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A Decline of Eurasian Watermilfoil in Minnesota Associated with the Milfoil Weevil, *Euhrychiopsis lecontei*

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ABSTRACT

The native milfoil weevil, *Euhrychiopsis lecontei* Dietz, is a candidate biological control agent for the exotic Eurasian watermilfoil (*Myriophyllum spicatum* L.) in northern North America. Declines of Eurasian watermilfoil populations have been associated with the weevil but many of these examples are poorly documented. We report the first documented decline of Eurasian watermilfoil in Minnesota due to the milfoil weevil. Eurasian watermilfoil in Cenaiko Lake declined from 123 ± 45 g dm/m^2 (±2SE) in July 1996 to 23 ± 14 g/m^2 in September 1996 and remained at <5 g/m^2 in 1997. It increased to 44 g/m^2 in June and July of 1998, but declined to 12 ± 10 g/m^2 in September 1998; the decline persisted through 1999. Biomass of other aquatic macrophytes increased while milfoil biomass decreased and other macrophytes remained >95 g/m^2, or more than 90% of plant biomass in 1997, and >200 g/m^2 and >85% of plant biomass in 1998. In July 1996, milfoil weevil densities in Cenaiko Lake were the highest yet observed in Minnesota (103 ± 42/m^2; 1.6 per stem), but declined with decreasing milfoil density, from 8.1/m^2 in September 1996 to below detection in September 1997; weevil density increased to over 2 per stem in September 1998. Densities of two herbivorous lepidopterans, *Acentria ephemerella* and *Parapoynx* sp. increased after the decline of Eurasian watermilfoil, however, these insects were associated primarily with *Ceratophyllum*, *Potamogeton* and *Zosterella* and did not appear to be the main cause of the decline. A decline in stem and leaf carbohydrates of milfoil and an increase in sediment ammonium accompanied the decline of Eurasian watermilfoil. These observations indicate that *Euhrychiopsis lecontei* can reach adequate densities to effect persistent declines in Eurasian watermilfoil in Minnesota, but the lack of declines in other lakes indicates that more research is needed.

Key words: Myriophyllum spicatum, *Euhrychiopsis lecontei*, biological control, herbivory, weeds, Eurasian watermilfoil, aquatic macrophyte control.

INTRODUCTION

Eurasian watermilfoil (*Myriophyllum spicatum* L.) is an exotic nuisance plant that has infested lakes across North America and is considered one of the most troublesome aquatic weeds (Smith and Barko 1990). Since it was first reported in Minnesota in 1987, Eurasian watermilfoil has spread to over 90 waterbodies in Minnesota (Exotic Species Programs 1999). In addition to inhibiting recreation and impeding boat traffic (Smith and Barko 1990), significant declines in macrophyte species diversity and richness have been attributed to Eurasian watermilfoil infestations (Madsen et al. 1991). Millions of dollars are spent annually on Eurasian watermilfoil control (Sheldon and Creed 1995). As with many exotic species, natural declines of the weed have been noted 10-20 years after invasion. Declines of Eurasian watermilfoil have been attributed to sediment nutrient depletion, poor water clarity, disease and herbivory (Carpenter 1980, Barko et al. 1994, Nichols 1994, Creed and Sheldon 1995, Lillie 1996, Creed 1998), nevertheless, in most instances data are anecdotal or were collected after the decline occurred.

Interest in biological control with native and naturalized insects has increased, because sustained control of Eurasian watermilfoil with traditional approaches, such as herbicides and harvesting, is costly and sometimes controversial (Nichols 1991, Cooke et al. 1993). Three herbivorous insects have been associated with Eurasian watermilfoil declines and are considered to have some potential for biological control: the moth *Acentria ephemerella* (Denis & Schiffermüller) (=*Acentria nivea* (Olivier)) a naturalized Pyralidae, the indigenous midge *Cricotopus myriophylli* Oliver and the indigenous weevil *Euhrychiopsis lecontei* (Dietz) (=*Eubrychiopsis lecontei*) (e.g., Painter and McCabe 1988, Kangasniemi et al. 1993, Creed and Sheldon 1995, Sheldon 1997a). All three taxa are present in Minnesota and Wisconsin (Newman and Maher 1995). Although all three taxa may have some potential to control milfoil, prior research (Creed and Sheldon 1995, Sheldon and Creed 1995, Sheldon 1997b) suggests that *E. lecontei* is the most promising control agent; documented declines and suppression of Eurasian watermilfoil have been attributed to weevil activity (Creed and Sheldon 1995, Sheldon and Creed 1995, Sheldon 1997b).

