Overview and Introduction to Harmful Algal Blooms in Mississippi Waters

A Report by

Dan Holiday, Adrienne Russell, and D. Jay Grimes
September 2007

The Gulf Coast of Mississippi consists of approximately 85 miles of sand shores interspersed by 3 major bays (Bay St. Louis, Biloxi Bay and Pascagoula Bay) and numerous bayous consisting of approximately 72,000 acres of coastal wetlands. The Pearl, Tchoutacabouffa, Biloxi, Escatawpa and Pascagoula Rivers are the primary fresh water inputs from the mainland. These nutrient-rich outflows influence the productivity of the relatively eutrophic, shallow waters of the Mississippi Sound between the Gulf Island National Seashore barrier island chain (Cat, Ship, Horn, and Petit Bois Islands in Mississippi and Dauphin Island in Alabama) and the coastal mainland (Mississippi Bight). Further influence from the Mobile Bay and Mississippi River via Lake Pontchartrain and Borgne greatly contribute to the nutrient and sedimentation levels of the Mississippi Sound. For these reasons, phytoplankton blooms in Mississippi coastal waters are highly diverse and primarily dominated by euryhaline species, with occasional high concentrations (500,000 to >1,000,000 cells/l) of Chlorophytes and Cryptophytes originating from freshwater sources during sustained and seasonal rain events.

Officially there has been only one major harmful algal bloom (HAB) event affecting Mississippi waters, an extensive *Karenia brevis* bloom in 1996 that was present in Mississippi waters south of the barrier islands and throughout most of Mississippi Sound. Currents, winds and storm events can push *K. brevis* from its normal high-salinity, oligotrophic environment off the mid to southern Florida coast far to the northwest into Mississippi waters. For example, sampling done by the Gulf Coast Geospatial Center (GCGC) after Hurricane Katrina showed the presence of *K. brevis* in numbers exceeding 50,000 cells/l during the month of October from stations to the north and south of Horn Island. During September and October of 2005, public reports and inquiries by fisherman were received on the Gulf Coast Research Laboratory website, reporting and asking for explanations of unusually high fish kills and respiratory problems while fishing on or near the north shores of Horn Island. The MS Department of Marine Resources (MDMR), in response to calls from the public, also conducted sampling in October 2005. On October 5, some of the samples collected by the MDMR had high levels of *Prorocentrum* spp. (6,300 cells /l) and one sample had *Karenia* spp. at 140 cells/l. On October 18, a follow-up sample contained *Prorocentrum* at 6,000 cells/l and *Karenia* at 963 cells/l. These public reports clearly alluded to a potential prolonged *K. brevis* HAB event following Hurricane Katrina and temporally corresponded to reports of bloom events on the Florida panhandle and in Mobile Bay, AL. Infrastructure damage due to the hurricane prevented any official actions on these data.

In nearby Louisiana and Alabama waters, reports of *Pseudo-nitzschia* species in near shore water samples are common during winter and spring months. These counts have exceeded 80,000 cells/l in Mississippi waters. This diatom is responsible for production of domoic acid, but the production of this toxin can be at low levels in spite of high population counts. Thus far, high toxic levels have not been reported in Mississippi samples, but the ability of this organism to thrive in these waters makes it a potential threat.

HAB Monitoring Efforts and HAB Research in Mississippi

Mississippi DMR and DEQ Water Sampling Programs

The MDMR conducts regular sampling of water in and around oyster beds and those samples are processed for fecal indicator bacteria by the Gulf Coast Research Laboratory. They also sample and test oyster meats according to the National Shellfish Sanitation Program guidelines. The Mississippi Department of Environmental Quality (MDEQ) conducts a beach monitoring program, testing for fecal indicator bacteria contamination. The beach monitoring samples are collected and tested by the same GCRL group that tests oysters and oyster growing waters. In March 2007, the MDMR initiated a Marine Biotoxin Contingency Plan for all marine and estuarine shellfish growing areas in Mississippi. Under this plan, discrete samples are collected and sent to the Alabama Department of Public Health for identification and numeration of potential harmful phytoplankton species. The new contingency plan is appended at the end of this overview.

