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## **The Susceptibility for Recreational Boating to Serve as a Vector for Harmful Algal Blooms**

John Michael Thraen

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## **Abstract**

To combat the spread of invasive and nuisance aquatic species, recreational boating has many recommendations and regulations in place for boaters to properly treat their boats in between uses in separate locations. While protection procedures are in place for preventing the spread of invasive mussel and plant species, there is no sufficient procedure in regard to the spread of cyanobacterial species. The objective of this study was to investigate whether common drying procedures employed in these treatment practices were capable of additionally preventing the spread of cyanobacterial species so as they may help prevent the spread of algal blooms throughout different freshwater bodies. This study employed the use of microscope slides simulating common recreational boat hull materials and the exposure they would experience during extended use in conditions of algal blooms, after which different drying treatments were applied. These drying treatments were used to analyze the effectiveness of commonly suggested drying periods for other nuisance species upon these cyanobacterial species and were analyzed afterwards through the culturing of the slide contents to determine whether or not the cells were still capable of growing into a new population. This study demonstrated that the widely used 5-day drying duration had no effect on the ability for the cyanobacterial species to grow in comparison to the cultures that experienced no such drying period, and in addition found that there was no significant difference in taxa richness found in the cultures. These results imply that the currently widely used 5-day drying method may not be sufficient for the prevention of the spread of cyanobacterial species. Revised policy is needed to better stop recreational boaters from cross contaminating separate water bodies with cyanobacteria.

Montclair State University

The Susceptibility for Recreational Boating to Serve as a Vector for Harmful Algal  
Blooms

By

John Michael Thraen

A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of

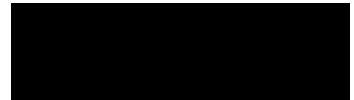
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THE SUSCEPTIBILITY FOR RECREATIONAL BOATING TO SERVE AS A  
VECTOR FOR HARMFUL ALGAL BLOOMS

A THESIS

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## **Introduction**

When it comes to boating, a common issue that the practice of using boats in multiple separate bodies of water has been known to lead to is the spread of aquatic nuisance and invasive species (Dalton and Coltrell, 2013). Invasive Quagga and Zebra mussels can use boats as a vector either by attaching boat hulls or by being retained in ballast water and other compartments of boats (Dalton and Coltrell, 2013). Mussels that are not physically or chemically removed from boats can become established in other areas when boats are transported among water bodies (Nebraska Public Power District, 2021). Transporting boats among water bodies without washing can spread other non-native organisms such as plants and zooplankton (Connecticut Department of Environmental Protection, 2005). Invasive cyanobacterial species are also of concern when it comes to freshwater bodies, as they too are capable of easily establishing and taking over the communities of freshwater habitats globally (Mehner et al, July 2010). To combat spreading invasive species, proper boat treatment guidelines have been developed and at times enforced throughout the United States by federal, state, and independent agencies.

### Overview of Current Recreational Boating Treatment Processes:

The current recreational boating treatments and cleaning procedures vary among different states and regions within the United States. To provide a full outlook on current recommendations and procedures in place in the US, a summary of the rules and regulations is included in Table 1.

Table 1: Overview of recreational boating treatment regulations and recommendations by state. State level as well as federal level recommendations and regulations are incorporated for each state.

State	Boat Treatment Recommendations and Regulations
Alabama	Alabama has adopted recommendations from the United States Department of Agriculture (USDA) requesting that boaters in the state dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007).
Alaska	Alaska has adopted recommendations from the United States Department of Agriculture (USDA) requesting that boaters in Alaska dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). Alaska’s Department of Fish and Game recommends using the Clean-Drain-Dry technique for preventing invasive species’ spread, with the drying duration stated as allowing the boat and gear to “completely dry” in between uses (Alaska Department of Fish and Game).
Arizona	Arizona has adopted recommendations from the United States Department of Agriculture (USDA) requesting that boaters in Arizona dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). Arizona has laws in place that require boaters to have their boats dried and inspected in between uses in separate bodies of water when they are known by the state to host invasive species, and the required drying duration varies by season with a 1-week long period from May through October and at least 18 days for the remainder of the year (Raney, 2018).
Arkansas	Arkansas has adopted recommendations from the United States Department of Agriculture (USDA) requesting that boaters in Arkansas dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). Arkansas has laws in place requiring boaters to remove drain plugs after using the boats to prevent the spread of water outside of the boating location (Thurston, 2020).



California	California has adopted recommendations from the United States Department of Agriculture (USDA) requesting that boaters in the state dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state's freshwater bodies (United States Department of Agriculture, 2007). California has additionally required boaters to follow a minimum 5-day drying period before introducing a boat to a different water body, and additionally recommends drying the boats for up to a month during the colder months of the year (California Department of Fish and Game, 2008).
Colorado	Colorado requires boaters to follow the Clean-Drain-Dry procedure between outings, and additionally has enforced the removal of drain plugs so as to prevent water from being transported by trailered boats after use (Colorado Parks and Wildlife)
Connecticut	Connecticut has adopted United States Department of Agriculture (USDA) recommendations, requesting that boaters in the state dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state's freshwater bodies (United States Department of Agriculture, 2007). Connecticut's Department of Environmental Protection recommends that boaters dry their boats for at least 2 and preferably 5 days between uses and has illegalized the transportation of plant life by trailered boats with the ability for the state to issue fines to those found in violation of said law (Connecticut Department of Environmental Protection, 2005).
Delaware	Delaware's Division of Fish and Wildlife Enforcement has recommended its boaters to dry their boats "for as long as possible" as a means of preventing the spread of species (Delaware Division of Fish and Wildlife Enforcement, 2011).
Florida	Florida's Department of Environmental Protection recommends that boats be kept dry in between outings, and has employed educational outreach programs to help prevent the state's boaters from spreading invasive species (Florida DEP, 2007)
Georgia	Georgia's Department of Natural Resources' Wildlife Resources Division has recommended the use of the Clean-Drain-Dry technique between outings, and additionally recommends using a drying duration of 5 days (Georgia Department of Natural Resources Wildlife Resources Division).
Hawaii	Hawaii's state government has the authority to regulate ballast water discharge within the state's waters and has been conducting oversight and regulatory actions for the purpose of preventing the spread of invasive and nuisance species through boating activity (Hawaii Department of Land and Natural Resources).

Idaho	Idaho has implemented inspection stations surrounding its water bodies that are used to prevent boaters from incidentally spreading nuisance and invasive species (Invasive Species of Idaho). The state has also implemented an identification sticker program for its Invasive Species Fund, where the states boaters are required to fund programs focused upon the prevention of invasive species' spread through the requirement of purchasing these stickers for their boats in order for them to be able to access the state's bodies of water (Idaho Parks and Recreation).
Illinois	Illinois has adopted recommendations from the United States Department of Agriculture (USDA) requesting that boaters in Illinois dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state's freshwater bodies (United States Department of Agriculture, 2007). The state has recommended its boaters to follow the Clean-Drain-Dry procedure with a drying period of at least 5 days being specified (Illinois Department of Natural Resources).
Indiana	Indiana has followed recommendations from the United States Department of Agriculture (USDA) that had recommended that boaters in Indiana dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state's freshwater bodies (United States Department of Agriculture, 2007). Indiana's Department of Environmental Management has recommended for its boaters to allow their vessels to dry for at least 5 days in between uses in separate water bodies for the purpose of preventing the spread of invasive species (Indiana Department of Environmental Management, 2012/2008).
Iowa	Iowa has adopted recommendations from the United States Department of Agriculture (USDA) requesting that boaters in the state dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state's freshwater bodies (United States Department of Agriculture, 2007). Iowa's state government recommends its boaters follow the Clean-Drain-Dry treatment technique, and they likewise recommend drying boats for at least 5 days in between uses (Iowa Department of Natural Resources, 2019).
Kansas	Kansas has followed the United States Department of Agriculture (USDA) recommendations that boaters in the state dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state's freshwater bodies (United States Department of Agriculture, 2007).
Kentucky	Kentucky has adopted recommendations from the United States Department of Agriculture (USDA) that state that boaters in Kentucky dry their equipment for at least 1 week in order to prevent the spread of mussel

	species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007).
Louisiana	Louisiana has adopted recommendations from the United States Department of Agriculture (USDA) stating that boaters in the state dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007).
Maine	Maine has laws in place illegalizing the movement of aquatic plants through boat transport and has likewise required boaters to drain all compartments of their boats after use, and the state has the authority to place fines on those found to introduce species to new locations (Boat-ed, August 2018).
Maryland	Maryland recommends boaters to follow the Clean-Drain-Dry procedure, and states that the minimum drying period for boats should be at least 2 days in between outings (Maryland Department of Natural Resources, 2019).
Massachusetts	Massachusetts’s Department of Conservation and Recreation has indicated that boats in between uses should be dried “completely” in between outings as a means to prevent invasive species’ spread (Massachusetts Department of Conservation and Recreation, 2008).
Michigan	Michigan’s recommendations have been adopted from the United States Department of Agriculture (USDA), requesting that boaters in Michigan dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). Michigan has laws in place through its Resources and Environmental Protection Act of 1994 requiring boaters to both clean and drain out their watercraft before leaving the area of use and giving the state the authority to fine those found in violation of this law (Michigan State Government, 1994). The state also requires its boaters to follow the standard Clean-Drain-Dry technique with a drying period of a minimum of 5 days in between uses (Michigan Department of Natural Resources, 2019).
Minnesota	Minnesota has adopted recommendations from the United States Department of Agriculture (USDA), requesting that boaters in Minnesota dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). Minnesota’s Department of Natural Resources has recommended the state’s boaters to have their boats dry for 5 days in between uses (Minnesota Department of Natural Resources).

Mississippi	Mississippi has recommendations adopted from the United States Department of Agriculture (USDA) requesting those in the state dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007).
Missouri	Missouri has adopted recommendations from the United States Department of Agriculture (USDA) that has stated that boaters in the state dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). There are recommendations in place in Missouri for its boaters to dry their watercraft for at least 5 days, and specifically should add on another days’ worth of drying for each rainy day that occurs during the drying duration frame (National Park Service, 2021).
Montana	Montana has laws in place requiring its boaters along with out of state boaters going to its waters to go through proper inspection at state run stations surrounding water bodies. The state recommends using the Clean-Drain-Dry technique, with the drying duration it indicates being so that the boat and gear are both dried “thoroughly,” and the state has also implemented laws illegalizing the transport of invasive species by boat transportation (Montana Fish Wildlife and Parks).
Nebraska	Nebraska has followed recommendations from the United States Department of Agriculture (USDA) that request that boaters in Nebraska dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). Nebraska has legislated the requirement for boaters to undergo inspections and follow the Clean-Drain-Dry technique in order to access the state’s waters (Nebraska Game and Parks, 2016). Additionally, the state runs an Aquatic Invasive Species stamp program, where boats are required to be properly identified with stamps that the boaters purchase, and the funds acquired through said stamp program are then used in the state’s actions towards handling aquatic invasive species (Nebraska Game and Parks, AIS Stamp 2016).

Nevada	Nevada has adopted recommendations from the United States Department of Agriculture (USDA) that request that boaters in the state dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). Nevada has an aquatic invasive species decal program in place that is used to fund the actions the state’s Department of Wildlife takes towards handling invasive species, with the decals being required to be purchased for all boats in the state. The state also recommends following the Clean-Drain-Dry procedure and using a drying period of either 5 to 7 days during the summer or up to at least 27 days during the fall and winter (Nevada Department of Wildlife).
New Hampshire	New Hampshire requires by law that boaters remove all debris, including animals and vegetation, and in addition for boaters to drain out the compartments of their boats before they transport their boats away from any body of water (New Hampshire Fish and Game). The state also encourages its boaters to use the Clean-Drain-Dry procedure after every outing (New Hampshire Fish and Game).
New Jersey	New Jersey’s Department of Environmental Protection recommends for boaters to clean off and drain their boats after outings and recommends for the boats to be “dried fully” with a timeframe of at least 4 to 6 hours in sunlight before further uses (New Jersey Department of Environmental Protection; Division of Fish and Wildlife, 2016). Other groups have recommendations akin to the common 5 days drying period, such as the Lake Hopatcong Foundation which recommends following the Clean-Drain-Dry procedure with the standard 5-day duration (Odgers, 2021).
New Mexico	New Mexico has laws in place that require boaters to have their boats inspected and if necessary decontaminated in between uses at state run stations surrounding their bodies of water (Dominguez, 2018)
New York	New York has followed recommendations from the United States Department of Agriculture (USDA) that ask that boaters in the state dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). New York’s Department of Environmental Conservation notes that boaters should dry off their vessels for periods of 5 to 7 days in between uses (New York Department of Environmental Conservation).
North Carolina	North Carolina’s Wildlife Resources Commission recommends the state’s boaters to clean off all of their watercraft and equipment in between uses, and in addition suggests the Clean-Drain-Dry technique with the drying duration allowing the boats to be dried “thoroughly” (North Carolina Wildlife Resources Commission).

