Changes in abundance of *Gloeotrichia pisum* across three macrophyte species in a Canadian lake

Tristan Kosciuch [Corresp., 1], **Audrey Normand** [1], **Loic R Gauthier** [1]

[1] Department of Biology, McGill University, Montreal, Quebec, Canada

Corresponding Author: Tristan Kosciuch
Email address: tristan.kosciuch@mail.mcgill.ca

Epiphytes play an important role in the productivity of aquatic systems but their relationships to macrophytes are complex. We describe the abundance of a little known epiphytic cyanobacteria, *Gloeotrichia pisum*, on three macrophytes species: *Myriophyllum spicatum*, *Ceratophyllum demersum*, and *Elodea canadensis* in Lac Hertel, Quebec, Canada. We find that *C. demersum* and *M. spicatum* have higher abundances of *G. pisum* than *E. canadensis*, and that there is significant variation in G. pisum abundance across sampling sites in *M. spicatum*. While not significant, *E. canadensis* showed similar variation in *G. pisum* across shallow sites as *M. spicatum*.

Title: Changes in abundance of *Gloeotrichia pisum* across three macrophyte species in a Canadian lake

Authors: Tristan Kosciuch[1], Audrey Normand[1], Loïc Renaud Gauthier[1].

[1]Department of Biology, McGill University, Montreal, Quebec, Canada.

Corresponding author: Tristan Kosciuch[1]

Email address: tristan.kosciuch@mail.mcgill.ca

Keywords: Rivulariaceae, Macrophyte epiphyte interactions, Limnology

Abstract: Epiphytes play an important role in the productivity of aquatic systems but their relationships to macrophytes are complex. We describe the abundance of a little known epiphytic cyanobacteria, *Gloeotrichia pisum*, on three macrophytes species: *Myriophyllum spicatum*, *Ceratophyllum demersum*, and *Elodea canadensis* in Lac Hertel, Quebec, Canada. We find that *C. demersum* and *M. spicatum* have higher abundances of *G. pisum* than *E. canadensis*, and that there is significant variation in G. pisum abundance across sampling sites in *M. spicatum*. While not significant, *E. canadensis* showed similar variation in *G. pisum* across shallow sites as *M. spicatum*.

Introduction

Aquatic epiphytes are sessile primary producers that grow on aquatic macrophytes. These epiphytes can sustain a collection of herbivores distinct from what feeds directly on macrophytes and can generate more primary productivity than their host macrophytes (Cattaneo & Kalff, 1980; Pelton, Levine & Braner, 1998). Thus, understanding how ecological disturbance influences an epiphyte community is useful for understanding change in aquatic ecosystems.

A major cause of ecosystem disturbance in the Anthropocene are human-mediated invasive species. One example is *Myriophyllum spicatum*, an aquatic macrophyte that has spread to the Americas, Africa, and Australia from its native range in Eurasia. *Myriophyllum spicatum* covers tens of thousands of hectares where it has been introduced and has been tied to declines in native macrophyte abundance and richness (Eiswerth, Donaldson & Johnson, 2000). In Canada, *M. spicatum* was first observed in 1961(~~4~~). *Myriophyllum spicatum* has been established as an abundant macrophyte in a small North American lake: Lac Hertel in Quebec, Canada, (45.5450407 ºN, 73.155265º W) since at least 2008 (Kovach, Velghe & Lechowicz, 2008). Lac Hertel is part of the McGill University Gault Nature Reserve (GNR), an ongoing research location since the 1800s. In August 2018, while conducting work for a field course, we noticed

that *M. spicatum* in Lac Hertel was covered by an epiphytic cyanobacteria, *Gloeotrichia pisum* (Thuret ex Bornet & Flahault 1886). We were surprised by the abundance of *G. pisum*; some *M. spicatum* had *G. pisum* completely covering almost every available surface (Fig S1). *Gloeotrichia pisum* appears to be resistant to many macroinvertebrate grazers, likely due to its hard outer sheath (Cattaneo, 1983). Because of this resistance to macroinvertebrate grazers any *M. spicatum* related changes in *G. pisum* abundance relative to other epiphytes might change macroinvertebrate communities and thus have downstream effects on predatory fish.

