Accomplishments: 1996-present

1996: Growth Regulation of Toxic Dinoflagellates

Studies on growth regulation in dinoflagellates have defined the molecular mechanism by which the dinoflagellate cell cycle is phased to the diel cycle. Phasing is accomplished by an inhibitory signal in response to blue light. The blue light receptor in dinoflagellate cells has not yet been identified, but the signaling pathway appears to be dependent on cAMP, a signaling molecule involved in transmitting blue light signals in higher plants. Cell cycle regulatory mechanisms in toxic dinoflagellates will yield useful probes to study the dynamics of harmful algal blooms. Additional investigations carried out this year addressed the role of marine biotoxins in regulating growth dynamics in the ciguatera dinoflagellate community. Ciguatera associated toxins have been identified to elicit allelopathic effects against other co-occurring dinoflagellate species. Results of these studies will provide insight into mechanisms initiating ciguateric reef conditions.

Contact: Fran Van Dolah

1997: HPLC-Mass Spectrometry of Polyether Toxins

Highly sensitive and efficient HPLC-MS analyses protocols have been developed for rapid identification and quantification of brevetoxin (PbTx), ciguatoxin (CTX), and okadaic acid (OA), as well as their analogs. This methodology has been optimized to characterize and quantitate these toxins accurately in subnanomolar concentrations. Chromatographic methods as front ends to mass spectrometry have been developed to concurrently allow matrix independent analyses of these toxins. These methods are being implemented to circumvent tedious multiple extractions/pre-purification steps making for more rapid and efficient testing protocols.

Contact: Peter Moeller

1998: Growth Control of Harmful Algal Blooms

Research on the biochemical pathways that control growth of red tide algae provides a new means to understand the processes that initiate harmful algal blooms and to evaluate measures to control growth of harmful algae. These pathways are amenable to chemical and biological intervention, such as that applied to inhibit growth of terrestrial plants. Current research efforts focus on the Florida red tide dinoflagellate, *Gymnodinium breve*, and the ciguatera associated dinoflagellates. Diel phasing of the cell cycle has been characterized in both laboratory cultures and field populations of the Florida red tide dinoflagellate, and the light dependent cues that couple the cell cycle to the diel cycle have been identified. The molecular regulators of the cell cycle have been shown to be sensitive to inhibition by a drug developed to inhibit growth of cancer cells. Studies on allelochemical interactions within the ciguatera dinoflagellate assemblage have identified a novel growth inhibitory compound produced by *Prorocentrum lima* and active against other dinoflagellates. Liquid chromatography-mass spectrometry has determined that this compound is unrelated to okadaic acid, the biotoxin produced by *P. lima*. Structural characterization and mode of action of this compound are currently are currently being addressed with LC-MS and nmr.

Contact: Fran Van Dolah

1999: Evidence of Diarrhetic Shellfish Poison Along the Coast of Maine

An extensive field survey conducted along the coast of Maine for diarrhetic shellfish poison activity in blue mussels yielded positive results with the protein phosphatase 2A activity. This is consistent with the contamination with okadaic acid or related congeners. Phytoplankton populations from these areas containing contaminated mussels were dominated by *Dinophysis norvegica*, a known toxic species. Two additional known toxic species of *Dinophysis* were also found in low numbers: *Dinophysis acuminata* and *D. rotunda*. However, all plankton samples were negative for phosphatase inhibitory activity. Examination of the epiphytic communities from areas with toxic mussels revealed the first occurrence of the toxic dinoflagellate *Prorocentrum lima* reported from the Northeast. Analysis of epiphytic samples rich in *Prorocentrum lima* were phosphastase inhibitory active. Subsequent analysis of these samples using LC-MS/MS showed the production of dinophysis toxin-1 (DTX-1) by wild populations of *P. lima*. Additional analyses are underway to determine which okadaic acid congener is responsible for the activity found in the blue mussels. This study has provided the first evidence of DSP toxins in U.S. coastal waters. *Contact: Steve Morton*

2000: Biomonitoring Hawaiian Green Sea Turtles (Eretmochelys Imbricata) For The Presence Of The Tumor Promoter Okadaic Acid

Fibropapillomas are begin tumors, which in sea turtles are increasing in occurrence. These tumors are fibrous masses of tissue often growing on the eyes, mouth or flippers and on occasions internally in lungs and kidney. These tumors can impede seeing and feeding, are at times fatal and are a threat to recovering species. A joint project with NMFS-Hawaii laboratory was initiated to biomonitor green sea turtles in regions where fibropapillomas are prevalent using the newly developed blood collection card sampling method. The monitoring is conducted for okadaic acid, a dinoflagellate toxin that is a known first stage tumor promoter. To optimize detection limits, a fluorescent microplate assay has been optimized for use with the rapid blood extraction from the collection cards. Regular sampling and analysis is continuing and positive samples will be related to the disease state of the animals. Blood collection cards have permitted the biomonitoring of toxin in living animals and has greatly increased sampling ease in the field. *Contact: Stacie Dover*

Joint Russian-US Survey of the Black Sea Identifies *Prorocentrum Lima*

A joint US-Russian collaboration sponsored by the US Civilian Research and Development Foundation identified a HAB species responsible for diarrheic shellfish poisoning in the Black Sea. Phytoplankton and shellfish were collected throughout the entire Russian coastline of the Black Sea by a joint US-Russian team comprised of scientists from CCEHBR, the Shirshov Institute of Oceanology, and Moscow State University. The dinoflagellate *Prorocentrum lima*, which produces okadaic acid, was identified growing in association with macroalgae, which dominates many parts of the subtidal zone. *P. lima* appeared to have a substrate preference in favor of *Dictyota dichotoma* and *Padina pavonica*, both species non-endemic to the Black Sea, as compared to the dominant indigenous macrophyte species *Cystoseira barbata* and *Cladophora albida*. *P. lima* cells were also identified in mussel stomachs by microscopic examination. Other potentially toxic phytoplankton species (*Dinophysis* and *Pseudonitzschia*) were also observed in low numbers. Further analysis of toxin burden in mussels collected from the Utrish Center for Marine Biotechnology and Aquaculture will be carried out at the Charleston laboratory. Enumeration of preserved phytoplankton communities samples will be examined by Russian partner scientists. Although many toxin producing dinoflagellates are known to be present in Russian coastal waters, there are no regulations regarding contamination in seafood and no statistics available on human poisonings. *Contact: Steve Morton*

