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Final Report: ECOHAB: Control of Harmful Algal Blooms using Clay

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Objective:

Harmful algal blooms (HABs), or red tides, result from the rapid proliferation and accumulation of certain microalgal species in surface waters, most of which can have profound, devastating, and deleterious effects on public health, aquaculture, tourism, and aquatic ecosystems. Although the significance and recurrence of these and other red tide phenomena would seem to justify bloom control as a high-priority topic for research, virtually no focused investigation on control has been conducted in the United States for nearly 40 years (Boesch, et al., 1997). Instead, the primary objectives of past and ongoing research on *Karenia brevis*, and other HABs in the United States, have been to obtain a fundamental understanding of the biological, chemical, physical, and ecological processes underlying blooms and their impacts. Notably, the gap in research addressing the control of marine species is in stark contrast to work conducted in the terrestrial environment, where human efforts to control insects, diseases, and weed species are more common. The reasons for this lack of effort are many (Anderson, 1997). The lack of research progress is not due to an absence of strategies worth exploring, but rather to a preference on the part of the HAB community and in general, to pursue fundamental research to better understand bloom phenomena. The rationale for this one-sided approach has been that fundamental understanding will be essential for man to ever manage or mitigate blooms (i.e., we cannot control what we do not understand). However, understanding is a subjective concept, and after decades of research on HABs, most scientists still have pressing questions to answer and more details to resolve. Hence, research into proactive bloom control and mitigation has remained a secondary or tertiary priority.

To date, the most promising strategy for controlling red tides in the field is the application of suspended clay particles over the bloom to flocculate and settle the algal cells (Anderson 1997; Boesch, et al., 1997). This method has been investigated and effectively used during red tide events in Japan (Shirota, 1989) and South Korea (Na, et al., 1996; Bae, et al., 1998; Choi, et al., 1998; Choi, et al., 1999) to protect vital mariculture operations. Due to its effectiveness and practicability, clay dispersal has become a part of South Korea's management scheme. Furthermore, clay minerals were deemed suitable for this purpose because they are naturally occurring particles that are commonly found in marine systems, abundant, readily available, relatively inexpensive, and easy to use. Moreover, clay addition is thought to have a low probability of causing environmental damage. For these reasons, we pursued the research program presented here to investigate the potential application of clay control in the United States, primarily focusing on locally available clays against problematic HAB species in U.S. coastal waters.

The general objectives of this research project were to use laboratory cultures, aquaria, and mesocosms to: (1) determine the removal efficiencies of selected clays on three HAB species found in U.S. waters (the Florida red tide dinoflagellate *Gymnodinium breve*, the New York brown tide chrysophyte *Aureococcus anophagefferens*, and fish-killing *Pfiesteria*-like dinoflagellates; (2) determine the variability in cell removal efficiencies due to such factors as species differences, growth stage, and cell density; (3) determine whether cell removal efficiencies can be improved by addition of alum or polymeric coagulants; (4) investigate changes in water chemistry following clay treatment including the release or removal of nutrients such as phosphorus and nitrogen, release of radioactivity, trace metals, and other toxicants; (5) investigate toxin release and uptake by clays during the flocculation process; (6) investigate the use of clays to mitigate the impacts of aerosolized brevetoxins; (7) conduct larger scale aquarium and mesocosm studies to examine the flocculation efficiencies on natural plankton assemblages and to assess the impacts of sedimented biomass and toxins on benthic organisms; (8) evaluate the engineering requirements, economic costs, and environmental clearances that must be addressed if this control strategy is to be applied to blooms in natural waters; and (9) design a pilot program for field application of this bloom mitigation strategy.

Summary/Accomplishments (Outputs/Outcomes):

Removal Efficiencies of Selected Clays on Three HAB Species Found in U.S. Waters (*G. breve*, *A. anophagefferens*, and *Pfiesteria*-like dinoflagellates). To date, we have tested more than 25 different clay minerals from domestic sources for their removal abilities against *K. brevis* (formerly *G. breve*), *A. Anophagefferens*, and *Pfiesteria piscicida*. In general, clays such as montmorillonite and a polymer-treated (cationic) kaolinite were highly effective (>90 percent) at removing *K. brevis* and *P. piscicida* from suspension within the 2.5-hour duration of the experiments. Conversely, none of the clays tested against *A. anophagefferens* achieved greater than 35 percent removal efficiency (RE). We then found that by gently agitating the cell culture after the clay slurry was added, essentially dispersing the clays throughout the medium instead of layering them near the surface, cell RE for *A. anophagefferens* increased to approximately 80 percent relative to the unmixed suspension (using the same clay at the same loading). Additional experiments revealed that by prolonging the flocculation time (up to 7 hours) and increasing the salinity of the medium used to prepare the clay slurry, cell RE could be improved by a factor of two from the initial surveys. These latter findings have led us to speculate that the removal of this tiny species with clay is limited by the encounter (or collision) rates between clays and cells in our quiescent system. Therefore, methods that introduce water motion such as brief agitation, or that prolong the time for flocculation, appear to enhance overall cell removal. Moreover, we further hypothesized that cell removal is highly dependent on the size of the clay particles introduced to the system relative to the size of the organism (i.e., the closer the clay particle size is to the cell size, the better the RE). We believe that this hypothesis can explain the improvement in cell removal when higher salinity water was used to prepare the clay slurry. With the use of more saline water in the clay slurry dispersed during the treatment, the clay particles left the surface layer much sooner and entered the cell culture as smaller particles due to the lack of a density difference between the slurry and the cell suspension. Furthermore, brief agitation and homogenization of the clay-cell suspension may have not only increased interparticle contacts, but decreased the size of the intercepting clay particles due to the shearing of larger clay aggregates.

In experiments with *K. brevis*, the removal ability of clay minerals far exceeded those of several nonclay minerals (e.g., diatomaceous earth, apatite, and volcanic glass). Additional tests using phosphatic clays from various locations in central Florida and from different processing stages revealed slight variations of removal ability at a loading rate less than 0.10 g/L. However, their effectiveness became uniform above this value. This clay also showed the ability to kill cells as a result of direct physical contact with the organism. Cell mortality increased with increasing clay loading and with longer contact with the algae. Likewise, up to 95 percent of the *P. piscicida* cells trapped in the settled flocs were killed using a cationic polymer-treated kaolinite, H-DP.

The focus of our future work will be on the Florida red tide organism, *K. brevis*, and on the clay minerals that have shown the highest removal ability, namely the montmorillonite-rich phosphatic clay, other pure montmorillonites, and H-DP. More work will be conducted on H-DP to understand how salinity affects its removal ability. In addition, our design of a dispersal apparatus in future larger scale experiments also will consider some of our empirical findings such as minimizing clay particle size to increase residence time and promote particle contacts, maximizing interparticle contacts through turbulent clay dispersal, and controlling clay particle charge in the slurry and upon dispersal over the bloom.

