

Summaries of Wildlife Research Findings **2022**



Minnesota Department of Natural Resources
Division of Fish and Wildlife
Wildlife Populations and Research Unit

SUMMARIES OF WILDLIFE RESEARCH FINDINGS 2022

Edited by:
Seth Goreham
Michelle Carstensen
Michael A. Larson
Nicole Davros
Bruce Davis



Minnesota Department of Natural Resources
Division of Fish and Wildlife
Wildlife Populations and Research Unit
500 Lafayette Road, Box 20
St. Paul, MN 55155-4020
(651) 259-5202

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For more information contact: DNR Information Center
500 Lafayette Road St. Paul, MN 55155-4040
(651) 296-6157 (Metro Area)
1 888 MINNDNR (1-888-646-6367)

TTY (651) 296-5484 (Metro Area)
1 800 657-3929
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Forest Wildlife Populations and Research Group

1201 East Highway 2
Grand Rapids, Minnesota 55744
(218) 327-4432



SPRUCE GROUSE AS INDICATORS OF BOREAL FOREST CONNECTIVITY

Charlotte Roy, Julia Ponder¹, and Cody Aylward²

SUMMARY OF FINDINGS

We completed a 4-year study (2019-2022) to examine spruce grouse (*Canachites canadensis*) occupancy of conifer forest patches, responses to timber harvest, and genetic connectivity in the boreal forest of northern Minnesota. We conducted a pellet survey of forest stands during late winter/early spring and documented the presence/absence of spruce grouse and other forest wildlife species, as it related to various landscape and forest attributes and climate variables. We examined changes in spruce grouse survival and space use in response to timber harvest with radio-marked birds. We also collected feathers from hunters and during capture efforts to examine landscape connectivity for spruce grouse using landscape genetic methods. Our occupancy results indicated that forest management practices that promote dense vegetation structure may benefit spruce grouse, especially a dense mid-canopy layer (5.0 – 15.0 m). The mid-canopy layer was not as important for the other wildlife species we examined. Our telemetry data indicated that spruce grouse have lower survival after timber harvest, but that most harvested conifer stands were avoided prior to harvest, likely due to the sparse midstory vegetation structure in many of these mature stands. We suggest that reduced spruce grouse survival after timber harvest may be due to indirect effects of timber harvest, such as harvest-related changes in predator behavior or predator space use (e.g., edge effects, changes in predator density in nearby stands) rather than habitat loss. Finally, our genetic data indicated that spruce grouse exist as a single interconnected population in Minnesota, with coniferous forest land cover and lower average temperatures during spring dispersal being the best predictors of gene flow. Therefore, climate change could potentially threaten the persistence of the single interconnected population.

OBJECTIVES

1. We determined whether occupancy of forest stands by spruce grouse and other wildlife species (i.e. ruffed grouse, snowshoe hare) was related to landscape-level, stand-level, and climate variables.
2. We determined spruce grouse responses to timber harvest by radio-marking spruce grouse and examining survival and space use before and after timber harvest.
3. We determined current boreal forest connectivity for spruce grouse using a landscape genetic approach.

METHODS

Occupancy

We used fecal pellet surveys to examine occupancy of conifer stands by spruce grouse, ruffed grouse (*Bonasa umbellus*), and snowshoe hares (*Lepus americanus*) as part of the annual spruce grouse survey during 2019-2022 (Roy et al. 2022). We built single-species multi-season

¹The University of Minnesota College of Veterinary Medicine, St. Paul, Minnesota, USA

²The University of Minnesota, College of Food, Agriculture, and Natural Resource Sciences

occupancy models to examine detection, occupancy and extinction, and multi-species occupancy models to examine co-occurrence of spruce grouse and ruffed grouse.

We used a stepwise approach to construct occupancy models, guided by *a priori* hypotheses for each single fixed-effect predictor. We subsequently built hypothesis-driven multivariate occupancy models using predictors that outperformed a null model (i.e., a model with no fixed-effects predictors). We examined landscape-scale, stand-scale, and climate variables as predictors of occupancy (Table 1). We also examined numerous detection covariates related to observers and survey conditions, including observer type (i.e., citizen volunteer, permanent DNR or cooperator staff, or seasonal DNR technician), the number of observers, survey conditions (0-10, with 10 representing optimal survey conditions), the extent of snow cover (i.e., none, partial, complete), survey date, and whether pellets were detected on snow or bare ground. We used the survey route as a random intercept to account for the sampling design of 67 routes comprised of 4-5 circular transects centered on road-based points (Roy et al. 2022). We constructed all single-species dynamic (i.e., multi-season) occupancy models in R package 'ubms' using the `stan_colext` function (Kellner et al. 2021).

We also constructed multi-species occupancy models to examine environmental conditions associated with species co-occurrence in late winter/early spring. We focused this analysis on spruce grouse and ruffed grouse. We used the most-supported models for each species from single-species occupancy models, then tested several hypotheses of co-occurrence. We expected co-occurrence to be associated with mixed coniferous/deciduous forest at a landscape scale. We constructed multi-species occupancy models in the R package 'unmarked' using the `occuMulti` function (Fiske and Chandler 2011) but did not include random intercepts because this program did not support random effects.

Responses to Timber Harvest

Study area

We had 2 focal study areas in northwestern Minnesota—Red Lake Wildlife Management Area (RLWMA) in Lake of the Woods and Roseau Counties, and another near Big Falls in the Littlefork MNDNR Forestry work area in Koochiching County (Fig. 1). We identified stands scheduled for harvest by working with wildlife managers and foresters. Eighteen black spruce stands (*Picea mariana*, 11–145 ac, 4–59 ha) and 17 jack pine (*Pinus banksiana*) stands (9–43 ac, 4–17 ha) were identified for harvest near RLWMA (C. Tucker, pers. comm.). An additional 43 black spruce stands (3–221 ac, 1–89 ha) were identified in the Littlefork Forestry area (B. Feldt and J. Rengo, pers. comm.), along with 87 black spruce stands (0.1–79 ac, 0.04–32 ha) and 10 jack pine stands (1.6–11 ac, 0.6–4.5 ha) identified by Koochiching County Land and Forestry (N. Heibel, pers. comm.). A few additional red pine (*Pinus resinosa*, $n = 4$) and white spruce (*Picea glauca*; or mixed spruce ($n = 13$) stands were planned for harvest but given a lower priority than black spruce and jack pine stands in the study. We added spruce top harvests and a birch pole harvest adjacent to jack pine stands during the study based on new permits and conversations with managers and foresters. Some stands were harvested after the study concluded.

Field methods

We captured spruce grouse with noose poles (Zwickel and Bendell 1967) in areas near planned timber harvest. We located birds for capture by broadcasting the female cantus call in spring (Fritz 1979, Boag and McKinnon 1982, Schroeder and Boag 1989, Whitcomb et al. 1996, Lycke et al. 2011), working with volunteers with trained dogs when weather was conducive, locating spruce grouse with other radio-marked birds or while walking in the woods, or when birds came to roads for grit. We fitted spruce grouse with necklace-style VHF transmitters (A-3950) from

Advanced Telemetry Systems® (Roy et al. 2016).

We used homing techniques to obtain GPS coordinates and habitat data for bird locations throughout the year. Birds were tracked 1-2 times weekly to characterize home ranges and survival before and after timber harvest. Locations were obtained throughout the day (excluding night) from within a few meters of the birds.

Additionally, we surveyed transects for spruce grouse pellets during winter and early spring to locate spruce grouse for capture in stands scheduled for harvest and to examine spruce grouse use of these stands. Pellet surveys were conducted >3 days after snowfall, unless harvest was imminent, and a survey had to be completed earlier. Otherwise, we surveyed each stand ≥ 3 times (Huggard 2003). We determined transect lengths based on timber stand area and sampled at a rate of 10 m/ac (25 m/ha). We placed transects systematically through timber harvest areas with a starting point on the harvest boundary. We spaced transects ≥ 150 m apart (Evans et al. 2007). We searched 1 m on either side of the transect for spruce grouse pellets (Evans et al. 2007, Schroeder and Vander Haegen 2014, Roy et al. 2020) and also recorded grouse observed, tracks, and ruffed grouse pellets (Huggard 2003) both on and off transect. Every 100 m along the transect, we measured habitat characteristics in a habitat plot (Huggard 2003), collecting the same data as collected at capture sites and at telemetry locations to characterize habitat in the stand. Specifically, we recorded GPS coordinates, overstory stand type, the center tree species, center tree circumference, distance to the nearest tree, nearest tree species and circumference, and the number of live trees in a 3.6-m radius from the center tree (40.7 m² plot), which is similar in size to the 1/100-ac fixed-radius plot that is used in Cooperative Stand Assessment Field Procedures (MNDNR 2001). At 3.6 m from center in the 4 cardinal directions, we collected spherical densiometer readings (Fiala et al. 2006, Paletto and Rosi 2009, Baudry et al. 2014) and measured shrub density in 1-m² plots. We selected these habitat measurements based on vegetation attributes that differed between stands where spruce grouse were observed and where they were not observed (Potvin and Courtois 2006), or other attributes that predicted spruce grouse presence or occupancy, including stem density, shrub cover, basal area, and canopy cover (Huggard 2003, Ross et al. 2016).

Analysis

For each individual, we determined whether ≥ 1 location occurred ≤ 500 m from a harvested stand. We used harvest date to categorize locations as pre-harvest or post-harvest. We categorized all locations for an individual as non-harvest if none overlapped a harvested stand.

We calculated 90% minimum convex polygons (MCPs) with R package 'adehabitatHR' (Calenge 2015). We chose 90% to be restrictive of outliers because a small number of birds made short-term (i.e., 2-3 weeks) movements to irregular locations that were not seasonal or harvest-related movements. We compared home range size in pre-harvest, post-harvest, and non-harvest treatments. We predicted that pre-harvest home ranges would be larger than post-harvest home ranges due to loss of available habitat.

To assess harvest effects on space use, we calculated the proportion of each MCP that overlapped a harvested stand and the proportion that overlapped a 500-m buffer area surrounding a harvested stand. We predicted more overlap between harvest areas and buffer areas in pre-harvest MCPs than post-harvest MCPs.

We fit Kaplan-Meier curves in the R package 'survival' to examine the impact of harvest on survival (Therneau et al. 2015). We estimated the probability of survival for >1 yr for pre-, post-, and non-harvest home ranges. We predicted that survival probability would be higher for pre- or non-harvest treatments than post-harvest.

Landscape Genetics

We collected hunter-submitted feathers from throughout northern Minnesota and feathers from birds captured from the Big Falls study area and RLWMA to obtain genetic samples. This genetic information can be linked to spatial information to examine the connectivity of the landscape for spruce grouse. Areas that share greater connectivity will be similar genetically, whereas areas with restricted connectivity will become more genetically dissimilar over time. We identified 3 putative populations: Superior Uplands, Big Falls study area, and RLWMA (Fig. 2).

We obtained unambiguous genotype data for 7 microsatellite loci from the University of Minnesota Genomics Center (Table 2). Microsatellites are highly variable, neutral (non-coding) genetic loci that are useful for studies of population structure (i.e., differences) and in landscape genetic applications. We used a non-spatial method in program STRUCTURE to assess genetic structure (Pritchard et al. 2000) with the default admixture and correlated allele frequency model (Falush et al. 2003). We summarized the results with StructureHarvester (Earl and von Holdt 2012) and determined best-supported K values with log likelihood scores and also using the ΔK approach (Evanno et al. 2005).

We used a landscape genetics approach based on causal modeling to examine environmental drivers of genetic connectivity for spruce grouse (Cushman et al. 2006). We constructed resistance surfaces for each of our hypothesized landscape predictors (Table 3), such that areas we hypothesized would facilitate gene flow had low resistance, and areas that we hypothesized would impede gene flow had high resistance. We tested two categorical resistance surfaces representing land cover: 1) coniferous forest (combining the wooded wetland and evergreen forest categories from the National Land Cover Dataset [Dewitz and USGS 2021]), and another using 2) spruce grouse preferred stand types based on the literature (i.e., combining jack pine, black spruce, and tamarack [*Larix laricina*] forest) from the GAP Land Cover Dataset (USGS 2011; Robinson 1969; Pietz and Tester 1982; Allan 1985; Anich et al. 2013). We also tested 5 continuous resistance surfaces representing the influence of humans (road density), mean annual snowfall (NOAA 2023), mean spring temperature (PRISM 2023), and two types of stand age resistance. In the first type of stand age resistance we considered, resistance increased with older stand age. In the second, resistance increased with greater deviance from age 40-yr because previous work suggested spruce grouse occupancy declines with forest succession, but that occupied stands were approximately 40-yr-old (Ross et al. 2016). We gave any land categorized as non-forest (i.e., lacking a stand age value) the highest value of resistance (R_{\max}).

We used the Maximum Likelihood Population Effect (MLPE) approach to construct landscape genetics models (Clarke et al. 2002). Genetic distance was the response, and resistance distances were predictors to determine which environmental variables were strong predictors of genetic distance. We used the `dist_euclidean` function in the R package 'gstudio' to calculate genetic distance between individuals (Dyer 2012). We used Circuitscape 5 to calculate resistance distance between individuals for each resistance surface (Anantharaman et al. 2020). We included a random intercept effect in each model for each of the 3 regions due to the clustered sampling design. We determined the R_{\max} value that resulted in the model with the strongest predictive performance for each variable using the corrected Akaike's Information Criterion (AIC_c ; Burnham and Anderson 2002). We used a "null" model based on a uniform resistance surface where every cell had a value of 1 as a standard with which to compare other candidate models. If more than one R_{\max} value outperformed the null model, we used only the most-supported R_{\max} value (based on AIC_c) for further modeling. Finally, we built multivariate models by combining all subsets of landscape variables receiving more support than the null model.

We predicted functional connectivity across the study area using the parameters from the most-supported model. We used the Raster Calculator tool in ArcGIS (ESRI 2011) to make a composite resistance surface raster based on the intercept and β coefficients of predictors in the most-supported model. We summed the intercept and the product of each resistance surface and their respective β coefficients in the most-supported model. We then normalized the values in this raster to a 1-100 scale to conduct circuit-based connectivity mapping. We mapped functional connectivity using Circuitscape (Koen et al. 2014; Aylward et al. 2020) and also Omniscape (Landau et al. 2021), which has the potential to produce more biologically realistic scenarios of connectivity but also has different assumptions.

RESULTS

Occupancy

The most-supported detection model for spruce grouse and ruffed grouse included observer type and survey condition, with spruce grouse detection also influenced by snow extent and ruffed grouse detection influenced by survey date (Table 4). Detection probability was greater for partial snow cover than complete or no snow cover, and detection probability was highest for seasonal technicians and lowest for citizen volunteers. For spruce grouse and ruffed grouse, detection probability increased with improving survey condition, but detection probability was consistently high for snowshoe hare and influenced only by observer type. Detection probability was 0.62 (0.58-0.67) for spruce grouse, 0.69 (0.66-0.72) for ruffed grouse, and 0.96 (0.95-0.97) for snowshoe hare.

Spruce grouse occupancy was best explained by land cover and forest structure (Table 4). The most-supported multi-season occupancy model included mid-canopy density (+) (i.e., LiDAR 5-15 m) and deciduous forest land cover (-) (Fig. 3). Models with tree density (+) and stand age (-) also outperformed a null model but were not included in multivariate models due to correlations with mid-canopy density. Spruce grouse extinction probability included the effects of deciduous forest land cover (+) and road density (+). Ruffed grouse occupancy was influenced by red pine (-), which also increased the probability of extinction, along with tree density (+) and mixed forest (-). In contrast, snowshoe hare occupancy was related to tree density (+), with extinction influenced by snowfall (-) and the density of lower canopy (i.e., LiDAR 1.37-5 m) (-).

Multi-species occupancy modeling indicated that co-occurrence of spruce grouse and ruffed grouse was associated with mixed coniferous/deciduous forest (+) (Table 5). Models with stand-scale variables were not more supported than a model without co-occurrence predictors.

Responses to Timber Harvest

We radio-marked 107 spruce grouse to examine responses to timber harvest, with 62 near Big Falls and 45 at RLWMA. Thirteen focal timber stands near Big Falls and 18 stands at RLWMA were harvested during the study. Additionally, 4 spruce top harvests and 1 birch pole harvest were executed in focal stands in the Big Falls area. In Big Falls, we completed pellet surveys and accompanying habitat measurements along transects in 20 stands and detected spruce grouse pellets in 3 stands. At RLWMA, we found spruce grouse pellets in 6 of 18 surveyed stands.

Overlap of MCPs with harvested areas was low in both study areas and was near 0 at RLWMA (Table 6). Near Big Falls, overlap decreased after harvest, but few birds overlapped harvested stands. More individuals overlapped with 500-m buffer zones around harvested stands, facilitating more robust statistical analysis. In both study areas overlap of MCPs with buffer zones decreased post-harvest relative to pre-harvest levels. Survival of individuals with an MCP overlapping a harvested stand was lower post-harvest than pre-harvest and was lower than for individuals in the non-harvest treatment (Table 7).

Landscape Genetics

The model representing a single interconnected spruce grouse population had the greatest log-likelihood. Land cover, stand age, and climate variables were included in ≥ 1 model that was more supported than the null model. However, the most-supported model included coniferous forest land cover and temperature during spring dispersal (Tables 8, 9).

Circuitscape and Omniscape produced similar maps of functional connectivity with high gene flow indicated in the northwestern and northeastern portions of the study area (Fig. 4). Movement corridors were more apparent in Circuitscape than Omniscape, but both analyses emphasized coniferous forest stands as areas of greater gene flow. Omniscape indicated several areas of no flow, corresponding to regions >7 km from source areas. For example, Red Lake and the Mesabi Range had low flow in both Circuitscape and Omniscape maps. Otherwise, the region was characterized by relatively well-connected flow, with higher flow in coniferous forest stands.

DISCUSSION

Occupancy

Our models suggest mid-canopy density was the key stand-scale determinant of spruce grouse occupancy and persistence, which is likely related to cover from predators and thermal protection (Thompson and Fritzell 1988). Forest structure may be a stronger determinant of spruce grouse habitat selection than stand type, which may explain the widely varying descriptions of stand types used by spruce grouse in the literature (Boag and Schroeder 1992, Lycke et al. 2011, Anich et al. 2013). In the Great Lake States, spruce grouse prefer areas of black spruce with some jack pine interspersed, but they have also been reported using balsam fir (*Abies balsamea*), tamarack, eastern hemlock (*Tsuga canadensis*), and northern white cedar (*Thuja occidentalis*; Robinson 1969, Pietz and Tester 1982, Anich et al 2013). In Wisconsin, spruce grouse used closed canopy coniferous uplands near lowland conifer swamps, especially mature black spruce-tamarack swamps and jack pine 16–29 years old (Anich et al. 2013). Similarly in Minnesota, but with a modest sample size, spruce grouse used lowland conifers with black spruce and mixed bogs during the breeding season and used jack pine during winter (Pietz and Tester 1982). Various successional stages are used throughout spruce grouse range, including early-successional (Boag and Schroeder 1992), mid-successional (Ross et al. 2016), and mature forest (Anich et al. 2013). We also found deciduous forest cover to be a key factor limiting the range of spruce grouse (Casabona et al. 2022).

The strongest predictors of both occupancy and local extinction probabilities for spruce grouse were landscape-scale land-cover variables and mid-canopy density (5-15 m). Temperature was a predictor in the most-supported multivariate extinction model but had a small effect relative to land cover, road density, and canopy structure. Road densities were positively related to spruce grouse extinction probability. Road densities may be positively associated with predator density, which can affect prey species occurrence (Boan et al. 2014). Road density may also be correlated with hunter accessibility and localized harvest pressure near roads. Areas with fewer roads may promote spruce grouse persistence.

Spruce grouse and ruffed grouse occurrence aligns with a coniferous-deciduous forest gradient with co-occurrence most probable in the mixed coniferous/deciduous transition zone. The land cover-based gradient coincides with the large-scale latitudinal transition from northern boreal forests to southern deciduous forests. This transition zone is expected to shift northward as climate warms and deciduous forests replace boreal forests (Taylor et al. 2017). Our work suggests that ruffed grouse are likely to replace spruce grouse as a result of changing habitat conditions in the study area.

Responses to Timber Harvest

Timber harvest reduced survival of spruce grouse. Timber harvest has negative demographic effects in several bird species, usually through an increase in predator activity in the edges created by harvest (Yahner and Mahan 1997, Duguay et al. 2000). Importantly, most spruce grouse occurred near harvested stands, but spruce grouse were rarely within harvested stands even before harvest. Therefore, reduced survival near harvested stands might occur indirectly through edge effects or from predator displacement from harvested stands producing greater predator densities in nearby stands used by spruce grouse. However, we did not collect data on predator responses to timber harvest in this study to evaluate such explanations.

We predicted spruce grouse would avoid harvested stands after harvest, due to the loss of cover, but did not anticipate that harvested stands would be avoided before harvest. This avoidance suggests that many harvested stands were not suitable for spruce grouse before harvest. Many stands mature enough to harvest in Minnesota may lack the vegetation structure necessary to support spruce grouse. Spruce grouse declined with forest succession in a New York study (Ross et al. 2016). Timber harvest may increase spruce grouse mortality but may also be necessary at some level to promote regeneration of stands with denser vegetation structure that supports spruce grouse.

At the home-range scale, MCPs initially overlapped harvested stands but exhibited large decreases in overlap post-harvest (from 81 to 56% at the Big Falls study area and 41 to 16% at RLWMA), although 95% CIs overlapped. This reduction in overlap is likely explained by higher predator activity near the edges of harvested stands (Yahner and Mahan 1997). However, in Quebec, spruce grouse remained in residual strips after harvest, seemingly unaffected by edge effects (Potvin and Courtois 2006). Our study area had abundant residual habitat, which might partially explain the differences in spruce grouse responses between this study and the Quebec study.

Landscape Genetics

The spruce grouse population in Minnesota currently exists as a continuous population with no distinct spatial demes. Distinct spatial demes would indicate that gene flow is restricted and that barriers to connectivity and movement exist that fragment the population into separate subpopulations. Gene flow was positively related to spring temperature during dispersal and coniferous forest land cover. Two approaches to connectivity modeling gave complementary perspectives of genetic connectivity, highlighting greater potential for gene flow in the northeast and northwest than central portions of the study area. Omniscape suggested more continuous gene flow than Circuitscape, but both indicated several partial barriers to gene flow, including Red Lake and the Mesabi Range. Forest management to promote gene flow through conifer forest cover may influence the connectivity of this region for spruce grouse with climate change. Our data indicate the central part of the study area may be most vulnerable to climate change impacts to gene flow. The cooler northwestern and northeastern portions of spruce grouse range in Minnesota may provide climate refugia and maintain some spruce grouse gene flow and connectivity (Huntingford and Lowe 2007; Anderson et al. 2020). This research highlights climate change and associated changes in conifer forest cover as important considerations for spruce grouse population connectivity at their southern range periphery.

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Table 1. Variables used in occupancy modeling for spruce grouse, ruffed grouse and snowshoe hare in northern Minnesota during 2019-2022. Data sources included National Land Cover Data (NLCD), unpublished MNDNR data, fecal pellet surveys (Roy et al. 2022), habitat surveys, Light Detection and Ranging (LiDAR) data, National Oceanic and Atmospheric Administration (NOAA) snowfall data, and PRISM Climate Group temperature data (<https://prism.oregonstate.edu/>).

Category	Name	Description	Source
Land cover	Deciduous forest	Percent deciduous forest land cover \leq 5-km buffer	NLCD
Land cover	Mixed forest	Percent mixed conifer/deciduous forest land cover in 5-km buffer	NLCD
Land cover	Coniferous forest	Sum of wooded wetland + conifer forest land cover in 5-km buffer	NLCD
Roads	Road density	Linear road length in 5-km site buffer	MNDNR
Stand type	Balsam fir	Binary variable for balsam fir comprising >30% transect	Pellet surveys
Stand type	Black spruce	Binary variable for black spruce comprising >30% transect	Pellet surveys
Stand type	Jack pine	Binary variable for jack pine comprising >30% transect	Pellet surveys
Stand type	Red pine	Binary variable for red pine comprising >30% transect	Pellet surveys
Stand type	Tamarack	Binary variable for tamarack comprising >30% transect	Pellet surveys
Stand type	Deciduous forest	Binary variable for deciduous spp. comprising >30% transect	Pellet surveys
Stand structure	Stand age	Mean stand age along transect	MNDNR
Stand structure	Tree density	Number of trees \leq 3.6 m from habitat plot center	Habitat data
Stand structure	LiDAR 0-1.37 m	Proportion LiDAR returns 0 – 1.37 m	LiDAR
Stand structure	LiDAR 1.37-5 m	Proportion LiDAR returns 1.37 – 5 m	LiDAR
Stand structure	LiDAR 5-10 m	Proportion LiDAR returns 5 – 10 m	LiDAR
Stand structure	LiDAR 10-15 m	Proportion LiDAR returns 10 – 15 m	LiDAR
Stand Structure	LiDAR >15 m	Proportion LiDAR returns >15m	LiDAR
Climate	Snowfall	Cumulative Jan + Feb snowfall	NOAA
Climate	Temperature	Mean daily high temperature in Jan and Feb	PRISM

Table 2: Microsatellite loci analyzed by University of Minnesota Genomics Center for our use in a landscape genetic approach for spruce grouse in northern Minnesota during 2014-2021. We did not retain all 16 loci in the analysis because of difficulties calling alleles when >2 products amplified or alternatively because of possible linkage to coding regions (i.e., ≤ 2 alleles) at some loci.

Locus	Source	Retained in analysis	Reason for exclusion
BG03	Wang et al. 2012	No	Low diversity
BG04	Wang et al. 2012	No	Low diversity
BG15	Wang et al. 2012	Yes	
BG21	Wang et al. 2012	No	Low diversity
BG26	Wang et al. 2012	No	Low diversity
BG29	Wang et al. 2012	No	Low diversity
BG94	Wang et al. 2012	No	Low diversity
CUAAGG37	Gibson et al. 2005	Yes	
LLSD2	Piertney and Dallas 1997	Yes	
LLSD6	Piertney and Dallas 1997	No	Ambiguous alleles
TTD4	Caizergues et al. 2001	No	Ambiguous alleles
TTD6	Caizergues et al. 2001	Yes	
TUD7	Segelbacher et al. 2000	No	Ambiguous alleles
TUT1	Segelbacher et al. 2000	Yes	
TUT3	Segelbacher et al. 2000	Yes	
TUT4	Segelbacher et al. 2000	Yes	

Table 3. We considered categorical and continuous variables in a landscape genetic approach for spruce grouse in northern Minnesota during 2014-2022. We hypothesized that coniferous forest and spruce grouse stand types would have lower resistance for spruce grouse gene flow in northern Minnesota during 2014-2022 than other land cover types, that greater road density would have higher resistance, and that stand age would either have (a) a positive linear relationship resistance or (b) a parabolic relationship with minimal resistance at age 40-yr and increasing resistance with greater deviance from 40 yr. We also hypothesized that areas with more average snowfall or lower average spring temperatures would have lower resistance.

