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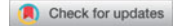
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Response of the invasive alga starry stonewort (*Nitellopsis obtusa*) to control efforts in a Minnesota lake

Wesley J. Glisson^{a,b}, Carli K. Wagner^{a,b}, Steven R. McComas^c, Kevin Farnum^d, Michael R. Verhoeven^{a,b}, Ranjan Muthukrishnan^{a,b}, and Daniel J. Larkin^{a,b}

^aDepartment of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, 135 Skok Hall, 2003 Upper Buford Circle, St. Paul, MN;

^bMinnesota Aquatic Invasive Species Research Center, University of Minnesota, 135A Skok Hall, 2003 Upper Buford Circle, St. Paul, MN;

^cBlue Water Science, 550 Snelling Avenue S, St. Paul, MN; ^dKoronis Lake Association, P.O. Box 333, Paynesville, MN

ABSTRACT

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Starry stonewort (*Nitellopsis obtusa*), an invasive green macroalga in the family Characeae, has recently been found for the first time in several Midwestern states. This aquatic invasive species is of increasing concern to management agencies, lakeshore property owners, and other stakeholders. Starry stonewort has proven difficult to control, partly due to its ability to reproduce via bulbils (asexual reproductive structures). There has also been a lack of applied research addressing the efficacy of current management practices for controlling starry stonewort. We examined the effects of mechanical and algaecide treatments on starry stonewort biomass, bulbil density, and bulbil viability by monitoring treated areas and untreated reference locations concurrent with management implemented on Lake Koronis in Minnesota. Chelated copper algaecide applications alone and in combination with mechanical harvesting significantly reduced starry stonewort biomass, but algaecide treatment alone failed to reduce the capacity of starry stonewort to regenerate via bulbils. A second, granular algaecide application following an initial treatment with liquid algaecide did not further reduce biomass in any treated area and was associated with a substantial increase in bulbil density in an area treated with algaecide alone. Bulbil viability was greatest in the area treated only with algaecide (86%) and an untreated reference area (84%) and was lowest in an area treated with both mechanical harvest and algaecide (70%). The ability of starry stonewort to regenerate and persist following algaecide treatment is concerning. Multi-pronged management incorporating both chemical and mechanical approaches may improve outcomes of starry stonewort control efforts.


KEYWORDS

Algaecide; aquatic plant management; bulbil; Characeae; chelated copper; invasive species; macroalgae; mechanical removal

Control and management of aquatic invasive plants is challenging because many factors can influence treatment efficacy. As a result, a wide variety of approaches have been developed to achieve more effective control of aquatic invasive plants (Madsen 1993, Gettys et al. 2014, Hussner et al. 2017). Identifying control strategies for a species with little history of applied research or management can be difficult, as approaches that have been effective for other target species may have limited efficacy. Even closely related species can respond quite differently to the same treatments (Parks et al. 2016). Thus, it is particularly important to evaluate efficacy of management in the case of newly discovered or understudied invasive species, for which early treatment

efforts are valuable opportunities to learn and update approaches to management.

In North America, starry stonewort (*Nitellopsis obtusa* [N.A. Desvaux] J. Groves) is an introduced macroalga in the family Characeae that is native to Europe and Asia. Starry stonewort was first found in the United States in the 1970s in the St. Lawrence River in New York (Geis et al. 1981) and then in the St. Clair–Detroit River system in Michigan 5 yr later (Schloesser et al. 1986). In just the past 5 yr, the species has been newly recorded in 5 US states (Pennsylvania, Indiana, Wisconsin, Vermont, and Minnesota) and Ontario, Canada (Kipp et al. 2017). New occurrence records and dense infestations have caused concern among lake

CONTACT Wesley J. Glisson  wjglisson@gmail.com

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users and resource managers (Pullman and Crawford 2010). Starry stonewort can produce dense beds and surface mats that interfere with boating and recreation, particularly at shallow depths. The ecological effects of starry stonewort invasion have received little investigation to date but there is evidence of negative effects on native aquatic plants; Brainard and Schulz (2016) found that native macrophyte species richness and abundance were negatively correlated with starry stonewort biomass in New York lakes. Moreover, starry stonewort may have a higher rate of carbon fixation (from HCO_3^-) in high pH conditions compared to other Characeae (Smith 1968), such as *Tolypella intricata*, which is native to the Great Lakes region. Higher rates of carbon fixation than native Characeae species could provide starry stonewort a competitive advantage in high pH lakes of the Midwest and Great Lakes region. Starry stonewort also appears to be exploiting novel niche space in the United States relative to its native range (Escobar et al. 2016), where it already exhibits fairly broad tolerance of environmental conditions (Rey-Boissezon and Auderset Joye 2015). Hence, there is cause for concern about the impacts of starry stonewort invasion, and research on the control of this introduced species is needed to guide management efforts.

Effective control of aquatic invasive plant species requires knowledge of individual species' biology (Hussner et al. 2017). For example, sexual reproduction has not been observed in populations of starry stonewort in North America due to an apparent absence of female individuals (Sleith et al. 2015). Instead, starry stonewort reproduction has been asexual, via the alga's nodes. Starry stonewort nodes are present aboveground along the stem and along rhizoids under the sediment, where they occur as specialized structures called bulbils (Bharathan 1983, 1987). Starry stonewort bulbils are white, multicellular, star-shaped structures (from which the species gets its common name) connected via rhizoids that help anchor starry stonewort in the substrate. Because new starry stonewort sprouts from bulbils (Bharathan 1987), management strategies need to target these structures to achieve effective control.

Another aspect of starry stonewort's biology that poses challenges for control is that, as an alga, it lacks a true vascular system (Raven et al. 2005). Hence, starry stonewort bulbils, which form beneath the sediment, are not connected by vascular tissue to aboveground structures. This limits the efficacy of herbicide

treatment for starry stonewort control. For example, systemic herbicides that rely on transport through vasculature may not be able to translocate through starry stonewort to reach bulbils. Furthermore, even contact herbicides that do not rely on transport, but rather physical contact, may not be able to reach unexposed bulbils beneath the sediment. The capacity of herbicides to reach bulbils will limit treatment efficacy if bulbils can persist and remain viable following treatment.

