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Effect of light on macrophyte sprouting and assessment of viable seedbank to predict community composition

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ABSTRACT

The role of the aquatic macrophyte seedbank in sustaining macrophyte communities in lakes is understudied. Therefore, a controlled laboratory experiment was conducted using sediment from two Minnesota lakes. Lake Ann had historically good water clarity and a high diversity of native macrophytes, whereas Lake Riley had historically poor water clarity, few natives, and abundant invasive macrophytes because of a common carp (*Cyprinus carpio* L.) infestation. The experiment was designed 1) to assess the germination response under different light treatments including a treatment representative of a lake with good water clarity, a treatment representative of a lake with poor water clarity, and a maximum germination treatment (treated with gibberellic acid), and 2) to compare the taxa observed sprouting to the taxa growing in the lakes. There was no significant difference in germination among light treatments, but germination was higher in the maximum germination treatment. The most frequent species observed in the seedbanks were chara (*Chara* spp. L.), curlyleaf pondweed (*Potamogeton crispus* L.), and wild celery (*Vallisneria americana* Michx.). Seventeen species were observed sprouting in Lake Riley and 16 in Lake Ann. Under maximum germination conditions, Lake Riley had a viable seed density of 2,916 ± 1,828 seeds per square meter and a viable chara spore density of 1,033 ± 698 spores per square meter . Lake Ann had a viable seed density of 1,100 ± 440 seeds per square meter and viable chara spore density of 13,833 ± 2,825 spores per square meter. The observed viable seedbanks reflected the macrophyte community growing in the lakes. In Lake Ann, every species observed in the seedbank experiment was growing in the lake. In Lake Riley, all but two species observed in the seedbank were also growing in the lake. Overall, the study demonstrated that by germinating propagules from a lake seedbank, managers can evaluate the viable macrophyte taxa present and the potential for recruitment from the seedbank.

Key words: lake seedbank, native aquatic plant restoration, propagule recruitment, water clarity.

INTRODUCTION

Macrophytes play an integral role in aquatic littoral zones. Macrophytes stabilize sediment and sequester nutrients, maintaining water clarity and reducing the potential for harmful algal blooms and other water quality impairments (Dennison et al. 1993, Horppila and Nurminen 2003, Bakker et al. 2010). Diverse, heterogeneous aquatic plant communities also provide habitat for invertebrates and fish communities (Valley et al. 2004, Cross and McInerny 2006). When large-scale disturbances occur in a lake that affects macrophytes, such as benthivorous fish damage or high nutrient levels, the macrophyte community is often reduced in abundance and composed of dense monocultures of low-light tolerant invasive plants because of poor water clarity and growing conditions (Chase and Knight 2006, Bajer et al. 2009). Subsequently, the limited macrophyte population further impairs ecosystem function and water quality because of reduced capacity to sequester nutrients, reduced sediment suspension, and reduced habitat for other aquatic organisms (Scheffer et al. 1993, Hansson et al. 1998). As a result, these systems have poor recreational value for lake users and provide poor habitat for aquatic organisms.

Therefore, the management and restoration of aquatic macrophytes is often a primary goal when aiming to improve water quality (Scheffer et al. 1993, Hilt et al. 2006, Bakker et al. 2013). The restoration of native macrophyte communities is a dynamic process that requires multiple years of active management (Hilt et al. 2006). Restorative actions often include nutrient reduction, nutrient sequestration, and invasive species control (Bakker et al. 2013, Cooke et al. 2016). These mechanisms aim to improve growing conditions for native macrophytes by decreasing competition with nonnative macrophytes and algae and by increasing the water clarity, allowing for efficient photosynthesis.

When water clarity is improved through management actions, such as a benthivorous fish removal or alum treatment, the native macrophyte community often increases in abundance and diversity (Bajer et al. 2009, Bajer and Sorensen 2015, Spears et al. 2016). Conversely, when management of invasive fish and/or macrophytes occurs without an increase in water clarity, the native macrophyte community may show a marginal increase in abundance and species diversity (Bakker et al. 2013, Knopik 2014, JaKa 2015). Therefore, it appears that to restore native macrophyte communities, water clarity must be maintained at a high-enough level throughout the summer growing season

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to facilitate the recruitment of native macrophyte species to the lake (Chambers and Kalff 1985, Doyle and Smart 2001, Bakker et al. 2013) or the success of transplants (Knopik and Newman 2018).

The recruitment of new species has been documented in lakes that have undergone a water clarity improvement. However, it is often unknown if the recruitment is due to growth from existing low-density source populations or the seedbank (Bakker et al. 2013). Native macrophytes mainly propagate through clonal growth and fragmentation, but they do still produce seeds or spores (Santamaría 2002, Boedeltje et al. 2003). However, the seeds/spores that are produced are generally not thought be a large contributor to macrophyte community propagation and recruitment (Boedeltje et al. 2003). Although propagation through clonal growth is most common, in some systems the source populations of submersed aquatic vegetation may be absent because of low clarity or benthivorous fish disturbance. The absence of a macrophyte community may lead to the role of the seedbank being more influential in the restoration of lake vegetation (De Winton et al. 2000, Pollux 2011, Bakker et al. 2013). If vegetation has been absent for a number of years fewer viable propagules may be present, or they may be buried deep in the sediment (Westcott et al. 1997, Baldridge and Lodge 2014). In order to assess response to restoration efforts, it is important to assess propagule availability and recruitment within the lake (Bakker et al. 2013).

