Team BREATHE

Thesis Proposal

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ABSTRACT

Harmful algal blooms negatively affect estuarine ecosystems, such as the Chesapeake Bay, diminishing dissolved oxygen levels in the water, releasing toxins, and blocking sunlight needed by submerged aquatic vegetation (SAVs). In order to mitigate these negative effects we plan to create a clay-flocculant mixture that is able to eliminate the algal blooms, neutralize their toxin, and re-grow the SAVs they destroy. Our optimum mitigation mixture is based on a variety of factors outlined in this paper, including efficacy of the mixture and the cost and availability of the materials. We plan to use experimental laboratory and field research to determine the most effective components of our mixture for removal efficiency. Based on our data, future researchers will be able to adapt our mixture to other aquatic and marine environments and other species of algae.

Section 1: INTRODUCTION

Harmful algal blooms are massive upwellings of algae that have increased both in frequency and in severity all over the world due to increased human presence near bodies of water. These bodies can be both freshwater and marine (Glibert et al. 2005). Harmful algal blooms (HABs) occur when specific conditions arise that are conducive to one particular alga dominating over all other species. The algae then multiply to unhealthy levels for that particular ecosystem, thus creating “bloom” conditions. While algae at normal levels are a very important component of every aquatic community, HABs disrupt the delicate balance of the ecosystem in which they occur. The rampant increase in HABs is often the result of eutrophication, which is the rising level of nutrients in an ecosystem that is often caused by increased human presence

(Anderson et al. 2003). The conditions most important to bloom formation are temperature, salinity, water column stability, and the ratio of nitrogen and phosphate in the water.

HABs have been occurring naturally for thousands of years. However, the combination of increased human population along the shores of bodies of water and the trend of global warming has led to HABs becoming more prevalent (Glibert et al. 2005). The increase in HAB occurrences is a major societal and environmental problem because HABs have a detrimental effect on the economy, the environment, and on people who directly interact with the water.

HABs are detrimental to the environment because they often strip the water of dissolved oxygen (DO), which other organisms need to survive. An environment that has < 2 mg DO/L, which is lower than normal levels of dissolved oxygen (~7 DO/L, at 20 degrees C), is characterized as hypoxic (Rabalais, 1994). Hypoxic conditions occur when aerobic bacteria decomposing the algal cells use dissolved oxygen at a faster rate than the flora in the ecosystem can replace it. Areas where dissolved oxygen is critically low are not able to support life, and become known as “dead zones,” where neither flora nor fauna can exist.

The Chesapeake Bay is just one body of water that is having problems with emerging new “dead zones” exacerbated by increasing hypoxic conditions. Often, these hypoxic conditions emerge as a result of HABs (Sellner et al. 2003). Furthermore, HABs often lead to a huge reduction in submerged aquatic vegetation (SAVs) in the affected area (Kemp et al. 2005). SAVs are underwater grasses that grow naturally in a healthy aquatic ecosystem and provide dissolved oxygen to the water by photosynthesizing throughout the daylight hours. SAVs are, therefore, nature’s defense against the formation of “dead zones.” As a result, HABs not only strip the water of dissolved oxygen through decomposition or nocturnal bloom respiration, but they also

severely limit the ability of the ecosystem to heal itself by causing massive SAV death (Kemp et al. 2005).

There are two approaches to dealing with HABs: preventative and *ex-post facto*. While a preventative approach is preferred because it treats the problem before the occurrence of a bloom, it is often much more difficult to implement because it may rely heavily on firmer environmental regulations in the Chesapeake Bay watershed. Consequently, until a successful preventative approach is designed and implemented, mitigation, an *ex-post facto* approach, is an important strategy that allows people to try to limit HAB impacts after the HAB is in place.

While a great range of mitigation techniques has been utilized globally to help suppress and deal with HABs, few mitigation techniques are both economically feasible and environmentally safe. Realizing this opportunity in HAB research, our team seeks a method to mitigate an HAB in the Chesapeake Bay that meets both of these criteria. After considering other mitigation approaches and techniques, our team decided to base our method on a successful clay flocculation model that has been used in other parts of the world to mitigate blooms (Sengco et al. 2004). We chose a clay flocculation model because clays and sediments are easily obtained, making the method economically feasible. Additionally, clays and sediments are naturally occurring materials, making the method environmentally safe.

Our main goal as a team is to develop a clay mixture that is most effective at mitigating a *Microcystis aeruginosa* HAB in the Chesapeake Bay. *Microcystis aeruginosa* is a cyanobacterium and a prominent bloom former in the upper tributaries of the Chesapeake Bay that is becoming more prevalent. In addition to the increasingly large role it is playing in the Bay's ecosystem, *M. aeruginosa* is also an extensively studied species of cyanobacteria. Apart from possibly stripping the water column of healthy levels of dissolved oxygen and causing

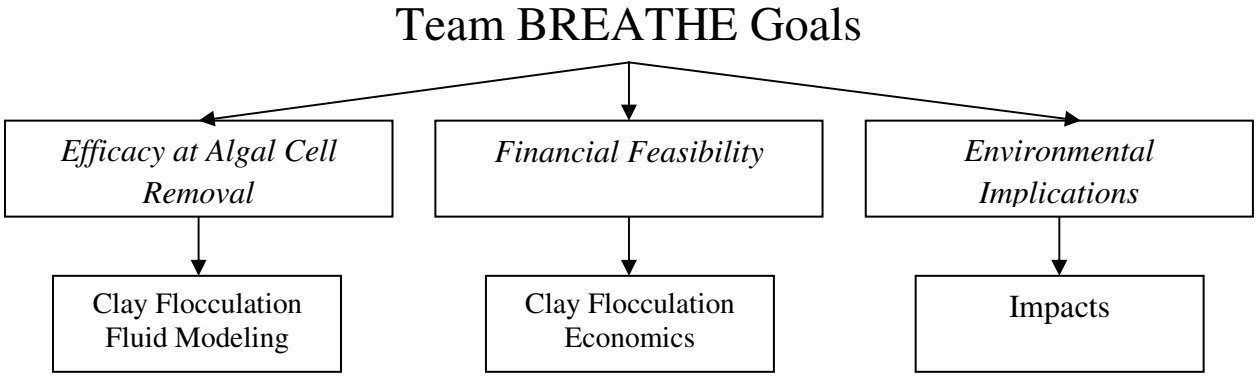
massive SAV death, *M. aeruginosa* blooms also release toxins into the water in about one-third of all blooms (The Fish and Wildlife Institute, 2005). Toxin release has a negative effect on the organisms living in the Bay and on the people depending on the Bay, either for their livelihood or for their personal enjoyment. Furthermore, *M. aeruginosa* blooms are typically very dense and therefore cause great losses to the tourism industry (Anderson et al. 2000).

