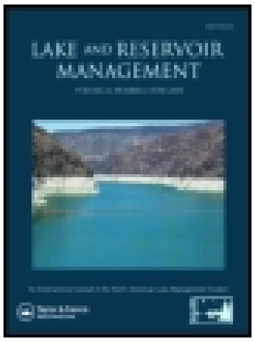

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Evaluation of large-scale low-concentration 2,4-D treatments for Eurasian and hybrid watermilfoil control across multiple Wisconsin lakes

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ABSTRACT

Nault ME, Barton M, Hauxwell J, Heath E, Hoyman T, Mikulyuk A, Netherland MD, Provost S, Skogerboe J, Van Egeren S. 2017. Evaluation of large-scale low-concentration 2,4-D treatments for Eurasian and hybrid watermilfoil control across multiple Wisconsin lakes. Lake Reserv Manage. 00:00–00.

Herbicides have been utilized for decades for nonnative milfoil control; however, limited literature is available examining large-scale herbicide applications, especially for commonly used herbicides such as 2,4-D (2,4-dichlorophenoxy acetic acid). Twenty-three lakes were studied pretreatment and posttreatment to monitor large-scale and low-concentration (lakewide rate: 73–500 μg/L) 2,4-D dissipation and degradation patterns, and determine the efficacy and selectivity of these treatments for Eurasian watermilfoil (*Myriophyllum spicatum*; EWM) and hybrid watermilfoil (*Myriophyllum spicatum* × *M. sibiricum*; HWM) control. Measured mean surface concentrations averaged throughout the initial 2 weeks after treatment ranged from 119 to 544 μg/L. In addition, the threshold for irrigation of plants which are not labeled for direct treatment with 2,4-D (<100 μg/L by 21 d after treatment) was exceeded in 18 of the 28 treatments. Calculated 2,4-D half-lives ranged from 4 to 76 d, and herbicide degradation was generally observed to be slower in oligotrophic seepage lakes. Year of treatment reductions in milfoil frequency ranged from 4 to 100%, with sustained multi-year control observed in some lakes. While good year of treatment control was achieved in all lakes with pure EWM populations, significantly reduced control was observed in the majority of lakes with HWM populations. Several native monocotyledon and dicotyledon species also showed significant declines posttreatment, with variation in recovery observed over time. Although target species control was achieved with some of these treatments, variation in herbicide persistence, reduced control in many HWM populations, and nontarget impacts to certain native plants demonstrate the need for additional research and field studies.

KEYWORDS
Aquatic herbicide; aquatic plant management; chemical control; invasive species; *Myriophyllum spicatum*; *Myriophyllum spicatum* × *sibiricum*; native macrophytes; 2,4-dichlorophenoxy acetic acid

Eurasian watermilfoil (*Myriophyllum spicatum*; EWM) is an invasive aquatic plant native to Europe, Asia, and northern Africa that was first introduced to Wisconsin in the 1960s and is currently known to be present in approximately 650 lakes and flowages throughout the state (WDNR 2016). It has become a widespread concern throughout the United States and Canada (Aiken et al. 1979, Couch and Nelson 1985), with some milfoil populations exhibiting dense canopy growth, impeding recreational activities, outcompeting native species, and reducing property values (Smith and Barko 1990, Madsen et al. 1991, Boylen et al. 1999, Horsch and Lewis 2009).

The recent recognition and widespread geographic distribution of hybrid watermilfoil genotypes (*M. spicatum* × *M. sibiricum*; HWM) in waterbodies across the United States (Moody and Les 2002, Moody and Les 2007, Sturtevant et al. 2009, Zuellig and Thum 2012) and Canada (Borrowman et al. 2014, Grafe et al. 2015) further complicates the understanding of both the ecology and management of invasive milfoils (hereafter the term "milfoil" will refer to both EWM and HWM populations). While there have been several laboratory studies conducted comparing the growth and control of various EWM and HWM strains (e.g., Poovey et al. 2007, Glomski and Netherland 2010, LaRue et al.

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Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/ulrm.

2013b, Taylor et al. 2017), there is very little literature available on the differences observed between these populations in natural field settings (but see Parks et al. 2016, Thum et al. 2017).

Although there have been a variety of management techniques investigated for milfoil control (e.g., mechanical harvesting, biocontrol, hand-removal, bottom barriers), lake managers in Wisconsin have primarily relied on auxin-mimic herbicides, especially 2,4-D (2,4-dichlorophenoxy acetic acid), which is viewed as a selective and cost-effective management tool. In more recent years, 2,4-D and a variety of other auxin-mimic and systemic herbicides (e.g., triclopyr, fluridone) have been applied on a large scale (generally defined as >5% surface area) in efforts to provide long-term lakewide control of the target invasive species. The value in relating measured herbicide concentrations to long-term efficacy and selectivity following large-scale milfoil management efforts has been demonstrated in research with the aquatic herbicide fluridone (Getsinger et al. 2002, Madsen et al. 2002, Pedlow et al. 2006, Valley et al. 2006, Wagner et al. 2007, Parsons et al. 2009) and triclopyr (Netherland and Jones 2015). However, very little literature is currently available on the effects of large-scale management utilizing 2,4-D.

Efficacy and selectivity of 2,4-D is a function of concentration and exposure time (CET) relationships. Green and Westerdahl (1990) describe several short-term CET scenarios (12–72 h of exposure to 500–2000 μg/L) that can occur following various operational treatments. Subsequent research by Glomski and Netherland (2010) demonstrated that long-term exposures (>14 d) to 2,4-D concentrations as low as 100 μg/L can result in milfoil control. Prior field research has been conducted to demonstrate efficacy and selectivity of 2,4-D following treatment of plots in larger waterbodies (Carpentier et al. 1988, Parsons et al. 2001, Wersal et al. 2010), but these studies were likely influenced by rapid dispersion of herbicide from treatment sites. A recent controlled field study examining whole-lake low-concentration (application rate: 275 and 500 μg/L) 2,4-D treatments on 2 northern Wisconsin lakes resulted in multi-year lakewide control of EWM; however, reductions in both frequency and biomass of several nontarget native monocotyledon and dicotyledon species were observed (Nault et al. 2014). Both of these treatments also resulted in much longer than predicted 2,4-D half-lives.

The continued spread of milfoil within invaded lakes, as well as new introductions of milfoil to waterbodies across the landscape, has resulted in an increase in permit and grant funding requests submitted to the Wisconsin Department of Natural Resources (WDNR) to chemically control milfoil at a large scale using 2,4-D. As with any large-scale chemical treatment, both the positive and negative effects of this type of treatment strategy are anticipated to occur at a lakewide scale.

To better understand the efficacy and selectivity of these herbicide treatments, we assessed the results of 28 large-scale, low-concentration 2,4-D treatments permitted in 23 Wisconsin lakes between 2008 and 2016. The specific objectives of the study were to (1) collect and analyze data regarding actual 2,4-D concentration and exposure times across various lakes, (2) collect and analyze data on short- and long-term control of EWM and HWM populations, (3) collect and analyze data on native plant community responses, and (4) develop recommendations for improving control of milfoil while minimizing nontarget impacts.

