MOTE / FMRI COOPERATIVE RED TIDE RESEARCH PROGRAM

FWC Contract No. 99013
Amendment for September 1, 2001 through November 30, 2002

PROGRESS REPORT
FOR THE FIRST REPORT PERIOD

October 31, 2001

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MOTE PROJECTS:

Mote Marine Laboratory Technical Report No. 788
TABLE OF CONTENTS

Page No.
Table of Contents ................................................................. i
List of Figures ................................................................. ii
List of Tables ................................................................. ii
Participants ................................................................. iii

INTRODUCTION .............................................................................. 1

SUMMARY OF ACTIVITIES FOR THE FIRST PROGRESS PERIOD ................. 1

I. Red Tide (HAB) Monitoring ...................................................... 1
   a) Sarasota Area Transects .............................................. 1
   b) Florida Keys Area Monitoring ........................................ 4
      Introduction ............................................................... 4
      Sampling Stations ...................................................... 5
      Results ............................................................... 6
   c) Process Cruise .......................................................... 7
   d) HAB Event Monitoring ................................................ 7

II. Ecology of HABs ..................................................................... 7
   a) Pigment Analyses ....................................................... 7
   b) Karenia brevis Detector (Breve Buster) ....................... 7
   c) Satellite Data Validation ......................................... 9

III. Health Effects of HABs ......................................................... 9
   a) Aerosolized Toxins Associated with Human Occupational Exposure ... 9

IV. Socioeconomic Aspects of HABs ............................................. 10
   a) Assess Clay as a Means to Remove HAB Toxins from Water .......... 10
      Introduction ............................................................... 10
      Methods ............................................................... 10

V. Public Information and Education .......................................... 12

VI. Anticipated Activities for the Second Report Period ...................... 12

REFERENCES ............................................................................. 12
LIST OF FIGURES

Figure 1(a). Vertical cross-sections of Sarasota transect CTD profiles from 9/26/01. ....................................................... 2

Figure 1(b). Vertical cross-sections of Sarasota transect CTD profiles from 10/15/01. ....................................................... 3

Figure 2. Density contours and K. brevis abundance from data collected during Leg A (September 20-26, 2001) of the 2001 ECOHAB Process Cruise. Density contours were created using surface flow-through data (monitored every 15 seconds during the duration of the cruise) while classed cell counts were determined from discrete samples. ............ 5

Figure 3. Locations of Florida Keys Red Tide Monitoring Stations. ..................... 8

Figure 4. Experimental design. Aerial view of limnocorral placement. ................. 11

LIST OF TABLES

Table 1. Station Coordinates of Florida Keys Red Tide Monitoring Stations. .......... 5

Table 2. Results of monthly samplings at stations in the Florida Keys. ................. 6
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INTRODUCTION

Potent neurotoxins produced by the Florida red tide cause massive fish kills, contaminate shellfish and cause severe irritation to the respiratory system of marine mammals. Public health also is affected through exposure to the air-borne toxins and ingestion of contaminated shellfish. A Federal initiative is underway through ECOHAB-FL to determine the factors that control the initiation and development of harmful algal blooms (HAB’s) in the Gulf of Mexico, applying this information toward predictive models. Although the ECOHAB program provides a basis for HAB monitoring and prediction, it is lacking in many aspects of continuous data collection along the southwest Florida coast, does not address critical issues regarding the fate and effects of toxins on marine mammals and on public health and does not provide for management strategies into control and mitigation or for disseminating information to the public. This cooperative research effort between Mote Marine Laboratory (MML) and the Florida Marine Research Institute (FMRI) was undertaken to supplement the ECOHAB-FL program providing information to address issues of special interest to the State of Florida.

This amendment to FWC Contract No. 99013 represents the third year continuation of a cooperative program between MML and the State of Florida FWC-FMRI. This amendment period began on September 1, 2001 and continues through November 30, 2002. This project focuses on five major areas of study including: 1) Red Tide (HAB) Monitoring, 2) Ecology of HAB’s, 3) Health Effects of HAB’s, 4) Socioeconomic Aspects of HAB’s and 5) Public Education and Outreach. Research activities within each category are summarized below.