One way our team will take the traditional clay flocculation model and expand it is by addressing the need to maximize algal cell removal while benefiting the environment. In order to maximize algal cell removal, our team will mix local clays and sediments with a flocculant, which is a compound that will improve clay-algae bonding and will increase cell removal efficiency with lower loading amounts of clay. We currently anticipate using chitosan as our flocculant because it is a naturally occurring compound found in crab and lobster shells that has been shown to increase algal removal efficiency (Zou et al. 2006). Our team will expand the current clay flocculation model by also integrating other components into the mixture that will benefit the environment. They will help alleviate the effects of the HAB by eliminating toxins and restoring SAVs to the affected area. In order to aid in toxin elimination, we will add an environmentally friendly substance into our mixture that will largely neutralize the microcystin toxin produced by the *M. aeruginosa* bloom. Secondly, in order to aid in SAV restoration efforts, we will integrate SAV seeds into the mixture. These seeds will encourage growth on the nutrient rich clay-algae aggregate on the bottom after the clay-algae mixture has settled.

The theory behind the efficiency of our mixture is that the clay particles will aggregate with the algal cells with the aid of the flocculant. This heavy aggregate will outweigh the buoyancy of the algal cells and will begin to sink to the bottom. As this aggregate sinks through the water column, it will continuously pick up algal cells in a process called “sweep floc,” thus

removing large amounts of the bloom. When the aggregate sinks to the aphotic bottom, the algae will decay over time and the nutrients will be released into the environment. To prevent these nutrients from being released into the water, we will be adding SAV seeds to the mixture, which will use these high levels of nutrients for their germination and their growth. Therefore, our mixture will not only mitigate the bloom, but it will also restore SAVs to the area, which will make the ecosystem healthier and better able to cope with blooms in the future. Therefore, our approach is not only an *ex-post facto* method of dealing with the bloom, but it is also a potentially preventative mixture, because blooms are less likely to occur in healthy areas with normal levels of SAV growth (Rabalais, 2002).

Team BREATHE plans to develop a clay mixture that will be able to both mitigate and prevent *Microcystis aeruginosa* blooms in the Chesapeake Bay. In order to accomplish this broad goal, the team has split into subgroups that will concentrate on specific aspects of the mitigation process and its effect on the surrounding ecosystem. The main goal consists of three different concentrations: mixture efficacy in terms of algal cell removal, financial feasibility, and environmental implications. Each subgroup seeks to contribute to one of these concentrations.



The clay flocculation subgroup and the fluid modeling subgroup will work together to create a clay-flocculant mixture that is most effective at removing algal cells from the water column. The flocculation subgroup will test the mixture experimentally in the laboratory and in

the field, while the modeling subgroup will devise mathematical models of clay flocculation and bloom dynamics to predict and corroborate experimental results.

The next concentration, financial feasibility, will be addressed by both the clay flocculation subgroup and the economics subgroup. These subgroups will seek a flocculation mixture that is both cost effective and acceptable to the public. Possible ways of addressing this issue for each subgroup are to use locally found sediments and materials and to understand public reactions to algal bloom mitigation efforts.

Lastly, the impacts subgroup will address the third concentration, environmental implications. The goal of the final mixture is not only to remove algal cells from the water, but also to restore the environment to a healthier state than before in order to deter the occurrence of future blooms. *M. aeruginosa* can release toxins upon cell death, which have negative impacts on the flora and fauna living in the Chesapeake Bay (Ross, 2005). The impacts subgroup will seek to analyze toxin production and neutralize or eliminate the toxin from the water column.

Harmful algal blooms also negatively impact the environment by causing massive SAV death by blocking sunlight from underwater grasses. SAV are vital to a healthy aquatic ecosystem, because they restore dissolved oxygen to the water column and assimilate excess nutrients. To address this, the impacts subgroup will also seek the species or mixture of species of SAV that grow best under *M. aeruginosa* bloom conditions so that SAV seeds can be incorporated into the mixture. The incorporation of SAV seeds will thereafter restore SAV growth and in turn, absorb excess nutrients in the water, improving the ecosystem.

RESEARCH QUESTIONS

What is the most efficient, financially feasible, and environmentally safe clay flocculation mixture that can mitigate a *Microcystis aeruginosa* harmful algal bloom in the Chesapeake Bay?

Sub-questions

- What clay mixture is both cost-efficient and effective at removing *Microcystis aeruginosa?*
- How can we model the natural and clay flocculant mitigated life cycles of *M. aeruginosa*? What do these models tell us about the ideal composition for the clay mixture for the most effective mitigation of algal blooms?
- How can we use an algal bloom aggregate to aid in SAV restoration? How can we couple SAV restoration with algal bloom mitigation?
- What can we incorporate into the clay mixture to prevent the negative effect of cyanobacterial toxin release from *M. aeruginosa* blooms?
- How does the general public (college students and other groups) react to mitigation efforts towards harmful algal blooms in the Chesapeake Bay?

Each of these sub-questions will be studied and addressed by the group in order to answer the main question.

Section 2: LITERATURE REVIEW

Clay Flocculation

Microcystis aeruginosa is a cyanobacterium, or photosynthetic algae, responsible for producing many of the harmful algal blooms (HABs) in the upper Chesapeake Bay. Blooms

reoccur each year in the Bay because cells originating from past blooms endure winter in a dormant state within the benthic sediment of the Chesapeake. Following the spring thaw, these cells transition back to an active state and create a new population (Reynolds, 1981). In terms of competitive success, *M. aeruginosa* enjoys higher levels of success because of its unique gas vesicles, allowing it to dominate over other species (such as green algae). These pockets of air within the algal cell are able to direct the vertical migration of the organism in the water column by regulating the gas content, which in turn regulates buoyancy (Ganf, 1982). Thermal stratification in eutrophied bodies of water, which are those rich in nutrients such as the Chesapeake Bay, contribute to ideal environmental conditions for *M. aeruginosa* because they are capable of large daily vertical migrations. Their gas vesicles allow the *M. aeruginosa* cells to sink below the euphotic (light) zone in order to absorb inorganic molecules (nitrogen and phosphorus) and rise again into the euphotic zone in order to carry out photosynthesis (Ganf, 1982). Changes in water nutrient content and the abiotic factors of the environment also contribute to *M. aeruginosa* dominance over other species. Cyanobacterial blooms are associated with nutrient-rich water (high phosphorus to nitrogen ratios), alkaline (basic) conditions, low salinity, and slow current.

