INTRODUCTION

Freshwater amphipods, an important component of food webs in prairie pothole wetlands, have declined in recent decades (Anteau and Afton 2006, 2008*a*, *b*, Huener 2011). Prairie pothole amphipods (hereafter "amphipods"; primarily *Gammarus lacustris* and *Hyalella azteca*) consume course particulate organic matter, algae, and bacteria from the benthos and aquatic macrophytes, and are consumed by amphibians, fish, waterfowl, and other invertebrates (Mathias and Papst 1981, Olenick and Gee 1981, Brown and Fredrickson 1986, Benoy et al. 2002, Strand et al. 2008). Amphipods reach densities of thousands of individuals per square meter in some wetlands (Mathias and Papst 1981, Wen 1992, Pickard and Benke 1996), where they may be a particularly important wildlife food resource. Declines in amphipods have been linked in particular to declines in the continental population of lesser scaup (*Aythya affinis*), which specialize on amphipods as a food resource during migration and breeding (Lindeman and Clark 1999, Anteau and Afton 2004, 2006, 2008*a*, *c*, 2009*a*, 2011, Huener 2011).

Numerous wetland and community characteristics may influence amphipod occurrence and abundance in the Prairie Pothole Region (PPR). Amphipods occur primarily in semi-permanent and permanent wetlands, as they lack a desiccation-resistant winter stage (Murkin and Ross 2000) and have limited dispersal ability. Water chemistry (e.g. temperature, pH) and toxin levels (e.g. pesticides from agricultural run-off) must remain within physiological tolerance limits. Vertebrate and invertebrate predators may impact amphipod occurrence and abundance. Fish in particular may factor in amphipod declines, as fish occurrence in prairie pothole wetlands has increased in recent decades due to a combination of increased wetland connectivity, changing precipitation patterns, and human introduction (Hanson et al. 2005, Anteau and Afton 2008*b*, McLean et al. 2016). Aquatic macrophytes, on the other hand, may provide cover to escape or hide from predators. Increased turbidity due to presence of bottom-feeding fish or agricultural run-off, along with algae growth promoted by nutrient run-off, may impact amphipods directly via changing water temperature and oxygen content, or indirectly via impacting aquatic vegetation. Landscape level factors, such as the presence of buffer strips - the strip of upland vegetation between the wetland edge and adjacent cultivated crop - may mitigate effects of agriculture on amphipods.

Despite the decline in amphipod populations, their habitat requirements are understudied. To our knowledge, Anteau et al. (2011) is the only prior study to examine the relationship between amphipods and community/habitat characteristics in a large number of prairie pothole wetlands. In a study of 283 PPR wetlands in North Dakota, Minnesota, and Iowa, Anteau et al. (2011) found amphipod density to be related to relative fish density, relative submerged aquatic vegetation density, levels of organic mineral suspended solids (*G. lacustris* only), and width of upland vegetation buffer (*H. azteca* only). Relevant fish groups included fathead minnows, other small fish (*H. azteca* only), large Cypriniformes (*H. azteca* only), and other large fish.

In our study, we aim to further explore habitat characteristics associated with amphipod occurrence and density, including fish and aquatic plant communities, water quality and clarity, and landscape-scale characteristics (buffer width extent, percentage of watershed composed of cropped land cover). Previous studies of similar factors using random-site selection techniques (Anteau and Afton 2008*b*, Anteau et al. 2011) were limited by the relatively small proportion of basins containing high amphipod densities, with *G. lacustris* and *H. azteca* densities of ≤5 m^{-3} in 92% and 61% of wetlands surveyed, respectively. To explore habitat characteristics associated with especially high-density basins (which may provide exceptional habitat for migrating waterfowl), we scouted for study sites containing high amphipod densities in the year prior to our study, and surveyed them in addition to randomly selected wetlands. Additionally, we will examine amphipod abundance/occurrence in relation to relative density of submerged aquatic vegetation at the species- to genus-level. Star duckweed (*Lemna trisulca*) and large-leafed

density wetlands because conclusions in previous studies in the Prairie Pothole region (Anteau and Afton 2008*b*, Anteau et al. 2011) were limited by the limited number of high-density basins found via random sampling techniques (M. Anteau, personal communication). High density wetlands were identified via preliminary scouting of 105 wetlands in western Minnesota in 2018, guided by knowledge of local land managers, landowners, and aerial imagery. The 2019 field season prioritized sampling of wetlands with the highest amphipod density in each geographic region. For each high density wetland, we randomly selected a Type 4 or 5 wetland ("inland deep freshwater marsh or inland open water") of similar size (+/- 25% area), within the high density basin's geographic region, from the National Wetlands Inventory Circular 39 wetlands classification layer (Minnesota Department of Natural Resources, Shaw and Fredine 1956) Geographic regions were defined by creating 15-mile circular buffers around each basin's centroid, and merging overlapping buffers (Figure 1). Reference wetlands were included to reduce potential influence of subconscious bias in our choice of wetlands and to ensure a range of amphipod densities (including basins without amphipods) for assessment.

Surveying amphipods, waterfowl, and wetland characteristics

We visited basins, working from south to north as much as possible, during each of three sampling periods.

First, as soon as possible after ice-out (April-May), we surveyed for amphipods and waterfowl. Our survey timing was aimed to capture amphipods prior to or shortly after the appearance of visible juveniles (which is followed by mortality of adults) and to survey waterfowl during migration. For waterfowl, our goal was to focus on migrating scaup (*Aythya affinis* and *A. marila*) and mallard (*Anas platyrhynchos*) breeding pairs in early spring because scaup are known to rely on amphipods as a food source, mallards are abundant in Minnesota, and both species are easy to detect. However, because it added minimal extra time in the field, we collected data on all waterfowl. We will analyze their abundance or presence/absence of additional species in relation to amphipod abundance if sample sizes permit.

We surveyed waterfowl immediately upon arrival to the site. When possible, we conducted surveys from the roadside. When necessary, observers walked in towards the wetland (preferably) or surveyed from canoes positioned just inside the ring of emergent vegetation surrounding the wetland. We used the independent double-observer method to collect data (Pagano and Arnold 2009). Two observers simultaneously scanned the wetland with binoculars and a spotting scope from one best vantage point and documented any birds observed for a fixed 10 minutes. Bird species and social group type (Table 1) were recorded in a data table and on a map of the wetland. Observers did not communicate and used various methods to ensure their actions did not provide useful information to the other observer, such as mock data recording, delayed recording, and mock or prolonged use of binoculars. After 10 minutes, observers communicated and recorded which waterfowl groups they both observed and missed, making use of maps, without changing the original data in their data tables. (Preservation of original data will allow for detectability modeling.) Observers also recorded estimated percentage of the wetland visible from their position, precipitation (light or none), estimated cloud cover (<50% or >50%), and air temperature and wind speed measured with a hand-held weather meter (Kestrel 3000).