In this study, we aimed to assess the effect of improved water clarity and improved light quantity on the germination of macrophyte propagules from lake seedbanks. First, we were interested in estimating the seedbank in two Minnesota lakes, one with a diverse plant community and the other with a historically limited plant community because of low clarity and carp disturbance, to understand if the seedbank reflects the current occurrence of macrophytes in a lake. Second, we sought to determine the role of light intensity on the germination of macrophyte propagules. Understanding how the seedbank of a lake responds to lake restoration efforts, such as water clarity improvement, is key as managers plan for desired outcomes, including a more stable, diverse macrophyte community.

MATERIALS AND METHODS

Lake characteristics and seedbank collection

Sediment was collected from Lake Ann (DOW ID 10001200) and Lake Riley (DOW ID 10000200). Both lakes are in Chanhassen, MN, in Carver County, west of the Twin Cities within the Riley Purgatory Bluff Creek Watershed District. Lake Ann has a small (100 ha) drainage basin with $< 10\%$ agriculture and residential development, and the lake shore is predominantly undeveloped and forested parkland. The lake is a dimictic, mesotrophic lake with a diverse macrophyte community and good water clarity (mean August Secchi depth: 1.8 m). In Lake Ann, 17 to 21 species have been regularly observed in 2011 to 2014. The lake area is 48 hectares with a maximum depth of 12.2 m. Lake Riley is a eutrophic lake with larger drainage basin (720 ha), more residential development (40%), and 50% natural open space. Lake Riley has a historically diminished macrophyte population that has been steadily improving because of lake management actions over the last several years (carp reduction, invasive macrophyte control, and alum treatment). The lake area is 120 hectares with a maximum depth of 15.1 m. The mean August Secchi depth was 0.5 m prior to the 2016 alum treatment. In Lake Riley, 7 to 10 species were regularly observed in 2011 to 2013, soon after common carp (*Cyprinus carpio*) removal and curlyleaf pondweed (*Potamogeton crispus* L.) control, and 12 to 15 species have been regularly observed between the years of 2014 to 2016, after Eurasian watermilfoil (*Myriophyllum spicatum* L.) control (2015 to 2016) and alum treatment (2016).

To collect the sediment for the treatments, seven transects were marked randomly around each lake using ArcGIS. Transects were uploaded to a Garmin GPS 76[1] and a boat was navigated to each site. At each transect, four sediment core samples were obtained at a 1.0-m depth using a 10.0-cm diameter PVC coring device; the top 5.0 cm of the sediment core was collected. The sediment samples from each lake were homogenized to reduce the heterogeneity of seedbank distribution in lake and then stored in a dark refrigerator at 4 C until the experiment was ready to begin.

Germination treatments

Sediment samples were washed with well water over a coarse sieve to remove large material, such as twigs and cobbles, and vegetative structures and buds, such as curlyleaf pondweed turions. After this material was removed, 200 ml of sediment was spread in a layer over a medium of 200 ml sterilized sand in 19.0 by 19.0 by 6.0–cm trays (Galatowitsch and van der Valk 1994) and covered with 3.0 cm of water (Boedeltje et al. 2002, Baskin and Baskin 2014). A total of 45 trays were created for each lake. Fifteen additional trays were used as controls to ensure that contamination in the growing room did not occur. Control trays consisted of 200 ml steam-sterilized sand and 200 ml sterilized lake sediment.

Trays (15 per treatment from each lake) were allocated to one of three germination treatments of varying light intensity and the experiment ran for 16 wk. Trays were illuminated with Heliospectra lights that emit nine wavelengths of light that can each be individually adjusted to control the light precisely. The lights produce 380-, 400-, 420-, 450-, 520-, 630-, 660-, and 735-nm wavelengths in addition to 5700 K white LED lights that are similar to sunlight. Curtains were placed between different light treatments to eliminate the effect of other light sources on the propagules.

To assess the extent of the viable seedbank in Lakes Ann and Riley, 15 of the trays for each lake were used to assess viability using the seedling emergence method to maximize germination (Boedeltje et al. 2002). To maximize germination, the trays were exposed to a series of environmental conditions known to induce germination in aquatic plants. The photosynthetically active radiation (PAR) was set to 800 μmol s^{-1} per square meter and a 15-h light, 9-h dark photoperiod (Coble and Vance 1987, Boedeltje et al. 2002). All the wavelengths of light and white light were kept at equal intensity set to maintain the 800 µmol s^{-1} per square meter PAR. The temperature ranged between 21 and 23 C (Boedeltje et al. 2002), and water levels remained at approximately 3.0 cm in the trays throughout the testing period (Boedeltje et al. 2002, Baskin and Baskin 2014). Gibberellic acid was applied to the trays once at the onset of the experiment to reach a concentration of 0.3 mM, to induce sprouting (Tuckett 2010, Baskin and Baskin 2014).

