionally 38% shared what they caught with friends and family, and 31% sold what they caught.

Different areas that the brochures (Figure 4.4) were distributed to had a variable result in the amount of surveys that were initially left and the ones that were still there when the follow-up survey was conducted (Table 4.6). For all of the areas, except Williams Boulevard (near Lake Pontchartrain), there was an employee that said they did not throw away any brochures. Louisiana Sea Grant also posted the brochure on their website

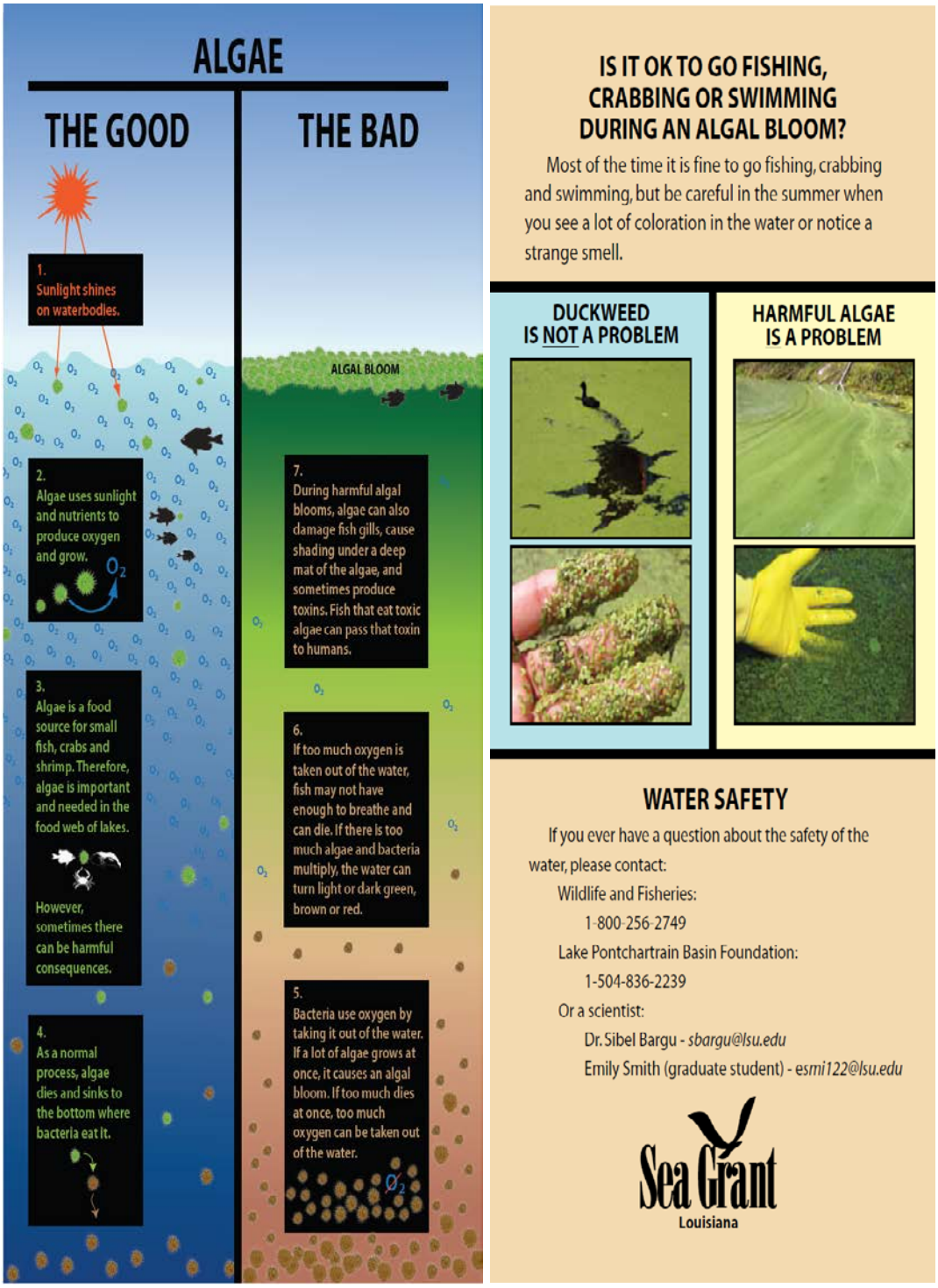


Figure 4.4. Educational brochure created from the initial interview data. Both the information provided and the visuals chosen were picked based on what information people did not know.

(http://www.seagrantfish.lsu.edu/pdfs/Algae_brochure.pdf) to increase amount of people that would see it, but they did not have a way to track the number of times it was viewed.

The majority of the participants (94%) had not seen the brochure prior to the follow-up survey. However, after the participant was allowed to look over the brochure, all of them agreed that the brochure was easy to understand. The two participants that had seen the brochure previously both indicated that they picked up the brochures because they looked interesting. A difference from the initial surveys is that a majority of participants had heard of a HAB previously (72%). When asked what information was new to them from the brochure, the highest percent of 28% learned that algae produces oxygen followed closely by 25% that noticed not all algae was bad or was bad only when there was too much of it. Sixteen percent learned that it can cause health problems to either people or fish.

Table 4.6. Number of brochures distributed by area and number remaining during follow-up surveys. * No way to verify that these were not thrown away.

	Distributed	Remaining
Frenier Landing	25	20
Madisonville	50	10
Rigolets Marina	100	20
Williams Blvd.	50	0*
Des Allemandes	50	40

Discussion

As CyanoHABs continue to expand around the world, the importance of educating the public so that they know what to look for and what to do when they see one

or experience symptoms of exposure is critical. This study revealed that a large percentage of the participants had extremely superficial understanding of algae, and almost no knowledge of harmful algal blooms. They all recognized the term “algae”, but could not explain it further accurately.

When comparing the experience levels to their answers, there was a general trend that the expert level would give an answer, that wasn’t “I don’t know” for an open-ended question. An example was for the question: “Have you caught a “bad” fish before?” The more experienced were willing to admit that they had caught a “bad” fish before, where the intermediates and novice either did not want to admit this had happened or had not recognized that they had indeed caught a “bad” fish before. Education level was not collected for this interview, but it may have been interesting to see if there was a correlation between education and experience level.

The educational brochure was mainly distributed by a method that may be out of date for information distribution. Most of our society these days gets their information from the internet and rarely turn to the printed version of the same information. In order to potentially increase awareness of HABs and their effect on society, a larger outreach in the more technical world may be needed. A current attempt to engage a larger audience is through the use of apps on phones or tablets. The development of an app to report possible HABs, would give the public more ownership of the problem and potentially increase awareness and education.

The visual component provided some insightfulness into how people perceive their environment. There was a much higher percent of people that would not go fishing in the extremely contaminated looking pictures (see H in Figure 4.2 for example) but the

small percent that would fish in any condition indicated a lack of knowledge about changes in water conditions affecting fish. Many of the participants also did not recognize that two of the photos they were shown were of Lake Pontchartrain, which could stem from survey anxiety.

Some biases that could exist include a mistrust of the researcher and media exposure from the Deepwater Horizon Oil Spill. There are multiple studies that show participants can have a bias towards the researcher for a variety of reasons (i.e., race, space between the two people, gender, age, etc.) (Kolstø 2001), including mistrust of scientists in general (Mooney and Kirshenbaum 2009; Hmielowski et al. 2013). The Deepwater Horizon Oil Spill was largely visible in the media for several months and therefore had an effect on the visual component because several of the participants thought some of the pictures were of oil in water.

