ABSTRACTS OF ORAL PRESENTATIONS

GULF OF MEXICO HABS SESSION

THE EFFECT OF NITROGEN SOURCE ON THE GROWTH AND TOXICITY OF SPECIES OF THE GENUS *PROROCENTRUM*

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The environmental factors that select for growth of harmful algal species is not well known, but some studies suggest that utilization of organic nutrients may be a way that harmful algae can out compete faster growing species. This study investigated the effect of nitrogen source on the growth and toxin production of four potentially harmful dinoflagellates of the genus *Prorocentrum*: two planktonic species, *P. minimum and P.* cf. *scutellum*, one semi-planktoninc/epibenthic species, *P. mexicanum* and one benthic species, *P. hoffimannianum*.

Experiments were carried out in triplicate batch cultures in modified L1-Si media (Guillard & Hargraves 1993) using five different sources of nitrogen (N) at an initial concentration of 50 µM. Nitrogen was delivered as: (1) nitrate, (2) ammonium, (3) urea, (4) l-glutamic acid, and (5) high molecular weight dissolved organic nitrogen (DON, concentrated from Mobile Bay waters using tangential flow ultra-filtration). Growth rates were monitored as cell numbers and *in vivo* fluorescence over periods of 10–28 days, depending on the growth rates of the individual species.

Results indicate that none of these species were capable of growing on the natural DON as a sole source of nitrogen. Although there were slight variations in some replicates, all species achieved equal growth on nitrate, ammonium and urea; growth on l-glutamic acid was generally lower. All species were also tested for okadaic acid (OA) production. *Prorocentrum hoffmannianum* was the only species to show significant OA production. OA production in this species was found to vary with nitrogen source and with growth stage/rate. Preliminary results (to be completed by the Symposium) suggest that OA production by *P. hoffnannianum* is greater in late log and stationary phase when grown on inorganic nutrients, and greater in log phase when grown on organic nutrients as the sole N source.

GYMNODINIUM BREVE RED TIDES IN THE GULF OF MEXICO: ANALYSIS OF BREVETOXINS AND METABOLITES IN SHELLFISH

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Red tides of the marine dinoflagellate *Gymnodinium breve* occur regularly in the lower latitudes of the Gulf of Mexico (southwest Florida and south Texas) and infrequently in northern Gulf waters. *G. breve* produces lipophilic neurotoxins (brevetoxins) which are responsible for marine animal mortalities, non-fatal human health effects, and localized economic recession. Human health effects include respiratory distress from exposure to seawater aerosols containing brevetoxins, and food poisoning (neurotoxic shellfish poisoning, NSP) from consumption of brevetoxin-contaminated shellfish. Analyses of shellfish from red tide events and from experimental exposures show that brevetoxins are readily accumulated and metabolized by shellfish. The metabolites identifed by liquid chromatography / mass spectrometry (LC/MS) include diastereomers of cysteine-conjugated brevetoxin-3, their oxidation products and a thio-glycerol adduct. These molluscan metabolites of brevetoxins retain toxicity to mammalian test systems and are slowly eliminated from shellfish tissues in situ. The only officially recognized method for NSP shellfish analysis, mouse bioassay, underestimates the toxicity of brevetoxin-contaminated shellfish because the toxin metabolites are not recovered using the official protocol. More efficient extraction techniques and in vitro or instrumental methods (e.g. cytotoxicity, sodium channel competitive binding, radioimmunoassay, LC/MS) show ppb level sensitivity for the brevetoxins and their metabolites. These methods are currently used for a more complete characterization of brevetoxin metabolism in shellfish, and will be further refined for application in NSP HAB events.

PSEUDO-NITZSCHIA SPP IN THE NORTHERN GULF OF MEXICO: OVERVIEW AND RESPONSE TO INCREASING EUTROPHICATION

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Pseudo-nitzschia spp. are extremely abundant on the Louisiana shelf in the extended plume of the Mississippi River. Abundances often exceed 10^6 cells/liter and were sometimes greater than10^7 cells/liter, especially in the spring when river flow is high. In fact, it is frequently the most abundant diatom. *Pseudo-nitzschia* spp. do not occur as often or at such high abundances in lower salinity estuaries, but peak abundances can still exceed 10^6 cells/liter. *Pseudo-nitzschia* spp. have been observed at salinities from 0.5-36 ppt and growth can be sustained in cultures of some clones down to 6 ppt.

