Environmental factors influencing biomass and bulbil production of *Nitellopsis obtusa* in Lake Koronis, Stearns County, Minnesota

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ABSTRACT

Starry stonewort [*Nitellopsis obtusa* (Desvaux) J. Groves] is a green macroalga in the family Characeae that is native to Eurasia. In the United States it has invaded many waterbodies across the northern tier from New York to Minnesota. In Minnesota, starry stonewort often grows later into the growing (September to December) season than most native species; however, the environmental factors that influence this life history trait are not well understood. Starry stonewort was harvested every 3 wk from May to November in 2020 and 2021 from four locations in Lake Koronis, Stearns County, MN. Light, water pH, and water temperature were also collected to correlate these variables to starry stonewort growth. Rhizoid biomass had weak negative correlations with temperature ($r_s = -0.19$) and pH ($r_s = -0.14$). Bulbil biomass had weak negative correlations with temperature ($r_s = -0.24$), pH ($r_s = -0.31$), and light transmittance ($r_s = -0.11$). Bulbil density had weak negative correlations with temperature ($r_s = -0.23$), pH ($r_s = -0.29$), and light transmittance ($r_s = -0.10$). Bulbil production was highest between October and November of each year. Bulbil biomass and density was lower in 2020 (4.6 g m^{-2} and 1,229 bulbils m^{-2}) than in 2021 (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 157,000 bulbils m^{-2}. The ability of starry stonewort to grow in dense mats and produce large quantities of bulbils contributes to the difficulty of controlling infestations. Bulbils are a method of spatial and temporal distribution that can allow for recolonization of previously treated areas.

Key words: abiotic, algae, bulbil, life history, light transmittance, pH, starry stonewort, temperature.

INTRODUCTION

Starry stonewort [*Nitellopsis obtusa* (Desvaux) J. Groves] is a green macroalga native to Eurasia in the family Characeae (Groves 1919). Starry stonewort can grow from the sediment to 30 to 120 cm in the water column (Larkin et al. 2018). Branchlets form from the main stem at the nodes in whorls of five to eight branchlets with each branchlet consisting of two or three segments with a total branchlet length up to 9 cm (Larkin et al. 2018). Starry stonewort is dioecious, and as such, gametangia are produced on separate plants. Gametangia arise solitarily or in pairs at the nodes of branchlets. The antheridia are orange to bright red, while the oogonia are bright red to light green and almost spherical in shape (Groves 1919, Boissezon et al. 2017, Larkin et al. 2018). In North America, oogonia bearing starry stonewort have not been observed (Sleith et al. 2015, Larkin et al. 2018). However, starry stonewort was found with oogonia present during lake surveys in Ontario, Canada (Harrow-Lyle et al. 2023). The presence of both antheridia and oogonia-bearing starry stonewort in North America could change the life history strategy of this species and allow for spread via sexual reproduction. Vegetative reproduction currently occurs through development of star-shaped bulbils, from which the common name is derived, that serve as a form of sexual reproduction and allow for dispersion through space and time (Bharathan 1987).

Invasion of North America began around 1978 in the St. Lawrence River where starry stonewort was found throughout the littoral zone, with the greatest abundance at 3 to 5 m. Although starry stonewort has been seen in eutrophic lakes, it occurs most frequently in oligotrophic and mesotrophic water bodies (Stewart 2004, Rey-Boissezon and Auderset Joye 2015, Schneider et al. 2015). Starry stonewort is typically found in areas that are protected from strong currents, have high calcium levels and high conductivity, and are neutral to basic pH (Rey-Boissezon and Auderset Joye 2015, Midwood et al. 2016, Sleith et al. 2018).