Methods

Study sites

Lakes were located throughout Wisconsin, USA (Fig. 1), and were chosen based upon research needs outlined by WDNR and U.S. Army Corps of Engineers Engineer Research and Development Center, as well as an interest in participation by lake association groups and management consultants. Lakes ranged from 5.4 to 577.7 ha in size, and from 3.4 to 25.9 m in depth. Lakes varied in trophic status from oligotrophic to eutrophic, and were mostly classified as drainage (inlet and outlet present) or seepage (no inlet or outlet present). During the growing season, lake water clarity (Secchi depth) ranged from 1.0 to 7.3 m, conductivity from 59 to 557 μS/cm, and pH from 7.3 to 8.9. Additional lake-specific characteristics are presented (Table 1).

Herbicide application and concentration monitoring

We defined large-scale treatments as those in which the total quantity of 2,4-D applied to the lake was sufficient to dissipate to a concentration that could affect aquatic plants lakewide (>100 μg/L; Glomski and Netherland 2010). In some lakes, the herbicide was applied over the entire lake surface at the lakewide

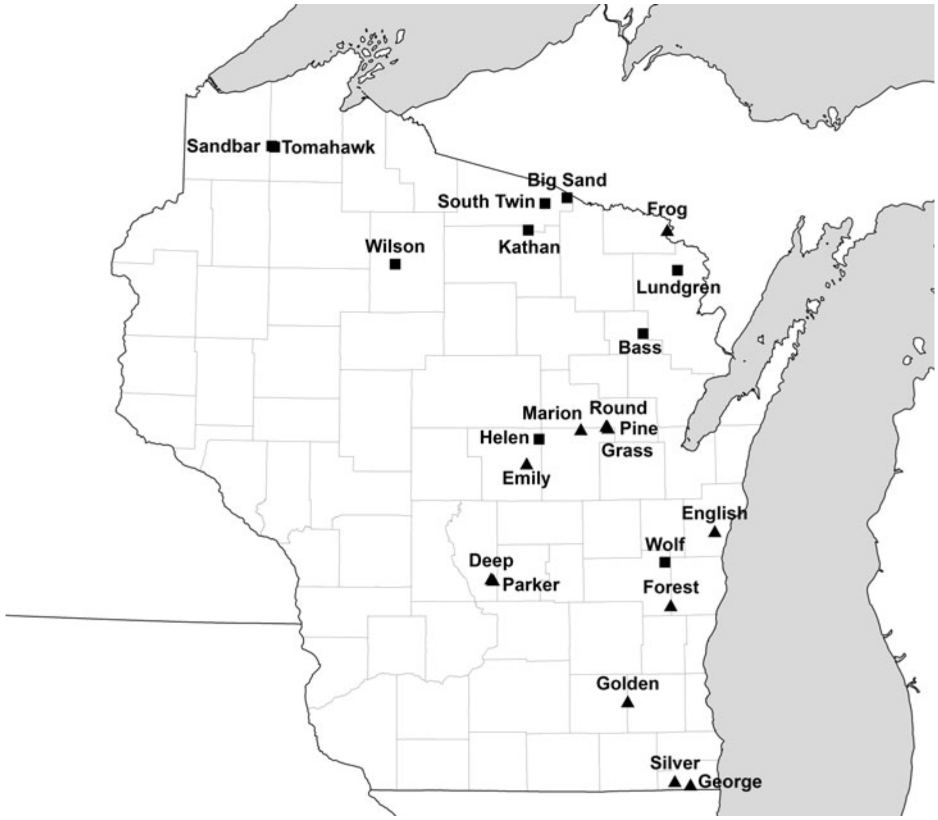

Figure 1. Location of 23 Wisconsin lakes monitored for herbicide concentration exposure times and aquatic plant community responses following large-scale 2,4-D treatments. Squares = EWM; Triangles = HWM.

Table 1. Summary of the characteristics of 23 Wisconsin lakes monitored for herbicide concentrations and aquatic plant community responses following large-scale 2,4-D treatments.

Lake	County	Lake area (ha)	Maximum depth (m)	% Littoral[1]	Lake type	Trophic status	pH[2]	Conductivity (μS/cm)[2]	Secchi (m)[3]
Tomahawk	Bayfield	53.1	12.8	84	Seepage	Mesotrophic	8.7	65	4.5
Sandbar	Bayfield	51.3	14.9	52	Seepage	Oligotrophic	7.6	59	5.6
South Twin	Vilas	254.0	13.1	49	Drainage	Mesotrophic	7.7	101	3.5
Kathan	Oneida	79.4	4.6	97	Drainage	Eutrophic	7.3	73	1.3
Wilson	Price	140.8	3.4	94	Drainage	Eutrophic	7.7	106	1.0
Wolf	Fond du Lac	30.4	14.3	38	Seepage	Mesotrophic	8.3	506	2.5
Helen	Portage	35.9	6.1	100	Seepage	Oligotrophic	8.8	325	3.8
Big Sand	Vilas	577.7	17.1	55	Drainage	Eutrophic	8.2	79	4.0
Lundgren	Marinette	12.2	18.9	31	Seepage	Oligotrophic	8.1	125	7.3
Bass	Oconto	5.4	3.4	96	Drained	Mesotrophic	7.8	129	2.3
English	Manitowoc	20.5	25.9	24	Seepage	Eutrophic	8.9	371	2.4
Forest	Fond du Lac	20.5	9.8	90	Seepage	Mesotrophic	8.6	221	4.5
Frog	Florence	7.1	6.4	100	Seepage	Mesotrophic	8.9	140	4.4
Silver	Kenosha	208.8	13.4	82	Drainage	Mesotrophic	8.8	457	2.6
Deep	Adams	13.5	14.9	38	Seepage	Mesotrophic	7.8	222	4.0
Round	Shawano	10.9	11.9	29	Spring	Mesotrophic	8.5	368	2.7
Grass	Shawano	36.3	15.8	44	Drainage	Mesotrophic	8.2	337	2.4
Pine	Shawano	87.9	10.7	52	Drainage	Mesotrophic	8.2	294	3.1
Emily	Portage	43.5	11.0	48	Seepage	Mesotrophic	8.6	284	3.1
Parker	Adams	22.9	11.0	84	Seepage	Oligotrophic	8.1	278	4.2
Golden	Waukesha	102.0	13.4	79	Spring	Oligotrophic	8.2	406	4.9
George	Kenosha	27.9	4.9	65	Drainage	Eutrophic	8.5	557	1.2
Marion	Waupaca	46.8	3.7	100	Drainage	Eutrophic	8.8	462	2.5

[1]Calculated from survey prior to large-scale treatment.
[2]Mean of surface measurements from April to October.
[3]Mean calculated for May–September from 2000 through year prior to treatment.

Table 2. Herbicide application details for 23 Wisconsin lakes (28 treatments) monitored for herbicide concentrations and aquatic plant community responses following large-scale 2,4-D treatments.

