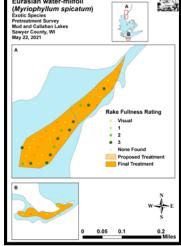
Eurasian water-milfoil (*Myriophyllum spicatum*) Pre and Posttreatment Surveys Mud and Callahan Lakes (WBIC: 2434800/2434700) Sawyer County, Wisconsin







2021 treatment areas

Pretreatment EWM density and distribution

Eurasian water-milfoil (Berg 2007)

Project Initiated by:

The Callahan and Mud Lakes Protective Association, Lake Education and Planning Services, LLC, and the Wisconsin Department of Natural Resources (Grant AEPP-610-20)





Callahan Lake facing east from western shoreline

Surveys Conducted by and Report Prepared by:

Endangered Resource Services, LLC Matthew S. Berg, Research Biologist St. Croix Falls, Wisconsin May 22 and July 7, 2021

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INTRODUCTION:

Callahan Lake (WBIC 2434700) (138 acres) and Mud Lake (WBIC 2434800) (464 acres) form a 602-acre drainage system created by an 8ft dam on the north fork of the Chief River in north-central Sawyer County, Wisconsin in the Town of Round Lake (T41N R7W S27/28 and 33/34). Callahan has a maximum depth of 18ft and an average depth of 11ft, while Mud has a maximum depth of 15ft and an average depth of 6ft. The lakes are mesotrophic in nature, and water clarity is good with summer Secchi readings averaging approximately 9.5ft in 2021 (WDNR 2021). The lakes' bottom substrate is primarily sand along the shoreline before transitioning to a sandy muck at most depths over 7ft (Bush et al. 1968) (Figure 1).

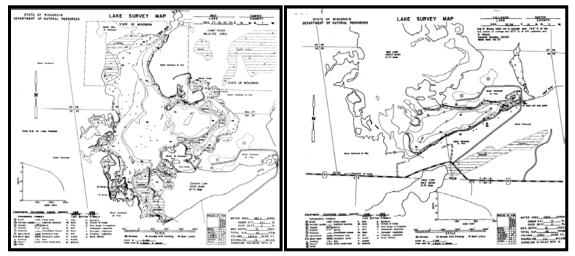


Figure 1: Mud and Callahan Lakes Bathymetric Maps

STUDY BACKGROUND AND RATIONALE:

Eurasian water-milfoil (*Myriophyllum spicatum*) (EWM) is an exotic invasive plant species that was first identified in Callahan and Mud Lakes in the fall of 2005. Following initial whole lake point-intercept surveys in 2008 by Jeremy Williamson (JW), the Callahan and Mud Lakes Protective Association (CMLPA) and the Sawyer County Land and Water Conservation Department (SCLWC - Kristi Maki) used a 2009 Wisconsin Department of Natural Resources (WDNR) rapid response grant (AIRR-060-09) to hire Ayres Associates (Tiffany Kleczewski) to write the lakes' original Aquatic Plant Management Plan (APMP) that outlined herbicide applications to control the infestation (Kleczewski 2009). Since the APMP's approval by the WDNR, these small-scale treatments have occurred periodically based on low intensity delineation surveys by the applicator and/or the SCLWC.

Using our 2020 late-summer bed mapping survey, Lake Education and Planning Services, LLC (LEAPS – D. Blumer) and the CMLPA decided to treat three beds totaling 12.69 acres in 2021. Prior to 2020, pre and posttreatment macrophyte surveys were not conducted on the lakes due to the small size of the treatments and limited budgets. However, in both 2020 and 2021, these surveys were requested to gather baseline data on the density and distribution of both EWM and native species in the beds, and to determine the effectiveness of the treatment. This report is the summary analysis of the 2021 pre and posttreatment field surveys conducted on May 22 and July 7, 2021.

METHODS: Pre/Post Herbicide Surveys:

LEAPS provided treatment area shapefiles, and we generated pre/post survey points based on the size and shape of the proposed treatment areas. The 50-point offset sampling grid at 33m resolution approximated to the minimum of 4 pts/acre required by WDNR protocol for pre/post treatment surveys (Appendix I).

The survey sample points were uploaded to a handheld mapping GPS (Garmin 76CSx) and located on the lake. At each point, we recorded the depth and bottom substrate and used a rake to sample an approximately 2.5ft section of the bottom. EWM was assigned a rake fullness value of 1-3 as an estimation of abundance (Figure 2), and we also recorded visual sightings of EWM within six feet of the sample point. Because visual sightings are not calculated into the pre/post statistical formulas, we only assigned a rake fullness value for non-EWM plants. A cumulative rake fullness value was also noted.

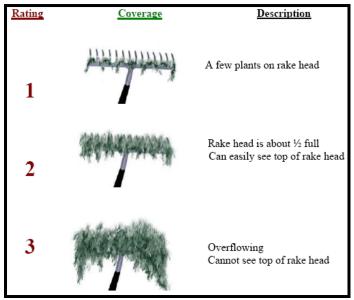


Figure 2: Rake Fullness Ratings

We entered all data collected into the standard WDNR APM spreadsheet (Appendix II). Data was analyzed using the linked statistical summary sheet and the WDNR pre/post analysis worksheet (UWEX 2010). For pre/post differences of individual plant species as well as count data, we used the Chi-square analysis on the WDNR pre/post survey worksheet. For comparing averages (mean species/point and mean rake fullness/point), we used t-tests. Differences were determined to be significant at p<0.05, moderately significant at p<0.01 and highly significant at p<0.001.

RESULTS AND DISCUSSION: Finalization of Treatment Areas:

Initial expectations were to treat three beds covering 12.69 acres (2.11% of the lakes' surface area). After the pretreatment found Eurasian water-milfoil in each area, it was decided to maintain the treatment as originally planned (Figure 3) (Appendix I).

Application occurred on May 26th with Northern Aquatic Services (Dale Dressel - Dresser, WI) applying liquid 2,4-D (Amine 4) at a rate of 3-4ppm (127.23 total gallons) (Table 1). At the time of treatment, the reported water temperature was 62°F, and the air temperature was 52°F. Wind speeds were clocked at 5-7mph out of the northwest.

Table 1: Early-season EWM Treatment SummaryMud and Callahan Lakes, Sawyer CountyMay 26, 2021

Bed Number	Proposed Treatment Area (acres)	Final Treatment Area (acres)	Change in Acreage (+/-)	Chemical, Rate, and Total Volume
1A	7.39	7.39	0.00	2,4-D (Amine 4) – 3ppm – 62.96gal.
1B	3.94	3.94	0.00	2,4-D (Amine 4) – 4ppm – 36.16gal.
2	1.36	1.36	0.00	2,4-D (Amine 4) – 4ppm – 25.11gal.
Total	12.69	12.69	0.00	2,4-D (Amine 4) – 3-4ppm – 127.23gal.

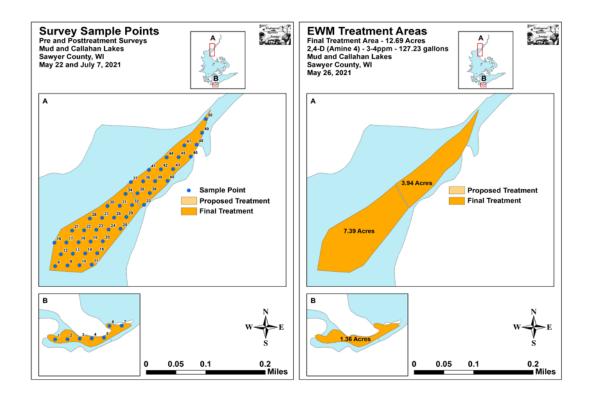


Figure 3: Pre/Post Survey Points and EWM Treatment Areas Eurasian Water-milfoil Pre/Post Herbicide Survey:

All survey points occurred in areas between 4.0ft and 8.0ft of water. Within the beds, plants grew at a mean and median depth of 5.5ft during the pretreatment survey, and these values were unchanged posttreatment (Table 2). Most plants were established over a thin sandy muck (Figure 4) (Appendix III).

