2023

Seasonal Starch Allocation in Starry Stonewort (Nitellopsis obtusa) Harvested from Lake Koronis, MN, and Development of Non-Chemical Management Recommendations

Alyssa M. Haram
Minnesota State University, Mankato

Recommended Citation

Seasonal Starch Allocation in Starry Stonewort (*Nitellopsis obtusa*) Harvested from Lake

Koronis, MN, and Development of Non-Chemical Management Recommendations

By

Alyssa M. Haram

Thesis Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

In

Biology

Department of Biological Sciences

Minnesota State University, Mankato

Mankato, Minnesota 56001, United States of America

February 2023

Examining Committee Approval/Signatures:

_____   _____

Chairperson of the Examining Committee   Date

(Dr. Ryan M. Wersal)

_____   _____

Examining Committee Member   Date

(Dr. Matthew Kaproth)

_____   _____

Examining Committee Member   Date

(Dr. Gray Turnage)

ACKNOWLEDGEMENTS

ABSTRACT

Starry stonewort (*Nitellopsis obtusa*) (Desvaux in Loiseleur) J. Groves is an invasive macro alga that can take over entire water columns and outcompete native species. Previous research has quantified seasonal life history and phenology but there is no research quantifying carbohydrate allocation which was the aim for Chapter II. Management efforts to control *Nitellopsis obtusa* have been limited to stressing the thalli and have not been able to directly target the reproductive bulbils on larger scales. Smaller scale efforts such as the use of hand pulling can be used but hand pulling is not realistic for larger infestations. Chapter III was conducted to test the effects of clipping and harvesting stress on *N. obtusa* to give a baseline on the effect of stress on the production of bulbils and the regrowth of thalli. Management needs to stress the growth of *N. obtusa* as much as possible to reduce the production of bulbils and limit the spread of current infestations in waterways. Both studies utilized samples harvested from Lake Koronis, Minnesota, USA from 2020 and 2021 to quantify starch allocation patterns in *N. obtusa* and to grow reserve cultures of *N. obtusa*. Starch was quantified using the Amylase/Amyloglucosidase method through a commercially purchased STA20 starch assay kit from Sigma Aldrich (Sigma Aldrich, St. Louis, Missouri). The starch data was then compared to environmental variables such as light transmittance (%), pH, and water temperature (℃) via mixed procedures models in SAS. The seasonal average low and high points of starch storage were observed to be June (3.3%) and April (24.9%) for thalli biomass then June (21.0%) and November (73.0%) for bulbils. These bulbils spread easily, spread rapidly, can store a lot of energy (73% starch), and are a major concern for the invasion of *N. obtusa*. Thalli biomass (g) was about 97.0% lower than reference thalli biomass after four clipping treatments and bulbil density (N m^{-2}) was about 46.0% lower than reference bulbil density after one clipping treatment.

Suggesting that mechanical clipping stress opens the water column to induce bulbil sprouting and reduce the total bulbil bank in the sediment. Mechanical clipping treatments would then be recommended to be used in conjuncture with other management methods to stress the alga as much as possible to reduce total biomass and bulbil production starting in June.

TABLE OF CONTENTS

CHAPTER I

STARRY STONEWORT [*Nitellopsis obtusa*] LITERATURE REVIEW

Current Distribution

Starry stonewort (*Nitellopsis obtusa* (Desvaux in Loiseleur) J. Groves) is an aquatic macro-alga that is native to Europe and Asia (Alix et al. 2017). Although *N. obtusa* is a prohibited invasive species in North America, it is an endangered species in its native range of Eurasia. *Nitellopsis obtusa* is IUCN red listed as near threatened, threatened, and vulnerable in different parts of Europe to Japan (Wood 1965; Soulié-Märsche et al. 2002; Urbaniak 2003; Naz et al. 2010; Kato et al. 2014; Alix et al. 2017). In North America, *N. obtusa* is an aquatic invasive species that was first recorded in New York, USA in 1978 and had spread to the Laurentian Great Lakes by 1983 (Karol and Sleith 2017). Since then, *N. obtusa* became an established invasive species in Indiana, Michigan, Minnesota, New York, Pennsylvania, Vermont, Wisconsin, and parts of Canada (Kipp et al. 2021).

In Minnesota, 22 waterways are currently listed as infested with *N. obtusa* by the Minnesota Department of Natural Resources (Table 1; MNDNR). Because of how similar looking charophytes are, it is possible that *N. obtusa* is established in other waterbodies and remains undetected (Escobar et al. 2016). This is supported by how *N. obtusa* had established in an area of over 100 ha in Lake Koronis by the time it was discovered in 2015 (MNDNR 2020; Escobar et al. 2016).

To predict vulnerability to future invasion in lakes of the upper Midwest, a habitat suitability model was run on *N. obtusa* and showed that all of the lakes in the models had substantial vulnerability to invasion by *N. obtusa* (Muthukrishnan et al. 2018). The suitability for

N. obtusa in lake habitats was found to be positively linked to lake depth and chlorophyll *a* concentration (Muthukrishnan et al. 2018), but this may also be due to the possibility that depth and chlorophyll *a* concentrations are indicators of increased opportunities of transmission by anthropogenic causes.

Taxonomy and Morphology of *Nitellopsis obtusa*

Nitellopsis obtusa is a dioecious green macroalgae in the family Characeae. *Nitellopsis obtusa* is also known by the common name starry stonewort, which comes from the star shaped bulbils it produces. For means of identification; *N. obtusa* is a green alga with white lower axial nodes. The main axis can be 0.7-2.0 mm diameter in width (Larkin et al. 2018). *Nitellopsis obtusa* has 5-7 branchlets in a whorl that grow to be 2 cm long (Naz et al. 2010). The entire algae can grow to 30-120 cm tall (Larkin et al. 2018). *Nitellopsis obtusa* is very similar to *Nitella transluscens* (Pers.) Agardh except that *N. obtusa* lacks terminal branchlet corona, which are small extensions at the top of the thalli, and *N. transluscens* lacks bract-cells and bulbils (Wood and Imahori 1965; Naz et al. 2010).

In North America only antheridia (male individuals) of *N. obtusa* have been observed until recently. In 2022 gametangia (female) bearing individuals have been discovered in two lakes in Ontario, Canada (Harrow-Lyle et al. 2022). Though in the United States only antheridia bearing individuals have been identified. Antheridia are an orange or reddish color which form on the branchlet nodes (Larkin et al. 2018). In it is native range, oogonia are reddish or green and reproduce sexually with antheridia (Groves 1919; Corillion 1957; Krause 1997; Bailly and Schaefer 2010; Mouronval et al. 2015; Kabus 2016; Boissezon et al. 2017; Larkin et al. 2018). *Nitellopsis obtusa* grows white, star-shaped bulbils from their rhizoid nodes and green bulbils from their main axis and branchlet nodes (Larkin et al. 2018).

Nitellopsis obtusa Reproduction and Spread

Because only antheridia bearing *Nitellopsis obtusa* individuals are currently found in the United States, the species likely reproduces clonally in the invaded range (Mann et al. 1999; Sleith et al. 2015; Larkin et al. 2018). Reproduction of *N. obtusa* is primarily asexually through its white star shaped bulbils, which can remain dormant in the soil (Pokrzywinski et al 2020a). These bulbils, along with fragments, act as the only known form of reproduction in the United States (Larkin et al. 2018). Bulbils develop from July to October and their development is dependent on light intensity (Langangen 2007; Naz et al. 2010). Previous research has shown that in Minnesota, *N. obtusa* produces more biomass when there is less light available later in the season (Carver 2022). Though this may not be a growth preference for lower light intensities but that *N. obtusa* is able to utilize a later season niche that has a lower light availability (Glisson et al. 2022).

Because *N. obtusa* is primarily found in areas of human development and not in low human impact areas, *N. obtusa* is most likely spread by anthropogenic vectors and not by endozoochory or epizoochory (Sleith et al. 2015). This suggests that boaters and fishermen act as vectors of spread for *N. obtusa* whenever bulbils or biomass become attached to equipment or gets trapped in boat ballast. Instances of long distance spread by *N. obtusa* is thought to be by boaters who do not comply with requirements to inspect and remove invasive materials from their watercraft, ballast, and trailer after exiting an infested waterbody (Rothlisberger et al. 2010, Cimino and Strecker 2018; Glisson et al. 2020). The bulbils of *N. obtusa* are produced at high rates and are small so they are easily spread by boating (Midwood et al. 2016). Because of this the bulbils are a major concern for the continued spread of *N. obtusa*.

Impacts of *Nitellopsis obtusa*

Nitellopsis obtusa is a problematic invasive because it creates recreational and ecological issues by forming dense mats of vegetation called packing, which is when a population fills all available optimal and nonoptimal growth habitats (Pullman and Crawford 2010; Pokrzywinski et al. 2020a). *Nitellopsis obtusa* impacts native fish populations because its packing growth pattern can inhibit spawning (Sleith et al. 2015) and because lakes with *N. obtusa* may risk oxygen depletion in the autumn as large mats of *N. obtusa* decompose (Kipp et al. 2014; Brainard and Schulz 2017). *Nitellopsis obtusa* also outcompetes native vegetation by physically excluding them from plant beds (Pullman and Crawford 2010; Kipp et al. 2014; Sleith et al. 2015). Besides negative ecological impacts, there are also negative recreational and economic impacts from *N. obtusa*. Some of the recreational impacts are from the density of the *N. obtusa* packing growth pattern making shallow lake areas unsuitable for swimming. The packing behavior also makes it difficult for recreational boating as algae gets stuck in boat propellers and motors thus hindering boat movement.

Economically, aquatic invasive plant management costs the United States close to $1 billion per year (Getsinger et al 2014). A study done by Zipp et al. (2019) calculated that a lake that is invaded with *Myriophyllum spicatum* (Eurasian watermilfoil) will cause an average property reduction value of $1,400 per house, which comes from an approximated 8% reduction in property value. Considering these costs, the probability that a boater would avoid visiting an invaded lake is 11% more than they would avoid visiting a noninvaded lake (Zipp et al. 2019).

Due to the economic, ecological, and recreational impacts caused by *N. obtusa*, there is an impetus to manage infestations and try to eliminate *N. obtusa* from North American waters. More research is needed on how to best manage *N. obtusa* by understanding the species

phenology so management can be as efficacious as possible. Further research is also needed on the long term impacts of *N. obtusa* management in aquatic systems to better understand the interactions of *N. obtusa* and the water chemistry as well as on the community dynamics in the aquatic ecosystem.

Tables

Table 1. Waterbodies with confirmed infestation of *Nitellopsis obtusa*.

County	Waterbody	Year Infested
Beltrami County		
	Upper Red Lake	2016
	Cass Lake	2016
	Moose Lake	2016
	Turtle Lake	2016
	Wolf Lake	2018
	Beltrami Lake	2019
	Mississippi River	2021
	Pimushe	2021
	Bemidji	2022
	Turtle River Lake	2022
Cass County		
	Leech	2021
	Bowen	2022
	Thunder	2022
Hennepin		
	Medicine	2018
Stearns		
	Carnelian	2020
	Grand	2017
	Koronis	2015
	Rice	2016
Pope		
	Minnewaska	2017
Wright		
	West Sylvia	2016
	Pleasant	2018
Multiple Counties		
	Winnibigoshish	2016

CHAPTER II

STARCH ALLOCATION

Introduction

Nitellopsis obtusa (Desvaux in Loiseleur) J. Groves, is a dioecious green macroalgae in the family Characeae and is also known by the common name starry stonewort, which comes from the star shaped bulbils it produces (Larkin et al. 2018). In North America *N. obtusa* is a newer species, first invading the Eastern United States in 1974 and since spreading to the upper Midwest and into the lower Canadian territories (Larkin et al. 2018). Previously in North America only antheridia (male) bearing *N. obtusa* individuals were thought to exist (Mann et al. 1999; Sleith et al. 2015; Larkin et al. 2018). Recently gametangia (female) bearing *N. obtusa* individuals were found in Ontario, Canada and have only been reported in two lakes (Harrow-Lyle et al. 2022). At this point, there are no current reports of gametangia bearing individuals in the United States so the only known reproduction is done asexually (Larkin et al. 2018). Asexual reproduction by *N. obtusa* is done through white star shaped vegetative propagules known as bulbils, which can remain dormant in the soil (Pokrzywinski et al 2020a). These bulbils are attached to below ground thalli biomass that is a transparent extension (rhizoid) of the thalli (Larkin et al. 2018). The thalli is the body of the alga (Larkin et al. 2018).