Control of starry stonewort by current treatment approaches has proven difficult. Copper-based algacides, including copper sulfate (CuSO_4) and chelated copper formulations, are contact herbicides widely used for algae control (Lembi 2014). Whereas these copper compounds have been used to manage starry stonewort in the United States, anecdotal observations indicate that these compounds may not achieve complete or sustained control of starry stonewort (Pullman and Crawford 2010). Mechanical harvesting has also been used for starry stonewort control, but anecdotal reports indicate that starry stonewort can regrow quickly following mechanical harvesting (Pullman and Crawford 2010). Compounding uncertainty about treatment effectiveness is a lack of research in this area; previous reports (i.e., Pullman and Crawford 2010) are qualitative and do not include a robust examination of treatment outcomes. We know of no published studies that have systematically evaluated outcomes of chemical or physical treatment options for starry stonewort management. Moreover, the few studies that have assessed the effect of treatment on other Characeae species either examined nontarget treatment effects (Hofstra and Clayton 2001, Wagner et al. 2007, Kelly et al. 2012), or were conducted in agricultural fields with limited application to natural lake systems (e.g., Pal and Chatterjee 1987, Guha 1991). This is a critical knowledge gap. The efficacy of current starry stonewort treatment practices must be addressed to better guide management decisions.

Observations from previous treatment efforts, combined with knowledge of starry stonewort biology, suggest that control of this species may be difficult, particularly because starry stonewort bulbils may persist and remain viable following treatments. We used a pilot treatment project for starry stonewort on Lake Koronis, the first lake in Minnesota found to have starry stonewort, to examine the response of starry stonewort to treatment by chelated copper algacides and mechanical harvesting. We implemented a

before-after-control-impact monitoring design in the field and laboratory tests of bulbil viability to evaluate management efficacy. Specifically, the objectives of our study were to evaluate the effects of mechanical and algaecide treatments on (1) starry stonewort biomass, (2) bulbil density, and (3) bulbil viability.

Study site

Lake Koronis is a 1201 ha lake on the border of Meeker and Stearns counties in central Minnesota that is part of the North Fork Crow River watershed (Fig. 1). The lake is classified as slightly eutrophic, with a Trophic State Index (Carlson 1977) of 54 (total phosphorus = 0.031 mg/L), and has a maximum depth of 40.2 m. Starry stonewort was discovered in Lake Koronis on 18 August 2015. The Minnesota Department of Natural Resources (MNDNR) conducted several surveys to delineate the extent of the infestation and found that, as of September 2015, it covered an area of ~100 ha.

Materials and methods

Treatments

In summer and fall of 2016, 3 infested areas of Lake Koronis were treated for starry stonewort control. These areas were designated for treatment by the Koronis Lake Association because they had large infestations of starry stonewort that interfered with navigation and recreational use. This ongoing treatment effort provided an opportunity to examine the subsequent response of starry stonewort. Hence, the 3 treated areas were the basis for our analysis and comprised the following: (1) a mechanically harvested channel (hereafter, mechanical area), (2) an area treated only with algaecide (algaecide area), and (3) an area that was first mechanically harvested and then treated with algaecide (mechanical + algaecide area; Fig. 1). To assess the efficacy of starry stonewort treatments, we also examined a 3.4 ha area invaded by starry stonewort that did not receive any treatment (untreated reference area) and compared this area to the treated areas. No algaecide or mechanical treatments were previously conducted in any of the treatment or reference areas that we evaluated.

Treatments were applied by independent contractors under the direction of the Koronis Lake Association. The mechanical area consisted of a 430 m

linear channel (approximately 10 m wide) extending from a public water access that was mechanically harvested on 10 August 2016 using an Eco Harvester (Lake Weeder Digest LLC, New Hope, MN; Fig. 1). The Eco Harvester is a single-manned aquatic plant harvesting vessel that uses a large rotating drum designed to uproot plants and feed them onto a conveyor that pulls plants out of the water. The mechanical + algaecide area consisted of a separate 1.5 ha starry stonewort infested area that was mechanically harvested between 11 August and 9 September 2016 to completely cover the area (Fig. 1). This area and an adjacent unharvested 1.1 ha area (Fig. 1) were treated on 21 September 2016 with a liquid chelated copper formulation (Cutrine-Plus; copper ethanolamine complex, mixed; liquid) at 54.5 L/ha. Copper concentrations were measured at 1 h following this application with a colorimeter (Series 1200, LaMotte Company, Chestertown, MD). Average copper concentrations were 0.37 ppm at the surface and 0.45 ppm at the lake bottom. A second application was conducted in both the algaecide and mechanical + algaecide areas on 11 October 2016 with a granular formulation of the same compound (Cutrine-Plus; copper ethanolamine complex, mixed; granular) at 41.2 kg/ha. This second, granular treatment, was performed with the goal of destroying starry stonewort bulbils and remaining biomass by targeting the lake bottom. Average copper concentrations at 1 h following the granular application were 0.16 ppm at the surface and 0.15 ppm at the lake bottom. Treatments previously performed by MNDNR near the public water access in 2015 and 2016 were located >50 m from the mechanical treatment area, ≥ 1 km from the algaecide and mechanical + algaecide treatment areas, and >600 m from the untreated reference area and are thus presumed to have had no influence on these treatment areas. The untreated reference area was located >500 m from the algaecide and mechanical + algaecide treatment areas (Fig. 1). PLM Lake and Land Management Corporation (Brainerd, MN) applied algaecide treatments, and Dockside Aquatic Services (Mendota Heights, MN) performed mechanical harvesting.