Thus, we asked whether *G. pisum* growth was indeed higher on *M. spicatum* than on two abundant native macrophytes in Lac Hertel: *Elodea canadensis* and *Ceratophyllum demersum*. To also test whether *G. pisum* abundance was structured by depth and space, we worked at five nearshore and five deep sites evenly spaced around Lac Hertel. We found that in Lac Hertel, *G. pisum* is more abundant on *M. spicatum* and *C. demersum* than on *E. canadensis*. We also found that *G. pisum* abundance varied significantly across sampling sites in shallow *M. spicatum*. In addition, *G. pisum* on *E. canadensis* showed a similar variation across shallow sites as *M. spicatum* although this was not significant.

Methods

Study site

Lac Hertel is a small (0.3 km^2) meso-oligotrophic post-glacial lake fed by three inlet streams and drained by one outlet stream. All water inflow is from area protected by the reserve. The lake is shallow and approximately 66% of the lake bottom is covered by macrophytes (Rooney & Kalff, 2003). *Gloeotrichia pisum* is known to prefer oligotrophic systems such as Lac Hertel (Hudon, Cattaneo & Gagnon, 2009).

Sample collection

We sampled macrophtyes and *G. pisum* on August 29 and 30th, 2018 from five nearshore and five deep sites around Lac Hertel (Figure 1). We included a depth component because we

hypothesized that differences in light availability due to depth could impact *G. pisum* abundance independently of macrophyte species identity. No species protected under provincial, national, or international regulations were sampled, intentionally or unintentionally.

We waded to collect from shallow sites, and snorkeled to collect from deep sites. On average, our shallow depth was 0.5m ± 0.16 (mean ± standard deviation), deep depth was 1.8m ± 0.57. At all sites, we tried to pick three specimens of each macrophyte species by hand. If three individuals of a species could not be found at a site after ~5 minutes of searching, we took only the specimens we had been able to find. Samples were placed in separate plastic bags and brought to the GNR Stearn Teaching Laboratory for analysis.

Quantifying G. pisum abundance

In the laboratory, individual macrophytes were analyzed for *G. pisum* abundance by TK, AN, and LRG. Each person analyzed no more than one sample per macrophyte species per site to limit observer differences. To quantify *G. pisum* abundance on macrophytes we scraped *G. pisum* from macrophyte leaves and stems using dissecting probes and tweezers (Fig S1). This technique left the underlying macrophyte intact. For most *E. canadensis* samples, we removed *G. pisum* from the entire macrophyte. For *E. canadensis* with highly abundant *G. pisum*, we subsampled a 20cm portion from the middle of the sample as cleaning the entire macrophyte was not feasible. For *M. spicatum* and *C. demersum*, we scraped *G. pisum* from 20 cm long sections taken from the middle of each macrophyte. After about half our samples were processed, we reduced this length to 15 cm due to field course time constraints. For those interested, we took detailed measures of the distribution of *G. pisum* on our macrophytes beyond sampling *G. pisum* from the middle 15 or 20 cm of macrophyte and provide these results in the supplemental material (Fig S2, Table S1). We then separately weighed the wet mass of *G. pisum* and the cleaned macrophyte section (the whole macrophyte for most *E. canadensis*, 20 or 15cm section for *M. spicatum* and *C. demersum*) to the nearest 0.001 gram. To facilitate comparisons to published results on epiphyte abundance

in other systems, we obtained wetmass:drymass conversion ratios for our macrophyes and *G. pisum*, and these are reported in the supplemental material (Table S2).