We sampled amphipods at 8 sample points per wetland (Figure 2). Sample points were placed on 8 evenly spaced transects across the wetland. Transects were oriented north-south or east-west, whichever direction was closest to perpendicular to the longest axis of the basin. On each transect, a sample point was placed either 5 m or 50 m from shore. The position of the first point (northern/eastern-most or western/southern-most transect, 5 m or 50 m from shore, measured from the west/south shore or the north/east shore) was randomly selected. Points were distributed from there by altering 5 m and 50 m from shore on adjacent transects, and by switching shores every 2 transects. Amphipods were sampled using dip nets (1200 micron mesh) from canoes or sport boats (hereafter ‘boats’). At each point, water depth was measured to the nearest 5 cm using depth markings on a dip net handle lowered vertically into the water. If water was too deep for sampling (>120 cm), we moved along the transect gradually toward shore until water was shallow enough for sampling. A benthic sample was taken by lowering a D-frame dip net vertically into the water, sweeping it along the top 1-2 cm of sediment for 0.5 m, and raising it vertically. Water column samples were taken in an undisturbed area (e.g. other side of the boat) with a modified D-frame or round-frame dip net, with the net frame bent perpendicular to the net handle. The net was lowered vertically to a depth 30 cm above the sediment (corresponding to the top of the area sampled by the benthic dip net), shifted horizontally to avoid disturbed water, and raised vertically to collect a sample. Samples were stored in 1-L plastic bottles or whirl-paks with 99% ethanol at a ratio of 30% sample volume to 70% ethanol volume. Differences in net frame area between D-frame and round nets were measured and incorporated into amphipod density calculations.

We sampled wetlands for fish in June-July. Fish abundance and presence was indexed via catch in one gill net (70’ length, comprised of seven, 10’ panels of different mesh sizes: 0.75”, 1”, 1.25”, 1.5”, 1.75”, 2”, 3”) and 3 mini-fyke nets (6.5mm bar mesh with 4 hoops, 1 throat, 7.62 m lead, and a 0.69 m x 0.99 m rectangular frame opening into the trap) set overnight (12-24 hours) The gill net was set along one of the transects used for amphipod surveys (randomly selected), with the smaller mesh end anchored 10 m from shore or the innermost ring of emergent vegetation when relevant. Mini-fyke nets were also set along randomly-selected transects, with the lead secured to shore or the innermost ring of emergent vegetation. Nets were set perpendicular to shore with the bag end anchored off-shore. Following net retrieval, fish were sorted by species, weighed (total mass of each species), and counted. Field crews subsampled nets if the time required to process the fish in the net was estimated to be greater than 30 minutes. In these cases, fish >30 cm were sorted to species, counted, and weighed. Smaller fish (majority of the catch) were grouped together and weighed. A subsample equivalent to ≥20% of the weight was retained and processed (total weight of each species and species-specific counts).

We visited wetlands a third time (July-early August) for aquatic vegetation surveys, waterfowl surveys aimed to incorporate waterfowl broods, and collection of water chemistry data. Timing of this visit was aimed to capture mature vegetation that could be identified to species, in addition to waterfowl broods. For summer waterfowl surveys, we followed the same methods used to survey waterfowl in spring.

We modeled vegetation survey methods after Minnesota DNR Shallow Lakes survey protocols (Perleberg et al. 2019). In brief, we surveyed sample points distributed across each basin according to the point-intercept method (grid of sampling points; Madsen 1999) from boats. We surveyed a minimum of 10 points per basin, with an additional point for every 1-acre increase in size above 10 acres (+/-10%) up to a maximum of 50 points. At each point, we collected the following five measurements: water depth, relative biomass of floating vegetation, relative biomass of submerged vegetation, and presence/absence of floating and submerged plant taxa. Plants were identified to species-level except in the case of macroalgae (*Chara* spp.), naiads (*Najas* spp.) and some narrow-leaved pondweeds (*Potomogeton* spp.). Water depth was measured using a weighted rope with depth markings. Floating vegetation biomass was indexed visually in a 1-m^2 sample area next to the boat on a 0-to-4 scale (0 = no floating vegetation, 1 = 1-25% area covered with floating vegetation, 2 = 26-50% covered, 3 = 51-75% area covered, 4 = 76-100% area covered), and presence of all floating plant taxa in the sampling area was recorded. Submerged vegetation was sampled by dragging a plant rake 1.5 m along the wetland bottom alongside the boat. The rake was raised and biomass indexed on a 0-4 scale (0 = no vegetation on rake, 1 = 1- 25% of prong area filled with vegetation, 2 = 26%-50% filled, 3 = 51%-75% filled, 4 = 75-100% filled). All living plant taxa on the rake were recorded. Type of emergent vegetation was recorded for each of the shore-adjacent points in the grid. Field crews recorded the dominant emergent vegetation taxon (covering >50% of the area filled with emergent vegetation) in a 1-m wide swathe between the boat and shore, along the transect created by the gridded sampling points. The boat was moved closer to shore as needed. Emergent vegetation was classified to broad group: cattail, sedge, or rush/bulrush.

We collected sediment samples for analysis of pyrethroid concentrations, total organic carbon, and grain size. We collected wetland sediments following procedures described in Shelton and Capel (1995). We collected sediment samples from a minimum of 5 randomly-generated points within 3 meters from shore (McMurry et al. 2016), adding an additional point for each 20-acre increase in wetland size for basins >50 acres. We collected approximately 192 mLs from the top 5 cm of the bottom substrate at each sampling location using a stainless steel sediment corer, and transported samples to a field processing station in a chilled stainless steel bowl. The samples were processed following the procedures of Radtke (2005). In brief, we homogenized each sample with a clean stainless steel spatula, subsampled it (if necessary), and separated it into two 500-mL amber glass jars: one for pyrethroid analysis, and one for total organic carbon and grain size analysis.

