2022

Environmental Effects on Biomass Allocation and Small Plot Evaluations of Aquatic Pesticides for Control of Nitellopsis obtusa (Starry Stonewort) Collected from Lake Koronis in Stearns County, Minnesota

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Environmental Effects on Biomass Allocation and Small Plot Evaluations of Aquatic Pesticides for Control of *Nitellopsis obtusa* (Starry Stonewort) Collected from Lake Koronis in Stearns County, Minnesota

By

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A Thesis Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

In

Biology

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Chapter I

Background of *Nitellopsis obtusa* (Starry Stonewort)

Species Description

Nitellopsis obtusa (starry stonewort) is a green macro alga native to Eurasia in the family Characeae (Groves 1919). *Nitellopsis obtusa* can grow from the sediment to 30-120 cm in the water column with a slender to robust axis approximately 0.7-2mm in diameter, depending on phenology and growing conditions (Larkin et al. 2018). Branchlets form from the main stem at the nodes in whorls of 5-8 branchlets with each branchlet consisting of 2-3 segments with a total length up to 9 cm. *Nitellopsis obtusa* is dioecious and on the nodes of the branchlets gametangia appear in pairs or in some cases solitarily. The antheridia are 0.8-1.5 mm in diameter and orange to bright red in color, while the oogonia are bright red to light green and almost spherical in shape (Groves, 1919, Boissezon et al. 2017, Larkin et al. 2018). The oogonia have yet to be observed in North America (Sleith et al. 2015, Larkin et al. 2018).

Nitellopsis obtusa also forms star-shaped bulbils, which the common name is derived from, as a way of asexual reproduction as well as a propagule for spatial and temporal dispersion. White bulbils form beneath the sediment along the nodes of the rhizoid, and green bulbils form along the main axes and branchlet nodes (Bharathan 1987). While *N. obtusa* is able to reproduce both vegetatively and sexually it appears to undergo vegetative reproduction more frequently in both its native and invasive ranges (Larkin et al. 2018). Although a growing season with warm and sunny conditions could stimulate sexual reproduction (Boissezon et al. 2017), this has not been observed in North American populations (Sleith et al. 2015, Larkin et al. 2018). There are a few hypotheses surrounding why this is the case including poor environmental conditions preventing oogonia formation, only antheridia plants survived introduction to North

America, and distinct ecotypes maybe suppressing reproductive structures (Larkin et al. 2018). However sexual reproduction could be a strategy to ensure the production of long-lived, resistant propagules (Boissezon 2014). Gyrogonites, the oospores found in sediments, can lie in a dormant state and persist for extended periods of time within lake sediments (Bonis and Grillas 2002). These gyrogonites can be ingested by waterfowl and carried long distances to new bodies of water to start new populations (Bonis and Grillas 2002).

Habitat

Nitellopsis obtusa has been seen in eutrophic lakes but are most frequently observed in oligotrophic and mesotrophic conditions (Ozimek and Kowalczewski 1984, Hargeby 1990, Blindow 1992, Królikowska 1997, Bennett et al. 2001, Stewart 2004, Rey-Boissezon and Joye 2015, Schneider et al. 2015). In its native range it is found in areas of low light intensity, typically 4 to 8m depths, however it can grow in as little as 1m and up to 30m depths (Olsen 1944). The areas of lakes they are typically found in are protected from strong currents, have high calcium levels, high conductivity, a neutral to basic pH, and a low to moderate coverage (Zaneveld 1940, Olsen 1944, Simons and Nat 1996, Królikowska 1997, Soulié-Märsche et al. 2002, Boissezon 2014, Auderset Joye and Rey-Boissezon 2015, Rey-Boissezon and Joye 2015). *Nitellopsis obtusa* is tolerant of saline conditions and can survive up to 17 ppt for up to a week (Simons and Nat 1996, Winter et al. 1999). Even though it can tolerate shifts in salinity, it is unable to survive and reproduce in water bodies with salinity consistently higher than 5 PSU.

It can grow in large monospecific mats varying in density generally in the preferred conditions mentioned above (Olsen 1944, Stewart 2004, Rey-Boissezon and Joye 2015). While these mats are usually dominated by *N. obtusa* there have been documented cases of co-occurrence with a variety of species (Olsen 1944, Best 1987, Blindow 1992, Kato et al. 2005, Hilt et al. 2010). In its nonnative range it has been found in a variety of habitats ranging from inland ponds to bays in the Laurentian Great Lakes, as well as a variety of substrates from a rocky/sandy bottom to a muddy bottom (Sleith et al. 2015). In its invaded range, *Nitellopsis obtusa* grows in similar calcareous, neutral to basic pH conditions as its native range, however it is found more in mesotrophic to eutrophic systems (Larkin et al. 2018). Similar to the native populations, the invasive populations grow in large monospecific mats with little co-occurrence of other macrophytes (Larkin et al. 2018). In Prequi'ile Bay, Lake Ontario, dock density, low wave action, and proximity to marinas were good predictors of *N. obtusa* presence (Midwood et al. 2016).

Distribution and Invasion

The native range of *N. obtusa* is a disjointed distribution extending from Western Europe to Japan and as far south as Myanmar (Soulié-Märsche et al. 2002). Its invaded range of North America began around 1978 in the St. Lawrence River where it was seen to be growing throughout the littoral zone, but with the greatest abundance at 3 to 5 m (Geis et al. 1981). Geis et al. (1981) suggests that *N. obtusa* first arrived in ship ballast water. It has since spread through New York primarily to waters near Lake Ontario and the St. Lawrence River (Sleith et al. 2015). In 1983 it was found in the St. Clair-Detroit River system at depths of 0.9 to 3.4 m and current velocities of 0 to 51.8 cm/s (Schloesser et al. 1986). As of May 2017, over half of the counties in the southern Lower Peninsula of Michigan have populations of *N. obtusa*, as well as one unconfirmed sighting in Millecoquins Lake in the Upper Peninsula during 2014 (Midwest Invasive Species Information Network (MISIN) 2017). The current invasive range includes the St. Lawrence River, the St. Clair-Detroit River system, Lake Ontario, Lake Erie, Lake Huron, Michigan's Lower Peninsula, New York, Vermont, Pennsylvania, northern Indiana, Wisconsin, and as of 2015 Minnesota (Mills et al. 1993, Sleith et al. 2015, Midwood et al. 2016, MISIN 2017). According to the Minnesota DNR the first occurrence of *N. obtusa* in Minnesota was in Lake Koronis in 2015 and has spread to 20 other lakes. The likely cause of overland dispersal in the U.S. are boats and boating equipment transporting the bulbils and vegetative fragments of *N. obtusa* (Larkin et al. 2018). Sleith et al. (2015) surveyed 20 lakes lacking boat launches within heavily *N. obtusa* invaded areas and did not detect presence of the species.

Ecological Impacts

Nitellopsis obtusa has been seen to lower macrophyte species richness in multiple lakes as its biomass increased at various depths (Brainard and Schulz 2017, Harrow-Lyle and Kirkwood 2022). Heavy stands of starry stonewort may limit fish spawning habitat as well as reduce the long-term viability of benthic organisms via oxygen depletion during senescence (Brainard and Schulz 2017).

Research Needs

There is insufficient data on the phenology of *N. obtusa* in both its native and invaded ranges as well as the ecological niches it fills in the invaded range. A study by Glisson et al. (2022) found that starry stonewort has a late season growth pattern however the environmental factors associated with these patterns are not yet understood. A better understanding of bulbil longevity and desiccation tolerance is needed to assess over-land dispersal. The efficacy of chemical treatments and other control methods has been researched by multiple groups with results showing moderate control of starry stonewort (Glisson et al. 2018, Pokrzywinski et al. 2021, Wersal 2022). More research on control methods and phenology of starry stonewort is needed to form more concise management recommendations. So, in order to determine resource allocation patterns in *N. obtusa* and how it responds to management this project covers:

Phenology

1) Investigate the seasonal phenology of *N. obtusa* over two growing seasons to determine statistical relationships between environmental factors and growth.

H_1: Aboveground biomass of *Nitellopsis obtusa* in Lake Koronis will be greatest in late summer (August) due to increased temperatures and improved light availability.

H_2: Subterranean bulbil production in Lake Koronis will be greatest in late summer (August) to early fall (September)

Management

Lake Koronis has an operational control program designed to reduce the abundance of *Nitellopsis obtusa* as part of this program the areas that do not undergo management and serve as a non-treated reference population. Therefore, I will test the hypothesis of what happened if managers did nothing.

H_3: Biomass and bulbil densities will be higher in areas that do not undergo routine management.

H_4: The herbicide diquat has activity on *N. obtusa* and applications under normal management scenarios will result in less biomass.

