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Note

Annual copper treatments provide within-year control of starry stonewort in Lake Koronis, Minnesota

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INTRODUCTION

Starry stonewort (*Nitellopsis obtusa*) is a nonnative freshwater green macroalga (charophyte) in North America that has become a major management concern (Geis et al. 1981, Sleith et al. 2015, Alix et al. 2017, Larkin et al. 2018). Since its first documentation in the St. Lawrence River in 1974 (Karol and Sleith 2017), starry stonewort has spread across inland lakes and major waterways primarily through human activity (e.g., recreational boating; Geis et al. 1981, Midwood et al. 2016, Brainard and Schulz 2017). This spread is concerning because starry stonewort can form dense beds (Brainard and Schulz 2017), which interfere with the recreational use of lakes, displace native aquatic plants, reduce fish habitat, and change invertebrate communities (Pullman and Crawford 2010, Sleith et al. 2015, Brainard and Schulz 2017, Larkin et al. 2018).

Different pesticide formulations may control starry stonewort, yet few field trials have corroborated these laboratory findings (Wersal 2022). Also, no field studies have evaluated long-term impacts to starry stonewort, native aquatic plants, and bulbils. Bulbils are small, asexually produced structures that recolonize aboveground growth after treatment. There is no known strategy to control starry stonewort bulbils (Carver et al. 2023) though mesocosm trials have shown success of mechanical "clipping" to be effective in reducing bulbil densities (Haram and Wersal 2023). To our knowledge, two publications have evaluated the effectiveness of small-scale herbicide treatments in the field, both in Lake Koronis, Minnesota. Glisson and colleagues (2018) found that chelated copper algaecide significantly reduced starry stonewort biomass within a single year in small plots, 1.5–3.4 ha plots (<2% of littoral area). Similarly, Carver (2023) and colleagues treated small, 6–40 ha plots (0.5–3.3% of littoral area) of starry stonewort with copper and diquat, finding that only copper reduced aboveground biomass and neither herbicide reduced bulbil densities. We build on this literature by evaluating a larger, two-year (2018–2019) algacidal treatment in Lake Koronis. Our study objectives were to 1) evaluate treatment effectiveness at reducing starry stonewort aboveground biomass, frequency of occurrence, and bulbil densities in the lake sediments and 2) evaluate nontarget treatment effects on native submerged aquatic plant taxa.

MATERIALS AND METHODS

Lake Koronis is a 1,201-ha lake in Stearns County, Minnesota. The maximum depth of Lake Koronis is 40 m, and 40% of the lake is littoral (476 ha). Starry stonewort was discovered in Lake Koronis in 2015 covering 21% (100 ha) of this littoral area. In response, the Koronis Lake Association and Minnesota Department of Natural Resources (MNDNR) treated and harvested small plots (<2% of littoral area) of starry stonewort from 2015 to 2017 with limited, temporary control success (Glisson et al. 2018). From 2018 to 2019, the Koronis Lake Association and MNDNR treated starry stonewort on a larger scale (up to 15% of lake littoral area, 71 ha) with liquid chelated copper (Cutrine-Plus®; copper ethanolamine complex, mixed)[1] at 1 mg L^{-1} twice per year in July and in August/September.

We evaluated the effects of the copper-based algaecide treatments on starry stonewort and native submerged aquatic plant frequency of occurrence by comparing changes in plant distribution within two large treatment plots and one reference plot. We estimated plant frequency of occurrence in each plot using the point intercept survey method (Madsen 1999). Sampling grids were based on proposed treatment regimens for each year with sampling points in depths up to 3.7 m, which includes habitat for all common submerged taxa in Lake Koronis. We collected plants from one rake toss at each sampling point spaced 70 m apart within each treatment plot (36 points in 64 ha in 2018 and 39 points in 21 ha in 2019 and 2020) and 50 m apart within the reference plot (23 points in 8 ha). We timed surveys before treatment ("Pre"), after the first treatment in July ("Post-1"), after the second treatment in August/September ("Post-2"),

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and one year after treatment the following July ("1-YAT" in 2019 and 2020; 1-YAT in 2018 is Pre in 2019). All plant taxa recovered on a rake were identified to species or genera following Crow and Hellquist (2000a, b) (sago pondweed, *Stuckenia pectinata*, was included with narrowleaf pondweeds, *Potamogeton* spp., due to inconsistent classification).

