# Aquatic Plant Treatment Evaluation Protocol

**Definitions** for lake terms included in this guidance document can be found in Appendix A: A Glossary of Some Common Terms Used Around Lakes

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## Contents



# Purpose and Applicability

This protocol is designed to evaluate the efficacy and impacts to native aquatic plant of an herbicide treatment or any other ecosystem manipulation (but from here on called a chemical application or treatment). The following protocol can be used to monitor whole-lake or small-scale aquatic plant management (APM) treatments on lakes. This guidance is recommended for DNR funded AIS grant projects or where performance results are desired (i.e. for scientific or financial accountability). This protocol is generally written for Eurasian water-milfoil (EWM) but can be adapted for other aquatic invasive plants. This protocol may also be adapted to evaluate non-herbicide controls.

Recent research conducted in Wisconsin has shown that herbicides disperse rapidly throughout a lake and that the area affected by herbicides is likely larger than the targeted treatment areas. If the proposed treatment will cover more than 5% of the lake area then a target whole-lake pesticide concentration should be calculated and included in the APM permit application. It is likely the herbicide will disperse over the entire lake, whether or not that is intended. In keeping with this tendency of herbicides to disperse quickly and widely, the areas of plants that might be affected by the herbicide will likely extend beyond the polygons of target species, and should be included in the pre- and post- aquatic plant community evaluation. If the targeted treatment areas will cover more than 10% of the lake area or will impact the entire lake, the directions below for whole-lake treatments should be followed. Monitoring of specific treatment areas (polygons) is relevant for "spot treatments" in APM projects in which the targeted treatment areas will not cover more than 10% of the lake area in total.

This protocol assumes that the lake group has an approved APM Plan in place with specific goals for the invasive and native plant species in the lake. For example, a goal of reducing the density of targeted treatment beds by one rake fullness category (for example, from an average rake

fullness of 2.5 to 1.5, measured a year after treatment) might be appropriate (see the DNR Baseline Aquatic Plant Monitoring Protocol p. 16-17 for description of rake fullness/plant density). Additionally, the group should choose a percent decrease in frequency of occurrence of the target species of at least 80% (measured a year after treatment) as a goal for whole-lake restoration projects. For an overall long term goal, a reduction of the target species to less than 10% of the littoral zone is reasonable. An acceptable native response is no significant net loss over the course of the project, and ideally some gain.

Aquatic plant management goals should be considered when determining the timing of the post-treatment survey. A post-treatment survey conducted the same year as a treatment will primarily serve to evaluate seasonal plant injury and may be appropriate if the goal is short-term nuisance relief in specific and small-scale treatment areas. However, if long-term invasive species control and lake restoration are the primary goals, then a year without large-scale treatment and an additional post-treatment survey(s) in a year(s) following a treatment will serve to evaluate project goals and additional years of post-treatment monitoring will determine the longevity of control.

We are aware that this approach necessitates several visits to the lake. This work is necessary to assess the overall success of treatments at reducing invasive species and at enhancing native species. As we continue to collect data and learn how each lake responds to specific treatment strategies, we hope and expect that we will be able to cut back on the intensity of annual evaluations and provide general best management practices. For now, rigorous quantitative data collection will help us understand the efficacy and selectivity of controlling invasive aquatic plants under a variety of herbicide formulations and use patterns.

Reporting recommendations are provided at the end of each step in the protocol. These are provided as a basis for improving consistency in analysis and reporting and can be used to interpret and discuss pre- and post-treatment results, to make next season's management recommendations and to compare effectiveness across different lakes. **Raw data should always be submitted with the reporting recommendations listed under each step below.** Submit the Excel files found in Appendix C and D of the Aquatic Plant Management Guide to both DNRBaselineAquaticPlants@wisconsin.gov and to your regional DNR Lake/APM Coordinator. Please refer to the detailed description below as a guide on how to conduct an APM treatment evaluation.

# Year 1: Season Before Treatment

1. *Confirm invasive species identification as needed.*
    a. Check DNR website (http://dnr.wi.gov/lakes/invasives/AISByWaterbody.aspx) to determine if the invasive species has already been verified on the lake.

    b. If not, collect five plant specimens and confirm the identity with the regional DNR AIS coordinator. These plants may be collected in the summer/fall before the treatment year or spring just before treatment, but must be confirmed before treatment takes place.

    c. For EWM, consult with regional DNR AIS coordinator to determine if specimens have been genetically tested for hybridity.

    d. **Reporting Recommendations:**
        i. Approval of invasive species verification by regional DNR AIS coordinator. The DNR coordinator will ensure that this information is included in the DNR SWIMS database for new AIS populations.
        ii. Genetic testing results should be sent to both DNRBaselineAquaticPlants@wisconsin.gov and to your regional DNR Lake/APM Coordinator.