Lake	Date(s) treated	Applied [2,4-D] in treated areas (μg/L acid equivalent)	% Lake surface area treated	Lakewide target [2,4-D] (μg/L ae)	Historical milfoil treatments?
Tomahawk	20-May-2008	500	100	500	No
Sandbar	25-May-2011	275	100	275[‡]	No
	21-Jun-2013	300	100	300[‡]	Yes
South Twin	30-May-2009	1750	27	100	Yes
	25-May-2010[†]	2500	26	240	Yes
	07-Jun-2016	2300	26	350[‡]	Yes
Kathan	13-May-2010[†]	500	59	277	No
Wilson	24-Apr-2012	1700; 1000[*]	32	331	Yes
Wolf	04-Jun-2014	2200	25	300[‡]	Yes
Helen	27-May-2014	4000	10	300[‡]	Yes
Big Sand	20-May-2010	2100	8	73	Yes
Lundgren	03-May-2016	870	36	300[‡]	No
Bass	27-May-2014	375	100	375	Yes
English	17-May-2010	2000	27	300[‡]	No
	01-May-2012	3750	28	347[‡]	Yes
Forest	26-May-2011	600	32	300[‡]	Yes
	12-Apr-2012	1000	43	365[‡]	Yes
Frog	13-May-2010	250	100	250	Yes
Silver	08-May-2013	1500	50	350	No
Deep	05-Jun-2013	350	100	350[‡]	No
Round	25-Apr-2012	3500	17	349[‡]	Yes
Grass	25-Apr-2012	1800	27	352[‡]	Yes
Pine	29-May-2013	2050	30	352[‡]	Yes
Emily	13-May-2015	4000	15	350[‡]	Yes
Parker	12-May-2015	4000	17	350[‡]	Yes
Golden	04-Jun-2013	2000; 1500[**]	14	242	No
George	23-May-2013	1400	57	350	Yes
Marion	13-May-2013	3100	8	350	Yes

[†]Herbicide application occurred over two consecutive days.
[*]One site treated at 1700 μg/L and two sites treated at 1000 μg/L.
[**]Liquid application at 2000 μg/L and granular at 1500 μg/L.
[‡]Epilimnetic target concentration.

target concentration, while in others herbicide was applied at higher rates only to areas densely populated with milfoil, assuming that dissipation would occur off these sites and lakewide low concentrations would be achieved (Table 2). Lakewide 2,4-D target concentrations ranged from 73 to 500 μg/L. In deeper lakes, thermal stratification was determined prior to treatment and only epilimnetic waters were used in volumetric calculations for dosing. The aquatic herbicide 2,4-D (2,4-dichlorophenoxy acetic acid, dimethylamine salt) was applied as a liquid formulation (DMA 4 IVM, Weedestroy AM40, or Havoc Amine) using a powered injection system with weighted drop hoses to inject the herbicide ~1–2 m below the water's surface. One exception to this was the English Lake 2012 treatment, which used the LittLine deep-water injection system, an application system that reportedly minimizes herbicide diffusion by delivering the herbicide closer to the bottom of the lake. Golden Lake also used granular 2,4-D (Navigate, butoxyethyl ester) in some milfoil areas and the liquid amine formulation in other areas. Herbicide treatments occurred between April and June while surface water temperatures were still cool (11–20 C). Herbicide applied in spring while water temperatures are still cool can allow a window of application that targets early emerging exotic plants and minimizes exposure to many dormant native plants, and thus may reduce likelihood of plant injury (Skogerboe and Getsinger 2006). Early-season applications also minimize plant biomass decomposition and dissolved oxygen depletion, and initially allow for slower herbicide degradation resulting in longer contact times.

Partnering with lake residents, we used an integrated sampler to collect upper water column (0–3 m) samples at 3–7 locations on each lake to quantify 2,4-D degradation rates and verify herbicide dissipation within the lake. To monitor vertical mixing in stratified lakes, we used a Van Dorn sampler to collect water samples at approximately one-half and three-fourths of the maximum lake depth. We collected water samples at each location at approximately 1, 3, 5, 7, 14, 21, 28, and 35 days after treatment (DAT), with actual collection dates slightly varying across lakes due to inclement weather, volunteer availability, and herbicide persistence. We preserved samples with muriatic acid to prevent microbial degradation of 2,4-D after collection and stored samples at 5 C until analyzed. Analyses of 2,4-D concentrations were conducted at the University of Florida Center for Aquatic and Invasive Plants or the Wisconsin State Lab of Hygiene using an enzyme-linked immunosorbant assay (ELISA; Fleeker 1987). Herbicide concentrations are reported here as 2,4-D acid equivalent (ae).

Statistical analysis was performed in the R environment for statistical computing (version 3.1.2; R Core Team 2014). We averaged the herbicide concentrations across upper water column sample sites for each sampling date. Linear models were fit to the concentration data over time for each application, and the response variable was log-transformed where necessary to satisfy the assumptions of linearity. In scenarios where the linear and log-linear models were both statistically significant ($P < 0.05$) and fit about equally well (R^2 values within 0.1), the linear model was chosen for its ease of interpretation. We used the estimated y intercept as an approximation of the initial lakewide concentration, and the slope parameter to describe the overall rate of degradation, estimated half-life of 2,4-D, and the number of DAT that lakewide concentrations were greater than the irrigation standard of 100 μg/L for each treatment. Linear models were used to analyze the relationship between estimated 2,4-D half-lives and within lake water quality variables (e.g., Secchi depth, pH, and conductivity). Unpaired t-tests were used to analyze the effect of lake type (e.g., seepage or drainage) on estimated 2,4-D half-lives, as well as the effect of previous 2,4-D exposure on degradation rates. Linear models were used to analyze the relationship between milfoil control and within-lake water quality variables, as well as the measured herbicide concentration during the first 2 weeks after treatment. Unpaired t-tests were used to analyze the effect of milfoil genetics (e.g., EWM or HWM) on milfoil control.

Aquatic plant surveys

We conducted lakewide pretreatment and posttreatment aquatic plant surveys following a grid-based point-intercept approach (Madsen 1999, Hauxwell et al. 2010), which has been shown to be appropriate for comparative studies (Mikulyuk et al. 2010). We recorded species presence/absence and water depth at each site on a geo-referenced sampling grid. Grid spacing resolutions were based on the equations given in Mikulyuk et al. (2010) and ranged from 20 to 80 m, resulting in 59–902 sample points (Table 3). At sites shallower than 5 m, we lowered a double-headed rake (0.34 m wide, 14 tines per head) on an adjustable pole vertically through the water column to the sediment surface, rotated it twice, and then pulled it straight out of the water. At sites deeper than 5 m, we used a similar rake head attached to a rope to collect plants. The maximum depth of plant colonization was determined for each lake, and all sites that were less than or equal to that depth were sampled. Plants retrieved on the rake, as well as plant fragments detached from the bottom, were identified to species level following Crow and Hellquist (2000a, 2000b), with the exception of macroalgae (i.e., *Chara* and *Nitella*), which were identified to genus level. Milfoil plants were collected from each lake and sent to the Annis Water Resources Institute at Grand Valley State University for genetic internal transcribed spacer (ITS) sequencing to determine whether milfoil populations were composed of HWM strains or pure-strain EWM (Moody and Les 2007, Zuellig and Thum 2012). The number of milfoil plants analyzed on each lake varied, and was primarily determined by collecting all visibly distinct phenotypes that were observed (based on leaflet number, leaf rigidity, color, etc.). In some lakes a single representative milfoil sample was analyzed (in instances where only one distinct phenotype was visually observed) while other lakes had numerous individual samples analyzed (up to several hundred individual plants in the cases of English, Frog, and Silver lakes, which were part of other research studies with more intensive within-lake sampling). EWM lakes were categorized as ones where only pure EWM strains were detected and HWM lakes had all or at least a portion of their milfoil population genetically confirmed as containing hybrid strains. Surveys were conducted between July and September to most accurately assess both native and nonnative plants. We also conducted supplemental pretreatment surveys on some lakes in the early spring, recording only whether milfoil was present at each survey point. Littoral frequencies of occurrence were calculated as the number of sites where a species was present, divided by the number of sites less than or equal to the maximum depth of plant colonization. Milfoil populations were tested for significant differences in littoral frequency of occurrence between the survey immediately before treatment and all years

Table 3. Aquatic plant community point-intercept survey summary statistics for 23 Wisconsin lakes monitored for herbicide concentrations and aquatic plant community responses following large-scale 2,4-D treatments.

