MITIGATION OF HARMFUL ALGAL BLOOMS WITH CLAY: A TEST OF THE EFFECTS OF REPEATED APPLICATIONS ON JUVENILE SOFT-SHELL CLAMS, *Mya arenaria*

M.-C. Archambault[2], M. Bricelj[1], and J. Grant[2]
[1]Institute for Marine Biosciences, National Research Council of Canada, Halifax, NS B3H 3Z1
[2]Oceanography Department, Dalhousie University, Halifax, NS B3H 4J1

An apparent global increase in harmful algal blooms (HABs) has prompted research on control and mitigation strategies to reduce ecological and economic impacts. Inert clays have been used to effectively remove dinoflagellate bloom species from the water column at Asian fish farms and their use is being tested experimentally in the USA. Potential repercussions revolve around the increasing flux of suspended particles to the bottom in the case of a depositional flow environment, where animals such as juvenile suspension-feeding bivalves could suffocate and/or starve due to burial. The objectives of this study were to determine the effects of a sedimented layer of phosphatic clay (IMC-P), resulting from single vs. repeated clay applications, on survival and growth of a commercially important, infaunal, suspension-feeding bivalve, the soft-shell clam *Mya arenaria.* The rationale for this experiment is that repeated clay application is likely to be required over the duration of a HAB for effective mitigation under field conditions. Previous work on the hard clam, *Mercenaria mercenaria*, showed no significant growth inhibition by sedimented clay (Archambault et al., submitted) but only tested the effects of a single clay application.

Juvenile *M. arenaria* held in sand were subjected to the following parallel experimental treatments during two sequential trials: a) single addition of clay [1 g dry weight (DW) l^{-1} of clay] to a bloom of a representative non-toxic dinoflagellate species (*Heterocapsa triquetra*), b) multiple (10) clay-*H. triquetra* additions (0.25 g DW l^{-1}) at 2-day intervals over 3 wks after an initial application of 1 g DW l^{-1}, c) a control with no clay addition.

Experiments were conducted in 3 recirculating raceways, where low flow (<2 cms^{-1}) allowed complete sedimentation of clay, thus simulating a low-energy field scenario. Clams suffered no mortalities, and were able to rapidly regain contact with the overlying water column and resume feeding even when subjected to repeated clay applications. However, preliminary analysis indicates that multiple clay applications resulted in a significant reduction in *Mya* tissue growth rates, ranging from 8 to 20% relative to controls. The single clay treatment showed only a small reduction in tissue growth rate (2% in trial 1) or even an increase in growth (10% in trial 2) compared to the respective controls. In both clay treatments, 93% of the suspended clay at 1 g DW l^{-1} was removed from the water column within 20 minutes. During subsequent clay applications of 0.25 g DW l^{-1} in the multiple clay treatment, IMC-P removal ranged between 50-63% within 20 minutes. The depth of the deposited clay layer averaged 0.7 cm with the single application, and attained 3 cm with multiple clay applications by the end of the experiment. Repeated clay delivery and bottom organic enrichment (due to dinoflagellate sedimentation at the time of clay application) did not result in sediment hypoxia. Although *M. arenaria* grows well in fine-grained sediments in its natural habitat, this study indicates that under an extreme-case scenario of 10 repeated clay deposition events, small but significant growth inhibition may occur in this species. The number of applications required for effective mitigation remains to be established under varying field conditions. However, fewer clay applications (~2-3) than the maximum number tested here will likely be considered in bloom treatment efforts in the U.S. and worldwide, due to economic and logistical considerations. Our future work will investigate effects of sedimented clay/dinoflagellates on other potentially vulnerable epifaunal suspension-feeders (e.g. scallops) and on deposit-feeders, which may benefit from bottom organic enrichment.

SEAPORT: IMPROVING BIOTOXIN MANAGEMENT THROUGH CITIZEN INVOLVEMENT

S. M. Conrad, S. Etheridge, and S. Hall
FDA Washington Seafood Laboratory, 8301 Muirkirk Road, Laurel, MD 20708, USA

Monitoring for marine biotoxins is made more challenging by the patchy and ephemeral distribution of the phytoplankters that produce them. The cost of sampling at adequate temporal and spatial density, coupled with the intrinsic limits of toxicity testing (both cost and time delay), place a significant burden on agencies responsible for seafood safety. Employing networks of field observers, primarily volunteers equipped with portable microscopes, to give advance warning and help focus toxicity testing effort can significantly improve the effectiveness and reduce the cost of marine biotoxin management programs.