2. *Establish baseline information about aquatic plant community.*
    a. Contact DNR Science Services (DNRBASELINEAQUATICPLANTS@wisconsin.gov) to request standard baseline point-intercept (P-I) grid information and GPS sampling points. In the near future P-I grids and associated files for individual lakes will be available for download on the DNR Lake Pages.

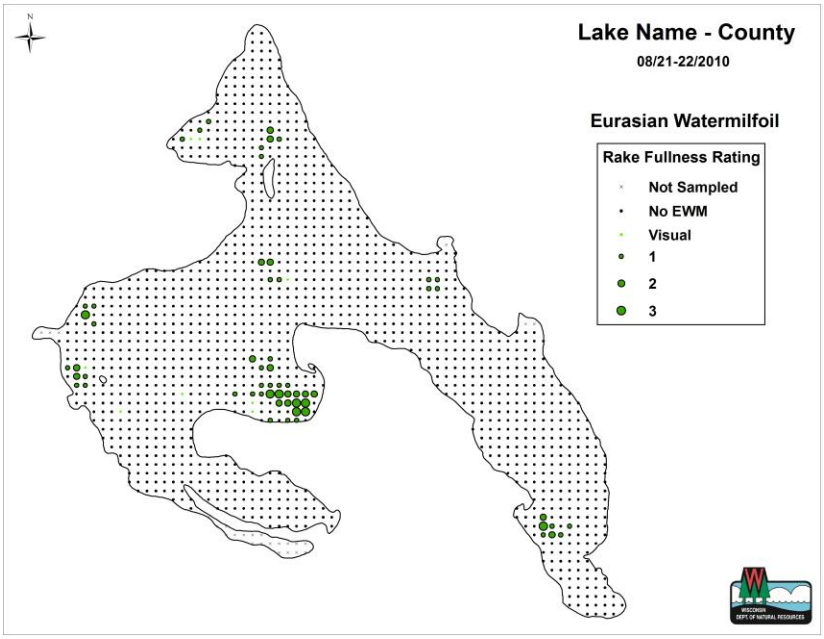
    b. Perform a whole-lake P-I survey to characterize the entire plant community if it has not been done during a year of no chemical treatment within the past 5 years.
        i. A P-I survey should be scheduled when native plants are well established, generally mid-July through mid-August.
        ii. Details on the protocol for conducting plant surveys can be found in Appendix B: Recommended Baseline Monitoring of Aquatic Plants in Wisconsin: Sampling Design, Field and Laboratory Procedures, Data Entry and Analysis, and Applications

    c. If a whole-lake treatment is being planned, the whole-lake P-I survey above can be used as pre-treatment data and treatment polygon P-I surveys (see Year 1, step 4 below) can be omitted.

    d. The bed mapping for the chemical treatment permit application (see Year 1, step 3 below) may not be needed if the herbicide treatment will cover the entire lake.

e. **Report Recommendations**:
   i. Baseline Whole-Lake Point Intercept Survey Table: Littoral Frequency of Occurrence for all species (this is calculated automatically in the "Stats" worksheet of Appendix C: Aquatic Plant Survey Data Workbook as *Frequency of occurrence at sites shallower than maximum depth of plants*.
   ii. AIS Map: Rake fullness (1, 2, 3, visuals) for all aquatic invasive plants (see example, Figure 1.).
   iii. Native Species Maps: In order to assess species interactions and potential impacts on non-target plants, map other plants as appropriate (such as wild rice, other common plants, species of concern, other water-milfoils – consult APM plan or local DNR lake manager). You may need a separate map for each species that is found at > 10% frequency (of points). See the DNR Baseline Aquatic Plant Monitoring Protocol (p.34-46) or the following You Tube video for directions on making aquatic plant distribution maps with different types of GIS software.

Figure 1. Map of EWM distribution from whole-lake point intercept survey.