Lake	Maximum depth of plant colonization (m)[1]	% Vegetated[1,2]	Species richness[1]	Milfoil % frequency[1,2]	Year milfoil reported	Genetic identification	Large-scale treatment year(s)	Aquatic plant surveys	# Sample points[3]	Distance between points (m)
Tomahawk	6.8	86	21	40	2004	EWM	2008	2006–2015	427	35
Sandbar	7.2	84	19	42	2004	EWM	2011; 2013	2007–2015	324	40
South Twin	5.0	94	26	21	2001	EWM	2009; 2010; 2016	2008–2011; 2013–2016	622	64
Kathan	2.9	96	30	49	2004	EWM	2010	2007–2015	203	65
Wilson	2.4	55	25	12	2002	EWM	2012	2011–2012; 2014–2015	225	78
Wolf	4.6	72	9	18	1994	EWM	2014	2013–2016	328	31
Helen	6.1	89	14	3	2008	EWM	2014	2013–2016	229	40
Big Sand	4.6	88	37	8	1990	EWM	2010	2006; May & Aug 2010; 2011	902	80
Lundgren	5.2	92	16	42	2014	EWM	2016	2015–2016	309	20
Bass	3.0	98	20	0‡	2002	EWM	2014	2011; May & Aug 2014	59	30
English	4.9	66	7	92	2008	HWM	2010; 2012	2006; May & Aug 2010; 2011–2012	211	30
Forest	7.6	92	19	27	1992	HWM*	2011; 2012	2008; May & Aug 2011; 2012–2013; 2015	184	33
Frog	5.2	93	12	16	2002	HWM	2010	2005; 2009–2012	125	30
Silver	6.7	87	18	53	1994	HWM*	2013	2006; 2012–2014	491	65
Deep	6.4	82	17	46	2008	HWM*	2013	2012–2016	148	30
Round	6.7	83	10	19	1992	HWM	2012	2010; 2012–2013; 2015	174	25
Grass	4.6	92	23	53	1994	HWM	2012	2010; 2012–2013; 2015	233	40
Pine	5.2	65	10	14	1994	HWM	2013	2010; 2013; 2015	398	47
Emily	6.4	78	30	8	1993	HWM	2015	2014–2016	351	35
Parker	7.6	88	15	9	1995	HWM*	2015	2014–2016	249	30
Golden	9.9	75	19	34	1995	HWM	2013	May & Aug 2013; 2014	565	46
George	1.7	76	10	78	1977	HWM	2013	2011; May & Aug 2013	85†	60
Marion	3.2	99	17	65	2000	HWM	2013	2012–2013	122	61

[1]Calculated from survey prior to treatment.
[2]Calculated for points within the littoral zone (shallower than maximum depth of plant colonization).
[3]Based upon Mikulyuk et al. 2010 except for (†).
‡Milfoil % frequency of occurrence too low to measure utilizing point-intercept methodology.
*Population also contains EWM strains.

posttreatment using Pearson's chi-squared test. Native plant species with at least a 10% littoral frequency of occurrence in either pretreatment or posttreatment surveys were tested for significant differences between the summer immediately before treatment and summer posttreatment.

Results and discussion

Herbicide dissipation and degradation

Lakewide target concentrations ranged from 73 to 500 μg/L and measured mean surface concentrations averaged throughout the initial 2 weeks of treatment (1–14 DAT) ranged from 119 to 544 μg/L (Table 4). In lakes where only areas populated with milfoil were treated at higher concentrations, herbicide dissipation from the treatment sites into surrounding untreated waters was rapid (within 1 d), and lakewide low-concentration equilibriums were reached within the first few days after application. This rapid dissipation of herbicide off of treatment areas is important for resource managers to consider in planning, as treating numerous targeted areas at a "localized" scale may actually result in low concentrations capable of

Table 4. Linear model parameters describing regression of 2,4-D concentration (μg/L) of lakewide means of all surface samples versus days after treatment. Log-transformed parameters were back-transformed; the slope parameter describes a multiplicative change in herbicide concentration.

Lake	Intercept	Slope	Adjusted R^2	F	P	2,4-D target (μg/L)	Measured 1–14 DAT mean (μg/L)	Measured 1–14 DAT maximum (μg/L)	Measured 1–14 DAT minimum (μg/L)	Calculated 2,4-D half-life (days)	# Days >100 μg/L
Tomahawk‡	623	−0.98 03296733	0.99	1658	< 0.001	500	544	926	370	35	93
Sandbar 2011	392	−6.25	0.92	94	< 0.001	275	350	467	285	31	47
Sandbar 2013	372	−4.76	0.86	32	0.005	300	340	491	248	39	57
South Twin 2009	141	−3.86	0.97	208	< 0.001	100	119	164	74	18	11
South Twin 2010	552	−19.04	0.97	90	0.011	240	399	513	272	14	24
South Twin 2016	350	−11.14	0.90	53	< 0.001	350	288	590	120	16	22
Kathan	191	−7.19	0.78	26	0.002	277	155	353	46	13	13
Wilson	347	−11.4	0.89	67	< 0.001	331	285	481	39	15	22
Wolf	330	−15.59	0.99	719	< 0.001	300	237	370	100	11	15
Helen‡	180	−0.99	0.21	3	0.102	300	174	250	85	99	84
Big Sand	206	−8.95	0.84	28	0.006	73	155	781	5	11	12
Lundgren	553	−3.66	0.97	347	< 0.001	300	530	690	430	76	124
Bass	372	−18.66	0.92	59	0.002	375	253	470	77	10	15
English 2010‡	597	−0.87	0.66	9	0.059	300	254	432	28	5	12
English 2012‡	583	−0.94	0.22	3	0.116	365	373	770	193	12	30
Forest 2011	339	−6.92	0.85	42	< 0.001	300	298	417	249	24	35
Forest 2012	402	−6.62	0.90	85	< 0.001	365	355	472	277	30	46
Frog	336	−11.34	0.95	120	< 0.001	250	270	357	136	15	21
Silver	370	−11.60	0.84	43	< 0.001	350	314	472	168	16	23
Deep	423	−1.58	0.02	1	0.313	350	409	662	304	134	204
Round	349	−3.65	0.40	6	0.040	349	325	456	132	48	68
Grass	313	−5.61	0.65	16	0.005	352	280	500	164	28	38
Pine	291	−5.00	0.90	62	< 0.001	352	258	435	86	29	38
Emily	303	−3.51	0.78	29	0.001	350	270	740	110	43	58
Parker	221	−2.71	0.87	49	< 0.001	350	206	300	85	41	45
Golden‡	497	−0.88	0.89	41	0.003	242	256	845	30	5	13
George	525	−42.53	0.81	22	0.009	350	319	864	11	6	10
Marion	295	−15.35	0.93	92	< 0.001	350	195	499	6	10	13

‡Concentrations log-transformed for regression.

having lakewide impacts as the herbicide dissipates off of the treatment sites. If the percentage of treated areas to overall lake surface area is >5% and targeted areas are treated at relatively high 2,4-D concentrations (i.e., 2000–4000 μg/L), then anticipated lakewide concentrations after dissipation should be calculated to determine the likelihood of lakewide effects. This is especially true if a thermocline has formed, as the volume into which the treatments are dissipating may be greatly reduced.