Biomass and bulbil sampling

In the summer and fall of 2016, we sampled starry stonewort biomass and bulbil density and collected bulbils for laboratory evaluations of viability. We measured starry stonewort biomass prior to any treatments

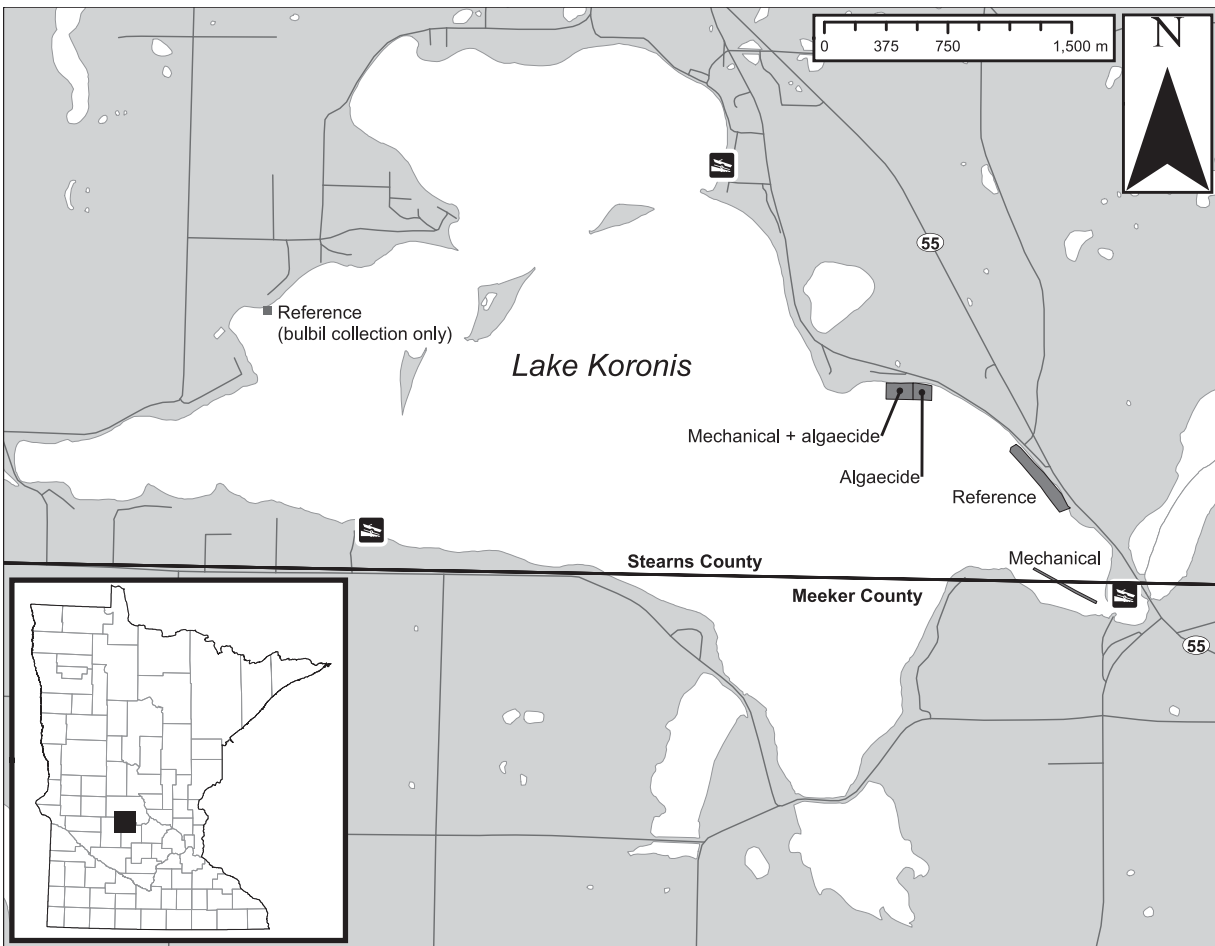


Figure 1. Map of starry stonewort (*Nitellopsis obtusa*) infested areas sampled July–October 2016 on Lake Koronis in Minnesota.

(19 Jul 2016 for treatment areas and 26 Jul 2016 for the untreated reference area) with grids of points distributed at 40 m spacing throughout each sampled area. Because our sampling comprised a uniform grid and treatment areas differed in size, we sampled different numbers of points in each area (mechanical, $n = 10$ points; algaecide, $n = 6$; mechanical + algaecide, $n = 8$; untreated reference, $n = 15$). At each point, we collected starry stonewort biomass by lowering a 7-tine rake (15 cm wide) attached to a telescoping pole to the lake bottom, making 3 rotations, and then pulling the rake and attached biomass to the surface (vertical rake method following Johnson and Newman 2011). We brought these samples to the lab, dried the samples to constant mass at room temperature in front of a fan, and weighed each sample. The vertical rake method can overestimate abundance for some aquatic plant species (Johnson and Newman 2011) and it is likely that we ensnared starry stonewort biomass from a greater area than that covered by the rake. Nonetheless, starry stonewort abundance values are comparable

among samples in our study. We repeated this sampling procedure on 13 September, 7 October, and 28 October 2016 (all areas were sampled on all dates, except for the mechanical area, which was not sampled on 7 Oct). We estimated bulbil density using the 7 October and 28 October 2016 starry stonewort biomass samples, for which we counted all bulbils in each sample following drying. The vertical rake method was not designed to sample bulbils and may overestimate or underestimate bulbil density due to a number of potential factors (algal biomass, phenology, etc.); however, no accepted method exists and the vertical rake method provided an efficient and consistent option.

On 28 October 2016, we collected bulbils for viability testing. Bulbils were collected from the algaecide, mechanical + algaecide, and untreated reference areas, as well as a second untreated reference location. We haphazardly collected bulbils throughout each sampling area using 2 spins of a 14-tine rake (33 cm wide). We sampled until we were confident that we had collected ≥ 100 bulbils from each area (5–15 rake

samples per area); however, bulbils were often small and obscured by plant material, so exact counts could not be determined in the field. Low bulbil density in the untreated reference area necessitated collection at a second untreated reference location ≥ 3.5 km from the algaecide and mechanical + algaecide areas (Fig. 1). We collected bulbils for viability testing at separate locations from sample points for bulbil density and biomass. We placed bulbils in plastic bags in a cooler for transport and returned the samples to the lab.