Variable	Description	Source
Coniferous forest	Categorical: Wooded wetland and evergreen forest land cover	NLCD ^a
Spruce grouse stand types	Categorical: Black spruce, jack pine, and tamarack land cover	GAP ^b
Road density	Continuous: Linear length of roads/km ²	MNDNR ^c
Stand age	Continuous: Yr since harvest	MNDNR ^c
Snowfall	Continuous: Mean annual snowfall	NOAA ^d
Temperature	Continuous: 30-yr temperature during Mar-Apr	PRISM ^e

^a Dewitz J, United States Geological Survey [USGS]. 2021. National Land Cover Database (NLCD). 2019. Products (ver. 2.0, Jun 2021). USGS data release. <https://doi.org/10.5066/P9KZCM54>

^b U. S. Geological Survey [USGS]. 2011. Gap Analysis Program, 20160513, GAP/LANDFIRE National Terrestrial Ecosystems. <https://doi.org/10.5066/F7ZS2TM0>

^c Minnesota Department of Natural Resources

^d National Oceanic and Atmospheric Administration [NOAA] 2023. National Gridded Snowfall Analysis. <https://www.nohrsc.noaa.gov/snowfall>

^e PRISM Climate Group (PRISM) 30-Yr Normals. 2022. Oregon State University. <https://prism.oregonstate.edu>

Table 4. Multi-season occupancy model coefficients for detection, occupancy, and extinction in the most-supported models for spruce grouse, ruffed grouse, and snowshoe hare in northern Minnesota during 2019-2022. We used a positive stepwise approach, limiting each model parameter to ≤ 3 predictors.

Parameter or predictor	Coefficient (95% Confidence interval)
Spruce grouse detection	
Survey condition	0.46 (0.30 – 0.63)
(Observer type) technician	0.06 (-0.30 – 0.42)
(Observer type) volunteer	-1.41 (-2.02 – -0.82)
(Snow extent) none	0.25 (-0.18 – 0.68)
(Snow extent) partial	0.79 (0.41 – 1.17)
Spruce grouse occupancy	
Deciduous (5 km)	-0.88 (-1.44 – -0.38)
Light Detection and Ranging (LiDAR; 5-10 m)	1.37 (0.72 – 2.11)
LiDAR (10-15 m)	1.06 (0.49 – 1.69)
Spruce grouse extinction	
Deciduous (5 km)	0.91 (0.33 – 1.53)
Road density (5 km)	1.04 (0.29 – 1.82)
Ruffed grouse detection	
Survey condition	0.46 (0.31 – 0.62)
Date	0.28 (0.12 – 0.43)
(Observer type) technician	0.63 (0.32 – 0.95)
(Observer type) volunteer	-0.22 (-0.69 – 0.23)
Ruffed grouse occupancy	
Red pine	-1.77 (-2.97 – -0.60)
Ruffed grouse extinction	
Mixed forest (5 km)	-1.17 (-2.11 – -0.36)
Red pine	1.86 (0.55 – 3.24)
Tree density	0.87 (0.26 – 1.53)
Snowshoe hare detection	
(Observer type) technician	1.03 (0.21 – 1.90)
(Observer type) volunteer	-1.33 (-2.07 – -0.59)
Snowshoe hare occupancy	
Tree density	1.75 (0.05 – 3.98)
Snowshoe hare extinction	
LiDAR (1.37-5 m)	-2.27 (-3.47 – -1.24)
Snowfall	-0.78 (-1.49 – -0.12)

Table 5. Multi-species occupancy modeling of spruce grouse as related to a coniferous forest type, ruffed grouse occupancy with a deciduous forest type, and co-occurrence with a mixed forest type (mix). Models with a significant co-occurrence predictor are bolded and underlined, non-significant (ns) relationships are indicated, and Akaike's Information Criterion (AIC_c) is provided.

Predictors (Coniferous / co-occur / deciduous)	2019		2020		2021	
	AIC _c	Coefficient	AIC _c	Coefficient	AIC _c	Coefficient
None	1003.6	NA	604.1	NA	1020.5	NA
<i>Landscape-scale</i>						
Coniferous / mix / deciduous	989.5	+ / ns / ns	590.9	+ / ns / ns	<u>1015.3</u>	<u>+ / + / ns</u>
<i>Stand-scale</i>						
Black spruce / mix / deciduous	1004.6	ns / ns / ns	609.0	ns / ns / ns	1025.1	ns / ns / ns
Jack pine / mix / deciduous	998.6	+ / ns / ns	599.9	+ / ns / ns	1023.4	ns / ns / ns
Black spruce / balsam fir / deciduous	1002.9	ns / ns / ns	608.0	ns / ns / ns	1025.2	ns / ns / ns
Jack pine / balsam fir / deciduous	995.3	+ / ns / ns	600.8	+ / ns / ns	1024.1	ns / ns / ns

Table 6. Percent overlap of spruce grouse minimum convex polygon (MCP) home ranges with harvested timber stands and 500-m stand buffers pre- and post-harvest in northern Minnesota during 2019-2022.

Study Area	Pre-Harvest (95% CI)	Post-Harvest (95% CI)
Big Falls		
% MCP in harvested stand	12 (2 – 22)	7 (4 – 10)
% MCP in harvest buffer	81 (57 – 100)	56 (42 – 70)
Red Lake Wildlife Management Area		
% MCP in harvested stand	0 (0 – 1)	0 (0 – 0)
% MCP in harvest buffer	41 (8 – 74)	16 (0 – 37)

Table 7. Number of spruce grouse home ranges (n) and deaths observed for each harvest category in the survival analysis and the restricted mean estimated survival time and 95% confidence interval (CI) for each category. Mean survival (days) was restricted to ≤ 1 yr (365 days) for harvest home range survival analysis.

Harvest category	n	Deaths	Survival in days (95% CI)
Non-harvest	50	13	307 (278 – 336)
Pre-harvest	23	1	349 (319 – 379)
Post-harvest	53	21	258 (220 – 296)

Table 8. Comparisons of landscape genetics models for spruce grouse in northern Minnesota during 2014-2022 using the change in Akaike's Information Criterion (ΔAIC_c). Variables in models included spruce grouse preferred stand types (black spruce, jack pine, and tamarack; BJT), snowfall, stand age, spring temperature, and coniferous forest and wooded wetland land cover (conifer cover). Models with road density did not perform better than the null model so are not included here.

Model	df ^a	logLikelihood	AIC _c	ΔAIC_c	Weight ^b
Conifer cover + temperature	6	-5767.68	11547.36	0.00	0.73
Conifer cover + snowfall + temperature	7	-5767.72	11549.46	2.10	0.26
Conifer cover + stand age + temperature	7	-5771.25	11556.50	9.14	0.01
Conifer cover + BJT + temperature	7	-5774.17	11562.36	15.00	0.00
Conifer cover + stand age + snowfall	7	-5785.21	11584.43	37.07	0.00
Conifer cover + BJT + snowfall	7	-5787.29	11588.58	41.22	0.00
Conifer cover + snowfall	6	-5788.60	11589.21	41.85	0.00
Conifer cover	5	-5801.39	11612.78	65.42	0.00
Snowfall + temperature	6	-5800.54	11613.08	65.72	0.00
Conifer cover + stand age	6	-5801.42	11614.84	67.48	0.00
Temperature	5	-5803.25	11616.50	69.14	0.00
Conifer cover + BJT + stand age	7	-5803.74	11621.48	74.12	0.00
BJT + temperature	6	-5805.27	11622.55	75.19	0.00
Conifer cover+ BJT	6	-5805.36	11622.73	75.37	0.00
Stand age + temperature	6	-5806.36	11624.73	77.37	0.00
BJT + snowfall	6	-5817.15	11646.30	98.94	0.00
Stand age + snowfall	6	-5824.71	11661.42	114.06	0.00
Snowfall	5	-5831.64	11673.29	125.93	0.00
BJT + stand age	6	-5841.35	11694.70	147.34	0.00
BJT	5	-5846.06	11702.12	154.76	0.00
Stand age	5	-5849.97	11709.94	162.58	0.00
Null model	4	-5851.48	11710.96	163.60	0.00

^a Degrees of freedom

^b Model weight from model selection

Table 9. Coefficient values of coniferous forest land cover and spring temperatures from the most-supported landscape genetics model used to parameterize a composite resistance surface for connectivity mapping for spruce grouse in northern Minnesota during 2014-2022.

Variable	R _{max}	Beta (95% Confidence intervals)
Intercept	--	2.509 (2.403 – 2.616)
Coniferous forest land cover	100	0.004 (0.003 – 0.005)
Spring temperature	100	0.005 (0.004 – 0.006)

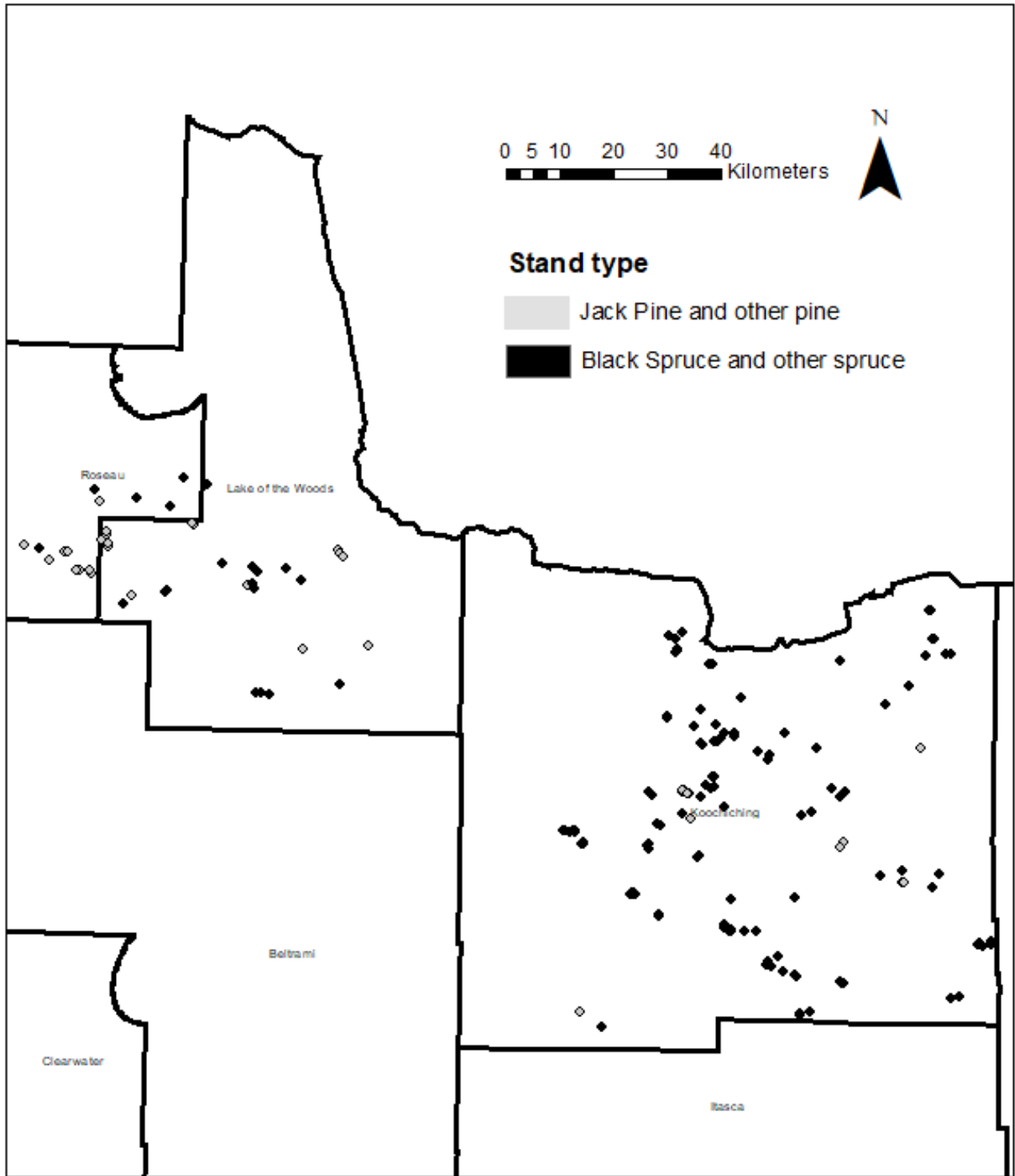


Figure 1. The spruce grouse timber harvest study area in northern Minnesota during 2019-2022 with 2 focal areas, one in Lake of the Woods and adjacent Roseau County with more jack pine than black spruce stands scheduled for harvest and another centered on Big Falls in Koochiching County which had more black spruce than jack pine stands scheduled for harvest.

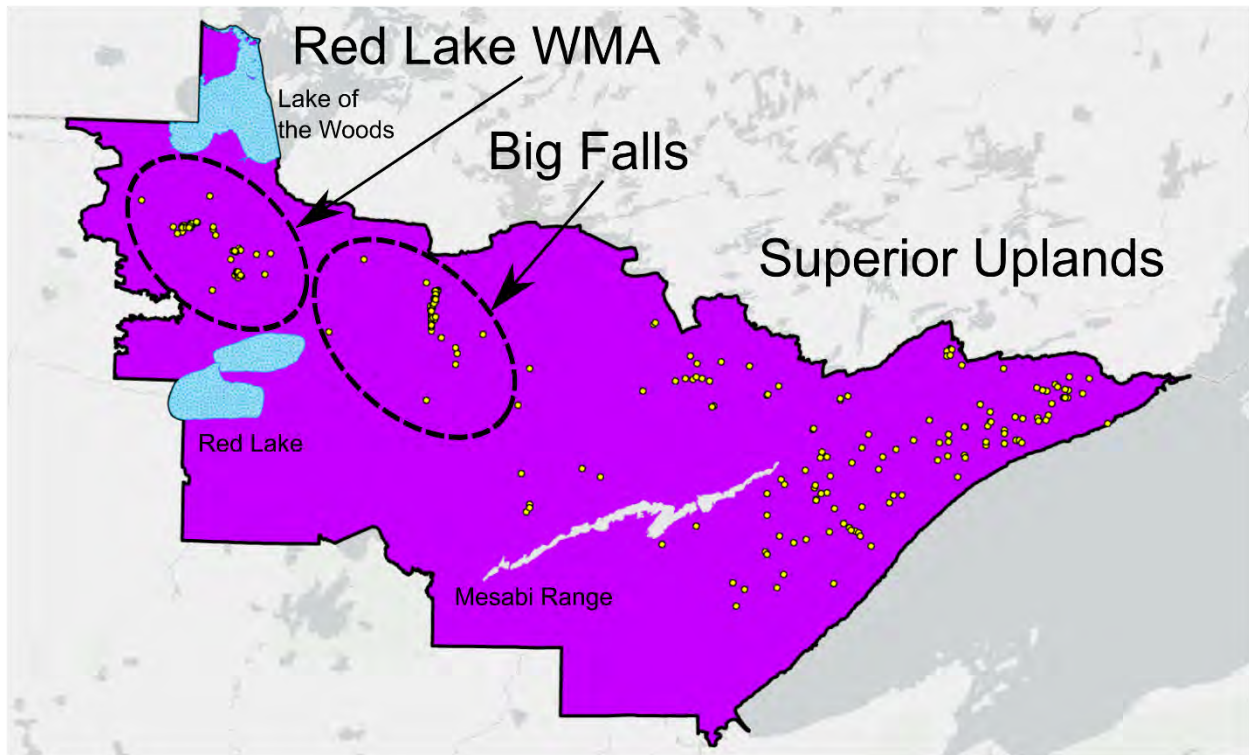


Figure 2. Areas sampled (yellow dots) for spruce grouse feathers to use in genetic analysis within the 2 timber-harvest study areas (Red Lake Wildlife Management Area [WMA] and Big Falls) and also locations that hunters sampled from these areas and the Superior Uplands in northern Minnesota during 2014-2021.

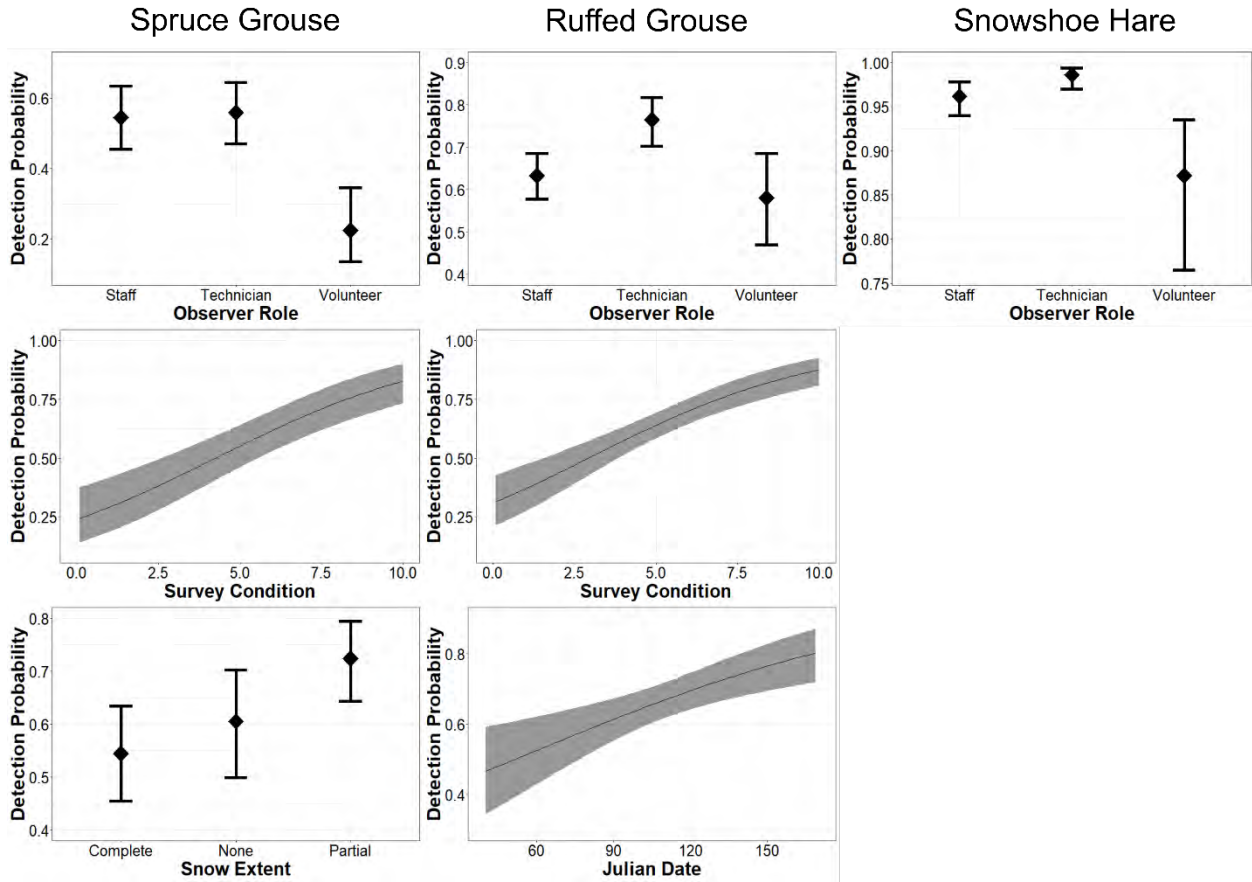


Figure 3. Marginal effects of predictors in most-supported detection models for spruce grouse, ruffed grouse, and snowshoe hares in northern Minnesota during 2019-2022.

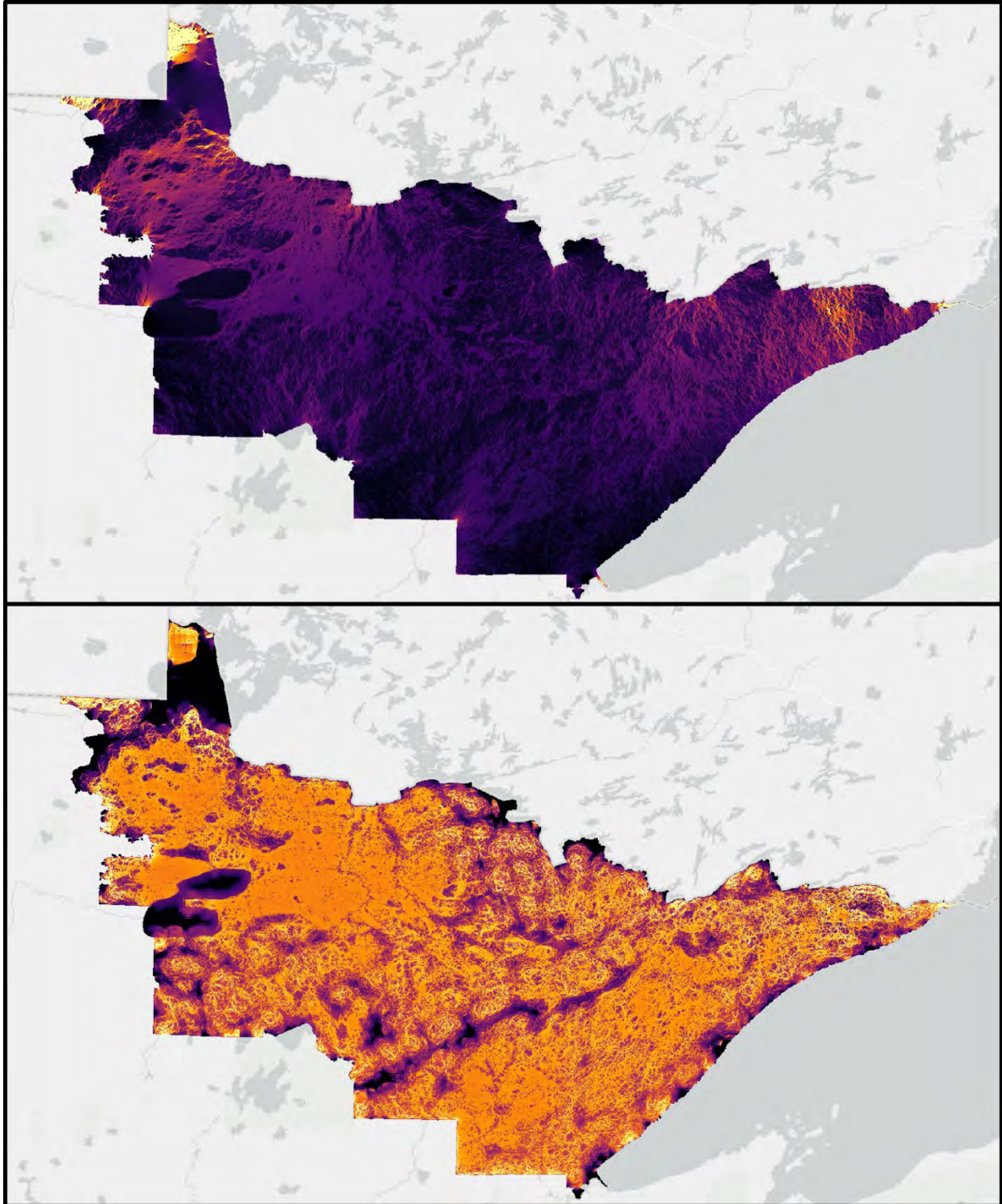


Figure 4. Circuitscape prediction of cumulative gene flow using a resistance surface parameterized from the most-supported landscape genetics model (top). Omniscape prediction of normalized gene flow using the same resistance surface, the proportion of coniferous forest cover within a 5-km radius of a source-strength layer, and a moving window size of 7 km (bottom). In both the top and bottom panels, yellow/orange indicates more gene flow, purple indicates less flow, and black represents no flow.

Wildlife Health Program
Wildlife Populations and Research Group
5463 - C West Broadway
Forest Lake, Minnesota 55025
(651) 296-5200



PER- AND POLYFLUOROALKYL SUBSTANCE (PFAS) IN WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) AND POTENTIAL CONSUMPTION RISKS TO MINNESOTA HUNTERS

Kelsie LaSharr¹, Deanna Scher², Sophie Greene³, Michelle Carstensen¹, Barb Keller¹, James Kelly²

SUMMARY OF FINDINGS

Game meat harvested in areas with environmental contamination may pose health risks to hunters and associated consumers. This study was undertaken to assess exposure levels of per- and polyfluoroalkyl substances (PFAS) in white-tailed deer (*Odocoileus virginianus*) harvested near three known or suspected areas of PFAS contamination. Liver samples from 116 hunter-harvested white-tailed deer were collected and analyzed for PFAS analytes ($n = 40$). Twenty PFAS analytes were detected at least once, and all livers contained measurable levels of perfluorooctanesulfonate (PFOS), specifically. The eight analytes found at highest concentrations included PFOS (max 96.0 ng/g), 6:2 FTS (max 48.4 ng/g), N-EtFOSE (max 13.4 ng/g), PFBA (max 9.14 ng/g), NFDHA (max 5.75 ng/g), PFOA (max 4.84 ng/g), PFHxS (max 2.78 ng/g), and PFHpA (max 2.42 ng/g). There were significant differences in PFAS detection frequencies and concentrations between sites and deer life stage/sex, with adult males having significantly higher concentrations of total perfluorinated compounds and total PFAS compared to adult females. When the liver PFOS concentrations reached a concentration threshold of 20 ng/g, the paired muscle sample from the same individual was also analyzed for 40 PFAS analytes ($n=17$ muscle samples). PFAS levels in the liver were approximately two orders of magnitude higher than the paired muscle tissue. This data is important for determining possible public health measures, including localized consumption advisories.

INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS), also known as “forever chemicals”, are anthropogenic derived compounds that have been manufactured and used worldwide since the 1930s; PFAS are found in non-stick coatings in cookware and food packing, lubricants, adhesives, paints, cleaning products, surfactants, herbicides and insecticides, textile stain and water repellants, and firefighting foam (MPCA Blueprint 2021; Death et al. 2021; Route et al. 2014). The characteristics that make these compounds useful, including resistance to breaking down in the presence of heat, oil, and water, also leads to environmental persistence and harmful bioaccumulation in people, livestock, and wildlife (Houde et al. 2011; Death et al. 2021). PFAS exposure has been documented in both humans and wildlife and is linked to negative impacts on the liver, lungs, kidney, endocrine and immune systems, and overall health and behavior (Dykstra et al. 2020; Stahl et al. 2011; Weiss et al. 2009; Tartu et al. 2014). Of all PFAS compounds, perfluorooctanesulfonate (PFOS) has received significant attention to date given elevated levels found ubiquitously throughout the environment; PFOS has also been documented at concerning levels in wildlife since 2001 (Giesy and Kannan et al. 2001). The

¹ Minnesota Department of Natural Resources

² Minnesota Department of Health; St. Paul, MN, USA

³ Minnesota Pollution Control Agency; St. Paul, MN, USA

highest levels of PFOS concentrations in wildlife (and humans) are found in the liver and blood/serum, as PFOS has an affinity for proteins (Butt et al. 2010; Houde et al. 2011; Jones et al. 2003). The most significant route of PFAS exposure in humans is through dietary intake such as food and water (Death et al. 2021) and is likely the same for wildlife (Giesy and Kannan 2001; Falk et al. 2012). As such, it is important to document the levels of PFAS that people may be consuming, especially if they are harvesting wildlife near known or suspected contamination sites.

Concern for PFAS exposure in human populations that consume wildlife has been increasing in recent years (DeSilva et al. 2021; Guillette et al. 2020; Fair et al. 2019). In Minnesota, PFAS consumption advisories have been established for fish, driven by PFOS levels (MPCA Blueprint 2021). No wild game samples from known PFAS contamination sites have been evaluated to determine if levels of PFAS pose a health risk to humans via wild game consumption (as well as risks to wildlife in the contamination areas). Community concerns regarding PFAS exposure through consumption of wild game led nearby states, Wisconsin and Michigan, to test white-tailed deer (*Odocoileus virginianus*) in areas where environmental PFAS contamination had occurred. After discovering high levels of PFOS in deer liver (range: Not Detected - 6,080 ppb) and muscle samples (range: Not Detected – 548 ppb), the Michigan Department of Health and Human Services issued a “Do Not Eat” advisory in 2018 for any part of a deer taken within five miles of Wurtsmith Air Force Base in Iosco County, Michigan (MI PFAS Report, 2021). In 2020, the Wisconsin Department of Natural Resources tested deer from the JCI/Tyco Fire Training Center in Marinette County, Wisconsin and detected PFOS in 20 deer livers (3.8 – 92 ng/g) and one muscle sample (2.67 ng/g); a similar “Do Not Eat” advisory was issued for deer livers taken nearby (WI PFAS Report, 2020). In 2021, community concerns prompted Maine state agencies to analyze eight deer harvested in/around Fairfield due to local contamination from land application of waste materials (MDIFW and MCDC, 2022). PFOS levels in the liver and muscle ranged from 5.5 – 809 ng/g and 1.4 – 43.5 ng/g respectively.

