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The Ability of an Aquatic Invader to Uptake Nutrients in an Upstream Estuarine Environment: Implications for Reducing the Intensity and Frequency of Massive Fish Kills in Florida

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The Ability of an Aquatic Invader to Uptake Nutrients in an Upstream Estuarine Environment: Implications for Reducing the Intensity and Frequency of Massive Fish Kills in Florida

by

Melissa L. Kerr

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Science and Policy School of Geosciences College of Arts and Sciences University of South Florida

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Keywords: Water Quality, Toxic Dinoflagellates, Water Hyacinth, Estuarine

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ABSTRACT

The purpose of this study was to assess whether nutrient absorption rates by water hyacinths are affected by low-salinity levels. In a controlled experiment, water hyacinths demonstrated the ability to absorb a significant amount of nutrients in low-saline waters while maintaining a slowed growth rate and shortened life span. Nutrient rates were reduced by an average of 36% in ammonia nitrogen and 48% in reactive phosphorus in the tanks of 4.45 parts per thousand (ppt) salinity. Growth rate in the experimental tanks of 4.45 ppt was observed at 33% slower than that of the control. The high salinity comparison tank of 7.0 ppt experienced complete mortality after three days.

Phytoremediation practices through the use of an aquatic invader, water hyacinths, can be used to reduce large-scale fish kills along the eastern U.S.’s major estuarine systems, focusing on Florida’s waters. Toxic dinoflagellates and other harmful algal blooms have been plaguing the contributing waterways of North Carolina and the Chesapeake Bay and are the main causes of these massive fish kills. The characteristics and trends that these upstream estuarine systems are following could serve as a warning for Florida.

An analysis of Florida’s fish kill database, as well as patterns and trends of the fish kills in North Carolina and the Chesapeake Bay, were used to determine areas at an increased risk for toxic dinoflagellates and harmful algal blooms to occur. Areas are
proposed for water hyacinths to be implemented in a controlled method to reduce massive fish kills in Florida’s waters.
CHAPTER 1:
INTRODUCTION AND PROBLEM

Fish kills along the eastern coast of the continental United States are increasing in frequency and intensity causing damage to our ecosystems and crippling our seafood supply (Paerl et al., 2014a). A phytoremediation practice utilizing an aquatic invasive plant can be used to reduce fills kills along the eastern U.S.’s major estuarine systems, in particular in Florida’s waters.

This study aimed to assess whether nutrient absorption rates by water hyacinths are affected by low-salinity levels. This study focused on the fish kill trends and patterns of toxic dinoflagellates and other harmful algal blooms of the Chesapeake Bay and the Pamlico-Neuse Estuary in North Carolina. Fish kill data and trends in Florida were examined to determine the areas at risk for the devastation that haunted the waters of North Carolina and the Chesapeake Bay. These two estuaries are the largest estuarine environments along the eastern coast and the largest suppliers of seafood to the eastern coast. “Coastal watersheds support more than one half of the world’s human population and are experiencing unprecedented urban, agricultural, and industrial expansion” (Paerl et al., 2014a, p.243). The characteristics of these systems are experiencing an increase in intensity and frequency of fish kills that could have serious implications for Florida and other similar estuarine systems.
Problem:

It is necessary to improve the water quality of these vital estuarine systems in order to reduce massive fish kills, focusing on those fish kills caused by lesser known toxic dinoflagellates and harmful algal blooms (HABs). Toxic dinoflagellates and harmful algal blooms, other than the well known red tide, are not as widely studied or monitored due to the elusive characteristics they possess, but are an increasing problem due to the threats of climate change and human activities on these estuarine systems. An aquatic invasive species that Floridians know all too well, water hyacinths, has been a controversial issue to many but can be used as a phytoremediation method to improve the water quality of upstream estuarine environments, the preferred habitats of toxic dinoflagellates and HABs. Removing the stimulant or trigger for these will reduce the number of outbreaks and thereby reduce the frequency and intensity of fish kills. Implementation of these plants can be used to reduce the risk for an outbreak of toxic dinoflagellates or HABs to occur and cause a massive fish kill. Areas at a greater risk for these outbreaks were determined and the effectiveness of these plants to absorb nutrients in those areas were analyzed for potential future implementation in a controlled method. This study was designed to assess the effectiveness of water hyacinths to absorb nutrients in a low-saline environment similar to those environments experiencing an increase in intensity and frequency of fish kills.

Eutrophication:

Water quality degradation is an ever-increasing problem facing the world, putting certain bodies of water at a greater risk. The quality of water can be affected by
deforestation, urban growth, and livestock/agricultural activities (Codd, 2000). In estuarine systems, their “high nutrient loads, long residence times, and their shallow nature (generally <5 m in depth) make these systems highly sensitive to nutrient inputs and susceptible to the detrimental systems of nutrient over-enrichment, including excessive algal production and harmful algal blooms, bottom water hypoxia, and fish kills” (Paerl et al., 2014b, p. S32). These activities cause nutrient over-enrichment of the water. With an increasing population and a decrease in space available, there has been a huge increase in the density of coastal development. More than one half of the world’s population lives in coastal water- and airsheds (Paerl et al., 2014a). An increase in development, decrease in vegetation, and altered surface water flows are causing our coastlines to deteriorate.

Eutrophication (hypertrophication) is caused by nutrient loading of a water body from an increase in development and population, especially around coastal areas, leading to an increase in harmful algal blooms and outbreaks of toxic dinoflagellates (Codd, 2000). Shallow water and lagoonal estuaries are areas that remain mostly stagnant and are at a greater risk for degraded water conditions (Paerl et al., 2014b). Degraded, nutrient-rich waters are the major stimulants for toxic dinoflagellates and HABs. These conditions can cause an outbreak or bloom that can produce toxic dinoflagellates such as *Pfiesteria piscicida* and *Prorocentrum minimum*, which are the major cause of large-scale fish kills along the eastern U.S. (Codd, 2000; McCord, 2000). Nutrient loading of a waterway is one of the characteristics that put certain estuarine systems at risk for an increase in frequency and intensity of fish kills. Degradation of
water quality by these activities can lead to a phenomenon called anthropogenic eutrophication.

Anthropogenic eutrophication is a human caused condition from the excess buildup of nutrients in water sources from human activities. These excess nutrients, mainly phosphorus and nitrogen, accelerate the growth of algae and algae-like organisms. This accelerated growth (or “blooms”) consumes oxygen in the water, and reduces sunlight in the water column. In addition, some toxic forms can cause marine life to become sick or die. Algal blooms have even been linked to sea lion deaths in California and manatee deaths in Florida as well as the cause of thousands of dead fish during a massive fish kill in Old Tampa Bay in 2008 (National Academy of Sciences, 2000; Salinero, 2008).

Toxic Dinoflagellates:

Many of these toxic algal blooms are caused by micro-organisms called dinoflagellates. Dinoflagellates are flagellate eukaryotes that are marine plankton, but also common in fresh water habitats (Wang, 2008). These dinoflagellates have the ability to produce toxins. These toxins can affect the nervous system (neurotoxins), the liver (hepatotoxins), the skin (dermatoxins), and potentially kill people from drinking contaminated water, eating food that had been exposed to the toxins, direct contact with the water, or inhaled through aerosolized toxins (Wang, 2008; Codd, 2000). Consumption of seafood that has been contaminated by the toxins of the dinoflagellates can also cause various seafood poisoning syndromes (Wang, 2008). Unlike the red tide dinoflagellates, *Karenia brevis*, which is seen as a more natural phenomenon and
commonly misused to name all harmful algal blooms, the toxic blooms causing great concern are of a different variety.

These dinoflagellates are smaller in size than the one associated with red tide, and are frequently overlooked on water quality monitors. These dinoflagellates have been known to be heterotrophic, able to survive through photosynthesis as well as becoming parasitic and feeding off marine life (Burkholder, 1998). They are not only primary producers and grazers, but they are also “major causative agents of harmful algal blooms” (Wang, 2008, p. 1). Toxic dinoflagellates such as *Pfiesteria piscicida* and *Prorocentrum minimum*, as well as other harmful algal blooms have been plaguing Chesapeake Bay and the Pamlico-Neuse Estuary in North Carolina for years and are posing a major threat to Florida’s waters (Kaier, 2002).
CHAPTER 2: 
BACKGROUND AND LITERATURE REVIEW

Our Major Estuarine Systems:

Water quality trends and fish kill data from Chesapeake Bay and Pamlico-Neuse Estuary can serve as a precursor warning for Florida. Their fish kill trends and locations can help identify areas along Florida’s coasts that are at the greatest risk for an increase in intensity and frequency of fish kills caused by toxic dinoflagellates and other similar toxic species.

Chesapeake Bay and the Pamlico-Neuse Estuary were chosen because they are the largest estuarine systems along the Eastern coast and supply the majority of seafood to the eastern U.S. (Paerl et al., 2014a; Codd, 2000; McCord, 2000). Characteristics of these upstream estuarine systems are similar to many waterways along the coast of Florida. Florida is home to many estuaries, lagoons, and bays. Both Chesapeake Bay and Pamlico-Neuse Estuary are plagued by toxic dinoflagellates and other harmful algal blooms, and similar species are beginning to be identified in Florida’s coastal waters. These regions are also among the areas at greatest risk for coastal nutrient loading, and thereby at risk for an increase in occurrence and intensity of fish kills by toxic dinoflagellates and harmful algal blooms. The first species of toxic dinoflagellate to be examined will be the ones impacting the waters of the Pamlico-Neuse Estuary in North Carolina.
**Pamlico-Neuse Estuary: *Pfiesteria piscicida***

The “cell from hell”, also known as the “phantom fish killer”, has been plaguing the coastal waters of North Carolina (Burkholder and Glasgow, 1997). Scientifically known as *Pfiesteria piscicida*, this fish killer has been responsible for multiple massive fish kills, habitat loss and public health outbreaks for over two decades. It was discovered in the Albemarle Pamlico estuary on North Carolina’s coast, and has since been spotted in the Chesapeake Bay and Florida’s coastal waters (Burkholder, 1998). This dinoflagellate has been the center of controversy since its discovery in 1988 due to its complex life cycle. Its complex lifestyle has not been widely studied and has made this organism difficult to identify. *Pfiesteria piscicida* changes its stage in life cycle after a fish kill has occurred, making it very difficult to catch in the act (Rublee et al., 2005). Some of the organism’s life stages look like many other estuarine dinoflagellate species leading to the cause of many massive fish kills being falsely identified (Rublee et al., 2005).

