Factors Influencing the Control of Eurasian Watermilfoil With Native Or Naturalized Insects

Fifth Status Report for 1999-2001

BY

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Introduction

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is an exotic aquatic weed that often interferes with recreation (Smith and Barko 1990), inhibits water flow, impedes navigation, (Grace and Wetzel 1978) and will displace other aquatic macrophytes (Madsen et al. 1991). It was first reported in Minnesota in 1987 and occurred in over 120 Minnesota waterbodies by fall 2000 (Exotic Species Program 2001).

Recent work on the biological control of Eurasian watermilfoil has focused on the indigenous weevil *Euhrychiopsis lecontei* (Dietz) (= *Eubrychiopsis lecontei*). This work work suggests that *E. lecontei* is the most promising control agent (Creed and Sheldon 1995, Sheldon and Creed 1995, Creed 1998, Newman and Biesboer 2000). The weevil is native to Minnesota and Wisconsin (Newman and Maher 1995, Jester et al. 1997) and is highly specific to watermilfoils (Solarz and Newman 2001). Sheldon and O'Bryan (1996), Newman et al. (1996, 1997), Mazzie et al. (1999) and Newman et al. (2001b) describe the life history and development times of the weevil.

Although declines of milfoil in several lakes have been related to the occurrence of *E. lecontei* (Sheldon and Creed 1995, Lillie 1996, Newman and Biesboer 2000, Creed 1998), it is clear that at many sites in Minnesota, weevil densities do not get high enough to effect control (Newman et al. 1996, Newman et al. 1998, Newman and Biesboer 2000). Fish predation may be one factor limiting populations in some lakes (Sutter and Newman 1997, Newman and Biesboer 2000). Getsinger et al. (*in press*) provide a good overview of the potential use of the weevil for control of milfoil.

The aim of this project is to monitor a set of milfoil populations for potential declines, determine factors that may be limiting control agent densities and their effectiveness in the field, determine the effects of fish on weevil augmentations and determine if chronic effects such as sediment quality or competition with native plants is responsible for declines of milfoil associated with herbivores. This report summarizes our methods and collection efforts in 2001 and presents preliminary results of our research through 2001.

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Methods

Semi-permanent Transect Sites:

During the summers of 1993 and 1994, we initiated selection of semi-permanent sampling sites, which can be repeatedly sampled at fixed locations (Newman and Ragsdale 1995). The sites were Lake Auburn (Carver Co.; T116N; R24W; S10), Otter Lake (Anoka and Ramsey Co.; T30-31N; R22W; S3-4, S35-36), Cedar Lake (Hennepin Co.; T29N; R24W; S29) and Smith's Bay of Lake Minnetonka (Hennepin Co.; T117N; R23W; S10,11). At each site, 5 transects, 30 m apart, were run from near shore (0.5 m depth) toward the plant limit. At Lake Auburn and Cedar Lake, the transects were extended to 50 m from the shoreward starting point, in approximately 2.5 m depth at Auburn and 5 m depth in Cedar. Semipermanent stations were marked along the transect at 10 m intervals with fluorescent floats that were attached to bricks and suspended 0.5-1m beneath the surface. At Otter Lake, the transects were extended 100 m from shore, in approximately 2 m depth. At Smith's Bay, transects were started 100 m from shore and run to 4.5 m depth, approximately 0.8 km from shore, with 5 sampling stations along each transect approximately geometrically spaced. Distances from shore determined from GPS data were: 100m, 200m, 370m, 585m and 805m. These stations were marked with floating milfoil buoys.

In summer 1996, we noticed a dense population of weevils at Cenaiko Lake (Anoka Co.; T31N; R24W; S26). We therefore sampled this lake in July and September as a new site to be regularly sampled. We ran 3 or 4 transects, west to east across the north end of the lake, with sampling stations every 30 m. This resulted in 25-32 samples on each date (21-30 with plants; deep stations were deleted from the analysis). At Lake Auburn transects were sampled at 10 m intervals (stations) , resulting in 6 samples per transect, or 30 samples. At Otter Lake samples were taken at each 20m sampling station, resulting in 5-6 samples per transect or 27 samples. At Cedar (30) and Smiths Bay (25), all stations were sampled, however, several stations in Cedar Lake were deeper than the plant limit (>7m) and these are excluded if no plants occurred there during the season. In 1997 sampling occurred twice: in late June to early July and in mid-September. In 1998, three lakes (Auburn, Cenaiko and Smith's Bay) were sampled thrice, in June, late-July or early August and in September. Otter and Cedar were sampled in June and September. Samples were alternately taken 2m from each side of each station on successive sampling dates to minimize sampling disturbance. In 1999, two lakes (Cenaiko, and Smith's Bay) were sampled thrice, in June, late-July or early August and in late August. Auburn and Cedar were sampled in June and late August and Otter was sampled in June and early August. In 2000, four lakes were sampled three times (Auburn, Cenaiko, Otter and Smith's Bay), in June, July and August and Cedar Lake was sampled twice, in June and August. Twenty-four to thirty samples were collected at each lake on each date.

In 2001, four lakes (Auburn, Cenaiko, Otter and Smith's Bay) were sampled three times, in June, late July and late August. Cedar was sampled in June and August. Twenty-five to thirty samples were collected at each lake on each date. These samples have been processed for wet mass, but invertebrates have not been sorted and enumerated and dry mass has not been calculated.

At each sampling station, plant biomass and invertebrate samples were taken from 0.1 m^2 quadrats (all plant material was clipped at sediment interface and immediately placed in a sealable bag underwater). Sediment cores were also collected at shallow, medium and deep stations along 3 transects (transects 1, 3 and 5 at all but Cenaiko, where 1-3 were sampled) at each site.

A set of water column parameters were measured in the open water (>5.5m depth and >100 m from the bed) at each site on each sampling date. Secchi depth and surface conductivity were measured and a water sample (combined surface and Secchi depth sample) was collected for pH, alkalinity and chlorophyll a determination. A light (Photosynthetically active radiation = PAR, Li-Cor LI-189 with LI-192SA quantum sensor), temperature and oxygen (YSI 50B) profile was taken at 0.5 m depth increments from surface to bottom.

Alkalinity was determined by titration in the field. For chlorophyll, 500 ml of water were filtered through a 1.2 mm glass fiber filter, the filter was placed on dry ice and returned to the laboratory and frozen until analysis. Chlorophyll was extracted and measured spectrophotometrically (APHA 1989). Sediment cores were stored on ice and returned to the laboratory. Within 48 hr the top 15 cm of sediment was homogenized. A 5 ml sediment subsample was dried at 105 ˚C for 24-48 hrs and then weighed to obtain bulk density (g dry mass ml^{-1}). The dried sediment was then ashed at 550 ˚C for 4 hrs to obtain percent organic matter ([AFDM dry $mass^{-1}$] X 100). Pore water was extracted from the remaining sediment by centrifugation, acidified to < pH 2 and stored in the refrigerator. In 2001 we further extracted the spun sediment with 2M KCl (shaken for 1 hr) to determine exchangable nitrogen. The extract was filtered and acidified. Within seven days, the NH_3 concentration was determined for both pore water and KCl extracted fractions by selective electrode (APHA, 1989). These results should allow us to evaluate McComas’s (1999) hypothesis that nuisance levels of milfoil should only appear in sediments with high total nitrogen (e.g., > 3 mgN/L) whereas native plants should dominate in lower nitrogen sediments.

Biomass samples were rinsed of invertebrates and invertebrates were picked (endophytic and external on milfoil and from the wash water) from all samples; weevils and Lepidoptera were enumerated. Milfoil stems were counted and the average maximum stem length determined. Plants were separated, identified to species, spun for 15 sec in a salad spinner and wet mass was recorded. These samples were dried (105 ˚C for 48h) and weighed or were frozen for later dry mass determination.

Because the relatively infrequent sampling of these sites (2 or 3 times per summer) does not provide very good resolution of weevil population dynamics, we initiated a biweekly weevil survey in Lake Auburn 1998 and in 1999 added Cenaiko and Smiths Bay to our weevil surveys. In 2000 we added Otter to our survey sites and we conducted bi-weekly surveys at Auburn, Cenaiko, Otter and Smith’s Bay in 2000 and 2001. For each survey, 5-8 stems (top 50 cm) of milfoil were collected at each of 15-18 stations every other week (at Cenaiko we often were unable to find milfoil at some stations). At sites with lower densities of weevils we have been collecting 7 or 8 stems to increase our power to detect weevils. Weevils and lepidoptera were removed from the samples, which were scanned at 8X magnification, and enumerated by life stage. Results were expressed as numbers per basal stem. Single weevil surveys were also conducted in Christmas Lake (Hennepin County) and Snail Lake (Ramsey County) during 2001.

