



Exhibit AF

Article Regarding Loomis Lake in Washington



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Effect of Fluridone on Macrophytes and Fish in a Coastal Washington Lake

JENIFER K. PARSONS^{1,2}, A. COUTO³, K. S. HAMEL⁴ AND G. E. MARX¹

ABSTRACT

Loomis Lake, a long narrow shallow lake on the coast of Washington State, had a submersed plant community dominated by the invasive non-native species Eurasian watermilfoil (*Myriophyllum spicatum* L.) and egeria (*Egeria densa* Planch.). In 2002, the whole lake was treated with the liquid formulation of the aquatic herbicide fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl]-4(1*H*)-pyridinone). We monitored aquatic plant frequency of occurrence and biomass before herbicide application (2002) and for three years after the treatment (2003 to 2005). The fish population was assessed one year prior to herbicide treatment (2001) and three years post treatment (2005). Prior to domination by invasive macrophytes, the lake had a diverse native plant community with low growing species in the deep water providing open water. During that time the lake supported a

stocked rainbow trout (*Oncorhynchus mykiss* Walbaum) and warmwater fishery. As invasive macrophytes took over, the native plant richness decreased, the trout stocking program ceased, and small yellow perch (*Perca flavescens* Mitchell) dominated the fish community. The herbicide treatment resulted in a significant reduction in frequency (86% for egeria, 84% for Eurasian watermilfoil) and biomass (98% for egeria, 99% for Eurasian watermilfoil) of the invasive species for three years. The native submersed plant community was also significantly reduced for the study duration. We attributed this to fluridone use at a nonselective rate and poor light penetration caused by wind-induced sediment entrainment. After treatment the growth of largemouth bass (*Micropterus salmoides* Lacepede) and pumpkinseed sunfish (*Lepomis gibbosus* Linnaeus) increased. In addition, the abundance of small yellow perch decreased while abundance of larger pumpkinseed sunfish increased.

Key words: *Egeria densa*, Eurasian watermilfoil, herbicide, largemouth bass, *Myriophyllum spicatum*, pumpkinseed sunfish, yellow perch.

¹Washington Department of Ecology, 15 W Yakima Ave, Ste 200, Yakima, WA 98902.

²Corresponding author; e-mail jenk461@ecy.wa.gov.

³Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia 98501-1091.

⁴Washington Department of Ecology, Mail Stop 47600, Olympia, WA 98504. Received for publication May 19, 2008 and in revised form November 3, 2008.

INTRODUCTION

To better understand the impacts of aquatic herbicides on nontarget plants under local conditions, the Washington De-

partment of Ecology (WDOE) has conducted monitoring studies for each of the herbicides allowed for use in Washington. Prior to inception of this project, fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl]-4(1*H*)-pyridinone) combined with follow-up spot treatment methods had been used to achieve control and occasional eradication of Eurasian watermilfoil (*Myriophyllum spicatum* L.) from specific waterbodies in the state. However, no data on impacts to nontarget plants or fish had been collected during those projects. The purpose of this project was to provide those data.

Loomis Lake, the study site, is a 69-ha lake located in the coastal dunes of southwestern Washington (Figure 1). It is

