

## Research Article

## Fecundity of a native herbivore on its native and exotic host plants and relationship to plant chemistry

Michelle D. Marko<sup>1,2,\*</sup> and Raymond M. Newman<sup>1</sup><sup>1</sup>Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota, St. Paul, MN, 55108, USA<sup>2</sup>Biology Department, Concordia College, Moorhead, MN 56562, USA

\*Corresponding author

E-mail: [marko@cord.edu](mailto:marko@cord.edu)

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**Editor's note:**

This study was first presented at the special session on aquatic invasive species at the 33rd Congress of the International Society of Limnology (SIL) (31 July – 5 August 2016, Torino, Italy) (<http://limnology.org/meetings/past-sil-congress/>). This special session has provided a venue for the exchange of information on ecological impacts of non-native species in inland waters.

**Abstract**

The host range expansion of the specialist milfoil weevil, *Euhrychiopsis lecontei*, from the native *Myriophyllum sibiricum* (northern watermilfoil) to invasive *M. spicatum* (Eurasian watermilfoil) is one of the few examples of a native insect herbivore preferring, growing and surviving better on a nonindigenous host plant than it does on its native host plant. The milfoil weevil's preference for the nonindigenous plant can be induced during juvenile development or through exposure to Eurasian watermilfoil as an adult. We evaluated how the fecundity of the milfoil weevil was affected over time by juvenile and adult exposure to the native, invasive and invasive × native hybrid milfoils and whether fecundity was correlated with host plant quality. Weevils reared on Eurasian watermilfoil laid more eggs than those reared on northern or hybrid watermilfoils. When weevils were collected from and exposed to milfoils collected directly from a lake, Eurasian-reared weevils had higher fecundity and greater preference for Eurasian over northern watermilfoil. When weevils were reared on and allowed to oviposit on milfoils grown in a common environment, the differences in fecundity and preference for Eurasian over northern or hybrid watermilfoils diminished. In Eurasian-northern experiments, milfoil weevils laid more than 80% of their eggs on Eurasian watermilfoil, but that value decreased when plants from common environments were used. Despite this preference, most weevils continued to use both hosts indicating that a complete host switch is unlikely. Weevils showed no oviposition preference between Eurasian and hybrid milfoils. The milfoil weevil had higher fecundity on Eurasian watermilfoil, which had a higher concentration of carbon, polyphenols and lignin than did northern watermilfoil, which had a higher concentration of ash. The milfoil weevil's preference for Eurasian watermilfoil was affected by changes in plant chemistry, and ash appeared to act as a deterrent to oviposition on northern watermilfoil. The milfoil weevil can modify its response based on host-plant chemistry.

**Key words:** *Euhrychiopsis lecontei*, *Myriophyllum spicatum*, *Myriophyllum sibiricum*, hybrid milfoil, specialist herbivore, oviposition preference and performance, host plant chemistry

**Introduction**

Specialist herbivores are adapted to the defenses of their host plants. Behavioral and physiological adjustments employed by specialists may facilitate acceptance of chemically similar novel host plants,

including nonindigenous invasive species (Keane and Crawley 2002; Craig and Itami 2008). These new associations may lead to a host range expansion or even a host-switch (Singer et al. 1993; García-Robledo and Horvitz 2012). The co-evolutionary history of the specialist insect and its native host plant may lead to the native host plant being better

defended against the specialist herbivore than the nonindigenous species, which had no previous experience with the specialist (Joshi and Vrieling 2005). However, nonindigenous host plants would still contain defenses against its co-evolved herbivores. Therefore, the success of an herbivore on native and nonindigenous host plants is ultimately mediated by the relative quality of these plants in a given region (Behmer 2009).

Basic nutritional quality of host plants, such as carbon, nitrogen and mineral content, as well as chemical defenses, play roles in the success of an herbivore. Nitrogen typically has a positive effect on insect fecundity (Herms and Mattson 1992), but carbon constituents (such as carbohydrates and lipids) and minerals have varied effects on fecundity (Awmack and Leather 2002). Carbohydrates and lipids can benefit herbivores, but their overall effect on insect performance depends on the age, sex and biotype of the insect as well as the chemical interactions among compounds (See review by Awmack and Leather 2002). Defensive compounds also have varied effects on herbivores. Adapted specialists tend to be undeterred by host plant defenses, but exceptionally high concentrations can still decrease insect performance (Bernays and Chapman 1994). The integrated effects of plant nutrients and defenses ultimately work together to determine herbivore preference and performance at all life stages (Behmer 2009).

One measure of an insect's success on a host plant is its fecundity, which can be affected at each developmental stage. As a larva, the insect must obtain adequate nutrition and tolerate or circumvent plant defenses. Larval feeding can influence adult feeding preference (Solarz and Newman 1996), duration of development, and pupal and adult size and survival (Stamp and Casey 1993). Adult feeding can influence its survival, fecundity (Hopkins and Ekbom 1999) and the success of the subsequent generation through maternal choice effects (Fox et al. 1995). Adult females must decide whether to spend time foraging for their own growth or for that of their offspring (Mayhew 2001; Scheirs and DeBruyn 2002; García-Robledo and Horvitz 2012). When larvae and adults feed on the same high quality host plants fecundity is high, while the opposite is true for low quality hosts (Wheeler and Center 1997; Awmack and Leather 2002). However, switching a larva from a high to low quality host, or *vice versa*, can have little or long-term impacts on adult fecundity (Hopkins and Ekbom 1999; Ekbom and Popov 2004; Diamond and Kingsolver 2010). For insects with longer-lived adults, adult feeding can mitigate the effects of a low quality larval host plant (Hopkins and Ekbom 1999).