Weevils collected from the surveys in 1999 were examined for pathogens (Oien and Ragsdale 1993). Samples were put in PBS with azide and squashed. A 10 microliter sample of each squashed tissue was then placed on a slide with a coverslip and examined under a compound microscope in phase contrast. Infection was defined as protozoan, microsoridia, or saprophytic fungi present in individuals of each stage. Those results are presented in Newman et al. (2001b).

Survey Sites:

We conducted broader scale (whole lake or bay) surveys in August at 5 sites: Lake Calhoun Hennepin Co.; T28-29N; R24W; S4,5,32,33), Lake Harriet (Hennepin Co.; T28N; R24W; S8,9,16,17), Lake of the Isles (Hennepin Co.; T29N; R24W; S32,33) and Shady Island (Hennepin Co.; T117N; R23W; S26) and Grays Bay (Hennepin Co.; T117N; R22W; S8) in Lake Minnetonka. At each lake, plant community structure was determined with plant hook surveys along 12-15 transects, water quality was recorded and a set of biomass samples was collected.

Localized sites in each of these lakes were sampled quantitatively for milfoil, invertebrates and site characteristics. At two of these sites (Gray’s Bay and Shady Island), 3 transects were run perpendicular to shore and 3 stations, based on depth (e.g., 2, 3 and 4 m), were sampled along each transect in August. At Calhoun, Lake of the Isles and Harriet, 5 transects with 5 stations on each transect were sampled in June and August. At each station $0.1m^2$ quadrat samples were taken for plants and invertebrates. Sediment cores were sampled at the intermediate depth station along each transect. Open-water water quality samples were taken and processed in the same manner as the permanent transect sites. Samples were processed as above for plant mass by species, weevil abundance, and sediment characteristics. The data have not yet been enumerated and summarized for these samples.

At these waterbodies, we also conducted whole lake or bay surveys. The extent of surfaced (matted) or visible milfoil was mapped by navigating along the edge of the matted milfoil (contiguous milfoil that reaches the surface and blocks ability to see beneath the surface) around the lake or bay while continuously recording our position with a GPS unit (Trimble Pathfinder Basic Plus). If very little milfoil was matted, this was noted and the extent of visible (seen beneath the surface) milfoil was mapped. At most lakes we mapped visible milfoil because surface matting was not extensive around the entire lake. The extent of matted or visible milfoil coverage (and thus area of nuisance level) was determined by subtracting the area encompassed by the differentially corrected GPS coordinates (calculated by Pfinder program) from the lake and littoral (DNR 15 ft contour) surface areas. These results have not yet been mapped.

To quantitatively determine the extent of milfoil coverage, a set of 10-15 transects, perpendicular to shore, was located around the lake or bay in a stratified random manner (i.e., 1 transect located within each 1/10 of the lake shoreline circumference). Along each transect, observations were made from shore (0.5 m depth) to the plant limit at 5 to 6 stations, at 7.5, 15, 30, 60, or 90m intervals to the depth of the plant limit. At steeper transects the shorter intervals were used, at long and gently sloping transects, the longer intervals were used. Transects were laid with a measuring rope and marked with jugs attached to bricks; the shoreward and offshore positions were recorded with a GPS unit. At each observation point, visible milfoil (% coverage) and other plant occurrence was recorded, plant height determined and plant disk (depth at which a Secchi disk disappears; Crowell et al. 1994) was measured within a $1m^2$ area around the marker jug. Depth was recorded by dropping a plant hook vertically; plant species found on the plant hook or the jug rope and brick were also recorded and milfoil was examined for weevils and given a weevil damage rating (0-5). These data provide an estimate of milfoil and other plant coverage and frequency of occurrence around the lake as well as a relative estimate of weevil damage or occurrence.

Semi-quantitative estimates of plant density and weevil abundance were determined along a stratified subset of 5 of the transects with modification of a grapple hook method of Jessen and Lound (1962; see Newman et al. 1994 for discussion of this approach). At each sampling point 3 or 4 grapple throws were collected and rated for plant occurrence (Jessen and Lound 1962); these data provide species occurrence and relative density estimates for each species. The milfoil collected on each throw was scanned for the presence of weevils and visually assigned a damage rating (0-5). Thus for these 5 transects, we have both visual estimates of plant occurrence and density as well as the semiquantitative plant hook estimates. The whole-lake survey data have have not yet been tabulated or analyzed for this report.

Weevil Introduction/Manipulation:

Our aim was to determine the effects of artificial introduction of weevils, *Euhrychiopsis lecontei*, on the density and condition of Eurasian watermilfoil and other macrophytes during a single growing season by introductions of weevils at replicated sites in fish exclosures and open areas. This should allow us to determine if fish predation may be limiting the success of prior introductions to open areas. To exclude fish, 3m X 3m cages were constructed with PVC pipe and fitted with 1/2" bar nylon mesh netting. The netting was attached to 1m high cross supports and was connected to cylinder floats that allowed the netting to extend to the surface from 1m to 2.25m maximum depth; the tops and bottoms of the cages were open. Ten cages were fitted with mesh on all four sides (complete enclosures) and 10 cages were fitted with two mesh panels that each covered 1.5 sides (i.e., a total of 3m or 1/4 of the cage was open); the open cages served as controls by permitting fish entry.

In July 1999 20 sites were located in milfoil beds in the NE bay of Cedar Lake in water ≤ 2.2m deep and marked with floats. The cages were placed over each float such that the float was in the center of each cage; the frames dropped straight to the bottom and the cylinder floats keep the mesh taut to the surface). Cage bottoms were pushed into the sediment and weighted with bricks. Two plant biomass samples ($0.1m^2$ quadrat samples) were collected from each cage prior to stocking. Cages were then fished to remove fish trapped within the cages. Cages (open or closed) and treatment (stocked or not stocked with weevils) were assigned to the sites in a stratified random block design. One hundred and fifty adult weevils (adults and the apical tips they were collected from, which contained some larvae and eggs), collected from Cenaiko Lake, were stocked into each cage designated to receive weevils (5 closed and 5 open cages). Care was taken to ensure that adults moved onto the live milfoil and the meristems were attached to milfoil plants to ensure that associated larvae and eggs also had access to the live plants. In August, the cages were resampled for biomass and weevils. In 1999 the cages were sampled for plants and weevils (2 samples per cage) in June and were stocked with 150 weevils in July; biomass was sampled again in late August. The samples within each cage (for pre and post stocking samples) were averaged and statistical analyses were performed treating each cage as a true replicate. The experiment was repeated in summer 2000. More effort was placed at removing fish and the weevils were collected from Smith's Bay of Lake Minnetonka.

At approximately biweekly intervals, cages were examined and counts of visible weevils (eggs, larvae, pupae and adults) were made by examining 100 to 150 stems during a 15 min period. Larval occurrence was estimated based on recent stem damage. Any fish observed in the closed cages were enumerated and angling and minnow traps were used to remove these fish. In 2000 we regularly removed any fish that invaded the cages.

We conducted the experiment a final time in 2001, and were able to start much earlier than previous years. Cages were sampled for plant biomass (duplicate $0.1 m^2$ quadrat samples) in late May and stocked with 175 adult weevils (larvae and eggs associated were also stocked but not enumerated) between 5 June and 15 July (2 stockings). As above, biweekly surveys were conducted, fish were regularly removed, and biomass was again sampled in early September.

In addition to the manipulation in Cedar, we also conducted fish manipulations in Cenaiko Lake and in Otter Lake. For these experiments we added sunfish (ca., 10-15 cm) to enclosed (2mX2m) cages. In Cenaiko Lake unstocked closed and open cages were used as controls; in Otter Lake closed controls were used. There were 4 reps of each treatment at each lake.

Effects of plant community:

We established a new set of plant manipulation plots in Otter Lake and Lake Auburn in 2001 At each lake we established 20 plots marked by 2mx2m pvc quadrats. The plots were sampled for plant biomass prior to manipulation. In five plots no plants were removed, in 5 plots all plants were removed and in the other plots either all native plants or all Eurasian watermilfoil was removed. Approximately every three weeks visual surveys (means of 16 0.5x0.5 cells) of plant coverage were conducted and in September, two biomass samples were taken from each plot. We also collected sediment cores from each plot in Otter Lake. These data have not yet been entered into the computer or analyzed.

