Characterization of Rain and Stormwater Nitrogen Inputs to the Mississippi Sound: A Landscape Approach

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CHARACTERIZATION OF RAIN AND STORMWATER NITROGEN INPUTS TO THE MISSISSIPPI SOUND: A LANDSCAPE APPROACH

by

Joshua Michael Allen

A Thesis
Submitted to the Graduate School
of The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Master of Science

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August 2014
ABSTRACT
CHARACTERIZATION OF RAIN AND STORMWATER NITROGEN INPUTS TO THE MISSISSIPPI SOUND: A LANDSCAPE APPROACH

by Joshua Michael Allen

August 2014

Urbanization in coastal watersheds is becoming an increasingly important issue in the world. Increased impervious surface cover, a factor of urbanization, has drawn more attention to stormwater runoff as a source of contamination to receiving waters. In this study, nitrogen species from rain and stormwater were analyzed across three different landscape types along the Mississippi Sound (hardened, residential, and pristine), as well as from drainage pipes that flow directly into the Mississippi Sound. Nitrogen stable isotopes were used to trace the stormwater nitrogen to the surface waters and biota within the Sound.

The objective of this study was to determine the contribution of rainwater and stormwater to the nitrogen pool of Mississippi coastal waters, and to the biota present within those waters. All landscape types were found to have variable nitrogen sources in addition to rainwater nitrogen. Stormwater $^{15}$NH$_4$ values were always enriched relative to rainwater $^{15}$NH$_4$ values. Stormwater in pristine sites consistently had the lowest concentrations of ammonium, nitrate, and phosphate. Surface water nutrient concentrations were generally higher in the more developed areas compared to waters near the pristine landscape.
ACKNOWLEDGMENTS

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CHAPTER I

INTRODUCTION

Increasing urbanization has become a major issue for developed societies worldwide. The past century has shown a marked increase in population size, especially in urban areas, and there is no sign of it slowing down (Walsh 2000). According to the United Nations Department of Economic and Social Affairs (UNDESA) World Population Prospects 2011 revision, the population residing in urban areas in 2050 is likely to be the same as the total world population of 2002. In 2011, 78% of the world’s population resided in urban areas, which is expected to increase to 86% by 2050. In addition to this, the world population is also expected to increase by 72% from 2011 to 2050 (UNDESA 2011). These numbers suggest that all population increase during this time will be concentrated in urban areas, as well as movement from rural to urban areas. In order to support these population increases, the areal extent of developed urban environments has and will continue to increase as well.

Most urban centers are located near waterways and coastal areas, usually causing a decline in water quality of the receiving waters. Two of the prominent factors involved in degrading water quality due to urban land use are sewage and stormwater runoff (Walsh 2000). Increases in impervious surface cover of the land, a factor of urbanization, have recently brought more attention to stormwater runoff as a source of contamination to receiving waters. Stormwater can be a source of pathogenic bacterial contamination (Schiff and Kinney 2001) as well as a suite of other pollutants including organics, metals (Pitt et al. 1995; Foster et al. 2000), suspended solids, and dissolved nutrient species, such as phosphorus, and nitrogen (Chui 1997; Graves et al. 1998; Brezonik and
Rainwater dissolved inorganic nitrogen (DIN) and stable nitrogen isotope values have been characterized in many studies (Freyer 1978; Paerl and Fogel 1994; Russell et al. 1998; Xiao and Liu 2002), but much less is known about the dynamics of nitrogen within stormwater (Dillon and Chanton 2005).

In recent years, isotopes have proven useful in determining nitrogen sources in aquatic systems. The largest sources of allochthonous nitrogen to coastal systems include riverine sources, oceanic sources, wastewater effluent, groundwater seepage, atmospheric deposition, and stormwater runoff. Historically, eutrophication has been linked to large wastewater loadings; however, wastewater treatment technologies are becoming more advanced. Wastewater has been incorporated into municipal wastewater systems in most areas and has subsequently shown a reduction in wastewater nutrient loading in many developed areas (Greening et al. 1997; Dillon et al. 2007). In some cases of reduced wastewater nutrient loading, stormwater and atmospheric deposition have moved to the forefront as the largest sources of anthropogenic nutrient loading and have thus become the focus of management strategies to minimize their impacts on eutrophication of coastal waters.

Stormwater nutrient concentrations can be quite variable over time due to various climatic influences such as rainfall intensity and duration, the period of dry weather before a rain event (antecedent dry weather period), and the controversial first flush phenomenon of initial stormwater runoff (Gupta and Saul 1996; Bertrand-Krajewski et al. 1998; Lee et al. 2002). As mentioned above, increased impervious surfaces also play a major role in stormwater nutrient loading. When rain falls onto soft, undeveloped surfaces, it can be used by plants or infiltrate the soil. This lessens the potential for
stormwater to reach receiving waters, as the runoff does not become severe until the infiltration capacity is overcome. In developed areas, rooftops, sidewalks, and streets increase the impervious surface area, resulting in increased runoff in a shorter time period (USEPA 1983).

As the watershed of the Mississippi Sound becomes more developed and populated, the resultant outcome is increased anthropogenic nutrient loading. In 2001, Bowen and Valiela used historic data to determine that the total nitrogen load delivered to the watershed of Waquoit Bay, Massachusetts has doubled in the last century. They noted that fertilizer application had reduced, thus reducing the load from NH$_4^+$, but NO$_x$ load had increased due to fossil fuel emissions, a factor driven by urbanization (Bowen and Valiela 2001). Generally, increased nutrient loading in urban areas can be due to many processes including atmospheric deposition, stormwater runoff, sewage disposal practices, and groundwater seepage, but fertilizer applications and farming practices in rural and suburban areas can also be major contributors (Greening et al. 1997).

Identifying and quantifying these individual sources has proven to be a difficult endeavor due to the fact that many processes work in tandem with one another. However, stable isotopes have been shown to be excellent natural tracers for the various chemical and physical processes that occur in natural systems. If nitrogen sources have distinct isotopic signatures, one could use mass balance calculations to determine the proportion of nitrogen in a system from each source. Therefore, sources of nutrients and organic matter could be delineated by their distinct isotopic ratios.

In order for these sorts of analyses to be possible, it is important to adequately characterize each nitrogen source, otherwise the dependability of mass balance
calculations can be diminished due to overlapping isotopic values from multiple sources. Wastewater nitrogen is typically increased in the heavy isotope, $^{15}$N ($\delta^{15}$N $\sim$ +10 to +25 per mil), due to coupled nitrification/denitrification reactions used in the treatment process (Cifuentes et al. 1988; Desimone and Howes 1996; Dillon et al. 2007). Groundwater nitrogen is also typically elevated in the heavy isotope due to coupled nitrification/denitrification reactions, and can also be enriched due to wastewater contamination from septic tanks, sprayfields, and shallow sewage disposal wells. As the majority of this elevated $^{15}$N is due to natural processes, the isotopic values are generally not quite as high as those of wastewater. Though hypoxic and anoxic conditions could potentially introduce a confounding influence on nitrification/denitrification reactions, these events are uncommon and short-lived in the Mississippi Sound and should be of little concern (Rabalais 1992).

The isotopic values of rainwater nitrate (NO$_3^-$) and ammonium (NH$_4^+$) are typically light, ranging from -12 to 0 per mil (Paerl and Fogel 1994; Dillon and Chanton 2005), but occasionally reach as high as +8 per mil (Russell et al. 1998). As rainwater transitions to stormwater in developed areas, the flow over heated impervious surfaces causes loss of NH$_4^+$ due to volatilization of gaseous ammonia (NH$_3$). The lighter nitrogen isotope, $^{14}$N, is preferentially volatilized over the heavier $^{15}$N for thermodynamic reasons, resulting in isotopic enrichment of the remaining NH$_4^+$ pool. For example, in a highly developed watershed in Southwest Florida, decreases in stormwater NH$_4^+$ concentrations as great as 50% and enrichment in $\delta^{15}$N as high as +24 per mil compared to rainwater $\delta^{15}$N have been consistently observed (Dillon and Chanton 2005). During dry periods, large amounts of nitrous oxides (NO$_x$) and phosphates (PO$_4$) tend to
accumulate on surfaces from dry deposition (Bergametti et al. 1992; Gupta and Saul 1996; Burian et al. 2001). Entrainment of NO\textsubscript{x} into stormwater on impervious surfaces results in higher concentrations of NO\textsubscript{3}\textsuperscript{−} compared to rainwater. In a 2005 study by Dillon and Chanton, the majority of the rain events sampled showed an increase in stormwater NO\textsubscript{3}\textsuperscript{−} concentration relative to rainwater. The increase was rapid and larger than the decrease seen in NH\textsubscript{4}\textsuperscript{+} concentration, suggesting there was an additional NO\textsubscript{3}\textsuperscript{−} source as opposed to nitrification of NH\textsubscript{4}\textsuperscript{+}. The entrainment of NO\textsubscript{x} usually results in enrichment of \(\delta^{15}\text{N}\); however, these changes are much more subtle than those of NH\textsubscript{4}\textsuperscript{+} (Dillon and Chanton 2005).

