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Neurotoxin in a Louisiana estuary: quantitative analysis of domoic acid in gulf menhaden (*Brevoortia patronus*) and qualitative modeling of links in a shark nursery

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**NEUROTOXIN IN A LOUISIANA ESTUARY: QUANTITATIVE ANALYSIS OF
DOMOIC ACID IN GULF MENHADEN (*BREVOORTIA PATRONUS*) AND
QUALITATIVE MODELING OF LINKS IN A SHARK NURSERY**

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Oceanography and Coastal Sciences

By
Ross P. Del Rio
B.S. Louisiana State University, 2007
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ABSTRACT

Harmful algal blooms are an increasing problem for coastal waters world-wide. The diatom genus, *Pseudo-nitzschia*, is of particular concern in Louisiana, due to the potential for several species to produce the neurotoxin domoic acid (DA). While trophic transfer of DA to consumers has repeatedly occurred along the California coast, little is known about trophic transfer of recently detected DA in the Gulf of Mexico. In this study, the presence of DA in gulf menhaden (*Brevoortia patronus*) and the potential for trophic transfer to higher order consumers was investigated. In addition, the effects of this transfer and other algal toxins that threaten Louisiana's coastal food webs were evaluated. DA quantification in water and fish tissue samples was determined by Enzyme-Linked Immunosorbent Assay (ELISA). Food web effects of algal toxins were analyzed through the use of a qualitative modeling technique, loop analysis. The results of the toxin assay illustrated that low-levels of DA exist in both water and tissue samples, with a significant correlation between the two ($n = 25$, $p = 0.025$, significance level of 0.05). The effects of HABs on the entire food web showed the possibility of trophic cascades. This is the first documentation of a DA vector in the entire Gulf of Mexico and confirms DA contamination in food webs of coastal Louisiana. Through the use of qualitative modeling, present and future threats posed by phycotoxins to coastal food webs can be assessed, providing resource managers valuable information to aid in mitigation of their negative consequences.

CHAPTER 1
NEUROTOXIN IN A LOUISIANA ESTUARY: QUANTITATIVE ANALYSIS OF
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REVIEW

INTRODUCTION

Harmful algal blooms (HABs) can be defined as events when species of algae that are capable of causing negative environmental problems accumulate to “sufficient” levels for these problems to occur due to their abundance, morphology, or toxin production (e.g. Glibert et al. 2005a). HABs have become an escalating problem in coastal marine communities, due to increased nutrient loading (Glibert et al. 2005b). One region where coastal eutrophication is occurring, and thus where the threat of HABs is high, is Louisiana, in the northern Gulf of Mexico. The chief potential HAB threat in Louisiana is the pennate diatom *Pseudo-nitzschia*, which produces the neurotoxin domoic acid (DA). A major consideration when assessing the risk of toxin-producing HABs is to identify vector species that transmit toxin within a food web. In Louisiana, an abundant, filter-feeding fish, the gulf menhaden (*Brevoortia patronus*), may act as a vector of DA in marine food webs. One important tool, in investigating the effects of HABs on an entire food web, is qualitative modeling. This technique can allow researchers to assess ecosystem response to perturbations like HABs. The present study uses both a quantitative assessment of the potential for gulf menhaden to be a DA vector, and a qualitative model to better understand the threat posed to Louisiana coastal food webs by blooms of *Pseudo-nitzschia* spp. and other HAB species.

DOMOIC ACID (DA)

Phycotoxins, toxic compounds produced by algae, are one of the biggest threats of HABs since they are capable of injuring both humans and marine organisms. DA is a phycotoxin produced by diatoms in the genus *Pseudo-nitzschia*, which produce neurotoxic effects through

ingestion. DA was first extracted from a red alga *Chondria armata* and described by Takemoto and Daigo (1958) as a water-soluble, tricarboxylic amino acid. Research into the toxicity of DA has revealed that it mimics the excitatory neurotransmitter glutamate in the brain (Debonnel et al. 1989), and it has a high binding affinity at ionotropic receptors, which results in a fractionally opened ion channel that resists closing (Ramsdell 2007). DA has also been found to have both pre- and post-synaptic effects (Brown & Nijjar 1995). Its pre-synaptic action is to continuously release glutamate, which opens ionotropic receptors in the natural way, but at unnatural levels; while its post-synaptic functionality binds to voltage-regulated calcium channels, holding them partially open for an indefinite span of time (Brown & Nijjar 1995). This increased neuron activity causes many of the neurological symptoms associated with DA intoxication. The resistance to the conformational change that would expel DA is what allows an unregulated level of calcium ions to enter the neurons, and eventually causes neuronal swelling and necrosis (Berman et al. 2002, Ramsdell 2007). When this occurs in the hippocampus, the ability to store short-term memory is degraded, in addition to other neurological impairments (Teitelbaum et al. 1990), thus earning the name Amnesic Shellfish Poisoning (ASP) in human cases (Wright et al. 1989), and since they are not known to get amnesia, Domoic Acid Poisoning (DAP) for other animals. Exposure to DA can lead to other problems as well, such as gastrointestinal symptoms, cardiac arrhythmias, and spinal cord problems (Perl et al. 1990). Unfortunately, the harmful effects of DA were first discovered after a major ASP event in Canada in 1987 (Bates et al. 1989, Wright et al. 1989).

Significant research was not conducted on DA until nearly 30 years after its first description. This was instigated by a major poisoning event that occurred in Canada in 1987. Over 100 people were hospitalized after consuming cultivated blue mussels (*Mytilus edulis*) from Prince Edward Island (Bates et al. 1989, Wright et al. 1989, Perl et al. 1990). Most victims

experienced vomiting, abdominal cramps, diarrhea, debilitating headaches, and short-term memory loss; some people needed to be admitted to Intensive Care Units due to seizures, comas, copious respiratory secretions, or blood pressure problems; and three individuals died (Perl et al. 1990). At the time, the causative compound was unknown, but through mouse bioassay and spectroscopic analysis, DA was found in some of the victims' uneaten food (Perl et al. 1990) and on mussel cultivation beds (Wright et al. 1989). This outbreak was the first recognized human intoxication from DA, and led to the discovery of the first microalgal group that could produce such a toxin (Bates et al. 1989, Wright et al. 1989, Perl et al. 1990).

Bates et al. (1989) determined the organism responsible for the contamination of the Prince Edward Island mussels to be the diatom *Nitzschia pungens* (now known as *Pseudo-nitzschia multiseries*). A series of observations led Bates et al. (1989) to identify *P. multiseries* as the organism responsible for the intoxication event. First, the toxic event co-occurred with a massive bloom of phytoplankton dominated by *P. multiseries*. Second, the DA producing macroalgal species in the area, *Chondria baileyana*, is rarely encountered and is too small to reach high enough biomass to contaminate the mussels. Third, the digestive tracts of the mussels had large quantities of *P. multiseries*. Fourth, mouse bioassays performed with extracts from *P. multiseries* cultures produced the same symptoms seen in the human poisonings. Finally, DA was confirmed to be present by high-performance liquid chromatography (HPLC) from mussel and phytoplankton samples. Together, these observations and experiments culminated in the identification of *Pseudo-nitzschia* as the first diatom group to produce the toxin DA (Bates et al. 1989). Following the 1987 Canadian event, another species of *Pseudo-nitzschia*, *P. australis* was identified as the causative organism in several DAP events, characterized by seabird and marine mammal mortalities, that occurred in Monterey Bay, California (Work et al. 1993, Scholin et al. 2000). Currently, there are 11 species of *Pseudo-nitzschia* that are known to produce DA

(Moestrup & Lundholm 2007), but new ultra-sensitive detection technologies have led other researchers to propose that all species of *Pseudo-nitzschia* can produce DA (Wells et al. 2005).

PSEUDO-NITZSCHIA

The reason DA has become a problem in the ocean is because it is produced by species in the ubiquitous pennate diatom genus *Pseudo-nitzschia* (Bates et al. 1989, Wright et al. 1989). These diatoms are common in both coastal and oceanic environments (Silver et al. in review). *Pseudo-nitzschia* exists in a variety of environmental conditions making them well-adapted to live in environments that can experience variable temperatures, salinities, or light levels. *Pseudo-nitzschia*'s presence in samples throughout the year (Dortch et al. 1997) and in monthly samples (Bargu et al. unpublished) from coastal Louisiana, an area with a subtropical climate, indicates that it can survive in a wide range of temperatures. In addition to temperature, variable salinity does not seem to affect *Pseudo-nitzschia*'s survivability. A study by Thessen et al. (2005) on the effects of salinity on *Pseudo-nitzschia* growth and toxin production showed that *Pseudo-nitzschia* has a wide range of salinity, both naturally and in culture (1 to >35 psu). Another reason that *Pseudo-nitzschia* is well-suited for life in dynamic habitats is its tolerance of low-light environments. Rines et al. (2002) noted an occurrence of *Pseudo-nitzschia* at about 5 m that was physically forced to near bottom (~30 m) for a few days due to winds allowing lower salinity water to enter their study site, a fjord in the San Juan Islands in Washington, USA. Samples taken while near the bottom revealed long, motile, healthy chains of *Pseudo-nitzschia*. Additionally, Rines et al. (2002) also noted *Pseudo-nitzschia*'s ability to exist with other species of phytoplankton. The researchers observed an association of *Pseudo-nitzschia* with *Chaetoceros socialis* which has also been noted in previous studies (Alldredge & Gotschalk 1989, Fryxell et al. 1997). This association may have several benefits for *Pseudo-nitzschia* spp., such as providing a protective micro-habitat within the *Chaetoceros* spines, offering a means to stay in

the upper portions of the water column, or keeping cells close together so sexual reproduction can occur. Because of its ability to tolerate complex environments, *Pseudo-nitzschia* is a common and, potentially, dangerous member of plankton communities all over the world.

Proper identification of which *Pseudo-nitzschia* species are present is important to understand the DA threat level, since not all species in the genus are toxic. Currently, identification of species is based on morphological differences seen through the use of electron microscopy, and more recently, molecular methods have been developed. Several species-specific probes have been developed that bind to the large subunit ribosomal RNA (LSU-rRNA) of *Pseudo-nitzschia* species. Two methods have been developed for attaching the probes: whole-cell and sandwich hybridization (Scholin et al. 1999, Miller & Scholin 1998, Miller & Scholin 1996). Even though probes give the opportunity to conduct species-specific cell enumeration, the use of the molecular probe technology remains limited since these probes are not available for all species of *Pseudo-nitzschia*, and, due to strain variability, are not applicable to all locations where *Pseudo-nitzschia* is found (Parsons et al. 1999). Until accurate and broadly applicable identification techniques are developed, researchers will have to rely on problematic and less precise traditional methods of *Pseudo-nitzschia* identification.

Toxic *Pseudo-nitzschia* has been discovered in oceanic communities thereby joining coastal regions in being under the threat of DA. Some of these oceanic regions have been the focus of iron-fertilization experiments in an attempt to sequester carbon from the atmosphere in algal biomass that will sink to the bottom of the ocean. *Pseudo-nitzschia* responds quickly to iron addition (de Baar et al. 2005, Marchetti et al. 2006), so enrichment experiments could cause large blooms. Nearly a dozen species of *Pseudo-nitzschia* produce DA (Moestrup & Lundholm 2007) at varying levels, but since the potency of each cell is relatively low, blooms larger than 10^5 cells/L are required for ecosystem effects (Rines et al. 2002). Silver et al. (in review) found

DA in samples taken from previous iron enrichment experiments and several naturally iron-limited sites that could be used for future iron enrichment. Cell counts of *Pseudo-nitzschia* from previous iron enrichment experiments show that blooms are larger than the 10^5 threshold described by Rines et al. (2002). Iron fertilization experiments may inadvertently cause serious ecological problems since large blooms of toxic *Pseudo-nitzschia* seem to be caused by the addition of iron.

The reason for DA production remains one of the largest unknown aspects of *Pseudo-nitzschia* biology. There have been several hypotheses proposed, and they indicate multiple uses for this toxin. One possible reason is that the chemical structure of DA makes it a good trace metal chelator especially for iron and copper. DA could be secreted to bind extra-cellular iron increasing its bio-availability, or to bind extra-cellular copper in order to reduce its availability (Rue & Bruland 2001). Another potential use for DA is to act as a grazing deterrent. Bargu et al. (2006) showed that krill (*Euphausia pacifica*) exhibited a reduced feeding rate in the presence of dissolved DA. The plausibility of both of these suggestions shows the complexity of the problem since there is no identification of a single reason for DA production.

TROPHIC TRANSFER OF DA

It is evident that DA can be vectored through the food web to higher trophic levels, as shown by the 1987 Canadian poisoning, which can be most effectively accomplished through short food chains. Since DA is water soluble (Takemoto & Daigo 1958) and exists in an aquatic medium, it is depurated quickly. Thus, short food chains are essential for large concentrations of toxin to reach upper trophic levels in short time periods. Several previous studies confirm that short food chains do exist in natural environments for the transfer of DA (Bates et al. 1989, Wright et al. 1989, Perl et al. 1990, Wekell et al. 1994, Lefebvre et al. 1999, Scholin et al. 2000, Bargu et al. 2002, Lefebvre et al. 2002a, Lefebvre et al. 2002b).

In many instances, toxic *Pseudo-nitzschia* is directly fed upon by organisms that can be prey items for top predators. One example is the 1987 Canadian poisoning, where humans consumed filter-feeding mussels that had ingested toxic *Pseudo-nitzschia* (Perl et al. 1990, Wright et al. 1989, Bates et al. 1989). Since the 1987 event, other major DA poisonings have occurred, especially in Monterey Bay, California (e.g. Work et al. 1993, Scholin et al. 2000), and many other vectors were identified to contain high DA levels including northern anchovies (*Engraulis mordax*), Dungeness crabs (*Cancer magister*), market squid (*Loligo opalescens*), krill (*Euphausia pacifica*), and razor clams (*Siliqua patula*) (Wekell et al. 1994, Lefebvre et al. 1999, Scholin et al. 2000, Bargu et al. 2002, Lefebvre et al. 2002a, Bargu et al. 2008). With such a large variety of potential vectors that are common prey items for large predators, there are many opportunities for DA to contaminate large high trophic level consumers.

Some of the identified vector species represent direct linkages for DA to large top predators. Bargu et al. (2002), found euphausiids (krill) to consume toxic *Pseudo-nitzschia*, and accumulate DA to levels greater than the FDA consumption limit ($20 \mu\text{g DA g}^{-1}$). Euphausiids are an important food source for squid, baleen whales, and seabirds (Bargu et al. 2002), and are capable of vectoring DA to large consumers (Lefebvre et al. 2002b, Bargu et al. 2008)

Filter-feeding fishes are one of the most important vectors of DA because they can accumulate extremely high levels of DA, and are commonly consumed by upper trophic level predators. In Monterey Bay, California, the poisoning events described by Work et al. (1993) and Scholin et al. (2000), identified northern anchovies, a filter-feeding fish, as the vector of DA to higher order predators. Lefebvre et al. (2002a) investigated the potential DA transmission for northern anchovies and Pacific sardines (*Sardinops sagax*) in Monterey Bay, California. Concentrations in fishes were related to blooms of toxic *Pseudo-nitzschia* because DA was detected in fishes whenever there was a bloom, but only during the bloom, which indicates fast

depuration of DA. Lefebvre et al. (2002a) also showed that fish accumulated DA levels well above the FDA consumption limit ($20 \mu\text{g DA g}^{-1}$ tissue), and reached a maximum of $1815 \mu\text{g DA g}^{-1}$ tissue for anchovies and $728 \mu\text{g DA g}^{-1}$ tissue for sardines. These planktivorous fishes are capable of vectoring extremely high amounts of DA, making them dangerous for higher trophic level predators during *Pseudo-nitzschia* blooms.

Since the 1987 human poisoning event, several DAP events have caused the contamination of higher trophic level consumers as well. One group of high trophic level marine predator that has been the victim of DAP events is seabirds. In September 1991, a mass stranding of brown pelicans (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) occurred along the northern shore of Monterey Bay, California. Through mouse bioassay of stomach content extracts from affected birds and analysis with high-performance liquid chromatography with an ultra-violet detector (HPLC-UV) (Work et al. 1993), DA was found to be the causal agent of the unusual seabird stranding. Subsequently, Work et al. found DA in plankton and northern anchovy samples taken from the bay. They then used scanning electron microscopy on plankton and stomach contents samples of anchovies and affected birds, and identified *Pseudo-nitzschia australis* as the dominant phytoplankter in all samples. This study provided the first account of DAP in an area outside of Atlantic Canada. It also established another species of *Pseudo-nitzschia* as a DA producer, *P. australis*, and another species, northern anchovy, as a vector of DA (Work et al., 1993). The results of this study confirm that DA is not only a human health threat, but also affects high trophic level marine predators.

DA also affects marine mammals in much the same way as seabirds. In May and June of 1998, over 400 California sea lions (*Zalophus californianus*) were found dead across many central California beaches. At the same time as the strandings occurred, plankton samples, treated with several molecular probes specific to toxic *Pseudo-nitzschia* species, revealed a large

bloom of *Pseudo-nitzschia australis*, a known DA producer (Scholin et al., 2000). In a concurrent study by Lefebvre et al. (1999), DA from *P. australis* was identified as the causative compound, and vectored by northern anchovies to California sea lions. Anchovy samples and fecal samples from some affected sea lions during the *Pseudo-nitzschia* bloom found DA and indicated *P. australis* as the responsible species. Their analyses left little doubt that *P. australis* was the causative organism (Lefebvre et al., 1999). The amount of damage caused by this DAP event highlights the need to understand the extent of DA contamination in marine food webs.

DA has been shown to contaminate many animals and multiple food webs, since it is a phycotoxin that is easily transferred. For example, Lefebvre et al. (2002b) investigated if DA could reach many different marine organisms including Pacific sanddab (*Citharichthys sordidus*), chub mackerel (*Scomber japonicus*), albacore tuna (*Thunnus alalunga*), petrale sole (*Eopsetta jordani*), jack smelt (*Atherinopsis californiensis*), walleye surfperch (*Hyperprosopon argenteum*), humpback whale (*Megaptera novaeangliae*), and blue whale (*Balaenoptera musculus*). This study revealed that all of the species tested, except petrale sole and walleye surfperch, had detectable levels of DA in their tissues or feces. Significantly, the discovery of DA in sanddab tissue, a benthic predator, confirms the presence of DA in benthic food webs, indicating that the threat of DA is greater than previously thought. Because DA contaminates both benthic and pelagic species, this study clearly demonstrates how pervasive DA is in marine food webs. Since trophic transfer is the major intoxication pathway, identification of vectors is essential to understanding the threat of DA for marine communities.

DA AND PSEUDO-NITZSCHIA IN LOUISIANA COASTAL WATERS

Louisiana coastal waters are home to several HAB species that are commonly found including *Pseudo-nitzschia* spp., *Dinophysis* spp., *Ceratium* spp., etc. Due to the input of nutrients from the Mississippi River (Turner & Rabalais 1994), Louisiana coastal waters are

eutrophic, which may increase the frequency and intensity of HABs (Parsons et al. 2002). Since the 1910s, *Pseudo-nitzschia* spp. have been found in Louisiana coastal waters, and recently, toxic species have been identified (Dortch et al. 1997, Parsons et al. 1999, Pan et al. 2001). The combined efforts of Parsons et al. (1999) and Pan et al. (2001), document two toxic species of *Pseudo-nitzschia*, *P. pseudodelicatissima* and *P. multiseriata* in the phytoplankton community of Louisiana. Two species occasionally reported as toxic, *P. delicatissima* and *P. pungens*, and two non-toxic species, *P. subfraudulenta* and *P. sp. cf. Nitzschia americana*, also occur. Evidence also suggests an increase in *Pseudo-nitzschia* abundance due to coastal eutrophication of the Louisiana continental shelf. Sediment cores taken for *Pseudo-nitzschia* abundance, revealed cell abundance increases are correlated with increases in nutrient loading of the Mississippi River (Parsons et al. 2002). With the combination of increasing eutrophic conditions and the presence of toxic species, there is the potential for blooms of toxic *Pseudo-nitzschia* with negative effects for the coastal marine communities of Louisiana.

In Louisiana coastal waters, one of the most abundant fishes is the planktivorous gulf menhaden which may act as a vector of DA. Gulf menhaden are estuarine-dependent as juveniles and only moving offshore in the winter as adults to spawn (Lassuy 1983, Ahrenholz 1991). Their life history places them in coastal waters during the times of the year when blooms of *Pseudo-nitzschia* are expected (Dortch et al. 1997). Not only are they present at the most critical times for HAB formation, but they are also one of the most abundant fishes in the area, so they are highly likely to ingest DA. According to the 2007 stock assessment report (Vaughan et al. 2007), Louisiana provides 52% of the juvenile abundance for the entire gulf menhaden stock. As a member of the Order Clupeiformes, gulf menhaden are closely related to a previously identified vector of DA, the northern anchovy. Gulf menhaden form dense schools, and filter the water column during feeding, frequently consuming toxic phytoplankton when present. Durbin and

Durbin (1975) found Atlantic menhaden (*Brevoortia tyrannus*) filtration rates to be dependent on mouth diameter and swimming speed, higher rates were possible, up to $34.8 \text{ l fish}^{-1} \text{ min}^{-1}$.

Filtration rates can be so substantial that McHugh (1967) claimed that if all of the Atlantic menhaden caught in the Chesapeake Bay fishery were all in the bay at the same time, they could strain the entire volume of the Virginia portion of the bay in one day.

The potential of gulf menhaden to be a DA vector is enhanced since they are commonly preyed upon by high trophic level consumers. In other states, organisms also found in coastal Louisiana have been shown to accumulate algal toxins through their diet. Hinton and Ramsdell (2008) investigated gut contents from a 2004 mass bottlenose dolphin (*Tursiops truncatus*) mortality event in the Florida Panhandle. Gulf menhaden was the major dietary component, and toxin analysis revealed high levels of the dinoflagellate toxin, brevetoxin. Thus, bottlenose dolphins, a common animal in Louisiana estuaries, are already known to be susceptible to algal intoxication potentially via gulf menhaden.

Gulf menhaden are also major components of the diets of several coastal sharks found in Louisiana (Snelson et al. 1984, Hoffmayer & Parsons 2003, Bethea et al. 2004). Bethea et al. (2004) investigated the diets of four species, Atlantic sharpnose (*Rhizoprionodon terraenovae*), blacktip (*Carcharhinus limbatus*), finetooth (*Carcharhinus isodon*), and spinner (*Carcharhinus brevipinna*) sharks along the Florida panhandle. Gulf menhaden were a major component of the diet of all four sharks. Similar diets were found by Hoffmayer and Parsons (2003) for Atlantic sharpnose, blacktip, and finetooth sharks in Mississippi Sound. Bull sharks (*Carcharhinus leucas*), also prey on gulf menhaden in the Indian River Lagoon in Florida (Snelson et al. 1984). In one instance, one of the bull sharks had six whole gulf menhaden in its stomach, indicating that when available, they can consume large quantities of gulf menhaden. Piscivorous fishes such as sharks are not the only upper trophic level organisms at risk for exposure to DA in Louisiana

coastal waters. The official state bird of Louisiana, the brown pelican (*Pelecanus occidentalis*), is also a major consumer of gulf menhaden (Ahrenholz 1991, U.S. Fish and Wildlife Service 2008), and is sensitive to DA, as described in Work et al. (1993). With so many top predators consuming a common prey item, the effects of DA on Louisiana coastal food webs could be larger than expected. As a vector for other algal toxins, and its filter-feeding behavior allowing them to ingest *Pseudo-nitzschia*, gulf menhaden is an ideal potential vector of DA to higher trophic organisms in Louisiana coastal estuaries.

QUALITATIVE MODELING THEORY

Qualitative modeling is an important technique for large, complex systems where gathering all of the necessary data for quantitative modeling may not be logistically or monetarily feasible. The study of HABs is one such example. The logistics of sampling are complicated since HABs occur on short time scales and are patchy in distribution. Food web effects are difficult and expensive to demonstrate since they require specialized techniques performed by highly trained individuals. Loop analysis, a type of qualitative modeling, provides a convenient method for analyzing the food web effects of events like HABs since it is a rapid and inexpensive way for investigators to assess ecosystem dynamics. Through this methodology researchers are able to evaluate effects of HABs, assess the relative vulnerability of coastal waters, or forecast responses to HAB events.

Qualitative modeling is a relatively new technique that is useful in determining the indirect effects, feedback, and influence of perturbations, and can be used to evaluate the stability of a system of interest. The practical application for this type of modeling in ecology was outlined by Levins (1966); modelers try to achieve three goals: reality, generality, or precision, but can only maximize two of the three. While quantitative models maximize reality and precision, qualitative models maximize reality and generality. This gives qualitative

modeling the advantages of realistically describing the direct interactions in the system and that they can be applied to many systems.

Loop analysis uses only the sign of the direct interactions (+,-,0) to describe the general behavior of the constituents, or nodes, and the stability of the system (May 1971). Since loop models are based on the signs of the direct interactions, they can be constructed with only a general knowledge of the natural history of the system in question. Loop models are graphic representations of the direct pair-wise interactions within the system, and are analyzed by counting feedback loops (positive or negative) that describe indirect interactions. These are pathways that return to their origin without going through another constituent twice (Rossignol et al. 2001). One advantage of loop analysis is that the models also account for indirect interactions present since feedback loops travel all possible pathways in the model. In addition to stability, ecosystem responses to sustained perturbations (presses) (*sensu* Bender et al. 1984) can also be evaluated by manipulating the interactions within the system. The flexibility and relative ease of conducting loop analysis makes this technique an important step in answering many ecological questions.

By using loop analysis, the larger ecosystem-wide effects of HABs can be examined. With this technique, the responses of all levels of the food web to a HAB can be assessed by their net number of positive and negative feedback loops influencing constituents of the model. Loop models can also be easily modified to accommodate new discoveries in the interactions between the components of the model, so the results can be as accurate as possible. Loop analysis also allows for the examination of multiple types of HABs by easily creating new models to suit the particular direct interactions of particular HAB types. It is because of its many applications that loop analysis has been established as an important tool in the study of HABs.

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CHAPTER 2

GULF MENHADEN (*BREVOORTIA PATRONUS*): A POTENTIAL VECTOR OF DOMOIC ACID IN COASTAL LOUISIANA FOOD WEBS

INTRODUCTION

A harmful algal bloom (HAB) is an accumulation of algal biomass to levels “sufficient” enough to foster a negative impact on the environment through either its morphology, toxin production, or sheer biomass (Glibert et al. 2005a). Studies have shown that eutrophication of coastal water bodies is exacerbating this issue, since rising nutrient levels are suspected to increase the intensity and frequency of these types of algal blooms (Glibert et al. 2005b). Consequently, there is growing concern over the threat HABs pose to the environment and humans, such as marine animal deaths, closures of fisheries, and human health issues. For marine food webs, one of the most prominent problems is that algal toxins can be easily transferred to higher trophic level consumers by filter-feeding vectors causing contamination at all trophic levels. The most commonly identified vectors are shellfish, however, in many cases, filter-feeding fishes can be just as, or even more, effective at spreading toxins (Wright et al. 1989, Bargu et al. 2002, Lefebvre et al. 2002a). For many coastal systems, these vectors have yet to be adequately described, even for well-studied HAB types.