North Dakota	North Dakota recommends that its boaters follow the Clean-Drain-Dry procedure, specifying that boats should be fully dry before being introduced to separate bodies of water after use (North Dakota Game and Fish, 2019).
Ohio	Ohio has implemented recommendations from the United States Department of Agriculture (USDA) that request that boaters in Ohio dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). Ohio also recommends following the Clean-Drain-Dry procedure and has through its Department of Natural Resources made efforts in educational outreach to help prevent boaters from spreading invasive species (Ohio Department of Natural Resources, 2021).
Oklahoma	Oklahoma has adopted recommendations from the United States Department of Agriculture (USDA) which request that boaters in Oklahoma dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). Oklahoma recommends the Clean-Drain-Dry procedure along with its own spin off, the Check-Drain-Clean-Dry technique (National Park Service, 2019). These recommendations involve using a drying duration of at least 5 days between uses (National Park Service, 2019).
Oregon	Laws in Oregon require boaters to have their watercraft inspected at state run inspection stations wherever they are run, and the state also uses the Clean-Drain-Dry technique for its boaters (Oregon State Government). The state also uses an Aquatic Invasive Species permit program in which boaters are required to acquire said permits in order to use boats in the state (Oregon State Government).
Pennsylvania	Pennsylvania has followed recommendations made by the United States Department of Agriculture (USDA) that requested that boaters in Pennsylvania dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). Pennsylvania recommends that boaters allow their boats to dry off so that they are “dry to the touch” and then give an additional 2-day long drying period (Pennsylvania Fish and Boat Commission).
Rhode Island	Rhode Island recommends its boaters to clean off and dry out their boats after use in water bodies hosting invasive species and has run educational outreach programs to help in controlling the spread of invasive species (Rhode Island Department of Environmental Management).

South Carolina	South Carolina has laws in place preventing the spread of aquatic nuisance species with the authority to apply fines and imprisonment upon those found in violation of said laws and has also recommended that boaters dry their boats off for at least 5 days in between outings (South Carolina Fishing).
South Dakota	South Dakota has implemented recommendations from the United States Department of Agriculture (USDA) which asked that boaters in South Dakota dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state's freshwater bodies (United States Department of Agriculture, 2007). South Dakota has set up inspection stations surrounding its water bodies' boat launches to help prevent nuisance species from being transported by boats, and the state has additionally recommended the Clean-Drain-Dry technique with a 5-day long drying duration frame (South Dakota Least Wanted).
Tennessee	Tennessee has adopted recommendations from the United States Department of Agriculture (USDA) which suggested that boaters in Tennessee dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state's freshwater bodies (United States Department of Agriculture, 2007). Tennessee's Wildlife Resources Agency has recommended an "Inspect-Drain-Dispose-Rinse-Dry" procedure for treating boats after use that is similar to the Clean-Drain-Dry technique, with its recommended drying period being at least 5 days (Tennessee Wildlife Resources Agency).
Texas	Texas recommends drying boats for at least a weeklong period in between uses in separate locations (Texas Parks and Wildlife).
Utah	Utah has implemented inspection stations at its boat launches for preventing the spread of nuisance species, and the state's Division of Wildlife Resources has recommended that its boaters follow the Clean-Drain-Dry technique with the drying duration frame allowing the boats to "dry completely" (Utah Division of Wildlife Resources).
Vermont	Vermont has adopted recommendations from the United States Department of Agriculture (USDA) which ask that boaters in Vermont dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state's freshwater bodies (United States Department of Agriculture, 2007). Vermont has laws in place illegalizing the spreading of nuisance species through recreational boat transportation (Vermont Fish and Wildlife Department, Aquatic). The state also recommends for its boaters to follow the Clean-Drain-Dry procedure with a 5-day drying duration in between uses in its waters (Vermont Fish and Wildlife Department, Stop)

Virginia	Virginia has followed recommendations given by the United States Department of Agriculture (USDA) which ask for boaters in the state to dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). Virginia has recommended that its boaters should clean their boats of debris, including attached organisms, after use, and that the boats should be allowed to dry “completely” for at least a day long period before being used again (Virginia Department of Wildlife Resources, 2022).
Washington	Washington has recommended the use of the Clean-Drain-Dry procedure for its boaters in between uses, with a 2-day long drying period being specified (Washington Department of Fish and Wildlife). The state also has laws in place illegalizing the transportation of nuisance species along with requiring boaters to drain out the compartments of their watercraft before transporting them away from boat launches (Washington State Parks). Additionally, the state runs a permit program for nuisance species that requires boaters to purchase said permits in order to be able to legally boat in the state (Washington State Parks).
West Virginia	West Virginia follows recommendations made by the United States Department of Agriculture (USDA) which ask that boaters in the state dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). West Virginia has laws in place preventing the unpermitted introduction of invasive species into public waters, and the state’s Division of Natural Resources has recommended its boaters to clean and drain out their boats in between uses (West Virginia Division of Natural Resources).
Wisconsin	Wisconsin follows recommendations made by the United States Department of Agriculture (USDA) which have indicated that boaters in the state should dry their equipment for at least 1 week in order to prevent the spread of mussel species known to have invaded some of the state’s freshwater bodies (United States Department of Agriculture, 2007). Wisconsin additionally has laws in place requiring the cleaning and draining of boats before transportation away from boat launches (Wisconsin Department of Natural Resources).



Wyoming	Wyoming has laws in place that requires boats brought into the state from March through November, along with boats that had been exposed to waters known to host invasive mussels, to be inspected at state run inspection stations before use in the state’s waters (Wyoming Game & Fish Department). The state recommends its boaters to use the Clean-Drain-Dry technique, and additionally runs an aquatic invasive species permit program, where boaters are required to acquire aquatic invasive species seals for their boats, and the funds acquired through purchasing said seals are then used in paying for actions taken by the state towards the control of invasive aquatic species (Wyoming Game & Fish Department).
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For boat treatment processes in between uses in separate bodies of water, the common practice when it comes to cleaning boat treatments is the widely applied and oftentimes regulated “Clean-Drain-Dry” technique (Wildlife Forever). The general process when it comes to this preventative method for the spread of nuisance species is that in between uses boats should be in some way cleaned off to remove organisms and other debris that may have attached to them, drained out to remove the ballast water that they took in during use, and finally the drying period in which the boats are allowed time to dry out as to kill off the aquatic nuisance species so any not properly removed from the first two steps would be less likely to be viable for spawning if introduced to a new environment (Wildlife Forever). Recommendations for how to stop the spread of aquatic nuisance species through properly treating, cleaning, and drying a boat between uses exist in every state within the United States, with several of them also having legislation in place to regulate boating processes as to stop particular invasive species from spreading within the state’s waters. There are additionally outlines made by federal agencies that have an effect on individual states’ boaters, with one example being the United States Department of Agriculture’s Forest Service recommending for boats to be dried for at least a week between uses as a means for the prevention of the spread of invasive mussel species, with the added note that 21 days is the most ideal for those species as they have been found to have their veliger fully die

off after three weeks (United States Department of Agriculture, 2007). With the Quagga and Zebra mussels being of particular concern for aquatic invasive species in the country, this outline from the United States Department of Agriculture (USDA) Forest Service has been applied to 27 states that had been identified to have at least one water body that hosts populations of these species (United States Department of Agriculture, 2007) (Table 1).

The most common recommendation for drying duration for the purpose of preventing invasive species and nuisance species from spreading is a timeframe of 5 days in between uses in separate water bodies. Independent and state-run groups have discussed this specific drying duration for 17 US states (Table 1). Some other states have agencies recommending longer drying durations based on time of year, as seen for Arizona (Raney, 2018) and California (California Department of Fish and Game, 2008), and some states have shorter recommendations in place such as Maryland's 2-day timeframe minimum (Maryland Department of Natural Resources, 2019). Another common recommendation for boat drying is the broader recommendation that the boat is allowed to dry "completely" or "thoroughly," oftentimes after which the agency recommends that further time, normally 1 to 2 days, is given for drying to ensure any potentially attached nuisance species have died off, and this sort of duration has been regulated or recommended for 11 states in the US.

While many of the states in the US only have simple recommendations when it comes to treating boats as to prevent the spread of nuisance species, several have laws established at the state level in order to enforce proper cleaning and drying treatments for recreational boaters. Arizona's Game and Fish Commission have noted that boaters are required by law to properly clean and then dry their boat "completely", along with having their boat inspected before using their boats in a body of water after use in locations known to host invasive species (Raney,

2018). The drying duration enforced by the state varies by the season due to how weather and temperature conditions can influence drying's capability of killing off retained organisms, with the requirement being a 1 week-long period during the summer season, May through October, and at least 18 days for the remainder of the year (Raney, 2018). In total, there are 16 states in the US that have state level legislation in place focused upon preventing the spread of nuisance eukaryotic organisms through either boating activity or transportation of recreational boats (Table 1).

A common method that has been established in many states as a means of controlling the spread of invasive species is the use of state run boat inspection stations, wherein boaters within those states are required to have their boats examined and when necessary treated at said stations as a means to remove and prevent nuisance and invasive species from traveling through the transportation of recreational boats. As of 2018, 19 states in the US have some form of inspection station program in place for checking and cleaning recreational boats according to the US Department of Agriculture (United States Department of Agriculture, National). Wyoming in particular has legislated that all boats brought into the state from March through November need to be inspected by a state-run inspection station before being used (Wyoming Game & Fish Department). These station programs serve to prevent the spread of nuisance species through investigating boats for attached organisms along with making sure that the compartments of boats are properly drained before and after their use.

Another common method of preventing and contributing to the control of aquatic nuisance and invasive species is the use of an enforced state funding program through the use of identification stickers for boats in the state. States that have this sort of program in place require all boaters to have purchased an id related to the control of invasive species, either on its own or

included with registering the boat with the state. There are 6 states in the US that have this sort of requirement in place (Table 1). This sort of requirement allows states in which it is enforced to fund their investigations and studies focusing on the control of nuisance and invasive species, while simultaneously serving as a means of educational outreach as the average boater in the state is introduced to these issues when registering their boat with the state.

There are also cases seen where there are variable recommendations and regulations present upon the same location. New Jersey is one such example of this, with its DEP currently recommending only 4-6 hours of drying after cleaning (New Jersey Department of Environmental Protection; Division of Fish and Wildlife, 2016) despite other independent agencies recommending the standard 5 days (Odgers, 2021) (Table 1). This sort of discrepancy may at times lead to confusion on what proper precautions should be considered, particularly in this instance as the larger overseeing organization appears to recommend the shorter drying duration.

#### Recreational Boating and Harmful Algal Blooms:

The standard 5-day drying period has mostly been recommended to slow the spread of invasive mussel species (California Department of Fish and Game, 2008). The cleaning processes seen throughout the country additionally appear to be primarily in response to both these mussel species along with nuisance plant species, as indicated by how so many different agencies recommend and often regulate the removal of any attached wildlife seen on boats after use. While these treatment processes may be effective at handling the spread of eukaryotic organisms, there is little information regarding their effectiveness for stopping the spread of prokaryotic species such as cyanobacteria.. While there are significant regulations and recommendations present for the treatment and prevention of invasive and nuisance plants and

animal species, there does not seem to be prevalent information regarding the capability for cyanobacterial species to spread by the same recreational boating vector. There has already been suspicion related to the drying duration in terms of its effectiveness towards the mussel species it has been used to handle (Zook and Phillips, 2012), and with the current understanding on cyanobacterial species indicating a strong ability to survive drying and desiccation events (Singh, 2018), a question can be brought up on whether or not the current treatments utilized in the prevention of the spread of nuisance species through recreational boating are effective in preventing cyanobacteria from spreading.