The following sections include a listing of institutions and individuals active in HAB-related projects in Mississippi waters.

University of Southern Mississippi
Gulf Coast Research Laboratory, Ocean Springs, MS

Dr. Greg Carter

Dr. Carter has an active interest in the use of remote sensing technology towards the detection and prediction of HAB species in the northern Gulf of Mexico (GOM). His research interests include the remote sensing of vegetation and coastal aquatic systems, including biophysical influences on radiative properties, invasive species detection, and uses of remote sensing technologies toward assessment of biodiversity and ecosystem function in aquatic, coastal, and terrestrial environments.

Dan Holiday

Ph.D. graduate student with Greg Carter and Dr. Hugh MacIntyre of the Dauphin Island Research Lab (DISL), Dan is interested in the use of MODIS and SeaWiFS data in the detection and prediction of HAB events in Case II waters. He and Dr. Carter are presently working with the GOM EPA in a collaborative grant with Dr. Richard Gould, Jr., Naval Research Laboratory at Stennis Space Center and Dr. MacIntyre at DISL. This project includes collection of nutrient data and phytoplankton populations in the Mississippi Sound, analysis of nutrient data, use of MODIS and SeaWiFS reflection and algorithm

data products, and modeling of the ecological conditions leading to HABs formation in the northern GOM. His research interests include the development of probabilistic predictive models using correlative satellite and insitu data parameters.

Dr. D. Jay Grimes
Dr. Grimes is a microbial ecologist and has an active research program focused on the biology and ecology of estuarine and marine bacteria. He is especially interested in the ecology of pathogenic vibrios and is currently using remote sensing (MODIS) and real-time molecular detection (e.g., real-time PCR) to examine environmental factors that determine the distribution and abundance of *Vibrio parahaemolyticus* (*Vp*), *V. vulnificus* (*Vv*) and other *Vibrio* spp. in coastal habitats. Both *Vp* and *Vv,* as well as most other vibrios, co-exist with plankton, marine invertebrates, and marine plants. One of Grimes' graduate students, Adrienne Russell, is focusing her Ph.D. research on *Vp* relationships with plankton including HAB species.

Dr. Crystal Johnson
Dr. Johnson is a marine microbiologist with special interest in *Vibrio parahaemolyticus* (*Vp*) and *V. vulnificus* (*Vv*) collected from the Mississippi Gulf Coast. She is examining the relationship between environmental parameters such as sea surface temperature and *Vp* and *Vv* densities in oysters, sediment, and overlying waters. She is particularly interested in the rare *Vp* strains that carry pathogenicity genes expressing thermostable direct hemolysin (*tdh*) and tdh-related hemolysin (*trh*). She is also interested in examining the role of Type III Secretion Systems in the relationships between vibrios and other organisms that share the same niche.

Adrienne Russell, M.A.
Adrienne Russell is a Ph.D. student with Dr. Grimes and is working currently as a Research Associate in Marine Microbiology. Adrienne entered into the field of microbiology with a background in plant systematics, plant ecology, and marine invertebrate taxonomy. She is working as a marine microbiologist with special interest in *Vibrio parahaemolyticus* and its association with a variety of substrates and microhabitats, including phytoplankton, zooplankton, sediment, oysters, and other macroinvertebrates.

University of Southern Mississippi
Department of Marine Science, Stennis Space Center, MS

Dr. Vernon Asper
Dr. Asper is the Director of the Underwater Vehicles Technology Center of the National Institute of Undersea Science and Technology program. NIUST is made up of three divisions: the Ocean Biotechnology Center & Repository (OBCR), the Seabed Technology Research Center (STRC), and the Undersea Vehicles Technology Center (UVTC). The overall goal of the program is the development of viable technologies involving interactive communication, systems command and control, data assimilation and processing, and artificial intelligence appropriate for the operation/deployment of ROVs and AUVs. He and Dr. Lohrenz are actively pursuing the use of underwater

vehicles equipped with instrumentation such as the breve-buster for optical discrimination of phytoplankton species.

