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Long-term studies of crayfish-invaded lakes reveal limited potential for macrophyte recovery from the seed bank

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Abstract: In situ seed banks are a promising avenue for restoration of plant communities damaged by anthropogenic changes. The invasive rusty crayfish (*Orconectes rusticus*) has had well documented effects on macrophytes in north temperate lakes. To understand mechanisms of macrophyte recovery from the seed bank, we examined seed banks from lakes with different durations of exposure to *O. rusticus* and compared these findings to long-term macrophyte surveys. We cored littoral-zone sediments at sediment depths of 0–5, 5–10, and 10–15 cm to test whether deeper layers hold viable seeds that can be used to restore macrophytes. The seed bank in uninvaded lakes contained a higher number and richness of viable seeds than did the seed bank in invaded lakes. Number and richness of viable seeds decreased with sediment depth in all lake categories, and most seeds were found in the top 10 cm. Among all lakes, at most, 33% of the species recorded in field surveys of standing vegetation were represented in the seed bank. We conclude that the seed bank has only modest potential to contribute to natural recovery. Natural regeneration of the macrophyte community is more likely to rely upon vegetative propagules from remnant stands in invaded lakes, especially from very soft-bottom areas avoided by crayfish. Our results emphasize the importance of preventing destruction of macrophytes, especially in lakes without habitats likely to constitute a refuge for macrophytes.

Key words: *Orconectes rusticus*, germination assay, restoration, north temperate lakes, *Potamogeton*, *Najas flexilis*, *Chara*

Multiple anthropogenic stressors, including eutrophication, sedimentation, acidification, and addition of nonnative species, can reduce aquatic vegetation. Once such disturbances cease, seed banks can contribute to natural recovery of the plant community. Seed banks for grassland and wetland ecosystems have been studied under a variety of circumstances (e.g., duration and nature of disturbance; Stroh et al. 2012), but much less is understood about seed banks for submerged macrophytes in lakes. A lake seed bank may be less abundant than those from terrestrial and semi-aquatic environments because submerged aquatic plants have a lower propensity for sexual reproduction and rely more upon production of vegetative structures for propagation and spread (Muenscher 1936, Haag 1983, Kautsky 1988). However, most aquatic macrophytes retain the ability to flower and set seed, and a few species reproduce exclusively by sexual means (McFarland and Rogers 1998). Therefore, increased understanding of aquatic plant seed banks could guide efforts to restore macrophytes once a disturbance ends.

In the north temperate lake region of Wisconsin and Michigan, the landscape comprises a patchwork of lakes

with different histories of exposure to the nonnative rusty crayfish (*Orconectes rusticus*), which has well documented deleterious effects on aquatic plants. High densities of rusty crayfish are associated with decreases in standing macrophyte abundance and species richness (Wilson et al. 2004). A survey of 57 lakes in Wisconsin revealed that sites were less likely to have macrophyte cover as crayfish density increased (Roth et al. 2007). Moreover, species richness of the seed bank from an invaded lake was depauperate compared to that from an uninvaded reference lake (Rosenthal et al. 2006). A reduction in macrophyte abundance has indirect and direct effects on higher taxonomic levels (Wilson et al. 2004) and implications for physicochemical processes (Carpenter and Lodge 1986). Restoration of the plant community is an important step for reestablishing normal ecosystem function after a disturbance, but the location, abundance, and viability of macrophytes seeds are largely unknown.

The vertical distribution of viable seeds in lake sediments has implications for restoration. Depth of seeds in the sediment can be an indicator of seed age (Leck 1989, Bekker et al. 1998), and previous investigators have used

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sediment depth to examine the history of seed deposition (Bonis and Lepart 1994, Xiao et al. 2010, Lu et al. 2012). However, rates of sediment accumulation in littoral zones of lakes can be highly variable because of microhabitat conditions, and accumulation rates can range from 0.2 to 3.6 mm/y (Benoy and Kalff 1999). Deeper sediment layers in invaded lakes may contain seeds deposited before the destruction of standing aboveground vegetation by *O. rusticus*. If so, a possible restoration strategy would be to overturn sediments in the littoral zone to encourage germination of deeper, in situ seeds by exposing them to the conditions required for germination.

Development of effective restoration strategies requires information on unaffected reference ecosystems, knowledge of the causes and consequences of degradation, and knowledge of recovery processes (Clewell and McDonald 2009). The first 2 requisites usually are satisfied in the course of studies on effects of invasive species, but few investigators examine the recovery process in a way that is informative for restoration. The time frame of disturbance and the resulting effects are important for planning and prioritizing restoration actions. We studied lakes in 3 *O. rusticus*-exposure categories: uninvaded, invaded in the past 24 y, and invaded >24 y ago.