We counted bulbils in the lab and physically separated them from rhizoids. We examined bulbils for signs of sprouting, and did not observe sprouting in any of the bulbils used in our experiment. We placed bulbils from each sampling area into separate 11.4 L plastic tanks filled with 2 cm of topsoil overlain with fine-grained play sand to keep the sediment from entering the water column. We pressed each bulbil lightly onto the sediment surface and filled the tanks with dechlorinated water to a depth of 8 cm above the substrate. Water chemistry was within the range of northern tier lakes in which starry stonewort has been observed (Sleith et al. 2015, Midwood et al. 2016): pH = 8.65, conductivity = 253 $\mu\text{S}/\text{cm}$, alkalinity = 159 mg/L as CaCO_3 , hardness = 145.4 mg/L as CaCO_3 , total phosphorus = 0.042 mg/L, and total nitrogen = 0.34 mg/L. We maintained tanks under a 14 h/10 h light/dark schedule with multi-spectrum lights (RX30, Heliospectra AB; Göteborg, Sweden). We covered tanks with 50% black shade cloth to limit light intensity. Photosynthetically active radiation (PAR) at the water's surface, beneath the shade cloth, was 8 $\mu\text{mol}/\text{m}^2/\text{s}$. Mean temperature in the lab over the course of the experiment was 19.9 C, and mean water temperature in the tanks was 17.8 C. The total number of bulbils evaluated for each sampling area was: algaecide, $n = 363$ (2 tanks: $n = 100, 263$); mechanical + algaecide, $n = 223$ (2 tanks: $n = 100, 123$); and untreated reference, $n = 100$ (1 tank). One tank from each sampling area was planted on 28 October 2016 and one additional tank each for the algaecide and mechanical + algaecide areas were planted on 31 October 2016. The bulbil viability experiment began on 31 October 2016.

We checked bulbils for sprouting every 1–7 d for a total of 12 weeks (84 d). Bulbil viability was confirmed when we observed the emergence of a new shoot from a bulbil (i.e., sprouting). We used our own previous observations of bulbil sprouting and additionally followed Bharathan (1987) as a visual guide. Newly

sprouting material was often conspicuously green (i.e., photosynthetic), which made determination of sprouting unequivocal. Occasionally, bulbils sank into the substrate before sprouting; these sprouting events were identified when green shoots emerged above the substrate. Once we observed a bulbil sprouting, we removed that bulbil from the tank to avoid duplicate counting. On the final day of the experiment, along with our regular examination, we used a fine-mesh strainer to sift through the substrate to collect and examine any remaining bulbils. We were not able to recover all bulbils that we had initially placed in tanks. Based on our observations, unrecovered bulbils were likely to have broken apart or decomposed over the course of the experiment; thus, we considered unrecovered bulbils as not viable.

Data analysis

Biomass

We examined differences in starry stonewort biomass among treatments using a before-after-control-impact (BACI) framework (Green 1979, Stewart-Oaten et al. 1986). Under this framework, we sought to determine whether the change in starry stonewort biomass in response to treatments significantly differed from changes in starry stonewort biomass that occurred naturally, as measured in the untreated reference area. Because treatments were implemented as pilot tests, each treatment was conducted in a single location and was not randomly assigned to a location, nor replicated. In order to take advantage of the data from Lake Koronis and make inferences about each treatment, sample points within each area were considered individual replicates, though we acknowledge that these points are not true replicates (Hulbert 1984, Stewart-Oaten et al. 1986). First, we used the BACI approach to examine overall treatment outcomes across the entire study. For this analysis, we included biomass data from sampling dates prior to any treatments being performed and from the final sampling date, after all treatments had been performed (Table 1, Fig. 2). Then, to more closely inspect outcomes of individual treatments, we separately analyzed biomass data for (1) before and after the mechanical harvest, (2) before and after the first (liquid) algaecide treatment, and (3) before and after the second (granular) algaecide treatment (Table 1, Fig. 2). We examined treatments in this manner to isolate the effects of individual management actions

