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Assessing watermilfoil invasion effects on native macrophyte communities in North American lakes using a novel approach for macrophyte sampling

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Abstract – Aquatic invasive species are among the greatest threats to freshwater biodiversity. The aim of this study was to understand the effects of two invasive watermilfoil species (*Myriophyllum heterophyllum* Michx. and *Myriophyllum spicatum* L.) on native macrophyte communities and to assess community response to a range of invasion intensities as well as examine the influence of canopy types. We hypothesized that some communities would be more sensitive to invasion, and that some canopy species would facilitate watermilfoil presence. We used a novel approach to give better representation of the 3D aspect of the community which involved employing a modified quadrat approach to sample at two Connecticut lakes. Results show that watermilfoil invasion has a significant negative effect on native species richness. Floating canopy does not vary with invasion intensity, but submerged canopy does. One species, (*Utricularia purpurea* Walter), was associated with high native species richness and rarely occurred with invasive species. The results identify potential species that are disproportionally threatened by invasive species, as well as identifying invasion indicator species. The examination of canopy effects is uncommon in aquatic invasion ecology, and this study suggests that this aspect may have significant effects on resilience to invasion and overall community dynamics.

Keywords: *Myriophyllum spicatum* / *Myriophyllum heterophyllum* / ecology / freshwater / invasive species

Résumé – Évaluation des effets de l'invasion du myriophylle en épi sur les communautés de macrophytes indigènes dans les lacs d'Amérique du Nord grâce à une nouvelle approche d'échantillonnage des macrophytes. Les espèces aquatiques envahissantes sont parmi les plus grandes menaces pour la biodiversité d'eau douce. L'objectif de cette étude était de comprendre les effets de deux espèces envahissantes de myriophylle (*Myriophyllum heterophyllum* Michx. et *Myriophyllum spicatum* L.) sur les communautés de macrophytes indigènes et d'évaluer la réponse des communautés à une série d'intensités d'invasion ainsi que d'examiner l'influence des types de canopée. Nous avons émis l'hypothèse que certaines communautés seraient plus sensibles à l'invasion et que certaines espèces de la canopée faciliteraient la présence du myriophylle. Nous avons utilisé une nouvelle approche pour donner une meilleure représentation de l'aspect 3D de la communauté, ce qui a impliqué l'utilisation d'une approche de quadrat modifiée pour échantillonner deux lacs du Connecticut. Les résultats montrent que l'invasion de myriophylle en épi a un effet négatif important sur la richesse des espèces indigènes. La canopée flottante ne varie pas avec l'intensité de l'invasion, mais la canopée submergée le fait. Une espèce, (*Utricularia purpurea* Walter), a été associée à une grande richesse en espèces indigènes et s'est rarement retrouvée avec des espèces envahissantes. Les résultats permettent d'identifier les espèces potentielles qui sont menacées de façon disproportionnée par les espèces envahissantes, ainsi que d'identifier les espèces indicatrices d'invasion. L'examen des effets de la canopée est peu courant dans l'écologie des invasions aquatiques, et cette étude suggère que cet aspect peut avoir des effets importants sur la résilience aux invasions et sur la dynamique globale de la communauté.

Mots clés : *Myriophyllum spicatum* / *Myriophyllum heterophyllum* / écologie / eau douce / espèces envahissantes

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1 Introduction

Aquatic plants produce some of the most severe biological invasions, since they are usually harder to control than terrestrial species (Les and Mehrhoff, 1999). Water milfoil invasion is a serious problem worldwide because of its impact on biodiversity, recreation, property values, and natural resources (Eiswerth *et al.*, 2000; Pimentel *et al.*, 2005; Havel *et al.*, 2015). Eurasian watermilfoil (*Myriophyllum spicatum* L.), native to Europe, Asia, and Northern Africa (Smith and Barko, 1990), occurs globally and is considered to be the 'most invasive' aquatic species in North America (Les and Mehrhoff, 1999). Variable-leaf watermilfoil (*Myriophyllum heterophyllum* Michx.) is native to the South-eastern United States (Les and Mehrhoff, 1999), but has become highly invasive in parts of north America, as well as eight European countries and China (Gross *et al.*, 2020).

Milfoil invasion is often a result of disturbance, but studies have also documented its colonization in undisturbed systems (Madsen, 1999). Lakes in temperate areas are particularly prone to milfoil invasion due to the high levels of natural disturbance from freeze-thaw, drought, storms, and other sources (Capers *et al.*, 2007). Biotic factors such as native species density (Capers *et al.*, 2007), presence of herbivore species (Qiu *et al.*, 2019), and propagule pressure (Levine, 2000; Lockwood *et al.*, 2005) potentially influence invasion resistance. *Myriophyllum spicatum* occurs in shallow water (1–4 m), grows quickly (Smith and Barko, 1990), is a strong light competitor, and often rapidly colonizes disturbed areas (Madsen *et al.*, 1991). It was also found to exhibit a higher resistance to highly toxic copper (Cu^{2+}) than other ecologically important freshwater plants such as *Stratiotes aloides* L., *Elodea canadensis* Michx. and *Lemna trisulca* L., with up to 8% of total chlorophyll converted to Cu-chlorophyll at the observed 2 μmol l^{-1} (Küpper *et al.*, 1996).