At least 6 species have been observed in this region, including *P. pseudodelicatissima*, *P. delicatissima*, *P. multiseries*, *P. pungens* (2 varieties), *P. cf. americana*, and *P subfraudulenta*, and there may be one or more unknown species. Five of the species observed in Louisiana waters have been shown to produce domoic acid (DA) in field samples or laboratory cultures elsewhere. Clones of *P. pseudodelicatissima,* but not clones of *P. delicatissima,* isolated from the Louisiana shelf, produce DA. In this area DA has been measured in plankton samples from the field, including samples taken directly over oyster beds. Despite the presence of abundant and toxic *Pseudo-nitzschia*, there have been no known incidents of Domoic Acid Poisoning (DAP) reported from the northern Gulf of Mexico.

Nonetheless, several lines of evidence provide strong proof that *Pseudo-nitzschia* spp. are stimulated by high nutrient inputs, thereby increasing the potential for DAP incidents in areas where eutrophication is increasing.

1. *Pseudo-nitzschia* spp. are more abundant in the spring when Mississippi River flow and nutrient inputs are highest and in the areas directly influenced by the Mississippi River. Univariate and multivariate statistical analyses of species, nutrient availability, and other environmental conditions show that individual *Pseudo-nitzschia* species are stimulated by different conditions.
2. There has been a large documented increase in nutrient inputs from the Mississippi River since the 1950's. Historical phytoplankton data (1950's, 1970's, 1990's) show a large increase in *Pseudo-nitzschia* abundance over that time. Further, the abundance of *Pseudo-nitzschia* in sediment cores taken from the Louisiana shelf has increased up core even more rapidly than other indicators of eutrophication.
3. Nutrient additions were made to microcosms (N, P, or Si and all combinations) of natural populations taken from the shelf at different seasons. In those microcosms where a response was observed, *Pseudo-nitzschia* spp. were either the only species to respond by rapid growth within 12-24 hours of nutrient addition (2 out of five microcosm experiments) or one of several species responding (2 out of five microcosms). The nutrient or nutrient combination stimulating growth was highly variable, including P alone (1 microcosm), N, P, and Si in combination (2 microcosms), or primarily N with P secondary (1 microcosm).

VARIABLE BREVETOXIN PRODUCTION IN *GYMNODINIUM BREVE* ATTRIBUTABLE TO GROWTH CONDITIONS AND STRAIN DIFFERENCES

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Blooms of the dinoflagellate *Gymnodinium breve* can produce sufficient concentrations of brevetoxin to negatively impact coastal ecosystems, human health, and local economies. Assessment of risks associated with *G. breve* blooms and subsequent brevetoxin exposure requires better understanding of factors governing bloom development and toxin production. Although the complete biosynthetic pathway for brevetoxin has not been elucidated, reports in the literature suggest some degree of geographic or strain-specific variability in total cellular brevetoxin concentration, ranging from 7 to 17 pg $cell^{-1}$ (Baden and Tomas, 1988; Hua et al., 1996).

During Fall 1999, a *G. breve* bloom impacted a 150 mile stretch of the Florida panhandle shoreline. In samples collected from16 locations on 3 fall sampling dates, *G. breve* abundance ranged from 0.1 - 20 x 10^6 L^{-1}. Using HPLC methods, we measured total brevetoxin (PbTx 1, 2, 3, 6) concentrations that greatly exceeded those reported in the literature. In shelf waters off Pensacola and Navarre beaches and in estuarine waters of Cinco Bayou and Santa Rosa Sound, total brevetoxin ranged from 47 to 67 pg $cell^{-1}$ (n=5), 59 to 126 pg $cell^{-1}$ (n=3), and 12 to 63 pg $cell^{-1}$ (n=8), respectively.

Brevetoxin production and accumulation in response to nitrogen, phosphorus, and light availability were examined in laboratory cultures using three *G breve* strains; the Piney Island (courtesy of FMRI) and Charlotte Harbor (courtesy of NOAA) strains isolated from the central west Florida coast, and the Pensacola Beach strain (EPAJR1) isolated from the Fall 1999 panhandle bloom. When grown under standard conditions with f/2 nutrient concentrations, total brevetoxin concentrations in Piney Island and Charlotte Harbor strains ranged from 10 to 20 pg $cell^{-1}$, whereas the Pensacola Beach strain produced about 30 pg $cell^{-1}$ five months following isolation. However, by reducing nitrate (<10 μM) or phosphate (<0.6 μM), by substituting urea (<5 μM), or by lowering irradiance (30 μmol quanta m^{-2} s^{-1}), total brevetoxin content increased to >100 pg $cell^{-1}$ in the Piney Island and Charlotte Harbor strains.