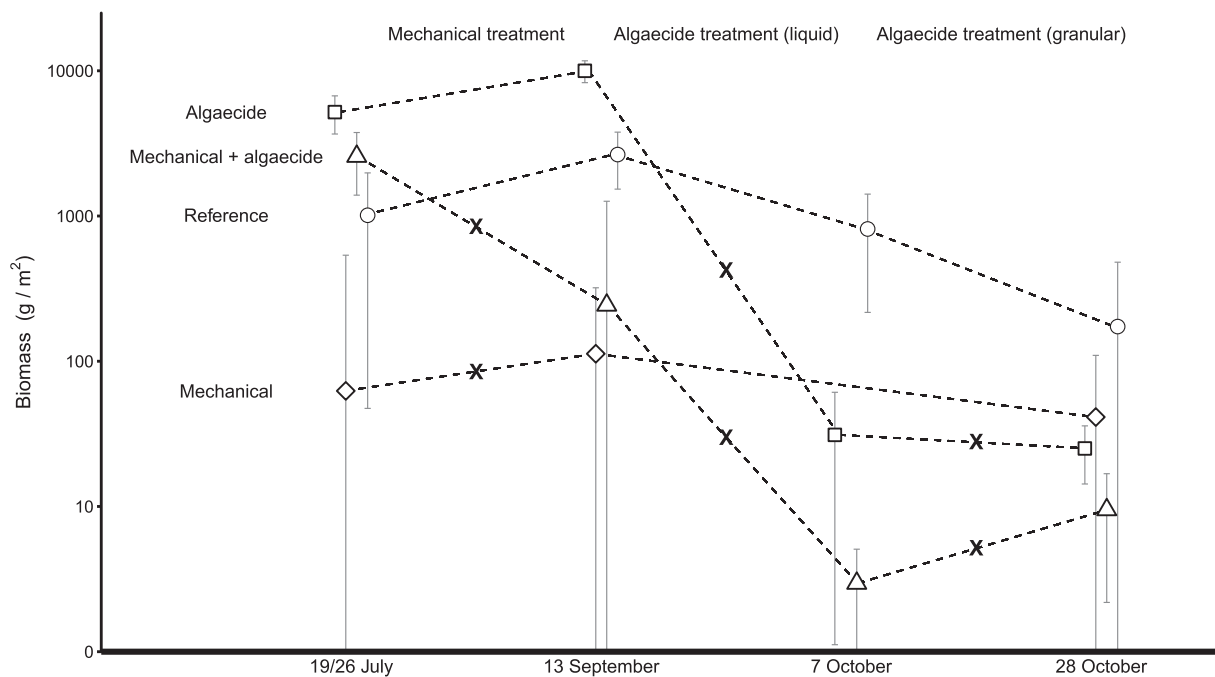


Figure 2. Starry stonewort (*Nitellopsis obtusa*) biomass July–October 2016. Biomass data are natural-log transformed. Each treatment area is represented with a different symbol. An X indicates that an area received the treatment designated at the top of the plot. Symbols and error bars are means ± 1 SE.

in areas where multiple treatments were applied. For each of these individual analyses, we only included the treatment areas targeted with a given treatment and compared them to the untreated reference area.

We analyzed biomass data using linear mixed effects (LME) models with the nonlinear mixed-effects (nlme) package in R, version 3.3 (Pinheiro et al. 2017, R Core Team 2017), with point-level starry stonewort biomass (g/m^2) as the response variable. Predictor variables included sampling period (i.e., before or after treatment), treatment type (up to 4 levels: mechanical, algaecide, mechanical + algaecide, and untreated reference), and a sampling period \times treatment interaction. In all models, we included sampling point as a random effect to account for repeated sampling of points over time (i.e., repeated measures). We natural-log transformed biomass data prior to analysis; this improved normality and resulted in greater homogeneity of variance among treatment types and sampling periods, as measured by the Fligner–Killeen test (Conover et al. 1981). Because there were some sampling points without starry stonewort, we added the minimum biomass value in the dataset ($2.26 \text{ g}/\text{m}^2$) to all observations prior to natural-log transformation. For the analysis of biomass before and after the mechanical harvest, we combined data

for the 2 mechanically harvested areas (mechanical and mechanical + algaecide). For the analysis of biomass before and after the first (liquid) algaecide treatment, we included data from the 2 sampling dates prior to algaecide treatment for the algaecide and untreated reference areas (Table 1, Fig. 2); hence, we included a random effect for sampling date in this model (within which the sampling point random effect was nested). Because we sampled an unbalanced number of points across sampling areas, we used Type III analysis of variance (ANOVA) to assess significance of our interaction term. A significant sampling period \times treatment type interaction would indicate differences among treatments in terms of changes in biomass over time. To determine whether changes in biomass in the treatment areas differed from those in the reference area (and differed among treatment areas), we calculated the least-squares means for each sampling period \times treatment type combination and used Tukey's honest significant differences (Tukey's HSD) tests of the least-squares means.

Bulbil density

We tested for differences in the change in bulbil density among treatments using the same BACI framework as for biomass. Because we first measured bulbil density

Table 1. Before-after-control-impact (BAC) analysis of starry stonewort (*Nitellopsis obtusa*) biomass during management from July to October 2016 on Lake Koronis in Minnesota. Each row shows the mean biomass (± 1 SE) of the treatment area before and after treatment (g/m^2), the change in biomass (g/m^2), the percent change in biomass, and the comparison of change in starry stonewort biomass in the treatment area versus the untreated reference area. *P* values with an asterisk (*) indicate significant biomass change ($P < 0.05$) based on Tukey's honest significant differences test.

Treatment examined	Sampling period		Treatment area	Biomass before (g/m^2)	Biomass after (g/m^2)	Biomass change (g/m^2)	Percent change	<i>P</i>
	Before	After						
All	19 Jul	28 Oct	Mechanical + algaecide	1144 (475)	157 (68)	-987	-86%	0.248
			Mechanical	5250 (1183)	16 (7)	-5234	-100%	0.004*
			Algaecide	6109 (1525)	30 (11)	-6079	-100%	0.013*
			Reference	3623 (967)	807 (307)	-2816	-78%	—
Mechanical	19 Jul	13 Sep	Mechanical (combined)	2969 (753)	977 (438)	-1992	-67%	0.155
			Reference	3623 (967)	4527 (1124)	+904	+25%	—
First (liquid) algaecide	13 Sep	7 Oct	Mechanical + algaecide	1598 (1020)	2 (2)	-1596	-100%	<0.001*
	19 Jul, 13 Sep	7 Oct	Algaecide	8385 (1291)	59 (30)	-8626	-99%	<0.001*
	26 Jul, 13 Sep	7 Oct	Reference	4075 (733)	1992 (599)	-2083	-51%	—
Second (granular) algaecide	7 Oct	28 Oct	Mechanical + algaecide	2 (2)	16 (7)	+14	+700%	0.018*
			Algaecide	59 (30)	30 (11)	-29	-49%	0.272
			Reference	1992 (599)	807 (307)	-1185	-59%	—

Initial biomass data for the untreated reference area were collected on 26 July. Mean biomass (± 1 SE) in the before sampling period of the first (liquid) algaecide treatment is the mean biomass from both dates prior to the first (liquid) algaecide treatment.

after the mechanical treatment and the first (liquid) algaecide treatment, we could not compare bulbil density before and after all treatments were performed. However, we were able to test for evidence of a change in bulbil density from before to after the second (granular) algaecide application (7 Oct and 28 Oct 2016, respectively). We used a LME model with bulbil density ($\text{bulbils}/\text{m}^2$) as the response variable and sampling period, treatment type (3 levels: algaecide, mechanical + algaecide, and untreated reference; the mechanical area was not included because it was not sampled on 7 Oct), and sampling period \times treatment interaction as predictor variables. We included sampling point as a random effect and used Type III ANOVA to assess significance of the interaction term, which would indicate differences among treatments in terms of change in bulbil density over time. We used Tukey's HSD of the least-squares means of each sampling period \times treatment type combination to determine whether changes in bulbil density in the treatment areas differed from those in the reference area (and differed among treatment areas).