Influence of milfoil genotype and rearing sediment on weevil performance:

The results of this experiment were presented in our June 2000 report and are not repeated here.

Fecundity of weevils on northern and Eurasian watermilfoil:

During summer 2001 we assessed the fecundity (mean eggs laid per day) of weevils on the native northern watermilfoil and the exotic Eurasian watermilfoil. Female weevils collected from either northern or Eurasian watermilfoil were placed in containers with either Eurasian or northern watermilfoil and new eggs were counted daily.

Weevil development with temperature and initial modelling:

Previous research determined the number of degree days required for milfoil weevil development (Mazzei et al. 1999). Temperature monitoring in several lakes has since been used to assess potential for weevil population development and for additional modelling.

Degree days above 10 ˚C (DD) were determined for two lakes (Auburn and Smith's Bay) that were monitored with temperature data loggers (Optic StowAway, Onset Computer, Pocasset, MA) from April or May through October 1996, 1998 and 1999. Temperatures were recorded every 0.5 hr at 0.75m depth and the surface. These results were used to estimate number of generations and potential population growth at the field sites. Data logger failure and loss resulted in no data prior to June (Auburn) or July (Smith's Bay) in 1997 and no surface temperature at Auburn in 1999. .

A stage structured model of weevil development with temperature was developed by grad student Darren Ward. The model is a stage structured model with plausible values for egg-adult survival (Newman et al., 1997; Mazzei et al., 1999), development time (Mazzei et al., 1999), and daily fecundity (Sheldon and O’Bryan 1996). Adult life span and the length of the pre-reproductive adult stage were estimated by finding the strongest correlations with observed relative population stage structure in field populations; it was set at values that correlated fairly well with field observations (from weevil surveys) for relative stage composition in Smith's Bay in 1999. The parameter estimates that provided the strongest correlations were: an average adult life expectancy of 125 DD, length of the pre-reproductive adult stage of 50 DD, and 0.9 female eggs/female/25 DD. At typical summer temperatures there are about 15 DD per day. Additional modifications were made to the model in Fall 2001 and the results of those modifications will be presented in our June report.

Results and Discussion

Semi-permanent transect sites:

Milfoil biomass in Cedar Lake remained high during 2001, similar to 1997-2000 (Table 1). Milfoil biomass at Lake Auburn continued to increase from the low densities of 1998-1999 (Fig. 1) to around 1600 g wet/m^2 during 2001. Nevertheless, milfoil remained suppressed well below densities found from 1994-1997.

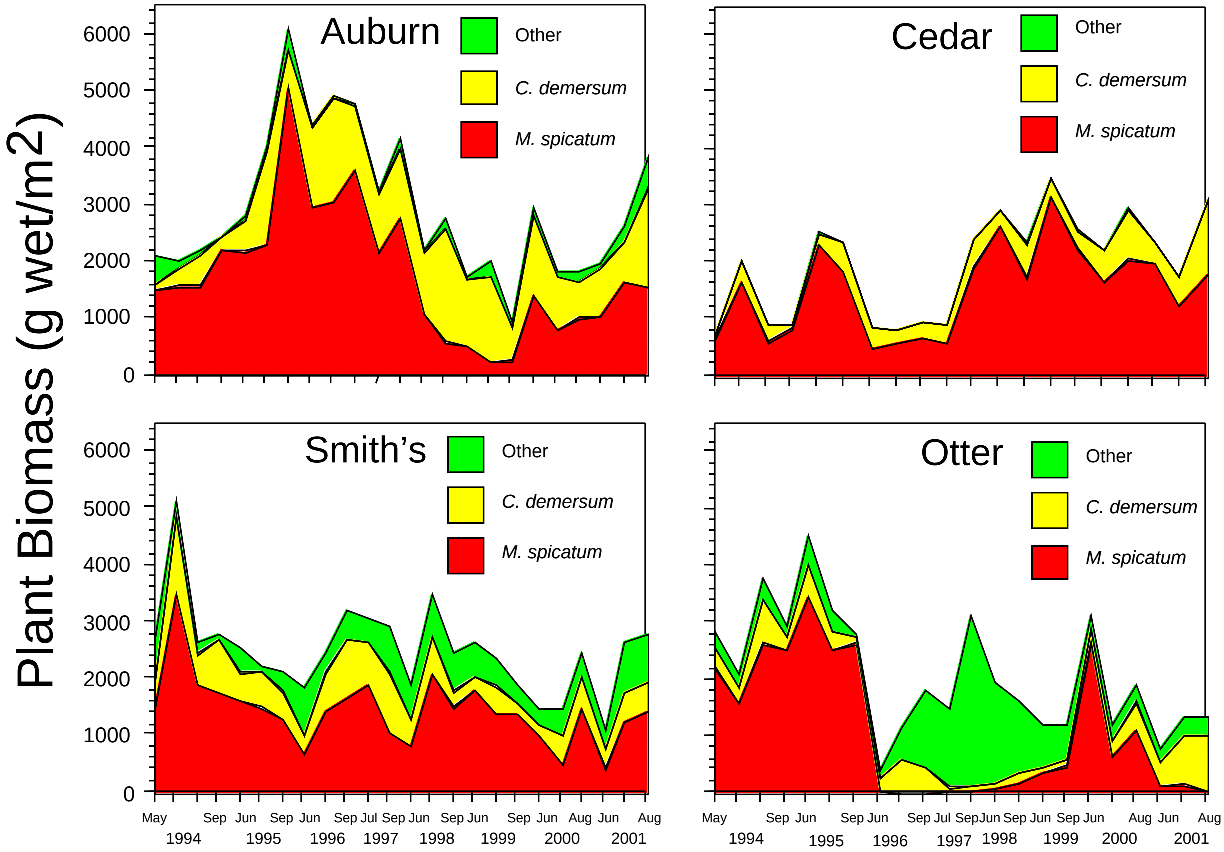

Fig. 1. Total plant biomass (Eurasian watermilfoil, coontail and other non-milfoil biomass; g wet/m^2) at the four permanent transect sites from May 1994 - August 2001.

Milfoil in Smith’s Bay started at a low density in June (400 g/m^2 wet) but as in 2000, increased to a more moderate density of 1440 g wet/m^2 in August, similar to previous years (Fig. 1). The higher milfoil density was mainly due to a high densities at the deepest three stations (>1400 g wet/m^2); density at the two shallowest stations remained low even in August. Milfoil declined dramatically at Otter Lake from 116 g wet/m^2 in June to 24 g/m^2 in August. This was a continuation of the decline that started after June 2000 when milfoil biomass was 2650 g/m^2. This two-year decline is clearly associated with weevils (see below). Changes in milfoil biomass at our sites (Fig. 1) are not due to regional changes; there was little concordance among the sites.

Non-milfoil biomass was higher at all lakes in August 2001 than in previous years (Table 2). The contribution of the non-milfoil plant community remained moderate at all sites except Cedar Lake; Eurasian watermilfoil contributed ≤ 56% of the biomass at Auburn and ≤45% at Smith’s Bay (Table 3). With the decline of milfoil at Otter Lake, it’s contribution dropped from 20% of plant biomass in June to 5% in August. Eurasian watermilfoil biomass remained high at Cedar Lake and contributed ≥ 60% of the plant biomass there and coontail composed over 90% of the non-milfoil biomass. It should be noted that as in 2000, at the shallowest station at Smith’s Bay, northern watermilfoil dominated Eurasian watermilfoil. The total number of species in each lake decreased slightly in Auburn (5 in July and August) and Cedar (2-3 species) and remained relatively high at Otter and Smith’s Bay (11-15 species) (Table 3). Similar trends were seen for numbers of species per sample with 3 or more per sample at Otter and Smith’s Bay but < 2 per sample at Cedar Lake (Table 2).

Sediment and water quality data have not yet been entered into the computer or summarized. The KCl extraction appears to have worked and is providing reasonable numbers. Secchi depths were similar to, but slightly lower than 2000.

Weevil densities have not been enumerated from the biomass samples. Weevil survey results are presented in a later section.

Table 1. Biomass ± 1SE (g wet/m^2) of Eurasian watermilfoil at the four sampling sites in 1994-2001. n = number of samples. Dry biomass (g/m^2 ± 1SE) is presented for 1995-2001.

