JUST, Vol. VI, No. 1, 2018
Trent University

The effect of Eurasian watermilfoil metal accumulation on the activity of milfoil weevil populations in Sudbury, Ontario

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Abstract
This paper explores patterns of metal accumulation in Eurasian watermilfoil (*Myriophyllum spicatum*), and determines whether populations of milfoil weevils (*Euhrychiopsis lecontei*) in the Sudbury region are visibly affected by these patterns. To investigate this relationship, milfoil patches in six Sudbury lakes, and one control lake (Baptiste Lake, near Bancroft, ON) were sampled for sediment and plant chemistry, as well as for milfoil weevil damage as a measure of weevil activity. Comparisons of sediment, milfoil root, and milfoil stem metal concentrations among the seven lakes found sediment metal concentrations of Cd, Cu, Ni, and Pb, were all lowest in the control lake, Baptiste. The greatest differences were found in Cu and Ni concentrations, which were approximately fifteen to eighty times higher in Sudbury. Milfoil metal concentrations in the root and stem were not strongly correlated to sediment concentrations indicating that total metal concentrations are not reflective of the available metal in the Sudbury Lakes. Weevil damage was weakly correlated with Cd/Zn ratios, but uncorrelated with all stem metal concentrations. Principal Components Analysis confirmed that metal accumulation in Eurasian milfoil could not predict milfoil weevil damage. It is unclear whether milfoil weevils are simply unaffected by metal contamination in their host plant, or whether the effect is too small to detect with this study design.

Keywords
Milfoil weevils — Eurasian watermilfoil — Biological control — Metal accumulation — Sudbury.

1. Introduction

1.1 Management of Eurasian Watermilfoil

Myriophyllum spicatum, more commonly known as Eurasian watermilfoil (EWM), is an exotic submerged aquatic plant known by lake users as a major nuisance species in North America (Van Driesche, et al., 2002). Since the 1960s, the species has become widespread in most lakes across the southern and central regions of the province (Van Driesche, et al., 2002; Boylen, et al., 1999). This spread is helped by the fact that the plant reproduces rapidly, forming large monoculture stands in densities as great as 250 stems/m^2 (Borrowman, 2012). When established, EWM dominates the ecosystem and subsequently alters and suppresses native macrophyte communities. This reduces the diversity and abundance of native fish and macroinvertebrate populations (Van Driesche, et al., 2002; Boylen, et al., 1999, Borrowman, et al., 2014). Large patches of EWM can also have visible impacts on water and sediment chemistry, by reducing dissolved oxygen levels in soil, nutrient availability, and water movement (Borrowman, et al., 2014). Aside from the considerable effects EWM has on ecosystem organisms, large patches are a source of considerable frustration for boaters, anglers, swimmers, and property owners.

EWM is particularly difficult to manage. It is highly pervasive as it reproduces mainly through fragmentation, allowing it to spread quickly within and between lakes (Hamel, 2014). Mechanical harvesting techniques are labour intensive and often exacerbate the spread of EWM by creating small plant fragments (Boylen et al., 1996). Herbicide treatments are difficult to control, with studies showing that commonly used herbicide treatments can have negative effects on non-target organisms, potentially further degrading the ecosystem (Poovey et al., 2004; Harrahy, et al., 2014; Borrowman, et al., 2014).

One alternative to the mechanical and chemical control of milfoil, is the use of biological control methods, the most notable of which is the milfoil weevil (*Euhrychiopsis lecontei*), a native aquatic beetle from the Curculionidae family. The milfoil weevil only consumes plants in the Myriophyllum genus, but is also entirely dependent on these plants for habitat and reproduction (Newman, 2004). Weevils damage milfoil plants by removing stem tissue as larvae; this stem tissue removal can result in reduced buoyancy and increased mortality (Creed and Sheldon, 1995). Traditionally, northern watermilfoil (*Myriophyllum sibericum*) served as the host for milfoil weevils, thereby dictating their distribution, which is limited to northern USA and southern Canada (Newman, 2004). However, the emergence of EWM in many lake ecosystems has allowed weevils to expand their host range to include the exotic species (Painter and McCabe, 1988; Creed and Sheldon, 1993).