One of the more commonly researched HAB categories are blooms of the pennate diatom *Pseudo-nitzschia*. It has a worldwide distribution and is present in both coastal and oceanic habitats. First seen in Canada (Bates et al. 1989, Wright et al. 1989), toxic *Pseudo-nitzschia* was then found in California (Work et al. 1993, Scholin et al. 2000), and now, has been documented in the Gulf of Mexico (Dortch et al. 1997), South America (Sar & Ferrario 2002), the United Kingdom (James et al. 2005) and Australia (Takahashi et al. 2007) (Fig. 2.1). Species in this genus are capable of producing the neurotoxin, DA, a water-soluble, tri-carboxylic amino acid (Takemoto & Daigo 1958). While not all species in the genus produce DA, new evidence using

ultra-sensitive detection assays, like Enzyme-Linked Immunosorbent Assay (ELISA), reveals that all species may be capable of producing DA to some degree (Wells et al. 2005). Thus, DA may be more of a problem than originally hypothesized.

Since first being isolated from the red alga *Chondria armata* (Takemoto & Daigo 1958), DA has been widely accepted as a neurotoxin that can affect both humans and marine animals. The toxin is absorbed through the lining of the stomach and intestines, and transported to the brain where it acts on neurons, known as Amnesic Shellfish Poisoning (ASP) in humans and Domoic Acid Poisoning (DAP) in other animals (Debonnel et al. 1989, Brown & Nijjar 1995, Ramsdell 2007). Human victims of DA intoxication experience symptoms such as abdominal cramps, nausea, and diarrhea, which can progress to seizures, short-term memory loss, or death (Perl et al. 1990). In Canada in 1987, an outbreak of ASP led to the hospitalization of hundreds of people and the death of three following the consumption of mussels that had been contaminated with DA by *Nitzschia pungens* (currently known as *Pseudo-nitzschia multiseriata*) (Bates et al. 1989, Perl et al. 1990). Since that episode, there has not been another serious outbreak of ASP; however, there have been several cases of marine animal mortalities. The west coast of the United States has experienced many DAP events where marine mammals and/or seabirds have been intoxicated after consuming filter-feeding anchovies and sardines containing toxic *Pseudo-nitzschia australis* (Work et al. 1993, Scholin et al. 2000). Because of these events, concern over the threat of DA has been increasing in regions where *Pseudo-nitzschia* has been found abundant.

With eutrophication of Louisiana coastal waters, due to the input from the Mississippi River (Turner & Rabalais 1994), increasing numbers of *Pseudo-nitzschia* have also been observed in this region since the 1950s, but they have been present since the 1910s (Parsons et al. 2002). Research conducted in this region, thus far, has only quantified its presence, identified

toxic species, or tested the effects of varying environmental parameters on *Pseudo-nitzschia* (Dortch et al. 1997, Parsons et al. 1999, Pan et al. 2001, Parsons et al. 2002, Thessen et al. 2005). Nothing has yet been done to characterize the extent of its role in marine food webs in the Gulf of Mexico. Previous examinations of the *Pseudo-nitzschia* community in Louisiana coastal waters revealed six different species with two confirmed DA producers, *Pseudo-nitzschia multiseries* and *P. pseudodelicatissima*, two potentially toxic species, *P. delicatissima* and *P. pungens*, and two non-toxic species, *P. subfraudulenta* and *P. sp. cf. Nitzschia americana*, in abundances that can reach 10^8 cells l^{-1} on the Louisiana continental shelf and 10^5 cells l^{-1} Terrebonne Bay, an inshore estuary (Dortch et al. 1997, Parsons et al. 1999, Pan et al. 2001). Although presence of *Pseudo-nitzschia* has been well documented in Louisiana's coastal waters, limited studies on its ecology make it imperative to understand how DA is vectored through Louisiana's marine food webs.

In order for algal toxins to be efficiently conveyed through food webs, the structure of the toxin, the length of the food chain, and the type of the vector are important. Because DA is water soluble and depurated quickly, it requires short food chains and bloom conditions to be passed to higher trophic levels effectively. In previous instances, DA has been found to move from water to many types of primary consumers, such as krill (*Euphausia pacifica*), razor clams (*Siliqua patula*), blue mussels (*Mytilus edulis*), or northern anchovies (*Engraulis mordax*), and then be directly consumed by humans and/or animals (Bates et al. 1989, Wright et al. 1989, Perl et al. 1990, Wekell et al. 1994, Lefebvre et al. 1999, Scholin et al. 2000, Bargu et al. 2002, Lefebvre et al. 2002a, Lefebvre et al. 2002b). As a result, organisms that are a part of these short food chains are the most potent vectors of DA. Furthermore, the life history of vector organisms can have an additional effect on efficient toxin transfer. The most commonly identified vectors of algal toxins are shellfish (Bates et al. 1989, Wright et al. 1989, Wekell et al. 1994), however

due to a sessile life history, their ability to take up toxin is limited by their location and the density of the HAB species in that specific area. Filter-feeding fishes, however, appear to represent the most efficient pathway for DA transmission (Lefebvre et al. 2002b). They have the ability to actively find blooms and can, potentially, ingest higher amounts of toxic phytoplankton than shellfish. In addition to their ability to accumulate toxin, filter-feeding fishes provide a direct link from primary producers to large, higher trophic level, secondary consumers. Thus, toxins can contaminate the upper levels of food webs in only two linkages. As an example, in California, northern anchovies were identified as the DA vector in the mass mortality events of brown pelicans (*Pelecanus occidentalis*) and Brant's cormorants (*Phalacrocorax penicillatus*) (Work et al. 1993) and California sea lions (*Zalophus californianus*) (Scholin et al. 2000), each in Monterey Bay. Since filter-feeding fishes represent the most efficient pathway for DA transfer to higher trophic level predators, regions with a high abundance of these types of fishes could be more susceptible to an ASP/DAP event.

Based on previous work in the region and the presence of an abundant potential vector, Louisiana coastal waters are ideal for an outbreak of DA. In Louisiana, the gulf menhaden (*Brevoortia patronus*) occupies a similar niche to the species that threaten California food webs (Work et al. 1993, Lefebvre et al. 1999, Scholin et al. 2000, Lefebvre et al. 2002b), since it, too, is a filter-feeding, Clupeid fish. The gulf menhaden is an abundant, estuarine-dependent fish (Lassuy 1983) that strains the water column at a potentially high rate (Durbin & Durbin 1975) which would allow them to accumulate large amounts of DA. Gulf menhaden are one of the most abundant fishes in the northern Gulf of Mexico, with Louisiana providing up to 52 percent of the juvenile abundance for the nation's second largest fishery in terms of landings (Vaughan 2007). They are also identified as a major prey item for several upper trophic level predators that are common in Louisiana estuaries, such as brown pelicans, bottlenose dolphins (*Tursiops*

truncatus), and several species of shark (Hildebrand 1963, Snelson et al. 1984, Ahrenholz 1991, Hoffmayer & Parsons 2003, Bethea et al. 2004, Barry et al. 2008, U.S. Fish and Wildlife Service 2008), so DA transmission to top predators could possibly occur in Louisiana estuaries.

One estuary that is at particularly high risk is Terrebonne Bay, Louisiana. Moderately high amounts of *Pseudo-nitzschia* (10^5 cells l^{-1}) have been documented in its waters and the adjacent water mass (Dortch et al. 1997), and, like most Louisiana estuaries, there is a high abundance of gulf menhaden that reside in the bay. Based on previous research and knowledge concerning DA and its vectors, the goal of the present study is to identify whether the gulf menhaden can act as a vector of DA in Terrebonne Bay, Louisiana.

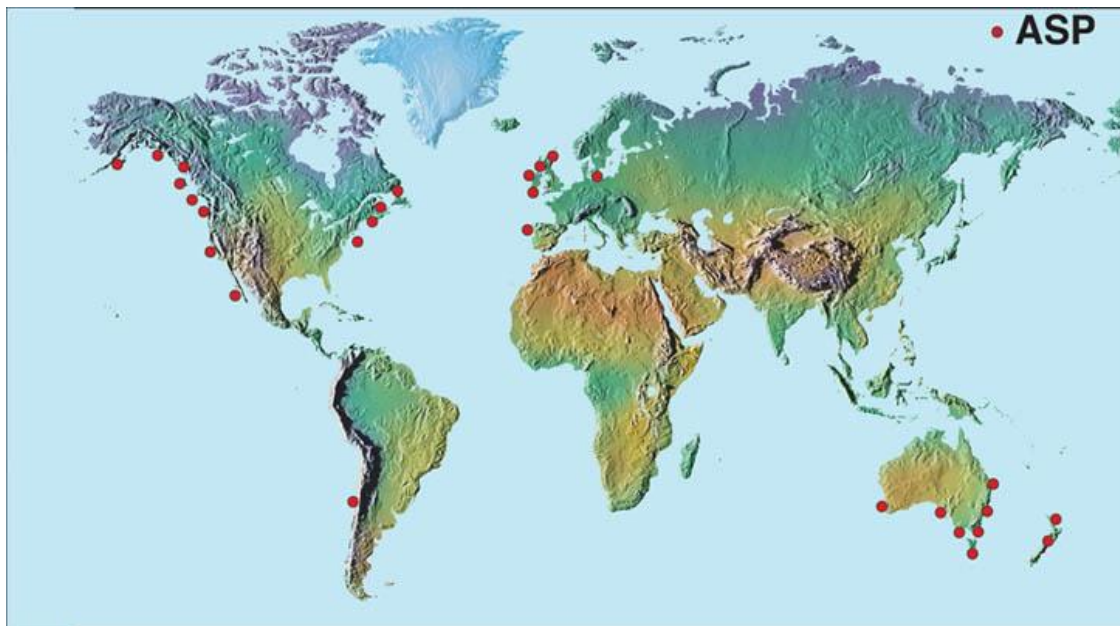


Figure 2.1. A map created by Woods Hole Oceanographic Institute of the distribution of Amnesic Shellfish Poisoning events around the world as of October 2008.

MATERIALS AND METHODS

Description of the Study Site: Terrebonne Bay, Louisiana, USA

The present study was conducted in Terrebonne Bay, Louisiana (29°09'N, 90°38'W), which is a typical representation of a Louisiana estuary (Fig. 2.2). It is bordered on the south by

barrier islands: Isles Dernieres, Timbalier Island, East Timbalier Island, and has three large connections to the open Gulf of Mexico: Whiskey Pass, Little Pass Timbalier, and a combination of Cat Island Pass and Wine Island Pass (Inoue & Wiseman 2000). Terrebonne Bay is a micro-tidal environment spanning 1761 km², generally with shallow, turbid waters (USEPA 1999). Because the bay receives little freshwater input, has a small tidal range, and demonstrates a diurnal tide, wind likely has the greatest impact on water movement (Marmer 1954, Prager 1992). In addition to typical physical characteristics of a Louisiana estuary, the nekton community in Terrebonne Bay closely resembles those of other estuaries along the Louisiana coast. Terrebonne Bay hosts diverse assemblage of nekton, commonly with high numbers of gulf menhaden, making this site an ideal place to conduct the present study.

Field Sampling and Initial Processing

In order to answer the question whether gulf menhaden can be a vector of the neurotoxin DA in Terrebonne Bay, Louisiana, water and fish samples were collected from that area to determine *Pseudo-nitzschia* cell presence and abundance, and DA concentration. Because these fish spawn offshore in winter (Ahrenholz 1991), field trips were conducted monthly from July 2007 to September 2007 and from April 2008 to June 2008 to ensure that gulf menhaden would be found in Terrebonne Bay. Sampling sites were chosen based on areas with a high probability of catching fish or observed gulf menhaden feeding (Fig. 2.2). When a suitable sampling site was found, a 186 m long gillnet was deployed, allowed to soak for 30 minutes, and then retrieved. The monofilament gillnet was composed of 6 panels of stretch mesh, ranging from sizes 7.6 cm (3.0”) to 14.0 cm (5.5”), in steps of 1.3 cm (0.5”). If time would not allow for a deployment of the gillnet, a 1.83 m (6’) diameter cast net with 0.95 cm (3/8”) mesh was also used to sample the fish. After the net was deployed, whole water samples were taken by placing a clean 2.0 liter Nalgene bottle in the surface-water. Also, concentrated plankton samples for species

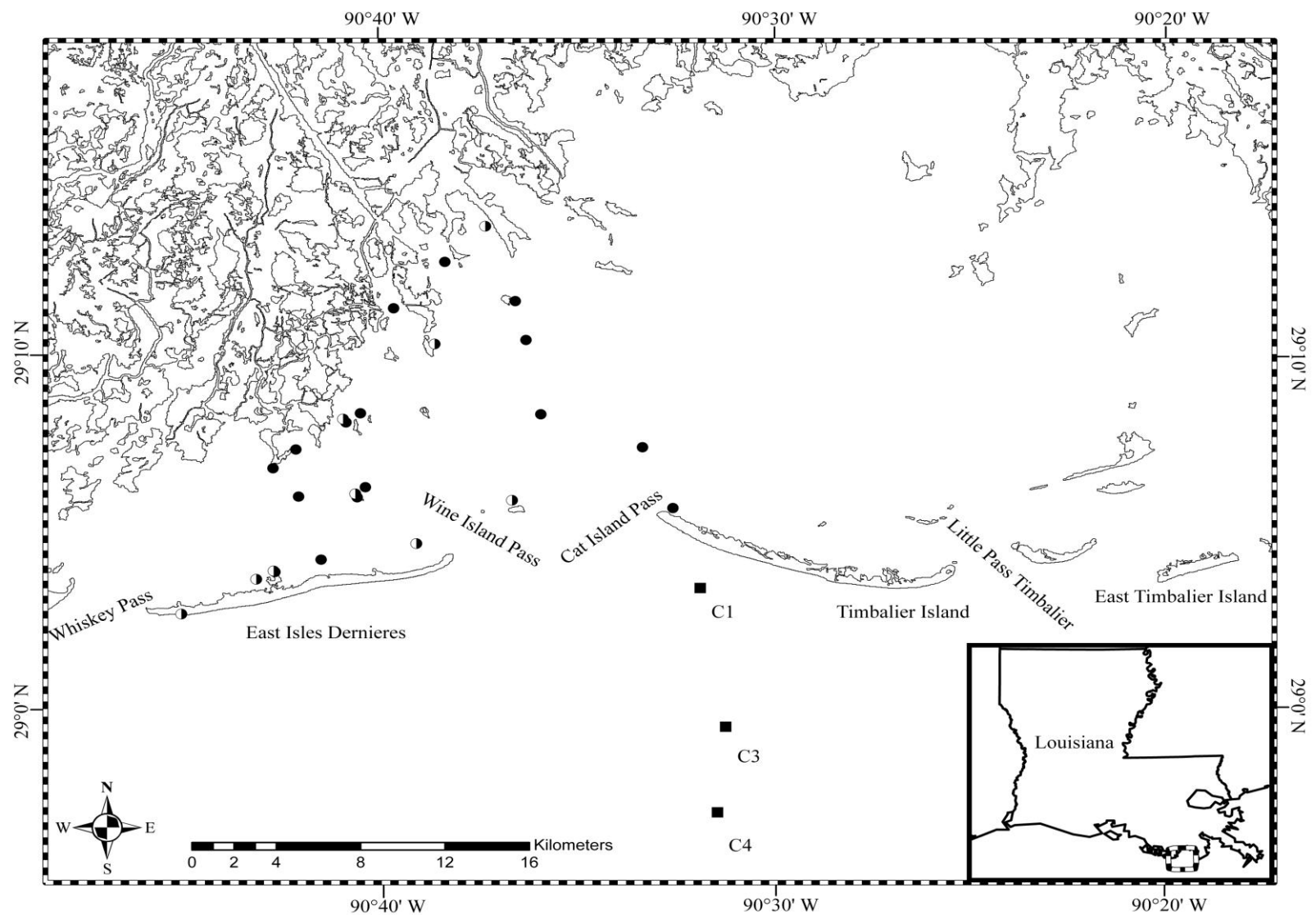


Figure 2.2. Map of sampling locations in Terrebonne Bay, Louisiana. Dark circles are 2007 sampling locations, half-white, half-black circles are 2008 sampling sites, and black squares are coastal sampling stations.

identifications were obtained by vertical net tows using a 30 μm mesh size plankton net (Aqua Research Instruments). The plankton net was lowered vertically into the water until it was no longer visible and then was retrieved; this process was repeated three times to ensure adequate sampling of the plankton. Subsamples of whole water and the net tows were archived in a 2% Glutaraldehyde solution immediately after collection, to be used in microscope observations of cell abundance and community composition, and were kept on ice in the field, and then refrigerated in the lab until needed for analysis. Other subsamples of whole water were kept on ice and brought to the lab for processing for Chlorophyll *a* (Chl. *a*) and particulate and dissolved toxin analyses. Meanwhile, water temperature ($^{\circ}\text{C}$), salinity (ppt), and dissolved oxygen (mg l^{-1}) were recorded *in situ* from a YSI-85 environmental meter; average depth (m) was recorded from the vessel's depth finder; water clarity (cm) was measured by secchi disc; and qualitative habitat type (e.g., mud, sand, oyster shell, etc.) was determined by visual observation. After the net was retrieved, caught gulf menhaden were measured to fork length (FL) and humanely euthanized in an ice water slurry. All by-catch were identified, measured to FL or total length (TL), and released. At the field station, 12 gulf menhaden were chosen at random from each sample set to be used in toxin or stomach contents analyses, and kept on ice until brought to the lab, where they were transferred to a -20°C freezer until analysis. The remaining fish were donated to another researcher.

Laboratory Procedures

Chlorophyll *a* Analysis

Chl. *a* concentrations were determined for samples taken from Terrebonne Bay in order to determine the total phytoplankton biomass. Fifty ml of whole water was filtered through a 25 mm diameter, 0.7 μm GF/F filter (Whatman), which was stored in a 15 ml centrifuge tube, covered with aluminum foil, and frozen at -20°C until processed. Extraction of Chl. *a* was

accomplished by adding 10 ml of 90% acetone (Sigma-Aldrich) to the filter, vortexing for 1 minute, and storing at -20°C for 24 hours. Extracted samples were allowed to reach room temperature and then centrifuged for 10 minutes with a relative centrifugal force (RCF) of 1399 g before the analyses. Chl. *a* concentrations were read on a Turner 10-AU fluorometer in low light to minimize photodegradation.

Domoic Acid (DA) Analysis

Enzyme-linked Immunosorbent Assay (ELISA) was used to quantify DA concentrations in water and fish samples. This method was chosen because of its very low detection limit (~10 pg DA ml⁻¹) and its status as one of the newest technologies available for toxin detection. A commercial DA ELISA kit (ASP direct cELISA kit) available from Biosense Laboratories through Abraxis LLC (USA) was used for the toxin assay.

DA EXTRACTION FROM WATER SAMPLES

Both particulate (DA contained within a cell) and dissolved (extra-cellular DA) toxins were measured in water samples collected from Terrebonne Bay. For particulate toxin samples, two replicates of whole water, taken from the same container, were filtered through three 25 mm diameter, 0.7 µm GF/F filters (Whatman) until it clogged (approx. 195 - 500 ml). The filters were then stored in 2 ml microcentrifuge tubes, the volume filtered was recorded, and filters were frozen at -20°C until analysis. The filtrate was collected in a 50 ml centrifuge tube and frozen to be used in dissolved toxin analysis. Unlike dissolved DA samples, particulate samples were subjected to extraction steps prior to the ELISA procedure. The extraction procedure for particulate DA, according to the ELISA protocol (Biosense Laboratories), involved placing the filter(s) in a 15 ml centrifuge tube, thawing the samples at room temperature for 1 hour, adding 10 ml of 20% methanol (MeOH), and vortexing the samples for 1 minute. Following the MeOH extraction, the filters were sonicated with a Misonix Sonicator 3000, equipped with a microtip,

for 2 minutes. This step was included to ensure that all cells contained on the filters were ruptured, releasing DA into the extraction solution. The samples were then centrifuged for 10 minutes at 3000 RPM with a RCF of 1399 g in a Thermo Electron Corp. IEC Centra CL2 centrifuge, and the supernatant was passed through a 0.22 μ m syringe filter into a clean 15 ml centrifuge tube to remove any remaining filter particles. Dissolved samples were filtered through a 0.22 μ m syringe filter into a clean 50 ml centrifuge tube to remove any particles since the DA was already free in solution. Finally, all samples were stored temporarily at -20°C until needed.

DA EXTRACTION FROM GULF MENHADEN VISCERAL TISSUE SAMPLES

Gulf menhaden gastro-intestinal tracts (GI), excluding the liver, were analyzed for presence of DA. Frozen gulf menhaden were thawed and approximately 5 - 6 fish were randomly chosen from each set to be dissected. Prior to dissection, each fish was measured to FL and weighed. Using scissors, dissection of the fish was performed by cutting from the anus through the pectoral girdle, along the keel. Once the body cavity was open, the esophagus was cut at the anterior end as high as possible, and the intestine was severed posterior as close to the anus as possible, freeing the entire GI.

The dissected fish GI(s) were combined and homogenized in a plastic beaker until smooth using a Biohomogenizer with a 1.4 and 2.5 cm generator (Biospec). However, the gulf menhaden's tough pyloric stomach, made up of the pyloric caecae and gizzard, could not be homogenized smooth, so those pieces were coarsely shredded, in order to include the stomach contents in the homogenate, and removed after homogenization. Four grams (or less when samples did not contain 4 g) of the homogenate was weighed on a Mettler balance, as the Biosense ELISA protocol specifies. Homogenate was then placed in a clean 50 ml centrifuge tube and 50% MeOH was added in a 1:4 weight to volume ratio, 1 part viscera weight to 4 parts MeOH. Since this step was dependent on the weight of the homogenate, samples weighing less

were taken into account. After this step, the homogenate was vortexed, sonicated, filtered, and stored in the same manner as the water samples.

TOXIN ANALYSIS

Prior to performing the ELISA for water and tissue samples, a spike and recovery experiment was conducted to determine the extraction efficiencies for each sample type. This procedure entailed adding a $0.01 \mu\text{g g}^{-1}$ spike of DA standard (Sigma-Aldrich) to a clean filter or homogenate before it was extracted. The remaining protocol was performed as described above. For tissue, since samples free of DA contamination were unavailable, another subsample from the same homogenate was prepared without a DA spike as a control, in order to assess the background DA level. After the results were obtained, the final DA number of the control sample was subtracted from the final DA value of the spiked sample, and that amount was divided by the spike amount in order to determine the percentage of the spike that was detected by the assay. Extraction efficiencies were 96.8% for water and 127% for gulf menhaden.

After the assay was verified to give reasonable values, all samples were analyzed following the protocol included in the kit (Appendix I). Each sample was run in duplicate at several dilutions in order to reduce possible additional matrix effects (interference from components of the sample other than the compound of interest). Using a micro-plate ELISA photometer, absorbance of the sample was read at a wavelength of 450 nm. After the raw photometric data was gathered, those numbers were entered into a macro developed by Biosense Labs for Microsoft Excel to give a DA concentration (pg ml^{-1}). For water samples, extraction solution volumes and the volumes of the samples filtered were integrated into the calculations to get the DA concentrations in ng DA l^{-1} . To get the concentration of DA per unit of fish mass, the macro numbers were inserted into the following formula:

$$\text{mg DA kg}^{-1} \text{ tissue} = \mu\text{g DA g}^{-1} \text{ tissue} = \frac{\text{macro number} * \text{extraction volume} * 1 \mu\text{g}/1,000,000 \text{ pg}}{\text{Mass of homogenate}}$$

***Pseudo-nitzschia* Cell Enumerations and Phytoplankton Community Composition**

Counts of *Pseudo-nitzschia* cells in Terrebonne Bay water, where the fish samples were collected, were attempted in order to estimate their abundance during the study. However, efforts to count *Pseudo-nitzschia* in these water samples were unsuccessful and are likely the result of a large, mixed assemblage of phytoplankton that made it difficult to find *Pseudo-nitzschia* under the light microscope. Instead, whole water samples from near-shore areas of the Louisiana continental shelf adjacent to Terrebonne Bay were counted. These samples were taken from a monthly sampling effort in coastal Louisiana conducted by researchers at Louisiana Universities Marine Consortium (LUMCON), in which the Bargu lab at Louisiana State University also participates, from the three stations nearest to Terrebonne Bay (C1, C3, and C4) (Fig. 2.2). Only the three closest stations from the transect sampled were counted because this is approximately the maximum range for the gulf menhaden fishery in coastal Louisiana (Vaughan 2007). The cell counts were conducted on samples from the experimental months as well as the month prior to sampling for the present study.

Pseudo-nitzschia cell counts were done using a gridded Sedgewick-Rafter 1 mm² counting chamber using a Zeiss Axio Observer - A1 inverted microscope with epifluorescence capability. *Pseudo-nitzschia* spp. cells were counted until 200 cells were reached, then the number of grids counted was recorded. For cell counts between 10 and 200 cells, the entire counting chamber was searched. If less than 10 cells were counted, 5 – 20 mls of the sample was stained with proflavin (Sigma-Aldrich), filtered, and recounted using epifluorescence microscopy on the Zeiss inverted microscope.

At the same time, a qualitative assessment of the major components of the plankton community in the sample was also recorded to gain a better understanding of Terrebonne Bay plankton community structure. For all of the water samples taken in Terrebonne Bay and at the

near-shore stations, common plankton groups were recorded, and the four most common categories were determined. The categories were created on the basis of a relative numerical percentage, then ranked as either rare ($>1\%$), present ($1\% \leq x \leq 10\%$), common ($10\% \leq x \leq 50\%$), or dominant ($< 50\%$) in the sample.

Gulf Menhaden Gut Contents Analyses

Gulf menhaden gut contents were examined in order to assess their composition and identify the presence of *Pseudo-nitzschia*. For gut content analyses, 5 fish from each sampling event were chosen at random and measured to FL and weighed. The fish were dissected in the same manner as for the toxin analysis, and the weight of the visceral mass was recorded. Finally, the contents of the entire GI were extruded and collected in a 25 ml scintillation vial to be preserved in a 2% Glutaraldehyde solution and kept refrigerated until analyses.

For the analysis of the community composition of the gulf menhaden gut contents, sample dilution was necessary due to the amount of debris in the GI of these filter-feeding fish. Initially, 1.0 ml of the gut contents solution, taken from each of three fish chosen at random from each selected sampling set, was put separately into different Petri dishes and diluted with water until the bottom of the dish was covered. The sample matter was allowed to settle for 20 minutes and then inspected at 20 X and 40 X magnifications, repeating this entire procedure two more times. The community composition of the gut contents was classified in the same manner as the water samples.

Because *Pseudo-nitzschia* was infrequently seen under light microscopy, this method was not adequate to conclusively confirm its presence for each sample or identify species of the cells encountered. While *Pseudo-nitzschia* can be seen through a light microscope, it can only give a rough estimate as to the identity of a species through its overall shape. Absolute *Pseudo-nitzschia* species identification is based on morphological features that can only be examined

with an electron microscope. As a result, the gut contents samples had to undergo a cleaning procedure to remove organic matter and be examined under transmission electron microscopy (TEM) (see below).