Macrophyte surface area to mass ratio

During a preliminary analysis of our data, we realized the macrophyte's surface area would be important to understand the amount of *G. pisum* on each sample. This is because macrophyte surface area may be a better predictor of *G. pisum* than macrophyte mass given *G. pisum* is an epiphyte and grows on the exterior surface of macrophytes. Therefore, in August 2019 we resampled macrophytes from Lac Hertel to obtain surface area:mass ratios that could be used to convert 2018 macrophyte mass to surface area if significant differences in surface area:mass across species were found. We introduce error is by sampling across years, but believe we improve our analysis and prevent having to extrapolate from published data on other watersheds. The only paper we could find that tested macrophyte leaf surface area measures through time did not show large differences (Balci & Kennedy, 2003) although they were not explicitly testing for temporal effects. Unfortunately, we were only able to collect five ~15 cm long samples of *E. canadensis* and *M. spicatum* from a single site ~1.5 meters deep off the Lac Hertel research pier. *C. demersum* was not found at this site at the time. We were unable to sample from the same locations as 2018 because we did not have access to a boat and were only given permission to sample from the research pier.

The number of leaves, length of stem, and wet mass for each sample was then recorded. Five randomly selected leaves and two sections of stem were then placed on a gridded plane and photographed using a Huawei P20 Pro cameraphone mounted 12 inches above the sample (Fig S3). ImageJ (Rueden et al., 2017) was used to measure surface area.

For *E. canadensis*, the area tool in ImageJ was used to calculate the surface area (cm^2) of one side of each leaf, and this was multiplied by two to obtain the leaf's total surface area. Stem diameter was averaged across the two stem samples and multiplied by pi to get circumference,

assuming a circle. The average leaf surface area of the five leaves were then multiplied by the number of leaves in the original sample, and the average stem circumference was multiplied by the length of the stem. The sum of the leaf and stem surface areas was then divided by the wet mass to obtain the surface area:mass ratio.

For *M. spicatum* surface area, five randomly selected leaves and two portions of stem were photographed the same way as for *E. canadensis*. To measure the surface area of each leaf, the length, diameter, and number of leaflets on the leaf's central stem was recorded. Then five leaflets evenly distributed along the leaf were selected, and the length and diameter of these leaflets was recorded. Leaflet area was then calculated as length x middle diameter of each leaflet x pi and was averaged across five leaflets evenly distributed along the length of the leaf. The surface area of each leaf was calculated as the average surface area of the leaflets times the number of leaflets plus the surface area of the central stem (the average of two measurements of the central stem's diameter x the length of the central stem x pi). This total was divided by the sample mass to obtain the surface area:mass ratio.

Analysis

We first tested for differences in the 2019 surface area:mass ratio of *M. spicatum* and *E. canadensis* using student t test to see if we should macrophyte use surface area as a covariate in our model instead of macrophyte mass. We found no significant difference ($p = 0.238$) and therefore used the ratio of *G. pisum* wet mass: macrophyte wet mass (henceforth mass:mass) in our analysis.

To test for differences in mass:mass across macrophyte species, sites, and depth, we ran generalized linear models (GLMs) with a quasi poisson error structure and log link function in R version 3.6.1 (*R: A language and environment for statistical computing*, 2019). We assumed a quasi poisson to improve the distribution of model residuals and account for our mass:mass being 0 bounded. Depth, site, and macrophyte species were all categorical predictors, depth being split

into deep or shallow. We built a full model that included all main effects and interactions, but remove the three way interaction of depth, site, and macrophyte species if it was not significant (from Type III ANOVA on model outputs) and Chi-square comparison of the full and reduced models showed no significant advantage to including the three way interaction. We calculated Type III sum of squares to asses the significance of our model's terms. Both models were also compared to a null hypothesis of mass:mass ~ 1 using a Chi-square test. *Post hoc*, for the best model, we investigated the effects of species, depth, and site using contrasts with Tukey correction with the *emmeans* and *pairs* functions in the R package *emmeans* (Lenth et al., 2019). Contrasts test for significance of a predictor variable or interaction while accounting for the effects of other predictors in the model.

We were unable to find *C. demersum* at any of our shallow sites, so for our analysis we excluded *C. demersum* to prevent unbalanced tests but conducted a separate GLM of mass:mass ~ macrophyte species assuming a quasipoisson distribution and including *C. demersum* from deep sites to help interpret our results. We report these findings at the end of our results.