SUMMARY OF ACTIVITIES FOR THE FIRST PROGRESS PERIOD

I. RED TIDE (HAB) MONITORING:

a) Sarasota Area Transects

Two transects were conducted during this reporting period on the following dates: September, 26 and October, 15, 2001. These transects included profiling casts of a SeaBird SBE-19 CTD that included sensors for the determination of dissolved oxygen, chlorophyll fluorescence, and photosynthetically active radiation (PAR). The resulting transect cross-sections are illustrated in Figures 1(a) and 1(b). As indicated by the density cross-sections the water column was well mixed during each transect yet nearshore surface fluorescence features were apparent in both. Cell counts from these inshore stations showed that the high chlorophyll a fluorescence signals were attributed to high abundances of Karenia brevis. It should also be noted that the water column density values associated with these blooms were within the apparent optimal sigma-t range of 21.5 to 23.0 kg m⁻³.
Figure 1(a). Vertical cross-sections of Sarasota transect CTD profiles from 9/26/01.
Figure 1(b). Vertical cross-sections of Sarasota transect CTD profiles from 10/15/01.
Brevetoxins were monitored in water and air from several transects and surveys during the first project period. The dates and samples collected are listed below. The samples are in the process of analysis and data processing.

- **9/4/01 Transect from MML to Boca Grande**  
  Samples collected; Aerosols, whole water, and intra and extra cellular water.

- **9/6/01 Transect from MML to North Longboat Key**  
  Samples collected; Aerosols, whole water, and intra and extra cellular water.

- **9/10/01 Diel Study 1 mile offshore MML**  
  Samples collected every 4 hours for 36 hours; Toxins in whole water and intra-cellular and extra-cellular toxins.

- **9/26/01 ECOHAB Transect**  
  Samples collected; whole water.

- **10/15/01 ECOHAB Transect**  
  Samples collected; whole water.

**b) Florida Keys Area Monitoring**

**Introduction:**

The Florida Keys Red Tide Monitoring Project began in December 1999. Method validation and establishment of stations was completed by early February 2000. Regular data were obtained from the Lower Keys Transect at approximately monthly intervals from February 2000 through March 2001, along with temperature and salinity measurements. In February 2001, stations in the Key West area and along the entire Lower Keys in Florida Bay were established, and weather permitting have been monitored bi-weekly since (Figure 3). In addition, measurements of temperature, salinity, pH, and dissolved oxygen have been recorded at each station since April 2001 using a Hydrolab Datasonde 4a. In addition to the monthly sampling protocols, the Project Team responds to reports of possible red tides throughout the Keys to verify the presence of *K. brevis* and, if present, determine cell concentrations. A “Marine Observer Network” has been established as part of the Marine Ecosystem Event Response and Assessment (MEERA) Project with funding from the Florida Keys National Marine Sanctuary, which provides reports of discolored water or fish kills that might indicate bloom development.
Sampling Stations:

![Sampling Stations Diagram]

