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# Note

# Survival and vegetative regrowth of Eurasian and hybrid watermilfoil following operational treatment with auxinic herbicides in Gun Lake, Michigan

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## INTRODUCTION

Eurasian watermilfoil (*Myriophyllum spicatum* L.; EWM) is an invasive aquatic plant that is widespread in inland lakes of the northern tier of the United States. It is commonly managed to alleviate negative economic and environmental impacts, including the formation of dense stands of submerged vegetation that extend from the lake bed to the surface, impairing recreational uses; affecting water movement, nutrient cycling, and sedimentation; reducing property values; and potentially altering habitats used by native species (Smith and Barko 1990, Zhang and Boyle 2010). Management costs of invasive aquatic plants in the United States can exceed $100 million annually (Rockwell 2003, Pimentel et al. 2005). In Michigan, where the present study was conducted, roughly $24 million are spent annually on herbicidal control of aquatic invasive plants, much of which is focused on EWM (MDEQ 2013).

The auxinic herbicides 2,4-D and triclopyr have been used extensively to selectively control EWM (e.g., Getsinger et al. 1982, 1997, Parsons et al. 2001, Wersal et al. 2010). However, herbicide treatments do not typically eradicate EWM from lakes. Even when control efforts result in undetectable levels of EWM for months to years, reestablishment of EWM commonly occurs (Netherland 2014).

The source(s) of regrowth following auxinic herbicide treatments are unclear. It is possible that regrowth occurs via reestablishment from untreated area(s) of the lake or from other lakes. Similarly, it is possible that regrowth occurs via recruitment from a viable seedbank, as EWM can flower profusely and produce viable seeds (Hartleb et al. 1993, Xiao et al. 2010, LaRue et al. 2013a). Alternatively, regrowth may occur from treated plants that are not completely killed, such as sprouting from root crowns or axillary meristems on surviving shoots. Distinguishing among sources of regrowth when assessing herbicide treatment efficacy could be important for informing decisions about management strategies and/or site-specific tactics. For example, if regrowth occurs from seed, management strategies should include tactics to reduce seed production and/or exhaust the seed bank to improve long-term control. If regrowth occurs via fragment recolonization from untreated areas, different spatial management strategies may be required. And, if regrowth arises from incomplete kill of treated plants, then different herbicides and/or application patterns may be required. However, in our experience, monitoring of the source(s) of regrowth is not routinely included in herbicide evaluations of EWM.

In this case study, we monitored quadrats established in treated areas of Gun Lake, Michigan, following treatment by auxinic herbicides. Our goal was to document the source(s) of regrowth throughout the remainder of the summer season following herbicide treatment, and into the following spring before the next management cycle began.

## MATERIALS AND METHODS

Gun Lake is located in both Barry and Allegan counties, Michigan, and has a surface area of 1,119 ha and a mean depth of 3 m. Watermilfoil in Gun Lake has been treated with herbicides since at least 1996, using primarily a combination of 2,4-D ester, 2,4-D amine, and triclopyr; occasionally, diquat dibromide has been applied in small areas.

Numerous areas of Gun Lake were treated with the auxinic herbicides triclopyr (granular trimethylamine salt; Renovate OTF[1]) and 2,4-D (granular dimethylamine salt; Sculpin G[2]) on May 26, 2015. The target concentration for

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Table 1. Description of treatment areas where monitoring quadrats were established, including the identifications of plants found in the nearest point from the point-intercept surveys, and the composition of plants from which vegetative regrowth was collected. The target concentration for 2,4-D treatments was 2.0 mg $L^{-1}$ ae (2 ppm ae) and 1.0 to 1.5 mg $L^{-1}$ ae for triclopyr (1.0 to 1.5 ppm ae). Pre, from pretreatment point-intercept survey; Post, from posttreatment point-intercept survey. EWM; Eurasian watermilfoil; HWM, hybrid watermilfoil; Mix, Eurasian and hybrid watermilfoil; —, no plants in point-intercept survey.

