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Exposure of blue crab *(Callinectes sapidus)* to modified clay treatment of *Karenia brevis* as a bloom control strategy

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Highlights

- Clay does not impact mortality or reflexes compared to untreated bloom conditions.
- The use of clay to control red tide will likely not affect blue crab populations.
- Clay destroyed algae cells but failed to remove all toxins from the water column.

Abstract

Harmful algal blooms (HABs) of the toxic marine dinoflagellate *Karenia brevis*, commonly called red tides, are an ongoing threat to human health and marine ecosystems in Florida. Clay flocculation is a standard control strategy for marine HABs in China and Korea and is currently being assessed for use in the United States. We evaluated the effects of a PAC-modified clay called Modified Clay II on mortality, eyestalk reflexes, and righting reflexes of 48 adult blue crabs *(Callinectes sapidus)*. Crabs were exposed to clay alone ($0.5\,g\ L^{-1}$), untreated *K. brevis* (1×10^{6} cells L^{-1}), or a combination of *K. brevis* and clay for eight days. Clay treatment reduced cell concentrations in the water column by 95% after 24h. We detected no significant differences in mortality, righting reflexes, or eyestalk reflexes between treatments. Our results indicate that the clay alone is not harmful to adult crabs at typical treatment concentrations within the measured time frame, and that treatment of *K. brevis* with this clay appears to have a negligible impact on crab mortality and the reflex variables we measured. These results suggest that Modified Clay II may be a viable option to treat *K. brevis* blooms without impacting adult blue crab populations. Additional controlled experiments and field tests are needed to further evaluate the impact of clay on natural benthic communities.

Previous Next

Keywords

HAB; Harmful algae; Red tide; Bloom control; Brevetoxin; Florida

1. Introduction

1.1. Karenia brevis

Harmful algal blooms (HABs) of the toxic marine dinoflagellate *Karenia brevis*, a natural phenomenon known as red tide, are an almost annual occurrence on the west coast of Florida, USA (Walshetal., 2006; Stumpfetal., 2008). *K. brevis* populations are monitored by the Florida Fish and Wildlife Conservation Commission, and their database shows that HABs of this species most frequently initiate in the summer and fall months and most frequently affect the coast between Hillsborough and Collier counties (Fig. 1, FloridaFish and Wildlife Conservation Commission(2021)). These data also show that *K. brevis* blooms vary in frequency, distribution, and intensity from year to year, and the variable nature of this phenomenon makes it difficult to predict, mitigate, and control.

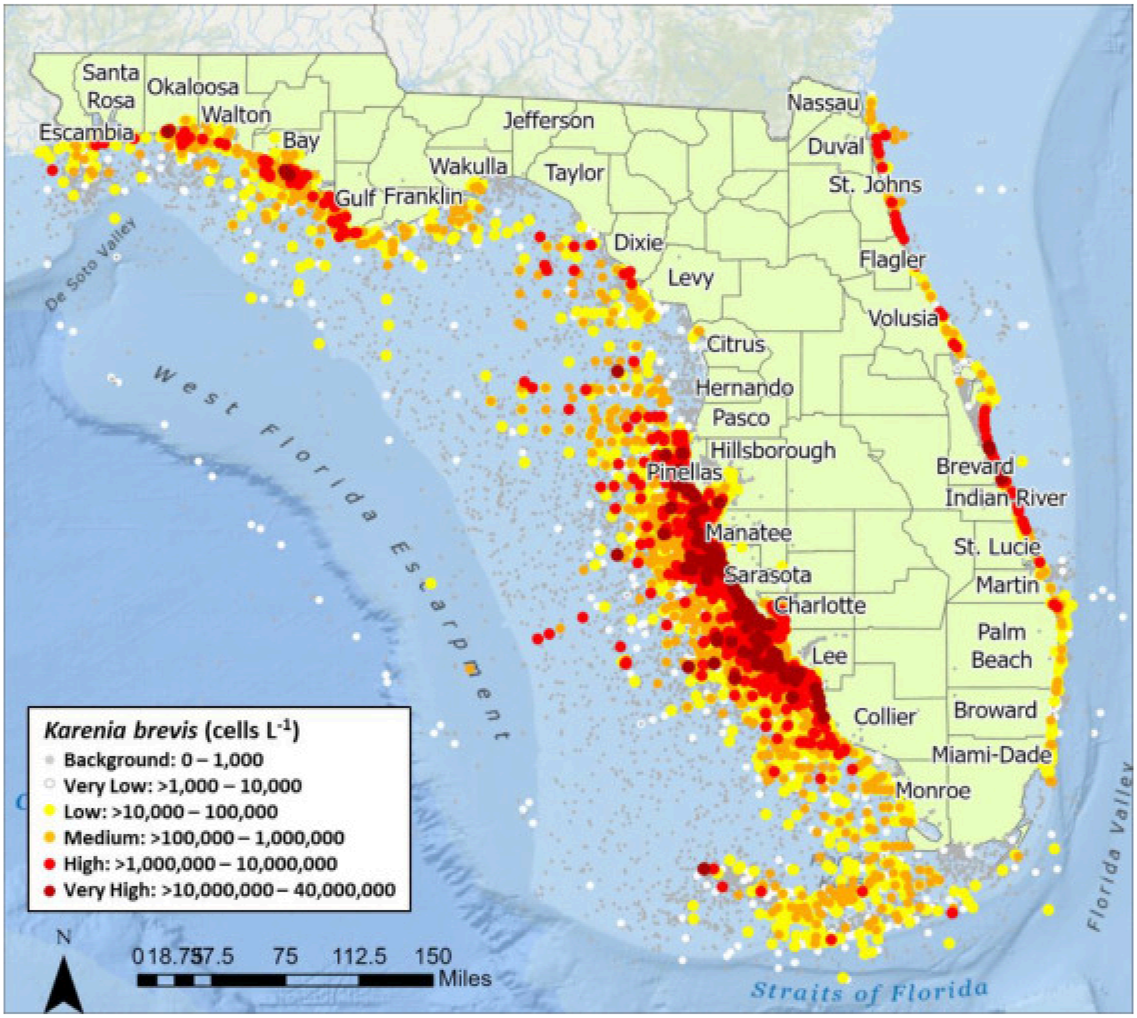

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Fig. 1. Cumulative map of *K. brevis* cell counts taken from 1990 to 2021 illustrating relative geographic extent and intensity of *K. brevis* blooms in Florida waters. Data provided by the Florida Fishand Wildlife Conservation Commission(2021).