Our work in Minnesota has focused on three aspects of the biological control of Eurasian watermilfoil: autecology of *E. lecontei* (host choice, developmental performance, and life history aspects), effects of the weevil on Eurasian watermilfoil in controlled and confined conditions, and longer term

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assessment of control agent and plant community responses at repeatedly sampled field sites. We have learned that *E. lecontei* is highly specific to watermilfoils for oviposition choice and that once the weevil is reared on or exposed to Eurasian watermilfoil it prefers Eurasian over other watermilfoil species (Solarz and Newman 1996, Solarz 1998). The weevil's developmental rate, survival and size are as good or better on Eurasian watermilfoil than on the native host of *E. lecontei*, northern watermilfoil (*M. sibiricum* Kom. = *M. exalbescens* Fern.; Newman et al. 1997). Furthermore, *E. lecontei* develops rapidly depending on temperature; development from egg to adult is completed in <25 days at 25C and typically 60% of eggs laid developed to adult (Newman et al. 1997). The lower developmental threshold appears to be about 10C and egg to adult development requires 309 degree days above 10C (Mazzei et al. 1999); up to 5 generations can be completed in a summer at typical Minnesota lake temperatures. A larva mines 15 cm of Eurasian watermilfoil stem to complete development and although the rate of damage depends on temperature, total damage per larva is constant across temperatures (Mazzei et al. 1999).

Euhrychiopsis lecontei caused significant declines of Eurasian watermilfoil in tank experiments, resulting in reductions in both stem and root mass in 4 weeks (Newman et al. 1996). The larval stem mining also resulted in significant reductions in root and stem carbohydrate levels and stocks (Newman et al. 1996), supporting the suggestion that weevil-induced declines of Eurasian watermilfoil may be due to reductions in stores of carbohydrates needed to survive the winter (Creed and Sheldon 1995). Despite these promising results, Newman et al. (1996) noted that no significant declines of Eurasian watermilfoil, clearly attributable to the weevil or other insects, had occurred in Minnesota as of summer 1995. We speculated that weevil densities were not high enough to effect declines. Based on our tank experiment and observations of others, we suggested that densities of 200 to 300 weevils per m^2 or 2 to 4 weevils per stem might be required for control. Sutter and Newman (1997) suggested that predation by sunfish may be one factor limiting weevil densities.

In this report we document a dramatic and sustained decline of Eurasian watermilfoil in Minnesota that can be attributed to stem-mining damage by weevil larvae. We also consider mechanistic factors that may have facilitated the decline and its persistence.

MATERIALS AND METHODS

We have been regularly monitoring plant community and biocontrol agent densities at four lake sites since 1992. In summer 1996 we began to monitor Cenaiko Lake (Anoka Co., MN; T31N; R24W; S26), a 12 ha man-made lake (Z_m = 10 m) along the Mississippi River, as a potential site for weevil introduction or augmentation. Reports to the Minnesota Department of Natural Resources (MN DNR) indicated that extensive beds of matted Eurasian watermilfoil had covered the northern portion of the lake for several years (C. H. Welling, MN DNR, pers. comm.); Eurasian watermilfoil was first reported from Cenaiko in 1992. In early July 1996, we noticed fairly extensive beds of matted Eurasian watermilfoil, but also noticed that it was heavily damaged (similar to milfoil in our tank experiment, Newman et al. 1996) and harbored a high density of the milfoil weevil, *Euhrychiopsis lecontei*. In mid-July we marked four sampling transects across the northern third of the lake (the only extensive littoral area in the lake). We also mapped the extent of milfoil coverage around the lake (Trimble Pathfinder Basic Plus global positioning system (GPS); post-processing differential correction with MCOR300). We marked and sampled three transects in September 1996, July and September 1997, and June, July, and September 1998.

Transects extended from shore to shore across the northern third of the lake from east to west. Starting and ending points were haphazardly chosen at the beginning and near the ends of the plant beds, but transects covered similar areas on each sampling date. Sampling stations were marked with floating jugs every 30 m along the transects; on each date from eight to 11 stations were sampled on each transect for a total of 25 to 32 samples. A >4 m channel cut through two of the transects, typically resulting in two to three stations with no plants. These samples were deleted from the analysis because it was clear that they were beyond the normal plant depth limit.

