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The Use of 2,4-D for Selective Control of an Early Infestation of Eurasian Watermilfoil in Loon Lake, Washington

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ABSTRACT

A patchy distribution of Eurasian watermilfoil (*Myriophyllum spicatum* L.) in Loon Lake was treated with the herbicide 2,4-D during July 1998. Aquatic plant biomass and frequency data were collected before treatment, and six weeks and one year after treatment. Aqueous concentrations of 2,4-D increased to 1 to 2 mg/l within one day of treatment, and were below detection limits by one week after treatment. Macrophyte data were analyzed to assess the herbicide's impacts on Eurasian watermilfoil as well as the rest of the aquatic plant community. Results showed a significant decrease in Eurasian watermilfoil biomass and frequency in treated areas 6 weeks after treatment, which continued through the one year post-treatment samples. No other plant species were significantly affected by the herbicide application.

Key words: aquatic plants, herbicides, biomass, frequency, *Myriophyllum spicatum* L., native plants.

INTRODUCTION

Eurasian watermilfoil is not native to North America, and is considered to be highly invasive in temperate climates outside its native range. It will tolerate a wide range of environmental conditions, and tends to quickly grow to the surface in the spring where it branches extensively and forms a dense mat (Nichols and Shaw 1986, Smith and Barko 1990, Madsen 1998). It is extremely difficult to control after it has been introduced to a waterbody due to its effective means of spreading through fragmentation and stolons (Aiken et al. 1979, Smith and Barko 1990, Madsen and Smith 1997). Dominance of a waterbody by Eurasian watermilfoil causes both environmental and economic impacts. Environmental impacts include a reduction in the biodiversity and frequency of native aquatic plant species, and impacts to the water quality such as lowered dissolved oxygen and changes to the nutrient cycling in the littoral zone (Aiken et al. 1979, Nichols and Shaw 1986, Frodge et al. 1991, Madsen et al. 1991). These impacts can alter the habitat value for invertebrates, fish and waterfowl (Newroth 1985, Dibble and Harrel 1997, Dibble et al. 1997). The dense vegetation at the surface also creates a nuisance for boaters and a swimming hazard (Newroth 1985, Smith and Barko 1990). This can lead to a reduction in the tourism important to many lake communities (Slipke et al. 1998). The burden of paying for control efforts usually falls on government agencies or local communities, and can run to hundreds of thousands of dollars.

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In Washington State, Eurasian watermilfoil is the most widespread invasive aquatic weed with known populations in more than 100 lakes and rivers (Parsons 1998). Once a population has grown beyond just a few plants, we have found that Eurasian watermilfoil is nearly impossible to eradicate using physical methods such as hand pulling or placement of benthic barriers. In small lakes (less than 150 ha), herbicides have been used on a whole lake basis to eradicate the plant or at least provide control for several years (Parsons 1998). However, until recently, no tool was permitted in Washington to manage intermediate levels of this plant in larger lakes where treating the entire littoral zone is not feasible.

The herbicide 2,4-D (2,4-dichlorophenoxy acetic acid) has been used in other parts of the United States to effectively and selectively treat small Eurasian watermilfoil patches (approximately 10,000 acres per year are treated with 2,4-D for Eurasian watermilfoil control in the United States (Lembi 1996)). Several studies have demonstrated the selectivity of 2,4-D against Eurasian watermilfoil at low doses and short exposure times (Killgore 1984, Miller and Trout 1985, Carpentier et al. 1988, Green and Westerdahl 1988, Bird 1993). Broad-leaf dicotyledonous species such as Eurasian watermilfoil are more susceptible to 2,4-D than narrow leaf monocots (Lembi 1996, Madsen 2000). The herbicide works by mimicking the plant hormone auxin. This affects the plant's respiration and food reserves, and causes excessive growth, cell division, and death (Christopher and Bird 1992, Sprecher and Netherland 1995). Once applied in the environment, 2,4-D tends to rapidly dissipate depending on the degree of water movement, temperature, pH, and the substrate present (Joyce and Ramey 1986). Both UV light and microorganisms living in the water and sediments will convert the herbicide to carbon dioxide, water, and chlorine (Aly and Faust 1964, Hemmett and Faust 1969, Joyce and Ramey 1986).

In 1998 the State Legislature directed the Department of Ecology to conduct a demonstration project using the herbicide 2,4-D on a pioneering population of Eurasian watermilfoil in Loon Lake, located in northeast Washington (Figure 1). The objective of this study was to document the impact of 2,4-D on the aquatic plant community in Loon Lake, and to test its effectiveness at controlling a pioneering Eurasian watermilfoil population.