We measured near-surface water temperature and pH (Hach HQ40d multimeter) and water turbidity (Hach 2100P turbidimeter). For chlorophyll-*a* measurements, we used a 60 mL syringe and Swinnex-sylte filter holder to filter a measured volume of water (60-240 mL) through a glass microfiber filter (Whatman 1822-025 GF/C 25-mm circular filter with 1.2 μm pore size). The filter was shaded from light and wrapped in stored in aluminum foil. We collected approximately 25mL of water into a plastic centrifuge tube for total phosphorous testing. Water samples and chlorophyll filters were transferred back to the lab on ice and frozen until analysis.

Amphipod processing and identification

For each wetland, we combined the contents of all benthic sample bottles before picking out invertebrates. We picked invertebrates from 25% (volumetric) of the sample at a time until at least 400 invertebrates were obtained, or the entire sample had been picked. We followed the same procedure with water column samples, but water column samples never included >400 invertebrates, and so were always picked entirely.

To process the samples, we filtered ethanol from sample bottles over a 500μm sieve. We poured bottle contents into a plastic tub, added water until the sample could be thoroughly stirred, poured the homogenized sample onto a subsampling apparatus (rectangular wooden frame containing 500-μm metal mesh), and rinsed it further with water. We floated the sample/mesh in shallow water (below the level of the wooden frame) to aid in dispersing the sample evenly across the mesh with a plastic spoon. To split the sample into 25% portions, we fitted a frame containing outlines of four grid squares to the subsampling apparatus and randomly selected a grid square for processing. Contents of the grid square were transferred to a tray using a plastic spoon and floated in water. The subsample was inspected with a 3x lighted magnifier and all invertebrates removed. Amphipods were stored separately from other invertebrates, and all invertebrates were stored in 70% ethanol.

Amphipods were identified (*Hyalella azteca, Gammarus lacustris,* or *Crangonyx* spp.) using a stereomicroscope (Laxco, 6.5-53x magnification) with a digital monitor attachment. We measured amphipod length along a curved line following the dorsal edge of the amphipod, from the base of the first antennae to the base of the telson, using Tcapture software (Tucsen Phototonics Co.) Other invertebrates are being stored for future identification.

Wetland buffers and watershed land use

Wetland buffer strips (also known as filter strips) are defined as the strip of upland vegetation between the wetland edge and adjacent cultivated crops. We assessed buffer strip coverage using remove techniques. Briefly, we used ArcMap tools to construct a buffer zone (30 m width; Sweeney and Newbold 2014) around each wetland edge. Using unsupervised cluster analysis and 2019 NAIP imagery (infrared, 1-m resolution), we then quantified land cover (cropped vs non-cropped) within the 30-m buffer zone. This allowed for the quantification of 1) the proportion of shoreline with a buffer strip ≥30 m wide, and 2) cultivation disturbance in the immediate upland area.

Land use/land cover characteristics in the upland area surrounding each wetland were evaluated at a watershed scale. We used 1-m resolution digital elevation models and ArcHydro tools to delineate the immediate upland catchment associated with each study wetland; these catchment perimeters were very similar to those corresponding with DNR level 08 automated catchments. We then used the 2016 national land cover dataset, 2017 MDA cropland data layer, and 2019 NAIP imagery to quantify dominant land use/land cover types within the wetland catchment area.

Objective 3: Identify habitat characteristics and waterfowl occurrence/abundances associated with amphipod occurrence and abundance

Experimental design, study area, and site selection

Objective 3 of our study focuses on evaluating amphipod stocking efficacy in the prairie-pothole and forest-transition regions of Minnesota. We used a before-after/control-impact study design, such that each stocked wetland was paired with a nearby control wetland of similar size and the same management type (MNDNR Wildlife Management Area, US Fish and Wildlife Service Waterfowl Production Area, or privately owned), and we sampled amphipod density in wetlands both before and after stocking. Wetlands were incorporated into the study in pairs, and the choice of which wetland to stock was randomized whenever possible. The decision was made non-randomly when accessibility for stocking (which required a snowmobile) was only possible on one wetland. All wetlands had semi-permanent or permanent hydrology and at least 1 m maximum depth (to avoid amphipod winterkill). Wetland pairs were chosen based on recommendations from managers, private landowner volunteers, and preliminary scouting.

Objective 3 is a three-year study (fall 2017 through fall 2020). We stocked wetlands in each winter of the study: 10 wetlands in winter 2017-18, 5 wetlands in 2018-19, and 7 wetlands in winter 2019-20. Excepting the first year of the study (see below), for each wetland and its associated control wetland, we sampled amphipod density in the fall (August-October) prior to stocking (“before” sample). We stocked wetlands the following winter (see below), and sampled amphipod population density in each fall following stocking through fall 2020 (“after” samples). Thus winter 2017-18 basins will have 3 after-stocking samples, winter 2018-19 basins with have 2 after-stocking samples, and winter 2019-20 basins will have 1 after-stocking sample, by the end of the study. Habitat characteristics (fish communities, water chemistry, and aquatic vegetation) are assumed to be stable throughout the three years, and are only sampled once per wetland in a year after stocking. Spring and summer waterfowl surveys are also only conducted in one year following stocking. In this research summary, we describe results through December 31, 2019.

Amphipod stocking

We hired a private contractor (Lincoln Bait, LLC) to stock locally collected *G. lacustris*. Amphipods were stocked in winter at the contractor’s recommendation for maximum amphipod survival and ease of collection. In winter, *G. lacustris* cluster on the underside of the wetland’s surface ice and begin mate-guarding (precopulatory amplexus). Amphipods were collected using a remote operated, underwater vehicle towing a net beneath the ice surface. Amphipods were held in tanks (<1 week) prior to stocking. On the day of stocking, amphipods were transported to the wetland in water-filled coolers. We stocked amphipods at a rate of 2 gallons/acre of wetland area, based on the contractor's recommendation. Coolers contained 10,871 +/- 4,441 (standard deviation) amphipods per gallon, based on four subsamples. Thus, we stocked at a rate of 6.4 +/- 5.4 amphipods per m^2.

Coolers were dragged onto the ice on a sled via snowmobile. We stocked amphipods by collecting them in a pitcher and pouring them into holes drilled through the ice. A pump connected to a hose, drawing from a second hole, was used to gently flush amphipods away from the hole after pouring. We divided amphipods among multiple holes: two holes for wetlands <10 acres, with an additional stocking hole added for every 10-acre increase in wetland size. The contractor spread stocking sites across the wetland, but all sites were away from shore (deep enough that water had not frozen to the substrate), and where ice was safe for snowmobile access.