Conclusions

Communication is the key to having a more scientific literate society. However, the methods for communicating effectively are changing constantly. As scientists, we need to start making a larger effort to communicate science to the public. Many years ago, Waterman reported on the first decade of the NSF saying, "Progress in science depends to a considerable extent on public understanding and support of a sustained program of science education and research," (Waterman 1960). This idea is still true today. CyanoHABs are increasingly a problem and the public needs to hear from scientists about how to handle them. The challenge for scientists is to actively work at including outreach as an integral part of the research verses being supplemental.

References

Bargu, S., J. White, C. Li, J. Czubakowski, and R. Fulweiler. 2011. Effects of freshwater input on nutrient loading, phytoplankton biomass, and cyanotoxin production in an oligohaline estuarine lake. *Hydrobiologia* 661:377-389.

Bauer, M. E. 2006. Harmful algal research and response: A human dimensions strategy, Pages 58. Woods Hole Oceanographic Institute, Woods Hole, MA, National Office for Marine Biotoxins and Harmful Algal Blooms.

Brown, B. A., J. M. Reveles, and G. J. Kelly. 2005. Scientific literacy and discursive identity: A theoretical framework for understanding science learning. *Science Education* 89:779-802.

Carmichael, W. W. 2001. Health Effects of Toxin-Producing Cyanobacteria: 'The CyanoHABs'. *Human & Ecological Risk Assessment* 7:1393.

Chorus, I., and J. Bartram. 1999, Toxic cyanobacteria in water a guide to their public health consequences, monitoring, and management. London; New York, E & FN Spon.

Codd, G. A. 1995. Cyanobacterial toxins: Occurrence, properties and biological significance. *Water Science and Technology* 32:149-156.

Codd, G. A., J. Lindsay, F. M. Young, L. F. Morrison, and J. S. Metcalf. 2005. Harmful cyanobacteria: From mass moralities to management measures, Pages 1-23 *in* J. Huisman, H. C. P. Matthijs, and P. M. Visser, eds. Harmful cyanobacteria. Netherlands, Springer.

DeBoer, G. E. 2000. Scientific Literacy: Another look at its historical and contemporary meanings and its relationship to science education reform. *Journal of Research in Science Teaching* 37:582-601.

Fleming, L., E. Jerez, W. B. Stephan, A. Cassedy, J. Bean, A. Reich, and B. Kirkpatrick. 2007. Evaluation of Harmful Algal Bloom Outreach Activities. *Marine Drugs* 5:208-219.

Garcia, A. C., S. Bargu, P. Dash, N. N. Rabalais, M. Sutor, W. Morrison, and N. D. Walker. 2010. Evaluating the potential risk of microcystins to blue crab (*Callinectes sapidus*) fisheries and human health in a eutrophic estuary. *Harmful Algae* 9:134-143.

Heise, H. A. 1949. Symptoms of hay fever caused by algae. *Journal of Allergy* 20:383-385.

Heisler, J., P. M. Glibert, J. M. Burkholder, D. M. Anderson, W. Cochlan, W. C. Dennison, and Q. Dortch. 2008. Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae* 8:3-13.

Hmielowski, J. D., L. Feldman, T. A. Myers, A. Leiserowitz, and E. Maibach. 2013. An attack on science? Media use, trust in scientists, and perceptions of global warming. *Public Understanding of Science*.

Huisman, J., and F. Hulot. 2005. Population Dynamics of Harmful Cyanobacteria, Pages 143-176 *in* J. Huisman, H. Matthijs, and P. Visser, eds. Aquatic Ecology Series, Springer Netherlands.

Ibelings, B. W., and I. Chorus. 2007. Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health: A review. *Environmental Pollution* 150:177-192.

Jöhnk, K. D., J. E. F. Huisman, J. Sharples, B. E. N. Sommeijer, P. M. Visser, and J. M. Stroom. 2008. Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biology* 14:495-512.

Kolstø, S. D. 2001. Scientific literacy for citizenship: Tools for dealing with the science dimension of controversial socioscientific issues. *Science Education* 85:291-310.

Kuhar, S. E., K. Nierenberg, B. Kirkpatrick, and G. A. Tobin. 2009. Public Perceptions of Florida Red Tide Risks. *Risk Analysis* 29:963-969.

Laugksch, R. C. 2000. Scientific literacy: A conceptual overview. *Science Education* 84:71-94.

Lewitus, A. J., L. B. Schmidt, L. J. Mason, J. W. Kempton, S. B. Wilde, J. L. Wolny, B. J. Williams. 2003. Harmful algal blooms in South Carolina residential and golf course ponds. *Population and Environment* 24:387-413.

Mooney, C., and S. Kirshenbaum. 2009, Unscientific America: How Scientific Illiteracy Threatens our Future. New York, New York, Basic Books.

National Science Education Standards (NSES). 1996. National Academy of Sciences. National Academy Press, Washington, D.C.

Nierenberg, K., M. M. Byrne, L. E. Fleming, W. Stephan, A. Reich, L. C. Backer, E. Tanga. 2010. Florida red tide perception: Residents versus tourists. *Harmful Algae* 9:600-606.

Nierenberg, K., J. Hollenbeck, L. E. Fleming, W. Stephan, A. Reich, L. C. Backer, and R. Currier. 2011. Frontiers in outreach and education: The Florida red tide experience. *Harmful Algae* 10:374-380.

Paerl, H., R. Fulton, P. Moisander, and J. Dyble. 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *The Scientific World Journal* 1:76-113.

Pilotto, L., P. Hobson, M. D. Burch, G. Ranmuthugala, R. Attewell, and W. Weightman. 2004. Acute skin irritant effects of cyanobacteria (blue-green algae) in healthy volunteers. *Australian and New Zealand Journal of Public Health* 28:220-224.

Prewitt, K. 1983. Scientific Illiteracy and Democratic Theory. *Daedalus* 112:49-64.

Qin, B., G. Zhu, G. Gao, Y. Zhang, W. Li, H. Paerl, and W. Carmichael. 2010. A Drinking Water Crisis in Lake Taihu, China: Linkage to Climatic Variability and Lake Management. *Environmental Management* 45:105-112.

Roy, E., J. White, E. A. Smith, S. Bargu, and C. Li. *2013*. Estuarine Ecosystem Response to Three Large-Scale Mississippi River Flood Diversion Events. *Science of the Total Environment*:374-387.

Smith, E.A., S. Bargu and C. Weidert (*in review*). Potential for Oyster Contamination from Freshwater Cyanobacterial Toxins in Coastal Louisiana. Submitted to *Marine Pollution Bulletin.*

Stone, D., and W. Bress. 2007. Addressing public health risks for cyanobacteria in recreational freshwaters: The oregon and vermont framework. *Integrated Environmental Assessment and Management* 3:137-143.

Waterman, A. T. 1960. National Science Foundation: A Ten-Year Résumé. *Science* 131:1341-1354.

White, J. R., H. W. Fulweiler, C. Y. Li, S. Bargu, N. D. Walker, H. H. Twilley, and S. E. Green. 2009. Mississippi River Flood of 2008: Observations of a Large Freshwater Diversion on Physical, Chemical, and Biological Characteristics of a Shallow Estuarine Lake. *Environmental Science & Technology* 43:5599-5604.