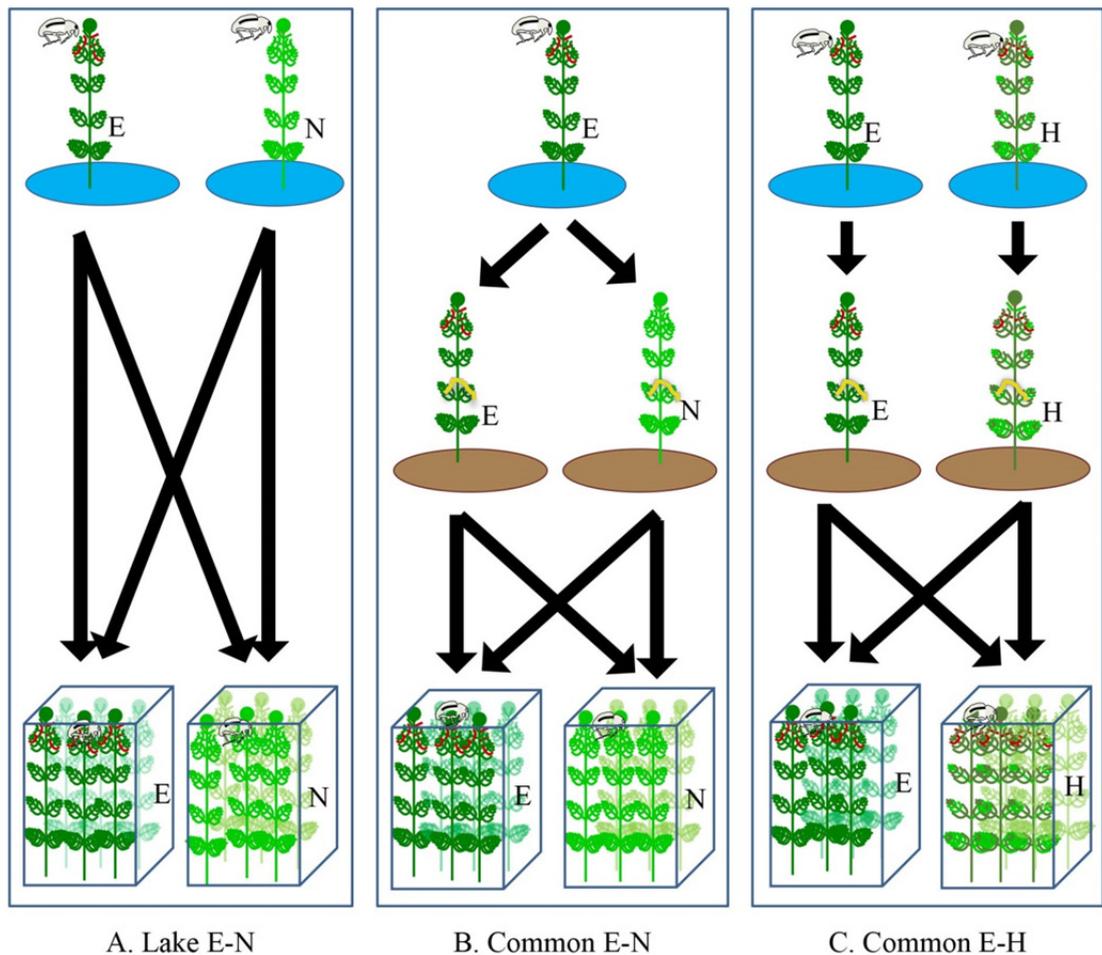
The specialist herbivore *Euhrychiopsis lecontei* (Dietz, 1896) (Coleoptera: Curculionidae) (hereafter, milfoil weevil) feeds on watermilfoils, *Myriophyllum* spp. (Haloragaceae), and has expanded its host range from its native host northern watermilfoil, *Myriophyllum sibiricum* Kom., to the invasive Eurasian watermilfoil, *M. spicatum* L (Solarz and Newman 1996). Not only does the milfoil weevil feed on both plants, but it prefers and often performs better on Eurasian watermilfoil (Solarz and Newman 2001). Milfoil weevils have shorter development times and greater survival (Solarz and Newman 2001; Roley and Newman 2006; but see Tamayo and Grue 2004 for contrast), and greater short-term oviposition rates on Eurasian watermilfoil than on its native host plant (Sheldon and Jones 2001). Weevil performance on the *M. spicatum* × *M. sibiricum* hybrid milfoils (hereafter, hybrid) may be variable (Roley and Newman 2006; Borrowman et al. 2015). Furthermore, exposure to Eurasian watermilfoil as an adult can induce a preference for Eurasian watermilfoil (Solarz and Newman 1996, 2001), but it is unclear when that preference develops and how exposure as either a juvenile or adult influences fecundity. In this study, we address the following hypotheses: Insect feeding as a juvenile or adult affects fecundity on native, invasive or hybrid host plants; host plant chemistry explains fecundity of the milfoil weevil; and fecundity changes over time in relation to these factors.

## Methods

### *Study organisms*

The milfoil weevil is found in Minnesota on its native host plant northern watermilfoil, the nonindigenous invasive Eurasian watermilfoil and their hybrid. The fully aquatic weevil can complete 3–5 generations per summer and overwinters in leaf litter at the shoreline of a lake (Newman et al. 2001). One to several eggs are laid on the apical meristems of a watermilfoil plant (Sheldon and O'Bryan 1996, Sheldon and Jones 2001). Larvae eat the meristem, then mine about 15 cm of the stem, exit the stem and pupate in a chamber 0.5 to 1 m below the apical meristem (Sheldon and O'Bryan 1996, Newman 2004). Approximately 21 d (depending on temperature) are required for development from egg to adult (Mazzei et al. 1999). The adult lifespan of the weevil is unknown, though it can live in the laboratory for more than 100 d (Sheldon and O'Bryan 1996).

Eurasian watermilfoil and Eurasian-reared weevils were collected from Lake Auburn (Carver Co., MN, USA; 44°52'N; 93°41'W) where Eurasian watermilfoil dominates the plant community. Northern watermilfoil



**Figure 1.** Diagram showing the experimental design with the top portion of each panel indicating the collection of weevils from Lake populations and whether females were placed directly into experimental aquaria (A: Lake E-N), or placed in a common environment so the emergent females could be mated then randomly assigned treatments (B: Common E-N or C: Common E-H). E = Eurasian watermilfoil, N = northern watermilfoil, H = Hybrid watermilfoil.

and northern-reared weevils were collected from Christmas Lake (Hennepin Co., MN, USA; 44°53'N; 93°32'W) where northern watermilfoil is abundant and Eurasian watermilfoil grew sporadically at the time of collection. Hybrid watermilfoil and hybrid-reared weevils were collected from Otter Lake (Ramsey Co., MN, USA; 45°7'N; 93°2'W) (Roley and Newman 2006; Moody and Les 2007).

*Oviposition performance and preference*

To determine the effect of juvenile rearing plant on fecundity, we collected adult weevils from lake populations of either Eurasian or northern watermilfoil (Lake Population Eurasian-Northern) or we reared weevils from egg to adult on each of Eurasian, northern or hybrid watermilfoils (Common Environment

Eurasian-Northern, Common Environment Eurasian-Hybrid). The effect of adult feeding and oviposition preference on fecundity was analyzed by allowing adult weevils from the three taxa to oviposit and feed on either Eurasian watermilfoil, northern watermilfoil, or hybrid watermilfoil (no choice treatments) or a combination of Eurasian watermilfoil and either northern or hybrid watermilfoil (two species choice experiment) (Figure 1). The no-choice treatments allowed us to determine the effect of oviposition plant on egg production. The choice treatment allowed us to determine oviposition preference. Together, both experiments allowed us to determine the effect of larval-rearing plant on oviposition performance and preference and the effect of single vs. mixed diet on oviposition performance.

Experiments were conducted in 9.5-l aquaria that were divided in half, with no water exchange between halves. Hereafter, aquarium refers to each 4.75-l half aquarium. Aquaria were filled with well water or tap water (dechlorinated) and aerated one day. Aquaria were kept under fluorescent lights (ca. 50  $\mu\text{mol s}^{-1} \text{m}^{-2}$  PAR at the surface) on a 16:8 h light cycle (light: dark) between 25–30 °C. The following day, six watermilfoil stems (each 20 cm, apical meristem included) of the oviposition treatment plant were placed in an aquarium along with one mated female weevil. Stem bases were weighted with to stand vertically. Each day, stems were evaluated for feeding and oviposition. Stems with eggs or damage were replaced. Stems with no apparent damage or eggs were replaced at least once every three days with stems that were recently collected. Approximately 10 weevils were used per treatment. Fecundity from all ovipositing weevils was included in the results. Weevils that never laid eggs were discounted in the experiment and weevils in some treatments were prone to “escaping” the aquaria before oviposition and have few replicates.