These results suggest that physiological responses to growth limiting conditions may enhance brevetoxin production, and that there may be genetic differences between strains that result in variable toxin production. Understanding and modeling stressor-response relationships, such as the effects of environmental conditions on growth and toxin production, will be important to predicting bloom toxin concentrations, toxicity, and ecological effects.

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ALGICIDAL BACTERIA ACTIVE AGAINST *GYMNODINIUM BREVE*: USE OF MOLECULAR TECHNIQUES TO ASSESS CHANGES IN MICROBIAL COMMUNITIES FOLLOWING THE INTRODUCTION OF BACTERIA

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A growing number of studies have suggested that algicidal bacteria may play a role in naturally regulating the development and termination of harmful algal blooms (HABs). Interest in such microbes has been further enhanced by their potential use as part of a HAB management strategy. Our laboratory has isolated two bacterial strains from the west Florida shelf that are lethal to *Gymnodinium breve*, a bloom-forming, toxic dinoflagellate responsible for severe economic losses in this region through its impacts on fisheries and tourism.

Phylogenetic analysis of the entire 16S rDNA sequence for both algicidal bacteria indicates that one strain (41-DBG2) is most closely related to *Cytophaga latercula* (92% similar) within the Cytophaga-Flavobacterium-Bacteroides (CFB) phylum, while the other strain (ANSW2-2) is most closely related to *Alteromonas macleodii* (99% similar) of the γ-Proteobacteria. We have analyzed available sequences for algicidal bacteria isolated from different locations and found that most cluster together within these two taxa. Our results indicate that algicidal bacteria from each of these two phylogenetic groups may be descendents of an ancestor that could have evolved the means to exploit algal-derived organic matter.

Fluorescent in-situ hybridization (FISH) with strain-specific oligonucleotide probes is currently being used to enumerate algicidal bacteria in laboratory time-course experiments, as well as for their detection in field populations. In addition, denaturing gradient gel electrophoresis (DGGE) is being used to assess changes in microbial communities associated with *G. breve* following the introduction of algicidal bacteria to algal cultures. Use of these two complementary techniques have indicated the following in experiments employing algicidal bacterium strain 41-DBG2:

1. Following inoculation into bacteria-containing *G. breve* cultures at 10^3 cells/mL, 41-DBG2 reaches densities of over 10^6 cells/mL within 2-3 days, after which its growth rate declines markedly. Little decrease in numbers occurs within 10 days of inoculation, suggesting that conditions remain favorable for the subsistence of 41-DBG2 over the short-term.
2. Total bacterial numbers increase as *G. breve* cells die and lyse, due likely to the release of organic nutrients from the algal cells into the medium.
3. The microbial assemblage associated with *G. breve* changes following the addition of 41-DBG2, with some phylotypes disappearing and others appearing throughout the killing event.
4. Algicidal activity of introduced bacteria seems to vary according to the target alga's physiological status, with resistance to attack decreasing with declining algal growth rate.

Our data suggest that algicidal bacterium 41-DBG2 may be able to fill a vacant niche (or displace another organism from its niche) within a *G. breve* culture, quickly reaching a high density and eventually causing death of the culture. Use of the above molecular approaches will aid in determining whether a similar process occurs over the course of a natural *G. breve* bloom.

GYMNODINIUM BREVE IN THE WESTERN GULF OF MEXICO: TWO YEARS OF COASTAL SAMPLING OFF THE TEXAS COAST

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We established a sampling program in Texas coastal waters to examine near shore waters (<15 km) for the presence of *G. breve* and related species. The first two years of sampling noted *G. breve* from the waters inside 15 km in <10% of the samples. During this period, significant red tide events occurred along the Texas coast near Brownsville and the Louisiana border. In all cases, the routine sampling (twice per month) did not provide advance warning of the bloom. The first event in Oct. 1999 off of Brownsville, TX may have been related to introdution of offshore populations (>15 km) during strong northeasterly winds. This bloom advected to the south over a two week period. An offshore transect that found nutrient-rich water advecting onto the shelf in association with an anticyclonic feature. This mesoscale feature resembled Loop Current intrusions and could have provided the offshore source waters for the bloom. There was no surface manifestation of this intrusion on the shelf.

A July, 2000 bloom moved north up the coast from Brownsville, TX and may have been transported in a coastal upwelling plume that extended from Tamulipas, Mexico to near Corpus Christi, Texas. However, this upwelling event was associated only with temperature reduction at the surface (T>26 ° C) and had no apparent nutrient enrichment (Fig. 1). A subsequent red tide off Sabine, Texas occurred near the Louisiana border (Aug-Sept. 2000). This bloom was primarily offshore and impacted the coastal zone only for a brief period in late August south of Galveston. A brief analysis of the historical circulation data suggests the seasonal migration of the coastal current convergence could be responsible for maintaining this bloom in a gyre like feature in the northern shelf. The historical record of *G. breve* blooms notes similar complex dynamics and suggests the seasonal coastal current patterns may play a fundamental role on determining whether blooms propagate onshore and in which direction they are transported.