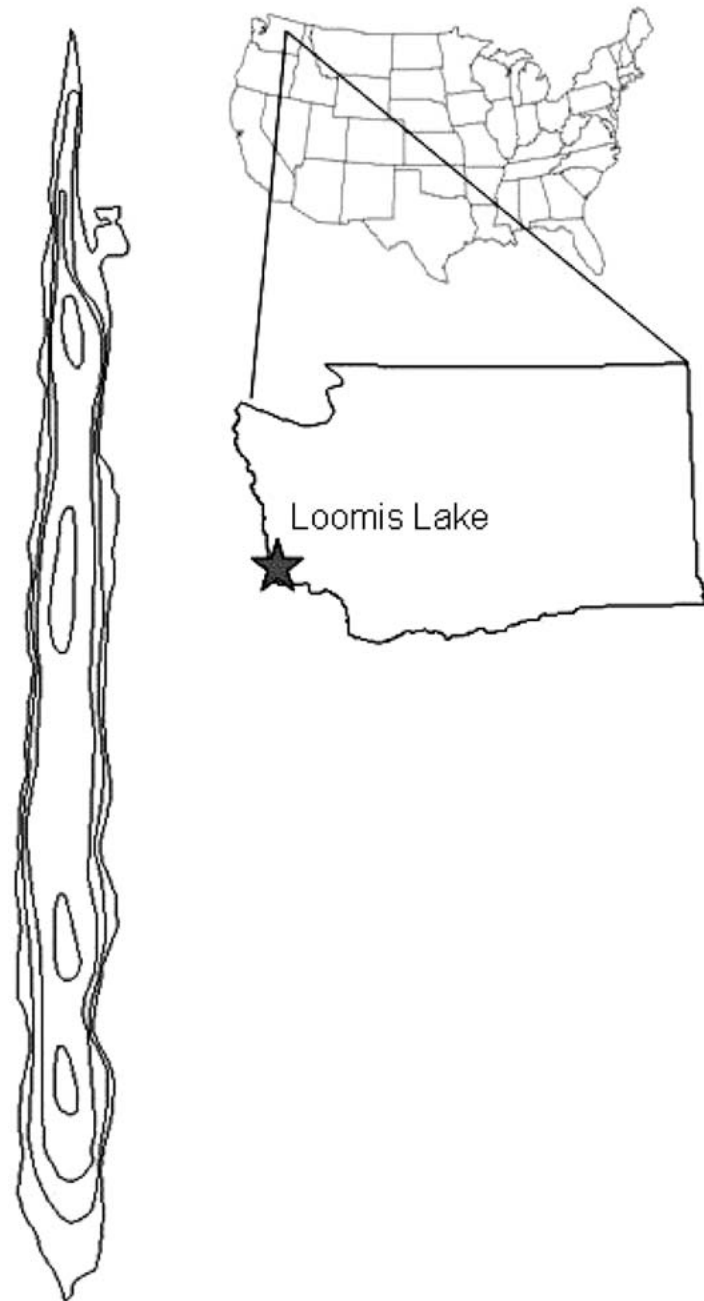


Figure 1. Loomis Lake, Washington. Depth contour intervals are 0.9 m.

on a peninsula, with the Pacific Ocean about 1 km to the west and Willapa Bay about 1 km to the east. The climate is wet and cool, with frequent strong storms during the winter. The lake is 3.2 km long by 250 m wide, with 6.9 km of shoreline. Development of the shore is limited to several houses and a public boat launch with a fishing dock on the west shore. The maximum depth is 3 m with an average depth of 1.5 m (Bortleson et al. 1976). Tannins stain the water, and high levels of nutrients result in a eutrophic classification (O'Neal et al. 2001). The shallow nature of the lake prevents development of a thermocline.

Aquatic plants historically grew throughout Loomis Lake, although in deeper water they were limited to low-growing plant-like macroalgae prior to the invasion by non-native species. Eurasian watermilfoil was first observed in the lake in 1996 and egeria (*Egeria densa* Planch.) was found in 1999. Both species are invasive non-native plants in Washington State. Eurasian watermilfoil has been in Washington since at least 1965 and is currently present in many waterbodies throughout the state. Egeria was first found in Washington in the early 1970s and is currently found in more than 20 lakes in the western part of the state (Parsons 2007). When introduced outside their native range, both species can dominate the submersed plant community to the detriment of native plant diversity, fish and wildlife habitat, water quality, flood control, recreation, and aesthetics (Nichols and Shaw 1986, Smith and Barko 1990, Madsen et al. 1991, Wells and Clayton 1991, Boylen et al. 1999, Valley and Bremigan 2002, California Department of Boating and Waterways 2006). Loomis Lake was no exception; within a few years these two species dominated the submersed plant community, forming a surfacing canopy throughout.

Loomis Lake was historically stocked with rainbow trout (*Oncorhynchus mykiss* Walbaum). However, as invasive macrophytes filled the lake the quality of the fishery declined, so stocking was eliminated from 2000 to 2002. In 2003 limited stocking resumed, was suspended in 2004, and resumed again in 2005. In addition to the rainbow trout, introduced warmwater species included yellow perch (*Perca flavescens* Mitchill), largemouth bass (*Micropterus salmoides* Lacepede), pumpkinseed sunfish (*Lepomis gibbosus* Linnaeus), black crappie (*Pomoxis nigromaculatus* Lesueur), brown bullhead (*Ameiurus nebulosus* Lesueur), and bluegill (*Lepomis macrochirus* Rafinesque). Two native species, sculpin (*Cottus* sp.) and three-spine stickleback (*Gasterosteus aculeatus* Linnaeus) were also present (Mueller 1998).

In 2002, the U.S. Army Corps of Engineers, Seattle District, and WDOE jointly funded this study on the use of fluridone to control both the Eurasian watermilfoil and egeria in Loomis Lake. The WDOE monitored macrophytes pre-treatment (2002) and for three years post-treatment (2003 to 2005) to assess the herbicide's effectiveness at controlling the two target invasive species as well as impacts on native vegetation. In addition, the Washington Department of Fish and Wildlife conducted a fish population assessment of Loomis Lake one year prior to treatment (2001), and three years after the herbicide treatment (2005). These assessments determined species richness, relative abundance (as measured by catch rates), and growth rates, all of which may be affected by the density and abundance of macrophytes.

MATERIALS AND METHODS

Herbicide

The typical scenario for a whole-lake fluridone treatment is to maintain low concentrations for a long time period. Concentration and exposure time (CET) requirements to control Eurasian watermilfoil are well documented (Getsinger and Netherland 1997, Netherland et al. 1997). To achieve selective Eurasian watermilfoil control, the recommended CET is 4 to 5 ppb for 60 days (Cockreham and Netherland 2000, Madsen et al. 2002, Pedlow et al. 2006, Wagner et al. 2007). When this study began, less work had been done on CET requirements for egeria than for Eurasian waterfoil. Laboratory studies indicated that concentrations of 8 to 12 ppb were lethal to egeria (Cockreham and Netherland 2000). To substantiate this, we used the SePRO Corporation PlanTEST® method to determine the target CET. This required that egeria from Loomis Lake be sent to the corporate lab for individual susceptibility tests. Results recommended a CET of 12 ppb for a minimum of 8 weeks for control. This treatment rate is above what Netherland et al. (1997) recommended for selective control, therefore die-off of native plant species, while not desired, was expected.