Sampling Date	Auburn	n	Cedar	n	Otter	n	Smith's Bay	n
5/19-6/3/94	1474 ± 326	10	610 ± 289	18	2208 ± 332	21	1470 ± 320	14
7/1-7/11/94	1570 ± 297	16	1642 ± 523	18	1589 ± 231	27	3478 ± 399	16
8/12-8/19/94	1581 ± 224	15	601 ± 207	15	2626 ± 472	14	1886 ± 328	16
9/14-9/21/94	2205 ± 350	19	824 ± 188	24	2510 ± 557	9	1767 ± 386	14
6/07-6/27/95	1999 ± 324	30	2307 ± 631	23	3444 ± 336	27	1618 ± 289	25
dry	280 ± 43		245 ± 67		312 ± 33		158 ± 28	
7/31-8/15/95	2277 ± 417	19	1821 ± 797	10	2526 ± 385	15	1481 ± 245	25
dry	267 ± 46		172 ± 79		171 ± 29		149 ± 28	
9/18-9/29/95	5044 ± 752	17	479 ± 173	17	2629 ± 323	18	1281 ± 178	25
dry	551 ± 94		37 ± 13		194 ± 23		113 ± 15	
6/12-6/24/96	2959 ± 402	30	568 ± 200	30	21 ± 8	27	665 ± 144	25
dry	306 ± 40		59 ± 24		2 ± 1		46 ± 10	
7/30-8/9/96	3035 ± 619	27	665 ± 219	30	1 ± 1	27	1415 ± 256	25
dry	390 ± 82		62 ± 20		0 ± 0		176 ± 36	
9/12-9/19/96	3622 ± 469	30	574 ± 174	30	0 ± 0	27	1656 ± 393	25
dry	361 ± 49		50 ± 14		0 ± 0		156 ± 40	
6/27-7/17/97	2134 ± 321	30	1906 ± 341	28	24 ± 22	26	1880 ± 327	25
dry	294 ± 46		210 ± 40		3 ± 3		296 ± 55	
9/8-9/18/97	2786 ± 400	30	2646 ± 502	29	4 ± 4	27	1055 ± 170	25
dry	321 ± 49		271 ± 55		0 ± 0		100 ± 18	
6/8-6/18/98	1080 ± 168	30	1690 ± 360	31	79 ± 52	27	815 ± 164	25
dry	130 ± 18	30	213 ± 52	31	7 ± 4	27	105 ± 21	25
7/27-8/3/98	581 ± 133	30					2103 ± 475	25
dry	67 ± 16	30					286 ± 65	25
9/8-9/16/98	530 ± 76	30	3146 ± 514	29	181 ± 44	27	1487 ± 338	25
dry	48 ± 7	30	367 ± 63	29	15 ± 4	27	172 ± 40	25
6/15-6/22/99	202 ± 50	30	2238 ± 393	28	355 ± 113	27	1806 ± 289	25
dry	24 ± 7	30	252 ± 50	28	25 ± 8	27	155 ± 32	25
7/29-8/3/99					483 ± 101	27	1358 ± 289	25
dry					36 ± 8	27	189 ± 44	25
8/23-8/25/99	253 ± 83	30	1632 ± 237	30			1362 ± 320	25
dry	25 ± 9	30	105 ± 15	30			106 ± 26	25
6/6-6/23/00	1392 ± 263	30	2045 ± 321	29	2652 ± 340	27	981 ± 318	25
dry	208 ± 39	30	219 ± 38	29	331 ± 42	27	109 ± 37	25
7/11-7/19/00	783 ± 200	30			607 ± 82	27	501 ± 150	25
dry	115 ± 32	30			45 ± 7	27	77 ± 22	25
8/23-8/29/00	1007 ± 152	30	1988 ± 305	29	1098 ± 136	27	1474 ± 346	25
dry	91 ± 14	30	175 ± 28	29	90 ± 14	27	162 ± 40	25
6/18-6/25/01	1022 ± 199	30	1213 ± 267	29	116 ± 34	27	408 ± 107	25
7/17/-7/30/01	1641 ± 279	30			138 ± 58	25	1211 ± 290	25
8/23-8/30/01	1549 ± 289	30	1798 ± 398	25	24 ± 11	27	1438 ± 381	25

Table 2. Mean number of species per sample (Spp/S) ± 1SE and non-milfoil biomass (B; g wet /m^2) at the 4 sampling sites in 1994-2001. Number of samples is given in Table 1.

Sampling Date	Auburn		Cedar		Otter		Smith's Bay	
	Spp/S	B	Spp/S	B	Spp/S	B	Spp/S	B
5/19-6/3/94	3.80±0.47	670	1.33±0.28	75	4.76±0.19	600	3.29±0.22	1231
7/1-7/11/94	3.63±0.29	444	1.83±0.28	370	4.37±0.29	520	3.75±0.35	1604
8/12-8/19/94	3.00±0.28	647	1.53±0.26	282	5.57±0.39	1126	3.13±0.42	765
9/14-9/21/94	3.11±0.37	268	1.46±0.19	54	4.89±0.61	431	3.50±0.39	975
6/07-6/27/95	2.23±0.22	822	1.43±0.20	214	4.70±0.21	1065	3.64±0.30	877
7/31-8/15/95	3.37±0.26	1789	1.70±0.15	516	4.27±0.30	642	2.68±0.24	703
9/18-9/29/95	2.18±0.18	1058	1.41±0.17	337	2.44±0.34	135	2.80±0.20	856
6/12-6/24/96	2.93±0.24	1450	2.10±0.22	248	5.19±0.25	434	4.32±0.36	1159
7/30-8/9/96	2.78±0.31	1186	1.43±0.18	270	4.19±0.20	1171	3.88±0.41	1017
9/12-9/19/96	2.50±0.20	1166	1.57±0.16	307	3.93±0.28	1798	3.88±0.32	1531
6/27-7/17/97	2.97±0.14	1435	1.82±0.14	460	4.31±0.29	1516	4.16±0.39	1162
9/8-9/18/97	2.63±0.17	1500	1.59±0.09	235	4.81±0.26	3180	3.64±0.27	1863
6/8-6/18/98	2.43±0.18	1158	1.74±0.81	637	5.37±0.24	1835	5.32±0.43	1038
7/27-8/3/98	2.97±0.23	2197					5.00±0.44	1385
9/8-9/16/98	2.40±0.12	1258	1.62±0.12	296	4.74±0.39	1423	4.32±0.38	969
6/15-6/22/99	3.07±0.16	1806	1.86±0.13	326	4.52±0.31	825	4.60±0.37	810
7/29-8/3/99					5.33±0.30	720	3.72± 0.31	973
8/23-8/25/99	1.93±0.13	679	1.37±0.09	570			2.92± 0.33	534
6/6-6/23/00	3.17±0.19	1597	1.62±0.10	919	4.33±0.28	471	3.44±0.39	458
7/11-7/19/00	2.70±0.20	1090			4.59±0.24	595	4.48±0.45	949
8/23-8/29/00	2.30±0.12	852	1.62±0.10	354	4.33±0.21	778	4.00±0.36	979
6/18-6/25/01	2.77±0.21	971	1.52±0.11	495	4.44±0.23	628	4.00±0.35	663
7/17/-7/30/01	2.40±0.11	996			2.92±0.22	1189	3.96±0.32	1387
8/23-8/30/01	2.80±0.16	2314	1.80±0.08	1303	3.81±0.27	1293	3.60±0.28	1342

Table 3. Percentages of total plant wet biomass that was Eurasian watermilfoil (±1SE) and number of species (N) collected at each site. These are the average percentage found in the samples and are thus not equal to total mean milfoil biomass/plant biomass.