<u>Factors Affecting Cell Removal by Clays and the Mechanism of Clay-Cell Flocculation</u>. Laboratory experiments were conducted to determine how factors such as particle concentration, particle size, surface chemical properties, cell swimming speed, and culture salinity can influence overall cell removal efficiency. From these empirical studies, a working understanding and model of clay-cell aggregation can be formulated and tested.

In our studies, removal of any given algal species from a wide range of taxa generally increases with increasing clay concentration for all types of clay minerals. However, these cell-removal trends varied from linear to sigmoidal or hyperbolic as loading increased. The case was not the same for increasing algal concentrations treated with a given clay mineral. In a survey using Florida phosphatic clay from IMC Phosphates, Inc., one of the largest phosphate producers in Florida (IMC-P2) against a wide variety of algal species from several taxonomic groups (e.g., Dinophyceae, Chrysophyceae, Bacillariophyceae), cell removal showed increasing, decreasing, and constant trends as phosphatic clay loading increased. The result was species-dependent. For example, *K. brevis* displayed increasing cell removal with increasing cell concentration (at a given clay loading). Eighty percent or higher cell removal was not seen until cell concentration exceeded approximately 1,000 cells/mL. Other species with increasing trends and high cell removal included *Heterosigma akashiwo*, *Alexandrium tamarense*, and *Akashiwo sanguinea* (formerly *Gymnodinium sanguineum*). With respect to patterns of cell removal, there was no correlation between RE and algal taxa, cell size (i.e., projected cross-sectional area), or swimming speed. However, a correlation was found between the RE of motile cells and the predicted particle collision frequency between clays and cells based on established physicochemical models. The latter parameter incorporates both cell size and swimming speeds. Finally, we determined that phosphatic clay can remove two species in a mixed culture differentially. When *K. brevis* (high RE) was mixed in different proportions with *Prorocentrum micans* (low RE) and treated with IMC-P2, *K. brevis* removal remained constantly high and *P. micans* remained low. In fact, the removal of *K. brevis* slightly increased in the presence of *P. micans*, which may indicate that even inefficient removal of a co-occuring algae may influence how *K. brevis* is removed. Similar results were found with *K. brevis* mixed with Skeletonema costatum, a cosmopolitan diatom species.

Using the ZetaPALS system at Clarkson University (NY), the net surface charge and electrophoretic mobility (EPM) was directly measured for a number of algal species and clay minerals. With the EPM, the surface (or zeta) potentials of both marine algae and clays (suspended in freshwater and seawater) were calculated and compared. Based on this analysis, there were no significant differences among the EPMs and zeta potentials of marine algae that could explain their differential removal by a given clay (e.g., phosphatic clay). Although there were vastly different EPMs for the clay minerals suspended in freshwater (salinity = 0), ranging from net positive charge (e.g., polymer-treated [cationic] kaolinite) to highly negative charge (e.g., bentonite), there were no significant differences among the clays when they were suspended in seawater (salinity = 29). In fact, differences among the EPM's of clay vanished when salinity was as low as 1. Therefore, based on this analysis, the varying ability of different clay minerals to remove a given algal species (e.g., *K. brevis*) could not be explained by simply measuring the surface charge. However, when these results were combined with a kinetic study of clay-cell aggregation with clay particles of uniform size, differences among the RE of clay minerals were clearly apparent.

Tracking the removal of clay particles and algal cells over time, the sinking loss of *K. brevis* closely followed the loss of phosphatic clay and bentonite, both resulting in high RE (>96 percent). In the case of *K. brevis* and untreated kaolinite, the sinking loss of both particles initially was in tandem, but diverged after the first 0.5 hours in the experiment, leading to cell removal greater than 50 percent. This divergence suggested possible escape of cells from the falling aggregates. These studies have demonstrated that the flocculation process responsible for clay-cell removal can be conceptualized using established concepts of physicochemical aggregation. However, our present understanding of the clay-cell flocculation model remains incomplete due to the high degree of complexity inherent in this system (e.g., particles with various sizes, behavior, surface chemistry, salinity, and hydrodynamics). Nevertheless, these studies have begun to elucidate some of the important features of the process that can be translated to larger scales. Presently, our working model for clay-algae removal involves direct flocculation between the organisms and the clays to form fast-sinking aggregates. Then, more cells are removed from suspension as these aggregates fall and sweep through the water column, entraining cells in the process ("sweep floc").

In studies with *P. piscicida*, changing the salinity of initial culture medium and the salinity of the initial clay slurry produced changes in the overall cell removal. In both cases, when the salinity of the culture medium was increased from 15 to 30 or when the salinity of the clay slurry was increased from 0 to 30, cell removal declined drastically. One explanation may be that higher salinity led to higher clay-clay flocculation, which then influenced their sinking rates and loss from the system. As the clays sink from the culture more quickly, they have less opportunity to interact with the organisms.

<u>Use of Coagulants and Flocculants To Enhance Cell Removal in Mesocosm Tanks Using</u>

Clays. Chemical coagulants and flocculants are used in water and wastewater treatment to promote the aggregation and settling of very fine particles. We tested these additives in combination with clays to determine whether they can further enhance cell removal by increasing the "stickiness" of clays. Of the chemicals tested, polyaluminum hydroxy-chloride (PAC) (Superfloc™ 9001, Cytec Industries) showed the best results in combination with the most effective clays used with *K. brevis*, *P. piscicida*, and *A. anophagefferens*, respectively.

In additional trials, the experiments were conducted in larger volumes (e.g., 2.5-m plexiglass core tubes with a 9-cm inner diameter). Unfortunately, the PAC-treated clay rapidly flocculated with itself, forming large, baseball-sized aggregates that quickly fell to the bottom. Within the first few minutes, the clays were deposited and cell removal was understandably low. After a number of repeated trials using alternative dispersal strategies, the best results were obtained using highly diluted PAC dispersed in seawater, which was first added to the culture and allowed to diffuse through the upper water column before the clays were added. The two to five-fold improvement in RE using this method was confirmed during a series of mesocosm experiments conducted in Corpus Christi, TX, during an actual *K. brevis* bloom. Treatments were performed in six fiberglass tanks (530 L each) filled with bloom water. Using only 4 ppm of PAC and 0.10 g/L of phosphatic clay, the results were very promising (RE >81 percent).