#### *Lake population experiments*

The Lake Population Eurasian-Northern experiment (Lake E-N) was conducted in 2001 (Figure 1A). Females were allowed to oviposit for 15 d. Oviposition rates for at least two weevils per treatment were continued indefinitely in order to estimate egg production over time and observe patterns of egg production.

#### *Common environment experiments*

Common Environment experiments were conducted with Eurasian and northern watermilfoils in 2002 (Figure 1B, Common E-N) and for Eurasian and hybrid watermilfoils in 2009 (Common E-H-1) and 2012 (Common E-H-2; Together Common E-H) (Figure 1C). Common environment experiments were designed to reduce the potential influence of sediment type on plant chemistry by growing the watermilfoils in 0.38-m<sup>3</sup> outdoor tanks (1.32 m × 0.69 m × 0.71 m, l × w × d) with homogenized sediment (Marko et al. 2008). The sediment was collected from Lakes Auburn and Christmas for Common E-N and Lakes Auburn, Otter and Lyseng (Clay Co., MN, USA; 46°57'N; 96°13'W) for Common E-H. Sediments were combined with a sand:loam (1:1) mix, homogenized, added to each tank to a depth of 4–7 cm and allowed to settle for 24 h before each tank was filled with tap water (Marko et al. 2008). The water was allowed to equi-

librate for 24 h and dechlorinating drops were added to neutralize any remaining chlorine. Two single-species tanks were planted with 200 stems, 20–30 cm long, for each of Eurasian, northern, or hybrid watermilfoils. Plants were allowed to root and grow for two weeks and maintained as described by Marko et al. (2008).

Common Environment experiments addressed the effect of weevil age and source population on fecundity (Solarz and Newman 2001) by using weevils of the same age (Figure 1). Ten adult weevils were collected from lake populations of Eurasian, northern or hybrid watermilfoils, as described above. Adults laid eggs and were removed after 14 d. Newly emerged adults were collected, placed on a stem of their juvenile rearing plant, and observed mating. Females were collected, laid their first egg, weighed (to the nearest 0.01 mg) and randomly placed into prepared experimental aquaria. Oviposition trials were limited to 11 d, based on the observation for Lake E-N females where egg production patterns were apparent within that time.

#### *Statistical analyses*

Weevil fecundity, quantified as the mean number of eggs laid female<sup>-1</sup> day<sup>-1</sup> (oviposition rate), was analyzed in two ways. First, to show broad patterns and summarize the data from all experiments, we used an analysis of covariance to compare oviposition rates using experiment (Lake or Common), rearing plant, and oviposition plant as explanatory variables, and initial weevil mass as a covariate (GLM procedure, SAS/STAT 9.1, SAS Institute, Inc., Cary, N.C.). Second, to look at egg deposition over time, we used a Poisson mixed effects model (NLMIXED procedure) to separately analyze the number of eggs laid female<sup>-1</sup> as a function of rearing plant, oviposition plant and time. Each experiment was analyzed separately. Fixed effects were rearing plant (for E-N experiments: Eurasian or northern watermilfoil; for E-H experiments: Eurasian or hybrid watermilfoils), oviposition plant (for E-N experiments: Eurasian, northern, or both watermilfoils; for E-H experiments: Eurasian, hybrid or both watermilfoils), weevil mass and day (repeated measures analysis). The random effect was variation in oviposition rate among females. The initial model included the following parameters:

$$\log_e(\text{No. eggs}) = b_0 + b_1d + b_2m + b_3r + b_4e + b_5n + b_6re + b_7rn + b_8dr + b_9de + b_{10}dn + b_{11}dre + b_{12}drn + \varepsilon$$

where  $b_0$  is the mean intercept,  $d$  is day of the experiment (~ 15 days),  $m$  is weevil mass,  $r$  is rearing

plant,  $e$  and  $n$  are oviposition plants. We tested for: trends over time, differences between rearing plants, differences between oviposition plants, rearing plant by oviposition plant interactions and differences in slopes. The best models were found by successively removing the term that decreased the Akaike information criteria (AIC) value most (Weisberg 2005). The NLMIXED procedure provides a hypothesis test for each parameter. Differences observed for oviposition rate based on juvenile rearing plant in the Lake E-N experiment may also reflect differences due to source population, species or plant chemistry whereas differences observed between rearing plants in Common Environment experiments should only reflect host plant species and chemistry.

Oviposition preference between Eurasian and northern watermilfoil or Eurasian and hybrid watermilfoil was determined as the mean proportion of eggs laid on Eurasian watermilfoil when weevils were presented with two watermilfoils. We used a logistic mixed effects model (NLMIXED procedure), assuming a binomial distribution. The initial model for weevils given a choice of oviposition plant included the following parameters:

$$\log_e(\text{No. eggs}) = b_0 + b_1d + b_2m + b_3r + b_4dr + \varepsilon$$

where terms are as defined above. The best fit model was determined by AIC.

A Pearson correlation was performed to determine whether mean egg production was related to initial female mass. An analysis of variance (GLM procedure) was used to determine if female mass was affected by juvenile rearing plant, oviposition plant and their interaction. A significant difference in oviposition plant would indicate a mass difference between treatments despite the random assignment of weevils to treatments. A significant difference in juvenile rearing plant should be the result of a physiological response by weevils to their rearing plant.

#### *Host plant chemistry*

To determine whether realized weevil fecundity and oviposition preferences were influenced by plant chemistry we collected Eurasian and northern watermilfoil stems from Lakes Auburn and Christmas in July and August 2001 to correspond to host plant chemistry from Lake E-N. The chemical analyses were run in parallel with Marko et al. (2008). Watermilfoils were collected in early and late August in 2002 from insect-free tanks to correspond to host plant chemistry from Common E-N. All stems were placed in Ziploc<sup>®</sup> bags and frozen at  $-20^\circ\text{C}$  until analyzed. Frozen stems (6 to 20 cm, including apical meristem) were lyophilized and ground to a fine powder by either

mortar and pestle or by pulverizing and homogenizing for 10 minutes in a mixer mill.