The objective of this study was to examine the effectiveness of commonly used boat treatment processes as a means of preventing cyanobacterial species from spreading to new bodies of water. In particular, this study focused upon whether or not the drying duration of 5 days was sufficient in preventing the spread of cyanobacterial species. We hypothesized that drying for 5 days would reduce the spread of cyanobacteria. The sufficiency of drying treatments was investigated with three separate timeframes: a 0-day control , a 5-day Short treatment, and a 10-day Long treatment, with the study focusing on testing the capability for cyanobacteria to grow after undergoing each of the three treatments. Biofilm on the boat hull materials was then collected and incubated to analyze its growing potential. We hypothesized that more cyanobacterial growth would occur for the control group compared to the Short and Long treatment groups, and that the Short treatment group would experience greater growth than the Long treatment group. Additionally, the study aimed to investigate the colonization rates of cyanobacteria on various hull materials (Polyethylene, Aluminum, Fiber Plastic, and Steel). The ultimate goal is to provide policy recommendations to better regulate recreational boating activities to prevent further spreading of HABs through recreational boating.

## Methodology

### Pilot Study:

The pilot study utilized a tank in a Montclair State University greenhouse to simulate a HAB event in a freshwater lake with periphyton samplers (Wildco, Yulee, FL) holding various untreated hull materials: Polyethylene (P), Aluminum (A), Fiber Plastic (F), and Steel (S). These four hull materials are most commonly used in the construction of recreational boats based on the description provided by a major boat manufacturer (SHM Group, 2018). Two common cyanobacteria, *Anabaena flosaquae* and *Microcystis aeruginosa*, were introduced into the tank to simulate an on-going HAB event with a target total cyanobacterial density of 80,000 +/- 10,000 cells/mL (Figure 1). Since the pilot study was conducted during the wintertime, the greenhouse air temperature was set at 80 °F, resulting in the tank water temperature ranging from 11.4 °C to 19.5 °C (Figure 1).

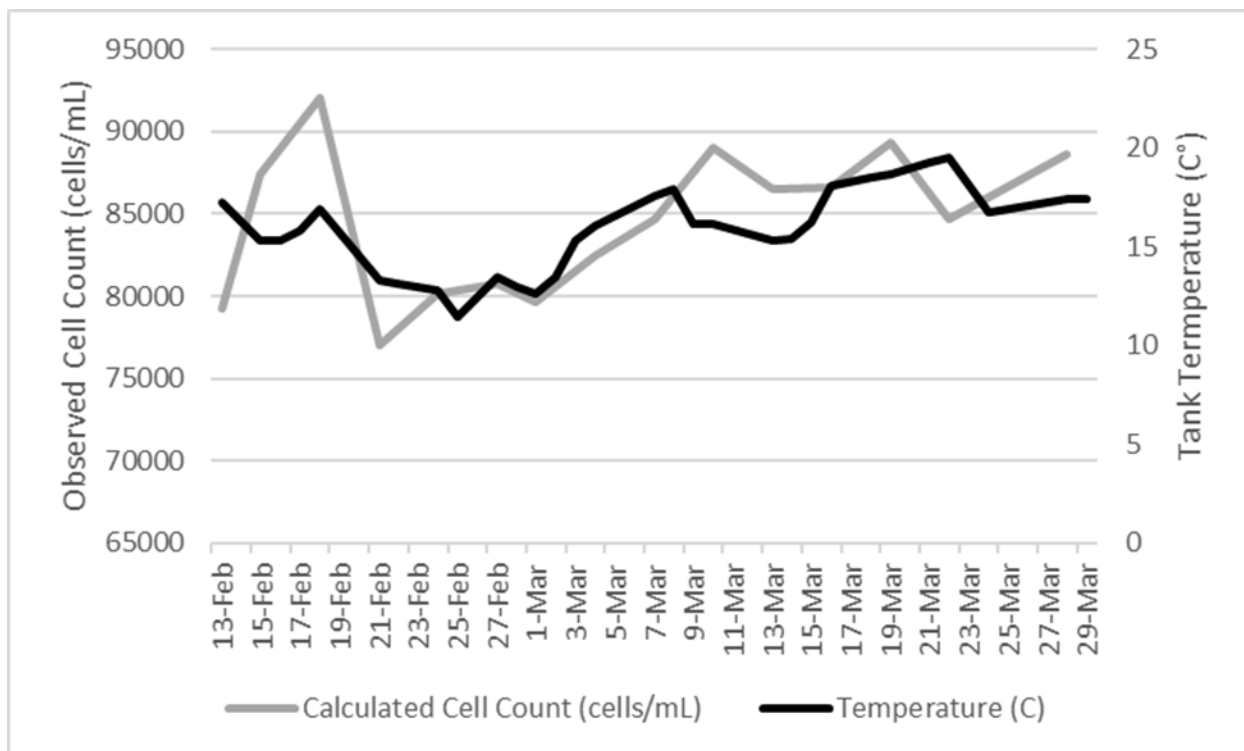


Figure 1: Greenhouse Tank Temperature and Cell Count Conditions during Pilot Study

A total of 12 periphyton samplers attached to a floating island rig system of pvc pipe (1.25 m x 0.9 m) with raised edges adhered to flotation devices were suspended 0.5 m below the water surface (Figure 2). Each sampler hosted 8 slides with 2 of each material being included. Among the 12 periphyton samplers, a total of 24 slides per material were tested in each trial. The experimental setup was kept in HAB conditions for a 2-week exposure period. After that, the slides were removed from the samplers and left to dry in accordance with the treatment durations of 0 (control), 5 (short), or 10 (long) days. After the designated treatment duration, the contents on the 8 slides of the same treatment (material x drying duration) (Table 2) were washed with 180 mL of DI water using soft paint brushes and composited into one culture flask with approximately 7% cyanobacteria BG-11 freshwater solution 50x (Sigma-Aldrich, St. Louis, MO). Cyanobacterial cell density was observed at the time immediately following the exposure period for the control group or the experimental drying duration for the two drying treatments (Day 0/D0), and then again and after a 15-day incubation period in an environmental chamber (Day 15/D15).



Figure 2: Photo of Floating Island Rig System with Periphyton Samplers

Table 2: Summary of treatments: replicates for each of the 4 hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T) and 3 drying durations (control/C, short/S, long/L) are included. Each replicate has a count taken immediately following its designated experimental drying duration (D0) as well as for after 15-day in the environmental chamber (D15)

		Drying Duration		
		Control (C)	Short (S)	Long (L)
Hull Materials	Polyethylene (P)	PC	PS	PL
	Aluminum (A)	AC	AS	AL
	Fiber Plastic (F)	FC	FS	FL
	Steel (T)	TC	TS	TL



### Field Study:

A field study was conducted at the Spruce Run Reservoir, Clinton, New Jersey, using the same sampling rig system that was used in the greenhouse setting trials. In order to keep the sampling rig relatively stationary within the reservoir and avoid drifting away from the study site, 3 cinder block anchors were attached to the top frame. Additional flotation devices were also included to maintain the target 0.5 meter water depth.

The same experimental process as the pilot study was conducted in the field study, however due to the lack of duplicate trials the content of each slide was cultured individually without being composited. A total of 8 replicates per treatment (material x drying duration) were included in the field study.

### Laboratory Procedure

Cyanobacteria in the water samples were identified and enumerated under a brightfield microscope (Leica CME Binocular Microscope) using a nanochamber, a modified Palmer-Maloney counting chamber (0.08 mL; Phycotech, St Joseph, MI). Three replicates were performed for each sample. Each replicate was counted under 400x magnification until reaching either 200 natural units or 40 field of views. The cell density in cells/mL was then converted into densities per area of hull (cells/cm<sup>2</sup>). Cyanobacteria were identified to the lowest taxonomic level possible using references including *Freshwater algae of North America: ecology and classification* (Wehr et.al, 2015), *Cyanoprokaryota-1. Teil/Part 1: Chroococcales* (Komárek & Anagnostidis 2008), *Cyanoprokaryota-2. Teil/Part 2: Oscillatoriales* (Komárek & Anagnostidis 2008), and *Cyanoprokaryota-3. Teil/Part 3: Heterocytous Genera* (Komárek, 2013).

### Data Analysis Process

The data collected in this study was analyzed through the use of Analysis of Variance (ANOVA) tests, including 6 two-way ANOVA conducted upon the cell densities immediately after the experimental drying durations (D0) and after the 15-day incubation period (D15) for the cell densities of the pilot and field study datasets as well as the taxa richness taken from the field study. Further analysis was conducted through one-way ANOVA, where in each either the 3 drying durations, those being the 10-day drying long-treatment group, the 5-day drying short-treatment group, and the 0-day drying control group, or the four hull materials of polyethylene, aluminum, fiber plastic, and steel were compared. The two-way ANOVA were conducted using R-Studio's car library functions, and the one-way ANOVA were conducted using the XLMiner Analysis ToolPak upon google sheets. The predictor variable was either the drying duration applied to the hull materials in the case when the 3 drying treatments were compared or the hull material when the 4 hull materials were being compared, and in both cases the response variable was the cell densities that had developed either immediately after the experimental drying duration, D0, or after the cyanobacterial contents were held in the environmental chamber for a 15-day incubation period, D15. The analysis upon the taxa richness data collected in the field study was analyzed similarly with the taxa richness found upon the hulls being the response variable instead of the cell densities. A total of 6 two-way ANOVA and 48 one-way ANOVA were conducted in this study, for which there were 1 two-way and 8 one-way ANOVA for each of D0 and D15 for the cell densities of the pilot and field studies as well as the taxa richness of the field study.

Post-hoc analyses were applied to the data only when the ANOVA demonstrated the presence of a significant difference. When the comparisons focused on differences between the 2 drying treatments and the control, Dunnett's tests through the DescTools library of Rstudio were

applied to determine the differences in either cell density or taxa richness between each of the 2 treatments and the control. When the ANOVA focused upon the 4 hull material varieties undergoing 1 of the 3 drying durations determined that a significant difference was present, Tukey HSD post-hoc analysis was performed through R to determine which hull materials were honestly significantly different in either cell density or taxa richness from one another.

## **Results**

### Pilot Study

The two-way analysis of variance indicated that no influence of the hull material and drying durations combined was identifiable immediately after the experimental drying durations ( $p = 0.9994$ ), nor was a difference in cell densities found to be caused by the drying durations alone ( $p = 0.761$ ) (Table 3). The one-way analysis of variance upon the 3 drying durations applied in the pilot study also indicated that no significant difference in cell densities had developed between them ( $p = 0.867$ ) (Table 4). Further one-way analysis of variance conducted upon each of the 4 hull materials individually found similar conclusions. No statistically significant difference in means was identified between the 3 different drying durations for the polyethylene ( $p = 0.986$ ), aluminum ( $p = 0.751$ ), fiber plastic ( $p = 0.970$ ), and steel ( $p = 0.840$ ) hulls. While some level of variance was observed between the control group and the hull materials that had experienced drying durations (Figure 3), this study could not identify a difference in the cell densities observed immediately after the drying durations, indicating that the drying treatments applied to the boat hulls were not sufficient in preventing the development of a cyanobacterial community upon the hulls' surfaces.

Table 3: Results of Two-Way Analysis of Variance (ANOVA) on cyanobacterial cell density of the pilot study immediately after the experimental drying durations (D0). Treatments include the 3 drying durations (control/C, short/S, long/L) and the 4 tested hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T). Results are organized to display the  $X^2$  value, the degrees of freedom (df), and the p-value for analyzing the cell density data under each of the conditional variables alone and incorporating both. \* indicates statistically significant.

Conditional Variables	$X^2$	df	p
Drying Durations	0.545	2	0.761
Hull Materials	38.614	3	*<0.001
Drying Duration*Hull Materials	0.314	6	0.999

Table 4: Results of one-way Analysis of Variance (ANOVA) on cyanobacterial cell density of the pilot study immediately after the experimental drying durations (D0). Treatments include the 3 drying durations (control/C, short/S, long/L) and the 4 tested hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T). Data sets were organized to show results comparing the 3 drying durations as a whole and for each hull material, and to compare the 4 hull materials after having undergone each of the 3 drying durations. \* indicates statistically significant.