METHODS AND MATERIALS

Site Description. Loon Lake is located in the mountainous northeast corner of Washington State about 100 km (60 miles) north of Spokane at latitude 48°3' 20" north and longitude 117°38' 30" west (Figure 1). It is 445 hectares (1,100 acres) with 12.7 km (7.9 miles) of shoreline at an elevation of 726 m (2,381 feet). The maximum depth is 30.5 m (100 feet), mean depth 14 m (46 feet) (Dion et al. 1976). It is an oligo-mesotrophic lake with moderate levels of nutrients and generally good water clarity (Smith et al. 2000). Loon Lake hosts a diverse plant and animal community, with at least 28 species of aquatic macrophytes growing to 6.7 m (22 feet) deep (Table 1). According to regional fisheries biologists, species present include rainbow trout (*Oncorhynchus mykiss*), eastern brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), lake trout (Mackinaw) (*Salvelinus namaycush*), kokanee salmon (*Oncorhynchus nerka*) and warm water species such as largemouth bass (*Micropterus salmoides*), pumpkinseed sunfish (*Lepomis gibbosus*), and yellow perch (*Perca flavescens*) (C. Vail, Regional Fish Biologist, personal communication). The shoreline is about 85% developed with seasonal and year-round residences. The remaining 15% is mostly wetlands.

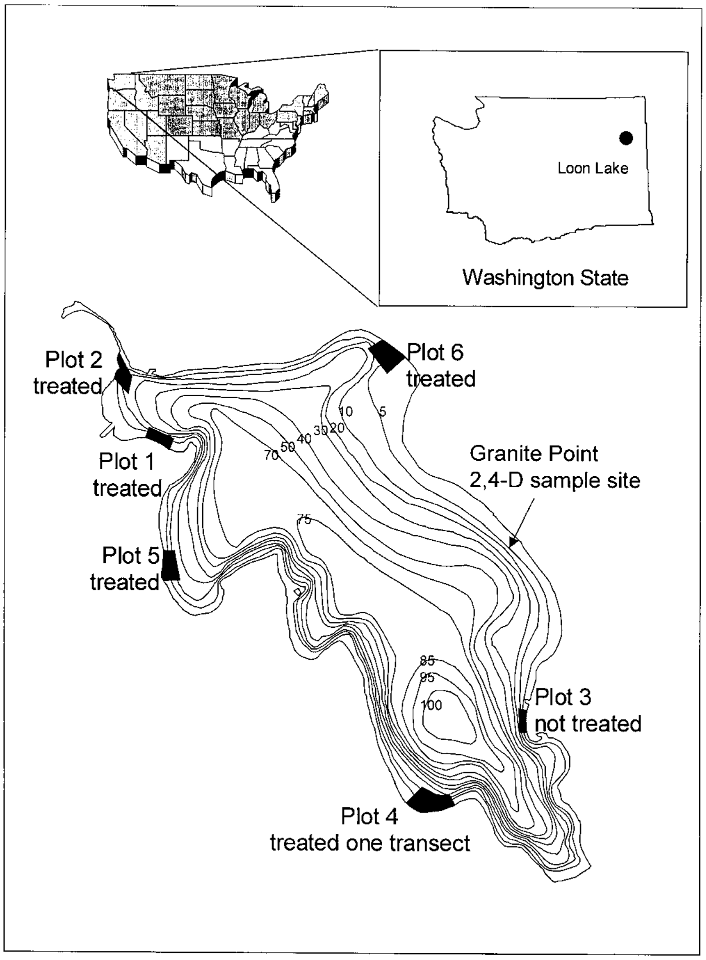

Figure 1. Loon Lake, Washington. Location of 6 study plots. Depth contour intervals marked in feet.

Eurasian watermilfoil was first found in Loon Lake in September 1996, at which time its distribution was limited to the northwest corner and a few other scattered patches around the lake. In the summer of 1997, diver hand-pulling and benthic barriers were used in an attempt to reduce the population. However, by the end of the summer it was evident that the Eurasian watermilfoil was continuing to spread beyond a level that divers could contain. At the time of this study the Eurasian watermilfoil was spreading, but still limited to small patches within about 24 ha, mainly in the northern half of the lake and in water less than three m deep.

