Arresting the Spread of Eurasian Watermilfoil in Lake Superior

Final Report

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2. Executive Summary

Michigan Technological University was funded by the Environmental Protection Agency (EPA) through a Great Lakes Restoration Initiative (GLRI) Grant (GL-00E01291) to conduct a multi-faceted project involving the control, detection, prediction, and outreach about the invasion of Eurasian Watermilfoil (*Myriophyllum spicatum*) in Lake Superior. This project was in part motivated by observations since the mid 2000's, of populations of the aquatic invasive species Eurasian watermilfoil (*Myriophyllum spicatum* or EWM) invading and spreading along the northern Great Lakes shorelines of Michigan's Upper Peninsula (Lakes Huron, Michigan and Superior). EWM is a prolific and costly invader with a complex invasion history that left unmanaged, impacts ecological and economic aspects of local communities through its effects on local ecosystems, fisheries, recreation, and aesthetics. Small communities along the Upper Peninsula's Great Lake shorelines, such as those along the Keweenaw waterway in the Keweenaw Peninsula, rely on nearshore waterways, ecosystems, and fish and bird habitat that enable the tourist industry and support quality of life for residents. The pattern of invasions and how best to control them is increasingly understood elsewhere, but relatively unknown in the northern Great Lakes such as Lake Superior where a colder climate, larger waterbody mass with episodic intense circulations, and critical fish, bird and plant habitat make this a unique problem, especially for local communities confronting invasion in their local waters.

In this project, we worked closely with small communities to learn from their experience attempting to control EWM (Les Cheneaux Islands, Lake Huron) and assisted two local communities (Torch Lake Township and Chassell Township) in the Keweenaw Waterway within the Keweenaw Peninsula (Figure 1) as they were just beginning their efforts to control recently detected invasions. While the approaches taken by LCI included the common management techniques of harvesting by machine and biological control using weevils, Torch Lake and Chassell Townships were planning to use herbicides, which are becoming an increasingly popular management option in the region. The major goals of our project were to learn from local community experiences, examine environmental conditions where EWM flourishes, monitor the effectiveness of control approaches such as herbicides, develop tools to better detect new invasions of EWM, model likely paths of hydrological transport of EWM propagules to predict future invasions for early detection, educate the public on invasive species and the importance of native macrophytes, and share what we learned by making the results of this experience available for others to use as they confront invasions in their waters.

Major outcomes associated with each primary Task of this project include:
Task A – Efficacy of Control: We found that herbicide treatment in Pike and Torch Bays of the Keweenaw Waterway met the objective of targeted, species-specific management of IWM using a treatment that reduced IWM apparently without harming off target macrophytes. As IWM biomass decreased through time, particularly in locations treated by herbicide, it appeared not to alter the overall structure of the macrophyte community. However, we did observe some increases in IWM biomass at untreated locations, and variable patterns of growth between the study sites that could be due to differential effects of colder water temperatures and deeper water depths in 2014-2016, and the presence of hybrid IWM in the study locations.

Task B – Remote Sensing and Mapping: We found that satellite imagery could serve as a good screening tool for identifying areas of surface vs. submerged aquatic vegetation, and that UAV-based imagery could provide a method for mapping SAV species such as EWM at high resolution. In addition, different species of aquatic plants sampled had distinct spectral profiles, and therefore we may be able to differentiate EWM from other species of submerged aquatic vegetation. Together, the satellite imagery, UAV-based based imagery, and spectral profile data provide another tool for mapping the extent of EWM and other SAV species, and could be used for monitoring areas undergoing treatment. We also found that it is possible to determine how much of a surveyed area contains vegetation using ultra high resolution side-scan sonar, although efforts to identify EWM based on "textures" in the collected acoustic data has been marginally successful.

Task C – Hydrodynamic Modeling: Using the linked hydrodynamic-particle trajectory modeling, we predict the local hydrodynamic condition play an important role in influencing the dispersal and settling of EWM fragments, which is critical to understand the invasion process and predicting areas of future EMW invasions. Regions such as the embayments of Isle Royale and Apostle Islands that are characterized by naturally restricted circulation that favor the retention of EWM fragment are highlighted as critical regions to pre-emptively survey to detect early potential prolific success of EWM.

Task D – Data Portal and Outreach: We created a web-based information clearinghouse designed to contain links to credible and relevant resources for managing EWM in the Great Lakes region. The "living document" is hosted at http://www.mtri.org/eurasian_watermilfoil.html. The web page includes extensive information on EWM biology, its invasive properties, ecological impacts, and spread, while also describing the mapping and modeling tools demonstrated through this project. We also conducted six public outreach presentations, conducted 16 presentations of results, and held three teacher professional workshops on invasive species.

3. Project Overview/Background

Eurasian watermilfoil (*Myriophyllum spicatum* or EWM) is among the most common and aggressive invasive aquatic plants in the Northern United States (Thum et al. 2012) and it is capable of altering the habitat structure, community dynamics, and ecosystem processes of lakes and rivers through both direct and indirect pathways (reviewed by Schultz & Dibble 2012). EWM entered North America sometime between the 1880s and the 1940s (Eiswerth et al. 2000). It is now extremely wide spread and has been detected in Michigan waters since 1961(Voss & Reznicek 2012), but only recently discovered in the upper Great Lakes, specifically western Lake Superior, around 1998 (USGS 2017) and by MI DNR in the Les Cheneaux waterways of Lake Huron (Fig. 1) in 2002 (GLCR 2016). EWM was first reported locally in the Keweenaw Waterway through the Keweenaw Peninsula of South Central Lake Superior (Fig. 1) in 2012.

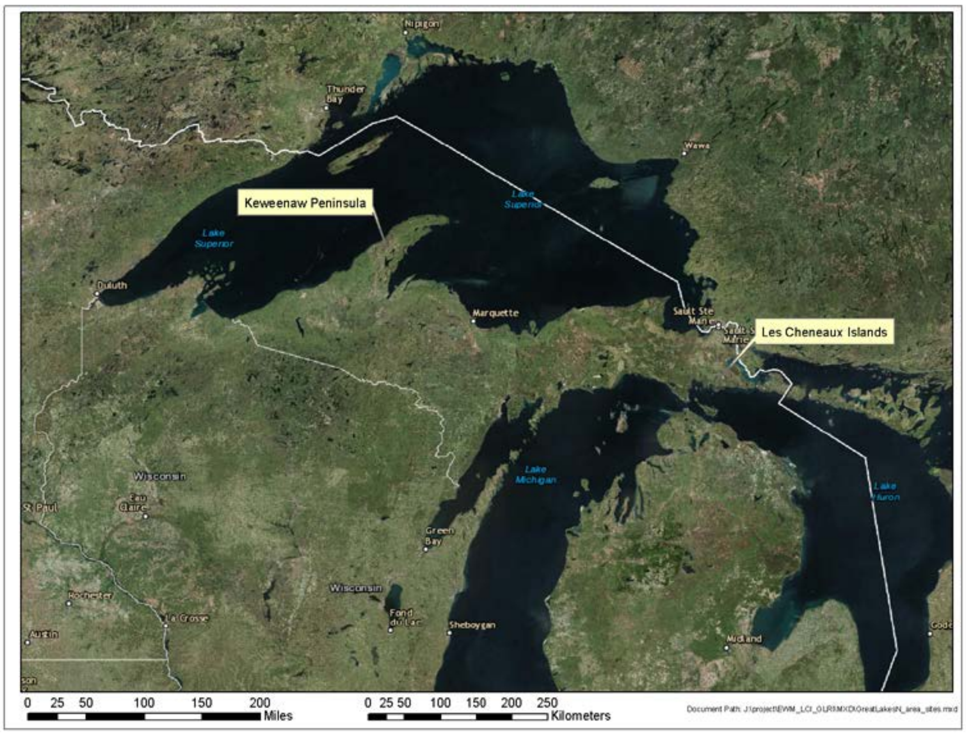


Figure 1. Regions in the upper Great Lakes of US. where project activities were focused on the Keweenaw Peninsula and the Les Cheneaux Islands of Michigan.

Small communities along the Upper Peninsula's Great Lake shorelines, such as those along the Keweenaw waterway and the Les Cheneaux Islands (LCI), rely on nearshore waterways, ecosystems, and fish and bird habitat that enable the tourist industry. Left unmanaged, invasive EWM can severely impact the ecosystems and the financial viability of local communities. Dense surface weed canopies can reduce water movement and oxygen levels and inhibit the sunlight penetration needed by desirable native plants, thus indirectly impacting fish and other aquatic organisms especially important for local tribal subsistence and commerce as well as broad recreation and tourism. In addition to effects on local ecosystems and fisheries, EWM may cause economic impacts for Great Lakes harbor and coastal communities, reducing real estate values and the tourist-associated economy. In the LCI, 34% of waterfront properties have been

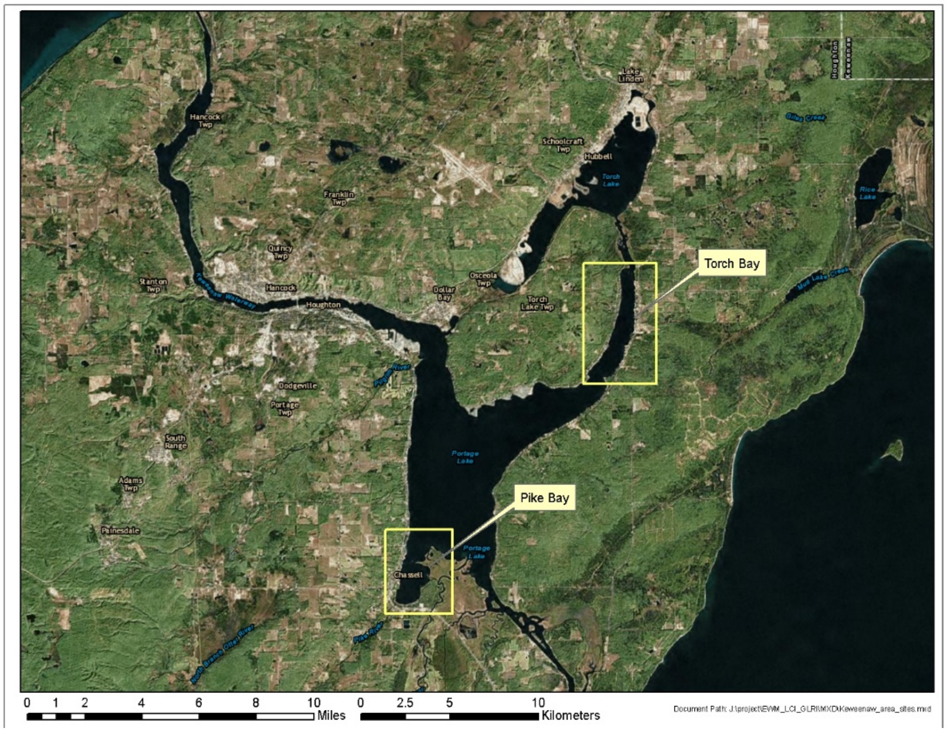


Figure 2. Local water bodies of the Keweenaw Waterway in the Keweenaw Peninsula where our intensive environmental and Eurasian Watermilfoil monitoring was conducted (2014-2016). The locations of Torch Bay and Chassell Bay (Pike Bay) Townships are noted by the yellow boxes.

adversely affected by dense EWM growth, and the estimated taxable value of twenty percent of township properties has been reduced due to degraded aesthetic values (personal communication-Clark Township, Supervisor, 2012).

The invasiveness or distribution of an invading species can be influenced by the abiotic (e.g., physical attributes such as water chemistry) and biotic (e.g., what other species of aquatic vegetation are present) characteristics of the habitat being invaded (Theoharides and Dukes 2007). The colder climate, larger waterbody mass with episodic intense circulations, and critical fish, bird and plant habitat make the upper Great Lakes unique. Indeed, the pattern of growth and establishment of EWM invasions can be temporally and spatially variable such as the initial proliferation followed by declines in EWM prevalence observed in 2014 after the severe winter of 2013-14 in the Les Cheneaux Islands region (GLCR 2016) and in the Keweenaw Waterway (unpublished data).

Herbicides are becoming an increasingly popular management option in the region, but herbicide resistance can increase rapidly in invasive aquatic plants, as has been identified for *Hydrilla* in the southeastern USA (Michel et al. 2004). In EWM, hybridization between native and nonindigenous species also has been linked to increased invasiveness (Moody and Les 2002), as well as reduced sensitivity to herbicides (LaRue et al. 2012, Thum et al. 2012, Zuellig and Thum 2012) and biological control agents such as weevils (Johnson et al. 2000). Communities in the Great Lakes region deciding upon strategies to manage invasions of EWM are typically small with limited financial resources to battle such an aggressive invader. When conducting research to identify efficient and cost-effective management approaches, these communities can be overwhelmed by the extensive and largely un-reviewed information available on the internet and through other management and outreach sources. The Les Cheneaux experience and lessons learned will serve to inform other Great Lakes communities who may be able to arrest the infestation with properly applied research, but only if those other communities can find the relevant information.

In this study we monitored the dynamics and effectiveness of control of EWM in patches scheduled to be treated by local communities: Chassell Township and Torch Lake Townships (Fig. 2). We integrated a suite of approaches to address the issue linking direct field monitoring of macrophytes with remote sensing, including aerial and satellite imagery as well as underwater acoustic imagery to identify and map areas of invasion; along with advanced hydrodynamic modeling to enable the prediction of future locations of possible invasions. The methodology and numerical predictive approach of this study was designed to inform and enable communities to "get out in front" of EWM infestations and thereby have a substantial positive impact on arresting its spread. This effort will improve the health of Great Lakes ecosystems by providing new and efficient methodology to detect, predict, and arrest the spread of EWM and by so doing will preserve natural flow through these shallow regions of great economic and natural value.

Understanding current invasion dynamics and how to control invasive species such as EWM is made more critical as we attempt to predict the effects of climate change, which are already being realized. Warming of the Great Lakes will continue to decrease lake levels and reduce ice cover, thus increasing light penetration and productivity (Kling et al. 2003), all of which will enhance the proliferation of EWM. Projected increases in overland flow and extreme discharge events will likely increase nutrient input in to receiving waters thus fueling growth rates of these unwanted aquatic invasives. Best available data suggest that the Midwest will experience average increases of summer temperatures of 3°F within decades and potentially 10°F by the end of this century (http://www.epa.gov/climatechange/impacts-adaptation/midwest.html). Warmer waters will allow EWM to begin growth earlier and have a longer growing season, suggesting that control measures may need to begin earlier in the year and continue later into the fall (Rahel & Olden 2008). Thus, under climate change influence it will be even more critical that we are better prepared to preemptively respond by arming ourselves with increased ability to detect and forecast emergence hot spots of EWM and be better informed to implement best management practices to control its impact and spread.

This study was designed to address the issues outlined above by focusing on the following major tasks:

Task A - Eurasian Watermilfoil Environmental Associations and Efficacy of Control Agents: We used direct assessment of submerged aquatic plants and environmental sampling to develop an improved understanding of the baseline associations between invasive milfoil and environmental conditions, and monitor the dynamics and effectiveness of control of EWM in patches treated by local communities: Chassell Township and Torch Lake Townships.

Task B. Remote sensing and mapping: Remote sensing, including aerial and satellite imagery as well as underwater acoustic imagery, was examined for its utility in quantitatively and efficiently assessing the distribution and composition of submersed aquatic vegetation including EWM. Remote sensing based methods can provide an assessment of both the regional extent of EWM—so managers could identify newly invaded areas at an early stage—and the local outcomes of EWM treatments without intensive field monitoring.

Task C. Hydrodynamic Modeling: In order to better predict sites of future invasion by characterizing likely paths of water transport of propagules, we conducted advanced next-generation, hydrodynamic modeling (Xue et al. 2015, 2016) to resolve the nearshore regions of the upper Great Lakes in sufficient detail to determine the general flow conditions within the Les Cheneaux Islands, Keweenaw Waterway and similar regions across the upper Great Lakes. This level of hydrodynamic predictive capacity is critical for our understanding of potential dispersal paths of EWM and other invasive propagules that foster their proliferation.

Task D. Data Portal and Outreach: There is a need for a centralized clearing house that integrates science from previous and the current study and provides relevant, vetted management information relevant to the Great Lakes region. To help communities better identify efficient and cost-effective management approaches, we developed a centralized clearing house web portal (http://www.mtri.org/eurasian_watermilfoil.html) that integrated science from previous and the current study and provides relevant, vetted management information relevant to the Great Lakes region. Such a resource will accelerate and improve the management of this invasive species and provide small communities management options that can be implemented within the constraints of their financial resources. We also actively and directly reached out to local communities to present our findings and discuss their unique management issues and approaches, presented findings to professional societies, and worked with school teachers and students to educate them about invasive species.

4. Project Tasks

Task A. Eurasian Watermilfoil Environmental Associations and Efficacy of Control Agents

Task A.1. Problem Definition/Background

Two bays on Portage Lake —Pike Bay, located in Chassell, Michigan (47°01'N 88°30'W), and Torch Bay, located in the Torch Lake township (47°06'N 88°24'W)—were scheduled for treatment in 2014 by the respective communities as a response to recent EWM invasion (KISMA 2013). The decisions to treat with herbicides was arrived at by both Townships after the standard process of public meetings, open discussion period, and voting to support the formation of Special Assessment Districts to fund the treatments (Figure A.1). Portage Lake is part of the Keweenaw waterway that is directly connected to Lake Superior on the southeast and northwest sides of the Keweenaw Peninsula. The waterway is composed of approximately 50% Lake Superior water, with the remainder originating from local streams and rivers, principally the Sturgeon River that flows into Pike Bay and from Torch Lake that flows into Torch Bay (Spain et al. 1969, Churchill et al. 2004).

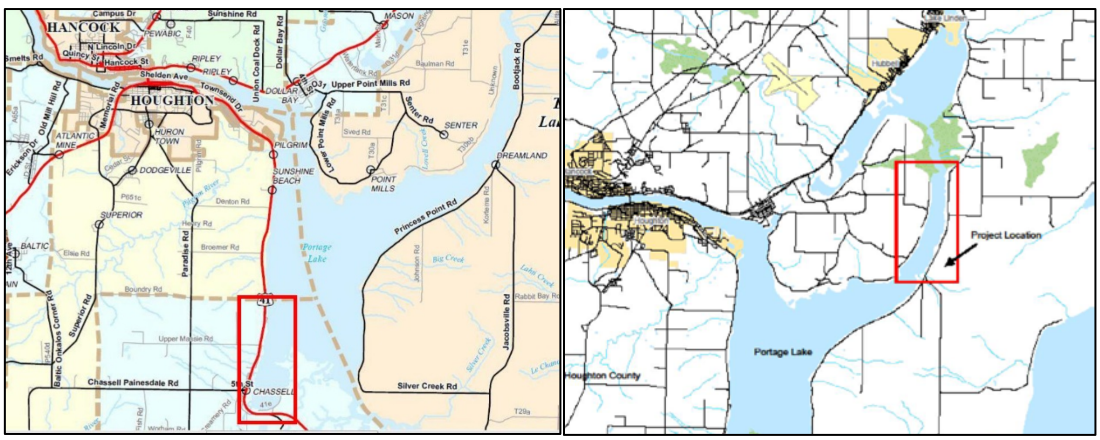


Figure A.1. Red rectangles outline special assessment districts for the treatment of Eurasian Watermilfoil in the Keweenaw Waterway the Pike Bay (left) within the Chassell Township, Michigan. (Many Waters, LLC. 2013); and Torch Bay (right) within the Torch Lake Township, Michigan (Many Waters, LLC. 2014).

Task A.2. Methods

To assess the associations of Eurasian Watermilfoil distribution with environmental conditions and the responses of target and non-target aquatic macrophytes to selective management, we conducted an integrated survey of the macrophytes and their environment before and after herbicide treatments were implemented. We conducted both qualitative and quantitative surveys of aquatic macrophytes in two areas where broad-scale herbicide treatment was planned (Torch Bay and Pike Bay, Michigan) to coincide with the boundaries of the Special Assessment Districts set up for the control on Eurasian Watermilfoil in each Township (Figure A.1). In addition to the bays scheduled for treatment, we chose an untreated waterway that contained EWM—Torch Cuts (47°08'N 88°24'W), a channel through which water in Torch Lake drains into Portage Lake—to monitor concurrently with the treated bays. Torch Cuts is upstream from Torch Bay, so herbicide contamination from the Torch Bay treatment was not a concern.

Task A.3. Sampling Design

We structured the environmental surveys based on a line-intercept sampling design along predetermined transects to gather data on macrophyte species and abundance, and environmental conditions over broad spatial scales (Madsen 1999, Smith et al 2012). Surveys were conducted before and after the treatment with herbicide; the application of which was determined by the respective Townships as per their Special Assessment District (SAD), their contracted consultant and applicator (Professional Lake Management), and MiDEQ permit specifications (QAPP- GL-00E01291, 2014). We used a Geographic Information System covering the known regions of infestation to establish transects located perpendicularly to shore (Figure A.2) and terminating at 3m water depth unless vegetation was detected. EWM had not been detected in our study areas at water depths greater than 4 m (Many Waters 2013) and macrophytes were not detected deeper than 3 m in any of our surveys. Operational transect length ranged from 15.25 m to 183 m perpendicular to the shore depending on bottom slope and depth, with between 2 and 13 sampling points in each. Distances between sampling locations were evenly spaced every 15.25 m (50 feet) throughout the length of each transect from shore to the end of the transect. Coordinates of the sample transects and sampling points along transects were based in UTM system for ease of point location in the field (Madsen 1999) following a boat mounted Global Positioning System (GPS).

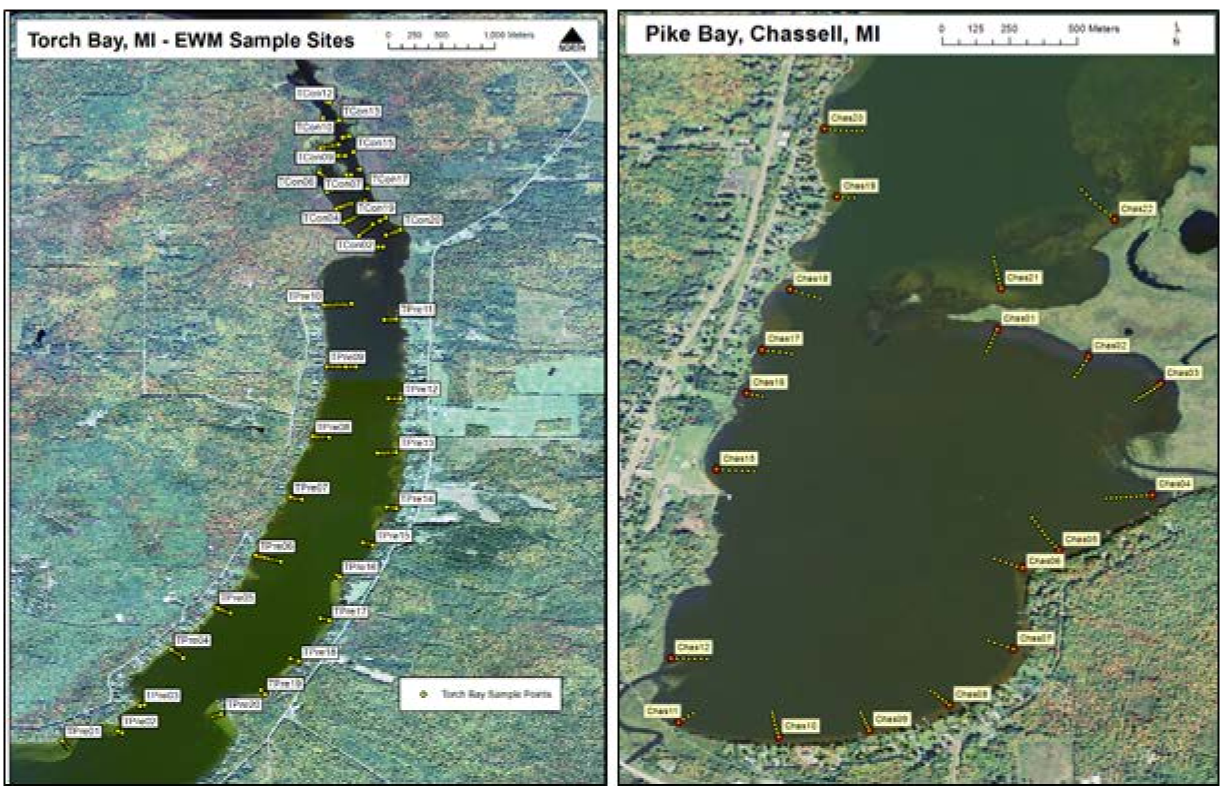


Figure A.2. Sampling transects defined for Torch Bay (left) and Chassell (Pike) Bay (right). Individual transects are shown in yellow.

Task A.3.1. Macrophyte Surveys

We sampled aquatic vegetation for relative abundance, biomass, and taxonomic composition (e.g., species) by collecting three qualitative rake toss samples and three quantitative rake twist samples in different directions (off the port beam, starboard beam, and bow of the boat) from each starting point of the transect. Vegetation was also collected using both methods at a single point every 15.25 m along each transect until the water depth reached 3.0 m.

For each rake toss sample, we recorded the level of rake fullness (1-4; Figure A.3). This metric of rake fullness is the same relative scale used by the local communities during the initial stages of their assessment and development of their SADs (Many Waters, LLC 2013). Relative densities of aquatic macrophyte species were visually assessed within an aquatic macrophyte assessment site (AMAS) defined as within a 3 m radius of the sample point. We followed the procedures recommended by the Michigan Department of Environmental Quality (MDEQ 2005) to estimate the aerial coverage of each species within each assessment site by recording the relative density of each macrophyte species as 1 of 4 percentage categories as follows:

1. = found: one or two plants of a species found in an AMAS, equivalent to less than 2% of the total AMAS surface area.
2. = sparse: scattered distribution of a species in an AMAS, equivalent to between 2% and 20% of the total AMAS surface area.
3. = common: common distribution of a species where the species is easily found in an AMAS, equivalent to between 21% and 60% of the total AMAS surface area.
4. = dense: dense distribution of a species where the species is present in considerable quantities throughout an AMAS, equivalent to greater than 60% of the total AMAS surface area.

Figure A.3. Relative metric of aquatic macrophyte abundance retrieved during rake tosses. Many Waters, LLC. Photo: USFS Ottawa National Forest.

