The Treat / No-Treat Aquatic Contact Herbicide Study of 2019—2021 Second Year Interim Report <u>Table of Contents</u>

1. Study Description

2. Pickerel Bay Study Area—ProcellaCOR

Chart 1 Pickerel Bay Treated Areas Chart 2 Pickerel Bay Un-Treated Areas Data & Notes

3. Echo Bay Study Area—Aquastrike

Chart 3 Echo Bay Treated Areas Chart 4 Echo Bay Un-Treated Areas Data & Notes

4. Control Bays 1,2 & 3—Untreated

- Chart 5 Control Bay 1 Chart 6 Control Bay 2 Chart 7 Control Bay 3 Data & Notes
- 5. Interim Conclusions

The Treat / No-Treat Aquatic Contact Herbicide Study of 2019 – 2021 Second Year Interim Report

Study Description:

In the summer of 2017, the Tomahawk Lake Association proposed to the Wisconsin Department of Natural Resources a study of contact type chemical herbicides (ProcellaCOR) and AquaStrike) for the control of Eurasian Water Milfoil (EWM) in the Tomahawk Lake watershed of Oneida County. The study identified two segregated areas (bays) in which one herbicide per Bay was to be tested for efficacy in the control of EWM, and also identified three small isolated bays in the east side of the lake to be used as untreated control bays for comparative purposes. After consideration by the DNR, in August 2018 the WDNR approved the study.

The first year of the study called for the application of the two chemical herbicides to be made in early June 2019, followed by three years of monitoring the results of the applications through the use of aquatic plant point intercept surveys. Each of the two treated bays were subdivided into (1)"treated areas" and (2)"un-treated areas" within the bays, which were immediately adjacent to the treated areas. This was done to better understand the effects on areas surrounding the treated areas following those applications.

In the initial year there were three-point intercept surveys called for in the treated areas:

 Pre-treatment - done prior to the herbicide application in early June. (Sept 2018)

June 9^{th, 2019} herbicide application

- 2. Post Treatment done eight weeks following the treatment application.
- 3. End of Year (EOY) done in early September 2019.

In addition, aquatic plant point intercept surveys were mandated within the protocol for the three bays in the control areas. In the control bays which received no chemical applications, the following point intercept surveys were performed:

Pre-treatment - done in early July 2019

End of Year - done in early September 2019

Included in the pages that follow, are consolidated data charts which reference the growth trends to the plant communities over the course of the growing seasons following the chemical applications. Identified are a number of aquatic plant indicators which give a picture of the trends within the plant community over time. Also included are chi-square analysis charts for changes in plant presence of the individual plant species within each area.

Finally, there are short articles containing "Data and Notes" for all of the bays included in the study, which may give some insights into both trends and the factors that influenced those trends.

In order to better understand the dynamics which are in play for the initial year of the study coupled with growth trends of the year, this Second Year Interim Report will include the first years "Data & Notes" for each of the study areas, and also the Second years "Data & Notes" which will be <u>highlighted in blue text</u>.

Ned Greedy The Tomahawk Lake Association, Inc.

Data Collected for Year to Year Comparisons Of Plant Community Dynamics:

During the course of the Treat – No-Treat & Aquatic Contact Herbicide Study a number of specific environmental indicators are monitored for each of the seven specific areas within the study. These indicators are generated within the UW/Extension Lakes Aquatic Plant Management Guide workbook (appendix C), when data from specific point intercept surveys done in these areas are introduced into each of the individual areas work books.

The indicators utilized within this study are:

- 1. For individual species:
 - Frequency of occurrence at sites shallower than maximum depth of plants
 - Number of sites where species found
 - Average Rake fullness
- 2. Plant Community Summary:
 - Total number of sites visited
 - Total number of sites shallower than maximum depth of plants
 - Frequency of occurrence at sites shallower than maximum depth of plants
 - Simpson diversity Index
 - Maximum depth of plants
 - Average number of species per site
 - Average number of native species per site
 - Species richness
 - Floristic quality index (FQL)

The areas included within the Treat – No-Treat & Aquatic Contact Herbicide Study are:

Pickerel Bay:

- Pickerel Bay Treated Areas (see charts 1 & 1-2)
- Pickerel Bay Un-Treated Areas (see charts 2 & 2-2)

Echo Bay:

- Echo Bay Treated Areas (see charts 3 & 3-2)
- Echo Bay Un-Treated Areas (see charts 4 & 4-2)

Control Bays:

- Control Bay #1 (see charts 5 & 5-2)
- Control Bay #2 (see charts 6 & 6-2)
- Control Bay #3 (see charts 7 & 7-2)

The protocols for the Treat / No-Treat and Aquatic Contact Herbicide Study call for the collection of data in the following sequence:

 Pre-treatment point intercept surveys of all areas within the study to establish a baseline for use in comparison with subsequent surveys. This initial PI survey was done in early September 2018.

Date of the first and only herbicide application done within the study - June 9, 2019.

- Post-treatment point intercept survey of all areas within the study, beginning the process of building a comparative history focused on the effects or lack of effects of the treatments that each study area experienced. This survey was completed six plus weeks following the herbicide applications.
- 3. End of Year (EOY) 2019-point intercept survey of all areas within the study at the end of the growing season of the initial year of the study. This survey was completed at the beginning of September 2019.
- 4. End of Year (EOY) 2020-point intercept survey of all areas within the study at the end of the second growing season. This survey was completed in early September 2020.
- 5. End of Year (EOY) 2021-point intercept survey of all areas within the study at the end of the third growing season this survey will be completed in early September 2021.

Interim reports at the end of the first and second growing seasons, and a final report at the end of the third growing season.