CHAPTER 5: CONCLUSIONS

The desire for humans to live near shorelines has always been great and will continue to increase over time (Gillis 2012). So it is our job as scientists to not only study the environmental impacts brought on by human habitation, but to look at how these impacts can be managed with ongoing habitat utilization. Human interactions with the coast began as a convenient way to have food available and later for travel (Smith 1863; Gillis 2012). Over the years manipulations were made to the coastal systems by humans for their benefit without concern for nature. The detrimental impact of these manipulations to the coastal system has become an area of focus for both scientists and managers in order to reverse the damage that has been caused. Estuaries, which are located along these coasts, are economically very important areas when it comes to fisheries because they provide important habitat for many species for all or part of their lives (De Sylva et al. 1962; Chubb and Potter, 1986, 1984; Schindler and Vallentyne 2008; Dando 2011). One impact from human activities is increasing eutrophication which promotes significant increase of phytoplankton biomass and possibly cyanobacteria harmful algal blooms (CyanoHABs), reducing the water quality of these estuaries. CyanoHABs have been observed in other parts of the country and world and are now being commonly found in Louisiana estuarine and freshwaters.

The management of waterways in south Louisiana was originally intended to prevent flooding of established cities and settlements (Lane et al. 1999; Lane et al. 2001; Lane et al. 2006). Later management efforts were expanded to try and correct the broader problem of separating the natural freshwater source to many estuaries and wetlands. The unintended consequence of diverting freshwater to prevent downstream

flooding into estuaries in large amounts has been the increased nutrient loads that stimulate excess phytoplankton growth, leading to hypoxia and other harmful effects. Flood prevention diversions, such as the Bonnet Carré Spillway (BCS) in Lake Pontchartrain, usually have the most unpredictable outcomes due to the non-regular pattern of their openings. In Lake Pontchartrain, there have been drastically different results each time the BCS has been opened in regards to volume of water, nutrient and sediment input, duration of the opening, timing of the opening, and the subsequent biological response of the primary producers (Turner et al. 2004; White et al. 2009; Bargu et al. 2011; Roy et al. 2013). The overarching goal of this dissertation was to study the dynamics of cyanobacteria bloom dynamics in South Louisiana. In the first study, these dynamics were studied in the context of a freshwater diversion (used for flood prevention only) opening, the Bonnet Carré Spillway, into Lake Pontchartrain and the corresponding phytoplankton community response. In the most recent opening in 2011, the hydrology and nutrient dynamics of the estuary and the phytoplankton responses were different than any other previous openings. Some of the needed elements, such as nutrient availability, were present for a large CyanoHAB to occur in the summer of 2011, but other factors like the amount of water and subsequent short resident time, flushing rate, turbidity and light limitation likely prevented any toxic cyanobacteria species from blooming. In the following year, the water column was more stable but this time nitrogen was very limited compare to the previous year. Nitrogen limitation would not prevent nitrogen-fixing species such as *Anabaena* from blooming, but *Anabaena*, likely coming from a seed population in the northern tributaries, was not present in high enough abundance for a bloom to occur that year. Even though dominant cyanobacteria species

in the estuary were previously found to be toxin producing, both studied years did not have any detectable particulate toxins, and dissolved toxins were only present at low levels.

In the second study, Breton Sound Estuary was the study site, which is connected to the Mississippi River through the Caernarvon diversion that was built for regulation of salinity in order to promote oyster bed growth and has been very successful in this (Chatry et al. 1983; Lane et al. 1999). However, the openings of this diversion have also allowed toxic cyanobacteria species to proliferate and travel successfully into the estuary where oyster beds are located. When we looked at the native oyster population of Breton Sound, we found cyanotoxins already present in their tissue. Afterwards, we conducted a laboratory experiment where we fed oysters to toxin producing species of *Anabaena* and found that they not only consumed the cells, but then retained some amount of toxin in their viscera. Oysters are now the second economically important Louisiana species to have cyanotoxins found in their tissue, with the first being blue crabs from Lac Des Allemandes (Garcia et al. 2010). Since oysters are heavily consumed by humans, establishing monitoring programs for seafood contamination in Louisiana is very much needed, specifically concerning freshwater cyanotoxins.

In estuaries that are used for recreation and fisheries, the need for public education about these environments is also vital. Residents should be made aware of how their daily interactions can alter their environment and be aware that there could be possible hazards for them that can originate from these interactions. For example, learning about basic differences between common algae and harmful ones can help prevent exposure to these toxins and possible contamination. The final research chapter

sought to learn how much fishermen knew about their environment when it came to algae in general and specifically HABs. The fishermen had very basic knowledge about their environment, but did not know specifics in terms of HABs. When follow-up surveys were conducted after the educational brochure was distributed, most of the fishermen had not seen the brochure previously. However, when they had an opportunity to look over the material they were able to acquire knowledge about HABs that they previously were unaware.

These three chapters have explored estuaries from a research perspective with diversions and how they might contribute to increased CyanoHAB blooms; an ecological standpoint with oysters potentially being contaminated by cyanobacterial toxins; and an outreach component for how humans perceive their environment concerning algae and CyanoHABs. The value of looking at a single issue from multiple angles is discovering how closely all of these different components are connected. Many studies only look at one part of an issue (i.e. toxicity in oysters) and never put that information into a larger context (i.e. overall seafood contamination and its effects on humans). It is important to bring together aspects of natural and social sciences to give a better understanding of how diversions can affect not only the estuaries they are connected to, but ultimately how these changes will affect the people that live near these estuaries. My range of experience, from working with fishermen to collecting water samples in Lake Pontchartrain, has let me discover that people want to understand their environment, specifically when events such as diversion openings occur. As scientists, it is our duty to be a part of their education especially when there are plans in place (State Master Plan of Louisiana) that include more diversions to be built. If everyone (residents, scientists and

managers) is aware of how diversions can change the environment, then more informed decisions can be made for the future.

References

Bargu, S., J. White, C. Li, J. Czubakowski and R. Fulweiler 2011. Effects of freshwater input on nutrient loading, phytoplankton biomass, and cyanotoxin production in an oligohaline estuarine lake. *Hydrobiologia* 661: 377-389. doi: 10.1007/s10750-010-0545-8

Chatry, M., R. J. Dugas, and K. A. Easley 1983. Optimum salinity regime for oyster production on Louisiana's state seed grounds. *Contributions in Marine Science* 26: 81-94.

Chubb, C. F. and I. C. Potter 1986. Age, growth and condition of the Perth herring, Nematolosa vlaminghi (Munro) (Dorosomatinae), in the Swan Estuary, south-western Australia. *Marine and Freshwater Research* 37: 105-112.

Chubb, C. F. and I. C. Potter 1984. The reproductive biology and estuarine movements of the gizzard shad, *Nematalosa vlaminghi* (*Munro*). *Journal of Fish Biology* 25: 527-543.

Dando, P. R. 2011. Site fidelity, homing and spawning migrations of flounder *Platichthys flesus* in the Tamar estuary, South West England. *Marine Ecology-Progress Series* 430: 183-196. doi: 10.3354/meps09116

De Sylva, D., C. Shuster and F. Kalber 1962. Fishes and ecological conditions in the shore zone of the Delaware River estuary: with notes on other species collected in deeper water. In Fishes and ecological conditions in the shore zone of the Delaware River estuary: with notes on other species collected in deeper water, 164. Newark: Dept. of Biological Sciences, University of Delaware.

Garcia, A. C., S. Bargu, P. Dash, N. N. Rabalais, M. Sutor, W. Morrison and N. D. Walker 2010. Evaluating the potential risk of microcystins to blue crab (*Callinectes sapidus*) fisheries and human health in a eutrophic estuary. *Harmful Algae* 9: 134-143. doi: 10.1016/j.hal.2009.08.011

Gillis, J. R. 2012. The Human Shore. Chicago: The University of Chicago Press.