***Pseudo-nitzschia* Species Identification**

Confirmation of the species of *Pseudo-nitzschia* identified in gulf menhaden and water samples was undertaken to investigate if those species are the ones that are known to produce DA. For *Pseudo-nitzschia* species identification, gut contents of gulf menhaden containing the highest concentrations of DA in their tissue (April 2008 Set 1) and gut contents from gulf menhaden from the date when the water had the highest DA concentration (April 2008 Set 2) were prepared for TEM. These samples were chosen because they had the highest DA concentrations found in this study; therefore, they provided the best opportunity to discover which species of toxic *Pseudo-nitzschia* were responsible for contaminating the fish. The corresponding water samples from Terrebonne Bay for April 2008 Set 1 and 2, and the April monthly sampling water were also prepared for TEM in order to identify the species of *Pseudo-nitzschia* present in the environment, and if these species match those found in gulf menhaden.

In order to confirm the presence of *Pseudo-nitzschia* and identify them to species level in Terrebonne Bay water and gulf menhaden gut contents, an additional cleaning method was used to target diatoms specifically. The cleaning step began with filtering a volume of sample through a 0.8 µm ATTP Isopore polycarbonate membrane filter (Millipore) on a custom-made filtration manifold. The sample was then rinsed three times with 3.0 ml of MilliQ water to remove excess salts. To remove organic matter from the samples, one or two drops of saturated potassium permanganate (KMnO₄), enough to cover the filter, was added with the vacuum off and allowed to sit for 1 hour. Next, the KMnO₄ was filtered through, and 3.0 ml of 12N HCl was added and allowed it to sit for a few minutes, in order to remove any remaining KMnO₄. This was filtered

and repeated until the sample was clear. Then, 3.0 ml of HCl was left to sit for 2 hours with the vacuum off. After filtering the HCl, the sample was rinsed twice with 3.0 ml of MilliQ water and 15 ml of MilliQ water was added for a final rinse. To ensure proper cleaning of the samples, this entire process was repeated for each sample. After cleaning, the filter was submerged in 0.5 ml of MilliQ water in a microcentrifuge tube and resuspended in solution. The water was affixed onto a 100 mesh copper grid with a formvar carbon support film. Finally, the samples were placed in a dessicator to dry before viewing them on a Jeol 100CX TEM.

Images of *Pseudo-nitzschia* frustules included whole or pieces of the diatom frustule, and when possible, a spot skewed from center, the central nodulus, and a close-up of the poroids were taken in order to facilitate the identification of species. From the TEM pictures, morphological characteristics, transapical axis (width), a count of striae and fibulae contained within 10 μm , the number of poroids in 1 μm , the number of rows of poroids, the existence of subdivisions within the poroids, and the presence of a central nodulus were measured. The dimensions were then compared to those of *Pseudo-nitzschia* spp. summarized in Bates (2007) and established in primary literature (e.g. Skov et al. 1999, Lundholm et al. 2003) for confirmation of species identity.

RESULTS

Environmental Characterization of Sampling Area

Over the course of the study, a total of 25 gillnet or cast net sets were collected at various locations throughout Terrebonne Bay, Louisiana. Sixteen sets were conducted from July through September 2007, and 9 sets from April to June 2008. For each of the sets, global positioning satellite (GPS) coordinates and a suite of environmental parameters were recorded. All the environmental parameters, including GPS location and substrate type, for each set are summarized in Table 2.1. Over the entire study interval, the variation in each environmental

Table 2.1. Summary of environmental parameters during field sampling. N.D. = No Data

Date	Set	Area Description	Latitude	Longitude	Depth (m)	Turbidity (cm)	Temp (°C)	Salinity (ppt)	DO (mg/L)	Substrate Type
9-Jul-07	1	Lake Peltó Sulphur Mine	29° 06.28' N	90° 40.466' W	2.41	105	32.3	25.7	12.2	Mud
10-Jul-07	2	Timbalier Island Western End	29° 05.685' N	90° 32.598' W	2.42	70	33	30.4	7.3	Sandy Clay
10-Jul-07	3	Terrebonne Bay	29° 07.422' N	90° 33.377' W	N.D.	N.D.	31.9	26.5	6.3	N.D.
10-Jul-07	4	Terrebonne Bay	29° 10.483' N	90° 36.356' W	2.62	N.D.	31.7	21.3	8.9	N.D.
11-Jul-07	5	Terrebonne Bay	29° 04.212' N	90° 41.599' W	N.D.	N.D.	31.7	25.4	9.2	N.D.
11-Jul-07	6	Lake Peltó, North of Bodwin Cutoff	29° 07.356' N	90° 42.246' W	1.14	47	32.7	24.2	6.5	Detritus and Mud
11-Jul-07	7	Bay Coon Road	29° 11.388' N	90° 39.745' W	1.16	N.D.	32.6	19.9	6.7	N.D.
7-Aug-07	8	Terrebonne Bay, Upper Range Marker	29° 11.593' N	90° 36.631' W	1.33	18	32.3	18.1	7.2	Clay w/ Shell
8-Aug-07	9	Terrebonne Bay	29° 08.362' N	90° 35.977' W	2.56	60	32.2	23	6.7	Clay w/ Shell
9-Aug-07	10	Last Island at Old Camp Pass	29° 03.859' N	90° 42.780' W	2.44	110	30.7	27.4	5	N.D.
9-Aug-07	11	Timbalier Bay	29° 06.011' N	90° 42.175' W	2.31	55	32.3	26.6	5.53	N.D.
9-Aug-07	12	Timbalier Bay	29° 08.130' N	90° 40.964' W	N.D.	40	33.1	24.6	5.63	N.D.
21-Sep-07	13	Lake Peltó	29° 08.39' N	90° 40.593' W	1.99	45	26.9	25.3	5.91	Clay and Mud
21-Sep-07	14	Lake Peltó	29° 06.822' N	90° 42.830' W	1.8	70	27.4	25.2	7.2	Caly and Shell
21-Sep-07	15	Lake Peltó Sulfur Mine	29° 05.991' N	90° 40.676' W	1.93	60	27.7	27.6	6.95	Clay and shell
21-Sep-07	16	Near Houma Navigation Channel	29° 12.713' N	90° 38.433' W	1.43	40	28.2	19.2	7.5	Silt
16-Apr-08	17	Lake Peltó	29° 03.654' N	90° 43.259' W	2.48	90	20.4	24.9	8.9	Sandy Clay
16-Apr-08	18	Sulphur Mine	29° 06.091' N	90° 40.726' W	2.22	65	20	23.5	9.1	Shelly Clay
16-Apr-08	19	Bay St. Elaine	29° 08.229' N	90° 41.037' W	1.77	30	19	17.4	9	Clay w/ Some Shell
18-Apr-08	20	Bay Chaland	29° 13.730' N	90° 37.399' W	1.25	35	19.9	14.4	8.7	Mud w/ Some Shell Fragments
18-Apr-08	21	Upper Terrebonne Bay	29° 10.370' N	90° 38.695' W	1.68	31	19.8	19	7.7	Clay
19-May-08	22	North of Last Island	29° 04.671' N	90° 39.163' W	2.73	45	26.5	18.8	7.2	N.D.
6-Jun-08	23	Lake Peltó behind Last Island	29° 03.892' N	90° 42.816' W	2.89	61	29.4	18.9	8	Clay
6-Jun-08	24	GOM (front side of Last Island)	29° 02.657' N	90° 45.176' W	2.28	50	30.4	19.2	9.59	Sand
6-Jun-08	25	Wine Island	29° 05.909' N	90° 36.715' W	2.13	55	31	18.6	8.3	Mud w/ Clay and Shell

parameter was relatively low, and mean values (followed by standard deviation) were 29.1 ± 3.3 °C for water temperature, 21.5 ± 3.3 ppt for salinity, 7.5 ± 1.3 mg l⁻¹ for dissolved oxygen (DO), 2.4 ± 1.4 m for depth, and 56.3 ± 23.5 cm for turbidity. The highest salinity, turbidity, and DO were found in July 2007, while the minimum turbidity and DO corresponded to the maximum temperature in August 2007 (Fig. 2.3). Additionally, the minimum temperature and salinity were observed in April 2008.

Chl. *a* concentrations throughout the study revealed a system that had moderately high levels for much of the year. The mean Chl. *a* concentration in Terrebonne Bay over the whole study period was 20.31 ± 11.2 µg l⁻¹, with a maximum of 47.67 µg l⁻¹ found in June 2008 and a minimum of 7.32 µg l⁻¹ found in August 2007 (Fig. 2.4).

Description of Fish Landings during Field Sampling

Over the entire study, a total of 23 different fish or crustacean species were caught with gulf menhaden being the most frequently encountered. In total, 481 gulf menhaden, representing 48.7% of the total catch (Fig. 2.5) with a mean catch per unit effort (CPUE) of 13.6 ± 18.7 , were caught in the sampling effort. While the highest yield came in May 2008, with 137 fish, sets 2 and 5 in July 2007, and set 16 in September 2007 yielded none. Although gulf menhaden were caught in gillnets of all mesh sizes, 79% were found in the smallest mesh size (7.62 cm).

However, despite mostly being caught in the smallest mesh of the gillnet, the mean FL for gulf menhaden in this study was 19.2 ± 2.0 cm (Fig. 2.6). This length corresponds to fish two to three years old (Lassuy 1983), indicating that, on average, these were adult fish since gulf menhaden mature at age 1. Descriptive statistics for gulf menhaden catch is shown in Table 2.2. In addition to gulf menhaden, several other species of fishes or crustaceans, including blue crabs (*Callinectes sapidus*), gaff topsail (*Bagre marinus*), hardhead catfish (*Arius felis*), and

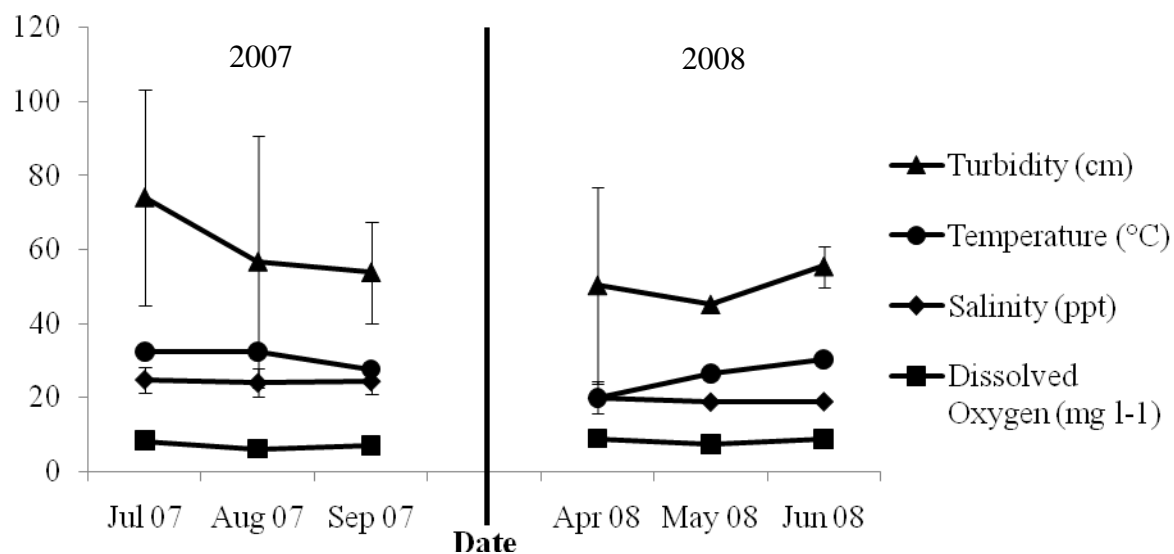


Figure 2.3. Time series of mean values for salinity (ppt), dissolved oxygen (mg l⁻¹), turbidity (cm), and temperature (°C). Error bars represent standard deviation.

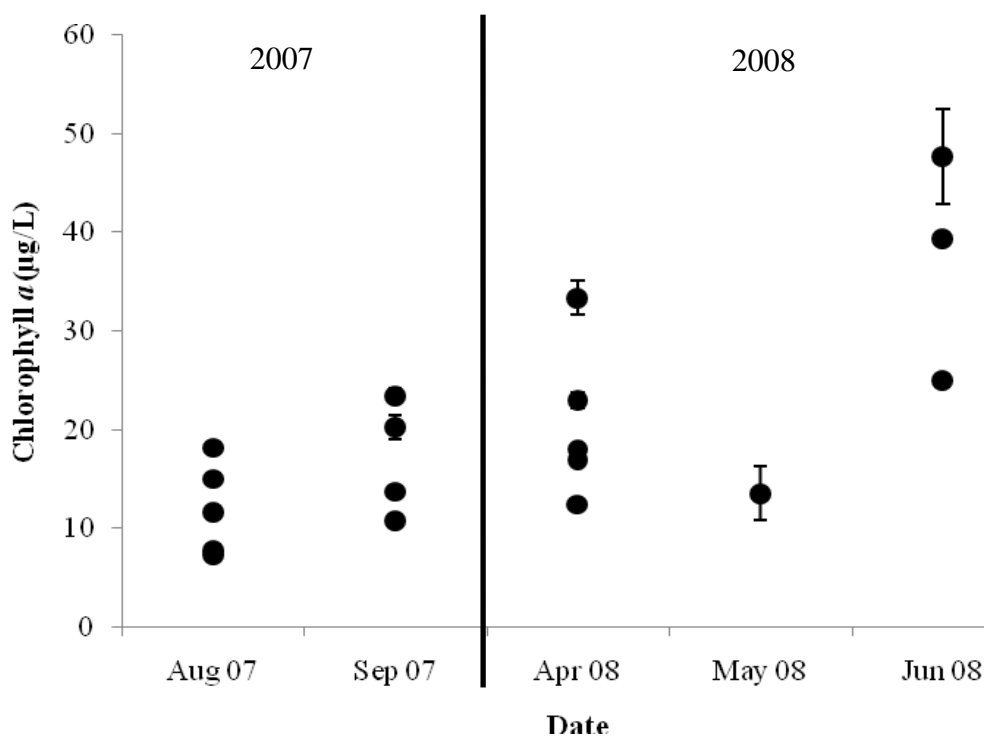


Figure 2.4. Chlorophyll *a* (µg l⁻¹) in Terrebonne Bay water samples during the sampling seasons. Each point represents a sampling site, see Fig. 2 for locations.

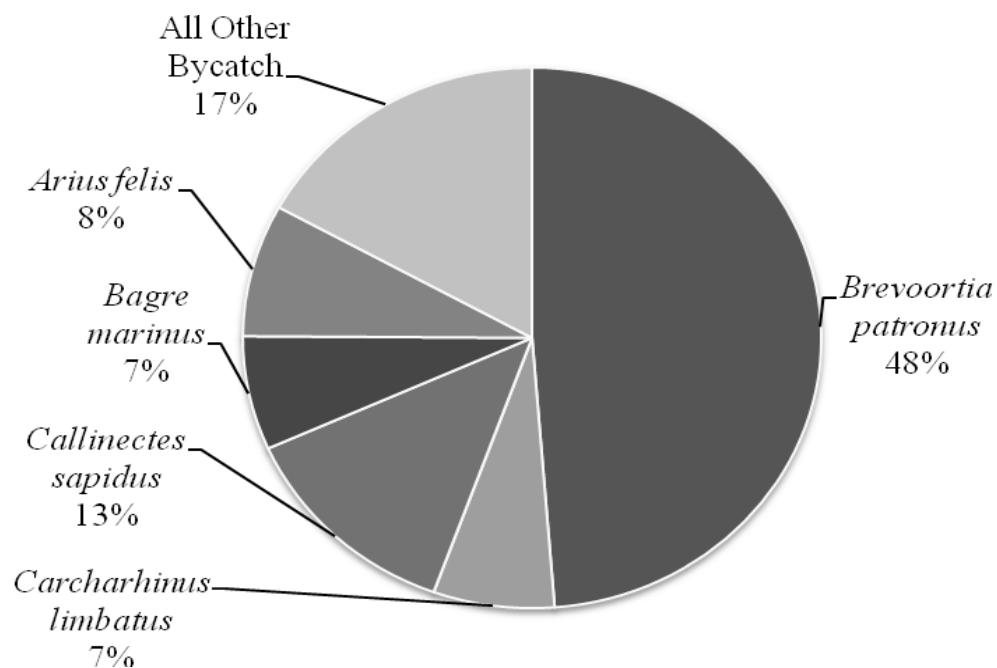


Figure 2.5. Percent landings of major groups of nekton from all 25 field sampling events using cast and gill-nets.

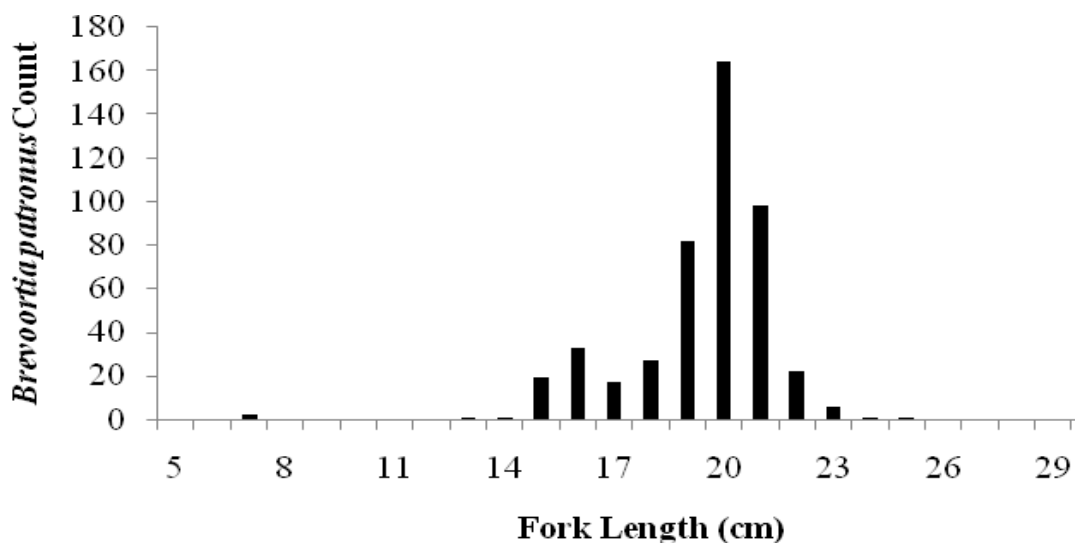


Figure 2.6. Numbers of *Brevoortia patronus* caught in gillnets or cast nets throughout sampling by size, FL.

Table 2.2. Summary of *Brevoortia patronus* catch data throughout sampling period. Values are means with standard deviations are in parenthesis. FL = fork length measurement, CPUE = catch per unit effort, and N.D. = No Data.

Date	Set	Number Caught	Gear Type	FL (cm)	Wet Weight (g)	CPUE
9-Jul-07	1	1	Gill Net	16	80.4	0.67
10-Jul-07	2	0	Gill Net	N.D.	N.D.	N.D.
10-Jul-07	3	6	Cast Net	N.D.	N.D.	N.D.
10-Jul-07	4	7	Cast Net	15.7 (1.25)	77 (23.47)	N.D.
11-Jul-07	5	0	Cast Net	N.D.	N.D.	N.D.
11-Jul-07	6	19	Gill Net	15.8 (1.31)	78.2 (21.55)	0.91
11-Jul-07	7	2	Cast Net	6.75 (0.35)	12.7 (0.89)	N.D.
7-Aug-07	8	1	Gill Net	16	71.7	0.57
8-Aug-07	9	5	Gill Net	17.2 (3.29)	123.8 (107.83)	1.42
9-Aug-07	10	6	Cast Net	15.5 (2.16)	78 (35.02)	N.D.
9-Aug-07	11	5	Cast Net	15.5 (0.35)	71.7 (5.02)	N.D.
9-Aug-07	12	6	Cast Net	15.4 (0.38)	70.6 (5.3)	N.D.
21-Sep-07	13	97	Gill Net	19.7 (1.33)	170.1 (42)	69.29
21-Sep-07	14	4	Gill Net	18.7 (1.71)	142.4 (50.63)	3.88
21-Sep-07	15	13	Gill Net	20.8 (.99)	210.1 (43.58)	12.75
21-Sep-07	16	0	Gill Net	N.D.	N.D.	N.D.
16-Apr-08	17	2	Gill Net	20.2 (1.06)	185.4 (27.02)	1.92
16-Apr-08	18	19	Gill Net	20.4 (1.23)	193.7 (56.53)	19.8
16-Apr-08	19	7	Gill Net	19.3 (1.35)	156.4 (43.93)	7.87
18-Apr-08	20	1	Gill Net	22.5	286.3	1.2
18-Apr-08	21	4	Gill Net	19.9 (1.31)	174.6 (44.4)	4.76
19-May-08	22	137	Gill Net	19.3 (1.3)	157.7 (36.21)	23.54
6-Jun-08	23	64	Gill Net	19.4 (1.6)	161.8 (52.11)	38.79
6-Jun-08	24	66	Gill Net	19.7 (1.29)	169.6 (40.06)	38.15
6-Jun-08	25	9	Gill Net	20.5 (0.53)	195.8 (20.88)	2.97

blacktip sharks (*Carcharhinus limbatus*), were caught in gillnets during the sampling periods (Fig. 2.5). A summary table of all bycatch species, number caught, and sizes is displayed in Appendix II.

DA in Water and Gulf Menhaden

Results from the ELISA confirm the presence of DA both in water samples and gulf menhaden visceral tissue. DA was found in most of the Terrebonne Bay samples over the entire

study period with the frequency of detection at 72% for particulate water samples, 45% for dissolved samples, and 96% for gulf menhaden visceral tissue. Particulate DA in water samples from the entire study ranged from none detected to 43.4 ng l⁻¹ (Fig. 2.7a) and dissolved DA, taken only in 2008 water samples, ranged from none detected to 1.11 ng l⁻¹ (Fig. 2.7b). Plots of particulate DA concentration versus the environmental parameters during sampling indicate the seasonality of the sampling periods. A plot of particulate DA versus temperature showed the range of temperatures where toxin was detected to be from 20 to 33.5 °C (Fig. 2.8a). DA was mostly detected at the higher end of the range; however, the highest DA concentrations were seen at lower temperatures in Spring when blooms of diatoms are likely to occur. The graph of particulate DA and salinity revealed that toxin was found within a typical estuarine salinity range from 17 to 28 ppt with a spike in DA concentration at around 25 ppt (Fig. 2.8b). The depiction of particulate DA as a function of turbidity, a measure of water clarity, revealed DA to be most prevalent at lower levels of clarity, ranging from 25 to 60 cm. However, higher levels of DA were seen in less turbid waters (Fig. 2.8c). When particulate DA was plotted as a function of Chl. *a*, an overall trend of increased DA concentration and frequency of detection was seen with lower values of Chl. *a*. Most DA was detected within a range of 10 to 25 µg Chl. *a* l⁻¹ (Fig. 2.9).

DA concentrations from gulf menhaden visceral tissue ranged from none detected to 0.31 µg g⁻¹ (Fig. 2.10). In all sample types, particulate, dissolved or gulf menhaden visceral tissue, the maximum DA concentrations were found during April 2008 (Figs. 2.7 & 2.10). When mean particulate DA in water was compared with mean DA in gulf menhaden visceral tissue, the data appeared to be correlated, despite a large discrepancy in the May 2008 sample (Fig. 2.11). A simple linear regression was run across all of the data points and found that there was a correlation with particulate DA in the water and DA in gulf menhaden ($n = 25$, $p = 0.025$, $\alpha = 0.05$). However, a better correlation was found when the data from May 2008 was excluded ($n =$

24, $p = 0.01$, $\alpha = 0.001$). These results show that there is a correlated relationship between particulate DA in water and gulf menhaden visceral tissue DA that is predictive at a 0.05 level, but the relationship is not causal.

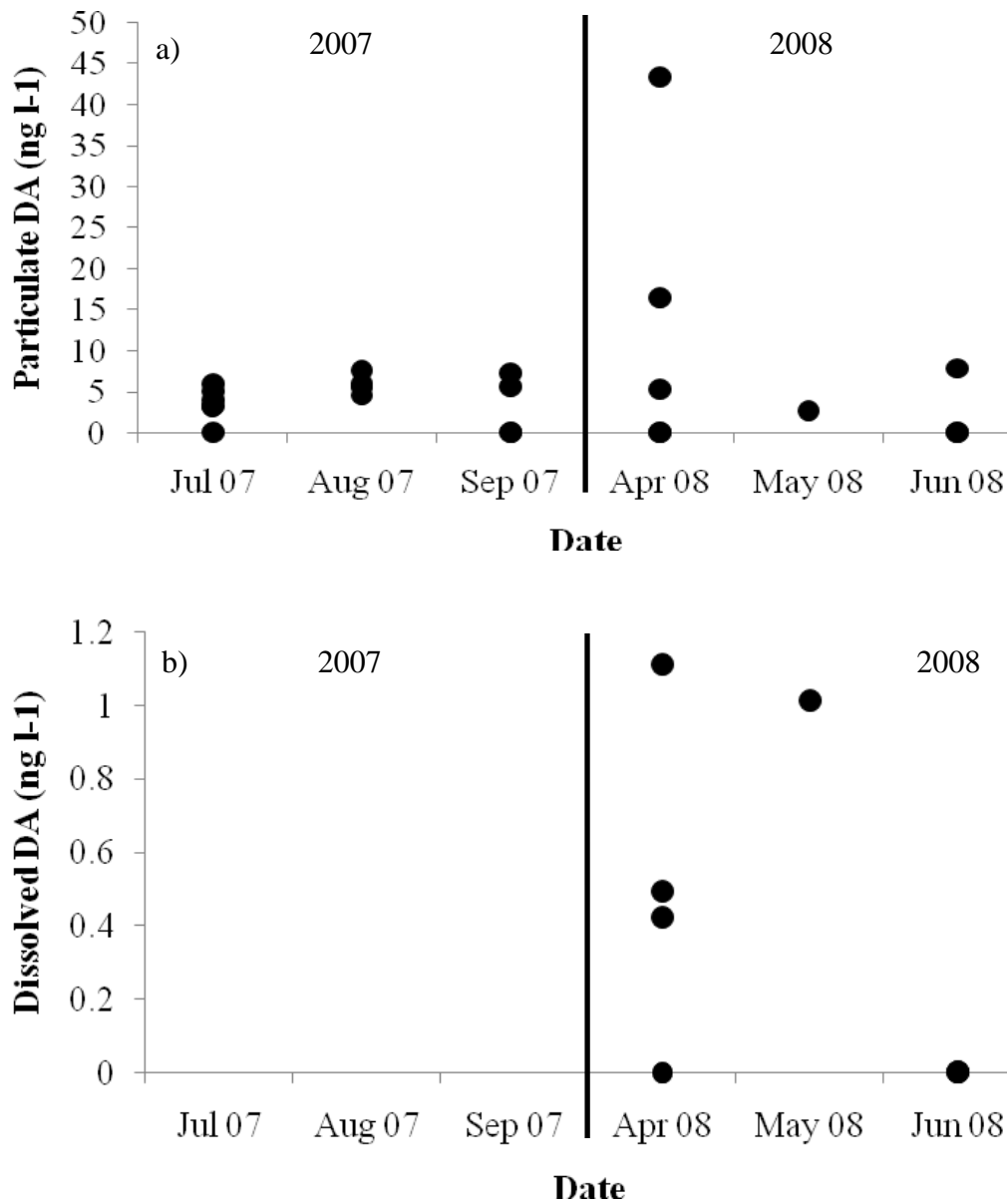


Figure 2.7. From Terrebonne Bay, Louisiana a) particulate domoic acid in water samples (ng l⁻¹), and b) dissolved domoic acid in water samples (ng l⁻¹).

