Evaluation of the Efficacy of Herbivorous Insects to Control Eurasian Watermilfoil Growth in Lake Bonaparte, New York

2009 Report

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Cover Photos
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Weevil larvae and Moth caterpillar
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We submit this report, Evaluation of the Efficacy of Herbivorous Insects to Control Eurasian Watermilfoil Growth in Lake Bonaparte, New York authored by R. L. Johnson, Toner J. A., Johnson, J. D., Sledziona, N., Pyne N. E., Riggs J. M. and Christina Killourhy, May 2010 to the Lake Bonaparte Conservation Club in fulfillment of our 2009 contract.

Executive Summary

In 2009, we conducted research on the effectiveness of aquatic herbivores to control the growth of Eurasian watermilfoil (*Myriophyllum spicatum*) a major plant weed pest in Lake Bonaparte. This effort is a continuation of Cornell University research begun in 1991 that focuses on herbivores that eat watermilfoil, their populations and any damage they cause to the plant. The Department of Ecology and Evolutionary Biology at Cornell University has monitored herbivore populations and damage to watermilfoil in Lake Bonaparte since 2003.

2009 Major Findings

- Weevil (*Euhrychiopsis lecontei*) densities at location S7A in the summer of 2009 at 1.5 weevils per apical stem were significantly greater ($p < 0.0001$) than the summer of 2008 at 0.45 weevils per apical stem where introduction of adult weevils by Cornell University occurred July 2008. The 2009 analysis includes herbivore densities reported on 6/15, 7/10 and 7/31/2009 (Tables 1, 2, 3).

- Weevil (*Euhrychiopsis lecontei*) densities at location S9 in the summer of 2009 at 0.73 weevils per apical stem were significantly greater ($p < 0.006$) than the summer of 2008 at 0.43 weevils per apical stem where introduction of adult weevils by Cornell University occurred July 2008. The 2009 analysis includes herbivore densities reported on 6/15, 7/10 and 7/31/2009 (Tables 1, 2, 3).

- Weevil (*Euhrychiopsis lecontei*) densities at location S8 in the summer of 2009 at 0.03 weevils per apical stem were not significantly different ($p < 0.566$) than the summer of 2008 at 0.013 weevils per apical stem where introduction of weevil eggs/larvae by EnviroScience, Inc. occurred June 2008. The 2009 analysis includes herbivore densities reported on 6/15, 7/10 and 7/31/2009 (Tables 1, 2, 3).

- Moth (*Acentria ephemerella*) showed high densities in 2009 at sites S2A and S7 locations where Cornell University added adult weevils in 2009. Additionally, location S2 and S7A located in the same bays had persistent high moth populations through the summer (Table 1, Figures 7a, 8a, 11a, 12a).

- Moth (*Acentria ephemerella*) has been recorded at high densities in another high population bluegill lake where Cornell University has added 25,000 adult weevils in one location. This may suggest a high watermilfoil herbivore population by adding either weevils or moths may result in a higher population of all herbivores, the weevil, moth and others or too many for bluegills to eat.

- Bluegill (*Lepomis macrochirus*) appears to consume a significant number of watermilfoil herbivores as indicated by analysis of their stomach contents from a single sampling at location S7 on 7/31/2009. The weevil larvae appear to be particularly venerable and may be partially due to not having legs.

Recommendations

- Future augmentation with the weevil (*Euhrychiopsis lecontei*) in Lake Bonaparte should be by adding adult weevils at the start of summer rather than adding very risky weevil eggs/larvae that have up to a 70% loss without fish predation (Cornell University and University of Minnesota weevil research). In light of the new stomach prey data from bluegills in this report, the loss from predation by bluegill of slow moving legless weevil larvae on augmented watermilfoil clusters could be very great.

- Consider conducting an aquatic plant survey in the future that describes the plant community and estimates the abundance of individual plant species at specific locations on the lake.

- Consider requesting an estimate of the fish population, especially sunfish, for Lake Bonaparte by electrofishing or trap netting using the NYDEC approved protocol to estimate an all fish catch per unit effort of the littoral zone.

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Introduction

We, at the Cornell University Research Ponds in the Department of Ecology and Evolutionary Biology, conducted research on Lake Bonaparte in 2009 under a contract with the Lake Bonaparte Conservation Club. The Lake Bonaparte Conservation Club's long-term goal is to reduce the impacts of the invasive exotic Eurasian watermilfoil (*Myriophyllum spicatum*) on recreational uses as well as the macrophyte community of the lake. Our objective in 2009 was to assess any differences in resulting herbivore population numbers that might arise by the augmentation of a different life stage of the Eurasian watermilfoil herbivore *Euhrychiopsis lecontei* in 2008 and 2009. Specifically in 2008, we compared the company EnviroScience, Inc.'s Middfoil® process of introducing EnviroScience Eurasian watermilfoil with eggs and newly hatched larvae to Lake Bonaparte to Cornell University's release of adult weevils to the lake. In 2009, we are accessing the 2008 augmented locations for any change in herbivore populations from 2008 and we are adding weevil adults to two locations in an attempt to increase populations of herbivores at those locations.

In 2003, we began measuring the densities of watermilfoil herbivores at nine locations within the lake. In 2002 and 2003, the company EnviroScience, Inc. chose to add eggs and larvae of E. lecontei to six of the locations and reserve three locations as controls with no additions. We continued to measure populations at those nine locations through 2009, with the exception of EnviroScience's control C, a rock in the lake that would have little chance of being weevil habitat. We replaced control C in 2003 with Cornell control D, a remote location distanced very far from any augmented locations. Population estimates made from our field data collection from 2003 – 2008 show little or no increase in weevil populations at EnviroScience, Inc., augmented locations above the control locations that did not receive weevil eggs and larvae. Some variability or unpredictability of measurement data is common in most, if not all, lake ecosystems such as Lake Bonaparte. However, because of our multi-year monitoring some trends begin to emerge, especially related to insect population densities and the damage they cause.

The most interesting question is why from 2002-2008 the EnviroScience Inc. augmentation program at Lake Bonaparte has not resulted in any significant increase in an overall lake-wide weevil population greater than the population that would be predicted if the lake had not been augmented with eggs/larvae of *Euhrychiopsis*. Most lakes in New York State that have not had eggs/larvae added to them have a much greater weevil population lake-wide (Johnson et al. 2008). Additional data included in the EnviroScience, Inc. report (January 2008) to The Lake Bonaparte Conservation Club shows very little evidence of weevil presence or weevil feeding in the lake at augmentation or after.

This report will suggest an additional possible reason, to reasons suggested already in Johnson et al. 2009, why this massive augmentation of eggs/larvae by EnviroScience, Inc., occurring annually since 2002 (LaMere 2008) has not resulted in a greater population of weevils in the lake.

Lastly, we recorded in 2009 a great increase in the population of the indigenous moth *Acentria ephemerella* at locations where we added large numbers of weevil adults. We have observed this before in a lake where we augmented about 25,000 weevil adults in one location. This may have important implications about augmenting herbivore populations and indicate a direction to make biological control of Eurasian watermilfoil more effective.

Methods

Eurasian watermilfoil herbivore and watermilfoil damage surveys

In 2009, we conducted measurements of watermilfoil herbivore populations and herbivore damage to watermilfoil at our historical sampling locations as well as specific locations selected and established in 2008 by EnviroScience, Inc. and Cornell University to add *E. lecontei*. We also measured in 2009 all the previous year's locations and an additional location S2A (Figure 1). Figure 1 shows the GPS locations as UTM coordinates where EnviroScience, Inc., augmented eggs/larvae of the weevil in previous years and the historical EnviroScience control locations A and B, as well as the Cornell Control D location (a replacement for EnviroScience Control C).

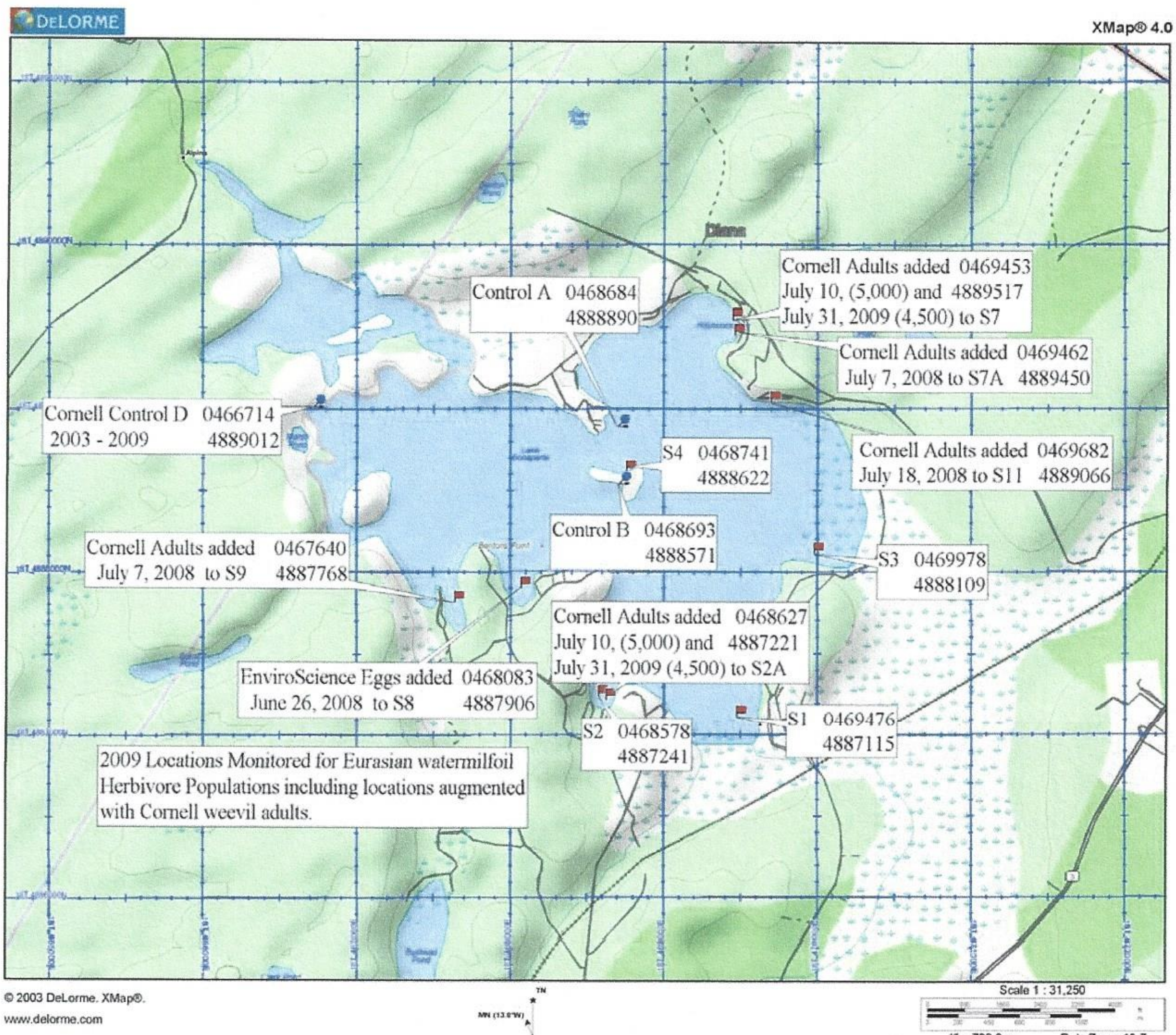


Figure 1. 2009 Lake Bonaparte map showing locations where we made population estimates and augmented with adult weevils by Cornell University and additional locations where we only made population measurements in 2009. Coordinates for locations are UTM (NAD27 datum and true north).

Additionally, we show the locations of the 2008 experiment from which we measured herbivore population densities where EnviroScience, Inc. added weevil eggs/larvae at location S7 and location S8, while we added adult weevils at location S7A and S9 all in 2008.

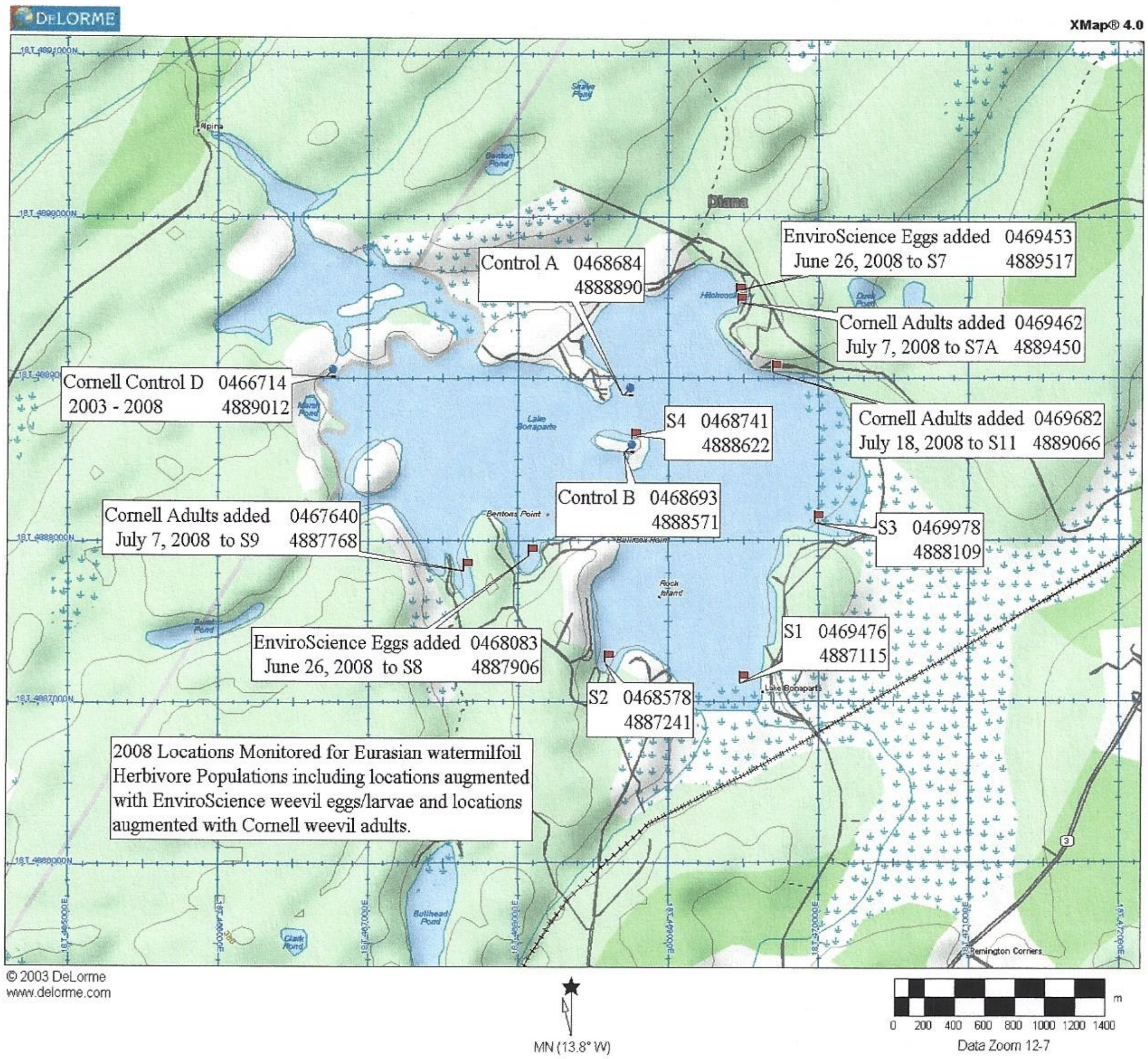


Figure 2. 2008 Lake Bonaparte map (Johnson et al. 2009) showing locations augmented with eggs/larvae by EnviroScience, Inc., and locations augmented by Cornell University with adult weevils where we made population measurements in 2008. We also made measurements at the additional locations on the map in 2008. Coordinates for locations are UTM (NAD27 datum and true north).