At each sampling station, plant biomass and invertebrate samples were taken by SCUBA from 0.1 m^2 quadrats. All plant material was clipped at sediment interface and immediately placed in a sealable bag underwater. Sediment cores (4 cm diameter, collected by hand), and samples of milfoil plants with roots for carbohydrate analysis were also collected from three stations along three transects (usually every third station if plants were present) by the divers. All samples were immediately placed on ice. The carbohydrate samples were drained of water and immediately frozen upon return to the lab. Biomass samples were refrigerated (<4C) until processing. The samples were rinsed of invertebrates. Invertebrates were picked with forceps (endophytic and external on milfoil at 2× magnification and from the wash water at 8× magnification) from all samples and weevils (larvae, pupae and adults) and Lepidoptera were enumerated. Basal stems were counted for all milfoil plants and missing and damaged meristems were counted as a proportion of the total. Plants were separated, identified to species (nomenclature follows Borman et al. 1997), spun for 15 seconds in a salad spinner and weighed to obtain wet mass. These samples were frozen individually by species for later dry mass (dm) determination. Frozen plants were thawed, dried at 105C for 48 h and weighed.

A set of water column parameters was measured in the open water (>7 m depth and >200 m from the bed) 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 47 mm glass fiber filter, the filter was placed on dry ice and returned to the laboratory. Within 30 days, chlorophyll was extracted and measured spectrophotometrically (APHA 1989). Sediment cores were stored on ice and returned to the laboratory.

Within 48 h, the top 15 cm of sediment or the entire core if <15 cm, was homogenized; a clay layer in Cenaiko lake often precluded collecting cores longer than 10cm. A 5 ml sediment subsample was dried at 105C for 48 h and then weighed to obtain bulk density (g dm/ ml). The dried sediment was subsequently ashed at 550C for 4 h to obtain percent organic matter ([AFDM/dm] × 100). Pore water was extracted from the remaining sediment by centrifugation, acidified to <pH 2 and stored in the refrigerator. Within seven days, the NH_4 concentration was determined by selective electrode (APHA 1989). Water quality and sediment characteristics are summarized in Table 1.

The milfoil plants collected for carbohydrate analysis were rapidly thawed to room temperature, separated into roots and into stems with attached leaves, and dried at 100C for 90 min followed by several hrs of drying at 70C (Raguse and Smith 1965). Flowering plants were uncommon and flowers were not included in the analysis. The plant organs from each sample were pooled into roots, stems and leaves, weighed, and ground in a Wiley mill to pass through a 0.5 mm screen. The ground tissues were collected and dried for one hour at 70C to remove any remaining moisture, placed into petri dishes sealed with parafilm, and stored in the freezer until analysis.

Both total nonstructural carbohydrates and total sugars were determined for the root, stem and leaf samples using the methods of Smith (1969) with the following modifications: amyloglucosidase (EC 3.2.1.3—Sigma Chemical Co., St. Louis, MO) was used to digest starch present in the samples and the 3,5 dinitrosalicyclic acid method was used to detect reducing sugars. All analyses were performed in triplicate. Enzyme solution was prepared daily. Sugars were extracted in 80% ethanol. Glucose was used as the standard reducing sugar. Percent starch in samples was calculated by subtraction of percent total sugars from percent total nonstructural carbohydrates.

We also conducted a stem survey for weevil density in early September 1998. Six stations along each of three transects were sampled by collecting five milfoil stems (top 50 cm of a plant) at each station and placing them in sealable bags. The stems were examined under 8× magnification and the number of eggs, larvae, pupae and adults were enumerated (similar to Jester et al. 1997). Densities are reported as mean number per stem based on the n = 18 sample stations.

RESULTS

It is likely that the density of Eurasian watermilfoil in Cenaiko Lake was much higher earlier in the summer and in previous years than when we first sampled in July 1996. Park personnel reported dense mats of flowering Eurasian watermilfoil. Because of the heavy insect damage and poor physiological condition of the milfoil, some flowering plants remained but most of the plants showed signs of weevil damage (darkened, mined stems); some areas where flowering and densely matted plants likely existed were occupied by only damaged milfoil stems with few leaves remaining. Based upon GPS mapping, approximately 5 ha of the 12 ha lake were covered with Eurasian watermilfoil; most plants were in the shallower north basin because the depth quickly dropped to >4 m around the southern end of the lake. Eurasian watermilfoil extended to 3.4-m depth but was most abundant in water <2.9 m.