3. *Identify and map plant beds proposed for treatment*
    a. The initial whole-lake P-I survey is unlikely to identify every location of an invasive species. During the summer/fall prior to the chemical treatment, map the proposed treatment areas of the target species and identify these polygons using GPS to outline the beds.

    b. Possible strategies for mapping include:
        i. Use a meander search (boat out from shore to the maximum rooting zone and then head back to shore, a short ways down the shore from where you started) to find beds.
        ii. If clarity is good (to the depth of rooted plants) and the EWM bed is topped out, identification can be visual but must be augmented with rake pulls to verify species identification and find the edges of the bed. Under glare conditions, brown polarized sunglasses are helpful.
        iii. If visibility is limited, SCUBA, underwater video, hydroacoustic sonar readings, or an Aqua-View Scope are all highly recommended to make a complete assessment of the beds.
        iv. Look for plant fragments wind-rowed on shore as an indication that plants may be growing off shore from this point.

    c. Qualitatively characterize target species density of individual treatment polygons. Plant bed density categories are:
        i. highly scattered
        ii. scattered
        iii. dominant
        iv. highly dominant
        v. surface matting plants
        vi. If the target species is not found in beds large enough to map (< 40' diameter) suggested density categories are:
            1. small colonies
            2. clumps
            3. single plants

    d. In order to secure a chemical treatment permit, the applicant must know the specific acreage, average depth and treatment area locations (mapped polygons).

    e. **Report Recommendations**
        i. Map of treatment areas for the target species
            1. Polygons representing beds of target species treatment areas
            2. Identify beds using numbers or letters (see example, Figure 2.)
        ii. Table: Report information about treatment polygons (see example Table 1 below), including acreage, average depth, volume, substrate, number of polygon sampling points (see Year 1, step 4 below), target herbicide application rate and target species density.
        iii. Electronic files: Provide electronic (GIS) shapefiles to regional DNR Lake/APM Coordinator.

Figure 2. Map of proposed EWM polygon treatment areas

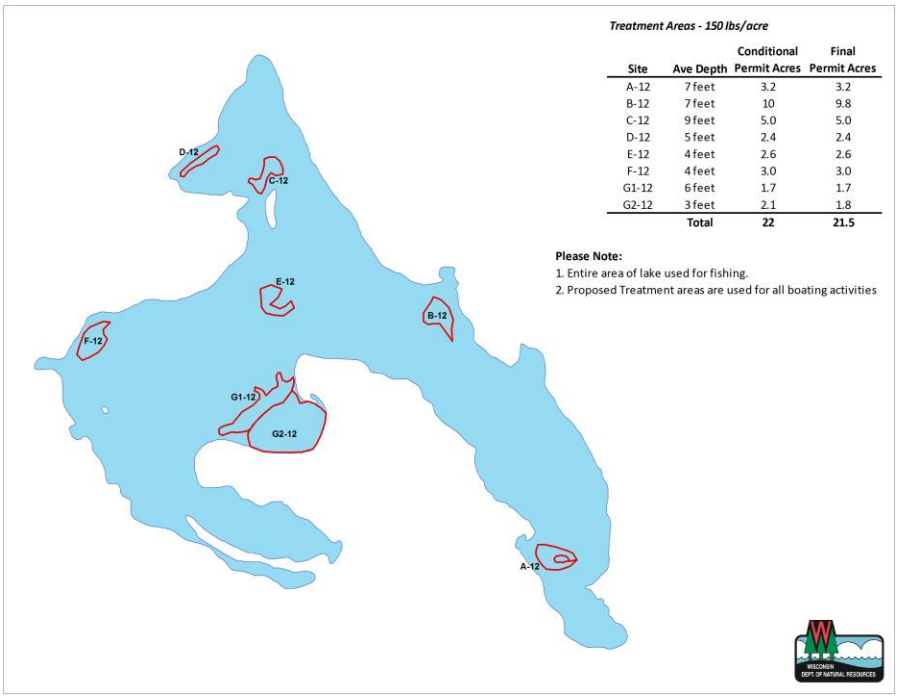



**Table 1.** Treatment Polygon Data (example data)

| EWM Beds | Acreage | Mean depth | Volume (acre-ft) | **Substrate** | P-I points | Target Application Rate (ppm) | EWM Density |
|---|---|---|---|---|---|---|---|
| a | 1.2 | 4 | 4.8 | Sand | 35 | 3 | scattered |
| b | 0.8 | 8 | 6.4 | Muck | 25 | 4 | dominant |
| c | 5.6 | 5 | 28 | Sand/Muck | 50 | 4 | dominant |
| d | 12.3 | 6 | 37.8 | Muck | 100 | 4 | highly dominant |
| e | 2.4 | 7 | 16.8 | Muck | 40 | 3 | scattered |