Lane, R., J.W. Day and J. N. Day 2006. Wetland surface elevation, vertical accretion, and subsidence at three Louisiana Estuaries receiving diverted Mississippi River water. *Wetlands* 26: 1130-1142. doi: 10.1672/0277-5212(2006)26[1130:WSEVAA]2.0.CO;2

Lane, R., J. Day Jr, G. P. Kemp and D. K. Demcheck 2001. The 1994 experimental opening of the Bonnet Carre Spillway to divert Mississippi River water into Lake Pontchartrain, Louisiana. *Ecological Engineering* 17: 411-422. doi: http://dx.doi.org/10.1016/S0925-8574(00)00170-1

Lane, R., J. Day and B. Thibodeaux 1999. Water quality analysis of a freshwater diversion at Caernarvon, Louisiana. *Estuaries and Coasts* 22: 327-336. doi: 10.2307/1352988

Roy, E., J. White, E. A. Smith, S. Bargu and C. Li 2013. Estuarine Ecosystem Response to Three Large-Scale Mississippi River Flood Diversion Events. *Science of the Total Environment*: 374-387.

Schindler, D. W. and J. R. Vallentyne 2008. The Algal Bowl; Overfertilization of the World's Freshwaters and Estuaries. Canada: Houghton Boston Printers.

Smith, A. 1863. An Inquiry into the Nature and Causes of the Wealth of Nations.

White, J. R., H. W. Fulweiler, C. Y. Li, S. Bargu, N. D. Walker, H. H. Twilley and S. E. Green 2009. Mississippi River Flood of 2008: Observations of a Large Freshwater Diversion on Physical, Chemical, and Biological Characteristics of a Shallow Estuarine Lake. *Environmental Science & Technology* 43: 5599-5604.

REFERENCES

Amorim, Á. and V.T. Vasconcelos. 1999. Dynamics of microcystins in the mussel Mytilus galloprovincialis. *Toxicon* 37, 1041-1052.

Anderson, D., P. Glibert, and J. Burkholder. 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. *Estuaries* 25: 704-726.

Backer, L., and D. McGillicuddy. 2006. Harmful algal blooms. *Oceanography* 19: 94.

Banks, P. 2012. Oyster Stock Assessment Report of the public oyster areas in Louisiana. In: Fisheries, L.W.a. (18).

Barbier, E. B., E. W. Koch, B. R. Silliman, S. D. Hacker, E. Wolanski, J. Primavera, E. F. Granek, et al. 2008. Coastal Ecosystem-Based Management with Nonlinear Ecological Functions and Values. *Science* 319: 321-323.

Bargu, S., J. White, C. Li, J. Czubakowski and R. Fulweiler 2011. Effects of freshwater input on nutrient loading, phytoplankton biomass, and cyanotoxin production in an oligohaline estuarine lake. Hydrobiologia 661: 377-389.

Bauer, M. E. 2006. Harmful algal research and response: A human dimensions strategy, Pages 58. Woods Hole Oceanographic Institute, Woods Hole, MA, National Office for Marine Biotoxins and Harmful Algal Blooms.

Beaugrand, G., F. Ibanez, and P. C. Reid. 2000. Spatial, seasonal and long-term fluctuations of plankton in relation to hydroclimatic features in the English Channel, Celtic Sea and Bay of Biscay. *Marine Ecology Progress Series* 200:93-102.

Boyer, G. 2008. Cyanobacterial Toxins in New York and the Lower Great Lakes Ecosystems. *In* H. K. Hudnell [ed.], Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs, vol. 619, Advances in Experimental Medicine and Biology, 153-165. Springer New York.

Boney, A.D. 1976. Phytoplankton. Great Britain: The Camelot Press Ltd, Southhampton.

Bouvy, M., R. Molica, S. De Oliveira, M. Marinho, and B. Beker. 1999. Dynamics of a toxic cyanobacterial bloom (Cylindrospermopsis raciborskii) in a shallow reservoir in the semi-arid region of northeast Brazil. *Aquatic Microbial Ecology* 20:285-297.

Breitburg, D.L. and G.F. Riedel. 2005. Marine Conservation Biology: The Science of Maintaining the Sea's Biodiversity. Island Press, Washington D.C.

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

Brown, B. A., J. M. Reveles, and G. J. Kelly. 2005. Scientific literacy and discursive identity: A theoretical framework for understanding science learning. *Science Education* 89:779-802.

Brown, C., E. Corcoran, P. Herkenrath, and J. E. Thonell. 2006. Marine and Coastal Ecosystems and Human Well-Being; A Synthesis Report Based on the Findings of the Millennium Ecosystem Assessment. United Nations Environment Programme, Nairobi.

Carmichael, W. 2008. A world overview—One-hundred-twenty-seven years of research on toxic cyanobacteria—Where do we go from here? In Cyanobacterial harmful algal blooms: State of the science and research needs, 105-125: Springer.

Carmichael, W. 2001. Health Effects of Toxin-Producing Cyanobacteria: 'The CyanoHABs'. *Human & Ecological Risk Assessment* 7: 1393.

Chatry, M., R.J. Dugas, and K.A. Easley. 1983. Optimum salinity regime for oyster production on Louisiana's state seed grounds. *Contributions in Marine Science* 26, 81-94.

Chorus, I. and J. Bartram. 1999. Toxic cyanobacteria in water a guide to their public health consequences, monitoring, and management. E & FN Spon, London; New York.

Chubb, C.F. and I.C. Potter. 1984. The reproductive biology and estuarine movements of the gizzard shad, Nematalosa vlaminghi (Munro). *Journal of Fish Biology* 25, 527-543.

Chubb, C.F. and I.C. Potter. 1986. Age, growth and condition of the Perth herring, Nematolosa vlaminghi (Munro) (Dorosomatinae), in the Swan Estuary, south-western Australia. *Marine and Freshwater Research* 37, 105-112.

Cloern, J. E. 1999. The relative importance of light and nutrient limitation of phytoplankton growth: a simple index of coastal ecosystem sensitivity to nutrient enrichment. *Aquatic Ecology* 33:3-15.

Codd, G.A. 1995. Cyanobacterial toxins: Occurrence, properties and biological significance. *Water Science and Technology* 32, 149-156.

Codd, G.A., J. Lindsay, F.M. Young, L.F. Morrison, and J.S. Metcalf. 2005. Harmful cyanobacteria: From mass moralities to management measures. In: Huisman, J., Matthijs, H.C.P., Visser, P.M. (Eds.), *Harmful cyanobacteria*. Springer, Netherlands, pp. 1-23.

Cook, P. L., D. P. Holland, and A. R. Longmore. 2010. Effect of a flood event on the dynamics of phytoplankton and biogeochemistry in a large temperate Australian lagoon. *Limnology and Oceanography* 55:1123.

Czubakowski, J. 2010. Estuarine phytoplankton response to annual and manipulated river inputs. M.S. Thesis, Baton Rouge: Louisiana State University.

Dando, P.R. 2011. Site fidelity, homing and spawning migrations of flounder Platichthys flesus in the Tamar estuary, South West England. *Marine Ecology-Progress Series* 430, 183-196.

Das, A., D. Justic, M. Inoue, A. Hoda, H. Huang and D. Park 2012. Impacts of Mississippi River diversions on salinity gradients in a deltaic Louisiana estuary: Ecological and management implications. *Estuarine, Coastal and Shelf Science* 111: 17-26. doi: http://dx.doi.org/10.1016/j.ecss.2012.06.005

De Sylva, D., C. Shuster, and F. Kalber. 1962. Fishes and ecological conditions in the shore zone of the Delaware River estuary: with notes on other species collected in deeper water. Dept. of Biological Sciences, University of Delaware, Newark, p. 164.