We estimated the effect of the treatments on plant frequency of occurrence using Bayesian binomial generalized linear models (GLMs). Bayesian GLMs are better suited for the quasi-completely separated plant presence data here (where plants were either fully present or absent in all sampled sites within some treatment groups) that can complicate model fitting with standard maximum likelihood estimation (package "arm" in R, Gelman 2020; R Development Core Team 2020). Plant presence on a sampling rake was modeled in a before-after-control-impact (BACI) design with a logit link as a function of plot ("Reference" = 0, "Treatment" = 1) and timing ("Pre" = 0 with dummy variables for "Post-1," "Post-2," and "1-YAT") with an interaction between plot and timing that estimates the treatment effects at each posttreatment time point (i.e., the relative between-plot difference in change from Pre to Post-1, Post-2, and 1-YAT). We also tested for a linear trend in starry stonewort frequency of occurrence using a model of starry stonewort presence as a function of continuous time (i.e., year + [day of year −1]/365), plot, and an interaction between continuous time and plot. Native submerged taxonomic richness (i.e., the count of native submerged taxa retrieved on a rake) at a point was modeled in a BACI design with negative binomially distributed error and a log link.

We measured starry stonewort biomass using the spinning rake method (Skogerboe et al. 2008) before treatment ("Pre") and after the second treatment ("Post-2") in the treatment and reference plots. To estimate treatment effects on biomass, we fit a BACI design model with Tweedie distributed error ($p = 1.8$, estimated using maximum likelihood estimation of average biomass or biomass $\sim$ 1) and a log link (package "tweedie" in R; Dunn 2022). We fit a second model to compare the change in biomass from pretreatment 2018 ("Pre") to pretreatment 2019 ("1-YAT") to test for a carryover effect of the 2018 treatment.

We estimated bulbil densities in the sediment using a Ponar grab (232 cm^2 sampling area) before the treatments and again after each treatment in the reference ($N = 12$) and treatment plots ($N = 22$). Models to estimate the treatment effect were structured with a BACI design and a negative binomial error distribution. We fit a second model to estimate a 1-YAT carryover effect from the 2018 treatment.

This before-after-control-impact ("BACI") design assumes that both the reference and treatment plots would have changed in the same way over time without treatment (e.g., both plots would have increased, decreased, or would not have changed). To test this assumption, we compared the reference plots in 2018 and 2019 and referenced historical starry stonewort trends and phenology in Lake Koronis to explore whether dynamics were shared between plots. For example, bulbil densities in the sediment are spatially patchy and unpredictable over time (Glisson et al. 2021), which could increase the probability that observed treatment effects are due to random dynamics and violate assumptions that underly BACI design.

RESULTS

Treatment did not reduce starry stonewort frequency of occurrence. Over the two years of treatment, starry stonewort frequency of occurrence decreased in both the treatment plot, from 97 to 72%, and the reference plot, from 91 to 83% (reference slope = −0.53, SE = 0.18, $P = 0.003$), but the decline did not differ between plots (time*treatment interaction coefficient = 0.0002, SE = 0.0008, $P = 0.82$). The year-to-year trends of this decline were similar between plots and years, with no significant treatment effects and no carryover effect for either treatment in the subsequent year (Figure 1a; Table 1).

Treatment reduced starry stonewort biomass within years relative to the reference plot. Starry stonewort biomass declined in both treatment and reference plots in both years but declined to a greater extent in the treatment plots in each year (Figure 1b). As a result, treatment effects on starry stonewort biomass were large and negative in both years (2018: −96%, coefficient = −3.15, SE = 0.83, $P < 0.001$; 2019: −99.97%, coefficient = −8.01, SE = 0.78, $P < 0.001$). Despite same-year effects on biomass, there was no 1-YAT effect for the 2018 treatment in 2019 (coefficient = 0.16, SE = 0.50, $P = 0.74$) (i.e., comparing "2018: Pre" to "2019: Pre" in Figure 1b).

