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Simulated mechanical control of *Nitellopsis obtusa* under mesocosm conditions

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Abstract

Management efforts to control starry stonewort [*Nitellopsis obtusa* (Desvaux in Loiseleur) J. Groves] have been limited to stressing the thalli and have not been able to directly target the reproductive bulbils. Smaller-scale efforts such as the use of hand pulling can be employed, but hand pulling is not realistic for larger infestations. This research was conducted to test the effects of clipping stress on *N. obtusa* to provide a baseline for the effect of stress on the production of bulbils and the regrowth of thalli. Mesocosms were set up under greenhouse conditions to test the effects on *N. obtusa* of simulated mechanical harvesting once, twice, and four times per growing season. Different seasonal timing and frequency of clipping treatments will remove different amounts of thalli biomass. The four-clipping treatment always reduced thalli biomass in this study at both 16 and 52 wk after treatment (WAT) compared with the nontreated reference, but there was no difference among clipping treatments at 52 WAT. At 16 WAT, one clipping reduced bulbil density by 44% (Trial 1) to 50% (Trial 2), two clippings reduced bulbil density by 28% (Trial 2) to 52% (Trial 1), and four clippings reduced bulbil density by 22% (Trial 2) to 88% (Trial 1). At 52 WAT, bulbil densities were 69% and 93% lower than those of the nontreated reference Trials 2 and 1, respectively. Results from this study indicate that clipping may be effective for *N. obtusa* control and could impact bulbil production.

Introduction

Starry stonewort [*Nitellopsis obtusa* (Desvaux in Loiseleur) J. Groves] is a problematic macroalga, because it creates recreational and ecological issues by forming dense mats of vegetation that fill all available optimal and nonoptimal growth habitats (Pokrzywinski et al. 2020; Pullman and Crawford 2010). The growth of *N. obtusa* might negatively impact native fish populations, because its growth pattern may inhibit spawning, although further research is needed to confirm this (Kipp et al. 2014; Pullman and Crawford 2010; Sleith et al. 2015). Lakes with dense infestations may be at risk of oxygen depletion in the autumn, as large mats of *N. obtusa* senesce and decompose (Brainard and Schulz 2017; Kipp et al. 2014), which could also be detrimental to fish populations. 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 Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Ginn et al. 2021; Kipp et al. 2014; Pullman and Crawford 2010; Sleith et al. 2015). The mats of *N. obtusa* have also been shown to reduce invertebrate diversity in the water column, disrupting the bottom chain of the food web (Harrow-Lyle and Kirkwood 2021).

Nitellopsis obtusa can grow from the sediment to 30 to 120 cm in the water column (Larkin et al. 2018). "Thalli" is the term for the aboveground biomass of *N. obtusa*. The thallus is made up of a single strand of cells attached end to end and is about 0.7 to 2 mm in diameter (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 to three segments with a total length up to 9 cm (Larkin et al. 2018). *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). Current management techniques for *N. obtusa* are unable to effectively manage vegetative spread.

To date, management strategies for *N. obtusa* include mechanical control, which is either hand pulling, driver-assisted suction harvesting (Pokrzywinski et al. 2020; B Steckart, personal communication, September 7, 2018), or mechanical harvesting (Pokrzywinski et al. 2020); and chemical control, which is the application of either copper-based algaecides or the herbicides diquat and/or endothall (Carver and Wersal 2022; Glisson et al. 2018; Larkin et al. 2018; Pokrzywinski et al. 2020; Wersal 2022). Chemical control is effective at reducing thalli biomass but is unable to reliably and consistently reach belowground structures such as rhizoids or

Management Implications

Seasonal timing and clipping frequency will impact the amount of *Nitellopsis obtusa* (starry stonewort) biomass that is harvested during each clipping event. Based on this small-scale, greenhouse trial, mechanical clipping may offer longer-term control of *N. obtusa* in areas that can be clipped at least four times per growing season. However, harvesting is generally a slow process, as a regular harvester can cut and remove plants at a rate of about 0.4 to 1.6 ha d^{-1} depending on the machine and plant density. Therefore, harvesting an entire 300-ha littoral zone infestation would take anywhere from 202 to 810 d. Smaller high-traffic areas or lake access points that would allow for repeated clippings would likely have the greatest chance of success, especially at reducing bulbil production, based on this trial. One clipping per growing season should only be used as an option for immediate nuisance relief. Clipping integrated with other management tactics is recommended to maximize stress of *N. obtusa* thalli production and to reduce the bulbil bank. Further 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.

bulbils (Carver and Wersal 2022). Water-level drawdowns are one method proposed for *N. obtusa* management, but are not applicable to many midwestern glacial lakes that do not have water-level control structures (Larkin et al. 2018; Menninger 2011).