1.2 Biological Control of Eurasian Watermilfoil in Sudbury

Due to the theoretical and documented success of milfoil weevils as biological control agents of EWM (Menninger, 2011; Jester, et al., 2000), the insects have been used commercially in the management of EWM infested waters. However, these programs have not always been able to deliver predictable results (Newman, 2004; Miller, et al., 2011). One such example is the attempted control of EWM in Sudbury, Ontario. The Sudbury Lakes region has a three year EWM control plan initiated by the City of Sudbury in 2011; during this period, several EWM patches at six different lakes (Grant, Long, McFarlane, Richard, Simon, and St. Charles) were stocked with 145,700 weevils (Enviroscience, 2013). Native weevil populations were augmented by weevil eggs taken from individuals at two Sudbury Lakes, including Richard and St. Charles, as well as one Haliburton Lake, Baptiste (Borrowman, pers. comm.). After each year of the program, stocked sites were surveyed for weevil damage, EWM stem density, and weevil density (Enviroscience, 2013). The treatment program initially had only limited effects on EWM densities, but has since seen some reductions (Monet, 2014). However, these reductions in EWM density were not as great as expected when compared to previous weevil augmentation programs. The failure has been blamed on low weevil densities; if weevil population densities are too low (less than about two weevils per stem), weevils are unlikely to be able to consistently control EWM patches effectively (Monet, 2014).

In general, there are several observed and hypothesized reasons for the failure of augmented weevil programs to produce weevil densities great enough to control EWM. These limitations to weevil populations include EWM patch depth and distance from shore, temperature, lake nutrient levels, plant nutrient quality, overwintering survival, and predation (Miller, et al., 2011; Tamayo, et al., 2000; Jester, et al., 2000, Newman and Inglis, 2009; Ward and Newman, 2006). However, an alternate reason for the lack of success in Sudbury could be due to a local factor, namely elevated metal concentrations in the tissues of plants resulting in a reduction in weevil grazing. Studies exploring metal uptake in EWM have found that the plant has high metal adsorption capabilities, making it a good candidate for the removal of metals from both sediments and water (Yabanh, et al., 2014; Keskinkan, et al., 2003). The potential for metal accumulation in Sudbury's EWM populations raises concerns about possible trophic effects on milfoil weevils.

Although metal contamination can appear to affect life history traits in insect herbivores, it is also clear that the implications of metal accumulation on plant-herbivore interactions is dependent on the accumulation characteristics of the plant species, and the resistance of the herbivore to the effects of metal exposure (Thiebault, et al., 2010; van Ooik, 2008; Kay and Haller, 1986). It is therefore important to thoroughly investigate the accumulation of metals in EWM, and the consequences of weevil exposure to these plants. To understand and explore this relationship, this study examines patterns of metal accumulation in EWM, and determines whether populations of milfoil weevils in Sudbury Lakes are visibly affected by these patterns.

2. Methods

2.1 Study Sites

The study was based on field data and observations from six lakes in the Sudbury region, and one control lake. These lakes host two separate biotypes of EWM (Borrowman, et al., 2014). Biotype 1 (EWM1) is the more common biotype that is slightly taller than biotype 2 (EWM2), which is typically more branched than EWM1 (Sager, pers. comm.). EWM2 also tends to be found in lakes with lower alkalinity; perhaps this is a reason for their abundance in the Sudbury region (Borrowman, et al., 2014). It is possible that these biotypes will behave differently in regards to their accumulation of metals; thus, of the six study lakes, three have EWM1 patches (Long, McFarlane, Ramsey), while the remaining three have EWM2 patches (Hannah, Richard, St. Charles). Genetic testing of the biotypes in each lake occurred in a previous study by Borrowman, et al. (2014). The lakes were also selected to include some that were stocked by Enviroscience Inc., as part of the milfoil weevil program. In each of the six lakes, three EWM patches with areas of at least 10m2 were selected for sampling. Several of these were the same patches used in the recent milfoil control program.

Baptiste Lake, in the Bancroft area, was used as a control lake. Like the Sudbury Lakes, some of the shoreline of Baptiste is developed, however the lake does not have a significant history of toxic metal deposition. Baptiste has known populations of both EWM1 and EWM2, as well as an existing milfoil weevil population making it a useful comparison to the Sudbury Lakes. Two patches of EWM1, and one patch of EWM2 were identified in Baptiste Lake; all three patches were sampled.