The initial herbicide treatment took place on June 28, 2002, using the liquid fluridone formulation (Sonar AS®). This treatment date was chosen because plants are typically still in a pattern of rapid spring growth in the wet cool climate of Loomis Lake, and it is at the end of the rainy season so herbicide dilution would be minimized. Approximately every two weeks from mid-July until the end of August water samples from three sites were tested for fluridone concentration using FasTEST® (an enzyme-linked immunosorbent assay (Netherland et al. 2002). Results of these tests indicated that additional herbicide was periodically required to maintain the target concentration of 12 ppb. The herbicide was added on July 11, July 30, August 14, and August 28 (T. McNabb, Aquatechnex, 2002, pers. comm.).

We did not expect the herbicide treatment to fully eradicate the egeria and Eurasian watermilfoil because previous experience had shown some form of follow up treatment is nearly always required. The Aquatic Plant Management Plan for Loomis Lake identified diver hand pulling as the follow-up method of choice to remove surviving invasive plants (Envirovision 1998). If the recovering plants were too numerous or widely scattered for diver hand pulling to be practical, additional herbicide treatments were considered.

Aquatic Plants

Aquatic plant biomass and frequency of occurrence data were collected in June of each year; once before the herbicide treatment (2002) and for three years after treatment (2003 through 2005). Species richness was also determined by listing all aquatic plant species observed, both at sample points and any new ones observed between points.

The point-intercept method was used to gather presence-absence data for species frequency of occurrence as per Madsen (1999). We created a 50 by 50 m grid covering the whole lake using a Geographic Information System. Each grid in-

tersection was a sample point, provided as UTM (Universal Transverse Mercator) coordinates. In 2002, the grid produced 251 sample points; from 2003 to 2005 the grid was modified slightly and resulted in 238 points. A Geographical Positioning System (GPS) unit was used to locate the points in the field. At each point macrophyte data were collected by using a sampling rake. The rake was deployed twice at each point, and all species collected were recorded. The data were analyzed using Chi square two-by-two analysis for the species present in at least 10% of samples during at least one sampling event.

Biomass samples were collected by a SCUBA diver from 50 points randomly selected from the frequency data grid. At each point the diver collected the sample using a 0.1-m² frame placed on the sediment (Madsen 1993). All above-ground plant matter was collected and placed in a mesh bag. The samples were sorted by species and dried in a forced air oven at 60 to 70 °C to a constant weight and weighed to 0.01 g accuracy. Analysis of variance was performed on log transformed data to check for significant differences before and after treatment. The resultant p-values were adjusted using a Bonferroni post-hoc test to adjust for multiple comparisons.

Water transparency data were also collected during each visit using a standard 20-cm secchi disk.

Fish

The fish community was surveyed in June of 2001 and 2005 following methods outlined by Bonar et al. (2000). Sampling occurred during the evening hours to maximize the type and number of fish captured. Fish were captured using three sampling techniques: electrofishing, gill netting, and fyke-netting. The electrofishing unit consisted of a Smith-Root SR-16s electrofishing boat, with a 5.0GPP pulsator unit. Experimental gill nets, 45.7 m long by 2.4 m deep, were constructed of four sinking panels (two each at 7.6 m and 15.2 m long) of variable-size (1.3, 1.9, 2.5, and 5.1 cm stretch) monofilament mesh. Fyke (modified hoop) nets were constructed of five 1.2-m diameter hoops with two funnels, and a 2.4-m cod end (6 mm nylon delta mesh). Attached to the mouth of the net were two 7.6-m wings, and a 30.5-m lead.

The 2001 survey spanned four days (June 12-15) and the 2005 survey spanned three (May 31-June 2). Each survey consisted of 12 electrofishing sections, 6 to 8 gillnet sections, and 6 to 8 fyke net sections (6 each in 2001; 8 each in 2005). Electrofishing sample locations were selected from a map by dividing the entire shoreline into 400-m sections, numbering them consecutively, and randomly choosing them without replication. The electrofishing boat was maneuvered slowly through the shallows (1 to 3 m deep) for 600 sec in each section. Gill nets were fished perpendicular to the shoreline; the small-mesh end was tied off to shore, and the large-mesh end was anchored off shore. Fyke nets were fished perpendicular to the shoreline as well. The lead was tied on shore, and the cod-end was anchored off shore, with the wings anchored at approximately a 45° angle from the net lead. Fyke nets are fished with the hoops 0.3 to 0.5 m below the water surface, which sometimes requires shortening the lead. Both nets types were set at dusk and retrieved in the morning, with

soak time recorded as “net nights” (1 net set over 1 night = 1 net night).

With the exception of sculpin (family Cottidae), all captured fish were identified to species level. Most fish were measured to the nearest millimeter (mm) total length, and weighed to the nearest gram (g). Fish <70 mm were not weighed due to inadequate scale precision. To reduce handling stress on fish, where large numbers (>200) of obviously similar-sized fish were collected simultaneously, a subsample was measured to the nearest millimeter and weighed to the nearest gram. The remaining fish were counted and the subsampled data expanded. Weights were then assigned using a length-weight regression formula. Scales were taken from five individuals of each warmwater game species per centimeter size class (>70 mm) for aging purposes. Total and incremental lengths at annulus formation were back-calculated using the Fraser-Lee method with y-axis intercepts specified by Carlander (1982).

The species composition expressed as the number of fish captured was determined by counting all the fish in a given species. Species composition expressed as weight percentage (%w) of fish captured was determined by dividing the total weight of fish of a given species by the total weight of the sample. All fish, including young-of-the-year, were used to determine biomass and species composition; the impact of young-of-the-year fish on data analysis was minimal due to sample timing (early June for both surveys).