A combined treatment of algaecide and mechanical pulling decreased *N. obtusa* bulbil density in Lake Koronis (Glisson et al. 2018). Similarly, a mechanical groomer system reduced *N. obtusa* biomass in the treated areas (Brandt 2017). Although previous mechanical control methods have been used on *N. obtusa*, none have quanitified effectiveness or how often these methods are needed to be implemented in a season to produce 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. In other harvesting studies, plants that were repeatedly clipped produced less biomass and fewer vegetative reproductive structures (Fox et al. 2002; Turnage et al. 2019).

The objective of the study was to develop clipping frequency recommendations for *N. obtusa* in the greenhouse. It was hypothesized that repeated clipping of *N. obtusa* would result in longer-term reduction in thalli biomass and especially a reduction in bulbil density. The overarching goal was to compare the ability of clipping treatments at different time frequencies and seasonal growth so that managers might employ this technique to reduce bulbil density. The goal was not to directly compare absolute reductions in thalli biomass, given that the different clipping regimens varied in their seasonal timing and, by default, would remove different amounts of biomass. Thalli biomass is compared between clipping treatments as a point of reference, however, with the expectation that frequent clippings should reduce biomass more at short- and long-term periods after treatment than less frequent clippings.

Materials and Methods

This study was performed under greenhouse conditions at Minnesota State University, Mankato from August 23, 2021, to October 3, 2022. *Nitellopsis obtusa* biomass 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, 14111 Scottslawn Road, Marysville, OH 43041) at a rate of 2 g L^{-1} sediment and topped with sand (creating an average soil surface height of 10 cm). For transplantation of *N. obtusa*, thalli were wrapped in small balls and placed on top of the sand in each container. The mesocosms were filled with 94.6 L of tap water (52-cm water depth) treated with sodium thiosulfate to remove chlorine (approx. 1:1 ratio of $Na_2S_2O_3$ to Cl_2) and then all mesocosms were covered in shade netting (60% light reduction) to aid in the early stages of growth. *Nitellopsis obtusa* was grown in either a greenhouse under ambient light conditions or a lab under artificial light using a 12:12 h light:dark cycle. Water temperature in the mesocosms averaged 22 C ± 0.1 SE, and pH was circumneutral. The water was aerated with a compressed-air system to add CO_2 to the water column in the mesocosms. All propagation methods were adapted from Wersal (2022).

Nitellopsis obtusa was allowed to establish for 1.5 mo (or until thalli reached the water surface, approx. 52.5 cm from the soil surface), after which 11 containers of *N. obtusa* were moved to each treatment mesocosm (for a total of 176 containers per trial). Mesocosms were subjected to an ambient light cycle, aerated with a compressed-air system, and filled with 94.6 L of water (63.5-cm depth) treated with sodium thiosulfate to remove chlorine. Three clipping treatments were applied and compared with a non-treated reference over a 4-mo period. All treatments used four mesocosms (replicates), and the study was repeated twice. Trial 1 was conducted from August 22, 2021 to August 22, 2022; Trial 2 was conducted from October 3, 2021 to October 3, 2022. 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 column until late June or early August (Carver 2022); mechanical treatments to remove thalli biomass would not be applied until the third month of the growing season. To mimic this, the one-clipping treatment was applied in the third month, the two-clipping treatment was applied in the second and fourth months, and the four-clipping treatment was applied every month. Therefore, the initiation of each clipping treatment differed 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 harvester could reasonably cut in a season. Biomass was clipped approximately 15 cm above the sediment surface. The clipping was chosen to simulate a harvestor cutting depth of 1.8 m (Aquarius Systems 2010). For example, if *N. obtusa* was growing to a height of 2 m and was clipped, there would be 20 cm of *N. obtusa* remaining above the sediment.