2.2 Field Sampling

Each patch was subject to the same sampling protocol, divided into three main components. Firstly, three surface sediment samples were taken. When possible, these samples were taken at a range of 0.5-2m in depth along a transect perpendicular to the shoreline, ensuring the patch was accurately represented. Sediment samples were collected only from a depth in which the EWM roots were buried. Secondly, milfoil root samples were taken. Approximately ten plant roots were sampled along the same transect as the sediment samples. This was then followed by the collection of milfoil stems. Milfoil stem samples served two purposes: assessing weevil activity and metal content. Patch weevil activity was used as a proxy for weevil abundance, which is a challenging and unreliable metric (Alwin, et al., 2010). This assessment of weevil activity, as described by Borrowman, et al. (2014), was consistent with the EWM control program. In this protocol, each stem was inspected for damage caused by weevil larvae or adults. This assessment required the sampling of the top 50 cm of 10 stems along three transects perpendicular from the shoreline.

2.3 Laboratory Techniques

Composite sediment and plant samples were prepared for metal analysis. Plant roots and stems were rinsed with deionised water and oven dried for 48 hours at approximately 60°C. Sediment samples were drained and air-dried. All samples were ground and pulverized using a SPEX Sample Prep 8000D mixer/mill steel ball mill grinder. Following this, 0.2 g of each sample was digested in 2.5mL of concentrated HNO3 at 110°C for approximately six hours. Metal content was analysed by inductively coupled plasma mass spectroscopy (ICP-MS).

3. Data Analysis

Patch-level stem, root, and sediment metal concentrations of Cd, Cu, Ni, Pb, Zn, Ca, and Fe (independent variables), as well as a patch-level damage score, and percent damage (dependent variables), were prepared for analysis. Percent damage scores were determined by the presence or absence of weevil damage on each EWM stem, as described by Borrowman et al. (2014). A damage score was also calculated to take into account the degree of damage observed on this stem; this was a subjective measure. Signs of minimal weevil damage were awarded one point, signs of more extensive weevil damage were awarded two points, clear signs of larval damage were awarded one additional point, and finally, stems with pupal chambers were awarded three points. Although subjective, this metric was only intended to provide a richer description of weevil activity that accounted for the degree of damage observed on EWM stems. All data was analyzed using Statistica 12. Tests for normality (Shapiro-Wilk; p>.05) found that most data variables were positively skewed and were consequently transformed with either a square root or natural log transformation.

One-way ANOVA tests and Tukey HSD post hoc tests were used to determine if there were significant differences (α = .10) in metal concentrations or weevil damage between lakes. Independent t-tests (α = .10) were used to investigate the effect of weevil stocking and EWM biotype on EWM metal accumulation and weevil activity. Pairwise correlations (α = .10) were used to examine patterns of metal accumulation in EWM, and particularly, to observe the relationship between this accumulation and weevil activity. Finally, Principal Component Analysis (PCA) was used to observe underlying patterns in the data. Due to the exploratory nature of the study, a 90% significance level was used in each statistical test.

4. Results

4.1 Lake Milfoil Accumulation Patterns

The mean values of stem, root, and sediment concentrations are listed in Table 1. Sediment metal concentrations of Cd, Cu, Ni, and Pb, were all lowest in the control lake, Baptiste. The greatest differences between Baptiste Lake and the Sudbury Lakes were found in Cu and Ni concentrations, which were approximately fifteen to eighty times higher in Sudbury. There were no significant differences in sediment Zn, Ca, and Fe concentrations among the different lakes. The differences in metal concentrations among lakes were not as great in the roots and stem, as they were in the sediment. Although no differences in Zn concentrations were found in the sediment, there were significant, but inconsistent differences of Zn in the roots between Baptiste and Long, as well as in the stems between Ramsey and McFarlane.

Correlation tests across all lakes found that, in general, sediment metal concentrations had some bearing on metal uptake in EWM. Cu and Pb concentrations in roots were found to be significantly related to levels in the sediment (r=.67, p=.001; r=.46, p=.036), however, all other relationships were insignificant. In the stems, Cu, Ni and Pb levels were significant related to sediment concentrations (r=.52, p=.018; r=.58, p=.007; r=.40, p=.078). There were significant relationships between root and stem concentrations of all metals; the strongest correlations were seen in Zn (p=.76, p<.001), Cu (p=.72, p<.001), and Pb (p=.64, p=.002), while differences were also observed in Cd (r=.59, p=.010), and Ni (r=.52, p=.021).

