Lake Koronis, Stearns County

2015- 2020 Lake Vegetation Management Plan Final Report

Report by the Invasive Species Program - Division of Ecological and Water Resources
Minnesota Department of Natural Resources

Prepared by:
Division of Ecological and Water Resources
Minnesota Department of Natural Resources
April 23, 2021

Project Details

Lake: Lake Koronis (DOW# 73020002)

Lake Surface Area: 2,968 acres

Littoral Area: 1,176 acres

County: Stearns County

Dates of Surveys: 2015- 2020

Date of Report: April 23, 2021

Observer[s]: **Surveyor[s]**: Minnesota Department of Natural Resources (MN DNR) Divisions of Fisheries and Ecological &Water Resources (Invasive Species Program (ISP) and Lake Ecology Unit (LEU): **2020-** Stephanie Simon, Emelia Hauck Jacobs. **2019-** Christine Jurek, Emelia Hauck Jacobs, Stephanie Simon, Wendy Crowell, Tina Fitzgerald**,** Emelia Holman, Timothy Paquin and Gary Montz (marcroinvertebrates). **2018-** Christine Jurek, Emelia Hauck Jacobs, Stephanie Simon, David Wick, Aliesha Bradford, Caroline Fazzio and Gary Montz. **2017-** Stephanie Simon, Josh Knopik, Donna Perleberg, Courtney Sullivan, Angela Les. **2016-** Christine Jurek, Courtney Millaway, Ty Riihiluoma, Tim Plude. **2015-** Christine Jurek, Courtney Millaway, Scott Mackenthun and Brad Carlson.

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Report Details

C. Jurek, E. Hauck Jacobs and G. Montz. 2021. Lake Koronis, Stearns County 2015- 2020 Lake Vegetation Management Plan Final Report. Minnesota Department of Natural Resources, Division of Ecological and Water Resources, Invasive Species Program, 1035 South Benton Drive, Sauk Rapids, MN 56379. 43 pp.

Project Summary

This document outlines the management and assessment of *Nitellopsis obtusa* (starry stonewort) in Lake Koronis, Stearns County. This study is the first large- scale (greater than 15% of littoral area) effort to manage starry stonewort in Minnesota. Starry stonewort is a freshwater green alga that is invasive in North America and produces characteristic star-shaped bulbils (asexual reproductive structure). Because little is known about management of starry stonewort, it is important to continue to monitor, assess and review the outcomes of management.

In 2018, a Lake Vegetation Management Plan (LVMP) was developed for Koronis Lake in coordination with the Lake Koronis Association, to expand management and monitoring efforts beyond 15% of the littoral area of the lake. As part of the LVMP (2018-2020), we evaluated starry stonewort and the native plant communities' distribution and abundance through lake-wide point-intercept surveys, sampled for biomass pre and post-treatment, evaluated bulbil abundance post-treatment and studied the impacts to macroinvertebrates following algaecide (copper- based pesticide) treatments. Over time with continued monitoring, the DNR and our partners can monitor the plant communities in Lake Koronis and evaluate possible responses to invasive aquatic plant management via herbicide and mechanical control.

Lake Description

Lake Koronis is a 2,968- acre lake located one mile south of the town of Paynesville in Stearns County, MN. The lake has two invasive plant species: starry stonewort (*Nitellopsis obtusa*) and curly-leaf pondweed (*Potamogeton crispus*). Starry stonewort was first identified in the lake in 2015, although it is unknown when it first appeared in the lake. Observations to date in Lake Koronis have indicated that starry stonewort growth accelerates in late June/early July and remains present in the lake at varying densities until its senescence during the fall. These observations are comparable to other phenology studies conducted in both Michigan (Nichols et al., 1988) and Minnesota.

The maximum depth of water in Lake Koronis is 132 feet, and 39% of the lake is classified as littoral (areas of water depth between 0 to 15 feet, where aquatic plants are most likely to grow). Lake Koronis is within the North Central Hardwoods Forested Ecoregion and is considered a eutrophic lake with an average water clarity of 7.8 feet, total phosphorus mean of 34.9 µg/L, and chlorophyll-a mean of 14.6 µg/L (RMB Environmental Labs, Inc. [RMB], 2021). Based on long-term data collected between 2001 and 2020 (RMB, 2021), Secchi disk readings (water clarity) indicated no significant trend (Figure 1), although both chlorophyll-a (Figure 2) and total phosphorus (Figure 3) have decreased. Data retrieved at [RMB Environmental Laboratories lake database](https://www.rmbel.info/data/) (https://www.rmbel.info/data/).

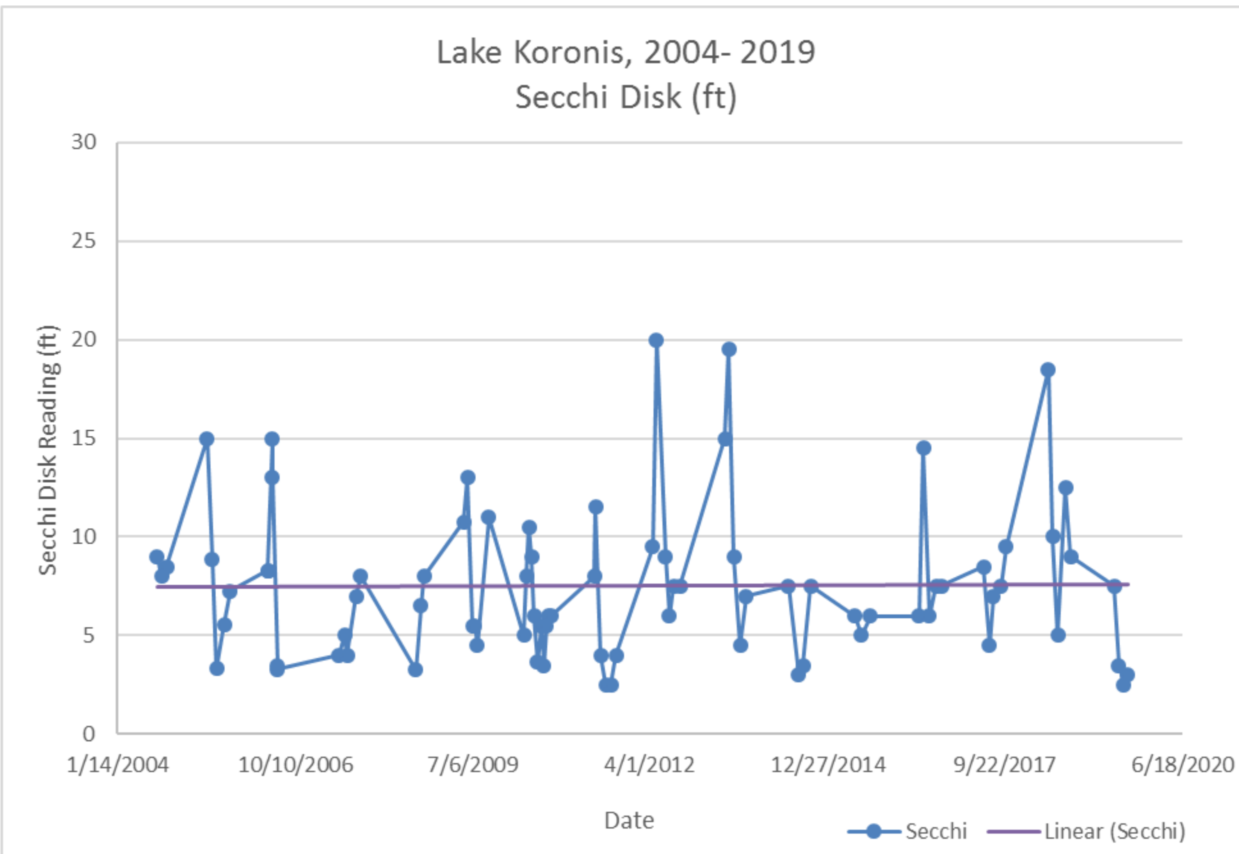

Figure 1. Trend analysis of Secchi disk readings (feet) in Lake Koronis, Stearns County (DOW# 73020002) indicate no significant trend between 2004 and 2019 (RMB Lakes Database, 2019).

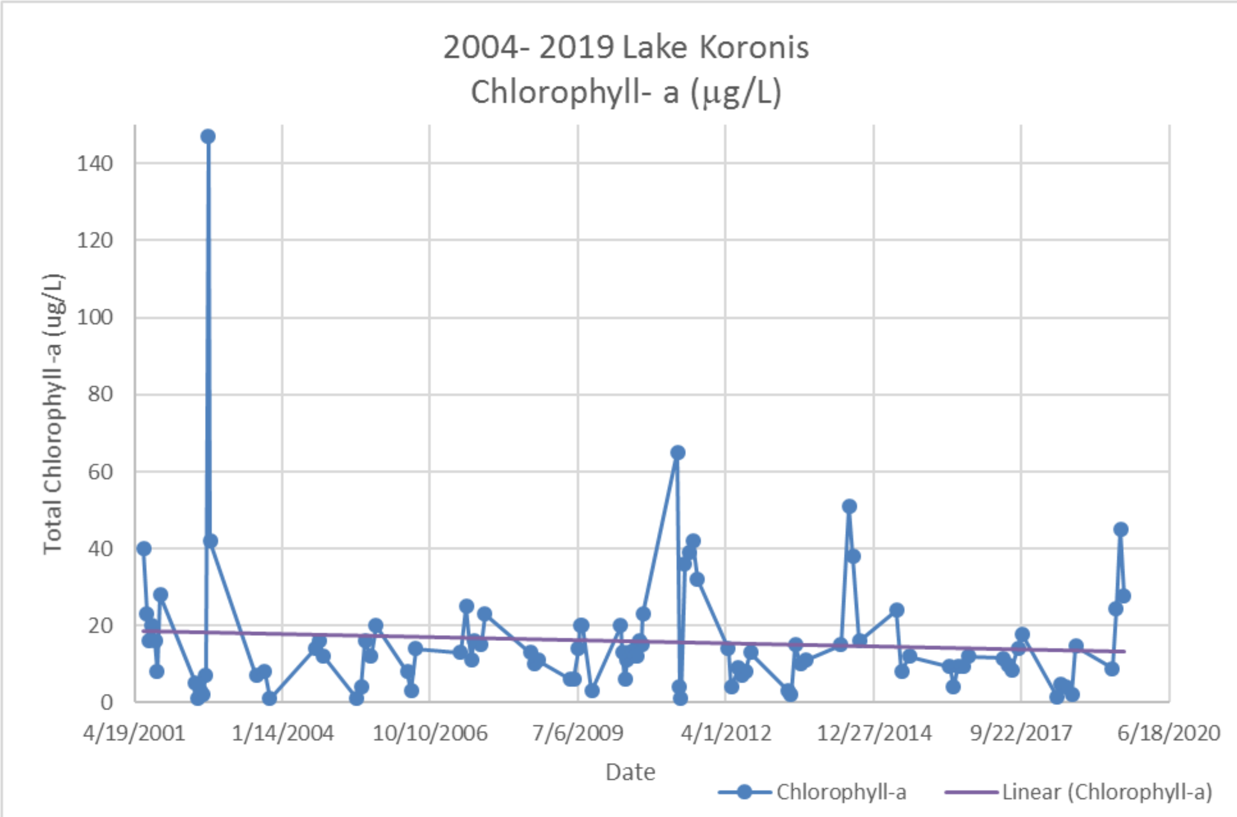

Figure 2. Trend analysis of chlorophyll-a (µg/ L) in Lake Koronis, Stearns County (DOW# 73020002) indicating a decrease between 2001 and 2019 (RMB Lakes Database, 2019).

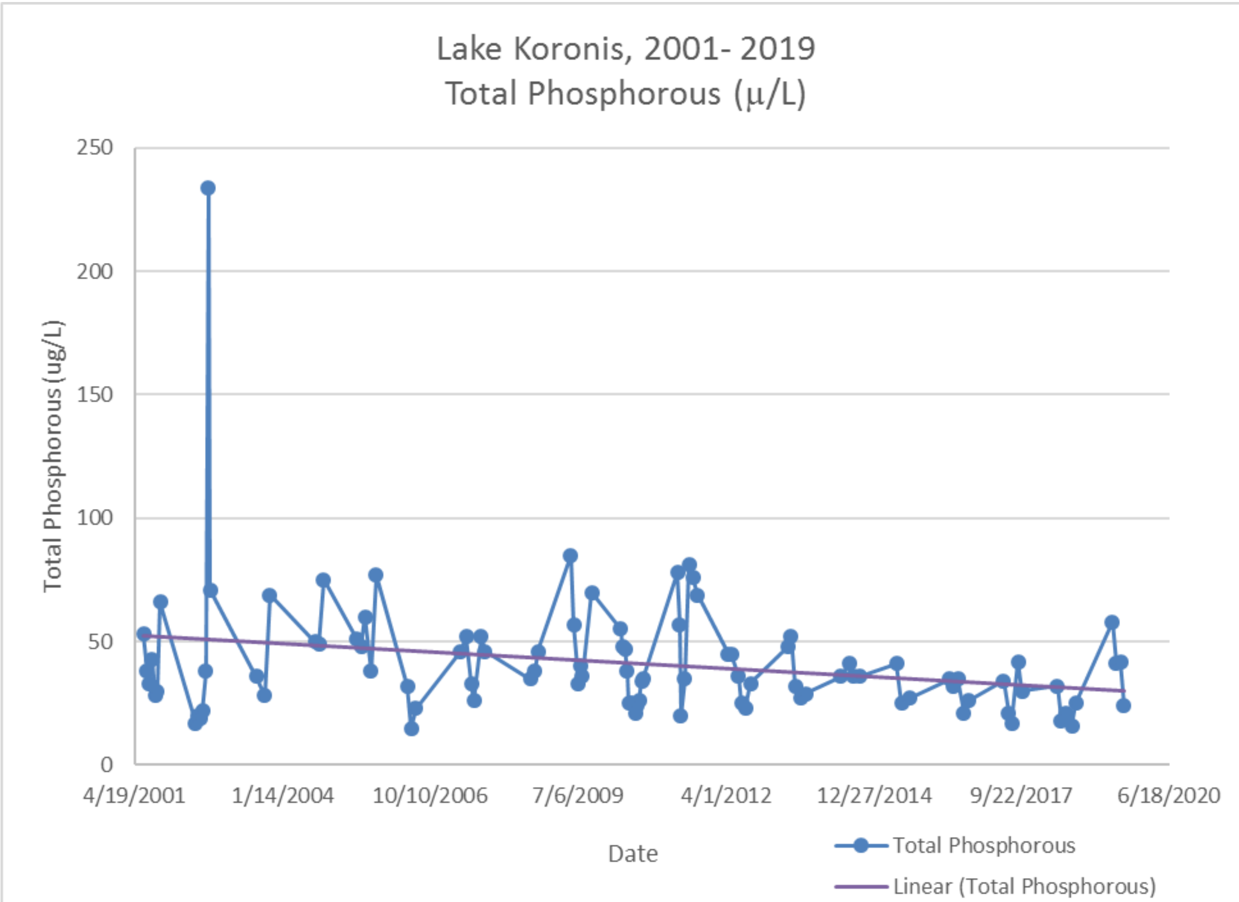

Figure 3. Trend analysis of total phosphorus (µg/ L) in Lake Koronis, Stearns County (DOW# 73020002) indicating a decrease between 2001 and 2019 (RMB Lakes Database, 2019).