In laboratory tests using the same core tubes described above (i.e., modified settling columns made from 2.5-m plexiglass with a 9-cm diameter), we also performed experiments using phosphatic clays alone (no PAC) with *K. brevis* to further investigate particle dynamics in space and time. We were able to take discrete samples at four depth intervals along the 2.0-m water column at specific times during the entire flocculation and sedimentation process. Cell concentration was determined by *in vivo* fluorescence, while clay concentration was measured concurrently by turbidity and spectrophotometry. We observed a strong correlation between cell loss at each depth with increasing clay concentration at that depth as the clay pulse arrived. The highest cell removal was always found 0.25 m from the surface, where the highest clay concentration was observed soon after dispersal. As the clay sank, a pulse of turbidity was detected at subsequent depths (0.75, 1.25, and 1.75 m from the surface), but the magnitude of the pulse (or the amount of clay) progressively decreased with increasing depth as the clay pulse was diluted across the entire water column. The clay pulse arrived at the lowest depth (1.75 m), regardless of the clay concentration, within 60 minutes of surface addition. As the amount of clay decreased with depth, cell removal also decreased. At a clay loading of 80 g/m^2 (or 0.04 g/L in this column), RE dropped from 75 percent at 0.25 m to only 25 percent 1.75 m from the surface. At 200 g/m^2 (or 0.10 g/L), RE started at 85 percent at 0.25 m and decreased to only 80 percent at 1.75 m. Finally, RE was uniform at 91 percent from surface to depth when 500 g/m^2 (or 0.25 g/L) was dispersed. These findings suggest that to maintain sufficiently high cell removal in the water column, the scaling factor for loading of clay must consider the depth of the water column. Furthermore, these results indicate the possible means of dispersing clay to maximize its effectiveness. First, the effective concentration must be maintained throughout the entire depth of the water column, where the target organisms are found to ensure removal. This means that dispersal methods may have to be adjusted to the intervals when the organisms are concentrated within a narrow band near the surface, or that dispersal may have to be added in pulses or beneath the surface. These issues will be addressed in the next phase of the project.

Resuspension properties of untreated phosphatic clay and PAC-treated clay also were tested in flow using a 17-m racetrack flume at WHOI. Cultures of *Heterocapsa triquetra* were used in lieu of *K. brevis* due to potential problems with toxins. The target cells and phosphatic clay were placed in a fenced portion and allowed to settle over the test bed. The material was allowed to consolidate for 3 hours, after which the layer was subjected to increasing flow. Flow speeds causing initiation of transport, bed-load transport, and full resuspension were noted and documented on video. The experiment was repeated for a clay-cell layer aged for 9 and 12 hours, and again for PAC-treated clays. Results indicated that the longer the material is allowed to consolidate, the higher the flow speed required for resuspension. Moreover, the addition of PAC lowered these values, suggesting that the material was more "fluffy" and thus easier to resuspend.

<u>Examination of Changes in Water Chemistry Following Clay Treatment, Including the Release or Removal of Nutrients Such as Phosphorus and Nitrogen, Release of Radioactivity, Trace Metals, and Other Toxicants</u>. The focus of this analysis was on the three most promising clay samples for removal of *K. brevis*, *P. Piscicida*, and *A. anophagefferens*: IMC-P2, Wyoming bentonite (WB-B), and H-DP. Our first task was to determine whether these clays (and the flocculant, PAC) added inorganic nutrients into the seawater medium. We used Sargasso seawater with low nutrient content as the base medium. In terms of nitrate, WB-B released significant amounts of this nutrient, while IMC-P2 and H-DP did not. Likewise, PAC added alone did not release nitrate to the medium. With respect to ammonium concentration, none of the clays tested (or PAC) added significant amounts of this nutrient into the seawater. However, phosphatic clay released significant amounts of phosphate into the seawater, as expected. The addition of WB-B, H-DP, and PAC did not significantly contribute to the seawater phosphate concentration. Finally, WB-B significantly increased the concentration of silicate in the system, while the two other clays and PAC did not.

In a second study, the above experiments were repeated using Sargasso seawater containing a known amount of each nutrient (i.e., "spiked"). Furthermore, the H-DP and IMC-P2 also were tested in combination with PAC (at 5 ppm). With respect to nitrate (40 μM spike), all of the clay and clay + PAC treatments significantly reduced the amount of dissolved nitrate. However, the greatest reduction was observed with PAC-treated phosphatic clay, which saw a change by two orders of magnitude (i.e., from 4 μM to 0.046 μM). Although both untreated and PAC-treated H-DP decreased the amount of the nitrate spike, there was no significant difference between the two. Similarly, all of the clays (with and without PAC) were able to decrease the amount of ammonium spike (4.5 μM) in the medium, mostly to about one-half of the ammonium concentration. Again, the greatest reduction was achieved by PAC-treated phosphatic clay, which produced a decrease of one order of magnitude in ammonium concentration (i.e., from 4.5 μM to 0.45 μM).

For phosphate, 2 μM were added to the ambient seawater. In this case, the highest reduction in dissolved phosphate was observed with the use of untreated and PAC-treated H-DP. H-DP alone decreased the amount of phosphate by a factor of 5.5, while H-DP with PAC combined decreased the amount by a factor of 9. The application of WB-B also saw a reduction in phosphate, but only by a factor of 2.5 in this experiment. The use of phosphatic clay alone resulted in the reduction of phosphate content by a factor of 1.2. However, when PAC was added to phosphatic clay, phosphate concentration decreased by a factor of 3.6 in the final suspension. The reduction on phosphate content produced by PAC-treated IMC-P2 was greater than that of WB-B bentonite without PAC. Lastly, the amount of silicate added to the system was negligible (0.003 μM) in our experiment. As in the previous experiment where no spike was added, WB-B contributed a significant amount of silicate into the surrounding medium, while H-DP and IMC-P2 did not. Moreover, the use of PAC with the two latter clays did not significantly add or remove silicate, but there was a high degree of variability associated with the measurements made for H-DP + PAC.

These results demonstrated that specific clays can both absorb and release certain inorganic nutrients. Based on our results, the most important consideration in using phosphatic clay is the release of excess phosphate. Although the addition of this nutrient to the system is a cause for concern with respect to possibly initiating a bloom, we have not fully investigated the possible outcomes. For example, a bloom may not occur in the system due to this released phosphate if another nutrient such as nitrate/ammonium is limiting. Nevertheless, we also found that the amount of phosphate released by phosphatic clay can be controlled through the use of PAC. Therefore, our attention will be focused on finding the best means of combining clay and PAC for field dispersal and understanding the consequences of adding PAC into the system.