OBJECTIVES

1. Determine if white-tailed deer harvested in Minnesota near areas of known or suspected areas of PFAS contamination have detectable or even elevated levels of PFAS.
2. If PFAS levels are elevated in white-tailed deer taken near areas of concern, determine if a consumption advisory is warranted for people consuming venison.

To assess the need for PFAS consumption advisories for deer in Minnesota, Minnesota Department of Natural Resources (MNDNR), Minnesota Pollution Control Agency (MPCA), and Minnesota Department of Health (MDH) coordinated to collect hunter-harvested deer samples. PFAS exposure may have potential lethal and sublethal effects on white-tailed deer, but those measurements or findings were beyond the scope of this study.

METHODS

Sample Collection

Three sampling sites (Lake Elmo, Duluth, and Camp Ripley Training Center) were selected for known or suspected PFAS contamination (Figure 1). MPCA and MDH have been monitoring PFAS concentrations in environmental samples in the Lake Elmo area for more than two decades in response to PFAS releases from a local 3M manufacturing facility (and subsequent waste disposal sites) that has produced PFAS since the late 1940s; local levels of PFOS are so elevated, that “Do Not Eat” advice has been issued for some fish caught in lakes and streams in the drainage basin below these point sources (Li and Gibson 2022, MPCA Blueprint 2021). Environmental PFAS contamination has been documented near the Duluth International

Airport/Duluth Air Force base following many years of firefighting foam product use that contain PFAS – contamination was high enough that some nearby private wells needed to be replaced or treated (MPCA Blueprint 2021). The environmental and background PFAS levels at Camp Ripley had not been documented at the time of the study, but it was suspected that PFAS contamination had likely occurred at some point in the military base’s history through the use of firefighting foam. Given the increased concern for current PFAS levels at Lake Elmo site, when compared to the other two sites, we intentionally increased the sample size for that location.

Samples were collected between 2020 and 2022 from hunter-harvested or culled deer intended for human consumption; depending on the study site, sample collections were either voluntary or a mandatory requirement for a restricted hunt opportunity. Liver and muscle samples analyzed in this study were collected under varying circumstances, but always between September and March. As these samples were provided by hunters, it was impossible to attain a standardized, equal collection at each study site regarding age and sex. Hunters at Lake Elmo and Duluth sites, as well as staff at Camp Ripley who removed deer from airstrips, (hereafter all referred to as “hunters”) were given sampling kits and asked to collect samples in the field immediately following harvest. Sampling kits included pre-labeled Ziplock® bags and whirlpacks; hunters were asked to submit an intact liver, a 1-inch x 1-inch x 1-inch section of skeletal muscle, a front incisor (tooth) for aging, and provide information about the deer: when and where it was harvested, age (fawn, yearling or adult), and sex (male, female). Completed sampling kits were collected by site coordinators and mailed back to DNR staff. Samples were stored at -20C until DNR staff prepped samples for laboratory submission.

Given PFAS levels are typically higher in the liver than skeletal muscle (Chen et al. 2018, Müller et al. 2011), we first screened livers and any deer with elevated levels of PFAS in the liver (one predetermined cut-off, >20ng/g ww PFOS), would result in additional testing of the paired deer muscle. The deposition pattern of PFAS in liver tissue is unknown; thus, we took a sub-sections from the caudal lobe, the medial portion of the liver, and lateral lobes and combined them into a single whirlpack, equaling to ~100g of liver sampled per deer. Samples were frozen and stored at -20C prior to submitting to the laboratory for analysis.

Laboratory Procedures

Samples were submitted to SGS AXYS Analytical Services Ltd. in Sidney, B.C., Canada (SGS AXYS) and analyzed using Method MLA-110 Rev. 02 Ver. 08. Tissue samples were homogenized and a representative 2g were analyzed. After addition of isotopically labelled surrogate standards, the sample was extracted with methanolic potassium hydroxide solution, with acetonitrile, and finally with methanolic potassium hydroxide solution, each time collecting the supernatants. The supernatants were combined, treated with ultra-pure carbon powder and evaporated to remove methanol. The resulting solution was diluted with water and cleaned up by solid phase extraction (SPE) on a weak anion exchange sorbent. The eluate was spiked with recovery standards and analyzed by liquid chromatography/tandem mass spectrometry (LC-MS/MS). Contaminant concentrations are presented in wet weight (ww).

Deer incisor teeth, when provided by hunters, were aged at Matson’s Laboratory (Manhattan, MT, USA) using cementum annuli.

Statistical Analyses

Descriptive statistics and visual plots were created to assess PFAS occurrence, magnitude, and distribution in deer livers and muscle tissue overall and by study site. These results were presented for both individual PFAS and PFAS groupings (e.g., perfluorinated sulfonates).

Scatterplots and Pearson correlation coefficients looked at potential linear correlation between analytes.

In univariable analyses, the outcomes of interest were binary (detection yes/no), or continuous (concentration) if the detection frequency was sufficient. Two potential explanatory variables (study site and deer life stage-sex) were assessed to look for relationships with these outcomes. Chi-squared tests were used for the binary outcome. To assess differences in concentration, 1-way ANOVA (after log transformation as analyte concentrations were right-skewed) or the non-parametric Kruskal-Wallis test were used. The Kruskal-Wallis test was preferred when group sample sizes were small. Due to the high number of left-censored values for many individual analytes, the ability to conduct statistical tests on concentration differences by explanatory factors was limited to commonly-detected analytes and analyte groupings.

The influence of life stage-sex group on PFAS concentrations was first evaluated separately by site due to significant differences in the presence and magnitude of PFAS between sites. As the individual observations are not independent, but rather correlated by site, we used a linear mixed effects model to explore whether age-sex categories were a significant predictor of total perfluorinated compounds and total PFAS. Models used a site-specific random intercept with life stage-sex category included as a fixed effect. Deer with unknown age or sex and yearlings were excluded due to small sample size. PFAS concentrations were log-transformed to ensure residuals were normally distributed. All statistical tests were based on a significance level of $p \leq 0.05$. All analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Liver and muscle samples were collected from 116 deer in Lake Elmo ($n = 59$), Duluth ($n = 26$) and Camp Ripley ($n = 31$) study site. Seventy-two percent were adults, 17% were fawns, 4% were yearlings, and 8% were deer of unknown life stage. Fifty-five percent of deer were females, 38% were males, and 7% were deer of unknown sex. Hunters self-reported age and sex of their harvested deer and exact age was analyzed by cementum annuli for 15 deer (range 0-10 years, median = 2 years).

Concentrations of PFAS in White-Tailed Deer Livers

Twenty of the 40 PFAS compounds were detected in at least one of the 116 liver samples (Table 1). Of these, the five analytes with the highest detection frequencies across all study sites were PFOS (100%), PFDA (66%), PFBA (62%), PFUnA (56%), and N-EtFOSE (47%). Eight analytes were found in at least one sample above 1 ng/g: PFBA (range: 0.83-9.14 ng/g), PFHpA (range: 0.14-2.42 ng/g), PFOA (range: 0.11-4.84 ng/g), PFHxS (range: 0.10-2.78 ng/g), PFOS (range: 0.15-96.0 ng/g), 6:2 FTS (range: 0.53-48.4 ng/g), N-EtFOSE (range: 0.87-13.4 ng/g), and NFDHA (range: 0.21-5.75 ng/g). PFBA and PFOS were the only analytes with a median concentration > 1 ng/g.

The forty analytes were placed into chemical structure groups, which can breakdown at different rates or are used in different products, which can help explain how they got into the environment. First dividing the analytes into per- vs. poly-fluoro, followed by consideration of the polar part of the chemical (sulfonate, acid, etc.). There were no detections in four groups: perfluorinated ether sulfonates, polyfluorinated acids, polyfluoroalkyl chloroether sulfonates, and polyfluoroalkyl ether acids, but the number of analytes in these groups only ranged from 1-3 (Table 2). Of the seven chemicals in groups with no detections, PFEESA, ADONA, 9Cl-PF3ONS, and 11Cl-PF3OUdS are considered "replacement" PFAS, while 3:3FTCA, 5:3FTCA, and 7:3FTCA are degradation products. PFCAs (perfluorinated carboxylates) and PFSAs (perfluorinated sulfonic acids) were further broken out by carbon chain length (Table 2). No short-chain PFSAs were found in the 116 samples but all short-chain PFCAs were detected.

Differences in Composition of PFAS Chemicals by Site

At the Lake Elmo site, fifteen analytes were detected in deer liver samples. PFOS, PFBA, and N-EtFOSE had the highest detection frequencies and concentrations (Table 1). Higher detection frequency and concentrations of PFBA were seen at the Lake Elmo site compared to the other sites; PFBA was previously produced near this site and is a common groundwater contaminant in the area (ATSDR 2008). Lake Elmo is only site in which PFOSA was detected; there was also a high 6:2 FTS concentration outlier. At the Camp Ripley site, seventeen analytes were detected. PFOS, PFDA, and PFUnA had the highest detection frequencies (Table 1) and PFOS concentrations were highest at this site. Camp Ripley was the only site with detectible PFPeA, PFNS, and PFDS concentrations. Finally, at the Duluth site, twelve analytes were detected (Table 1). PFOS, 6:2 FTS, and PFDA/PFUnA were the most frequently detected with a high PFOS outlier. There was a statistically significant difference in detection frequency between sites for all of the analytes that had a high enough detection frequency to formally test: PFBA ($\chi^2=49.7$, $df=2$, $p<.0001$), PFNA ($\chi^2=8.6$, $df=2$, $p<.0132$), PFDA ($\chi^2=9.0$, $df=2$, $p<.0113$), PFUnA ($\chi^2=7.9$, $df=2$, $p<.0191$), 6:2-FTS ($\chi^2=26.1$, $df=2$, $p<.0001$), N-EtFOSE ($\chi^2=73.4$, $df=2$, $p<.0001$) and PFOS was not examined because 100% of all samples had detections.

Looking across sites at major PFAS groupings, the Duluth site had visually notable lower “total perfluorinated compound” and “total PFAS” concentrations compared to the other two sites (Figure 2). While Camp Ripley had the highest total perfluorinated compound median and mean concentrations, Lake Elmo had the highest total PFAS mean and median concentrations due to the added contribution of polyfluorinated compounds. Detection frequency was adequate to formally test for differences in mean concentration between sites for PFOS, PFDA, PFUnA, total perfluorinated compounds, and total PFAS concentrations using one-way ANOVA. There was a statistically significant difference in log PFOS concentrations ($F=18.7(2)$, $p<.0001$), log total perfluorinated chemical concentrations ($F=40.1(2)$, $p<.0001$) and log total PFAS concentration ($F=34.7(2)$, $p<.0001$) between sites. Difference in log total polyfluorinated chemical concentrations was also significant when comparing Duluth versus Lake Elmo ($F=17.9(1)$, $p<.0001$).

Differences in Composition of PFAS Chemicals by Age and Sex

Since both age and sex have been previously shown to be important predictors of PFAS in humans and other animals (Li et al. 2018, Smithwick et al. 2005) we assessed differences in PFAS concentration by life stage-sex groupings. The influence of deer life stage and sex on PFAS concentrations was evaluated separately by site due to the significant differences in PFAS concentrations between sites demonstrated above (Figure 3). Unknown age and/or sex were excluded from analysis. At Camp Ripley, no statistically significant differences in concentration between adult females, adult males and female fawns were seen for total PFAS ($H(2)=0.29$, $p=.8655$), short-chain PFCAs ($H(2)=2.3$, $p=.3207$), long-chain PFCAs ($H(2)=5.0$, $p=.0837$), or long-chain PFSA ($H(2)=0.11$, $p=0.9476$). At the Duluth site, adult male deer had higher mean and median liver concentrations of total PFAS, short-chain PFCAs, and long-chain PFCAs compared to other life stage-sex groups upon visual inspection. Comparing adult females to adult males, there was a statistically significant difference in concentration for total PFAS ($H(1)=11.2$, $p=.0008$), short-chain PFCAs ($H(1)=6.9$, $p=.0085$), long-chain PFCAs ($H(1)=13.0$, $p=.0003$), and short-chain PFSA ($H(1)=9.9$, $p=.0017$). At the Lake Elmo site, no major differences in concentration were seen between life stage-sex group for total PFAS and long-chain PFSA. Comparing adult females, adult males, and male fawns, there was no statistically significant difference in concentration for total PFAS ($H(2)=2.2$, $p=.3278$) or long-chain PFSA ($H(2)=1.6$, $p=.4415$). However, there was a statistically significant difference for short-chain PFCAs ($H(2)=8.2$, $p=.0167$) and long-chain PFCAs ($H(2)=19.4$, $p<.0001$).

To evaluate the influence of life stage-sex categories on PFAS concentrations for the entire sample while accounting for cluster-correlation within sites, we created mixed effects models for total perfluorinated compounds and total PFAS. Adult males were found to have significantly higher concentrations of both total perfluorinated compounds and total PFAS compared to adult females. While no significant differences were seen for fawns, the small sample sizes likely limited statistical analysis for this life stage.

Concentrations of PFAS in White-Tailed Deer Muscle

Seventeen deer (14.7%) exceeded our predetermined threshold of concern of >20 ng/g ww PFOS in the liver, which triggered analysis of the paired muscle samples for the same suite of 40 PFAS compounds. All muscle samples had detectable levels of PFOS, (reporting limit= 0.1 ng/g), but all muscle samples were less than 2 ng/g PFOS (n=17, 0.15-1.24 ng/g), a very large reduction in PFAS load when compared to the associated livers (Table 4). The other compounds found in these muscle samples were also detected at low levels: PFBA (n=3, range 0.91-1.94 ng/g), PFHxS (n=2, 0.29-0.45 ng/g), 6:2 FTS (n=1, 2.31 ng/g) and NFDHA (n=2, 0.21-0.65 ng/g).

DISCUSSION

In the U.S., there are hundreds of thousands of industrial and commercial sites with ongoing or historic use of PFAS (US EPA 2023). These sites pose potential threats as on-going sources of PFAS release into drinking water and local ecosystems, including fish and game species. Full environmental remediation at existing sites, many of which have yet to be identified, is not feasible or practicable in most cases because the state of the science on treatment technology is in an early stage and the need for multi-media advanced technologies is largely cost prohibitive. As such, it is important to understand the levels of PFAS in fish and wild game near known or suspected PFAS contamination sites, which can pose health risks to the species themselves and those that consume them.

We found that all deer in this study were exposed to PFAS, with PFOS being most common (100%). While short-chain PFAAs are as or more persistent and mobile in the environment than their long-chain homologues, short-chain compounds are not assumed to be bioaccumulative in wildlife due to their shorter half-lives in animals (Brendel et al. 2018). However, all short-chain PFCAs were detected at least once, with PFBA having the highest median and upper percentile concentrations of any analyte after PFOS. This demonstrates that ongoing exposure to short-chain PFAS in the environment can exceed their elimination half-lives in biota. There were significant differences in the composition of PFAS compounds in deer livers at the different study sites, likely reflecting the distinct PFAS contamination sources. This is the first report of PFBA, 6:2 FTS, and NFDHA detected in deer muscle tissue samples. These PFAS were either not analyzed for or not detected in reports from other states (MDHHS 2021; WI PFAS 2020; MDIFW and MCDC, 2022). PFDA, which was found in the majority of the deer muscle samples in Maine, was not found in any of the muscle samples from this study. Future studies should employ an expansive analyte list to capture the broad range of PFAS that may be present in wild game.

The small and unequal sex-life stage groupings within each site limited our ability to fully assess differences in PFAS concentrations by age and sex. However, comparisons that could be made demonstrated significant differences between adult males and adult females; the former having higher concentrations of total perfluorinated compounds and total PFAS. Across the entire 2021 Minnesota deer hunting season, the ratio of male to female deer harvested was 63:37, and of the 182,994 harvested deer, 86.4% were adults (MNDNR 2021). When developing future study

designs, there should be more direct communication with hunters throughout the sampling season about desired samples in order to ensure a more even distribution of sex and age. Of note, no significant difference was found in liver PFOS levels between male and female deer harvested in Michigan from 2018 to 2020 (MDHHS 2021).

The PFOS levels in white-tailed deer in this study were similar to or less than other studies of white-tailed deer in the northern Great Lakes region. Our PFOS liver results (0.23-96.0 ng/g) were comparable to Wisconsin white-tailed deer livers (3.8-92 ng/g) (WIDNR & WDHS 2020), and significantly less than 2018 Michigan white-tailed deer livers (ND – 6,080 ng/g) (MDHHS 2021). Similarly, PFOS concentrations in our white-tailed deer muscle samples (0.15-1.24 ng/g) were similar to Wisconsin deer muscle samples (PFOS ND-2.67 ng/g) (WIDNR & WDHS 2020), and significantly lower than Michigan 2018 deer muscle samples (ND – 548 ng/g) (MDHHS 2021).

When comparing these cervid PFOS liver findings to liver concentrations reported in other ungulates around the world, the PFOS levels reported here were higher: roe deer in Germany (1.3-67.5 ng/g; Falk et al. 2012), caribou from Canada (0.02-4.4 ng/g, Roos et al. 2022), caribou in Greenland (0.07-28.3 ng/g, Roos et al. 2022), caribou in Sweden (0.01-14 ng/g, Roos et al. 2022), chamois from Austria (ND– 4.6 ng/g; Riebe et al. 2016), and moose from Norway (range 0.18–0.39 ng/g, Hanssen et al. 2019), and northern Canada (0.34 ng/g ww. Larter et al. 2017). The proximity of deer sampled in our study to known or likely point sources is a big contributor to the higher concentrations of PFAS in this study (Landberg et al. 2019; Stock et al. 2007; Skaar et al. 2019).

Concentrations of PFAS in the muscle of deer harvested from these sites were approximately two orders of magnitude lower than those in the liver. Similar relationships were observed in Maine between paired liver and muscle PFAS concentrations, with a 4-fold to 51-fold difference in PFOS levels (MDIFW and MCDC, 2022). For Wisconsin deer harvested in 2020, only one muscle sample (out of n=20 deer) had detectable levels of PFAS (PFOS = 2.67 ng/g), while the 20 livers had 6 detectable compounds (WI PFAS 2020). This pattern, where PFOS appears to be concentrated in the liver rather than skeletal muscle, follows similar findings from past studies in other animals, including caribou (*Rangifer tarandus*) and wolves (*Canis lupus*; Chen et al. 2018, Müller et al. 2011). PFOS in particular appears to significantly accumulate in the liver (Jones et al. 2003, Butt et al. 2010). However, previous work by Müller et al. (2011) emphasizes that muscle tissue contains 60-90% of the body burden of PFAS chemicals, where body burden is the concentration in the tissue multiplied by the tissue fraction against total body weight. So, while the liver may have higher concentrations, it is a small organ compared to the greater skeletal muscle, which is consumed at higher quantities and rates by both humans and other predators.

The major strength of this work is the diversity of sites, relatively large number of deer harvested, and extensive analyte list. There were also some limitations to this analysis. As these samples were provided by hunters, it was impossible to attain a standardized, equal collection at each study site regarding age and sex which limited our ability to conduct formal statistical testing of differences by age and life stage in many cases. While we expected to find PFAS in deer at Lake Elmo and Duluth due to previous efforts to characterize PFAS contamination in environmental media, we lacked baseline information from Camp Ripley which limits our ability to contextualize the results from this site. Deer were harvested at sites with suspected or known localized contamination and it is unknown to what extent they represent deer in other parts of Minnesota or areas of the country more broadly with no known local contamination. We expect that the deer collected near these sites represent high exposure

potential of deer to PFAS. Finally, this study was not designed to assess what effects these PFAS compounds may be having on deer health, behavior, or survival.

CONCLUSIONS

Both the risks and benefits of consuming wild game and fish, including the frequency and quantities at which they are consumed, must be carefully considered when evaluating these results. Eating wild game and fish is considered a healthy food option. Venison is one of the leanest and most nutrient-rich meats available (Valencak et al 2015). However, fish and game consumption can be potential pathway of human exposure to PFAS as well as other contaminants. For example, previous studies have found health hazards of consuming deer meat related to lead shot fragments in meat (Hunt et al. 2009), and pathogens in poorly processed and under-cooked meat (Rounds et al. 2012). At the time of this publication, there are no PFAS consumption advisories in Minnesota for white-tailed deer, but discussions between state agencies on this topic are on-going. Until formal risk assessments are conducted by state or federal agencies, those specifically concerned about PFAS in deer are currently advised by Minnesota DNR and MDH to avoid consuming the liver given the results found here and more broadly, the function of that organ to filter contaminants (Kicińska et al. 2019).

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Table 1. PFAS results for 116 white-tailed deer liver samples from September 2020 - December 2021 at three study sites in Minnesota. Ranges provided in ng/g, as wet-weight basis.

PFAS Compound	Lake Elmo Study Area (n=59 samples)		Camp Ripley Study Area (n=31 samples)		Duluth Study Area (n=26 samples)	
	Number of livers with detectable concentrations	Range	Number of livers with detectable concentrations	Range	Number of livers with detectable concentrations	Range
PFBA - Perfluorobutanoic acid	55	0.83 - 9.14	10	1.68 - 3.30	7	1.48 - 2.53
PFPeA - Perfluoropentanoic acid	0	-	11	0.21 - 0.44	0	-
PFHxA - Perfluorohexanoic acid	1	0.97	9	0.12 - 0.40	0	-
PFHpA - Perfluoroheptanoic acid	1	0.97	4	0.28 - 2.42	3	0.14 - 0.32
PFOA - Perfluorooctanoic acid	14	0.11 - 4.84	2	0.17 - 0.24	0	-
PFNA - Perfluorononanoic acid	21	0.11 - 0.41	21	0.10 - 0.40	11	0.11 - 0.58
PFDA - Perfluorodecanoic acid	38	0.10 - 0.32	26	0.12 - 0.60	12	0.10 - 0.45
PFUnA - Perfluoroundecanoic acid	29	0.10 - 0.18	24	0.11 - 0.29	12	0.10 - 0.21
PFDoA - Perfluorododecanoic acid	0	-	1	0.12	3	0.11 - 0.16
PFHxS - Perfluorohexane sulfonate	2	0.11 - 0.36	13	0.10 - 2.78	2	1.45 - 2.44
PFHpS - Perfluoroheptanesulfonic acid	3	0.12 - 0.15	6	0.12 - 0.62	1	0.64
PFOS - Perfluorooctane sulfonate	59	0.23 - 43.1	31	0.92 - 96.0	26	0.52 - 42.0
PFNS - Perfluorononanesulfonic acid	0	-	2	0.09 - 0.1	0	-
PFDS - Perfluorodecanesulfonic acid	0	-	1	0.138	0	-
6:2 FTS - 6:2 Fluorotelomer sulfonic acid	3	1.7 - 48.4	4	0.53 - 1.14	13	0.64 - 2.49
PFOSA - Perfluorooctane sulfonamide	5	0.10 - 0.12	0	-	0	-
N-EtFOSA - N-ethyl perfluorooctane sulfonamide	0	-	0	-	2	0.25 - 0.26
N-EtFOSE - N-ethyl perfluorooctane sulfonamido ethanol	51	1.02 - 13.4	2	0.87 - 1.05	2	1.01 - 1.24
PFMPA - Perfluoro-3-methoxypropanoic acid	1	0.72	0	-	0	-
NFDHA - Nonafluoro-3,6-dioxaheptanoic acid	2	0.24 - 0.96	3	0.45 - 5.75	0	-

The following PFAS compounds were analyzed for but not detected in these 116 deer liver samples: Perfluorotridecanoic acid (PFTrDA), Perfluorotetradecanoic acid (PFTeDA), Perfluorobutanesulfonic acid (PFBS), Perfluoropentane sulfonic acid (PFPeS), Perfluorodecane sulfonic acid (PFDoS), 4:2 Fluorotelomer sulfonic acid (4:2 FTS), 8:2 Fluorotelomer sulfonic acid (8:2 FTS), N-Methylperfluorooctanesulfonamide (N-MeFOSA), 2-(N-Methylperfluorooctanesulfonamido)acetic acid (MeFOSAA), N-ethylperfluorooctane sulfonamidoacetic acid (EtFOSAA), N-Methylperfluorooctanesulfonamidoethanol (N-MeFOSE), Hexafluoropropylene oxide dimer acid (HFPO-DA), 4,8-dioxa-3H-perfluorononanoic acid (ADONA), Ethanesulfonic acid (9Cl-PF3ONS), 11-chloroeicosfluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS), 3-Perfluoropropyl Propanoic acid (3:3 FTCA), 5:3 Fluorotelomer carboxylic acid (5:3 FTCA), Decanoic acid (7:3 FTCA), Perfluoro(2-ethoxyethane)sulphonic acid (PFEESA), Perfluoro(4-methoxybutanoic) acid (PFMBA).

Table 2. Per- and polyfluoroalkyl substance (PFAS) detections by chemical group.

Chemical Group (# of analytes in group)	Detected (at least once)	Not Detected
Perfluorinated acid ("PFCAs") (11)	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA	PFTrDA, PFTeDA
perfluorinated sulfonate ("PFSAs") (8)	PFHxS, PFHpS, PFOS, PFNS, PFDS	PFBS, PFPeS, PFDoS
perfluorinated sulfonamide (1)	PFOSA	--
perfluorinated ether acid (4)	PFMPA, NFDHA	HFPO-DA, PFMBa
perfluorinated ether sulfonate (1)	--	PFEESA
polyfluorinated acid (3)	--	3:3FTCA, 5:3FTCA, 7:3FTCA
polyfluorinated sulfonamide (4)	N-EtFOSA	MeFOSAA, EtFOSAA, N-MeFOSA
polyfluorinated sulfonamido alcohol (2)	N-EtFOSE	N-MeFOSE
polyfluorinated sulfonate (3)	6:2FTS	4:2FTS, 8:2FTS
polyfluoroalkyl chloroether sulfonate (2)	--	9Cl-PF3ONS, 11Cl-PF3OUdS
polyfluoroalkyl ether acid (1)	--	ADONA
PFCA and PFSA Detections by Carbon Chain Length		
Short-chain PFCAs (4)	PFBA, PFPeA, PFHxA, PFHpA	--
Short-chain PFSAs (2)	--	PFBS, PFPeS
Long-chain PFCAs (7)	PFOA, PFNA, PFDA, PFUnA, PFDoA	PFTrDA, PFTeDA
Long-chain PFSAs (6)	PFHxS, PFHpS, PFOS, PFNS, PFDS	PFDoS

Table 3. Mixed effects model of life stage-sex on PFAS liver concentrations

	Estimate	SE	p-value
Log total PFAS			
Intercept	1.93	0.46	.052
Adult male	0.39	0.17	.029
Fawn male	-0.22	0.28	.435
Fawn female	0.07	0.27	.787
Adult female	REF	--	--
Log total perfluorinated			
Intercept	1.64	0.50	.081
Adult male	0.48	0.19	.012
Fawn male	-0.22	0.30	.474
Fawn female	0.26	0.29	.382
Adult female	REF	--	--

*Yearlings were excluded from analysis due to small sample size.

Table 4. Comparing PFOS levels in liver and muscle for 17 white-tailed deer with elevated liver levels. (Concentrations provided in ng/g wet weight basis).