Management History

In 2015, Lake Koronis was the first confirmed waterbody in Minnesota with starry stonewort. It was found to be widely distributed during the initial rapid response survey; occurring at high densities in the bay near the public water access and extending into the main basin. Starry stonewort can form large, dense, monotypic mats and intermixes with native aquatic plants. To gain a better understanding of the distribution and abundance of starry stonewort over time, the MN DNR has been monitoring aquatic plant communities in Lake Koronis since its discovery. In addition, data and observations regarding its phenology; including the presence of starry stonewort and its bulbils year-round (Figure 4) had been recorded. This information will help us gain a better understanding of starry stonewort growth and the timing of management efforts.

Figure 4. Starry stonewort and bulbils found year- round in Lake Koronis taken by MN DNR on Lake Koronis (DOW# 73020002), Stearns County, Minnesota. From left to right, photos are from winter 2015 and summer 2019 respectively.

Between 2015 and 2017, small-scale pesticide treatments and mechanical harvesting occurred in less than 2% of the littoral area for starry stonewort. Various pesticides have been used, including combinations of granular chelated copper, salts of endothall (Hydrothol 191 ®), and chelated coppers (liquid/ granular). Mechanically pulled vegetation and starry stonewort were placed above the original high water level and transported offsite for disposal. In-lake monitoring data from treatments in 2016 indicated that using both liquid chelated copper algaecide and mechanical harvesting can significantly reduce starry stonewort biomass (Glisson et al., 2018) The second application of a granular copper did not further reduce biomass or bulbil viability (Larkin et al., 2018).

In 2018, the MN DNR and the Koronis Lake Association (KLA) developed a lake vegetation management plan (LVMP) to monitor the large-scale management of starry stonewort (greater than 15% of the littoral area). The LVMP goals included effective control of starry stonewort in management plots, reduce matting and nuisance conditions interfering with recreational use, increase native plant abundance and diversity, evaluate treatment effectiveness on bulbils, and achieve a limited to no net loss of sensitive freshwater macroinvertebrates.

Between 2015 and 2020, management included both small- scale and large-scale mechanical harvesting and copper treatments (liquid chelated copper; Table 1). During the large- scale management years, two treatments of liquid chelated copper formulation (Cutrine-Plus®; copper ethanolamine complex, mixed; liquid) were applied at 1 mg L^{-1} between July and October of each year. Management plots ranged in size from one acre to over 150 acres and were delineated based on starry stonewort abundance. Mechanical harvesting occurred from July to September between 2016 and 2020, ranging from 7 to 52 acres acres each year, extending from shore to depths of 4 feet using an Eco Harvester in designated areas. During 2020, approximately 145 acres was treated with copper and 52 acres were harvested (Figure 5).

During this project, we evaluated the copper concentration following the treatments to determine exposure of times. In 2018, copper concentrations were monitored in the largest copper plot prior to treatment (-0.5 hour.) and at 0, 1, 2, 3, 4, 9, 24, and 48 hours after treatment (HAT). Samples were collected near the bottom at 0.3 m above the sediment and submitted to Lonza Innovation & Technology Center for analysis using a Perkin Elmer® Optima[3] 8300 instrument. Mean copper concentrations decreased from 0.5 mg L^{-1} to 0.3 mg L^{-1} within 4 hours and dropped below 0.1 mg L^{-1} after 48 HAT. In 2019, copper concentrations were measured in small copper plots for comparison at 0, 1, 2, 3, 4, 5 and 24 HAT and submitted to Minnesota Department of Agriculture laboratory for analysis by plasma-mass spectrometry (Environmental Protection Agency Method 200.8). Mean copper concentrations decreased from 0.3 mg L^{-1} at 1-HAT to < 0.2 mg L^{-1} at 5-HAT.

Table 1. Invasive plant management summary. Characteristics and history of pesticide treatments for starry stonewort in Lake Koronis, Stearns County (DOW#73020002).

Date	Copper Treated (acres)	Mechanically Harvested (acres)	Delineated Beds (acres)
2015	4	0	200
2016	18	18	353
2017	15	7	272
2018	224 (2x)	43	376
2019	109 (2x)	41	576
2020	145 (2x)	52	600<

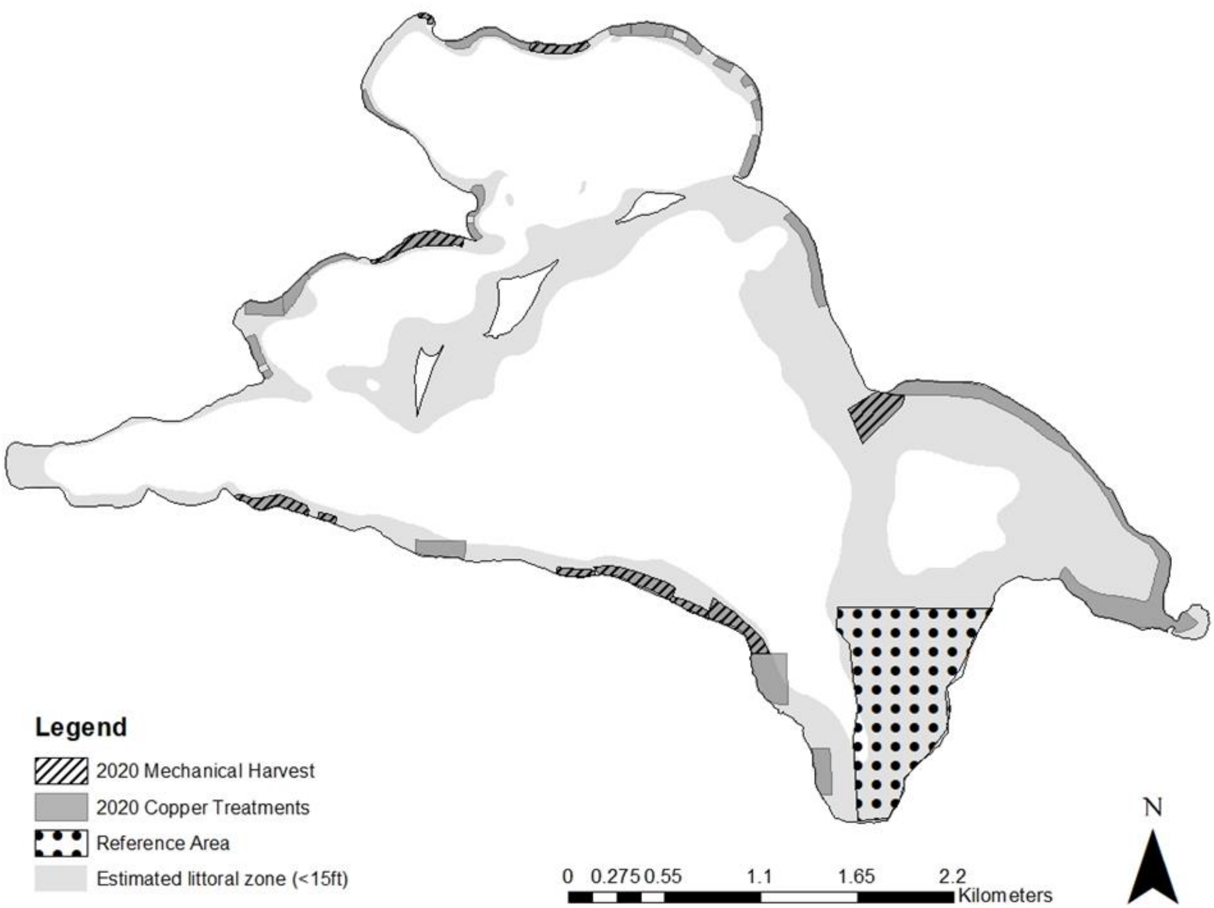

Figure 5. 2020 Locations of copper algaecide treatments, mechanical harvesting and reference plot from Lake Koronis (DOW# 73020002), Stearns County, Minnesota.

Survey Methods

Lake wide aquatic plant surveys

Point-intercept surveys were used to assess the distribution, frequency of occurrence and abundance of aquatic plant taxa in Lake Koronis. The MN DNR surveyors conducted annual point- intercept surveys from 2015- 2017 before management and from 2018- 2020 during lake wide management. Additional point-intercept data from September 2018 and 2020 was collected by Steve McComas with Blue Water Science (McComas, 2018 and 2020) and MN DNR Lake Ecology Unit (July 2017- 2020), which is included in this report. MN DNR surveyors used a point-intercept grid survey method developed by John Madsen in "Aquatic Plant Control Technical Note MI-02, 1999" and MN DNR for starry stonewort monitoring (MN DNR, 2017) to employ quantitative methods for measuring aquatic plant distribution and abundances (Madsen, 1999). Survey points were placed 130 meters apart using a Geographic Information System (GIS; Figure 6). A total of 331 points were sampled in depths up to 20 feet of water. In 2017 and 2018, additional sampling points were added to account for taxa observed at deeper depths and nearshore communities. A reference plot in a non-managed area was designated in 2015 and maintained to track natural variation over time, as well as to compare to managed areas (Figure 6).

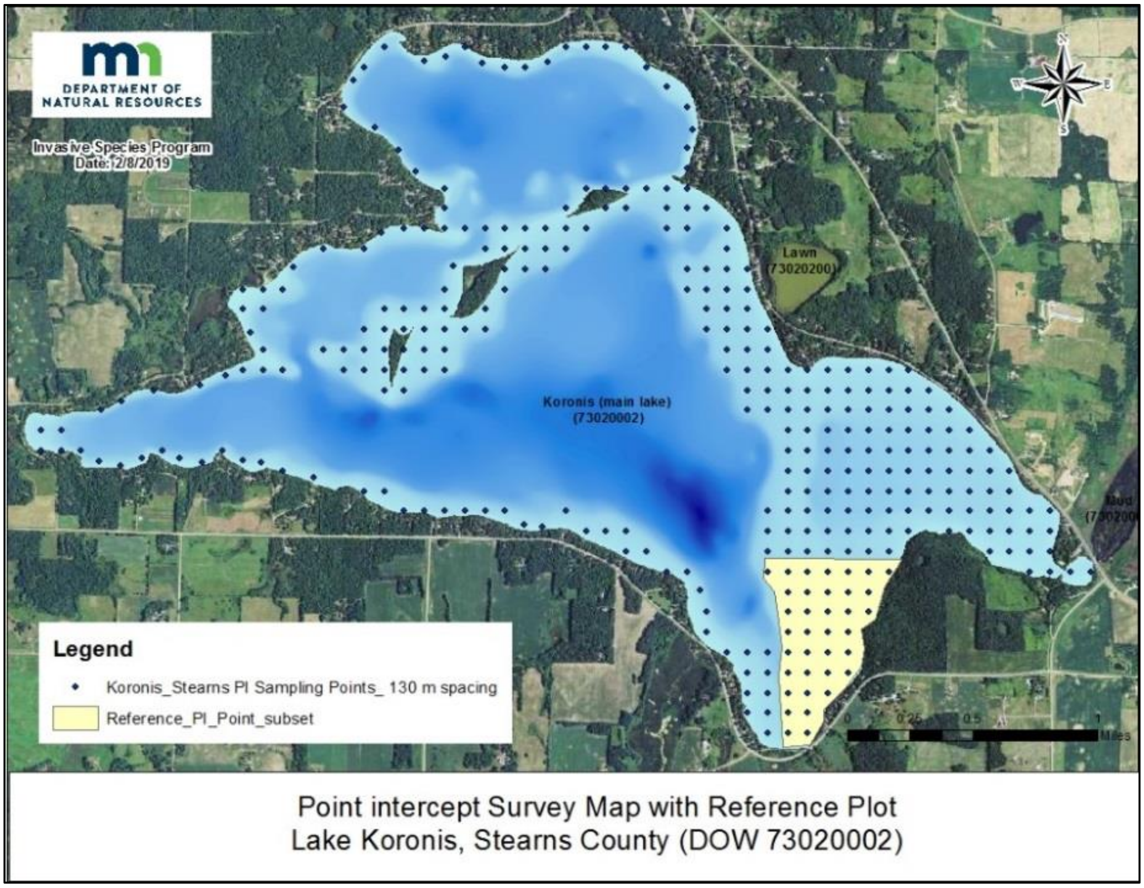

Figure 6. Point-intercept survey grid. Point-intercept survey map with reference plot from Lake Koronis (DOW# 73020002), Stearns County, Minnesota.

Plant samples were collected by throwing and dragging a double-sided rake along the lake bottom at each point. Each rake was given an abundance rating. The abundance rake rating are as follows: 1: sparse, 2: common/ frequent/ occasional, and 3: abundant/matted (Table 2). All plant taxa (submerged, floating-leaf, emergent and free floating) were recorded to species or genus during the survey following Crow and Hellquist (2000). Plant samples were assessed on the boat to determine species presence-absence and abundance. It is important to note that an abundance rating for starry stonewort is widely used as a surrogate to measure biomass since it is less labor-intensive. Despite this, in 2019, MN DNR invasive species staff collected biomass pre and post management. Frequencies of occurrence (i.e. how often a plant species was found in the lake) were calculated based on the littoral zone (the portion of the lake that is less than 15 feet in depth). Sample depths were rounded to the nearest whole number and recorded for each species. Maximum depths were calculated at the 95th percentile for all vegetated sampling points. A reference plot (154 acres) provided a standard for identifying changes that are greater than changes due to natural annual variation (Schindler, 1987; Carpenter, 1990). The reference plot was comprised of a subset of 36 point-intercept points which is comparable to Plot 1 (large treatment area of 157 acres) in terms of the substrate, depth and plant communities.