Our goal was to assess the potential for natural recovery of aquatic plants from lake seed banks, and we focused on lakes invaded by *O. rusticus*. We surveyed the aboveground macrophyte community to provide a quantitative reference point for comparison of aboveground and seed-bank species richness. We then used greenhouse germination assays of sediments collected from 3 sediment depths to test 3 hypotheses: 1) richness and abundance of viable seeds will be greater in lakes uninvaded by *O. rusticus* than in lakes with a history of invasion, 2) in uninvaded lakes, richness and abundance of viable seeds will decrease with sediment depth, and 3) in invaded lakes, richness and abundance of viable seeds will increase with sediment depth.

METHODS

Site description and field sampling

We studied 6 lakes in Vilas County, Wisconsin (USA), that are part of a larger multiyear survey of crayfish and macrophytes begun in 1987. The sites had substrate that could be sampled with a corer (sand or firm muck). We visited each sampling site 3 times (1987, 2001, 2011) to collect data on crayfish and aboveground abundance of macrophytes and once (2011) to collect sediments for seed germination assays. We used maps, landmarks, and global positioning system coordinates to ensure that the same location and water depth were sampled each time (Table 1).

We categorized the lakes on the basis of their duration of exposure to *O. rusticus* (Table 1). Following Kreps et al.

(2012), we considered a lake to be uninvaded if average trap catches of rusty crayfish were <10 male crayfish/trap and invaded if the crayfish populations exceeded this threshold. Below this threshold, effects of rusty crayfish on macrophyte standing stock are minimal (Wilson et al. 2004). The uninvaded lakes, Wild Rice and Upper Allequash, have always been below the threshold. In the transition lakes, *O. rusticus* reached invasive densities ≥ 10 (Little John) and 16 y (Plum) ago (DML, unpublished data). The invaded lakes, Presque Isle and Papoose, have had invasive densities of *O. rusticus* for ≥ 34 and 24 y, respectively (Capelli and Magnuson 1983, Olsen et al. 1991). Sampling in many of these lakes was infrequent around the time of *O. rusticus* invasion, so these are conservative estimates based on the date of the first record where trap catch was >10 male crayfish/trap.

We surveyed standing vegetation in late July to early August 1987, 2002, and 2011 with the line–intercept method (Brower et al. 1997). At each site in each lake, divers visually assessed the presence or absence of all macrophyte species crossing the vertical plane of a 0.10-m segment at 1-m intervals along a 25-m transect. Rare species (those occurring at just 1 site in a lake) without a positive identification were not included in our analyses. During the same periods, we used standard methods to sample rusty crayfish (i.e., 1 modified Gee minnow trap baited with 120 ± 10 g of beef liver and set overnight at each site) (Capelli and Magnuson 1983).

We collected sediments for seed-bank quantification from 2 to 3 sites in each lake (total sites = 17) in September 2011. At each site, we collected a core every ~ 3 m along a 25-m transect following the depth contour parallel to shore. We constructed a custom coring apparatus from 10.16-cm-diameter polyvinyl chloride (PVC) pipe that was capped at the top. We cut thin transverse slits at 5-cm intervals from the bottom edge of the pipe and resealed them with electric tape to facilitate dividing the sediment cores into depth strata. A rubber stopper inserted into the cap after the corer was pushed into the sediment created a sufficient vacuum to hold the sediments in place. We immediately divided each core into 3 depth strata (0–5, 5–10, and 10–15 cm) by inserting a sharp divider into each of the slits. We collected a total of 8 cores on each transect, for a total bottom area = 0.065 m^2 . We pooled the 8 subcores by depth to produce 3 composite samples for each site (total sample volume for each sediment layer = 3.2 L). The sediments were kept in dark storage at 4°C for 20 to 24 wk to simulate winter stratification (a condition important for breaking seed dormancy; Haag 1983).

Greenhouse germination assays

We sieved all sediments collected in the field to remove particles <250 μm and >4 mm to improve germina-

Table 1. Summary of study lakes and site characteristics.

Category	Lake	Coordinates (Lat/long)	Lakewide mean (SE) <i>Orconectes rusticus</i> /trap			Site	Site depth (m)
			1987	2002	2011		
Uninvaded	Upper Allequash	46.037°N, 89.629°W	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	AL08	0.75
						AL15	2.5
						AL17	1.7
	Wild Rice	46.070°N, 89.803°W	0.1 (0.1)	0.6 (0.2)	0.1 (0.1)	WR02	1.3
						WR07	0.9
						WR12	0.9
Transition	Little John	46.016°N, 89.640°W	0.0 (0.0)	17.6 (1.6)	16.5 (1.5)	LJ04	1.7
						LJ27	1.7
	Plum	46.997°N, 89.533°W	6.9 (2.4)	28.3 (2.8)	12.6 (2.1)	PL09	0.75
						PL13	3.1
						PL31	0.75
Invaded	Papoose	46.223°N, 89.771°W	37.9 (4.5)	23.0 (2.9)	39.5 (4.8)	PA02	2.7
						PA04	1.8
						PA24	1.8
	Presque Isle	46.176°N, 89.807°W	39.0 (6.3)	47.9 (4.8)	49.6 (3.6)	PI16	2.5
						PI21	2.5
						PI24	2.5

