

Final Abstract

Final Report Approved on December 11, 2023

M.L. 2021 Project Abstract

For the Period Ending June 30, 2024

Project Title: Starch Allocation Patterns of Invasive Starry Stonewort Harvested from Lake Koronis

Project Manager: Ryan Wersal

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Funding Source:

Fiscal Year:

Legal Citation: M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 06a

Appropriation Amount: \$101,000

Amount Spent: \$101,000

Amount Remaining: -

Sound bite of Project Outcomes and Results

Starry stoneworts allocate the majority of its energy to bulbils. Bulbils are easy to transport and can be long-lived in the sediment allowing for the spread and proliferation of this species in MN lakes. Management efforts need interrupt carbohydrate movement and to limit starch accumulation in bulbils.

Overall Project Outcome and Results

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. The current study utilized samples harvested from Lake Koronis, Minnesota, USA from 2020 and 2021 to quantify starch allocation patterns in starry stonewort. Starch was quantified using the amylase/amyloglucosidase method. Starch data were compared to light transmittance (%), pH, and water temperature (°C) via mixed procedures models in SAS. Seasonal average low and high points of starch storage were observed to be June (3.3%) and April (9.9%) for thalli biomass then June (41.7%) and November (54.6%) for bulbils. In regard to allocation patterns, above ground thalli reallocate carbohydrate stores to the below ground rhizoids in May. 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 its invaded range, bulbils are important for energy store, 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 starry stonewort. Water temperature and light availability were shown to negatively influence starch content of thalli. These findings suggest that in the invaded range, starry stonewort is capable of accumulating large amounts of starch later into the growing season. The energy reserves may give starry stonewort a competitive advantage over native species by allowing a longer growing season resulting in the production of more vegetative propagules and greater longevity in the environments it invades.

Project Results Use and Dissemination

Results from this project were presented at the Aquatic Plant Management Society annual conference in 2022 and 2023 (presentations attached). Additional presentations were given at Minnesota State University, Mankato and statewide DNR meetings. Results from this study were published in the Journal of Freshwater Ecology. The article was attached in tab 7.

Alyssa M. Haram & Ryan M. Wersal (2023) Seasonal starch allocation of starry stonewort (*Nitellopsis obtusa*) growing in Lake Koronis, MN, Journal of Freshwater Ecology, 38:1, 2211086, DOI: 10.1080/02705060.2023.2211086

To link to this article: <https://doi.org/10.1080/02705060.2023.2211086>



Environment and Natural Resources Trust Fund

M.L. 2021 Approved Final Report

General Information

Date: August 28, 2024

ID Number: 2021-017

Staff Lead: Mike Campana

Project Title: Starch Allocation Patterns of Invasive Starry Stonewort Harvested from Lake Koronis

Project Budget: \$101,000

Project Manager Information

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Project Reporting

Final Report Approved: December 11, 2023

Reporting Status: Project Completed

Date of Last Action: December 11, 2023

Project Completion: July 31, 2023

Legal Information

Legal Citation: M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 06a

Appropriation Language: \$101,000 the first year is from the trust fund to the Board of Trustees of the Minnesota State Colleges and Universities System for Minnesota State University, Mankato, to evaluate the starch allocation patterns of the invasive starry stonewort to identify weaknesses in the plant's growth that could be targeted for management.

Appropriation End Date: June 30, 2024

Narrative

Project Summary: Starry stonewort is a macro-algae that has invaded Minnesota lakes, though nothing is known about its starch allocation. These data can identify weak points in allocation strategy to enhance management.

Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.

Starry stonewort is a non-native macro-algae from Europe and western Asia that was introduced into Lake Koronis, MN in 2015, and has since spread to 14 other waterbodies. Unlike many of the native green macro-algae, starry stonewort can elongate into the water column, and in some cases reach plant lengths of 2 meters. Dense growth of starry stonewort can alter the community structure of aquatic habitats by extirpating native vegetation, thereby changing macro-invertebrate assemblages and ultimately fish assemblages. Starry stonewort interferes with boating and other recreational activities. Aquatic invasive species like starry stonewort have also resulted in declines in property values. Starry stonewort is anchored to bottom sediments by rhizoids. These rhizoids are important as they often contain bulbils, or are the point of bulbil production. Bulbils are starch-containing tissues used for overwintering and perennation. When conditions are conducive for growth, bulbils will sprout and grow a new plant. Although there are data on its impact as a non-native species, and how to manage it; to date, there are few studies on its life history characteristics, and no studies on phenology and resource (starch) allocation patterns for this species.

What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.