Clay flocculation is the most promising technique for the mitigation of harmful algal blooms because of its ability to maximize removal efficiency and minimize cost (Sengco, 2003). Although the clay particles themselves are capable of submerging an algal bloom, studies have shown the mitigation becomes much more effective upon the addition of a flocculant to the mixture. Clay particles alone require much higher rates of clay loading to immerse the HAB. Even though clay particles have a net negative charge, natural cations in the water neutralize their negative surface charges, thus making the dominant attractive forces between the clay

particles the weaker hydrogen bonds and van der Waals attractions. However flocculants help to circumvent these potential obstacles. Flocculants such as chitosan or polyaluminum chloride (PAC) are long organic molecules that bridge across clay particles to form a netted structure. This net acts as a blanket that effectively submerges the algal bloom by a process called "sweep floc." In this process, the combined weight of the mixture and the bloom travels through the water column and aggregates algal cells as the entire mass sinks to the benthos, the bottommost layer of the Bay (Sengco, 2005).

Modeling

In order to develop a mechanistic model of fluid dynamics, a large number of underlying factors have to be understood. These factors include the interactions between clay particles and flocculants, the interactions between many types of algae, and the interactions between the clay and the algae.

Models of clay flocculation have been developed in the past in application to wastewater treatment. Bouyer, Liné, and Do-Quang developed an experimental model that evaluated flocculant size as a function of viscous dissipation rate of kinetic energy and mixing history (Bouyer, 2001). However, the application of such a model to HAB mitigation occurs in a fundamentally different environment and seeks to achieve different goals. Water treatment occurs in a controlled environment. Consequently, many of the studies were carried under the assumption that mixing occurred, as aggregation occurs more effectively under such conditions (Bouyer & Denis, 2004). Most algal blooms, however, occur in still water. This means that turbulence-induced aggregation and breakup generally does not occur. Moreover, it will be difficult to ascertain the extent of the surface charges on natural particles, the factor that will

determine the particles' adhesiveness, given differences in pH, and the amount of dissolved constituents (e.g. organic molecules) in the water. One of the main goals of aggregation in water treatment is to create large flocculants that can easily be removed (Bouyer & Denis, 2004). This makes comparison difficult, as cleaning the water column of an HAB requires an even, blanket-like sweep floc that can maximize contact between the clay and algae without the benefit of mixing.

Considerable work has been done in the development of mud suspensions in estuarine conditions (Winterwerp, 1999; Winterwerp, 2002). These studies were conducted in a similar environment as our target and can provide a background on how clay will interact with other clay particles. It does not incorporate any other particles, however, which is the main interest of the present application.

Information on basic mixing in the tidal conditions of the Chesapeake Bay as well as settling characteristics such as velocity will be important to review for a more holistic overview of how particles are distributed in the Bay. A mathematical model simulating how the river flow into the Chesapeake Bay is affected by the tides shows that when tide is present, plumes, or columns of one fluid moving through another, tend to be found on the bottom whereas, in the case of no tides, plumes remain near the surface (Guo, 2007). A study using a recirculation column with an oscillating grid found that settling velocities of particles depends on the suspended sediment concentrations and turbulence level (Johansen, 1998).

The daily cycle of rise and fall within the water column, algal buoyancy, is key to *M. aeruginosa* behavior and is integral to this study. Previously developed models of this behavior and the factors they account for can be applied to our model as well (Wallace, 2000).

Clay-algae aggregation has been studied by a number of researchers. Much of the data collected by these researches will be adapted to frame and enrich models of algal mitigation through flocculation. For example, studies of collision rate through trajectory analyses yield encouraging results that could be adapted to fit our model (Han, 2001). However, collision is only half of the aggregation process. Once collision occurs, there is still a chance that sticking will not occur. In spite of this, it may be possible to form a mechanistic model of sticking probability through modeling the interaction of algal surface polysaccharides and the clay, which has been studied in the past as well (Lagaly, 1984; Labille, 2005).

However, if this is not feasible, we may have to rely on existing mathematical models of flocculation, in which the sticking coefficient is usually solved for after all other parameters are determined. A useful review of past flocculation models has been previously documented (Thomas, 1999). Models that incorporate both sticking probability and collision frequency are of greatest interest to this project, as both factors are quite important in clay-algae flocculation due to the dynamics of this system. More encompassing reviews of flocculation models, especially in application to marine environments, have been conducted. They not only consider flocculation and coagulation models but also discuss particle size distribution models, providing a slightly different approach (Jackson, 1998).

Impacts (SAVs)

Submerged aquatic vegetation, or SAVs, provide the ecological framework for the great diversity of life seen in the Chesapeake. Together the approximately twenty indigenous grasses of the bay work together to provide a source of shelter, food, and oxygen for the Bay's inhabitants. Historically, these grasses have been in such great abundance that the entire shallows

of the Bay would be blanketed with a lush green carpet made entirely of these grasses. However, due to the recently degradation of water quality in the Chesapeake Bay, SAV populations have decreased significantly in the last 30 years. Because SAV populations are so dependent on the water quality of the Bay, their abundance and diversity are excellent indications of the health of the water and level of pollution within the ecosystem. Excess sediments deposited in the Bay from local agriculture and human activities act together to block sunlight needed for SAV to grow. Nutrient runoff is also a leading cause of SAV degradation. Nutrients such as nitrogen and phosphorus, when in excess, create ideal conditions for algal growth. Explosion in algal growth can result in the appearance of blooms. These blooms, which consist of millions of individual algal cells, can significantly reduce light penetration, diminishing the growth of aquatic vegetation (Rabalais, 2002).