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Chapter II

Phenology and Biomass Allocation of *Nitellopsis obtusa* Collected from Lake Koronis in Stearns County, Minnesota

Introduction

Phenology is defined as the study of the seasonal timing of critical life stages in plants, whereby the allocation of biomass and other resources such as carbohydrates are fundamental aspects during these life stages (Wersal and Madsen 2018). In most cases, aquatic plants will display distinct seasonal patterns in biomass and carbohydrate allocation, wherein storage peaks, and then is depleted after plant growth has occurred (Madsen 1991). Phenological studies offer baseline data on the growth patterns of target species which is essential to creating management strategies for invasive species by identifying the optimum times in the plant's life cycle and implementing control methods during that time (Madsen 1991, Madsen 1993, Wersal et al. 2011, Wersal et al. 2013, Wersal and Madsen 2018).

Nitellopsis obtusa (Desv.) J. Groves (starry stonewort) is a green macro alga native to Eurasia in the family Characeae (Groves 1919). Starry stonewort can grow from the sediment to 30-120 cm in the water column with a slender to robust axis approximately 0.7-2mm in diameter, depending on growing conditions (Larkin et al. 2018). Branchlets form from the main stem at the nodes in whorls of 5-8 branchlets with each branchlet consisting of 2-3 segments with a total length up to 9 cm. Starry stonewort is dioecious and on the nodes of the branchlets gametangia appear in pairs or in some cases solitarily. The antheridia are 0.8-1.5 mm in diameter and orange to bright red in color, while the oogonia are bright red to light green and almost spherical in shape (Groves, 1919, Boissezon et al. 2017, Larkin et al. 2018). The oogonia have yet to be observed in North America (Sleith et al. 2015, Larkin et al. 2018).

Starry stonewort forms star-shaped bulbils, from which the common name is derived, as a way of asexual reproduction as well as an organ for spatial and temporal dispersion (Bharathan 1987). White bulbils form beneath the sediment along the nodes of the rhizoid, and green bulbils form along the main axes and branchlet nodes (Bharathan 1987). While starry stonewort is able to reproduce both vegetatively and sexually it appears to undergo vegetative reproduction more frequently in both its native and invasive ranges (Larkin et al. 2018). In its invaded range, starry stonewort grows in similar calcareous, neutral to basic pH conditions as its native range, however it is found more in mesotrophic to eutrophic systems (Larkin et al. 2018). These invasive populations grow in large monospecific mats with little co-occurrence of other macrophytes (Larkin et al. 2018).

Invasion of North America began around 1978 in the St. Lawrence River where starry stonewort was seen to be growing throughout the littoral zone, but with the greatest abundance at 3 to 5 m. It has been suggested that starry stonewort first arrived in ship ballast water (Geis et al. 1981). It has since spread through New York primarily to waters near Lake Ontario and the St. Lawrence River (Sleith et al. 2015). In 1983 it was found in the St. Clair-Detroit River system at depths of 0.9 to 3.4 m and current velocities of 0 to 51.8 cm s^{-1} (Schloesser et al. 1986). The current invaded range includes The St. Lawrence River, the St. Clair-Detroit River system, Lake Ontario, Lake Erie, Lake Huron, Michigan's Lower Peninsula, New York, Vermont, Pennsylvania, northern Indiana, Wisconsin, and as of 2015 Minnesota (Mills et al. 1993, Sleith et al. 2015, Midwood et al. 2016, MISIN 2017).

The first occurrence of starry stonewort in Minnesota was in Lake Koronis in 2015 and has since spread to 20 other lakes and the Mississippi River. The likely cause of overland dispersal in the U.S. are boats and boating equipment transporting the bulbils and vegetative fragments of starry stonewort (Larkin et al. 2018). Lakes lacking boat launches were surveyed within heavily starry stonewort infested areas and did not detect presence of the species (Sleith et al. 2015). The life history of starry stonewort in MN was first documented by Glisson et al (2022); however, a thorough assessment of phenology has not been done. Therefore, the objectives of this project were to 1) document the seasonal life history of starry stonewort growing in Lake Koronis, and 2) determine relationships between starry stonewort growth and seasonal environmental factors.

Materials and Methods

Study Location

The study was conducted on Lake Koronis, near Paynesville, MN (45.3298° N, 94.6986° W) during the growing seasons of 2020 and 2021. Lake Koronis is a 1,201-hectare lake with a maximum depth of 40 meters. Within the 476-hectare littoral zone macrophytes such as *Vallisneria americana* Michx., *Ceratophyllum demersum* L., *Lemna minor* L., and *Nymphaea odorata* Aiton can be found. Four plots were chosen as sampling locations on Lake Koronis based on moderate to high *N. obtusa* densities, distance from management plots, and water depth that was conducive for sampling (Figure 1) Plots 1, 3,5, and 6 were sampled while plots 2 and 4 were used as backups in case the other plots were unavailable.

Biomass Sampling

This study followed the phenology sampling methodology as outlined by Wersal and Madsen 2018. Thirty biomass samples were collected using a 0.018 m^2 PVC coring device (Madsen et al. 2007) every three weeks beginning in late April and continuing to ice cover during the growing seasons of 2020 and 2021. Samples were taken from the 4 corners of a boat and then the boat was allowed to drift to a new location within a plot, where 4 more samples were collected; this sampling methodology was repeated until 30 samples were harvested in each plot. Collected biomass samples were rinsed in a 19 L bucket with a 4 mm^2 mesh bottom to remove sediment from the plants and to retain bulbils. Once rinsed, samples were placed in a 3.8 L zip top bag and stored in a cooler, on ice, for transport back to the lab. At the lab samples were washed and separated into aboveground biomass, belowground biomass (rhizoids), and bulbils. Biomass samples were placed in paper bags and placed in a constant temperature oven at 48°C for at least 48 hours to dry completely. After the samples were dry, they were weighed to determine biomass (g DW m^{-2}) based on the area of the coring device. Bulbils were counted, and density determined for each sampling time across both seasons.

Environmental Sampling

Environmental data was recorded once every 3 weeks during biomass harvesting to determine relationships between environmental factors and *N. obtusa* growth. A LI-COR LI-1500 light meter was used to collect both ambient and submersed light in 0.5 m intervals from the water surface to the bottom sediment. The light profile was used to calculate light transmittance, with light transmittance being the percentage of light in the water column of the light available above the surface. Water temperature (°C) and pH measurements were made using a Hydrolab HL7 Sonde at a similar depth profile as LI-COR measurements. Additionally, temperature sensors (HOBO Pendants, Onset Computer Corporation), were deployed at three intervals (bottom, middle, and top of the water column) in the center of each plot by anchoring a large buoy to the bottom of the lake. The pendant sensors were affixed to the anchor chain of each buoy in each plot. The pendant sensors recorded temperature (°C) in one-hour intervals throughout both growing seasons. The buoys and pendant sensors were deployed from April to October per the permit issued by the Stearns County Sheriff's Department.

Data Analysis

Monthly averages for biomass, bulbil density, and environmental data were computed for each site and analyzed together. Data were analyzed by fitting mixed models using the mixed procedure method in SAS (Litell et al. 1996) to determine relationships between environmental factors and *N. obtusa* biomass and bulbil density. Above and belowground biomass and bulbil densities were included as the dependent variables. Water temperature, pH, submersed light, and light transmittance, and year were included as the independent variables in all models. Site was included as a random effect in the model to account for its influence on results. All terms included in the analyses were linear. Data are reported as means (± 1 SE) and analyses were conducted at an $\alpha \leq 0.05$ significance level and displayed graphically to show trends and relationships (Wersal et al. 2006, Wersal et al. 2011, Wersal et al. 2013).

Results

Environmental factors

Water temperature was highest between June and August in both years and steadily declined until sampling ended in November of each year (Figure 2). In 2020 pH had peaked at 8.6 in July and again in September with a yearly range of 8.0 to 8.6 (Figure 2). Meanwhile, in 2021 pH peaked at 8.6 in late June and slowly dropped to 5.8 in November with a yearly range of 5.6 to 8.6 (Figure 2). Light transmittance ranged between 15.8 to 45.8% indicating enough light was reaching the bottom sediments to sustain growth of submersed plants (Figure 2).

Seasonal Biomass

Both total and aboveground biomass were higher in 2020 than in 2021 with maximum total biomass being 230 g m^{-2} and 157 g m^{-2} respectively. Maximum aboveground biomass was 224 g m^{-2} and 196 g m^{-2}, respectively for 2020 and 2021 (Figures 3). Rhizoid biomass was lower in 2020 than in 2021 with a maximum of 0.50 g m^{-2} in 2020 and 0.71 g m^{-2} in 2021. Maximum biomass and bulbil density was achieved in early to mid-autumn in both years (Figures 3 and 4). Peak bulbil biomass was lower in 2020 than in 2021 at 4.6 g m^{-2} and 14.7 g m^{-2}, respectively (Figure 3). Bulbil density was lower in 2020 than in 2021 with a peak average density of 1,229 m^2 in 2020 and 5,211 m^2 in 2021 (Figure 4). Bulbil densities ranged from 0 to 156,944 m^{-2} with an average annual density of 1,537 m^{-2} (Figure 4).