4. *Conduct summer P-I survey in proposed treatment polygons*.
    a. In order to assess the potential non-target effect of treatment on natives, there must be a survey of all plant species before treatment. Since native species will be largely absent at the time of the spring pre-treatment bed mapping finalization (Year 2, step 2 below), the natives must be assessed the summer before treatment (mid-July through mid-August). This survey cannot be used as a measure of CLP frequency (see separate CLP Treatment Evaluation Guidance) as CLP will likely have senesced by this time of year.

    b. If greater than 10% of lake area will be treated then the treatment will be considered whole-lake. Then the whole-lake P-I survey (Year 1, step 2 above) can be used as pre-treatment data to evaluate treatment effectiveness and the polygon P-I surveys discussed below (c-d) can be omitted.

c. Intensive monitoring in some polygons may be warranted for evaluating the efficacy and plant community change associated with new management techniques or new species even if the treatment will not impact the whole-lake. Consult with your DNR Lake/APM Coordinator about whether or not an evaluation of a spot treatment is needed. If an evaluation is desired follow the recommendations below.

d. After defining the proposed treatment polygons (Year 1, step 3 above), perform a P-I survey (following Appendix B: Recommended Baseline Monitoring of Aquatic Plants in Wisconsin: Sampling Design, Field and Laboratory Procedures, Data Entry and Analysis, and Applications) at a sub-sample of points within the polygons using the following guidelines:
    i. Set up a grid of points over treatment polygons that will be evaluated. This can be done by using ArcMap, QGIS or another GIS program.
    ii. We suggest sampling at least 40 points in each treatment area to be evaluated if possible. These same points will be used again during the post-treatment assessment.
    iii. If the treatment area is greater than one acre we suggest increasing the number of sample points to match the level of change that you want to be able to detect.
    iv. For example, by sampling 40 points for the pre- and post-treatment survey, you will be able to detect a 50% or larger change in species frequency (of both natives and the target species) if the pre-treatment frequency of the species is at least 40%. It will be difficult to detect any amount of change if the pre-treatment frequency is less than 10%.
    v. Evaluation of individual treatment beds less than an acre in size will be difficult because the accuracy of common GPS units will not allow navigation to points that are this close together. A more accurate GPS unit will be needed if you want to evaluate very small treatment areas.

e. **Report Recommendations:**
    i. Treatment Map: Polygons to be treated and locations of all the points to be sampled within the polygon (polygon sub-sample points) (see examples, Figures 3 and 4).
    ii. AIS Map: Rake fullness (1,2,3, visuals) for all aquatic invasive plants within treatment polygon subsample points. This map will look like Figure 1, but for each polygon that will be evaluated.
    iii. Native Species Maps: In order to assess species interactions and potential impacts on non-target plants, map the plants in polygon subsamples as appropriate (such as wild rice, other common plants, species of concern, other water-milfoils). This map will look like Figure 1, but for each polygon. Consult DNR lake manager or APM plan.
    iv. Table: Frequency of occurrence for all species for each polygon that will be evaluated (use the "Stats" worksheet of Appendix C: Aquatic Plant Survey Data Workbook, see http://www.uwsp.edu:80/_layouts/download.aspx?SourceUrl=/cnr-ap/UWEXLakes/Documents/ecology/Aquatic%20Plants/Appendix-C.xls ).

v. These report recommendations should be coupled with post-treatment results and be made available to the regional DNR APM coordinator and Lake Association/District after evaluation.
vi. The regional APM coordinator will place the pre-post monitoring report in the SWIMS project for this AIS grant or project.