The proposed project will utilize laboratory techniques to quantify seasonal starch allocation patterns in starry stonewort form samples previously collected from two growing seasons on Lake Koronis, MN. The commercially available starch assay kit (Sigma Aldrich) will be used to separate starch from aboveground, rhizoid, and bulbil biomass. Data will elucidate seasonal patterns in starch allocation for starry stonewort and identify which structures are important for storage. This approach has been utilized by the principle investigator on other aquatic species such as parrotfeather, phragmites, Eurasian watermilfoil, and Cuban bulrush. Funding is being sought to support a graduate student for two summers to conduct the tissue analysis of starry stonewort harvested from Lake Koronis from two growing seasons. Funding will also support undergraduate wages in order to involve undergraduate students in the research process. Funds are being sought by the principle investigator for summer salary to manage the project and mentor both the graduate and undergraduate students. Funds will be needed to purchase the necessary starch kits, reagents, and supplies to conduct the analyses.

What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?

Having an understanding of starch allocation patterns in starry stonewort will allow for better management of this species. Knowing when starry stonewort begins growth and when it achieves maximum starch reserve is crucial in developing targeted management approaches. Management then occur during the times when starch reserves are at their lowest points making the plant more vulnerable to management techniques. Targeting starry stonewort during low points in its starch allocation curve will make managing this species easier and potentially more cost effective resulting in fewer impacts to Minnesota lakes and non-target species; and ultimately restore and conserve our water resources.

Project Location

What is the best scale for describing where your work will take place?

Statewide

What is the best scale to describe the area impacted by your work?

Statewide

When will the work impact occur?

During the Project

Activities and Milestones

Activity 1: Seasonal Starch Analysis

Activity Budget: \$101,000

Activity Description:

The proposed project will utilize laboratory techniques to quantify seasonal starch allocation patterns in starry stonewort from samples previously collected from two growing seasons on Lake Koronis, MN. We will conduct tissue analysis using the commercially available starch assay kit (Sigma Aldrich) to separate starch from aboveground, rhizoid, and bulbil biomass. We will then analyze the data to elucidate seasonal patterns in starch allocation for starry stonewort and identify which structures are important for storage. Knowing when starry stonewort begins growth and when it achieves maximum starch reserve is crucial in developing targeted management approaches. Management can then occur during the times when starch reserves are at their lowest points making the plant more vulnerable to management techniques. Targeting starry stonewort during low points in its starch allocation curve will make managing this species easier and potentially more cost effective resulting in fewer impacts to Minnesota lakes and non-target species; and ultimately restore and conserve our water resources.

Final findings will be presented at a regional or national conference and will be developed into a peer-reviewed journal article for publication in an appropriate journal.

Activity Milestones:

Description	Approximate Completion Date
Begin Starch Extraction and Quantification of Resource Allocation	June 30, 2022
Present Preliminary Findings at the Aquatic Plant Management Society annual meeting	July 31, 2022
Finish Starch Extraction, Data Analysis, and Begin Final Report	June 30, 2023
Prepare and Submit Journal Article	July 31, 2023
Present Final Findings at the Aquatic Plant Management Society annual meeting	July 31, 2023

Dissemination

Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENTRF Acknowledgement Requirements and Guidelines.

Results from this project will be disseminated both orally and in written form. The graduate student funded on this project will give at least one presentation at the national Aquatic Plant Management Society annual conference. Either the graduate student or Dr. Wersal will give additional presentations at statewide or regional scientific meetings as time or travel restrictions allow. These presentations will have regional to national reach and will impact researchers, managers, regulators, and in some instances lakeshore owners. Dr. Wersal will work closely with the Koronis Lake Association and the Minnesota Department of Natural Resources (DNR) - Aquatic Invasive Species staff to further refine management recommendations for starry stonewort in Minnesota based phenology and starch allocation patterns. The collaborative partnerships with lake associations and the Minnesota DNR will ensure that data are being applied in an effective manner to better focus management efforts in order to better protect, conserve, or enhance Minnesota's waterbodies. Finally, a peer-reviewed journal article will be developed and submitted to an appropriate journal for publication. A journal publication will allow the major results from this study to be accessible to researchers and managers for many years to come. With many journals, individual articles can be accessed by anyone via the internet so that private lakeshore owners can find scientifically sound information on starry stonewort.

The Environment and Natural Resources Trust Fund will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENTRF Acknowledgment Guidelines.

Long-Term Implementation and Funding

Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this work be funded?