Although nitrogen is historically considered to be the limiting nutrient in estuaries and coastal oceans, phosphorus is also an essential contributor to eutrophication and in some cases may limit primary production in coastal environments (Howarth 1988; Carpenter et al. 1998). Phosphorus is generally less abundant than nitrogen in precipitation; however, stormwater runoff is considered to be a major contributor of phosphorus to receiving waters (Hatt et al. 2004). PO\textsubscript{4} concentration increases as rainwater transitions to flowing stormwater due to entrainment and dissolution of particles, like that of NO\textsubscript{x}. In 2008, Dillon and Chanton saw an increase from rainwater PO\textsubscript{4} concentration from >2 \(\mu\text{M}\) to 4-12 \(\mu\text{M}\) in stormwater. Sources of phosphorus in the atmosphere include fine particles from rock and soil, living and dead organisms, as well as volatile compounds released from plants, the burning of fossil fuels, and fires (Newman 1995).

It has been shown that biolimiting nutrients can have very rapid turnover rates (>10 minutes) in the water column (Suttle and Harrison 1988; Suttle et al. 1990) and are
thus poor indicators of the trophic state of an ecosystem (Smith et al. 1981; Valiela et al. 1990). In the Mississippi Sound, dissolved inorganic nitrogen ([DIN] = [NH$_4^+$] + [NO$_3^-$] + [NO$_2^-$]) concentrations in surface waters are generally near or below detection limit (Dillon, unpublished data) and are not typically useful in identifying non-point nitrogen sources. Since the limiting nutrients are rapidly incorporated into vegetative and algal biomass, $^{15}$N values of primary producers, and thus primary consumers, can be used as proxies for water column nitrogen.

In cases of nitrogen loading to receiving waters, DIN is often studied while dissolved organic nitrogen (DON) is typically ignored (McClelland and Valiela 1998; Seitzinger et al. 2002; Dillon and Chanton 2005; Taylor et al. 2005). DON may contribute up to half of the total nitrogen (TN) load, although a large portion may be refractory (Antia et al. 1992; Taylor et al. 2005). DIN has the greatest impact on receiving waters because it is more readily available to primary producers and heterotrophic bacteria (Seitzinger et al. 2002). Despite this, an undetermined proportion of low-molecular weight compounds found in DON are expected to be biologically labile (Antia et al. 1992). Observing DON concentrations along with DIN concentrations in runoff may add further insight to implications of eutrophication.

In systems that are nitrogen limited, primary producers show little to no isotopic fractionation during uptake of DIN. In these cases, the incorporated DIN typically reflects the DIN assimilated during growth (Peterson and Fry 1987; Pennock et al. 1996; Derse et al. 2007). Macroalgae and phytoplankton depend on water column DIN in order to meet their nitrogen requirements for growth (Wallentus 1984). The nitrogen is rapidly assimilated to biomass in these organisms, and there is no evidence suggesting any
significant isotopic fractionation when DIN concentrations are low (Peterson and Fry 1987; Derse et al. 2007). Due to this lack of fractionation and sole use of DIN to meet nitrogen requirements, macroalgae and phytoplankton prove to be good indicators of short-term pulses of DIN into the system, such as those associated with stormwater.

Delineating nitrogen sources to a system was once a difficult task, but recently it has become increasingly common to do so by examining $\delta^{15}$N values in macroflora in order to trace nitrogen sources to coastal waters. In Australia, stable nitrogen isotopes of macroalgae were used to map sewage dispersal and impacts in coastal waters (Costanzo et al. 2001; Gartner et al. 2002). Corbett et al. (2000) have used seagrass and macroalgae nitrogen stable isotopes in conjunction with methane and radon concentrations in the water column of Florida Bay to evaluate areas with high rates of groundwater seepage to coastal waters. Stable nitrogen isotopes of mangroves and seagrass have also been used to determine sewage effluent dispersal (Udy and Dennison 1997; McClelland and Valiela 1998; Fry et al. 2000). Dillon and Chanton (2008) used stable nitrogen isotopes of seagrass and macroalgae to evaluate rainwater and stormwater inputs to Sarasota Bay, FL.

While the primary producers in a nitrogen limited system tend to reflect the isotopic composition of the DIN available for assimilation, primary consumers typically display an enrichment of $^{15}$N of about +3 to +5 per mil (Peterson and Fry 1987). The accepted average for $^{15}$N enrichment of vertebrate consumers relative to their prey is +3.4 per mil (Minigawa and Wada 1984; Peterson and Fry 1987; Cabana and Rasmussen 1994; Guzzo et al. 2011). A 2010 study by Fertig et al. showed $^{15}$N enrichment of +3 per mil in *Crassostrea virginica* tissue relative to seston in a tributary of the Chesapeake Bay,
a decline of 1 to 2.5 per mil from their pre-deployment values. Alternatively, Adams and Sterner (2000) concluded that trophic fractionation may be dependent on N availability, and when N is highly limited the $^{15}$N value of the consumer will be identical to that of the prey. Despite this, average trophic enrichment of $^{15}$N is fairly constant, and $^{15}$N values of a consumer with a known trophic level can be used to determine the nitrogen sources of its diet. It is hypothesized that oysters should prove to be very useful organisms to trace nitrogen sources in this study due to the fact that they are stationary filter feeders that utilize phytoplankton, zooplankton, and detrital material to satisfy nitrogen requirements, as well as the fact that they have the ability to thrive in Mississippi coastal waters. If the nitrogen sources delivered to the Sound have distinct isotopic signatures, stationary primary producers and consumers within the vicinity of each source will be able to be used to determine the extent to which this runoff affects the Sound.

DIN from rainwater and stormwater delivered to the Mississippi Sound will likely have distinct isotopic signatures, according to their sources. In this study, I characterized nutrient concentrations as well as the $^{15}$N values of NH$_4^+$ and NO$_3^-$ of rainwater and stormwater from several areas along the Mississippi Gulf Coast. Stormwater collection sites were chosen to be specific to varying levels of development along the coast. They span a land use/land cover gradient ranging from a natural undeveloped landscape type to areas with high development and increased hardened surfaces. Stormwater samples from larger drainage areas were collected from pipes that deliver stormwater directly to the Mississippi Sound. These samples are more characteristic of watershed level drainage and were used to more accurately describe stormwater that reaches the Sound.
I also sampled Mississippi Sound surface water and deployed biota (oysters, *C. virginica* and macroalgae, *Gracilaria*) along three transects and determined the nitrogen concentrations and $^{15}$N compositions following storm events. With this data, I can determine if rainwater and stormwater nitrogen is a major source of nitrogen for low trophic level organisms within the Sound. This data will be used to further evaluate the impacts of stormwater on coastal waters of the Mississippi Sound.

**Hypotheses and Objectives**

I hypothesized that the stormwater nutrient concentrations, as well as the $^{15}$N values of dissolved inorganic nitrogen (DIN), will vary across landscape types due to the differences in chemical and physical processes occurring during stormwater transport across the varying degrees of development and hardened surfaces. Furthermore, I tested the hypothesis that the more highly developed/hardened landscape type will have higher nitrogen loadings as compared to the other mixed land use areas. I also hypothesized that the stormwater from the hardened landscape type will have a larger zone of influence on the receiving waters of the Mississippi Sound due to the rapid delivery of nitrogen as a result of the lack of natural buffers, such as woodlands and marsh that exist in the undeveloped sites. In order to test these hypotheses, I measured concentration and isotopic values of various forms of nitrogen in stormwater, rainwater, and Mississippi Sound surface waters, as well as within deployed macroalgae and oyster samples placed along transects in the Mississippi Sound. The overall goal of this project was to determine the nitrogen contribution of rainwater and stormwater delivered to the nitrogen pool of Mississippi coastal waters, and to the biota found within those waters by completing the following objectives:
1. Measure nutrient concentrations and $^{15}\text{N}$ values of NH$_4^+$ and NO$_3^-$ from rain collected from two sites within the study area (Ocean Springs and Grand Bay National Estuarine Research Reserve) and from stormwater collected across three distinct landscape types along the Mississippi Gulf Coast (hardened, residential, and pristine), as well as stormwater from municipal drainage systems that flow directly to the Mississippi Sound (integrated).

2. Determine changes in nutrient concentrations and $^{15}\text{N}$ values of NH$_4^+$ and NO$_3^-$ in stormwater from three distinct landscape types, as well as from the integrated drainage pipes, as compared to rainwater.

3. Determine the fraction of stormwater delivered to the Sound which originates from each landscape type and how it reacts in surface waters—(i.e., is stormwater N removed quickly due to biological activity, or is it quickly advected into the Sound and changing nutrient dynamics some distance from shore?).