Initial culture studies on G. *breve* isolated in Oct. 99 showed a lower salinity tolerance between 23 and 25 PSU with a maximum growth rate of 0.3 –0.4 div d^{-1}. Ongoing experiments are evaluating the growth response over a wider range of light, salinity and temperature conditions.

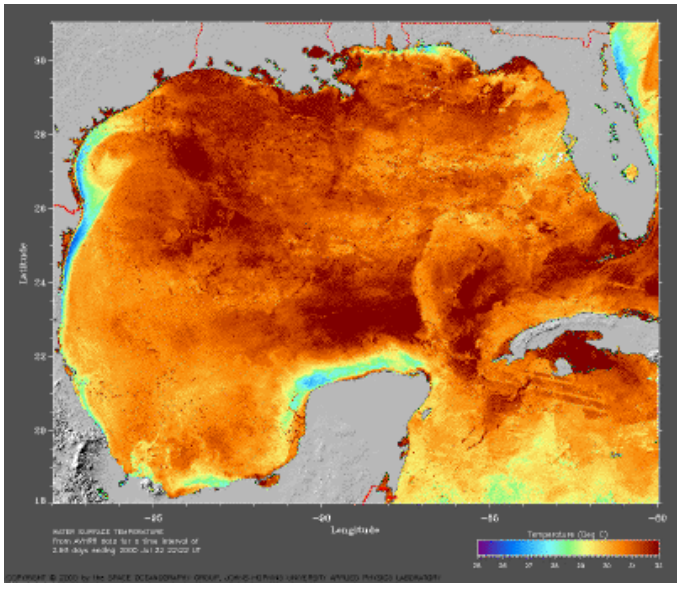

Fig. 1. Sea surface temperature image showing coastal upwelling along northern Mexico and

ABSTRACTS OF POSTERS

GULF OF MEXICO HABS SESSION

EFFECTS OF CLAY FLOCCULATION ON BREVETOXIN IN SEAWATER

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The Florida red tide has been a recurrent and serious problem along the West Coast of Florida. It is caused by the dinoflagellate, *Gymnodinium breve*, a single celled alga that can grow to extremely high cell densities sufficient to discolor the water. *G. breve* produces an array of highly potent neurotoxins commonly called brevetoxins. Episodes of red tide cause shellfish toxicity, mortalities of fish and other marine organisms, as well as respiratory irritation in humans and marine mammals. Studies at Woods Hole Oceanographic Institution (WHOI) have shown clay to be a promising approach to control red tide by the use of clay as a flocculent to aggregate cells and other particles into a floc that settles to the bottom. Poly-aluminum chloride (PAC) is a compound commonly added with clay to facilitate the production of a floc. Studies were conducted at Mote Marine Laboratory to determine the effects of clay flocculation on brevetoxin in seawater. Two concentrations of clay were used with and without the addition of PAC. Two concentrations of *G. breve* were also used, 5 and 10 million cells per Liter. These red tide levels represent naturally occurring blooms. The higher loading of clay tested represented the amount used in the field pilot study (500 g/M^2) conducted in cooperation with WHOI in March 1999. The lower loading reflected the amount of IMCP clay currently being used in studies conducted at WHOI (0.25 mg/L).

These experiments were conducted in 4-L beakers with 3-L of 5×10^6 cells/L or 10×10^6 cells/L of diluted *G. breve* culture. The clay was added to each beaker as a wet slurry at the rate of 10g (high level; dry weight) or 0.75 g (low level; dry weight) IMCP clay in 100ml salt water. Each treatment was conducted in triplicate. For the experiments using PAC, it was added immediately after the addition of the clay in 10 ml of deionized water. After settling for 2.5 hours, a 10 ml sub-sample was collected for post flocculation cell counts and the rest of the samples was processed as described below for brevetoxin analysis.

The clarified culture above the settled clay (ca 2.8-L) was collected by siphon. The settled clay was then transferred to a 500 ml beaker. The *G breve* controls (no clay) were processed by extraction of the whole 3-L. The culture was extracted by using a C-18 disc (Ansys Diagnostics) and the toxin was eluted from the disc with methanol.