Dr. Steven Lohrenz
Dr. Lohrenz is active in several HAB research efforts, with a focus in hyperspectral remote sensing efforts and in-water optical instrumentation. Areas of interest include optical detection and assessment of harmful algal blooms with special interests in using hyperspectral data and autonomous underwater vehicle instruments, fine-scale distributions of photosynthetic pigments and associated microbial biomass and activity, development and application of models for description of primary production and phytoplankton distributions, transformation and transport of organic matter in coastal ecosystems, and single-cell techniques for characterization of growth and photosynthetic processes in phytoplankton.

Dr. Stephan Howden
Dr. Howden oversees the activities of the Central Gulf of Mexico Ocean Observing System (CenGOOS). This system is under development following Katrina, but will include moored observations of temperature, salinity, currents, and meteorological conditions at various locations offshore. A component of this effort also includes routine water sampling along a station line from St. Louis Bay to the buoy sites. A High Frequency radar array will provide surface current information. A CenGOOS autonomous glider pilot project has been leveraged by the Northern Gulf Institute for HABS research through purchase of a Brevebuster instrument bay for the glider from Mote Marine Laboratory. Collaboration with the Naval Research Laboratory will involve implementing coastal circulation models that can be refined through data assimilation of observing system information.

Dr. Karen Orcutt
Dr. Orcutt has done extensive work with nitrogen fixing cyanobacteria in both open ocean and brackish water systems. Her work involves environmental factors that contribute to algal bloom formation including harmful algal species. Her research interests include molecular ecology of marine microbes, genetic diversity of phytoplankton, marine nitrogen fixation and biogeochemical cycling. She also has developed nanostructured probes for nutrient acquisition and biosensing of trace analytes that may contribute to the development of harmful algal blooms.

Dr. Don Redalje
Dr. Redalje has an active interest in phytoplankton optics. His research interests include marine phytoplankton ecology, physiology, bio-optics, biochemistry, primary production and taxonomy with particular emphasis on the effects of environmental factors on carbon and nitrogen metabolism, cellular composition and growth at the species and population levels of both laboratory cultures and natural populations.

Dr. Kjell Gundersen
Dr. Gundersen has worked extensively on microplankton nutrient dynamics, including toxic species of filamentous cyanobacteria, in brackish and open

ocean waters. His research is focused on the role of phytoplankton growth dynamics, excretion of dissolved organic matter, and microbial respiration in ocean biogeochemistry. More recently, Dr. Gundersen applied novel fluorophores (nanocrystals) to tag nutrient stress proteins in single phytoplankton cells and, he is also actively pursuing the use of automated sensors in biogeochemical time-series applications.

University of Southern Mississippi
Department of Biological Sciences, Hattiesburg, MS

Dr. Timothy McLean
Dr. McLean is a molecular biologist applying molecular techniques to marine microbiology. He is taking a functional genomics approach to understanding the basic biology and ecology of *Karenia brevis*. He has created a *K. brevis* expression sequence tag (EST) library with approximately 6,000 unique genetic sequences. Using these sequences, microarrays have been created for use in determining gene expression profiles of these cells when cultured under different environmental conditions. In collaboration with researchers at the University of Miami and Florida International University who will measure various physiological parameters including toxin production from *K. brevis* cultures, Dr. McLean will make connections between the genetic profiles and cellular physiology under defined conditions. An additional interest that is being developed is to determine the level of genetic diversity or population structure that exists among cells within a bloom as well as between blooms.