With respect to trace metals, measurements were taken from the various clay samples at WHOI, as well as samples from the U.S. Environmental Protection Agency (EPA) and IMC Phosphates. For comparison, some additional data from various sources also were obtained and presented including values from the literature on Florida phosphorites (from Altschuler, 1980) and in-house analysis performed at IMC Phosphates. Overall, our measurements for As, Cd, and Fe were lower than those in other reports, while our Cr value was higher. For Cu, Mn, and Zn, we found higher amounts than those from other phosphatic clays measured by IMC Phosphates, but lower than those from Florida phosphorites and phosphorites from other regions. Hg, Ni, Pb, and Se measurements fell within the range of the other reported values, while our values for Ag and Sb were less than those from the available data sets.

Compared to reference sediments used by the EPA Laboratory in Gulf Breeze, FL, all of our measurements (i.e., means) were higher than those for the references. After considering the variability in samples, however, only the measurements for Al, Cd, Cr, and Mn were significantly different. When our values also were compared to those of "natural sediments" (Lewis, et al., submitted 2003), we found Cd, Cr, Ni, and Se elevated in phosphatic clays compared to natural sediments.

Finally, using sediment quality parameters proposed by McDonald, et al. (1996), we found that our values for As, Cu, Hg, Pb, and Zn were all below the Threshold Effects (TEL) and Probable Effects (PEL) Guideline Values. Ni was between the TEL and PEL, while Cd and Cr were both above the PEL. There were no suggested guidelines for Ag, Fe, Mn, Sb, Se, Sn, and Al.

With regards to radionuclides, samples of Florida phosphatic clay were analyzed at the Marine Chemistry and Geochemistry Department at WHOI for the amount of ^{266}Ra, ^{238}U, and ^{210}Pb. First, we found some variability among the samples tested. For example, samples obtained from the Nu-Gulf clay company had about one-third of the amount of each element compared to those obtained from IMC Phosphates, Inc., and about one-half of the amount of each element in samples provided by the Florida Institute for Phosphate Research (FIPR). These values then were compared to concentrations in sediment, as well as to U.S. EPA guidelines for effluents. The amount of ^{266}Ra in all phosphatic clay samples were higher than the concentration in sediment, while the amount of ^{210}Pb fell within the range of values found in sediments. Assuming full release of the elements from the clay to the surrounding medium, the amounts carried in phosphatic clay for ^{266}Ra and ^{238}U are lower than those for the effluent standards. For ^{210}Pb, samples from Nu-Gulf were lower than the effluent standards, while those from IMC Phosphates and FIPR were above the standard by a factor of 2 or 3. However, these are "worse-case" scenarios, because they assume all radioisotopes are released on contact with seawater. More tests are needed to determine the actual amounts of each element released into water by phosphatic clays.

<u>Investigation of Toxin Release and Aerosolized Brevetoxin Uptake by Clays During the Flocculation Process</u>. At Mote Marine Laboratory, controlled experiments focusing on the effect of clay treatment on intracellular and extracellular toxins have been completed. Briefly, results showed that the brevetoxins (e.g., PbTx-2) were removed with high efficiency. At 0.25 mg/L of phosphatic clay, 81 percent of brevetoxin was removed from the water column containing 5 x 10^6 lysed cells/L, 99 percent of brevetoxin was removed from the water column with 5 x 10^6 intact cells/L, and 99 percent of brevetoxin was removed from 1 x 10^7 intact cells/L (R. Pierce, unpublished data). The addition of PAC did not significantly improve toxin removal with clay alone. Other results found that the presence of clay reduced the amount of aerosolized toxin by 75 percent. These observations underscore the potential applicability of clay at mitigating the toxins alone. However, the strong affinity of these toxins for clay particles argues for the need to investigate the fate (e.g., permanence, degradation) and effects (e.g., bioavailability, potential for trophic transport) of brevetoxins associated with clay in the water column, and especially in the benthos. These experiments are underway in our ECOHAB renewal project recently funded through the National Oceanic and Atmospheric Administration (NOAA).

<u>Mesocosm Studies To Examine Flocculation Efficiencies on Natural Plankton Assemblages</u>. In October and November 2000, a series of experiments were conducted in Corpus Christi, TX, during a large *K. brevis* bloom in the region. Six fiberglass tanks (530 L each) were set up at the Texas State Aquarium along Corpus Christi Bay. Depending on the location and accessibility of bloom-rich water, the tanks were filled with water directly from the aquarium dock using a diaphragm pump, or from tanker trucks that carried water collected from another site. Removal experiments were performed using untreated and PAC-treated phosphatic clays. Cell removal was determined over a range of clay loadings, cell concentrations, and PAC concentrations. With each run, changes in water quality and chemistry were monitored including turbidity, temperature, salinity, dissolved oxygen (DO), pH, inorganic nutrients (i.e., nitrate, phosphate, ammonia, and silicate), toxins, chlorophyll-a, and total suspended solids (TSS). Samples also were taken for cell counts and plankton identification before and after treatment. Briefly, cell removal rates were comparable from

laboratory trials given the combination of clay loading and cell concentrations. Cell RE ranged from 50 percent to 85 percent. Likewise, PAC treatment increased cell removal. Turbidity increased predictably by a factor of three as clay was added and remained high throughout the short time period of the experiment (2.5 hours). There was no change in salinity, pH, and DO due to clay addition. Adsorption of nitrate/nitrite was observed, while ammonia appeared to be released and absorbed. As expected, some release of phosphate was found. There was no significant difference in the silicate concentration before and after clay addition. Finally, differential cell removal was observed among the four dominant species in the water in one set of replicated experiments: *K. brevis*, *Bacillaria* sp., and *Skeletonema* sp. were removed at about the same level, while Prymnesium sp. was not removed.

In October 2001, experiments were conducted in Sarasota Bay, FL, using three limnocorrals: two clay treatments (at 0.25 g/L), and one control. A marked area near the control limnocorral also served as a control site (i.e., ambient control). The limnocorrals (2-m diameter, 3-m depth) were deployed from an aluminum powerboat over a predetermined site to capture red tide-rich water. Bloom concentration ranged from 5 x 10^5 to 1 x 10^6 cells/L during the 1-day treatment. The limnocorrals were placed in a row, where water depth was approximately 3 m. The open bottoms (no cod end) were allowed to rest over the bottom and were secured with stakes around the perimeter. A series of sediment traps were placed inside the limnocorrals to capture flocculated materials. Sediment cores from the study site were collected just prior to the experiments for benthic faunal counts. The study ran for 4 hours following clay addition, although the limnocorrals were kept in place for 24 hours to allow most of the suspended material to settle over the bottom sediments and within the traps. Water quality parameters were monitored and water samples were collected and analyzed for bloom and plankton concentration, turbidity, toxin content, and inorganic nutrients. The material in the sediment traps was analyzed for toxin content, particle size composition, total solid composition, volatile solid composition, and biological oxygen demand (BOD). Finally, sediment cores were again taken after 96 hours to determine faunal composition in areas with and without clay.