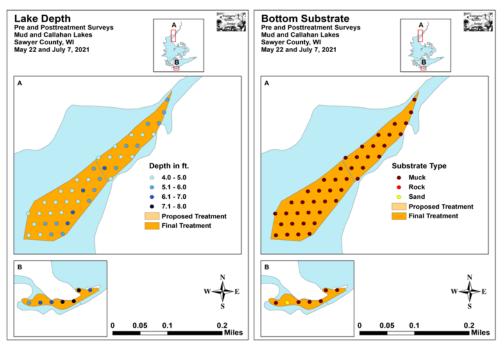


Figure 4: Treatment Area Depths and Bottom Substrate

Table 2: Pre/Posttreatment Surveys Summary StatisticsMud and Callahan Lakes, Sawyer CountyMay 22, 2021 and July 7, 2021

Summary Statistics:	Pre	Post
Total number of points sampled	50	50
Total number of sites with vegetation	50	50
Total number of sites shallower than the maximum depth of plants	50	50
Freq. of occur. at sites shallower than max. depth of plants (in percent)	100.0	100.0
Simpson Diversity Index	0.88	0.90
Mean Coefficient of Conservatism	6.4	6.4
Floristic Quality Index	25.8	33.3
Maximum depth of plants (ft)	8.0	8.0
Mean depth of plants (ft)	5.5	5.5
Median depth of plants (ft)	5.5	5.5
Average number of all species per site (shallower than max depth)	2.88	3.96
Average number of all species per site (veg. sites only)	2.88	3.96
Average number of native species per site (shallower than max depth)	2.40	3.88
Average number of native species per site (sites with native veg. only)	2.50	3.88
Species Richness	17	28

Mean Rake Fullness (veg. sites only)2.182.24The entire treatment area fell within the littoral zone, and plants were present at all points
during both the pre and posttreatment surveys (Figure 5) (Appendix IV). Total richness
increased sharply from 17 species pretreatment to 28 species posttreatment, while the
Simpson's Diversity Index ticked up from a high pretreatment value of 0.88 to a very
high 0.90 posttreatment. The Floristic Quality Index (another measure of native plant
community health) also jumped from 25.8 pretreatment to 33.3 posttreatment.

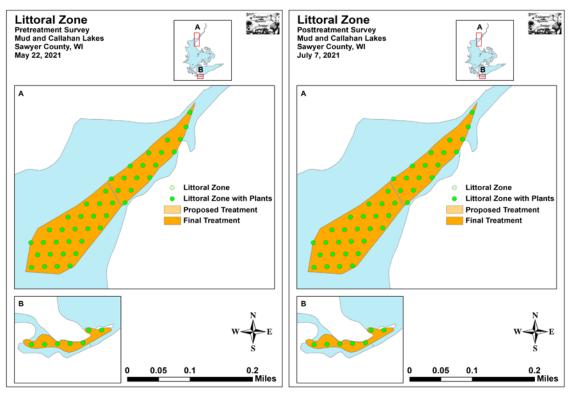


Figure 5: Pre/Posttreatment Littoral Zone

Mean native species richness at points with native vegetation demonstrated a highly significant increase (p < 0.001) from 2.50 species/point pretreatment to 3.88/point posttreatment (Figure 6). Total mean rake fullness also increased from a moderate 2.18 pretreatment to 2.24 posttreatment; however, this was not significant (p=0.32) (Figure 7) (Appendix IV).

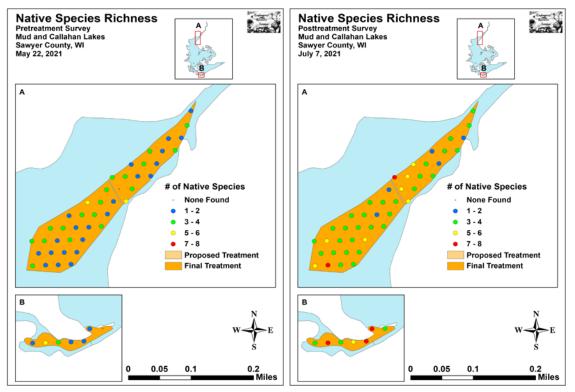


Figure 6: Pre/Posttreatment Native Species Richness

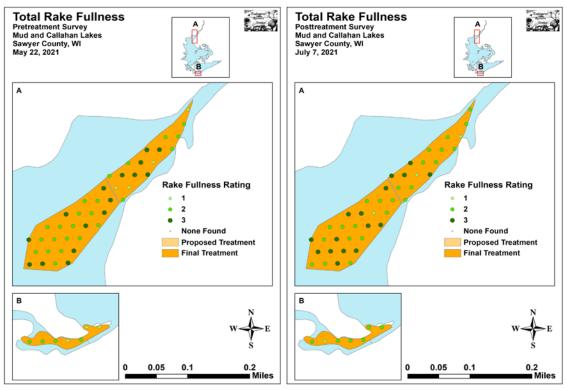


Figure 7: Pre/Posttreatment Total Rake Fullness

We found Eurasian water-milfoil dominated the majority of Bed A, but was scattered and patchy in Bed B. During the pretreatment survey, it was present in the rake at 24 points (48.00% coverage) with 15 additional visual sightings. We rated nine points a rake fullness of 3, four a 2, and the remaining 11 a 1 for a mean rake of 1.92. The 13 points with a rake fullness of 2 or 3 suggested 26.00% of the treatment area had a significant infestation (Figure 8) (Appendix V).

Posttreatment, we found EWM in the rake at four points (8.00% coverage) with one additional visual sighting. A single sample on a floating muck bog rated a 2 (2.00% significant infestation) while the other three samples and the visual sighting were represented by a single plant. This produced a mean rake fullness of 1.25 and suggested the treatment resulted in highly significant declines (p<0.001) in total distribution and visual sightings; a moderately significant decline (p=0.002) in rake fullness 3; and significant declines in rake fullness 1 (p=0.02) and total density (p=0.04) (Figure 9).

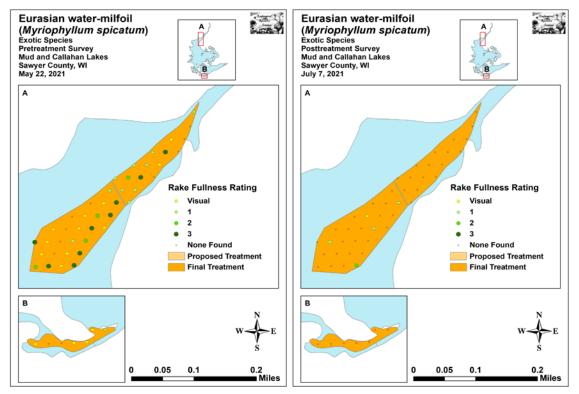
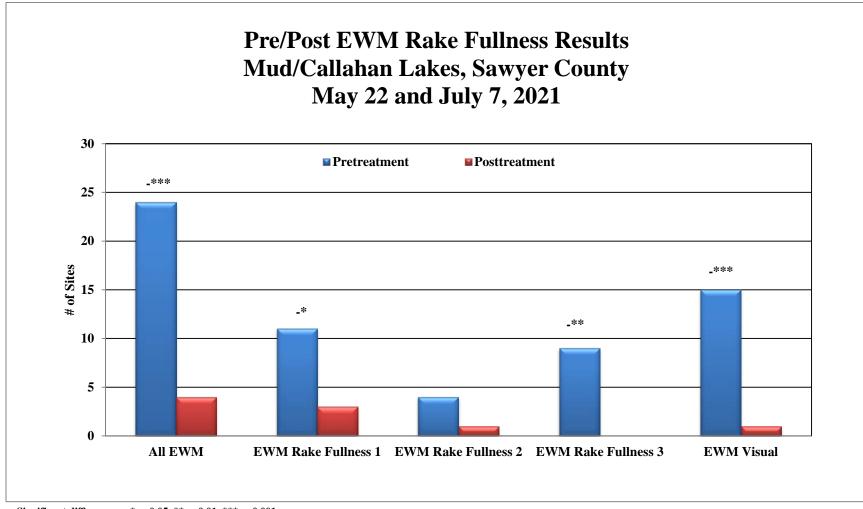


Figure 8: Pre/Posttreatment EWM Density and Distribution



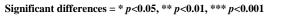


Figure 9: Changes in EWM Rake Fullness

Fern pondweed (*Potamogeton robbinsii*) was the most widely-distributed native species during the pretreatment survey (Figure 10) (Table 3). Present at 29 sites, it underwent a significant decline (p=0.02) in distribution to 17 sites posttreatment and slipped to become the fourth most common native species (Table 4). However, its slight decline in density from a mean rake fullness of 1.72 pretreatment to 1.65 posttreatment was not significant (p=0.38).

