NOAA-NATIONAL OCEAN SERVICE
CENTER FOR COASTAL ENVIRONMENTAL HEALTH AND BIOMOLECULAR RESEARCH
FY05 SIGNIFICANT ACCOMPLISHMENTS
MARINE BIOTOXINS PROGRAM

Volunteer Monitoring Group Identifies Bloom of *Pseudo-nitzschia* in North Carolina

The Southeastern Phytoplankton Monitoring Network (SEPMN) is a NOAA sponsored community program that serves to increase the awareness of constituent groups about many issues related to harmful algae and directly involves volunteers in coastal stewardship. In January 2005, the SEPMN increased its sampling sites to include 14 groups in North Carolina. Within the first month of sampling, a bloom of *Pseudo-nitzschia* was observed by students of First Flight High School in Kill Devil Hills, North Carolina. Preserved samples sent to the Marine Biotoxins Program were positively identified using scanning electron microscopy as *Pseudo-nitzschia pseudodelicatissima*, a potential producer of the toxin, domoic acid. Subsequent blooms samples as well as cultures of *P. pseudolicatissma* isolated from this bloom tested negative for domoic acid. The identification of *P. pseudodelicatissima* in North Carolina's waters is an excellent example of a volunteer monitoring program helping to develop a species list and record of distribution patterns, as well as alerting NOAA scientists to the presence of potentially harmful phytoplankton species.

Marine Biotoxins Program Contributes to National Plan for Marine Biotoxins and Harmful Algae

The US Science community has been guided by the National Plan for Marine Biotoxins and Harmful Algae for the past thirteen years. The Marine Biotoxins Program hosted a workshop to develop a new National Plan for Marine Biotoxins and Harmful Algae, and contributed substantially to the final report entitled: HARRNESS (*Harmful Algal Research and Response: A National Environmental Science Strategy 2005-2015*). The plan, published by the Ecological Society of America, was developed at the request and support of NCCOS to assemble a broad constituency of scientists and managers that would provide the groundwork shaping the next decade of Federal HAB investment. Accordingly, the process was based on the input of several hundred scientists and managers from a wide range of disciplines, who contributed to the extensive knowledge on which the science strategy is based. This plan will succeed the National Plan on Marine Biotoxins and Harmful Algae (1993) and serve as an important guide for research, monitoring, and response activities at all levels of government both in the United States and abroad.

A New Species of Dinoflagellate Identified from the Hawaiian Islands

Accurate identification is fundamental to understanding the ecology and distribution of any species within an ecosystem. Cultures isolated by researchers at the University of Hawaii at Hilo and representing the dominate dinoflagellate within the local benthic

community known for outbreaks of ciguatera fish poisoning (CFP) were sent to the Marine Biotoxins Program for identification. Using scanning electron microscopy, these cultures were described as a new species named *Ostreopsis tholus*. Since other species of *Ostreopsis* have been reported to be toxic, the potential for toxin production by this new species is currently being evaluated. The description of *O. tholus* will contribute to a better scientific understanding of the ecology of dinoflagellates associated with the human disease CFP in the Hawaiian Islands.

First Identification of Algal Toxins in Shellfish from the White Sea, Russia

People living on the Russian White Sea coast routinely harvest and consume mussels and oysters in the absence of any biotoxin testing. A collaboration between researchers of the Maine Biotoxins Program, the National Research Council of Canada, and the Shirshov Institute of Oceanology conducted the first phytoplankton survey along the White Sea coast of the Russian Federation. During this ecological survey, blooms of the toxin producing dinoflagellates, *Dinophysis acuminata* and *D. norvegica*, were observed. Blue mussels (*Mytilus edulis*) collected during this bloom tested positive for the presence of diarrheic shellfish poisoning activity using the protein phosphatase inhibition assay. Liquid chromatography-mass spectrometry confirmed the presence of several lipophilic shellfish toxin classes, including okadaic acid, dinophysistoxin-1, pectenotoxins, and yessotoxins. This study is the first to demonstrate the occurrence of algal toxins at this location and underscores the need for a monitoring program to insure the safety of shellfish for human consumption.

Trophic Transfer of PSP Toxins Through Gulf of Maine Zooplankton Community

The transfer of marine algal toxins by a range of phytoplankton-consuming vectors exposes organisms at higher trophic levels (including humans) to potentially dangerous toxin levels. The Marine Biotoxins Program, along with collaborators at U. Mass. Dartmouth and the Woods Hole Oceanographic Institution, completed a study examining the contribution of various zooplankton size classes to PSP toxin trophic transfer in the Gulf of Maine. A principal finding of this work was the ability of any grazer size class (64–100, 100–200, 200–500, >500 µm) to serve as an initial vector for introducing PSP toxins into the food web. In addition, the PSP toxin content of the toxic dinoflagellate *Alexandrium fundyense* varied considerably, leading to elevated toxin levels at low cell concentrations and vice versa. Overall, we found that the extent of PSP toxin transfer into zooplankton, and thus into higher trophic levels, is determined by multiple factors, including *A. fundyense* and grazer abundance, algal cell toxin content, and zooplankton community composition. These findings confirm the complexity of PSP toxin trophic interactions and serve to identify several factors that will influence predictive models of resource contamination by dinoflagellate toxins**.**