University of Mississippi
School of Pharmacy, Oxford, MS

Dr. Mark T. Hamann
A marine natural products chemist/pharmacologist and CSO of Triton BioPharma LLC; Dr. Hamann's group is actively engaged in the detection, fermentation, analysis and detailed NMR structure assignment of marine natural products and toxins. Dr. Hamann's group in collaboration with Al Place a UMBI is currently assigning the structure of the karlotoxins using detailed NMR studies. The karlotoxins are a group polyketide toxins produced by *Karlodinium veneficum* and are responsible for massive fish kills globally. This series of toxins are highly likely responsible for the fish kill's associated with *Pfiesteria piscidia.* Dr. Hamann's group is also investigating the use of super critical fluid carbon dioxide (SCF CO_2) for the cold pasteurization of oyster's and other seafood products. SCF also have significant utility in the dissolution and specific removal of marine toxins from food supplies.

Dr. Robert Doerksen
Dr. Doerksen is a computational medicinal chemist using molecular modeling to study toxin-target interactions of marine toxins. Dr. Doerksen's group uses atomistic and quantum mechanical models to help characterize previously uncharacterized toxins such as karlotoxin, to study the conformations and flexibility of toxin molecules and to model

their interactions with other small molecules such as cholesterol or with proteins in order to better define their mechanism of action and remediation approaches.

Dr. Takashi Tomioko
Dr. Tomioka's group is experienced in the synthesis of polyketide derived marine toxins. These synthetic materials can be utilized as standards for evaluation of environmental samples as well as characterizing the pharmacology and metabolic stability of marine toxins. Tomioka's group is currently working on the total synthesis of karlotoxin, and developing new synthetic methodologies/reagents to establish the practical supplying system of karlotoxin and related marine toxins for further biological investigation.

Dr. JianNan Peng
Dr. Peng is a research scientist and NMR spectroscopist working on the complete structure assignment including absolute configuration of the karlotoxin class of marine toxins.

Damaris Agathe F. Meujo
Ms. Meujo has an MS degree in both Biochemistry and Microbiology. She is currently a Ph.D. student working on drug discovery from marine microorganisms (*Actinomycetes*). She is also actively engaged in the use of supercritical fluid CO_2 to control the levels of pathogenic bacterial contaminant present in oysters and other seafood products.

Naval Research Laboratory, Stennis Space Center, MS

Dr. Richard Gould, Jr.
Dr. Gould is a lead research scientist in the Ocean Optics Section of the Naval Research Laboratory. Dr. Gould is actively involved in phytoplankton research, presently collaborating with Dr. Carter and Dan Holiday of the GCGC and Dr. MacIntyre of DISL and having collaborated with Dr. Richard Stumpf on several projects involving the use of SeaWiFS data for detection, monitoring and prediction of HABs in the Gulf of Mexico**.** His research interests include biological oceanography, all aspects of ocean remote sensing and ocean optics, optical classification of water masses, ecological indicators, harmful algal blooms, hypoxia, and predictive modeling of optical ocean properties.

MS Department of Marine Resources
Biloxi, MS

Bradley Randall
Bradley Randall is Biological Program Coordinator for the Shellfish Bureau of Mississippi's Department of Marine Resources. He helped develop the Marine Biotoxin Contingency Plan (appended to this document) and is working hard to better connect all of the MS HABs players. Before joining the MDMR, Randall was a shellfish biologist at USM's GCRL.

Private Companies

Dr. Cynthia Moncreiff
Formerly a marine botanist for the GCRL and now a scientist with the Gulf Coast office of Anchor Environmental LLC, Dr. Moncreiff's research interests include taxonomy and ecology of phytoplankton, with a focus on harmful algae blooms. She is also interested in the ecology of wetland environments and seagrass communities. Professionally, she is actively involved with seagrass mapping efforts in coastal Mississippi, and in remediation investigations for Anchor. The 1996 *Karenia brevis* bloom event and subsequent drought year phytoplankton bloom events were documented by Dr. Moncreiff and her staff during her time at the GCRL.