SAVs improve water quality dramatically by absorbing excess nutrients, trapping excess sediments, maintaining oxygen level in the water, preventing erosion by stabilizing the benthos, and provide habitats for Chesapeake Bay wildlife. One study conducted by the Virginia Institute of Marine Science showed that crabs where 30 times more abundant in areas populated by SAV beds compared to unvegetated areas (Moore, 2004).

In 1978, SAV population of the Chesapeake Bay dropped to 10% of historic levels. Environmentalists have been making extensive efforts in more recent years to restore SAV populations in the Chesapeake Bay, indicating how important these organisms are to the health of the ecosystem. Currently the restoration goal of the Chesapeake Bay Program is to establish 185,000 acres of SAV beds by year 2010 (Bradley, 2008).

With the tremendous environmental focus on the restoration of SAV in the Chesapeake Bay, the incorporation of SAV seeds into our algal mitigation plan will make our project very

attractive for testing in the Chesapeake Bay. Because underwater grasses are so effective at absorbing excess nutrients in the water and restoring oxygen levels, the incorporation of SAV seeds into our mitigation should reverse the hypoxic effect of algal blooms. The improvement of the water quality by SAV will prevent excess algal growth from reoccurring, making our mitigation plan a long-term solution to the detrimental occurrence of harmful algal blooms.

Impacts (Toxin)

Studies show that the most potent and widespread variant of toxin released by *Microcystis aeruginosa* is microcystin-LR, abbreviated MC-LR (Hoeger et al. 2002; Carmichael, 2001). MC-LR refers to the specific structural aspects of the toxin that differentiates it from other toxins. Specifically, MC refers to the toxin secreted by *Microcystis aeruginosa*, while LR refers to the amino acids leucine and arginine, which are unique to this toxin (Carmichael, 2001).

Previous research has linked the presence of this enterotoxin, which is a toxin that is synthesized and resides inside the cell, to possible competitive benefits. Data from the Hoeger study showed that the microcystin was effective in killing *Daphnia pulicaria*, a predator of the algae. The death of these water fleas could provide enough of a competitive evolutionary advantage to select for these more potent strains of algae. Another advantage of the toxin is the possibility that it may act as an intercellular signal (Dittman et al. 2001).

Besides its effects on *Daphnia*, MC-LR has many other attributes that are harmful to the ecosystem. Research has also shown that presence of the toxin has the potential to reduce root length and increase peroxidase activity in plants, thereby inhibiting their defensive mechanisms. This would be extremely detrimental to our group's SAV restoration programs. In higher organisms, MC-LR affects the liver by binding to adenosine receptors located throughout the

organ. Once bound, the toxin disrupts the normal structure and function of the affected areas, causing cirrhosis and tumors. In terms of overall health, presence of the toxin in a body can cause diarrhea, sore throat, vomiting, blisters, and rash.

Current research has discovered many possible methods of toxin neutralization. Many of these methods, including halogenation and ozonation, utilize the chemical properties of various substances to attack the toxin. When these products attack the toxin, they alter existing chemical structures. These alterations prevent the toxin from acting as it normally does, thus mitigating the effects of toxin release. Halogenation involves treating water containing MC-LR with diatomic halogen molecules such as bromine or chlorine. Ozonation employs a similar process by using ozone instead of halogens, which yields a much greater success rate. These processes, although effective, often yield harmful byproducts and high costs (Jungmann, 1992). Another potential method of mitigation involves utilizing the principle of competitive inhibition. Due to the fact that MC-LR relies on adenosine receptor sites to attack the liver, it has been hypothesized that if these receptors are present in the water, MC-LR will bind to these receptors. This renders the toxin harmless to living organisms. A final method, and the one that is most pertinent to our research project as a whole, involves using clay particles to adsorb the toxin. These clay particles are incredibly effective, removing up to 81% of the toxin that is present in the water column (Perez & Aga, 2005).

Economics

The economical impacts of harmful algal blooms (HABs) are both diverse and widespread. Consequently, there are many ways of estimating the financial impact of such a bloom. There is a clear distinction between the economic and the scientific approaches to

assessing effects from HABs. Economists concern themselves primarily with changes in tangible financial values such as monetary losses that are consequences of HABs (Hoagland & Scatasta, 2006). Data gathered over the past few decades demonstrate the devastating economic impact of algal blooms. Studies estimate that losses from algal blooms reach as high as one billion dollars, averaging out to about $34 million to $82 million annually (Anderson et al. 2000).

These losses are spread out over several fields, such as public health, commercial fisheries, recreation/tourism, and monitoring/management. Public health impacts represent around 45 percent of economic losses, with commercial fisheries representing 37 percent. The rest is made up of impacts on recreation/tourism, and monitoring/management. Such estimates are very conservative in nature and therefore do not even account for economic multipliers, which could potentially triple this amount. They also do not reason effects on untapped resources, which are being prevented from harvesting due to toxicity resulting from HABs (Anderson et al. 2000)

Public health comprises a significant amount of economic impacts. Algal toxins are responsible annually for more than 60,000 intoxication incidents. In the past, the Centers for Disease Control and Prevention estimated that around twenty percent of all food-borne outbreaks result from seafood consumption, with half of this twenty percent resulting from algal toxin. This percentage has surely gone up due to the rise in algal blooms within the past several decades. Other studies show that contact with bloom water, exposure to aerosolized algal toxins, or consumption of contaminated seafood "results in six recognized human poisoning syndromes" (Dolah, Roelk, & Greene, 2001).

The Chesapeake Bay has experienced hypoxia levels as early as the 1930s, when bottom water quality was first investigated in deep channel areas. In 1997, a *Pfiesteria piscicida* bloom

occurred in several Chesapeake Bay tributaries, causing health problems for both marine life and for humans in the region (Magnien, 2001). It was estimated that about 50,000-80,000 menhaden were killed, and although menhaden are not consumed, public attention was still heavily drawn to the *Pfiesteria* bloom (Hoagland et al. 2002). This provided a useful study of the dynamics between science, public perceptions, and policy.