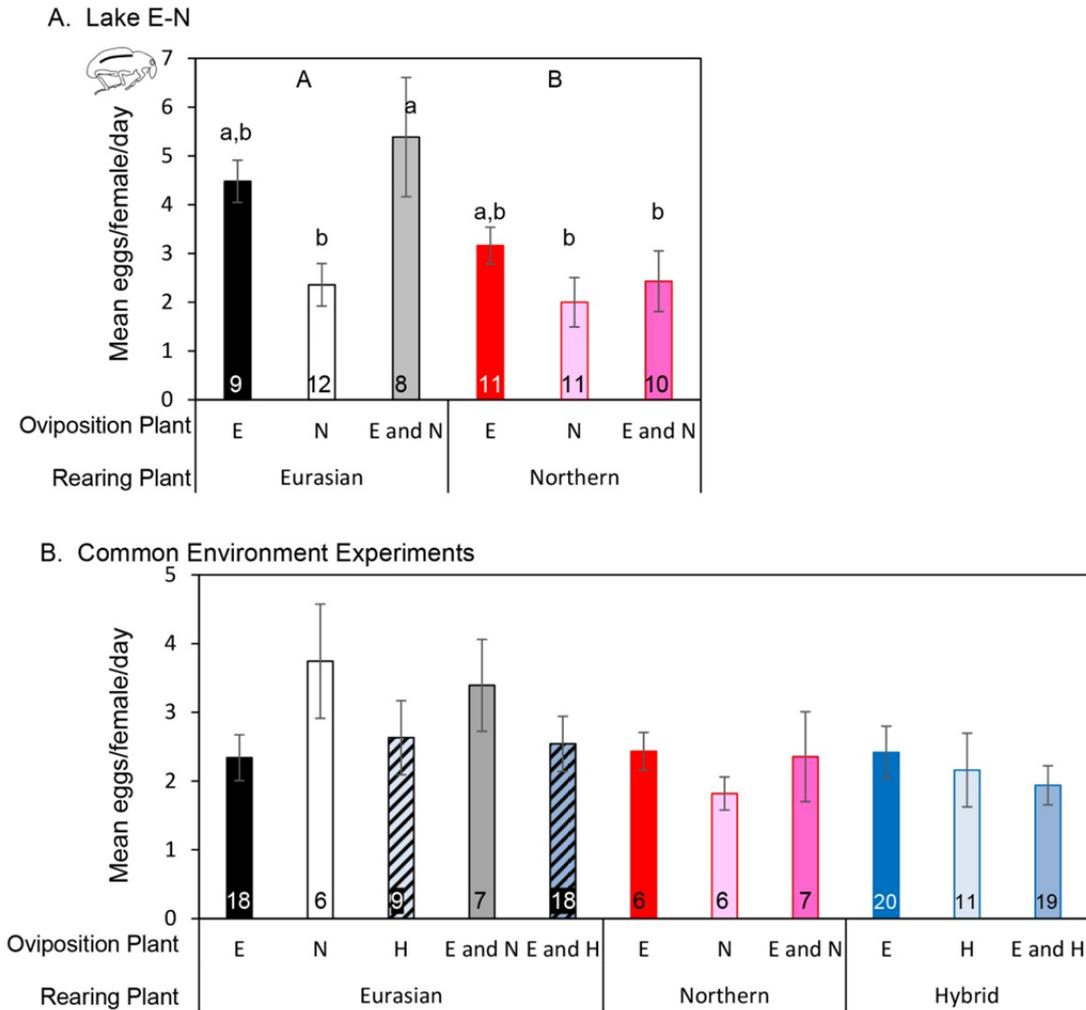
Milfoil stems were analyzed for carbon and nitrogen content with a Costech ECS 4010 (Costech Analytical) by the University of Nebraska School of Biological Sciences. Phosphorus was measured using the acid extraction method and colorimetric analysis with an Auto Analyzer 3 (AA3, Bran-Luebbe, Germany) by the University of Nebraska School of Biological Sciences.

Dried plant samples were also analyzed for concentrations of various carbon fractions (Ryan et al. 1990) at the Center for Water and the Environment (Natural Resources Research Institute, University of Minnesota, Duluth, Minnesota) according to forest products techniques (Ryan et al. 1990). Due to the quantity of material needed for carbon fraction analysis, only a limited number of samples were analyzed, though each sample was a composite of more than ten stems. Samples were analyzed for ash, nonpolar extractives (NPE: fats, oils, waxes and chlorophylls), water-soluble compounds (WS: simple sugars, soluble phenolics, amino acids), acid-soluble compounds (AS: polysaccharides, polypeptides, nucleic acids) and insoluble material, which consists of lignin and other indigestible materials, hereafter lignin. The water-soluble component was further analyzed for phenolics (Folin-Denis method) measured as tannin equivalents and simple sugars measured as percent glucose equivalents. The acid-soluble component was further analyzed for polysaccharides (cellulose, hemicellulose, starch) measured as percent glucose equivalents. Lignin is the material remaining after all extractions. All carbon fractions are presented as percent ash-free dry mass.

#### *Statistical analyses*

Analyses of variance were used to analyze the effect of plant source (Lake vs. Common) and plant species and their interaction on the chemical constituents found in the top 6 to 20 cm of the plant stems. When the factor plant source was significant (including C:N ratio with  $p = 0.051$ ), a 1-way ANOVA (GLM procedure) for each experiment was performed with plant species as factor. Two-way ANOVAs were used to analyze the effect of plant source and plant species for carbon fraction chemical constituents. When significant differences among treatments were found, the ANOVAs were followed by Tukey's Honest Significant Difference (HSD) test adapted for unequal sample sizes (Day and Quinn 1989).

To directly compare weevil performance data with chemical analyses, we used Pearson correlations.



**Figure 2.** Mean ( $\pm 1$  standard error) number of eggs laid per female per day for (A) Lake E-N and (B) Common Environment experiments. Replicates per treatment are listed at the base of each histogram. Oviposition plant is the plant that mated females were presented with. Rearing plant is the species the females were reared on from egg to adult. In Lake E-N, significant differences between rearing plants are indicated with capital letters ( $P < 0.05$ ). Significant differences among oviposition plants are indicated by lower case letters ( $P < 0.01$ ). In Common Environment, differences among oviposition plants were not significant. Associated ANCOVAs are presented in Table S1.