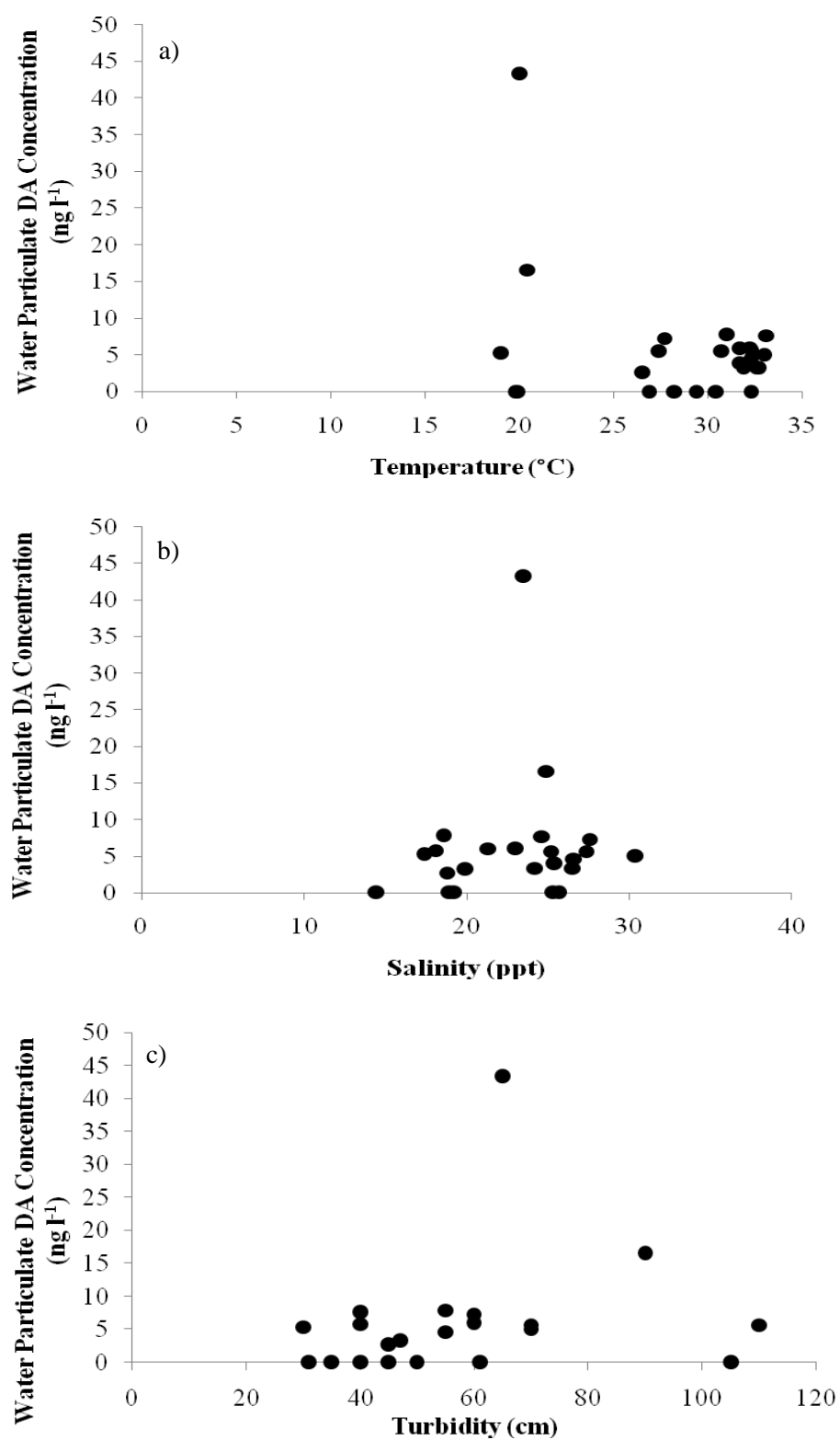


Figure 2.8. Particulate domoic acid plotted against environmental parameters a) temperature (°C), b) salinity (ppt) and c) turbidity (cm), from Terrebonne Bay from all sampling locations in the entire study.

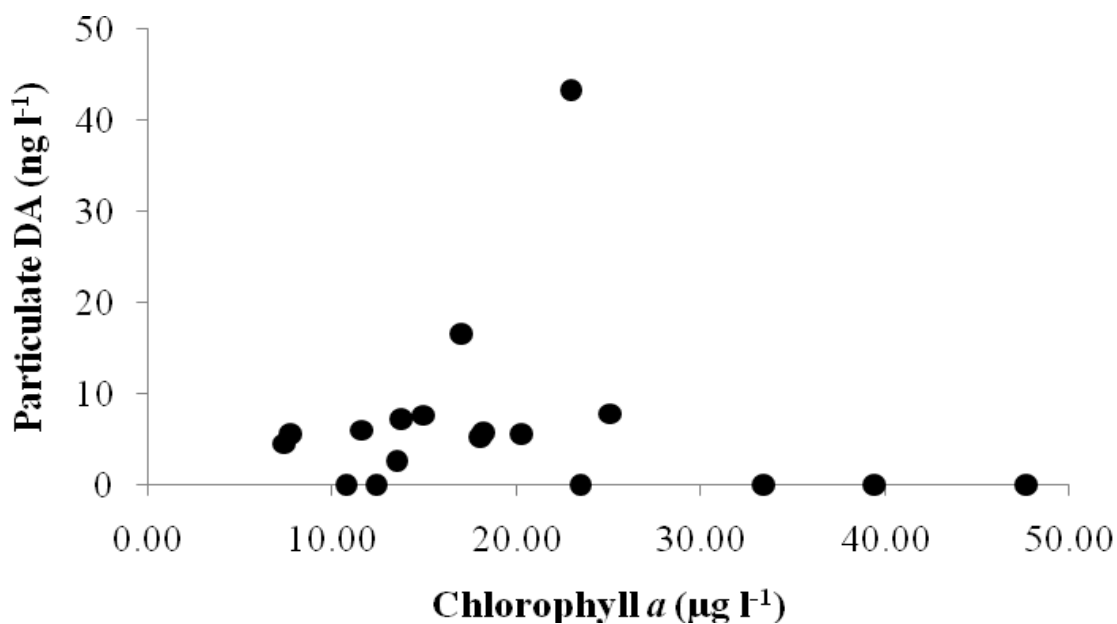


Figure 2.9. Terrebonne Bay particulate domoic acid as a function of Chlorophyll *a* from all samples.

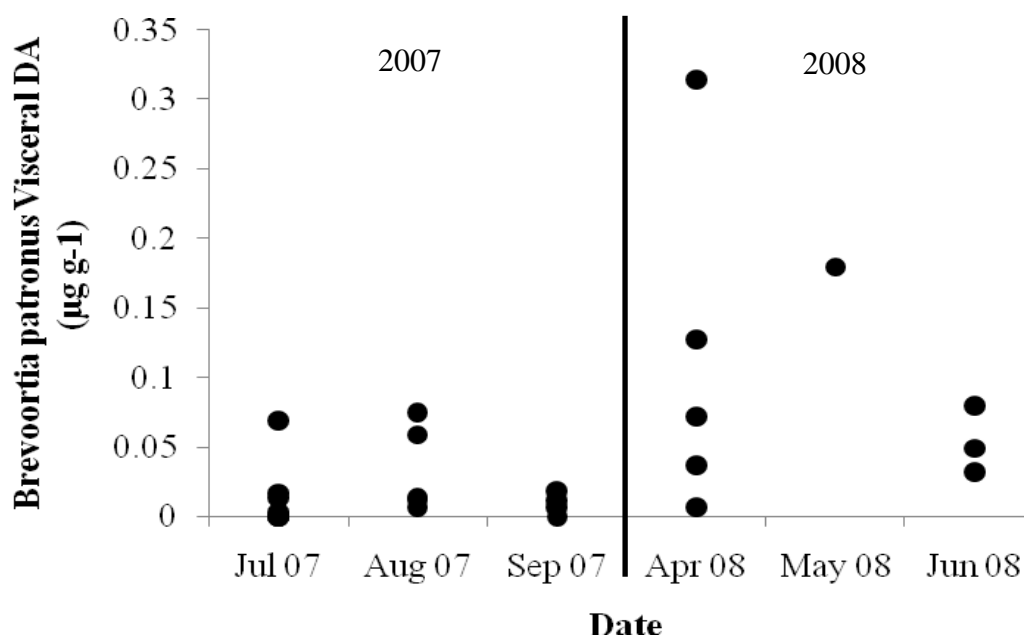


Figure 2.10. *Brevoortia patronus* visceral tissue domoic acid (µg g⁻¹) concentrations from Terrebonne Bay, Louisiana over the entire study period.

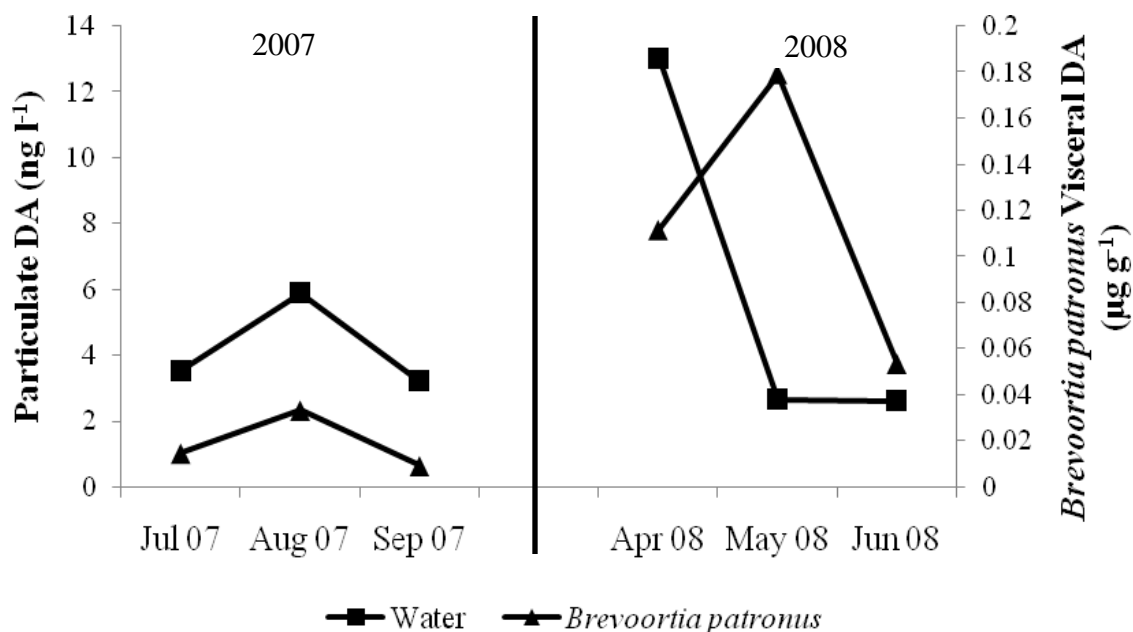


Figure 2.11. Time scale of mean *Brevoortia patronus* visceral tissue domoic acid plotted against mean particulate domoic acid for each month of the entire study period.

Pseudo-nitzschia Cell Abundances

Cell counts of coastal waters, just outside of Terrebonne Bay (Fig. 2.2), revealed the presence of *Pseudo-nitzschia* spp. throughout the entire study period. Direct cell counts of Terrebonne Bay water, where all of the fish samples were caught, were unsuccessful due to low *Pseudo-nitzschia* abundances present in high abundances of other phytoplankton in the same size range as *Pseudo-nitzschia*. Therefore, methods to yield a countable sample, through concentration in order to increase *Pseudo-nitzschia* abundance or filtration in an effort to reduce the amount of other phytoplankton, were not successful. *Pseudo-nitzschia* was present in every month sampled from coastal Louisiana LUMCON stations, except for station C1 in August 2007, but it was present at the other two stations sampled in that month. Counts revealed *Pseudo-nitzschia* abundance ranged from none to 2.06×10^6 cells l⁻¹, but the highest DA values in Terrebonne Bay corresponded to only moderate cell numbers (5×10^4 cells l⁻¹) in the coastal

waters. A plot of the cell abundances over time reveals slight variability, falling between the range of 1×10^4 and 1×10^6 cells l^{-1} , although the counts were transformed by adding 1 to all values to account for the lack of cells in the sample collected from August 2007 at Station C1 (Fig. 2.12).

Qualitative Community Composition of Water Samples

A qualitative examination of the plankton community composition was performed in samples collected over the entire study. The results were plotted over the entire study period using a qualitative scale as they relate to presence in the sample where 1 = rare, 2 = present, 3 = common, and 4 = dominant (Fig. 2.13). The analysis of Terrebonne Bay water samples revealed the most dominant plankton groups to be centric diatoms, followed by pennate diatoms, dinoflagellates, and zooplankton, including copepods, copepod nauplii, and tintinnids (Fig. 2.13a). When comparing all of the samples, centric diatoms were the most prevalent component of the plankton community, and zooplankton was the least common. Dinoflagellates were shown to be a more prominent member of the plankton in 2008 than 2007. Pennate diatoms were found to be present in all samples, but did not make up a significant portion of the community in any month sampled. The May 2008 sample showed a decrease in all categories of plankton with centric and pennate diatoms and dinoflagellates making up equivalent portions of the plankton community.

Similar to the Terrebonne Bay water samples, analysis of coastal waters revealed the major components of the plankton to be centric and pennate diatoms, dinoflagellates, and zooplankton, consisting of copepods, copepod nauplii, and tintinnids. Additionally, centric diatoms were the most important component of the community, and zooplankton was the least important (Fig. 2.13b). Dinoflagellates were seen in most of the samples, but were only an appreciable constituent in April and May 2008. By investigating the samples, pennate diatoms

were shown to be present slightly more in the coastal samples than in Terrebonne Bay. March 2008 saw a peak in pennate diatoms, dominated by *Pseudo-nitzschia*, where they represented the most dominant portion of the plankton community on the Louisiana shelf.

When compared to each other, there were a few trends between the Terrebonne Bay and coastal samples. Centric diatoms were clearly the most prevalent group of plankton in Terrebonne Bay; however the coastal samples had a more even distribution of the major groups of plankton. Despite the evenness of the plankton community, the relative dominance of the groups was much more variable in the coastal waters than in Terrebonne Bay. One difference in the data between the two locations was that March 2008 was sampled in the coastal waters, but not in Terrebonne Bay. This is because the sampling effort in Terrebonne Bay was designed to sample gulf menhaden as well as the water, and gulf menhaden are still offshore in March. Interestingly, the data show this month to be the only one where centric diatoms were not the most dominant group in the plankton community; it was dominated by pennate diatoms, namely *Pseudo-nitzschia*.

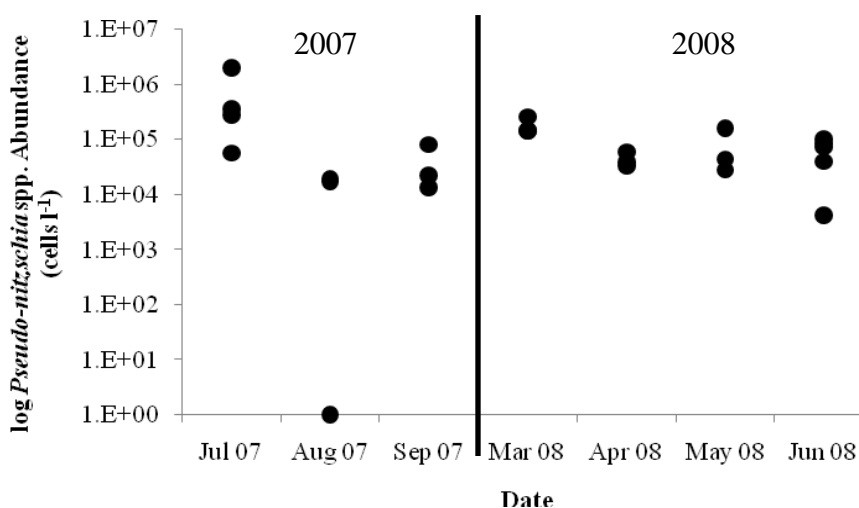


Figure 2.12. *Pseudo-nitzschia* cell abundances in coastal waters just outside Terrebonne Bay, Louisiana. The abundances were plotted on a logarithmic scale and transformed by adding 1 to all values.

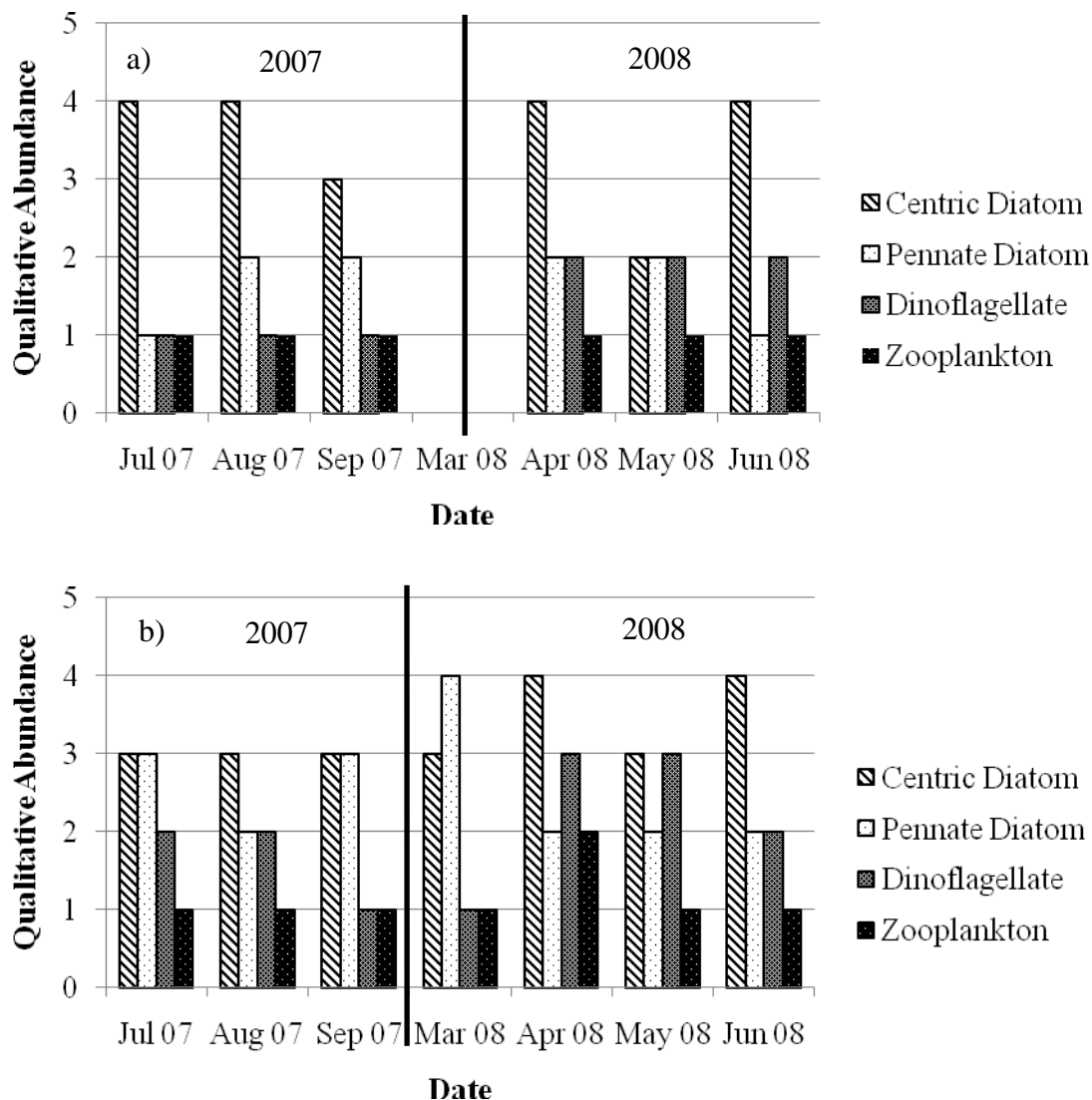


Figure 2.13. Qualitative community composition of the four major constituents for a) Terrebonne Bay and b) near-shore water samples for the entire study period.

Stomach Contents Analysis of Gulf Menhaden

A total of seven fish stomachs were examined for the presence of *Pseudo-nitzschia* spp., as well as other planktonic groups that fish might have consumed. The samples were chosen from April 2008 set 17 and set 18, and June 2008 set 25. April set 17 was chosen because it had the highest gulf menhaden tissue DA concentration, and April set 18 had the highest water

particulate DA concentration. June set 25 was chosen because it had moderate DA values in water and fish samples, to contrast the higher April DA concentrations, and to provide more temporal scaling, since both of the other samples were taken in April.

From each set, a maximum of three stomachs were examined on three separate occasions using a light microscope, however April 2008 set 17 only had one specimen to examine. Zooplankton was the most prevalent food item in set 17 of April 2008, followed by centric diatoms, pennate diatoms, and finally dinoflagellates. The dominant members of the qualitative groups were Copepods, *Prorocentrum* spp., *Navicula* sp., and *Rhizosolenia* spp. (identified by an end spike only), respectively (Plate 2.1).

April 2008 set 18, located 4 miles away from set 17, had a different diet composition with centric diatoms as the most dominant group. Dinoflagellates were the next most abundant, followed by pennate diatoms, and zooplankton comprising only a small part of the diet. *Rhizosolenia* spp. (identified by an end spike only), other centrals, *Prorocentrum* spp. *Protoperidinium* spp., *Pleurosigma* spp., and copepods were the main constituents of their groups in the diet (Plate 2.2).

June 2008 set 25 had a diet composition different from both of the April 2008 samples. Dinoflagellates were the main gut component found for this set, followed by centric diatoms, pennate diatoms, and zooplankton. The most prevalent members of the dinoflagellate community were *Prorocentrum* spp., and *Ceratium* spp., while *Rhizosolenia* spp. (identified by an end spike only) and *Coscinodiscus* spp. were the most frequently seen centric diatoms. Common pennate diatoms were *Pleurosigma* spp. and *Navicula* spp., and copepods and tintinnids made up the majority of the zooplankton component of the diet (Plate 2.3).

Attempts to conclusively verify the presence of *Pseudo-nitzschia* spp. in the viscera of gulf menhaden using light microscopy were unsuccessful, however *Pseudo-nitzschia* frustules

were found in all gut contents examined. Therefore, TEM was used to confirm the presence and identify the species of *Pseudo-nitzschia* in the gut contents. The majority of diatom frustules found in gut contents of gulf menhaden were broken which was likely a result of the gizzard, which grinds food particles.

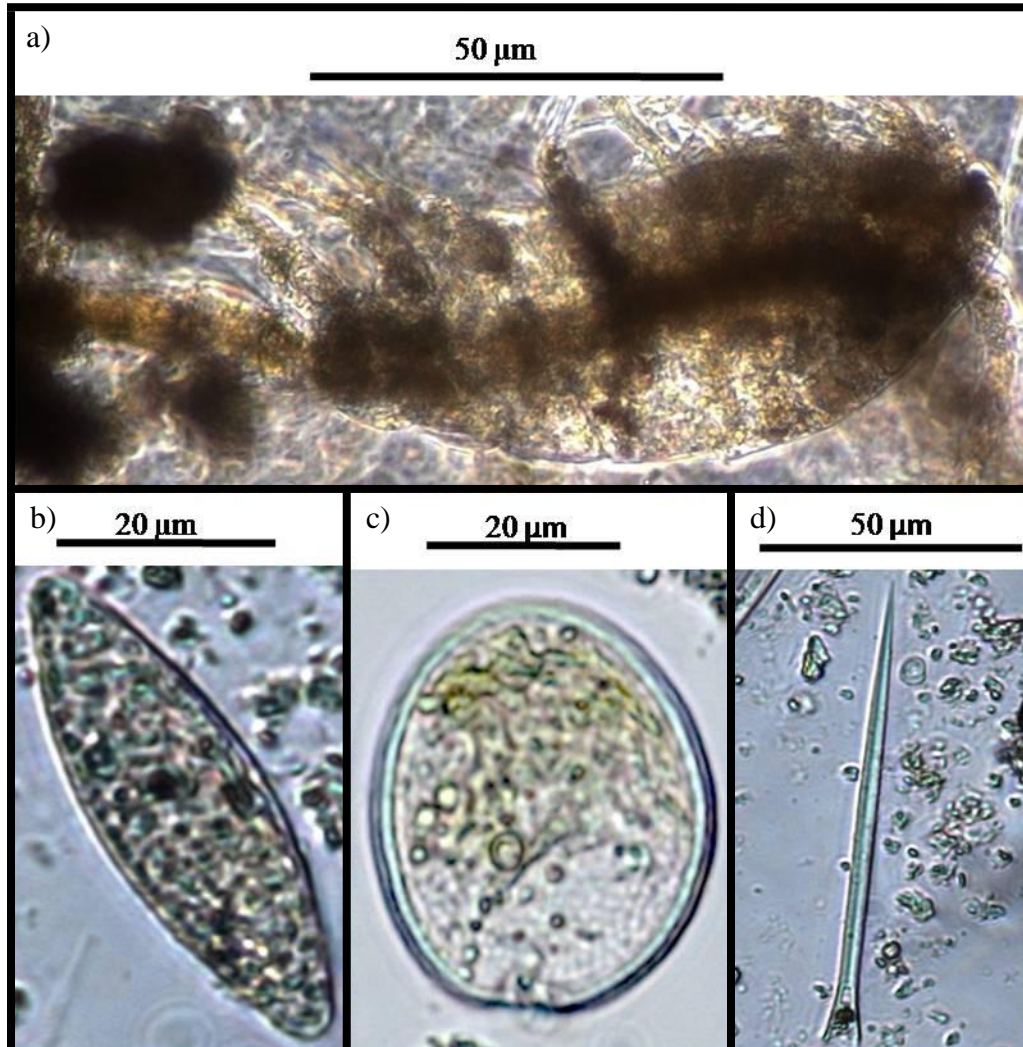


Plate 2.1. Photographs of the most prevalent organisms, a) copepods, b) *Navicula* sp., c) *Prorocentrum* sp., and d) *Rhizosolenia* sp. end spike, from the main constituents of the gut contents of *Brevoortia patronus* from the April 2008 set 17.