Bulbil viability

Lastly, we assessed bulbil viability based on data from the laboratory sprouting experiment. Each bulbil had a response of either *sprouted* (sprouted by the end of the experiment) or *unsprouted* (did not sprout by the end of the experiment). We used the summed counts of sprouted and unsprouted bulbils from each treatment type as the response variable in a generalized linear model (GLM) with binomial errors. We used treatment type as a categorical predictor variable (3 levels: algaecide, mechanical + algaecide, and untreated reference). With this model, we tested for differences in the proportion of viable bulbils among treatment areas. Additionally, as a metric for starry stonewort recovery potential via bulbils, we calculated the product of the proportion of bulbils sprouted from each area and bulbil density on the final sampling date (28 Oct 2016); this metric has units of viable bulbils/ m^2 .

Results

Biomass

Change in starry stonewort biomass over the course of the study (from before to after all treatments) significantly differed by treatment type (sampling period \times

treatment type interaction: $P < 0.001$, $X^2 = 21.993$, $df = 3$). Both the algaecide treatment alone (algaecide area) and the combined mechanical + algaecide treatment resulted in significantly greater biomass reduction than observed in the untreated reference area (Table 1, Fig. 2). Mechanical treatment alone did not result in significantly greater reduction in biomass than the untreated reference area, though we note that biomass in the mechanical area was initially much lower than in the reference area (Table 1, Fig. 2). Among treatments, reduction in starry stonewort biomass was significantly greater in the algaecide area and the mechanical + algaecide area compared to the mechanical area ($P = 0.002$ and $P < 0.001$, respectively; Table 1, Fig. 2).

To examine the effects of individual management actions, we analyzed change in starry stonewort biomass separately for each treatment: (1) mechanical harvest, (2) first (liquid) algaecide treatment, and (3) second (granular) algaecide treatment. Change in starry stonewort biomass from before to after mechanical harvest did not significantly differ from the untreated reference area when data from both mechanically harvested areas were combined (mechanical and mechanical + algaecide; Table 1, Fig. 2). However, we did observe an overall reduction in biomass among these areas (Table 1) and a large biomass reduction in the mechanical + algaecide area (Fig. 2).

Change in starry stonewort biomass from before to after the first (liquid) algaecide treatment significantly differed by treatment type (sampling period \times treatment type interaction: $P < 0.001$, $X^2 = 23.134$, $df = 2$). Reduction in starry stonewort biomass was significantly greater in both the algaecide-only area and the mechanical + algaecide area, compared to the untreated reference area (Table 1, Fig. 2).

Lastly, change in starry stonewort biomass from before to after the second (granular) algaecide treatment significantly differed by treatment type (sampling period \times treatment type interaction: $P = 0.039$, $X^2 = 6.472$, $df = 2$), with significantly greater biomass reduction in the untreated reference area compared to the mechanical + algaecide area (Table 1, Fig. 2). Given that the granular algaecide treatment was intended to reduce biomass, this result was unexpected, but should be interpreted with caution given that remaining biomass in the treated areas was very low at this time—and thus our ability to detect changes in biomass concomitantly low. Change in starry stonewort biomass

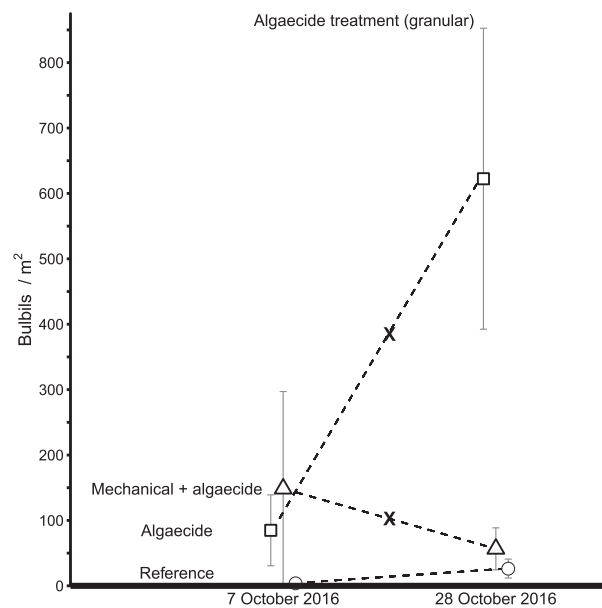


Figure 3. Starry stonewort (*Nitellopsis obtusa*) bulbil density before and after the second (granular) algaecide treatment. Each treatment area is represented with a different symbol. An X indicates that an area received the granular algaecide treatment. Symbols and error bars are means ± 1 SE.

did not significantly differ between the algaecide and untreated reference areas (Table 1, Fig. 2).

Bulbil density

For the analysis of bulbil density, there was a significant interaction between sampling period and treatment type ($P = 0.002$, $X^2 = 12.941$, $df = 2$), indicating that change in bulbil density differed among treatments from before to after the granular algaecide treatment. The area treated with algaecide alone had a significantly greater *increase* in bulbil density than the untreated reference and mechanical + algaecide areas ($P = 0.005$ and $P = 0.002$, respectively; Fig. 3). There was no difference in change in bulbil density between the mechanical + algaecide and untreated reference areas ($P = 0.458$; Fig. 3).

Bulbil viability

Bulbils from all sampling areas began sprouting within 7 d (Fig. 4). At the conclusion of the experiment (12 weeks), 85.7% of bulbils had sprouted from the algaecide area, 84.0% from the untreated reference area, and 70.4% from the mechanical + algaecide area. Bulbil sprouting did not significantly differ between the algaecide and untreated reference areas ($P = 0.675$,



Figure 4. Sprouted starry stonewort (*Nitellopsis obtusa*) bulbils from the bulbil viability experiment. Pictured bulbils are ~5 mm in diameter.

deviance = 20.493, $df = 2$; Fig. 5a). Bulbil sprouting was significantly lower in the mechanical + algaecide area than both the algaecide and untreated reference areas ($P < 0.001$ and $P = 0.011$, respectively; Fig. 5a).