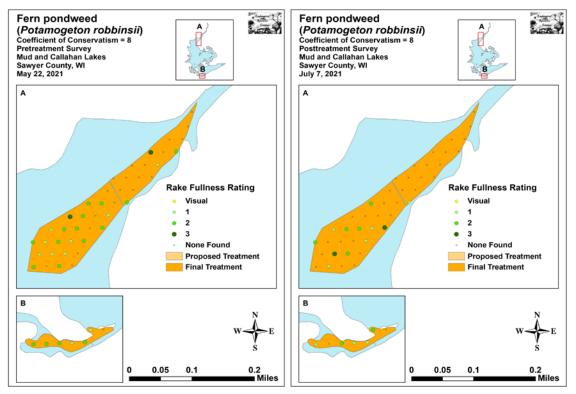


Figure 10: Pre/Posttreatment Fern Pondweed Density and Distribution

We identified Flat-stem pondweed (*Potamogeton zosteriformis*) as the second most common native species pretreatment and the most common posttreatment. In May, it was located at 18 sites with a mean rake fullness of 1.06 (Figure 11). By July, it had undergone highly significant increases (p < 0.001) in both distribution (39 sites) and density (mean rake of 1.72).

Common waterweed (*Elodea canadensis*) was the third most common native species during both the pre and posttreatment surveys. Found at 16 sites with a mean rake fullness of 1.38 pretreatment, it underwent a significant posttreatment increase (p=0.02) in distribution to 28 points. This was accompanied by a non-significant increase (p=0.18) in density to a mean make fullness of 1.54 (Figure 12).

Table 3: Frequencies and Mean Rake Sample of Aquatic MacrophytesPretreatment Survey - Mud and Callahan Lakes, Sawyer CountyMay 22, 2021

Species	Common Name	Total	Relative	Freq. in	Freq. in	Mean	Visual
species	Common Name	Sites	Freq.	Veg.	Lit.	Rake	Sites
Potamogeton robbinsii	Fern pondweed	29	20.14	58.00	58.00	1.72	0
Myriophyllum spicatum	Eurasian water-milfoil	24	16.67	48.00	48.00	1.92	15
Potamogeton zosteriformis	Flat-stem pondweed	18	12.50	36.00	36.00	1.06	0
Elodea canadensis	Common waterweed	16	11.11	32.00	32.00	1.38	0
Potamogeton amplifolius	Large-leaf pondweed	16	11.11	32.00	32.00	1.50	0
Ceratophyllum demersum	Coontail	11	7.64	22.00	22.00	1.45	0
Utricularia vulgaris	Common bladderwort	9	6.25	18.00	18.00	1.11	0
	Filamentous algae	7	*	14.00	14.00	1.29	0
Myriophyllum verticillatum	Whorled water-milfoil	6	4.17	12.00	12.00	1.33	0
Potamogeton praelongus	White-stem pondweed	3	2.08	6.00	6.00	1.33	0
Bidens beckii	Water marigold	2	1.39	4.00	4.00	1.00	0
<i>Chara</i> sp.	Muskgrass	2	1.39	4.00	4.00	1.00	0
Nuphar variegata	Spatterdock	2	1.39	4.00	4.00	2.00	0
Potamogeton epihydrus	Ribbon-leaf pondweed	2	1.39	4.00	4.00	1.00	0
Brasenia schreberi	Watershield	1	0.69	2.00	2.00	1.00	0
Nymphaea odorata	White water lily	1	0.69	2.00	2.00	1.00	0
Potamogeton gramineus	Variable pondweed	1	0.69	2.00	2.00	1.00	0
Potamogeton natans	Floating-leaf pondweed	1	0.69	2.00	2.00	1.00	0

* Excluded from relative frequency analysis

Table 4: Frequencies and Mean Rake Sample of Aquatic Macrophytes
Posttreatment Survey - Mud and Callahan Lakes, Sawyer County
July 7, 2021

Creation	Common Nomo	Total	Relative	Freq. in	Freq. in	Mean	Visual
Species	Common Name	Sites	Freq.	Veg.	Lit.	Rake	Sites
Potamogeton zosteriformis	Flat-stem pondweed	39	19.70	78.00	78.00	1.72	0
Ceratophyllum demersum	Coontail	29	14.65	58.00	58.00	1.34	0
Elodea canadensis	Common waterweed	28	14.14	56.00	56.00	1.54	0
Potamogeton robbinsii	Fern pondweed	17	8.59	34.00	34.00	1.65	0
Potamogeton amplifolius	Large-leaf pondweed	14	7.07	28.00	28.00	1.79	0
Potamogeton praelongus	White-stem pondweed	8	4.04	16.00	16.00	1.50	0
Utricularia vulgaris	Common bladderwort	8	4.04	16.00	16.00	1.13	0
Bidens beckii	Water marigold	5	2.53	10.00	10.00	1.40	0
Myriophyllum sibiricum	Northern water-milfoil	5	2.53	10.00	10.00	1.20	0
Myriophyllum spicatum	Eurasian water-milfoil	4	2.02	8.00	8.00	1.25	1
Myriophyllum verticillatum	Whorled water-milfoil	4	2.02	8.00	8.00	1.25	0
Nuphar variegata	Spatterdock	4	2.02	8.00	8.00	2.50	0
Nymphaea odorata	White water lily	4	2.02	8.00	8.00	1.00	0
Potamogeton epihydrus	Ribbon-leaf pondweed	4	2.02	8.00	8.00	1.50	0
Vallisneria americana	Wild celery	4	2.02	8.00	8.00	1.25	0
Heteranthera dubia	Water star-grass	3	1.52	6.00	6.00	1.33	0
Potamogeton pusillus	Small pondweed	3	1.52	6.00	6.00	1.00	0
Brasenia schreberi	Watershield	2	1.01	4.00	4.00	1.50	0
Najas flexilis	Slender naiad	2	1.01	4.00	4.00	1.00	0
Potamogeton natans	Floating-leaf pondweed	2	1.01	4.00	4.00	1.00	0
Spirodela polyrhiza	Large duckweed	2	1.01	4.00	4.00	2.00	0
Chara sp.	Muskgrass	1	0.51	2.00	2.00	1.00	0
Lemna minor	Small duckweed	1	0.51	2.00	2.00	1.00	0
Myriophyllum heterophyllum	Various-leaved water-milfoil	1	0.51	2.00	2.00	1.00	0
Potamogeton gramineus	Variable pondweed	1	0.51	2.00	2.00	2.00	0

Table 4 (continued): Frequencies and Mean Rake Sample of Aquatic Macrophytes
Posttreatment Survey - Mud and Callahan Lakes, Sawyer County
July 7, 2021

Species	Common Name	Total	Relative	Freq. in	Freq. in	Mean	Visual
Species	Common Name	Sites	Freq.	Veg.	Lit.	Rake	Sites
Potamogeton richardsonii	Clasping-leaf pondweed	1	0.51	2.00	2.00	1.00	0
Potamogeton vaseyi	Vasey's pondweed	1	0.51	2.00	2.00	2.00	0
Sparganium emersum	Short-stemmed bur-reed	1	0.51	2.00	2.00	2.00	0
	Freshwater sponge	1	*	2.00	2.00	1.00	0
	Filamentous algae	1	*	2.00	2.00	1.00	0

* Excluded from relative frequency analysis

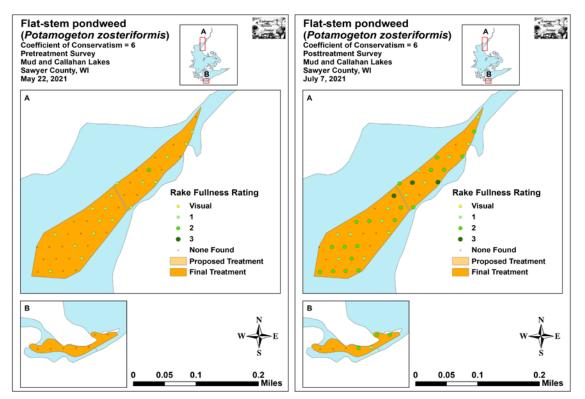


Figure 11: Pre/Posttreatment Flat-stem Pondweed Density and Distribution

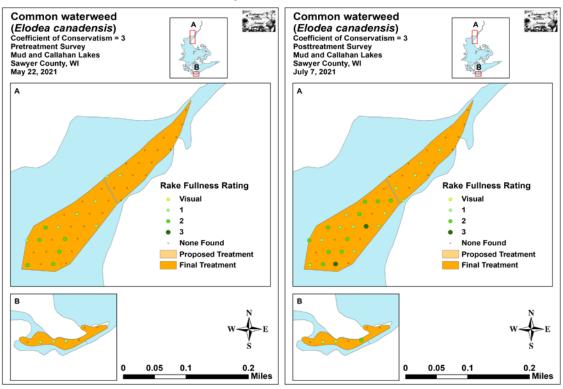
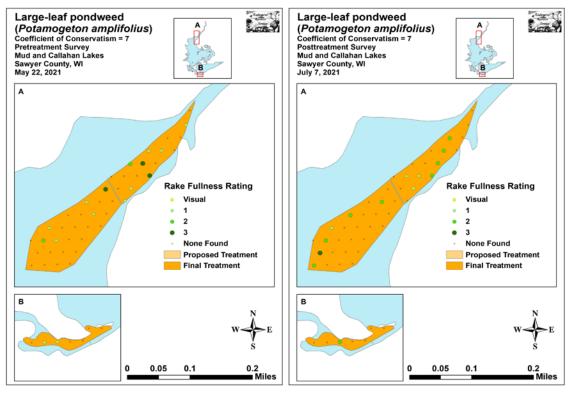