We sampled in 2009, 9 of our established historical herbivore sampling locations in Lake Bonaparte along with the additional locations established in 2008 and 2009 to evaluate the effectiveness of two different methods of adding *E. lecontei*. The nine historical locations we have sampled since 2003 are S1 (known alternatively as S1, S1A, S6, or S6A), S2 (S2, S2A, S2B), S3, S4, S7 (S7, S18), S11, Control A, Control B and Cornell Control D. We included data collected from previous sampling years in the results and discussion section (Johnson et al. 2009). We summarized the herbivore population densities recorded during 2009 at all locations in tables and graphs that follow in the results and discussion section of this report. For populations reported in 2008 see Johnson et al. 2009.

We used in 2009 all historical and the specific locations S7, S8 ,S9, S7A and S2A to contrast and evaluate any differences in resulting herbivore populations that might arise by adding adult or different weevil life stages. The Cornell University treatments tested and evaluated in 2009 included previous treatments in 2008 and new treatments in 2009 where we augmented 5,000 adult weevils on July 10 at each of the two locations S7 and S2A. On July 31, we added 4,500 additional adult weevils to each location S7 and S2A. We sampled all the experimental locations on June 10 and July 31 to determine herbivore population densities before the augmentations on those days.

At each sampling location, we randomly collected a series of aquatic plant samples using a grapple hook formed by connecting the "heads" of two garden rakes back-to-back. In the boat, we blindly selected twenty-five watermilfoil stems from our "rake-toss" samples (no more than five from each rake toss) by choosing them from their basal ends. We then pinched off the top 25 cm of each stem, (the apical stem) for our sample. We placed each apical stem into an individually labeled plastic zipper bag and stored all samples in a cooler chest for transport to our laboratory.

In the laboratory, we refrigerated all samples until we microscopically examined each apical stem. Apical stems and herbivores are stored in the refrigerator for up to two weeks, and we froze any samples for later analysis that we could not examine within two weeks. At the time of examination, we placed each apical stem under a stereoscopic dissecting microscope. We dissected each stem and evaluated the entire sample, recording numbers and types of herbivores found, evidence of herbivore use (e.g., retreats, cocoons, or pupae chambers), and plant tissue damage (leaflet damage, stem mining, missing or grazed apical meristems).

For each apical stem sampled, we identified, counted and recorded all life stages (eggs, larvae, pupae and adults) of each herbivore species found. We qualified and quantified all watermilfoil tissue damage using a consistent scoring system we developed in our laboratory. Finally, we calculated the numbers of weevils and moths per apical stem, including individuals in all life stages. Using this standard protocol, we are able to determine which herbivores are responsible for particular types of damage and can assess the amount of plant damage caused by each herbivore.

Evaluation of consumed prey in small bluegill stomach and intestines

We examined the stomach contents of 28 small Lake Bonaparte bluegill collected from location S7 on 7/31/2009, where we augmented weevil adults in 2009. We identified and counted prey for each individual fish and recorded in Tables 4 and 5. We show in graphs the identification, number and percentage of prey consumed (Figures 16, 17 and 18) as well as fish size distribution (Figure 19).

Results and Discussion

In 2009, the locations described in the methods section and sampled by us to determine Eurasian watermilfoil herbivore densities we list below (Table 1).

Table 1. Mean numbers of weevils (all life stages – eggs, larvae, pupae and adults) and moths (eggs, larvae and pupae) recorded on milfoil apical stems and a mean damage rating for apical stems at Lake Bonaparte locations for 2009.

Lake Area	Plot	Date	No. of Apical Stems	Weevils per apical stem mean (SE)	Moths per apical stem mean (SE)	Damage Rating mean (SE)
Hotel Road	S1	6/15/2009	25	0.60 (0.17)	0.00	1.08 (0.17)
	S1	7/10/2009	25	0.72 (0.21)	0.16 (0.09)	1.48 (0.20)
	S1	7/31/2009	25	1.48 (0.27)	0.12 (0.07)	2.32 (0.24)
	S1	9/09/2009	25	0.40 (0.12)	0.08 (0.06)	2.28 (0.16)
Baron's Bay	S2	6/15/2009	25	0.12 (0.07)	0.16 (0.09)	1.56 (0.23)
	S2	7/10/2009	25	0.28 (0.14)	0.92 (0.19)	2.44 (0.18)
	S2	7/31/2009	25	0.44 (0.13)	0.36 (0.13)	2.36 (0.22)
	S2	9/09/2009	25	0.36 (0.13)	0.20 (0.08)	2.44 (0.12)
Baron's Bay	S2A	7/31/2009	25	1.00 (0.22)	7.48 (6.82)	3.24 (0.12)
	S2A	9/09/2009	30	0.53 (0.12)	0.36 (0.15)	2.70 (0.15)
Bulrush Bay	S3	6/15/2009	25	0.12 (0.09)	0.00	1.68 (0.21)
	S3	7/10/2009	25	0.00	0.04 (0.04)	1.92 (0.15)
	S3	7/31/2009	25	0.00	0.00	2.08 (0.15)
	S3	9/09/2009	25	0.04 (0.04)	0.00	2.32 (0.11)
Birch Bay	S4	6/15/2009	25	0.20 (0.08)	0.00	1.20 (0.17)
	S4	7/10/2009	25	0.08 (0.06)	0.00	1.36 (0.19)
	S4	7/31/2009	25	0.00	0.00	1.68 (0.21)
	S4	9/09/2009	25	0.00	0.00	1.08 (0.14)
Hitchcock Bay	S7	6/15/2009	25	0.60 (0.16)	7.24 (6.91)	1.80 (0.21)
	S7	7/10/2009	25	0.72 (0.23)	11.56 (10.56)	2.76 (0.14)
	S7	7/31/2009	25	0.64 (0.16)	0.48 (0.14)	2.92 (0.18)
	S7	9/09/2009	25	0.44 (0.14)	0.52 (0.15)	3.08 (0.14)
Hitchcock Bay	S7A	6/15/2009	25	0.84 (0.21)	0.20 (0.08)	1.92 (0.22)
	S7A	7/10/2009	25	2.40 (0.49)	1.12 (0.27)	2.64 (0.16)
	S7A	7/31/2009	25	1.16 (0.37)	0.08 (0.06)	2.64 (0.17)
	S7A	9/09/2009	25	0.48 (0.13)	0.20 (0.10)	2.88 (0.16)
Baldwin's Bay	S8	6/15/2009	25	0.04 (0.04)	0.04 (0.04)	0.92 (0.17)
	S8	7/10/2009	25	0.00	0.00	1.16 (0.19)
	S8	7/31/2009	25	0.04 (0.04)	0.00	0.48 (0.16)
	S8	9/09/2009	25	0.16 (0.12)	0.00	1.48 (0.22)
Tamarack Bay	S9	6/15/2009	25	0.88 (0.18)	0.04 (0.04)	1.36 (0.19)
	S9	7/10/2009	25	1.08 (0.20)	0.16 (0.09)	1.72 (0.20)
	S9	7/31/2009	25	0.24 (0.10)	0.08 (0.06)	2.40 (0.24)
	S9	9/09/2009	25	0.04 (0.04)	0.08 (0.06)	1.92 (0.19)
Sister's Island	S11	6/15/2009	25	0.28 (0.17)	0.08 (0.06)	1.52 (0.19)
	S11	7/10/2009	25	0.24 (0.10)	0.04 (0.04)	1.84 (0.21)
	S11	7/31/2009	25	0.12 (0.07)	0.00	1.84 (0.23)
	S11	9/09/2009	25	0.40 (0.13)	0.00	2.32 (0.19)
Control	A	6/15/2009	25	0.08 (0.06)	0.00	1.76 (0.23)
	A	7/10/2009	25	0.08 (0.06)	0.04 (0.04)	1.68 (0.18)
	A	7/31/2009	25	0.00	0.00	1.96 (0.14)
	A	9/09/2009	25	0.00	0.00	1.28 (0.20)
Control	B	6/15/2009	25	0.32 (0.14)	0.04 (0.04)	1.20 (0.20)
	B	7/10/2009	25	0.00	0.00	1.36 (0.19)
	B	7/31/2009	25	0.08 (0.06)	0.00	1.40 (0.17)
	B	9/09/2009	25	0.00	0.00	0.96 (0.20)
Cornell Control	D	6/15/2009	25	0.24 (0.09)	0.08 (0.06)	0.88 (0.24)
	D	7/10/2009	25	0.72 (0.20)	0.32 (0.13)	2.00 (0.20)
	D	7/31/2009	25	0.16 (0.09)	0.04 (0.04)	1.32 (0.20)
	D	9/09/2009	25	0.16 (0.07)	0.08 (0.08)	1.72 (0.21)

Table 1 above, shows the results of our 2009 microscopic screening of collected watermilfoil apical stems and lists the densities of two common watermilfoil herbivores, the aquatic weevil *Euhrychiopsis lecontei* and the aquatic moth *Acentria ephemerella*. The 2009 data shown below in Table 2 and 3 are the mean number of weevils per apical stem found on the three collection dates in the summer for all the locations we sampled. The densities of herbivores appear to be reasonable estimates of populations feeding on watermilfoil. The one exception is S1 (the bay at Hotel Road) where since the spring of 2007 very little watermilfoil can be found. In this large bay in 2007, we identified and notified the stakeholders of the presence of the exotic invasive macro algae *Nitellopsis obtusa*. From 2007 through 2009, this exotic plant overwhelms and limits watermilfoil growth within the bay. Without abundant watermilfoil in the bay, the thousands of weevils overwintering on shore entered the lake in 2007 through 2009, concentrating on the few watermilfoil plants present at S1, and abnormally increased the number of weevils per apical stem we measured.

To explore the statistical significance of the results obtained from the comparison of the two methods of augmenting the watermilfoil herbivore *E. lecontei* we used the nonparametric test Mann-Whitney (at <0.05 p) which tests if the medians of the collected data are equal or not. This statistical test showed a highly significant difference between the methods of adding the weevil to Lake Bonaparte for both paired locations in 2008 (Johnson et al. 2009). The adding of 4500 adults at S7A by Cornell resulted in a significantly ($P< 0.0034$) greater number of weevils (all life stages) found on the combined sampling dates 7/18 and 8/14/2008 than at location S7 where EnviroScience added 5400 eggs/larvae.

In 2008 at the Cornell location S9 where 4500 adults were added, the difference in the resulting number of weevils for the combined sampling dates 7/18 and 8/14/2008 when contrasted to S8 where EnviroScience added 4400 eggs/larvae was even larger, at a greater significance of ($P< 0.00001$). Herbivore data collected on 10/23/2008 (Johnson et al. 2009), were not included in the 2008 analysis, because the majority of weevils are overwintering and hibernating on shore by early fall (Johnson and Blossey 2002).

In 2009, weevil (*Euhrychiopsis lecontei*) densities at location S7A in the summer of 2009 at 1.5 weevils per apical stem were significantly greater ($p< 0.0001$) than the summer of 2008 at 0.45 weevils per apical stem where introduction of adult weevils by Cornell University occurred July 2008. The 2009 analysis includes herbivore densities reported on 6/15, 7/10 and 7/31/2009 (Tables 1, 2, 3). Weevil densities at location S9 in the summer of 2009 at 0.73 weevils per apical stem were significantly greater ($p< 0.006$) than the summer of 2008 at 0.43 weevils per apical stem where introduction of adult weevils by Cornell University occurred July 2008. The 2009 analysis includes herbivore densities reported on 6/15, 7/10 and 7/31/2009 (Tables 1, 2, 3).

At location S8, weevil densities in the summer of 2009 at 0.03 weevils per apical stem were not significantly different ($p< 0.566$) than the summer of 2008 at 0.013 weevils per apical stem where introduction of weevil eggs/larvae by EnviroScience, Inc. occurred June 2008. The 2009 analysis includes herbivore densities reported on 6/15, 7/10 and 7/31/2009 (Tables 1, 2, 3).

Of special interest the moth (*Acentria ephemerella*) showed high densities in 2009 at sites S2A and S7 locations where Cornell University added adult weevils in 2009 and additionally, location S2 and S7A located in the same bays had persistent high moth populations through the summer (Table 1, Figures 7a, 8a, 11a, 12a). The moth has been recorded at high densities in another high population bluegill lake where Cornell University added 25,000 adult weevils in one location. This may suggest a high watermilfoil herbivore population results in a higher population of all herbivores, the weevil, moth and others in spite of bluegill sunfish populations. Possibly the fish have more than enough to eat and herbivore populations increase.

Table 2. Descriptive statistics for 2009 and 2008 Lake Bonaparte's estimated weevil population (total weevil life stages per apical stem) counted from 25 stems for 6/15, 7/10 and 7/31/2010.

