TRANSFER OF SAXITOXINS WITHIN THE INDIAN RIVER LAGOON, FLORIDA FOOD WEB

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Since January 2002, 23 cases of Puffer Fish Poisoning (PFP) were reported in four states due to saxitoxin (Quilliam et al., 2002) traced back to southern puffer fish (*Sphoeroides nephelus*) originating from the northern Indian River Lagoon (IRL) on Florida's east coast (Bodager 2002). Saxitoxin was previously unknown in Florida marine waters (Landsberg et al., 2002). Because puffer fish were involved in PFP we have now routinely screened > 400 southern, 40 checkered (*S. testudineus*), and 40 bandtail puffer fish (*S. dorsalis*) for saxitoxins (STXs) statewide using the Ridascreen® STX ELISA kits (Usleber et al., 1991). Since April 2002 selected biota from the IRL have also been tested for STX distribution and prevalence within the food web. The geographical hot spot for STXs in the IRL is in the north from Titusville south to Melbourne. Approximately 18.1% of samples (n = 791) were below the detection limit of 1 µg STXeq/100g tissue, 27.4% were below 80µg STXeq/100g tissue, while the majority of samples, 54.5% contained moderate to high levels of STX in a range of tissues. Except for southern puffer fish, the muscle, skin, and mucus of which contain up to 5865.5 µg STXeq/100g tissue, STXs are present but below regulatory levels in the muscle of checkered puffer fish, Atlantic spadefish (*Chaetodipterus faber*), striped burrfish (*Chilomycterus schoepfi*), and porcupine fish (*Diodon hystrix*). Recreationally prized fish such as sheepshead (*Archosargus probatocephalus*), Gulf flounder (*Paralichthys albigutta*), southern kingfish (*Menticirrhus americanus*) and spotted sea trout (*Cynoscion nebulosus*), contained up to 35.9 µg STXeq/100g tissue in the skin and mucus. Commercially significant species such as blue crabs (*Callinectus sapidus*) had a maximum of 11.1 µg STXeq/100g tissue in the hepatopancreas and whole hard clams (*Mercenaria* sp.) a maximum of 17.2 µg STXeq/100g tissue. A maximum of 116.5 µg STXeq/100g tissue was found in polychaetes (*Glycera dibranchiata*), 14.2 µg STXeq/100g tissue in gastropods (*Urosalpinx cinerea)*, 1.1 µg STXeq/100g tissue in brittle stars (*Ophiothrix spiculata*), and up to 4301.2µg STXeq/100g tissue in small non-harvestable whole razor clams (*Ensis minor*). With the exception of puffer fish, concentrations do not presently pose significant threats to public health but they indicate the significant transfer of STXs within the IRL food web. Both natural bloom samples and clonal isolates of *Pyrodinium bahamense* from the IRL have tested positive for STXs (Landsberg et al., 2002). On Florida's west coast, where *P. bahamense* blooms are less frequent and likely reach less toxic biomass, STX concentrations are markedly lower in biota than those in the IRL. By comparison, STX concentrations in southern puffer tissues from Florida's west coast were no higher than 2735.5 µg STXeq/100g tissue in the skin and mucus when compared with up to 10111.8 µg STXeq/100g tissue in the gut contents of southern puffers from the IRL. In addition, STX levels in west coast sheepshead and flounder are normally below our detectable limit. We still have to confirm the major transfer route of STXs from *P. bahamense*, the most likely source of STX, into puffer fish. Small benthic filter-feeding bivalves, a significant component of the southern puffer fish diet, are likely vectors for STX transfer and will be tested for toxicity in the upcoming months. Comparison of analytical methods is also required.

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EFFECTS OF INHALED FLORIDA RED TIDE BREVETOXINS: AN INTERDISCIPLINARY STUDY IN OCEANS AND HUMAN HEALTH

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Florida red tides occur in the Gulf of Mexico and result from blooms of the marine dinoflagellate *Karenia brevis*. *K. brevis* produces highly potent polyether toxins known as brevetoxins that activate voltage sensitive sodium channels. The brevetoxins are associated with massive fish kills, marine mammal poisoning and human health problems. The severity of human health effects varies annually and temporally in coastal regions. Explanations for the variable toxicity include meteorological, biochemical and strain toxicity differences. Nine natural toxins have been identified and their structures are based on two different polyether backbones, brevetoxin-a (eg. PbTx-1) and brevetoxin-b (eg. PbTx-2). A number of smaller polyether compounds, the first of which is known as brevenal, also have been identified. The present study sought to examine the complex relationship between brevetoxin and brevenal concentration in coastal ocean water as measured by liquid chromatography-coupled mass spectrometry, the concentration and composition of toxin which becomes airborne as measured on particle filters, and the human health effects in occupationally-exposed individuals and recreational beachgoers. Six significant results have been achieved recently: (1) the relative concentrations of brevetoxin and brevenal vary over 10-fold in water and air depending on bloom stage. This is offered as one additional explanation for the low potency in blooms with high toxin concentrations, or for the high potency demonstrated in blooms with lower toxin concentrations. The non-toxic but competitive polyether antagonist brevenal reduces lethality in fishes at nM concentrations, prevents or subdues the bronchoconstrictor activity caused by inhaled brevetoxin at fM concentrations, and prevents the binding of toxin to site 5 on sodium channels at nM-□M concentrations; (2) the intratracheal LD_{50} for brevetoxin PbTx-3 in animals is 10 □g/kg, fully 25-fold more potent than previously demonstrated. The half-maximal bronchoconstriction concentration in air is 1 picogram/liter; (3) the particle size distribution of brevetoxin particles in air (a mix of salt and toxin) is 10 microns, indicating it will deposit primarily in the upper airways. A few nanograms of toxin per cubic meter of air is sufficient to elicit symptoms in people on the beach; (4) therapeutically, the effects of brevetoxin-induced bronchoconstriction can be relieved by histamine H1 antagonist diphenhydramine, or naphthoyl-brevetoxin derivatives; (5) brevetoxin exposure results in altered immune response by inflammatory cells (neutrophils) and also may cause decreased viability of macrophages in exposed individuals. This, when coupled to a decreased tracheal mucous velocity caused by toxin, can lead to increased contact time of toxin with pulmonary tissue as well as increase the likelihood of secondary pulmonary infections; and, (6) in collaboration with the CDC and FL Dept of Health, investigators have begun to evaluate potential biomarkers for assessing exposure. The concentration of toxin in air that causes human respiratory discomfort is orders of magnitude lower than the analytical capability of current measuring devices.

LINKAGES BETWEEN BIOCHEMICAL FLUXES AND MIGRATION BEHAVIOR IN POPULATIONS OF THE RED TIDE DINOFLAGELLATE, *Karenia brevis*

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Prior laboratory studies and modeling have explored the possibility that the migratory behavior of the red tide dinoflagellate, *Karenia brevis*, is influenced by cellular biochemical fluxes. To determine whether there were systematic differences in biochemical fluxes in different stages of migration in natural populations, we measured the incorporation of photosynthetically fixed inorganic ^{14}C into major subcellular end products of *K. brevis* during a bloom event in Florida coastal waters in 2001. Samples were incubated in simulated *in situ* conditions on board ship, and determinations were made of ^{14}C-incorporation into low molecular weight materials (LMW), lipid, carbohydrate+nucleic acids, and protein. Measurements were also made of incorporation of ^{14}C into the nitrogen transport amino acids, glutamine and glutamate. Carbon flux showed systematically higher proportions in carbohydrate+nucleic acids and lower proportions in protein in surface samples compared to that in deep samples. Nutrient enriched samples exhibited enhanced protein incorporation in both surface and deep populations and decreased incorporation into carbohydrate +nucleic acids. Therefore the ratio of protein/ carbohydrate+nucleic acids appeared to provide an index of nutrient status. The ratio of carbon flux into glutamine relative to glutamate in nutrient enriched samples increased as much as 3.8-7.4 times. The enhanced carbon flux into glutamine in response to nutrient addition is consistent with a GS-GOGAT pathway of amino acid assimilation. Our results suggest that the patterns of biochemical fluxes differ among different migrating subpopulations, but that the patterns are sensitive to nutrient status.

MICROBIAL INTERACTIONS ASSOCIATED WITH THE TOXIC DINOFLAGELLATE *Karenia brevis*

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Harmful blooms of the toxic dinoflagellate, *Karenia brevis*, occur annually in the Gulf of Mexico along the west Florida shelf. Recent investigations suggest that algicidal bacteria may play a key role in regulating *K. brevis* blooms. Our laboratory has isolated three bacterial strains algicidal to *K. brevis*, two of which have been identified and characterized previously (Doucette et al., 1999). A third strain (S03) is currently being described and appears to require direct contact with algal target cells in order to induce killing. Efforts using both classical and molecular techniques are also directed at assessing bacterial composition and succession within samples of the natural microbial assemblage collected during a 2001 *K. brevis* bloom, with an emphasis on algicidal taxa. Our research has shown that all three algicidal bacteria are able to kill *K. brevis* isolate C2, yet other *K. brevis* isolates, also originating from west Florida shelf waters, are resistant (NOAA-1 and C5). Based on earlier findings (Mayali and Doucette, 2002), we hypothesize that antagonistic, bacteria-bacteria interactions within these latter cultures confer this apparent resistance to algicidal attack and work is underway to characterize these interactions as well as isolate and identify the bacteria involved. Results from preliminary experiments indicate that a dissolved substance is not associated with this antagonistic activity. Describing details and the nature of these interactions may help to better elucidate the role of algicidal, and corresponding antagonistic, bacteria in regulating the growth of harmful species such as *K. brevis*. Such information is also essential for the critical evaluation of bacteria as a potential component of more comprehensive management strategies for these toxic bloom events.