K. brevis blooms have far-reaching impacts in the coastal environment and communities in Florida. *K. brevis* produce neurotoxins (brevetoxins), which can lead to impairment and mortality in many forms of marine life (Pierceetal., 2005; Abrahametal., 2006; Walshetal., 2006; Pierceand Henry,2008; Plakasand Dickey,2010; Pierceetal., 2011). HABs can also potentially lead to anoxic or hypoxic zones, and the combined toxic and hypoxic conditions resulting from *K. brevis* blooms can inflict mass mortalities in fish and other forms of life, including sharks, dolphins, manatees, sea turtles, and sea birds (Landsbergetal., 2002; Naaretal., 2007; Gannonetal., 2009; Shietal., 2012; DiLeoneand Ainsworth,2019; Turleyetal., 2022). In humans, ingestion of brevetoxins leads to neurotoxic shellfish poisoning (NSP), and inhalation of brevetoxins causes respiratory distress and airway constriction. Toxic aerosol is a widespread problem on coasts afflicted by *K. brevis*, which can significantly disrupt tourism and recreational activities (Watkinset al., 2008; Flemingetal., 2011). Declines of fish stocks, disruption of tourism and recreation, medical costs, and costs of beach cleanup result in the loss of millions of dollars to the local economy during each *K. brevis* HAB event (Hoagland etal., 2014; Adams,2017; Murphy,2018; Bechard,2020).

1.2. Bloom control

In Florida, USA, *K. brevis* bloom control strategies are a relatively new avenue of mitigation that are currently being researched and developed. Historically, *K. brevis* bloom management has focused on the development of environmental policies to reduce sources of nutrients that sustain these blooms (Sengco,2009; Heiletal., 2014). Other mitigation strategies include programs that monitor *K. brevis* populations, regulate seafood, remove fish kills, and educate the public (Sengco,2009). However, natural resource managers are currently lacking tools that directly control or suppress *K. brevis* blooms as they are occurring. To address this issue, multiple institutions have invested in the research and development of physical or chemical algicides, which are applied to an active bloom to remove cells and/or toxins (hereafter, referred to as bloom control).

A variety of HAB control materials and techniques are in development, but there are still many factors to be considered before these methods can be widely applied to an active bloom in open waters. The effectiveness of any bloom control technique at removing cells and toxins must be balanced against the costs of production and transportation of the control agent, feasibility of application over a large area, and minimization of negative impacts to the environment. In addition, the managers and officials responsible for using bloom control in public waters must consider public opinion (Sengco,2009). Bloom control is perhaps the least developed area of marine HAB research and management, and is arguably the most controversial, given the myriad stakeholders who use the coastal ecosystem for business and recreation and who want to ensure bloom control will not be detrimental to fisheries, ecosystems, or human health.

To date, there has yet to be a *K. brevis* bloom control method to gain public and/or regulatory acceptance. However, the intense *K. brevis* blooms that have afflicted Florida in the past decade have led to increased efforts in research circles to develop bloom controls that prioritize environmental safety. Following the 2018*K. brevis* bloom, which impacted over 150 miles of Florida coast and cost millions of dollars in damages (Murphy,2018; NOAA,2022), the state of Florida pledged $18 million to the creation of the Florida red tide mitigation & technology development initiative (RTMTDI), a collaborative research program between the Fish and Wildlife Conservation Commission and Mote Marine Laboratory to pursue development of *K. brevis* bloom control and mitigation strategies (S.B.1552, 2019). This program funds and investigates projects, including the one presented here, with the goal of finding the most efficient and benign methods for *K. brevis* bloom control in Florida waters.

1.3. Modified clay II as a bloom control

At this time, the most advanced and globally widespread method of marine HAB control is clay flocculation, which has been actively used in China and Korea for over 25 years, often on HABs over 100 km^2 in size. Among the various options for bloom control, clay is appealing because it is inexpensive to source and transport, is easily scalable over large areas, and has low environmental impacts, which has been demonstrated in the extensive laboratory experiments and field applications in China and Korea (Parketal., 2013; Yuetal., 2017; Songetal., 2021), as well as experiments taking place within the Florida RTMTDI program by the authors of this paper (currently unpublished).

In the process of clay flocculation, a clay-seawater solution (typically 5 - 20g m^{-2}) is sprayed over the surface of an affected area. As the clay particles travel through the water column, the particles interact with each other and with the bloom, lysing cells and aggregating (aka "flocculating") with cells and toxins (Pierceetal., 2004; Sengcoetal., 2005; Parketal., 2013; Segeretal., 2015; Yuetal., 2017; Liuetal., 2019; Songetal., 2021; Segeretal., 2022). The clay and cells create sinking aggregates (aka "floc"), clearing bloom biomass from the water column and depositing on the bottom sediments to be dispersed by natural currents. A variety of clay compounds have been developed for this purpose, and here we used the formulation known as Modified Clay II (MC II; described in detail in Yuetal., 2017). MC II is a kaolinite (silica-based) clay treated with polyaluminum chloride (PAC), a compound that coats clay particles and carries a positive charge, thereby allowing MC II to electrically attract particles that carry a negative charge, including *K. brevis* cells. The chemical and electrical properties of PAC-modified clay create microscopic net-like structures that capture algae cells as the clay falls through the water column, providing high cell removal efficiency (Pierceetal., 2004; Parketal., 2013; Yuetal., 2017; Songetal., 2021).