Study Area # 1 Pickerel Bay Treated with ProcellaCOR On June 9th, 2019

Chart #1 – Pickerel Bay Treated Area Chart #2 – Pickerel Bay Un-Treated Area 2020 Pickerel Bay Data & Notes

Chart #1 - 2020 Pickerel Bay Treated Points Trends				
Tomahawk Lake				
Oneida County				
number of Point Intercept sites = 49	Pre-treat (9/2018)	Post-treat (7/2019)	E.O.Y. (9/2019)	E.O.Y. 9/2020
Eurasian water milfoil specific				
Frequency of Occurance (shallower than Max. Depth of plants)	87.76	53.06	57.14	71.43
Number of sites where species found	43	26	28	35
Average rake fullness	1.37	1.12	1.32	1.66
Summery Statistics				
Total number of sites visited	49	49	49	49
Total number of sites shallower than max. depth of plants	49	49	49	49
frequency of occurance at sites shallower than max. depth of plants	100.00	100.00	100.00	100
Simpson Diversity Index	0.88	0.89	0.90	0.88
Maximum depth of plants	12.20	10.00	10.50	10
Average number of species per site (s.t.m.d.p.)	4.88	4.39	4.78	4.35
Average number of native species per site (s.t.m.d.p.)	4.20	3.86	4.20	3.63
Species Richness	19.00	22.00	23.00	17
Floristic Quality Index	29.70	29.46	30.70	24.5

Chart #2 - Pickerel Bay Un-Treated Points Trends				
Tomahawk Lake				
Oneida County				
number of Point Intercept sites = 38	Pre-treat Sept 2018	Post-treat july 2019	E.O.Y. Sept. 2019)	E.O.Y. Sept. 2020
Eurasian water milfoil specific				
Frequency of Occurance (shallower than Max. Depth of plants)	31.58	16.22	26.32	32.43
Number of sites where species found	12.00	6.00	10.00	12
Average rake fullness	1.08	1.00	1.50	1.25
Summery Statistics				
Total number of sites visited	38.00	38.00	38.00	38
Total number of sites shallower than max. depth of plants	37.00	37.00	38.00	37
frequency of occurance at sites shallower than max. depth of plants	100.00	100.00	100.00	97.37
Simpson Diversity Index	0.89	0.90	0.90	0.9
Maximum depth of plants	10.60	10.80	10.50	11
Average number of species per site (s.t.m.d.p.)	3.95	4.49	4.45	4.39
Average number of native species per site (s.t.m.d.p.)	3.60	4.15	4.16	4.08
Species Richness	14.00	23.00	17.00	19
Floristic Quality Index	22.46	31.34	25.25	26.87

2019 Pickerel Bay Data and Notes

2020 Pickerel Bay Data and Notes

The 2019 treat/no treat study which began with pretreatment aquatic plant surveys done in the fall of 2018 in the treated areas and in July 2019 in the control areas and that will continue over the following three seasons seeks to examine the efficacy of two contact type chemical herbicides segregated into two small bays in the western side of Tomahawk Lake. In Pickerel Bay, the chemical herbicide ProcellaCOR (Florpyrauxifen-benzyl) was applied to 11.1 acres at a rate of four PDUs per acre/foot based on an average depth of 8 feet. While ProcellaCOR is a systemic herbicide, similar to contact herbicides, it is extremely fast acting and is virtually gone from the water column within three hours following application. For the purposes of the Treat/No Treat Aquatic Herbicide Study, Pickerel Bay was divided into two parts in order to compare the areas which were treated with the herbicide and those areas which were un-treated but adjacent to the treated areas.

The first of three point intercept surveys (pre-treatment) in these two areas were done in September 2018, with the herbicide application taking place in the first week of June 2019. The post-treatment surveys were done eight weeks following the application in the end of July 2019, and the final "end of year" surveys were done in early September 2019

Included within these notes are comparative data charts for each of the two areas and also included are chi-square analyses for pre-treatment versus post-treatment and pre-treatment versus end of year surveys these were done to understand the effects of the ProcellaCOR

application after eight weeks following the application and after the full 2019 growing season (EOY).

Pickerel Bay Treated Areas:

Over the course of several years, Pickerel Bay had become infested with Eurasian water milfoil at spot locations, which were treated in some years with 2,4-D. Over the course of time the spot locations grew in both spatial area and plant density, and in 2017 and again in 2018 the infestation level on the western perimeter of the bay became extremely heavy and hampered recreational use for riparian owners and a commercial girls camp at the head of the bay. As the bay was heavily used for waterskiing by the camp, and for fishing by Lake users, the Eurasian Water Milfoil infestation began to routinely canopy and spread on the top of the water by 2018.

Pickerel Bay was chosen as one of the two herbicide test bays in the treat/ no treat study because it was segregated from the rest of the lake by a long shallow sandbar and very high highland areas surrounding the rest of the bay. This creates a "Bowl" which as much as possible segregates the bay from the rest of the lake.

Chart #1 - Pickerel Bay Treated Points:

Chart #1 - 2019 – Aquatic Plant Dynamics – Pickerel Bay – Treated Points Only notes the important data comparisons made in the three point intercept surveys performed during the 2019 growing season. They indicate the changes which took place in the treated areas following the application of the chemical aquatic herbicide ProcellaCOR.

The frequency of Occurrence for Eurasian Water Milfoil at sites shallower than the maximum depth of plants for these three-point

intercept surveys indicate that while the ProcellaCOR application had an immediate negative effect on Eurasian Water Milfoil (target species), the effect of the application was short-lived. The trend line is as follows:

Pre-treatment EWM Frequency of Occurrence	87.76
Post-treatment EWM Frequency of Occurrence	53.06
End of Year EWM Frequency of Occurrence	57.14

The same trend presented itself in the number of sites the species was found, indicating an initial reduction in the number of sites due to the treatment but that the progress made was short-lived:

Pre-treatment sites	43 of 49
Post-treatment sites	26 of 49
End of Year sites	28 of 49

Finally, similar results are noted in the average EV	VM Rake fullness:
Pre-treatment rake fullness	1.37
Post-treatment rake fullness	1.12
End of Year rake fullness	1.32

It would appear that the EWM plants within the treated area received an application of ProcellaCOR that was sufficient to "burn" the EWM plant foliage but was not sufficient to kill the plants completely. (As an aside, this is also a possible reason for the poor results experienced with AquaStrike in Echo Bay.) Considering the claims made by SePRO, the company which produces ProcellaCOR, that this herbicide effectively kills virtually 100% of EWM while being very friendly to the native plant community, one would have to say that the results in EWM control within the treated areas of Pickerel Bay with this herbicide application were disappointing in the least. In looking at chart #1's summary statistics for all plants it would appear that the total plant community was less affected by the application of ProcellaCOR then Eurasian Water Milfoil specifically. The Frequency of Occurrence at sites shallower than maximum depth of plants remained unchanged at 100%. The Simpson Diversity Index trended very slightly higher in each of the surveys and the average number of species per site dipped slightly at eight weeks but recovered strongly by the end of the year. The average number of native species per site dipped slightly at eight weeks from 4.20 to 3.86 but returned to 4.20 by the end of the year. Species richness increased from 19.0 pretreatment to 23.0 at the End Of Year survey. Conversely, the floristic quality index showed an overall increase from 29.7 pretreatment to 30.70 at the end of year survey. These figures tend indicate that SePRO's claim that ProcellaCOR was indeed friendly to the native plant community was correct.

