

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

Second 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 now occurs in 90 lakes and six streams in Minnesota (Exotics Species Programs 1999).

Recent work on the biological control of Eurasian watermilfoil has focused on the indigenous weevil *Euhrychiopsis lecontei* (Dietz) (= *Eubrychiopsis lecontei*). This work suggests that *E. lecontei* is the most promising control agent (Creed and Sheldon 1994a, 1995, Sheldon and Creed 1995, Newman et al. 1996, Sheldon 1997, Creed 1998). 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 1996). Sheldon and O'Bryan (1996) and Newman et al. (1996, 1997a) 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 *in press*, 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 et al. 1999, Newman and Biesboer *in press*). Fish predation may be one factor limiting populations in some lakes (Sutter and Newman 1997, Newman and Biesboer *in press*).

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 disruption of milfoil nutrient stores or competition with native plants is responsible for declines of milfoil associated with herbivores. This report summarizes our methods and collection efforts in 1999 and presents results of our research through 1999.

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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.

At each sampling station, plant biomass and invertebrate samples were taken from 0.1 m² quadrats (all plant material was clipped at sediment interface and immediately placed in a sealable bag underwater). Sediment cores, water samples (for chlorophyll a) and samples of milfoil (for carbohydrate analysis) 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. Within seven days, the NH₄ concentration was determined by selective electrode (APHA, 1989). The milfoil plants collected for carbohydrate analysis were placed on ice and frozen as soon as they were returned to the laboratory.

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 frozen for later dry mass determination.

We sampled our regular transect sites in 5 lakes. 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. Twenty-four to thirty samples were collected at each lake on each date. These samples have been processed, but invertebrates have not been sorted and enumerated.

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 are now doing bi-weekly surveys at Auburn, Cenaiko, Otter and Smith's Bay. 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 were removed from the samples, which were scanned at 8X magnification, and enumerated by life stage. Results were expressed as numbers per basal stem.

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, microsporidia, or saprophytic fungi present in individuals of each stage.

Survey Sites:

We conducted broader scale (whole lake or bay) surveys in August or early September 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. We initiated but did not complete a survey at Pierson Lake due to logistical constraints. 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 collected.

Localized sites in each of these lakes were sampled quantitatively for milfoil, invertebrates and site characteristics in mid to late August. At each of these sites (except Calhoun and Harriet), 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. At Calhoun and Harriet 5 transects with 5 stations on each transect were sampled. At each station 0.1m² quadrat samples were taken for plants and invertebrates. Sediment cores and milfoil roots and shoots (for carbohydrate analyses) 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. Weevil densities have not yet been enumerated 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 Pathfinder program) from the lake and littoral (DNR 15 ft contour) surface areas.

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 1m² 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. All of the whole-lake survey data have been entered into the computer and estimates have been calculated, however, they have not 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 enclosures and open areas. This should allow us to determine if fish predation may be limiting the success of prior introductions to open areas (see Newman et al. 1997b). 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 1998 20 sites were located in milfoil beds in the NE bay of Cedar Lake in water 2.2m deep and marked with floats. Two plant biomass samples (0.1m² quadrat) were collected 3m from each float (on opposite sides). These samples were designed to be 1-2 m outside of the cage area to minimize the effects of sampling on the plants in the caged area. One week later, 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. Cages were then electrofished 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. Eighty 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 September 1998 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.

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 was used to remove these fish. We were not entirely successful at removing fish from some cages and this may have influenced the results.

Influence of milfoil genotype and rearing sediment on weevil performance:

Because previous work indicated that weevils perform better on different milfoil species (Newman et al. 1997a), other studies have shown that plant genotype and nutritional status can affect biocontrol agent performance (Newman et al. 1998), and because we have seen substantial variation in weevil densities amongst lakes, we conducted an experiment to determine the effects of milfoil genotype (lake source) and milfoil rearing sediment on weevil performance. This experiment, which was a modification of the one conducted in 1998 by Ramona Johnson (see Newman et al. 1999), was conducted by Joanna Watson.

The experiment was set up as a 2 sediment by 2 genotype factorial. Milfoil plants and lake sediment were collected from two Minnesota lakes which have contrasting weevil populations; Cedar Lake, Hennepin Co. and Otter Lake, Ramsey Co. Fifteen cm long cuttings were placed in stock tanks containing either Cedar or Otter sediment, resulting in the two plant genotypes being reared on both lake sediments. The plants were allowed to root and grow for 2-3 weeks until they reached 35 cm. One of eight female weevils, collected from Lake Auburn, was introduced to the meristem of a plant and allowed to oviposit. The egg and plant were then transplanted to a 45 cm tall clear plastic tube containing 5 cm of the original growing sediment. The tubes were placed in a 27 °C environmental chamber (16h daylength) and observed daily for weevil development. Development to each stage was recorded based on criteria of Newman et al. (1997a). Newly eclosed weevils were removed and weighed and stem diameters were measured above and below the pupal case holes.

Plants and sediment were also analyzed for nutrient content. Individual plants were sectioned into top 20 cm, bottom stem, leaves, and roots and then frozen with liquid nitrogen. The plants were then sent to Dr. David Spencer the Exotic & Invasive Weed Research Unit at UC Davis for analysis of carbon, nitrogen and phenolics. The sediment was analyzed for NH_4^+ , organic content and bulk density with previously described methods.

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.

Results and Discussion

Semi-permanent transect sites:

Milfoil biomass in Cedar Lake was similar in 1999 to 1997-1998 (Table 1). Milfoil biomass at Lake Auburn continued its decline from high levels in the mid 1990s; it declined from 1998 to 1999, to the lowest values we have seen (202 g wet/m² in June; Fig. 1). Part of this decline may have been due to poor water clarity, however June native plant biomass was as high as previous years (Table 2) and milfoil biomass increased to 250 g/m² in September. No weevil damage was evident in 1999 and no weevils were found in the biweekly weevil surveys.

Milfoil in Smith's Bay was relatively high in early summer 1999 but decreased to more moderate densities of <1400 g wet/m² in August, similar to previous years. The high milfoil density was mainly due to a high densities at the deepest two stations; density at the three shallower stations remained low during the summer. Milfoil continued to increase at Otter Lake in 1999, from <200 g wet/m² in fall 1998 to 480 g wet/m² in August 1999. These are the highest densities we have seen since the catastrophic decline in 1996, but Eurasian watermilfoil still remained 40% of total plant biomass (Table 3), well below pre-decline densities. However, native plant density declined at Otter Lake suggesting competitive pressure from Eurasian watermilfoil. Changes in milfoil biomass at our sites (Fig. 1) are not due to regional changes; there was little concordance among the sites.