Conceptually, if clay flocculation was used to treat *K. brevis* blooms, then cells and brevetoxins would be delivered to bottom sediments; therefore, researchers and managers must consider potential impacts to organisms living at or near the bottom of the water column, such as seagrass, shellfish, mollusks, crustaceans, and benthic fish. A body of previous research has been conducted in which a non-target organisms were exposed to a various clay compounds and HAB species (Parketal., 2013; Yuetal., 2017; Zhangetal., 2020;

Songetal., 2021, 2022). Collectively, these studies conclude that clay alone is generally not harmful to non-target organisms at the concentrations used to treat blooms (typically 5 - 20g m^{-2}), and that clay treatment may reduce mortality compared to organisms exposed to an untreated bloom. However, there have been few studies on the potential impacts of clay treatment of *K. brevis* on animals (e.g., Lewisetal., 2003), and to our knowledge, this study is the first that investigates the combination of *K. brevis* with the MC II formula on animals. Given the numerous concerns and possible outcomes of adding non-endemic compounds to natural systems, it is imperative to investigate how MC II might potentially affect the marine food web, including recreational and commercial species that are important components of Florida's ecological and economic viability.

1.4. Research objectives

The goal of this experiment was to observe potential lethal and sublethal (behavioral) effects of Modified Clay II (hereafter referred to as clay) and *K. brevis* on a bottom-dwelling species with ecological and economic importance in Florida. It should be noted that this study was not conducted to assess the toxicity of *K. brevis* to blue crabs, the cell or toxin removal efficiency of MC II, or the impact of clay treatment on water quality, since these aspects have been studied separately our research group as a part of the Florida RTMTDI program (currently unpublished). However, we will present cursory results of some of these topics as they relate to our specific questions outlined below.

Blue crabs *(Callinectes sapidus)* were chosen for this study for their importance in the marine food web and significance as a fishery species. Blue crabs are a vital component of the benthic marine food web and are known as a dominant prey source for marine fish, birds, and mammals, and can also serve as keystone species by consuming herbivores that affect habitat structure (e.g., *Littoraria irrorate*, which feeds on marsh grass; Boudreauand Worm,2012). Blue crab is also a valuable fishery species throughout the Gulf of Mexico and Eastern United States, with over a century of history in Florida (Steeleand Bert,1998). In 2020 alone, commercial landings of blue crab in Florida were reported at over 3000 t, with a worth of over 13 million USD (NOAAFisheries,2021). News articles have reported blue crabs appearing in fish kills associated with recent *K. brevis* blooms (Allen,2018; O'Brien,2021) and blue crabs were identified in 29 fish kills in the Florida Fish and Wildlife database in 2018 alone (FloridaFish and Wildlife Conservation Commission,2023a).

The objective of this study, based on Lewisetal.(2003) and other previous studies conducted with various clays and animals, outlined in Parketal.(2013), Yuetal.(2017), and Songetal.(2021), was to determine if clay increased mortality or caused behavioral changes in blue crab. Specifically, we hypothesized that mortality and reflex impairment of blue crabs exposed to clay-treated *K. brevis* bloom would be less than or equal to crabs exposed to an untreated *K. brevis bloom.*

2. Methods

2.1. Experimental laboratory

This study took place at Mote Marine Laboratory in Sarasota, FL. Blue crabs *(Callinectes sapidus)* were collected between July and October of 2020 from Sarasota Bay at Bird Key Park using baited crab traps (FL Special Activity License 20–2204-A-SR). A total of 48 crabs were collected, 39 male and 9 female, with a mean carapace length of 14cm (± 1.5cm). According to historic records, *K. brevis* blooms typically initiate offshore in the summer months and achieve bloom density (> 100,000 cells/L) between June and October (Walshetal., 2006). When this study took place, according to the state database, *K. brevis* cell concentrations at west coast sampling sites were not above background levels (0 – 1000) at any time, and most cell counts were zero (FloridaFish and Wildlife Conservation Commission,2023c).

2.2. Experimental setup

Crabs were randomly assigned to individual 20L tanks (42cm length, 27cm height, and 22cm width) containing filtered and ozonated seawater supplied by an indoor flow through system. Tanks were arranged in random positions within two rows within a raceway and randomly assigned to treatments. Each tank was treated as an experimental unit in statistics. Additionally, separating individuals prevented potential mortality and stress through interaction. Each tank was aerated with Penn Plax air pumps via two tubes without air stones (20cm length, 0.5 inner diameter). Before the experiments, during acclimation, water was allowed to overflow the tanks into the raceway; for the duration of the experiments, flow was turned off and tanks were static to prevent loss of cells or clay from the tanks. Crabs were allowed to acclimate to laboratory conditions for a minimum of 24h before experiments began. Crabs were fed with frozen shrimp provided daily, and uneaten food and solid waste were removed daily using small aquarium dip nets. Due to limited space, only eight tanks could be tested at a time; therefore, this study was conducted over seven experiments that ran between July and October in 2020.

2.3. Experimental design

Tanks were randomly assigned to one of four treatments: seawater only, clay only, *Karenia* only, or clay-treated *Karenia* (Fig. 2, n = 12 tanks per treatment). Crabs were exposed to their respective treatments for two consecutive periods of four days (96 h each), for a total treatment time of eight days (192 h total). After four days, tanks were given a complete water change and were re-dosed with culture and clay in the same concentrations described below. Because water flow remained off for the duration of the experiments, a water change was necessary to prevent dissolved ammonia waste from building to toxic levels, which can induce stress in crabs (Weihrauch, 2004).

