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Chemical Management Strategies for Starry Stonewort: A Mesocosm Study

Kaytee Pokrzywinski, West Bishop, Christopher Grasso, Kaitlin Volk, September 2021
and Kurt Getsinger

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Chemical Management Strategies for Starry Stonewort: A Mesocosm Study

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Abstract

US Environmental Protection Agency (USEPA) approved algaecides and herbicides are frequently utilized to manage nuisance algae and aquatic macrophytes. However, there is limited information available on the effectiveness of these products for the management of starry stonewort. Thus, the goal of this research was to discern effective chemical control products for later growth stages of starry stonewort using mesocosm studies. Eleven treatments were evaluated using various combinations of four copper-based products, endothall, diquat, and carfentrazone – all with USEPA registrations for use in aquatic sites. To assess treatment efficacy, water quality, photophysiology, biomass changes, and bulbil viability were evaluated. Nine of the eleven treatments yielded lower dissolved oxygen concentrations and higher specific conductance when compared to the control. Photophysiological response varied by condition, but seven of eleven treatments resulted in significantly lower fluorescent and maximum fluorescent yield. Five of these also exhibited significantly lower average photosynthetic yields, with combination treatments resulting in more drastic decreases. Ten of the eleven treatments had significantly less biomass compared to the control when measured via wet weight; however, only four treatments were significant when measured via dry weight. Lastly, all conditions utilizing copper-based products significantly reduced bulbil viability while non-copper products had no impact.

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Preface

The work reported herein was conducted by the US Army Engineer Research and Development Center (ERDC), Environmental Laboratory (EL), Vicksburg, MS, and SePRO Corporation. The project was funded by the Aquatic Plant Control Research Program (APCRP) under Funding Account Code U4368767, AMSCO Code 075098. Mr. Warren Lorentz was the Acting Technical Director, and Dr. Christine VanZomeren was the Acting Program manager.

At the time of publication of this report, Dr. Michael Rowland was Acting Chief of the Environmental Processes Branch; Mr. Warren Lorentz was Acting Chief of the Environmental Processes and Engineering Division; Dr. Brandon Lafferty was the Deputy Director of EL, and Dr. Jack Davis was the Acting Director.

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1 Introduction

1.1 Background

Starry stonewort is a macroalga in the family Characeae that is native to Europe and Asia. In its native range, starry stonewort is a desired species, though many populations are in decline (Larkin et al. 2018 and references therein). The first confirmed introduction in the United States was the St. Lawrence River system in New York in the 1970s (Geis et al. 1981; Karol and Sleith 2017). Since its introduction into the United States, starry stonewort has become highly invasive and is now documented in hundreds of lakes across the northern tier states as well as Ontario, Canada (Kipp et al. 2019). In the current state of knowledge, ecological niche modeling has suggested high suitability of starry stonewort invasion throughout 29 states from the Great Plains through the Mid-Atlantic and Northeastern ecoregions (Escobar et al. 2016; Sleith et al. 2018). An increased understanding of effective management strategies for this new invader is critical to limiting its spread across North America.

Starry stonewort is a dioecious green macroalgae, and only male individuals have been described in the United States; therefore, reproduction in this region occurs asexually via star-shaped bulbils (MAISRC 2019). As such, starry stonewort distribution primarily occurs from bulbils and fragments. Bulbils can be found at vegetative nodes, though many are concentrated on the rhizoids or *root-like* structures of the macroalga. Maximum starry stonewort biomass and bulbil density typically occurs during late summer/early fall, but bulbils have been documented year round (Nichols et al. 1988). While some minimal vegetative biomass can persist throughout the winter, most of the starry stonewort re-establishment in the spring/summer is likely from the overwintering of bulbils (Midwood et al. 2016; Larkin et al. 2018).

The large vegetative single-cells in starry stonewort are primarily comprised of water, which allow for a rapid and energetically inexpensive means of increasing overall biovolume (Goldstein and van de Meent 2015). This condition may allow them to outcompete vascular aquatic plants for light, nutrients, and space (Coops 2002) and support pioneer establishment in new areas within invaded water bodies (Lambert-Servien et al. 2006). Once established, starry stonewort can form monocultures of dense

mats (up to 7 m* in depth) that outcompete submersed native vegetation (Sleith et al. 2015; Larkin et al. 2018). As such, starry stonewort can directly impact fish habitat and spawning activities, water chemistry, and biogeochemical cycles (Pullman and Crawford 2010; Larkin et al. 2018). Alterations in water quality including low redox potential, anoxia, and nutrient release from the sediments, may also directly or indirectly limit habitat suitability for co-occurring species (Brainard and Schulz 2017). Moreover, dense starry stonewort surface mats can negatively impact boating and other recreational activities (MAISRC 2019).

Limited options are available for the effective control of starry stonewort in North America. While mechanical harvesting, diver-assisted suction harvesting (DASH) and manual removal have been used with moderate success, these strategies tend to be high in cost and limited in scope. These processes may also promote dispersal of fragments and bulbils and thereby increase distribution (Larkin et al. 2018). Benthic barriers and fall/winter lake draw-down events have also been tried with limited success.[†] While use of barriers/drawdowns are best deployed as a means of preventing early stage growth, their effectiveness has yet to be demonstrated on large-scale infestations. Chemical management of starry stonewort has demonstrated some opportunity for success in the field, though more data are needed to understand the impact of growth stage, biovolume and bulbil density on treatment efficacy — along with the duration of control. Documented field or mesocosm scale results are either non-existent to date or limited by confounding factors and/or a constrained number of tested products, showing mixed efficacy (Glisson et al. 2018). Since herbicides have been successfully used for controlling other algal species, including charophytes, more chemical testing on starry stonewort is warranted.