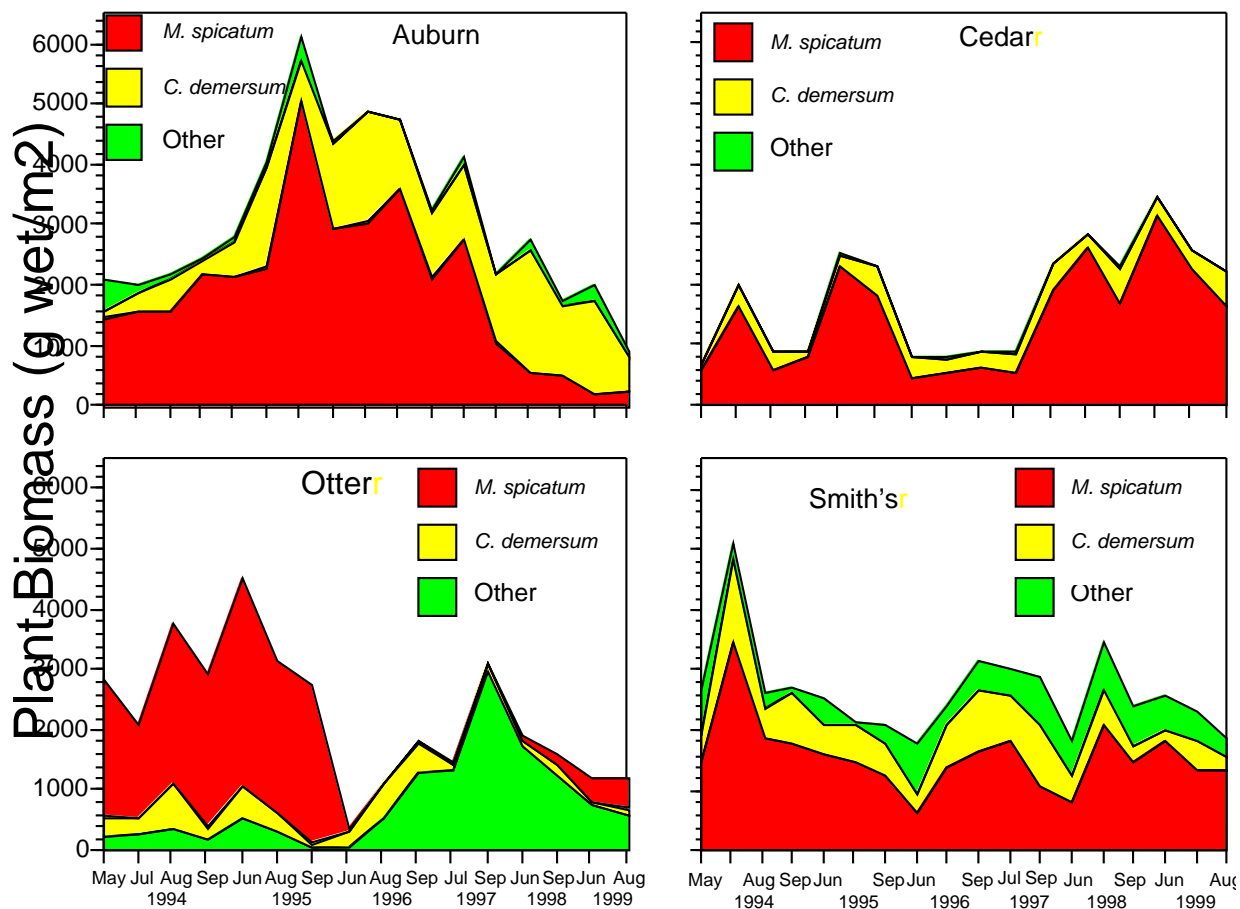


Fig. 1. Total plant biomass (Eurasian watermilfoil, coontail and other non-milfoil biomass; g wet/m²) at the four permanent transect sites from May 1994 - August 1999.

Table 1. Biomass \pm 1SE (g wet/m²) of Eurasian watermilfoil at the four sampling sites in 1994-1999. n = number of samples. Dry biomass (g/m² \pm 1SE) is presented for 1995-1999.

Sampling Date	Auburn	n	Cedar	n	Otter	n	Smith's Bay	n
5/19-6/3/94	1474 \pm 326	10	610 \pm 289	18	2208 \pm 332	21	1470 \pm 320	14
7/1-7/11/94	1570 \pm 297	16	1642 \pm 523	18	1589 \pm 231	27	3478 \pm 399	16
8/12-8/19/94	1581 \pm 224	15	601 \pm 207	15	2626 \pm 472	14	1886 \pm 328	16
9/14-9/21/94	2205 \pm 350	19	824 \pm 188	24	2510 \pm 557	9	1767 \pm 386	14
6/07-6/27/95	1999 \pm 324	30	2307 \pm 631	23	3444 \pm 336	27	1618 \pm 289	25
dry	280 \pm 43		245 \pm 67		312 \pm 33		158 \pm 28	
7/31-8/15/95	2277 \pm 417	19	1821 \pm 797	10	2526 \pm 385	15	1481 \pm 245	25
dry	267 \pm 46		172 \pm 79		171 \pm 29		149 \pm 28	
9/18-9/29/95	5044 \pm 752	17	479 \pm 173	17	2629 \pm 323	18	1281 \pm 178	25
dry	551 \pm 94		37 \pm 13		194 \pm 23		113 \pm 15	
6/12-6/24/96	2959 \pm 402	30	568 \pm 200	30	21 \pm 8	27	665 \pm 144	25
dry	306 \pm 40		59 \pm 24		2 \pm 1		46 \pm 10	
7/30-8/9/96	3035 \pm 619	27	665 \pm 219	30	1 \pm 1	27	1415 \pm 256	25
dry	390 \pm 82		62 \pm 20		0 \pm 0		176 \pm 36	
9/12-9/19/96	3622 \pm 469	30	574 \pm 174	30	0 \pm 0	27	1656 \pm 393	25
dry	361 \pm 49		50 \pm 14		0 \pm 0		156 \pm 40	
6/27-7/17/97	2134 \pm 321	30	1906 \pm 341	28	24 \pm 22	26	1880 \pm 327	25
dry	294 \pm 46		210 \pm 40		3 \pm 3		296 \pm 55	
9/8-9/18/97	2786 \pm 400	30	2646 \pm 502	29	4 \pm 4	27	1055 \pm 170	25
dry	321 \pm 49		271 \pm 55		0 \pm 0		100 \pm 18	
6/8-6/18/98	1080 \pm 168	30	1690 \pm 360	31	79 \pm 52	27	815 \pm 164	25
dry	130 \pm 18	30	213 \pm 52	31	7 \pm 4	27	105 \pm 21	25
7/27-8/3/98	581 \pm 133	30					2103 \pm 475	25
dry	67 \pm 16	30					286 \pm 65	25
9/8-9/16/98	530 \pm 76	30	3146 \pm 514	29	181 \pm 44	27	1487 \pm 338	25
dry	48 \pm 7	30	367 \pm 63	29	15 \pm 4	27	172 \pm 40	25
6/22/99	202 \pm 50	30	2238 \pm 393	28	355 \pm 113	27	1806 \pm 289	25
dry	24 \pm 7	30	252 \pm 50	28	25 \pm 8	27	155 \pm 32	25
8/3/99					483 \pm 101	27	1358 \pm 289	25
dry					36 \pm 8	27	189 \pm 44	25
8/25/99	253 \pm 83	30	1632 \pm 237	30			1362 \pm 320	25
dry	25 \pm 9	30	105 \pm 15	30			106 \pm 26	25

The contribution of the non-milfoil plant community remained high at all sites except Cedar Lake; Eurasian watermilfoil contributed 50% of the biomass at Auburn and Otter and 60% at Smith's Bay. Eurasian watermilfoil biomass remained high at Cedar Lake and contributed > 80% of the plant biomass there. The total number of species remained high at Otter and Smith's Bay but declined at Auburn and Cedar (Table 3). Similar trends were seen for numbers of species per sample (Table 2).

Table 2. Mean number of species per sample (Spp/S) \pm 1SE and non-milfoil biomass (B; g wet /m²) at the 4 sampling sites in 1994-1999. Number of samples is given in Table 1. Dry biomass for all plants is given in Appendix I.

