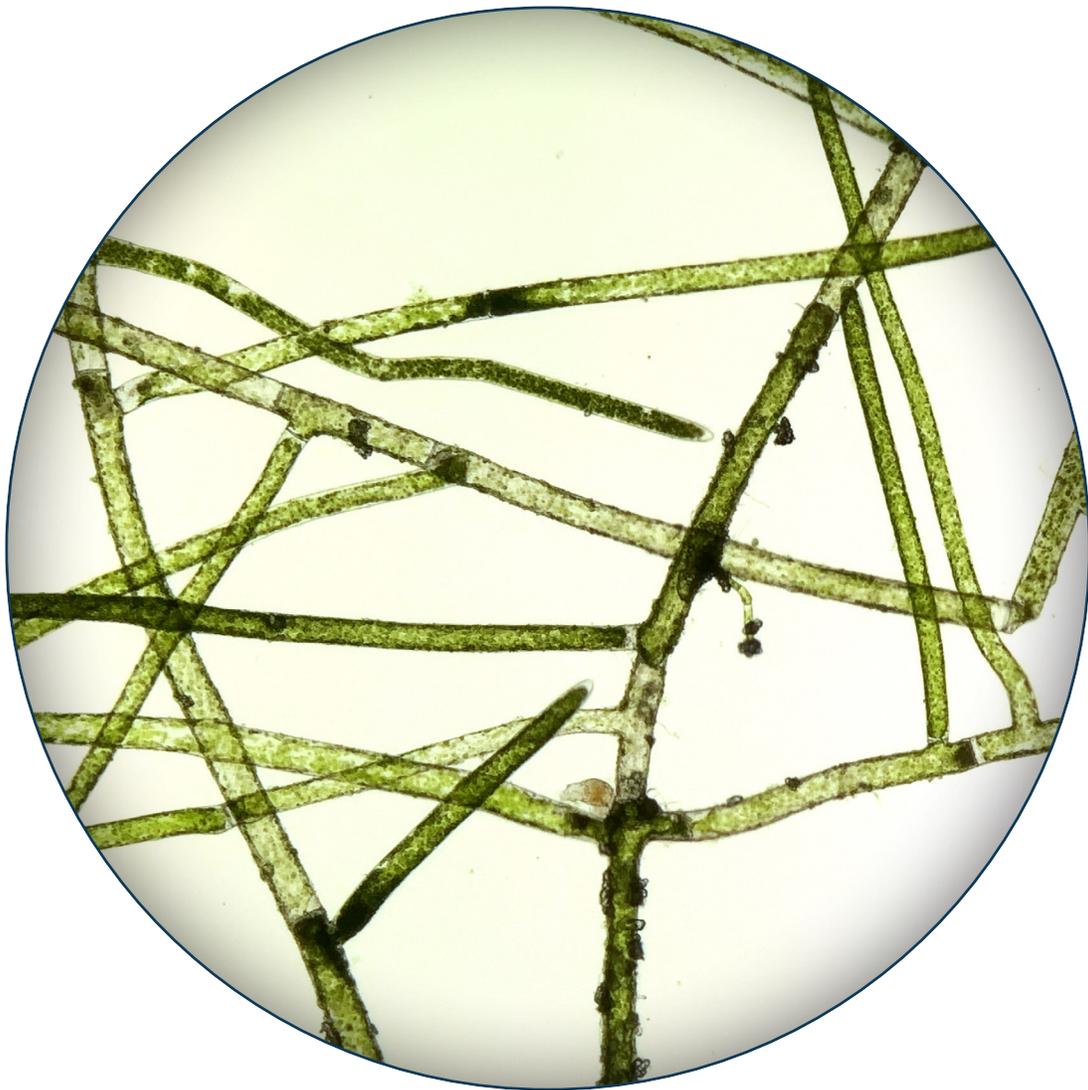


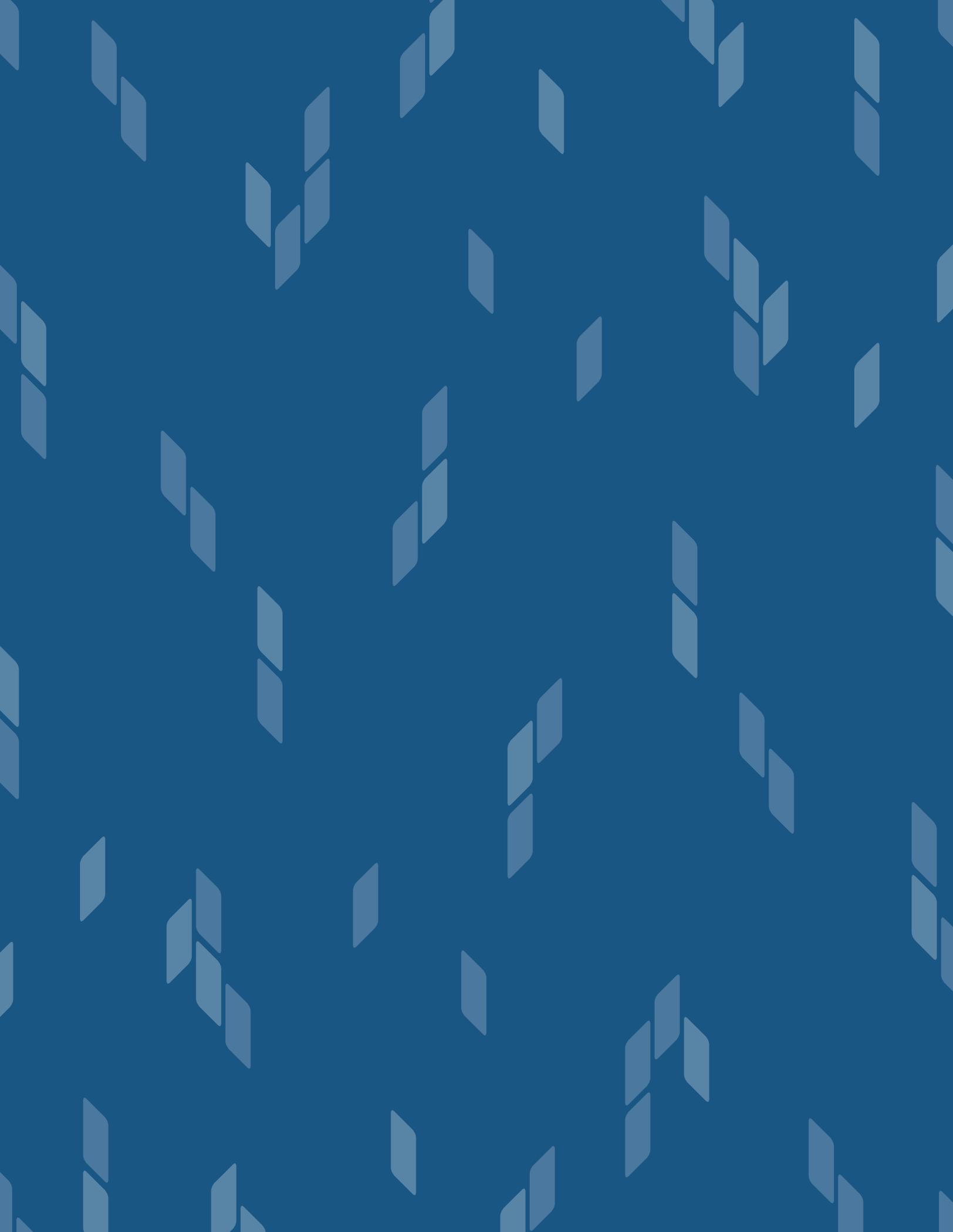
# Naturalake Biosciences PondZilla Pro Study

*Pithophora* Testing in 2020



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# Study Summary

- Pond water samples with *Pithophora* were treated, comparing the performance of three copper algaecides alone vs copper algaecides with PondZilla Pro vs an untreated control
- At 7 DAT, only copper algaecides with PondZilla Pro exhibited whitening on edges of the *Pithophora*
- At 10 DAT, all samples treated with algaecides exhibited visible cellular damage
- All samples treated with PondZilla Pro resulted in lower measurable biomasses compared to those with algaecide only