2nd Year Chart #1 Pickerel Bay Treated Points Data & Notes: In late August of 2020, an "End of Year" Point Intercept Survey was performed in Pickerel Bay, to continue to examine the effects of the ProcellaCOR Aquatic Plant Herbicide application done in June of 2019. The data set from this P.I. survey was added to those created from the three surveys done in 2019, so that long term data trends could be developed that would accurately describe the effects of the herbicide treatment on the plant community. With the addition of the 2020 End of Year survey data set to the three survey data sets developed in 2019, the picture of the dynamics of plant growth within Pickerel Bay become clearer. (see Chart 1):

EWM: prior to the initial ProcellaCOR treatment in June 2019, the frequency of occurrence of EWM in the treated areas of Pickerel Bay

was 87.76. Six weeks following the treatment date the frequency of occurrence had fallen to 53.06. The EWM Pickerel Bay treated area frequency of occurrence in the sites shallower than the maximum depth of plants then began to rebound, and by the end of year 2019-point intercept survey EWM frequency of occurrence had risen to 57.14. In the end of year 2020 survey the EWM frequency of occurrence had rebounded further to 71.43. This trend of a small reduction in frequency of occurrence immediately following the treatment and then a steady rebound in frequency of occurrence through the end of year 2020 was mirrored in the number of sites where EWM was found over the course of the two growing seasons, (43 pre-treatment sites/26 posttreatment sites/28 end of year 2019 sites/35 end of year 2020 sites), as well as in plant density as described as average Rake fullness (1.37 pretreatment /1.12 posttreatment/1.32 end of year 2019/1.66 end of year 2020.)

<u>All Plant Species:</u> The Treat/No-Treat Aquatic Contact Herbicide Study protocols not only called for the examination of the effects on the target species (EWM) in the treated areas but also the effects of the application on the native plant community in the treated areas. As stated in the first- year interim report, the producer of ProcellaCOR claimed that the effects of ProcellaCOR on the non-target native species would be very limited. The addition of an additional growing season seems to continue to bear out the manufacturers claims.

While the summary of statistics developed for the primary indexes include some minor variations from survey to survey (see chart 1) there is very little change over the four survey data sets. The Chi Square analysis for the Pickerel Bay treated areas indicate virtually no significant changes from pre-study survey (9/2018) through the End of Year survey (9/2020). Only the target species – EWM showed a slightly significant reduction in species presence. No other changes in the presence within the native plant community was deemed significant.

Chart #2 - Pickerel Bay Un-Treated Points:

Chart #2 - 2019 – Aquatic Plant Dynamics – Pickerel Bay – Un-Treated Points Only tends to show that the un-treated portions of Pickerel Bay experienced much the same effects from the ProcellaCOR application as did the treated areas. In the case of Eurasian Water Milfoil, Frequency of Occurrence, the number of sites where the species was found, and the Average Rake fullness ratings for EWM followed the same pattern of an initial partial control after eight weeks, followed by a rebound in spatial and density growth by the End of Year.

Summary statistics for all plants again indicated only minimal changes to this pattern. These changes occurred in Species Richness and the Floristic Quality Index, where the post treatment survey which occurred eight weeks following the application date showed significant increases, but which fell back by the end of the year. These two indices however did show a net increase in species richness from 14 to 17, and in the Floristic Quality Index from 22.46 pre-treatment to 25.25 by the end of year survey in September 2019. Again, these statistics seem to validate ProcellaCOR's stated claim that it has little or no effect on non-target native plants.

The Chi Square analysis for the treated areas within Pickerel Bay Pickerel Bay indicates that Eurasian Water Milfoil showed "highly significant", and also "highly significant" Najas flexilis and Najas guadalupensis. While these changes for Eurasian Water Milfoil are correct, the changes for the two Najas species were due to misidentification by the surveyor for these two plant species during the surveys. The Chi Square analysis for the untreated points indicated that while there was no significant change within Eurasian Water Milfoil, there were "highly significant" changes for the two Najas species identified. Again these changes were due to surveyor error in identification of these two species.

In the final analysis for Pickerel Bay one would have to say that SePRO's claim that ProcellaCOR was "kind to natives" was indeed verified, but their claim to the almost total effectiveness against Eurasian Water Milfoil was considerably overstated.

2nd Year Chart 2 Pickerel Bay Un-Treated Points: In addition to the 2020 end of year point intercept survey done in the treated areas of Pickerel Bay, a 2020 End of Year Point Intercept Survey was also done in the un-treated areas in the remainder of Pickerel Bay. The untreated areas within Pickerel Bay were surveyed to understand the effects of any bleed over of ProcellaCOR into the untreated areas of the bay. The results of the addition of end of year September 2020 survey data into "Chart #2 - treated Pickerel Bay Un-Treated Points Trends" provides the following perspectives:

EWM: Frequency of Occurrence in the adjacent untreated areas of Pickerel Bay following the June 2019 application of ProcellaCOR showed a significant reduction immediately following the treatment followed by a rapid rebound by the end of year 2019 survey. Frequency of occurrence at the end of year 2020 survey in the untreated areas had rebounded and surpassed frequency of occurrence levels in the pretreatment 2018 survey. This dynamic trend is consistent with the other treated and untreated areas within the study, where EWM Frequency of Occurrence levels show an immediate reduction following the herbicide application which then progresses to a steady increase in Frequency of Occurrence levels throughout the remainder of the growing season. This pattern continues throughout the second year of the study (end of year 2020 survey). By the end of the 2020 growing season virtually all of the indexes had returned to EWM Pre-Treatment (September 2018) levels.

All Plant Species: The summary statistics for all plants noted on "Chart #2- Pickerel Bay Un-Treated Points Trends" remain virtually unchanged from the pretreatment September 2018 survey compared to the end of year September 2020 survey. This would indicate that the ProcellaCor Treatment made in June 2019 had virtually no effect on the long-term aquatic plant community in the untreated portions of Pickerel Bay. The chi Square analysis indicates virtually no significant changes from pre-application levels through the end of the second year of the study in the species present. Only Elodea canadensis (13 to 25 sites) and Potamogeton robbinsii (32 to 24 sites) indicated any significance to the number of sites present.

While we have no theories concerning the lack of success of the ProcellaCOR application in Pickerel Bay, we do find it curious that ProcellaCOR applications in nearby lakes at virtually the same time and under virtually the same environmental conditions had near perfect Eurasian Water Milfoil control results. The only conclusions that we are able to draw at this time are that the concentration of ProcellaCOR put down during the application was insignificant to effectively kill the Eurasian Water Milfoil plants. Our herbicide applicator assures us that this was not the case.

