### INTRODUCTION

The purpose of this report is to relay information regarding the Eurasian water milfoil (EWM) herbicide treatment that was completed on the Lower Eagle River Chain of Lakes 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. The frame of reference begins with the spring pretreatment surveys completed during May 2008, but calls on data collected during the summer of 2007 (pretreatment survey). The report goes on to discuss the condition of the EWM in each lake following the herbicide application. The data used in that section was 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 each lake-specific section. Once agreed upon by the Unified Lower Eagle River Chain of Lakes Commission (ULERCLC) 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 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. There are two different methods of evaluation 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 was 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 ULERCLC successfully applied for an Aquatic Invasive Species Established Infestation Grant and implemented this protocol starting with the 2008 spring pretreatment survey. A quantitative assessment of the treatment was made by collecting data at 682 point-intercept sample locations on the Lower Eagle River Chain of Lakes (Appendix A). At these locations, EWM presence and rake fullness were documented as well as water depth, and substrate type. A rake fullness rating of 1-3 was used to determine abundance of EWM at each location (Table 1).

**Table 1. Rake Fullness Ratings.** These ratings are given for EWM during the spring and summer surveys; and for native plants during the summer surveys.

Rating	Coverage	Description
1	Minnin	A few plants on the rake
2	****	Rake head is about half full
3		The rake head is overflowing

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 this 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 are susceptible to the dicot-specific active ingredient in the granular herbicide Navigate<sup>®</sup> (2,4-D).

#### 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 the Lower Eagle River Chain of Lakes, 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 the 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 variation.

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 less than eight 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 lake-wide analysis. A 20-meter spacing (resolution) between sub-sample locations is considered the closest that hand-held GPS technology can effectively allow. Because many of the 2008 treatment areas on the Lower Eagle River Chain of Lakes were relatively small, only a few sub-sample locations could be placed within their boundaries using this resolution. There were 38 out of 61 treatment sites on the Lower Eagle River Chain of Lakes that had less than eight sub-sample locations. These data must be combined so they can be analyzed appropriately.

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 inter-annual 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 - 05/20/08, 05/21/08, and 05/22/08

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. These areas were accepted by the ULERCLC and the WDNR, and considered the *final* treatment areas. These locations were then provided to the herbicide applicator.

On the first day of the survey, Watersmeet Lake and the Eagle River below Yellow Birch Lake were visited. The weather conditions were sunny and windy. On the second day Scattering Rice, Cranberry, and Catfish Lakes were surveyed. It was sunny with a light wind. On the third day of the survey Voyageur, Eagle, Yellow Birch, Duck, Lynx, and Otter Lakes were visited. The weather conditions were cloudy with little to no wind. Overall, viewing EWM in the Lower Eagle River Chain of Lakes from the surface was relatively effortless because of the clarity of the water during this time of the year. An aqua scope and submersible video camera were used to aid in the survey. The ambient air temperature was 50°F, 46°F, and 60°F respectively.

The final treatment areas (Maps 1-10) were essentially the same as defined in the conditional permit. The most notable exceptions were on Catfish and Otter Lakes. On Catfish Lake, an expansion of Cat-C and the addition of Cat-D added 3.3 acres to the treatment (Map 2). Expansion of Duck-A and Duck-C caused 3.8 additional acres to be added after the pretreatment survey. All treatments occurred between May 28 and June 2, 2008.

# Post Treatment & Peak biomass EWM Survey – 08/19/08, 08/20/08, 08/21/08, and 08/25/08

During this survey, all treatment areas were visited to determine the efficacy of the chemical application. On the first day, Cranberry and Catfish Lakes were visited and the conditions were mostly sunny and breezy. On the second day of the survey Catfish, Voyageur, Eagle, and Scattering Rice Lakes were visited. The conditions were sunny with a light wind. Duck, Lynx, Otter and Yellow Birch Lakes were visited on the third day. The weather conditions were cloudy with a light wind. The last day of the survey Watersmeet Lake and Eagle River were surveyed and the conditions were sunny with a light wind. During the late summer, the EWM has reached its peak biomass, so the plants have nearly reached the surface, making viewing the plant optimal. However, the low water clarity of the chain at this time of the year made viewing deep water occurrences (> 8 feet) difficult. All point-intercept sample locations were also revisited during this survey and data were collected in the same manner as during the pretreatment survey. Native plant frequencies were also documented at the sub-sample locations during this survey for comparison with past and future summer surveys.

The Eagle River Chain contains an established population of EWM. The criteria used to evaluate success of an herbicide treatment for this level of infestation are different than for a newly discovered, pioneer infestation. Please note that the following criteria for success is based on standards created by Onterra and are not outlined within the Aquatic Plant Management Plans developed in 2006 for each respective lake.

The success of the herbicide treatments are evaluated in multiple ways. Qualitatively, a successful treatment on a particular site includes 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 in 2008 needs to decrease by one level of density as described above for an individual site. The definition of each density rating is as follows:

**Scattered** If the target plant occurs in an area that can be enclosed by some geographic boundary, such as a shoreline and a depth contour or in a small bay and that exotic's aerial coverage does not meet the density descriptions described below, then that area would be labeled as "scattered".