Quantitative estimates of macrophyte abundance and composition were conducted using the vertical twist rake method (Johnson and Newman 2011). At each sample point, a long-handled double-sided rake was lowered vertically to the bottom and rotated 3 times on its axis to collect plant material from within the circular area (0.125 cm^2). Retrieved plant material for each sample was placed into plastic bags labeled with the collection site information (water body, site, date, time, transect and sample point identifiers, latitude and longitude, method of collection, name of collector). Upon return to the lab the samples were sorted by plant species, spun in a large salad spinner to remove water and reduce drying time, and oven dried at 55°C (Wetzel and Likens 2000, Johnson and Newman 2011) to a constant weight for at least 48 h. Dried samples were weighed and results were converted to species-specific dry mass per square meter (g/m^2).

Task A.3.2. Myriophyllum Identification

As an initial verification of watermilfoil identification prior to herbicide treatment, five watermilfoil growing shoots were collected, sealed in a plastic bag, placed on ice and shipped overnight to the "Aquagen" laboratory at Grand Valley State University for genetic identification using genomic DNA fingerprinting technique (Thum et al. 2006). Twenty-one watermilfoil samples were sent for genetic identification post treatment. As we surveyed aquatic macrophytes in this region we determined that Eurasian Watermilfoil (EWM) could not be distinguished visually from the hybrid genotype so we refer to unknown genotypes as invasive watermilfoil (IWM).

Task A.3.3. Environmental Sampling

At each sample transect we collected environmental data including water depth, water transparency, water chemistry and substrate composition. At each station, we measured water temperature, conductivity, pH, turbidity, and dissolved oxygen using a YSI 6960 V2 sonde at the surface and at 0.5m increments in the water column until the bottom was reached. Water samples were collected at a depth of 0.5 m to analyze dissolved organic carbon (DOC), total dissolved nitrogen (TDN), soluble reactive phosphorus (SRP), phytoplankton chlorophyll a (Chl-a,) plankton ash-free dry mass (AFDM), total phosphorus (TP), and total dissolved phosphorus (TDP). At the deepest point of each transect, water clarity was assessed using a Secchi disk, and vertical light profiles were measured using a Li-Cor LI193SA spherical underwater quantum sensor to calculate extinction coefficients.

Aquatic soils (sediment) were collected along line-intercept transects at the same sampling stations where macrophytes and environmental samples were collected. Soils were collected to a depth of 20 cm using a Large Bore Sediment Corer (Aquatic Research Instruments, Hope, ID). Upon collection, soils were placed into a plastic sealed bag and frozen upon returning to campus. In the lab, soils were thawed and dried to a constant weight in an oven at 110°C. Once dried, soils were weighed to calculate bulk density using the mass of the dried sample divided by the volume of the sample. Soil organic matter (SOM) was determined by ashing the soils in a muffle oven at the Michigan Tech Soils Laboratory to remove all organic matter.

Task A.3.4. Herbicide application

The Chassell and Torch Lake townships established special assessment districts for the treatment of EWM and contracted a state-certified aquatic plant management service to control the EWM population in their respective waterbodies. After assessment, the contracted technician made the decision to use combinations of 2,4-D and triclopyr for their selectivity and the presence of Vasey's pondweed (*Potamogeton vaseyi*), a threatened macrophyte in Michigan. Product selection, timing of product use, and the locations these products were applied as per the applicator and the Townships.

Pike Bay Treatment: Pike Bay underwent a complete littoral zone treatment with Sculpin G, a granular formulation of 2,4-D, to a concentration of 2.5-3 ppm on June 24th, 2014 followed by a subsequent spot treatment 30 days later. In 2015, the technician returned and spot treated new areas and retreated a few other areas on July 22nd and August 18th using Renovate OTF, a granular formulation of triclopyr (Table A.1, Figure A.4).

Torch Bay Treatment: Torch Bay had lower EWM infestation; therefore, it was initially spot treated on July 24th, 2014 using Sculpin G and Renovate OTF. A follow-up spot treatment with Sculpin also occurred later that summer on September 3rd. The technician then returned in 2015 on August 18th and September 28th to treat new areas and retreat a few previously treated areas with Renovate Max G—a product that contains both 2,4-D and triclopyr—in addition to Sculpin G and Renovate OTF applications (Table A.1, Figure A.4).

Table A.1. Dates, herbicides types, application rates, and total areas treated in 2014 and 2015 in the Keweenaw waterway to control EWM. Torch Cuts was used as an untreated comparison; therefore, no herbicide was applied in that waterbody.

Location	Date	Herbicides	Rate (lbs/ac)	Area Treated (ac)
Pike Bay	24-Jun-14	Sculpin G	160	78.75
	24-Jul-14	Sculpin G	160	2
	22-Jul-15	Renovate OTF	270	0.44
	18-Aug-15	Renovate OTF	270	4.75
Torch Bay	24-Jul-14	Sculpin G	160	11.75
		Renovate OTF	160	11.75
	3-Sep-14	Sculpin G	200	8.5
		Renovate OTF	200	0.5
	22-Jul-15	Sculpin G	320	3.37
		Renovate OTF	370	0.74
		Renovate Max G	320	1
	18-Aug-15	Sculpin G	320	4
		Renovate OTF	270	2.25
	28-Sep-15	Sculpin G	320	2
		Renovate OTF	270	2
Torch Cuts	n/a			

Table A.2. Dates the waterbodies were sampled for vegetation, water chemistry, and physical properties and the number of weeks relative to the initiation of the treatment program and the most recent herbicide application. *Abbreviated surveys were conducted in Torch Bay and Cuts on 11 October 2014; therefore, these data are not included in the final analyses.

Location	Survey dates	Week post treatment program initiation	Weeks post herbicide application
Pike Bay	18-21 Jun. 2014	0	0
	11-14 Aug. 2014	7	2.5
	1-3 Jun. 2015	49	44.5
	3-5 Aug. 2015	58	2
Torch Bay	8-10 Jul. 2014	0	0
	*11 Oct. 14	11	5
	4-8 Jun. 2015	45	39
	3-4 Oct. 2015	62	1
Torch Cuts	7-11 July 2014	n/a	n/a
	*11 Oct. 14	n/a	n/a
	8-10 Jun. 2015	n/a	n/a
	10-11 Oct. 2015	n/a	n/a

Pike Bay was assessed 1 week prior to herbicide application then again 7, 49, and 58 weeks after the 2014 treatment. The 58 week sampling occurred after the initiation of the treatment program, the bay had been spot treated 2 weeks prior (Table A.2). Torch Bay was assessed 2 weeks prior to the herbicide treatment, 11, 45, and 62 weeks after the initiation of the treatment program. The 62 week survey occurred 1 week after a spot treatment in 2015. Sampling in the untreated Torch Cuts occurred concurrently with Torch Bay sampling (Table A.2). Due to unexpected circumstances, the October 11th surveys of Torch Bay and Torch Cuts was abbreviated; therefore, it was not included in the analyses.

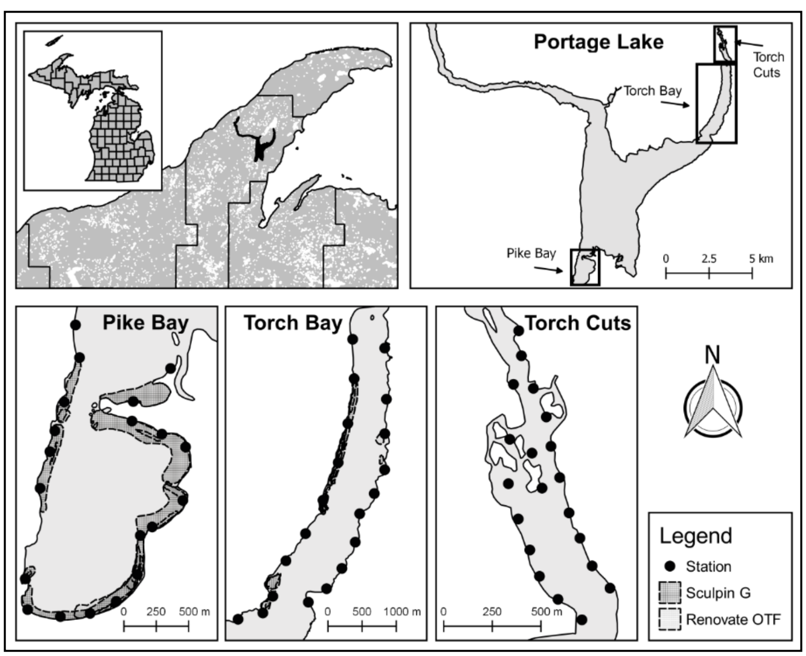


Figure A.4: Location of the three study sites within the Keweenaw Waterway. The black points represent sampling stations where water chemistry and quality were assessed. These points also served as the initial points for the vegetation sampling transects that ran perpendicular to the shore every 15.25 m to a depth of 3 meters. Areas shaded with the checkered or dotted patterns depict where Sculpin G and Renovate OTF were applied, respectively.

Task A.3.5. Analysis

Because each body of water was treated differently and had different environmental characteristics, they were analyzed separately. Each township's objective was to eradicate EWM from their respective waterbody so we first analyzed each bay as whole. Since the contracted herbicide applicator treated at their discretion and only spot treated in some areas, we then used our pretreatment data to assess: 1) if areas where we detected EWM in the pre-treatment surveys were subsequently treated by the applicator and 2) if there was a decrease in the biomass, relative dominance, and frequency of EWM in the areas that were treated by the applicator. We then examined if EWM spread into untreated areas and increased in biomass there during the duration of the study. We predicted that: 1) herbicide would reduce the frequency and biomass of EWM in the waterbodies undergoing treatments while the untreated waterbody would have no change in EWM biomass; 2) EWM biomass would be correlated with water chemistry and quality; and 3) we would see a change in the macrophyte community when EWM was removed from the system.

A goal of this study was to determine the relationship between EWM and water chemical and physical properties; therefore, we compared water chemistry and quality in areas where EWM was present versus where it was absent. Regression was then used to determine the relationship between EWM biomass and the water properties. Lastly, we assessed the vegetative community using Non-metric multidimensional Scaling Ordination (NMDS) in PC ORD (McCune and Mefford 2006) to determine the patterns in macrophyte community assemblages present before and after treatments. Due to unexpected circumstances, Torch Cuts and Torch Bay sites were unable to be fully sampled during the second assessment, so neither are included in the analyses. Full sampling did occur for the 3rd and 4th surveys (Table A.2). Analysis of Variance (ANOVA), linear regression, and chi-square analyses were completed using JMP version 13.0.0 (SAS Institute, Cary, North Carolina, USA). Outcomes/Products: Baseline Macrophyte Communities and Environmental Associations with Eurasian Watermilfoil.

Task A.4. Outcomes/Products

Task A.4.1. Environmental parameters of the bays

The majority of the physical and chemical water properties measured during the surveys were relatively similar through time and between the two sample areas (Table A.3). There were no statistical differences in water physical property values throughout the water column; therefore, these values were averaged at each station and used for further analysis. There were no correlations between watermilfoil biomass and any of the physical and chemical properties of the waterbodies, except for total organic carbon ($r^2 = 0.09$, $p = 0.03$), ash-free dry mass of the plankton in the water column ($r^2 = 0.11$, $p < 0.0001$), and total phosphorus ($r^2 = 0.13$, $p = 0.01$) each of which were positively correlated with watermilfoil biomass. This effect may be because IWM grows in patches with high biomass of submerged aquatic vegetation, which may release or accumulate organic carbon and suspended particles and plankton that have high phosphorus content.

All of the other water chemistry and physical properties did differ significantly between survey dates at each site (Table A.4). The patterns of change do not suggest any influence of the herbicide treatments or the presence of IWM on these environmental parameters and are most likely due to seasonal fluctuations.

Task A.4.2. Sediment relationships among sites

Our results show that the aquatic soils that we sampled varied considerably between lakes and within lakes. The bulk density averaged across all samples was 1.0 g/cm^3, and ranged between 0.08 – 1.90 g/cm^3. The organic matter content was strongly correlated with bulk density of the soil (Figure A.5) and averaged 7.5% (0.1 – 64.0%) across all samples. Pike Bay, in general, had much sandier soils with high average bulk densities (1.4 g/cm^3) and low average organic matter contents (1.9 %). Torch Lake tended to have finer textured and mucky soils that had lower average bulk densities (0.8 g/cm^3) and greater average organic matter content (11.1%).

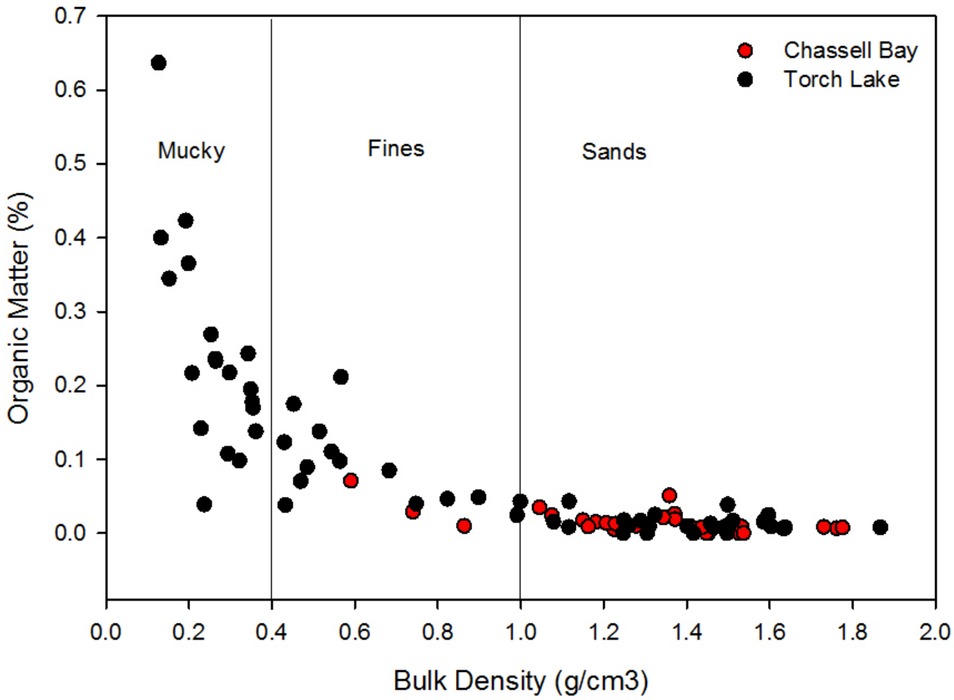


Figure A.5. Correlation between bulk density and organic matter content in the top 20 cm in Torch Lake and Pike Bay (Chassell Bay). Vertical lines indicate the approximate separation between sandy, fine textured and mucky soils.

Table A.3: Summary of the physical and chemical water properties in each surveyed waterbody. Values are averages across all sampling points with SE in parenthesis derived from pooling all station sampling points across time within the waterbody.

Water-body	Area (ha)	Secchi Depth (m)	Cond. (ms cm^{-1})	Temp (°C)	pH	Turb. (NTU)	DO (%)	DO (mg L^{-1})	DOC (mg C L^{-1})	TDN (mg N L^{-1})	SRP (µg P L^{-1})	Chl-α (µg m^{-3})	AFDM (mg L^{-1})	NH_4 (µg N L^{-1})	TDP (µg P L-1)	K_Δ (m^{-1})
Pike		2.01	0.11	19.86	7.96	0.44	100.11	9.17	7.76	0.31	2.79	6.07	1.82	8.36	13.62	-1.33
Bay	182	(0.05)	(0.001)	(0.29)	(0.03)	(0.21)	(0.64)	(0.06)	(0.22)	(0.01)	(0.19)	(0.36)	(0.10)	(0.81)	(0.92)	(0.03)
Torch		2.03	0.12	17.12	7.98	1.23	98.48	9.52	5.96	0.31	2.48	4.40	1.70	8.47	7.09	-1.54
Bay	293	(0.06)	(0.001)	(0.30)	(0.05)	(0.14)	(0.33)	(0.06)	(0.26)	(0.01)	(0.20)	(0.55)	(0.10)	(0.91)	(0.67)	(0.03)
Torch		2.05	0.14	17.40	7.89	2.02	93.37	8.93	6.58	0.31	2.27	3.61	1.75	8.43	7.13	-1.51
Cuts	96	(0.07)	(0.001)	(0.33)	(0.04)	(0.16)	(0.43)	(0.07)	(0.20)	(0.01)	(0.37)	(0.25)	(0.13)	(0.63)	(0.78)	(0.03)

Notes: Values are means (SE). Abbreviations: DO = dissolved oxygen, TDN = total dissolved nitrogen, SRP = Soluble reactive phosphorus, Chl-α = phytoplankton chlorophyll α, AFDM = plankton ash-free dry mass, TDP = Total dissolved phosphorus. K_Δ is the photosynthetically active radiation (PAR) diffuse attenuation coefficient estimated using the Beer-Lambert Law equation.

Table A.4: The physical and chemical water properties in each surveyed waterbody. Values are averages within the waterbody during a sampling survey with SE in parenthesis. Survey dates are presented in Tables A.1 and A.2. Values with the same letter denote those that are not statistically different (p = 0.05)

Water-body	Survey	Secchi Depth (m)	Cond. (ms cm^{-1})	Temp (°C)	pH	Turb. (NTU)	DO (%)	DO (mg L^{-1})	DOC (mg C L^{-1})	TDN (mg N L^{-1})	SRP (µg P L^{-1})	Chl-α (µg m^{-3})	AFDM (mg L^{-1})	NH_4 (µg N L^{-1})	TP (µg P L^{-1})	TDP (µg P L^{-1})
Pike Bay	1	1.72 a	0.10 a	18.66 a	8.29 a	1.23 a	97.63 a	9.10 a	9.88 a	0.37 a	2.71 ab	4.96 a	2.28 a	12.38 a	26.60 a	18.44 a
		0.03	0.00	0.35	0.09	0.62	1.99	0.14	0.12	0.01	0.41	0.60	0.20	1.78	1.67	1.90
	2	2.64 b	0.12 b	21.95 b	8.10 ab	-1.07 b	97.82 a	8.57 b	5.67 b	0.23 b	2.39 a	5.36 a	2.09 ab	4.90 b	18.36 bc	9.34 b
		0.07	0.00	0.22	0.08	0.52	1.89	0.16	0.12	0.00	0.32	0.57	0.12	0.82	0.79	0.92
	3	1.70 a	0.10 c	16.25 c	7.38 c	1.25 a	101.65 ab	9.91 c	9.15 c	0.35 a	3.76 b	5.91 ab	1.18 c	7.80 b	20.73 bc	13.08 b
		0.04	0.00	0.17	0.04	0.27	1.18	0.12	0.21	0.01	0.42	0.41	0.15	0.85	1.42	1.12
	4	2.01 c	0.12 d	20.60 d	7.98 b	1.54 a	104.28 b	9.36 a	6.35 d	0.27 c	2.32 a	8.05 b	1.73 b	11.65 a	15.34 c	3.22 c
		0.09	0.00	0.16	0.08	0.29	1.98	0.16	0.24	0.01	0.29	0.98	0.25	0.87	1.41	0.45
Torch Bay		1.70 a	0.11 a	21.10 a	8.42 a	2.02 a	99.21 a	8.81 a	7.39 a	0.34 a	2.58 a	6.33 a	2.20 a	12.51 a	18.96 a	9.38 a
	1	0.05	0.00	0.14	0.07	0.44	0.96	0.07	0.13	0.01	0.58	1.44	0.11	1.41	1.40	0.97
		1.94 b	0.11 a	15.88 b	7.44 b	0.76 b	99.51 a	9.85 b	6.87 b	0.35 a	1.91 a	2.72 b	1.24 b	4.65 b	13.33 b	4.27 b
	3	0.04	0.00	0.19	0.04	0.25	0.68	0.07	0.15	0.00	0.00	0.26	0.15	0.36	0.69	0.51
		2.60 c	0.13 b	14.81 c	8.06 c	0.82 b	97.03 a	9.82 b	2.97 c	0.21 b	3.09 a	4.30 ab	1.52 b	11.39 a	20.80 a	5.24 b
	4	0.07	0.00	0.13	0.07	0.19	0.51	0.06	0.11	0.01	0.00	0.20	0.10	0.70	1.34	0.43
Torch Cuts		1.54 a	0.13 a	21.80 a	8.39 a	1.97 ab	94.11 a	8.25 a	8.20 a	0.38 a	3.25 a	6.04 a	2.41 a	11.41 a	19.09 ab	9.95 a
	1	0.06	0.00	0.20	0.16	0.39	1.03	0.07	0.17	0.01	1.27	0.44	0.19	0.94	1.53	1.31
		1.89 b	0.13 b	17.73 b	7.51 b	1.14 a	96.99 b	9.24 b	6.88 b	0.33 b	2.08 a	2.35 b	1.31 b	5.67 b	15.33 a	4.60 b
	3	0.06	0.00	0.19	0.05	0.20	0.64	0.06	0.23	0.01	0.17	0.18	0.27	0.22	1.30	0.68
		2.60 c	0.15 c	13.44 c	7.77 b	3.04 b	89.90 c	9.26 b	4.96 c	0.24 c	1.67 a	2.80 b	1.55 b	14.92 c	20.56 b	5.70 b
	4	0.07	0.00	0.13	0.03	0.42	0.61	0.13	0.12	0.01	0.00	0.13	0.11	0.58	1.15	0.51

Notes: Values are means (SE). Abbreviations: DO = dissolved oxygen, DOC = dissolved organic carbon, TDN = total dissolved nitrogen, SRP = soluble reactive phosphorus, Chl-α = phytoplankton chlorophyll α, AFDM = plankton ash-free dry mass, TP = total phosphorus, TDP = total dissolved phosphorus.

Task A.4.3. Pre-treatment Macrophyte Communities

Surveys of the aquatic macrophyte communities revealed a relatively diverse array of species in the three study sites (Table A.5). Of particular note is that Pike Bay supports a population of Vasey's pondweed, *Potamogeton vaseyi*, a species classified as threatened in Michigan.

Table A.5: Species list of macrophytes found in each sampling sites across all sampling dates.

Pike Bay	Torch Bay	Torch Cuts
Aquatic moss	*Bidens beckii*	*Aquatic moss*
Bidens beckii	*Ceratophyllum demersum*	*Bidens beckii*
Ceratophyllum demersum	*Chara spp.*	*Brasenia schreberi*
Chara spp.	*Elodea canadensis*	*Ceratophyllum demersum*
Elodea canadensis	*Eriocaulon aquaticum*	*Chara spp.*
Eriocaulon aquaticum	*Invasive Myriophyllum spp.*	*Elodea canadensis*
Invasive Myriophyllum spp.	*Najas flexilis*	*Eriocaulon aquaticum*
Littorella uniflora	*nuphar variegata*	*Invasive Myriophyllum spp.*
Najas flexilis	*Nymphaea odorata*	*Isoetes spp.*
nuphar variegata	*Potamogeton amplifolius*	*Myriophyllum heterophyllum*
Nymphaea odorata	*Potamogeton epihydrus*	*Najas flexilis*
Potamogeton alpinus	*Potamogeton gramineus*	*Nuphar variegata*
Potamogeton amplifolius	*Potamogeton illinoensis*	*Nymphaea odorata*
Potamogeton epihydrus	*Potamogeton perfoliatus*	*Potamogeton amplifolius*
Potamogeton foliosus	*Potamogeton praelongus*	*Potamogeton epihydrus*
Potamogeton friesii	*Potamogeton pusillus*	*Potamogeton gramineus*
Potamogeton gramineus	*Potamogeton robbinsii*	*Potamogeton illinoensis*
Potamogeton perfoliatus	*Sparganium fluctuans*	*Potamogeton perfoliatus*
Potamogeton praelongus	*Stuckenia filiformis*	*Potamogeton praelongus*
Potamogeton pusillus	*Stuckenia pectinata*	*Potamogeton pusillus*
Potamogeton pusillus	*Utricularia vulgaris*	*Potamogeton pusillus*
Potamogeton robbinsii	*Vallisneria americana*	*Potamogeton robbinsii*
Potamogeton vaseyi		*Ranunculus reptans*
Ranunculus reptans		*Schoenoplectus acutus*
Sparganium fluctuans		*Sparganium fluctuans*
Stuckenia filiformis		*Stuckenia pectinata*
Stuckenia pectinata		*Utricularia vulgaris*
Utricularia vulgaris		*Vallisneria americana*
Vallisneria americana		

We detected relatively patchy distributions of IWM during our surveys of the three study waterbodies although it was broadly present in all of our sampling areas, and in general was found in locations where the overall biomass of submerged aquatic vegetation was highest (Figure A.6). On average, total plant biomass in plots with IWM was 48 g/m^2, while it was 17 g/m^2 in plots without IWM. However, the higher biomass in plots with IWM cannot be attributed to the addition of this species, as it only comprised on average 19% of the biomass in these plots (Figure A.6). Similarly, transects where IWM was present generally had high higher species richness of aquatic vegetation (Figure A.7). Together, our data suggest that IWM invades sites with overall more favorable conditions for macrophyte growth, and presence of IWM per se is not associated with lower occurrence of other macrophyte species in this study area. It may be the case that these sites where IWM invaded previously had greater biomass and/or species richness of native species, however, that can't be determined by available data from before the invasion.

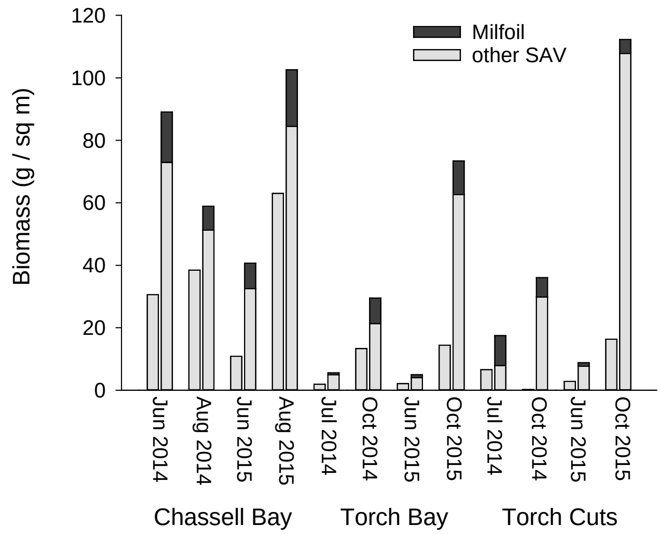


Figure A.6: Comparison of IWM (milfoil) and biomass of all other submerged aquatic vegetation averaged across all sampling points in each bay on each sampling period.