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MULTIVARIATE ANALYSIS OF HAB ORGANISM OCCURENCE ON A CROSS-SHELF TRANSECT IN LOUISIANA

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The Louisiana Shelf is a highly eutrophic system impacted by the outflow of the Mississippi and Atchafalaya Rivers. Several HAB organisms, and many other potentially harmful organisms are commonly found at certain times and under certain conditions on the Louisiana shelf. Increases in nutrients and nutrient pulses have been linked to HAB events in many places including the northern Gulf of Mexico, exemplified by increases in *Pseudo-nitzschia* spp. concurrent with increases in nutrients to the Louisiana shelf since the 1950's. Also, the current ratio of Si:N approximates 1 in the Mississippi River, which creates the possibility for Si to become limiting on the shelf, which is potentially a more favorable condition for non-siliceous flagellates, some of which could be harmful.

HAB organisms are a threat to coastal Louisiana and it is important to understand what conditions and environmental variables influence their distribution along the Louisiana Shelf. A long time series from 1990-1998 of phytoplankton counts and environmental sampling were taken monthly along a cross-shelf transect influenced by the outflow of the Mississippi River. Counts of certain HAB or potential HAB species or groups from surface samples were investigated along with environmental variables utilizing canonical correlation analysis. Organisms included in the analysis were: *Alexandrium monilatum, Ceratium tripos, Dinophysis caudata, Dinophysis ovum/acuminata, Gymnodinium sanguineum, Heterocapsa triquetra, Heterosigma akashiwo, Karenia brevis, Katodinium* spp., *Lingulodinium polyedrum, Mesodinium rubrum, Prorocentrum micans, P. compressum, P. gracile, P. mexicanum, P. minimum, P. scutellum, Pseudo-nitzschia* spp., and *Scrippsiella* spp. The environmental variables used in the analysis were: station (to describe inshore vs. offshore), season, salinity, temperature, dissolved oxygen, nitrate, phosphate, and silicate.

Initial results of canonical correlation analysis of data from 1990-1998 highlighted several relationships between some species and environmental variables. *Pseudo-nitzschia* spp. and *Dinophysis ovum/acuminata* were positively related with spring, high NO_3, and high O_2, conditions and negatively related with summer and high temperatures. *Alexandrium monilatum* and *Pseudo-nitzschia* spp. were positively related with fall, hi salinity, and low SiO_3 conditions and negatively with summer. *Prorocentrum compressum* was positively related to summer, high SiO3, and low salinity and negatively with fall. *Dinophysis caudata, Prorocentrum micans, P. compressum,* and *Pseudo-nitzschia* spp. were positively related to spring and lower salinity. While this analysis does not perfectly describe the conditions for all of the potentially harmful species on the Louisiana shelf, it does give insight into the controlling factors of the distribution for some of the more commonly occurring HAB or potential HAB organisms.

SUBLETHAL EFFECTS OF THE TOXIC DINOFLAGELLATE *Karenia brevis* ON COPEPOD BEHAVIOR

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Karenia brevis blooms are most frequently found off the west coast of Florida, to a lesser extent elsewhere in the Gulf of Mexico, and on one occasion, as far north as North Carolina. Increasing attention is being paid to the interactions between copepods and harmful algal species, with copepods as a potential factor in bloom dynamics through grazing or avoiding toxic cells and/or selecting for non-toxic cells. While toxin accumulation in copepods is generally noted during blooms and has been seen under some laboratory conditions, depuration is generally rapid, but sometimes incomplete. However, vectorial transfer of toxins through copepods to higher trophic levels has been demonstrated both in the field and in laboratory studies. What is not generally appreciated is the effect of toxic phytoplankton on the suite of behavioral defenses that copepods routinely use to avoid predation (e.g., diel vertical migration and escape responses). During sublethal exposure to HAB species, vectorial toxin transfer could be magnified if copepods were more vulnerable to predation. Given the important role that copepods play in marine food webs, aspects of this scenario warrant study. To this end, we examined feeding, mortality and behavior of copepods at ecologically relevant concentrations of dissolved brevetoxins (PbTx-2) and *K. brevis* cells.

At the highest *Karenia brevis* concentrations offered, *Temora turbinata* displayed similar grazing rates on *K. brevis* and the control dinoflagellate. In mortality experiments, dissolved purified brevetoxin (PbTx-2) did not affect survival in either *T. turbinata* or *Centropages hamatus*. In contrast, mortality in both species increased in a dose-dependent manner upon exposure to whole *K. brevis* cells. For *C. hamatus*, the LD_{50} for mortality was lower at 2.4×10^5 cells L^{-1} (95% CI = $5.2 \times 10^4 - 5.6 \times 10^5$ cells L^{-1}), whereas the *T. turbinata* LD_{50} was 7.1×10^6 cells L^{-1} (95% CI = $2.6 \times 10^6 - 7.3 \times 10^7$ cells L^{-1}). Further experiments with *T. turbinata* suggest that adverse sublethal behavioral effects are apparent after 24 hr exposure to either dissolved purified brevetoxin (PbTx-2) or whole *K. brevis* cells. Swimming behavior (rate of change in direction, net-to-gross displacement ratio) was negatively affected at a dissolved brevetoxin concentration of 2×10^{-5} g L^{-1}. A cell concentration of 5×10^6 cells L^{-1} was required to negatively alter swimming behavior (swimming speed, rate of change in direction). Photobehavior was affected in a dose-dependent manner, with loss of photosensitivity beginning at a dissolved brevetoxin concentration of 2×10^{-6} g L^{-1}. Cell concentrations as low as 1×10^5 cells L^{-1} resulted in loss of photosensitivity, but severe behavioral alteration was not apparent until 5×10^6 cells L^{-1}. As both swimming behavior and photobehavior are involved in predator avoidance, their alteration upon exposure to sublethal concentrations of dissolved brevetoxins and *K. brevis* cells may mean that exposure of this copepod species to blooms could result in increased predation risk. Future studies will investigate *C. hamatus* behavior upon exposure to sublethal levels of dissolved brevetoxin and whole cells. Similar experiments will also be done with *Acartia tonsa*, a species known to be relatively insensitive to *K. brevis.*

PRIMARY PRODUCTIVITY BY THE TOXIC FLORIDA RED-TIDE DINOFLAGELLATE, *Karenia brevis*: EVALUATION OF A BIO-OPTICAL MODEL WITH LABORATORY AND FIELD DATA

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The Florida red-tide dinoflagellate, *Karenia brevis*, frequently forms large blooms in the coastal waters of the Southeastern USA and throughout the Gulf of Mexico, and can contribute significantly to the annual production of these areas. Accordingly, understanding the primary productivity of this key HAB species is integral to understanding the community-level processes of these water bodies. Previous work has established oxygenic and fluorometric photosynthesis versus irradiance (P/E) parameters, photoprotective capabilities, pigmentation dynamics, absorption characteristics, carbon acquisition rates, and the spectral irradiance of the ambient light field in both laboratory and *in situ* bloom conditions. However, to date there have been no attempts to collect all of these disparate pieces of data into a coherent bio-optical model of *K. brevis* productivity. We will present a wavelength resolved model that is parameterized from laboratory-/field-derived data and validated against both simulated *in situ* and *in situ* field measurements. Results from model optimization routines will be discussed.

SEAGRASS AS A ROUTE OF BREVETOXIN EXPOSURE IN THE 2002 RED TIDE-RELATED MANATEE MORTALITY

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Brevetoxins produced by *Karenia brevis*, the Florida red tide dinoflagellate, have previously been associated with mass mortalities of manatees in Florida. In 1982, three weeks after the dissipation of a red tide bloom, 39 manatees died and others exhibited debilitation and uncoordinated motility. Incidental ingestion of toxic filter feeding tunicates attached to seagrass was believed to be the primary source of brevetoxin. A red tide bloom in 1996 resulted in fewer sick animals but 149 deaths. In this case, manatees were dying in areas with high concentrations of red tide cells and environmental conditions conducive to aerosolization of brevetoxin. These events suggest that manatees can be exposed to brevetoxins through both ingestion and inhalation routes.

In mid-February of 2002, high levels of *K. brevis* were present in southwest Florida. In mid-March, three weeks after cell counts had dropped to low levels, manatee deaths greatly increased along the coasts of Sarasota, Charlotte, Lee, and Collier counties. With no active red tide and a noted absence of pulmonary lesions like those seen in 1996, ingestion was the hypothesized route of primary exposure. The 2002 mortality event ended in early May.

Guided by the location of initial manatee carcass recoveries, we selected four sites and one control in Charlotte County to determine if there was residual brevetoxin remaining in the system. From March 28 through August 15, 2002, water, sediment and seagrass samples were collected every two weeks and analyzed for brevetoxins using a competitive enzyme-linked immunosorbent assay (ELISA). From each site, one set of seagrass samples was analyzed whole. A second set was scraped for epiphytes and detritus. The scraped seagrass was then rinsed vigorously with tap water and separated into blades, sheaths, and rhizomes/roots.