After allowing the clay to settle for 24 hours, the clay was transferred to centrifuge tubes and dewatered by centrifugation at 2800 rpms for 4 minutes. The clay pellet was extracted with an ultrasonic probe in acetone. After sonication the clay was centrifuged and the acetone collected. The acetone was evaporated to dryness and transferred to a vial in 3 ml of methanol for HPLC analysis.

Seven different treatments are presented. Clay, with and without PAC, at a high (Hi) and Low (Lo) loading as well as 5 and 10 million cells per liter cultures were tested. The treatments were as follows: 1) High clay loading with *G. breve* at 5 mc/L, 2) Same as 1 with the addition of PAC, 3) Low clay loading with *G. breve* at 5mc/L, 4) Same as 3 with the addition of PAC, 5) Low clay loading with lysed *G. breve* cells at 5mc/L, 6) Low clay loading with *G. breve* at 10 mc/L, and 7) Same as 6 with PAC. Figures 1 and 2 show cultures immediately after the addition of clay, and two and a half hours later, respectively.

For all treatments of live *G. breve* cells, the percent reduction of brevetoxin was greater than 94%. When used with lysed cells, the clay was only able to remove 80 % of the toxin from the lysed culture. In the absence of cells, this represents a situation where the clay is binding extracellular toxin, suggesting that

both the physical flocculation of cells as well as the binding of toxin occurs during the clay settling process.

Low loading of clay worked as well, if not better, than high loading. The addition of PAC was not significant. These data show that clay is a highly effective method for removal of brevetoxin from culture in the laboratory. Future studies will involve natural blooms in enclosed mesocosms.

PHYSIOLOGY AND CELL CYCLE PROGRESSION OF *GYMNODINIUM BREVE* UNDER LIGHT AND NITRATE LIMITATION

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Reports of blooms of *Gymnodinium breve*, a brevetoxin-producing dinoflagellate, date back centuries, yet information on the physiology of this organism is lacking in the published literature. The primary objective of this study is to describe the physiological properties of laboratory cultures of *G. breve* in response to conditions of light and nitrate limitation. Additionally, the intraspecific variability in these physiological responses is addressed using several geographic isolates of this species.

Laboratory cultures of *G. breve* (Pensacola, Piney Island and Charlotte Harbor isolates) are grown over a range of irradiances from 16 - 360 _E m^{-2} s^{-1} and two nitrate concentrations, ~5 and ~50M. Measured physiological parameters include population growth rates, photosynthesis-irradiance (P-I) curves, cellular carbon and nitrogen, and photopigment content and composition.

We are also investigating the regulation of cell cycle progression by growth-limiting factors in cultures of *G. breve* and its relationship to toxin production. Olson and Chisholm (1986) have demonstrated that both nitrogen and light limitation resulted in an extension of the G1 phase of the cell cycle in the dinoflagellate *Amphidinium carteri*. Toxin production in two other toxic dinoflagellates has been shown to be coupled directly to specific phases of the cell cycle (Taroncher-Oldenburg et al 1997; Pan et al 1999). However, to our knowledge, the relationship between limiting conditions, cell cycle progression, and toxin production has not been previously addressed for any brevetoxin-producing microalgae.

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BIOMARKER LIPIDS IN RED TIDE (*GYMNODINIUM BREVE*) BLOOMS ALONG THE NORTHWEST FLORIDA COAST

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The ability to characterize phytoplankton communities and algal blooms using lipids as biomarkers requires knowledge of their distribution and taxonomic significance. Such an approach would have application, for example, in distinguishing and tracking certain dinoflagellates such as the toxic species *Gymnodinium breve*, which is responsible for red tide events in the Gulf of Mexico. To explore this possibility, the lipids of over forty laboratory-cultured dinoflagellates, including three different isolates of *G. breve*, and a number of representatives of other eukaryotic algal classes such as the Bacillariophyceae, Haptophyceae, and Raphidophyceae, have been examined for the presence of chemotaxonomically useful fatty acids and sterols. A dense bloom (over 20 million cells/L) of *G. breve* in the fall of 1999 in the near shore waters of the Gulf of Mexico from Destin to Pensacola in northwest Florida provided an opportunity to compare the lipids of the field-collected samples with those found in laboratory cultures.

Extracted lipids were fractionated into different classes (neutral, glyco-, and phospho-lipids) prior to conversion to gas chromatography/mass spectrometry (GC/MS)-amenable fatty acid methyl esters (FAMEs) and sterol-trimethylsilyl (TMS)/acetate derivatives. The bloom of *G. breve* was found to contain two principal 4-methyl sterols, (24*S*)-4α-methylergosta-8(14),22-dien-3β-ol (ED) and its 27-*nor* derivative (NED), recently described by Faraldos and Giner (1998). The bloom sample was also found to contain the highly unsaturated long chain fatty acid, octacosaoctaenoic acid (28:8), recently discovered in dinoflagellates by Mansour et al. (1999).