**Figure 3. Map of EWM polygon treatment areas and sub-sample P-I points.**

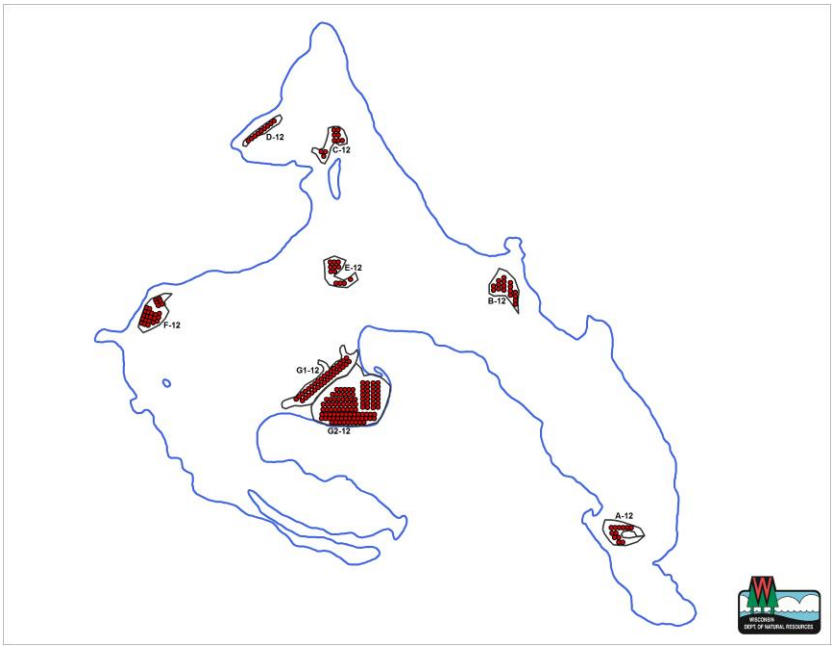
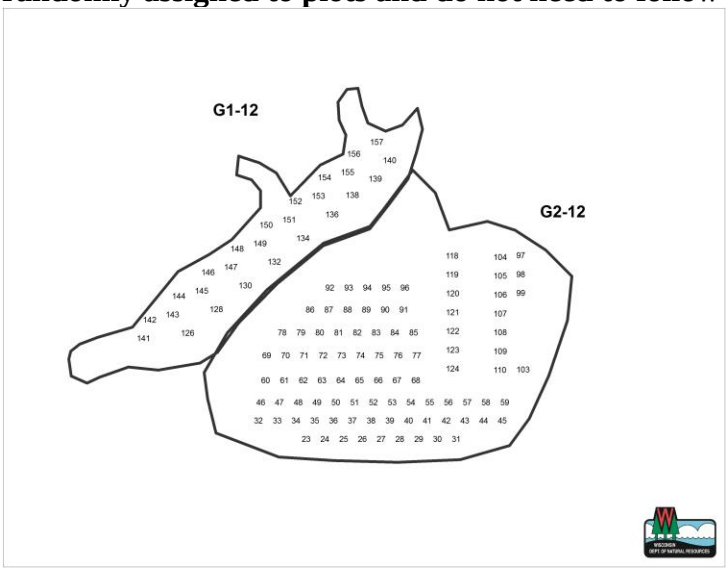



**Figure 4. Map of sub-sample P-I points within a treatment polygon. Points can be randomly assigned to plots and do not need to follow a standard grid pattern.**



# Year 2: Early Season Treatment and Monitoring

1. *Contact the State Lab of Hygiene for a herbicide concentration monitoring kit*
    a. Herbicide concentration monitoring will complement the aquatic plant evaluation data and help determine appropriate adaptations to future herbicide treatments for AIS control. Herbicide concentration monitoring kits should be ordered well in advance of treatment.

    b. Herbicide concentration monitoring should be done for whole-lake treatments.

    c. Herbicide concentration data may also be collected on large-scale or spot treatment projects where treatment evaluation is desired and plant data is being collected.

    d. See step 5.a. below for herbicide concentration monitoring protocol

2. *Spring finalization of treatment polygons*
    a. Verify that the invasive species is growing and finalize treatment polygon areas. Plants may be small and sparse this time of year. Underwater visual/video and sporadic rake pulls in the middle and edges of the proposed polygon are highly recommended. Adjust the delineation of the treatment area, if necessary.

    b. **Report Recommendations: Map and Table:**
        i. Update final treatment polygon maps and tables (from Year 1, step 3 above) as needed.
        ii. Include herbicide concentration monitoring points on final treatment maps.

3. *Determine depth of stratification if conducting a whole-lake herbicide treatment*
    a. If the lake is considered deep (check the DNR Lake Pages) or it may stratify then a temperature profile at the deepest point in the lake should be collected prior to treatment.
    b. Ideally the treatment will take place after the lake has stratified in order to minimize the amount of herbicide needed to reach the whole-lake target concentration.
    c. Measuring the temperature profile of the lake just before a whole-lake treatment will allow an accurate estimate of the volume of water (epilimnion) that needs to be treated.