**Algaecides Alone**

**VS.**

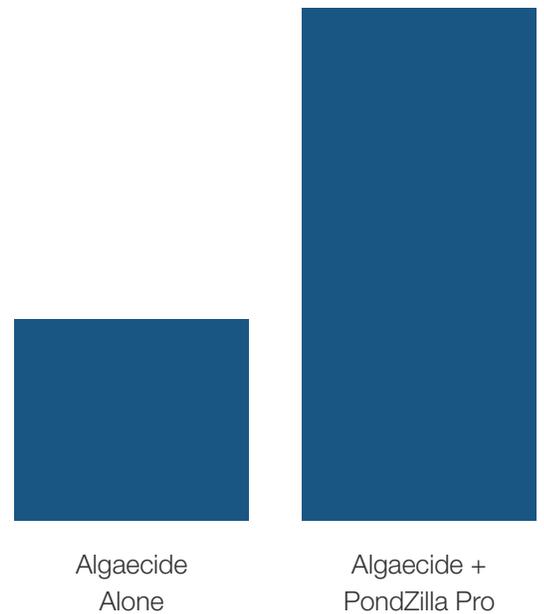
**Algaecides + PondZilla**

**VS.**

**Control**

**Algaecides with PondZilla Pro performed 327% better on average vs algaecides alone\***

**10 DAT**



*\*comparing average biomass reduction*

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## Abstract

*Pithophora* is a common filamentous green alga that is found in a variety of water resources. It can often be problematic and is difficult to treat and control. *Pithophora* blooms form dense mat structures and have thick cell walls that limit algaecide penetration and efficacy. In addition, the cells can often be coated in carbonate crystals or attached diatoms that further reduce penetration and efficacy of algaecide treatments. *Pithophora* also forms resting structures called akinetes. The structures are packed with food reserves and have thicker cell walls that allow for survival when conditions become unfavorable. These structures also allow for rapid regrowth of vegetative filaments when favorable conditions return.

Improving algaecide efficacy through improving the absorption and the degradation of dead akinetes was the focus in the development of PondZilla Pro. PondZilla Pro is a biocatalyst that is designed to be mixed with algaecides when treating *Pithophora*. It does not kill or damage *Pithophora* on its own. Increasing the amount of algaecide that penetrates the akinetes should result in enhanced control of the *Pithophora*. More complete degradation of the dead resting structures that aid in regrowth should result in enhanced control of *Pithophora*. Visual observations in laboratory tests and field tests have reported increased damage to *Pithophora* filaments and longer lasting control when algaecides were mixed with PondZilla Pro. To better quantify the impact of PondZilla Pro, a controlled laboratory experiment was designed to measure the change in damage to filaments and overall reduction in biomass attributed to the addition of PondZilla Pro.

## Objective

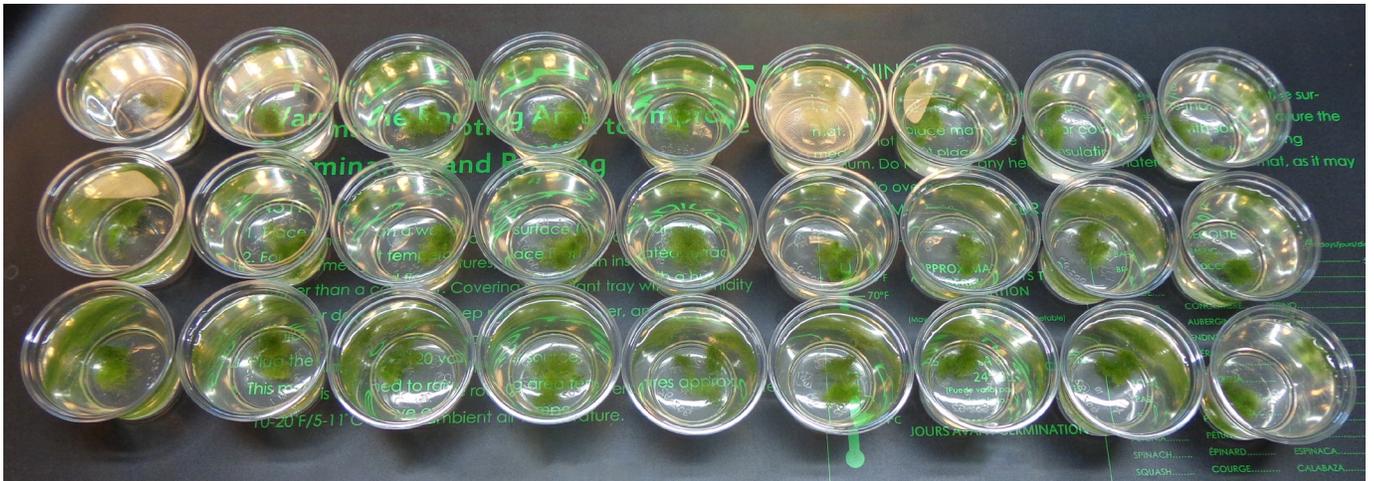
To measure how the addition of PondZilla Pro effects treatment efficacy of *Pithophora* when combined with copper algaecides.