It is thought that these bulbils may serve as a major sink for carbohydrates, most notably for the storage of starch. Starch is an insoluble stored form of excess chemical energy created from photosynthesis. Other forms of energy storage by Carbon in macro alga include hydrosoluble glycogen and water soluble β-1,3-glucans which can be stored inside the chloroplast, cytoplasm, chloroplast endoplasmic reticulum vesicles, periplastid compartments, and cytoplasmically localized vacuoles (Hildebrand et al. 2013; Hildebrand et al. 2017). Algae

can also utilize lipid storage for energy (Vitova et al. 2015). Understanding how a species stores carbon and how much it stores is important for understanding the biology of the species, especially for algae since carbon is necessary for growth and efficient growth is a major selective pressure for continued survival (Hildebrand et al. 2017).

During the daytime, or times of high productivity, plants and algae store excess carbon to create starch stores (MacNeill et al. 2017). At night, or times of low productivity, plants and algae use the stored starch to perform respiration in the absence of photosynthesis (MacNeill et al. 2017). In times of stress, such as drought, salt stress, or freezing temperatures, plants utilize starch to provide energy for regrowth or to mitigate the effects of low productivity periods (MacNeill et al. 2017). Understanding the amount of starch stored seasonally can give insight to how alga can respond to stress situations. Starch allocation patterns can also be paired with environmental variables to predict seasonal changes of energy storage (Wersal et al. 2011). Because *N. obtusa* has been noted to persist much later into the growing season than other aquatic species, it is important to understand what mechanism the algae is utilizing to fill this open late season niche (Glisson et al. 2022).

To better understand this, starch allocation patterns can be used to quantify seasonal phenology. Phenology is the study of seasonal timing of critical life stages in living organisms and the environmental conditions of their habitat, such as seasonal changes in biomass and starch allocation in response to light availability (Wersal and Madsen 2018). Seasonal changes in aquatic photosynthetic organisms like plants and algae usually show energy storage peaks in summer or fall and storage depletion in the spring after plant growth has occurred (Madsen 1991; Wersal et al. 2011). Understanding phenology allows for a better understanding of ecological

dynamics within the plant community, especially dynamics between invasive species, and to give

management recommendations when there is seasonal low points in stored energy.

Understanding phenology can also help explain species niche space dynamics.

Phenological studies of alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.),

waterhyacinth (*Eichhornia crassipes* (Mart.) Solms), Eurasian watermilfoil (*Myriophyllum*

spicatum L.), hydrilla (*Hydrilla verticillata* (L. f.) Royle), curlyleaf pondweed (*Potamogeton*

crispus L.), Brazilian elodea (*Egeria densa* Planch.), parrotfeather (*Myriophyllum aquaticum*

(Vell.) Verdc.), and phragmites (*Phragmites australis* (Cav.) Trin. ex Steud.) are examples of

previous research that have examined the seasonal carbohydrate allocation patterns of pest

species to better understand their seasonal development patterns (Weldon and Blackburn 1968;

Madsen 1993, 1997; Madsen and Owens 1998; Woolf and Madsen 2003; Pennington and Sytsma

2009; Wersal et al. 2011; Wersal et al. 2013).

The purpose of this study was to quantify seasonal starch allocation patterns of *N. obtusa*,

and to determine the influence of environmental variables on starch allocation to better define

phenological patterns of *N. obtusa*. Currently there is only one study documenting seasonal life

history and one study quantifying seasonal phenology of *N. obtusa* (Glisson et al. 2022; Carver

and Wersal *In press*). There are no studies that have quantified the starch allocation at the

individual level. Specifically, I tested the hypothesis that the largest sink for available starch

would occur in bulbil tissues throughout the year.

Materials & Methods

Study site and sampling design

The study utilized samples that were harvested from four existing locations of *N. obtusa*

in Lake Koronis, Stearns County, Minnesota (45.31°N, -94.68°W) (Carver 2022). Lake Koronis

is a 1,201-hectare mesotrophic to eutrophic lake with a maximum depth of 40 meters. Four plots

(3.3 m to 8.1 m ha) were chosen as sampling locations on Lake Koronis based on moderate to

high *N. obtusa* densities, distance from management plots, and water depth (0.5–3 m) that was

conducive for sampling based on the accessible depth of the PVC coring device used. At each

plot 30 samples were collected every three weeks from May to November in 2020 and 2021

(Carver 2022).

Additionally, Carver (2022) recorded environmental data once every 3 weeks during

biomass harvesting. 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. 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.

Starch analysis

Harvested *N. obtusa* samples were separated into thalli biomass, rhizoid biomass, and

bulbil biomass. During 2020 sample processing, an additional biomass type was recognized

(immature bulbil biomass) and was then added as an additional biomass type for 2021 samples.

Immature bulbils were identified as bulbils that were not fully white or star shaped. Samples

were placed into labeled paper bags and dried at 48 °C for at least 48 hours in a forced air oven.

Once dry, biomass samples were composited so that biomass samples 1-10 represented starch tissue sample 1; biomass samples 11-20 represented starch tissue sample 2; and biomass samples 21-30 represented tissue sample 3. Compositing biomass in this manner ensured that adequate tissue sample is available for the starch analysis procedure and ensured proper replication of sample analysis (Wersal et al. 2011; Wersal et al. 2013). Only rhizoid biomass from 2021 was analyzed due to insufficient quantities in 2020.

After compositing biomass, samples were ground using a Cyclone Sample Mill (UDY 146 Corporation, Fort Collins, CO) to pass through a #40 mesh screen (1 mm). Approximately 50 mg of the ground sample was transferred into plastic whirl-pack bags for storage and preparation for the starch analysis. Starch extraction and determination was conducted using the Amylase/Amyloglucosidase method through a commercially purchased STA20 starch assay kit from Sigma Aldrich (Sigma Aldrich, St. Louis, Missouri, Wersal et al. 2011; Wersal et al. 2013; Clarke et al. 2023). In addition to *N. obtusa* samples, wheat starch standards included with the kits were tested to determine the reliability of the starch data. Wheat standards of 89% purity were used to determine the average percent recovery of 92.7% from the analysis. Standard curves were developed to ensure that the starch data were within the range of what the kits can detect, and to assess the relative accuracy of the data (Wersal et al. 2011; Wersal et al. 2013). The starch content in the bulbil biomass was too high for accurate quantification of the starch assay method so bulbil samples were diluted at a 1:2 water to sample ratio before sample digestion. The calculated starch values were then adjusted after measuring the absorption to correct for the dilution. This ensured that the sample absorbance would be within the 540nm using a LAMBDA™ Bio UV/Vis Spectrometer (PerkinElmer, Waltham, MA, USA) range after digestion. The standard curve R^2 was 0.98 as determined by linear regression. The precision of

the assays was determined by the percent difference of duplicate samples with a mean percent difference of 9.75 ± 1.07 (± 1 SE).

Statistical analysis

Monthly averages of percent starch for thalli, rhizoids, and bulbils were computed for each site. Data was analyzed by fitting mixed models using the Mixed Procedures Model in SAS (SAS Institute Inc, Cary, NC, USA; Littell et al., 1996; Wersal et al. 2011; Wersal et al. 2013). Starch content from individual structures was included as dependent variables. Environmental factors included were light transmittance, pH, and temperature as independent variables in all models. Year was included as a random effect in the model and site was included as a repeated measure to account for their influence on the results. Significant rates of increase and decrease for each biomass type were then confirmed using a Repeated Fitted Mixed Measures ANOVA in SAS JMP Pro (SAS Institute Inc, Cary, NC, USA). Month and site were accounted for as covariates. Significant differences were further analyzed with a Tukey's LSD post hoc. All analyses were conducted at the $\alpha \leq 0.05$ significance level.

Results

The starch content in the bulbils of *N. obtusa* peaked from July-October (t=-1.88, p=0.06; Figure 1a) with an average starch content of 52.4% ± 0.9 SE (maximum of 73.7%, and a minimum of 21.0%). Thalli starch peaked from October to November (t=1.99, p=0.04; Figure 1b) with an average starch content of 4.7% ± 0.2 SE (maximum of 24.9%, and minimum of 0.03%). Rhizoid starch peaked in late June to early July and steadily declined until September (t=2.61, p=0.01; Figure 1c) with an average starch content of 5.9% ± 0.4 SE (maximum of 13.6% and minimum of 2.5%).

Thalli starch storage was negatively influenced by temperature (t=-5.18, p<0.01) and light transmittance (t=-2.11, p=0.03), while pH had no relationship (t=0.087, p=0.38; Table 1). Rhizoid starch was negatively influenced by water temperature (t=-3.72, p<0.01) and positively influenced by water pH (t=3.23, p<0.01), and showed no relationship with light (t=0.13, p=0.89). Immature and mature bulbil starch had no relationship with any environmental factor measured in this study.

Discussion

Bulbils stored starch up to 73% of total dry biomass showing that *N. obtusa* allocates most of its energy reserves to vegetative reproductive biomass. Comparing to previous research only 16.3% maximum total starch is found in *M. aquaticum* stolons (Wersal et al. 2011). Common reed (*P. australis*) only contains up to 20% starch in the below ground biomass (Wersal et al. 2013). The winter buds of *Vallisneria americana* (Michx.) can reach TNC levels of 46% (Titus and Adams 1979). While the total starch content of Cuban bulrush [*Oxycaryum cubense* (Poepp. & Kunth) Lye] peaked at 3.7% (Clarke et al. 2023). Therefore the vegetative reproductive structures of *N. obtusa* contain much more starch than other aquatic invasive species. Colonies of *N. obtusa* can also produce up to 156,944 bulbils m^{-2} with monthly peak dry bulbil biomass being up to 14.7 g m^{-2} (Carver 2022). Because *N. obtusa* bulbils can store so much energy, are produced, rapidly, and in high numbers management should continue to focus on limiting bulbil production to limit the spread of *N. obtusa*.

It should be noted that samples for this study were not collected from December to March, so it is uncertain if the starch in the thalli declines or if stays constant during those months when lake ice is present. It is possible that the starch allocated in the thalli gets used slowly while the plant is under ice, since the available resources would be limited, though there

is no research to support this. Rhizoid starch increases after thalli starch decreases, suggesting that above ground thalli convert starch to mobile sugars and transports this energy to below ground rhizoids in May. How *N. obtusa* does this is uncertain as there are no vasculature tissues but future research should look at the mode of energy transport to better understand how this relationship works. Carbohydrates are then transferred from rhizoids to bulbils from July to early October as rhizoid starch decreases and the bulbil starch increases. The timing of the starch peak in the bulbil biomass from July-October also relates to a decrease of thalli starch during this time suggesting that late summer to early fall is a time of heavy resource allocation for this plant. These findings also explain why *N. obtusa* is able to persist so late into the year as noticed in previous studies (Glisson et al. 2022). Because there is the capacity to store up to 25% starch in the thalli tissues, this storage in thalli may be an important factor in allowing aboveground biomass to persist into winter far longer than other species of submersed vegetation.

The findings from this study linking thalli starch production negatively to available light transmittance confirm findings in *N. obtusa*'s native range that it does not grow well in eutrophic lake systems that have low water clarity (Larkin et al. 2018). As light availability increases *N. obtusa* likely uses stored starch to grow biomass to develop a surface canopy. This is supported by previous phenology work also finding a positive correlation between *N. obtusa* biomass production and light transmittance (Carver 2022). Other environmental factors influencing growth are water temperature or pH. The negative relationship between thalli starch and temperature suggests that *N. obtusa* starch development is dependent on seasonal water temperature changes. Previous research showed that *N. obtusa* had a temperature tolerance between 14.0 and 28.0 °C in its native range and between 18.2 and 25.4 °C in its invaded range (Larkin et al. 2018). In this study the water temperature ranged between 7.5 and 26.5 °C with

viable green thalli showing a widened tolerance to cooler temperatures than observed previously (Carver 2022).

Water temperature is an important ecological factor since it influences photosynthetic rates, regulates phenology, and regulates resource allocation (Madsen 1991). In this study it is not certain if the decreasing water temperature drove the increase of thalli starch or if they coincided. But temperature did have the strongest correlation with *N. obtusa* starch accumulation. Previous research showed that *N. obtusa* occupied a late season niche space with a peak in thalli biomass influenced by decreasing water temperature (Glisson et al. 2022; Carver 2022). This shows that *N. obtusa* allocates high amounts of free carbohydrates to produce biomass as well as to store energy late into the growing season (October to November). This has been seen before in other invasive species such as hydrilla (*H. verticillata*) which peaks in biomass and in total nonstructural carbohydrates from October to December (Owens and Madsen 1998).