Reports of "cold-like symptoms, skin problems, and generally poor health," associated with contact with the algae, as well as reports of menhaden with skin abnormalities and lesions, created an atmosphere bordering on hysteria (Magnien, 2001). The public's general reaction was so negative that the Governor of Maryland closed down several Chesapeake tributaries that were sources of recreation and fishing. It was estimated that the outbreak cost the seafood industry $46 million due to the "halo effect," where the public heard of the menhaden contamination and abstained from consuming any seafood (Anderson et al. 2000). The state of Maryland tried to avoid this by spending half a million dollars on promotional efforts to try to decrease such effects on the market (Hoagland et al. 2002).

The public sector has held generally negative views towards algal blooms due to their effects on human health as well as the aquatic environment. In addition to this, massive economic losses have been calculated throughout the years, increasing such negative views. Throughout the past, economic data has been calculated very conservatively, so actual economic impacts may be much higher than reported amounts. Algal blooms are very important not only in the scientific sector, but the economic and public sectors as well.

Section 3: METHODOLOGY

Team BREATHE plans to develop a clay mixture that will be able to both mitigate and prevent *Microcystis aeruginosa* blooms in the Chesapeake Bay. The mixture will incorporate seeds of submerged aquatic vegetation (SAV) — vegetation that grows on the waterbed — and neutralizers for *M. aeruginosa* toxins that are sometimes released when the algae die. Furthermore, the mixture must be both environmentally friendly and financially feasible.

The team plans to use a research design that uses multiple methods. We will incorporate both quantitative and qualitative methodologies to answer our research questions. Our quantitative approach will involve data collection in a controlled laboratory environment and will progress to implementation in the field — the Chesapeake Bay. The clay flocculation and impacts subgroups will develop and test the mixture in a laboratory setting using jars and tanks before testing the final mixture in the field. The modeling subgroup will also use a quantitative approach to model clay flocculation and bloom dynamics. While most of the subgroups will use a quantitative approach, the phenomenon of harmful algal blooms cannot be understood entirely without looking at public reaction to algal blooms and bloom mitigation. For this reason, the economics subgroup will use a qualitative approach, conducting surveys of public opinion. By using both a quantitative and a qualitative approach, our team will address the full extent of the algal mitigation process.

Drawbacks of the research design will be present both in the quantitative and in the qualitative methodologies. However, these drawbacks should not significantly affect our results. In the quantitative methodology, the transition from laboratory testing to field testing will likely be the greatest concern. In a laboratory setting, there is much greater ability to control a number of variables that cannot be controlled in a field setting. To address this issue, a more gradual

transition from laboratory testing to field testing will be implemented through the use of an intermediate step where testing will occur in microcosms in the field, which will allow for more control over external factors. Microcosms are enclosed areas of water, which can maintain similar water conditions throughout experimentation. When testing in open water, other factors, such as water flow, differing topography, and varying weather conditions, may affect results.

Drawbacks of the qualitative methodology mostly arise from the fact that surveys and interviews often lack external validity. One of the main problems that the economics subgroup will face in gathering data from surveys and interviews will be finding a representative population. Since the subgroup will concentrate mostly on interviews, the number of people they need to interview to get an accurate representation of the public's views on algal bloom mitigation will be a main concern. Interviews will provide more in-depth responses but may not represent the views of the entire Chesapeake Bay-watershed population.

SUBGROUP METHODOLOGIES

Clay Flocculation

The flocculation subgroup seeks the most efficient and financially feasible mixture to mitigate *M. aeruginosa* harmful algal blooms in the Chesapeake Bay. First, the focus will be on local sediments and clays available naturally in the Chesapeake Bay watershed area. We will do research on the sediments naturally available in the areas where *M. aeruginosa* blooms frequently occur. Then, different flocculants, which are compounds that enhance clay aggregation, will be researched, especially those that are inexpensively available and naturally found in the Chesapeake Bay. Currently, chitosan is being investigated as a potential flocculant. Chitosan is a compound that is naturally found in the exoskeletons of Chesapeake Bay blue crabs

and has been previously shown to greatly improve removal rates (Zou et al., 2006). We will first obtain two strains of *M. aeruginosa*; one that has been known to produce toxins in the past and one that has produced no toxins. These strains will be obtained from the University of Tennessee. The first goal will be to grow these cultures in the lab in BG11media and to create an inoculum that resembles bloom-like conditions. Once this is done, the algae will be grown in jars and tanks, as well as put under light cycles to simulate the diel periodicity of the sun's role in the natural environment.

Data collection will focus entirely on quantitative experimental methods. For testing the efficiency of algal cell removal using the mixture, an experimental methodology used by Gang Pan in his research on clay efficiency for flocculation will be employed (Pan et al., 2006). In the laboratory, we will collect data on removal rates by first measuring settling rates of various clays and sediments in jars of water by measuring light penetration and then measuring settling rates of the same clays in the presence of algae. After obtaining data on initial suspension times, loading rates, and removal rates, we will add a flocculant, such as chitosan, to the clay mixture and measure the removal rates. We will use a spectrophotometer or fluorometer to determine the cell concentrations in the water before and after addition of our clay mixture. We will also rely on the compilation by Andersen on *Algal Culturing Techniques* to grow algae in the laboratory (Lorenz, Friedl & Day, 2005) as well as a paper by van der Westhuizen on growing *Microcystis aeruginosa* in the lab (van der Westhuizen and Eloff, 1985).

After we obtain data from the laboratory experiments, we will test our clay-chitosan mixture on natural bloom samples, and then later, in microcosms, or enclosed environments, in the Chesapeake Bay. We will follow a similar experimental design, but will likely encounter large colonies or surface scums, rather than the single cell cultured populations we expect in the

initial laboratory experiments. We will be applying our mixture to a natural algal bloom in replicated 4-foot tubs. We will collect data on cell concentrations before and after clay mixture addition to measure removal efficiency. After obtaining data in the microcosms, we will move to testing our mixture in open water conditions in the Bay, following a similar methodology. Through this approach, we will get quantifiable data that will allow us to answer the sub-question: what clay mixture is both cost-efficient and effective at removing *M. aeruginosa*?