**Figure 3.** Locations of Florida Keys Red Tide Monitoring Stations.

**Table 1.** Station Coordinates of Florida Keys Red Tide Monitoring Stations.

<table>
<thead>
<tr>
<th>Station</th>
<th>Location</th>
<th>Latitude(N)</th>
<th>Longitude(W)</th>
<th>Depth(ft)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT04</td>
<td>Summerland Key</td>
<td>24.64009°</td>
<td>81.45865°</td>
<td>6</td>
</tr>
<tr>
<td>RT08</td>
<td>N. of Sawyer Key</td>
<td>24.78364°</td>
<td>81.56958°</td>
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</tr>
<tr>
<td>RT10</td>
<td>Marathon</td>
<td>24.70714°</td>
<td>81.12505°</td>
<td>~15</td>
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<td>Key West - Offshore</td>
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</tr>
<tr>
<td>RT12</td>
<td>Key West - Hawk Channel</td>
<td>24.46773°</td>
<td>81.78203°</td>
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</tr>
<tr>
<td>RT13</td>
<td>Key West - Harbor</td>
<td>24.51067°</td>
<td>81.80447°</td>
<td>18</td>
</tr>
<tr>
<td>RT14</td>
<td>Key West - Northwest Channel</td>
<td>24.55421°</td>
<td>81.81337°</td>
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<tr>
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<td>RT16</td>
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<tr>
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<td>24.64097°</td>
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<td>14</td>
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<td>Key West - Florida Bay</td>
<td>24.68048°</td>
<td>81.90799°</td>
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<tr>
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<td>Smith Shoals</td>
<td>24.71724°</td>
<td>81.92366°</td>
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<td>RT20</td>
<td>NE. of Marquesas</td>
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<td>82.02452°</td>
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<tr>
<td>RT21</td>
<td>N. of Marquesas</td>
<td>24.71731°</td>
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<td>24.73238°</td>
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<td>RT23</td>
<td>NW. of Saddlebunch Keys</td>
<td>24.75000°</td>
<td>81.74417°</td>
<td>33</td>
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<td>RT24</td>
<td>NW. of CudJoe Key</td>
<td>24.76532°</td>
<td>81.65648°</td>
<td>27</td>
</tr>
</tbody>
</table>
Results:

Results from the regular monthly samplings are shown in Table 2. All stations showed no signs of *K. brevis* present during sampling. Although water quality measurements were taken at multiple depths for each station, only surface water measurements are shown below.

Table 2. Results of monthly samplings at stations in the Florida Keys.

<table>
<thead>
<tr>
<th>Date</th>
<th>Station</th>
<th>Temp(°C)</th>
<th>Sal(ppt)</th>
<th>DO(mg/L)</th>
<th>pH</th>
<th>Cells/L</th>
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<td>35.61</td>
<td>5.83</td>
<td>8.58</td>
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<td>35.92</td>
<td>5.62</td>
<td>8.51</td>
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</tr>
</tbody>
</table>
c) **Process Cruise**
ECOHAB-FL Process Cruises were undertaken from September 20-26 (leg-A), and October 20-26, 2001 (leg-B) aboard the R/V Suncoaster. High abundances of *K. brevis* were identified on the west Florida continental shelf between Tampa Bay and Charlotte Harbor before each leg of the cruise therefore little time was spent searching for a bloom patch. Leg-A focused on issues of cell cycle while the emphasis of leg-B was bio-optics. Each cruise was comprised of diel studies as well survey components.

*Karenia Brevis* distributions during leg-A were primarily restricted to neashore waters although a patch was identified greater than fifty nautical miles offshore of Charlotte Harbor (Figure 4). The highest cell abundances were identified in waters having sigma-t values of less than 22.5 kg m⁻³.

Brevetoxins concentration and distribution was determined in water and air samples during the 7-day research cruise aboard the R/V Suncoaster (September 20 to September 26, 2001). Samples were collected at 4-hour intervals including in conjunction with two 24-hour diel studies. These data are in the process of analysis and interpretation.

d) **HAB Event Monitoring**
Three surveys were conducted during this reporting period in response to a HAB event first identified in August 2001. Pre-cruise surveys were conducted before each leg of the 2001 ECOHAB Process cruise on the following dates: September 6, October 12, and October 20, 2001. Patches of *K. brevis* were identified during each survey which not only helped direct the initial search on board the R/V Suncoaster but helped track the bloom over the course of time.

II. **ECOLOGY OF HABs:**

a) **Pigment Analyses**
Filters for HPLC pigment analyses were collected during all surveys, transects and ECOHAB Process Cruises during the reporting period. HPLC analyses of the collected samples is currently underway.