The positive correlation between pH and rhizoid starch may suggest a potential pH vulnerability of the rhizoid biomass to extreme pH conditions with respect to inorganic carbon availability. This effect is most likely due to the effects of water pH on the form of available carbon in the water for photosynthesis (Chen and Durbin 1994). When the pH of the water decreases to become more acidic the available carbon shifts from higher carbonate concentrations to higher carbon dioxide and bicarbonate concentrations (Moazami-Goudarzi and Colman 2012). The changes in pH levels affect the form of available carbon in the water and then change the rates of carbon uptakes based on species specific photosynthetic pathways (Moazami-Goudarzi and Colman 2012). Previous work on other charophyte species have shown a growth preference for moderate to high pH waters which aligns with the increase of starch

accumulation in higher pH conditions of this study (Pełechaty et al. 2014; Simons and Nat 1996; Midwood et al. 2016; Sleith et al. 2018). With the positive correlation between rhizoid starch accumulation and pH in the current study it might be assumed that *N. obtusa* may prefer carbon dioxide and bicarbonate forms of carbon for uptake in the water column. There is no current research measuring the specific form of carbon uptake in *N. obtusa* though other research looking at a related Chara species found that the plasma membranes of Chara can transport both carbon dioxide and bicarbonate via H+ -ATPase (Ray et al. 2003). Showing additional preference to pH conditions around 8.2-8.8 based on the carbon uptake (Ray et al. 2003).

In the invaded range of Minnesota, *N. obtusa* has not been found in water bodies south of Medicine Lake, Hennepin County, MN (45.01°N, -93.42°W) since 2018 (USGS 2022). This is likely due to the poor water quality of lakes in Southern Minnesota. The average southern Minnesota lake has a higher pH range (8.2-9.0 southern regions versus 7.2-8.3 northern regions) and a lower average depth (1.9-3.4 m southern regions versus 2.5-10.5 m northern regions) which increases seasonal water temperature (Heiskary and Wilson 2008). Suggesting that *N. obtusa* spread may be limited to lakes with deeper, cooler water, and lakes with a circumneutral pH.

Data from this study elucidates reasons for why *N. obtusa* is an invasive species that can persist so late into the growing season. The ability to store up to 73% starch in the bulbils may suggest that *N. obtusa* can maintain a persistent bulbil bank under the sediment for multiple years. This has been seen with the invasive hydrilla (*H. verticillata*) which has reproductive tubers that can remain viable in the sediment for up to four years (Van and Steward 1990). The tubers of *H. verticillata* have a comparable amount of stored starch to *N. obtusa* with about 50-60% starch throughout the growing season (Madsen and Owens 1998). Bulbils of *N. obtusa*,

which have high levels of stored energy and possibly long viability are small and are easily transported from lake to lake via boating (Sleith et al. 2015). This spread from lake to lake has been consistent and rapid. In Minnesota *N. obtusa* has been spreading to one to three new water bodies each year since 2015 (MNDNR 2020). The recent discovery of *N. obtusa* gametangia in North America may induce changes in how *N. obtusa* acts as an invasive species in its invaded range as a competitor and in how it spreads (Harrow-Lyle et al. 2022, Molofsky et al. 2014). So, the impacts of *N. obtusa* may become more drastic or harder to manage due to the additional ability to now reproduce sexually in the invaded range. Because of this, *N. obtusa* is an invasive species of concern and more research is needed on its ability to store energy, reproduce, and spread.

Tables

Table 1. Mixed Procedures Model in SAS output for each environmental factor compared to different *Nitellopsis obtusa* biomass types via the Mixed Procedures Model in SAS. N=228.

Biomass Type	Environmental Factor	t-value	p-value
Thalli	Temperature	**-5.18**	**<0.01**
	pH	0.87	0.38
	Light Transmittence	**-2.11**	**0.03**
Rhizoid	Temperature	**-3.72**	**<0.01**
	pH	**3.23**	**<0.01**
	Light Transmittence	0.13	0.89
Mature Bulbils	Temperature	-0.63	0.52
	pH	1.04	0.30
	Light Transmittence	1.62	0.10
Immature Bulbils	Temperature	-0.34	0.73
	pH	-0.08	0.93
	Light Transmittence	-1.52	0.13

Figures
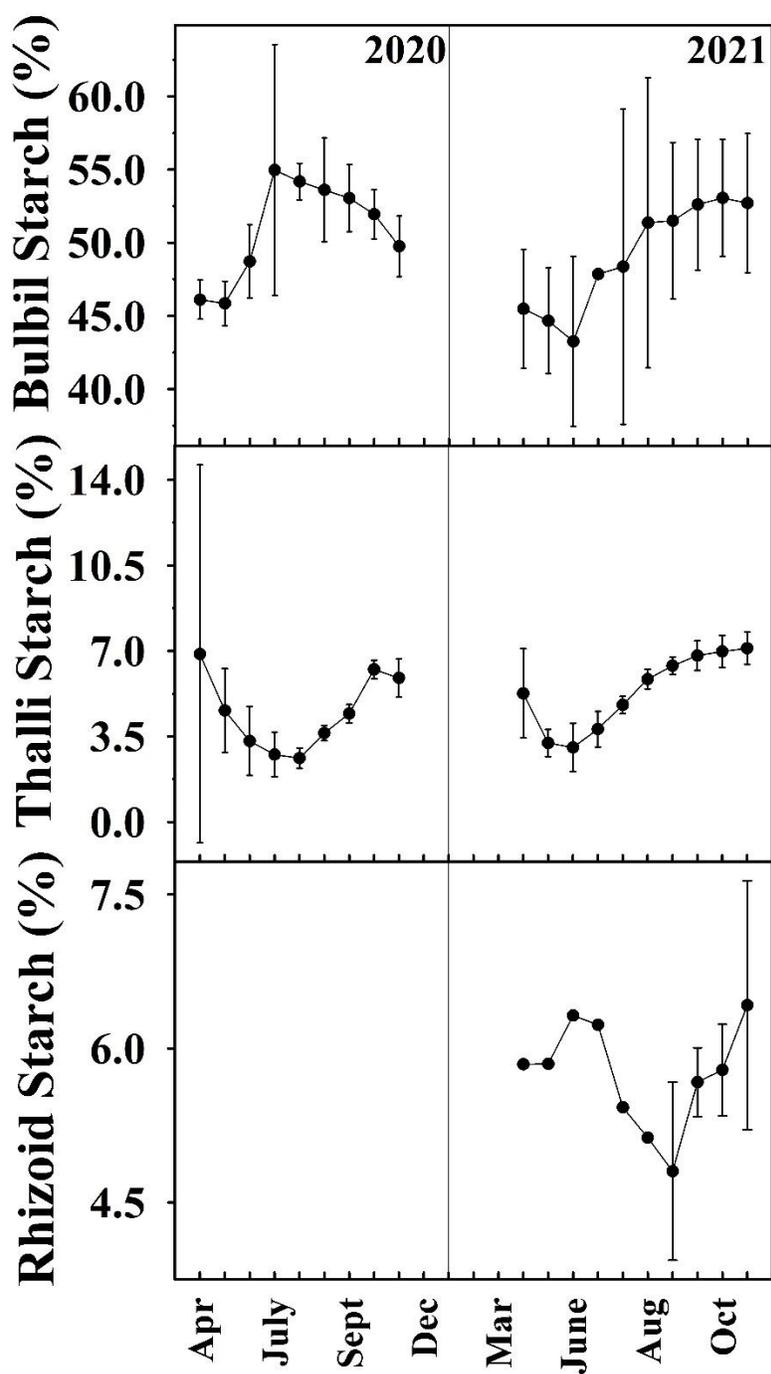
Figure 1. Seasonal percent starch of different tissue types of *Nitellopsis obtuse* from 2020 to 2021; starch from bulbil biomass (a), thalli biomass (b), and rhizoid biomass (c). Error bars represent ± 1 SE.

CHAPTER III

SIMULATED MECHANICAL CONROL FOR *Nitellopsis obtusa*

Introduction

Starry Stonewort (*Nitellopsis obtusa* (Desvaux in Loiseleur) J. Groves) is a

problematic macro-alga because it creates recreational and ecological issues by forming dense

mats of vegetation that fills all available optimal and non-optimal growth habitats (Pullman and

Crawford 2010; Pokrzywinski et al. 2020). The growth of *N. obtusa* impacts native fish

populations because its growth pattern can inhibit spawning (Sleith et al. 2015) and because

lakes with dense infestations may be at risk of oxygen depletion in the autumn as large mats of

N. obtusa decompose (Kipp et al. 2014; Brainard and Schulz 2017). The thalli of *N. obtusa* also

outcompete native vegetation by physically excluding them from plant beds and have even been

shown to outcompete other invasive species such as *Myriophyllum spicatum* (Pullman and

Crawford 2010; Kipp et al. 2014; Sleith et al. 2015; Ginn et al. 2021). The mats of *N. obtusa* also

have been shown to reduce invertebrate diversity in the water collumn disrupting the bottom

chain of the food web (Harrow-Lyle and Kirkwood 2021).

Thalli is the term for the above ground biomass of *N. obtusa*. The thalli is made up of a

single strand of cells attached end to end and are about 0.7 to 2 mm in diameter (Larkin et al.

2018). The thalli of *N. obtusa* form 5-7 branchlets in a whorl that grow to be 2 cm long (Naz et

al. 2010). *Nitellopsis obtusa* produces vegetative propagules called bulbils that are white star

shaped stuctures that form under the sediment on rhizoid nodes (Larkin et al. 2018). The rhizoids

are filamentous strands of clear biomass that grow underneath the sediment (Larkin et al. 2018).

In North America, bulbils and fragmentation are the predominant form of spread for *N. obtusa*

(Larkin et al. 2018). Throughout most of North America only male gametangia bearing *N. obtusa*

individuals have been identified (Mann et al. 1999; Sleith et al. 2015; Larkin et al. 2018) which suggested spread in the invaded range was only done through vegetative means. In 2022 two water bodies in North America have found female gametangia bearing individuals (Harrow-Lyle et al. 2022) suggesting there may be a shift from predominately vegetative reproduction to sexual reproduction of *N. obtusa* in the invaded range. The product of sexual reproduction in *N. obtusa* are fertilized zygotes called oospores (Larkin et al. 2018). Oospores of *N. obtusa* can be long lived and drought resistant (Boissezon et al. 2018). Current management techniques for *N. obtusa* are unable to effectively manage vegetative spread, so the introduction of sexual reproduction may make management even less effective due to the longevity of *N. obtusa* oospores (Boissezon et al. 2018).

To date, management strategies for *N. obtusa* include mechanical control, which is either hand-pulling, driver assisted suction (DASH) harvesting (Bradley Steckart, pers. comm., September 7, 2018; Pokrzywinski et al. 2020b), mechanical harvesting (New York State, December 2017; Pokrzywinski et al. 2020b); and chemical control, which is the application of either copper based algaecides or the herbicides diquat and/or endothall (Glisson et al. 2018; Larkin et al. 2018; Pokrzywinski et al. 2020b; Wersal 2022; Carver and Wersal 2022). Chemical control is effective at reducing thalli biomass but is unable to reach below ground structures such as rhizoids or bulbils (Carver and Wersal 2022). Water level drawdowns are a proposed method of *N. obtusa* management however it is not applicable to many midwestern glacial lakes that do not have water level control structures (Menninger 2011, Larkin et al. 2018).

For *N. obtusa*, methods such as harvesting and cutting need to be carefully implemented to not leave fragments or bulbils behind after management. These fragments could be dispersed as the boat or diver moves and could possibly accelerate the spread of *N. obtusa* (Larkin et al.

2018). Because bulbils grow mostly at the lower axial nodes, it is less likely mechanical

harvesting will spread bulbils during management. Also, mechanical harvestors can only cut 1.8

meters deep (Aquarius Systems 2010) while *N. obtusa* has been reported to grow in depths of

0.4-14.0 meters in its native range (Korsch et al. 2008; Janauer et al. 2010; Larkin et al. 2018). In

small scale invasions, precise methods such as hand pulling and suction harvesting, which collect

the algal fragments, can reduce biomass of *N. obtusa* (Larkin et al. 2018). However in larger

infestations hand pulling and suction harvesting are not feasible.

Previous mechanical control methods have been used on *N. obtusa* but none have

quanitified effectiveness or how often these methods have to be implemented in a season to see

specific results (Glisson et al. 2018). Because *N. obtusa* has been difficult to manage under field

conditions with both pesticides and mechanical control techniques, the purpose of this study was

to evaluate the effects of simulated harvesting on *N. obtusa* under mesocosm conditions. It is

possible that *N. obtusa* will have a limit to the amount of cutting stress it can handle before

regrowth and/or bulbil production is inhibited. This is supported by *Hydrilla verticillata* (L. f.)

Royale producing fewer and smaller tubers, producing less rhizomes, and having less total

biomass in repeatedly clipped plots of *H. verticillata* than in non clipped and less frequently

clipped plots (Fox et al. 2002). Therefore, was hypothesized that one clipping would be enough

to reduce thalli biomass and bulbil density of *N. obtusa* in this small scale trial.