Results

Surface area:mass

All mean values are reported as mean ± standard deviation. *E. canadensis* had an average surface area:mass ratio of 138.54 ± 22.12 cm^2/g and *M. spicatum*'s average was 122.00 ± 11.50 cm^2/g. Surface area:mass ratios were not significantly different between *E. canadensis* and *M. spicatum* ($p = 0.238$). Also, all five 2019 samples of *M. spicatum* had *G. pisum* present on the macrophyte, while none of the 2019 *E. canadensis* sample did. The average *G. pisum*:macrophyte mass:mass ratio for 2019 *M. spicatum* was 0.13 ± 0.14 g/g.

Mass:mass ratio

The full model with all interaction terms did not have a significant three way interaction ($p = 0.816$) and Chi-square test did not indicate it provided a better fit than the model without the

three way interaction ($p = 0.817$) so the three way interaction term was dropped from subsequent analysis.

We found a significant effect of depth ($p = 0.026$), macrophyte species ($p < 0.001$), and the depth:site interaction ($p < 0.001$) on mass:mass ratio (Table 1). These results are consistent with a strong species effect, a weak depth effect independent of sampling location, and a sampling location effect that is depth dependent.

All post-hoc P values in this paper are reported with Tukey correction already applied. Post-hoc contrasts revealed that *E. canadensis* (0.123 ± 0.214 g g^{-1}, mean ± standard deviation) had a lower mass:mass ratio than *M. spicatum* (1.608 ± 1.237 g g^{-1}), although this was marginally significant ($p = 0.076$). Depth was also not significantly different post-hoc ($p = 0.751$). Sites one, two, three, and five differed in mass:mass ratio across depth, but the direction of change was not consistent. Site one and five had more abundant *G. pisum* at shallow depths, but sites two and three had more abundant *G. pisum* at deep depths.

No deep sites had significantly different mass:mass ratios, but shallow sites two and five were significantly different ($p = 0.023$). We then repeated this depth ~ site contrast adding the species term. This revealed no significant differences across sites for deep *E. canadensis* or *M. spicatum*, no significant differences across sites for shallow *E. canadensis*, but numerous differences across sites for shallow *M. spicatum*. For all differences across sites for shallow *M. spicatum* except one (site two - site 3 which was not a significant difference for either *M. spicatum* or *E. canadensis* ($p = 0.885$ and $p = 0.993$, respectively)), the direction of change for shallow *E. canadensis* was the same for that of shallow *M. spicatum*, suggesting this spatial effect in shallow water may transcend macrophyte species (Table 2).

C. demersum

Deep C. demersum had an average mass:mass ratio of 1.544 ± 0.550 g g^{-1}. The quasipoisson GLM of mas:mass ~ species which included deep *C. demersum* revealed a

significant effect of species on mass:mass ($p < 0.001$). Contrasts revealed both *C. demersum* and *M. spicatum* to have higher mass:mass estimated marginal means than *E. canadensis* ($p < 0.001$) but no significant differences between *C. demersum* and *M. spicatum* ($p = 0.989$).

Discussion

We asked whether the invasion of *M. spicatum* could alter the epiphyte community in Lac Hertel by favoring the growth of Gloeotrichia pisum more than other common native macrophytes. The evidence for facilitation of *G. pisum* by *M. spicatum* is mixed. *M. spicatum* maintains more *G. pisum* than the native *E. canadensis* but similar amounts as the native *C. dermersum*, at least in deep waters. However if *M. spicatum* has been replacing *E. canadensis* as it invades, then *G. pisum* is likely more abundant because of the invasion of *M. spicatum*.

We noticed that macrophytes with abundant *G. pisum* appeared to have less macroinvertebrates such as freshwater amphipods and pond snails. We kept a collection of these invertebrates that we found as we scraped *G. pisum* from our macrophytes: almost all macroinvertebrates came from samples with minimum *G. pisum*, particularly from shallow, *G. pisum* free *E. canadensis*.