Temperature, salinity, DO, and pH exhibited nearly identical patterns through time for both the control and the experimental site, suggesting that the addition of phosphatic clay did not affect these parameters. However, a noticeable reduction in DO saturation was observed at all sites on the second day after the clay addition, although the change was more pronounced in the two clay-treated limnocorrals. This decrease may be a result of oxygen depletion due to an anticipated increase in microbial activity in the mesocosms and the minimal exchange of water across the limnocorrals. The results from the analysis of BOD showed a slight increase in BOD in the clay treated limnocorrals, which further supports this hypothesis.

The removal efficiencies of *K. brevis* in the treated limnocorrals were 60 percent (treatment 1) and 62 percent (treatment 2) 4 hours after clay treatment. These values are smaller than laboratory trials at comparable clay and cell concentrations. On closer inspection, the removal efficiencies for the clay-treated limnocorrals after 1 hour were both 73 percent at the surface. After 2 hours, the removal values were 66 percent and 81 percent. These data suggest two possibilities: (1) *K. brevis* may be able to escape and return to the surface within 4 hours; or (2) the cells from the bottom of the mesocosms that were not captured by the clay were able to migrate to the surface. Another possible explanation may be the presence of other phytoplankters that could bind clay and reduce the amount of material that can interact with *K. brevis*.

Results showed that most of the brevetoxins in the control limnocorral remained in the water column 24 hours after clay addition, while all of the brevetoxins in the treated limnocorrals were associated with the clay floc collected in the sediment traps. These results agreed with the measured removal efficiency of *K. brevis* during the study, which suggested that toxin removal was associated with removal of intact cells. Although laboratory experiments exhibited more efficient removal of toxins from water than was observed in the field (90 percent toxin removal versus 70 percent, respectively), methods developed during laboratory studies proved to be applicable for mesocosm field studies. Moreover, the settled material within the sediment traps still retained brevetoxins after 96 hours.

Surface turbidity quickly decreased after treatment, eventually reaching initial background levels after 22 hours.

The addition of phosphatic clay to the limnocorrals increased total settled solids by 1,426 percent, volatile settled solids by 527 percent, and the BOD rate by 31 percent. BOD rates were similar between the ambient site (8.0 mg/L) and the control limnocorral (8.8 mg/L). As in the previous limnocorral study, the concentration of phosphate increased following clay treatment in the two limnocorrals, especially near the surface. The results for nitrate and nitrite were inconsistent. Therefore, it is difficult to conclude what the effect of clay treatment would be on NO_3^+/NO_2^+ content. The concentration of ammonium throughout the entire study was below the detection limit of the analysis system.

Finally, information on the number and composition of the benthic infauna were obtained from 56 cores. Representing 11 taxa, 285 animals were collected before clay was added and 494 animals were collected 96 hours after dispersal. The number of animals collected from the two controls increased by 56 percent (209 to 326), while the number from the two clay-treated areas increased by 121 percent (76 to 168). Oligochaetes were numerically dominant. As infaunal particulate feeders, oligochaetes typically are tolerant of low levels of DO. Polychaetes comprised the bulk of the remainder of the fauna. Notably absent from the infauna were the molluscs and crustacean groups, which typically are quite abundant in Florida coastal waters. Finally, summary statistics illustrated the poor state of the benthic fauna over the course of the study highlighted by: (1) very few species, maximum of four at a site; (2) low abundance; and (3) very low diversity (typical range expected for Shannon Index = 2.5 ~ 4.0).

There were several complications related to the assessment of impacts on the benthic fauna. Field observations at the application site noted that the subtidal benthos exhibited a coating of bottom algae approximately 1.0 cm thick (algal mat) prior to the initiation of the study. The layer occurred throughout the area encompassing the ambient, control, and treatment sites. The algal cover often is observed in the shallow warm waters of this area in late summer, but the expanse of coverage was atypical, possibly related to the extensive red tide. The bottom appeared to be anoxic beneath the algal mat, as exhibited by the black color of the underlying sediment. The effect of the algal mat on the benthic infauna became evident after the processing of the samples. The benthic infauna was depauperate as compared to "normal" bay fauna. It is likely that the fauna taken from the study were associated with the surface of the algal mat or the very near surface of the sediment. The anoxic state of the sediment was not conducive to a healthy benthos. Therefore, it is likely that the benthic community was already stressed and depleted in numbers prior to the study. As a result, the effects of clay application on the benthic biota were indeterminate.

Examination of Impacts of Clay/PAC Treatments on Benthos. Experiments conducted at the National Research Council of Canada have been completed and a manuscript bearing the results has been submitted for publication. In summary, a series of 2-week experiments were conducted to determine the impact of fully sedimented and resuspended clay-cell aggregates on the survival and growth of juvenile hard clams, *Mercenaria mercenaria*. Experiments were performed in a recirculating flume using the nontoxic dinoflagellates *H. triquetra* and *P. micans* and phosphatic clay (no PAC). Flow regimes simulated two extreme conditions, representing end members of a continuum expected in the field, where: (1) low flow (~ 2 cm/sec) allowed complete settling and formation of a sediment layer; and (2) high flow (~ 14 cm/sec) maintained complete particle resuspension. No clam mortalities occurred in either treatment. The fully sedimented treatment produced by a single-clay application showed no significant differences in shell or tissue growth compared to controls (no sediment layer), and clams rapidly resumed siphon contact with the overlying water. In contrast, a significant growth effect (~ 90 percent reduction in shell and tissue growth compared to no-clay controls) occurred in trials with clay maintained in suspension for 2 weeks at 0.25 g/L. These results suggest that clay applications in the field are likely more detrimental to clams under flow conditions leading to prolonged *in situ* resuspension of clay than under conditions that promote rapid sedimentation. The magnitude of impacts is thus dependent on the flow regime and the duration of exposure to resuspended clay.

Additional benthic impact experiments at the U.S. EPA Gulf Breeze Laboratory (FL) have been completed and a publication of the results is planned. In brief, the objective of these studies was to use standard EPA sediment toxicology procedures to determine acute and chronic toxicities of clay/cell flocs following the treatment of *K. brevis*. The toxicity of phosphatic clay, with and without PAC, was assayed using four benthic organisms in 4- to 28-day exposures. The organisms tested were *Ampelisca abdita* (infaunal amphipod), *Cyprinodon variegatus* (sheepshead minnow), *Leptocheirus plumulosus* (infaunal amphipod), and *Palaemonetes pugio* (grass shrimp). Several tests also were conducted in the presence of intact and lysed cells of *K. brevis*. Clay and flocculant alone were not lethal to these juvenile fish and epibenthic and infaunal invertebrates following both acute or chronic exposures. Furthermore, the chronic and acute toxicities of the settled clay/flocculant/*K. brevis* cell aggregates were not significantly different from the toxicity of the *K. brevis* cells alone, suggesting that the use of this bloom control method may not result in toxicity to these types of organisms above that naturally occur during a red tide event.