Location	Year	# Stems	Mean	Median	Treatment Mean	St Dev	SE Mean
A	2009	75	0.053	0.0	0.000	0.226	0.026
B	2009	75	0.133	0.0	0.045	0.445	0.051
D	2009	75	0.373	0.0	0.269	0.712	0.082
S1	2009	75	0.933	1.0	0.806	1.155	0.133
S2	2009	75	0.280	0.0	0.194	0.583	0.067
S2A	2009	55	0.745	0.0	0.653	0.927	0.125
S3	2009	75	0.040	0.0	0.000	0.257	0.030
S4	2009	75	0.093	0.0	0.045	0.293	0.034
S7	2009	75	0.653	0.0	0.522	0.937	0.108
S7A	2009	75	1.467	1.0	1.209	1.968	0.227
S8	2009	75	0.027	0.0	0.000	0.162	0.019
S9	2009	75	0.733	0.0	0.642	0.890	0.103
S11	2009	75	0.213	0.0	0.105	0.599	0.069
A	2008	75	0.040	0.0	0.000	0.197	0.023
B	2008	75	0.013	0.0	0.000	0.116	0.013
D	2008	75	0.107	0.0	0.045	0.352	0.041
S1	2008	75	0.507	0.0	0.343	1.032	0.119
S2	2008	75	0.453	0.0	0.373	0.703	0.081
S3	2008	75	0.000	0.0	0.000	0.000	0.000
S4	2008	75	0.000	0.0	0.000	0.000	0.000
S7	2008	75	0.187	0.0	0.105	0.512	0.059
S7A	2008	75	0.453	0.0	0.328	0.827	0.096
S8	2008	75	0.013	0.0	0.000	0.116	0.013
S9	2008	75	0.427	0.0	0.284	0.888	0.103
S11	2008	75	0.147	0.0	0.060	0.485	0.056

Table 3. Mann – Whitney statistical test of significance comparing 2009 to 2008 at sampled locations to test any difference in populations of weevils (all life forms and eggs/larvae vs. adults).

Location	2009	2008	p-Value	Sig at P<0.05
S1	0.933	0.507	0.0027	Yes
S2	0.28	0.453	0.091	
S2A	0.745	NA		
S3	0.04	0		
S4	0.093	0		
S7	0.653	0.187	0.0001	Yes
S7A	1.467	0.453	0.0001	Yes
S8	0.027	0.013	0.566	
S9	0.733	0.427	0.006	Yes
S11	0.213	0.147	0.459	
A	0.053	0.04	0.703	
B	0.133	0.013	0.029	Yes
D	0.373	0.107	0.003	Yes

Location	2009	2009	p-Value	Sig at P<0.05
S2A vs S2	0.745	0.28	0.0013	Yes

We have included some additional information in this report that we feel has bearing on trying to improve the efficacy of biological control agents in Lake Bonaparte to control the growth of Eurasian watermilfoil. Reasons for the recorded low density of insects remain unclear, but it could be due to a variety of factors such as overwintering habitat for the weevils, fish predation or a lack of feeding material. Relatively speaking, there are much less dense stands of watermilfoil at Lake Bonaparte than there are for example at Chautauqua Lake (Johnson et al. 2008), which may make it harder for weevil population numbers to build up in Lake Bonaparte. Sparse milfoil stands may make it difficult for weevils to hide from fish, and could mean less viable material for egg laying substrate and resulting feeding by weevil larvae.

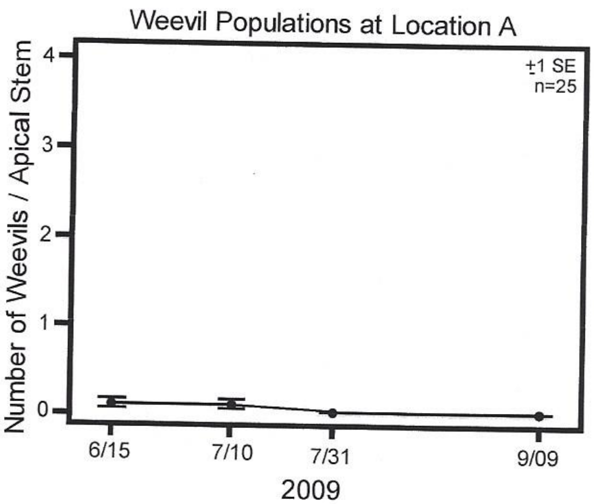
Adult weevils however move and spread out very quickly. Johnson et al. 2008 shows how weevils spread out from shore during a typical year in Chautauqua Lake. By the third week of June, weevils had traveled approximately 1.3km from their nesting spots on shore to reach milfoil beds in the middle of the bay, and weevils were at the same densities from the middle of the bay to the shore. This indicates that adult weevil populations move early in the season, very fast and disperse widely after overwintering in an effort to produce more than one generation of weevils during the growing season of watermilfoil. This cannot take place with the introduction of eggs/larvae into Lake Bonaparte in late June or early July because of the long length of egg hatching and development to the adult stage.

Another reason to consider as a cause of no increases in weevil numbers by augmentation to Lake Bonaparte may be simply the act of adding eggs/larvae of the weevil *E. lecontei,* but not considering normal losses of proceeding to adults. LaMere 2008 reports greenhouse experiment losses of 47-70 % of a sub-sample of EnviroScience eggs/larvae placed into Lake Bonaparte on July 5, 2007. This sub-sample was not placed into the lake or exposed to bluegill predation. This is very similar to the total loses in weevil populations from the egg to adult stages of 20-70 % reported by others studying weevil life cycles (Mazzei et al. 1999, Newman et al. 1997).

In our 2009 report of 2008 research, we suggested the need to consider the losses experienced by EnviroScience's method of attaching milfoil clusters containing eggs/larvae to the lake's milfoil and their hope of having newly hatched larvae move to the lake milfoil. LaMere 2008 reported, well after augmentation in 2008, that he observed EnviroScience milfoil clusters or baggie ties from the previously attached clusters still attached to Lake Bonaparte milfoil, but little or no weevil damage to the attached lake's milfoil. Our 2003 report (Johnson et al. 2003) made the same observation and included photos. We again on July 18, 2008 while sampling EnviroScience augmented location S7 recovered the EnviroScience baggie tie on Lake Bonaparte milfoil but observed no tied-on EnviroScience milfoil cluster in their baggie tie or any larval weevil damage to the Lake Bonaparte plant. These observations suggest that this may be the part of the Middfoil® process where large losses occur. To expect transfer of EnviroScience larvae to the lake's milfoil but having the transfer poorly executed by any hatched eggs is very possible. The 2007 greenhouse experiment (LaMere 2008) looked specifically at the transfer of larvae to new milfoil but observed very little movement.

Bluegill (*Lepomis macrochirus*) appears to consume a significant number of watermilfoil herbivores as indicated by analysis of their stomach contents of a single sampling at location S7 on 7/31/2009 (Tables 4 and 5). Our microscopic analysis reports a mean of 3 weevils per bluegill and 1.1 moth larvae per bluegill (Table 4). Weevil larvae appear to be particularly venerable and this may be partially due to not having legs. Our 2009 data of bluegill stomach contents of prey may suggest a plausible reason for the lack of damage to Lake Bonaparte watermilfoil by EnviroScience's weevil larvae after hatching on the EnviroScience attached watermilfoil clusters. A nearby bluegill attracted to the bright green watermilfoil cluster (See photos in Johnson et al. 2003) laden with eggs from EnviroScience, tied to Lake Bonaparte watermilfoil, at the time of weevil egg hatching would consume all the larvae noticed. Because the weevil larvae do not have legs and only a few could mine into the EnviroScience cluster stems or surrounding lake stems, most would likely be consumed. With EnviroScience clusters holding between 250 to 300 eggs, weevil losses would be very large and may explain additional losses leading to the often ineffectiveness of this method of augmentation.

EnviroScience Control



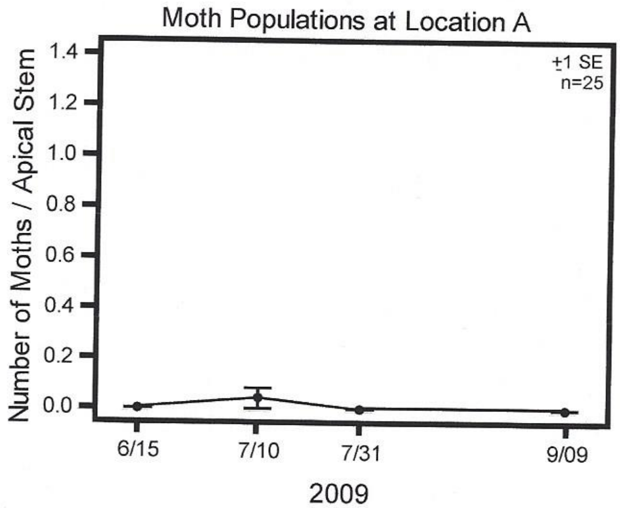


Figure 3a. Mean weevil numbers ± 1 SE (includes all life stages, eggs, larvae, pupae and adults) recorded at location A per 25cm length apical stem (n=25 apical stems searched unless noted). Mean moth numbers ± 1 SE (includes the life stages eggs, larvae, and pupae) recorded at location A per 25cm length apical stem (n=25 apical stems searched unless noted).

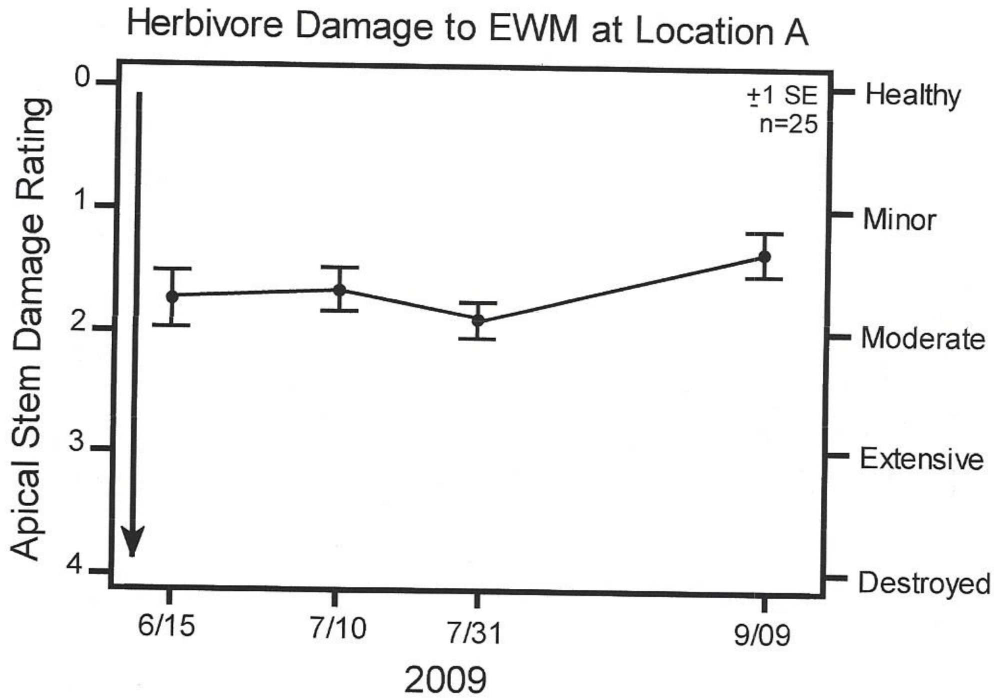


Figure 3b. Herbivore damage to 25cm length apical stems of Eurasian watermilfoil at location A shown as a mean rating ± 1 SE of herbivore damage to 25 apical stems. Herbivore damage ranges from 0 = no damage to 4 = apical tip destroyed.

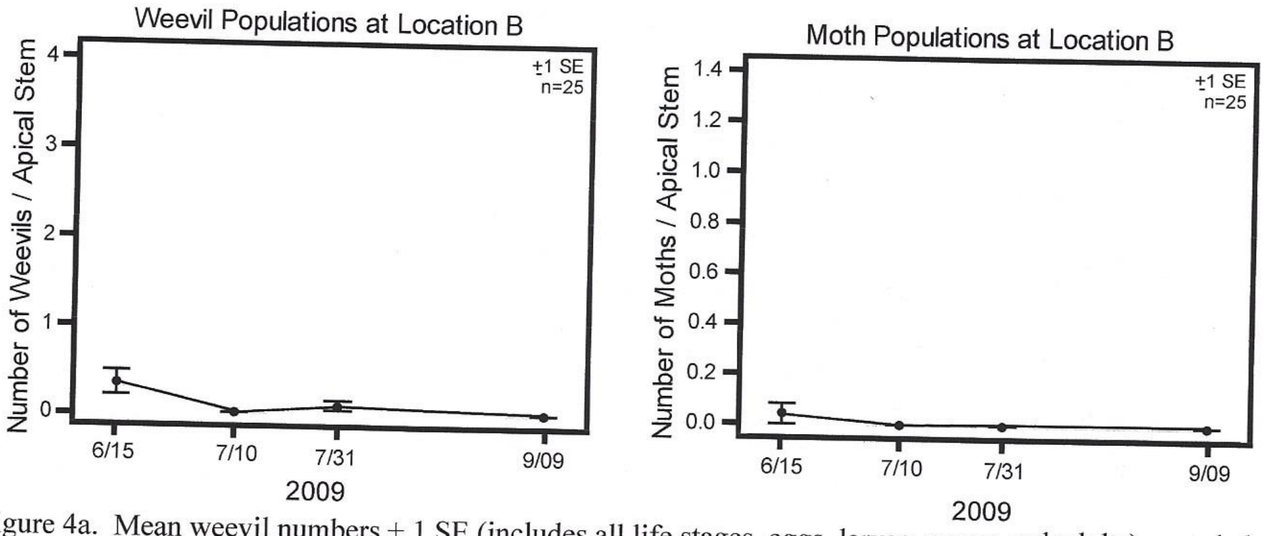


Figure 4a. Mean weevil numbers ± 1 SE (includes all life stages, eggs, larvae, pupae and adults) recorded at location B per 25cm length apical stem (n=25 apical stems searched unless noted). Mean moth numbers ± 1 SE (includes the life stages eggs, larvae, and pupae) recorded at location B per 25cm length apical stem (n=25 apical stems searched unless noted).

