Is There a Relationship Between Aquatic Vegetation and Amphipod Stocking Mortality?

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Amphipod occurrence and abundance has declined in the Prairie Pothole Region (PPR). Wildlife managers have stocked prairie pothole wetlands with *Gammarus lacustris* since 2018 to establish self-sustaining populations. *G. lacustris* have been detected in only 2 of 22 stocked basins, suggesting that they have died or failed to reproduce in most stocking attempts. Aquatic plant surveys were conducted in stocked wetlands and compared to data from surveys of wetlands with naturally occurring populations to understand if *G. lacustris* would be expected to survive in stocked wetlands based on characteristics of the aquatic vegetation communities. SAV coverage was significantly higher in stocked basins (P = 0.03) and is likely positively correlated with *G. lacustris*. Floating biomass (P < 0.01) and floating coverage (P < 0.01) were significantly higher in stocked basins. Floating species were only found in 5% of natural wetlands and 33% of stocked wetlands. Communities were similar apart from a few species including *Lemna trisulca* which is known to have a positive relationship with *G. lacustris* abundance. These preliminary results suggest that aquatic plant communities may be correlated with lack of *G. lacustris* establishment in some stockings, but not most.

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Introduction

The Prairie Pothole Region (PPR) serves as an important migration habitat for waterfowl breeding in the Boreal Forest and the Arctic such as Lesser Scaup *Aythya affinis*, Wigeon *Mareca*, Green-winged Teal *Anas carolinensis*, Canada Geese *Branta canadensis,* and Snow Geese *Chen caerulescens*. The amphipod *G. lacustris* is often found in wetland benthic littoral zones, usually within five meters of the surface in vegetated areas (Qvenild et al. 2020). Known as herbivores or detritivores, amphipods serve as important trophic links throughout the food web (Berenzina 2011; Wilhelm and Schindler 2011). *G. lacustris* and similar species *Hyalella azteca* are known to be a central part of wetland ecosystems in the upper Midwest, USA (Kantrud et al. 1989). However, recent research suggests that amphipod occurrence and abundance has declined in the Prairie Pothole Region (Anteau and Afton 2008).

For over a hundred years, amphipod stocking activities have occurred on a global scale. Deksbach (1952) gives examples of *G. lacustris* transplants in the Siberian waters in the early years of the 20th century to serve as a prey source for fishes. Since 2018, Wildlife managers have stocked 22 wetlands in the Prairie Pothole Region with *G. lacustris* to establish self-sustaining populations.

As of spring 2020, *G. lacustris* have been detected in only 2 of 22 stocked basins post-stocking, suggesting that they have died or failed to reproduce in most stocking attempts. The objective in this project was to compare the submerged and floating aquatic plant communities in stocked wetlands to the plant communities in wetlands where *G. lacustris* exist naturally (“natural wetlands”), as part of an effort to assess whether stocked wetlands are like those where *G. lacustris* are known to survive. The following metrics, for both wetland types were compared: (1) floating and submerged aquatic vegetation coverage and average biomass, (2) species richness, and diversity indices, and (3) plant community structure. Where stocked wetlands fall outside the range of values associated with natural *G. lacustris* populations, habitat characteristics may have contributed to *G. lacustris’s* failure to establish.

Methods

Study Area

Wetlands were surveyed in the western half of Minnesota, encompassing the PPR and forest-transition zone (around Bemidji, Fergus Falls, and St. Cloud MN). All wetlands were near the harvest sites and existing natural wetlands. Most sites were on State Wildlife Management Areas, with some sites on private property and federal Wildlife Production Areas. Plant communities in 12 of the 22 stocked wetlands were surveyed.

Field Collection

Surveys were conducted at specified points across the wetland using the established point-intercept method (Madsen 1999). The number of points varied depending on the size of the wetland. Minimum points per wetland was 10, and maximum number of points was 60 with an error margin of 10%.

Project leaders generated survey maps using MN DNR Shallow Lakes Software. At each survey point six different measurements were taken. To avoid bias during surveying, the side of the boat used for all data collection was decided prior to the start of each survey by flipping a coin (e.g. heads = right side, tails = left side). Data for each vegetation type was collected differently.