Canonical correspondence analysis (CCA) ordination was used to examine the relationship between plant species composition and environmental gradients (e.g., depth of water, water chemistry) using PC-ORD 7.0 (Gleneden Beach, OR, USA). All aquatic vegetation was relativized to the maximum value in the data set. CCA of only pretreatment data from 2014 indicate that Torch Lake and Pike Bay are distinctive floristically (Figure A.8). The most common aquatic plants found in Torch Lake were pondweeds (*Potamogeton perfoliatus, Potamogeton gramineus L.*), and there was also a high percentage of bare areas, whereas Pike Bay had a greater coverage of *Ceratophyllum demersum, Elodea canadensis,* and *Myriophyllum spicatum*, with little bare ground. EWM coverage was also much larger in Chassell compared to Torch Lake. Pike Bay was associated with greater total organic carbon (TOC), Total dissolved phosphorus, and low soil organic matter, while Torch lake was the opposite (Figure A.8).

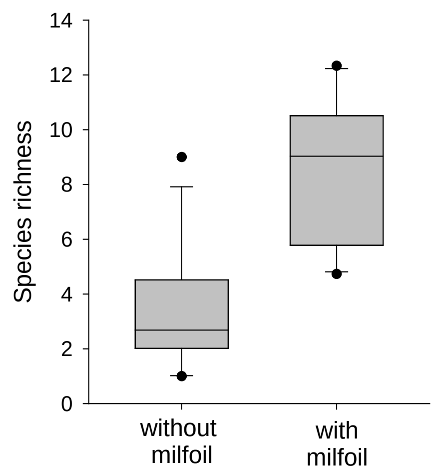


Figure A.7: Species richness observed on sampling transects with and without IWM (milfoil) across all sampling bays and dates

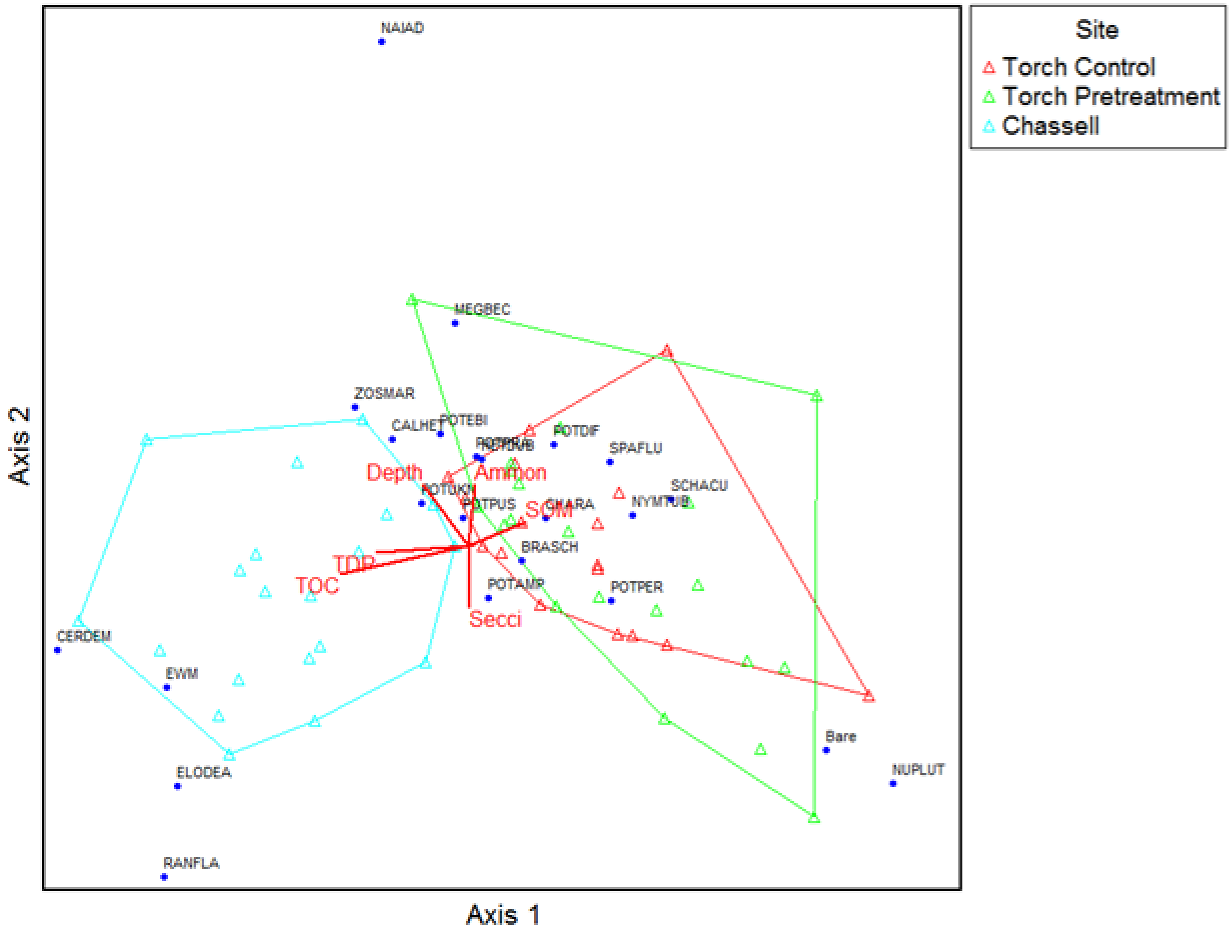


Figure A.8. Multivariate analysis of aquatic macrophyte abundance and composition, and environmental parameters surveyed in Pike Bay (Chassell), Torch Lake, and Torch control site in 2014 (pretreatment).Red arrows indicate environmental variable associated with the vegetation (total organic carbon (TOC), total dissolved phosphorus (TDP), water depth (Depth), ammonium (Ammon), soil organic matter (SOM), secci (secci depth). Plant species are indicated as blue dots and values shown are the Canonical correspondence analysis loadings along Axes 1 and 2 for *Myriophyllum spicatum L.* (EWM), *Potamogeton amplifolius* (POTAMP), *Chara* spp. (CHARA), *Potamogeton Richardson* (POTPER), *Ceratophyllum demersum* (CERDEM), *Vallisneria amercana* (ZOSMAR), *Elodea Canadensis* (ELODEA), *Potamogeton robbinsii* Oakes (CALHET), *Potamogeton gramineus* L. (POTDIF), *Schoenoplectus acutus* Muhl. ex Bigelow (SCHACU), *Megalondonta beckii* Torr. ex Spreng. Greene. (MEGBEC), *naiad* spp. (NAIAD), *Eriocaulon aquaticum* (HETDUB), *Potamogeton epihydrus* (POTEBI), *Nuphar variegate* (NUPLUT), *Ranunculus flammula* (RANFLA), *Sparganium fluctuans* (SPAFLU), *Potamogeton pusillus* L. (POTPUS), *Potamogeton* unknown species (POTUKN), *Brasenia schreberi* J.F. Gmel.(BRASCH), *Nymphaea odorata* Aiton (NYMTUB), *Potamogeton praelongus* Wulfen (POTPRA).

Task A.4.4. *Myriophyllum* identification.

Genetic analysis verified two of the five initial watermilfoil samples as Eurasian watermilfoil; however, the other three shoots were determined to be hybrids (*M. spicatum* × *M. sibericum*) between Eurasian watermilfoil and the native northern watermilfoil (*M. sibericum*). Of the 21 samples genetically identified post-treatment, 19 shoots were determined to be hybrids with only 2 verified as Eurasian watermilfoil. Because the hybrids were so prevalent in the bays, we henceforth refer to the watermilfoil sampled during surveys as invasive watermilfoil (IWM).

Task A.5. Responses to Efforts to Control Eurasian Watermilfoil

Task A.5.1. Invasive watermilfoil management

Frequency of IWM detection and treatment. Following an exceptionally cold winter and higher than normal lake levels, populations of IWM did not flourish in Torch Bay in 2014, but did in Pike Bay. As a result, the Torch Bay waterbody was less extensively treated with herbicides in 2014 as was Pike Bay (Table A.2).

During the 2 years of herbicide treatments in Pike Bay, 34.5% of the initial sampling points (n = 171) had IWM present prior to the initiation of the whole-littoral zone herbicide treatment (Figure A.3). Of the 171 total sampling points, 69.6% (119 sampling points) fell within 10 m of the initial herbicide treatment, and contingency analysis showed that the majority of the sampling points with IWM detected in the Bay (86.4%) were initially treated by the applicator (χ^2 =19.153, $p > 0.0001$). Seven weeks after the first herbicide treatment and 2.5 weeks after a follow up spot treatment, 31% of the treated points still had IWM present, and 13% (7 sampling points) of points not included in the initial treatment had IWM present. One year after the initiation of the herbicide treatments, we detected IWM in 22% of the sampling points, and after the final herbicide treatments in year 2, IWM was detected in 26% of the treated points. The proportion of IWM treated when detected decreased to 82.22% (χ^2 =8.16, $p = 0.006$), 83.33%, (χ^2 =5.56, $p = 0.033$,), and 76.32% ($\chi^2 = 2.57$, $p > 0.13$) throughout the study during sampling times 2, 3, and 4, respectively, with sampling 4 showing a non-significant likelihood that if IWM was detected it was treated. There was a decrease in likelihood during each sampling because IWM was detected in new sampling points that were not treated by the applicator. Across the two years of treatment in all sampling points (treated and untreated), IWM frequency decreased from being present in 34.5% of the sampling points to 23.9%.

In Torch Bay, only 4% of the 168 total sampling points had IWM present. Because the intensity of IWM invasion was not widespread, the herbicide applicator chose to spot treat the bay. There were a total of 11 sampling points within 10 m of herbicide treatments, and if watermilfoil was detected there was a significant likelihood ($\chi^2 = 7.74$, $p = 0.0072$) that it was treated during the first herbicide application.. The decision by the certified applicator to spot treat later in the season pushed the second sampling effort into the fall. The plants were beginning to senesce and there was a lack of a sampling crew; therefore, only a partial follow-up survey was conducted. We detected IWM in only one (9%) of the sampling points that underwent herbicide treatment during the third (45 week) sampling. Of the points not treated (n = 158), three had IWM present during the third sampling period. The herbicide applicator applied additional spot treatments throughout the bay, which included 15 sampling points that were not initially treated with herbicide. IWM was detected in 19% (5 sampling points) during the 4th sampling period. If watermilfoil was detected in year 2 (3rd and 4th sampling) it was unlikely to have been treated (p = 0.09 and 0.12, respectively) based on the distribution of these spot treatments. Across the two years of treatment in all sampling points (treated and untreated), IWM frequency increased from being present in 4% of the total number of sampling points to 7%.

Torch Cuts did not have any herbicide treatments during the study. Of the 147 points sampled in the Torch Cuts, 19% (28 sampling points) had IWM at the onset of the study. This detection frequency decreased to 6% during the third sampling period then again to 4% of sampling points at the completion of the study.

Response of Invasive watermilfoil to treatment efforts: Because the aquatic invasive species management district's objective to eradicate IWM from the bays, we first analyzed each bay as a whole. There was a significant decrease in watermilfoil biomass across Pike Bay (Figure A.9), and the dominance of watermilfoil in the bay significantly decreased from 22.42 ± 2.96% pretreatment to 5.46 ± 1.09%. The sampling points that were not subject to herbicide treatment showed no significant change in the IWM biomass (p = 0.23) or dominance (p = 0.83) throughout the study. On the other hand, in the areas that were treated, there was a significant change in IWM biomass (p = 0.0004). The biomass decreased from 7.75 ± 1.38 g m^{-2} prior to treatment to 4.62 ± 1.22 g m^{-2} during the second sampling effort. One year after the initiation of the treatments, the IWM

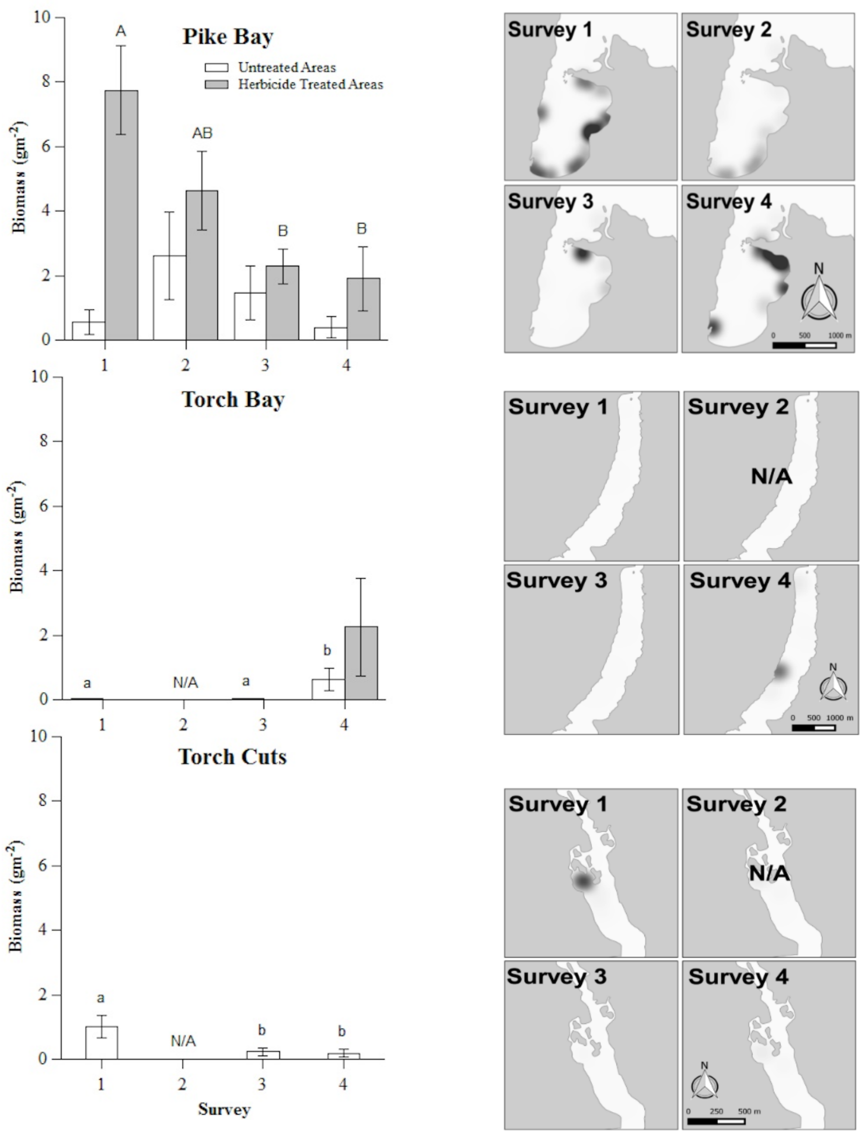


Figure A.9. IWM biomass in treated and untreated areas in each of the waterbodies throughout the study. Bars with different letters are statistically different (p<0.05). In the maps, darker areas represent areas in the bay that have more IWM biomass.

biomass was 2.29 ± 0.54 g m^{-2} then further decreased to 0.4 ± 0.32 g m^{-2} during the 4^{th} sampling period. The dominance of IWM in the macrophyte communities decreased significantly ($p < 0.0001$) throughout the study. However, there was a major resurgence of IWM biomass in the northern part of the bay (Figure A.9). This area had a population of Vasey's pondweed, *Potamogeton vaseyi*, a species classified as threatened in Michigan, so the applicator limited herbicide applications in those areas in 2015 and then heavily treated in 2016. There was also reestablishment of IWM in the southwest corner of the bay, which may be due to reinvasion or regrowth of IWM that survived initial herbicide treatments.

There was low incidence of watermilfoil present in Torch Bay; however, there was a significant increase in watermilfoil biomass throughout the study (Figure A.9). There was a significant increase in watermilfoil biomass in the untreated sections of Torch Bay ($p = 0.017$) from 0.03 ± g m^{-2} to 0.6 ± 0. g m^{-2} at the end of the study as IWM invaded new, untreated areas. In the treated areas, watermilfoil remained at less than 3 g m^{-2} and composed no more than 10.5% of the macrophyte community. There was no significant change in the dominance of watermilfoil in the macrophyte communities, which ranged from 2.27 ± 1.04% at the highest to 0.53 ± 0.9% at the lowest ($p = 0.43$).

Watermilfoil biomass was relatively low in the untreated Torch Cuts, however, there was a significant decrease in watermilfoil biomass as the study progressed ($p = 0.01$ and 0.04 for biomass and dominance respectively; Figure A.9). At the beginning of the study milfoil represented 5.01 ± 1.49% of the macrophyte community, and decreased to 0.28% dominance) by the end of the study.

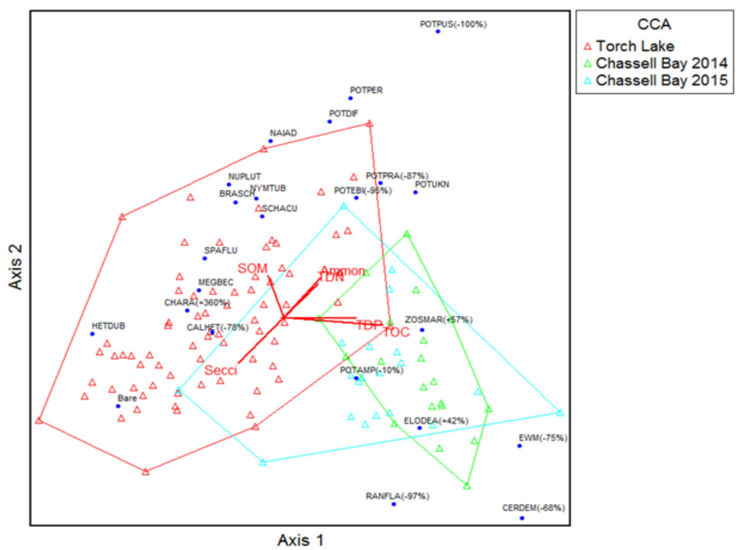


Figure A.10. CCA analysis of changes in plant communities after treatment. The (%) indicates direction and magnitude of individual species change in biomass from 2014 to 2015 in Pike Bay.

Macrophyte community response to IWM management: A major question regarding herbicide treatment is the potential impacts on the native plant communities and the environment. Analysis of plant and environmental data from 2014 and 2015 provide an initial response to IWM treatment (Figure A.10). Torch Lake 2014/2015 data indicate that macrophytes showed some changes in species composition from year to year without any treatments having occurred, but the percent changes can be misleading because of the small abundances of plants found in Torch Lake (a small absolute change can be a large percentage change). However, large changes were detected in Pike Bay after treatment (Figure A.10). As noted above, biomass of *Myriophyllum spicatum* (EWM, and/or hybrids) dropped approximately 75% from 2014 to 2015 sampling periods across all transects. However, several other plant species also dropped, including a 68% drop in *Ceratophyllum demersum*, a 97% drop in *Ranunculus flammula*, and a 10% drop in *Potamogeton amplifolius.* However, not all plants decreased following treatments; for example, biomass of *Elodea canadensis* increased 42% and *Zostera marina* increased 57%. The amount of bare ground also increased after treatment (+500%).

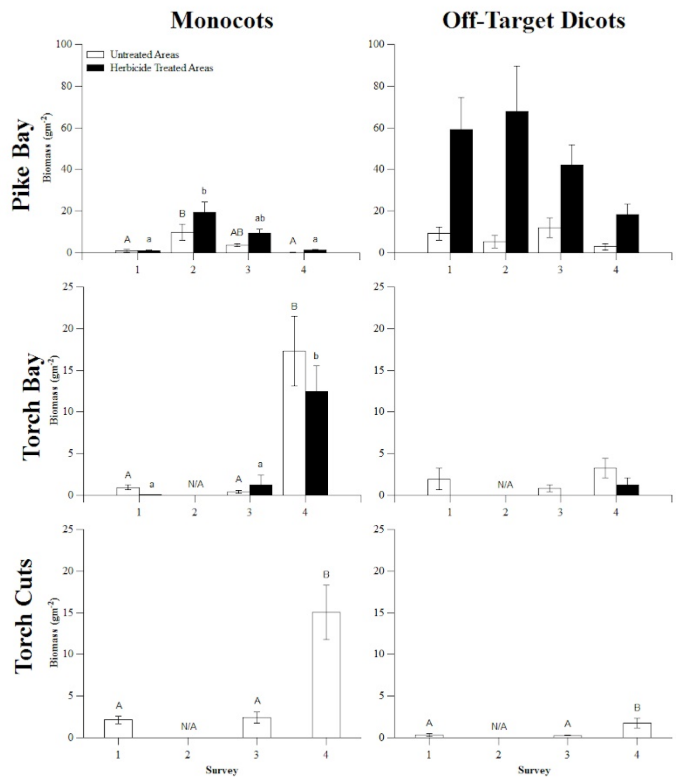


Figure A.11. Effects of herbicide treatment on non-target monocots and dicots over the course of the study.

The areas that were treated generally had more vegetation than those untreated; therefore, no comparisons were made between treated and untreated areas. Instead we examined changes in macrophytes through time in all the waterbodies. We analyzed monocots separately from dicots because the herbicide 2,4-D targets dicots such as Eurasian watermilfoil, while monocots are less sensitive to this herbicide. Although there were fluctuations in biomass through time, the patterns of the fluctuations do not suggest that the off-target species were influenced by the herbicide treatments (Figure A.11). Some species did show variation throughout the study. The monocot biomass, primarily *Potamogeton* spp., *Elodea canadensis* and *Vallisnaria americana*, was relatively low, ranging from approximately 1 g m^{-2} at the start of the study to a high of 16.4 ± 3.7 g m^{-2} [1] during the first survey period compared to the dicot biomass, but did significantly change throughout the study ($p < 0.01$; Figure A.11). The dicot biomass was composed primarily of *Ceratophyllum demersum*, which is in a sister group and the same clade as the eudicots therefore combined with the dicots for this study. Other dominant dicots included *Bidens beckii* and *Ranunculus reptans.* Dicot biomass fluctuated from 44.1 ± 10.89 g m^{-2} at the start of the study to a low of 13.7 ± 3.5 g m^{-2} at the end but those changes through time were not significant ($p = 0.074$).

In Torch Bay, a similar suite of plants was detected, albeit with lower biomasses than Pike Bay. The monocots did fluctuate significantly from less than 1 g m^{-2} at the beginning of the study to 15.33 ± 0.81 g m^{-2} ($p < 0.0001$; Figure A.11). The dicots remained at approximately 1.5 g m^{-2} throughout the study ($p = 0.26$).

In Torch Cuts we detected increases through time for both the monocot and dicot groups ($p < 0.0001$, and $p = 0.0025$, respectively). The monocot biomass was 2.14 ± 0.17 g m^{-2} during the first survey and was statistically the same one year later (2.43 ± 0.07 g m^{-2}). The monocot biomass, mostly composed of *Vallisnaria americana*, with *Potamogeton* spp., and *Elodea Canadensis* also present, increased to 15.08 ± 0.56 g m^{-2} at the conclusion of the study (Figure A.11). The dicot biomass, mostly *Ceratophyllum* *demersum*, also increased from 0.35 ± 0.17 g m^{-2} and 0.28 ± 0.07 g m^{-2} during surveys 1 and 3 respectively to 1.7 ± 0.56 g m^{-2} at the conclusion of the study.

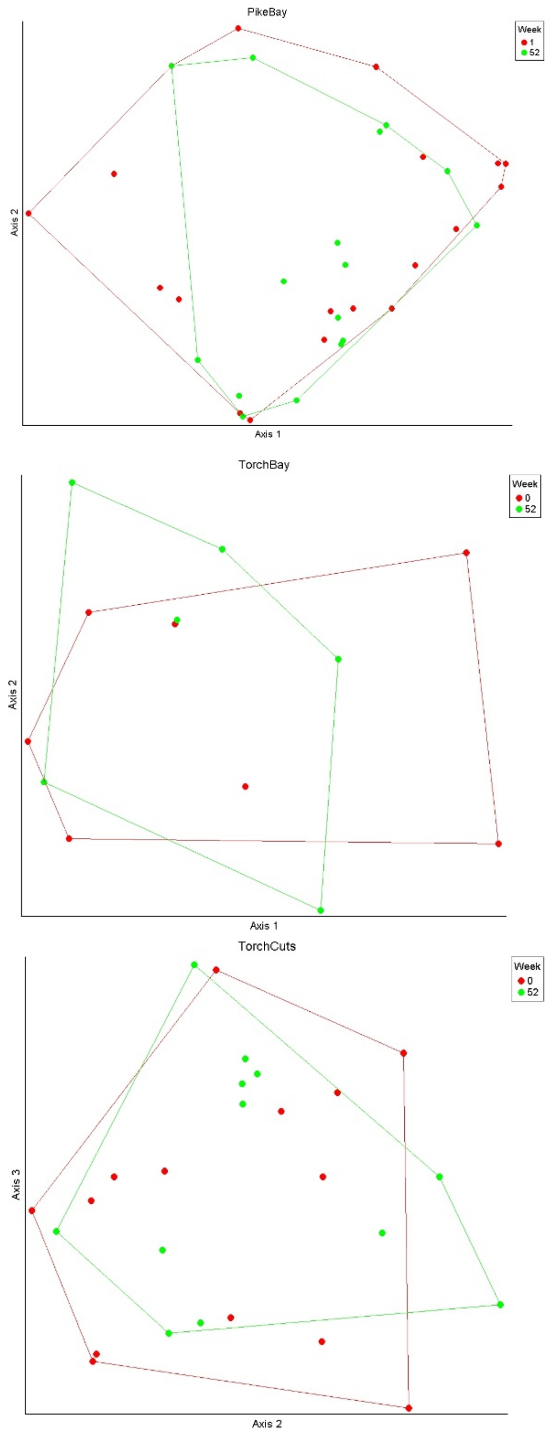


Figure A.12. NMDS analysis of changes in Pike Bay, Torch Bay, and Torch Cuts plant communities from pre-treatment (2014) to the 1 year follow up in 2015. Axes 1 and 2 explained 61.6%, 82%, and 38% of the variation for plots from top to bottom.

Non-metric multidimensional scaling ordination (McCune and Grace 2006) was conducted to determine patterns in macrophyte community assembly before and after treatment. The Pike Bay vegetative community resulted in a 3-dimensional solution with a final stress of 10.81 and instability criteria of 0.0 after 53 iterations. The cumulative r^2 was 0.775 with axes 1 and 2 explaining the most variation r^2 of 0.302 and 0.314 (Figure A.12, top pane). Invasive *Myriophyllum* was had a strong positive correlation with axis 1 (r = 0.777) while *Vallisnaria americana* was negatively correlated with that axis (r = -0.533). *Ceratophyllum demersum* was strongly negatively correlated with axis 2 (r = -0.869) while *Potamogeton gramineus* shown the strongest positive correlation (r = 0.296). There was no obvious successional trajectory when the pre-treatment macrophyte community was compared to the one-year follow up survey. The Pike Bay macrophyte community did not appear to change over the course of the study.