The first occurrence of starry stonewort in Minnesota was in Lake Koronis in 2015. It has since spread to 28 other lakes as well as the Mississippi River (Minnesota Department of Natural Resources 2024). The likely cause of overland dispersal in the United States is boats and boating equipment transporting the bulbils and vegetative fragments of starry stonewort (Larkin et al. 2018). Lakes lacking boat launches within areas of high starry stonewort occurrence have lacked the presence of starry stonewort, suggesting that boats and boating equipment may be a major vector for spread and transport of this species (Sleith et al. 2015). The life history of starry stonewort in Minnesota was first documented by Glisson et al. (2022). In most cases,

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aquatic plants will display distinct seasonal patterns in biomass and carbohydrate allocation, wherein energy stores peak and then are depleted after plant growth has occurred (Madsen 1991). It has been stated that starry stonewort fills a late season (September to December) niche (Glisson et al. 2022) in that it grows longer in areas where native species have senesced; however, there has been no explanation for why this might be. Therefore, it was hypothesized that starry stonewort in Lake Koronis grows later into the season (September to December) because of delayed growth initiation and its relationship with higher water temperatures. The objectives of this research were to 1) quantify seasonal biomass allocation patterns of starry stonewort over two growing seasons and 2) determine correlations between environmental factors (light, temperature, and pH) and starry stonewort growth.

MATERIALS AND METHODS

Site description

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-ha lake with a maximum depth of 40 m. Within the 476-ha littoral zone, macrophytes such as *Vallisneria americana* Michx., *Ceratophyllum demersum* L., *Lemna minor* L., and *Nymphaea odorata* Aiton can be found. Four plots (33,000 to 81,000 m^2) were chosen as sampling locations on Lake Koronis based on moderate to high starry stonewort densities, distance from management plots, and water depth (0.5 to 3 m depth contours) that was conducive for sampling based on the accessible depth of the PVC coring device used (Figure 1). The sampling depth was also based on the maximum depth of starry stonewort growth (2.7 m) in Lake Koronis as reported by Glisson et al. (2022).

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 3 wk beginning in late April and continuing to ice cover during the growing seasons of 2020 and 2021. Samples were taken from the four corners of a boat, and then the boat was allowed to drift to a new location within a plot, where four 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. At the laboratory, samples were washed and separated into thalli (aboveground stems with branchlets) biomass, rhizoid biomass, and bulbils. Biomass samples were placed in paper bags and placed in a constant temperature oven at 48 C for at least 48 h 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 was determined for each sampling time across both seasons.

Environmental sampling

Environmental data were recorded once every 3 wk at the center of each plot and one pelagic site during biomass harvesting to determine relationships between environmental factors and starry stonewort growth. A LI-COR LI-1500 light meter[1] 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 the percent light transmittance through the water column. Water temperature (C) and pH measurements were made using a Hydrolab HL7 multiparameter sonde[2] at a similar depth profile as LI-COR measurements. Additionally, temperature sensors[3] 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 1 h 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[4] (Litell et al. 1996) to determine influences of environmental factors on starry stonewort biomass and bulbil density. Thalli biomass, rhizoid biomass, and bulbil densities were included as the dependent variables. Water temperature, pH, submersed light, 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. Additionally, Spearman's rank correlation (0.0 to 1.0) was used to better show the correlations between plant metrics and environmental metrics (water temperature, percent light transmittance, and pH) because it does not make assumptions about the frequency distribution of variables (Hauke and Kossowski, 2011). Correlation strength was defined as no correlation (0 to 0.1), weak correlation (0.1 to 0.4), moderate correlation (0.4 to 0.6), strong correlation (0.6 to 0.9), or perfect correlation (0.9 to 1.0; Dancey and Reidy 2004). Correlation analyses were conducted using Morpheus.[5] Data are reported as means ($\pm$ 1 SE), and analyses were conducted at an $\alpha \leq 0.05$ significance level and displayed graphically to show correlation strength. A similar approach was utilized for Cuban bulrush (*Oxycaryum cubense*) (Clarke et al. 2023).