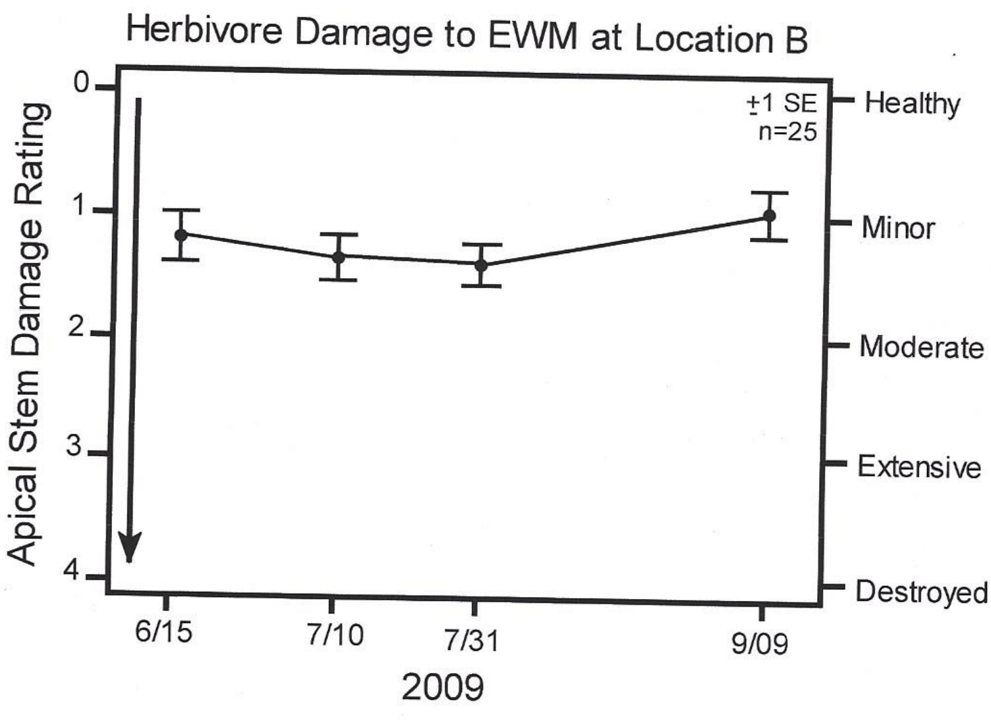
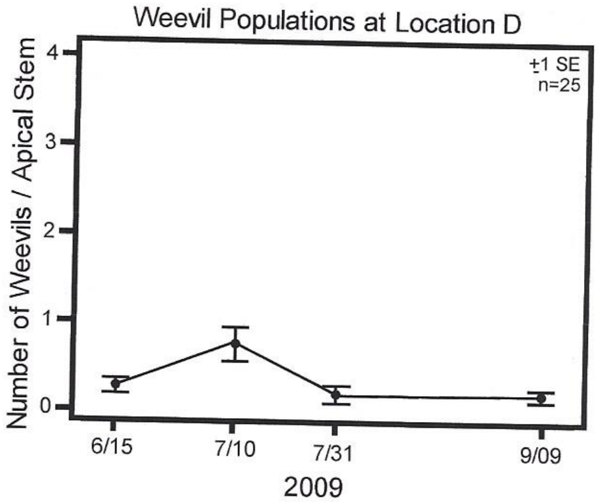


Figure 4b. Herbivore damage to 25cm length apical stems of Eurasian watermilfoil at location B shown as a mean rating ± 1 SE of herbivore damage to 25 apical stems. Herbivore damage ranges from 0 = no damage to 4 = apical tip destroyed.

Cornell Control



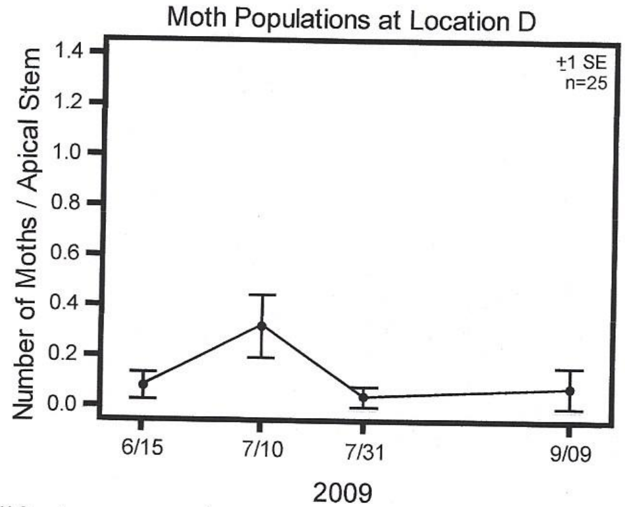


Figure 5a. Mean weevil numbers ± 1 SE (includes all life stages, eggs, larvae, pupae and adults) recorded at location D per 25cm length apical stem (n=25 apical stems searched unless noted). Mean moth numbers ± 1 SE (includes the life stages eggs, larvae, and pupae) recorded at location D per 25cm length apical stem (n=25 apical stems searched unless noted).

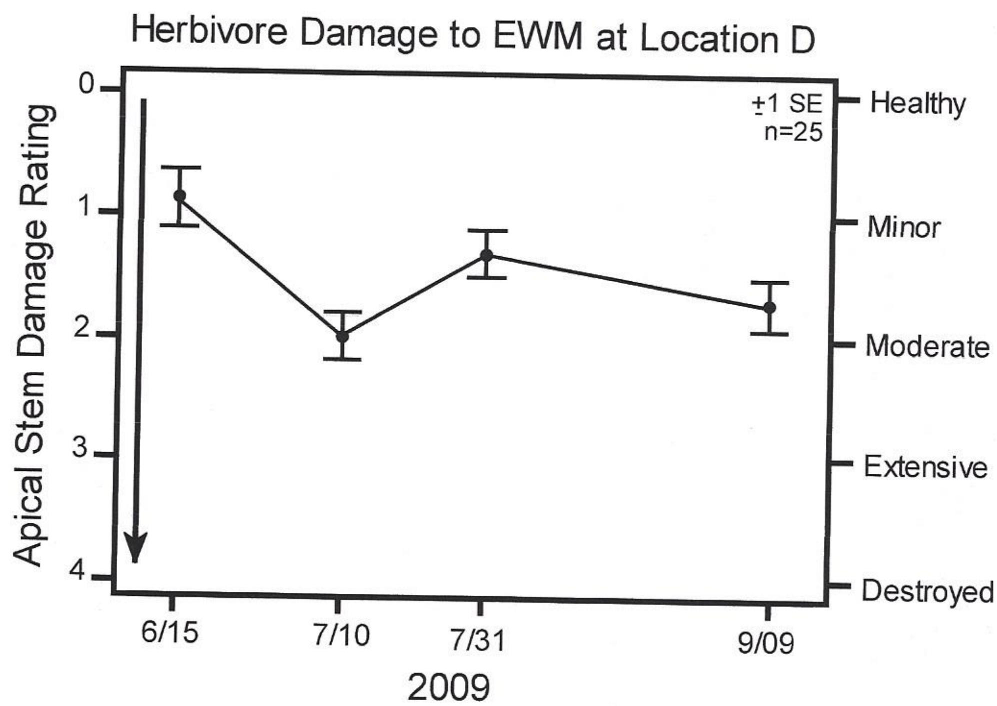
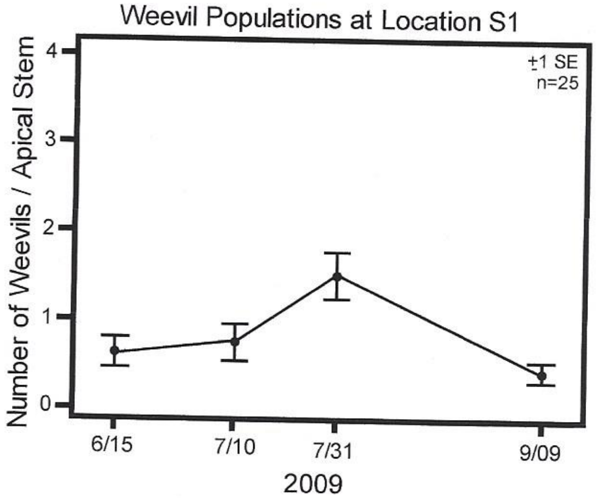


Figure 5b. Herbivore damage to 25cm length apical stems of Eurasian watermilfoil at location D shown as a mean rating ± 1 SE of herbivore damage to 100 apical stems. Herbivore damage ranges from 0 = no damage to 4 = apical tip destroyed.



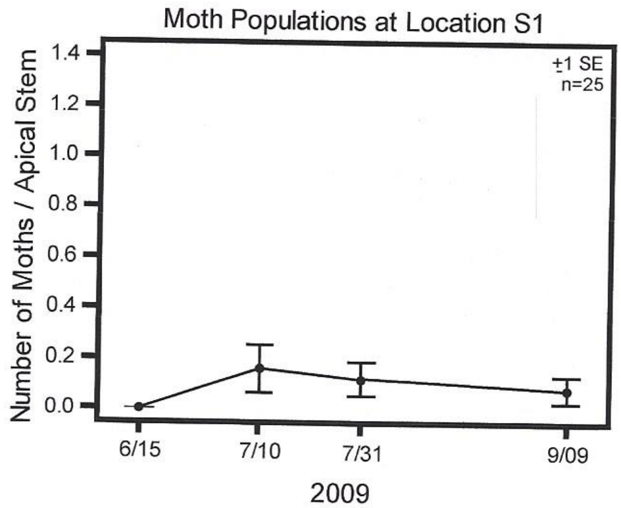


Figure 6a. Mean weevil numbers ± 1 SE (includes all life stages, eggs, larvae, pupae and adults) recorded at location S1 per 25cm length apical stem (n=25 apical stems searched unless noted). Mean moth numbers ± 1 SE (includes the life stages eggs, larvae, and pupae) recorded at location S1 per 25cm length apical stem (n=25 apical stems searched unless noted).

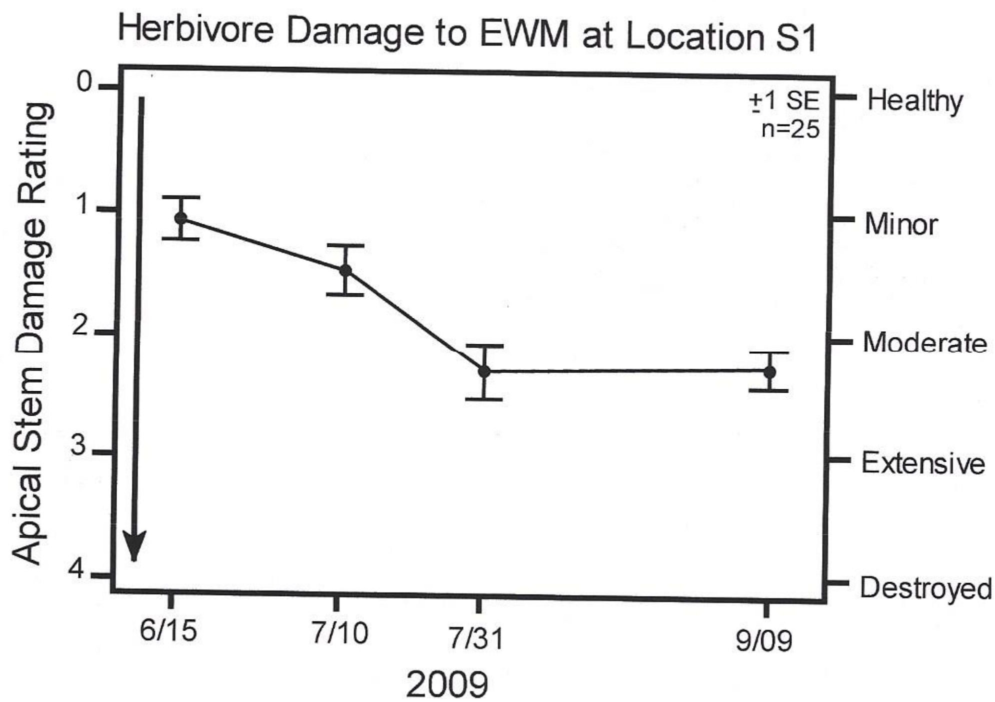


Figure 6b. Herbivore damage to 25cm length apical stems of Eurasian watermilfoil at location S1 shown as a mean rating ± 1 SE of herbivore damage to 25 apical stems. Herbivore damage ranges from 0 = no damage to 4 = apical tip destroyed.

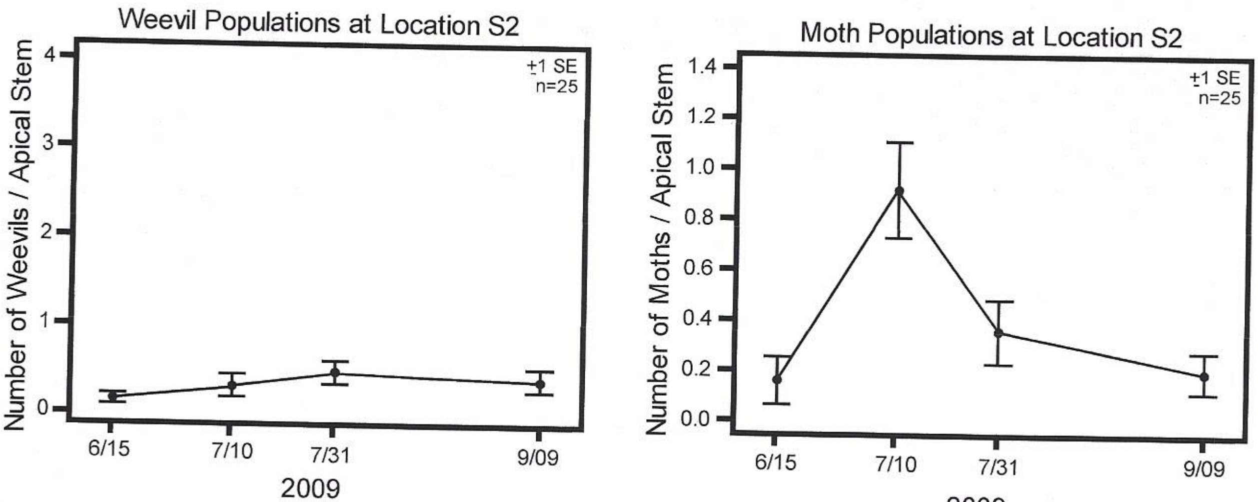


Figure 7a. Mean weevil numbers ± 1 SE (includes all life stages, eggs, larvae, pupae and adults) recorded at location S2 per 25cm length apical stem (n=25 apical stems searched unless noted). Mean moth numbers ± 1 SE (includes the life stages eggs, larvae, and pupae) recorded at location S2 per 25cm length apical stem (n=25 apical stems searched unless noted).