b) **Karenia brevis Detector (Breve Buster)**
The instrument was implemented in flow-through mode on board the R/V Suncoaster during the October leg of the 2001 ECOHAB Process Cruise. The similarity index for relative *K. brevis* abundance and absorption spectra for the discrimination of colored dissolved organic material (CDOM) were simultaneously monitored during the cruise. These data will compliment the information obtained using profiling bio-optical instrumentation.
Figure 4. Density contours and *K. brevis* abundance from data collected during Leg A (September 20-26, 2001) of the 2001 ECOHAB Process Cruise. Density contours were created using surface flow-through data (monitored every 15 seconds during the duration of the cruise) while classed cell counts were determined from discrete samples.
c) Satellite Data Validation
Remote sensing at MML focused on the acquisition and processing of satellite images. A considerable amount of effort was expended investigating the availability of high resolution SeaWiFS images. Not only is it required that images correspond to cruise dates but such images need to be free of clouds as well. MML used the Seadas software and images provided by NASA to process satellite derived chlorophyll a values for regions where K. brevis abundance was high. Ground truth information exists for these images since the dates were chosen according to ECOHAB-FL cruise dates.

III. HEALTH EFFECTS OF HABs:
Numerous anecdotal reports exist about the human respiratory effects for exposure to aerosolized K. brevis. However, little has been published measuring the effects on the human respiratory system. The Center for Disease Control and Prevention, the Florida Department of Health, the University of Miami School of Public Health and MML conducted the first phase of a study to assess the changes on human respiratory function in people who are occupationally exposed to red tide. On September 7-11, 2001 eleven Sarasota County lifeguards volunteered to participate in the study. The study was conducted daily over the five-day period. A brief health history questionnaire was completed as well as pre and post shift symptoms questionnaires. Pre and post shift spirometry and nasal and throat swabs were also obtained on participants. The non-exposure component of the study, which will be involve the same 5-day study during no red tide, is planned for May 2002.

a) Aerosolized Toxins Associated with Human Occupational Exposure
Brevetoxins were monitored in air and water on Lido Beach and Siesta Key Beach in Sarasota, Florida, during a red tide episode, from September 7-11, 2001. Three collection locations were established at beach level adjacent to lifeguard stations on Lido Beach and one location with three samplers was established for Siesta Beach; two ground-level air samplers and one placed about 3 m up on the lifeguard stand.

Water samples were collected in 1-L glass bottles. Brevetoxins were extracted by passing the water through a C-18 solid-phase extraction disk under vacuum (Ansys Technologies, Inc.; Lake Forest, CA). The C-18 disks were then rinsed with RO water to remove any remaining salts and eluted with methanol, according to the method of (Pierce et al., 1992). Concentrated samples were injected in methanol onto a Shimadzu SPD-M6-A diode array HPLC. The mobile phase was 1 ml per minute isocratic 85:15 methanol:water using a 250x4.6 mm 5μm C-18 column. Detection was from 200-300 nm with quantitation at 215 nm.

The air samplers were fitted with a 8”x10” glass fiber filter (Whatman EPM2000; Maidstone, England) and allowed to run for approximately 5 hours. As the filters were collected from the samplers, they were placed in aluminum foil and cut in half. One-half to MML for processing solvent extraction and HPLC analyses, and the other half to UNC Wilmington for direct ELISA analyses and for supercritical fluid extraction and HPLC analyses. For
transport to MML, the filters were placed in glass jars, covered with dichloromethane (DCM) according to the method of Pierce et al., 1990. The glass fiber filters were extracted at MML using a Soxhlet apparatus, in DCM. Samples were allowed to extract overnight. The DCM was evaporated using a rotation evaporator. Samples were transferred to vials using methanol for HPLC analysis. HPLC analysis was conducted as described for the water samples above.

IV. SOCIOECONOMIC ASPECTS OF HABs:

a) Assess Clay as a Means to Remove HAB Toxins from Water

Introduction:
This study was designed to assess the removal efficiency of locally-available phosphatic clay against *K. brevis* and its toxins, during a natural red-tide bloom in Sarasota Bay (FL), and, to evaluate the possible adverse impacts of the settled clay and toxins on benthic fauna and associated water quality. Brevetoxin fate studies were funded partially by this project, water quality and benthic studies were funded through a separate source.

Methods:
The study area was located in Sarasota Bay adjacent to MML’s bay dock (water depth down to 3 m). This site was experiencing a red-tide bloom of moderate to high concentrations that had begun more than one month prior to this study. Cell counts made at the time of the experiment gave a range of $5 \times 10^5$ to $1 \times 10^6$ cells/L.