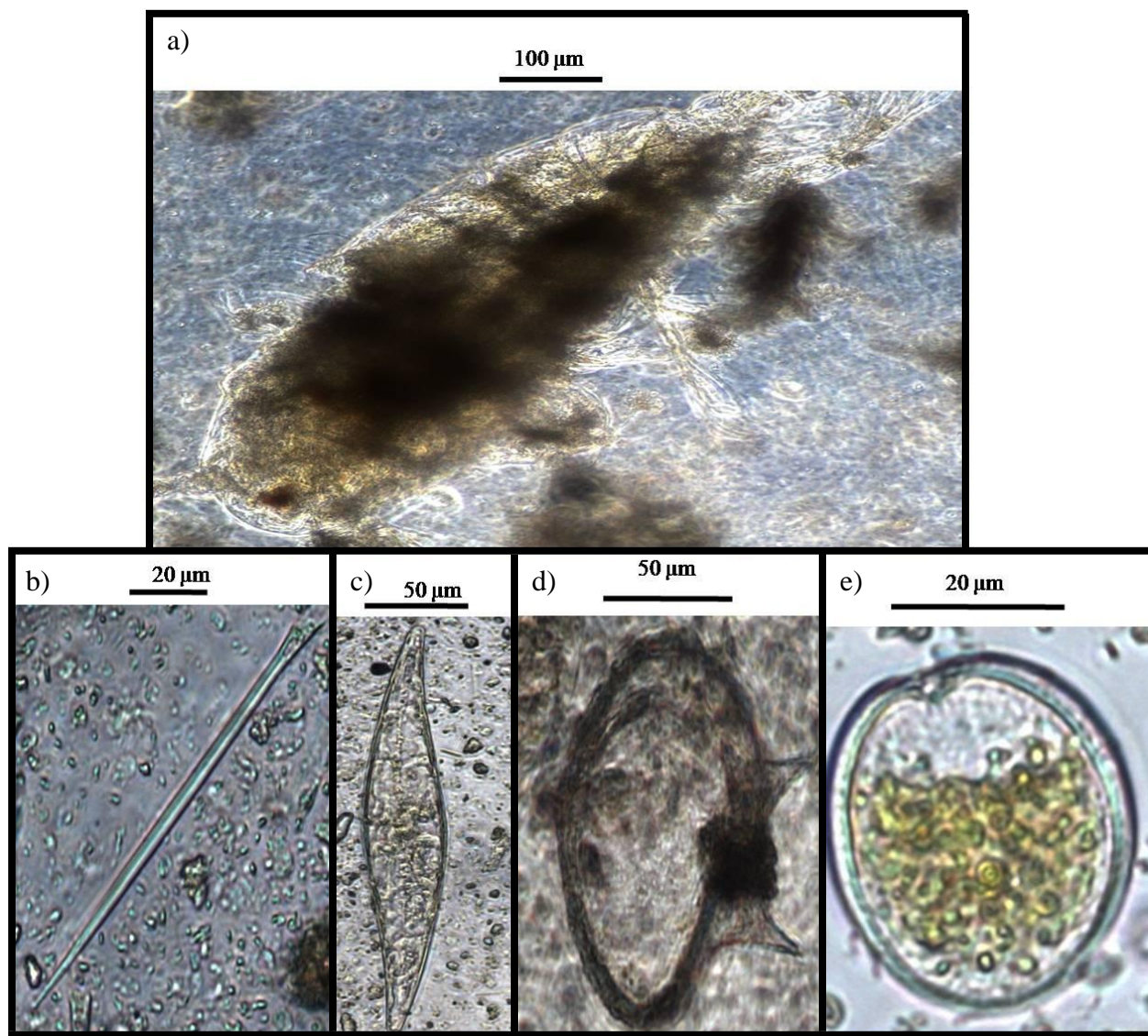


Plate 2.2. Images of a) copepods, b) *Rhizosolenia* sp. end spike, c) *Pleurosigma* sp., d) *Protoperidinium* sp., e) *Prorocentrum* sp. which represent the most common examples of the major components of the plankton community in *Brevoortia patronus* gut contents from April 2008 set 18.

Species Identification

Both Terrebonne Bay and coastal water samples and stomach contents of gulf menhaden were examined under TEM in order to confirm the presence and determine the species of *Pseudo-nitzschia*. Terrebonne Bay water samples from April 2008 sets 17 and 18 were examined for *Pseudo-nitzschia* presence and speciation. April 2008 set 17 contained one species, *P.calliantha*, which had a transapical axis of 2.24 µm, 29 stria and 15 fibula in 10 µm, 5 poroids

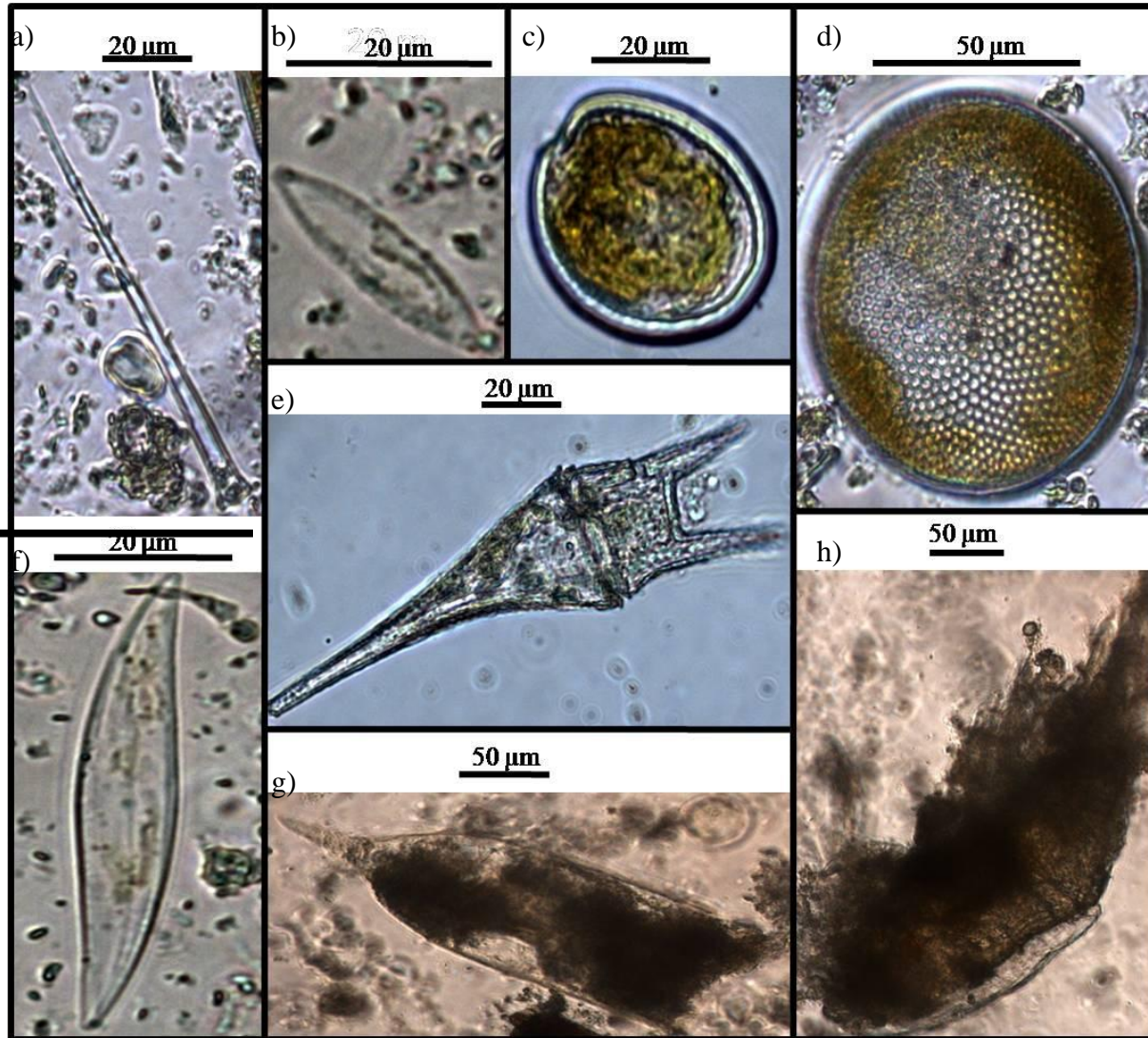


Plate 2.3. Photographs from *Brevoortia patronus* gut contents from June 2008 set 25. These photos are examples of the most prevalent organisms from each major group of the gut contents, a) *Rhizosoleia* sp. end spike, b) *Navicula* sp., c) *Prorocentrum* sp., d) *Coscinsdiscus* sp., e) *Ceratium* sp., f) *Pleurosigma* sp., g) Tintinnids, and h) copepods.

in 1 μm arranged in one row, a central nodulus was present, and the poroids were subdivided (Fig. 2.14). The sample from April 2008 set 18 also had only one species of *Pseudo-nitzschia* present, *P. pseudodelicatissima*. The specimen had a transapical axis of 1.67 μm , 32 stria in 10 μm , 20 fibula in 10 μm , 5 poroids in 1 μm arranged in a single row, and no subdivisions within the poroids (Fig. 2.15).

Water samples from coastal Louisiana stations, C1, C3, and C4, for the month of April were also analyzed for *Pseudo-nitzschia* species identification. *Pseudo-nitzschia* was present at all of these stations and TEM of the samples revealed the presence of *Pseudo-nitzschia pseudodelicatissima* to be the dominant species and *P. americana* was also found. The specimen of *P. pseudodelicatissima* had a transapical axis of 2.29 μm , 33 stria in 10 μm , 19 fibulae in 10 μm , and the poroids were arranged in one row with 6 in 1 μm (Fig. 2.16a). *P. americana* had a transapical axis of 1.79 μm , with 30 stria 10 μm , 20 fibulae in 10 μm , and the poroids are arranged in 2-3 rows with 9 in 1 μm (Fig. 2.16b).

Gulf menhaden stomach contents from April 2008 sets 17 and 18, revealed three different species of *Pseudo-nitzschia*. April 2008 set 17 contained only one species of *Pseudo-nitzschia*, *P. calliantha*. An analysis of morphometric characteristics showed that this specimen had a transapical axis of 1.8 μm , 38 stria in 10 μm , 16 fibula in 10 μm , 5 poroids in 1 μm in 1 row, and poroids divided into lesser segments (Fig. 2.17). The samples from April 2008 set 18 had three different species present in the gut contents: *P. calliantha*, *P. pungens*, and *P. pseudodelicatissima*. The most abundant of these species was *P. calliantha*, with *P. pungens* and *P. pseudodelicatissima* found in equally lower abundance. The samples of *P. calliantha* had a range of transapical axes from 1.67 – 2.23 μm , but all had 40 stria in 10 μm , 20 fibula in 10 μm , 5 poroids in 1 μm arranged in one row, and subdivided poroids (Fig. 2.18a). Another species of *Pseudo-nitzschia* was found to be *P. pungens*, had a transapical axis of 2.23 μm , 20 stria and 20 fibula in 10 μm , 3 poroids in 1 μm arranged in 2 rows (Fig. 2.18b). The final observed species of *Pseudo-nitzschia* from April 2008 set 18 was *P. pseudodelicatissima*. There was a transapical axis of 1.4 μm , 50 stria and 10 fibula in 10 μm , 8 square poroids in a single row in 1 μm (Fig. 2.18c).

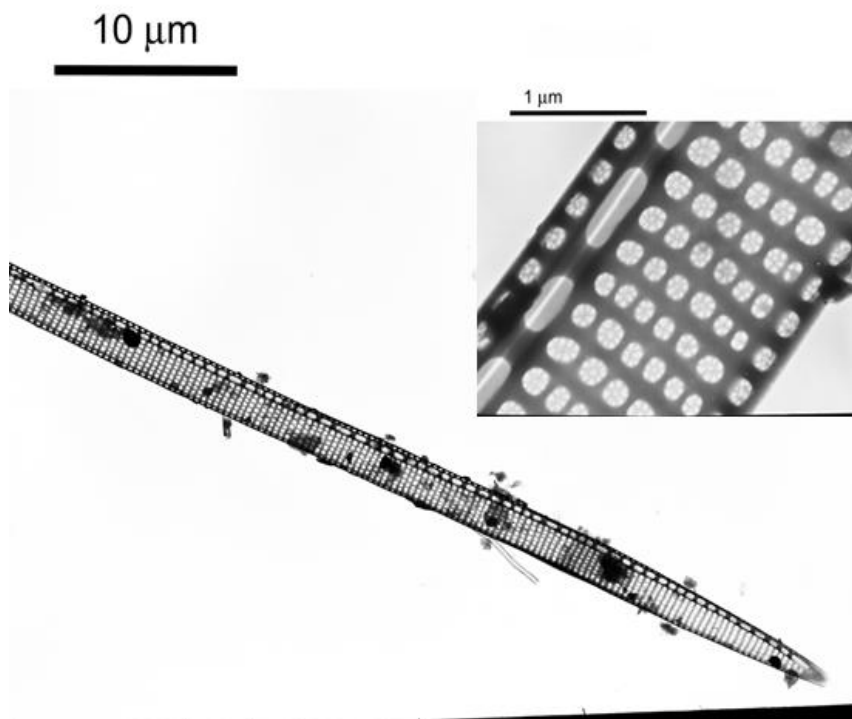


Figure 2.14. *Pseudo-nitzschia calliantha* from Terrebonne Bay water from April 2008 set 17. Width = 2.24 µm, Stria = 29 in 10 µm, Fibulae = 15 in 10 µm. The poroids are arranged in one row with 5 in 1 µm, and are subdivided in the interior, and a central nodulus is present. The inset image is to show the pore division structure.

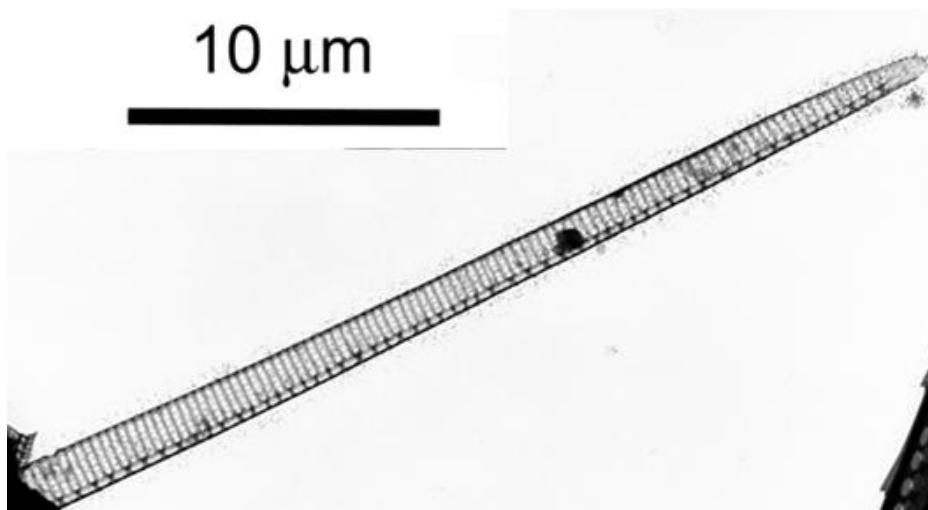


Figure 2.15. *Pseudo-nitzschia pseudodelicatissima* from Terrebonne Bay water from April 2008 set 18. Width = 1.67 µm, Stria = 32 in 10 µm, Fibulae = 20 in 10 µm. The poroids are arranged in one row with 5 in 1 µm.

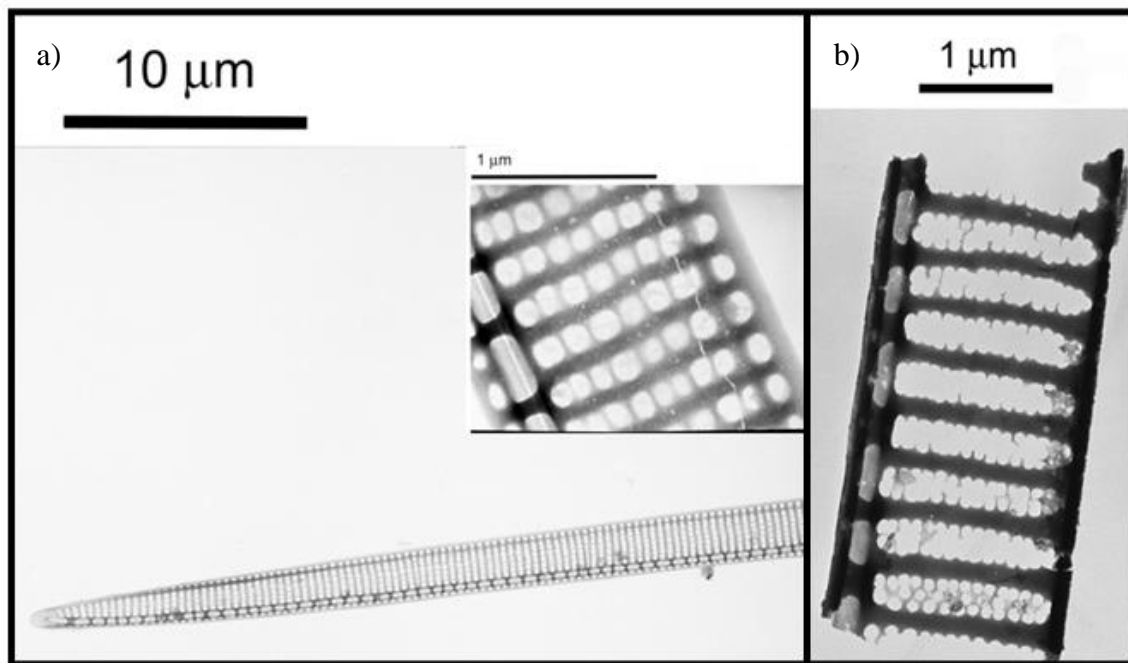


Figure 2.16. *Pseudo-nitzschia* specimens from coastal Louisiana water from April 2008 a) *Pseudo-nitzschia pseudodelicatissima*, Width = 2.29 µm, Stria = 33 in 10 µm, Fibulae = 19 in 10 µm. The poroids are arranged in one row with 6 in 1 µm. Inset photo is to show the poroid structure, b) *Pseudo-nitzschia americana*, Width = 1.79 µm, Stria = 30 in 10 µm, Fibulae = 20 in 10 µm. The poroids are arranged in 2-3 rows with 9 in 1 µm.



Figure 2.17. *Pseudo-nitzschia calliantha* from *Brevoortia patronus* gut contents from April 2008 set 17. Width = 1.8 µm, Fibulae = 16 in 10 µm, Stria = 38 in 10 µm. The poroids are arranged in one row with 5 in 1 µm, and are subdivided in the interior.

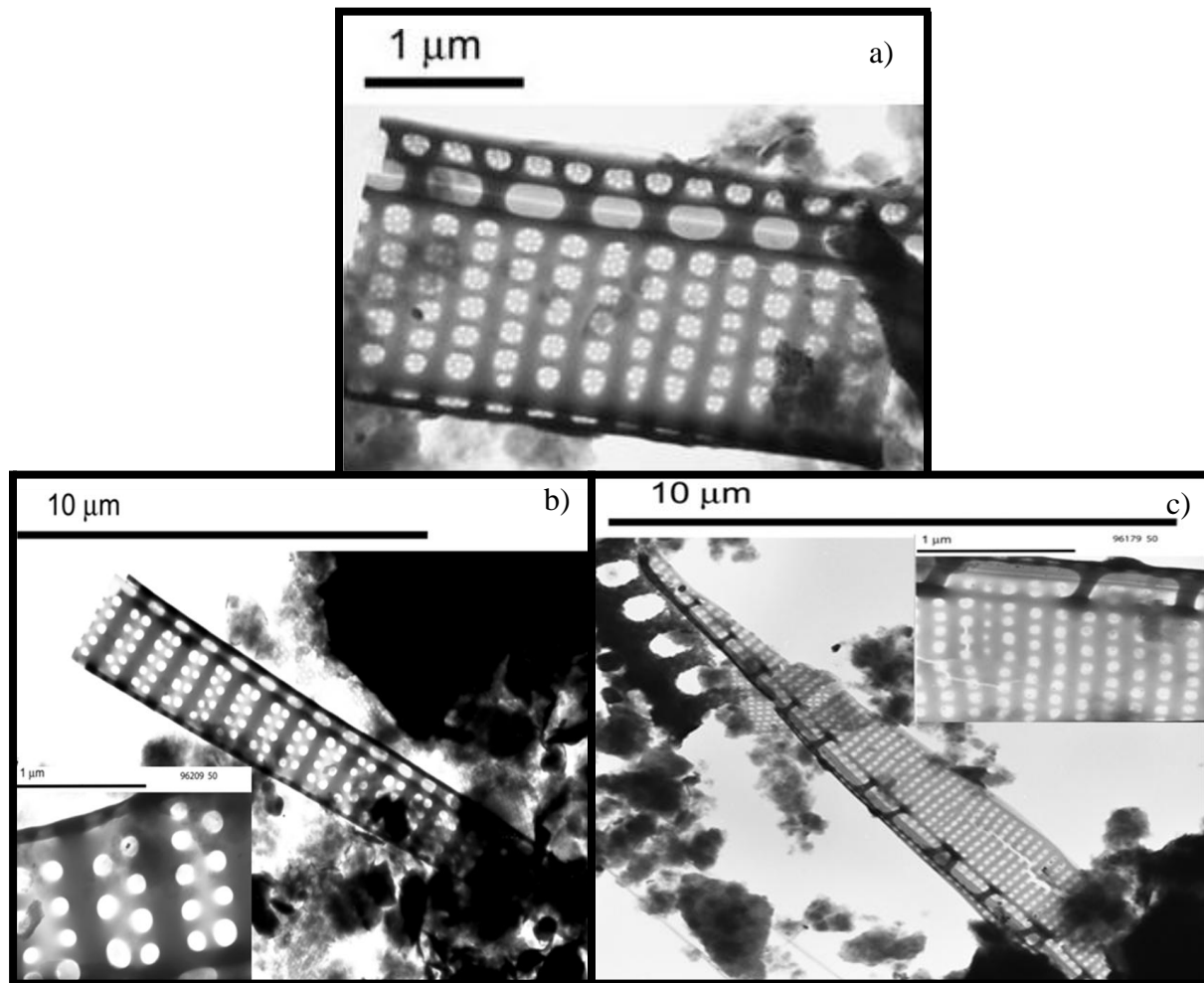


Figure 2.18. *Pseudo-nitzschia* species from *Brevoortia patronus* gut contents from April 2008 set 18 a) *Pseudo-nitzschia calliantha*, Width = 1.81 μm , Stria = 40 in 10 μm , Fibulae = 20 in 10 μm . The poroids are arranged in one row with 5 in 1 μm , and are subdivided in the interior, b) *Pseudo-nitzschia pungens*, Width = 2.23 μm , Stria = 20 in 10 μm , Fibulae = 20 in 10 μm . The poroids are arranged in two rows with 3 in 1 μm , c) *Pseudo-nitzschia pseudodelicatissima*, Width = 1.4 μm , Stria = 70 in 10 μm , Fibulae = 10 in 10 μm . The poroids are arranged in one row with 8 in 1 μm .

DISCUSSION

The goal of the present study was to examine whether gulf menhaden, *Brevoortia patronus*, can act as a potential vector of DA to higher trophic level consumers in Louisiana coastal waters. There is evidence that DA is capable of contaminating food webs in the northern Gulf of Mexico. Samples from a bottlenose dolphin mortality event were found to contain

measurable amounts of DA (Fire pers. comm.). However due to degradation of the dolphins prior to sampling, a vector was not identified. This is the first study to identify a potential DA vector in the Gulf of Mexico, and represents one of the major milestones in understanding the potential food web impacts of DA in Louisiana. Previously published research on *Pseudo-nitzschia* in Louisiana revealed that species of this genus has been present since the 1910s and increasing in abundance since the 1950s, including toxic species, and can reach high abundances during blooms (Dortch et al. 1997, Parsons et al. 1999, Pan et al. 2001, Parsons et al. 2002). The results of these studies indicate that Louisiana has the potential for ASP/DAP events; however no vector for the transmission of DA has yet been identified.

There are several conditions that must be met in order for transmission of DA to occur in Louisiana. First, *Pseudo-nitzschia* must exist in the area and be capable of producing DA. Second, primary consumers such as gulf menhaden must consume *Pseudo-nitzschia* and take up the toxin. In order for *Pseudo-nitzschia* to exist in the area, the physical conditions must meet its biological needs. *Pseudo-nitzschia* has been documented as a part of the Louisiana plankton community since the 1910s (Parsons et al. 2002), and the results of this study show *Pseudo-nitzschia* is currently present in coastal Louisiana. There are several physical parameters, including temperature, salinity, and turbidity, that can have an impact on *Pseudo-nitzschia* presence in Louisiana coastal waters. In the present study, *Pseudo-nitzschia* was found at all times sampled at, generally, moderate abundances, despite the somewhat variable nature of physical conditions on the Louisiana coast. Louisiana has a subtropical climate, characterized by warm summers and periodically cold winters, thus allowing for temperature fluctuations in coastal water bodies over the course of a year. Regardless of temperature, *Pseudo-nitzschia* was found during all sampling times, occurring over a range of temperatures from 19 – 33 °C. This finding is consistent with those of Dortch et al. (1997) who discovered a seasonal cycle of

abundance with *Pseudo-nitzschia* always being present in the plankton community over a five-year period.

The Mississippi River is another potential controlling factor on Louisiana's coastal phytoplankton community. The discharge of freshwater onto the Louisiana continental shelf by the Mississippi River plays a key role in the salinity regime of coastal water bodies. While salinity is known to alter phytoplankton community composition, *Pseudo-nitzschia* presence appeared unaltered and was found over a wide range of salinities (15 – 30 ppt.). These findings are also in agreement with a study by Thessen et al. (2005), who found *Pseudo-nitzschia* to exist both naturally and in culture from 1 to >35 psu.

The Mississippi River discharge of freshwater also carries a substantial amount of fine sediments which mix into coastal bays and estuaries, such as Terrebonne Bay, through tides and winds (Marmer 1954, Prager 1992, Inoue & Wiseman 2000). This influx of sediments and the shallow depths of coastal bays, contribute to an environment that is capable of becoming highly turbid. However, in the present study *Pseudo-nitzschia* was found to live in a large range of water clarity (18 – 105 cm), which agrees with the findings of Rines et al. (2002) that shows *Pseudo-nitzschia* can survive in low-light situations. This study, in combination with previous research, shows that Louisiana waters provide an inhabitable environment for *Pseudo-nitzschia* to exist.

Results from this study illustrated that DA was generated by *Pseudo-nitzschia* in Terrebonne Bay and surrounding coastal waters. The water particulate DA concentrations found in this study, up to 43.4 ng l⁻¹, were lower than previous ASP/DAP events around the world (Bates et al. 1989, Wright et al. 1989, Work et al. 1993, Scholin et al. 2000), and analysis of the community composition may have provided an explanation for low toxin presence in the water. Chl. *a* analysis combined with microscopic examination of Terrebonne Bay water samples

showed a large, mixed assemblage of phytoplankton, with *Pseudo-nitzschia* comprising only a small part, potentially diluting the DA levels. However, since gulf menhaden are fish and not sessile organisms, like other effective DA vectors, their mobility allow them to move into and out of the bay which can allow for uptake of DA from coastal sources as well. In addition, the results of gut contents analysis showed that gulf menhaden also consumed copepods, which could be another pathway for menhaden to take-up toxin. Despite the low levels of DA detected in water, gulf menhaden have several potential pathways to accumulate toxin and threaten Louisiana coastal food webs.