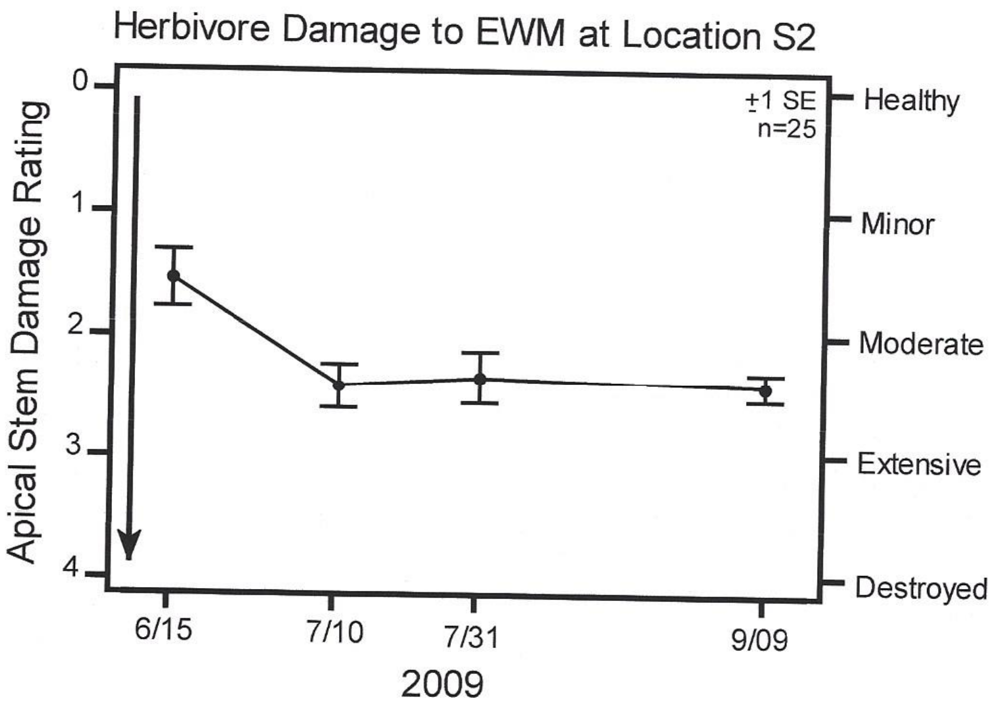


Figure 7b. Herbivore damage to 25cm length apical stems of Eurasian watermilfoil at location S2 shown as a mean rating ± 1 SE of herbivore damage to 25 apical stems. Herbivore damage ranges from 0 = no damage to 4 = apical tip destroyed.

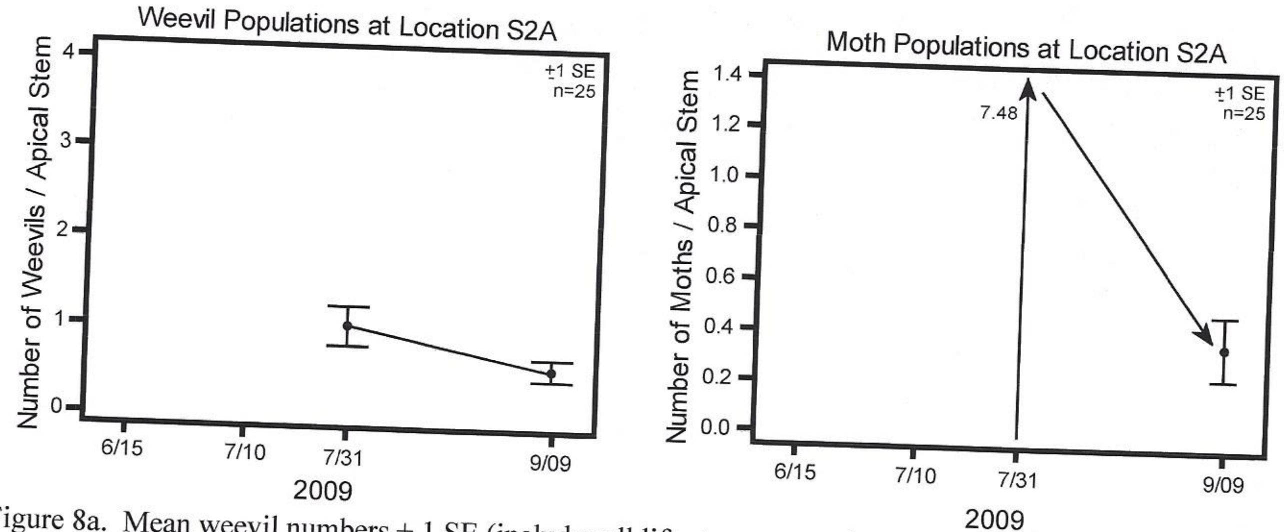


Figure 8a. Mean weevil numbers ± 1 SE (includes all life stages, eggs, larvae, pupae and adults) recorded at location S2A per 25cm length apical stem (n=25 apical stems searched unless noted). Mean moth numbers ± 1 SE (includes the life stages eggs, larvae, and pupae) recorded at location S2A per 25cm length apical stem (n=25 apical stems searched unless noted).

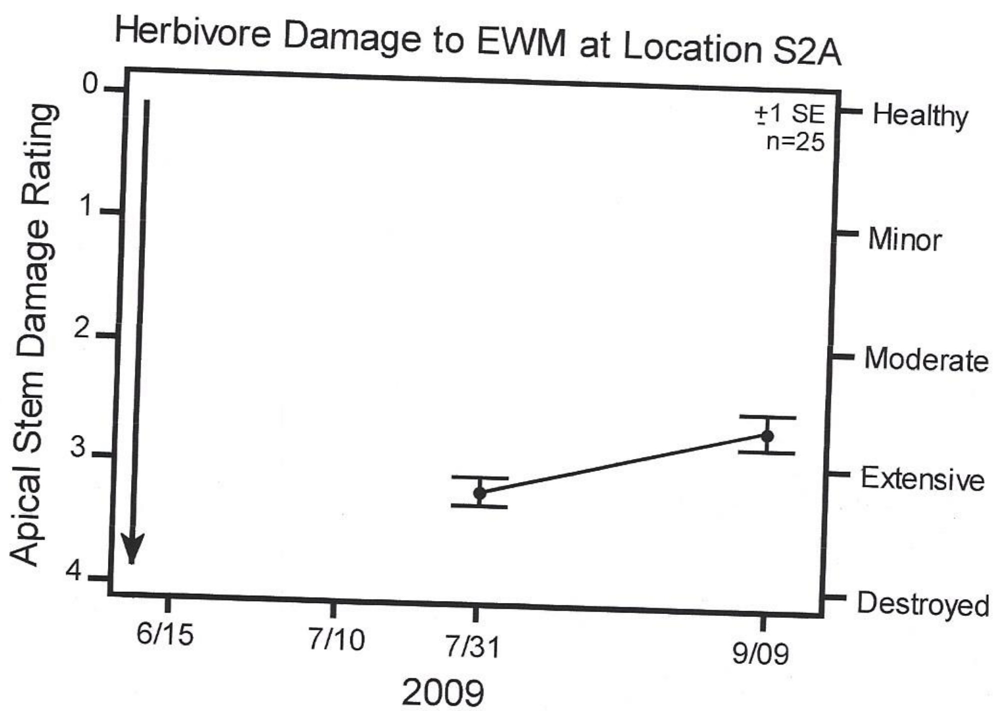
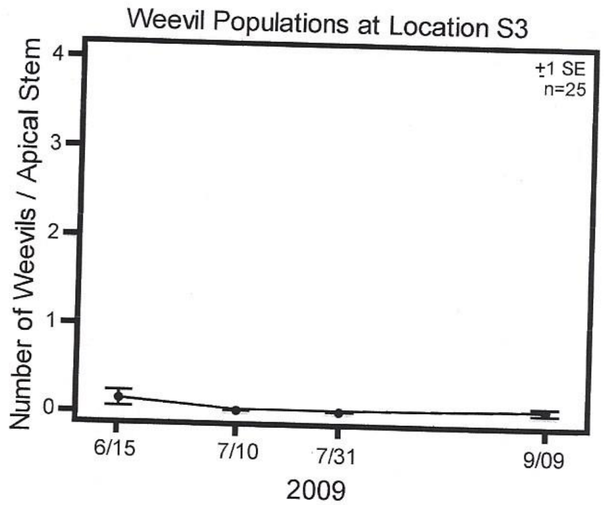


Figure 8b. Herbivore damage to 25cm length apical stems of Eurasian watermilfoil at location S2A shown as a mean rating ± 1 SE of herbivore damage to 25 apical stems. Herbivore damage ranges from 0 = no damage to 4 = apical tip destroyed.



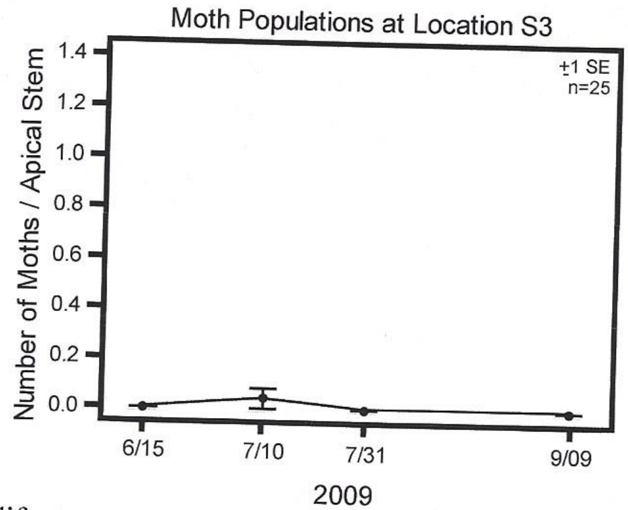


Figure 9a. Mean weevil numbers ± 1 SE (includes all life stages, eggs, larvae, pupae and adults) recorded at location S3 per 25cm length apical stem (n=25 apical stems searched unless noted). Mean moth numbers ± 1 SE (includes the life stages eggs, larvae, and pupae) recorded at location S3 per 25cm length apical stem (n=25 apical stems searched unless noted).

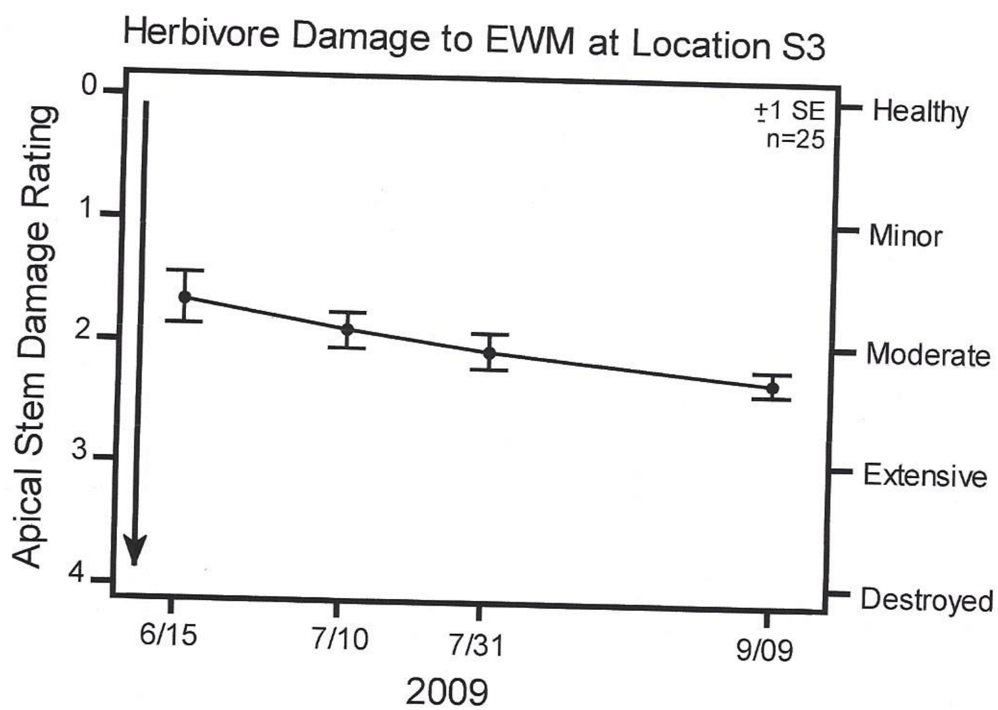
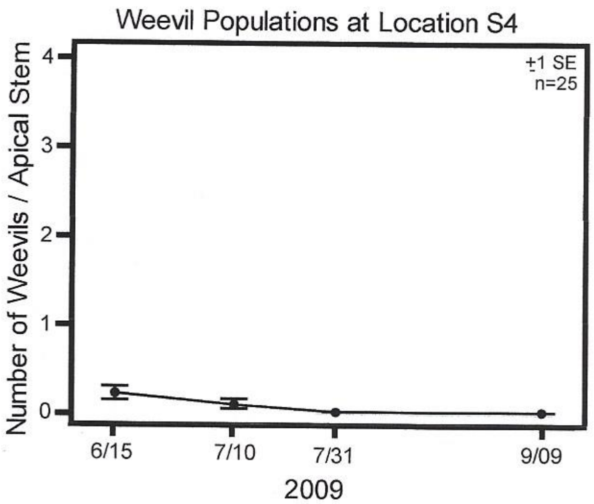


Figure 9b. Herbivore damage to 25cm length apical stems of Eurasian watermilfoil at location S3 shown as a mean rating ± 1 SE of herbivore damage to 25 apical stems. Herbivore damage ranges from 0 = no damage to 4 = apical tip destroyed.



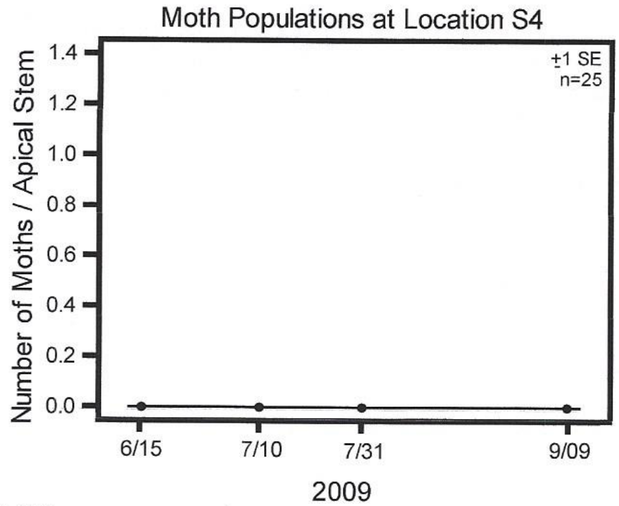


Figure 10a. Mean weevil numbers ± 1 SE (includes all life stages, eggs, larvae, pupae and adults) recorded at location S4 per 25cm length apical stem (n=25 apical stems searched unless noted). Mean moth numbers ± 1 SE (includes the life stages eggs, larvae, and pupae) recorded at location S4 per 25cm length apical stem (n=25 apical stems searched unless noted).