Results from this project will have direct implications for how starry stonewort is managed nationwide. Presentations and journal publications will be developed and made available to the resource managers, lake associations, and the public. Stakeholder meetings with state agencies, such as the Minnesota Department of Natural Resources (MN DNR), lake associations, watershed districts, and federal agencies will continue in order to redefine management strategies for this species. Future in-field management projects will be developed based on results from this project with funds sought from the MN DNR, aquatic invasive species prevention program, Federal grants, and private companies.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount	\$ Amount Spent	\$ Amount Remaining
Personnel										
Student Personnel		Student Personnel Summer			7.65%	0.3		\$17,294	-	-
Graduate Student		Graduate Student Summer			7.65%	0.3		\$13,456	-	-
Dr. Ryan Wersal		Project Director			19%	0.3		\$42,631	-	-
							Sub Total	\$73,381	\$73,381	-
Contracts and Services										
							Sub Total	-	-	-
Equipment, Tools, and Supplies										
	Tools and Supplies	Cost per item # of item - STA20 Starch Assay Kits- \$206.00 (50 kits), Sulfuric Acid Reagent-\$475.00 (3 bottles), Reagent Alcohol-\$149.00 (3 bottles), Demethyl Sufoxide-\$810.00 (3 bottles), Pipette Tips-\$150.00 (9 containers), Test Tubes-\$494.00 (4 boxes), Test Tube Racks-\$56.00 (5 racks), Centrifuge Tubes -\$85.60 (5 boxes), Centrifuge Tube Caps-\$12.50 (3 boxes)	Materials and supplies to complete the research					\$18,392	\$18,392	-
							Sub Total	\$18,392	\$18,392	-
Capital Expenditures										
		Udy Cyclone Sample Mill	Plant samples will be ground using a Cyclone Sample Mill. Approximately 50 mg of the ground sample will be transferred into plastic centrifuge tubes for storage					\$5,127	\$5,127	-

			and preparation for the starch analysis							
							Sub Total	\$5,127	\$5,127	-
Acquisitions and Stewardship										
							Sub Total	-	-	-
Travel In Minnesota										
							Sub Total	-	-	-
Travel Outside Minnesota										
	Conference Registration Miles/ Meals/ Lodging	Aquatic Plant Management Society Annual Meetings	The meeting attendees include academic researchers, state and federal agency personal, local and state resource managers, watershed district personnel, and pesticide applicators. These data would be applicable to all individuals currently managing starry stonewort in their respect regions	X				\$3,500	\$3,500	-
							Sub Total	\$3,500	\$3,500	-
Printing and Publication										
	Publication	Publication costs	Publication of results					\$600	\$600	-
							Sub Total	\$600	\$600	-
Other Expenses										
							Sub Total	-	-	-
							Grand Total	\$101,000	\$101,000	-

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
Travel Outside Minnesota	Conference Registration Miles/Meals/Lodging	Aquatic Plant Management Society Annual Meetings	<p>The Aquatic Plant Management Society has an annual meeting each year and is very inviting to graduate student presentations. The focus of the proposed project aligns with the Society's mission and goals and therefore would be of interest to Society members and meeting participants. Furthermore the meetings are generally well attended by government agency personnel and resource managers who would benefit from data generated from the proposed study to aid in starry stonewort management. The meeting is also a good venue for graduate students to build their professional network.</p> <p>Travel to the meeting will be to facilitate a formal presentation (oral or poster) of the proposed project outcomes to a wider scientific and management orientated audience.</p>

Non ENRTF Funds

Category	Specific Source	Use	Status	\$ Amount	\$ Amount Spent	\$ Amount Remaining
State						
			State Sub Total	-	-	-
Non-State						
			Non State Sub Total	-	-	-
			Funds Total	-	-	-

Attachments

Required Attachments

Visual Component

File: [598386ad-1e7.pdf](#)

Alternate Text for Visual Component

The attached visual is a map of the locations on Lake Koronis where seasonal biomass was harvested from a previous project. The seasonal biomass samples are the ones to be analyzed for starch content during the proposed study....

Supplemental Attachments

Capital Project Questionnaire, Budget Supplements, Support Letter, Photos, Media, Other

Title	File
Research Addendum	6756bb01-338.pdf
Background Check Certification Form	f6dc52b4-9c4.pdf
APMS Presentation	0a8ca6f7-521.pptx
Seasonal starch allocation of starry stonewort (<i>Nitellopsis obtusa</i>) growing in lake Koronis, MN	10251dea-5d3.pdf
Final APMS Presentation	c328a02f-e6e.pdf

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

I changed the typo in the activities and milestone title.

Additional Acknowledgements and Conditions:

The following are acknowledgements and conditions beyond those already included in the above workplan:

Do you understand and acknowledge the ENRTF repayment requirements if the use of capital equipment changes?

Yes

Do you agree travel expenses must follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?

Yes, I agree to the Commissioner's Plan.

Does your project have potential for royalties, copyrights, patents, sale of products and assets, or revenue generation?

No

Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?

N/A

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?

N/A

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

No

Work Plan Amendments

No Amendments Entered

Status Update Reporting

Final Report Approved: December 11, 2023

Project Status: Project Completed

Final Status Update September 14, 2023

Date Submitted: July 31, 2023

Date Approved: August 31, 2023

Overall Update

The project as proposed has been completed with the final presentation given at the Aquatic Plant Management Society meeting on July 26, 2023.