Table 2. Quantitative rake abundance ranking (0-3) used to estimate plant abundance for each species based on rake coverage and/or visual observation (MN DNR). A zero (0) ranking indicates no target plants were retrieved or observed in a sample.

Abundance Ranking	Rake Coverage	Description
1		Sparse; plants covering <25% of the rake head
2		Common; plants covering 25%-75% of the rake head
3		Abundant; plants covering >75% of the rake head

For statistical analysis of lake wide point-intercept data, we used a chi-square analysis to compare pre-management to post management (September 2015 & September 2020). September surveys were used for comparison since that is when starry stonewort is at its peak biomass. Change was measured as the difference between the number of occurrences of a given taxa between years.

<u>Biomass (wet-weight) measurements</u>

In 2019, MN DNR conducted pre and post-treatment biomass monitoring to evaluate the impacts from management on both native and non-native taxa. These surveys were used to evaluate the impacts of copper, mechanically harvested, combined management and reference plots; moreover, to evaluate if management can reduce biomass or matting conditions. To document any reductions in biomass pre and post-management, we measured wet weight biomass in 2019. MN DNR staff followed methods developed by Bickel and Perrett in "Precise determination of aquatic plants wet mass using a salad spinner"(Bickel & Perrett, 2016). Biomass was sampled in the large copper treatment (Site 1), mechanically harvested plot (Site A1), combined management plot (Site 4N) and the reference plot (Site 4; Figure 7). Sampling points were placed 40 to 130 meters apart depending on the size of plot using ArcGIS software. When points were determined too deep in the field, additional points were created. We utilized a 12- inch fixed rake to collect aquatic plant taxa. The rake was placed onto the bottom sediments and then turned five times to collect plants. We separated starry stonewort from native taxa before we rinsed plants with a wash bucket and placed them into a commercial salad spinner to remove excess water. After spinning the plant material 20 times in a commercial salad spinner, the plants were weighed using a digital scale. Any fragments of starry stonewort not detectable on the scale were recorded as <1 gram.

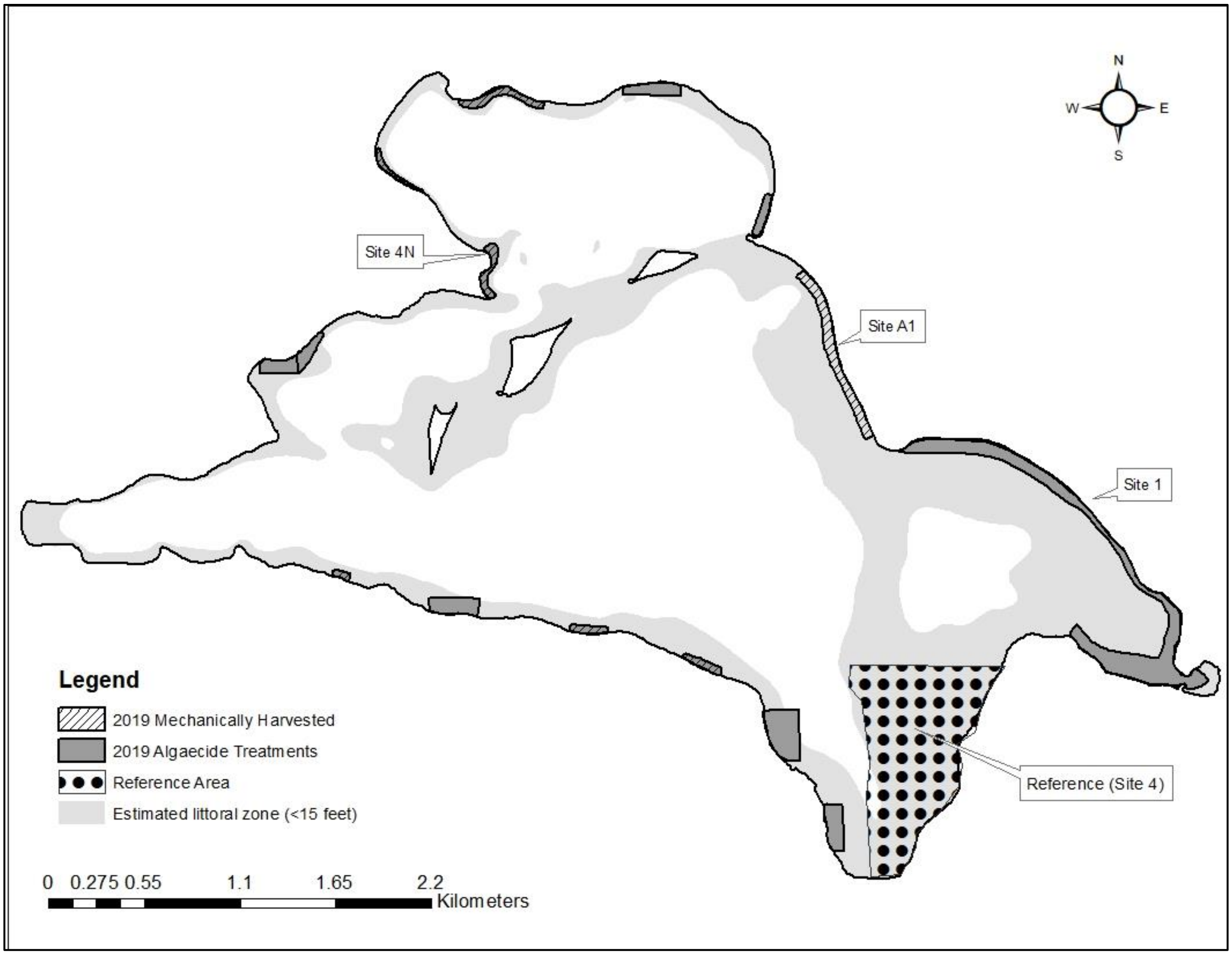

Figure 7. Biomass sampling map. Biomass was sampled in the large copper (algaecide) treatment (Site 1), mechanically harvested (A1), combined management (Site 4N) and the reference (Site 4) in Lake Koronis, Stearns County (DOW# 73020002). Points were placed 130 meters apart in copper plot (N= 10), 130 meters in reference plot (N= 10), 40 meters in the combined management area (N= 10), and 80 meters in mechanical harvested area (N= 11).

Bulbil assessment

In 2018 and 2019, we evaluated the effect of copper on bulbils before treatment and following both the initial and second treatment. We sampled at 7 to 10 days prior to treatment and 7 to 10 days post-treatment. Sample points were set with grids of points distributed at 65 m spacing for the large copper plot (Plot 1) and 40 m for both the small plots (Plots 5 and 6) and reference plot in depths from 5 to 8 feet (Figure 8). Bulbils were sampled in the largest copper plot in 2018 and in two small copper plots the following year. As this study was in conjunction with our macroinvertebrate study, the sampling points were identical (Figure 8). The reference plot was sampled each year to provide bulbil abundance within a non-managed plot. Bulbil samples were collected using a petite Ponar grab (232 cm^2). We removed any aquatic plants and debris from the outside of the closed grab before emptying the contents into a wash bucket with a 600 micron mesh screen. We preserved the samples with reagent denatured ETOH and bulbils were later sorted under a dissecting microscope. Since one or two grabs were collected at a site depending on the amount of material, bulbil counts were standardized to one Ponar grab, converted into densities (number of bulbils per m^{-2}) and comparative statistics for each sampling event were calculated. We estimated the average treatment effect on bulbil densities using a generalized linear model (R Development Core Team 2020). We used treatment timing (either the initial or second treatment of the season), year (2018 or 2019), and plot ID to account for seasonal, annual, and plot-specific variation among the four treatments. Since the treatment site changed between years, we were unable to observe treatment effects longitudinally from 2018 through 2019.

Macroinvertebrate study

We analyzed the impacts to macroinvertebrates as copper has been shown to impact different aquatic invertebrate taxa. We focused on total mollusks (predominantly freshwater snails, Gastropoda), amphipods (Amphipoda), caddisflies (Trichoptera), and midges (Chironomidae). Samples were collected within the large copper treatment plot (Plot 1) in 2018 and two smaller treatment plots (Plot 5 and 6) in 2019 and the reference plot (2018 &2019; Figure 8). Samples were taken in depths from 5 – 8 feet. Sampling occurred before and after each copper treatment for a total of four sample periods. These sampling dates were within 7 – 10 days of proposed copper application and 7 – 10 days post- treatment. At each site, one to two petite Ponar grab samples were taken, depending on total amount of material collected. The material was then sieved through a wash bucket with 600 micron mesh. All samples were preserved in the field with reagent denatured ETOH. In the lab, invertebrates were sorted using a stereo dissecting microscope, identified to the lowest practical taxonomic level and counted. For our statistical comparison of major invertebrate groups and taxa abundance, we used the Mann-Whitney Rank Sum test ($p < 0.050$).

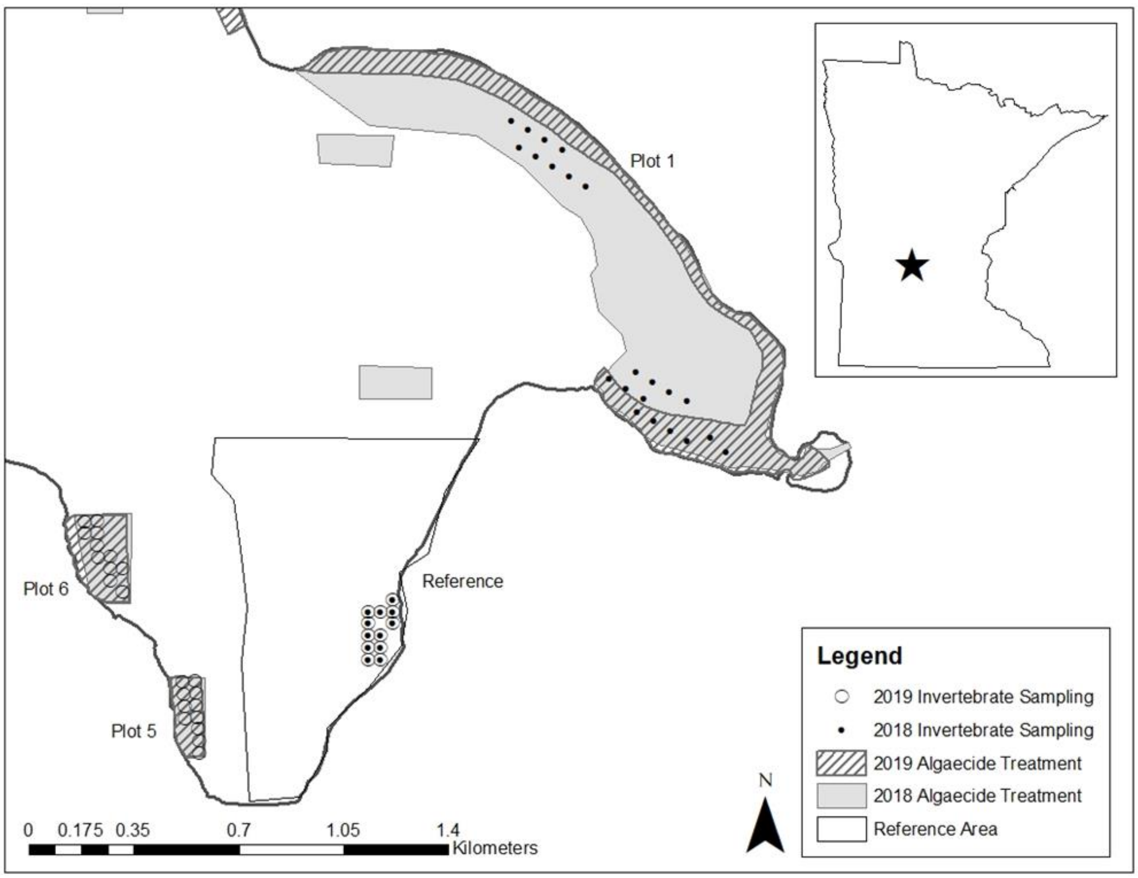

Figure 8. Bulbil sampling map. In 2018 and 2019, bulbils were sampled in Lake Koronis, Stearns County (DOW# 73020002) within a copper (algaecide) treatment (plot 1) in 2018 and in two small copper treatments (plots 5, 6) the following year. The reference plot provided data on starry stonewort bulbils abundance. Bulbil samples were collected using a petite Ponar grab.

Results and Discussion

Aquatic plant communities

Lakewide frequency of starry stonewort in Lake Koronis varied from 17% to 30% during early infestation years (2015 to 2017) to 66% in 2020. Overall, starry stonewort continues to re-grow in both managed plots and un-managed plots. An increase in frequency of occurrence of starry stonewort occurred prior to lake wide management, during management years (Figure 9, Appendix A) and within the reference plot (non-managed area; Figure 10). It is important to point out that some declines in native taxa were additionally observed in the reference plot (Table 6 and Table ***7***). This may be attributed to the direct competition between native aquatic plants and starry stonewort since this invasive taxa increased by 80% over the 5 years in the non-managed reference plot.