RESULTS AND DISCUSSION

Both total and thalli biomass were higher in 2020 than in 2021 with highest total biomass being 203 and 163 g DW m^{-2}, respectively (Figure 2). High points in thalli biomass in 2020 and 2021 were 197 g DW m^{-2} and 149 g DW m^{-2}, respectively, and accounted for $>90\%$ of total annual biomass during both years. Rhizoid biomass was not a major component with respect to biomass allocation because it

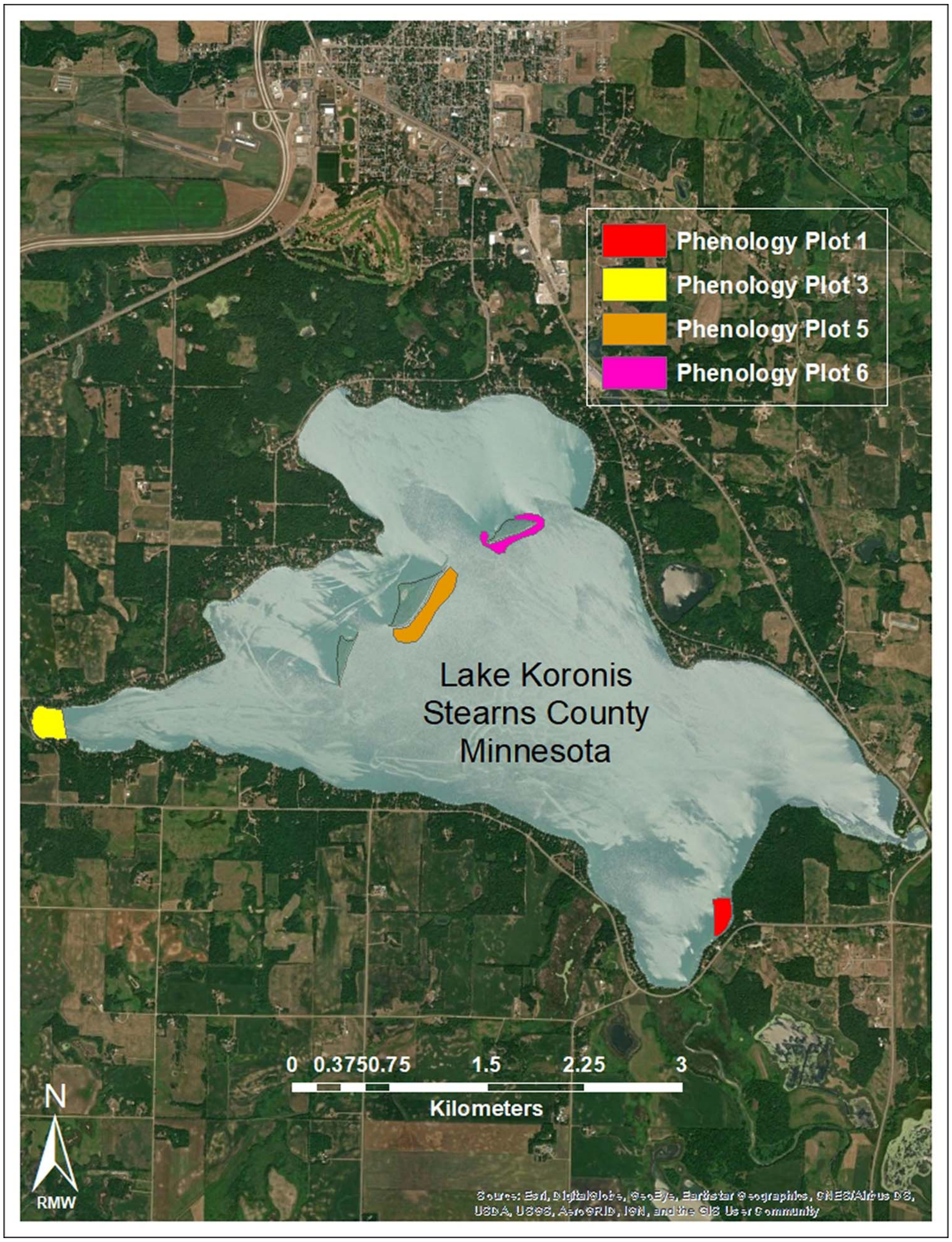

Figure 1. Sampling plots located in Lake Koronis, Stearns County, MN, where starry stonewort was harvested in 2020 and 2021.

represented < 1% of total biomass each year. Bulbil biomass ranged from 0.5 and 14.7 g DW m^{-2} and like rhizoid biomass accounted for < 1% of total biomass.