Floating Vegetation

A 1 x 1 m sampling frame made of PVC pipe was placed next to the boat. The total cover of floating and floating-leaved vegetation within the quadrant was assessed using a biomass scale of 0-4 (Table 1) and recorded on a datasheet. Within the same 1 x 1 m area, all floating and floating-leaved plants were identified and recorded on the data sheet. Only living plants were identified. All plants were identified down to the species level except for some *Lemna* species.

Submergent Vegetation

The depth reading was recorded by lowering a weighted, marked rope into the water. A plant rake was dropped into the water, dragged for 1.5 m along the wetland bottom, and then pulled up to the boat. Once the rake was retrieved, the relative biomass of submerged vegetation was ranked for a density estimate, using a 0-4 biomass scale (Table 1). Submerged plants were identified and recorded. Only living plants were identified/recorded. All plants were identified down to the species level with the exceptions of *Chara* species, some *Najas* species, and some *Utricularia* species.

Emergent Vegetation

At the end of each transect line, surveyors faced the shoreline in the direction of the transect, and, using a 1-m-wide reference, observed the emergent vegetation. The dominant species present (cattail, bulrush, grass, sedge, or “other”) was recorded. If a plant was unidentifiable in the field, it was placed in a Whirl-Pak with a small amount of water. Unidentified plants were brought to the lab for further identification. If a point was unable to be surveyed due to depth, impassible emergent vegetation, or algae mats, it was marked as “point not surveyed.”

Table 1. Relative density rating for submerged aquatic vegetation and floating vegetation .

Relative Density Ratings for Aquatic Plants	
0	No plants
1	1-25% of area filled with plants
2	25-50% of area filled with plants
3	50-75% of area filled with plants
4	75-100% of area filled with plants

Data Analysis

Using program R, both floating and submerged aquatic vegetation coverage and average biomass was calculated between the two basin types. With coverage representing the percent of basin covered by vegetation. Independent T-Tests (two-tailed alpha = 0.05) were run to compare coverage and biomass calculations between the submerged and floating plants in stocked wetlands (n = 12) compared to natural wetlands (n = 37).

Common plants were found by separating plant species present in ≥ 50% of total basins of at least one wetland type. Average species richness, Shannon Wiener Diversity Index and Simpson’s Index of Diversity were calculated from each basin sampled. The Shannon-Weiner Diversity Index was calculated using $H = -\Sigma p\ i * \ln(p\ i)$. Simpson’s diversity was found using $D = \Sigma ni(ni-1) \ / \ N(N-1)$. Independent T-Tests (two-tailed= 0.05) were run to compare species diversity between the submerged and floating plants by wetland type.

Results

In total, 567 samples were analyzed encompassing 49 basins. Average submerged vegetation (SAV) biomass did not show a significant difference between the wetlands (P = 0.96; Table 2). SAV cover (P = 0.03), floating biomass (Figure 1; P < 0.01), and floating cover (Figure 2; P < 0.01) were found to be statistically significant. Floating species found included Watershield *Brasenia schreberi*, White Water Lily *Nymphaea odorata*, and Yellow Water Lily *Nuphar* *variegata*. Floating species were found in 5% of natural wetlands and 33% of stocked wetlands. Common plant communities were seen throughout both wetland types.

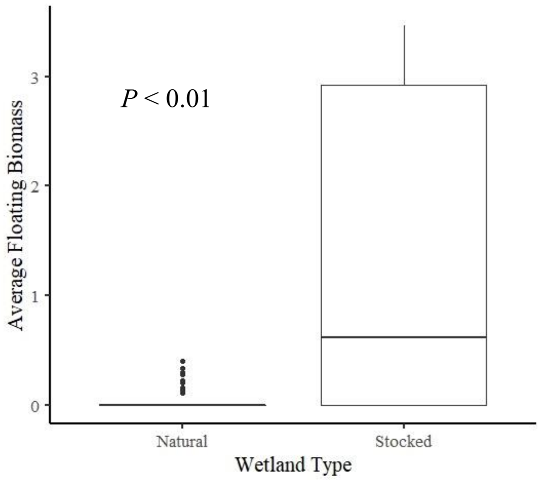

Figure 1: Average floating vegetation biomass in relation to wetland type. There was a significant difference in floating biomass between the wetland types.

