Herbicide Treatment Analysis for *Potamogeton crispus*- Curly-leaf pondweed (CLP)

Bone Lake, Polk County WI (WBIC: 2628100)

June, 2021

Surveys, mapping, and analysis provided by Ecological Integrity Service Amery, Wisconsin
Herbicide Application provided by Lake Restoration, Inc. Rogers, Minnesota

Analysis Summary

On May 17 and 24, 2021 13.07 acres of curly-leaf pondweed (CLP) (*Potamogeton crispus*) was treated with endothall. The same beds treated in previous years were adjusted somewhat to reflect changes made in the plant management plan. A pretreatment survey was conducted in April and a posttreatment survey was conducted in June during peak growth in other areas of the lake. A chi-square analysis showed that the reduction in frequency of occurrence within the treatment beds was statistically significant (71.6% to 19.8%; $p=2.6X10^{-15}$). A chi-square analysis comparing frequencies from the 2020 post treatment (same beds) survey and the 2021 post treatment survey showed no significant reduction occurred (it actually increased slightly). A 2020 pretreatment frequency comparison to 2021 pretreatment frequency chi-square showed no significant decrease. An analysis of the native plant species showed a reduction in one species, forked duckweed (*Lemna minor*) following the 2021 treatment. The frequency of CLP since 2014 has increased slightly a and the density of CLP in all treatment beds has decreased slightly since 2014. The turion analysis showed a small increase in the mean turion density of all beds compared to 2020. This was expected due to reduced treatment area within three beds compared to 2020. A bed mapping survey resulted in 30.35 acres of CLP beds dense enough and near the surface to potentially cause nuisance/navigation issues.

Introduction

On May 17 and May 24, 2021, a total of 13.07 acres of curly-leaf pondweed beds (five different beds) were treated with endothall. The permit was to treat 18.1 acres, but two beds (beds 3 and 4) were not treated because treatment couldn't be completed prior to warm water temperatures and substantial growth of native plant species. These beds were changed from 2020 due to changes in the management plan.

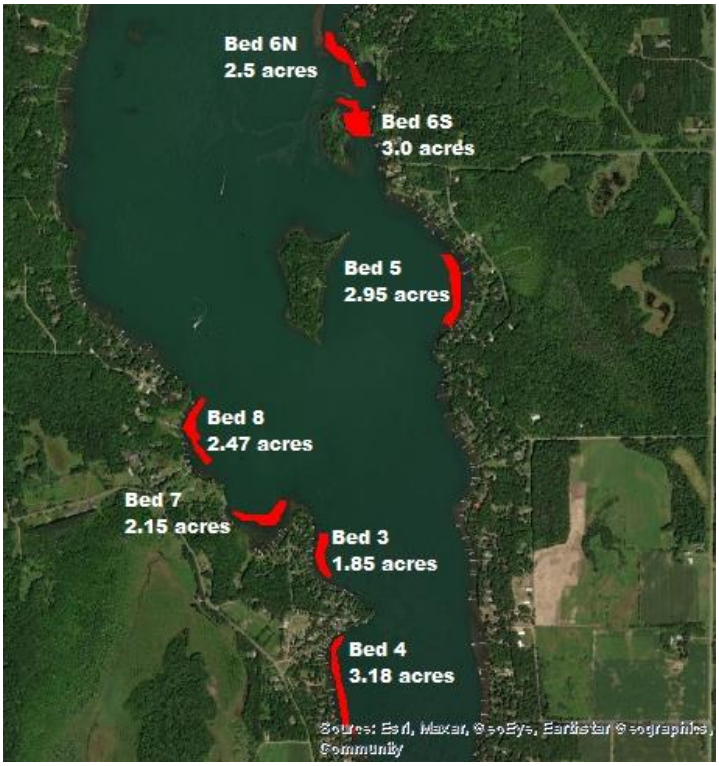

Figure 1: Map showing Bone Lake 2021 treatment beds.

Bed	Area (acres)	Mean Depth	Acre-feet	Herbicide Target Concentration	Date treated	Water temperature (°F)[1]	Wind conditions at treatment (mph/direction
3*	1.85	7.4	13.69	2.5 ppm	Not treated	n/a	n/a
4*	3.18	8.6	27.35	2.5 ppm	Not treated	n/a	n/a
5	2.95	8.2	24.19	2.5 ppm	5/17/21	58	5-7/SE
6-N	2.50	5.6	14.00	2.5 ppm	5/17/21	58	5-7/SE
6-S	3.00	6.3	18.90	2.5 ppm	5/17/21	58	5-7/SE
7	2.15	7.4	15.91	3.0 ppm	5/24/21	63	3-5/SW
8	2.47	6.5	16.06	3.0 ppm	5/24/21	63	3-5/SW
Total	18.1		130.09				

Beds 3 and 4 were not treated in 2021.

[1] Treatment day conditions of water temp. and wind conditions were reported by herbicide applicator.

Table 1: Summary of Bone Lake 2021 treatment bed parameters and treatment conditions.

Methods

To prepare for and analyze the treatment, two surveys are conducted following the protocol outlined in 2009 by the Wisconsin DNR. The first survey is referred to as a pretreatment survey. This involves going to predetermined GPS coordinates within the proposed treatment area. A high-definition underwater camera as well as a rake is used to determine the presence of CLP at each sample point. Density is not measured as the plants are typically very small and density is very subjective and variable. The presence of CLP is simply determined. There are also points checked outside of the bed delineation to assure the boundary is correct (not recorded unless CLP was present).