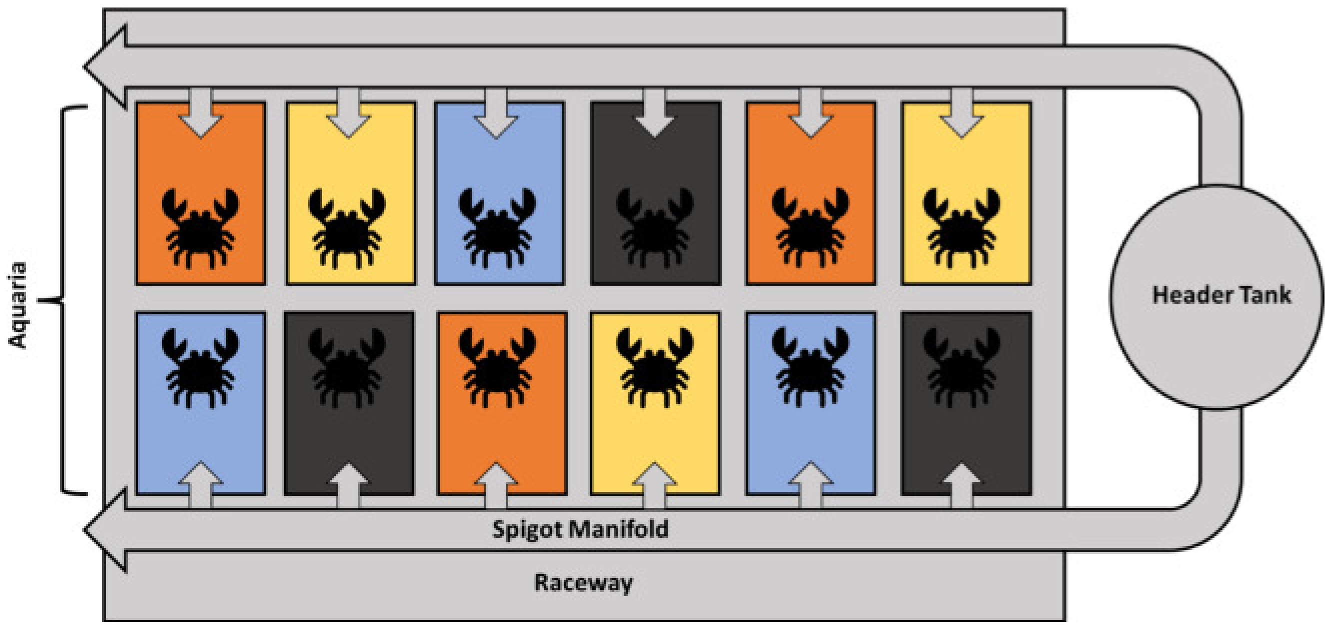

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Fig. 2. Conceptual diagram of the experimental setup. Water was supplied to tanks via spigots from a single header tank. Water was allowed to overflow the tanks during acclimation and drain into the raceway, and flow was turned off during the experimental period. Tanks were randomly assigned to one of four treatments: seawater only (blue), clay only (yellow), *Karenia* only (orange), or clay-treated *Karenia* (gray).

K. brevis culture was added to *Karenia* only and clay-treated *Karenia* tanks to achieve treatment concentrations of 1×10^6 cells L^{-1}, which has been used as a target in previous *K. brevis* exposure studies (Lewis et al., 2003; Gravinese et al., 2018, 2019) and is representative of a "high" intensity bloom according to the Florida Fish and Wildlife Conservation Commission (2023b). Mean initial cell concentrations were measured at 1023,333 ± SE 55,683 cells L^{-1} on day 1 and 923,333 ± SE 90,265 cells L^{-1} on day 5. *K. brevis* was cultured at Mote in L1 media (enriched f/2 media from

Guillard and Ryther, 1962) at 24°C, salinity 32–34, and a 12-hour light-dark cycle at 50–60 µmol m^{-2} s^{-1}.

Modified Clay II was added to clay only and clay-treated *Karenia* tanks. MC II was provided by the Institute of Oceanology Chinese Academy of Science, Qingdao, China. The base kaolinite has a median grain size (D50) of 23.5µm and the following chemical composition: silicone dioxide (SiO_2): 45.6%; aluminum oxide (Al_2O_3): 31.2%; potassium oxide (K_2O): 1.8%; iron (III) oxide (Fe_2O_3): 0.9%; calcium oxide (CaO): 0.1%; magnesium oxide (MgO): 0.2%; sodium oxide (Na_2O): 0.02%; sulfur trioxide (SO_3): 1.1%; other inorganics (LOI): 16.1%. Clay was applied one hour after culture was applied. For application, clay was mixed with seawater in a squeeze bottle and sprayed on the surface of tanks to achieve a final concentration of 0.5 g L^{-1}. It should be noted that this concentration is a relatively high, as high *K. brevis* cell removal can be achieved at 0.1 g L^{-1} of MC II (unpublished data), but a higher concentration was chosen as it better reflects the clay deposition expected from clay treatment of natural waters in actual practice, compared to these shallow aquarium tanks.

Environmental parameters were monitored for stasis to ensure the drivers of stress on the crabs were the *K. brevis* and clay, and not a confounding effect of water quality. Water quality parameters, including temperature, salinity, pH, and dissolved oxygen, were measured daily using a calibrated multimeter (ProDSS YSI). Ammonia was monitored using Salifert aquarium test kits (0.5 – 2.0 ppm limit). To monitor daily cell populations, 10 mL integrated samples were taken from the water column at the center of the tank with a pipette. These samples were preserved with Lugol's iodine solution to conduct cell counts on a Sedgewick rafter slide under a compound light microscope. Toxins were measured in addition to cell populations, since toxins were the mechanism by which the *K. brevis* would induce stress and mortality in the crabs in this experiment. To measure toxins, integrated 100 mL water samples were taken from the water column at the center of the tank with a pipette at the beginning and end of each dosing period (days 0, 4, 5, and 8). Brevetoxin types and concentrations were analyzed by liquid chromatography-mass spectrometry using a ThermoFinnigan AqA HPLC/MS (methods described in Pierce et al., 2011). For this study, we measured the two parent toxins produced by *K. brevis*, BTx-1 and BTx-2 (intracellular toxins), and the two major degradation products (extracellular toxins) of BTx-2, which are BTx-3 and BTx-2-CA (extracellular toxins). The parent toxins create a suite of other products that were not included in analysis due to the lack of standards and inconsistent alterations in mass spectrometric detector response. For our instrument, the limits of detection were 54 ng L^{-1} for BTx-1, 23 ng L^{-1} for BTx-2, 9 ng L^{-1} for BTx-3 and 14 ng L^{-1} for BTx-2-CA.

Trends in cell concentrations, total toxins, and water quality parameters were examined with multivariate generalized linear models (GLMs) to determine if treatments had an impact on these measurements, and to ensure water quality was in stasis and not having a confounding effect on mortality and reflexes of the crabs apart from experimental treatments. If data did not meet assumptions of normality and variance of a Gaussian distribution, alternative distributions were explored.