The effect of treatment on starry stonewort bulbil densities in the sediment was inconclusive. Here the within-year change in the reference plot was not consistent between years (Figure 1c), increasing over the course of the year in 2018 and decreasing in 2019. A consistent annual phenology in the reference plot could have strengthened inference, implying that a similar phenology could have occurred in the treatment plot without treatment. In 2018, bulbil densities increased similarly in both plots, and there was no treatment effect (coefficient = −0.09, SE = 0.50, $P = 0.86$). In 2019, bulbil densities increased in the treatment plot and did not change in the reference plot, and there was a positive treatment effect (+234%, coefficient = 1.2, SE = 0.45, $P = 0.007$). There was a negative 1-YAT effect (−73%, coefficient = −1.29, SE = 0.41, $P = 0.001$) where densities increased in the reference plot and decreased in the treatment plot from Pre-2018 to Pre-2019. Treatment effects on bulbils should be interpreted with caution given the dynamic nature of bulbil densities in lake sediments.

Since native species richness appeared to be changing over longer timescales (and without a clear annual phenological pattern or long-term linear trend), it was not appropriate to make direct comparisons with BACI-designed linear models to estimate individual treatment effects. Instead, it appeared that native richness declined in 2018 and then increased by the end of 2019 in both plots, but minimum richness occurred one year earlier in the treatment plot (Figure 1d). Although frequency of occurrence was variable over the study period for native submerged taxa, there were no consistent negative treatment effects (Table 1). Many taxa increased from 2019 to 2020 (e.g., muskgrass, *Chara* spp.; coontail, *Ceratophyllum demersum*; sago pondweed and narrowleaf pondweeds; wild celery, *Vallisneria americana*; water stargrass, *Heteranthera dubia*; and Richardson's pondweed, *Potamogeton richardsonii*; Table 1) as reflected in the native richness results.

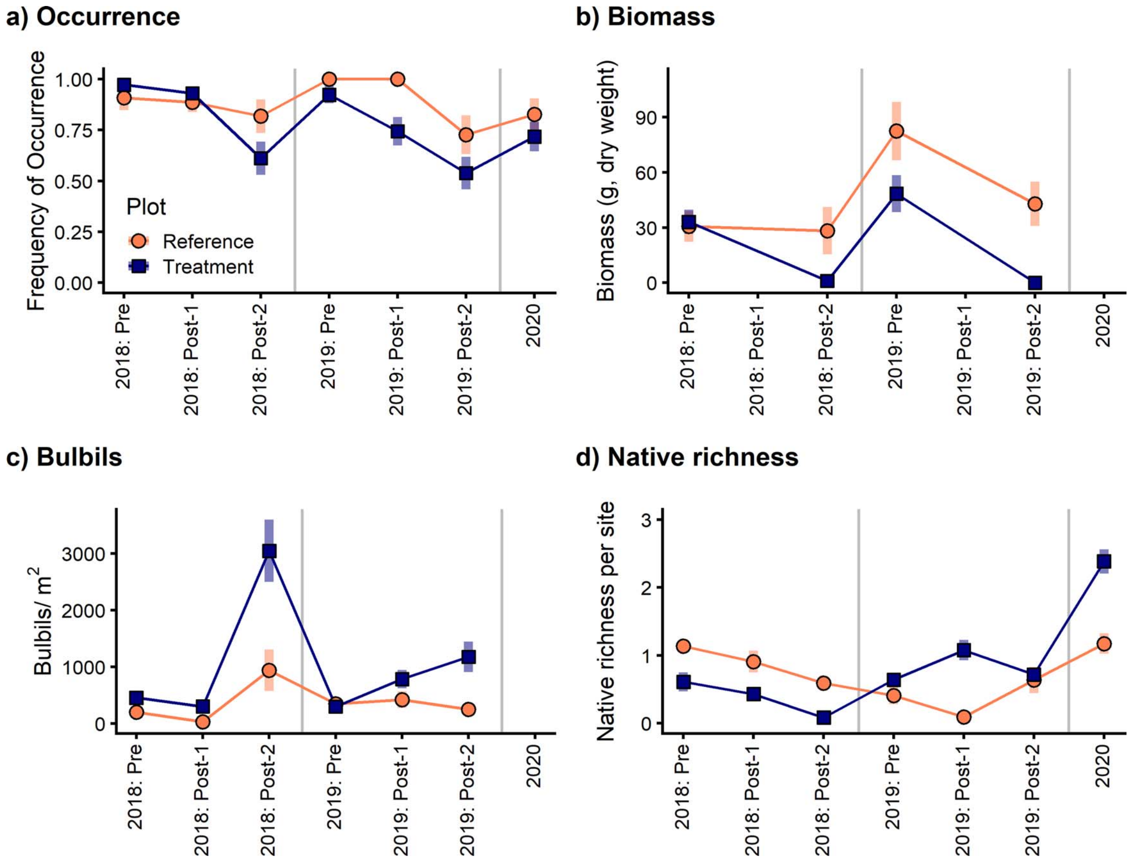

Figure 1. Starry stonewort frequency of occurrence (a), biomass (b), and bulbil densities in the lake sediment (c) and native richness per site (d) (average count of native submersed taxa on each rake toss) in treatment (navy lines and squares) and reference (orange lines and circles) plots from 2018 to 2020 (± 1 S.E.).