DeBoer, G. E. 2000. Scientific Literacy: Another look at its historical and contemporary meanings and its relationship to science education reform. *Journal of Research in Science Teaching* 37:582-601.

Dortch, Q. & S. Achee. 1998. Lake Pontchartrain 1997 algal bloom: identification, toxicity, and similar occurrences elsewhere in Louisiana coastal waters. In Malek-Wiley, R. R. (ed.), Clean enough? Lake Pontchartrain Basin Foundation, New Orleans: 19–20.

Dortch, Q., M. L. Parsons, N. N. Rabalais, and R. E. Turner. 1999. What is the threat of harmful algal blooms in Louisiana coastal waters? *in* L. P. Rozas, J. A. Nyman, C. E. Proffitt, N. N. Rabalais, D. J. Reed, and R. E. Turner, eds., The Symposium Recent Research in Coastal Louisiana: Natural System Function and Response to Human Influence. LA, Louisiana Sea Grant College Program.

Downing, J. A., S. B. Watson, and E. McCauley. 2001. Predicting Cyanobacteria dominance in lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 1905-1908.

Duan, S., M.A. Allison, T.S. Bianchi, B.A. McKee, A.M. Shiller, L. Guo, and B.E. Rosenheim. 2014. Biogeochemical dynamics at Major River-Coastal Interfaces: Linkages with Global Change. Cambridge University Press, New York.

Engström-Öst, J., S. Repka, and M. Mikkonen. 2011. Interactions between plankton and cyanobacterium Anabaena with focus on salinity, growth and toxin production. *Harmful Algae* 10: 530-535.

Fischer, W. J., I. Garthwaite, C. O. Miles, K. M. Ross, J. B. Aggen, A. R. Chamberlin, N. R. Towers, and D. R. Dietrich. 2001. Congener-Independent Immunoassay for Microcystins and Nodularins: *Environmental Science & Technology*, v. 35, p. 4849-4856.

Fleming, L., E. Jerez, W. B. Stephan, A. Cassedy, J. Bean, A. Reich, and B. Kirkpatrick. 2007. Evaluation of Harmful Algal Bloom Outreach Activities. *Marine Drugs* 5:208-219.

Fogg, G. and B. Thake. 1987. Algal Cultures and Phytoplankton Ecology. The University of Wisconsin Press, Madison, Wisconsin.

Frost, B.W. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnology and Oceanography* 17 (6) 805-815.

Garcia, A. C., S. Bargu, P. Dash, N. N. Rabalais, M. Sutor, W. Morrison, and N. D. Walker. 2010. Evaluating the potential risk of microcystins to blue crab (*Callinectes sapidus*) fisheries and human health in a eutrophic estuary. *Harmful Algae* 9:134-143.

Gillis, J. R. 2012. The Human Shore. Chicago: The University of Chicago Press.

Gilpin, L., K. Davidson, and E. Roberts. 2004. The influence of changes in nitrogen: silicon ratios on diatom growth dynamics. *Journal of Sea Research* 51: 21-35.

Glibert, P. M., D. M. Anderson, P. Gentien, E. Graneli, and K. G. Sellner. 2005. The global, complex phenomena of harmful algal blooms.

Harding Jr, L. W., M. E. Mallonee, and E. S. Perry. 2002. Toward a Predictive Understanding of Primary Productivity in a Temperate, Partially Stratified Estuary. *Estuarine, Coastal and Shelf Science* 55: 437-463.

Hastings, R. W. 2009. The Lakes of Pontchartrain: Their History and Environments: Jackson, MS, University Press of Mississippi, 319 p.

Heise, H. A. 1949. Symptoms of hay fever caused by algae. *Journal of Allergy* 20:383-385.

Heisler, J., P. M. Glibert, J. M. Burkholder, D. M. Anderson, W. Cochlan, W. C. Dennison, and Q. Dortch. 2008. Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae* 8:3-13.

Hmielowski, J. D., L. Feldman, T. A. Myers, A. Leiserowitz, and E. Maibach. 2013. An attack on science? Media use, trust in scientists, and perceptions of global warming. *Public Understanding of Science*.

Hopkinson, C., and J. Vallino. 1995. The relationships among man's activities in watersheds and estuaries: A model of runoff effects on patterns of estuarine community metabolism. *Estuaries* 18: 598-621.

Huisman, J., and F. Hulot. 2005. Population Dynamics of Harmful Cyanobacteria, Pages 143-176 *in* J. Huisman, H. Matthijs, and P. Visser, eds. Aquatic Ecology Series, Springer Netherlands.

Hupp, C., C. Demas, D. Kroes, R. Day, and T. Doyle. 2008. Recent sedimentation patterns within the central Atchafalaya Basin, Louisiana. *Wetlands* 28: 125-140.

Ibelings, B.W. and I. Chorus. 2007. Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health: A review. *Environmental Pollution* 150, 177-192.

Ibelings, B. and K. Havens. 2008. Cyanobacterial toxins: a qualitative meta–analysis of concentrations, dosage and effects in freshwater, estuarine and marine biota. In: Hudnell, H.K. (Ed.), Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Springer New York, pp. 675-732.

IPCC. 2007. Working Group III Report. Climate Change 2007*in* U. N. E. Programme, ed. New York.

Jochens, A. E., T. C. Malone, R. P. Stumpf, B. M. Hickey, M. Carter, R. Morrison, J. Dyble, et al. 2010. Integrated Ocean Observing System in Support of Forecasting Harmful Algal Blooms. *Marine Technology Society Journal* 44: 99-121.

Jöhnk, K. D., J. E. F. Huisman, J. Sharples, B. E. N. Sommeijer, P. M. Visser, and J. M. Stroom. 2008. Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biology* 14:495-512.

Karjalainen, M., J. Engstrom-Ost, S. Korpinen, H. Peltonen, J.P. Paakkonen, S. Ronkkonen, S. Suikkanen, and M. Viitasalo.2007. Ecosystem consequences of cyanobacteria in the northern Baltic Sea. *Ambio* 36, 195-202.

Kennish, M. J. 2002. Environmental threats and environmental future of estuaries. *Environmental Conservation* 29:78-107.

Kingsford, R. T. 2000. Ecological impacts of dams, water diversions and river management on floodplain wetlands in Australia. *Austral Ecology* 25: 109-127.

Kolstø, S. D. 2001. Scientific literacy for citizenship: Tools for dealing with the science dimension of controversial socioscientific issues. *Science Education* 85:291-310.

Kuhar, S. E., K. Nierenberg, B. Kirkpatrick, and G. A. Tobin. 2009. Public Perceptions of Florida Red Tide Risks. *Risk Analysis* 29:963-969.

Kurien, J. 1998. Traditional Ecological Knowledge and Ecosystem Sustainability: New meaning to Asian coastal proverbs. *Ecological Applications* 8: S2-S5.

Landsberg, J. H. 2002. The Effects of Harmful Algal Blooms on Aquatic Organisms. *Reviews in Fisheries Science* 10: 113-390.

Lane, R., J. Day and J. N. Day. 2006. Wetland surface elevation, vertical accretion, and subsidence at three Louisiana Estuaries receiving diverted Mississippi River water. *Wetlands* 26: 1130-1142. doi: 10.1672/0277-5212(2006)26[1130:WSEVAA]2.0.CO;2

Lane, R., J. Day Jr, G. P. Kemp and D. K. Demcheck 2001. The 1994 experimental opening of the Bonnet Carre Spillway to divert Mississippi River water into Lake Pontchartrain, Louisiana. *Ecological Engineering* 17: 411-422. doi: http://dx.doi.org/10.1016/S0925-8574(00)00170-1

Lane, R., J. Day, B.D. Marx, E. Reyes, E. Hyfield, and J. Day. 2007. The effects of riverine discharge on temperature, salinity, suspended sediment and chlorophyll a in a Mississippi delta estuary measured using a flow-through system. *Estuarine Coastal & Shelf Science* 74, 145-154.