In shallow sites, we believe that the growth of *E. canadensis* is a good indicator for the amount of *G. pisum* found on both *E. canadensis* and *M. spicatum*. At shallow site five, which had the highest levels of *G. pisum* on both *E. canadensis* and *M. spicatum*, *E. canadensis* was sparse and obviously less abundant than places like shallow site three, where there were dense *E. canadensis* beds and very little *G. pisum*. The similarity of the spatial pattern of *G. pisum* in shallow *M. spicatum* and *E. canadensis* could be related to the density of *E. canadensis* growth, perhaps dense beds of *E. canadensis* also inhibit *G. pisum* on *M. spicatum*.

It may be that *E. canadensis* can allelopathicly inhibit *G. pisum* growth. Allelopathy is common in aquatic systems (Gross, 2003), and *M. spicatum*, *C. demersum*, and *E. canadensis* have all been shown to have allelopathic affects towards a number of different epiphytes although

226 none have been tested for allelopathy towards *G. pisum* (Gross, 2003; Hilt, 2006). Deepwater *E.*
227 *canadensis* was typically growing below a region on *M. spicatum* and *C. demersum* that we
228 called the "bottom length unaffected" (BLU, Figure S2, Table S1). It may be possible that deep
229 *E. canadensis* was too deep to support *G. pisum*, but that shallow samples suppress *G. pisum*
230 through allelopathic inhibition or some other means. Further testing is needed.
231 Here we have shown that an invasive species, *M. spicatum*, supports higher abundance of
232 *G. pisum* than at least one abundant native macrophyte which *M. spicatum* may be replacing. As
233 *M. spicatum* and other invasive macrophytes continue to become more abundant around the
234 world, this study highlights how the impact of macrophyte invasion may be quantified beyond the
235 replacement of native macrophytes.
236

237 **Acknowledgements**

238 Much thanks goes to Dr. Yoel Stuart, Dr. Urs Kalibitzer, and Dr. Anna Hargreaves who provided
239 feedback that improved the manuscript. Also thanks goes to Dr. Alessandra Giani and Dr. Irene
240 Gregory-Eaves who helped identify *Gloeotrichia pisum*. This work was conducted as part of a
241 field course, and we would like to thank Dr. Anna Hargreaves, Dr. Rowan Barret, Dr. Simon
242 Reader, David Hunt, and the Gault Nature Reserve staff for their contributions to the course.
243

244 **References**

Aiken SG, Newroth PR, Wile I. 1979. The biology of Canadian weeds: 34. *Myriophyllum spicatum* L. *Canadian Journal of Plant Science* 59:201–215. DOI: 10.4141/cjps79-028.

Balci P, Kennedy JH. 2003. Measures of Plant Surface-areas for Eurasian Watermilfoil and Water Stargrass. *J. Aquat. Plant Manage.*:4.

Cattaneo A. 1983. Grazing on epiphytes. *Limnology and Oceanography* 28:124–132. DOI: 10.4319/lo.1983.28.1.0124.

Cattaneo A, Kalff J. 1980. The relative contribution of aquatic macrophytes and their epiphytes to the production of macrophyte beds1. *Limnology and Oceanography* 25:280–289. DOI: 10.4319/lo.1980.25.2.0280.

Eiswerth ME, Donaldson SG, Johnson WS. 2000. Potential Environmental Impacts and Economic Damages of Eurasian Watermilfoil (*Myriophyllum spicatum*) in Western Nevada and Northeastern California [1]. *Weed Technology* 14:511–518. DOI: 10.1614/0890-037X(2000)014[0511:PEIAED]2.0.CO;2.

Gross EM. 2003. Allelopathy of aquatic autotrophs. *Critical reviews in plant sciences* 22:313–339.

Hilt S. 2006. Allelopathic inhibition of epiphytes by submerged macrophytes. *Aquatic Botany* 85:252–256.