BENTHIC RESPONSE TO APPLICATION OF PHOSPHATIC CLAY FOR THE REMOVAL OF *Karenia brevis* FROM THE WATER COLUMN, SARASOTA BAY, FLORIDA

J. K. Culter[1], M. R. Sengco[2], J. Sprinkel[1], R. H. Pierce[1] and D. M. Anderson[2]
[1] Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, Florida 34236-1096, USA
[2]Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA

Public perception is that red tide blooms on the west Florida coast are becoming both more frequent and more persistent in duration. Whether or not this is true remains to be established. The blooms are caused by the toxic dinoflagellate, *Karenia brevis*. Release of brevetoxins as a result of normal cell growth and divisions as well as cell breakage may reach levels that are toxic to both fish and invertebrates at sufficiently high cell concentrations. It is certain that red tide blooms have an economic impact due to losses in tourism and fishing revenues. Public concern over the effects of red tide is resulting in pressure to develop control measures.

Clay suspensions are used in South Korea to precipitate harmful algae from the water column when blooms occur in the vicinity of shellfish aquaculture areas. The characteristics of the clay suspension are such that algal cells adhere to the particulates and settle to the bottom.

The present study was undertaken as one component in examining the utility of phosphatic clay to precipitate *K. brevis* (as well as other plankton) from the water column, and to focus on the fate and impacts of the settled clay-dinoflagelate-plankton floc on the benthos. Potential impacts include: change in the surface sediment grain-size distribution, release of brevetoxins at the benthic boundary layer, and the increase of organic matter at the benthic boundary layer. A small-scale pilot study was conducted in May of 2003 utilizing controlled phosphatic clay applications in 5 enclosures (clay-pens) over natural substrate in Sarasota Bay at a depth of ~ 3.7 m. The area consisted of a gently sloping bottom of clean (i.e. low silt/clay fraction and low organics), medium to fine quartz sand, with some shell material. The low silt/clay content was considered to be advantageous in terms of detecting any added clay. The clay-pens consisted of a circular plastic coated hog wire mesh (diameter ~3.0 ft.) lined with fiberglass window screen, and a thin walled rigid fiberglass tube that was temporarily placed within the screen enclosure to confine the clay floc so that all or the bulk of the material would settle on the intended target benthos. Clay slurry (~10% solids in seawater, = 0.5 kg dry wt) was applied to five clay-pens. Five identical pens were left untreated as controls. After settlement the rigid liner was removed, with the screened enclosure remaining to limit currents and prevent disturbance from large mobile epifauna and bottom feeding fishes. Benthic fauna were samples four days prior to clay application and again 15 days after application. In addition, a mix of *Mercenaria mercenaria* juveniles (obtained as aquaculture seed stock) and wild mollusks were placed into each clay-pen just prior to treatment. Temperature, salinity, pH, and dissolved oxygen were monitored for treatment, control, and ambient conditions. Tidal effects on these parameters were observed but there were no treatment effects. The clay floc settled rapidly and there was no difference in turbidity between control and treatment clay-pens one hour after application. After settlement the clay floc was clearly visible as a fine surface layer on the bottom. A one-way ANOVA comparing the faunal abundance of control and treatment clay-pens showed no significant difference in numbers of organisms collected. However, there was a significant difference between pre- and post-treatment organism abundance for both control and treatment site fauna indicating a significant effect, possibly due to disturbance during clay-pen installation and sampling. The results of the survival of *M. mercenaria* juveniles indicate the possibility of a treatment effect. The overall survival of *Mercenaria* within the control pens was 47% while the survival within the treatment pens was 15%. All of the treated pens exhibited a lower number surviving treatment than their control counterparts. The effect was significant ($n=5$, $p=0.045$, non-parametric t-test). There were complications, however, due to portunid crab predation within the clay-pens.