Chelated copper-based algaecides/herbicides are commonly used products for controlling nuisance algae (filamentous, planktonic, and macroalgae) and submersed macrophytes in US water bodies (AquaPlant 2019). Chelation is designed to improve stability of the copper ion in solution and

* For a full list of the spelled-out forms of the units of measure used in this document, please refer to *US Government Publishing Office Style Manual*, 31st ed. (Washington, DC: US Government Publishing Office 2016), 248-52, <https://www.govinfo.gov/content/pkg/GPO-STYLEMANUAL-2016/pdf/GPO-STYLEMANUAL-2016.pdf>.

[†] Mischelle Nault. Personal communication. WI DNR. May 2018.

alter charge properties of resulting copper complexes to passively penetrate cell membranes (Stauber and Florence 1987; Strauss and Tucker 1993; Mastin and Rodgers 2000). Increased algaecidal efficacy has been documented with chelated formulations compared with copper sulfate (Bishop and Rodgers 2012; Calomeni et al. 2014; Iwinski et al. 2016). Recent chelated copper-algaecide formulations have shown significantly better control of nuisance algal species in both the laboratory and field (Bishop et al. 2015; Bishop et al. 2018). Additionally, non-copper products (diquat dibromide, endothall, carfentrazone-ethyl) have been used in field applications for macroalgae with some success, though more robust evaluations are needed (GLMRIS 2012; Oliveira et al. 2016; Minnesota DNR 2019). Combining copper algaecides with non-copper products has shown increased efficacy on submersed macrophytes than either product used alone (Sutton et al. 1970; Sutton et al. 1971; Pennington et al. 2001) but needs further evaluation on macroalgae. Chemicals selected for this study included several classes of active ingredients with differing modes of action (Table 1). All products evaluated in this study are US Environmental Protection Agency (USEPA)-registered for use in aquatic sites containing starry stonewort and included non-copper, multiple different chelated coppers, and numerous combinations of these formulations. These products were selected based upon documented reports of efficacy against algae and submersed macrophytes in operational field programs and to provide a broad comparison of the different products commercially available.

1.2 Objectives

The overall goal of this research was to discern effective chemical control mechanisms for mature growth stages of the invasive macroalga, starry stonewort.

Specific objectives were to accomplish the following:

1. Measure the responses of starry stonewort to exposures of algaecides and aquatic herbicides alone and/or in combination.
2. Compare effectiveness of treatments through time on photosynthetic yield and total biomass.
3. Indirectly assess bulbil viability via metabolic activity in treatments compared with non-treated controls.

1.3 Approach

This effort utilized mesocosm studies to compare multiple chemical products alone and in combinations for control of starry stonewort. All products evaluated have previously been used by water resource management agencies and commercial vendors, with some level of efficacy reported in field scenarios. Scaled bioassays (such as mesocosms) with algae have been specifically designed to increase the predictive ability of success in field sites (Bishop and Rodgers 2012; Bishop et al. 2015; Bishop et al. 2017; Bishop et al. 2018). Trials conducted at the mesocosm scale allow for multiple replicates of each treatment and repeated studies to compare efficacy among many products in the absence of confounding factors that are commonly observed in the field. In these trials, chemical treatments occurred on a population of starry stonewort from Wisconsin, using late summer growth, a time when notable biomass is easily identified and when the highest bulbil production is predicted prior to winter decline.

1.4 Purpose and scope

This technical report was prepared under the work-unit “Starry Stonewort: A Weed Gone Wild,” funded by the Aquatic Plant Control Research Program. The purpose of this study was to objectively compare multiple treatment options for controlling the invasive macroalga *Nitellopsis obtusa* (Desv. in Loisel.) J. Groves, commonly known as starry stonewort. Multiple trials were conducted in a controlled environment setting to discern the chemical products that are most likely to provide rapid control of starry stonewort in the field. Additionally, the impact of these treatment strategies was also evaluated on the asexual resting structures, or bulbils, of this macroalga as a means of assessing its potential to recover from treatments in a field setting.

2 Materials and Method

2.1 Experimental design

Whole, mature starry stonewort specimens and lake water were collected from Pike Lake, Hartford, WI (approximate Global Positioning System coordinates: 43.304037, -88.328692) and transported to a private outdoor research facility in close proximity to the lake on August 13 and 14, 2019 (WI DNR Chapter NR40 Permit #IS-2019-05). Two consecutive studies were conducted at the facility 24 hours apart to assess the efficacy of chemical products on late-season starry stonewort growth and bulbil viability. For each study, 48 mesocosms (19 L capacity) were filled with approximately 15 L of lake water (pH 6.5-7.0) and 50 g +/- 2 g (wet weight) of starry stonewort (including intact rhizoids and bulbils). Notable debris was physically removed from starry stonewort biomass, and rhizoids were gently rinsed with lake water to remove sediments prior to placement in each mesocosm, where specimens were allowed to acclimate overnight prior to treatment.

A total of six independent chemical products, five chemical combinations and a no-treatment control were evaluated in each trial (n=4 per condition, per trial) (Tables 1 and 2). Ten pre-treatment specimens were dried for biomass comparisons, and 30 bulbils were collected for pre-treatment viability determination. Treatments occurred over two consecutive days where Trial 1 was treated on August 14, 2019, and Trial 2 was treated on August 15, 2019. Both days were partly cloudy, and air temperatures ranged from 72 to 75 °F during treatment, with approximately 4 hours of daylight remaining at time of treatments. Each chemical treatment was applied at the rate listed in Tables 1 and 2 and was the same for each trial, with the exception of carfentrazone-ethyl (hereon referred to as carfentrazone) where a ten-fold higher rate was used in Trial 2 (2 ppm). Note the granular formulation of copper EDA (copper EDA-G) was used alone where the liquid formulation was used in combination trials. Stock solutions of diquat and carfentrazone were pre-diluted ten-fold in lake water to enable more even diffusion within each mesocosm. To ensure an even application, each chemical was pre-homogenized in a working solution containing 15 mL of lake water. For combination treatments, both products were added to the same working solution prior to treatment. Products were applied just below the water surface in each mesocosm.