In addition to assessing the total viability of the seedbanks in Lake Ann and Lake Riley, the effect of water clarity and light quantity on submersed aquatic vegetation germination in lake seedbanks was assessed by exposing the seedbank to one of two levels of light intensity using Heliospectra RX30 Research Lights.[2] Fifteen trays were exposed to a light condition representative of a clear lake at 1.0-m depth and the remaining 15 trays were exposed to a light condition representative of a turbid lake at 1.0-m depth. For this experiment, a "clear lake" was defined as having an August Secchi depth of 1.5 m or greater, and a "turbid lake" was defined as an August Secchi depth of less than 1.5 m.

Field observations with a spectroradiometer were collected in six lakes of varying clarity in the Riley Purgatory Bluff Creek Watershed District at mid-day on a day with no cloud cover and little wind. These observations indicated that high-clarity lakes had an average light intensity of 650 µmol s^{-1} per square meter at 1.0-m depth and low-clarity lakes 125 µmol s^{-1} per square meter at 1.0 m. The light spectrum observed at 1.0 m was very similar among the lakes, and therefore the wavelengths remained at the same ratios for each treatment and only the overall intensity of the light was altered. Thus, in the high-clarity treatment the Heliospectra lights were set at a PAR of 650 µmol s^{-1} with an equal intensity of all nine wavelengths. In the low-clarity treatment the lights were set at 125 µmol s^{-1} PAR with an equal intensity of all nine wavelengths. For both treatments a 15 h light : 9 h dark photo period was used. In the good- and low-clarity treatments, the water temperature was consistently between 21.0 and 23.0 C in the trays, and the dissolved oxygen was consistently between 7.0 and 9.0 mg L^{-1}.

Trays were checked weekly and new propagule sprouts were identified to species and recorded. Any observed sprouting from the sediment was counted as germinated and was considered viable (Boedeltje et al. 2002). Propagule sprouts were removed from the trays after being counted to prevent them from being counted multiple times. If needed, propagules were transplanted into an environmental chamber for continued growth to confirm species identification. When trays were checked for sprouting, temperature, and dissolved oxygen readings were made using a YSI Pro ODO dissolved oxygen meter[3] every week at midday when the lights had been on for at least 5 h.

After the experiment was concluded, a subsample of five trays from each treatment were examined to enumerate the seedbank to evaluate the number and species of propagules that did not germinate (Bernhardt et al. 2008). Sediment was sifted through 1.0-, 0.5-, 0.25-, 0.125-, and 0.053-mm sieves stacked on each other to sort the sample by grain size and more easily find all ungerminated propagules. The sediment in each sieve was visually inspected and propagules were picked and identified to genus or species (depending on the morphological characteristics) with the use of a Nikon stereo microscope.

Last, the viable seedbank was compared to the plants observed growing in the lake through point-intercept surveys (50-m grid) to evaluate if the seedbank is representative of the existing plant community. The species observed sprouting were ranked by most abundant (rank = 1) to least abundant and the species observed during the surveys were ranked by most abundant (rank = 1) to least. These values were plotted on a scatterplot for each lake to evaluate if the most abundant sprouts were also the most abundant plants observed in the lake.

Sediment analysis

Part of the homogenized sediment from Lakes Ann and Riley was analyzed for soil characteristics, specifically dry bulk density and organic-matter content. To determine the dry bulk density and organic matter content, five 10.0-ml subsamples of sediment from each lake were obtained using a modified syringe. The subsample was placed in a crucible that was weighed and recorded prior to the sediment being added. The subsamples were dried in an oven at 100 C for 48 h. After drying, the samples were weighed and the dry bulk density was calculated as grams per dry milliliter. The samples were then placed in a muffle furnace for 3 h at 500 C to combust the organic content in the sediment. The samples were cooled and promptly weighed. The organic matter content was obtained by subtracting the mass of the sediment after the muffle furnace from the mass of the sediment after the drying oven; percent organic matter was then calculated as a percentage of the dry sediment mass. The five subsamples of dry bulk density and organic matter content for each lake were averaged to obtain a mean dry bulk density and organic matter content for the sediment (Galatowitsch and van der Valk 1994).

Statistical analysis

We compared the germination of seeds from the seedling emergence method to the abundance and richness of propagule sprouts in the two light treatments. We also compared the viable seedbank to the observed species growing in the lakes. The Lake Riley and Lake Ann seedbanks were assessed for several metrics. The number of total propagule sprouts, number of propagules per species, number of species germinated, the average total viable seeds per tray and per square meter lake bottom, and the average viable seeds per tray and per square meter for each species was calculated. Additionally, the metrics of average viable propagule density per tray and per square meter was separated into vascular plants that produce seeds and the nonvascular plant spores from chara (*Chara* spp.) to understand the composition of the lake seedbank, because chara was a large contributor to the total propagule count in each lake. The average viable propagules per square