Characterization of free and esterified sterols from laboratory cultures of *G. breve* has confirmed the predominance of these two sterols. ED and NED were shown also to be the primary sterols of the closely related dinoflagellates, *Gymnodinium mikimotoi* and *Gymnodinium galatheanum.* The wider distribution of this sterol pattern is consistent with the known close relationship between *G. breve* and *G. mikimotoi* (Haywood et al. 1996, Leblond et al. 2000). However, these sterols were also found as components of more complex sterol profiles in other members of the *Gymnodinium/Peridinium/Prorocentrum* (GPP) taxonomic group, thus limiting their biomarker potential.

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THE PHYLOGENETIC RELATIONSHIP OF THE RED TIDE DINOFLAGELLATE *GYMNODINIUM BREVE* TO OTHER MEMBERS OF THE GENERA *GYMNODINIUM* AND *GYRODINIUM*.

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Phylogenetic relationships between the red-tide dinoflagellate *Gymnodinium breve* and other members of the genera *Gymnodinium* and *Gyrodinium* have not been studied at the molecular level. *G. breve* is most noted for its production of brevetoxin, which has been linked to extensive fish kills, marine mammal mortalities, neurotoxic shellfish poisoning, and respiratory irritation caused by aerosolized toxin (Steidinger et al. 1997). The phylogeny of four isolates of *G. breve* and of seventeen other members of the genera *Gymnodinium* and *Gyrodinium* was determined by comparison of small subunit (SSU) rRNA genes. The sequences of the *G. breve* isolates differed from each other by less than 0.3%. In addition, sequences were obtained from twenty-three other members of the class Dinophyceae, including several members of the *Gymnodinium/Peridinium/Prorocentrum* (GPP) complex, and *Coolia* and *Fragilidium* species isolated in this laboratory. The rRNA sequences of the *G. breve* isolates were aligned with these and other previously published full-length dinoflagellate sequences. Phylogenetic analyses indicate that *G. breve* is closely related to the morphologically similar dinoflagellates *Gymnodinium mikimotoi* and *Gymnodinium galatheanum*. The phylogenetic positions of *G. breve*, *G. mikimotoi*, and *G. galatheanum* are further supported by data that show these three species possess sterols unlike other dinoflagellates (Leblond and Chapman, 2000).

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EFFECTS OF CLAY FLOCCULATION OF THE FLORIDA RED TIDE DINOFLAGELLATE (*GYMNODINIUM BREVE*) ON BENTHIC ORGANISMS

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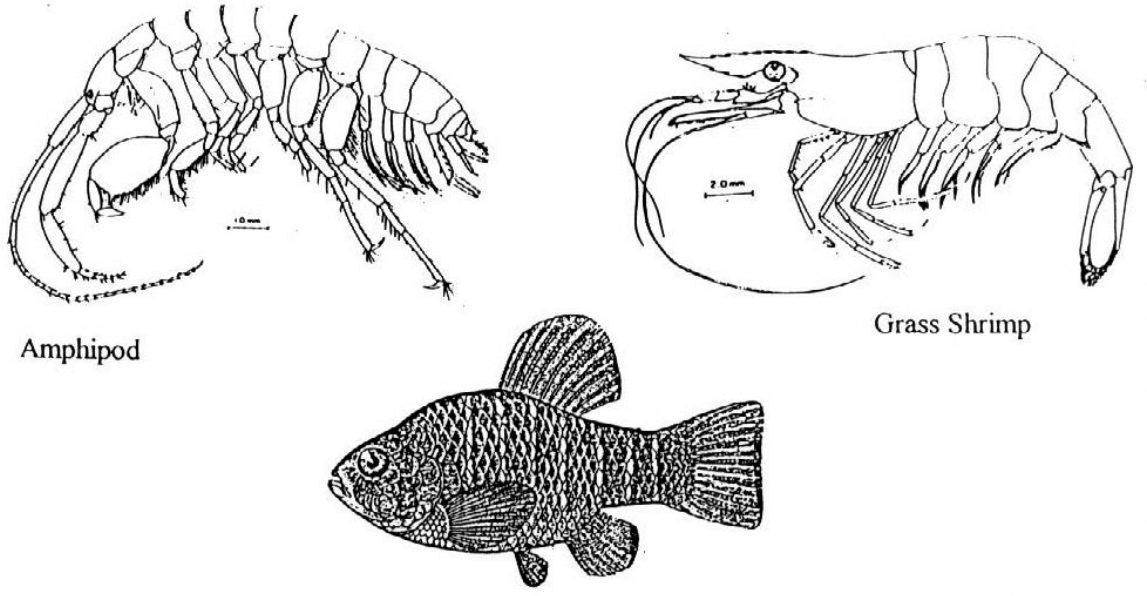
Evaluating the feasibility of controlling red tide using clay flocculation is part of an ECOHAB- funded project. One aspect for the feasibility and future application of clays is the determination of potential negative environmental impacts. The removal of toxin-containing dinoflagellates from the water column may result in acute or chronic toxicity to benthic organisms. Using EPA-approved standard toxicity test methods, we designed a study to determine the acute and chronic toxicities of settled *G. breve* cells, clay and coagulant to several species of marine life (see below). The laboratory-conducted solid phase and pore water bioassays were of 4 to 28 days duration. The test species included *Cyprinodon variegatus* (sheepshead minnow), *Palaemonetes pugio* (grass shrimp), *Leptocheirus plumulosus* (amphipod), and *Ampelisca abdita* (amphipod). These species were exposed to the clay, coagulant and *G. breve* alone and in binary and ternary combinations at three treatment levels. Effects on organism survival and reproduction were determined and reported as LC50 values, no effect concentrations, and the lowest effect concentration. In addition to the laboratory bioassays, we will determine benthic quality below a natural red tide event by analyzing sediment toxicity, chemical quality and benthic community composition. The results of the acute and chronic toxicity evaluations will be discussed in this presentation as well as their relevance to the *in-situ* remediation of red tide events.