Catch-per-unit-of-effort (CPUE) data, which measures the number of fish in each species collected with a given sampling gear over a standardized unit of time, was used to compare the relative abundance of stocks between lakes or over time. For electrofishing, the results were expressed as the number of fish caught per hour. The CPUE for gill nets and fyke nets is expressed as the number of fish caught per net-night. An average CPUE (across sample sections) with an 80% confidence interval was calculated for each species and gear type. The CPUE of stock length and sub-stock length fish was calculated separately (stock length is approximately 20 to 26% of the all-tackle, world record length for each species, and often correlates to age of maturity (Gablehouse 1984). Analyses of means for CPUE data were calculated using the Mann-Whitney rank-sum test with $\alpha = 0.05$.

To evaluate fish growth in the years immediately adjacent to the herbicide treatment, growth rate assessments were conducted using incremental length-at-age data (growth that occurred between annulus formation). Incremental length-at-age data was considered superior to total length-at-age data because the former provides growth data specific to one year, whereas the latter is the cumulative growth of a fish's entire lifespan and is therefore influenced by previous years' growth rates. To limit the potential influence of age effects on growth (different growth rates inherent to different age fish), comparisons were limited to like age-classes.

For each calendar year, mean incremental lengths-at-age for each cohort were produced. From these data a cumulative mean for each age-class was calculated spanning 1997 to 2004 (growth data from a 1997 Loomis Lake fish inventory (Mueller 1998) was included.) The mean incremental length-at-age for each cohort in each calendar year was then compared to the cumulative incremental length-at-age for

the corresponding age-class and expressed as a percent difference. For example, the mean incremental length of age-1 largemouth bass from 2001 (64.27 mm) was compared to the mean incremental length of all age-1 largemouth bass from 1997 to 2004 (77.24 mm) and expressed as a percent difference (-16.8%). A comparison of means to determine a statistically significant difference between these data sets was conducted using the Mann Whitney rank sum test per Klumb et al. (1999).

RESULTS AND DISCUSSION

Herbicide

The average herbicide concentration varied between 10 and 26 ppb throughout the treatment period (Figure 2). This was above the target CET normally used for Eurasian watermilfoil control, and only dipped below the target concentration for egeria during a short period at the end of July. An additional water sample collected October 30, 2002, showed the fluridone persisted into autumn, with a concentration of 10 ppb at that time (T. McNabb, Aquatechnex, 2003, pers. comm.).

Aquatic Plants

During routine monitoring before the submersed plant community became dominated by egeria and Eurasian watermilfoil, 16 submersed and floating-leaved aquatic plant species had been observed (Table 1; pre-2000 column). In 2002, just before the herbicide treatment, the number of species was reduced to 12; four pondweed species were not found. At that time the invasive species were growing to the surface nearly throughout the lake. They were the most frequently encountered species (Table 2) and accounted for 91% of the plant biomass (Table 3). Other studies have also documented declines in native macrophyte species richness corresponding to dominance by invasive macrophytes (Madsen et al. 1991, Trebitz et al. 1993, Boylen et al. 1999). Thus the egeria and Eurasian watermilfoil were dominating the plant community to the extent that several native plants were elim-

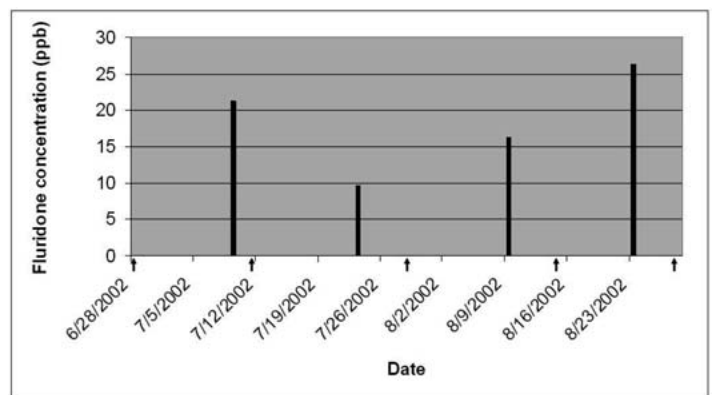


Figure 2. Fluridone concentration results from FasTEST® for Loomis Lake, WA. Arrows indicate dates when herbicide was added to maintain levels at or above the target concentration of 12 ppb.

TABLE 1. SUBMERSED (S) AND FLOATING LEAVED (FL) AQUATIC PLANT SPECIES AND YEAR OF OBSERVATION IN LOOMIS LAKE, WA. HERBICIDE TREATMENT BEGAN THREE DAYS AFTER THE 2002 INVENTORY.