Deer ID	Study Area	Sex	Age Class	PFOS concentration in liver	PFOS concentration in skeletal muscle
PFAS_0041	Duluth	Male	Adult	42.0	0.22
PFAS_0105	Camp Ripley	Male	Adult	65.0	0.42
PFAS_0108	Camp Ripley	Female	Adult	26.7	0.43
PFAS_0116	Camp Ripley	Female	Fawn	72.7	0.37
PFAS_0119	Camp Ripley	Female	Adult	22.0	0.29
PFAS_0122	Camp Ripley	Unknown	Unknown	96.0	1.24
PFAS_0123	Camp Ripley	Unknown	Unknown	41.7	0.55
PFAS_0125	Camp Ripley	Female	Adult	20.6	0.30
PFAS_0166	Lake Elmo	Male	Adult	40.9	0.38
PFAS_0167	Lake Elmo	Female	Adult	20.5	0.22
PFAS_0168	Lake Elmo	Female	Adult	43.1	0.94
PFAS_0190	Lake Elmo	Female	Adult	23.1	0.42
PFAS_0205	Lake Elmo	Male	Adult	27.0	0.15
PFAS_0207	Lake Elmo	Male	Adult	21.8	0.19
PFAS_0242	Lake Elmo	Male	Unknown	28.2	0.42
PFAS_0246	Lake Elmo	Unknown	Unknown	21.9	0.47
PFAS_0248	Lake Elmo	Female	Adult	32.4	0.45

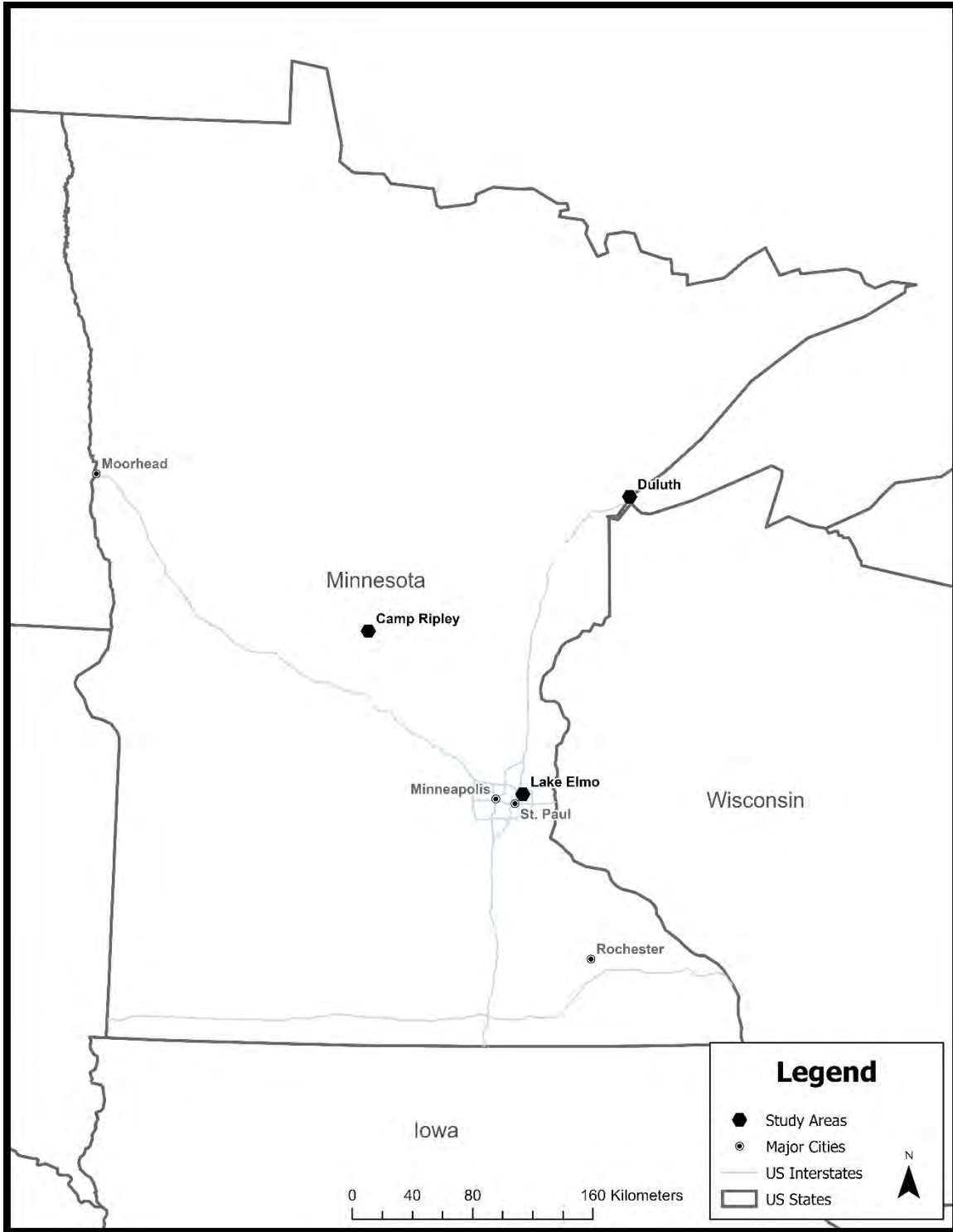
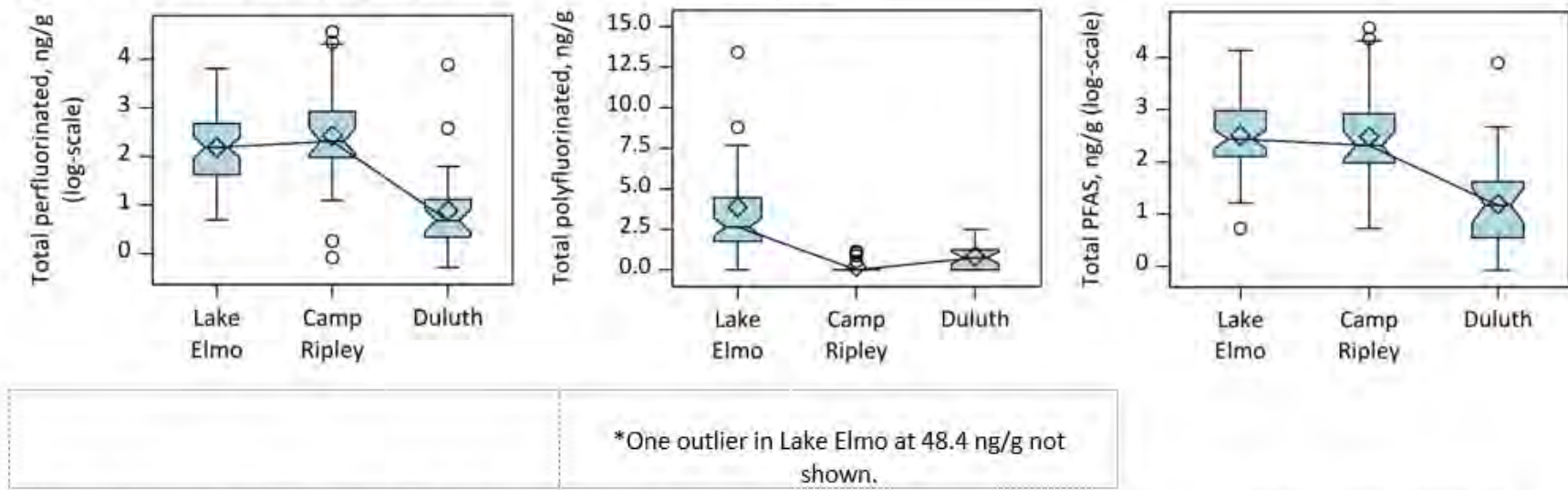


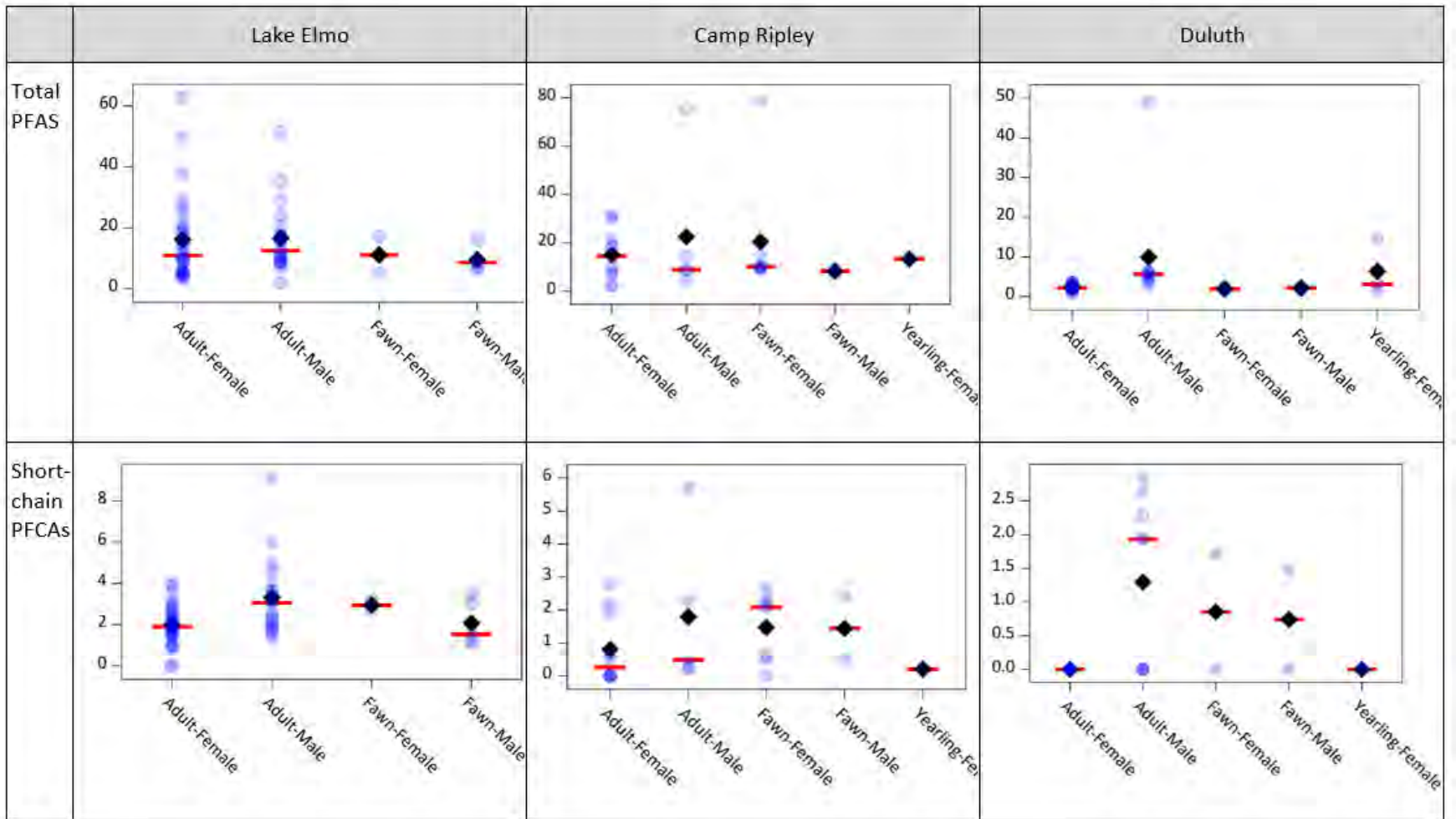
Figure 1 – Location of three study areas, Lake Elmo, Duluth, and Camp Ripley, for our collection of white-tailed deer livers during 2020 and 2021 to assess PFAS levels.

Figure 2. Boxplots of analyte group liver concentrations by study site¹

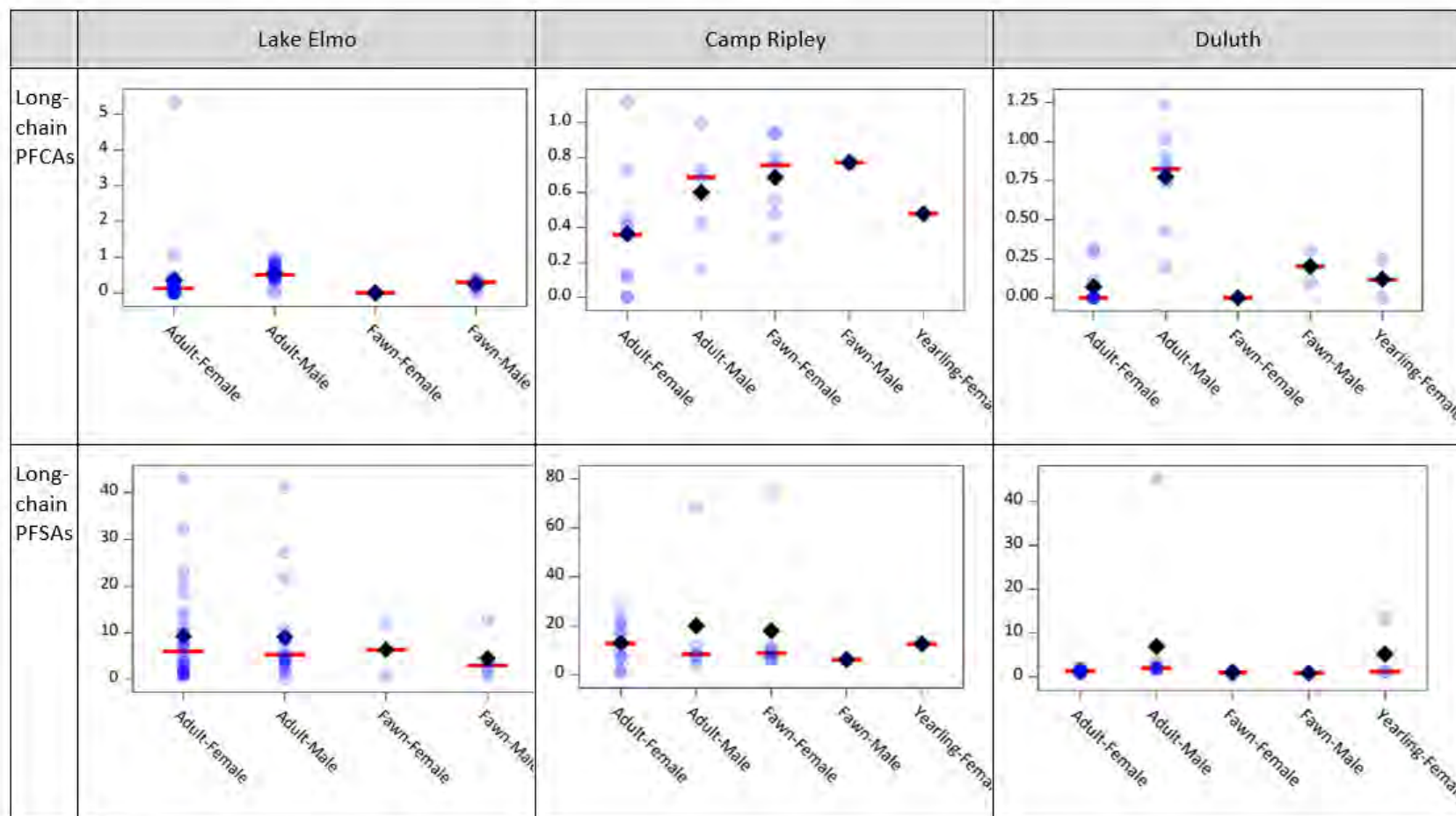


¹ Mean=diamond, median=line. Notches that do not overlap indicate statistically significant difference in median concentration.

Figure 2. Boxplots of analyte group concentrations by hunting Area. Total perfluorinated and polyfluorinated and total PFAS concentrations are also shown in boxplots below, in regular scale or log-scale (to improve visual assessment of differences in distributions when outliers are present). Differences in chemical class concentrations by site are seen. The Duluth site had notably lower “total perfluorinated compound” and “total PFAS” median concentrations compared to the other two sites. Camp Ripley had the lowest polyfluorinated compound concentrations. The lack of overlap of the notches in the boxplots indicates statistically significant differences in chemical group median concentrations.



Continued next page



¹ Mean=diamond, median=line

Figure 3. At the Lake Elmo site, no major differences in concentration were seen between life stage-sex group for total PFAS and long-chain PFSAs. Comparing adult females, adult males, and male fawns, there was no statistically significant difference in concentration for total PFAS ($H(2)=2.2$, $p=.3278$) or long-chain PFSAs ($H(2)=1.6$, $p=.4415$). However, there was a statistically significant difference for short-chain PFCAs ($H(2)=8.2$, $p=.0167$) and long-chain PFCAs ($H(2)=19.4$, $p<.0001$). At Camp Ripley, no statistically significant differences in concentration between adult females, adult males and female fawns were seen for total PFAS ($H(2)=0.29$, $p=.8655$), short-chain PFCAs ($H(2)=2.3$, $p=.3207$), long-chain PFCAs ($H(2)=5.0$, $p=.0837$), or long-chain PFSAs ($H(2)=0.11$, $p=0.9476$). At the Duluth site, adult male deer had higher mean and median liver concentrations of total PFAS, short-chain PFCAs, and long-chain PFCAs compared to other life stage-sex groups upon visual inspection (Figure 3). Comparing adult females to adult males, there was a statistically significant difference in concentration for total PFAS ($H(1)=11.2$, $p=.0008$), short-chain PFCAs ($H(1)=6.9$, $p=.0085$), long-chain PFCAs ($H(1)=13.0$, $p=.0003$), and short-chain PFSAs ($H(1)=9.9$, $p=.0017$).

Wetland Wildlife Populations and Research Group
102 – 23rd Street Northeast
Bemidji, Minnesota 56601
(218) 308-2282



Restoring Wetland Invertebrates to Revive Wildlife Habitat: A Study of Wetland Characteristics and Stocking to Support Prairie Pothole Amphipods

Report prepared by Megan Fitzpatrick¹

Project collaborators:

Co-Principal Investigators: Danelle Larson², Michael Anteau³, Carl Isaacson⁴, Emily Schilling⁵

Graduate Students who completed MS Theses: Jake Carleen⁴, Breanna Keith⁴

Undergraduates who completed research projects/write-ups/posters: Michael Bieganeck⁵, Demey DeJong⁵, Alaina Taylor⁴

SUMMARY OF FINDINGS

Freshwater amphipods are an important but declining component of food webs in prairie pothole wetlands, with particular influence on the continental scaup population. The broad goal of this study was to obtain information that would help natural resource practitioners manage for amphipods and their consumers in the Prairie Pothole Region (PPR) of Minnesota. We had three objectives. Our first objective was to identify wetland characteristics associated with amphipod abundance, with the aim of informing wetland management for amphipods. Our second objective was to identify associations between waterfowl abundance and amphipod abundance, to better understand the importance of amphipods to waterfowl. Our third objective was to evaluate the effectiveness of amphipod stocking (conservation translocation) as a management technique to establish self-sustaining *Gammarus lacustris* amphipod populations in wetlands where they did not previously exist. For Objectives 1 and 2, we measured amphipods, waterfowl, fish, aquatic vegetation, water chemistry/clarity, sediment pyrethroid (insecticide) concentrations, buffer strip width, and watershed-scale landcover characteristics in >45 Minnesota prairie pothole wetlands. We found that amphipod abundance decreased with increasing abundance of several fish species, especially black bullheads. Amphipod abundance was also negatively associated with pyrethroid abundance, which was in turn negatively associated wetland buffer strip coverage (proportion of wetland shoreline surrounded by upland vegetation buffer strip at least 30 m wide). On the other hand, aquatic plant diversity and biomass were positively related to amphipod abundance. Preliminary analysis suggests that amphipod density was related to scaup density, and these data require further analysis. For Objective 3, we conducted a before-after/control impact (BACI) study of *G. lacustris* stocking. Though *G. lacustris* had high survival between collection and release (>98%), stocking established a *G. lacustris* population at only 5% of sites (n=19). Improved outcomes might be achieved via a habitat-based modeling approach to selecting candidate basins for stocking, or by stocking recently restored or enhanced wetlands.

¹Minnesota Department of Natural Resources, Wetlands Wildlife Population and Research Group, 102 23rd St. NE, Bemidji, MN 56601

²U.S. Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Road, La Crosse, WI 54603, USA

³U.S. Geological Survey, Northern Prairie Wildlife Research Center, 8711 27th St SE, Jamestown, ND, 58401, USA

⁴Bemidji State University, Department of Environmental Studies, Sattgast Hall 109, Bemidji, MN, 56601

⁵Augsburg University, 2211 Riverside Avenue, Minneapolis, MN 55454, USA 3

Overall, our results suggest that management actions to increase aquatic plant diversity, remove fish, prevent fish invasion of remaining amphipod-rich basins, and reduce pesticide levels in wetlands should increase amphipod populations on the landscape and improve habitat for migrating scaup. For example, managers might consider drawdowns, removal of hydrological connections between wetlands, installation of fish barriers, piscicide treatments, agricultural practices allowing for reduced pesticide use, and 30-m buffer zones of non-cultivated vegetation around wetlands. Stocking may contribute to increased amphipod abundance on the landscape if paired with such wetland enhancement activities, or with preliminary assessments to select wetlands with ideal amphipod habitat.

INTRODUCTION

Freshwater amphipods are an important component of food webs in prairie pothole wetlands. Prairie pothole amphipods (hereafter “amphipods”; primarily *Gammarus lacustris* and *Hyaella azteca*) consume coarse particulate organic matter, algae, and bacteria from 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. However, amphipods have declined in recent decades across the Prairie Pothole Region (PPR) (Anteau and Afton 2006; 2008a, b; Huener 2011). 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 (Anteau and Afton 2004, 2006, 2008a, 2009a, 2011; Huener 2011).

Numerous wetland and community characteristics may influence amphipod occurrence and abundance in the PPR. Amphipods are restricted primarily to semi-permanent and permanent wetlands, as they lack a desiccation-resistant winter stage (Murkin and Ross 2000) and have limited dispersal ability (Swanson 1984). Aquatic macrophytes may be positively related to amphipod abundance, as macrophytes may provide foraging substrate, provide cover to avoid predators, increase system productivity and therefore abundance of amphipod food resources, or indicate other factors supporting both macrophytes and amphipods (such as water quality). In prior study of prairie pothole amphipods, Anteau et al. (2011) found that amphipod densities were positively related to submerged aquatic vegetation density. On other hand, fish in prairie pothole wetlands may factor in amphipod declines. Prior studies have found negative relationships between fish abundance and amphipod abundance in prairie pothole wetlands (Anteau et al. 2011, Janke 2016), and fish occurrence in prairie potholes 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 2008b, Wiltermuth 2014, McCauley et al. 2015, McLean et al. 2016). Fish may directly predate amphipods, compete with amphipods for food, or indirectly influence amphipods by stirring up bottom sediments while foraging (i.e., benthivorous fish), creating turbid, nutrient-rich water and shifting basins from a macrophyte-dominated to an algae-dominated state (Scheffer et al. 1993). Shifting basin conditions may impact amphipods directly via changing water temperature and oxygen content, or indirectly via impacting aquatic vegetation.

Impacts of intensive agriculture in the PPR may also influence amphipods in prairie pothole wetlands. In addition to increased connectivity and potential for fish invasion due to consolidation drainage, sedimentation and nutrient run-off from farm fields contributes to turbidity and algal growth in PPR wetlands, and insecticides entering wetlands (via runoff or drift) can directly impact populations of aquatic invertebrates (Li et al. 2017). Surrounding

landcover, such as the presence of buffer strips - the strip of non-cultivated upland vegetation between the wetland edge and adjacent cultivated crop - may mitigate impacts of agriculture.

Information about which habitat characteristics promote abundant amphipod populations will help management organizations more effectively protect, restore, and enhance wetlands for amphipods and consumers like lesser scaup. In our study, we explored habitat characteristics associated with amphipod abundance in Minnesota prairie pothole wetlands, including fish and aquatic plant communities; water quality and clarity; levels of pyrethroid pesticides; and landscape-scale characteristics related to agricultural impacts on wetlands, including buffer width extent and percentage of watershed composed of cropped land cover (**Objective 1**). To further understand the relationship between waterfowl, amphipods, and underlying habitat characteristics, we also carried out spring migratory waterfowl surveys and summer waterfowl brood surveys at our study wetlands (**Objective 2**). Importantly, previous studies of similar factors using random-site selection techniques (Anteau and Afton 2008b, Anteau et al. 2011) were limited by the relatively small proportion of basins containing high amphipod densities, with low *G. lacustris* and *H. azteca* densities of $\leq 5 \text{ m}^{-3}$ in 92% and 61% of wetlands surveyed, respectively. To incorporate especially high-density basins into our study, we scouted for sites containing high amphipod densities in the year prior to our study and included them in addition to randomly selected wetlands.

A third objective in our study was to assess the efficacy of amphipod stocking (conservation translocation; Seddon et al. 2014) as a wildlife management technique for establishing persistent amphipod populations (**Objective 3**). Conservations groups and private landowners have been stocking *G. lacustris* for over 20 years as a method of providing forage for fish and waterfowl (Mathias and Papst 1981; B. Thoele, Lincoln Bait LLC, personal communication). However, the persistence of amphipods post-stocking has not been evaluated.

Stocking may be a particularly appropriate management activity for amphipods due to their limited means of dispersal. Unlike many aquatic invertebrates, which can fly during some point in their life cycle, amphipods are limited to dispersal via animals (Swanson 1984) or hydrological connections between wetlands (Keith 2021). Some aspects of amphipod biology may support their persistence in stocked (e.g. maternal egg/brood care), whereas other aspects (e.g. tendency toward local adaptation) may present challenges (Jourdan et al. 2019). Consequently, we evaluated whether amphipod stocking created persistent populations of amphipods using a before-after/control-impact study design. We also measured habitat characteristics (fish and plant communities, water chemistry) in study wetlands to understand what factors support stocking success. To document potential influences on waterfowl, we conducted spring and summer waterfowl surveys at stocked basins in comparison to randomly-selected reference basins.

We have published components of this research project as two graduate student theses and two papers (Keith 2021, Carleen 2022, Keith et al. 2022, Larson et al. 2022). We anticipate publishing five additional papers (including one recently submitted: Fitzpatrick et al., unpublished).

OBJECTIVES

- 1. Identify habitat characteristics associated with amphipod occurrence and abundance in Minnesota prairie-pothole and forest-transition zone wetlands, including fish and plant communities, water quality and clarity, sediment pyrethroid levels, and landscape-scale characteristics (buffer width extent, percentage of watershed composed of cropped land cover).**

We hypothesized that:

- a. amphipod abundance would be positively related to aquatic macrophyte diversity and biomass
- b. amphipod abundance would be negatively related to abundance of fathead minnows (*Pimephales promelas*), brook stickleback (*Culaea inconstans*), and benthivorous fish,
- c. amphipod abundance would be negatively related to anthropogenic disturbances from agriculture: pyrethroid concentrations, watershed-level crop intensity, stream connectivity, and turbid-state conditions.

2. Identify associations between waterfowl occurrence/abundance and amphipod occurrence/abundance in spring and summer, to better understand the importance of amphipods to waterfowl.

We hypothesized that:

- a. spring waterfowl abundance would be higher in basins with higher amphipod abundance, especially for lesser scaup
- b. summer brood abundance would be higher in basins with higher amphipod abundance

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

We hypothesized that:

- a. new populations of *G. lacustris* would usually be established at stocked basins after one generation (to be measured after reproduction and senescence/mortality of stocked amphipods, approximately 9 months following stocking)
- b. *G. lacustris* densities would increase over time (up to three years post-stocking), with the expectation that several years would be required for population growth
- c. Establishment of stocked amphipods might be impacted by aquatic macrophytes diversity and biomass, and fish abundance (as described in Objective 1 hypotheses)

METHODS

Objectives 1 and 2: Habitat characteristics and waterfowl associations of prairie pothole amphipods

Study design, study area, and site selection

Our study focused on permanent and semi-permanent wetlands in the prairie-pothole and forest-transition regions of Minnesota. Study sites were located in four clusters to reduce travel time while incorporating a range of latitudes: near the cities of Windom, St. Cloud, Fergus Falls, and Detroit Lakes, MN (Fig. 1). We set a 200-acre maximum limit on wetland size for logistical reasons (limiting time spent per wetland to maximize sample size of wetlands).