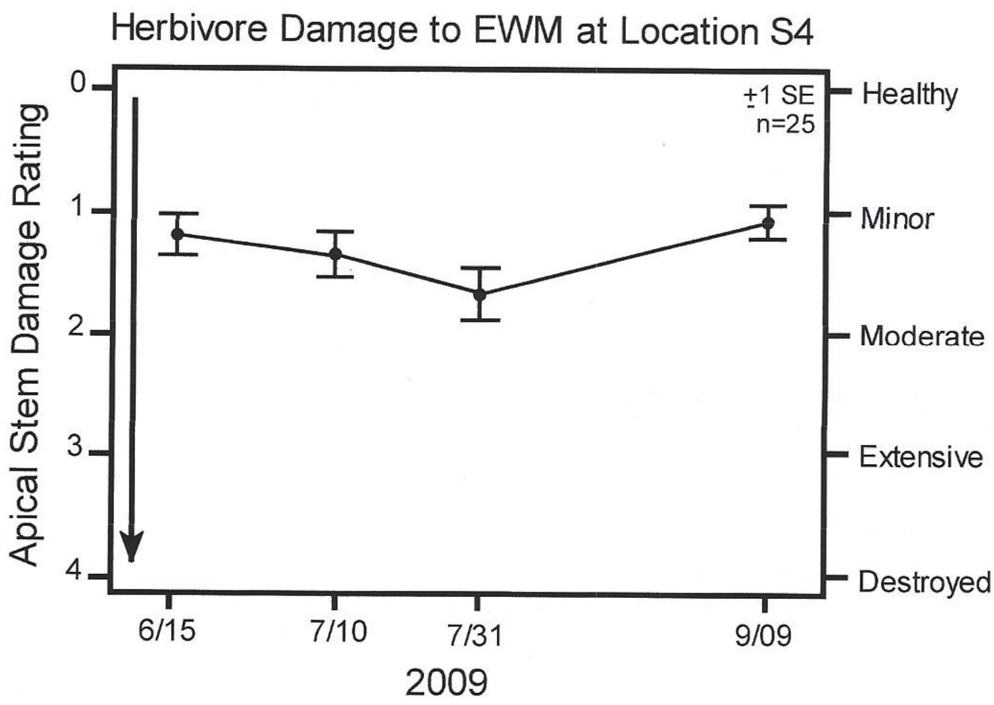
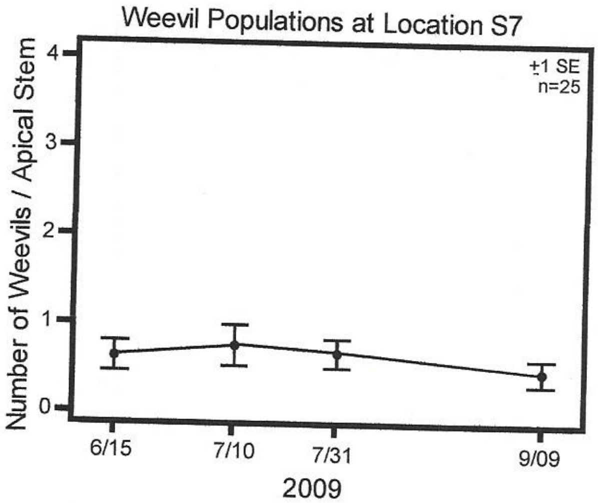


Figure 10b. Herbivore damage to 25cm length apical stems of Eurasian watermilfoil at location S4 shown as a mean rating ± 1 SE of herbivore damage to 25 apical stems. Herbivore damage ranges from 0 = no damage to 4 = apical tip destroyed.



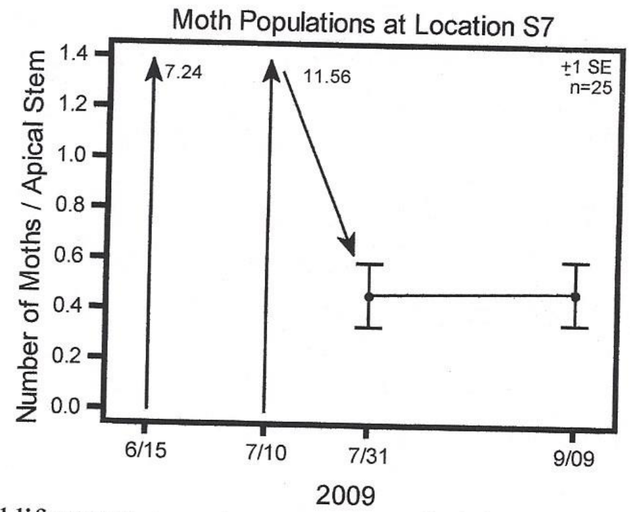


Figure 11a. Mean weevil numbers ± 1 SE (includes all life stages, eggs, larvae, pupae and adults) recorded at location S7 per 25cm length apical stem (n=25 apical stems searched unless noted). Mean moth numbers ± 1 SE (includes the life stages eggs, larvae, and pupae) recorded at location S7 per 25cm length apical stem (n=25 apical stems searched unless noted).

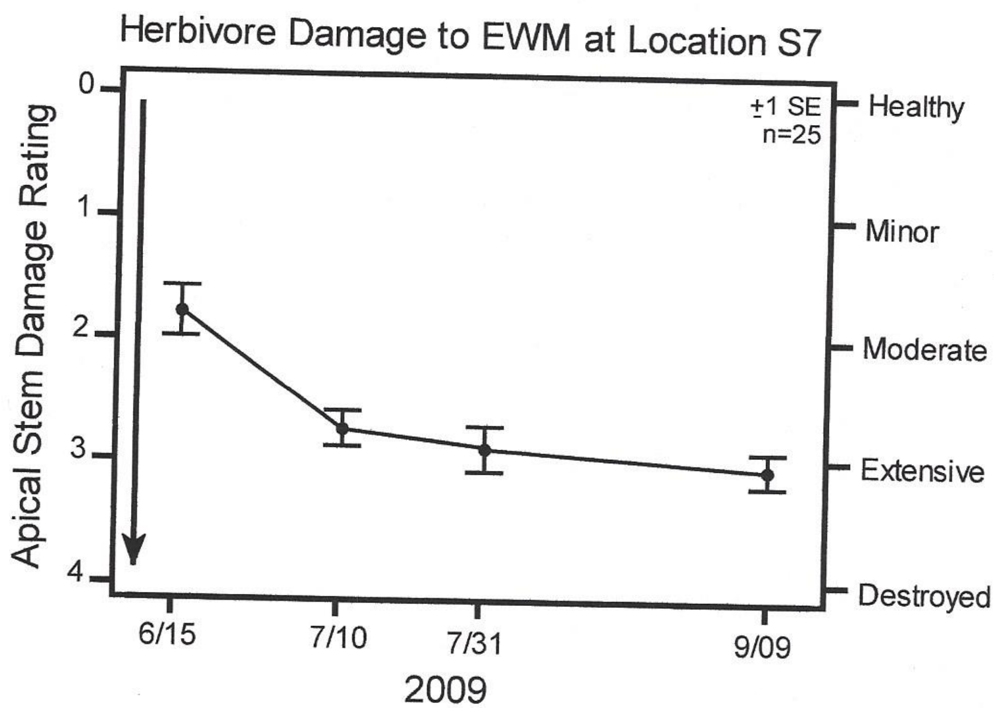


Figure 11b. Herbivore damage to 25cm length apical stems of Eurasian watermilfoil at location S7 shown as a mean rating ± 1 SE of herbivore damage to 25 apical stems. Herbivore damage ranges from 0 = no damage to 4 = apical tip destroyed.

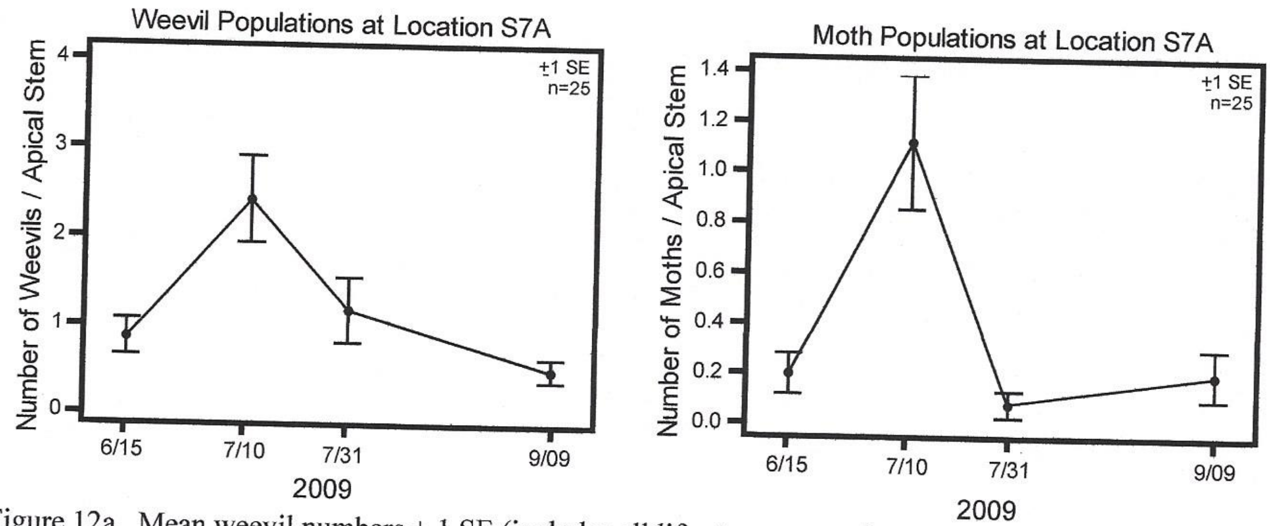


Figure 12a. Mean weevil numbers ± 1 SE (includes all life stages, eggs, larvae, pupae and adults) recorded at location S7A per 25cm length apical stem (n=25 apical stems searched unless noted). Mean moth numbers ± 1 SE (includes the life stages eggs, larvae, and pupae) recorded at location S7A per 25cm length apical stem (n=25 apical stems searched unless noted).

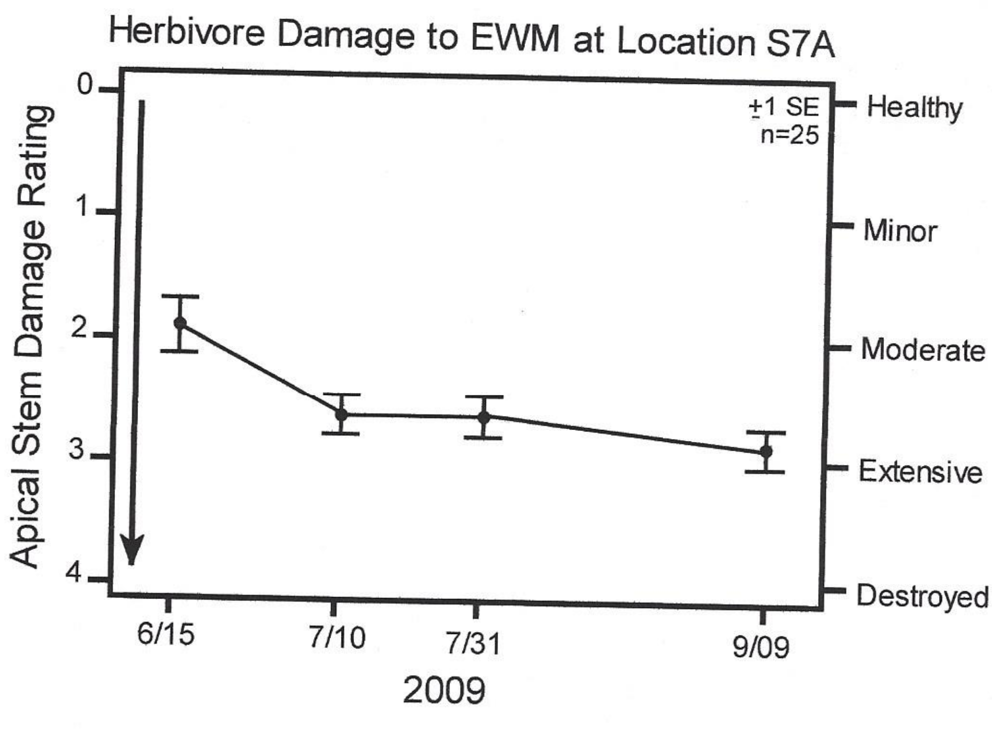
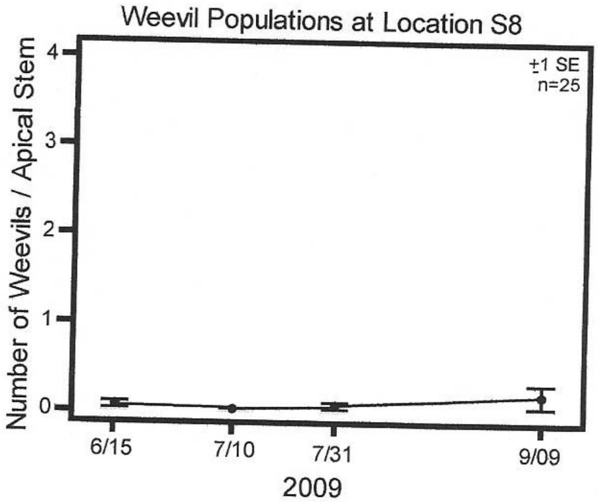


Figure 12b. Herbivore damage to 25cm length apical stems of Eurasian watermilfoil at location S7A shown as a mean rating ± 1 SE of herbivore damage to 25 apical stems. Herbivore damage ranges from 0 = no damage to 4 = apical tip destroyed.



