Relationship between the invasive Eurasian milfoil (*Myriophyllum spicatum* L.) and macrophyte diversity across spatial scales

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Abstract

The effect of the invasive macrophyte *Myriophyllum spicatum* L. on native macrophyte diversity was studied across 21 lakes at small (1 quadrat) and large (18 quadrats) scales and in mesocosms. No relationship was observed between *M. spicatum* and native richness at either spatial scale, or native evenness at small scales; however, at large scales native evenness was negatively correlated with the cover of *M. spicatum* in lakes. This suggests that *M. spicatum* can grow in lakes with other dominant species, contrary to predictions that invasive species will outcompete other dominant species. While macrophyte communities were mainly distinguished by environmental and spatial variables, *M. spicatum* was a significant predictor of the remaining variation in community composition. Within lakes *M. spicatum* occurred in deep water apart from other species; however, competition studies in mesocosms did not find sufficient evidence that this isolation was due to superior competitiveness.

Résumé

L’effet de l’espèce envahissante *Myriophyllum spicatum* L. sur les macrophytes indigènes a été étudié à deux échelles spatiales dans 21 lacs, soit à l’échelle réduite du quadrat et à plus grande échelle (18 quadrats), de même que sous conditions expérimentales en serre. Aucune corrélation n’a été observée entre *M. spicatum* et la richesse spécifique des macrophytes indigènes aux deux échelles spatiales considérées, ainsi qu’entre l’abondance de *M. spicatum* et l'abondance relative des espèces indigènes lorsqu’étudiées à échelle réduite. Par contre, un effet négatif significatif a été décelé entre *M. spicatum* et l'abondance relative des macrophytes indigènes à grande échelle. Ces résultats suggèrent que *M. spicatum* peut croître dans les lacs en présence d’autres espèces compétitives, contrairement aux prédictions que les espèces envahissantes vont éliminer les espèces compétitives. De plus, une relation réelle existe entre *M. spicatum* et la composition des communautés végétales, mais elle a été constatée seulement après que les effets environnementaux et spatiaux furent considérés. Contrairement aux autres espèces inventoriées, *M. spicatum* existe principalement dans les portions profondes des lacs; cependant, les études de compétition en serres n’ont pas conclu que *M. spicatum* prolifère en zone plus profonde à cause de l’exclusion d’autres macrophytes.

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Chapter 1. General introduction

Freshwater biodiversity

Freshwater ecosystems have high conservation value due to the large number of plant and animal species that they support (Dudgeon et al., 2005). However, several threats exist to freshwater biodiversity, including overexploitation of resources, pollution, altered flow, degraded habitat and the introduction of invasive species (Dudgeon et al., 2005). One group of species that inhabit freshwater systems are macrophytes, plants that carry out their entire lifecycle within standing water (Riemer, 1984).

Macrophytes are important in freshwater ecosystems as habitat for epiphytes, macroinvertebrates and fish (Kelly & Hawes, 2005; Schultz & Dibble, 2011) due to the diverse environment they provide across size scales, from fine branching on individual plants to large beds of macrophyte communities (Chambers et al., 2007). In fact, the majority of freshwater aquatic life lives in and around macrophyte habitat (Chambers et al., 2007).

The structural characteristics of macrophytes also contribute to regulating water flow, temperature and light, reducing sediment resuspension and removing nutrients from the water column (Chambers et al., 2007; Strayer, 2010). Lastly, macrophytes influence water chemistry by producing allelopathic chemicals, increasing dissolved oxygen and pH through photosynthesis and reducing dissolved oxygen through decomposition (Chambers et al., 2007; Strayer, 2010; Schultz & Dibble, 2011).

Lake characteristics in turn influence macrophyte growth through available light, wave disturbance, shoreline slope, sediment type and trophic status. Macrophytes can tolerate a range of nutrient conditions but are limited by their preference for alkalinity and pH, with different groups of species in: softwater (pH < 7.5), hardwater (pH > 7), brownwater (pH < 5.5 and zero alkalinity), and saline water (high pH & alkalinity) (Lacoul & Freedman, 2006). Connectivity is also an important predictor of species composition, but only for species with poor dispersal traits as lakes in a watershed are expected to contain similar long distance dispersers, with poor dispersers present in a few adjacent lakes (Lacoul & Freedman, 2006).

Invasive macrophytes

A growing proportion of the species richness and biomass in fresh water systems of North America and Europe consists of exotic species (Strayer, 2010), some of which become so widespread and abundant that they are also considered invasive (Colautti & MacIsaac, 2004). Exotic plants entered the Great Lakes through a combination of deliberate introductions, attachment to boats and escape from aquaria and gardens (Mills et al., 1993). Their potential for abundant growth and tolerance of eutrophic conditions has led to more dense habitat and increased water clarity (by slowing water and competing with phytoplankton) (Strayer, 2010; Schultz & Dibble, 2011). Currently in Ontario eight invasive floating and submerged aquatic plants are present and six others are considered as potential invaders (Lui et al., 2010). Eurasian watermilfoil (*Myriophyllum spicatum* L.) is among the most invasive and is now widespread throughout Ontario.

M. spicatum is native to Eurasia and Greenland. This species was first introduced to North America in the Chesapeake Bay area in the late 19th century, possibly through shipping ballast, and was first observed in Canada in 1961 in Rondeau Provincial Park, Lake Erie (Aiken et al., 1979). *M. spicatum* has spread throughout the Great Lakes and St. Lawrence River, parts of British Columbia and much of the United States (Lui et al., 2010);

however, it has not yet established in all suitable habitat in North America (Strayer, 2010). It grows best in eutrophic lakes with salinity up to 10 ppt in both acidic (pH 5.4) and alkaline (pH 9-10) water. The plant colonizes using vegetative fragments, establishing first in water depths of 2-3 m and spreading to depths of 0.5 – 3.5 m, up to 5 m. It grows up to 7 m long, producing a large biomass that shades out other plants, displacing them within 2-3 years of establishment (Aiken et al., 1979). *M. spicatum* is also allelopathic, with negative effects on fish larvae and epiphytes (Schultz & Dibble, 2011).

Thesis rationale and objectives

Species richness

Native-invasive relationships across scales

A growing body of literature suggests that the relationship between invasive species and native biodiversity is scale dependent. In a meta-analysis of invasive terrestrial plants and native plant richness, the effect of invasive plants on native richness was found to decrease with increasing spatial scale (Powell et al., 2011). Similarly, a variety of terrestrial plant communities have shown native-exotic richness relationships that change from negative to positive with increasing spatial scale (Fridley et al., 2007). More generally, in a review of ecology studies that considered spatial scale 90% of studies found different results at different spatial scales (Sandel & Smith, 2009).

At small scales the native-invasive species relationship is expected to be negative due to competition for space as habitat conditions are uniform and unlikely to support many different species. Negative relationships may occur when: native species occupy all niches (competitive exclusion), high native richness increases the likelihood of a native species occurring that resists invasion (sampling effects), or plants do not compete but available space is a limiting factor (neutral species assembly). On the other hand, a positive relationship may occur if native species facilitate invasive species growth, especially in stressful environments (Fridley et al., 2007).

At large scales a greater number of species are expected to be present (Rørslett, 1991; Dodson et al., 2000) and the native-invasive species relationship should be positive as habitat is variable enough that no species occupies the entire area and individuals cannot interact with all their neighbors (Fridley et al., 2007). Positive relationships may occur when: habitat heterogeneity selects for different species in different habitats, dispersal and/or disturbance reintroduces species to an area, and native and invasive species are suited for similar habitat conditions (termed 'biotic acceptance') (Fridley et al., 2007). Even if native species are outcompeted, they will likely find refuge habitat to persist in, preventing a negative relationship from being observed (Powell et al., 2011).

Macrophyte richness at fine spatial scales

Macrophyte studies at small scales (individual quadrats or point samples) have usually observed negative relationships between individual invasive macrophytes and native richness (Table 1-1). Studies comparing richness between plots dominated by an invasive and plots lacking the invasive have observed lower richness with *Cabomba caroliniana* A. Gray (submerged) (Lyon & Eastman, 2006), *Elodea canadensis* Michx. (submerged, invasive in New Zealand), *Egeria densa* Planch. (submerged)*, Hydrilla verticillata* (L.f.) Royle (submerged) (de Winton & Clayton, 1996), *M. spicatum* (submerged) (Boylen et al., 1999; Kovalenko et al., 2010), *Hydrocotyle ranunculoides* L.f. (floating, invasive in Europe)*, Ludwigia grandiflora* Michx. (floating)*, Myriophyllum aquaticum* (Vell.) Verdc.

(submerged / floating) (Stiers et al., 2011) and *Impatiens glandulifera* Royle (herbaceous) (Hulme & Bremner, 2005). When richness has been compared across a gradient of invasive abundance, lower richness has been observed in plots with greater abundance of *E. canadensis* (Mjelde et al., 2012), *Urochloa subquadripara* (Trin.) R.D. Webster (emergent graminoid) (Michelan et al., 2010) and *M. spicatum* (Olson et al., 2012), although no relationship was observed between richness and cover of *Alternanthera philoxeroides* (Mart.) Griseb (emergent herbaceous) (Bassett et al., 2012) (Table 1-1).

Macrophyte richness at broad spatial scales

At larger scales (100 m transects or combined samples across a waterbody) differing results have been observed between native richness and individual invasive macrophytes. The presence of *H. verticillata* within 100 m transects was associated with lower richness (Thomaz et al., 2009), richness was negatively correlated with abundance of *Phalaris arundinacea* L. (emergent graminoid) (Houlahan & Findlay, 2004), cumulative richness curves for quadrats invaded by *U. subquadripara* (Michelan et al., 2010) and *I. glandulifera* (Hulme & Bremner, 2005) had lower richness than curves for quadrats not invaded by the invasive species, and lakes containing *Elodea* spp. (*E. canadensis* and *Elodea nuttallii* (Planch.) H. St. John not differentiated) had lower richness than lakes without *Elodea* spp. (O'Hare et al., 2012). On the other hand, no correlations have been observed between richness and the invasion of *E. canadensis* over time in a lake (Mjelde et al., 2012), the frequency of occurrence of *M. spicatum*, *Najas minor* All. (submerged) and *Potamogeton crispus* L. (submerged) in coastal wetlands (Trebitz & Taylor, 2007) the biomass of *M. spicatum* and *H. verticillata* in river segments (Rybicki & Landwehr, 2007) or the abundance of *Lythrum salicaria* L. (herbaceous), *Hydrocharus morsus-ranae* L. (floating) or

Rhamnus frangula L. (shrub) in wetlands (Houlahan & Findlay, 2004). Lastly the presence of *U. subquadripara* within 100 m transects was associated with higher richness (Thomaz et al., 2009) (Table 1-1).

Studies of native species richness and the richness of all exotic species have also found a trend of negative to neutral relationships at small scales, and neutral to positive relationships at larger scales. A negative relationship was observed between native and exotic macrophyte richness at small scales in a river delta (Santos et al., 2010) and wetlands (Chen et al., 2010). No relationship between native and exotic richness has been observed from quadrats to counties in lakes (Capers et al., 2007) and streams (Quinn et al., 2011). Lastly, a positive correlation between native and exotic richness has been observed at the wetland scale (Houlahan & Findlay, 2004; Chen et al., 2010) (Table 1-1).

Species composition

Macrophyte community composition at fine spatial scales

In contrast to species richness, some macrophyte studies suggest that invasive species are associated with changes in community composition at both small and large scales, while other studies find no relationship. At small scales, the co-occurrence of individual native species with *U. subquadripara* was always less than expected by chance (Thomaz & Michelan, 2011) and particular species have been associated with *M. spicatum* presence (Olson et al., 2012). The species composition of quadrats invaded by *U. subquadripara* was different from those not invaded based on DCA scores (Michelan et al., 2010) and CCA scores for native and exotic submerged macrophytes (grouped together, not individual species) were also arranged on different sides of an ordination plot (Quinn et al., 2011). Species occupying the same depth as *E. canadensis* have moved into deeper water or become locally extinct (Mjelde et al., 2012) and analysis of similarity (ANOSIM) found different species compositions in non-invaded quadrats than in those containing *A. philoxeroides* (Bassett et al., 2012), *H. ranunculoides* and *L. grandiflora*, but not *M. aquaticum* (Stiers et al., 2011). However, no association was found between *C. caroliniana* and individual species when comparing their abundance distributions in non-metric multidimensional scaling (NMS) (Lyon & Eastman, 2006) (Table 1-2).

Macrophyte community composition at broad spatial scales

At larger scales the presence of *Elodea* spp. (O'Hare et al., 2012) and *M. spicatum* (Mikulyuk et al., 2011) have been significant predictors of native community composition. This relationship appears to be due to positive associations, not negative associations, as studies that considered pairwise species interactions found 16/66 species to be positively associated with *M. spicatum* presence, and 1/66 species negatively associated (Nichols & Buchan, 1997), while 22/41 species were positively associated with *U. subquadripara* presence, and 3/41 were negatively associated (Thomaz & Michelan, 2011). On the other hand, exotic wetland species display a more random distribution, as the inclusion of exotic species in community comparisons increased the similarity between wetlands by 10-17% (Chen et al., 2010) (Table 1-2).

Species evenness

Species diversity in communities is a reflection of both the number of species present (species richness) and the relative abundance of species (evenness) (Washington, 1984; Smith & Wilson, 1996). Evenness can be computed as the ratio of the effective number of species (given their abundance) to the total number of species present (Tuomisto, 2012). Unlike richness, species evenness is not always expected to increase asymptotically with spatial scale (Wilson et al., 1999). Sites that have local dominant species may have greater evenness at larger scales due to habitat heterogeneity or randomness in species composition (Hillebrand et al., 2008). On the other hand, anthropogenic factors that homogenize communities (such as high nutrient additions) could lead to dominance of a few plants at large spatial scales and lower evenness (Hillebrand et al., 2008).

Declines in evenness are important because they change the relative importance of interspecific and intraspecific competition among plants and may change the relative abundance of plant functional traits in a community (Hillebrand et al., 2008). For instance, a meta-analysis of forest productivity found that evenness explained almost three times more variation in productivity than species richness (Zhang et al., 2012). While predatory invasive species are expected to reduce species richness (Fridley et al., 2007), competitive invasive plants will more likely change species evenness, at least at the outset of invasion (Valéry et al., 2009). Invasive species are expected to increase native evenness in one of two ways, either by reducing the abundance of common species, or by driving rare species locally extinct (Powell et al., 2011).

Despite the importance of evenness, it has been reported less than other measures of diversity in ecology studies. In a review of freshwater biodiversity studies between 2000 and 2010, diversity metrics (such as evenness) were considered in 13% of studies, while richness and community composition were considered in 36% and 31% of studies, respectively (Stendera et al., 2012). With respect to macrophytes, the relationship between native species evenness and invasive species has been reported twice at small scales. Lower evenness

(Shannon equitability) was observed in plots containing *C. caroliniana* compared to non-invaded plots (although invasive biomass was included in the calculation) (Hogsden et al., 2007), while higher evenness (Simpson's evenness) was observed in plots containing *I. glandulifera* compared to non-invaded plots (excluding invasive biomass in the calculation) (Hulme & Bremner, 2005). At small scales *M. spicatum* is often the dominant species (Bosch et al., 2009; Ginn, 2011) and may be influencing native evenness. Although not studied directly, it appears that at larger scales evenness is not related to invasive species, as no change in habitat complexity (based on plant fractal dimensions) was observed after *M. spicatum* removal (Kovalenko et al., 2010) and native species appear equally likely to be dominant as exotic species in lakes (Capers et al., 2009) and wetlands (Houlahan & Findlay, 2004) (Table 1-3). Evenness at larger scales appears related to nutrient conditions, as both native and exotic macrophytes were more likely to be dominant in shallow, high nutrient lakes (Capers et al., 2009) and a decline in evenness with increasing nutrients was observed at large spatial scales (2,400 m^2) in Danish coastal macroalgal communities (Middelboe & Sand-Jensen, 2004).

Thesis objectives

It appears that native macrophyte richness at small scales is negatively correlated to both the abundance of individual invasive macrophyte species and the number of exotic species, but has either no relationship or is positively associated with invasive species / exotic richness across an entire waterbody. While native richness at the waterbody scale may not be related to invasive abundance, there may still be an effect of invasive species on native species community composition or evenness. Studies of community composition are beginning to enter the literature (ten of the twelve above were published since 2010) and show various results at both small and large scales. Even fewer studies have investigated invasive effects on macrophyte evenness.

This thesis investigated the relationship between native submerged and floating macrophyte communities and the invasive aquatic *M. spicatum* at small (1 m^2 quadrat) and large (18 1 m^2 quadrats) scales in lakes of the Rideau Valley Watershed. Abundant populations of *Myriophyllum* sp. have been reported within the Rideau River as early as 1968 (Department of Energy and Resources Management, 1968). Two other biological gradients in lakes were also of interest. First, the presence of invasive zebra mussels (*Dreissena polymorpha* Pallas) was included as they are currently spreading through lakes of the Rideau Valley Watershed (Sarah MacLeod, Rideau Valley Conservation Authority *pers. comm.*) and act as ecosystem engineers to improve macrophyte habitat (Zhu et al., 2006). Second, the cover of charophytes (macroscopic filamentous algae) was included as charophyte meadows have been found to be negatively associated with the extent of invasive macrophyte colonization in oligotrophic New Zealand lakes (de Winton et al., 2012).

Chapter two considered macrophyte communities in terms of species richness and species evenness at small and large scales in lakes and chapter three considered macrophyte communities in terms of community composition at small and large scales in lakes and competition in greenhouse mesocosms. It was hypothesized that competition from *M. spicatum* would reduce native richness, increase native evenness and select for different species communities, with a smaller effect size at large scales due to a decreasing effect of competition. Zebra mussels were hypothesized to increase richness and evenness and shift community composition to macrophytes intolerant of turbid water through improvements in water clarity. Lakes with large charophyte meadows were hypothesized to experience less colonization by *M. spicatum* and support different plant communities than lakes with fewer charophytes. Competition by charophytes (which form dense beds) was expected to reduce species richness and increase evenness.

Table 1-1. Summary of invasive vs. native macrophyte richness studies reviewed in this paper arranged from small to large scale. Abbreviations for waterbody include: D = delta, L = lake, R = riparian edge, S = stream/river, W = wetland. Abbreviations for growth form include: A = all, E = emergent, F = floating, S = submerged. Symbols in effect include: (n/a) = no effect, (-) = negative effect, (+) = positive effect, (-/+) = negative or positive effect.

Author	Location	Waterbody	Area Sampled	Species	Growth Form	Effect	Comments
Lyon and Eastman (2006)	MA, USA	L	Rake sampling point	*Cabomba caroliniana*	S	(-)	Lower richness in invaded quadrats
Santos et al.(2011)	CA, USA	D	Rake sampling point	*Egeria densa*	S	(-)	Negative relationship between native and non-native species richness
Boylen et al. (1999)	NY, USA	L	0.25 m^2 quadrat	*Myriophyllum spicatum*	S	(-)	Decline in mean native richness with colonization of *M. spicatum*
Mjelde et al. (2012)	Norway	L	0.25 m^2 quadrat	*Elodea canadensis*	S	(-)	Mean quadrat richness declined where *E. canadensis* had max abundance (2-4 m) and increased at other depths
Chen et al.(2010)	IL, USA	W	0.25 m^2 quadrat	Exotic species	E	(-)	Negative native-exotic richness relationship
Michelan et al. (2010)	Brazil	L	1 m^2 quadrat	*Urochloa subquadripara*	E	(-)	Negative relationship with native richness and invasive biomass
Hulme and Bremner (2006)	England	R	1 m^2 quadrat	*Impatiens glandulifera*	E	(-)	Lower richness in plots containing *I. glandulifera* than in removal plots

Table 1-1. (Continued)

Author	Location	Waterbody	Area Sampled	Species	Growth Form	Effect	Comments
deWinton and Clayton (1996)	New Zealand	L	4 sediment cores in 2 m^2 quadrat	*Elodea canadensis* *Egeria densa* *Hydrilla verticillata*	S	(-)	Seed bank analysis found lower richness in cores of invaded quadrats
Chen et al.(2010)	IL, USA	W	4 x 0.25 m^2 quadrats	Exotic species	E	(+)	Positive native-exotic richness relationship
Capers et al. (2007)	CT, USA	L	2 m diameter sampling point	Invasive species	S	(n/a) (-/+)	No correlation between native and invasive richness, but some individual lakes had negative correlations (16/60 lakes) or positive correlations (7/60 lakes)
Stiers et al. (2011)	Belgium	L	4 m^2 quadrat	*Hydrocotyle ranunculoides* *Ludwigia grandiflora* *Myriophyllum aquaticum*	F E S	(-)	Lower richness in invaded quadrats
Quinn et al. (2011)	Australia	S	0.25 m wide transect across stream	Exotic species	A	(n/a)	No correlation between native and exotic richness
Kovalenko et al. (2010)	MN, USA	L	5 x 1m lines	*Myriophyllum spicatum*	S	(-)	Lakes treated with herbicide. Native richness increased in a lake where *M. spicatum* never re-established, but richness did not increase in a lake where it did re-establish
O'Hare et al. (2012)	Scotland	L	20 rake sampling points	*Elodea canadensis* *E. nuttallii*	S	(-)	Lower richness in lakes with *Elodea* spp.
Chen et al.(2010)	IL, USA	W	20 x 0.25 m^2 quadrats	Exotic species	E	(+)	Positive native-exotic richness relationship

Table 1-1. (Continued)

Author	Location	Waterbody	Area Sampled	Species	Growth Form	Effect	Comments
Olson et al. (2012)	WI, USA	L	9 m^2 visual observation, 3 m long rake pull	*Myriophyllum spicatum*	S	(-/+)	*M. spicatum* presence positively associated with species richness (but *M. spicatum* dominance positively associated with wave action and native richness negatively associated with wave action)
Michelan et al. (2010)	Brazil	L	7-15 x 1 m^2 quadrats	*Urochloa subquadripara*	E	(-)	Lower richness for cumulative richness curves of invaded quadrats
Hulme and Bremner (2006)	England	R	12 x 1 m^2 quadrats	*Impatiens glandulifera*	E	(-)	Lower richness for cumulative richness curves of invaded quadrats
Bassett et al. (2012)	New Zealand	W	25 m^2 quadrat	*Alternanthera philoxeroides*	E	(n/a)	No relationship between native richness and invasive cover
Quinn et al. (2011)	Australia	S	3 x 0.25 m wide transects	Exotic species	A	(n/a)	No correlation between native and exotic richness
Boylen et al. (1999)	NY, USA	L	36 m^2 quadrat	*Myriophyllum spicatum*	S	(-)	Decline in native species with *M. spicatum* colonization
Trebitz and Taylor (2007)	Great Lakes, USA	W	Entire littoral zone along 100 m of shoreline	*Myriophyllum spicatum* *Najas minor* *Potamogeton crispus*	S	(+)	Presence (and dominance for *M. spicatum*, other species never dominant) of invasive submerged plants associated with higher native richness

Table 1-1. (Continued)

Author	Location	Waterbody	Area Sampled	Species	Growth Form	Effect	Comments
Mjelde et al. (2012)	Norway	L	623-789 x 0.25 m^2 quadrats (156-197 m^2)	*Elodea canadensis*	S	(n/a)	No significant decline in whole lake richness after colonization by *E. canadensis*
Thomaz et al. (2009)	Brazil	L	Transect 80-100 m x 10 m	*Urochloa subquadripara* *Hydrilla verticillata*	E S	(+) (-)	*U. subquadripara* associated with higher richness, *H. verticillata* associated with lower richness
Trebitz and Taylor (2007)	Great Lakes, USA	W	5-7 x entire littoral zone along 100 m of shoreline	*Myriophyllum spicatum* *Najas minor* *Potamogeton crispus*	S	(n/a)	No correlation between native richness and invasive frequency of occurrence
Houlahan and Findlay (2004)	ON, Canada	W	Mean wetland area = 66.7 ha	Exotic species *Phalaris arundinaceae* *Lythrum salicaria* *Hydrocharus morsus-ranae* *Rhamnus frangula*	E E E F E	(+) (-) (n/a)	Positive relationship between native and exotic richness. Negative relationship between *P. arundinaceae* and richness. No relationship between *L. salicaria*, *H. morsus-ranae* or *R. frangula* abundance and native richness
Rybicki and Landwehr (2007)	MD/VA, USA	S	0.3 - 14.5 km^2 surveys (mean = 6.5 km^2)	*Myriophyllum spicatum* *Hydrilla verticillata*	S	(n/a)	Richness not significantly correlated to biomass of either *M. spicatum* or *H. verticillata*
Quinn et al. (2011)	Australia	S	6-9 sites containing 3 transects 0.25 m wide	Exotic species	A	(n/a)	No correlation between native and exotic richness

Table 1-1. (Continued)

Author	Location	Waterbody	Area Sampled	Species	Growth Form	Effect	Comments
Capers et al. (2007)	CT, USA	L	1 - 8 transects of 10 x 2 m diameter samples (31-251 m^2), plus visual and rake surveys	Invasive species	S	(n/a)	No correlation between native and invasive richness
Capers et al. (2007)	CT, USA	L	Counties containing 4 - 22 lakes	Invasive species	S	(n/a)	No correlation between native and invasive richness

Table 1-2. Summary of invasive vs. native macrophyte community composition studies reviewed in this paper arranged from small to large scale. Abbreviations for waterbody include: D = delta, L = lake, R = riparian edge, S = stream/river, W = wetland. Abbreviations for growth form include: A = all, E = emergent, F = floating, S = submerged. Symbols in effect include: (n/a) = no effect, (-) = negative effect, (+) = positive effect, (-/+) = negative or positive effect.