The Torch Bay vegetative community resulted in a 2-dimensional solution with a final stress of 8.77 and instability criteria of 0.0 after 79 iterations. The cumulative r^2 was 0.820 with axes 1 explaining the most variation (r^2 = 0.652) followed by axis 2 (r^2 = 0.168; (Figure A.12, middle). *Eriocaulon septangulare* had the strongest positive correlation with axis 1 (r = 0.312) while *Nymphaea odorata had* the strongest negative correlation (r = -0.727). *Potamogeton gramineus* was strongly negatively correlated with axis 2 (r = -0.507) while *Chara* spp. shown the strongest positive correlation (r = 0.548). Like Pike Bay, there was no obvious successional trajectory when the pre-treatment macrophyte community was compared to the one-year follow up survey, suggesting little over all change over the course of the study.

The Torch Cuts vegetative community resulted in a 3-dimensional solution with a final stress of 12.91 and instability criteria of 0.0 after 45 iterations. The cumulative r^2 was 0.543 with axes 2 explaining the most variation (r^2 = 0.215) followed by axis 3 (r^2 = 0.165; Figure A.12, bottom). Axis 1 followed with an r^2 of 0.163. *Ceratophyllum demersum* had the strongest positive correlation with axis 2 (r = 0.333) while *Nymphaea odorata* had the strongest negative correlation (r = -0.593). *Nuphar variegata* was strongly negatively correlated with axis 2 (r = -0.351) while *Vallisnaria americana* had the strongest positive correlation (r = 0.437). Like the other water bodies, there was no obvious successional trajectory when the pre-treatment macrophyte community was compared to the one-year follow up survey suggesting little over all change over the course of the study.

Species diversity fluctuated throughout the study (Table A.6), but the pattern does not suggest herbicide treatments had a major effect on the richness and diversity within the aquatic macrophyte community.

Table A.6. Species richness (N) and diversity represented as Simpson's index for the three waterbodies Pike Bay, Torch Bay, and Torch Cuts analyzed as whole waterbodies, just the treated sample points or just the untreated points.

			Entire Waterbody		Treated Points		Untreated Points	
Waterbody	Sample Date	Weeks post-treatment initiation	Species Richness	Simpson's index (1-D)	Species Richness	Simpson's index (1-D)	Species Richness	Simpson's index (1-D)
Pike Bay	June 20 14	0	16	0.24	14	0.23	11	0.31
	August 2014	7	17	0.44	17	0.4	13	0.64
	June 2015	49	17	0.3	15	0.3	11	0.41
	August 2015	58	22	0.52	22	0.47	14	0.74
Torch Bay	July 2014	0	17	0.79	11	0.79	16	0.77
	June 2015	45	13	0.53	8	0.5	13	0.6
	October 2015	62	16	0.51	14	0.55	15	0.47
Torch Cuts	July 2014	*n/a*	20	0.82				
	June 2015	*n/a*	20	0.79				
	October 2015	*n/a*	23	0.87				

Task A.6. Major Conclusions and Study Constraints

The objective of targeted, species-specific management of IWM is to select a treatment that will reduce IWM without harming off target macrophytes. In Pike Bay, the treatments significantly reduce the amount of IWM, while the IWM biomass in the untreated areas was unchanged or increased in some areas. In Torch Bay where the IWM was spot treated, there was an overall increase of IWM, albiet the population remained at low loves for the 2 years of monitoring described here. There is no evidence that the treatments in 2014 and 2015 altered the overall plant community of the bays.

When treating, it's important that all areas of IWM are treated, so new invasions and spread do not occur. At the onset of the study, if IWM was detected, there was a high likelihood that it was initially treated. As time progressed the likelihood of detected IWM being treated with herbicide decreased to the point where it was no longer significant. This is due to the spread and new establishment of IWM in areas in the waterbodies that were not previously invaded. Because the certified applicator treated the bays according to the herbicide label, it is possible that these new invasions were not treated to avoid exceeding the recommended application restrictions. An alternate possibility is that IWM may have initially gone undetected by the applicator, or the invasion was below the treatment threshold. The frequency of IWM did remain low throughout the study, so perhaps other treatment options could have been used to supplement herbicide application to assist in IWM eradication.

We observed variable and divergent patterns of milfoil emergence growth through time at Torch Bay and Pike Bay, the two sites of recent infestations of IWM in the Keweenaw Waterway. While both sites were in the early stages of establishment at the start of the project, one site (Pike Bay) became relatively more heavily invaded while the other site (Torch Bay) showed minor regrowth, perhaps due to differential effects of colder water temperatures and deeper water depths in 2014-2016. Therefore, herbicides were applied more extensively in Pike. In Pike Bay, we observed annual regrowth of milfoils following herbicide treatment and increasing frequency of detection of IWM hybrid genotypes vs. pure *M. spicatum* from pre-treatment in 2014 to post-treatment in 2015. This differential treatment of Torch Bay limited the effectiveness of considering Torch Bay and Pike Bay as "replicate" systems to track the growth and treatment of invasive milfoil. In reality, this variable and spatially differential growth of invasive milfoil may be one of the unique aspects of the behavior of this invasive species in the cold and extreme waters of the Northern Great Lakes region. The role of hybridization on treatment effectiveness and growth of IWM in the northern Great Lakes is part of ongoing research by our group.

Task A.7. Other related projects

Task A.7.1. Effects of stamp sands on the structure of Macrophyte communities in the Keweenaw Waterway

One of the defining physical features of the Keweenaw Waterway are large deposits of mining residue known as stamp sand from historic copper mining activities. Stamp sands contain high concentrations of heavy metals that exhibit toxicity and have a jagged, irregular structure that make them a harsh habitat for rooting plants and burrowing animals. Elevated heavy metal concentrations could have negative impacts on lakeshore communities, particularly submerged aquatic macrophytes that exhibit susceptibility to heavy metal toxicity. In collaboration with this GLRI project and with additional support from the Summer Undergraduate Research Fellowship program and the Ecosystem Science Center at Michigan Tech, Ryan Van Goethem (BS Biology 2015, now MS student in Biological Sciences), led a study in summer 2015 to characterize macrophyte and watermilfoil species response to environmental gradients to assess the effects of stamp sands on macrophyte communities. This study tested 2 hypotheses: 1) stamp sands alter diversity of macrophyte communities and 2) the frequency of watermilfoil species growing on stamp sands sites will be different from those growing in non-stamp sites. We used a balanced study design to conduct point sampling of 15 sites on stamp sand deposits and 15 non-stamp sand sites on

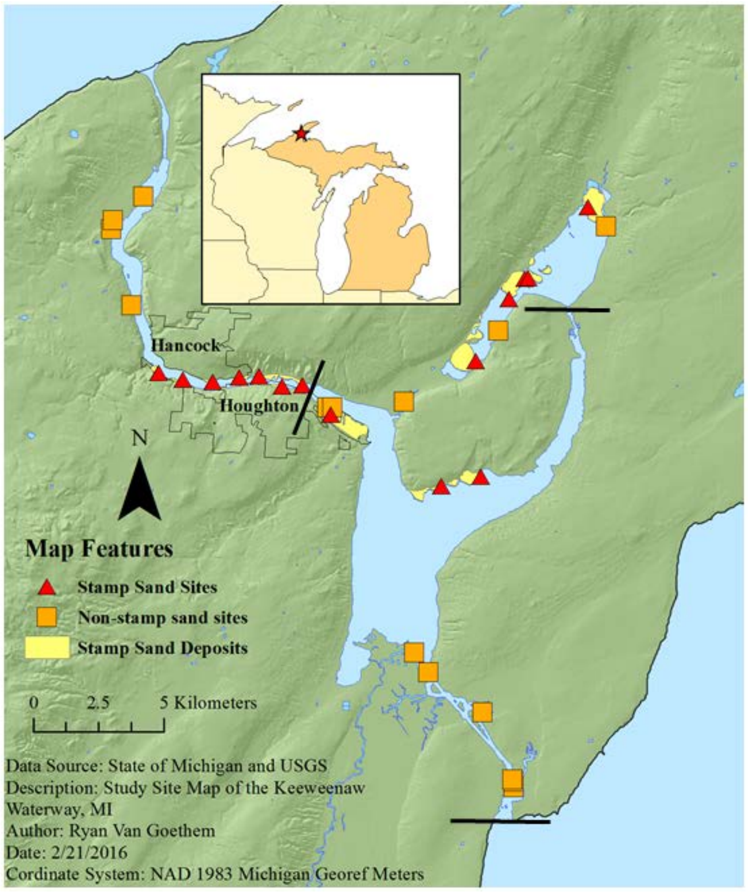


Figure A.13: Distribution of study sites in the interior of the Keweenaw Waterway, MI which bisects the Keweenaw Peninsula connecting Lake Superior on both sides. The waterway has natural subdivisions, labeled, and sectioned by the black bars. Study sites by site type are depicted, along with areas of terrestrial stamp sand deposits at historic deposition sites.

native sediments (Figure A.13). We constrained our site selection prior study by performing a survey of the waterbody in June – July 2015 using visual inspection by boat, aerial photography, and prior knowledge to capture spatial extents of macrophyte communities. Stamp sands sites were selected from surveyed macrophyte communities that were present presumably on benthic deposits, randomly selecting one site per unique deposit determined by stamp mill records (Forgrave 2009; Kerfoot et al. 1994) using ArcGIS. Non-stamp sand sites were randomly selected from the remaining macrophyte communities with a required minimum 100m spacing of sites using ArcGIS. In late July-early August 2015 we visited the 30 sites in the waterway to collect water chemistry, macrophyte, sediment samples, and general limnological characteristics following methods described in the EPA-approved QAPP.

Indices of community diversity and structure were derived from fixed-area biomass sampling of macrophyte species. Species richness (R), Shannon's diversity (H'), Shannon's evenness (J'), and average site coefficients of conservatism (SCOC) were calculated for each site for comparisons. Coefficients of conservatism for Michigan were retrieved from a current plant identification guide (Skawinski, 2014) and computed as: SCOC =(∑ Bij*COCij)/n. In this equation B refers to dry biomass, COC refers to coefficient of conservatism, n refers to number of samples at a site, and ij refers to data pairs. Site 21 was removed from the data set as the macrophyte sampling captured an extensive un-separable mix of detrital Potamageton robbinsii and living, violating sampling method assumption of living shoot only collection. All indices were assessed for normality amongst analysis groups using Shapiro-Wilks goodness of fit tests and justly transformed to meet normality assumptions of t tests using JMP® Pro (SAS Institute Inc., 2015).

Comparing indices of the community and diversity between SS and NSS sites such as dry biomass, *R*, *H'*, *J'*, and COC showed no significant differences between site type (TableA.7). In addition, we observed no difference in presence of non-native *Myriophyllum* species between stamp sand and non-stamp sand sites

(Fishers Exact Test N=16, p=1.00).

Table A.7: Macrophyte community diversity (Means ± SE) of Keweenaw Waterway, MI

Group	n	Biomass (g/m^2)	Species richness	Shannon's Diversity	Shannon's Evenness	Av. Coefficient of Conservatism
Stamp Sand	15	18.94 ± 7.36	3.00 ± 0.26	0.53 ± 0.11	0.45 ± 0.08	4.94 ± 0.75
Non-stamp Sand	14	16.06 ± 6.21	3.14 ± 0.36	0.53 ± 0.11	0.45 ± 0.07	5.57 ± 0.51
t-test		p = 0.831†	p = 0.750	p = 0.986	p = 0.999	p = 0.492

†: Natural log transformed to fit assumptions of normality

Community structure of macrophytes at sites was examined post-hoc by multidimensional multivariate analyses such as Non-metric Multidimensional Scaling (NMS) and Multiple Response Permutation Procedure (MRPP) in PC-ORD v6.20 (McCune & Mefford, 2011). A whole waterway ordination was resolved first to investigate the data. This ordination resolved from 116 iterations to a two-dimensional solution, stress of 20.40, and final instability < 0.00001 (Figure A.14). 70.3% of variance of structure was

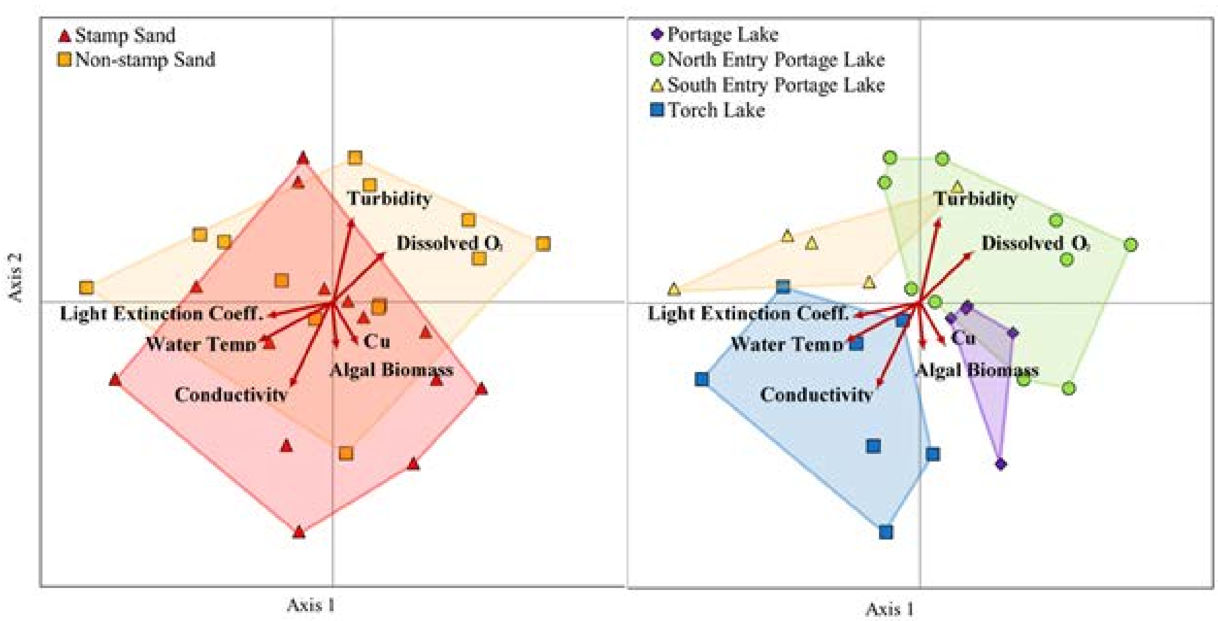


Figure A.14: NMS ordination of study sites depicting community structure of species composition in a two-dimensional solution grouped by site type (left) and waterway subdivisions (right). Environmental variables with high Pearson Correlations (R^2 > 0.200) are scaled by relative magnitudes.

captured by the ordination axes and MRPP of raw data showed a non-significant difference in community structure between sites with and without stamp sands, heterogeneity within groups was about equal to heterogeneity by chance ($p = 0.382$, $A = 0.001$). Waterbody classification of North Entry Portage Lake, Portage Lake, South Entry Portage Lake, and Torch Lake (Figure A.13) were significantly different by MRPP with heterogeneity within groups slightly less than chance ($p = 0.015$, $A = 0.0343$). Significant environmental correlations ($p < 0.05$) were water conductivity, water temperature, light extinction coefficient, turbidity, and optical dissolved oxygen (Figure A.14).

Because of the large differences observed in macrophyte communities among the different regions of the Keweenaw Waterway, we posited that local scale analysis of a subset of data would allow an analysis of a less heterogeneous species pool to detect measureable effects of stamp sands on macrophytes. The North Entry Portage Lake waterbody subset of sites was used as it had the highest sample size. Ordination of this local subset yielded a drastically different results than sites across the waterway, with clear separation between stamp sand and non-stamp sand sites for species compositions in ordination space ($p = 0.028$) (Figure A.15). *Potamogeton praelongus*, *P. gramineus*, *P. spirillus*, *P. richardsonii* and *Vallisneria americana* were significantly correlated with ordination axis. Stamp sand areas with increased sand and Cu, Mn, and Zn show a dominance of *Potamogeton gramineus*, and environmental variables with high Pearson Correlations (R2> 0.200) were metals (Cu, Zn, Mn, As, Cr), turbidity, percent sand of sediment (Sand), total phosphorus of water (TP), water pH, sediment Ash-Free Dry Mass (AFDM), and chlorophyll a (Chl a) scaled by relative magnitudes.

In conclusion, this study found that, although stamp sands deposits were mostly devoid of macrophytes, when present site type did not explain changes in community diversity, structure, or invasibility across all waterway subdivisions. Heavy metals and sediment texture did help explain changes of macrophyte communities on stamp sands at the scale of waterway subdivision, but not at the scale of the whole waterway, likely because of wider gradients in environmental conditions and plant communities among these sub-regions of the Keweenaw Waterway. In this waterway, historic disturbances may interact with species presence and propagation to create patterns in macrophyte community structure at different scales.

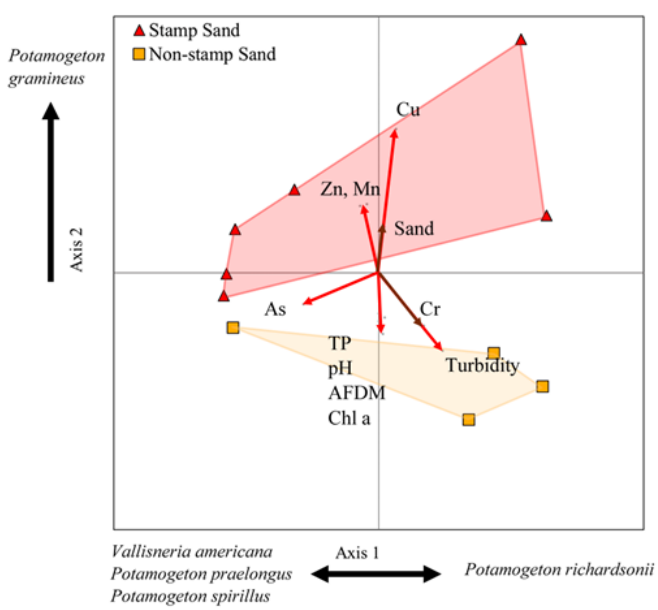


Figure A.15: NMDS ordination of study sites in the North Entry Portage Lake, MI depicting community structure of species composition in a two-dimensional solution grouped by site type.

The results of this analysis were presented at the 2016 summer meeting of the Association for the Sciences of Limnology and Oceanography in Santa Fe, New Mexico, as well as at the 2016 meeting of the Midwest Aquatic Plant Management Society in Grand Rapids, MI (see 3.4.3. Outcomes/Products below). We are also in the process of preparing a manuscript reporting these results for submission to the *Journal of Great Lakes Research.*

Task A.7.2. Effects of Eurasian watermilfoil and nutrients on algal assemblages - a mesocosm study

The capacity of *M. spicatum* to form dense surface mats also can have undesirable consequences for aquatic ecosystems including a decrease in sunlight available to native primary producers, such as macrophytes, attached algae, and phytoplankton, and reduced water movement, leading to lowered rates of surface-oxygen diffusion (Schultz and Dibble 2012). In addition, *M. spicatum* is capable of inhibiting growth of various algal taxa, particularly cyanobacteria, through allelopathy (Körner and Nicklisch 2002). Nutrient availability in a system can control the abundance and community composition of primary producers, including both native and invasive macrophytes as well as algal assemblages (Sand-Jensen and Borum 1991). Cyanobacteria, for example, prosper in the nutrient-rich conditions associated with eutrophication, but typically are a poor food source for grazing zooplankton as they are capable of producing toxins harmful to other algae and zooplankton (Carmichael 2001). Furthermore, *M. spicatum* flourishes in nutrient-rich conditions. Understanding the interaction between eutrophication and invasion of *M. spicatum* is vital because these stressors are expected to have opposite effects on algal assemblages, yet invasion of *M. spicatum* often is facilitated by nutrient loading.

To unravel interacting effects of non-native plants and nutrient enrichment on assemblages of primary producers, Jade Ortiz (BS Biology 2015), led an outdoor mesocosm experiment to simulate various nutrient loading and *M. spicatum* invasion scenarios in collaboration with this GLRI project and with additional support from the Summer Undergraduate Research Fellowship program at Michigan Tech. We tested two hypotheses concerning how these stressors could influence algal assemblages. First, we hypothesize that nutrient additions would alter biomass, productivity, and community composition of primary producers; we predicted that nutrient additions would lead to an increase in the biomass of all primary producers and in total primary productivity. Secondly, we hypothesized that *M. spicatum* presence would alter the biomass and composition of primary producers, but these changes would not affect overall primary productivity. We predict that the primary producer biomass would shift towards macrophytes and away from algae because macrophytes are good competitors for nutrients and light. We also predicted that the presence of *M. spicatum* will alter the community composition of algal assemblages to favor epiphytic species by creating habitat (i.e., attachment structure) while inhibiting the growth of cyanobacterial species.

We conducted a two-way factorial experiment to test effects of nutrient loading and presence of *M. spicatum* on algal assemblages. The experiment was conducted in twelve 1470-L 2-m diameter plastic stock tanks (Pride of the Farm, Houghton, Iowa) in the outdoor experimental mesocosm facility at the Great Lakes Research Center at Michigan Technological University, Houghton, Michigan, USA, during August 2014.Treatments were assigned randomly; 3 mesocosms received additions of high concentrations of nutrients relative to concentrations typically observed in the Keweenaw waterway, 3 received additions of potted M. spicatum, 3 received additions of both high nutrient and M. spicatum, and 3 served as controls without additions of *M. spicatum* or high nutrients. Mesocosms were situated under a 50% shade cloth, which was elevated 2 m above their surface. The experiment was conducted over 30 days (5 August – 4 September 2014).

Our results demonstrated that the presence of *M. spicatum* in conjunction with high concentrations of available nutrients can alter the composition, biomass, and productivity of aquatic algal communities. Concentrations of chlorophyll α, a proxy for biomass of algal communities,were consistently higher in high-nutrient treatments than the low-nutrient treatments until day 25 of the experiment, indicating algal communities were bolstered by nutrient additions. On day 30, phytoplankton-community biomass sharply declined in the high-nutrient treatment with M. spicatum, decreasing to 57-67% lower than the low-nutrient treatments (Figure A.16).

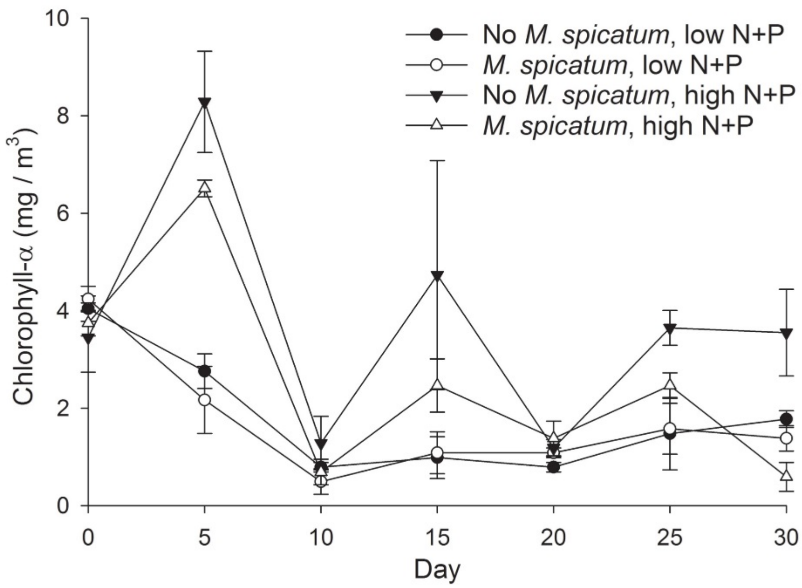


Figure A.16: Algal responses represented as concentrations of water column chlorophyll-α in 5-day increments throughout the duration of the 30-day experiment. Data points represent treatment means on that day ± standard error (SE).

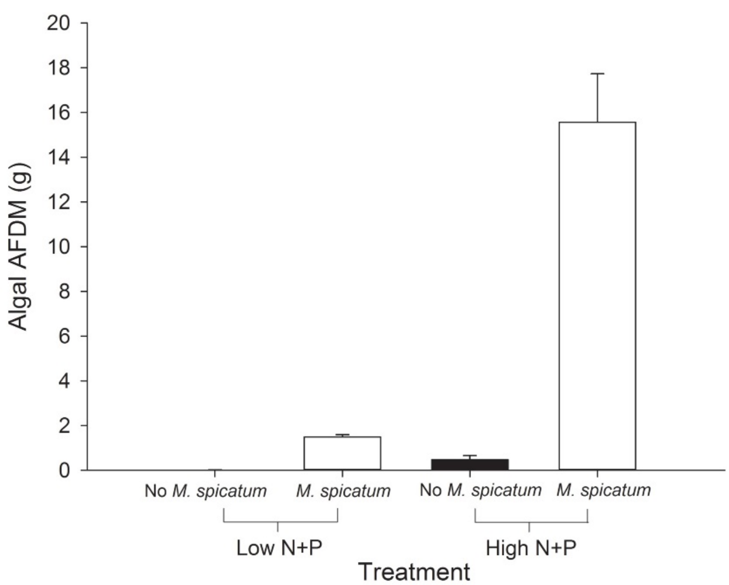


Figure A.17: Means ± standard error (SE) of final biomass of attached algae in each mesocosm treatment group measured as ash-free dry mass (AFDM) on experimental day 30.

We attribute this abrupt decline to nutrient competition between suspended and attached algae in those treatments. By the end of the 30-day experiment, biovolume of attached (e.g., *Cladophora* spp. and *Bulbochaete* spp.) was an average of 35× higher in mesocosm treatments containing *M. spicatum* than those that did not and an average of 11× higher in high-nutrient treatments as compared with low-nutrient treatments (Figure 3.17). Growth of attached algal taxa was facilitated by the surface area provided by the presence of *M. spicatum*, and was not observed in treatments without *M. spicatum*.

The large increases in attached algae in treatments with *M. spicatum* had striking consequences for system productivity. Dissolved-oxygen concentrations were 4-20% higher in the high-nutrient treatments until day 15; during the second half of the experiment DO declined in the high-nutrient treatments without M. spicatum, but remained high in the treatments with both high nutrients and *M. spicatum* (Figure A.18).