Lakewide regression models relating mean 2,4-D concentration at surface sites to DAT were statistically significant ($P < 0.05$) for all lakes except English, Deep, and Helen (Fig. 2; Table 4). English Lake is a small (20.5 ha) and very deep (25.9 m) seepage lake, which may have affected lakewide herbicide dissipation patterns. Deep and Helen showed almost no herbicide degradation over the monitoring period, and thus appropriate degradation models could not be generated. The adjusted R^2 for significant models ranged from 0.40 to 0.99. Calculated 2,4-D half-lives from statistically significant models ranged from 4 to 76 DAT, and the threshold for irrigation of plants which are not labeled for direct treatment with 2,4-D (<100 μg/L by 21 DAT) was exceeded in 18 of the 28 treatments. The 100 μg/L threshold is also ecologically relevant to milfoil control, as previous studies have indicated that this low concentration can achieve milfoil control if exposure times are extended (Glomski and Netherland 2010). In lakes where vertical mixing was monitored, significantly lower concentrations were measured at middle and bottom sampling sites (approximately one-half and three-fourths of the maximum depth), indicating that thermal stratification impeded vertical mixing of the herbicide into deeper waters (Fig. 3). These findings are consistent with other large-scale chemical applications monitored to date, and indicate that application rate calculations for large-scale treatments may be more accurate if based on the volume of water above the thermocline rather than the volume of the whole lake (Netherland et al. 2002, Nault et al. 2014, Netherland and Jones 2015). If

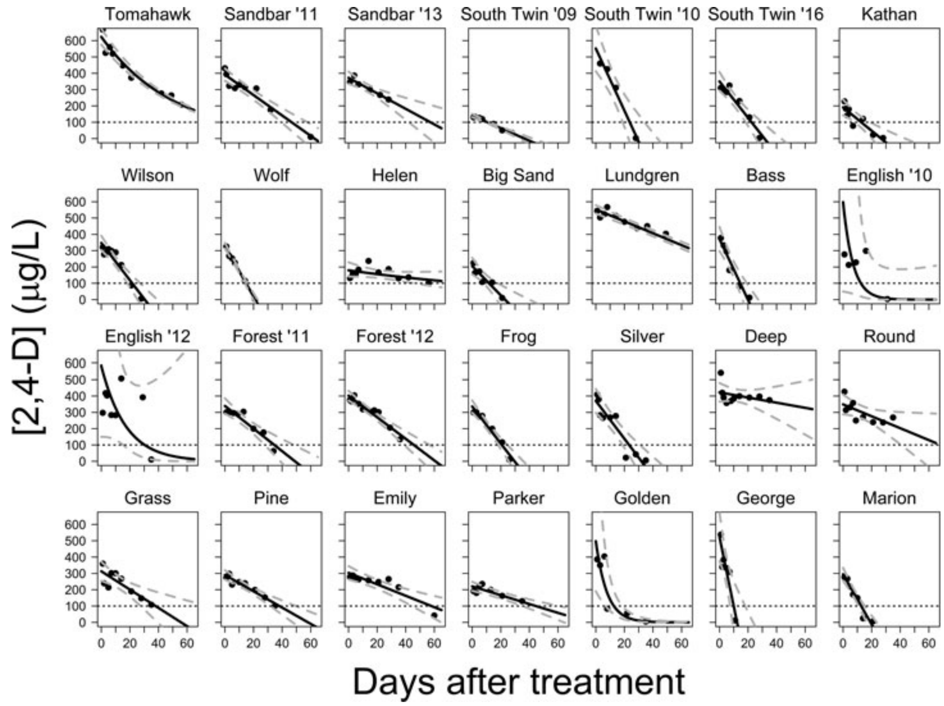

Figure 2. Linear models describing relationship between mean [2,4-D] across surface sites to days after treatment in 23 Wisconsin lakes (28 treatments); data have been (log) back-transformed where necessary. Solid circles indicate mean measured concentration, solid black line indicates the model, dashed gray lines indicate 95% confidence intervals, and the irrigation threshold of 100 μg/L is indicated by a horizontal dotted line.

stratification is present but not accounted for in volumetric dosing calculations, actual lakewide concentrations will likely exceed the target. If thermal stratification is anticipated but is absent or not fully formed at time of treatment, actual lakewide concentrations will likely be lower than target.

Previous studies have indicated that 2,4-D degradation in water is highly variable depending on

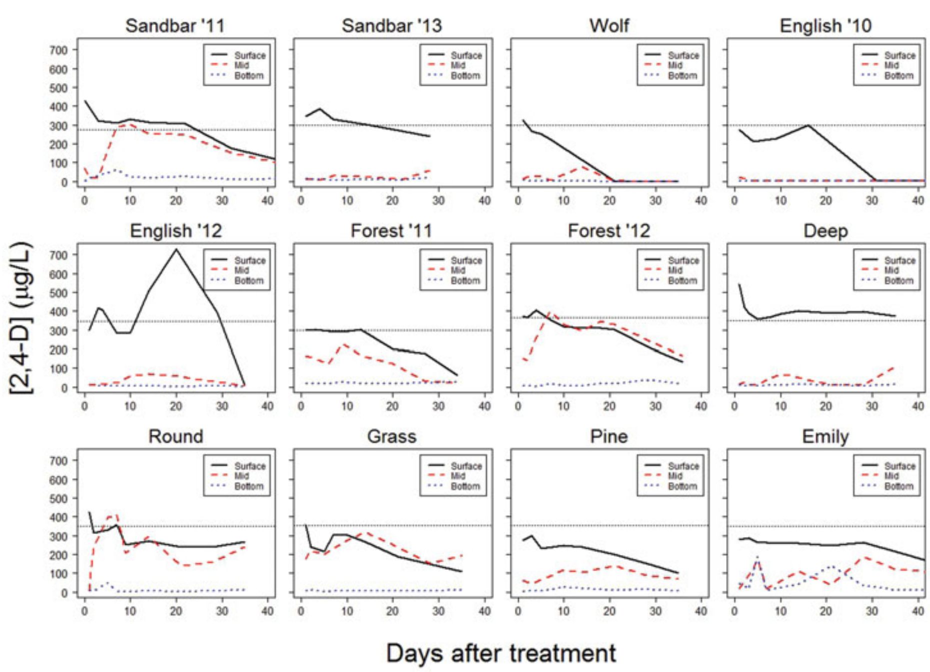

Figure 3. Herbicide concentration data over time for surface (mean), mid-depth, and bottom sites. Lakewide epilimnetic target concentrations indicted by a horizontal dotted line.