There were multiple significant relationships between the seasonal biomass of starry stonewort and the environmental factors measured (Table 1). Total biomass was positively related to temperature ($p<0.01$) and light transmittance ($p<0.01$). Total biomass had significant negative relationships with pH ($p<0.01$). Aboveground biomass had significant positive relationships to temperature ($p<0.01$) and light transmittance ($p<0.01$). The significant negative relationships for aboveground biomass were pH ($p<0.01$). There were no significant relationships between rhizoid biomass and any of the environmental factors tested, in fact rhizoid biomass comprised less than 0.9% of total biomass on average. Bulbil biomass was negatively related to pH ($p<0.01$). Bulbil density was also negatively influenced by pH ($p<0.01$). However, bulbil density was positively related to light transmittance ($p=0.04$).

Discussion

This study corroborates similar findings stating that starry stonewort has late season growth patterns in its invaded range, particularly, low biomass early in the year, an increase through the summer, and a peak in the fall (Geis et al. 1981, Schloesser et al. 1986, Nichols et al.

1988, Glisson et al. 2022). This study did not continue to sample through the winter underneath the ice, however green tissues has been observed underneath the ice in Minnesota lakes (Glisson et al. 2022). Data from this study indicated that the growth cycle of starry stonewort is dependent on water temperature and light transmittance; both factors affecting when peak biomass occurs and when senescence begins. Biomass peaked when temperature ranged from 7.4-8.8 °C in 2020 and 12.5-15.2 °C in 2021. Unlike most macrophytes, the growth of charophytes like starry stonewort has been seen to be affected by pH and even shows preference to growing in areas of moderate to high pH (Pełechaty et al. 1990, Simons and Nat 1996).

Bulbil biomass and density followed a seasonal pattern of abundant bulbils early in the year with declines in early summer as bulbils were sprouting and resources allocated to aboveground growth (Figure 4). Beginning in July of each year bulbil densities began to increase, and bulbils were being produced until the end of the sampling period in autumn. In the 2021 season there was a much higher increase in bulbil production from the 2020 season. Bulbils are able to persist in the sediment for multiple years and as such could replenish the current population present for multiple years (Glisson et al. 2022). Given the littoral zone in Lake Koronis infested with starry stonewort is 324 ha there is an average of 4,979,880,000 bulbils produced each year with a possible maximum of 508,498,560,000 bulbils produced. The amount of bulbils produced each year and those already in the sediment will take several years of management to reduce the propagule bank to an unsustainable level or at the very least be able to reduce management efforts to a minimum.

Starry stonewort has a late season peak in biomass implying a potential niche this species could occupy; allowing coexistence and limited competition with native species (Glisson et al. 2022). However, other species such as *Hydrilla verticillata* (L.f.) Royle have similar late season peaks in biomass and turion production but is an aggressive invasive species (Owens and Madsen 1998). Bulbil production provides the biggest obstacle for management as current chemical treatments effective on aboveground biomass have little to no effect on bulbils already in the sediment, and bulbils provide the ability for rapid regrowth after treatment or the year following (Glisson et al. 2018).

The current study indicates that management activities in May and early June would target less aboveground biomass and times when bulbil production is limited. Future research should focus on species specific treatments of starry stonewort that affect bulbil biomass or bulbil production as well as the effects starry stonewort has on native plant populations and whether or not it fills a previously empty niche.

Acknowledgements

This project is funded in part by the State of Minnesota through its Department of Natural Resources and in part by a federal grant to the Minnesota DNR from CFDA #15.662 (Aquatic Invasive Species Grants to Great Lakes States) under award F17AP00352. We would like to thank Alyssa Haram, Allison Squires, Alyssa Anderson, Amber Fistler, Maxwell Gebhart, Sara Ademi, Frank Rogers, and Vincent McKnight for their tireless efforts in data collection in both the lab and field. Additional logistical support was provided by Koronis Lake Association, Kevin Farnum, and Blue Water Science.

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Table 1. Solutions for fixed effects of the mixed procedures model analyzing *Nitellopsis obtusa* biomass and environmental factors.

Tissue	Effect	t-value	p-value
Total Biomass	Temperature	6.33	<0.01
	pH	-3.95	<0.01
	Light Transmittance	3.12	<0.01
Aboveground Biomass	Temperature	6.56	<0.01
	pH	-3.54	<0.01
	Light Transmittance	3.05	<0.01
Rhizoid Biomass	Temperature	-1.94	0.05
	pH	-1.05	0.29
	Light Transmittance	1.50	0.13
Bulbil Biomass	Temperature	-0.46	0.64
	pH	-3.31	<0.01
	Light Transmittance	1.40	0.16
Bulbil Density	Temperature	-0.39	0.69
	pH	-4.18	<0.01
	Light Transmittance	1.99	0.04

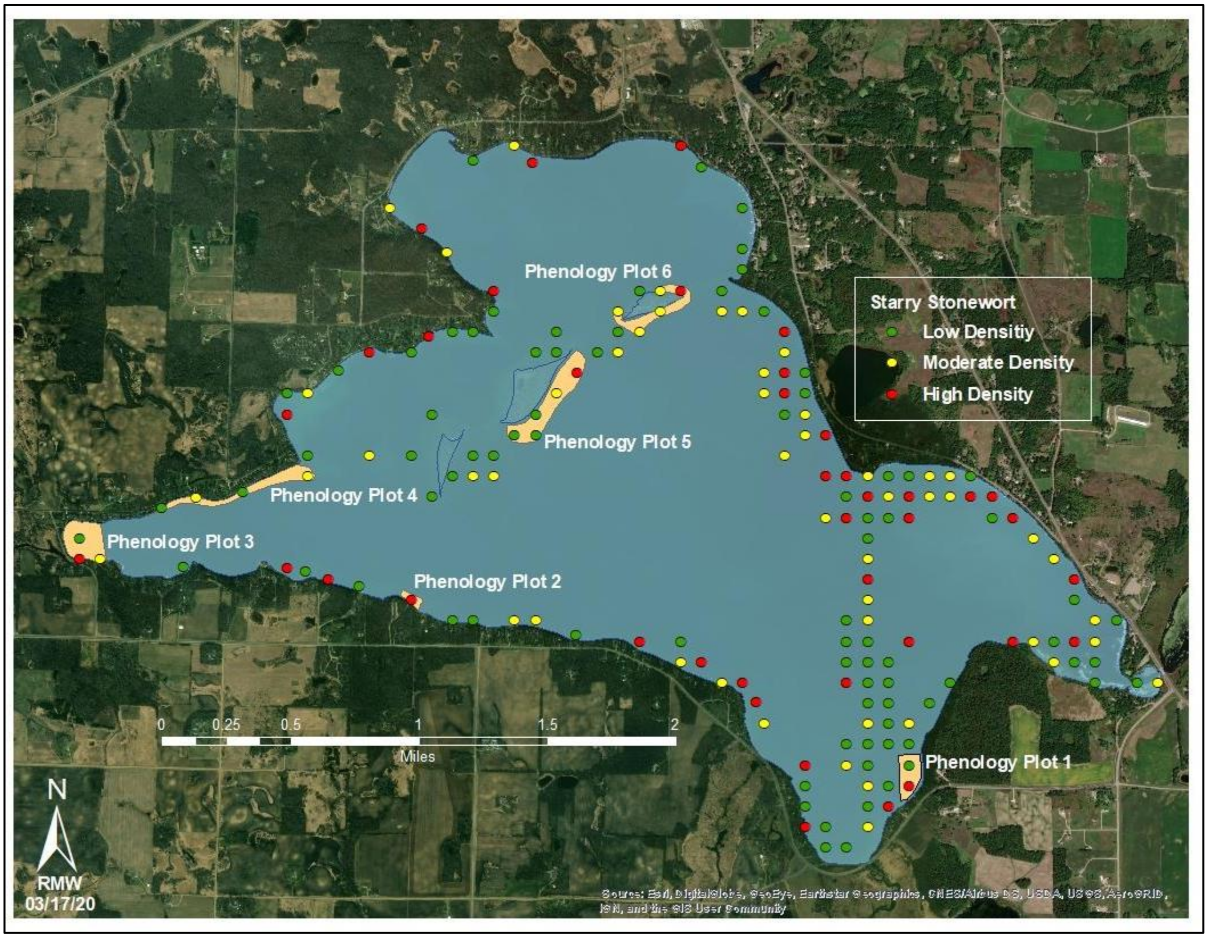

Figure 1. Phenology sample plots for Lake Koronis during the 2020 and 2021 growing seasons. Plots 1, 3, 5, and 6 were sampled while plots 2 and 4 were used as backups in case the other plots were unavailable.