Mississippi Department of Marine Resources (DMR)
Mississippi Marine Biotoxin Contingency Plan
For All Marine and Estuarine Shellfish Growing Areas

March 1, 2007

A. Emergency Shellfish Sampling And Assay Program
   a) Department of Marine Resources (DMR) personnel will collect routine water samples quarterly when the areas are opened to harvest or prior to opening by boat throughout the season from indicator stations, to be assayed for the presence of toxic algal blooms by DMR, Gulf Coast Research Laboratory (GCRL), Alabama Department of Public Health (ADOPH) or other appropriate personnel.
   b) DMR personnel will make field observations by boat during routine water sampling trips of shellfish growing areas. Areas of discolored water suspected to be from a bloom of dinoflagellate phytoplankton will be noted and GPS coordinates recorded.
   c) Reports to the DMR of marine biotoxins being detected in the adjacent waters of bordering states will intensify monitoring efforts.
   d) Over flights of Mississippi's shellfish growing areas may be conducted as deemed necessary. Flight paths will include as many active reef areas as possible. DMR personnel will conduct all flight observations.
   e) Areas of discolored water suspected to be from blooms of dinoflagellate phytoplankton will be noted and GPS coordinates recorded.
   f) DMR personnel will investigate possible dinoflagellate phytoplankton blooms reported by other reliable sources.
   g) DMR or other appropriate personnel will investigate indications of a potential bloom by collecting water samples for analysis where deemed appropriate.
   h) DMR, GCRL, ADOPH or other appropriate personnel will make identifications and cell counts per liter where appropriate.
   i) The HAB sample stations by area are (See Figure 1):
      i. Area 1 - W/S 1-7
      ii. Area 2 - W/S 2-14C and 2-15B
      iii. Area 3 - W/S 3-7
      iv. Area 4 - W/S 4-2B, 4-8 and 4-9
      v. Area 5 – W/S 5-11
      vi. Area 6 - W/S 6-10 and 6-15
      vii. Area 7 - W/S 7-2 and 7-3
      viii. Area 8 - W/S 8-14

B. Close Growing Areas And Embargo Shellfish
   a) Identification of the dinoflagellate *Karenia brevis (Gymnodinium breve)* in water column exceeding 5,000 organisms per liter will immediately trigger a closure of affected shellfish-growing areas in Mississippi. Oyster areas that are affected will remain closed to harvest until concentrations drop below 5,000 cells per liter.
   b) Identification of plankton as one of the dinoflagellates *Prorocentrum minimum, Gonyaulax monilata (Alexandrium monilatum),* or the blue-green algae *Oscillatoria erythrer* will warrant no special actions regarding shellfish.
   c) In the event that unknown biotoxin-producing organisms are encountered, technical assistance from the Food and Drug Administration (FDA) and others will be sought in determining closing and re-opening criteria.