	F	p	F crit	df
C, S, L	0.143	0.867	3.285	33
PC, PS, PL	0.014	0.986	5.143	6
AC, AS, AL	0.300	0.751	5.143	6
FC, FS, FL	0.031	0.970	5.143	6
TC, TS, TL	0.179	0.840	5.143	6
PC, AC, FC, TC	4.374	*0.042	4.066	8
PS, AS, FS, TS	5.804	*0.021	4.066	8
PL, AL, FL, TL	3.120	0.088	4.066	8

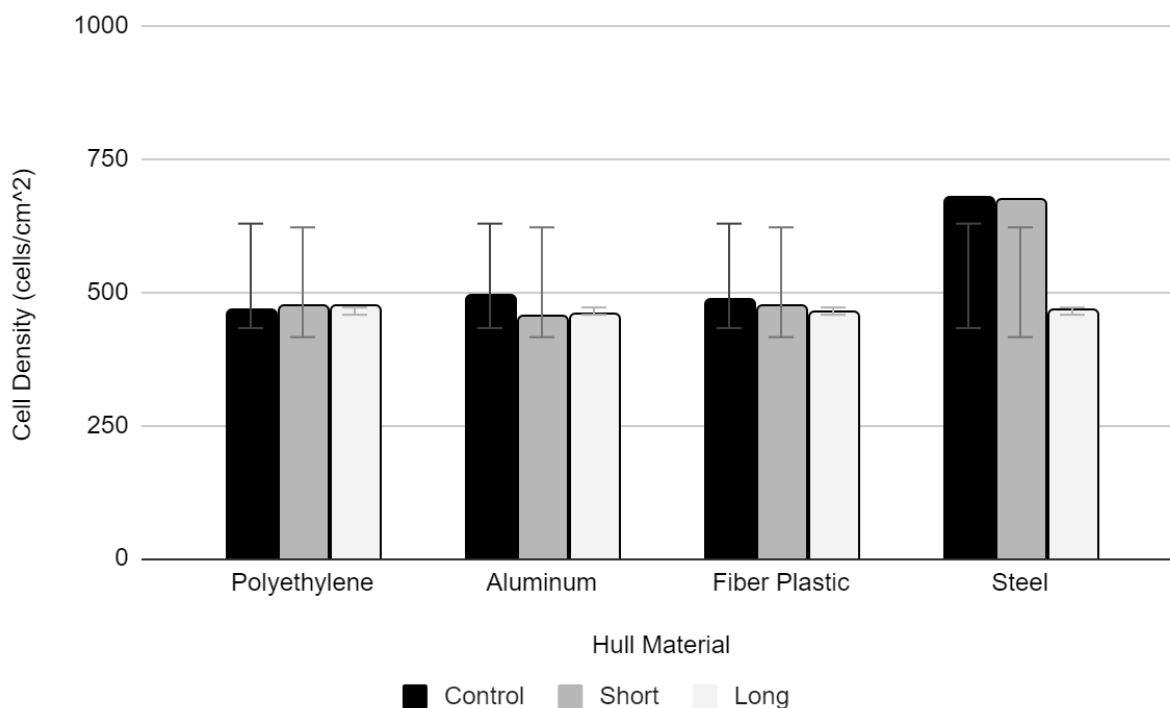


Figure 3: Display of the average cyanobacterial cell densities (cells/cm<sup>2</sup>) immediately following the designated experimental drying duration (D0) found in the pilot study. The 4 hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T) were treated with 3 experimental drying durations (control/C, short/S, long/L).

Analysis conducted upon the cell densities after having undergone the 15-day incubation period found similar results to those seen immediately after the experimental drying durations for the pilot study. The two-way analysis of variance again found that the combination of drying treatment and hull variety had no significant effect ( $p = 1.000$ ), and that there was no difference caused by the drying durations alone ( $p = 0.334$ ) (Table 5). The one-way analysis also found that overall no significant difference in the cyanobacterial cell densities had grown upon the boat hulls between the 3 drying durations applied in this study after the incubation periods were conducted ( $p = 0.631$ ) (Table 6). This pattern again was also identified when investigating each of the 4 materials individually, with no significant differences in cell densities arising between the 3 drying durations for polyethylene ( $p = 0.686$ ), aluminum ( $p = 0.215$ ), fiber plastic ( $p =$

0.780), and steel ( $p = 0.816$ ). A general pattern for lower densities with greater drying times did occur in this study, but the data acquired in the pilot study failed to indicate that either of the 2 drying durations were able to reduce the cyanobacterial cell densities after the experimental incubation period. These results demonstrate that both the 5-day drying period and the 10-day drying period failed to reduce the capability for cyanobacteria to reestablish blooms when reintroduced to a new location.

Table 5: Results of Two-Way Analysis of Variance (ANOVA) on cyanobacterial cell density of the pilot study after the 15-day incubation period (D15). Treatments include the 3 drying durations (control/C, short/S, long/L) and the 4 tested hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T). Results are organized to display the  $X^2$  value, the degrees of freedom (df), and the p-value for analyzing the cell density data under each of the conditional variables alone and incorporating both. \* indicates statistically significant.

Conditional Variables	$X^2$	df	p
Drying Durations	2.191	2	0.334
Hull Materials	53.221	3	* $<0.001$
Drying Duration*Hull Materials	0.305	6	1.000

Table 6: Results of one-way Analysis of Variance (ANOVA) on cyanobacterial cell density of the pilot study after the 15-day incubation period (D15). Treatments include the 3 drying durations (control/C, short/S, long/L) and the 4 tested hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T). Data sets were organized to show results comparing the 3 drying durations as a whole and for each hull material, and to compare the 4 hull materials after having undergone each of the 3 drying durations. \* indicates statistically significant.

	F	p	F crit	df
C, S, L	0.466	0.631	3.285	33
PC, PS, PL	0.401	0.686	5.143	6
AC, AS, AL	2.007	0.215	5.143	6
FC, FS, FL	0.259	0.780	5.143	6
TC, TS, TL	0.210	0.816	5.143	6
PC, AC, FC, TC	6.478	*0.016	4.066	8
PS, AS, FS, TS	8.216	*0.008	4.066	8
PL, AL, FL, TL	3.998	0.052	4.066	8

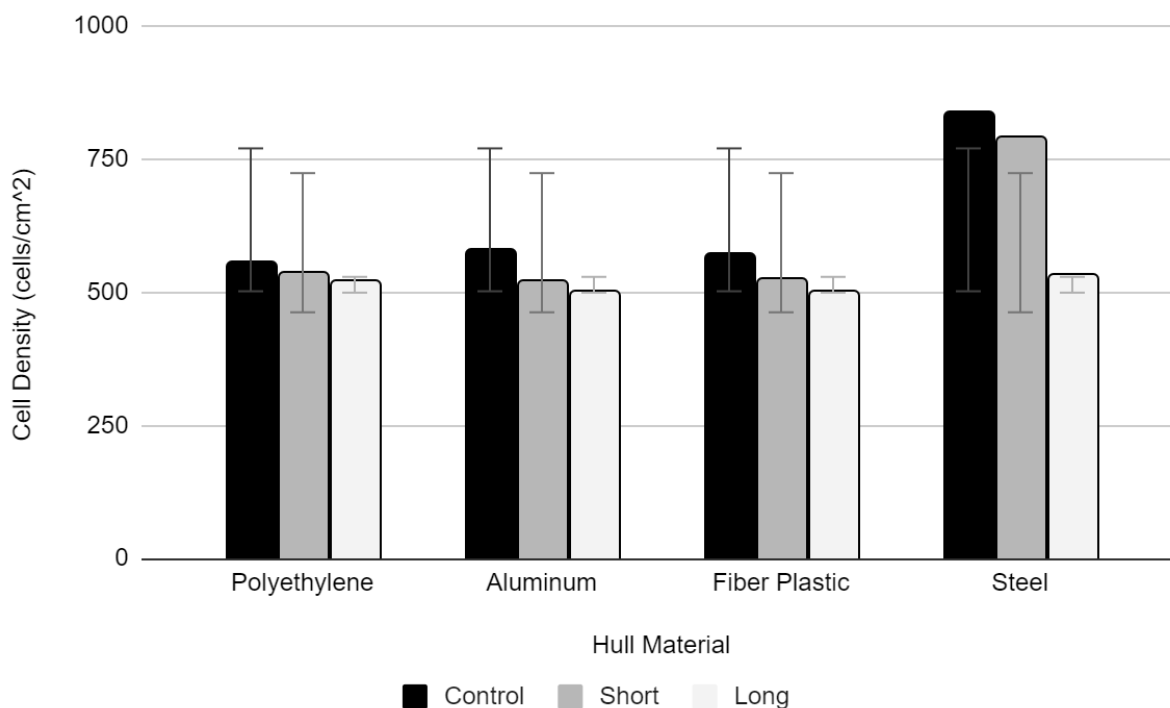


Figure 4: Display of the average cyanobacterial cell densities (cells/cm<sup>2</sup>) after the 15-day incubation period (D15) found in the pilot study. The 4 hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T) were treated with 3 experimental drying durations (control/C, short/S, long/L).

Investigations upon the 4 hull materials' effects upon the developing and reestablishing of cyanobacterial species demonstrated significant differences between the tested materials immediately after the experimental drying durations. For the two-way analysis, while it was observed that no difference occurred between the 3 drying durations nor between both variables, a significant difference in cell densities was identified in it between the 4 hull varieties ( $p < 0.001$ ) (Table 3). For the one-way ANOVA, it was observed that there was a significant difference present between the 4 hull varieties both for those that had undergone no drying ( $p = 0.042$ ) and for those that had undergone the 5-day drying duration ( $p = 0.021$ ) (Table 4). Tukey HSD analyses were applied to the cell densities of the 4 materials under these conditions, and for the hulls of the control group, it was determined that the materials were in actuality not honestly

significantly difference from one another, and that despite the significant result found in the one-way analysis of variance that none of the hulls resulted in significantly different densities of cells immediately after the exposure period (Table 7). The post-hoc analyses on the hulls that had dried for 5 days indicated that the cell densities of polyethylene, aluminum, and fiber plastic were each not significantly different from one another, having pairwise p-values of 0.993 between polyethylene and aluminum, 1.000 between polyethylene and fiber plastic, and 0.996 between aluminum and fiber plastic (Table 8). This analysis did indicate significant differences in cell densities immediately after the 5-day drying duration for the steel hulls compared to polyethylene ( $p = 0.033$ ), aluminum ( $p = 0.048$ ), and fiber plastic ( $p = 0.035$ ). Based on the trend observed for the short 5-day drying duration and the cell densities that were observed upon the hulls immediately after said duration, it was seen that the average density upon the steel hulls was greater than that upon each of the other hulls (Figure 3). With the results of the analyses and these trends, the pilot study demonstrated that the steel hulls were capable of hosting a greater density of cyanobacteria as identified by the densities taken immediately after the experimental drying duration for the hulls that had undergone 5 days of drying.

Table 7: Results from Tukey HSD Post-Hoc analysis on cyanobacterial cell density of the 4 hull materials (Polyethylene/P, Aluminum/A Fiber Plastic/F, Steel/T) of the control group immediately after the experimental drying duration for the pilot study. \* indicates statistically significant

D0	PC	AC	FC	TC
PC	-	-	-	-
AC	0.960	-	-	-
FC	1.000	0.974	-	-
TC	0.057	0.113	0.063	-



Table 8: Results from Tukey HSD Post-Hoc analysis on cyanobacterial cell density of the 4 hull materials (Polyethylene/P, Aluminum/A Fiber Plastic/F, Steel/T) of the short group immediately after the experimental drying duration for the pilot study. \* indicates statistically significant.

D0	PS	AS	FS	TS
PS	-	-	-	-
AS	0.993	-	-	-
FS	1.000	0.996	-	-
TS	*0.033	*0.048	*0.035	-

The effect of the hull material upon the re-establishment of cyanobacterial through the 15-day incubation period was found to be similar to that seen upon the cell densities observed upon the hulls immediately after the experimental drying durations. The two-way analysis of variance applied to these datasets found that after the incubation period there was a significant difference in the cell densities between the 4 hull materials ( $p < 0.001$ ) (Table 5). It was determined through the one-way ANOVA that there was a significant difference in the cell density upon at least 2 of the hull materials after the 15-day incubation period both for the hulls of the control group that had experienced no drying following the exposure period ( $p = 0.016$ ) and for the hulls that had went through the 5-day drying procedure ( $p = 0.008$ ) (Table 6). Post-hoc analysis was conducted upon both these datasets through Tukey HSD, and for the control group it was found through pairwise comparisons that the steel hulls had cell densities develop significantly different from polyethylene ( $p = 0.025$ ), aluminum ( $p = 0.041$ ), and fiber plastic ( $p = 0.025$ ) (Table 9). The remaining comparisons indicated that polyethylene was not significantly different in its developed cell density after the 15-day incubation period compared to both aluminum ( $p = 0.0983$ ) and fiber plastic ( $p = 1.000$ ), and it additionally indicated that aluminum's cell densities were not significantly different from fiber plastic ( $p = 0.983$ ). Analysis upon the hulls dried for 5 days found that the steel hulls had significantly different cell densities following the 15-day incubation period compared to polyethylene ( $p = 0.033$ ),

aluminum ( $p = 0.048$ ), and fiber plastic ( $p = 0.035$ ) (Table 10). It was also found that polyethylene was neither different from aluminum ( $p = 0.993$ ) nor fiber plastic ( $p = 1.000$ ), and that aluminum was not different from fiber plastic ( $p = 0.996$ ) in terms of cell densities at this time point after having dried for 5 days. Trends observed in the average cyanobacterial cell densities that had developed after the 15-day incubation period demonstrated that the steel hulls held the highest cell densities both for the control group and for the 5-day drying group (Figure 4), which when related to the results found in the statistical analyses indicated that for this study steel had the largest re-established cyanobacterial community in comparison to each of the other 3 materials, which were not found to be different from one another in their hosted cyanobacterial community size.

