

INTRODUCTION

The purpose of this report is to relay information regarding the Eurasian water milfoil (EWM) herbicide treatment that was completed on Enterprise Lake during May 2008. It includes a description of the methods used to evaluate the treatment and the criteria used to determine if it was successful. Its frame of reference begins with the spring pretreatment surveys completed during May 2008. The report goes on to discuss the condition of the EWM in the treatment areas following the herbicide application. The data used in that section were collected during August 2008 (summer post treatment survey). During that same time, the 2008 peak biomass survey was completed to gather information used in creating the 2009 proposed treatment areas, which are discussed near the end of the report. Once agreed upon by the Enterprise Lake Protection and Rehabilitation District and the Wisconsin Department of Natural Resources (WDNR), the proposed treatment areas will be used to obtain a conditional treatment permit for the May 2009 treatment.

TREATMENT MONITORING

Determining the success or failure of chemical treatments on Eurasian water milfoil (EWM) is often a difficult task because the criteria used in determining success or failure is ambiguous. Most people involved with EWM management, whether professionals or laypersons, understand that the eradication of EWM from a lake, or even a specific area of a lake, is nearly, if not totally, impossible. Most understand that achieving control is the best criteria for success. Similar to the 2007 treatment report, two different methods of evaluation were used to understand the level of control that was achieved by the chemical treatment. A qualitative assessment was determined for each treatment site by collecting spatial data with a sub-meter Global Positioning System (GPS), in addition to, comparing detailed notes from the pre- and post treatment observations.

Quantitative monitoring of the treatments were completed following protocols disbursed by the Wisconsin Department of Natural Resources (WDNR) in April 2007. This protocol calls for the monitoring of target plants (EWM) and native plants before and after treatments. Pretreatment surveys are completed the summer before treatment and the spring of the treatment. Post treatment surveys are completed the summer following treatment and the next spring following the treatment. The Enterprise Lake P&R District successfully applied for an Aquatic Invasive Species (AIS) Education, Planning, and Prevention Grant and implemented this protocol starting with the 2008 spring pretreatment survey. A quantitative assessment of the treatment was begun by collecting data at 137 point-intercept sample locations on Enterprise Lake (Appendix A). At these locations, EWM presence and rake fullness were documented as well as water depth and substrate type. Native plant abundances were also determined at each plot during the post treatment surveys, that summer. Due to budget limitations of the Enterprise Lake P&R District, a prioritized treatment was conducted in 2008. Of the 137 sub-sample locations sampled, 43 were contained within the areas that were treated.

Statistical Analysis of Pre- and Post Treatment Survey Data

Scientists often rely on the use of statistical analysis to understand whether the observed differences in nature are merely a product of chance or can be attributed to a particular factor. In the case of the pre- and post treatment monitoring surveys completed on Enterprise Lake, the

particular factor we are concerned with is the herbicide treatment. The desired result is a decrease in EWM within the treatment areas. The amount of EWM within a treatment site is measured with the sub-sampling surveys and expressed in terms of percent frequency of occurrence. The EWM frequency is a percentage of sub-sampling sites that contain EWM relative to the total sub-sampling sites in the treatment area. For example, if a treatment site has 20 sub-sampling locations and 5 of those locations contained EWM, then the EWM frequency would be 25%.

As a part of the treatment monitoring, the sub-sampling sites are visited before and after the treatments to produce the pre- and post treatment data. By comparing those data, we can see if there is more, less, or the same amount of EWM before and after the treatment. As mentioned above, the desired result is to have less EWM after treatment. If there is a difference between the pre- and post treatment data, statistical analysis is used to determine if the difference is sufficient to be attributed to the treatment or if the difference may have occurred randomly. If the difference is sufficient, it is considered to be *significantly different*, if it is not sufficient, it is considered to be *insignificantly different*. In the end, a significant difference can be attributed to some factor, while an insignificant difference can only be attributed to random chance.

With guidance from WDNR Integrated Sciences, a Chi-square distribution analysis ($\alpha = 0.05$) was used to determine if the quantitative data collected before the treatment are statically different from the data collected after the treatment. The alpha value is set such that we consider the results statistically significant when the test is 95% confident that the results are truly different and non-random.