Additionally, the influence of wind patterns and current flow also plays a role in the movement of *Pseudo-nitzschia* and domoic acid in the environment. For example, wind and current direction data from the Wave-Current-Surge Information System (WAVCIS) for coastal Louisiana may provide an explanation for the discrepancy between high April DA values and low *Pseudo-nitzschia* abundance in coastal Louisiana. WAVCIS showed wind direction was from the Southwest, and wave direction was from the Southeast during sampling which could have forced coastal Gulf of Mexico water into Terrebonne Bay causing more DA to be detected from more *Pseudo-nitzschia* in the bay, and less *Pseudo-nitzschia* abundance in the coastal water. The wind and current information also supports that cell counts of samples from coastal water provide an adequate estimate of abundance within the bay, because the wind and tidal influence of water flow in Terrebonne Bay exchanges bay water with that of the near-shore Gulf easily, so the plankton communities would be similar (Marmer 1954, Prager 1992, Inoue & Wiseman 2000).

Identification of the responsible phytoplankton species is an important consideration when investigating HABs, especially *Pseudo-nitzschia* blooms since not all species in the genus produce DA. Moestrup and Lundholm (2007) have listed 11 species of *Pseudo-nitzschia* as

known DA producers from around the world. In Louisiana, the work of Parsons et al. (1999) and Pan et al. (2001) have identified two toxic species: *P. multiseriata* and *P. sp. cf. pseudodelicatissima*, and two potentially toxic species: *P. delicatissima* and *P. pungens*. Our results indicate the presence of another potentially toxic species, *P. calliantha*, in both Terrebonne Bay and gulf menhaden. Known in the Gulf of Mexico from Tampa Bay and Apalache Bay in Florida (Lundholm et al. 2003), this is the first identification of *P. calliantha* in Louisiana. Previously, *P. sp. cf. pseudodelicatissima* was identified as the dominant species in Louisiana coastal waters, but in the present study *P. calliantha* was most dominant followed by *P. pseudodelicatissima*. These two species of *Pseudo-nitzschia* closely resemble each other; however the divisions within the poroids separate the two species. Further identification is needed using molecular methods for absolute species confirmation. Nevertheless, the results of the present study show toxic species of *Pseudo-nitzschia* exist in the area and are actively producing DA.

Results of the present study showed that DA contamination of Louisiana coastal food webs occurred in gulf menhaden via consumption of toxic *Pseudo-nitzschia*. In several previous studies, gulf menhaden have been described as filter-feeders (Lassuy 1983, Deegan 1986, Ahrenholz 1991). This type of feeding strategy results in consumption of bulk suspended material in the water column; therefore, if *Pseudo-nitzschia* is present, it would likely be consumed. A gut contents analysis was performed on gulf menhaden in order to verify *Pseudo-nitzschia* consumption. The results from that investigation showed when *Pseudo-nitzschia* was present in the environment, they were also found in the diet of gulf menhaden, relevant to water concentrations. This outcome was to be expected since the community of phytoplankton in Louisiana coastal waters is so large and diverse (Dortch et al. 1997), and, at the times sampled, *Pseudo-nitzschia* was not very abundant. Regardless, this analysis confirmed the presence of *P.*

calliantha and *P. pseudodelicatissima*, confirmed DA producers (Martin et al. 1990, Lundholm et al. 1997, Pan et al. 2001), and *P. pungens*, a species that has been found toxic in some studies and non-toxic in others (Bates et al. 1993, Rhodes et al. 1996), in gulf menhaden gut contents. The identification of these species of *Pseudo-nitzschia* in the gut contents of gulf menhaden demonstrates that DA contamination of these fish is possible.

The presence of DA in gulf menhaden revealed that these fish do, indeed, contain toxin. Other fishes from the same taxonomic order as gulf menhaden, but from other regions, have been shown to accumulate DA to levels far above FDA safety limits (Lefebvre et al. 2001). While the results of the present study do not reach levels above food safety limits, the presence of DA, up to $0.31 \mu\text{g g}^{-1}$, confirms gulf menhaden's status as a potential DA vector and represents a potential threat to Louisiana coastal food webs. Moreover, DA in gulf menhaden visceral tissue was found to be significantly correlated to DA in water samples in the present study. This relationship implies that whenever DA is found in Louisiana coastal waters, one would expect it to be found in gulf menhaden. This is important when large blooms of toxic *Pseudo-nitzschia* occur, or cellular toxicity increases, then DA levels in gulf menhaden would also be elevated. Because low levels of DA were detected in all months sampled, continuous low-level exposure could be also a problem for Louisiana coastal food webs. The effects of chronic exposure have not been investigated for fishes, but, in California sea lions, have been linked to reproductive failure and epilepsy (Goldstein et al. 2007, Goldstein et al. 2009).

Various aspects of gulf menhaden life history may enhance its potential to be an effective vector of DA. Some of these characteristics are filtration rate, abundance, and mobility. While feeding efficiency is unstudied for gulf menhaden, particularly on toxic *Pseudo-nitzschia*, a study by Durbin and Durbin (1975) on Atlantic menhaden (*Brevoortia tyrannus*) may provide insight. They concluded that filtration rate is dependent on mouth diameter and swimming speed, and

found Atlantic menhaden could filter up to $34.8 \text{ l min}^{-1} \text{ fish}^{-1}$. With such a high filtration rate, gulf menhaden would potentially be able to consume nearly 3.5 million cells of *Pseudo-nitzschia* $\text{min}^{-1} \text{ fish}^{-1}$, if the average abundance of *Pseudo-nitzschia* from the present study ($10^5 \text{ cells l}^{-1}$) was encountered, potentially transferring a large dose of DA. However, this circumstance has yet to be investigated by researchers. Since it has been established by this study that they do carry toxin, the high abundance of gulf menhaden in coastal Louisiana provides many opportunities for DA to be taken up and, potentially, transfer large amounts of DA into the food web. Additionally, gulf menhaden have the ability to spread DA through the food web to other communities over a large area since they are highly mobile fish and are not bound to one location. Thus, the potential for gulf menhaden to transfer DA is greatly enhanced due to its life history characteristics.

Gulf menhaden have been identified in many other studies as an important prey item for upper trophic level predators such as brown pelicans (*Pelecanus occidentalis*) (Ahrenholz 1991, US Fish and Wildlife Service 2008), bottlenose dolphins (*Tursiops truncatus*) (Hinton & Ramsdell 2008), and many species of coastal shark (Snelson et al. 1984, Bethea et al. 2004, Barry et al. 2008). A project to study this potential for several shark species is already underway, and preliminary data indicates that sharks do contain measurable amounts of DA in their tissue (Del Rio et al. unpublished). As a common prey item, gulf menhaden have the ability to transfer DA to these important apex predators, providing a direct link for DA to contaminate food webs.

The present study raises several points of interest about the transmission of DA through food webs in the northern Gulf of Mexico. First, this study has positively identified gulf menhaden as a potential vector of DA in Louisiana coastal water bodies. Furthermore, a statistically significant correlation between DA in water and fish was found, giving some predictive ability. Second, the high abundances of *Pseudo-nitzschia* in Louisiana coastal waters,

and the identification of a potential DA vector indicates the potential importance of toxic *Pseudo-nitzschia* as a food resource. Despite the presence of toxic *Pseudo-nitzschia*, there have not been any other vectors of DA confirmed in this region or any insight into food web effects. However, findings from this study suggest the potential for other DA vectors to exist. In order to fully understand the threat DA poses to Louisiana food webs, further research is needed not only to better understand the efficiency of gulf menhaden as a DA vector to higher trophic levels, but also to investigate the capability of other potential vectors of DA in Gulf of Mexico food webs.

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CHAPTER 3

QUALITATIVE MODELING OF HARMFUL ALGAL BLOOMS IN COASTAL LOUISIANA SHARK NURSERY FOOD WEBS

INTRODUCTION

As global eutrophication of coastal waters has increased in recent years, so has the frequency and intensity of harmful algal blooms (HABs) (Glibert et al. 2005a). HABs generally refer to occurrences when species of algae that are capable of producing negative ecological effects, due to toxin production, their total biomass, or morphology, accumulate to “sufficient” levels where strong negative effects occur (Glibert et al. 2005b). Some harmful algal species are capable of producing powerful toxins that cause fish kills (Landsberg 2002), marine mammal strandings (Scholin et al. 2000), shellfish intoxication (Wekell et al. 1994), and even, human health problems (Wright et al. 1989, Bates et al. 1989, Perl et al. 1990). Therefore, areas undergoing increased nutrient loading could have an increased susceptibility to HABs.

Coastal Louisiana is experiencing eutrophication from Mississippi River discharge, and is one region that is potentially at risk for HABs (Turner & Rabalais 1994). These blooms can be consumed by filter-feeding organisms potentially introducing phycotoxins into food webs. In coastal Louisiana, the gulf menhaden (*Brevoortia patronus*) is the most abundant potential vector of algal toxins, since Louisiana produces half of the juvenile recruits that support the second largest fishery in the U.S. in terms of landings (Vaughan 2007). These fish represent direct links to higher trophic level consumers in coastal Louisiana’s estuarine environments, and therefore, may act as an effective vector of algal toxins.

Harmful algal blooms of three basic types pose potential threats to Louisiana’s coastal estuaries including Ciguatera Fish Poisoning (CFP), Neurotoxic Shellfish Poisoning (NSP), and Domoic Acid Poisoning (DAP). None of these HAB toxins have been documented as problematic in Louisiana coastal and estuarine waters, but there is the potential for all three. The

first two HAB types, CFP and NSP, are theoretical risks since the phytoplankton species responsible do not occur in abundance in coastal Louisiana at the present. The algae that causes DAP, however, is commonly found in the phytoplankton community of coastal Louisiana (Bargu unpublished). Whatever the likelihood of occurrence, the potential severity of the ecological consequences of HABs responsible for CFP, NSP, and DAP warrant the attention of researchers and resource managers.

The first HAB type, CFP, is caused by blooms of the epiphytic dinoflagellate *Gambierdiscus toxicus*. It produces gambiertoxin, a precursor for the toxin ciguatera, which is metabolized after ingestion by herbivorous fishes (Yasumoto & Murata 1993). Traditionally believed to be passed by herbivorous reef fishes and their predators, new evidence shows an alternate, more pelagic, pathway where planktivorous fishes can spread CFP (Randall 1958, Murata et al. 1990). *G. toxicus* is hosted by free-floating mats of *Sargassum* spp. and consumed by zooplankton, which are, in turn, eaten by filter-feeding or zooplanktivorous fishes, and the toxin is passed up trophic levels (Bomber et al. 1988, Kelly et al. 1992). It is this alternate pathway that most directly threatens Louisiana's coastal communities. *Sargassum* spp., common in the Gulf of Mexico, is capable of carrying *G. toxicus* into Louisiana near-shore waters and coastal bays. Then the large population of gulf menhaden can, potentially, spread ciguatera throughout the food web, where once in the food web, ciguatera is an ichthyotoxin (Lewis 1992) that bio-accumulates as it travels from prey to predator (Landsberg 2002). Recent evidence also suggests that with rising ocean temperatures, *G. toxicus*, a tropical species, could extend its range into the subtropics, and that the numerous oil production platforms off the Louisiana coast may provide suitable habitat for attached algae on which *G. toxicus* can grow (Villareal et al. 2007). Therefore, Louisiana is likely at risk of CFP events, if *G. toxicus* becomes more prevalent in the

area and abundant filter-feeding fishes, like gulf menhaden, are suitable vectors for dispersing the toxin throughout food webs.

Another potential threat to coastal Louisiana food webs is the dinoflagellate *Karenia brevis*, which produces the neurotoxin, brevetoxin, and causes NSP. *K. brevis* is widely distributed in the northern Gulf of Mexico, and Louisiana, near the center of its range, has not typically experienced the negative NSP effects (Tester & Steidinger 1997, Brown et al. 2006). Ecologically, NSP is one of the most destructive HAB types known, and in nature, brevetoxin makes its way into food webs via many pathways. It may be transmitted by consumption of intermediate consumers; but it can also be taken up directly by gill tissue or through respiration (Woodcock 1948, Pierce 1986, Landsberg 2002). Because there are many ways to become intoxicated with brevetoxin, *K. brevis* blooms are devastating to coastal marine communities and often manifested as massive fish kills, but also as marine mammal and invertebrate mortality events (Gunter et al. 1947, Gunter et al. 1948, Flewelling et al. 2005, Hinton & Ramsdell 2008).

Because of the highly destructive nature of *K. brevis* blooms, their effects on Louisiana coastal waters remain a concern. *K. brevis* is a rare component of Louisiana phytoplankton communities. One hypothesis is that the lower salinity of Louisiana's coastal waters, due to the influence of Mississippi River water, reduces the likelihood of *K. brevis* blooms, but does not prevent its occurrence (Brown 2006). In fact, in 1997, a bloom of *K. brevis* in Breton Sound, Louisiana, demonstrated that HABs of *K. brevis* are possible although unusual (Dortch et al. 1998), and since that bloom, *K. brevis* has only been rarely encountered in Louisiana waters (Bargu pers. comm.). Because NSP is common elsewhere in the Gulf of Mexico, and highly destructive to coastal food webs, it remains a concern for Louisiana.

Another confirmed HAB type that threatens coastal Louisiana food webs is responsible for DAP. This type of algal poisoning event is caused by diatoms in the genus *Pseudo-nitzschia*

that can produce the neurotoxin domoic acid (DA). Several DAP incidents, involving mass seabird and marine mammal mortality events (Work et al. 1993, Scholin et al. 2000) have occurred in Monterey Bay, California. In these examples, filter-feeding, clupeid fishes have been the DA vector to higher trophic level consumers. With the world-wide distribution of *Pseudo-nitzschia* species, areas that have a high abundance of filter-feeding organisms could be at risk for outbreaks of DAP.

Pseudo-nitzschia spp. are ubiquitous members of the Louisiana phytoplankton community and their presence has been known since the 1910's (Parsons et al. 2002). Six species of this genus have been identified in Louisiana: two toxic species, *Pseudo-nitzschia pseudodelicatissima* and *P. multiseriata*, two occasionally toxic species, *P. pungens* and *P. delicatissima*, and two non-toxic species *P. subfraudulenta* and *P. sp. cf. Nitzschia americana*. Abundances of *Pseudo-nitzschia* in Louisiana can become large, up to 10^8 cells/L in coastal waters and up to 10^5 cells/L in Terrebonne Bay (Dortch et al. 1997). In Louisiana, abundant oysters and gulf menhaden (Vaughan 2007) are functionally similar to the mussels and filter-feeding fishes implicated in previous ASP/DAP events, and the results of Chapter 2 show gulf menhaden can act as a vector of DA (Del Rio in prep). Thus, the presence of *Pseudo-nitzschia* and an abundance of potential filter-feeding vectors in Louisiana raise concerns for future consequences of this HAB type.

A qualitative modeling approach (Levins 1966, May 1971, Bender et al. 1984) was used to evaluate the effects of HABs in Louisiana coastal food webs. Qualitative models emphasize generality and realism and can be used to study the effects of HABs on an entire food web. Loop analysis, a type of qualitative model, are graphical representations of the direct and indirect interactions and can be developed with only a general knowledge of the natural history of the system. They use the sign of direct pair-wise interactions in a community interaction matrix

consisting of positives (+1), negatives (-1), or neutrals (0) to understand the behavior and stability of systems at or near equilibrium (May 1971). The potential two-way interactions include predator-prey (1, -1), commensalism (1,0), or ammensalism (-1,0) (Rossignol et al. 2001). These direct interactions form longer feedback loops that connect many species in indirect interactions. Loop model analyses evaluate the net number of positive and negative feedback loops acting on each constituent, or node, in the model. Feedback loops are pathways that return to their node of origin without going through any node twice (Rossignol et al. 2001). Loop analysis examines all of the pathways within a system, including indirect effects, important characteristics of a system that can overwhelm direct interactions, potentially involving multiple species. Loop analysis can also be used to examine ecosystem responses to long-term perturbations, or presses (*sensu* Bender et al. 1984). Press experiments are useful when working with large, complex systems where temporal, logistical, or budgetary constraints hinder quantitative modeling. Currently our understanding of food web effects of HABs is constrained by inadequate information for quantitative analysis, thus, the benefits of qualitative modeling are obvious. Because HABs occur at varying temporal and spatial scales, the logistics of adequately sampling them are difficult. Analysis of the ecology of HABs is also challenging because the techniques used to understand their different effects must be carried out by highly trained individuals. Essentially, loop analysis provides an easy and inexpensive means of predicting some of the harmful effects of HABs, like food web contamination, vulnerability of systems to HABs, or forecasting of future changes.

The present study utilizes loop analysis to evaluate the effects of three different HABs on a shark nursery food web in Louisiana. Terrebonne Bay is a major shark nursery for several species including, most commonly, blacktip (*Carcharhinus limbatus*), Atlantic sharpnose (*Rhizoprionodon terraenovae*), finetooth (*Carcharhinus isodon*), and bull (*Carcharhinus leucas*)

sharks (de Silva et al., 1999; Condrey et al., 2000; Thompson et al., 2001; Neer et al., 2007).

Although, there are many harmful algal species that could severely alter this ecosystem, the focus of the present study is on two potential blooms, *Gambierdiscus toxicus* and *Karenia brevis*, and one identified ubiquitous group, *Pseudo-nitzschia* spp. Loop analysis was used to address how the Terrebonne Bay food web would respond to the effects of these HABs.

MATERIALS AND METHODS

Description of the Study Area

This study was conducted in Terrebonne Bay, Louisiana, which is a typical southern Louisiana estuary. It is micro-tidal with generally shallow, turbid waters. Terrebonne Bay is bordered on the south by three barrier islands, Isles Dernieres, Timbalier Island, East Timbalier Island, and opens through three large passes onto the Gulf of Mexico (Whiskey Pass, Little Pass Timbalier, and a combination of Cat Island Pass and Wine Island Pass) (Inoue & Wiseman 2000). The bay now receives little freshwater input from the Mississippi River due to levee construction (USEPA 1999) and flushing is driven by tides and wind patterns (Prager 1992, Inoue & Wiseman 2000) with winds being the dominant physical force determining water movement (Marmer 1954). Most freshwater input is from direct precipitation and the annual freshwater inflow is $4.7 \times 10^5 \text{ m}^3 \text{ hr}^{-1}$ (USEPA 1999).

Data Acquisition

Dietary information on the species found within Terrebonne Bay was gleaned from several sources to establish the direct pair-wise interactions in the system. The diets of the four most common shark species was determined from the primary literature (Snelson et al. 1984, Castro 1993, Hueter 1994, Cortes 1999, Hoffmayer & Parsons 2003, Bethea et al. 2004, Barry et al. 2008) and diets of shark prey were determined by searching Fishbase (www.fishbase.org), and summarizing general life history knowledge. Interactions of harmful algal species with the

components of the model were also determined from primary literature (Kelly et al. 1992, Lewis 1992, Jones et al. 1995, Edmunds et al. 1999, Dizer et al. 2001, Lefebvre et al. 2001, Lincoln et al. 2001, Landsberg 2002, Bargu et al. 2003, Lewis et al. 2003, Lundholm et al. 2005, Cohen et al. 2007, Naar et al. 2007, Villareal et al. 2007, Bejarano et al. 2008, Hinton & Ramsdell 2008, Prince et al. 2008). However, the vast numbers of predator-prey interactions necessitated aggregation into broader groups, with the exception of sharks since they were the focus of the study. Initially, 20 nodes were identified: YOY Atlantic sharpnose, juvenile Atlantic sharpnose, adult Atlantic sharpnose, YOY blacktip, juvenile blacktip, juvenile bull, YOY finetooth, juvenile finetooth, pelagic carnivorous fishes, epibenthic carnivorous fishes, Squid, Planktivorous fishes, Crustaceans, Gastropods, Bivalves, Echinoderms, Infauna, Zooplankton, Harmful phytoplankton, Non-harmful phytoplankton.

Model Construction

Overall

An initial overall model was constructed to show major direct interactions in the Terrebonne Bay food web. This model utilized the broadest descriptions of all the data acquired, and followed a basic food web design for a shark nursery with several age-classes of sharks as apex predators, several intermediary consumers, and phytoplankton as primary producers. Since most harmful algal species occur in a mixed assemblage in Terrebonne Bay, the effects of HABs were generalized in the overall model. However, the size and complexity of the initial loop model necessitated simplification to 12 nodes that was accomplished by aggregating several nodes (see below). The second, reduced model was reconstructed after aggregation, in the same style as previously described.

Ciguatera Fish Poisoning

The model for CFP was based on the overall aggregated model, but with some differences. The dissimilarities originated from no direct interaction between *Gambierdiscus toxicus* and non-harmful phytoplankton, zooplankton predation, and *G. toxicus* being directly consumed by benthic feeders (Randall 1958, Kelly et al. 1992, Landsberg 2002). All upper trophic level interactions remained unchanged from the overall model.

Neurotoxic Shellfish Poisoning

The NSP model was also created by changing the interactions of the overall reduced model. Since nearly all groups included in the model are sensitive to brevetoxin, negative direct effects were applied to all nodes (Gunter 1947, Gunter et al. 1948, Landsberg 2002). Because of the magnitude of *K. brevis* abundance during blooms, a positive press was applied to *K. brevis* and a negative press was applied to non-harmful plankton to simulate *K. brevis* eclipsing all other planktonic species in the analysis.

Domoic Acid Poisoning

Like the other experimental models, the domoic acid poisoning model originated from the interactions in the overall aggregated model. However, negative effects were applied to zooplankton and planktivorous fishes (Lefebvre et al. 2001, Bargu et al. 2002), and removed from other phytoplankton. All other interactions were unaltered from the overall model.

Model Aggregation

Models were simplified to facilitate analyses. The regular equivalence function of UCINET 6.0 software (2002) was used to condense the nodes by grouping some based on similarity of positive, direct interactions within the food web (Luczkovich et al. 1993, Borgatti et al. 2002, Metcalf et al. 2008). The UCINET program identified similar nodes that could be combined to reduce model complexity. For example, half of the shark species included

gastropods in their diet, the other half did not. The regular equivalence function recognized these differences and created two groups of sharks based on the presence or absence of gastropods. However, some of the simplifications were not acceptable, due in part to the lack of consideration given to negative interactions. Aggregations were accepted or rejected based on how the groupings fit with general life history characteristics of the nodes involved or under consideration. When aggregations were deemed acceptable, the new reduced models were used to evaluate the effects of the three different HAB types on the system by pressing harmful algal blooms.

Qualitative Modeling

To model these food web interactions, five different signed digraphs were developed in PowerPlay v. 2 software (Westfahl et al. 2002) to generate general community interaction matrices, and analyzed with an algorithm by Dambacher et al. (2006) written for Maple software (Version 12, Waterloo Maple Inc., © 1981 - 2008). The signed digraphs are graphical representations of the direct interactions of food webs with arrows between the nodes indicating the type of interaction (positive or negative). The positive (+1), negative (-1), and neutral (0) interactions are placed into a community interaction matrix, which is the numerical representation of the system, and used by the algorithm to input the food web, to check model stability, and generate predictions for press perturbations in the loop analysis.

Model Stability and Loop Analysis

Maple software (Maple 2008) and the qualitative modeling algorithm, provided by (Dambacher et al. 2006) were used to conduct the loop analysis. Loop modeling is based on the assumptions that the system is at or near equilibrium, that there are no extinctions or additions to the system and the perturbations are small enough to avoid altering system stability (Dambacher & Ramos-Jiliberto 2007). Within the limits of these assumptions, the first Routh-Hurwitz

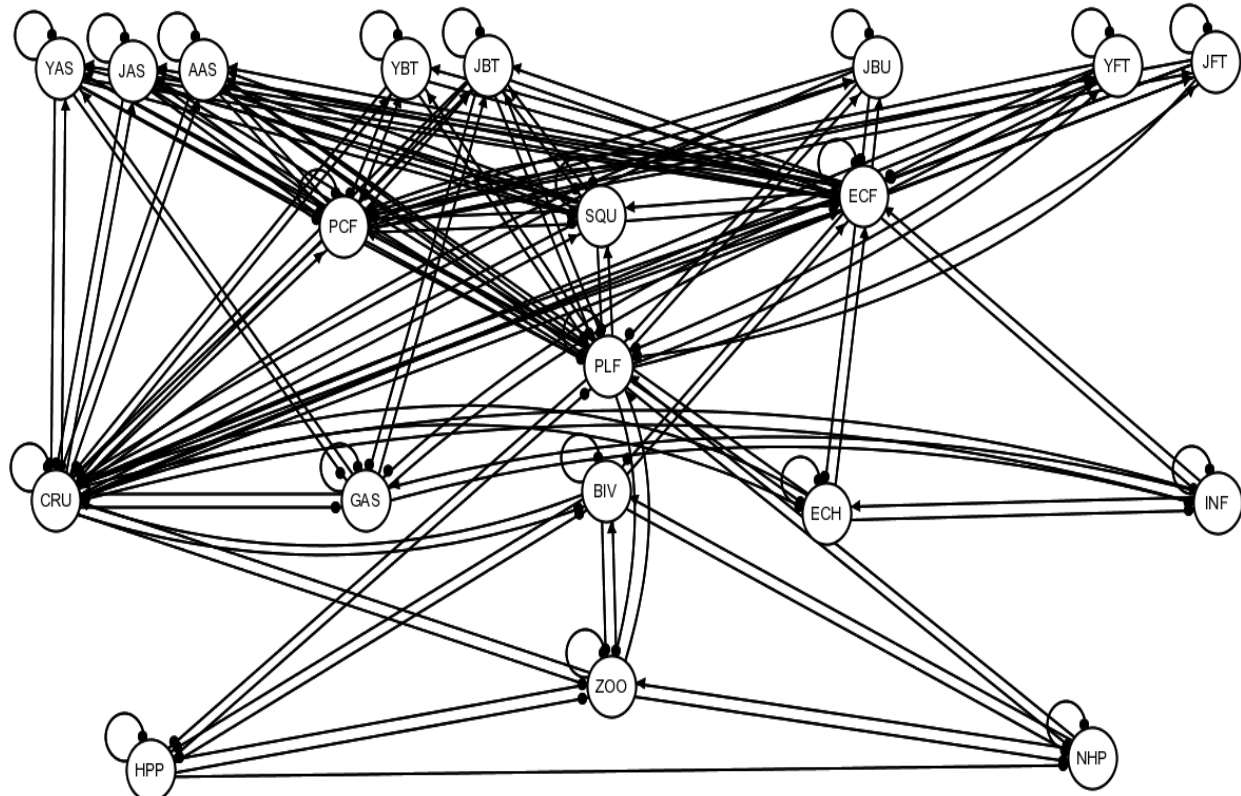
criterion is satisfied if negative feedback is present at all levels of the system. The second Routh-Hurwitz criterion is satisfied if lower level feedback dominates higher level feedback. Stable models meet both criteria and are Class I, and those that meet only the first criterion are Class II and are conditionally stable (Dambacher et al. 2003). Predictive or experimental output from a loop analysis is in the form of an adjoint matrix, which represents the net number of positive or negative feedback loops for the column j acting upon the row i , and can be used to estimate the effects of perturbations on the system.

A perturbation is an alteration in abundance of one or more model constituents. Perturbation experiments are used to investigate system responses, as a whole, to a disturbance. One type of perturbation is a “press” where the abundance of one or more constituents is changed in a sustained manner to a new level to see the responses of the unaltered constituents (Bender et al. 1984). Other short-term perturbations, termed “pulses” are more ephemeral and not suitable for loop analysis. The adjoint matrix shows the system’s responses to a positive press upon any particular node by reading “the effect to i from j ” (Rossignol et al. 2001). Negative presses can be examined by reversing all signs in the negatively pressed column, and multiple presses can be assessed by summing the column values of positively and/or negatively pressed variables across rows. Since loop analysis is a qualitative method, the actual values in the adjoint matrix should not be used to estimate the magnitude of change, but only to indicate the direction of change.