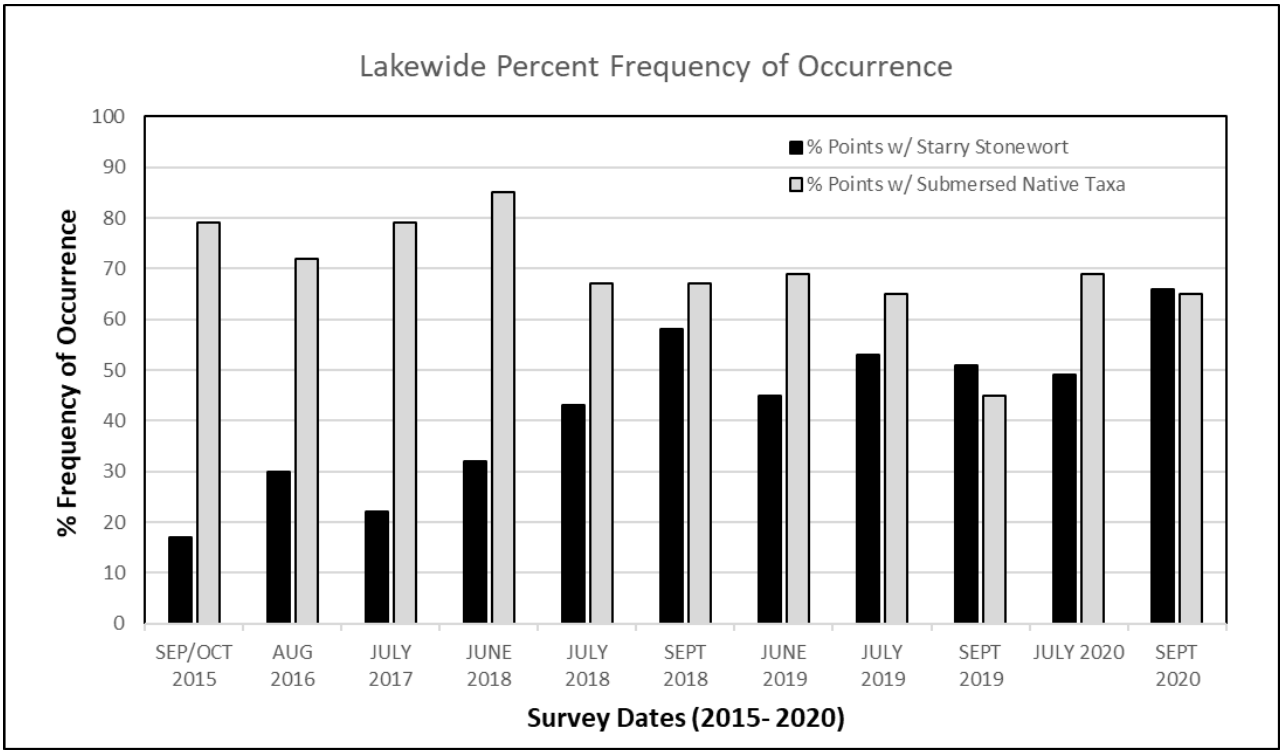

Figure 9. Lake wide plant frequency of occurrence between 2015 and 2020. Percent frequency of occurrence for submersed native taxa and starry stonewort pre and post-management in Lake Koronis, Stearns County (DOW# 73020002).

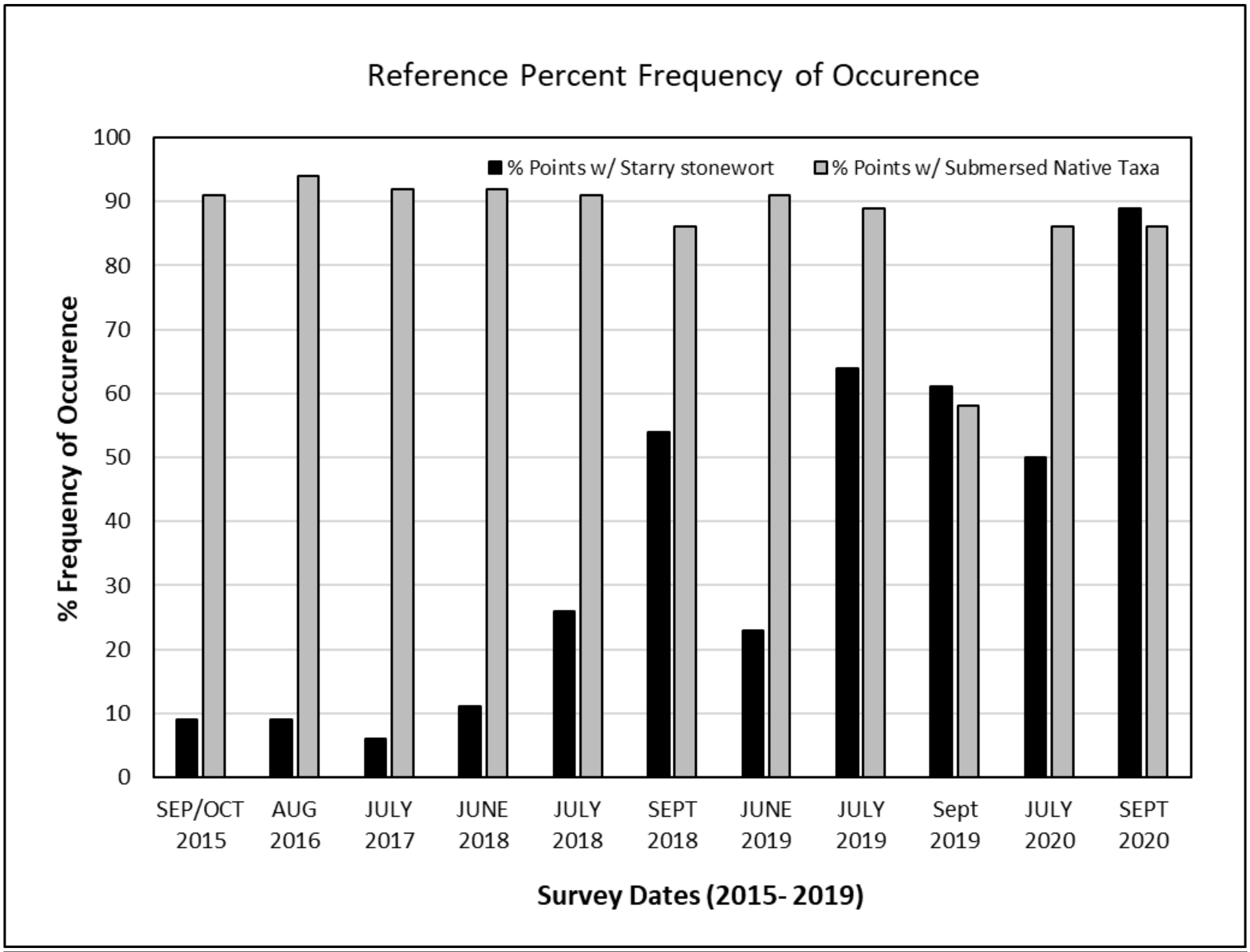

Figure 10. Reference plant frequency of occurrence between 2015 and 2020. Percent frequency of occurrence for submersed native taxa and starry stonewort in a non-managed plot in Lake Koronis, Stearns County (DOW# 73020002).

Based on lake wide point-intercept surveys between 2015 and 2020, the maximum depth recorded of starry stonewort growth in Lake Koronis was 19 feet (Table 3; Simon & Perleberg, 2017) and observed matting conditions were at seven feet (2015). Starry stonewort was most commonly found at depths of six feet across survey years. Other species observed at this depth include wild celery and muskgrass. Lake Koronis has two non-native submerged taxa (starry stonewort and curly-leaf pondweed) and up to 17 submersed native taxa (Table 3). The most common submerged aquatic plant taxa found in Lake Koronis are muskgrass (*Chara* sp.), coontail (*Ceratophyllum demersum*), narrow-leaf pondweed groups (*Potamogeton* spp.) and wild celery (*Vallisneria americana*; Table 4). Less common species (<1 %) include river pondweed (*Potamogeton nodosus*) and floating-leaf pondweed (*Potamogeton natans*). Filamentous algae is common during the summer in Lake Koronis and has shown declines in frequency of occurrence following copper treatments during both 2018 (32%), 2019 (18%) and 2020 (1%).

Table 3. Lake wide summary of point-intercept metrics (2015-2020) for Lake Koronis, Stearns County (DOW# 73020002). Values shaded in grey were calculated from littoral depth range. Taxa includes submerged and floating-leaf vegetation.

Date Surveyor	**Sept/Oct 2015** MN DNR	**August 2016** MN DNR	**July 2017** MN DNR	**June 2018** MN DNR	**July 2018** MN DNR	**Sept 2018** MN DNR	**June 2019** MN DNR	**July 2019** MN DNR	**Sept 2019** MN DNR	**July 2020** MN DNR	**Sept 2020** MN DNR
Total # Points Sampled	331	325	351	297	365	332	321	336	303	348	330
Max Depth of growth (95%) in feet	10	10.1	11	12.9	12.4	11	13.3	11	10.5	10	11
Max Depth of Starry Stonewort – feet	12.1	12.2	19.0	15.4	14.5	12.0	16.0	12.5	11.5	12.0	15.0
# Points in Littoral (0-15 feet)	297	287	304	280	301	297	292	305	294	309	293
% Points w/Submersed Native Taxa	79	72	79	85	67	67	69	65	45	69	65
% Points w/Submersed Non-native Taxa	18	30	24	36	43	58	55	55	45	54	68
Mean Submersed Native Taxa/Point	1.5	1.1	1.5	1.1	1.0	0.9	1	1.1	0.6	1.2	1.0
# Submersed Native Taxa	14	14	15	9	14	11	12	16	10	17	14
# Submersed Non-Native Taxa	2	1	2	2	1	1	2	2	2	2	2
Mean Abundance of Starry Stonewort	1.7	1.7	1.7	1.7	1.8	1.7	1.3	1.8	1.6	1.8	2.1
% Points w/ Starry Stonewort	17	30	22	32	43	58	45	53	51	49	66
% Points w/ Abundant Starry Stonewort	24	44	1	15	17	21	1	19	13	22	43
% Points w/ Common Starry Stonewort	20	21	72	43	48	28	24	42	33	37	19
% Points w/ Sparse Starry Stonewort	56	35	27	42	35	51	74	38	54	41	38
% Points w/ Muskgrass and Starry Stonewort	4	12	11	18	12	28	20	23	16	28	32
% Points w/ Muskgrass	61	59	57	72	42	51	45	42	28	43	44
Mean Abundance of Muskgrass	2.1	1.8	1.7	1.9	1.8	1.5	1.6	1.7	1.4	1.7	1.7

Table 4. Lake wide point-intercept plant frequency of occurrence for submersed and floating vegetation within the littoral zone (0-15 feet) in Lake Koronis (DOW# 73020002), Stearns County. Values shaded in light brown represent lake wide management (2018 and 2019). Abbreviations include Lake Ecology Unit (LEU), Invasive Species Program (ISP), and BWS (Blue Water Science). **Species not recorded in this table, although less than 5% frequency of occurrence, include:** *Elodea Canadensis, Heteranthera dubia, Najas species, Nitella species, Potamogeton illinoensis, Potamogeton freisii, Potamogeton praelongus, Potamogeton robbinsii, Potamogeton zosteriformis, Zannichellia palustris, Nuphar variegata, Nymphaea odorata.*

Taxonomic Name	**Common Name**	**SEP/OCT 2015**	**AUG 2016**	**JULY 2017**	**JUNE 2018**	**JULY 2018**	**SEPT 2018**	**JUNE 2019**	**JULY 2019**	**SEPT 2019**	**JULY 2020**	**SEPT 2020**
Surveyor		MN DNR (ISP)	MN DNR (ISP)	MN DNR (LEU/ISP)	MN DNR (ISP)	MN DNR (LEU/ISP)	MN DNR (ISP)	MN DNR (ISP)	MN DNR (LEU/ISP)	MN DNR (ISP)	MN DNR (LEU/ISP)	BWS
SUBMERSED NON-NATIVE												
Nitellopsis obtusa	Starry stonewort	17	30	22	32	4	58	49	53	51	49	66
Potamogeton crispus	Curly-leaf pondweed	2	0	3	6	0	0	13	3	2	11	5
SUBMERSED NATIVE												
Ceratophyllum demersum	Coontail	14	15	8	15	20	16	13	14	14	11	13
Chara sp.	Muskgrass	61	59	57	72	42	51	45	42	28	43	44
Myriophyllum sibiricum	Northern watermilfoil	15	5	0	0	1	1	2	1	0	1	1
Potamogeton richardsonii	Clasping-leaf pondweed	6	4	2	1	4	2	1	2	2	2	3
Potamogeton spp.	Narrowleaf pondweed	4	0	31	19	19	0	8	11	0	17	4
Stuckenia pectinata	Sago pondweed	17	10	28	0	3	11	24	21	9	27	23
Vallisneria americana	Water celery	21	16	7	0	6	5	0	3	4	4	6

Using a chi-square analysis, starry stonewort and the most dominant native species were compared prior to management (2015) and following three years of management (2020). Overall, the invasive taxa significantly increased lake wide from 17% (Sept. 2015) to 66% (Sept. 2020; Table 5). During this same period, the percent occurrence of submersed native taxa lake wide reduced from 79% to 65%. Muskgrass, northern water-milfoil, and wild celery significantly declined over the last six years (Table 4). In general, native pondweeds and coontail did not show any apparent trends. Between 2015 and 2020, the mean submersed native taxa per point decreased from 1.5 to 1.0 (Table 5). Similar decreases in the frequency of native submersed aquatic plants such as northern water-milfoil and wild celery also occurred in the reference plot (Table 6 and Table ***7***), with the exception of muskgrass which only indicated a slight decline of 6% over the 5 years.

Table 5. Comparison of point-intercept metrics for both lakewide and reference between 2015 and 2020 in Lake Koronis, Stearns County (DOW# 73020002).

Area	**Date**	**Sept/Oct 2015**	**Sept 2020**
	Surveyor	MN DNR	MN DNR
Lakewide	% Points w/ Submersed Native Taxa	79	65
	% Points w/ Submersed Non-native Taxa	18	68
	Mean Submersed Native Taxa/ Point	1.5	1.03
	% Points w/ Starry Stonewort	17	66
	% Points w/ Muskgrass	61	44
Reference	% Points w/ Submersed Native Taxa	91	86
	% Points w/ Submersed Non-native Taxa	9	89
	Mean Submersed Native Taxa/ Point	1.4	1.2
	% Points w/ Starry stonewort	9	89
	% Points w/ Muskgrass	86	80

Table 6. Reference plot summary of point-intercepts metrics for Lake Koronis, Stearns County (DOW#73020002). Values shaded in grey were calculated from littoral depth range. Taxa includes submerged and floating-leaf vegetation. Abbreviations include Lake Ecology Unit (LEU), Invasive Species Program (ISP), and BWS (Blue Water Science).