Aquatic plant invasions have cascading effects on aquatic communities and common impacts include changes in nutrient load, water clarity, habitat availability, sediment load, and plant community structure (Van Donk and Otte, 1996; Gergs and Rothhaupt, 2015). Milfoil invasion has the capacity to reshape aquatic communities. In particular, Eurasian milfoil can increase community biomass (Bosch *et al.*, 2009), increase water nutrient concentrations (Tan *et al.*, 2018), alter light availability to other species (Smith and Barko, 1990), which in turn influences water temperature, dissolved oxygen concentration, and plant species composition (Strayer, 2010). Milfoil invasion suppresses native plant species, leading to reduction in diversity (Madsen *et al.*, 1991; Boylen *et al.*, 1999). Despite the well documented negative ecological impacts of invasion, some research indicates a positive impact with *M. heterophyllum* associated with high native macrophyte richness (Thum and Lennon, 2010). The majority of these studies have either investigated impacts on native species diversity, or overall diversity, but never analysed and compared both aspects of macrophyte diversity within the same study. It is currently recognised that in studies investigating impacts of invasive species, it is imperative that both aspects of species diversity are investigated separately (Bernard-Verdier and Hulme, 2015).

A major challenge in aquatic macrophyte lake sampling has been identifying and employing approaches that can effectively sample canopy as well as submerged species, but at the same time being able to differentiate the position in the water column (Madsen *et al.*, 2007). A traditional quadrat (a frame applied from above) would collapse the forest-like submerged canopy, making observation of the plants underneath difficult (Madsen, 1999; Madsen *et al.*, 2007; Madsen and Wersal, 2017), therefore necessitating the need for an alternative approach to macrophyte lake sampling.

This work examines macrophyte community responses to invasion and outlines species of concern, possible indicator species, and characteristics of communities at different degrees of invasion. Very importantly, it develops a new methodology for aquatic plant surveys and explores the interaction between invasion and canopy type and their effects on native species.

We hypothesized that, (1) increasing invasion intensity has a negative impact on the native as well as the total (overall) species richness, (2) native species demonstrate varied responses to increasing invasion intensity, and (3) the type of canopy influences invasion dynamics by either facilitating or hindering the process.

2 Materials and methods

Two small lakes in Goshen, Connecticut, USA, were surveyed in 2016. 'Lake 1' (41.855020N, −73.256685W) and 'Lake 2' (41.813904N, −73.239412W) are both partially residentially developed and have wooded wetland areas along the unoccupied shore. Lake 1 has a surface area of 0.17 km^2 with a maximum depth of 9.14 m, while Lake 2 is 0.27 km^2 with a maximum depth of 3.35 m (Capers and Selsky, 2005; June-Wells and Hart, 2012). Thirty-three aquatic plant species are known to be present in Lake 1 (Sabina Perkins, personal communication, 4 March 2016). The Connecticut Agricultural Experiment Station (CAES) identified 19 aquatic plant species (CAES, 2005) in Lake 2. Both lakes have been invaded by *M. spicatum*, and Lake 1 additionally has *M. heterophyllum* present.

2.1 Sampling method

Sampling was conducted daily between 10:00 and 16:00 from 7 June 2016 to 6 September 2016. A novel, 3-D quadrat design (Fig. 1) was used to allow the best possible observation with minimum damage to the plant community and thus allowing the accurate recording of both the floating as well as submerged vegetation. This 3-D quadrat consisted of a one metre long telescopic pole with a weighted anchor at the base that allowed the quadrat to be stabilised in the water. A rotating arm (1 m long) at the top allowed for the exact delineation of the 1 m radius area to be sampled accurately. Sampling took place along regularly spaced transects in which species' presence/absence was recorded, thus combining point-intercept and line transect methods (Madsen and Wersal, 2017). Transects were oriented perpendicular to the shore, with 2-m diameter circular quadrats (Capers *et al.*, 2007), spaced seven metres apart centre-to-centre. Due to the difficulty of maintaining such a sampling system in field conditions