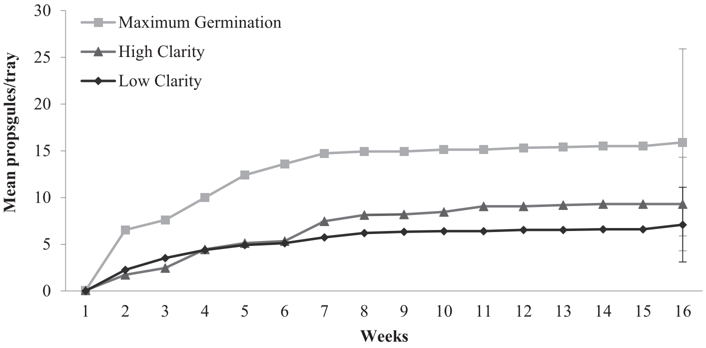

Figure 1. Lake Riley mean cumulative germination (propagules per tray) under the three treatment conditions.

meter of lake bottom was determined by taking the average of the count of propagules in each tray and dividing by 40 cm^2 to obtain the average propagules per square centimeter. The sediment core volume (10.0-cm diam and 5.0-cm depth) was 395 cm^3 representing a surface area of 79 cm^2. Thus, the 200 cm^3 of sediment in each tray represents a surface area of 40 cm^2. The propagules per square centimeter value was then multiplied by 10,000 to obtain the propagules per square meter.

All statistical analysis was conducted using R statistical software version 3.3.2 (R Core Team 2013). Results were considered statistically significant when $P < 0.05$. To assess the effect of the treatment on the number of propagules counted for Lake Ann and Lake Riley samples, Poisson log-linear models were used as the data followed Poisson distributions. The models were used to evaluate the total count of propagules as well as the count of each species observed germinating. A Poisson log-linear model was also used to evaluate the significance of the difference between the species richness observed in each treatment.

RESULTS

Lake Riley

The maximum germination treatment had 16 taxa, the high-clarity treatment had 13 taxa observed sprouting, and 9 taxa were observed sprouting in the low-clarity treatment (Table 1). These differences were not significant ($P > 0.05$). The total number of propagules germinated was approached by Week 8 and was reached by Week 16 (Figure 1).

Under maximum germination conditions, the average number of propagules germinated per tray at the end of the experiment was 15.9 ± 6.2 propagules per tray (Figure 1). Therefore, the mean number of viable propagules was 3,950 ± 1,561 propagules per square meter. The mean number of vascular seeds germinated per tray was 11.6 ± 7.3 seeds per tray or 2,916 ± 1,828 seeds per square meter. The mean number of chara spores germinated per tray was 4.1 ± 2.8 spores per tray or 1,033 ± 698 spores per square meter (Figure 2). In the maximum germination conditions, curlyleaf pondweed had the greatest number of viable seeds observed with an average of 6.3 ± 3.1 seeds per tray or 1,583 ± 776 seeds per square meter (Figure 2, Table 1). We are confident that these were from seeds because all visible turions had been removed from the sediment. Chara was the second most common propagule (Figure 2, Table 1). Wild celery (*Vallisneria americana* Michx.) also had a high number of seeds, at an average of 2.6 ± 1.6 seeds per tray or 650 ± 399 seeds per square meter (Figure 2, Table 1).

The Lake Riley sediment samples had no significant difference between the maximum germination, high-clarity, and low-clarity treatments. For the high-clarity treatment, the average germination by 16 wk was 9.3 ± 1.7 propagules per tray or 1,783 ± 444 propagules per square meter (Figure 1). The mean number of vascular seeds germinated per tray was 4.5 ± 1.3 seeds per tray or 1,116 ± 312 seeds per square meter. The mean number of chara spores

Table 1. Total number of propagules germinating from Lake Riley sediment for the maximum germination, high-clarity, and low-clarity treatments based on 3 L of collected sediment per treatment.

		Lake Riley			Lake Ann		
Species	Species Abbreviation	Maximum Germination	High Clarity	Low Clarity	Maximum Germination	High Clarity	Low Clarity
Ceratophyllum demersum L.	Cdem	10	8	3	1	2	3
Chara spp.	Char	62	40	49	830	801	911
Elodea canadensis Michx.	Ecan	3	5	1	0	0	0
Heteranthera dubia (Jacq.) MacMill.	Hdub	1	1	0	0	0	0
Lemna minor L.	Lmin	1	2	0	0	2	0
Lemna trisulca L.	Ltri	0	0	0	2	0	0
Myriophyllum spicatum L.	Mspi	1	1	0	0	0	0
Najas guadalupensis (Spreng.) Magnus	Ngua	1	0	0	2	0	0
Nelumbo lutea Willd.	Nlut	0	0	0	1	0	0
Nyphar varigaeta Engelm. ex. Durand	Nvar	1	0	0	1	0	0
Potamogeton crispus L.	Pcri	95	26	46	14	20	18
Potamogeton pusillus L.	Ppus	13	8	5	5	5	11
Potamogeton nodosus Poir.	Pnod	9	1	3	1	0	1
Potamogeton richardsonii (Benn.) Rydb.	Pric	0	0	0	2	1	1
Potamogeton robinsii Oakes	Prob	1	1	0	0	1	0
Potamogeton zosteriformis L.	Pzos	4	3	3	1	2	0
Ranunculus longirostris Godr.	Rlon	9	0	0	1	0	0
Stuckenia pectinata (L.) Boerner	Spec	39	4	1	5	5	0
Vallisneria americana Michx.	Vame	3	20	22	32	38	29