These experiments were conducted using three, clear, polyvinyl limnocorals with open ends (diameter=2 m, height=3 m). They were deployed from a small boat with the help of 2 or 3 divers. The open bottoms were then anchored over the test plots to insure that the they receive clay/plankton treatment. The limnocorals were arranged linearly, parallel to the shore, and were equally spaced about 2 m apart (Figure 5). Two limnocorals were treated with clay, and the other served as a control. The open area near the control was also monitored for changing ambient conditions.

Based on previous laboratory trials and a consideration of the ambient cell concentration at the time of the experiment, a suitable clay loading of 0.25 g/L (final) was determined. This concentration translated to 1.4 kg of phosphatic clay (dry weight) per limnocoral. The material was soaked in filtered seawater overnight, processed in a blender, and then filtered through a 63-micron metal sieve. The resulting slurry was dispersed into the limnocorals using a submersible pump with a hose and nozzle attachment. Aggregation was allowed to proceed for 4 h.
Water quality, *K. brevis* concentration and toxin concentration were monitored before and after the addition of clay. The methods are described in the following section. The limnocorals were kept in place for 24 h to allow for the undisturbed settling of the clay. In addition, benthic core samples were collected from each of the four sites one day before the limnocorals were deployed, and 7 days after clay application to assess the impact on benthic fauna.

Water samples were collected before clay addition, and at 1, 2 and 4 h after clay addition to determine cell counts, pigments, toxin concentration, species composition, turbidity and inorganic nutrients. Tygon tubing was lowered near the center of each limnocorral to 0.5 m and 2.5 m depth, at which 4 L of water were withdrawn using a vacuum pump. Subsamples were then processed.

Water samples were collected again at 24 h (just before the limnocorals were removed) and again at 96 h. For *K. brevis* counts, water samples were fixed with an iodine solution and the cells were enumerated using a hemocytometer. Brevetoxins from water samples were recovered by elution through a C-18 solid-phase-extraction disk and extracted off the disk into methanol. The volume was reduced to 3 ml for HPLC-DAD analyses. Toxin analyses was also performed from samples obtained from 5-L sediment traps placed near the bottom of the limnocorals.

Three 5-L traps were placed within each limnocorral and at the ambient site to collect the flocculated material for toxin analyses. These traps were retrieved at 4, 24 and 96 h, respectively, to assess the amount of toxins associated with the clay floc and to observe toxin degradation over time. The 5-L sediment traps were allowed to stand for 24 h as the clay continued to settle. The supernatant liquid was carefully poured off and the remaining wet clay was transferred to 50 ml centrifuge tubes and dewatered by centrifugation at 2800 rpm for 4 min. The clay pellet was extracted three times using an ultrasonic probe in acetone. The acetone was evaporated and transferred to a vial in 3 ml of methanol for HPLC analysis.
V. PUBLIC INFORMATION AND EDUCATION:

The public information portion of this project is a collaborative project between MML, S.T.A.R.T., and FMRI. Information and education has been provided directly to the public through a special HAB internet web site providing background information about red tides and their effects on the environment as well as public health. During the recent red tide event, MML and FMRI personnel have shared information and were in constant contact with the National Center for Disease Control (CDC) and the Florida Department of Health (DOH) for toxin aerosol reports and with local media to provide accurate information to the public for education and public health issues. In addition, we have provided Red Tide Fact Sheets to the County Health Departments, public media and local citizens groups.

VI. ANTICIPATED ACTIVITIES FOR THE SECOND REPORT PERIOD:


2. Continue monitoring near-shore Sarasota areas including cell counts, water quality profiles, pigments, toxins, bioaccumulation and toxin aerosol.

3. Monitor for red tide bloom passage through the Florida Keys.

4. Continue assessing the use of clay as a medium for mitigation and control of red tide blooms.

5. Apply LC-MS and develop capability for analysis of brevetoxins and major metabolites produced by marine organisms.

REFERENCES