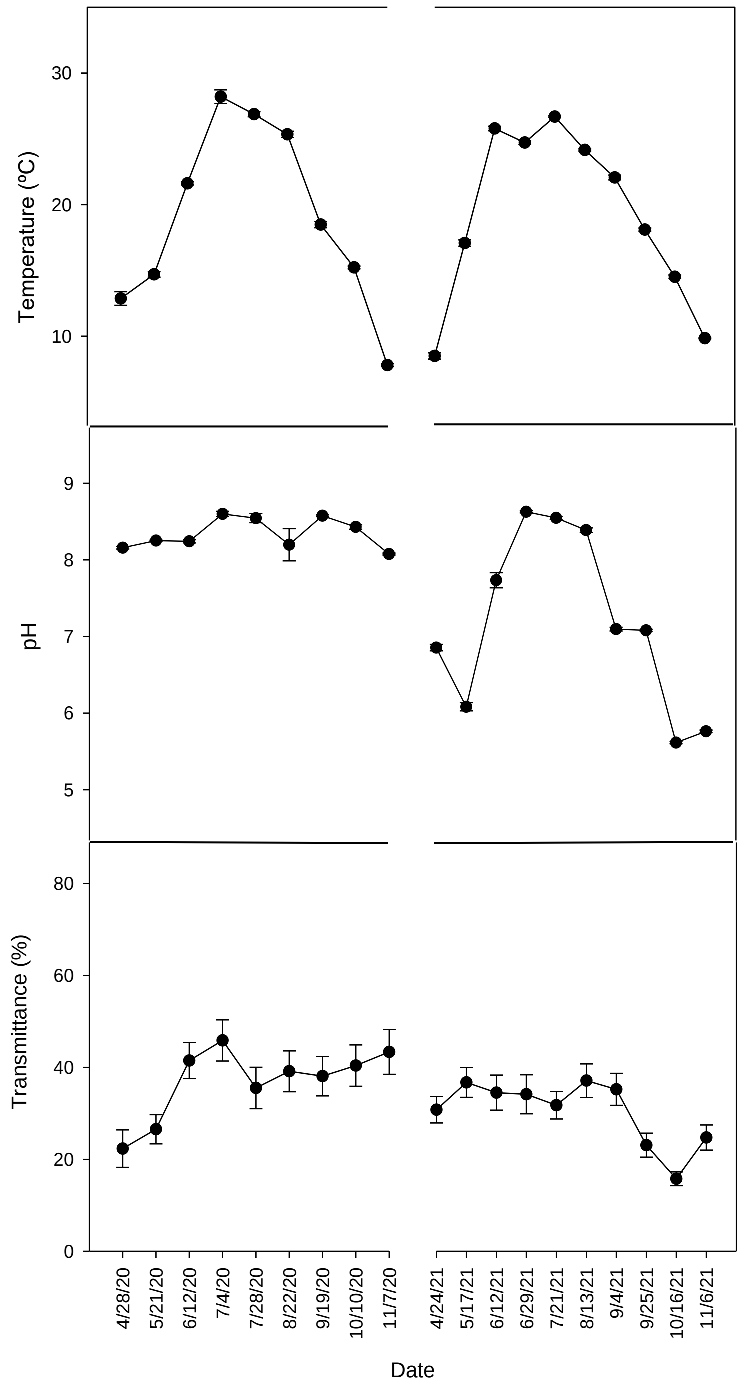

Figure 2. Mean (± 1 SE) seasonal fluctuations of temperature (°C), pH, and light transmittance (%) measured from 4 plots on Lake Koronis in Stearns County, Minnesota from April to November 2020 and 2021.

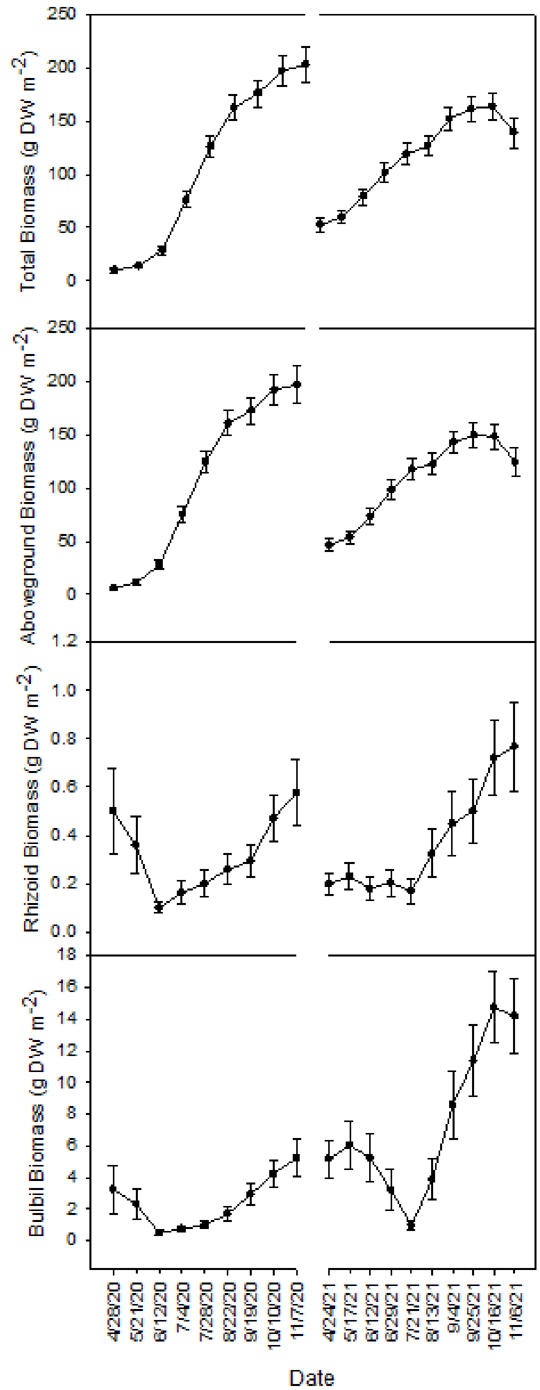

Figure 3. Mean (± 1 SE) total, aboveground, rhizoid, and bulbil biomasses of *Nitellopsis obtusa* harvested from 4 plots on Lake Koronis in Stearns County, Minnesota from April to November 2020 and 2021.

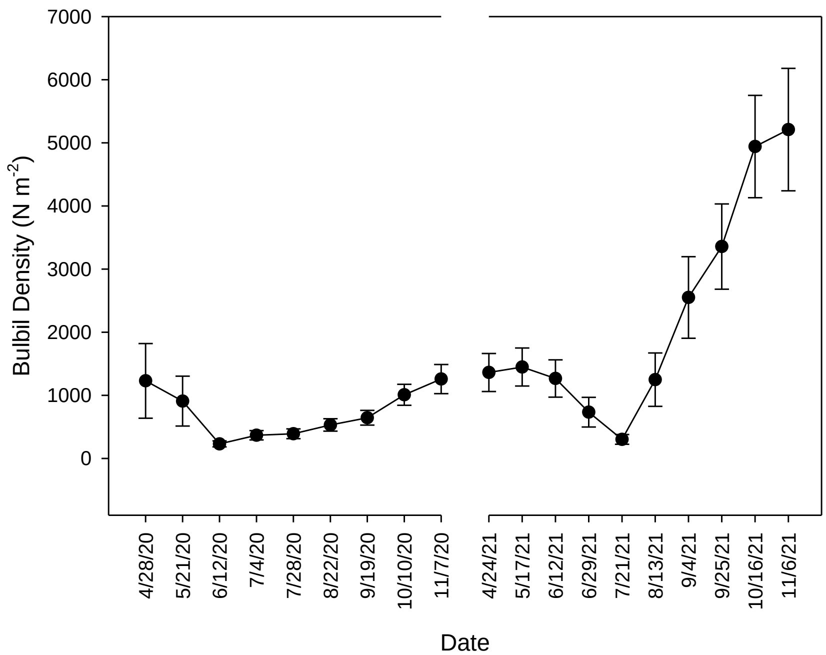

Figure 4. Mean (± 1 SE) bulbil density of *Nitellopsis obtusa* harvested from 4 plots on Lake Koronis in Stearns County, Minnesota from April to November 2020 and 2021.

Chapter III

Small Plot Evaluations of Aquatic Pesticides for Control of Starry Stonewort (*Nitellopsis obtusa*) in Lake Koronis, MN

Introduction

Nitellopsis obtusa (starry stonewort) is a green macroalga native to Eurasia in the family Characeae (Groves 1919). Starry stonewort forms star-shaped bulbils, which the common name is derived from, as a means of asexual reproduction and for spatial and temporal dispersion. White bulbils form beneath the sediment along the nodes of the rhizoid, and green bulbils form along the main axes and branchlet nodes (Bharathan 1987). While starry stonewort is able to reproduce both vegetatively and sexually it appears to undergo vegetative reproduction more frequently in both its native and invasive ranges (Larkin et al. 2018). Reductions in macrophyte species richness has been observed at various depths and locations in the invaded range of starry stonewort (Brainard and Schulz 2017, Harrow-Lyle and Kirkwood 2022).