Herbicide Application. Treatment occurred on the morning of July 8, 1998 using the granular 2,4-D formulation Aqua-Kleen® (2,4-D BEE (butoxy ethyl ester), 19% acid equivalent). A total of 2,722 kg was applied over approximately 24 ha containing Eurasian watermilfoil (112 kg/ha of product, 21 kg/ha acid equivalent) using a granular blower and cyclone spreaders mounted on an airboat. This application rate was calculated to attain the 1 to 2 mg/l target concentra-

TABLE 1. AQUATIC PLANT SPECIES IN LOON LAKE, WA. GROWTH FORMS ARE INDICATED BY E, EMERGENT; F, FLOATING-LEAVED; AND S, SUBMERSED. TYPE IS DEFINED AS M, MONOCOT; AND D, DICOT.

Scientific name	Common name	Growth form	Type
Brasenia schreberi Gmel.	watershield	f	d
Ceratophyllum demersum L.	coontail	s	d
Chara sp.	muskgrass	s	macroalgae
Eleocharis sp.	spikerush	s	m
Eleocharis palustris (L.) R. & S.	common spikerush	e	m
Elodea canadensis Rich.	common elodea	s	m
Fontinalis sp.	aquatic moss	s	moss
Megalodonta beckii Greene	water marigold	s	d
Myriophyllum sibiricum Kom.	northern watermilfoil	s	d
Myriophyllum spicatum L.	Eurasian watermilfoil	s	d
Najas flexilis (Willd.) R. and S.	common naiad	s	m
Nitella sp.	stonewort	s	macroalgae
Nuphar polysepala Engelm.	spatterdock	f	d
Nymphaea odorata Ait.	fragrant waterlily	f	d
Polygonum amphibium L.	water smartweed	f	d
Potamogeton amplifolius Tucker.	large-leaf pondweed	s	m
Potamogeton gramineus L.	grass-leaved pondweed	s	m
Potamogeton illinoensis Morong.	Illinois pondweed	s	m
Potamogeton natans L.	floating leaf pondweed	s	m
Potamogeton praelongus Wulf.	whitestem pondweed	s	m
Potamogeton richardsonii (Ben.) R.	Richardson's pondweed	s	m
Potamogeton robbinsii Oakes	Robbins' pondweed	s	m
Potamogeton sp.	thin leaved pondweed	s	m
Potamogeton zosteriformis Fern.	eel-grass pondweed	s	m
Ranunculus aquatilis L.	water buttercup	s	d
Schoenoplectus sp. Syn. *Scirpus* sp.	bulrush	e	m
Stuckenia pectinata (L) Börner Syn. *Potamogeton pectinatus* L.	sago pondweed	s	m
Utricularia vulgaris L.	bladderwort	s	d
Vallisneria americana Michx.	water celery	s	m
Zosterella dubia (Jacq) Small Syn. *Heteranthera dubia* (Jacq) MacM.	water star-grass	s	m

tion for 24 to 48 hours recommended for severe injury or complete control of Eurasian watermilfoil (Green and Westerdahl 1988). Treatment took place in early July to coincide with the window during which Eurasian watermilfoil is most susceptible to control efforts (May to July in northern latitudes) (Madsen 1997).

Water samples were collected before treatment, and three hours, one day, three days, one week, two weeks and three weeks after treatment for analysis of 2,4-D concentrations. Four areas treated with 2,4-D were chosen for sample collection; three within aquatic macrophyte data collection plots, and one at Granite Point where Eurasian watermilfoil was growing, but no plot was located (Figure 1). At each site, duplicate samples were taken from the upper and lower one third of the water column at the treatment area center, and at 50 m and 100 m from the lakeward edge of the treatment area. All collection site locations were recorded with positions from a Global Positioning System (GPS) Unit. The samples were frozen and shipped to the analytical chemistry laboratory at the U.S. Army Engineers Lewisville Aquatic Ecosystem Research Facility in Lewisville, TX. Residue analyses were conducted using approved procedures (APHA 1976) with a detection limit of 0.005 mg/l.

Aquatic Plant Community. The aquatic plant community was assessed before treatment and at six weeks and one year after treatment using the following three methods: 1) biomass sampling in six study plots; 2) line intercept frequency sampling for six study plots; and 3) point intercept frequency sampling for the whole lake. These three different methods were used to evaluate in-plot versus whole-lake changes, and species abundance (biomass) versus diversity and distribution (frequency) within the plots.