Karenia brevis cells were either absent or present at very low levels (max. 2,670 cells/L) for the entire sampling period. Brevetoxin concentrations in the water column were typically below 1 mg/L and toxin in sediments averaged 12-17 ng/g dry wt. All grass components consistently tested positive for brevetoxins, but the highest concentrations were detected in the grass scrapings (up to 3,130 ng/g dry wt.). Based on the dry weight of each component, the contribution of the scrapings was 46-97% of the total toxin in the seagrass, with a median value of 89%. A subset of grass and scrapings was also analyzed by LC-MS and PbTx-3 was confirmed at an average ratio of 30% of the total brevetoxin measured by ELISA. Limpets were abundant in the epiphytic community, and samples of these limpets also tested positive for brevetoxin. Seagrasses continued to test positive for brevetoxins up until August 1, 2002.

These results confirm the stability of brevetoxins in the environment in the absence of an ongoing *K. brevis* bloom, and demonstrate the chronic risk of manatees to brevetoxins through an indirect exposure route. That physical and biological concentration of brevetoxins on and in seagrass can occur post-red tide bloom was unconfirmed in previous manatee mortality events. Based on necropsy findings and brevetoxin analyses of the urine and tissues of the 2002 carcasses, a total of 34 manatee deaths were attributed to brevetoxicosis. This is the first time that a significant number of manatee deaths have been positively related to the ingestion of brevetoxin via seagrass and associated epiphytes subsequent to a red tide event.

ASSESSING THE BIOAVAILABILITY AND UPTAKE OF DISSOLVED HUMIC SUBSTANCES BY THE HAB SPECIES *Karenia brevis* USING RADIOISOTOPIC TECHNIQUES

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Blooms of some HAB dinoflagellates species initiate in coastal regions characterized by high concentrations of colored dissolved organic material (CDOM), suggesting that these compounds are potentially bioavailable and may serve as both a carbon (C) and/or nitrogen (N) source. Measuring the direct uptake of dissolved humic substances by phytoplankton has been confounded by the polymictic nature of these compounds. The bioavailability of humic compounds to the HAB dinoflagellate *Karenia brevis* was addressed via two radioisotopic techniques: measurement of the uptake of ^{125}I-labelled humic acids extracted from the Peace River, a river implicated in supporting *K. brevis* blooms on the west Florida shelf, and measurement of the uptake of laboratory synthesized 'model' humic compounds which have been labeled in either the carbon or nitrogen moieties with ^{14}C during their synthesis. *Karenia brevis* took up a ^{125}I-labeled Peace River humic acid fraction at rates ranging from 1.0 - 2.2 pg cell^{-1} hr^{-1}, with the highest uptake rates observed in darkness. A similar range of uptake rates were observed with ^{125}I-labelled IHSS standard Suwannee River humic acids. Comparatively, *Dunaliella tertiolecta* and *Skeletonema costatum* took up the same labeled fractions at rates of 0.5 – 1.0 and 0.01 – 0.02 pg cell^{-1} hr^{-1} respectively. Stoichiometric calculations utilizing measured humic acid uptake rates and N content and a typical *K. brevis* bloom concentration suggest that the maximum amount of N available to a moderate *K. brevis* bloom from humic sources on the west Florida shelf is approximately 0.04 μM N L^{-1} d^{-1}. Assuming average growth rates and reported N:Chl ratios, the same bloom would have a N demand of 1.0 μM N L^{-1} d^{-1}. This suggests that, although humic fractions of estuarine CDOM are actively taken up by *K. brevis*, they are not a significant source of N to *K. brevis* blooms.

CELL-CONCENTRATION DEPENDENCE IN NET GROWTH OF *Alexandrium monilatum*

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The red tide dinoflagellate, *Alexandrium monilatum*, was experimentally inoculated over a range of initial cell concentrations into seawater containing a natural plankton community. In the presence of potential predators and competitors, positive net growth of the dinoflagellate population did not occur unless its initial cell concentration exceeded a threshold of 300-400 cells ml^{-1}. However, in filtered seawater, growth of the dinoflagellate was unrelated to cell concentration. The relationship between cell concentration and growth in whole seawater appeared to be due to inhibition of predation as initial cell concentration increased. A suite of hypothetical interactions between *A. monilatum* and a generalized predator were modeled and fit to the experimental data. To fit the experimental data with realistic parameter values, models had to include: a realistic carrying capacity for *A. monilatum*, mortality of *A. monilatum* that was related to contact probability with the predator, and growth of the predator that was independent of *A. monilatum* concentration (prey switching). This modeling exercise demonstrated that the unusual cell-concentration-dependence of *A. monilatum* net growth could be described by simple models with straightforward predator-prey interactions. The models constrained the potential ways in which the dinoflagellate and predator could interact and provided parameter values for growth and mortality rates of this hypothetical predator.

It is novel to suggest that dinoflagellate populations must exceed a cell concentration threshold to escape predation. By implication, red-tide initiation may have to be preceded by aggregation of dinoflagellate cells to levels exceeding such a threshold for positive net growth. Our results suggest that within such aggregations, mortality of the dinoflagellate would be reduced, allowing positive net growth and further accumulation of cells.

CHARACTERISTICS OF THE *Karenia brevis* DIEL VERTICAL MIGRATION BASED ON LABORATORY OBSERVATIONS

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Several years of laboratory observations on the cell division, physiology, biochemistry and behavior of *Karenia brevis* are combined to provide alternate diel vertical migration scenarios based on unequal and equal daughter cell formation. These scenarios form the basis of the programmed behaviors that are used to direct field-deployed *K. brevis* population mimics. Pertinent observations include: 1) cell division occurs throughout the occupied water column; 2) the strength of the daylight surface aggregation increases after a large cell division the previous night; 3) repeated (2-3 times) isolation of the noon surface aggregate, that occurs after a large cell division the previous night, to inoculate new water columns eventually yields a quantized cell division in a *K. brevis* population; 4) under the tested experimental conditions, approximately half the population in a water column aggregates at the surface by noon after a quantized cell division the previous night; 5) cells in the post-quantized, noon, surface aggregate can contain much less lipid and chlorophyll and somewhat less protein and carbohydrate than cells remaining deeper in the water column; 6) at night, cell sub-populations at all water depths return to the same average physiological potential and to the same average biochemical content; 7) some clones of *K. brevis* exhibit a nutrient-related chemotaxis that increases with nutrient deprivation and during the daylight part of a diel cycle; and, 8) cells require three days between divisions under standard laboratory growth conditions. Both alternate scenarios incorporate cell differences related to cell age (time since the last cell division) to explain observations 1-3. The alternate scenarios diverge in the explanation of observations 4-8 depending on whether cell division yields unequal or equal daughter cells. In the former case, initial cell composition strongly influences subsequent water column position, while in the latter case, initial water column position and conditions strongly influence subsequent water column position. Since water column position determines environmental exposure and physiological rates, cell growth follows complex patterns both within and between the alternate scenarios. Though present evidence tends to support many aspects of the equal daughter cell scenario, the exact nature of the daughter cell formation remains uncertain. A recently developed physical-biological model examines the influence of selected *K. brevis* behavioral patterns on bloom dynamics in a water column characterized by three-dimensional shear.

HARMFUL ALGAL BLOOMS IN FLORIDA's MARINE WATERS: THE NEW MILLENIUM

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Florida's marine and estuarine waters continue to experience new and sustained impacts from HAB events. Since early 2002, with more than 20 cases of saxitoxin (STX) food poisoning caused by the consumption of toxic southern puffer fish (*Sphoeroides nephelus*) recreationally harvested from the Indian River Lagoon (IRL) (Quilliam et al. 2002), STXs were confirmed in *Pyrodinium bahamense* for the first time in the U.S. (Landsberg et al. 2002). In the IRL, southern puffer fish remain highly toxic, while trace or below regulatory levels of STXs have been confirmed in a wide variety of fish and invertebrates including commercially harvested hard clams *Mercenaria* sp. Non-harvestable small razor clams, *Ensis minor*, with up to 4300 µg STX eq./100g tissue, are of interest for their potential risk as toxic prey (Abbott et al., this conference). In 2001, preceding the puffer fish toxicity outbreak by six months, a significant chronic mortality of more than 30 dolphins (M. Stolen, Hubbs-Sea World, pers.comm.) occurred in the general area of the IRL where high STX concentrations have now been found. An unusual mortality of seven manatees in the IRL in late 2001/early 2002 (E. Haubold, FWC, pers.comm.) was of concern and potentially significant in that STXs were confirmed in manatee stomach contents containing toxic tubeworms. To what extent STXs may chronically affect marine mammal health in the IRL is unknown. Florida now has all major groups of HABs with a potential to affect public health, cause economic losses, and impact ecological resources. Historically, *Karenia brevis* red tides have caused significant threats to the public from Neurotoxic Shellfish Poisoning or from aerosolized brevetoxins. In spring 2002 and 2003, an unusual time of year for blooms to occur, more than 100 endangered manatees died from brevetoxicosis in southwest Florida. In 2003, possibly for the first time, more than eight domestic dogs roaming on the beach were reportedly affected by exposure to brevetoxins during a highly concentrated red tide event. Urine samples repeatedly sampled from two dogs, independently submitted to two veterinary clinics, tested positive for Pbtx-2 eq. by ELISA; one dog for at least three weeks post exposure (Flewelling, unpubl. data). Although not verified and only visually confirmed in one case, dogs were considered exposed to brevetoxins via consumption of dead fish or from the ingestion of highly toxic foam at the seawater/beach interface. In spring 2002, a mortality of more than 20 lesser scaup *Aythya affinis* was also attributed to brevetoxicosis with toxin concentrations of almost 16,000 ng/g PbTx-2 eq. by ELISA in the gastrointestinal contents with less toxin present in the lungs, liver, and kidney (Flewelling, unpubl. data). These incidents demonstrate the persistent and expanding nature of brevetoxin impacts as well as signaling possible effects from other *Karenia* species now known in Florida's waters (Haywood and Steidinger, unpubl.data). Recent blooms of toxic *Pseudo-nitzschia pseudodelicatissima* along Florida's west coast signal potential concerns for Amnesic Shellfish Poisoning outbreaks. Ciguatera continues to be attributed to benthic *Gambierdiscus toxicus* along Florida's reef tract, but a potential role for *Prorocentrum* or *Ostreopsis* toxins in this food poisoning complex has not yet been explored. Eleven ichthyotoxic species, including *Alexandrium monilatum*, *Gymnodinium pulchellum*, *Karenia brevis*, *Karenia mikimotoi*, and *Chattonella* sp., have varying impacts statewide. Low and essentially benign concentrations of *Pfiesteria piscicida* and *P. shumwayae* were monitored in less than 4% of sites statewide with no evidence thus far for *Pfiesteria*-associated fish kills. *Trichodesmium* is present in high concentrations along Florida's coasts and has been implicated as a nutrient source for *Karenia* red tides, while *Lyngbya majuscula* causes frequent bloom mats in inshore bay areas. Continued networking and surveillance activities through the Florida HAB Task Force established in 1997 have ensured in-state investigations and monitoring for potential HAB events. Management plans need continuous reappraisal to address the changing scope and impacts associated with HABs in Florida's waters.