To confirm initial exposure concentrations of products, surrogate mesocosms (without macroalgae present) were filled to the same volume and treated in the same manner as experimental mesocosms. The water in each mesocosms was homogenized before sampling to get an accurate aqueous concentration of the active ingredient(s). Treatments were intentionally left static to mimic worst-case field exposure conditions. Inverted conical tubes were used in triplicate to sample the concentration in the surrogate mesocosms. Aqueous total copper samples were preserved, 1% v/v, with trace metal grade nitric acid (Fisher Scientific, Inc. A509) and filtered (0.22 µm Whatman GF/F glass microfiber) prior to analysis. Copper was measured using inductively coupled plasma-optical emission spectrometry (ICPE 9000; Shimadzu Corporation), including a matrix-matched calibration curve from serial dilution of a 1000 mg Cu/L standard (Fisher Scientific, Inc. SC194; APHA 2005). Copper samples were analyzed at the SePRO Research and Technology Campus and were all within 10% of their target rate (Whitakers, NC).

Mesocosms were monitored daily for 5 days after treatment (DAT) and harvested on the sixth day. The relatively short algaecide exposure period was employed to maintain integrity of water quality and macroalgae in the non-treated mesocosms. Efficacy was evaluated through water quality and photochemical changes, wet weight, dry weight, and bulbil viability.

Table 1. Independent chemical products evaluated in starry stonewort mesocosm trials.

<i>Product</i>	<i>Trade Name</i>	<i>Active Ingredient(s)</i>	<i>Mode of Action</i>	<i>% Active Ingredient</i>	<i>Concentration</i>
Copper EDA/TEA	Nautique	Copper Ethylenediamine Complex (13.2%) Copper Triethanolamine Complex (14.9%)	Inhibitor of enzyme production, cell division, photosystem II activity, ATP production and respiration	9.1% Copper	1 ppm
Copper EDA-G	Komeen Crystal	Copper Ethylenediamine Complex (50%)		17.5% Copper	1 ppm
Copper EDA	Komeen	Copper Ethylenediamine Complex (22.9%)		8% Copper	1 ppm
Copper EAs	Captain XTR	Copper Ethanolamine Complexes (28.2%)		9.1% Copper	1 ppm
Carfentrazone	Stingray	Carfentrazone-Ethyl	Protoporphyrinogen oxidase (PPO) enzyme inhibitor	21.30%	0.2 ppm and 2 ppm
Diquat	Reward	Diquat Dibromide	Photosystem I inhibitor	37.30%	0.370 ppm
Endothall	Hydrothol 191	Mono(N,N-dimethylalkylamine) salt of Endothall	Protein phosphatase inhibitor, cellular membrane disruption	53%	1.5 ppm

Nautique, Komeen, Komeen Crystal, Captain XTR and Stingray are all registered trademarks of SePRO Corporation
Reward is a registered trademark of Syngenta Group Company
Hydrothol 191 is a registered trademark of United Phosphorous, Limited.

Table 2. Combination chemical products evaluated on starry stonewort.

<i>Product Combination</i>	<i>Concentration</i>	
	<i>Product 1</i>	<i>Product 2</i>
Diquat + Endothall	0.370 ppm	1.5 ppm
Copper EDA/TEA + Endothall	1 ppm	1.5 ppm
Copper EDA + Diquat	1 ppm	0.370 ppm
Copper EAs + Carfentrazone	1 ppm	0.200 ppm
Copper EAs + Endothall	1 ppm	1.5 ppm

2.2 Water quality

Water chemistry was determined using a ProDSS hand-held multi-parameter water quality meter (YSI Inc., Yellow Springs, OH) equipped with optical dissolved oxygen, conductivity/temperature, and total algae sensors. Water quality information was collected from each mesocosm immediately before treatment (after 24-hour acclimation period) and 5 DAT. The following parameters relevant to these studies that were collected during each sampling event include temperature (°C), specific conductance (SPC) (μScm^{-1}), chlorophyll a ($\mu\text{g/L}$) and optical dissolved oxygen (DO) (mg/L). Data were retrieved using the software KorDSS v 1.6.6.0 (YSI Inc.) and exported to Prism8 v 7.04 (GraphPad, San Diego, CA) for analysis. For each trial, statistical differences between the control group and chemical treatments were determined using a one-way analysis of variance (ANOVA) with a Dunnett's Post-hoc test for multiple comparisons at an alpha level of 0.05.

2.3 Photochemistry

An underwater chlorophyll fluorometer (Diving PAM-II; Walz, Germany) was used to determine in situ changes in the photosynthetic activity of starry stonewort. The Diving PAM-II applies a pulse-modulated measuring light for the selective detection of the photosynthetic yield of photosystem II. From this instrument, the chlorophyll fluorescence yield (F) and maximal yield (Fm') were measured, and the photosynthetic yield Y(II) was calculated using the following equation:

$$Y(\text{II}) = (\text{Fm}' - \text{F}) / \text{Fm}'$$

In these trials, the fluorometer measured the efficiency of photosystem II in the light-adapted state only, to provide a comparison of photosynthetic yield and overall fluorescence between non-treated and chemically treated specimens. A total of five to seven representative filaments were individually measured from each mesocosm before treatment and five DAT. The software WinControl-3 v 3.29 (Walz) was used to retrieve the data, and the data were analyzed in Prism8 (GraphPad). For each study, statistical differences between the control group and all treatments were determined using a one-way ANOVA with a Dunnett's test for multiple comparisons at an alpha of 0.05.