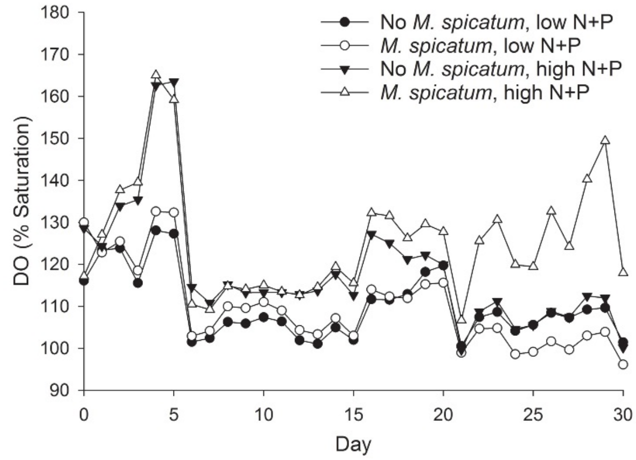


Figure A.18: Daily means ± standard error (SE) of dissolved oxygen reported as percent saturation in each mesocosm treatment group.

Although cyanobacterial species (e.g., *Anabaena* spp. and *Microcystis* spp.) composed 11-13% of the biomass in high-nutrient treatments without M. spicatum, those same taxa composed < 1% of the biovolume in those treatments with *M. spicatum*. An non-metric multidimensional scaling ordination of the algal communities over the experiment had a 2-dimensional solution with a cumulative r2 was 0.816 with axis 1 explaining the most variation (r^2 = 0.524) followed by axis 2 (r^2 = 0.291). The successional trajectories of the communities that received high-nutrient additions were divergent from those with low-nutrient additions. Movement in the ordination space indicates both communities moved along axis 1 as the populations of *Synedra* grew and *Cryptomonas* decreased, but with divergence on axis 2. The low-nutrient treatments exhibited a community increasingly dominated with *Oscillatoria* through time, whereas the high-nutrient treatment had less *Oscillatoria* and more *Cladophora* (Fig. A.19). The successional shifts in both treatments appear to correspond with increases in light transmittance and decreases in nutrients, conductivity, and dissolved oxygen (Fig. A.19). The high-nutrient community was more associated with an increase in pH through time than the communities in the low-nutrient treatment.

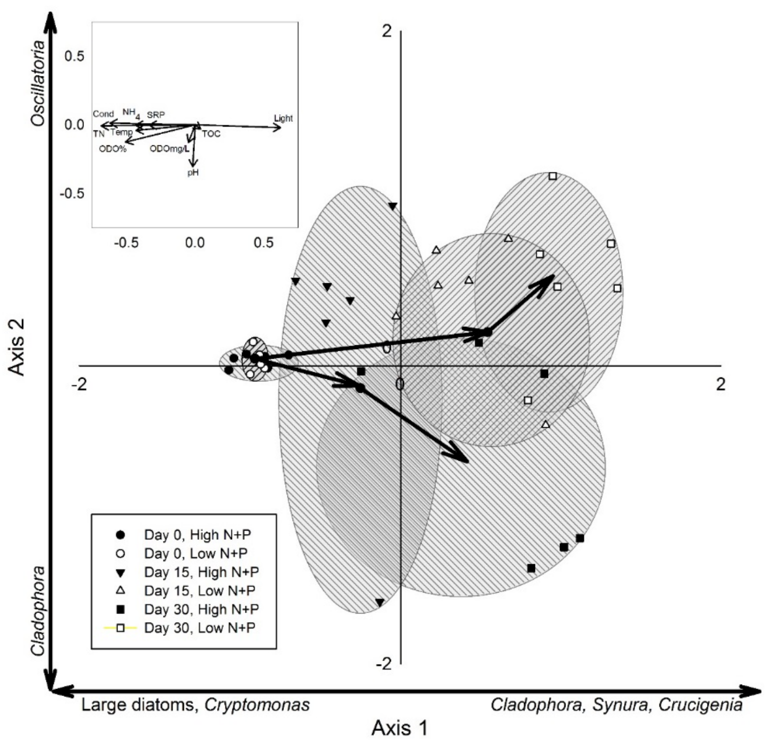


Figure A.19: Non-metric multidimensional scaling (NMDS) ordination of algal communities in high- and low-nutrient treatments through time. Ovals were added visually to cluster like treatment points by day.

This study emphasizes the importance of considering the interactive effects of stressors in order to better anticipate and manage ecosystem consequences of simultaneous species invasions and nutrient pollution. Moreover, it suggests that a key effect of EWM invasion on lake ecosystems may be by facilitating the growth of attached algae, particularly since EWM has a higher surface area than most of the native macrophytes that growth in regional lakes. The results of this research was presented at the 2015 meeting of the Society for Freshwater Science in Milwaukee, WI and a manuscript describing these results is currently in review at *Freshwater Science.*

Task A.7.3. Laboratory Trial of Efficacy and Specificity of (Mycoleptodiscus terrestris) fungus as biocontrol.

Other than herbicides, a range of management activities have been proposed to control EWM ranging from hand and mechanical harvesting (Boylen et al. 1996), to biocontrol using the native aquatic watermilfoil weevil (*Euhrychiopsis lecontei*; Creed and Sheldon 1995), to benthic barriers (Boylen et al. 1996), and replanting of native vegetation (Boustany 2003) to occupy open habit cleared by herbicide treatment or disturbance. However, most tests of these control methods treated them as single control measures, despite the fact that a common approach in restoration is to use combination of control treatments to combat invasive species (e.g. Creed and Sheldon 1995, Boylen et al. 1996, Boustany 2003). Multiple alternative measures to control EWM and been proposed and tried and often with variable degrees of success. (*Mycoleptodiscus terrestris* (Mt) is a pathogenic fungus that is suggested to be an effective control for invasive Eurasian watermilfoil (Nelson and Shearer 2005). The effects of Mt on native macrophytes are not well-studied nor is it well-known how Mt impacts the Eurasian watermilfoil x Northern watermilfoil (M. sibiricum) hybrid (Ellstrand and Schierenbeck 2006).

Figure A.20: Experimental array of native and invasive macrophytes planted in replicate jars of individuals of the same species.

Methods: We conducted a small-scale factorial laboratory experiment to study the effects of Mt on the hybrid, as well as four native macrophyte species: *Elodea canadensis*, *Potamogeton robbinsii*, *Myriophyllum heterophyllum*, and *Vallisneria Americana*. Plants were rinsed, measured, and weighed prior to planting in glass jars in monospecific groups of three and replicated nine times (Fig. A.20) Each jar received one of three randomly selected treatments: control (1 ml/L heat treated Mt inoculum), low dosage (0.5 ml/L of active Mt inoculum and 0.5 heat treated Mt), and high dosage (1 ml/L of active Mt inoculum). The Mt culture was produced by USDA lab and supplied to Dr. Ashley Moerke, Lake Superior State University who collaborated with us on this project. Heat treated Mt inoculum was autoclaved for ~1 hour and added to experimental jars not receiving live inoculum to account for additional nutrients in the solution that could affect growth. The condition of plants in each replicate was monitored weekly, and one individual was destructively sampled from each jar biweekly to track change in wet and dry biomass; treatment effects on growth were examined as difference in individual weight (W_t-W_0).

At the conclusion of the experiment after 6 weeks we detected no significant effects of Mt on the growth of native or hybrid macrophytes (Figure A.21, RMANOVA, $F_{2,30}$=1.09, P=0.35). However, the species did grow differentially (P<0.0001) and hybrid watermilfoil showed relatively high rate of mass increase during the experiment. Although we found no indications that Mt affected hybrid growth, the efficacy of the fungus is suggested to be highest when used as an integrated control with herbicide-based treatments (Nelson and Shearer 2005). In addition, larger scale studies are warranted to determine the effectiveness of Mt against the hybrid when applied alone and in combination with herbicides.

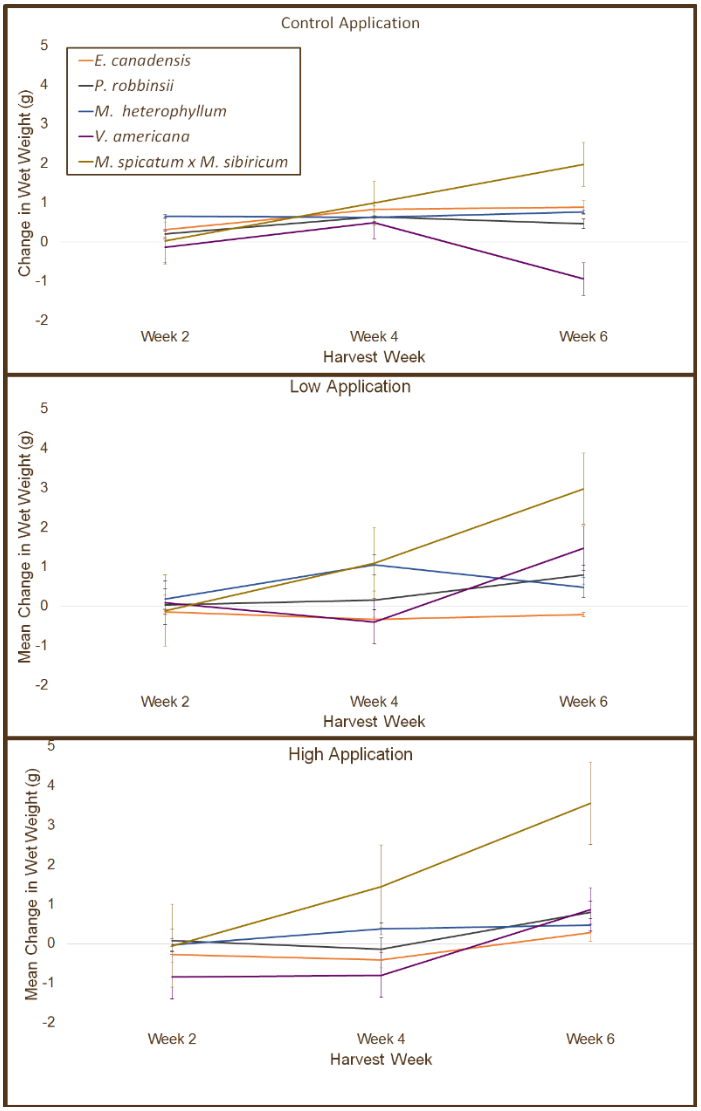


Figure A.21: Mean change in wet weight (g) with standard error bars for each destructive harvest of individuals from the a.) control (1.0 ml/L heat treated Mt inoculum), top; b.) low (0.5 ml/L of active Mt inoculum + 0.5 heat treated Mt), middle; and high dosage treatments (1 ml/L active Mt inoculum), bottom.

Task B. Remote sensing and mapping

Task B.1. Optical mapping

Task B.1.1. Problem Definition/Background

A regional assessment of the extent of EWM establishment and the effects of different control efforts within the upper Great Lakes is crucial for the development of a rigorous invasive species management plan. However, many shoreline communities in danger of EWM invasion lack the resources for a field-based aquatic vegetation monitoring program. High-resolution imagery provides a tool for monitoring EWM and other species of management interest across larger areas than would be feasible with traditional field monitoring, given that the target has spectral features or spatial patterns that make it distinguishable from other vegetation.

Through a previous US EPA Great Lakes Restoration Initiative grant project (Grant GL-00E00561-0), Michigan Tech Research Institute (MTRI) successfully mapped submerged aquatic vegetation (predominantly *Cladophora* algae) across most of the nearshore zone of the lower four Great Lakes using Landsat imagery, achieving an overall classification accuracy of > 83% (Brooks et al. 2015, Shuchman et al. 2013). This mapping was performed using a water-depth-invariant retrieval algorithm for bottom reflectance developed from a previously validated water column correction technique (Lyzenga 1978, 1981; Lyzenga et al. 2006). Given appropriate multispectral imagery and training data, these bottom reflectance index images can be processed to produce classified maps of lake bottom type (i.e., bare substrate, types of vegetation) based on differences in reflectance characteristics between the types. Here, we applied the Shuchman et al. method adapted from Lyzenga to very-high-resolution WorldView-2 8-band multispectral (MS) satellite imagery rather than Landsat data to perform more detailed classification using field-measured reference endmembers and attenuation coefficients. Due to the limitations of approaches based on currently available satellite imagery (spatial resolution, cloud cover, competing task requests), we also completed an initial effort demonstrating the suitability of imagery collected from unmanned aerial vehicles (UAVs) for mapping EWM extents.

Task B.1.2. Methods

Task B.1.2.1. Field Spectra Collection

In situ spectral data were collected in Torch Bay and Pike Bay in mid-June and mid-August, 2015, for use as ground truth in algorithm development and validation. GPS coordinates were associated with all measurements. Collection points were located along vegetation transects so that they could be associated with vegetation field data.

Figure B.1: Photo from Les Cheneaux Islands fieldwork of the hexacopter carrying an Ocean Optics STS spectrometer configuration to collect spectral profile data and imagery from a boat-based platform.

An ASD FieldSpec 3 full spectrum (UV/VNIR/SWIR) (Analytical Spectral Devices, Inc., Boulder, CO, USA) spectrometer was used to collect in situ measurements of surface reflectance above optically deep water and shallow water of different substrate types (submerged EWM and other common submerged macrophytes, bare sediment). Above-water spectra of the same aquatic macrophytes were also collected, immediately after removing the plants from the water. The Fieldspec 3 collects a continuous spectrum of 512 bands ranging from 350-2500 nm. The measurements were made with the instrument's bare fiber optic cable, which has a 25 degree field of view. For water and substrate measurements, the optic was held approximately 0.5 m above the water surface in a nadir viewing direction, producing an IFOV diameter on the water's surface of 22 cm. For above-water macrophyte spectra, a monospecific plant sample was arranged to cover 100% of the background surface. An 8 degree FOV foreoptic was attached and held at nadir approx. 20 cm from the target, producing an IFOV diameter of 3 cm. To capture the spectral variation within the sample, the foreoptic was moved slowly over the surface of the sample while recording spectra. Relative reflectance (the reflectance factor) was calculated in real time by the RS^3

software used to operate the FieldSpec 3 based on the downwelling irradiance measured from a Spectralon reference panel. Each recorded spectra was an average of 10 measurements, and 30 spectra were recorded for each target. Sky conditions were documented, and when cloud cover was present or conditions were otherwise sub-optimal, white reference measurements were made before and after spectral collection for each target.

Spectral measurements were also collected with Ocean Optics STS series modular spectrometers (Ocean Optics, Dunedin, FL, USA) mounted on a UAV platform (Figure B.1). These sensors have an optical resolution of 1.5 nm and a VNIR range from 350-1100 nm. Relative reflectance was recorded as the ratio between a skyward-pointing sensor with a cosine corrector foreoptic and nadir-pointing sensors. The flying height selected, 12 m, was the lowest height that did not cause the downwash from the UAV rotors to disturb the surface of the water. OceanOptics fibers also have a 25 degree FOV, for water surface projected IFOV with a diameter of 5.3 m. Due to this large spot size, we took care to inspect spectral collection points from a boat beforehand to confirm that we were capturing a large, fairly homogenous patch of substrate. Due to the limit on UAV flight time imposed by battery life, 9 spectra were recorded per point with 15 scans averaged per spectra.

Task B.1.2.2. Satellite-based Mapping

Task B.1.2.2.1. Torch Bay satellite methods and results

A WorldView-2 satellite image collected on September 4th, 2014 that covered Torch Bay provided the needed remote sensing data to demonstrate the capability to map areas of emergent and surface aquatic vegetation vs. subsurface aquatic vegetation. Relatively cloud-free satellite imagery of specific regions of the Great Lakes is fairly uncommon, and this image was the one collected closest to the project's fieldwork dates (primarily in 2015). Using aerial photographs and 2015 field data, it was possible to identify areas of surface and subsurface vegetation; differences in open

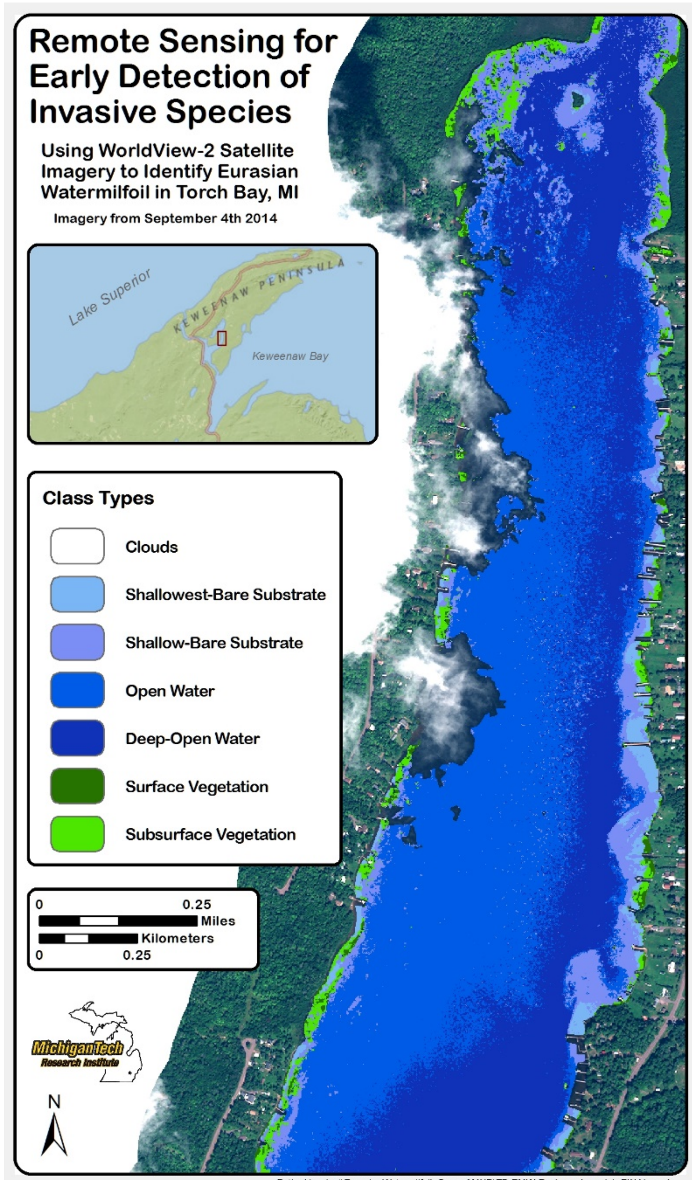


Figure B.2: Final map of submerged aquatic vegetation created for the Torch Bay project area using WorldView-2 satellite imagery.

water reflectance appeared correlated with shallow to deeper water (see Figure B.2.). A pixel-based supervised classification process was used to map the cover types shown in Figure B.2 using ENVI v.4.8 software. A cloud mask was created to remove areas of cloud cover from analysis, and the classification area was restricted to areas within the Torch Bay waters through manual digitizing of an image-specific lake boundary. This demonstrated that satellite imagery could be used as a screening tool for finding areas more likely to be EWM (the "subsurface vegetation" class), but species-level mapping did not appear possible with WorldView-2's spectral and spatial resolution given the strong masking of SAV spectral patterns by the continually tannin-stained water of the Keweenaw Waterway.

Task B.1.2.2.2. Les Cheneaux Satellite Methods and Results

Because the low clarity of the tannin-stained Keweenaw Waterway limits the utility of multispectral satellite imagery and sets it apart from many other areas of invasive EWM growth, we selected a secondary study area to evaluate the utility of such imagery in clear-water conditions. A GeoEye-1 commercial satellite image of the Les Cheneaux archipelago with four spectral bands (blue, 450-510 nm; green, 510-580 nm; red, 655-690 nm; NIR, 780-920 nm; 1.84 m resolution) and a finer panchromatic band (450-800 nm, 0.46 m resolution) was acquired on 12 July 2012 and used for the project. This coincides with a season of very high EWM growth in LCI, with a floating vegetation mat of over 400 acres and a much larger area of submerged beds. The total GeoEye-1 image area is 337 km^2 with 7.3% cloud cover. The water level of the study area at the collection time was 176.0 m (IGLD 85), approximately 0.6 m below the long-term July average for Lakes Michigan-Huron. The image processing approach was similar to that taken for the Keweenaw Waterway, but in the absence of ground truth data, we used an unsupervised classification method to map spectrally separable lake bottom types, and each

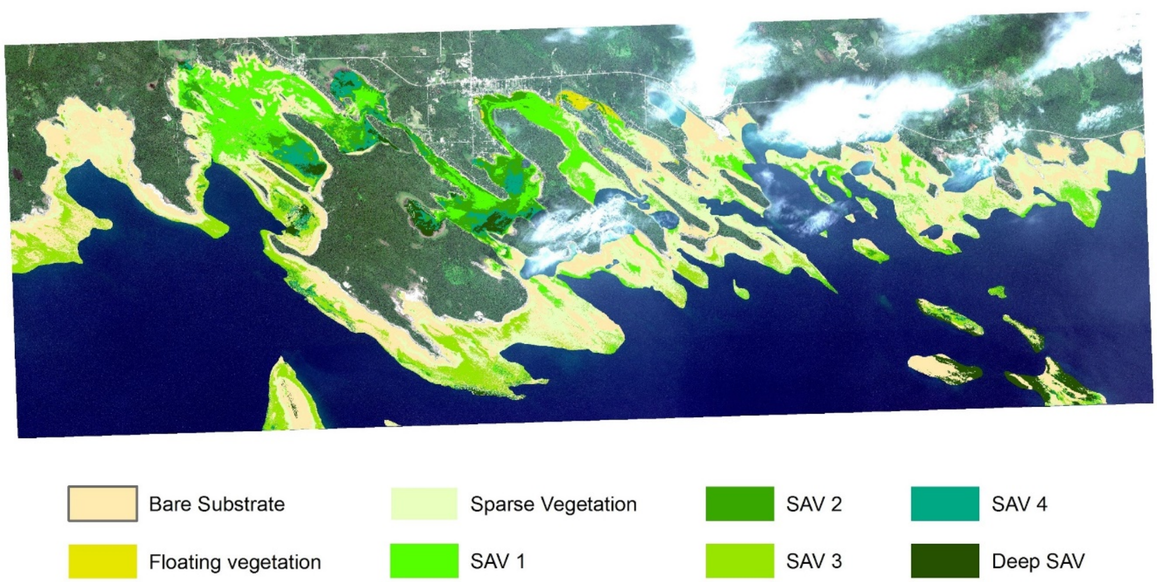


Figure B.3: Classified 2012 map of spectrally distinct submerged aquatic vegetation (SAV) types in the Les Cheneaux Islands archipelago in northern Lake Huron. On the ground observations indicate that classes SAV 1 and SAV 2 correspond to mixed SAV species with a significant EWM component and a dense near-monoculture of EWM, respectively.

type was assigned a probable identity based on trained image interpretation. In the initial classification, an issue arose where some single classes appeared to correspond to one bottom type on the west side of the large image and a different type on the east side due to differences in viewing geometry. This issue was resolved in the final classification by applying a regionally tuned dark pixel subtraction method. The final classification included floating vegetation, four distinct types of submerged aquatic vegetation cover, a mixed “deep water/dark SAV” class, sparse SAV, and bare substrate (Figure B.3). Comparing this map to the Les Cheneaux Watershed Council’s observations from 2012, the SAV 1 and SAV 2 classes are likely dominated by EWM, especially SAV 2. These results support the potential of satellite imagery for predicting the extent of EWM in new areas where extensive field validation data are unavailable.

Task B.1.2.3. Aerial Mapping with Unmanned Platforms

Given the brief time periods of maximum EWM visibility when imagery collection is ideal, imaging techniques that could be rapidly deployed when needed, such as during periods of rapid EWM growth and/or during periods of clearer water, would be of great utility, as well as potentially being more flexible and cost-effective when surveying for small, newly established populations. An aerial platform also mitigates some of the drawbacks of using commercial satellites for invasive species applications, e.g., uncertainty of image acquisition due to competing satellite tasking demands from a large group of worldwide users, the high frequency of cloud cover in the region, and the cost of commercial imagery.

For this project, we demonstrated the collection of field spectra and aerial photos of submerged aquatic vegetation using an unmanned aerial vehicle platform in August 2015. A small quadcopter (a DJI Phantom 2 Vision with 12 mp camera) was used to collect initial aerial photos of areas with SAV visible from the side of the research vessel to evaluate the utility of aerial-based imaging to identify SAV over larger areas. Figure B.4 shows how the quadcopter could successfully collect imagery with visible areas of SAV, as well as a photo of its operations, taken at the Pike Bay study area.

Figure B.4: Example of the type of imagery able to be collected via a small quadcopter to find areas of SAV (left) with a field photo of the UAV being flown (right); the ability to identify areas of SAV was confirmed through these initial data collections.

Figure B.5: Example of nadir-view image taken over an area of predominantly EWM in the Pike Bay area.

To follow up on this initial effort, a hardware configuration including the two Ocean Optics spectrometers described above, a small camera, and a GPS receiver were flown from a larger Bergen hexacopter to obtain spectral profiles of areas visually confirmed as largely dominated by one species of SAV and to photograph the area being profiled. Figure B.5 shows an example of one of the nadir-viewing images that were taken; these images were used to create example classifications of the main aquatic cover types that could be seen in the images.

Four examples of the 5-mp natural color images like the one in Figure B.5 were imported into the eCognition object-based image classification software to show how centimeter-scale maps of dominant aquatic vegetation could be created. A field visit to this area of Pike Bay had confirmed that EMW was the predominant aquatic vegetation type visible near the water's surface. Figure B.6 is an example of part of the Fig. B.5 image run through the standard eCognition object-based supervised classification routine. Reference areas of known bottom types were manually delineated in each image as the basis for the classification. The resulting polygons show areas dominated by EWM (green), areas that are predominantly open water (blue), and areas of vegetation with lower reflectance in deeper water that are likely to be

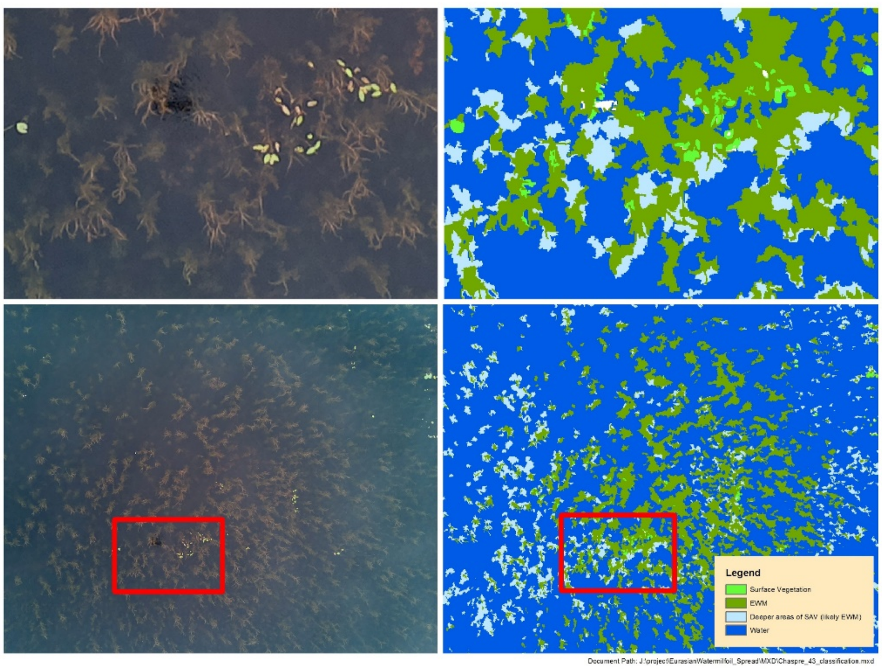


Figure B.6: Classification mapping results for the same image area of Figure X2 where dark green = EWM, bright green = surface vegetation, blue = water, and grey = deeper areas of SAV that are likely EWM.