| | | | Point-intercept | | Composition of Plants in Quadrat | |
|---|---|---|---|---|---|---|
| Treatment Area | Size (ha) | Herbicide (product) | Pre | Post | EWM | HWM |
| 14 | 0.4 | Triclopyr (Renovate OTF) | EWM | HWM | 10 | 0 |
| 15 | 0.2 | Triclopyr (Renovate OTF) | EWM | Mix | 7 | 0 |
| 18 | 0.4 | Triclopyr (Renovate OTF) | EWM | — | 5 | 1 |
| 21 | 1 | Triclopyr (Renovate OTF) | Mix | Mix | 1 | 7 |
| 24 | 0.4 | Triclopyr (Renovate OTF) | Mix | Mix | 10 | 0 |
| 31 | 0.2 | 2,4-D (Sculpin G) | Mix | Mix | 12 | 0 |
| 34 | 0.3 | 2,4-D (Sculpin G) | Mix | HWM | 2 | 0 |
| 35 | 0.8 | Triclopyr (Renovate OTF) | HWM | HWM | 0 | 4 |
| 38 | 1.6 | 2,4-D (Sculpin G) | HWM | — | 0 | 5 |

2,4-D was 2.0 mg $L^{-1}$ ae (2 ppm ae), and 1.0 to 1.5 mg $L^{-1}$ ae for triclopyr (1 to 1.5 ppm ae). Specific application rates of the granular products (in kg $ha^{-1}$) were determined in the field by the application company based on water depths in the treatment areas, according to the product labels. In total, 20 areas of the lake ranging in size from 0.1 to 1.4 ha were treated with triclopyr, and seven areas of the lake ranging in size from 0.2 to 1.6 ha were treated with 2,4-D. We focused our study on nine of these areas based on site characteristics that were conducive to scuba (see Table 1; Figure 1; locations of other treatment areas are available from the authors upon request). Each of these areas had either been previously treated as recently as 2014, or were located adjacent to areas that were treated as recently as 2014.

We conducted shoreline point-intercept vegetation surveys before and after treatment on Gun Lake on 15 May and 8 September 2015, respectively. A total of 417 points were surveyed using established waypoints along the shoreline (Figure 1), and we determined frequency of occurrence and abundance at each sampling point using a rake toss method as in Parks et al. (2016). Briefly, we estimated the abundance at each grid point using a semiquantitative index based on visual assessment of the amount of watermilfoil on the rake: 1 = no living watermilfoil present on the rake, 2 = < 5% of the rake tines were covered with watermilfoil, 3 = 5 to 25% of the rake tines were covered with watermilfoil, 4 = 25 to 50% of the rake tines were covered with watermilfoil, and 5 = > 50% of the rake tines were covered with watermilfoil. The mean score of two rake tosses, one from each side of the boat, was used as the abundance at each grid point.

Because EWM, northern watermilfoil (*Myriophyllum sibiricum* Komarov), and hybrid watermilfoil are difficult to accurately distinguish in the field, a representative plant was taken from each point where watermilfoil was found for genetic identification using an assay based on internal transcribed spacer DNA sequence (ITS; Thum et al. 2006, Grafé et al. 2015). Genetic identifications were performed because EWM and hybrid watermilfoil can exhibit different responses to auxinic herbicides (LaRue et al. 2013b, Parks et al. 2016). At survey points where qualitatively distinct phenotypes based on leaf rigidity, pinnae number, and color were observed, one plant per distinct phenotype was collected for genetic identification.

We used an exact McNemar test for dependent proportions to test whether EWM and hybrids significantly decreased in treated and untreated areas (Hollander et al. 2014). We also tested for differences in the degree to which lake-wide EWM versus hybrid watermilfoil frequencies of occurrence changed following treatment using Zelen's exact test (Hollander et al. 2014).