Scientific name	Common name	Growth form	Pre-2000	2002	2003	2004	2005
<i>Ceratophyllum demersum</i>	coontail; hornwort	s	√	√			√
<i>Egeria densa</i>	egeria	s	√	√	√	√	√
<i>Elodea sp.</i>	common elodea	s	√	√			√
<i>Hydrocotyle ranunculoides</i>	water-pennywort	fl	√	√	√	√	√
<i>Myriophyllum spicatum</i>	Eurasian watermilfoil	s	√	√		√	√
<i>Najas sp.</i>	water-naiad	s	√	√		√	√
<i>Nitella sp., Tolypella sp.</i>	macro-algae	s	√	√		√	√
<i>Nuphar polysepala</i>	yellow waterlily	fl	√	√	√	√	√
<i>Nymphaea odorata</i>	fragrant waterlily	fl	√	√			√
<i>Potamogeton amplifolius</i>	large-leaf pondweed	s	√			√	
<i>Potamogeton ephedrus</i>	ribbon-leaf pondweed	s	√			√	√
<i>Potamogeton natans</i>	floating leaf pondweed	fl	√				
<i>Potamogeton praelongus</i>	whitestem pondweed	s				√	√
<i>Potamogeton richardsonii</i>	Richardson's pondweed	s	√	√			√
<i>Potamogeton sp.</i>	thin leaved pondweed	s	√			√	√
<i>Potamogeton zosteriformis</i>	eelgrass pondweed	s	√	√	√	√	√
<i>Utricularia sp.</i>	bladderwort	s	√	√		√	√

inated from the lake or had become so rare that they were not observed using any of our sampling methods.

One year after treatment (YAT), in June 2003, the submersed and floating-leaved plant community had changed substantially. Four sparsely distributed submersed or floating-leaved species were recorded (Table 1), 75% of sample points contained no plants (Table 2), and total plant biomass was reduced by 97% (Table 3). Egeria and a small amount of eelgrass pondweed were the only submersed plants found. Most of the egeria had black stems and leaves, with only a green inner stem to indicate it may be viable. A large die off of native plant species during the year of treatment was expected because of the concentration of fluridone administered to control egeria. Concentrations of 10 ppb or higher with an exposure time of several months will significantly reduce many native species (Netherland et al. 1997, Welling et al. 1997, Wagner et al. 2007). However, we expected to see greater recovery by one YAT.

A corresponding difference one YAT was a reduction in water clarity. Because Loomis Lake is long, narrow, shallow,

TABLE 2. AQUATIC PLANT FREQUENCY OF OCCURRENCE DATA CHI-SQUARE ANALYSIS RESULTS FOR COMMON SPECIES. NUMBERS ARE THE PERCENT OF SAMPLES WHERE THE PLANT WAS FOUND IN LOOMIS LAKE, WA, JUNE 2002 TO 2005.

Plant	% present			
	June '02	June '03	June '04	June '05
Egeria	59	16*	0.4*	8*
Eurasian watermilfoil	82	0*	0*	13*
Coontail	16	0*	0*	5*
eelgrass pondweed	56	2*	3*	20*
Elodea	33	0*	0*	0.4*
water-naiad	0.8	0	18*	32*
macroalgae	2	0	47*	52*
No Plants	3	75*	42*	30*

*significantly different from pretreatment frequency of occurrence (June 2002). Significance level corrected for multiple comparisons is $p < 0.017$.

and subject to strong winds that accompany frequent storms off the Pacific Ocean, the fetch is the entire 3.2 km length of the lake. Notable sediment entrainment occurred when plant death left large areas of the lake bed unprotected. This was evidenced in the Secchi depth, which averaged a tannin-stained 1.5 m in summers 1997 and 1999 prior to treatment (Smith et al. 2000, O'Neal et al. 2001), but fell to a muddy-looking 0.6 m during sample collection one YAT. Loomis Lake is also prone to periodic algae blooms, which may have contributed to the reduction in water clarity. Decreases in water clarity have corresponded with other whole lake fluridone treatments, especially in nutrient-rich lakes where planktonic algae take advantage of reduced competition from macrophytes and in lakes where the CET of fluridone was high enough to reduce native plant growth (Valley et al. 2006, Wagner et al. 2007). Thus, it was likely a combination of the long exposure to relatively high levels of fluridone along with reduced water clarity one YAT that prevented native macrophytes from recovering quickly from the herbicide treatment. By June 2004 (two YAT) the Secchi depth had improved to pretreatment values (1.7 m).

Healthy looking egeria was found at a few sample points two YAT (2004; Tables 2 and 3). We also noticed egeria patches outside the sample points, especially at the north end of the lake where sediment is deep and flocculent. A SCUBA diver was contracted to hand-pull the recovering plants in August 2004. The diver pulled some egeria, but after a short time in the water, realized the amount of egeria present was more than could be controlled within the project budget (D. Freeland, ACE Diving, 2004, pers. comm.). Although Eurasian watermilfoil was not collected at any sample points in June 2004, it was spotted by the diver in August, and a few plants were removed. By June 2005 (three YAT) both Eurasian watermilfoil and egeria samples were collected in frequency of occurrence (Table 2) and biomass (Table 3). We had hoped the combination of fluridone and hand pulling would eradicate the two invasive weeds, as has been the case with Eurasian watermilfoil in other Washing-

TABLE 3. AQUATIC PLANT MEAN DRY WEIGHT BIOMASS WITH STANDARD DEVIATION IN PARENTHESES AND BONFERRONI ADJUSTED ANOVA RESULTS FROM COMMON SUBMERSED SPECIES IN LOOMIS LAKE, WA, JUNE 2002 TO 2005.