Study Area # 2 Echo Bay Treated with Aquastrike On June 9th, 2019

Chart #3 – Echo Bay Treated Area Chart #4 – Echo Bay Un-Treated Area 2020 Echo Bay Data & Notes

Chart #3 -Echo Bay Treated Points Trends				
Tomahawk Lake				
Oneida				
Number of Point Intercept Sites = 57	Pre-treat Sept 2018	Post-treat July 2019	E.O.Y. Sept 2019	E.O.Y. Sept 2020
INDIVIDUAL SPECIES STATS:	EWM	EWM	EWM	EWM
Frequency of occurrence at sites shallower than maximum depth of plants	92.59	58.18	67.31	77.78
Number of sites where species found	50	32	35	42
Average Rake Fullness	2.69	2.13	2.44	1.98
present (visual or collected)	present	present	present	present
SUMMARY STATS:				
Total number of sites visited	58	58	58	57
Total number of sites with vegetation	54	51	52	53
Total number of sites shallower than maximum depth of plants	54	55	52	54
Frequency of occurrence at sites shallower than maximum depth of plants	100	92.73	100	100
Simpson Diversity Index	0.87	0.89	0.9	0.88
Maximum depth of plants (ft)**	14.3	16.5	14.1	15.5
Average number of all species per site (shallower than max depth)	3.67	2.91	3.39	3.11
Average number of native species per site (shallower than max depth)	2.78	2.35	3.03	2.33
Species Richness	21	20	22	21
Floristic Quality Index (FQI)	30.41	28.22	29.89	28.85
**SEE "MAX DEPTH GRAPH" WORKSHEET TO CONFIRM				

Chart #4 - Echo Bay Un-Treated Area Trends				
Tomahawk Lake				
Oneida				
Number of Point Intercept Sites = 31	Pre-Treat Sept 2018	Post-Treat July 2019	E.O.Y. Sept 2019	E.O.Y. Sept 2020
INDIVIDUAL SPECIES STATS:	ewm	ewm	ewm	ewm
Frequency of occurrence at sites shallower than maximum depth of plants	87.5	20.0	30.0	46.67
Number of sites where species found	7.0	2.0	3.0	7
Average Rake Fullness	1.3	1.0	1.0	1.29
present (visual or collected)	present	present	present	present
SUMMARY STATS:				
Total number of sites visited	26.0	26.0	26.0	31
Total number of sites with vegetation	8.0	10.0	8.0	12
Total number of sites shallower than maximum depth of plants	8.0	10.0	10.0	15
Frequency of occurrence at sites shallower than maximum depth of plants	100.0	100.0	80.0	80
Simpson Diversity Index	0.9	0.9	0.9	0.9
Maximum depth of plants (ft)**	17.7	17.8	14.3	17.7
Average number of native species per site (shallower than max depth)	2.9	2.3	2.9	2
Average number of native species per site (veg. sites only)	3.3	2.6	3.6	2.73
Species Richness	16.0	15.0	14.0	13
Floristic Quality Index (FQI)	26.7	22.5	21.4	26.44
**SEE "MAX DEPTH GRAPH" WORKSHEET TO CONFIRM				

2019 Echo Bay Data & Notes

2020 Echo Bay Data & Notes

The 2019 Treat / No-Treat study which began with pretreatment aquatic plant surveys done in the fall of 2018 in the treated areas, and in July of 2019 in the control areas and that will continue over the following three seasons, seeks to examine the efficacy of two "contact type" chemical herbicides segregated into small bays in the western side of Tomahawk Lake.

In Echo Bay the herbicide identified by the brand-name AquaStrike (a combination of Endothall & Diquat) was applied to 14 acres in the spring of 2019. In Pickerel Bay the herbicide ProcellaCor (Florpyrauxifen – benzyl) was applied to 11.1 acres, also in the spring of 2019. Comparative data sets for both Echo Bay treated areas as well as Echo Bay untreated areas are included in these notes along with Chi-Square analysis files.

Within the 14 acre treatment area within Echo Bay, the contact herbicide AquaStrike aquatic plant herbicide was applied. Aqua strike is a mixture of 28.6% die potassium salt of Endothall combined with 10.6% of Diquat dibromide. The remaining 60.8% of the mixture is noted as other ingredients. Aquastrike was applied at 1.5 gallons per acre foot to 14 acres based on an average depth of 10 feet.

For each of the two areas within Echo Bay(treated and untreated), three point intercept surveys were performed, including a pretreatment survey done in September 2018, a post treatment survey done in the last week of July 2019, and an end of year survey done in September 2019. Included within these notes are comparative data charts for each of the two areas. Also included are Chi-Square analysis for Pre-Treatment versus Post-Treatment, and Pre-Treatment versus End of Year surveys. These were done to understand the effects of the AquaStrike application after eight weeks following application, and after the full 2019 growing season (EOY).

Echo Bay Treated Area:

The Echo Bay treated area began to become heavily infested with Eurasian water milfoil in 2017 and 2018. During those growth periods EWM began to take over the surface of the treated area and plant density became a real nuisance to navigation. Riparian owners and Lake users became concerned that the infested areas were not usable for recreational purposes, and that some docks within the bay were not approachable by any motorized means. The Echo Bay area was included in the Treat/ No-Treat study because the layout of the bay allowed for complete segregation of the herbicide application, and minimal effects of weather-related environmental factors on the application.

Chart #3 - Echo Bay Treated Points Areas Data & Notes

The Echo Bay Treated Points Trends point out the important data comparisons made in the three point-intercept surveys made during the 2019 growing season which indicate the changes which took place following the application of the chemical aquatic herbicide AquaStrike. While the pretreatment survey was taken in September 2018, the application of the herbicide actually took place in the first week of June in 2019. Eight weeks following the herbicide application the posttreatment point intercept survey was taken in late July. The third and final End Of Year point intercept survey was taken in September 2019.

The <u>frequency of occurrence for Eurasian Water Milfoil</u> at sites shallower than the maximum depth of plants for these three-point intercept surveys indicate that while the AquaStrike application seemed to have an immediate negative effect on Eurasian water milfoil (the target species), the effect of the application was short-lived. The trend line is as follows:

Pre-Treatment Survey EWM Frequency of Occurrence	92.59
Post-Treatment Survey EWM Frequency of Occurrence	62.75
End of Year Survey EWM Frequency of Occurrence	67.31

In the same manner the number of sites where the species was found indicated an initial significant drop in foliage spatial area and plant stem density followed by a somewhat less significant rebound:

Pre-Treatment sites	50
Post-Treatment sites	32

In much the same manner, average EWM Rake fullness indicated this same pattern:

Pre-Treatment rake fullness	2.69
Post-Treatment rake fullness	2.13
End of Year rake fullness	2.44

Anecdotal Visual examination of the treated areas by the surveyor notes that while the initial response to the herbicide treatment seemed to have a positive effect on the removal of Eurasian water milfoil from the water column, over time and particularly within the month of August Eurasian water milfoil plant foliage returned to the surface and in many cases spread out horizontally on the surface.