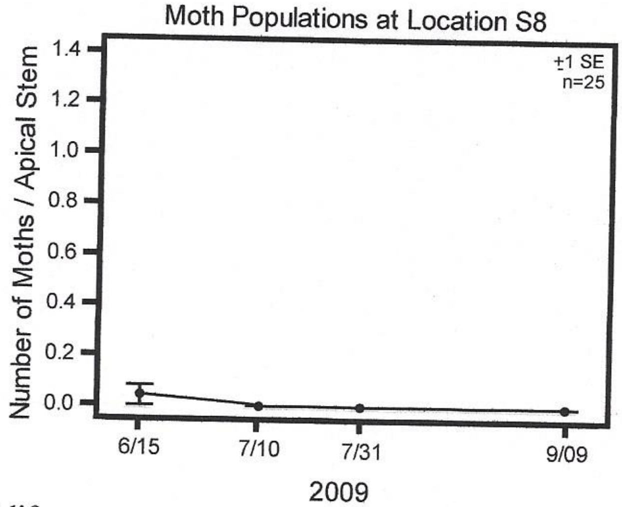


Figure 13a. Mean weevil numbers ± 1 SE (includes all life stages, eggs, larvae, pupae and adults) recorded at location S8 per 25cm length apical stem (n=25 apical stems searched unless noted). Mean moth numbers ± 1 SE (includes the life stages eggs, larvae, and pupae) recorded at location S8 per 25cm length apical stem (n=25 apical stems searched unless noted).

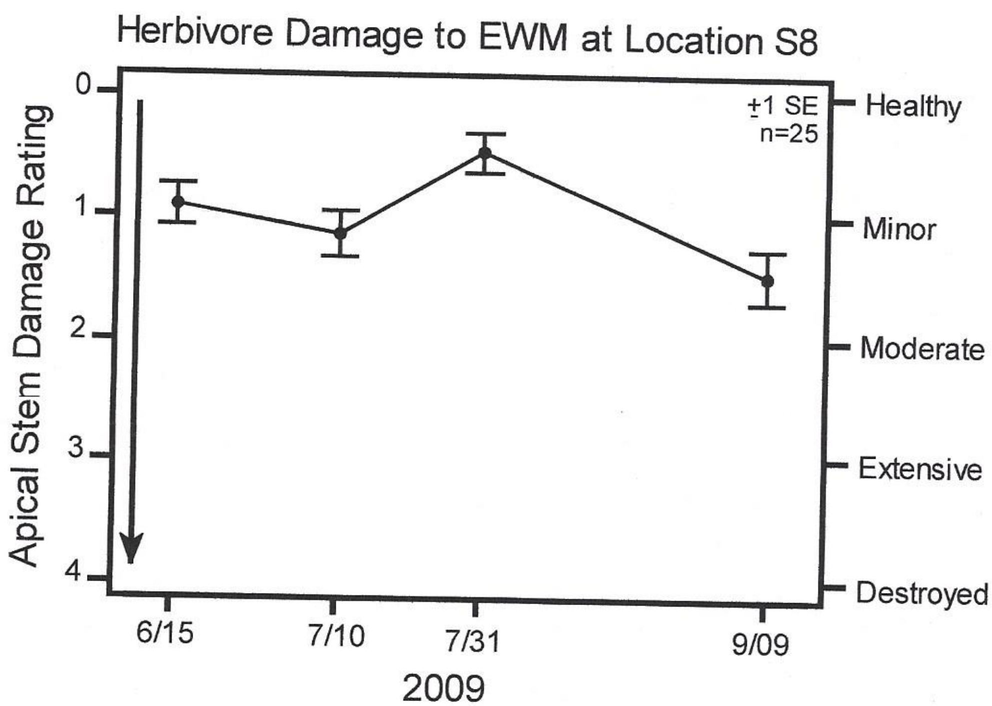
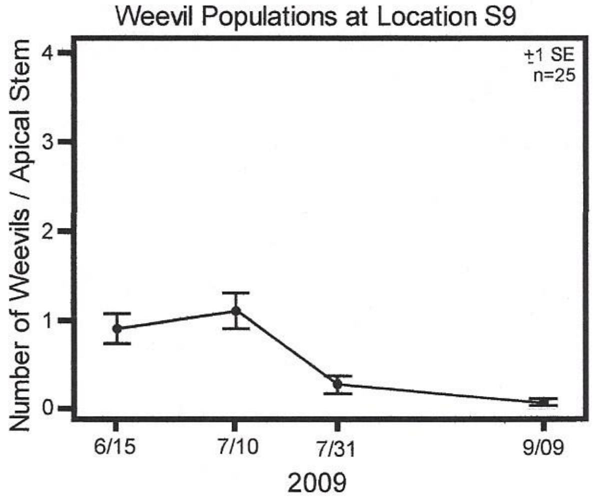


Figure 13b. Herbivore damage to 25cm length apical stems of Eurasian watermilfoil at location S8 shown as a mean rating ± 1 SE of herbivore damage to 25 apical stems. Herbivore damage ranges from 0 = no damage to 4 = apical tip destroyed.



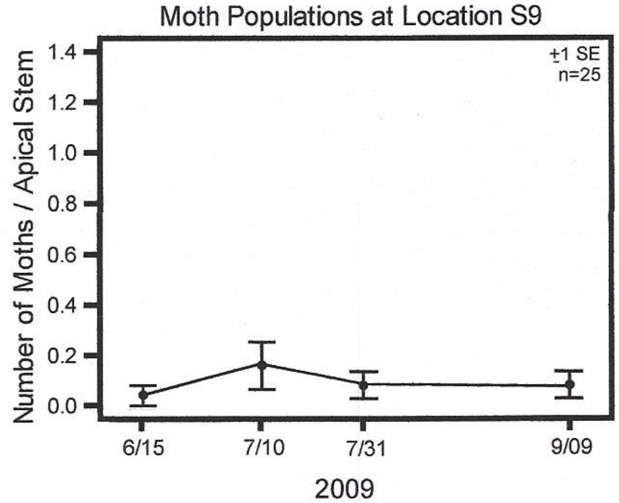


Figure 14a. Mean weevil numbers ± 1 SE (includes all life stages, eggs, larvae, pupae and adults) recorded at location S9 per 25cm length apical stem (n=25 apical stems searched unless noted). Mean moth numbers ± 1 SE (includes the life stages eggs, larvae, and pupae) recorded at location S9 per 25cm length apical stem (n=25 apical stems searched unless noted).

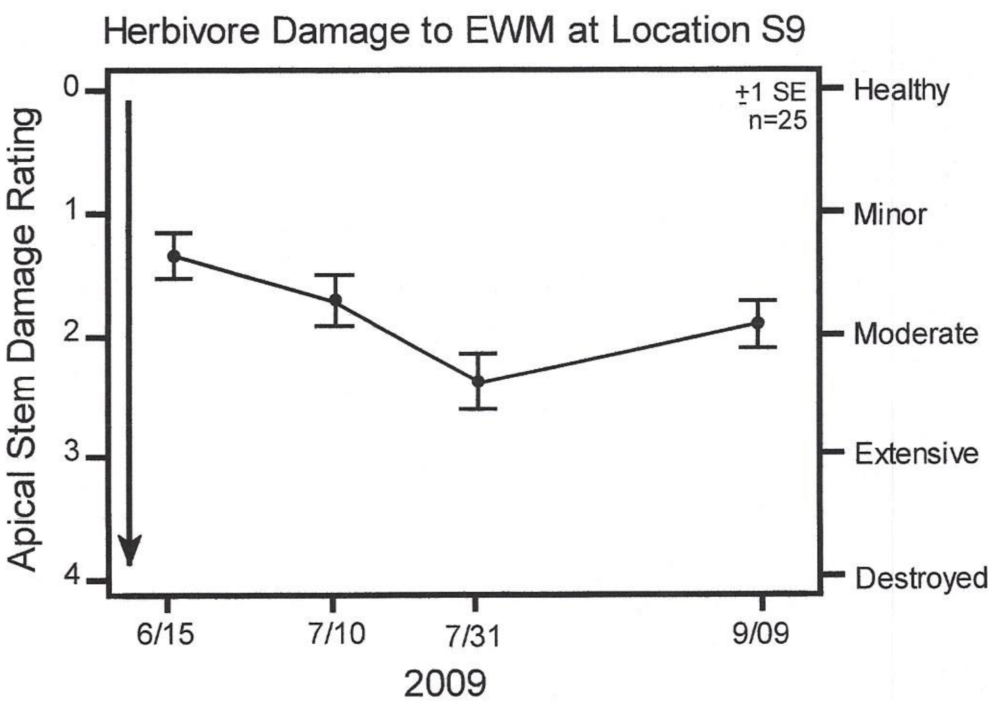
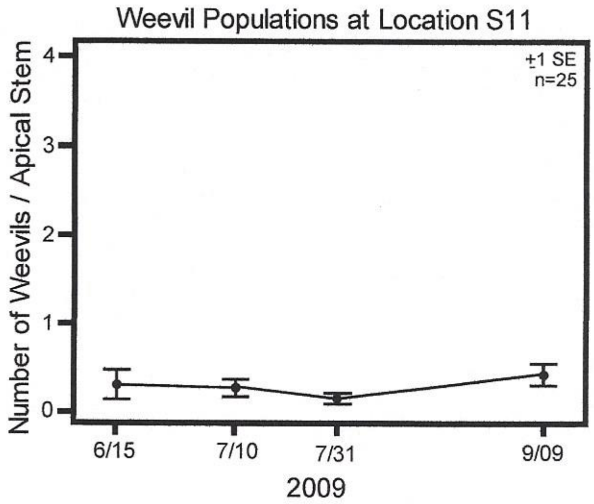


Figure 14b. Herbivore damage to 25cm length apical stems of Eurasian watermilfoil at location S9 shown as a mean rating ± 1 SE of herbivore damage to 25 apical stems. Herbivore damage ranges from 0 = no damage to 4 = apical tip destroyed.



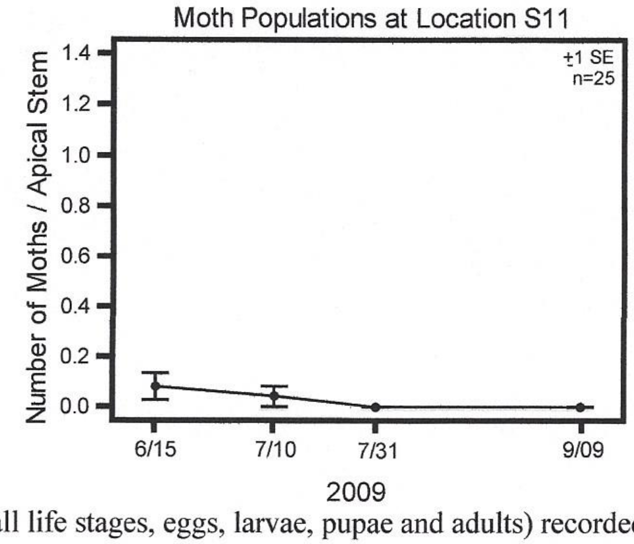


Figure 15a. Mean weevil numbers ± 1 SE (includes all life stages, eggs, larvae, pupae and adults) recorded at location S11 per 25cm length apical stem (n=25 apical stems searched unless noted). Mean moth numbers ± 1 SE (includes the life stages eggs, larvae, and pupae) recorded at location S11 per 25cm length apical stem (n=25 apical stems searched unless noted).

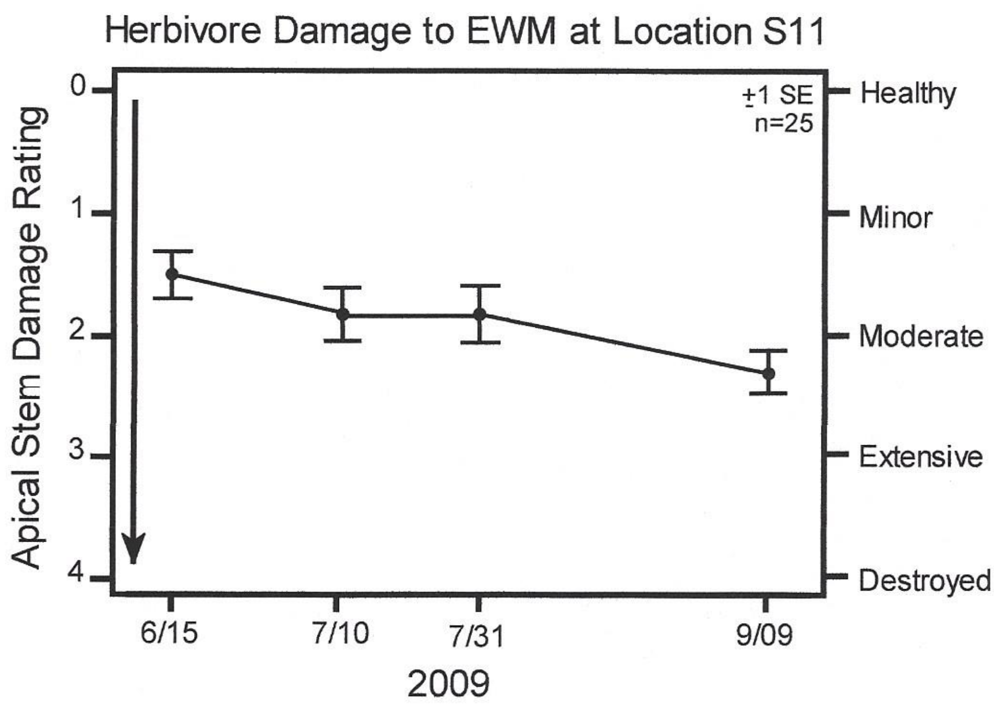


Figure 15b. Herbivore damage to 25cm length apical stems of Eurasian watermilfoil at location S11 shown as a mean rating ± 1 SE of herbivore damage to 25 apical stems. Herbivore damage ranges from 0 = no damage to 4 = apical tip destroyed.