4.2 Lake Weevil Damage Patterns

Weevil damage was found in each lake, but was not significantly different among the lakes (Damage Score, F(6,14)=1.13, p=.40; % Damage, F(6,14)=0.72, p=.64). Both weevil damage metrics were highly variable in most lakes, with standard deviations often close to, or larger than the mean value. Despite this variation, some small correlations between weevil damage score and metal concentrations were observed. Ni concentrations in EWM roots was correlated with damage score (r=-.40, p=.08), as were root Zn concentrations (r=-.46, p=.04). Sediment Fe concentrations exhibited some correlation with the weevil damage score (r=.42, p=.06), while Cd/Zn ratios were slightly correlated with damage score (r=.39, p=.09). Weevil damage score was not significantly correlated with any other variable. Percent damage was not found to correlate significantly with any recorded variable.

4.3 Effects of Stocking and Biotype

The effect of the three-year weevil-stocking program on Sudbury Lakes was not visible in the data set. Independent t-tests showed that there were no significant differences in damage score (t(19)=0.17, p=.86) or percent damage (t(19)=0.44, p=.67) between stocked and unstocked patches. Independent t-tests showed there was no significant difference between biotypes, EWM1 and EWM2 in damage score (t(19)=1.04, p=.31) or percent damage (t(19)=0.61, p=.55). However, there were significant differences in metal sediment concentrations between patches dominated by EWM1, rather than EWM2 plant communities. Concentrations of Cd (t(19)=-2.18, p=0.04), Cu (t(19)=-2.84, p=0.01), Ni (t(19)=-2.69, p=0.02), Pb (t(19)=-3.48, p=0.002), and Zn (, t(19)=-1.90, p=0.07) were all significantly greater in EWM2 patches than EWM1 patches. Differences between biotype patches was mostly restricted to sediment chemistry although slightly significant differences in stem Cd concentrations were found; EWM1 plants had on average 2.28 μg/g of Cd, compared to EWM2 plants that contained 1.61μg/g (t(18)=1.76, p=0.10). There was no significant difference in Cd/Zn ratios between the biotype patches (t(18)=0.75, p=.46).

5. Metal/Weevil Interactions and PCA

To simplify the PCA, not all variables examined in previous tests were used. Sediment and stem concentrations of Cd, Cu, Ni, Pb, and Zn were analyzed as predictor variables, along with both weevil damage indices. The sediment and stem variables were chosen, as they are essential components of the hypothesized EWM metal accumulation, and the relationship of this accumulation with milfoil weevil activity. The PCA created 12 synthetic factors, the first three of which accounted for 78% of the variation in the entered variables; factor 1 accounted for 43%, while factor 2 and 3 accounted for 19% and 17% respectively. Factor 1 is strongly correlated with all stem and sediment chemistry concentrations, and most strongly correlated with sediment Cu concentrations (r=-.903), and is most weakly correlated with percent weevil damage (r=.03). Factor 2 is weakly correlated with both weevil damage indices, and moderately correlated with stem and sediment chemistry concentrations. However, factor two separates sediment and stem chemistry values. Factor 3 is weakly correlated with sediment and stem metal concentrations, apart from stem Zn concentrations (r=-.45), but is very strongly correlated with both damage score (r=.91) and percent damage (r=.86). The plotting of lake patches on these same axes (factor 1 vs 2, and factor 1 vs 3) indicates that patches are inconsistent, in terms of metal contamination, metal accumulation, and weevil activity, with other patches on the same lake (Figure 1). The lower of the two graphs, which plots factor 1 (correlated with lake contamination) against factor 3 (correlated with weevil activity), appears to have greater in-lake variation between patches, than the upper graph that plots factor 1 against factor 2 (correlated with stem and sediment metal accumulation).

6. Discussion

6.1 Patterns of metal accumulation in EWM

Cu and Ni sediment concentrations were above the severe effect level, as stated by Ontario's sediment quality guidelines, in all Sudbury Lakes. These two elements were also concentrated highly in the roots and stems of EWM, particularly Ni that retained stem concentrations greater than the severe effect level sediment concentrations. Although sediment concentrations cannot be strictly applied to plant concentrations, they do give an indication as to how serious metal concentrations are in EWM. Zn and Cd are greater in the stems than in roots, indicating that they accumulate in the stems of the plants. Although toxic in large quantities, Zn is an essential nutrient. The accumulation by plants is therefore unsurprising, particularly as sediment concentrations of Zn are not elevated above the severe effect level in any of the study lakes. Cd is known to replace Zn in plants (Garg, et al., 1997), thus explaining its similar accumulation patterns in the stem of the plants. Along with Zn, Cd is not elevated above the severe effect level, however both elements have concentrations above the lowest effect level in all Sudbury sediments, and Sudbury EWM stems.