Author	Location	Waterbody	Area Sampled	Species	Growth Form	Effect	Comments
Lyon and Eastman (2006)	MA, USA	L	Rake sampling point	*Cabomba caroliniana*	S	(n/a)	No association between *C. caroliniana* and individual species
Mjelde et al. (2012)	Norway	L	0.25 m^2 quadrat	*Elodea canadensis*	S	(-)	Species that occupied the same depths as *E. canadensis* moved into deeper water or were not observed
Thomaz and Michelan (2011)	Brazil	L	1 m^2 quadrat	*Urochloa subquadripara*	E	(-)	Co-occurrence of individual species with invasive always lower than expected by chance
Michelan et al. (2010)	Brazil	L	1 m^2 quadrat	*Urochloa subquadripara*	E	(-/+)	Different DCA scores for invaded and native sites
Stiers et al. (2011)	Belgium	L	4 m^2 quadrat	*Hydrocotyle ranunculoides* *Ludwigia grandiflora* *Myriophyllum aquaticum*	F E S	(-/+) (-/+) (n/a)	Native species composition didn't differ between invaded/uninvaded plots of *M. aquaticum*, but did for *H. ranunculoides* and *L. grandiflora* using ANOSIM
O'Hare et al. (2012)	Scotland	L	20 rake sampling points	*Elodea canadensis* *E. nuttallii*	S	(-/+)	Presence of *Elodea* spp. significant predictor of submerged macrophyte community in CCA

Table 1-2. (Continued)

Author	Location	Waterbody	Area Sampled	Species	Growth Form	Effect	Comments
Chen et al.(2010)	IL, USA	W	20 x 0.25 m^2 quadrats	Exotic species	E	(n/a)	Exotic species increased similarity of wetland species by 10-17%
Quinn et al. (2011)	Australia	S	0.25 m wide transect across stream	Exotic species	A	(-/+)	Exotic and native submerged species groups on opposite ends of CCA ordination
Olson et al. (2012)	WI, USA	L	9 m^2 visual observation, 3 m long rake pull	*Myriophyllum spicatum*	S	(-/+)	*M. spicatum* presence associated with presence of some species and absence of others
Bassett et al. (2012)	New Zealand	W	25 m^2 quadrat	*Alternanthera philoxeroides*	E	(-/+)	Quadrats without invasive had different species composition from quadrats with invasive using ANOSIM
Mikulyuk et al. (2011)	WI, USA	L	44-3098 x 0.4 m^2 rake sampling points (mean = 122 m^2)	*Myriophyllum spicatum*	S	(-/+)	Presence of *M. spicatum* significant predictor of macrophyte community in RDA
Thomaz and Michelan (2011)	Brazil	L	80-100 m x 10 m transect	*Urochloa subquadripara*	E	(+)	Co-occurrence of species with invasive almost always higher than or same as expected by chance
Nichols and Buchan (1997)	WI, USA	L	Species lists from lake records	*Myriophyllum spicatum*	S	(+)	*M. spicatum* had high association with sixteen species

Table 1-3. Summary of invasive vs. native macrophyte evenness studies reviewed in this paper arranged from small to large scale. Abbreviations for waterbody include: D = delta, L = lake, R = riparian edge, S = stream/river, W = wetland. Abbreviations for growth form include: A = all, E = emergent, F = floating, S = submerged. Symbols in effect include: (n/a) = no effect, (-) = negative effect, (+) = positive effect, (-/+) = negative or positive effect.

Author	Location	Waterbody	Area Sampled	Species	Growth Form	Effect	Comments
Bosch et al. (2009)	NY, USA	L	0.25 m^2 quadrat	*Myriophyllum spicatum*	S	(-)	*M. spicatum* dominant at 1.5-3.5m depths
Ginn (2011)	ON, Canada	L	Rake or ponar sampling point	*Myriophyllum spicatum*	S	(-)	*M. spicatum* displaced *Chara* spp. at depths below 3.5 m
Hogsden et al. (2007)	ON, Canada	L	1 m^2 quadrat	*Cabomba caroliniana*	S	(-)	Lower Shannon equitability in *C. caroliniana* sites (including *C. caroliniana*)
Hulme and Bremner (2006)	England	R	1 m^2 quadrat	*Impatiens glandulifera*	E	(+)	Higher Simpson evenness in *I. glandulifera* sites (not including *I. glandulifera*)
Kovalenko et al. (2010)	MN, USA	L	5 x 1 m lines	*Myriophyllum spicatum*	S	(n/a)	No change in habitat complexity with loss of *M. spicatum*
Houlahan and Findlay (2004)	ON, Canada	W	Visual survey (mean area = 66.7 ha)	Exotic species	E	(n/a)	Proportion of dominant native and exotic species was not different
Capers et al. (2009)	CT, USA	L	1 - 8 transects of 10 x 2 m diameter samples (plus visual and rake surveys)	*Myriophyllum spicatum* Seven other invasive species	S	(n/a)	No significant difference between probability of a native vs. invasive becoming dominant

Chapter 2. Richness and evenness in macrophyte communities: effects of *Myriophyllum spicatum* L., *Dreissena polymorpha* Pallas and charophytes across spatial scales

Abstract

Aim To study the relationship between native macrophyte diversity (richness and evenness) and the invasive macrophyte *Myriophyllum spicatum* L. in lakes at small (1 quadrat) and large (18 quadrats) scales. It was hypothesized that competition by *M. spicatum* would extirpate rare native species from lakes and reduce the abundance of dominant native species, lowering native richness and increasing native evenness.

Location Lakes of southeastern Ontario, Canada.

Methods Submerged and floating macrophyte communities were surveyed in 1 m^2 quadrats across 21 lakes. The relationship between native richness or Simpson's evenness and total cover of *M. spicatum* was studied across lake communities (large scale) and for quadrats within individual lakes (small scale) using linear models. The effect of zebra mussels (*Dreissena polymorpha* Pallas) and charophytes (two taxa with uneven distributions across the watershed) and habitat gradients were also included in the models.

Result Native richness was negatively related to charophyte cover at small scales and positively related to zebra mussel presence at large scales. Native evenness was not related to *M. spicatum,* charophytes or zebra mussels at small scales and negatively related to *M. spicatum* cover at large scales. Lake location (subwatershed and longitude) was an important predictor of both native richness and evenness at the lake scale.

Main conclusions Despite accounting for the degree of invasion of *M. spicatum*, the results do not support the hypothesis that competition by an invasive species reduces native richness or increases native evenness at either large or small scales (entire lake *vs*. quadrat).

Introduction

Freshwater ecosystems have high conservation value due to the large number of plant and animal species they support (Dudgeon et al., 2005). While drivers of freshwater biodiversity have been well studied, the effect of stressors on macrophytes (aquatic plants) has received less attention (Stendera et al., 2012). Several threats exist to freshwater biodiversity, including resource exploitation, pollution, altered flow, degraded habitat and the introduction of invasive species (Dudgeon et al., 2005).

In addition to the effect of stressors, biodiversity patterns are also affected by spatial scale (Sandel & Smith, 2009). Reviews of terrestrial plant studies at different scales found that at larger scales individual invasive plant species had less impact on native richness (Powell et al., 2011) and native-exotic richness relationships changed from negative to positive (Fridley et al., 2007).

A similar pattern exists for macrophytes: native macrophyte richness at small scales (e.g. individual quadrats) has been found to be negatively correlated to both individual invasive macrophyte species (Boylen et al., 1999; Lyon & Eastman, 2006; Michelan et al., 2010; Stiers et al., 2011; Mjelde et al., 2012) and exotic species richness (Chen et al., 2010; Santos et al., 2010; but see Capers et al., 2007; Quinn et al., 2011). However, at larger scales (transects to entire water bodies) the relationship between native macrophyte richness and individual invasive macrophytes has differed among studies, with examples of relationships that were negative (Houlahan & Findlay, 2004; Hulme & Bremner, 2005; Thomaz et al., 2009; Michelan et al., 2010; O'Hare et al., 2012), non-significant (Houlahan & Findlay, 2004; Rybicki & Landwehr, 2007; Trebitz & Taylor, 2007; Mjelde et al., 2012) or positive (Thomaz et al., 2009).

Species diversity consists of both the number of species present (species richness) and the relative abundance of species (species evenness) (Washington, 1984; Smith & Wilson, 1996); however, freshwater diversity studies report species evenness far less frequently than species richness (Stendera et al., 2012). Species richness is expected to decline with predatory invasive species (Fridley et al., 2007); however, competitive invasive species, like plants, are more likely to change species evenness (Valéry et al., 2009). Invasive species are expected to increase native evenness by either reducing the abundance of common species or by driving rare species locally extinct (Powell et al., 2011).

Few studies have investigated invasive effects on native macrophyte evenness. At small scales, the removal of the riparian invasive *Impatiens glandulifera* Royle resulted in lower evenness of the remaining native and exotic species (Hulme & Bremner, 2005). At larger scales evenness does not appear to be related to invasive species, as no change in habitat complexity was observed after *Myriophyllum spicatum* L. removal (Kovalenko et al., 2010) and native species were equally likely to be dominant as exotic species in lakes (Capers et al., 2009) and wetlands (Houlahan & Findlay, 2004).

In addition to *M. spicatum*, charophytes (macroscopic filamentous algae) and zebra mussels (*Dreissena polymorpha* Pallas) are other important organisms to consider regarding macrophyte biodiversity. Invasive macrophyte colonization has been negatively associated with charophyte cover in oligotrophic New Zealand lakes (de Winton et al., 2012). Zebra mussels are ecosystem engineers that improve macrophyte habitat (Zhu et al., 2006), and are associated with shifts in the Bay of Quinte, Ontario from turbidity tolerant macrophyte species to turbidity intolerant species (Leisti et al., 2012).

This study investigated the relationship in lakes between native submerged and floating macrophyte communities and three aquatic organisms: the invasive macrophyte *M. spicatum,* charophytes and zebra mussels. The purpose of the study was to answer three questions: (1) how do richness and evenness of native macrophytes vary with the cover of *M. spicatum*, cover of charophyte meadows and presence/absence of zebra mussels? (2) Does this relationship change at small (1 m^2) and large (whole lake) spatial scales? (3) Does this relationship change after accounting for abiotic habitat variables and lake location?

Methods

Data collection

Lakes were situated within two subwatersheds of the Rideau Valley Watershed in southeastern Ontario, Canada: the Tay Subwatershed (*n* = 9) and the Rideau Lakes Subwatershed (*n* = 12) (Figure 2-1). Lakes were surveyed once each between June 26th and August 16th, 2012 during the peak growing season. Single surveys have been done in other studies (Trebitz & Taylor, 2007; Capers et al., 2009; Mikulyuk et al., 2011) as macrophytes are often present throughout the growing season (as discussed in Mikulyuk et al., 2010). Three transects per lake were chosen to be away from cottages (to avoid shoreline disturbance), along flat shorelines (to avoid shorelines with no shallow region) and as far apart as possible while still surveying lakes in a single day (up to 2 km).

At each transect, submerged and floating macrophytes were surveyed at six 1 m x 1 m quadrats distributed evenly from shore to the deepest point where plants could be observed using a Plexiglas water viewer. Records were made of the percent of substrate covered by charophytes, native and invasive macrophyte species as well as the presence of zebra mussels. Species that were difficult to identify using the viewer were collected with a hard garden rake and identified *in situ* or saved for later identification. Family, species names and native/non-native status follow Brouillet et al (2010) and species identification followed Crow & Hellquist (2000).

M. spicatum was distinguished from the native *Myriophyllum sibiricum* Kom. as plants with 12 or more leaflets on a side and plants with less than 12 leaflets per side, respectively (Crow & Hellquist, 2000). While hybridization between *M. spicatum* and *M. sibiricum* has occurred in the watershed (Borrowman, 2012), it is not possible to distinguish

M. spicatum from *M. spicatum* x *sibiricum* without molecular analysis (Moody & Les, 2007).

Charophytes were treated as a single group to explore the relationship between charophyte meadows and macrophyte diversity, as in de Winton et al. (2012), although four species have been identified in the Rideau River (Makkay et al., 2008). While charophytes have been identified to species level in some macrophyte studies (de Winton & Clayton, 1996; Mjelde et al., 2012; O'Hare et al., 2012), it is not uncommon for studies to limit taxonomic resolution to the genus level (Lyon & Eastman, 2006; Michelan et al., 2010; Ginn, 2011) or family level (Trebitz & Taylor, 2007).

Other variables that may influence macrophyte communities are trophic status (lake algal productivity), available light, lake connectivity (Lacoul & Freedman, 2006) and habitat area (Rørslett, 1991; Dodson et al., 2000). To measure the concentration of total phosphorus and chlorophyll-*a* in the littoral zone, surface water samples were collected at the second quadrat from shore. Concentration of total phosphorus was measured by the City of Ottawa Surface Water Quality Laboratories while the concentration of chlorophyll-*a* was estimated using spectrophotometry after filtration through a 1.2 μm glass fibre filter and extraction in 95% ethanol (Wintermans & DeMots, 1965). Conductivity was measured using a Hydrolab Minisonde near shore and at the deepest quadrat (both at subsurface and above substrate) for each transect. To estimate water clarity Secchi depth was measured once per lake in deeper water towards the centre of the lake. To estimate length of the littoral zone, the distance between shore and the deepest point of plant growth was measured using quadrat co-ordinates plotted in Google Earth. Estimates of littoral length were strongly correlated with measurements made using meter tape or an electronic range finder ($b = 1.07$, $R^2 = 1.00$, $F(1,$

7) = 2065, $p < 0.001$, Figure S-1). Lake area was measured from satellite maps in ArcGIS Explorer.

Variables considered

Lake species richness, S, was calculated as the number of native macrophyte species present in a lake or quadrat. Lake species evenness, $E_{1/D}$, was calculated as the ratio of Simpson's diversity, D^{-1}, to maximum diversity (S) (Smith & Wilson, 1996):

$$E_{1/D} = 1 / (D * S)$$

Where,

$$D = \sum_{(s = 1 : S)} p_s^2$$

$$p_s = x_s / \sum x$$

x = estimated percent cover of the s^{th} species in all lake quadrats

To account for the variation in minimum possible evenness values at low species richness (Tuomisto, 2012) quadrat species evenness, $E_{1/D}$, was calculated as above, with the additional step of rescaling evenness to range between zero and one. Rescaling was done by subtracting the theoretical minimum evenness ($1/S$) and dividing by the theoretical range in evenness ($1 - 1/S$), termed 'ranged evenness' (Tuomisto, 2012).

Independent variables related to the hypotheses were the mean cover of *M. spicatum*, mean cover of charophytes and presence of zebra mussels. Other independent variables considered were: the mean concentration of chlorophyll-*a,* mean concentration of total phosphorus and mean conductivity (a measure of ions in water) to account for trophic status and water chemistry; Secchi depth and max depth of plant growth to account for light availability; latitude, longitude and subwatershed identity to account for lake location; and, mean littoral length and lake area to account for habitat area.

Statistical analysis

Statistical analyses were performed using R version 2.15.1 (R Core Team, 2012). To correct for extreme values, variables showing outliers were transformed using a $\log_{10}(x)$ transformation or $\log_{10}(x+1)$ transformation if a variable contained zeros. To reduce the number of variables considered, Principal Components Analysis was performed on all continuous abiotic habitat variables. The first three principal components and subwatershed (a nominal variable) were used to describe the habitat gradient across lakes.

To compare quadrat level richness or evenness against quadrat cover of *M. spicatum* or charophytes, separate Spearman rank correlations were computed for each lake. To compare relationships between native macrophyte diversity (lake richness, lake evenness or within lake correlations) and lake biological variables (*M. spicatum* cover, charophyte cover and zebra mussel presence) Spearman rank correlations were computed (*M. spicatum* cover and charophyte cover) and a Mann-Whitney U-test was performed (zebra mussel presence). To account for lake habitat gradients when comparing the relationship between macrophyte diversity and biological variables subwatershed identify and the first three components from PCA of lake habitat variables were included in a linear model of macrophyte diversity against biological variables. To visualize the relationship between macrophyte diversity and biological variables in the linear model, partial regression plots were made using the car package in R (Fox & Weisberg, 2011).

In addition to studying the effect of biological variables on native richness or evenness, a separate linear model was created for the lake scale to find the most parsimonious model predicting native macrophyte richness/evenness. The model with the lowest value of the Bayesian Information Criterion (BIC) was selected from the set of models containing all combinations of 1-5 variables using the leaps package in R (Lumley, 2009).

Results

Lake characteristics

Zebra mussels were observed in ten lakes (nine in the Rideau Lakes Subwatershed) and not observed in eleven lakes (eight in the Tay River Subwatershed). Mean quadrat cover of *M. spicatum* ranged from 0% to 22% in lakes and mean quadrat cover of charophytes ranged from 0% to 73% in lakes.

Lake littoral regions were basic (pH 8.5 to 9.1) with low nutrients, relatively low conductivity and high Secchi depth (> 2 m), while both littoral length and surface area were variable (Table 2-1). Principal components analysis of lake latitude, longitude and seven physical and chemical lake variables explained 75% of the variation in lake characteristics in the first three principal components (Figure 2-2). The largest PC1 component loading coefficients were associated with nutrients and habitat area (total phosphorus, -0.50; littoral length, -0.47; chlorophyll-*a*, -0.41). The largest PC2 component loading coefficients were associated with geographic location (latitude, -0.53; longitude, -0.53). Finally, the largest PC3 component loading coefficients were associated with lake size and water clarity (maximum plant depth, 0.65; Secchi depth, 0.43; lake surface area, 0.45). Coefficients for conductivity were moderate for PC1 and PC2 (-0.31 and -0.33, respectively).

Species composition

A total of 35 submerged/floating macrophyte species were observed in the Rideau Valley Watershed: 32 native vascular macrophytes, 2 invasive vascular macrophytes (*M. spicatum* and *Hydrocharis morsus-ranae* L.) and charophytes (Table 2-2). The three most common taxa observed were *Vallisneria americana* Michx., charophytes and *Najas flexilis*

(Willd.) Rostk. and W.L.E. Schmidt (92%, 81% and 81% of transects, respectively). The three least common species observed were *Potamogeton nodosus* Poir., *Isoëtes echinospora* Durieu and *Utricularia minor* L. (5%, 3% and 3% of transects, respectively). The invasive species *M. spicatum* was observed in 63% of transects. The invasive species *H. morsus-ranae* was observed in 20% of transects, but only at low abundance and was not considered further.

Within individual lakes, native macrophyte species richness ranged from 11 to 22 species and native species evenness ranged from 0.06 to 0.41. Within-lake Spearman rank correlations between quadrat-scale native richness/evenness and quadrat-scale *M. spicatum* cover/charophyte cover varied from positive to negative among lakes.

Richness

At the quadrat scale, mean Spearman rank correlations, r_S, of native richness, S, vs. *M. spicatum* cover, I, or charophyte cover, C, were not significantly different from zero ($r_{S\ \mathrm{S\text{-}I}}$: $t(11) = 0.56$, $p = 0.585$; $r_{S\ S\text{-}C}$: $t(20) = -0.40$, $p = 0.691$). However, $r_{S\ S\text{-}C}$ became more negative in lakes with a greater total cover of charophytes, both in single regression ($r_S(20) = -0.59$, $p = 0.005$, Figure 2-3b) and after accounting for: PC1-3, subwatershed, zebra mussel presence/absence and *M. spicatum* cover ($F(1, 13) = 5.43$, $p = 0.037$, Figure 2-3d). Residuals met assumptions for homoscedasticity and normality and no lakes appeared to have a disproportionate influence on the results (Figure S-2). On the other hand, $r_{S\ S\text{-}I}$ did not have a significant relationship with the total cover of *M. spicatum*, either alone ($r_S(11) = -0.42$, $p = 0.178$, Figure 2-3a) or after accounting for other independent variables ($F(1, 4) = 0.32$, $p = 0.601$, Figure 2-3c).

At the lake scale, native species richness showed a marginally significant relationship with charophyte cover ($r_S(20) = -0.41$, $p = 0.064$), but did not show significant relationships with either cover of *M. spicatum* ($r_S(20) = 0.12$, $p = 0.596$) or zebra mussel presence/absence ($U(11, 10) = 40.50$, $p = 0.319$) ().

Native species richness showed a significant multivariate relationship with zebra mussel presence/absence at the lake scale after accounting for subwatershed, PC1-3, *M. spicatum* cover and charophyte cover with greater macrophyte richness in lakes where zebra mussels were observed ($F(1, 13) = 5.29$, $p = 0.039$, Table 2-3). Neither cover of *M. spicatum* nor cover of charophytes showed significant relationships with macrophyte richness in the full model (Table 2-3). Residuals met assumptions for homoscedasticity and normality; however, two lakes appeared to have a disproportionate influence on the results (Figure S-4). Excluding these lakes did not change the model results or pattern of residuals (Table S-1, Figure S-5).

The most parsimonious lake model explaining native macrophyte richness included chlorophyll-*a*, zebra mussel presence/absence and subwatershed (Figure S-6). An interaction effect between chlorophyll-*a* and either zebra mussels or subwatershed was suggested when macrophyte richness was plotted against chlorophyll-*a* for each combination of zebra mussel presence/absence and subwatershed (Figure S-7). In a linear model predicting macrophyte richness, the significant predictors were chlorophyll-*a*, subwatershed, zebra mussel presence/absence and the interaction between chlorophyll-*a* and zebra mussels. The interaction between chlorophyll-*a* and subwatershed was not significant and excluded from the model. The full model was significant ($F(4, 16) = 12.27$; $p < 0.001$) and explained 75% of the variation in macrophyte richness (Table 2-4). Residuals were homoscedastic but appeared right skewed and strongly influenced by one lake (Figure S-8); however, removal of Loon Lake resulted in similar model results with normally distributed residuals (Table S-2, Figure S-9). Partial regression plots showed greater species richness in lakes having greater concentrations of chlorophyll-*a*, invaded by zebra mussels and situated in the Tay River subwatershed (Figure 2-4). Substituting littoral total phosphorus for chlorophyll-*a* also yielded a significant model ($R^2 = 0.64$, adjusted $R^2 = 0.56$, $F(4,16) = 7.24$, $p = 0.002$) with p values for individual variables less than 0.06.

Evenness

At the quadrat scale, the mean Spearman rank correlation of ranged native evenness vs. *M. spicatum* cover, $r_{S\ E\text{-}I}$, was not significantly different from zero ($t(11) = 1.24$, $p = 0.242$), and the mean Spearman rank correlations of ranged native evenness vs. charophyte cover, $r_{S\ E\text{-}C}$ was also not significantly different from zero ($t(20) = -0.20$, $p = 0.844$). Furthermore, no significant relationships were found between $r_{S\ E\text{-}I}$ and whole lake *M. spicatum* cover or $r_{s\ E\text{-}C}$ and whole lake charophyte cover, either alone ($r_S(11) = -0.08$, $p = 0.804$; $r_S(20) = 0.03$, $p = 0.913$, respectively) or after accounting for the other independent variables: PC1-3, subwatershed, zebra mussel presence/absence, *M. spicatum* cover and charophyte cover ($F(1, 4) = 1.79$, $p = 0.252$; $F(1, 13) = 0.06$, $p = 0.810$, respectively; Figure 2-5).

Whole lake native evenness did not show significant bivariate relationships with any of the biological variables considered: cover of *M. spicatum* ($r_S(20) = -0.35$, $p = 0.125$), cover of charophytes ($r_S(20) = 0.34$, $p = 0.137$), or zebra mussel presence/absence ($U(11, 10) = 43.00$, $p = 0.426$) ().

At the lake scale, native evenness showed a marginally significant multivariate relationship with the cover of *M. spicatum* after accounting for PC 1-3, subwatershed, zebra mussels and charophyte cover with lower evenness in lakes containing greater cover of *M. spicatum* (*F*(1,13) = 3.59; *p* = 0.081, Table 2-5). Residuals met assumptions of homoscedasticity and normality and no lakes appeared to strongly influence the results (Figure S-11). Neither the cover of charophytes or zebra mussel presence/absence showed a significant relationship with native macrophyte evenness under the full model (Table 2-5).

The most parsimonious lake model explaining native macrophyte evenness contained *M. spicatum* cover, littoral length and longitude (Figure S-12). Littoral length and longitude were significant predictors of native evenness (littoral length: *F*(1, 17) = 5.88, *p* = 0.027; longitude: *F*(1, 17) = 8.90, *p* = 0.008, Table 2-6) and *M. spicatum* cover was marginally significant (*F*(1, 17) = 4.15, *p* = 0.058, Table 2-6). The full model was also significant (*F*(4, 16) = 4.92, *p* = 0.009) and explained 55% of the variation in evenness (Table 2-6). Residuals met assumptions for homoscedasticity and normality (Figure S-13). One site (Noble’s Bay) appeared to have a strong influence on the results and removal of the site resulted in littoral length ceasing to be a significant predictor in the model (Table S-3, Figure S-14). Partial regression plots showed greater evenness in eastern lakes with shorter littoral lengths and lower cover of *M. spicatum* (Figure 2-6). Littoral total phosphorus was not significant when used in place of littoral length (*F*(1, 17) = 0.861, *p* = 0.366), nor was the model significant (R^2 = 0.28, *F*(3, 17) = 2.19, *p* = 0.126).

Discussion

The association of *M. spicatum*, charophytes and zebra mussels with macrophyte richness and evenness was: (1) negative for charophytes with native richness at small scales only, (2) positive for zebra mussels with native richness at large scales, and (3) negative for *M. spicatum* with evenness at large scales only. Evenness at small scales was unrelated to either *M. spicatum* or charophytes (zebra mussels were only considered as present or absent in lakes). Lake location, whether indicated by subwatershed or longitude, was important in explaining differences in both lake-wide native richness and evenness.