2.4 Biomass assessment

One day before treatment and 6 DAT, starry stonewort material was collected from each mesocosm using a fine mesh net. Biomass specimens were gently blotted on a paper towel to remove excess water and were weighed using a calibrated digital balance (± 0.01 g). At harvest, after wet weight was obtained, each specimen was placed into a paper bag and allowed to air dry until baking at 160 °C for at least 72 hours. After drying, each specimen was weighed to the nearest tenth of a gram. Wet weight and dry weight were used to determine the overall effectiveness of each product, and a wet weight-to-dry weight ratio was calculated. A one-way ANOVA (alpha 0.05) was used to determine differences between the non-treated control and chemically treated macroalgae specimens.

2.5 Bulbil viability

Indirect bulbil viability was determined (via a metabolic assay) on pre-treated bulbils prior to chemical applications (n=30) and bulbils obtained at harvest (n=8-10 per treatment, per trial) using the tetrazolium assay. Briefly, bulbils were incubated overnight in lake water containing 1%, 2,3,5 triphenyl tetrazolium chloride (TTC), pH 7.0 (modified slightly from Verma and Majee 2013) and imaged the following morning. No pre-conditioning or washing steps were performed (e.g., scarification). A positive TTC signal appears as red/dark pink and denotes metabolically active *viabile* bulbils whereas non-viable bulbils have little to no color shift and appear white. Bulbil viability was assessed categorically as follows: white bulbils = 0, white bulbils with red/pink tips = 1, red/pink bulbils with white tips = 2, and bulbils that were completely red/pink = 3.

3 Results

3.1 Water chemistry

3.1.1 Exposure confirmation

Due to no differences between trials, copper concentrations for each product were pooled between experiments. Copper EDA* averaged 106.8%, copper EAs 96.1%, copper EDA-G 106.0%, and copper EDA/TEA 95.1% of targeted nominal copper exposures. Due to the proximity to the nominal value, the target concentration was used in the analyses and to make comparisons between treatments.

3.1.2 Temperature

After acclimation and before chemical treatment, the mesocosm temperature averaged 19.7 °C and 18.8 °C in Trials 1 and 2, respectively (Tables 3 and 4). The minimum temperature throughout both trials was 18.6 °C while the maximum temperature was 30.1 °C. Given that the trials had similar temperature profiles, they were analyzed together to increase the power of the analysis. A two-way mixed-model ANOVA was used with treatment and time as factors (alpha 0.05). There was no significant difference in temperature between treatments; however, there was an overall significant temperature increase throughout the study ($p < 0.001$).

Table 3. Minimum, maximum and average water temperatures in mesocosms throughout Trial 1.

Trial 1		Temperature (°C)		
Date	Time (DAT)	Min	Max	Mean
8/14/19	0	19.4	20.0	19.7
8/15/19	1	22.2	27.4	24.8
8/16/19	2	21.6	25.1	23.2
8/17/19	3	21.8	26.8	24.0
8/18/19	4	21.7	24.4	22.8
8/19/19	5	24.8	30.1	27.7

* The abbreviations in this sentence are defined in Table 1.

Table 4. Minimum, maximum and average water temperature in mesocosms throughout Trial 2.

Trial 2		Temperature (°C)		
Date	Time (DAT)	Min	Max	Mean
8/15/19	0	18.6	19.3	18.9
8/16/19	1	19.7	23.3	21.1
8/17/19	2	22.7	26.8	24.6
8/18/19	3	23.2	25.6	24.5
8/19/19	4	25.3	29.6	27.8
8/20/19	5	24.8	27.7	26.2

3.1.3 Dissolved oxygen (DO)

It is well known that oxygen is a critical component to healthy aquatic ecosystems (Wetzel 1975). The oxygen-carrying capacity of water is far less than that of air and is strongly associated with temperature (Medina et al. 2018). In general, low dissolved oxygen concentrations can suggest poor plant/macroalgae health and a decline in plant/macroalgae biomass within aquatic communities from both a decrease in photosynthetic activity as well as bacterial-mediated degradation of senescing biomass. Overall, DO in the pre-treatment collections was in excess of 10 mg/L or 95% saturation (data not shown). The average control condition across both trials had DO levels of 11.1 mg/L, ranging from 8.79 mg/L to 12.86 mg/L (Figure 1A). All treatments exhibited a statistically significant average decrease in DO ranging from 7.6 mg/L (31.5% decrease, copper EDA/TEA) to 3.7 mg/L (66.7% decrease, copper EDA/TEA + endothall) ($p < 0.05$); however, carfentrazone ($p = 0.118$) and diquat ($p = 0.591$) did not negatively impact DO and had average concentrations of 12.3 mg/L and 10.3 mg/L, respectively (Figure 1A). With the exception of copper EAs, all combination treatments showed lower DO levels (3.7 to 4.7 mg/L, 66.7 % to 57.7 % decrease) than each product alone (4.2 mg/L to 12.3 mg/L, 62.2 % decrease to 10.8 % increase). Mesocosms that received copper products also displayed lower DO concentrations (4.2 mg/L to 7.6 mg/L, 62.2 % to 31.5 % decrease) than other products (diquat, carfentrazone, and endothall) (6.0 mg/L to 12.3 mg/L, 45.9% decrease to 10.8% increase) (Figure 1A).