Lane, R., J. Day, and B. Thibodeaux. 1999. Water quality analysis of a freshwater diversion at Caernarvon, Louisiana. *Estuaries and Coasts* 22, 327-336.

Laugksch, R. C. 2000. Scientific literacy: A conceptual overview. *Science Education* 84:71-94.

Lehman, P., G. Boyer, M. Satchwell, and S. Waller. 2008. The influence of environmental conditions on the seasonal variation of *Microcystis* cell density and microcystins concentration in San Francisco Estuary. *Hydrobiologia* 600, 187-204.

Lenihan, H.S., F. Micheli, S.W. Shelton, and C.H. Peterson. 1999. The Influence of Multiple Environmental Stressors on Susceptibility to Parasites: An Experimental Determination with Oysters. *Limnology and Oceanography* 44, 910-924.

Lewitus, A. J., L. B. Schmidt, L. J. Mason, J. W. Kempton, S. B. Wilde, J. L. Wolny, B. J. Williams. 2003. Harmful algal blooms in South Carolina residential and golf course ponds. *Population and Environment* 24:387-413.

Martin, J. F. 2002. Emergy valuation of diversions of river water to marshes in the Mississippi River Delta. *Ecological Engineering* 18: 265-286.

Metcalf, J.S. and G.A. Codd. 2009. Cyanobacteria, neurotoxins and water resources: Are there implications for human neurodegenerative disease? *Amyotrophic Lateral Sclerosis* 10, 74-78.

Miller, M.A., R.M. Kudela, A. Mekebri, D. Crane, S.C. Oates, M.T. Tinker, M. Staedler, W.A. Miller, S. Toy-Choutka, C. Dominik, D. Hardin, G. Langlois, M. Murray, K. Ward, and D.A. Jessup. 2010. Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters. *PLoS ONE* 5, 1-11.

Mize, S. and D.K. Demcheck. 2009. Water quality and phytoplankton communities in Lake Pontchartrain during and after the Bonnet Carré Spillway opening, April to October 2008, in Louisiana, USA. *Geo-Marine Letters* 29: 431-440.

Moisander, P.H., E. McClinton III, and H.W. Paerl. 2002 Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. *Microbial Ecology* 43:432-442.

Mooney, C., and S. Kirshenbaum. 2009, Unscientific America: How Scientific Illiteracy Threatens our Future. New York, New York, Basic Books.

National Science Education Standards (NSES). 1996. National Academy of Sciences. National Academy Press, Washington, D.C.

Negri, A.P. and G.J. Jones. 1995. Bioaccumulation of paralytic shellfish poisoning (PSP) toxins from the cyanobacterium Anabaena circinalis by the freshwater mussel Alathyria condola. *Toxicon* 33, 667-678.

Nierenberg, K., M. M. Byrne, L. E. Fleming, W. Stephan, A. Reich, L. C. Backer, E. Tanga. 2010. Florida red tide perception: Residents versus tourists. *Harmful Algae* 9:600-606.

Nierenberg, K., J. Hollenbeck, L. E. Fleming, W. Stephan, A. Reich, L. C. Backer, and R. Currier. 2011. Frontiers in outreach and education: The Florida red tide experience. *Harmful Algae* 10:374-380.

Oberemm, A., J. Becker, G.A. Codd, and C. Steinberg. 1999. Effects of cyanobacterial toxins and aqueous crude extracts of cyanobacteria on the development of fish and amphibians. *Environmental Toxicology* 14, 77-88.

Otsuka, S., S. Suda, R. Li, M. Watanabe, H. Oyaizu, S. Matsumoto, and A.M.M. Watanabe. 1999. Characterization of morphospecies and strains of the genus Microcystis (Cyanobacteria) for a reconsideration of species classification. *Phycological Research* 47, 189-197.

Paerl, H. 1988. Nuisance Phytoplankton Blooms in Coastal, Estuarine, and Inland Waters. *Limnology and Oceanography* 33:823-847.

Paerl, H., N.S. Hall, B.L. Peierls, K.L. Rossignol, and A.R. Joyner (2013). Hydrologic Variability and Its Control of Phytoplankton Community Structure and Function in Two Shallow, Coastal, Lagoonal Ecosystems: The Neuse and New River Estuaries, North Carolina, USA. *Estuaries and Coasts* pp 1-15.

Paerl, H., and J. Huisman. 2008. Blooms Like It Hot. *Science* 320:57-58.

Paerl, H., R. Fulton, P. Moisander, and J. Dyble. 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *The Scientific World Journal* 1:76-113.

Paerl, H., and D. Justić. 2011. 6.03 Primary Producers: Phytoplankton Ecology and Trophic Dynamics in Coastal Waters.

Paerl, H., H. Xu, M. J. McCarthy, G. Zhu, B. Qin, Y. Li and W. S. Gardner 2011. Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): The need for a dual nutrient (N & P) management strategy. *Water Research* 45: 1973-1983. doi: 10.1016/j.watres.2010.09.018

Parsons, T., Maita, Y., Lali, C. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford.

Piazza, B.P. and M.K. La Peyre. 2007. Restoration of the annual flood pulse in Breton Sound, Louisiana, USA: habitat change and nekton community response. *Aquatic Biology* 1: 109-119.

Pilotto, L., P. Hobson, M. D. Burch, G. Ranmuthugala, R. Attewell, and W. Weightman. 2004. Acute skin irritant effects of cyanobacteria (blue-green algae) in healthy volunteers. *Australian and New Zealand Journal of Public Health* 28:220-224.

Pinckney, J.L., H.W. Paerl, M.B. Harrington, and K.E. Howe. 1998. Annual cycles of phytoplankton community-structure and bloom dynamics in the Neuse River Estuary, North Carolina. *Marine Biology* 131:371-381.

Pinckney, J.L., J.L. Wee, A. Hou, and N. Walker. 2009. Phytoplankton community structure responses to urban effluent inputs following Hurricanes Katrina and Rita. *Marine Ecology Progress Series* 387: 137-146.

Prewitt, K. 1983. Scientific Illiteracy and Democratic Theory. *Daedalus* 112:49-64.

Qin, B., G. Zhu, G. Gao, Y. Zhang, W. Li, H. Paerl, and W. Carmichael. 2010. A Drinking Water Crisis in Lake Taihu, China: Linkage to Climatic Variability and Lake Management. *Environmental Management* 45:105-112.

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

Råbergh, C.M.I., G. Bylund, and J.E. Eriksson. 1991. Histopathological effects of microcystin-LR, a cyclic peptide toxin from the cyanobacterium (blue-green alga) *Microcystis aeruginosa* on common carp (*Cyprinus carpio*). *Aquatic Toxicology* 20, 131-145.

Rabalais, N., R. J. Diaz, L. A. Levin, R. E. Turner, D. Gilbert, and J. Zhang. 2010. Dynamics and distribution of natural and human-caused hypoxia. *Biogeosciences* 7:585-619.

Rabalais, N., R. E. Turner, Q. Dortch, D. Justic, V. Bierman, Jr., and W. Wiseman, Jr. 2002. Nutrient-enhanced productivity in the northern Gulf of Mexico: past, present and future. *Hydrobiologia* 475-476: 39-63.

Rabalais, N., R. E. Turner, D. Justić, Q. Dortch, W. Wiseman, and B. Sen Gupta. 1996. Nutrient changes in the Mississippi River and system responses on the adjacent continental shelf. *Estuaries* 19:386-407.