The main confounding variable to be dealt with is the major difference between the laboratory environment and the natural field environment. No matter how effective the clay-chitosan mixture is in the laboratory, there is a chance that, due to differing conditions in the field, the clay flocculation will not yield the same results. Some of the factors that will differ in the field include food web interactions that are not present in the laboratory, weather conditions, topographical variation, size distributions of *Microcystis*, and alterations in water flow. Also, the laboratory-cultured algae may have different physiological properties than the algae found in a natural algal bloom. For example, toxin content or the surface carbohydrate layers may be considerably different. Often, strains that have been cultured in laboratory conditions for many generations have different genotypes than strains that are found in the field. To address this issue, we will attempt to collect *M. aeruginosa* samples from a natural bloom in the Chesapeake Bay and then use these algal cells to test our mixture in the laboratory. There have also been problems with creating bloom-like conditions in a laboratory environment, so we will anticipate this difficulty when growing our algal cells in the laboratory.

Our subgroup anticipates creating a clay flocculant mixture that can successfully mitigate *M. aeruginosa* harmful algal blooms in a way that is both efficient in terms of cell removal and is

financially feasible. These results will contribute to the overall team goal of creating a clay mixture to mitigate a harmful algal bloom in the Chesapeake Bay.

Modeling

The first step in modeling the efficacy of clay flocculation on harmful algal blooms is to develop a model of clay-cyanobacteria flocculation. The model will consist of a set of mathematical equations that describe clay-algae behavior. Ultimately, the input to the model will be clay mixture composition and concentration, and the output will be the amount of algal bloom removed. For example, a mixture of different weights of a clay mixture, 60% montmorillonite and 40% kaolinite (two types of clays) with 500 ppm chitosan (the flocculant) can be tested, and a regression model can be produced that would predict how much of the algal bloom will be removed as a function of the amount of clay and flocculant used.

A model of bloom dynamics and clay-algae flocculation will allow the team to predict the behavior of clay mixtures in bloom conditions. It will also allow us to focus our experimental efforts on mixtures that are already potentially effective, saving the team time and resources. Because a model is less resource-intensive, the team will be able to undertake more in-depth studies of a few clay mixtures that are theoretically predicted to work well (Lagaly, 1984).

The only data necessary for the first part of the model development process will be background information on clay and algal properties, including physical, chemical, and biological factors. Much of this information is still being researched, so published studies will be a major source of pertinent data.

The next step in the development of the model will be to expand the first part of our model to create a more comprehensive model, which would model the life cycle of a *M. aeruginosa* bloom, both naturally and after the addition of clay. Harmful algal blooms will

naturally die on their own due to a number of factors, and the purpose of clay flocculation is to speed up the process of bloom decline. The model of the natural life cycle will be the control in this part of the process, to be compared to the model of the mitigated life cycle. Ideally, the mitigated life cycle of the algal bloom will be shorter, justifying clay mitigation efforts.

We will use quantitative and qualitative data from the Bay on the factors that will affect bloom initiation, mitigation, and dissipation. These will include water temperature, salinity, sunlight, flow velocity, algal species present, and other factors that affect bloom dynamics. We will be able to find these data from studies and reports, the MD Eyes on the Bay website, and from the results of the flocculation subgroup's experiments.

There are two different approaches we could take to build a mathematical model of the algal life cycle. The first approach is a statistical one in which we would accumulate the aforementioned data from previous studies and perform statistical analysis to create a fitting model. This would be an inductive approach. The second approach would be the deductive one that takes into account the biological, chemical, and physical factors that are involved in bloom development. With this approach, we would start with a large number of assumptions to simplify the model and then remove assumptions to develop a more complex model so that it more closely resembles reality. This approach would involve substantial in-depth studying of all the processes involved in bloom development.

Because our purpose is to develop a model that takes into account as many factors as possible, any variables we have failed to account for previously will be included in improved versions of the model, eliminating any confounding variables as model development progresses.

Impacts (SAV)

To answer the team's research questions, this subgroup will determine which species of SAV will be best suited to the conditions under which *M. aeruginosa* harmful algal blooms are most likely to form. In order to do this, the subgroup will look at germination and growth rates of different SAV species, light penetration in the water column, salinity of the water, type of sediment at the site of planting, and how the plants will respond to the clay, flocculant, and decaying algal cells. The subgroup will choose a manageable number of SAV species with which to run our experiments and collect seeds from various regional sources. From the collected seeds, the most viable seeds will be identified. The qualifications for viability vary by species.

However, a good indicator of viability is a rigid seed coat (Orth et al., 2003). Before the development of the clay mixture, tests will be run to determine the germination rates of selected SAV species, which will help the team choose a species that will grow within the necessary timeframe. Germination tests will be run by planting SAV seeds in the appropriate sediment contained in small jars and observing the seeds for the appearance of the cotyledon (Orth et al. 2003). The cotyledon is part of the seed's embryo, which generally becomes a seedling's leaves.

Once the clay flocculant is developed, the sub-group will simulate the field environment in the lab in large tanks and then incorporate SAV seeds into the flocculant. After mitigation, the subgroup will determine which SAV was most viable. This conclusion will be based on a variety of factors, such as the amount of bed biomass or percent of vegetative cover, as well as which species most successfully removes excess nutrients from the water column and increased dissolved oxygen levels (Moore, 2004).

Sediment type, dissolved oxygen and nutrient levels in the experimental design are potential confounding variables. SAVs may not necessarily be the cause of variations in these

aspects of the estuarine environment. In the chosen design, impacts of these confounding variables should be limited, since work will be conducted in a controlled laboratory setting.

The anticipated result is that the presence of SAV growth following the clay flocculation process will help to restore the health of the environment. The growing SAVs utilize excess nutrients released by the decaying algal cells and will help to restore normal levels of dissolved oxygen in the water column (Benson, O'Neil, & Dennison, 2007) and lower nutrient levels, thereby reducing the recurrence of harmful algal blooms.

Impacts (Toxin)

The subgroup will compare a control group of algae without a toxin-mediating product with a toxin-mediating product. The subgroup will first conduct further research to compile potential neutralizing agents for Microcystin-LR (MC-LR), which is a leucine-arginine variant of the toxin associated with *M. aeruginosa*, and will then test the absorbance rates of these agents in a controlled aquarium environment. The variables that will be altered will be the presence and concentrations of the different types of neutralizing agents.

A promising potential neutralizing agent is dried, powdered liver. The toxin MC-LR causes the most damage to living organisms by binding to liver cells. The binding specificity with which the toxin binds to the liver means that the proteins in the liver could bind to the toxin in the solution and thus prevent it from mixing throughout the ecosystem. Due to the fact that the liver particles are an organic product, adding the liver to the flocculant will produce few negative effects on the environment.