The second survey is referred to as the post-treatment survey. This survey involves going to the same GPS coordinates as the pre-treatment survey and doing a rake sample at the point. If any CLP is on the rake, the density of the CLP is recorded (see Figure 2 for reference). All other species are also recorded from the rake sample to assess potential damage to the native plants.

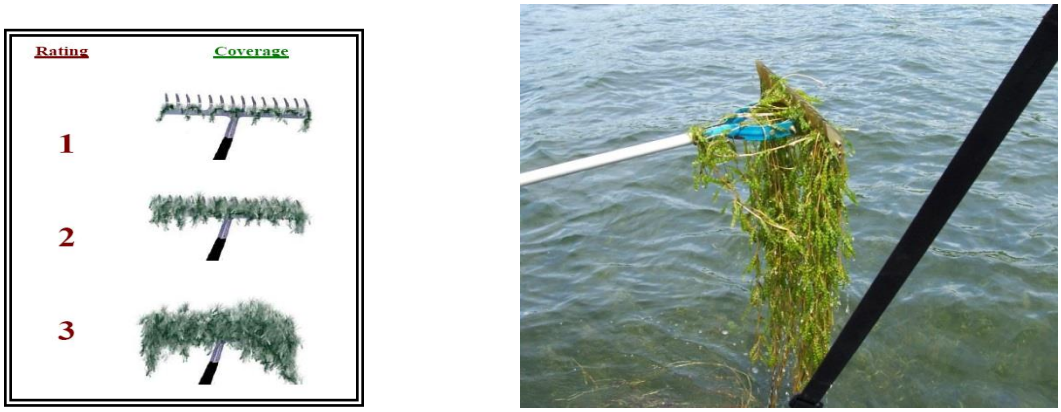

Figure 2: Density rating system and example CLP rake sample.

When the surveys are complete, the frequency of occurrence is determined as well as the mean density for each bed and all beds combined. The frequency of occurrence for each native plant species sampled is also calculated. Chi-square analysis is then used to determine if the change in frequency is statistically significant ($p<0.05$). With successful treatment, the chi-square analysis shows that the frequency of CLP is significantly reduced and the native plants are not significantly reduced.

The comparison for reduction is evaluated in three ways. First, the result from the previous year's post-treatment survey is compared to the present year's post-treatment survey. This reflects long-term effectiveness. As more treatments are done in annual succession, these frequency values can become very similar since the CLP growth is reduced so much. This can make it appear the treatment is not progressing successfully since the frequency appears to not be reduced. Each year, turions that germinate in the fall/winter create new growth. The result may be a low frequency in the post-treatment survey, but with turion germination, high-frequency CLP growth can result in the following spring.

Second, to reflect the effect the treatment has on new spring growth, a second comparison is done. This compares the frequency of CLP in the spring, pre-treatment survey to the post-treatment results in that same year. This shows what the CLP growth was just before treating and the result after treatment.

The third evaluation is comparing the pretreatment survey of the previous year(s). Since the spring growth will reflect CLP growth after turion germination, a reduction in the pretreatment frequency can show an overall reduction in CLP due to reduced turion germination, thus reflecting an overall reduction in CLP and its turions.

In the end, we want to see a statistically significant reduction when comparing the pre-treatment frequency to the post-treatment frequency. We would also like to see a consistent frequency reduction from year to year, depending on how low it is in both the pre- and post-treatment surveys of successive years. If the frequency in any post-treatment survey is very low (less than 10% as an example), then lowering it, even more, may be difficult. Turions can remain viable for several years, which can affect reduction amounts achieved.

To reflect potential future growth and the cumulative success of treatments, a turion analysis is conducted in the fall following treatment. This analysis involves going to sample points near the middle of the CLP bed (assuming this will reflect the highest density). At each sample point, a sediment sampler is lowered to the lake sediment and a sediment sample is obtained. Two samples are obtained from each side of the boat at each location. The samples are then sifted with a screened bucket to isolate the turions. The turions are then counted and the density of turions is calculated in turions/square meter. Consistently successful treatments should show a trend of reduced turion density each year. This way we know the treatments are killing plants before turion production, resulting in an overall reduction in CLP in those beds.

a.

b.

c.

Figure 3: Pictures showing turion density methods. a shows sediment sample; b shows separation; c shows separated turions.

Results

A pre-treatment survey was conducted in April 2021 followed by a post treatment survey conducted in early June 2021. The CLP frequency of occurrence of these two surveys were compared as well as to the post treatment survey from 2020 to determine effectiveness. Table 1 summarizes frequency and density data. The frequency from the post treatment survey in 2020 was adjusted for beds 6,7 and 8 to account for portions of the beds not treated in 2021 and the frequency and density of all beds does not include beds 3 and 4.

Bed	2021 Pre-treatment FOO	2021 Post treatment FOO	2020 pre-treatment FOO[#]	2020 Post treatment FOO[#]	2021 post treatment mean density	2020 post treatment mean density[#]
3*	52.6%	52.6%	50%	0%	0.84	0.00
4*	45.7%	42.9%	43.8%	6.25%	0.46	0.06
5	64%	0%	68%	28%	0	0.32
6N	70%	10%	66.7%	22.2%	0.1	0.50
6S	76%	36%	78.9%	26.3%	0.6	0.47
7	68.4%	42.1%	70%	0%	1.0	0.00
8	77.7%	14.8%	84.6%	0%	0.22	0.00
All treated beds (5,6N,6S,7 and 8)	71.6%*	19.8%*	74%	14.8%	0.36*	0.24

Beds 3 and 4 were not treated in 2021 because of a treatment window with appropriate wind conditions was not available prior to active growth of native plants. All treated bed FOO and density does not include these two beds in the calculation.