Plant	Biomass (g/m ²)			
	June 2002	June 2003	June 2004	June 2005
Egeria	157.7 (263.9)	9 (16.5)*	0.5 (3.4)*	3.15 (17.3)*
Eurasian watermilfoil	129.4 (165.5)	0*	0*	0.5 (1.6)*
Coontail	1.3 (6.4)	0	0	0.3 (1.5)
eelgrass pondweed	13.2 (23.8)	0*	0.2 (0.8)*	1.1 (3.5)*
Elodea	3.56 (12.6)	0*	0*	0*
water-naiad	0	0	0.07 (0.2)	0.2 (0.3)*
macroalgae	0.07 (0.34)	0	0.9 (3.5)	18.7 (43)*
Total of all plants	313.9 (256.7)	9 (16.5)*	1.7 (4.9)*	26 (45.9)*

*significantly different from pretreatment biomass (June 2002) at $p \leq 0.05$.

ton lakes; however, the degree of plant recovery, along with the poor water clarity, made diver hand pulling impractical.

Although eradication was not realized, the treatment achieved 100% control of Eurasian watermilfoil one YAT that lasted into early summer two YAT. The treatment also provided control of egeria for the duration of the study. There was a 73% reduction of frequency and 94% reduction in biomass of egeria one YAT. These numbers were higher two YAT (>99% reduction in frequency and biomass), likely due to our overestimation of the viability of egeria remaining on the sediment during the one YAT sampling. By three YAT Eurasian watermilfoil, as well as egeria, were present in samples, though they were still significantly reduced compared with pretreatment (Eurasian milfoil, 84% reduction in frequency and >99% biomass reduction; egeria, 86% reduction in frequency and 98% biomass reduction). In 2006, herbicides were again used to control the recovering Eurasian watermilfoil and egeria.

At two YAT, native macrophyte richness was beginning to recover, and by three YAT, 15 submersed and floating leaved species were found (Table 1). Floating-leaved pondweed was the only species historically present in the lake that was not seen after the treatment. We did not see large-leaf pondweed three YAT but found a very small patch two YAT. Whitestem pondweed was not known from the lake prior to the treatment, but was present two and three YAT.

In spite of recovering species richness, the frequency of sample points with no plants was significantly higher than pre-treatment for three YAT (Table 2). Common elodea, eelgrass pondweed, and coontail, all common before treatment, were significantly reduced for three YAT (Tables 2 and 3). Common elodea was the slowest of the three to show signs of recovery. It was absent from samples until three YAT, and then frequency of occurrence was reduced 99% compared with pretreatment data (it was not collected in biomass samples). Frequency of coontail was reduced 69% three YAT. Other studies have also found that elodea and coontail are susceptible to fluridone at the CET used in Loomis Lake and are reduced or eliminated after treatment (Netherland et al. 1997, Smith and Pullman 1997, Poovey et al. 2004, Harmon et al. 2005, Valley et al. 2006, Wagner et al. 2007). Eelgrass pondweed is susceptible to fluridone at concentrations >20 ppb, and intermediately susceptible at

concentrations <20 ppb (Hauxwell and Wagner in prep). For the majority of this study, fluridone concentrations were between 10 and 20 ppb (Figure 2). Also, plants that produce vegetative reproductive structures recover more quickly from fluridone treatments (Netherland et al. 1997), and eelgrass pondweed produces leafy turions (Haynes and Hellquist 2000). Thus the combination of its higher tolerance to fluridone, the dormant turions, and the fact that it was the most common native plant prior to treatment likely enhanced the recovery of eelgrass pondweed above that of most other native species.

The two most common submersed species two and three YAT were macroalgae and water-naiad. Their frequency of occurrence and biomass were significantly higher than prior to treatment (Tables 2 and 3). These were also shown to recover most quickly from fluridone in mesocosm experiments (Netherland et al. 1997) and increased quickly after some whole-lake treatments (Crowell et al. 2006), although in a different study, Wagner et al. (2007) showed naiad species took three to five years to recover from whole lake fluridone treatments. Naiad is an annual plant that relies on seed for reproductive success (Wingfield et al. 2004), so its recovery was likely driven by seed germination. Macroalgae have demonstrated tolerance to fluridone in other studies (Welling et al. 1997; Hauxwell and Wagner, in prep.); therefore we suspect the absence of this plant from samples one YAT was due more to growth inhibition from turbidity than direct impacts by the herbicide. After the water cleared, the macroalgae colonized much of the area opened up by the vascular plant removal.

Fish

Nine fish taxa were collected from Loomis Lake in 2001. In 2005, bluegill were missing from the sample, for a total of eight taxa (Table 4). Largemouth bass, pumpkinseeds, and yellow perch dominated the species composition of both surveys. Combined, they account for 97% of the abundance by number and 89% of the weight in 2001, and 94% of the abundance by number and 76% of the weight in 2005 (Table 4). With the possible exception of the hatchery-planted rainbow trout, whose abundance is independent of the lake environment, no other taxa had sufficient samples sizes to assess.

TABLE 4. FISH SPECIES COMPOSITION BY WEIGHT PERCENTAGE (%W) AND NUMBER (#) FOR FISH COLLECTED FROM LOOMIS LAKE, PACIFIC COUNTY, WA, IN JUNE 2001 AND JUNE 2005.