## Methods

9oz clear plastic cups were labeled and mass was recorded. The pond water from a Madison, WI pond was collected filtered through a 0.45um pore filter before freezing. The filtered water was thawed and used to make 1:8 SD11 media. The cups were filled with 200ml each of the 1:8 SD11 media. Pieces of *Pithophora* were blotted on paper towels to remove excess water. 0.100g±0.003g of green *Pithophora* was added to each cup. The mass of the cups with media and algae were recorded. Products were added to 1ml of water removed from the cup of corresponding number, products were added, the mixture was resuspended five times to mix and added to the corresponding cup in a dropwise manner randomly across the surface of the cup. The cups were photographed and then placed on a Vivosun heat mat controlled by a Vivosun controller set to 85°F. The tops of the cups were about 14 inches under the T4 cool fluorescent (6w) grow light. The lights were on a 12h:12h light:dark cycle. To prevent excessive evaporation, the cups were covered loosely with plastic wrap. Every day for the 10 day test period, the cups were uncovered, photographed and 20ml of media was exchanged with fresh media. At the end of the test period, the cups were drained of most of the media and placed uncovered back on the mat. The liquid in the cups was evaporated for seven days before the final mass of the cups containing dry algae was recorded. Dry algal biomass was determined by subtracting the final cup and algal mass from a sacrificial set prepared initially.

## Table of Treatments

Cup #	Treatment
1, 2, 3	PondZilla Pro
4, 5, 6	8% Copper
7, 8, 9	Argos
10, 11, 12	Citrine Plus
13, 14, 15	Argos + PondZilla Pro
16, 17, 18	8% Copper + PondZilla Pro
19, 20, 21	Citrine Plus + PondZilla Pro
22, 23, 24	Control
25, 26, 27	Control - Sacrifice (7/27/2020)