[#] 2020 frequency and density data adjusted to include only points within the 2021 treated portions.

Table 2: Frequency and mean density statistics for each bed from surveys in 2020 and 2021.

The herbicide treatment in 2021 resulted in a significant reduction in CLP frequency (all beds) from before treatment to after treatment (71.6% to 19.8%). Density from pretreatment to post treatment is not evaluated as the plants are so small in the early spring. There was rather significant growth of CLP after treatment in beds 6S and 7, which is not desirable.

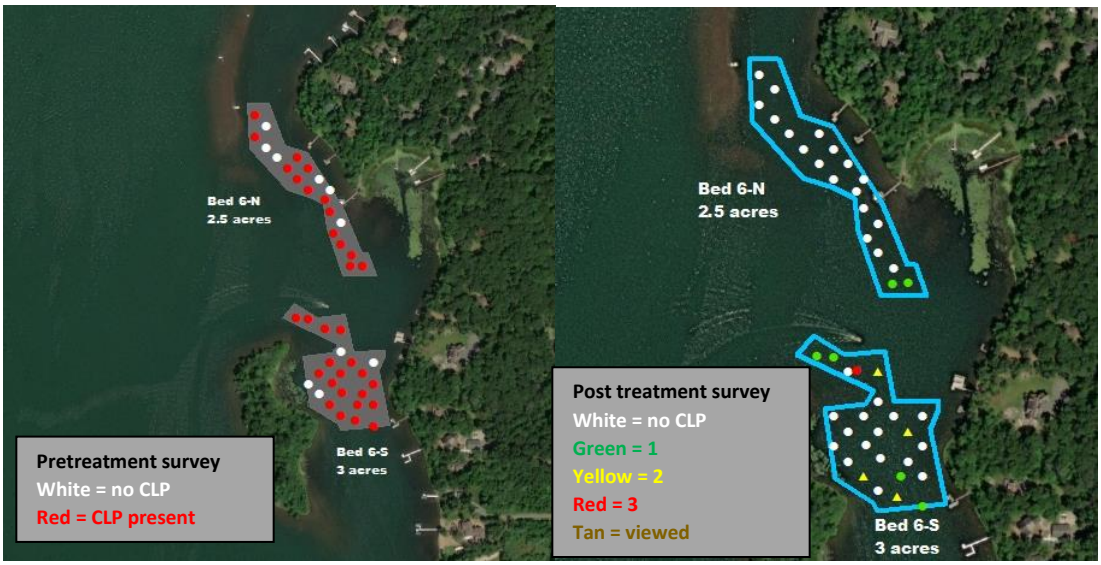

Figure 4: Pretreatment and post treatment CLP growth in Bed 6N and 6S, 2021.

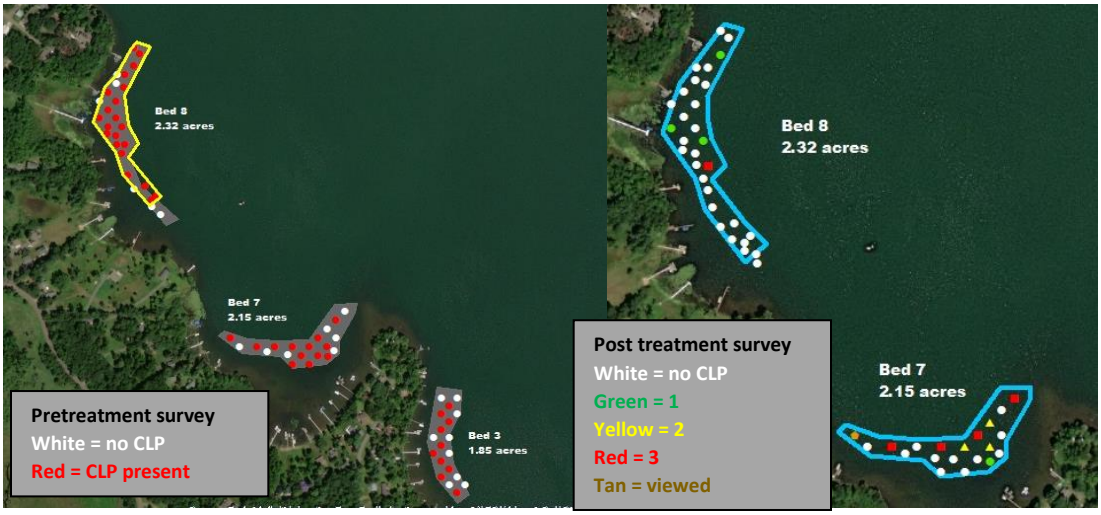

Figure 5: Pretreatment and post treatment CLP growth in Beds 7 and 8, 2021. Note the red and yellow in Bed 7 on the right (after treatment).

Figure 6: Pretreatment and post treatment CLP growth in Bed 5, 2021.

Beds 3 and 4 were not treated. There are wind criteria that must be met to allow treatment. When the winds finally subsided to the level that meets the criteria, the native plants were too far along in growth and treatment was cancelled to protect those plants.