tion by reducing sample volume (ter Heerdt et al. 1996). We measured % organic content as loss on ignition at 550°C from triplicate 5- to 8-mL sediment samples collected before sieving (Steinman et al. 2007). We assigned sediment samples randomly to tanks ($n = 51$) in a greenhouse. Tanks were clear 22.7-L containers (0.1 m² surface area) containing 5 cm of autoclaved and rinsed sand (Play Sand; Quikrete Companies, Inc., Atlanta, Georgia), covered by the lake sediments and 10 to 12 cm of aerated well water. Four control tanks contained only sand to test whether the sand base contained seeds. Three additional control tanks contained sand and 1.5 L of the silt/clay sediments (<250 µm) removed by sieving to check for small seeds or spores that might have been lost during sediment processing. No germination occurred in any control tank, so we do not report further on either kind of control tank. We maintained all tanks under full-spectrum metal halide lights (14 h:10 h light:dark cycle) at ambient temperatures ranging between 21 and 26°C. The composition of the sediment samples differed among sites and depths, so the final volume of sediment added to each tank after sieving also varied. We recorded sediment height in each tank after lake sediments were added.

We ran germination assays for 10 wk. To aid in identification of plants, we promoted foliage growth by adding 1.5 mL of Leaf Zone Aquarium Plant Food (0.10% chelated Fe, 3.0% soluble potash; Mars Fishcare, Mars, Pennsylvania) to each tank after the number of germinating

seeds reached a plateau (~week 8). These nutrients do not affect germination (Albano Pérez et al. 2011). We identified and removed seedlings once diagnostic features (e.g., leaves, stipules, stems, and seed coats) became clear enough for identification to species or genus with the aid of keys by Crow and Hellquist (2006a, b). We cultivated some seedlings after the end of the experiment until diagnostic features were sufficiently developed.

Data analysis

We ran all analyses in R (version 2.15.1; R Project for Statistical Computing, Vienna, Austria). We analyzed lakewide mean species richness from field surveys with a 2-way analysis of variance (ANOVA) with lake category and year as factors. We used linear models with sediment depth and lake category as factors to analyze the lakewide means for seed species richness and count (seedling count was transformed as $\log[\text{count} + 1]$ to meet the assumption of normality). The uninvaded lakes and the 0–5-cm sediment depth were designated as the reference level within their respective factors against which the other levels were tested. We tested for potential influence of site-specific factors on seed richness and abundance with multiple linear regressions for each response variable against water depth of collection site, sediment height in the germination tanks, and % organic matter of the sediment sample. We further explored the relationships between duration

of exposure to *O. rusticus* and seed richness and count with Pearson's correlation tests for each response variable at each sediment depth against time since invasion. Each comparison met the assumption of normality according to Shapiro-Wilk tests, but we $\log(x)$ -transformed the data for presentation.

We calculated Sørensen's similarity coefficient to describe the similarity between the seed bank and standing vegetation in the field for each lake category. The coefficient is calculated as $S_s = 2c/(a + b)$, where a is the number of species in the seed bank, b is the number of species in the field surveys, and c is the number of species in common. We could not assign any 1 sediment depth layer to a specific time period, so we pooled species data from all 3 sediment depths in each lake category and compared this value to the field surveys over time. We analyzed S_s with a randomized complete block ANOVA with lake category blocked by year.

RESULTS

Invasion by *O. rusticus* significantly affected standing macrophyte species richness among lake categories. The highest richness occurred in uninvaded lakes (ANOVA, $F_{2,9} = 59.86, p < 0.0001$; Fig. 1). Transition lakes showed a slight decrease in macrophyte species richness between 1987 and 2002 (the period during which rusty crayfish densities increased in these lakes), but overall, changes over time were not significant ($F_{2,9} = 2.20, p = 0.17$). All plant surveys in the invaded lakes were conducted after crayfish densities were high, and they reveal consistently low macrophyte species richness (Fig. 1).