Pretreatment samples (one container from every mesocosm) 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 h to determine biomass (g dry weight [DW] pot^{-1}) for each tissue type. The number of bulbils was recorded before drying. At 16 wk after treatment (WAT), half the containers in each mesocosm were harvested in the same manner as pretreatment samples. The remaining containers in all mesocosms were harvested at 52 WAT. The posttreatment sampling occurred at 16 wk from the first clipping (Trial 1: December 13, 2021; Trial 2: January 24, 2022) and 52 wk from the first clipping (Trial 1: August 22, 2022; Trial 2: October 3, 2022). It is acknowledged that sampling in this manner might result in less biomass being present in the two- and four-clipping treatments compared with the one-clipping treatment, because the multiple-clipping treatments would be clipped closer to the 16 WAT sampling time and would not have the same amount of recovery time as the one-clipping treatment. The 16 WAT and 52 WAT sampling times were chosen to assess the short-term (in-season) and longer-term efficacy of the clipping treatments and followed the sampling frequency of other simulated harvesting studies (Turnage et al. 2019). 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 h. The mass for each clipping was then weighed to give an estimate of how much biomass was removed during each harvesting event and to determine whether harvesting was offering longer-term reductions in thalli biomass.

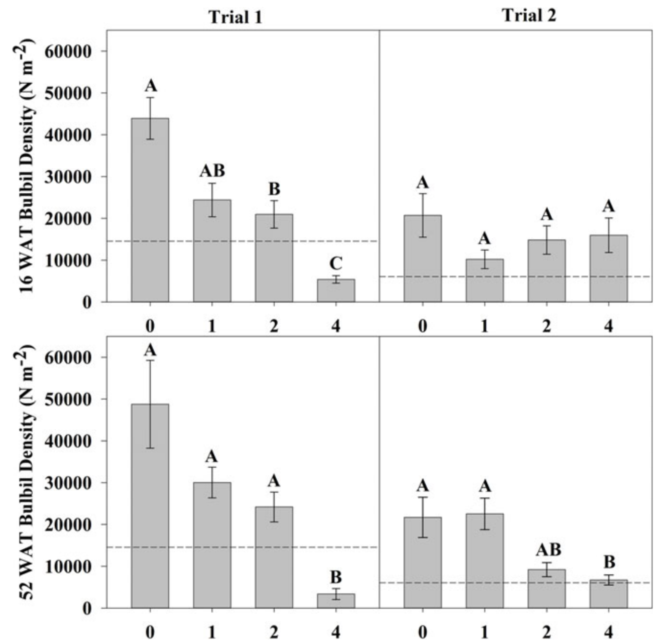

Figure 1. Mean (± 1 SE) bulbil density (N m^{-2}) for the clipping treatments (0, 1, 2, 4) at 16 and 52 wk after initial treatment (WAT) for both trials. 16 WAT Trial 1: $F = 22.57$, $P < 0.01$; 16 WAT Trial 2: $F = 0.65$, $P = 0.59$; 52 WAT Trial 1: $F = 18.04$, $P < 0.01$; 52 WAT Trial 2: $F = 6.67$, $P < 0.01$. Bars sharing the same letter are not different according to a Dunn's all-pairwise comparison at an $\alpha \leq 0.05$ significance level. Dashed line represents mean pretreatment bulbil density (Trial 1 = 14,568 bulbils m^{-2}; Trial 2 = 6,082 bulbils m^{-2}).

Statistical Analysis

To minimize the inherent varibility when working with *N. obtusa*, the lowest-performing replicate in each treatment was excluded from statistical analysis; therefore, all analyses were done using three replicates for each treatment. A Shapiro-Wilk normality test confirmed that data were not normally distributed for all variables ($P < 0.01$; Statistix 10 Analytical Software, 2105 Miller Landing Road, 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). 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 biomass (Trial 1: $H = 0.55$, $P = 0.91$; Trial 2: $H = 6.09$, $P = 0.09$) or between bulbil density (Trial 1: $H = 1.53$, $P = 0.72$; Trial 2 $H = 4.41$, $P = 0.23$). Initial clipped biomass harvested from each treatment was also not significantly different ($H = 1.61$, $P = 0.22$). There was a difference between trials for thalli biomass and bulbil density at both 16 and 52 WAT, so data were analyzed using a Kruskal-Wallis test within WAT and for each trial. The Dunn's all-pairwise comparison post hoc test was used to determine differences. All statistical tests were conducted using an $\alpha \leq 0.05$ significance level.