Sampling Date	Auburn	N	Cedar	N	Otter	N	Smith's Bay	N
5/19-6/3/94	65% ±10%	9	67% ±11%	4	80% ± 6%	9	64% ±10%	8
7/1-7/11/94	79% ± 6%	9	67% ± 9%	4	75% ± 5%	9	72% ± 6%	11
8/12-8/19/94	74% ± 6%	9	61% ±13%	3	75% ± 6%	11	81% ± 5%	11
9/14-9/21/94	91% ± 6%	9	87% ± 5%	4	83% ± 6%	11	71% ± 8%	9
6/07-6/27/95	72% ± 7%	7	82% ± 7%	3	79% ± 4%	9	61% ± 5%	10
7/31-8/15/95	58% ± 7%	7	58% ± 6%	2	80% ± 7%	9	63% ± 6%	11
9/18-9/29/95	81% ± 7%	5	38% ± 5%	2	95% ± 1%	6	63% ± 7%	10
6/12-6/24/96	70% ± 7%	7	57% ± 7%	5	7% ± 5%	9	33% ± 6%	10
7/30-8/9/96	56% ± 8%	7	59% ± 9%	5	0.1% ± 0.1%	10	56% ± 7%	11
9/12-9/19/96	69% ± 6%	8	73% ± 6%	4	0% ± 0%	9	49% ± 7%	10
6/27-7/17/97	53% ± 13%	10	82% ± 9%	3	1.2% ± 2.3%	12	54% ± 14%	12
9/8-9/18/97	60% ± 13%	8	88% ± 9%	2	0.2% ± 0.3%	13	40% ± 14%	11
6/8-6/18/98	42% ± 5%	11	79% ± 5%	4	4% ± 2%	15	37% ± 6%	15
7/27-8/3/98	24% ± 4%	12					49% ± 8%	16
9/8-9/16/98	34% ± 4%	7	82% ± 6%	4	20% ± 5%	13	50% ± 8%	13
6/15-6/22/99	14% ± 4%	7	82% ± 6%	3	30% ± 6%	13	61% ± 7%	12
7/29-8/3/99					40% ± 5%	14	53% ± 8%	13
8/23-8/25/99	36% ± 7%	6	85% ± 6%	2			61% ± 8%	12
6/6-6/23/00	43% ± 6%	9	75% ± 7%	5	81% ± 5%	12	49% ± 9%	13
7/11-7/19/00	37% ± 6%	9			53% ± 4%	15	40% ± 8%	15
8/23-8/29/00	55% ± 6%	6	77% ± 6%	3	63% ± 5%	9	50% ± 8%	13
6/18-6/25/01	52% ± 6%	10	77% ± 6%	2	20% ± 5%	15	35% ± 8%	14
7/17/-7/30/01	56% ± 6%	5			9% ± 4%	11	42% ± 7%	14
8/23-8/30/01	40% ± 6%	5	59% ± 8%	2	5% ± 3%	12	42% ± 8%	12

Cenaiko Lake

The suppression of milfoil biomass at Cenaiko Lake (Fig. 2) continued in 2001 (Table 5). Milfoil biomass did not exceed 61 g wet/m^2 or 5% of total plant biomass. Native plant biomass was similar to 2000 but the mean number of species remained lower than other years with only 6-7 total species being found on each sampling date. The extremely high early and mid-summer water levels (up to 2m above normal) appeared to suppress plant growth of all species except coontail. It is unclear what the implications are for 2002.

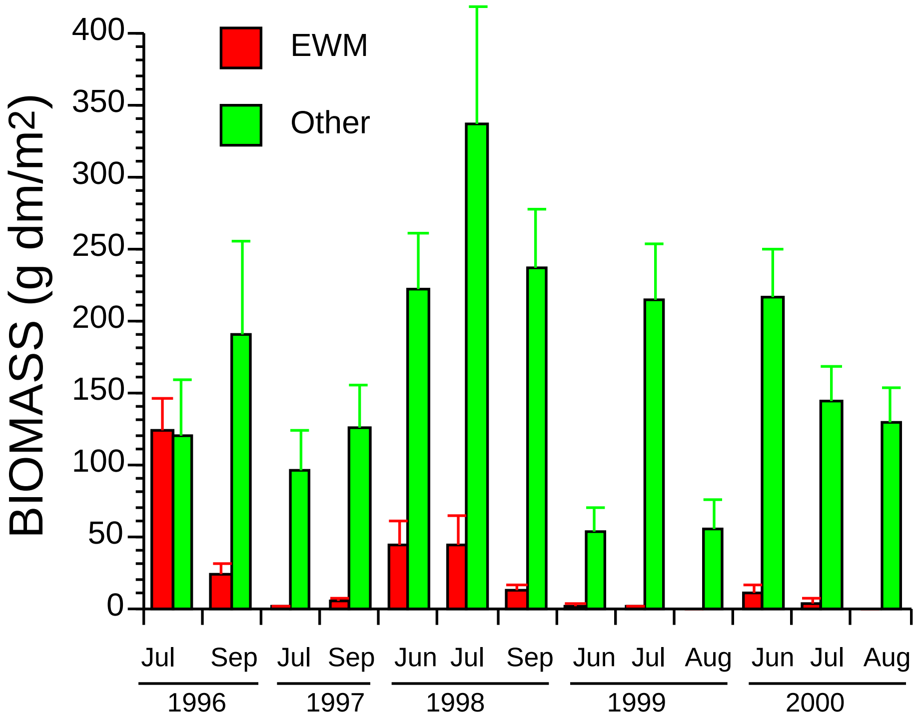

Fig. 2. Dry biomass of milfoil (EWM) and non-milfoil plants at Cenaiko Lake, 1996-2000. There was a significant decline of milfoil between July and September 1996 and July 1997 and a significant increase in native plants. Milfoil was present but not found in August 1999 samples. N > 20 samples on each date. Dry mass has not yet been tabulated for 2001.

Table 5. Biomass (g wet/m^2) of all plants (Total), Eurasian watermilfoil (MSP), the dominant plants (coontail (CRT), *Heteranthera* (HET), *Potamogeton zosteriformis* (PZS), *Chara* (CHA) and *Potamogeton amplifolius* (PAM)), non-milfoil biomass, total number of species and mean percentage of biomass that was Eurasian watermilfoil in Cenaiko Lake 1999-2001. N=22-27 samples per date.

Date	Total	MSP	CRT	PZS	HET	CHA	PAM	N Sp	nonEWM	%MSP
6/24/99	718	10	484	4	62	2	111	11	708	7.5%
1 SE	214	7	186	3	56	2	91		215	5.0%
8/2/99	2019	9	1243	0	290	0	237	10	2010	0.7%
1 SE	381	6	387	0	100	0	164		382	0.5%
8/26/99	875	0	538	2	72	0	92	5	842	0.0%
1 SE	376	0	378	1	46	0	62		362	0.0%
6/29/00	1873	76	1185	1	202	189	136	9	1798	6.2%
1 SE	324	33	327	1	107	87	91		324	4.1%
7/20/00	1354	26	890	0	214	61	128	8	1328	7.7%
1 SE	234	15	228	0	109	37	87		237	4.8%
8/30/00	1456	1	932	444	1	30	18	8	1455	0.1%
1 SE	255	1	251	191	1	26	15		255	0.1%
6/26/01	366	24	294	10	0	0	7	7	343	2.5%
1 SE	139	23	145	5	0	0	7		136	2.4%
7/30/01	1279	61	987	0	0	0	0	7	1218	5.3%
1 SE	418	34	372	0	0	0	0		411	3.8%
8/27/01	1591	1	1311	13	0	0	105	6	1590	4.0%
1 SE	346	1	351	8	0	0	75		346	4.0%

Bi-weekly weevil surveys

Densities at Lake Auburn were moderate in June and July, but weevils were not found in our samples in August and September (Table 6). Weevils were seen near our sampling sites at this time, but it is clear that late summer densities were quite low. Similarly, at Cenaiko Lake, mid-to-late summer weevil densities dropped to quite low levels. This decline may have been due to the high June and July water levels, however, we also noted more sunfish at Cenaiko in 2001. Weevil densities were low to moderate at Smith’s Bay and weevils persisted throughout the summer. Weevil densities were high at Otter Lake and persisted at high density throughout the summer (Fig 3). Total densities ranged from 0.2 to 0.8 per stem and adults were present throughout the summer suggesting low adult mortality at Otter in 2001. The persistently higher adult populations rival those seen at Cenaiko during the major decline. Weevil damage was clearly responsible for the milfoil decline at Otter during 2000-2001. *Parapoynx* was present only at Otter Lake (and Auburn in 2000) and appeared primarily in the late summer and fall when densities approached 0.2/stem. None were found in July and August. *Acentria* was also absent from Lake Auburn and Smith’s Bay in 2001 and was generally most abundant early and late in the season but not in mid summer (probably in egg and undetected early instar stages during mid-summer). Densities at Otter never exceeded 0.05/stem but high densities (> 0.1/stem) were found at Cenaiko in late May and mid September. Similar patterns were seen in 2000. The low densities of caterpillars at Otter in May through August (<0.05/stem) suggests they were not instrumental in the milfoil decline. Although the number of observations is low, it appears that the caterpillars are not common in lakes or years with low weevil densities, further suggesting that sunfish may be limiting populations of potential control agents.

Table 6. Density of weevil life stages (per stem), total weevils per stem and density of the caterpillars *Acentria* (Acent) and *Parapoynx* (Parap) from the bi-weekly weevil surveys.