The six study plots set up for the biomass and line intercept frequency sampling were established based on knowledge of the Eurasian watermilfoil distribution from a spring 1998 survey (Winterowd and Lamb In Press). Four plots to be treated with herbicide were located in areas with the densest known growths of Eurasian watermilfoil, and two plots were located in areas thought to be free of Eurasian watermilfoil for the no-treatment control (Figure 1). Although for scientific purposes it would have been preferable to establish control plots in areas with Eurasian watermilfoil, this was not done to accommodate the project's goal of treating all Eurasian watermilfoil within the lake. One transect from the untreated plot 4 ended up too close to a treatment area to be considered untreated. Therefore, plot 4 was split during statistical analyses and the treated transect was added to the treatment group.

Within each plot, two 100-m transects marked at 1-m intervals were established within the area of plant growth (less than 6.7 m deep). The transect lines were perpendicular to shore unless the zone of plant growth was too narrow, in which case they were curved. Careful notes and GPS points were used to ensure that the transects were in the same locations during all three data collection efforts.

Biomass. During all three sampling periods, ten biomass samples were collected in each plot (five along each transect line). The sample points were located at stratified-random distances along and away from the transect lines; each sample was located randomly at 20-m intervals and between 1 to 5 m away from the line.

Sample collection and processing followed methods described by Madsen (1993). The samples were collected by a diver using SCUBA gear and a 0.1 m^2 frame made of PVC pipe. The diver placed the frame on the sediment at the predetermined sample site and collected all shoot biomass within the frame. Plants were placed in a mesh bag, carried to a nearby boat, and transferred to a labeled plastic bag. On shore the samples were rinsed, trimmed to remove any remaining roots, sorted by species, and placed into preweighed and numbered paper bags. Samples were allowed to air dry until the end of each four-day sample period when the paper bags were returned to the lab and dried in a forced air oven at 60C to a constant weight. They were then reweighed to 0.01 g accuracy. The resulting data were entered into a relational database and analyzed both as individual species and grouped as monocots or dicots. A one way Analysis of Variance (ANOVA) was used for the analysis after performing a $\log_{10} + 1$ transformation to approximate a normal distribution. The resultant p-values were adjusted using a Bonferroni correction to account for multiple comparisons. Post-hoc analysis determined which of the comparisons were significant.

Line Intercept Plant Frequency. This method utilized the same transect lines used for biomass sampling (see previous section), and the data were gathered simultaneously. All species observed crossing the vertical plane made between the transect line and the lake bottom were recorded by a snorkeler at one m intervals (Madsen 1999). Data were gathered the length of the transect where the plants could be seen from the surface or with a quick dive.

The data were entered into a relational database and Chi-square two-by-two analyses (Zar 1984) were performed on species present in at least 5% of the sample intervals. Comparisons of the presence-absence data were made separately for the treated and untreated plots. Three combinations of the sample dates were analyzed: before treatment (June 1998) with 6 weeks after treatment (August 1998), before treatment with one year after treatment (June 1999), and 6 weeks after treatment with one year after treatment. The probability was adjusted using a Bonferroni correction to account for multiple comparisons.

Point Intercept Plant Frequency. Plant samples were gathered at points on a 50 × 50 m grid developed for the littoral zone using a Geographical Information System (GIS) (Madsen 1999). A GPS was used to find these points as Universal Transverse Mercator (UTM) coordinates in the field. At each point two samples were gathered from the starboard side of the boat. If the sample site was in shallow water, the plant species were recorded from an area of approximately 1 m^2 using visual observation. In deeper water, plants were sampled using two metal leaf rakes bolted back-to-back with the handles removed and replaced with a 30-m marked rope. This sampler was thrown twice, sampling approximately 0.5 m^2 each time. All recovered species were recorded.

Data were analyzed using the same methods used for the line-intercept frequency data (see previous section). Only species observed in at least 15 samples were included.

RESULTS AND DISCUSSION

Herbicide Application. No herbicide residue was detected in the water samples collected before treatment. Results from the 2,4-D residue analysis from samples collected up to one week after treatment are provided in Table 2. The herbicide concentrations increased to the targeted level of between 1 to 2 mg/l in treated areas within one day of treatment, then generally diminished by 3 days after treatment and were not detectable by 1 week after treatment or thereafter. Plot 5 had the lowest concentrations, probably due to springs located in the area that enhanced herbicide dissipation. There was little herbicide detected off-site in samples collected 100 m from the treatment areas. This pattern of rapid dissipation is typical for aquatic 2,4-D applications (Lim 1976, Killgore 1984, Carpentier et al. 1988). However, the Eurasian watermilfoil in all areas where 2,4-D residues were measured were exposed to a concentration and exposure time sufficient to cause severe injury or to provide complete control (at least 1 mg/l for 36 hours, or 2 mg/l for 24 hours) (Green and Westerdahl 1988).