North Carolina houses about 2.3 million acres of estuary making it the largest on the eastern coast (Rublee et al., 2005). *Figure 2.1* displays the large area that this estuary covers as well as the area affected by the outbreaks shown by the blackened areas. The state is heavily dependent on its fishing industry and the ecotourism revenue it brings in with its beautiful coastal waters (Oversight Hearing on Pfiesteria, 1997). Between 1990 and 1997, North Carolina lost one billion fish and shellfish due to these outbreaks (Oversight Hearing on Pfiesteria, 1997).
Pfiesteria piscicida is a heterotrophic dinoflagellate, that when triggered causes harmful micro-algae blooms causing massive fish kills (Glasgow et al., 2001). Pfiesteria typically lives in its dormant or photosynthetic life stage similar to many other dinoflagellates. However, when exposed to environmental stimulants it reverts to its toxic form and begins feeding on the aquatic life (Glasgow et al., 2011). The toxic form of Pfiesteria piscicida is mainly triggered by degraded water quality. Excess nutrients, in particular phosphorus, waste water, algae, and the presence of a large school of fish stimulate the dinoflagellate and cause it to show its toxic form (University of Maryland Center for Environmental Science, 2015; Pinckney et al., 2000; Burkholder and Glasgow, 2001; Kaiser, 2002). A combination of these paired with their environmental
preferences, discussed later, make the perfect setting for an outbreak to occur. Without nutrient stimulation, the presence of fish, and the ideal outbreak conditions, this dinoflagellate reverts to the stage in its life cycle where it is benign and relies on photosynthesis for nutrition (University of Maryland Center for Environmental Science, 2015). Their benign form typically settles in the sediments along the bottom of the water column making it harmless to fish, people, and the ecosystem (Pinckney et al., 2000).

Research and examination conducted by multiple researchers and labs on the North Carolina Pfiesteria piscicida outbreaks have attributed the cause of these outbreaks to anthropogenic nutrient loading of the waterways, a non-point source of pollution (Burkholder and Glasgow, 2001). This organism needs to be taken as a serious threat before the outbreaks spread. These toxic dinoflagellates are a worldwide problem causing massive fish kills, but these fish kills are often mistakenly attributed to other dinoflagellates or other harmful algal blooms (Rublee, 2005). There has already been an increase in frequency and documented sightings of Pfiesteria up and down the eastern U.S. coast making the issue even more of a concern (Rublee, 2005).

About seventy-five percent of the outbreaks in North Carolina in the early 1990’s came from areas where the water had high nutrient levels (Burkholder and Glasgow, 2001). Researchers found that these water ways were getting polluted from runoff of cropland. They also sourced it from poorly treated waste water mainly from animal waste from the hog farms that populated much of that region of North Carolina (Burkholder and Glasgow, 2001). The waters in these areas are warm, mostly stagnant and shallow, making them ideal conditions for Pfiesteria. In addition, large schools of fish migrate to that area every summer. The presence of large schools of fish coupled
with nutrient stimulation makes yearly outbreaks a trend (Glasgow et al., 2001; Burkholder and Glasgow, 1997).

There is also the climatic influence on *Pfiesteria* outbreaks. With climate change and coastal waters getting warmer, there is an increase in activity of *Pfiesteria*. In addition, with an increasing global population and a decrease in farmable land, there is a greater need and reliance on aquaculture. Aquaculture is the farming of seafood and is characterized by “growing” sea life in controlled environments held in tanks or enclosures. This typically produces high nutrient waters in warmer shallow areas with reduced turbidity, the perfect conditions for a *Pfiesteria* outbreak (Burkholder, 1998; Moestrup et al., 2014; Kaiser, 2002). This could have a huge negative impact on our fisheries industry and our economy.

Environmental preferences when coupled with nutrient enrichment are the major cause of the fish kills in North Carolina (Burkholder and Glasgow, 2001; Rublee et al., 2005). Pfiesteria outbreaks are linking water pollution with fish health and human health and attributing the cause to human activities (Burkholder, 1998; Kaiser, 2002). We are essentially creating this problem that may have never existed were it not for our disruption of the ecosystems.

**Chesapeake Bay: *Prorocentrum minimum***

Similar to the outbreaks in North Carolina, an increase in development and agriculture along the coastline of Maryland is causing an increase in nutrient inputs into the Chesapeake Bay and a dramatic increase in the average number of harmful algal blooms occurring each year (Li et al., 2015). Plagged mainly by *Prorocentrum minimum*
and now recently *Pfiesteria piscicida*, massive fish kills and shellfish kills are becoming more common and more intense. Sickness and death among seabirds and marine mammals have also been increasing, disrupting the food chain and causing some areas to become anoxic (Li et al., 2015).

*Prorocentrum minimum* has a simpler lifecycle than that of *Pfiesteria*. It is able to live in a wide range of habitats, from cold temperate brackish waters to warm tropical waters, but prefers more shallow estuarine waters (Faust and Gulledge, 1999). This dinoflagellate is much smaller in size than *Karina brevis*, and varies in size from oval to triangular and heart-shaped. Its varying shape and small size is typically over-looked in water quality samples which also tend to focus on the more common threats like red tide. This dinoflagellate is also an active swimmer, and produces a hepatotoxin responsible for the majority of fish kills in the Chesapeake Bay (Li et al., 2015). In addition to producing a toxin that can kill fish directly it also kills them indirectly. This dinoflagellate consumes all the nutrients in the water until it can no longer sustain itself, begins to die off, and during its decomposition all the oxygen in the water is consumed, causing marine life to suffocate and die (Li et al., 2015).

*Pfiesteria piscicida* is also thriving in the Chesapeake Bay with its nutrient rich waters. In 1997, *Pfiesteria* killed thousands of fish as well as affected the health of the public. Local papers were filled with similar stories of the accounts of fishermen of the Pamlico-Neuse in North Carolina (University of Maryland Center for Environmental Science, 2015). This massive fish kill cost the seafood industry tens of millions of dollars, demonstrating the enormous effect these outbreaks are having on our economy (University of Maryland Center for Environmental Science, 2015).
This “cell from hell” has not been identified as the culprit in recent fish kills in the Chesapeake Bay; however, researchers ran into the same problems that those in North Carolina did. They found that the organism itself and the toxin it produces are only around for a short while. The toxin is only present in the water for as long as the fish kill is occurring, which typically is only a few hours (University of Maryland Center for Environmental Science, 2015). It is possible that more recent fish kills have been the result of *Pfiesteria* in the Chesapeake. *Prorocentrum*, however, is still present in the Chesapeake and has in fact increased in intensity and frequency (University of Maryland Center for Environmental Science, 2015).

*Prorocentrum minimum* can take on a brownish color when alive and has earned the nickname “mahogany tide” which leads to many false assumptions about their outbreaks and its association with red tide (McCord, 2000). Being that this dinoflagellate prefers shallow estuarine waters, its ideal home is in direct line for the effects of nutrient loading from nearby runoff. The occurrence of this bloom is more directly related to the effects of human activities than that of “red tide” that tends to occur out in cool, salty, waters. Giving it the name “tide” also leads to the assumptions that it is a more regularly occurring event. That is natural, especially since a bloom seems to occur each spring. However, this can be attributed to the influx of nutrients into the bay’s waters from agriculture runoff which is in full production that time of year (Li et al., 2015).

The most common dinoflagellates to plague the Chesapeake waters are *Prorocentrum minimum* and *Karenia brevis*, better known as red tide. However, *Prorocentrum minimum* is not as widely studied or monitored as red tide. This lack of
monitoring makes it an even bigger threat for the Chesapeake and other areas at risk for *P. minimum* to occur.

Although *P. minimum* has “been present in the Chesapeake Bay for decades, often causing mahogany tides…this year is worse than others” (McCord, 2000). Harmful algal blooms are increasing in frequency and intensity across the globe following the increase in nutrient inputs into our water bodies (Li et al., 2015). The Philippines, an area characterized by high nutrient levels from high human development and aquaculture practices, has been enduring major environmental changes, in particular a massive fish kill in 2002 that was attributed to the toxic dinoflagellate *Prorocentrum minimum* (San Diego-McGlone et al., 2008). This demonstrates again how human activity is creating nutrient enriched areas that are also poorly flushed and shallow, thereby creating the perfect habitat for toxic dinoflagellates and other harmful algal blooms to occur. This poses the threat of an increase in major fish kills to occur affecting our food supply, the ecosystem and public health.

**Trends in Fish Kill Occurrence:**

In 2015, a study conducted by the University of Maryland Center for Environmental Science determined that over the past 20 years algal blooms have “increased in frequency in the Bay and is a warning that more work is needed to reduce nutrient pollution entering the Bay’s waters” (University of Maryland Center for Environmental Science, 2015, p.1). Anthropogenic eutrophication caused HABs have been an ongoing problem with the Chesapeake Bay just as with the Pamlico-Neuse. However, due to increased population, development, and agricultural production it is
getting worse. On-going efforts have been occurring in the Bay in an attempt to restore it to a healthy ecosystem, but nutrient pollution stands in the way. “We’re seeing this all over the world. More blooms, more often, lasting longer. In many places these trends are consistent with increased nutrient loads” states Pat Gilbert, professor at the University of Maryland Center for Environmental Science’s Horn Point Laboratory (University of Maryland Center for Environmental Science, 2015, p.2).

In 2009, another massive fish kill plagued the waters of the Neuse River. However, no public warnings or statements were issued for this event, and many fish kill events tend to occur without public warnings or statements (North Carolina Riverkeepers and Waterkeeper Alliance, 2012). Documentation of these fish kills tends to be only available from locals’ reports to the fish kill hotline as well as other local organizations trying to make these matters public, such as the North Carolina Riverkeepers and Waterkeeper Alliance (North Carolina Riverkeepers and Waterkeeper Alliance, 2012).

The North Carolina Riverkeepers and Waterkeeper Alliance site is dedicated to keeping the public informed of issues pertaining to the health of North Carolina’s waters such as the potential dangers of water pollution. The site also serves as a database for locals to post pictures of fish kill sightings as well as serving as a platform to get the water quality degradation problem from hog farms the attention it needs to prevent more fish kills from occurring (North Carolina Riverkeepers and Waterkeeper Alliance, 2012). Figure 2.2 depicts the areas at highest risk of toxic blooms along North Carolina’s Neuse River. From local reports, the site found that upstream estuarine waters are waters that are listed as impaired according to the Neuse River Basinwide Water
Quality Plan of 2009. These impaired waters are nutrient rich from nearby hog farms and also the location of fish kills in 2010 (North Carolina Riverkeepers and Waterkeeper Alliance, 2012).

These patterns in North Carolina correlate with the habitat characteristics of the fish kills in Chesapeake Bay. Dr. Pat Gilbert and her team collected water quality data from 1991 through 2008 which showed that the average number of *Prorocentrum minimum* has doubled, explaining the increased frequency in of fish kills in the bay. She also noted an increase in other harmful blooms over those years suggesting an impairment in water quality (University of Maryland Center for Environmental Science, 2015).
As seen in Figure 2.3, the team at the University of Maryland Center for Environmental Science found a pattern in water quality that led to the increase in blooms.