3.1.4 Specific conductance (SPC)

SPC is a useful general measure of how well water can conduct electricity. Conductivity is known to increase with an increase in the amount and mobility of dissolved ions. This is primarily caused by the breakdown of

organic and inorganic compounds, such as those found in macrophytes, macroinvertebrates, sediment, and road runoff. As these compounds breakdown into ions, they conduct electricity because they hold a charge when dissolved in water (Murphy 2007). Therefore, greater SPC values in chemically treated mesocosms than non-treated control values can be indicative of biomass decomposition. In both trials, the controls had SPC levels between $618.8 \mu\text{Scm}^{-1}$ and $650.9 \mu\text{Scm}^{-1}$ with an average of $639.6 \mu\text{Scm}^{-1}$ (Figure 1B). All treatment conditions showed a statistically significant increase in SPC over the control ranging from $687.7 \mu\text{Scm}^{-1}$ (7.5% increase) to $713.1 \mu\text{Scm}^{-1}$ (11.5 % increase) ($p < 0.05$), again with the exception of carfentrazone ($634.4 \mu\text{Scm}^{-1}$, $p = 0.992$) and diquat ($649.4 \mu\text{Scm}^{-1}$, $p = 0.750$) (Figure 1B). There was a slight increase in SPC in products used in combination ($697.3 \mu\text{Scm}^{-1}$ to $713.1 \mu\text{Scm}^{-1}$, 9.0 % to 11.5 % increase) over products used alone ($687.7 \mu\text{Scm}^{-1}$ to $699.2 \mu\text{Scm}^{-1}$, 7.5% to 9.3% increase).

Figure 1. Water chemistry information 5 DAT for both mesocosm trials combined. Bars represent the average dissolved oxygen (A) and specific conductance (B). Error bars denote the standard error of the mean (SEM) ($n=7-8$). Statistical significance is denoted by asterisks where *; $p < 0.05$.

3.2 Photophysiology

Photosynthesis is an essential process required for metabolic growth in aquatic plants and algae. Products having differing modes of action can have differential impacts on photosynthetic yield and the overall ability of the plant or algae to recover from exposure to such products. In the current studies, the fluorescence yield of the non-treated control mesocosms was between 81 relative fluorescence units (RFUs) and 824 RFUs with an average of 350 RFUs, the maximal fluorescence yield was an average of 457 and ranged between 90 RFUs and 1221 RFUs, and the photosynthetic yield was an average of 0.21 ranging from 0.015 to 0.576 (Figure 2). The impact of each chemical product (alone or in combination) to photophysiology was more variable.

Several products showed no significant decrease in the variable fluorescence yield (F) when compared to the controls, including copper EDA/TEA ($p < 0.05$ – increased fluorescence), copper EDA-G ($p = 0.999$) carfentrazone ($p = 0.982$) and diquat ($p = 0.569$). Fluorescence yields in these products ranged from 294 RFUs to 465 RFUs (Figure 2A). All other products showed a statistically significant decrease in the fluorescence yield ($p < 0.05$) (3 RFUs to 144 RFUs) where copper EAs + endothall (3 RFUs, 99% decrease from control) had the lowest average F values followed by copper EDA/TEA + endothall (11 RFUs, 97% decrease), copper EAs (29 RFUs, 92% decrease), copper EAs + carfentrazone (31 RFUs, 91% decrease), diquat + endothall (51 RFUs, 85% decrease), endothall (87 RFUs, 75 % decrease) and copper EDA + diquat (144 RFUs, 59% decrease). Once again, the product combinations showed greater decreases (3 RFUs to 144 RFUs, 99% to 59% decrease) in measured parameters over the individual products alone (87 RFUs to 465 RFUs, 75% decrease to 2% increase), with the exception of copper EAs (alone and in combination), which had low fluorescence yields in all treatment scenarios (3 RFUs to 31 RFUs).

Maximal fluorescence yields mimicked the variable fluorescence yields (F) but were higher, owing to the application of the saturation pulse. Several products showed an increase or no significant change in the light-adapted maximal fluorescence yield (F_m') when compared to the controls, which included copper EDA/TEA ($p < 0.05$), copper EDA-G ($p = 0.539$) carfentrazone ($p = 0.999$) and diquat ($p = 0.678$). Maximal fluorescence yields in these products ranged from 378 RFUs to 742 RFUs (Figure 2B). All other products showed statistically significant decreases in the maximal fluorescence yields ($p < 0.05$) (7 RFUs to 233 RFUs, 98% to 49% decrease

from control) where copper EAs + endothall (7 RFUs, 98% decrease) had the lowest average F_m' values followed by copper EDA/TEA + endothall (18 RFUs, 96% decrease), copper EAs (40 RFUs, 91% decrease), copper EAs + carfentrazone (45 RFUs, 90% decrease), diquat + endothall (68 RFUs, 85% decrease), endothall (127 RFUs, 72% decrease) and copper EDA + Diquat (233 RFUs, 49% decrease). The individual products also showed higher F_m' values (127 RFUs to 742 RFUs, 72% decrease to 62% increase) over the combination products (7 RFUs to 68 RFUs, 98% to 85% decrease), with the exception of copper EAs (Figure 2B).