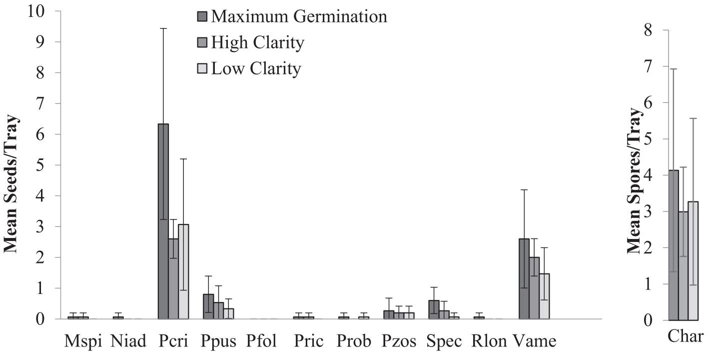

Figure 2. Lake Riley average germination (seeds per tray or spores per tray) under the maximum germination, high-clarity, and low-clarity treatment conditions for the most abundant species observed. Abbreviations are located in Table 1.

germinated per tray was 2.7 ± 1.4 spores per tray or 667 ± 341 spores per square meter. In the high-clarity condition, chara was the most frequent species observed (Figure 2, Table 1). The second most abundant species was curlyleaf pondweed, with an average germination of 1.7 ± 0.63 seeds per tray or 433 ± 158 seeds per square meter (Figure 2, Table 1). Wild celery was also observed at an average 1.3 ± 0.6 seeds per tray or 333 ± 152 seeds per square meter (Figure 2, Table 1). The remaining species were all observed in low frequencies.

The Lake Riley sediment samples exposed to the low-clarity treatment had an average germination per tray of 7.1 ± 2.7 propagules per tray or 2,167 ± 683 propagules per square meter (Figure 1). The mean number of vascular seeds germinated per tray was 5.4 ± 2.6 seeds per tray or 1,350 ± 653 seeds per square meter. The mean number of chara spores germinated per tray was 3.3 ± 2.3 spores per tray or 817 ± 574 spores per square meter. In the low-clarity conditions, chara was the most abundant observed at an average of 3.3 ± 2.3 spores per tray or 816 ± 574 spores per square meter (Figure 2, Table 1). Curlyleaf pondweed was also abundant, with an average of 3.1 ± 2.1 seeds per tray or 767 ± 533 seeds per square meter (Figure 1, Table 1). Wild celery was also common, with an average of 1.5 ± 0.8 seeds per tray or 367 ± 212 seeds per square meter (Figure 1, Table 1). The remaining species observed were in low abundance.

Table 2. Total ungerminated seeds found in the subsample of five Lake Riley and five Lake Ann trays from each treatment.

	Lake Riley			Lake Ann		
Species[1]	Maximum Germination	High Clarity	Low Clarity	Maximum Germination	High Clarity	Low Clarity
Mspi	1	0	0	3	0	2
Niad	1	2	2	1	2	0
Pamp	0	1	0	0	0	4
Pcri	0	2	1	0	2	1
Ppus	2	0	1	0	0	0
Prob	0	1	0	1	0	0
Pzos	0	0	1	2	0	0
Spec	0	0	0	0	0	0
Zpal	0	2	3	0	0	0

[1] Mspi, *Myriophyllum spicatum* L.; Niad, *Najas* spp.; Pamp, *Potamogeton amplifolius*; Pcri, *Potamogeton crispus*; Ppus, *Potamogeton pusillus* L.; Prob, *Potamogeton robinsii*; Pzos, *Potamogeton zosteriformis* L.; Spec, *Stuckenia pectinata* (L.) Boerner; Zpal, Zannichellia palustris.

The results of the Poisson model indicated no significant difference between the different treatment types for the Lake Riley samples. Specifically, there was no difference in the total number of propagules, species diversity, or species abundance (all $P > 0.05$). All taxa were that were observed germinating in the maximum germination conditions, apart from two species (*Potamogeton richardsonii* (A.Benn.) Rydb. and *Potamogeton robinsii* Oakes), were also observed growing in the lake during macrophyte surveys. Two taxa, wild celery and water stargrass (*Heteranthera dubia* (Jacq.) MacMill.), which were not present in previous years, appeared in Lake Riley during the summer of 2016.

The Lake Riley seedbank enumeration yielded few additional propagules that had not germinated in the maximum germination conditions. The maximum germination conditions yielded the fewest propagules that had not germinated, and the good- and low-clarity conditions had a slightly higher abundance of propagules observed in each subsample (Table 2). An average of 1.3 ± 0.42 propagules per tray for the maximum germination condition was counted. The good- and low-clarity treatments had a slightly higher number of remaining seeds with an average of 5.0 ± 2.3 propagules per tray and 6.1 ± 2.5 propagules per tray, respectively. No species were found as propagules that had not also been observed germinating. The average dry bulk density of the collected Lake Riley sediment was 0.53 ± 0.34 g ml^{-1} and the average organic matter content was 13% ± 4.5%.