Its current invaded range includes the St. Lawrence River, the St. Clair-Detroit River system, Lake Ontario, Lake Erie, Lake Huron, Michigan's Lower Peninsula, New York, Vermont, Pennsylvania, northern Indiana, Wisconsin, and as of 2015 Minnesota (Mills et al. 1993, Sleith et al. 2015, Midwood et al. 2016, MISIN 2017). The first known occurrence of starry stonewort in Minnesota was in Lake Koronis in 2015 and has spread to 18 other lakes and the Mississippi River (MNIWL 2022). The likely cause of overland dispersal in the U.S. are boats and boating equipment transporting the bulbils and vegetative fragments of starry stonewort (Larkin et al. 2018). Lakes lacking boat launches were surveyed within heavily starry stonewort infested areas and did not detect presence of the species (Sleith et al. 2015).

Starry stonewort like other macrophytes pose multiple challenges for management (Madsen 1993, Hussner et al. 2017, Glisson et al. 2018). The importance of understanding the efficacy of control methods is imperative for the management of invasive species in a cost-effective manner while still achieving management goals. Currently sufficient research is lacking on the effective management strategies for starry stonewort. Mechanical harvesting has seen limited success as starry stonewort regrew rapidly after harvesting events (Pullman and Crawford 2010, Glisson et al. 2018).

Commonly used pesticides for control of starry stonewort and other algae include copper-based algaecides (Lembi 2014, Glisson et al. 2018, Wersal 2022). Copper algaecides can differ in efficacy and in the species targeted based on the formulation. Such as, chelated copper formulations having increased efficacy on planktonic and filamentous algae when compared to copper salt formulations (Bishop and Rogers 2012, Calomeni et al. 2014, Iwinski et al. 2016, Pokrzywinski et al. 2021).

The efficacy of herbicide applications is limited by concentration and exposure times (CET) leading to decreased efficacy of those products. To overcome CET issues herbicide combinations have been utilized to reduce the exposure time needed for effective control (Madsen et al. 2010). Improved efficacy for copper and non-copper combinations on submersed macrophytes have also been documented (Sutton et al. 1970, Sutton et al. 1971, Pennington et al. 2001, Pokrzywinski et al. 2021). However, underground bulbils can survive treatment from contact herbicides and grow into new aboveground structures.

Diquat has seen efficacy on multiple taxa of green algae, such as *Selenastrum capricornicum*, with sensitivity being species specific. (Phlips et al. 1992, Peterson et al. 1997). Numerous filamentous green algae, including *Cladophora glomerata*, have also shown sensitivities to diquat at ≤ 1 mg L^{-1} (Robson et al. 1976). More recently diquat has shown efficacy in small-scale trials on starry stonewort (Wersal 2022); where diquat and herbicides containing diquat led to >95% biomass reductions four weeks after treatments.

Other studies reported activity of copper, non-copper, and pesticide combinations on starry stonewort (Pokrzywinski et al. 2021, Wersal 2022). A lab-based study as well as a field study on Lake Koronis of copper algaecides found similar effective control of starry stonewort (Glisson et al. 2018, Glisson et al. 2022a). All of these studies have shown promising results for the chemical control of starry stonewort using copper and other herbicides in combination with copper, and as such scaling up to and corroborating field trials is appropriate. Therefore, the objectives of this field demonstration are to 1) verify copper efficacy in small plots at the lake scale; 2) evaluate efficacy of diquat applied alone or in combination with copper under field conditions to control starry stonewort. To our knowledge this is the first field evaluation of diquat for use on starry stonewort.

Materials and Methods

Site Description

The field demonstration took place on Lake Koronis, near Paynesville, MN (45.3298° N, 94.6986° W) during the growing seasons (June to September) of 2020 and 2021. Lake Koronis is a 1,201-ha lake with a maximum depth of 40 meters and a littoral zone that encompasses 476 ha. Lake Koronis has had starry stonewort for the longest known period of time (since 2015) in Minnesota and has a well-established population in regard to biomass and bulbils. Within the 476-hectare littoral zone macrophytes such as *Vallisneria americana, Ceratophyllum demersum, Lemna spp., Potamogeton spp.,* and *Nymphaea odorata* can be found. Currently, starry stonewort is present in 324 ha or 68% of the littoral zone. In 2020 approximately 51 ha were undergoing management for starry stonewort via copper triethanolamine complex (25 ha), diquat (15 ha), and copper triethanolamine complex + mechanical pulling (11 ha). In the 2021 roughly 55 ha were treated for starry stonewort via copper triethanolamine complex (40 ha), diquat + copper triethanolamine complex (6 ha), and copper triethanolamine complex + mechanical pulling (9 ha). Six plots were established in 2020 and 2021 as part of the operational management program for starry stonewort in order to evaluate chemical treatments (Table 1).

2020 Field Demonstration

Prior to herbicide applications, 15 biomass samples were randomly harvested from two copper plots, two diquat plots, and two reference plots using a PVC coring device (Madsen et al. 2007). Pretreatment sampling occurred on 8 July 2020. Harvested samples were placed into individually labeled Zip-loc bags and stored on ice for transport to the Aquatic Weed Science Lab at Minnesota State University, Mankato for post-processing. In the lab, samples were rinsed and sorted to aboveground biomass and bulbils. Bulbils were counted at the time of biomass sorting to estimate density (N m^{-2}). Afterwards each tissue type was put into labeled paper bags and dried in a forced air oven at 48 C for at least 48 h to obtain g DW m^{-2} for aboveground biomass. Following the pretreatment assessment, copper (copper ethanolamine complex) or diquat was applied by a licensed applicator to the plots during the week of 13 July 2020. Water samples were collected at the time of application, 1 hour after treatment (HAT), 3 HAT, 6 HAT, 24 HAT, and 48 HAT from both diquat plots. Water samples were shipped to Pace Analytical (Minneapolis, MN) for diquat residue determination. Residues were used to estimate exposure time of starry stonewort to diquat. All diquat residue samples from Lake Koronis were combined to model the overall diquat exposure. Copper residues were not collected as part of this study. At four (12 August 2020) and eight (12 September 2020) weeks after treatment (WAT), 15 biomass samples were collected from each plot in a similar fashion as pretreatment samples to assess post treatment efficacy. Samples were collected and processed in a similar manner to the pretreatment samples.

2021 Field Demonstration

In summer of 2021 two plots were chosen for copper treatment, two plots for copper + diquat treatments, and two plots for non-treated references. Sampling and processing methods in 2021 were similar to those in 2020. Pretreatment sampling for the 2021 season occurred 1 July, 4 WAT occurred 10 August, and 8 WAT occurred 5 September. After the pretreatment assessment, copper (copper ethanolamine complex) and copper + diquat was applied by a licensed applicator to the plots during the week of 12 July. Water samples were not taken for residue determination during the 2021 season as general water exchange patterns were established in the treatment plots during the 2020 season.

Statistical Analysis

Biomass data did not meet the assumption of normality according to a Shapiro-Wilk test. Therefore, data were subjected to a Kruskal-Wallis test within plant tissue type, sampling times, and year. If a significant treatment effect was detected means were separated using a Dunn's All-Pairwise Comparison test. All analyses were conducted at the $\alpha \leq 0.05$ significance level. Additionally, an exponential decay regression model was used to model diquat dissipation over time in order to estimate an overall diquat half-life for the herbicide treatments conducted in 2020.

Results and Discussion

2020 Field Demonstration

At the start of the 2020 season plots had similar levels of aboveground biomass and similar bulbil densities. Aboveground biomass was more effectively controlled by treatments of copper algaecide where a 96% ($p<0.001$) reduction was seen by 8 WAT (Figure 1). Bulbil production in the copper plots had increased from 105.6 m^{-2} during the pretreatment sampling to 524.1 m^{-2} by the 8 WAT sampling period (Figure 2). Bulbil production was prolific at the end of this season and preventing or limiting this excessive production of bulbils is imperative for the control of this species.

Applications of diquat showed no reductions in aboveground biomass during the 2020 season (Figure 1). By 4 WAT aboveground biomass in the diquat treated plots increased by 198% when compared to the reference plots (Figure 1). Throughout the study period there were no significant changes in bulbil density in the diquat treated plots (Figure 2). Diquat residue analysis indicated that water exchange and diquat dilution was rapid within treated plots (Figure 3). Under the assumption that the target concentration of 0.37 mg L^{-1} was achieved, 52% of the diquat was lost by 1 HAT and 98% was lost by 6 HAT. The estimated half-life of diquat was <2 h among all treated plots on Lake Koronis.

The efficacy of diquat is greatly affected by the concentration exposure time (CET) relationships, or the length of time a lethal dose of the herbicide is maintained near target plants. In lab trials the 0.37 mg L^{-1} concentration of diquat with a 12 h exposure time reduced starry stonewort biomass (Wersal 2022). During the field demonstration water exchange and diquat dilution limited this exposure time to 2 h thereby impacting the efficacy of diquat. The efficacy of future pesticide applications will be dependent on the rapid dilution and off target movement of that herbicide. Additional water exchange studies are needed to characterize bulk water flow in more areas of the lake. This information will be crucial in developing more effective treatment recommendations, and better timing of application. Additional research is needed on other copper formulations, herbicides, and pesticide combinations.