This pattern of an initial negative response followed by a relatively rapid rebound of plant foliage growth was replicated <u>within the native plant community</u> as well. Overall frequency of occurrence at sites shallower than the maximum depth of plants dropped from 100% in the pretreatment survey to 92.73% in the July post treatment survey to 100% in the end of year survey. The Simpson diversity index showed no post-treatment drop and went from pretreatment 0.87 to posttreatment 0.89 to 0.90 in the end of year survey. While the number of all species per site went from a pre-treatment value of 3.67 to a value of 2.91 in the posttreatment survey, the end of year survey indicated a rebound up to 3.39 in September. In much the same way the average number of native species per site shallower than maximum depth went from 2.78 in the pretreatment survey to 2.35 in the posttreatment survey, and increased to 3.03 in the end of year survey. The species richness trend went pretreatment 21, posttreatment 20 and end of year of 22. Finally the floristic quality index indicated a pretreatment FQI of 30.41 to a Post-Treatment FQI of 28.22, and an End of Year FQI of 29.89.

Seemingly all of these indexes displayed the same basic pattern of an initial negative effect on the aquatic plants present followed by a substantial rebound at the end of the year. An overall assessment of this application of AquaStrike

aquatic plant herbicide in the treated areas of Echo Bay would be that the herbicide did not meet the anticipated expectations.

2nd Year Chart #3 Echo Bay Treated Areas Data & Notes: In late August 2020 an End of Year Point Intercept Survey was performed in Echo Bay to continue to examine the effects of the Aquastrike Aquatic Plant Herbicide application done in June 2019. The data set from this point intercept survey was added to those created from the three surveys done in 2019 so that a long-term data trend could be developed that would accurately describe the effects of the herbicide treatment on the plant community. With the addition of the 220 end of year survey data set to the three survey data sets developed in 2019 the picture of the dynamics of plant growth within Echo Bay become clear. (See chart 3)

EWM: Prior to the initial aqua strike treatment in June 2019 the frequency of occurrence of EWM in the treated areas of Echo Bay was 92.59. Six weeks following the treatment date, the frequency of occurrence in the treated areas of Echo Bay had fallen to 58.18, indicating that the Aquastrike treatment had been marginally effective in reducing EWM in the treated areas. However, the effects of the treatment was short-lived, as by early September the frequency of occurrence had rebounded to 67.31 in the end of year 2019 survey. This pattern continued during the summer of 2020, with the End of Year 2020 survey indicated that EWM frequency of occurrence had rebounded to 77.78.

The other indicators of EWM plant development mirrored the pattern that had been established with frequency of occurrence in that while the pretreatment number of sites where the EWM was found (50 sites) fell to just 32 sites six weeks following the treatment. Again this reduction in the number of sites where the species was found was short-lived as the end of year 2019 survey indicated that the number of sites had increased back to 35 sites and by the end of year 2020 survey the number of sites had rebounded to 42 sites. Average Rake fullness that was noted at 2.69 in the pretreatment survey in 2019 had dropped to 2.136 weeks following the date of treatment, but had rebounded to 2.44 at the end of year 2019 survey the end of year 2020 survey showed that the average Rake fullness had fallen to 1.98 indicating that while the EWM infestation in the treated areas of Echo Bay increased through the growing season of 2020 the average plant density of EWM at the sites surveyed had decreased somewhat.

All Plant Species: The overall plant growth dynamics within the treated areas within Echo Bay, which includes both native plants as well as EWM, would indicate that while some relatively minor negative effects occurred within the plant community following the aqua strike application, that these negative effects were short-lived. The overall patterns observed throughout the first and second growing seasons would indicate that the herbicide had a marginal effect on the plant community following the application, and that in the majority of plant species observed the rebound to pretreatment levels was steady and rapid. The trends from pretreatment levels at the beginning of 2019 to the end of year 2020 indicate that the Aquastrike application had virtually no long-term affect in the treated areas of Echo Bay.

Chi Square analysis indicates that the majority of changes within species presence were positive in nature and were not significant. Notable exceptions were in Najas flexilis which went from 22 to 9 sites (deemed somewhat significant) and in Potamogeton robbinsii which went from 33 to 13 sites (highly significant). The writer would offer the theory that as EWM increased in spread within the treated areas of Echo Bay, and had in many areas come to and spread horizontally across the water surface, that species which occupy areas lower the water column would be negatively affected from lower light transmission and increased stem density of EWM.

Chart #4 – Echo Bay Un-Treated Area

The Echo Bay Un-Treated Points Trends point out the important data comparisons made in three point-intercept surveys made in areas adjacent to the treated areas within Echo Bay during the 2019 growing season. These surveys indicate the changes which took place following the application of the chemical aquatic herbicide AquaStrike. While the pretreatment survey was taken in September 2018, the application of the herbicide actually took place in the first week of June in 2019. Eight weeks following the herbicide application the post-treatment point intercept survey was taken in late July. The third and final End of Year point intercept survey was taken in September 2019. The Un-Treated areas were

surveyed to ascertain what if any bleed over of the AquaStrike herbicide was experienced in the areas adjacent to the areas where the herbicide was applied, and what effects on the plant community did any bleed over have.

The <u>frequency of occurrence for Eurasian Water Milfoil</u> at sites shallower than the maximum depth of plants for these three-point intercept surveys indicate that the AquaStrike application did indeed bleed into the untreated areas within Echo Bay. However, because only 8 of the 26 sites in the Un-Treated area survey were shallower than the maximum depth of plants, and the pre-treatment survey noted 7 of the 8 sites included EWM, the Frequency of Occurrence for EWM in the pre-treatment survey seemed very high. The margins of the treated areas within Echo Bay tend to have steep sides and subsequently, the points just outside of the treated areas may have experienced the bleed over while the greater majority of the points did not have EWM plants to take in the dissipated herbicide.