Richness

Both positive and negative relationships were observed at the quadrat level between native richness and *M. spicatum* / charophyte cover within individual lakes. The correlations did not vary with the total cover of *M. spicatum* in lakes, even after accounting for other variables. Within lake native – exotic richness correlations in Connecticut lakes also varied between positive and negative (Capers et al., 2007), and no relationship was observed between native and invasive richness at small scales across waterbodies (Capers et al., 2007; Quinn et al., 2011). Similarly, native species richness was not found to be a significant predictor of *M. spicatum* abundance at small scales, although it was associated with *M. spicatum* presence (Olson et al., 2012). While lower richness at small scales dominated by invasive submerged macrophytes has been observed in studies, these studies compared sampling sites that were heavily invaded to sampling sites that were not invaded, rather than examining a gradient of invasive abundance (de Winton & Clayton, 1996; Boylen et al., 1999; Lyon & Eastman, 2006; Mjelde et al., 2012). The results suggest that with random quadrat selection, which may be more reflective of the entire lake community, no relationship exists between invasive cover and native richness.

Unlike *M. spicatum,* within-lake correlations between native richness and charophyte cover declined with the lake-wide mean cover of charophytes and remained significant after accounting for other habitat variables. Charophytes grow best in oligotrophic, hard water lakes (Kufel & Kufel, 2002), which were typical of the study region, while *M. spicatum* prefers more eutrophic conditions (Aiken et al., 1979), so the habitat conditions surveyed favored charophytes rather than *M. spicatum*. Nevertheless, several lakes had abundant *M. spicatum* beds in deep water, allowing a range of *M. spicatum* cover to be considered. Charophytes also differ from *M. spicatum* as charophytes form dense beds at the lake bottom (Kufel & Kufel, 2002), while *M. spicatum* forms a dense canopy near the water surface (Aiken et al., 1979). It may be that at small scales, native richness is related to competition for available substrate rather than competition for light.

No correlation was observed between native richness at the lake scale and the cover of *M. spicatum* or charophytes, in agreement with most other studies of submerged invasive species (Capers et al., 2007; Rybicki & Landwehr, 2007; Trebitz & Taylor, 2007; Quinn et al., 2011; Mjelde et al., 2012). Lower richness was observed in lakes invaded by *Elodea canadensis* Michx.; however, in that study *E. canadensis* was associated with higher nutrient lakes (maximum TP was 931.7 ug/L vs. maximum in this study of 26 ug/L) (O'Hare et al., 2012), so the lack of a relationship in this study may be due to sampling only in oligo-mesotrophic lakes. The results support the idea that native species are able to persist in lakes despite the abundant growth of other species (Rørslett, 1991; Capers et al., 2009; Powell et al., 2011).

Charophytes have been associated with good macrophyte habitat conditions (clear, low trophic state, hard water) (Kufel & Kufel, 2002); however, there is no evidence in this study that they are associated with greater macrophyte richness.

Richness at the lake scale increased with the presence of zebra mussels after accounting for other habitat variables. Zebra mussels were also associated with increased macrophyte richness in a comparison of pre and post-invasion communities (Zhu et al., 2006). Zebra mussels have been observed to improve habitat conditions for macrophytes, increasing water clarity and facilitating colonization across a greater lake area (Higgins & Zanden, 2010; Leisti et al., 2012). In this study however, Secchi depth was not a significant predictor of species richness. The range of Secchi depth was similar to that in another study where macrophyte richness increased with zebra mussels, although that study did not test the effects of Secchi depth directly (Zhu et al., 2006). Zebra mussels also improve macrophyte habitat by increasing sediment organic content through nutrients secreted in their waste (Higgins & Zanden, 2010); however, sediment samples were not collected in this study to compare sediment nutrients in lakes inhabited and not inhabited by zebra mussels.

The positive relationship between richness and chlorophyll-*a* in this study likely reflects the role of nutrients as a limiting variable for plant and algal growth in these lakes. Similarly, in a study of Danish lakes, submerged macrophyte richness was variable in lakes with less than 50 ug/L total phosphorus and declined in more eutrophic lakes, while the concentration of chlorophyll-*a* increased steadily with trophic status (Jeppesen et al., 2000). The results support the prediction of the productivity-diversity relationship of plant communities. In stressful, low nutrient conditions, increases in nutrients should result in greater richness as more stress intolerant species are able to persist in the community but are not yet able to outcompete stress tolerant species (Grime, 1973; Michalet et al., 2006). At higher nutrient concentrations however, a negative relationship would be expected between macrophyte richness and algal abundance (Scheffer et al., 1993).

The interaction between zebra mussel presence and chlorophyll-*a* may indicate a greater importance of zebra mussels than water column nutrients in predicting native richness. In the absence of zebra mussels measures of nutrients in the water may be a good indicator of total nutrients available to macrophytes in both the sediment and water column. In the presence of zebra mussels however, nutrients in the water column may not reflect nutrients available in the sediment due to zebra mussels filtering the water column and secreting wastes into the sediments (Higgins & Zanden, 2010).

Greater richness was observed in the more isolated western subwatershed (Tay River) than the eastern subwatershed (Rideau Lakes), which is connected to the Rideau Canal. The difference in richness may be due to an unmeasured habitat gradient between the two watersheds, or to dispersal limitations preventing species in the western subwatershed from colonizing suitable habitat in the eastern subwatershed. Other studies have found evidence for dispersal effects, as macrophyte communities were more similar in nearby lakes than in more separated lakes (Capers et al., 2010; Mikulyuk et al., 2011). The community composition of the two subwatersheds will be considered in chapter three.

Evenness

At the quadrat scale, within-lake correlations between ranged native evenness and either *M. spicatum* cover or charophyte cover varied from positive to negative across lakes. Neither correlation was significantly related to the mean lake-wide cover of *M. spicatum* or charophytes, even after accounting for other habitat variables. This differs from the observation in riparian plots that plant species evenness (excluding the invasive *I. glandulifera*) was higher in invaded plots than exclusion plots, which was attributed to other dominant species exploiting gaps in plots where *I. glandulifera* was removed (Hulme & Bremner, 2005). Littoral regions are exposed to disturbance from waves and variable water levels. A lack of correlation between evenness at the quadrat level and competition from *M. spicatum* or charophytes may be due to sufficient habitat disturbance preventing competition from structuring macrophyte communities at small scales (Huston, 1999).

Lake native species evenness was negatively related to cover of *M. spicatum*, but not to charophytes or zebra mussel presence. The lack of a relationship between zebra mussel presence and macrophyte evenness has been previously reported (Zhu et al., 2006). On the other hand, the results do not support the prediction that native evenness will be greater in invaded habitats due to suppression of strong native competitors and local extirpation of weak native competitors (Powell et al., 2011). While it is possible that more even communities are resisting invasion, experimental studies with prairie grasses have either found no relationship between evenness and invasion (Emery & Gross, 2007; Mattingly et al., 2007), or have found evenness-resistance relationships to be species specific for both the native and invading species (Losure et al., 2007). On the other hand, factors leading to high *M. spicatum* cover may also support competitive native species. This supports the description of *M. spicatum* as a 'matrix dominant' (Trebitz & Taylor, 2007), defined as an abundant species associated with other competitive species (Frieswyk et al., 2007). It has been observed that native species are just as likely as exotic species to be dominant in lakes and wetlands (Houlahan & Findlay, 2004; Capers et al., 2009). The results of the present study suggest that at large scales native and exotic species may become abundant in the same water bodies and not at the expense of the other. Such a positive association may be due to dominant native and exotic species both benefiting from similar habitat conditions at large scales, which is believed to be one reason for a positive relationship between native and exotic richness among terrestrial plants at large scales (Fridley et al., 2007 and references therein).

Native evenness was found to be lower in lakes with higher *M. spicatum* cover, longer littoral lengths and further to the west. In a Connecticut study, individual macrophyte species were more likely to become dominant in shallow, high productivity lakes (Capers et al., 2009) although evenness was not measured. Similarly, a decline in total evenness with nutrients was observed at large spatial scales (2,400 m^2) in Danish coastal macroalgal communities (Middelboe & Sand-Jensen, 2004). Although littoral length was positively correlated with littoral total phosphorus in PCA, total phosphorus was not a significant predictor of native evenness when substituted for littoral length. Lower evenness in longer littoral regions may instead be related to increased wave disturbance to macrophyte beds far from the shoreline. Macrophytes vary between strong and weak dispersal species (Lacoul & Freedman, 2006) and wave disturbance would support the fragmentation and spread of strong dispersing species compared to poor dispersing species. The greater richness in the western subwatershed compared to the eastern subwatershed may have been due to species unable to disperse to the eastern subwatershed. If true, it would also support the lower evenness in the western subwatershed due to the presence of both strong and weak dispersing species. The distribution of species across the study area will be explored in chapter three.

Summary

At small scales, the richness of native macrophytes decreased with the cover of charophytes and at large scales increased with the presence of zebra mussels, both benthic organisms. No relationship was observed between native richness and *M. spicatum* cover. Evenness of native macrophytes showed no relationship with *M. spicatum* or charophytes at small scales and declined with the cover of *M. spicatum* at large scales. Accounting for habitat gradients and lake location in the models either improved or did not change the strength of the relationships between native macrophyte richness/evenness and *M. spicatum*, charophytes and zebra mussels.

From a conservation perspective neither the invasive species *M. spicatum* or zebra mussels, nor native charophyte species exhibited strong negative effects on macrophyte richness or evenness in low nutrient lakes. The association of *M. spicatum* with lower lake wide native evenness suggests that *M. spicatum* dominance is a response to conditions that also promote dominance among native species. Greater exposure to physical disturbance from waves or boats may be the cause of decreased evenness, although further study is required. While charophyte beds at small scales were associated with low native richness, this trend disappeared at the lake scale, suggesting that lake habitat is complex enough to accommodate beds of both charophytes and other macrophytes.

Tables

Table 2-1. Summary of continuous independent lake variables and transformation required for statistical analysis. * represents no transformation.

	Units	Minimum	Maximum	Median	Mean	Standard Error	*N*	Transformation
Myriophyllum spicatum cover	%	0	22	3	4	1	21	$\log_{10}(x+1)$
Charophyte cover	%	0	73	5	13	4	21	$\log_{10}(x+1)$
Chlorophyll-*a*	µg/L	0.91	6.41	1.49	2.02	0.28	21	$\log_{10}(x)$
Littoral TP	µg/L	4	26	9	10	1	21	$\log_{10}(x)$
Conductivity	µS/cm	64	265	200	184	11	21	*
Secchi depth	m	2.1	6.5	4.3	4.6	0.3	20	*
Max macrophyte depth	m	2.2	5.5	4.4	4.2	0.2	21	*
Mean littoral length	m	37	402	111	139	20	21	$\log_{10}(x)$
Lake surface area	ha	19	6482	290	764	313	21	$\log_{10}(x)$

Table 2-2. Macrophytes and charophytes observed across 63 transects and 21 lakes in the Rideau Valley watershed. * indicates invasive macrophytes.

Family	Species	Lakes (*N*)	Transects (*N*)	
Alismataceae	*Sagittaria cuneata* E. Sheld.	3	3	
Araceae	*Lemna minor* L.	3	5	
	Lemna trisulca L.	11	27	
	Spirodela polyrhiza (L.) Schleid.	10	19	
	Wolffia sp. Horkel ex Schleid.	5	9	
Asteraceae	*Bidens beckii* Torr. ex Spreng.	12	21	
Cabombaceae	*Brasenia schreberi* J.F. Gmel.	4	5	
Ceratophyllaceae	*Ceratophyllum demersum* L.	20	44	
Characeae	*Chara* spp. L.	21	51	
Haloragaceae	*Myriophyllum sibiricum* Kom.	16	32	
	Myriophyllum spicatum L.	16	40	*
Hydrocharitaceae	*Elodea canadensis* Michx.	18	46	
	Hydrocharis morsus-ranae L.	9	13	*
	Najas flexilis (Willd.) Rostk. and W.L.E. Schmidt	21	51	
	Vallisneria americana Michx.	21	58	
Isoëtaceae	*Isoëtes echinospora* Durieu	2	2	
Lentibulariaceae	*Utricularia intermedia* Hayne	2	4	
	Utricularia minor L.	2	2	
	Utricularia vulgaris L.	6	9	

Table 2-2 (Continued)

Family	Species	Lakes (*N*)	Transects (*N*)
Nymphaeaceae	*Nuphar variegata* Durand	12	17
	Nymphaea odorata Aiton	19	39
Pontederiaceae	*Heteranthera dubia* (Jacq.) MacMill.	19	43
Potamogetonaceae	*Potamogeton amplifolius* Tuck.	9	17
	Potamogeton friesii Rupr.	16	30
	Potamogeton gramineus L.	15	28
	Potamogeton illinoensis Morong	15	32
	Potamogeton natans L.	7	10
	Potamogeton nodosus Poir.	2	3
	Potamogeton perfoliatus L.	5	7
	Potamogeton pusillus L.	17	44
	Potamogeton richardsonii (A. Benn.) Rydb.	6	11
	Potamogeton robbinsii Oakes	17	45
	Potamogeton zosteriformis Fernald	20	46
	Stuckenia pectinata (L.) Börner	17	28
Ranunculaceae	*Ranunculus aquatilis* L.	14	28

Table 2-3. Results from linear model of native macrophyte richness using PC1-3 of lake habitat variables, subwatershed, cover of *Myriophyllum spicatum*, cover of charophytes and presence/absence of zebra mussels in 21 lakes. $R^2 = 0.55$, adjusted $R^2 = 0.30$, $F(7, 13) = 2.25$, $p = 0.099$.

	Coefficient	Type III Sum Sq	*DF*	*F*	*p*
PC1	-0.30	3.29	1	0.65	0.436
PC2	0.19	0.80	1	0.16	0.698
PC3	-0.43	2.87	1	0.56	0.466
Subwatershed (Tay River)	3.93	40.52	1	7.96	0.014
$\log_{10}$(*M. spicatum* cover)	0.10	0.02	1	< 0.01	0.952
$\log_{10}$(charophyte cover)	-0.90	3.71	1	0.73	0.409
Zebra mussels (present)	3.86	26.92	1	5.29	0.039
Residuals		66.18	13		

Table 2-4. Results from linear model of macrophyte richness with lowest BIC value among all models with one to five variables. Linear model of richness as a function of $\log_{10}$(chlorophyll-*a*), zebra mussel presence/absence, subwatershed and interaction between chlorophyll-*a* and zebra mussel presence/absence. $R^2 = 0.75$, adjusted $R^2 = 0.69$, $F(4, 16) = 12.27$, $p < 0.001$.

	Coefficient	Type III Sum Sq	*DF*	*F*	*p*
$\log_{10}$(chlorophyll-*a*)	12.83	47.75	1	20.03	< 0.001
Zebra mussel (presence)	5.36	57.93	1	24.30	< 0.001
Subwatershed (Tay)	2.71	19.48	1	8.17	0.011
$\log_{10}$(chl-*a*) * zebra mussel	-10.82	22.47	1	9.42	0.007
Residuals		38.15	16		

Table 2-5. Results from linear model of native macrophyte evenness using PC1-3 of lake habitat variables, subwatershed, cover of *Myriophyllum spicatum*, cover of charophytes and presence/absence of zebra mussels in 21 lakes. $R^2 = 0.47$, adjusted $R^2 = 0.18$, $F(7, 13) = 1.63$, $p = 0.212$.

	Coefficient	Type III Sum Sqr	*DF*	*F*	*p*
PC1	0.01	0.00	1	0.55	0.473
PC2	-0.02	0.01	1	2.74	0.122
PC3	0.01	0.00	1	0.13	0.720
Subwatershed (Tay)	-0.03	0.00	1	0.59	0.457
$\log_{10}$(*M. spicatum* cover)	-0.10	0.02	1	3.59	0.081
$\log_{10}$(charophyte cover)	-0.01	0.00	1	0.10	0.752
Zebra mussels (present)	0.03	0.00	1	0.35	0.567
Residuals		0.07	13		

Table 2-6. Results from linear model of native macrophyte evenness with lowest BIC value among all models with one to five variables. Linear model of evenness as a function of *Myriophyllum spicatum* cover, littoral length and longitude in 21 lakes. $R^2 = 0.44$, adjusted $R^2 = 0.34$, $F(3,17) = 4.40$, $p = 0.018$.

	Coefficient	Type III Sum Sq	*DF*	*F*	*p*
$\log_{10}$(*M. spicatum* cover)	-0.07	0.02	1	4.15	0.058
$\log_{10}$(littoral length)	-0.14	0.02	1	5.88	0.027
longitude	0.23	0.04	1	8.90	0.008
Residuals		0.07	17		

Figures

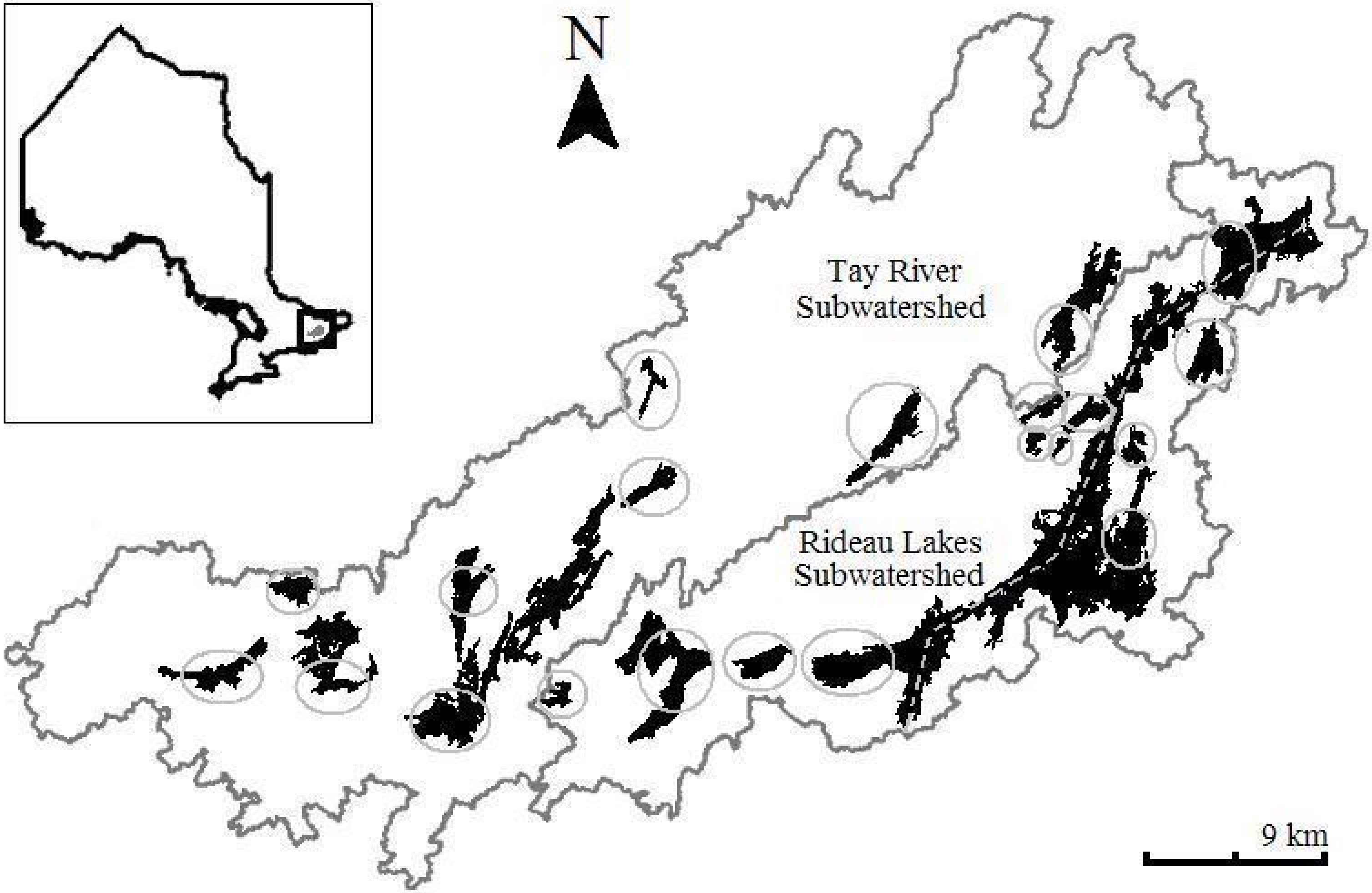

Figure 2-1. Twenty-one lakes surveyed during the study. Circles indicate location surveyed in each lake. Dashed line indicates route of Rideau Canal. Subwatersheds delineated as described in Ahmed (2010). Figure created using ArcGIS Explorer and datasets from OMNR (2010a, 2010b) and Statistics Canada (2011).

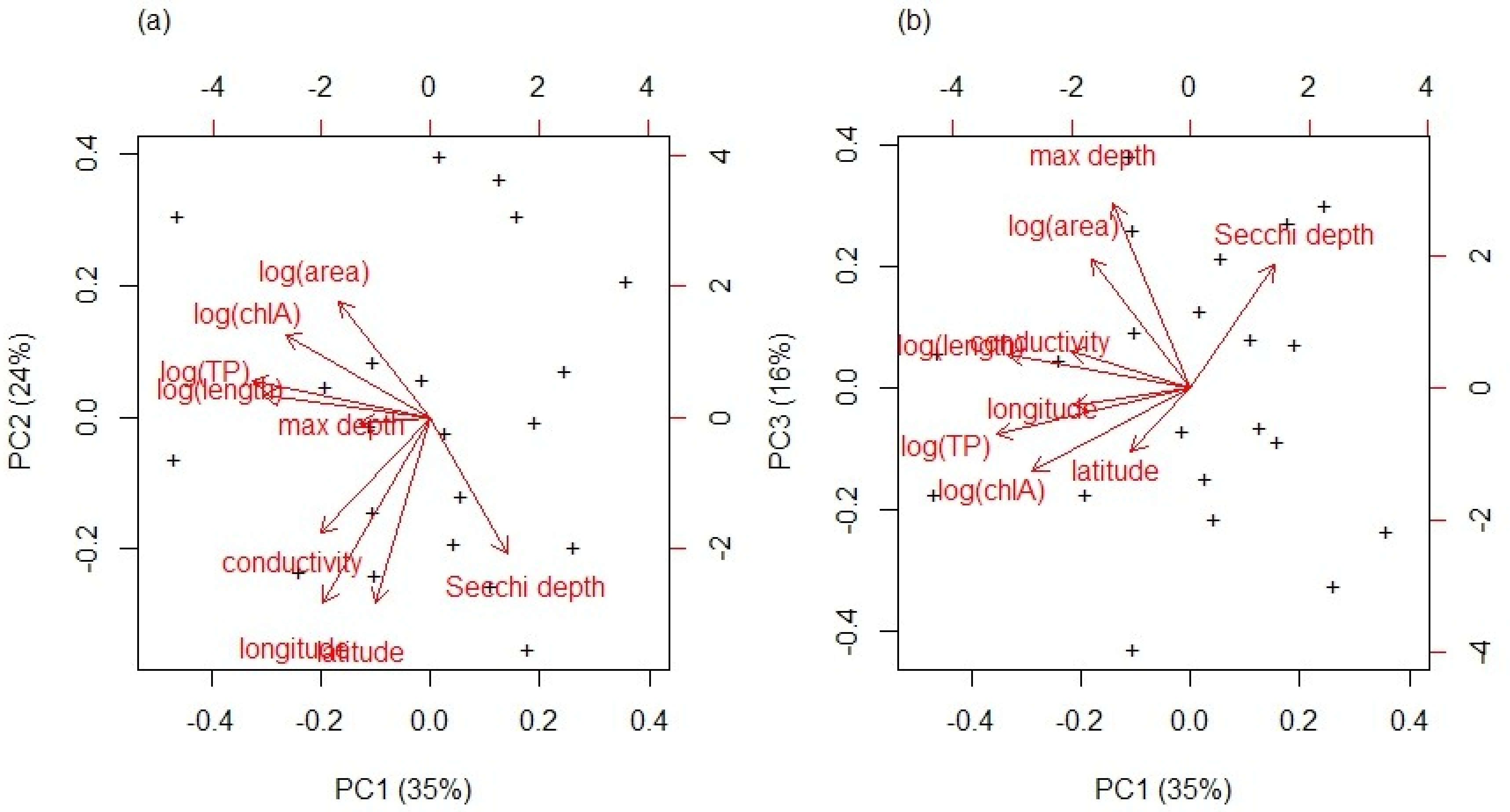

Figure 2-2. Biplots of results from principal components analysis (PCA) of habitat variables in 21 lakes. (a) PC2 vs. PC1, (b) PC3 vs. PC1.