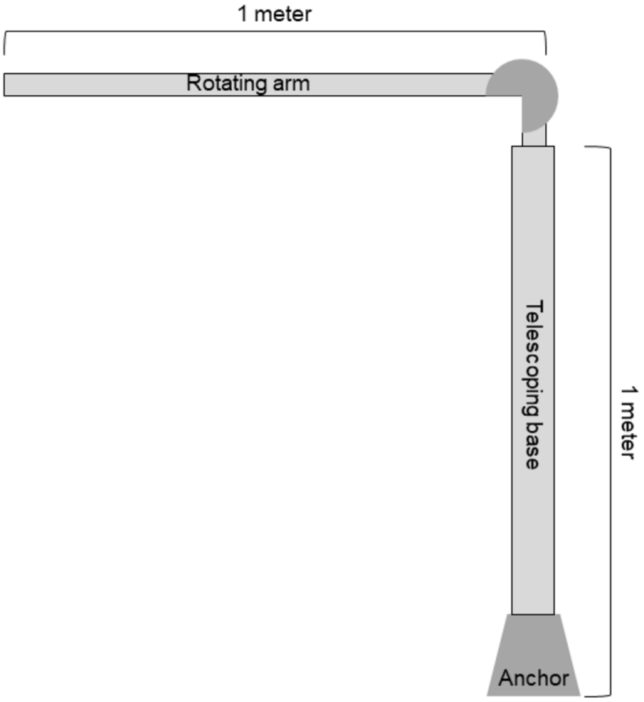

Fig. 1. Quadrat used for survey. Consists of a small anchor holding an upright PVC tube (1 m tall) with a smaller tube inside to allow the quadrat to telescope and pivot around this central axis. A 90° joint connects the telescoping base to another PVC tube (1 m) which acts as the radius of the quadrat. Depth measurements are marked on the base to allow easy depth measurement. The anchor is a one-gallon plastic bucket filled with concrete.

(Madsen, 1999), the distance of seven metres was chosen because it could be measured with two kayak lengths. An in-water snorkelling observer recorded the data within the quadrats as described before (Capers, 2000).

2.2 Data recording

Quadrat number, GPS coordinates recorded with a GPS receiver, date/time, water depth, dominant floating canopy species, dominant submerged canopy species, species presence/absence, and invasion intensity was recorded. Canopy dominance was determined by estimating the percent cover of each species in the quadrat, recording the species with the most cover 'dominant'. Invasion intensity was determined by estimating percent cover of invasive species and recorded on a scale of one to five. Greater than 50% cover is categorized as invasion level five, 21–50% is four, 5–20% is three, <5% is two, and lack of milfoil presence categorised as level one (Capers *et al.*, 2007).

2.3 Data analysis

Pearson correlations discerned relationships between invasion level and water depth. Generalized linear models (hereafter, GLMs) fitted to a Poisson distribution identified factors affecting overall and native species richness in the two lakes (Capers *et al.*, 2007). The ability of a GLM to address contributions of multiple variables while giving a significance value for each was the reason it was used for this analysis and are often used in aquatic community ecological studies (Louca *et al.*, 2009, 2014). The Poisson distribution was selected as it provides a more appropriate model to fit the count data (in this case species richness) (Warton *et al.*, 2016). Principal Coordinates Analysis (PCO), using the LabDSV package in R (Roberts and Roberts, 2016) was used to identify patterns or clusters of spatial association of macrophyte species. Only species that were recorded in at least 4 quadrats were included in the analysis. Since the PCO analysis used binary presence/absence data, the similarity scores reflect how often species co-occur.

Utricularia purpurea Walter and water marigold (*Bidens beckii* Torr. ex Spreng.) were selected in this study as focal species due to their ecological sensitivity and conservation status. *B. beckii* is threatened in Connecticut (Lichvar, 2012), and is listed as endangered or extirpated in other states. *U. purpurea* became a species of interest after exhibiting noteworthy relationships within this study (see Results). GLMs, with data fitted to a binary distribution (since we analysed presence/absence data), were used to analyse potential relationships between this species' presence, invasion intensity, and depth.

Chi-square tests identified whether patterns of canopy dominance changed with invasion intensity. If invasion effect was significant, we used Kruskal-Wallis tests (Stiers *et al.*, 2011), to discern differences in species richness under each canopy. We used pairwise Mann-Whitney tests to further examine where the differences lie.

GLMs and PCO analysis were performed in R 3.6.0. Figures were produced using Microsoft Excel 2013 and Minitab 17.

3 Results

In total, 78 total quadrats were recorded, 63 on Lake 1 and 15 on Lake 2. A total of 33 aquatic plant species were recorded, 30 at Lake 1 (one of which was not possible to identify to species level) and 10 in Lake 2. The average sampling depth was 94.4 cm at Lake 1 and 103.5 cm at Lake 2, with an overall water depth ranging from 50 to 150 cm. The average overall (OSR) and native species richness (NSR) at Lake 1 was 8.0 and 7.1 species, respectively. At Lake 2, OSR and NSR was 5.4 and 4.6, respectively. The highest OSR was seen at invasion level 2 (average 8.1 species) and the NSR was seen at invasion level 1 (average 7.7 species).

Correlation analysis demonstrated a positive relationship between water depth and invasion intensity ($r(76) = 0.325$, $p = 0.004$). The relationship between invasion intensity and species richness is described with GLMs (Tabs. 1 and 2), taking variability due to depth and location into account in the model as covariates (Lake 1 or 2). There was a significant effect of 'lake' effect on both OSR (GLM, Odds ratio = 2.479, df = 62, $p < 0.001$, Tab. 1) and NSR (GLM, Odds ratio = 2.185, df = 62, $p = 0.001$, Tab. 2), which highlights macrophyte community differences between the lakes. NSR was also significantly affected by high (invasion level 5)

Table 1. GLM results of OSR relationships with level of invasion, depth, and lake.

Invasion Intensity	*N*	Odds ratio (B)	Std. Error	*P*
Model (intercept)	2	12.034	1.656	<0.001*
Invasion intensity	78	−0.266	0.222	0.231
Depth (cm)	78	−0.008	0.016	0.605
Lake	63+15	−2.459	0.711	<0.001*

*Indicates significant effects.