Fish Species	Scientific Name	Species Composition			
		2001		2005	
		(%w)	(#)	(%w)	(#)
Yellow perch	<i>Perca flavescens</i>	45.56	1178	35.88	242
Largemouth bass	<i>Micropterus salmoides</i>	41.35	116	26.12	49
Rainbow trout	<i>Oncorhynchus mykiss</i>	7.74	16	22.80	25
Pumpkinseed	<i>Lepomis gibbosus</i>	2.34	100	13.88	355
Brown bullhead	<i>Ameiurus nebulosus</i>	2.43	4	0.36	2
Bluegill	<i>Lepomis macrochirus</i>	0.25	5		
Black crappie	<i>Pomoxis nigromaculatus</i>	0.16	3	0.53	5
Sculpin	<i>Cottus spp.</i>	0.15	13	0.41	8
Three-spine stickleback	<i>Gasterosteus aculeatus</i>	0.01	4	0.02	3

Stock lengths for the three most abundant species in Loomis Lake are 20 cm for largemouth bass, 8 cm for pumpkinseed, and 13 cm for yellow perch (Anderson and Neumann 1996). We collected CPUE data for stock length largemouth bass, pumpkinseed, and yellow perch (Table 5), and data for sub-stock length fish (excluding stock length and above; Table 6). The stock length data show a significant decline in gill-netted largemouth bass ($P = 0.0084$) and a significant increase in electrofished ($P = 0.0078$) and fyke-netted ($P = 0.001$) pumpkinseeds in 2005 compared with 2001. The CPUE data for sub-stock length fish showed a significant decline of fyke-netted largemouth bass ($P = 0.0102$), and both electrofished ($P < 0.0001$) and fyke-netted ($P = 0.006$) yellow perch. The sub-stock length gill-net CPUE samples for all

three species were very small in both surveys, which is probably due to the inefficiency of gill nets in capturing small fish.

Both the CPUE and species composition data show the fish community in 2001 was comprised of high numbers of small fish (yellow perch), typical for lakes with high plant density throughout the lake (Dibble et al. 1997). Although the CPUE of stock length yellow perch did not change significantly after the herbicide treatment, the number of sub-stock length yellow perch declined significantly. Conversely, stock length pumpkinseeds increased post-treatment, with no significant change in sub-stock length pumpkinseeds. The decline in small yellow perch may be attributed to increased largemouth bass predation (Guy and Willis 1991) because decreased macrophyte density is known to improve

TABLE 5. AVERAGE CATCH PER UNIT EFFORT FOR STOCK SIZE FISH COLLECTED AT LOOMIS LAKE, WA, JUNE 2001 AND 2005 WITH 80% CONFIDENCE INTERVAL IN PARENTHESES.

	electrofishing (#/hour)		gill netting (#/net night)		fyke netting (#/net night)	
	2001	2005	2001	2005	2001	2005
largemouth bass	7.0 (2.3)	7.4 (3.8)	2.7 (0.9)	0.4 (0.2)*	0	0
pumpkinseed	9.0 (4.2)	27.2 (8.9)*	0.3 (0.3)	0.3 (0.3)	0.3 (0.3)	12.9 (3.2)*
yellow perch	75.0 (15.4)	89.3 (26.1)	5.8 (1.6)	3.1 (1.3)	5.0 (3.9)	3.3 (2.1)

*significantly different from 2001 CPUE ($p \leq 0.05$).

TABLE 6. AVERAGE CATCH PER UNIT EFFORT FOR SUB-STOCK SIZE FISH (EXCLUDING STOCK SIZE AND LARGER) COLLECTED AT LOOMIS LAKE, WA, JUNE 2001 AND 2005 WITH 80% CONFIDENCE INTERVAL IN PARENTHESES.

	electrofishing (#/hour)		gill netting (#/net night)		fyke netting (#/net night)	
	2001	2005	2001	2005	2001	2005
largemouth bass	29.0 (9.3)	15.0 (4.8)	0.3 (0.3)	0	4.3 (2.0)	0.3 (0.2)*
pumpkinseed	22.5 (11.5)	48.5 (17.2)	0	0	5.7 (3.2)	12.5 (3.2)
yellow perch	398.9 (41.0)	7.5 (3.3)*	1.5 (1.4)	0	26.0 (13.7)	0.1 (0.2)*

*significantly different from 2001 CPUE ($p \leq 0.05$).

predator efficiency (Crowder and Cooper 1982, Hayse and Wissing 1996, Dibble et al. 1997). The changes we observed in the size structure of the yellow perch population, from one with many small fish to one with fewer but larger fish, is a well known result of increased predation (Novinger and Legler 1978, Tonn and Paskowski 1986). However, the increase in pumpkinseed abundance is unexpected because largemouth bass would be expected to prey on them as well, unless they preferentially feed on yellow perch over pumpkinseeds. The increase in pumpkinseed abundance could also be caused by reduced competition for resources with the decline in yellow perch. Resource utilization overlap occurs in small fish of both species feeding on zooplankton (Hubert and Sandheinrich 1983, Mittelbach 1984, Lott et al. 1996).

A potentially confounding factor to the CPUE data is the effect the removal of vegetation may have had on the capture efficiency of the various collection gears. Unfortunately, published research on the subject is limited. Bayley and Austen (2002) found that the catch efficiency of a boat electrofisher was related to the percentage of a lake covered by macrophytes. Gill-net efficiency can be affected by twine diameter and color, both of which alter the net's visibility (Hansen 1974, Jester 1977), which could also be affected by the presence or absence of macrophytes. Weaver et al. (1993) found differences in catch rates of fyke-nets fished at multiple sites with a range of macrophyte density and species composition, although whether the results reflect differences in fish abundance at each site or differences in gear efficiency is unclear. Anecdotal reports from the 2001 survey of Loomis Lake suggests that the macrophyte density may have prevented the lead lines of both net types from laying flush on the lake bottom. Thus we do not know how much the presence or absence of macrophytes may have altered catch efficiency. Adding further complexity, alterations in the catch efficiency for each gear type may have species-specific impacts.