Richardson, K. 1997. Harmful or Exceptional Phytoplankton Blooms in the Marine Ecosystem. *In* J. H. S. B. a. A. J. Southward [ed.], *Advances in Marine Biology*, 31, 301-385. Academic Press.

Roberts, H. H. 1998. Delta switching: early responses to the Atchafalaya River diversion. *Journal of Coastal Research*: 882-899.

Roblin, R. 2008. Water quality modeling of freshwater diversions in the Pontchartrain Estuary. M.S. Thesis, New Orleans: University of New Orleans.

Rocha, C., H.M. Galvão, and A.B. Barbosa. 2002. Role of transient silicon limitation in the development of cyanobacteria blooms in the Guadiana estuary, south-western Iberia. *Marine Ecology Press Series* 228: 35-45.

Roy, E., N. T. Nguyen, S. Bargu, and J. R. White. 2012. Internal loading of phosphorus from sediments of Lake Pontchartrain (Louisiana, USA) with implications for eutrophication. *Hydrobiologia* 684: 69-82.

Roy, E., J. White, E. A. Smith, S. Bargu, and C. Li. 2013. Estuarine Ecosystem Response to Three Large-Scale Mississippi River Flood Diversion Events. *Science of the Total Environment*:374-387.

Rozas, L. P., T. J. Minello, I. Munuera-Fernández, B. Fry, and B. Wissel. 2005. Macrofaunal distributions and habitat change following winter–spring releases of freshwater into the Breton Sound estuary, Louisiana (USA). *Estuarine, Coastal and Shelf Science* 65:319-336.

Schindler, D. W., and J. R. Vallentyne. 2008, The Algal Bowl; Overfertilization of the World's Freshwaters and Estuaries. Canada, Houghton Boston Printers.

Sivonen, K., and G. Jones. 1999. Cyanobacterial toxins *in* I. Chorus, and J. Bartram, eds. Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. Great Britain, St. Edmundsbury Press.

Small, C., and R. J. Nicholls. 2003. A Global Analysis of Human Settlement in Coastal Zones. *Journal of Coastal Research* 19: 584-599.

Smith, A. 1863. An Inquiry into the Nature and Causes of the Wealth of Nations.

Smith, E.A., S. Bargu and C. Weidert (*in review*). Potential for Oyster Contamination from Freshwater Cyanobacterial Toxins in Coastal Louisiana. Submitted to *Marine Pollution Bulletin.*

Snedden, G. A., J. E. Cable, C. Swarzenski, and E. Swenson. 2007. Sediment discharge into a subsiding Louisiana deltaic estuary through a Mississippi River diversion. *Estuarine, Coastal and Shelf Science* 71: 181-193.

Srivastava, A. K., P. Bhargava, R. Thapar, and L. C. Rai. 2008. Salinity-induced physiological and proteomic changes in Anabaena doliolum. *Environmental and Experimental Botany* 64: 49-57.

Stone, D., and W. Bress. 2007. Addressing public health risks for cyanobacteria in recreational freshwaters: The oregon and vermont framework. *Integrated Environmental Assessment and Management* 3:137-143.

Strom, D. and M. Thompson. 2000. Biological Implications of Stable Salinity Gradients within the Context of the St. Lucie Estuary, Florida. In: Protection, F.D.o.E., Program, S.D.W.Q. (Eds.).

Sun, J., and D. Liu. 2003. Geometric models for calculating cell biovolume and surface area for phytoplankton: *Journal of Plankton Research*, v. 25, p. 1331-1346.

Tonk, L., K. Bosch, P.M. Visser, and J. Huisman. 2007. Salt tolerance of the harmful cyanobacterium Microcystis aeruginosa. Inter-Research.

Tsujimura, S. and T. Okubo. 2003. Development of Anabaena blooms in a small reservoir with dense sediment akinete population, with special reference to temperature and irradiance. *Journal of Plankton Research* 25, 1059-1067.

Turner, H. 1985. Parasites of eastern oysters from subtidal reefs in a Louisiana estuary with a note on their use as indicators of water quality. *Estuaries and Coasts* 8, 323-325.

Turner, R. E., Q. Dortch, D. Justic, and E. M. Swenson. 2002. Nitrogen loading into an urban estuary: Lake Pontchartrain (Louisiana, U.S.A.). *Hydrobiologia* 487:137-152.

Turner, R. E., Q. Dortch, and N. N. Rabalais. 2004. Inorganic nitrogen transformations at high loading rates in an oligohaline estuary. *Biogeochemistry* 68:411-422.

USEPA. 1993. Methods of chemical analysis of water and wastes, *in* USEPA, ed., Cincinnati, Environmental Monitoring Support Laboratory.

USGS. 2012. Water resources data for the United States, Water Year 2012: U.S. Geological Survey Water. Data Report: WDR,US-2012, site 295124089542100

Valiela, I., J. L. Bowen, and J. K. York. 2001. Mangrove Forests: One of the World's Threatened Major Tropical Environments. *BioScience* 51: 807-815.

VanZomeren, C., J.R. White and R.D. DeLaune. 2013. Ammonification and denitrification rates in coastal Louisiana bayou sediment and marsh soil: Implications for Mississippi river diversion management. *Ecological Engineering* 54:77-81.

Vilhena, L. C., I. Hillmer, and J. Imberger. 2010. The role of climate change in the occurrence of algal blooms: Lake Burragorang, Australia. *Limnol. Oceanogr* 55:1188-1200.

Vörösmarty, C. J., P. Green, J. Salisbury, and R. B. Lammers. 2000. Global water resources: vulnerability from climate change and population growth. *Science* 289: 284.

Walter, R. C., R. T. Buffler, J. H. Bruggemann, M. M. M. Guillaume, S. M. Berhe, B. Negassi, Y. Libsekal, et al. 2000. Early human occupation of the Red Sea coast of Eritrea during the last interglacial. *Nature* 405: 65-69.

Waterman, A. T. 1960. National Science Foundation: A Ten-Year Résumé. *Science* 131:1341-1354.

Wheelock, K. 2003. Pulsed river flooding effects on sediment deposition in Breton Sound estuary, Louisiana, University of Rhode Island.

White, J. R., H. W. Fulweiler, C. Y. Li, S. Bargu, N. D. Walker, H. H. Twilley, and S. E. Green. 2009. Mississippi River Flood of 2008: Observations of a Large Freshwater Diversion on Physical, Chemical, and Biological Characteristics of a Shallow Estuarine Lake. *Environmental Science & Technology* 43:5599-5604.

White, S.H., L.J. Duivenvoorden, L.D. Fabbro, and G.K. Eaglesham. 2006. Influence of intracellular toxin concentrations on cylindrospermopsin bioaccumulation in a freshwater gastropod (*Melanoides tuberculata*). *Toxicon* 47, 497-509.

Wu, K., and Y. J. Xu. 2007. Long-term freshwater inflow and sediment discharge into Lake Pontchartrain in Louisiana, USA. *Hydrological Series Journal* 52: 166-180.

APPENDICES

APPENDIX 1: IRB APPLICATION AND APPROVAL

Application for Exemption from Institutional Oversight

Institutional Review Board
Dr. Robert Mathews, Chair
131 David Boyd Hall
Baton Rouge, LA 70803
P: 225.578.8692
F: 225.578.6792
irb@lsu.edu
lsu.edu/irb

Unless qualified as meeting the specific criteria for exemption from Institutional Review Board (IRB) oversight, ALL LSU research/ projects using living humans as subjects, or samples, or data obtained from humans, directly or indirectly, with or without their consent, must be approved or exempted in advance by the LSU IRB. This Form helps the PI determine if a project may be exempted, and is used to request an exemption.