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 \pm 0.47	670	1.33 \pm 0.28	75	4.76 \pm 0.19	600	3.29 \pm 0.22	1231
7/1-7/11/94	3.63 \pm 0.29	444	1.83 \pm 0.28	370	4.37 \pm 0.29	520	3.75 \pm 0.35	1604
8/12-8/19/94	3.00 \pm 0.28	647	1.53 \pm 0.26	282	5.57 \pm 0.39	1126	3.13 \pm 0.42	765
9/14-9/21/94	3.11 \pm 0.37	268	1.46 \pm 0.19	54	4.89 \pm 0.61	431	3.50 \pm 0.39	975
6/07-6/27/95	2.23 \pm 0.22	822	1.43 \pm 0.20	214	4.70 \pm 0.21	1065	3.64 \pm 0.30	877
7/31-8/15/95	3.37 \pm 0.26	1789	1.70 \pm 0.15	516	4.27 \pm 0.30	642	2.68 \pm 0.24	703
9/18-9/29/95	2.18 \pm 0.18	1058	1.41 \pm 0.17	337	2.44 \pm 0.34	135	2.80 \pm 0.20	856
6/12-6/24/96	2.93 \pm 0.24	1450	2.10 \pm 0.22	248	5.19 \pm 0.25	434	4.32 \pm 0.36	1159
7/30-8/9/96	2.78 \pm 0.31	1186	1.43 \pm 0.18	270	4.19 \pm 0.20	1171	3.88 \pm 0.41	1017
9/12-9/19/96	2.50 \pm 0.20	1166	1.57 \pm 0.16	307	3.93 \pm 0.28	1798	3.88 \pm 0.32	1531
6/27-7/17/97	2.97 \pm 0.14	1435	1.82 \pm 0.14	460	4.31 \pm 0.29	1516	4.16 \pm 0.39	1162
9/8-9/18/97	2.63 \pm 0.17	1500	1.59 \pm 0.09	235	4.81 \pm 0.26	3180	3.64 \pm 0.27	1863
6/8-6/18/98	2.43 \pm 0.18	1158	1.74 \pm 0.81	637	5.37 \pm 0.24	1835	5.32 \pm 0.43	1038
7/27-8/3/98	2.97 \pm 0.23	2197					5.00 \pm 0.44	1385
9/8-9/16/98	2.40 \pm 0.12	1258	1.62 \pm 0.12	296	4.74 \pm 0.39	1423	4.32 \pm 0.38	969
6/15-6/2/99	3.07 \pm 0.16	1806	1.86 \pm 0.13	326	4.52 \pm 0.31	825	4.60 \pm 0.37	810
7/29-8/2/99					5.33 \pm 0.30	720	3.72 \pm 0.31	973
8/23-8/25/99	1.93 \pm 0.13	679	1.37 \pm 0.09	570			2.92 \pm 0.33	534

Sediment bulk density and organic content at each of the lakes were similar to previous years (Table 4). Sediment ammonium decreased in June 1999 from 1998, but generally increased to high levels in late summer relative to pre-1998 values. Water clarity remained low in Lake Auburn, but not as low as in 1998. Water clarity during 1999 in Otter and Smith's Bay was similar to 1998, while clarity in Cedar Lake decreased to values more typical of the mid-1990's (1.6-2.6m) compared the the high clarity seen in 1998.

Table 3. Percentages of total plant wet biomass that was Eurasian watermilfoil ($\pm 1SE$) 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. Dry mass percentages were similar (Appendix I).

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

Table 4. Sediment characteristics (bulk density, percent organic matter, sediment pore water ammonium and water column characteristics in 1995-1999 at the four permanent transect sites. Sediment samples were collected from shallow, moderate and deep stations along transects 1, 3 and 5 (n=9). Secchi depth (SD), chlorophyll a (Chl-a; pooled surface and SD sample) and light and temperature profiles were taken in deep water > 100 m from the plant bed. Temperature is at 1m depth and 10% PAR depth is the depth at which light intensity was 10% of surface light (presented as the range which encompassed the 10% value). *Water quality data for Cedar in late July 1998 was collected for the weevil introductions and sediment was not analyzed.

Lake/Date	Bulk Dens. (g dm/ml)	NH ₄ (mg/L)	% Organic	Chl-a (mg/m ³)	SD (m)	Temp (°C 1m)	10% PAR Depth (m)	Plant Limit (m)
Auburn								
6/15/95	0.60	3.96	11.34	9.5	2.3	20.7	2.5-3.0	3.0
2se	0.15	0.91	3.73					
8/1/95	0.49	4.00	10.69	13.9	1.4	26.0	1.5-2.0	3.0
2se	0.18	1.24	4.39					
9/26/95	0.45	4.40	12.67	8.0	2.0	14.8	2.5	3.0
2se	0.13	1.96	4.05					
6/13/96	0.41	3.08	16.0	2.9	4.2	25.1	3	3.0
2se	0.11	1.66	8.6					
7/31/96	0.42	5.81	13.6	12.8	2.4	23.3	1-1.5	3.0
2se	0.17	1.52	4.7					
9/12/96	0.38	2.68	13.7	8.8	2.4	21.2	2.5-3.0	3.0
2se	0.14	0.95	4.3					
6/23/97	0.59	1.93	25.64	11.2	1.2	24.5	2.0	3.4
2se	0.22	0.56	16.79					
9/8/97	0.48	4.42	12.30	16.6	1.4	22.4	1.5-2.0	3.4
2se	0.14	1.46	3.27					
6/8/98	0.23	11.82	11.91	14.4	1.9	18.8	1.5-2.0	
2se	0.08	4.07	4.43					
7/28/98	0.45	20.09	9.52	41.2	0.7	25.7	0.5-1.0	
2se	0.27	3.68	4.25					
9/9/98	0.44	37.72	11.86	36.4	1.1	21.9	1.0-1.5	
2se	0.15	12.57	4.59					
6/22/99	0.50	2.79	13.62	9.4	1.8	22.4	2	
2SE	0.16	1.06	3.80					
8/23/99	0.44	10.98	11.64	11.0	1.5	23.1	1-1.5	
2SE	0.12	1.81	4.23					
Cedar								
6/28/95	0.62	3.90	13.73	10.2	4.5	24.0	4.5	4.0
2se	0.36	1.63	6.00					
8/3/95	0.45	7.27	16.41	16.3	1.2	26.7	1.0-1.5	3.1
2se	0.33	1.39	7.40					
9/28/95	0.43	6.06	21.56	27.5	0.8	14.8	1.0-1.5	3.1
2se	0.36	1.98	7.38					
6/18/96	0.57	3.78	13.3	1.1	5.5	24.6	3.5-4.0	6.5
2se	0.38	1.34	6.3					
8/1/96	0.42	3.86	19.0	4.5	1.9	23.8	2.5-3.0	3.1
2se	0.38	1.59	7.5					
9/16/96	0.41	5.12	18.5	5.3	2.8	20.1	2-2.5	3.1
2se	0.37	1.63	6.9					
7/8/97	0.54	3.97	12.89	9.6	2.5	21.0	3.0-4.0	6.0
2se	0.40	2.87	5.97					
9/11/97	0.42	5.69	15.76	0.8	3.7	22.0	3.0-3.5	6.4
2se	0.33	2.26	6.31					
6/18/98	0.31	4.01	18.35	2.1	4.7	22.6	4.5-5.0	
2se	0.30	1.99	5.27					
7/24/98*	N.A.	N.A.	N.A.	1.3	4.7	26.0	4.5-5.0	
9/16/98	0.29	34.77	18.68	6.9	2.6	23.4	2.5-3.0	
2se	0.30	18.72	4.78					
6/23/99	0.51	4.68	16.15	5.3	2.6	25.6	3.5	
2SE	0.36	1.68	8.79					
8/24/99	0.36	12.35	12.14	17.6	1.6	22.9	2-2.5	
2SE	0.34	3.87	3.37					