Age data were collected on 53 largemouth bass, 24 pumpkinseeds, and 62 yellow perch in 2001, and 44 largemouth bass, 36 pumpkinseeds, and 48 yellow perch in 2005. Combined growth rate data for all three species indicate improved population growth for the two years subsequent to the herbicide treatment (Figure 3). For four years prior to the herbicide treatment (1997-2000) the combined growth rate (measured as incremental lengths-at-age) of these three species was 0.3% below the mean. After treatment (2003 and 2004), the combined growth rate was 7.9% above the mean. When considering individual species cohorts, incremental length-at-age data for 2004 for age-1 and age-2 largemouth bass and age-1 pumpkinseed were significantly higher than average ($P = 0.0018$ to <0.0001). Other studies have also shown that largemouth bass and sunfish (bluegill and redear sunfish) growth increased with total macrophyte removal (Dibble et al. 1997). Age-1 largemouth bass from 2003 and age-1 yellow perch from both 2003 and 2004 showed no significant difference from the mean of all three surveys ($P \geq 0.05$). Other age-classes had insufficient data to analyze.

When considering combined growth and abundance data, fish growth typically has an inverse relationship to fish abundance; fewer fish mean reduced competition for resources and increased growth for the remaining individuals (Swingle and Smith 1941, Partridge and DeVries 1999, Sass et al. 2004,

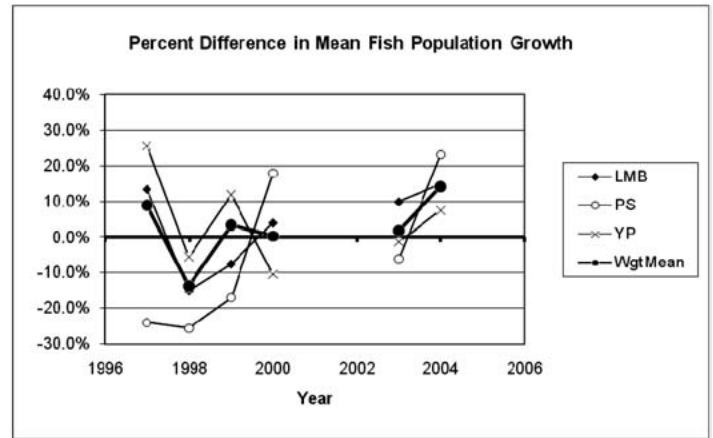


Figure 3. Percent difference in cohort growth per year measured as incremental lengths-at-age for largemouth bass (LMB), pumpkinseed sunfish (PS) and yellow perch (YP) collected from Loomis Lake in June 2001 and 2005. Data compare the mean incremental lengths-at-age for Loomis Lake. The weighted mean (Wgt Mean) of all three species is also shown.

Aday et al. 2005, Headley and Lauer 2008). Our findings appear to disagree with this. In this study, largemouth bass exhibited increased growth post-treatment (compared to pretreatment growth) despite essentially unchanged abundance. Pumpkinseed showed significant increases in both growth and abundance post-treatment. Yellow perch showed no significant growth increase post-treatment (again, compared to pretreatment growth) despite significantly reduced relative abundance.

The impact of vegetation removal on fish abundance, growth, survival, and diversity has mixed results in literature (Maceina et al. 1991, Bettoli et al. 1993, Olson et al. 1998, Pothoven et al. 1999, Unmuth et al. 1999). Most found significantly increased growth in at least some age classes of some species. Changes in abundance were highly variable depending on species, sampling gear, and predator-prey relationships. Bettoli et al. (1993) discovered significant alterations in species richness and diversity as prey species adapted to highly vegetated habitats gave way to those that prefer less vegetation. They also found that although some changes could be explained by species life histories, others could not. In a study similar to Loomis Lake, Pothoven et al. (1999) followed two lakes given whole-lake Sonar treatments and compared results to three control lakes. Largemouth bass and bluegill exhibited increased growth rates, but showed mixed results for abundance, depending on species, age, and gear type.

Fish response to vegetation removal appears to be highly dependent on the underlying ecosystem dynamics. The density of macrophytes before and after removal, the difference in density as a result of removal, and the persistence of the removal all appear to be factors. Fish life histories and predatory and competitive pressures, both intra- and inter-specific, also play a role.

In conclusion, the fluridone treatment at Loomis Lake achieved a significant reduction in frequency (86% for egeria; 84% for Eurasian watermilfoil) and biomass (98% for egeria; 99% for Eurasian watermilfoil) for three YAT. Be-

cause the CET was above that which will achieve species selectivity, the herbicide treatment also significantly reduced native plant frequency of occurrence and biomass for the same time period. This allowed the lake to become more turbid for one YAT. The fish community experienced a decline of small fish, which had become abundant due to dense vegetation prior to treatment. After treatment, the growth of largemouth bass and pumpkinseed and abundance of stock size pumpkinseed increased. Whole-lake herbicide treatments that reduce native plant cover in shallow nutrient rich systems run the risk of producing an algae-dominated system. The native plant community composition and tolerance to herbicide CET, as well as the potential for algae and sediment entrainment, should be considered before use to avoid potential long term impacts to the lake biota.

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