Another set of benthic impact studies was performed in conjunction with a recent limnocorral experiment in Sarasota, FL. These experiments were discussed in the previous section.

Evaluate the Engineering Requirements, Economic Costs, and Environmental Clearances That Must be Addressed if This Control Strategy is to be Applied to Blooms in Natural Waters, and Design a Pilot Program for Field Application of This Bloom Mitigation Strategy. In addition to considering its efficacy and environmental impacts, the decision to implement a clay control program also must consider the feasibility and cost of mounting an effort in larger, more complex, and dynamic systems. Unfortunately, there is very little information in the literature regarding cost and specific project designs from earlier Japanese and Korean experiments. Although some additional technical information has been obtained from personal communications with Korean colleagues, they have not provided sufficient detail to replicate their experiments locally. Therefore, it has been necessary to develop a "conceptual" treatment protocol for the United States guided by previous research and experience to identify the practical and engineering requirements at each step of a large-scale treatment. The following sections present the major steps involved in planning and executing a possible field program. Some of these ideas were generated through discussions with engineers at the JAD Enterprises (Macon, GA, USA) and Grain Processing Corporation (Muscatine, IA, USA). Wherever possible, cost estimates are provided.

Phosphatic Clay Acquisition and Transport to Treatment Site. Our first task was to calculate the required amount of clay for the operation. Clearly, the amount will be dictated by various factors including the treatment scale (i.e., volume or surface area), loading rate, and the number of repetitions. The treatment scale will depend on the aerial and depth distribution of the bloom, which can be determined by aerial and shipboard surveys. The target area can be further defined by the presence of priority sites (e.g., aquaculture sites, marina, public beaches). Once a treatment "volume" is calculated from the surface area and depth distribution of the bloom, this value can then be multiplied by the "optimal" loading rate (in mass per unit volume) as defined by empirical trials. Based on current data, the optimal loading rate of phosphatic clay against *K. brevis* ranges from 0.10 to 0.50 g/L. Finally, the required amount of clay can be multiplied by the number of times the treatment might be repeated during the operation. In Korea, it is not uncommon to prescribe two or three dispersals over a given area.

IMC Phosphates has offered us their clays at no cost (J. Keating, personal communication). However, the costs involved will be in labor and transportation to the treatment site, most likely along the Gulf Coast of Florida. According to the American Freight Company, which represents a consortium of freight truck companies in Florida, the cost of hiring a truck to move clay between Bartow and Sarasota or Charlotte Harbor currently is set at $799 (as of February 25, 2003). Each truck has a weight capacity of 45,000 lbs with a 3,500-ft^2 cargo space and 9-ft clearance. Using this information, the cost of transporting the predicted amount of clays from source to treatment site can be estimated. The cost of treatment increases linearly with the number of repetitions called for at each loading rate. The treatment cost also grows linearly with increasing clay concentrations. Finally, there is a clear cost advantage to securing and using clay samples with higher solids content in terms of shipping cost. For example, the cost of shipping enough dry phosphatic clay to treat a 62-acre area three times with 0.50 g/L each, is about $45,000. In the same table, the cost of shipping enough wet phosphatic clay at 3 percent solids, for the same treatment scale, loading and frequency, is $1.5 million. At higher scales of treatment (e.g., 1 to 5 square miles), the cost of shipping alone approaches potentially prohibitive values.

In addition to collecting the clay from its source and its transport to the treatment sites, we considered the intermediate steps that could affect the cost of the operation, and expedite steps such as clay preparation and dispersal. Because we anticipated that the cost of collecting and transporting the clay can be maximized by selecting samples with the highest solid content, we explored the possibility of further drying the clay at the collection site or at some processing facility before delivery to the treatment site. Apparently, the technology exists to allow "flash drying" of wet clay to a desired moisture content (B. Dahlquist, JAD Enterprises, personal communication). This procedure may allow us to deliver more clay solids. However, we do not know at this time whether such a procedure is feasible or cost-effective, given the anticipated clay demand. In addition, the clay's dryness can significantly affect its dispersal in water, which can affect its removal ability. Therefore, it will be necessary to investigate this drying procedure further to assess its effectiveness, practicality, and cost to the operation.

Based on our experience working with larger volumes (e.g., in flumes or field mesocosms), clay slurry preparation beginning with rock-sized clumps of clay is a tedious, labor-intensive, rate-limiting step which requires soaking the clay for several hours, manual and mechanical breakage of softened clay, blending, vigorous mixing, and sieving. Commercially available machines for making clay slurries, such as those from Korea or the United States, call for finely sized clays. Therefore, another crucial step between collection and delivery is the crushing or pulverization of the clays to make it suitable for rapid slurry preparation as described in the following section. Again, the technology for this process is widely available, although it may be necessary to have a clay company perform this task. This step also may be coupled with the flash drying process described previously (B. Dahlquist, JAD Enterprises, personal communication). This is another area that we currently are investigating with regards to practicality and cost. However, this may be one step in the operation that may not be as readily avoided or disregarded as the drying procedure.

Preparation and Dispersal of Phosphatic Clay Slurry. The next set of engineering challenges involves the efficient processing of phosphatic clay for shipboard dispersal. We first considered the addition of dry clay powder to the surface of the bloom as this appeared to be the easiest, fastest, least labor-intensive and most cost-effective method. Although the intermediate step of preparing a clay slurry would be omitted, it would still be necessary to grind the clay and find a means of dispersing it over the sea surface. We investigated this in the laboratory by adding dried, crushed, and sifted clay powder to the surface of the cell culture (data not shown). Cell removal efficiency was only 20 percent at 0.25 g/L, and less than 40 percent at 0.50 g/L, for both IMC-P1 and IMC-P2, compared to greater than 90 percent for their respective slurries. We observed clumping of the clay powder at the surface, resulting in large, visible aggregates that quickly fell through the medium. We also found that the dried and crushed powder was difficult to disperse in either freshwater or seawater, even with prolonged soaking and agitation over several days. It appeared that when the mineral crystals are collapsed by dehydration, it becomes difficult to reintroduce water into the lattices. Hence, the removal efficiencies for these "reconstituted" clays were approximately 60 percent at 0.25 g/L and 85 percent at 0.50 g/L. Although these results were higher than using dried powder, these were still lower than diluted clay slurries at the same concentrations. Therefore, both of these findings strongly suggest that using fully dried and crushed phosphatic clay directly or in suspension would not be suitable for this treatment effort. Incidentally, phosphatic clay at 70 percent solids content (e.g., IMC-P7) adequately softened after several hours of soaking in water and produced a fine slurry with sufficient mechanical processing (e.g., blending, rotor mixing). More importantly, the removal ability of the clay was comparable to clays with higher moisture levels. Therefore, there may be a dryness limit at which phosphatic clays would remain effective and workable.