Table 9: Results from Tukey HSD Post-Hoc analysis on cyanobacterial cell density of the 4 hull materials (Polyethylene/P, Aluminum/A Fiber Plastic/F, Steel/T) of the control group after the 15-day incubation period for the pilot study. \* indicates statistically significant.

D15	PC	AC	FC	TC
PC	-	-	-	-
AC	0.983	-	-	-
FC	1.000	0.983	-	-
TC	*0.025	*0.041	*0.025	-

Table 10: Results from Tukey HSD Post-Hoc analysis on cyanobacterial cell density of the 4 hull materials (Polyethylene/P, Aluminum/A Fiber Plastic/F, Steel/T) of the short group after the 15-day incubation period for the pilot study. \* indicates statistically significant.

D15	PS	AS	FS	TS
PS	-	-	-	-
AS	0.993	-	-	-
FS	1.000	0.996	-	-
TS	*0.033	*0.048	*0.035	-

### Field Study:

The cell density data collected in the field study was analyzed in the same manner as the pilot study, with a total of 2 two-way and 16 one-way analyses of variance (ANOVA) being conducted to compare both the effectiveness of the 3 drying durations used in this study as well as the 4 hull varieties that were simulated. For the cell densities observed immediately following the experimental drying durations, the two-way ANOVA indicated that no significant differences in cell densities occurred between the 4 hull materials and the 3 drying durations ( $p = 0.997$ ), but for the drying durations alone significant differences in cell densities was identified between them ( $p = 0.012$ ) (Table 11). From the one-way ANOVA, it was also found that a significant difference in cell densities was present between the 3 drying durations applied to the hulls ( $p = 0.008$ ) (Table 12). Further analysis upon the 4 hull types demonstrated when viewed individually, none of the hull materials were found to have a significant difference in cell densities between the 3 drying durations at this timeframe. All of polyethylene ( $p = 0.263$ ), aluminum ( $p = 0.344$ ), fiber plastic ( $p = 0.114$ ), and steel ( $p = 0.627$ ) did not have the significant difference in densities between the 3 drying treatments that was observed in the hulls as a whole. Post-hoc analysis was conducted through a Dunnett's Test upon the cell densities found after the experimental drying durations to identify which of the drying treatments differed from the control. The cyanobacterial cell densities upon the hulls that had dried for 5 days were determined as significantly different from the control ( $p = 0.005$ ), while those that had dried for 10 days were not found to host significantly different densities from the control at that time ( $p = 0.595$ ). Average cell densities observed at this point for the field study indicated that the 5-day duration trended to hosting lesser cyanobacteria in comparison to the control group (Figure 5). These results indicate that immediately following the experimental drying duration, the 5-day

drying treatment had caused the boat hulls to hold significantly less cyanobacterial cells than that seen in the control.

Table 11: Results of Two-Way Analysis of Variance (ANOVA) on cyanobacterial cell density of the field study immediately after the experimental drying durations (D0). Treatments include the 3 drying durations (control/C, short/S, long/L) and the 4 tested hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T). Results are organized to display the  $X^2$  value, the degrees of freedom (df), and the p-value for analyzing the cell density data under each of the conditional variables alone and incorporating both. \* indicates statistically significant.

Conditional Variables	$X^2$	df	p
Drying Durations	8.911	2	*0.012
Hull Materials	0.804	3	0.848
Drying Duration*Hull Materials	0.555	6	0.997

Table 12: Results of one-way Analysis of Variance (ANOVA) on cyanobacterial cell density of the field study immediately after the experimental drying durations (D0). Treatments include the 3 drying durations (control/C, short/S, long/L) and the 4 tested hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T). Data sets were organized to show results comparing the 3 drying durations as a whole and for each hull material, and to compare the 4 hull materials after having undergone each of the 3 drying durations. \* indicates statistically significant.

	F	P	F crit	Df
C, S, L	5.372	*0.008	3.204	45
PC, PS, PL	1.553	0.263	4.256	9
AC, AS, AL	1.203	0.344	4.256	9
FC, FS, FL	2.784	0.114	4.256	9
TC, TS, TL	0.492	0.627	4.256	9
PC, AC, FC, TC	0.065	0.978	3.490	12
PS, AS, FS, TS	0.992	0.429	3.490	12
PL, AL, FL, TL	0.711	0.564	3.490	12

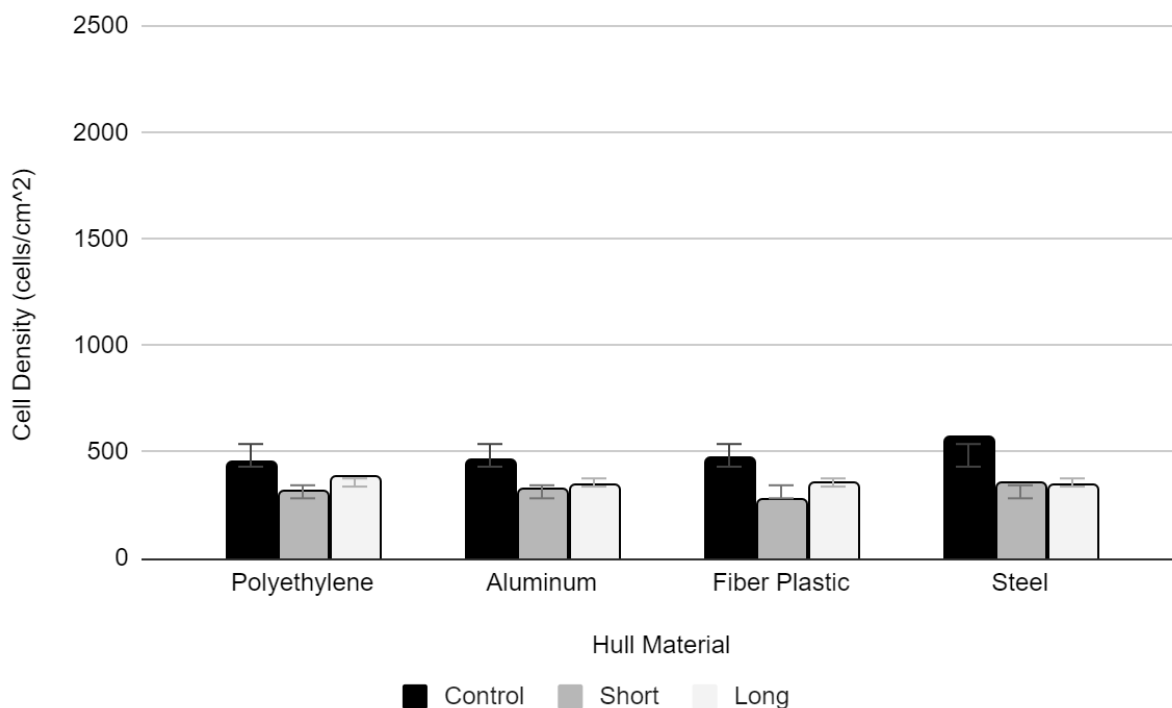


Figure 5: Display of the average cyanobacterial cell densities (cells/cm<sup>2</sup>) immediately following the designated experimental drying duration (D0) found in the field study. The 4 hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T) were treated with 3 experimental drying durations (control/C, short/S, long/L).

As for the cell density data recorded after the 15-day incubation period was conducted upon the cyanobacterial contents of the tested hulls, the two-way analysis of variance again identified that while there was no difference in means between both the drying durations and the hull varieties ( $p = 0.613$ ), the drying durations were determined to have the hulls host significantly different cell densities ( $p < 0.001$ ) (Table 13). The results of the one-way ANOVA also found that the 3 drying durations as a whole allowed for significantly different re-established cyanobacterial cell densities ( $p < 0.001$ ) (Table 14). Analyses upon the 4 hull types individually indicated that significant differences in the densities of the re-established cyanobacterial blooms existed between the 3 drying durations for the polyethylene ( $p = 0.001$ ), fiber plastic ( $p = 0.030$ ), and steel hulls ( $p = 0.050$ ). The aluminum hulls were not found to have

significant differences arise from the 3 drying durations after the 15-day incubation period ( $p = 0.074$ ). The comparisons for the 3 drying durations as a whole as well as for polyethylene, fiber plastic, and steel individually were further analyzed through Dunnett's post-hoc analyses. This analysis upon the 3 durations as a whole indicated that the 5-day drying duration did not result in significantly different re-established cell densities compared to the control ( $p = 0.853$ ), while the 10-day duration did result in a significant difference ( $p < 0.001$ ) (Table 15). Polyethylene hulls individually had no difference between the 5-day duration and the control identified ( $p = 0.980$ ) but did have one identified between the 10-day and the control ( $p = 0.001$ ). The fiber plastic hulls did not have a difference from the control develop in the 5-day duration ( $p = 0.585$ ) but did have said difference between the control and the 10-day duration ( $p = 0.021$ ). Steel hulls likewise demonstrated no significant difference in cell densities after the incubation period between the control and those dried for 5 days ( $p = 0.662$ ), and in contrast to the pattern seen overall between the 3 drying durations did not hold significantly different cell densities between its control and 10-day drying groups ( $p = 0.124$ ). The trends in average cell densities after the 15-day incubation period displayed that the long, 10-day drying treatment groups tended to host lower re-established densities in comparison to the control (Figure 6), which when applied to the differences seen in the data analyses indicates that the 10-day drying duration resulted in a significantly lower re-established density of cyanobacterial cells in comparison to the control. These results show that the 5-day drying duration failed to reduce the density of a re-established cyanobacterial community in comparison to not drying the hulls at all, while drying for 10 days succeeded in reducing the re-established cyanobacterial community.

Table 13: Results of Two-Way Analysis of Variance (ANOVA) on cyanobacterial cell density of the field study after the 15-day incubation period (D15). Treatments include the 3 drying durations (control/C, short/S, long/L) and the 4 tested hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T). Results are organized to display the  $X^2$  value, the degrees of freedom (df), and the p-value for analyzing the cell density data under each of the conditional variables alone and incorporating both. \* indicates statistically significant.

Conditional Variables	$X^2$	df	p
Drying Durations	38.010	2	*<0.001
Hull Materials	29.466	3	*<0.001
Drying Duration*Hull Materials	4.473	6	0.613

Table 14: Results of one-way Analysis of Variance (ANOVA) on cyanobacterial cell density of the pilot study after the 15-day incubation period (D15). Treatments include the 3 drying durations (control/C, short/S, long/L) and the 4 tested hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T). Data sets were organized to show results comparing the 3 drying durations as a whole and for each hull material, and to compare the 4 hull materials after having undergone each of the 3 drying durations. \* indicates statistically significant.

D15	F	p	F crit	df
C, S, L	12.233	*<0.001	3.204	45
PC, PS, PL	16.056	*0.001	4.256	9
AC, AS, AL	3.522	0.074	4.256	9
FC, FS, FL	5.292	*0.030	4.256	9
TC, TS, TL	4.265	*0.050	4.256	9
PC, AC, FC, TC	28.786	*<0.001	3.490	12
PS, AS, FS, TS	1.026	0.416	3.490	12
PL, AL, FL, TL	3.828	*0.039	3.490	12

Table 15: Results of Dunnett's method analyses conducted upon the cyanobacterial cell density recorded in the field study after the 15-day incubation period (D15). Treatments compared include the 3 drying durations (control/C, short/S, long/L) as well as the 4 hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T). The comparisons of the drying durations are shown as control vs short (CS) and control vs long (CL), of which the p-value for each is included. \* indicates statistically significant.