CHANGES IN THE COMPOSITION OF BREVETOXINS FOLLOWING CLAY FLOCCULATION FROM SEAWATER MEDIA

R. H. Pierce, M. S. Henry, P. C. Blum, and S. L. Hamel
Mote Marine Laboratory, Sarasota, FL 34236, USA

Harmful algal blooms (HABs) occur worldwide affecting marine life and public health through seafood-borne illnesses and exposure to toxin-containing marine aerosol. This study was undertaken to investigate the fate of brevetoxins (from the toxic dinoflagellate, *Karenia brevis*) removed from seawater culture media with settled phosphatic clay. Concentrations of the most abundant brevetoxins were monitored in association with both clay and those remaining in the water column over a 14-day period. This study was conducted with lysed (ruptured) *K. brevis* cells to ensure that all of the toxins were extra-cellular, available for adsorption to the clay particles. The experimental design included three treatments: 1) lysed control cultures of *K. brevis* with no clay added, to observe brevetoxin degradation in the presence of ruptured cellular debris, 2) clay added to lysed cultures to observe brevetoxin degradation in association with clay particles that have settled to the bottom, and 3) clay plus natural sediment added to lysed culture to assess the role of natural microflora on toxin degradation. Laboratory cultures of 5×10^6 cells/L of *K. brevis* were obtained from the Mote Marine Laboratory red tide culture facility. Flocculation experiments were performed using phosphatic clay (IMC-P2) (International Mining Corporation [IMC], Bartow, FL) following the work of Sengco et al. (2001). The cells were lysed with ultra-sonication and 500mL of the lysed culture was added to 1-L beakers. Natural sediment was obtained from Sarasota Bay in an area frequently experiencing red-tide blooms. Triplicate 500-mL samples from the original culture were analyzed to determine the initial brevetoxin content. Thereafter, triplicate sets of beakers from each treatment were recovered for toxin analyses at intervals of 2, 5, 9 and 14 days. Toxin analysis was performed by LC-MS using standard brevetoxins obtained from Dan Baden at UNC, Wilmington. In the original culture, the most abundant toxins were: PbTx-2 (11µg/L), PbTx-3 (1.5 µg/L) and a trace of PbTx-1 (<0.03 µg/L). In the lysed culture with no clay or sediment, PbTx-2 was totally degraded by day 14 and PbTx-3 remained at about the original concentration. In association with settled clay, PbTx-2 diminished to 2 µg/L by day 9 and then remained at that level through day 14. In the presence of natural sediment with clay, PbTx-2 diminished more rapidly to 1 µg/L by day 9 and to 0.5 µg/L at day 14, while the PbTx-3 concentration remained about the same throughout the 14-day study.

References:

Sengco, M.R., A. Li, K. Tugend, D. Kulis, and D.M. Anderson. 2001. Removal of red- and brown-tide cells using clay flocculation. I. Laboratory culture experiments with *Gymnodinium breve* and *Aureococcus anophagefferens*. Mar. Ecol. Prog. Ser. 210: 41-53.

SHELLFISH AS VECTORS FOR INTRODUCTION OF HARMFUL ALGAE, WITH EMPHASIS ON THE TOXIC DINOFLAGELLATES *Karenia brevis* AND *Alexandrium monilatum*

S. E. Shumway[1], S. E. Pate[2], J. J. Springer[2], and J. M. Burkholder[2]
[1]Department of Marine Sciences, University of Connecticut, Groton, CT 06340, USA
[2]Center for Applied Aquatic Ecology, North Carolina State University, Raleigh, NC 27606, USA