Amphipod sampling

We sampled amphipods in each stocked wetland, and its associated control, in the fall (August-October) of each year, beginning the year before the wetland was stocked. We sampled amphipods along transects that radiated out from the center of the wetland (Figure 2). Wetlands <10 acres had two transects, and a transect was added for each 10-acre increase in wetland size. The first transect was placed a randomly selected bearing (0-359), and the remaining transects were distributed evenly around the wetland (e.g. two transects 180° apart, three transects 120° apart, etc.) On each transect, amphipods were sampled at a site 5 m from shore and a site 50 m from shore.

Amphipods were sampled using D-frame dip nets (1200 micron mesh) from boats. At each point, water depth was measured to the nearest 5 cm using depth markings on the dip net handle, which was lowered vertically into the water. If water was too deep for sampling (>120cm), we moved along the transect gradually toward shore until water was shallow enough for sampling. We then used the net to sweep horizontally along the top 1-2 cm of sediment for a distance equal to the water depth, and then brought the net to the surface at a 45 degree angle. (That is, we sampled the benthos and water column together.) Samples were stored in 1L plastic bottles or whirl-paks with 99% ethanol at a ratio of 30% sample volume to 70% ethanol volume.

One exception to the sampling procedure was that we did not conduct fall before-stocking sampling in the first year of the study. Amphipods informally sampled in winter, prior to stocking, via drilling an ice augur through the ice and into the water, such that water with amphipods washed onto the ice surface.

Amphipod processing and identification

Amphipods were processed and identified as in Objectives 1 and 2, with the following exceptions. Rather than combining sample matter from all sampling points in a wetland, each sampling point was processed separately. We picked invertebrates from 25% (volumetric) of the sample at a time until at least 100 invertebrates were obtained, or the entire sample had been picked.

Waterfowl surveys, fish surveys, water chemistry, and aquatic vegetation

For Objective 3, we will survey waterfowl, fish, water chemistry, and aquatic vegetation at all wetlands following the methods described for Objectives 1 and 2.

PRELIMINARY RESULTS

Objectives 1 and 2: Identify habitat characteristics and waterfowl occurrence/abundances associated with amphipod occurrence and abundance

Amphipod occurrence/abundance

We found amphipods in 42 of 49 wetlands surveyed in 2019. As expected based on previous studies in the prairie pothole region, the most common species were *H. azteca* (n = 39 wetlands) and *G. lacustris* (n = 25 wetlands). In all but 3 wetlands where *G. lacustris* were present, *H. azteca* were also present. We also identified a small number of *Crangonyx* amphipods (identified to genus only) in 9 wetlands, where they co-occurred with *G. lacustris*, *H. azteca*, or both. A small number of amphipods (<1%) could not be identified to genus due to damaged or missing body parts.

In basins with amphipods, total densities (summed across species, averaged across depths and sample points) ranged from 1.4 to 2,061.32 amphipods per cubic meter of water, or 1.5 to 2,203.0 amphipods per m^2 of wetland area (Figure 3). For all taxa, most amphipods were found in benthic sweeps (bottom 0.3 m of water), with densities ranging up to 7,140.6 amphipods per cubic meter (all taxa combined). Water column sweep density ranged up to 60% of benthic density (maximum 785.4 amphipods per cubic meter; all taxa combined).

Amphipod relationships to fish

We found fish in 41 of 49 wetlands surveyed in 2019. A total of 23 species were sampled, with the most common species being fathead minnows (*Pimephales promelas*; n = 33 wetlands), brook stickleback (*Culaea inconstans*; n = 25 wetlands), and black bullheads (*Ameiurus melas; n* = 16 wetlands). In basins with fish, counts ranged from 6 to 2,586 fish sampled and biomass ranged from 14 g to 35,550 g. Probability of *G. lacustris* occurrence decreased significantly with increasing fish biomass (Figure 4; $\chi^2 = 4.70$, $df = 48$, $p = 0.03$). However, preliminary linear regression models assessing fish abundance or biomass as an explanatory variable for *G. lacustris* or *H. azteca* abundance were not significant ($p > 0.05$). Analyzing fish by primary feeding characteristic with redundancy analysis revealed significant, negative associations of amphipod community to benthivores, but not with planktivores or piscivores (F=6.46, df = (1,45), $p < 0.01$). Results will be reassessed with a larger sample size of wetlands following the 2020 field season.

Amphipod relationships to aquatic vegetation

Emergent vegetation data have not yet been analyzed. Preliminary analysis of submerged and floating aquatic vegetation indicated that *H. azteca* densities were positively correlated to submerged plant prevalence. However, *G. lacustris* were not associated with submerged vegetation prevalence, relative biomass, or species richness. The amphipod community was significantly predicted by the vegetation community according to redundancy analysis. Benthic counts of amphipods (all species together) were positively associated with relative density of narrow-leaved pondweeds (*Potamogeton* spp.) and northern watermilfoil (*Myriophyllum sibiricum*). *G. lacustris* counts were positively associated with star duckweed (*Lemna trisulca*). Amphipoda were negatively associated with common bladderwort (*Utricularia macrorhiza*), a carnivorous plant.

Amphipod relationships to water chemistry

Preliminary analyses evaluating relationships between water chemistry and amphipod abundance did not reveal distinct trends; overall, both species of amphipods appeared to exist at a wide range of densities across a wide gradient of turbidities, nutrient concentrations, and pH levels. Data will be reanalyzed with a larger sample size of basins following the 2020 field season.

Amphipod relationships to sediment characteristics and pyrethroids

Analysis of sediment characteristics and pyrethroid levels are on-going in BSU labs, following delays due to COVID-19.

Amphipod relationships to buffer widths and watershed scale land use

Preliminary landscape-level results showed that *H. azteca* were positively associated with upland buffer strip coverage (Figure 5), whereas neither *H. azteca* nor *G. lacustris* exhibited a strong relationship with watershed-scale land use/land cover (Figure 6). We will continue examining these trends with a larger sample size of basins following the 2020 field season.

Waterfowl relationships to amphipods

In April-May 2019, we carried out spring waterfowl surveys at 48 of the 49 basins sampled for amphipods. Waterfowl were not detected at 5 basins. We counted 250 waterfowl (duck, goose, or swan) social groups (e.g. pairs, lone individuals, flocked males, groups), including 1830 adults and 191 indicated breeding pairs in the other 43 basins. Canada geese and mallards were the most common species (occurred on the most basins). Scaup occurred in 10 basins, primarily with high amphipod densities (Figure 7). Mallards occurred on 26 basins, but did not show an obvious trend in occurrence or abundance related to amphipods (Figure 7).