Materials & Methods

This study was performed under greenhouse conditions at Minnesota State University,

Mankato from August 23rd 2021 to October 3rd 2022. Biomass of *N. obtusa* was harvested from

historically nontreated plots in Lake Koronis, Stearns County, Minnesota (45.31°N, -94.68°W)

and used to establish greenhouse stock cultures. After harvest, *N. obtusa* was transplanted into

0.473 L plastic containers filled with sediment amended with Osomocote Fertilizer (19-6-12,

Scotts-Sierra Horticultural Products Company, 14111 Scottslawn Rd., Marysville, OH 43041) at

a rate of 2 g L^{-1} sediment, and topped with sand (making the average soil surface height 10 cm).

To transplant *N. obtusa*, thalli were wrapped in small balls and were placed on top of the sand in

each culture. The tanks were filled with 94.6 L of tap water (52 cm water depth) and were treated

with sodium thiosulfate to remove chlorine (about 1 to 1 ratio of $Na_2S_2O_3$ to Cl_2) and tanks were

covered in shade netting (60% light reduction) to aid in the early stages of growth. These

cultures were grown in either a greenhouse under ambient light conditions or a lab under

artificial light using a 12:12 hour light:dark cycle. Water temperature in the tanks where about 22

°C ± 0.1 SE and pH was circumnuetral. Cultures were aerated with a compressed air system to

add CO_2 to the water collumn in the tanks. All propagation methods were adapted from Wersal

2022.

After letting *N. obtusa* cultures establish for 1.5 months (or until thalli reached the water

surface, approximately 52.5 cm from soil surface), 11 *N. obtusa* cultures were moved to each

treatment mesocosm (for a total of 198 cultures per trial). These mesocosms were subjected to an

ambient light cycle, aerated with a compressed air system and were filled with 94.6 L of water

(63.5-cm depth). Three clipping treatments were applied and compared to a non-treated reference

over a four-month period. All treatments used four mesocosms (replicates) and the study was

repeated twice. Trial two started about six weeks after trial one started. The treatments were: (1)

a nontreated reference; (2) clipping once per growing season; (3) clipping every other month

(bimonthly, two clippings total); and (4) clipping every month (four clippings total).

Clipping treatments were timed to mimic when mechanical treatments would be applied

in the field. In Minnesota *N. obtusa* does not top out in the water collumn until late June or early

August (Carver 2022). To be able to remove thalli biomass, mechanical treatments would not be applied until the third month of the growing season. To mimic this the one clipping treatment was applied on the third month, the two clipping treatment was applied on the second and fourth month, and the four clipping treatment was applied every month. Therefore the initiation of each clipping treatment was different in an attempt to capture the optimal time to harvest in the field.

Treatments were chosen based on previous simulated harvesting trials (Turnage et al 2019), and modified to simulate the rate at which a harvestor could reasonably cut in a season. Biomass was clipped approximately 15 cm above the sediment surface. This clipping was determined to simulate a harvestor cutting depth of 1.8 meters (Aquarius Systems 2010) of 4.5 meters of *N. obtusa* biomass. The depth of 4.5 meters was estimated from Lake Koronis having established populations of *N. obtusa* in depths between 0 to 4.5 meters and was supported by the average depth of *N. obtusa* growth occuring between 4 and 8 meters of depth in its native range (Olsen 1944, Carver 2022).

Pretreatment samples (1 container from each treatment tank) were harvested the day before the first clipping. Harvested *N. obtusa* was seperated into thalli biomass, rhizoid biomass and bulbil biomass, placed in labeled paper bags, and dried in a forced air oven at 48 °C for at least 48 hours to determine g DW pot^{-1} for each tissue type. The number of bulbils were recorded before drying. At 16 weeks after treatment (WAT), half the containers in each mesocosm were harvested in the same manner as pretreatment samples. The remaining pots in all mesocosms were harvested 52 WAT. Additionally, during each harvesting event, clipped biomass was removed from mesocosms to simulate harvester-boat operation in a field setting. Harvested biomass was put into labeled paper bags and dried in a forced air oven at 48 °C for at least 48 hours. The mass for each clipping was then weighed to give an estimate of how much biomass

was removed during each harvesting event, and if harvesting was offering longer-term reductions in thalli biomass.

Statistical Analysis

In order to minimize the inherent varibility when working with *N. obtusa* the lowest performing replicate in each treatment was excluded from statistical analysis; threfore all analyses were done using three replicates for each treatment. Shapiro-Wilks normality test was used to determine the data was nonparametric for all statistics ($p<0.01$ for all data; Statistix 10 Analytical Software, 2105 Miller Landing Rd, Tallahassee, FL 32312). Therefore, a Kruskal Wallis test was used to test for trial effects and clipping effects on thalli biomass and bulbil density (Statistix 10 Analytical Software, 2105 Miller Landing Rd, Tallahassee, FL 32312). To account for different start times for each clipping treatment a Kruskal Wallis test was used to compare treatment differences in the pretreatment biomass. At pretreatment there was no significant difference between thalli clipping treatments (trial one: $H=0.55$, $p=0.91$; trial two: $H=6.09$, $p=0.09$) or between bulbil clipping treatments (trial one: $H=1.53$, $p=0.72$; trial two $H=4.41$, $p=0.23$). Initial clipped biomass harvested from each treatment was also not significantly different ($H=1.61$, $p=0.22$). There was a trial difference for thalli biomass and bulbil density across both 16 WAT and 52 WAT so data were analyzed using a Kruskal Wallis test within WAT and for each trial. The Dunn's All-Pairwise comparison posthoc test was used to determine differences. All statistical tests were conducted using an $\alpha \leq 0.05$ significance level.

Results & Discussion

At 16 WAT trial one showed a significant difference after two clipping treatments compared to the non treated reference bulbil density (112 days; $H=32.01$, $p<0.01$) but trial one

showed no significant difference between treatments (H=1.98, p=0.58; Figure 1). At 16 WAT

one clipping reduced bulbil density by an average of 41% (trial one reduced by 44%, trial two

reduced by 38%), two clipping reduced bulbil density by an average of 51% (trial one reduced

by 52%, trial two reduced by 50%), and the four clipping treatment reduced bulbil density by an

average of 90% (trial one reduced by 88%, trial two reduced by 93%). Clipping four times per

growing season reduced bulbil density, on average, by about 46% (trial one reduced by 23% and

trial two reduced by 69%) after 52 weeks from initial treatment (WAT) (trial one H=29.00,

p<0.01; trial two H=15.54, p<0.01; Figure 1). The four clipping treatment mesocosms were at or

below the pretreatment density of 14,568 N m^{-2} for trial one and 6,083 N m^{-2} for trial 2. Showing

that mechanical harvesting once per month during the growing season stresses *N. obtusa* enough

to prevent regrowth of bulbils after a full year. This is possibly due to the clipping and

subsequent harvesting opening the water column to light penetration that potentially induced

bulbil sprouting after each clipping. At 52 WAT the one clipping reduced bulbil density by an

average of 23% across treatments (trial one reduced by 51%, trial two increased by 4%) and two

clipping reduced bulbil density by an average of 43% across treatments (trial one reduced by

28%, trial two reduced by 58%).

At 16 WAT it also only took one to two clipping treatments to significantly reduce thalli

biomass (trial one H=37.13, p<0.01; trial two H=30.72, p<0.01; Figure 2). At 16 WAT the one

clipping reduced thalli biomass by an average of 51% (trial one reduced by 17%, trial two

reduced by 85%), two clipping reduced thalli biomass by an average of 69% (trial one reduced

by 52%, trial two reduced by 86%), and four clippings reduced thalli biomass by 86% (trial one

reduced by 90%, trial two reduced by 82%). At 52 WAT it only took one to two clipping and

harvesting treatments to reduce thalli biomass (g DW) compared to the untreated reference (trial

one H=39.76, p<0.01; trial two H=38.05, p<0.01; Figure 2). All clipping treatments were less

than the pretreatment after 52 WAT besides the nontreated reference. At 52 WAT the one

clipping treatment reduced thalli biomass by an average of 91% (trial one reduced by 84%, trial

two reduced by 98%), two clipping reduced thalli biomass by an average of 98% (trial one

reduced by 97%, trial two reduced by 99%), and four clippings reduced thalli biomass by 97%

(trial one reduced by 97%, trial two reduced by 98%).

Previous research measuring the regrowth after a clipping regime on flowering rush

(*Butomus umbellatus* L.), an invasive aquatic plant in Minnesota lakes, found that biweekly

(every two weeks) clippings consistently reduced above ground biomass after 52 WAT, while

monthly clippings only sometimes had a significant difference from non treated mesocosms

(Turnage et al. 2019). Comparitively mechanical harvesting is needed less frequently to reduce

above ground biomass for *N. obtusa* than it is for *B. umbellatus*. This supports previous work

that suggests that a species tolerance to mechanical clipping will be determined by the amount of

belowground biomass present (Turnage et al. 2019). Mature individuals of *Juncus ingens* (N. A.

Wakef.) were reportedly able to survive clipping stress more often than immature individuals due

to the amount of belowground biomass avaiable to promote regrowth (Middleton 1990; Mayence

et al. 2010). Clipping directly targets above ground biomass, so individuals or species with more

below ground biomass may be more tolerant to clipping stresses than those with less

belowground biomass. There is a lot less propagule biomass for *N. obtusa* bulbils and rhizoids

(15.4 g m^{-2}) compared to *B. umbellatus* rhizome buds (4,000 g m^{-2}) though propagule density for

N. obtusa (156,944 N m-2) is much higher than for *B. umbellatus* (4,800 N m^{-2}; Turnage et al.

2019; Carver 2022). Because *N. obtusa* has less below ground biomass, this suggests clipping

may be a viable management option in smaller infestations where four clipping treatments can be applied in one growing season.

In this controlled mesocosm study each consecutive clipping removed on average 39% of the amount of biomass previously harvested suggesting that clipping treatments become less efficient every consecutive clipping (Table 1). Interestly, it was observed during the four clipping treatment, that *N. obtusa* started to grow horizontally in the water column beneath the clipping height after the second clipping. It is not certain if *N. obtusa* stopped growing out of the top nodes and only grew sideways as a physiological response, or if there are other mechanisms that would cause this growth pattern. More research would be needed to test if there is an adaptation response or if the growth was coincidental. Lateral growth may be a form of morphological plasticity that has been observed in other macro algaes such as brown algae (Charrier et al. 2012). The plasticity of the brown algae *Fucus gardneri* (P. C. Silva) allows individuals to change their size, shape, and bendability in response to extreme storm waves that remove individuals from their attachment points (Koehl 1984; Koehl and Alberte 1988; Blanchette 1997; Dudgeon and Johnson 1992; Charrier et al. 2012). This is also seen in the kelp species *Nereocytis luetkeana* (K. Mertens) that can change morphology in a span of 4 to 5 days to limit water movement drag (Koehl et al. 2008; Charrier et al. 2012). If lateral growth is an adaptation response of *N. obtusa*, then resource managers will need to consider this when utilizing repeated clipping as a control mechanism.

Overall, these data suggest mechanical clipping could be used for nuisance relief if the area is small enough to be clipped at least four times per growing season. Clipping four times per growing season was enough to stress *N. obtusa* to reduce bulbil density and thalli biomass. Populations of *N. obtusa* may respond differently to clipping in field conditions where there is

more variability in bulbil densities (0 bulbils m^{-2} to 156,944 bulbils m^{-2}) (Carver 2022) suggesting this study should be tested in field sites prior to recommendation as an operational control technique.A single harvestor on average can clip about 0.4 to 1.6 ha per day; therefore, a harvestor can only clip about 48 to 192 ha once per growing season, 24 to 96 ha twice per growing season, and only 12 to 48 ha four times per growing season (Greenfield et al. 2004).

   If clipping is to be implemented as a method to reduce thalli biomass and bulbil density, then it would be most effective in smaller treatment sites such as lake access points, high traffic areas like boat lanes, or in swimming areas that have small populations of *N. obtusa* that are near harvester offload points. The timing for mechanical clipping treatments should also be carefully planned to avoid disturbing fish spawning or harm native fish and fauna (Wile 1978; Mikol 1985; Booms 1999). Harvesting can easily trap invertebrates and fish that get entangled on macrophytes so harvesting should be used responsibly to avoid removing too many off target species (Booms 1999). It is recommended to integrate clipping with other management tactics to maximize stress of *N. obtusa* thalli production and to reduce the sediment bulbil bank. Though futher research should be done to test the response of *N. obtusa* growth to harvesting treatments done in a field setting to account for variables that could not be captured in this study.

Tables

Table 1. Values of reduction of removed biomass, average harvested biomass, and harvested biomass SE at each clipping treatment harvest time. Reduction of biomass removed represents the reduction from the previous to the current harvest of biomass at each treatment.

