2015

Effect of the Herbicide Atrazine on Phytoplankton, Water Quality, and Ecosystem Functions in Louisiana Estuaries

Alexis Victoria Starr
Louisiana State University and Agricultural and Mechanical College

EFFECT OF THE HERBICIDE ATRAZINE ON PHYTOPLANKTON, WATER QUALITY, AND ECOSYSTEM FUNCTIONS IN LOUISIANA ESTUARIES

A Thesis

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
Master of Science

in

The Department of Environmental Sciences

by
Alexis V. Starr
B.S., Louisiana State University, 2013
August 2015

Acknowledgements

I would like to express my deepest gratitude to my Co-Advisiors Dr. Sibel Bargu and Dr. Vince Wilson. Dr. Bargu's passion and energy always kept our work interesting. Throughout process, she was always supportive, pushed me to do my best work, and encouraged me to think creatively as well as analytically. Dr. Wilson always asked the toughest questions, encouraging me to apply my knowledge to the "big picture". I would also like to thank my committee members, Dr. Crystal Johnson and Dr. Ron Delaune for their insight and support. Special thanks to Dr. Kanchan Maiti and Puspa Adhikari, for their input and training on the dissolved oxygen portion of the study, and Dr. Melissa Baustian, Jamal Mathrin, and Amy Hernandez for their assistance in the atrazine field sampling and analysis. I would also like to thank my parents, Elaine and Peter Starr, my siblings, Ashton and Peter Starr, and my grandmothers, Betty Parker and Julia Starr for their unyielding love and support in everything I do. Finally I would like to thank the Louisiana Water Research Institute for funding this project.

Table of Contents

Abstract

Pesticides are used primarily for agricultural purposes in the US and while these chemicals provide many benefits, the inherent toxicity of the compounds pose a substantial risk to the environment. These chemicals may enter water bodies in areas with a high proportion of agricultural land use through surface run off, ground water discharge, and erosion, and negatively impact non-target aquatic organisms. As a result, Louisiana's estuaries may be vulnerable to a variety of compounds, including the herbicide atrazine. Atrazine is used extensively throughout the Midwest and has been known to enter the Mississippi River through surface runoff and ground water discharge. The River transports the compound downstream to the delta, where it is discharged into Louisiana's coastal estuaries. Due to the high amount of sugarcane production in the southeastern part of the state, atrazine also has the potential to enter these systems indirectly through agricultural runoff, and adversely affect native aquatic organisms. Because it is a photosynthesis inhibitor, phytoplankton communities may be especially susceptible to atrazine exposure. The phytoplankton stress response in these systems may be critical because phytoplankton form the base of the food web and are essential to the production of the entire ecosystem. The purpose of this study was to determine the extent of atrazine contamination in Louisiana's estuaries, and its effect on local phytoplankton stress response. Field samples were taken under low and high flow and nutrient conditions from Breton Sound and Barataria Estuary. The results showed that atrazine was consistently present in these systems at low levels. Local phytoplankton from Barataria Estuary were also grown in microcosm and exposed to an atrazine dilution series under low

and high nutrient conditions to determine the phytoplankton stress response. The treatment groups that received 5 ppb and 50 ppb atrazine treatments under high nutrient conditions exhibited an extended lag phase and entered into the exponential growth phase several days after the control groups. Overall, communities in nutrient enriched treatment groups exhibited higher growth response, oxygen production, and were healthier than non-enriched groups, indicating that atrazine exposure may induce a stress response in phytoplankton communities under low nutrient conditions.

Chapter 1: Introduction

Agricultural, industrial, and residential activities are a major source of pollution worldwide. Pesticides are used extensively for agricultural purposes and their use is necessary to control nuisance organisms and increase crop yields to support the rising global population. 5.6 billion lbs of pesticides are used annually throughout the world, while 1.6 billion pounds are used in the United States alone (Alavanja, 2009). Pesticides are classified based on the pest in which they are designed to control, and include insecticides, rodenticides, herbicides, and fungicides. Due to the growing human population and demand for food, the amount of pesticides used is expected to increase in the near future (Mensah et al., 2014).

Herbicides, chemical compounds used to control or kill unwanted vegetation, lead all other pesticides groups in the amount produced, the area treated, and the value from sale (Mensah et al., 2014). They are also the most commonly used type of pesticide in the United States (Virginia Cooperative Extension, 2009) and employ a variety of mechanisms to inhibit their target organism and include photosynthesis inhibiting compounds, cell metabolism inhibitors, hormone inhibitors, cell division inhibitors and lipid synthesis inhibitors (Radosevich, 2007; Perez, 2011). While herbicides control unwanted weeds, due to their inherent toxicity, they can also adversely affect non-target organisms or travel to non-target locations. They may enter water bodies through agricultural runoff and ground water discharge, and pose an immediate concern for aquatic life. Heavily farmed areas, such as the Mississippi River Basin, may be especially vulnerable to adverse effects to water quality, due to the high use of pesticides in the area. Approximately 300 billion kg of synthetic

compounds, which are used in agricultural, consumer, and industrial products, enter freshwater systems annually (Mensah et al., 2014). These compounds have been found to alter phytoplankton biomass, species richness, and community composition, which may result in food web modifications and alterations in nutrient recycling and energy flow (Perez, 2011). In extreme cases of contamination, un-intentional kills of fish, frogs, birds, and mussels have been reported in these systems (Reigart and Roberts, 1999; USEPA, 2002; Fishel, 2005; Nesheim et al, 2005). The Mississippi River Basin is one of the most intensely farmed areas in the United States and may be likely to experience these water quality issues.

The Mississippi River Basin is the third largest river basin in the world and the largest in the United States. It drains approximately 42 percent of the continental United States and contains one of the most productive agricultural regions in the world (Burkart & James, 1994). Over the past 100 years, flood control structures have isolated the Mississippi River from many of Louisiana's coastal wetlands. To combat wetland loss, the state has developed a freshwater diversion plan to reintroduce sediment and fresh water into these areas by mimicking the natural flood events of the Mississippi River (Turner, 2009). Along with freshwater and sediment, these diversion structures also introduce nutrients and pollutants into these systems. Due to the high amount of agriculture in the Mississippi River Valley, pesticides are a major type of pollutants that can be introduced into Louisiana's estuaries.

Agriculture has been the dominant land use in the Mississippi River Basin over the past 200 years and is one of the greatest contributors to non-point source nutrients and pollutants in rivers, lakes, and other water bodies of the United States

(USEPA, 1994). Agricultural runoff, due to rainfall, and groundwater discharge carry these pollutants to the Mississippi River where they are transported downstream and discharged into Louisiana's estuaries. The state also has a high proportion of agricultural land use itself, further exasperating the problem as runoff enters these coastal water bodies from treated sugarcane, corn, sorghum, and wheat fields. Louisiana is one of the largest producers of sugarcane in the United States, making its coastal estuaries particularly susceptible to elevated levels of chemicals, such as the herbicide atrazine (USDA, 2014).

Atrazine, which is often used in sugarcane production, is the most widely used agricultural herbicide in the United States. It was first manufactured in 1959 and is still used today because it is cost effective and efficient (USEPA, 2002). It is applied pre-emergence and post-emergence to control annual broadleaf and grass weeds (Solomon et al., 1995). It is a relatively persistent, mobile compound that does not tend to bioaccumlate and is subjected to biotic and abiotic degradation (Khan, 1978; Graymore et al., 2001; Weiner et al, 2004).

Atrazine is the number one contaminant in the streams of the Midwest, and concentrations in those streams have exceeded the United States Environmental Protection Agency (USEPA) maximum contaminant level of 3 ppb in many cases (Lerch and Blanchard 1995, Lerch et al. 1995, Thurman et al. 1996, Clark and Goolsby, 2000). It has been found at levels ranging between 0.02 -1000 ppb in waters directly adjacent to treated fields (Pereira et al., 1992; Readman et al., 1993). Many estuarine systems have been found to contain detectable levels of atrazine, but these levels are often considerably lower than levels reported in corresponding rivers, due

to dilution and degradation (Graymore et al., 2001). Concentrations within these water bodies are generally episodic, correlate with high flow conditions and as a result typically peak in spring and early summer following application. Atrazine was detected in every sample collected April to June 1991 from the Mississippi and its major tributaries in concentrations ranging from 0.29-3.2 ppb from (USEPA, 2002).

Due to the chemical properties of the compound, atrazine tends to persist in the environment over long periods of time with an environmental half-life of 244 days at a pH of 4 and at 25 °C (Li et al., 1972). It has a low volatilization potential (vapor pressure=2.89 x 10^{-7} mm at 25 °C, Henry's Law constant=2.48 x 10 atm m^3 $mol^{-1)}$ from surfaces and water, a moderate water solubility (33 μg/ml at 22 °C), small K_d (0.19-2.46) and K_{oc} (25.3-155.0), indicating that it will favor movement in the dissolved state from treated surface soils to subsurface water during rain events or following irrigation (Ciba-Geigy Corporation, 1994; Solomon et al., 1995), that it is not likely to adsorb strongly with sediments, and is expected to partition moderately from the water column. The environmental fate of atrazine is largely influenced by its *s*-triazine ring, which contributes to its slow photolysis and hydrolysis rates, and also limits microbial degradation (Li et al., 1972; Khan, 1978, Howard, 1991). As a result, concentrations in receiving water bodies are often dependent on hydraulic residence time and dilution of the compound. This also indicates that chemical degradation may play a larger role than biodegradation in the breakdown of atrazine in the environment. Its long residence time in the water column may result in the prolonged exposure of non-target aquatic organisms to the compound.

Although atrazine is a photosynthesis inhibitor and does not bioaccumulate, it can be still moderately toxic to fish and invertebrates (Jantenen et al., 2008; Solomon et al., 2008; Kabra et al., 2014). The chronic effect values for freshwater fish species ranged from 88.3 to 430 ppb, while estuarine fish species had a chronic effect of 2,542 ppb. The chronic effect values for freshwater and estuarine invertebrates ranged from 159 to 3,500 ppb and 123-20,900 ppb, respectively (USEPA, 2001). However, atrazine concentrations of 20 ppb have been found to significantly reduce the reproduction and diet of the bluegill and adversely affect the species richness and abundance of several insects (USEPA, 2002). Adverse effects to aquatic ecosystem structure and function can be found at concentrations of 15 ppb and above (USEPA, 2002). The EPA has drafted freshwater acute and chronic atrazine water quality criteria of 350 ppb and 12 ppb, respectively, and saltwater (acute and chronic) criteria of 760 ppb and 26 ppb (USEPA, 2001). Acute and chronic criteria exceedances have been found in watersheds with a high proportion of agricultural land use in ambient water in the spring, corresponding to herbicide application and rainfall events, indicating that some of these species may be adversely affected (LDEQ, 1998). While atrazine has been found to be moderately toxic to fish and invertebrates, it has been found to be somewhat non-toxic to birds. The LD_{50} for the mallard duck was measured to be greater than 2,000 ppm, while no effect was observed in the bobwhite quail at levels exceeding 5,000 ppm (EXTOXNET, 1996).

Based on its method of action, primary producers, such as phytoplankton, may be the most sensitive to atrazine exposure. Atrazine acts as a photosynthesis inhibitor by competing with plastoquione II at its binding site on the D1 protein, blocking the

electron transport from photosystem II (Moreland and Hill, 1964). This mechanism has been defined in terrestrial plants, and is assumed to be similar in aquatic primary producers (Weiner et al., 2004). Alterations of the phytoplankton growth rate, community structure and primary productivity, which may be brought on by atrazine exposure, may induce responses in higher trophic levels and reduce ecosystem productivity. Concentrations as low as 1-10 ppb, which are regularly found in many water bodies, have been found to affect photosynthesis in phytoplankton (Lakshminarayana, 1992). At higher concentrations (10-20 ppb) death of susceptible species occurs and the community has been found to shift towards more resistant species. At levels reaching 500 ppb, photosynthesis was almost completely inhibited and severe reductions in biomass were noted (deNoyelles, 1982; Graymore et al., 2001).

Sensitivity to atrazine has been found to vary between phytoplankton phyla (Guazon, et al., 1996). Certain species have been found to be negatively impacted by atrazine levels as low as 1 ppb, while others have been resistant to concentrations up to 1000 ppb (Stratton, 1984; Bester et al., 1995). The most sensitive group is the chlorophytes, followed by cyanophytes, cryptomonads, dinophytes, and euglenophytes (Abou-Waly et al., 1997; Tang et al., 1997). Phytoplankton response to atrazine is dependent on a variety of factors including species tested, atrazine treatment concentrations, and endpoints measured (Weiner et al., 2004). Cell size, pigment profile, differences in cell uptake, binding and metabolism may conttribute to the differences in a species' sensitivity to the compound.

The purpose of this study was to quantify the amount of the herbicide atrazine entering into Louisiana estuaries via the Mississippi River and agricultural runoff under different flow and nutrient regimes (Spring and Summer) (Chapter 2). The Louisiana phytoplankton growth response and oxygen production under acute exposure conditions to varying levels of atrazine was also assessed (Chapter 3). Phytoplankton collected from Barataria Estuary were maintained in microcosms and exposed to an atrazine dilution series. The dilution series was designed to mimic peak atrazine levels that have occurred in many tributaries, lakes, and other water bodies throughout the United States during the spring. The field data was then used to determine the acute risk of atrazine to the Louisiana native estuarine phytoplankton community. Some studies have been conducted to determine the levels of atrazine in Louisiana waterways. However, these studies focus on small tributaries and streams rather than larger estuarine systems. Additionally, several studies have been conducted on individual phytoplankton species and communities in other estuarine habitats. However, the results of those studies cannot be extrapolated to make general statements about atrazine in Louisiana estuarine ecosystems due to the inherent variability in phytoplankton community structure and the wide range in environmental variables within estuarine systems.

References

Abou-Waly, H., Abou-Setta, M.M., Nigg, H.N., Mallory, L.L. 1991. Growth Response of freshwater algae, Anabaena flos-aquae and Selenastrum capricornutum to atrazine and hexazinone herbicides. Bull. Environ. Contam. Toxicol. 46: 223-229.