Aquatic Plant Community Biomass. A total of 24 species were found in the biomass samples. Whitestem pondweed and Richardson's pondweed were combined due to difficulty in distinguishing the two species; several of these plants were observed with intermediate characteristics which led us to suspect hybridization. Plant distribution was very patchy, with several samples containing no measurable plant matter, and other samples with as many as seven different species. Total biomass per sample ranged from 0.1 to 1,396.4 g/m^2 dry weight, with an average of 37 g/m^2 dry weight. The most abundant and commonly collected plant was Robbins' pondweed, found in 48% of the biomass samples. Table 3 lists the mean biomass for the most common species collected, divided into pretreatment, six weeks post-treatment, one year post-treatment and treated versus untreated plots.

Eurasian watermilfoil demonstrated the only significant decrease in plant biomass by six weeks after treatment, with a 98% reduction during this time interval. One year after treatment the biomass in treated plots was still reduced by 87% compared to pretreatment levels (Table 3). Killgore (1984) had similar results on Lake Osoyoos in north-central Washington, where there was a 91% reduction in Eurasian watermilfoil biomass 28 days after treatment, and an 86% decrease by 84 days after treatment using a similar application rate of the same 2,4-D formulation. Getsinger and Westerdahl (1984) achieved 60 to 70% Eurasian watermilfoil control by 28 days after treatment, and a 50 to 60% reduction in the original biomass by day 56 when using a similar application rate (22 kg acid equivalent/ha) of a different 2,4-D formulation (14-ACE-B) in Lake Seminole, Florida. The rebound in Eurasian watermilfoil biomass experienced in these other studies, and evident in this study by one year after treatment, indicate that additional measures will need to be taken to

Table 2. Aqueous 2,4-D residue levels (mg/L) in Loon Lake, WA, 1998. All values are averages of two analyses per sample, and the average of duplicate samples at each collection site. Time intervals are after treatment. Sample ID designation: U—from the upper ⅓, L—from the lower ⅓ of the water column. N.D. is not detectable.

Sample area	Sample location	Sample ID	3 hours	1 day	3 days	1 week
Plot 2	treatment area	U3	0.676	1.377	0.613	N.D.
		L3	0.931	1.577	1.236	N.D.
	50 m outside	U3	0.039	0.052	0.100	N.D.
		L3	N.D.	0.074	0.300	N.D.
	100 m outside	U3	N.D.	0.162	N.D.	N.D.
		L3	N.D.	N.D.	0.157	N.D.
Plot 5	treatment area	U3	0.282	N.D.	N.D.	N.D.
		L3	1.262	N.D.	N.D.	N.D.
	50 m outside	U3	N.D.	N.D.	N.D.	N.D.
		L3	N.D.	N.D.	N.D.	N.D.
	100 m outside	U3	N.D.	N.D.	N.D.	N.D.
		L3	N.D.	N.D.	N.D.	N.D.
Plot 6	treatment area	U3	0.235	0.628	N.D.	N.D.
		L3	0.130	1.421	N.D.	N.D.
	50 m outside	U3	0.218	0.259	0.739	N.D.
		L3	N.D.	0.735	0.743	N.D.
	100 m outside	U3	N.D.	0.110	0.522	N.D.
		L3	N.D.	N.D.	N.D.	N.D.
Granite Point not located with in a sample plot	treatment area	U3	1.817	0.374	N.D.	N.D.
		L3	1.427	0.181	N.D.	N.D.
	50 m outside	U3	N.D.	N.D.	N.D.	N.D.
		L3	N.D.	N.D.	N.D.	N.D.
	100 m outside	U3	N.D.	N.D.	N.D.	N.D.
		L3	N.D.	N.D.	N.D.	N.D.

maintain reduced Eurasian watermilfoil biomass in Loon Lake. Other researchers have reached similar conclusions. In Minnesota 2,4-D has been widely used to attempt to control Eurasian watermilfoil spread. They have concluded that it may slow the spread of Eurasian watermilfoil through the lake, but will not halt it (Crowell 1999). Gibbons and Gibbons (1985) reached a similar conclusion in a study using a different formulation of 2,4-D in the Pend Oreille River in northeast Washington. They found that two applications of the herbicide over the growing season produced better Eur-

Table 3. Mean biomass (g/m^2) and Bonferroni adjusted ANOVA results (P-value) from selected species in treated and untreated plots, Loon Lake, WA 1998-1999. Significantly different comparisons are indicated by letters.