Number of Clippings per Growing Season	Harvest Number	% Biomass Removed	Average Biomass Removed at Harvest (g)	SE Biomass Removed at Harvest
One	1	x	4.0	1.2
Two	1	x	2.8	0.8
	2	31.1	0.8	0.4
Four	1	x	1.5	0.8
	2	43.3	0.6	0.2
	3	44.2	0.3	0.1
	4	39.2	0.1	0.04

Figures
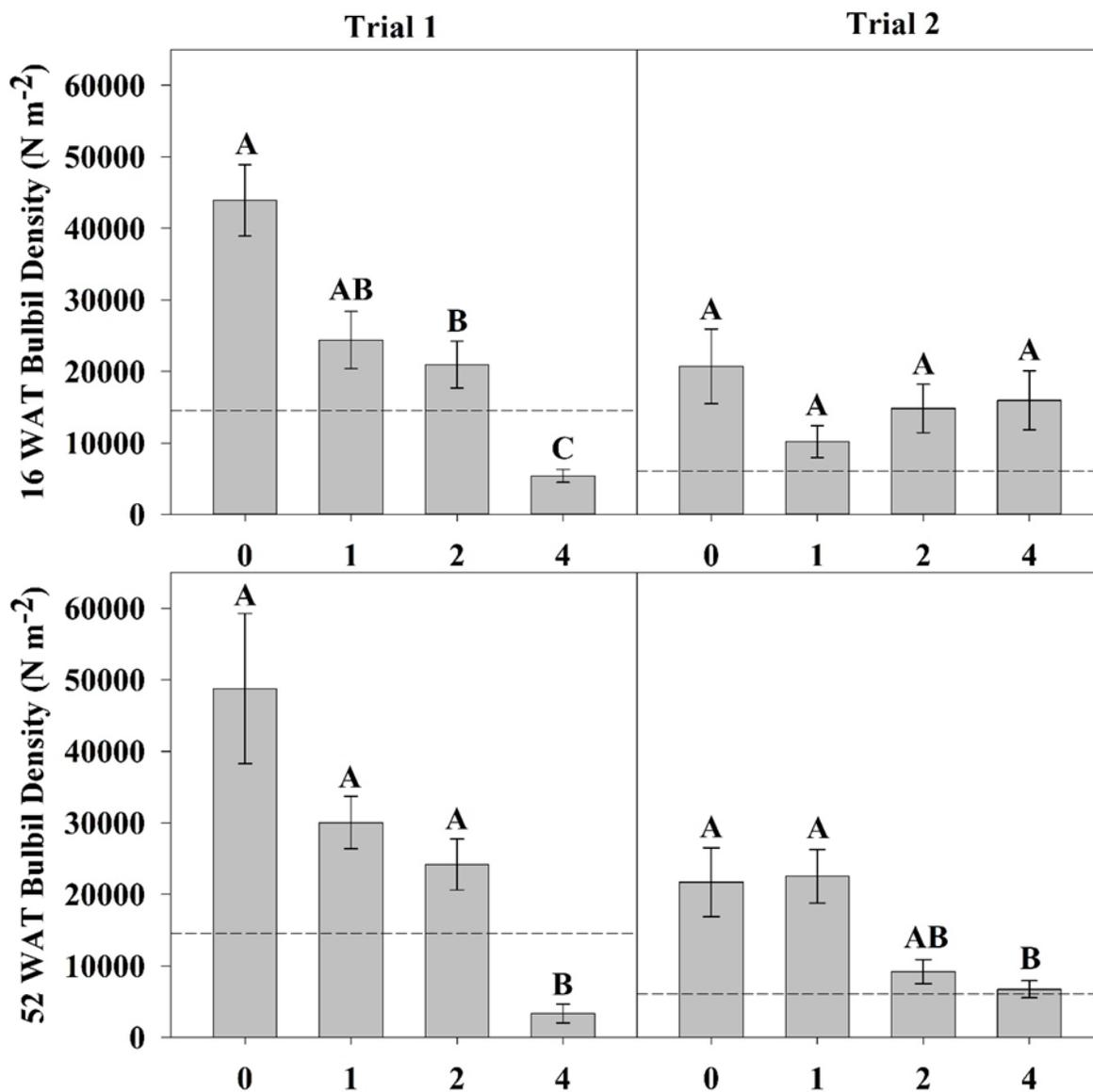
Figure 1. Mean (± 1 SE) bulbil density (N m^{-2}) for the clipping treatments (0, 1, 2, 4 clip) at 16 weeks after initial treatment (WAT) and 52 WAT for both trials. 16 WAT trial one H=22.57, p<0.01; 16 WAT trial two H=0.65, p=0.59; 52 WAT trial one H=18.04, p<0.01; 52 WAT trial two F=6.67, p<0.01. Dashed line represents mean pretreatment bulbil density (trial one=14,568 N m^{-2}, trial two=6082 N m^{-2}).
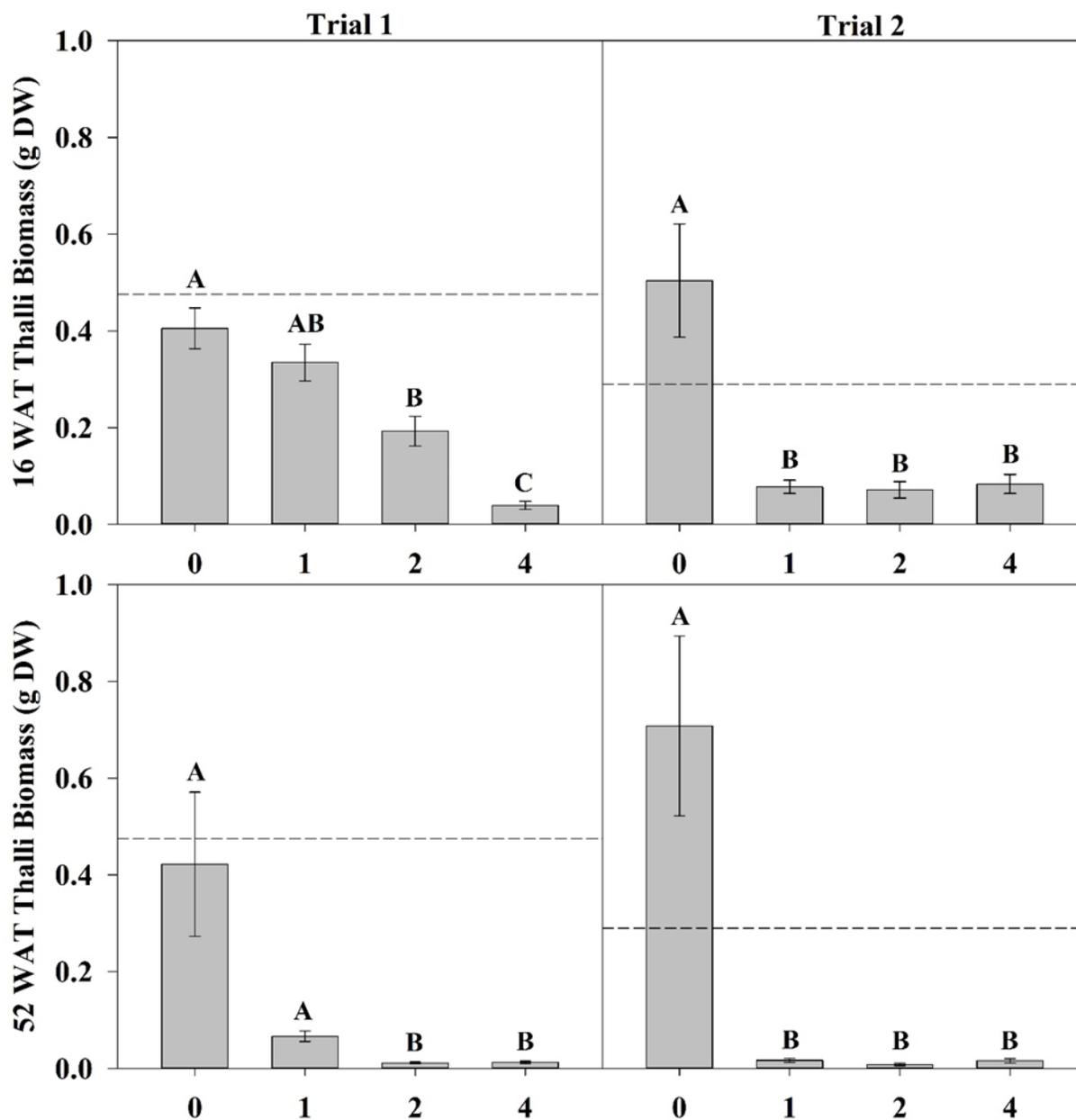
Figure 2. Mean (± 1 SE) thalli biomass (g DW) for the clipping treatments (0, 1, 2, 4 clip) at 16 weeks after initial treatment (WAT) and 52 WAT for both trials. 16 WAT trial one H=32.55, p<0.01; 16 WAT trial two H=20.16, p<0.01; 52 WAT trial one H=36.33, p<0.01; 52 WAT trial two H=30.89, p<0.01). Dashed line represents mean pretreatment thalli biomass (trial one=0.48 g DW, trial two=0.29 g DW).

CHAPTER IV

CONCLUSIONS

Chapter II

The seasonal average low and high points of starch storage were observed to be June

(3.3%) and April (24.9%) for thalli biomass then June (41.7%) and November (54.6%) for

bulbils. Water temperature and light availability were shown to negatively influence starch

content of thalli. Regarding allocation patterns, above ground thalli reallocates carbohydrate

stores to the below ground rhizoids in May. Future research is needed to determine the mode of

energy transfer. It is hypothesized that carbohydrates are then transported to bulbils from July

through September as rhizoid starch decreases and the bulbil starch increases. Ultimately, the

carbohydrates are converted to starch and stored in bulbils (21.0-73.7%). In the invaded range,

N. obtusa bulbils are important for energy storage, vegetative reproduction and spread, as well as

temporal distribution. These bulbils spread easily, spread rapidly, can store a lot of energy (73%

starch), and are a major concern for the invasion of *N. obtusa*. These findings also suggest that *N.*

obtusa can persist in a late season niche due to a temperature and light transparency tolerance

that allow high levels of starch allocation later in the growing season that other aquatic

vegetation cannot utilize.

Chapter III

Management efforts to control *N. obtusa* have been limited to stressing the thalli and

have not been able to directly target the reproductive bulbils on larger scales. Smaller scale

efforts such as the use of hand pulling can be used but hand pulling is not realistic for larger

infestations. Management needs to stress the growth of *N. obtusa* as much as possible to reduce

the production of bulbils and limit the spread of current infestations in waterways. Thalli

biomass (g) was 97% lower than reference thalli biomass after four clipping treatments and

bulbil density (N m-2) was 46% lower than reference bulbil density after four clipping treatments. Mechanical clipping treatments may be useful for integration with other management techniques to further stress *N. obtusa* for reduction of total biomass and bulbil production.

Management Recommendations

These results provide lake management groups and resource managers a better understanding of mechanical control of *N. obtusa* and when to initiate management of *N. obtusa* infestations. Harvesters can cut and remove plants at a rate of 0.4 to 1.6 ha per day depending on the machine, plant density, and distance to off-load sites (Greenfield et al. 2004). Lake Koronis is a large lake with approximately 324 ha of *N. obtusa* (Carver 2022) and one whole lake harvest event would take 202 to 810 days to complete; however, the typical growing season in Minnesota is May to August (120 days). Therefore, mechanical harvesting would only be feasible in smaller infested areas like high traffic boat lanes or lake access points to allow for a minimum of four clippings per growing season. Unless managers can apply four clipping treatments per growing season no long-term effects on thalli reductions should be expected. Even with two clipping treatments in a growing season the bulbil bank will persist, and likely expand, so an integrated approach should be utilized to stress *N. obtusa* as much as possible to limit the production of bulbils. One clipping per growing season should only be used as an option for immediate nuisance relief. Management personnel should initiate herbicide treatments in June to synchronize chemical applications to *N. obtusa* seasonal times of low starch storage in bulbil and thalli biomass. Based off these findings it is not recommended to use mechanical treatments during this time since thalli biomass is seasonally low and does not peak until late November to October (Carver 2022). From this research it is recommended to apply management methods that can reach biomass in deeper water depths in the early growing season (such as chemical

treatments in June), and then apply additional mechanical treatments in high traffic priority areas later in the growing season. Applying stress and limiting energy allocation to the bulbils should be a management priority to limit the continued spread of *N. obtusa*.

Literature Cited

Alix MS, Scribailo RW, Weliczko CW (2017) *Nitellopsis obtusa* (Desv.) J. Groves, 1919 (Charophyta: Characeae): new records from southern Michigan, USA with notes on environmental parameters known to influence its distribution. BioInv. Rec. 6(4):311-319.