Figure 12: Pre/Posttreatment Common Waterweed Density and Distribution

Large-leaf pondweed (*Potamogeton amplifolius*) was the fourth most widely-distributed native species pretreatment and the fifth most common posttreatment (Figure 13). Present at 16 sites with a mean rake fullness of 1.50 pretreatment, it underwent a non-significant decline (p=0.66) in distribution posttreatment to 14 sites. Its increase in density to a mean rake of 1.79 was also not significant (p=0.14).





Found at 11 sites with a mean rake fullness of 1.45, Coontail (*Ceratophyllum demersum*) was the fifth most widely-distributed native species in the pretreatment survey (Figure 14). Posttreatment, following a highly significant increase (p<0.001) in distribution, it became the second most common species in the study area. Despite this expansion, its mean rake fullness declined to 1.34 posttreatment; however, this was not significant (p=0.28).

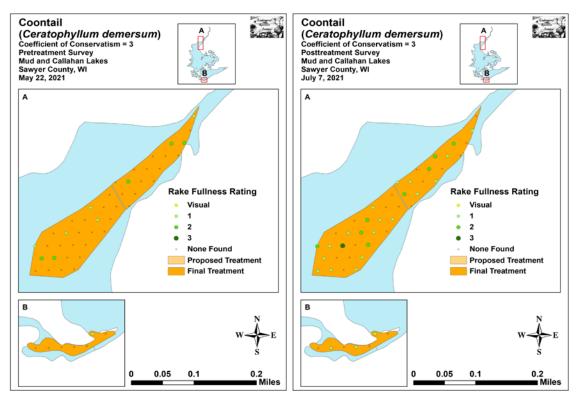
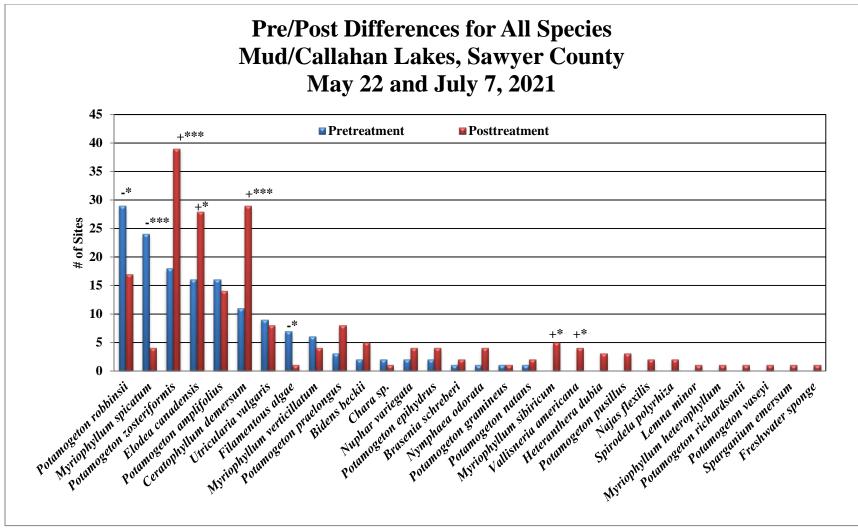


Figure 14: Pre/Posttreatment Coontail Density and Distribution

Eurasian water-milfoil was the only species that showed a highly significant decline posttreatment. In addition to Fern pondweed, filamentous algae also experienced a significant decline in distribution (p=0.03). Flat-stem pondweed and Coontail were the only species that showed highly significant expansions posttreatment, but, along with Common waterweed, Northern water-milfoil (*Myriophyllum sibiricum*) and Wild celery (*Vallisneria americana*) also demonstrated significant increases (p=0.02/p=0.04) in distribution (Figure 15) (Maps for all native species from the pre and posttreatment surveys can be found in Appendixes VI and VII).



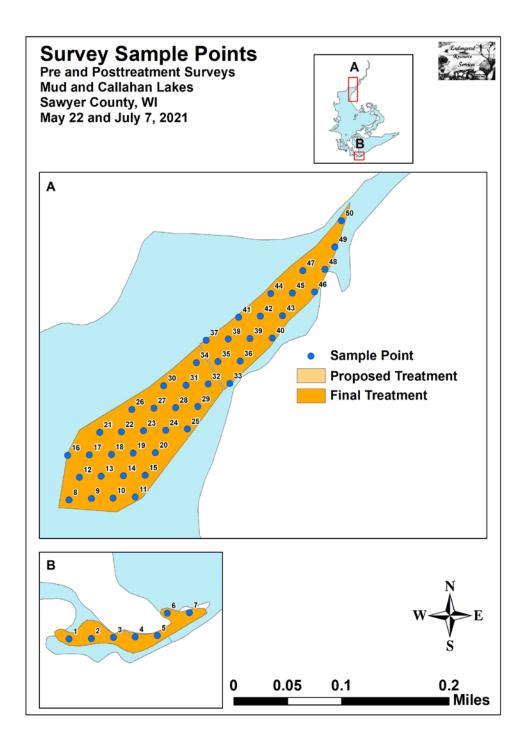
Significant differences = * *p*<0.05, ** *p*<0.01, *** *p*<0.001

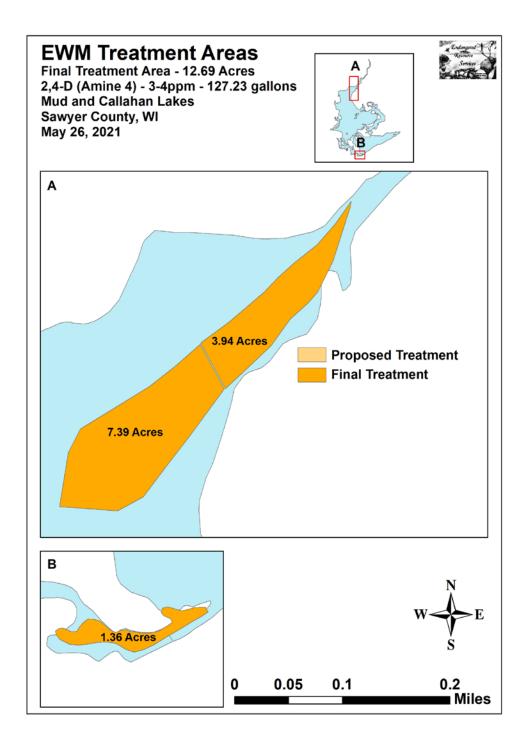


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Appendix I: EWM Pre/Post Survey Sample Points and Treatment Areas