PHYSIOLOGY AND ECOLOGY OF MACROALGAL BLOOMS (GREEN TIDES) ON CORAL REEFS OFF NORTHERN PALM BEACH COUNTY, FLORIDA (USA)

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Since 1990 coral reefs in 20 to 50 m depths off northern Palm Beach County have experienced an unprecedented succession of macroalgal blooms involving the genera *Codium* and *Caulerpa*. Previous work has established that: 1) blooms of *Codium isthmocladum,* which initially expanded on these reefs in the early 1990's, are seasonal and grow from late spring through fall with peak biomass in late summer, 2) blooms of *Caulerpa verticillata* and *Caulerpa racemosa*, which appeared in 1997, and the non-native *Caulerpa brachypus* (Pacific native) that invaded the reefs in 2001, are now competing with the seasonal blooms of *C. isthmocladum*, and 3) blooms of both *C. isthmocladum* and *Caulerpa* spp. appear to be supported by nitrogen derived from land-based sewage discharges. Our research in 2003 involved comparative studies of seasonal growth patterns, fluorescence yield, tissue biochemistry, and the potential for herbivores to control these "Green Tides". Analysis of quarterly digital underwater video transects from our two monitoring stations at the Princess Anne (PA) and North Colonel's Ledge (NCL) indicated that, unlike *C. isthmocladum*, some *Caulerpa* spp. are capable of year-round growth. In January/February 2003 when reef temperatures were ~ 20 ^{o}C, biotic cover at both stations was dominated by *C. verticillata* (58 % at NCL, 62 % at PA) with relatively low cover of *C. racemosa* (9.1 % at NCL, 5% at PA) and *C. brachypus* (1 % at NCL, 19 % at PA). Following increased water temperatures to > 20 ^{o}C in late-February, the invasive *C. brachypus* developed extensive blooms in early March coincident with extreme low tides and increased concentrations of ammonium in the water column. By late April, *C. brachypus* was the dominant alga at both sites, accounting for 63 % cover at NCL and 72 % cover at PA. The molar C:N, C:P, and N:P ratios of *Caulerpa* spp. in the winter sampling averaged 16.8, 487, and 32.5 at NCL and 14.2, 504, and 35.6 at PA. Stable nitrogen isotope values (d ^{15}N) of *Caulerpa* spp. during winter averaged 5.56 o/oo at NCL and 5.18 o/oo at PA, values characteristic of enrichment with sewage nitrogen. Measurements of fluorescence yield (Fv/Fm) in *Caulerpa racemosa*, *C. mexicana*, and *C. prolifera* consistently approached the maximum value for PS II activity (a value of 0.8); in comparison, high but less consistent values were observed in *Codium isthmocladum*, *Caulerpa verticillata*, and *C. brachypus,* particularly in July 2003 when upwelling resulted in temperatures as low as 14 ^{o}C at NCL. Comparative studies of fluorescence yield in macroalgae from oligotrophic Bahamian waters showed lower values of Fv/Fm, suggesting that the high fluorescent yield values of bloom species on northern Palm Beach County's reefs are indicative of eutrophic conditions. *In situ* bioassays of bloom species palatability to resident icthyofauna indicated that although *C. brachypus* is highly palatable to grazing fish, its high net productivity after grazing losses allows it to be a persistent and often dominant component of the reef biota. Other bloom species, including *C. isthmocladum*, *C. racemosa*, *C. mexicana*, and *C. prolifera*, are generally not preferred diets for grazing icthyofauna.

LIPID COMPOSITION OF *Karenia brevis* BLOOMS IN THE GULF OF MEXICO

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In the Gulf of Mexico, recurring *Karenia brevis* blooms lead to significant health and economic impacts. *K. brevis* is one member of a small group of dinoflagellates, related morphologically and by DNA-based phylogenetic analysis, that synthesize the carotenoid, gyroxanthin diester, in place of the more widely distributed peridinin. While this novel photopigment has been proposed as a biomarker, especially for remote-sensing imaging technologies, to detect the emergence of *K. brevis* blooms, other chemicals such as sterols and triglycerides, respectively, with potential to report the distribution and physiological condition of *K. brevis* are required. Recent work from our laboratories characterizing the lipids of dinoflagellates has confirmed that *K. brevis*, together with those its close relatives, *Karenia mikimotoi* and *Karlodinium micrum*, lacking peridinin, possesses a relatively simple sterol profile comprised of two unusual primary 4-methyl sterols, (24*S*)-4a-methyl-5a-ergosta-8(14),22-dien-3b-ol (ED) and its 27-*nor* derivative (NED). An October 1999 *K. brevis* bloom in the waters of the northwest Gulf of Mexico provided an opportunity to examine the usefulness of these sterols and other lipids as indicators of *K. brevis* in phytoplankton communities. Lipid extracts of filtered bloom samples, fractionated to separate free and esterified sterols, were examined by GC/MS of trimethylsilyl ether derivatives. ED and NED were the major sterols found in all bloom samples. Fatty acids found in lipid fractions containing membrane phospholipids, chloroplast-associated glycolipids, and storage triglycerides, respectively, differed significantly. The glycolipid fraction was found to contain octadecapentaenoic acid [18:5(n-3)], a fatty acid commonly associated with dinoflagellates. The phospholipid fraction was found to contain small amounts of the recently described highly-unsaturated fatty acids, octacosaoctaenoic acid [28:8(n-3)] and octacosaheptaenoic acid [28:8(n-6)]. Fatty acids from the triglyceride fraction were more abundant than those associated with glycolipids and phospholipids. These results were found to closely resemble cultured *K. brevis*. They will be compared to a more recent Fall 2002 bloom.

ANALYSIS OF EXPRESSED SEQUENCE TAGS (ESTs) FROM THE DINOFLAGELLATE *Karenia brevis*

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Sequencing of cDNA libraries to generate expressed sequence tags (ESTs) is an effective means of gene discovery. Our objective was to sequence cDNA clones from *Karenia brevis* cells and to identify putative genes for future genome-wide functional analysis studies.

Dinoflagellate genomes vary widely in a species specific manner, but in general are characterized as having a large DNA content, up to 40 times that of the human; however, little is known about complexity. The *K. brevis* haploid genome contains approximately 100 pg/cell or ~1 x 10^{11} base pairs. Although the total number of distinct genes is unknown for any dinoflagellate genome, approximately 6000 genes have been reported for other protists. Thus to gain insight into the genetic information carried by *K. brevis*, a cDNA library was constructed from cells in logarithmic growth phase and 8000 5´-end sequence tags were established. Total RNA (1.1 mg) was isolated from exponentially growing *K. brevis* cells using Qiagen RNeasy columns and the cDNA library was constructed from mRNA in the lZapII expression vector. For EST analysis, *E. coli* host strain XL1-Blue MRF' was infected with the lambda phage and *in vivo* excision of the pBluescript SK(-) phagemid from the lZAP II vector was performed with the ExAssist helper phage. The excised phagemid was then transformed in *E. coli* SOLR strain and plated on LB-ampicillin agar. Individual colonies were grown and purification of pBluescript DNA was performed with QIAprep Miniprep Kits using a QIAvac 96 Top Plate system (Qiagen, Valencia, CA). Sequencing was performed using the universal T3 and/or T7 primers. Nucleotide sequences obtained were then compared to non-redundant GenBank sequence database using the basic local alignment search tool program (BLAST) in its version for nucleotides (BLASTN) and aminoacids (BLASTX).

To expedite the screening process, we used an implemented EST pipeline to automate the high throughput EST data analysis process. This integrated pipeline enabled large batch submission of sequences and automated procedures included sequence phredding/phrapping, quality control (i.e. elimination of short sequences, empty vectors and those containing too many unreadable bases), NCBI BLAST submission and redundancy calculations. Preliminary results for a total of 1392 5´-end sequence tags indicated that 74% were similar to registered sequences when compared to the GenBank sequence database. Classification of these homolgs into functional categories revealed that 11% are involved in metabolism, 5% transcription, 2% cell growth, 5% defense, 3% communication, 6% transport, 4% energy, 3% protein synthesis, 10% membrane/structural, and 13% DNA/nuclear. The remaining sequences were defined as novel ESTs. This, as well as future sequence information, serves as a powerful source for genome-wide functional analyses of *K. brevis* and investigations into the pathways that control the growth of harmful algae.