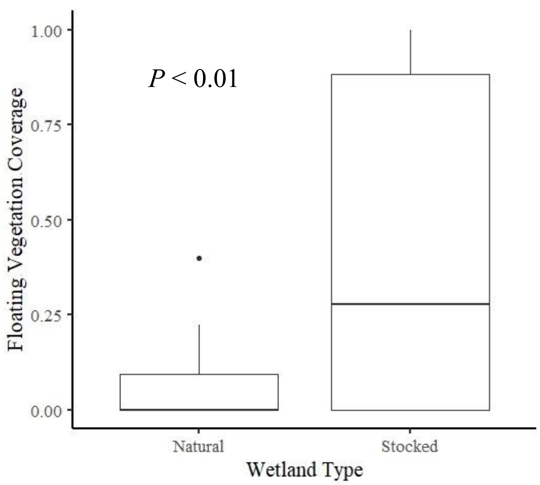

Figure 2: Average floating vegetation coverage in relation to wetland type. There was a significant difference in floating coverage between the wetland types.

Coontail *Ceratophyllum demersum* was the dominant species in both wetland types. It was found in 95% of natural basins and 75% of stocked (Figure 3.). When found coverage was high (57% natural and 56% stocked). Muskgrass *Chara* spp. was another common species and was found in 51% of natural and 66% of stocked basins. Star Duckweed *Lemna trisulca* and Slender Naiad *Najas flexilis* had vast differences per basin type. Star Duckweed was found in 62% of natural basins and 8% of stocked while Slender Naiad was found in only 8% of natural and 58% of stocked (Figure 3).

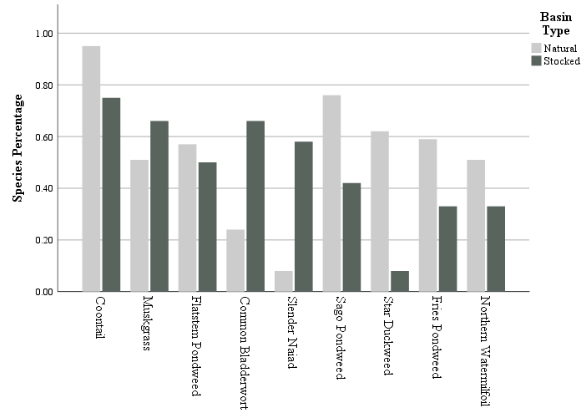

Figure 3. Percentage of basins with common species present (Common species = ≥ 50% of total basins sampled of at least one wetland type). Stocked and natural wetlands were similar except for a few species including Star Duckweed.

The Simpson's Index of Diversity was slightly higher in stocked basins (Table 3) indicating that stocked basins are slightly less diverse. The greater the value, the lower the diversity. The Shannon-Weiner Index of Diversity was slightly higher in stocked basins (Table 3) indicating the opposite. The greater the value, the higher the diversity. Both the Simpson's (Figure 4; $P = 0.41$) and Shannon-Weiner Index of Diversity ($P = 0.37$) did not show a significant difference between the wetlands.

Table 2. Maximum depth, coverage, and biomass per wetland type.

Variable	Natural	(SD)	Stocked	(SD)
SAV Coverage	0.84	(0.24)	0.99	(0.02)
SAV Average Biomass	1.96	(0.83)	1.95	(0.73)
Floating Coverage	0.05	(0.09)	0.43	(0.46)
Floating Average Biomass	0.06	(0.11)	1.29	(1.50)
Average Max Depth	7.50	(3.23)	8.33	(5.99)

Discussion

SAV biomass did not show a significant difference between the wetlands while SAV coverage was significantly higher in stocked basins. Biomass and coverage offer different uses to invertebrates. High coverage is important because it provides essential habitat in multiple areas of the basin. SAV is important in aquatic ecosystems due to the organic matter it produces (Strayer and Malcom 2007). This organic matter is an important food source for macroinvertebrates. While analyzing habitat influences on *G. lacustris* and *H. azteca*, in the Upper Midwest Anteau et al. (2011) found a positive correlation between densities of SAV and amphipod species. This provides evidence that SAV did not negatively influence survival.