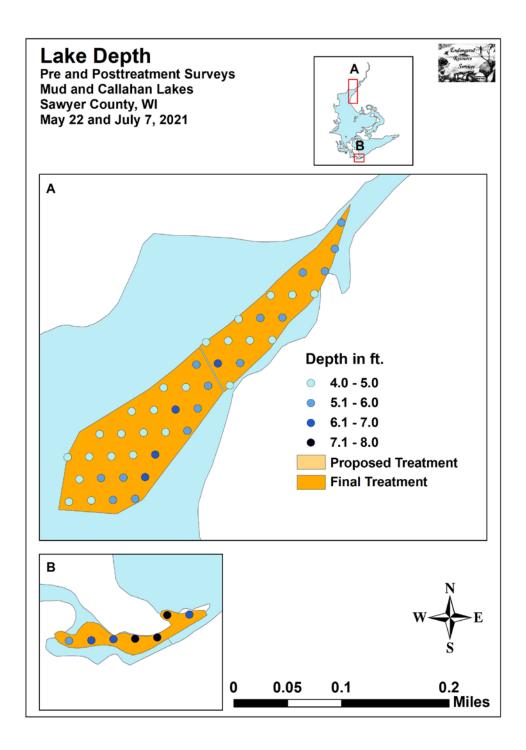


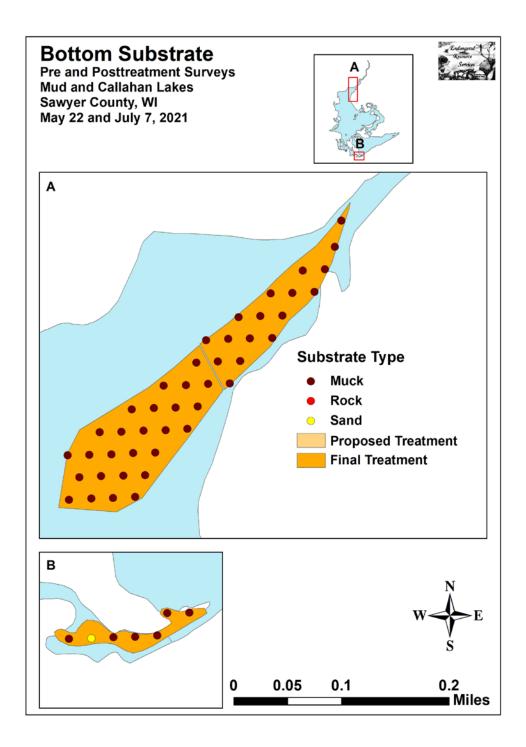


Appendix II: Vegetative Survey Datasheet

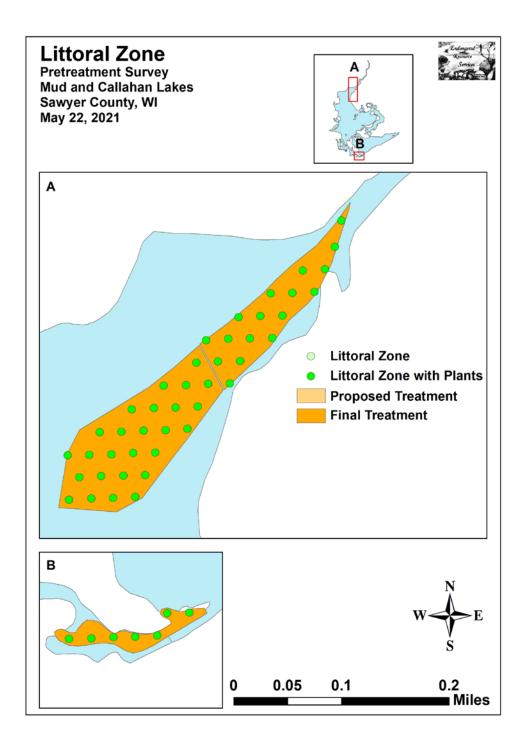
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Site #	Depth (ft)	Muck (M), Sand (S), Rock (R)	Rake pole (P) or rake rope (R)	Total Rake Fullness	EWM	EWM	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
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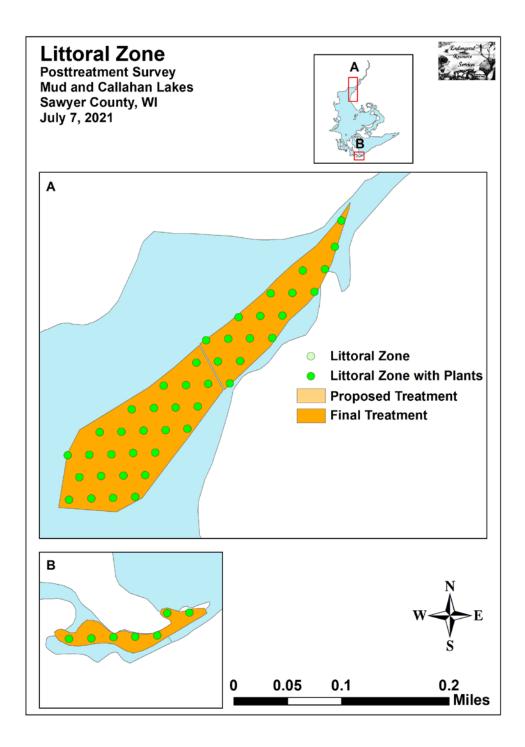
Appendix III: Pre/Post Habitat Variables

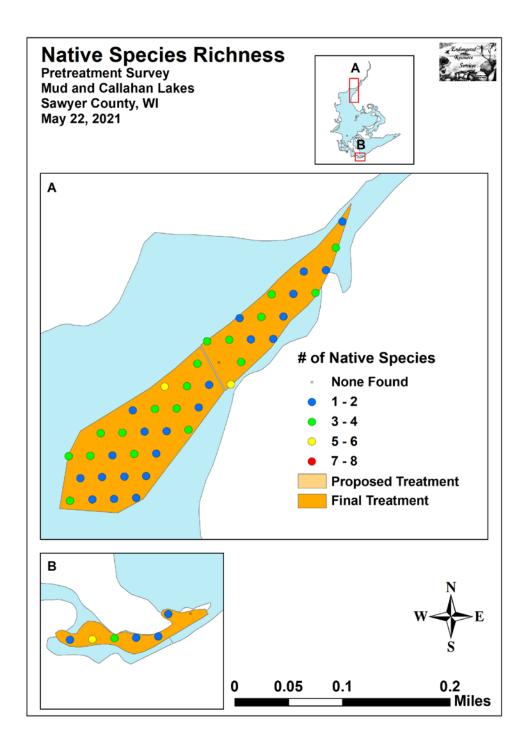


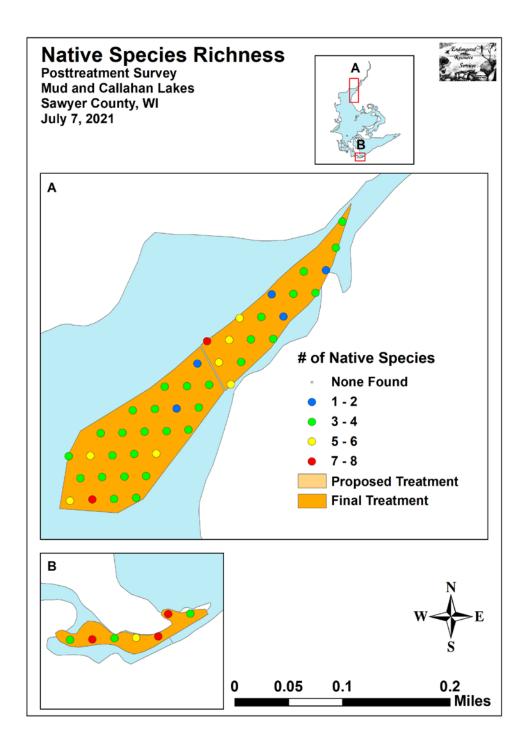


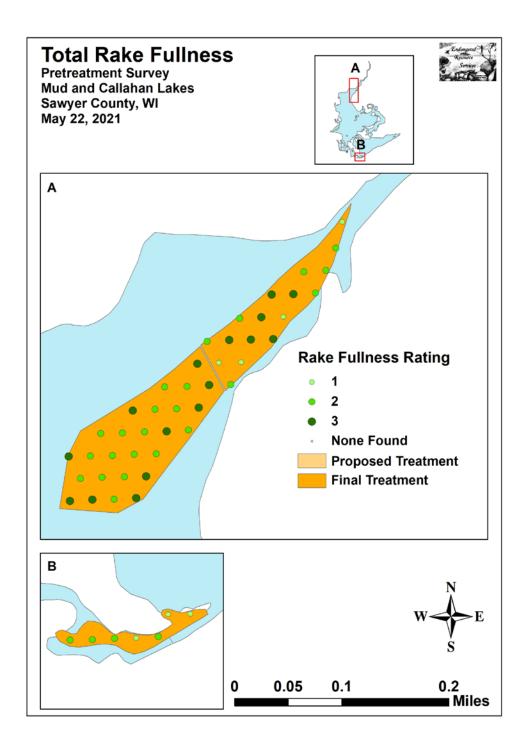
Appendix IV: Pre/Post Littoral Zone, Native Species Richness and Total Rake Fullness