However, bulbil densities were high with the highest average densities of 1,229 m^{-2} and 5,211 m^{-2} in 2020 and 2021, respectively (Figure 2). Annual bulbil densities ranged from 0 to 157,000 m^{-2} with an average annual density of 1,537 m^{-2} across all plots. In Minnesota the native aquatic plant sago pondweed [*Stuckenia pectinate* (L.) Börner] produces 23 to 105 tubers m^{-2} (Wersal et al. 2006), and the non-native plant triploid flowering rush (*Butomus umbellatus* L.) produces 297 to 442 rhizome buds m^{-2} (Marko et al. 2015). The observed bulbil densities for starry stonewort in the current study are like those produced by hydrilla [*Hydrilla verticillata* (L.F.) Royle]. Dioecious hydrilla on average produces 20 to 900 subterranean turions m^{-2} (Netherland 1997), and monoecious hydrilla on average produces 200 to 1,228 subterranean turions m^{-2} (Harlan et al. 1985). Prolific bulbil production is of concern because they are easily moved, and boat traffic has been shown to be a major predictor of spread (Midwood et al. 2016).

Bulbil densities followed a bimodal pattern in that there were abundant bulbils in the sediment early in the year (April) with declines beginning in June as bulbils sprouted and resources were allocated to thalli growth. Beginning in July of each year, bulbil densities began to increase and were produced until the end of the sampling period (November). The bimodal pattern of bulbil densities corroborates other observations on Lake Koronis (Glisson et al. 2022). Bulbils can persist in the sediment for multiple