Lake	date	eggs	larvae	pupae	adults	total	Acent	Parap
Cenaiko								
	5/16/00	0.1952	0.0229	0.0000	0.0000	0.2181	0.2762	0.0000
	5/30/00	0.0397	0.0159	0.0069	0.0000	0.0625	0.1905	0.0000
	6/13/00	0.1190	0.0883	0.0488	0.0756	0.3318	0.1584	0.0000
	6/29/00	0.2476	0.0556	0.0397	0.0238	0.3667	0.0508	0.0000
	7/11/00	0.3214	0.0347	0.0208	0.1141	0.4911	0.1141	0.0000
	7/24/00	0.7393	0.0208	0.0069	0.1181	0.8851	0.0417	0.0000
	8/10/00	0.5417	0.0917	0.0000	0.0167	0.5667	0.0083	0.0000
	8/24/00	0.0822	0.0519	0.0065	0.0652	0.2058	0.0465	0.0000
	9/7/00	0.0278	0.0324	0.0379	0.0866	0.1847	0.1554	0.0000
	9/20/00	0.0000	0.0694	0.0000	0.0478	0.1173	0.0556	0.0000
	10/3/00	0.0000	0.0368	0.0000	0.0083	0.0451	0.0000	0.0000
	5/21/01	0.0833	0.0000	0.0000	0.0000	0.0833	0.8068	0.0000
	6/6/01	0.6893	0.0000	0.0000	0.1857	0.8750	0.1250	0.0000
	6/18/01	0.0500	0.0000	0.0000	0.0000	0.0500	0.0000	0.0000
	7/3/01	0.0343	0.0000	0.0000	0.0000	0.0343	0.0100	0.0000
	7/19/01	0.0000	0.1268	0.0000	0.0000	0.1268	0.0250	0.0000
	7/30/01	0.0000	0.0000	0.0000	0.0125	0.0125	0.0250	0.0000
	8/15/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	8/27/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	9/5/01	0.0104	0.0000	0.0000	0.0000	0.0104	0.0625	0.0000
	9/18/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.1472	0.0000
Auburn								
	5/19/00	0.0267	0.0267	0.0000	0.0000	0.0533	0.0000	0.0000
	6/1/00	0.0000	0.0218	0.0000	0.0079	0.0298	0.0000	0.0000
	6/15/00	0.0139	0.0278	0.0000	0.0000	0.0417	0.0000	0.0000
	6/27/00	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	7/10/00	0.0000	0.0000	0.0069	0.0347	0.0417	0.0000	0.0000
	7/25/00	0.1528	0.0000	0.0069	0.0556	0.2153	0.0000	0.0000
	8/9/00	0.0368	0.0515	0.0515	0.0294	0.1691	0.0000	0.0000
	8/28/00	0.0000	0.0000	0.0000	0.0074	0.0074	0.0000	0.0000
	9/12/00	0.0000	0.0208	0.0062	0.0123	0.0394	0.0000	0.0149
	9/28/00	0.0000	0.0000	0.0000	0.0139	0.0139	0.0000	0.0000
	5/10/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	5/24/01	0.2562	0.0139	0.0000	0.0309	0.3009	0.0000	0.0000
	5/30/01	0.1847	0.0000	0.0000	0.0000	0.1847	0.0000	0.0000
	6/13/01	0.0069	0.0139	0.0139	0.0308	0.0655	0.0000	0.0000
	6/28/01	0.0278	0.0139	0.0000	0.0000	0.0417	0.0000	0.0000
	7/9/01	0.0278	0.1389	0.0139	0.0139	0.1944	0.0000	0.0000
	7/23/01	0.0000	0.0123	0.0270	0.0139	0.0532	0.0000	0.0000
	8/8/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	8/20/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	9/11/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	9/27/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Table 6. Continued.

Lake	date	eggs	larvae	pupae	adults	total	Acent	Parap
Otter								
	6/5/00	0.1940	0.1321	0.0500	0.0821	0.4583	0.0250	0.0000
	6/22/00	0.1395	0.2027	0.0580	0.0804	0.4806	0.0268	0.0089
	7/5/00	0.0000	0.0403	0.0079	0.0079	0.0575	0.0000	0.0000
	7/18/00	0.0000	0.0074	0.0074	0.0000	0.0147	0.0000	0.0000
	8/2/00	0.0218	0.0000	0.0069	0.0218	0.0506	0.0069	0.0000
	8/16/00	0.0074	0.0147	0.0000	0.0000	0.0221	0.0000	0.0000
	8/29/00	0.0000	0.0441	0.0074	0.0515	0.1029	0.0000	0.0000
	9/13/00	0.0000	0.0394	0.0278	0.0231	0.0903	0.0000	0.0000
	9/26/00	0.0000	0.0069	0.0764	0.1042	0.1875	0.0000	0.0000
	5/21/01	0.3268	0.0000	0.0000	0.1250	0.4518	0.0000	0.0000
	6/4/01	0.2225	0.0000	0.0000	0.1789	0.4015	0.0417	0.0147
	6/21/01	0.5345	0.0407	0.0000	0.0663	0.6415	0.0074	0.0000
	7/5/01	0.4117	0.1354	0.0851	0.1634	0.7955	0.0202	0.0000
	7/16/01	0.1119	0.0000	0.0000	0.2608	0.3727	0.0000	0.0000
	8/1/01	0.1027	0.0469	0.0000	0.1007	0.2502	0.0000	0.0000
	8/13/01	0.1507	0.0306	0.0000	0.0512	0.2324	0.0000	0.0000
	8/28/01	0.0515	0.1922	0.0000	0.0221	0.2658	0.0074	0.0000
	9/5/01	0.1128	0.1553	0.0131	0.1063	0.3875	0.0378	0.0069
	9/17/01	0.0278	0.2750	0.0486	0.2935	0.6449	0.0069	0.1918
	10/2/01	0.0193	0.0432	0.0288	0.1211	0.2124	0.0455	0.0481
Smith's								
	5/25/00	0.2867	0.0267	0.0000	0.0000	0.3133	0.0000	0.0000
	6/8/00	0.2095	0.1429	0.0095	0.0000	0.3619	0.0000	0.0000
	6/21/00	0.2519	0.0824	0.0429	0.0167	0.3938	0.0583	0.0000
	7/3/00	0.0810	0.0369	0.0000	0.0000	0.1179	0.0000	0.0000
	7/19/00	0.0167	0.0250	0.0111	0.0417	0.0944	0.0000	0.0000
	8/4/00	0.2604	0.0702	0.1339	0.0274	0.4919	0.0000	0.0000
	8/15/00	0.0472	0.0750	0.0074	0.0389	0.1685	0.0000	0.0000
	8/23/00	0.0919	0.1100	0.0726	0.0871	0.3361	0.0085	0.0000
	9/6/00	0.0250	0.0880	0.0000	0.0591	0.1721	0.0000	0.0000
	9/19/00	0.0000	0.0167	0.0000	0.0167	0.0333	0.0000	0.0000
	5/15/01	0.0000	0.0000	0.0000	0.0083	0.0083	0.0000	0.0000
	5/31/01	0.0241	0.0000	0.0000	0.0333	0.0574	0.0000	0.0000
	6/11/01	0.2287	0.0083	0.0000	0.0095	0.2466	0.0000	0.0000
	6/25/01	0.0222	0.0000	0.0000	0.0274	0.0496	0.0000	0.0000
	7/10/01	0.0000	0.0482	0.0240	0.0000	0.0722	0.0000	0.0000
	7/23/01	0.0000	0.0639	0.0307	0.0000	0.0946	0.0000	0.0000
	8/8/01	0.0250	0.1480	0.0194	0.0083	0.2008	0.0000	0.0000
	8/24/01	0.0148	0.0917	0.0083	0.0000	0.1148	0.0000	0.0000
	9/13/01	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