To evaluate lethal and sublethal responses to treatments, we monitored mortality and conducted reflex tests daily using a binary data system. In this experiment, we measured righting reflex and eyestalk reflex daily, modeled after the pesticide exposure experiment conducted on blue crabs by Schroeder-Spain et al. (2018). Crabs were handled inside the tanks and were not handled for more than five minutes each day to minimize stress. The righting reflex was measured by using forceps to flip the crab on its dorsal side. Presence of the reflex (value of 1) was noted if the crab righted itself in ≤ 5 s; absence of the reflex (value of 0) was noted if the crab failed to right itself after > 5 s. The eyestalk reflex was measured by touching the forceps to the eyestalks. Presence of the reflex (1) was noted if the crab retracted both eyestalks; absence of the reflex (0) was noted if the crab did not retract one or both eyestalks in response to touch. Active, responsive crabs were considered alive (1). Crabs were considered dead (0) if they were inactive, limp, and unresponsive to both reflex tests.

2.4. Statistical analyses

To analyze crab mortality, we used Cox proportional hazard models (Cox, 1972) using the “survival” and “survminer” packages in R (R Core Team, 2020). Cox models are a method of modeling survival as a function of one or more variables, in which the primary assumption is that the risk of mortality between groups is constant and proportional over time. The base formula structure for the hazard function h(t) is:

$$h(t) = h_0(t) \times \exp(b_1 x_1 + \ldots + b_p x_p)$$

In this formula, t represents survival time, x represents covariates, b represents the effect size of covariates, and h_0 is the value of the hazard when all covariates equal zero. Cox models have been used in previous studies to analyze crab mortality in response to stressors (Gravinese et al., 2018; Tankersley and Wieber, 2000). Sublethal responses of the crabs were examined using multivariate generalized linear models (GLMs) using logistic regression. Variables considered in these models, apart from time and treatment, included crab sex, crab size, experimental trial, tank placement, and water quality parameters; however, these covariates were not found to have any significant effect on mortality and were not included in the final model presented here.

In our model exploration process, to compare between GLMs and select the distribution and formula structure that best represented each response variable, we used Akaike's Information Criteria (AIC) scores (Akaike, 1973), a measure of model predictive error, in which lower scores indicates a better fit to the data. All statistical analyses were conducted in the statistical program R (R Core Team, 2020).

3. Results

3.1. Changes in experimental environment

Water quality parameters remained within narrow ranges and showed little variation within or between treatments, with pH as the notable exception (Supplementary Tables 1 – 2, Supplementary Fig. 1). Temperature ranged between 22 – 26 °C, salinity between 34 – 37, and dissolved oxygen remained above 6.0 mg/L, falling within observations of the ranges blue crabs occupy in nature (Fisher, 1999). In clay only and clay-treated *Karenia* tanks, application of clay caused pH to drop significantly (GLM, $p < 0.0001$). On day 1, mean pH of clay only tanks was 7.25 (0.07 SE) compared to a pH of 7.86 (0.03 SE) in seawater tanks. After this initial drop, pH of clay tanks gradually increased over time and returned to ambient pH of the seawater tanks after 72 h. Lower levels of pH have been shown to affect survival rates of larval blue crab (Tomasetti et al., 2018), but did not appear to have an effect on mortality and reflexes of our adult crabs. Despite the observed effect on pH, water quality parameters did not contribute significant effects to models of trends in cell concentrations, toxins, mortality, or reflexes.

Cell concentrations in clay-treated *Karenia* tanks decreased by 95% after 24 h and 98% after 48 h. In models, cell concentrations were significantly lower in clay-treated *Karenia* tanks compared to *Karenia* only tanks (GLM, $p < 0.0001$, Supplementary Table 3 - 4). The percent removal efficiency of clay was 93% after 24 h (Fig. 3).

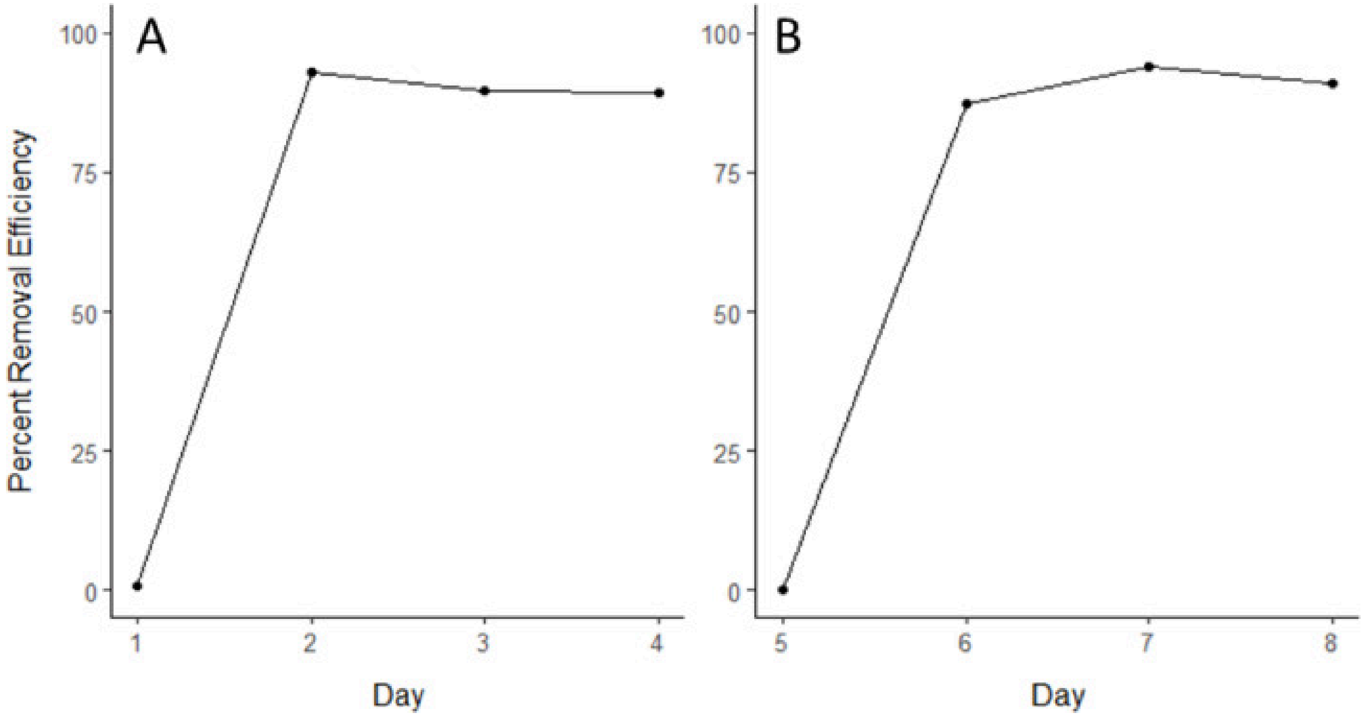

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Fig. 3. Percent removal efficiency of *Karenia brevis* cells over time from tanks treated with clay subtracted from untreated tanks. Plots are separated by measurements taken during the first dosing period on 1 - 4 (A) and the second dosing period on days 5 - 8 (B).