Previous studies of PPR amphipods and their habitat characteristics (Anteau and Afton 2008b, Anteau et al. 2011) were limited by the small number of high-density basins found via random sampling (M. Anteau, US Geological Survey, personal communication). Consequently, we incorporated wetlands with preliminarily known, high amphipod densities (hereafter “high-density wetlands”) into this study. 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. For each high-density wetland, we randomly selected a Type 4 or 5 (“inland deep freshwater marsh” or “inland open water”) wetland of similar size (\pm 25% area of high-density wetland) in the study region (within 15 miles of any high-density wetland) from the National Wetlands Inventory Circular 39 wetlands classification layer (Shaw and Fredine 1956, Minnesota Department of Natural Resources).

We also incorporated randomly selected wetlands to ensure a range of amphipod densities (including basins without amphipods) for assessment. Our original goal was for half of the study wetlands to be known high-density wetlands and half to be randomly selected wetlands. Due to COVID-19 pandemic restrictions against overnight travel in 2020, we sampled all remaining high-density wetlands within day trip distance of our Bemidji office (Detroit Lakes cluster) and additional randomly selected wetlands. Overall, we sampled 65 wetlands for amphipods and habitat characteristics, including 48 in 2019 (24 known high-density wetlands and 24 randomly selected wetlands) and 17 in 2020 (4 high-density and 13 randomly selected).

Our site selection methods for 2019 and 2020, which focused on a mix of randomly selected basins and basins known to contain high amphipod density, yielded a small sample size of wetlands with scaup ($n=10$). Consequently, we extended the Objective 2 portion of the study for a third year with a different site selection method, focused on increasing our sample size of basins with migratory scaup. We scouted for scaup in our study area (Detroit Lakes and Fergus Falls clusters due to on-going COVID-19 travel restrictions) during peak scaup migration and stopped to conduct a formal waterfowl survey if scaup were sighted. We planned driving routes to scout a large number of accessible basins potentially used by scaup based on size and proximity to roads, with priority given to basins on public land, using the National Wetlands Inventory Circular 39 dataset (Shaw and Fredine 1956, Minnesota Department of Natural Resources). Local Wildlife Area staff also suggested basins and alerted us to potential sights when they saw scaup during their spring work. We scouted over 110 basins in 2021, detected scaup in 25 basins, and returned to 19 basins for amphipod surveys as soon as possible after completion of waterfowl surveys (Fig. 2).

General field survey timing

Each basin was surveyed for amphipods, habitat characteristics, and waterfowl in one year (2019 or 2020). Some additional basins were surveyed for amphipods and waterfowl only in spring 2021.

In 2019 and 2020, we visited each basin three times to achieve appropriate phenological timing for each type of survey. We sampled amphipods and waterfowl as soon as possible after ice-out each year (April-May). After the initial spring visit to survey amphipods, we returned to as many basins as possible to survey each type of wetland characteristic and waterfowl broods (June-August). For our Objective 2 extension in 2021, we scouted and surveyed waterfowl in April in accordance with peak scaup migration timing through the study area and returned as soon as possible afterward to survey amphipods (May-June).

Amphipod survey methods

Amphipod sampling methods are described in (Keith 2021, Carleen 2022, Keith et al. 2022, Larson et al. 2022). In short, we sampled amphipods at 8 sample points per wetland (Fig. 3a). Sample points were placed on 8 evenly spaced, parallel 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, or the innermost ring of emergent vegetation when emergent vegetation was present. 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 μm 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. A water column sample was then taken in an undisturbed area with a D-frame or round-frame dip net, modified such that the net frame was 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 and returned to the lab for invertebrate picking and identification.

Wetland characteristics survey methods

We sampled wetlands for fish in June-July of each year. Our fish sampling methods are described in Carleen (2022). In short, species-specific fish abundance and biomass 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 miniature 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 emergent vegetation was present. Miniature 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 offshore. Following net retrieval, fish were sorted by species, weighed (total mass of each species), and counted. We also measured fish length to the nearest cm using a measuring board (up to 50 individuals per species). 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 $\geq 20\%$ of the weight was retained and processed (total weight of each species, species-specific counts and lengths).

We visited wetlands a third time (July-early August) for collection of aquatic vegetation data, water chemistry measurements, and sediment samples for pyrethroid analysis. Aquatic vegetation survey and water chemistry measurement methods are described in Keith (2021), Carleen (2022), and Larson et al. (2022). In brief, we modeled vegetation survey methods after Minnesota DNR Shallow Lakes survey protocols (Perleberg et al. 2019). 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 ($\pm 10\%$) 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 aquatic vegetation (SAV), 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 (*Potamogeton* spp.). Water depth was measured at each sample point using a weighted rope with depth markings. Floating vegetation biomass was indexed visually in a 1-m² 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 at the nearest shore was recorded for each of the shore-adjacent points in the grid. Field crew 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 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.

Sediment sampling and pyrethroid analysis techniques are described in detail in Keith (2021). In brief, we collected wetland sediments following procedures described in Shelton and Capel (1995), 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. Following Radtke (2005), we collected approximately 192 mL of sediment 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. We homogenized each sample with a clean stainless steel spatula, subsampled it (if necessary), and separated it into two 500-mL amber glass jars. Samples were transported to the lab in a portable freezer and kept frozen at -20°C until analysis.

Waterfowl survey methods

We surveyed migratory waterfowl in spring when we visited wetlands to survey amphipods (April-May), and we surveyed waterfowl broods in summer when we visited wetlands to sample vegetation, water, and sediment (July-August). In 2021, we sampled additional wetlands for migratory waterfowl (April).

Our primary waterfowl survey goals were to count migrating scaup (*Aythya affinis* and *A. marila*) and mallard (*Anas platyrhynchos*) breeding pairs (spring) and mallard broods (summer) 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 surveyed all waterfowl at each wetland.

For both pair surveys and brood surveys, we surveyed waterfowl immediately upon arrival to the site (2019-2020) or upon detection of scaup (2021). 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 waterfowl (ducks, geese, swans) observed for a fixed 10 minutes. Waterfowl 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 notes indicating which waterfowl groups each person detected and missed, making use of maps. Observers added notes rather than changing original data in data tables to allow for detection probability 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).

Amphipod processing and identification

We processed dip net samples and identified amphipods in the lab following field work. Methods are described in depth elsewhere (Keith 2021, Carleen 2022, Keith et al. 2022, Larson et al. 2022). In brief, 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.

To process the samples, we filtered ethanol from sample bottles over a 500 μ m 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- μ m 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 were 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 trinocular stereomicroscope (Laxco, 6.5-53x magnification) with a digital camera and 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 the camera's Tcapture software (Tucsen Phototonics Co.)

Pyrethroid extraction and analysis from sediment samples

Keith (2021) describes pyrethroid extraction and analysis in depth. In brief, 50 mL subsamples of sediment were prepared for extraction by thawing and oven-drying. Keith (2021) extracted pyrethroids from each subsample by combining 10 g of dried sediment with 25 mL of acetonitrile and allowing it to settle, passing the supernatant through a through a conditioned carbon-based solid phase extraction cartridge (Supelco Envi-Carb, 500mg, 6 mL), evaporating the solvent from extract with a nitrogen blanket (Thermo Scientific Reacti-Vap evaporator system), and dissolving the remaining residue in a 1:1 ratio of methanol and deionized water. The extract was then analyzed for pyrethroids using a 96-well plate enzyme-linked immunosorbent assay (ELISA) (Eurofins Abraxis, PN 500204), following the kit's standard operating procedure. The kit's lowest detection limit was 0.40 ng/g dried sediment.

Wetland buffers and watershed land use

We used GIS techniques to quantify the proportion of each wetland's shoreline with an upland vegetation buffer strip greater than 30 m wide (Sweeney and Newbold 2014), and proportions of the watershed composed of various land use/land cover types, for use in modeling amphipod abundance.

GIS work to quantify wetland buffer strip extent and watershed land use/land cover was conducted in ArcMap (ESRI). Keith (2021) describes GIS methods in depth. In short, to analyze buffer strips, we extracted raster cells from 2019 NAIP color-infrared aerial imagery (1-m resolution) from a 50m buffer zone around the wetland edge and used unsupervised cluster analysis to classify cells as cropped or not cropped. We used the 2016 National Landcover Dataset, 2017 MDA cropland data layer, and 2019 NAIP imagery to quantify dominant land use/land cover types within Level 9 catchment boundaries generated for each wetland using the Minnesota DNR AutoCatchment geodatabase (https://mnatlas.org/metadata/dnr_watersheds_dnr_level_09_auto_catchments.html).

Data analysis

The relationship between amphipods and fish, plants, water chemistry, pyrethroid levels, and landscape level characteristics were analyzed in a variety of ways (Keith 2021, Carleen 2022, Larson et al. 2022). For most subprojects, total count (per wetland) of *H. azteca* or *G. lacustris* was modeled as a function of the habitat characteristic of interest, using generalized linear mixed models with negative binomial distribution, with volume of water swept for amphipods as an offset variable. Amphipod species were modeled separately because they may respond differently to environmental variables. Amphipod density is likely a function of numerous wetland characteristics. Consequently, covariates to the focal predictor variables of interest were added or removed to improve model fit and evaluation of the relationship between predictor and response variable. (E.g., aquatic vegetation biomass was used as a covariate in models with fish counts as a focal predictors).

The relationships between waterfowl and amphipods have been preliminarily explored via graphs and will be further analyzed via generalized linear modeling (migratory waterfowl, waterfowl indicated pair, and waterfowl brood abundance) and occupancy modeling (migratory waterfowl, waterfowl indicated pair, and waterfowl brood presence/absence), with amphipod abundance as a predictor variable. Wetland size and shape will be explored as covariates. We will focus on migratory scaup and mallard migrants/pairs/broods, but will examine any species with sufficient sample size, along with broader taxonomic and ecological groups (dabbling ducks, diving ducks, all ducks), for relationships with amphipod abundance.

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

Experimental design, study area, and site selection

Objective 3 focuses on evaluating amphipod stocking efficacy in depressional wetlands at sites within the prairie-pothole and forest-transition regions of Minnesota (Figure 4). 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 each wetland was sampled for amphipods before and after stocking.

Site selection criteria included at least two similarly-sized wetlands with semi-permanent or permanent hydrology, at least 1 m maximum depth (to avoid amphipod winterkill), area less than 50 acres (to allow statistical replication within the project budget), and reasonable accessibility. Wetlands were chosen based on recommendations from managers, private landowner volunteers, and preliminary scouting. The choice of which wetland to serve as the control was randomized whenever possible, with a few exceptions for logistical reasons (Table 2).

We stocked wetlands at 19 sites, and there were 41 wetlands total in the study. Two sites had more than one stocked basin (Table 2). In these cases, *G. lacustris* densities were averaged

across stocked basins at the site to obtain a single value for statistical analysis in relation to control basin values.

Objective 3 was originally designed as a three-year study (fall 2017 through fall 2020). *G. lacustris* were only observed post-stocking at a single site during this timeframe, so the study was extended for an additional two years (through fall 2022), in case stocked *G. lacustris* existed in low numbers and required additional reproductive cycles to reach observable population sizes.

We stocked wetlands in each of three winters. We stocked 10 wetlands in winter 2017-18 (“cohort 1” of wetlands), 5 wetlands in 2018-19 (“cohort 2”), and 7 wetlands in winter 2019-20 (“cohort 3”) (Table 2). We usually sampled amphipod density in the fall (August-October) prior to stocking (“before” sample). We stocked the wetlands the following winter, and sampled amphipod population density in each fall following stocking through fall 2020 (“after” samples). For the project extension, we sampled cohort 1 in Fall 2021, and cohort 2 and 3 in Fall 2022. Thus, cohort 1 basins had 4 after-stocking samples, cohort 2 had three after-stocking samples, and cohort 3 had two after-stocking samples by the end of the study.

We also sampled wetland characteristics that might influence *G. lacustris* establishment (fish communities, water chemistry, and aquatic vegetation) at each site. Habitat characteristics were assumed to be stable throughout the three years and were only sampled once per wetland after stocking, in 2019 or 2020. We did not sample habitat characteristics at all wetlands due to travel restrictions associated with the COVID-19 pandemic but sampled as many wetlands as possible. We also conducted spring and summer waterfowl surveys were in one year following stocking.

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) at the contractor’s facility 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. Based on volumetric subsamples collected from coolers at the stocking sites, we estimated that this value equated to an average of 10 individuals per m² wetland surface area (range: 2.1-15.7*m²).

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 3). 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 randomly selected location along the transect within 5 m of shore, and at another randomly selected location along the transect greater than 5m from shore.

Amphipods were sampled using D-frame dip nets (1200 µm mesh) from boats. At each point, we measured water depth 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 this sampling procedure was that we did not conduct before-stocking sampling using dip nets for the first year of the study (cohort 1 before-stocking surveys). In this first year, amphipods were sampled in winter, shortly prior to stocking, using a propane-powered ice augur. We drilled two side-by-side holes in the ice and moved snow away from the area to create a depression. We placed a sieve (bucket with bottom removed and a mesh bag inserted) over one of the holes. We then inserted the ice augur bit into the water of the second hole and ran it for 15 seconds. The augur drew water onto the ice surface, and it flowed out through the sieve. A subsequent experiment showed that the volume of water raised in 15 seconds varied substantially variable. Consequently, we switched to using sweep nets during ice-free seasons to obtain more precise estimates of amphipod density.

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.

Video monitoring

Due to low rates of *G. lacustris* establishment, we opportunistically monitored *G. lacustris* at a small stocked wetland near our office (Site 14; 3.3 acres) using an underwater video camera (MarCum VT7106 PanCam Wi-Fi Camera System with RT-9 tablet module) in winter 2020 to assess potential for mass mortality following stocking. We observed *G. lacustris* immediately following release, one time per day over the next three days, and then one time per week until 5 weeks post-release. We ceased underwater monitoring after 5 weeks due to field work restrictions imposed by the COVID-19 pandemic.

At each site visit, we lowered the camera into the release hole and rotated it to observe *G. lacustris* swimming and clinging to the underside of the ice. We also checked new locations within 20 m of the release hole beginning one week after release to look for dispersal. In addition, we cut a larger hole (approximately 0.6 x 0.6m) in the ice near (6m) the release hole on the day of release and used a sweep net to check mortality and relative density through time (weeks 1-4 following release). Holes were kept open by covering with foam insulation and were reopened as needed.

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

We surveyed waterfowl, fish, water chemistry, and aquatic vegetation following the methods described for Objectives 1 and 2.

Statistical methods

We have statistically analyzed results from the first three years of the study. We used a Welch's two-sample t-test to test whether stocking increased *G. lacustris* densities in comparison to densities at the associated control basins (Smith 2002). We conducted a non-parametric Welch's t-test due to differing sample size and non-homogeneity of variance between pre- and post-stocking surveys (Bartlett's test yielding $K^2 = 2.95$, $df = 1$, $p = 0.085$). The response variable was the difference in amphipod densities between the stocked and control basins at each site at each timepoint (D_{pik}). That is,

$$D_{pik} = R_{pik} - C_{pik}$$

(after Smith 2002, equation 3) where R_{pik} is *G. lacustris* density (m^{-3}) in stocked basin p ($p = 1, 2, \dots, 19$ sites) at time ik , where i represents period (before or after stocking) and k represents time within period ($k = 1$ within $i =$ before and $k = 1, 2, \text{ or } 3$ within $i =$ after). C_{pik} is *G. lacustris* density in control basin p at time ik .

The predictor variable was period (before or after stocking). We log-transformed the response variable to improve normality. Model estimates (difference in D between post-stocking and pre-stocking, associated confidence interval endpoints) were back-transformed for reporting.

To test whether *G. lacustris* densities increased through time (up to three years) following stocking, we fitted a one-way ANOVA with *G. lacustris* densities from post-stocking samples as a function of time since stocking (A1 = first fall, approximately 9 months, after stocking; A2 = second fall post-stocking; A3 = third fall post-stocking).

We used $\alpha = 0.15$ as evidence of statistical significance of stocking effectiveness.

RESULTS

Objectives 1 and 2: Habitat characteristics and waterfowl associations of prairie pothole amphipods

Amphipod occurrence and density

We sampled 65 wetlands for amphipods and habitat characteristics in 2019-2020. We found amphipods in 89% of wetlands, with densities ranging from 0-2044 amphipods per cubic meter of water (Fig. 5). *H. azteca* was the most commonly occurring species (85% of wetlands), followed by *G. lacustris* (55% of wetlands) and *Crangonyx* (25% of wetlands). Similarly, *H. azteca* was frequently the species occurring in highest density (66% of wetlands with amphipods; maximum density 1996 individuals/ m^3), followed by *G. lacustris* (29% of wetlands, maximum density 991 individuals/ m^3) and *Crangonyx* (3% of wetlands, maximum density 91 individuals/ m^3). *H. azteca* occurred in all study regions. *G. lacustris* were not found in the Windom region and were most common in the northern Fergus Falls and southern Detroit Lakes regions.

The low occurrence of *Crangonyx* species precluded most statistics.

Amphipod relationships with fish

We surveyed fish communities in 59 wetlands (Carleen 2022). (Carleen 2022) discusses results in depth. In brief, fish occurred in 81% of surveyed basins, with counts ranging up to 2631 (median 185). Three species occurred commonly enough to be modeled in relationship to amphipods: fathead minnow, brook stickleback, and black bullhead (*Ameiurus melas*).

Amphipod counts (*G. lacustris* or *H. azteca*) were modeled as a function of each fish species count (fathead minnow, brook stickleback, and black bullhead), using generalized linear mixed models with negative binomial distribution, including volume of water swept in amphipod samples as an offset variable. Covariates included submerged aquatic vegetation biomass, basin pH, and basin size for *G. lacustris* counts, and submerged aquatic vegetation biomass for *H. azteca* counts. Two basins were removed from analysis as outliers with high populations of both *G. lacustris* and fathead minnows (see discussion).

Both species of amphipods had a strong negative relationships with black bullhead counts. In particular, *G. lacustris* and black bullhead co-occurred in only one basin, precluding generalized linear modeling. A redundancy analysis of amphipod counts in relation to fish feeding guilds also showed a significant negative association between amphipod counts and benthivorous fish counts (which were driven primarily by black bullhead counts; $F_{1,55} = 6.04$, $p = 0.003$).

Both amphipod species were negatively related to brook stickleback counts, and *G. lacustris* counts were negatively related to fathead minnow counts. *H. azteca* was positively related to fathead minnow counts, though with weak effect size and marginal significance.

Amphipod relationships with aquatic vegetation

(Larson et al. 2022) analyzed the relationships between amphipod counts and aquatic plant communities for wetlands sampled in 2019 (n=49 wetlands). In brief, we modeled amphipod counts (*G. lacustris* or *H. azteca*) as a function of SAV biomass, macrophyte diversity (Simpson diversity), and prevalence of emergent vegetation along wetland shoreline, with volume of water swept for amphipods as an offset variable, using generalized linear mixed models with negative binomial distribution. We also conducted a redundancy analysis to test relationships between amphipod abundance and prevalence of the most common aquatic macrophyte and algae taxa: the eight most common SAV species, cattail (*Typha* spp.), and filamentous algae.

We found that abundances of both amphipod species were positively related to Simpson diversity, with *G. lacustris* having a particularly strong relationship. *H. azteca* also had a significant, negative quadratic relationship with SAV biomass, with abundance maximized at moderate biomass. *G. lacustris* had a negative but weak relationship with SAV biomass, and neither species had a significant relationship with *Typha* prevalence. Covariates included maximum water depth (positive relationship), wetland size (negative), total phosphorous (positive), and an index of suspended sediment (positive) for *G. lacustris*, and pH for *H. azteca* (positive).

In RDA, *G. lacustris* abundance was strongly associated with star duckweed (*Lemna trisulca*) abundance. *H. azteca* abundance was positively related to prevalence of several taxa: sago pondweed (*Stuckenia pectinata*), Fries' pondweed (*Potamogeton friesii*), flatstem pondweed (*Potamogeton zosteriformis*), northern watermilfoil (*Myriophyllum sibiricum*), and filamentous algae. Both species were negatively related to cattail and common bladderwort (*Utricularia macrorhiza*) prevalence.

Amphipod relationships with anthropogenic disturbance metrics: pyrethroids, wetland trophic status, hydrologic connectivity, buffer coverage, and land use

We sampled pyrethroid levels in 56 wetlands. Pyrethroids occurred in 44% of wetlands, with concentrations ranging from 0.45 to 2.25 ng /g dried sediment.

G. lacustris and *H. azteca* abundance were modeled as response variables in generalized linear models (binomial distribution) as a function of sediment pyrethroid concentration, wetland trophic status (clear-water or turbid, as determined by a k-means cluster analysis incorporating submerged aquatic vegetation coverage, total phosphorous, chlorophyll a, and turbidity),

hydrologic connectivity to a permanent stream (binomial), upland buffer coverage, and proportion of watershed composed of cropped land use. Covariates included aquatic plant diversity, water chemistry, wetland size, and wetland depth.

G. lacustris and *H. azteca* occurred across a wide range of agricultural intensities, but abundance related to some environmental predictor variables. In best-performing models, abundances of both species were negatively related to pyrethroid concentration but positively related to watershed-level crop abundance. *G. lacustris* was also negatively associated with connectivity to stream systems, and positively associated with wetland turbid state.

To explore factors related to pyrethroid presence in wetlands, we also created logistic regression models of pyrethroid occurrence as a function of landscape-level factors: proportions of crop, forest, and grassland land cover types, and buffer coverage. In our best-performing model, probability of pyrethroid occurrence was negatively related to buffer coverage and forested land cover.

Associations between waterfowl occurrence/abundance and amphipod occurrence/abundance

We surveyed amphipods and spring waterfowl in 85 wetlands ($n = 48$ in 2019, $n=18$ in 2020, and $n=19$ in 2021). The most commonly occurring waterfowl species was Canada geese, followed by mallards, blue-winged teal, scaup, and ring-necked ducks (Table 3). Amphipod densities ranged from 0 to 2370 amphipods/m³ water.

Wetlands containing scaup appeared to have higher amphipod density than those without (Figure 6a; 75% of wetlands with scaup having densities greater than 41 amphipods/m³), and this pattern will be formally analyzed for significance. The distribution of amphipod densities in basins with and without mallards were more similar (Figure 6b) but will also be analyzed.

We surveyed waterfowl broods in 65 wetlands in 2019 and 2020. In preliminary data exploration, waterfowl broods did not show obvious patterns associated with amphipods (Fig. 7). However, this pattern will be reassessed with basin size and shape as an additional predictor.

Objective 3:

Condition of amphipods prior to release

Most *G. lacustris* were actively swimming and appeared alive and uninjured while in coolers immediately prior to release on the stocked basin. In pre-release spot checks ($n = 22$ samples from coolers at 7 stocked basins), we observed a mean mortality rate of 2. Less than 1% were alive but visibly injured. Approximately 25% were paired in precopulatory amplexus.

Amphipod abundance

We conducted a total of 56 amphipod surveys at the site-level (19 pre-stocking surveys and 37 post-stocking surveys; Table 2). Post-stocking, we detected *G. lacustris* in stocked basins at only 1 of the 19 sites. On average across all sites, the stocked-control difference values were 0.44 more *G. lacustris**m⁻³ after stocking (85% confidence interval: 0.07 to 0.93 *G. lacustris**m⁻³). Though amphipod density was statistically higher after stocking ($t = 1.84$, $df = 27.6$, $p = 0.08$, Cohen's $D = 0.57$, moderate treatment effect size), this result was driven by the single site where post-stocking *G. lacustris* were detected (Site 3, Quistorff WMA; Fig. 8; Table 2). The one-way ANOVA of post-stocking samples indicated no significant difference in *G. lacustris* density among the first, second-, and third-years following stocking ($F_{2,34} = 1.81$, $p = 0.18$).

At the site where *G. lacustris* were established (Site 3, Quistorff WMA, which contained two stocked basins and one control basin), no *G. lacustris* were detected prior to stocking in any

basin. After stocking, the two stocked basins reached maximum *G. lacustris* densities of 35.2 m⁻³ (31.7 m⁻²) and 2.5 m⁻³ (2.1 m⁻²), while *G. lacustris* remained absent in the control.

Most (17 of 19) sites did not contain *G. lacustris* prior to stocking, per the study design (Fig. 8; Table 2). At two sites (Sites 15 and 18), *G. lacustris* were not visually detected in the sweep nets during pre-stocking field sampling but were later detected in under microscopy at low densities (<20 m⁻³). (Fig. 8; Table 2). Despite being present in the pre-stocking samples, *G. lacustris* were not detected post-stocking.

We found two additional amphipod taxa (*H. azteca* and *Crangonyx* spp.) in the study basins during pre- and post-stocking periods. *Hyalella azteca* was present in 95% of basins (0-1,500 individuals/m³). Fourteen (35%) basins contained *Crangonyx* spp. (0-605 individuals/m³).

At the wetland where we opportunistically monitored *G. lacustris* post-stocking using an underwater camera, we saw *G. lacustris* in high numbers on the underside of the surface ice, with smaller numbers swimming in the water column and near the wetland bottom, throughout the first week following translocation. We found small numbers of *G. lacustris* at other augur holes. We saw declining numbers of amphipods in weeks 2-5 of monitoring. There was no evidence of extensive amphipod mortality in the videos or in winter dip net samples, but our methods cannot rule out this possibility.

Characteristics of stocked wetlands

We surveyed fish on 14 stocked and 11 control wetlands in 2019 and 2020. The most common species found were black bullhead, fathead minnows, and dace spp. (*Chrosomus* spp., grouped due to difficulty of differentiating in the field), followed by central mudminnows and brook sticklebacks (*Umbra limi*). Fish abundance ranged widely, from fishless to >1000 fish. Among stocked basins, the Site 3 basins where *G. lacustris* were found post-stocking were fishless, as was an additional basin (“Swan”, Site 13, Anderson WPA) where *G. lacustris* were not found post-stocking.

We surveyed 12 stocked wetlands for aquatic vegetation. (Because aquatic vegetation surveys did not begin until 2020, when COVID-19 travel restrictions limited our sampling ability, we focused on surveying stocked wetlands rather than controls.) Commonly occurring species were coontail (*Ceratophyllum demersum*), muskgrass (*Chara* spp.).

In a preliminary analysis, Taylor (unpublished) compared aquatic vegetation of stocked wetlands to aquatic vegetation of 37 wetlands with naturally occurring (not stocked) populations of *G. lacustris* that were surveyed as part of Objective 1. Floating vegetation coverage (proportion of sample points with floating vegetation) was variable in stocked wetlands (average 0.43% ± standard deviation 0.46), but was often higher than in wetlands with naturally occurring *G. lacustris* (average 0.05 ± 0.09). Submerged aquatic vegetation coverage was more similar between stocked wetlands (0.99 ± 0.02) and wetlands with naturally occurring *G. lacustris* (0.84 ± 0.24). However, some species-level differences existed between the two groups. For example, star duckweed occurred in 8% of stocked basins but 62% of basins with naturally occurring *G. lacustris* populations.

Waterfowl counts have not been analyzed in a BACI context, given the minimal difference in amphipod density (the hypothesized driver of differences in scaup and other waterfowl use) between stocked and control wetlands.