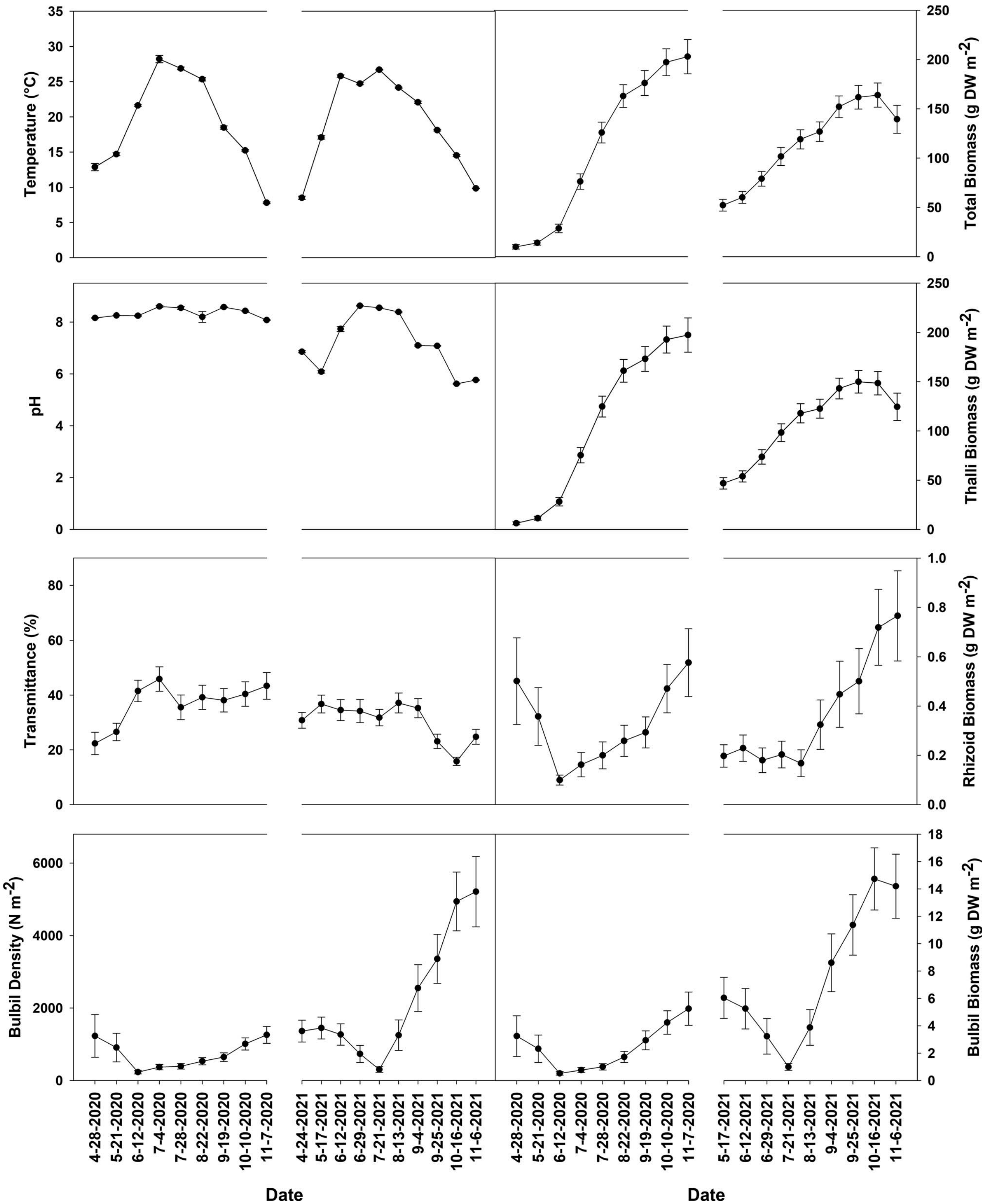

Figure 2. Mean (± 1 SE) environmental factors, bulbil density, total, aboveground, rhizoid, and bulbil biomass of starry stonewort measured from four plots on Lake Koronis in Stearns County, MN, from April to November 2020 and 2021.

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

Tissue	Effect	t value	P value	Spearman's rank[1] correlation
Total biomass	Temperature	6.33	< 0.01	0.10
	pH	−3.95	< 0.01	−0.01
	Light transmittance	3.12	< 0.01	−0.01
Thalli biomass	Temperature	6.56	< 0.01	0.10
	pH	−3.54	< 0.01	0.01
	Light transmittance	3.05	< 0.01	0.01
Rhizoid biomass	Temperature	−1.94	0.05	−0.19
	pH	−1.05	0.29	−0.14
	Light transmittance	1.50	0.13	0.01
Bulbil biomass	Temperature	−0.46	0.64	−0.24
	pH	−3.31	< 0.01	−0.31
	Light transmittance	1.40	0.16	−0.11
Bulbil density	Temperature	−0.39	0.69	−0.23
	pH	−4.18	< 0.01	−0.29
	Light transmittance	1.99	0.04	−0.10

[1]Correlation strength was defined as no correlation (0 to 0.1), weak correlation (0.1 to 0.4), moderate correlation (0.4 to 0.6), strong correlation (0.6 to 0.9), or perfect correlation (0.9 to 1.0. All analyses were conducted using Morpheus.[5]

years and as such will result in population longevity in Lake Koronis (Glisson et al. 2022).

Environmental factors influenced starry stonewort growth, and multiple significant, albeit weak, correlations between seasonal biomass of starry stonewort and environmental factors were observed (Table 1). Total and thalli biomass were positively correlated with temperature (r_s = 0.10). Total and thalli biomass were not correlated to pH or light transmittance (Table 1). Rhizoid biomass had weak negative correlations with temperature ($r_s = -0.19$) and pH ($r_s = -0.14$). Bulbil biomass had weak negative correlations with temperature ($r_s = -0.24$), pH ($r_s = -0.31$), and light transmittance ($r_s = -0.11$). Bulbil density had weak negative correlations with temperature ($r_s = -0.23$), pH ($r_s = -0.29$), and light transmittance ($r_s = -0.10$) (Table 1).

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. 2014, Simons and Nat 1996, Midwood et al. 2016, Sleith et al. 2018). In 2020, pH was highest (8.6) in July and 8.6 in late June 2021 (Figure 2). In looking at long-term pH data (1950 to 2019, $n = 616$) collected by the Minnesota Pollution Control Agency in Lake Koronis, the mean pH during this period was 7.9 ± 0.4 SD with a minimum of 5.2 and maximum of 8.7. Observationally, the low pH values in Lake Koronis corresponded to times when lake turnover was occurring and times of lower water levels in Minnesota.