The correlation between plant metal content and sediment metal content was poor. Only Cu and Pb root concentrations were correlated with sediment concentrations, while Cu, Ni, and Pb stem concentrations were correlated with sediment levels. This is likely because the total metal content of most lakes in Sudbury does not represent the total quantity of metals available for plant uptake. Metal availability in Sudbury Lakes has been steadily decreasing over the past several decades (Belzile & Morris, 1995). Historical sulphur emissions have resulted in sulphur-enriched lake sediments (Belzile & Morris, 1995). Sulphur reduction in these sediments has increased ambient sediment pH, leading to the increased adsorption of metals by iron oxyhydroxides in oxic sediments (Belzile & Morris, 1995). This means that total metal concentrations in Sudbury Lakes are likely not reflective of the available metal in the same lakes, resulting in insignificant correlations between sediment metal concentrations, and plant metal concentrations. Variation in sediment chemistry and metal mobility could therefore have significant consequences on the accumulation of metals in EWM plants from lake to lake, explaining some of the inter-lake accumulation variability.

Another possible explanation for the insignificant correlations between sediment and plant metal concentrations could be due to competitive exclusions within the plant. A study by Miller et al. in 1983 found that concentrations of metals within the submerged aquatic plant Eriocaulon aquaticum did not correlate with concentrations of metals found in the surrounding sediment. They hypothesized that this may be

Table 1. *Displays the mean stem, root, and sediment metal concentrations (µg/g) in each of the study lakes, as well as the p-value of a one-way independent ANOVA test conducted to determine differences between the lakes. The results of a Tukey HSD post-hoc test are also displayed.*

Variables		Baptiste	Hannah	Hannah	McFarlane	McFarlane	McFarlane	St. Charles	df (Effect,Error)	F-value	p-value	Post-Hoc (Tukey HSD; p <.10))
STEM	Cd	0.91	2.2	2.68	1.83	3.05	1.22	1.96	6,13	4.36*	0.012	Bap <Long, Ram; Rich <Ram
	Cu	23.39	106.23	82.28	90.09	72.42	44.68	79.58	6,13	3.57*	0.026	Bap <McF, Han
	Ni	43.45	164.33	152.8	121.33	136.47	128.2	131.5	6,13	2.72*	0.061	Bap <Long, Han
	Pb	0.65	3.21	1.98	1.24	0.83	0.74	4.9	6,13	8.16*	<.001	Bap, Ram, Rich <Han; Bap, McF, Ram, Rich <St.C
	Zn	62.07	62.98	126.6	109.62	75.58	63.35	124.9	6,13	3.45*	0.029	Bap <Long
ROOT	Cd	0.31	1.39	2.58	1.38	0.97	0.59	2.74	6,12	6.35*	0.003	Bap, Rich <Long, St.C
	Cu	17.22	209	127.3	107.37	87.73	59.53	320.83	6,14	13.65*	<.001	Bap <Long, McF, Ram, Han; Rich <Han; Bap, Long, McF, Ram, Rich <St.C
	Ni	7.79	356.33	295.3	442.5	164.67	171.03	496.17	6,13	5.18*	0.006	Bap <McF, Han; Bap, Ram, Rich <St.C
	Pb	5.23	8.35	7.95	4.36	6.56	3.26	17.75	6,14	4.5*	0.01	Bap, McF, Rich <St.C
	Zn	58.12	58.62	106.18	113.4	45.97	59.1	126.35	6,14	4.1*	0.014	Ram, Bap <St.C; Ram <McF
SEDIMENT	Cd	1.07	5.1	3.67	1.88	1.8	3.72	3.2	6,14	3.2*	0.034	Bap <Han
	Cu	23.13	1428.33	309	135.5	493.5	544.5	695	6,14	14.12*	<.001	Bap <Long, Ram, Rich, Han, St.C; McF <St.C; Long, McF, Rich, Ram <Han
	Ni	22.75	1626.67	520.33	376.5	608.67	1428	811.5	6,14	8.21*	<.001	Bap <Long, Ram, Rich, Han, St.C; Long, McF <Han
	Pb	37.75	98.63	30.95	10.72	38.75	39.83	57.6	6,14	5.42*	0.004	Bap, Long, McF, Rich <Han; McF <St.C
	Zn	126.02	173.5	197.33	96.87	134.25	203.17	163.23	6,14	1.16	0.381	-
	Ca	4896.67	3635	4350	2001.67	3410	4071.67	2836.67	6,14	2.04	0.127	-
	Fe	17033.33	19950	12298.33	9933.33	21785	11190	11316.67	6,14	1.17	0.377	-