2001: Initiation of the South Carolina Phytoplankton Monitoring Network

The inaugural year for South Carolina Phytoplankton Monitoring Network began with great enthusiasm and the opening of a new home page http://www.chbr.noaa.gov/CoastalResearch/SCPMN/SCPMNmain.htm. This community outreach p rogram consists of high school marine science and biology classes monitoring local waters for the presence of possible harmful algal species. Teachers participating in the network attended a workshop on algal identification and sampling techniques. Currently, 12 teachers and approximately 170 students are actively sampling local waters for harmful algae. Based on the observations of these groups, a number of potentially harmful species have been detected in South Carolina, some for the first time. These include representatives of the genera *Prorocentrum, Pseudo-nitzschia, Heterosigma,* and *Akashiwo*. Additional community groups will be added to the network during the next year to extend coverage of this program along the coast of South Carolina. *Contact: Steve Morton*

Toxicogenomics: A Global Approach to Assessing Marine Toxins Exposure and Effects

Toxin exposure almost always causes changes in gene expression, either directly, due to the specific interaction of a toxic agent with its receptor, or indirectly due to the induction of intracellular signaling cascades. Toxicogenomics is the application of DNA arrays to identify a specific pattern of gene expression induced by a particular toxicant. Once a "signature" gene response is identified, this information may be useful for elucidating a toxic mode of action and may potentially yield biomarkers of exposure unique for a particular toxicant or class of toxicants. This year the Marine Biotoxins Program co-organized a workshop on "Toxicogenomics and Nanotechnologies: New Frontiers for Mycotoxins and Phycotoxins" (June 22-23, 2001; Tufts University Bedford, MA) and carried out preliminary studies to determine the suitability of this approach for algal toxin exposure. Changes in gene expression in brains and livers of mice exposed to brevetoxin were studied. Several genes were found to be induced in response to this toxin class. Ongoing studies will determine the dose/response and time course of genetic responses and compare gene induction "signatures" of different algal toxin classes.
Contact: Fran Van Dolah

2002: cDNA Library Provides Molecular Tools to Understand HAB Formation

Understanding the mechanisms that control the growth and toxicity of dinoflagellates has long been hampered by our lack of insight into their molecular biology, stemming from the lack of molecular tools needed for such investigations. Development and screening of a cDNA library containing expressed gene sequences from the Florida red tide dinoflagellate, *Karenia brevis,* was therefore initiated this year to provide some of these tools. This project has yielded novel insights into the intracellular signaling pathways, cell cycle control, and stress response mechanisms present in this dinoflagellate species. To date, 1150 *K. brevis* expressed sequence tags (ESTs) have been sequenced. Of these, 36% have high homology to known genes in the GenBank database. Using these sequence data, we have developed probes for known cell cycle regulatory proteins to study the mechanisms controlling the growth phase of bloom formation and for stress proteins involved in adaptation/survival of *K. brevis* cells as they are exposed to changing water column conditions. Understanding cellular regulation is a prerequisite to developing truly predictive models or species-specific control strategies.
Contact: Fran Van Dolah

Transfer of Receptor Assay Technology to SW African Countries Initiated

The southwest African countries of South Africa, Namibia, and Angola have either historical or recently emerging problems with one or more groups of marine algal toxins. These countries have requested assistance through the U.N. International Atomic Energy Agency (IAEA) in establishing capabilities for receptor assay-based detection of algal toxins in seafood products. A project planning meeting was held at IAEA Headquarters in Vienna, Austria to develop a regional technical cooperation proposal for the transfer of the Marine Biotoxins Program's receptor assay technology to each of these three African countries. This project will be modeled after an ongoing IAEA-sponsored program in SE Asia, with the African end-users visiting the CCEHBR laboratory next year for training and returning to their home institutions to begin conducting the assays. An inter-calibration study coordinated through our Program will follow, and then receptor assays will be implemented as a component of their respective toxin monitoring programs, which are either well established (S. Africa) or currently being developed. Acquisition of receptor-based technology will be of immediate benefit to each of our African partners, given their rapidly growing fishery and aquaculture industries along with the accompanying demands for biotoxin testing of products for export to world markets.

Contact: Fran Van Dolah

Volunteers Monitor Harmful Phytoplankton Along South Carolina Coast

The South Carolina Phytoplankton Monitoring Network (SCPMN) began its second year of existence with over 34 groups monitoring state coastal waters for potentially harmful algal species. A total of over 50 sampling sites from all coastal counties of South Carolina are monitored each week. Volunteer groups are composed of both middle and high school students, state park personnel, and citizen environmental groups. This NOAA sponsored community program serves to increase the awareness of constituent groups about the many issues related to harmful algae and directly involves volunteers in coastal stewardship. In the SCPMN's first year of existence, volunteers observed three potentially toxic algae, including *Pseudo-nitzschia*, *Dinophysis*, and *Prorocentrum lima*. Observation and identification of phytoplankton along the South Carolina coast will be useful in developing a species list and record of distribution, as well as alerting NOAA scientists to the presence of potentially harmful species at the many sampling sites.

Contact: Steve Morton