d) Shellfish-growing waters will be closed immediately and harvested contaminated shellfish shall be returned to the waters upon the presence of marine biotoxin-producing organisms in numbers sufficient to cause a health risk.
e) Authority to close waters or embargo shellfish rests in:
    i. Mississippi Code of 1972, § 49-15-3. Definitions.
        1. "(e) "Illegal oysters" means:"
            a. "(i) All untagged shell stock;"
            b. "(ii) Shell oysters obtained from uncertified shops or dealers or from an unlicensed catcher;"
            c. "(iii) Oysters obtained from waters not declared safe and sanitary by the department, except those oysters caught by the commission for re-laying or under private leases pursuant to Section 49-15-27;"
            d. "(iv) Shucked oysters obtained from uncertified shops or repackers."
    ii. Mississippi Code of 1972, § 49-15-15. Powers and duties of commission.
        1. (1.)(a) "To exercise full jurisdiction and authority over all marine aquatic life and to regulate any matters pertaining to seafood, including cultivated seafood;"
        2. (1)(b) "To adopt, promulgate, amend or repeal, after due notice and public hearing, in accordance with the Mississippi Administrative Procedures Law and subject to the limitations in subsection (2) of this section, rules and regulations authorized under this chapter, including, but not limited to, rules and regulations necessary for the protection, conservation or propagation of all seafood in the waters under the territorial jurisdiction of the State of Mississippi…"
        3. (1)(n) "To develop a resource management plan to preserve seafood resources and to ensure a safe supply of these resources."
    iii. Mississippi Code of 1972, § 49-15-21 (2.) – "…The enforcement officers may seize at any time aquatic life caught, taken or transported in a manner contrary to the laws of this state, and may confiscate and dispose of the same. …"
    iv. Mississippi Code of 1972, § 49-15-36 (3) – "… The department may limit the sale of oysters for human consumption…"
    v. Mississippi Code of 1972, § 49-15-44. Sale or possession of illegal oysters prohibited; penalties. - "The commission shall prohibit the sale or possession of illegal oysters. It is unlawful for any person, firm or corporation to possess or to engage in the sale of oysters not certified in this state, or to shuck or repack for sale any illegal oysters, unless that person, firm or corporation possesses a bill of sale, valid permit or affidavit of another state, properly dated, evidencing the legality of the sale or possession of the oysters in that state. Any person in possession of illegal oysters shall be subject to civil or criminal prosecution and shall be fined not less than One Hundred Dollars ($100.00) or punished as provided in Section 49-15-63."
    vi. TITLE 22 PART 1
        1. Chapter 08 Shellfish Area Openings and Closings
            a. 102 – "If closure is necessitated by any other polluting event, which threatens imminent peril to public health,

closure will be immediate and any oysters or other shellfish taken which have been subjected to such pollution as determined by the MDMR shall be returned to the water."

2. Chapter 12 Adulterated, Misbranded, or Unlabeled Shellfish and Products
    a. 100 – "It shall be unlawful for any person within the state of Mississippi to produce, harvest, provide, purchase, sell, offer, possess, or expose for sale, or have in possession with intent to sell, any raw shellfish and shellfish products which are adulterated, misbranded, or unlabeled and no person shall hold or pack shellfish under conditions whereby the shellfish may become adulterated."
    b. 100.01 – "Any adulterated, misbranded, mislabeled, or unlabeled shellfish or shellfish products may be impounded by the direction of the MCMR or as hereby authorized the MDMR, its Executive Director, Director of Marine Fisheries, the Program Coordinator or other MDMR designee in charge of molluscan shellfish handling and processing, or Marine Patrol Officer."
    c. 100.02 – "Seizure and disposal of such shellfish products shall be by the direction of the MCMR, or as hereby authorized the MDMR, its Executive Director or Marine Patrol Officer."
3. Chapter 21 Penalties
    a. 103 – "Any person in possession of shellfish in violation of any chapter of this Part shall be subject to confiscation and disposal of such shellfish by direction of any designated Marine Patrol Officer of the MDMR."

C. Prevent Harvesting of Contaminated Species.
    a) The harvest of shellfish contaminated by the presence of marine biotoxin-producing organisms in numbers sufficient to cause a health risk will be prevented by the resulting closure of affected waters.

D. Provide For Product Recall.
    a) Title 22, Part 1, Chapter 08 Shellfish Area Openings and Closings, 102 – "If closure is necessitated by any other polluting event, which threatens imminent peril to public health, closure will be immediate and any oysters or other shellfish taken which have been subjected to such pollution as determined by the MDMR shall be returned to the water."

E. The DMR will immediately disseminate information on the occurrences of toxic algal blooms and/or toxicity in shellfish meats to adjacent states, the shellfish industry, and local health agencies by the most appropriate effective communication means available.