In July-August, we carried out surveys at all 49 basins. We observed waterfowl in 36 basins, including 23 basins with waterfowl broods. Mallards and "unknowns" (observers could not identify to species) were the most common species of adults and broods in summer. Number of broods did not show an obvious trend in occurrence or abundance related to amphipods (Figure 7).

Spring and summer survey outcomes will be reassessed with larger sample size following the 2020 field season.

Objectives 3: Evaluate the effectiveness of amphipod stocking as a management technique to establish self-sustaining amphipod populations and improve waterfowl habitat

As of December 2019, we had stocked 15 wetlands with *G. lacustris*. At two sites, 2-3 stocked basins were associated with the same control basin, such that we had 12 control basins. Objective 3 amphipod counts are currently being incorporated into our project database for analysis. *G. lacustris* were not found in any basins prior to stocking, with the exception of one site (Old Red Lake Trail Wildlife Management Area) stocked in the first year of the study. *H. azteca* were found in some basins, and their densities will be analyzed in the future. However, *G. lacustris* have not been found in any basins post-stocking, with the exception of Old Red Lake Trail WMA.

Spring waterfowl surveys were conducted at all 27 basins in spring 2019, and fish surveys were conducted at 12 basins. These data remain to be analyzed.

An additional seven wetlands were stocked with amphipods in winter 2019-20. We will conduct spring waterfowl surveys at these basins, aquatic vegetation and summer waterfowl surveys at all basins, and fish surveys at all remaining basins, in spring-summer 2019-20.

DISCUSSION

Objectives 1 and 2: Identify habitat characteristics and waterfowl occurrence/abundances associated with amphipod occurrence and abundance

Our preliminary data suggest that our method of including of preliminarily-scouted basins will allow us to assess relationships between habitat characteristics and amphipod density in high-density basins. 53% of our 2019 study wetlands contained amphipods at densities greater than 100 individuals per cubic meter.

Preliminary results suggest that fish generally have a negative relationship with amphipod density, whereas plants have a positive relationship. Our findings that *G. lacustris* are less likely to occur in wetlands with higher fish biomass, and that benthivorous fish counts are negatively associated with amphipod counts, align with Anteau et al.'s (2011) findings that fish at high densities are negatively correlated with amphipod densities. Benthivores may consume amphipods directly, or affect amphipods indirectly by stirring up wetland turbidity (Anteau et al. 2011). While previous studies have documented a positive relationship between relative density of submerged aquatic vegetation and amphipods, our preliminary data suggest that particular species, including northern watermilfoil, narrow-leaved pondweeds, and star duckweed may play a role in supporting high amphipod populations. On the other hand, negative correlations with common bladderwort may indicate that this carnivorous plant depresses amphipod populations via predation. Interestingly, our preliminary finding that *H. azteca*, but not *G. lacustris*, densities are related to buffer strip coverage matches Anteau et al.'s (2011) results. This may indicate differing tolerances for pesticide or sediment run-off between the two species, or that *G. lacustris* simply don't occur in landscapes where agriculture is intense enough that upland vegetation has much impact on water quality. However, we caution that all these data are highly preliminary, and will be reassessed with an increased sample size after a second field season.

In waterfowl surveys, scaup appeared to occur primarily in basins with high amphipod densities (>400 amphipods per cubic meter in benthic samples). These preliminary results concur with earlier studies finding a positive relationship between scaup occurrence and amphipod density during migration and breeding (Lindeman and Clark 1999, Anteau and Afton 2009*b*) However, with a such a small sample size of basins with scaup (n = 10), at least one additional field season is needed to establish whether this pattern is consistent. Water column amphipod densities will be incorporated into density values for comparison to Anteau and Afton's (2009*b*) finding that probability of scaup foraging peaks at 26 amphipods per cubic meter.

Objectives 3: Evaluate the effectiveness of amphipod stocking as a management technique to establish self-sustaining amphipod populations and improve waterfowl habitat

Stocked *G. lacustris* have not been detected post-stocking in any of our wetlands. It is possible that stocked amphipods have 100% mortality within the year due to predation, other aspects of habitat quality, or stress associated with translocation. However, monitoring of newly stocked wetlands via underwater camera in January 2020 indicates that stocked amphipods remain alive under the ice in high numbers for at least 7 days following stocking. Amphipods may be persisting in basins at low, undetectable levels and appear in future years. Monitoring of stocked wetlands will continue for at least another year. Additionally, habitat characteristics in stocked wetlands will be compared to wetlands where *G. lacustris* naturally occur (Objective 1) to assess potential reasons for stocking challenges.

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Table 1. Social groups counted by observers during spring and summer waterfowl surveys in Minnesota in 2019.

Social group	Description
Lone male	Single isolated male without visible associated female
Lone female	Single isolated female without visible associated male
Lone unknown	Single isolated bird of unknown sex. For sexually monomorphic species and lone birds where species is unknown.
Pair	One male and one female of the same species in close association
Flocked males	2-4 male individuals of same species in close association
Group	Any other group of adults[1]
Brood hen with brood	Brood associated with an adult female
Brood	Brood without associated adult
Pair with brood	Two adults closely associated with brood. Only for geese and swans.
Lone Unknown with Brood	One adult with a brood. Only for geese and swans (sexually monomorphic).
Unknown	Group cannot be classified because species or sex cannot be identified

[1]One female associated with two males is considered a pair and a lone male.