Total toxins (sum of BTx-1, BTx-2, BTx-3, and BTx-2-CA) significantly decreased over time in both treatments (GLM, $p < 0.0001$). We found no significant differences in total toxins between *Karenia* only tanks and clay-treated *Karenia* tanks at any time point (GLM, $p > 0.05$, Fig.4).

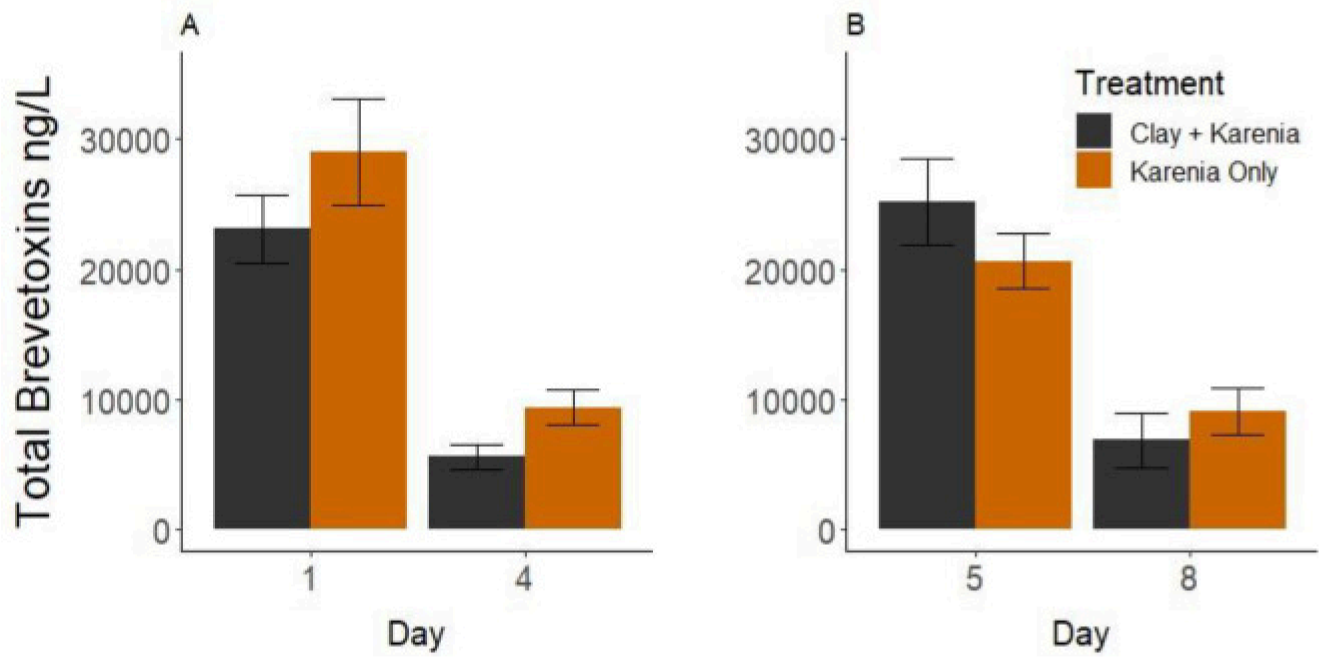

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Fig. 4. Mean ± SE concentrations of sum of BTx-1, BTx-2, BTx-3, and BTx-2-CA from tanks containing either *K. brevis* only or *K. brevis* treated with clay (n=12 tanks per treatment). Plots are separated by measurements taken during the first dosing period on days 1 and 4 (A) and the second dosing period on days 5 and 8 (B).

3.2. Mortality and reflexes

Out of the 48 crabs tested, 2 mortalities occurred in seawater only tanks, 1 in clay only tanks, and 1 in *Karenia* only tanks. No mortalities occurred in the clay-treated *Karenia* tanks, and no mortalities occurred in any treatment within the first 48 h of exposure. The survivorship analysis found no significant differences among treatments (Cox model, $p > 0.05$, Table 1 and Fig. 5). There were also no significant differences observed among treatments for either the eyestalk reflex or the righting reflex (GLM, $p > 0.05$, Tables 2 and 3).

Table 1. Summary of Cox survivorship model of *Callinectes sapidus* survivorship as a function of time and treatment.

Response variable	**Distribution**	**Formula**	**Degrees of Freedom**
Survival (Alive or Dead)	**Binomial**	***y*=day+treatment**	**4**
Coefficients	**Hazard Ratio (exp(coef))**	**Standard Error**	**Probability > z (P-value)**
Day	0.9645	0.0210	0.0857
Clay only	0.7817	0.1433	0.0857
Karenia only	0.8689	0.1422	0.3229
Seawater	0.8701	0.1422	0.3314