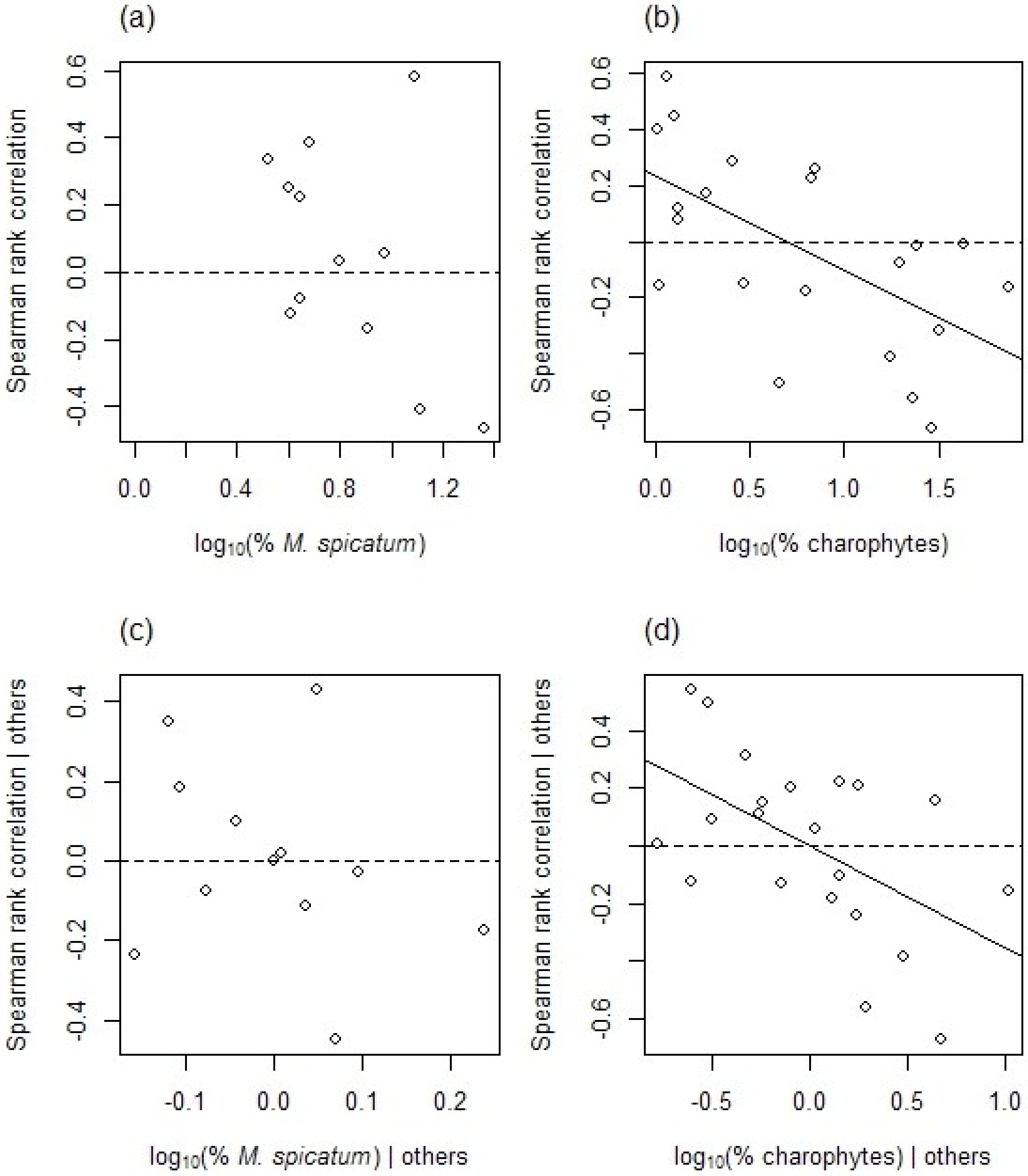

Figure 2-3. Scatter plots of (a) Spearman rank correlation (within-lake quadrat richness *vs.* *Myriophyllum spicatum* cover), $r_{S\ S\text{-}I}$, *vs.* mean lake *M. spicatum* cover, *I*, and (b) Spearman rank correlation (within-lake quadrat richness *vs.* charophyte), $r_{S\ S\text{-}C}$, *vs.* mean lake charophyte cover, *C*. Partial regression plots of (c) $r_{S\ S\text{-}I}$ *vs.* *I* after accounting for PC1-3, subwatershed, zebra mussel presence/absence and charophyte cover, and (d) $r_{S\ S\text{-}C}$ *vs.* *C* after accounting for PC1-3, subwatershed, zebra mussel presence/absence and *M. spicatum* cover. *N* = 12 lakes (a, c), 21 lakes (b, d).

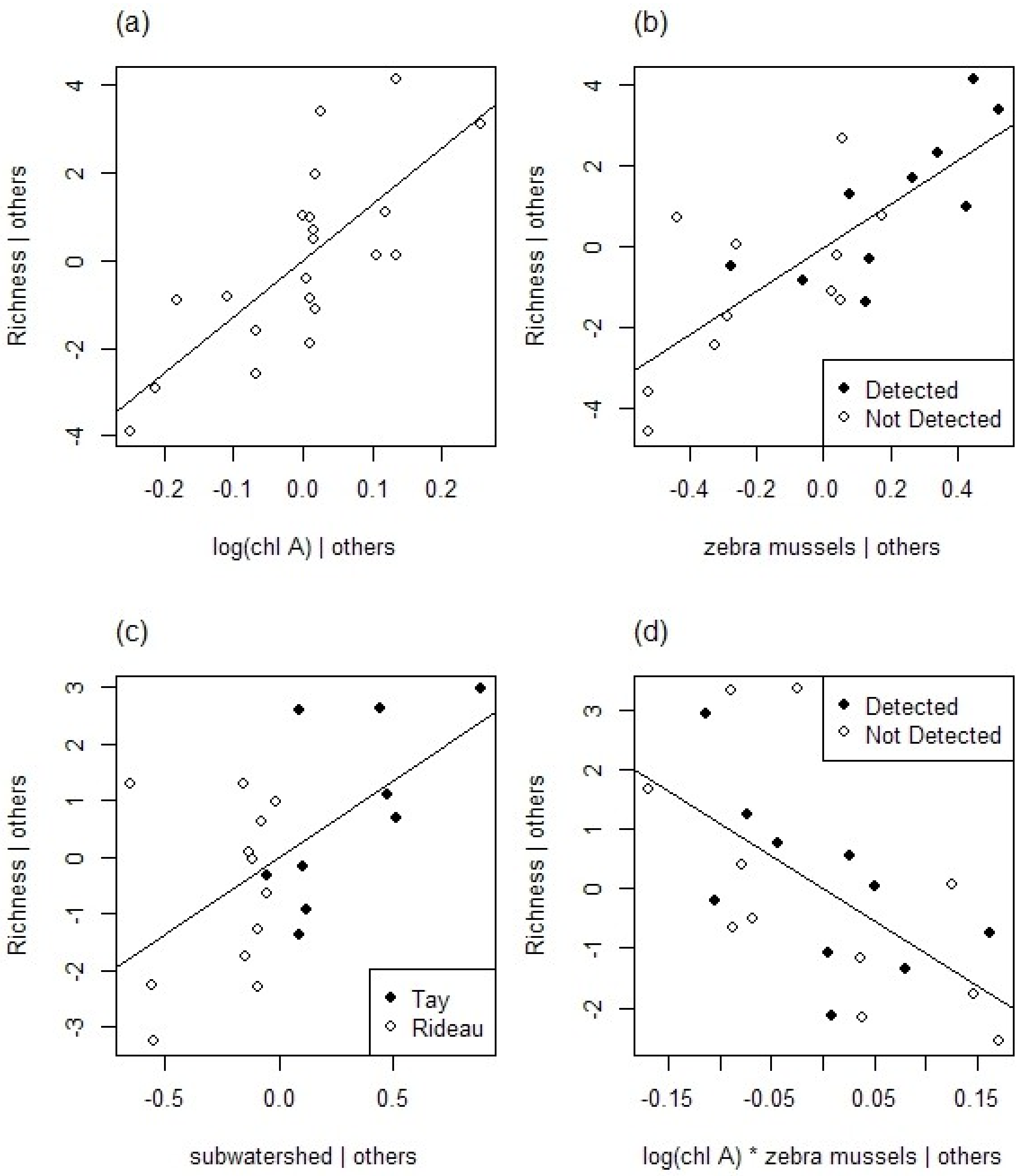

Figure 2-4. Partial regression plots from linear model of native macrophyte richness against (a) $\log_{10}$(chlorophyll-*a*), (b) zebra mussel presence/absence, (c) subwatershed type and (d) the interaction between $\log_{10}$(chlorophyll-*a*) and zebra mussel presence/absence for 21 lakes after accounting for the other three variables (Table 2-4).

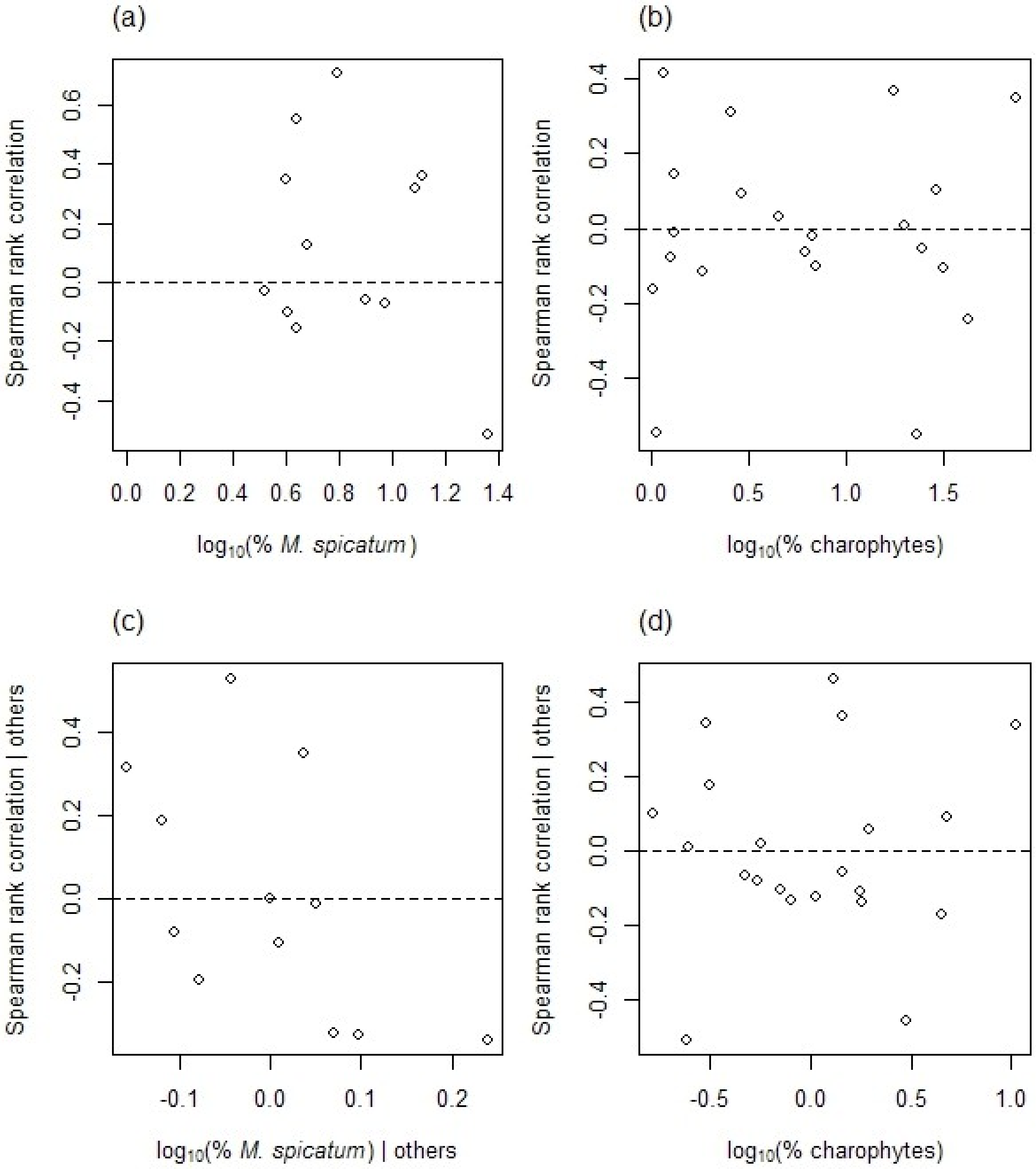

Figure 2-5. Scatter plots of (a) Spearman rank correlation (within-lake quadrat ranged evenness *vs. Myriophyllum spicatum* cover), $r_{S\ E\text{-}I}$, against mean lake *M. spicatum* cover, *I*, and (b) Spearman rank correlation (within-lake quadrat ranged evenness *vs.* charophyte cover), $r_{S\ E\text{-}C}$, against mean lake charophyte cover, *C*. Partial regression plots of (c) $r_{S\ E\text{-}I}$ *vs. I* after accounting for PC1-3, subwatershed, zebra mussel presence/absence and charophyte cover, and (d) $r_{S\ E\text{-}C}$ *vs. C* after accounting for PC1-3, subwatershed, zebra mussel presence/absence and *M. spicatum* cover. *N* = 12 lakes (a, c), 21 lakes (c, d).

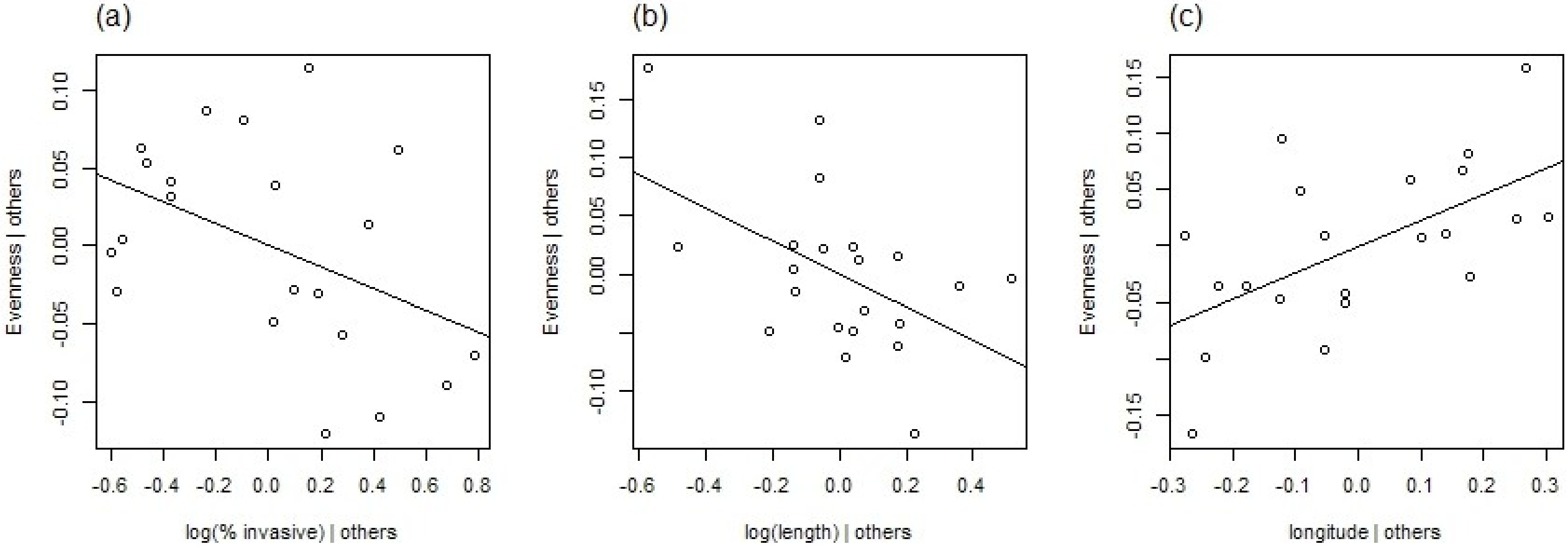

Figure 2-6. Partial regression plots from linear model of native macrophyte evenness against cover of: (a) *Myriophyllum spicatum*, (b) mean littoral length and (c) longitude in 21 lakes after accounting for the other two variables (Table 2-6).

Chapter 3. Effects of the invasive Eurasian watermilfoil (*Myriophyllum spicatum* L.) on native macrophyte communities in lakes and macrophyte performance in mesocosms

Abstract

Aim To study the relationship between macrophyte community composition and *Myriophyllum spicatum* L. cover in lakes at small (1 quadrat) and large (18 quadrats) scales and in greenhouse mesocosms. It was hypothesized that competition by *M. spicatum* on native species would differ among species, leading to separate macrophyte communities.

Location Lakes of southeastern Ontario, Canada and greenhouse at Environment Canada.

Methods Submerged and floating macrophyte communities were surveyed in 1 m^2 quadrats across 21 lakes. The relationship between native community composition and cover of *M. spicatum* was studied using ordination methods, clustering and indicator species analysis. In the greenhouse *M. spicatum*, and three native species were grown together in 67 L mesocosms. The competitive effect of native species on *M. spicatum* dry weight was studied using linear models.

Result *M. spicatum* was a significant predictor of community composition at the lake scale and was isolated from other species at the quadrat scale. In mesocosms competition by native species with *M. spicatum* was not significantly different among species; however, the trend was similar to the association between *M. spicatum* and species in lake quadrats.

Main conclusions The results from the mesocosms do not support the hypothesis that competition by *M. spicatum* leads to different macrophyte communities despite the isolation of *M. spicatum* at small scales in lakes. At the lake scale, where competition has less influence, the relationship between community composition and *M. spicatum* cover suggests similar habitat preferences between *M. spicatum* and some native species.

Introduction

Biodiversity patterns are known to differ with spatial scale (Sandel & Smith, 2009), in particular the relationship between native and exotic plant richness (Fridley et al., 2007). On the other hand, relationships between macrophyte community composition and invasive species show variable patterns across scales. For instance, at small scales competition was not an important driver of macrophyte community composition in a field exclusion experiment (Chambers & Prepas, 1990), but appeared to be important in a long term study (Mjelde et al., 2012). Studies comparing invaded and non-invaded quadrats have found both significant (Michelan et al., 2010; Stiers et al., 2011) and non-significant (Lyon & Eastman, 2006; Stiers et al., 2011) differences in community composition. At lake scales, the presence of invasive macrophyte species has been a significant predictor of community composition (Mikulyuk et al., 2011; O'Hare et al., 2012); however, a comparison of communities across a range of invasive cover was not tested.

The purpose of the study in this chapter was to compare patterns of native macrophyte community composition against the variation in cover of the invasive submerged macrophyte *Myriophyllum spicatum* L., cover of charophytes (macroscopic filamentous algae) and the presence of zebra mussels (*Dreissena polymorpha* Pallas), in the field at small and large scales. To compliment the field study and test whether patterns of species composition in lakes can be explained by competition from *M. spicatum* a subset of native species were grown in greenhouse mesocosms with *M. spicatum*.

The study lakes were chosen from the approximately 65 lakes of the Rideau Valley Watershed of eastern Ontario (Department of Energy and Resources Management, 1968). The majority of lakes are found within two subwatersheds at the headwaters of the Rideau

Valley Watershed, the Tay River Subwatershed and the Rideau Lakes Subwatershed. The Rideau Lakes Subwatershed contains the Rideau Canal, which connects Lake Ontario to the Ottawa River (Department of Energy and Resources Management, 1968). This subwatershed can be further divided into lakes that contain the canal and lakes not directly connected to the canal. Additionally, within the watershed two major biological gradients of interest are present. Zebra mussels have become established in half of the lakes (Sarah MacLeod, Rideau Valley Conservation Authority, *pers. comm.*) and have been associated elsewhere with changes in macrophyte communities from turbidity tolerant species to turbidity intolerant species (Leisti et al., 2012). Charophytes vary in cover across the lakes from absent to comprising large beds (*pers. obs.*) and have been found to be inversely related to invasive macrophyte colonization in oligotrophic New Zealand lakes (de Winton et al., 2012).

Methods

Lake communities

Macrophyte communities were studied using the same dataset as Chapter 2. The collection of lake data and treatment of variables is described in Chapter 2: Methods. An additional variable was used to describe the macrophyte communities: the wetland macrophyte index (WMI). This index assigns macrophyte communities a score from one (poor habitat) to five (good habitat) according to the tolerance of disturbance and habitat range of macrophyte species in the community (Croft & Chow-Fraser, 2007).

Detrended Correspondence Analysis and variable scaling

The statistical program R version 2.15.1 (R Core Team, 2012) and the package vegan (Oksanen et al., 2012) were used to study macrophyte community composition in lakes. Differences in community composition at the quadrat and lake scale were studied using detrended correspondence analysis (DCA) of the relative cover of all species present in greater than 5% of quadrats or lakes, respectively. Although the numbers of species and lakes were similar, ordination methods based on correspondence analysis are suitable for any ratio of species to sites (ter Braak & Verdonschot, 1995). Correlations between quadrat site scores and water depth were computed using the function 'ordisurf' and fit onto the DCA ordination as a generalized additive model. A *p*-value was not computed using the generalized additive model as its validity has not been formally established (Simpson, 2011). Correlations between lake site scores and individual variables (zebra mussel presence/absence, subwatershed identity, chlorophyll-*a*, total phosphorus, conductivity, littoral length, lake surface area, max depth of plant colonization, Secchi depth, latitude and longitude) were computed using the function ‘envfit’ and fit onto the DCA ordination as vectors (continuous variables) or centroids (nominal variables).

Cluster Analysis

Because lakes appeared clustered in the DCA ordination, separate agglomerative hierarchical clustering of lakes and quadrats was also performed. Clustering was performed using Bray-Curtis dissimilarity (Bray & Curtis, 1957) of the relative cover of species in greater than 5% of lakes or quadrats, respectively. Groups were merged using the average linkage method. The final number of groups was chosen as the group number that minimized the mean *p*-values of species indicator values computed from indicator species analysis (McCune & Grace, 2002). Indicator species analysis was also used to assess the importance of individual species in defining each group (Dufrene & Legendre, 1997). The method assigns species to the group where they have the highest product of proportional abundance and relative frequency of occurrence, termed an indicator value (*IV)*. The significance of this value was tested by comparing indicator values to the distribution obtained by randomizing lake groups 1000 times (McCune & Grace, 2002).

Partial Constrained Correspondence Analysis

The effect of *M. spicatum*, charophytes and zebra mussels on lake community composition after accounting for lake habitat gradients (PC1-3) and subwatershed was investigated using partial constrained correspondence analysis (pCCA) with the same species * site data from DCA analysis excluding invasive species and charophytes. The significance of both variables and axes was tested using an ANOVA like permutation test (Legendre et al., 2011).

Greenhouse experiment

Plant collection and propagation

Plant material was collected in the summer of 2012 from Westport Sand Lake (44.679883°N, 76.428294°W) and consisted of charophytes, *Elodea canadensis* Michx., *M. spicatum*, *Potamogeton illinoensis* Morong and *Ranunculus aquatilis* L.. Charophyte and *R. aquatilis* cuttings failed to propagate, so plant material of *Ceratophyllum demersum* L. and *Vallisneria americana* Michx. was purchased from PetsMart during the winter as both species were also present in the study system (original collection site unknown). Stems from all species were grown in a greenhouse during the fall and winter. Stems were grown in pots containing various mixtures of potting soil, sand and solid fertilizer and grown submersed in 38 L plastic containers (25 cm high * 31 cm wide * 38 cm long) with CO_2 enrichment until beginning the experiment.

Experimental setup

Lake mesocosms were simulated at the National Wildlife Research Centre greenhouse of Environment Canada for 87 days between Feb 4th, 2013 and May 1st, 2013 using 16 opaque 67 L tubs (42 cm diameter * 41 cm high) sterilized with 5% bleach. A sediment layer for each tub was created using five 0.8 L pots (10 cm high * 8.8 cm wide * 8.8 cm long) filled with a 1:1 mixture by volume of sand and potting soil and 0.42 g of solid fertilizer (6-12-6, Miracle-Gro Houseplant Food Spikes). Tanks were filled with tap water and allowed to settle for five days before adding plants. In each tank, 10 cm apical cuttings of *E. canadensis*, *P. illinoensis*, *C. demersum* and *M. spicatum*, and a similarly sized rosette of *V. americana* were planted into individual pots. One additional 10 cm apical cutting of *M. spicatum* was also planted into each pot with native species. Tanks were individually enriched with CO_2 throughout the experiment using yeast cultures (5 mL yeast, 250 ml sugar and 1.5 L water in a sealed 2 L bottle with air-line tubing attached to an air-stone; www.thekrib.com). *C. demersum* floated out of its pots early in the experiment leaving *M. spicatum* stems without a competitor, so those pots were excluded. At the end of the experiment, all above ground plant material was collected, separated by species and pot, dried and weighed.

Maintenance

Partial water changes were performed weekly (7 L removed and tap water added to fill the tank). Fresh yeast cultures were established bi-weekly (www.thekrib.com). To account for differences in CO_2 enrichment between tanks, yeast cultures were moved between tanks every 3-4 days. Algae were skimmed from the surface of the tanks as needed. To check on plants not visible due to turbidity and slow plant growth, a 16 L water change was performed on day 32 instead of the normal 7 L water change.

Tank monitoring

To document water conditions across tanks during the experiment, water temperature and pH were measured weekly using a Model IQ150 handheld pH/mV/temperature meter (Spectrum Technologies, Inc.) in all tanks during the afternoon prior to water changes. Due to increasing turbidity, Secchi tube depth was measured weekly beginning on day 33 as the depth at which a Secchi disk disappeared in a glass graduated cylinder (4.7 cm diameter) filled with tank water. Carbonate hardness and concentration of phosphate was measured weekly in all tanks using aquarium test kits (API Aquarium Pharmaceuticals, Mars Fishcare). Light intensity directly above tanks was measured during an overcast day, a sunny day and a day with moderate clouds using a LI-6400XT Portable Photosynthesis System (LI-COR).

Statistical analysis

To compare the relative competitive performance, *CP*, of macrophytes in the tank mesocosms, the percent change in *M. spicatum* dry weight between *M. spicatum* control pots and pots with competitors was computed as:

$$CP_{ij} = (P_{0j} - P_{ij}) / P_{0j}$$

where CP_{ij} is the relative competitive performance of species *i* in the j^{th} tank, P_{0j} is the dry weight of *M. spicatum* alone of the j^{th} tank, and P_{ij} is the dry weight of *M.* spicatum grown with species *i* in the j^{th} tank (Gaudet & Keddy, 1995). *M. spicatum* failed to establish in control pots of three tanks, and those tanks were excluded from calculations of *CP*. The difference in mean *CP* of competitors was tested using analysis of variance.