2021 Field Demonstration

Aboveground biomass was similar in all plots during the pretreatment sampling event (p=0.41). By 4 WAT the copper plots showed a 78% reduction in aboveground biomass when compared to the reference plots (Figure 4). Regrowth was observed by WAT however, biomass was still lower than non-treated reference plots. Copper applications resulted in an 82% reduction in bulbil densities by 4 WAT, potentially due to heavy damage to aboveground structures, preventing the allocation of resources to bulbil production (Figure 5). Though, by 8 WAT bulbil production had recovered when compared to untreated reference. Combinations of copper + diquat resulted in 75% reduction of aboveground biomass by 4 WAT (Figure 4). Unlike in the copper plots, there was no indication of regrowth in the combination plots at 8 WAT. The combination plots had greater bulbil densities than either the copper or reference plots at 1985.2 m^{-2} (Figure 5). By 4 WAT the bulbil density had more than doubled (5035.2 m^{-2}) in these plots and stayed near that level by 8 WAT. This wide discrepancy is due to the high spatial variability seen in bulbil production.

While management of algae has been successful for decades using copper compounds the bulbils of starry stonewort have shown to be problematic (Glisson et al. 2018). This is due to the inability of copper algaecides to reduce the viability of starry stonewort bulbils either by inhibiting sprouting or the direct destruction of bulbils (Glisson et al. 2018). A variety of copper formulations were found to have negatively affected the viability of bulbils, but this was not confirmed viability via sprouting experiments (Pokrzywinski et al. 2021). The ability of starry stonewort to recover from management via bulbils is cause for serious consideration when developing management plans. As we currently know there are no workable strategies to prevent bulbil formation in established populations of starry stonewort.

Observations in this study corroborated the findings of another study on Lake Koronis in that a single algaecide application was not enough to prevent regrowth or regeneration via bulbils of starry stonewort (Glisson et al. 2018). An integrated approach to management may be the best option until application timing can be optimized (Glisson et al. 2018). An understanding of water exchange is needed, as well as a thorough analysis of starry stonewort's seasonal phenology (Glisson et al. 2022b) over multiple years to optimize the timing of treatment and subsequently the efficacy of treatment.

The overall impacts of management must be weighed against the decision to do nothing. When evaluating management, the assumption is erroneously made that doing nothing is environmentally neutral. In dealing with non-native aquatic species, the environmental consequences of doing nothing may be high, possibly even greater than the effects of management (Madsen 1997). Unmanaged, these species can have severe negative effects on water quality, native plant distribution, abundance and diversity, and the abundance and diversity of aquatic insects and fish (Madsen 1997). If left unmanaged, the dense growth of starry stonewort will likely extirpate native aquatic plants from those areas (Larkin et at. 2018). Starry stonewort has been seen to reduce the species richness of macrophytes in several lakes within its invaded range (Brainard and Schulz 2017, Harrow-Lyle 2022). It has been speculated that infestations of starry stonewort may limit fish spawning habitat, as well as limit the long-term viability of benthic organisms (Brainard and Schulz 2017). These impacts are cause for concern for managers and stakeholders alike.

Lake Koronis has a 476 ha littoral zone of which 324 ha have starry stonewort. No more than 55 ha were managed for starry stonewort in the 2021 season leaving 269 ha of starry stonewort unmanaged. In the current study one application of copper or copper + diquat was enough for nuisance management when compared to non-treated reference areas, rather than the alternative of no management which will lead to an increased population in future years. Management projects should focus on maintaining current levels of starry stonewort by treating at least once a year only if multiple treatments are not feasible. However, multiple treatments should be used to reduce starry stonewort levels until more effective control methods are found or until a more accurate timing for treatment can be utilized. Research should focus on the efficacy of various copper formulations as well as combinations with other pesticides. While also investigating the hydrological properties of the water bodies that are to be treated.

Acknowledgements

Funding for this project was provided by the U.S. Army Engineer Research and Development Center (Federal award number W912HZ-21-2-0025) awarded under the *Cooperative Ecosystem Studies Units* (*CESU*) Great Lakes Northern Forest unit. Additional funding was provided by the Minnesota Department of Natural Resources under Project R29G20201700352, and in part by a grant to the Minnesota DNR from the Great Lakes Restoration Initiative under award F17AP00352. We would like to thank Alyssa Haram, Allison Squires, Alyssa Anderson, Amber Fistler, Sara Ademi, Frank Rogers, Ashley Kasper, Kari Solfest, and Vincent McKnight for their tireless efforts in data collection in both the lab and field. We would also like to thank Christine Jurek, Keegan Lund, and Kylie Cattoor with the Minnesota DNR for their assistance in field sampling. Additional logistical support was provided by Koronis Lake Association, Kevin Farnum, and Blue Water Science.

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Table 1. Herbicide and treatment rates for starry stonewort control in small plots in Lake Koronis.

Year	Plot	Hectares	Average Depth (m)	Copper Rate (mg/L)	Diquat Rate (mg/L)
2020	2B	3.1	2.0		0.37
2020	3B	2.3	2.2		0.37
2020	A4	1.3	1.4	1.0	
2020	13	1.3	1.2	1.0	
2021	13	1.3	1.2	1.0	
2021	6B	1.7	2.6	1.0	
2021	2B	3.1	2.0	1.0	0.37
2021	3B	2.3	2.2	1.0	0.37

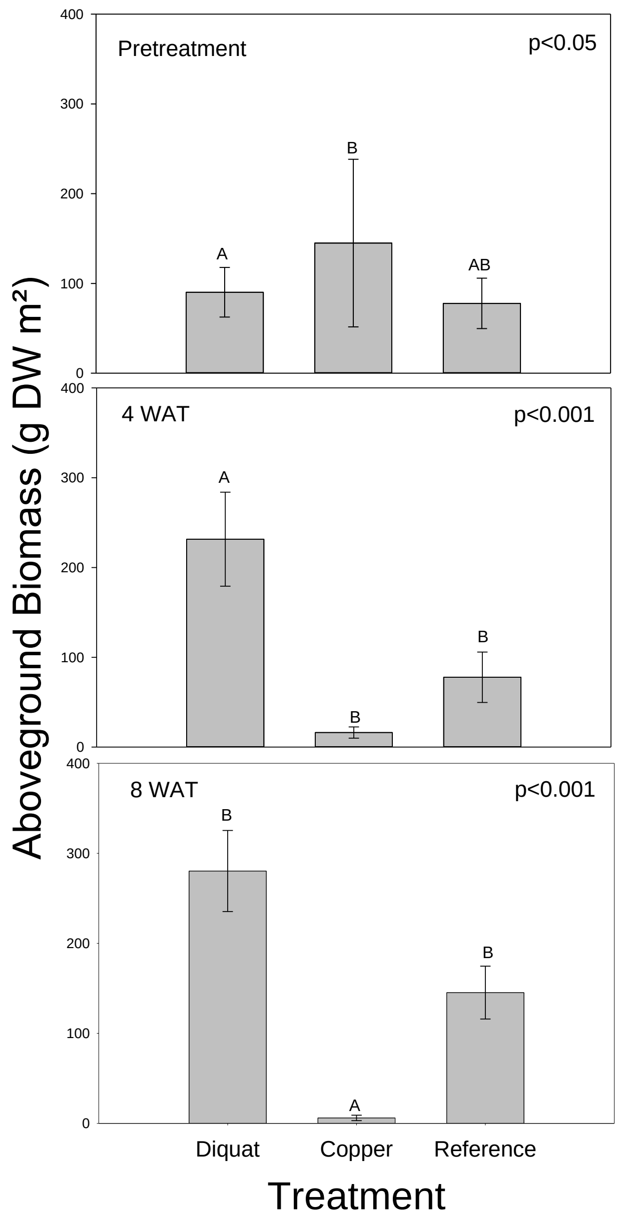

Figure 1. Mean (± 1 SE) aboveground biomass of starry stonewort at pretreatment, 4 weeks after treatment (4 WAT), and 8 weeks after treatment (8 WAT) with select herbicides, summer 2020. Bars with the same letter are not different according to a Dunn's all-pairwise comparison test at an $\alpha \leq 0.05$. All analyses were conducted within sampling time.