The number of sub-sample sites within a treatment area must be considered when evaluating the treatment impacts on that particular site. A higher sample size (N), leads to more credible results and conclusions. In general, sites containing 6 or less sub-sample locations are not considered sufficient for analysis; however, those data are considered valuable when pooled (combined) with the other sub-sample sites within the lake for the lakewide analysis. A 20-meter spacing (resolution) between sub-sample locations is considered the closest that hand-held GPS technology can accommodate effectively. Two of the three areas treated on Enterprise Lake in 2008 were relatively small, so only a few sub-sample locations could be placed within their boundaries using this resolution. Therefore only one of the 2008 treatment sites could be evaluated for statistical significance on a site-specific basis.

The caveat to all of this is that we assume that the differences observed were caused by the herbicide treatment, but truly, without having comparable data from a non-treatment site (control group), this cannot be absolutely certain. For example, was the reduction in EWM caused by interannual variations caused by competitive dynamics between species, fluctuating water levels, natural plant cycles, or changes due to climatic conditions? Without a true experimental design that uses a control site, we cannot absolutely answer that question. In the end, it is impractical to take the risk of not treating a colony of EWM within a lake just to make sure that the results of the studies are scientifically sound; therefore making the educated-assumption that the difference is caused by the herbicide treatment is reasonable.

Pretreatment Survey – 05/09/08, 05/12/08, & 05/14/08

The purpose of this survey was to refine the treatment areas used in the conditional permit (based on the 2007 peak biomass survey) to more accurately and effectively coordinate the control method.

The weather conditions on all three days were sunny and windy. The EWM was difficult to view from the surface, especially in deep water, due to the stained water and the windy conditions. The surface water temperature was approximately 52°. The ambient air temperature was between 44° and 50° on all three days. The use of an aqua scope and underwater camera were used to help observe EWM occurrences and determine colony extents.

Site D-08 & E-08 These proposed sites were dropped from the treatment plan as very little EWM was observed within these areas during this survey. I was theorized that during this winter, the plants within these shallow water sites died due to freezing (Map 1).

Site F-08 & G-08 These two sites were observed to be a part of a large colony of scattered EWM extending into 12 feet of water or more. Site F-08 was slatted for treatment at 150 lbs/ac because of its depth (Map 1).

Site H-08 During the 2007 peak-biomass survey, the only plants located within this northern bay were contained in the southwest portion of the site (Map 1). However, the spring pretreatment showed that scattered EWM existed throughout this bay. In 2007, this site was treated at 100 lbs/acre. Due to the lack of long term success observed, this site was recommended to be treated at 150 lbs/acre (Map 1).

Site I-08, L-08, & K-08 The EWM within these sites was observed to be largely the same as during 2007. The only exception was in site K-08, where a few plants were observed growing to the north of the treatment area, which was modified to encompass those plants. These relatively isolated sites were proposed to be treated at 100 lbs/acre (Map 1).

Site J-08 No EWM was discovered within this site during this survey and therefore was not proposed for treatment (Map 1).

Site M-08 & N-08 These two sites were found to be contained within a larger scattered EWM colony. This entire area was proposed for treatment (Map 1, M-08).

Site A-08, B-08, & C-08 The EWM within these three sites was found to be largely the same as during the 2007 peak biomass survey. Only a slight modification was made to B-08 (Map 1).

The Enterprise Lake P&R District and the Wisconsin Department of Natural Resources (WDNR) were informed of the expanded treatment recommendations. The WDNR approved all areas for treatment; however, the Enterprise Lake P&R District's approved budget did not allot funding for this amount of treatment. The Enterprise Lake P&R District prioritized three areas for treatment in 2008 (Map 2). These sites were chosen based on proximity to public access sites and high traffic areas.

Post Treatment & Peak biomass EWM Survey – 07/21/08

During this survey, all treatment areas were visited to determine the efficacy of the chemical application. The conditions were mostly sunny with a slight breeze. At this time of year the EWM has reached its peak biomass, so the plants have nearly reached the surface, making viewing optimal. All point-intercept sample locations were also revisited and data were collected in the same manner as during the pretreatment survey. Native plant occurrences were also documented at the sub-sample locations during this survey for comparison with past and future summer surveys. **Please note:** that although only 43 sub-sample locations were contained within the areas treated in 2007, all 137 locations were sampled for use in determining future treatment efficacy.