Results and Discussion

At 16 WAT, Trial 1 showed a significant difference after two clippings compared with the nontreated reference bulbil density (112 d; $H = 32.01$, $P < 0.01$), but Trial 2 showed no significant difference between treatments ($H = 1.98$, $P = 0.58$; Figure 1). At 16 WAT, one clipping reduced bulbil density by 44% (Trial 1) to 50% (Trial 2), two clippings reduced bulbil density by 28% (Trial 2) to 52% (Trial 1), and four clippings reduced bulbil density by 22% (Trial 2) to 88% (Trial 1). At 52 WAT, bulbil densities were 69% and 93% lower than that of the nontreated reference for Trials 2 and 1, respectively (Trial 1: $H = 29.00$, $P < 0.01$; Trial 2: $H = 15.54$, $P < 0.01$; Figure 1). The four-clipping treatment resulted in bulbil densities that were at or below the pretreatment density of 14,568 bulbils m^{-2} for Trial 1 and 6,083 bulbils m^{-2} for Trial 2. This is possibly due to the clipping and subsequent harvesting opening the water column to light penetration that induced bulbil sprouting after each clipping.

Different seasonal timing and frequency of clipping treatments will by default remove different amounts of thalli biomass, and as expected, the four-clipping treatment always reduced thalli biomass in this study at both 16 and 52 WAT compared with the nontreated reference (Figure 1), but there was no difference among clipping treatments at 52 WAT (Figure 2). At 16 WAT, one clipping reduced thalli biomass by 17% (Trial 1) to 85% (Trial 2), two clippings reduced thalli biomass by 52% (Trial 1) to 86% (Trial 2), and four clippings reduced thalli biomass by 82% (Trial 2) to 90% (Trial 1). At 52 WAT, *N. obtusa* biomass in the one- and two-clipping treatments was still less than biomass in the nontreated reference (Trial 1: $H = 39.76$, $P < 0.01$; Trial 2: $H = 38.05$, $P < 0.01$; Figure 2). Biomass in all clipping treatments was below pretreatment levels at 52 WAT except in the nontreated references. The duration of the study for both trials included winter months (nonoptimal growth conditions), which influenced the 52 WAT data, in that the combination of clipping and natural senescence caused reduced or no growth during the winter months. This is made evident by both the nontreated reference bulbil density and thalli biomass not increasing through time (Figures 1 and 2). It does illustrate the potential to clip natural populations later in the season to reduce overwintering biomass. Under this scenerio, the *N. obtusa* population would enter winter stressed by the cutting