The biomass of Eurasian watermilfoil declined from 122.9 g dm/m^2 ± 44.6 (2SE) in July 1996 to 23.4 ± 13.8 g/m^2 in September 1996 (Figure 1). The decline continued in 1997 with biomass of Eurasian watermilfoil at 0.9 ±1.3 g/m^2 in July and <5 g/m^2 in September 1997. Eurasian watermilfoil increased to 44 ± 40 g/m^2 in July 1998, but declined to 12 ± 10 g/m^2 in September 1998. Weevil density in July 1996 was among the highest we have seen in Minnesota at 103 ± 42/m^2 (1.6 per stem; Table 2). Weevil densities declined with the decreased density of Eurasian watermilfoil in 1997, however, the densities of two generalist herbivores, *Acentria ephemerella* and *Parapoynx* sp. increased; these caterpillars were most commonly associated with coontail (*Ceratophyllum demersum* L.),

TABLE 1. SEDIMENT CHARACTERISTICS (BULK DENSITY, PERCENT ORGANIC MATTER AND SEDIMENT PORE WATER AMMONIUM) AND WATER COLUMN CHARACTERISTICS IN 1996-1998 AT CENAIKO LAKE. SEDIMENT SAMPLES WERE COLLECTED FROM THREE STATIONS ALONG THREE TRANSECTS (N = 9). SECCHI DEPTH (SD), CHLOROPHYLL A (CHL-A; POOLED SURFACE AND SD SAMPLE) AND LIGHT AND TEMPERATURE PROFILES WERE TAKEN IN DEEP WATER >200 M FROM THE PLANT BED. TEMPERATURE IS AT 1 M DEPTH AND 10% PAR DEPTH IS THE DEPTH AT WHICH LIGHT INTENSITY WAS 10% OF SURFACE LIGHT (PRESENTED AS THE RANGE THAT ENCOMPASSED THE 10% VALUE). SEDIMENT CHARACTERISTICS INCLUDE AN ESTIMATE OF TWICE THE STANDARD ERROR (2SE).

Date	Bulk dens. (g dm/ml)	NH_4-N (mg/L)	% Organic	Chl-a (mg/m^3)	SD (m)	Temp (C)	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%					

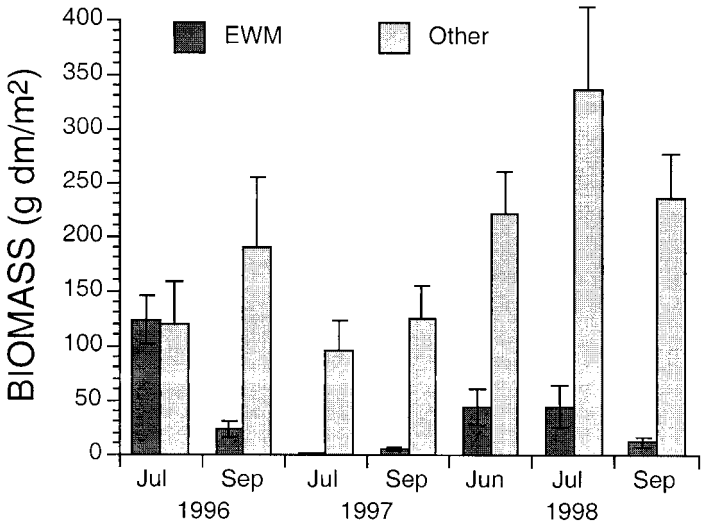

Figure 1. Dry biomass (+1SE) of Eurasian watermilfoil (EWM; g/m²) and non-milfoil (other) plants at Cenaiko Lake, 1996-1998. There was a significant decline of milfoil between July and September 1996 and July 1997 but no change in total biomass of other plants (Tukey's HSD; $\alpha = 0.05$). N > 20 samples on each date.

Zosterella (=*Heteranthera*) *dubia* Small and *Potamogeton zosteriformis* Fern. Weevil densities remained low in 1998 with the low occurrence of milfoil, however, the stem survey in early September revealed a high density per stem, with over 2.7 larvae, pupae and adults per stem.

While the biomass of Eurasian watermilfoil declined, the biomass of other aquatic macrophytes increased, from 120.0 ± 76.0 m² in July 1996 to 189.8 ± 129.6 g/m² in September 1996 and 125.3 ± 60.6 in September 1997, and remained >200 g/m² in 1998 (Figure 1). Eurasian watermilfoil had declined from 63% of total plant biomass in July 1996 to <7% of plant biomass in 1997, and remained <16% of total plant biomass in 1998 (Table 3). Although the mean number of plant species per sample remained low during 1996 and 1997 (2.1 to 2.5 species per sample) the total number of species increased from 5 to 8 in 1996 to 9 on each date in 1997. In 1998, 8 to 11 species were found on each date and the mean number of species per sample increased to 3 ± 0.4. Coontail was the dominant non-milfoil plant (Table 3), however it did not exceed an average of 75% of native plant biomass on any date.