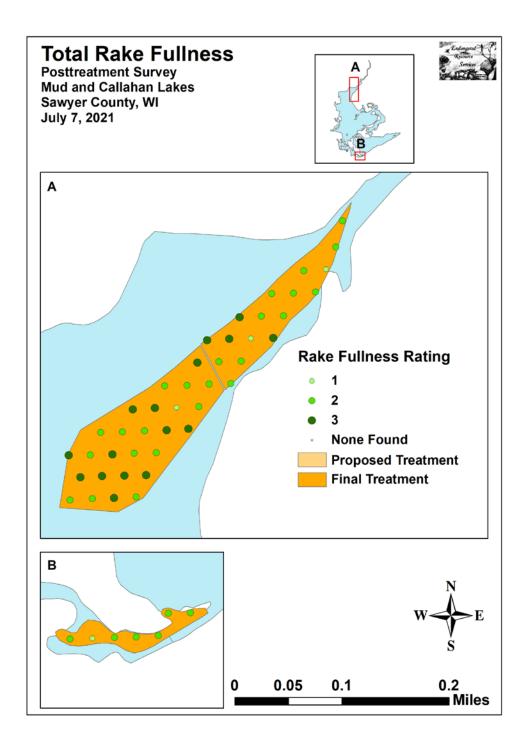




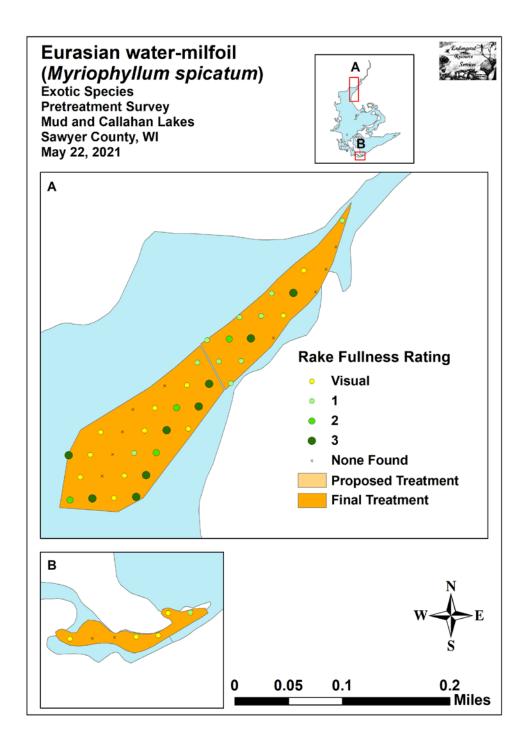


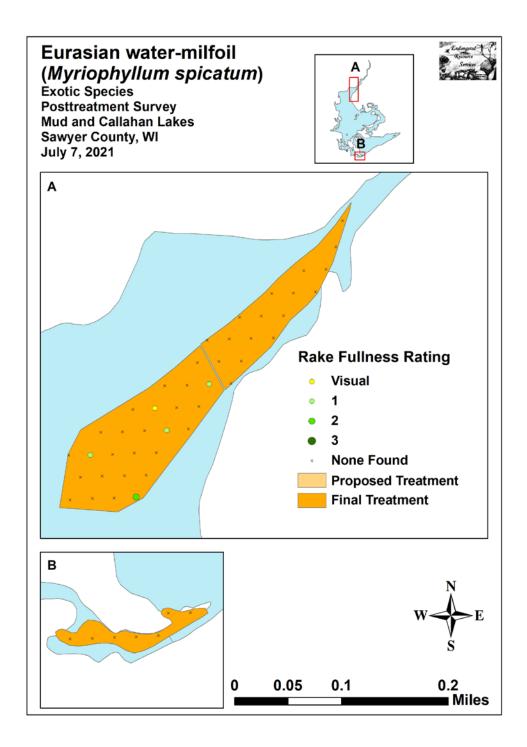




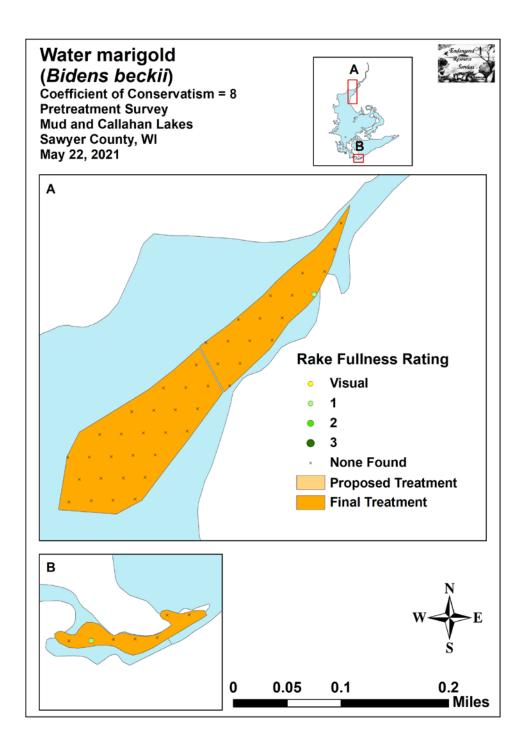


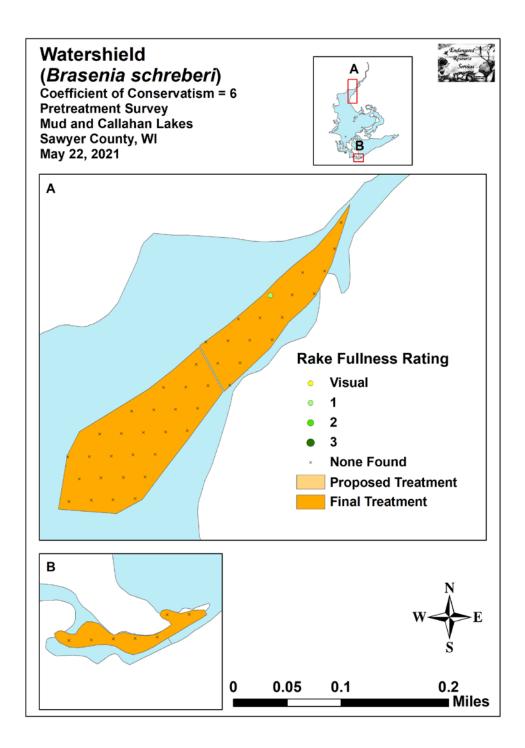
Appendix V: EWM Pre/Posttreatment Density and Distribution