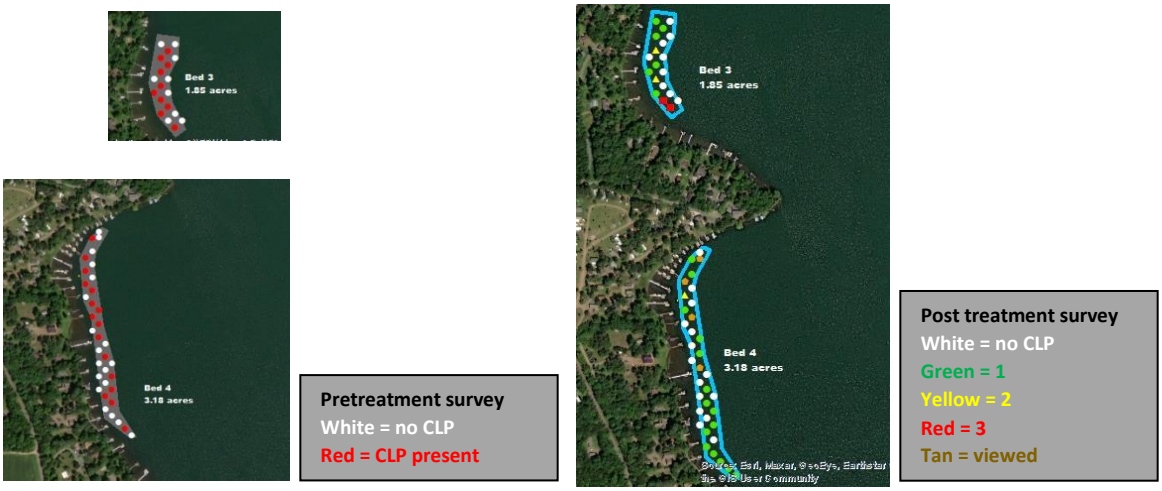

Figure 7: Pretreatment and post treatment CLP growth in Beds 3 and 4, 2021. These two beds were NOT treated with herbicide in 2021.

Figure 8 is a graph showing the change in frequency within each bed from the pretreatment survey 2021 to post treatment survey 2021. Note the higher FOO in beds 6S and 7, which were treated. Beds 3 and 4 were not treated so a higher FOO and the similar values between pretreatment and post treatment. Potential navigation issues/nuisance CLP was reduced in all beds except Bed 7 and somewhat in Bed 6S. Bed 7 had enough CLP after treatment to lead to navigation issues for boats.

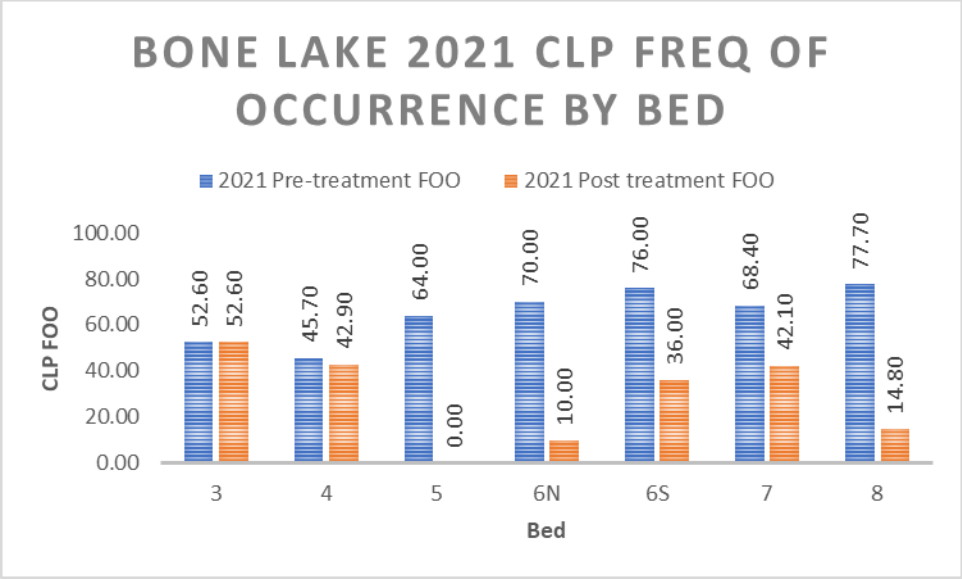

Figure 8: Graph showing the frequency of CLP within each bed from pretreatment and post treatment surveys in 2021.

The pre and post treatment frequency of CLP was similar between 2020 and 2021. There was a slight decrease in the pretreatment FOO, which may be due to the successful treatment in 2020, especially in Beds 7and 8 where the post treatment frequency in 2020 was 0% in both of these beds. Figure 9 shows the FOO from the pre and post treatment surveys in 2020 and 2021.

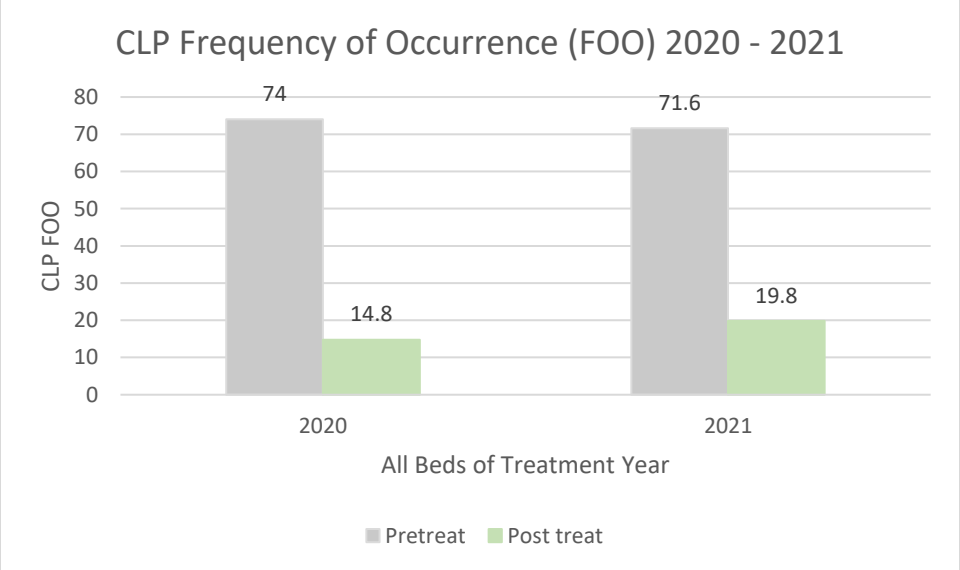

Figure 9: Graph comparing the pretreatment and post treatment CLP frequency in all beds from 2020 and 2021.