Lake Ann

Lake Ann had a total propagule count that was much greater than Lake Riley, largely because of a high abundance of chara spores. In the maximum germination conditions, 14 taxa were observed, in the good-clarity treatment 10 taxa were observed and in the low-clarity treatment 7 taxa were observed. The maximum germination was approached by Week 12 and was complete by Week 16 in Lake Ann.

Under maximum germination conditions the average total germination after 16 wk was 59.7 ± 11.1 propagules per tray or 14,933 ± 2,771 propagules per square meter (Figure 3). The mean number of vascular seeds germinated

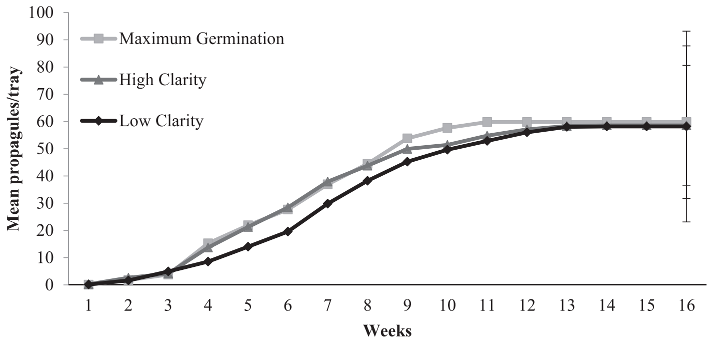

Figure 3. Lake Ann mean cumulative germination (propagules per tray) under the three treatments.

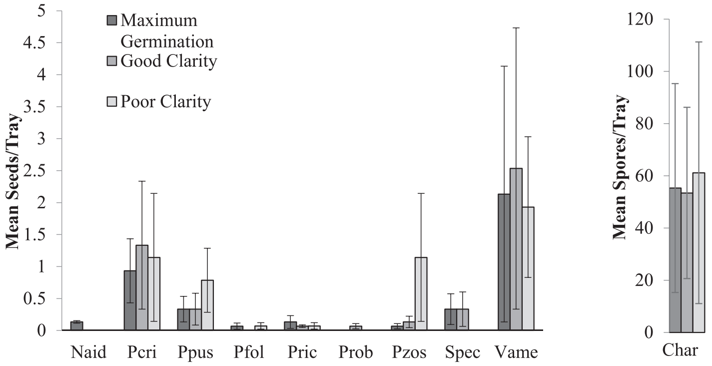

Figure 4. Lake Ann average germination (seeds per tray or spores per tray) under the maximum germination, high-clarity, and low-clarity treatment conditions for the most abundant species observed. Abbreviations are located in Table 1.

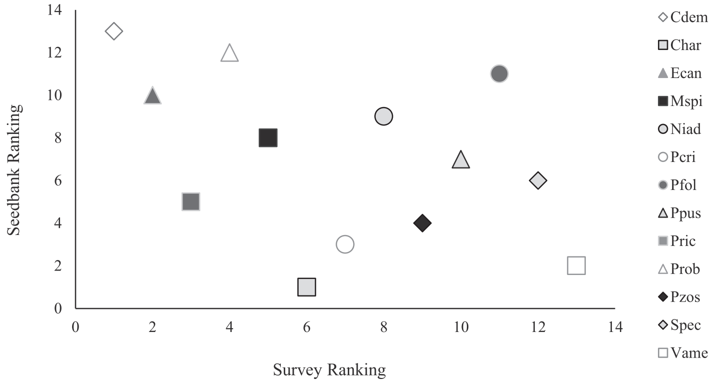

Figure 5. Lake Ann ranking of species sprouting from the seedbank compared to species observed during point-intercept surveys. Species with a rank of 1 were the most abundant.

per tray was 4.4 ± 1.6 seeds per tray or 1,100 ± 408 seeds per square meter. The mean number of chara spores germinated per tray was 55.3 ± 11.3 spores per tray or 13,833 ± 2,825 spores per square meter (Figure 4, Table 1). The second most abundant species was wild celery, observed at an average of 2.1 ± 1.2 seeds per tray or 533 ± 296 seeds per square meter (Figure 4, Table 1). Curlyleaf pondweed was observed at an average of 0.9 ± 0.4 seeds per tray or 233 ± 142 seeds per square meter (Figure 4, Table 1). The remaining species observed were all at low abundances.

The Lake Ann sediment samples exposed to high-clarity and low-clarity conditions had a similar level of germination relative to the maximum germination conditions; the difference between treatments was not significant ($P > 0.05$) For the high-clarity treatment, the average germination per tray in each treatment was 58.6 ± 13.4 propagules per tray or 14,000 ± 3,351 propagules per square meter (Figure 3). The mean number of vascular seeds germinated per tray was 4.5 ± 1.8 seeds per tray or 1,125 ± 446 seeds per square meter. The mean number of chara spores germinated per tray was 53.4 ± 12.6 spores per tray or 13,350 ± 3,149 spores per square meter (Figure 4, Table 1). Chara was the most abundant taxa. The second most abundant species was wild celery with an average of 2.5 ± 1.1 seeds per tray or 633 ± 275 seeds per square meter (Figure 4, Table 1). Curlyleaf pondweed was also present at high levels at an average of 1.3 ± 0.8 seeds per tray or 333 ± 193 seeds per square meter (Figure 4, Table 1). The remaining species were all in low abundances.