The trend line for the 8 points is as follows:

Pre-Treatment Survey EWM Frequency of Occurrence	87.5
Post-Treatment Survey EWM Frequency of Occurrence	20.0
End of Year Survey EWM Frequency of Occurrence	30.0

In the same manner the number of sites where the species was found indicated an initial significant drop in foliage spatial area and plant stem density followed by a somewhat less significant rebound:

Pre-Treatment sites	7
Post-Treatment sites	2
End of Year sites	3

Average EWM Rake fullness indicated this same pattern:

Pre-Treatment rake fullness	1.29
Post-Treatment rake fullness	1.0
End of Year rake fullness	1.0

This pattern of an initial negative response followed by a relatively minor rebound of plant foliage growth was replicated <u>within the native plant community</u> as well. Overall frequency of occurrence at sites shallower than the maximum depth of plants dropped from 100 in the pretreatment survey to 100 in the July post treatment survey to 80 in the end of year survey. The Simpson diversity index showed no post-treatment drop and went from pretreatment 0.90 to posttreatment 0.92 to 0.92 in the end of year survey.

While the number of all species per site went from a pre-treatment value of 3.63 to a value of 2.56 in the post-treatment survey, the end of year survey indicated a rebound up to 3.20 in September. In much the same way the average number of native species per site shallower than maximum depth went from 2.88 in the pretreatment survey to 2.33 in the posttreatment survey, and rebounded to 2.90 in the end of year survey. The species richness trend went pretreatment 16, posttreatment 15 and end of year of 14. Finally the floristic quality index indicated a pretreatment FQI of 26.67 to a Post-Treatment FQI of 22.45, and an End of Year FQI of 21.36.

Seemingly all of these indexes displayed the same basic pattern of an initial negative effect on the aquatic plants present followed by a substantial rebound at the end of the year. An overall assessment is that the application of AquaStrike aquatic plant herbicide in the treated areas of Echo Bay had a bleed over effect on the untreated areas adjacent to the treated area margins, but because the water depth in those areas drops off quickly, few of the un-treated adjacent sites at greater distance from the treated area margins were effected.

2nd Year Chart #4 Echo Bay Un-Treated areas Data & Notes:

As stated in the first Year Interim Report, the Un-Treated areas within Echo Bay contained relatively few point intercept locations shallower than the maximum depth of plants. That total increased however over the course of the 2020 growing season. The margins of the treated areas within Echo Bay tend to have steep sides and subsequently, the points just outside of the treated areas may have experienced aquatic herbicide bleed over while the greater majority of the

points did not have EWM plants to take in the dissipated herbicide. During the course of 2020 the aquatic plant community in the steep sided areas seemingly had some success in repopulating the adjacent areas to the treated polygons. **EWM:** The frequency of occurrence for EWM in these areas increased as did the number of sites where the species were found and as did the average Rake fullness within those sites. While the number of sites rebounded from to pretreatment levels (7 sites) the frequency of occurrence rebounded to a lesser degree (46.67).

<u>All Plant Species:</u> While the frequency of occurrence of all plants at sites shallower than the maximum depth of plants remained at s lower level of 80 than the pretreatment level of 100, the maximum depth of plants grew to 17.7 feet. The Simpson diversity Index remained at .9 and the floristic quality index rebounded to 26.44 which is approximates the 26.7 FQI prior to the treatment been made.

Chi Square analysis for the Echo Bay untreated areas indicates that there were no significant changes in species presence from the pretreatment survey (9/2018) to the end of year survey taken in 9/2020)

Study Area # 3 Control Bays 1,2 & 3 Un-Treated On July 6th, 2019

Chart #5 – Control Bay #1 Chart #6 – Control Bay #2 Chart #7 – Control Bay #3 2020 Control Bays Data & Notes

Chart # 5 Treat / No-Treat Control Bay #1 Trends Through 2020			
Tomahawk Lake			
Oneida	Pre-season 7/2019	E.O.Y. 9/2019	E.O.Y. 9/2020
INDIVIDUAL SPECIES STATS:	EWM	EWM	EWM
Frequency of occurrence at sites shallower than maximum depth of plants	31.43	24.14	44.83
Number of sites where species found	11	7	13
Average Rake Fullness	1	1	1.38
present (visual or collected)	present	present	present
SUMMARY STATS:			
Total number of sites visited	35	35	35
Total number of sites with vegetation	27	24	26
Total number of sites shallower than maximum depth of plants	35	29	29
Frequency of occurrence at sites shallower than maximum depth of plants	77.14	82.76	89.66
Simpson Diversity Index	0.88	0.9	0.9
Maximum depth of plants (ft)**	22.7	16.6	14.6
Number of sites sampled using rake on Rope (R)	5	14	12
Number of sites sampled using rake on Pole (P)	30	21	23
Average number of all species per site (shallower than max depth)	1.8	2.17	2.45
Average number of all species per site (veg. sites only)	2.33	2.63	2.73
Average number of native species per site (shallower than max depth)	1.49	1.93	2
Average number of native species per site (veg. sites only)	2.08	2.33	2.32
Species Richness	15	17	18
Species Richness (including visuals)	15	17	18
Floristic Quality Index (FQI)	23.25	25.5	26.19
**SEE "MAX DEPTH GRAPH" WORKSHEET TO CONFIRM			

Chart # 6 Treat / No-Treat Control Bay #2 Trends Through 2020			
Tomahawk Lake			
Oneida	Pre-season 7/2019	E.O.Y. 9/2019	E.O.Y. 9/2020
INDIVIDUAL SPECIES STATS:	EWM	EWM	EWM
Frequency of occurrence within vegetated areas (%)	41.94	61.61	41.94
Frequency of occurrence at sites shallower than maximum depth of plants	34.21	45.71	37.14
Relative Frequency (%)	15.1	17.4	15.1
Relative Frequency (squared)	0.02	0.03	0.02
Number of sites where species found	13	16	13
Average Rake Fullness	1.08	1.38	1.38
#visual sightings			
present (visual or collected)	present	present	present
SUMMARY STATS:			
Total number of sites visited	44	44	44
Total number of sites with vegetation	31	31	31
Total number of sites shallower than maximum depth of plants	38	35	35
Frequency of occurrence at sites shallower than maximum depth of plants	81.58	88.57	88.57
Simpson Diversity Index	0.87	0.87	0.9
Maximum depth of plants (ft)**	22.1	19.5	19.4
Number of sites sampled using rake on Rope (R)	14	19	20
Number of sites sampled using rake on Pole (P)	30	25	24
Average number of all species per site (shallower than max depth)	2.26	2.63	2.4
Average number of all species per site (veg. sites only)	2.77	2.97	2.71
Average number of native species per site (shallower than max depth)	1.92	2.17	2.06
Average number of native species per site (veg. sites only)	2.52	2.53	2.4
Species Richness	15	17	18
Species Richness (including visuals)	15	17	18
Floristic Quality Index (FQI)	23.78	24.25	25.71
**SEE "MAX DEPTH GRAPH" WORKSHEET TO CONFIRM			