The chi-square analysis comparing the pretreatment to the post treatment frequency shows a statistically significant reduction ($P<0.001$). Comparison of changes in frequency of occurrence from various surveys from 2020 to 2021 as well as 2014 to 2021 were not found to be statistically significant (Table 3). The reduction that occurred after treatment meets the goal of reducing nuisance CLP and reducing navigation issues within these beds.

Survey comparison	Change	P value	Significant (P<0.05)
2021 Pre to 2021 Post	-	$2.6X10^{-15}$	Yes
2020 Post to 2021 Post	+	0.32	No
2020 Pre to 2021 Pre	-	0.67	No
2014 Pre to 2021 Pre	-	0.48	No

Table 3: Chi-square analysis results comparing various surveys.

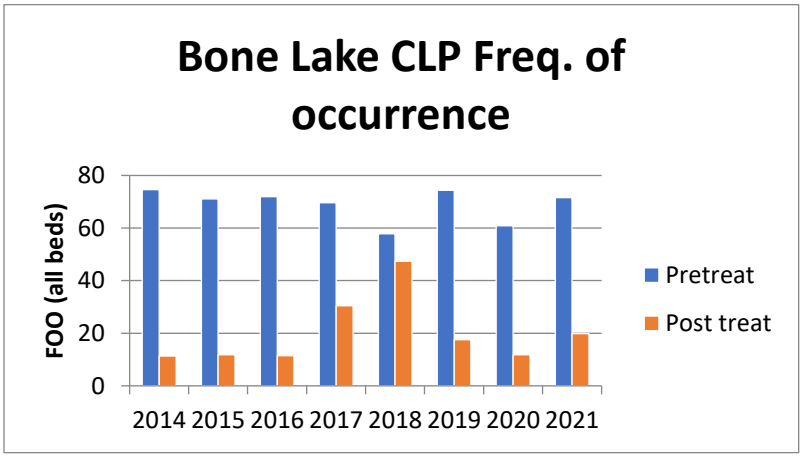

Figure 10: Graph showing FOO of pretreatment and post treatment surveys from 2014-2021.

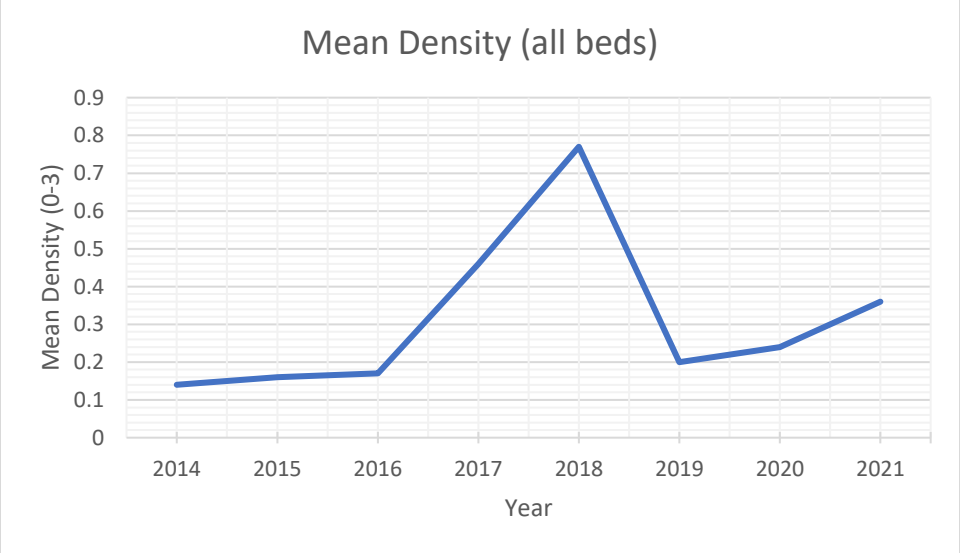

Figure 11: Graph showing long-term change in post treatment mean density (all beds).

Arguably the most effective analysis of long-term CLP reduction is to evaluate the change in CLP frequency from annual pretreatment surveys. This is due to the life cycle of CLP. If a CLP reaches maturity in the summer, it produces a reproductive structure known as a turion. When the plant dies, the turion settles into the sediment and germinates into a new CLP plant in late summer/early fall. This plant will then be present in the pretreatment survey before treatment. If a treatment effectively kills a

CLP plant, it cannot produce turions. This reduces the number of new plants present in the early spring, thus reducing the frequency of the CLP within a bed. With several subsequent effective treatments, the number of turions should go down, and be reflected by lower frequency value in the early spring pretreatment survey.

Figure 12 shows that the FOO in pretreatment surveys increased in 2019 due to no treatment in 2018. It is slightly decreasing in 2020 and 2021, but not immensely. The 2014 to 2020 comparison using a chi-square analysis shows the change is not statistically significant.