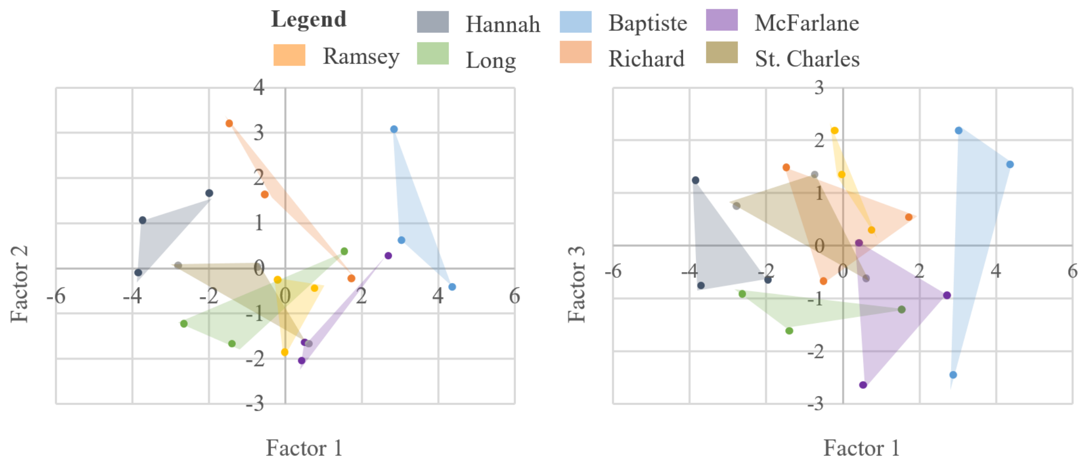

Figure 1. *A projection of the patches at each of the seven study lakes on factor-planes for factors 1 and 2, as well as factors 1 and 3. Triangular shapes join points of the same lake together to illustrate the variability and overlap in the data.*

because the abundance of metals in the sediment could lead to competitive exclusions by other more common metals already absorbed by the plant (Miller, et al., 1983). However, caution should be used when applying Miller's hypothesis to EWM. A recent study by a group of Belgian researchers found that EWM was capable of accumulating almost twice the amount of Cu found in any EWM plants in the Sudbury area (Lesage, et al., 2008). However, it may be possible that since its initial establishment, speciation of EWM in the Sudbury region has improved its ability to exclude metals. A review of speciation in plants exposed to mine tailings found that plants could develop reproductive barriers, selections that reduce gene flow and maladaptive breeding with non-tolerant populations, within one hundred generations of the initial exposure (Hendry, et al., 2007). Therefore, it is possible that, given its presence in the Sudbury region for almost four decades, EWM has developed traits that allow it to exclude quantities of metal from its roots, resulting in lower accumulation concentrations than unexposed plants.

The hypothesis that EWM speciation could exclude metals, protecting the plant from the negative effects of metal contamination, could help to explain the differences noted in the sediment metal content of the two biotypes. Perhaps EWM2 has adapted to tolerate harsher and more toxic conditions than EWM1 plants by excluding metals from the plant. This hypothesis could help explain why, despite the fact that EWM plants spread so quickly and easily, the two biotypes of EWM are only known to co-occur on one lake (Baptiste), and that EWM2 occurs more frequently in Sudbury, than it does elsewhere in the province. However, a much wider experiment must be done before any conclusions on this subject are made.