**Density** = 1 (**Dominant**) When the colony boundaries are distinct and the exotic appears to be at roughly 50 percent aerial coverage, it would be given a density = 1 rating (D=1).

**Density** = 2 (**Highly Dominant**) These colonies have exotic aerial coverage clearly exceeding 50 percent. The exotic is obviously the dominant species in these colonies, but there is no surface matting.

**Density** = 3 (Surface Matting) This rating would be reserved only for the densest colonies. In these colonies, exotic aerial coverage approaches 100 percent and the plants are canopied and matted on the surface. Boating in these areas may be difficult due to the mass of exotic plants at the surface.

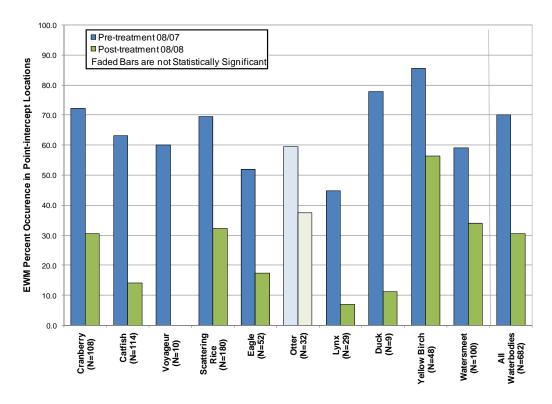
Quantitatively, a successful treatment on a specific site would include a significant reduction in EWM frequency following the treatments as exhibited by at least a 50% decrease in EWM frequency based upon the sub-sampling. In other words, if the EWM frequency of occurrence before the treatment was 80%, 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 survey, 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. Please note that these recommendations are provided within the following sections.

## **CHAIN-WIDE SUMMARY AND CONCLUSIONS**

During the summer before the 2008 treatment on the Lower Eagle River Chain of Lakes, 69.9% of the point-intercept locations contained EWM and 30.6% contained EWM after the treatment indicating a statistically significant, 56.2% reduction in EWM occurrence within the 2008 treatment areas (Figure 1). Yellow Birch Lake and Otter Lake were the only two lakes that did not meet the quantitative success criteria of having a reduction of EWM occurrence greater than 50%. Otter Lake was also the only waterbody that was not shown to be significantly different (p=0.20) (Figure 1). Voyageur had the greatest reduction in EWM occurrence (100%), followed by Duck (85.7%), Lynx (84.6%), and Catfish (76.4%).

A rake fullness rating of 1-3 was used to determine abundance of EWM chain-wide. Figure 2 displays the number of point-intercept locations exhibiting each of the rake fullness ratings within the areas treated throughout the Lower Eagle Chain of Lakes. The figure shows that out of the 477 locations that contained EWM before the treatment, 213 had a rake fullness of greater than one (Figure 2). After the treatment, only 30 contained a rake fullness rating of greater than one (Figure 2).



**Figure 1. EWM percent occurrence in point-intercept locations displayed by lake.** Please note only those treatment sites with eight or more point-intercept locations are displayed on the graph. Statistical significance is determined by Chi-square distribution analysis (alpha = 0.05).

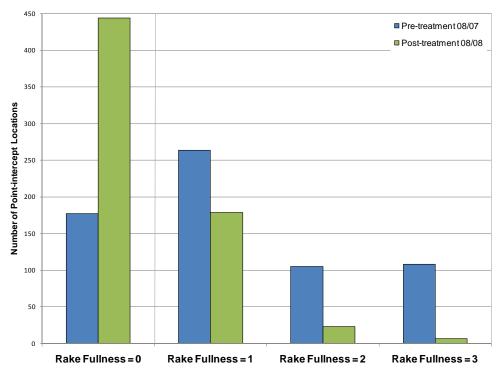


Figure 2. EWM rake fullness distribution chain-wide.

Although it is never the intent of the treatments to impact native species, it is important to remember that these non-target impacts can only be considered in the context of the areas treated and not on a *lake-wide* basis. In other words, the impact of the treatments on a non-target species in the treatment areas cannot be extrapolated to the entire population of that plant within the lake, unless it is only found in locations where there is EWM. The same cannot be said for EWM, because by targeting EWM within the lake, it is intentionally being impacted on a lake-wide basis. One may claim that an impact to non-target natives may leave a 'hole' where pioneer infestations of EWM can take hold. The herbicide currently being used on the chain (Navigate®: 2,4-D) is broad-leaf (dicot) specific and as long as a particular treatment site is not dominated by broad-leaf natives, native monocots (which most aquatic plants are) will provide ample competition to ward off the non-native threat.

Overall only three plants, northern water milfoil, water marigold, and Illinois pondweed were found to have a statistically significant decline within the treatment areas on the Lower Eagle River Chain (Figure 3). Northern water milfoil occurrence reduced slightly by approximately 6% and water marigold reduced even less by 1.5% within the treatment areas. Both these species are dicots and are susceptible to the herbicide application. Herbicide application on the chain occurred in May before the majority of our native plants should be actively growing, adding to the selectivity of the herbicide.