Paired 1-$m^2$ quadrats were established in nine different areas of the lake that were treated with auxinic herbicide. We excluded treated areas located in canals in the northwest portion of the lake, and a narrow bay on the north side of the lake, because of logistical difficulties associated with scuba diving and visibility. Within each treated area selected for monitoring, we determined the exact locations of quadrats by randomly choosing coordinates on a 10 by 10–m grid in the center of the treated area. One member of each pair of quadrats was randomly chosen to be sampled throughout the monitoring period and in these quadrats new growth was removed during sampling. The other quadrat in each treatment area served as an unmanipulated reference that we used to visually confirm that any regrowth observed in our sampled quadrats was indeed viable if left undisturbed.

Beginning 2 wk after treatment in 2015, quadrats were qualitatively monitored weekly by snorkeling to determine if any visible regrowth was apparent. Once regrowth appeared, two scuba divers recorded the number of surviving plants (i.e., plants with mature stems that had survived treatment and were erect) in each quadrat. Once per week from 1 July through 4August 2015 (total of 6 wk), two scuba divers counted and removed all new regrowth in the sampled quadrats. Every observed form of plant regrowth was classified as 1) seedling, 2) fragment, 3) shoot, or 4) root. These forms can be distinguished from one another in the field. Seedlings are small plants with immature leaves, small root crowns, and possibly cotyledons. Fragments will have roots coming from multiple nodes, and usually will have decaying stem tissue toward the bottom of the plant. In contrast, new shoots will arise either from axillary buds on existing stems (shoot growth) or established root crowns (root growth). Because different quadrats had different numbers of surviving plants, we