THE ASSOCIATION OF ALGICIDAL BACTERIA AND RAPHIDOPHYTE BLOOMS IN SOUTH CAROLINA BRACKISH STORMWATER DETENTION PONDS

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Over the past 3 years, raphidophyte blooms have been documented with notable frequency in brackish stormwater detention ponds along the South Carolina coastal zone. During bloom events in 2002 and 2003, we investigated temporal fluctuations of algicidal bacteria against raphidophyte species (*Heterosigma akashiwo* and *Chattonella subsalsa*) abundance using the microplate most probable number (MPN) method. A total of 168 strains of algicidal bacteria have been isolated from raphidophyte blooms. In 2002, an increase in *C. subsalsa* algicidal bacteria from 2 MPN ml^{-1} to 103 MPN ml^{-1} was noted in response to a bloom of *C. subsalsa* (2.5 X 10^3 cells ml^{-1}). Subsequently, the abundance of *C. subsalsa* decreased to 14 cells ml^{-1}. A second bloom of *C. subsalsa* followed (1.1 X 10^3 cells ml^{-1}) during which *C. subsalsa* algicidal bacteria increased from 52 MPN ml^{-1} to 131 MPN ml^{-1}. A similar response was noted with *H. akashiwo* algicidal bacteria where abundance estimates increased from 18 MPN ml^{-1} to 97 MPN ml^{-1} associated with a decrease in *H. akashiwo* abundance from 1.6 X 10^3 cells ml^{-1} to 373 cells ml^{-1}. High population densities of *H. akashiwo* algicidal bacteria (> 100 MPN ml^{-1}) were noted in several other Kiawah Island pond samples associated with raphidophyte bloom events. In the summer of 2003, *C. subsalsa* and *H. akashiwo* were noted at low levels (< 5 cells ml^{-1}) and the numbers of algicidal bacteria targeting these two flagellates also appeared at low densities (< 2.2 MPN ml^{-1}) in the same ponds. In addition, bioassay experiments often indicated a stimulatory effect of antibiotic addition on raphidophyte growth (e.g. Fig. 1). These results suggest that algicidal bacteria may play an important role in the initiation and termination of raphidophyte blooms in brackish stormwater detention ponds.

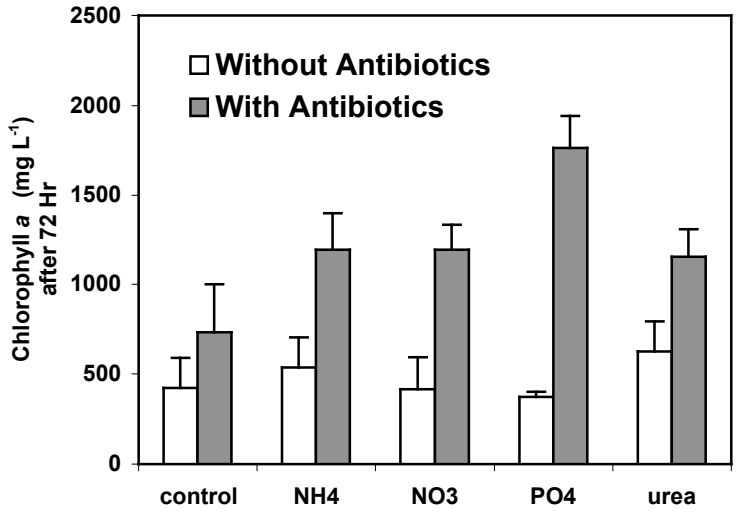

Figure 1. Bioassay results using water collected from *Chattonella subsalsa* bloom.
Comparison of 72-hr chlorophyll in nutrient treatments without (white) and with (gray) antibiotics.

BEHAVIOR AND INTERNAL CELLULAR STATE OF *Karenia brevis* UNDER VARIOUS LIGHT AND TEMPERATURE CONDITIONS

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Karenia brevis is a photosynthetic dinoflagellate responsible for many HAB events in the Gulf of Mexico. Behavior is an integral part of the life history of *K. brevis.* The species undergoes a diel vertical migration where cells typically aggregate at the surface during the day and spread throughout the water column at night. Swimming characteristics, combined with physical factors, dictate the distance a cell can move in the water column, influencing not only environmental exposure (light, nutrients) but also influencing horizontal movement as the cell's relative position in the water column can expose it to varying flow regimes. Three clones of *K. brevis* - Manasota, Apalachicola, and Jacksonville - were examined under a range of light intensities and temperatures that correspond to the viable range of the organism. These clones were chosen because they were isolated from different geographic areas on Florida's panhandle, west coast and east coast, and because work by Schaeffer et al. (2002) suggested these clones have different photosynthetic capabilities. Cultures were grown under standardized conditions and then incubated in a radial photosynthetron for six or twelve hours under constant and changing light. Subsamples taken from each light level were 1) videotaped and analyzed with Expert Vision Motion Analysis package for swimming characteristics, 2) analyzed on the Pulsed-Amplitude-Modulated-Fluorometer for production, and 3) stained and evaluated on the flow cytometer for liposome content. Swimming speeds at 22°C for all three clones demonstrate an unusual response where speeds initially decrease with increasing light (50-300 umol quanta/m2/s), but then increase at intermediate light (631-1126 umol quanta/m2/s) before diminishing at high light (>1300 umol quanta/m2/s). While all three clones showed the same trend, Apalachicola tended to swim the fastest at all light levels, whereas Jacksonville tended to swim the slowest. Production decreased at lower temperatures. Results indicate an increase in liposome content with increasing light. This type of comparative data provides insights into the resource allocation within the cells by examining their swimming capabilities with respect to their photosynthetic capabilities. Observations detailing aggregation patterns among *K. brevis* clones in laboratory cultures have shown distinct surface patterns in the three clones examined. Further work will investigate intraspecific differences in swimming speed and liposome and chloroplast distribution, and what this might mean for the patterns described.

References:

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APPLICATION OF A SPECIES-SPECIFIC *Karenia brevis* LSU rRNA PROBE ALONG THE TEXAS GULF COAST, USA

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Blooms of the brevetoxin producing dinoflagellate, *Karenia brevis*, occur in coastal waters throughout much of the Gulf of Mexico and are responsible for marine mammal mortalities and fish kills, as well as severe socio-economic impacts in this region. Methods for the detection of *K. brevis* cells and discrimination from morphologically-similar species in mixed natural communities are necessary for both monitoring and research purposes. We have recently developed a species-specific LSU rRNA oligonucleotide probe that distinguishes *K. brevis* from the morphologically similar and often co-occurring dinoflagellate, *K. mikimotoi*, in laboratory cultures using a whole-cell hybridization approach. This probe has also been successfully applied to preserved field samples from the west Florida shelf, showing strong labeling of cells identified microscopically as *K. brevis*. One of our present aims is to test the suitability of the *K. brevis* probe for use in other coastal areas of the Gulf of Mexico (e.g., Texas), including its application for discriminating against frequently observed morphological variants of *K. brevis* that may actually represent a different taxon or taxa.

As part of this study, the *K. brevis*-specific probe (Kbprobe-7) was applied to four "*K. brevis*-like" cultures isolated from Corpus Christi Bay, TX during the winter of 2002 (B1, C9, C18, C15) and one isolate from Nueces Bay, TX (NBK) sent in the blind by the Texas A&M laboratory to NOAA, Charleston. Prior to shipping, cultures were fixed using a modified saline ethanol fixative (Miller and Scholin, 2000) with 10% formalin added. A culture known to be *K. brevis* (NOAA-1) was fixed simultaneously at NOAA, Charleston in the same way to serve as a positive control for both the probe and the fixation protocol. Kbprobe-7 was applied to all cells using a whole cell hybridization/FISH protocol. Only one of the isolates tested (NBK), in addition to the *K. brevis* positive control, yielded a positive signal, whereas the remaining four Corpus Christi Bay isolates were clearly negative. Positive and negative control probes applied to each isolate gave the expected results. Sequence data from the nuclear ribosomal ITS region made available by the Texas A&M laboratory following the probe experiment indicated that the four negative isolates were, in fact, *K. mikimotoi*-like, while the single positive isolate was *K. brevis* and thus consistent with all probe results. Sequencing of the LSU rDNA is now underway to provide supporting phylogenetic data. Our findings represent the first successful application of probe Kbprobe-7 for distinguishing *K. brevis* from closely related dinoflagellates in Texas coastal waters, and also confirm the effectiveness of our fixation protocol. These results also indicate that use of this probe has a strong potential for incorporation into existing and planned efforts to monitor the occurrence of *K. brevis* in this region.