Alavanja, M. 2009. Pesticides Use and Exposure Extensive Worldwide. Environ Health. 2009; 24(4): 303-309.

Bester, K., Huhnerfuss, H., Brockmann, U., Rick, H.J. 1995.Biological effects of the triazine herbicide concentration on marine phytoplankton. Arch. Environ. Contam. Toxicol. 29, 277-283.

Burkart, M. R., James, D. E. 1999. Agricultural-nitrogen contributions to hypoxia in the Gulf of Mexico. Environ. Qual. 28: 850-859.

Ciba-Geigy Corporation. 1993. Label for Aatrex Nine-0. EPA Registration Number 100-585. Ciba Crop Protection, Greensboro, NC, USA.

Clark, G. M., D. A. Goolsby. 2000. Occurrence and load of selected herbicides and metabolics in the lawn Mississippi River. Sci. Tot. Environ. 248: 101-113.

deNoyelles F., Kettle, W.D., Sinn, D.E. 1982. The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. Ecology 63(5):1285-93.

Ellgehausen, H., Guth, J.A., Esser, D.O. 1980. Factors determining the bioaccumulation potential of pesticides in the individual compartments of aquatic food chains. Ecotoxicol. Environ. Saf. 4:134.

EXTOX NET. 1996. Atrazine. Extension Toxicology Network, Pesticide Information Profiles. June 1996. Web. http://extoxnet.orst.edu/pips/atrazine.htm

Fishel, F.M., 2005 Pesticide Toxicity Profiles. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Gunkel, G., Streit, B. 1980. Mechanisms of a herbicide (atra- zine, s-triazine) in a freshwater mollusc (*Ancylus fluviatilis* Mull.) and a fish (*Coregonus fera* Jurine). Water Res. 14:1573–1584.

Guanzon, N.G., Fukuda, M., Nakahara, H., 1996. Accumulation of agricultural pesticides by three freshwater microalgae. Fish. Sci. Health 62 (5): 690-697.

Howard, P., 1991. Handbook of environmental fate and exposure data for organic chemicals, vol. 3. Lewis, Chelsea, MI.

Jantunen, A.P.K., Tuikka, A., Akkanene, J., Kukkonen, J.V.K. 2008.Bioaccumulation of atrazine and chlorpyrifos to *Lumbriculus Variegatus* from lake sediments. Exotox. Environ. Saf.71: 860-868.

Kabra, A., Ji, M.K., Choi, J., Kim, J.R., Govindwar, S., Jeon, B. 2014.Toxicity of atrazine and its bioaccumulation and biodegradation in a green microalga, *Chlamydomonas Mexicana. Environ. Sci. Poll. Res* 21:*12270-12278*

Khan, S.U. 1978. Kinetics of hydrolysis of atrazine in aqueous fulvic acid solution. Pestic. Sci. 9:39–43

Klaasen, H.E., Kadoum, A. 1979. Distribution and retention of atrazine and carbofuran in farm pond ecosystems. Arch. Environ. Contam. Toxicol. 8:345–353.

Knuesli, E., D. Berrer, G. Depuis and H. Esser.1969. S-tria- zines. In P.C. Kearney and D.D. Kaufman, eds., *Degradation of Herbicides.* Marcel Dekker, New York, NY, USA, 51–70.

Lakshminarayana, J.S.S, O'Neill, H.J., Jonnavithula, S.D., Leger, D.A., Milburn, P.H.1992. Impact of atrazine-bearing agricultural tile drainage discharge on planktonic drift of a natural stream. Environ. Poll., 76: 201–210.

Lerch R. N., Blanchard, P. E. 1995. Regional-scale factors affecting herbicide contamination of northern Missouri streams. Practices, Systems, and Adoption, 3:175-178.

Lerch, R. N., Donald, W. W., Li, Y. X., Alberts, E. E. 1995. Hydroxylated atrazine degradation products in a small Missouri stream. Environ. Sci. Techn. 29: 2759-2768.

Li, G.C., Feldbeck. G. 1972. Atrazine hydrolysis as catalyzed by humic acids. Soil Sci. 114:201–209.

Louisiana Department of Environmental Quality. 1998. 1998 Atrazine Activities for the Upper Terrebonne Basin. Office of Water Resources, Louisiana Department of Environmental Quality, Baton Rouge, LA.

Lynch, T.R., Johnson, H.E, Adams, W.J. 1982. The fate of atrazine and a hexachlorobiphenyl isomer in naturally-derived model stream ecosystems. Environ. Toxicol. Chem. 1:179–192.

Macek, K.J., Buxton, K.S., Sauter, S., Gnilka, S., Dean., J.W. 1976. Chronic toxicity of atrazine to selected aquatic invertebrates and fishes. EPA-600/3-76-047. U.S. Environmental Protection Agency, Duluth, MN.

Metcalf, R.L., Sanborn, R. 1975. Pesticides and environmental quality in Illinois. Ill. Nat. Hist. Surv. Bull.31:377.

Moreland, D.E., Hill, K.L., 1964. Interference of herbicides with the Hill reaction of isolated chloroplasts. Weed Sci. 15, 229-236.

Nesheim, O.N., Fishel, F.M., Mossler, M.A. 2005. Toxicity of Pesticides UF/IFAS EDIS Document PI-13.

Pereira, W.E., Rostad, C. E., Leiker, T. J. 1990. Distribution of agrochemicals in the lower Mississippi River and its tributaries. Sci. Tot. Environ. 97/98: 41-53.

Pérez, G.L., Vera, M.S., Miranda, L. 2011. Effects of Herbicide Glyphosate and Glyphosate-Based Formulations on Aquatic Ecosystems, Herbicides and Environment, Andreas Kortekamp (Ed.), ISBN: 978-953-307-476-4, InTech.

Radosevich, S.R., Holt, J.S., Ghersa, C.M., 2007. Ecology of Weeds and Invasive Plants: Relationship to Agriculture and Natural Resource Management, 3rd edition.Wiley-Interscience, Hoboken, USA.

Reigart, J.R., Roberts, J.R. 1999. Recognition and management of pesticide poisonings, 5th edition. United States Environnemental Protection Agency Publication EPA-735-R-98-003.

Readman, J.W., Albanis, T.A., Barcelo, D., Galassi, S., Tronczynski, J., Gobrielides, G.P. 1993. Herbicide contamination of Mediterranean estuarine waters: results from a MED POL pilot survey. Mar. Pollut. Bull. 26(11):613-9.

Solomon, K, Baker, D.B., Richards, R.P., Dixon, K.R., Klaine, S.J., La Point, T.W., Kendall, R.J., Weisskopf, C.P., Giddings, J.M., Geisy, J.P., Hall. L.W., Williams, W.M. 1995. Ecological risk assessment of atrazine in North American surface waters. Environ. Tox. and Chem. 15: 31-76.

Solomon, K., Carr, J., Du Preez, L., Giesy, J., Kendall, R., Smith, E., Van Der Kraak, G. 2008. Effects of atrazine on fish, amphibians, and aquatic reptiles: a critical review. Crit. Rev. Tox. 38(9): 721-72.

Stratton, G.W. 1984. Effects of the herbicide atrazine and its degradation products alone, and in combination, on phototrophic organisms. Arch. Environ. Contam. Toxicol. 13:35-42.

Tang, J., Sigfried, B.D., Hoagland, K.D., 1997. Glutathione-s-transferase and in vitro metabolism of atrazine in freshwater algae. Pest. Biochem. Physiol. 155-161.

Thurman, E.M., Goolsby, D.A., Aga, D.S., Pomes, M.L., Meyer, M.T. 1996. Occurrence of alachlor and its sulfonated metabolite in rivers and reservoirs of the Midwestern United States-the importance of sulfonation in the transport of chloroacetanilide herbicides. Environ. Sci. and Techn. 30: 569-574.

Turner, R.E. 2009. Doubt and the values of ignorance-based world view for wetland restoration: Coastal Louisiana. Estuaries and Coasts 32:1054-1068.

U.S. Department of Agriculture. 2014. U.S. Sugar Cane Production. U.S. Department of Agriculture Economic Research Service. https//www.ers.usda.goc/topics/crops/sugar-sweeteners/background.aspx.

U.S. Environmental Protection Agency. 1994. National water quality inventory – 1992 report to congress. U.S. Environmental Protection Agency, 841-R-94-001, Washington, D.C.

U.S. Environmental Protection Agency. 2001. Draft Ambient Aquatic Life Water Quality Criteria for Atrazine. Office of Water, U.S. Environmental Protection Agency, Washington D.C.

U.S. Environmental Protection Agency. 2002. Summary of atrazine in EPA Region 6 surface waters. U.S. Environmental Protection Agency Region 6. Dallas, TX.

U.S. Geological Survey. 1994. U.S. Geological Survey Open File Report. 94.376.

U.S. Geological Survey. 2006. Pesticides in the nation's streams and ground water, 1992-2001. U.S. Department of the Interior, U.S. Geological Survey.

U.S. Geological Survey. 2009. Monitoring the Water Quality of the Nation's Large Rivers. National Stream Quality Accounting Network. Web. http://water.usgs.gov/nasqan

Virginia Cooperative Extension. 2009. Pesticides and Aquatic Animals: A Guide to Reducing Impacts on Aquatic Systems. Fisheries and Wildlife Services, Virginia Tech.

Weiner, J., DeLorenzo., Fulton., M. 2004. Relationship between uptake capacity and different toxicity of the herbicide atrazine in selected microalgal species. Aq. Tox. 68: 121-128.

Chapter 2: Atrazine Concentrations of Breton Sound and Barataria Estuaries for Spring and Summer Months of 2014

Abstract

Atrazine is a pre-emergence and post-emergence herbicide used to control annual broadleaf weeds and grasses. It is often found in rivers, lakes, and other water bodies throughout the United States in concentrations exceeding USEPA maximum contaminant level of 3 ppb. Louisiana estuaries are susceptible to elevated atrazine levels due to input from the Mississippi River and non-point sources, such as agricultural run-off. Atrazine levels were determined in two Louisiana estuaries, Breton Sound Estuary and Barataria Estuary. Three locations were sampled in each estuary in late spring and summer to evaluate the effects of river flow and pesticide application on atrazine levels. Atrazine was consistently present at low levels in both estuaries. However, Breton Sound Estuary exhibited higher atrazine levels than Barataria Estuary, over the time period sampled, most likely due to differences in the area's land use and hydrological characteristics. The presence over the three-month period suggests that aquatic organisms located in the two estuaries may be negatively impacted by chronic atrazine exposure. To determine the potential risk to estuarine organisms, atrazine levels in Louisiana estuaries should be measured year round to determine the impact of chronic vs acute exposure.

Introduction

Agriculture has been the dominant land use in the Mississippi River Basin over the past 200 years and is one of the greatest contributors to non-point source pollutants and nutrients in rivers, lakes, and other water bodies of the United States (EPA, 1994). The Mississippi River flows 3782 km from its source at Lake Itasca to the Gulf of Mexico. Its watershed drains 3.2 million km^2, which is approximately 42% of the continental US (Burkart & James, 1994). In the early 1900s, flood control structures were constructed and isolated Louisiana's coastal wetlands from the Mississippi River. To counteract wetland loss, the state of Louisiana developed a fresh water diversion plan. The purpose of these diversions is to mimic the natural flooding events of the Mississippi River, and allow for the reintroduction of freshwater and sediment into coastal wetlands. The use of diversions to restore wetlands has been somewhat controversial, as the project has raised questions about the effect of high nitrogen levels on aquatic ecosystems (Swarzenski et al., 2008; Turner, 2009). The freshwater diversions are now transporting millions of gallons of water that contain nutrients and pollutants into wetlands and estuaries through siphoning, pumping, and by cutting through lower levees. Pesticides are a type of pollutants that can be introduced by Mississippi River water into Louisiana's coastal wetlands and estuaries, however, the effect of pesticides, on estuarine dynamics has not been fully addressed.

Pesticides are often used in the production of corn, sugarcane, and many other crops throughout the Midwest and other states in the Mississippi Valley. Louisiana is one of the nation's largest producers of sugarcane (USDA, 2014). In 2009, over

400,000 acres in the state were harvested for sugarcane. The sugarcane industry has expanded westward and northward in recent years, as returns for competing crops have decreased. The state's production of sugarcane has also expanded due to the employment of high yielding sugar cane species and the investment in new harvesting techniques. An increase in agriculture, often leads to an increase in pesticide use, and therefore an increase in agricultural runoff to estuaries and other water bodies. The application of pesticides has increased 40-fold over a thirty year period from 1946 through 1976 in the United States (Gianessi 1992). Atrazine, which is often used in sugarcane production, is used extensively as a pre-emergence and post-emergence herbicide to control weeds. Atrazine is the number one contaminant in the streams of the Midwest, and its concentrations in Midwestern streams in many cases exceeded the USEPA maximum contaminant level of 3 ppb (Lerch and Blanchard 1995, Lerch et al. 1995, Thurman et al. 1996, Clark and Goolsby 2000). Mississippi River water contains high levels of atrazine and is an environmental concern (USEPA 1994). Atrazine loads of up to 2,000 kg day^{-1} in the Mississippi River have been reported in 1989 and 1992 near the river mouth (Pereira et al. 1990, USGS 1994). The annual herbicide load (1991 through 1997) from the Mississippi River Basin to the Gulf of Mexico ranged from approximately 450 t in 1992 to 1,920 t in 1993 (USGS, 2006). Mass transport (east to west along the Louisiana coast) for atrazine ranged from 800-3,000 kg day^{-1}, which compares with the amount in the Mississippi River. However, still very little is known about concentrations and the total amount of atrazine entering estuaries as agricultural run-off from Louisiana watersheds and drainage basins through diversions. The purpose of this study was to quantify the amount of

the herbicide atrazine entering into estuaries via the Mississippi River and agricultural runoff under different flow and nutrient regimes (Spring and Summer).