Water pH will influence the forms of inorganic carbon (carbon dioxide, carbonic acid, bicarbonate, and carbonate) available for use by submersed plants or photosynthesis (Bornette and Puijalon 2011). Roughly half of all submersed plants can use bicarbonate (HCO_3^-) as an additional source of in organic carbon (Hussner et al. 2016, Dülger and Hussner 2017). Bicarbonate is typically more available then CO_2 especially at pH above seven (Hussner et al. 2016). As pH in Lake Koronis increased (above eight) later in the growing season, bicarbonate would have been the predominate form of inorganic carbon available to starry stonewort. The increase in bicarbonate may have triggered the allocation of resources to starch formation and accumulation within starry stonewort at the expense of new biomass production. Long-term exposure of milfoil (*Myriophyllum*) species to CO_2 enrichments resulted in starch accumulation in leaves and more biomass allocation to roots (Dülger and Hussner 2017). It could also explain why starry stonewort is able to produce so many bulbils per growing season, though additional studies are needed to elucidate the ecophysiology of starry stonewort, especially photosynthesis and carbohydrate allocation. Though it was determined recently that starry stonewort can store starch in bulbils between 21.0 and 73.7% of dry weight depending upon time of year and growing conditions (Haram and Wersal 2023).

Light availability may be the primary environmental factor limiting submersed macrophyte colonization and biomass (Barko et al. 1986). Light transmittance during the growing season in Lake Koronis ranged from 15.8 to 45.8% indicating enough light was reaching the bottom sediments to sustain growth of submersed plants (Figure 1). In its native range it is found in areas of low light intensity, typically 4-to-8-m depths, but it can grow in water up to 30-m depths (Olsen 1944). In the current study, light availability was not a limiting factor. Often increased light availability is correlated with increases in water temperature, which would explain the late season growth of starry stonewort in Minnesota.

Water temperature was highest between June and August in both years and steadily declined until sampling ended in November of each year (Figure 1). Water temperature influences plant physiology, especially photosynthetic rates, and can regulate phenology and resource allocation (Madsen 1991). Starry stonewort has a late season peak in biomass, which is influenced by increasing water temperature, which implies a potential niche this species could occupy, possibly allowing coexistence and limited competition with native species (Glisson et al. 2022). However, other species such as hydrilla have similar late season peaks in biomass and turion production, but it is an aggressive invasive species that is listed as a federal noxious weed in the United States (Owens and Madsen 1998). Hydrilla and Eurasian watermilfoil (M*yriophyllum spicatum* L.) are two submersed plants, capable of creating thick beds that limit water movement and light penetration, and ultimately reduce habitat complexity within the systems they invade (Crooks 2002).

Starry stonewort has a similar growth habitat and exhibits characteristics that would allow it to become an ecosystem engineer like hydrilla and Eurasian watermilfoil, most notably the ability to develop a dense surface canopy that restricts light penetration, water movement, and gas exchange; and the production of bulbils. Starry stonewort 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). Dense beds 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). Bulbil production is currently the biggest obstacle for curtailing the spread of this species in North America. Given the littoral zone in Lake Koronis infested with starry stonewort is 324 ha, there is an average of about 15 million bulbils ha^{-1} produced each year with a possible maximum of roughly 1.5 billion ha^{-1}. The number of bulbils produced each year and those already in the sediment will take many years of management to reduce the propagule bank to a sustainable level or at the very least be able to reduce management efforts to a minimum. Future research should focus on the development of specific management strategies for starry stonewort that affect bulbil production as well as the effects starry stonewort has on native plant populations and whether it fills a previously empty niche.

SOURCES OF MATERIALS

[1]LI-COR LI-1500 light meter, LI-COR, Lincoln, NE.

[2]Hydrolab HL7 multiparameter sonde, OTT HydroMet, Sterling, VA.

[3]HOBO Pendant, Onset Computer Corporation, Bourne, MA.

[4]SAS9.4, SAS Institute, Cary, NC.

[5]Morpheus, https://software.broadinstitute.org/Morpheus, Broad Institute, Cambridge, MA.

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