numerous factors such as microbial presence, temperature, nutrients, light, oxygen, organic content of substrate, pH, and whether or not the water has been previously exposed to 2,4-D or other phenoxyacetic acids (Howard et al. 1991). Reports of 2,4-D half-lives in the literature have ranged from 15 d in aerobic aquatic environments up to 41–333 d in anaerobic environments (EPA RED 2005). The rate of herbicide degradation in this study was generally observed to be slower in oligotrophic seepage lakes. Estimated 2,4-D half-lives from statistically significant models was positively correlated with water clarity (Secchi depth), with clearer lakes exhibiting slower 2,4-D breakdown versus more turbid lakes ($P = 0.003$, $R^2 = 0.31$). Seepage lakes exhibited significantly longer 2,4-D half-lives than drainage lakes (unpaired t-test, $P = 0.005$), although estimated degradation rates in drainage lakes are also likely influenced by the rate of herbicide movement out of the waterbody. Previous historical use of 2,4-D may also be an important variable to consider, as microbial communities that are responsible for the breakdown of 2,4-D may potentially exhibit changes in community composition over time with repeated use (de Lipthay et al. 2003, Macur et al. 2007). We did not observe a significant difference in degradation rates in lakes with previous 2,4-D exposure versus those that had never been exposed (unpaired t-test, $P = 0.407$). However, additional research on the factors which control microbial breakdown of 2,4-D is warranted, including studies that examine the number of times a waterbody has previously been exposed, and the scale of previous exposures.

The long exposure times observed with many of these large-scale 2,4-D treatments may also have potential adverse effects on other nontarget aquatic organisms; however, limited literature is available on the effects of low-concentration chronic exposure to commercially available 2,4-D formulations (EPA RED 2005). A recent laboratory study conducted by DeQuattro and Karasov (2016) observed that fathead minnows (*Pimephales promelas*) exposed for 28 d to 50 μg/L of two different commercial formulations of 2,4-D (DMA 4 IVM and Weedestroy AM40) had decreases in larval survival and tubercle presence in males, suggesting that these formulations may exert some degree of chronic toxicity or endocrine-disruption which has not been previously observed when testing pure compound 2,4-D. The 2,4-D half-lives and exposure times observed in this field study were much longer than those reported by previous studies, and suggest that additional laboratory and field studies may be warranted to examine potential effects of long-term exposure of various aquatic biota to low concentrations of 2,4-D.

Efficacy and longevity of milfoil control

Year of treatment reductions in milfoil littoral frequency from pretreatment to posttreatment ranged from 4 to 100%, with sustained multi-year control observed in some lakes (Figs. 4 and 5). Milfoil exhibited statistically significant decreases in frequency across all lakes monitored except Frog, Marion, Round, and the English 2012 treatment. For Bass, EWM littoral frequency of occurrence was too sparse to measure utilizing the point-intercept methodology. There was a significant negative linear relationship between milfoil control and pH ($P = 0.005$, $R^2 = 0.24$) as well as milfoil control and conductivity ($P = 0.006$, $R^2 = 0.24$), consistent with the findings reported in Frater et al. (2017). The degradation of 2,4-D in the environment is largely dependent on pH, with lower pH levels inhibiting microbial degradation, and thus resulting

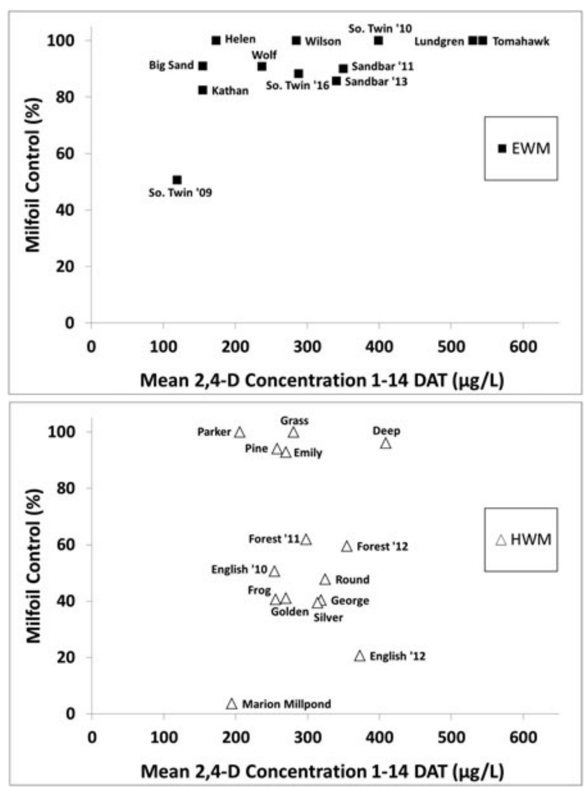

Figure 4. Milfoil control versus mean lakewide 2,4-D concentration during the first 2 weeks of treatment. Solid squares indicate pure EWM populations and open triangles indicate HWM populations.

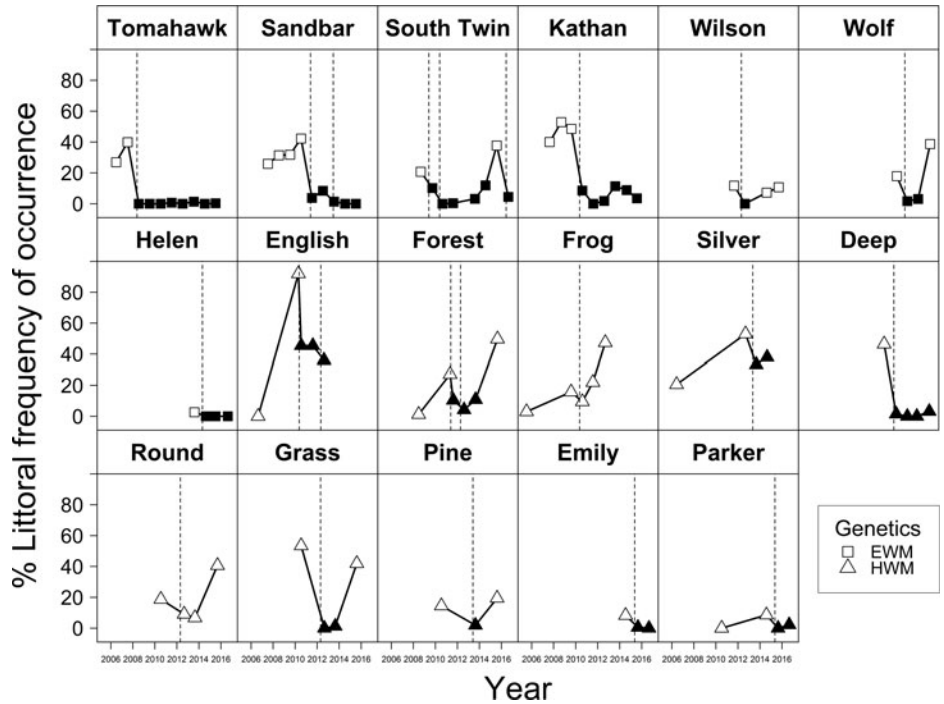

Figure 5. Milfoil littoral frequency of occurrence over time for lakes with multiple (≥2) years of posttreatment surveys. Large-scale 2,4-D treatment dates displayed as vertical dashed lines and statistically significant decreases from pretreatment year indicated by solid filled symbols. Squares = EWM; Triangles = HWM.

in longer exposure times (and increased likelihood of target species control) in large-scale treatments. In lakes where year of treatment milfoil control was achieved, the longevity of control ranged from 2 to 8 yr. However, it is important to note that milfoil was not "eradicated" from any of these lakes, and is still present even in those lakes which have sustained very low frequencies over time.