To examine the relationship between the dry weight of competitor species and *M. spicatum* when grown together a linear model was tested for competitor dry weight vs. *M. spicatum* dry weight blocked by species. The association between individual competitor species and *M. spicatum* in lake quadrats was tested using Chi-square tests with Yate's continuity correction for the number of quadrats where the two species were present/absent.

Results

Community composition

At the quadrat scale, detrended correspondence analysis of macrophyte communities separated species along a depth gradient (adjusted $R^2 = 0.28$), with deep quadrats at the left of the ordination and two groups of shallow quadrats along the right of the ordination. *M. spicatum* was situated near the deepest quadrats, with a clear separation from other species. Charophytes were situated at the opposite end of the ordination between the two groups of shallow quadrats (Figure 3-1). Clustering of quadrats represented the original dissimilarity matrix well (Cophenetic $r = 0.90$) and quadrats were optimally clustered in 21 groups. Each group had only one significant indicator species ($p < 0.05$), with the exception of a group containing both *Potamogeton amplifolius* Tuck. and *Bidens beckii* Torr. ex Spreng. On the other hand, clustering quadrats into only three groups separated charophytes ($IV = 0.99$, $p = 0.001$), then *M. spicatum* ($IV = 0.98$, $p = 0.001$) from the majority of other macrophytes. The charophyte group also contained *P. illinoensis* ($IV = 0.33$, $p = 0.001$) and *Utricularia vulgaris* L. ($IV = 0.10$, $p = 0.014$), while the *M. spicatum* group contained *C. demersum* ($IV = 0.28$, $p = 0.032$).

At the lake scale, seven variables showed significant correlations with lake scores from detrended correspondence analysis (DCA) of macrophyte communities: conductivity, WMI, littoral length, subwatershed, longitude, max depth of plant colonization and zebra mussel presence/absence (Table 3-1). Macrophyte communities were separated along the first DCA axis according to subwatershed, with communities in the Rideau Lakes subwatershed having higher conductivity and containing zebra mussels. Macrophyte communities were separated along the second DCA axis according WMI scores, with higher WMI scores in shallow communities with short littoral regions (Figure 3-2).

Agglomerative hierarchical cluster analysis of lakes using macrophyte communities organized lakes into three main groups (Figure S-15). The groups separated lakes according to their presence in: (1) the Tay River Subwatershed, (2) the Rideau Lakes Subwatershed within the Rideau Canal, and (3) the Rideau Lakes Subwatershed outside of the Rideau Canal (Fisher's exact test for count data, $N = 21$, $p = 0.002$). Indicator species analysis identified several species associated with a particular lake cluster (Table 3-2). Lakes in the Tay River Subwatershed (group one) were more likely to have large-leaved macrophytes, like *Potamogeton robbinsii* Oakes, *P. amplifolius* and *Nuphar variegata* Durand. Lakes connected to the Rideau Canal (group two) were more likely to have small floating, or submerged floating species, like *Lemna trisulca* L., *Spirodela polyrhiza* (L.) Schleid. or *C. demersum*. Lastly, lakes in the Rideau Lakes Subwatershed not strongly connected to the Rideau Canal (group three) were more likely to have hard-water plants, like charophytes and *P. illinoensis*. *M. spicatum* was not strongly associated with any group of lakes (Figure S-16).

After accounting for habitat gradients (PC1-3) and subwatershed, the cover of *M. spicatum* was a significant predictor of lake community composition in partial constrained correspondence analysis (pCCA) (pseudo $F(1, 13) = 1.80$, $p = 0.032$, Figure 3-3). Neither charophyte cover nor zebra mussel presence/absence showed significant effects ($p > 0.05$). The first axis was significant (pseudo $F(1, 13) = 1.83$, $p = 0.024$), had an eigenvalue of 0.17 and explained 11% of the variation in community composition. The second and third constrained axes were not significant ($p > 0.05$).

Greenhouse experiment

The average tank condition was turbid, with slightly acidic pH, room temperature water and low light intensity (Table 3-3). While early propagation tanks experienced algae blooms and phosphate concentrations as high as 2,000 – 5,000 ug/L, competition tanks did not have visible algae layers after the first two weeks and phosphate concentrations were always below the detectable limit of the test kit (250 ug/L).

Species biomass was variable across tanks (Figure 3-4) and a linear model of competitor biomass against *M. spicatum* biomass blocked by species found that competitor biomass was negatively related to *M. spicatum* biomass ($F(1, 44) = 16.56$, $p < 0.001$) and that *V. americana* maintained a greater biomass than either *E. canadensis* or *P. illinoensis* ($F(2, 44) = 26.46$, $p < 0.001$). The full model explained half of the variation in competitor biomass ($R^2 = 0.59$, adjusted $R^2 = 0.56$, $F(3, 44) = 21.27$, $p < 0.001$) and residuals met assumptions of normality and homoscedasticity (Figure S-17).

Chi-square tests with Yates' continuity correction of the presence and absence of competitor species in lake quadrats found that *M. spicatum* was positively associated with *E. canadensis* (odds ratio = 2.55, $c^2(1, N = 375) = 17.58$, $p < 0.001$), not significantly associated with *V. americana* (odds ratio = 1.00, $c^2(1, N = 375) = 0.00$, $p = 1.000$) and negatively associated with *P. illinoensis* (odds ratio = 0.40, $c^2(1, N = 375) = 7.84$, $p = 0.005$). Mean competitive performance of species against *M. spicatum* in tank mesocosms declined from 0.45 ± 0.14 (*SE*) for *E. canadensis*, to 0.14 ± 0.15 (*SE*) for *V. americana* and 0.08 ± 0.12 (*SE*) for *P. illinoensis* (Figure 3-5), but was not significantly different between species ($F(2, 36) = 2.11$, $p = 0.137$).

Discussion

At the quadrat scale the large separation of *M. spicatum* from other species in deep water suggests a negative association between *M. spicatum* and most other deep water macrophytes. Clustering of quadrats into 3 groups resulted in a significant indicator value for only *C. demersum* in the *M. spicatum* group of quadrats; however, this disappeared when quadrats were clustered at their optimum level (21 groups). *C. demersum* is another deep water species that has been observed with *M. spicatum* in other studies as well (Bosch et al., 2009; Olson et al., 2012). The results differ from observations in Great Lakes wetlands where *M. spicatum* coexisted with neighboring species, although the minimum sampling area in that study was 100 m long (Trebitz & Taylor, 2007). On the other hand, the results are similar to a long-term study of macrophyte distributions in a Norwegian lake where *E. canadensis* became dominant at 3-4 m depths (the centroid for *M. spicatum* was at 3.2 m in DCA for this study) and either displaced sub-dominant species to deeper water or reduced their abundance (Mjelde et al., 2012). Other studies in streams, wetlands or shallow lakes have also found changes in communities in the presence of dominant species (Michelan et al., 2010; Stiers et al., 2011; Bassett et al., 2012).

The mesocosm experiment found significant declines in native biomass as *M. spicatum* grew more abundantly, suggesting that competition for resources occurred among the macrophytes studied. The competitive ability of species grown with *M. spicatum* followed the same trend as their association with *M. spicatum* in quadrats. *E. canadensis*, the most competitive of the three species, has been positively associated with *M. spicatum* in other studies as well (Croft & Chow-Fraser, 2007; Olson et al., 2012). Despite the observed trend between competitive ability and association with *M. spicatum* in quadrats, no significant difference was found in competitive ability among the three native species. The mesocosm results are therefore not sufficient to reject claims that competition does not structure submerged macrophyte communities (Chambers & Prepas, 1990). *M. spicatum* may instead be isolated at deeper quadrats due to a greater tolerance for mechanical disturbance (such as wave action) and low light than other species have (Olson et al., 2012; Zhu et al., 2012).

At the lake scale *M. spicatum* was not a significant indicator of lake groups using indicator species analysis, suggesting that *M. spicatum* is not restricted to particular lake types within the watershed. However, *M. spicatum* became a significant predictor of community composition after accounting for habitat gradients and subwatershed in partial CCA. This suggests that while habitat effects are stronger in structuring macrophyte communities there is also a secondary effect of *M. spicatum*. This supports other studies that also found invasive submerged species to be important predictors of community composition at the lake scale (Mikulyuk et al., 2011; O'Hare et al., 2012).

Different macrophyte communities were observed in deep and shallow quadrats, in agreement with the general observation of changes in macrophyte communities with depth (Sculthorpe, 1967). The ordination of deep water quadrats along a single gradient and shallow water quadrats towards two points suggests that deep water habitat is more uniform than shallow water habitat. In deep water light availability is likely the limiting habitat variable, while in shallow water light is more plentiful and other factors, such as substrate, trophic status and disturbance can all influence macrophyte community composition (Lacoul & Freedman, 2006).

While zebra mussels were correlated with lakes scores in DCA, zebra mussels ceased to be an important predictor of community composition after accounting for subwatershed and habitat gradients in partial CCA. Community composition after zebra mussel invasion was found to shift towards turbidity intolerant species in a shallow bay (87% < 5 m deep), but not in deeper bays with smaller littoral regions (22 – 39% < 5 m deep) (Leisti et al., 2012). Although the entire littoral area of lakes in the current study was not measured, lake morphometry appeared more similar to the deeper bays studied in Leisti et al. (2012) than the shallow bay. The lakes in this study may therefore have been too deep for improvement in habitat by zebra mussels to be more important than other factors affecting community composition.

An important factor affecting community composition was the geographic distribution of lakes, which was associated with differences in community composition using both DCA and cluster analysis. Subwatershed and longitude were correlated with lake DCA scores and clustering of lakes by species composition separated lakes into three geographic areas. This agrees with the results of other studies, where spatial variables were found to be important predictors of community composition (Capers et al., 2010; O'Hare et al., 2012; but see Mikulyuk et al., 2011). In this case, the significant indicator species from each lake group suggest different habitat conditions among the lakes, and not dispersal limitation, which was hypothesized in chapter two. The submerged floating species *C. demersum* and *L. trisulca*, and the small floating species *S. polyrhiza* were significant indicators of lakes in the Rideau Canal, which experiences frequent boat traffic and wave disturbance. Conversely, large-leaved species (which are sensitive to physical disturbance) like *P. robbinsii, P. amplifolius* and *N. variegata* were significant indicators of lakes in the

Tay River Subwatershed, which does not contain the same level of boat traffic as the Rideau Canal. Additionally, the increase in lake WMI scores towards lakes in the Tay River Subwatershed also suggests macrophyte habitat with less disturbance in this region. Lastly, species associated with hard water, like charophytes and *P. illinoensis* were significant indicators of non-canal lakes in the Rideau Lakes Subwatershed, which have greater concentrations of calcium than lakes in the Tay River Subwatershed (21 – 35 mg/L *vs.* 10 – 20 mg/L, respectively; www.rvca.ca).

The correlation of lake variables with site scores from DCA identified two gradients separating macrophyte communities. The first gradient had a high correlation with conductivity and separated high conductivity lakes containing zebra mussels in the Rideau Lakes Subwatershed from low conductivity lakes without zebra mussels in the Tay River Subwatershed. The second gradient had a high correlation with lake WMI scores and separated better macrophyte habitat lakes with short, shallow littoral zones from poorer macrophyte habitat lakes with longer, deeper littoral zones. Other macrophyte studies have identified similar gradients of conductivity, disturbance, water clarity and habitat area (Toivonen & Huttunen, 1995; Capers et al., 2010; Mikulyuk et al., 2011; O'Hare et al., 2012) and support the observed distribution of macrophyte communities in this study.

Summary

The results of this study suggest that the overall pattern of macrophyte community composition is driven by habitat and lake location. However, after accounting for macrophyte habitat, *M. spicatum*, but not charophytes or zebra mussels, showed a significant effect on the remaining variation in macrophyte community composition at the lake scale. It remains unclear if the relationship between *M. spicatum* and macrophyte communities is due to competition with other species or a greater tolerance for wave disturbance and low light in deeper water.

From a conservation perspective, the protection of a variety of macrophyte communities in the study region would best be accomplished by recognizing the different habitat requirements of communities in each subwatershed. The lack of association between *M. spicatum* and a particular lake group suggests that *M. spicatum* has the potential to colonize deep water regions in additional lakes across the watershed.

Tables

Table 3-1. Correlation of lake variables with lake scores from detrended correspondence analysis of macrophyte communities. Significant correlations indicated by *.

Variable	R^2	p
conductivity	0.60	0.001 *
Wetland Macrophyte Index (WMI)	0.53	0.002 *
$\log_{10}$(littoral length)	0.42	0.007 *
subwatershed	0.38	0.001 *
longitude	0.35	0.021 *
maximum plant depth	0.33	0.034 *
zebra mussel presence	0.25	0.011 *
$\log_{10}$(littoral total phosphorus)	0.23	0.102
$\log_{10}$(lake surface area)	0.09	0.431
$\log_{10}$(chlorophyll-*a*)	0.05	0.642
latitude	0.04	0.700
Secchi depth	0.01	0.942

Table 3-2. Results of indicator species analysis to identify macrophytes associated with a single group of lakes. Group one mostly in Tay River Subwatershed. Group two mostly part of Rideau Canal. Group three mostly in Rideau Lakes Subwatershed apart from the canal. Significant ($p < 0.05$) indicator values (*IV*) denoted with *. Invasive species in bold.

Group	Species	Code	*IV*	*p*
1	*Bidens beckii* Torr. ex Spreng.	BIDBECK	0.37	0.366
	Brasenia schreberi J.F. Gmel.	BRASCHR	0.44	0.081
	Heteranthera dubia (Jacq.) MacMill.	HETDUBI	0.49	0.488
	Hydrocharis morsus-ranae **L.**	HYDMORS	0.26	0.611
	Myriophyllum spicatum **L.**	MYRSPIC	0.48	0.273
	Nuphar variegata Durand	NUPVARI	0.67	0.048 *
	Potamogeton amplifolius Tuck.	POTAMPL	0.55	0.036 *
	Potamogeton friesii Rupr.	POTFRIE	0.34	0.903
	Potamogeton perfoliatus L.	POTPERF	0.56	0.054
	Potamogeton pusillus L.	POTPUSI	0.89	0.025 *
	Potamogeton robbinsii Oakes	POTROBB	0.81	0.001*
	Utricularia vulgaris L.	UTRVULG	0.15	0.905
2	*Ceratophyllum demersum* L.	CERDEME	0.75	0.013 *
	Elodea canadensis Michx.	ELOCANA	0.61	0.067
	Lemna minor L.	LEMMINO	0.21	0.390
	Lemna trisulca L.	LEMTRIS	0.91	0.001 *
	Potamogeton richardsonii (A. Benn.) Rydb.	POTRICH	0.18	0.836
	Potamogeton zosteriformis Fernald	POTZOST	0.71	0.093
	Ranunculus aquatilis L.	RANAQUA	0.83	0.004 *
	Spirodela polyrhiza (L.) Schleid.	SPIPOLY	0.88	0.011 *
	Vallisneria americana Michx.	VALAMER	0.62	0.104
	Wolffia sp. Horkel ex Schleid.	WOLSP	0.07	1.000
3	*Chara* spp. L.	CHASP	0.85	0.001 *
	Myriophyllum sibiricum Kom.	MYRSIBI	0.62	0.303
	Najas flexilis (Willd.) Rostk. and W.L.E. Schmidt	NAJFLEX	0.44	0.885
	Nymphaea odorata Aiton	NYMODOR	0.59	0.086
	Potamogeton gramineus L.	POTGRAM	0.43	0.434
	Potamogeton illinoensis Morong	POTILLI	0.77	0.002 *
	Potamogeton natans L.	POTNATA	0.45	0.151
	Sagittaria cuneata E. Sheld.	SAGCUNE	0.15	0.661
	Stuckenia pectinata (L.) Börner	STUPECT	0.41	0.821

Table 3-3. Summary of tank water parameters: pH, temperature, Secchi tube depth, carbonate hardness, surface photosynthetically active radiation (PAR). Min, median and max values are from all tanks and measurement days. Mean and standard error values are from the mean of each tank (*N* = 16).

	Units	Min	Median	Max	Mean	SE
pH	*	5.90	6.90	8.40	6.87	0.02
Water temperature	^{o}C	19.9	25.0	35.0	24.9	0.1
Secchi tube	m	0.12	0.21	0.31	0.21	0.01
Carbonate hardness	ppm	54	107	161	113	1
Surface PAR	μMol*m^{-2*}s^{-1}	127	300	1003	334	18

Figures

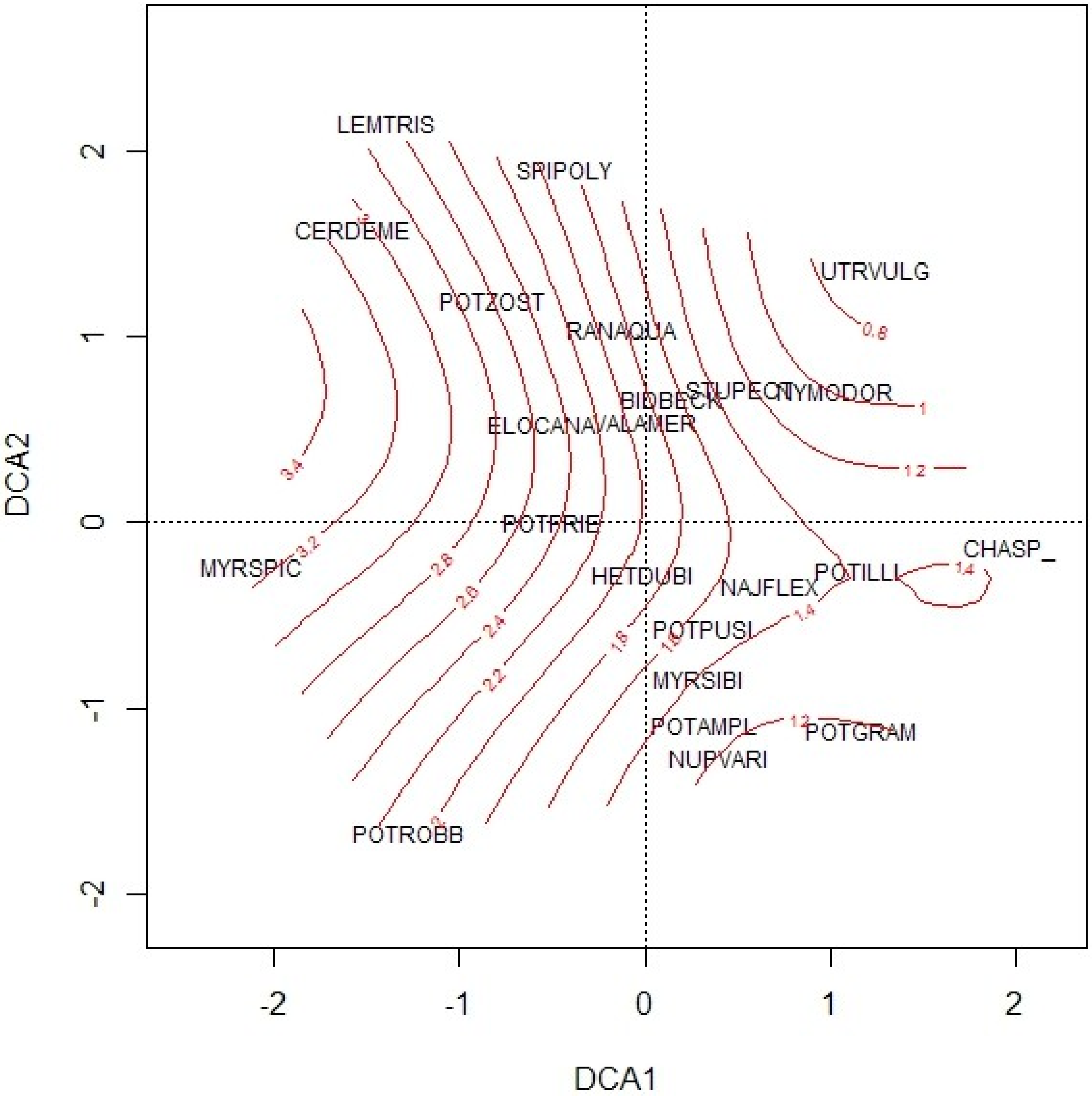

Figure 3-1. Ordination plot from detrended correspondence analysis (DCA) for relative cover of the 23 macrophyte species present in > 5% of 375 quadrats from 21 lakes (quadrats not shown). Red curves represent depth gradient across quadrats (adjusted R^2 = 0.28). Eigenvalues for first two axes = 0.80, 0.68. Decorana values for first two axes = 0.82, 0.63. Axis lengths for first two axes = 4.08, 3.71. Species codes listed in Table 3-2.

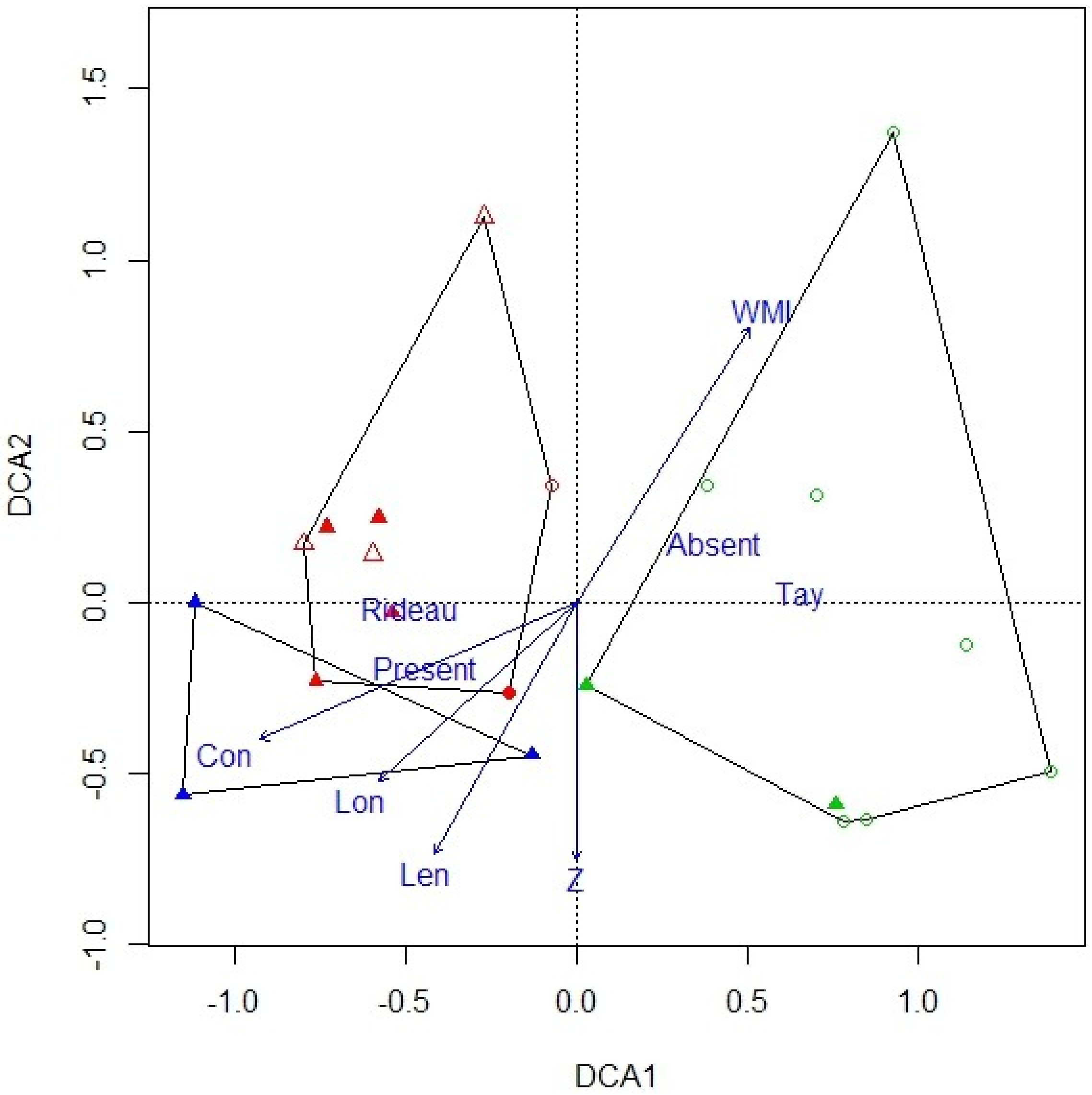

Figure 3-2. Ordination plot from detrended correspondence analysis (DCA) for relative cover of 31 macrophyte species present in > 5% of 21 lakes (species not shown). Decorana values for first two axes = 0.46, 0.21; eigenvalues = 0.46, 0.29; axis lengths = 2.54, 2.02. Blue vectors and centroids indicate the magnitude and direction of significant correlations between individual environmental variables and site scores: *Con*, conductivity; *Len*, littoral length; *Lon*, longitude; *Present/Absent*, zebra mussels present/absent; *Rideau/Tay*, Rideau Lakes/Tay River Subwatersheds; *WMI*, lake wetland macrophyte index; *Z*, max plant depth. Symbols include: *circles*, Tay River subwatershed; *triangles*, Rideau Lakes subwatershed; *clear symbols*, zebra mussels absent; *filled symbols*, zebra mussels present. Grouping of lakes according to cluster analysis: *blue (bottom left)*, lakes mainly in Rideau Lakes Subwatershed within Rideau Canal; *red (top left)*, lakes mainly in Rideau Lakes Subwatershed outside of Rideau Canal; *green (right)*, lakes mainly in Tay River Subwatershed.