Materials and Methods

Site Selection

The Caenarvean freshwater diversion site in Breton Sound, Louisiana, was chosen as the first site to quantify the amount of Atrazine entering into the estuary from the Mississippi River. Breton Sound is located southeast of New Orleans, Louisiana and is composed of approximately 247,105 acres of fresh and brackish wetlands (Fig 1a). The estuary contains several large lakes throughout its upper areas and opens up into Breton Sound coastal waters. Breton Sound receives Mississippi River water via the Caernarvon freshwater diversion, located at the northernmost point of the estuary. The diversion structure is one of the largest in southern Louisiana and discharges freshwater into the estuary at a maximum rate of approximately 7981 cfs, but has averaged approximately 1907 cfs since 2001 (USGS, 2015). The discharge rate is regulated according to river height, simulates natural seasonal flow trends, and manages salinity in portions of the estuary. Intermittent pulsing up to the maximum flow from December to June occurs to benefit oyster production and sediment delivery to the estuary, and may also impact the amount of atrazine entering the area at a given time.

Barataria Estuary, Louisiana, was chosen as the second sampling site to evaluate atrazine inputs as a result of agricultural runoff and the Mississippi River via the Davis Pond Diversion (Fig. 1b). The Davis Pond diversion is located in St.

Charles Parish approximately 15 miles upstream of New Orleans. The structure is built into the mainline Mississippi River levee and diverts freshwater and the accompanying nutrients and sediment into Barataria at a rate of 10,650 cfs (USGS, 2001). Lake Cataouatche and Lake Salvador, subsegments of Barataria Estuary, were chosen for the Barataria sampling sites. Lake Cataouatche and Lake Salvador are approximately 9280 acres and 44,800 acres, respectively (EPA, 2005). The Davis Pond structure diverts water from the Mississippi River east of the town of Luling into a 9190 acre marsh located between Highway 90 and Lake Cataouatche. The water disperses throughout the marsh before entering Lake Cataouatche on the northwest side of the lake. From there, the water then flows from Lake Cataouatche into Lake Salvador. The upper Barataria Estuary consists of crop-grass lands, primarily sugar cane, bottom land hardwood forests, cypress swamps, and coastal marshes. These habitats range from fresh to salt water. Precipitation is a major fresh water input in the area. As a result, atrazine and other chemicals may enter the upper Barataria Estuary through agricultural runoff associated with the sugarcane fields and the Mississippi River.

Field Sampling and Analysis

Three stations located in both Breton Sound Estuary and Barataria Estuary were sampled to determine seasonal variation (spring and summer) in atrazine concentrations (Fig.1 and Fig. 2). Breton Sound was sampled during May, June, and August of 2014, while Barataria was only sampled during June and August of 2014. The three points within each estuary were sampled at varying distances from each

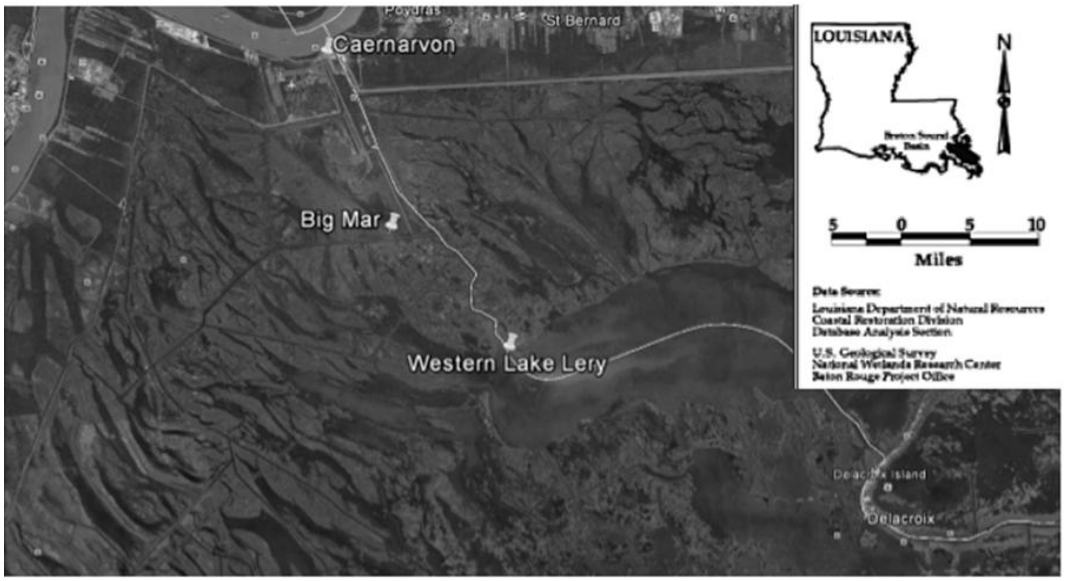
diversion (Table 1). Chlorophyll *a* (Chl *a*) was determined for the Breton Sound and Barataria stations as a measure of phytoplankton biomass. Surface water samples were taken at each site in 2 liter plastic Nalgene bottles and transported on ice to the lab. Upon arrival, 25 ml sub-samples of surface water were filtered through 25 mm GF/F filters and placed in the freezer at -20 °C until extraction. The filters were extracted for 24 h in 90% aqueous acetone at -20 °C and subsequently analyzed for Chl *a* using a Turner fluorometer (Model 10-AU, Turner Industries, Modesto, CA) (Parsons et al., 1984). Fifty ml of each water sample was vacuum-filtered through 0.45 µm membrane filters upon return to the laboratory and analyzed for DRP (Method 365.1; USEPA, 1993), NO_3-N (Method 353.2; USEPA, 1993) and NH_4-N (Method 350.1; USEPA, 1993) on a Seal Analytical (Mequon, Wisconsin) AQ2+ discrete analyzer using standard colorimetric methods. A YSI EXO2 water quality sonde was used at each sampling location to determine salinity.

Atrazine Analysis

Water samples were collected in 2 liter Nalgene bottles and transported to the laboratory on ice. Water samples designated for atrazine analysis were stored in a refrigerator at 4 °C overnight, and analyzed the following morning. Atrazine was extracted from the water samples using liquid-liquid portioning with methlylene chloride (dichloromethane) and exchanged to hexane. The extract was concentrated to 1 ml using an N-EVAP. An Agilent 7683 Automated liquid sampler was used to inject 2µl of 500 ml/ml extract into a Hewlett Packard 6890 Gas Chromatograph (GC) with an RTX 5MS 30 M x 0.25 mm x 0.25 µm capillary column installed.

Helium, used as a carrier gas, flowed throw a split-less mode inlet at 1.0 ml/min and 1 °C. The oven temperature was set to 80 °C with a hold time of 2 minutes, was set to increase 30 °C/min until a temperature of 190 °C was reached. After the oven reached 190 °C, it was set to increase 8 °C/min until the temperature of 300 °C was reached.

A.



B.

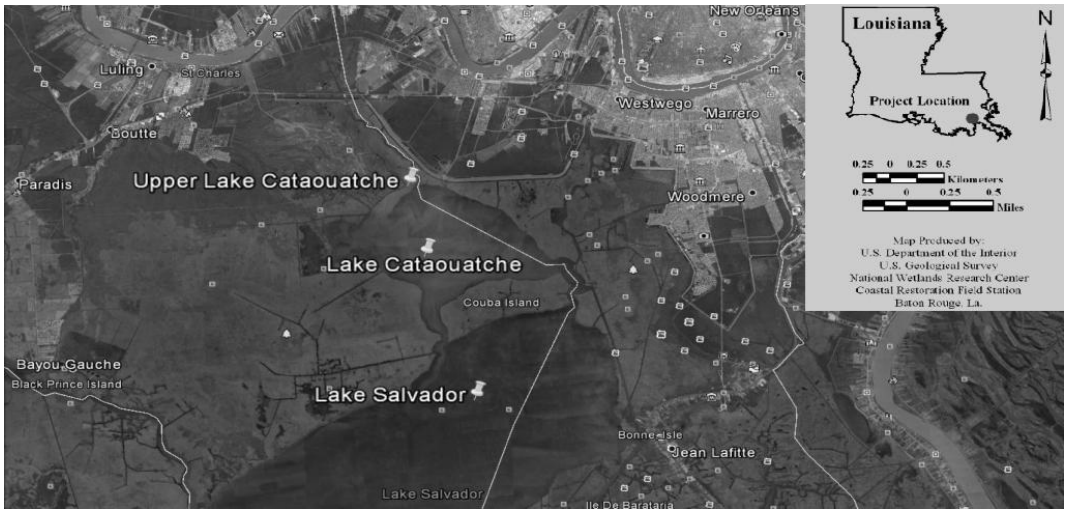


Figure 1A: Represents May, June, and August sampling sites located in Breton Sound. Pins indicate the points sampled at the Caernarvon outfall, Big Mar, and Western Lake Lery. B**:** Represents June and August sampling locations in the Upper Barataria Basin. Pins indicate the points sampled downstream from the Davis Pond Diversion, in Lake Cataouatche and Lake Salvador.

Once the oven reached 300 °C, it was held at that temperature for 5 minutes. The flow then continued through a 280 °C transfer line to a Hewlett Packard 5973 mass selective detector in selective ion monitoring mode, with a source temperature of 230 °C and a quadruple temperature of 150 °C. The ions (m/z) monitored for atrazine were 172.95, 200.05, 211.05, 215.05, and the retention time was 9.13 minutes. Single point external quantitation was performed using the 215.05 ion (m/z) with 200.05 and 172.95 as qualifiers at 35% and 172%, respectively, against an analytical standard of 0.20pm. A second injection of all samples, using the same initial GC parameters, but a different detection mode, allowed for a full scan confirmation of positive atrazine samples. This was done using a Hewlett Packard MSD that monitors ions between 50-450 with a minimum detection limit of 0.01 ppb.

Results

Salinity, Chl *a*, and nutrient levels differed between Breton Sound Estuary and Barataria Estuary and varied over time within each estuary. Chl *a* levels in Breton Sound ranged from 30-100 μgl^{-1} for the month of May and increased in June and August to 40-245 μgl^{-1}. Chl *a* levels were consistently lower in Barataria Estuary than in Breton Sound Estuary. The June Barataria Chl *a* concentrations ranged from 4.0-10 mgl^{-1} while the August Chl *a* measurements ranged from 2.5-10.5 mgl^{-1}. The salinity between sampling sites of the two estuaries was similar and ranged from 0.17-0.47 psu. Nitrogen levels varied month to month for each sampling site in both Breton Sound and Barataria Estuary. In Breton Sound, NO_3 and NH_4 levels ranged

from 0.03-1.08 mg Nl^{-1} and 0.02-.06 mg N l^{-1}, respectively, over the months sampled. NO_3 (0.14-8.25 mg Nl^{-1}) and NH_4 (0.6-4.1 mg N l^{-1}) concentrations exhibited more variation in Barataria Estuary than in Breton Sound Estuary.

Atrazine concentrations were found to be higher in Breton Sound for all months sampled. The Breton Sound Sampling sites also exhibited more variation in atrazine levels than Barataria Estuary. Atrazine concentrations in Breton Sound varied both temporally and spatially (Fig. 2). Atrazine levels in both Big Mar and Lake Lery for the month of May were measured below the detection limit (0.01 ppb), while the atrazine concentration at the Caernarvon Outfall, the closest sampling site to the diversion, was 0.42 ppb. Atrazine concentrations decreased over time at the Caernarvon Outfall sampling site and increased significantly overtime at the Lake Lery site. Atrazine peaked at the Big Mar site in June at 0.4 ppb and decreased in the month of August to 0.22 ppb.

Lake Cataouatche had the highest atrazine concentration of the Barataria Estuary sampling sites in June at 0.24 ppb (Fig. 3). In the Upper Lake Cataouatche and Lake Salvador sampling sites, atrazine concentrations in June were measured at 0.1 ppb and 0.2 ppb, respectively. The atrazine concentrations stayed constant in Lake Cataouatche between June and August. However, atrazine levels in Upper Lake Cataouatche and Lake Salvador increased to 0.23 ppb and 0.24 ppb, respectively.

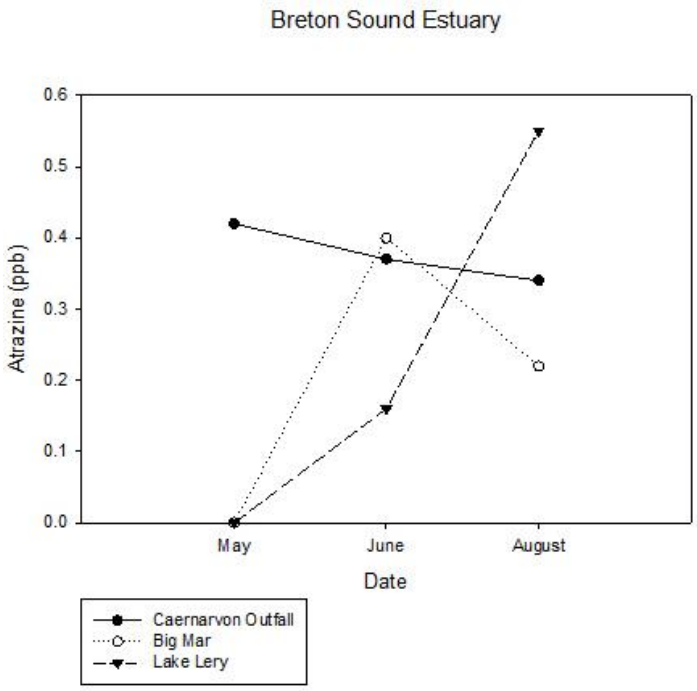


Figure 2: Atrazine concentrations at the Breton Sound sampling sites for the months of May, June, and August. May atrazine levels for Big Mar and Lake Lery fell below the detection limit and therefore are not reported.