Figure 2.3: Distribution of Prorocentrum minimum in the Chesapeake Bay
(Figure adapted from Li et al., 2015)

The black triangles in the image to the left of Figure 2.3 represent areas experiencing a high occurrence of *P. minimum*. These areas are mostly located in the upstream tributaries; areas characterized by being warm, calm, and nutrient rich. These areas marked by the black triangles are also areas characterized by having a low salinity represented in red in the image to the right (Li et al., 2015). These characteristics of the bay make for favorable conditions for eutrophic waters, the best suited environment for *P. minimum* and other HABs to grow (University of Maryland Center for Environmental Science, 2015). Like *Pfiesteria*, *P. minimum* needs a nutrient stimulant for an outbreak to occur. These types of upstream estuarine environments are
those that *P. minimum* and *pfisteria* prefer, especially with a nutrient stimulant from the surrounding agricultural and farmland.

These characteristics and nutrient ratios have been shown to be similar to those along the south west Florida shelf, making Tampa Bay an area at risk for an outbreak of toxic dinoflagellates and other HABs (University of Maryland Center for Environmental Science, 2015). With an increase in human development and the effects of climate change, more and more of Florida’s water bodies are becoming the perfect habitat for these blooms/outbreaks to occur, making fish kills more common and more intense.

According to *Figure 2.4*, Tampa Bay along with other water bodies in Florida, are already experiencing a pattern of eutrophic conditions (Li et al., 2015).

*Figure 2.4: Map of Eutrophic Water Bodies*
(Figure adapted from Li et al., 2015, pg. 4)
What This Could Mean for Florida’s Waters:

Massive fish kills are something that Florida has seen before. Like North Carolina and Chesapeake Bay, Florida has experienced the effects that nutrient enriched waters can cause. Many of Florida’s major coastal water bodies, such as Tampa Bay, Sarasota Bay, St. John’s River, Charlotte Harbor, and Indian River, have similar characteristics to the Pamlico-Neuse Estuary and the Chesapeake Bay. These characteristics paired with similar threats from nutrient pollution from an increase in development and agriculture has made these water bodies areas at risk for an increase in intensity and frequency of fish kills. As seen in Figure 2.5, this is the distribution of *Pfiesteria* along the eastern coast as of 2001. The areas in red are locations where *Pfiesteria* has been confirmed during a major fish kill. The black circles represent locations where potential strains of *Pfiesteria* have been documented through standardized fish bioassays. Areas with stars represent locations where the toxic dinoflagellate has been detected using molecular probes. The bold red circle represents the area where the organism was first identified (Burkholder and Glasgow, 2001). Figure 2.5 shows the increasing problem toxic dinoflagellates could pose on the eastern coast, and what this could mean for those at risk coastal areas and our fish/seafood supply.
Florida Case Studies:

In 1997, Jacksonville’s St. Johns River was impacted from the same toxic dinoflagellates found in the Chesapeake Bay and Pamlico-Neuse Sound (Hollingsworth, 1997). The Florida Marine Research Institute determined that the sick fish found floating in the St. Johns River displaying their open sores was caused by the toxic dinoflagellate *Pfiesteria piscicida* or a very similar organism (Hollingsworth, 1997). Researchers, including Burkholder who identified the species in North Carolina, have confirmed that it is not *Pfiesteria* but a toxic species that looks and acts similarly. Although *Pfiesteria* was not the culprit in this massive fish kill, a similar and still unknown toxic dinoflagellate was the killer in the St. Johns River that year, demonstrating the lack of knowledge surrounding this new threat to Florida’s waters (Hollingsworth, 1997; Moestrup et al.,...
2014; Li et al., 2015). Just as with the cases in Maryland and North Carolina, the cause for the increase in fish kills has been linked to large amounts of runoff from agriculture fertilizers and farmland (Hollingsworth, 1997; Li et al., 2015; Moestrup et al., 2014). Although the exact species of toxic dinoflagellate has proven difficult to identify, the problem plaguing Florida’s waters, as well as those of North Carolina and Maryland, is increasing in frequency and intensity. An unprecedented influx of excess nutrients from plant and animal agriculture runoff are degrading our waters and creating the perfect habitat for the dinoflagellates to bloom (Paerl et al., 2014a).

Then in 2008, another massive fish kill occurred in the coastal waters of Florida. Thousands of dead catfish and menhaden were seen floating in Old Tampa Bay, causing motorists crossing the Courtney Campbell Parkway to complain (Salinero, 2008). In addition, a second fish kill was reported just north near Safety Harbor. Scientists of the Florida Fish and Wildlife Research Institute investigated the fish kills and determined that it was not the well known red tide, *Karena brevis*, but an unidentified toxic algae. It was reported that many toxic algae had been in bloom in Old Tampa Bay recently (Salinero, 2008). Although the exact culprit was not identified, it did bring to light once again the increase in occurrence of HABs and other toxic dinoflagellates. The upper part of the bay is the perfect environment for an algal bloom to occur. The waters there are calm, shallow, and not well circulated, similar to the conditions of the Pamlico-Neuse Estuary as with the inland tributaries of the Chesapeake Bay (Salinero, 2008).

In order to protect Florida’s water bodies that are at risk for an outbreak of toxic dinoflagellates or other HABs, a phytoremediation method is necessary to improve the
quality of water and remove the stimulant of these organisms causing the increase in intensity and frequency of fish kills.

**Florida Massive Fish Kill Patterns:**

Florida has been seeing an increase in intensity, frequency, and duration of massive fish kills over time. Utilization of Florida’s fish kill database, compiled and run by Florida Fish and Wildlife and Fish and Wildlife Research Institute, has given insight into the patterns occurring over the last decade and what this could mean for the future state of our waters (Florida Fish and Wildlife Conservation Commission, 2016a). Florida Fish and Wildlife (FWC) have a fish kill database containing all the calls/reports they receive to their “Fish Kill Hotline” regarding fish kills in the state of Florida. It is a statewide marine fish kill hotline mainly reported by local citizens with each report not always being confirmed or observed by scientifically trained individuals. FWC does perform follow ups on certain events but is unable to verify each account directly (Florida Fish and Wildlife Conservation Commission, 2016a). With that being said, there is a possibility that multiple people called in the same fish kill, as well as the possibility that the species and number affected are not complete or accurate.

The categories used for reporting ranged from: fish kill, mortality (bird, manatee, shark, crustacean, etc.), abnormal appearance, discolored water, or injured. The causes for the reports could range from: algae bloom, abnormal appearance, boat collision, cold stress, deformities, disease, high salinity, hurricane/storm, invasive species, injured, lesions, low dissolved oxygen, parasites, pollution, red tide, sewage spill, and sores. However, the cause was not listed with the majority of reports. The data
for each report recorded the date, city, county, call category, water body name, specimen count, and any comments which typically listed the species involved (Florida Fish and Wildlife Conservation Commission, 2016a).

In Figures 2.6, 2.7, and 2.8, fish kills of 1000 or more reported dead were considered to be massive. The reports typically reported mortality numbers around 100 or less, 1000, 2000, 3000, or occasionally more than 3000 dead specimens. Figure 2.6, 2.7, and 2.8 display the relative location, marked by different colored circles, of reported fish kills that had a mortality over 1000. The circles were used to represent each individual call. Some of these fish kills reported cover a large distance and the descriptions of the locations tended to be vague and not very descriptive. However, Figure 2.6, 2.7, and 2.8 serve as a visual representation of the number of fish kills reported that year and the location of those events. The years chosen to be examined were 10 years ago in 2006, 5 years ago in 2011, and the previous year of 2016. Each year was mapped separately to give a general idea of the trend and location patterns of massive fish kills occurring in Florida’s waters. These maps were developed using ESRI world imagery (ESRI, 2017).
Figure 2.6: Fish Kill Reports for 2006
(Melissa Kerr, map created January 2017)
Figure 2.7: Fish Kill Reports for 2011
(Melissa Kerr, map created January 2017)
As seen in *Figures 2.6, 2.7, and 2.8*, these maps give a visual representation of the areas at risk in Florida. They show the locations where the majority of massive fish
kills have occurred and they seem to be increasing in intensity and frequency over the years. In 2011 and 2016, Florida experienced some massive fish kills that spanned large areas and lasted for multiple days. These large-scale events were cross-referenced with local news reports.

2006 Fish Kills:

In this year, there were not any major massive fish kills that extended over a large area or had duration of multiple days. There was a total of 1,184 reports to the fish kill hotline, 53 of which were considered to be massive fish kills (over 1,000 reported dead). Out of those 53 considered massive, 10 of those were events with over 3,000 reported dead. The range of reported mortality from those 10 events ranged from 3,000-40,000 specimens reported dead.

2011 Fish Kills:

In the beginning of 2011, the Indian River Lagoon experienced a massive fish kill that killed over 30 different species spanning an area of 50 miles (Waymer, 2016). The cause of this fish kill is believed to be a toxic algal bloom other than the common red tide. The conditions of the lagoon during that time were characterized by high nitrogen levels, low dissolved oxygen levels, and high turbidity levels, the perfect conditions for a toxic dinoflagellate outbreak or HAB to occur (Waymer, 2016). This single event received 26 reports to the fish kill database and lasted almost two weeks.

Five months later, an area just south of the Indian River Lagoon in Port St. Lucie experienced another massive fish kill that received 14 reports. This report was
verified but no known cause was determined other than low dissolved oxygen levels (Killer, 2011).

Then in late September through early October another massive fish kill struck the eastern coast of Florida in the Banana River Lagoon, north of the Indian River Lagoon. “This is one of the largest kills on record in the Banana River Lagoon, I’ve documented more than 15 species ranging from mullet, puffers, and whiting to sheepshead, snook, trout, and redfish” stated outdoors journalist Ted Lund to CapMel: Conservation News. (CapMel Staff, 2016). The cause of this fish kill was more than likely attributed to the extremely warm temperatures that year as well as runoff from fertilizer applications in the area that triggered a brown algae bloom to occur (CapMel Staff, 2016).

There was a total of 1,395 reports to the fish kill hotline, 111 of which were considered to be massive fish kills. Out of those 111 considered massive, 7 of those were events with over 3,000 reported dead. The range of reported mortality from those 7 events ranged from 3,000-1,000,000 specimens reported dead. More massive fish kills occurred in 2011 than in 2006, with fewer of those events being over 3,000. However, a much larger range of those reported dead occurred in 2011.

2016 Fish Kills:

The trend of increasing intensity and duration of massive fish kills continued in 2016 when 2011’s record was broken with “an estimated hundreds of thousands of [dead fish were] floating belly up in brackish, polluted water as far as the eye can see” stated a report from CNN in March of 2016 (Gray, 2016). From March 18th to March
25th, 73 reports were called in to the fish kill hotline spanning for miles from Banana River Lagoon down to Indian River Lagoon. This fish kill not only affected the fish but also killed the oysters and half of the seagrass of that area (Gray, 2016). Submerged aquatic vegetation like seagrass plays a vital role in the health of a water body. They are excellent water cleaners and negatively affected by nutrient loaded waters and poor water quality. The cause of this massive fish kill event was a depletion of dissolved oxygen in the water caused by a toxic algal bloom and brown tide triggered by a combination of factors (Gray, 2016). This area as well as much of Florida had received very heavy rainfall during its normal dry season due to the influence of an El Niño event. This rainfall caused large amounts of pollutants and fertilizer to runoff into the water. In addition, it was unusually warm that time of year (Gray, 2016).