	Untreated plots				Treated plots			
Species	June 98 n = 15	Aug 98 n = 15	June 99 n = 15	P-value	June 98 n = 45	Aug 98 n = 45	June 99 n = 45	P-value
	- - - - - - - - - - - - - biomass (g/m^2) - - - - - - - - - - - - -				- - - - - - - - - - - - - biomass (g/m^2) - - - - - - - - - - - - -			
Chara	10.39	17.60	7.22	0.631	1.33	14.46	2.37	0.084
Elodea canadensis	29.63	19.49	23.47	0.650	5.42	8.04	17.46	0.684
Megalodonta beckii	5.08	20.81	0.79	0.294	1.38 ab	8.37 a	0.48 b	0.013
Myriophyllum sibiricum	7.16	4.87	0.00	0.114	2.55	1.20	0.21	0.161
Myriophyllum spicatum	0.00	0.01	2.01	0.134	6.58 a	0.16 b	0.83 ab	0.009
Najas flexilis	0.00	1.20	0.01	0.180	0.03	0.54	0.22	0.112
Potamogeton amplifolius	12.03	35.73	11.39	0.574	7.44	20.20	10.91	0.367
Potamogeton gramineus	0.00	0.00	0.00	—	0.46	2.06	0.16	0.092
Potamogeton praelongus + *Potamogeton richardsonii*	4.05	31.14	1.49	0.097	3.36	4.35	3.24	0.819
Potamogeton robbinsii	21.20	16.34	29.62	0.864	86.57	59.84	43.98	0.857
Potamogeton zosteriformis	2.25	0.01	0.98	0.394	0.27	0.13	0.60	0.514
Utricularia vulgaris	0.00	0.00	0.00	—	0.51	1.75	7.60	0.410
Vallisneria americana	0.41 ab	3.92 a	0.10 b	0.031	0.31 a	4.75 b	0.15 a	0.007
Zosterella dubia	0.30	0.57	0.37	0.798	0.18	1.04	0.06	0.068

n = number of samples.

asian watermilfoil control than a single application at both low and high herbicide concentration levels. That application frequency may also improve results at Loon Lake.

Among the other dicotyledonous species, water marigold was the only one to show a significant change in any of the three comparisons for the treated and untreated plots (Table 3). It decreased significantly in the treated plots between August 98 and June 99 ($p = 0.013$). This is probably a seasonal effect, since most aquatic plants in temperate climates attain peak biomass toward the end of summer (Westlake 1965).

None of the monocot species' biomass was affected by the herbicide. Water celery was the only one to change significantly, with an increase in August relative to the two June samples (Table 3). This pattern was also demonstrated by the majority of other species, and is most likely due to seasonal growth patterns. Exceptions to this pattern were common elodea, Robbins' pondweed, and eel-grass pondweed. These species showed a decrease in August (possibly due to early growth and senescence compared to the species that peaked in August), or an increase in biomass over time. In a study where Eurasian watermilfoil was the dominant species before treatment, Miller and Trout (1985) observed that native species, especially the algae muskgrass and the monocot common naiad, increased after treatment when compared to control areas. Similarly, Sprecher et al. (1998) observed no significant reduction in the monocot sago pondweed when exposed to up to 2 mg/l 2,4-D for 24 hours.

When the flowering vascular plants were grouped as either monocots or dicots neither group showed a significant difference between the dates ($p > 0.05$) (Table 4). The herbicide's selectivity for dicots would lead one to expect this group to decrease in treated plots. The peak dicot biomass in August increased less in the treated plots than in the untreated plots, but this was partially due to the significant decrease in Eurasian watermilfoil over this time period.

Line Intercept Plant Frequency. Species were recorded and analyzed at a total of 2,475 transect intervals for all observation periods (before and after treatment, treated and untreated plots). A total of 24 different species were identified on the transect surveys. Large-leaf pondweed was the most frequently observed plant, and several, such as water smartweed and Richardson's pondweed were uncommon in the transects. Two species, northern watermilfoil and water marigold, were combined due to the difficulty experienced in differentiating them under water. Along many of the transects the species assemblage was diverse, with many 1-m intervals containing up to 7 different species. Twelve percent of intervals contained no plants. These were mostly located in areas where the transects crossed benthic barriers placed the previous year for Eurasian watermilfoil control, or in areas of sandy substrate.