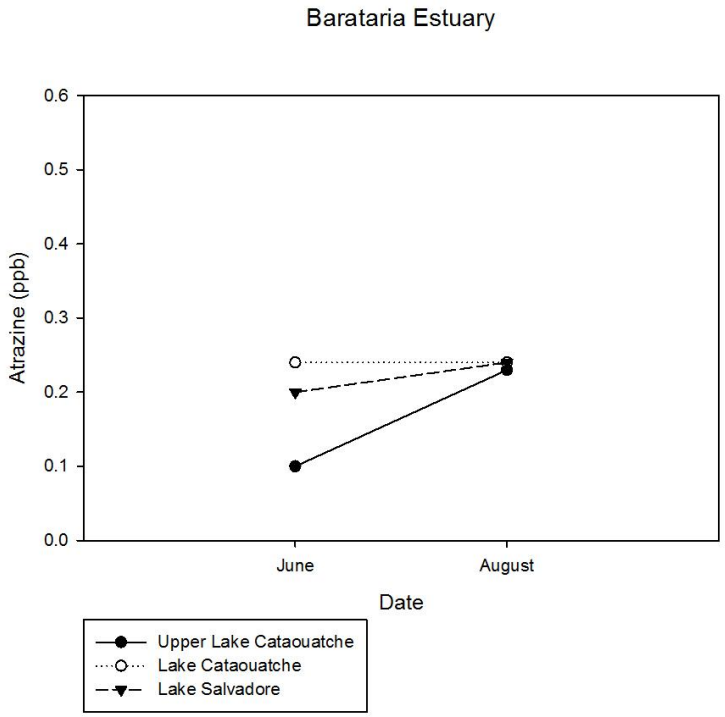


Figure 3: Atrazine concentrations for the Barataria Basin sampling sites for the months of June and August.

Table 1: Depicts distance to the Caernarvon Diversion and atrazine concentrations measured in Breton Sound Estuary during May, June, and August of 2014. Values measured below the detection limit of 0.1 ppb are denoted as "bdl".

Month	Station	Distance to Diversion (km)	Atrazine(ppb)
May	Caernarvon Outfall	2.5	0.42
	Big Mar	4.5	bdl
	Lake Lery	8	bdl
June	Caernarvon Outfall	2.5	0.37
	Big Mar	4.5	0.4
	Lake Lery	8	0.16
August	Caernarvon Outfall	2.5	0.34
	Big Mar	4.5	0.22
	Lake Lery	8	0.55

Table 2: Depicts distance to the Davis Pond Diversion and atrazine concentrations measure in Barataria Basin during June and August of 2014.

Month	Station	Distance to Diversion (km)	Atrazine(ppb)
June	Upper Lake Cataouatche	10.8	0.1
	Lake Cataouatche	14.6	0.24
	Lake Salvadore	22.6	0.2
August	Upper Lake Cataouatche	10.8	0.23
	Lake Cataouatche	14.6	0.24
	Lake Salvadore	22.6	0.24

Discussion

Ambient water quality data indicates that there is a widespread occurrence of atrazine at low levels in agriculturally influenced water bodies, with strong seasonal peaks in the spring (USEPA, 2002). Atrazine was consistently found in both Breton Sound Estuary and Barataria Estuary for the late spring and summer months sampled. However, the two estuaries exhibited different patterns of seasonal variation of atrazine present in surface water. Breton Sound generally had higher atrazine levels

for the months of June and August of 2014 than Barataria Estuary. The concentration of atrazine at each Breton Sound sampling site varied from month to month, while atrazine levels in Barataria were more stable over time and space. Differences in atrazine levels between the two estuaries may be associated with differences in diversion discharge, land use of surrounding areas, and the sampling site's distances to the diversion.

Barataria exhibited more variation in river diversion discharge than Breton Sound, but was more stable in atrazine concentrations. The discharge rate and daily discharge fluctuations of the two diversions may have impacted the amount of atrazine present in each estuary. The two diversions had a similar average discharge rate over the May-August sampling period. However, the two diversions exhibited different patterns in daily discharge fluctuations. Over the four month period, the average discharge for the Caernarvon Diversion was approximately 150-200 cfs. Daily fluctuations in the discharge of the Caernarvon Diversion generally ranged from approximately 0 to 500 cfs (USGS, 2015). Four days in May, one day in June, and two days in August of 2014 had discharge rates greater than 500 cfs. The Davis Pond also exhibited daily fluctuations in discharge and an average rate of 150-200 cfs for the months of June and August. The Davis Pond Diversion had several days in the month of June with significantly higher discharge rates than the average (up to 10,000 cfs), while the discharge was somewhat constant over the month of August. The slight variation in atrazine concentrations between Barataria sampling sites for the month of June and the consistent atrazine levels at approximately 0.24 ppb for the month of August, indicate that fluctuations in the Davis Pond Diversion discharge

may affect the variation of atrazine levels between stations in Barataria Estuary. However, this does not seem to be the case in Breton Sound, as diversion discharge rate was more stable over time but atrazine concentrations varied much more from month to month at each station.

While the Davis Pond Diversion river discharge rate was more unstable for the month of June than the Caernarvon Diversion river discharge, both diversion's mean discharge over the time period sampled was very similar. Because there was no substantial difference in the discharge rates of the two diversions, it cannot be said that the differences in atrazine concentrations between Breton Sound and Barataria are due to differences in discharge. Other factors, including the difference in land use and hydrologic features, may play a key role in the differences in atrazine levels between the two estuaries.

Breton Sound is a remnant of the abandoned St. Bernard Mississippi River deltaic lobe and is approximately 676,400 acres in size (CWPPRA and USGS, 2014). The Breton Sound Estuary consists of 184,000 acres of wetlands and 51,300 acres of public land (LDEQ, 2014).The principal hydrologic features of Breton Sound include the Mississippi River and its natural levees. Levees constructed as flood control structures prevent the annual input of freshwater and the nutrients and sediment associated with it. Major freshwater inputs into the Sound are limited to the diversions at Caernarvon, White's Ditch, Bohemia, and Bayou Lamoque, as well as the abandoned delta distributaries of Bayou Terre aux Boeufs and River aux Chenes (CWPPRA and USGS, 2014).

Barataria Estaury has different hydrological characteristics and land uses than Breton Sound. The Barataria contains approximately 1,565,000 acres composed of swamp, fresh marsh, intermediate marsh, brackish marsh, and saline marsh (LDEQ, 2014). In the Barataria-Terrebonne basin agriculture is a major land use. Sugarcane production in the basin totals over 295,000 acres while soybeans and grain production make up 12,000 acres and 7,000, acres respectively (LDEQ, 2014). Due to the sugarcane, corn, and other crops grown in the area, it is likely that atrazine is used pre-emergence and post-emergence to control broad-leaf and grass weeds. Non-point source runoff from agricultural lands has the potential to carry atrazine to the estuary. The amount of atrazine entering the estuary and the distribution input varies spatially and is dependent on rainfall, pesticide application, and the season. Atrazine is typically applied as a pre-emergence herbicide 6-8 weeks before crops are planted. Depending on the crops planted this usually falls between April 1 and May 1. If used post emergence, atrazine may be applied as late as June or July. Therefore, atrazine may enter the surface waters of Barataria Estuary through agricultural runoff during the late spring and through the majority of summer.

Atrazine is relatively persistent and does not strongly associate with soil or sediment particles (USEPA, 2002). Due to its slow breakdown, water column concentrations in closed aquatic systems may become elevated, especially those in watersheds with a large proportion of agricultural land use. Because Breton Sound contains significantly less agricultural lands than Barataria, it was expected that atrazine would be higher in the surface waters of Barataria. However, this was not the case. Atrazine levels in Breton Sound were higher overall than concentrations

measured in Barataria. In some cases atrazine levels found in Breton Sound were almost double of those measured in Barataria. Surface water atrazine levels were most likely lower in Barataria due to dilution. The Barataria Estuary is over twice the size of Breton Sound. Lake Cataouatche (9,280 acres) and Lake Salvador (44,800 acres) in Barataria are significantly larger than Big Mar (2,040 acres) and Lake Lery (USGS, 2015; LDWF, 2010). The larger amount of water present in the lakes sampled in Barataria give them a greater potential to dilute pollutants such as atrazine. The hydrologic isolation of Breton Sound could also reduce atrazine dilution. Breton's natural levees and man-made flood control structures limit freshwater input to the system further slowing the dilution process. Distance of the sampling sites to the diversions may have also played a role in the dilution of atrazine and therefore the amounts measured. The Barataria sampling sites were several times further from the Davis Pond Diversion than the Breton Sound sampling sites were to Caernarvon. Therefore, the atrazine present in the surface water had more time disperse when traveling from the Davis Pond diversion to the sampling stations.

While there was variation in atrazine concentrations within and between the two estuaries sampled, atrazine was consistently found to be present at low levels in both Breton Sound Estuary and Barataria Estuary over the time period sampled. All atrazine levels remained below the USEPA maximum containment level of 3 ppb. However, because atrazine was present over the four-month period, aquatic organisms may be negatively impacted due to chronic exposure. To determine the risk to aquatic organisms in these two estuaries, the sites in Breton Sound and Barataria should be sampled year round for atrazine levels and further examined to

determine the impact of acute vs chronic atrazine exposure to estuarine organisms. Photosynthetic organisms, such as phytoplankton, form the base of the food chain and are essential players in trophic level dynamics of estuarine ecosystems. However, limited data is available regarding the effects of atrazine on Louisiana estuarine phytoplankton communities.

References

Barataria-Terrebonne National Estuary Program. 2014. CCMP Part Three: The Technical Supplement. Barataria-Terrebonne Action Plans. EM-11 Reduction of Agricultural Pollution. Thibodaux, LA.

Burkart, M. R., James, D. E. 1999. Agricultural-nitrogen contributions to hypoxia in the Gulf of Mexico. Environ. Qual. 28: 850-859.

Coastal Wetlands Planning, Protection and Restoration Act Program, U.S. Geological Survey. 2014. The Breton Sound Basin. Coastal Wetlands Planning, Protection and Restoration Act. http://www.lacoast.gov.

Clark, G. M., D. A. Goolsby. 2000. Occurrence and load of selected herbicides and metabolics in the lawn Mississippi River. Sci. Tot. Environ. 248: 101-113.

Gianessi, L. 1992. U.S. pesticide use trends: 1966-1989, resources for the future, quality of the Environment Division, Washington, D.C.

Lerch R. N., Blanchard, P. E. 1995. Regional-scale factors affecting herbicide contamination of northern Missouri streams. Practices, Systems, and Adoption, 3:175-178.

Lerch, R. N., Donald, W. W., Li, Y. X., Alberts, E. E. 1995. Hydroxylated atrazine degradation products in a small Missouri stream. Environ. Sci. Techn. 29: 2759-2768

Louisiana Department of Wildlife and Fisheries. 2010. Big Mar Caernarvon: Waterbody Management Plan Series, Part VI-A. Louisiana Department of Wildlife and Fisheries, Office of Fisheries, Inland Fisheries Division, District 8.

Parsons, T.R., Maita, Y., Lalli, C.M. 1984. A manual of chemical and biological methods for seawater analysis. 173:285-295.

Pereira, W.E., Rostad, C. E., Leiker, T. J. 1990. Distribution of agrochemicals in the lower Mississippi River and its tributaries. Sci. Tot. Environ. 97/98: 41-53.

Swarzenski, C.M., Doyle, T.W., Fry, B., Hagris, T.G. 2008. Biogeochemical response of organic-rich freshwater marshes in the Louisiana delta plain to chronic river water influx. Biogeochem. 90:49-63.

Thurman, E.M., Goolsby, D.A., Aga, D.S., Pomes, M.L., Meyer, M.T. 1996. Occurrence of alachlor and its sulfonated metabolite in rivers and reservoirs of the Midwestern United States-the importance of sulfonation in the transport of chloroacetanilide herbicides. Environ. Sci. and Techn. 30: 569-574.

Turner, R.E. 2009. Doubt and the values of ignorance-based world view for wetland restoration: Coastal Louisiana. Estuaries and Coasts 32:1054-1068.

U.S. Department of Agriculture. 2014. U.S. Sugar Cane Production. U.S. Department of Agriculture Economic Research Service. https//www.ers.usda.goc/topics/crops/ sugar-sweeteners/background.aspx

U.S. Environmental Protection Agency. 1993. Method 350.1: Determination of Ammonia Nitrogen by Semi-automated Colorimetry. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory. Cincinnati, OH.

U.S. Environmental Protection Agency. 1993. Method 353.2: Determination of Nitrate-Nitrite Nitrogen by Semi-automated Colorimetry. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory. Cincinnati, OH.

U.S. Environmental Protection Agency. 1993. Method 365.2: Determination of Phosphorus by Semi-automated Colorimetry. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory. Cincinnati, OH.

U.S. Environmental Protection Agency. 1994. National water quality inventory – 1992 report to congress. U.S. Environmental Protection Agency, 841-R-94-001, Washington, D.C.

U.S. Environmental Protection Agency. 2002. Summary of atrazine in EPA Region 6 surface waters. U.S. Environmental Protection Agency Region 6. Dallas, TX.

U.S. Environmental Protection Agency. 2005. Lake Cataouatche TMDLs for Dissolved Oxygen and Nutrients. Water Quality Protection Divison, 68-C-02-108, FTN Associates, LTD. Little Rock, AR.

U.S. Geological Survey. 1994. U.S. Geological Survey Open File Report. 94.376.

U.S. Geological Survey. 2015. National Water Information System: Web Interface. U.S. Geoloigcal Survey. http://waterdata.usgs.gov/nwis.

U.S. Geological Survey. 2006. Pesticides in the nation's streams and ground water, 1992-2001. U.S. Department of the Interior, U.S. Geological Survey.