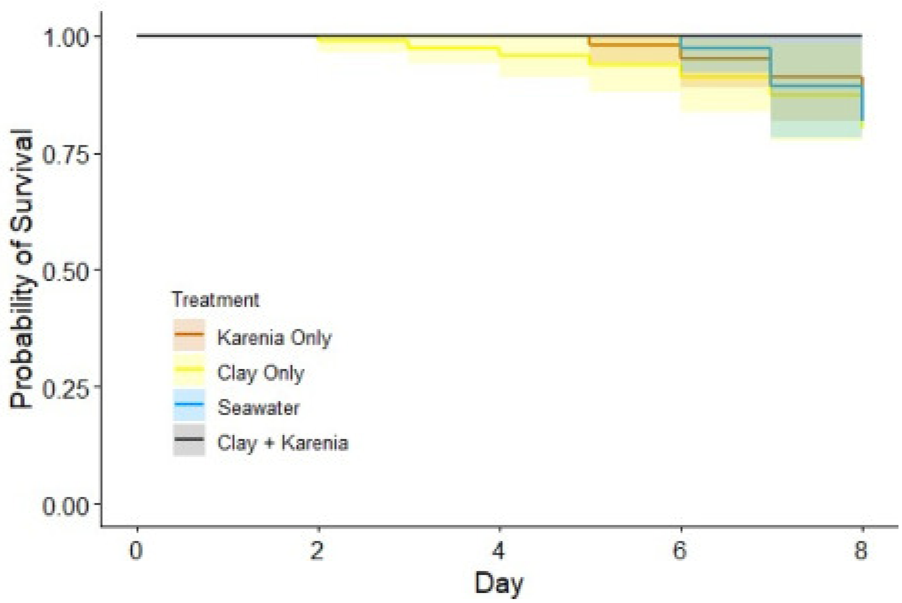

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Fig. 5. Cumulative survivorship of *Callinectes sapidus* in tanks containing seawater only, clay only, *K. brevis* only, or *K. brevis* treated with clay (CK) over eight days. Lines represent means of the model and shading represents 95% CI (n=12 tanks per treatment). No significant differences were detected among treatments ($p > 0.05$).

Table 2. Summary of Generalized Linear Model of *Callinectes sapidus* eyestalk reflex as a function of day and treatment over eight days.

Response variable	**Distribution**	**Formula**	**Degrees of Freedom**
Eyestalk Reflex (Pass or Fail)	**Binomial**	***y*=day+treatment**	**321**
Coefficients	**Estimate**	**Standard Error**	**Probability > z (P-value)**
Day	0.1669	0.0704	0.0176
Clay only	0.4986	0.5262	0.3434
Karenia only	0.8882	0.4720	0.0599
Seawater	−0.6900	0.5855	0.2386

Table 3. Summary of Generalized Linear Model of *Callinectes sapidus* righting reflex as a function of day and treatment over eight days.

Response variable	**Distribution**	**Formula**	**Degrees of Freedom**
Righting Reflex(Pass or Fail)	**Binomial**	***y*=day+treatment**	**399**
Coefficients	**Estimate**	**Standard Error**	**Probability > z (P-value)**
Day	0.3654	0.1311	0.0053
Clay only	17.942	1699	0.9916
Karenia only	17.330	1699	0.9919
Seawater	17.571	1699	0.9918

4. Discussion

4.1. Effects of clay on experimental subjects

In this study, we investigated the potential impacts of Modified Clay II treatment of *K. brevis* on blue crab *(C. sapidus)*, an ecologically and commercially important species in Florida. Supporting our initial hypothesis, mortality and reflexes in crabs were not significantly different in clay-treated *Karenia* tanks compared to *Karenia* only tanks (Fig.5, Tables 1 – 3). Our study can be most closely compared to Lewisetal.(2003), which to our knowledge is the only other study that examined the impact of modified clay treatment of *K. brevis* on marine animals, using a formulation of phosphatic clay. In that study, researchers found that mortality of amphipods *(Leptocheirus plumulosus, Ampelisca abdita)*, grass shrimp embryos

(Palaemonetes pugio), and sheepshead larvae *(Cyprinodon variegatus)* in clay-treated *K. brevis* tanks were not significantly different from mortalities in untreated *K. brevis* tanks, similar to the findings presented in this paper. Additionally, our results fall in line with other previous research with modified clays, which indicate clay has negligible impacts on animal survival (Parketal., 2013; Yuetal., 2017; Zhangetal., 2020; Songetal., 2021, 2022). We conclude a 0.5 g L^{-1} dosage of MC II combined with bloom-level densities of *K. brevis* had zero to negligible impacts on the lethal and sublethal responses of crabs taken within the time frame of this study. As stated previously, the clay loading concentration used in this study is significantly higher than levels that are known to be effective at removing *Karenia* cells with modified clay (ex. 0.1 - 0.2 g/L; Yuetal., 2017). Our results here are thus conservative, based on an initial clay loading that is as much as five times higher than is needed for effective cell removal, but which is representative of a clay loading that might occur in a practical application where multiple doses might be needed.

This study contributes to ongoing research of bloom control technologies as one of the few investigations on the impact of clay treatment of *K. brevis* on non-target organisms, and to our knowledge, is the first animal study conducted using MC II in combination with *K. brevis*. Our findings suggest that MC II treatment of a natural bloom may not be more harmful to adult blue crab populations than untreated bloom conditions. Further, because the use of clay will reduce growth and spread of the bloom, the crab mortalities typically seen in fish kills from prolonged exposure to *K. brevis* (FloridaFish and Wildlife Conservation Commission,2023a) could be prevented. Therefore, crab populations could see fewer mortalities and may recover more quickly with treatment than if the bloom were allowed to persist untreated. Any form of bloom control would be most effective at preserving fish and shellfish populations if treatment can be applied before the bloom reaches high intensities, perhaps multiple times throughout the bloom event.

4.2. Effects of clay on experimental environment

This study did not focus on the impact of MC II on water quality or the effectiveness of clay on cell and toxin removal since these aspects are being examined in past and future work within our research team. But we do find it pertinent to provide a brief overview of these findings in the context of the goals of this paper. Cell removal capabilities of the clay reported in this study (Fig.3) are in line with preliminary experiments of MC II conducted by our research team, and extensive literature describing the removal capabilities of various clay types on different algae species (Pierceetal., 2004; Sengcoand Anderson,2004; Parketal., 2013; Yuetal., 2017; Songetal., 2021). Other previous studies reported high removal rates of toxins with various clays and algae species (Pierceetal., 2004 (90% decrease in brevetoxins with phosphatic clay); Segeretal., 2015 (100% decrease in prymnesins with bentonite clay); Yuetal., 2017 (97% decrease in microcystins with modified clay); Segerand Hallegraeff,2022 (60 - 90% decrease in microcystins with various clays)). Other water quality characteristics, including pH, nutrients, and organic matter, may also have an impact on the ability of clay to remove cells and toxins (Sengcoetal., 2005; Segeretal., 2015; Liuetal., 2019). In examining total toxins (sum of the four toxin analogs), we did not observe a significant difference between treatments in this study (Fig.2). Although we were not attempting to examine the effectiveness of clay on toxins, this finding may be of interest to examine further in future work. We did observe a significant decline in total toxins in both treatments over time, which has several potential explanations. First, conversion between the parent and metabolite toxins is not one-to-one, and parent toxins were likely converted to a suite of other metabolites over time (Abrahametal., 2006; Rothetal., 2007; Pierceetal., 2011) which are not normally measured using our current HPLC procedures. It is also possible that some toxins were degraded (Kieberetal., 2010) or sequestered in the clay floc (Pierceetal., 2004). Other possible routes of toxin loss may be through the organisms, where toxins may accumulate or depurate (Hintonand Ramsdell,2008), or through aerosolization (Pierceetal., 2005). These potential routes of toxin fate are of interest but were outside the scope of this study and will be considered in future research within our team.