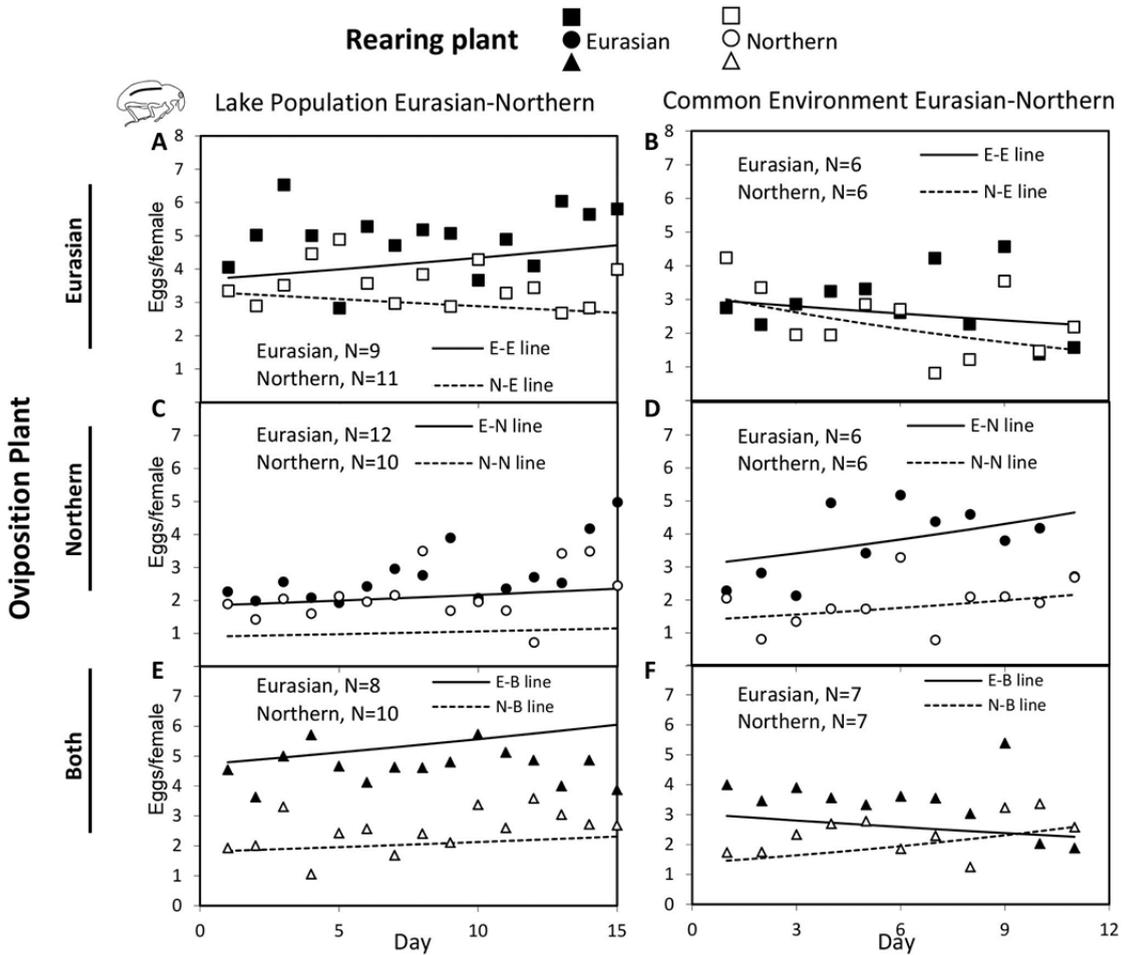
We summarized plant chemical data and weevil performance data to correspond with rearing or oviposition plant. Elemental and atomic ratios from both host species were averaged by year for plants from the first and second collections (2 species by 2 years by 2 collections = 8 data points). The oviposition rate was averaged by experiment and rearing plant or oviposition plant to correspond to the plant chemistry from the two collections. Oviposition rates were averaged to correspond with plant samples for carbon fraction analyses. Due to limited plant chemistry samples, only five points were used to correlate ash, lignin, polyphenol and acid-solubles with egg production. Therefore, Pearson correlations

between egg production and carbon fractions should be considered exploratory. Initial weevil mass was tested for correlation with carbon constituents from the first collections ( $n = 4$ ). Common E-H milfoils were not chemically analyzed due to limited resources.

## Results

### *Oviposition performance*

In the summary analysis of Lake E-N, Eurasian-reared females had the highest fecundity when presented with Eurasian or Eurasian and northern watermilfoils, laying an average of  $4.5 \pm 0.43$  and  $5.4 \pm 1.22$  eggs day<sup>-1</sup>, respectively (Figure 2A). A maximum of 20 eggs was



**Figure 3.** Mean number of eggs per female by day and the modeled response based on the Poisson mixed effects model for Lake E-N no-choice and choice oviposition experiments (A, C, and E) and Common E-N no choice and choice experiments (B, D, and F). Points within each panel are the mean number of eggs/female for females reared on either Eurasian (filled markers) or northern watermilfoil (open markers) and the plant they were placed on for oviposition: Eurasian (squares), northern (circles) or both species (triangles). Lines are based on the best fit Poisson mixed effects model for no-choice and choice data limited to the first 11 or 15 days of the experiment (values from Table S2). N = number of weevils per treatment.

laid by one female and up to 12 eggs were laid on one meristem. Females typically laid more than one egg per stem ( $2.0 \pm 0.10$  eggs stem<sup>-1</sup>). Fecundity of lake-reared weevils was significantly affected by both juvenile rearing plant and oviposition plant (Table S1). Of the females allowed to oviposit for longer than 15 d, the three longest-lived Eurasian-reared weevils laid an average of  $187 \pm 52$  eggs over an average lifespan of 34 days and northern-reared weevils laid  $127 \pm 13$  eggs over an average lifespan of 39 days. One Eurasian-reared weevil placed onto Eurasian watermilfoil produced 288 eggs in 44 days.

In common environment experiments, the summary ANCOVAs did not explain mean fecundity for any treatment, but the explanatory variable rearing plant

was significantly different for Common E-N with Eurasian weevils generally laying more eggs than northern-reared weevils (Figure 2, Table S1). Weevils laid an average of  $69 \pm 5$  eggs over an average lifespan of 16 days; due to experiment constraints hybrid weevils were not allowed to oviposit as long as in Lake E-N.

A more detailed assessment of oviposition rate revealed the importance of duration, juvenile rearing plant and adult oviposition plant on egg production (Table S2). Based on the parameter estimates, the modeled response of weevils was presented along with the data for each treatment for all experiments (Figures 3 and 4). Some generalities in oviposition rates were observed. Similar to the summary analysis,



distribution,  $b_0 = 0$  would indicate 50% selected Eurasian watermilfoil). In Common E-N, Eurasian- and northern-reared weevils both preferred Eurasian watermilfoil and laid 79% of their eggs on it (Table S3). However, the preference for Eurasian watermilfoil decreased over time and the proportion of eggs laid on Eurasian watermilfoil decreased over the course of the experiment from 86% to 69%. In Common E-H, Eurasian- and hybrid-reared weevils expressed no preference for Eurasian or hybrid watermilfoils (replicate 1:  $df = 16$ ,  $t = 1.43$ ,  $p > 0.1$ ; replicate 2:  $df = 19$ ,  $t = 0.22$ ,  $p > 0.1$ ).

#### Weevil mass

Females collected from lake populations of Eurasian watermilfoil were significantly heavier ( $\bar{x} = 1.48 \pm 0.041$  mg;  $N = 27$ ) than weevils collected from lake populations of northern watermilfoil ( $\bar{x} = 1.33 \pm 0.026$  mg;  $N = 32$ , Table S4). In Lake E-N, female mass ranged from 1.02 mg to 2.02 mg. However, that difference was not observed for weevils in Common E-N (Eurasian-reared:  $1.30 \pm 0.061$  mg,  $N = 19$ ; northern-reared:  $1.30 \pm 0.038$  mg,  $N = 19$ ) ( $df = 1$ ,  $F = 0.01$ ,  $p > 0.1$ ). In Common E-H-1, weevils reared on Eurasian watermilfoil had greater mass than hybrid-reared weevils (Eurasian-reared:  $1.79 \pm 0.086$  mg,  $N = 12$ ; hybrid-reared:  $1.50 \pm 0.057$  mg,  $N = 23$ , Table S4) but not Common E-H-2 (Eurasian-reared:  $1.54 \pm 0.054$  mg,  $N = 27$ ; hybrid-reared:  $1.36 \pm 0.061$  mg,  $N = 27$ , Table S4).