All these factors working together made for the perfect environment for a toxic dinoflagellate or HAB to occur. With climate change these factors will increase putting these areas at a higher risk and creating more new areas at risk for massive fish kills to occur. The time to do something is now, our waters and economy cannot continue to take hits like this. “It (will be) hard to recover. You never fully recover” stated a local fisherman in the CNN report (Gray, 2016).

Other smaller scale fish kills occurred over the course of that year, in addition to a red tide occurrence that killed thousands of fish along Florida’s western coast affecting Pinellas, Manatee, and Sarasota counties (Richmond, 2016). However, none to the extent of the one that plagued the eastern coast.

Out of the 1,804 total reported events to the fish kill hotline that year, 122 of those were considered to be massive fish kills. Compared to the 1,395 total reports for
2011 out of which 111 were massive fish kills and the 1,184 in 2006 that had 53 that were massive fish kills (Florida Fish and Wildlife Conservation Commission, 2016a). Out of those 122 massive fish kills for 2016, 9 of those were events with over 3,000 reported dead. The range of reported mortality from those 9 events ranged from 3,000-2,000,000 specimens reported dead.

It is clear that massive fish kills are increasing in occurrence as well as intensity and duration. However, the database does not account for the possibility of duplicate calls or any inaccuracy in reporting with every event not being verified. Replicated reporting could make these large-scale events look like multiple smaller ones. For instance, there could have been 10 reports called in with an estimation of 2,000 specimens dead per report; when in actuality it could be one massive event of a mortality of 20,000.

**Models:**

Phytoremediation is a natural method to clean up an environment and can be more effective than industrial and mechanical methods. This practice uses living plants for the removal, degradation, or containment of contaminants in soils, sludges, sediments, surface water and groundwater (United Nations Environment Programme, 2016). The plant to be examined and used as a hydroponic zone is the controversial water hyacinth.
Water Hyacinths:

Called a non-native and invasive species, water hyacinths (seen in Figure 2.9), scientifically called *Eichhornia crassipes*, have been cause for much controversy in the south-eastern United States. Originally found in Brazil, it has made its way into the waters of the U.S. and many other countries (Florida Fish and Wildlife Conservation Commission, 2016b). It was introduced into Florida’s waters in the 1880s and has exploded in numbers since. When left uncontrolled, the plants can overtake waterways and choke out the native species. Many efforts by the Florida Department of Environmental Protection and the U.S. Army Corps of Engineers have been made to reduce their numbers. It has even been made illegal in Florida to possess this plant without a special permit (United States Department of Agriculture, 2016; Florida Fish and Wildlife Conservation Commission, 2016b).

A water hyacinth is a perennial aquatic plant that has broad thick leaves and free-floats at the water’s surface with its long feather-like roots suspended below as seen in Figure 2.10. It can grow to a height of one meter and grows pink to lavender
flowers. The roots are long and feather like and the plant can grow up to twice its size in less than two weeks (Florida Fish and Wildlife Conservation Commission, 2016b).

Water hyacinths are a very hardy plant that can grow in rivers, lakes, and ponds and prefer waters that are nutrient-enriched but can survive in waters with low nutrient levels (Department of ecology: State of Washington, 2016). Water hyacinths do not tolerate a high salinity environment. They do not grow higher than 40 degrees North and 45 degrees South latitude. The plants are in full bloom in the summer and early fall, which coincides with high nutrient input trends in the Chesapeake Bay and the Pamlico-Neuse Estuary (Department of ecology: State of Washington, 2016). Water hyacinths cannot withstand temperatures below freezing but can survive unaffected by a drought for short periods of time, living only in moist soil (Rezania et al., 2015). They can reproduce both sexually from a seed as well as vegetatively by budding (Department of ecology: State of Washington, 2016).

Figure 2.10: *Eichhornia crassipes*
(Melissa Kerr, photos taken October 2016)

This plant has one of the highest growth rates of any known plant and can survive in a relatively wide range of habitats. Its temperature tolerance ranges from 54
degrees Fahrenheit to 95 degrees Fahrenheit, but prefers around 86 degrees. Its pH tolerance is estimated to be between 5.0 to 7.5 (Fish and Wildlife Conservation, 2016).

**Why Water Hyacinths:**

The plant’s remarkable growth rate is the reason people call it a pest. When water hyacinth growth goes unchecked and overtakes a body of water it can lower the dissolved oxygen concentrations, add excess plant material to the bottom of waterways, dam up the flow of waterways, and decrease biodiversity of the natural flora and fauna (Fish and Wildlife Conservation, 2016). However, when properly utilized and controlled, a waterway can benefit from this plant’s untapped abilities without degrading the ecosystem.

Water hyacinths have been shown to greatly reduce the nutrient levels in water bodies; in fact it is in nutrient over-enriched waters that these plants thrive. The main nutrients absorbed are nitrogen and phosphorus. These are the main culprits in non-point source pollution of our waters, and typically come from surrounding agricultural land (Department of ecology: State of Washington, 2016; Wang et al., 2012; Wetland Solutions Inc., 2013). In addition, they are also effective in the removal of suspended solids, biochemical oxygen demand (BOD), organochlorides, and heavy metals (Department of ecology: State of Washington, 2016).

Unlike other similar native plants that are known to improve water quality, like water lettuce, water hyacinths are much hardier. They can tolerate low salinity levels, short periods of drought, a wider range of habitats and environmental conditions as well as being able to live in heavily degraded water (United State Department of Agriculture,
Many studies have been conducted on the potential benefits of water hyacinths and its water purification properties in freshwater; however, very few studies have been conducted in estuarine waters or have examined its potential application to reduce fish kills.

**Freshwater Study 1:**

There is much controversy about the presence of water hyacinths in our waterways; however, the plant’s nutrient absorption capabilities cannot be debated. Many studies have been conducted in China and other Asian countries due to water hyacinth’s prolific growth there and the extreme eutrophication issue of most of their water bodies. Around 54% of China’s lakes are eutrophic (Wang et al., 2012). One of those studies looked at the effects of water hyacinths for nutrient removal in Baishan Bay of Lake Dianchi in China (Wang et al., 2012). Lake Dianchi had become a large shallow eutrophic lake due to degradation from human activities. They chose water hyacinths as their remediation method because it improves the quality of water from decreasing the level of pollutants as well as decreasing the turbidity (Wang et al., 2012). In addition, the water hyacinth is also found to thrive in polluted and eutrophic water (Wang et al., 2012). In this study, water hyacinths were implemented attached to mats in different areas of the bay. Water quality analysis during the course of the study revealed that the water quality improved in areas around the water hyacinth mats. This study served as an example in showing how these plants can be used for nutrient removal in eutrophic lakes (Wang et al., 2012).
**Freshwater Study 2:**

A similar study was conducted in the fresh spring water of Kings Bay, located just north of Tampa Bay, by the Florida Springs Institute in collaboration with the Kings Bay Springs Alliance, Save the Manatee Club, and Stetson University. This study was carried out in an attempt to restore Kings Bay by killing the algae degrading the bay’s water quality (Wetland Solutions Inc., 2013).

The Kings Bay Phytoremediation Project (KBAM) began in 2012 where water hyacinths and water lettuce, a plant with similar nutrient absorbing characteristics to water hyacinths, were corralled together by floating PVC-pipe cages and placed along Kings Bay (Wetland Solutions Inc., 2013). Utilizing 12 cages containing roughly 50 relatively mature plants harvested near the project site, they hoped to reduce the concentration of nutrients in the water, thereby reducing the algae in the waters and improving the quality of water. Improved and reinforced cages were needed because the original design was unable to withstand the effects of strong storms and allowed some of the plants to escape. These cages comprised of pvc-pipes with mesh netting that extended two to three feet above the water line. These cages were anchored to the river bottom. This reinforced design has not lost any plants since its implementation over the course of the three-year study (Wetland Solutions Inc., 2013). However, since water hyacinths can reproduce by seeds there is a chance that they can spread outside their PVC-pipe cages. The Kings Bay study figured that any seeds that escaped and grew were likely eaten by manatees who have been known to eat water hyacinths (Wetland Solutions Inc., 2013).
According to a progress report released in 2013, researchers found that due to a combination of events a viable plant community was not able to establish, and therefore no measurable impact on improving the water quality was made (Wetland Solutions Inc., 2013). They believe that due to the manatees feeding on the plants in this area and high salinity levels, a plant community was not able to establish (Wetland Solutions Inc., 2013). However, the progress report did not include critical data, such as the exact salinity levels that potentially caused the plants to be unable to establish, the age of the plants used, whether an incubation period was included, or the characteristics of the reference site.

In the beginning of 2016, they ended the project and began the removal of the plants and cages. Although the study did not produce any measurable impacts on the water quality, this study did address the real-world application of implementing water hyacinths in a growth controlled enclosure. The King’s Bay study demonstrated that the plants can exist in a waterbody without escaping and taking over the habitat.

**Other Freshwater Studies:**

In other studies, researchers tested the effectiveness of water hyacinths in treating wastewater by removing heavy metals, organic and inorganic pollutants (Rezania et al., 2015). These studies attempted to use water hyacinth for its pollutant absorption qualities since controlling the plant’s growth has not proven very effective in the past and the plant has been spotted in most countries now, spanning across North America, Europe, Asia, and Australia (Wang et al., 2012; Rezania et al., 2015). With increasing population and urbanization, more waste and pollutants are being dumped
into our water bodies. Wastewater treatment is very expensive and can cause environmental damage. Eutrophic lakes now account for “54%, 53%, 28%, 48% and 41% of lakes in Asia, Europe, Africa, North America and South America” respectively (Wang et al., 2012). This is why phytoremediation techniques are vital to improving water quality and thereby reducing the frequency and intensity of fish kills. These other studies demonstrate that water hyacinths can be used to improve the water quality of polluted rivers, municipal waste water, and eutrophic lakes and conclude that water hyacinths can be used in a wide application of waste waters and greatly improve the water quality by reducing many nutrient levels by as much as 45% (Rezania et al., 2015). Water hyacinths have been shown to reduce levels of heavy metals and organic and inorganic nutrients in freshwater bodies.