Greenhouse assay of seedling species richness and seedling count revealed significant differences among lake categories and over time (Fig. 2A, B). Species richness was significantly lower at 10–15 cm than at 0–5 cm (linear

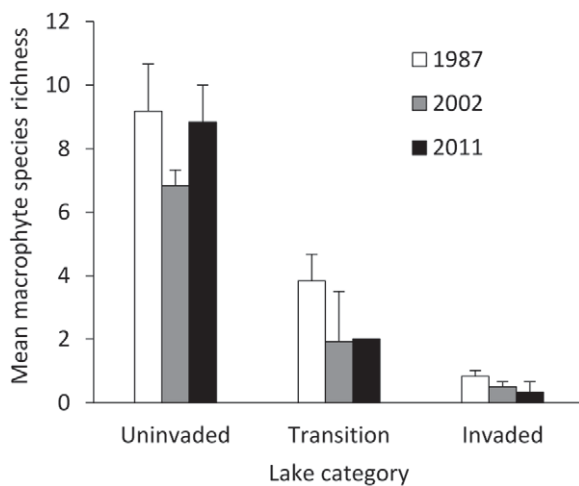


Figure 1. Mean macrophyte species richness (+1 SE) from field surveys for each lake category at 3 time points.

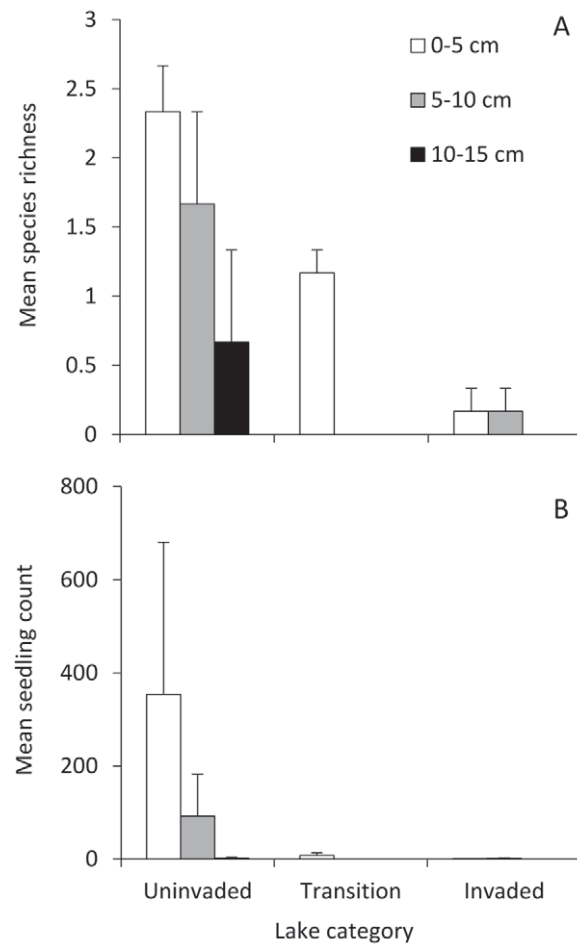


Figure 2. Mean (+1 SE) species richness (A) and seedling count (B) of germinating seeds from the greenhouse assays among lakes in each invasion category and at 3 sediment depths.

model: $t = -3.40, p < 0.01$; Fig. 2A), but did not differ between 0–5 and 5–10 cm ($t = -1.36, p = 0.21$). Richness was significantly greater in uninvaded than in transition ($t = -2.38, p = 0.04$) and invaded lakes ($t = -4.42, p < 0.01$). None of the depth \times lake-category interactions were significant (all $p > 0.06$). Site-specific factors did not influence species richness (multiple linear regression: % organic matter, $t = -0.49, p = 0.63$; sediment height, $t = 0.07, p = 0.95$; water depth, $t = -1.63, p = 0.13$). The count of germinated seedlings was significantly lower at 10–15 than at 0–5 cm (linear model: $\log[\text{count} + 1]$ -transformed data, $t = -3.11, p = 0.01$; Fig. 2B), but did not differ between 0–5 and 5–10 cm ($t = -1.21, p = 0.26$). Seedling count was significantly lower in transition ($t = -2.34, p = 0.04$) and invaded lakes ($t = -3.58, p < 0.01$) than in uninvaded lakes. None of the depth \times lake-category interactions were significant (all $p > 0.06$). Seedling count decreased significantly with increasing water depth (multiple linear regression: $\log[\text{count} + 1]$ -transformed data, $t = -2.27, p = 0.04$), but was

not affected by % organic matter ($t = -0.38$, $p = 0.71$) or sediment height ($t = -0.17$, $p = 0.87$).

Seed species richness at 0–5 cm was correlated with time since invasion ($r = -0.89$, $p = 0.02$; Fig. 3A, Table 2), but seedling count at 0–5 cm was not correlated with time since invasion ($p = 0.28$). None of the correlations between time since invasion and seedling richness or seedling count were significant at greater sediment depths (all $p > 0.13$; Fig. 3B, Table 2).