RESULTS

General HAB Model

The initial model included 20 nodes (Fig. 3.1), and was too large to yield results. However, it was simplified by aggregating similar nodes (Fig. 3.2) to produce a more manageable model (Fig. 3.3) of 12 nodes, emphasizing lower trophic levels and aggregating



	YAS	JAS	AAS	YBT	JBT	JBU	YFT	JFT	PCF	ECF	SQU	PLF	CRU	GAS	BIV	ECH	INF	ZOO	HPP	NHP
YAS	-1	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0
JAS	0	-1	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0
AAS	0	0	-1	0	0	0	0	0	1	1	1	1	1	0	0	1	0	0	0	0
YBT	0	0	0	-1	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0
JBT	0	0	0	0	-1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0
JBU	0	0	0	0	0	-1	0	0	1	1	0	1	1	0	0	0	0	0	0	0
YFT	0	0	0	0	0	0	-1	0	1	1	0	1	1	0	0	0	0	0	0	0
JFT	0	0	0	0	0	0	0	-1	1	1	0	1	1	0	0	0	0	0	0	0
PCF	-1	-1	-1	-1	-1	-1	-1	-1	-1	1	1	1	1	0	0	0	0	0	0	0
ECF	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	0	1	1	1	1	1	1	0	0	0
SQU	-1	-1	-1	0	-1	0	0	0	-1	1	-1	1	1	0	0	0	0	0	0	0
PLF	-1	-1	-1	-1	-1	-1	-1	-1	-1	0	-1	-1	0	0	0	0	0	1	-1	1
CRU	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	0	-1	1	1	1	1	1	0	0	0
GAS	-1	0	0	0	-1	0	0	0	0	-1	0	0	-1	-1	0	0	1	0	0	0
BIV	0	0	0	0	0	0	0	0	0	-1	0	0	-1	0	-1	0	0	1	-1	1
ECH	0	0	-1	0	0	0	0	0	0	-1	0	0	-1	0	0	-1	1	0	0	0
INF	0	0	0	0	0	0	0	0	0	-1	0	0	-1	-1	0	-1	-1	0	0	0
ZOO	0	0	0	0	0	0	0	0	0	0	0	-1	-1	0	-1	0	0	-1	-1	1
HPP	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	-1	0	0	-1	-1	0
NHP	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	-1	0	0	-1	-1	-1

Figure 3.1. Overall model of the Terrebonne Bay, Louisiana food web showing all interactions with Community Matrix. Abbreviations in the model are YAS = YOY Atlantic sharpnose, JAS = Juvenile Atlantic sharpnose, AAS = Adult Atlantic sharpnose, YBT = YOY blacktip, JBT = Juvenile blacktip, JBU = Juvenile bull, YFT = YOY finetooth, JFT = Juvenile finetooth, PCF = Pelagic carnivorous fishes, ECF = Epibenthic carnivorous fishes, SQU = Squid, PLF = Planktivorous fishes, CRU = Crustaceans, GAS = Gastropods, BIV = Bivalves, ECH = Echinoderms, INF = Infauna, ZOO = Zooplankton, HPP = Harmful phytoplankton, NHP = Non-harmful phytoplankton.

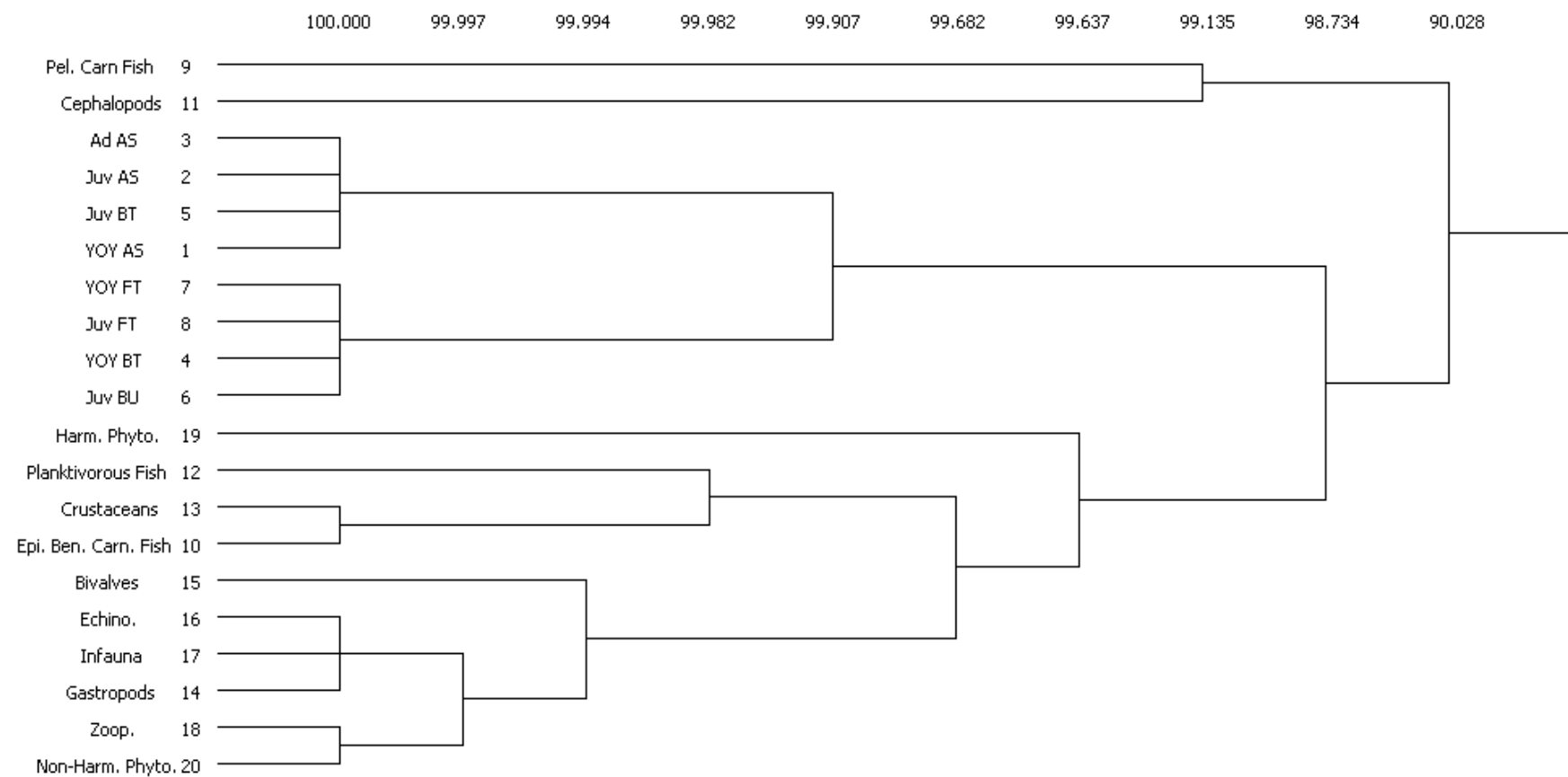
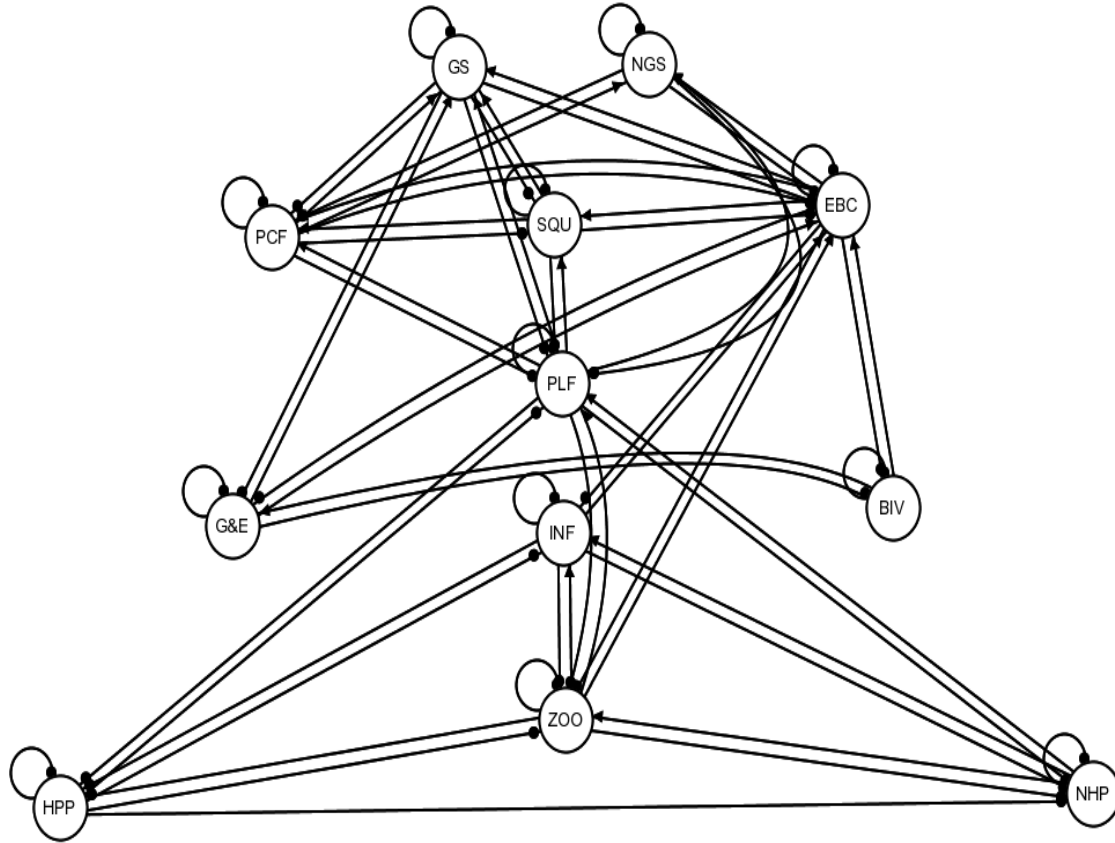


Figure 3.2. Ucinet model aggregation groupings of initial 20 nodes. Life history stages of four shark species were simplified to two nodes of gastropod and non-gastropod feeding sharks.



	GS	NGS	PCF	EBC	SQU	PLF	G&E	BIV	INF	ZOO	HPP	NHP
GS	-1	0	1	1	1	1	1	0	0	0	0	0
NGS	0	-1	1	1	0	1	0	0	0	0	0	0
PCF	-1	-1	-1	1	1	1	0	0	0	0	0	0
EBC	-1	-1	-1	-1	-1	0	1	1	1	1	0	0
SQU	-1	0	-1	1	-1	1	0	0	0	0	0	0
PLF	-1	-1	-1	0	-1	-1	0	0	0	1	-1	1
G&E	-1	0	0	-1	0	0	-1	0	1	0	0	0
BIV	0	0	0	-1	0	0	0	-1	0	1	-1	1
INF	0	0	0	-1	0	0	-1	0	-1	0	0	0
ZOO	0	0	0	-1	0	-1	0	-1	0	-1	-1	1
HPP	0	0	0	0	0	-1	0	-1	0	-1	-1	0
NHP	0	0	0	0	0	-1	0	-1	0	-1	-1	-1

Figure 3.3. Overall aggregated model. Abbreviations in the model are GS = Opportunistically gastropodivorous sharks, NGS = Non-gastropodivorous sharks, PCF = Pelagic carnivorous fishes, EBC = Epibenthic carnivores, SQU = Squid, PLF = Planktivorous fishes, G&E = Gastropods and echinoderms, BIV = Bivalves, INF = Infauna, ZOO = Zooplankton, HPP = Harmful phytoplankton, NHP = Non-harmful phytoplankton.

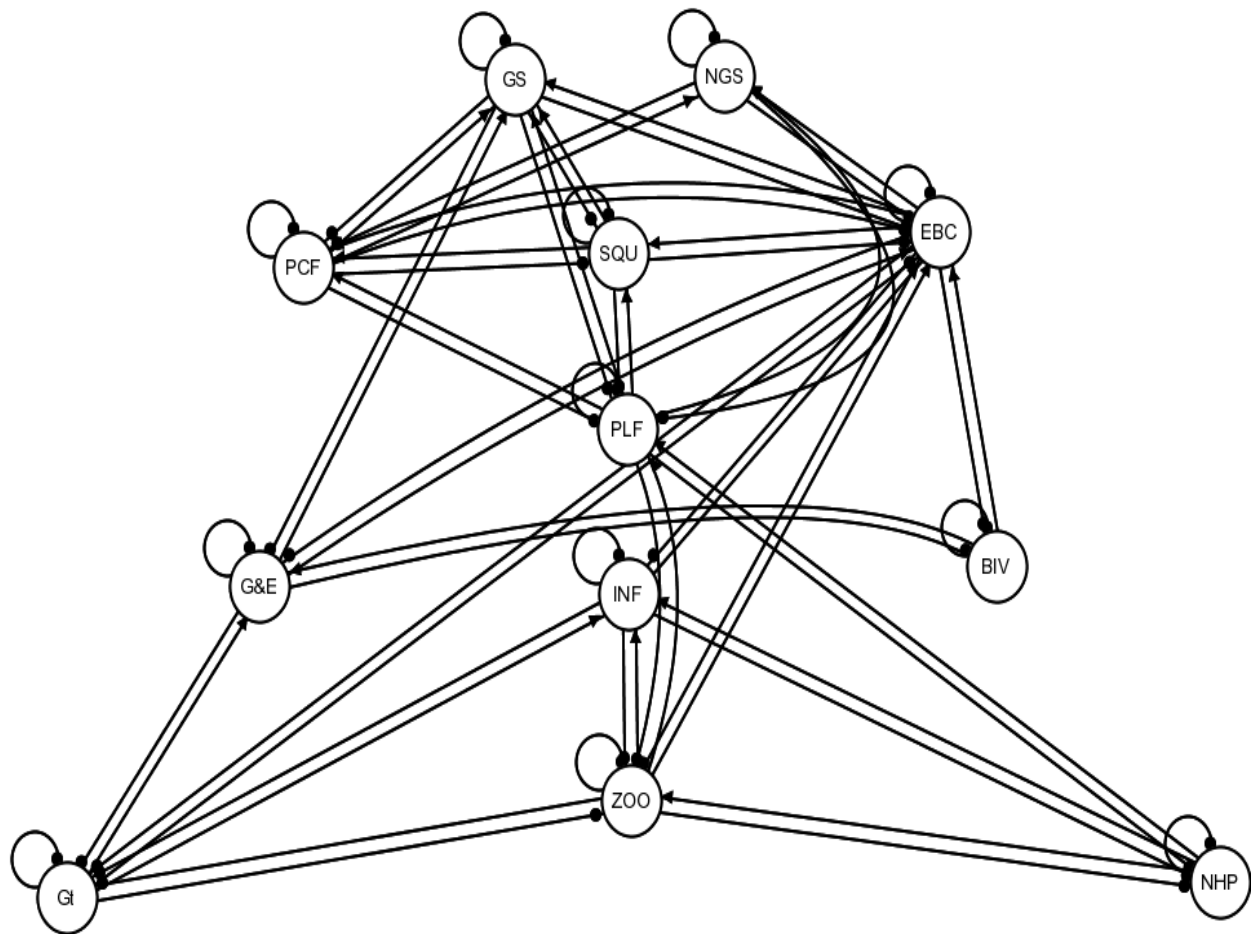
	GS	NGS	PCF	EBC	SQU	PLF	G&E	BIV	INF	ZOO	HPP	NHP
GS	6	0	0	-24	6	24	3	-12	-21	66	-156	78
NGS	-4	10	5	-24	1	24	-2	-12	-26	76	-176	88
PCF	-4	-5	5	6	1	-6	-2	3	4	-14	34	-17
EBC	0	0	0	0	0	0	0	0	0	15	-30	15
SQU	-2	5	-5	-12	8	12	-1	-6	-13	38	-88	44
PLF	0	0	0	-30	0	30	0	-15	-30	75	-180	90
G&E	-3	0	0	12	-3	-12	6	6	18	-48	108	-54
BIV	-4	10	5	-84	16	69	-2	-27	-86	226	-536	268
INF	3	0	0	-12	3	12	-6	-6	-3	33	-78	39
ZOO	0	0	0	15	0	-15	0	0	15	-30	90	-45
HPP	4	-10	-5	99	-16	-84	2	42	101	-271	641	-313
NHP	0	0	0	0	0	0	0	0	0	0	-15	15

Figure 3.4. Adjoint matrix from the overall aggregated model. Abbreviations in the model are GS = Opportunistically gastropodivorous sharks, NGS = Non-gastropodivorous sharks, PCF = Pelagic carnivorous fishes, EBC = Epibenthic carnivores, SQU = Squid, PLF = Planktivorous fishes, G&E = Gastropods and echinoderms, BIV = Bivalves, INF = Infauna, ZOO = Zooplankton, HPP = Harmful phytoplankton, NHP = Non-harmful phytoplankton.

shark nodes. This second, aggregated general model met both stability criteria: there was negative feedback at all levels and lower level feedback dominated upper levels, thus, it was a Class I model. When a positive press on harmful phytoplankton was applied (Fig. 3.4), sharks, squid, planktivorous fishes, and bivalves were negatively affected and only gastropods, echinoderms, and zooplankton benefitted from the HAB. The responses from pelagic carnivorous fishes, epibenthic carnivores, infauna, and non-harmful phytoplankton were neutral (Table 3.1).

Ciguatera Fish Poisoning Model

The third model, developed to address the influence of Ciguatera Fish Poisoning on the Terrebonne Bay food web by placing negative effects on zooplankton and epibenthic carnivores (Fig. 3.5), yielded similar results to the initial model, with a few differences. The CFP model met the stability criteria, and was a Class I model (Fig. 3.6). A positive press on *G. toxicus* led to an enhancement of gastropods and echinoderms, bivalves, and non-harmful phytoplankton.



	GS	NGS	PCF	EBC	SQU	PLF	G&E	BIV	INF	ZOO	Gt	NHP
GS	-1	0	1	1	1	1	1	0	0	0	0	0
NGS	0	-1	1	1	0	1	0	0	0	0	0	0
PCF	-1	-1	-1	1	1	1	0	0	0	0	0	0
EBC	-1	-1	-1	-1	-1	0	1	1	1	1	-1	0
SQU	-1	0	-1	1	-1	1	0	0	0	0	0	0
PLF	-1	-1	-1	0	-1	-1	0	0	0	1	-1	1
G&E	-1	0	0	-1	0	0	-1	0	1	0	0	0
BIV	0	0	0	-1	0	0	0	-1	0	1	1	1
INF	0	0	0	-1	0	0	-1	0	-1	0	0	0
ZOO	0	0	0	-1	0	-1	0	-1	0	-1	-1	1
Gt	0	0	0	0	0	-1	0	-1	0	-1	-1	0
NHP	0	0	0	0	0	-1	0	-1	0	-1	0	-1

Figure 3.5. Ciguatera Fish Poisoning model and Community Matrix. Abbreviations in the model are GS = Opportunistically gastropodivorous sharks, NGS = Non-gastropodivorous sharks, PCF = Pelagic carnivorous fishes, EBC = Epibenthic carnivores, SQU = Squid, PLF = Planktivorous fishes, G&E = Gastropods and echinoderms, BIV = Bivalves, INF = Infauna, ZOO = Zooplankton, Gt = *Gambierdiscus toxicus*, NHP = Non-harmful phytoplankton.

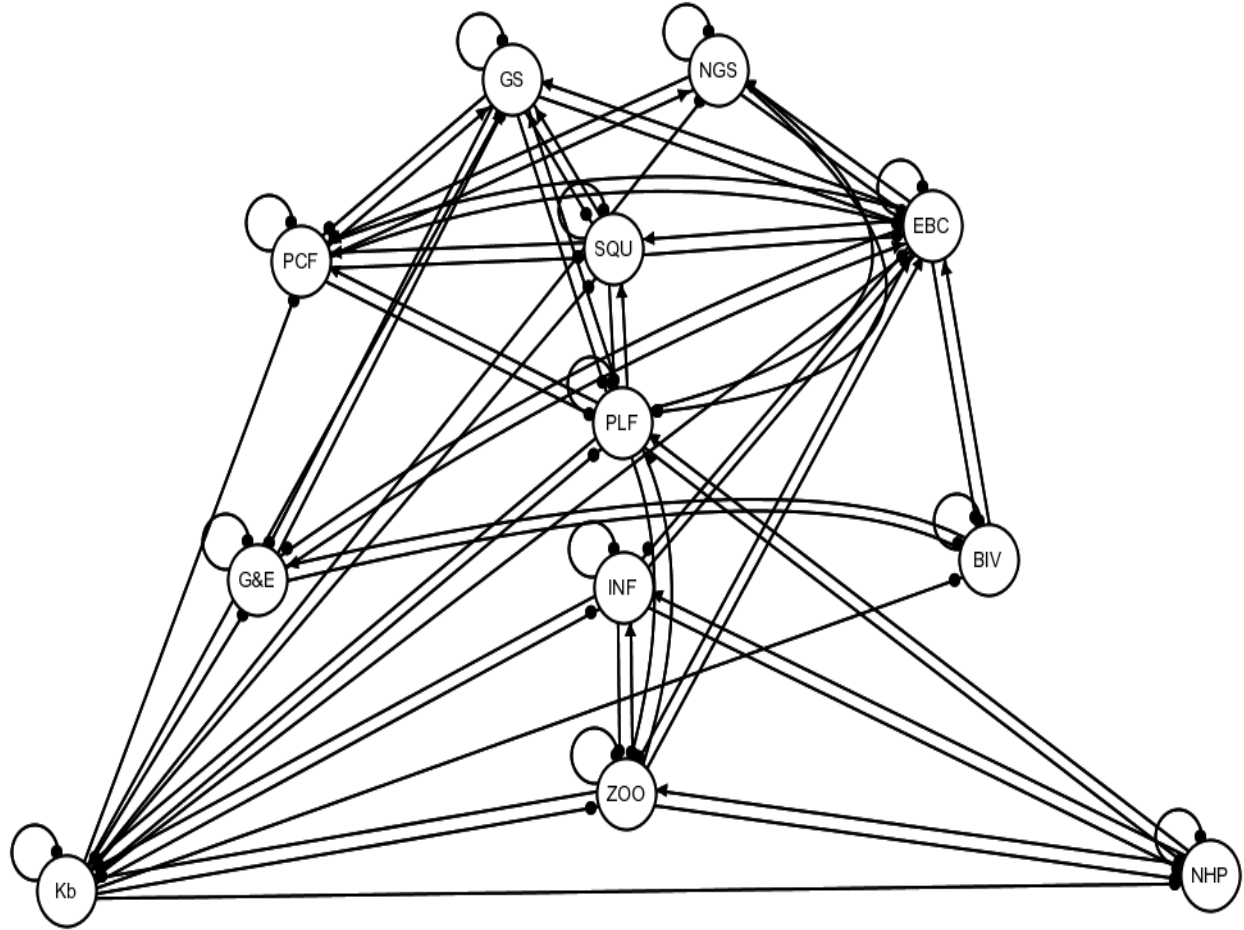
	GS	NGS	PCF	EBC	SQU	PLF	G&E	BIV	INF	ZOO	Gt	NHP
GS	339	-130	-65	5	79	190	136	-95	141	-31	67	64
NGS	-222	412	206	142	-82	-76	32	38	174	214	-286	176
PCF	-225	-174	255	33	111	-114	-60	57	-27	69	-105	12
EBC	-57	38	19	209	19	-266	76	133	285	209	-209	76
SQU	-111	206	-239	71	301	-38	16	19	87	107	-143	88
PLF	60	-136	-68	-100	-212	304	16	-152	-84	-64	28	88
G&E	-12	-64	-32	-208	-140	304	88	-152	-120	-352	496	-200
BIV	99	-42	-21	-51	15	114	-156	285	-207	-231	411	168
INF	69	26	13	-1	121	-38	-164	19	519	143	-287	124
ZOO	-231	250	125	43	269	-418	212	-133	255	691	-655	140
Gt	201	-182	-91	7	-163	266	-220	-133	-213	-317	641	-184
NHP	72	-72	-36	108	-72	0	-72	0	36	-396	216	288

Figure 3.6. Ciguatera Fish Poisoning model Adjoint Matrix. Abbreviations in the model are GS = Opportunistically gastropodivorous sharks, NGS = Non-gastropodivorous sharks, PCF = Pelagic carnivorous fishes, EBC = Epibenthic carnivores, SQU = Squid, PLF = Planktivorous fishes, G&E = Gastropods and echinoderms, BIV = Bivalves, INF = Infauna, ZOO = Zooplankton, Gt = *Gambierdiscus toxicus*, NHP = Non-harmful phytoplankton.

Negative responses were seen from non-gastropodivorous sharks, pelagic carnivorous fishes, epibenthic carnivores, squid, infauna, and zooplankton. Opportunistically gastropodivorous sharks and planktivorous fishes showed a neutral response (Table 3.1).

Neurotoxic Shellfish Poisoning Model

The fourth model, developed to examine the effects of Neurotoxic Shellfish Poisoning on the Terrebonne Bay food web, produced strong ecosystem responses to this HAB type. Due at least in part to NSP's direct negative effects on virtually all nodes in the model (Fig. 3.7). It met both of the stability criteria and was a Class I model. A positive press, simulating a bloom of *Karenia brevis*, (Fig. 3.8), revealed only one node with a neutral response, namely gastropods and echinoderms, and only two of the twelve nodes showed a positive response to an increase in *K. brevis*. Pelagic carnivorous and planktivorous fishes showed a positive effect, nevertheless, all other groups had a negative response to a positive press on this HAB (Table 3.1).



	GS	NGS	PCF	EBC	SQU	PLF	G&E	BIV	INF	ZOO	Kb	NHP
GS	-1	0	1	1	1	1	1	0	0	0	-1	0
NGS	0	-1	1	1	0	1	0	0	0	0	-1	0
PCF	-1	-1	-1	1	1	1	0	0	0	0	-1	0
EBC	-1	-1	-1	-1	-1	0	1	1	1	1	-1	0
SQU	-1	0	-1	1	-1	1	0	0	0	0	-1	0
PLF	-1	-1	-1	0	-1	-1	0	0	0	1	-1	1
G&E	-1	0	0	-1	0	0	-1	0	1	0	-1	0
BIV	0	0	0	-1	0	0	0	-1	0	1	-1	1
INF	0	0	0	-1	0	0	-1	0	-1	0	-1	0
ZOO	0	0	0	-1	0	-1	0	-1	0	-1	-1	1
Kb	0	0	0	0	0	-1	0	-1	0	-1	-1	0
NHP	0	0	0	0	0	-1	0	-1	0	-1	-1	-1

Figure 3.7. Neurotoxic Shellfish Poisoning model with Community Matrix. Abbreviations in the model are GS = Opportunistically gastropodivorous sharks, NGS = Non-gastropodivorous sharks, PCF = Pelagic carnivorous fishes, EBC = Epibenthic carnivores, SQU = Squid, PLF = Planktivorous fishes, G&E = Gastropods and echinoderms, BIV = Bivalves, INF = Infauna, ZOO = Zooplankton, Kb = *Karenia brevis*, NHP = Non-harmful phytoplankton.