Survey Metrics	**SEPT/OCT 2015**	**AUG 2016**	**JULY 2017**	**JUNE 2018**	**JULY 2018**	**SEPT 2018**	**JUNE 2019**	**JULY 2019**	**SEPT 2019**	**JULY 2020**	**SEPT 2020**
Surveyor	MN DNR	MN DNR	MN DNR	MN DNR	MN DNR	MN DNR	MN DNR	MN DNR	MN DNR	MN DNR	BWS
Total # Points Sampled	36	36	36	36	36	36	36	36	36	36	36
Max Depth of Growth (95%) in feet	7.3	7.7	8	12.1	9.1	8.4	NA	NA	NA	8	9
# Point in Max Depth Range	29	30	30	32	29	31	30	30	30	33	32
Max Depth of SSW in feet	8.6	7.5	8	15.4	11.9	9	9	11	9	8	7
# Points in Littoral (0-15 feet)	35	35	36	36	35	35	35	36	36	36	35
% Points w/ Submersed Native Taxa	91	94	92	92	91	86	91	89	58	86	86
% Points w/ Submersed Non-Native Taxa	9	9	6	11	26	54	26	67	61	56	89
Mean Submersed Native Taxa/ Point	1.4	1.1	1.2	1.1	1.2	1	1.2	1.4	0.7	1.2	1.2
# of Submersed Native Taxa	6	7	4	4	6	4	3	7	4	6	6
# of Submersed Non-native Taxa	1	1	1	1	1	1	2	2	1	2	1
Mean Abundance of Starry Stonewort	2.3	1.7	2	1.8	1.4	1.6	1.3	1.4	1.5	0.8	1.5
% Points w/ Starry Stonewort	9	9	6	11	26	54	23	64	61	50	89
% Points w/ Muskgrass	86	86	75	86	80	80	80	69	50	72	80

Table 7. Reference plot point-intercept plant frequency of occurrence for submersed and floating vegetation within the littoral zone (0-15 feet) in Lake Koronis, Stearns County (DOW# 73020002).

Taxonomic Name	**Common Name**	**SEP/OCT 2015**	**AUG 2016**	**JULY 2017**	**JUNE 2018**	**JULY 2018**	**SEPT 2018**	**JUNE 2019**	**JULY 2019**	**SEPT 2019**	**JULY 2020**	**SEPT 2020**
Surveyor		MN DNR (ISP)	MN DNR (ISP)	MN DNR (LEU/ISP)	MN DNR (ISP)	MN DNR (LEU/ISP)	MN DNR (ISP)	MN DNR (ISP)	MN DNR (LEU/ISP)	MN DNR (ISP)	MN DNR (LEU/ISP)	BWS
SUBMERSED NON-NATIVE												
Nitellopsis obtusa	Starry stonewort	9	9	6	11	26	54	23	64	61	50	89
SUBMERSED NATIVE												
Ceratophyllum demersum	Coontail	0	3	0	3	6	3	3	19	8	6	3
Chara sp.	Muskgrass	86	86	75	86	80	80	80	69	50	72	80
Myriophyllum sibiricum	Northern watermilfoil	14	3	0	0	3	0	0	0	0	0	0
Potamogeton spp.	Narrowleaf pondweed	14	0	11	22	23	0	0	11	0	14	3
Stuckenia pectinata	Sago pondweed	11	9	31	0	0	14	40	22	11	22	20
Vallisneria americana	Water celery	11	9	0	0	3	0	0	0	0	0	0

Within the year of treatment during 2018 and 2019, we used a subset of the whole lake point intercept sampling points to assess various control methods towards starry stonewort and non-target native species. All plots for copper and copper + mechanical were combined to compare in June (pre-treatment) and September (post-treatment). A non-managed reference was designated as the control for our study. For our analyses, we used a chi-square test ($P \leq 0.05$, Madsen 1999) to detect changes in the frequency of starry stonewort or native submersed species for copper, copper + mechanical and the reference plot. Following management, significant increases in starry stonewort frequency were only observed in the reference plot (Figure 11). This suggests that although the growth and frequency of starry stonewort increased in the management plots, management actions had likely suppressed starry stonewort within the treated areas. In managed plots, native plant frequency significantly declined with the exception of the algaecide plots in 2019 (Figure 12), which this treatment was comparably smaller than 2018.

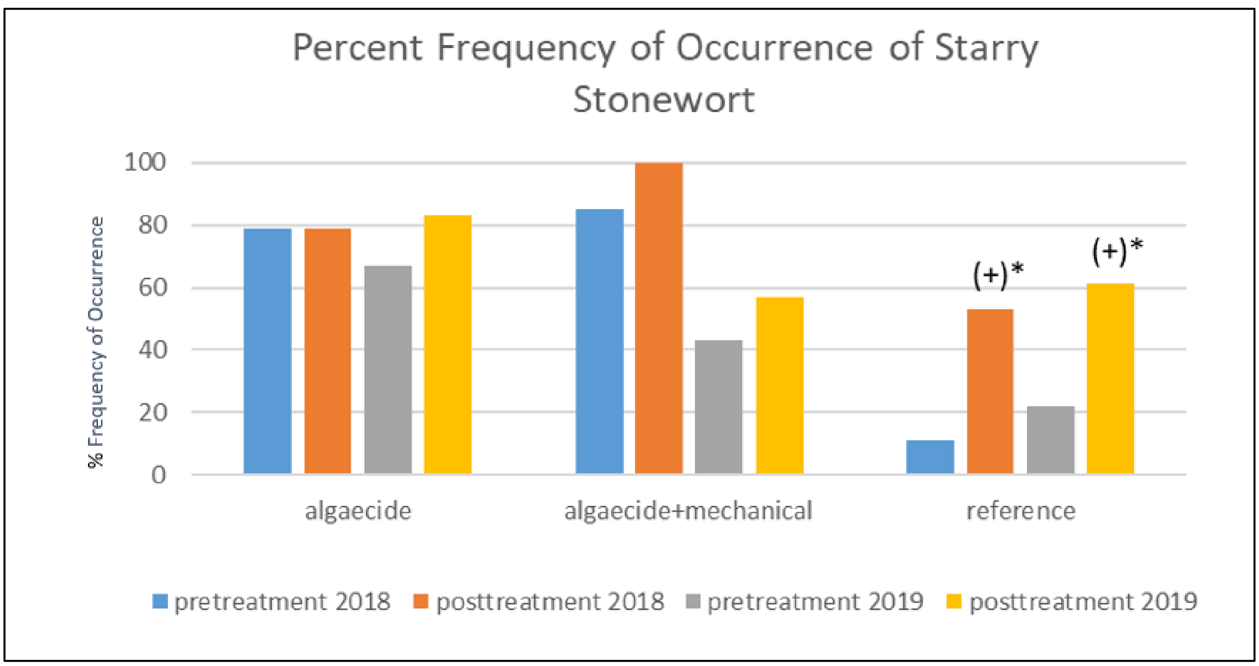

Figure 11. Percent frequency of occurrence of starry stonewort pre-treatment and post-treatment in 2018 and 2018 within copper (algaecide), copper + mechanical and reference plots in Lake Koronis, Stearns County (DOW# 73020002).

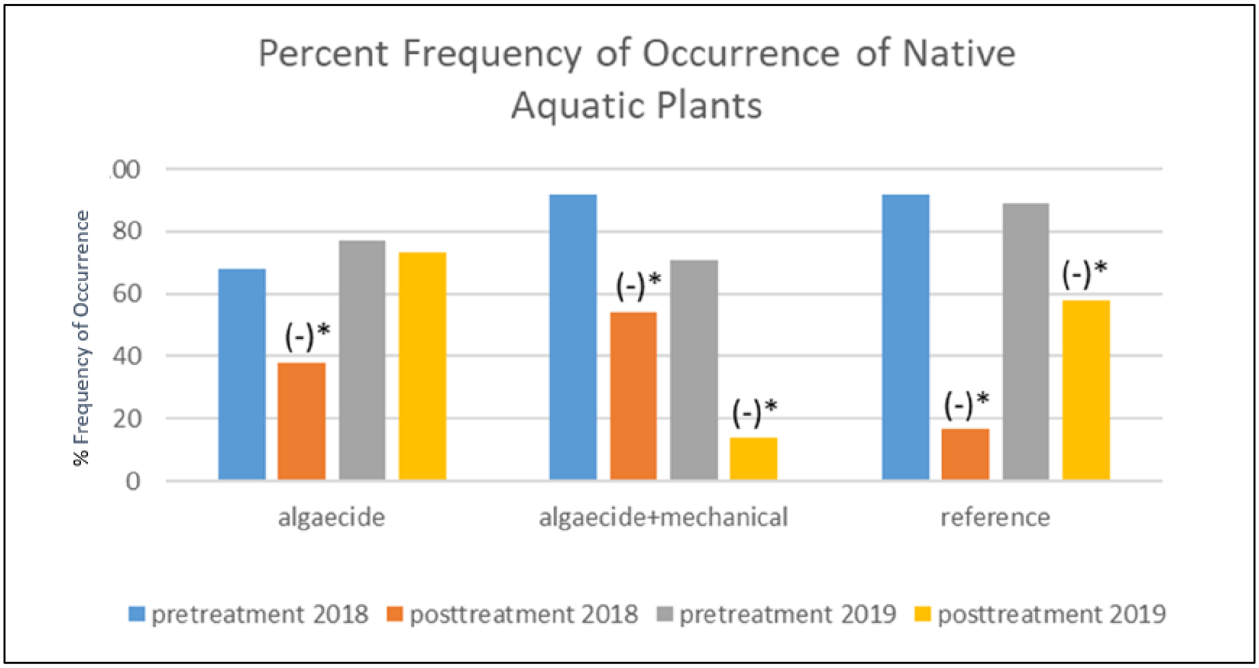

Figure 12. Percent frequency of occurrence of native aquatic plants pre-treatment and post-treatment in 2018 and 2018 within copper (algaecide), copper + mechanical and reference plots in Lake Koronis, Stearns County (DOW# 73020002).

Evaluation of biomass (wet-weight) measurements

A reduction of the abundance (biomass) of starry stonewort was achieved during a single growing season (2019). We sampled biomass using wet-weight measurements (Figure 13). Reductions in biomass occurred in all management plots during 2019 (Figure 14). For our statistical analysis, we used a Wilcoxon 2-sample rank sum test to analyze differences in native plants and starry stonewort both pre and post management. Significant biomass (g/m^2) reductions of starry stonewort were observed in both the copper and copper+ mechanical plots after the initial and second treatment. However, biomass did not change between the initial and second treatments (copper P = 0.898, copper + mechanical (P = 0.260). This may suggest that the method and/or timing of treatment was not as effective during the second treatment or more likely the biomass had already been reduced considerably from the initial treatment and therefore less evident after the second treatment. The mechanically harvested plot (A1) also showed significant declines post-management (two weeks post-harvest). During the same period, starry stonewort increased in the reference plot, but not at a significant level. Biomass of native aquatic plants were so minimal that the data was not analyzed.

Figure 13. Vegetation biomass (wet- weight measurements) sampling in Lake Koronis, Stearns County (DOW# 73020002). MN DNR surveyors used method developed by Bickel and Perrett (2015).

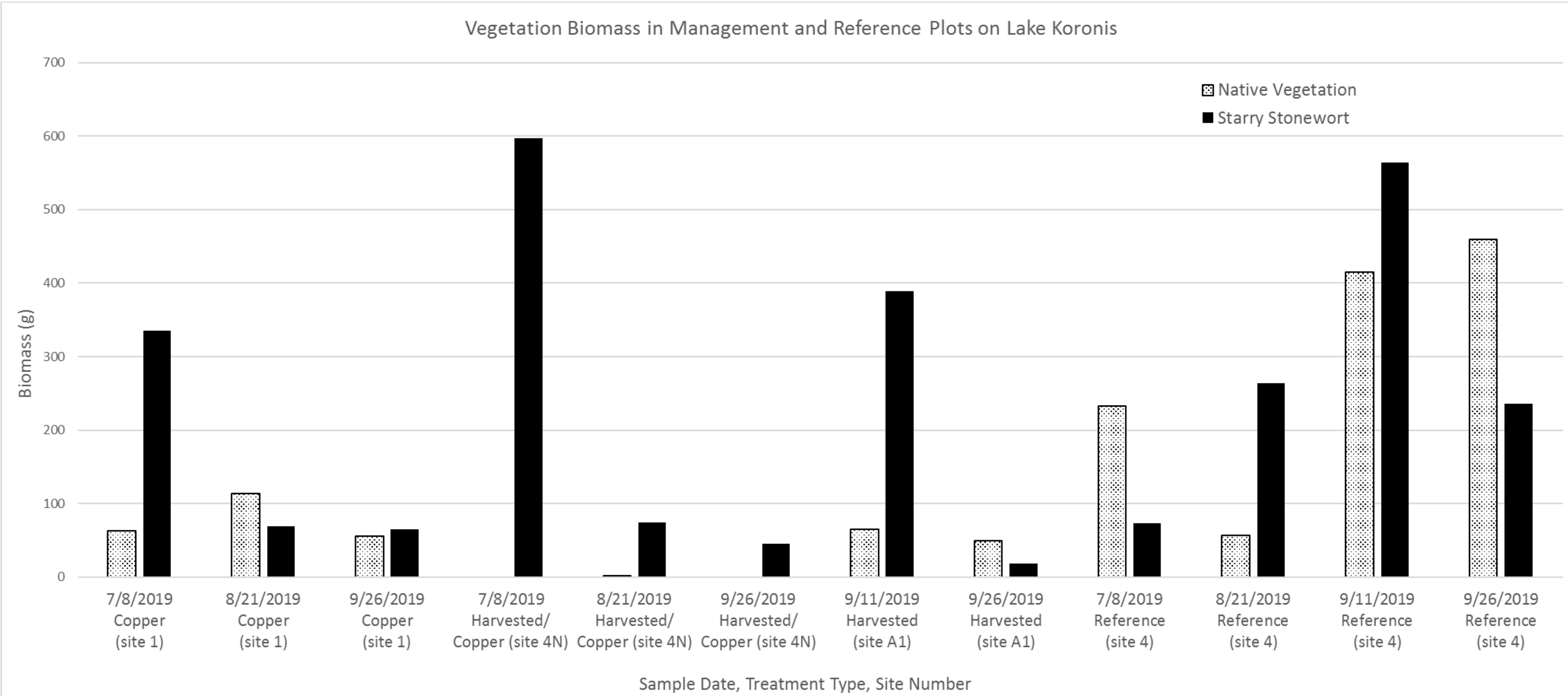

Figure 14. Vegetation biomass measurements in Lake Koronis, Stearns County (DOW# 73020002). Biomass was sampled in the large copper (algaecide) treatment (Site 1), combined management area (Site 4N), mechanically harvested area (A1) and the reference (Site 4). Copper treatments in 2019 occurred on August 5 and September 9 in Plot 1 & August 7 and September 10 for Plot 4N (combined management plot). Mechanical harvesting occurred between pre and post sampling dates in for harvested plots. Sample size varied based on size of management plots: copper plots (N= 10), reference plots (N= 10), copper and mechanical harvested plots (N= 10), and mechanical harvested plots (N= 11).