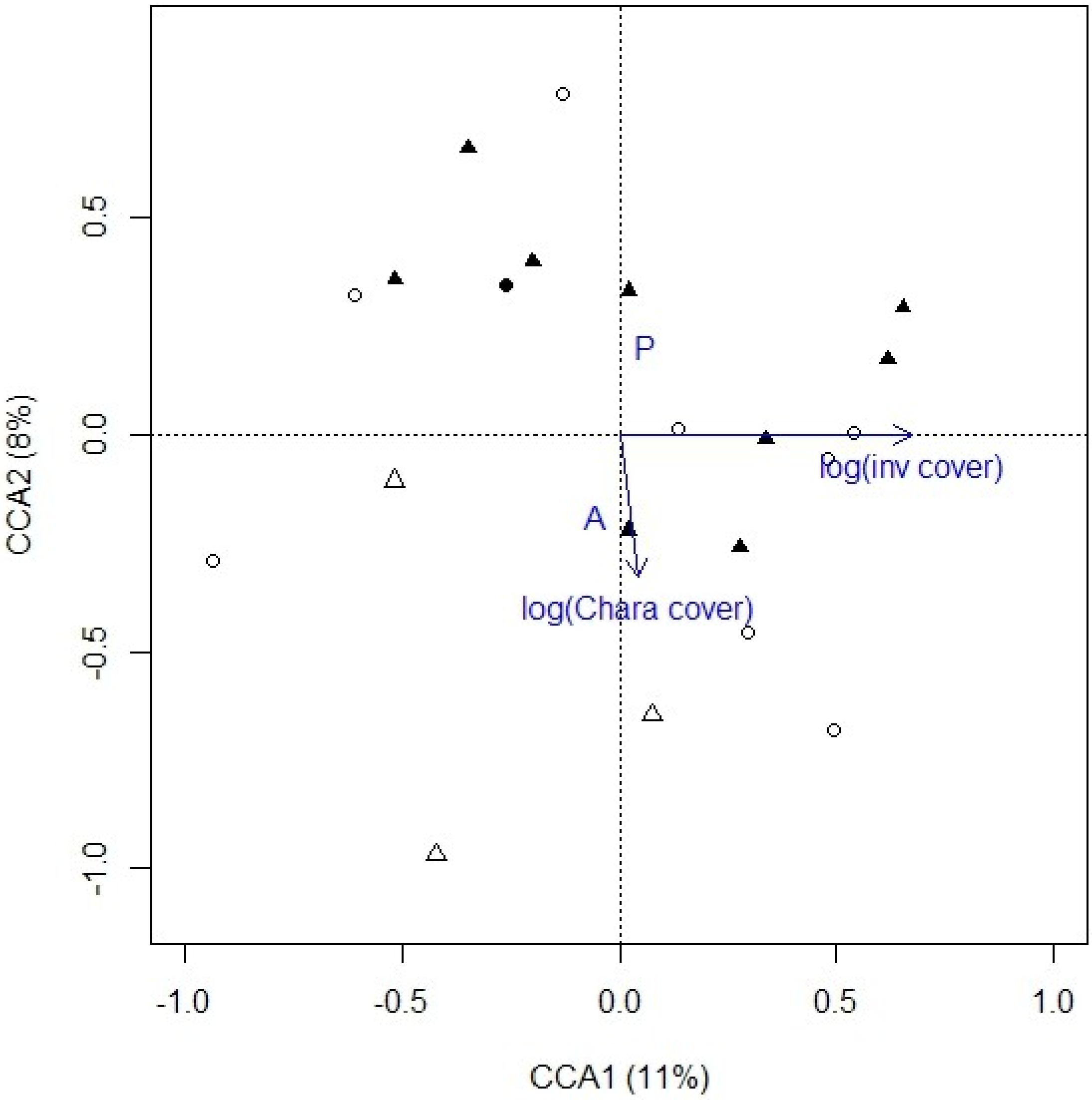

Figure 3-3. Ordination plot from partial constrained correspondence analysis (pCCA) for relative cover of 28 native macrophyte species present in > 5% of 21 lakes (species not shown) showing the response scores for the first two constrained axes with scaling by sites. Constraining variables are displayed as vectors for $\log_{10}$(*Myriophyllum spicatum* cover), *log(inv cover)*; and $\log_{10}$(charophyte cover), *log(Chara cover)*; and factors for zebra mussel presence/absence, *P/A*; according to biplot scores for the first two constrained axes. Covariables (not shown) are PC 1-3 and subwatershed. Percent values along axes represent % of variance in response scores explained by that axis. Eigenvalues = 0.17 (CCA1), 0.13 (CCA2). Proportion of constrained eigenvalues = 0.41 (CCA1), 0.31 (CCA2). *Circles*, lakes in Tay River Subwatershed; *triangles,* lakes in Rideau Lakes Subwatershed; *clear symbols*, lakes where zebra mussels absent; *filled symbols,* lakes where zebra mussels present.

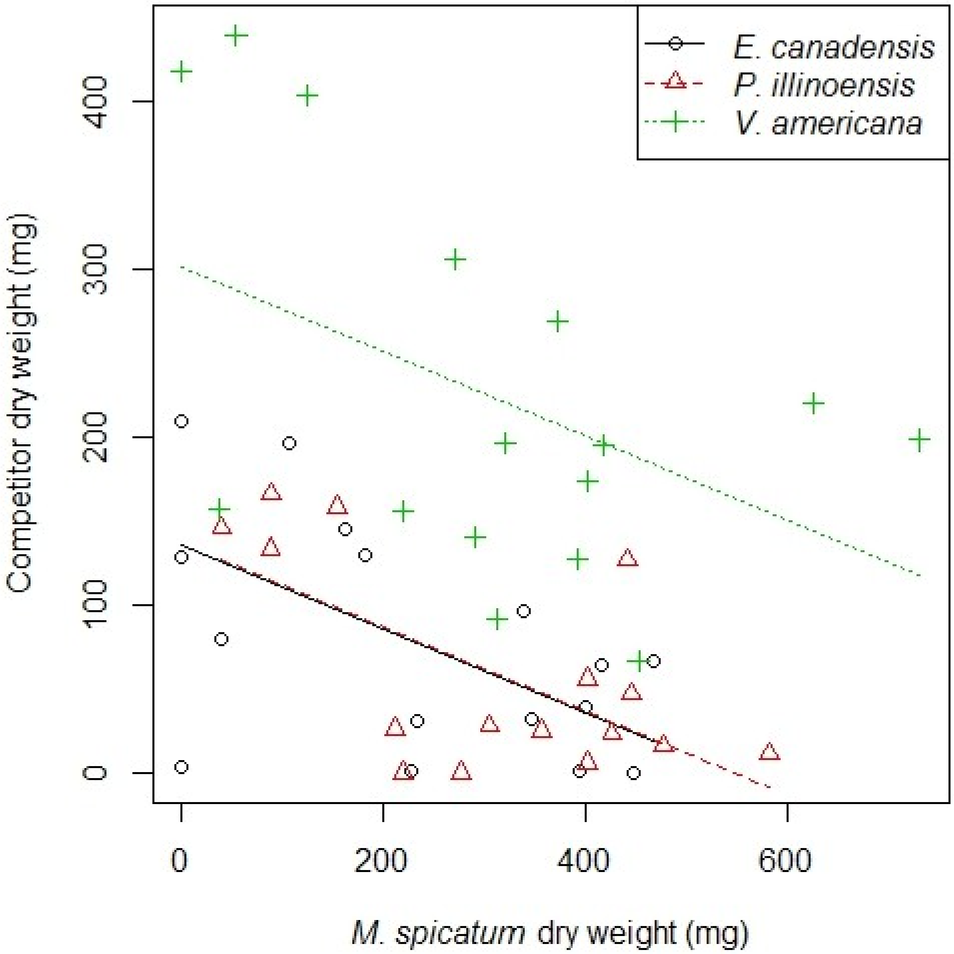

Figure 3-4. Plot of competitor species dry weight (*Elodea canadensis, Potamogeton illinoensis* or *Vallisneria americana*) vs. *Myriophyllum spicatum* dry weight when grown together in tank mesocosms ($N = 48$).

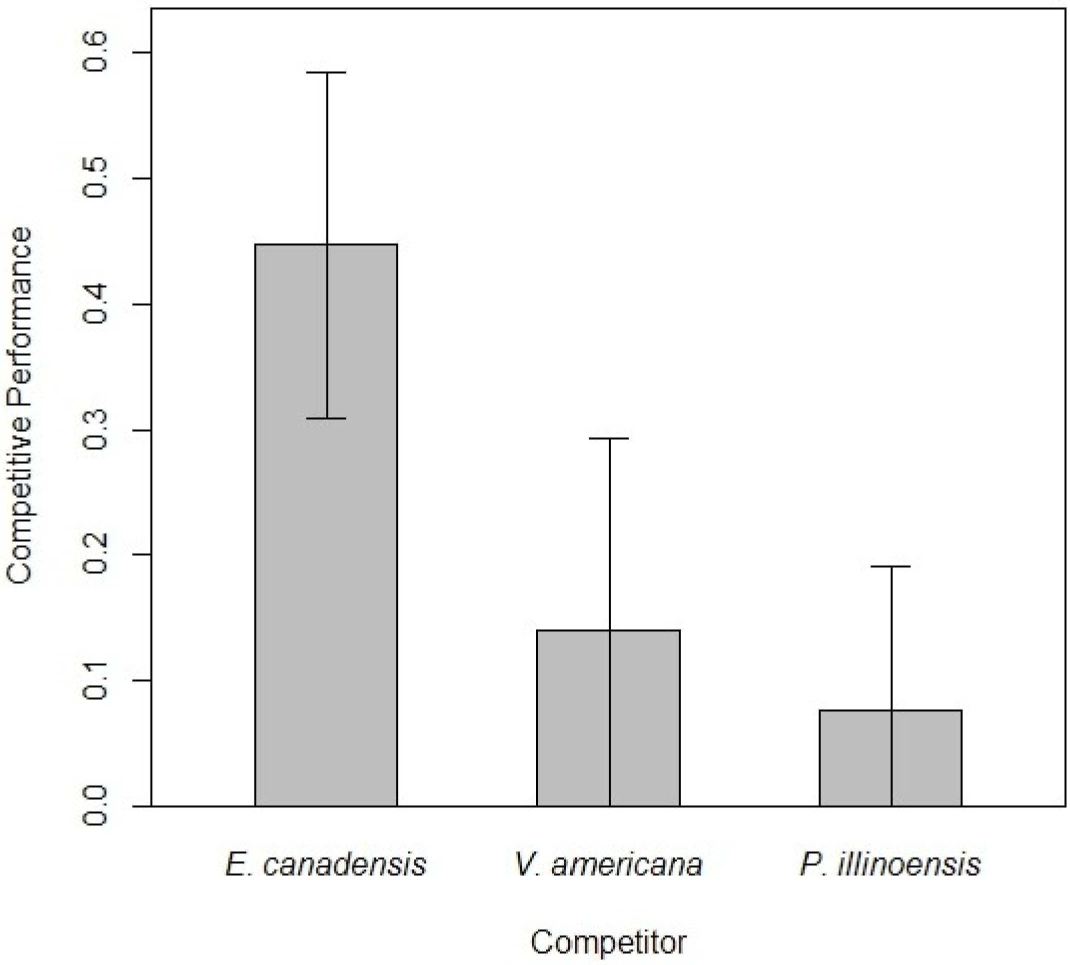

Figure 3-5. Bar plot of mean ± standard error of relative competitive performance of different native species growing with *Myriophyllum spicatum*. Competitors are: *Elodea canadensis* (*n* = 13), *Vallisneria americana* (*n* = 13) and *Potamogeton illinoensis* (*n* = 13). Means are not significantly different ($p > 0.05$).

Chapter 4. General conclusion

This study examined the relationship between the invasive macrophyte *Myriophyllum spicatum* L. and macrophyte diversity at different spatial scales after accounting for habitat gradients.

Previous research on invasive macrophytes has mainly focused on the presence/absence of invaders and native species, with the finding of negative relationships between invaders and richness at small scales, and positive or no relationships at larger scales. The purpose of this study was to include measures of macrophyte cover to more accurately describe the degree of invasion in lakes and its effect on native macrophyte diversity in the form of evenness and community composition, in addition to species richness. It was hypothesized that a higher cover of *M. spicatum* would lead to greater competition with native macrophytes and result in increased macrophyte evenness, different community composition and reduced species richness. The study was performed by surveying macrophyte communities in the littoral regions of 21 lakes in the Rideau Valley Watershed and performing a small competition experiment in mesocosms between *M. spicatum* and native macrophytes.

Chapter two examined the effect of *M. spicatum* on native macrophyte richness and evenness in lakes after accounting for lake habitat. Contrary to expectations, native macrophyte evenness declined in lakes with greater cover of *M. spicatum*; however there was no relationship at the quadrat scale. On the other hand, *M. spicatum* was not related to native richness at either scale. The significance of this research is to present evidence that invasive macrophyte effects on native macrophyte communities can be detected at the level of species evenness, but not species richness. Furthermore, lakes with the highest cover of

M. spicatum had the lowest evenness, suggesting that *M. spicatum* is growing in lakes with other dominant native species and not replacing them.

Chapter three examined the composition of macrophyte communities in the Rideau Valley Watershed with respect to *M. spicatum*. Furthermore, the association between individual native macrophtyes and *M. spicatum* in quadrats was compared to the competitive ability of those species against *M. spicatum* in mesocosms. While the main drivers of macrophyte community composition in lakes were due to environmental and spatial effects, *M. spicatum* was a significant predictor of the remaining variation in community composition. Within lakes, *M. spicatum* was found apart from other species in the deepest quadrats. It was unclear if this isolation was due to competition or a tolerance for more adverse habitat conditions (e.g. low light, wave disturbance). While there was a trend in greenhouse mesocosms for greater competitive effects by species that were more strongly associated with *M. spicatum* in the field, the competitive effects were not significantly different between species. The significance of these results is to present preliminary trends for an effect of competition by *M. spicatum* on native macrophyte communities; however, a greater number of species need to be grown in mesocosms and compared to field conditions to adequately test this relationship.

Further research could add to this study in several ways. The study involved a deep water invasive macrophyte (*M. spicatum*) in low nutrient, basic lakes. The inclusion of lakes containing shallow water invasive species and a greater range of nutrients and pH levels would test the generality of this study’s findings. Second, another survey after several years would be informative to track the spread of *M. spicatum* across lakes and the potential changes in macrophyte communities in response to the timing of invasion. Finally, the mesocosm experiment was limited to a small number of native species; however, a larger experiment with more species would better test the significance of a possible relationship between species competitive ability and association with *M. spicatum.*

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Chapter 5. Appendix

Tables

Table S-1. Results of linear model for lake scale native richness after excluding lakes with high values for Cook's Distance (Round Lake and Loon Lake). $R^2 = 0.64$, adjusted $R^2 = 0.41$, $F(7, 11) = 2.80$, $p = 0.062$.

	b	SE	*t*	*p*
(Intercept)	14.96	2.77	5.39	< 0.001
PC1	-0.08	0.33	-0.25	0.809
PC2	0.14	0.49	0.27	0.790
PC3	-0.43	0.48	-0.90	0.390
subwatershed (Tay River)	3.99	1.63	2.45	0.033
$\log_{10}$(*Myriophyllum spicatum* cover)	-0.25	1.68	-0.15	0.884
Zebra mussel (presence)	4.53	1.91	2.38	0.037
$\log_{10}$(charophyte cover)	-1.66	1.03	-1.62	0.134

Table S-2. Results of linear model excluding lakes with high values for Cook's Distance (Loon Lake) for lake wide native richness vs. chlorophyll-*a*, zebra mussel presence, subwatershed and the interaction between chlorophyll-*a* and zebra mussel presence. $R^2 = 0.84$, adjusted $R^2 = 0.79$, $F(4, 15) = 19.22$, $p < 0.001$.

	b	SE	*t*	*p*
(Intercept)	10.95	0.83	13.27	< 0.001
$\log_{10}$(chlorophyll-*a*)	12.47	2.41	5.16	< 0.001
Zebra mussel (presence)	6.27	0.97	6.45	< 0.001
subwatershed (Tay River)	3.75	0.88	4.25	< 0.001
$\log_{10}$(chlorophyll-*a*) * zebra mussel	-10.28	2.97	-3.46	0.003

Table S-3. Results of linear model excluding lakes with high values for Cook's Distance (Noble's Bay) for lake wide native evenness vs. *Myriophyllum spicatum* cover, littoral zone length and longitude. $R^2 = 0.43$, adjusted $R^2 = 0.33$, $F(3, 16) = 4.09$, $p = 0.025$.

	b	SE	*t*	*p*
(Intercept)	13.17	5.95	2.21	0.042
$\log_{10}$(*Myriophyllum spicatum* cover)	-0.09	0.03	-2.82	0.012
$\log_{10}$(littoral length)	-0.06	0.06	-0.97	0.344
longitude	0.17	0.08	2.17	0.045

Figures

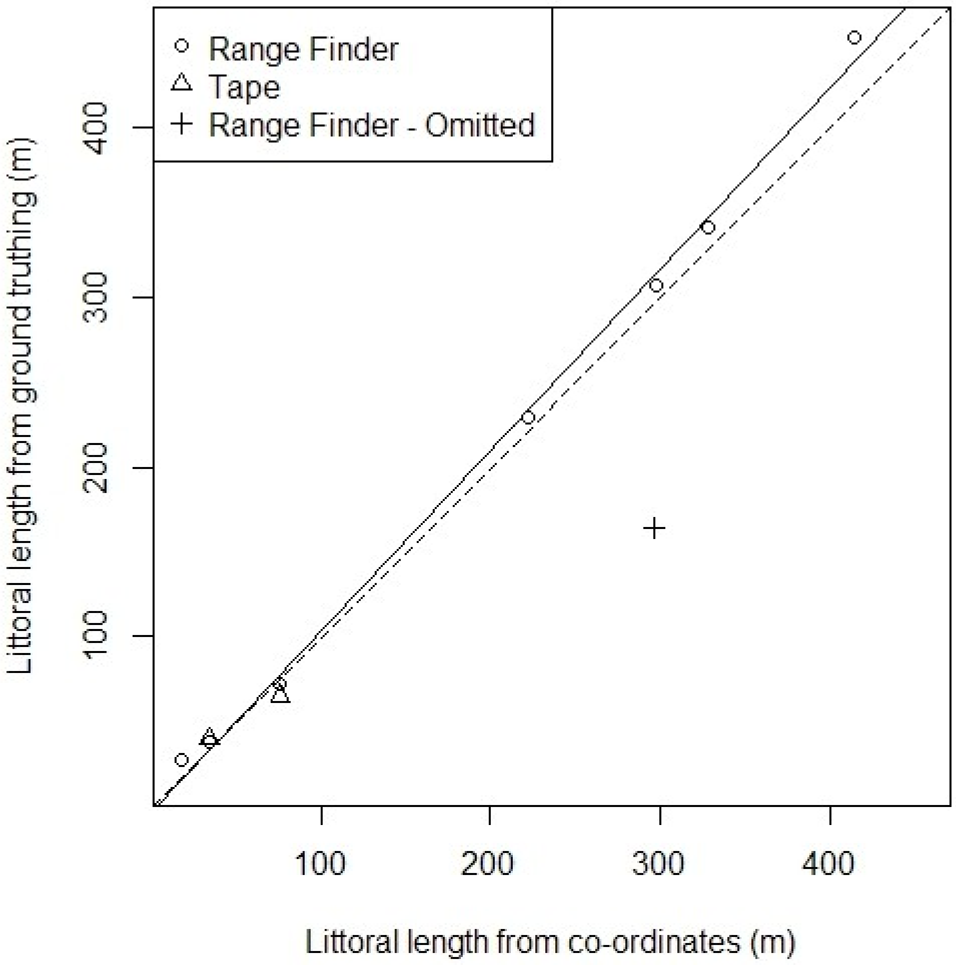


Figure S-1. Scatter plot of ground-truthed littoral length versus littoral length estimated from latitude and longitude. Dashed line represents 1:1 relationship. Solid line represents trend line excluding omitted value. $R^2 = 1.00$, $b = 1.07$, $F(1, 7) = 2065$, $p < 0.001$.

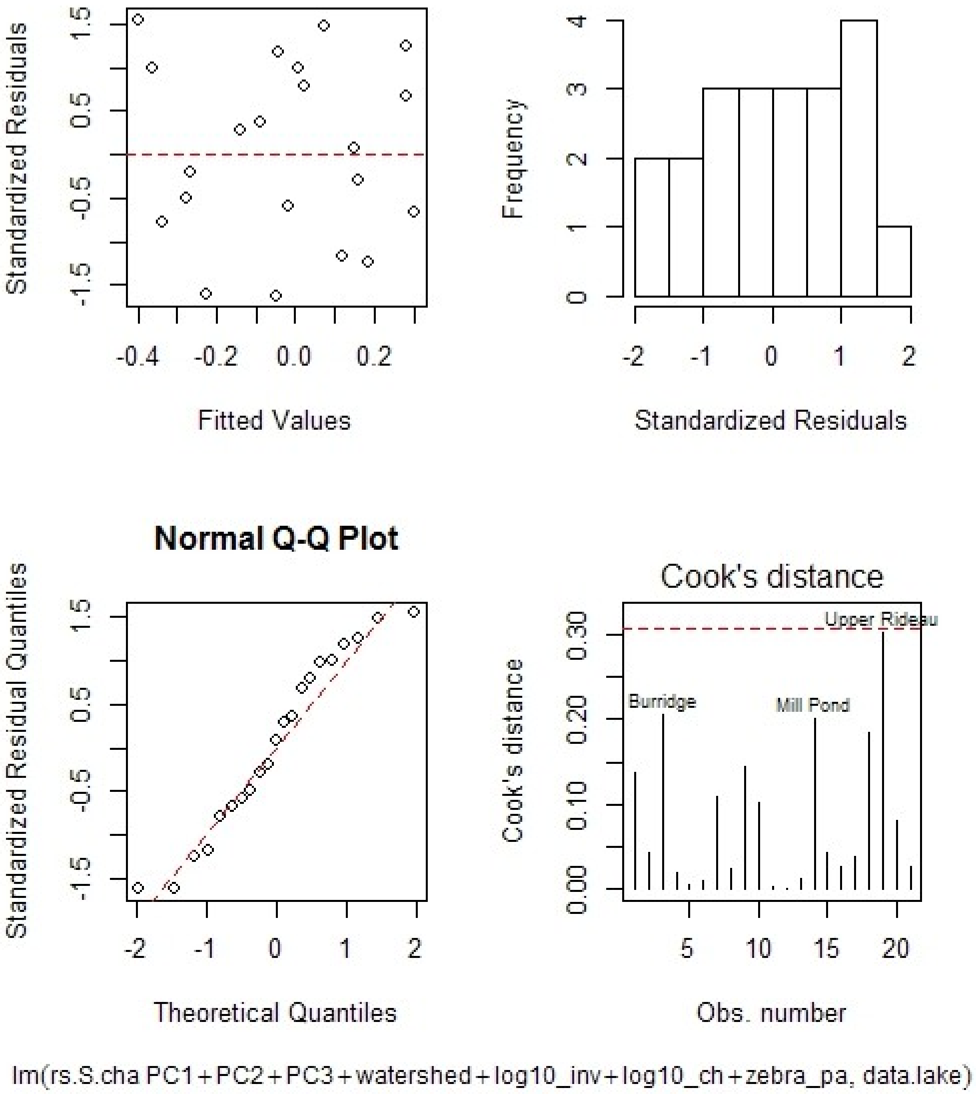


Figure S-2. Residual analysis of linear model for Spearman rank correlation (quadrat level native richness vs. charophyte cover) vs. lake wide charophyte cover, PC1, PC2, PC3, *Myriophyllum spicatum* cover, subwatershed and zebra mussel presence.

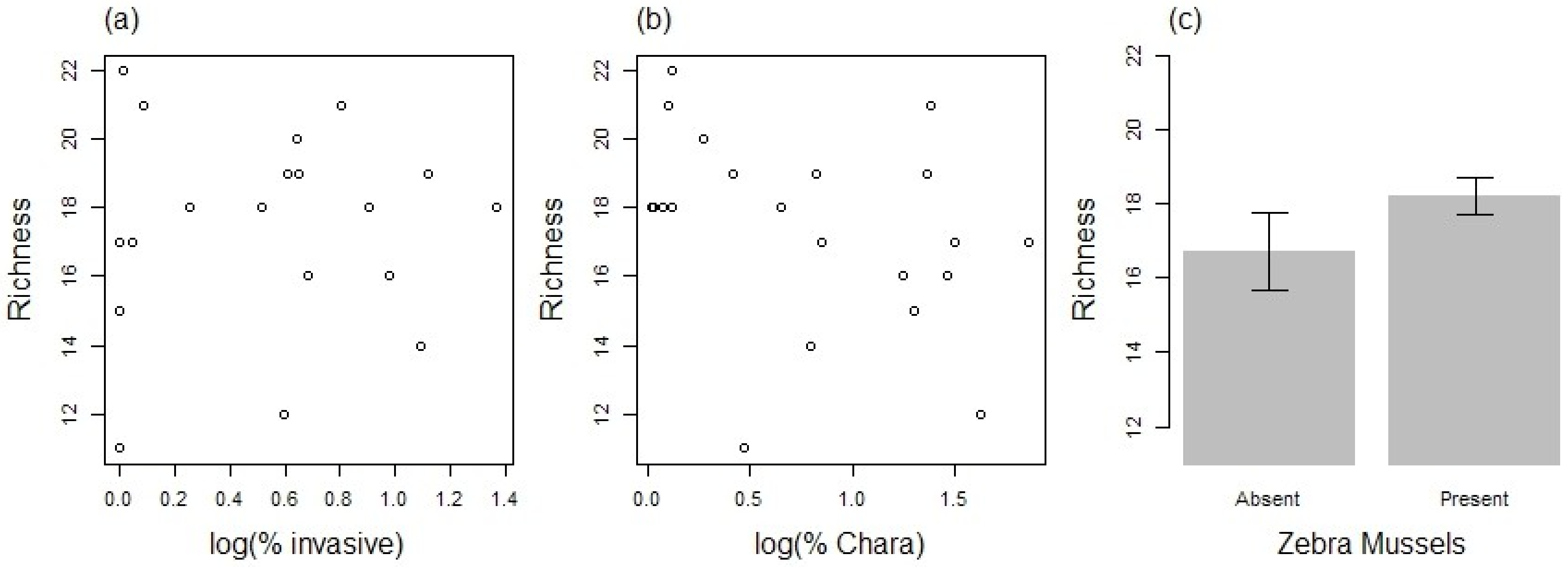


Figure S-3. Scatter plots of native macrophyte richness vs. (a) Myriophyllum spicatum cover, and (b) charophyte cover in 21 lakes, and (c) bar plot of mean +/- standard error of macrophyte richness vs. zebra mussel presence/absence (n = 11, 10). No significant difference in richness with any variable ($p > 0.05$).