F. The DMR will coordinate control actions taken by other state or federal agencies involved.

MS Department of Marine Resources
(228) 374-5000

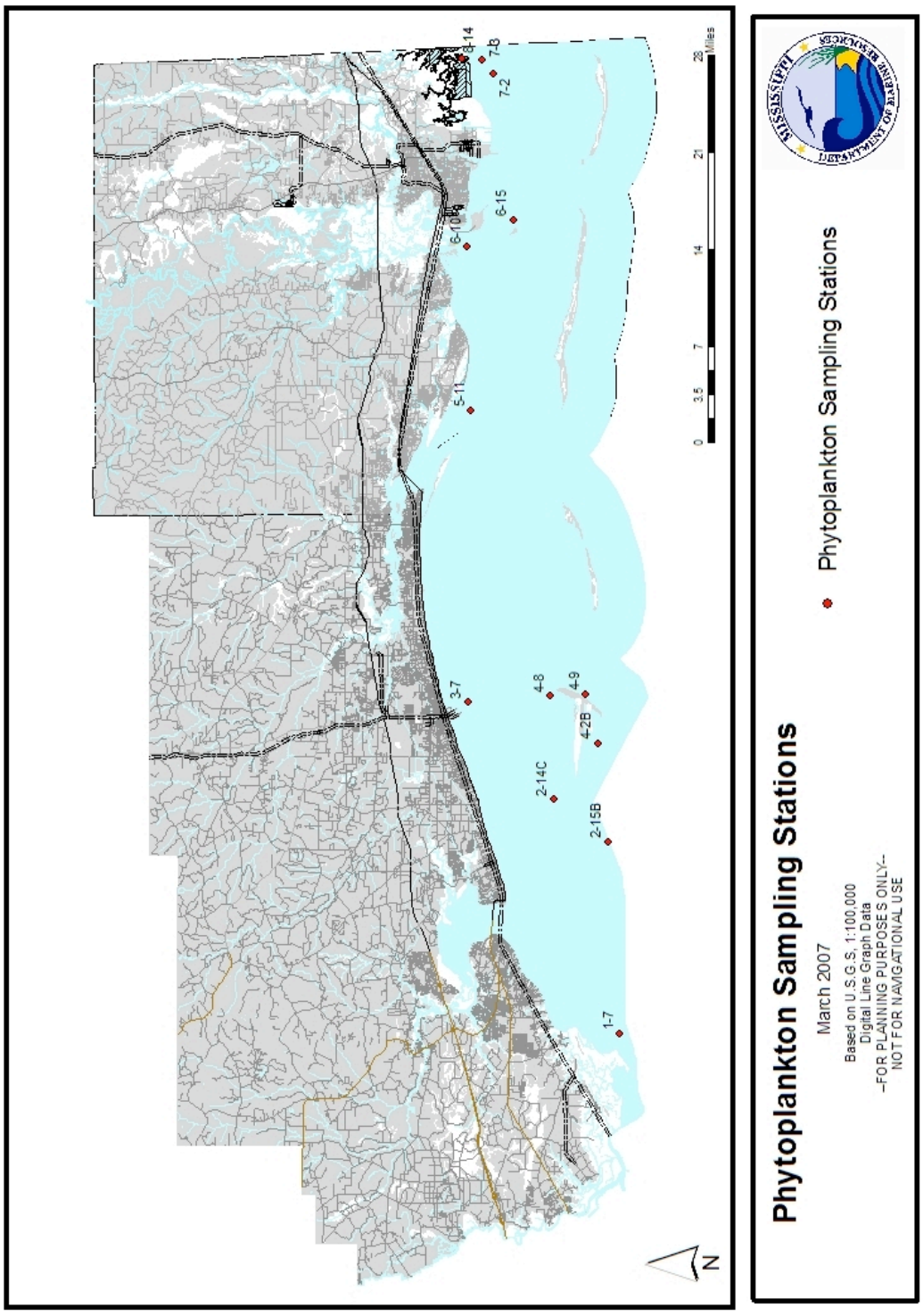

Figure 1. Phytoplankton Sampling Stations