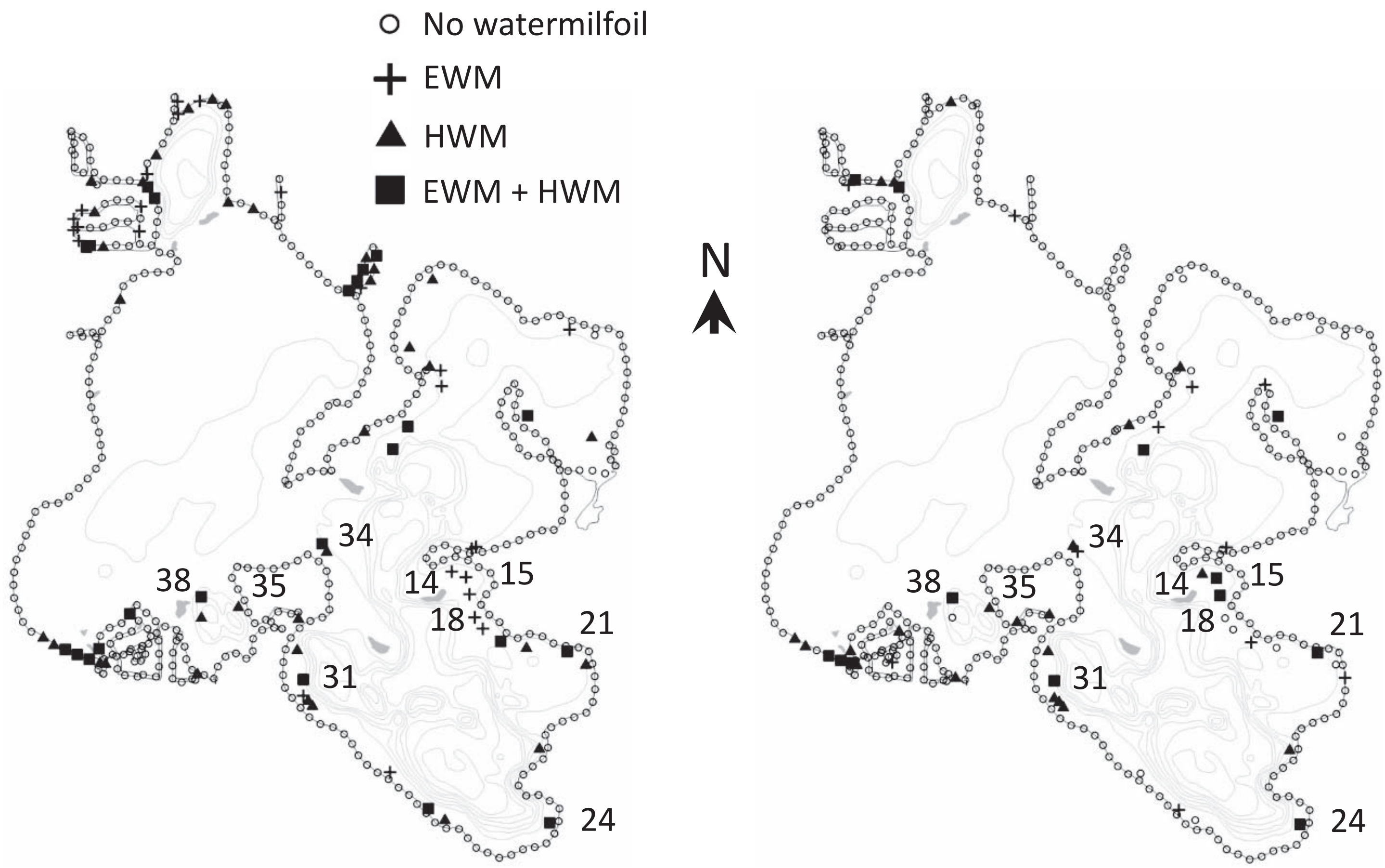


Figure 1. Map of Gun Lake showing the shoreline point-intercept sampling points and plant identifications for pretreatment (left) and posttreatment (right) surveys. The approximate locations of treatment areas where monitoring quadrats were established are numbered as in Table 1. Open circles indicate intercept point where watermilfoil was not found; +, intercept point where Eurasian watermilfoil (EWM) was found; solid triangle, intercept point where hybrid watermilfoi (HWM) was found; solid square, intercept point where both Eurasian and hybrid watermilfoil were found. Light gray lines represent depth contours; the first contour represents 1.5 m (5 feet), and the remaining contours represent 3-m intervals (10 feet). A more detailed map is available online at http://www.dnr.state.mi.us/SPATIALDATALIBRARY/PDF__MAPS/INLAND__LAKE__MAPS/BARRY/GUN__LAKE.PDF. Accessed on Monday, March 13, 2017.

converted counts of regrowth forms into per capita regrowth by dividing counts by the number of surviving plants occurring in each quadrat.

We revisited our quadrats in the spring of 2016 on 16 May and 23 May to visually confirm whether plants from 2015 were still present and viable, as well as to look for any evidence of seedlings. Our study areas were treated again with auxinic herbicides at the end of May, at which point we stopped monitoring our quadrats.

## RESULTS AND DISCUSSION

Based on point-intercept pre- and post-treatment surveys, frequency of occurrence significantly decreased for both EWM and hybrid watermilfoil (see Figure 1). When considering only intercept points located in directly treated areas, pre- and posttreatment frequencies of occurrence decreased from 52 to 27% for EWM (McNemar's test, $P <$ 0.001), and from 65 to 35% for hybrids (McNemar's test, $P <$ 0.001). In contrast to treated areas, pre- and posttreatment frequencies of occurrence did not decrease significantly in areas that were not treated with auxinic herbicides: from 4 to 2% for EWM (McNemar's test, $P = 0.17$), and from 5 to 3% for hybrids (McNemar's test, $P = 0.17$). Furthermore, EWM and hybrids did not differ significantly in their respective lake-wide reductions in frequencies of occurrence (Zelen's test, $P = 0.67$). Thus, it is clear that both EWM and hybrids were significantly and similarly impacted by the auxinic herbicide treatments.

Snorkeling surveys of established quadrats found that the treated plants were severely injured after treatment; most plants in treated beds were lying on the sediment surface and appeared to be dead. However, at 4 wk posttreatment, we observed that many of the same injured plants had survived, and their stems were once again erect. By the end of our sampling in August 2015, plants in treated areas had clearly reestablished (see details below), and visual surveys in May 2016 confirmed that plants were still clearly present and viable in our quadrats.