**Water Hyacinths, a green solution:**

Others have also found water hyacinths to be a potential solution to our environmental problems. In a local newspaper article, Florida entrepreneurs George Boynton (board builder) and Bruce Prezzavento (chemist) discussed the amazing ability of water hyacinths to remove excess nutrients from Florida’s rivers and canals as well as their proposal to harvest the plants to then be turned into a high-grade potent fertilizer to be sold to farmers (Gruss, 2011). They found that by burning the plants and using the ash-like material, called biochar, as fertilizer it can increase their crop yields. Biochar does not run off from the fields like many other fertilizers on the market do when it rains, therefore greatly decreasing the amount of fertilizer used, which makes happy farmers, and less nutrient runoff which makes happy environmentalists (Gruss, 2011).
Biochar is almost as potent as chicken waste but much less harmful. This could potentially help lessen the use of harmful fertilizers and therefore reduce the amount of nutrients flowing into our waterways. However, their plan has yet to take off pending government funding as of 2011 with no current status update (Gruss, 2011). Water hyacinths can be used for: wastewater treatment, production of bioenergy, manufacturing of fertilizer, and paper products (Rezania et al., 2015). Their application can have endless benefits. The plants can be utilized while living as well as after they die.

Water hyacinths have numerous uses that are still being discovered today (Bolenz, 1990). The plants fibers can be used as material to make things from baskets, to crates and even furniture as seen in . There are even popular online sites that sell products made from water hyacinth fibers such as www.wayfair.com and www.zulily.com. Despite their controversial past, it is possible to look forward and see the many benefits these plants can offer when practiced in a controlled method.

**Water Hyacinths and Brackish Waters:**

There have been many studies conducted on the effectiveness of water hyacinths in freshwater; however, the effectiveness of water hyacinths to uptake excess nutrients in a low-salinity estuarine system has not been as widely studied.

**Nutrient and Plant Growth Study 1:**

One of the few salinity studies was conducted in France to test the effects salinity has on water hyacinth growth and the plants’ potential to improve the water
quality of Berre Lagoon. This lagoon has salinity levels that range from 2-14 parts per thousand (ppt) (De Casabianca and Laugier, 1995). This study examined water hyacinth production at different salinities as well as the plants’ ability to recover after saline shock in hopes of demonstrating the potential application of water hyacinths to treat waste water in a semi-saline environment.

The De Casabianca and Laugier study was conducted in a greenhouse utilizing six tanks of different salinity levels and monitored for 28 days. A second portion of the study was conducted in tanks with freshwater flow-through to test the plants’ ability to recover from saline shock (De Casabianca and Laugier, 1995). The researchers took into account nutrient limitation and added nutrients at the beginning and at the end of the experiment to eliminate nutrient availability as a limiting factor (De Casabianca and Laugier, 1995).

De Casabianca and Laugier (1995) concluded that nitrogen nutrient level reduction was negatively linked to salinity level but did not provide any data on the rate of nutrient absorption or by how much the nitrogen levels were reduced (De Casabianca and Laugier, 1995). The De Casabianca and Laugier study also demonstrated the plants’ ability to grow in low-saline waters. The plants in the low salinity tanks had production rates that doubled in growth at the conclusion of the study (De Casabianca and Laugier, 1995). In the second stage of the study, the tanks had a flow-through system of freshwater to examine the plants’ ability to recover from a saline shock. Those with mid salinity levels did not experience positive growth but remained alive. Those in high salinity levels experienced necrosis and irreversible damage. Despite no positive growth in mid to high salinity levels, the plants’ survival in the mid-
salinity level demonstrated the potential application for these plants to improve waste water in low saline conditions for “purification can be effective with a production close to zero” (De Casabianca and Laugier, 1995, pg. 42). It also demonstrated the type of ecosystem these plants can survive in with the plant’s ability to recover from a mid-range salinity shock.

**Nutrient and Plant Growth Study 2:**

A similar study was conducted in Waccamaw River in South Carolina, a freshwater river that experiences tidal fluctuations and saltwater intrusion, to assess the plants’ growth rate under these conditions (Rotella, 2010). This South Carolina study was part of a graduate thesis and served as a guideline for designing my experiment and aided in the selection of variables to be tested.

Water hyacinths are established and currently managed in South Carolina (Rotella, 2010). The section of the Waccamaw river studied was freshwater, had low oxygen levels, a low nutrient system, and the lower reaches of the study area were estuarine from tidal influxes (Rotella, 2010). The Waccamaw River is joined by the Atlantic Intracoastal Waterway and then empties into the Winyah Bay in Georgetown, SC (Rotella, 2010). Eighteen cages constructed from netting and PVC piping were used in the upper, middle, and lower portions of the section of the Waccamaw River being studied. Each cage was fixed to the river bottom to account for tidal changes and storms and each cage was populated with six young water hyacinths (Rotella, 2010).

The experiment ran for one month and ten plants from each cage were randomly selected for analysis. The plants’ absolute growth change was determined by new
leaves, new offshoots (new buds), root length, longest leaf length, widest leaf width and stem base diameter (Rotella, 2010). This growth change showed biomass almost doubling, with the majority of the biomass being in the roots and leaves. The greatest increase in biomass came from the plants further upriver with 0.0 ppt salinity (Rotella, 2010). Those plants grown in greater salinity found little to no growth and some died or were irreversibly damaged. This South Carolina study concluded that the greater the salinity, the greater the decrease in plant productivity, although their results did show some growth of plants in water at 3.0 ppt (Rotella, 2010).

However, the site had low concentrations of nutrients necessary for the growth of water hyacinths, perhaps causing the low growth rate in plants at higher salinity. Water quality was also not analyzed and instead examined through nutrient accumulation by the plants. This South Carolina graduate study mainly addressed the effects of salinity on the growth rate of water hyacinths in a natural environment that experiences salt water influxes and served as a framework for selecting a salinity range to test water hyacinth’s nutrient absorption rates as well as the parameters to measure plant growth.

**Salinity Level Tolerance Study:**

Another graduate thesis study looked at the effects salinity can have on the growth of water hyacinths and its possible application for a method of control of the species (Cheng, 2004). This study served as a guideline for my experiment’s parameters and salinity levels to be tested. Cheng’s study was conducted on water hyacinths collected from a tidal marsh and marina in California with salinity levels of 0.1 parts per thousand (ppt). The plants were kept in 15 liter tanks inside a greenhouse to
assess at what salinity levels water hyacinth growth was negatively affected (Cheng, 2004).

Two trials were conducted for one week each. Each tank had quarter strength nutrient water and salinity concentrations of 0.5 ppt, 2.25 ppt, 4.5 ppt, 9 ppt, and 18 ppt. To determine growth, leaf number, length, width and plant color were measured. Leaf number was counted visually and any new leaf bud was considered as positive growth and any leaf that rotted or wilted was considered as negative growth (Cheng, 2004). Leaf length was measured from the base of the root up till the apex of the leaf of the largest leaf and width was measured from the widest points on the largest leaf. Leaf color had two categories, green being healthy and yellow being unhealthy, utilizing the Munsell Color Chart for Plant Tissue (Cheng, 2004).

Cheng’s greenhouse study found that all the plants in a salinity solution showed some wilting on the larger leaves. The plants in 9 and 18 ppt showed drastic changes in leaf color, number and tissue stability. New shoots in tanks of salinity above 4.5 ppt started to wilt towards the end of the experiment.

Cheng concluded that those plants in a salinity level lower than 4.5 ppt were not negatively affected by the salinity. Leaf number and color of those plants in 4.5 ppt showed a slight decrease in leaf number, but not as drastic as those in higher salinities (Cheng, 2004). Cheng also found that the younger plants were more greatly affected by the salinity levels than the larger more mature plants (Cheng, 2004). This study served as a framework to choose a salinity level to test as well as parameters to measure plant growth in my experiment.
**Salinity Recovery Study:**

Another graduate thesis study was conducted in the San Francisco Bay Delta assessing the recovery from salinity induced stress on water hyacinths (Wunder, 2005). This study was similar to that of De Casabianca and Laugier (1995) where they looked at the plants’ ability to survive after a saline shock of 28 days, and looked at the ability of the plant to recover from salinity induced stress based on these factors: exposure duration, salinity level, and recovery time (Wunder, 2005). Salinity levels used were 5, 6, and 7 ppt for 7, 14, 21, and 28 day periods of exposure. Each plant was allowed 7 to 14 days to recover in non-saline water. Wet plant weight and leaf chlorophyll were used to indicate the plants’ health.

Wunder’s study found that the base level needed for water hyacinth mortality with no recovery time would occur in a salinity level of 7 ppt or greater exposed for 21 days. This was similar to the findings of the study conducted in France (Wunder, 2005; De Casabiana and Laugier, 1995). Wunder’s study also determined that the plants’ ability to recover decreased the greater the salinity level and the greater the exposure. Recovery was seen, however, in new buds/growth but not in the original existing leaves of the plants in those of a salinity level less than 7 ppt if allowed recovery time (Wunder, 2005). This again correlates with the findings of the France lagoon study in the tanks that had a freshwater flow-through system as a second stage to their salinity study (De Casabianca and Laugier, 1995). Leaf chlorophyll was not a conclusive indicator and wet plant weight also created some issues with the saline water weighing more than freshwater (Wunder, 2005).
However, the ability of water hyacinths to recover after a saline shock is of great importance for its application to estuarine environments to combat fish kills. These environments can be affected by tidal changes and storms which could potentially bring in higher salinity levels. Therefore, the ability of the plant to recover from one of these events is vital to its effectiveness at reducing nutrient levels. This study demonstrated the potential application of water hyacinths in low-saline estuarine environments as well as providing a guideline of salinity levels to test and duration of the experiment.

Gaps in Knowledge:

The previously mentioned studies by Wunder (2005), Cheng (2004), Rotella (2010), and De Casabianca and Laugier (1995), examined the effects that different levels of salinity had on the growth rate of water hyacinths in both natural and controlled environments. The ability of the plant to recover from salinity influxes was also studied for the plants’ application in tidal ecosystems or areas that can be influenced by storm surge. However, these studies did not address the effectiveness of the plant’s nutrient absorption rates in those brackish environments. It was not determined if the rates are slowed or possibly not as effective as those nutrient absorption rates in freshwater. The studies also had many limiting factors that could have altered their results such as nutrient availability and temperature fluxes.

In addition, some of these studies conducted in natural environments were in areas not necessarily favored by toxic dinoflagellates like *Pfiesteria piscicida* and *Prorocentrum minimum* or other harmful algal blooms. The areas at greatest risk for an increase in frequency and intensity of fish kills are much shallower,
calmer, warmer, and more nutrient rich than those used in the majority of the previously mentioned studies. These studies, however, did give potential real-world application to the survival of the plants in low-brackish waters but not its application for nutrient absorption rates in those or similar environments.