Eight taxa germinated in greenhouse assays: *Najas flexilis* (% occurrence among all tanks = 25.5%), *Chara* spp. (17.6%), *Isoetes* spp. (13.7%), *Potamogeton robbinsii* (5.9%), *Potamogeton zosteriformis* (2.0%), *Zosterella dubia* (2.0%), *Eriophorum* sp. (2.0%), and an unidentified dicot (2.0%). Seed species richness was much lower than standing vegetation species richness. At most, 33% of the spe-

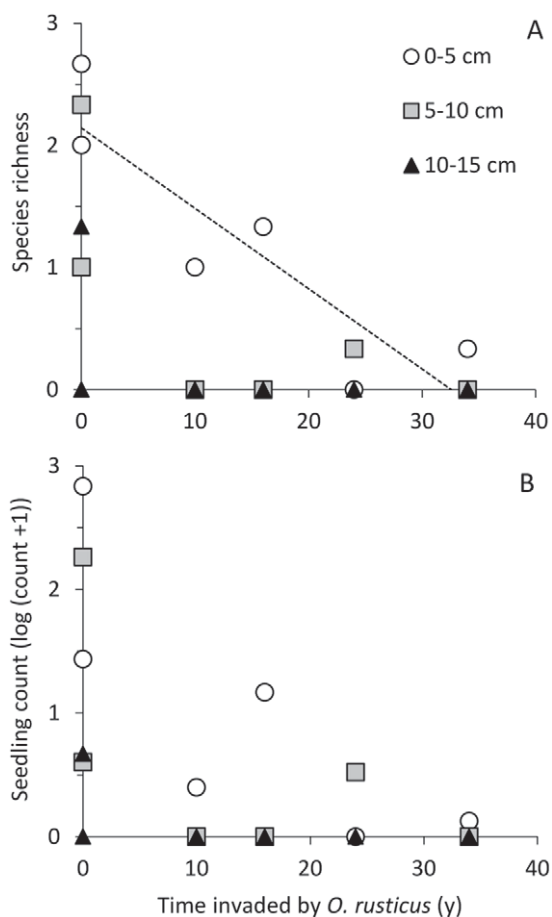


Figure 3. Mean seed species richness (A) and log(seedling count) (B) of germinating seeds as a function of time since invasion by *Orconectes rusticus* for each of the 3 sediment depths. Each datum represents a lake. Analyses were done with Pearson's correlation tests. Lines of best fit (dashed line) from linear regression are included to illustrate significant correlations between species richness at 0–5 cm sediment depth and time since invasion.

Table 2. Results of Pearson's correlation tests between each response variable and the time since invasion by *Orconectes rusticus*. Bold indicates $p < 0.05$.

Response variable	0–5 cm		5–10 cm		10–15 cm	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Species richness	-0.89	0.02	-0.69	0.13	-0.51	0.30
Seedling count	-0.53	0.28	-0.51	0.30	-0.51	0.30

cies recorded in the field were in the seed bank for a given lake category (Table 3). The Sørensen similarity coefficient for the standing vegetation and seed bank differed among the lake categories. Similarity was greater in uninvaded and transition lakes than in invaded lakes (randomized block ANOVA: $F_{2,4} = 26.1$, $p < 0.01$; Table 3). Species that were common in the field surveys but absent in the germination assays included *Ceratophyllum demersum*, *Elodea canadensis*, *Myriophyllum* spp., *Potamogeton amplifolius*, *Potamogeton gramineus*, *Potamogeton richardsonii*, *Sagittaria* spp., and *Vallisneria americana* (Table 4).

DISCUSSION

Our results support the hypotheses that richness and abundance of viable seeds will be greatest in lakes with less exposure to *O. rusticus* and at shallower sediment depths. However, we found no evidence that the richness and abundance of viable seeds increases with sediment depth in invaded lakes. The pattern of overall decreases in richness and abundance of viable seeds in lakes invaded by *O. rusticus* mirrors the documented negative effects of

Table 3. Similarity of species from field surveys of standing vegetation (Field) and from the seed bank that germinated under greenhouse conditions (SB). All 3 sediment depths were pooled among the seed-bank samples. Sørensen's coefficient was calculated based on 2011 values for SB vs 1987, 2002, and 2011 field data.

Category	Year	Field	SB	Found in field and SB	Sørensen's coefficient
Uninvaded	1987	24		6	0.39
	2002	18		6	0.48
	2011	19	7	6	0.46
Transition	1987	12		3	0.40
	2002	6		1	0.22
	2011	6	3	2	0.44
Invaded	1987	3		0	0.00
	2002	1		0	0.00
	2011	1	2	0	0.00

Table 4. Species list for aquatic macrophytes in each lake category, including surveys of standing vegetation over time (I = 1987, II = 2002, and III = 2011) and the results of germination assays for each depth (A = 0–5 cm, B = 5–10 cm, C = 10–15 cm). Species in bold germinated in the laboratory and were found in field surveys. R indicates rare species that appeared in only one of the sites in that lake category. – indicates species not present.