The regrowth that we observed in the quadrats consisted of regrowth from shoots, roots, and fragments (Figure 2). We found between 2 and 12 plants in the manipulated

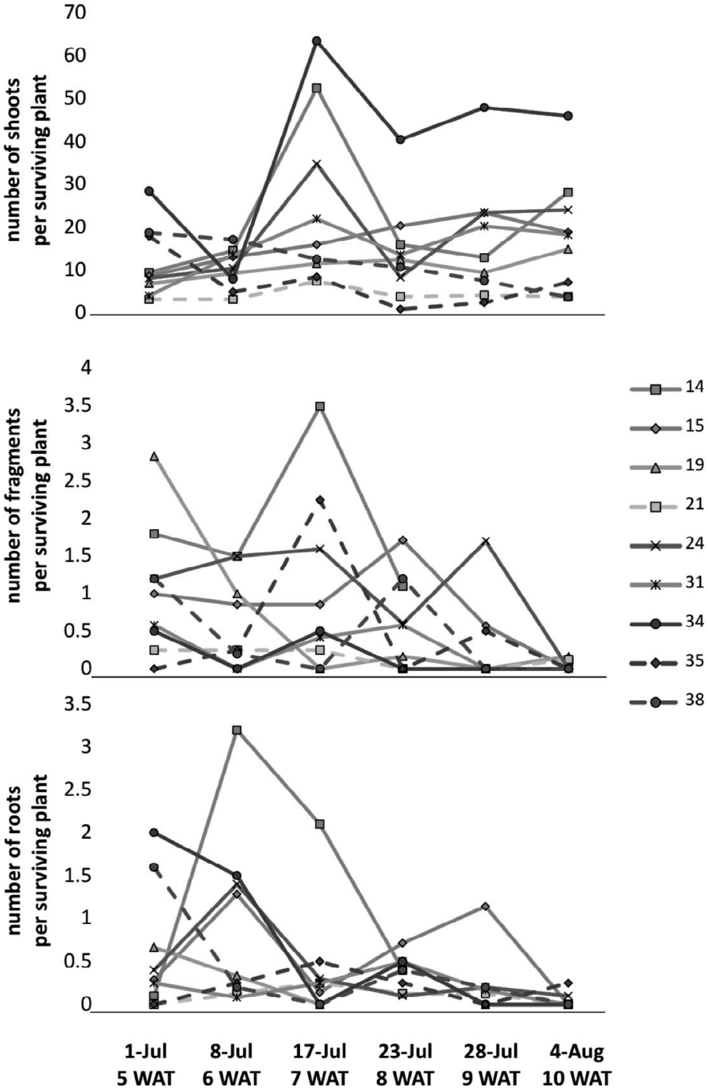


Figure 2. Per capita vegetative regrowth of watermilfoil across six sampling times in nine quadrats located in different treatment areas of Gun Lake, Michigan, following treatment with auxinic herbicides in 2015. (Top) Number of branches per surviving plant sprouting from axillary buds (shoots). (Middle) Number of vegetative fragments per surviving plant settling to and rerooting on the bottom of the quadrats (fragments). (Bottom) Number of shoots per surviving plant sprouting from root crowns (roots). The legend indicates quadrats referred to in Table 1; solid lines apply to quadrats dominated by Eurasian watermilfoil; dashed lines apply to quadrats dominated by hybrid watermilfoil. WAT indicates weeks after treatment.

quadrats in 2015, and genetic analysis of these plants based on the ITS restriction identifications indicated that each quadrat was composed entirely or nearly entirely of either Eurasian or hybrid watermilfoil following treatment (see Table 1). In noting the composition of our study quadrats, we do not intend to imply any differences in survival rates between EWM and hybrids in treatment locations, as we do not have pre- and posttreatment comparisons of the composition of plants in our quadrats. In addition, we did not perform genetic identifications on the fragments that rerooted in the quadrats, so we do not know whether they were similar or different in composition compared to surviving plants.