4. *Conduct Early Season Treatment.*
    a. It is best to conduct the treatment in early spring, when water temperatures are near 60 degrees, for several reasons.
        i. Many studies have shown that the chemical herbicides are effective and longer lasting at temperatures normally found in lakes just after ice-off.
        ii. It is best to treat before the native plant species are actively growing, so that they are minimally affected by the chemical.

b. Therefore, treatment should occur in spring, when the target species is actively growing throughout the proposed treatment areas (optimally around 6 inches tall).

c. If optimal conditions for treatment have not occurred prior to May 31, consult with the DNR to confirm if treatments may go forward. It is possible that treatments are unnecessary or would be detrimental to the native plant community if conducted too late in the season.

d. **Report Recommendations:** Chemical applicators must submit a treatment record (DNR Form 3200-111) within 30 days of treatment to the regional DNR Lake/APM Coordinator. This should include the type and quantity of herbicide used and the size and location of each treatment area (in GIS format).

5. *Monitor herbicide concentration in the lake following treatment*
    a. Detailed protocols can be found in the Whole Lake Herbicide Monitoring Fact Sheet.
        i. This monitoring could be done by trained volunteers.
        ii. If the treatment is not a typical whole-lake treatment with 2,4-D or endothall herbicide or is taking place in a unique lake-type (flowage), consult with the regional DNR Lake/APM Coordinator to discuss an appropriate herbicide concentration monitoring plan.

    b. **Report Recommendations:**
        i. Map illustrating the herbicide concentration collection points and treatment areas.
        ii. Concentration by exposure time graph

6. *Mid-late summer post-treatment P-I Survey to evaluate seasonal control and impacts*
    a. Aquatic plant management goals should be considered when determining the timing of the post-treatment survey.
        i. A post-treatment survey the same year as a treatment (year 2) will only serve to evaluate seasonal plant control/injury, while a survey in a year following (year 3) and without treatment will serve to evaluate long-term plant control. Additional years of post-treatment monitoring without additional treatments will help determine the longevity of AIS control. Post-treatment surveys in year 3 and beyond are recommended; especially for whole-lake treatments and treatments larger than 10 acres.

    b. A post-treatment survey to evaluate seasonal control and impacts can be scheduled when native plants are well established, generally mid-July through mid-August.
        i. For the post-treatment survey, repeat the P-I for all species in the treatment polygons, as was done the previous summer.
        ii. For whole-lake treatments, a full lake-wide P-I survey should be conducted (see Year 3 below).

c. To compute the significance of results from the pre- and post-treatment surveys (pre-treatment survey in summer of Year 1 and post-treatment survey in summer of Year 2 and/or Year 3) see Appendix D - Compute Pre & Post Data Workbook (Excel).

d. **Report Recommendations:**
   i. AIS Map: Rake fullness (1,2,3, visuals) for all aquatic invasive plants within polygon subsample points.
   iv. Native Species Map: Other non-target plants as appropriate (such as wild rice, other common plants, species of concern, other water-milfoils). You may need a separate map for each species or group of species.
   v. Table: Frequency of occurrence for all species, including invasive species, for all polygon subsample points (use the "Stats" worksheet of Appendix C: Aquatic Plant Survey Data Workbook, see Appendix C - Aquatic Plant Survey Data Workbook (excel)
   vi. Table: Report the number of sites where each species was found pre- and post-treatment and how the frequency changed with treatment using the pre/post Chi Square evaluation (see example, Table 3.) See Appendix D - Compute Pre & Post Data Workbook (Excel).
   vii. Graph: Create bar graph of pre and post-treatment results for all species, noting significant changes (see example, Figure 5).
   viii. Graph: Report rake fullness of target species for pre- and post-treatment. Include average rake fullness (see example Figure 6.).
   ix. If evaluating multiple treatment polygons then report steps i through vii above for each polygon that was evaluated.
   x. Text: Summarize results from this survey and compare them with the results from the pre-treatment survey in order to:
      1. evaluate the effectiveness on target plants,
      2. evaluate any harm or benefit to native plants
      3. revisit target levels of control from your lake management plan and update the plan for the future.
   xi. If necessary, identify next year's potential treatment areas for target plants (follow year 1, step 2 above).