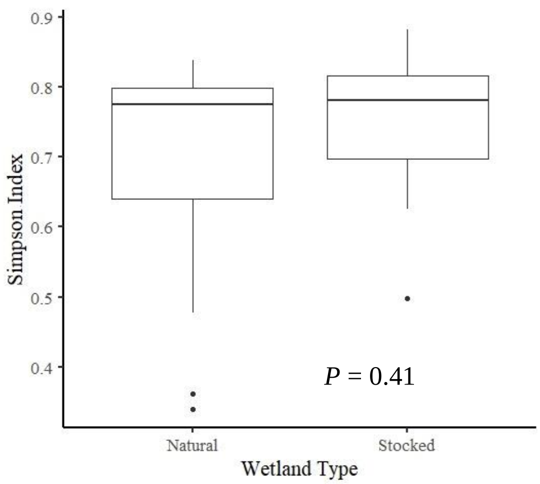

Figure 4: Simpson's Index of Diversity by wetland type. There was not a significant difference in diversity between the wetland types.

Table 3. Average species richness and diversity per wetland type.

Variable	Natural	(SD)	Stocked	(SD)
Species Richness	6.02	(2.07)	7.50	(3.80)
Shannon Index	1.47	(0.38)	1.59	(0.43)
Simpson Index	0.71	(0.13)	0.75	(0.11)

When comparing floating vegetation by basin type, it is observed that floating vegetation collected from the stocked basins are significantly higher in biomass and coverage compared to those collected from natural basins. Even though floating vegetation was statistically different, 66% of stocked and 95% of natural basins did not have floating vegetation indicating that floating vegetation presence may have only influenced lack of *G. lacustris* survival in some stockings. Due to shading, basins with more floating vegetation may have less SAV coverage, which may affect *G. lacustris* directly through habitat loss or indirectly through changes in water chemistry (i.e., hypoxic conditions) (Verdonschot and Verdonschot 2014).

Aquatic plant diversity was statistically the same between wetland types. Simpson's diversity indices overlapped between basin types. Stocked ranged from 0.50 to 0.88 while natural ranged from 0.34 to 0.83. Shannon diversity overlapped as well with stocked having a greater minimum and maximum value than natural basins. Even though diversity indices overlapped, diversity and richness still seemed to be low in both basin types. Lesica (1993) found plant species richness that ranged from 6 to 11 in pothole prairie ponds on the Blackfeet Indian Reservation in north-central Montana Average species richness in natural basins was 6.02 and 7.50 in stocked. Lesica (1993) also found community diversity (Shannon) that ranged from 1.64 to 2.26. Average plant diversity (Shannon) in natural basins was 1.47 and 1.59 in stocked.

Common plant communities were observed with Coontail and Muskgrass seen consistently independent of wetland type while species like Star Duckweed and Slender Naiad varied. Star Duckweed was found in 62% of natural basins and 8% of stocked. Meyers (1982) found a positive relationship between Star Duckweed and amphipod species. Samples of Duckweed were found to contain high densities of both amphipod and gastropod species (Meyers 1982). Similar research from 1970, analyzing aquatic plant-macroinvertebrate relationships, found that Star Duckweed had the greatest mean weight (2,059 mg) of invertebrates per 100 g of plant matter (Krull, 1970). Species like Water Star Grass *Heteranthera dubia* (1,530 mg), Coontail (1,510 mg), and Common Waterweed *Elodia canadensis* (1,117 mg) followed. Krull also found that samples of Star Duckweed had the highest number of different taxonomic groups of organisms. Indicating that Star Duckweed may provide substantial habitat for macroinvertebrates including *G. lacustris*.

Previous unpublished research from 2019, studying the relationship between amphipod density and macroinvertebrates in the PPR, found a positive correlation between density of invertebrates and the density of amphipods (Chalberg and Morris 2019). Because of this correlation. Chalberg and Morris (2019) suggest sampling wetland macroinvertebrate communities before introducing amphipods into a system.

Aquatic plant diversity, coverage, and biomass were similar between stocked and natural wetlands. These preliminary results suggest that aquatic plant communities may be correlated with lack of *G. lacustris* establishment in some stockings, but not most. However, other factors (e.g. presence/absence of Star Duckweed) and floating vegetation may have contributed. Future work should investigate factors related to macroinvertebrate community structure to better understand if stocking amphipods is warranted. Density of stocked *G. lacustris* should also be reevaluated to make sure they are being stocked accurately and efficiently.

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