Table 2. GLM results of NSR relationships with level of invasion, depth, and lake.

Invasion intensity	*N*	Odds ratio (B)	Std. Error	*P*
Model (intercept)	2	11.562	1.656	<0.001*
Invasion intensity	78	−0.526	0.206	0.001*
Depth (cm)	78	−0.007	0.015	0.593
Lake	63 + 15	−2.459	0.711	<0.001*

*Indicates significant effects.

invasion intensity (GLM, Odds ratio $= -1.909$, df $= 14$, $p = 0.035$). No significant effect of depth or species richness was found.

Principal Coordinates Analysis (PCO) was used to identify communities or species spatial associations within Lake 1 (Fig. 2). Only data from Lake 1 were used to avoid the confounding factor of location and the relatively small sample size from Lake 2. Principal Component 1 (PC1) explained 24% of the variability in species associations, whereas PC2 explained 20% of the variability. Visually, species can be separated into 2 spatial clusters, with the cluster that includes the two invasive *Myriophyllum* species associated with positive PC2 values, whereas a distinct macrophyte species association which is characterised by native species is has negative PC2 values (Fig. 2).

3.1 Floating canopy

Four species formed the dominant floating canopy. In cases where there no floating species were present, it was recorded as ‘no canopy’. Variegated pond lily (*Nuphar variegata* Durand) canopy was associated with the highest species richness (mean 7.8 native species, 8.7 overall species), ‘no canopy’ had the lowest (mean 4.6 native species, 5.5 overall species), and water shield (*Brasenia schreberi* J.F.Gmel.), American white waterlily (*Nymphaea odorata* Aiton), and broad-leaved pondweed (*Potamogeton amplifolius* Tuck.) had similar intermediate relationships with species richness (Fig. 3).

Table 3. List of all species sampled in the two lakes with average occurrence at each lake.

Species	Abbreviation	Proportion of sites in lake present	
		Lake 1	Lake 2
Bidens. beckii	B.bec	0.35	0.00
Brasenia. schreberi	B.sch	0.71	0.00
Ceratophyllum demersum	C.dem	0.03	0.25
Chara sp.	Char.	0.27	0.08
Eleocharis acicularis	E.ec	0.02	0.00
Eriocaulon aquaticum	E.aq	0.03	0.00
Elodea canadensis	E.can	0.27	0.75
Eleocharis robbinsii	E.rob	0.40	0.00
Heteranthera dubia	H.dub	0.00	0.08
Myriophyllum alterniflorum	M.alt	0.08	0.00
Myriophyllum heterophyllum	M.het	0.73	0.00
Myriophyllum spicatum	M.spic	0.14	0.92
Nymphoides cordata	N.cor	0.13	0.00
Najas flexilis	N.flex	0.32	0.00
Najas guadalupensis	N.guad	0.00	0.67
Nymphaea odorata	N.odo	0.86	0.00
Nymphoides variegata	N.var	0.52	0.00
Potamogeton amplifolius	P.amp	0.37	1.00
Potamogeton bicupulatus	P.bic	0.17	0.00
Pontederia cordata	P.cord	0.06	0.00
Potamogeton epihydrus	P.epi	0.06	0.00
Potamogeton foliosus	P.fol	0.29	0.00
Potamogeton gramineus	P.gram	0.03	0.00
Potamogeton illinoensis	P.ill	0.25	0.00
Potamogeton obtusifolius	P.obt	0.05	0.00
Potamogeton robbinsii	P.rob	0.43	1.00
Potamogeton zosteriformis	P.zos	0.02	0.25
Sagittaria graminea	S.gram	0.16	0.58
Scirpus sp.	Scir.	0.08	0.00
Utricularia geminiscapa	U.gem	0.05	0.00
Utricularia macrorhiza	U.mac	0.02	0.00
Utricularia purpurea	U.pur	0.70	0.00
Vallisneria americana	V.am	0.37	0.00

The abbreviations included here are used in Figure 2.

Chi-square tests tested differences in frequency between each dominant canopy species and invasion intensity, using the lowest invasion intensity as a baseline reference group. No differences in frequency of dominant floating canopy species at no invasion compared to frequency at higher invasions was observed: [invasion level 2 ($X^2 = 1.323$, df $= 4$, $N = 20$, $p = 0.857$), invasion level 3 ($X^2 = 2.042$, df $= 4$, $N = 20$, $p = 0.728$), invasion level 4 ($X^2 = 7.504$, df $= 4$, $N = 20$, $p = 0.112$), invasion level 5 ($X^2 = 8.319$, df $= 4$, $N = 20$, $p = 0.081$)].

3.2 Submerged canopy

Nine dominant submerged canopy (DSC) species were recognized originally. The species *Eleocharis acicularis* (L.) Roem & Schult*, Elodea canadensis* Michx and *Najas flexilis* (Willd.) Rostk. & W.L.E. Schmidt were only dominant in one quadrat, therefore they were removed from the analysis. When there was complete codominance between variable-leaf and *M. spicatum*, it was recorded as ‘*Myriophyllum*’.