EWM (grey). These mapping results showed that high-resolution UAV-based imagery could be used to identify areas of SAV, potentially down to the species level for at least areas dominated primarily by one species. With the spectral profiles showing that different SAV species had distinct spectral profiles, we concluded that multispectral UAV-based mapping would be a promising tool for performing this kind of SAV mapping for larger areas as part of field inventory and monitoring programs for invasive aquatic plants.

Figure B.7: The hexacopter UAV platform with Ocean Optics-based LPR system mounted, ready for data collection over water. The small UAV launch/landing platform attached to the research vessel, first-person viewer screen, and UAV controller can also be seen.

Figure B.7 shows the hexacopter platform that was used to collect the Ocean Optics data and accompanying nadir images. This provided a steady, heavier lift platform that could deploy the Ocean Optics hardware at heights of approximately 12-15 m while also collecting natural color imagery. It was also a test for its capability as a platform for planned future imaging with a tunable multispectral camera.

Figure B.8 provides an example of spectral profile data collected for aquatic macrophytes that were taken out of the water to determine if they have distinct reflectance profiles. These data showed that EWM could have spectral profiles different than other SAV species (such as Elodea, leafy pondweed, large-leaf pondweed, spatterdock, and others). The widths of the spectral bands captured by WorldView-2 are also represented.

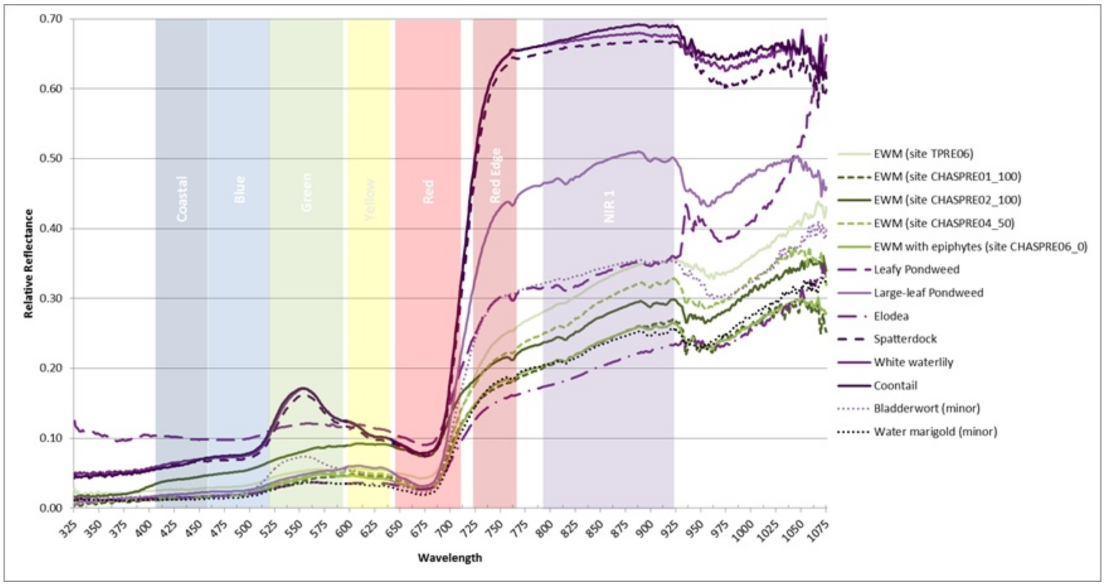


Figure B.8: Example of spectral reflectance profiles measured with an ASD spectroradiometer for macrophytes taken out of the water, demonstrating that species had different reflectance characteristics.

When collecting spectral profile data from the side of the research vessel, at approximately 0.5 m above the water's surface, it was also possible to collect distinct spectral profiles for bottom areas dominated by different species (see Figure B.9). Together, these "out of water" and "in water" spectra showed that it should be possible to map EWM vs. other macrophytes using airborne imaging platforms that could collect the needed spectral bands. An additional step in this process was collecting spectral profile data from a height representing a UAV-based mapping platform. Figure B.10 shows spectral profiles collected via the Lightweight Portable Radiometer (LPR) platform that used the two Ocean Optics sensors to profile three macrophytes. The blue vertical lines show the blue to red edge range of visible light that can be expected to be useful for mapping submerged aquatic vegetation. EWM (in red) appears different from large leaf pondweed and white water lilies, particularly in the 500-600 nm range.

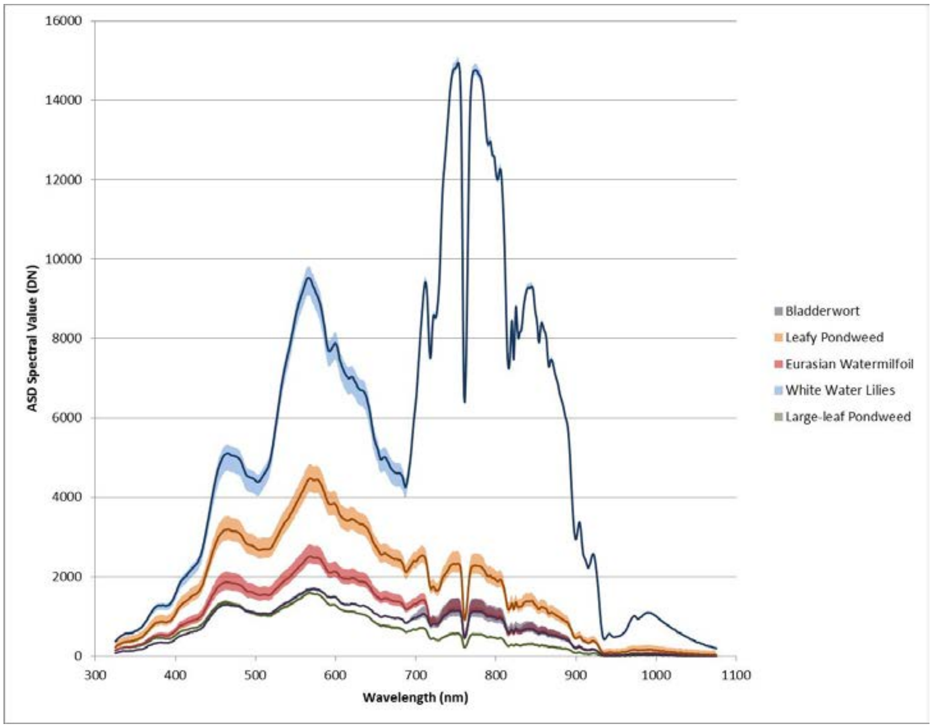


Figure B.9: In-water spectral profiles collected over areas dominated by different macrophytes also showing different spectral profiles by species.

Task B.1.3. Major Conclusions

A major conclusion was that satellite imagery could serve as a good screening tool for identifying areas of surface vs. submerged aquatic vegetation, with the SAV areas potentially serving as a tool for predicting where EWM would be more likely to occur. Another conclusion was that UAV-based imagery could provide a method for mapping SAV species such as EWM at high resolution, with the UAVs able to be deployed from a small research vessel. A third conclusion was that the aquatic plants sampled did have distinct spectral profiles. These profiles could be identified in spectroradiometer data collected from plants removed from the water, of plants still in the water from the side of the research vessel, and via a

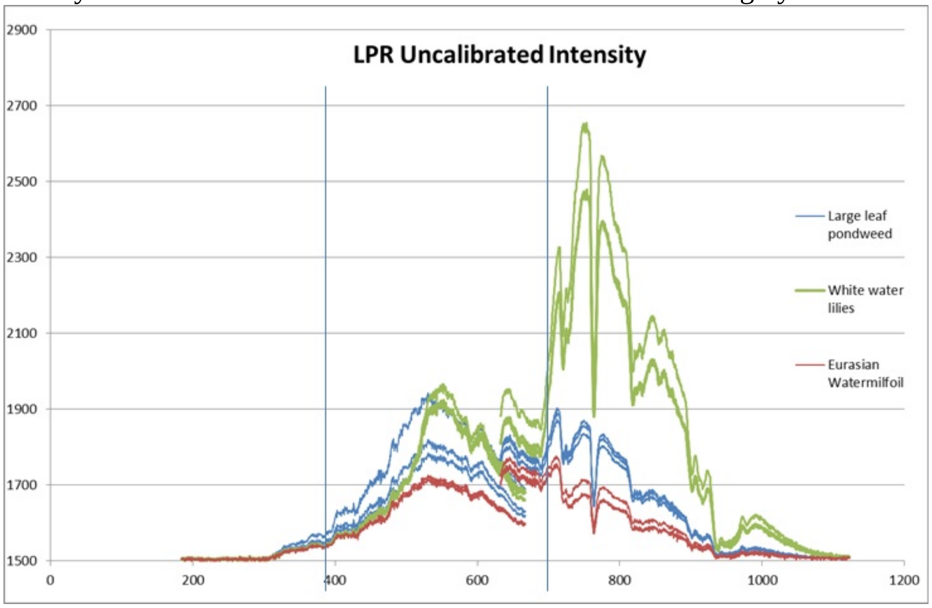


Figure B.10: Spectral profiles of macrophytes collected from the hexacopter platform using the Ocean Optics spectrometers.

UAV-based light-weight portable radiometer system. Together, the satellite imagery, UAV-based based imagery, and spectral profile data provide another tool for mapping the extent of EWM and other SAV species, and could be used for monitoring areas undergoing treatment.

Task B.1.4. Constraints

The satellite, UAV, and spectroradiometer remote sensing tools did reveal several constraints that make their successful deployment dependent on careful data collection. Water clarity is one constraint, where large amounts of sediment, chlorophyll, or color dissolved organic matter can make it difficult to see into the water. Waters in Torch Bay had more sediment in the water on the days that the UAVs were being flown, with clearer waters in Pike Bay proving more amenable to UAV-based sensing. For satellite-based sensing, frequent cloud cover in the Great Lakes can be an issue, and commercial satellites can be tasked to other priorities, making availability an issue. With more sensors being launched (such as the WorldView-3 satellite), this is less of an issue than it has been, but should still be considered. With EWM hybridizing with native northern milfoil, it is probably not possible to distinguish EWM from its hybridized form using multispectral imagery, but a larger concern may be identifying northern watermilfoil from EWM. The opportunity to compare the spectral profiles of both species was not available through this project but should be evaluated in future efforts.

Task B.2. Sonar

Task B.2.1. Problem Definition/Background

This project compares direct measures of aquatic macrophyte biomass with ultra high resolution Side Scan Sonar (SSS) acoustic data to test the efficacy of remotely sensing and differentiating macrophyte species. Initially, SSS acoustic data used was collected in surveys conducted in 2014 and 2015 along areas under treatment for EWM in both Pike Bay and Torch Bay using an Ultra-high resolution EdgeTech (4125), dual frequency Side Scan Sonar. This effort was then also extended to 2016 to capture an additional year of data and analysis. If the biomass and acoustic data dictate that this is an effective approach to discriminating vegetation textures, advances could be made in remote monitoring the advancement or disappearance of a selected species from the waterways.

A multi-step analysis was conducted in order to determine if a EWM texture can be identified and differentiated from other aquatic macrophytes in the remote sensing acoustic data (RSAD). This analysis works towards answering the following questions:

1. What was the total area acoustic remotely surveyed within Pike Bay and Torch Bay, respectively?

2. In the resulting RSAD for each location, is it possible to identify and isolate vegetation textures, thereby finding the estimated total area of vegetation within the survey area?

3. Is it possible for EWM to be isolated by its texture from all other identified vegetation? If so, can this texture be qualitatively and quantitatively tested for accuracy, allowing for its duplication and application in other EWM based projects?

Task B.2.2. Methods

Data Source and Organization: The side-scan sonar surveys were conducted using an Ultra-high resolution EdgeTech (4125) Side Scan Sonar deployed from our 22-foot long S/V Polar survey vessel. Acoustic data used was collected in surveys conducted in 2014 and 2015 along areas planned for or under treatment for EWM in both Pike Bay and Torch Bay. This effort was then also extended to 2016 in Pike Bay to capture an additional year of data and analysis. The 2014 and 2015 data will be reported on first, with the 2016 data and analysis following. In addition, two sets of aquatic macrohpyte biomass data were collected through twist rake surveys in both locations, around where EWM grew in abundance. Summary Maps for all four surveys can be found in Appendix B.

The first Twist Rake Data (TRD), in week 0 of 2014, recorded the amount of vegetation in the region prior to herbicide treatment. The second TRD, in week 6, recorded the amount of vegetation in the region after the herbicide treatment. The same process for TRD acquisition was conducted in 2015, giving data for weeks 52 and 58, pre and post treatment respectively. The RSAD was collected near the same date the week 0 TRD was obtained, and therefore the analysis was performed using only the week 0 TRD. An additional third field season in 2016, provided the opportunity to conduct two additional missions in Pike Bay with the IVER3, fully autonomous underwater vehicle (AUV) equipped with the advanced EdgeTech 2205 interferometric SSS system in addition to the use of the conventional Side Scan Sonar, providing multiple sets of RSAD.

Task B.2.3. Analysis

Geospatial analysis was done using ArcMap10.2.2 and then later ArcMap10.3.1, in the WGS84, UTM Zone 16N datum. Using ModelBuilder, each RSAD was reclassified, converted from a Raster to a Polygon, and then dissolved. The dissolved RSAD were then Merged and Unioned separately. Merging allowed the Total Survey Area to be found, yet areas of overlap existed, causing a double count to occur when calculating the Total Survey Area. Performing a Union on the dissolved images created polygon segments within the overlapped regions, giving the actual Total Survey Area. Most vegetation has unique architecture based on leaves or leaflets and branching pattern, and with growth pattern ranging from emergent, to low growing and prostrate. For RSAD this knowledge tells us to look for identifying shadows and that the returns will exhibit certain behaviors. In the case of vegetation that is emergent, it could be assumed that the RSAD returns for this type will have taller or thicker shadows. Additionally, knowing the RSAD texture of unvegetated substrate helped to isolate those textures which are different from vegetation. Joining the RSAD with the TRD was performed using ArcMap by importing individual TRD Excel Sheets using the Excel to Table Conversion Tool. Once added, these Standalone Attribute Tables were joined to their respective spatial points in their geodatabases. Spatial points could only be utilized in the analysis if they intersected the RSAD. Of the total available data from 2014 and 2015, only Pike Bay 2015 had almost all of the RSAD intersecting with the TRD spatial points, as well as the most numerous points containing EWMCT vegetation values greater than zero. This can be seen in Figure B.11.

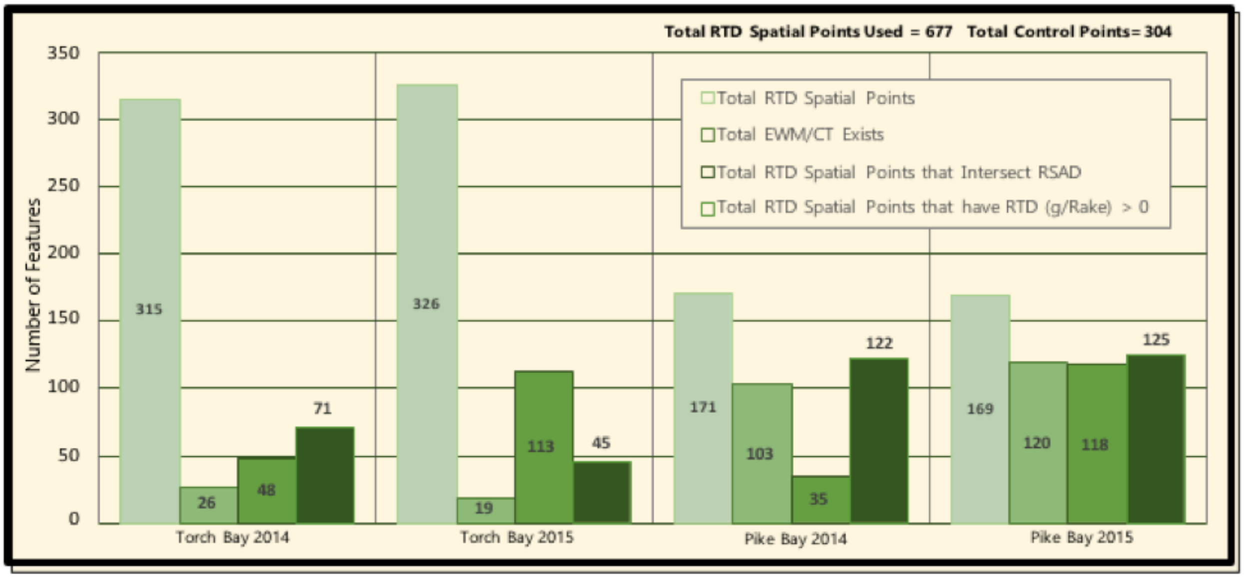


Figure B.11. Summary of RTD spatial points with respect to RSAD and vegetation by survey location and year.

Using data available for Pike Bay 2014 we developed an initial description of how EWM appears within the RSAD. The RSAD texture shown below, the texture around EWM differs from both the areas without vegetation and other areas with different vegetation (Figure B.12). Each time this texture was observed, there were EWM values greater than zero. Therefore, it was concluded that the regions identified as having EWM displayed an elongated and shadowed texture.

Figure B12 overlapped RSAD and macrophyte sampling points along transect 16 in Pike Bay 2014 indicating EWM presence (green) and absence (red) matching texture and pattern in sonar returns. This elongated nature can be seen in both RSAD returns shown above, with the latter more clearly featuring the consistent shadowed, elongated texture, and the transition from EWM to other vegetation types.

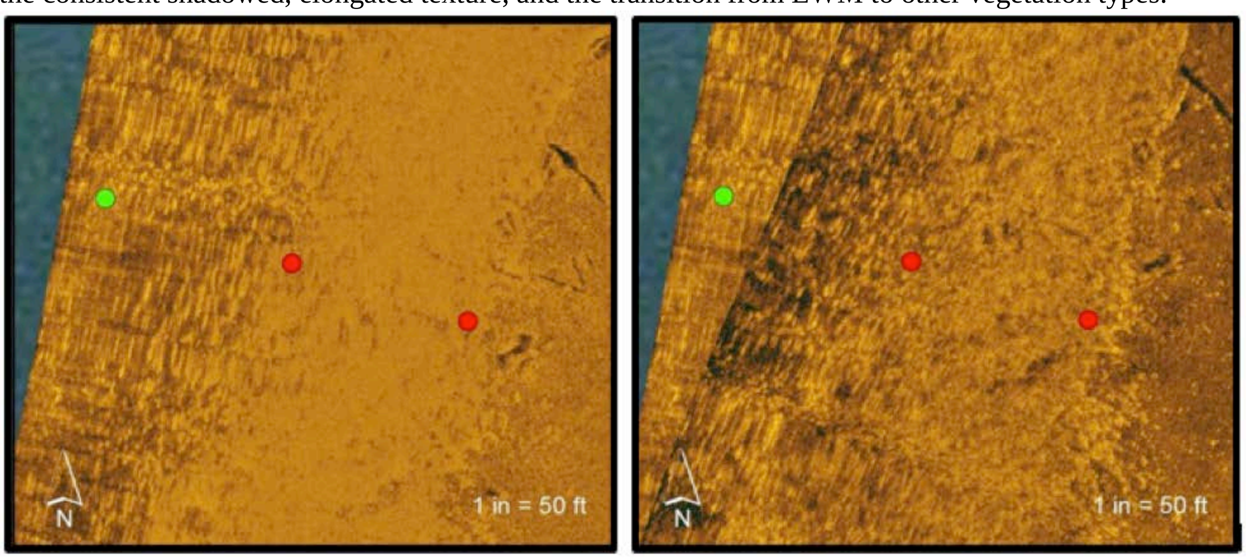


Figure B.12. Pike Bay 2014 - Transect 16 Indicating EWM Existence (Green) using overlapping RSAD.

Task B.2.4. Outcomes/Products

As discussed in the previous section, the total survey area was determined for each year using Unioned polygons created from the RSAD. For each of the three years in which surveys were conducted, it was possible to identify areas within the RSAD that were composed of vegetation based on texture. These areas that were determined to have vegetation were assigned polygons that were then used to determine the total area of vegetation. Tables B.1 and B.2 below show the total areas of acoustic remote surveys, site location and year.

Table B.1. Total Survey and Vegetation Area for Pike Bay

2014	Total Survey Area	289,618 m^2
		0.3 km^2
	Total Vegetation Area	64,924 m^2
		0.1 km^2
2015	Total Survey Area	1,228220 m^2
		1.2 km^2
	Total Vegetation Area	296,106 m^2
		0.3 km^2
2016	Total Survey Area	487,047 m^2
		0.5 km^2
	Total Vegetation Area	82,391 m^2
		0.1 km^2

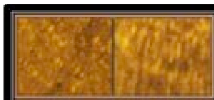
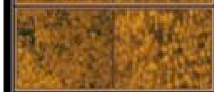
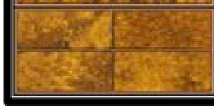
Table B.2. Total Survey and Vegetation Area for Torch Lake

2014	Total Survey Area	1,745,248 m^2
		1.7 km^2
	Total Vegetation Area	478,076 m^2
		0.5 km^2
2015	Total Survey Area	2,025,575 m^2
		2.0 km^2
	Total Vegetation Area	729,394 m^2
		1.7 km^2

Acoustic Signatures

In comparison to EWM, CT is not an emergent species, and therefore returns for this species should not indicate the same height as EWM. As this was the case, areas with a greater CT presence saw a shift from the apparent elongated pattern in denser EWM regions to a more rounded or circular pattern with fewer dense shadows. Despite these initial textural differences, a distinction could still be made between returns showing EWM and CT coexistence versus other vegetation, giving rise to a basic assumption about the texture to be expected with EWMCT existence. This texture, dubbed the Testing Hypothesis (TH) Texture, is described in Table B.3 below.

Table B.3. TH Texture Defined using RSAD for Example Textures

	EWM is Elongated
	CT is Circular
	EWMCT have Fluffy, Dense and Shadowed Textures
	Non EWMCT Texture

The following SSS images (Figures B.13-B.17) provide examples of the various acoustic signatures associated with a variety of vegetation types and combinations.

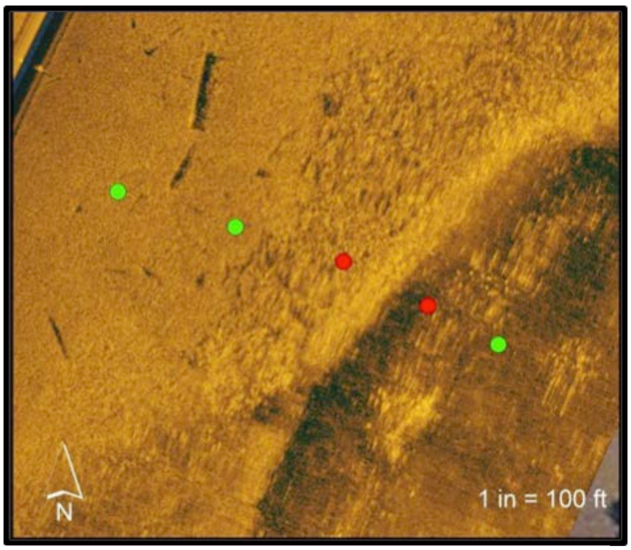


Figure B.13. Torch Bay 2014 - Transect 17 Indicating Incorrectly (Red) and Correctly (Green) Identified Features.