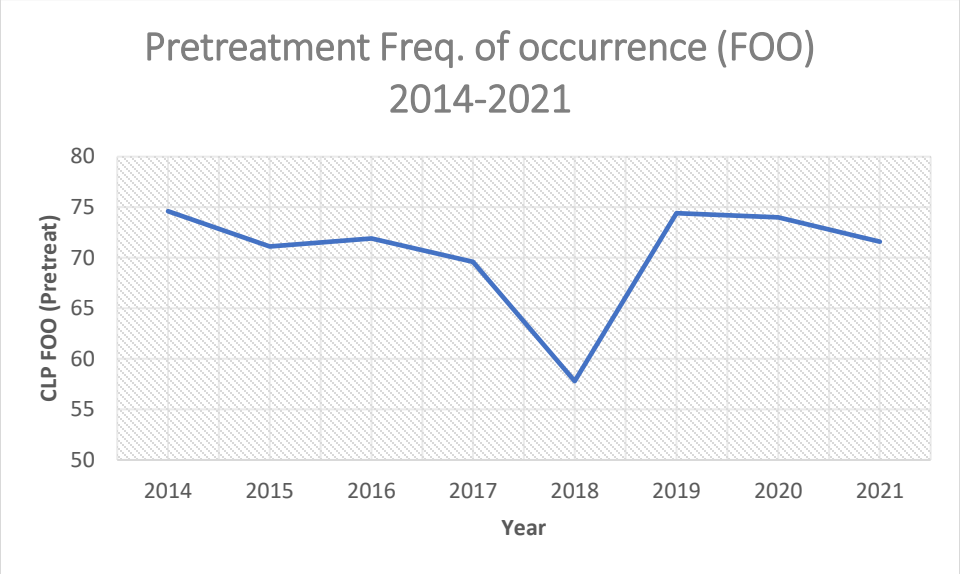

Figure 12: Graph showing long-term pretreatment frequency of CLP (all beds).

Estimated Phosphorus Reduction

Phosphorus reduction through CLP management is of interest to the Bone Lake District. As part of the Aquatic Plant Management Plan, the amount of phosphorus that was reduced due to treatment of the CLP was estimated. To estimate this amount, the area that was treated that actually had CLP growing was determined. It is assumed that the CLP would grow to a similar density that was used in a 2010 CLP nutrient study. This amount was reduced by the CLP that remained after treatment. The estimate is the gross phosphorus in kg, but not all of this phosphorus would likely be available and will settle into the sediment. Based upon the 2021 CLP data, the estimate for phosphorus reduction was ***9.9 kg***, which is ***0.31%*** of the estimated total phosphorus load that occurs in a typically year into Bone Lake[2].

Native species evaluation

An effective herbicide treatment not only results in a reduction in the target species, but with little or no reduction in any native species. The comparison of the post treatment surveys from 2020 and 2021 showed a statistically significant reduction in the native species *Lemna trisulca* (forked duckweed). It is possible this is due the herbicide, but it may also be due to natural growth variation from year to year.

[2] From a 2018 nutrient analysis conducted on Bone Lake.

Also, forked duckweed is free floating and is not rooted. It is usually sampled attached to other plants unless growing rather thick on the lake bottom, so may be prone to sampling variation. No other native species were shown to have a significant reduction.

Species with reduction	P value	Significance
Lemna trisulca-Forked duckweed	$2.3X10^{-4}$	Very ($p<0.001$)

Table 4: Chi-square analysis results to evaluate native species within the treatment beds.

The most recent Bone Lake Aquatic Plant Management Plan added the evaluation of native plant species within designated sensitive areas (established by the Wisconsin DNR) that are adjacent to CLP treatment beds. The baseline native species data was collected in June 2021. There is no data to compare to, however this baseline will be used in future CLP herbicide management analysis.

Turion Analysis

A turion analysis was conducted on Oct. 13, 2021. The same sample points were used as in all past turion analysis in order to monitor changes in turion density. With changes in bed treatment boundaries, some points are outside of the 2021 treatment area. This data can still be helpful to monitor changes in turions in the areas outside of the treatment areas.

The analysis shows that bed 8 has the highest mean turion density of 272 turions per square meter (in sediment). This is followed closely by bed 6 with 232. The lowest was in a bed that has not been treated for a few years (bed 2). Bed 7 was quite low at just under 72 turions/square meter, which has not been treated as many years as beds 3-5 (figure 13). The turions density is shown in maps that follow (figure 14).

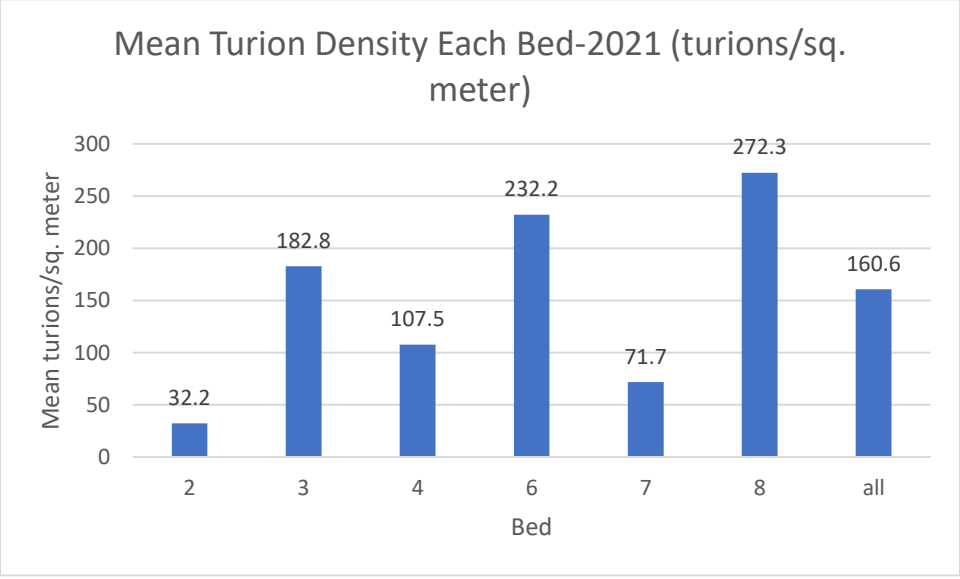

Figure 13: Graph showing the turion density within each bed from the October, 2021 turion analysis.