In comparison to pure EWM populations, significantly diminished control of milfoil was observed in the majority of lakes with confirmed HWM populations (unpaired t-test, $P = 0.007$). Year of treatment reductions in EWM littoral frequency ranged from 51 to 100% (mean = 89%), while HWM reductions ranged from 4 to 100% (mean = 59%). EWM control was correlated with the mean concentration of 2,4-D measured during the first 2 weeks of treatment, with increasing lakewide concentrations resulting in increased EWM control ($P = 0.06$, $R^2 = 0.2462$). In contrast, there was no significant relationship observed between HWM control and mean concentration of 2,4-D ($P = 0.91$, $R^2 = -0.08$). In lakes where good (>60%) year of treatment control of HWM was achieved, 2,4-D degradation was slow, and measured lakewide concentrations were sustained at >100 μg/L for longer than 31 d. In addition to reduced year of treatment efficacy, the longevity of control was generally shorter in lakes that contained HWM versus EWM, suggesting that HWM may have the ability to rebound quicker after large-scale treatments than pure EWM populations. However it is important to keep in mind that HWM is a broad term for multiple different strains, and variation in herbicide response and growth between specific genotypes of HWM has been documented (Taylor et al. 2017). In the current study we used a binary categorization of EWM versus HWM lakes, and did not further examine the relative within-lake composition of these populations, nor the individual genotypes which were present within the population. Future studies should further examine whether the relative composition of specific milfoil genotypes might influence both short- and long-term efficacy.

Recent laboratory studies have shown that certain strains of HWM exhibit more aggressive growth and are less affected by 2,4-D (Glomski and Netherland 2010, LaRue et al. 2013b), while other studies have not seen differences in overall growth patterns or treatment efficacy when compared to pure EWM (Poovey et al. 2007). Recurrent hybridization has been documented (Zuellig and Thum 2012), and further research is needed to better understand how different strains of hybrid milfoil are affected by herbicides. Field studies conducted in the Menominee River Drainage in northeastern Wisconsin and upper peninsula of Michigan observed hybrid milfoil genotypes more frequently in lakes that had previous 2,4-D treatments, suggesting possible selection of more tolerant hybrid strains over time (LaRue 2012). A recent study conducted in Houghton Lake, Michigan, on herbicide efficacy in a mixed community of EWM and HWM observed better within-season control of EWM versus HWM, adding evidence that observations of reduced control of HWM versus EWM seen in certain laboratory studies may also be applicable in the field (Parks et al. 2016). In Wisconsin, ∼150 waterbodies tested to date have had populations of milfoil genetically confirmed as HWM, and further statewide and regional testing is ongoing. Additional large-scale treatments conducted in spring 2013 using a low-concentration combination of 2,4-D and endothall on English Lake, and fluridone on Frog Lake, resulted in more effective seasonal control of HWM than the previous large-scale 2,4-D treatment on both systems (Heath et al. 2014). Low-concentration fluridone treatments have also been recently implemented on Silver Lake, as well as the Cloverleaf Chain (Round, Grass, and Pine) with effective milfoil control being reported posttreatment, and long-term data collection currently in progress. These case studies suggest that alternative herbicide strategies and other integrated techniques should be further explored for controlling HWM populations that are not responding to 2,4-D treatments.

LaRue et al. (2013a) also showed that certain HWM strains can be sexually viable under both laboratory and natural conditions, which may also have important management implications. An HWM strain present in another long-term study lake, Loon Lake, Shawano County, Wisconsin, has been observed forming winter turions (Skogerboe J, pers. comm.), a genetic trait that is absent from pure EWM populations, but present in native northern watermilfoil (*M. sibiricum*). Hybrid vigor has been well demonstrated with agricultural and aquaculture activities (Bartley et al. 1997, Schierenbeck and Ellstrand 2009), but the role of hybridization in relatively isolated natural aquatic systems is not well understood, especially between native and introduced species.

Development of herbicide resistance has been observed with several invasive aquatic plant species such as with fluridone use on hydrilla (*Hydrilla verticillata*; Michel et al. 2004, Arias et al. 2005, Puri et al. 2006) and diquat on dotted duckweed (*Landoltia punctata*; Koschnick et al. 2006). Given the documentation of resistance in these species to other herbicides, it is possible that other invasive aquatic plant species may develop resistance to the herbicide 2,4-D. Reduced fluridone sensitivity has been observed in both field and laboratory studies with a HWM genotype from Townline Lake in central Michigan, however the mechanism contributing to the increased tolerance is not yet well understood (Berger et al. 2012, Thum et al. 2012, Berger et al. 2015). A recent mesocosm study by Netherland and Willey (2017) demonstrated that HWM strains from Frog and English lakes showed a greater tolerance to 2,4-D when compared to another strain of HWM and EWM. In the future, resource managers should consider conducting genetic pretreatment screening of target populations to better understand among- and within-population variation, as well as changes in population genetic composition after chemical control (Moody et al. 2008). In addition to currently utilized ITS sequencing and amplified fragment length polymorphism (AFLP) analysis techniques (Zuellig and Thum 2012), recently developed polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) screening may make sampling for hybridity less technically demanding and more cost effective in the future (Grafe et al. 2015).

Native plant community response

Several native monocotyledon and dicotyledon species showed significant declines during the first year posttreatment (Table 5). On each lake, 0–7 native species exhibited a significant decline posttreatment (mean = 3.6) and 0–2 species exhibited a significant increase (mean = 0.7). Specifically, northern watermilfoil, slender naiad (*Najas flexilis*), water marigold (*Bidens beckii*), and several thin-leaved pondweeds (e.g., *Potamogeton pusillus, P. strictifolius, P. friesii*, and *P. foliosus*) showed highly significant declines in the majority of the lakes monitored. In addition, Variable/Illinois pondweed (*P. gramineus*/*P. illinoensis*), flat-stem pondweed (*P. zosteriformis*), fern pondweed (*P. robbinsii*), and sago pondweed (*Stuckenia pectinata*) also declined in many lakes. Ribbon-leaf pondweed (*P. epihydrus*) and water star-grass (*Heteranthera dubia*) declined in the lakes where they were found. Mixed effects of treatment were observed with wild celery (*Vallisneria americana*) and southern naiad

Table 5. Pearson's chi-square analysis of pretreatment versus posttreatment for all native species >10% littoral frequency of occurrence (in either survey). Statistically significant differences are indicated by directional arrows (↓ = decrease; ↑ = increase; n.s. = not significant) and number of arrows correspond to magnitude of statistical change (↓ = $P < 0.05$; ↓↓ = $P < 0.01$; ↓↓↓ = $P < 0.001$).