We summarize research on harmful algal species that have been shown to pass intact through the digestive tract of shellfish, with emphasis on *Karenia brevis* and *Alexandrium monilatum* which form toxic blooms in the Gulf of Mexico. Successfully invading species are difficult or impossible to eradicate; therefore, potential vectors of such species should be carefully examined in efforts to minimize bioinvasive species dispersal. Introduction of HAB species via cargo ship ballast water has received considerable attention in recent years, but other potential modes of transfer mostly have been ignored. One obvious vector for the possible transfer of HAB species is molluscan shellfish (e.g. oysters, clams, mussels). Some harmful dinoflagellates survive ingestion, gut passage, and egestion by bivalve molluscs. Dinoflagellates potentially may be introduced into new areas through molluscan feces if cells are able to survive passage through the digestive tract, thus rendering molluscs prime vectors for transfer of harmful algal species. Viable cells within the feces can also influence the recurrence and duration of toxic blooms. If live dinoflagellate cells or cysts are transported into areas via shellfish and their feces, the cells may be able populate or re-populate the site. We microscopically examined fecal material from shellfish species *Crassostrea virginica* (eastern oyster), *Mercenaria mercenaria* (northern quahog), *Argopecten irradians* (bay scallop) and *Perna viridis* (green-lipped mussel) after exposure to harmful or potentially harmful algae to determine whether cells remained viable after passage through the digestive tract. Feces produced by the shellfish were gently removed and washed several times with 0.22-µm filtered seawater to remove free dinoflagellate cells and debris. The viability of the cells within the feces was determined by inoculating rinsed, intact fecal strands (n = 5) into flasks containing sterile growth media and into flasks containing 0.22-µm filtered natural seawater. Dinoflagellate cell densities were quantified at 7-day intervals to assess whether the cells within the feces had been viable. If cysts were detected within the feces, they were removed using a micromanipulator (100 cysts per replicate; n = 5). The cysts were placed into conductive media for growth, and into 0.22-µm filtered natural seawater. Excystment and subsequent zoospore motility were observed at 1-hour intervals over 24 hours. A viability stain was also used on washed feces and extracted cells and cysts from fecal material as a second test for cell viability. Cells and/or cysts of toxic strains of *Pfiesteria piscicida* and *Pfiesteria shumwayae* were structurally intact and viable after passage through the digestive tract of eastern oysters, northern quahogs, bay scallops, and/or green-lipped mussels. Cells of *Karenia brevis, Alexandrium monilatum, Karenia mikimotoi* and *Prorocentrum minimum* were also structurally intact, and additional tests of viability are ongoing. Overall, the data indicate that molluscs are potential vectors for the dispersal of toxic dinoflagellates, and could aid in increasing the geographical range of toxic species.

CHEMICAL CHARACTERIZATION OF THE ALGISTATIC FRACTION OF BARLEY STRAW INHIBITING *Microcystis aeruginosa*

T. Waybright[1], D. E. Terlizzi[2], and M. Drew Ferrier[1]
[1]Hood College, Frederick, MD 21701, USA
[2]Maryland Sea Grant Extension Program, Baltimore, MD 21202, USA

Using barley straw for algal growth management has become routine in parts of the U.S. after gaining wide acceptance in the U.K. Nevertheless, there are conflicting reports of its effectiveness *in situ,* and recent laboratory studies have shown that, in addition to inhibition, stimulation is observed in species of marine and freshwater algae. Limited research conducted to identify algistatic agents in barley straw has suggested oxidized phenolics or free radicals from their photodecomposition are the inhibitory components.

To isolate and identify the inhibitory components of barley straw extract, a microplate assay system was developed using *Microcystis aeruginosa. M. aeruginosa* has been consistently inhibited by barley straw extracts in studies conducted in our laboratory and by others. The 24-well plate assay utilizes *in vivo* fluorescence monitoring for determination of chlorophyll levels in each 2-ml culture. Barley straw extracts used in this study were aerobically decomposed for 2-3 days up to 6-7 months, with inhibition present in all of the aqueous samples tested.

One difficulty in a bioactivity-guided fractionation study is preparing enough material for assays and for analysis. This study involved extracting kilogram-sized samples of straw, yielding multiple grams of extract for testing. Initially, samples of freeze-dried extract were reconstituted in water/ethanol (1:1), refrigerated overnight, and centrifuged to separate the supernatant from precipitated constituents. This step reduced the sample mass by half, but concentrated the active components into the supernatant. The precipitate showed no activity. Next, a portion of the supernatant was percolated through a bed of Polyamide CC6 resin (for irreversible adsorption of larger polyphenolic compounds such as tannins), which resulted in the loss of activity. Additional supernatant was then passed through a filter with a nominal molecular weight cutoff of 1000, which resulted in much less growth inhibition when compared with the unfiltered material. An aliquot of supernatant was further passed through filters with nominal MW cutoffs of 3000 and 10,000 to further classify the approximate size of the molecule responsible for the growth inhibition.

Results to date suggest the inhibitory component(s) are polyphenolics of a larger size than previously reported Structural characterization of the algistatic fractions by HPLC/MS, coupled with UV/Fluorescence/ELSD detectors is underway and these results will also be reported.