TEST SPECIES



Amphipod

Grass Shrimp

Sheepshead Minnow

MOLECULAR CHARACTERIZATION OF *GYMNODINIUM BREVE* STRAINS FROM THE TEXAS SHORE (GULF OF MEXICO) USING RIBOSOMAL DNA INTERNAL TRANSCRIBED SPACERS AND 18S REGIONS

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The toxic dinoflagellate *Gymnodinium breve* is known to be a major species involved in bloom events in the Gulf of Mexico (Steidinger & Penta, 1999). It produces potent toxins that may result in human illness or death due to shellfish poisoning (Saunders et al., 1997). The occurrence of bloom events has significantly increased along the Texas shore during the last decade (T. Villareal, pers. comm.) but their origin remains poorly understood. For these reasons, the characterization of *G. breve* populations from Texas contributes to a better knowledge of the major species involved in bloom events in this region.
Three strains of *G. breve* were collected from the South Texas coast during the 1999 red tide event and the 18S ribosomal RNA gene (rDNA) and internal transcribed spacer (ITS) regions have been targeted to identify these isolates at the intra-specific level. The sequences we obtained for the ITS and 18s regions were compared with known sequences of *G. breve* strains isolated from the Florida coast.

The internal transcribed spacers and the 18S rDNA regions were amplified by polymerase chain reaction (PCR) and sequenced. Aligned nucleotide sequences of the 3 isolates of *G. breve* including the complete ITS and the 18S rDNA region showed that the size of both regions were conserved in the 3 isolates and were 600 and 1700 bp respectively. The 18S and ITS nucleotide sequences obtained for the 3 strains of *G. breve* from the Texas coast were aligned. A very high homogeneity was observed between the 3 isolates and for both genes. Similarly, the consensus sequences obtained for the 18S and ITS regions were aligned with the corresponding regions for a *G. breve* strain isolated from the Florida coast. The aligned sequences showed homogeneity of both regions among these different isolates from the Gulf of Mexico.
The present study based on the ITS and 18S regions has shown a high homogeneity between different strains of *G. breve* isolated from the Gulf of Mexico. The usefulness of both ITS and 18S regions as genetic markers for *G. breve* will be discussed.