References:

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CELLULAR STRESS RESPONSES OF *Karenia brevis* TO HEAT AND OXIDATIVE STRESSES: IDENTIFICATION AND RESPONSE CHARACTERIZATION OF STRESS PROTEINS AND ANTIOXIDANT ENZYMES

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The environmental conditions under which *Karenia brevis*, the Florida red tide dinoflagellate, often exists and thrives are remarkably variable and seemingly non-ideal for bloom growth and maintenance. The primary line of defense against any factor inducing stress is at the biochemical level, underlying all effects at higher organizational levels; however, little is known about the cellular mechanisms by which *K. brevis* adapts to adverse and/or changing environmental conditions. The induction of stress proteins (Hsps) and/or antioxidant enzymes and measurements of photosynthetic efficiency (F_V/F_M) are commonly used indicators of cellular stress. To date, no published studies have identified any stress proteins or antioxidant enzymes in *K. brevis*. The current study identifies Hsp 60, chloroplast small heat shock protein (chlshsp), mitochondrial small heat shock protein (mitoshsp), manganese superoxide dismutase (Mn SOD), and iron superoxide dismutase (Fe SOD) in laboratory cultures of the Wilson isolate of *K. brevis*. These 5 proteins represent 2 superfamilies of stress proteins, chaperones (Hsp 60) and low molecular weight (LMW) Hsps (chlshsp and mitoshsp), and 1 superfamily of antioxidant enzymes, superoxide dismutases (Fe SOD and Mn SOD). *K. brevis* shows differential induction of a subset of these proteins in response to different stressors: mitoshsp is induced by heat stress whereas the chlshsp is oxidatively induced. Furthermore, the chlshsp responds differentially to various sources of oxidative stress (hydrogen peroxide, lead, or increased light). Light stress results in changes in F_V/F_M at levels that do not result in induction of chloroplast-specific stress proteins. In contrast, stressors that do not selectively target the photosynthetic machinery (all stressors excluding increased light) result in the induction of the stress proteins and antioxidant enzymes that are not paralleled by a decrease in F_V/F_M, suggesting that expression of these proteins represents a more immediate stress response and thus a more sensitive indicator of general cellular stress in *K. brevis*. These results provide, for the first time, evidence of stress proteins and antioxidant enzymes functioning in the adaptive mechanisms of *K. brevis* and, with further research, may provide a sensitive indicator of bloom health.

TOXICITY OF THE CHAIN FORMING DINOFLAGELLATE, *Alexandrium monilata* ISOLATED FROM THE GULF OF MEXICO WITH PRELIMINARY STRUCTURAL DETERMINATION OF A NOVEL NON-POLAR TOXIN

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Cultures of *Alexandrium monilata*, isolated from a red tide off Mississippi, were examined for the possible production of bioactive compounds. The strain, AM01, was grown in batch culture until mid-log growth phase and harvested. The resulting cell mass was extracted using an elutropic series of increasing polarity using 5 different solvents. Each extract was tested for activity using different live animal and cell based assays. Two distinctive toxic fractions were observed; a polar soluble fraction and a non-polar soluble fraction. The polar fraction was tested for saxitoxin-like activity using the STX receptor binding assay. This assay was negative therefore this species of *Alexandrium* does not produce saxitoxin or any saxitixon-like compound.

Structural determination of the icthyotoxic non-polar fraction is currently underway. This fraction was purified using a several step process using TLC and HPLC. Mass spectral analysis using both LC-MS and MALDI -MS of this purified compound yielded a molecular ion of 790 amu. Proton and carbon NMR structural analysis demonstrate a macrolide-like compound with four exo-cyclic double bonds. This compound has a nominal molecular formula of $C_{47}H_{98}O_8$. This new toxic compound is compared to known toxins produced by other species of *Alexandrium*.

DOES NITROGEN REGENERATION FROM THE N_2 FIXING CYANOBACTERIA *Trichodesmium* spp. FUEL *Karenia brevis* BLOOMS IN THE GULF OF MEXICO?

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Blooms of the toxic dinoflagellate, *Karenia brevis,* occur in the oligotrophic waters of the eastern Gulf of Mexico where known nitrogen (N) sources are insufficient to support observed biomass accumulations. Large *K. brevis* blooms frequently co-occur or occur subsequent to blooms of the N_2 fixing cyanobacteria, *Trichodesmium* spp. *Trichodesmium* alleviate N limitation where they occur by using atmospheric N_2. Much of the recently fixed N_2 is regenerated as NH_4^+ and dissolved organic N (DON). This regenerated N is then available to support the growth of other cells. We hypothesized that N regenerated from N_2 fixation provides the N necessary to support blooms of *K. brevis* in the Gulf of Mexico, and have conducted a combination of field and laboratory investigations to demonstrate a viable nutritional link and to quantitatively assess the role of *Trichodesmium* in providing N to support the growth of *K. brevis*. Preliminary results demonstrated that *Trichodesmium* fix N_2 at high rates with more than 50% of this new N released as NH_4^+ and DON, that *K. brevis* has a high affinity for reduced N sources and can extracellularly degrade some organic compounds. In addition to these indirect lines of evidence, we have conducted a number of studies to establish direct links between *Trichodesmium* and *K. brevis* production. In the field, stable isotopes were used to trace the uptake of $^{15}N_2$, its regeneration into dissolved N and its subsequent uptake into *K. brevis* biomass. Dialysis bags containing *Trichodesmium* were suspended in gas-tight incubation bottles containing *K. brevis* and $^{15}N_2$ enriched water. We observed that regenerated ^{15}N label (as NH_4^+ and DON) passed through the dialysis bag and was taken up by *K. brevis*. In the laboratory, we have established continuous cultures of *Trichodesmium*, grown them at 3 different growth rates and determined that N released from *Trichodesmium* cultures could fuel *K. brevis* growth by directing the outflow from continuous cultures of *Trichodesmium* into recently isolated cultures of *K. brevis* growing on medium without added N. With these experiments we demonstrate that N released from *Trichodesmium* can support the growth of *K. brevis*.

EXPERIMENTAL BIOACCUMULATION OF ICHTHYOTOXIC BREVETOXINS IN HEALTHY FISH

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Brevetoxins and ciguatoxins are potent neurotoxins produced respectively by *Karenia* spp. and *Gambierdiscus* spp. These two kinds of marine toxins present a similar chemical nature (trans-fused polyether rings) resulting inactivation of the voltage sensitive sodium channel by a specific interaction of the toxins with the site 5 of the α-subunit of the sodium channel. Ciguatoxins are transferred up the food chain to carnivorous fish usually without any signs of toxicity to contaminated fish. However, it is well known that fish are very susceptible to brevetoxins during blooms of *Karenia brevis*. During such events, brevetoxins are present in the water resulting in massive fish kills. Fish can potentially be exposed to brevetoxins by absorption of soluble toxin across the gills, by ingestion of *K. brevis* cells, or by transfer of brevetoxins through the food web. By feeding two species of coastal fish: Croakers (*Micropogonius undulatus*) and Pinfish *(Lagodon rhomboides)* with highly contaminated shellfish (total brevetoxin: 1.8 μg/g of shellfish tissue) collected in Florida, we demonstrated bioaccumulation of brevetoxins in tissue of the fish without any signs of toxicity or disease in the fish. Up to 4.2 μg of total brevetoxins per gram of fish tissue were found to be associated with the stomach and the intestine but significant amounts were also present in the muscles and skin of the exposed fish. The total amount of toxins in the body of some fish (up to 96 μg) was several magnitudes higher than what is known to kill fish when toxins are present in seawater (LD_{50} 4 hours: 6 ng/ml)

The toxin profile in the fish organs was similar to the profile in the clams used in the feeding experiments. In these experiments, fish were fed toxic clams for 2 consecutive weeks and then fed non-toxic clams for another 2 weeks. During the 4 weeks of the experiments, brevetoxins exposed fish were as healthy as the control fish fed only with non-toxic clams. When exposed to both toxic and non-toxic clams, fish could not discriminate between the two, and were feeding on all shellfish. These results strongly suggest that fish have the potential to accumulate non lethal concentrations of toxins in the wild after a red tide, when toxins are no longer present in the water but are concentrated in shellfish. This hypothesis was partially indicated but not confirmed by the detection of high concentration of brevetoxins by ELISA in tissues of dead striped burrfish (*Chilomycterus schoepfi*) collected in Florida after a recent red tide. Several days to a few weeks after a local red tide, a mortality of striped burrfish occurred in southwest Florida. Internal organs including liver, gills, and muscle as well as intestinal content, were found to contain high concentrations of brevetoxins (up to approximately 6500 ng/g PbTx-2 equivalents by ELISA), and fish had been feeding on small bivalves. Although this mortality indicated that fish were exposed to lethal concentrations of brevetoxins, the presence of brevetoxins (but as yet uncharacterized for potentially non-toxic metabolites) in the muscle of these fish does not necessarily indicate any potential risk to human consumers. Thus far we have no evidence for the accumulation and persistence of high concentrations of brevetoxins in the muscle of healthy fish during or after natural bloom events. Additionally, routine sampling of a range of fish species with different feeding habits indicates no or minimal accumulation of brevetoxins in the muscle, while mouse bioassays to date have not indicated the presence of toxic metabolites in the muscle. To ascertain to what extent these experimental data can be extrapolated to natural field situations and their potential as a human health risk needs further study. It must be determined 1) the condition of accumulation of brevetoxin in fish in the wild, 2) the level of contamination in healthy fish and 3) the toxicity of the fish after red tide.

AN INITIAL ASSESSMENT OF GYROXANTHIN-RADIOLABELING AS A TOOL FOR DETERMINATION OF *Karenia brevis* CARBON-SPECIFIC GROWTH RATES *IN SITU*

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Determination of *in situ* growth rates of HAB-forming species is critical to an accurate description of bloom dynamics but there are currently few reliable methods of directly determining growth rates on natural populations. We are examining the use of radiolabeling of the biomarker pigment gyroxanthin to determine growth rates of *Karenia brevis*. We compared photopigment-radiolabeling derived rates to those determined by time-course measurements of cell numbers and chl *a*.