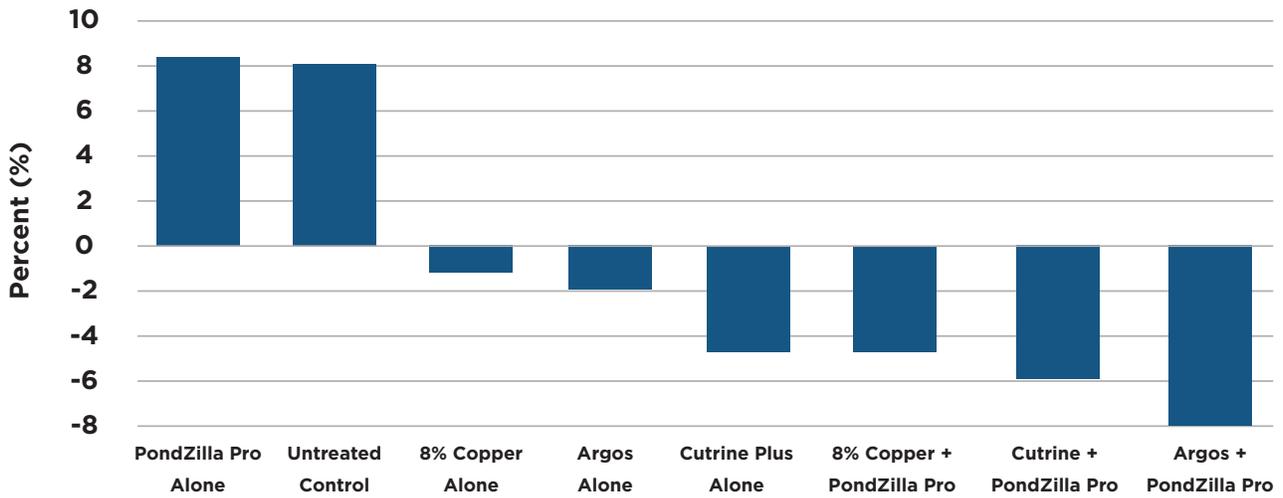
## 0 Days After Treatment

Treated algae placed on heat mat in lab under grow light. Left column has cups number 1, 2, 3, then going by column to the right are cups 4, 5, 6, then next right column are cups 7, 8, 9, etc. Sacrifice 25, 26, 27 (far right) right after treated algae were placed on heat mat. Sacrificed cups were left on heat mat for seven days before final dry mass of algae was determined. All cups started with dark green clumps of *Pithophora* to a mass of  $0.100 \pm 0.003\text{g}$ .

## Post Treatment Visual Analysis

Cup #	Treatment	7 DAT	10 DAT
1	PZP	-----	Dark green/growing
2	PZP	-----	Dark green/growing
3	PZP	-----	Dark green/growing
4	8% Copper	-----	White edges, slightly pale green, some attached growth
5	8% Copper	-----	White edges, slightly pale green, some attached growth
6	8% Copper	-----	White edges, slightly pale green, some attached growth
7	Argos	-----	White edges, slightly pale green, some attached growth
8	Argos	-----	White edges, slightly pale green, some attached growth
9	Argos	-----	White edges, slightly pale green, some attached growth
10	Citrine Plus	-----	White edges, slightly pale green, some attached growth
11	Citrine Plus	-----	White edges, slightly pale green, some attached growth
12	Citrine Plus	-----	White edges, slightly pale green, some attached growth
13	Argos + PZP	-----	Slightly pale green, white edges, some brown filaments
14	Argos + PZP	White/brown edges	Slightly pale green, white edges, some brown filaments
15	Argos + PZP	White/brown edges	Slightly pale green, white edges, some brown filaments
16	8% Copper + PZP	White/brown edges	Slightly pale green, some white edges
17	8% Copper + PZP	-----	Slightly pale green, some white edges
18	8% Copper + PZP	-----	Slightly pale green, some white edges
19	Citrine Plus + PZP	White/brown edges	Slightly pale green, some white edges
20	Citrine Plus + PZP	-----	Slightly pale green, some white edges
21	Citrine Plus + PZP	White/brown edges	Slightly pale green, some white edges
22	Control	-----	Dark green/growing
23	Control	-----	Dark green/growing
24	Control	-----	Dark green/growing