Chart #7 Treat / No-Treat Control Bay #3 Trends Through 2020			
Tomahawk Lake			
Oneida	Pre-season 7/2019	E.O.Y. 9/2019	E.O.Y. 9/2020
INDIVIDUAL SPECIES STATS:	EWM	EWM	EWM
Frequency of occurrence within vegetated areas (%)	18.18	45.45	45.45
Frequency of occurrence at sites shallower than maximum depth of plants	18.18	45.45	45.45
Relative Frequency (%)	5.3	10.1	11.2
Relative Frequency (squared)	0	0.01	0.01
Number of sites where species found	8	20	20
Average Rake Fullness	1	1.05	1.5
present (visual or collected)	present	present	present
SUMMARY STATS:			
Total number of sites visited	44	45	45
Total number of sites with vegetation	44	44	44
Total number of sites shallower than maximum depth of plants	44	44	44
Frequency of occurrence at sites shallower than maximum depth of plants	100	100	100
Simpson Diversity Index	0.87	0.9	0.88
Maximum depth of plants (ft)**	10.9	10	9.8
Number of sites sampled using rake on Rope (R)	0	1	1
Number of sites sampled using rake on Pole (P)	44	44	44
Average number of all species per site (shallower than max depth)	3.41	4.5	4.05
Average number of all species per site (veg. sites only)	3.41	4.5	4.05
Average number of native species per site (shallower than max depth)	3.23	4.05	3.59
Average number of native species per site (veg. sites only)	3.23	4.14	3.59
Species Richness	20	22	16
Species Richness (including visuals)	20	22	16
Floristic Quality Index (FQI)	27.53	29.24	25.05
**SEE "MAX DEPTH GRAPH" WORKSHEET TO CONFIRM			







2019 Control Bays Data & Notes.

2020 Control Bays Data & Notes

In October of 2016, Kevin Gauthier of the Wisconsin Department of Natural Resources began to put forth what came to be called the "do-nothing" option for the control of Eurasian Water Milfoil (EWM) in localized areas in the states Lakes and reservoirs. This option was based upon observations made by Lake managers from Northern Wisconsin who had noted that in many cases Lakes which had become infested with EWM and had been treated with aquatic herbicides (notably 2,4-D) over a period of time had fared no better than comparable Lakes which had received no treatments of any kind in the control of EWM. The "donothing" theory, while not part of a specific study in support of the theory, was backed up by data collected and discussed by these Lake managers. The "donothing" option was also presented at the March 2016 Wisconsin Lakes convention in Stevens Point in the presentation by the DNR on integrative AIS control strategies.

The theory is that localized infestations of EWM within a lake, if left alone with no treatment or control efforts, will grow from its initial infestation to a spatial size and density that out competes the natural plant species , and establishes EWM as the dominant plant species within the site. However, after outgrowing the natural carrying capacity for EWM within the site, the infestation level will fall back to a level lower than the natural carrying capacity. This cycle may be repeated a number of times, but the long-term outcome is that EWM will seek its natural carrying capacity level within the plant community, and that level will be at or below the levels experienced in the lakes which had been treated with chemical herbicides for the removal of EWM. In addition, that level will be relatively stable in the long-term, and it will be a level that is acceptable to most if not all Lake users.

The 2019 Treat / No-Treat study which began with pretreatment aquatic plant surveys done in the fall of 2018 in the treated areas, and in July of 2019 in the control areas, and that will continue over the following three seasons, offers an opportunity to test the efficacy of this theory. The Treat/No Treat Study also

seeks to examine the efficacy of two "contact type" chemical herbicides segregated into two small bays in the western side of Tomahawk Lake.

In Echo Bay the herbicide identified by the brand-name Aquastrike (a combination of Endothall & Diquat) was applied to 14 acres in the spring of 2019. In Pickerel Bay the herbicide ProcellaCor (Florpyrauxifen – benzyl) was applied to 11.1 acres, also in the spring of 2019. Along with these two segregated bays, the study calls for data collection in three specific protected Bay's on the eastern side of Windy Point. The three control bays were selected because they are protected from weather and current related environmental factors, and because their layout approximates the spatial layout of the two "herbicide applied" study bays. It is these three bays that may well yield data that will provide some clarity to the donothing theory. The last Chemical applications to these three bays took place in May of 2016.

The "pre-treatment" points in all three bays were surveyed in July of 2019 rather than the previous fall of 2018, as the site coordinates for the prescribed survey sites were not plotted until June of 2019.

The three years of Aquatic Plant surveys in the control areas will take place in early September each year, and will represent a "point in time" comparison at the end of each growing season for 2019, 2020 and 2021. Particular attention will be given to Eurasian Water Milfoil changes, which should yield trends of EWM over time. It will be these trends which should indicate whether the No-Treat or Donothing concept is active. In addition, the trends which will be established for the native plants will indicate if the Eurasian Water Milfoil infestations in these bays are having positive or negative effects on the aquatic plant community in general.

Significant changes which took place in the three control bays in the 2019 growing season. (timeframe is July 16th to Mid-September)

Control Bay #1:

EWM frequency of occurrence at sites shallower than the maximum depth of plants decreased from 31.43 in July to 24.14 in September. This is the only bay of the three which indicated a decrease in frequency of occurrence. The number of sites where EWM was found decreased from 11 to 7. EWM Rake fullness indicated no change.

Frequency of occurrence for all plants at sites shallower than the maximum depth of plants increased from 77.14 to 82.76. The Simpson diversity Index went from .88 TO 0.9. The average number of all species per site increased from 1.8 to 2.17 and the average number of native only species per site increased from 1.49 to 1.93. Species richness increased from 15 to 17, and at the floristic quality index increased from 23.25 to 25.5.

The Chi-Square analysis for Control Bay #1 indicates that species presence changes which took place within the bay in this time frame were insignificant in all but two cases, being Heteranthera dubia and Valisneria americana, both of which were deemed only slightly significant. The reduction in the frequency of occurrence for Eurasian water milfoil was deemed as "not significant."

2nd Year Data & Notes for Control Bay #1

<u>EWM</u>: Following the initial year of the study(2019), which indicated a modest reduction in Eurasian Water Milfoil Frequency of Occurrence in this untreated control Bay over the course of the initial growing season, indications from the End Of Year survey in 2020 reflect substantial growth of EWM during the growth season of 2020. Frequency of occurrence increased from 24.14 at the end of 2019 to 44.83 at the end of the 2020 growing season. The number of sites where the species was found increased from 7 to 13 and the average Rake fullness increased from 1.0 to 1.38. The growth exhibited in the 2020 season seems to represent a rebound to EWM levels noted at the beginning of the 2019 season. Chi Square analysis notes that the changes in Myriophyllum spicatum presence within the bay from the beginning of the study to the end of the second year were not significant.