Numerically, per capita vegetative regrowth was highest from shoots (mean of 16.2 shoots per plant across all quadrats and sampling dates), followed by fragments that had settled in the quadrat (mean of 0.6 fragments per plant across all quadrats and sampling dates), and finally by new shoots establishing from root crowns (mean of 0.4 fragments per plant across all quadrats and sampling dates) (Figure 2).

Some quadrats had more vegetative regrowth than others. In particular, four quadrats had a large pulse of vegetative regrowth from axillary buds on shoots approximately 17 July (between 4 and 5 wk after treatment). However, the underlying causes for these differences are unclear. There was no obvious relationship between the extent of per capita regrowth and treatment method (2,4-D vs. triclopyr). Interestingly, the per capita extent of vegetative regrowth was qualitatively higher for quadrats dominated by EWM compared to quadrats dominated by hybrid watermilfoil.

We did not observe any signs of regrowth from seedlings in our quadrats in either 2015 or 2016. Instead, regrowth came primarily from vegetative growth on surviving plants. However, genetic diversity and differentiation from within and among lakes suggests that sexual reproduction and subsequent recruitment of seedlings plays a role in the establishment of watermilfoil populations (Zuellig and Thum 2012; R. A.Thum, unpublished data). It is possible that seed germination and seedling recruitment is spatially and/or temporally variable. We therefore recommend that similar monitoring for seedlings be conducted on managed lakes whenever possible.

Our study demonstrates the value of monitoring sources of regrowth as part of watermilfoil management programs, but future studies of regrowth could be improved and expanded. First, we did not have the resources to measure the achieved herbicide concentrations in treated areas, which is neither required nor routine for watermilfoil control with auxinic herbicides in Michigan. It is possible that the achieved concentration-exposure times in our study quadrats were only sufficient to injure EWM, as opposed to control it, as predicted by the concentration and exposure time relationship for EWM presented by Green and Westerdahl (1990). Indeed, the treatment areas were small relative to the overall size of Gun Lake, which may lead to rapid dilution and dissipation of the herbicide (Getsinger et al. 2000, Poovey et al. 2004). Since the extent and sources of regrowth may vary with the concentration and exposure time of plants, future studies of regrowth should include measurements of the actual herbicide concentrations achieved, and search for any patterns between regrowth and exposure. Second, we did not quantitatively compare sampled quadrats to reference quadrats, nor did we quantify regrowth in treated versus untreated areas. These comparisons in future studies could help better quantify the extent of regrowth, which could help inform management decisions aimed at reducing the likelihood and extent of regrowth. Finally, future studies could consider whether different biotypes or genotypes exhibit different tendencies for regrowth.

In conclusion, through underwater monitoring of nine quadrats located in auxin herbicide–treated areas in Gun Lake, Michigan, we observed recovery and survival of severely injured plants. Vegetative regrowth from these plants occurred in the forms of axillary shoot formation, resprouting from root crowns, and rooting of settled vegetative fragments. Vegetative regrowth responses first became evident approximately 4 wk after treatment and appeared to be the main sources of the regrowth of EWM and hybrid watermilfoil following treatment with auxinic herbicides in Gun Lake. While the exact source(s) of regrowth following herbicide treatment may vary from lake to lake and over time, this study demonstrates that severely injured plants, judged as having been killed when visually assessed up to 4 wk after treatment, can survive auxinic herbicide treatment (at recommended levels in Michigan) and produce new shoots. We therefore recommend that, although labor intensive, EWM management plans include field monitoring of efficacy and regrowth whenever possible in order to best inform adaptive management decisions. Over the long term, implementing such studies in multiple lakes and across multiple treatments may help to establish patterns of regrowth of different lineages of watermilfoil in different environmental settings.

## SOURCES OF MATERIALS

[1]Renovate OTF, SePRO Corporation, 11550 North Meridian Street, Suite 600, Carmel, IN 46032.

[2]Sculpin G, SePRO Corporation, 11550 North Meridian Street, Suite 600, Carmel, IN 46032.

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