Effect of Herbicide Atrazine on Phytoplankton, Water Quality, and Ecosystem Functions in Louisiana Coastal Wetlands

Basic Information

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Problem and Research Objectives

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.

Principal Findings and Significance

Methodology:

Atrazine levels were measured in Breton Sound Estuary for the months of May, June, and August and in Barataria Estuary during June and August. The three points within each estuary were sampled at varying distances from the closest river diversion. Chlorophyll a, nutrients and environmental conditions were examined together with atrazine levels. 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.

Field 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. 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.

Experimental Setup for Laboratory Growth Experiments:

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 μE $m^{-1}s^{-1}$ 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). For oxygen production experiment, 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 sensitive of <0.5%, a detection limit of 0.05 μM and a negligible analyte consumption rate of 5-50x10^{-4} nmol hr^{-1}.

Results and Discussion:

Atrazine was consistently measured in Breton Sound and Barataria Estuaries over the months sampled (Table 1 and 2). 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.

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 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

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 (Figure 1,3). Under these treatments, the community experienced an extended lag phase, and entered the exponential phase several days after the control groups. 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 (Figure 1, 2, 3, and 4). 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.

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.

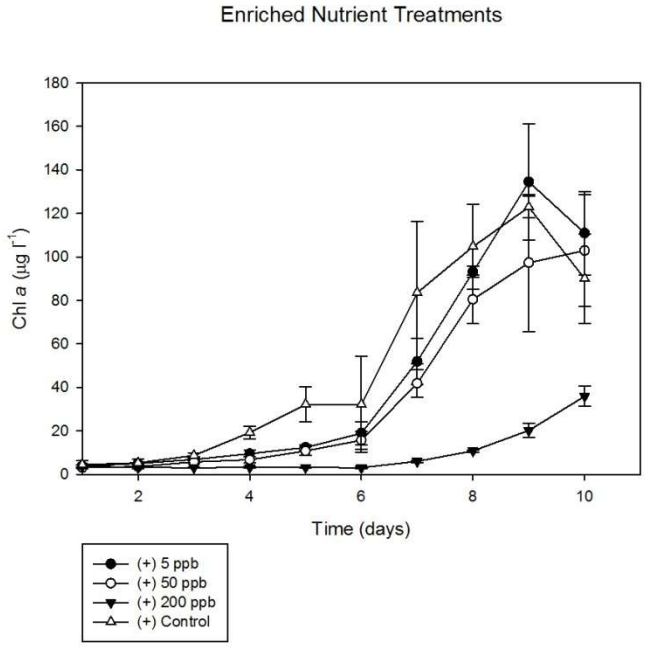

Figure 1: The chlorophyll *a* (µg l^{-1}) concentrations of the nutrient enriched (+) and control group (n = 3) with (+) 5 ppb, (+) 50 ppb, and (+) 200 ppb of atrazine.

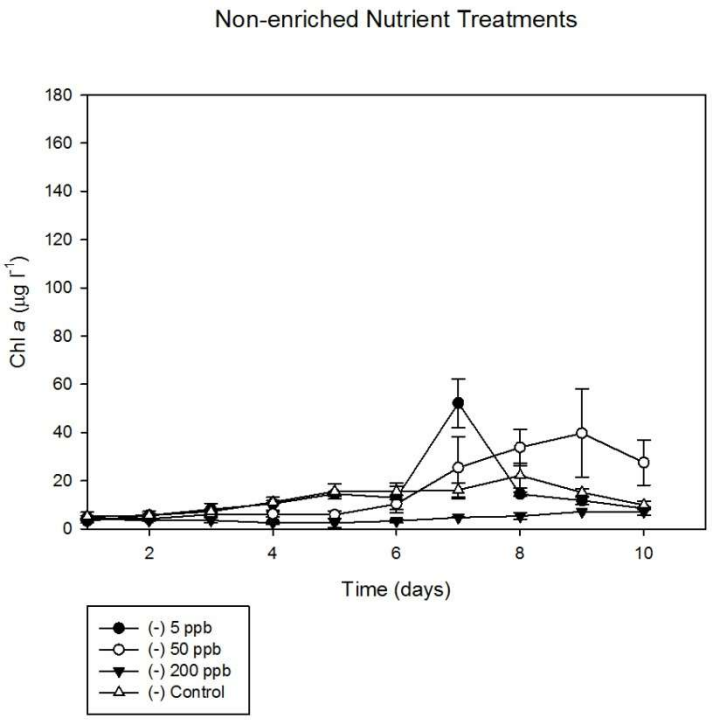

Figure 3: The chlorophyll *a* (Chl *a*, µg l^{-1}) concentration of the non-enriched (-) control group and replicates treated with 5 ppb, 50 ppb, and 200 ppb of atrazine. The measurements were taken over a ten-day period.