As outlined within the Enterprise Lake Aquatic Plant Management Plan – Draft (May 2008), success of the herbicide treatments would be evaluated in multiple ways. Qualitatively, a successful treatment on a particular site would include a reduction of EWM density as demonstrated by a decrease in density rating (e.g. highly dominant to dominant). In terms of a treatment as a whole, at least 75% of the acreage treated that year would decrease by one level of density as described above for an individual site.

Quantitatively, a successful treatment on a specific site would include a significant reduction in EWM frequency following the treatments as exhibited by at least a 50% decrease in EWM frequency based upon the sub-sampling. In other words, if the EWM frequency of occurrence before the treatment was 80%, the post treatment frequency would need to be 40% or lower for the treatment to be considered a success for that particular site. Evaluation of the treatment-wide effectiveness would follow the same criteria based upon pooled sub-sample data from all treatment sites. Further, there would be a noticeable decrease in rake fullness ratings within the fullness categories of 2 and 3. Preferably, there would be no rake tows exhibiting a fullness of 2 or 3 during the post treatment surveys.

During this field visit, a peak biomass EWM survey was conducted to provide an accurate account of all EWM locations within the lake to aid in coordinating the 2009 management actions. These recommendations are provided within this section.

Site M-08 Only a few EWM locations were observed within this site after the treatment (Map 3). EWM occurrence from the 32 point-intercept sub-sample locations contained within this site was reduced from 18.8% before the treatment to 3.1% after the treatment. This equates to a statistically significant 83.3% reduction ($p = 0.04$). This site is not proposed for treatment in 2009.

Site B-08 A scattered occurrence of EWM was observed within this site after the treatment (Map 3). This is largely consistent with what was observed in 2007 (Map 1). Only 5 sub-sample locations were contained within this site; which is not a large enough sample size for analysis. Site B-09 is proposed for treatment in 2009, which includes Site B-08 (Map 3).

Site F1-08 This site targeted a dense EWM colony found within F-08 (Map 2). Of the six sub-sample locations contained within this treatment site, one contained EWM before the treatment and one contained EWM after the treatment displaying no change in occurrence.

However, a sample size of six is truly too small for analysis. The proposed Site B-09 also encompasses this treatment site.

CONCLUSIONS AND RECOMMENDATIONS

Before the treatment on Enterprise Lake, 16.3% of the point-intercept locations contained EWM and 9.3% contained EWM after the treatment indicating a 42.9% reduction in EWM occurrence within the 2008 treatment areas. Although a reduction was observed, it was not found to be statistically significant using the Chi-square analysis and may be a result of random variation. As stated above, the EWM within Site M-08 was reduced by a statistically significant 83.3%, but when the data was pooled with Site B-08 and F1-08, the treatment-wide results were not significant.

A rake fullness rating of 1-3 was used to determine abundance of EWM at each location. Figure 1 displays the number of point-intercept locations exhibiting each of the rake fullness ratings within the areas treated on Enterprise Lake. Before and after treatment, all of 37 locations containing EWM had rake fullness ratings of 1 (Figure 1). This shows that the colonies treated on Enterprise Lake were not comprised of dense EWM before treatment and densities did not increase following treatment.