Figure 14: Maps of turion density within beds 2-8, from the Oct. 2021 turion analysis. White shows no turions, green up to 100, yellow up to 300, orange up to 700 and red more than 700. The density is listed near the sample point.

The turion density increased slightly from 2020 to 2021 for all beds combined (separated by period of time beds have been treated). This is likely due to the reduction in treatment areas in beds 6, 7 and 8. The reduced treatment would allow more CLP to grow and produce turions that would show up in the October samples. Bed 7 had some dense CLP growth after treatment in 2021, but the turion density did not change much. This may be due to sampling variation since turions are not likely to be evenly distributed within an area of CLP growth.

As the graph in figure 15 shows, beds 2-5 had a pretty significant mean turion density. Since beds 3 and 4 were not treated in 2021, an increase in turion density would be expected.

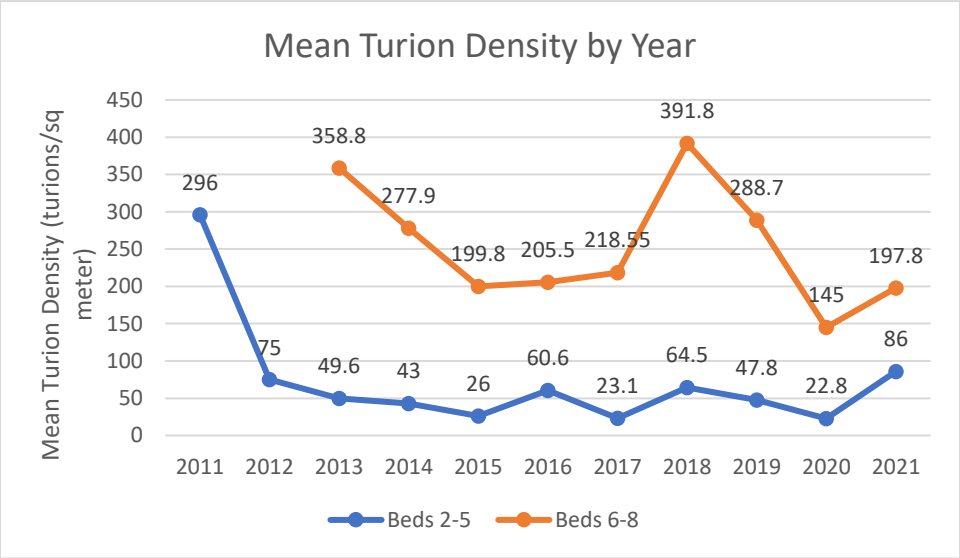

Figure 15: Graph showing long-term turion density separated into older treatment beds (2-5) and more recent treatment beds (6-8). In 2021, the size of beds 6-8 were made smaller to accommodate changes in the Aquatic Plant Management Plan.

2021 Bed Mapping

The CLP beds are mapped annually (inside and out of treatment beds). The focus on CLP management has changed some based upon the Bone Lake Aquatic Plant Management Plan update. The main objective is to reduce CLP nuisance and navigation issues, but treating certain beds. The beds mapped in 2021 reflect beds that are dense enough to delineate a border, with areas of CLP having an estimated density of 2 or greater and at least 50% of the bed with aerial coverage of CLP[3]. These bed density criteria may lead to nuisance levels of CLP, which could affect lake use such as navigation.

Table 5 shows the area of each bed mapped and Figure 16 is a map showing the location of each bed. The bed sizes ranged from a high of 16.3 acres (Bed A in northern most bay of Bone Lake) to low of 0.05 acres. Nineteen total beds were mapped, four of which occurred within or just adjacent to treatment beds from 2021 (Beds E, H, I and J which were within or adjacent to treatment beds 6S, 7 and 8 respectively). This increased growth adjacent to treatment beds is due to stopping treatment in portions of these beds that were historically treated. This increase in growth shows the result of

[3] This criterion is described in the Aquatic Plant Management Plan.

stopping treatment in these beds and can reflect the difference herbicide use makes in these nuisance beds. If these beds are treated again, this expansion may need to be considered.

Bone Lake CLP Bed-2021	Area (acres)
A	16.3
B	3.33
C	0.36
D	0.35
E	1.49
F	0.87
G	1.37
H	0.63
I	0.59
J	0.1
K	0.31
L	1.19
M	0.25
N	0.86
O	0.05
P	0.06
Q	1.16
R	0.2
S	0.88
Total	**30.35**

Table 5: Bone Lake CLP beds resulting from the June 2021 bed mapping survey.