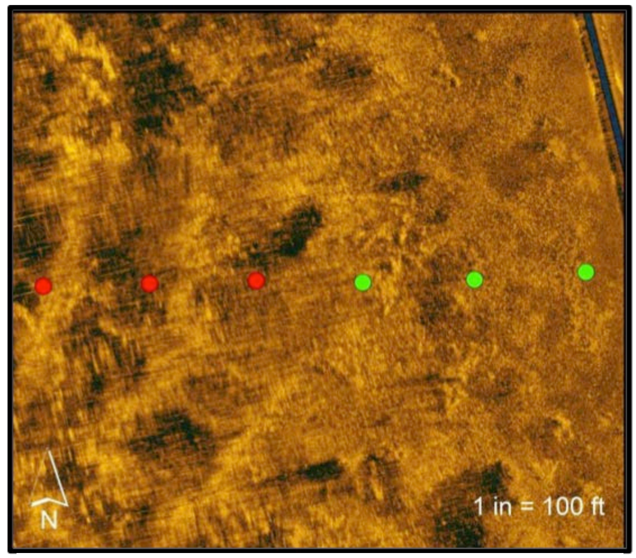


Figure B.14. Torch Bay 2015 - Transect 10 Indicating CT Existence (Green)

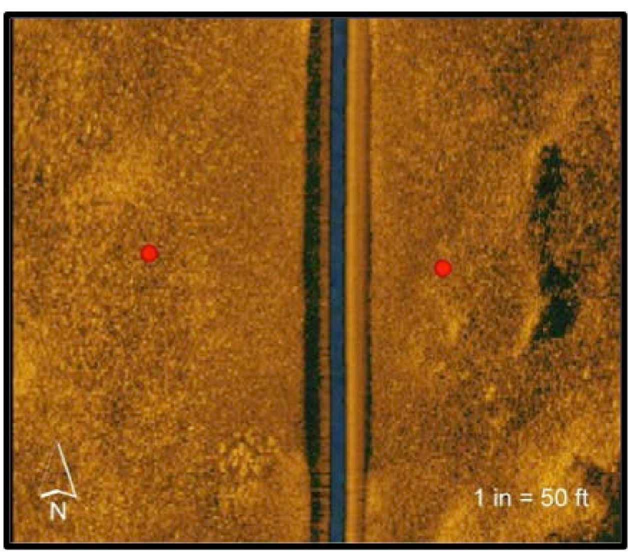


Figure B.15. Torch Bay 2015 - Transect 9 Indicating No EWMCT Existence (Red)

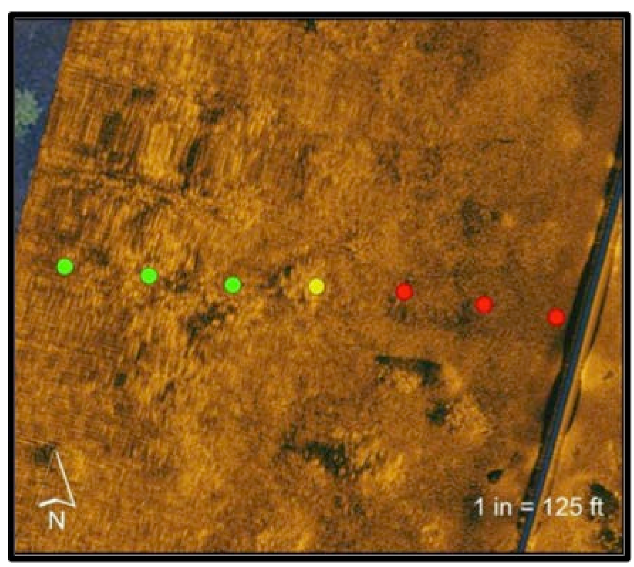


Figure B.16. Torch Bay 2015 - Transect 8 Indicating EWMCT Existence (Green) and TH Score of 0.5 (Yellow)

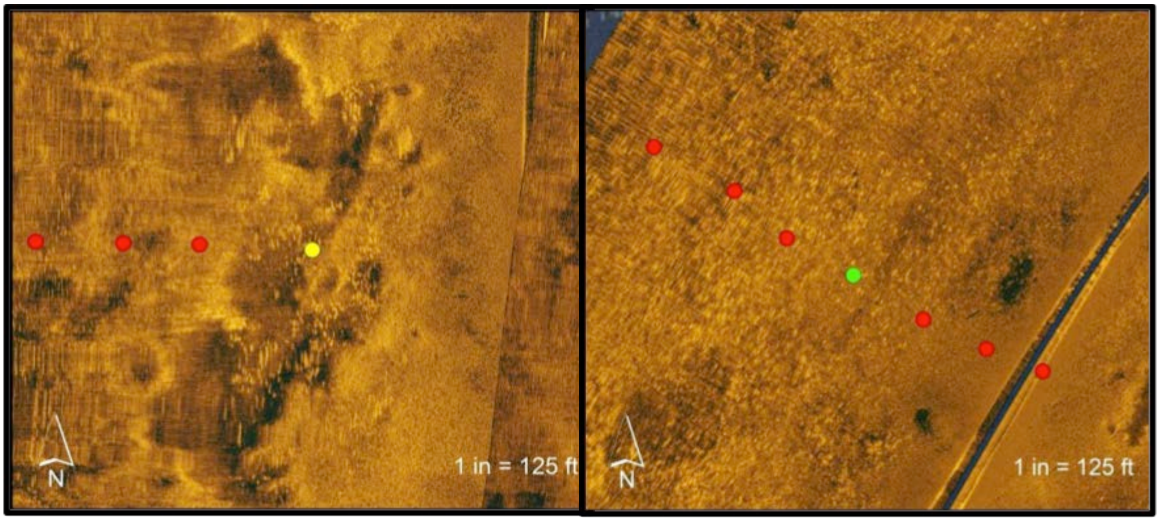


Figure B.167 Torch Bay 2014 - Transect 9 (Left) Indicating Feature with Eelgrass Existence (Yellow); Torch Bay 2015 - Transect 5 (Right) Indicating Feature with EWM existence (Green)

Task B.2.5. Major Conclusions

It is possible to determine the total area surveyed in an RSAD return as well as how much of that area contains vegetation. At this point, the ability to identify EWM textures in RSAD has been marginally successful. Furthermore, a defined EWMCT acoustic texture was isolated and studied through the creation of the Testing Hypothesis (TH) Texture and associated TH Score. Evaluation of the accuracy of this analysis was possible through the creation of the Overall Evaluation Score. While the lack of a large number of comparative sample points hindered the robustness of this analysis, some progress was made in isolating the texture EWMCT exhibited in RSAD. Through further identification of the TH Texture, it was discovered that a set of valid RSAD returns can be used to isolate EWM and/or CT. It is believed that with a greater number of RTD spatial points and valid RSAD returns, the ability to fine-tune this TH Texture is possible. In this way, not only will the overall accuracy of this analysis increase, but also the difficulties in over-identifying this TH Texture can be possibly reduced or resolved.

Task B.2.6. Constraints

A low number of usable RTD spatial points in 2014 and 2015 and the poor quality of some RSAD returns were the two main constraints on this analysis. There were no RTD spatial points to compare with the RSAD analysis in Pike Bay in 2016.

For Torch Bay 2014, there were a total of 315 spatial points, 147 of which were Control Points. Torch Bay 2015 had 157 Control Points out of the 326 total RTD spatial points. Pike Bay did not have any Control Points, which meant that out of 981 RTD spatial points only 677 could be used in the analysis. Unfortunately, most of the RSAD returns in these surveys had too many artifacts, hindering the analysis. For example, Torch Bay had fewer than 100 of the RTD spatial points which intersected the RSAD, despite there being a greater number of collected points, and even fewer of them had EWM or CT. This highlights the importance of having enough sample points, but also that the quality of the RSAD is crucial to conducting texture analysis. Essentially, the fact that RTD spatial points exist and might overlap the RSAD does not mean they are necessarily useful to analysis.

In the actual analysis conducted in Torch Bay 2014 and 2015, there were a total of 263 points analyzed, however only 40 RTD spatial points had values greater than zero for EWMCT. This meant that 84%, or 223 of the total features, were not used in the analysis. That being said, 11% or 29 features were not accessible due to either being repeats or not intersecting the RSAD.

Task C. Hydrodynamic Modeling

Task C.1. Problem Definition/Background

Because removal of EWM is difficult and costly once it is established, prevention is the best management approach (Parkinson et al. 2011). EWM spreads primarily by fragmentation, where the plant breaks apart naturally (auto-fragmentation) or by external forces (i.e., by waves and currents), and a single small EWM fragment can grow into a full plant. Once a fragment reaches a new habitat, it must encounter suitable environmental conditions to grow and invade. Therefore, understanding the invasion process and predicting areas of future *M. spicatum* invasions requires integrating knowledge of the biological and ecological constraints on establishment with hydrodynamic and anthropogenic transport mechanisms.

New, next generation, numerical hydrodynamic FVCOM models of Lake Superior and coupled Lakes Michigan-Huron were collaboratively under further development at both Michigan Tech and at NOAA/GLERL. Working FVCOM models are operating in both locations at previously unprecedented resolution in both the horizontal and vertical. Michigan Tech has invested heavily in creating this next generation of Great Lakes predictive capability by providing for both a new supercomputer, *"Superior"* within the GLRC and by investing in new faculty to further the creation of these models. It is hypothesized that the unique, naturally restricted circulation through the Les Cheneaux Islands is a critical component to the prolific success of EWM in that region. Should this hypothesis be true, similar, not yet affected regions of the upper Great Lakes may also be identified using these tools based upon their respective flow regimes under a variety of natural forcings. Similarly, knowledge of residence time, water temperature and flow through the region is critically important for the efficient and effective application of herbicides and anticipating future changes in management in response to regional climate change. These models will also be useful in identifying the times of proper application and dispersal.

As an example of this existing new capability, Figure C.1 provides a sample of the resolution achievable within Lake Superior and the Keweenaw Waterway. Isle Royale (National Park Service) and the Apostle Islands of Lake Superior are clearly other regions of significant concern with respect to EWM. Similar resolution is achievable in these additional areas as is indicated below for the Keweenaw Waterway.

To help address above questions, we have applied Lake Michigan-Huron and Lake Superior hydrodynamic models with locally refined ultra high model resolution of 50~100 m in the regions of interest: i.e. Les Cheneaux island complex of Lake Huron, Isle Royale (National Park Service) and the Apostle Islands of Lake Superior. In our model configuration, the lakewide model simulation allows for resolving the background circulation pattern, combined with the local ultra high-resolution simulation for the detailed hydrodynamic conditions in the regions of EWM concern. The simulations were conducted for late spring (May) to late fall (November) for year 2011 as case studies after one month model spin-up (April) with a focus on the circulation and thermal structure during the summer seasons in the regions of EWM concern. Using the lake-wide model, we were able to predict dispersal paths, propensities of the EWM fragments towards other coastal regions within Lake Superior. Figures C2 and C3 provide the high-resolution model mesh grids that accurately resolve the details of the coastal complexities.

To make model simulation as accurate as possible, we used realistic meteorological forcing to drive hydrodynamic model. The surface boundary condition of FVCOM consists of momentum and heat flux at each surface mesh element. The momentum and heat fluxes are calculated internally in FVCOM using surface meteorological data from the Climate Forecast System Reanalysis (CFSR, NCAR 2015) and the internally calculated water temperature. As a global, high resolution, coupled atmosphere-ocean-land surface-sea ice system, the CFSR was designed to provide the best estimate of the state of these coupled domains by including (1) coupling of atmosphere and ocean during the generation of the 6 hour guess field, (2) an interactive sea-ice model, and (3) assimilation of satellite radiances by the Grid-point Statistical Interpolation scheme and all available conventional and satellite observations. Preliminary analysis of the CFSR output indicates a product far superior in most respects to the reanalysis of the mid-1990s (Saha et al. 2010)

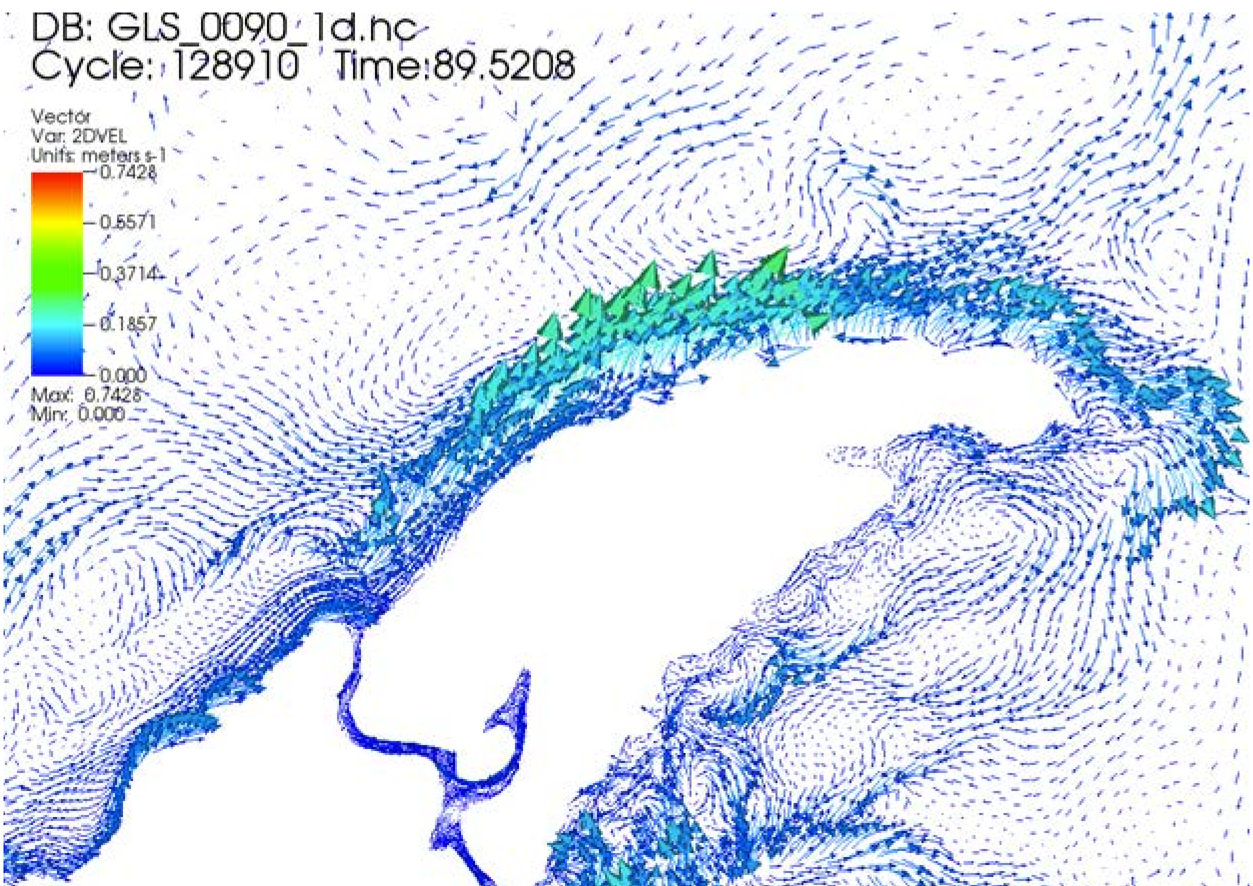


Figure C.1. Finite Volume Coastal Ocean Model (FVCOM) simulated water surface flow vectors for a standard wind forcing event

The CFSR meteorological output is used to as the driving forcing for Lake Superior FVCOM model. The CFSR forcing is retrieved at hourly temporal resolution and ~20 km horizontal resolution from the gridded reanalysis data. The hydrodynamic simulation driven by CFSR forcing for Lake Superior and Lake Michigan-Huron was shown to provide robust simulations of the circulation patterns and thermal structure, mainly due to its accurate representation of wind pattern and radiation fields (Xue et al. 2015). With a focus on spatial and temporal estimates of EMW dispersal, the model is applied to Lake Superior for the ice-free seasons. The model configuration follows Xue et al. (2015).

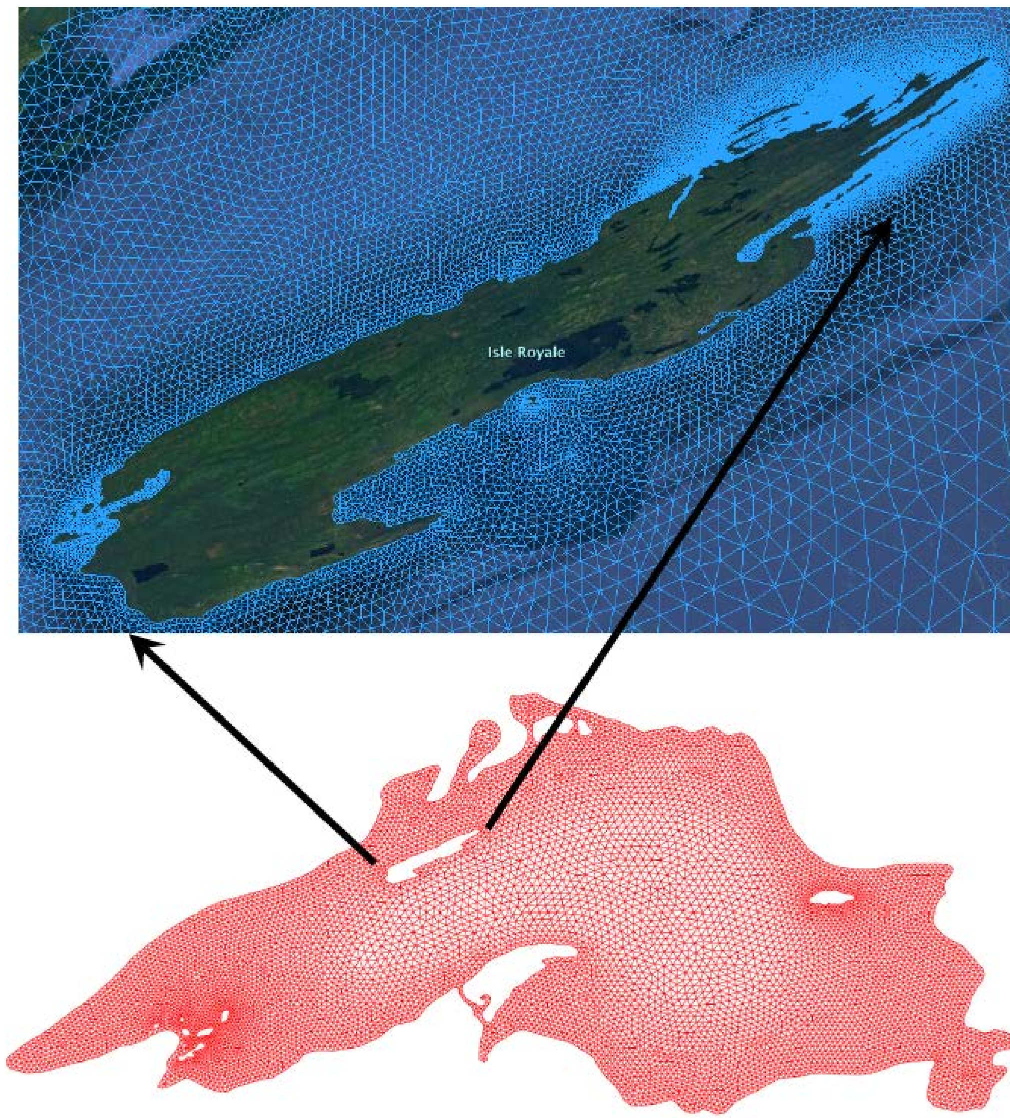


Figure C2: the model grid of Lake Superior and locally refined high resolution grids around Isle Royale.

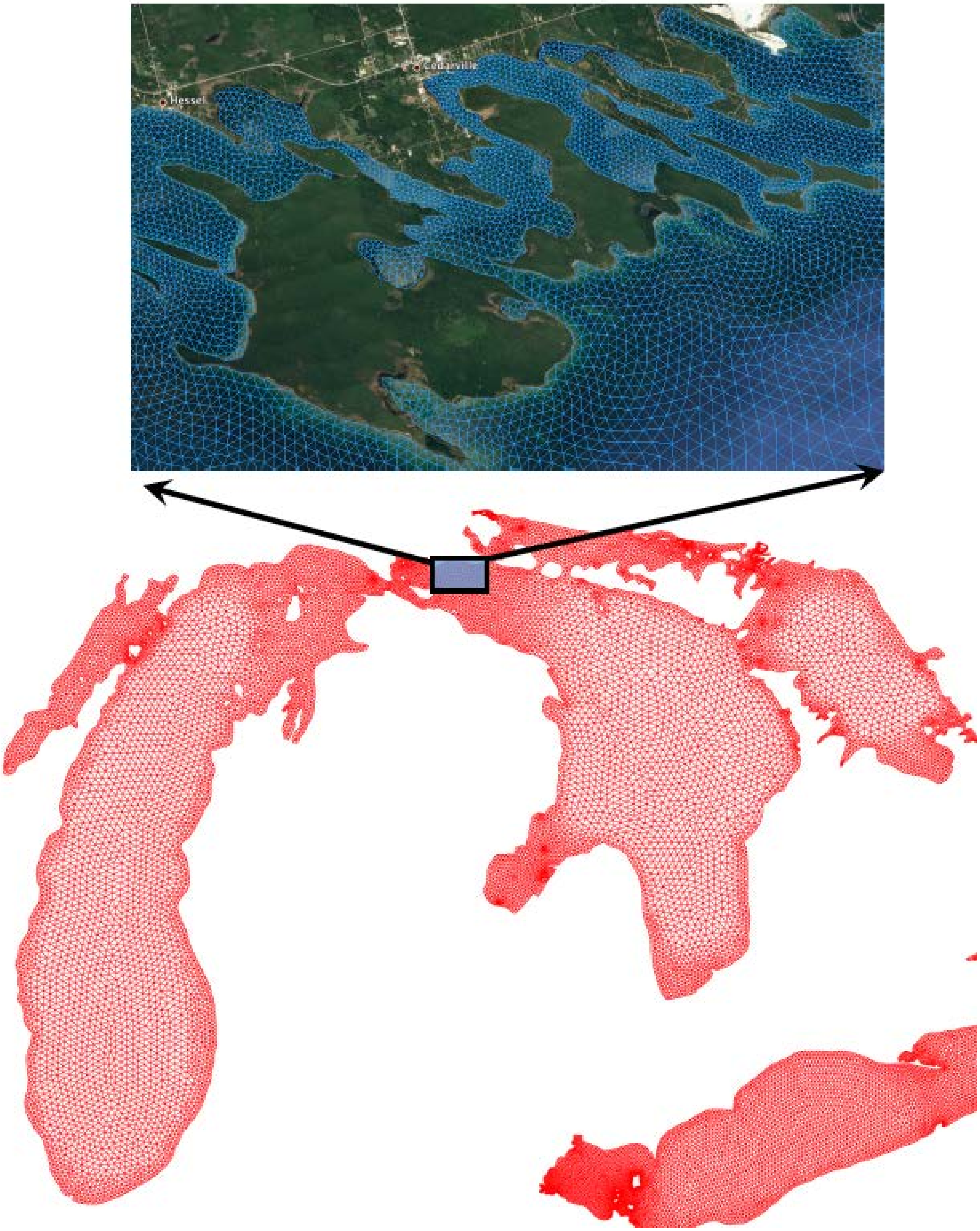


Figure C.3: The model grid of Lake Michigan-Huron and locally refined high resolution grids in the Les Cheneaux Islands.

Task C.2. Outcomes/Products

We simulated detailed water circulation under realistic meteorological forcing such as surface wind, solar radiation, and cloud coverage, which allowed us to further conduct the linked Lagrangian particle trajectory model simulation. The particles represented EWM stem fragments, and the model simulated their movements with currents within and around the target region. This linked hydrodynamic and particle model will provide the framework for integrating EWM fragments introduction and dispersal with information on ecological conditions and EWM tolerances to predict not only where invaders may spread, but more importantly, where EWM are likely to establish new populations in the coastal Great Lakes.

By examining their flow trajectories, we identified the impact of hydrodynamics on the settling and dispersal of the EWM fragments. We found that the unique, naturally restricted circulation through the Les Cheneaux Islands, which favored the retention of EWM fragments, is likely a critical component to the prolific success of EWM in that region (Fig. C.4). Similarly, we conducted hydrodynamic and particle model simulations for Isle Royale from May to November 2011 as a case study. We found the inland harbors and bays of Isle Royale are the most favorable places for EWM settling if fragments are introduced by boating activities (Fig. C.4), suggesting that these locations warrant careful monitoring for EWM invasions.

Using lake-wide models, we can also predict likely paths of EWM dispersal towards other coastal regions. We identified the boat launch locations along the coast of Lake Superior and simulated the possible dispersal of EWM fragments under a scenario when EWM fragments are introduced by boating activities at these locations. Results shows a wide dispersal of fragments along the coasts. A closer look reveal that there are several places particularly favor the aggregation of fragments including Keweenaw Bay, Whitefish Bay, Duluth Bay etc. In addition, although there are not many fragments retained in Apostle Islands in this case, a flood of fragments passed through the apostle islands during the simulation period. One of the reasons for little fragments retained within Apostle Islands is because the present model grids for this region are not sufficiently high to resolve the all the details of the shorelines. This hypothesis is supported by our modeling experiments around the Isle Royale: When the model have sufficiently high grid resolution , the fragments in the simulation are seen to settle in the embayments (Fig

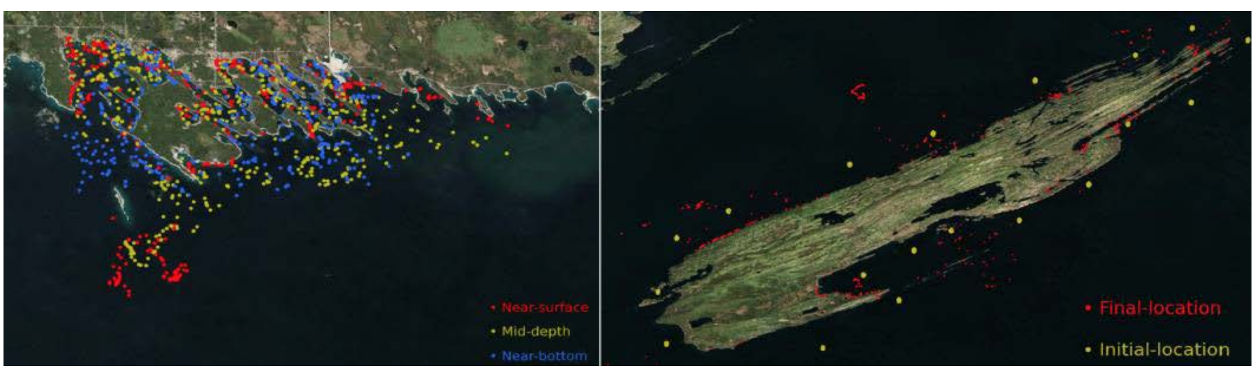


Figure C.4: Left: Model results show the high retention rate of EWM fragments within the Les Cheneaux Island complex due to its unique, naturally restricted circulation. Right: Modeled EWM fragment dispersal and settling (red) within the inland harbors and bays under a process-oriented scenario assuming fragments are introduced near Isle Royale (yellow).

C.5) , while fragments can easily spread to the open water with little retention when simulation was conducted with coarser model grid resolution, which artificially smoothen the coastline (not shown).