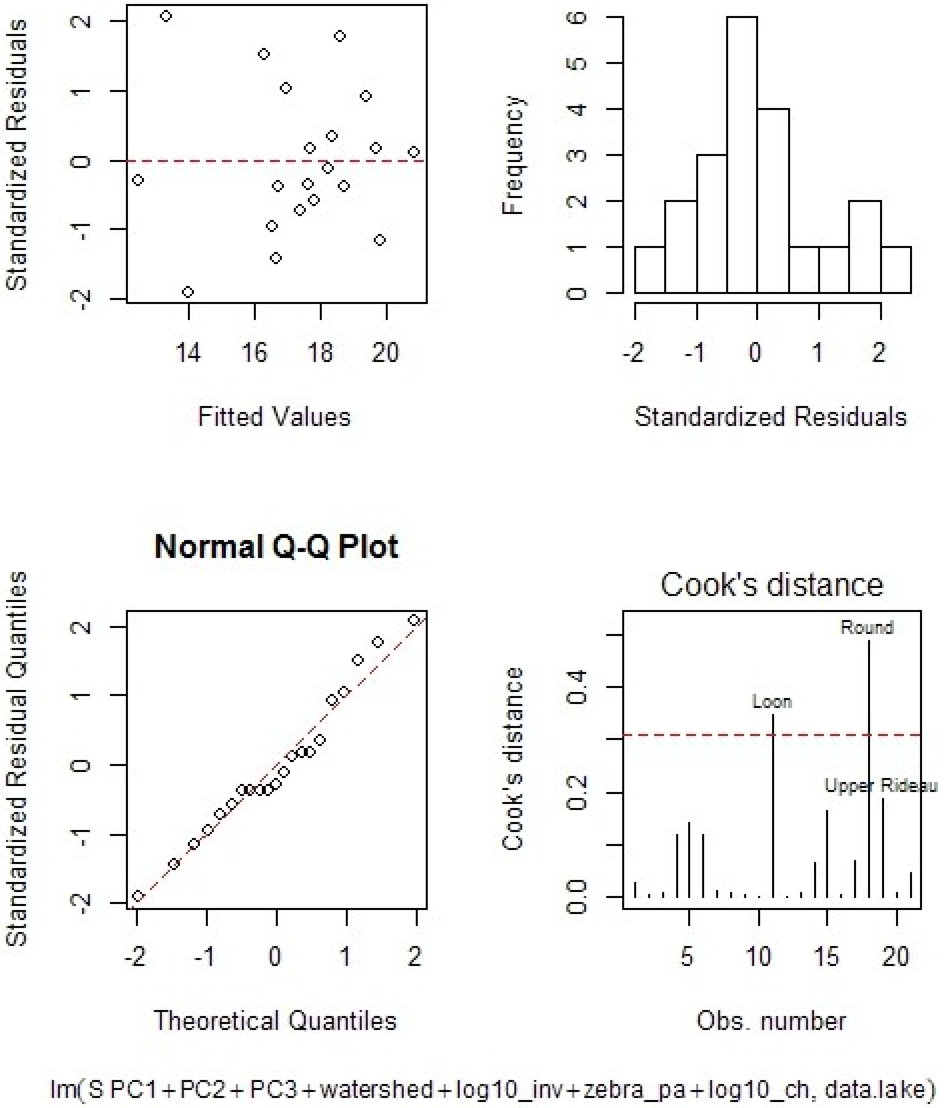


Figure S-4. Residual analysis for linear model at the lake scale of native richness vs. PC1, PC2, PC3, subwatershed, *Myriophyllum spicatum* cover, charophyte cover and zebra mussel presence.

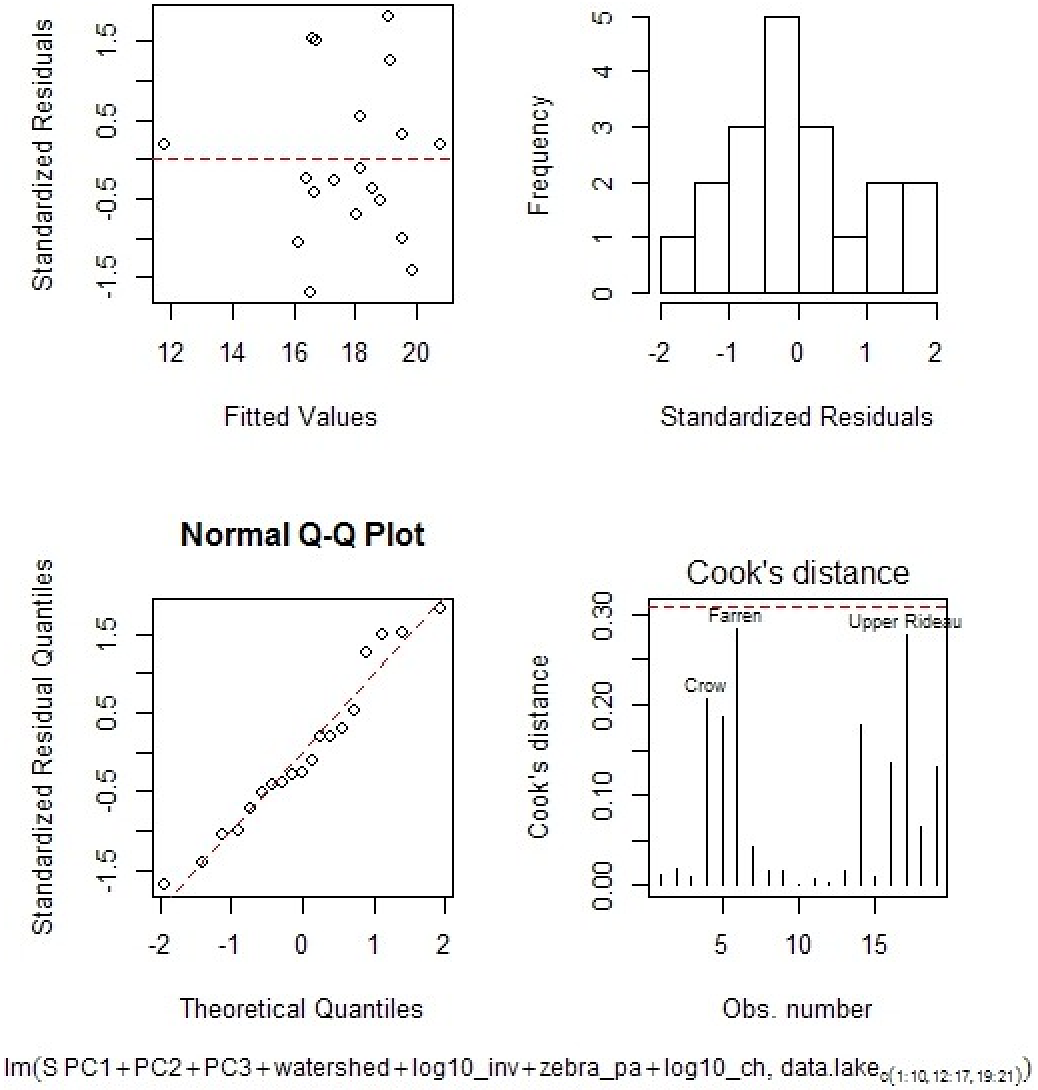


Figure S-5. Residual analysis after excluding lakes with high values for Cook's Distance (Round Lake and Loon Lake) from linear model at the lake scale of native richness vs. PC1, PC2, PC3, subwatershed, *Myriophyllum spicatum* cover, charophyte cover and zebra mussel presence.

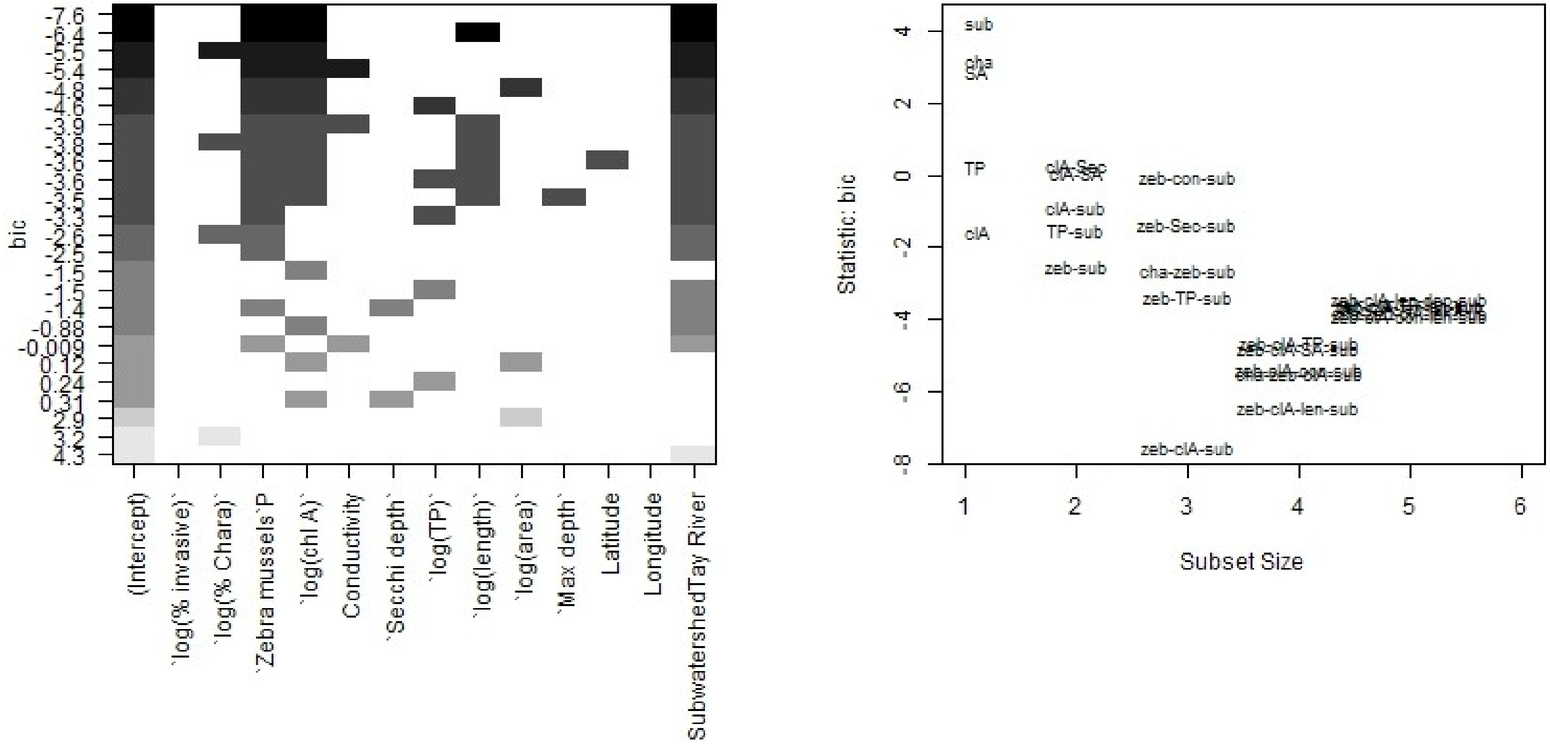


Figure S-6. Result of comparison of native richness models using the BIC statistic, showing the five models with lowest BIC values each for combinations of one to five variables in 21 lakes. (a) Ranking of models from highest to lowest BIC value. Shaded boxes indicate variables included in each model. (b) Plot of model BIC value vs. number of variables. Text indicates variables included in each model.

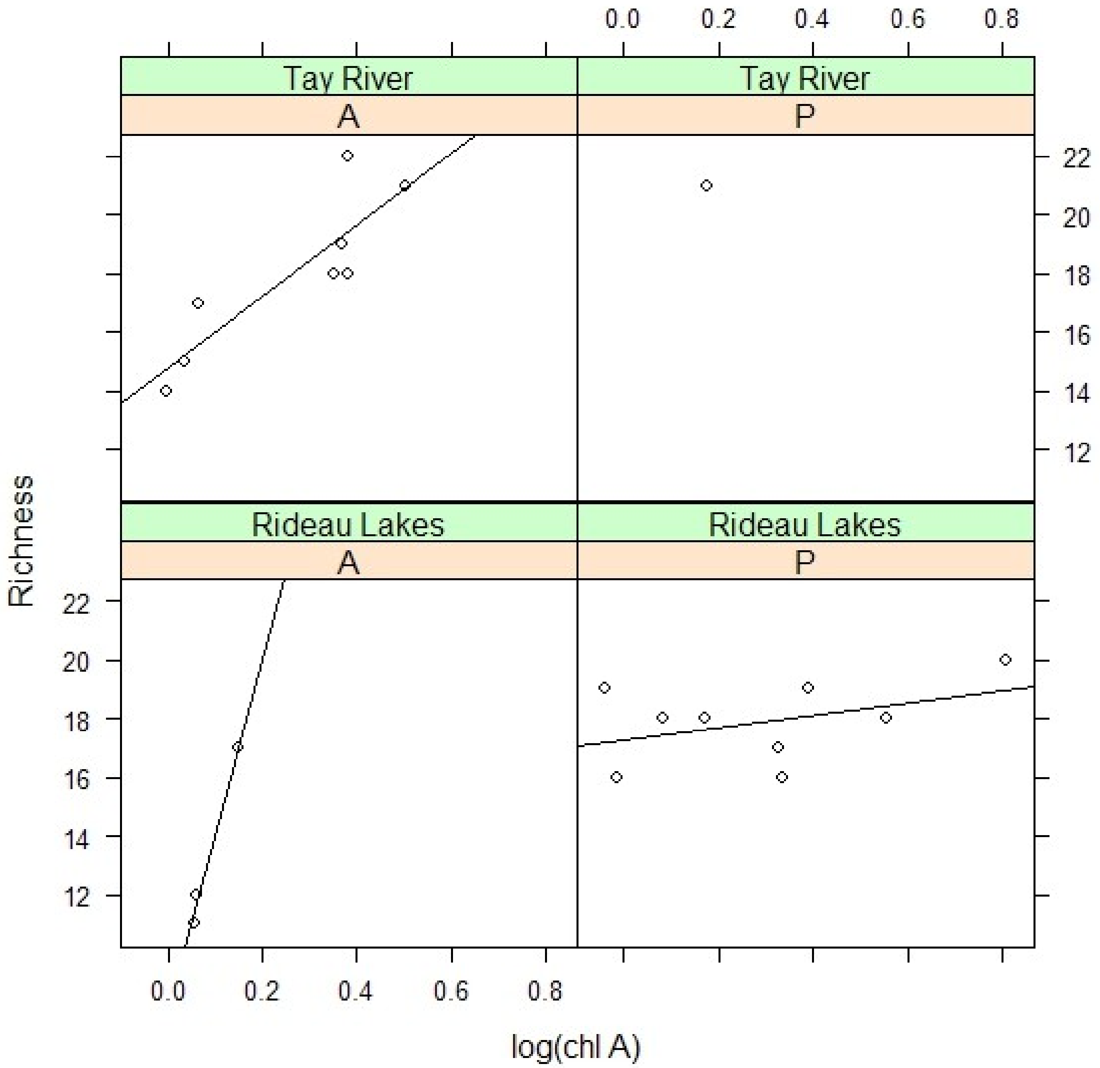
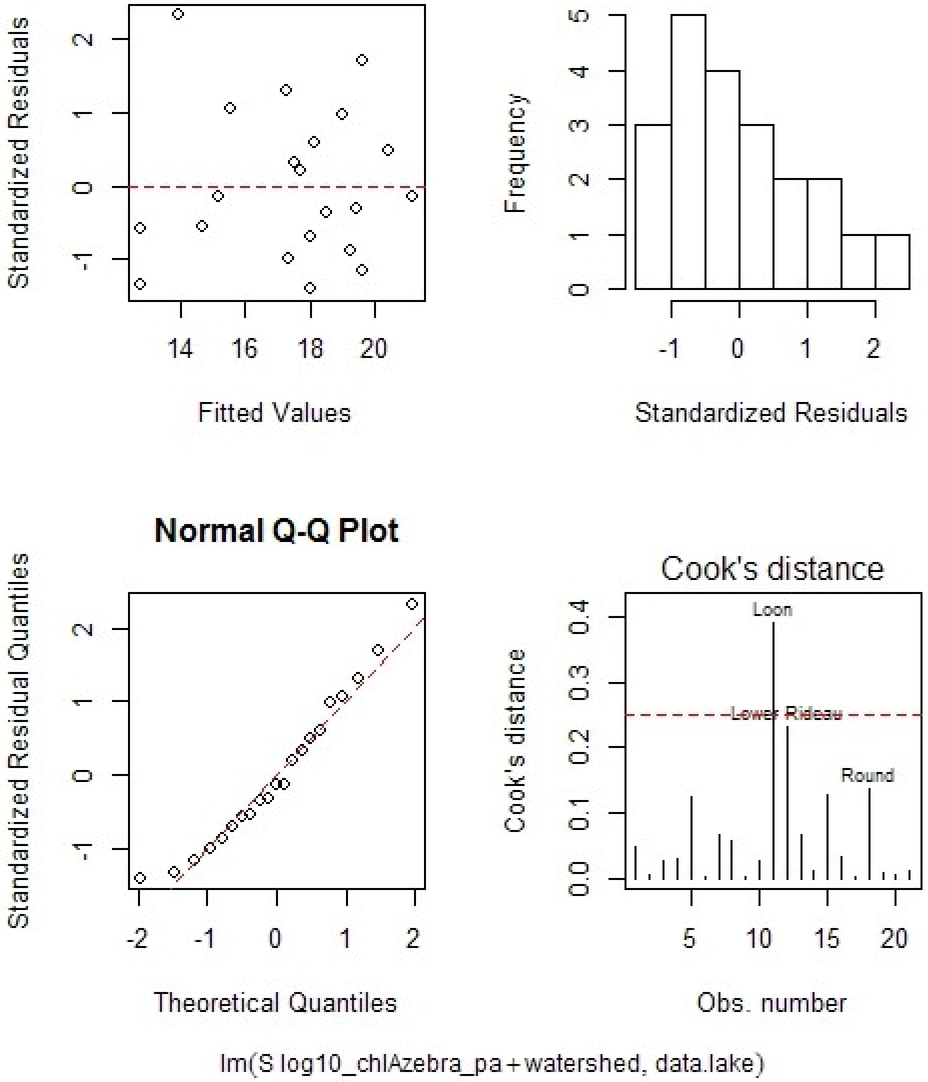


Figure S-7. Scatter plot of native macrophyte richness vs. $\log_{10}$(chlorophyll-*a*), for lakes in the Tay River and Rideau Lakes Subwatersheds, where zebra mussels were present, P, or absent, A. $N = 21$ lakes.



Normal Q-Q Plot

Cook's distance

Figure S-8. Residual analysis for linear model of lake wide native richness vs. chlorophyll-*a*, zebra mussel presence, subwatershed and the interaction between chlorophyll-*a* and zebra mussel presence.

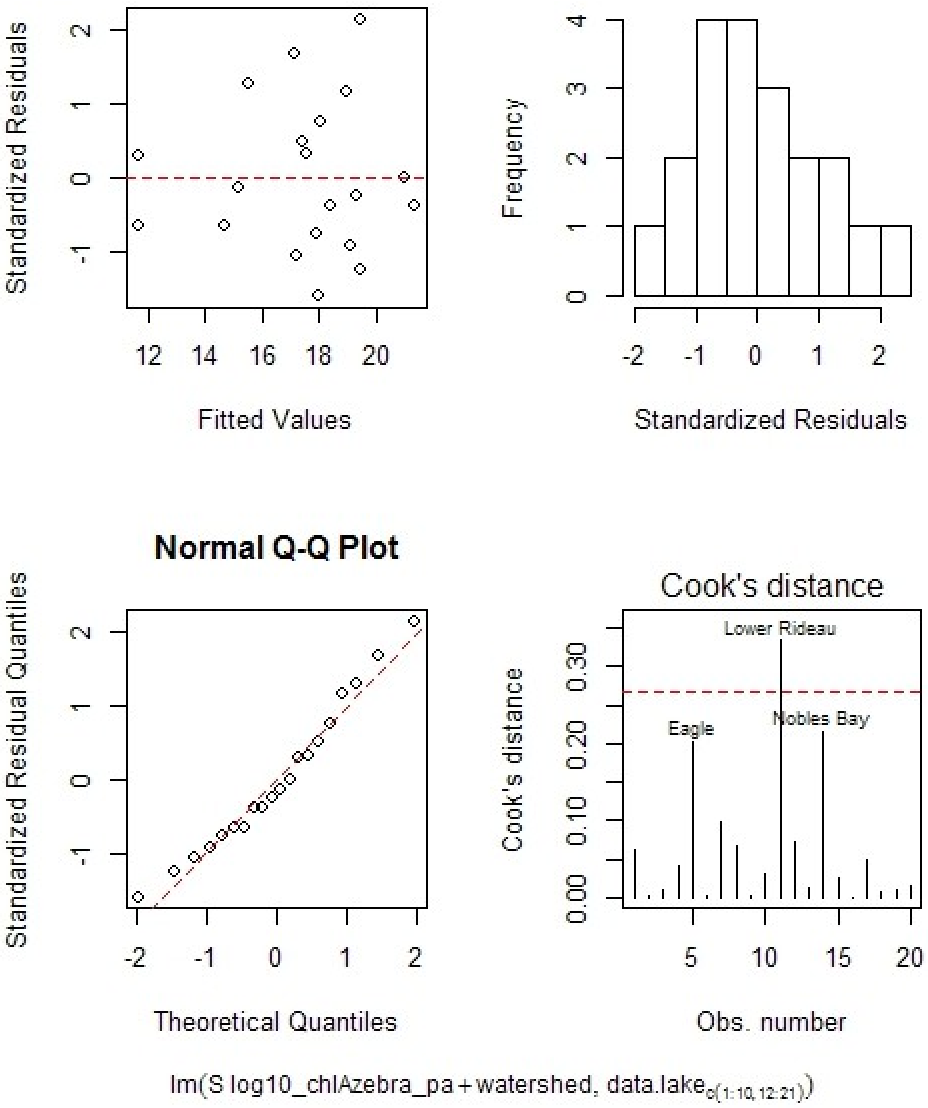


Figure S-9. Residual analysis of linear model excluding lakes with high values for Cook's Distance (Loon Lake) for lake wide native richness vs. chlorophyll-*a*, zebra mussel presence, subwatershed and the interaction between chlorophyll-*a* and zebra mussel presence.

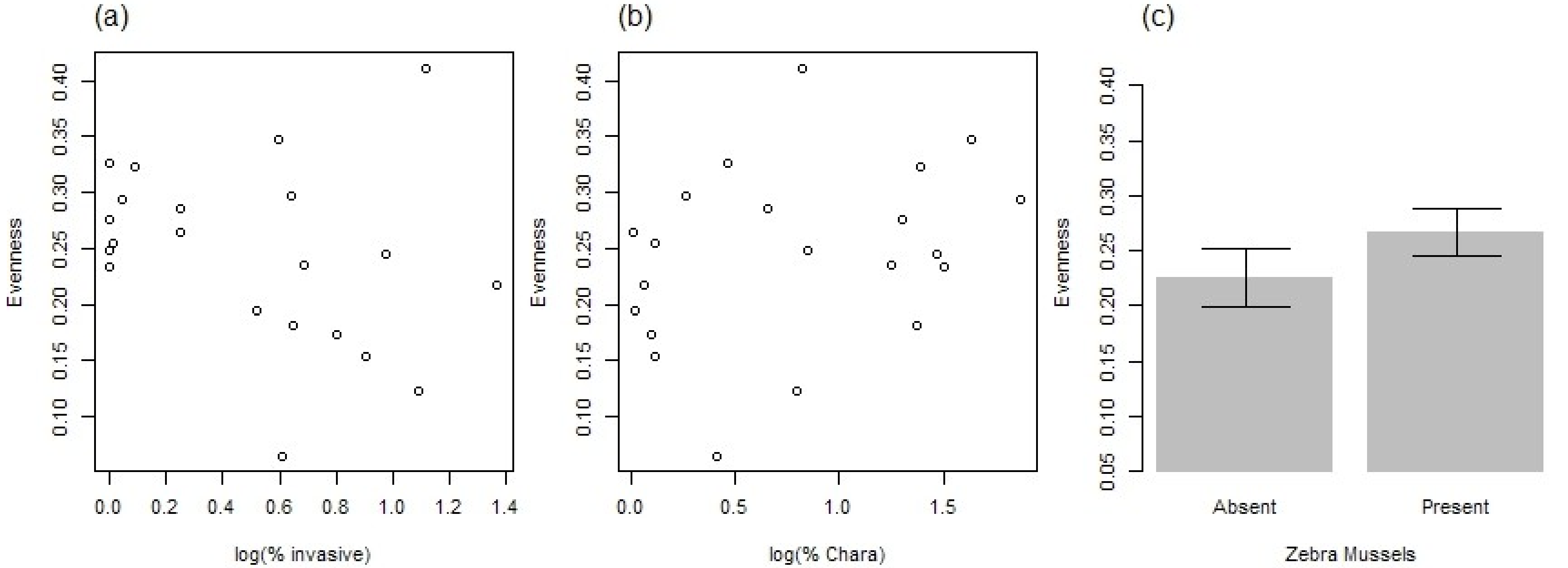


Figure S-10. Scatter plots of native macrophyte evenness vs. (a) Myriophyllum spicatum cover, and (b) charophyte cover in 21 lakes, and (c) bar plot of mean +/- standard error of macrophyte evenness vs. zebra mussel presence/absence (n = 11, 10). No significant difference in richness with any variable ($p > 0.05$).