	Tomahawk	Sandbar '11	South Twin '09	Kathan	Wilson	Wolf	Helen	Lundgren	Frog	Silver	Deep	Emily	Parker	Marion
B. beckii	—	—	↓↓↓	—	—	—	—	↓↓	—	—	—	—	—	—
B. schreberi	—	—	—	n.s.	—	—	—	↓↓	—	—	—	—	—	—
C. demersum	—	—	n.s.	n.s.	↓↓↓	n.s.	—	—	—	n.s.	—	—	—	n.s.
Chara spp.	n.s.	n.s.	↓↓↓	n.s.	—	↓	↑	↑	n.s.	n.s.	n.s.	↑	n.s.	—
E. acicularis	—	n.s.	—	—	—	—	—	—	—	—	—	—	—	—
E. canadensis	↓↓↓	n.s.	n.s.	n.s.	—	—	—	—	—	—	—	—	—	↓↓↓
H. dubia	—	—	↓↓↓	—	—	—	—	—	—	—	—	—	—	—
M. sibiricum	—	—	↓↓↓	—	—	—	↓↓↓	—	—	—	↓↓	—	—	—
N. flexilis	↓↓↓	↓↓	n.s.	↓↓↓	—	—	↓↓↓	↓↓	↓↓↓	n.s.	—	↓↓↓	↓↓↓	↓↓↓
N. guadalupensis	—	—	—	—	—	—	↓↓↓	—	—	↑↑↑	—	—	↑↑↑	—
N. marina[*]	—	—	—	—	—	—	—		—	↑↑↑	—	—	—	—
Nitella spp.	—	—	—	↓↓↓	—	—	—	—	—	—	n.s.	↑	—	↓↓↓
N. odorata	—	—	—	—	—	—	—	—	—	—	—	n.s.	—	—
P. amplifolius	↓↓↓	—	—	—	—	—	—	n.s.	n.s.	—	n.s.	—	—	—
P. epihydrus	—	—	—	↓↓↓	—	—	—	—	—	—	—	—	—	—
P. foliosus	—	—	—	—	—	—	—	↓↓↓	—	—	—	—	—	—
P. friesii	—	—	—	—	—	—	—	—	—	—	—	↓↓↓	—	↓↓↓
P. gramineus/P. illinoensis[**]	↓	↓	n.s.	—	—	—	↓↓↓	n.s.	—	n.s.	—	n.s.	↓↓↓	—
P. praelongus	—	—	n.s.	—	—	—	—	n.s.	—	—	—	—	—	↑
P. pusillus	↓↓↓	↓↓↓	↓	↓↓↓	—	—	—	—	n.s.	—	—	—	—	—
P. richardsonii	—	—	n.s.	—	—	—	—	—	—	—	—	—	—	—
P. robbinsii	↓	—	n.s.	—	↓↓↓	—	—	—	—	—	—	—	—	—
P. strictifolius	—	—	—	↓↓↓	—	—	—	—	↓↓↓	—	—	—	—	—
P. zosteriformis	—	—	n.s.	↑	↓↓↓	—	—	—	—	—	↓↓↓	↓↓↓	—	—
S. pectinata	—	—	—	—	—	↓	↓↓↓	—	—	n.s.	—	—	↓↓↓	↓↓↓
U. vulgaris	—	—	—	n.s.	—	—	—	—	—	—	—	—	—	—
V. americana	↓↓↓	↓↓↓	↓↓↓	↑	—	n.s.	—	—	—	↑	—	n.s.	n.s.	—
# native spp sig increase	0	0	0	2	0	0	1	1	0	2	0	2	1	1
# native spp sig decrease	7	4	6	5	3	2	5	4	2	0	2	3	3	5
net increase/decrease	−7	−4	−6	−3	−3	−2	−4	−3	−2	+2	−2	−1	−2	−4

[*]considered non-native in Wisconsin.
[**]*P. gramineus* and *P. illinoensis* (& hybrids) combined for analysis.

(*Najas guadalupensis*), with some lakes showing significant declines posttreatment and other lakes showing increases. Muskgrasses (*Chara* spp.) exhibited either no change or a significant increase after treatment in the majority of lakes, which has also been observed in previously published studies (Miller and Trout 1985, Nault et al. 2014).

In lakes monitored for multiple years posttreatment, the native species that exhibited declines showed variation in recovery, with some lakes showing sustained multi-year reductions, and others exhibiting more rapid recovery back to pretreatment frequencies. The magnitude of native impacts varied across treatments, with some lakes exhibiting highly significant sustained declines across numerous native species and others exhibiting relatively smaller and shorter declines across a select few species. Certain native species (e.g., *P. pusillus, N. flexilis, M. sibiricum*) were completely absent from some lakes during the posttreatment survey, but were again detected during the survey year following treatment, presumably recovering from a seedbank or rootstock that was present. Although the exact mode of action of 2,4-D is not fully understood, the herbicide is traditionally believed to target broad-leaf dicotyledon species, with minimal effects generally observed on numerous monocotyledon species, especially in terrestrial applications (WSSA 2007). However, observed declines of several monocotyledon species in the current study suggests that long exposure times of 2,4-D in large-scale aquatic treatments may reduce selectivity. Continued long-term monitoring of these large-scale treatments will provide additional insight as to whether native species are able to recover and reestablish quicker than the invader is able to recolonize.

These native species impacts are different than those reported by other studies conducted to date in Wisconsin (Helsel et al. 1996, Cason and Roost 2011) and the western United States (Parsons et al. 2001, Wersal et al. 2010), which did not find major significant differences in natives pre– and post–2,4-D treatment. Helsel et al. 1996 reported initial reductions of several species (e.g., coontail [*Ceratophyllum demersum*], common waterweed [*Elodea canadensis*], variable-leaf watermilfoil [*Myriophyllum heterophyllum*], and wild celery [*Vallisneria americana*]); however, these species recovered by 10–12 weeks posttreatment. Cason and Roost (2011) reported a significant decline in *M. sibiricum* after treatment, and also declines in *N. flexilis* and *Potamogeton* spp., however they attributed these declines to "other factors" because the latter taxa were considered tolerant to 2,4-D. These previous studies conducted seasonal comparisons of native aquatic plants between late spring (May/Jun) and late summer (Aug/Sep), which may have been confounded by the phenology of many native aquatic plant species emerging and thriving later in the growing season in northern temperate inland lakes. In addition, all these previously conducted studies examined granular ester formulations of 2,4-D, whereas the current study examines liquid amine 2,4-D applications.

Conclusions

Any successful restoration treatment will minimize unintended impacts while maximizing the level of control of the target species. While high target impact and no collateral damage is often the stated goal of treatment plans, our current study shows this expectation may be unrealistic in many cases. Although multi-year lakewide milfoil control was achieved with some of these low-concentration lakewide applications, variation in herbicide persistence, observations of reduced control in some hybrid genotypes, nontarget impacts to certain native plants, and uncertain long-term biotic and abiotic effects demonstrate the need for additional research, monitoring and field studies.

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