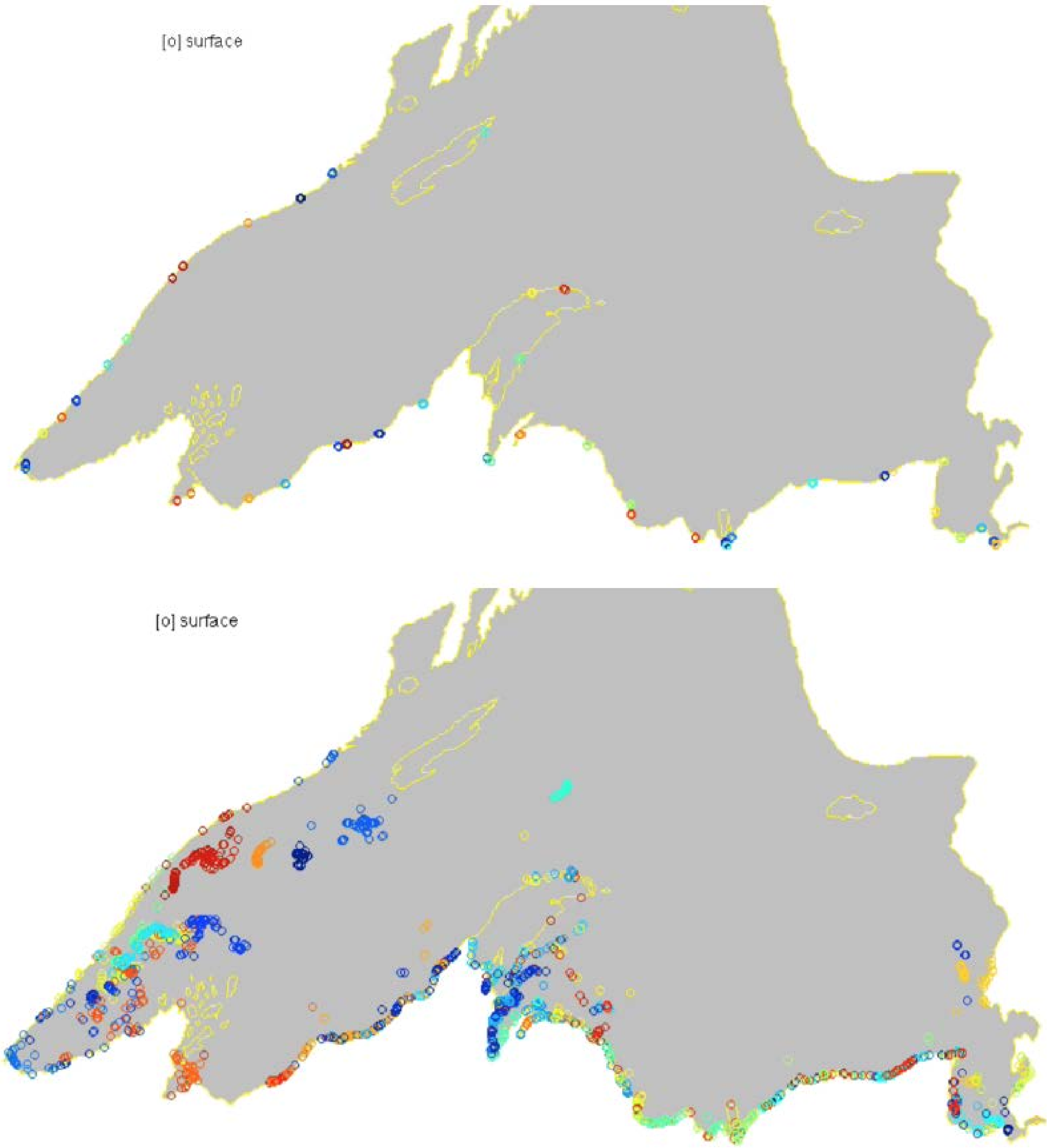


Figure C.5: The initial locations of simulated particles (fragments) around the identified the boat launch locations along the coast of Lake Superior. (upper panel); the dispersal of the simulated particles (fragments) with water circulation during the summer (lower panel).

Task C.3. Major Conclusions

Using the linked hydrodynamic-particle trajectory modeling, we have found the local hydrodynamic condition play an important role in influencing the dispersal and settling of EWM fragments, which is critical to understand the invasion process and predicting areas of future EMW invasions. Regions that are characterized by naturally restricted circulation that favor the retention of EWM fragment are critical regions to the prolific success of EWM. Featuring such characteristics, not yet affected regions of the upper Great Lakes should receive extra monitoring attention. In particular, results suggest the embayments of Isle Royale and Apostle Islands warrant careful monitoring for EWM invasions. However, the results from hydrodynamic-particle trajectory modeling alone should not be used independently as indicators of EWM invasion. Rather, the results should be used as a component to be integrated into our multi-facet analysis with observation information, experiments of EWM bio-kinetics for a comprehensive assessment.

Task C.4. Constraints

Thus far, our understanding of the spread of EWM is mainly related to hydrodynamics throughout the lake. However, results can be improved by adding biological processes to particles (fragments) tracking model to give a more realistic representation of EWM spread. For example, we can upgrade the model developed in the previous sections by attaching environmental variables to each particle to simulate the growth and decay processes of the fragments, hereafter referred to as Property-Carrying Particle-tracking model (PCPM). In other words, each particle will have biological activities based on the attached environment variables, such as light, temperature, water depth and nutrient availability, etc. The detailed empirical equations to describe the biological kinetics must be partially based on literature research completed with previous GLRI funding and from field and lab work as new proposed work. The framework of the coupled bio-physical particles tracking model can be easily built by following Xue et al. (2016).

Task D. Data Portal and Outreach

Task D.1. Outcomes/Products

Task D.1.1. Invasive Eurasian Watermilfoil Web Page

The project team used the experience, data and infestation history of the broader Great Lakes and local Keweenaw communities to guide a variety of outreach activities designed to help alleviate confusion and aid other Great Lakes communities to make effective and timely decisions with regard to managing EWM invasions. First, we created a web-based information clearinghouse designed to contain links to credible and relevant resources for managing EWM in the Great Lakes region. The content of this website was based on the results of our literature review, as well as outputs from the current project including remote sensing maps of all target areas and results of herbicide testing. The "living document" is hosted at http://www.mtri.org/eurasian_watermilfoil.html and the entry page is shown at Figure D.1.

The web page includes extensive information on EWM biology, its invasive properties, ecological impacts, and spread, while also describing the mapping and modeling tools demonstrated and developed through this project. Related ongoing research provides the opportunity to maintain and extend the page with updated EWM research results, new citations, and further information on EWM treatment efforts around the Great Lakes and beyond.

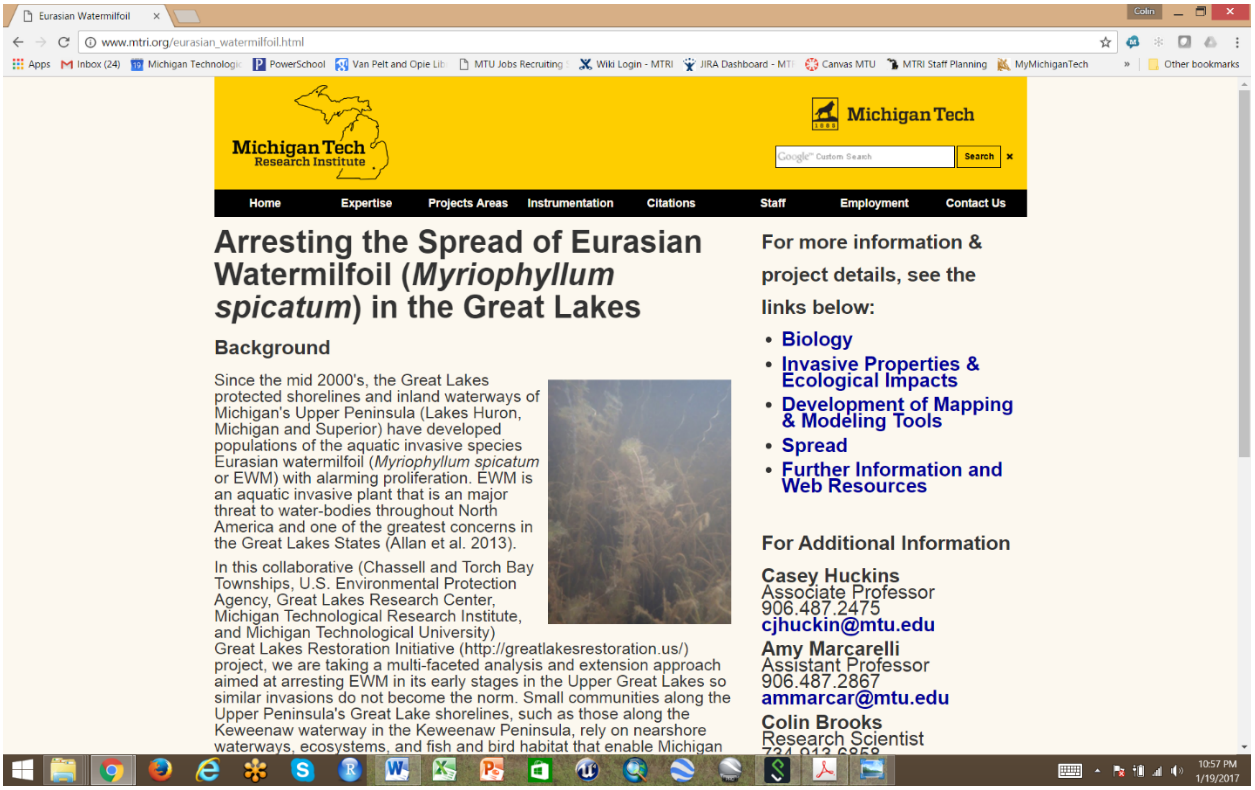


Figure D.1: The entry page for the Eurasian watermilfoil information clearinghouse web page created for this project to enable information sharing.

Task D.1.2. Presentations - Oral

Juneau, Kevyn J., Huckins, Casey J., Marcarelli, Amy M., Chimner, Rodney A., Brooks, Colin N., Xue, Pengfei, Meadows, Guy A., "Arresting the spread of Eurasian watermilfoil (*Myriophyllum spicatum*) in the Keweenaw Waterway.", Science in the Northwoods, USGS, UWisc, WDNR, USFS, Boulder Junction, WI. (October 2014).

Juneau, Kevyn J., Huckins, Casey J., Marcarelli, Amy M., Chimner, Rodney A., Brooks, Colin N., Xue, Pengfei, Meadows, Guy A., "Ecological Response to Eurasian Watermilfoil Management in a Lake Superior Coastal Waterway", Annual Conference on Great Lakes Research, International Association for Great Lakes Research, Burlington, VT, (May 28, 2015)

Juneau, Kevyn J., Huckins, Casey J., Marcarelli, Amy M., Chimner, Rodney A., Brooks, Colin N., Xue, Pengfei, Meadows, Guy A., "Environmental Response to Eurasian Watermilfoil Management in a Lake Superior Coastal Waterway", Annual Meeting, Ecological Society of America, Baltimore, MD. (August 2015).

Juneau, Kevyn J., Huckins, Casey J., Marcarelli, Amy M., "Innovative and Multifaceted Control of Invasive Eurasian and Hybrid Watermilfoil Using Integrated Pest Management Principles", Sixth Annual Northern Great Lakes Invasive Species Conference, Marquette, MI, Oral. (October 22, 2015).

Juneau, K., and C. Huckins. "Control, Monitoring, and Effects of Eurasian Watermilfoil". Oral Presentation. Lake Superior Environmental Monitoring Collaborative, US Environmental Protection Agency, Houghton, MI. (19 March 2015).

Juneau, K. (2015, Apr 24). Advancing Ecologically Based Pest Management Decisions into Natural Areas. Invited talk. Biology Department Seminar Series. Michigan Technological University, Houghton, MI.

Huckins, Casey , Amy Marcarelli, Kevyn Juneau, Rodney Chimner, Colin Brooks, Pengfei Xue, Guy Meadows, Erika Hersch-Green. "Collaboration and Challenges with Prevention, Control, and Management of Invasive Eurasian Watermilfoil" 76th Midwest Fish & Wildlife Conference, Grand Rapids, MI. (January, 2016).

Marcarelli, Amy, Casey Huckins, Kevyn Juneau, Colin Brooks, Rodney Chimner, Erika Hersch-Green, Guy Meadows. "Integrated management of nonnative and hybrid Eurasian watermilfoil in the Portage Waterway of the Upper Peninsula of Michigan" 36th Annual Meeting of the Midwest Aquatic Plant Management Society, Grand Rapids, MI. (March 2016).

Brooks, Colin N., Grimm, Amanda G., Huckins, Casey J., Marcarelli, Amy M., Van Goethem, Ryan, Dobson, R J., "Evaluating the spread and control of Eurasian watermilfoil through remote sensing technologies", Annual Conference on Great Lakes Research, International Association for Great Lakes Research, Guelph, ON, Canada, Oral. (June 2016).

Brooks, Colin, Grimm, Amanda, Huckins, Casey J., Marcarelli, Amy M., "Development of a spectral-based algorithm for mapping and monitoring of Eurasian watermilfoil (*Myriophyllum spicatum*) in the Great Lakes region from an unmanned aerial vehicle platform", Annual meeting, Ecological Society of America, Ft Lauderdale, FL, Oral. (August 2016).

Juneau, K., Huckins, C. Marcarelli, A. Invasive Watermilfoil Response to Control Efforts in a Lake Superior Coastal Waterway. Upper Midwest Invasive Species Conference, La Crosse, Wi. (19 October 2016).

Task D.1.3. Presentations - Poster

Ortiz, Jade E., Marcarelli, Amy M., Juneau, Kevyn J., Huckins, Casey J., "Invasive *Myriophyllum spicatum* and Nutrients Interact to Influence Phytoplankton Communities", Annual Meeting, Society for Freshwater Science, Milwaukee, WI, Poster. (May 20, 2015). (also presented at 3 MTU events).

Juneau, K., A. Marcarelli, C. Huckins, R. Chimner, C. Brooks, "Environmental response of a Lake Superior coastal waterway to Eurasian watermilfoil management". Poster presentation. Ecological Society of America Annual Meeting, Baltimore, MD. (13 August 2015).

Leguizamon, Carmen, Kevyn Juneau, Casey Huckins, and Ashley Moerke. A Laboratory Study of *Mycoleptodiscus terrestris* Fungus as a Tool for Integrated Control of Eurasian Watermilfoil and its

Effect on Native Macrophytes. Midwest Aquatic Plant Management Society, Grand Rapids, MI (March 2016).

Van Goethem, R., Amy Marcarelli, Kevyn Juneau, and Casey Huckins. Legacy Disturbance In A Lake Littoral Zone: Effects Of Stamp Sands On The Structure Of Macrophyte Communities In The Keweenaw Waterway Of MI. Midwest Aquatic Plant Management Society, Grand Rapids, MI (March 2016).

Van Goethem, Ryan R., Marcarelli, Amy M., Juneau, Kevyn J., Huckins, Casey J., "Legacy disturbance effects in a lake littoral zone: effects of stamp sands on structure of macrophyte communities in the Keweenaw waterway of MI", Summer Meeting, Association for the Sciences of Limnology and Oceanography, Santa Fe, NM, Poster. (June 7, 2016).

Task D.1.4. Published Manuscripts

Xue. P, D.J. Schwab, S. Hu (2015), An Investigation of the Thermal Response to Meteorological Forcing in a Hydrodynamic Model of Lake Superior, J. Geophys. Res. Oceans, 120, 5233–5253,, doi: 10.1002/2015JC010740

Xue, P., J. Pal, X. Ye, J. Lenters, C. Huang, and P. Chu, 2016: Improving the Simulation of Large Lakes in Regional Climate Modeling: Two-way Lake-atmosphere Coupling with a 3-D Hydrodynamic Model of the Great Lakes. J. Climate. doi:10.1175/JCLI-D-16-0225.1, in press.

Ortiz, J. E., Marcarelli, A. M., Juneau, K. J., Huckins, C. J. Invasive *Myriophyllum spicatum* and nutrients interact to influence algal assemblages. Freshwater Science. (Submitted)

Task D.1.5. Public Outreach

Initial planning and coordination of the project began during the spring and summer of 2014 when several team members met with representatives from partnering Townships and the aquatic plant control specialist (PLM, contracted by Townships) to discuss the status and issues of invasive aquatic macrophytes in their local waters and their plans for treatment of EWM. These discussions informed their plans for treatment and our understanding of treatment goals, and responses to treatments.

Dec. 19, 2014 – Over view of research and restoration activities including, “USEPA-Great Lakes Restoration Initiative MTU Grant: Arresting the Spread of Eurasian Watermilfoil in Lake Superior” to MI State Representative Mike Latti and MTU Board of Control.

May 13-14, 2014 –Three representative team members to meet with citizens of Cedarville, Mi and members of the Les Cheneaux Watershed Council to learn from each other, discuss our project, inform our QAPP development and approach, and help guide their ongoing efforts attempting to control EWM in the Les Cheneaux Islands, Lake Huron.

Aug. 17, 2014 - USEPA-Great Lakes Restoration Initiative, MTU Grant: Arresting the Spread of Eurasian Watermilfoil in Lake Superior. Four of our team members presented project overview to US Senator Levin at MTU.

July 9, 2015 - Town Forum presentation and community outreach session, Cedarville, Michigan. Huckins, C., K. Juneau, A. Grimm, G. Meadows. . Control, Monitoring, and Ecology of Eurasian Watermilfoil – imbedded within Communities of Important Native Macrophytes. Les Cheneaux Watershed Council. Community Center, Cedarville, Michigan.

Huckins, C., A. Marcarelli. (17 Sept 2015). "Ecology and management of Eurasian Watermilfoil in lake littoral zones". Educational and outreach presentation to Michigan Tech CE 4905 – Senior Project Design course in Environmental Engineering. Houghton, MI.

August 5, 2016. Dr. Casey Huckins and graduate students Ryan Van Goethem and Taylor Zallek (both conducting graduate research on invasive Eurasian Watermilfoil) MTU Dept. of Biological Science presented a display of live invasive and native aquatic plants and littoral zone fish to the public at the Keweenaw Water Festival. We estimate that up to 70 people observed from the display.

Task D.1.6. Teacher Workshops:

As part of the Education/Outreach program based out of MTU and aimed at education about aquatic invasive species, three teacher professional development workshops on invasive species have been conducted.

Teacher workshops were held across the state of Michigan to educate teachers and thus their students about invasive species such as Eurasian Watermilfoil. These workshops were originally motivated and supported by this EPA-GLRI project and they were directly funded by our Michigan Invasive Species Grant project (MISPG IS14-2005) that was also motivated by this GLRI project.

a) Grand Learning Network, a Great Lakes Stewardship Initiative (GLSI) hub in the Lansing area in central lower Michigan. (2-day teacher workshop for 24 teachers on June 22-23, 2015 at the Bengel Wildlife Center, near Lansing)

b) Both the West Michigan Great Lakes Stewardship Initiative (WMGLSI) hub in the Muskegon area and the Lake Superior Stewardship Initiative (LSSI) hub serving four counties in the western Upper Peninsula, conducted one-day teacher professional development workshops on invasive species. The LSSI-sponsored invasive species workshop took place on June 16, 2016, at the Great Lakes Research Center at Michigan Technological University. The first half (morning) of the workshop focused on terrestrial invasive species, with a presentation and field trip led by Dr. Sigrid Resh (see Project Image 8), director of the Keweenaw Invasive Species Management Area. The afternoon focused on aquatic invasive species, with a presentation and field to Pike Bay led by Dr. Casey Huckins, PI on this project. Each teacher received a copy of the Field ID Guide to Invasive Plants in Michigan's Natural Communities as well as, copies of the presenters' powerpoint presentations to adapt for classroom use.

c) Thirteen teachers attended the WMGLSI-sponsored workshop held August 3, 2016 at Duck Lake State Park. Again the morning half of the workshop focused on terrestrial invasive species and the afternoon half of the workshop focused on aquatic species.

Fourteen teachers attended a half-day session on Aquatic Invasive Species held at Michigan Technological University and led by Dr. Casey Huckins, MTU Dept. of Biological Science. who provided an overview of aquatic invasive species and led a field trip to Pike Bay to demonstrate milfoil research sampling procedures (July 7, 2015).

Graduate student Brian Danhoff, from the MTU Dept. of Biological Sciences presented on Invasive Species for a STEM Tour at Michigan Tech University for a total of 40 elementary school students and their teachers (June 1st, 2016, September 27th, 2016).

5. References

American Public Health Association (APHA). 2005. Standard methods for the examination of water and wastewater, 21st edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.

Brooks, C., Grimm, A., Shuchman, R., Sayers, M., & Jessee, N. (2015). A satellite-based multi-temporal assessment of the extent of nuisance Cladophora and related submerged aquatic vegetation for the Laurentian Great Lakes. *Remote Sensing of Environment*, *157*, 58-71.

Carmichael, W. W. 2001. Health effects of toxin-producing cyanobacteria: "The CyanoHABs". Human and Ecological Risk Assessment 7:1393-1407.

EdgeTech. 2012. 4125 Series Dual Frequency Side Scan Sonar System User's Manual. Document No. 990-4125MAN-1000 Rev. D. 101 pp.

Eiswerth M. E., S. G. Donaldson, W. S. Johnson. 2000. Potential environmental impacts and economic damages of Eurasian watermilfoil (*Myriophyllum spicatum*) in western Nevada and northeastern California. Weed Technology 14:511-518.

Great Lakes Coastal Resilience Planning Guide (GLCR). 2016. Les Cheneaux Watershed Council Combats Invasive Species to Preserve Recreational Opportunities. Accessed online 15 Dec 2015 at http://greatlakesresilience.org/stories/michigan/les-cheneaux-watershed-council-combats-invasive-species-preserve-recreational-0

Forgrave, M. (2009). Copper Country Explorer. http://www.coppercountryexplorer.com/

Johnson, Robert L. and Van Dusen, Peter J. and Toner, Jason A. and Hairston, Nelson G. (2000) Eurasian watermilfoil biomass associated with insect herbivores in New York. Journal of Aquatic Plant Management, 38, pp. 82-88.

Kerfoot, C., Lauster, G., & Robbins, J. A. (1994). Paleolimnological study of copper mining around Lake Superior: Artificial varves from Portage Lake provide a high resolution record. *Limnology and Oceanography*, *39*(3), 647–669. doi:10.4319/lo.1994.3

Kling GW, et al. 2003. Chapter Three: Ecological Vulnerability to Climate Change: Aquatic Ecosystems. in Confronting climate change in the Great Lakes Region. Union of Concerned Scientists, Ecological Society of America. http://www.ucsusa.org/greatlakes/.

Körner, S., and A. Nicklisch. 2002. Allelopathic growth inhibition of selected phytoplankton species by submerged macrophytes. Journal of Phycology 38:862-871.

Johnson JA, Newman RM. 2011. A comparison of two methods for sampling biomass of aquatic plants. Journal of Aquatic Plant Management 49:1-8.

Johnson RL, Van Dusen PJ, Tone JA, Hairston NG. 2000. Eurasian watermilfoil biomass associated with insect herbivores in New York. Journal of Aquatic Plant Management 38: 78–81.

LaRue EA, Zuellig MP, Netherland MD, Heilman MA, Thumal RA. 2012. Hybrid watermilfoil lineages are more invasive and less sensitive to a commonly used herbicide than their exotic parent (Eurasian watermilfoil). Evolutionary Applications 6: 462-471.

Lyzenga DR, Malinas NP, Tanis FJ. 2006. Multispectral Bathymetry Using a Simple Physically Based Algorithm. IEEE Transactions on Geoscience and Remote Sensing 44: 2251-2259.

Lyzenga DR. 1981. Remote sensing of bottom reflectance and water attenuation parameters in shallow water using aircraft and Landsat data. International Journal of Remote Sensing 2: 71-72.

Lyzenga DR. 1978. Passive remote sensing techniques for mapping water depth and bottom features. Applied Optics 17: 379-383.

Madsen, J. (1999). Point intercept and line intercept methods for aquatic plant management. Aquatic plant control technical note U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.: 16.

Many Waters, LLC. 2014. "Aquatic Plant Analysis and Management Approaches: Torch Bay, Houghton County, MI". Many Waters, LLC, Iron River, MI.

Many Waters, LLC. "2013 Qualitative Assessment of Eurasian watermilfoil *Myriophyllum spicatum* (including *Myriophyllum sibericum x spicatum*) of Pike Bay – Chassell Township, Houghton County, MI". 2013. Many Waters, LLC, Iron River, MI.

McCune B, Grace JB (2002) Analysis of Ecological Communities. Gleneden Beach, OR: MjM Software Design.

McCune, B., & Mefford, M. J. (2011). PC-ORD. Gleneden Beach, Oregon: MjM Software Design.

Michel A, Arias RS, Scheffler BE, Duke SO, Netherland M, Dayan FE. 2004. Somatic mutationmediated evolution of herbicide resistance in the nonindigenous invasive plant hydrilla (Hydrilla verticillata). Molecular Ecology 13: 3229-3237.

Michigan Department of Environmental Quality. 2005. Procedures for aquatic vegetation surveys. https://www.michigan.gov/deq/0,4561,7-135-3313_3681_3710-81012--,00.html

Moody ML, Les DH. 2002. Evidence of hybridity in invasive watermilfoil (Myriophyllum) populations. Proceedings of the National Academy of Sciences USA 99: 14867–14871.

Parkinson, H., J. Mangold, J. Jacobs, J. Madsen, and J. Halpop (2011), Eurasian Watermilfoil.

Rahel FJ, Olden JD. 2008. Assessing the Effects of Climate Change on Aquatic Invasive Species. Conservation Biology 22: 521-533.

Saha, S. et al. (2010), The NCEP Climate Forecast System Reanalysis, Bull. Amer. Meteor. Soc., 91, 1015-1057.

Sand-Jensen, K., and J. Borum. 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquatic Botany 41:137-175.

Schultz, R., and E. Dibble. 2012. Effects of invasive macrophytes on freshwater fish and macroinvertebrate communities: the role of invasive plant traits. Hydrobiologia 684:1-14.

Shuchman RA, Sayers MJ, Brooks CN. 2013. Mapping and monitoring the extent of submerged aquatic vegetation in the Laurentian Great Lakes with multi-scale satellite remote sensing. Journal of Great Lakes Research Vol. 39, Supplement 1, pp 78-89. http://dx.doi.org/10.1016/j.jglr.2013.05.006.

Skawinski, P. M. (2014). *Aquatic Plants of the Upper Midwest: A photographic field guide to our underwater forests* (2nd ed.). Wisconsin, USA.

Smith, Craig S., Kurt D. Getsinger, Angela G. Poovey, William F. James, Michael D. Netherland, R. Michael Stewart, Mark A. Heilman, Scott McNaught, Anthony Groves, Pam Tyning, and Paul Hausler. 2012. Selective Control of Eurasian Watermilfoil in Houghton Lake, Michigan: 2002–2006. USACE ERDC/EL TR-12-15

Theoharides, K. A., and Dukes, J. S. 2007. Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. New Phytologist 176:256-273.

Voss E. G., A. A. Reznicek. 2012. Field manual of Michigan flora. The University of Michigan Press, Ann Arbor, MI.

Wetzel, R.G., Likens, G.E. 2000. Limnological Analyses. 3rd ed. New York (NY): Springer Science+Business Media Inc. 429 p.

Xue, P., D. J. Schwab, and S. Hu (2015), An investigation of the thermal response to meteorological forcing in a hydrodynamic model of Lake Superior, *Journal of Geophysical Research: Oceans, 120*, 5233-5253.

Xue. P., D. J. Schwab, R. W. Sawtell, M. J. Sayers, R. A. Shuchman, G. L. Fahnenstiel (2016), A Particle-tracking Technique for Spatial and Temporal Interpolation of Satellite Images Applied to Lake Superior Chlorophyll Measurements, Journal of Great Lakes Research (in revision).

Zuellig, M. P., and R. A. Thum. 2012. Multiple introductions of invasive Eurasian watermilfoil and recurrent hybridization with northern watermilfoil in North America. J. Aquat. Plant Manage. 50:1-19.