Aquarius Systems (2010) Aquatic Weed Harvesters. Aquarius-Systems. http://www.aquarius-systems.com/Pages/53/aquatic_weed_harvesters.aspx/. Accessed March 16, 2021.

Bailly G, Schäfer O (2010) Guide illustré des Characées du nord-est de la France. Conservatoire botanique national de Franche-Comté.

Blanchette CA (1997) Size and survival of intertidal plants in response to wave action: a case study with *Fucus gardneri*. Ecol. 78:1563-1578.

Booms TL (1999) Vertebrates removed by mechanical weed harvesting in Lake Keesus, Wisconsin. J. Aquat. Plant Manage. 37:34–36.

Boissezon A, Joye Dam Garcia T (2018) Temporal and spatial changes in population structure of the freshwater macroalga *Nitellopsis obtusa* (Desv.) J.Groves. Bot. Let. 165(1):103-114.

Brainard AS, Schulz KL (2017) Impacts of the cryptic macroalgal invader, *Nitellopsis obtusa*, on macrophyte communities. Freshw. Sci. 36(1):55-62.

Carver PJ (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. MS dissertation. Mankato, MN: Minnesota State University. 51 p.

Charrier B, Le Bail A, de Reviers B (2012) Plant Proteus: brown algal morphological plasticity and underlying developmental mechanisms. Trends Plant Sci. 17(8):468-477.

Chen CY, Durbin EG (1994) Effects of pH on the growth and carbon uptake of marine phytoplankton. Marine Eco. Progr. Series 109:83-94.

Cimino SA, Strecker AL (2018) Boater knowledge and behavior regarding aquatic invasive species at a boat wash station. Northwest Sci. 92:224– 233.

Clarke M, Wersal RM, Turnage G (2023) Seasonal phenology and starch allocation patterns of Cuban bulrush (*Oxycaryum cubense*) growing in Mississippi, USA. Aquat. Bot. 186:103627.

Corillion R (1957) Les Charophycées de France et d'Europe occidentale. 37.

Dudgeon SR, Johnson AS (1992) Thick vs thin: thallus morphology and tissue mechanics influence differential drag and dislodgment of 2 codominant seaweeds. J. Exp. Mar. Biol. Ecol. 165:23-43

Escobar LE, Qiao H, Phelps NB, Wagner CK, Larkin DJ (2016) Realized niche shift associated with the Eurasian charophyte *Nitellopsis obtusa* becoming invasive in North America. Sci. Rep. 6(1):1-5.

Fox AM, Haller WT, Cuda JP (2002) Impacts of Carbohydrate depletion by repeated clipping on the production of subterranean turions by dioecious Hydrilla. J. Aquat. Plant Manage. 40: 99-104.

Getsinger KE, Dibble JH, Rodgers Jr, Spencer D (2014) Benefits and costs of controlling nuisance aquatic plants and algae in the United States. CAST Commentary. 12 pp.

Ginn BK, Dias EFS, Fleischaker T (2021) Trends in submersed aquatic plant communities in a large, inland lake: impacts of an invasion by starry stonewort (*Nitellopsis obtusa*). Lake Reservoir Manage 37: 199-213.

Glisson WJ, Wagner CK, McComas SR, Farnum K, Verhoeven MR, Muthukrishnan R, Larkin DJ (2018) Response of the invasive alga starry stonewort (*Nitellopsis obtusa*) to control efforts in a Minnesota lake. Lake Reserv. Manage. 34(3): 283-295.

Glisson WJ, Wagner CK, Verhoeven MR, Muthukrishnan R, Contreras-Rangel R, Larkin DJ (2020) Desiccation tolerance of the invasive alga starry stonewort (*Nitellopsis obtusa*) as an indicator of overland spread risk. J. Aquat. Plant Manage. 58: 7–18.

Glisson WJ, Muthukrishnan R, Wagner CK, Larkin DJ (2022). Invasive *Nitellopsis obtusa* (Starry stonewort) has distinctive late-season phenology compared to native and other invasive macrophytes in Minnesota, USA. Aquat. Bot. 176:103452.

Greenfield BK, David N, Hunt J, Wittmann M, Siemering G (2004) Aquatic pesticide monitoring program review of alternative aquatic pest control methods for California waters. Oakland, CA: San Francisco Estuary Institute. 106 p.

Groves J (1919) Notes on *Lychnothamnus braun*. J. Bot. 57:125-129.

Harrow-Lyle TJ, Kirkwood AE (2021) Pervasive changes to the lower aquatic food web following *Nitellopsis obtusa* establishment in a large, shallow lake. Freshwater Bot 67(3): 533-541.

Harrow-Lyle TJ, Ginn BK, Kirkwood A, Melles SJ (2022) First report of female gametangia in the invasive macroalga starry stonewort (*Nitellopsis obtusa*) in North America. Bot. https://doi.org/10.1139/cjb-2022-0096.

Heiskary S, Wilson B (2008) Minnesota's approach to lake nutrient criteria development. Lake Reserv. Manage. 24(3):282-297.

Hildebrand M, Abbriano RM, Polle JEW, Traller JC, Trentacoste EM, Smith SR, Davis AK
(2013) Metabolic and cellular organization in evolutionarily diverse microalgae as related
to biofuels production. Current Op. Chem. Bio. 17(3):506-514.

Hildebrand M, Manandhar-Shrestha K, Abbriano R (2017) Effects of chrysolaminarin synthase
knockdown in the diatom *Thalassiosira pseudonana*: Implications of reduced
carbohydrate storage relative to green algae. Algal Research 23: 66-77.

Janauer GA, Schmidt-Mumm U, Schmidt B (2010) Aquatic macrophytes and water current
velocity in the Danube River. Ecol. Eng. 36(9):1138-1145.

Kabus T (2016) *Nitellopsis obtusa*. Arbeitsgruppe Characeen Deutschlands (Ed.),
Armleuchteralgen. Die Characeen Deutschlands, Springer Verlag, Berlin, Heidelberg.
505-514.

Karol KG, Sleith RS (2017) Discovery of the oldest record of *Nitellopsis obtusa* (Charophyceae,
Charophyta) in North America. J. Phycol. 53(5):1106-1108.

Kato S, Kawai H, Takimoto M, Suga H, Yohda K, Horiya K, Higuchi D, Sakayama H (2014)
Occurrence of the endangered species *Nitellopsis obtusa* (Charales, Charophyceae) in
western Japan and the genetic differences within and among Japanese populations.
Phycolog. Research 62: 222–227.

Kipp RM, McCarthy M, Fusaro A (2014) *Nitellopsis obtusa*. USGS Nonindigenous Aquatic
Species Database. US Geological Survey, Gainesville, Florida.

Kipp RM, McCarthy M, Fusaro A, Pfingsten IA (2021) *Nitellopsis obtusa* (Desvaux in
Loiseleur) J. Groves, (1919): U.S. Geological Survey, Nonindigenous Aquatic Species
Database, Gainesville, FL, and NOAA Great Lakes Aquatic Nonindigenous Species
Information System, Ann Arbor, MI.

https://nas.er.usgs.gov/queries/GreatLakes/FactSheet.aspx?SpeciesID=1688. Access
Date: 2/16/2021

Koehl MAR (1984) How do benthic organisms withstand moving water. Am. Zool. 24:57-70.

Koehl MAR, Alberte RS (1988) Flow, flapping, and photosynthesis of Nereocystis luetkeana – a functional comparison of undulate and flat blade morphologies. Mar. Biol. 99:435-444.

Koehl MAR, Silk WK, Liang H, Mahadevan L (2008) How kelp produce blade shapes suited to different flow regimes: a new wrinkle. Integr. Comp. Biol. 48:834-851.

Korsch H, Raabe U, van de Weyer K (2008) Verbreitungskarten der Characeen Deutschlands. Rostocker Meeresbiologische Beiträge 19:57-108.

Krause W (1997) Süsswasserflora von Mitteleuropa: Charales (Charophyceae). Gustav Fischer Verlag, Jena, Germany.

Langangen A (2007) Charophytes of the Nordic countries. Saeculum. ANS. 102 pp.

Larkin DJ, Monfils AK, Boissezon A, Sleith RS, Skawinski PM, Welling CH, Cahill BC, Karol KG (2018) Biology, ecology, and management of starry stonewort (*Nitellopsis obtusa*; Characeae): A Red-listed Eurasian green alga invasive in North America. Aquat. Bot. 148:15-24.

Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. Gary (NC): SAS Institute Inc.

MacNeill GJ, Mehrpouyan S, Minow MA, Patterson JA, Tetlow IJ, Emes MJ, Raines C (2017) Starch as a source, starch as a sink: the bifunctional role of starch in carbon allocation. J. Exp. Bot. 68(16):4433-4453.

Madsen JD (1991) Resource allocation at the individual plant level. Aquat. Bot. 41:67–86.

Madsen JD (1997). Seasonal biomass and carbohydrate allocation in southern populations of

      Eurasian watermilfoil. J. Aquat. Plant Manage. 35:15–21.

Madsen JD, Owen CS (1998) Seasonal biomass and carbohydrate allocation in dioecious

      hydrilla. J. Aquat. Plant Manage. 36:138–145.

Mann H, Proctor VW, Taylor AS (1999) Towards a biogeography of North American

      Charophytes. Aust. J. Bot. 47:445-458.

Mayence CE, Marshall DJ, Godfree RC (2010) Hydrologic and mechanical control for an

      invasive wetland plant, *Juncus ingens*, and implications for rehabilitating and managing

      Murray River floodplain wetlands, Australia. Wetlands Ecol. Manage. 18:717-730.

Middleton BA (1990) Effect of water depth and clipping frequency on the growth and survival of

      four wetland plant species. Aquat. Bot. 37:189–196.

Midwood JD, Darwin A, Ho ZY, Rokitinicki-Wojcik D, Bragas G (2016) Environmental factors

      associated with the distribution of non-native starry stonewort (*Nitellopsis obtusa*) in a

      Lake Ontario coastal wetland. J. Great Lake Res. 42:348-355.

Mikol GF (1985) Effects of harvesting on aquatic vegetation and juvenile fish populations at

      Saratoga Lake, New York. J. Aquat. Plant Manage. 23:59–63.

[MNDNR] Minnesota Department of Natural Resources (2020) Infected Waters List.

      https://www.dnr.state.mn.us/invasives/ais/infested.html. Accessed: February 7, 2023.

Moazami-Goudarzi M, Colman B (2012) Changes in carbon uptake mechanisms in two green

      marine algae by reduced seawater pH. J. Exp. Marine Bio. Ecol. 413:94-99.

Molofsky J, SR Keller, S Lavergne, MA Kaproth and MB Eppinga (2014) Human-aided

      admixture may fuel ecosystem transformation during biological invasion: Theoretical and

      experimental evidence. Ecology and Evolution 4(7):899-910

Mouronval JB, Baudoin S, Borel N, Soulié-Märsche I, Klesczewski M, Grillas P (2015) Guide des Characées de France méditerranéenne. Office National de la Chasse et de la Faune Sauvage.

Muthukrishnan R, Sleith RS, Karol KG, Larkin DJ (2018) Prediction of starry stonewort (*Nitellopsis obtusa*) invasion risk in upper Midwest (USA) lakes using ecological niche models. Aquat. Bot. 151:43-50.

Naz S, Diba NJ, and Zaman M (2010) *Nitellopsis obtusa* (desv.) J. groves: A new charophytic record for Bangladesh. Bangl. J. Plant. Taxon. 17(2): 203.

Olsen S (1944) Danish Charophyta. Chronological, ecological and biological investigation. In: Biologiske Skrifter. København, pp 1–240

Owens CS, Madsen JD (1998) Phenological studies of carbohydrate allocation in Hydrilla. J. Aquat. Plant Manage. 36:40-44.

Pełechaty M, Pronin E, Pukacz A (2014) Charophyte occurrence in *Ceratophyllum demersum* stands. Hydrobiologia. 737:111-120.

Pennington TG, Sytsma MD (2009) Seasonal changes in carbohydrate and nitrogen concentrations in Oregon and California populations of Brazilian egeria (*Egeria densa*). Invas. Plant Sci. Manage. 2:120–129.

Pokrzywinski K, Sartain B, Greer M, Getsinger K, Fields M (2020a) Optimizing conditions for *Nitellopsis obtusa* (starry stonewort) growth and bulbil germination in a controlled environment. Aquat. Bot. 160:103163

Pokrzywinski KL, Getsinger KD, Steckart B, Midwood JD (2020b) Aligning research and management priorities for *Nitellopsis obtusa* (starry stonewort). US Army Corps of

Engineers Engineer Research and Development Center. Aquatic Plant Control Research
Program. 37 p.