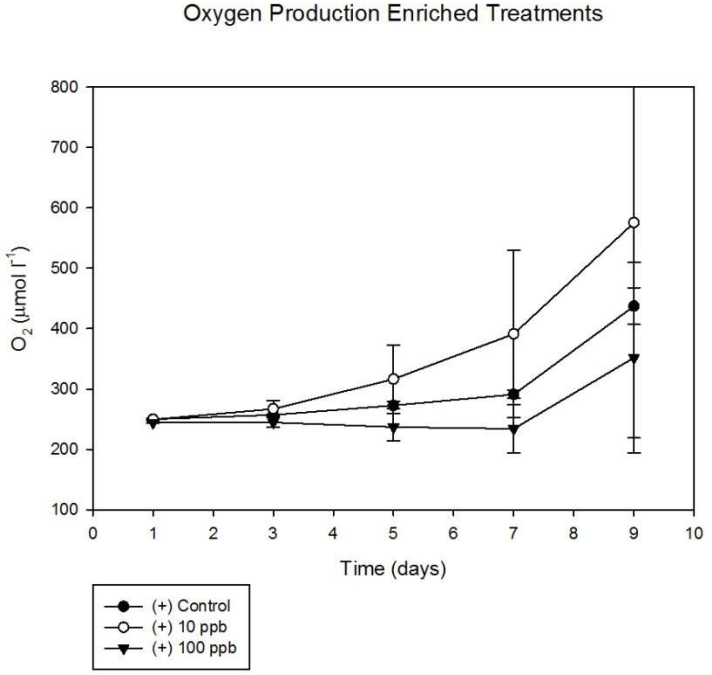

Figure 3: The oxygen production (µmol l^{-1}) of enriched (+) replicates. Sample treatments were designated as control with no atrazine addition, 10 ppb, and 100 ppb atrazine. Oxygen levels were recorded every other day over a ten day period.

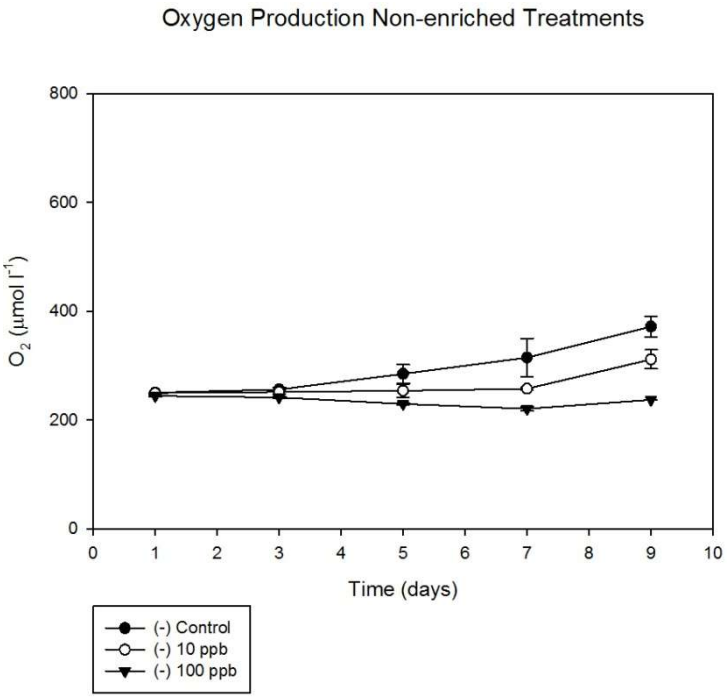

Figure 4: The oxygen production (µmol $l^{-1)}$ of non-enriched (-) replicates. Sample treatments were designated as no (control), 10 ppb, and 100 ppb atrazine. Oxygen levels were recorded every other day over a ten-day period.

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