Chi-square tests identified a significant difference in canopy species frequency between a baseline of no invasion

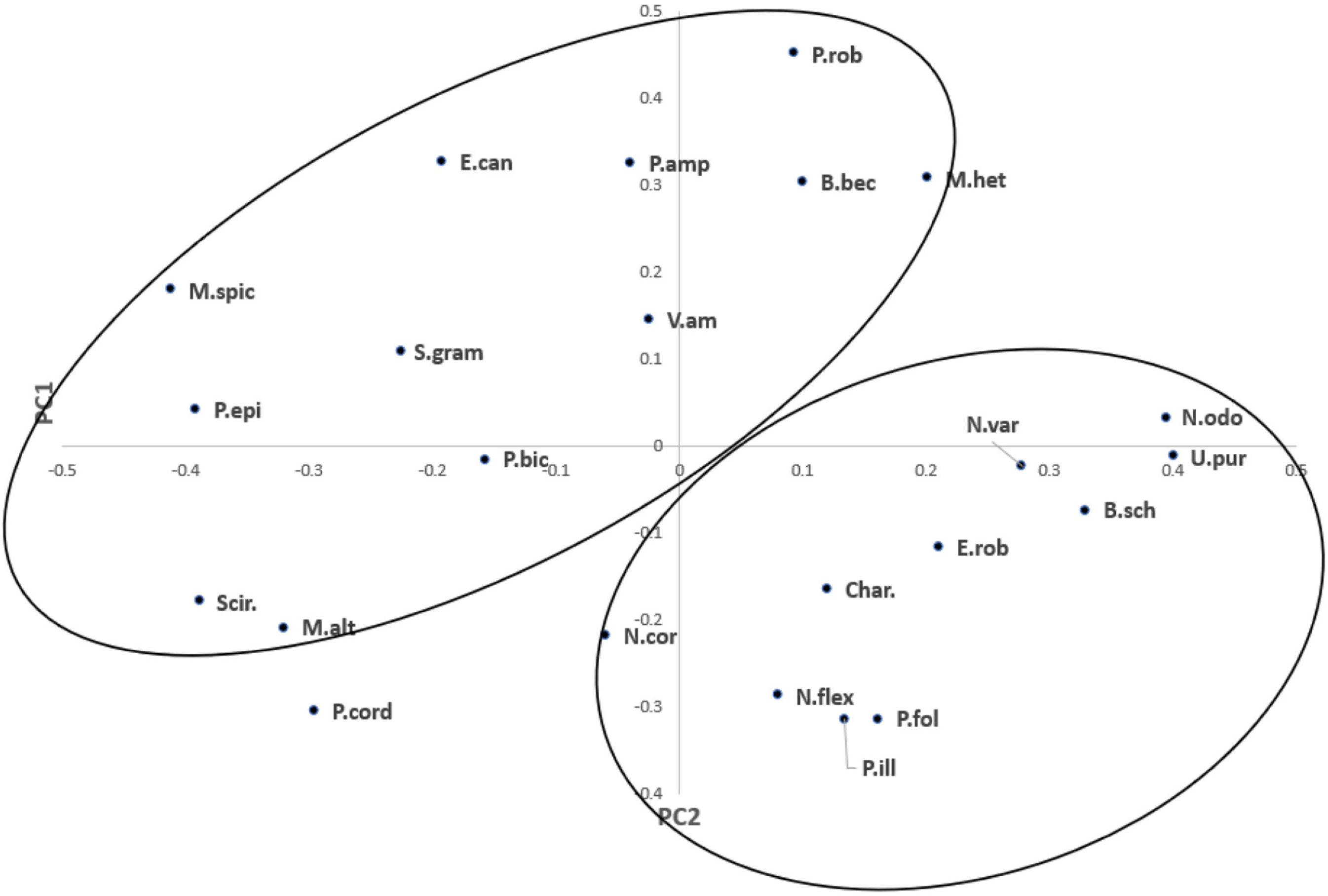

Fig. 2. PCO biplot depicting the proportion present at sampling sites in each lake of all macrophytes sampled (min used in analysis, $N=3$). The corresponding species name for the species acronyms are given in Table 3.