Pullman GD, Crawford G (2010) A decade of starry stonework in Michigan. Lake Line,
Summer. 36-42.

Ray S, Klenell M, Choo KS, Pedersen M, Snoeijs P (2003) Carbon acquisition mechanisms in
Chara tomentosa. Aquat. Bot. 76(2):141-154.

Rothlisberger JD, Chadderton WL, McNulty J, Lodge DM (2010) Aquatic invasive species
transport via trailered boats: what is being moved, who is moving it, and what can be
done. Fisheries 35:121–132.

Simons J, Nat E (1996) Past and present distribution of stoneworts (Characeae) in The
Netherlands. Hydrobiologia. 340:127–135.

Sleith RS, Havens AJ, Stewart RA, Karol KG (2015) Distribution of *Nitellopsis obtusa*
(Characeae) in New York, U.S.A. Brittonia 97(2):166-172.

Sleith RS, Wehr JD, Karol KG (2018) Untangling climate and water chemistry to predict
changes in freshwater macrophyte distributions. Ecolog. Evolut. 8:2802-2811.

Soulié-Märsche I, Benammi M, Gemayel P (2002) Biogeography of living and fossil *Nitellopsis*
(Charophyta) in relationship to new finds from Morocco. J. Biogeog. 29:1703–1711.

Titus JE, Adams MS (1979) Comparative carbohydrate storage and utilization patterns in the
submersed macrophytes, *Myriophyllum spicatum* and *Vallisneria americana*. Americ.
Midland Nat. 102:263-272.

Turnage G, Madsen JD, Wersal RM, Byrd JD (2019) Simulated mechanical control of flowering
rush (*Butomus umbellatus*) under mesocosm conditions. Invas. Plant Sci. Manage. 12:
120–123.

Urbaniak J (2003) *Nitellopsis obtusa* (Desv. In Loisel) J. Groves 1919. In: Schubert H, Blindow I

    (eds), Charophytes of the Baltic Sea. The Baltic Marine Biologists Publication, No. 19.

    A.R.G. Ganter Verlag Kommanditgesellschaft, Königstein, pp 216–222

[USGS] United States Geological Survey. (2022). Nonindigenous Aquatic Species database,

    Nitellopsis obtusa. Nonindigenous Aquatic Species Program, Gainesville, FL.

Van TK, Steward KK (1990) Longevity of monoecious Hydrilla propagules. J. Aquat. Plant

    Manage. 28:74–76.

Vitova M, Bisova K, Kawano S, Zachleder V (2015) Accumulation of energy reserves in algae:

    from cell cycles to biotechnological applications. Biotech. Advanc. 33(6):1204-1218.

Weldon LW, Blackburn RD (1698) Herbicidal treatment effect on carbohydrate levels of

    alligatorweed. Weed Sci. 16:66–69.

Wersal RM (2022) Small-scale evaluations of select pesticides for development of management

    recommendations for starry stonewort (*Nitellopis obtusa*). J. Aquat. Plant Manage. 60:10-

    15

Wersal RM, Cheshier JC, Madsen JD, Gerard PD (2011) Phenology, starch allocation, and

    environmental effects on *Myriophyllum aquaticum*. Aquat. Bot. 95(3): 194-199.

Wersal RM, Madsen JD (2018) Designing and using phenological studies to define management

    strategies for aquatic plants. J. Aquat. Plant Manage. 56(s):83-89.

Wersal RM, Madsen JD, and Cheshier JC (2013) Seasonal Biomass and Starch Allocation of

    Common Reed (*Phragmites australis*) (Haplotype I) in Southern Alabama, USA. Invas.

    Plant Sci. Manage. 6:140–146.

Wile I (1978) Environmental effects of mechanical harvesting. J. Aquat. Plant Manage. 16:14–

    20.

Wood RD (1965) Monograph of the Characeae. In: Wood RD, Imahori K (eds), A revision of the
Characeae, Vol. 1. Verlag von J. Cramer, Weinheim, pp 1–904

Wood RD, Imahori K (1965) A revision of the Characeae. Part-I. Monograph of the Characeae.
Verlag Von, J. Cramer, Weinheim. pp. 349-355.

Woolf TE, Madsen JD (2003) Seasonal biomass and carbohydrate allocation patterns in Southern
Minnesota curlyleaf pondweed populations. J. Aquat. Plant Manage. 41:113–118.

Zipp KY, Lewis DJ, Provencher B, Vander Zanden MJ (2019) The spatial dynamics of the
economic impacts of an aquatic invasive species: An empirical analysis. Land Econ.
95(1):1-8.

APPENDIX

Starch Percent Recovery

Table 1. Wheat starch standards ran in conjunction with biomass of *Nitellopsis obtusa*.

Type	Mass	% Starch	% Purity	% Recovery
Wheat	15.00	84.15	89.00	94.55
Wheat	12.00	76.97	89.00	86.49
Wheat	13.00	79.72	89.00	89.57
Wheat	14.00	75.69	89.00	85.04
Wheat	10.00	71.67	89.00	80.53
Wheat	18.00	73.87	89.00	83.01
Wheat	16.00	84.97	89.00	95.47
Wheat	12.00	68.75	89.00	77.25
Wheat	16.00	73.33	89.00	82.39
Wheat	14.00	78.29	89.00	87.97
Wheat	16.00	88.59	89.00	99.54
Wheat	10.00	86.60	89.00	97.30
Wheat	10.00	74.25	89.00	83.43
Wheat	16.00	79.78	89.00	89.64
Wheat	12.00	94.61	89.00	106.30
Wheat	10.00	87.07	89.00	97.84
Wheat	10.00	88.70	89.00	99.66
Wheat	12.00	80.73	89.00	90.71
Wheat	12.00	78.29	89.00	87.96
Wheat	10.00	80.68	89.00	90.65
Wheat	10.00	87.34	89.00	98.13
Wheat	15.00	79.45	89.00	89.26
Wheat	15.00	80.67	89.00	90.64
Wheat	11.00	135.52	89.00	152.27
Wheat	11.00	72.97	89.00	81.99
Mean % Starch:		82.51	Mean % Recovery:	92.70

Starch Percent Difference

Table 2. Percent difference of duplicate samples ran on biomass samples of *Nitellopsis obtusa*.

Month	Year	Pheno	Tissue	Mass	Starch	% Difference
August	2020	1	Thalli Dup	51.00	1.25	
August	2020	1	Thalli	54.00	1.38	9.53
August	2020	1	Thalli Dup	53.00	2.54	
August	2020	1	Thalli	51.00	2.67	4.64
August	2020	1	Thalli Dup	54.00	4.94	
August	2020	1	Thalli	54.00	4.56	7.72
July	2020	1	Thalli Dup	51.00	9.48	
July	2020	1	Thalli	56.00	8.89	6.24
July	2020	1	Thalli Dup	51.00	1.96	
July	2020	1	Thalli	52.00	2.12	7.41
July	2020	1	Thalli Dup	50.00	2.64	
July	2020	1	Thalli	54.00	2.54	3.64
July	2020	1	Thalli Dup	53.00	2.59	
July	2020	1	Thalli	53.00	2.39	8.05
July	2020	1	Thalli Dup	55.00	4.37	
July	2020	1	Thalli	54.00	4.46	1.96
July	2020	1	Thalli Dup	50.00	3.08	
July	2020	1	Thalli	56.00	2.28	25.98
June	2020	1	Thalli Dup	51.00	12.36	
June	2020	1	Thalli	57.00	9.80	20.71
June	2020	1	Thalli Dup	55.00	12.15	
June	2020	1	Thalli	54.00	13.72	11.48
June	2020	1	Thalli Dup	52.00	10.10	
June	2020	1	Thalli	52.00	9.99	1.08
May	2020	1	Thalli Dup	51.00	16.31	
May	2020	1	Thalli	55.00	13.67	16.20
May	2020	1	Thalli Dup	52.00	13.14	
May	2020	1	Thalli	55.00	10.70	18.55
May	2020	1	Thalli Dup	52.00	10.73	
May	2020	1	Thalli	50.00	7.83	27.06
November	2020	1	Thalli Dup	51.00	5.09	
November	2020	1	Thalli	52.00	4.76	6.56
November	2020	1	Thalli Dup	50.00	4.44	
November	2020	1	Thalli	53.00	6.66	33.35
November	2020	1	Thalli Dup	52.00	4.99	
November	2020	1	Thalli	57.00	8.20	39.12
October	2020	1	Thalli Dup	50.00	5.67	
October	2020	1	Thalli	50.00	6.25	9.39
October	2020	1	Thalli Dup	51.00	4.93	

October	2020	1	Thalli	52.00	5.10	3.31
October	2020	1	Thalli Dup	53.00	4.48	
October	2020	1	Thalli	55.00	4.94	9.36
October	2020	1	Thalli Dup	54.00	4.62	
October	2020	1	Thalli	51.00	7.47	38.16
September	2020	1	Thalli Dup	52.00	5.21	
September	2020	1	Thalli	53.00	5.08	2.48
September	2020	1	Thalli Dup	54.00	4.58	
September	2020	1	Thalli	54.00	4.18	8.71
September	2020	1	Thalli Dup	57.00	4.95	
September	2020	1	Thalli	58.00	4.83	2.38
September	2020	1	Bulbil Dup	56.00	63.84	
September	2020	1	Bulbil	58.00	61.77	3.23
September	2020	1	Thalli Dup	52.00	3.72	
September	2020	1	Thalli	54.00	2.82	24.17
September	2020	1	Bulbil Dup	56.00	61.77	
September	2020	1	Bulbil	58.00	63.83	3.23
April	2020	3	Thalli Dup	50.00	18.72	
April	2020	3	Thalli	53.00	19.67	4.86
April	2020	3	Bulbil Dup	56.00	58.76	
April	2020	3	Bulbil	56.00	59.29	0.90
August	2020	3	Thalli Dup	54.00	2.95	
August	2020	3	Thalli	50.00	3.08	4.20
August	2020	3	Thalli Dup	51.00	3.80	
August	2020	3	Thalli	51.00	4.10	7.33
October	2020	3	Thalli Dup	52.00	5.60	
October	2020	3	Thalli	53.00	6.45	13.14
September	2020	3	Thalli Dup	50.00	2.78	
September	2020	3	Thalli	51.00	2.94	5.17
November	2020	5	Bulbil Dup	54.00	23.32	
November	2020	5	Bulbil	50.00	51.73	2.98
November	2020	5	Bulbil Dup	54.00	53.31	
November	2020	5	Bulbil	50.00	51.73	2.97
April	2020	6	Thalli Dup	52.00	7.50	
April	2020	6	Thalli	58.00	7.87	4.62
August	2020	6	Thalli Dup	51.00	1.08	
August	2020	6	Thalli	52.00	1.37	21.22
August	2020	6	Thalli Dup	51.00	1.50	
August	2020	6	Thalli	52.00	1.90	20.91
July	2020	6	Thalli Dup	53.00	2.15	
July	2020	6	Thalli	52.00	2.67	19.60
July	2020	6	Thalli Dup	52.00	4.58	
July	2020	6	Thalli	51.00	4.29	6.35

Month	Year		Type			
June	2020	6	Thalli Dup	54.00	2.44	
June	2020	6	Thalli	51.00	2.52	3.43
June	2020	6	Thalli Dup	51.00	3.00	
June	2020	6	Thalli	57.00	3.46	13.31
June	2020	6	Thalli Dup	54.00	3.19	
June	2020	6	Thalli	53.00	4.55	29.88
June	2020	6	Bulbil Dup	52.00	52.07	
June	2020	6	Bulbil	50.00	52.42	0.65
May	2020	6	Thalli Dup	51.00	3.71	
May	2020	6	Thalli	55.00	5.18	28.44
November	2020	6	Thalli Dup	50.00	8.11	
November	2020	6	Thalli	51.00	9.09	10.76
November	2020	6	Thalli Dup	52.00	5.02	
November	2020	6	Thalli	52.00	4.38	12.80
October	2020	6	Thalli Dup	55.00	5.96	
October	2020	6	Thalli	52.00	5.79	2.83
October	2020	6	Thalli Dup	52.00	4.32	
October	2020	6	Thalli	56.00	6.42	32.69
September	2020	6	Thalli Dup	53.00	3.39	
September	2020	6	Thalli	53.00	3.40	0.27
September	2020	6	Thalli Dup	54.00	3.30	
September	2020	6	Thalli	53.00	2.71	17.87
September	2020	6	Thalli Dup	50.00	5.41	
September	2020	6	Thalli	54.00	5.39	0.30
September	2020	6	Bulbil Dup	68.00	63.76	
September	2020	6	Bulbil	56.00	64.15	0.61
September	2020	6	Bulbil Dup	68.00	63.76	
September	2020	6	Bulbil	56.00	64.15	0.61
August	2021	1	Thalli Dup	56.00	5.19	
August	2021	1	Thalli	56.00	6.68	22.37
May	2021	1	Thalli Dup	51.00	4.84	
May	2021	1	Thalli	56.00	5.32	9.11
May	2021	1	Green Bulbil Dup	59.00	26.02	
May	2021	1	Green Bulbil	60.00	21.04	19.14
October	2021	1	Green Bulbil Dup	59.00	21.59	
October	2021	1	Green Bulbil	60.00	21.27	1.46
November	2021	1	Green Bulbil Dup	54.00	40.70	
November	2021	1	Green Bulbil	58.00	38.95	4.30
June	2021	1	White Bulbil Dup	52.00	59.33	
June	2021	1	White Bulbil	52.00	60.35	1.69
September	2021	1	White Bulbil Dup	52.00	68.72	
September	2021	1	White Bulbil	52.00	70.33	2.30
April	2021	1	Thalli Dup	56.00	27.50	