Table 4 Continued

Otter								
6/26/95	0.42	3.27	20.26	5.6	3.0	30.0	3.5-4.0	4.0
2se	0.18	1.43	7.23					
8/10/95	0.39	4.66	24.44	12.5	2.5	24.7	1.5-2.0	4.0
2se	0.26	1.77	9.49					
9/30/95	0.38	2.76	25.07	3.7	1.1	14.5	1.0-1.5	4.0
2se	0.26	1.34	11.34					
6/20/96	0.47	4.86	23.5	8.5	1.9	21.1	1.5-2.0	3.5
2se	0.34	1.67	10.2					
8/6/96	0.27	3.54	27.5	4.8	2	26	2-2.5	4.0
2se	0.16	0.88	8.6					
9/17/96	0.33	3.77	24.9	8.0	1.5	17.9	1.5-2.0	4.0
2se	0.24	1.76	9.5					
7/2/97	0.33	1.89	26.42	9.9	1.3	21.1	2.0-2.5	3.5
2se	0.21	1.09	8.17					
9/15/97	0.29	5.88	27.47	4.8	2.1	21.0	2.0-2.5	3.5
2se	0.16	2.61	9.52					
6/10/98	0.18	10.51	24.24	2.9	2.6	17.8	4.5-5.0	
2se	0.11	3.55	8.54					
9/10/98	0.24	27.47	24.36	1.6	4.0	21.1	3.5-4.0	
2se	0.11	9.40	7.55					
6/21/99	0.24	3.37	27.31	15.5	2.7	24.5	2.5	
2SE	0.07	0.83	8.34					
7/29/99	0.22	9.58	25.37	13.4	2.1	26.4	2	
2SE	0.12	3.02	8.61					
Smith's								
6/29/95	0.59	5.18	11.81	4.0	3.9	23.7	5.0	5.0
2se	0.25	3.40	4.62					
8/16/95	0.28	4.06	12.86	7.5	2.1	24.9	3.5-4.0	5.0
2se	0.14	0.97	3.71					
9/18/95	0.31	4.25	12.50	10.7	2.1	14.7	2.5	5.0
2se	0.15	0.77	3.98					
6/24/96	0.36	1.13	13.9	3.7	3.7	20.6	3.5-4.0	5.0
2se	0.22	0.32	4.7					
8/8/96	0.37	2.61	17.6	1.3	3.4	24.4	4.5-5.0	5.0
2se	0.21	1.01	5.3					
9/19/96	0.32	2.43	19.1	3.2	3.5	20.1	3.0-3.5	5.0
2se	0.18	0.90	14.3					
7/15/97	0.34	2.44	9.29	1.6	3.5	22.2	4.5-5.0	5.0
2se	0.17	0.80	3.48					
9/18/97	0.31	2.94	14.10	5.3	2.4	20.9	2.5-3.0	5.0
2se	0.17	1.21	4.74					
6/15/98	0.35	3.35	11.50	1.6	3.6	21.0	4.0-4.5	
2se	0.19	1.98	4.22					
8/4/98	0.34	9.32	11.76	4.0	2.9	23.6	3.5-4.0	
2se	0.16	3.27	3.59					
9/15/98	0.30	26.00	13.55	4.3	2.7	22.5	3.0-3.5	
2se	0.14	5.87	3.40					
6/16/99	0.34	2.21	12.71	4.3	3.7	20.8	4	
2SE	0.18	0.40	4.08					
8/4/99	0.37	11.54	10.32	4.8	2.6	26.1	4.5-5	
2SE	0.22	8.83	3.84					
8/25/99	0.30	9.71	10.63	7.2	2.9	24.7	4	
2SE	0.16	3.24	3.52					

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

Cenaiko Lake

The decline in milfoil biomass at Cenaiko Lake continued in 1999, to below sample detection in August (Table 5, Fig. 2), although milfoil was present in the lake. Native plant biomass was generally lower than previous years and the mean number of species was lower than other years although 10 species were present in June and early August. Water clarity was lower than 1998 and more similar to 1997 (Table 6). Sediment ammonium levels remained higher than during the initial decline in 1996-1997.

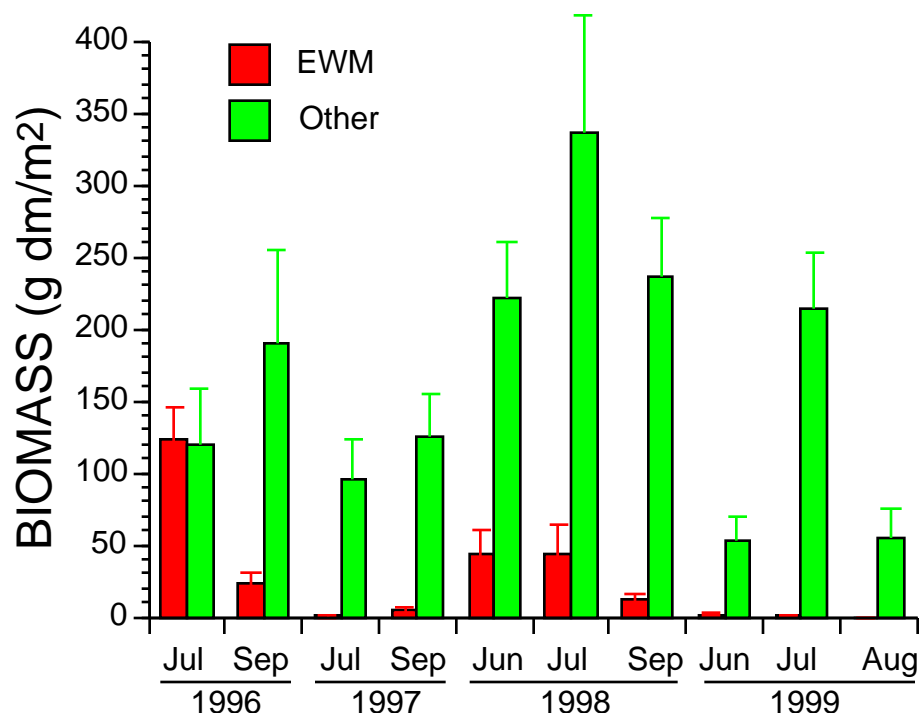


Fig. 2. Dry biomass of milfoil (EWM) and non-milfoil plants at Cenaiko Lake, 1996-1999. There was a significant decline of milfoil between July and September 1996 and July 1997 and a significant increase in native plants. Milfoil was present but not found in August 1999 samples. $N > 20$ samples on each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

Table 5. Biomass (g dm/m²) of all plants, Eurasian watermilfoil (MSP), the dominant plants (coontail (CRT), *Heteranthera* (HET), and *Potamogeton amplifolius* (PAM)), non-milfoil biomass, mean number of species per sample and mean percentage of biomass that was Eurasian watermilfoil in Cenaiko Lake 1999. $N=24-26$ samples per date.

Date	Total	MSP	CRT	HET	PAM	N Spec	nonMSP	%MSP
6/24/99	54	1.3	32.2	3.0	12.3	1.88	52.4	7.9%
1 SE	17	0.9	12.0	2.5	10.7	0.17	17.1	5.2%
8/2/99	215	1.1	124.5	26.7	34.1	2.60	213.5	1.0%
1 SE	40	0.8	37.5	9.7	23.6	0.22	40.2	0.7%
8/26/99	55	0.0	30.2	5.0	6.7	1.46	55.0	0.0%
1 SE	20	0.0	20.1	3.4	4.4	0.12	20.1	0.0%

Table 6. Sediment characteristics (bulk density, percent organic matter, sediment pore water ammonium and water column characteristics in 1996-1999 at Cenaiko Lake. Sediment samples were collected from shallow, moderate and deep stations along transects 1, 2 and 3 (n=9).

Date	Bulk Dens. (g dm/ml)	NH ₄ (mg/L)	% Organic	Chl-a (mg/m ³)	SD (m)	Temp (°C 1m)	10% PAR Depth (m)	Plant Limit (m)
7/22/96	1.23	0.60	1.5%	1.34	5.0	25.4	4.5-5.0	3.4
2se	0.22	0.54	0.5%					
9/5/96	1.22	0.67	2.4%	5.61	4.0	25.7	5.0	3.4
2se	0.23	0.40	1.1%					
7/16/97	1.10	1.63	2.5%	4.54	2.3	27.6	3.5	3.0
2se	0.20	0.67	0.6%					
9/17/97	0.96	2.87	2.5%	1.60	2.3	21.3	2.0-2.5	3.0
2se	0.18	1.65	0.5%					
6/16/98	0.98	2.37	2.2%	2.41	3.8	23.7	5.5-6.0	3.4
2se	0.18	0.66	0.5%					
7/29/98	0.97	4.98	2.3%	2.41	4.4	25.9	4.5-5.0	3.4
2se	0.16	2.31	0.7%					
9/14/98	1.12	6.08	1.7%	3.21	3.0	23.8	3.5-4.0	3.2
2se	0.12	4.90	0.5%					
6/24/99	1.12	1.12	1.76	1.3	2.7	24.3	3.5-4.0	
2SE	0.24	0.24	0.82					
8/2/99	1.14	2.09	1.29	3.5	2.7	27.4	3-3.5	
2SE	0.17	0.78	0.40					
8/26/99	1.22	4.20	1.30	2.1	3.1	24.3	3-3.5	
2SE	0.14	1.27	0.45					

Bi-weekly weevil surveys

The bi-weekly weevil surveys showed that the disappearance of weevils in Lake Auburn in July 1998 (Newman et al. 1999) persisted through 1999; no weevils were found in the 1999 surveys. Moderate to high weevil densities (total 0.5 per stem) persisted throughout the summer at both Smith's Bay and Cenaiko Lake (Fig. 3). Densities were generally higher at Cenaiko Lake than Smith's Bay. Eggs were not found after late August or early September. We did not start our Cenaiko surveys until mid-June and these weevils are likely the progeny of the overwinter generation which had disappeared in Smith's Bay around mid-June (Fig. 3). At least 3 generations are obvious in the stage frequencies.