## PERCENT BIOMASS GROWTH



## Results

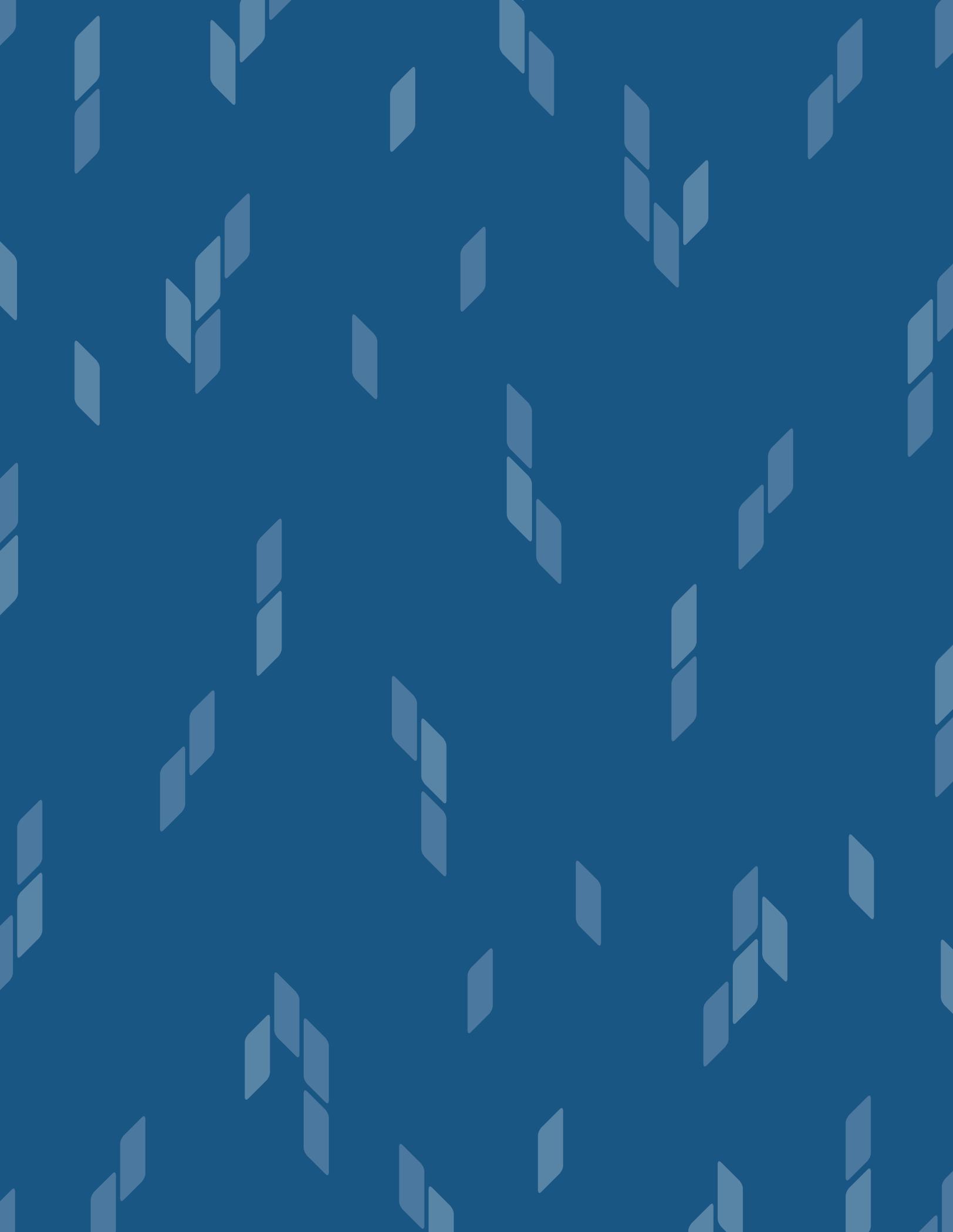
Both the PondZilla Pro alone and untreated control resulted in increased biomass after 10 DAT. All copper algaecides resulted in a slight decrease in *Pithophora* biomass after 10 DAT. All copper algaecides with PondZilla Pro resulted in decreased biomass compared to the untreated control, PondZilla Pro alone and algaecide alone treatment samples.

Only algaecide with PondZilla Pro treated samples show visible cellular damage at 7 DAT. AT 10 DAT, algaecide samples with PondZilla Pro showed significantly more visible cellular damage compared to algaecide samples without PondZilla Pro.

## Conclusion

In this study, it is shown that PondZilla Pro alone does not damage or kill the green filamentous algae, *Pithophora*. Copper algaecides alone were effective at providing some level of control and reducing the biomass of *Pithophora*. Copper algaecides improved response time, overall control, and reduced more biomass when combined with PondZilla Pro. PondZilla Pro can be used as an effective biocatalyst to improve treatments of *Pithophora* with copper algaecides.





Study crafted and completed by

# Naturalake Biosciences