**Table 3**. Pre-post statistical comparison using chi-square test of independence.

| **Table 3. Wonder Lake** | | | | | |
|---|---|---|---|---|---|
| pre-treatment survey total points | 85 | | | | |
| post-treatment survey total points | 85 | | | | |
| | PRE | POST | p | Significant change | Increase/Decrease |
| Elodea canadensis | 52 | 54 | 0.75155 | **n.s.** | **+** |
| Myriophyllum spicatum | 80 | 15 | 0.00000 | *** | **-** |
| Myriophyllum sibiricum | 75 | 21 | 0.00000 | *** | **-** |
| Najas flexilis | 75 | 75 | 1.00000 | **n.s.** | **no change** |
| Nitella sp | 29 | 30 | 0.87200 | **n.s.** | **+** |
| Potamogeton robbinsii | 26 | 35 | 0.15012 | **n.s.** | **+** |
| Vallisneria americana | 73 | 69 | 0.40818 | **n.s.** | **-** |

**Figure 5.** Littoral frequency of occurrence from before to after treatment. * Indicates statistically significant difference ($p<0.05$).

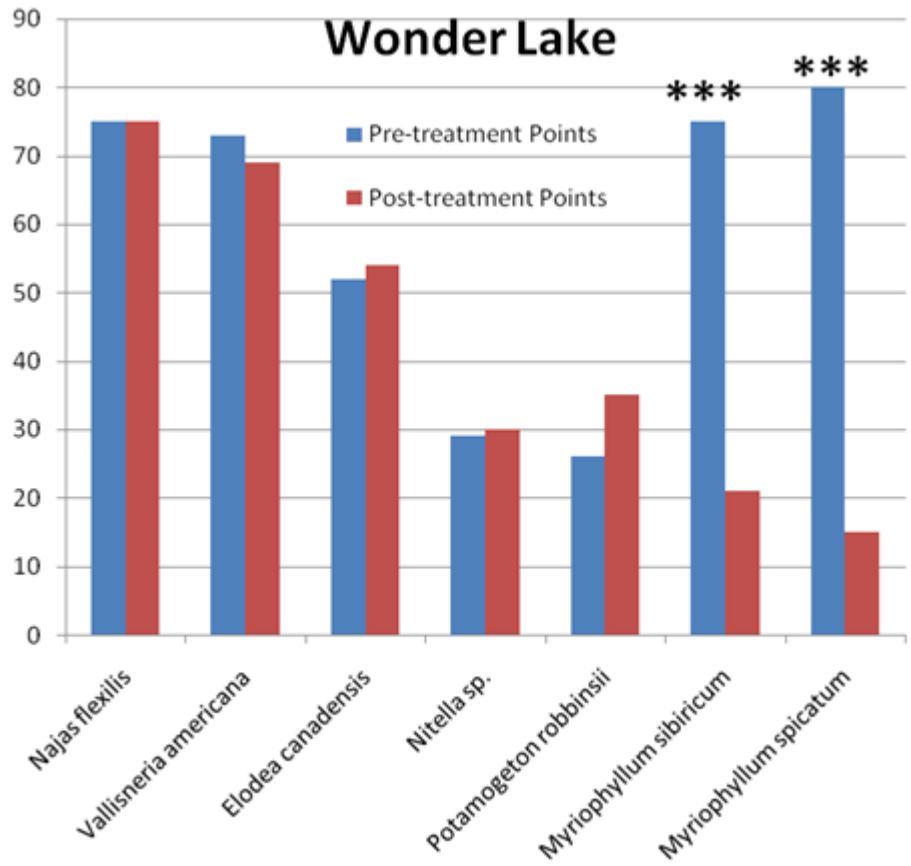
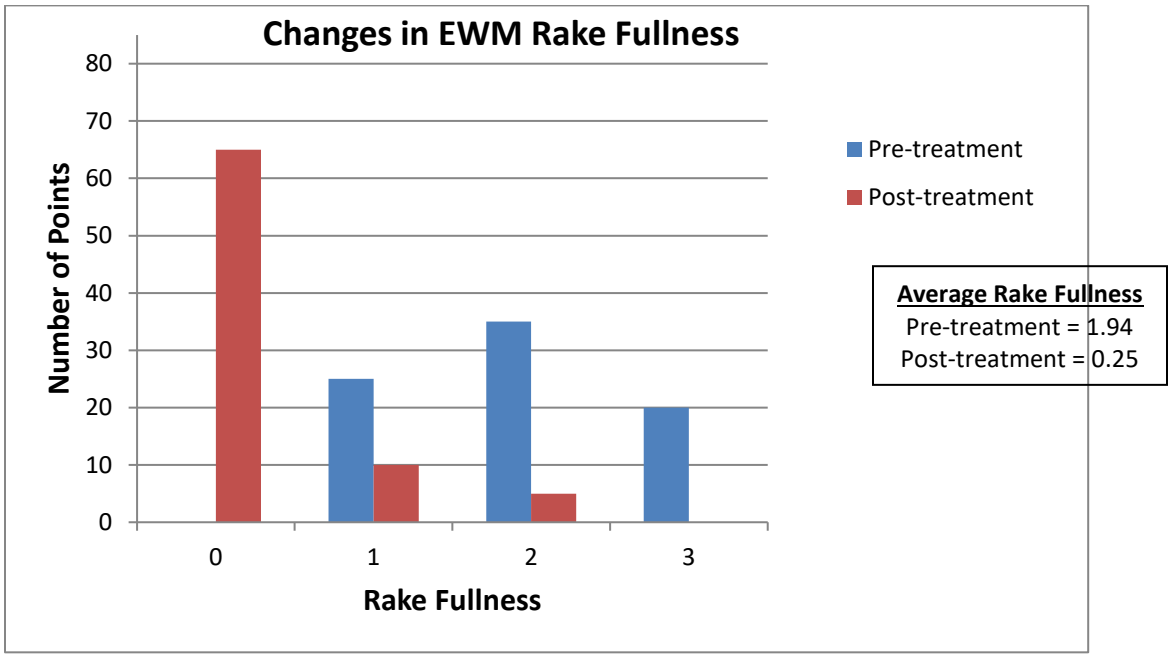