<u>All Plant Species</u>: The summary statistics for the overall plant community within the bay indicate a slow and steady increase in the diversity of the plant community over the first and second year. Frequency of occurrence steadily increased from 77.4 in July 2019 to 89.66 at the beginning of September in 2020. Simpson diversity Index increased from .88 2.90 average number of all species per site increased from 1.8 to 2.45 species richness increased from 15 in July 2019 to 18 in early September 2020 and the floristic quality index increased from 23.25 to 26.19 over the course of the first two seasons of the study. Chi Square analysis reported presence changes of individual species within the bay were insignificant with the exception of Potamogeton zostriformis, which showed a slightly significant increase over the two seasons. Overall Chi Square analysis indicates virtually no significant changes from the initial pre-study point intercept surveys in July 2019 through the end of year point intercept survey done in 2020.

Control Bay #2:

EWM frequency of occurrence for sites shallower than the maximum depth of plants increased from 34.21 in July to 45.71 in September. The number of sites where the species was found increased from 13 in July to 16 in September and the average Rake fullness of EWM increased from 1.08 in July to 1.38 in September.

Frequency of occurrence for all plants at sites shallower than the maximum depth of plants increased from 81.58 to 88.57. The Simpson diversity Index remained unchanged at .87. The average number of all species per site increased from 2.26 in July to 2.63 in September, and the average number of native species per site increased from 1.92 to 2.17. Species richness increased from 15 to 17 and the floristic quality index increased from 23.78 to 24.25.

The Chi-Square analysis for Control Bay #2 indicates that species presence changes which took place within the bay within this timeframe were insignificant in all but one case, being Najas flexilis which experienced a slightly significant decrease. The increase in Eurasian water milfoil was deemed "not significant".

2nd Year Data & Notes for Control Bay #2

EWM: EWM frequency of occurrence for sites shallower than the maximum depth of plants fell slightly to 37.14 in the end of year 2020 survey this

number was supported in a reduction of sites where the species was found in 2020 to 13 from the 16 sites found at the end of 2019. Average Rake fullness remain the same at 1.38 from the end of year 2019 survey. The average Rake fullness of 13 in the end of year survey in 2020 reflects no change from the average Rake fullness in the initial study survey in July 2019. The Chi Square analysis for Control Bay #2 indicates no significant change in species presence for Myriophyllum spicatum over the first two growth seasons of the study.

All Plant Species: The summary statistics for the overall plant community within Control Bay #2 indicate the same slow and study increase in the diversity the plant community over the first and second year of the study. Frequency of occurrence at sites shallower than maximum depth of plants increased from 81.582 88.57 from the July 2019 two end of year 2020 growth periods the Simpson diversity index increased from .87 2.90 the average number of species per site increased from 2.26 to 2.4 species richness increased from 15 to 18 and the floristic quality index increased from 23.78 to 25.71. The Chi Square analysis for Control Bay #2 indicates that species presence within the bay over the two seasons study. Showed no significant changes with the exception of Potamogeton gramineus, which showed a slight significant increase over the two seasons. All in all Control Bay #2 exhibited virtually no significant changes from prestudy levels through the and of the second year of the study.

Control Bay #3:

EWM frequency of occurrence at sites shallower than the maximum depth of plants increased from 18.18 in July to 45.45 in September. The number of sites where the species was found increased from eight in July to 20 in September and the average Rake fullness for EWM increased from 1.0 to 1.05.

Frequency of occurrence for all plants at sites shallower than the maximum depth of plants was 100%. The Simpson diversity Index increased from 0.87 in July to 0.90 in September. The average number of all species per site increased from 3.41 in July to 4.50 in September. The average number of

native species per site went from 3.23 in July to 4.05 in September. Species richness increased from 20 to 22 and the floristic quality index increased from 27.53 in July to 29.24 in September.

The Chi-Square analysis for Control Bay #3 indicates that significant species presence changes took place within the bay during this timeframe, including a somewhat significant increase in EWM, a slightly significant increase in Nitella and a somewhat significant increase in Potamogeton foliosus. The changes which took place within the remaining species within the aquatic plant community were deemed as insignificant.

2nd Year Data & Notes for Control Bay #3

EWM: EWM frequency of occurrence for sites shallower than the maximum depth of plants showed no changes from FOO levels seen at the end of year survey in 2019. However, EWM frequency of occurrence in the initial year of the study (2019) had shown substantial increases in FOO of EWM, indicating that while the pre-study survey in July 2019 through the end of year survey of 2020 showed a substantial increase in frequency of occurrence 18.18 to 45.45, this increase was experienced in the initial year of the study (2019). While the second year frequency of occurrence remained the same as the end of year survey from year one, average Rake fullness increased significantly from 1.05 to 1.50 in the 2020 growing season. The Chi Square analysis indicates a significant change in species presence for EWM over the course of the two year growing seasons. However, there was no change in species presence for Myriophyllum spicatum in the 2020 growing season.

<u>All Plant Species</u>: The summary statistics for the overall plant community within Control Bay #3 indicates a slight decrease in plant diversity within this Bay. While frequency of occurrence at sites shallower than maximum depth of plants remained at 100 the Simpson diversity Index of .88 trended slightly downward from the end of 2019. Species richness fell to 16 from 20 at the beginning of the study in July 2019. And the floristic quality index fell to 25.05 from the initial 27.53. The Chi Square analysis for Control Bay #3 indicates minimal significant changes from pre-study levels through the end of the second year. The significant changes which have taken place within Bay #3 have indicated increases in species presence. The one highly significant change was to Vallisneria americana, which increased from eight sites in the initial point intercept survey in July 2019 to 25 sites in the end of year 2020 survey.

The overall trends in plant growth within the three Control Bays within the Treat / No-Treat Study tend to indicate a general increase in total plant diversity and species richness during the first two seasons of the three-year study. This would indicate an overall increase in the health of the aquatic plant communities within these bays.

The trend lines within Eurasian Water Milfoil have followed those exhibited by the total plant community in that frequency of occurrence for EWM in each of the three bays has increased over the two growing seasons. In general, the number of sites where the species is found, and the average Rake fullness indicators tend to exhibit an overall relatively minor increase in plant growth and plant health as well.

Chi Square analysis for each of the three bays would indicate that changes in species presence are largely insignificant.