4. Determine if stormwater is a major N source for primary producers (Gracilaria macroalgae) or primary consumers (eastern oysters) in the Sound and, if so, determine the distance in which the stormwater N can be traced in the Sound.
CHAPTER II

METHODS

Study Area

The Mississippi Gulf Coast has varying degrees of development in the southern portion of Mississippi bordering the Mississippi Sound. The Sound is bordered by a barrier island chain, consisting of five islands that separate it from the Gulf of Mexico. Major freshwater inputs to the Sound include major rivers (Pearl and Pascagoula Rivers) and local freshwater inputs from tidal creeks. Rain and stormwater was collected from sites along the Mississippi Gulf Coast, and transects were established in the Mississippi Sound for surface water and biota sampling.

Rainwater was collected at two sites along the Mississippi Gulf Coast. The first was located at the Gulf Coast Research Lab (GCRL) in Ocean Springs and is designated as RCL. The second rainwater site was located at the Grand Bay National Estuarine Research Reserve (GBNERR) and is designated as RCGB (Figures 1 and 2).

Two levels of stormwater samples were examined. The first of these levels consisted of a gradient of very specific sub-watershed landscape types that are common to the Mississippi Gulf Coast: completely hardened areas (highly developed), semi-hardened areas (residential and moderate commercial development), and unhardened areas (undeveloped or pristine). Three sampling sites were located within each landscape type, with an additional residential site located near the developed sites in Biloxi. The stormwater sampling sites are shown in Figures 1 and 2 and listed below.
Figure 1. Map of Mississippi Sound and coastline. (Top) Stormwater and rainwater sampling sites broken down by landscape type. (Bottom) Raster image of the coast displaying developed areas in a red gradient.

Figure 2. Map layout of each study area separated with individual stormwater sites labeled.
1. Hardened landscape – downtown Biloxi site (SWH1), Mississippi Coast Coliseum site (SWH2), and Edgewater Mall site (SWH3).
2. Residential landscape – three sites in Ocean Springs residential areas (SWR1, SWR2, and SWR3), and one site in a Biloxi residential area (SWR4).
3. Pristine landscape – three sites in the GBNERR (SWP1, SWP2, and SWP3).

Sites were chosen based on their appearance to receive stormwater runoff from the landscape type of interest during storm events.

The second level of stormwater samples came from three pipes that drain directly into the Mississippi Sound. These sites are referred to as integrated stormwater (ISW) and were located in two sites on Biloxi Beach (ISW1 and ISW2), as well as one site in Ocean Springs on East Beach (ISW3) (Figures 1 and 2). This level of sampling provides stormwater that is more characteristic of large watersheds and longer evolution times, as opposed to the more discrete drainage boundaries described in the first level.

The sites for analysis of Mississippi Sound biota and surface water were located along three 1 km transects within the Sound. One transect was designated to each landscape type: hardened landscape in Biloxi, residential landscape in Ocean Springs, and pristine landscape in Grand Bay. Each transect was divided into three stations, for a total of nine stations overall. The designations are as follows: Biloxi – B1 (closest to shore), B2, and B3 (farthest from shore); Ocean Springs – OS1, OS2, and OS3; and Grand Bay – GB1, GB2, and GB3 (Figure 3).
Field Methods

Rainwater Sampling

Total monthly rainfall within the study area is fairly consistent with slightly increased amounts during the summer and lesser in the month of October (Table 1). The mean annual precipitation in the area is 165 cm per year, resulting from an average range of 70 to 90 thunderstorms per year (NOAA NWS). Due to this rate of consistency, we attempted to collect twelve *significant* storm events (>3 cm of rainfall) in each year of the two year study. Samples were collected monthly, when possible, in order to characterize the seasonal differences of the evolution of nitrogen species from rainwater and subsequent stormwater. The minimum parameter of 3 cm of rainfall to define a *significant* storm was chosen as it provides the volume of rainwater to each rain collector that is necessary to conduct the suite of chemical analyses presented herein. Although

*Figure 3.* Map layout of transect sample sites in each of the study areas. Biloxi – Hardened, Ocean Springs – Residential, Grand Bay – Pristine.
rainfall amounts are fairly constant throughout the year on average, storms along the Gulf Coast are also quite sporadic. Due to this nature, a large degree of flexibility existed in sampling times, although every effort was made in order to supply a good representation of seasonality that may exist within the rainfall and stormwater nitrogen concentration and isotopic composition. NOAA’s National Weather Service, the Weather Channel, and Weather Underground were all used in order to provide precipitation forecasts for the study period.

Table 1

*Historic Average Monthly and Annual Rainfall Totals for Biloxi, MS*

<table>
<thead>
<tr>
<th>Month</th>
<th>Rainfall (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>15.44</td>
</tr>
<tr>
<td>Feb</td>
<td>13.92</td>
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<tr>
<td>Mar</td>
<td>15.65</td>
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<td>Nov</td>
<td>12.29</td>
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<tr>
<td>Dec</td>
<td>12.42</td>
</tr>
<tr>
<td>Annual Mean</td>
<td>164.69</td>
</tr>
</tbody>
</table>

Note. Source – NOAA NWS.

Rainwater from each storm event sampled was collected in two mechanical rain collectors (Loda Electronics, model 2005) located at each of the rainwater sampling sites described above. Each rain collector is equipped with a sensor, triggered by rain drops,
that uncovers the collection bucket by a mechanical arm. Before each storm event, the collection buckets were acid washed and placed in each mechanical collector. When the rain ceased and the sensor dried, the bucket was covered again until it could be retrieved. Actual precipitation rates and weather conditions were obtained from on-site weather stations at both GCRL and GBNERR.

When the storm event was complete, the rain water was collected in 1L acid washed Nalgene polyethylene bottles, placed on ice, brought to the lab, and filtered through precombusted glass fiber filters (Whatman GF/F) housed in an acid washed 47mm glass filter holder (Millipore). The filtered rain water was collected in 30ml and 1L acid washed Nalgene polyethylene bottles, as well as muffled 22ml glass vials with septa tops, then frozen until nutrients (ammonium, NH$_4^+$; nitrate, NO$_3^-$; nitrite, NO$_2^-$; dissolved organic nitrogen, DON; and soluble reactive phosphorus, PO$_4^{3-}$) and the $^{15}$N composition of NH$_4^+$ and NO$_3^-$ could be measured as described below. The samples were filtered and frozen as soon as possible upon completion of the storm event. The rain temperature was inferred from the air temperature measured by the weather stations during the storm event.

*Stormwater Sampling*

Stormwater samples were collected directly from open and flowing stormwater drains or ditches located at each site. The hardened sites have distinct boundaries and engineered stormwater flow paths. Although these sites drain smaller areas than the sites within other landscape types, the boundaries offer a sampling advantage in that the stormwater collected is representative of stormwater that has only flowed over impervious surfaces. The ability to determine accurate stormwater flow paths is
decreased in the less developed sites due to the increasingly diffusive nature of stormwater drainage when transitioning from engineered areas to undeveloped ones. As previously stated, the residential and pristine sites appear to receive runoff primarily from the landscape type of interest (personal observation).

Samples were collected in duplicate 1L acid washed Nalgene polyethylene bottles directly from the flowing stormwater. Samples were then placed on ice and returned to the lab and filtered through precombusted glass fiber filters (Whatman GF/F) housed in an acid washed 47mm glass filter holder (Millipore). The filtered stormwater was transferred to 30ml and 1L acid washed Nalgene polyethylene bottles and to muffled 22ml glass vials with septa tops. The samples were then frozen until nutrients and $^{15}$N composition could be measured.

All hardened and residential sample sites are located within 12 miles of the rain collector at GCRL (RCL). Due to this proximity, these areas have a high probability of being impacted by the same storm event. This provided the ability to make comparisons between stormwater from these locations to the precipitation collected at GCRL. The GBNERR rain collector (RCGB) was used to compare the pristine stormwater sites to precipitation.

*Mississippi Sound Biota and Surface Water Sampling*

The transect study was limited to the 2011 sampling season. Six transect dates were sampled for the Biloxi and Ocean Springs sites, while four transect dates were sampled for the Grand Bay sites. Due to distance and time constraints, Grand Bay could not be sampled on the same day as Biloxi and Ocean Springs.
Surface water and biota samples collected at each station of the previously mentioned transects were analyzed in order to determine if stormwater nitrogen can be traced into the local food web and, if so, how far the nitrogen can be traced offshore. In order to accomplish this, surface water samples were collected in duplicate acid washed Nalgene polyethylene bottles, placed on ice, and returned to the lab to be filtered through precleaned glass fiber filters in an acid washed 47mm glass filter holder. The filtered surface water was then transferred to 30ml and 1L acid washed Nalgene polyethylene bottles and to muffled 22ml glass vials with septa tops. Living biota samples were also deployed at each station. The $^{15}$N composition of the biota was monitored throughout the study as described below. The samples were housed in flow-through substrate cages at each of the sites.