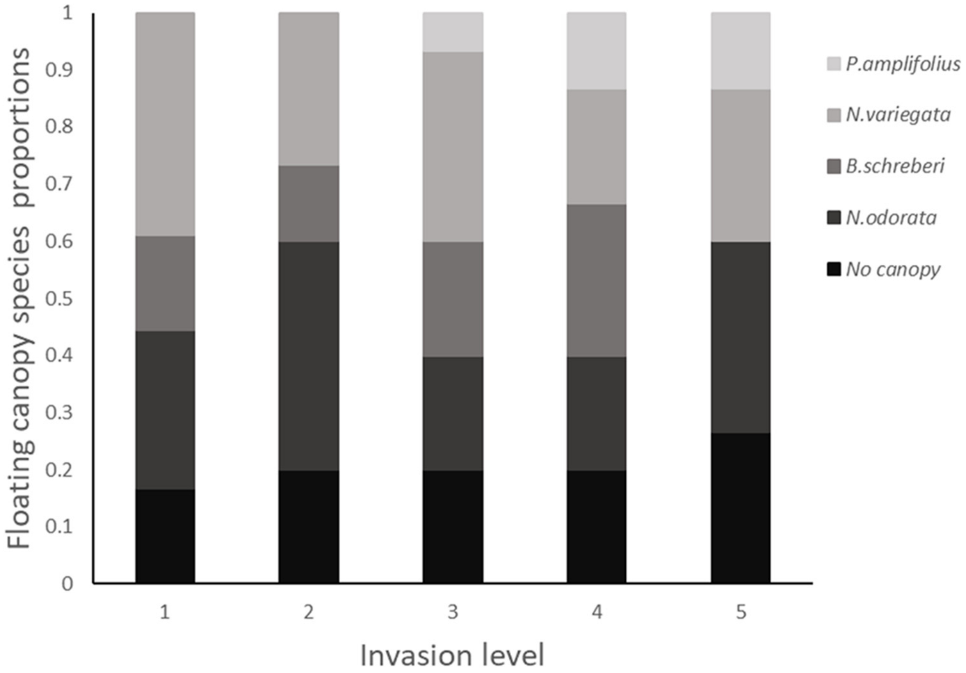

Fig. 3. Bar charts showing frequency of dominance of each floating canopy species the five different invasion intensities. There is little change in dominant canopy types across invasion intensities. The main difference is the appearance of *P. amplifolius* as a dominant canopy species at invasion levels 3, 4, and 5.

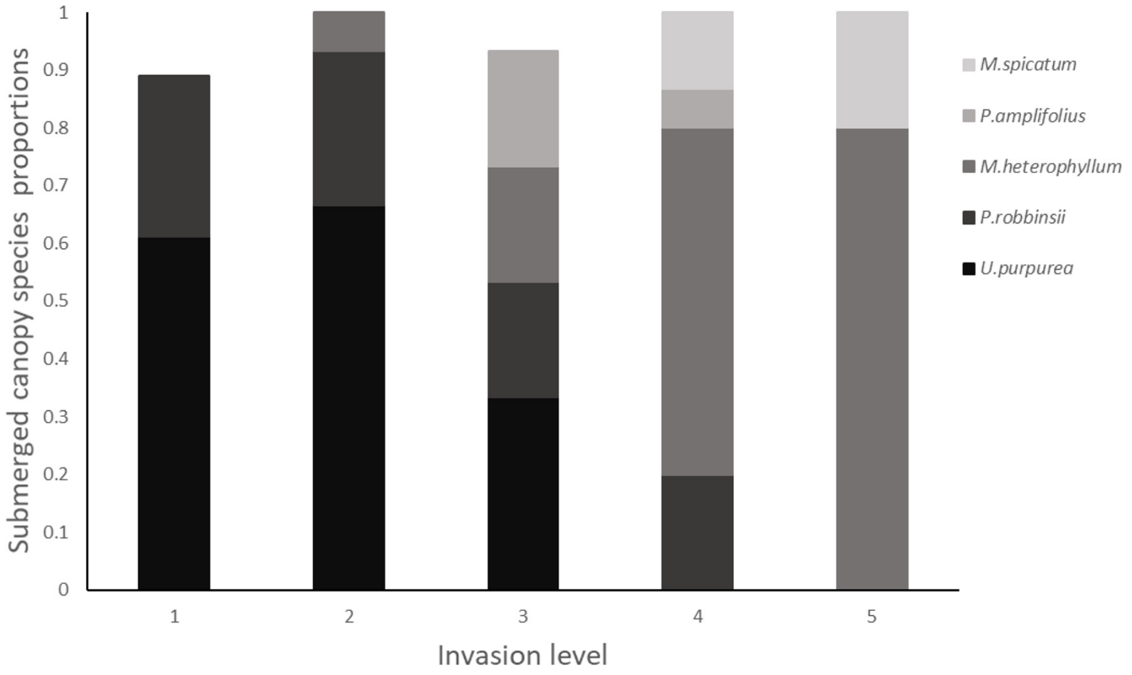

Fig. 4. Bar charts showing frequency of dominance of each submerged canopy species across the five invasion intensities. There is a visually obvious difference in frequency of canopy types at different invasion levels. Invasion levels 1 and 2 are dominated by *P. robbinsii* and *U. purpurea*, while invasion levels 3, 4, and 5 are increasingly dominated by *Myriophyllum* species, although the native species *P. amplifolius* and *P. robbinsii* persist until invasion level 5.

Table 4. Pairwise Mann-Whitney tests for OSR under each set of submerged canopy species.