#### Host plant chemistry

Eurasian watermilfoil contained a greater percentage of carbon than northern watermilfoil and similar amounts of nitrogen and phosphorus (Table S5, Figure 5 A, C, and E). The differences observed in carbon content between species led to significantly higher C:N ratio in Lake E-N Eurasian watermilfoil ( $df = 1$ ,  $F = 5.46$ ,  $p = 0.0285$ ), but no difference in C:N was observed for Common E-N Eurasian or northern watermilfoil ( $df = 1$ ,  $F = 0.09$ ,  $p > 0.1$ ) (Figure 5B). Nitrogen and phosphorus content did not differ between species (Table S5). Phosphorus content was lower in watermilfoils from lake populations than plants grown in the common environment. The difference among experiments in phosphorus content was reflected in lower C:P and N:P ratios in plants from the common environment (Figure 5 D and F).

Differences in carbon content were further investigated with the carbon fraction analysis. Nonpolar extractables (fats, oils, lipids) and sugar content were higher in plants from the common environment than in lake populations (Table 1). In contrast,

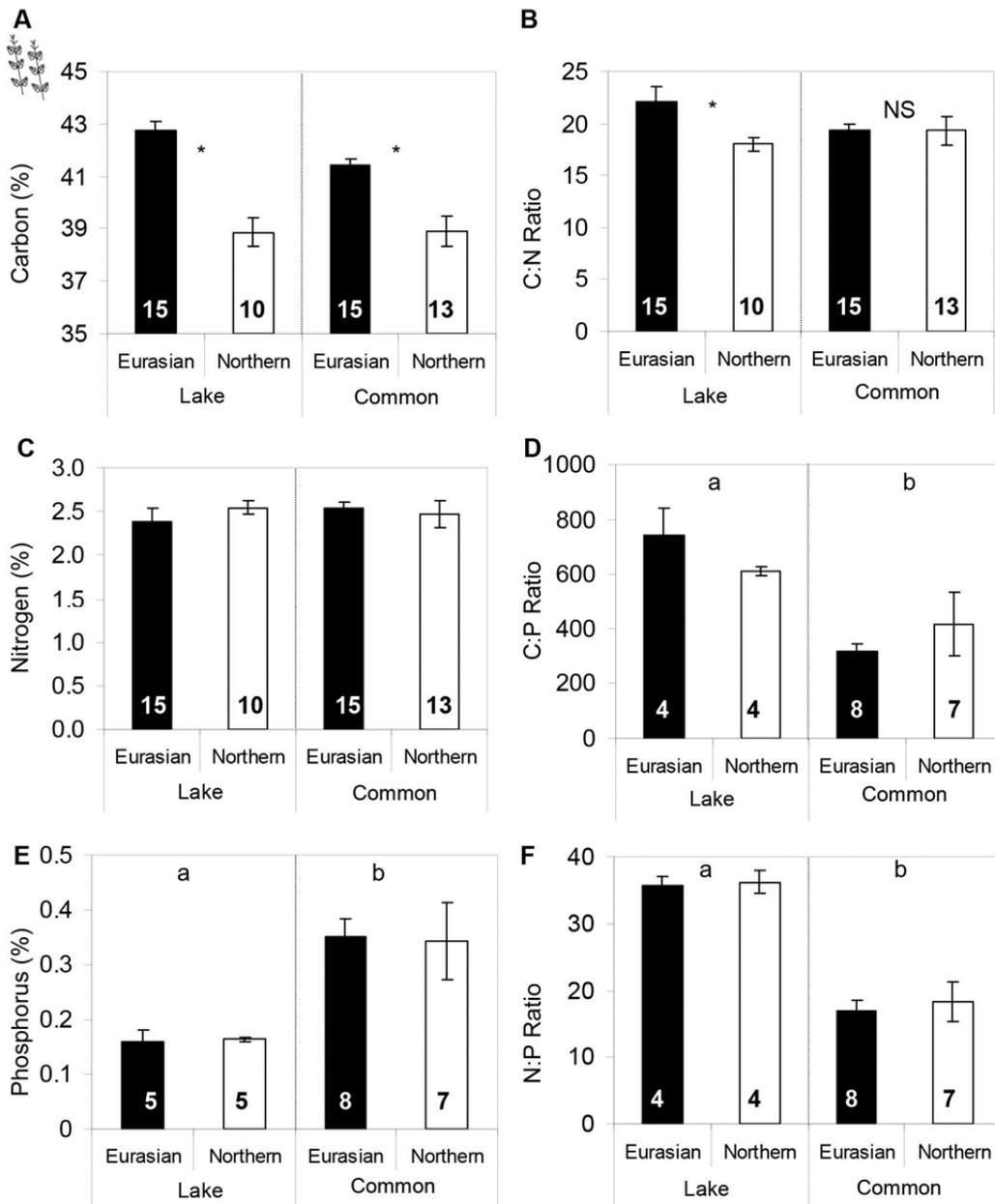
polysaccharide content was higher in plants from lake populations. Differences in carbon constituents between species were observed for percent ash, acid solubles, polyphenols, lignin, and lignin:nitrogen ratio (Table 1). Percent ash was higher in northern watermilfoil than Eurasian watermilfoil. Polyphenols, lignin, and lignin:nitrogen ratio were higher in Eurasian than in northern watermilfoils.

To explore comparisons of weevil performance with plant chemistry, correlations were calculated with oviposition rate and weevil initial weight and the chemical constituents that were significantly different between species: carbon content, C:N, ash, acid soluble chemicals, polyphenols, lignin and lignin:N (Table 1 and Table S5). Oviposition rate was positively correlated with carbon ( $r = 0.89$ ,  $p = 0.041$ ) and negatively correlated with ash ( $r = -0.92$ ,  $p = 0.027$ ). Female mass was positively correlated with lignin content ( $r = 0.95$ ,  $p = 0.050$ ) and lignin:N ratio ( $r = 0.91$ ,  $p = 0.086$ ).

#### Discussion

The native specialist herbivore *E. lecontei* had higher fecundity on the nonindigenous Eurasian watermilfoil than on the native northern watermilfoil and preferred the nonindigenous species to its native host plant. This extends previous studies that showed weevil performance (development rate, mass and survival) to be higher on Eurasian watermilfoil than on northern watermilfoil and the influence of rearing plant on performance (Newman et al. 1997; Roley and Newman 2006; Solarz and Newman 2001). However, unlike previous studies that demonstrated *E. lecontei* development rate and survival on hybrid watermilfoil was intermediate or better than performance on the parental plants (Roley and Newman 2006; Borrowman et al. 2015), we found that oviposition rates did not differ for weevils reared on or exposed to Eurasian or hybrid watermilfoils. These results confirm our hypothesis that both larval rearing plant and adult feeding plant affect fecundity, although we did not find differences between fecundity of weevils exposed to Eurasian and hybrid watermilfoils.