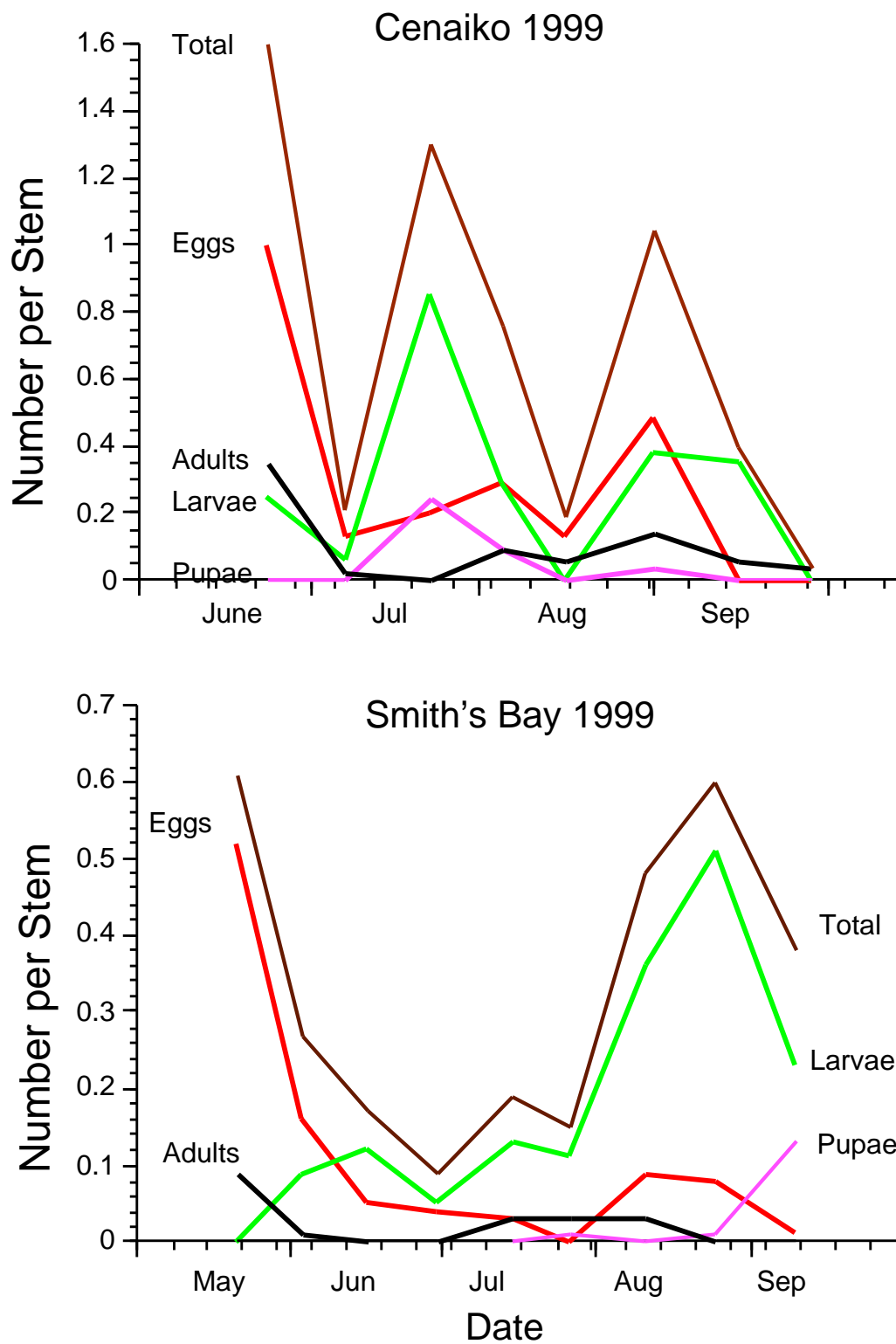


Fig. 3. Biweekly density (number per milfoil stem) of weevil life stages at Cenaiko Lake and Smith's Bay in 1999.

Survey sites:

Eurasian watermilfoil biomass decreased relative to 1998 at Grays Bay and Shady Island, but increased at Lake of the Isles where it composed > 80% of plant biomass (Table 7). Non-milfoil biomass decreased at Shady Island and Lake-of-the-Isles. Water clarity was better in 1999 at all three sites (Table 8) but sediment ammonium decreased from the high levels of 1998.

Table 7. Total plant and milfoil biomass (g dry/m²) and mean percent of plant biomass that was Eurasian watermilfoil at the three survey sites in summer 1995-1998. N= 9 samples at all sites.

Lake	Date	Total Plant Biomass (g/m ²)	Milfoil Biomass (g/m ²)	% Milfoil (of biomass)	Secchi Depth (m)
Gray's Bay	8/30/95	209.4	194.0	94.0%	2.0
	SE	55.3	53.2	3.8%	
	9/4/96	309.0	49.5	30.9%	1.9
	SE	132.1	21.1	12.7%	
	8/15/97	323.7	99.7	37.3%	3.5
	SE	43.0	29.6	10.6%	
	8/25/98	420.0	294.3	58.5%	2.3
	SE	61.8	40.8	6.9%	
	8/12/99	270.0	117	27.2%	3.1
SE	67.0	37	6.7%		
Shady Island	9/12/95	259.8	215.1	83.6%	1.8
	SE	42.8	37.3	4.8%	
	9/4/96	262.2	158.6	70.5%	2.3
	SE	45.5	30.6	10.8%	
	8/28/97	432.9	175.6	47.4%	2.4
	SE	45.8	47.5	12.5%	
	8/27/98	339.6	139.2	42.6%	1.9
	1 SE	59.4	57.7	15.2%	
	8/6/99	100.4	40.3	41.1%	2.2
1SE	28.0	19.0	14.2%		
Lake of the Isles	9/14/95	62.5	58.3	90.1%	0.5
	SE	20.6	22.6	5.0%	
	8/30/96	199.7	169.2	74.6%	1.1
	SE	74.0	74.1	10.1%	
	8/14/97	31.9	9.9	22.4%	1.4
	SE	10.4	5.3	8.6%	
	8/31/98	28.2	14.0	36.9%	0.3
	1 SE	4.7	6.1	12.2%	
	8/16/99	51.8	49.3	88.3%	0.5
1SE	14.8	14.5	4.4%		

Milfoil biomass at Harriet was high and water clarity was poorer than in 1997 and 1998 (Table 9). Milfoil composed over 88% of plant biomass at Harriet with a density of 157.2 ± 25.4 g dm/m². Milfoil density was much lower at Lake Calhoun (7.6 ± 3.7 g dm/m²) and composed about 10% of total plant biomass, however total plant biomass was also low (43 g dm/m²), perhaps due to the relatively poor water clarity (Table 9).

Table 8. Sediment characteristics (bulk density, percent organic matter, sediment pore water ammonium concentrations) and water column characteristics in 1995-1997 at the three survey sites. Three sediment samples from the intermediate depth were collected at each site.