Our metric for starry stonewort recovery potential (viable bulbils/m²) was 24 × greater in the algaecide area compared to the untreated reference area, 13.4 × greater in the algaecide area compared to the mechanical + algaecide area, and 1.8 × greater in the mechanical + algaecide area compared to the untreated reference area (Fig. 5b).

Discussion

To our knowledge, this is the first study to report outcomes of *in situ* algaecide and mechanical treatments aimed at controlling starry stonewort and reducing its capacity to regenerate via bulbils. Chelated copper algaecide treatment and mechanical + algaecide treatment substantially reduced starry stonewort biomass. However, treatments did not eliminate the capacity of starry stonewort to regenerate via bulbils. Algaecide treatments alone did not reduce starry stonewort bulbil viability, and regardless of treatment, ≥70% of bulbils sprouted in our experiment. Furthermore, bulbil density substantially and significantly increased in the area treated with algaecide alone, a pattern not observed in an untreated reference area or areas that were also mechanically harvested. There was also no evidence that the second (granular) algaecide treatment further reduced starry stonewort biomass, nor the capacity of starry stonewort to regenerate via bulbils. These findings suggest high potential of starry stonewort to

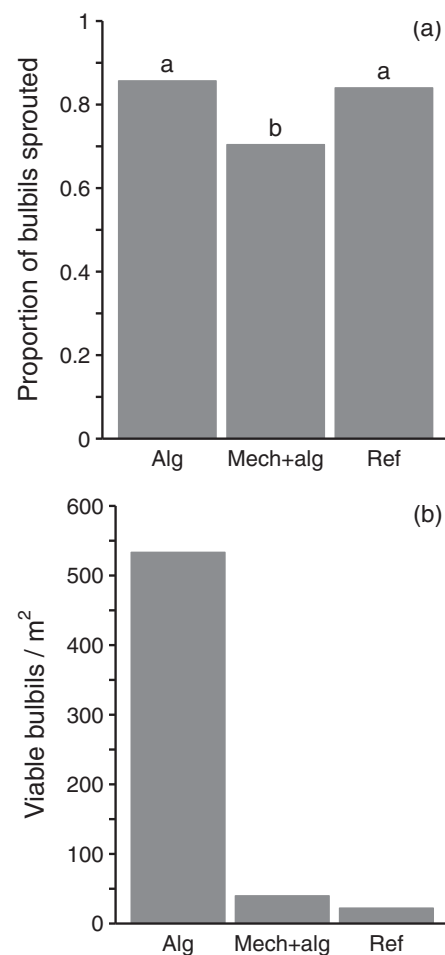


Figure 5. The proportion of starry stonewort (*Nitellopsis obtusa*) bulbils that sprouted from each treatment area (a), and starry stonewort recovery potential via bulbils following each treatment (b). Alg = algaecide treatment area, Mech + alg = mechanical + algaecide treatment area, Ref = untreated reference area. For proportion of bulbils sprouted (a), different letters indicate significant differences between treatment areas determined using a generalized linear model with binomial errors ($P < 0.05$).

regenerate and persist via bulbils following algaecide treatment. The viability and density of bulbils following algaecide treatment is concerning and has implications for starry stonewort control that necessitate further investigation.

An important caveat of our study is that it was conducted in one lake over a single growing season. Furthermore, treatments were applied as large-scale pilot tests of alternative management options rather than being implemented as part of a designed experiment. As a result, treatments were not randomly assigned to experimental units, treatments were not replicated, and our replicate samples were not entirely independent; thus, treatments could have been confounded by unaccounted-for differences in environmental conditions in each area. These factors can limit the conclusions drawn from BACI analyses like the ones employed on our study (Stewart-Oaten et al. 1986, Underwood 1994). However, our findings reflect the outcomes of actual, hectare-scale, management efforts and provide valuable insights for future management, but should be interpreted in light of their limitations and viewed as a case study that illustrates patterns for further investigation.

Copper compounds have been used to successfully manage algae for decades (Netherland 2014) and provided substantial reductions of starry stonewort biomass in the present study, but failed to reduce the viability of starry stonewort bulbils. Failure of algaecide treatments to reduce bulbil viability could be because chelated copper simply does not destroy bulbils or inhibit sprouting. However, we consider this unlikely given the observed efficacy of algaecide treatments for destroying aboveground biomass and unpublished reports of effective bulbil control by copper compounds in laboratory trials. It is more likely that bulbils were not exposed to sufficient concentrations of chelated copper for sufficient lengths of time due to the physical barrier created by overlying sediment. Sufficient exposure is likely difficult to achieve when targeting bulbils under realistic *in situ* conditions. For example, Kelly et al. (2012) found that chelated copper did not prevent germination of oospores of the Characeae genera *Nitella* and *Chara* that were beneath the substrate. Similar results have been found with other aquatic plant species; for example, contact herbicides had little impact on growth and production of underground propagules (tubers) of hydrilla (*Hydrilla verticillata*; Steward 1969, Joyce

et al. 1992). Following treatments with contact and systemic herbicides, underground propagules (turions) of curly-leaf pondweed (*Potamogeton crispus*) also remained viable at levels consistent with untreated lakes (Johnson et al. 2012). Thus, while our study is the first to document this pattern in starry stonewort, our findings are consistent with prior research on control of other submersed macrophytes that produce belowground asexual reproductive structures.

Chelated copper compounds that destroy bulbils or reduce bulbil viability *ex situ* may have limited effect on bulbils *in situ*. Laboratory studies evaluating effects of algaecides on starry stonewort bulbils should account for overlying sediment that protects bulbils in lakes (and realistic algaecide concentrations at or below the sediment) in order to better mimic field conditions. Depth profiles of starry stonewort bulbils beneath the sediment have not (to our knowledge) been reported, but *Chara* bulbils were at highest density 10–12 cm below the sediment surface and found at depths up to 29 cm (van den Berg 1999).

