

NOAA-NATIONAL MARINE FISHERIES SERVICE
SOUTHEAST FISHERIES SCIENCE LABORATORY IN CHARLESTON

FY96 SIGNIFICANT ACCOMPLISHMENTS

MARINE BIOTOXINS PROGRAM

Recent studies of PSP toxigenesis suggest that both dinoflagellates, as well as certain bacteria associated with these algae, are capable of synthesizing PSP toxins. This has been addressed using both laboratory and field based studies. Reintroduction of bacteria isolated from toxic, but not nontoxic strains of *Alexandrium tamarense* was determined to increase PSP production in axenic *Alexandrium* cultures. Field studies conducted in collaboration with the Department of Fisheries and Oceans (Canada) during a red tide bloom in the lower St. Lawrence River estuary determined that bacteria grown from size excluded isolates produced PSP toxins. Continuing investigations will focus on how bacterial-algal interactions influence PSP toxigenesis.

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.

Receptor-based assays for marine biotoxins have been further optimized this year. The assay for domoic acid has been modified to utilize a cloned glutamate receptor, and addition of glutamate decarboxylase to sample extracts has been demonstrated to be useful in removing potential interference due to glutamate. The assay has been utilized successfully by the NMFS Seattle Laboratory for field studies on domoic acid production in diatoms. Receptor assays were demonstrated at the NATO Advanced Study Institute on Harmful Algal Blooms, held in Bermuda, May 28-June 6. Feasibility of using receptor assays for shipboard monitoring of algal blooms was demonstrated on a research cruise aboard the R/V Pelican in the Gulf of Mexico, Sept 6-15.

A rapid and efficient preparative purification protocol for maitotoxin has been developed using a novel combination of preparative electrophoresis and size exclusion media. Using this technique as a front end to a mass spectrometer (universal detector) promises a huge savings in time, personnel, and money in our ongoing efforts to provide preparative amounts of MTX for the preparation of standards and antisera. This new technology is currently being tested and optimized for ciguatoxin and other seafood toxins.

Gambierdiscus toxicus extracts have yielded several highly unsaturated pigments that quench β emissions and interfere with isotopic based assays that are used to monitor the presence of ciguatera related toxins isolated from dinoflagellates. The isolation and characterization of these pigments (peridinen and analogues) is complete. Modifications of bioassay guided fractionation procedures have been put in place in an effort to obviate the problems these agents present.

A new assay technology has been developed for algal toxins. Reporter gene assay have been established using the c-fos response element linked to the coding region for firefly luciferase and this approach has been published (Anal. Biochem.). This method is very effective for measuring ciguatoxins and should permit high capacity monitoring of the toxin in small (< 1 g) finfish samples. A second reporter gene assay has been developed for *Pfiesteria* toxin in collaboration with the NIH and North Carolina State University; it uses the same construct stably expressed in a different cell line.

The hazards of marine toxin exposure to specific groups of seafood consumers have been further elucidated, this time investigating the potential hazard to consumers who may receive repeated subsymptomatic exposures to amnesic shellfish poison (domoic acid). A battery of tests including toxicokinetics, scored symptomatology, working memory assessment and neurodegeneration analysis were used as endpoints to evaluate enhanced toxicity to four repeated exposures to domoic acid. Experiments included both four subsymptomatic and four symptomatic doses and were conducted in two strains of mice, one of which is highly susceptible to drug induced seizures. Repeated exposures did not produce enhanced toxicity in any of the four endpoints, indicating that each exposure is an independent event that is nonadditive. These results were presented in September to Canadian and U.S. regulatory officials.