Chapter 3: The Effect of Atrazine on Louisiana Estuarine Phytoplankton Growth and Oxygen Production

Abstract

Atrazine is a triazine herbicide used to control annual broadleaf and grass weeds. The compound is frequently detected in water bodies, such as lakes, rivers, and streams, throughout the United States. Because atrazine is used extensively for agricultural purposes in the Mississippi River Valley and the state of Louisiana, it is likely that atrazine may enter Louisiana estuaries via the Mississippi River and from non-point sources, due agricultural runoff. Atrazine is known to inhibit photosynthesis and as a result, primary producers in aquatic ecosystems, such as phytoplankton, may be negatively impacted by elevated levels of atrazine. Phytoplankton are essential in estuarine ecosystems, as they form the base of the food web. Therefore, high atrazine levels may adversely affect the productivity of the entire estuarine ecosystem if the phytoplankton community is severely impacted. The purpose of this study was to determine the effects of atrazine on Louisiana estuarine phytoplankton growth response and oxygen production. A dilution series of 5 ppb, 50 ppb and 200 ppb of atrazine was applied to estuarine phytoplankton under high and low nutrient conditions. Phytoplankton were grown in culture and sampled daily over a 10-day period to determine the change in biomass related to growth. Oxygen production was also measured over the time period in phytoplankton exposed to low (10 ppb) and high (100 ppb) atrazine treatments. These treatments were further divided into nutrient enriched and non-enriched groups. The results showed that atrazine greatly inhibited phytoplankton growth and oxygen production in low nutrient conditions. The phytoplankton exposed to atrazine in high nutrient

conditions, were able to "bounce-back" after an extended acclimation period. Similarly, the communities grown under high nutrient conditions grew more rapidly and produced higher levels of oxygen over the 10-day period than the low nutrient treatment groups. There was a greater stress response in the non-enriched treatment group brought on by the combined effect of atrazine exposure and a lack of sufficient nutrients. High levels of stress on phytoplankton may adversely impact entire aquatic ecosystem functions because they form the base of the food chain and are major primary producers. If phytoplankton biomass or photosynthetic rate is inhibited, or there is a shift in the community structure, the species richness and productivity of the system as a whole may suffer.

Introduction

Approximately 1.8 billion people worldwide are involved in agriculture, with the majority using pesticides to increase crops yields (Alavanja, 2002). These chemicals are also used commercially, residentially, and in public health programs to prevent the spread of vector borne diseases. A pesticide is a substance used for destroying insects or other organisms harmful to cultivated plants or animals and include fungicides, herbicides, insecticides, and rodenticides (Merriam-Webster, 2014). 5.6 billion lbs. of pesticides are used worldwide annually, while 1.6 billion lbs. are used in the United States alone (Alavanja, 2002). Herbicides, a subclass of pesticides that suppresses or kills unwanted vegetation, are the most commonly used pesticide in the United States (Virginia Cooperative Extension, 2009).

Herbicides protect against crop losses and allow for more efficient food production. However, many are toxic to non-target organisms and their toxicity is not always limited to the area in which they are applied. Herbicides act through various mechanisms and can inhibit hormones, cell division, photosynthesis, pigment synthesis, lipid synthesis, and cell metabolism (Radosevich, 2007, Mensah et al., 2014). They have been known to adversely affect non-target organisms such as soil microorganisms, bees and other pollinators, amphibians, fish, birds, plants, and phytoplankton (Reigart and Roberts, 1999; USEPA, 2002; Fishel, 2005; Nesheim et al, 2005). Due to their extensive use in agriculture, it is not uncommon to find herbicides in the surface waters located in agricultural areas (Deneer, 2000). These chemicals enter systems through various transport processes, including agricultural runoff, which are dependent on drainage patterns, chemical properties of the herbicide, rainfall, microbial activity, and application rate (Larramendy and Soloeski, 2014). They can affect water quality and ecosystem functions by altering plant and phytoplankton biomass, species richness, and community composition resulting in food web modifications and alterations in nutrient recycling and energy flow (Perez, 2011). The presence of these compounds in terrestrial and aquatic ecosystems has become an important environmental issue worldwide.

Many developing countries around the world have not developed standards for herbicides. In the Unites States, under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the carcinogenic risk of every pesticide, used commercially and residentially, is assessed using genotoxicity studies and short-term mutagenic assays (Fenner-Crisp, 2001; Alavanja and Bonner, 2005). The United

States Environmental Protection Agency (USEPA) has used this information to set a maximum acceptable contaminant level for the majority of pesticides in drinking water. However, no standards for aquatic organisms have been developed. Exposure to these chemicals could adversely affect human health and estuarine ecosystems (USEPA, 1996).

Atrazine is a triazine herbicide used to control annual broadleaf and grass weeds in a variety of crops, including corn, sorghum, and sugarcane (Solomon et al., 1995), and it is frequently detected in rivers, lakes and estuaries. The environmental half-life of atrazine in microcosm studies ranges from 30 to 120 days (Cunningham et al., 1984; Kemp et al., 1985; Glotfelty et al., 1998; Pinckey et al., 2002). Due to the *s*-triazine ring, microbial biodegradation is minimal (Howard, 1991). Atrazine's long residence time in the water column may result in the prolonged exposure of aquatic organisms to the compound and estuaries can accumulate atrazine through acute and chronic loading events (Jones et al, 1982; Insensee, 1987; Glotfelty et al., 1998; Pinckney, 2002). Atrazine has been known to reduce primary productivity (Solomon et al., 1995; Graymore et al, 2001), which in turn impacts the ecosystem's community structure as a whole. Because atrazine is known to inhibit photosynthesis, it has the potential to directly affect phytoplankton communities in water column. Negative effects on photosynthesis of various phytoplankton and submerged vascular plants are reported at levels as low as 1 to 10 ppb (Kemp et al., 1985; Lakshimrayana et al., 1992), levels that are commonly found in many water bodies.

Atrazine, alachlor, and other chemicals are used extensively in the Midwest and throughout other states located in the Mississippi River Valley (Pereira et al,

1990). The annual herbicide load (1991 through 1997) from the Mississippi River Basin to the Gulf of Mexico ranged from approximately 450 t (1992) to 1,920 t (1993) (USGS, 2006). Mass transport (east to west along the Louisiana coast) for atrazine ranged from 800-3,000 kg day^{-1}, which is comparable with the amounts detected in the Mississippi River (USGS, 1994). The Mississippi River contains high concentrations of essential nutrients, such as nitrogen (N) and phosphorus (P), which are required for phytoplankton growth and algal bloom formation, achieves peak flow during the spring due to snowmelt and surface runoff into its tributaries (Turner and Rabalais, 1994; Snedden et al., 2007; Hyfield et al., 2008). As a result, phytoplankton blooms occur in late spring through early fall in the receiving estuaries due to increased nutrient delivery, with seasonal community composition shifts commonly observed (Murrell et al. 2007; Thronson et al. 2008, Bargu et al. 2011). Two large diversion structures, the Caernarvon Diversion structure and the Davis Pond Diversion structure, have been operating in southeastern Louisiana for the past two decades and control the input of nutrient loaded freshwater into Breton Sound Estuary and Barataria Estuary, respectively. The biomass and community composition of phytoplankton in Breton Sound and Barataria Estuaries are dependent on changes in temperature and by factors are highly influenced by the river and other nonpoint source inputs: nutrient availability, salinity, water mixing and contamination levels. Sugarcane, which is grown on the natural levee of the Mississippi River deltaic plane, has been an important contributor to Louisiana economy for a long period of time. However, like Mississippi River water itself, major source of pollutants impacting the

water quality (excess of nutrients, pesticide levels etc.) of this watershed is the runoff from these sugarcane fields.

Phytoplankton are critically important pelagic primary producers in estuarine ecosystems and their communities are composed of numerous species that exhibit a range of physiological responses to environmental stressors. Responses of phytoplankton to various atrazine concentrations can be especially important to higher trophic levels since their growth and abundance can determine the potential productivity of the entire ecosystem (Wissel and Fry 2005). While atrazine at low levels has been found to have minimal effects on some specific phytoplankton communities (Pinckney et al, 2002), it has been also found to reduce phytoplankton growth at 5-20 ppb (Solomon et al, 1995). Certain phytoplankton species have also been found to be more susceptible to atrazine exposure. Specifically, chlorophytes have been found to be more susceptible to atrazine than diatoms and cyanobacteria (Whitacre, 2011). Cells with a larger surface to volume ratio have been found to incorporate more atrazine and in general are more sensitive to the compound (Weiner et al, 2000). Even though there is literature investigating the impact of atrazine on individual phytoplankton species and communities, there is no information available for the phytoplankton community that resides in Louisiana estuaries. Due to input from the Mississippi River and the high proportion of agricultural land use in southern Louisiana, atrazine has the potential to enter estuaries via river water and agricultural runoff.

The goal of this study was to determine the Louisiana phytoplankton growth response and oxygen production under acute exposure conditions to varying levels of

atrazine. Phytoplankton collected from Barataria Estuary were maintained in microcosms and exposed to an atrazine dilution series. The dilution series was designed to mimic peak atrazine levels that have occurred in many tributaries, lakes, and other water bodies throughout the United States during the spring.

Materials and Methods

Estuarine Water Collection

The toxicity of atrazine was assessed by using a native phytoplankton community from Barataria Estuary in Louisiana. Two-20 L Nalgene carboys were used to collect estuarine surface water samples from Lake Salvador, located in Upper Barataria, in June 2014. Field samples taken from this location were found to contain background atrazine of 0.2 ppb. The Lake Salvador water was filtered through a 100 μm mesh sieve to separate zooplankton and particulates.

Preparation of Atrazine Stock Solution:

Pestanal® Sigma-ALDRICH atrazine was placed in deionized water to form a 10 ppm atrazine stock solution. Because atrazine has a moderate solubility in water (30 ppm at 20 °C), the solution was placed on a hot plate with a magnetic stirrer where it was heated at 23 °C and mixed with magnetic stirring rods for the 24 hour period prior to the experiment to ensure the atrazine was fully dissolved (USEPA, 2015).

Experimental Setup

Samples were initially divided into two groups, with (+) and without (-) nutrient enrichments (Fig. 1). Nutrients were initially added to the enriched treatment group according to the ratios outlined in DY-V media instructions. Each group was then further divided by atrazine treatments. Each sample contained the same volume of non-enriched filtered estuarine water ((-) FEW) or enriched filtered estuarine water ((+) FEW) solution to ensure the initial concentration of phytoplankton was approximately the same for all flasks at the start of the experiment. The growth experiment atrazine treatment groups consisted of 5 ppb, 50 ppb, and 200 ppb atrazine, while the oxygen production experiment atrazine treatment groups consisted of 10 ppb and 100 ppb atrazine. For each experiment, two control groups containing only phytoplankton with no atrazine addition in the nutrient enriched and non-enriched groups were used. Sterilized Pyrex flasks were used in the growth experiment. For the oxygen production experiment, sterilized glass bottles (300 ml) with glass penny head stoppers were used. The test media volume was 300 ml to ensure no air remained in the bottles. All experimental flasks and bottles were kept at 24 °C on a 12:12 h light:dark cycle with cool white fluorescent lights at an irradiance of 85 $\mu E\ m^{-1}s^{-1}$ for a period of 10 days.

For the growth experiment, 10 ml water subsamples were taken from each flask over a 10-day period to determine daily changes in phytoplankton biomass. Each subsample was filtered through a 25 mm GF/F filter and stored in the freezer at -20 °C until extraction. The filters were then extracted for 24 h in 90% aqueous

acetone at -20 °C and subsequently analyzed for Chl a using a Turner fluorometer (Model 10-AU) (Parsons et al., 1984).

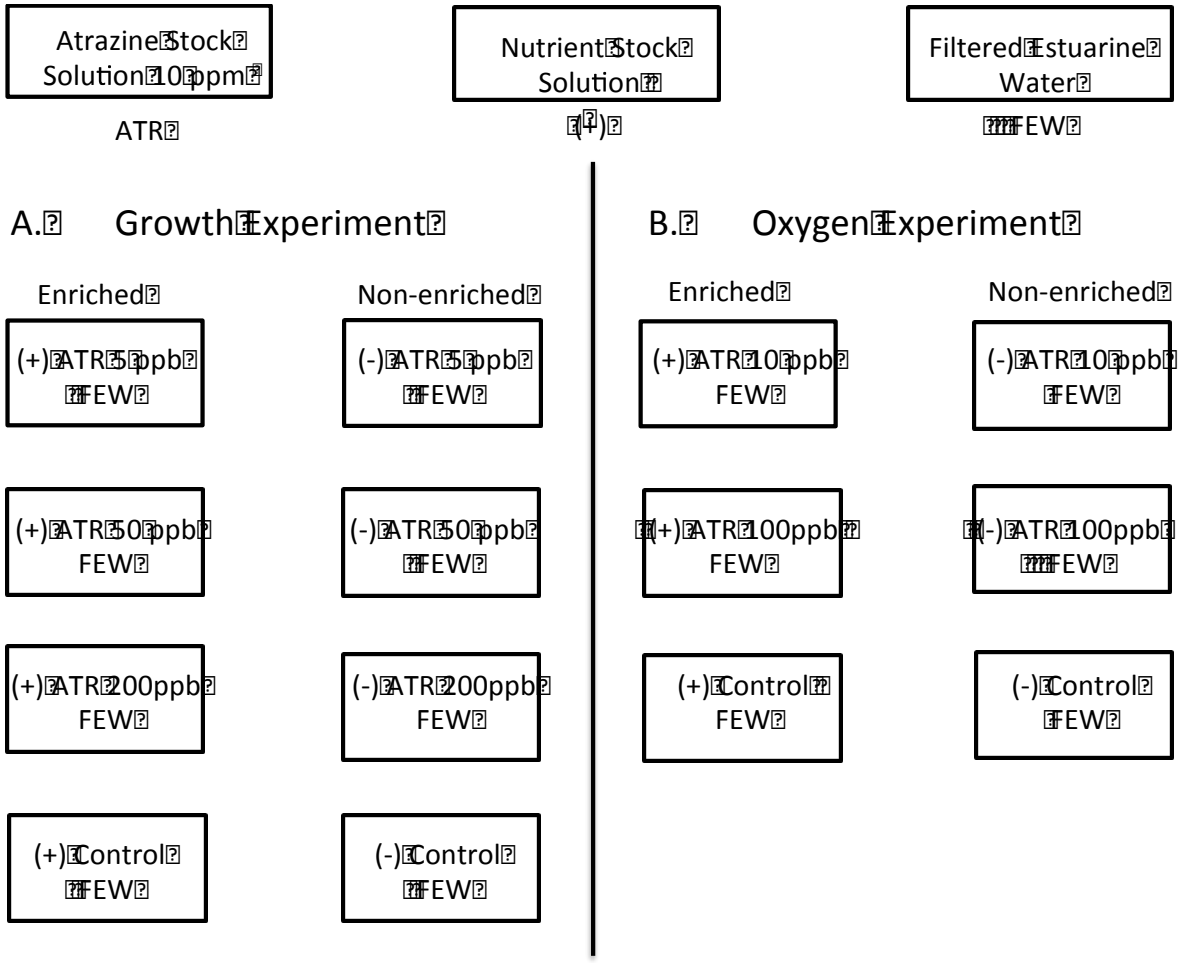


Figure 1: Summary of stock solutions and treatment compositions used in the growth and oxygen production studies. Each treatment consisted of three replicates. Atrazine and nutrients were added initially to the appropriate treatments. The enriched treatment is designated by (+), while the non-enriched treatment is designated by (-).