Month	Year	Site	Type			
April	2021	1	Thalli	56.00	23.95	12.91
June	2021	3	Thalli Dup	55.00	1.82	
June	2021	3	Thalli	54.00	1.71	6.19
September	2021	3	Thalli Dup	57.00	4.58	
September	2021	3	Thalli	58.00	4.52	1.23
October	2021	3	Green Bulbil Dup	57.00	23.41	
October	2021	3	Green Bulbil	57.00	23.10	1.32
November	2021	3	Green Bulbil Dup	54.00	53.12	
November	2021	3	Green Bulbil	54.00	54.06	1.74
May	2021	3	White Bulbil Dup	58.00	53.71	
May	2021	3	White Bulbil	52.00	56.15	4.34
November	2021	3	Green Bulbil Dup	54.00	53.11	
November	2021	3	Green Bulbil	54.00	54.05	1.73
November	2021	5	Thalli Dup	55.00	8.06	
November	2021	5	Thalli	59.00	8.08	0.19
June	2021	5	Thalli Dup	52.00	1.67	
June	2021	5	Thalli	52.00	1.38	17.65
April	2021	5	Thalli Dup	54.00	4.20	
April	2021	5	Thalli	50.00	3.34	20.55
September	2021	5	Green Bulbil Dup	50.00	34.24	
September	2021	5	Green Bulbil	50.00	34.12	0.37
April	2021	5	White Bulbil Dup	50.00	65.13	
April	2021	5	White Bulbil	50.00	66.42	1.94
November	2021	5	White Bulbil Dup	52.00	58.38	
November	2021	5	White Bulbil	50.00	59.68	2.19
November	2021	5	Green Bulbil Dup	54.00	40.69	
November	2021	5	Green Bulbil	58.00	38.94	4.30
September	2021	6	Thalli Dup	52.00	8.86	
September	2021	6	Thalli	59.00	7.62	14.05
September	2021	6	Green Bulbil Dup	54.00	26.30	
September	2021	6	Green Bulbil	54.00	28.78	8.62
September	2021	6	Green Bulbil Dup	52.00	50.42	
September	2021	6	Green Bulbil	52.00	49.10	2.63
July	2021	6	Thalli Dup	51.00	4.42	
July	2021	6	Thalli	52.00	3.64	17.52
September	2021	6	Green Bulbil Dup	52.00	50.42	
September	2021	6	Green Bulbil	52.00	49.09	2.62
May	2021	All	Rhizoid Dup	62.00	5.92	
May	2021	All	Rhizoid	58.00	5.78	2.40
September	2021	All	Rhizoid Dup	52.00	5.64	
September	2021	All	Rhizoid	50.00	5.54	1.75

| | Mean % Difference (± 1 SE): | 9.75 ± 1.07 |

Starch Standard Curve
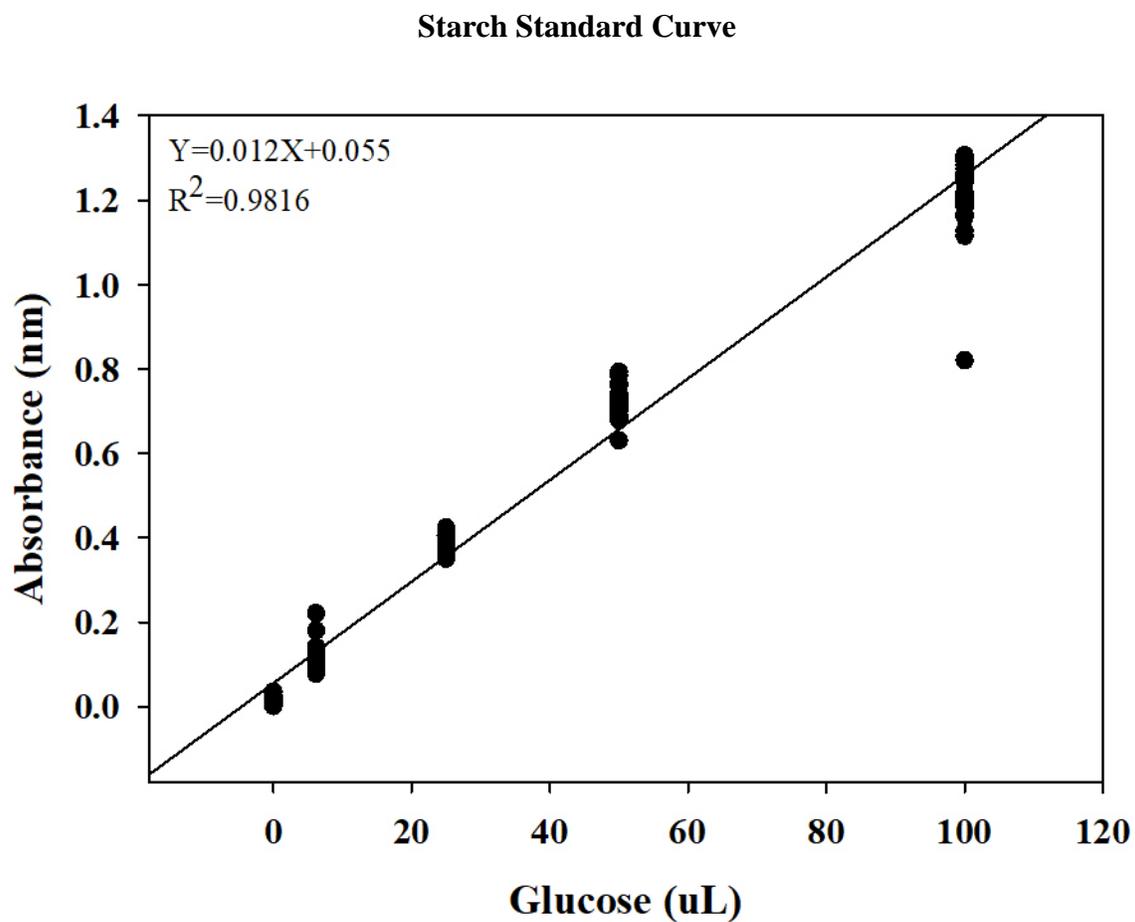
Figure 2. Combined standard curve absorbances (nm) ran in conjunction with biomass samples of *Nitellopsis obtusa*.

Mesocosm Light Intensity
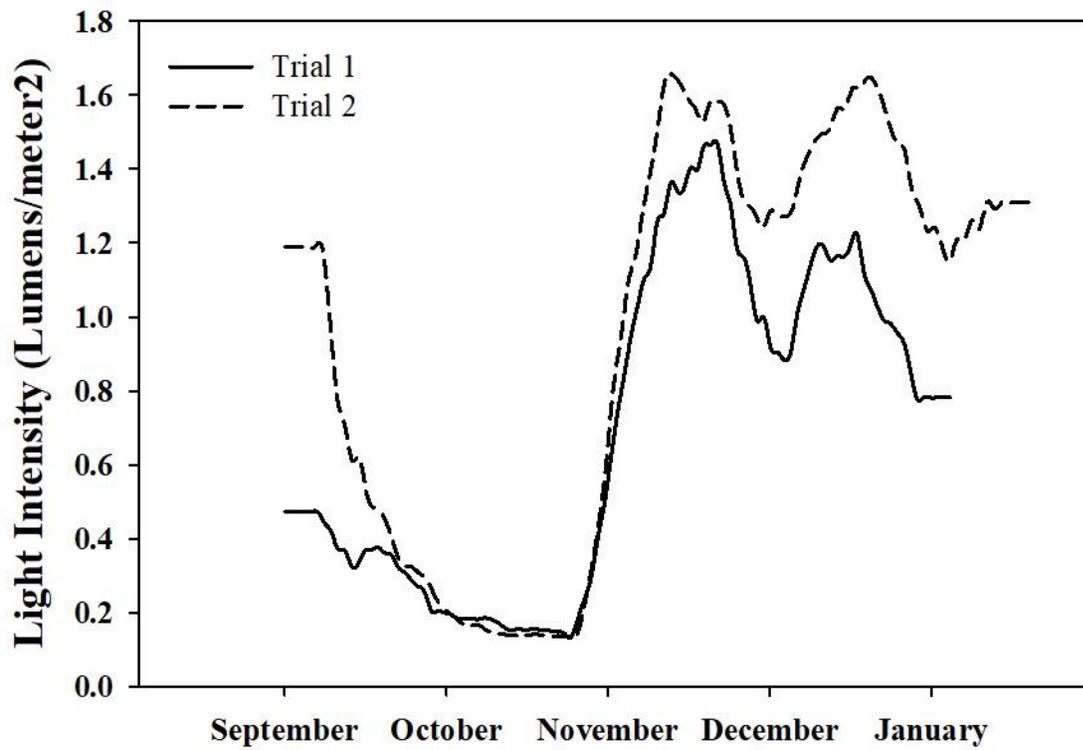
Figure 3. Mean light intensity of trial 1 and trial 2 controlled growth study tanks averaged across September 2021 to October 2022 measured hourly by HOBO sensors.

Mesocosm Temperature
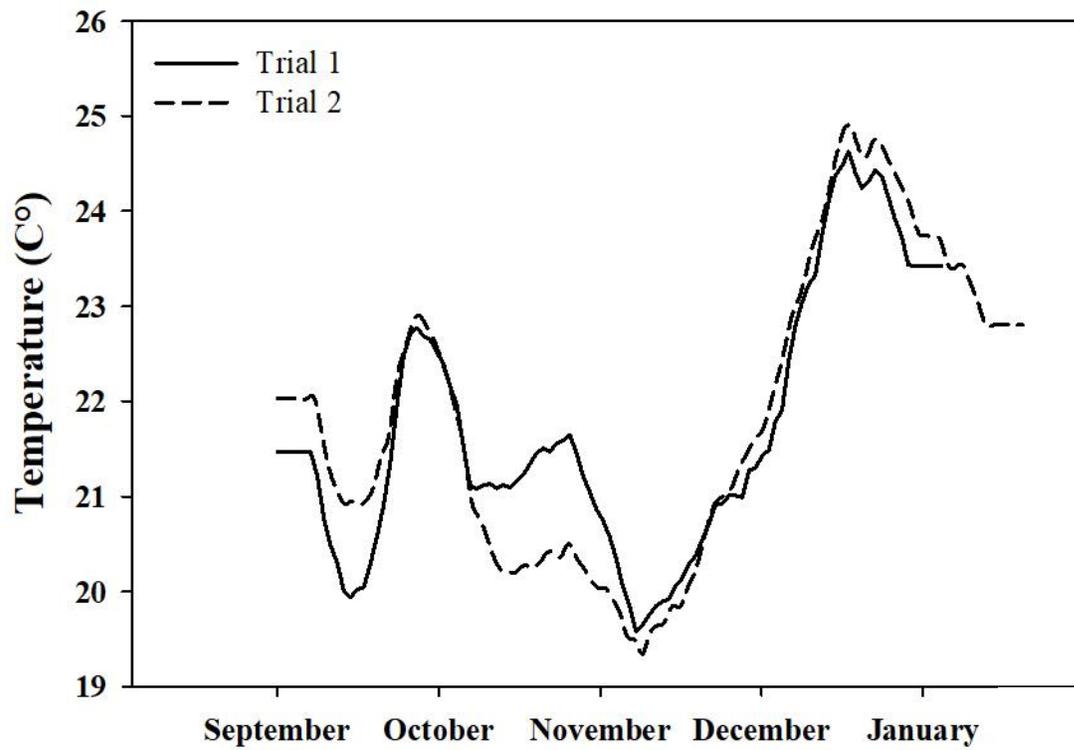
Figure 4. Mean temperature of trial 1 and trial 2 controlled growth study tanks averaged across September 2021 to October 2022 measured hourly by HOBO sensors.