The potential for rapid, post-treatment recovery of starry stonewort by viable bulbils would be exacerbated by increased bulbil density. Hence, our finding that bulbil density significantly and substantially increased following granular algaecide application is concerning. We did not examine the causes of increased bulbil density in our study, but there are several explanations for our findings. For example, our results may be influenced by our ability to sample bulbils using the vertical rake method; this method was developed to sample aboveground biomass and may not accurately or precisely capture variation in bulbil density. Factors such as the amount of aboveground biomass, natural phenology (e.g., senescence and rhizoid formation), and overlying sediment may affect the number of bulbils collected in a vertical rake sample. Nonetheless, redistribution of resources to rooting and reproductive structures following injury or damage is a well-documented phenomenon in plants (McNaughton 1983, Trumble et al. 1993, Lennartsson et al. 1997, Hawkes and Sullivan 2001, Schwachtje et al. 2006) and a similar process may drive the shifts in bulbil density we observed. For example, compensatory root production following substantial loss of aboveground biomass (as we observed in our treatments) has been shown in the invasive aquatic plant, alligatorweed (*Alternanthera philoxeroides*; Schooler et al. 2007). Moreover, stimulation of growth and reproduction

following herbicide application—particularly at low doses—has been shown in numerous plant and alga species (Tiwari et al. 1981, Cedergreen et al. 2007, Cedergreen 2008, Calabrese and Blain 2009, Velini et al. 2010). Low algaecide exposure to starry stonewort rhizoids and bulbils beneath the sediment could have stimulated bulbil production through a direct growth-stimulation response. Alternatively, resources could have been reallocated through internal signaling to belowground biomass and reproduction following injury to aboveground structures. Chemical signaling following plant injury is well documented (Karban and Myers 1989, Walling 2000, Heil and Silva Bueno 2007) and, despite the lack of vasculature, intercellular transport of ions does occur in Characeae through plasmodesmata (Spanswick and Costerton 1967, Allen 1980, Franceschi et al. 1994). In addition, *Chara* spp. can take up and translocate nitrogen and phosphorus between aboveground and belowground structures (Littlefield and Forsberg 1965, Vermeer et al. 2003). Hence, nutrients, chemical compounds, and/or electrical signals stimulating bulbil growth may be able to travel through starry stonewort from exposed aboveground parts of the alga to belowground structures.

It is also possible that reductions in aboveground biomass could have created conditions that stimulated bulbil production from residual biomass. Removal of conspecific (same-species) neighboring plants can increase plant population growth rates by increasing propagule survival and growth (Gustafsson and Ehrlén 2003). Increased access to nutrients or light following aboveground biomass reduction may also have stimulated starry stonewort bulbil production. This effect has been shown in other Characeae; for example, increased light (UV-B radiation) from very low to ambient levels caused a substantial increase in the production of *Chara aspera* bulbils (de Bakker et al. 2001).

Mechanical harvesting was generally associated with better outcomes in terms of potential for reinvasion by bulbils. The mechanical harvest appeared to counter the increase in bulbil density observed in the algaecide-only treatment, as we observed no increase in bulbil density for the area that was mechanically harvested prior to algaecide treatments. These differences may be related to a large, rapid reduction in biomass in the algaecide-only area; prior to the initial algaecide treatment, biomass in the algaecide area was much greater (by >9 kg/m²) than biomass in the mechanical + algaecide area. This substantially greater biomass

was then rapidly reduced to levels similar to those in the mechanical + algaecide area (Table 1, Fig. 2). Such a large, rapid reduction in biomass may have stimulated bulbil production—by chemical signaling, reallocation of resources, and/or increased access to light or nutrients—to a greater degree in the algaecide area than in the mechanical + algaecide area, where comparable biomass had not accumulated. Furthermore, an increase in bulbil production in fall and winter, following senescence and biomass loss (Nichols et al. 1988), appears to be a natural component of starry stonewort phenology (McComas SR, Blue Water Science, Jun 2017, unpubl. data). Hence, sudden substantial losses of biomass associated with algaecide treatment may stimulate early onset of bulbil production. In other words, the large increase in bulbil density we observed in the algaecide area compared to the mechanical + algaecide area may have represented a hastening of an otherwise natural process rather than a net increase in bulbil production. Year-round sampling of starry stonewort biomass and bulbil density is needed to elucidate these patterns and clarify net effects of algaecide treatment on bulbil production.

An initial mechanical harvest to reduce biomass, followed by algaecide treatment of residual biomass, may be a means to reduce starry stonewort without triggering bulbil production. Our findings of lower bulbil density and reduced bulbil viability in the area that was initially mechanically harvested is encouraging for starry stonewort management (though high viability of starry stonewort bulbils remains a concern). Repeated mechanical and algaecide treatments may be a means to exhaust starry stonewort resources and bulbils over time. However, it should also be noted that harvesters can facilitate spread of aquatic invasive plants within water bodies (Anderson 2003, Hussner et al. 2017), and mechanical harvesting can be inefficient for small or low-density infestations. For small-scale starry stonewort infestations, manual hand-removal may be a better option. Continued hand-pulling of small starry stonewort infestations could reduce populations over time while engaging lake associations, volunteers, and other stakeholders in removal efforts.

Our study highlights the challenges associated with starry stonewort control efforts, particularly in large, dense infestations like the one in Lake Koronis. Therefore, measures should be taken to reduce starry stonewort spread in order to avoid dependence on difficult, costly, and resource-intensive management

efforts. Where large infestations have established, starry stonewort is likely to persist for the foreseeable future and realistic, sustainable goals (e.g., reducing abundance and minimizing risk of spread) should be pursued.

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ORCID

Wesley J. Glisson  <http://orcid.org/0000-0002-2540-3696>

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