6.2 Weevil Activity

Correlation tests found that weevil damage was weakly correlated with Ni and Zn root concentrations, as well as Fe sediment concentrations and Cd/Zn ratios. The results of these correlation tests are somewhat conflicting, and quite inconclusive. Although much lower in EWM stems than in sediment, Ni concentrations were still greater than the severe effect level sediment guidelines. Studies on the toxicity of nickel to aquatic macroinvertebrate herbivores are scarce. Lethal concentrations of Ni in water have been determined for several aquatic macroinvertebrates (Beasley & Kneale, 2002). Some benthic macroinvertebrates can tolerate Ni water concentrations as high as 400ppm, while other more sensitive organisms such as caddisflies struggle to tolerate 0.25ppm (Beasley & Kneale, 2002). Very high concentrations of Ni (6500μg/g) in a plant from the mustard family (Streptanthus polygaloides) also caused dietary shifts in invertebrate herbivores. Clearly, there is significant variation in Ni tolerance among insects. Therefore, it is impossible to predict whether Ni concentrations observed in Sudbury EWM plants have a visible effect on weevil damage. Although there was a significant correlation between Ni root concentrations and the weevil damage score, that correlation was not shared between the weevil damage score and Ni stem concentrations (r=.05, p=.84). The lack of correlation undermines the root-weevil correlation findings. The relationship between Zn root concentrations and the weevil damage score was also not reciprocated in Zn stem concentrations (r=-18, p=.44). However, the correlation between the weevil damage score and Fe sediment concentrations may support the hypothesis that weevils are affected by metal toxicity. As mentioned above, the presence of iron oxyhydroxides in oxic soils can limit the availability of metals to EWM plants, which likely leads to a reduction in EWM metal uptake. Therefore, a positive correlation between iron oxyhydroxides and weevil damage would be expected if the study's hypothesis were true. However, Fe sediment concentrations describe total iron, rather than any particular species of the element, rendering this correlation rather ambiguous.

The weak relationship between the Cd/Zn ratio and weevil damage, is the only correlation that really supports the hypothesis of this study. Although Cd concentrations in the stem of Sudbury EWM plants are all below the severe effect level for aquatic sediments, invertebrates including Ephemeroptera, Plecoptera, and Trichoptera have been found to accumulate Cd at 600 to 30,000 times greater than their ambient water concentration (Spehar, et al., 1978). This means it is reasonable to consider that the Cd concentrations found in EWM stems, could have negative effects on milfoil weevils. Despite this observation, overall, the correlations between weevil damage metrics and metal sediment, stem, and root concentrations were inconclusive. There is only minimal evidence in these results to suggest metal accumulation in EWM has a significant effect on weevil activity.

Although inconclusive, the correlation tests do indicate that there is a significant amount of noise in the EWM-weevil relationship. This observation is supported by the results of the Principal Components Analysis. Factor 1 was strongly correlated with metal contamination, but was weakly correlated with weevil damage; similarly, Factor 3 was strongly correlated with weevil damage, but weakly correlated with the independent variables. Figure 1, which plots the lake patches on these axes, showed significant inter-lake variability in EWM metal accumulation and weevil damage. A good example of this variability is illustrated in the lower of the two graphs in Figure 1. Patch 1 and 3 in Baptiste Lake have similar metal contamination levels and milfoil damage levels; although patch 2 has similar metal contamination levels, almost no weevil damage was recorded. Clearly, a large percentage of the variation in weevil damage cannot be explained by EWM sediment or stem chemistry. Overall, the results of the PCA indicate that the metal accumulation in EWM is not a good predictor of milfoil weevil damage. It is possible to infer from this conclusion that metal accumulation in EWM has little to no visible effect on milfoil weevils.

There are several possible reasons for this lack of effect. Firstly, it is possible that milfoil weevils are simply unaffected by the observed levels of contamination in EWM stems. Secondly, there is a possibility that milfoil weevils have developed resistance to high metal concentrations and is supported by pre-existing knowledge that invertebrates have been found to adapt to contaminated host plants. The autumnal moth (Epirrita autumnata), which feeds on mountain birch trees (Betula pubescens. Czerepanovii), developed tolerance to Cu and Ni contamination during an experiment investigating the effect of metal pollution on the relationship between the host plant and the herbivore (van Ooik, 2008). Finally, there is a possibility that the small sample size and inaccuracies in both the measured variables and study design affected the ability of this study to detect a significant effect.

7. Acknowledgments

Thanks to Kyle Borrowman and his summer staff at Enviroscience Inc. for generously helping with fieldwork and guidance during the start of the project, Hayley Murray and Janel Sauder for assisting with fieldwork in Sudbury in September, and Hayley Murray and Tanner Liang for their guidance and assistance with lab work. Additionally, thank you to Dr. Graeme Spires and Troy Maki at Laurentian University for analytical services and the City of Sudbury and Dr. Stephen Monet for covering the analytical costs.

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