For low-clarity conditions, the total germination was an average of 58.1 ± 17.1 propagules per tray. The mean number of germinated propagules was 16,286 ± 4,266 propagules per square meter in the low-clarity treatment (Figure 3). The mean number of vascular seeds germinated per tray was 4.0 ± 1.7 seeds per tray or 1,000 ± 428 seeds per square meter. The mean number of chara spores germinated per tray was 61.1 ± 16.9 spores per tray or 15,286 ± 4,218 spores per square meter. The most abundant species was chara (Figure 4, Table 1). The second most abundant species was wild celery with 1.9 ± 0.8 seeds per tray or an average of 482 ± 210 seeds per square meter (Figure 4, Table 1). Curlyleaf pondweed was also abundant with an average of 0.8 ± 0.1 seeds per tray or 286 ± 196 seeds per square meter (Figure 4, Table 1).

The results of the Poisson model indicated no significant difference between the different treatment types for the Lake Ann samples. Specifically, there was no difference in the total number of sprouts, species diversity, or species abundance. All taxa were that were observed germinating in the maximum germination conditions were also observed growing in the lake during macrophyte surveys.

The Lake Ann seedbank enumeration yielded few additional propagules that had not germinated. An average of 2.3 ± 1.4 propagules per tray remained from the maximum germination treatment (Table 2). An average of 1.3 ± 1.0 propagules per tray remained from the high-clarity condition (Table 2). An average of 2.3 ± 1.7 propagules per tray were counted from the low-clarity condition (Table 2). No species were found as propagules that had not also been observed as sprouts. Lastly, the average dry bulk density of the Lake Ann sediment was 0.72 ± 0.12 g ml^{-1} and the average organic matter content was 21 ± 5%.

Overall, the seedbanks of Lakes Ann and Riley were distinct despite being within the same watershed. The Lake Ann seedbank had a higher number of chara spores in all treatments relative to the Lake Riley seedbank and the difference was significant ($P < 0.05$, *t* test). The vascular seed count was similar among the two lakes for each treatment and there was no significant difference in the vascular seed counts between the two lakes or among the three treatments.

DISCUSSION

Although germination was higher in the maximum germination treatment for both lakes, there was high variability and no significant increase in germination with that treatment for either lake relative to the good- and low-clarity treatments ($P > 0.5$). Despite the findings in other studies (Dugdale et al. 2001, Sederias and Colman 2007), in this study light quantity did not appear to have a significant effect on the germination of propagules from a lake seedbank. Other studies have suggested that temperature and burial depth in the sediment are also key factors in dormancy breaking and germination (Baskin and Baskin 2014). In our experiment, these other factors may have been more critical to germination than light.

Interestingly, the timing of the germination was variable between the two lakes, despite them having similar compositions of taxa present (Figures 1 and 3). Germination was observed in both lake sediments beginning at Week 2. However, the germination in the Lake Riley sediments leveled off roughly after 8 wk, whereas in Lake Ann germination continued through Week 12. Overall, Lake Ann had a much higher viable propagule count in all treatments relative to Lake Riley, because of the many chara spores in the sediment in Lake Ann (Figure 4). However, there was no difference in the number or species of vascular seeds counted in the trays between both lakes, despite Lake Ann having a greater level of macrophyte diversity. Our study demonstrates that the seedbanks of lakes, even within the same drainage, can be variable regarding the abundance of propagules, such as is the case with chara.

These results provide important information that will guide future studies on macrophyte seedbanks. Light intensity did not have an effect on germination; however, it is likely an important factor promoting propagule growth and development into a mature plant (Jarvis and Moore 2008). Further evaluation of seedling survival in different light conditions is needed, such as evaluating responses in lower PAR conditions, such as 25 μmol s^{-1} where the light may be under the compensation point for the plant.

Assessment of the seedbank also provides useful information to lake managers regarding invasive species management. Prior to the sediment collection in 2016, in Lake Riley, curlyleaf pondweed was treated with endothall herbicide for three consecutive years in May of 2014, 2015, and 2016 and the control efforts were successful at reducing the abundance of curlyleaf pondweed growth in Lake Riley. The fall turion densities in the sediment declined significantly from 61 ± 20 turions per square meter in 2012 to 2 ± 1.4 turions per square meter in 2015 ($P < 0.05$; Dunne and Newman 2017). Suppression or depletion of turions is an important strategy for curlyleaf pondweed control but it is difficult to achieve (Madsen and Crowell 2002, Johnson et al. 2012). In the Lake Riley seedbank samples, curlyleaf pondweed was consistently one of the most abundant species sprouting, with an estimated viable seed density of 175 seeds per square meter in the sediment as compared to the viable turion density of 2 turions per square meter in 2016. There is clearly still an abundant and viable propagule source that managers will need to be aware of as they manage this plant over the next several years; recruitment of curlyleaf pondweed from seed may be more common than generally thought. McFarland and Rogers (1998) also found viable curlyleaf pondweed seeds, but their densities were much lower.