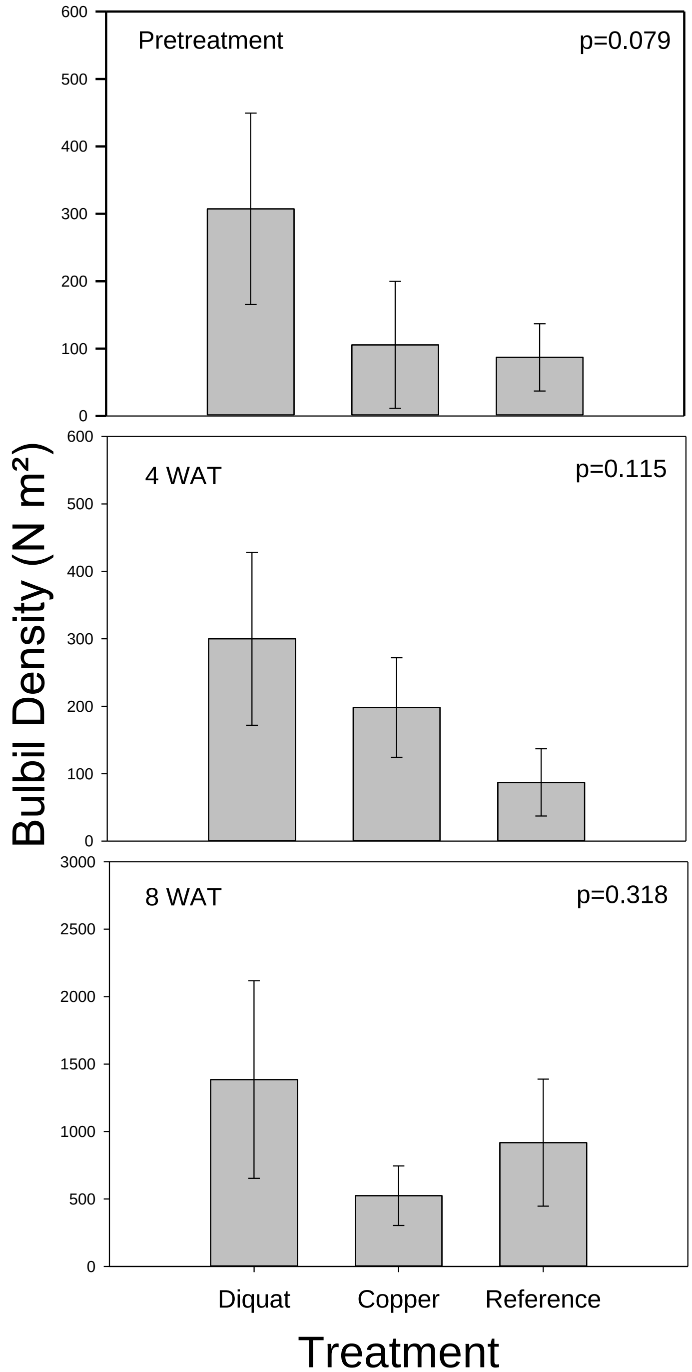

Figure 2. Mean (± 1 SE) bulbil density of starry stonewort at pretreatment, 4 weeks after treatment (4 WAT), and 8 weeks after treatment (8 WAT) with select herbicides, summer 2020. Bars with the same letter are not different according to a Dunn's all-pairwise comparison test at n α≤0.05. All analyses were conducted within sampling time.

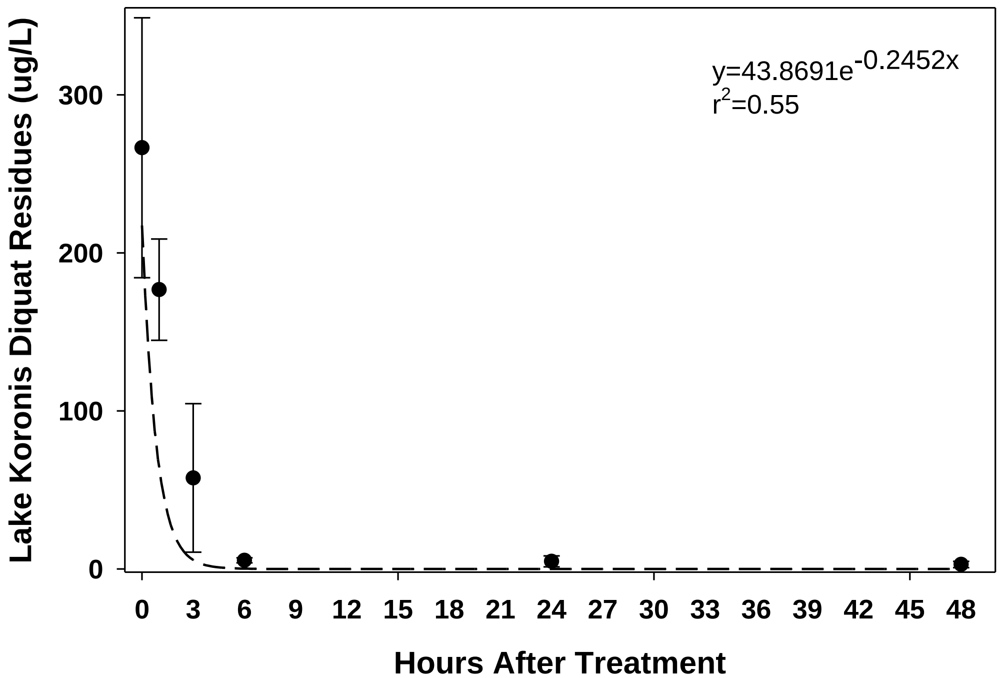

Figure 3. Exponential decay model of mean (± 1 SE) diquat residues from four plots in Lake Koronis following applications made on July 15, 2020 for starry stonewort control.

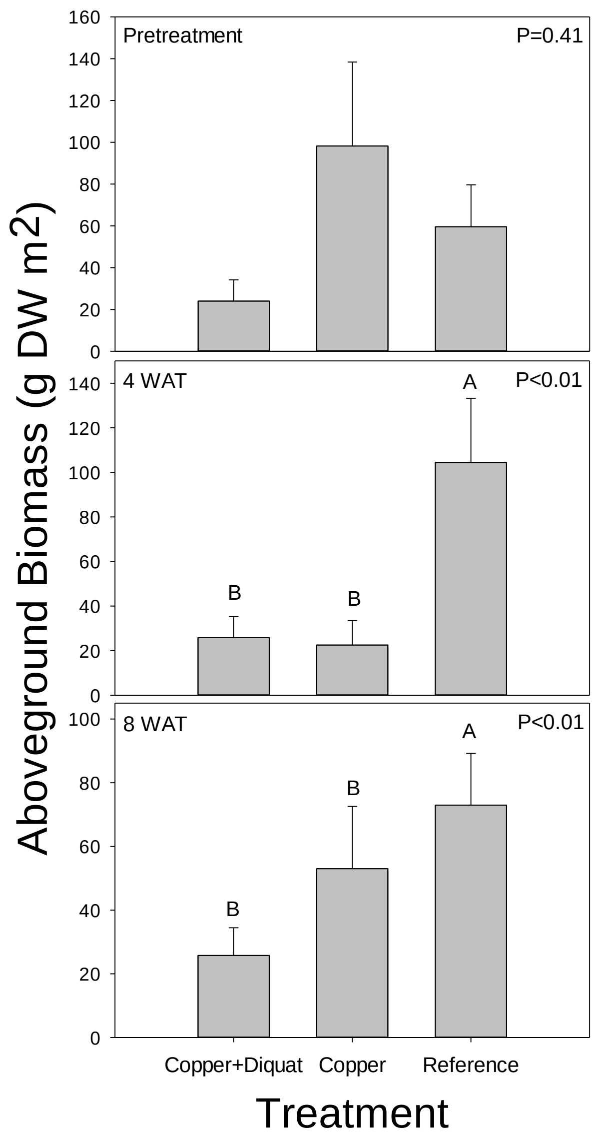

Figure 4. Mean (± 1 SE) aboveground biomass of starry stonewort at pretreatment, 4 weeks after treatment (4 WAT), and 8 weeks after treatment (8 WAT) with select herbicides, summer 2021. Bars with the same letter are not different according to a Dunn's all-pairwise comparison test at n $\alpha \leq 0.05$. All analyses were conducted within sampling time.