	M. heterophyllum		*M. spicatum*		*P. amplifolius*		*P. robbinsii*	
	W	*p*	W	*p*	W	*p*	W	*P*
M. spicatum	322.5	0.369						
P. amplifolius	285.0	0.404	18.5	0.118				
P. robbinsii	437.0	0.563	50.0	0.860	48.0	0.449		
U. purpurea	365.0	<0.001*	21.5	0.002*	42.0	0.226	203.0	0.002*

*Indicates significant relationships.
Significance adjusted for ties. *M. heterophyllum n = 22*, *M. spicatum n = 5*, *P. amplifolius n = 4*, *P. robbinsii n = 15*, *U. purpurea n = 26.*

and invasion levels three ($X^2 = 23.633$, df = 5, $N = 20$, $p < 0.001$), four ($X^2 = 84.548$, df = 5, $N = 21$, $p < 0.001$), and five ($X^2 = 133.262$, df = 5, $N = 21$, $p < 0.001$), demonstrating that invasion intensity did have a significant relationship with submerged canopy structure (Fig. 4).

OSR differed significantly between the submerged canopy species (Kruskal-Wallis, H = 21.22, df = 5, $p = 0.001$). Mann-Whitney Pairwise tests (Tab. 4) showed that there was a significant difference in OSR between *M. heterophyllum* × *U. purpurea*, *M. spicatum* × *U. purpurea*, and *Potamogeton robbinsii* Oakes × *U. purpurea*. Canopies dominated by *U. purpurea* had the highest OSR (average 8.8 species) and NSR (average 8.2 species). *Myriophyllum spicatum* had the lowest OSR (average 6.0 species) and NSR (average 5.0 species).

There was also a significant difference in NSR under different submerged canopies (Kruskal-Wallis, H = 28.36, df = 5, $p < 0.001$). Pairwise Mann-Whitney tests of differences in NSR between submerged canopy types identified significant differences between *U. purpurea* canopies and almost every other submerged canopy (Tab. 5). *P. amplifolius* × *U. purpurea* was not significant (Mann-Whitney W = 31.0, $p = 0.0578$).

3.3 Focal species: *B. beckii*

Analysis of *B. beckii* was done on data from Lake 1 because *B. beckii* does not appear in Lake 2. It was found most frequently at invasion level 3 (frequency = 0.500) and was most common under a *N. odorata* floating canopy (frequency = 0.500) and a *P. robbinsii* submerged canopy (frequency = 1.000). GLM analyses with presence/absence data of *B. beckii* investigated the relationship between this species' presence, invasion intensity, and depth. It was found that *B. beckii* presence cannot be predicted by invasion intensity but is significantly influenced by depth. The presence of this species was positively correlated with depth (GLM, $N = 63$; Odds ratio B = 0.055; $p = 0.004$). After

Table 5. Mann-Whitney Pairwise tests showing species that have significantly different relationships with NSR.

	M. heterophyllum		*M. spicatum*		*P. amplifolius*		*P. robbinsii*	
	W	p	W	p	W	p	W	p
M. spicatum	318.0	0.536						
P. amplifolius	281.5	0.272	18.5	0.118				
P. robbinsii	425.0	0.838	51.0	0.929	48.5	0.418		
U. purpurea	322.0	$<0.001^*$	17.0	$<0.001^*$	31.0	0.058	200.0	0.002^*

*Indicates significant relationships.

Significance adjusted for ties. *M. heterophyllum n = 22*, *M. spicatum n = 5*, *P. amplifolius n = 4*, *P. robbinsii n = 15*, *U. purpurea n = 26.*

removing depth from the GLM, the relationship with invasion did not change.

3.4 Focal species: *U. purpurea*

Analysis of *U. purpurea* was carried out on species presence/absence data from Lake 1 because *U. purpurea* is not present in Lake 2. *U. pupurea* was found most commonly (frequency = 1.000) at invasion level two, under a floating canopy of *N. odorata* (frequency = 0.864) and a submerged canopy of *M. heterophyllum* (frequency = 0.636). The presence patterns of *U. purpurea* were examined with a GLM, which identified invasion level five as a significant negative predictor (GLM, N = 12, Odds ratio B = −2.364, p = 0.018) of *U. purpurea* presence. No other invasion levels nor depth were significant predictors of *U. purpurea* presence.

4 Discussion

4.1 Methodology

This study describes a modified portable quadrat that allows for efficient, fine-resolution, low-impact, single-observer study of submerged macrophyte systems in three-dimensional space. This quadrat design is useful for short-term study where installation of a permanent quadrat is impractical. It is non-destructive, thus it is well suited to use in sensitive habitats or, in this case, habitats where disturbance can encourage invasion. Alternate methods such as rake sampling (Buchan and Padilla, 2000) create patches of disturbed ground which milfoil is likely to colonize. We therefore suggest that such non-destructive sampling approaches that consider the three-dimensionality of the aquatic environment should be employed for macrophyte sampling and surveys in lakes. The addition of a meter-ruler on the vertical pole would allow the exact positioning of each sampled macrophyte, if such fine-scale information is required.

This study also examines the effects of including invasive species in species richness counts, with the impacts on species richness analysed twice, with and without invasive species. The overall conclusions drawn from analysis of these two counts produce different results. A significantly negative impact on species richness was found only when the invasive species were excluded. This is important to note when comparing across studies in invasion ecology literature, as significant impacts of invasive species can be masked by including them in the community analysis.