The photosynthetic yields were slightly different than either the fluorescence or maximal fluorescence yields. Interestingly, copper EDA/TEA with an average $Y(II)$ of 0.35 along with copper EDA + diquat with a $Y(II)$ of 0.36 both showed a significant increase of 67% and 71%, respectively, in photosynthetic yield over the control condition at 0.2 ($p < 0.05$) (Figure 2C). Copper EDA-G ($p = 0.646$), carfentrazone ($p = 0.985$), diquat ($p = 0.999$) and Endothall ($p = 0.999$) all showed no changes in average photosynthetic yield ranging from 0.18 to 0.25 when compared to the control. All other test conditions showed a significant decrease ($p < 0.05$) in average photosynthetic yields in the following rank: Diquat + endothall showed the greatest yields in this group with a $Y(II)$ of 0.09 (57% decrease from control), followed by copper EA alone and in combination with carfentrazone with average $Y(II)$ s of 0.06 (71% decrease), and lastly copper EDA/TEA + endothall and copper EAs + endothall which had no measurable average photosynthetic yields (Figure 2C). In general, the combination treatments showed greater decreases in photosynthetic yields when compared to the products alone, with the exception of copper EDA + diquat.

Figure 2. Photophysiology five DAT for both mesocosm trials combined. Bars represent the average fluorescence yield (A), maximal fluorescence yield (B), and photosynthetic yield (C). Error bars signify the SEM (n=7-8). Statistical significance is denoted by asterisks where *; $p < 0.05$.

3.3 Biomass changes

All treatments were evaluated for efficacy by wet weight immediately following harvesting. All products resulted in a significant decrease in wet

weight when compared to the control (48 g), except Carfentrazone that had an average wet weight of 43 g ($p = 0.388$) (Figures 3–4). Diquat had the least impact after carfentrazone ($p < 0.05$) at 40 g (17% decrease). All other products demonstrated greater decreases in masses ranging from 27 g (44% decrease, copper EDA-G) to 37 g (23% decrease, copper EAs) ($p < 0.05$). There were no differences in wet weight when comparing products used alone or in combination, except when carfentrazone and diquat were applied. Formulations containing copper EDA (including copper EDA/TEA, copper EDA-G and copper EDA + diquat) showed the greatest decrease in overall biomass ranging from 27 g to 28 g (44% to 42% decrease). Although still statistically significantly lower mass than the controls, when copper EDA formulations were combined with endothall, more biomass was retained (35 g, 27% decrease from control and 25% increase in biomass from copper EDA formulations used alone).

Dry weight revealed very different relationships than wet weight. Only three treatments had a statistically significant change in biomass ($p < 0.5$) when compared to the control of 4.5 g: Copper EDA-G at 3.5 g (22% decrease from the control), and copper EDA/TEA + endothall and copper EDA + diquat both at 3.4 g (24% decrease). All other treatments did not show a significant change in dry weight from the control: Copper EDA/TEA at 3.9 g ($p = 0.604$), copper EAs at 3.6 g ($p = 0.087$), carfentrazone at 4.7 g ($p = 0.995$), diquat at 4.3 g ($p = 0.999$), endothall at 4.0 g ($p = 0.814$), diquat + endothall at 4.0 g ($p = 0.621$), copper EAs + carfentrazone at 4.1 g ($p = 0.875$), and copper EAs + endothall at 3.8 g ($p = 0.353$). There was no trend in products that were administered alone or in combination.

Figure 3. Images of representative starry stonewort biomass changes 6 DAT with control products in Trial 1.

Figure 4. Biomass changes 6 DAT for both mesocosm trials combined. Bars represent the average wet weight (A) and dry weight (B) for all tested mesocosms. Error bars represent the SEM of both trials combined. Statistical significance is denoted by asterisks where *; $p < 0.05$.

3.4 Bulbil viability

Bulbils were indirectly evaluated for their viability following each trial using the TTC assay. All of the pre-treatment bulbils ranked between 2 and 3 showing greater than 90% viability (data not shown). Bulbils obtained from control conditions were also ranked as 2 (3 out of 20) or 3 (17 out of 20). All treatments having copper-based products (alone and in combination) had bulbils that primarily ranked as 0, where bulbils treated with copper EDA/TEA, copper EDA-G, copper EDA/TEA + endothall, copper EDA + diquat and copper EAs + carfentrazone were exclusively reported as 0 (20 per treatment). Furthermore, bulbils exposed to copper EAs were almost all exclusively ranked as 0, except two bulbils that ranked

as 2 (out of 20). Last, 19 bulbils treated with copper EAs + endothall were reported as 0's and one as a 3 (Figure 5).

Figure 5. Bulbil viability 24 hours after harvesting. In A, representative TTC assay images. In B, bars represent the average rank 0 to 3, where lower values indicate white, dead bulbils and higher values indicate pink, active/viable bulbils (B inset). Error bars represent the SEM.

4 Discussion

Several strategies have been employed for controlling starry stonewort in the field; however, current control methods have had minimal impacts on established populations and many treatment strategies are anecdotal (Wisconsin DNR 2015; Hackett et al. 2017; Larkin et al. 2018). As a result, this study evaluated chemical control strategies, alone and in combination, in mesocosms containing late-season starry stonewort growth. Scaled bioassays of this type have been previously employed to predict the efficacy of chemical products in field trials for nuisance species like the filamentous mat-forming algae *Lyngbya wollei* and *Pithophora varia* (Bishop and Rodgers 2012; Bishop et al. 2015; Bishop et al. 2017; Bishop et al. 2018). To assess efficacy of each tested product, water quality, photophysiology, and biomass changes as well as bulbil viability were evaluated. The information presented in this study may help elucidate some of the confounding and variable results observed during field demonstrations. Such a tiered evaluation approach (small scale mesocosms to large field verifications) has proven invaluable in developing techniques for successfully managing other problematic submersed plants including Eurasian watermilfoil and hydrilla (Netherland and Getsinger 2018).