DISCUSSION

Objectives 1 and 2: Habitat characteristics and waterfowl associations of prairie

pothole amphipods

Our study revealed associations between amphipod abundance and several habitat characteristics, with implications for management actions that could be taken to support populations of amphipods in prairie pothole wetlands.

Our results suggest that diverse SAV communities with at least moderately abundant biomass benefit amphipods in prairie pothole wetlands. Both *G. lacustris* and *H. azteca* abundances were positively related to aquatic plant diversity, as predicted (Larson et al. 2022). *H. azteca* abundance was also positively related aquatic vegetation biomass (maximized at moderately high biomass levels). *G. lacustris* abundance was negatively related to SAV biomass; however, this statistical pattern may have occurred because *G. lacustris* were also more abundant in deeper wetlands, which have (proportionally) more area lacking plants due to low light levels. Macrophytes may directly support amphipods (providing food, cover, or other ecosystem benefits), or macrophyte communities and amphipods may benefit from similar habitat conditions (such as water quality).

Contrary to our hypothesis, neither amphipod species displayed increased abundance with increased prevalence of emergent vegetation around shorelines. However, confounding variables related to water quality and probability of fish occurrence may have influenced this result. The most common taxon of emergent vegetation in our study was cattail (*Typha* spp.), likely the widespread hybrid *Typha x. glauca*. *Typha x. glauca* is particularly likely to dominate other aquatic plant species in wetlands impacted by nutrient loading and consolidation drainage. Nutrient loading may shift wetlands toward a turbid state, which we found to be negatively related to amphipod abundance (especially *H. azteca*) (Keith 2021). Consolidation drainage increases wetland potential for fish invasion by increasing wetland connectivity, and we found a negative relationship between abundance of both amphipods species and several fish species (Carleen 2022).

In particular, both *G. lacustris* and *H. azteca* abundance were strongly, negatively related to black bullhead abundance. Black bullheads are benthivores that may directly predate on amphipods or affect them indirectly via feeding activities that stir up bottom sediment, increase water turbidity, decrease macrophyte abundance and diversity. This result aligns with previous work finding negative relationships between prairie pothole amphipods and other benthivorous fish species (Anteau et al. 2011), and with our finding of a negative relationship between amphipod abundance and aquatic macrophyte diversity. We also found a negative relationship between amphipod abundance and brook stickleback abundance, likely due to predation. This pattern has not been documented previously for brook stickleback, which tend to occur at lower abundances than black bullheads or fathead minnows in the PPR, but readily colonize prairie potholes (Carleen 2022).

We found that the more prevalent fathead minnow had a negative relationship with *G. lacustris* abundance when fathead minnows occurred at high abundances (quadratic relationship). Anteau (Anteau et al. 2011) found a similar pattern in prairie pothole wetlands. Fathead minnows may directly predate on amphipods and/or competing with them for detrital food resources (Carleen 2022). *H. azteca* showed a positive relationship with fathead minnow abundance, but the effect was weak.

Carleen (2022) removed two basins from statistical analysis as outliers. These basins had high densities of both fathead minnows and *G. lacustris*, contrary to the pattern seen in the other 57 basins. Other researchers have observed similar, rare outliers in other areas of the PPR (M. Anteau, US Geological Survey, personal communication). These basins may be in a transition state (increasing fathead minnow population and with decreasing *G. lacustris* population to follow in the near future) or contain a mitigating environmental factor allowing the two species to

co-exist in high abundance. A mitigating factor was not apparent in our data, but research focusing on a larger sample size of such anomalous basins might produce information useful for mitigating the impacts of fish invasions on *G. lacustris* (Carleen 2022).

In addition to fish abundance, amphipod species were negatively related to pyrethroid concentrations. Pyrethroids concentrations detected in this study were lower than lab-derived lethal concentrations (LC₅₀) for *H. azteca*, but our results align with mesocosm experiments that showed declining amphipod abundance at low pyrethroid concentration (Hua and Relyea 2019). Low concentrations may impact amphipods via sublethal effects and/or changes to food webs (Hua and Relyea 2019, Keith 2021). Alternatively, pyrethroid values measured in this study may be the remains of earlier, higher-concentrations pulse inputs that had started to break down before our sampling period. Pyrethroid levels may also be indicators of other types of agricultural disturbance with potential to impact amphipods in the area surrounding the wetland (e.g., other types of pesticides, nutrient and sediment run-off).

In our associated exploration of pyrethroid occurrence across the landscape, we found that pyrethroid occurrence was negatively related to buffer strip coverage (proportion of each wetland's shoreline with an upland vegetation buffer strip greater than 30 m wide) and abundance of forested landcover in the wetland's watershed. Buffer strips are particularly effective at removing hydrophobic compounds like pyrethroids from runoff (Zhang et al. 2010). Combined with our finding that amphipod abundance is negatively associated with pyrethroid concentrations, the negative relationship between pyrethroid occurrence and buffer strip coverage suggests that amphipods may benefit from presence of buffer strips 30 m wide. Forested landcover was negatively correlated with cropped landcover and may reflect reduced pesticide use in the watershed.

Contrary to our hypotheses, abundance of both amphipod species was positively related to proportion of cropped landcover in the wetland watershed. Previous studies have found positive, or no, relationship between aquatic invertebrate metrics and land use surrounding wetlands. Janke et al. (2019) proposed that productive wetlands (more likely to support higher populations of invertebrates like amphipods) are more likely to occur in more productive local areas of the landscape, which are in turn more likely to be farmed. This confounding factor may explain the positive relationship. It is also possible that amphipod abundance in the Minnesota PPR has been influenced by historical land use not captured in our study (Hanson et al. 2012).

With regard to waterfowl associations, our preliminary data exploration suggests a positive association between scaup occurrence and amphipod density, as predicted. This pattern will be analyzed formally via generalized linear modeling of abundance and occupancy modeling of presence/absence.

Though scaup were only detected in only a single basin lacking amphipods, numerous basins containing amphipods were not used by scaup. Basin size (open water area) has been found to influence probability of scaup wetland use in previous work (Anteau and Afton 2009b) and will be incorporated into further analysis of occupancy and abundance for scaup.

Mallard pairs and waterfowl broods did not show an obvious association with amphipod densities but will also be further analyzed with metrics of wetland size and shoreline as covariates. In addition to our two focal species, Canada geese, blue-winged teal, and ring-necked ducks were detected in a large enough number of wetlands to warrant further analysis.

Together, our results suggest that amphipods in Minnesota's prairie potholes would benefit from management actions to increase aquatic plant diversity, remove fish, prevent fish invasion of remaining amphipod-rich basins, and reduce disturbances related to intensive agriculture. Such management to increase amphipod populations on the landscape will likely improve habitat for

migrating scaup. Actions might include drawdowns (benefiting aquatic plants and removing fish), removal of hydrological connections, installation of fish barriers, piscicide treatments, maintenance of riparian buffer zones of non-cultivated vegetation, and changing agricultural practices to reduce the need for pyrethroid use.

Objective 3: Efficacy of amphipod stocking

In our study, *G. lacustris* stocking had limited success (as measured within 4 years post-stocking). *G. lacustris* established at 5% of sites (1 of 19 sites, or 2 of 22 recipient basins). Accordingly, *G. lacustris* densities did not increase significantly through time in the first 3 years following translocation, though *G. lacustris* did increase through time in the single basin where they were detected twice post-stocking. The overall change in *G. lacustris* density across the landscape ($< 1 G. lacustris \cdot m^{-3}$) is not likely to be pertinent to wildlife, though the maximum *G. lacustris* density reached at Site 3 ($35.2 m^{-3}$) may provide wildlife forage. Amphipod densities greater than approximately $26 m^{-3}$ promote efficient feeding by lesser scaup (Anteau and Afton 2009b).

It is unclear whether *G. lacustris* establishment was limited by impacts of the translocation process on survival and reproduction. Most (>98%) of *G. lacustris* were alive without visible injuries immediately prior to release, suggesting that translocation methods did not have a negative impact on amphipods. Opportunistic video monitoring and sweep net sampling did not reveal evidence of mass mortality immediately following stocking ($n=1$ wetland), and declining amphipod abundance in videos may have been the result of dispersal throughout the wetland. However, our monitoring methods could not rule out the possibility of widespread mortality, and we did not assess for non-lethal stress that could impact reproductive rates or long-term survival of translocated individuals.

Given the high survival rates of stocked amphipods from collection to release, it may be more likely that habitat characteristics of stocked wetlands limited *G. lacustris* establishment. Given the variation in aquatic plant and fish communities in stocked wetlands, different factors may have been limiting at different sites. For example, six of the fourteen wetlands sampled for fish contained black bullhead, with three sites (Sites 5, 11, and 15; “School”, “Polk”, and “Klondike”) having especially high abundance. Carleen (2022) found a particularly strong negative relationship between *G. lacustris* and black bullheads as part of Objective 1. Similarly, nine wetlands contained fathead minnows and/or brook stickleback, which had a negative relationship with *G. lacustris* abundance in Objective 1 work (Carleen 2022). Among basins surveyed for aquatic vegetation, few were found to contain star duckweed, an aquatic plant species found to be positively associated with *G. lacustris* in Objective 1 work (Larson et al. 2022). Additionally, floating vegetation coverage on six stocked wetlands was higher than that typically found Objective 1 wetlands with naturally occurring *G. lacustris* populations. Differences in aquatic vegetation may directly impact stocked *G. lacustris*, or signal other aspects of wetland conditions with a negative influence of establishment.

We did not conduct extensive preliminary surveys of candidate wetland characteristics prior to stocking for logistical reasons (time, expense, need to stock many basins for statistical replication). Thus, our experiment tested a methodology that would minimize time and expense for managers. However, our limited rate of amphipod establishment (5% of sites) suggests that surveys of wetland characteristics may be needed to achieve moderate or high rates of population establishment. A model relating amphipod density to wetland characteristics (based on Objective 1 work and potentially other prior research) could be useful in selecting basins for stocking. Wetlands for which a model predicts high *G. lacustris* abundance, but *G. lacustris* are not present, may be good candidates for stocking. Combining information from prior research

into a single model based on the fewest, most influential variables would help minimize the number of types of preliminary surveys needed.

Future research projects could also use a modeling approach to target experimental stocking basins, with the goal of minimizing habitat limitations and assessing for any negative impacts of the stocking process on amphipod survival and reproduction. Researchers should also consider increased sampling frequency post-stocking, beginning immediately after stocking with underwater video cameras and continuing with dip net sampling as soon as possible after ice-out. Our first post-stocking samples were usually collected nine months after stocking (September-October). At this time, parental *G. lacustris* in the PPR have mostly ended their annual lifespan, and the population is composed primarily of new recruits (Menon 1966, Biette 1969). Our timing was intended to assess comparative amphipod densities after a complete reproductive cycle, but this sampling regime left us unable to disentangle parental mortality from reproductive rates. Examination of females sampled in winter and early spring for occurrence of eggs and young in brood pouches (Biette 1969, Menon 1969, Wilhelm and Schindler 2000) could also yield useful information. Substantial decline in adult density prior to that observed in naturally occurring populations (e.g., mid-July; Menon 1966, Biette 1969) could indicate predation on adult *G. lacustris* or resource limitation, whereas widespread occurrence of unfertilized or malformed eggs or dead broods may indicate sublethal physiological stress in amphipods, potentially due to environmental conditions (Wiklund and Sundelin 2001) or translocation. High juvenile mortality could suggest predators that select smaller prey, including predation by adult *Gammarus* (Macneil et al. 1999).

Finally, we note that wetlands that have recently been restored or enhanced may be good candidates for *G. lacustris* stocking. Our work was partially inspired by an earlier stocking attempt (not part of this study) conducted by the MN DNR in a shallow lake in the Sauk Rapids Area (Smith Lake, Cokato, MN), where *G. lacustris* were detected post-stocking in multiple years. A key difference from our experimental wetlands is that *G. lacustris* were translocated into Smith Lake following enhancement activities (drawdown and installation of fish barriers). Though this example is only a single case, stocking recently restored basins may be promising. Future research could examine a group of paired, stocked and unstocked, restored wetlands to assess whether stocking increases *G. lacustris* colonization rate in relation to unstocked, restored wetlands.

In summary, our results suggest that amphipod stocking using the methods employed in this study (minimal preliminary surveys) result in a low rate of amphipod population establishment. However, stocking of recently restored wetlands, or a model-driven approach to selecting candidate wetlands for stocking, may provide a path forward, especially if paired with management actions suggested by Objective 1 to increase amphipod habitat availability and quality across the landscape.

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Table 1. Social groups recorded by observers during spring and summer waterfowl surveys in Minnesota prairie pothole wetlands (2019-2021).

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	Social group cannot be classified, because, e.g., species or sex cannot be identified

¹One female associated with two males was considered a pair and a lone male.

Table 2. Average *Gammarus lacustris* densities (individuals/m³) from ice augur (January 2018) and sweep net (all other timepoints) surveys in control and *G. lacustris*-stocked basins in the first three years of a before-after/control-impact study of amphipod stocking. Groups of basins ("cohorts") entered the study in 3 successive years. Each basin received one pre-stocking survey, *G. lacustris* stocking, and then 1-3 annual post-stocking surveys. *G. lacustris* were stocked into Cohort 1 in winter 2017-18, into Cohort 2 in winter 2018-19, and into Cohort 3 in 2019-20. Numbers followed by (B) indicate pre-stocking samples; all other samples are post-stocking. Empty cells indicate no amphipod sampling because basins had not yet entered the study. Values in units of *G. lacustris* per m² (horizontal area) are shown in brackets [] for non-zero values for comparison to estimated release rates (2.1-15.7 *G. lacustris**m⁻²).

Basin Cohort	Site ID	Site ownership	Treatment	Jan. 2018	Fall 2018	Fall 2019	Fall 2020
1	1	private	stocked ^f	0 (B)	0	0	N/A ^a
1	1	private	control ^f	0 (B)	0	0	N/A ^a
1	2	Old Red Lake Trail WMA	stocked ^{b,c}	0 (B)	0	0	0
1	2	Old Red Lake Trail WMA	stocked ^{b,c}	0 (B)	0	0	0
1	2	Old Red Lake Trail WMA	stocked ^{b,c}	0 (B)	0	0	0
1	2	Old Red Lake Trail WMA	control	0 (B)	0	0	0
1	3	Quistorff WMA	stocked ^b	0 (B)	4.4 [4.8]	0	35.2 [31.7]
1	3	Quistorff WMA	stocked ^b	0 (B)	0	2.5 [2.1]	0
1	3	Quistorff WMA	control	0 (B)	0	0	0
1	4	Randall WPA	stocked	0 (B)	0	0	0
1	4	Randall WPA	control	0 (B)	0	0	0
1	5	public access	stocked	0 (B)	0	0	0
1	5	public access	control	0 (B)	0	0	0
1	6	St. Olaf WMA	stocked	0 (B)	0	0	0
1	6	St. Olaf WMA	control	0 (B)	0	0	0
1	7	Succonix WMA	stocked	0 (B)	0	0	0
1	7	Succonix WMA	control	0 (B)	0	0	0
2	8	private	stocked		0 (B)	0	0
2	8	private	control		0 (B)	0	0

Basin Cohort	Site ID	Site ownership	Treatment	Jan. 2018	Fall 2018	Fall 2019	Fall 2020
2	9	private	stocked		0 (B)	0	0
2	9	private	control		0 (B)	0	0
2	10	Erskine Complex WPA	stocked		0 (B)	0	0
2	10	Erskine Complex WPA	control		0 (B)	0	0
2	11	private	stocked		0 (B)	0	0
2	11	private	control		0 (B)	0	0
2	12	private	stocked		0 (B)	0	0
2	12	private	control		0 (B)	0	0
3	13	Anderson WPA	stocked ^g			0 (B)	0
3	13	Anderson WPA	control ^g			0 (B)	1.1 [1.2]
3	14	private	stocked ^e			0 (B)	0
3	14	private	control ^e			0 (B)	0
3	15	Dunham WPA	stocked			0 (B)	0
3	15	Erickson WPA	control			5.9 (B) [4.5]	0
3	16	Gustafson WPA	stocked			0 (B)	0
3	16	Hagen WPA	control			0 (B)	0
3	17	Hubbel WMA	stocked ^e			0 (B)	0
3	17	Hubbel WMA	control ^e			0 (B)	0
3	18	Mee WPA	stocked ^f			0.9 (B) [1.1]	0
3	18	Mee WPA	control ^f			17.0 (B) [1.92]	0
3	19	private	stocked ^e			0 (B)	0
3	19	private	control ^e			0 (B)	0

^aSite 1 was not surveyed in 2020.

^bIn analyses, for sites with >1 stocked basin, *G. lacustris* densities were averaged across stocked basins to obtain a single value for comparison to the control basin in analyses

^cSite 2 was intended to contain two stocked and two control basins. An intended control basin accidentally stocked by field crews.

^dAn additional basin was stocked with amphipods at Site 3 and opportunistically included into the study.

^eNon-random selection of control versus stocked basin. Choice of control versus stocked basin was modified due to snowmobile access barriers between the basin and property access point.

^fNon-random selection of control versus stocked basin. Choice was modified to avoid releasing *G. lacustris* into basins where pre-existing *G. lacustris* were believed or known to exist.

^gNon-random selection of control versus stocked basin. *G. lacustris* were released into a basin with recent drawdown and fish barrier installation to increase the number of fishless stocked basins in the study.

Table 3. Number of study basins where waterfowl species were found in 2019-2021 spring waterfowl surveys.

Species	Number of wetlands
Canada Goose (<i>Branta canadensis</i>)	52
Mallard (<i>Anas platyrhynchos</i>)	50
Blue-winged Teal (<i>Spatula discors</i>)	39
Ring-necked Duck (<i>Aythya collaris</i>)	31
Scaup (<i>Aythya affinis</i> and <i>A. marila</i>)	31
Unknown ¹	29
Hooded Merganser (<i>Lophodytes cucullatus</i>)	23
Bufflehead (<i>Bucephala albeola</i>)	17
Trumpeter Swan (<i>Cygnus buccinator</i>)	14
Redhead (<i>Aythya americana</i>)	13
Canvasback (<i>Aythya valisineria</i>)	12
Northern Shoveler (<i>Spatula clypeata</i>)	6
Green-winged Teal (<i>Anas crecca</i>)	5
Gadwall (<i>Mareca strepera</i>)	4
Ruddy Duck (<i>Oxyura jamaicensis</i>)	4
Wood Duck (<i>Aix sponsa</i>)	4
American Wigeon (<i>Mareca americana</i>)	3
Common Goldeneye (<i>Bucephala clangula</i>)	3
Northern Pintail (<i>Anas acuta</i>)	3
Common Merganser (<i>Mergus merganser</i>)	2
Red-breasted Merganser (<i>Mergus serrator</i>)	2

¹Neither observer could identify to species

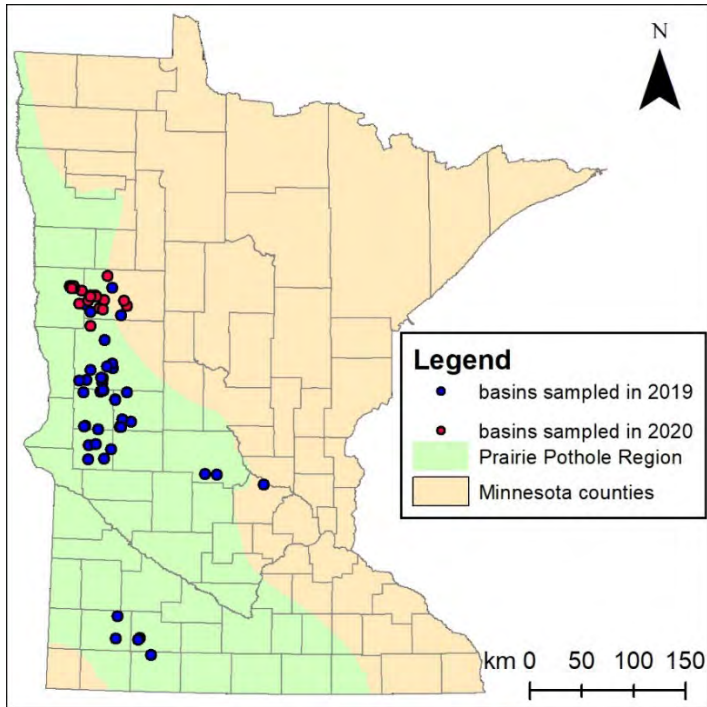


Figure 1. Basins surveyed for amphipod density, waterfowl, and habitat characteristics in spring-summer 2019 and 2020.

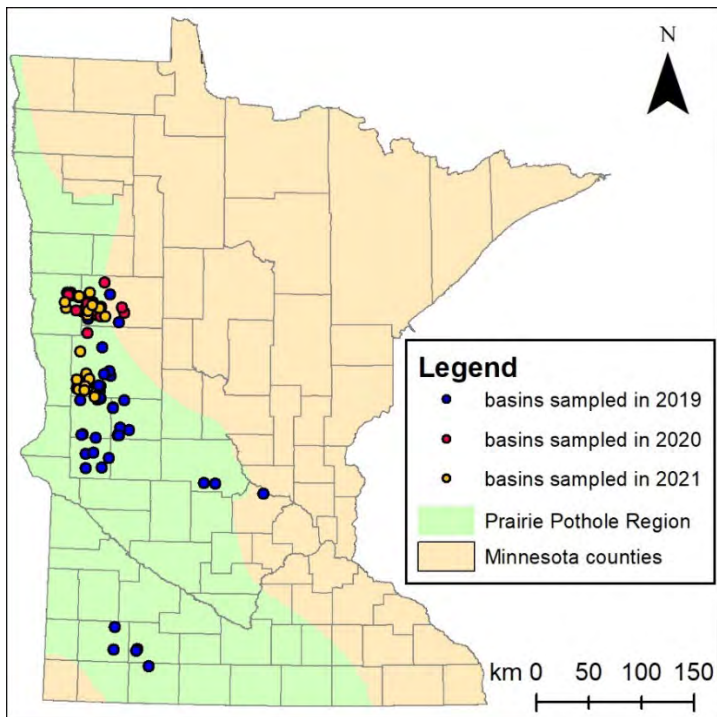


Figure 2. Basins surveyed for amphipod density and surveyed for waterfowl in 2019, 2020, and 2021. We surveyed migratory waterfowl (spring) in all three years and broods (summer) in 2019-2020 only.

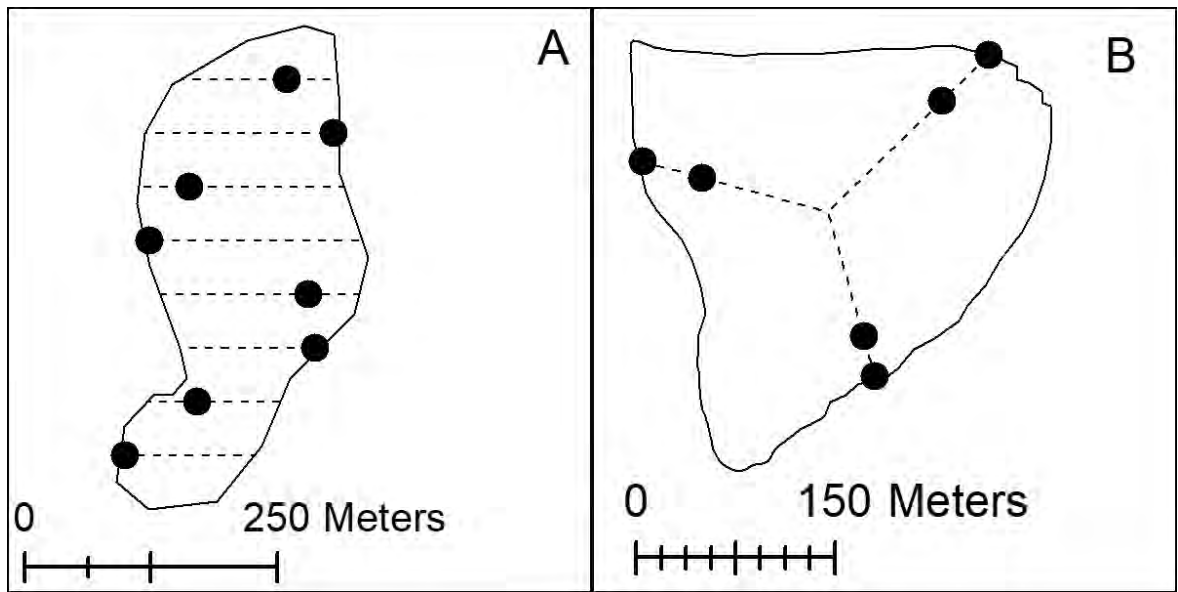


Figure 3. Amphipod dip-net sampling point arrangement for (A) Objectives 1 and 2, (B) Objective 3. Solid lines represent sample wetland outlines, dashed lines represent transects, and dots represent survey points.

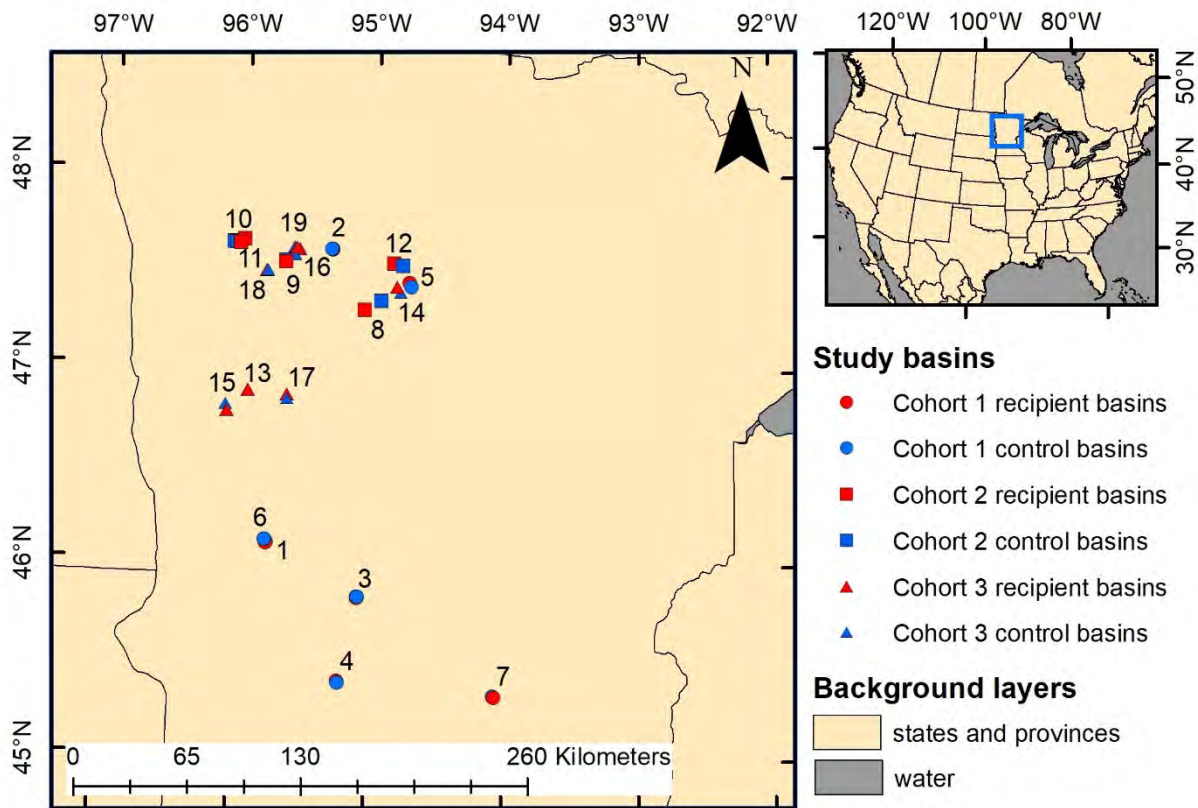


Figure 4. Map showing 19 study sites selected for a before-after/control-impact study of *Gammarus lacustris* stocking (conservation translocation) in western Minnesota, USA. Inset

map shows extent of larger map (blue rectangle). Each site includes one control basin and one to three *G. lacustris*-stocked basins. Basins entered the study in three “cohorts”. *G. lacustris* were translocated into cohort 1 in winter 2017-18, cohort 2 in winter 2018-19, and cohort 3 in winter 2019-20. Basins were surveyed for *G. lacustris* one time before stocking and up to four years following stocking.