A combination of host plant chemistry, behavioral and adult and juvenile physiological factors can influence oviposition rates over time (Awmack and Leather 2002). As juveniles, insects are influenced by maternal effects such as egg size and condition (Rossiter 1996; Wheeler 1996) as well host plant quality. For many insects, including the milfoil weevil, higher oviposition rates can be attributed directly to greater adult mass (Newman et al. 1997; Armbruster and Hutchinson 2002). However, in our experiments female mass was a significant factor in



**Figure 5.** Carbon, nitrogen, phosphorus content and C:N, C:P, and N:P molar ratios (mean  $\pm$  1 S.E.) for Eurasian (solid bars) and northern (open bars) watermilfoils collected from lake populations for Lake E-N and weevil free tanks for Common E-N. The number of samples per treatment are indicated within each bar. Significant differences between species within each experiment are indicated with an asterisk on F-tests for each experiment at  $P < 0.05$ . Significant differences between experiments (Lake vs. Common) are indicated by lower case letters above each experiment at  $P < 0.05$ . Data correspond to statistical analyses presented in Table S5.

only one of the four models (Common E-H-1). Therefore other factors, such as quality of rearing plant, condition of the egg, or host plant quality must affect the initial oviposition rates.

Larval rearing plant was also insufficient to explain the long-term oviposition rates observed in

these experiments. When weevils were reared on northern watermilfoil, they initially laid fewer eggs, however, they increased oviposition rates when presented with Eurasian and northern watermilfoils. Even the Eurasian-reared females had the highest oviposition rate when Eurasian and northern watermilfoils

**Table 1.** Means from carbon fractions analysis are listed for Eurasian and northern watermilfoils for the following chemical components: C, N, C:N molar ratio, ash, nonpolar extractives (NPE: fats, oils, waxes and chlorophylls), water-soluble fraction (WS: simple sugars, hydroxyphenols, amino acids), acid-soluble carbohydrates (AS: cellulose, hemicellulose, and starch), water-soluble simple sugars (WS sugars), acid-soluble sugars (AS sugars), water-soluble phenolics (polyphenols), lignin and lignin:N ratio (mg/g). All fractions are percentage by mass of Ash Free Dry Mass (AFDM). Significant differences for each model are indicated with a letter next to the variable in the first column. Significant differences for the parameters species and experiment are indicated for each variable next to appropriate F-value. Standard errors are listed in parentheses below the mean value.

Variable	Eurasian (N=6)	Northern (N=3)	Species	Experiment
			F	F
%C <sup>a,*</sup>	47.32 (1.11)	39.64 (2.62)	11.02*	
%N <sup>a</sup>	2.76 (0.41)	2.80 (0.06)	0.00	
%C:N <sup>a</sup>	21.58 (3.55)	16.54 (1.44)	0.87	
%Ash <sup>+</sup>	7.97 (0.946)	20.69 (5.77)	8.98*	0.15
%NPE <sup>****</sup>	11.25 (2.74)	12.29 (4.17)	0.87	125.9 <sup>****</sup>
%WS <sup>***</sup>	18.88 (2.41)	18.64 (2.99)	0.05	96.67 <sup>****</sup>
%AS <sup>****</sup>	52.30 (3.16)	59.92 (5.47)	67.29 <sup>***</sup>	271.8 <sup>****</sup>
%WS sugars <sup>*</sup>	5.65 (1.38)	4.20 (1.07)	1.50	17.00 <sup>**</sup>
%AS sugars <sup>*</sup>	27.70 (3.99)	27.18 (4.13)	0.02	12.74 <sup>*</sup>
%Polyphenols <sup>*</sup>	5.62 (0.405)	3.34 (0.38)	13.69 <sup>*</sup>	1.62
%Lignin <sup>*</sup>	17.58 (2.46)	9.15 (1.69)	10.15 <sup>*</sup>	8.23 <sup>*</sup>
Lignin:N <sup>a,**</sup>	7.58 (0.36)	3.87 (0.20)	45.10 <sup>**</sup>	

<sup>a</sup>df = 1, 4; all other model df = 2, 6

Significance at <sup>\*</sup>P<0.1, <sup>\*</sup>P<0.05, <sup>\*\*</sup>P<0.01, <sup>\*\*\*</sup>P<0.001, <sup>\*\*\*\*</sup>P<0.0001

were both present. Therefore, despite the clear demonstration of shorter development times and higher survival rates on Eurasian watermilfoil (Newman et al. 1997; Roley and Newman 2006; but see Tamayo and Grue 2004), females still laid eggs and received a benefit from being on northern watermilfoil. This confirms our hypotheses that adult feeding affects fecundity and that fecundity coincidentally changes over time. Maternal choice was not optimized solely to benefit offspring development and success. A large amount of variation has been observed in natural populations of the milfoil weevil on populations of different milfoil species (Borrowman et al. 2014; Tamayo and Grue 2004). A more detailed analysis of plant chemistry throughout the experiment is needed to determine how changes in plant quality affect oviposition rate with time.

Fecundity can be influenced or constrained by behavioral choice or physiological capability in response to host plant qualities (García-Robledo and

Horvitz 2012; Mayhew 2001). If the ovipositing females detected changes in plant quality, they may have altered egg or clutch size to provide offspring with an advantage (Ekbom and Popov 2004). Wheeler and Ordnung (2005) suggest that for the beetle *Oxyops vitiosa* Pascoe, 1870, adult feeding could determine fecundity, even “ameliorating” the effect of poor quality juvenile rearing plant. Ekbom and Popov (2004) found that the pollen beetle, *Brassicogethes aeneus* (Fabricius, 1775) (*Meligethes aeneus* in article), could determine host plant quality and then respond by laying fewer smaller eggs on lower quality host plants. Therefore, both physiological responses to host plant quality and behavioral responses to differences in host plant quality (even within a treatment) could affect oviposition rates.

Northern and Eurasian watermilfoils are very similar morphologically, genetically and chemically, except for higher polyphenol concentration in Eurasian watermilfoil and higher ash content in northern

watermilfoil (Marko et al. 2008; Moody and Les 2010). The host range expansion to Eurasian watermilfoil from its native northern watermilfoil seems to be facilitated by a combination of deterrence from the native host plant (Marko et al. 2008), attraction to the nonindigenous invasive species (Marko et al. 2005) and preference (Solarz and Newman 1996, 2001) for Eurasian watermilfoil when host plant quality was high.

### Preference

Solarz and Newman (2001) found that the exposure of adult weevils for two weeks to Eurasian watermilfoil induced a preference for Eurasian watermilfoil. Exposure to northern watermilfoil did not reverse that preference. In our experiments, both northern- and Eurasian-reared weevils found Eurasian watermilfoil attractive within the first day of exposure, suggesting that Eurasian watermilfoil is inherently more attractive than northern watermilfoil (Solarz and Newman 2001; Sheldon and Jones 2001; Marko et al. 2005). No preference between Eurasian or hybrid milfoils was found for Eurasian or hybrid-reared weevils corroborating performance data, which is variable depending on the hybrid milfoil strain (Roley and Newman 2006; Borrowman et al. 2015).