Substrate cages (Figure 4) were constructed using 6” schedule 40 PVC and 1cm mesh vinyl-coated steel hardware cloth. The hardware cloth was cut into sections measuring approximately 29cm x 50cm. Two 2 inch segments of the PVC were placed at either end of the hardware cloth, which was then wrapped around the PVC and zip-tied together to produce an open cylinder. The ends were capped by zip-tying hardware cloth that was cut to the diameter of the cylinder ends. An opening measuring approximately 12cm x 12cm was cut into the middle of the cage and zip-tied back into place, creating an access door. The cages were attached to concrete weights which were connected to a GCRL buoy to mark the location at the surface.
American oysters (*Crassostrea virginica*) were deployed in the substrate cages at each transect station monthly. Six oysters were placed in each cage and allowed to soak for two to four weeks and collected after a storm event. A subset of oysters from the same batch were sampled at the time of deployment to be measured for N content (%) and $^{15}$N composition (per mil). The oysters used for this study were obtained from the Auburn shellfish lab on Dauphin Island.

Red macroalgae (*Gracilaria sp.*) was also deployed at each station in clear plastic containers with holes in them to allow water to circulate. They were held near the surface by the buoy connected to each substrate cage, and were used to evaluate N assimilated by primary producers. The cages were collected after a five day soak period following a storm event as described by Costanzo et al. (2001). The sample was measured for N
content (%) and $^{15}$N composition (per mil). Red macroalgae samples were ordered from a vendor in Tampa, Florida, and kept in a tank filled with artificial seawater that was spiked with low levels of fertilizer as a nitrogen source. Time zero samples were measured for the original $^{15}$N composition before deployment.

Surface water samples were collected at each of the transect stations, as well as basic physical parameters (temperature, salinity, pH, and dissolved oxygen), at the time of retrieval. The samples were collected in 1L acid washed Nalgene polyethylene bottles through a pneumatic pump. The short transect lengths (1km) for this study were chosen based on a similar study in Sarasota Bay, where dissolved inorganic nitrogen (DIN) was either rapidly diluted or taken up by biologic activity within 1km from shore (Dillon and Chanton 2008).

**Laboratory Analysis**

**Analytical Methods**

All rainwater, stormwater, and surface water samples were analyzed for various nutrient concentrations in order to characterize all dissolved nitrogen forms present in each water type. $\text{NH}_4^+$ concentration was determined by the colorimetric method described by Bower and Holm-Hansen (1980). $\text{NO}_2$ and $\text{PO}_4$ were also measured colorimetrically (Strickland and Parsons 1972) while $\text{NO}_3^- + \text{NO}_2^-$ was measured with a Model 42i Thermo Scientific chemiluminescent NO$_x$ analyzer using the method described by Braman and Hendrix (1989). $\text{NO}_3^-$ concentrations were then determined as the difference in measurements between $\text{NO}_3^- + \text{NO}_2^-$ and $\text{NO}_2^-$. Total dissolved nitrogen (TDN) concentrations were measured from the 22ml glass vials using a Shimadzu TOC-V analyzer equipped with a Shimadzu TNM-1 total nitrogen measuring unit. DON
concentrations were calculated as the difference in concentrations of TDN and DIN 
([DON] = [TDN] – [DIN], where [DIN] = [NH₄⁺] + [NO₂⁻] + [NO₃⁻]).

All water samples with sufficient DIN concentrations (>2µM) were also analyzed for ¹⁵NH₄⁺ and ¹⁵NO₃⁻. Extraction of NH₄⁺ for this analysis was performed using the ammonium diffusion method described by Holmes et al. (1998). First, diffusion packets were constructed on an ethanol rinsed and dried sheet of aluminum foil placed on a few paper towels. Diffusion packets are composed of 35 µL of 4N H₂SO₄ on a 1 cm precombusted GF/D filter sealed between two acid washed Millipore 25 mm Teflon filters (10 µm pore size). A scintillation vial was used to seal the packet by placing it on top of the stacked filters and pressing while turning slightly. The paper towels beneath the foil provided padding, which allowed a seal to form between the Teflon filters.

The 1 L water samples for DI¹⁵N were thawed and brought to room temperature slowly in coolers to minimize volatilization of ammonium. After thawing, NaCl precombusted at 550ºC for four hours was added to each sample, if needed, to raise the salinity to 35. Addition of salt prevents the packets from bursting due to osmotic pressure differences (Holmes et al. 1998). The diffusion packets were then added to the sample and the pH was brought to 9.7 or greater by adding 300 mg of magnesium oxide (MgO) per 100 mL of sample. The caps were sealed immediately after addition of MgO to prevent loss of ammonia. The increase in pH converts NH₄⁺ to ammonia gas (NH₃) which is then scavenged as ammonia sulfate by the sulfuric acid within the diffusion packet.

The samples were then placed in a 40º C incubator shaker at 60 rotations per minute for 14 days. After the 14 day period, diffusion packets were removed from the bottles and rinsed with 10% HCl and DI water. They were then put into a dessicator for
two days with silica gel and an open container of concentrated sulfuric acid to remove any excess ammonia. The diffusion packets were then dried for an additional two to three days in a vacuum oven at 80°C. After drying, the packets were stored in scintillation vials placed in a sealed dessicator until analysis could be completed.

NO$_3^-$ was extracted from the samples using the modified diffusion method described by Sigman et al. (1997). This extraction method, when coupled with the ammonium diffusion method, has an analytical advantage, in that they can be performed on the same water sample sequentially. Once the ammonium had been extracted, the water samples were transferred to 1L acid washed glass bottles and evaporated to 20 – 50% of their original volume in a 95°C laboratory oven. Samples were then transferred to 500 mL acid washed bottles and a diffusion packet was added. Next, Devarda’s Alloy (75 mg per 100 mL initial sample volume) was added and the cap was sealed immediately. Devarda’s Alloy, which consists of 50% Cu, 45% Al, and 5% Zn, reduces NO$_3^-$ to NH$_4^+$. The samples were then placed in a 65°C oven for four days, which facilitates nitrate reduction and ammonium diffusion. After heating, the samples were placed on a 60 rpm shaker at room temperature for 14 days. Diffusion packets were then removed, rinsed with 10% HCl and DI water and stored as described above. After extraction of each nitrogen compound, $^{15}$N analysis was conducted using a Thermo Delta V Advantage stable isotope mass spectrometer coupled to a Costech elemental analyzer.

Oyster samples were shucked and the meat was rinsed with 10% HCl and deionized water. The shucked oysters were then frozen in a -80°C freezer and then freeze dried using a Labconco FreeZone 6 freeze dryer. Macroalgae samples were also rinsed with 10% HCl and deionized water, and then dried in a laboratory oven at 65°C. All dried
samples were then ground to a fine powder, placed in a centrifuge tube, rinsed with 10% HCl and three times with deionized water. After each rinse, the samples were shaken on a vortexer, centrifuged, and decanted. This cleaning technique causes no isotopic fractionation in the sample (Chanton and Lewis 2002). After the rinse cycles were completed, the samples were dried, reground, and analyzed for $^{15}$N values and percent N with a Thermo Delta V Advantage stable isotope mass spectrometer coupled to a Costech elemental analyzer.
CHAPTER III

RESULTS

Nutrient Composition and Nitrogen Isotopes

Rain and stormwater collection took place from November 30, 2010, to September 4, 2011, and again from November 27, 2012, to September 21, 2013. These will be referred to as the 2011 season and the 2013 season, respectively. The 2011 season yielded a total of 112.9 cm of precipitation in Ocean Springs, while the 2013 season brought a total of 118.5 cm of precipitation. In Grand Bay, precipitation totals were 113.5 cm for 2011 and 140.6 cm for 2013. Total precipitation for each collection season was similar, but the majority of the monthly totals differed greatly from 2011 to 2013, as seen in Figures 5 and 6.

Figure 5. Monthly precipitation for 2011 sampling year for Ocean Springs and Grand Bay (centimeters).
A total of 16 rain events were collected during the sampling seasons. Nine storms were collected in the 2011 season, and seven storms were collected during the 2013 season. Due to the somewhat unpredictable nature of rain events, many of the collection dates did not meet the 3 cm threshold we defined as a significant storm event. As a result of this, some sampling dates lacked enough collected rain volume for the full suite of analyses of rainwater samples. This also resulted in stormwater collection sites occasionally not flowing during collection attempts. As such, sample amounts did vary between some sites.

Ammonium

Rainwater ammonium concentrations (Figure 7) at the Ocean Springs GCRL site (RCL) during the study period varied from below detection (BD) to 39.20uM. At the Grand Bay site (RCGB), NH$_4^+$ concentrations varied from BD to 16.03uM. The high end of the range at RCL (39.20uM) did not have a corresponding collection from RCGB on
that date (1/5/11). The majority of 2011 samples and all of the 2013 samples had similar nutrient concentrations between the two rain collection sites. Rainwater NH$_4^+$ concentrations were highest from January to April in 2011. Similarly, the peak NH$_4^+$ concentration in 2013 occurred in February, but the values tended to be much higher in 2011.