In this study, significant changes in water chemistry were demonstrated following chemical treatment. The majority of products resulted in a significant decrease in DO with a concomitant increase in SPC – both early indicators of plant stress and injury. Low DO concentrations can suggest biomass declines in aquatic plant communities from both a decrease in photosynthetic activity, as well as, bacterial-mediated degradation of senescing biomass. For example, as algal and plant material decay, heterotrophic bacteria can rapidly consume the dissolved organic matter and drive oxygen levels down, thereby causing hypoxic (low oxygen) or even anoxic (no oxygen) conditions. Exact thresholds for hypoxia and anoxia vary between sources and waterbodies. Hypoxia refers to a level of DO below that required to sustain aquatic animals like fish or macroinvertebrates for an extended period of time (Weinke and Biddanda 2018). Generally, a waterbody is considered hypoxic when DO levels drop below 2 or 3 mg/L, but thresholds have also been set as high as 6.5 mg/L (Nurnberg 2002; Weinke and Biddanda 2018; Gulf Hypoxia 2018). Similarly, anoxia thresholds range from <2 mg/L to 0 mg/L depending on the organization (Nurnberg 2002; Weinke and Biddanda 2018). In this

study, the majority of chemical treatments could be considered as hypoxic-like conditions using the higher threshold of 6.5 mg/L, except specimens receiving copper EDA/TEA (7.6 mg/L) and copper EDA-G (6.6 mg/L), which both still displayed significantly decreased oxygen levels, and carfentrazone and diquat, which had no impact on dissolved oxygen (Figure 1A). Furthermore, starry stonewort specimens exposed to products associated with decreased oxygen levels often had collapsed internodal regions and were feathery in appearance, morphologies of which are typically associated with decaying biomass. Directions for using quick-acting algaecides are product specific and required labeling to mitigate aqueous hypoxic levels in field-scale applications. A significant increase in specific conductivity was also observed, which suggests there was successful infusion of each product into the associated biomass and supports that algal material was decaying in treatments having low DO levels (Figure 1B).

Photosynthesis and photorespiration are coupled processes in algae, where the products of photosynthesis (sugars and oxygen) are used for photorespiration, and the photorespiration product (carbon dioxide, along with light) is used for photosynthesis (Pedersen et al. 2013). An important consideration directly impacting the rate of photosynthesis and respiration is the diffusion boundary layer (DBL), or layer in which gas exchange can occur (Pedersen et al. 2013). Increased bacterial productivity on algal surfaces can directly impact the DBL and therefore limit gas exchange, including both the carbon dioxide and oxygen required for essential metabolic processes. This ultimately suggests that as material decays and heterotrophic bacteria increase, algae cannot meet their in situ oxygen or carbon dioxide demands to maintain positive growth. Therefore, as a means of assessing overall physiological health, photosynthetic yield on treated starry stonewort specimens was assessed. Overall, the fluorescence yield and maximal fluorescence yield of treated starry stonewort specimens displayed a similar pattern to that of DO levels (Figures 1 and 2) supporting this relationship between photorespiration, photosynthesis, and biomass decay. However, in some cases, there was actually an increase in the fluorescence yield and maximal fluorescence yield as with specimens treated with copper EDA/TEA and copper EDA-G. This likely has to do with the specific mode of action and penetrability of these chelated copper products. For example, the copper EAs formulation includes surfactants as an additive, which likely helped facilitate deeper penetration and therefore greater impacts to photosystem II. Specimens

that received copper EDA/TEA and copper EDA-G, formulations which lack these added penetrants, had more efficient photosynthetic yields and greater fluorescence yields, suggesting that these products may not have infused deep enough to reach those specific action sites. As such, it is likely that these copper products activated a different mode of action to elicit the observed starry stonewort biomass changes (e.g., cell wall integrity and/or free radical creation, among others). Furthermore, copper products that were applied in combination with the dimethylalkylamine salt of endothall had some of the greatest impacts on overall fluorescence and photosynthetic yields. This is not unexpected as one mode of action of endothall is to facilitate cellular membrane permeabilization or disruption, thereby allowing greater penetration of the copper products, which are known inhibitors of photosystem II. Moreover, the added effects of these two chemistries on aquatic plant control have been documented in previous research (Sutton et al. 1971; Pennington et al. 2001).

In comparison to other charophytes, which are comprised of 70%–80% water (Goldstein and van de Meent 2015), starry stonewort was comprised of approximately 90% water (determined using the wet:dry weight ratio [$\sim 9:1$]), since much of the biomass consists of water within a flaccid structure. This ratio would support a rapid and high degree of decrease in wet weight with an effective treatment that altered the cell permeability. All treatments evaluated (except carfentrazone) significantly decreased the wet weight at 6 DAT (Figure 3). The biovolume (estimated by wet weight in this study) is the primary concern from a management perspective as this species can increase rapidly and occupy much of the water column thereby outcompeting macrophytes for space and directly impeding fish and wildlife habitat. Additionally, recreational water uses (e.g., fishing, swimming, and boating) are commonly hindered by the overall biovolume of this relatively new invader. Therefore, the rapid decrease in wet weight observed in the effective treatments from this study would be of high value in restoring water resource uses through the reduction in starry stonewort biomass. More insight into biomass-algaecide-efficacy relationships would be useful in developing more effective chemical treatment strategies.