D15	CS	CL
C, S, L	0.853	*<0.001
PC, PS, PL	0.980	*0.001
FC, FS, FL	0.585	*0.021
TC, TS, TL	0.662	0.124

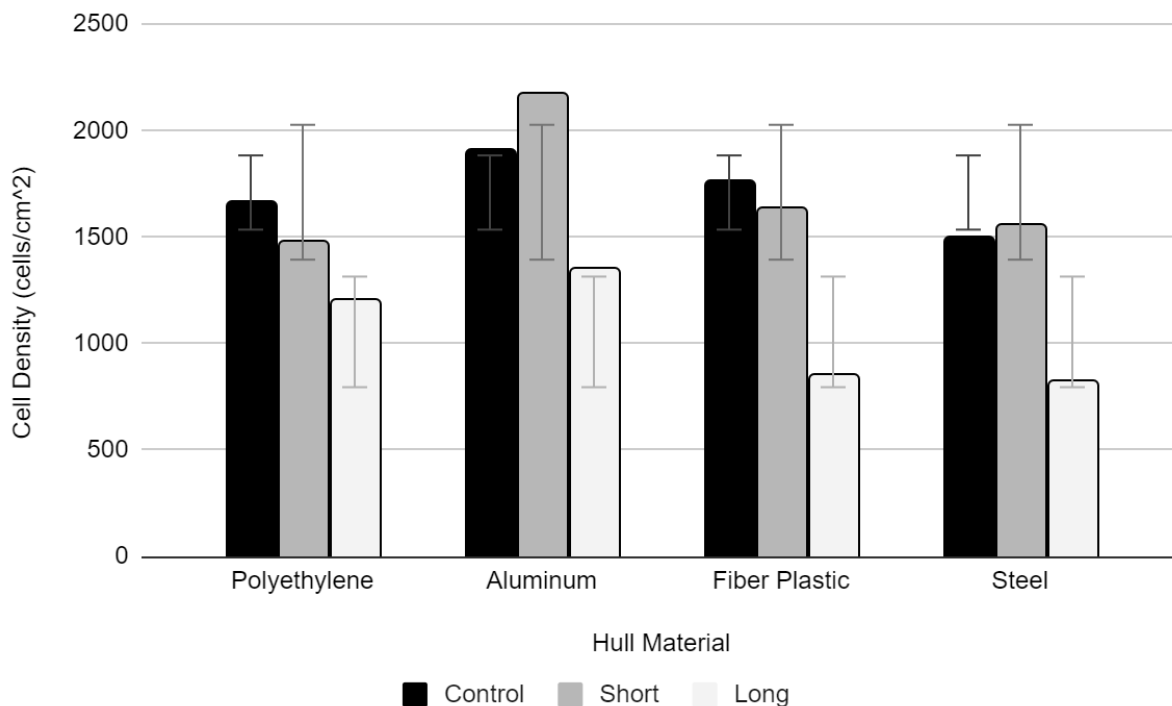


Figure 6: Display of the average cyanobacterial cell densities (cells/cm<sup>2</sup>) after the 15-day incubation period (D15) found in the field study. The 4 hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T) were treated with 3 experimental drying durations (control/C, short/S, long/L).

The 4 hull materials were observed as having little to no effect on the development of cyanobacterial cells found immediately after the experiment drying durations in comparison to each other. The two-way analysis of variance conducted upon the data taken immediately after the experimental drying durations found no difference in cell densities between the 4 hull varieties ( $p = 848$ ) (Table 11). The one-way ANOVA upon the 4 materials at each of the 3 drying durations also demonstrated no significant difference in means between the 4 materials after having undergone 10 days drying ( $p = 0.564$ ), 5 days ( $p = 0.429$ ), and no drying at all ( $p = 0.978$ ) (Table 14). Based on these results, it was shown that the hull materials tested in the field study did not result in a difference in developing cell densities.



The two-way analysis of variance conducted upon the field study data taken after the 15-day incubation period found that a significant difference in cell densities was present between the 4 hull varieties ( $p < 0.001$ ) (Table 13). The one-way ANOVA found that this difference in mean cyanobacterial cell densities was present between the hulls in the control group ( $p < 0.001$ ) and for those that had been dried for 10 days in the long treatment group ( $p = 0.039$ ). No statistically significant difference was identified in the one-way analysis of variance conducted upon the 4 hulls after the 5-day drying duration ( $p = 0.416$ ). The results of the control and 10-day drying groups were further analyzed through Tukey HSD post-hoc analyses. The post-hoc analysis upon the control group following the 15-day incubation period indicated that the steel hulls allowed for significantly different re-established cell densities in comparison to each of polyethylene ( $p = 0.01$ ), aluminum ( $p < 0.001$ ), and fiber plastic ( $p < 0.001$ ) (Table 16). It was additionally determined that aluminum was significantly different in its cell densities compared to both polyethylene ( $p < 0.001$ ) and fiber plastic ( $p = 0.015$ ), while polyethylene and fiber plastic were not found to be statistically distinct ( $p = 0.398$ ). The post-hoc analysis upon the hulls that had experienced 10 days of drying also demonstrated a significant difference present between the steel and aluminum hulls ( $p = 0.040$ ), while it did not find statistically significant differences between any of the other materials (Table 17). This analysis found no difference between polyethylene and aluminum ( $p = 0.254$ ), fiber plastic ( $p = 0.866$ ), nor steel ( $p = 0.677$ ), and it additionally found no difference between fiber plastic and both aluminum ( $p = 0.074$ ) and steel ( $p = 0.983$ ). It had been observed in the average cell densities determined after the 15-day incubation period that aluminum tended to hold higher densities compared to each of the other 3 materials, while steel hosted less (Figure 6). These trends along with the results from the data analyses demonstrated that the aluminum hulls were more susceptible to hosting cyanobacterial

communities that were capable of re-establishing, followed by both polyethylene and fiber plastic which were not statistically distinct from each other, and then steel was observed as being the least susceptible to carrying and re-establishing cyanobacterial communities.

Table 16: Results from Tukey HSD Post-Hoc analysis on cyanobacterial cell density of the 4 hull materials (Polyethylene/P, Aluminum/A Fiber Plastic/F, Steel/T) of the control group after the 15-day incubation period for the field study. \* indicates statistically significant.

D15	PC	AC	FC	TC
PC	-	-	-	-
AC	*<0.001	-	-	-
FC	0.398	*0.015	-	-
TC	*0.010	*<0.001	*<0.001	-

Table 17: Results from Tukey HSD Post-Hoc analysis on cyanobacterial cell density of the 4 hull materials (Polyethylene/P, Aluminum/A Fiber Plastic/F, Steel/T) of the long group after the 15-day incubation period for the field study. \* indicates statistically significant.

D15	PL	AL	FL	TL
PL	-	-	-	-
AL	0.254	-	-	-
FL	0.866	0.074	-	-
TL	0.677	*0.040	0.983	-

Investigations upon the taxa richness observed upon the hulls in the field study indicated through the two-way analyses of variance that immediately after the experimental drying durations there was no effect between both the drying durations and the hull varieties ( $p = 0.170$ ), nor was a difference in richness identified based on the drying durations alone ( $p = 0.563$ ) (Table 18). The results of the one-way ANOVA also found that no difference developed among the cyanobacteria immediately after the experimental drying durations ( $p = 0.583$ ). This was likewise found when analyzing the 4 hull materials individually, with no difference in taxa richness being linked to the 3 drying durations for polyethylene ( $p = 0.767$ ), aluminum ( $p = 0.323$ ), fiber plastic ( $p = 0.622$ ), and steel ( $p = 0.073$ ) (Table 19). With little variance being

observed in the average taxa richness between each of the 3 drying durations (Figure 7), it was determined that the drying treatments had no effect on the taxa richness that had developed immediately following the experimental drying durations compared to the control.

Table 18: Results of Two-Way Analysis of Variance (ANOVA) on the taxa richness of the field study immediately after the experimental drying durations (D0). Treatments include the 3 drying durations (control/C, short/S, long/L) and the 4 tested hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T). Results are organized to display the  $X^2$  value, the degrees of freedom (df), and the p-value for analyzing the cell density data under each of the conditional variables alone and incorporating both. \* indicates statistically significant.

Conditional Variables	$X^2$	df	p
Drying Durations	1.149	2	0.563
Hull Materials	2.234	3	0.525
Drying Duration*Hull Materials	9.064	6	0.170

Table 19: Results of one-way Analysis of Variance (ANOVA) on cyanobacterial taxa richness of the field study immediately after the experimental drying durations (D0). Treatments include the 3 drying durations (control/C, short/S, long/L) and the 4 tested hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T). Data sets were organized to show results comparing the 3 drying durations as a whole and for each hull material, and to compare the 4 hull materials after having undergone each of the 3 drying durations. \* indicates statistically significant.

	F	p	F crit	df
C, S, L	0.547	0.583	3.204	45
PC, PS, PL	0.273	0.767	4.256	9
AC, AS, AL	1.286	0.323	4.256	9
FC, FS, FL	0.500	0.622	4.256	9
TC, TS, TL	3.545	0.073	4.256	9
PC, AC, FC, TC	0.704	0.568	3.490	12
PS, AS, FS, TS	2.000	0.168	3.490	12
PL, AL, FL, TL	2.000	0.168	3.490	12

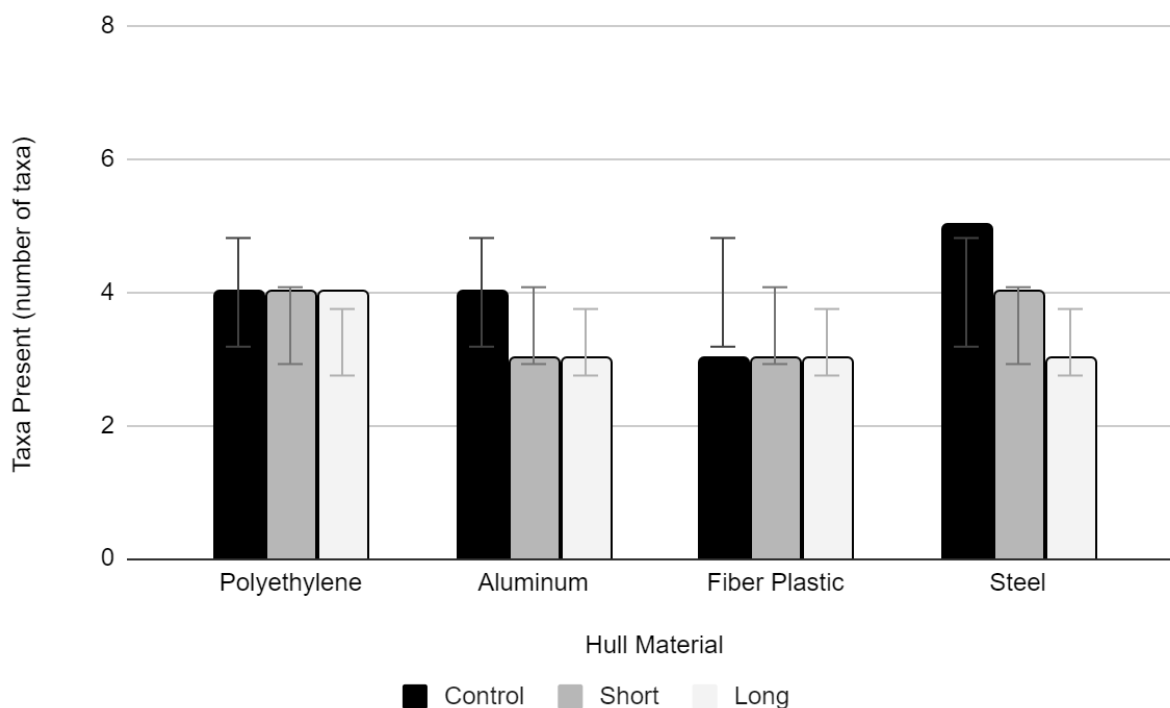


Figure 7: Display of the average taxa richness (number of taxa) immediately following the designated experimental drying duration (D0) found in the field study. The 4 hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T) were treated with 3 experimental drying durations (control/C, short/S, long/L).

The two-way analysis of variance conducted upon the richness taken after the 15-day incubation period found that while there was no combined influence of the drying durations and hull varieties ( $p = 0.673$ ), a difference in richness was present comparing the drying durations alone ( $p < 0.001$ ) (Table 20). The one-way ANOVA also indicated that significant differences in the cyanobacterial taxa richness developed between the 3 drying durations ( $p < 0.001$ ) (Table 21). Analyses upon the 4 hull materials individually found similarly significant results for the aluminum ( $p = 0.003$ ), fiber plastic ( $p = 0.004$ ), and steel hulls ( $p = 0.032$ ), while no such statistically significant difference in taxa development was found in the polyethylene hulls ( $p = 0.091$ ). The results of the drying durations as a whole as well as individually upon aluminum, fiber plastic, and steel hulls were further analyzed through the Dunnett post-hoc analysis method.