Starry stonewort bulbil assessment

Overall, results from the bulbil counts suggest copper treatments did not reduce bulbil density following treatments. Bulbil densities collected from the sediment were highly variable both spatially and among years, with mean bulbil densities ranging from 299 to 3,044 bulbils m^{-2} in the copper plots and 32 to 646 bulbils m^{-2} in the reference plot (Figure 15). Across all four of the treatments (i.e., the initial and second treatment from each year, 2018 and 2019), there was little evidence of a negative treatment effect on bulbil densities in the simplest model including a "treatment effect" that directly compares the change in bulbil densities with treatment between reference and copper treated plots (Count ~ Plot Type*Sampling Timing pre- or post-treatment). On average over the four treatments, bulbil densities were higher in copper treated plots ($P = 0.04$) and did not change significantly in reference plots ($P = 0.5$). However, it is difficult to rigorously account for these sources of variation and treatment-specific variation in treatment effect as models quickly became over fitted given our study design. Moreover, we conclude that our results support past findings (e.g., Gilsson et al. 2018) that copper treatments as currently administered likely are not reducing bulbil densities. We argue that further research is warranted to better understand if bulbil densities are more driven by temporal differences, plant physiology and phenology rather than by impacts from the copper treatments.

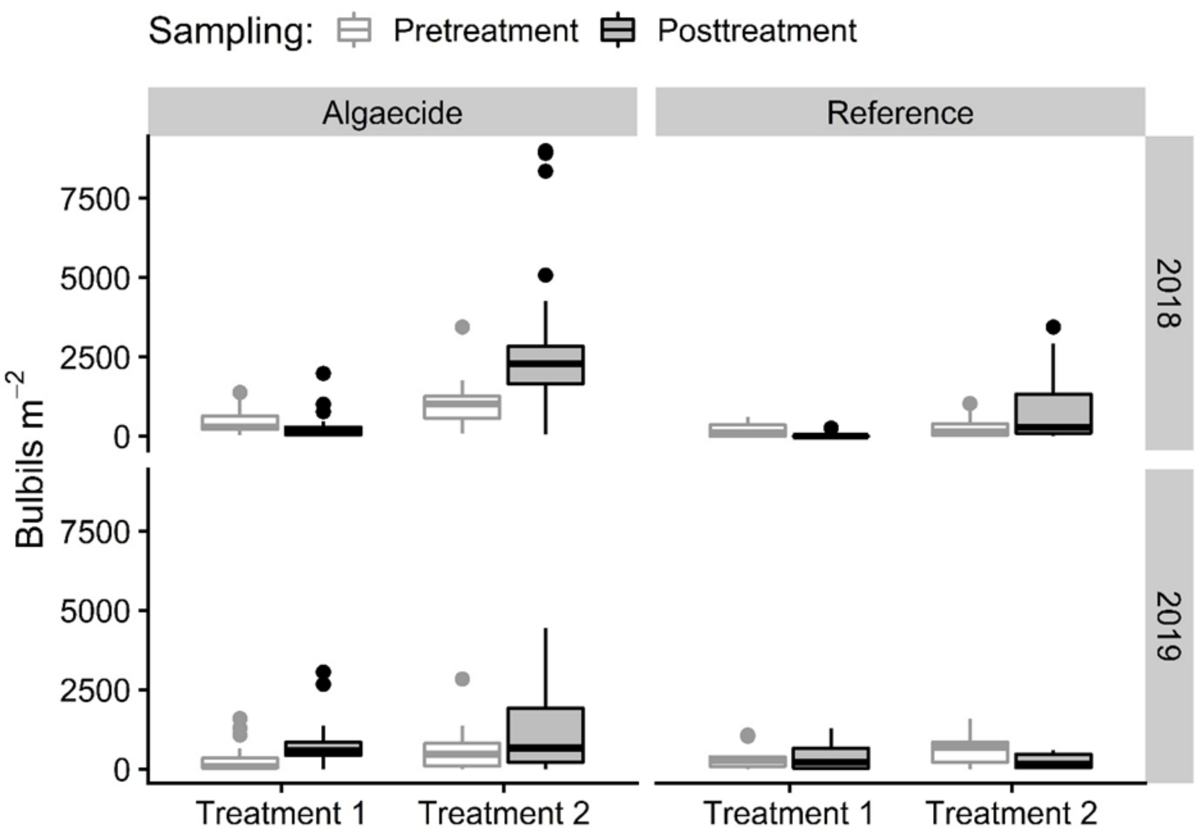

Figure 15. Bulbil densities (bulbils m-2) in the algaecide (copper) and reference plots for 2018 and 2019 in Lake Koronis, Stearns County (DOW# 73020002).

Macroinvertebrate analysis

In conjunction with this study, we examined the impacts to aquatic macroinvertebrates and determined that copper had a significant impact to macroinvertebrates in the algaecide plots, resulting in major reductions in abundances to Mollusca, several Trichoptera taxa, Amphipoda, and total invertebrates (G. Montz et al., Minnesota Department of Natural Resources, 2020). These reductions were observed in both years, with many of the groups declining after the first treatment. Abundances of Mollusca, Amphipoda, Trichoptera and total invertebrates declined significantly following the first treatment in 2018. The first copper treatment in 2019 had significant declines in many of the same groups, with the exception of total Trichoptera. In contrast, there was relatively little change in macroinvertebrate densities in the untreated reference plots.

Mollusca abundances were not significantly reduced either year after the second copper treatment; however, the abundance of this group was extremely low after the first copper application and had not recovered by the pre-treatment period of the second copper application.

The second application in 2018 occurred in late September, a time when water temperatures were declining. Eisler (1998) suggested that mortality of aquatic species is greatest under higher water temperatures. Bat et al. (2000) reported that toxicity of three metals (including copper) decreased with declining water temperatures for a common amphipod. Thus, it is possible that the reduced water temperatures in the fall application lessened the impacts of the copper treatments. Results from copper monitoring in the summer application showed that copper levels had declined rapidly within the first 24 hours; thus, the rapidly declining copper levels combined with cooler fall water temperatures may have reduced toxicity for some invertebrates.

The reductions seen in these invertebrate groups and taxa are not surprising and agree with impacts reported in literature. Snails and amphipods are two groups that are often reported as sensitive or moderately sensitive to copper (Brix et al. 2011, Eisler 1998, USEPA 2007, Phipps et al. 1995). Mastin and Rodgers (2000) concluded that the manufacturers' recommended application rates for three commercial copper products were close to 48 hour LC50's for four aquatic invertebrates studied, including the amphipod *Hyalella azteca*.

These data suggest that the copper treatments had significant negative impacts to the aquatic invertebrate taxa in Lake Koronis, with significant reductions occurring after the first copper application and persisting in some invertebrate taxa into fall. These impacts can be viewed as seasonal, as the invertebrate life cycles are short enough that they could be expected to repopulate areas in the coming summer, particularly in smaller area treatments. At this time, long-term negative impacts could not be evaluated because the length of the study. In a study of a small chain of lakes in Minnesota which received regular applications of copper over multiple years, Hanson and Stefan (1984) found multiple ecosystem impacts, including: a) copper accumulation in the sediments, b) tolerance adjustments of certain species of algae to higher copper sulfate dosages, c) shift of species from green to blue-algae and from game fish to rough fish, d) disappearance of macrophytes and e) reductions in benthic macroinvertebrates. However, the application rates in that study far exceeded that which was seen in the present study and may not be comparable.

Repetitive use of copper for regularly occurring situations in lakes, such as in the case of starry stonewort control in Lake Koronis, can have immediate and possibly long-term impacts to the lake benthic community. Resource managers should carefully consider the negative impacts to the invertebrates of the lake food web base when considering permits for large area or annual use of copper in lakes. Small treatment areas over a short term (one or two seasons) could help prevent long-term damage to the lake ecosystem.

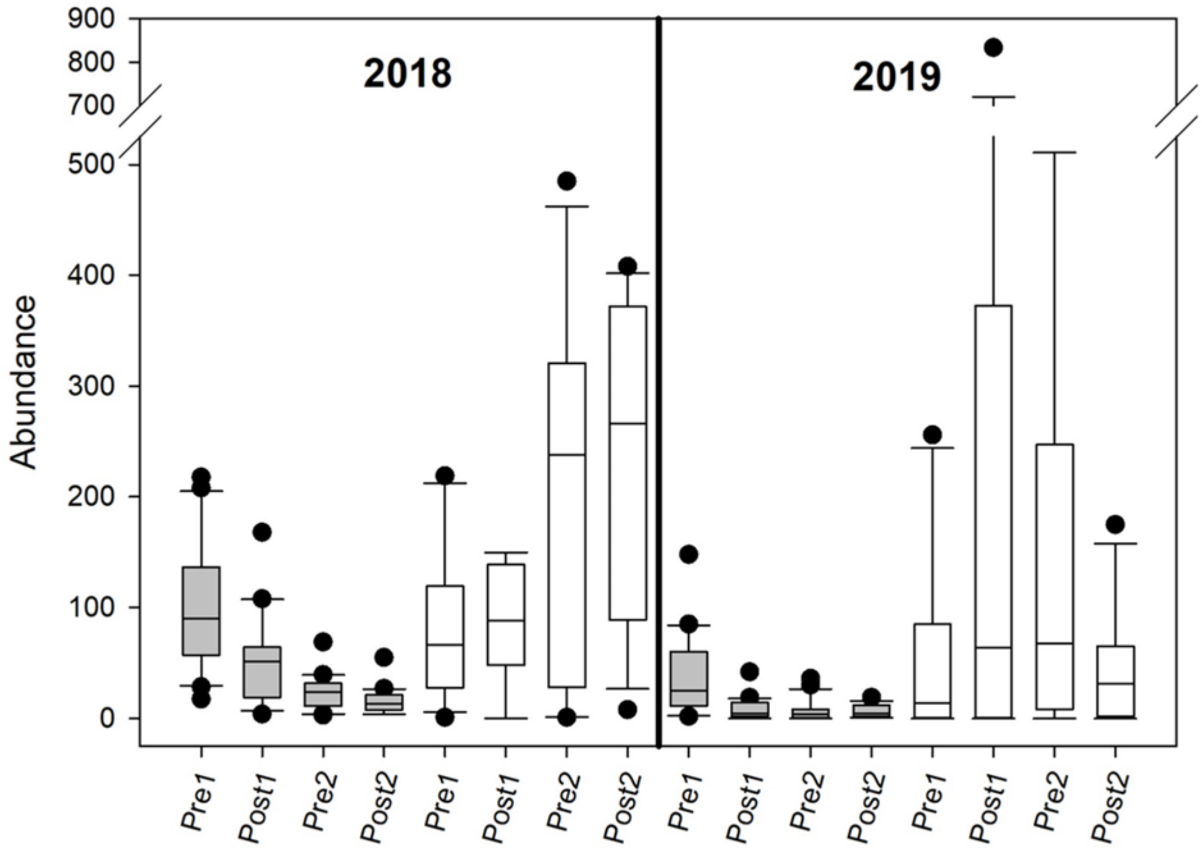

Figure 16. Mollusca abundance in pre- and post-treatments for treatment and reference sites within two copper (algaecide) treatments in Lake Koronis, Stearns County (DOW# 73020002) in 2018 and 2019. Shaded boxes are treatment samples, open bars are reference samples.

Discussion

This study evaluated partial- lake large-scale management of starry stonewort. Management efforts were unable to reduce the spread or frequency of starry stonewort lakewide, however biomass was suppressed and significantly reduced in the management plots through repeated copper treatments and harvesting. Additionally, we observed substantial decreases in the frequency of submersed native plants, specifically muskgrass. Increases in starry stonewort are likely due to a combination of the invasiveness of starry stonewort, its competitive advantage over native counterparts, non-selective management and environmental factors. Nonetheless, these efforts provided some nuisance relief of starry stonewort during the growing season, although annual re-growth remains problematic for lake managers. Bulbil density was not impacted during this study and will continue to promote the spread and persistence of starry stonewort in Lake Koronis.

Displacement through starry stonewort invasivion caused declines in the frequency of occurrence and abundance of native taxa, primarily muskgrass in Lake Koronis. The results of this report are consistent with invasion impacts research conducted by the University of Minnesota in Lake Koronis (Wagner et al., 2017); whereas significant effects of starry stonewort on native species were also observed. Starry stonewort has the ability to displace native vascular plants or algae because its ability to fill the water column at shallow depths, causing native plants or algae to become light limited (Larkin et al., 2018). Starry stonewort has shown to have a competitive advantage over muskgrass, since starry stonewort persisted at sampling points where both species co-existed in managed plots in this study. Starry stonewort was highly competitive over muskgrass species through displacement of muskgrass beds in non-managed areas during the research conducted above (Wagner et al., 2017). Non-selective management using copper also directly impacted its native counterpart (muskgrass), as well as damaged vascular plants. Unintentional removal of native aquatic plant taxa via mechanical harvesting could also be a contributing factor. Declines in water clarity and other environmental variables, such as precipitation (high water levels) and weather can have an impact on both invasive and native aquatic plant growth on an annual basis.

In terms of aquatic management goals, maintaining or lowering the frequency of occurrence of these invasive taxa has not been attainable to date. Moreover, management has not lead to the increase and diversity of native aquatic plants. Starry stonewort has shown temporary reductions in biomass across all management types, although re-growth of starry stonewort during the growing season remains problematic when managing this species. Moreover, the evaluation of the treatment effectiveness on bulbils (reproductive structures) did not indicate a reduction or a correlation between bulbils and copper treatments. In addition, past observations from bulbil sampling during the winter in Lake Koronis indicated that the bulbils persist in copper-treated plots through the winter. Since bulbil density was not impacted during this study, the continued spread and persistence of starry stonewort in Lake Koronis is likely to occur. It is important to consider the difficulty of managing a species with large, dense infestations. It may be more attainable to focus on reducing interference with recreation and access. Currently, implementing management strategies to reduce biomass through mechanical and/or copper means have proven to be an effective option for short-term relief, although careful consideration of timing and frequency and duration of copper treatments is important for minimizing harm to native biota. Our findings suggest that that the challenges with long-term population control are likely related to the species' bulbils, which we recommend as a key area of future research for understanding how to control starry stonewort over multiple years.