DISCUSSION

Copper algaecide treatment reduced starry stonewort biomass to a greater degree in the treatment plots, supporting past findings that treatment can provide same-year control. Moreover, the 2018 and 2019 treatments revealed that temporary biomass control may be possible without a reduction in the occurrence of native submerged plant taxa. However, as in past smaller treatments, treatment did not reduce the frequency of starry stonewort occurrence within or between years, likely because of limited effects of treatment on bulbil densities in the sediment (Figure 1c; Glisson et al. 2018, Carver et al. 2023).

Two key limitations to the study design arose from the differing needs of real-world management and ideal observational design. First, the 2019 treatment area was reduced to focus effort on nuisance management and for cost saving. Reducing the treatment area between years makes results from a direct comparison of the two years harder to interpret (e.g., testing for a carryover effect 1-YAT for the 2018 treatment). Second, statistically significant "treatment effect" coefficients captured both those differences in relative change between treatment and reference plots due to treatment as well as differences due to any other factors that could influence a differential response in the two plots (Shaffer and Johnson 2008). Regardless of these limitations, managers often target stronger and more obvious responses to treatment than what we estimate here.

The lack of long-term control with copper algaecide treatment may be due to starry stonewort's life history of producing bulbils that can reinitiate growth each summer from lake sediments (Midwood et al. 2016, Glisson et al. 2018, Pokrzywinski et al. 2021, Carver et al. 2023). Although our results were inconclusive, a lack of clear effectiveness does support the broader literature that bulbils are not effectively controlled by treatments that do not penetrate sediments. Incorporating starry stonewort phenology into management strategies could target treatments before bulbils grow prolifically (Glisson et al. 2021, Wersal and Carver 2022). Similarly, timing treatments prior to seed germination or after seed production of key native aquatic plant species (Madsen et al. 2016) could facilitate native aquatic plant growth and annual regeneration. This approach has been successful for treating nonnative curly-leaf pondweed (*Potamogeton crispus*) before turion formation (McComas and Stuckert 2000, Johnson et al. 2012) and flowering rush (*Butomus umbellatus*) management to reduce rhizome bud densities (Madsen et al. 2016). Both approaches can provide long-term control by reducing the propagules, but also providing selective control of the target species by properly timing the pesticide application. As suggested by Glisson and colleagues (2018), treatment or harvest prior to bulbil production over multiple, consecutive years may provide more effective control toward well-established populations

Table 1. Percent frequency of occurrence for submerged aquatic plants in Lake Koronis, Minnesota.

			2018			2019			2020
Common Name	Scientific Name	Plot	Pre	Post-1	Post-2	Pre	Post-1	Post-2	Pre
Starry stonewort	*Nitellopsis obtusa*	Ref.	91	89	83	100	100	73	83
		Treat.	97	93	61	92	74	54	72
Muskgrass	*Chara* spp.	Ref.	96	66	59	32	9	46	78
		Treat.	11	3	0	8	13	3	21
Coontail	*Ceratophyllum demersum*	Ref.	0	0	0	0	0	0	0
		Treat.	28	26	6	28	41	44	44
Sago pondweed/Narrowleaf pondweeds	*Stuckenia pectinata/Potamogeton* spp.	Ref.	5	7	0	9	0	0	22
		Treat.	14	10	3	13	21	5	87
Wild celery	*Vallisneria americana*	Ref.	5	2	0	0	0	5	0
		Treat.	3	1	0	13	15	13	8
Water stargrass	*Heteranthera dubia*	Ref.	0	0	0	0	0	0	0
		Treat.	0	0	0	3	10	5	5
Small pondweed	*Potamogeton pusillus*	Ref.	9	2	0	0	0	5	17
		Treat.	0	0	0	0	0	0	0
Richardson's pondweed	*Potamogeton richardsonii*	Ref.	0	2	0	0	0	5	0
		Treat.	0	0	0	0	3	3	8
White-stem pondweed	*Potamogeton praelongus*	Ref.	0	7	0	0	0	0	0
		Treat.	0	0	0	0	5	0	0
Curly-leaf pondweed	*Potamogeton crispus*	Ref.	0	0	0	0	0	0	0
		Treat.	0	0	3	0	0	0	8
Naiad	*Najas* spp.	Ref.	0	5	0	0	0	5	0
		Treat.	0	0	0	0	0	0	0
Flat-stem pondweed	*Potamogeton zosteriformis*	Ref.	0	0	0	0	0	0	0
		Treat.	0	0	0	0	0	0	8
Canadian waterweed	*Elodea canadensis*	Ref.	0	0	0	0	0	0	0
		Treat.	0	0	0	0	0	0	3