HAB control technologies have the potential to significantly reduce ecological impacts and economic losses in areas affected by *K. brevis* blooms. It is important to consider that the water quality changes induced by clay are temporary (i.e., turbidity, pH, etc.), and that the alternative to utilizing bloom control technologies is to allow an untreated bloom to run its course, thus allowing continuous damage to the environment over weeks or months. Shumwayetal.(2003) and Archambaultetal.(2004) expressed caution towards the use of clay for bloom control. In these studies, researchers exposed various invertebrates to yellow loess and phosphatic clay, respectively, without the addition of a toxic HAB species. Shumwayetal.(2003) reported reduced clearance rates in their various filter-feeding organisms exposed to relatively high concentrations of continuously suspended clay, and Archambaultetal.(2004) reported reduced growth rates in juvenile hard clams *(Mercenaria mercenaria)* exposed to continuously suspended clay and nontoxic algae. However, managers might consider the findings of Shumwayetal.(2003) and Archambaultetal.(2004) acceptable outcomes compared to the widespread economic and ecological damage inflicted by an untreated toxic bloom, recognizing also that the amount of clay being used in more recent work is considerably lower than was the case twenty years ago, nor is continuous resuspension a realistic scenario, except in very shallow waters.

4.3. Future directions

Further study is needed on the fate of *K. brevis* cells and toxins and on potential sublethal impacts of clay on the marine food web. As we are currently lacking a holistic understanding of how clay might affect the marine ecosystem as a whole, scientists, managers, officials, and the public are reluctant to apply clay on active blooms. Although the use of clay is prevalent in other parts of the world, further study is needed on its impacts in the United States and in Florida waters to determine if this is a safe and effective technology to use on *K. brevis* blooms. To that end, our team will continue our research of clay in iterative experiments of increasing physical scale. The next step in this process will be to conduct experiments in 1400L mesocosm tanks to test Modified Clay II for cell and toxin removal, changes in water quality and nutrients, and impacts on a benthic community representative of Sarasota Bay, which will include hard clam (Mercenaria campechiensis), sea urchin (Lytechinus variegatus), and blue crab (C. sapidus). In these experiments, we intend to further explore potential sublethal impacts of treatment and the fate of toxins by measuring respiration rates in organisms and measuring toxins in organs. Following these land-based experiments, our team plans to conduct experiments with MC II in field tests on natural *K. brevis* blooms to gain additional information on clay dispersal dynamics and gage practicality of clay application.

The overall goal of our work is advance the development of *K. brevis* bloom control in Florida by providing a foundation of study on the potential impacts of clay treatment on non-target species so that resource managers can make informed decisions on the use of bloom control technologies in their local waters. Our continued studies on the ecological impacts of clay will include additional important species, such as the macrobenthic and microbial communities that support the marine ecosystem. If clay proves to be a safe and effective bloom control method, this technique may be adapted to treat HABs across the nation.

Author declaration

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, without the written consent of the copyright-holder.

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- Each author has participated sufficiently in the work to take public responsibility for all the content.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix. Supplementary materials

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Recommended articles

Data availability

Data will be made available on request.

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2024, Harmful Algae

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Modified clay compounds are used globally as a method of controlling harmful algal blooms, and their use is currently under consideration to control *Karenia brevis* blooms in Florida, USA. In 1400 L mesocosm tanks, chemical dynamics and lethal and sublethal impacts of MC II, a polyaluminum chloride (PAC)-modified kaolinite clay, were evaluated over 72 h on a benthic community representative of Sarasota Bay, which included blue crab *(Callinectes sapidus),* sea urchin *(Lytechinus variegatus),* and hard clam *(Mercenaria campechiensis)*. In this experiment, MC II was dosed at 0.2 g L^{-1} to treat bloom-level densities of *K. brevis* at 1 × 10^6 cells L^{-1}. Cell removal in MC II-treated tanks was 57% after 8 h and 95% after 48 h. In the water column, brevetoxin analogs BTx-1 and BTx-2 were found to be significantly higher in untreated tanks at 24 and 48 h, while in MC II-treated tanks, BTx-3 was found to be higher at 48 h and BTx-B5 was found to be higher at 24 and 48 h. In MC II floc, we found no significant differences in BTx-1 or BTx-2 between treatments for any time point, while BTx-3 was found to be significantly higher in the MC II-treated tanks at 48 and 72 h, and BTx-B5 was higher in MC II-treated tanks at 24 and 72 h. Among various chemical dynamics observed, it was notable that dissolved phosphorus was consistently significantly lower in MC II tanks after 2 h, and that turbidity in MC II tanks returned to control levels 48 h after treatment. Dissolved inorganic carbon and total seawater alkalinity were significantly reduced in MC II tanks, and partial pressure of CO_2 (pCO_2) was significantly higher in the MC II-only treatment after 2 h. In MC II floc, particulate phosphorus was found to be significantly higher in MC II tanks after 24 h. In animals, lethal and sublethal responses to MC II-treated *K. brevis* did not differ from untreated *K. brevis* for either of our three species at any time point, suggesting MC II treatment at this dosage has negligible impacts to these species within 72 h of exposure. These results appear promising in terms of the environmental safety of MC II as a potential bloom control option, and we recommend scaling up MC II experiments to field trials in order to gain deeper understanding of MC II performance and dynamics in natural waters.