![Figure 7. Rainwater NH$_4^+$ concentrations ± standard deviation for Ocean Springs (RCL) and Grand Bay (RCGB).](image)

Rainwater δ$^{15}$NH$_4^+$ values ranged from -8.09 per mil to 5.98 per mil at RCL and from -5.96 per mil to 0.61 per mil at RCGB (Figure 8). The lowest and highest values for each site occurred on the same two dates, 7/24/11 and 4/3/13 respectively. Other than one relatively enriched peak RCL δ$^{15}$NH$_4^+$ value, the rest of the ammonium nitrogen stable isotope values were within the range generally seen in rainwater samples (~ -10 per mil to +8 per mil) (Russel et al. 1998; Dillon and Chanton 2005). Although the δ$^{15}$NH$_4^+$ values at RCL and RCGB occasionally diverged from one another on corresponding dates (range of difference = 0.43 to 5.37 per mil), they followed similar trends over time.
Stormwater $\text{NH}_4^+$ concentrations were generally higher in the 2011 season compared to the 2013 season, as was the case with rainwater $\text{NH}_4^+$. In 2013, stormwater $\text{NH}_4^+$ concentrations were typically depleted with occasional slight increases relative to rainwater $\text{NH}_4^+$ concentrations. In 2011, some dates exhibited increased stormwater $\text{NH}_4^+$ concentrations relative to rainwater, suggesting a source of $\text{NH}_4^+$ in the area. Other
collection dates had stormwater NH$_4^+$ concentrations that were similar to those of rainwater. Increases in stormwater NH$_4^+$ concentrations over both study years were most pronounced in the integrated stormwater sites, with roughly half of all measured sites over the study period displaying some increase of NH$_4^+$ concentration relative to rainwater.

Figure 9 displays stormwater NH$_4^+$ concentrations for all sites within each landscape type. NH$_4^+$ concentrations in stormwater ranged from below detection in all landscape types to 65.52µM for the hardened landscape (SWH1), 68.05µM for the residential landscape type (SWR1), 12.99µM for the pristine landscape type (SWP3), and 54.30µM for the integrated stormwater pipes (ISW2). Both hardened and residential peak values occurred on May 26, 2011. The second highest pristine NH$_4^+$ concentration also occurred on that date, 12.65µM at site SWP2. Rainwater NH$_4^+$ concentrations were below detection at both sites on that date, but the antecedent dry weather period (ADWP) was the highest on that date relative to all other collection dates (30 days of dry weather previous to the storm event).

Interestingly, NH$_4^+$ loss from rainwater to stormwater seemed to be greater and more prevalent in cooler months as opposed to warmer ones. Peak loss within all sites occurred in January to April 2011 or February of 2013. Summer months exhibited little or no change generally, which is unexpected due to increased chance of volatilization with increasing temperatures of hardened surfaces (roads, parking lots, rooftops, etc.).
Figure 9. Stormwater NH$_4^+$ concentrations ± standard deviation for sites within (a) hardened, (b) residential, (c) pristine, and (d) integrated drainage pipes.

Figure 10 displays the difference in stormwater and rainwater NH$_4^+$ concentrations for each landscape type. Positive values represent an increase relative to rainwater, whereas negative values denote decreases. Many of the samples from May 26, 2011 represented the peak increase for each individual site. Overall, the majority of samples in both years decreased in NH$_4^+$ concentration relative to rainwater for sites...
within the hardened and pristine landscape types. Residential sites show more small increases in 2013 than in 2011. Integrated drainage sites tended to increase in NH$_4^+$ concentration relative to rainwater more often than the three landscape types.
Figure 10. Difference between stormwater and rainwater NH$_4^+$ concentrations (stormwater – rainwater) for (a) hardened, (b) residential, (c) pristine, and (d) integrated drainage pipes. Ocean Springs rainwater NH$_4^+$ concentrations used for hardened, residential, and integrated pipes and Grand Bay NH$_4^+$ concentrations used for pristine sites.

Stormwater $\delta^{15}$NH$_4^+$ values (Figure 11) were almost always enriched relative to rainwater values. While rainwater values were typically negative, the vast majority of the stormwater samples were positive values. Values ranged from -7.51 to 16.05 per mil at the hardened landscape type, -2.09 to 17.89 per mil at the residential landscape, -3.54 to
7.54 per mil at the pristine landscape, and -2.93 to 19.41 per mil at the integrated
stormwater drainage sites. Values above 10 per mil were most frequently observed in the
residential and integrated drainage sites. The hardened and pristine sites tended to have
the lowest $\delta^{15}\text{NH}_4^+$ values.
Figure 11. δ¹⁵NH₄⁺ values for each site and rainwater for (a) hardened, (b) residential, (c) pristine, and (d) integrated drainage pipes.

Within individual collection dates, a few different overall trends were observed. NH₄⁺ concentrations and δ¹⁵NH₄⁺ values are shown for four individual storm dates in Figure 12. On 5/26/11, rainwater NH₄⁺ concentrations were below detection, while the stormwater samples were increased and exhibited positive isotopic values. On 7/24/11, stormwater NH₄⁺ concentrations remained very similar to the rainwater concentrations,
while the isotopic values were enriched in stormwater relative to rainwater. On 1/30/13, \( \text{NH}_4^+ \) concentrations in stormwater were increased relative to rainwater at two sites, and decreased at the rest while still exhibiting isotopic enrichment relative to rainwater samples. On 2/25/13, the trend was somewhat similar to 1/30/13, but the rainwater \( \text{NH}_4^+ \) concentrations were much higher and all stormwater sites were decreased in concentration and enriched isotopically.
Figure 12. $\text{NH}_4^+$ concentration and $\delta^{15}\text{NH}_4^+$ values for individual storm dates (a) 5/26/11, (b) 7/24/11, (c) 1/30/13, and (d) 2/25/13.

Surface water $\text{NH}_4^+$ concentrations varied over time, ranging from below detection to 12.44uM at B2 of the Biloxi transect, 20.85uM at OS2 of the Ocean Springs transect, and 6.61uM at GB1 of the Grand Bay transect (Figure 13). Ocean Springs transect $\text{NH}_4^+$ concentrations were almost always higher than the Biloxi transect, which
were taken on the same dates. There was no clear pattern of increasing or decreasing NH$_4^+$ concentrations along any of the transects, although the one relatively high NH$_4^+$ concentration at the Grand Bay transect was closest to shore. All other Grand Bay transect surface water NH$_4^+$ concentrations were near or below the detection limit (> 1µM).

The majority of transect δ$^{15}$NH$_4^+$ values ranged from approximately -4 to +5 per mil (Figure 14). At the Ocean Springs transect, the highest NH$_4^+$ concentrations and the highest $^{15}$N values were seen on 9/8/11, which was only a few days after Tropical Storm Lee. This storm delivered over 10 inches of rain between September 2 and September 5. The stormwater NH$_4^+$ concentrations collected on 9/2 and 9/4 were mostly below detection, possibly due to flushing of dry deposited nutrients before collection took place.
Figure 13. Transect NH$_4^+$ concentrations ± standard deviation for (a) Biloxi, (b) Ocean Springs, and (c) Grand Bay.
Figure 14. Transect $\delta^{15}\text{NH}_4^+$ values for (a) Biloxi, (b) Ocean Springs, and (c) Grand Bay.
Nitrate

Rainwater NO$_3^-$ concentrations differed between collection sites slightly more than NH$_4^+$ concentrations. RCL NO$_3^-$ concentrations ranged from 1.36uM to 30.32uM and from 1.36uM to 19.30uM at RCGB (Figure 15). As seen with the NH$_4^+$ concentrations, rainwater NO$_3^-$ concentrations tended to be much higher in 2011 than in the 2013 season. In the 2013 season, NO$_3^-$ concentrations followed a similar trend to NH$_4^+$ concentrations, peaking in the beginning of spring. In the 2011 season, the trend was not quite as visible due to high concentrations of NO$_3^-$ throughout, while NH$_4^+$ was more sporadic. The common trends between NH$_4^+$ and NO$_3^-$ concentrations suggest that NO$_3^-$ and NH$_4^+$ in rainwater may have common sources.

Figure 15. Rainwater NO$_3^-$ concentrations ± standard deviation for Ocean Springs (RCL) and Grand Bay (RCGB).

Rainwater $\delta^{15}$NO$_3^-$ values ranged from -7.53 per mil to 4.72 per mil at RCL and from -5.46 per mil to 1.74 per mil at RCGB (Figure 16). For the majority of the samples, these values also fell within the previously measured range of rainwater $\delta^{15}$NO$_3^-$ (~ -5 to
5 per mil) (Dillon and Chanton 2005). Between site variability in $\delta^{15}$NO$_3^-$ was greater than observed for $^{15}$NH$_4^+$ values.

![Graph A](image)

![Graph B](image)

*Figure 16.* Rainwater NO$_3^-$ concentrations ± standard deviation and $\delta^{15}$NO$_3^-$ values for (a) Ocean Springs and (b) Grand Bay.