For the oxygen production analysis, the dissolved O_2 concentrations were measured every other day using a Clark-type microelectrode sensor with a 100μm tip. The oxygen sensor chosen for this particular application has a response time <8 sec, a

stirring sensitivity of <0.5%, a detection limit of 0.05 µM and a negligible analyte consumption rate of 5-50x10^{-4} nmol hr^{-1}.

Table 1: A. Initial nutrient concentrations present in non-enriched treatment groups. B: Initial nutrient concentrations in the enriched treatment groups.

A. Nutrients	**Concentration in Estuarine Water Samples**
NO_3	9.43 x 10^{-5} M
NH_4	1.97 x 10^{-4} M
SRP	1.60 X 10^{-6} M
B. Nutrients	**Concentration in Enriched Estuarine Samples**
MES	1.02 x 10^{-3} M
$MgSO_4$	2.03 x 10^{-4} M
KCl	4.02 x 10^{-5} M
NH_4Cl	5.01 x 10^{-5} M
$NaNO_3$	2.35 x 10^{-4} M
Na_2 b-glycerophosphate	1.00 x 10^{-5} M
H_3BO_3	1.29 x 10^{-5} M
Na_2SiO_3• 9 H_2O	4.93 x 10^{-5} M
$CaCl_2$• 2 H_20	6.76 x 10^{-4} M

Mircoscopy

A gridded Sedgwick-Rafter slide was used to measure 1-ml subsamples of water from each sample preserved with Lugol's solution at different magnifications (100-400x) on a Zeiss Axio Observer-A1 inverted microscope with epiflourescence capability (Zeiss). Prior to examination, each subsample was inverted three times and allowed to settle for 35 minutes, then loaded onto the Sedgewich-Rafter slide. Cells were categorized by the following major groups: cyanobacteria, diatoms, chlorophytes, dinaflagellates, and flagellates.

Nutrient Analysis:

Fifty ml of each water sample were vacuum-filtered through 0.45 μm membrane filters and analyzed for DRP (Method 365.1; USEPA, 1993), NO_3-N (Method 353.2; USEPA, 1993) and NH_4-N (Method 350.1; USEPA, 1993) within 24 hours on a Seal Analytical (Mequon, Wisconsin) AQ2+ discrete analyzer using standard colorimetric methods.

Statistical Analysis:

All statistical analysis was carried out using SigmaPlot 11.0 software (Systat Software Inc., San Jose, CA, USA). T-tests and ANOVA were used to evaluate the significance of individual differences with a probability threshold of 0.05.

Results

For all enriched atrazine treatments, overall phytoplankton biomass increased over the 10-day period, although each treatment exhibited a different pattern (Fig. 1). The Chl *a* levels increased slightly, but statistically significantly, for the (+) 5 ppb and (+) 50 ppb treatment from day 1 to day 4 ($p<0.05$, t-test). Chl *a* began to increase exponentially on day 6 for both of these treatments and peaked on day 9 at 134.5 ± 26.6 μg l^{-1} for the (+) 5 ppb atrazine treatment and began to decrease afterward. For the (+) 50 ppb atrazine treatment, the Chl *a* increase slowed down significantly between days 8 and 10 but continued to steadily increase reaching its peak at a lower level of 102.9 ± 25.7 μg l^{-1} on day 10. The (+) 200 ppb treatment Chl *a* levels were lower than the other two treatment groups. However, the (+) 200 treatment was only significantly lower than the (+) 5 ppb and the (+) control groups ($p<0.001$, T-test).

Essentially no increase in Chl *a* occurred for the (+) 200 ppb treatment until day 6. After the 6 day, the Chl *a* concentration slowly increased to 35.5 ± 4.6 μg l^{-1} on day 10. The control group exhibited an earlier exponential increase in Chl *a* than the (+) 5 ppb and (+) 50 ppb atrazine treatments, which began on day 4. Chl *a* levels continued to increase from day 3 to day 9, where the Chl *a* levels peaked at 122.9 ± 4.9 μg l^{-1}, and started to decline on day 10, similar to the (+) 5 ppb treatment.

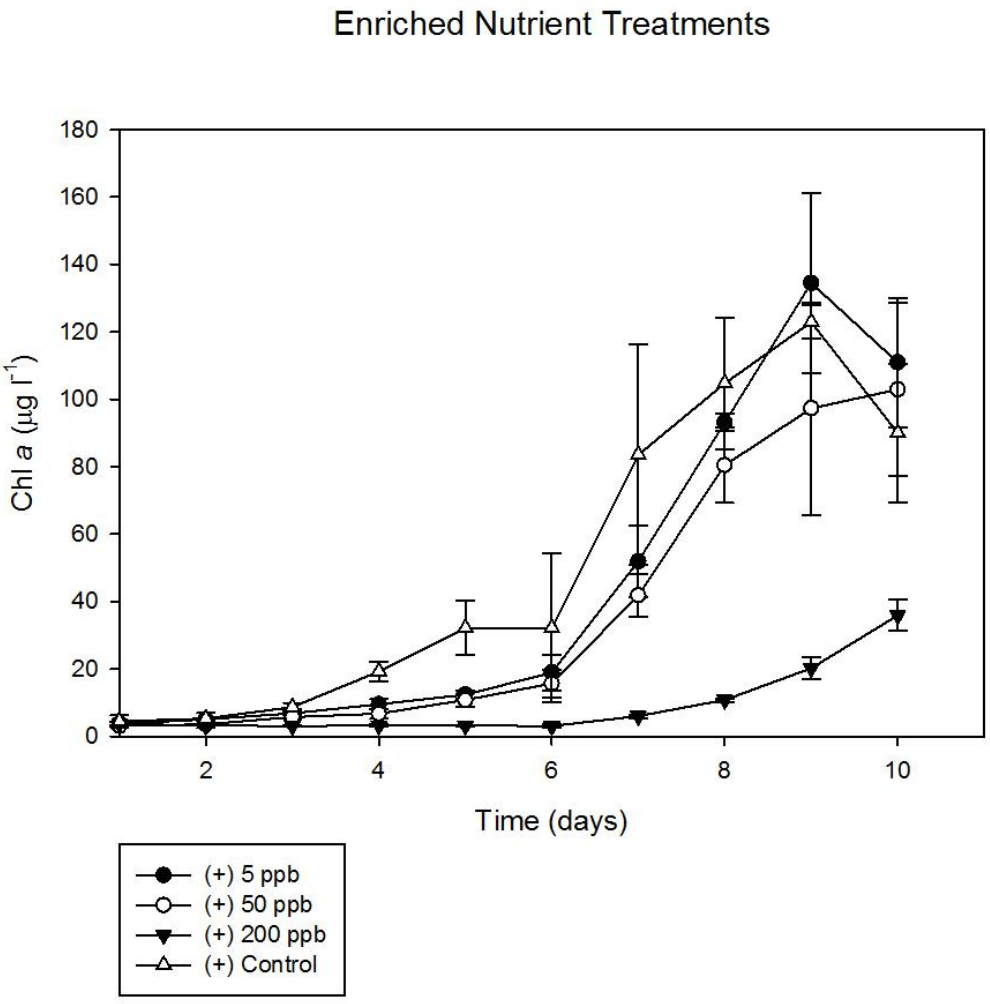


Figure 2: The chlorophyll *a* (μg l^{-1}) concentrations of the nutrient enriched (+) and control group (n = 3) with (+) 5 ppb, (+) 50 ppb, and (+) 200 ppb of atrazine.

Total biomass at the end of the 10-day period for the 5 ppb, 50 ppb, and 200 ppb treatment groups in nutrient enriched conditions were all significantly higher than their corresponding nutrient non-enriched treatment groups ($p<0.05$, T-test). The biomass in the enriched and non-enriched control groups were also found to be significantly different from each other ($p = 0.001$, T-test).

Similar to the enriched treatment groups, the only significant differences between the non-enriched groups were between the (-) 200 ppb and (-) 5ppb treatments and between the (-) 200 ppb and (-) Control ($p<0.001$, ANOVA). Chl *a* levels increased slowly between day 1 and day 5 for the (-) 5 ppb treatment group and exhibited an exponential growth pattern on day 6, similar to the enriched treatment group (Fig. 3). A significant increase in Chl *a* levels at 52.20 ± 10.1 μg l^{-1} occurred on day 7 ($p <0.05$, T-test), followed by a decline on day 8. The Chl *a* levels continued to steadily decrease from day 8 to day 10. The Chl *a* peak with a lower maximum and subsequent decline of (-) 5 ppb occurred two days earlier than the (+) 5 ppb treatment group. The (-) 50 ppb treatment group also exhibited an exponential growth pattern. The Chl *a* levels began to increase significantly on day 6, peaked at 39.7 ± 18.3 μg l^{-1} on day 9 and decreased between day 9 and day 10. The (-) 200 ppb treatment exhibited very little growth, increasing from 5.2 μg l^{-1} to 7.2 μg l^{-1} over the 10-day period. The Chl *a* concentrations of the non-enriched control increased exponentially, similar to nutrient enriched treatments throughout the experiment but the overall biomass was significantly lower than the enriched control ($p < 0.05$, ANOVA).

Over the course of the growth experiment, daily subsamples were also taken to determine qualitative community composition. During the first few days of the

experiment, chlorophytes, dinoflagellates, and cyanobacteria were the most observed groups. However, over the 10-day sampling period, the presence of cyanobacteria increased and the community appeared to shift more towards centric diatoms. Less chlorophytes and almost no dinoflagellates were observed in the final samples of day 10.

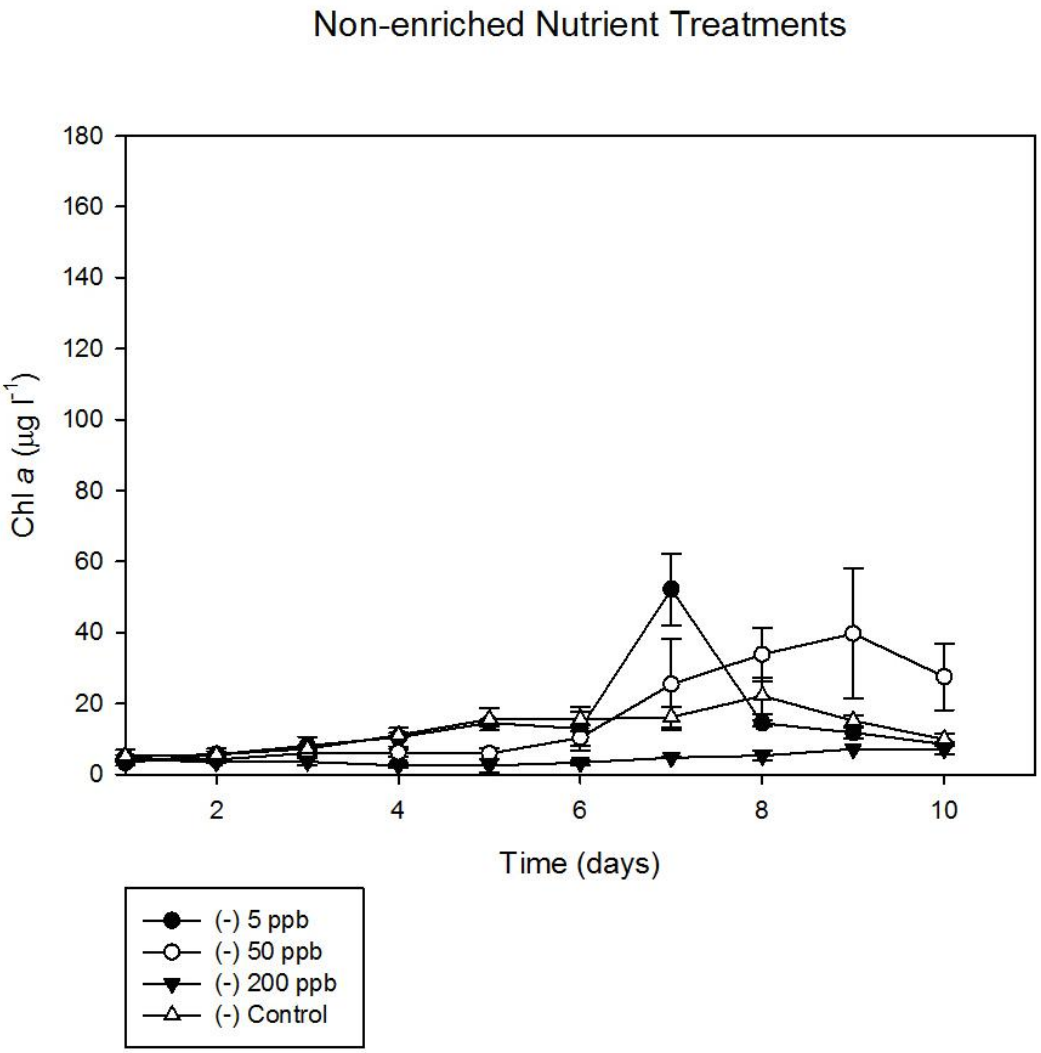


Figure 3: The chlorophyll *a* (Chl *a*, µg l^{-1}) concentration of the non-enriched (-) control group and replicates treated with 5 ppb, 50 ppb, and 200 ppb of atrazine. The measurements were taken over a ten-day period.