TABLE 4. MEAN BIOMASS (G/M^2) OF MONOCOTS AND DICOTS AND BONFERRONI ADJUSTED ANOVA RESULTS, LOON LAKE, WA 1998-1999.

Treated Plots	June 98	Aug 98	June 99	P-value
Monocots	104.98	102.48	77.38	.735
Dicots	11.77	13.40	12.31	.453
Untreated Plots				
Monocots	69.97	108.40	67.43	.874
Dicots	12.33	25.69	2.80	.284

Results from the Chi-square analysis in percent present for the three sample dates and P-values for the three comparisons are given for the most common species in Table 5 and Table 6. Eurasian watermilfoil showed a significant decrease in frequency in the treated plots between the pretreatment sample collection and both the six week post-treatment, and one year post-treatment sample collection. This is the only species that showed a significant decrease throughout both post-treatment collection periods. By one year after treatment there was a low frequency of Eurasian watermilfoil in the untreated plots, indicating that this species was continuing to spread. These data corroborate results from the biomass data.

The other common dicots along the transects were northern watermilfoil, water marigold and bladderwort. The combination of northern watermilfoil and water marigold showed a significant decrease in the one year post-treatment data (Tables 5 and 6). It is not known why this decrease occurred, but the fact that it was evident in both the treated and untreated plots would indicate that it was not a result of the herbicide, but probably due to different growing conditions between the years. The bladderwort showed just the opposite effect, with a significant increase one year post-treatment in the treated plots (Table 5). These results are similar to what was found with the biomass data.

Of the monocots, eel-grass pondweed showed a significant decrease in frequency by 6 weeks after treatment in the untreated plots (Table 6). This could be due to an early senescence of this species, since it usually grows and blooms early in the season (Borman et al. 1997). It was present again at a high frequency by June 1999. A similar pattern of higher biomass in the June samples is also present in the eel-grass pondweed data from the treated plots. Three species, common naiad, Robbins' pondweed and water celery were present at a significantly higher frequency during the August sampling than either of the June samplings in the treated plots (Table 5), probably due to a seasonal growth pattern. Other species (sago pondweed in treated plots and large leaf pondweed in untreated plots) showed a significantly higher frequency one year post-treatment (Tables 5 and 6). Again, this is likely due to factors other than the herbicide treatment, such as variability in growing conditions from year to year.

Point Intercept Plant Frequency. We sampled a total of 602 points and observed 28 different species during the point intercept frequency survey. As with the biomass data, whitestem pondweed and Richardson's pondweed were combined due to suspected hybridization. The collection frequency of Eurasian watermilfoil was not significantly different using this lake-wide sampling method among any of the sampling dates (Table 7), in contrast to what was found with the line-intercept survey method and the biomass data. This is probably due to the fact that treatment plots for the line intercept and biomass methods were located in areas known to contain the highest concentrations of Eurasian watermilfoil, and also were areas where the herbicide was applied. In contrast, the point intercept method sampled the entire littoral zone including areas where the Eurasian watermilfoil was sparsely distributed, and left untreated. The fact that Eurasian watermilfoil was

TABLE 5. MACROPHYTE FREQUENCY AND RESULTS FROM CHI-SQUARE ANALYSIS OF LINE-INTERCEPT DATA, 2,4-D TREATED PLOTS IN LOON LAKE, WA, 1998-1999.

	% present			P-value		
Species	June 98	Aug 98	June 99	June 98 with Aug 98	June 98 with June 99	Aug 98 with June 99
No plants	13	13	12	0.931	0.358	0.401
Chara	21	20	20	0.601	0.549	0.937
Elodea canadensis	5	6	7	0.248	0.175	0.833
Megalodonta beckii + *Myriophyllum sibiricum*	19	20	12	0.599	**0.001***	**0.000***
Myriophyllum spicatum	17	5	4	**0.000***	**0.000***	0.429
Najas flexilis	3	11	4	**0.000***	0.310	**0.000***
Potamogeton amplifolius	27	31	27	0.147	0.977	0.137
Potamogeton gramineus	10	12	12	0.259	0.344	0.957
Potamogeton robbinsii	17	30	20	**0.000***	0.171	**0.000***
Potamogeton zosteriformis	3	2	7	0.357	0.006	**0.000***
Stuckenia pectinata	8	7	14	0.487	**0.000***	**0.000***
Utricularia vulgaris	5	8	14	0.020	**0.000***	**0.000***
Vallisneria americana	3	17	5	**0.000***	0.082	**0.000***

*Bonferroni corrected significance of P ≤ 0.004.

only present in three to six percent of the samples from the whole littoral zone is evidence of its early stage of invasion.