Stormwater NO$_3^-$ concentrations were also generally higher in the 2011 season compared to the 2013 season (Figure 17). During the 2011 season, NO$_3^-$ concentrations were consistently high at all of the hardened sites until 9/2/2011 and 9/4/2011 collection dates (Tropical Storm Lee), during which they were very low or below detection. NO$_3^-$ concentrations in the rainwater on these dates were below 2uM at both rain collection
sites. In addition to low rainwater NO₃⁻ concentrations, the large amount of rainfall during that time period may have flushed away any dry deposited nutrients well before collection occurred.

Stormwater NO₃⁻ concentrations did not follow any noticeable seasonal trends. Peak values for each landscape type all occurred in the 2011 season on different dates. All landscape types ranged from below detection to 50.21uM at SWH3 on 7/24/2011, 78.50uM at SWR2 on 1/5/2011, 71.65uM at SWP1 on 4/4/2011, and 45.40uM at ISW3 on 5/26/2011. Aside from the high peak pristine NO₃⁻ concentration, the majority of the pristine sites had relatively low concentrations and were most often below detection. In the 2013 season, hardened and residential sites generally had higher NO₃⁻ concentrations than did the pristine and integrated sites.
B

C

Date

NO3 conc (uM)

Date

NO3 conc (uM)
Figure 17. Stormwater NO$_3^-$ concentrations ± standard deviation for sites within (a) hardened, (b) residential, (c) pristine, and (d) integrated drainage pipes.

Stormwater NO$_3^-$ concentrations relative to rainwater NO$_3^-$ concentrations were greatly variable by year and landscape type. Hardened sites show much larger increases in 2011 than in 2013, with few decreases in each year (Figure 18). Residential sites had one date with large increases in 2011, while the vast majority of dates in that year exhibited decreases. Increases were more frequent in 2013 for residential, pristine and integrated drainage pipes, but the highest increases were always seen in 2011.
Figure 18. Difference between stormwater and rainwater NO$_3^-$ concentrations (stormwater – rainwater) for (a) hardened, (b) residential, (c) pristine, and (d) integrated drainage pipes. Ocean Springs rainwater NO$_3^-$ concentrations used for hardened, residential and integrated pipes, and Grand Bay NO$_3^-$ concentrations used for pristine sites.

Stormwater $\delta^{15}$NO$_3^-$, in most cases, tended to be depleted relative to rainwater values (Figure 19). Some values, predominantly in the 2011 season, were uncharacteristically low ($<-5$ per mil). The lowest values for each landscape type were all found in samples from the 2011 season. For 2011, values ranged from -26.34 to 5.33
per mil at the hardened sites, -19.10 to 3.99 per mil at the residential sites, -23.83 to -0.51 per mil at the pristine sites, and -29.08 to 1.44 per mil at the integrated drainage sites. In the 2013 season, values ranged from -8.95 to 1.40 per mil, -13.33 to 5.72 per mil, -14.48 to 0.69 per mil, and -10.23 to 6.07 per mil, respectively.

The samples with very low $\delta^{15}NO_3^-$ values generally had either very low or very high extraction efficiencies. For instance, the lowest residential value (-19.10 per mil) at SWR4 on 4/4/11, the extraction efficiency was 980%. This suggests that there was possibly a breakdown of low molecular weight DON that was converted to NH$_4^+$ by the Devarda’s alloy and scavenged to the filter although it could be due to some other type of unknown analytical error. On the other hand, the lowest hardened sample value (-26.34 per mil) at SWH1 on 7/17/11 had extraction efficiencies of 12% and 16% for the duplicate samples and the standard deviation of $\delta^{15}N$ was 9.17. Low extraction efficiency can be caused by NO$_3^-$ “salting out” and not redissolving in the evaporation process. Although this may explain the low extraction efficiency, nitrate precipitating as nitrate salts does not generally cause fractionation of $^{15}N$ (Dillon, personal communication).
Surface water NO$_3$ concentrations were almost always below detection. All three stations at the Biloxi transect were below detection for every sampling date. In Ocean Springs, two of the collection dates exhibited detectable NO$_3$ concentrations for all three stations (Figure 20a). These concentrations were 0.68uM to 3.13uM for 7/20/11, which increased from the site closest to shore to the most offshore site. On 9/8/11, the opposite
trend was seen, with concentrations decreasing from 0.78uM to 0.55uM. Grand Bay transects had detectable NO$_3^-$ concentrations on only two dates as well, but displayed no trend in any increase or decrease relative to distance from shore (Figure 20b).

Isotopic values for surface water NO$_3^-$ were extremely negative (Figure 21), ranging from around -14 to -17 per mil. The samples from which these values were obtained were very low concentration (~ 1.5uM and below), which is too low for reliable measurements from the method used.
Figure 20. Transect NO$_3^-$ concentrations ± standard deviation for (a) Ocean Springs and (b) Grand Bay.
Figure 21. Transect $\delta^{15}$NO$_3^-$ values for (a) Ocean Springs and (b) Grand Bay.

Dissolved Organic Nitrogen

Excluding negative values due to differences in detection limits of analyses or potential analytical or collection error, rainwater DON concentrations ranged from 1.88μM to 30.53μM at RCL and from 3.78μM to 49.44μM at RCGB (Figure 22). The highest values at each site were observed on the same date (5/26/11). Four of the collection dates had only one of the two rain sites measured, and four dates had data from neither rain station. This was due to lack of rain on that date or loss of sample from cracked vials.
Figure 22. Rainwater DON concentrations for Ocean Springs (RCL) and Grand Bay (RCGB).

As explained with the rainwater samples, DON concentrations were sometimes unavailable due to either lack of NH$_4^+$, NO$_3^-$, or TDN data for a particular site due to sample loss. Calculated DON concentrations were also negative at times in the 2011 sampling period, due to some analytical error in one of the calculating factors. Overall, DON concentrations did not follow the same trend as NH$_4^+$ and NO$_3^-$, which were generally higher in 2011 than in 2013. For each landscape type, the peak DON concentration did occur in 2011, but the majority of the samples exhibited similar fluctuations over time. DON concentrations for all landscape types are shown in Figure 23.
Figure 23. Stormwater DON concentrations for sites within (a) hardened, (b) residential, (c) pristine, and (d) integrated drainage pipes.

Phosphate

PO_4\textsuperscript{3-} concentrations at each rain collection site were similar during each collection date, ranging from below detection at both sites to 2.21\textmu M and 2.15\textmu M at RCL and RCGB, respectively (Figure 24). Similar to the trends of NH_4\textsuperscript{+} and NO_3\textsuperscript{-}, PO_4 was also much higher in most collections of 2011 than the 2013 sampling dates.
Figure 24. Rainwater PO$_4$ concentrations ± standard deviation for Ocean Springs (RCL) and Grand Bay (RCGB).

Nearly all sites showed some increase in PO$_4$ concentration from rainwater to stormwater (Figure 26). Of the few decreases that were observed, all were below 2uM, the majority of which were below 1uM and could potentially be attributed to analytical variation or detection limits. The most consistent increases were seen in specific sites of each landscape type as opposed to seasonal trends. SWH1, SWP3, and ISW3 had the most consistent increases over time relative to sites within the same landscape type. All of the residential sites tended to have some sort of increase at every sampling date.

The residential landscape type dominated the total PO$_4$ inputs. All four residential sites peaked on 5/26/2011, once again corresponding to the highest ADWP. All three pristine sites also peaked on this sampling date. Concentrations ranged from below detection to 9.50uM at SWH1, 32.03uM at SWR3, 3.58uM at SWP3, and 19.26uM at ISW1 (Figure 25). The peak integrated drainage pipe value was not characteristic of any
other values, which otherwise tended to peak around 5μM. The pristine landscape type displayed the lowest overall PO₄ concentrations.
Figure 25. Stormwater PO₄ concentrations ± standard deviation for sites within (a) hardened, (b) residential, (c) pristine, and (d) integrated drainage pipes.
Figure 26. Difference between stormwater and rainwater PO₄ concentrations (stormwater – rainwater) for (a) hardened, (b) residential, (c) pristine, and (d) integrated drainage pipes. Ocean Springs rainwater PO₄ concentrations used for hardened, residential, and integrated pipes, and Grand Bay PO₄ concentrations used for pristine sites.

PO₄ concentrations were always detectable at every surface water transect site.

For the Biloxi and Ocean Springs transects, concentrations were comparable on each sampling date (Figure 27). Ocean Springs PO₄ concentration peaked on 8/9/11 at 2.48uM. Though the Biloxi transect was not sampled on this date, the peak PO₄
concentration in Biloxi coincided with the second highest \( \text{PO}_4 \) concentration for the Ocean Springs transect, 2.21µM and 2.09µM, respectively. The Grand Bay transect had the lowest \( \text{PO}_4 \) concentrations that were below the limit of detection (~ 0.5µM), but the sampling dates for this transect did not match up to the Biloxi and Ocean Springs transects, and these were also conducted at a different time of year. For this reason, the Grand Bay surface water samples cannot be compared to Biloxi and Ocean Springs.