– Applicant,Please fill out the application in its entirety and include the completed application as well as parts A-E, listed below, when submitting to the IRB. Once the application is completed, please submit two copies of the completed application to the IRB Office or to a member of the Human Subjects Screening Committee. Members of this committee can be found at http://research.lsu.edu/CompliancePoliciesProcedures/InstitutionalReviewBoard%28IRB%29/item24737.html

– A Complete Application Includes All of the Following:
(A) Two copies of this completed form and two copies of part B thru E.
(B) A brief project description (adequate to evaluate risks to subjects and to explain your responses to Parts 1&2)
(C) Copies of all instruments to be used.
*If this proposal is part of a grant proposal, include a copy of the proposal and all recruitment material.
(D) The consent form that you will use in the study (see part 3 for more information.)
(E) Certificate of Completion of Human Subjects Protection Training for all personnel involved in the project, including students who are involved with testing or handling data, unless already on file with the IRB. Training link: (http://phrp.nihtraining.com/users/login.php)
(F) IRB Security of Data Agreement: (http://research.lsu.edu/files/item26774.pdf)

1) Principal Investigator Emily Smith **Rank:** Graduate Student

Dept: Oceanography and Coastal Sci **Ph:** 225-733-7847 **E-mail:** esmi122@lsu.edu

2) Co Investigator(s): please include department, rank, phone and e-mail for each
*If student, please identify and name supervising professor in this space
Sibel Bargu, Assoc. Prof., 225-578-0029, sbargu@lsu.edu
Pam Blanchard, Assoc. Prof. 225-578-2297, pamb@lsu.edu

IRB# E5957 LSU Proposal #_____
✓ Complete Application
✓ Human Subjects Training

3) Project Title: Education and Public Outreach concerning harmful algal blooms

Study Approved By:
Dr. Robert C. Mathews, Chairman
Institutional Review Board
Louisiana State University
203 B-1 David Boyd Hall
225-578-8692 | www.lsu.edu/irb
Approval Expires: 4/2/2015

4) Proposal? (yes or no) no **If Yes, LSU Proposal Number** _____

Also, if YES, either ◯ **This application completely matches the scope of work in the grant**
OR ◯ **More IRB Applications will be filed later**

5) Subject pool (e.g. Psychology students) LA Licensed Fishermen (Sport and Commercial)
*Circle any **"vulnerable populations" to be used:** (children <18; the mentally impaired, pregnant women, the ages, other). Projects with incarcerated persons cannot be exempted.

6) PI Signature Emily S **Date** 3/7/12 (no per signatures)

**** I certify my responses are accurate and complete.** If the project scope or design is later changes, I will resubmit for review. I will obtain written approval from the Authorized Representative of all non-LSU institutions in which the study is conducted. I also understand that it is my responsibility to maintain copies of all consent forms at LSU for three years after completion of the study. If I leave LSU before that time the consent forms should be preserved in the Departmental Office.

Screening Committee Action: Exempted ✓ Not Exempted ____ Category/Paragraph ____

Reviewer Kristin A. Gansle KAG **Signature** Kristin A. Gansle **Date** 04/03/2012

Study Approved By:
Dr. Robert C. Mathews, Chairman
Institutional Review Board
Louisiana State University
203 B-1 David Boyd Hall
225-578-8692 | www.lsu.edu/irb
Approval Expires: 4/2/2015

Consent Script

I am a graduate student at LSU and am conducting research on fishermen surrounding Lake Pontchartrain and Lake Des Allemandes. The name of the study is Education and Public Outreach. I'm looking at information like frequency of fishing, type of fishing and experience with fishing in LA's lakes. I am only surveying licensed fishermen in the state of LA. The information gathered will be used to help educate others about the lake. All information used for this study will not be linked in any way to your name. Participation is completely voluntary. There is no risk to you. Would you like to participate?

After the interview is complete. Do you have any questions?

Please feel free to contact me if any questions arise (give LSU business card).

APPENDIX 2: INITIAL SURVEY

Date	Time	Location	Interview #
Gender: ☐ M or ☐ F	Age	☐ Commercial ☐ Sports Fisherman	
Experience of fishing (years)		How often do you go fishing? ☐ once a year ☐ once a month ☐ once a week ☐ more than once a week	
Type of fish:		Do you: ☐ eat what you catch? ☐ Sell? ☐ Share?	
Have you heard of ***algae***? Can you please describe and tell me what you know about this organism?			
Below (or on a separate page) are pictures of water that are different in color. Which of the picture(s) most closely resembles "colored water" have you seen before? Can you remember where you were when you saw this color of water? What time of year? From these pictures, in which type(s) of "colored water" would you <u>not</u> go fishing? (pictures attached)			
Have you ever caught "bad fish" or a fish that you would not eat? ☐ yes ☐ no If so, why did you think it was "bad"? ☐ taste ☐ visual cues ☐ other _____			
Have you ever gone to a doctor/hospital from eating bad fish/shellfish? ☐ yes ☐ no Did you ☐ catch or ☐ buy this fish? Where?			
Where do you get information (on fishing conditions) about the lake? ☐ Fellow fishermen ☐ news ☐ WLF ☐ other _____			
Have you ever seen dead fish in lake? ☐ yes ☐ no Did you quit fishing that day? ☐ yes ☐ no Did you contact anyone about (report) the dead fish? ☐ yes ☐ no Who?			
Is all algae "bad"? ☐ yes ☐ no Why? Are you familiar with the term "harmful algal blooms"? What does this mean?			

APPENDIX 3. FOLLOW-UP SURVEY

Date	Time	Location	Interview #
Gender: ☐ M or ☐ F	Age	☐ Commercial ☐ Sports Fisherman	
Experience of fishing (years)		How often do you go fishing? ☐ once a year ☐ once a month ☐ once a week ☐ more than once a week	
Type of fish:		Do you: ☐ eat what you catch? ☐ Sell? ☐ Share?	
Have you seen this public service document? (show example of document) ☐ yes ☐ no			
Was the information easy to understand? ☐ yes ☐ no			
Did you know about harmful algae before reading it? ☐ yes ☐ no			
What did you learn from the document?			

Interviewee's initials:
Date:

VITA

Emily Anne Smith was born to Victoria Weilepp Smith and Richard Vickers Smith in Biloxi, Mississippi and grew up in Thibodaux, Louisiana. She earned her first Bachelor of Science degree in Marine Biology in 2002 and the second in Biology Education in 2003 at the University of Southern Mississippi moved to Chattanooga, Tennessee where she worked as a middle school science teacher and a volunteer tutor helping students before and after school. After teaching a few years, Emily returned to earn her Masters of Education in secondary education with an emphasis in science and then decided to pursue a PhD.

She applied and was accepted into the Department of Oceanography and Coastal Sciences at Louisiana State University (LSU) under Dr. Sibel Bargu. Her dissertation topic was on harmful algal blooms specifically in the vanishing estuaries of south Louisiana. While enjoying the research side of academia, she decided to use her skills as a teacher and incorporate outreach into her dissertation. This experience has led Emily to pursue a career path that emphasizes outreach, to both the public and policy makers about important environmental issues. During her time at LSU, Emily has been active in more than just her research. She was the President and Social Chair of the Coast and Environment Graduate Organization in different years; President of the Graduate Student Association for LSU for one year; Served as a Graduate School Senator in LSU Student Government for 1.5 years and was awarded LSU's Graduate Student Leader of the Year in 2013. Her time at LSU has been a mix of research and outreach, something she desires to continue into her career.