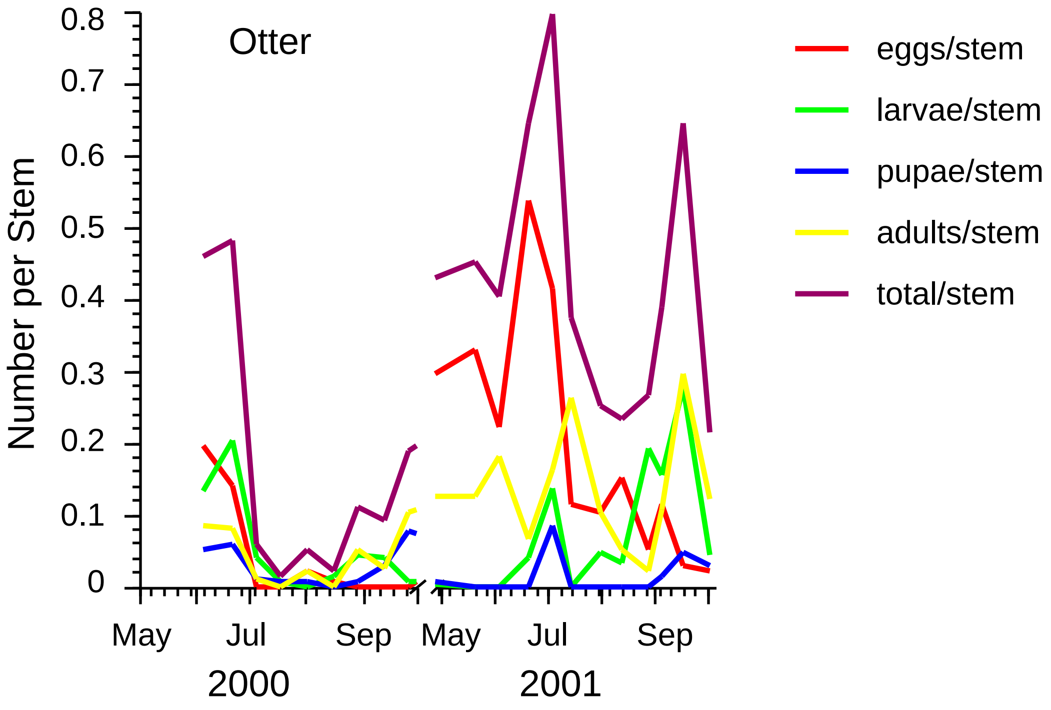

Fig. 3. Biweekly density (number per milfoil stem) of weevil life stages at Otter Lake in 2000-2001.

Survey sites:

These data have not be tabulated or analyzed.

Fecundity of weevils on northern and Eurasian watermilfoil:

These data are not fully analyzed, but consistent with Sheldon and Jones (2001), we found many more eggs were laid on Eurasian watermilfoil (3.5-4.5 per day) than northern watermilfoil (1.5-2.5 per day). The lower numbers are for weevils collected from northern watermilfoil, which suggests that in addition to plant preference (these were no choice experiments) prior feeding will affect egg production and subsequent fecundity. It thus appears that lifetime fecundity may be twice as high on Eurasian compared to northern watermilfoil (see also, Sheldon and Jones 2001).

Weevil Introduction/Manipulation:

The results of the 2001 experiments are not completely analyzed, but are consistent with the 2000 results (presented in Newman et al. 2001a), where we found a significant cage effect ($p< 0.05$) for larvae (more larvae in closed than in open cages) and a significant ($p <0.05$) cage and stocking effect for adults (more adults in closed cages and in stocked cages).

In 2001, there were significant fish (cage) effects on larvae and total weevils, as well as a significant stocking effect on all stages except eggs (Table 7). There was also a significant fish by time interaction for adults, indicating that weevil densities in open cages declined relative to closed cages over the course of the summer (Fig. 4). Weevil densities were highest in stocked, closed cages.

No significant effects of fish or stocking were found for milfoil biomass ($P>0.1$). Although weevil densities were higher in stocked, closed cages (Fig 4), this did not translate into any significant milfoil effect, despite the fact that we stocked in early June. More analysis should shed light into the possible reasons for this lack of an effect.

Table 7. Repeated Measures ANOVA P-values, for the Cedar 2001 cage visual survey data. Densities were log(x+1) transformed.*First sampling date omitted for pupae, no pupae were observed on that date

		Life Stage				
		Eggs	Larvae	Pupae*	Adults	Total
Factor	Fish	0.233	0.041	0.102	0.187	0.053
	Stocking	0.377	0.001	0.025	0.038	0.005
	Fish*Stocking	0.450	0.012	0.019	0.140	0.018
	Fish*Time	0.939	0.473	0.704	0.025	0.752
	Stocking*Time	0.988	0.003	0.544	0.043	0.007
	Fish*Stocking*Time	0.997	0.139	0.784	0.003	0.098

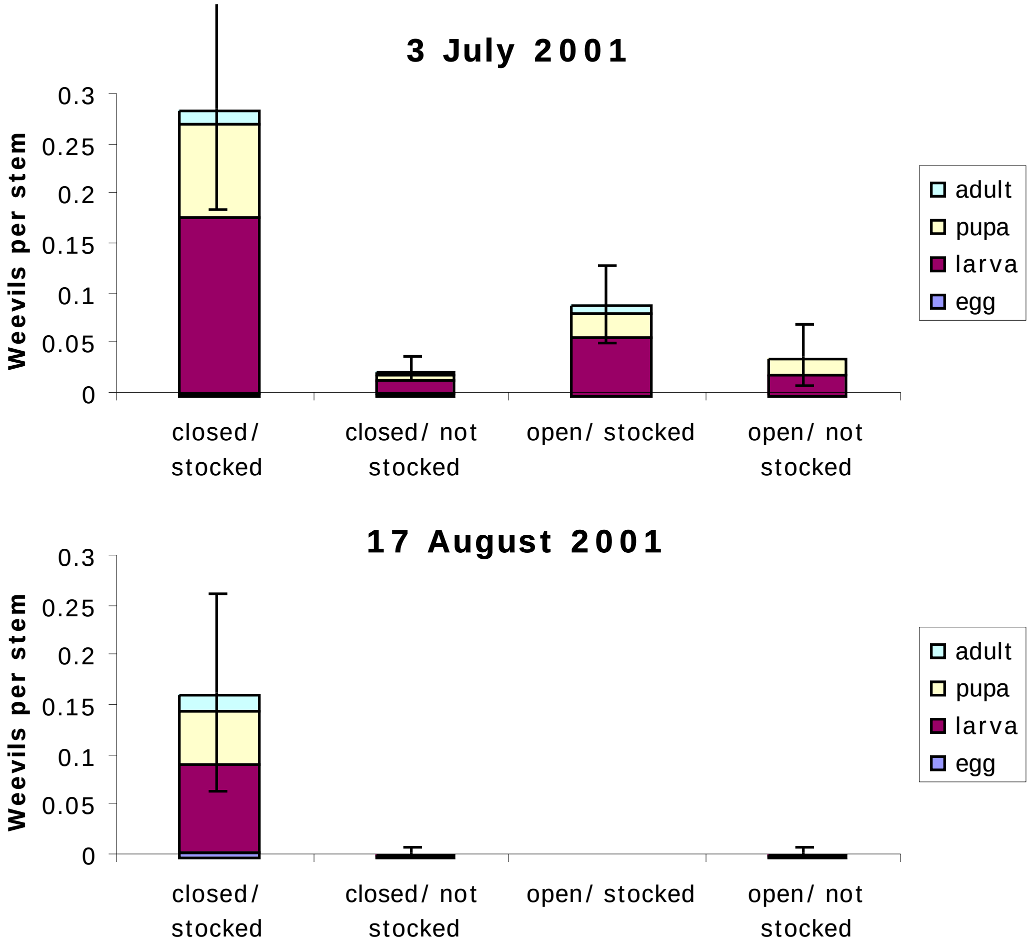

Figure 4. Examples of weevil densities in cages at Cedar Lake, 2001.

Weevil modelling:

Results of updated weevil models will be presented in June.

Summary

We have now documented two declines clearly attributable to weevil stem mining (Cenaiko and Otter). We also have evidence that weevil damage, at least in the shallower sites, at Lake Auburn and Smith's Bay have reduced milfoil abundance, but the persistence of these declines will depend on the persistence of weevil populations. The decline at Cenaiko Lake has persisted; milfoil remained at < 75g/m^2 during 2000 and 2001 and did not exceed 8% of total plant biomass. It is not certain what permits development of high weevil populations in Cenaiko Lake, however, low predation by sunfish appears to be a factor. All life stages persist throughout the summer and adult densities in September were as high as seen all summer, although densities were lower than previous years in 2001. It is unclear if this lower density was due to high water levels or an increasing sunfish population. Observations in 2002 should clarify this. The fish exclusion results in Cedar Lake further suggest that fish may be limiting weevil populations. If predation by sunfish is shown to be an important limiting factor, it may be feasible to explore fisheries enhancements to the sunfish population and size structure through enhancement of predator populations or fishing regulations. It would be particularly fortuitous if enhancing sport fishing populations would aid in the biological control of Eurasian watermilfoil.