![Graph A](image)

![Graph B](image)
Figure 27. Transect PO₄ concentrations ± standard deviation for (a) Biloxi, (b) Ocean Springs, and (c) Grand Bay.

Total Dissolved Nitrogen

Averaging each of the nitrogen species for each landscape type reveals the overall trends for each sampling season (Figure 28). Due to the large variation between sampling dates and within landscape types, these values inherently have large standard deviations. This is expected of data of this nature, and averages are used here simply to better visualize the relative contributions of the dissolved nitrogen species.
Figure 28. Average NH$_4^+$, NO$_3^-$, and DON concentrations and percent of total dissolved nitrogen load for each landscape type and rain in (a) 2011 and (b) 2013.

In the 2013 sampling period, average rainwater TDN was composed of roughly equal parts of NH$_4^+$, NO$_3^-$, and DON. All landscape types exhibited a decrease in NH$_4^+$ concentration relative to rainwater, with the pristine landscape having the lowest average...
concentration and the integrated drainage sites having the highest average concentration. The average NO$_3^-$ concentration of the pristine landscape was nearly identical to the average rainwater NO$_3^-$ concentration, with the hardened landscape showing only a slight increase compared to rainwater (5.94uM to 6.06uM). The residential landscape had the largest average NO$_3^-$ concentration of 9.30uM. The pristine landscape was composed mostly of DON and also had the largest DON concentration of all landscape types. All landscape types had an increase in DON relative to rainwater, the lowest of which occurred in the hardened landscape at 16.11uM from a rainwater concentration of 5.09uM. The residential landscape and integrated drainage pipes were very similar in DON concentration, nearly double that of the hardened landscape.

Biota

Tables 2 and 3 outline δ$^{15}$N and δ$^{13}$C values of macroalgae and oysters, respectively. Macroalgae δ$^{15}$N ranged from 2.58 to 8.57 per mil (average = 6.33 ± 1.41 per mil), while δ$^{13}$C ranged from -18.94 to -26.49 per mil (average = -21.46 ± 2.37 per mil). Oysters showed less variability, ranging from 9.34 to 10.83 per mil for δ$^{15}$N (average = 10.19 ± 0.50 per mil) and from -22.31 to -24.72 per mil for δ$^{13}$C (average = -23.42 ± 0.65 per mil).
Table 2

$\delta^{15}N$ and $\delta^{13}C$ Values for Macroalgae (Gracilaria)

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<th>$\delta^{13}C$</th>
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Table 3

$\delta^{15}N$ and $\delta^{13}C$ Values for Oysters (Crassostrea virginica)

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Figure 29. $^{13}$C and $^{15}$N biplot of macroalgae, oysters, and particulate organic matter

Carbon and nitrogen stable isotopes of particulate matter were also analyzed on six filters from Biloxi and Ocean Springs transect sites. Table 4 displays the $\delta^{15}$N and $\delta^{13}$C values of the particulates from the six filters. Mean $\delta^{13}$C was -25.94 ± 0.74 per mil, and mean $\delta^{15}$N was 5.06 ± 1.61 per mil with ranges from -24.71 to 26.85 per mil and 2.50 to 7.51 per mil respectively.
### Table 4

$\delta^{15}N$ and $\delta^{13}C$ Values for Particulates Filtered from Transect Surface Water Samples

<table>
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CHAPTER IV
DISCUSSION

Synopsis of Findings

Nutrient concentrations in stormwater varied greatly spatially and temporally. Average total nitrogen loads and trends from the 2011 season were quite different from those in the 2013 season. In 2011, the integrated drainage pipes dominated the average total dissolved nitrogen (TDN) load, followed by the hardened, residential, and pristine landscapes, respectively. Rainwater had the lowest average TDN load. In 2013, rainwater also had the lowest average TDN load, and integrated drainage pipes were highest, but it was followed by the residential landscape type. The hardened landscape type had a much lower average TDN concentration than all other landscape types.

\(\text{NH}_4^+\) and \(\text{NO}_3^-\) sources appear to exist in all landscape types. Stormwater \(\text{NH}_4^+\) concentrations were most often either lower or close to those of rainwater \(\text{NH}_4^+\), probably due to volatilization and combinations of nonpoint source loading in addition to volatilization. Stormwater \(\text{NO}_3^-\) concentrations were increased more often relative to rainwater. Increases in stormwater \(\text{NH}_4^+\) and \(\text{NO}_3^-\) concentrations relative to rainwater were less prevalent in the pristine landscape type compared to all others.

Stormwater nitrogen did not seem to be traceable into the Mississippi Sound. \(\text{NH}_4^+\) concentrations were usually detectable in Biloxi and Ocean Springs surface water, but due to the widely variable \(^{15}\text{NH}_4^+\) values they could not be definitively attributed to stormwater \(\text{NH}_4^+\). \(\text{NO}_3^-\) concentrations were usually below detection in surface water.
Rainwater

Due to the somewhat unpredictable nature of rain events, collection attempts occasionally did not produce enough rain for the whole suite of analyses, or they were cancelled due to lack of substantial rainfall in the study area. Also, many of the storm events within the study period were not well defined storms that could be tracked over time. Instead, they would develop within the Mississippi Sound, giving little time to prepare. The amount of time necessary to deploy clean buckets to the Grand Bay rain collector was a hindering factor in these cases. Quite often strong rain events in the area occurred at night, which we had determined to be too dangerous to collect due to hazardous driving and sampling conditions as well as the location of the Grand Bay rain collector being atop a high platform. Despite this, two sampling dates in the 2013 season were carried out at night in order to increase the amount of storms collected that year. Ideally, more storms would have been collected in both sampling years, but each season was represented in each year by at least one storm event.

Nutrient concentrations were similar between the Ocean Springs and Grand Bay rain collectors, suggesting the same storm impacted each of the study areas with very little atmospheric effect over the relatively short distance between the two rain collectors. As mentioned previously, the nutrient concentrations tended to be higher in 2011 than in 2013, especially in the case of NO$_3^-$ and PO$_4$.

Large atmospheric NH$_4^+$ sources are generally associated with agriculture and livestock farming (ApSimon et al. 1987), so storms originating over land should have higher rainwater NH$_4^+$ concentrations than those coming from the Gulf of Mexico. For the 2011 sampling period, the highest rainwater NH$_4^+$ concentrations were from storms that arrived from the northwest of the
sampling areas. However, storm origins and seasonal temperatures were very similar between the two years, and the highest rainwater NH$_4^+$ concentrations measured in 2013 were from a storm that originated southwest of the study area with minimal time over land in Louisiana. Only two sample dates, which were associated with Tropical Storm Lee in September of 2011, represented storms that had no land interaction before reaching the study area. In these two cases, concentrations were very low or below detection for NH$_4^+$, NO$_3^-$, and PO$_4^-$.

Due to the lack of obvious differences in physical parameters between the two years, differences in NO$_3^-$, PO$_4^-$, and DON from 2011 to 2013 are more difficult to explain. They did not exhibit a clear trend based on storm direction. The nutrient concentration differences between the two years are probably due to a variety of factors that could not be teased apart from the limited number of samples in this study.

Rainwater $\delta^{15}$NH$_4^+$ and $\delta^{15}$NO$_3^-$ values mostly fell within expected ranges of about -12 to +8 per mil and -5 to +5 per mil, respectively (Paerl and Fogel 1994; Russell et al. 1998; Dillon and Chanton 2005). One heavy $\delta^{15}$NH$_4^+$ value was measured in April of 2013 (5.98 ± 0.28 per mil). The average $\delta^{15}$NH$_4^+$ value between the two rain collectors was -2.10 ± 2.76 per mil (n = 21), and the average $\delta^{15}$NO$_3^-$ value was -1.93 ± 3.81 per mil (n = 18). In the 2005 study by Dillon and Chanton in Sarasota, Florida, $\delta^{15}$NO$_3^-$ values were closer to atmospheric on average and had a narrower range. The NO$_3^-$ sources in our sampling area seem to be more variable in origin.

Stormwater

Variability in stormwater nutrient concentrations can be attributed to a variety of factors, including the initial rainwater concentrations, aerial deposition (Zhang et al.
activities within the catchment, and the amount of imperviousness of the catchment (Mayer et al. 2002; Taylor et al. 2005). For $\text{NH}_4^+$, temperature of the ground’s surface may also play a role in the variability due to increased volatilization potential, but that was not shown in this study, likely due to the presence of $\text{NH}_4^+$ point and non-point sources in the study areas.

Another potentially large source of variation within this study is the time storm events were sampled relative to when the storm began. Since samples were collected by hand, as opposed to an auto-sampler, timing varied from storm to storm. The antecedent dry weather period may also play a role in this (Gupta and Saul 1996). During periods of dry weather, dry deposition of $\text{NO}_x$ and $\text{PO}_4$ is potentially increased and can accumulate over time. The first flush phenomenon suggests that the initial stormwater runoff will contain the highest pollutant concentrations and gradually decrease over time (Gupta and Saul 1996; Lee et al. 2002). These initial concentrations may be exacerbated by longer antecedent dry weather periods.