In a controlled environment, I established the nutrient absorption rates by the water hyacinths in a low-saline environment and determined if these rates are comparable to those in fresh water and therefore a viable option in phytoremediation. This following study will artificially replicate an upstream estuarine ecosystem, shallow, calm, low-saline, with over nutrient-enriched waters. These are the habitats ideal for a massive fish kill to occur as well as habitats favored by water hyacinths. These plants have mainly been used in studies in attempts to improve water quality and clarity in freshwater systems but not as a method to reduce fish kills in estuarine environments. The plants potential policy application to reduce the intensity and frequency of massive fish kills in at risk areas along Florida’s estuarine systems has not been looked at to my knowledge.

**Research Question:**

Are nutrient absorption rates (ammonia nitrogen and reactive phosphorus) by water hyacinths affected by low-salinity levels. The hypothesis of this study is that nutrient rates of the experimental tanks in 4.45 ppt would be similar and comparable to the rates of the control tank of 0.0 ppt and that salinity would not have a strong measureable effect on nutrient uptake rates.
CHAPTER 3:
GOALS, VARIABLES, AND METHODS

Purpose and Goal:

The purpose of this experiment was to test the effectiveness of nutrient absorption (ammonia nitrogen and reactive phosphorus) by water hyacinths in a controlled brackish water environment for its potential application as a phytoremediation method to reduce the intensity and frequency of massive fish kills in those environments.

The salinity level of the brackish water in other studies slowed the growth rate of the water hyacinths to help control their spread, making their application in the wild more practical. Nutrient absorption rates by the water hyacinths in brackish waters were similar to those rates found in freshwater environments.

The goal was to reduce the nutrient levels by 45% which is the EPA’s nutrient reduction goal for most eutrophic waters in the U.S. (Environmental Protection Agency, 2016). Starting nutrient levels had ammonia nitrogen at 2.5 mg/l and reactive phosphorus at 0.27 mg/l. These levels are well above the goal nutrient levels of many water bodies such as the Chesapeake Bay, with a nutrient goal of nitrogen at 1 mg/l and phosphorus of 0.05 mg/l (Santoro, 2007). This will be tested utilizing 375 liter tanks, five replicas (experimental tanks) with one control and one of a greater salinity for comparison in an indoor environment normally used for aquaculture.
The hosting facility was provided by the Florida Fish and Wildlife Conservation Commission. The hosting facility, seen in Figures 3.1 and 3.2, is the Florida Bass Conservation Commission also known as the Richloam Fish Hatchery located in the Richloam State Forest in Webster, Florida. A special thanks goes out to all those at the Richloam Fish Hatchery that made this all possible.

**Materials:**

- Seven, 375-liter tanks
- freshwater
- marine salt
- fertilizer (N & P source)
- about 56 individual water hyacinths
- UV lights

![Figure 3.1: Experiment Facility](image1)

![Figure 3.2: Tanks](image2)

(Melissa Kerr, photos taken October 2016)

**Methods:**

The following parameters were chosen to duplicate the environment in the Chesapeake Bay, Pamlico-Neuse Estuary, and similar estuarine water bodies in Florida
that have experienced the majority of toxic dinoflagellates and HABs that led to an increase in fish kills. As described earlier, these areas are located further inland along contributing waterways where the waters were shallower, warmer, and calmer. These areas are characterized by low-salinity brackish waters, part of the mixing zone upstream of estuaries, lagoons and bays. These are also the areas that receive the greatest influx of nutrients from runoff of plant and animal agriculture before being deposited into our larger water bodies. Nutrient rich waters are the perfect breeding grounds for toxic dinoflagellate and other harmful algal blooms as well as the preferred environment for water hyacinths to flourish. Nutrient loaded waters are also a growing concern for many water bodies across the globe, including Florida and the Tampa Bay (Paerl et al., 2014b).

The control and high salinity comparison tank had the same amount of plants and eutrophic conditions as the five experimental tanks. The five experimental tanks were all maintained at the determined salinity level and nutrient concentrations while the control had all the same parameters but used fresh water at 0.0 ppt (Florida Fish and Wildlife Commission, 2016). The same parameters were also used in the high salinity tank that had a salinity level maintained at 7.0 ppt. This allowed for multiple readings to determine the plant’s absorption rates and a freshwater control and high salinity tank for comparison.
Ecosystem Design:

1. Each tank was filled with 370 liters of fresh water obtained from the hatchery that treats onsite to meet aquaculture standards. Water quality standards are very strict to protect the health of the fish. Water must be free of contaminants and nutrients. No soil/sand substrate layer was used in the tanks to eliminate the possible absorption of nutrients by the soil and affect the nutrient absorption rate of the plants.

2. Marine salt from INSTANTOCEAN Marine Aquarium Sea Salt, model #SS1-160P was added to each tank excluding the control tank to achieve the predetermined salinity levels. The salinity level was maintained at 4.45 parts per thousand (ppt) for the five experimental tanks, 7.0 ppt. for the high salinity comparison tank, and 0.0ppt for the control tank. The average salinity of an upstream estuarine environment is around 4.45ppt, the appropriate middle range of low-salinity brackish waters (National Oceanic and Atmospheric
The average salinities of low-salinity brackish range from 0.05-7.0 ppt, mid-salinity from 7.0 to 14.0 ppt, and high-salinity brackish above 15 ppt (National Oceanic and Atmospheric Administration, 2008). Salinity level of 4.45 ppt is also the salinity level that showed the greatest success in the previously mentioned salinity studies that served as guidelines for developing this study.

3. The average pH of a brackish water ecosystem is about 7.2 to 7.5 and was maintained at those levels for the duration of the experiment (National Oceanic and Atmospheric Administration, 2008). Average dissolved oxygen (DO) levels were maintained above 5 mg/L which is the minimum standard according to the Florida water quality standards for healthy fish to live (Environmental Protection Agency, 2016). Artificial UV light was used as needed, as the tanks were located in an indoor aquaculture facility that received only partial, indirect natural light. The system was a closed system with no water flow, similar to the desired conditions where the majority of massive fish kills occur.

4. Artificial inorganic ammonium polyphosphate 10-34-0 manufactured by Growers Fertilizer Corporation, model #F336 was added to each tank to achieve the predetermined ammonia nitrogen and reactive phosphorus concentrations (N: 1.50 mg/l and P: 0.27 mg/l). Eutrophic water conditions were artificially created using standard ammonia polyphosphate fertilizers ((NH₄PO₃)ₙ). This fertilizer is used by the fish hatchery when zooplankton growth in the ponds is too low for the fish
to feed and need a boost to rapidly increase their growth. Nitrogen (N) levels above 1.5mg/l and Phosphorus (P) levels above 0.1mg/l are necessary to achieve eutrophication of a water body. According to the EPA, levels cannot exceed that amount if the water body discharges into a larger body of water (Environmental Protection Agency, 2016). Therefore, to achieve eutrophication without making the waters toxic, the tanks’ initial nitrogen concentration was 2.5mg/l and the phosphorus concentration at 0.27mg/l.

5. Eight water hyacinth plants were added to each tank. Plants were chosen that are relatively mature parent plants. This was defined as a plant with a healthy root system that had only one new bud sprouting from that parent plant. These new plants were separated from the parent plant to allow for individual assessment. Previous trials have demonstrated that young smaller plants that have not grown any offshoots/buds did not survive the transportation/transplant phase. The plants were obtained from local storm water ditches using a permit issued by the Florida Department of Agriculture and Consumer Services. According to code 5B-64.011, water hyacinths are listed as a prohibited aquatic plant requiring an application and permit to move such organisms regulated by the state of Florida, reference permit #2016-062 (United States Department of Agriculture, 2016).

6. An incubation period of three weeks was met to establish a stable ecosystem, and to eliminate any possible plant mortality from
transplant. The water of the tanks did not have any salt or fertilizer added to it so the plants could establish and stabilize after being transported to the testing facility.

7. Initial plant growth measurements were taken based on the criteria delineated below in the “plant growth monitoring” section.

8. Initial water quality measurements, delineated below in the “water quality monitoring” section, were taken from all tanks.

Timeline:

1. After the three-week incubation period, all the outlined parameters of plants growth and water quality were measured for initial readings.

2. The plants were then introduced into each tank.

3. Over the course of four weeks, the water quality of the tanks and plant growth were measured using the outline parameters and testing methods below in the “Methods” section.

4. At the conclusion of the experiment, the water was sent to the treatment facility at the hatchery. The water hyacinths were burned and their ashes used to fertilize the ground. These steps were necessary in obtaining the permit for these plants.

Monitoring:

Nitrogen (ammonia nitrogen) (NH₃-N)

1. EPA test method 350.1
2. Measured in mg/l

3. A 10ml sample was collected from each tank including the control tank at 14:00 twice a week on Tuesday and Friday for the duration of the experiment.

4. Using the salicylate nitrogen test method from the Hach Company, using a HACH DR/890 Colorimeter, the total ammonia nitrate level was determined and recorded for each sample.

**Phosphorus (orthophosphate) (PO$_4^{3-}$)**

1. EPA test method 365.3

2. Measured in mg/l

3. A 10ml sample was collected from each tank including the control tank at 14:00 twice a week on Tuesday and Friday for the duration of the experiment.

4. Using the ascorbic acid method from the Hach Method 8048 of the HACH Company, using a HACH DR/890 Colorimeter, the reactive (ortho) phosphate (phosphorus) level was determined and recorded for each sample.

**Salinity**

1. Measured in parts per thousand.

2. Salinity was obtained using a refractometer SR-6 manufactured by VITAL SINE.
3. Levels were maintained at 4.45 ppt (for the 5 experimental tanks), 7.0 ppt (for the high salinity comparison tank), and 0.0 ppt (for the control) and was adjusted with additional fresh water or marine salt accordingly.

**pH**

1. Measured on a scale of 0-14
2. A 50ml sample was collected from each tank including the control tank at 14:00 twice a week on Tuesday and Friday for the duration of the experiment.
3. pH was determined using a bench pH/conductivity meter, Oakton PC510 pH meter, manufactured 12/03, model # 68X090816.

**Dissolved Oxygen (DO)**

1. Will be measure in mg/l
2. Measurements were obtained using a dissolved oxygen instrument manufactured by YSI Environmental 2006, YSI 550A, and was calibrated weekly.

**Temperature**

1. Measured in degrees Celsius.
2. Measurements were obtained using the dissolved oxygen meter manufactured by YSI Environmental 2006, YSI 550A, and was calibrated weekly.

Turbidity
1. Measured in formazin attenuation units (FAU)
2. A 10ml sample was collected from each tank including the control tank at 14:00 twice a week on Tuesday and Friday for the duration of the experiment.
3. Turbidity was obtained using the absorptometric method from Hach Method 8237, HACH Company, using a HACH DR/890 Colorimeter, to determine the turbidity of each tank and recorded for each.

Plant Growth
1. Length, height, and width are recorded in millimeters.
2. Plant tissue color was recorded according to the Munsell Color Chart for Plant Tissue.
3. The growth rate of the water hyacinths was monitored by measuring plant height, plant width, root length, largest leaf length and width, number of wilted leaves, number of new leaves, and number of new buds (plants).
4. Plant height was obtained by measuring from the plant base to the tip of the tallest leaf tip.
5. Plant width was obtained by measuring from the farthest outreaching leaves from the leaf tip.