Lake/Date	Bulk Dens. (g dm/ml)	NH ₄ (mg/L)	% Organic	Chl-a (mg/m ³)	SD (m)	Temp (°C 1m)	10% PAR Depth (m)	Plant Limit (m)
Grays Bay								
8/30/95	0.10	6.75	34.1	6.1	2.0	25.2	3.0-3.5	3.5
2se	0.04	3.39	4.3					
9/4/96	0.12	3.29	21.3	2.1	1.9	26.2	3.0-3.5	3.5
2se	0.04	1.82	1.0					
8/15/97	0.10	4.90	35.4	3.5	3.5	22.6	4.0-4.5	4.1
2se	0.05	3.19	4.9					
8/25/98	0.10	29.13	33.7	3.5	2.3	25.1	3.0-3.5	3.3
2se	0.02	7.08	6.7					
8/12/99	0.07	10.96	27.6	4.3	3.1	25	4	
2se	0.01	6.24	3.9					
Shady Island								
9/12/95	0.14	3.74	23.9	8.8	1.8	21.0	2.0-2.5	4.5
2se	0.05	3.12	2.8					
9/4/96	0.42	1.44	10.1	7.5	2.3	25.1	3.0-3.5	3.5
2se	0.41	0.48	9.0					
8/28/97	0.09	4.49	27.2	2.4	2.4	23.9	3.0-3.5	4.7
2se	0.77	1.87	16.8					
8/27/98	0.69	10.93	10.8	5.9	1.9	24.6	3.0-3.5	4.4
2se	0.93	8.71	10.7					
8/6/99	0.20	6.64	14.3	5.6	2.2	25.8	3-3.5	
2se	0.13	2.65	2.3					
Lake of the Isles								
9/14/95	1.45	5.21	1.8	57.4	0.5	20.3	0.5-1.0	0.5
2se	0.36	4.36	1.1					
8/30/96	0.28	9.30	10.0	6.9	1.1	24.6	1.5-2.0	2.0
2se	0.08	5.32	6.7					
8/13/97	0.71	8.48	16.2	26.2	1.4	22.5	1.0-1.5	3.7
2se	0.58	0.88	20.0					
8/31/98	0.25	29.33	23.9	54.3	0.3	24.3	0.5-1.0	3.3
2se	0.28	19.07	19.0					
8/16/99	0.15	0.54	24.21	83.7	0.5	22.5	0.5-1.0	
2se	0.05	0.56	12.48					

Table 9. Water column characteristics of two additional survey sites.

Lake/Date	Chl-a (mg/m ³)	SD (m)	Temp (°C 1m)	10% PAR Depth (m)	Milfoil Limit (m)	Plant Limit (m)
Calhoun	9/24/97	7.2	3.1	18.9	2.5-3.0	4.7
	9/4/98	3.7	3.0	23.7	3.5-4.0	4.1
	9/21/99	17.1	1.6	18.5	2	
Harriet	10/9/97	4.5	> 5.4	17.3	3.0-3.5	5.2
	9/23/98	3.7	2.6	20.3	4.0-4.5	5.0
	9/24/99	7.5	2.6	17.5	3.5	

Weevil Introduction/Manipulation:

Milfoil density at the 20 Cedar Lake plots in June 1999 (prior to weevil stocking) ranged from 3112 ± 909 g wet/m² to 3810 ± 664 g wet/m² (508 g dry/m²) (Table 10); this was higher than these sites in 1998 and than our permanent transect sites in 1999. At the end of the experiment in late August, milfoil biomass declined to between 1512 ± 458 g wet/m² and 2551 ± 252 g wet/m². The mean number of species also declined.

Table 10. Wet and dry biomass (g/m² \pm 1SE) of Eurasian watermilfoil (MSP) and non-milfoil plants, %Eurasian watermilfoil and mean number of species per sample for the cage experiment. The June sample was taken 3 weeks prior to stocking and the September sample was taken 8 weeks after initial stocking. Two samples per cage were taken in July and 3 samples per cage in September. N=5 replicate cages per treatment. Open cages allow fish entry, closed cages do not. A total of 150 adult weevils were stocked into each stocked cage.

Date	Cage Type	Stocked	MSP	NonMSP	%MSP	Mean No. spp.
6/3/99	Open	No	3810 ± 664	424 ± 195	$89.9 \pm 4.1\%$	2.30 ± 0.34
	Dry		389 ± 59.12	36 ± 17.48	$91.3 \pm 4.2\%$	
6/3/99	Closed	No	3455 ± 495	149 ± 76	$95.5 \pm 1.3\%$	2.00 ± 0.16
	Dry		331 ± 37	8 ± 4	$96.3 \pm 0.9\%$	
6/3/99	Open	Yes	3112 ± 909	409 ± 187	$81.8 \pm 9.9\%$	2.50 ± 0.16
	Dry		321 ± 88	36 ± 16	$83.2 \pm 9.6\%$	
6/3/99	Closed	Yes	3252 ± 430	350 ± 151	$88.1 \pm 7.0\%$	2.50 ± 0.22
	Dry		346 ± 39	27 ± 10	$90.1 \pm 5.9\%$	
8/30/99	Open	No	2551 ± 252	363 ± 183	$87.9 \pm 5.8\%$	1.70 ± 0.20
	Dry		175 ± 22	22 ± 12	$89.3 \pm 5.9\%$	
8/30/99	Closed	No	1512 ± 458	174 ± 173	$92.5 \pm 7.4\%$	1.30 ± 0.20
	Dry		106 ± 33	13 ± 13	$92.2 \pm 7.8\%$	
8/30/99	Open	Yes	2241 ± 524	429 ± 311	$82.8 \pm 13.1\%$	1.80 ± 0.12
	Dry		153 ± 45	25 ± 17	$81.9 \pm 13.8\%$	
8/30/99	Closed	Yes	2062 ± 250	319 ± 132	$78.4 \pm 10.0\%$	1.80 ± 0.20
	Dry		140 ± 21	22 ± 9	$78.6 \pm 10.3\%$	

Weevil stocking appeared less successful than in 1998. Initially, higher densities of weevils were found in stocked vs non-stocked cages during bi-weekly visual surveys, but later in the summer higher densities of weevils were found in closed compared to open cages (Table 11). Few significant differences in weevil density were found. By the last date there were significantly ($P > 0.1$) more total weevils, and more larvae and pupae per stem in the stocked cages but no effect of cage type.

There was no significant effect of cage or stocking on milfoil biomass (all $P > 0.1$); biomass generally decreased in all the cages after stocking. The failure to build substantially higher weevil densities in stocked cages and the relatively late stocking date may have prevented any effect on the watermilfoil. There was also no evidence of carryover effects from stocking in 1998. Fish invasion was a persistent problem and we will redo the experiment once more in 2000 with an even

earlier stocking date.

Table 11. Visual counts (mean number per 100 stems and 1 SE) of weevils in stocked and unstocked cages (open and closed) at Cedar Lake in 1999. There were 5 reps of each treatment combination.

Date	Cage type	Stocked	Eggs	Larvae	Pupae	Adults	Total
7/23/99	Open	No	0.3	6.4	1.7	0.1	8.5
		1 SE	0.3	3.6	1.4	0.1	3.2
	Closed	No	0	3.9	1.1	0.7	5.6
		1 SE	0.0	2.4	0.5	0.3	2.5
	Open	Yes	1.2	5.1	2.3	0.8	9.3
		1 SE	0.5	1.5	1.6	0.5	3.6
8/5/99	Open	No	0.8	8.3	4.0	0.8	13.9
		1 SE	0.6	4.5	3.7	0.4	8.2
	Closed	No	0.5	4.1	1.6	4.1	10.4
		1 SE	0.3	1.0	0.7	2.9	3.9
	Open	Yes	0.4	2.3	0.3	0.4	3.3
		1 SE	0.4	1.5	0.2	0.3	2.2
8/17/99	Open	No	0.4	1.2	0.4	0.8	2.8
		1 SE	0.2	0.6	0.3	0.5	0.5
	Closed	No	0.3	8.7	0.8	0.5	10.3
		1 SE	0.2	3.8	0.2	0.4	4.0
	Open	Yes	0.7	1.2	0.8	0.5	3.2
		1 SE	0.3	0.6	0.5	0.5	0.9
Closed	Yes	0.9	8.8	2.1	1.5	13.3	
	1 SE	0.5	5.1	0.7	0.9	5.3	

Influence of milfoil genotype and rearing sediment on weevil performance:

Analysis of the sediment showed a significant effect (two-way ANOVA, $\alpha = .05$) of sediment source on bulk density, percent water, and organic content and a significant effect of plant source on NH_4^+ concentration. Cedar sediment had lower bulk density and higher organics than Otter sediment and Cedar plants appeared to result in lower ammonium concentrations (Table 12). Despite the big differences in sediment character, no significant effects were found for hatch, larval, pupal or egg to adult development for either sediment or plant source (Fig 4). There were also no significant differences in stem diameter at pupation. Factors such as bulk density and organic content can affect plant growth, however, we purposely grew the plants to similar size to eliminate plant size effects. The lack of a significant plant or sediment source effect on weevil development in 1999 suggests that plant size or stem diameter (not measured in 1998) may be more important than other measures of plant quality. We do not yet have the results of the chemical analyses.