Stem carbohydrate levels were relatively high in July 1996 (Table 4) but quickly declined to <2% in September and did not recover in 1997. Root carbohydrates were moderate in July 1996 but roots nearly disappeared from sediments and were impossible to recover in adequate quantity to analyze on subsequent sampling dates. Carbohydrate levels increased in 1998 but remained below the pre-decline levels and did not increase in fall as would be expected.

Water clarity was high in 1996 (Table 1) and declined in 1997, due to heavy rains that washed suspended clay into the lake; the decrease in clarity was not due to algae because chlorophyll levels remained low. Light did not appear to be limiting plant growth as adequate light for growth remained at 2-3 m. Sediment ammonium increased in 1997 and 1998 with the decrease in milfoil biomass. It should be noted that the sediment above the clay layer at Cenaiko is quite shallow and cores indicated low organic content and a relatively high bulk density (Table 1), both likely due to the high clay content of the basal sediments in this lake.

DISCUSSION

Smith and Barko (1996) provided several criteria for assessing macrophyte declines: duration, scope (species affected) and geographic extent. In the case of Cenaiko Lake the decline was of one species (Eurasian watermilfoil), had a duration of several years and was local, i.e., declines did not

TABLE 2. DENSITY (N/M² ± 2 SE AND N PER STEM) OF *EUHRYCHIOPSIS LECONTEI* (*E.L.*) LARVAE, PUPAE AND ADULTS, AND *ACENTRIA EPHEMERELLA* AND *PARAPOYNX* SP. AT CENAIKO LAKE IN 1996-1998. DENSITIES PER STEM (A BASAL MILFOIL STEM EMERGING FROM THE SEDIMENT) WERE ONLY CALCULATED FOR SAMPLES WITH EURASIAN WATERMILFOIL. *RESULTS OF THE SEPTEMBER 1998 STEM SURVEY (5 STEMS FROM EACH OF 18 STATIONS) ARE PRESENTED FOR COMPARISON AND THE TOTAL FOR THIS SURVEY INCLUDES EGGS.

Date	n	Larvae N/m²	Pupae N/m²	Adults N/m²	Total *E.l.* N/m²	*Acentria* N/m²	*Parapoynx* N/m²
7/22/96	29	49 ± 25	23 ± 11	32 ± 14	103 ± 42	18 ± 8	1 ± 2
per stem	26	0.92 ± 1.29	0.34 ± 0.46	0.38 ± 0.28	1.64 ± 1.97		
9/5/96	21	3 ± 2	1 ± 1	4 ± 4	8 ± 6	32 ± 20	0 ± 0
per stem	8	0.23 ± 0.26	0.01 ± 0.02	0.42 ± 0.52	0.65 ± 0.72		
7/16/97	26	2 ± 2	0 ± 0	0 ± 0	2 ± 2	9 ± 6	0 ± 0
per stem	3	0.39 ± 0.40	0.00 ± 0.00	0.00 ± 0.00	0.39 ± 0.40		
9/17/97	24	0 ± 0	0 ± 0	0 ± 0	0 ± 0	32 ± 20	2 ± 2
per stem	6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
6/16/98	25	0.4 ± 0.8	0 ± 0	0 ± 0	0.4 ± 0.8	18 ± 9.1	0.4 ± 0.8
per stem	15	0.004 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.004 ± 0.01		
7/29/98	25	0 ± 0	0 ± 0	1 ± 2	1 ± 2	2 ± 2	0.4 ± 0.8
per stem	12	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.04	0.02 ± 0.04		
9/14/98	25	0 ± 0	0 ± 0	0 ± 0	0 ± 0	6 ± 5	22 ± 20
per stem	3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
9/2/98*	—	—	—	—	—	—	—
per stem	18	1.98 ± 0.66	0.16 ± 0.11	0.61 ± 0.20	3.60 ± 0.84		