Hydrilla Confirmed as a Causative Link in the Neurotoxic Disease, Avian Vacuolar Myelinopathy

A disease known as avian vacuolar myelinopathy (AVM), first discovered in 1994, has caused repeated mortalities of bald eagles, great horned owls, and a variety of waterfowl

in the southeastern United States. AVM disease causes lesions in the white matter of the brain and spinal cord of affected animals and lesion pathology suggests a neurotoxin as the likely cause of this disease. Food chain transfer of AVM from herbivorous birds (coots) to top predators (bald eagles and great horned owls) has been established. Further, an association between occurrences of AVM, the vascular water weed *Hydrilla*, and an epiphytic cyanobacterium present on *Hydrilla* in AVM affected lakes has led to the hypothesis that cyanobacteria are the source of a neurotoxin causing AVM. This year, in a Marine Biotoxins Program collaboration with Clemson University and SC Department of Natural Resources, researchers extracted *Hydrilla* collected from AVM-affected ponds and unaffected ponds and examined the toxicity of partially purified extracts in laboratory reared-ducks. A methanol-soluble fraction of *Hydrilla* from AVM-affected ponds was found to cause characteristic AVM lesions in the ducks and confirmed this plant as a causative link in AVM. These findings will help to direct further studies aimed at identifying the toxin(s) causing AVM.

Toxicologic Characterization of Domoic Acid Isomers in New Zealand Shellfish

New Zealand scientists have discovered an unusual form of domoic acid, isodomoic acid C (isoDA C) in shellfish and established its source to be the DA producing diatom *Pseudo-nitzschia australis*. Because of the high levels found in shellfish and its potential threat to seafood consumers, assessing the toxic potential of this isomer was essential. IsoDA C, as well as several other isomeric forms of DA, were isolated and purified by scientists at the Cawthron Institute. In a joint analysis conducted at the Marine Biotoxins Program, Cawthron and NOAA scientists determined the potency of this new form of domoic acid. The isoDA C isomer bound to the same receptor site in the brain as DA, but with an affinity about 100-250 times lower. This information along with results from other tests will be used to provide a more accurate assessment of the potential for human health effects by these DA isomers.

Successful Interlaboratory Calibration Trial of the PSP Receptor Assay Completes Technology Transfer to Five SE Asian Nations

The transfer of a receptor binding assay (RBA) for paralytic shellfish poisoning (PSP) toxins to five SE Asian nations was completed as part of a U.N. IAEA-funded technical cooperation program. The RBA is considered a promising candidate for replacing live animal testing currently used worldwide as the regulatory testing procedure. Under the agreement, scientists from five countries received training on toxin detection methods during ~1 month fellowships at the Marine Biotoxins Program, followed by implemention the PSP RBA in their home laboratories. The transfer process concluded this year with a successful interlaboratory calibration exercise, verifying that each laboratory is capable of correctly utilizing this assay for PSP monitoring. The interlaboratory calibration exercise in SE Asia provided preliminary data for a formal international trial of the assay by the International Association of Official Analytical Chemists (AOAC) in FY06, which will assess its suitability for regulatory testing of shellfish for international trade.

Diagnostic Test for HAB Monitoring Developed and Transferred to South Korea

South Korea depends heavily on aquaculture for a large portion of fish supplying its domestic and export markets, with losses reported to exceed USD $90 million in a single year due to HAB events caused by the dinoflagellate, *Cochlodinium polykrikoides*. Management and mitigation of this problem have been elevated to a national priority. As part of a cooperative effort between NOAA and the Korean Ministry of Maritime Affairs and Fisheries, a rapid, diagnostic test was developed for *C. polykrikoides*. The Marine Biotoxins Program hosted scientists from the Korean National Fisheries Research and Development Institute for a two-month scientific exchange to transfer this assay technology. This automated, molecular probe-based test will be used by Korean coastal managers as a component of the country's routine monitoring program. Early warning of impending HAB activity made possible by this sensitive assay will provide an opportunity to implement mitigation techniques and reduce the potentially devastating economic impacts of *C. polykrikoides*.