Table 12. Sediment ammonium (mg/L), bulk density (g dm/ ml) and organic matter for sediment used in the plant and sediment source rearing experiment.

Sediment/Plant	NH_4^+	% water	bulk density	% organic
OtterSed/CedarPlant	0.72	46.13%	0.805	1.08%
OtterSed/OtterPlant	4.17	45.15%	0.823	0.92%
CedarSed/CedarPlant	1.26	75.38%	0.299	1.23%
CedarSed/OtterPlant	3.53	75.52%	0.281	1.26%

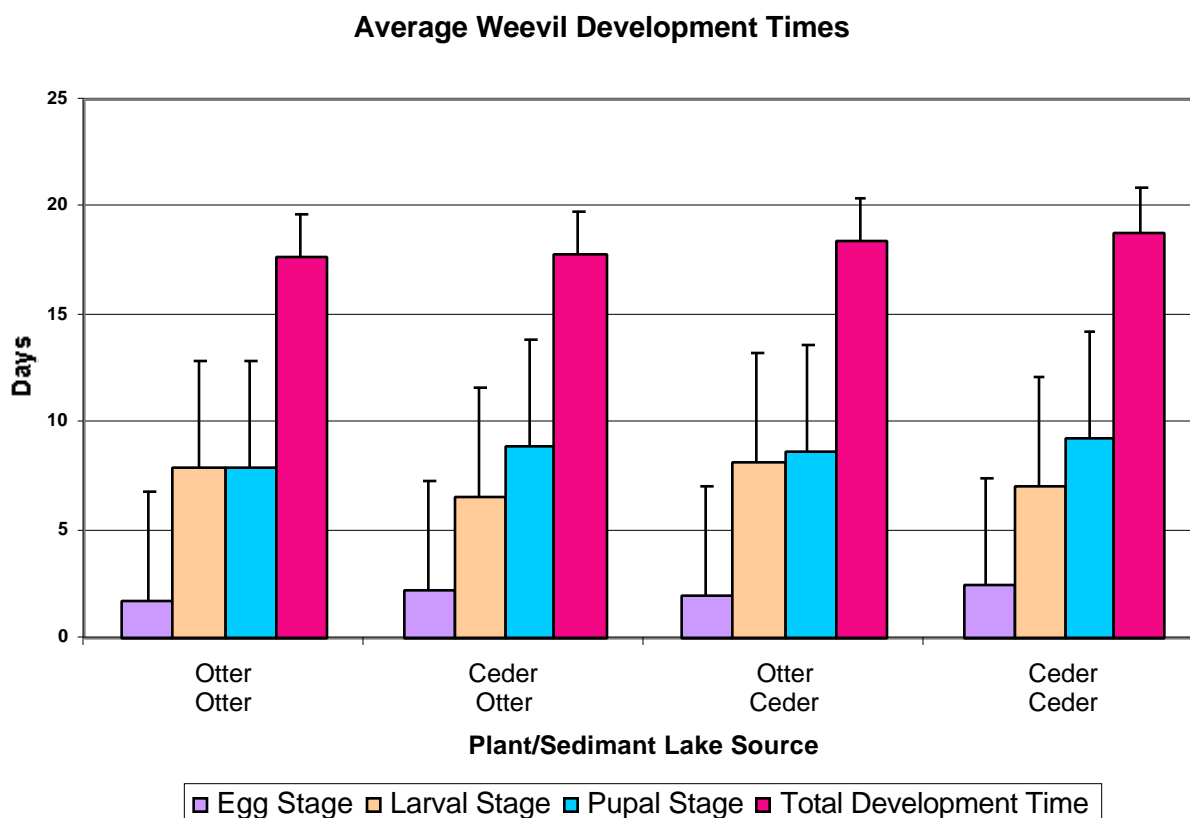


Figure 4. Influence of sediment and plant source (Cedar or Otter) on milfoil weevil development times. There was no effect of sediment or plant source on development time, survival or adult eclosion mass.

Overwinter assessments and pathogens:

Shoreline assessments were not conducted in 1999. Weevils were assessed for pathogens during the summer of 1999 from weevils collected in the weekly surveys. Pathogens were present in 14% of the weevils examined (Table 13). Cenaiko adults had a 54% incidence of infection and 50% of the Cenaiko pupae were infected. However, the degree of infection for all stages was very low and there was no evidence of pathogenic levels in any of the animals sampled. The results of prior overwinter assessments indicated little parasitism over winter (Newman et al. 1999).

Table 13. Incidence of pathogens (protozoa, microsporidia (*Nosema*), or saprophytic fungi) in individuals of each stage of weevils collected from Cenaiko Lake and Smith's Bay during summer 1999. N = number of individuals, Infect = infected (pathogen present).

	N	N Infect	% Infect
Cenaiko			
adult	13	7	53.85%
pupa	2	0	0.00%
larva	66	1	1.52%
total	81	8	9.88%
Smith's Bay			
adult	5	0	0.00%
pupa	14	7	50.00%
larva	85	11	12.94%
total	104	18	17.31%
Grand total	185	26	14.05%

Weevil modelling:

Thermistors at 0.75m depth in Lakes Auburn and Smith's Bay showed that in 1999 early-May minimum temperatures exceeded 10 °C in both lakes and by June temperatures averaged over 20 °C; temperature continued to increase to a peak of over 30 °C in early August and temperature declined rapidly in September from 25 to around 15 °C (Appendix II). Temperatures exceed 30 °C for several days in late July. To provide a conservative estimate of accumulated degree days, we only included data from mid-May to mid-September when mean daily temperatures were above 15 °C (and minima well above 10 °C). In both lakes more than 1700 degree days (>10 °C, the lower thermal threshold) were accumulated, indicating a potential for development of five generations (but see below).

The stage structure model was parameterized to match proportions of each life stage observed over the summer at Smith's Bay. 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 (25 °C) there are about 15 DD per day. Based on these estimates the mean generation time calculated from the life table was 450 DD (about 30 days at typical mid-summer lake temperatures). Based on the model, weevil populations increase over the 1500 DD of summer (about the number generally accumulated before September 1st in our lakes). Even though reproduction is continuous, there are obvious peaks and valleys in the abundance of each stage (Fig. 5), however, all stages increase in abundance over the summer. Note that there are only three peaks in the adult population, corresponding to the estimated average generation time. However, 4 generations are possible with slightly more degree days. Thus although the maximum number of generations possible in a summer (from progeny of the first adults) is 4-6 (Newman et al. 1999) it is likely that only 3 or 4 average generations are produced each year (see also Creed and Sheldon 1995, Sheldon and O'Bryan 1996). The last (4th) peaks of eggs and larvae may not mature or eggs may not be laid as egg laying appears to decline in field populations after late August or early September.

The correlation between predicted and observed population stage structure was most sensitive to average adult life expectancy and relatively insensitive to the length of the pre-reproductive adult stage and the oviposition rate. However, weevil density predicted was sensitive to the length of the pre-reproductive adult stage and the oviposition rate, and high correlations could be obtained for the population stage structure at with unreasonable predictions for population density (negative values or unreasonably high).