Hudon C, Cattaneo A, Gagnon P. 2009. Epiphytic cyanobacterium Gloeotrichia pisum as an indicator of nitrogen depletion. *Aquatic Microbial Ecology* 57:191–202. DOI: 10.3354/ame01344.

Kovach T, Velghe K, Lechowicz M. 2008. Aquatic macrophytes of Lac Hertel.

Lenth R, Singmann H, Love J, Buerkner P, Herve M. 2019. *Package 'emmeans.'*

Pelton DK, Levine SN, Braner M. 1998. Measurements of phosphorus uptake by macrophytes and epiphytes from the LaPlatte River (VT) using 32P in stream microcosms. *Freshwater Biology* 39:285–299. DOI: 10.1046/j.1365-2427.1998.00281.x.

R: A language and environment for statistical computing. 2019. Vienna, Austria: R Foundation for Statistical Computing.

Rooney N, Kalff J. 2003. Interactions among epilimnetic phosphorus, phytoplankton biomass and bacterioplankton metabolism in lakes of varying submerged macrophyte cover. *Hydrobiologia* 501:75–81.

Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW. 2017. ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics* 18:529. DOI: 10.1186/s12859-017-1934-z.

Figure 1

Sampling locations in Lac Hertel

Map data © 2020 Google.

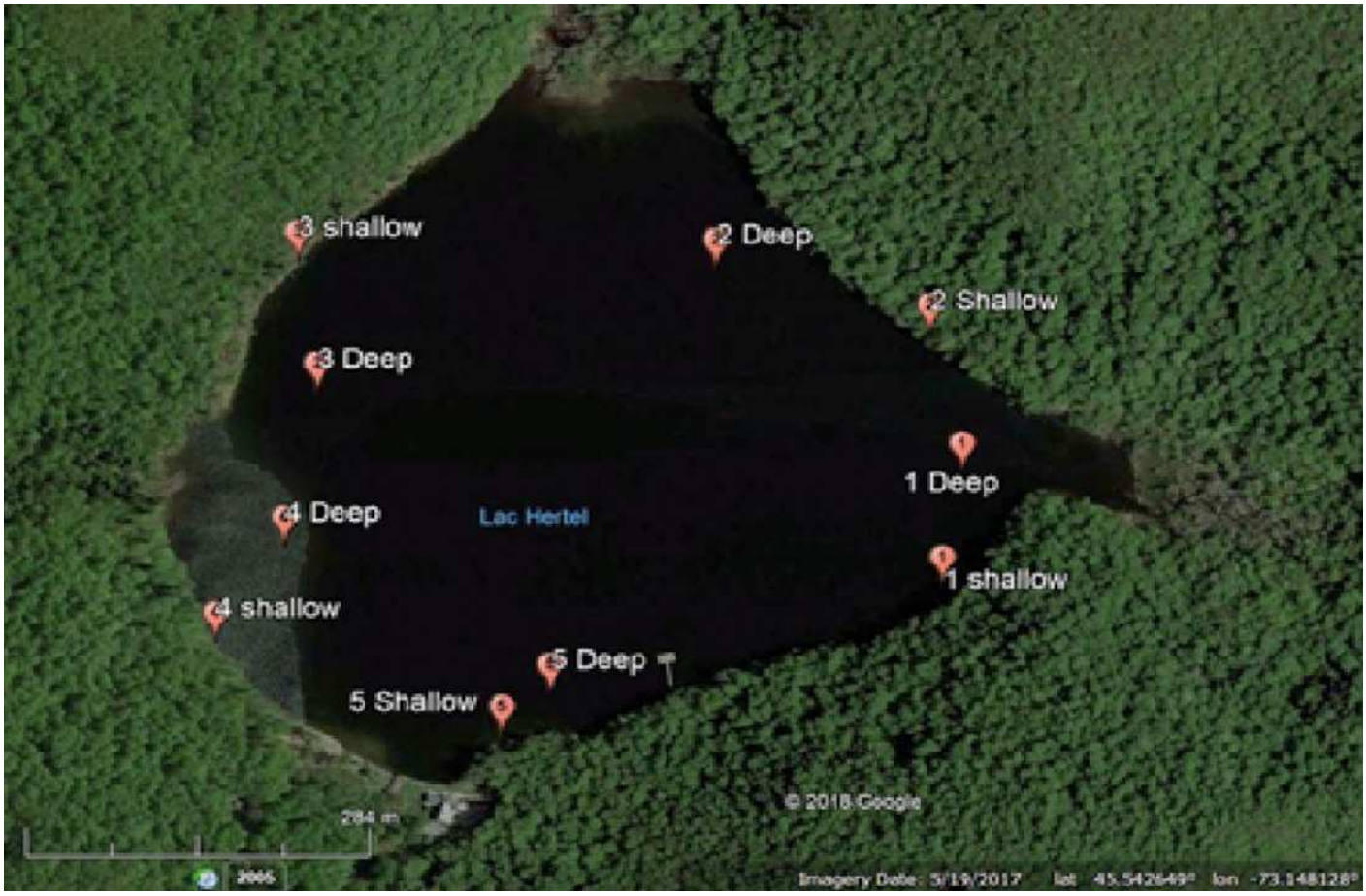

Table 1(on next page)

Model summary statistics for our final model.

A semi colon indicates the interaction between terms. Sum Sq: sum of squares, DF: degrees of freedom, LR Chisq: Chisquare likelihood ratio, F: F statistic, P: p value

Table 1. Model summary statistics for our final model. A semi colon indicates the interaction between terms. Sum Sq: sum of squares, DF: degrees of freedom, LR Chisq: Chisquare likelihood ratio, F: F statistic, P: p value

Term	Sum Sq	DF	LR Chisq	F	P
depth	1.774	1	5.167	5.167	0.028