Within the treatment areas, nine native monocots and two native dicots were found to have significantly increased within this time period (Figure 3). It is possible these plants are increasing because they are able to grow without competition from the EWM. The increase in Vasey's pondweed occurrence within the treatment areas by almost 10% is of particular interest because of this species' rarity in Wisconsin. Although its populations are secure globally, it is

rare or uncommon in Wisconsin and is considered a species of special concern in this state. Large-leaf pondweed also increased in frequency within the treatment areas and is of particular interest because it is a popular habitat for large predatory fish like northern pike and muskellunge.

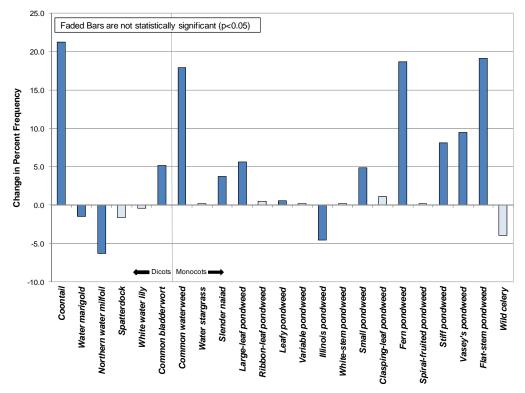


Figure 3. Native plant change in percent frequency with treatment areas from 2007 to 2008 chain-wide.

In 2007, less than one native species was found on average in each point-intercept sub-sample location on Yellow Birch Lake. In 2008, over 3.5 native species were found in each location on average. Although Yellow Birch Lake displayed the largest increase in species richness on the chain, all lakes were shown to increase their native species richness within the treatment areas. Some of the largest increases in species richness were on Yellow Birch, Watersmeet, and Scattering Rice Lakes, those with the greatest densities of EWM infestations. This shows that even a small reduction in EWM density can increase native species abundance, making the system healthier and more resistant to environmental perturbations.

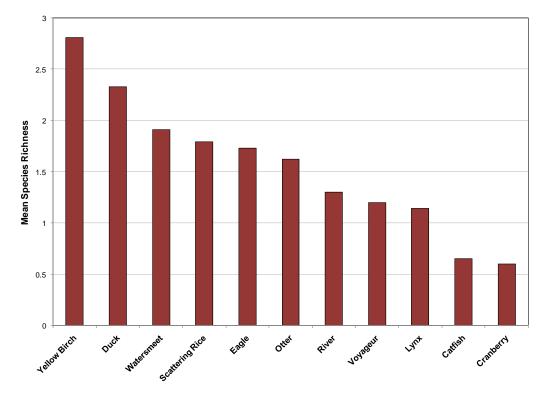


Figure 4. Mean change in native species richness from the point-intercept sub-sample locations from 2007 to 2008. Please note that species richness increased on all waterbodies during this period.

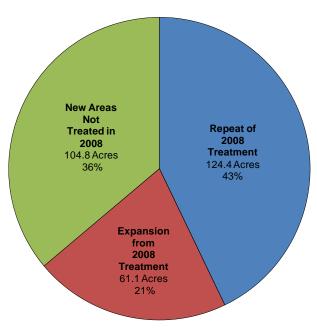


Figure 5. Common acreage comparison between 2008 treatment and proposed treatment for 2009.

In every lake there is at least a portion of a treatment area that was treated in 2008 that is proposed to be treated again in 2009. Approximately 43% of the treatment acreage recommended for 2009 is a repeat of areas treated in 2008 (Figure 5). That scenario is not uncommon in EWM management as dense areas often require multiple years of treatment to decrease the site's density. That being said, not all the lakes have new treatment areas proposed for 2009. Lynx and Voyageur Lakes do not contain newly discovered colonies of EWM that are completely independent from areas (Maps 13 and 17). treated in 2008 Watersmeet and Yellow Birch Lake comprise almost 70% of the new treatment areas proposed for 2009 (Maps 19 and 20). As mentioned in each of the lake-specific sections above, this is largely because an experimental treatment of a subset of the EWM warranting treatment was conducted in 2008. The remaining 21% of the proposed treatments for 2009 are new areas adjacent to 2008 treatment sites. These could be expanded populations since the 2007 peak biomass survey or could have been present, but went undetected during that survey.

Success of the 2008 herbicide treatment was observed on the Eagle River Chain in multiple ways. There were approximately 224 acres of EWM treated on the Lower Eagle River Chain of Lakes in 2008 (Maps 1-10). One hundred of the 224 acres (45%) treated in 2008 are not proposed for treatment in 2009. Additionally, as mentioned above, according the point-intercept survey there was a 56.2% reduction in EWM occurrence chain-wide (Figure 2) coupled with a large shift in rake fullness distribution towards lighter occurrences within those areas that continue to contain EWM. One of the greatest successes of the Eagle River Chain control program is the commitment by volunteers to aid in this process. Some volunteers aid in coordination of the project, some volunteers provide data to the professional ecologists relating to EWM occurrences, and others work to educate other stakeholders on the importance of the system. Continued volunteer commitment will be needed for long-term successes to continue.