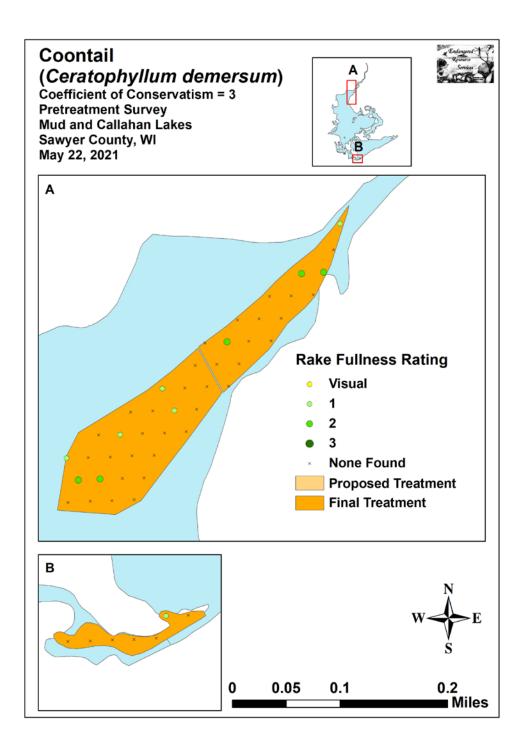


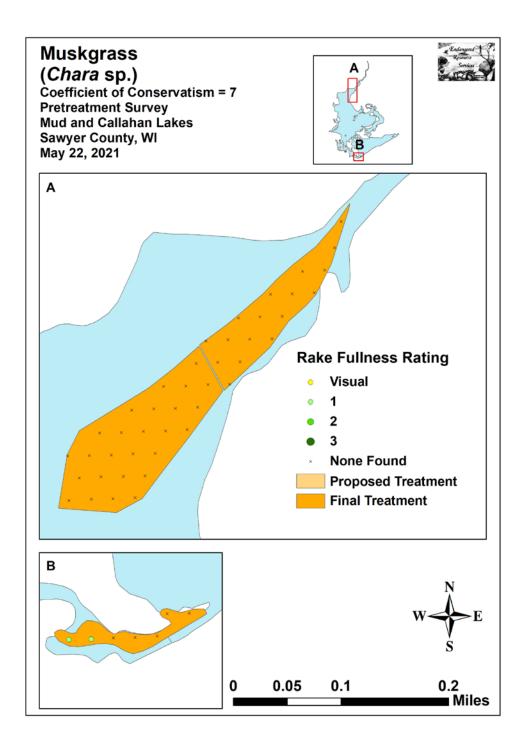


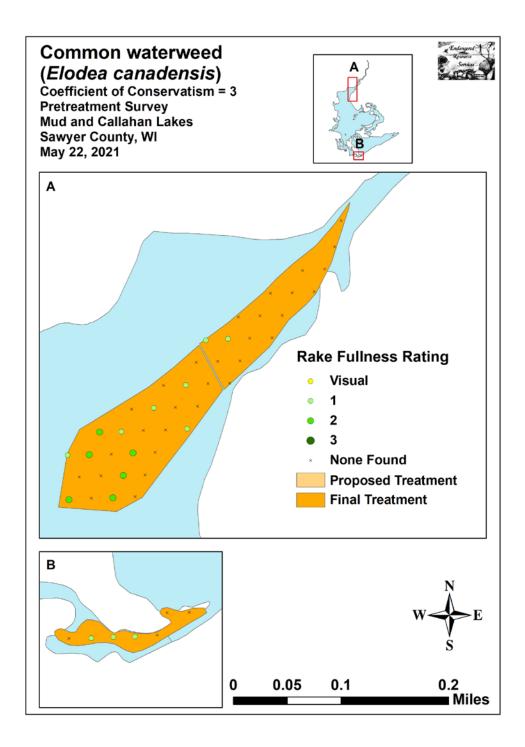
Appendix VI: Pretreatment Native Species Density and Distribution