We will test for the presence of toxins in the water using the Enzyme-Linked ImmunoSorbent Assay (ELISA) and then measure the concentrations of the toxin before and after the addition of the neutralizing agents by using high performance liquid

chromatography/tandem mass spectrometry (HPLC/TMS), which requires the use of specialized equipment available through the College Park US FDA laboratory and Dr. Jon Deeds. Other potential means of quantifying the amount of toxin in solution include spectrophotometry and pH. If we chose to use spectrophotometry, we would have to establish the standard molar absorptivity of the toxin by generating a plot of absorptions at different concentrations. From this reference, we could use the absorbance of our samples at a predetermined wavelength to measure the amount of toxin in solution. A methodology using pH would require similar preparation to have a reference for comparison.

Additionally, our group seeks to establish a healthy sentinel species for testing under a variety of adverse conditions. Potential sentinel species include fish, snails, mussels, clams, crayfish, frogs, newts, and salamanders. Snails are a favorable choice, as they are invertebrates native to the area that have a green gland that has the capacity to filter toxins from the blood stream. The best choice, however, appears to be juvenile freshwater clams. Shell growth in juvenile clams acts as an excellent indicator for the health of the ecosystem. This organism is favorable because it is an invertebrate and the measure of organism health is simple and straight-forward to measure. Other organisms will require their stress levels to be evaluated based on either the mortality of the snails or the amount of stress proteins present in the organisms. The organisms will be subjected to post-floc conditions both in the presence and in the absence of the toxin. The performance of our sentinel species will provide an indicator as to the effects that our treatment will have on the native fauna.

The extraneous variables the toxin subgroup will concentrate on are salinity, temperature, potential effects of the flocculant on the toxin, and the pH of the water. Each of these factors affects the growth of the algae, which in turn is reflected in the prevalence of the toxin in the area

of study. Additionally, these factors could alter the perceived efficiency of the mitigating agent by denaturing the toxin or altering the mitigating agent itself.

The subgroup predicts that at least one of the agents will be able to effectively decrease the concentrations of MC-LR present in the water column without adverse effects on the Bay ecology.

The toxins subgroup's immediate goals are to be able to successfully grow the toxic strain of M. aeruginosa and to establish environmental controls for optimal growth conditions. The same principles will be used to find an appropriate sentinel species. After this is completed and the flocculant mixture is finalized, the sentinel species will be used to test for any extraneous and harmful variables that could negatively affect native flora and fauna.

The subgroup hopes that at least one of the agents will be able to effectively decrease the concentrations of MC-LR present in the water column without adverse effects on the Bay ecology.

Economics

The economics sub-group will be gathering qualitative data through in-depth surveys, with some surveys targeting University of Maryland students and others residents more familiar with the bloom regions. These surveys will include two packets. The former will contain background information, as well as different proposals for dealing with harmful algal blooms. The latter will consist of survey questions asking the responders how they feel about clay mitigation in the Chesapeake Bay and their opinions on different methods.

Confounding variables may include a lack of external validity. This is why there is the inclusion of residents of the watershed familiar with cyanobacteria blooms. Students from the University of Maryland might not be representative of the population of the Chesapeake Bay

watershed area as a whole. Our survey method will also create obtrusive measures, which may skew our data, since participants are cognizant of the fact that their opinions are being recorded and observed. Surveys of outside groups may also be undertaken, pending identification of these organizations.

We will also concentrate on keeping the cost of the mitigation mixture low so that it has true potential of being utilized by the government to control blooms and to improve the Chesapeake Bay ecosystem.

CONTRIBUTIONS TO THE RESEARCH FIELD

Team BREATHE is introducing a new method to mitigate and to prevent *M. aeruginosa* blooms in an estuarine environment. Clay flocculation mitigation efforts have previously not been studied in an estuarine environment, although lake studies with clays and clays with flocculants look very promising in reducing cyanobacteria biomass. We are also looking at how SAV seeds can be incorporated in the mixture to aid with SAV restoration, as at least one freshwater study suggests that sedimented bloom biomass amended with SAV seeds can lead to successful grass growth and expansion. Finally, we will collect new data on the effects of clay mitigation on MC-LR toxin release in the Chesapeake Bay and for non-toxic strains, as well as possible benthic responses to sedimenting blooms and clay-flocculant mixtures. We will also fill in the gaps in the literature about the life cycle of *M. aeruginosa*.

Our results will be important contributions to the research field, because: 1) mitigating tidal-fresh cyanobacteria blooms in the region has not been successful in the past; 2) SAV restoration has never been incorporated into clay flocculation attempts in the region before and SAV growth from decomposing bloom biomass may foster new opportunities for ecosystem restoration; 3) toxin fate, through interactions with the clay and flocculant, or bloom fate,

through assayed benthic organism response, will aid in broader adoption of mitigation practices in the future; 4) modeled mitigation may be an important resource for other regions experiencing cyanobacteria blooms, providing an initial first cut at possibilities for reducing public health and ecosystem impacts with a model before investment in costly purchases of clays, flocculants, ship time, etc.; and 5) public reactions to possible mitigation have deferred field manipulations in other systems. The proposed survey results will inform regional resource managers of public support for field intervention, thereby alleviating government concerns or, alternatively, indicating progressive government education to inform possible concerned citizens of mitigation impacts.

This aspect of our project has the potential to permanently improve the water quality of the Bay and to restore a balanced ecosystem by limiting algal blooms from recurrence in the area. Since the lower portions of the Chesapeake Bay have recently seen a large drop in SAV population, our mitigation approach might aid us in receiving resource manager support and permission for field testing. We can also incorporate a known neutralizing agent in the flocculant mixture to maximize the efficiency of toxin absorption, thus preventing the negative effects of this particular algal toxin in the environment.

Section 4: TIMELINE

During the spring of team BREATHE's sophomore year, the team plans on being proactive so that as much time as possible may be spent in the lab and in the field over the course of the project. One of the first steps taken will be finalizing experimental methodology. From there, a relatively concrete budget can be created, based on what is necessary for these

experiments. To help finance this budget, the team will frequently apply for grants throughout the project's duration.