Consequently, dry weight is typically not as sensitive of a response parameter for algae over short time frames as it takes increased time to manifest based on degradation rates. Mesocosm studies, without sediments and associated bacterial populations, may also delay degradation compared with field treatment application rates (Bishop and

Rodgers 2011). Although there were some decreases in dry weight for treatments tested in this research, it was less pronounced (< 25% change) than other response parameters and less consistent with other efficacy assessment strategies including wet weight. However, it is predicted that this response would become more exaggerated over time, as is typical of field applications on macrophytes where time intervals greater than 2 months are commonly measured (MacDonald and Netherland 2018). The duration of this mesocosm study was not extended beyond 1 week owing to confounding factors that could have interfered with interpretation of the study, including increased water temperature, nutrient exhaustion, and declining DO concentrations among others.

To date, there are limited published studies that assess the impacts of herbicides and algaecides on starry stonewort bulbil viability. In the Great Lakes Region, there is a high innate viability (> 86%) (Glisson et al. 2018; Pokrzywinski et al. 2020) of natural bulbils and this is similar to the viability of untreated controls reported in this research (> 90%). These findings support the notion that starry stonewort populations rely on bulbil production for growth within an invaded waterbody and expansion to adjacent waterbodies. Management techniques that decrease the viability of bulbils are critical to managing the existing populations as well as limiting spread. This research evaluated the impact of chemical products on the viability of bulbils (attached to rhizoids) in a late-season starry stonewort population. All copper formulations, including combinations thereof, had a significant negative impact on bulbil viability as determined indirectly via the TTC metabolic assay, though these have yet to be confirmed through sprouting experiments. The decreased viability is likely due to the direct exposure to each product, rather than the potential translocation of the product via the rhizoids as these were primarily contact herbicides. For example, Glisson et al. (2018) used a different copper ethanolamine complex (copper EAs) algaecide formulation for direct application in areas of Lake Koronis, MN, at approximately half the concentration applied in this study. Glisson et al. (2018) found that after in-lake algaecide exposure, bulbil viability remained high. It is therefore possible that the sediments could directly interfere with binding of the copper to buried bulbils. Previous studies assessing the fate of copper algaecides applied to ponds found that the majority of copper was transferred from the water column to the sediment (Rader et al. 2019; Willis and Bishop 2016) in a matter of days and could be deposited deeper into the sediment through bioturbation (Liu et al.

2006). However, copper algaecides become diluted as they migrate into greater sediment depths and can react with the sediment to shift to a less bioavailable and effective form (Willis 2016). Further research is needed to understand how algaecides interact with the sediment and how this may affect their effectiveness. For sustained, operational control of starry stonewort, a greater understanding of how the available treatment options directly and indirectly impact the reproductive structures will be critical.

Overall, no one chemical treatment was consistently the most effective at reducing starry stonewort health as measured by water quality, photophysiology, biomass, and bulbil viability. Carfentrazone and diquat showed no significant effects on any measurement with the exception of a 17% decrease in wet weight for diquat, suggesting that they would be ineffective in controlling starry stonewort in the field. Copper EDA/TEA + endothall was the only treatment that had significant results in every variable assessed and had some of the most drastic effects in over half of these variables. Copper EAs, used alone and in combination, also had large negative impacts on all variables assessed except for dry weight. The combination treatment of copper EAs + carfentrazone performed similarly well as the lone copper EAs treatment, making it difficult to assess if carfentrazone actually played a role in the observed effects. Copper EDA/TEA and copper EDA treatments, on the other hand, performed better when used in combination with endothall and diquat, respectively, suggesting some synergistic or additive effects. The diquat + endothall treatment was also more effective than when each chemical was used individually, but showed no significant decrease in photosynthetic yield (Y(II)), dry weight, or bulbil viability.

5 Conclusions

With the documented spread of starry stonewort throughout the United States and the anticipated increase in its distribution across the Northern tier states and southern Canada, establishment of effective and scalable management techniques is crucial. Approved USEPA algaecides and herbicides for infested water resources provide an avenue to rapidly manage this species at numerous infested sites and at differing scales. This research identified candidate products and combinations thereof that have the potential to significantly control starry stonewort biomass and/or reproductive structures at field-scales. The most promising products were the copper-based algaecides. Although all copper products resulted in decreased biomass and a loss in bulbil viability the exact formulation may be important in conferring different modes of action that may limit the ability of starry stonewort to recover. These chemical management approaches have the potential to provide immediate restoration of water resource uses, prevent spread of viable fragments/bulbils and potentially minimize the potential for re-growth following application. In the future, top candidate products, such as copper EDA/TEA + endothall and copper EAs, identified in this research will be scaled to field trials to confirm efficacy and duration of control in operational treatment programs.

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14. ABSTRACT US Environmental Protection Agency (USEPA) approved algaecides and herbicides are frequently utilized to manage nuisance algae and aquatic macrophytes. However, there is limited information available on the effectiveness of these products for the management of starry stonewort. Thus, the goal of this research was to discern effective chemical control products for later growth stages of starry stonewort using mesocosm studies. Eleven treatments were evaluated using various combinations of four copper-based products, endothall, diquat, and carfentrazone – all with USEPA registrations for use in aquatic sites. To assess treatment efficacy, water quality, photophysiology, biomass changes, and bulbil viability were evaluated. Nine of the eleven treatments yielded lower dissolved oxygen concentrations and higher specific conductance when compared to the control. Photophysiological response varied by condition, but seven of eleven treatments resulted in significantly lower fluorescent and maximum fluorescent yield. Five of these also exhibited significantly lower average photosynthetic yields, with combination treatments resulting in more drastic decreases. Ten of the eleven treatments had significantly less biomass compared to the control when measured via wet weight; however, only four treatments were significant when measured via dry weight. Lastly, all conditions utilizing copper-based products significantly reduced bulbil viability while non-copper products had no impact.						
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