It should be noted that the resulting model was calibrated based on population stage structure, not density, so density predictions should not be taken literally. However, manipulation of the model will provide insight into sensitive life stages and factors limiting weevil populations. For example, average adult longevity of 125 DD corresponds to about 8 days at typical summer temperatures and will result in a population increase; adult longevity of 75 DD (5 days) can also result in a population increase (Fig 5), however, further decreases in adult longevity will result in stable (50DD) or declining populations. An increase of adult longevity from 75DD to 150 DD resulted in an 8 fold increase in fall adult density, underscoring the potential importance of female longevity to mid- and late-summer weevil densities.

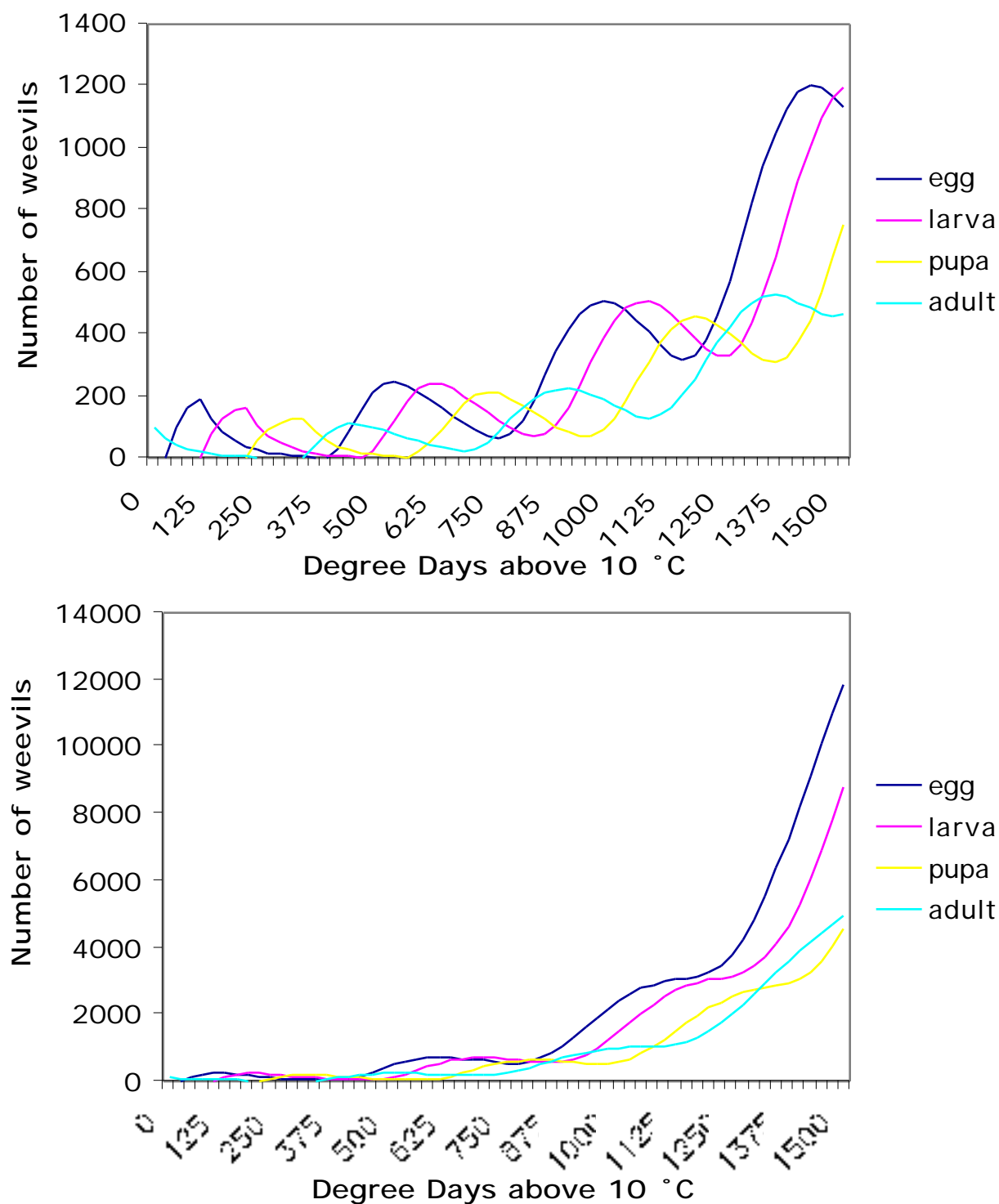


Figure 5. Predicted population density of egg, larvae, pupae and adults over a summer of 1500 degree days above 10 °C. Top: Based on average adult longevity of 75 DD (about 5 days). Bottom: Based on average adult longevity of 150 DD (about 10 days). Initial density of adults was 100 and hatch and pupal survival were 0.8, larval survival was 0.7 and egg laying was estimated at 0.9 female eggs/female/25 DD. Development times for each stage were estimated from temperature-development relationships given by Mazzei et al. (1999).

Summary

We have documented one decline that is clearly attributable to weevil stem mining, and have evidence that weevil damage, at least in the shallower sites, at Lake Auburn and Smith's Bay have reduced milfoil abundance. The decline at Cenaiko Lake has persisted; an increase in milfoil in early summer 1998 was met with high weevil populations and a subsequent decrease of milfoil and the decline persisted through the summer of 1999 with milfoil biomass remaining below 2g/m^2 (Newman and Biesboer, *in press*). It is not certain what permits development of such high weevil populations in Cenaiko Lake, however, low predation by sunfish appears to be a factor. 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 slower decline of Eurasian watermilfoil leveled at Smith's Bay; at the shallower sites milfoil remains suppressed and native plants have developed extensively. 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 which has not been maintained at the other lakes. In Cedar Lake, the improved water clarity and very low weevil densities resulted in a continued increase in Eurasian watermilfoil that persisted through the summer. Milfoil is increasing at Otter Lake, and may become dominant in 2000, but still remains well below historic highs; the slow increase may be due to a combination of plant competition and herbivore pressure.

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. 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 during 1993-1995, with sunfish increasing from 1993 to 1995. 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 if milfoil will continue suppressed in Lake Auburn, or if the milfoil will return in force with low weevils densities; the milfoil did not strongly rebound in 1999, despite the absence of weevils, however, it was not until 1995 that the milfoil fully recovered from the 1993 decline.

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). The potential importance of plant community response to stress imposed on Eurasian watermilfoil was addressed above. It is also clear that at many of our sites weevil populations have not built to adequate densities; Cenaiko Lake provides a clear example 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 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 and that milfoil genotype and sediment could affect weevil performance.

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. 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 at Cenaiko Lake and Lake Auburn in 1999. Milfoil increased in Otter Lake to 40% of total plant biomass, but is still <20% of its typical density in 1994-1995. In Smith's Bay, milfoil remained suppressed at the shallower sites with high non-milfoil biomass and much evidence of weevil damage, but remained dense at the deeper sites that show little evidence of weevil damage. Milfoil dropped below sample detection at Cenaiko Lake by September 1999. Native plants remain abundant at Cenaiko.
- Bi-weekly weevil surveys showed that weevils had disappeared from Lake Auburn in July 1998 and did not return in 1999 (a few have been found in 2000). Weevil densities at Cenaiko and Smith's Bay were relatively high and all stages persisted throughout the summer.
- The fish exclusion experiment gave only slight evidence that fish predation may limit weevil populations, however, we failed to keep fish out of the cages and may need to use higher stocking densities and earlier stocking times.
- In contrast to 1998, we found no evidence that weevil performance (developmental rate, size and survival) was influenced by rearing plant and rearing plant sediment, even though substantial differences in sediment chemistry existed. These results suggest that plant size and stem diameter, which may have been influenced by sediment and plant source, may be more important than internal plant quality.
- Weevil temperature-development models are useful for predicting trends and matching field observations. A stage based model suggests that 3 or 4 average generations are produced per year, that adult longevity of 50 to 125 degree days is required to sustain populations and that adult longevity is important to developing high weevil densities later in the summer.

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Appendix I. Abbreviations and dry mass of plants collected.

Key to plant abbreviations used in this report.

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