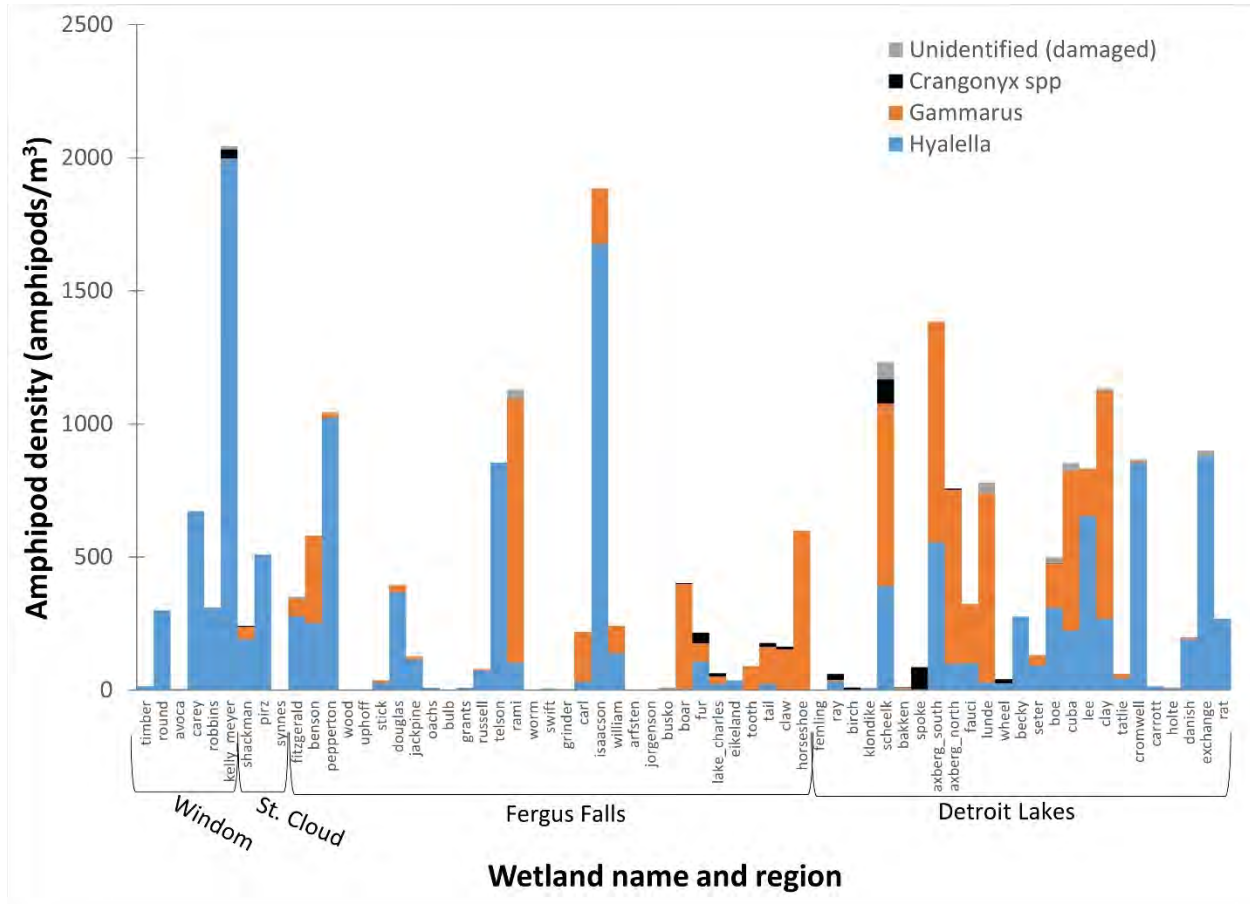


Figure 5. Amphipod density (amphipods per cubic meter of water) in Minnesota prairie pothole and forest transition zone wetlands sampled in 2019 and 2020. Densities of individual species (*Hyalella azteca*, *Gammarus lacustris*, and *Crangonyx* sp.) are indicated by colors within bars. Study basins were in four geographic clusters named for nearby population centers (Windom, St. Cloud, Fergus Falls, and Detroit Lakes). The basins are organized by latitude from south (left side of x-axis) to north (right side of x-axis).

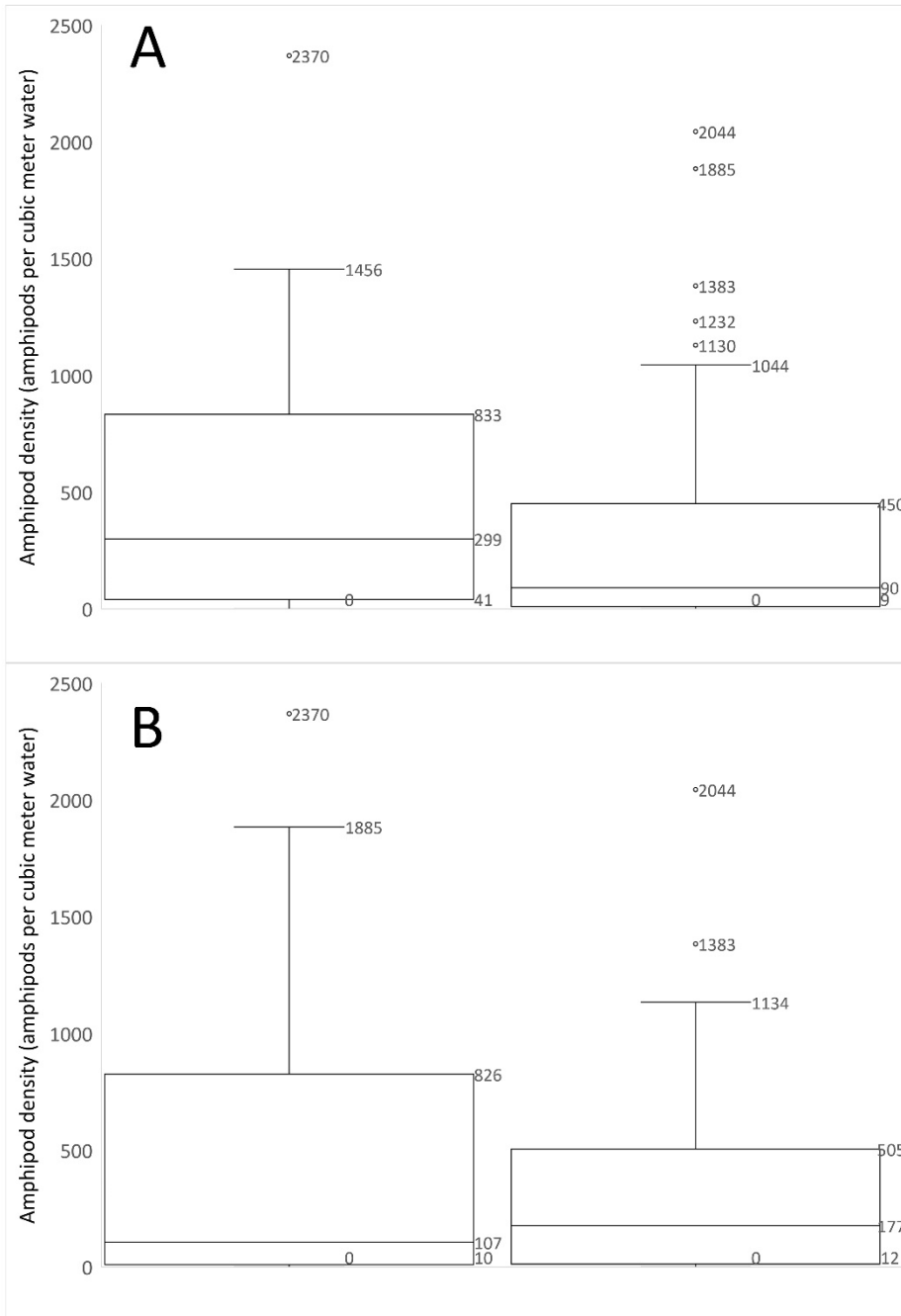


Figure 6. Box and whisker plots comparing densities of amphipods in wetlands where (A) scaup were and were not observed, and (B) mallards were and were not observed, in n=85 prairie pothole and forest transition zone wetlands in Minnesota. Outliers (values outside 1.5 times the interquartile range) are shown as points. The 25th, 50th, 75th, and local minima/maxima are labeled with amphipod density values.

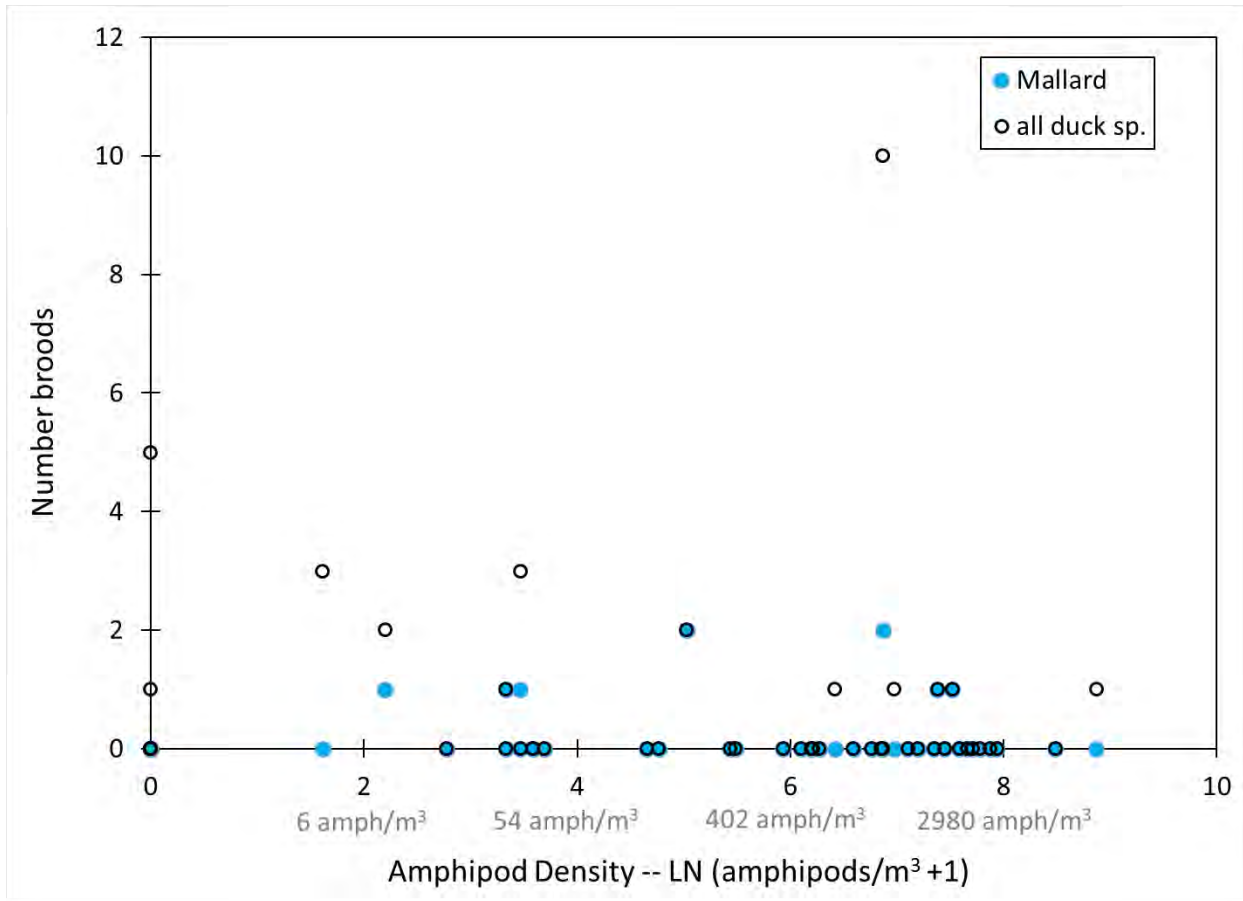


Figure 7. Number of waterfowl broods observed on prairie pothole and forest-transition zone wetlands of varying benthic-zone amphipod density in Minnesota in spring 2019. Mallard broods, a focal species, are highlighted with blue color. Amphipod densities are transformed to a log scale for visual clarity.

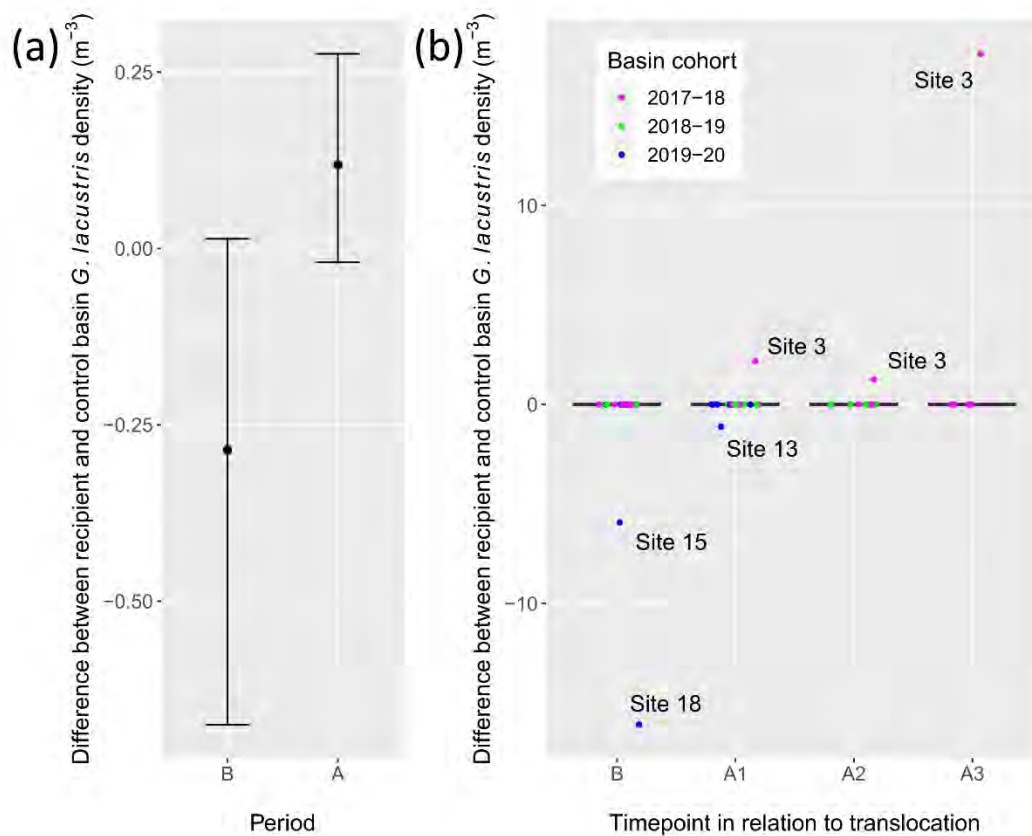


Figure 8. (a) Mean difference in *Gammarus lacustris* density between stocked and control basins (black dots; calculated as stocked minus control), with 85% confidence intervals, for the periods before (“B”) and after (“A”) *G. lacustris* stocking. Data were log-transformed to improve normality. Means and confidence intervals were back transformed for graphing. (b) Dot plot (x-jittered) showing difference in *G. lacustris* density between stocked and control basins at 19 sites through time before (“B”) and 1-3 years after (“A1”, “A2”, “A3”) stocking. The colored dots indicate “cohorts,” with cohort 1 stocked in winter 2017-18, cohort 2 stocked in winter 2018-19, and cohort 3 stocked in winter 2019-20. Black lines demarcate $y = 0$, where most points lie. Non-zero data points are labeled with site number.

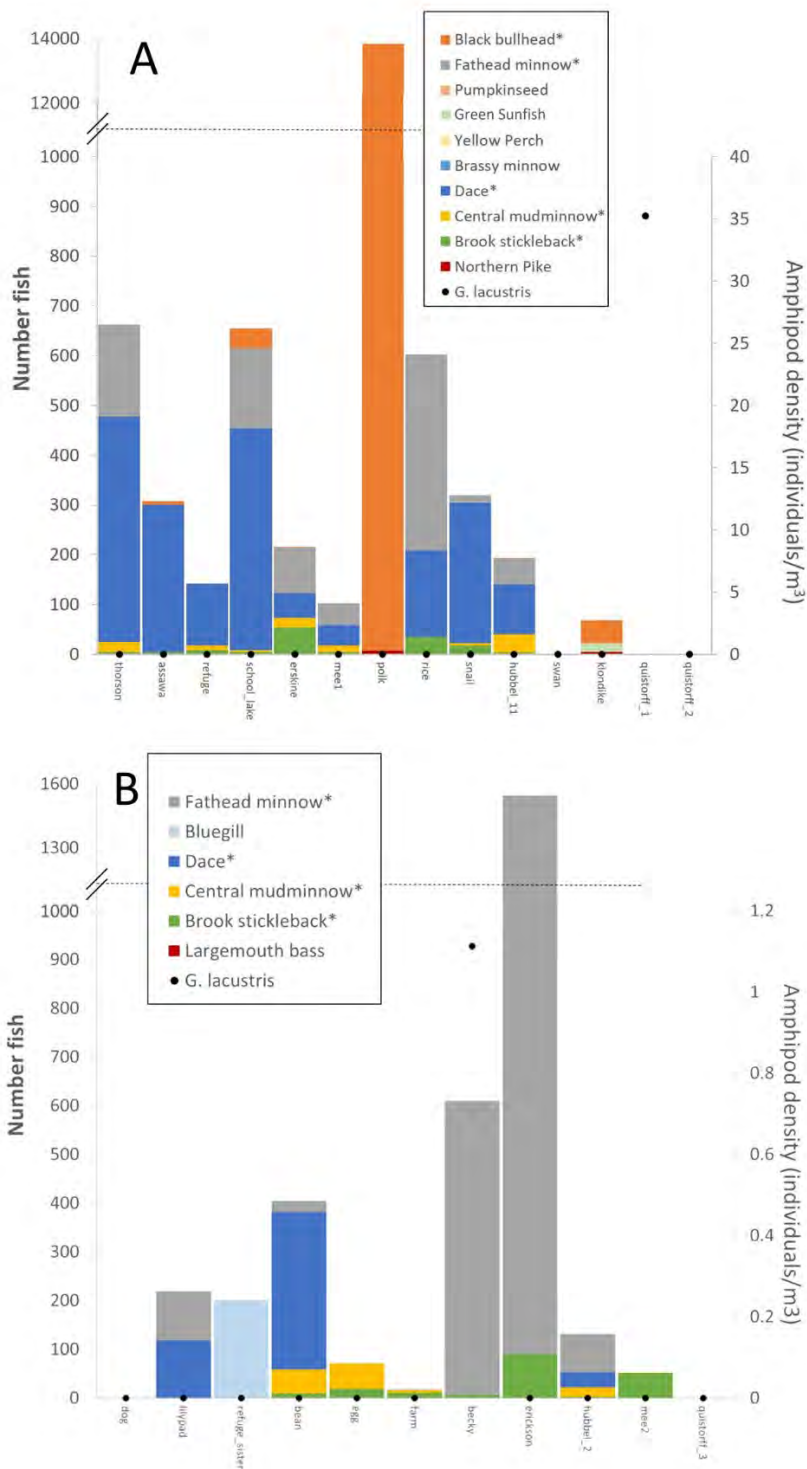


Figure 9. Counts of fish from fish surveys in (A) 14 stocked and (B) 11 control wetlands used in a BACI study of *G. lacustris* stocking. Fish were sampled in each wetland once post-stocking (summer 2019 or 2020) using three miniature fyke nets and one gill net deployed overnight (12-24 hours). *G. lacustris* density measured in dip net surveys in spring of 2020 are shown on a secondary axis.

Farmland Wildlife Populations and Research Group
35365 800th Avenue
Madelia, Minnesota 56062-9744
(507) 642-8478 Ext. 221



ASSESSING USE OF THERMAL INFRARED DRONES TO LOCATE AND CAPTURE WHITE-TAILED DEER FAWNS FOR MONITORING OF SURVIVAL AND CAUSES OF MORTALITY

Tyler R. Obermoller, Eric S. Michel, and Brian S. Haroldson

SUMMARY OF FINDINGS

Drones are growing in popularity and are used to locate individual animals, estimate populations, and monitor species such as rhinoceros, penguins, marine mammals, and chimpanzees. However, previous research has not used drones to locate individual wildlife with the intent of capture. Our goal was to assess the efficacy of using drones to locate and capture neonatal white-tailed deer (*Odocoileus virginianus*). During May–June 2021 and May 2022, we used a drone with a thermal-infrared and Red-Blue-Green (RGB) camera to locate and capture fawns in Wildlife Management Areas in Minnesota’s southern farmland region. We located and captured 75 and 82 neonatal fawns in 2021 and 2022, respectively. We flew the drone for 46.7 hours and covered 2,072.6 hectares (44.4 hectares per hour) in 2021, whereas we flew for 43.4 hours and 2,620.1 hectares (49.2 hectares per hour) in 2022. Our effort was 3.1 and 2.4 person-hours to capture each fawn in 2021 and 2022, respectively. In comparison to other common capture methods such as vaginal implant transmitters, ground searches, or doe behavior, using drones to locate and capture fawns was much more efficient and required fewer personnel. Although we found drones to be an efficient method, we recommend flying overnight or in cloudy conditions to avoid false positives.

INTRODUCTION

Neonatal survival is generally the most variable demographic parameter affecting population growth in ungulates (Gaillard et al. 2000). Understanding neonatal survival provides managers important information on annual recruitment and can help facilitate proactive management. White-tailed deer (*Odocoileus virginianus*) fawn survival rates, particularly during the first hunting season and winter, are largely unknown or are outdated in Minnesota (Brinkman et al. 2004, Grovenburg et al. 2011) but are used in the Minnesota Department of Natural Resources (MNDNR) annual deer population model (Michel and Giudice 2022). Using outdated vital rate information directly impacts model reliability which can affect subsequent management decisions for white-tailed deer in Minnesota (Michel and Giudice 2022). However, a major logistical challenge and financial constraint associated with monitoring juvenile survival rates is locating and marking young ungulates (White et al. 1972, Carstensen et al. 2003). If fawns can be located efficiently, marking and collaring requires minimal physical restraint and no chemical immobilization or capture traps (e.g., netted-cage traps, drop nets).

The most common neonatal fawn capture method in grassland regions is conducting opportunistic ground searches. Strategies include systematic searches through suspected fawn-rearing habitat or monitoring doe behavior as an indicator of fawn presence nearby (Downing and McGinnes 1969, Huegel et al. 1985, Carstensen et al. 2003, Grovenburg et al. 2011). Previous capture methods required numerous personnel, coordination of large search groups, and intensive search efforts. Pusateri (2003) reported 8-10 person-hours per fawn captured via ground searches in Michigan, compared to 5-214 person-hours per fawn using doe behavior (White et al. 1972, Carstensen et al. 2003, Huegel et al. 1985).

Using vaginal implant transmitters (VITs) is generally a more efficient means of capturing fawns compared to other methods. For example, capture success rates of fawns located from VITs implanted in dams was much greater (88% versus 15%) than using doe behavior to detect fawn presence (Carstensen et al. 2013). This increased efficiency reduced capture effort by up to 3.5 times that of using doe behavior (Carstensen et al. 2003). Similarly, Bishop et al. (2007) found capture success of mule deer (*O. hemionus*) fawns increased 57% when using VITs compared to using doe behavior. However, using VITs requires capturing adult females, which increases associated costs and can also result in unnecessary stress of adult females during capture.

Using thermal infrared (TIR) cameras can be efficient for identifying and subsequently capturing wildlife. One of the first wildlife studies to use TIR devices identified a polar bear (*Ursus maritimus*) and its tracks (Brooks 1972). Thermal imaging has been used to estimate populations of ungulate species such as moose (*Alces alces*; Millette et al. 2011), bison (*Bison bison*; Chrétien et al. 2015), elk (*Cervus canadensis*; Chrétien et al. 2015), and white-tailed deer (Croon et al. 1968, Wiggers and Beckerman 1993, Haroldson et al. 2003). Ditchkoff et al. (2005) also used TIR cameras mounted on the back of a 4-wheel drive vehicle to locate fawns. They conducted searches overnight to maximize the heat differential between fawn signatures and surrounding area. This technique required 3.3 person-hours per fawn encounter, 9.4 person-hours per fawn captured, and only 2–3 personnel to conduct the fieldwork. Although vehicle mounted TIR cameras are more effective than previous search methods, they are restricted to roads which ungulates typically avoid (Ward et al. 2004, Long et al. 2010, Anderson et al. 2013).

Drones used for civil applications offer new opportunities for wildlife managers (Shahbazi et al. 2014, Whitehead and Hugenholtz 2014). Recent studies show drones provide many advantages over traditional manned aerial surveys including lower disturbance and flight altitudes, higher quality images, and improved safety for pilots and biologists (Jones et al. 2006, Linchant et al. 2015, Christie et al. 2016). A TIR-equipped drone also has improved detection compared to ground-based TIR cameras because the aerial view minimizes obstruction from herbaceous ground cover, reducing search effort costs (Kissell and Nimmo 2011, Chrétien et al. 2015, 2016, Linchant et al. 2015, Christie et al. 2016, Witczuk et al. 2018). Recently, TIR-equipped drones were used to detect and count white-tailed deer in Quebec, Canada (Chrétien et al. 2016) and captive white-tailed deer in Alabama (Beaver et al. 2020). Thermal-infrared equipped drones were also used to detect roe deer (*Capreolus capreolus*) fawns in pastures to minimize mowing mortality (Israel 2011).

Neonatal fawn survival is affected by several factors, but predation is often the leading cause of mortality (Carstensen et al. 2009, Grovenburg et al. 2012, Severud et al. 2019). Coyote (*Canis latrans*) predation had the largest impact on fawn survival in the Northern Great Plains and South Dakota (Grovenburg et al. 2012). Additionally, fawn survival in south central Minnesota was last estimated almost 20 years ago and coyote track survey indices have increased 252% from 2000 to 2020 (J. Erb, MNDNR, pers. comm.). Therefore, the impact of coyote predation on fawn survival is outdated and has likely changed since it was last estimated. Other studies have reported additional sources of mortality including hunting, hypothermia, starvation, and vehicle collisions, and these may have important implications for population dynamics (Pojar and Bowden 2005, Burroughs et al. 2006, Kilgo et al. 2012).

Global Positioning System (GPS) collars are an essential tool to identify causes of mortality (Severud et al. 2015, Swanepoel et al. 2015) and also expand our understanding of animal movements (e.g., dispersals, migrations; (Nelson and Mech 1984, Purdon et al. 2018), resource use (Hebblewhite et al. 2005, Bista et al. 2023), behavior (Creel et al. 2008, Chimienti et al.