Batch cultures of *Karenia brevis* (Texas clone SP3) were grown at 2 light levels (80 and 130 mmol m^{-2} s^{-1}) with nitrate, ammonium, or urea (20 mM-N) as the N source. At 130 mmol m^{-2} s^{-1}, chl-based growth rates were 0.31 ± 0.02 d^{-1} on nitrate, 0.28 ±0.01 d^{-1} on ammonium and 0.26±0.05 d^{-1} on urea; there was no significant difference between growth rates on the three forms of N. Cells grew significantly more slowly at 80 mmol m^{-2} s^{-1} irradiance. Rates were 0.25 ± 0.03 d^{-1}, 0.18 ± 0.01 d^{-1} and 0.19 ± 0.02 d^{-1} for ammonium, nitrate, and urea cultures, respectively. Again, there was no significant difference between the nutrient treatments. A significant light x nutrient interaction was observed in that the light level had a significant effect on the growth rate of cells grown on nitrate.

Photopigment (Chl *a*)-based growth rates measured on batch cultures in exponential growth were not significantly different from the time series (chl a) growth rate measurements when the calculation method of Goericke & Welschmeyer (1992) was used. Gyroxanthin-based rates were more difficult to discern, because gyroxanthin concentrations per cell were low and growth rates were slow, hence it was difficult to get a good radiolabeled gyroxanthin signal (above background). When gyroxanthin rates could be calculated (in the fastest growing cultures) they agreed well with the chl a based rates (both radiolabeling and time course approaches). Future experiments will examine differences between growth rates of cells in semi-continuous culture (~balanced growth) and rates determined by the photopigment radiolabeling approach.

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PHOTOOXIDATION AND PHOTOINHIBITION IN THE RED TIDE DINOFLAGELLATE *Karenia brevis*

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Laboratory studies have identified the internal biochemical status of the cell as a determinant for the growth, reproduction, and possibly migratory behavior of *Karenia brevis*. The internal biochemical status of the cell is directly influenced by the cell's capability to photosynthesize. Understanding mechanisms that affect photosynthesis in *Karenia brevis* is crucial in identifying the organism's ability to use resources in local waters and to proliferate. Previous work included a quantized population in which all cells divided at night. At dawn, the same biochemical composition was found throughout the mesocosm, but by noon surface low lipid and subsurface high lipid subpopulations developed. This biochemical differentiation was thought to result from differential diel vertical migration by daughters with different resource allocations from the parent cell. An alternative hypothesis described the biochemical differentiation as a response to a light gradient. Here, photooxidation and photoinhibition mechanisms are thought to be primarily responsible for the cellular biochemical decreases observed at the surface while optimal production is thought to be responsible for cellular biochemical increases at depth. To investigate this alternative hypothesis cultures were grown in a 225L mesocosm to allow diel vertical migration for three days during 12 hour light/dark cycles. Prior to lights on (day 1) and in mid-afternoon (day 3), samples were removed from the mesocosm and incubated in a radial photosynthetron. Aliquots from the mesocosm and the photosynthetron were subsequently collected to determine pulsed amplitude modulate fluorometer (PAM-FL) electron transport, yield, chlorophyll, and lipid content for populations with (mesocosm) and without (photosynthetron) behavior. All lipid samples were analyzed by thin layer chromatography with a flame ionization detector Iatroscan using a multiple step separation technique. This technique focused on triacylglycerol storage lipids, 1,3 diacylglycerol, 1,2 diacylglycerol intermediates, and finally monogalactosyldiglyceride and digalactosyldiglyceride chloroplast membrane lipids which are likely targets for oxidation. PAM-FL measurements were compared to lipid content within the cells at different depths and times during the diel cycle.

THE DISTRIBUTION OF BREVETOXIN (PbTx-3) TO SPECIFIC LIPOPROTEIN FRACTIONS IN MOUSE BLOOD

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Brevetoxin, the neurotoxin produced by *Karenia brevis*, has had adverse effects on the environment, humans, and marine organisms for hundreds of years. Following consumption of shellfish infected with brevetoxins, or simply inhaling aerosolized brevetoxins, humans can show symptoms ranging from respiratory to digestive distress. Blood is an essential tissue to analyze when a human or animal is suspected of having brevetoxicosis because it serves to distribute toxins to the tissues. Many small molecules are bound to carrier proteins, such as albumin or specific binding proteins, or to larger lipoprotein particles in the blood. Lipophilic contaminants such as o',p'-DDT are known to accumulate in lipoprotein particles and because brevetoxin is also lipophilic, we have investigated the distribution of the brevetoxin congener PbTx-3 to blood lipoproteins. The first stage of the experiment was conducted using mouse plasma spiked with PbTx-3. This plasma was fractionated into different size lipoproteins by iodixanol gradient ultracentrifugation. Each fraction was then characterized and quantified by Lp(a) lipoprotein affinity agarose gel electrophoresis and radioimmunoassay (RIA). The PbTx-3 was restricted to only those gradient fractions confirmed to contain high-density lipoproteins (HDLs). None of the fractions containing low-density lipoproteins (LDLs) contained PbTx-3. We are currently analyzing the distribution of brevetoxin in lipoprotein fractions of blood from mice exposed to sublethal doses of PbTx-3. New information on the distribution of brevetoxins in blood and the process by which the toxin is delivered to tissues may permit more effective therapeutic measures to treat intoxication from brevetoxins and the related ciguatoxins.

AN ALGICIDAL BACTERIUM ACTIVE AGAINST *Karenia brevis*: INVOLVEMENT OF A DISSOLVED COMPOUND

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Cytophaga sp. (strain 41-DBG2) is a bacterium isolated previously in our laboratory and found to be algicidal against the brevetoxin producing dinoflagellate, *Karenia brevis*. In fact, many algicidal bacteria identified by other investigators also belong to the Cytophaga-Flavobacterium-Bacteroides (CFB) group. Algicidal activity may be induced either via direct attack of a target alga by the bacterium or indirect attack through release of a dissolved substance. Recent investigations have been aimed at obtaining insights as to the nature of 41-DBG2's killing activity. Results of experiments involving the co-culture of *K. brevis* (isolate C2) with strain 41-DBG2 indicate that this bacterium produces a soluble, heat-sensitive, algicidal compound with a molecular weight between 0.5 and 300 kDa (thereby eliminating viral-mediated activity) and capable of killing various algal species. Dilution of filtrate (<0.22 μm) from the above co-culture with filtrate from a co-culture of *K. brevis* (isolate C2) and the non-algicidal bacterium, *Cytophaga latercula*, illustrate a concentration-dependent killing by the dissolved 41-DBG2 algicidal agent upon re-inoculation with *K. brevis* (isolate C2) (Figure 1).

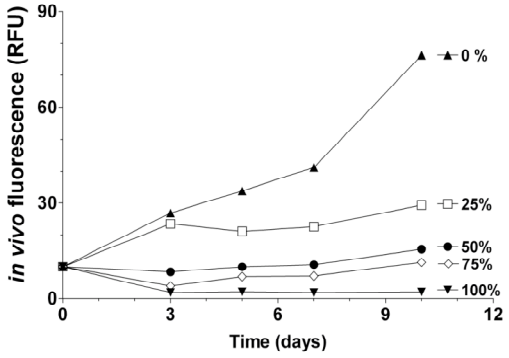

Figure 1. Growth of *Karenia brevis* (isolate C2) in diluted filtrates from *K. brevis*/41-DBG2-killed co-culture. Killed co-cultures were filtered (<0.22 μm), re-amended with *f/2* nutrients, and then diluted (100, 75, 50, 25%) with co-culture filtrates of *K. brevis*/*Cytophaga latercula* (non-algicidal). Negative controls (0%) were co-culture filtrates of *K. brevis*/*C. latercula* only, which showed normal growth of the re-inoculated *K. brevis* cells.

Algal taxa susceptible to lysis by the 41-DBG2 algicidal agent include the dinoflagellates *K. brevis* (strains C2 and Wilson), *K. mikimotoi*, *Gymnodinium simplex*, and the diatom *Skeletonema costatum.* Non-susceptible or resistant algal strains include selected *K. brevis* strains (NOAA-1, C5), the dinoflagellate *Akashiwo sanguinea*, the diatoms *Thalassiosira weissflogii* (CCMP 1336) and *Chaetoceros neogracile* (CCMP 1318), and the raphidophyte *Heterosigma akashiwo* (CCMP 1870). In the case of *K. brevis* isolates, work in our laboratory has demonstrated that differences in susceptibility reflect the ability of the resident bacterial assemblage to confer resistance by some yet to be determined mechanism.

Current work is aimed at using bioassay-guided fractionation (HPLC) of dialyzed culture filtrates to isolate and identify the 41-DBG2 algicidal agent. Subsequently, mode of action studies will be undertaken in order to better characterize this potentially novel compound.

CIGUATERA FISH POISONING ASSOCIATED WITH OIL PRODUCTION PLATFORMS ALONG THE TEXAS COAST

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Ciguatera Fish Poisoning (CFP) is the most common form of HAB intoxication known, and accounts for > 90% of the medical costs associated with HAB toxins in the U.S. The causative organism, *Gambierdiscus toxicus*, is endemic in tropical waters throughout the world, and is part of a benthic dinoflagellate assemblage that produces a variety of water- and lipid-soluble toxins. Polyether toxins produced by *G. toxicus* are transformed and biomagnified up the food web and accumulate as ciguatoxin (and its derivatives) in upper level predators such as barracuda, grouper, and jacks. Other toxins in the benthic dinoflagellate assemblage may accumulate as well and are suspected to play a secondary role in producing the diverse variety of symptoms that are typical of ciguatera.