	GS	NGS	PCF	EBC	SQU	PLF	G&E	BIV	INF	ZOO	Kb	NHP
GS	84	-20	-10	-106	44	136	42	-68	-64	294	-694	362
NGS	-40	100	50	-40	-30	80	-20	-40	-60	240	-520	280
PCF	-52	-60	65	43	18	-48	-26	24	17	-87	217	-111
EBC	0	0	0	0	0	0	0	0	0	190	-380	190
SQU	-20	50	-70	-20	80	40	-10	-20	-30	120	-260	140
PLF	16	-40	-20	16	-64	44	8	-22	24	-134	284	-112
G&E	-46	20	10	-46	-6	16	72	-8	26	-66	86	-58
BIV	-12	30	15	-107	48	62	-6	64	-113	243	-593	369
INF	42	-10	-5	-53	22	68	-74	-34	63	147	-347	181
ZOO	-8	20	10	-8	32	-22	-4	-84	-12	162	-142	56
Kb	4	-10	-5	99	-16	-84	2	42	101	-271	641	-313
NHP	0	0	0	0	0	0	0	0	0	0	-190	190

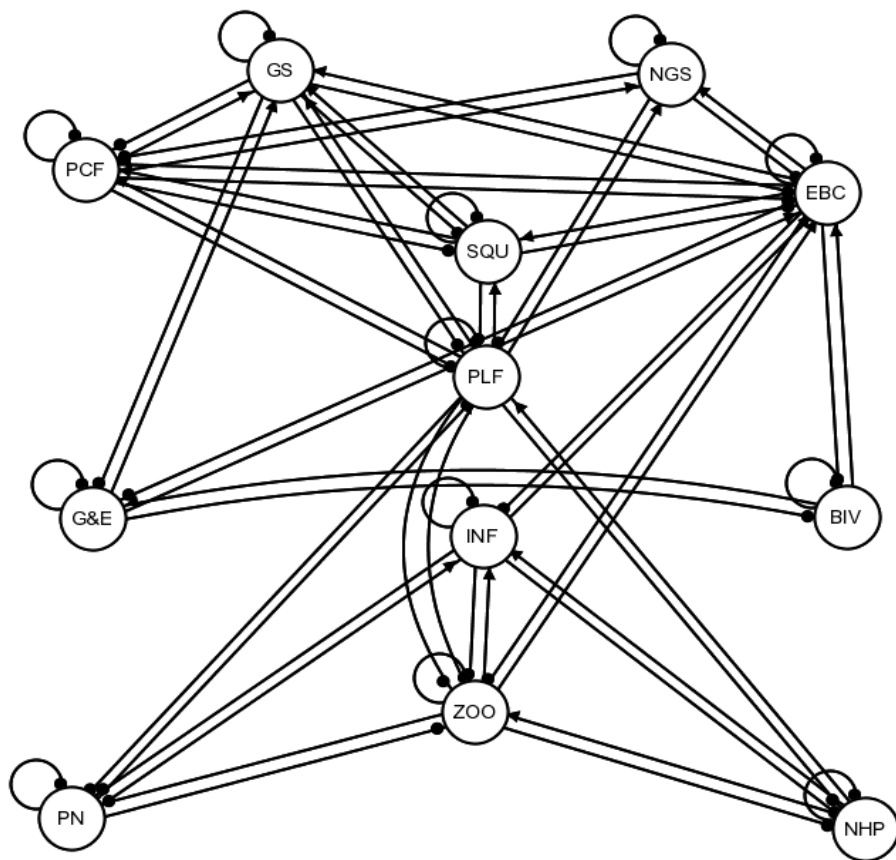
Figure 3.8. Neurotoxic Shellfish Poisoning model Adjoint Matrix. Abbreviations in the model are GS = Opportunistically gastropodivorous sharks, NGS = Non-gastropodivorous sharks, PCF = Pelagic carnivorous fishes, EBC = Epibenthic carnivores, SQU = Squid, PLF = Planktivorous fishes, G&E = Gastropods and echinoderms, BIV = Bivalves, INF = Infauna, ZOO = Zooplankton, Kb = *Karenia brevis*, NHP = Non-harmful phytoplankton.

Domoic Acid Poisoning Model

The final model was developed to assess the response to a Domoic Acid Poisoning event (Fig. 3.9) by negatively connecting *Pseudo-nitzschia* with planktivorous fishes and zooplankton. It met both of the stability criteria and was, therefore, a Class I model (Fig. 3.10). The four nodes that showed a beneficial response to a positive press on *Pseudo-nitzschia* bloom were epibenthic carnivores, infauna, and non-harmful phytoplankton. Likewise, five groups responded negatively to the bloom, including opportunistically gastropodivorous sharks, non-gastropodivorous sharks, planktivorous fishes, bivalves, and zooplankton. Finally, the three remaining nodes, pelagic carnivorous fishes, squid, and gastropods and echinoderms, (Table 3.1) showed neutral reactions.

DISCUSSION

This study offers some insight into the ecosystem effects of four HAB types in shark nursery food webs of Terrebonne Bay, Louisiana. Over all of the model runs, it was clearly shown that HABs have a large negative effect by changing the trophic structure of the food



	GS	NGS	PCF	EBC	SQU	PLF	G&E	BIV	INF	ZOO	PN	NHP
GS	-1	0	1	1	1	1	1	0	0	0	0	0
NGS	0	-1	1	1	0	1	0	0	0	0	0	0
PCF	-1	-1	-1	1	1	1	0	0	0	0	0	0
EBC	-1	-1	-1	-1	-1	0	1	1	1	1	0	0
SQU	-1	0	-1	1	-1	1	0	0	0	0	0	0
PLF	-1	-1	-1	0	-1	-1	0	0	0	1	-1	1
G&E	-1	0	0	-1	0	0	-1	1	0	0	0	0
BIV	0	0	0	-1	0	0	-1	-1	0	0	0	0
INF	0	0	0	-1	0	0	0	0	-1	1	1	1
ZOO	0	0	0	-1	0	-1	0	0	-1	-1	-1	1
PN	0	0	0	0	0	-1	0	0	-1	-1	-1	0
NHP	0	0	0	0	0	-1	0	0	-1	-1	0	-1

Figure 3.9. Domoic Acid Poisoning model and Community Matrix. Abbreviations in the model are GS = Opportunistically gastropodivorous sharks, NGS = Non-gastropodivorous sharks, PCF = Pelagic carnivorous fishes, EBC = Epibenthic carnivores, SQU = Squid, PLF = Planktivorous fishes, G&E = Gastropods and echinoderms, BIV = Bivalves, INF = Infauna, ZOO = Zooplankton, PN = *Pseudo-nitzschia* spp., NHP = Non-harmful phytoplankton.

	GS	NGS	PCF	EBC	SQU	PLF	G&E	BIV	INF	ZOO	PN	NHP
GS	112	-36	-18	-34	40	88	56	22	-44	98	-230	142
NGS	-48	120	60	32	-52	32	-24	8	-16	80	-128	96
PCF	-68	-74	85	25	28	-36	-34	-9	18	-29	83	-47
EBC	4	-10	-5	99	-16	-84	2	101	42	-27	153	-69
SQU	-24	60	-92	16	96	16	-12	4	-8	40	-64	48
PLF	16	-40	-20	-92	-64	152	8	-84	-76	136	-364	212
G&E	-60	28	14	-82	-4	40	92	10	-20	-22	-38	-2
BIV	56	-18	-9	-17	20	44	-94	133	-22	49	-115	71
INF	-8	20	10	46	32	-76	-4	42	160	-190	426	-106
ZOO	-12	30	15	-53	48	8	-6	-59	-126	325	-459	207
PN	4	-10	-5	99	-16	-84	2	101	42	-271	641	-313
NHP	4	-10	-5	99	-16	-84	2	101	42	-271	397	-69

Figure 3.10. Domoic Acid Poisoning model Adjoint Matrix. Abbreviations in the model are GS = Opportunistically gastropodivorous sharks, NGS = Non-gastropodivorous sharks, PCF = Pelagic carnivorous fishes, EBC = Epibenthic carnivores, SQU = Squid, PLF = Planktivorous fishes, G&E = Gastropods and echinoderms, BIV = Bivalves, INF = Infauna, ZOO = Zooplankton, PN = *Pseudo-nitzschia* spp., NHP = Non-harmful phytoplankton.

webs. From the overall model, the strong harmful response seen in both shark groups eases the predatory pressure from the apex predator on the system which could lead to major trophic restructuring. The CFP model had strong negative responses by upper trophic level consumers, and invertebrate epifauna were probably enhanced by the relaxation of predation pressure. NSP was characterized by the strongest reactions of all of the model runs indicating severe ecosystem effects. Finally, the DAP model also indicated a major re-organization of trophic relationships because of the strong negative responses at all trophic levels by sharks, planktivorous fishes, and zooplankton. The model predictions of presses on HAB types highlight the level of danger posed to coastal Louisiana food webs if conditions change to favor toxic plankton.

Qualitative modeling is a technique that is highly applicable to HAB research. In general, HAB ecology is of increasing interest and management concern, and this method can be useful in elucidating ecosystem-wide problems associated with HABs. The results of this study illustrate the substantial effects that HABs can have on coastal estuarine ecosystems. In all four models,

the shark community within Terrebonne Bay responded negatively to presses on toxic phytoplankton, which can have serious, ecological consequences for coastal communities. Myers et al. (2007) showed the indirect effects of a loss of apex predatory sharks on the U.S. Atlantic coast has caused a trophic cascade leading to a decline in shellfish due to the increase of

Table 3.1. Summary of the results of the model presses on harmful phytoplankton. Overall Reduced model = the effects of all examined HAB types, CFP = Ciguatera Fish Poisoning, NSP = Neurotoxic Shellfish Poisoning, DAP = Domoic Acid Poisoning. Responses were characterized, based on their relative value, as either strongly positive, strongly negative, or, if intermediate, neutral. Model outputs are results of positive presses on Harmful phytoplankton, except NSP which also includes a negative press on Non-harmful phytoplankton.

Model	Overall Reduced	CFP	NSP	DAP
Algal Type	Harmful Phytoplankton	<i>Gambierdiscus toxicus</i>	<i>Karenia brevis</i>	<i>Pseudo-nitzschia spp.</i>
Opportunistically gastropodivorous sharks	-156	67	-1056	-230
Non-gastropodivorous sharks	-176	-286	-800	-128
Pelagic carnivorous fishes	34	-105	328	83
Epibenthic carnivores	-30	-209	-570	153
Squid	-88	-143	-400	-64
Planktivorous fishes	-130	28	396	-364
Gastropods and echinoderms	108	496	144	-38
Bivalves	-536	411	-962	-115
Infauna	-78	-287	-528	426
Zooplankton	90	-655	-198	-459
Harmful phytoplankton	641	641	954	641
Non-harmful phytoplankton	-15	216	-380	397

mesopredatory elasmobranchs, especially cow-nosed rays (*Rhinoptera bonasus*). Since Terrebonne Bay is an important shark pupping and nursery ground for several species including large and small coastal species (de Silva et al. 1999, Condrey et al. 2000, Thompson et al. 2001, Neer et al. 2007), more frequent and intense HABs in that area could potentially reduce the numbers of apex predators in coastal systems across the northern Gulf of Mexico, affecting both adults moving into the system for pupping, and their offspring's success in the nursery. Through the use of loop analysis, resource managers and researchers can now assess both the direct and indirect effects of HABs on food webs that support shark nursery functions.

The results of the CFP model show *Gambierdiscus toxicus* blooms are generally detrimental, primarily to pelagic members of the Terrebonne Bay food web (Fig 3.6). The pelagic nature of the effects of CFP was expected because of the nature of ciguatoxin transmission (Randall 1958, Kelly et al. 1992), and a lack of knowledge of its effects on the benthos (Landsberg 2002). A few unexpected results were seen, namely opportunistically gastropodivorous sharks and planktivorous fishes had a neutral response to the HAB. As an apex predator, sharks are exposed to high levels of ciguatoxins due to bio-accumulation (Randall 1958), and since ciguatoxins are ichthyotoxins (Lewis 1992), a negative response is expected. Planktivorous fishes were also expected to be negatively affected by CFP since, again, ciguatoxin can be ichthyotoxic; however, the model results predicted otherwise. These irregularities could have resulted from not including unknown but important direct interactions in the model. The enhancements shown for gastropods and echinoderms, and bivalves were expected since, as shown by the direct interactions of the model, these benthic organisms do not play a role in pelagic food webs, but effects of ciguatoxin on these organisms due to settled out *G. toxicus* have not been investigated. One caveat for this model is that the effects of ciguatoxins on aquatic organisms have not been fully researched (Landsberg 2002); therefore, many direct

interactions in the model may need modification to generate more realistic models. Aside from the lack of knowledge regarding effects of ciguatoxin (Landsberg 2002), *G. toxicus* was difficult to accurately model because of the life cycle of ciguatoxin. Direct interaction with *G. toxicus* does not guarantee CFP; the toxin must be converted into the toxic form, so metabolism leading to ciguatoxin is an indirect pathway which is difficult to account for in a loop model since the models are based on direct interactions. Despite its indirect nature, realistic models of CFP can be constructed by assuming *G. toxicus* to be toxic. Although CFP is not a current problem in Louisiana, qualitative modeling allows for the forecasting of potential consequences if conditions in Louisiana become more conducive to *G. toxicus* blooms.

The second model made, NSP, was for another theoretically problematic HAB in Louisiana coastal waters (Fig. 3.8). Most nodes experienced strong negative responses to a positive press of *K. brevis* which is characteristic of these HABs, since brevetoxin is so potent that most organisms cannot escape the toxic effects. Blooms of *K. brevis* typically cause massive mortality events of marine animals affecting all levels of the food web (Gunter et al. 1947, Gunter et al. 1948, Landsberg 2002, Flewelling et al. 2005, Hinton & Ramsdell 2008). Some of the model predictions were somewhat unexpected for groups that have been shown to be sensitive to brevetoxin (Roberts et al. 1979, Naar et al. 2007); however, both pelagic carnivorous and planktivorous fishes responded positively and gastropods and echinoderms had neutral responses. These unexpected consequences may be due to the fact that predation pressure was alleviated by the reduction of shark populations, in the case of fishes, and the combination of declines in sharks and epibenthic carnivores for gastropods and echinoderms. Nevertheless, these unforeseen findings do not change the obvious overall trend that a bloom of *K. brevis* in Terrebonne Bay would be detrimental to the system.

The final model on DAP (Fig. 3.10) is the only one in this study dealing with a present threat to Terrebonne Bay food webs. Since coastal populations of filter-feeding organisms, namely gulf menhaden, are important prey in coastal Louisiana, the potential for DA pressing the food web is high. As shown by the model results, a bloom of toxic *Pseudo-nitzschia* in Terrebonne Bay could cause a major re-organization of trophic structure. The strong negative response by planktivorous fishes is consistent with Lefebvre's (2001) findings, where DA exposure caused planktivorous fishes to lose schooling ability, making them easier targets for predators. The decline in menhaden is also felt by the shark nodes in the model, as shown by the negative response of the sharks. This outcome was expected since planktivorous gulf menhaden make up the majority of the sharks' diets, and they represent the short food chain length that DA transfer needs to be effective (Snelson et al. 1984, Bethea et al. 2004). The positive response of the pelagic carnivorous fishes and epibenthic carnivores was surprising, but several explanations exist. The reduction of their predators, sharks, could have allowed these groups to flourish, or differential prey selection than the sharks (Fishbase) of Terrebonne Bay could have buffered them against the effects of DA. Additionally, the food chain lengths for these other predators may be longer than that of most sharks, so the accumulated DA concentrations in prey could be less. The other effects seen in the model results were expected, but as with the CFP model more research into the effects of DA on different members of the community will help to clarify the direct interactions in the model and provide a more accurate model. Evaluating the outcomes of a major DAP event should inform resource managers of the possible consequence of increasing eutrophication. The results of this study clearly show how detrimental HABs could be to Terrebonne Bay food webs. Harmful algal blooms can alter trophic structure that disrupts food webs.

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CHAPTER 4

NEUROTOXINS IN A LOUISIANA ESTUARY: QUANTITATIVE ANALYSIS OF DOMOIC ACID IN GULF MENHADEN (*BREVOORTIA PATRONUS*) AND QUALITATIVE MODELING OF LINKS IN A SHARK NURSERY: CONCLUSIONS

In the present study, I examined some of the potential risks posed to Louisiana coastal food webs by HABs. I first focused on the identification of a potential vector of domoic acid (DA) in coastal Louisiana water bodies. Second, I broadened the scope of my study by investigating the ecosystem-wide effects of domoic acid and other algal toxins that potentially threaten Louisiana coastal food webs. By exploring the problem of HABs in Louisiana on a micro and macro level, the present study evaluates how toxins may contaminate the food web and potential risks to consumers. While HABs are a present threat in Louisiana waters, to my knowledge this is the first study to examine trophic transfer of DA in this region.

In Chapter 1, pertinent literature for understanding the vector potential of gulf menhaden, and for qualitative modeling was reviewed. The goal of this chapter was to provide the reader with general background information before reading these studies, and to demonstrate the need for more information on these topics.

In chapter 2, the vector potential of gulf menhaden was assessed. It was discovered, for the first time in the Gulf of Mexico, that tissue of gulf menhaden contained DA, thereby making them a potential vector for DA to higher trophic level consumers. In addition, I found a positive correlation between DA in fish tissue and water particulate DA. Specifically, whenever toxic *Pseudo-nitzschia* spp. were present in water samples, DA was found in gulf menhaden. These results confirm the potential of gulf menhaden to be a vector of DA to Louisiana coastal food webs.

In Chapter 3, a broad assessment of the possible effects of three different HABs on a shark nursery food web was determined. The first food web model, Ciguatera Fish Poisoning (CFP), showed that upper trophic level consumers are vulnerable to ciguatera poisoning by their

negative responses. The Neurotoxic Shellfish Poisoning (NSP) model demonstrated how severe its problems can be since there were strong negative effects on almost all of the nodes. The final model evaluated the effects of Domoic Acid Poisoning (DAP), and illustrated harmful effects at several trophic levels. In all of these cases, the potential for trophic cascades, due to the loss of an apex predator in the system, is high since shark populations had some of the strongest negative responses shown by the models.

This study highlights the vulnerability of Louisiana coastal food webs to toxicity by HABs. The possibility of food web contamination by phycotoxins in Louisiana exists since gulf menhaden are very abundant, are a common prey item, and have the potential to be a DA vector. Additionally, gulf menhaden are filter-feeders, so they possess the ability to potentially transfer other algal toxins, like ciguatoxin or brevetoxin, into the food web. Therefore, Louisiana coastal systems could experience the negative consequences of HABs, such as trophic cascades, as shown by the qualitative modeling. The results of this study combine to show coastal Louisiana as one area that is at risk for serious ecological consequences due to HABs.

Not only are the findings presented in this study applicable to Louisiana, but they can have implications for other coastal systems around the world. Gulf menhaden are distributed throughout the Gulf of Mexico, so the potential for them to transfer toxins into food webs exists for coastal waters throughout the entire basin. There are also close relatives of the gulf menhaden, Atlantic menhaden that are very abundant along the Atlantic coast of the US that could possibly transfer toxin as well. Additionally, the technique of loop analysis is applicable to any ecosystem; one only needs basic life history information in order to conduct analyses, so the effects of HABs, or any other perturbation, on other food webs can be established. The results of the present study have brought new insight into aspects of HAB ecology that may aid other researchers as they investigate the global problem of HABs.

APPENDIX I

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) PROTOCOL

Theory

Enzyme-linked immunosorbent assay (ELISA) is a direct competition method where domoic acid (DA) in the sample competes with a DA protein coated onto the walls of a 96-well plate for an anti-DA antibody. True DA will bind to the antibody more readily, and will get washed out of the well; the leftover antibody bound to the well will be treated throughout the assay to be read photometrically. So the assay is read inversely, the lighter the color of the well means there was more DA in the sample because fewer antibodies remained in the well to generate color.

Preparation

Before the ELISA can be performed, there are several preparatory steps that must be completed. There are several reagents that must be prepared before the assay can be started. The first of which is the washing buffer where two of the supplied tablets are dissolved in 1.0 liter of DI water. The next reagent is the standard/sample buffer, and 10 ml of MeOH is added to 90 ml of washing buffer. The final reagent prepared prior to the assay is the antibody-HRP ovalbumin buffer. This buffer is prepared by adding 6 ml of washing buffer to the supplied vial of 60 mg of Ovalbumin. During the preparation of the reagents, the samples are allowed to come to room temperature.

In order to facilitate a more organized working station, 2 ml microcentrifuge tubes are set up for each sample and dilution as needed. The next step is to add 450 μ l of standard/sample buffer to each microcentrifuge tube. Then the samples are diluted by adding 50 μ l of sample to the proper microcentrifuge tube. It is not recommended to do a 1:1 test of a sample because of the risk of false positive results, so all samples are diluted to a minimum of 1:10. Some of the samples may need to be diluted more in order to give a reading in the working range of the assay

(10 pg DA ml⁻¹ – 300pg DA ml⁻¹), so 1:10 serial dilutions are done for those samples that need it. After the samples are diluted, the standard curve is prepared by serial dilution ranging in concentrations from 10,000 – 0.16 pg DA ml⁻¹. One 2 mL microcentrifuge is filled with 450 µl of standard/sample buffer, and nine others have 300 µl of standard/sample buffer put into them. Then, 50 µl of a supplied DA standard is pipetted into the first tube and vortexed for 10 seconds. Each subsequent tube receives 125 µl from the previous tube and is vortexed for 10 seconds.

Plate Set-up

After all of the reagents are prepared, the samples are diluted, and the standard curve is ready, the assay can begin. The first step is to assemble the supplied 96-well plate, and pre-rinse it by adding 300 µl of washing buffer to each well using a Fisherbrand e1200 multichannel pipettor, and letting it sit in the wells for 5-10 minutes. This pre-rinse step protects from over-estimations by removing any DA protein that may have fallen off of the well wall during shipping. After shaking the washing buffer out of the wells, the plate is ready to be filled with the standard curve and samples using an Eppendorf Research series 20-200 µl mechanical pipettor. The Amax (maximum absorbance) and blank wells are prepared by adding 50 µl of standard/sample buffer to all of the Amax and blank wells, and 50 µl of Antibody-HRP ovalbumin buffer to only the blank wells. Next, 50 µl of each standard is pipetted into their respective wells, followed by pipetting 50 µl of each sample into their designated wells.

Reaction, Color Development, and Reading

After the plate has been filled with the standard curve and samples, the next step is the competitive binding reaction. One ml of the Anti-DA-HRP conjugate from the kit is diluted in 5 mls of the Antibody-HRP ovalbumin buffer, vortexed for 1 minute, and this reagent is added to all of the wells except the blank wells. Then the plate was sealed and incubated at room temperature in the dark for 1 hour. After incubation, the plate is washed 4 times with 300 µl for

each well with washing buffer using a Statfax 2600 plate washer. The washing step ensures that all of the bound DA in the sample is washed out of the well. Then, 100 µl of the included TMB peroxidase is added to all of the wells with the multichannel pipettor, and incubated at room temperature in the dark for 15 minutes to allow color to develop. To stop the color developing reaction, 100 µl of 0.3M sulphuric acid (H_2SO_4) was added to all of the wells. Finally, after two minutes, the plate was read on a Thermo Electron Corporation Varioskan Flash plate reader.

APPENDIX II

SUMMARY OF ALL BYCATCH FROM GILL NET SAMPLING

Table A.1. Summary of all bycatch from gill net sampling. Mean values are reported with standard deviations in parenthesis.
PCL = precaudal length, FL = fork length, TL = total length, DW = disc width, CW = carapace width, STL = stretched total length,
N.D. = No Data.

Species	Gear Type	Number Caught	PCL (cm)	FL (cm)	TL/DW/CW (cm)	STL (cm)	Number Male	Number Female	Dominant Maturity State
<i>Callinectes sapidus</i>	Gillnet	126	N.D.	N.D.	15.2 (1.68)	N.D.	3	107	Adult
<i>Litopenaeus setiferus</i>	Gillnet	7	N.D.	N.D.	17.3 (1.89)	N.D.	N.D.	N.D.	N.D.
<i>Menippe mercenaria</i>	Gillnet	1	N.D.	N.D.	10	N.D.	N.D.	N.D.	N.D.
<i>Carcharhinus isodon</i>	Gillnet	17	56.9 (24.44)	63 (27.14)	71 (31.11)	78.4 (33.71)	13	3	YOY
<i>Carcharhinus leucas</i>	Gillnet	2	44.75 (0.35)	49.8 (0.35)	61	62.8 (0.35)	1	1	YOY
<i>Carcharhinus limbatus</i>	Gillnet	67	45.3 (8.09)	50.4 (9.51)	61 (10.8)	63.3 (11.9)	40	24	YOY
<i>Rhinoptera bonasus</i>	Gillnet	21	N.D.	N.D.	47.3 (6.54)	N.D.	12	8	YOY
<i>Rhizoprionodon terraenovae</i>	Gillnet	15	37 (15.55)	40.7 (16.63)	48.6 (19.8)	50.1 (19.85)	11	4	YOY
<i>Arius felis</i>	Gillnet	78	N.D.	31.7 (3.51)	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Bagre marinus</i>	Gillnet	67	N.D.	43.6 (6.85)	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Caranx hippos</i>	Gillnet	2	N.D.	86 (1.41)	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Cynoscion arenarius</i>	Gillnet	31	N.D.	22.7 (2.82)	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Cynoscion nebulosus</i>	Gillnet	6	N.D.	28.8 (8.96)	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Menticirrhus americanus</i>	Gillnet	1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Micropogonias undulatus</i>	Gillnet	2	N.D.	N.D.	13.8 (1.06)	N.D.	N.D.	N.D.	N.D.
<i>Peprilus alepidotus</i>	Gillnet	18	N.D.	15 (1.45)	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Peprilus berti</i>	Gillnet	8	N.D.	12.7 (0.84)	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Pogonias cromis</i>	Gillnet	3	N.D.	N.D.	56.5 (17.76)	N.D.	N.D.	N.D.	N.D.
<i>Rachycentron canadum</i>	Gillnet	2	N.D.	46.5 (16.3)	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Scomberomorus maculatus</i>	Gillnet	19	N.D.	45.2 (5.13)	N.D.	N.D.	N.D.	N.D.	N.D.
<i>Syngnathus sp.</i>	Gillnet	1	N.D.	N.D.	5.5	N.D.	N.D.	N.D.	N.D.
<i>Trachinotus carolinus</i>	Gillnet	11	N.D.	24.6 (5.19)	N.D.	N.D.	N.D.	N.D.	N.D.

VITA

Ross Del Rio was born in 1983 in Metairie, Louisiana, to Kathy and Ralph Del Rio. He graduated with honors from Brother Martin High School in New Orleans, Louisiana in 2002. He then attended Louisiana State University in Baton Rouge, Louisiana, where he earned his bachelor's degree in May 2007 in biological sciences with a concentration in marine biology, and three minors: chemistry, oceanography, and business administration. Upon graduation, Ross began his master's degree in the summer of 2007. He is now candidate for a Master of Science degree in the summer of 2009.