TABLE 3. BIOMASS (G DRY/M²) OF ALL PLANT SPECIES COLLECTED AT CENAIKO LAKE, PERCENT OF BIOMASS THAT WAS EURASIAN WATERMILFOIL (%MSP, BASED ON THE AVERAGE OF SAMPLE PERCENTS), NON-MILFOIL BIOMASS (NONMSP) AND MEAN NUMBER OF SPECIES PER SAMPLE (NO. SPP.). SPECIES ABBREVIATIONS ARE: CHA = *CHARA* SPP.; CRT = *CERATOPHYLLUM DEMERSUM*; MSP = *MYRIOPHYLLUM SPICATUM*; MGD= *MEGALODONTA* (=*BIDENS*) *BECKII*, NAJ = *NAJAS FLEXILIS*.; PAM = *POTAMOGETON AMPLIFOLIUS*; PCR = *POTAMOGETON CRISPUS*; PEC = *POTAMOGETON PECTINATUS*; PFO = *POTAMOGETON FOLIOSUS*; PNO = *POTAMOGETON NODOSUS*; ZOS = *ZOSTERELLA DUBIA*. N > 20 ON ALL SAMPLING DATES. IN JULY 1997, *POTAMOGETON ZOSTERIFORMIS* WAS PRESENT AT 0.1 G/M² AND IN JUNE 1998, *ELODEA CANADENSIS* AND *MYRIOPHYLLUM SIBIRICUM* WERE PRESENT AT 0.05 G/M².

Date	Total	MSP	% MSP	Non-MSP	CRT	MGD	NAJ	PCR	ZOS	PEC	CHA	PAM	PNO	PFO	No. Spp.
7/22/96	242.9	122.9	63.0%	120.0	95.5	0.0	0.2	0.8	0.3	2.2	20.7	0.0	0.0	0.0	2.5
1 S.E.	38.0	22.3	6.8%	38.0	34.4	0.0	0.2	0.4	0.3	1.2	20.2	0.0	0.0	0.0	0.2
9/23/96	213.1	23.4	34.6%	189.8	146.0	0.0	0.0	0.0	0.1	2.6	41.1	0.0	0.0	0.0	2.3
1 S.E.	63.9	6.9	7.8%	64.8	56.0	0.0	0.0	0.0	0.1	1.5	39.9	0.0	0.0	0.0	0.2
7/16/97	97.0	0.9	3.7%	96.1	81.6	0.0	1.5	2.0	0.9	5.8	0.0	0.0	3.4	0.8	2.1
1 S.E.	27.5	0.7	2.8%	27.4	28.2	0.0	1.5	0.7	0.5	2.6	0.0	0.0	2.8	0.7	0.2
9/17/97	130.0	4.7	6.5%	125.3	86.1	0.0	0.2	1.6	12.5	7.5	0.1	12.2	5.1	0.0	2.2
1 S.E.	30.3	2.1	3.6%	30.3	30.3	0.0	0.2	1.1	6.5	2.8	0.1	12.2	4.0	0.0	0.2
6/18/98	265.3	44.2	15.7%	221.1	158.4	0.0	11.7	13.4	12.8	1.4	13.4	1.3	8.7	0.0	3.0
1 S.E.	40.0	16.2	5.0%	38.4	40.7	0.0	10.2	9.6	7.0	0.5	7.9	1.3	8.7	0.0	0.2
7/29/98	380.4	44.4	14.0%	335.7	189.3	0.0	5.7	0.0	36.9	4.8	64.8	32.8	1.4	0.0	2.9
1 S.E.	82.7	20.0	4.7%	81.5	58.4	0.0	4.2	0.0	16.7	2.5	59.2	23.3	1.3	0.0	0.3
9/14/98	247.7	11.8	6.9%	235.9	142.3	0.4	9.1	0.2	40.2	15.7	4.6	17.0	6.4	0.0	3.0
1 S.E.	41.4	4.8	2.9%	42.0	43.0	0.4	6.6	0.1	13.8	7.1	4.6	14.4	6.4	0.0	0.2