6. Largest leaf length was measured from leaf base to leaf tip and leaf width measured from the widest part of the leaf.

7. Number of wilted leaves, number of new leaves/buds was counted and recorded.

8. The color of the leaves which was indicative of the plants health was determined using the Munsell Color Chart for Plant Tissue.

9. Measurements were taken at 14:00 at the beginning and at the end of the experiment.
CHAPTER 4:
RESULTS AND DISCUSSION

Water Quality:

The data collected from the five experimental tanks of 4.45 ppt showed improvement in the quality of water by the conclusion of the experiment. The control tank of 0.0 ppt showed the greatest improvement as expected. A greater than 45% reduction in nutrient levels of the control tank demonstrated the known ability of water hyacinths to improve the water quality in freshwater and served as a comparison to the rates of the experimental tanks at 4.45 ppt. Of the experimental tanks, ammonia nitrogen levels were reduced by an average of 0.9 mg/l, a 36% reduction. Reactive phosphorus levels were reduced by an average of 0.13 mg/l, a 48% reduction. Turbidity levels were reduced by an average of 18 FAU, a 67% improvement in water clarity.

The high salinity comparison tank showed no noticeable improvement in water quality for all the plants died after one week. The plants in the high salinity comparison tank began to wilt and turn brown after the first three days of the experiment. This aligns with previous studies confirming that water hyacinths cannot tolerate higher salinity levels of 7.0 ppt. In Figures 4.1, 4.2, and 4.3, one can see the difference in nutrient absorption rates of the experimental tanks of 4.5ppt in comparison to the control tank of 0.0 ppt. This difference is comparable to the rates of the control tank. The average of all
the experimental tanks (seen in the “Appendix” section) was used to compare to the rates of the control tank and the high salinity tank.

![Figure 4.1: Change in Ammonia Nitrogen Levels](image1)

![Figure 4.2: Change in Reactive Phosphorus Levels](image2)
As seen in Figures 4.1, 4.2, and 4.3, the nutrient absorption rate of the experimental tanks was on average slower and slightly less than that of the control tank. This was expected and likely due to the stressed health of the plants from the salinity as well as the initial shock of the introduction to the saline waters. In regards to the change in turbidity, all the tanks improved while the high salinity comparison tank worsened. This could be explained by the plants breaking down and decomposing in the tank towards the end of the experiment.

The improvement in water quality of the experimental tanks at 4.5ppt demonstrated the plants tolerance to low saline levels as well as the practicality of these plants being used in upstream estuarine environments. Although nutrient reduction levels were slower and less than the control tank of 0.0 ppt, nutrient levels were still lowered and neared nutrient level target goals for many similar estuarine environments as well as Tampa Bay (Janicki Environmental, Inc., 2011; Santoro, 2007).
**Plant Growth:**

At the beginning of the experiment, following the incubation period, all the water hyacinth plants were healthy and green. Health was determined by plant color according the Munsell Plant Tissue Color Charts as described in the “Methods” section previously. Those plants that did not survive the incubation period or experienced any negative effects on the health of the plants after the incubation period (any noticeable wilting, spots, impaired buoyancy, or change in color) were not used in the experiment. As discussed previously, different ages (young and mature) of plants were used in the first trial of the incubation period to see if age was a factor in survival rate. The young, smaller plants (without any off shoots/new buds) did not survive the incubation period. The larger mature plants that had formed off shoots/new buds all survived and were overall very healthy (off shoots/new buds were separated prior to the incubation period and experiment to assess each plant individually).

The plants used all had an average leaf color of 5GY 5/4 as seen in *Figure 4.4*. Leaf color of 5GY 5/4 was used as a base line for plant health; any deviation would be considered negative health. The GY stands for the hue, green-yellow. The numbers stand for the scale of value and scale of chroma. A decrease in value from the starting color of 5GY to a 2.5GY was considered an indicator of impaired health. The image below displays the plants leaf starting color creating the baseline to measure health. Any decrease in that value, movement up the chart in leaf color, to a value of 2.5GY or lower was considered to be impaired health.
As seen in Figure 4.5, the majority of the plants in each tank had a very high percentage of plants with leaves of 5GY 5/4 at the beginning of the experiment, indicating good plant health. The percentage represented by the red color indicates the plants in each tank that had a leaf color of 2.5GY and lower on the scale of value according to the Munsell Plant Tissue Color Book. Those belonging to this group were considered unhealthy.
At the end of the experiment there was a noticeable change in plant health by the experimental tanks as seen in Figure 4.6. The high salinity tank experienced total mortality by the end of the first week and was breaking down in the water by the conclusion of the experiment. The control tank, however, did not experience the same noticeable change in leaf color or mortality with about 85% of the leaves of the tank still maintaining a 5GY on the scale of value. This difference can likely be attributed to the stress the salinity was causing on the plants’ health, which was expected. Water hyacinths are perennial plants and can potentially live indefinitely as demonstrated by the plant health of the control tank (Fish and Wildlife Conservation, 2016). The plants in the five experimental tanks were beginning to die and break down in the water as shown by the increase in percentage of leaves of impaired health suggesting that these plants cannot survive past 28 days in a salinity of 4.45 ppt. A shorter plant lifespan,
while improving water quality, will help reduce the likelihood of any growth getting out of control.

![Figure 4.6: Ending Leaf Color: Plant Health](image)

Each plant began with an average leaf count of 8 and an average plant height and width of 28cm and 14cm respectively, at the beginning of the experiment. Largest leaf length averaged at 8cm with an average leaf width at 7cm, and had an average of 2 wilted leaves to start. The individual measurements can be seen in Figures 4.7, 4.8 and 4.9.

After the first 3 days, all the plants in the high salinity comparison tank were experiencing significant wilting and browning of all the leaves. The plants in the high salinity comparison tanks experienced impaired buoyancy, soft stems, and had lost bits of the feather-like roots that had fallen and sunk in the tank. After 7 days all the plants of the high salinity comparison tank had died and the roots and stems were breaking down in the water.
Plant growth was the highest in the control tank as was expected. As seen in Figure 4.7, the control tank averages of change in height and change in width were the highest. The average of each experimental tank was used and depicted as ‘replica’ in the following graphs. Again, the high salinity tank experienced complete mortality and was breaking down in the water and unable to be measured. The experimental tanks experienced a slower growth rate than those of the control tank as expected. A slower growth rate will help decrease the chances of the plants getting out of control or taking over the area, perhaps reducing the cost of maintaining these plants when implemented.

Figure 4.7: Average Change in Plant Height & Plant Width

Figure 4.8 and Figure 4.9 demonstrate that the control tank experienced the highest growth rate. Change in leaf number is represented by the average number of leaves gained or lost by each individual plant of each tank. The change in largest leaf
length and width is represented by the average centimeters gained by each individual plant. Figure 4.9 shows how largest leaf length and width were measured; a similar board was also used to measure plant height and width. These average measurements of each experimental tank were used and depicted as ‘replica’ in the following graphs.
The plants in the experimental tanks had similar but smaller growth rates than those of the plants in the control tank. The experimental plants experienced about a 33% smaller growth rate in terms of plant height and a 28% smaller growth rate in terms of plant width. They also had on average 3.4 fewer new leaves per plant than the control tank. The rates of these experimental tanks were slower than the control as was expected. It appears that high salinity can cause complete mortality of water hyacinths in a relatively short time and low salinity levels hinder the growth rate of these plants. This shortened lifespan and slower growth rate will help the plants not spread too fast when implemented. Water quality measurements and plant growth measurements can be seen in the “Appendices” section.

Discussion/ Analysis:

The outcomes from this study demonstrated the plants’ ability to absorb a substantial amount of nutrients (around 45% reduction rate of ammonia nitrogen and
reactive phosphorus) from the water, as well as the plants ability to maintain a positive but slowed growth rate.

Ammonia nitrogen levels of the experimental tanks were reduced to 1.6 mg/l on average and reactive phosphorus levels reduced to 0.14 mg/l on average. The Delaware Estuary nutrient goals as of 2007 were to achieve 0.05 mg/l of total phosphorus and 1 mg/l of total nitrogen with goals of maximum levels at 0.2 mg/l total phosphorus, and 3 mg/l of total nitrogen (Santoro, 2007). Tampa Bay also developed similar nutrient level goals in 2011, and divided the bay into sections each with its own total nitrogen (TN) and total phosphorus (TP) goals listed in Table 4.1 (Janicki Environmental Inc., 2011). These Delaware Estuary and Tampa Bay nutrient goals were nearly achieved in this study.

<table>
<thead>
<tr>
<th></th>
<th>TN</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hillsborough Bay</td>
<td>1.01 mg/L</td>
<td>0.45 mg/L</td>
</tr>
<tr>
<td>Lower Tampa Bay</td>
<td>0.74 mg/L</td>
<td>0.10 mg/L</td>
</tr>
<tr>
<td>Middle Tampa Bay</td>
<td>0.87 mg/L</td>
<td>0.29 mg/L</td>
</tr>
<tr>
<td>Old Tampa Bay</td>
<td>0.93 mg/L</td>
<td>0.31 mg/L</td>
</tr>
</tbody>
</table>

(Table adapted from Janicki Environmental Inc., 2011)

These goals are similar to those of the Delaware Estuary and were chosen as recommendations to protect the dissolved oxygen levels of the bay which is very important to preventing fish kills. These recommendations for nutrient concentrations were prepared by the Tampa Bay Nitrogen Management Consortium and prepared for the Tampa Bay Estuary Program as part of a management strategy to improve water quality of the bay and to protect the estuary and the life it supports (Janicki Environmental Inc., 2011).
The other goal of this study was to reduce the turbidity of the water by 60%, which was achieved with an average reduction of 65% for the experimental tanks. This is the amount of reduction achieved by similar studies as well as the goal of the Environmental Protection Agency (Environmental Protection Agency, 2016). This nutrient goal is the Environmental Protection Agency’s nutrient reduction goal for many water bodies, including the Chesapeake Bay (Environmental Protection Agency, 2016).