In oxygen production experiment, oxygen levels steadily increased in both nutrient enriched atrazine treatments over the time period sampled. For each treatment, oxygen levels were measured at the highest concentration on day 10. The (+) 10 ppb treatment group exhibited higher oxygen production over the time period measured, reaching 575 ± 356.4 µmol l^{-1}, while the (+) 100 ppb treatment had lower measured oxygen levels with maximum of 351.7 ± 158.1 µmol l^{-1}. The (+) control group oxygen levels fell between the two atrazine treatment groups, reaching a maximum of 437.15 ± 30.5 µmol l^{-1}at day 10. The oxygen concentrations of the control and the (+) 10 ppb treatments began to increase significantly on day 5, while the (+) 100 ppb began to increase on day 7.

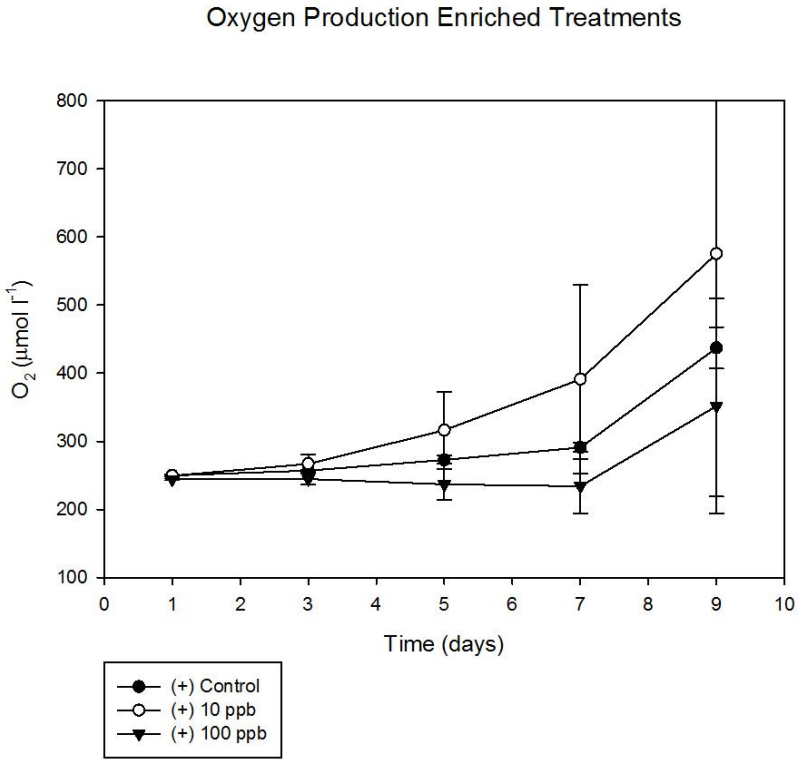


Figure 4: The oxygen production (µmol l^{-1}) of enriched (+) replicates. Sample treatments were designated as control with no atrazine addition, 10 ppb, and 100 ppb atrazine. Oxygen levels were recorded every other day over a ten day period.

Overall, all non-enriched atrazine treatments increased linearly over time, similar to the enriched treatments but at a lower rate. In the non-enriched group, the control exhibited the highest daily oxygen levels over the 10 day period, reaching 371.7 ± 18.9 µmol l^{-1} at day 10. The (-) 10 ppb treatment produced the second highest daily oxygen levels while the (-) 100 ppb treatment produced the lowest. The measured oxygen levels at day 10 for the (-) 10 ppb and the (-) 100 ppb treatments were 311.5 ± 17.5 µmol l^{-1} and 237 ± 0.07 µmol l^{-1}, respectively.

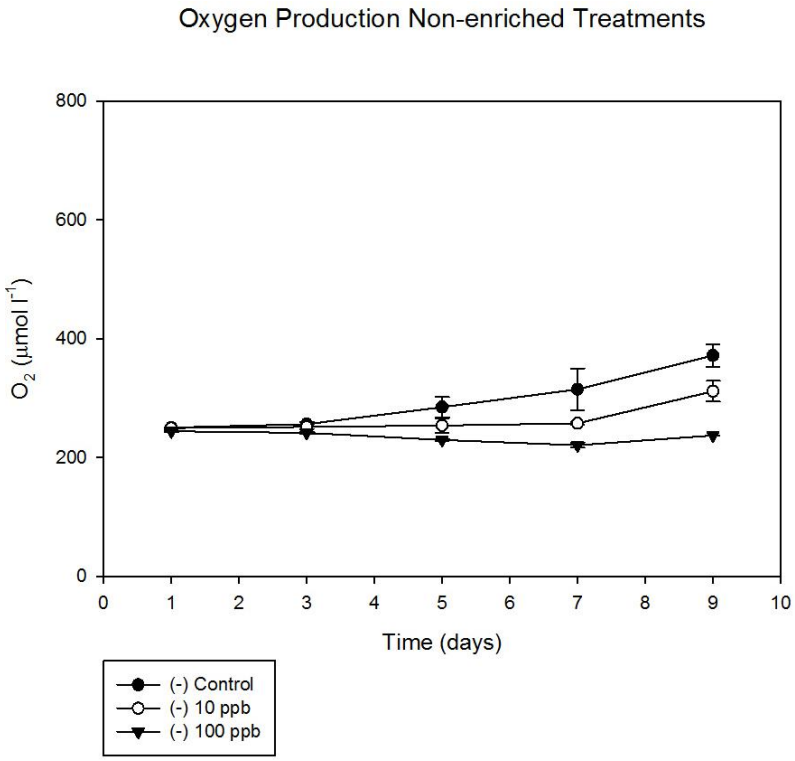


Figure 5: The oxygen production (µmol $l^{-1)}$ of non-enriched (-) replicates. Sample treatments were designated as no (control), 10 ppb, and 100 ppb atrazine. Oxygen levels were recorded every other day over a ten-day period.

Discussion

Many estuarine systems with a high proportion of agricultural land use have been subjected to increasing herbicide loading in recent years. These loading events have the potential to adversely affect aquatic primary producers such as phytoplankton. The phytoplankton community of Barataria Estuary, which is comprised largely of agricultural lands and receives input from the Mississippi River, was used to determine the effects of atrazine on Louisiana native phytoplankton growth response and oxygen production. High (200 ppb), medium (50 ppb), and low (5ppb) atrazine dilution levels were used to determine the phytoplankton growth response while low (10 ppb) and high (100 ppb) atrazine treatments were used in the oxygen production experiment. The atrazine treatment concentrations used were higher than the atrazine levels measured in Breton Sound and Barataria Estuaries in the late spring and summer of 2014, as described in the previous chapter. While the low atrazine treatments exceeded EPA maximum containment level of 3 ppb and the collected field data, they were lower than several atrazine concentrations previously measured throughout water bodies in Louisiana. In March and April of 2001, atrazine levels have been detected at concentrations as high as 15.1 ppb and 21.3 ppb in Big Creek, Louisiana, a subsegment of the Mississippi River (USEPA, 2002). Several water bodies within the Upper Terrebonne Basin were found to have chronically elevated levels of atrazine exceeding 12 ppb (USEPA, 2002). The mid (50 ppb) atrazine treatment reflects a worst case scenario, which may occur temporarily in small tributaries adjacent to agricultural lands as a result of large atrazine inputs due to runoff associated with heavy rainfall. The high atrazine treatments are somewhat unrealistic but have been used in other studies to determine

the median effective concentration (EC_{50}) of specific phytoplankton groups (Stratton, 1984; Solomon et al., 1995; Pennington and Scott, 2001). Much of the published data regarding the effect of atrazine on phytoplankton relates to the EC_{50} of specific species and data on mixed communities is limited. Therefore, these high concentrations were used to determine if a mixed phytoplankton communities reacted similarly to individual species when exposed to high atrazine levels. These levels were also used to determine if there was a significant difference between low, medium, and high atrazine treatments on growth response.

Because loading events, which bring high concentrations of atrazine to estuaries, also bring growth stimulating nutrients, the atrazine treatment groups were further divided into nutrient enriched and non-enriched treatment groups. The results show that there is a complex relationship between atrazine concentrations and nutrients. All enriched atrazine treatment groups grew significantly higher than their non-enriched counterparts over the 10-day period. Both the enriched and non-enriched atrazine treatment groups experienced a significantly longer lag phase than control groups. This may be due to the fact that phytoplankton exposed to atrazine have been known to experience diminished photosynthetic capability, cell growth, chlorophyll synthesis, and nitrogen synthesis (Hoagland et al., 1996). Overall, the enriched treatments exhibited more significant exponential growth phases and longer stationary phases than the non-enriched treatment groups. The phytoplankton growth was stunted for both the (+) 200 ppb and (-) 200 ppb treatment groups. However, at the end of the 10 day period, the (+) 200 ppb treatment group appeared to be exiting the lag phase began to increase, while the (-) 200 ppb treatment group remained

relatively constant. This indicates that the level of nutrients available in the system may play a role in phytoplankton tolerance to atrazine. All atrazine treatment groups with nutrient additions exhibited higher growth response and oxygen production then those in low nutrient conditions.

The oxygen concentration was used as a proxy for photosynthesis in the oxygen production experiment. Because phytoplankton community composition and size range were not determined quantitatively, the exact photosynthetic rate could not be determined, as different phytoplankton species produce various amounts of oxygen during photosynthesis and exhibit different photosynthetic rates (Gerber and Hader, 1995). However, because oxygen is a product of photosynthesis, it was assumed that oxygen levels measured would be proportional to the photosynthetic rate of the phytoplankton community. Oxygen production of estuarine phytoplankton have been found to range from 0.2 – 2,000 μmol l^{-1}day and is dependent on light conditions, depth, season, and other factors (Williams et al., 1979; Gazeau et al., 2007). The oxygen production of both the enriched and non-enriched treatment groups fell within this range. The dissolved oxygen and Chl *a* levels of the low (r=0.991), medium (r=0.887), and control (r=0.895) nutrient enriched treatment groups were strongly correlated, while there was no significant correlation in dissolved oxygen and the Chl *a* levels between the low (r=0.073), and medium (r=0.334) in non-enriched treatment groups (Pearson's Correlation Coefficient) The dissolved oxygen and Chl *a* concentrations for the non-enriched control group were, however, strongly correlated (r=0.749). Phytoplankton growth and productivity rely on a variety of factors including light, pH, temperature, and nutrient availability. Due to the complex nature

of phytoplankton, if one of these factors is perturbed, they may become stressed and the overall health of the community may be negatively affected. Chl *a* concentrations, as an indication of biomass, and the oxygen production of the phytoplankton community were used as indicators for their stress response to atrazine exposure and were expected to correlate. Atrazine inhibits photosynthesis by blocking electron transfer to PSI by binding to the second electron acceptor located in PSII (Steinback, 1981). As a result, it was hypothesized that oxygen production would be inversely proportional to atrazine exposure. It was also predicted that oxygen production would be higher in the nutrient enriched treatment groups due to the increase biomass. The strong correlation between the Chl *a* and dissolved oxygen levels of the nutrient enriched treatment groups suggests that these communities were only stressed by atrazine exposure, and were otherwise healthy. However, the non-enriched atrazine treatment groups did not exhibit a correlation between Chl *a* and dissolved oxygen concentrations, indicating that those communities were experiencing stress brought on by both the addition of atrazine and lack of sufficient nutrients. Overall, the oxygen production experiment conformed to these predictions as enriched treatment groups produced significantly more oxygen than their corresponding non-enriched treatment groups ($p<0.05$).

For the non-enriched groups, the control exhibited the highest oxygen production, while the (-) 100 ppb treatment group exhibited the lowest, as expected. However, for the enriched treatments, the (+) 10 ppb group exhibited the highest O_2 production, the (+) 100 ppb treatment group exhibited the lowest, and the control fell between the two. This unexpected result may be due to unseen factors such a slightly

different initial community composition, or a shift in the phytoplankton community composition. The low concentration (+10 ppb) of atrazine may have caused certain phytoplankton species, which were more resistant to atrazine, to out compete more susceptible species. This would cause a shift in the structure of the community and may affect the amount of oxygen produced over the 10-day period.

Over the course of the experiments, there seemed to be a slight shift in the composition of phytoplankton communities exposed to atrazine. As time went on, the community appeared to shift from chlorophyte and dinoflagellate dominated communities to communities dominated by cyanobacteria and centric diatoms. However, the exact cell numbers could not be quantified, but this trend is consistent with current literature. Studies have reported similar shifts in community composition and have indicated that diatoms are relatively atrazine resistant while chlorophytes are more susceptible (Pinckney et al., 2002). It is also known that diatoms and cyanobacteria exhibit a significantly lower percent of inhibition and a significantly higher EC_{50} than chlorophytes (Hoagland, 2008). Because only the overall trend in the community composition shift was obtained, more detail studies should be conducted to quantify the extent of community compositional shifts in Louisiana native phytoplankton species when they exposed to contaminants like atrazine.

Because atrazine is highly persistent in the water column and it was found to slow phytoplankton growth and inhibit oxygen production following acute exposures, determining the possible long-term risks to aquatic ecosystems would be very valuable within estuarine phytoplankton communities. A chronic atrazine exposure experiment would help determine if there is a significant impact to phytoplankton

growth response, oxygen production, and shift in community composition due to long term exposure. Chronic atrazine levels may cause the phytoplankton community to become more tolerant to atrazine exposure due to a shift to more resistant species. Phytoplankton form the base of the food web and are essential to ecosystem functions. A slight shift in phytoplankton community composition may affect trophic dynamics, energy transfer, nutrient cycles and species richness, leading to a less productive ecosystem overall.

References

Alavanja, M. 2009.Pesticides Use and Exposure Extensive Worldwide. Environ Health. 24(4): 303-309.

Alavanja M.C.R., Bonner, M. R. 2005. Pesticides and Human Cancers. Cancer Investigations;23:700–711.

Cunningham, J., Kemp, W., Lewis, M., Stevenson, J. 1984. Temporal responses of the macrophyte, *Potamogeton perfoliatus L*., and its associated autotrophic community to atrazine exposure in estuarinemicrocosms. Estuaries 7, 519–530.

Deneer, J., 2000. Toxicity of mixtures of pesticides in aquatic systems. Pest Management Science 56(6): 516-520.