occur regionally. The extensive damage we observed, associated with high densities of the milfoil weevil, provides strong evidence that the decline of Eurasian watermilfoil was due to stem mining by weevil larvae. Many of the plants in July 1996 had darkened, mined stems, and over 50% of the meristems were missing or damaged. Although pathogens may have assisted the decline, inspection of plant samples by Dr. Judy Shearer (US Army Engineer Research and Development Center, Vicksburg, MS) indicated few fungi and those that occurred were likely opportunistic saprophytes. Densities of the two other milfoil herbivores, *Acentria ephemerella* and *Parapoynx* sp. increased after the decline had started. Although *A. ephemerella* were found in samples with Eurasian watermilfoil in 1996, it appeared more strongly associated with coontail, particularly in 1997 when milfoil was not found in most samples that contained *A. ephemerella*. Johnson et al. (1998) reported declines of Eurasian watermilfoil associated with densities of *A. ephemerella* in excess of 100/m². Given the much lower densities of *A. ephemerella* than *E. lecontei* in July 1996 and *A. ephemerella*'s typical occurrence on other plants, we do not believe that *A. ephemerella* was a major cause of the decline; however, it may have partially caused the decline and may have prevented a resurgence of Eurasian watermilfoil in 1997. The partial resurgence of Eurasian watermilfoil in 1998 was accompanied by increased weevil densities and a subsequent decline of Eurasian watermilfoil to <12 g/m². During 1999 Eurasian watermilfoil densities remained below 1 g/m² (<10% of total plant biomass) and biweekly stem surveys revealed combined larvae, pupae and adult densities ranging form 0.07 per stem to 0.9 per stem over the summer (Newman et al. unpublished data).

TABLE 4. PERCENT (OF DRY MASS) SUGAR (TS), STARCH (STARCH) AND TOTAL NONSTRUCTURAL CARBOHYDRATES (TNC) ± 1SE FOR ROOTS, STEMS AND LEAVES AT CENAIKO LAKE IN 1996-1998. N = 6-9 IN JULY 1996 AND ALL OF 1998. IN SEPTEMBER 1996, ONLY 3 SAMPLES WITH USABLE MATERIAL WERE COLLECTED AND FEW ROOTS WERE OBTAINED. IN 1997 NO PLANTS WERE COLLECTED IN JULY AND 5 SAMPLES WERE COLLECTED IN SEPTEMBER, HOWEVER THE ROOT MASS OF THE COLLECTED PLANTS WAS TOO LOW FOR GOOD TNC DETERMINATION. VALUES WITH NO SE'S HAD ONLY ONE ESTIMATE.

Tissue	Date	% TNC	% TS	% Starch
Roots	Jul-96	8.2% ± 1.2%	0.9% ± 0.2%	7.4% ± 1.1%
	Sep-96	—	0.4% ± .	—
	Sep-97	—	0.8% ± .	—
	Jun-98	6.4% ± 0.7%	4.3% ± 1.3%	2.5% ± 0.5%
	Jul-98	6.1% ± 0.8%	3.0% ± 0.9%	3.3% ± 0.3%
	Sep-98	5.0% ± 0.5%	2.0% ± 0.4%	3.4% ± 0.5%
Stems	Jul-96	24.2% ± 3.9%	0.8% ± 0.1%	23.3% ± 4.0%
	Sep-96	1.6% ± .	0.4% ± 0.0%	1.2% ± .
	Sep-97	3.8% ± 0.19%	0.8% ± 0.1%	3.0% ± 0.2%
	Jun-98	13.2% ± 1.4%	8.8% ± 1.4%	4.4% ± 1.4%
	Jul-98	12.6% ± 1.7%	8.2% ± 1.4%	5.6% ± 1.6%
	Sep-98	9.8% ± 1.0%	7.7% ± 1.5%	2.1% ± 1.1%
Leaves	Jul-96	6.5% ± 1.3%	0.4% ± 0.00%	7.0% ± 1.1%
	Sep-96	3.9% ± .	0.4% ± 0.00%	3.5% ± .
	Sep-97	2.7% ± 0.3%	0.4% ± 0.00%	2.3% ± 0.3%
	Jun-98	3.4% ± 0.3%	0.4% ± 0.1%	3.0% ± 0.3%
	Jul-98	3.8% ± 0.4%	0.5% ± 0.1%	3.3% ± 0.4%
	Sep-98	4.7% ± 0.4%	0.9% ± 0.2%	3.8% ± 0.3%

A parallel decline in other aquatic macrophyte species was not observed, and in fact, populations of native plants increased. Although declines at several other lakes were noted in 1996, Eurasian watermilfoil increased in other lakes and no general patterns of decline occurred in the lakes of our region (Newman et al. 1998a). These observations along with relatively good water clarity indicate that the decline was not likely due to climatic or regional environmental factors.

Although sediment ammonium was quite low in 1996, and the sediment of Cenaiko Lake is generally poor, we do not believe sediment nutrient depletion was the cause for the decline. The poor sediment conditions, however, may have facilitated the weevil-induced decline. Sediment ammonium increased in 1997 and 1998, after the decline, and the low

levels of ammonium in 1996 may have reduced the ability of the plant to outgrow weevil damage.