It was found in the analysis of the 3 drying durations that the 5-day drying duration failed to significantly change the taxa richness compared to the control ( $p = 0.967$ ), but it did identify the richness observed in the hulls dried for 10 days as being significantly different than the control ( $p < 0.001$ ) (Table 22). The analysis upon the aluminum hulls determined similar results, with the 5-day duration not significantly changing the taxa compared to the control ( $p = 0.838$ ) while the 10-day duration succeeded ( $p = 0.006$ ). The fiber plastic hulls also followed this pattern, with the 5-day duration not being significantly different from the control ( $p = 1.000$ ) while the 10-day duration was ( $p = 0.006$ ). The steel hulls did also find that the 5-day drying duration was not distinct from the control ( $p = 0.838$ ), but it also failed to indicate a significant difference between the 10-day drying duration's taxa richness and the control ( $p = 0.060$ ). The trend in taxa richness had shown that the richness of the control group tended to be higher on average compared to the 10-day drying group (Figure 8), and this trend coupled with the results of the data analysis indicate that the 10-day drying duration significantly reduced the taxa richness that were capable of re-establishing after the 15-day incubation period.

Table 20: Results of Two-Way Analysis of Variance (ANOVA) on the taxa richness of the field study after the 15-day incubation period (D15). Treatments include the 3 drying durations (control/C, short/S, long/L) and the 4 tested hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T). Results are organized to display the  $X^2$  value, the degrees of freedom (df), and the p-value for analyzing the cell density data under each of the conditional variables alone and incorporating both. \* indicates statistically significant.

Conditional Variables	$X^2$	df	p
Drying Durations	49.624	2	* $<0.001$
Hull Materials	11.118	3	*0.011
Drying Duration*Hull Materials	4.024	6	0.673

Table 21: Results of one-way Analysis of Variance (ANOVA) on cyanobacterial taxa richness of the field study after the 15-day incubation period (D15). Treatments include the 3 drying durations (control/C, short/S, long/L) and the 4 tested hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T). Data sets were organized to show results comparing the 3 drying durations as a whole and for each hull material, and to compare the 4 hull materials after having undergone each of the 3 drying durations. \* indicates statistically significant.

	F	p	F crit	df
C, S, L	21.832	*<0.001	3.204	45
PC, PS, PL	3.171	0.091	4.256	9
AC, AS, AL	12.167	*0.003	4.256	9
FC, FS, FL	10.500	*0.004	4.256	9
TC, TS, TL	5.167	*0.032	4.256	9
PC, AC, FC, TC	3.190	0.063	3.490	12
PS, AS, FS, TS	0.952	0.446	3.490	12
PL, AL, FL, TL	1.636	0.233	3.490	12

Table 22: Results of Dunnett's method analyses conducted upon the cyanobacterial taxa richness recorded in the field study after the 15-day incubation period (D15). Treatments compared include the 3 drying durations (control/C, short/S, long/L) as well as the 4 hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T). The comparisons of the drying durations are shown as control vs short (CS) and control vs long (CL), of which the p-value for each is included. \* indicates statistically significant.

	CS	CL
C, S, L	0.967	*<0.001
AC, AS, AL	0.838	*0.006
FC, FS, FL	1.000	*0.006
TC, TS, TL	0.838	0.060

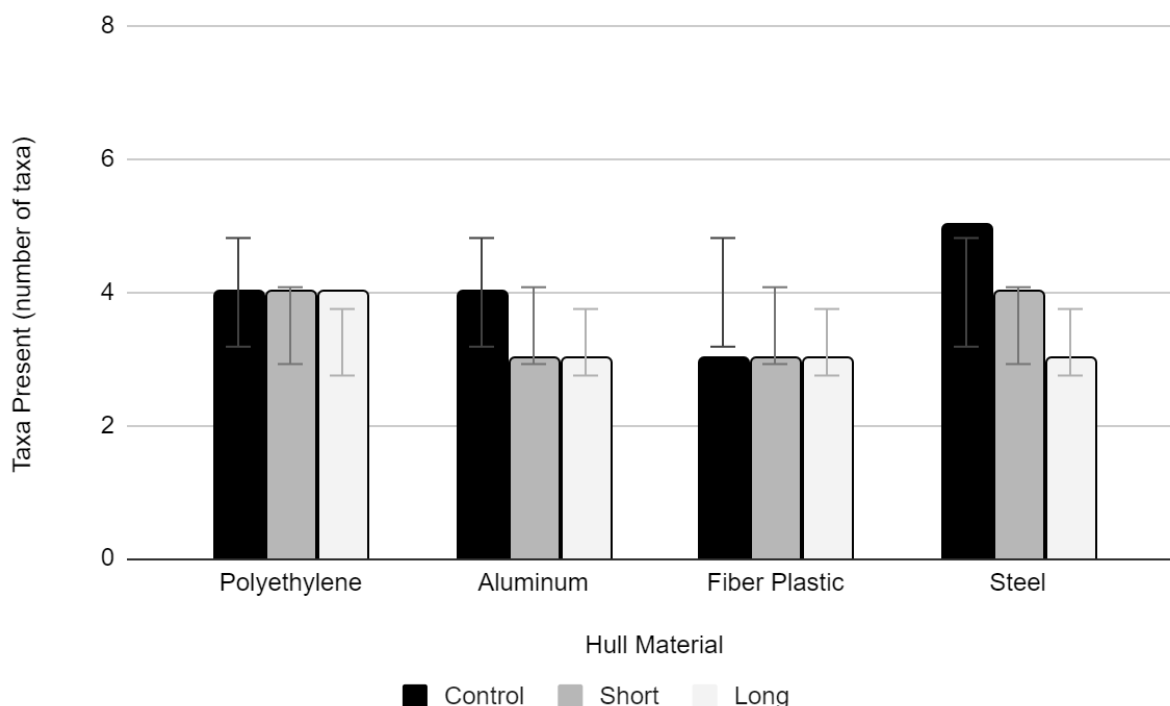


Figure 8: Display of the average taxa richness (number of taxa) after the 15-day incubation period (D15) found in the field study. The 4 hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T) were treated with 3 experimental drying durations (control/C, short/S, long/L).

Investigations upon the 4 hull materials in terms of taxa richness demonstrated that the hull variety had no impact upon the richness of the cyanobacteria that had developed upon it immediately following the experimental drying duration. The two-way analyses of variance (ANOVA) found that at this timeframe no significant difference could be identified between the cell densities of the 4 hull materials ( $p = 0.525$ ) (Table 18). The one-way ANOVA conducted upon the 4 materials after going through each of the 3 drying durations also failed to identify a significant difference in taxa, with a  $p$ -value of 0.568 in the control group, 0.168 for the hulls dried for 5 days, and 0.168 for the hulls dried for 10 days (Table 19). With no differences identified, it was concluded that the hull materials did not influence the diversity in taxa that had initially colonized their surfaces.

The analysis upon the taxa richness upon the 4 hull materials following the 15-day incubation period determined results similar to those observed immediately after the experimental drying durations. While the two-way analysis of variance (ANOVA) found a significant difference present between the 4 hull materials ( $p = 0.011$ ) (Table 20), the one-way ANOVA conducted upon the richness observed upon the 4 materials in the control group found that no significant difference was present between any of them ( $p = 0.063$ ) (Table 21). Analysis upon the short 5-day drying group found similar results ( $p=0.446$ ), as did the analysis upon the long 10-day drying group ( $p = 0.233$ ). These results found that none of the 4 hull materials had any sort of significant impact upon the taxa richness that were capable of re-establishing after the 15-day incubation period.

#### Comparing Results of Pilot and Field Studies:

In terms of drying durations, both the pilot and field study's results had demonstrated that the 10-day drying duration failed to differentiate the cell densities present immediately after the drying duration and after the 15-day incubation period in relation to the control group. The results of the 2 studies differed in comparing the 5-day drying duration and the control, with the pilot study finding no difference in cell densities while the field study identified the 5-day drying time as having reduced the cyanobacterial cell density. Following the 15-day incubation period, it was found that the 10-day drying duration significantly reduced the cyanobacterial density in the field study, while no such reduction was identified in the pilot study. Overall, both studies' results indicated that the 5-day drying duration was insufficient in preventing the spread of cyanobacteria.

As for comparing the hull materials, the pilot study identified a pattern for the steel hulls being the most susceptible for hosting cyanobacteria, while the field study found steel as the least



susceptible to host cyanobacterial cells while aluminum was the most likely to take on cells. With this discrepancy in mind, due to how the field study actually took place within a natural environment while the pilot study took place in an experimental tank housing only 2 taxa, it was thought that the field study's results were more reliable.

## **Discussion**

The results of this study have indicated that the longer drying durations had significantly reduced the cyanobacterial cell density that had developed upon the surfaces of the hull. This study's findings demonstrate a pattern that has been identified previously for cyanobacterial growth after drying. Focusing on the freshwater locations that had undergone drought conditions, Teferi et al (2014) had found that locations that had gone through such drying events had cyanobacterial communities less dense than the locations studied that hadn't been fully drained through desiccation. Just as this study had found that the longer duration had significantly lower cell densities and taxa richness colonizing hull surfaces, Teferi et al (2014) had determined that their study sites that went through a more drastic drought for longer time frames had a lower cyanobacterial cell density. This pattern was also observed in the study conducted by Bakker and Hill (2016), which identified that locations that had undergone "complete drying" would, after having the water of the location re-established, host a less dense and less diverse cyanobacterial community.

The currently available information on cyanobacteria has indicated that some taxa have a strong capacity to survive drying events (Singh, 2018). Our study had also demonstrated that cyanobacteria were capable of recolonizing and re-establishing blooms after long drying durations. This strong ability to survive such desiccation events could potentially be attributed to the ability for some cyanobacteria species to produce dormant, more resilient cell variants

(Nienaber, 2021). These dormant cells are capable of surviving conditions that other cyanobacteria cells may not normally be able to thrive in, and a particular variety of these dormant cells known as akinetes (Nienaber, 2021) has been known to be produced by the cyanobacteria genera, *Anabaena* and *Dolichospermum*. Given how these akinetes-producing genera were identified among all of the treatments both immediately after the drying durations and after the 15-day incubation period for the field study, and how it was found capable of establishing a bloom after all of the drying durations and treatments for both the pilot and field study, it is likely that these dormant cells may have influenced the growth of cyanobacteria post drying treatment. Additionally, adaptations have long been a part of the physiology of cyanobacterial species, as many groups of cyanobacteria are capable of surviving extended periods of drying (Potts, 2010); functions and mechanisms in their biology provide them the ability to prevent the harmful effects of extended periods of drying such as protein oxidation (Potts, 2010). These mechanisms may also be employed by the taxa that were observed in our study and could potentially be an answer as to why the long drying group had a lower diversity than the control and short treatments; cyanobacteria species without these survival mechanisms would not be able to withstand so that they may re-establish a bloom.

When it comes to the patterns observed on the hull materials and their susceptibility to cyanobacteria colonization, past research seems to indicate that of the hull materials used in this study steel would be most likely to host a cyanobacterial community. Previous investigations reported a positive relationship between iron and cyanobacterial growth (Dengg et al, 2022). Dengg et al (2022) demonstrated that iron serves as the limiting nutrient in the growth of the cyanobacteria species *Dolichospermum hermannii*, and that the growth rate of this species significantly increased as the iron content in the water increased. Steel, being an alloy

incorporating iron and carbon, would thereby be inferred to be the better material for cyanobacterial growth compared to the other three hull materials tested in this study. This is additionally likely when considering how aluminum has been previously determined to be toxic to cyanobacterial species, particularly when the water is acidic (Gensemer and Playle, 1999). Research onto the effects of aluminum when put into acidic water has demonstrated that aluminum has been capable of inhibiting the growth of cyanobacterial species (Pettersson et al, 2006)

However, while in an acidic environment aluminum has been demonstrated to serve as a toxic element towards cyanobacteria in the research conducted by Pettersson et al (2006) as well as in other studies discussed by Gensemer and Playle (1999), there has been another relationship identified between cyanobacteria and aluminum, specifically through the function of chlorophyll-a (Shi et al, 2015). Shi et al (2015) indicated that the growth of cyanobacteria *Synechococcus* had a positive relationship with aluminum indirectly through chlorophyll-a. Chlorophyll-a's function was increased when the aluminum content in the water column increased, and its function would lead to increased growth and colonization in the cyanobacteria (Shi et al, 2015). This association might have caused the increased colonization observed upon the aluminum hulls in our field study, particularly considering how it is known that the majority of New Jersey water bodies are alkaline. As for the pilot study, it is thought that the trend observed may have resulted from interactions between the iron within the steel hulls and the cyanobacteria based on iron being a limiting nutrient for cyanobacterial growth (Gonzales et al, 2018).