species	7.893	1	22.989	22.990	<0.001
site	1.356	4	3.949	0.987	0.425
depth:species	1.009	1	2.940	2.940	0.094
site:species	1.003	4	2.921	0.730	0.577
depth:site	14.613	4	42.562	10.641	<0.001
residuals	14.076	41			

Table 2(on next page)

Contrasts for shallow and deep sites split by species.

Estimates of difference in mass:mass are given on the log transformed scale. SE: standard error, P: p value with Tukey correction.

Table 2. Contrasts for shallow and deep sites split by species. Estimates of difference in mass:mass are given on the log transformed scale. SE: standard error, P: p value with Tukey correction.

Deep						
	M. spicatum			*E. canadensis*		
contrast	estimate	SE	P	estimate	SE	P
five - four	-0.053	0.356	.999	0.475	1.025	0.990
five - one	0.507	0.417	0.743	1.137	1.054	0.818
five - three	-0.554	0.322	0.421	3.774	9.617	0.995
five - two	-0.312	0.336	0.887	-1.086	1.109	0.865
four - one	0.560	0.414	0.658	0.661	1.253	0.985
four - three	-0.501	0.317	0.511	3.298	9.638	0.997
four - two	-0.258	0.332	0.937	-1.561	1.251	0.723
one - three	-1.061	0.384	0.046	2.637	9.645	0.999
one - two	-0.818	0.397	0.237	-2.222	1.353	0.470
three - two	0.243	0.294	0.923	-4.859	9.634	0.987
Shallow						
contrast	estimate	SE	P	estimate	SE	P
five - four	0.684	0.356	0.306	1.212	0.948	0.705
five - one	0.404	0.327	0.732	1.033	0.941	0.808
five - three	2.080	0.624	0.008	6.408	9.610	0.964
five - two	3.060	0.892	0.006	2.286	1.188	0.305
four - one	-0.280	0.386	0.951	-0.179	1.160	0.999
four - three	1.397	0.657	0.209	5.196	9.631	0.983
four - two	2.376	0.915	0.071	1.073	1.330	0.929
one - three	1.677	0.642	0.068	5.375	9.634	0.981
one - two	2.656	0.907	0.028	1.252	1.374	0.893
three - two	0.979	1.053	0.885	-4.123	9.647	0.993