Ciguatera-causing dinoflagellates are typically associated with coral reef ecosystems but have been noted in pelagic *Sargassum* communities as well. The Texas coast has no coral reef systems except for the Flower Gardens National Marine Sanctuary well offshore and is generally considered a low risk area. However, oil production platforms are common along the coast and extend out past the edge of the continental shelf. The only reported cases of ciguatera come from barracuda caught at these rigs. The toxin burden in the barracuda population and presence/absence of *G. toxicus* remains unknown. This is potentially a serious public health risk since barracuda are commonly eaten in Texas, and few physicians are familiar with ciguatera symptoms.

In order to provide baseline data for assessing the distribution of both toxic fish and *G. toxicus*, we conducted a fish toxicity survey and examined potential dinoflagellate substrates. Barracudas collected from oil production platforms were examined for ciguatoxins using the receptor binding assay and cytotoxicity assay. Substrate (mixed algae, barnacles and other benthic fouling organisms) were collected from platforms and examined for *G. toxicus*. In addition, *Sargassum* was collected as well when present. As part of the fish donation process, fishers volunteered information on fish consumption and related symptomology that permitted documentation of probable ciguatera cases in the past.

At the time of this writing, over 100 barracuda (0.8-21.3 kg) have been collected from over a dozen sites including the Flower Gardens National Marine Sanctuary. Initial assays indicate the presence of toxic fish from the rigs and from the Flower Gardens. *G. toxicus* and *Prorocentrum lima* have been identified from platform substrate. Two additional incidents of CFP have been found in the past 10 years, with one case involving multiple poisonings by relatively small fish (approximately 1 m, about 9-10 kg weight). Ciguatera appears to be endemic to Texas coast, although at this time we cannot determine if the toxins are incorporated locally, or are being transported via fish migrations.

LC/MS ANALYSIS OF BREVETOXINS AND THEIR METABOLITES IN THE EASTERN OYSTER (*Crassostrea virginica*)

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Brevetoxins from *Karenia brevis* are rapidly metabolized in the Eastern oyster (*Crassostrea virginica*), as evidenced by LC fractionation and *in vitro* assay of toxic oyster extracts (Dickey et al., 1999). In laboratory studies with pure brevetoxins (B-type, PbTx-2 and -3), we previously identified by LC/MS two of these metabolites, cysteine-PbTx and its sulfoxide (MH^+: *m/z* 1018 and 1034), as derivatives of PbTx-2 (Plakas et al., 2002). PbTx-2 is also metabolically reduced to PbTx-3 in the oyster. In the present study, we further explore brevetoxin metabolism in oysters naturally exposed to *K. brevis* red tide, by using LC/MS and *in vitro* assay. In addition to the previously identified metabolites of PbTx-2, we found a cysteine conjugate and its sulfoxide (MH^+: *m/z* 990 and 1006) with A-type backbone structure, as probable derivatives of PbTx-1. We also found glycine-cysteine-PbTx (*m/z* 1047 and 1075), glutamyl-cysteine-PbTx (*m/z* 1147), and glutathione-PbTx (*m/z* 1176 and 1204) conjugates with A- and B-type backbone structures. Amino acid-toxin conjugates can further react with fatty acids in oysters through amide linkage to form a series of fatty acid-amino acid-toxin conjugates. These fatty acid conjugates are apparent major contributors to the composite cytotoxic responses obtained in extracts of brevetoxin-contaminated oysters. Other brevetoxin compounds found in oysters were consistent with hydrolysis and oxidation/reduction reactions. Brevetoxins and metabolites observed in field-exposed oysters were confirmed in oysters exposed to *K. brevis* cultures in the laboratory. Of those analyzed, the previously identified cysteine-PbTx metabolite of PbTx-2 was by far of the highest relative abundance by LC/MS. Cysteine-PbTx and its sulfoxide are being evaluated as potential biomarkers for monitoring brevetoxin contamination in oysters.

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WILL OFFSHORE WASTEWATER DUMPING LEAD TO HARMFUL ALGAL BLOOMS IN THE GULF OF MEXICO?

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The Florida Department of Environmental Protection is looking to avoid what could be an environmental disaster for the Tampa Bay, Florida area, by dispersing millions of gallons of treated wastewater into the Gulf of Mexico. There are over 1.2 billion gallons of wastewater held in gypsum stacks and ponds at the defunct Mulberry Corporation's phosphate fertilizer plant located near Port Manatee, Florida. Heavy rainfall or hurricane conditions could cause water levels in the stacks and ponds to rise to a point that would lead to a breech in the gypsum stack and pond dikes unleashing this wastewater directly into the Bishop Harbor section of Tampa Bay.

A short-term measure was put in place to prevent the gypsum stacks from overflowing. Starting in January 2003, about 2 million gallons of treated wastewater began to be discharged in Bishop Harbor per day in an effort to lower water levels at the site. By February 2003 two HAB events had been documented, a *Heterosigma akashiwo* bloom followed by a *Prorocentrum minimum* bloom. Will the trend be the same for the Gulf of Mexico after treated, but still highly nutrient rich, wastewater is released there?

Every 4-6 days from late July through the end of November 2003, about 7.5 million gallons of treated wastewater will be shipped 100 miles offshore, where it will be sprayed into the Gulf of Mexico. Following the wastewater dispersal, water quality samples, including samples for harmful algal bloom analysis, are being collected. Analysis of monitoring efforts both in Bishop Harbor and the offshore dumping site will be presented alongside baseline data collected before the wastewater dumping began. Included will be results from culture studies on inoculum size and treated wastewater suitability conducted using *Karenia brevis*.

BIOMONITORING BLOOD BREVETOXIN IN STRIPED MULLET (*Mugil cephalis*) AFTER SUBLETHAL LABORATORY EXPOSURE TO *Karenia brevis*

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There is a critical need to simply and reliably monitor brevetoxins routinely in the blood of humans and aquatic animals. The blood collection card method in conjunction with the brevetoxin radioimmunoassay (RIA) has proven successful in determining the toxicokinetics of blood brevetoxin levels in laboratory mice. Most recently this method has successfully identified blood brevetoxin levels in bottlenose dolphins and West Indian manatees exposed to red tides. Our newest studies are using striped mullet as laboratory test animals to better define the kinetics of aqueous exposure to *Karenia brevis*. To do this we have exposed striped mullet to sublethal densities of *K. brevis* (~250 000 cells/liter) for 4, 8, 12, and 24 hours. At each time point a water sample was collected and the fish bled for further analysis by RIA. The RIA results indicate that blood brevetoxin levels increased to values significantly different from that of the controls at 8 to 12 hours of exposure ($p < 0.05$), this was followed by levels not significantly different from controls at 24 hours. Striped mullet were also exposed to a *K. brevis* culture with a known brevetoxin concentration of 0.5 nM. Even at low brevetoxin concentrations the RIA was able to detect significant amounts ($P < 0.05$) of brevetoxin in the blood of the mullet at 8 hours of exposure. This method of analysis, using RIA in conjunction with blood collection cards, has proven to be an effective method to detect blood brevetoxin in finfish exposed to brevetoxin via *K. brevis* even at concentrations as low as 0.5 nM.

IDENTIFICATION OF EUGLENOID ALGAE THAT PRODUCE ICHTHYOTOXIN(S)

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We report toxin production by eucaryotic freshwater microalgae by members of the genus *Euglena*. Fish mortalities (sheepshead minnows, catfish, striped bass, and tilapia) occurred in pond fish and when fish were exposed to unialgal isolates of two species of *Euglena [E. sanguinea* Ehrenberg and *E. granulata* (Klebs) Lemm.-see Figure 1]. Erractic swimming behavior of fish, as well as death of rat cell lines suggest the toxin may be a neuro-transmitter analogue. At least one toxic fraction has been isolated from unialgal isolates of both species, and is water-soluble. The toxin(s) is/are stable at –80° C for at least 60 days and are heat stable to 30° C.

Table 1. Fish killing potential of *Euglena* spp. in laboratory and field samples.

Date	Location	Fish killed	Sample Type	Algal Density (cells/mL)
July-August	field	striped bass	wild (*E. sanguinea)*	unknown
Early August	laboratory	talapia	wild (*E. sanguinea)*	unknown
August 23	laboratory	catfish	wild (*E. sanguinea)*	3,500*
November 7	laboratory	catfish	isolate (*E. sanguinea)*	982
December 13	laboratory	catfish	culture (UTEX LB2345)	1,220
January 15	laboratory	sheepshead	culture (UTEX LB2345)	1,345**

*sample also contained over 1,500 resting cysts
**sample also contained over 1,100 resting cysts

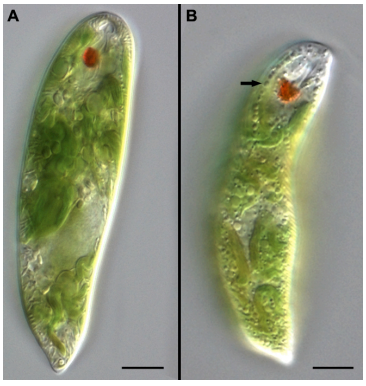

Fig.1. Toxin producing photosynthetic euglenoids. (A) *Euglena sanguinea* isolated from a freshwater pond in North Carolina. (B) University of Texas Culture Collection strain LB2345, now identified as *Euglena granulata*. One of the many rows of subsurface mucocysts is seen at the arrow. Bars, 10 µm.