While the effect of the drying duration may have been concluded as insufficient to handling the growth of the cyanobacterial taxa, for taxa richness, this study's results indicated

that a 10-day drying timeframe may indeed have a significant impact on a cyanobacterial assemblages on a boat hull. Previous studies have indicated that cyanobacteria have varied survivability to difference stresses based on cell varieties such as what is seen in *Dolichospermum* and *Anabaena* akinete varieties (Nienaber, 2021) and different homeostasis mechanisms involved in preventing the negative impacts of desiccation such as what's been observed by Potts (2010) in *Nostoc*. These differences between groups of cyanobacteria may contribute to the differing success in colonization based on drying duration, and these differences may have been what caused the decreased diversity found in the hulls dried for 10 days in comparison to the two other drying treatments.

### **Conclusion**

The results of this study indicate that the current commonly applied 5-day drying time on recreational boats for the purpose of preventing the spread of nuisance species is insufficient for preventing the spread of cyanobacteria. The longer 10-day drying duration showed a significant reduction in cell density and in the diversity of cyanobacterial taxa that colonized the hull materials' surfaces; however, the 10-day trying treatment was not able to prevent the spread of HAB. The field study indicated that of the 4 hull materials tested, steel was the least susceptible to colonization while aluminum developed the greatest cell density among the hull materials; steel hulls are a better choice than aluminum while selecting hull materials for preventing HAB. Future investigations into this topic may benefit from focusing on the other key components of the treatments applied to recreational boats in the work to prevent the spread of nuisance species, so as they may indicate where procedures in either the cleaning or draining processes may also require adjustment in order to answer the concern of boats serving as vectors for cyanobacteria. In cases when actions are taken to reduce the transmittance of HAB events through recreational

boating, it is also recommended that investigations into the required drying times of the cyanobacterial taxa that are of particular concern are treated sufficiently. The results of this study demonstrate a need for more analysis investigating recreational boating's contributions to the spread of cyanobacterial species, as based on its results there is a significant chance that the use of boats in separate bodies of water may inadvertently introduce cyanobacterial species to new locations and result in spreading HAB events.

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**Appendix A:**

Cell density (cells/cm<sup>2</sup>) data recorded immediately after the experimental drying durations from the field study. Data for the 4 hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T) and 3 drying durations (control/C, short/S, long/L) are included.

Treatment Replicate	<i>Synechococcus</i>	<i>Anabaena</i>	<i>Cylindrospermopsis</i>	<i>Chroococcus</i>	<i>Microcystis</i>	<i>Wonochinia</i>	<i>Aphanocapsa</i>	Total
PC1	12300	15800	16300	400	0	0	0	44800
PC2	11200	13900	14900	0	0	0	0	39900
PC3	8100	14000	15800	200	0	0	0	38000
PC4	5200	7700	8800	0	0	0	0	21700
PS1	9400	10000	11100	0	0	500	0	31000
PS2	9100	9800	7200	0	0	0	0	26100
PS3	9900	9500	8200	0	0	2500	0	30100
PS4	11300	5500	8400	0	0	0	0	25200
PL1	11400	11000	12100	0	0	4100	0	38500
PL2	10100	9400	13700	0	0	0	0	33100
PL3	7500	11300	12100	0	0	0	0	30800
PL4	6700	12100	10400	0	0	0	0	29100
AC1	10900	13200	15400	0	0	0	6500	46000
AC2	10200	13100	15300	0	0	0	0	38600
AC3	9100	13400	15200	0	0	0	0	37700
AC4	5800	6900	4200	0	0	0	0	16900
AS1	10500	9400	12200	0	0	0	0	32100
AS2	7700	6500	7700	0	0	0	0	21900
AS3	10100	10200	7600	0	0	0	0	27800
AS4	9600	7400	11500	0	0	0	0	28500
AL1	10900	11000	12800	0	0	0	0	34700
AL2	9100	13300	14800	0	0	0	0	37200
AL3	8700	11400	13300	0	0	4300	0	37800
AL4	6800	11900	11900	0	0	600	0	31200
FC1	14100	16700	15800	0	0	0	0	46600
FC2	12500	14100	14900	200	1200	0	0	42900
FC3	10000	12700	14200	0	0	0	0	36900
FC4	8900	9300	6200	0	0	0	0	24400
FS1	10100	6700	10700	0	0	0	0	27500
FS2	11700	10500	8000	0	0	0	0	30300
FS3	9000	12000	8100	0	0	1400	0	30500
FS4	10600	5900	8700	0	0	0	0	25200
FL1	10400	11800	13100	0	0	0	0	35300
FL2	9500	11500	14800	0	0	1900	0	37600
FL3	8200	11000	13500	0	0	2200	0	34800

FL4	7900	11900	12900	0	0	1700	0	34400
TC1	14500	13400	16000	0	10800	0	1600	56400
TC2	9500	13900	14100	0	0	600	0	38200
TC3	10400	13500	15800	0	4100	0	0	43800
TC4	6400	6800	2100	0	0	0	0	15300
TS1	11000	10600	11000	0	0	2300	0	34800
TS2	14000	13800	5000	0	0	0	0	32800
TS3	10200	12100	8300	0	0	400	0	30900
TS4	10100	7700	6900	0	0	1900	0	26600
TL1	9100	10600	14700	0	0	0	0	34400
TL2	9200	11500	13300	0	0	0	0	34000
TL3	8600	13200	15600	0	0	0	0	37400
TL4	7700	10900	14500	0	0	0	0	33100



## Appendix B

Cell density (cells/cm<sup>2</sup>) data recorded immediately after the 15-day incubation period from the field study. Data for the 4 hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T) and three drying durations (control/C, short/S, long/L) are included.

Treatment Replicate	<i>Synechococcus</i>	<i>Anabaena</i>	<i>Cylindrospermopsis</i>	<i>Chroococcus</i>	<i>Microcystis</i>	<i>Woronochinia</i>	<i>Aphanocapsa</i>	Total
PC1	53600	51200	55200	3400	2300	700	0	166400
PC2	62900	49900	56100	2300	0	600	0	171800
PC3	60400	43600	49000	2600	1700	0	2600	159900
PC4	67300	42200	58000	3700	1400	900	0	173300
PS1	60800	38900	47900	0	0	0	0	147600
PS2	61100	50500	49200	1600	1400	2100	0	165900
PS3	66700	37000	49000	2600	3000	2700	0	160900
PS4	65900	56600	64400	2800	0	1900	0	191500
PL1	53900	28200	37900	0	0	0	0	120000
PL2	50800	33200	38300	0	0	700	0	122900
PL3	55500	38400	39200	200	2000	0	0	135400
PL4	49900	36400	39400	0	600	0	0	126300
AC1	60700	58800	58800	3000	9200	900	0	191200
AC2	66000	53400	61000	5500	0	13100	0	198800
AC3	75800	58700	70100	1700	0	0	0	206300
AC4	85200	53300	61300	2100	6800	3000	0	211700
AS1	71300	73200	66400	300	4400	1900	0	217500
AS2	68800	52600	55900	1900	2500	0	0	181700
AS3	74300	53200	62100	3000	0	3500	0	196200
AS4	57200	44000	47200	2500	5000	2000	0	157900
AL1	61300	33600	39800	0	0	0	0	134700
AL2	65300	46700	51000	0	0	0	0	162900
AL3	63500	52800	60300	300	0	0	0	176800
AL4	71100	55700	58400	0	0	0	0	185200
FC1	56200	48500	58900	3300	3300	6200	0	176400
FC2	56100	49000	60600	1700	0	700	0	168100
FC3	72200	48400	59300	3800	4900	5300	0	193800
FC4	75000	41100	51300	2300	1800	3900	0	175300
FS1	70400	42800	43500	1200	2700	3100	0	163700
FS2	36000	25800	33100	1100	0	500	0	96500
FS3	73300	42000	53100	3400	1600	2300	0	175700
FS4	72300	48900	66200	4000	5200	3800	0	200300
FL1	33000	23600	28300	0	0	0	0	84800
FL2	51400	33600	34000	200	0	600	0	119900
FL3	38800	27600	29700	500	0	0	0	96700

FL4	57100	41100	43600	500	0	0	0	142300
TC1	53000	40100	52400	800	0	0	3500	149700
TC2	52700	34300	42100	500	0	0	0	129600
TC3	58200	41500	48000	3500	0	0	0	151100
TC4	65000	34700	38700	1100	1600	0	0	141100
TS1	60500	45200	48500	1300	0	0	0	155500
TS2	57700	50100	59300	1100	0	2200	0	170400
TS3	53000	41800	44600	1400	0	0	0	140800
TS4	59600	45100	55000	1900	2200	1200	0	164900
TL1	30600	25200	26600	0	0	0	0	82500
TL2	51500	33100	38400	0	0	0	0	123000
TL3	61500	46100	45500	0	0	1500	0	154700
TL4	17900	18600	18500	0	0	0	0	55000

### Appendix C

Cell density (cells/cm<sup>2</sup>) data taken immediately after the experimental drying duration of the pilot study. Data for the 4 hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T) and 3 drying durations (control/C, short/S, long/L) are included, one for each trial of the pilot study.

Treatment Replicates	<i>Microcystis</i>	<i>Anabaena</i>	Total
PC1	30990	15637	46628
PC2	10215	5746	15961
PC3	30893	17613	48505
PS1	31161	16149	47310
PS2	9003	4817	13820
PS3	28508	16527	45034
PL1	31773	15567	47340
PL2	8238	5627	13865
PL3	25930	16648	42577
AC1	31414	18085	49499
AC2	19222	13505	32727
AC3	30630	20171	50802
AS1	27425	18076	45502
AS2	13415	11839	25254
AS3	28561	18399	46960
AL1	27581	18128	45709
AL2	13910	7428	21338
AL3	25939	17715	43654
FC1	29902	18661	48564
FC2	7833	5897	13730
FC3	31238	20727	51965
FS1	29520	17952	47472
FS2	7293	4952	12245
FS3	28739	19609	48348
FL1	28577	17610	46187
FL2	6167	4817	10984
FL3	27576	16978	44553
TC1	47074	20665	67739
TC2	81030	27287	108317
TC3	46389	28725	75113

TS1	33627	33695	67322
TS2	78537	26040	104577
TS3	56513	30542	87055
TL1	24869	21851	46720
TL2	73956	23923	97879
TL3	53725	29073	82797

**Appendix D**

Cell density (cells/cm<sup>2</sup>) data taken after the 15-day incubation period from the pilot study. Data for the 4 hull materials (polyethylene/P, aluminum/A, fiber plastic/F, steel/T) and 3 drying durations (Control/C, Short/S, Long/L) are included, one for each trial of the pilot study.

Treatment Replicates	<i>Microcystis</i>	<i>Anabaena</i>	Total
PC1	34495	21217	55712
PC2	20347	15441	35788
PC3	34673	23492	58166
PS1	32847	20925	53772
PS2	15891	12245	28135
PS3	30727	20853	51580
PL1	34636	17477	52113
PL2	12425	9183	21608
PL3	27163	17633	44796
AC1	35413	22542	57955
AC2	23923	26479	50403
AC3	35772	23321	59092
AS1	30902	21201	52103
AS2	18412	18907	37319
AS3	30745	22872	53618
AL1	30860	19309	50170
AL2	13955	17070	31025
AL3	27267	18727	45994
FC1	33714	23365	57079
FC2	16431	15576	32007
FC3	35546	25009	60556
FS1	32056	20480	52536
FS2	15666	13820	29486
FS3	30944	23428	54372
FL1	31179	19024	50203
FL2	14045	12830	26875
FL3	29215	18466	47681
TC1	51082	32601	83683
TC2	87905	61877	149783
TC3	52645	45966	98611
TS1	37890	41009	78900

TS2	83191	56026	139217
TS3	65664	38894	104558
TL1	26798	26427	53226
TL2	74633	56721	131353
TL3	57864	37606	95470