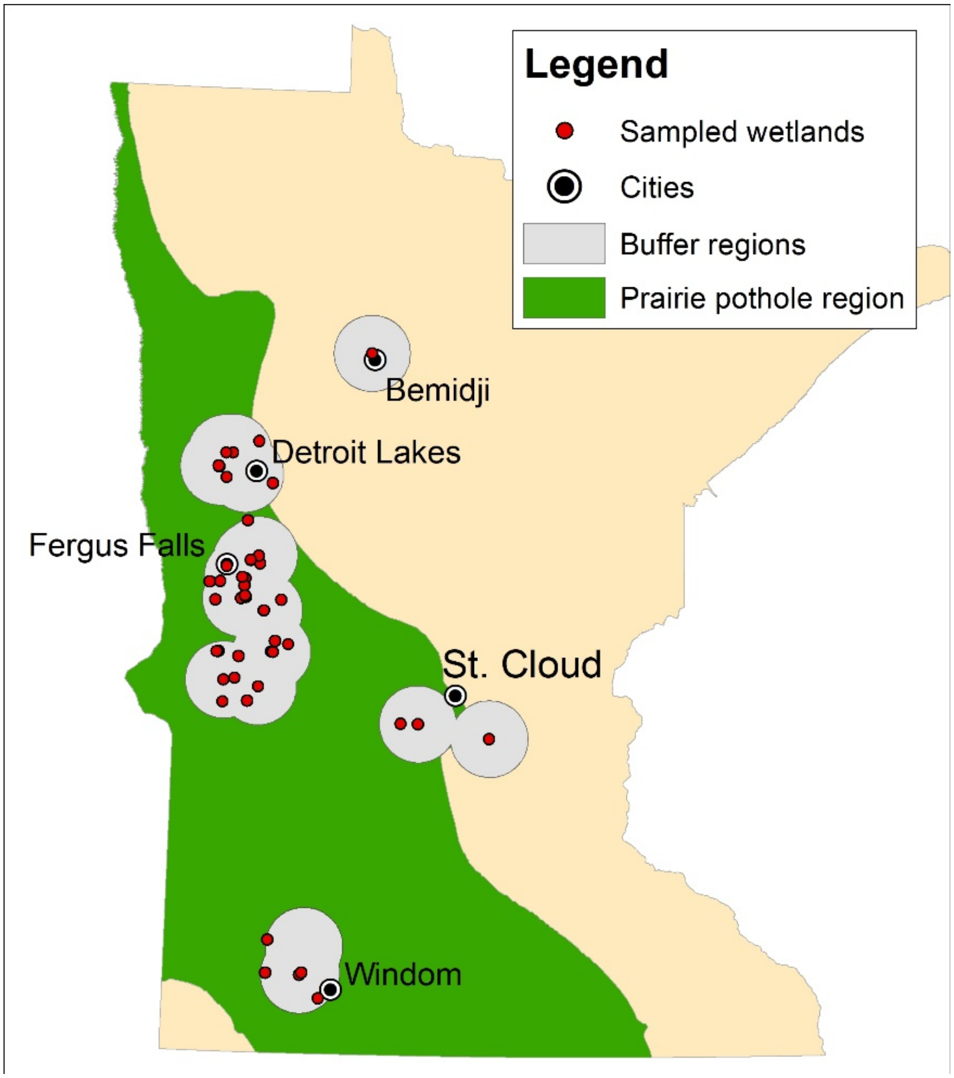

Figure 1. Prairie pothole and forest-transition zone wetlands sampled for amphipods, waterfowl, and habitat characteristics (fish communities, aquatic vegetation, soil and water properties) in 2019. Wetlands were primarily distributed in four sampling clusters near Windom, St. Cloud, Fergus Falls, and Detroit Lakes, MN. Study sites included wetlands of known high amphipod density and randomly selected wetlands. Randomly selected wetlands were chosen from merged 15-mile circular buffer zones around high density basins in each sampling cluster.

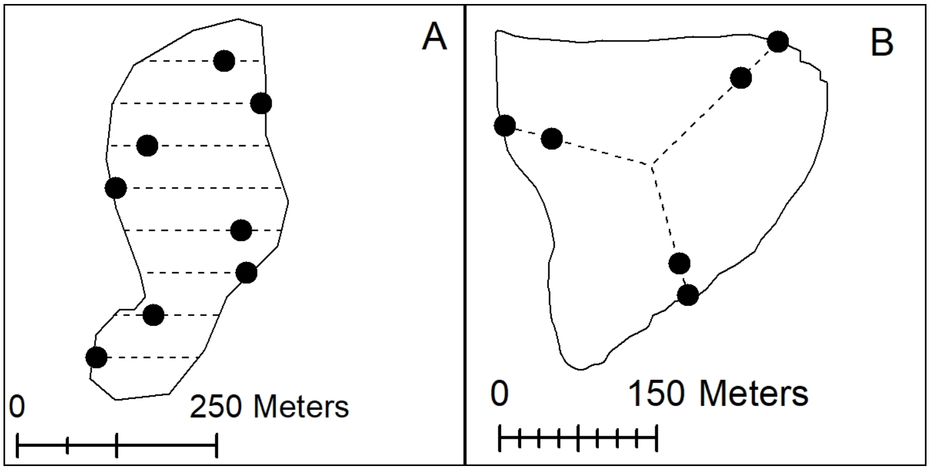

Figure 2. Amphipod dip-net sampling point arrangement for (A) Objectives 1 and 2, (B) Objective 3. Solid lines represent sample wetland outlines, dashed lines represent transects, and dots represent survey points.