To maximize its effectiveness, we concluded that phosphatic clay must be dispersed in a slurry form, similar to the methods used in Japan (Shirota, 1989) and South Korea (Bae, et al., 1998). In our initial experience working in larger volumes (e.g., flumes and mesocosms), slurry preparation and dispersal involved two separate, independent steps. Slurry preparation began by soaking and softening relatively dry clumps of clay (i.e., between 46-72 percent solids) in seawater. The clumps consisted of large, rocklike pieces taken directly from settling ponds. After several hours, the clumps could be broken by hand and processed using a blender. Softened clumps also were processed into slurries placing them in 20-gallon buckets with seawater and immersing a rotor attached to a power drill. The final step involved sieving the slurry to remove plant debris and large, unbroken pieces. In all, the preparation took several hours. For dispersing the clay slurry over the sea surface, we used various submersible pumps and power washers attached to a hose and adjustable spray nozzle. Constant mixing by hand was necessary to prevent settling of the clay. Clogging of the pumps and nozzles sometimes occurred. One advantage of this system is the low cost of the various dispersal equipment. However, the labor cost involved in slurry preparation would more than offset the savings from the dispersal equipment.

Presently, we are aware of several machines that can combine slurry preparation and dispersal. Clay suspensions can be prepared rapidly and with greater control over particle size and concentration, as well as the rate of dispersal. In South Korea, a seawater slurry from dry yellow loess is made using a specially designed machine. Raw seawater is pumped in and forced through angled channels along the walls of the mixing chamber to generate a vortex. Dry loess is then added to the mixing chamber. The finished slurry, containing particles less than 50 mm, flows into a second chamber through an overflow slot at the top of the mixing chamber. It is then pumped directly over the sea surface from boats or barges. The greatest advantage of this design is the incorporation of slurry preparation and dispersal. Unfortunately, there is no information available on the specifications of this device (e.g., capacity, mixing rate, dispersal rate), or on the exact procedures and quantities such as the clay-to-seawater mixing ratios. The Korean manufacturer offers an iron model at $4,300 and a stainless steel model at $10,800. The shipping cost from Korea to Florida was estimated at $1,000, excluding the tariff. The latest model of this machine includes a chamber where seawater is hydrolyzed by passing a current through it to produce a short-lived sodium oxychloride species (NaOCl) (H.G. Kim, personal communication). Loess then is added to the hydrolyzed water to produce the slurry bearing the cell-killing oxychloride species. The result is higher bloom mortality with less loess. This technology has not been tested for U.S. species. It is unknown whether this newer version can be purchased.

In the United States, a device is available from Grain Processing Corporation (Muscatine, IA) that also can prepare clay suspensions rapidly and precisely. Dispersal also is easily controlled. The machine can be hired on a per-day basis and the cost of shipping would be more reasonable than the cost of shipping a new sprayer from Korea; both this and the Korean machine are about the same size. They also require that the clays be of a certain small size that can be wetted relatively quickly. Although our contacts at JAD Enterprises have offered to design a machine specifically for our purposes, it may be more advantageous and costeffective to purchase or rent an existing machine, instead of producing one *de novo*. Obtaining a pre-existing device also means that the product could be field-tested and optimized more rapidly.

Typically, clay has only been directly and systematically added to the surface of the water column in previous reports and studies. The slurry is diluted as the particles flocculate and sink. We do not have any empirical evidence on how different dispersal methods can affect the overall efficacy of the clay treatment. For example, subsurface addition and/or pulsed addition may enhance removal by preventing excessive clay addition over a given area. Turbulent addition (i.e., injecting the clay to the water surface with a vigorous, more powerful flow) also may be considered to maximize the collision frequency between particles near the surface. Regardless of the method used, some attention must be given to the effective concentration (or the rate of dispersal) in a given parcel of water and the clay particle size entering the system. These will be the focus of upcoming field trials. At present, we are unaware of any efforts to apply PAC or other flocculants in the field, in conjunction with clay dispersal. Currently, most reports on the possible benefits of PAC-treatment have been restricted to laboratory trials and mesocosm experiments. At WHOI, some of our experiments in settling columns demonstrated that scaling up of the PAC loading and treatment did not produce the desired effect. When the clays were pretreated with PAC before addition to the column, we observed that the clays immediately formed large, baseball-sized aggregates, which immediately sank to the bottom, leaving behind most of the cells in suspension. To avoid this phenomenon, we subsequently added the PAC separately from the clay. Based on our preliminary findings, the best results were found when PAC was added to the seawater first to allow the flocculant to dilute and diffuse through the surface, which was followed by the clay slurry after a 20-minute delay. Although these latter experiments have shown promising results at these larger scales, more effort must be given to testing the dispersal methods at field treatment scale.

Ship and Crew Hiring. A significant portion of this operation, such as slurry preparation and dispersal, will be performed onboard ships and by the ship's crew. One way of minimizing the cost is to involve volunteers, including those who wish to use their private vessels for the purpose (e.g., local property owners, fishermen, aquaculture). Otherwise, some current ship hiring costs were provided by our colleagues at Mote Marine Laboratory (Sarasota, FL): their largest research vessel (46 ft) with a frame and full complement of equipment would cost about $1,100 per day. A smaller craft (27 ft), which has the necessary working area for clay dispersal and sample processing would cost $600 per day. Finally, the smallest vessel that would be appropriate for clay dispersal would cost about $300. This craft would have to unload the dispersal apparatus and return with sampling gear. Depending on the size and power, pleasure craft can be rented in the area for prices ranging from $600 to a few thousand dollars. Other considerations on the choice and number of ships will be the water depth at the treatment site, the surface area to be treated, the size and complexity of the dispersal apparatus or system, and the size of the crew.

Two shipping companies in the Florida region were contacted to inquire about barges and other vessels for clay transport and dispersal. With respect to barges, the Cashman Equipment Corporation provided the following examples (estimates as of April 10, 2003): (1) 220" x 60" x 14" (L x W x H), with a 3,500-ton capacity, at $1,000-$1,400 per day; (2) 250" x 72" x 16," 5,500-ton capacity, at $1,600-$1,800 per day; and (3) 300" x 100"x 18," 10,000-ton capacity, at $4,500 per day.