The subgroups that will be working in the laboratory should be able to run their initial experiments at this time. For example, the SAV sub-group can run the seed germination tests and the flocculation sub-group can run their tests on suspension times, both of which were discussed earlier. Approval from the University's IRB (Institutional Review Board) is required before the economics subgroup can begin to administer surveys. Therefore, that subgroup will finalize survey forms early in the semester, so that the IRB application may be submitted as soon as possible. The team will also begin appealing to the agencies regulating the Chesapeake Bay in hopes of gaining access to test the clay flocculant in the Bay. Other tasks which will be completed at this time include the completion of the team's website, as well as the writing and presentation of a thesis proposal. Laboratory work will be continued throughout the summer, with quantification of clay-flocculant-colony sedimentation rates.

Tentative Laboratory Schedule, Spring 2009

1) late February-early March: Analyze the concentrate collected without the copepods. Filter, look for algal cells under microscope, identify found algae, attempt preliminary growth trials
2) late February-early March: Filter collected seawater from Mattawoman, MD. Use vacuum filters in Dr. Gantt's lab to filter all of the water (0.7 micron filters)
3) late February-early March: Obtain algae through Steve Wilhelm (samples to be sent to Dr. Gantt's office)
4) early March: Prepare for Thesis Proposal Defense, update budget and schedule

5) early March-mid March: Set up culture chamber for the algae, work in Dr. Gantt's lab to set up an area where the algae can be grown and tested

6) mid March-April: Practice obtaining accurate data from cell counting techniques, create a regression for concentration of algae vs. number of cells based on cell counts, centrifuge for biomass, fluorometer, chlorophyll extraction

7) April-May: Start tesing clay and flocculant settling rates, obtain clays and flocculants and begin getting data for settling rates without algae, determine most efficient mixtures, fastest settling rates

8) April-May: Use data from settling rate experiments to determine which concentration/type of clay which will most effectively submerge a bloom

9) late May (if time permits): Attempt to grow algae to bloom conditions and concentrations, determine the best way to create bloom conditions in the lab (if the algae can be prompted to grow in mat form this is impressive), experiment the best ways to create conditions conducive to an algal bloom and colony formation

The fall of the team's junior year will be comprised mainly of further laboratory work, with the sub-groups moving on to the next stages of their respective experiments. At this stage, algae should be present in the SAV and flocculation sub-groups' experiments. The economics subgroup will begin to administer surveys and conduct interviews as soon as IRB approval is received. This semester the team will outline its final thesis, and begin drafting the first sections. Other tasks for this semester include presenting the team's research to date at the Gemstone fall colloquium.

By spring of junior year, the clay/flocculant mixture should be in its final form so that the impacts subgroup may include the fully developed clay/flocculant mixture in their experiments. Laboratory experiments will be nearly completed this semester. Pending access, field experiments will be executed, first in microcosms (small enclosures), and then in the Bay. The team will begin data analysis, continue drafting the thesis, and present at the University of Maryland's Undergraduate Research Day.

The following summer will see completion of field experiments, moving out of the microcosms and into chambers or corrals in open water. Data analysis will continue throughout the summer and into the fall, as will drafting of the thesis. Senior year will consist of thesis revision, leading to a final thesis presentation and defense.

Section 5: BUDGET

The team's projected funds are approximately $6,000 for the fiscal year 2009. This money will primarily come from our mentors' SERC stipend ($2,000), Dr. Gantt's stipend contribution of $1,250/semester, and potentially the remainder of SeaGrant's allocated funds for the Gemstone program ($4,000). Funds will probably increase during 2010 due to the cost of obtaining equipment like microcosms and a boat to test our mitigation mixture in the field.

Clay Flocculation

The budget for the clay flocculation subgroup will consist mainly of laboratory fees before implementation in the field and potential leasing/access to a fluorometer. Since our secured lab already contains most of the necessary equipment that we need for the maintenance of algal cultures, the main cost for this subgroup will be the monthly price of incubation in the culture chamber and autoclaving the glassware. We estimate that this will be approximately

$50.00 per month. Also, the delivery fee of the parent *M. aeruginosa* cultures will be minimal, but another added cost for this subgroup. The purchase of the chitosan and clay should, once again, be of minimal cost since it is likely that we can obtain free samples from quarries and simple collection from field sites in the watershed area.

Testing our mixture in the field will most likely be the most expensive aspect of this subgroup's budget, but it is difficult to estimate this cost so early in the course of our research. Leasing (>$1,000) of a laboratory fluorometer, or access on campus, may be a substantial cost, and certainly purchase of appropriate excitation and emission filters may be necessary for the fluorometer, at approximately $600.00. Refurbishing a microbiology hood for axenic handling of the growth and flocculation experiments would be highly desirable. The present hood has a considerably decreased capacity and requires a new filter ($1,000 estimated cost). The total budget estimate is approximately $3,000.

Modeling

The Fluid Modeling subgroup has a projected budget requiring no support, as they intend to seek permission to use the University of Maryland's computing resources.

Impacts (SAV)

The SAV subgroup also has a small minimal projected budget as the SAV seeds are being obtained either from the Maryland Department of Natural Resources, the National Plant Materials Center in Beltsville, Maryland, or Dr. Steve Ailstock of the Anne Arundel Community College soil department. Funds may be necessary for chambers or corrals during junior year ($750.00).

Impacts (Toxin)

The budget of the toxins/impacts subgroup is estimated at a minimum of $2,400 dollars. This could easily go up if some of the lab equipment needed cannot be easily found on campus. This includes access to a spectrophotometer, High Performance Liquid Chromatography (HPLC) machine or columns, a DO sensor and meter, and potentially a pH sensor and meter. The cost of maintenance of our sentinel species is estimated at $200 dollars for food and habitat. A majority of total expenses comes from the ELISA tests that we will need to establish the presence or absence of toxins from certain algal strains. Each ELISA test costs $400. Depending on the longevity of these tests, we estimate that we require at least 4-5 kits to satisfy our experimental needs. Other costs include liver products, lab fees and purchase of a toxic strain. This estimate is a rough figure subject to change based on the generosity of others.

Economics

Since this subgroup plans on creating the surveys online, the economics subgroup's budget will consist of travel expenses to and from local organizations in the region as well as conferences near the Bay. There is also the possibility of this subgroup using incentives in the form of gift cards if the survey is not completed by a sufficient number of individuals.

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