BREVETOXIN ANALYSIS IN SEAWATER, MAMMALIAN BODY-FLUID AND SHELLFISH HOMOGENATE BY COMPETITIVE ELISA

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A competitive Enzyme Linked Immuno-Sorbent Assay (competitive ELISA) was developed for analyzing brevetoxin (PbTxs). This assay is based on the activity of goat anti-brevetoxin antibodies obtained following immunization with KLH-PbTxs conjugate. The antibodies were used in combination with a multi-step signal amplification procedure for the detection of toxin. This procedure minimizes non-specific signal and background usually observed in complex matrices. Therefore, analysis can be performed in seawater, mammalian body fluid and shellfish homogenate without any extraction and/or purification steps. PbTxs analysis in liquid samples like seawater, urine and serum are performed without pretreatment, dilution or purification. The limit of quantification of PbTxs is $5x10^{-9}$ M in all the liquid samples. For shellfish monitoring, analysis are performed after homogenization of shellfish meat (5gr) with brevetoxin-ELISA buffer (200ml) and can be run on a single mollusk as well as on a pool of shellfish meat. Comparative quantification of PbTxs achieved in buffer, seawater, mammalian body fluid and shellfish homogenate spiked with the same amount of toxin (10ng/ml sample) varies no more than 5%. It is concluded that the matrix composition of the sample does not affect the performance of the assay. The limit of quantification of PbTxs was 10 micrograms by 100 grams of shellfish meat, that is 8 times more sensitive than the mouse bioassay. Because this assay is not affected by the matrix composition and can be performed in shellfish homogenate, it measures the real concentration of the brevetoxin present in the shellfish meat and not only the toxin fraction extracted by solvent in conventional assays. Therefore, this procedure can be used to prevent and diagnose human exposure to PbTxs and has the potential to replace the currently used mouse bioassay for monitoring PbTxs in shellfish.

HAB DISTRIBUTIONS IN ALABAMA COASTAL WATERS: 1998-1999

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In the late fall of 1996, a toxic bloom of *Gymnodinium breve* resulted in the closure of Alabama oyster reefs for the first time in history. As a result of this bloom, we initiated monthly monitoring of Mobile Bay and nearshore coastal water of the Gulf of Mexico to investigate the distributions of *G. breve* and other harmful algal species in the region. Our objectives were to monitor for a recurrence of *G. breve* and assess for the presence and distribution patterns of potentially harmful algal species.

During 21 months of sampling, there was no recurrence of *Gymnodinium breve* in Alabama coastal waters (Although, there was a *G. breve* bloom that moved across the Florida pan handle into the eastern most portion of Alabama coastal water during the fall/winter of 1999-2000, after our project had ended.). However, numerous potentially harmful algal species were observed. *Pseudonitzschia* sp., a potential domoic acid producing diatom, was observed year-round, over a wide range of salinities. Other potentially harmful dinoflagellates (e.g. *Ceratium* spp., *Prorocentrum* spp. and *Dinophysis caudata)* were also present in low numbers. We also observed high numbers of *Ceratium hircus* during the summer in waters of moderate salinity. A near unialgal bloom of *Prorocentrum minimum* reached concentrations of 2500 cells ml^{-1} in February 1999 and was associated with the Mobile Bay plume front.

The observed year-round presence of harmful algal species in Alabama coastal waters indicates a potential for harmful algal blooms to occur in this region.

HARMFUL ALGAL BLOOM OUTREACH

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St. Petersburg, FL 33701

The purpose of harmful algal bloom (HAB) outreach is to distribute consistently accurate information about red tides, especially those in progress, to the public. When citizens believe that a HAB threatens them, they want immediate information about what, where, when, and how. What occurred? Where and when did it occur? How are humans affected? How is the environment affected? What is being done to fix it? At Florida Marine Research Institute (FMRI), we have a multidisciplinary approach to disseminate accurate information: the Internet, printed material, and public presentations.

Our web page [http://www.fmri.usf.edu] has two target audiences -the general public and fellow scientists. The web page is divided into four subsections: Red Tide; Florida's Red Tide Status; Research Projects; and More Information. The basic format follows:

1) Red Tide
   a) What is Red Tide? What is a HAB?
   b) HAB Species
   c) Red Tide and Marine Animals
   d) Shellfish Poisoning
2) Florida's Red Tide Status
   a) Current status
   b) Historical Red Tide
3) Current Projects
   a) ECOHAB: Florida
   b) Estuarine Monitoring
4) More Information about Red Tides
   a) Photo Gallery
   b) HAB Species of the Month
   c) Links
   d) Select Publications and Glossary

The printed material that we produce includes color brochures, Frequently Asked Questions (FAQs), Fact Sheets (both general and technical), press releases, and a training manual (Steidinger and Penta 1999). The training manual was produced for resource and regulatory personnel investigating HABs. All printed information is freely given to the public. Most of it is available through our web site.

Presentations are done in person and on-line (via Power Point). We have presented at scientific conferences, high schools, college classes, and local business groups (e.g. charter boat captains, dive clubs, and anglers). We also give presentations to tour groups visiting FMRI (school groups, concerned citizens, and legislators). The Power Point presentations include slides and text and can be seen on the Internet or down loaded as a PDF file.

Steidinger, K.A. and H.L. Melton Penta. 1999. *Harmful Microalgae and Associated Public Health Risks in the Gulf of Mexico.* US EPA: Gulf of Mexico Program. EPA Grants #MX004729-95-0.