The longer and much less dramatic suppression of Eurasian watermilfoil continued at Smith's Bay. The June milfoil biomass (400 g wet/m^2) was the lowest we have seen there since sampling began in the early 1990's. Milfoil did increase through the summer to moderate levels and weevil densities failed to increase to previous levels. At the shallower sites milfoil remains suppressed and native plants dominate. Northern watermilfoil has returned to the shallowest stations. At deeper sites, with little evidence of weevil damage, Eurasian watermilfoil remains quite dense, but well beneath the surface. A key to success in both Cenaiko and Smith's Bay appears to be the summer-long persistence or increase in weevil density, particularly adults, which in the past, has not been maintained at the other lakes. In Cedar Lake, fair water clarity and the very low weevil densities resulted in a continued high density Eurasian watermilfoil that persisted through the summer. DNR fisheries surveys have consistently indicated a high density of bluegills at Cedar Lake (60-90 per trapnet) and the other lakes in the Minneapolis Chain-of-Lakes.

Milfoil increased greatly at Otter Lake in the spring of 2000, to a biomass similar to historic highs, but weevil populations increased and the milfoil declined and remained below 90 g dry/m^2 in 2000. The decline continued in 2001 with high weevil densities. By August, milfoil was <25 g wet/m^2 and 5% of total plant biomass. This delcine indicates that weevils can suppress the plant at Otter Lake. Climatic factors may have been generally favorable to weevils in 2000-2001 because these are the highest density of weevils we have observed at Otter. A more likely explanation is low sunfish densities. DNR Fisheries surveys in Otter in 1997 indicated a low density of bluegills (2.1 per trapnet) and even lower densities in 2001 (following a suspected winter kill).

The response of Lake Auburn remains puzzling. The early season decline of milfoil in 1998 was associated with relatively low weevil densities but much apparent damage (personal observation). However, for some reason the weevil population crashed and the poor light probably prevented regrowth of milfoil and other plants. Although no weevils were found in 1999 they returned in 2000 and although they did not reach high densities the population increased and persisted through the summer. It appeared that weevil populations might recover in 2001, but densities declined below detection in August and September. Due to poor visibility it is difficult to tell if sunfish populations are high, however surveys conducted by Pothoven (1996) in Cedar and Auburn suggest similar high densities of sunfish in both lakes during 1993-1995, with sunfish increasing from 1993 to 1995. DNR Fisheries surveys reported 62 bluegill per trapnet in Auburn in 1995; this density increased to 110 per trapnet in 2000. In some ways, the recent milfoil decline is similar to that observed in 1993; weevil populations declined in 1995 and milfoil increased to record levels. It remains to be seen weevil populations will recover and if milfoil will remain suppressed, at least below the high densities of the mid 1990s. If not, we suspect milfoil will continue its increase in 2002.

We will collate additional fisheries information to determine if there is any relationship between sunfish density and weevil densities. Unfortunately, the typical 5 or more years between fisheries surveys may not capture important changes in fish populations. For example, sunfish density in Cenaiko declined from 95 per trapnet in 1992 to 5 per trapnet in 1998.

It is possible that other herbivores in addition to the milfoil weevil are affecting milfoil populations. Johnson et al. (1998, 2000) have shown milfoil declines in New York associated with high densities of *Acentria*. They suggest that in many lakes *Acentria* may be more important than the milfoil weevil and they also suggested competition between *Acentria* and *Euhrychiopsis*. *Acentria* and *Parapoynx* have been at low densities in all of our lakes with the exception of Cenaiko Lake and, in 1996-1997, Otter Lake. The high densities in Otter Lake (20-100 per m^2) were noted the summer following the decline of milfoil when milfoil densities ranged from not detectable to <25 g wet/m^2. Most caterpillars were associated with plants other than Eurasian watermilfoil. Furthermore, although caterpillar were found in 2000 and 2001, the densities were low, particularly in mid summer when the greatest declines occurred. Thus, the caterpillars may be assisting with milfoil suppression following a decline but we have little evidence that they are initiating declines. Furthermore, if fish predation is limiting weevil densities it likely would limit caterpillar densities. We do not have high caterpillar densities in our lakes that have few weevils and high sunfish densities. More analysis of these interactions is required.

Two conditions are needed for successful biological control of weeds: adequate agent densities and a negative response of the target to the control agent (Newman et al. 1998). At sites with persistent control of milfoil, the native plant community has expanded. It is also clear that at many of our sites weevil populations have not built to adequate densities, although weevil densities in 2000 appeared higher in all lakes, and these populations appear to have at least contained milfoil growth in all except Cedar during 2000. Cenaiko Lake and now Otter Lake provide clear examples of the potential for high weevil populations and subsequent effects on milfoil. Given the potential for population increase in the summer, and the lack of a strong correlation between in-lake and onshore densities, it does not appear that overwinter populations are the main limiting factor (Newman et al. 2001) at least at Lake Auburn and Smith's Bay where detectible populations have been found in early summer each year. Fish exclusion experiments suggest that fish predation could be one important factor.

It is clear that we do not yet have adequate information to reliably predict if and when insects will cause declines in milfoil populations or if the declines will persist (Creed 2000). It is also clear that milfoil suppression can be obtained given adequate densities of weevils throughout the summer, and perhaps positive plant community response. On-going focused research should shed additional light on the factors that regulate weevil populations and their effects on plant communities. Once these factors have clearly been identified, management strategies, such as piscivore enhancement or water clarity improvements can be tested to determine their feasibility for enhancing the biological control of Eurasian watermilfoil.

Conclusions

- Declines in Eurasian watermilfoil biomass persisted through 2001 at Cenaiko Lake and native plants remain abundant. Milfoil declined dramatically during 2000-2001 at Otter Lake where it now composes ≤ 5% of total plant biomass. In Smith's Bay, milfoil remained suppressed at the shallower sites with high non-milfoil biomass and high weevil densities, but remained dense at the deeper sites that show little evidence of weevil damage. Milfoil continued to increase at Lake Auburn from the very low densities of 1999, but remained at moderate levels through the summer. Milfoil density remained high at Cedar Lake and composed ≥75% of plant biomass.

- Bi-weekly weevil surveys showed that weevils had disappeared from Lake Auburn in July 1998 and were absent in 1999, but returned to the lake in 2000 and persisted through 2001.

Weevil densities at Cenaiko and Smith’s Bay were low to moderate and all stages persisted throughout summer 2000. Weevils were abundant at Otter Lake in 2000 and very abundant in 2001. Weevil damage suppressed the high density of milfoil in early summer 2000 to < 25 g wet/m^2 by August 2001.

- The fish exclusion experiment in Cedar lake provided some evidence that fish predation may limit weevil populations and that milfoil is depressed with increasing weevil densities.

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Appendix I. Abbreviations and wet mass (g/m^2) of plants collected from 1994 through 2001.

Key to plant abbreviations used in this report.

CHA	*Chara* spp. (muskgrass)
CRT	*Ceratophyllum demersum* (coontail)
ELD	*Elodea canadensis* (Canada waterweed)
HET	*Heteranthera dubia* (mud plantain) = *Zosterella dubia*
LMR	*Lemna minor* (lesser duckweed)
LTR	*Lemna trisulca* (star duckweed)
MGD	*Megalodonta beckii* (water marigold)
MSI	*Myriophyllum sibiricum* (northern watermilfoil)
MSP	*Myriophyllum spicatum* (Eurasian watermilfoil)
NAJ	*Najas* spp.
NMP	*Nymphaea* spp.
NUP	*Nuphar* spp.
PAM	*Potamogeton amplifolius* (largeleaf pondweed)
PBE	*Potamogeton berchtoldi* (Berchtolds' pondweed)
PCR	*Potamogeton crispus* (curled pondweed)
PDI	*Potamogeton diversifolius*
PEC	*Potamogeton pectinatus* (sage pondweed)
PFO	*Potamogeton foliosus* (leafy pondweed)
PGR	*Potamogeton gramineus* (variable pondweed)
PIL	*Potamogeton illinoensis* (Illinois pondweed)
PNA	*Potamogeton natans*(floating leaf pondweed)
PNO	*Potamogeton nodosus* (river pondweed)
PRI	*Potamogeton richardsonii* (claspingleaf pondweed)
PRO	*Potamogeton robbinsii* (Robins' pondweed)
PSP	*Potamogeton spirillus* (snailedseed pondweed)
PZS	*Potamogeton zosteriformis* (flatstem pondweed)
RAN	*Ranunculus* spp. (white water buttercup)
SPO	*Spirodela polyrhiza* (greater duckweed)
VAL	*Vallisneria americana* (wild celery)
UTV	*Utricularia vulgaris* (bladderwort)