Species	Uninvaded						Transition						Invaded					
	Year			Depth			Year			Depth			Year			Depth		
	I	II	III	A	B	C	I	II	III	A	B	C	I	II	III	A	B	C
Chara spp.	x	x	x	x	x	–	R	–	R	x	–	–	–	–	–	–	R	–
Isoetes spp.	x	x	x	x	x	R	–	–	–	–	–	–	–	–	–	–	–	–
Najas flexilis	x	x	x	x	x	R	x	–	R	R	–	–	–	–	–	–	–	–
Potamogeton robbinsii	R	x	x	R	R	–	x	R	–	R	–	–	–	–	–	–	–	–
Potamogeton zosteriformis	R	x	x	R	–	–	–	R	R	–	–	–	–	–	–	–	–	–
Zosterella dubia	x	R	x	–	–	R	–	–	–	–	–	–	–	–	–	–	–	–
<i>Eriophorum</i> spp.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	R	–	–
Unknown dicot	–	–	–	–	–	R	–	–	–	–	–	–	–	–	–	–	–	–
<i>Bidens beckii</i>	x	–	x	–	–	–	R	–	–	–	–	–	–	–	–	–	–	–
<i>Brasenia schreberi</i>	R	R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Ceratophyllum demersum</i>	x	–	x	–	–	–	x	x	x	–	–	–	–	–	–	–	–	–
<i>Eleocharis</i> spp.	R	x	–	–	–	–	R	–	–	–	–	–	–	–	–	–	–	–
<i>Elodea canadensis</i>	x	x	x	–	–	–	x	x	x	–	–	–	–	–	–	–	–	–
<i>Myriophyllum</i> spp.	x	R	x	–	–	–	x	–	–	–	–	–	R	–	–	–	–	–
<i>Potamogeton alpinus</i>	x	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Potamogeton amplifolius</i>	R	x	x	–	–	–	R	–	–	–	–	–	x	x	x	–	–	–
<i>Potamogeton gramineus</i>	x	x	x	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Potamogeton praelongis</i>	–	–	R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Potamogeton pusillus</i>	–	–	x	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Potamogeton richardsonii</i>	R	x	R	–	–	–	R	R	–	–	–	–	–	–	–	–	–	–
<i>Sagittaria</i> spp.	x	–	x	–	–	–	R	R	–	–	–	–	–	–	–	–	–	–
<i>Scirpus</i> spp.	R	R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Sparganium</i> spp.	R	R	R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Vallisneria americana</i>	x	x	x	–	–	–	R	–	R	–	–	–	R	–	–	–	–	–
<i>Juncus</i> spp.	R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Nuphar</i> spp.	R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Nymphaea</i> spp.	R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Spirodela</i> spp.	–	R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Utricularia</i> spp.	R	–	R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Zizania</i> spp.	–	R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

O. rusticus on standing vegetation. Moreover, the decrease in richness scaled linearly with increasing length of time that the lake had been invaded. Across all lake categories, the vast majority of richness and abundance of viable seeds occurs in the top 10 cm of sediment. We identified limitations for natural recovery from the seed bank in lakes experiencing long-term (i.e., >24 y) invasion by *O. rusticus*. The seed bank in lakes invaded for a shorter period (i.e., 15–24 y) contained a few species that reflected present and past standing plant communities. Uninvaded lakes had the

highest richness and abundance of viable seeds in the sediments, but many species common in the field were not represented in the seed bank.

The species present in the standing vegetation often differ substantially from the species present in the seed bank because of differences among species in methods of propagation and duration of seed viability (Leck 1989, Matus et al. 2003). For example, annual plants have a strategy for higher seed production, whereas plants with longer-lived vegetative structures may rely upon turions or vegetative

reproduction (McFarland and Rogers 1998). The percentage of species in the standing vegetation represented in the seed bank differs across systems and among studies: 50–85% in wetland systems (Wetzel et al. 2001, Stroh et al. 2012) and 41–64% in lakes (Haag 1983, Kimber et al. 1995, McFarland and Rogers 1998, Rosenthal et al. 2006). Only 16% of the aboveground species along a lakeshore with variable flooding rates were detected in the seed bank (Grelsson and Nilsson 1991). Nevertheless, even with consideration of the potential variation in the relationship between aboveground species richness and seed bank richness, our results illustrated a strong impact of rusty crayfish on the seed bank. Sørensen's coefficient scores indicated that similarity between aboveground richness and viable seed richness was highest in the uninvaded lakes and that the invaded lakes had no species in common between the field and seed bank. The lowest non-0 coefficient of similarity was found in the transition lakes in 2002 (the first sampling year after invasion by *O. rusticus*).