of starry stonewort. Mechanical harvest has been shown to reduce bulbil densities in greenhouse mesocosms (e.g., "clipping"; Haram and Wersal 2023). The late season growth and treatment of starry stonewort may limit damage to native aquatic plant species that begin growing in late spring and early summer (Glisson et al. 2021). In addition, timing starry stonewort treatment after native aquatic plant seed production may improve selectivity and promote native regeneration. Furthermore, secondary fall treatments consistent with late-season phenology of starry stonewort could be used for more selective treatments (Glisson et al. 2021) and possibly influence bulbil production, reduce overwintering biomass, and reduce energy storage (Haram and Wersal 2023). Further research is needed to determine the effectiveness of potential fall integrated pest management strategies (Haram and Wersal 2023).

The recovery of the native aquatic plant community in both the treatment and reference plots could be due to the release from competition as starry stonewort frequency of occurrence declined slightly in both plots and biomass was reduced after treatment. Also, native species richness in the treatment plot recovered one year earlier than the reference plot. It is possible that the within-year effects of treatment on starry stonewort biomass accelerated native aquatic plant recovery. Starry stonewort was first detected near the treatment plot and did not proliferate in the reference area until 2018 and 2019. So, although it is possible that treatment is accelerating recovery, it is also possible that native plant responses to starry stonewort proliferation and slight decline may be delayed in the reference plot. Different responses in native species richness could also be due to slightly different native aquatic plant communities in the treatment and reference plots (Table 1). Future monitoring should continue to evaluate the stability and extent of the native aquatic plant community recovery in Lake Koronis, especially for muskgrass, which is sensitive to both copper treatment and competition with starry stonewort. Also, additional treatment and reference plots from other managed and unmanaged lakes will be needed to separate starry stonewort and treatment effects from effects due to differing invasion timelines, natural variation, and aquatic plant community succession.

Although nontarget effects on native submerged plants were limited, effects of use over multiple years, copper accumulation in sediments, and impacts to other aquatic biota remain a concern with the use of copper as a pesticide (Hanson and Stefan 1984, Larkin et al. 2018). MNDNR monitored nontarget impacts to aquatic macroinvertebrates during both years in conjunction with this study. Copper algaecide treatments were associated with major reductions in Mollusca, several Trichoptera taxa, Amphipoda, and total invertebrates relative to little change in the reference plot (G. Montz et al., Minnesota Department of Natural Resources, pers. comm.). It is worth noting that there are no invertebrate records before starry stonewort invasion to evaluate potential effects of starry stonewort on invertebrates. Recent studies have shown that alternative, more selective pesticides can reduce starry stonewort biomass in the laboratory (Pokrzywinski et al. 2021, Wersal 2022), though these results were not successful in the field (Carver et al. 2023). Integrated pest management could be used to design a treatment regime around the life history of starry stonewort, native aquatic plants, and invertebrate communities (Glisson et al. 2021, Haram and Wersal 2023).

The widespread population of starry stonewort in Lake Koronis highlights the value of early detection and rapid response. Indeed, rapid response treatments have appeared to limit the within-lake spread of several new infestations in Minnesota lakes. Like the management of Lake Koronis, these efforts were facilitated by active partnerships between the MNDNR, local governments, and lake associations. Additional research should evaluate the effectiveness of early detection and rapid response in these lakes—possibly preventing less tractable challenges like those observed in Lake Koronis.

SOURCES OF MATERIALS

[1]Cutrine-Plus®, copper ethanolamine complex, mixed, SePRO Corp., 11550 North Meridian Street, Suite 600, Carmel, IN 46032.

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