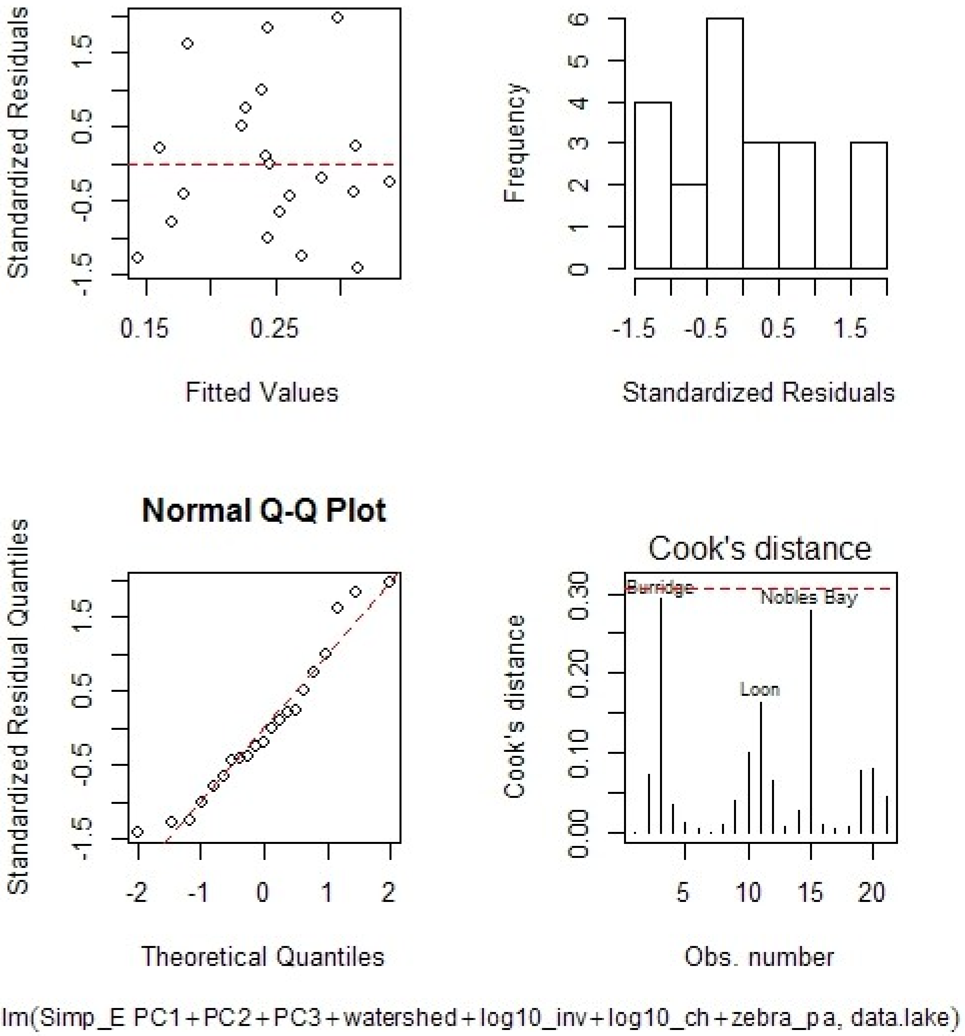


Figure S-11. Residual analysis of linear model for lake wide native evenness vs. PC1, PC2, PC3, subwatershed, charophyte cover, *Myriophyllum spicatum* cover and zebra mussel presence.

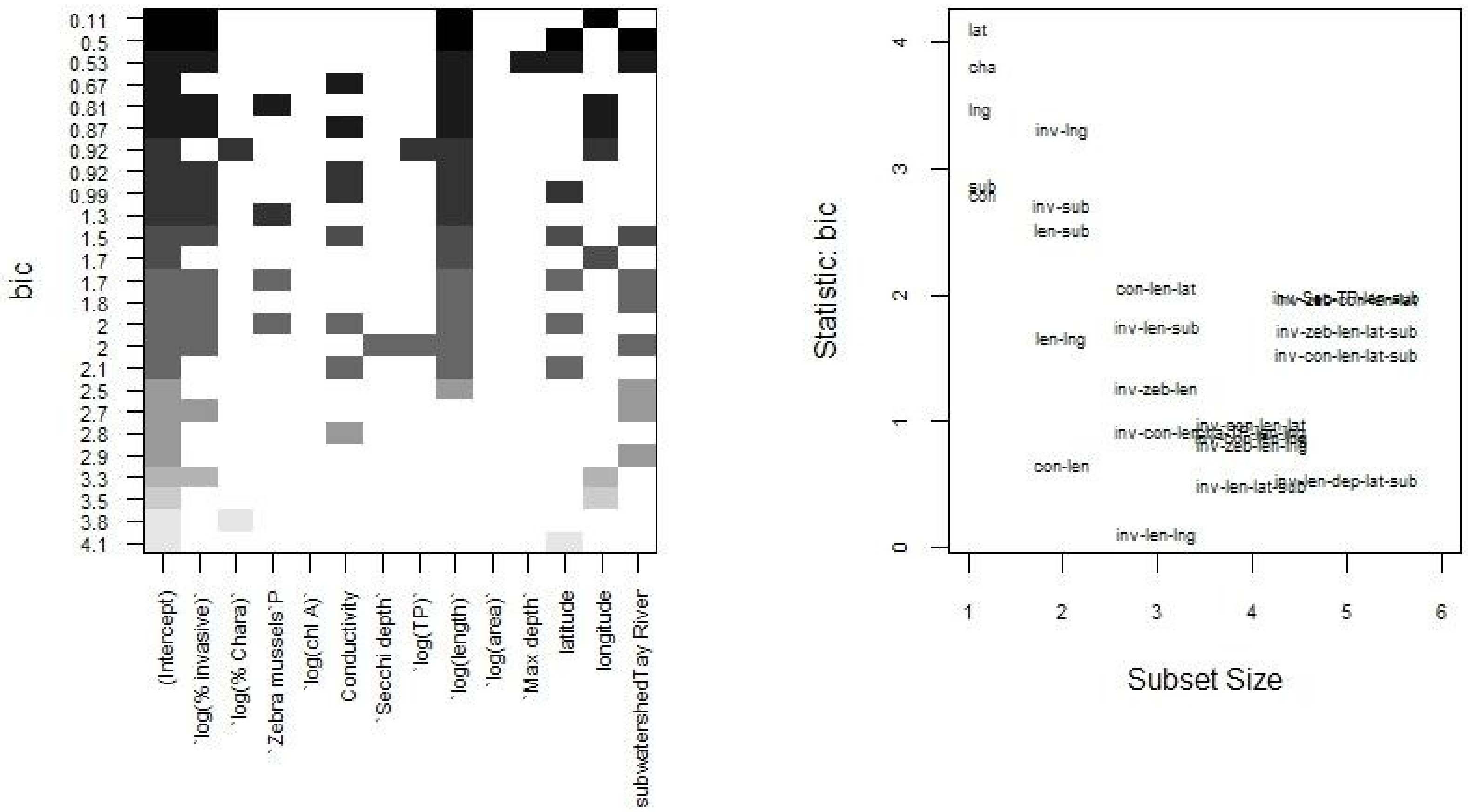


Figure S-12. Result of comparison of native evenness models using the BIC statistic, showing the five models with lowest BIC values each for combinations of one to five variables in 21 lakes. (a) Ranking of models from highest to lowest BIC value. Shaded boxes indicate variables included in each model. (b) Plot of model BIC value vs. number of variables. Text indicates variables included in each model.

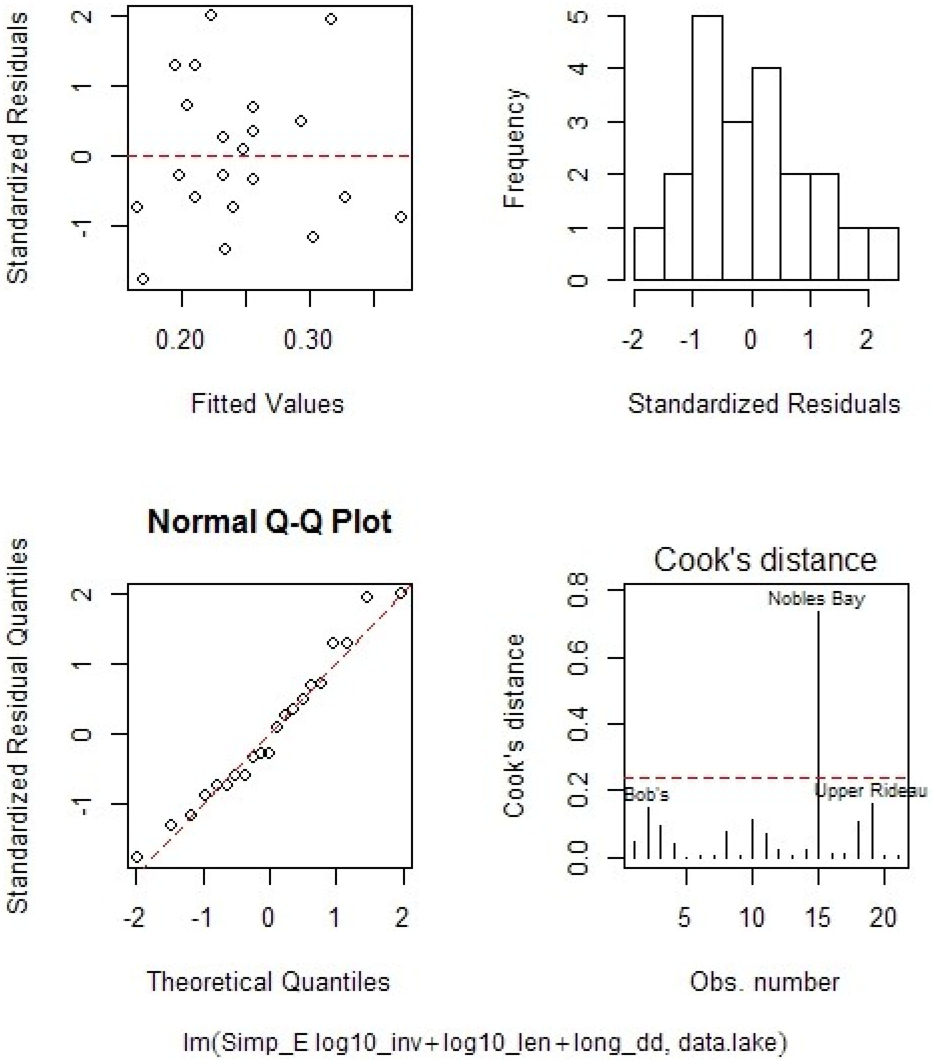


Figure S-13. Residual analysis of linear model for lake wide native evenness vs. *Myriophyllum spicatum* cover, littoral zone length and longitude.

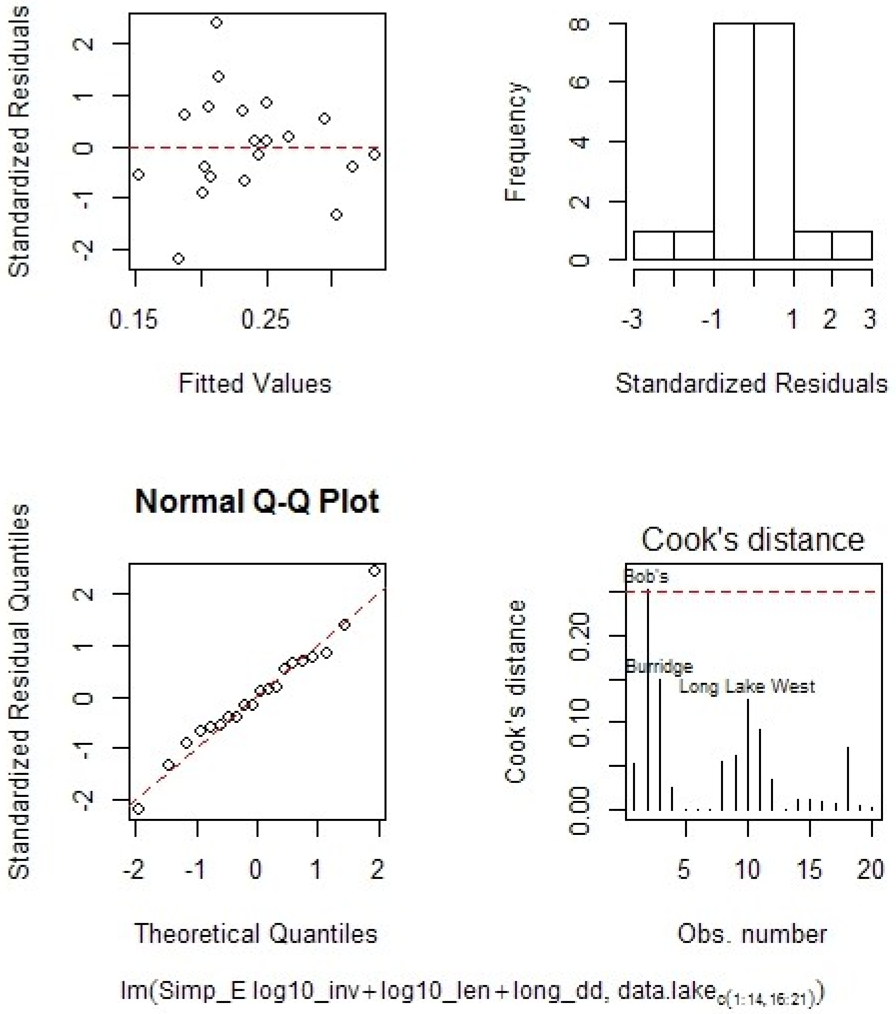


Figure S-14. Residual analysis excluding lakes with high values for Cook's Distance (Noble's Bay) for lake wide native evenness vs. *Myriophyllum spicatum* cover, littoral zone length and longitude.

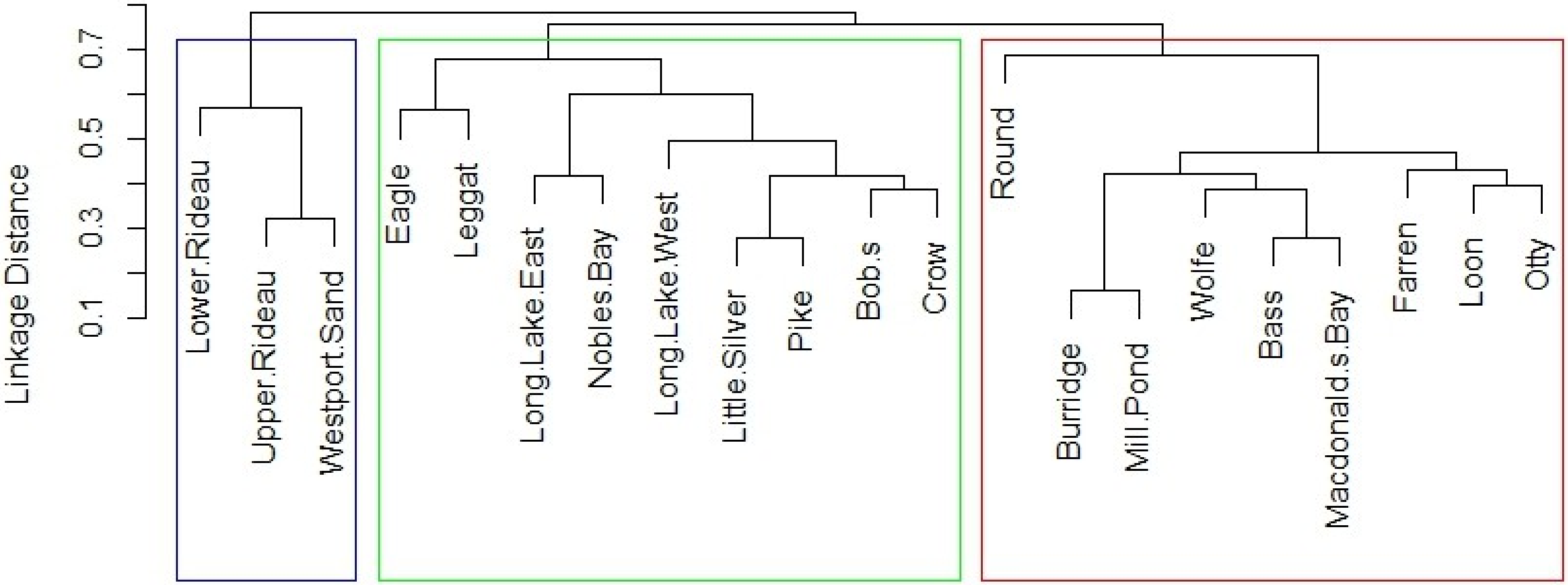


Figure S-15. Cluster dendrogram of 21 lakes based on relative cover of the 31 macrophyte species present in > 5% of lakes. Lakes grouped using average linkage of Bray-Curtis dissimilarities (Cophenetic correlation = 0.80). Box colors are blue, green, red from left to right and correspond to same lake groups in detrended correspondence analysis ordination (Figure 3-2).

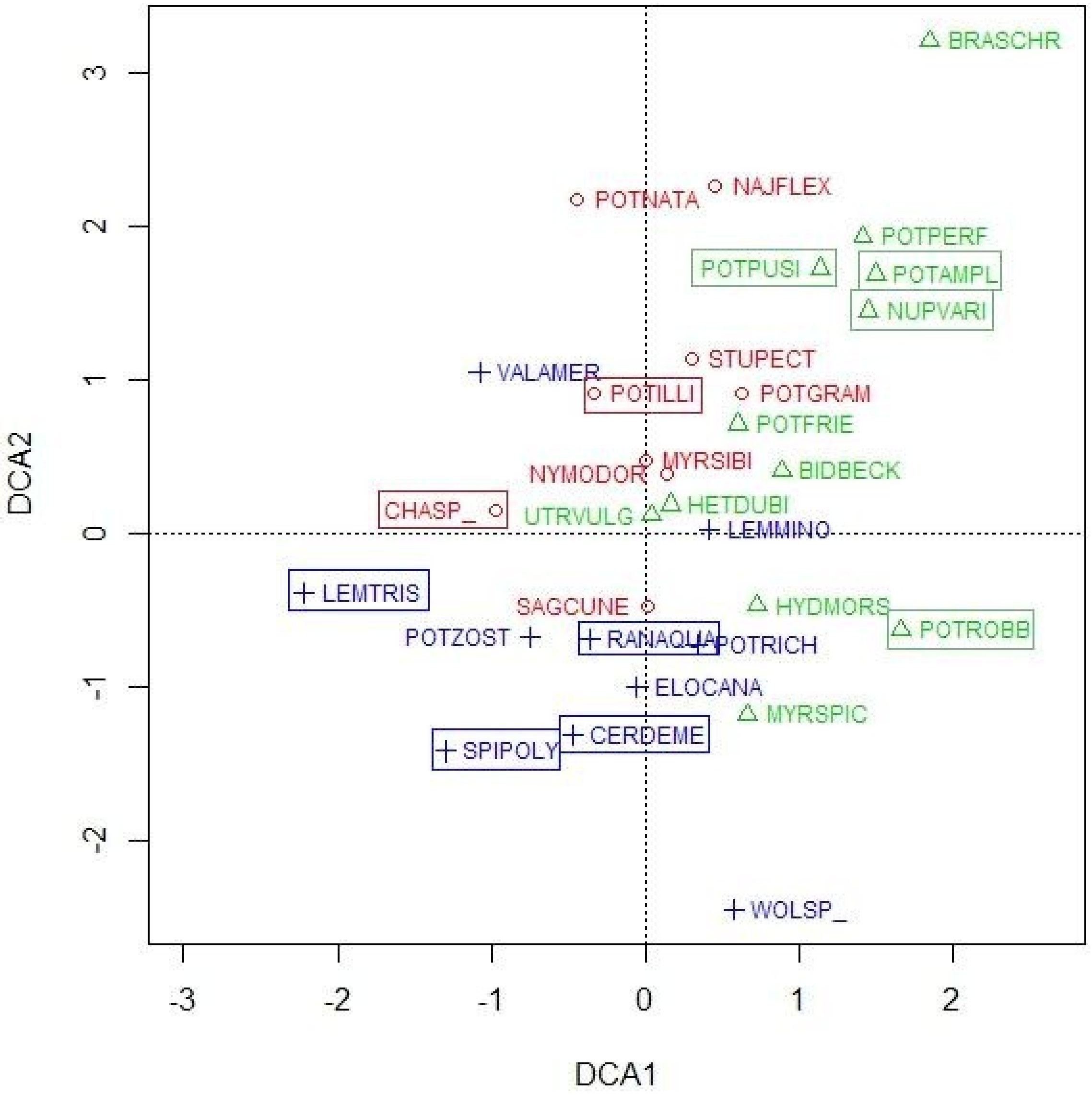


Figure S-16. Ordination plot from detrended correspondence analysis (DCA) for relative cover of 31 macrophyte species present in > 5% of 21 lakes (lakes not shown). Decorana values for first two axes = 0.46, 0.21; eigenvalues = 0.46, 0.29; axis lengths = 2.54, 2.02. Grouping of species according to indicator species analysis (Table 3-2) from three clusters of lakes (Figure S-15): *blue crosses*, lakes mainly in Rideau Lakes Subwatershed within Rideau Canal; *green triangles*, lakes mainly in Tay River Subwatershed; *re*d circles, lakes mainly in Rideau Lakes Subwatershed outside of Rideau Canal. Rectangles indicate significant indicators ($p < 0.05$). Species codes listed in Table 3-2.

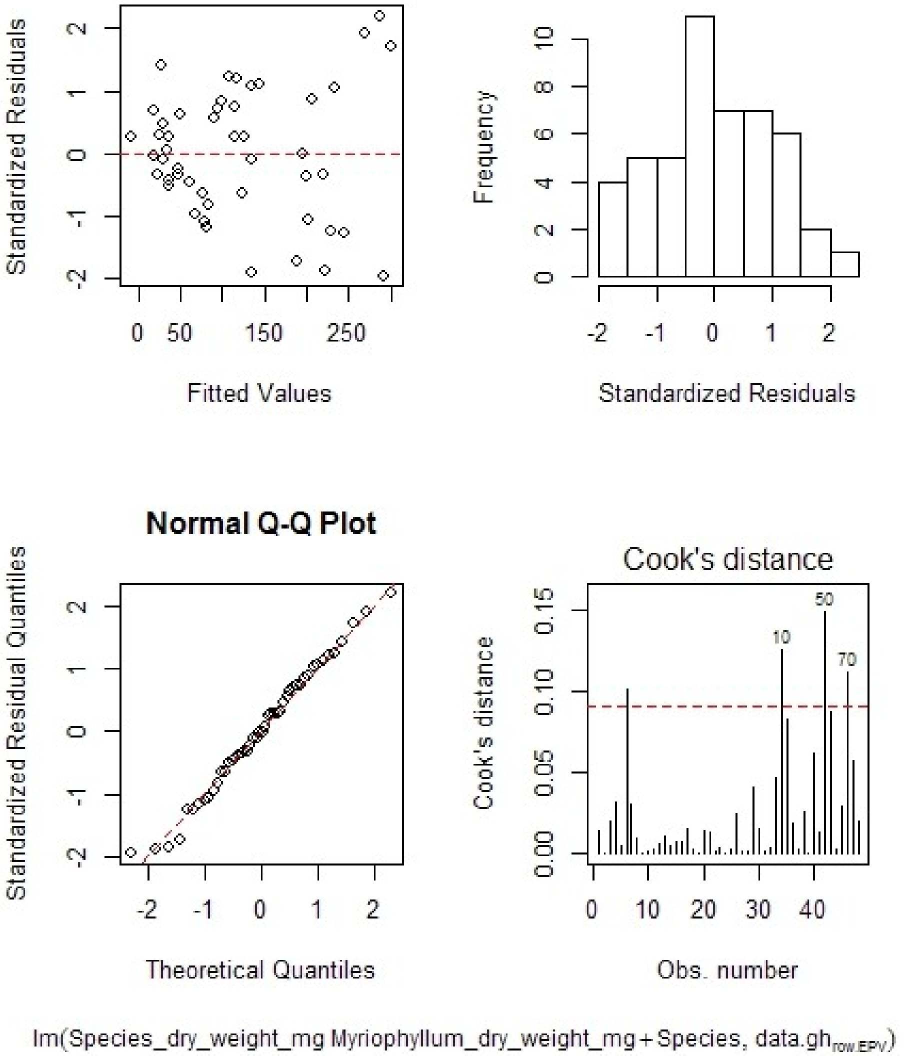


Figure S-17. Residual analysis for general linear model of competitor species dry weight (mg) vs. *Myriophyllum spicatum* dry weight (mg) and competitor species (*Elodea canadensis, Potamogeton illinoensis* and *Vallisneria americana*) when plants grown in tank mesocosms.