**Figure 6.** Changes in rake fullness scores and average rake fullness from before to after treatment.



# Year 3: Post-Treatment Evaluation: Efficacy and non-target impacts

1. *If a whole-lake treatment was conducted in Year 2, no whole-lake treatments should take place in Year 3.*
    a. Another whole-lake P-I survey should be completed in the year prior to another whole-lake treatment according to guidance.

2. *A post-treatment survey(s) to evaluate long-term control and native species impacts should be scheduled when native plants are well established, generally mid-July through mid-August.*
        a. Follow the reporting recommendations under Year 2, step 6(d) above using whole-lake P-I survey data if the treatment was whole-lake in scale.

<u>Additional treatments in following years</u>

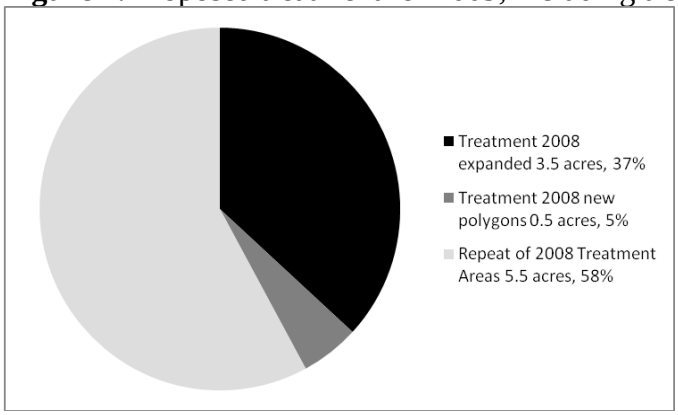
a. If further treatments are needed in a subsequent year, go back to Year 1, step 2 and repeat all steps through the end of Year 3, step 2.

b. If one or more polygons treated in Year 2 will be treated again in Year 3 the post-treatment survey results for those polygons in Year 2 can serve as the proposed treatment survey for the treatment to be done on them in Year 3.

c. If any proposed treatment polygons are different in any way from polygons already treated, the new polygons must be sampled as if they are brand new.

d. **Report Recommendations**:
    i. Graph or Table: In addition to the reporting recommendations from all the steps that will be repeated present a summary of acreages to be treated in the subsequent year, partitioned into repeated, expanded and new treatment areas (see example, Figure 7.).

**Figure 7.** Proposed treatment for 2009, including treatment areas in 2008.



## Year 4 and Beyond: Revising the aquatic plant management plan

1. A lake wide P-I survey should be repeated every five years to gauge overall lake community response. This survey should be done in a year without a whole-lake treatment.

2. Use the P-I results (and reporting recommendations above) to update the APM Plan.

3. Consult with a DNR lake coordinator to adjust your APM plan goals based on an evaluation of previous management.