Mapping of Paralytic Shellfish Poisoning Toxins in Sea Scallops Identifies a New Southern Limit for PSP in the Northeast US

The harvest of roe-on sea scallops offers a new higher-value market for commercial fisheries, yet opportunities are hampered by higher levels of toxin accumulation in roe than in muscle tissue. In collaboration with NMFS and representatives of the scallop fishing industry, the Marine Biotoxins Program completed the first year of a multi-year study to assess the potential for development of a roe-on sea scallop industry in the northeast US. Scallops in the US are currently marketed as adductor muscle only, due to the potential for paralytic shellfish poisoning (PSP) associated with roe, after an outbreak in 1989. The current study surveyed for both PSP toxins and domoic acid in sea scallops from Georges Bank (Massachusetts) to Delaware. The survey identified the occurrence of PSP toxins south of Cape Cod that was not previously recognized. Moreover, the massive red tide of 2005 has likely further extended the southern boundary of the causative HAB species. Toxin levels in the edible tissues were below regulatory levels at all but one station. The ongoing project will assess seasonal changes in toxin levels present and will test the suitability of ship-board assays for screening. Establishment of a roe-on sea scallop industry could open a $10 million export market to the EU.

Lipoproteins Determined to Transport Brevetoxins in Blood

Brevetoxins can reach high concentrations in blood, yet their fat soluble properties require a means to transport the toxins in the bloodstream. Scientists in the Marine Biotoxins Program have determined that brevetoxin associates with lipoproteins in the blood of laboratory animals and humans. Brevetoxin administered to mice by injection was found to distribute nearly exclusively in the HDL lipid fraction. This finding was confirmed for humans following development of an *in vitro* scintillation proximity assay using purified human lipoproteins and demonstrating real time binding of brevetoxin to the different lipoprotein classes. In humans, brevetoxins associate with each class of lipoproteins, yet show a preference for HDL. Lipoproteins normally serve to transport fat soluble substances such as cholesterol to the tissues for metabolic needs as well as to the liver for elimination. This new information represents a foundation for understanding the

process by which algal toxins are delivered to and removed from tissues, and provides valuable information on potential human susceptibility and therapeutic measures to treat intoxication.

First Time Analysis of Ciguatoxins in Blood

Human ciguatera fish poisoning (CFP) has the highest public health impact of all algal toxin-related syndromes, exceeding cost estimates for all the shellfish poisonings combined by more than twenty-fold. At present there is no means to confirm exposure in humans, as ciguatoxins have never been measured in body fluids of humans or experimental animals. Scientists in the Marine Biotoxins Program have reported successful detection of these toxins in the blood of mice exposed to each of the three ciguatoxin classes originating from the Caribbean, Pacific, and Indian Oceans. This method, which utilizes blood collection cards for sample collection, storage, and extraction, is designed for clinical application. Preliminary testing of a 20 sample subset from a 125 patient case:control study in the Southwest Pacific demonstrated that the method is applicable to humans.

Detoxification Mechanism and Key Urine Biomarker Identified for Brevetoxin

Definition of the body's defense mechanisms against toxins can provide valuable clues to better monitor exposure and reduce toxicity. Scientists in the Marine Biotoxins Program have traced the elimination of the two most common brevetoxins (PbTx-2 and PbTx-3) and determined by mass spectrometry that the former is rapidly transformed to a cystine conjugate metabolite (m/z 1080). This conjugate appears in blood within the first four hours after exposure and rapidly is filtered by the kidneys into the urine. This work identifies for the first time a brevetoxin metabolite and detoxification mechanism in mammals. These findings are important to public health officials and wildlife managers because they establish a biomarker for brevetoxin in urine as well as an exquisitely sensitive method for its analysis.

Domoic Acid Exposure Prior to Birth Determined to Cause Permanent Behavioral Deficits

Early developmental stages of life are susceptible to "silent" effects of toxins that may not manifest until later in life. In collaboration with Duke Medical School, neurobehavioral consequences of prenatal exposure to domoic acid have been determined. Pregnant female rats were dosed with domoic acid and the offspring tested during adolescence and adulthood for spontaneous exploratory behavior, locomotor activity, and habituation, as well as spatial learning and memory. Prenatal domoic acid exposure disrupted the normal sex-difference in spatial learning and diminished cognitive reserve supporting working memory. The results of this work provide the first indication of delayed "silent" effects from early-life exposure to domoic acid levels below the threshold levels that cause acute observable symptoms. This is a significant issue to high

exposure wildlife populations such as sea lions and sea otters, as well as human populations subsistent on shellfish in regions of endemic domoic acid contamination.

Domoic Acid Found to Cause Reproductive Failure in California Sea Lions

Extensive mortalities of California sea lions have occurred repeatedly in recent years as a consequence of domoic acid-producing toxic algal blooms along the California coast. The majority of affected animals are females, with a large percentage in their third trimester of pregnancy. The major pupping grounds for California sea lions are the Channel Islands. Here, hundreds of dead sea lion fetuses were reported concurrent with blooms of domoic acid producing diatoms. In a collaboration with the Marine Mammal Center and Sea World, the Marine Biotoxins Program confirmed maternal transfer of domoic acid to the fetuses. Domoic acid is a neurotoxin that causes seizures in both adult and newborn animals and causes female sea lions to abort their fetuses or to produce still-born pups. The impact of domoic acid on reproductive success raises the question of long term consequences of more frequent domoic acid producing diatom blooms on California sea lions.