Activity 1

The project resulted in 1 journal article and two presentations at national meetings. The journal article and presentations are attached.

(This activity marked as complete as of this status update)

Dissemination

The final presentation given at the Aquatic Plant Management Society meeting on July 26, 2023.

Status Update Reporting

Status Update June 1, 2023

Date Submitted: June 1, 2023

Date Approved: June 2, 2023

Overall Update

The overall results from this project indicated that the seasonal average low point of starch storage for bulbils was observed in June (41.7%) suggesting that the best time to initiate management might be in June. Preventing bulbil production is imperative as starry stonewort is a prolific bulbil producer and they can store up to 73% starch which illustrates the potential for longevity of these propagules in the sediment. Furthermore, as water temperature and light availability decreased later in the growing season in Lake Koronis, starch accumulation in thalli tissue increased. This indicates a tolerance to cooler and dark environments that many other Minnesotan native aquatic plant species do not have. These findings suggest that in the invaded range, starry stonewort is capable of accumulating large amounts of starch later into the growing season. The energy reserves may give it a competitive advantage over native species by allowing a longer growing season resulting in the production of more vegetative propagules and greater longevity in the environments it invades.

Activity 1

Milestone 3 has been completed and the starch extraction/quantification has been done. Starch content in the bulbils had an average starch content of $52.4\% \pm 0.9$ SE (maximum of 73.7%). Thalli starch was highest in April and October with an overall average starch content of $4.7\% \pm 0.2$ SE (maximum of 24.9%). Rhizoids had an average starch content of $5.9\% \pm 0.4$ SE (maximum of 13.6%). We are looking at other correlations with environmental factors. 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.

Milestone 2 will be completed July 24-27 at the 63rd Aquatic Plant Management Society Meeting in Indianapolis when I give the final presentation of the project. I am still working on the final presentation.

Milestone 1 has been completed and the journal article has been attached in tab 7.

Dissemination

Alyssa Haram presented the preliminary work at the Aquatic Plant Management Society annual conference in 2022. Ryan Wersal will give the final presentation at the same meeting in 2023. Ms. Haram also gave a presentation at Minnesota State University, Mankato that was open to the public via online access as part of her MS thesis defense. Ryan Wersal has met with the MN DNR and researchers from MAISRC on several occasions to discuss the future of starry stonewort management in MN. Ryan has also discussed this project and results within the Statewide AIS Taskforce meetings. There are ongoing projects on Lake Koronis to identify methods that are effective and could be used to target the application window suggested above.

Results from this study were published in the Journal of Freshwater Ecology. The article was attached in tab 7.

Alyssa M. Haram & Ryan M. Wersal (2023) Seasonal starch allocation of starry stonewort (*Nitellopsis obtusa*) growing in lake Koronis, MN, Journal of Freshwater Ecology, 38:1, 2211086, DOI: 10.1080/02705060.2023.2211086

To link to this article: <https://doi.org/10.1080/02705060.2023.2211086>

Status Update Reporting

Status Update December 1, 2022

Date Submitted: December 20, 2022

Date Approved: December 21, 2022

Overall Update

Ms. Haram (graduate student) is close to completing the starch analysis in the lab and is beginning to conduct data analysis for her thesis and final deliverable.

Activity 1

The milestones need to be re-ordered in Tab 5. As is Milestones 4 and 5 have been completed and we are well underway to completing Milestone 3. We are on track to complete the project as outlined.

Dissemination

Ms. Haram presented her preliminary work at the national Aquatic Plant Management Society Meeting this past July. Additionally, I have had several meetings with the MN DNR AIS staff to discuss this work, starry stonewort management, and future projects. Once the final samples have processed and data analyzed, Ms. Haram will draft the journal article for submission and the final results will be presented July 2023 at the national Aquatic Plant Management Society Meeting.

Status Update Reporting

Status Update June 1, 2022

Date Submitted: June 15, 2022

Date Approved: July 1, 2022

Overall Update

We have had some supply chain issues in acquiring the lab supplies for this project, but have made significant progress in analyzing starch content from the year 1 samples. Based on these preliminary results, starry stonewort utilizes the bulbils almost exclusively for starch storage and main energy reserve. Management will need to target and reduce bulbil production if long-term reductions in starry stonewort populations.

Activity 1

The graduate student (Alyssa Haram) has made great progress on the year 1 starry stonewort samples. She has preliminarily completed the majority of the starch extractions from the different plant tissues. She is currently reviewing the methodology and making some changes in order to accurately quantify the high starch concentration in the bulbil tissues. Ms. Haram is preparing an abstract for an oral presentation at the Aquatic Plant Management Society meeting in July 2022. The presentation would satisfy the reporting requirement for preliminary results.

Dissemination

The first opportunity for the dissemination of preliminary results will be at Aquatic Plant Management Society meeting in July 2022. We are currently working on abstract submission.