Fenner-Crisp, P.E. 2001. Risk-assessment and risk management: the regulatory process. In: Kreiger, R., editor. Handbook of Pesticide Toxicology. 2nd Ed. Academic; San Diego. 681-690.

Fishel, F.M. 2005. Pesticide Toxicity Profiles. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Gazeau, F., Middleburg, J., Loijens, M.M., Vanderborght, J., Pizay, M., Gattuso. 2007. Planktonic primary production in estuaries: comparison of ^{14}C, O_2, and ^{18}O methods. Aquatic Micro. Eco. 46: 95-106.

Gerber, S., Hader, D. 1995. Effects of enhanced solar irradiation on chlorophyll fluorescence and photosynthetic oxygen production of five species of phytoplankton. Microecology 16 (1): 33-41.

Glotfelty, D., Taylor, A., Isensee, A., Jersey, J., Glen, S. 1984. Atrazine and simazine movement to Wye River estuary. Environ. Qual. 13: 115–121.

Graymore, M., Stagnitti, F., Allinson, G. 2001. Impacts of atrazine in aquatic ecosystems. Environ. Intern. 26(7-8): 483-95.

Hoagland, K., Carder, J., Spawn, R. 1996. Effects of organic toxic substances. In: Stevenson, R., Bothwell, M., Lowe, R. (Eds.), Algal Ecology: Freshwater Benthic Ecosystems. Academic, San Diego. 469–496.

Howard, P. 1991. Handbook of environmental fate and exposure data for organic chemicals, vol. 3. Lewis, Chelsea, MI.

Hyfield, E.C.G.; Day, J.W.; Cable, J.E., Justic, D., 2008. The impacts of re-introducing Mississippi River water on the hydrologic budget and nutrient inputs of a deltaic estuary. Ecological Engineering, 32: 347–359.

UPAC. 1997. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford . XML on-line corrected version: http://goldbook.iupac.org (2006) created by M. Nic, J. Jirat, B. Kosata; updates compiled by A. Jenkins. ISBN 0-9678550-9-8.

Isensee, A., 1987. Persistence and movement of atrazine in a salt marsh sediment microecosystem. Bull.Environ. Cont. Tox. 39: 516–523.

Kemp, W.M., Boynton, W.R., Cunningham, J.J, Stevenson, J.C., Jones, T.W., Means, J.C. 1985. Effects of atrazine and linuron on photosynthesis and growth of the macrophyte, *Potamogeton perfoliatus L*. and *Myriophyllum spicatum L*. in an estuarine environment. Marine Environ. Res. 16: 25-280.

Jones, T., Kemp, W., Stevenson, J., Means, J. 1982. Degradation of atrazine in estuarine water/sediment systems and soils. Environ. Qual. 11: 632–638.

Lakshminarayana, J.S.S, O'Neill, H.J., Jonnavithula, S.D., Leger, D.A., Milburn, P.H.1992. Impact of atrazine-bearing agricultural tile drainage discharge on planktonic drift of a natural stream. Environ. Poll., 76: 201–210.

Larramendy, M., Soloneski, S., 2014. Pesticides: Toxic Aspects. Pesticides. InTech. DOI: 10.5772/56979

Merriam-Webster, 2014. "pesticide." Merriam-Webster online. Web. 8 April 2015.

Nesheim, O.N., F.M. Fishel and M.A. Mossler. 2005. Toxicity of Pesticides UF/IFAS EDIS Document PI-13.

Parsons, T.R., Maita, Y., Lalli, C.M. 1984. A manual of chemical and biological methods for seawater analysis. 173:285-295.

Pereira, W.E., Rostad, C. E., Leiker, T. J. 1990. Distribution of agrochemicals in the lower Mississippi River and its tributaries. Sci. Tot. Environ. 97/98: 41-53.

Pennington, P., Scott, G. 2001.Toxicity of atrazine to the estuarine phytoplankter Pavlova sp. (Prymnesiophyceae): increased sensitivity after long-term, low-level population exposure. Environ. Tox. Chem. 20(10), 2237-42.

Pérez, G.L., Vera, M.S. and Miranda, L. 2011. Effects of Herbicide Glyphosate and Glyphosate-Based Formulations on Aquatic Ecosystems, Herbicides and Environment, Andreas Kortekamp (Ed.), ISBN: 978-953-307-476-4, InTech.

Pinckney, J. L., Ornolfsdottir, E. B., Lumsden, S.E. 2002. Estuarine phytoplankton group-specific responses to sublethal concentrations of the agricultural herbicide, atrazine. Marine Poll. Bull. 44: 1109-1116.

Radosevich, S.R., Holt, J.S., Ghersa, C.M., 2007. Ecology of Weeds and Invasive Plants: Relationship to Agriculture and Natural Resource Management, 3rd edition.Wiley-Interscience, Hoboken, USA.

Reigart, J.R., Roberts, J.R. 1999. Recognition and management of pesticide poisonings, 5th edition. United States Environmental Protection Agency Publication EPA-735-R-98-003.

Stratton, G.W. 1984. Effects of the herbicide atrazine and its degradation productions, alone in combination, on phototrophic microorganisms. Arch. Envirn. Contam. Toxicol. 13 :35-42.

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

Solomon, K, Baker, D.B., Richards, R.P., Dixon, K.R., Klaine, S.J., La Point, T.W., Kendall, R.J., Weisskopf, C.P., Giddings, J.M., Geisy, J.P., Hall. L.W., Williams, W.M. 1995. Ecological risk assessment of atrazine in North American surface waters. Environ. Tox. and Chem. 15: 31-76.

Steinback, K. E. 1981. Identification of the triazine receptor protein as a chloroplast gene product. Proceed. National Academy Sci. 78(12): 7463-46

Thronson, A.M. 2008. Effect of variation in freshwater inflow on phytoplankton productivity and community composition in Galveston Bay, p.75.Texas. Master of Science Thesis, Texas A&M University.

Turner, R. E., Rabalais, N. N. (1994). "Coastal eutrophication near the Mississippi river delta." Nature 368: 619-621.

U.S. Environmental Protection Agency. 1993. Method 350.1: Determination of Ammonia Nitrogen by Semi-automated Colorimetry. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory. Cincinnati, OH.

U.S. Environmental Protection Agency. 1993. Method 353.2: Determination of Nitrate-Nitrite Nitrogen by Semi-automated Colorimetry. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory. Cincinnati, OH.

U.S. Environmental Protection Agency. 1993. Method 365.2: Determination of Phosphorus by Semi-automated Colorimetry. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory. Cincinnati, OH.

U.S. Environmental Protection Agency. 1996. Drinking water regulations and health advisories. Office of Water, U.S. Environmental Protection Agency 822-B-96-002, Washington, D.C.

U.S. Environmental Protection Agency. 2002. Summary of atrazine in EPA Region 6 surface waters. U.S. Environmental Protection Agency Region 6. Dallas, TX.

U.S. Geological Survey. 1994. U.S. Geological Survey Open File Report. 94.376.

Virginia Cooperative Extension, 2009. Pesticides and Aquatic Animals: A Guide to Reducing Impacts on Aquatic Systems. Fisheries and Wildlife Services, Virginia Tech.

Whitacre, D. 2011. Reviews of Environmental Contamination and Toxicology. U&niversity of Florida, Springer Science and Business Media. Paron, AR. Vol.214.

Williams, P., Raine, R. Bryan, J. 1979. Agreement between the ^{14}C and oxygen methods of measuring phytoplankton production: reassessment of the photosynthetic quotient. Ocean. Acta 2(4): 411-416.

Wissel, B. and B. Fry. 2005. Tracing Mississippi River influences in estuarine food webs of coastal Louisiana. Oecologia 144: 659-672.

Chapter 4: Conclusion

In the United States, approximately 857 million lbs of conventional pesticide active ingredient were applied in 2007 and 80% of US pesticide use during this time was in agriculture (USEPA, 2011). There are several types of pesticides, including insecticides, rodenticides, herbicides, and fungicides, named for their target organisms. While pesticides have many beneficial uses, they can also adversely affect the environment. Pesticides can be transported to non-targeted areas through surface runoff, leaching, erosion, and through other mechanisms (Larramendy and Soloeski, 2014). Watersheds that contain a high proportion of agricultural land use are especially susceptible to pesticide contamination due to runoff (LDEQ, 1998). As a result, Louisiana's estuaries may be vulnerable to elevated pesticides levels, such as the herbicide atrazine. Atrazine is used both pre-emergence and post-emergence to control annual broadleaf and grass weeds in corn, sugarcane, and sorghum production (Solomon et al., 1995). Atrazine is used extensively in the Midwest for corn production and as a result, may enter the Mississippi River through runoff. The river then carries the chemical down stream where it is discharged into Louisiana's estuaries and eventually into the Gulf of Mexico. Atrazine may also indirectly enter Louisiana estuaries as a result of the sugarcane industry located in the south eastern part of the state through surface runoff brought on by rainfall and storm events. Elevated atrazine levels may negatively impact local estuarine organisms, specifically, phytoplankton since atrazine is known to inhibit photosynthesis. The phytoplankton response to atrazine exposure at various concentrations can be especially important to higher trophic levels since their growth and abundance can determine the potential productivity of the entire ecosystem (Wissel and Fry, 2005).

The purpose of this study was to determine the extent of atrazine present in Louisiana estuaries due to agricultural runoff under different flow and nutrient regimes (Spring and Summer) and its effect on the growth response and oxygen production of the local phytoplankton community. Atrazine levels were measured in Breton Sound Estuary for the months of May, June, and August and in Barataria Estuary during June and August. Local phytoplankton were also collected from Barataria Estuary and grown in microcosm and exposed to an atrazine dilution series. The dilution series was designed to mimic peak atrazine levels that have occurred in many tributaries, lakes, and other water bodies throughout the United States during the spring.

Atrazine was consistently measured in Breton Sound and Barataria Estuaries over the months sampled. However, these levels were found to be significantly below the maximum contaminant level of 3 ppb set by EPA (USEPA, 2002) and the lowest atrazine treatments of 5 and 10 ppb used in the growth and oxygen production experiments. Acute atrazine levels in surface waters tend to peak in March and April due to the time of application and increased rainfall. The field samples used on this study were collected later in the year during May, June, and August of 2014. This suggests that the atrazine levels measured in this study were not indicative of peak concentrations as there was more time for the chemical to become diluted, degrade, adsorb, and be taken up by aquatic organisms. As a result, the Louisiana phytoplankton may be exposed to higher atrazine levels in March and April than the months sampled, which may potentially impact the phytoplankton community and the ecosystem as a whole during that time. Field samples were taken in large water

bodies where atrazine could become easily diluted. Louisiana streams and tributaries have consistently exhibited atrazine levels higher than EPA's maximum contaminant level (USEPA, 2002). As a result, phytoplankton communities located in these smaller water bodies may be more susceptible to the chemical as it is less likely to become diluted.

Based on the low atrazine concentration and high nutrient availability in both Breton Sound and Barataria Estuaries, it is likely that the native phytoplankton community would be able to recover from acute atrazine exposure at levels found in field samples. The results of the growth response and oxygen production experiments indicate that Louisiana phytoplankton could overcome low (5 ppb) and medium (50 ppb) atrazine exposure in high nutrient conditions. Under these treatments, the community experienced an extended lag phase, and entered the exponential phase several days after the control groups. As a result, these low acute levels present in the estuaries may only slightly delay phytoplankton blooms. However, due to the persistence of atrazine in the environment, it is likely that aquatic organisms are susceptible to chronic atrazine exposure in these estuaries. Chronic atrazine exposure at low levels may have a different effect than acute influxes on the phytoplankton community. Because phytoplankton are so sensitive to environmental factors, it is likely that the chronic presence of atrazine, even at low levels, may impact the community composition, as the phytoplankton are unable to properly acclimate. Over time, species may become more tolerant to atrazine due to chronic exposure. This may increase the chance of transferring the contaminant to higher trophic levels under acute exposure conditions. The native community may also experience a long-term

shift from more sensitive species, such as chlorophytes, to more resilient species, such as diatoms. This shift in composition has the potential to reduce species richness, alter food web dynamics, nutrient cycling, and energy flow between trophic levels.

As a result, further study should be conducted on field atrazine levels in Breton Sound and Barataria Estuaries as well as the response to Louisiana native phytoplankton to chronic atrazine exposure. Native phytoplankton communities should be used in further experimentation to determine the growth response, oxygen production, and extent of any community composition shifts associated with chronic atrazine exposure. Atrazine levels should also be monitored year round in these estuaries to determine the timing of peak acute exposure in estuarine systems and persistence in the environment to determine the extent of exposure in these systems.

References

Louisiana Department of Environmental Quality. 1998. 1998 Atrazine Activiteis for the Upper Terrebonne Basin. Office of Water Resources, Louisiana Department of Environmental Quality, Baton Rouge, La.

Solomon, K, Baker, D.B., Richards, R.P., Dixon, K.R., Klaine, S.J., La Point, T.W., Kendall, R.J., Weisskopf, C.P., Giddings, J.M., Geisy, J.P., Hall. L.W., Williams, W.M. 1995. Ecological risk assessment of atrazine in North American surface waters. Environ. Tox. and Chem. 15: 31-76.

U.S. Environmental Protection Agency. 2002. Summary of atrazine in EPA Region 6 surface waters. U.S. Environmental Protection Agency Region 6. Dallas, TX.

United States Environmental Protection Agency. 2011. Pesticides: Environmental Effects. Office of Pesticide Programs' Aquatic Life Benchmarks.

Wissel, B., Fry, B. 2005. Tracing Mississippi River influences in estuarine food webs of coastal Louisiana. Oecologia 144: 659-672.

Vita

Alexis Starr was born in Alexandria, Virginia in December of 1990. After graduating from West Potomac High School in 2009, she went on to attend Louisiana State University. She received her bachelor's degree in Coastal Environmental Science from the School of the Coast and Environment of Louisiana State University in May of 2013 and began graduate school in the fall of 2013. She started in the Environmental Science professional program, but switch to the thesis program after conducting summer research. She currently works for the Louisiana Department of Environmental Quality.