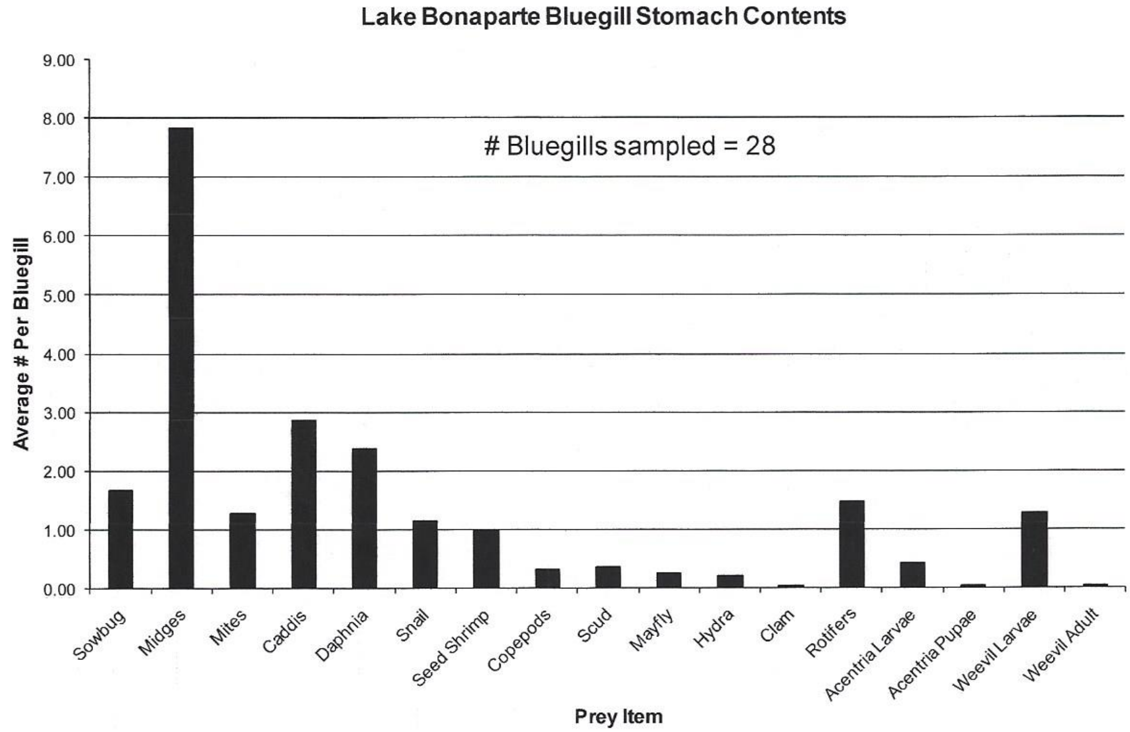


Figure 16. Mean number of identified prey found as food per bluegill sampled at location S7 on 7/31/2010.

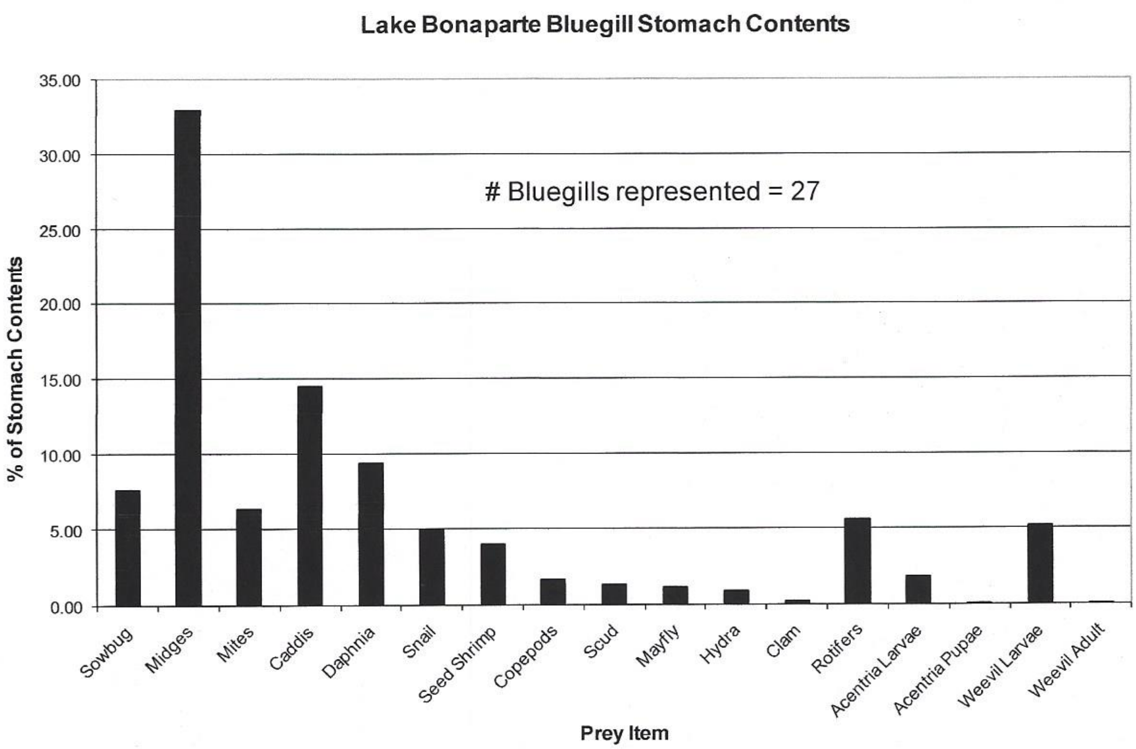


Figure 17. Percentage of each identified prey found in stomach contents per bluegill sampled at location S7 on 7/31/2010.

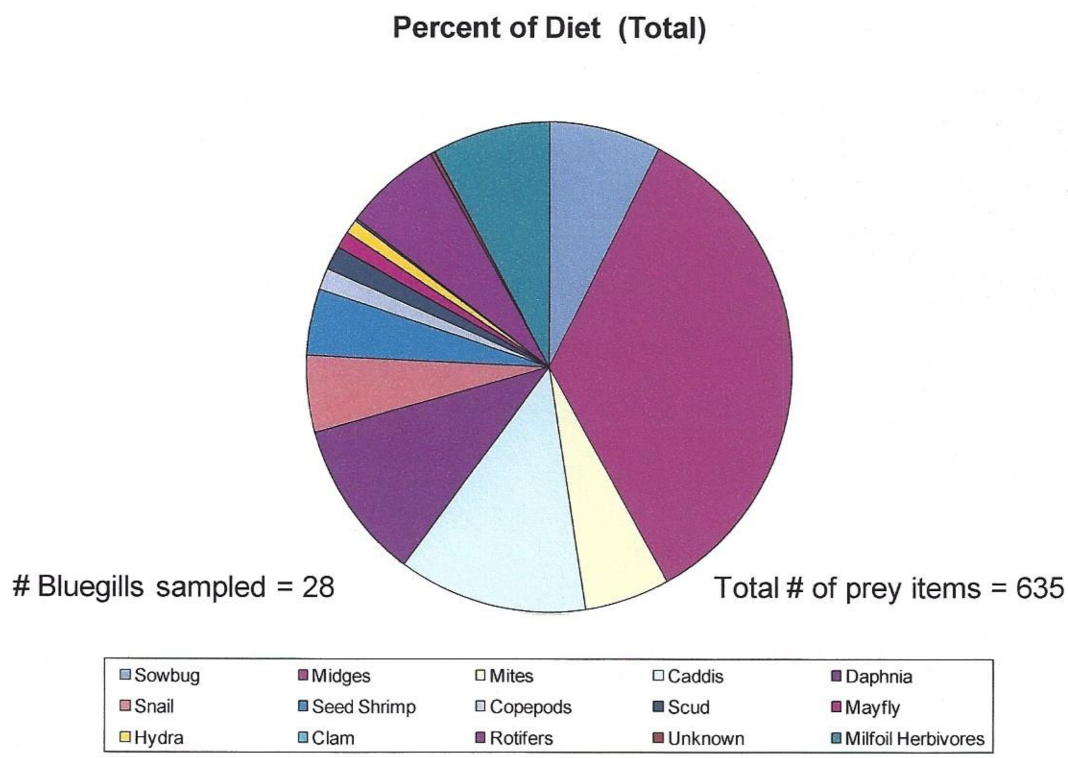


Figure 18. Percentage of the total diet of sampled bluegill on 7/31/2010 from location S7.

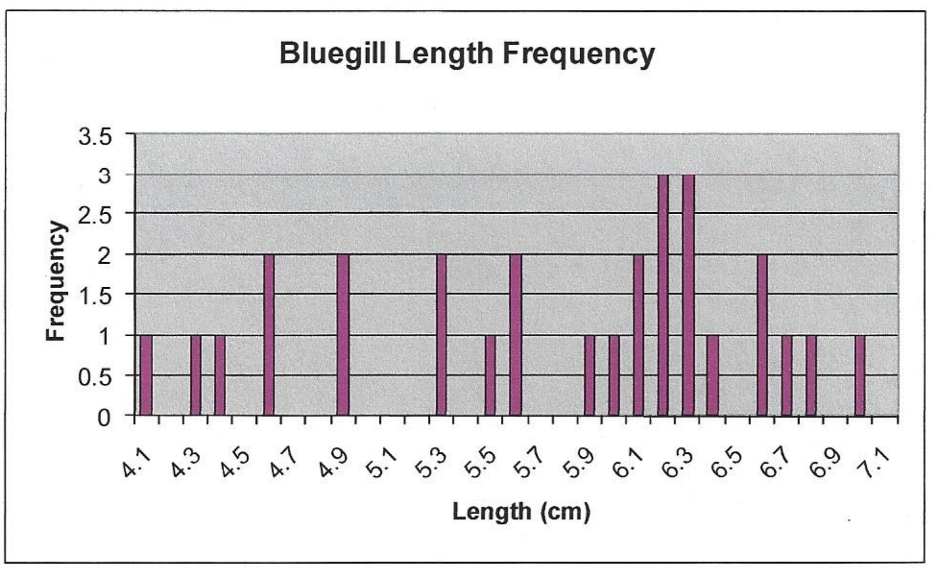


Figure 19. Size frequency distribution as length (cm) of sampled bluegills from location S7 on 7/31/2010.

Table 4. The numbers of herbivores (*Acentria* moth and *Euhrychiopsis* weevil) found in the stomachs and intestines of bluegills from location S7 on 7/31/2010.

	Stomachs Contents				Intestines Contents			Combined Intestines and Stomach (Total)			
Bluegill ID #	Acentria Larvae	Acentria Pupae	Weevil Larvae	Weevil Adult	Acentria Larvae	Weevil Larvae	Weevil Adults	Acentria Larvae	Acentria Pupae	Weevil Larvae	Weevil Adult
1	0	0	0	0	0	0	0	0	0	0	0
2	1	0	0	0	4	0	0	5	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	2	0	5	0	3	10	0	5	0	15	0
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
8	1	0	0	0	0	0	0	1	0	0	0
9	0	0	0	0	1	0	0	1	0	0	0
10	0	0	3	0	2	6	0	2	0	9	0
11	4	0	5	0	1	2	0	5	0	7	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	1	0	0	0	0	0	0	1	0	0
14	0	0	0	0	0	0	0	0	0	0	0
15	0	0	2	0	1	5	0	1	0	7	0
16	3	0	8	1	4	9	1	7	0	17	2
17	1	0	9	0	0	3	0	1	0	12	0
18	0	0	0	0	0	4	0	0	0	4	0
19	0	0	2	0	1	2	0	1	0	4	0
20	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	2	0	0	2	0	0	0
22	0	0	1	0	0	1	0	0	0	2	0
23	0	0	1	0	0	0	0	0	0	1	0
24	0	0	0	0	0	1	0	0	0	1	0
25	0	0	0	0	0	2	0	0	0	2	0
26	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0
								31	1	81	2
								Acentria	32	Weevils	83
								Per fish	1.14	Per fish	2.96

Table 5. The identification and numbers of all prey as bluegill food found in bluegills from location S7 on 7/31/2010 with table sorted by fish length (cm).

Bluegill #	Length (cm)	Sowbug	Midges	Mites	Caddis	Daphnia	Snail	Seed Shrimp	Copepods	Scud	Mayfly	Hydra	Clam	Rotifers	Acentria Larvae	Acentria Pupae	Weevil Larvae	Weevil Adult			
1	7	1	11	1	9	2	1	1	0	0	0	0	0	0	0	0	0	0			
3	6.8	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0			
5	6.7	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
2	6.6	0	8	2	3	0	3	2	2	0	0	0	0	0	5	0	0	0			
4	6.6	0	7	2	6	0	0	0	0	1	0	0	0	0	5	0	15	0			
9	6.4	9	14	3	3	2	0	2	0	0	0	0	0	0	1	0	0	0			% of total
10	6.3	6	6	1	7	2	4	0	0	0	0	0	0	0	2	0	9	0	Acentria	23	71.88
12	6.3	7	16	1	3	1	1	0	0	1	1	0	0	0	0	0	0	0	Weevils	66	79.52
16	6.3	0	9	0	4	8	1	3	0	0	1	2	0	0	7	0	17	2			
6	6.2	0	4	0	5	5	0	1	0	0	0	3	0	0	0	0	0	0			
17	6.2	2	7	0	2	2	1	0	0	0	0	0	0	0	1	0	12	0			
18	6.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0			
14	6.1	10	5	2	0	0	0	2	0	0	1	0	0	0	0	0	0	0			
15	6.1	0	5	0	6	0	2	1	0	1	1	0	0	0	1	0	7	0			
13	6	0	20	3	4	7	2	2	0	2	0	0	0	0	0	1	0	0			
11	5.9	1	11	0	2	0	0	0	0	0	0	0	0	0	5	0	7	0			
21	5.6	0	29	0	2	10	1	3	0	2	0	0	0	0	2	0	0	0			% of total
25	5.6	1	4	1	0	2	10	0	2	0	1	0	0	0	0	0	2	0	Acentria	7	21.88
23	5.5	3	0	17	2	3	3	3	0	0	0	0	0	0	0	0	1	0	Weevils	12	14.46
20	5.3	3	8	0	1	0	2	0	0	0	0	0	1	0	0	0	0	0			
22	5.3	0	10	0	3	2	0	2	0	1	0	0	0	0	0	0	2	0			
7	4.9	1	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0			
26	4.9	0	15	1	2	3	0	1	0	0	0	0	0	12	0	0	0	0			% of total
8	4.6	1	3	1	4	2	0	2	0	0	1	0	0	0	1	0	0	0	Acentria	2	6.25
19	4.6	1	7	0	3	6	0	2	0	1	0	0	0	0	1	0	4	0	Weevils	5	6.02
27	4.4	0	5	0	1	2	0	1	0	0	1	1	0	18	0	0	0	0			
24	4.3	1	9	0	5	7	0	0	0	1	0	0	0	0	0	0	1	0			
28	4.1	0	2	0	1	0	1	0	5	0	0	0	0	11	0	0	0	0			

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