These costs do not include the cost of renting a tugboat or offshore supply vessel (OSV) to tow the barges. In addition, this company and others similar to it keep their fleet based in Louisiana, and would take between 8-10 days to tow the barges to Florida. The barges offer large working areas and cargo room, but may present difficulties in stability and movement from site to site. Hence, this option is less appealing.

Ted Brown Marine Services in Pensacola, FL, proposed that OSVs would be the most suitable vessels for this purpose. OSVs were chosen in transporting both dry materials and wet drilling muds to drilling platforms in the Gulf. They often have large storage tanks to keep wet clays. They also are self powered with plenty of cargo and workspace. Their speed and stability offer suitable conditions for working. Most companies that rent OSVs also are located in LA, but there are a few in Tampa and the Gulf Coast of Florida. The cost estimates below include rental and crew who also have familiarity with navigating the shallower, coastal regions, where the treatment would likely occur. Therefore, the use of the OSV may be the best option. One estimate provided by the company is the following: 150'-170' with 500-1,000 ton capacity, at $5,000-$10,000 per day. Assuming that each ship can transport up to 1,000 tons (or 907.2 metric tons), the number of ships needed to disperse the required amount of clays was calculated. Then, the number of ships to hire was multiplied by the average cost of OSV rental (i.e., averaged at $7,500 per day) to get the total. As with the cost of transport by truck, the cost of ship hiring and dispersal also increases linearly with both the frequency of treatment at one loading rate, and with increasing loading rates. The cost of treating between 60 and 100 acres using dry clay, with respect to ship rental, is less than $23,000 for example, even with three treatments at 0.50 g/L each. However, using wet clay at 3 percent solids, we already can expect to spend this amount on OSV rental when treating only 10 acres twice with 0.25 g/L of phosphatic clay. There may be some time and effort in using wet clays due to lower demands in processing the clays for dispersal, but other logistical and practical considerations also will be accounted for, such as the need to move large volumes of slurry from truck containers to the OSV's holding tanks and storage of wet clays.

Finally, the values were combined to provide an estimate for the cost of clay treatment at various scales. As before, using dry clay instead of wet slurry will be more cost effective. Applying wet clay can be anywhere from 7 to 30 times the cost of working with dry clay. Depending on the scale, the combined costs can be as little at $9,000 to tens of thousands of dollars when the treatment scale grows to between 60 and 100 acres (with dry clay); if more than 100 acres, the costs climb to a few hundred thousand dollars. Finally, the cost of treating a 10-square mile area with at least three doses of dry clay at 0.50 g/L is conservatively estimated at more than $5 million. Ultimately, the decision on cost effectiveness will be based on a comparison between clay dispersal and the estimated losses of leaving a bloom untreated.

We emphasize that these cost estimates are highly conservative and approximate. Additional effort is needed to estimate costs associated with the use of commercial clays such as H-DP, which can be obtained and shipped in bags or in bulk. Through careful analysis, ways to decrease overall costs likely will become apparent.

Designing Pilot Study. A pilot study for clay dispersal has been designed and funded by NOAA through the ECOHAB program. Our first task was to choose an appropriate field site. During site selection, the maximum size (i.e., surface area) of the treatment area was balanced against the logistics of clay dispersal and the need to treat a sufficiently large region to create a sizeable plume that can lead to floc deposition at a reasonable distance from the application site. At this stage, we are considering treatment areas of several hundred m^2 or more (i.e., 15-20 m on each side). Other considerations include flow regime, rate of water exchange (i.e., access to red tide-rich waters), water depth, and the presence of intact benthic communities. Finally, the chosen site should be free from boat traffic and other recreational activities in a relatively unpopulated area.

Two estuarine areas have been selected: one in Sarasota Bay near Mote Marine Laboratory's main campus and the other in Charlotte Harbor, near Mote's Pine Island field station. These sites experience frequent *K. brevis* blooms, yet are about 100 miles apart such that blooms have occurred in one area, while absent from the other. Another critical criterion is that the sites are adjacent to Mote facilities for logistics of field sampling and each site encompasses diverse benthic communities for evaluating the possible impacts from clay applications.

The Sarasota Bay site is in an embayment bounded on the east by Mote Marine Laboratory property and on the north by a wetland preserve such that the amount of boat traffic and curious onlookers can be controlled within the study area. This is the area in which a permit for clay applications has been provided. The Charlotte Harbor site is located in Pine Island Sound to the East of the Mote field station and adjacent to uninhabited mangrove islands. Access to this area also can be restricted so that the pilot study can be implemented without interference from curious or inadvertent boaters.

Once a suitable red tide has been identified (e.g., >1 x 10^6 cells/L), the study will proceed as follows. Several days prior to the start of the experiment, the sediment profile and quality of the benthic community (abundance and composition) will be assessed using sediment and/or box cores. The target loading of phosphatic clay will be 400 g/m^{-2}, applied twice over a 24-hour interval, to maximize impacts (Korean fish farmers sometimes apply clay more than once at a given location). Clay will be pretreated as previously described. The clay slurry will be dispersed from a small ship or powerboat over a fixed area using a high-capacity, high-power submersible pump or an alternative model to be determined.

At the dispersal site, current meters will be strategically placed and an array of sediment traps (with baffles) will be placed along the bottom to collect settled flocs. The number and distribution of traps will be determined, while considering the *in situ* flow regime. Before the clay is dispersed, an initial survey of the study site will be conducted. Water quality parameters such as temperature, conductivity, DO, pH, light availability, turbidity (via the turbidity meter and/or transmissometer), and *in situ* fluorescence will be measured using a CTD array and other devices. Water samples will be collected at various depths across the study site and analyzed for total phytoplankton and zooplankton, bacteria, brevetoxin content, TSS, and various inorganic and organic nutrients. Immediately after clay dispersal, the plume will be tracked using the procedures developed and tested using one or two powerboats, depending on the anticipated dispersal rate of the material. The boat(s) will move in and out of the plume in a systematic fashion, along a predetermined grid pattern taking the same samples listed above. Additional water samples and turbidity measurements will be taken at 1 and 3 hours.

After 5 hours (or a suitable amount of time for settling), the experiment will be terminated and a final set of measurements and samples will be collected. Sediment traps will be recovered by divers and the floc analyzed for toxin content, total inorganic solids, total volatile (organic) solids, particle size distribution, and BOD. Some samples will be reserved to quantify viable *K. brevis* cells. Divers will return at 24, 96, and 144 hours to collect sediment and box cores for analysis to evaluate changes in the composition and abundance of benthic species.

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Type	Citation	Project	Document Sources
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