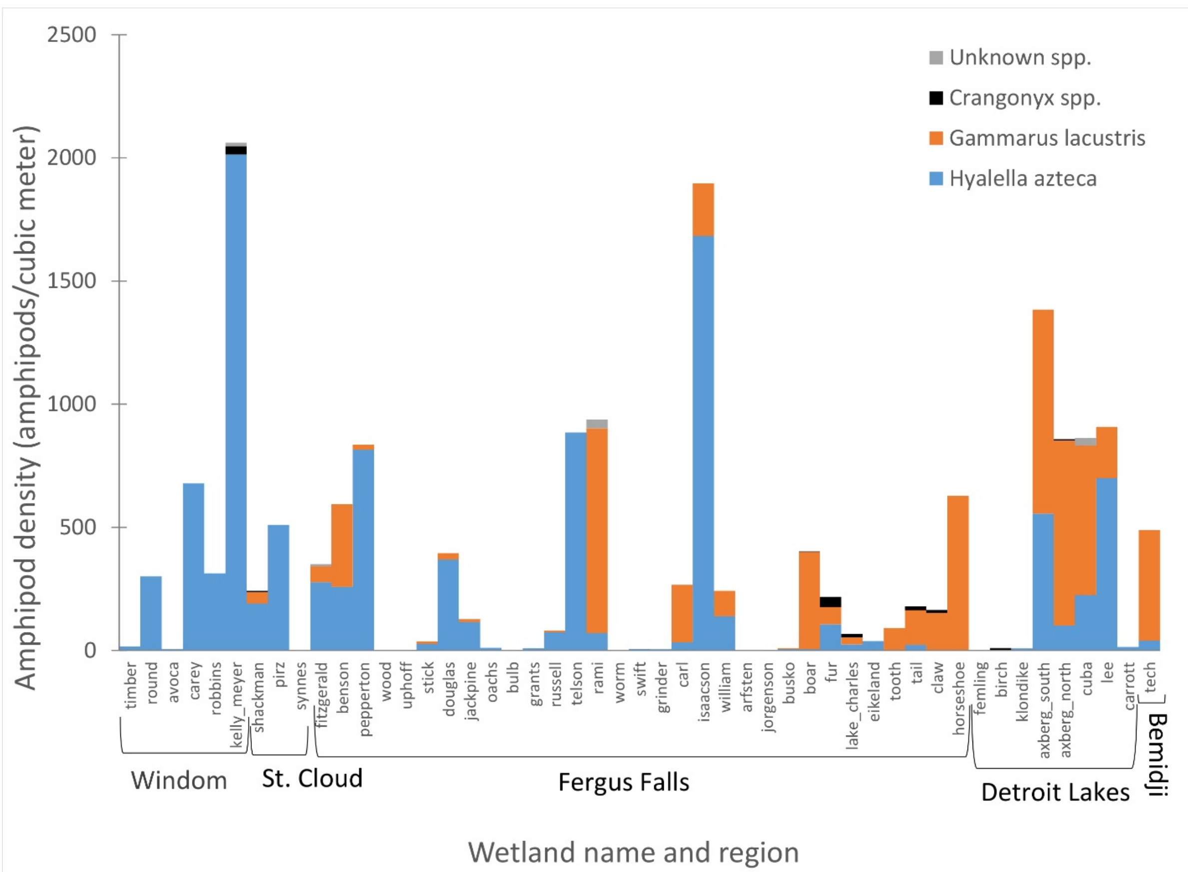

Figure 3. Amphipod densities by species (spp.) in prairie pothole and forest-transition zone wetlands in western Minnesota in spring 2019. Densities are calculated from eight benthic and eight water column dip net sweeps per wetland, accounting for water column depth. Wetlands were in geographic clusters named for nearby cities (Windom, St. Cloud, Fergus Falls, Detroit Lakes, Bemidji) and are ordered from lowest to highest latitude (left to right) on the x-axis.

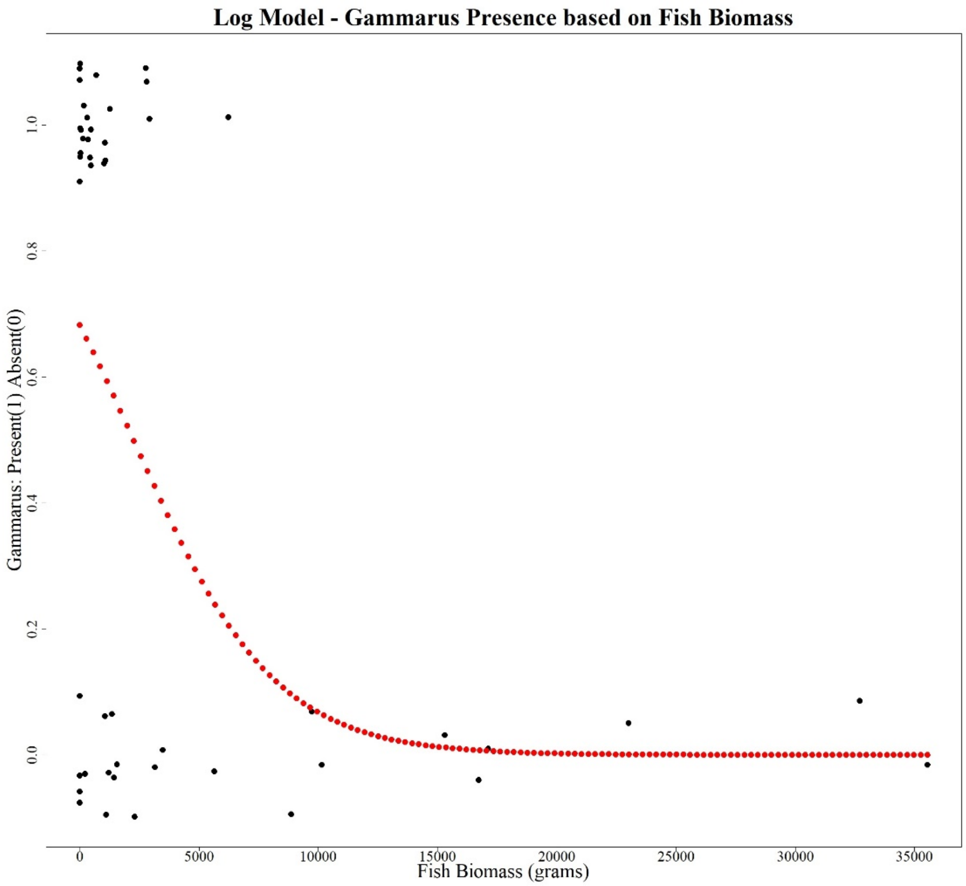

Figure 4. Relationship between probability of *G. lacustris* occurrence and total fish biomass in prairie pothole and forest transition-zone wetlands sampled for fish and amphipods in Minnesota in 2019. Red dots represent the best-fit logistic regression line. Probability of *G. lacustris* occurrence decreased significantly with increasing fish biomass ($\chi^2(48) = 4.70$, $p = 0.03$).

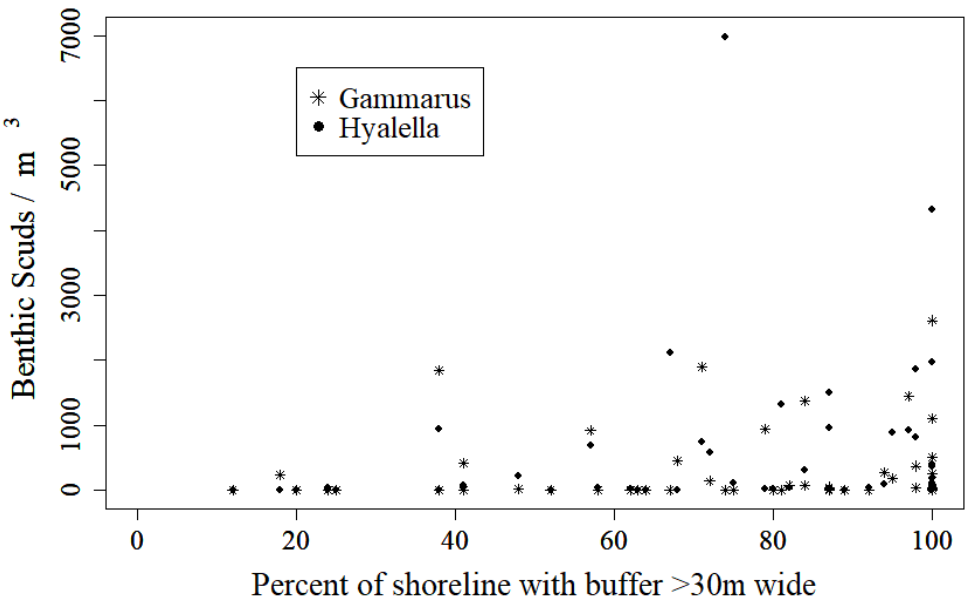

Figure 5. Relationship between benthic-zone amphipod density and percent of shoreline length with a buffer strip at least 30 m wide in 49 prairie pothole and forest-transition zone wetlands sampled in Minnesota in 2019.