Literature Cited

Bat, L., M. Akbulut, M. Culha, A. Gundogdu, and H.H. Satilmis. 2000. Effect of temperature on the toxicity of zinc, copper and lead to the freshwater amphipod *Gammarus pulex* (L. 1758). Turkish Journal of Zoology 24: 409 – 415.

Bickel, T. O., & Perrett, C. (2016). Precise determination of aquatic plant wet mass using a salad spinner. *Canadian Journal of Fisheries and Aquatic Sciences*, *73*(1), 1–4.

Brix, K.V., A.J. Esbaugh and M. Grosell. 2011. The toxicity and physiological effects of copper on the freshwater pulmonate snail, *Lymnaea stagnalis*. Comparative Biochemistry and Physiology, Part C 154: 261 – 267.

Carpenter, S. R. (1990). Largescale perturbations: Opportunties for innovation. *Ecology*, *71*, 2038–2043.

Crow G, Hellquist C. 2000a. Aquatic and wetland plants of northeastern North America. Vol. 1. Pteridophytes, Gymnosperms and Angiosperms: Dicotyledons. University of Wisconsin Press, Madison, WI. 480 pp.

Crow G, Hellquist C. 2000b. Aquatic and wetland plants of northeastern North America. Vol. 2. Angiosperms: Monocotyledons. University of Wisconsin Press, Madison, WI. 400 pp.

Eisler, R. 1998. Copper hazards to fish, wildlife and invertebrates: a synoptic review. U.S. Geological Survey, Biological Resources Division, Biological Science Report USGS/BRD/BSR-1997-0002. 98 pp.

Glisson, W. J., Wagner, C. K., McComas, S. R., Farnum, K., Verhoeven, M. R., Muthukrishnan, R., & Larkin, D. J. (2018). Response of the invasive alga starry stonewort (*Nitellopsis obtusa*) to control efforts in a Minnesota Lake. *Lake and Reservoir Management*, *34*(3), 283- 295.

Hanson M, Stefan H. 1984. Side effects of 58 years of copper sulfate treatment of the Fairmont Lakes, Minnesota. J. Am. Water Resour. Assoc. 20:889-900.

Larkin, D. J., Monfils, A. K., Boissezon, A., Sleith, R. S., Skawinski, P. M., Welling, C. H., & Karol, K. G. (2018). Biology, ecology, and management of starry stonewort (*Nitellopsis obtusa*; Characeae): A Red-listed Eurasian green alga invasive in North America. *Aquatic Botany*, *148* (2018), 15–24.

Madsen, J. D. (1999). Point Intercept and Line Intercept Methods for Aquatic Plant Management. In *Aquatic Plant Control Technical Note MI-02* (Issue February, pp. 1–16).

Mastin, B.J. and J.H. Rodgers, Jr. 2000. Toxicity and bioavailability of copper herbicides (Cleargate, Cutrine-Plus and copper sulfate) to freshwater animals. Archives of Environmental Contamination and Toxicology 39: 445 – 451.

McComas, S. R. (2018). *Lake Koronis, Stearns County, Point Intercept Survey, 2018.* Report prepared for the Koronis Lake Associatioin and Minnesota Department of Natural Resouces. 13pp.

McComas, S. R. (2020). *Lake Koronis, Stearns County, Point Intercept Survey, September 8-9, 2020.* Report prepared for the Koronis Lake Associatioin and Minnesota Department of Natural Resouces.

16 pp.

Nichols, S. J., Schloesser, D. W., & Geis, J. W. (1988). Seasonal growth of the exotic submersed macrophyte *Nitellopsis obtusa* in the Detroit River of the Great Lakes. *Canadian Journal of Botany*, *66*, 116–118.

Phipps, G.L., V.R. Mattson and G.T. Ankley. 1995. Relative sensitivity of three freshwater benthic macroinvertebrates to ten contaminants. Archives of Environmental Contamination and Toxicology 28: 281 – 286.

Simon, S. and Perleberg, D. (2017). *Aquatic vegetation of Lake Koronis (73-0200-02), Stearns County, Minnesota, 2015, 2016, 2017*. Minnesota Department of Natural Resources, Division of Ecological and Water Resources Division, Lake Ecology Unit, Brainerd, MN. 25 pp.

Minnesota Department of Natural Resources. (2017). *Starry stonewort monitoring surveys*. Minnesota Department of Natural Resources, Division of Ecological and Water Resources, Invasive Species Program, St. Paul. 4pp.

R Development Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Schindler, D. W. (1987). Detecting ecosystem responses anthropogenic. *Department of Fisheries and Oceans, Freshwater Institute*, *44* (1), 6–25.

Wagner C., D. Larkin, M. Weber. and P. Mulcahy. (2017). *Starry Stonewort Impacts* [Aquatic Invasive Species Webinar Series]. https://www.youtube.com/watch?v=0mYEH9D3osM&feature=emb_title

Appendix A: Lake Koronis, Stearns Co. Starry Stonewort Distribution Maps 2015-2020

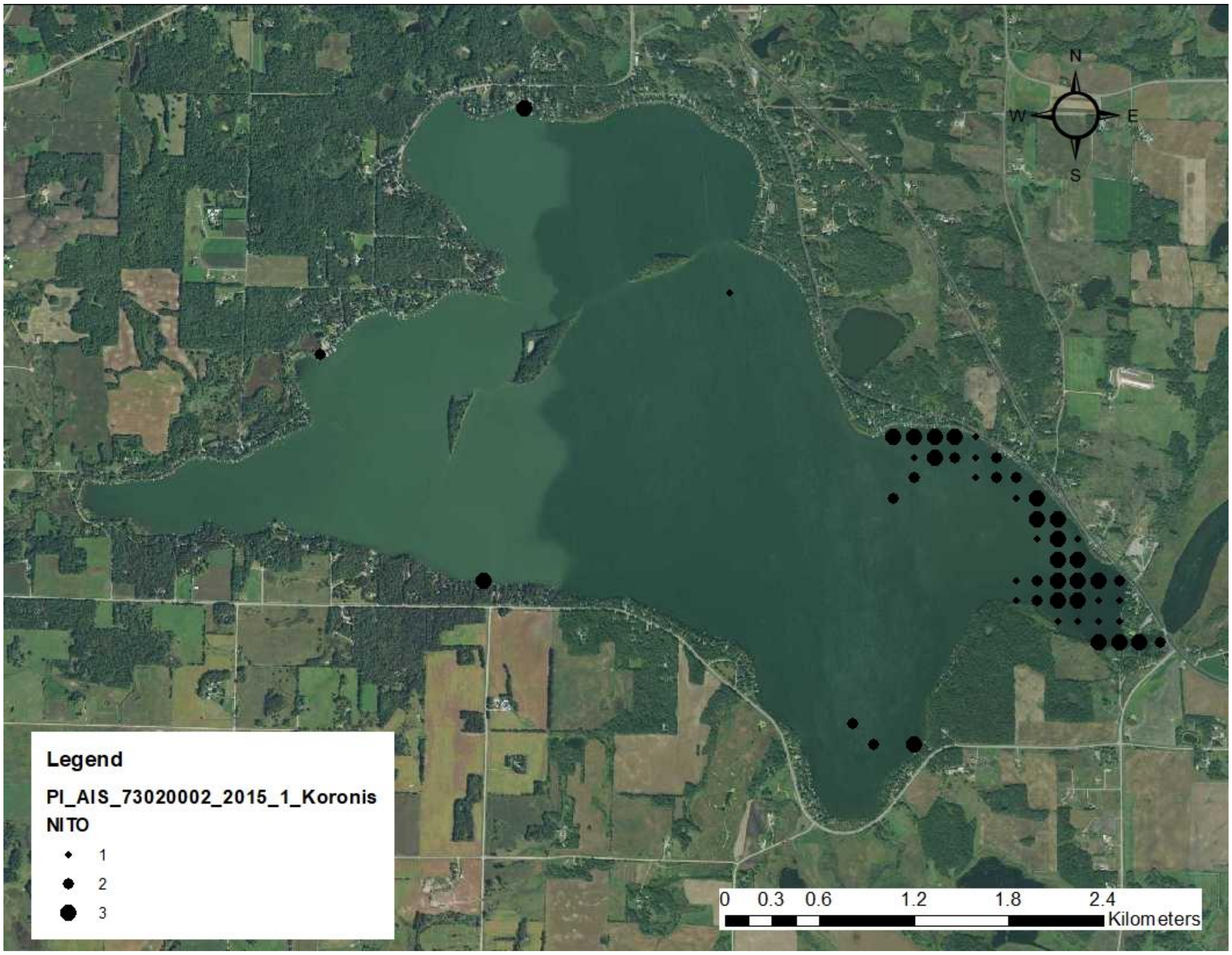

Figure A- 1. 2015 locations and abundance of starry stonewort on Lake Koronis (DOW# 73020002), Stearns County, Minnesota.

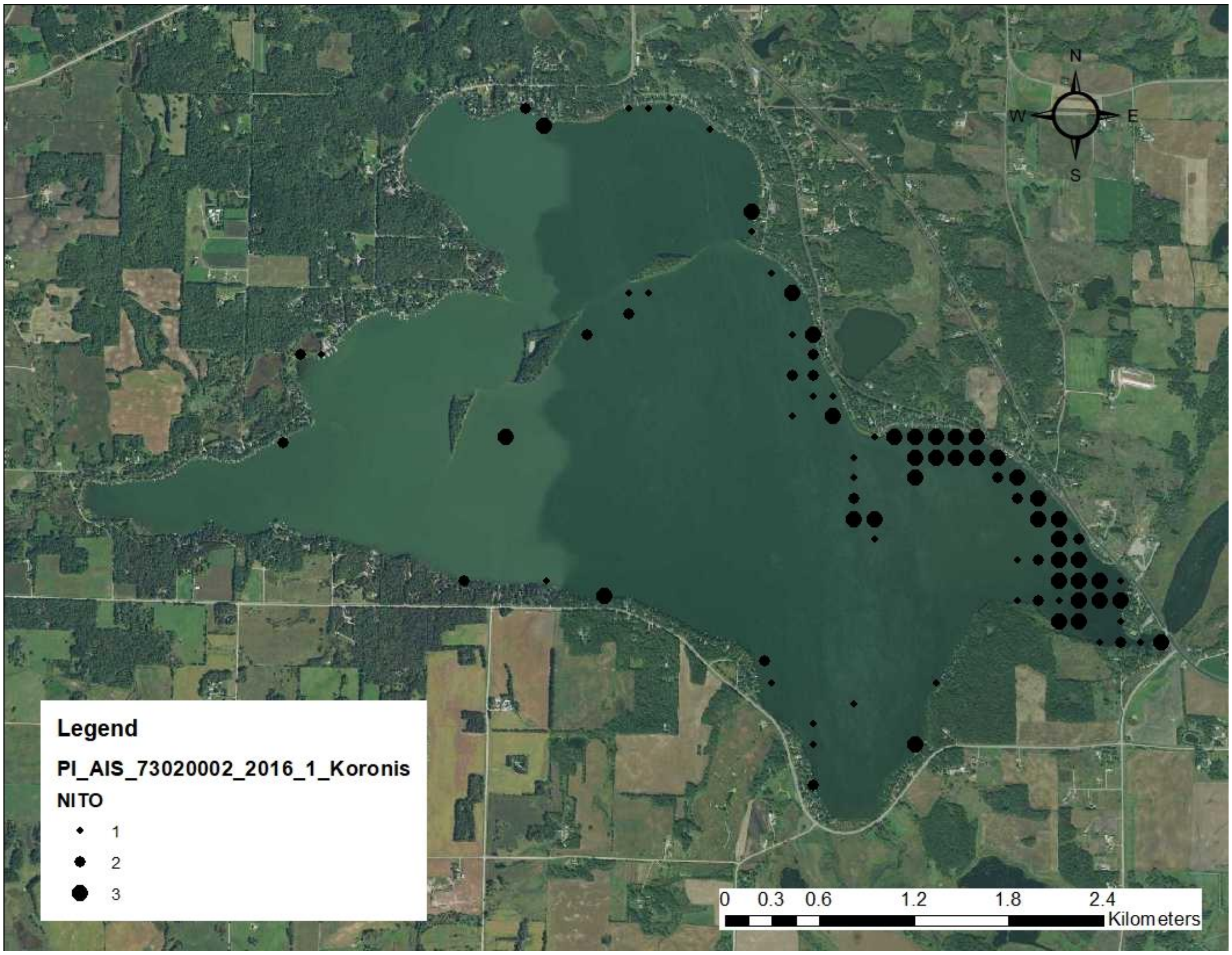

Figure A- 2. 2016 locations and abundance of starry stonewort on Lake Koronis (DOW# 73020002), Stearns County, Minnesota.