The species that appeared most frequently in our germination assays are ones that rely heavily or exclusively on seed, megaspore, or oospore production. *Najas flexilis*, the most common seed bank species in our study lakes, is an annual with no form of vegetative reproduction (Hutchinson 1975). *Najas flexilis* and *Chara* spp. were common in other published germination assays (Kimber et al. 1995, McFarland and Rogers 1998, Rosenthal et al. 2006). These species have high ecological importance because they provide forage for migrating waterfowl and habitat for macroinvertebrates (Krull 1970, Knapton and Petrie 1999).

Vegetative propagation is the dominant reproductive strategy for most of the common species in our field survey that did not appear in the greenhouse assays, including several species of *Potamogeton* (Muenscher 1936), *Ceratophyllum demersum* (Muenscher 1940), *E. canadensis* (Barrat-Segretain and Cellot 2007), *V. americana* (Korschgen et al. 1997), and *Myriophyllum* spp. (Les and Mehrhoff 1999). Based on the success of propagation from seed in other studies (Kimber et al. 1995, McFarland and Rogers 1998), we were surprised that neither *V. americana* nor *Sagittaria* spp. appeared in our greenhouse assay. However, seedlings of *V. americana* are rarely observed in temperate lakes, so it may be that seed production was lower in our study lakes in northern Wisconsin than in shallow lakes in central Wisconsin (Kimber et al. 1995). *Elodea canadensis* produces seeds at shallow depths (<1 m) and has appeared in previous greenhouse germination assays in low densities (Kimber et al. 1995). However, in >3 decades of observations in the lakes studied here, we have never observed *E. canadensis*, *C. demersum*, or *Sagittaria* spp. flowering at the collection depths of our study sites.

Germination for most aquatic plants occurs within 1 or 2 y of when the seed is set, and some species peak at 3 or 4 y (Hutchinson 1975). Thus, aquatic seeds tend to

be more transient than seeds from many terrestrial plants. Terrestrial species usually have transient (few years) or persistent (up to multiple decades) seed-viability strategies (Bekker et al. 1998). A review of European macrophytes revealed that persistence of propagules is transient (<1 y) to short term (Bakker et al. 2013). *Chara* spp. oospores can survive ≥ 4 y (Proctor 1967), and are more likely to persist in deeper sediments. One of the only species in the invaded-lake seed bank was *Chara* spp. at 5–10 cm. *Najas marina* seeds retain viability for >4 y when stored in wet or dry conditions (Agami and Waisel 1984). Given the modest estimates of seed longevity for aquatic plants, that we encountered so few viable seeds at greater sediment depths is not surprising. However, some species of macrophytes found in shallow lakes have regenerated from the seed bank >20 y after the loss of submerged vegetation (de Winton et al. 2000, Lu et al. 2012), and a *Nelumbo nucifera* seed germinated after >230 y (Hutchinson 1975).

Thus, natural recovery from the existing seed bank of a macrophyte community after cessation of a disturbance is most likely when species have only recently been eliminated (van der Valk and Pederson 1989). The seed bank of a fen meadow community represented the predisturbance diversity of a *Cirsio-Molinietum* plant community, but only if the disturbance occurred just a few years prior (Matus et al. 2003). Our examination of seed banks in lakes with known disturbance histories and long-term surveys of standing vegetation confirms that long-term invasions (>24 y) are detrimental to the standing aquatic vegetation and to the potential for natural recovery. The results from transition lakes suggest that invasion for only 15 y substantially diminishes the potential for natural recovery. Even in uninvaded (i.e., undisturbed) lakes, the diversity of species in the seed bank is not sufficient to recreate the aboveground plant community. Therefore, natural recovery would require a combination of regeneration from the seed bank and dispersal of seeds and vegetative propagules from other sources.

Vegetative recolonization from local remnant populations can provide a significant contribution to recovery following disturbance (Bakker et al. 2013). Water currents facilitate transport of relatively heavy vegetative diaspores (Kautsky 1988). Propagules (both seed and vegetative) also could arrive at a site via transport by birds and fish (Agami and Waisel 1984, Carpenter and Lodge 1986). In our invaded study lakes, dispersal of propagules from remnant stands, albeit gradual, may be the most promising avenue for natural recovery.