**Possible Variables:**

In addition to the salinity causing a variance in nutrient absorption rates, there could be some variance due to the changes in temperature and lack of direct natural sunlight. During the experiment, a cold snap occurred causing the temperatures of the waters to dip from 26°C to 19°C. The experiment was also conducted during the fall, while the plants are typically in full bloom during the summer months, perhaps causing a slower growth rate (Department of ecology: State of Washington, 2016). Another possible factor that could have influenced the plants’ nutrient absorption rates was that the plants were collected from stormwater ditches that normally get sprayed with herbicides to prevent their growth. The exact area these plants were harvested from had not received treatment although the surrounding areas had received treatment two months prior to collecting. Other water quality parameters remained constant during the duration of the experiment and posed no known negative effects on the plants’ nutrient absorption rates. These parameters were: pH at a 7.3, and dissolved oxygen at 13 mg/l.
Statistical Analysis:

The Fligner-Wolfe Test was chosen for a statistical comparison on the nutrient absorption data. This test works with non-normal distributions and nonparametric data as well as being used to compare data sets with a control set (SYSTAT Software Inc., 2009). The hypothesis of this study was that the nutrient rates of the experimental tanks in 4.45 ppt would be similar and comparable to the rates of the control tank of 0.0 ppt and that salinity would not have a strong measurable effect on nutrient uptake rates. The null hypothesis would then be that the nutrient rates of the experimental tanks have no relationship with the rates of the control experiment and that there is a strong measurable effect of salinity on the nutrient uptake rates. The high salinity tank was not used for analysis since it experienced complete mortality after three days and did not produce any useful data.

Table 4.2 shows the output results from the analysis on the nitrogen absorption data. This indicates that the experimental tanks did not differ significantly from the control group. A strong p-value of 0.200 indicates weak evidence against the null hypothesis, thereby rejecting the null hypothesis and accepting the alternative hypothesis that there is a strong relationship between the rates of the experimental tanks and the control tank and that there is only a small measurable effect of salinity on nitrogen uptake rates. A strong p-value is anything that is greater than 0.05.
Table 4.2: Nitrogen Statistics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>TANK$ (6 levels)</td>
<td>1 2 3 4 5</td>
</tr>
</tbody>
</table>

Fligner-Wolfe Test with Control = 1.000  
Size of control group : 9  
Size of treatment group : 45  
Fligner-Wolfe Statistic (FW) : 1,292.000  
Standardized Statistic : 1.283  
Two-Sided p-Value : 0.200

Table 4.3 also indicates that the experimental tanks did not differ significantly from the control group in regards to phosphorus absorption rates. A strong p-value of 0.518 indicates weak evidence against the null hypothesis, thereby rejecting the null hypothesis and accepting the alternative hypothesis that there is a strong relationship between the rates of the experimental tanks and the control tank and that there is only a small measurable effect of salinity on phosphorus uptake rates.

Table 4.3: Phosphorus Statistics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>TANK$ (6 levels)</td>
<td>1 2 3 4 5</td>
</tr>
</tbody>
</table>

Fligner-Wolfe Test with Control = 1.000  
Size of control group : 9  
Size of treatment group : 45  
Fligner-Wolfe Statistic (FW) : 1,265.000  
Standardized Statistic : 0.546  
Two-Sided p-Value : 0.518

In regards to turbidity, Table 4.4 indicates that the experimental tanks did not differ significantly from the control group. A strong p-value of 0.152 indicates weak
evidence against the null hypothesis, thereby rejecting the null hypothesis and accepting the alternative hypothesis that there is a strong relationship between the rates of the experimental tanks and the control tank and that there is only a small measurable effect of salinity on turbidity reduction rates.

Table 4.4: Turbidity Statistics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>TANKS (6 levels)</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Dependent Variable</td>
<td>T</td>
</tr>
<tr>
<td>Grouping Variable</td>
<td>TANKS</td>
</tr>
</tbody>
</table>

Fligner-Wolfe Test with Control = 1.000
Size of control group : 9
Size of treatment group: 45
Fligner-Wolfe Statistic (FW) : 1.299 000
Standardized Statistic : 1.432
Two-Sided p-Value : 0.152

The results of this experiment have provided a model for implementing these plants in upstream estuarine environments. This study showed that the plants are effective nutrient absorbers, despite the slowed rate and reduced life span, and can potentially be implemented in these particular environments that are more prone to fish kills caused by outbreaks of toxic dinoflagellates and other harmful algal blooms. A slowed growth rate and reduced life span were expected for it makes the management of these fast growing invasives much more manageable and cost effective.
CHAPTER 5: RECOMMENDATIONS AND CONCLUSION

Implementation of water hyacinths in a natural environment will be an environmental way to solve a problem that has proven difficult to prevent and monitor in addition to turning a problematic species into a solution. Dinoflagellates and harmful algal blooms, other than red tide, are not as easily prevented and not detected typically until it is too late for they can be very evasive and complex species.

The potential application of these plants in upstream estuarine waters will help protect species and ecosystems further down such as lagoons, sounds, bays, and the ocean. This is necessary for downstream protection values which the EPA defines as “those water quality criteria in flowing waters that ensure protection of designated uses in the downstream estuarine waters as required by the Clean Water Act under 40 CFR 131.10(b)” (Environmental Protection Agency, 2016). Upstream estuarine waters must be improved for the benefit of those downstream estuarine waters and the water bodies it flows into. With the increasing effects of climate change and human activities, our water systems will be stressed even further. Increased development will cause our waters to become warmer, shallower and experience decreased water flow; the perfect environment for toxic dinoflagellates and toxic algal blooms to occur (Paerl et al., 2014a). Certain areas are at a greater risk for these changes to occur. These at risk areas are identified on maps in the following section. These potential policy implications
aim to improve the water quality of the estuarine water bodies at risk for massive fish kills, thereby reducing the intensity and frequency of massive fish kills in Florida and other similar estuarine environments.

**Implementation Recommendations:**

Most of the water bodies affected by the massive fish kills in Florida displayed on the previously displayed maps of Florida Fish Kills in 2006, 2011, and 2016 in Chapter 2 are: lagoons, inlets, estuaries, tidal creeks, or canals; although some were located more isolated inland lakes. As seen in Figure 5.1 and 5.2 the areas that experienced the most massive fish kills according to the previously mentioned maps are estuarine water bodies surrounded by heavy coastal development, similar to North Carolina and Chesapeake Bay. The contributing waterways for these waterbodies are adding nutrients and pollution from runoff of the surrounding development and agricultural land (Janicki Environmental Inc., 2011). With increasing development and the effects of climate change, these water bodies will continue to suffer and more water bodies will become at risk for an increase in fish kills.

*Figure 5.1* shows the water bodies at risk in the northern region of Florida, Pensacola Bay, West Bay, Dead Lake, and St. John’s River. *Figure 5.2* shows the water bodies at risk in the central region of Florida: Tampa Bay, the Nature Coast, Charlotte Bay, Banana River/Lagoon, and Indian River/Lagoon. These water bodies were determined to be at risk based on the maps of Florida Fish Kills in 2006, 2011, and 2016 as well as based on having similar characteristics to the areas affected in North Carolina and the Chesapeake Bay.
Figure 5.1: Areas at Risk for an Increase in Massive Fish Kills North Florida Region
(Melissa Kerr, map created January 2017)
These water bodies outlined in blue are the areas that received the majority of massive fish kills as well as those areas that experienced major events lasting multiple days and spanning large areas (Figure 2.7, Figure 2.8 and Figure 2.9). These areas are also water bodies that are beginning to see an increase in massive fish kills over the
last ten years according to the Florida Fish and Wildlife 'Fish Kill Hotline' (Florida Fish and Wildlife Conservation Commission, 2016a). These are the areas at greatest risk for an increase in intensity and frequency of massive fish kills to occur based on the fish kill data as well as possessing characteristics preferred by toxic dinoflagellates and other harmful algal blooms. The outlined water bodies are areas where our attention needs to be focused to protect the state’s water bodies and economy that depends on them. The focus needs to be on the water ways contributing to these at risk water bodies. The pollution and nutrients carried by these contributing waterways are deposited into Florida’s bays, estuaries, and lagoons putting them into this at risk category.

I recommend, in order to prevent an increase in intensity and frequency of massive fish kills in Florida’s at risk water bodies, the focus needs to be on preventing the excess nutrients from reaching these water bodies. This can be achieved through the implementation of water hyacinths in a controlled method similar to the previously mentioned pvc-cage designs from the King’s Bay study.

Limitations:

This study demonstrated water hyacinths ability to absorb nutrients in a low-saline, nutrient rich environment. However, there were some limitations to this study for the experiment had to be conducted in a controlled environment indoors. It is very difficult to obtain a permit to test these plants in the natural environment; time and resources did not permit this option. Implementing these plants in the contributing water ways of Florida’s at risk water bodies could potentially decrease the intensity and
frequency of massive fish kills caused by toxic dinoflagellates and other harmful algal blooms.

Further research regarding the characteristics of contributing water ways could be used in determining the exact location to implement water hyacinths for the greatest improvement on water quality. In addition, a more comprehensive assessment of fish kill trends in Florida of reports that are all scientifically verified (unlike the Fish Kill Hotline where only some of the reports were scientifically verified) would give a better understanding of the trends and patterns occurring. This could potentially find more water bodies at risk and allow for improved monitoring of fish kills caused by toxic dinoflagellates and harmful algal blooms. Further research is also recommended on water hyacinths nutrient absorption capabilities in a natural estuarine environment. Literature is contradicting on these plants and the effects salinity has on its nutrient absorption capabilities is not widely studied, perhaps due to its controversial background. Real world application of my study in a natural estuarine environment could give insight into all that these ‘pests’ can truly offer.

**Conclusion:**

Using an aquatic invader to treat the contributing waters of the at risk waterbodies will help improve that water body’s water quality. This will thereby help to eliminate the nutrient trigger of toxic dinoflagellates and harmful algal blooms, thus reducing the intensity and frequency of massive fish kills. Toxic dinoflagellates and harmful algal blooms other than the well-known red tide are usually overlooked and not
widely monitored. They are very evasive and present a difficult challenge in even identifying the species after a fish kill has occurred.

Turning a controversial ‘pest’ into a green solution for an environmental, economic, and public health solution can be a practical and cost effective solution to managing massive fish kills. A policy framework for implementing water hyacinths as a phytoremediation method should be developed for Florida’s at risk water bodies.

Water hyacinths are hardy and adaptive plants. They are one of the fastest growing plants and can double their population in two weeks (United States Department of Agriculture, 2016). Water hyacinths are currently being managed in the state of Florida by being treated with regular herbicide sprayings (Florida Fish and Wildlife Conservation Commission, 2016b). These efforts could instead be used to collect the plants. This could potentially cut down on costs of producing and using the herbicide as well as the costs involved in training people to use them. Switching from treating to collecting will potentially provide a phytoremediation method to reduce massive fish kills is Florida’s at risk water bodies. Water hyacinths can be used where the native filtering plants are suffering to improve the water quality to a level tolerable by the less hardy native filtering plants, allowing native plants to thrive and re-establish.

This study demonstrated water hyacinth’s ability to uptake ammonia nitrogen and reactive phosphorus in a controlled brackish environment. It also showed how low-salinity levels reduce the lifespan of water hyacinths making their potential application more practical. An aquatic invader like water hyacinths can be used to combat the major culprits of massive fish kills in Florida’s at risk water bodies.
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DOI:10.1126


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APPENDICES

Appendix a: Nitrogen Data

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