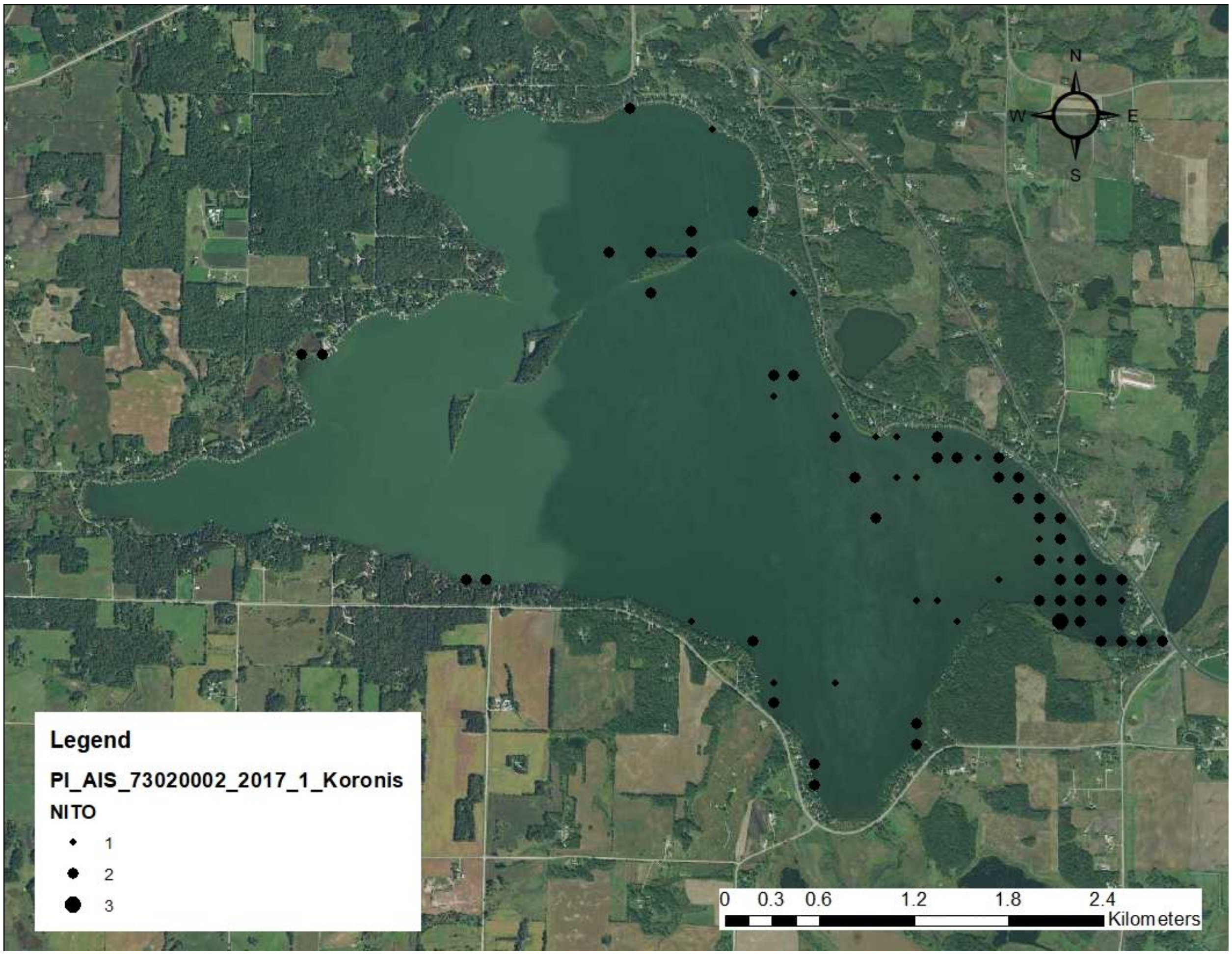

Figure A- 3. 2017 locations and abundance of starry stonewort on Lake Koronis (DOW# 73020002), Stearns County, Minnesota.

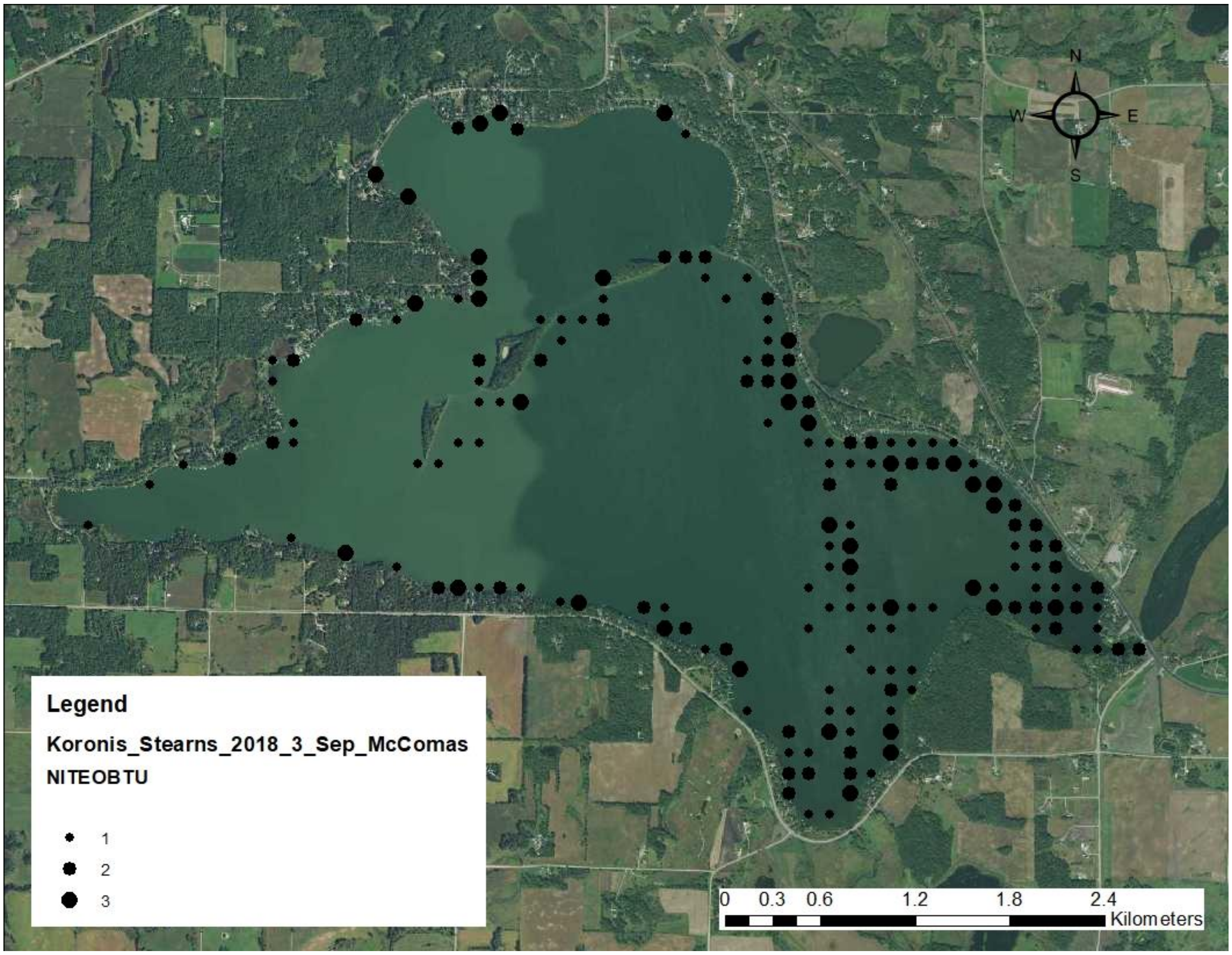

Figure A- 4. 2018 locations and abundance of starry stonewort on Lake Koronis (DOW# 73020002), Stearns County, Minnesota.

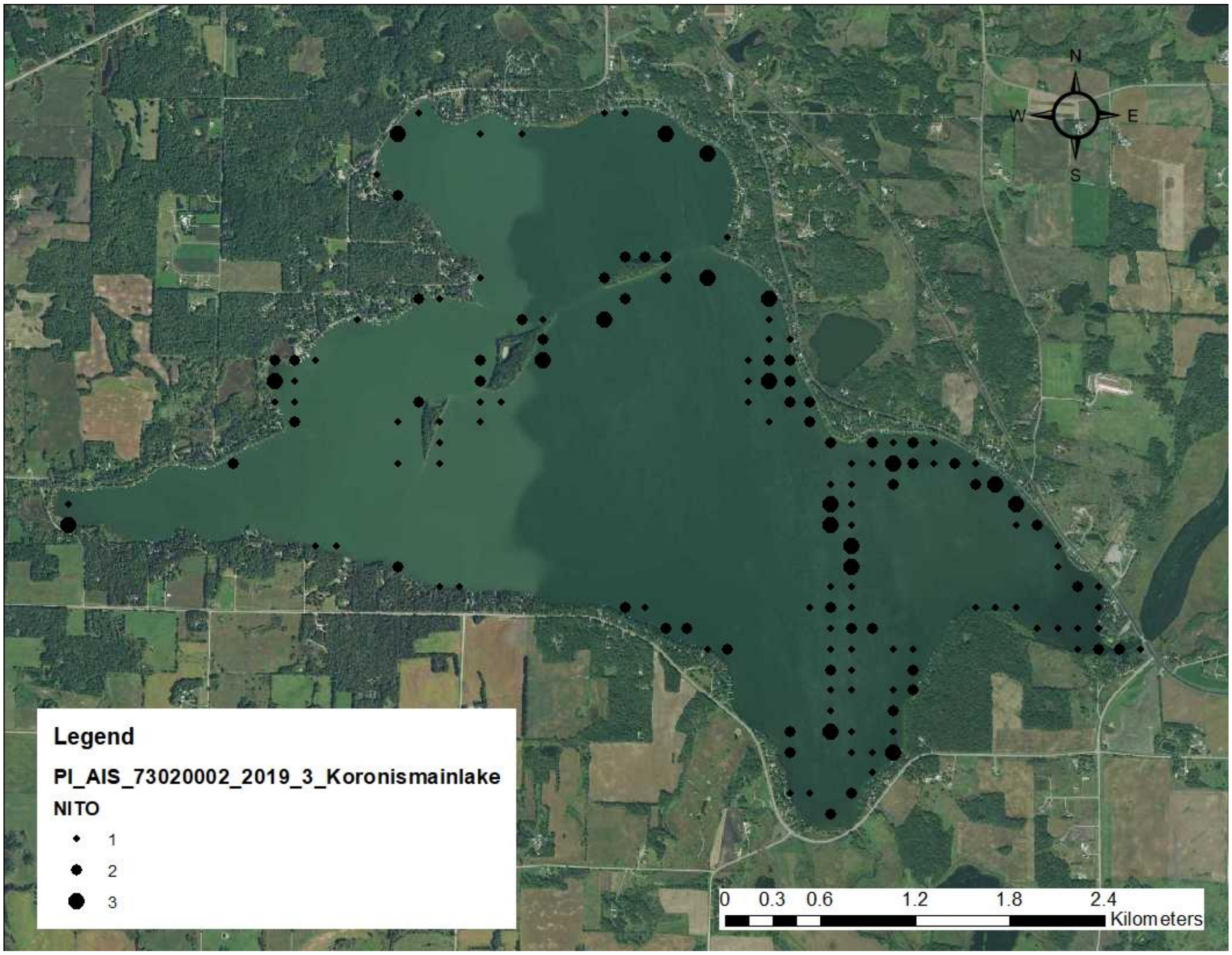

Figure A- 5. 2019 locations and abundance of starry stonewort on Lake Koronis (DOW# 73020002), Stearns County, Minnesota.

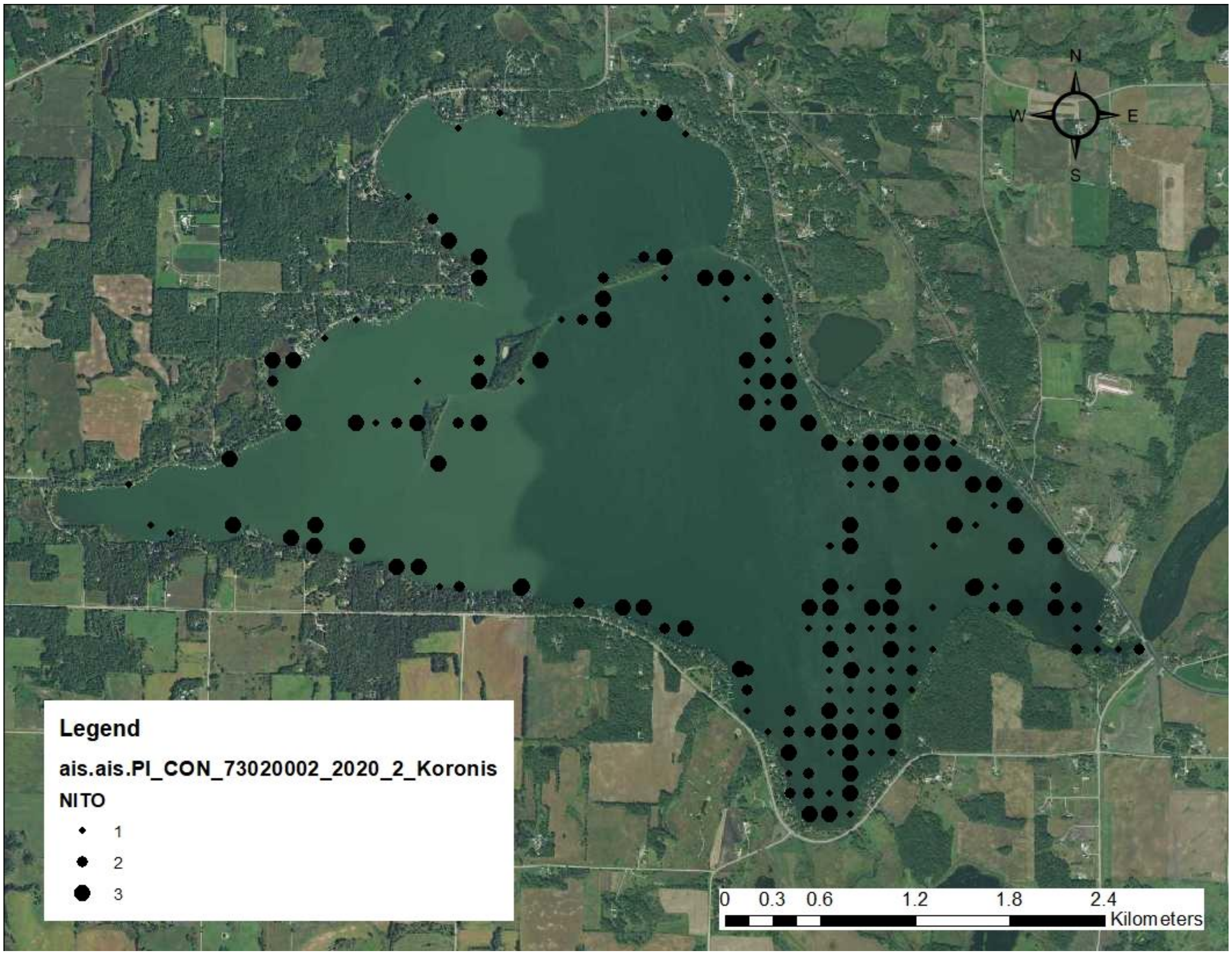

Figure A- 6. 2020 locations and abundance of starry stonewort on Lake Koronis (DOW# 73020002), Stearns County, Minnesota.