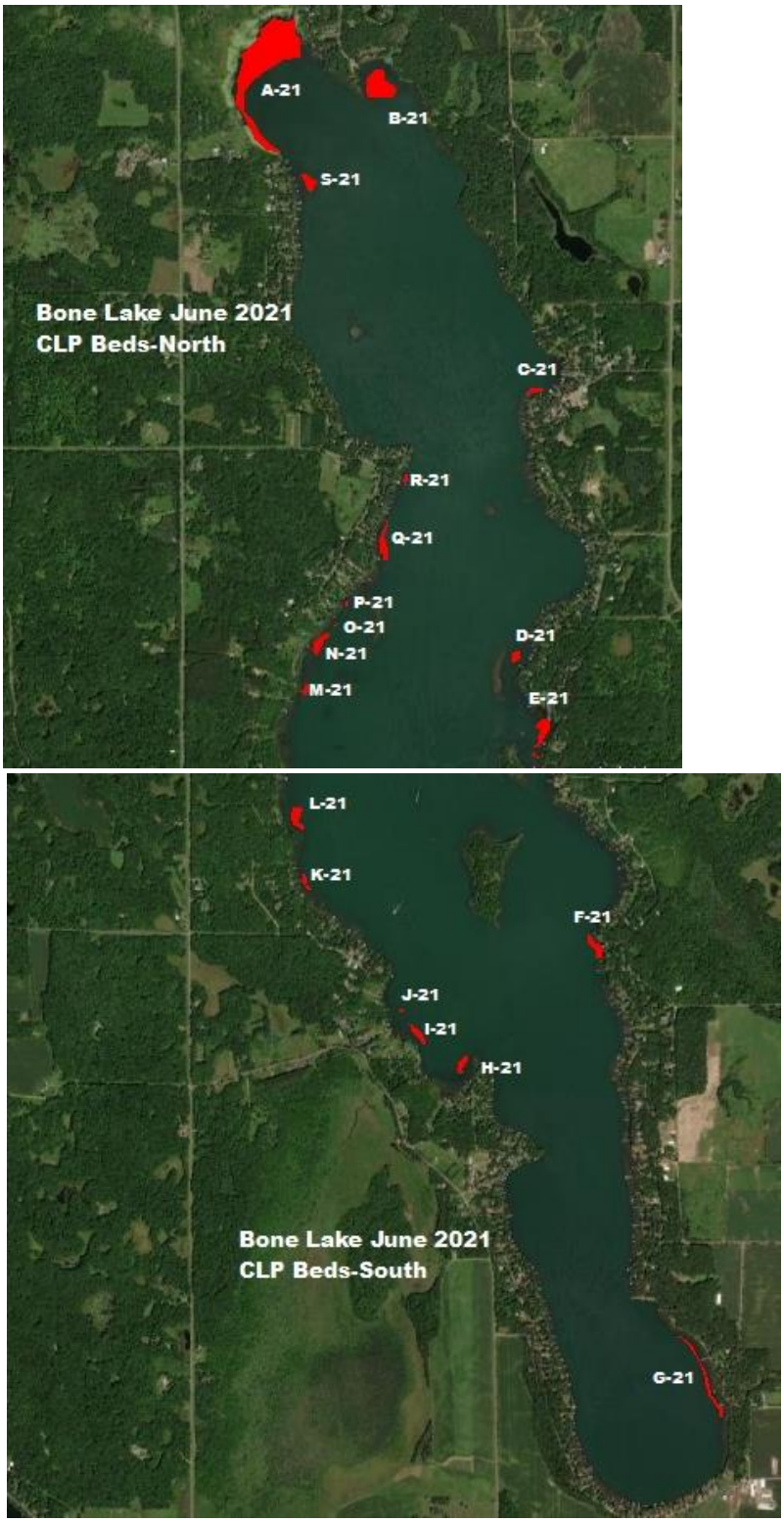

Figure 16: Bone Lake CLP bed map from June 2021.

Discussion

The main goal for CLP herbicide treatment on Bone Lake is to reduce nuisance CLP that can largely lead to navigation issues and other lake use. Based upon that goal, the 2021 treatment was mostly successful. The frequency of CLP went down in all beds. There was enough CLP remaining in Bed 7 to create navigation issues, and maybe to a small degree in bed 6S.

Beds 3 and 4 were not treated due to the late date that conditions were conducive for treatment. Too many native plants were actively growing. Since the pretreatment frequency was fairly low, the concern for native plant preservation took precedence and treatment was cancelled for these beds. This will likely lead to increase turion production and increased growth of CLP in 2022.

If long-term reduction can occur in some of the beds, it would help reach to goal of reducing nuisance CLP. Although the pretreatment frequency of occurrence was slightly lower in 2021, it is not a significant reduction. Long-term pretreatment frequency trends do not indicate significant progress in long-term CLP reduction. This can be due to limited treatment success, no treatment in 2018 as well as natural seasonal variation in CLP growth. The lowest pretreatment frequency was in 2018, but this could have been due to naturally reduced CLP growth in lakes that spring. Regardless, the 2021 pretreatment frequency was higher than 2018.

The bed mapping in 2021, resulted in 19 beds totaling 30.35 acres that are dense enough and have CLP close enough to the surface to be potential problem beds in regard to nuisance growth. Beds to be treated will be evaluated based upon their location, historical herbicide treatment, and potential effectiveness of treatment (some beds based upon shape, depth and exposure to wind would likely not result in adequate effective reduction from herbicide application).

The October 2021 turion analysis showed a slight increase in turion density within all beds combined. This was to be expected since beds 3 and 4 were not treated, and beds 6,7, and 8 were made smaller. This allowed for more CLP growth after treatment which would results in more plants creating turions. When the plant died off, the turions would be distributed into the sediment, resulting in higher turion counts.

References

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