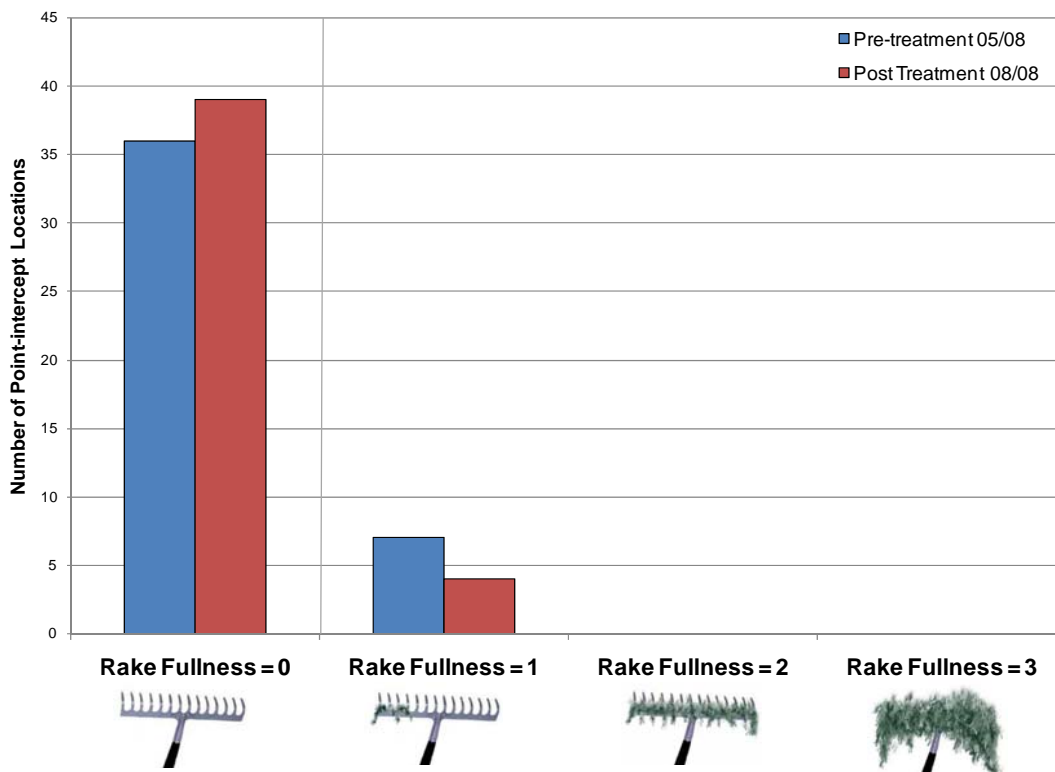


Figure 1. EWM rake fullness distribution within treated areas on Enterprise Lake.

While successes were documented in Site M-08, they were not in B-08 or F1-08. Based on the 2008 peak biomass survey, these sites were both parts of larger colonies (Map 3). While F1-08 was understood to consist of the densest part of F-08 (Map 2), Site B-08 was thought to contain

the entire EWM colony at that time. This could be a result of an expanded population since the 2007 peak biomass survey or the EWM could have been present, but went undetected during that survey. Regardless, this resulted in an incomplete treatment. Incomplete treatments are difficult to evaluate as there is a nearby source population of the exotic that can easily recolonize the area before the natives are empowered long enough to expand their population and reclaim the area.

The reality is that we are in line to retreat these two treatment areas in 2009. That scenario is not uncommon in EWM management as dense areas often require multiple years of the treatment to drastically decrease the site's density. While there wasn't a noticeable reduction in EWM density within Site B-08, the EWM within F1-08 appeared to be less than previously understood.

Due to the lifecycle of most of our region's native plants, they should be at very low biomass (or not even started growing yet) during the spring survey, and therefore are not monitored at that time of the year. Native plant frequencies are monitored during the summer post treatment surveys, when most of the plants are at their peak biomass. It is particularly important to monitor the broad-leaf (dicot) native aquatic plants, as these are the species that could be affected by 2,4-D. The Enterprise Lake P&R District is perusing a WDNR AIS Established Infestation Control Grant to help finance a multi-year project that along with monitoring the long-term efficacy of the treatments, also aims to understand the unintended effects on the system's native plant community.

As previously mentioned, Site B-08 and F1-08 were found to be part of a larger EWM colony, that consists of most of the western part of Enterprise Lake. Much of this area is contained within the productive and shallow slow-no-wake bay bounded by the lake's island. The EWM within this shallow bay is scattered, but a few relatively dense clumps exist within it (Map 3). As stated within the Enterprise Lake's Draft Aquatic Plant Management Plan, the EWM infestation within the lake is at an early stage, possibly still at a pioneer level. Therefore, an aggressive control approach has been proposed for 2009, specifically within the western area of the lake. The inner part of the bay is proposed to be treated with granular 2,4-D at 100 lbs/acre. The deeper, outer parts of this bay are proposed for treatment at 150 lbs/acre.

Because this bay is shallow and isolated compared with the rest of the lake, it is also conducive for a liquid 2,4-D treatment. This type of herbicide was used in 2007 on another northern Wisconsin lake with anecdotal evidence of an extremely successful treatment. It is important to note that the use of this formulation in northern Wisconsin continues to be experimental. As more information emerges, the potential for its use on Enterprise Lake will be better understood.

In total, 165 sub sample locations were sampled within Site B-09 during the summer of 2008. These data will serve as a valuable pretreatment survey to compare with a post treatment survey conducted during the summer of 2009. This type of data is the most comparable because it surveys the plants when they are at their peak growth (biomass). Native and non-native plant occurrences were noted during this survey.