The significant differences seen in the whole-lake frequency of common naiad and water celery were probably due to seasonal increases in seed or rhizome sprouting, since the highest frequency for both was seen in the August samples (Table 7). The increase of large-leaf pondweed in June 1999 could have been due to an annual fluctuation. A similar increase of this species was seen from the line intercept data in the untreated plots (Table 6).

In conclusion, the 2,4-D herbicide application in Loon Lake significantly reduced both the biomass and frequency of Eurasian watermilfoil in the treatment plots during the year of treatment. One year after treatment Eurasian watermilfoil frequency in treated plots remained significantly lower than pre-treatment levels. The other plant species growing in the lake did not show any significant reductions in biomass or frequency as a result of the herbicide treatment. Thus, the application rate and formulation of 2,4-D used in this study selectively controlled Eurasian watermilfoil in Loon Lake without significantly impacting the native aquatic plant species. However, one year after treatment the Eurasian watermilfoil was increasing again slightly, so continued management activities will be required to keep its growth in Loon Lake under control.

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TABLE 6. MACROPHYTE FREQUENCY AND RESULTS FROM CHI-SQUARE ANALYSIS OF LINE-INTERCEPT DATA, UNTREATED PLOTS, LOON LAKE, WA, 1998-1999.

	% present			P-value		
Species	June 98	Aug 98	June 99	June 98 with Aug 98	June 98 with June 99	Aug 98 with June 99
No plants	12	13	5	0.732	0.036	0.013
Chara	45	46	44	0.876	0.892	0.762
Elodea canadensis	10	18	15	0.053	0.217	0.450
Megalodonta beckii + *Myriophyllum sibiricum*	30	35	17	0.358	0.008	**0.000***
Myriophyllum spicatum	0	0	1	—	0.189	0.164
Najas flexilis	1	6	1	0.010	0.654	0.015
Potamogeton amplifolius	35	32	52	0.561	**0.004***	**0.000***
Potamogeton gramineus	1	5	1	0.080	0.474	0.015
Potamogeton robbinsii	5	15	17	**0.004***	**0.001***	0.647
Potamogeton zosteriformis	27	6	28	**0.000***	0.932	**0.000***
Stuckenia pectinatus	0	0	2	—	0.107	0.088
Utricularia vulgaris	1	0	0	0.189	0.280	0.737
Vallisneria americana	2	6	3	0.076	0.618	0.162

*Bonferroni corrected significance of P ≤ 0.004.

Table 7. Macrophyte frequency and results of Chi-square analysis on the point intercept frequency data, Loon Lake, WA, 1998-1999.

	% present			P-value		
Species	June 98 n = 195	Aug 98 n = 198	June 99 n = 20-9	June 98 with Aug 98	June 98 with June 99	Aug 98 with June 99
No plants	12	13	11	0.688	0.803	0.510
Brasenia schreberi	11	11	15	0.958	0.177	0.159
Ceratophyllum demersum	2	3	7	0.753	0.024	0.046
Chara	41	32	32	0.073	0.077	0.959
Elodea canadensis	19	27	29	0.068	0.031	0.747
Megalodonta beckii	14	17	13	0.528	0.673	0.286
Myriophyllum sibiricum	24	24	22	0.974	0.701	0.676
Myriophyllum spicatum	6	5	3	0.621	0.265	0.535
Najas flexilis	4	15	9	**0.000***	0.065	0.041
Potamogeton amplifolius	16	23	33	0.115	**0.000***	0.021
Potamogeton gramineus	5	7	4	0.300	0.694	0.148
Potamogeton natans	7	6	4	0.510	0.138	0.409
Potamogeton praelongus + *Potamogeton richardsonii*	15	20	13	0.206	0.670	0.087
Potamogeton robbinsii	28	34	33	0.154	0.245	0.777
Potamogeton zosteriformis	5	4	8	0.438	0.277	0.049
Stuckenia pectinata	2	4	4	0.372	0.293	0.876
Vallisneria americana	6	14	5	0.009	0.699	**0.002***
Zosterella dubia	5	10	6	0.063	0.636	0.152

*Bonferroni corrected significance of P ≤ 0.003.

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