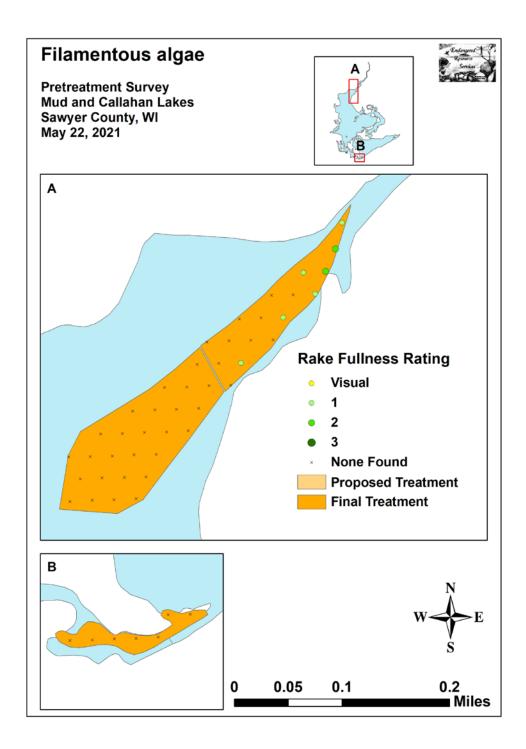


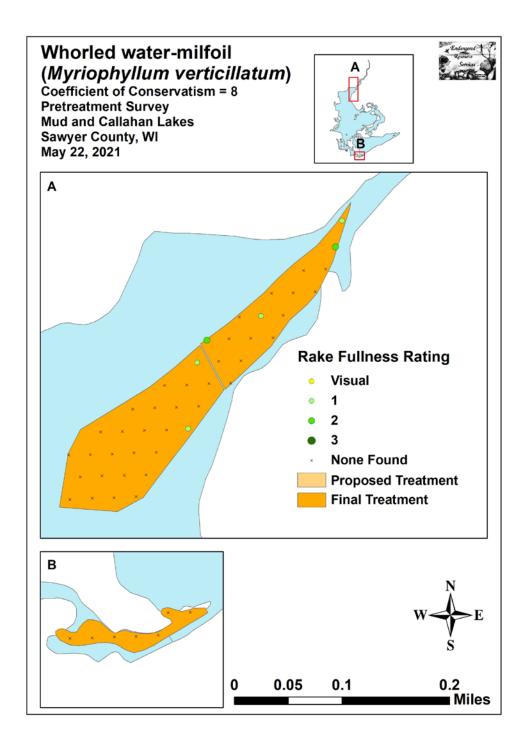


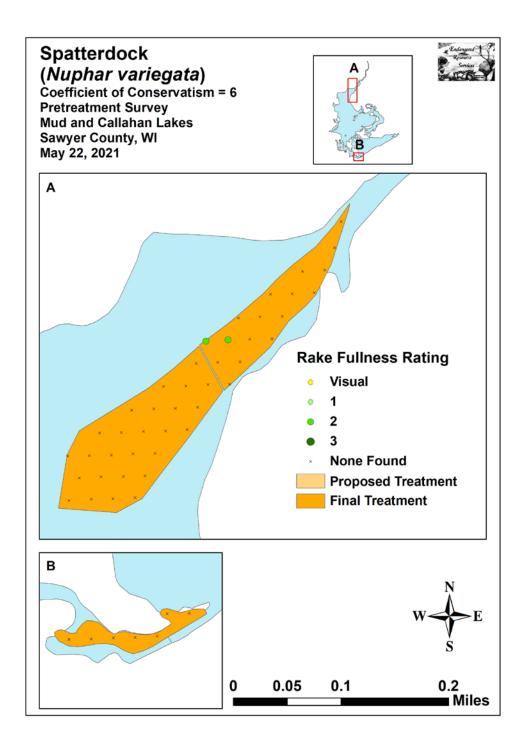


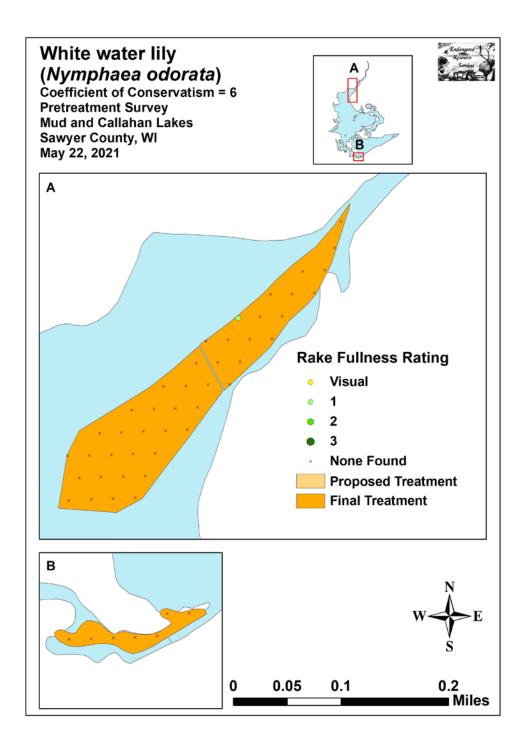


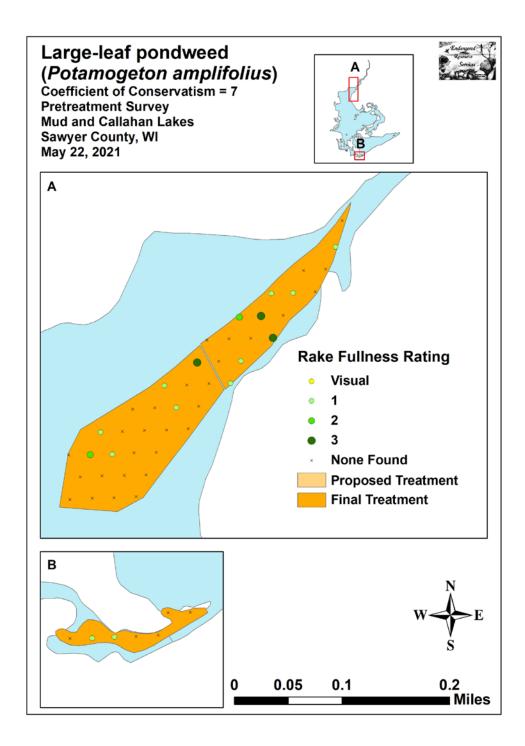


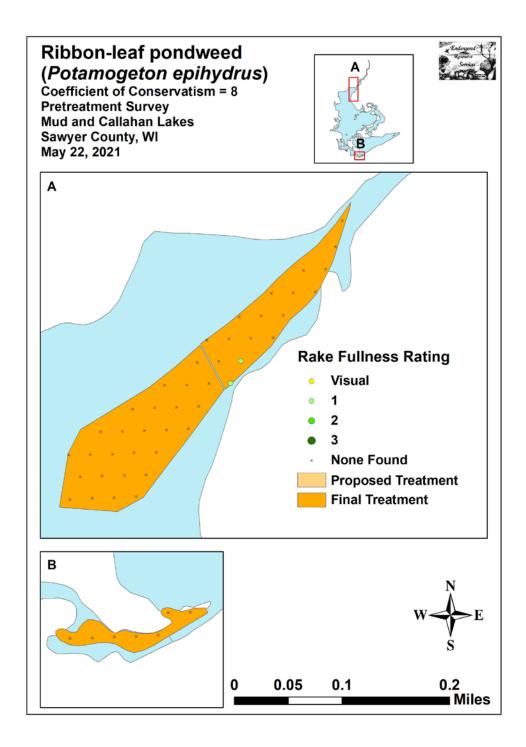


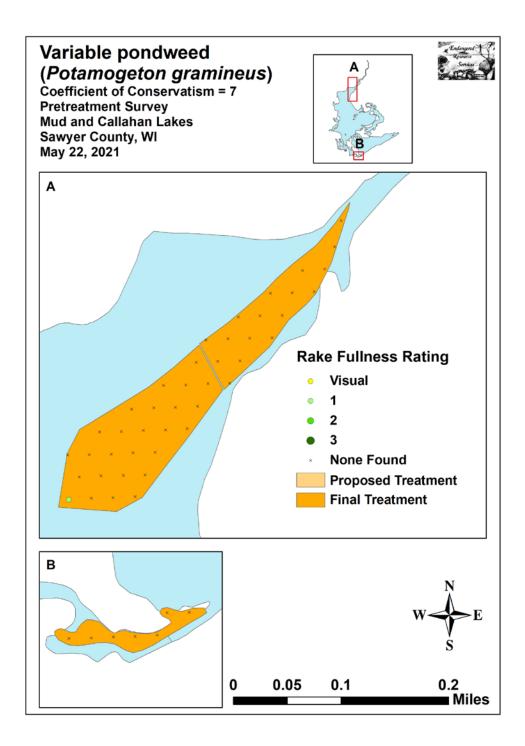


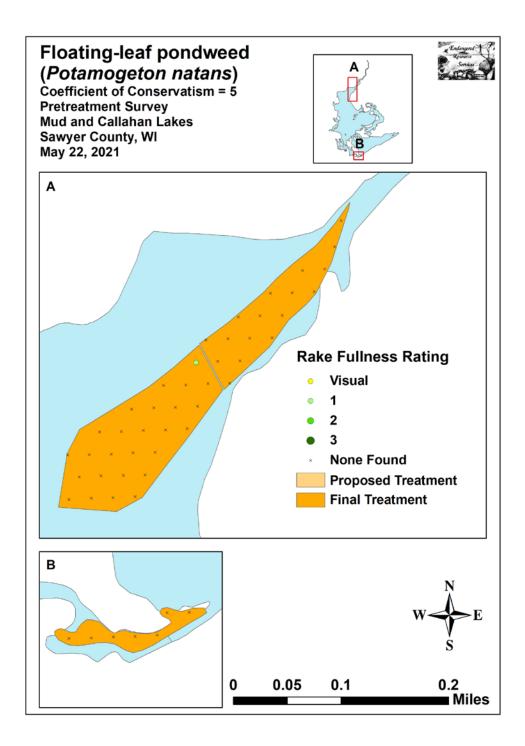


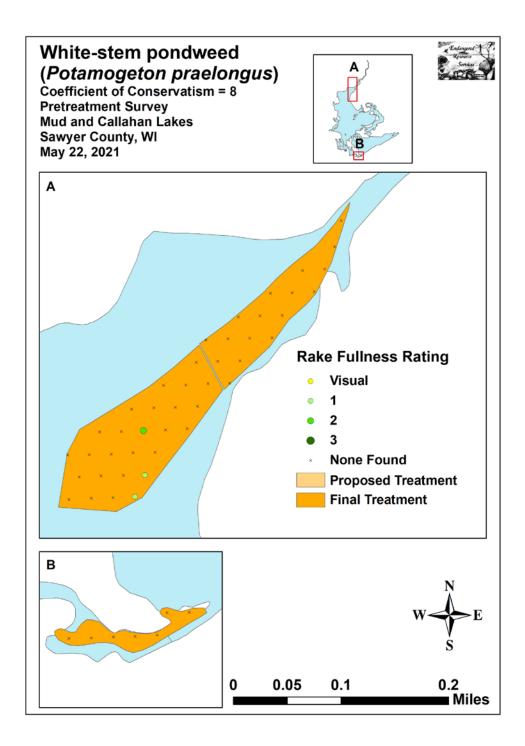


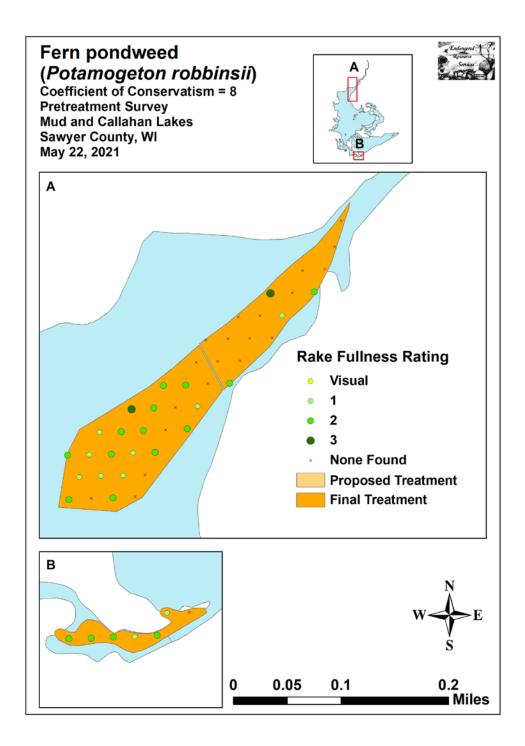


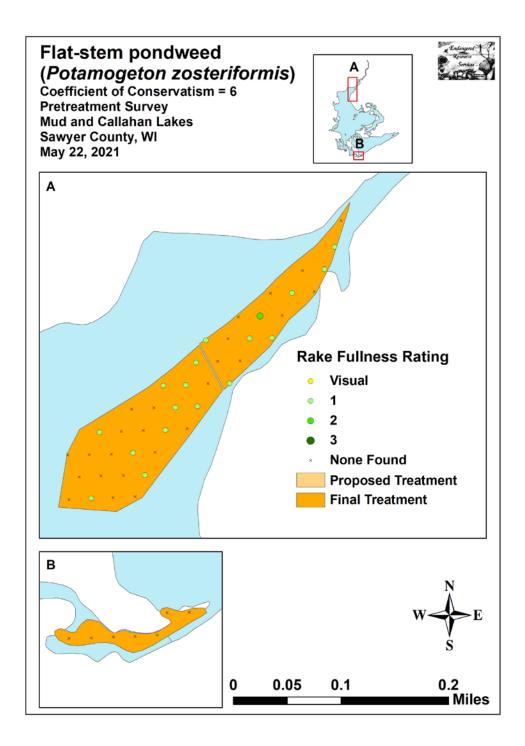


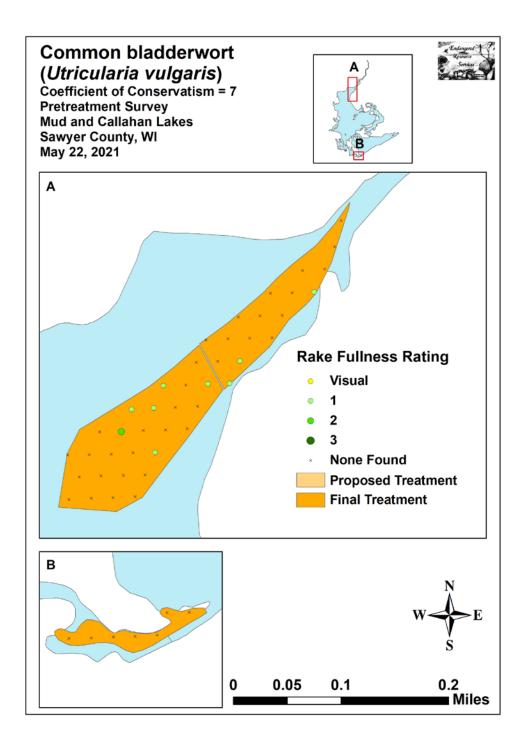












Appendix VII: Posttreatment Native Species Density and Distribution