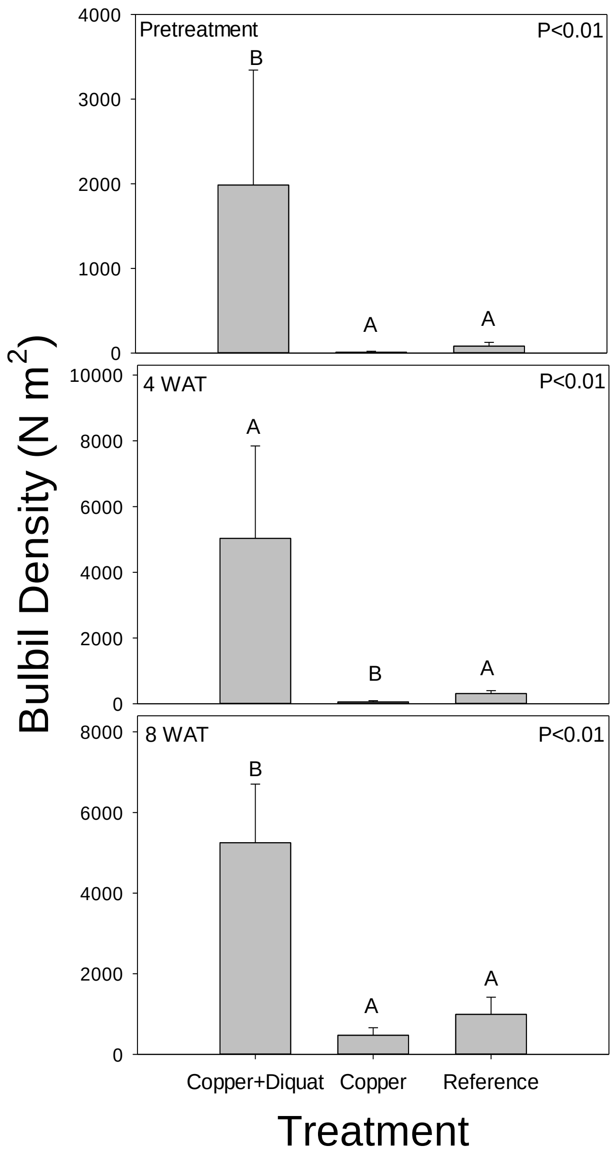

Figure 5. Mean (± 1 SE) bulbil density of starry stonewort at pretreatment, 4 weeks after treatment (4 WAT), and 8 weeks after treatment (8 WAT) with select herbicides, summer 2021. Bars with the same letter are not different according to a Dunn's all-pairwise comparison test at n $\alpha \leq 0.05$. All analyses were conducted within sampling time

Chapter 4

Conclusion and Management Recommendations

Phenology is the study of critical life stages in plants in relation to shifts in environmental factors during seasonal changes. Starry stonewort is a green macro alga in the family Characeae native to Eurasia. Much of the Midwestern United States has been invaded by this species. Starry stonewort has been seen to have late season growth patterns in its invaded range. This study found that this growth pattern was dependent upon water temperature and light transmittance both of which affect biomass production and senescence. It was hypothesized that peak biomass would occur in the late summer (August), however we observed biomass peak in November of 2020 and October of 2021 with 230 g m^{-2} and 157 g m^{-2} of total biomass, respectfully. Bulbil production was similarly hypothesized to peak in late summer (August) as well as early fall (September) and like biomass the bulbil production peak was later in the year. Bulbil production declined in the early summer as sprouting and aboveground growth occurred; but by July of each year bulbil production increased rapidly until the end of the sampling period in autumn. Peak bulbil biomass and density was lower in 2020 with 4.6 g m^{-2} and 1,229 bulbils m^{-2}, and in 2021 the biomass and density were 14.7 g m^{-2} and 5,211 bulbils m^{-2}. The average annual bulbil density was 1,537 bulbils m^{-2} and ranged from 0 to 156,944 bulbils m^{-2}. The ability of starry stonewort to grow in dense mats and produce large quantities of bulbils contribute to the difficulty of controlling infestations. Bulbils are a method of spatial and temporal distribution that can allow for recolonization of previously treated areas.

The chemical treatments and combinations of chemical treatments of starry stonewort has been under researched. As such, copper, diquat, and copper + diquat treatments were applied and evaluated on small plots in Lake Koronis, MN during the summers of 2020 and 2021. It was expected that areas not regularly undergoing management would have higher biomass and bulbil densities, but in 2020 the reference areas were not significantly different from areas treated with diquat. In 2020 the copper plots and in 2021 the copper and copper + diquat plots were seen to have less biomass and bulbil densities than the reference plots. Applications of copper had more than 90% reduction of aboveground biomass by 8 weeks after treatment in 2020. Bulbil densities were not affected by copper treatments in 2020. Diquat in 2020 was not effective at reducing aboveground biomass or bulbil density at 4 and 8 weeks after treatment. In the diquat plots, bulbil densities ranged from 33.3 ± 33.3 to 4266.7 ± 3963.3 bulbils m^{-2} depending upon sample time and site. The lack of diquat efficacy was contributed to water exchange resulting in a half-life of <2 h among all treated plots. In 2021, copper treatments had a 78% reduction in aboveground biomass at 4 weeks after treatment and 27% at 8 weeks. Bulbil density was also reduced by 4 weeks after treatment in the copper treated plots. While diquat alone did not have activity starry stonewort under normal management scenarios in 2020, the copper + diquat treated plots in 2021 had seen a reduction of 76% and 65% in aboveground biomass at 4 and 8 weeks after treatment, respectfully. The combination plots had shown no reductions in bulbil densities. All plots, regardless of the treatment applied, had seen regrowth by 8 weeks after treatment.

While starry stonewort has late season peaks in biomass can imply a potential niche for it to occupy, this notion should be approached with caution as other species such as *Hydrilla verticillata* also have such seasonal peaks and are aggressively invasive. The biggest obstacle for the management of this species is bulbil production as treatments for aboveground structures have little to no effect on the sediment bound bulbils. The bulbils will then allow for rapid regrowth after treatment or recolonization the year following treatment. Since starry stonewort is such a prolific bulbil producer and can regrow rapidly, new strategies are needed to target bulbil production, induce bulbil mortality, or gain long term control of aboveground biomass. This study found that one application of herbicides was not enough to prevent regrowth via bulbils of starry stonewort in Lake Koronis which was also seen by Glisson et al. (2018). Though, one treatment of copper or copper + diquat did provide short-term reduction of aboveground biomass, or nuisance control; which should be more preferable than the “do nothing option” which will lead to an increased population in the coming years. If left unmanaged starry stonewort will lower the species richness of other macrophytes in the lakes it has invaded and may even limit fish spawning areas and the long-term viability of benthic organisms. Managers and stakeholders should be concerned of these impacts caused by an infestation of starry stonewort.

An integrated management plan with phenological timing should be used to gain the best control of this species. Management activities in May and early June would target smaller amounts of aboveground biomass and a time when bulbil production is limited as shown in Figure 1. At least one treatment in a year should be used at a minimum to maintain growth below a nuisance level. Until researchers find more effective methods of control, multiple treatments should be used per year to reduce starry stonewort levels. Currently, copper and copper + diquat have been seen to be effective agents of control until better treatments are found. The hydrological properties of the water body being treated should be investigated to avoid exposure times of chemical treatments being limited and ineffective. Future research should be focused on the efficacy of various copper formulations, combinations of copper and other pesticides, and species-specific treatments of starry stonewort that affect bulbil biomass or bulbil productions.

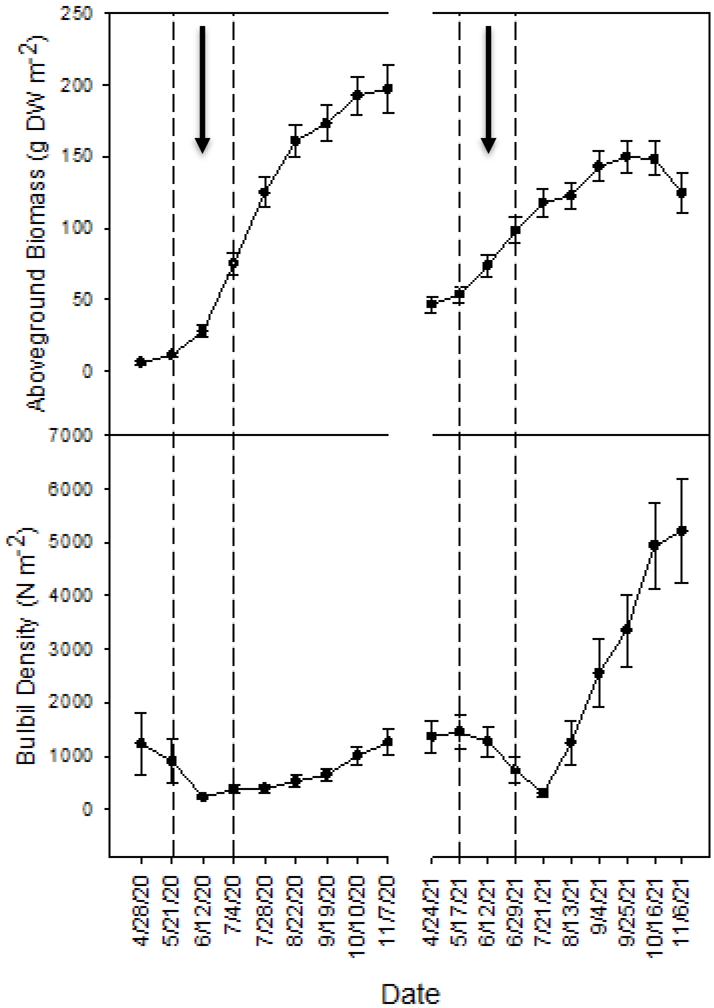

Figure 1. Recommended management times for *Nitellopsis obtusa* based on phenological timing. Arrows indicating ideal timing for treatments.