In Lake E-N, Eurasian and northern-reared weevils laid 82% of their eggs on Eurasian watermilfoil and increased their oviposition rate over time when exposed to both Eurasian and northern watermilfoils together. Initially, the weevils may have been attracted to Eurasian watermilfoil by chemical attractants (Marko et al. 2005) and high oviposition rates may have been sustained by a physiological response to higher quality rearing plants. For Common E-N, exposure to both host plants resulted in decreased preference for Eurasian watermilfoil regardless of rearing plant. In Common E-H, weevils did not distinguish between Eurasian and hybrid milfoils and oviposited equally on both species. In the common environment experiments, plant chemistry was similar between species, which could lead to habituation to and a greater acceptance of northern watermilfoil and no discernment between Eurasian and hybrid milfoils (Bernays and Chapman 1994).

### Host plant chemistry

Our findings indicate that plant chemistry, particularly carbon content, is important to the fecundity of the milfoil weevil, which confirms our initial hypothesis. Nitrogen content did not differ among treatments and was an unlikely cause of the differences in herbivore performance observed here (as also seen by Fischer and Fielder 2000). Although

there was a significant difference in phosphorus content between Lake E-N and Common E-N, higher phosphorus content in Common E-N did not result in larger weevils or higher oviposition rates. In fact, weevils laid fewer eggs in Common Environment experiments than in Lake E-N. Of the stoichiometric parameters, only carbon content differed significantly between species, being higher in Eurasian watermilfoil. Additionally, polyphenol and lignin content were also higher in Eurasian watermilfoil. Hybrid milfoil from Otter Lake, similar to that used in this study, had ash, lignin, polyphenol and C content similar to Eurasian watermilfoil (Marko and Newman, unpublished results). The higher carbon content in Eurasian and hybrid watermilfoils reflected greater concentrations of quantitative (polyphenols) and structural (lignin) defenses, which apparently had no negative impact on weevil fecundity. Insects can compensate for the presence of defensive chemicals when nutrient content is adequate (Behmer 2009) and modify their preference and performance based on their changing nutritional requirements (Simpson et al. 2015). The high level of preference by adult weevils for Eurasian watermilfoil could indicate that the milfoil weevil was optimally foraging to obtain good nutrition (Scheirs and DeBruyn 2002).

Polyphenols and lignin typically decrease herbivore performance in terrestrial and marine systems, particularly among generalists, though a wide range of responses have been observed (Ilkkoenen 2002; Foss and Rieseke 2003). Likewise in freshwater systems, phenolic compounds have a variety of effects depending on the herbivore and plant. Phenolic compounds in Eurasian watermilfoil deter consumption by the generalist snail *Radix swinhoei* Adams, 1866 (Li et al. 2004) and reduce the growth of the lepidopteran *Acentria* Stephens, 1829 (Choi et al. 2002) by negatively affecting gut microbiota (Walenciak et al. 2002). Given that the milfoil weevil did not exhibit a similar negative response, either this specialist weevil was not affected by these constitutive defenses that affect generalists (through coevolution), or other positive factors outweighed the negative effects of the constitutive defenses, making Eurasian watermilfoil a better host plant despite its defensive chemistry.

Ash is one chemical constituent that may make northern watermilfoil a lower quality host plant for the milfoil weevil. Ash, which includes minerals such as calcium carbonate, silica, potassium and magnesium (Aiken and Picard 1980), was higher in northern watermilfoil than in Eurasian watermilfoil and was negatively correlated with fecundity. These values are comparable to ash concentrations found in other aquatic plants (Spencer et al. 1997) and in a

related study (Marko et al. 2008). Although ash is not usually considered in studies of host plant quality, it has been negatively associated with the survival of the Florida apple snail, *Pomacea paludosa* (Sharfstein and Steinman 2001), and the beetle *Coleomegilla maculata* De Geer, 1775 (Lundgren and Wiedenmann 2004), and can wear down insect mouth parts (Stromberg et al. 2016). High ash content may yield fewer calories per gram food (Yufera et al. 1997), limit micronutrient availability (Lundgren and Wiedenmann 2004), or act as a deterrent through synergisms with defensive chemicals (Hay et al. 1994). Discernment among these hypotheses requires a more detailed feeding study with a single species milfoil containing high and low concentrations of C, N, polyphenols, lignin and ash.

### Implications for *E. lecontei* populations

Fecundity of *E. lecontei* females was significantly affected by rearing plant, oviposition plant, and time. Although the fecundity values we found fall within the range observed in other studies (Sheldon and Jones 2001), the greatest mean rate of egg production ( $5.4 \pm 1.22$  eggs female<sup>-1</sup> day<sup>-1</sup>) was higher than previous reports for mean number of eggs female<sup>-1</sup> day<sup>-1</sup>. Ten percent of females laid ten or more eggs on a single day. Given the range of fecundities observed, population growth rate can vary considerably, ultimately leading to variation in the weevils' ability to control Eurasian watermilfoil. A fecundity of 1.9 eggs female<sup>-1</sup> day<sup>-1</sup> was used in the model developed by Miller et al. (2011) to identify best management practices for stocking weevils in lakes. Altering the oviposition rate could impact both the population-density implications and the stocking pattern suggested by their model. Although there are many variables impacting the efficacy of biocontrol in a lake (Miller et al. 2011; Newman 2004), we have demonstrated that host plant quality could result in more than a two-fold increase in oviposition rate and may have a big effect on population size and control in the field.

Despite their preference for Eurasian watermilfoil, 28 of 32 weevils deposited an egg on northern watermilfoil, and 34 of 37 weevils laid an egg on hybrid watermilfoil. Furthermore, given that northern-reared weevils experienced a decrease in fecundity when northern watermilfoil was absent, it seems that a complete host shift is unlikely and that some weevils will remain on populations of northern watermilfoil (Solarz and Newman 1996). Our results support the suggestion by Sheldon and Jones (2001) that the co-occurrence of fluctuating Eurasian, northern, and hybrid watermilfoil populations within

a lake probably make it advantageous for weevils to be behaviorally plastic in their host preference. The greater preference for and performance on Eurasian watermilfoil by adults and juvenile weevils has contributed to the weevil's host range expansion to the introduced species. However, a complete host shift is unlikely because the weevil can modify its preference based on changes in host plant quality.

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### Supplementary material

The following supplementary material is available for this article:

**Table S1.** ANCOVA results for the effects of rearing plant, oviposition plant, their interaction and the covariate initial weight, on mean number of eggs female<sup>-1</sup> day<sup>-1</sup> for weevils collected directly from lake populations and those grown in a common environment on either Eurasian, northern or hybrid milfoils.

**Table S2.** Estimates and t-values for the non-linear mixed effects models with Poisson distribution using individual weevil as the random effect.

**Table S3.** Parameter estimates for Common E-N best-fit model in proportion of eggs laid on Eurasian watermilfoil for choice oviposition experiments.

**Table S4.** ANOVA results for the effects of source (rearing) plant, oviposition plant, their interaction on the initial mass for the milfoil weevil used in oviposition experiments.

**Table S5.** ANOVA results for the effects of species, experiment type and their interaction on carbon, nitrogen and phosphorus content and C:N, C:P and N:P.

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