Eurasian watermilfoil also occurs in Lake Ann and Lake Riley in relatively high abundances at certain locations in the lakes. However, this species had a low abundance in the seedbank based on sediment samples in both lakes. This finding is consistent with other studies on Eurasian watermilfoil propagation, indicating that this species may rely mainly on fragmentation and clonal growth for its propagation (Coble and Vance 1987, Madsen and Smith 1997). However, viable seeds were present and did sprout in Lake Riley sediments at low levels.

In addition to aiding the understanding of invasive species populations, by employing a germination study, lake managers can also better understand the potential for native species recolonization from the seedbank in a lake. Specifically, lake managers can determine the extent of species diversity present in the lake and what taxa have the potential to recolonize (Westcott et al. 1997, McFarland and Rogers 1998, Bakker et al. 2013, Baldridge and Lodge 2014). For example, in Lake Riley two species were observed sprouting in this experiment that had not been observed in Lake Riley during aquatic vegetation point-intercept surveys that occurred from 2011 to 2016. Richardson's pondweed (*Potamogeton richardsonii*) and Robbins' pondweed (*Potamogeton robinsii*) were observed as sprouts, and this indicates that there may be the potential for recruitment from the seedbank in Lake Riley. Additionally, several species that were observed in the seedbank from spring 2016 were only observed growing in Lake Riley after invasive species management and water clarity improvement, including floating leaf (*Potamogeton nodosus* Poir.) and flat stem pondweeds (*Potamogeton zosteriformis* L.) in 2015, and water stargrass (*Heteranthera dubia*) and wild celery in 2016. Our study suggests that these species may have been recruited from seed because of the improvement in growing conditions. In Lake Ann, there was a high diversity of taxa observed during the 2011 through 2014 survey years. In the Lake Ann seedbank all species observed sprouting were also observed during point-intercept surveys.

Some studies have found reduced or depleted propagule banks in lakes with long (20 to 30 yr) histories of disturbance from crayfish (Baldridge and Lodge 2014) or eutrophication and sedimentation (Westcott et al. 1997). Our results indicate that despite the long-term presence of carp and a limited plant community, a fairly diverse and viable seedbank remained in Lake Riley. Somewhat surprisingly, there was no detectable difference in seedling abundance or richness between the disturbed and relatively undisturbed lakes, indicating the value of a seedbank assessment.

Evaluation of the seedbank will also be helpful before transplantation efforts, to determine if transplantation may be needed and to determine which taxa to transplant. If propagules are present, there may be no need for transplantation, and if these propagules fail to recruit naturally, other factors, such as poor water clarity or benthivorous fish, may be limiting and also likely to limit the success of transplants (Knopik and Newman 2018).

The scatterplots evaluating the relationship between seedbank abundance and lake abundance showed no relationship (Figures 5 and 6). If there were a relationship between abundance in the seedbank and the lake, one would expect the points to align linearly with a slope of one; however this is not the case in either lake. There was no clear pattern of sprout density and observed plant density, indicating that high abundance in the seedbank does not equate to high abundance in the lake.

Overall, lake seedbanks can be variable in abundance and richness, and in our study the seedbanks appear to reflect

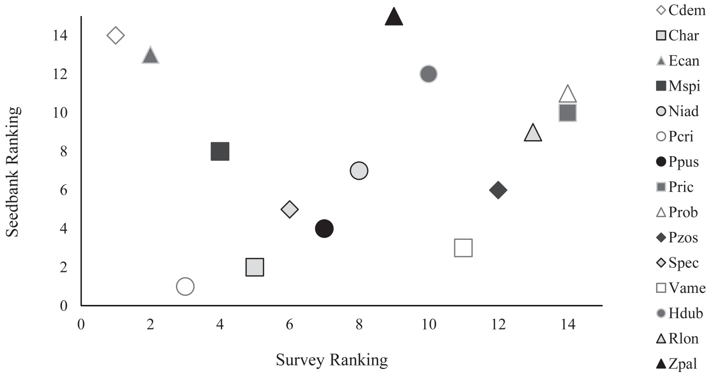

Figure 6. Lake Riley ranking of species sprouting from the seedbank compared to species observed during point-intercept surveys. Species with a rank of 1 were the most abundant.

the existing macrophyte community in the lakes. Moreover, water clarity and ranges of high light intensity did not impact the propagule germination of macrophytes in our study, but further investigation is warranted as to the effect of light on the survival of propagules to maturity.

SOURCES OF MATERIALS

[1]Garmin GPS 76, Garmin International, 1200 East 151st Street, Olathe, KS 66062-3426.

[2]RX30 Research Light, Heliospectra AB, Box 5401, SE-402 29 Göteborg, Sweden.

[3]YSI Pro ODO dissolved oxygen meter, YSI Incorporated, 1700/1725 Brannum Lane, Yellow Springs, OH 45387-1107.

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