Furthermore, the extensive weevil damage may have reduced milfoil carbohydrate stores, which suppressed the ability of Eurasian watermilfoil to recover in 1997, even with higher sediment ammonium. The plants were so severely damaged that root samples collected with the plants in late 1996 and 1997 were too small to accurately determine carbohydrate levels. Although we do not have a quantitative estimate of root mass, it appears that weevil damage may have reduced both carbohydrate levels and root mass as observed in a tank experiment (Newman et al. 1996). The weevil-induced decline at Cenaiko Lake may have been facilitated by poor sediment conditions that reduced the plant's ability to regrow and over-winter following severe weevil damage.

Two factors are essential to effective biological control of weeds: adequate densities of control agents and proper target weed response (Newman et al. 1998b). In July 1996, weevil density in Cenaiko Lake was the highest recorded in Minnesota since monitoring began in 1992 (Newman et al. 1998a). Weevil density may have been higher earlier in the season when the milfoil would have been less damaged. This watermilfoil decline suggests that weevil densities of $100/m^2$ or 1.5 per stem are adequate to control Eurasian watermilfoil; however, as noted above, the poor sediment conditions (low nutrients and organics) may have precipitated a decline at lower weevil densities than would be effective elsewhere. These weevil densities compare well with surveys in Wisconsin where densities of weevils ranged from zero to about 2 per apical stem; densities >1 per stem occurred in only 5 of 14 lakes surveyed (Jester et al. 1997). The Wisconsin results are not directly comparable to our estimates from biomass sampling (Table 2) because they used apical stems (vs our basal stems) and eggs were included (typically 30-50% of the total for high-density lakes, L. L. Jester, pers. comm.). However, our stem survey of all life stages in September 1998 indicated very high densities of weevils, 3.6 total per stem and 0.6 adults per stem, comparable to the highest densities reported elsewhere (Jester et al. 1997, Sheldon 1997b). This density of weevils is clearly adequate to cause a decline. Densities of ≥0.5 weevils per stem have been associated with milfoil declines and suppression in Vermont (Creed and Sheldon 1995, Sheldon 1997b). These observations suggest that densities of 1.5 weevils per stem or 100 per m^2 may be sufficient to cause declines in some instances, with declines occurring at even lower densities (e.g., 0.5 per stem, Sheldon 1997b).

Sutter and Newman (1997) suggested that predation by sunfish might be a factor limiting agent densities in some lakes. Sunfish were not commonly noted while sampling in Cenaiko Lake and although 1992 MN DNR survey records indicated high densities of hybrid sunfish, equal to densities of sunfish in lakes we have sampled that also have very low densities of weevils, the most recent DNR survey in 1998 indicated a very low density of sunfish. Thus, we have some evidence that the high weevil densities in Cenaiko Lake may have been possible due to low predation by sunfish, but more detailed investigation of this limiting factor is warranted. Clearly, identification of the factors that permit such high densities of weevils in Cenaiko Lake would enhance our ability to predict and effect control elsewhere.

The second important factor in successful control of weeds is a favorable target weed response. Suppression of milfoil growth and reductions in carbohydrate reserves were seen in Cenaiko. In addition, the native plant community remained intact and appeared to replace some of the damaged milfoil. The increased growth and biomass of the native plant community, probably aided by maintenance of good water clarity, may be essential to effective milfoil control, as has been seen for other terrestrial and aquatic weeds (Newman et al. 1998b). Our observations at other lakes in Minnesota suggest that a positive native plant community response to Eurasian watermilfoil damage may be essential for effective control.

Creed (1998) reviewed declines of Eurasian watermilfoil and concluded that damage by the milfoil weevil was likely responsible for a significant number of the declines. Unfortunately, most of the declines were poorly documented, lacking quantitative data on milfoil, other plant and weevil densities, along with information on water and sediment quality. Creed's (1998) broad-based correlation, along with the few well-documented studies (e.g., Creed and Sheldon 1995, Sheldon 1997b, this study), indicates that the weevil can cause declines. However, key issues remain regarding the extent and predictability of weevil-induced declines. Larger scale controlled field experiments with augmentations of agents are needed to demonstrate repeatable declines. More work providing quantitative data on milfoil, weevils and plant community response, along with identification of factors limiting weevil densities and their effects on plants is needed before milfoil weevils can be relied upon as effective biological control agents.

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