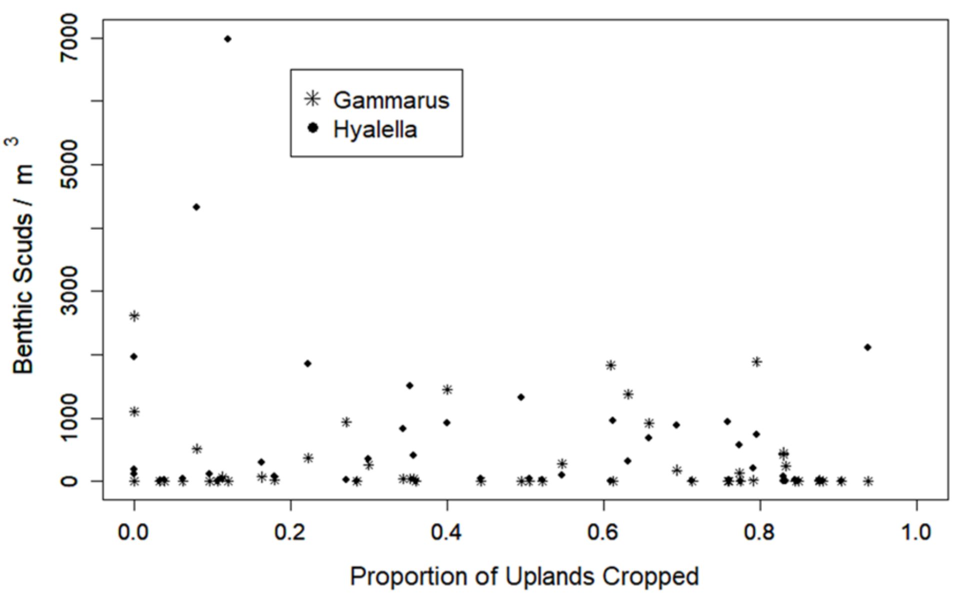

Figure 6. Relationship between benthic-zone amphipod density and proportion of immediate upland catchment with cropped landcover in 49 Minnesota prairie pothole and forest-transition zone wetlands sampled in 2019.

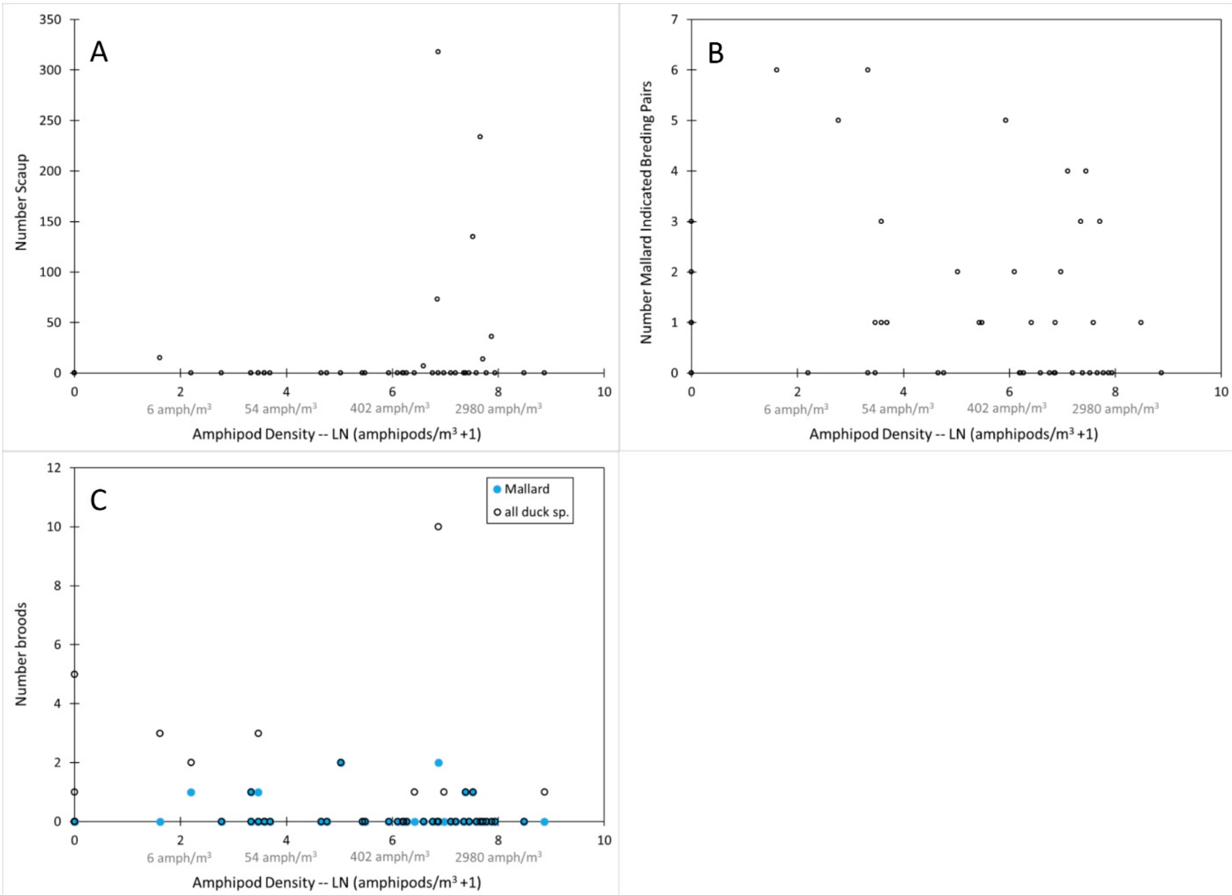

Figure 7. Number of (A) adult scaup (*Aythya affinis* and *A. marila*) in spring, (B) mallard (*Anas platyrhynchos)* indicated breeding pairs in spring, and (C) number of summer waterfowl broods observed on prairie pothole and forest-transition zone wetlands of varying benthic-zone amphipod density in Minnesota in spring 2019. Amphipod densities are transformed to a log scale for visual clarity.