In addition to spatial and temporal variability of stormwater nutrient concentrations alone, $\text{NH}_4^+$ dynamics between rain and stormwater were also variable. In some cases $\text{NH}_4^+$ concentrations decreased relative to rain, whereas in other cases stormwater $\text{NH}_4^+$ concentrations were the same or increased when compared to rainwater. Decreases in $\text{NH}_4^+$ concentrations in stormwater are attributed to volatilization, accompanied by an increase in $\delta^{15}\text{NH}_4^+$. Nearly all stormwater samples exhibited some increase in $\delta^{15}\text{NH}_4^+$ relative to rainwater $\delta^{15}\text{NH}_4^+$. Increased $\text{NH}_4^+$ concentrations in stormwater signifies additional $\text{NH}_4^+$ sources, entraining $\text{NH}_4^+$ subsequent to the evolution of rainwater to stormwater. Since $^{15}\text{N}$ was enriched even when the stormwater
NH$_4^+$ concentrations were similar to those of rainwater, the amount volatilized must have been similar to the amount input from additional sources.

Stormwater NO$_3^-$ concentrations did not seem to display any visible trends seasonally or between landscape types. In instances where NO$_3^-$ concentrations were increased relative to rainwater, NO$_x$ was probably being entrained from dry deposition. These samples could have been collected early within the storm period, but since stormwater flow rates and flow durations are not known, it is difficult to adequately determine. In cases of stormwater NO$_3^-$ concentration decrease relative to rainwater, denitrification is probably the dominant factor, although the majority of stormwater NO$_2$ concentrations were well below 1uM and did not seem to show any increases when NO$_3^-$ concentrations decreased.

Nitrogen isotopic values for stormwater nitrate did not follow the same trend as the similar Dillon and Chanton (2005) study. The stormwater samples in their study showed an enrichment of $\delta^{15}$NO$_3$ relative to rainwater. Most of the stormwater samples within this study exhibited a decrease in $\delta^{15}$NO$_3$ relative to rainwater. Also, a fair amount of the sample values were uncharacteristically low ($\lt -10$ per mil). These low nitrogen isotopic values could be due to breakdown of low molecular weight DON during the high temperature evaporation process. Mississippi River water samples with low DON concentrations run using the same method in our laboratory never exhibited these low values (Dillon, unpublished data). Further evidence for this is the fact that many of the $\delta^{15}$NO$_3^-$ extraction efficiencies were greater than 100%, suggesting additional nitrogen being scavenged onto the filter. Some extraction efficiencies were also low, occasionally in the range of 20% to 30%. Low extraction efficiencies suggest that a portion of the
NO\textsubscript{3} precipitates out of the sample as NO\textsubscript{3} salts and do not get redissolved in the final shaking step of the process. It is possible that the lighter $\delta^{14}$NO\textsubscript{3} redissolves preferentially to the $\delta^{15}$NO\textsubscript{3}, leading to a decrease in the nitrogen isotopic value.

Calculated stormwater DON concentrations were occasionally negative in 2011 samples. It is possible that detection limit differences in the DIN and TDN analyses played a role in this when DON was calculated, but a few of the values were too negative for this to be the case every time. In these cases, it is more likely that some unknown analytical error in the analysis of DIN or TDN was the dominant factor.

The overall stormwater nitrogen composition differed greatly from 2011 to 2013. The trends between each landscape type when averaged for each year were always lower for concentrations of NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3} in 2013, and higher for DON concentrations. The higher average DON concentrations in 2013 are likely due to the negative values in 2011, but it is unknown if that is the sole reason, as actual DON concentrations for some 2011 samples are unknown. In 2011, all landscape types exhibited a concentration increase in each of the nitrogen species relative to rainwater, aside from NH\textsubscript{4}\textsuperscript{+} and NO\textsubscript{3} in the pristine landscape type. In 2013, average concentrations showed no NH\textsubscript{4}\textsuperscript{+} sources in any landscape type compared to rainwater, and very little NO\textsubscript{3} increase. DON was consistently increased relative to rainwater in all landscape types during both years.

The yearly differences suggest that in 2011 there may have been a greater amount of nitrogen sources in the hardened, residential, and integrated drainage areas. It is also possible that first flush and ADWP effects could have played a role in these differences. Since samples were taken from each site at only one time point during each storm, first flush effects are unknown. If the majority of samples in 2011 were taken during very
early stormwater flow, while 2013 samples were taken later, first flush effects could potentially have had a large role in concentration differences.

In order to visualize how rain stormwater within the different landscape types related to one another, non-metric multidimensional scaling (NMDS) plots were created for each of the two study years as well as seasonally. Figure 30 displays four of the seasonal NMDS plots. Yearly plots had no separation between sample types. Likewise, the majority of the seasonal plots exhibited poor separation. Figure 30a, which is composed of two storms from spring 2011, did not reveal any trends based on rain or landscape type. Figure 30b, summer 2011 (four storms), showed very slight separation by the pristine and residential (res) landscape types. Due to variability within landscape types and between storms, NMDS plots were generally not effective in displaying differences in landscape types. Figures 30c and 30d best represented visual trends between sample types. Winter 2012 and summer 2013, comprising two and three storms, respectively, both revealed hardened, residential, and integrated drainage sites to be most similar. Rainwater and the pristine landscape type were very reasonably separated from the other sites in summer 2013. Pristine sites were also far separated for winter 2012, but there was not sufficient rainwater data to be plotted. For these two plots, the three sample types that are presumably most affected by anthropogenic point and non-point nitrogen sources were grouped near each other.
Figure 30. Seasonal non-metric multidimensional scaling (NMDS) plots using NH$_4$$^+$, NO$_3^-$, TDN, DON, and PO$_4$ concentrations for (a) Spring 2011, (b) Summer 2011, (c) Winter 2012, and (d) Summer 2013.
Transects

Surface water transects displayed a great degree of spatial and temporal variability. Grand Bay consistently had the lowest NH$_4^+$ and PO$_4$ surface water concentrations. NO$_3^-$ was always below detection in Biloxi, and near or below detection limits on most dates in Ocean Springs and Grand Bay. Nitrogen concentrations were almost always below the redfield ratio when compared to phosphorus concentrations, signifying a nitrogen limited system.

Surface water $\delta^{15}$NO$_3^-$ values were unreliable due to the concentrations being lower than the method requires (>2uM) (Sigman et al. 1997). Aside from one sample date, surface water $\delta^{15}$NH$_4^+$ values were relatively light (~ 0 ± 5 per mil) and did not resemble those of stormwater. However, shortly after Tropical Storm Lee, Ocean Springs surface water $\delta^{15}$NH$_4^+$ values ranged from 17.06 per mil (closest to shore) to 21.73 per mil (furthest from shore), with NH$_4^+$ concentrations greater than 15uM. Rainwater NH$_4^+$ concentrations were below detection during the storm. Although stormwater NH$_4^+$ values were below detection in the residential landscape during the tropical storm, it was likely due to heavy rains flushing dry deposited nutrients prior to the sampling effort. The isotopic values seen for surface water NH$_4^+$ after Tropical Storm Lee are indicative of stormwater or wastewater nitrogen (Cifuentes et al. 1988; Desimone and Howes 1996; Dillon and Chanton 2005; Dillon et al. 2007). Without knowledge of initial stormwater nitrogen concentrations or fecal indicators in the surface water, it is not possible to definitively determine the source of high $\delta^{15}$NH$_4^+$ values after this storm event.

The high $\delta^{15}$NH$_4^+$ values seen after Tropical Storm Lee suggest that during very heavy storm events, stormwater may possibly be able to be traced into the Sound and
should be further investigated. Unfortunately, due to the exceptionally strong storm conditions, no biota samples are available for the time period. The oysters and macroalgae $\delta^{15}$N values for all other sample dates showed little fluctuation over time.

Conclusions

In conclusion, the results of this study suggest that stormwater runoff may not contribute a traceable amount of nitrogen to the surface waters of the Mississippi Sound. However, further research should be conducted, especially during very strong storm conditions such as tropical storms and hurricanes, when enriched $\delta^{15}$NH$_4^+$ signals seem to be present in surface water. All landscape types, as well as integrated drainage pipes, appear to have variable nitrogen sources in addition to rainwater. Furthermore, $\delta^{15}$NH$_4^+$ values were always enriched in stormwater samples relative to rainwater even when stormwater concentrations increased, suggesting stormwater can be traced isotopically whether or not volatilization is the main driver of NH$_4^+$ concentrations in the stormwater. Although spatial and temporal variability is high, hardened and residential sites, along with integrated drainage pipes, tend to have much higher nutrient concentrations than pristine sites. In future research, nitrogen should be examined over the whole course of individual storms in order to determine first flush effects as well as how ADWP may play into entrainment of dry deposited nutrients. Additionally, larger, long-term substrate cages for biota should be utilized in order to increase sampling efficiency after unexpected storms.
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