The reduced species richness detected by our germination assays compared to the field surveys also may be an artifact of sampling effort or greenhouse conditions. The total area of our sediment cores at each site was 0.065 m², and the area surveyed using the line-intercept method was 0.125 to 0.25 m² (assuming that sediments 2.5 or

5 cm on either side of the line transect were captured in the survey). This 2- to 4-fold lower sampling intensity may decrease the chance of detection for seeds relative to aboveground species richness. A lack of suitable germination cues also could help explain why some species did not appear in the greenhouse assays (Stroh et al. 2012). McFarland and Rogers (1998) had good success germinating seeds in a low-alkalinity culture solution (but see Kimber et al. 1995). However, in most studies we encountered, investigators using the emergence method did not apply a chemical treatment to stimulate germination (e.g., Haag 1983, Bonis and Lepart 1994, ter Heerdt et al. 1996, Stroh et al. 2012). A follow-up to our study would be to conduct germination assays in field plots protected from crayfish instead of under greenhouse conditions (similar to Matsuzaki et al. 2009). Doing so would increase the volume of sediment tested and eliminate artifacts that may have prevented germination in the greenhouse.

We removed large particles (>4 mm) from sediments to exclude vegetative propagules (ter Heerdt et al. 1996). However, we also may have removed larger seeds that occur in the seed bank. *Ceratophyllum demersum* usually reproduces via fragmentation, but it also produces large, spiny fruits (Muenscher 1940). *Potamogeton amplifolius*, *P. praelongis*, and *E. canadensis* have seeds with maximum dimensions >4 mm (Haynes 2000, Haynes and Hellquist 2000). However, despite being common in the field, neither *P. amplifolius* nor *E. canadensis* was detected in the seed bank in other studies in which field enclosures (Wilson 2002) or greenhouse germination assays (Rosenthal et al. 2006) were done with unsieved sediments.

Site characteristics can influence rates of seed deposition and sedimentation, both of which directly affect seed-bank dynamics. The water depth of our field sites did not influence seed-bank species richness, but the number of germinating seedlings was significantly reduced at greater water depths. This result is not surprising because seed production decreases with increasing depth because of reduced irradiance and greater time required to grow to the surface, where most flowers are produced (Haag 1983). Based on the range of littoral-zone sedimentation rate estimates by Benoy and Kalff (1999), each of our 5-cm strata could represent 14 to 250 y of sediment accumulation. In lakes with invasive densities of crayfish and, therefore, low coverage of standing macrophytes, sedimentation rates are likely to be reduced (Benoy and Kalff 1999). Thus, a 5-cm sediment core from an invaded lake may represent many years of deposition from before invasion and could contain older seeds that have lost their viability. Our goal was not to pinpoint times of deposition, but rather to examine relative patterns of attenuation of seed viability in each lake. The reduced richness of viable species at greater sediment depths demonstrates that older, deeper sediments (i.e., >10 cm) cannot be used to rejuve-

nate a diverse plant community in invaded lakes. The implication of these factors is that sediments collected near macrophyte beds or in protected bays are more likely to contain viable seeds.

Our first surveys in the invaded lakes occurred after invasion, so we do not have pre-invasion data on macrophyte abundance in these lakes. However, the limnological and morphometric characteristics of these lakes are similar to those of our other study lakes, and we have no reason to think they would not previously have supported a diverse macrophyte community. In a lake with substrate and water-clarity characteristics similar to those of our invaded lakes, the number of submerged macrophyte species decreased from 12 to 4 after invasion (Rosenthal et al. 2006). Moreover, in one of our invaded lakes (Papoose), a mucky bay where crayfish are rarely found supports high macrophyte richness. This remnant stand of vegetation probably reflects the broader pre-invasion macrophyte community and could be a potential source of vegetative propagules for restoration if the crayfish invasion were reversed.

Management intervention often is necessary to reduce the source of disturbance and restore the system to a more desired state. The presence of invasive crayfish (*Procambarus clarkii*) in a Dutch lake inhibited the development of submerged macrophytes (van der Wal et al. 2013). In our invaded lakes, plans to restore the macrophyte community would have to begin with substantial reductions of the *O. rusticus* population. Strategies for controlling invasive crayfish include removal via trapping or trawling and biocontrol with predatory fish (Hein et al. 2007, Peters 2010). Rusty crayfish also can undergo natural population declines (Kreps et al. 2012). In a Wisconsin lake, the macrophyte community recovered, at least partly, within 1 decade after mechanical removal of *O. rusticus*, but the contribution of the seed bank relative to vegetative propagules from remnant macrophyte populations was not estimated (Hansen et al. 2013).

Our findings are relevant to the natural recovery of macrophytes in lakes after many kinds of disturbance, including invasion by other species (de Winton and Clayton 1996) and nutrient loading (Bakker et al. 2013). Because of the transient nature of lake seed banks, management efforts to reduce the source of disturbance in a timely manner are critical to the potential for natural recovery of macrophytes. Benefits of rapid action include increased likelihood of presence of viable seeds in the top sediment layer and preservation of extant remnant populations in the same water body that can contribute seeds and vegetative propagules.

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