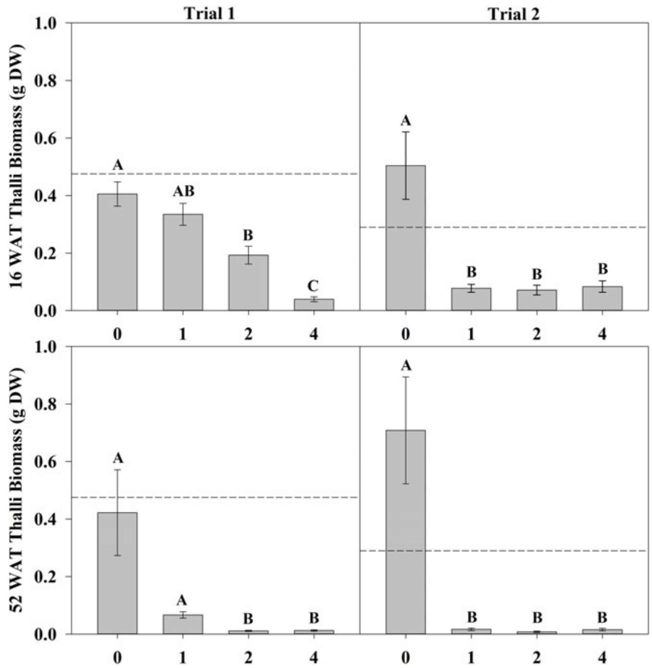

Figure 2. Mean (± 1 SE) thalli biomass (g dry weight [DW]) for the clipping treatments (0, 1, 2, 4) at 16 and 52 wk after initial treatment (WAT) for both trials. 16 WAT Trial 1: $F = 32.55$, $P < 0.01$; 16 WAT Trial 2: $F = 20.16$, $P < 0.01$; 52 WAT Trial 1: $F = 36.33$, $P < 0.01$; 52 WAT Trial 2: $F = 30.89$, $P < 0.01$). Bars sharing the same letter are not different according to a Dunn's all-pairwise comparison at an $\alpha \leq 0.05$ significance level. Dashed line represents mean pretreatment thalli biomass (Trial 1 = 0.48 g DW; Trial 2 = 0.29 g DW).

and possibly die during the winter months. If *N. obtusa* did survive clipping and then winter conditions, it would enter the next growing season with lower biomass and reduced energy stores, which could impact its seasonal growth paterns and therfore make subsequent management more effective.

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 2 wk) clippings consistently reduced aboveground biomass after 52 WAT, while monthly clippings only sometimes had a significant difference from nontreated mesocosms (Turnage et al. 2019). Comparatively, mechanical harvesting is needed less frequently to reduce aboveground biomass for *N. obtusa* than it is for *B. umbellatus*. This supports previous work suggesting that a species' tolerance to mechanical clipping will be determined by the amount of belowground biomass present (Turnage et al. 2019). Mature individuals of giant rush [*Juncus ingens* (N. A. Wakef.)] were reportedly able to survive clipping stress more often than immature individuals due to the amount of belowground biomass available to promote regrowth (Mayence et al. 2010; Middleton 1990). Clipping directly targets aboveground biomass, so individuals or species with more belowground biomass may be more tolerant to clipping stresses than those with less belowground biomass. There is much less propagule biomass for *N. obtusa* bulbils and rhizoids (15.4 g m^{-2}) compared with *B. umbellatus* rhizome buds (4,000 g m^{-2}), although propagule density for *N. obtusa* (156,944 N m^{-2}) is much higher than for *B. umbellatus* (4,800 N m^{-2}; Carver 2022; Turnage et al. 2019).

In this greenhouse study, each consecutive clipping removed approximately 30% to 45% of existing biomass (Table 1). Interestingly, it was observed during the four-clipping treatment

Table 1. Average biomass removal of *Nitellopsis obtusa* after successive clippings.[a]

Clipping treatment	Clipping number	Biomass removed		
		%	g	SE
Reference		0	0	0
One	1	–	4.01	1.24
Two	1	–	2.87	0.87
	2	31.13	0.89	0.42
Four	1	–	1.57	0.81
	2	43.33	0.68	0.20
	3	44.21	0.30	0.14
	4	39.23	0.12	0.04

[a]Trials 1 and 2 were combined to calculate biomass removal.

that *N. obtusa* started to grow horizontally in the water column beneath the clipping height after the second clipping. It is not certain whether *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 whether an adaptive response exists or the growth was coincidental. Lateral growth in *N. obtusa* may be a form of morphological plasticity, as has been observed in other macroalgae 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 (Blanchette 1997; Charrier et al. 2012; Dudgeon and Johnson 1992; Koehl 1984; Koehl and Alberte 1988). This is also seen in the kelp species *Nereocytis luetkeana* (K. Mertens), which can change morphology in a span of 4 to 5 d to limit water movement drag (Charrier et al. 2012; Koehl et al. 2008). If lateral growth is an adaptive 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 longer-term control in areas that can be clipped at least four times per growing season. Clipping four times per growing season was enough 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 before clipping is recommended as an operational control technique. A single harvester on average can clip about 0.4 to 1.6 ha d^{-1}; therefore, a harvester 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 harming native fish and fauna (Booms 1999; Mikol 1985; Wile 1978). Harvesting can easily trap invertebrates and fish that get entangled on macrophytes, so it should be applied responsibly to avoid removing too many off-target species (Booms 1999). Integrating clipping with other management tactics is recommended to maximize stress on *N. obtusa* thalli production and reduce the sediment bulbil bank. Further research should be done in a field setting to test the response of *N. obtusa* growth to harvesting treatments and account for variables that could not be captured in this study.

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