4.2 Invasion trends

The negative relationship between community species richness and increasing invasion intensity adds to the evidence base of negative impact of *Myriophyllum* species on native communities (Boylen *et al.*, 1996, 1999; Madsen, 1999). A study examining effects of *Myriophyllum* invasion over time (Boylen *et al.*, 1999) found that species richness declined as the invasion progressed. Other studies have demonstrated that the impact of *Myriophyllum* on native macrophytes is dependent on the local environmental conditions (Grudnik and Germ, 2013). In the present study, the highest invasion level had a significant negative effect on NSR while no other invasion level did. Some studies have discovered positive relationships of these species with community diversity. These inconsistencies in the literature may come from sampling bias, unexpected ecological relationships, or truly varying relationships of *Myriophyllum* in different study areas. However, the generally accepted consensus is that invasive *Myriophyllum* does negatively impact community diversity, and the present study adds support to that claim.

4.3 Species responses to invasion

The PCO analysis identified associations of species according to the frequency at which they co-occurred. This analysis provided useful insights when considering the individual species' invasion response. It is evident that some species co-occur with *Myriophyllum* while others do not. With this information comes the potential for developing indicator species for degree of invasion based on the presence of sensitive/tolerant species. For example, in the Lake 1 system, areas with a higher proportion of *N. variegata, B. schreberi,* and *U. purpurea* were less likely to be heavily invaded. Likewise, areas with high proportions of *P. amplifolius, E. canadensis,* and *P. robbinsii* tended to have higher invasion levels. *P. robbinsii* has shown tolerance to milfoil invasion in other studies (Madsen *et al.*, 1991). This contrasts previous evidence that these three species were negatively affected by milfoil invasion (Boylen *et al.*, 1999). This may be caused by different regions or different sampling techniques but is still noteworthy. It is more difficult to make claims about relationships of rarer species (encounter rate of species in Lake 1 is available in Tab. 3).

4.4 Canopy types

The use of canopy type as a character of invasion intensity is sparse in the literature with some recent evidence that the presence of native macrophyte species (including canopy species), does not tend to limit the colonisation success of invasive macrophytes (Louback-Franco *et al.*, 2019). This study examines floating and submerged canopy relationships with invasion intensity and overall/native species richness. The results indicate that there is no relationship between floating canopy and invasion intensity. However, there is an effect of submerged canopy on invasion. This is expected as the invasive species examined here are part of the submerged canopy. When examining submerged canopies in a species richness context, patterns emerge. In pairwise tests of the species richness associated with each canopy species, *U. purpurea* stands out as the submerged canopy that harbours significantly more native species richness than the other canopy species. This is supported by the Plant Canopy Hypothesis, which notes that the localized effects of certain aquatic plant species on water chemistry may be pronounced enough to denote areas with different dominant canopies as different habitats (Frodge *et al.*, 1990). As such, the concept of plant canopies should be incorporated into invasion studies more frequently, especially in the study of invasive species that themselves create new chemical environments.

4.5 Species of conservation interest

Bidens beckii is a species of interest because it is threatened in Connecticut and therefore its presence can slow down or even prevent active management. In this study, the only significant predictor of this species' presence was depth, not invasion with its abundance being highest at intermediate invasion intensity. This has implications for management. It appears that this species' niche overlaps with that of invasive *Myriophyllum*, so spot treatment of *Myriophyllum* beds is not a solution that minimizes impacts on this threatened species. Studies like the present one could be important for informing management of other aquatic systems seeking to manage for both threatened and invasive species.

Utricularia purpurea became a species of interest due to its co-occurrence with high native species richness and low occurrence with invasive species, and in this sense it can be used as an indicator species of fairly pristine macrophyte communities with high species richness and low occurrence of invasive species. A GLM analysis of this species investigating the influence of water depth and invasion intensity identifies the highest invasion level as the greatest predictor of its absence. Capers *et al.* (2007) note that native species density, not richness, has a negative relationship with invasive species presence. Furthermore, it has also been observed that macrophyte communities with higher species richness over-yielded, and thus had better invasion resistance (Petruzzella *et al.*, 2018). The dense mats formed by *U. purpurea* that rarely co-occurred with *Myriophyllum* in this study could further support this claim. Thus, presence of *U. purpurea* may be an indicator of lower invasion intensity, and its dominance as a canopy type could be used as an indicator of high native species richness.

5 Conclusion

The new modified sampling method introduced and used in this study allowed us to collect accurate data on both canopy and well as submerged canopy and subsequently assess impacts of invasive macrophytes separately on each group.

The present study provides support for results of previous studies, confirming the negative effect of invasive *Myriophyllum* species on native macrophyte communities. It also develops traditional quadrat designs to make single-observer sampling in fragile macrophyte communities easier, less destructive, and thus potentially more accurate. Species presence patterns reveal community types with different relationships with invasion. Relationships between canopy type and invasion and species richness show potentially useful results for characterizing communities. Some of the most important results come from the examination of species of conservation interest. Co-occurrence of a threatened species with beds of invasive species have implications for management. One submerged canopy species (*U. purpurea*) may be having significant interactions with invasive species. Further study on the interactions between *U. purpurea* and invasive *Myriophyllum* is desirable.

Conflict of interest

The authors declare that they have no conflict of interest.

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