2021, Pokrovsky et al. 2021), and home range size (Nelson and Mech 1984, Körtner et al. 2015). The addition of expandable bands on GPS collars allows researchers to monitor animals with changing body size shortly after birth until important biological life events (e.g., dispersal, overwinter survival, recruitment; Gaillard et al. 1998, Severud et al. 2015, Gilbertson et al. 2022). Past design iterations for neonatal ungulates required heavy GPS packages to meet increased location fix rates, leading to premature band expansion via increased stress on the expandable bands (Obermoller et al. 2018). Poor collar designs (i.e., poor stitching strength and band material) can lead to a loss of data through censored animals (DelGiudice et al. 2002, Severud et al. 2019). Subsequently, we collaborated with researchers from the University of Georgia to test 3 expandable GPS collar models for their retention and influence on behavior of captive fawns (see Wesner et al. 2022 for details). We then used results from our pen study along with existing data from our 2019 pilot field study (Obermoller et al. 2020, Obermoller et al. 2021) to suggest design modifications to the collar companies. Our suggestions were for improved stitching patterns, smaller battery housings, improved weight distribution, and smaller band circumference. Although our pen study helped with initial assessment and refinement of collar designs, a critical next step is to field test these expandable GPS collars on wild neonatal fawns.

In 2019 and 2020, we conducted a feasibility study using TIR drones and determined this method was an efficient technique to locate fawns (see Obermoller et al. 2021) but using this technology for capture efforts was still largely unknown. Thus, we continued our research to determine the feasibility of using TIR drones to assist with capturing in 2021 and 2022. We acknowledge comparisons among fawn search and capture techniques can be confounded by a variety of factors; however, the intent of our research was to provide information about the efficacy, benefits, and challenges associated with the use of TIR drones to locate neonatal white-tailed deer for capture. Additionally, we evaluated expandable GPS collars by determining retention rates of 2021 and 2022 band designs and assessing the GPS collar function (e.g., fix success rate, mean linear error) in grassland and forested cover types. Fawn captures can be logistically and financially prohibitive yet are necessary to collect the data needed for a variety of research and monitoring objectives (e.g., survival rates, causes of mortality, habitat use and selection).

OBJECTIVES

1. Evaluate the efficiency of using TIR-equipped drones to locate neonates for capture.
2. Validate performance of Vectronic GPS radiocollars on free-ranging white-tailed deer fawns.
3. Identify cost-effective approaches for long-term and large-scale monitoring of fawn survival and recruitment in Minnesota's farmland regions.

METHODS

Study Site

Our 7,219 km² study area consists of 4 deer permit areas (DPAs; 252, 253, 296, 299) in south central Minnesota, USA (Figure 1). Recently, deer densities have averaged 8–21 deer per square mile across the 4 DPAs (Michel and Giudice 2022). Row crop agriculture, largely corn (*Zea mays*) and soybeans (*Glycine max*), are the most abundant cover type accounting for 71% of the area, with grasslands (12%), urban/developed (7%), wetlands (5%), forest (3%), and open water (2%) encompassing the remaining area (Rampi et al. 2016). Within the study area, we focused our drone flights on Wildlife Management Areas (WMAs) maintained by the MNDNR. Study WMAs consisted largely of wetlands (37%) and grasslands (34%), but also

agriculture (corn food plots; 12%), open water (9%), and forest (7%; MNDNR 2009). The most common graminoids included smooth brome (*Bromus inermis*), reed canary grass (*Phalaris arundinacea*), big bluestem (*Andropogon gerardii*), switchgrass (*Panicum virgatum*), and Indian grass (*Sorghastrum nutans*). Common forbs included goldenrod (*Solidago spp.*), sweet clover (*Melilotus spp.*), aster (*Symphotrichum spp.*), and milkweed (*Asclepias spp.*).

Data Collection

We contracted with a drone company (Fines Aerial Imaging, St. Cloud, MN, USA) to create flight paths (Figure 2) and fly their drone at each of our sites in 2021 and 2022. The drone contractor created a flight path for each WMA at 60-m altitude with a 10% overlap. We used a DJI Matrice 300 RTK drone with dual thermal-infrared (DJI H20T: 640 x 512 pixels 13.5 mm focal length, 30 Hz Advanced Radiometric Thermal Camera) and Red-Green-Blue (RGB) camera (DJI H20T: 1920 x 1080 pixels, 6.8-120 mm focal length). The drone had a single housing with both thermal and zoom camera capabilities and therefore allowed for seamless transitions between cameras while in flight. We used the TIR camera to locate thermal signatures and the RGB camera to confirm fawns (Figure 3). We connected the ground control system to a 50-cm screen (via HDMI) to allow more observers to monitor thermal signatures.

Pilots flew drones through the preprogrammed flight path until we detected a suspected fawn. The pilot would then pause the preprogrammed flight path and manually direct the drone over the suspected fawn. Next, the pilot switched to the RGB camera and confirmed identification by modifying the zoom-scale of the camera rather than adjusting flight altitude of the drone. This procedure minimized auditory disturbance and stress-flight behavior of the fawn. The drone contractor recorded georeferenced video footage and photo-documented fawning site characteristics. We recorded the following for each fawn detection: number of fawns, time, activity (e.g., lying, standing, moving), habitat type, doe presence, thermal obstruction (from vegetation), and the location (e.g., latitude and longitude). We found diurnal conditions rapidly reduced our ability to locate thermal signatures and therefore began performing early morning (0200 – 0600) flights. In diurnal and sunny conditions, groundcover quickly illuminated with the TIR camera and caused many false positives. We could not confirm a suspected heat signature as a fawn during nighttime conditions (e.g., no/low sunlight for RGB camera); therefore, we recorded the coordinates of the heat signature and attempted to confirm via drone after sunrise or with a ground search.

We fit Vectronic Vertex GPS radiocollars (with 3-axis accelerometers to identify body movement; Vectronic Aerospace GmbH, Berlin, Germany) to neonatal fawns to closely monitor survival, reduce our response time for mortality investigations, and increase our ability to confidently identify causes of mortality. We examined carcass characteristics to determine depredated versus scavenged and the predator type. We searched the immediate area for other evidence such as predator hair, tracks, or scat. We also collected scat and saliva swabs (swabbing predator bite wounds on fawns) to use in DNA analysis to assign specific causes of mortality to a specific predator species (Obermoller et al. 2019). If mortality cause was unknown and sufficient carcass remained, we delivered it to the University of Minnesota's Veterinary Diagnostics Laboratory (VDL) for necropsy. We calculated Kaplan-Meier survival using the KMSurv package in Program R (R Core Team 2022).

We also conducted a 1-week trial to assess the efficacy of the GPS package by estimating the mean linear error and fix-success of transmitting locations by habitat type starting on 16 July 2021. We placed 10 collars in 2 habitat types: grassland habitat (0% average canopy cover) and forested (deciduous) habitat (94% average canopy cover). We selected these habitats because they were frequently used by our GPS-collared neonates and provided an assessment of the

collars' function in two vastly different cover types. We used a handheld GPS unit (Garmin GPSMAP 64s) to record an averaged location-fix until confidence reached 100% at the specific focal point for each cover type. We then placed a neonate GPS collar on the ground at the focal location to simulate a bedded fawn and reduce the effect behavior may have on collar error (Bowman et al. 2000). Next, we placed 4 neonate collars 5 m, 10 m, 15 m, and 20 m east of the focal location, respectively. Starting 5 m north of the focal location, we then placed another parallel row of 5 collars 5 m north of the first row. We then calculated the exact easting and northing coordinates for each collar from the focal location (Obermoller et al. 2018). Finally, we estimated the location error from the Euclidean distance between location-fixes and the exact location of each individual collar's location. We programmed the collars to send 1 location per hour during the week-long trial. We did not assess the location error of the collars in 2022 due to the GPS-collar technology being identical between years. We calculated the mean linear error for each collar and then compared error by cover type. We used a simple linear regression to compare mean Euclidean location error by cover type and collar ID.

RESULTS

2021

We conducted drone flights for 46.7 hours and covered 2,072.6 hectares (44.4 hectares per hour) at 17 WMAs. We worked 233.5 total person-hours with a mean crew size of 5 people and required a mean 3.1 person-hours to capture each fawn. We GPS-collared 75 fawns (38 males, 37 females); 73 fawns were located using the drone and 2 fawns were found opportunistically. Captured fawns were 5.3 ± 2.3 (SD) days old (range 0-11 days, $n = 75$) and weighed 4.5 ± 1.0 kg (range = 2.7-7.1 kg, $n = 73$). The hind leg length was 27.0 ± 1.6 cm (range = 21.1-30.2 cm, $n = 74$). We captured 76% (57) of fawns in grasslands, 15% (11) in wetlands, 5% (4) in forested habitats, 3% (2) in roadsides, and 1% (1) in standing crops. The doe was present for 28% of fawn captures. We documented 26 mortalities: 17 coyote kills, 5 health-related, 2 vehicle collisions, and 1 accident. The 1- and 3-month (summer) survival were 85.6% and 78.1%, respectively (Figure 4). The 6-month and 1-year survival were 71.8% and 66.2%, respectively.

We found retention issues with the expandable band for 23 of 73 (32%) collars by 1 year of age: 9 (40%) premature collar expansion, 7 (30%) caught on fences, and 7 (30%) with expandable bands breaking (Figures 5A-5D). The mean number of days the collars were retained was 150 days (± 115 , range = 2-359 days, $n = 23$). Collar retention was 88% at 3 months of age (spring/summer survival), declining to 59% ($n = 28$) at 1 year of age.

The mean linear location error of the GPS collars was 12.7 m (95% CL = 11.1, 14.4, range = 10-17 m, $n = 10$) in forested cover and 5.1 m (95% CL = 4.2, 5.9, range = 4-7 m, $n = 10$) in grassland cover. We found GPS location error was significantly different by cover type ($F_{1,18} = 85.6$, $p \leq 0.001$) and collar ID ($F_{3,16} = 40.4$, $p = 0.02$).

2022

We conducted drone flights for 43.4 hours and covered 2,620.1 hectares (49.2 hectares per hour) at 21 WMAs. We worked 197.9 total person-hours with a mean crew size of 5 people and required a mean 2.4 person-hours to capture each fawn. We GPS-collared 82 fawns (49 males, 33 females); 81 fawns were located using the drone and 1 fawn was found opportunistically. Captured fawns were 3.3 ± 1.9 (SD) days old (range 0-10 days, $n = 82$) and weighed 4.3 ± 1.1 kg (range = 2.0-8.3 kg, $n = 82$). The hind leg length was 26.6 ± 1.8 cm (range = 21-31 cm, $n = 82$). We captured 54% (44) of fawns in grasslands, 28% (23) in wetlands, and 18% (15) in forested habitats. The doe was present for 32% of fawn captures. We documented 47 mortalities: 36 coyote kills, 4 health-related, 4 vehicle collisions, and 3 harvest-related. The 1-

and 3-month (summer) survival were 68.4% and 55.2.1%, respectively (Figure 4). The 6-month and 1-year survival were 47.3% and 31.4%, respectively.

We found retention issues with the expandable band for 22 of 81 (27%) collars with similar expandable band issues: 12 (55%) caught on fences, 8 (36%) expandable bands breaking, and 2 (9%) premature collar expansion (Figures 5A-5D). The mean number of days the collars were retained was 160 days (± 94 , range = 13-333 days, $n = 22$). Collar retention was 91% at 3 months of age (spring/summer survival), declining to 41% ($n = 10$) at 1 year of age.

DISCUSSION

We found using TIR-equipped drones was an effective method to locate and then capture neonatal fawns for GPS-collaring and subsequent survival monitoring. We also found our method was more efficient than using TIR with a vehicle because we were not limited to searching near roads, which deer avoid (Ward et al. 2004, Long et al. 2010, Anderson et al. 2013; Table 1). Additionally, ground searches require extensive personnel effort and likely have a lower fawn detection rate compared to our method. The cost of ground searches is difficult to estimate because of the unknown number of individuals required to conduct ground searches and the extensive pre-fieldwork coordination efforts conducted by personnel. Vaginal implant transmitters (VITs) are also less efficient because they require adult capture, followed by ground searches after parturition to locate the fawn within a given area (Table 1). When a VIT is expelled, personnel are notified and then a capture team is launched, whereas we located and captured fawns instantaneously. We acknowledge that we are capturing slightly older fawns compared to those captured via the use of VITs. Therefore, our survival estimates may be biased because mortalities occurring within the first days of life may be missed (Gilbert et al. 2014, Chitwood et al. 2017), although estimating fawn age and entering them into the survival analysis based on age will help reduce bias (Kautz et al. 2019). Additionally, VITs are the more costly search method because they require capture and collaring of adults and insertion of the VIT, all of which require more personnel, time, equipment, and supplies (Carstensen et al. 2003). Capturing and GPS-collaring of adult females is unwarranted unless specific female monitoring objectives are stated.

The greatest challenge we encountered was thermal loading to the environment during diurnal hours. We found the sun quickly heated the ground and reduced the temperature differential between the vegetation and fawn thermal signatures (Israel 2011, Chrétien et al. 2016, Beaver et al. 2020). Detection of thermal signatures was adequate immediately after sunrise because the sun was still low on the horizon. However, our ability to detect fawns deteriorated as the sun rose and detection of thermal signatures was no longer possible about 3 hours after sunrise. On overcast days, we were able to detect fawns throughout the day. We therefore moved our operation start times to early morning hours (0200 to 0900), which resolved the thermal loading issue and increased our flight time under good conditions but made fawn confirmations more difficult due to low light conditions.

Forest canopy cover reduced ground visibility because TIR radiation was unable to penetrate through the forest canopy (Witczuk et al. 2018, Beaver et al. 2020). We also found that trees held residual heat from the previous day, which made locating fawn thermal signatures in forested areas more difficult (Steen et al. 2012, Beaver et al. 2020). The drone method may bias captures to grassland and scrub-shrub wetland habitats; however, most fawn capture techniques are inherently biased (Bishop et al. 2007, Chitwood et al. 2017) and our goal was to select areas that would maximize efficiency to locate fawns with the drone. Additionally, WMAs in southern Minnesota are primarily comprised of grasslands and wetlands. Thus, our captures were focused on the primary habitat type available to does and their fawns in our study area.

We found low fawn disturbance with all fawns remaining bedded and only a few lifting their head in response to the drone. Linchant et al. (2015) reviewed several drone studies and found no disturbances reported during drone flights. Although not directly related, Christie et al. (2016) showed lower disturbances compared to other aerial methods; therefore, drones may be an advantageous method for minimizing disturbance to wildlife in general.

During our drone flights, we observed a multitude of other wildlife species, including: coyotes with pups, raccoons (*Procyon lotor*), muskrats (*Ondatra zibethicus*), mallards (*Anas platyrhynchos*), ring-necked pheasants (*Phasianus colchicus*), wild turkey (*Meleagris gallopavo*), and many small mammals and other bird species. Although we were able to detect the thermal signatures of passerine species, we did not record them because they were not comparable to a fawn signature (i.e., there was little chance of misidentifying the thermal signature of a passerine as a fawn's thermal signature). Drones show immense promise for the future of wildlife detection and estimation and, for ungulates, can provide a higher search efficiency compared to previous methods.

The 2021 collars did not entirely meet our expectations for collar fit and retention due to issues with premature expansion. But in 2022, we had fewer collars (2 versus 9) prematurely expanding (compared to 2021) and therefore a better fit on the fawns in 2022. We did not make any design changes to the expandable band folds or stitching between years, but we hypothesize the collar company's modification to the connection point (to include a two-point attachment to the package; Figures 5A and 5D) may have better dispersed the weight of the package and subsequently reduced pressure on the expandable loops. Unfortunately, one of the consequences of the modification was added pressure to the expandable band-GPS package, leading to increased issues with the expandable band breaking (Figures 5C and 5D). Fawns retained more collars throughout spring and summer in 2022 but we had continued issues with fawns retaining collars to 1-year of age (recruitment) and other important movement milestones (e.g., migration, dispersal; Beier 1995, Grovenburg et al. 2011a, Gilbertson et al. 2022). We also experienced an unavoidable issue of collars getting caught on fences (Figure 5B). Grovenburg et al (2014) reported similar issues in their South Dakota study area dominated by agriculture and livestock, with 93% of their prematurely shed collars being the result of fencing snags. Given the combination of heavily fragmented areas and adaptability of white-tailed deer to these environments, such retention issues could be an ongoing issue for studies using expandable collars (Grovenburg et al. 2014, Obermoller et al. 2018).

The use of expandable GPS collars on neonatal fawns has led to a much better understanding of fawn behavior and improved mortality response time and subsequently, improved accuracy to identify specific causes of mortality (Cristescu et al. 2022). Global Positioning System collars can also allow for the collection of activity data and fine-scale location data with vastly reduced monitoring efforts compared to VHF collars (Pellerin et al. 2008, Kochanny et al. 2009, Obermoller et al. 2018). Despite the benefits, researchers need realistic collar retention expectations and careful consideration of their research objectives when determining their sample size. We recommend researchers consider their specific ecological questions (e.g., summer survival, migration, winter habitat use) and account for potential retention issues (e.g., fences, thick cover) when determining their sample size. Collar retention considerations should include but are not limited to ungulate species, habitat type, and time frame of objectives. We also recommend continual communication with collar companies and fellow researchers regarding collar issues and potential modifications in future years. This iterative process is invaluable for future research to help meet the sample size requirements needed to properly address study objectives and gather time-sensitive ecological data for species management.

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Table 1. List of methods used to locate and capture deer fawns (*Odocoileus* spp.) across various studies. Also shown is the year of the study, sample size (N), and effort (person-hours) from each study.

Capture method	Year	N	Effort	Study
Drone w/ thermal imaging (2021)	2021	75	3.1	Obermoller et al. (this study)
Drone w/ thermal imaging (2022)	2022	82	2.4	Obermoller et al. (this study)
Vaginal implant transmitter (VIT)	2003 - 2004	83	7 - 16	Bishop et al. 2007
Vaginal implant transmitter (VIT)	2001	20	60	Carstensen et al. 2003
Doe behavior	1997 - 1999	25	145 - 214	Carstensen et al. 2003
Doe behavior	1980 -1983	58	14.5 - 43.8	Huegel et al. 1985
Vehicle w/ thermal imaging	2004	26	3.3 - 9.4	Ditchkoff et al. 2005
Ground searching	1994 - 1996	35	30.6	Ballard et al. 1998

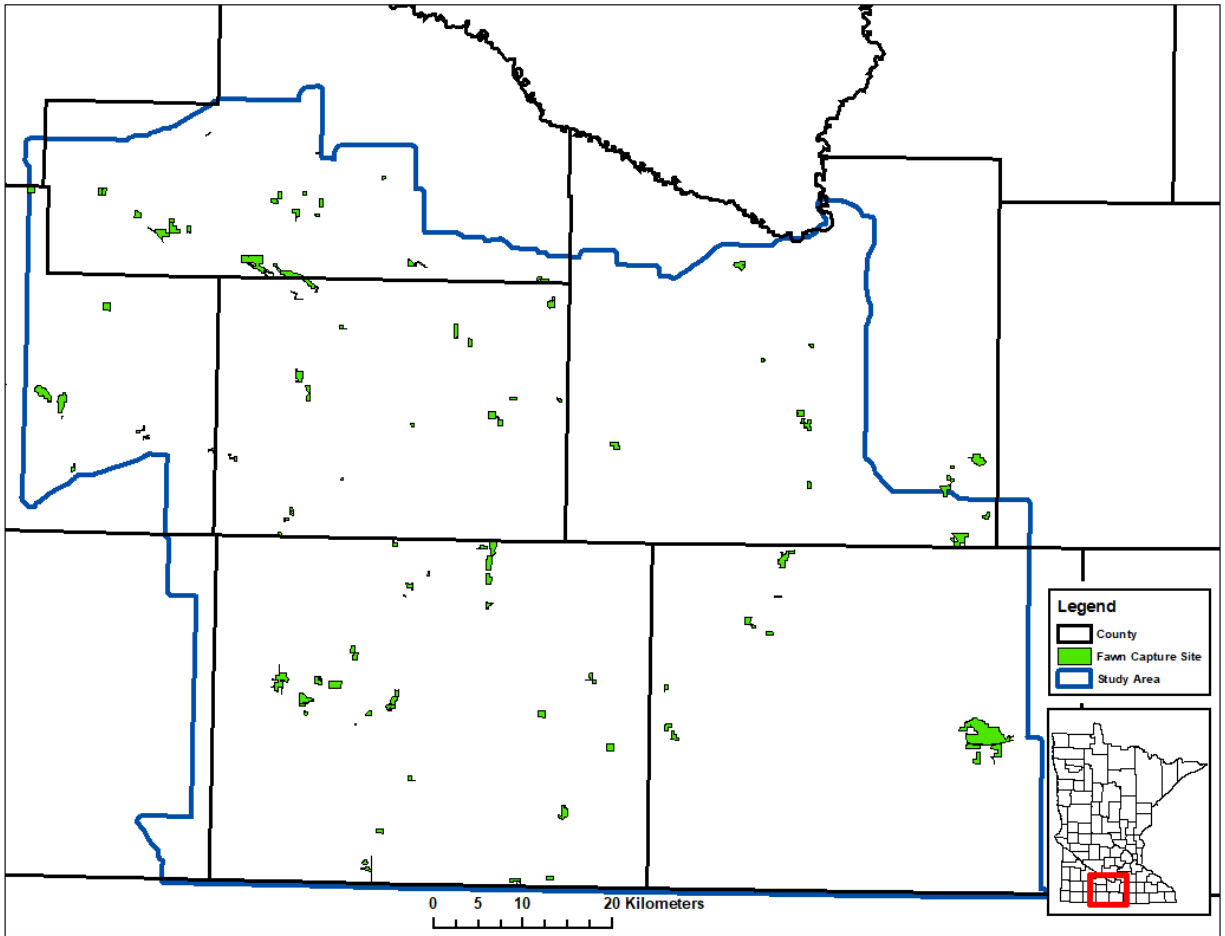


Figure 1. Study area including location of Wildlife Management Areas (green polygons) where white-tailed deer fawns were located and captured in south central Minnesota, USA during May-June 2021 and May 2022.



Figure 2. Flight path (yellow polylines) of the drone used to search for white-tailed deer fawns at Groebner Wildlife Management Area in south central Minnesota, USA, May-June 2021 and May 2022.

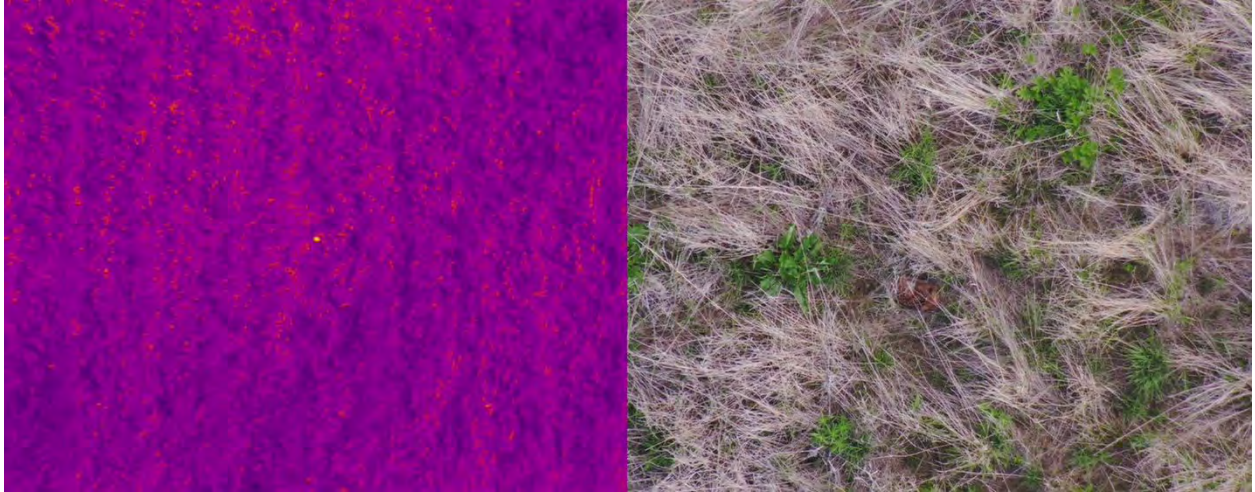


Figure 3. Thermal infrared image from a drone of a white-tailed deer fawn (left) and a zoomed-in image of the same fawn bedded in grass (right) in south central Minnesota, USA, May 2021.

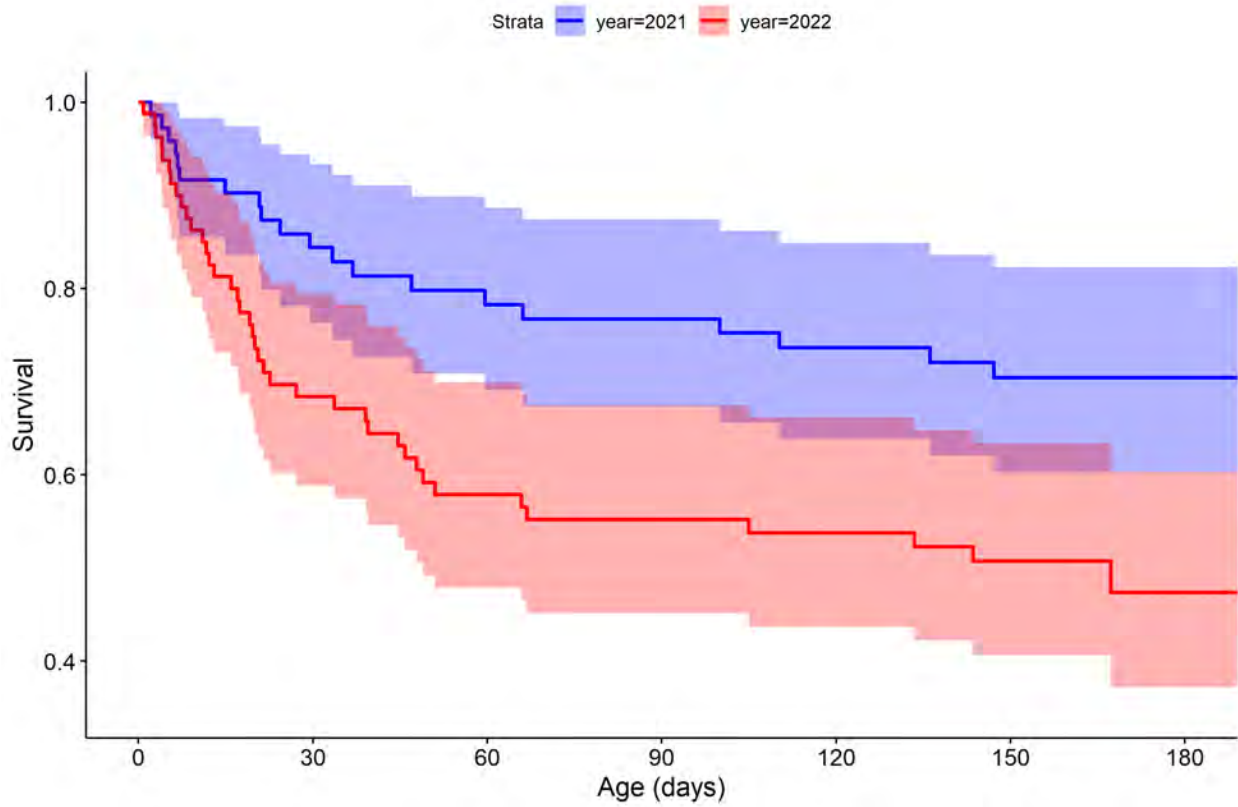


Figure 4. Cumulative daily survival (using Kaplan-Meier survival curve) of white-tailed deer fawns in south central Minnesota, USA, 2021 and 2022. Shaded areas represent 95% confidence intervals for the survival curves.



Figure 5. Vectronic Vertex MINI Globalstar expandable GPS collars deployed on white-tailed deer fawns in south central Minnesota, USA in 2021 and 2022. Panels depict: A) a collar that prematurely expanded, B) a collar caught on a fence, C) a collar initially caught on a fence that subsequently broke the expandable band, and D) a collar that got caught on vegetation and the expandable band broke.

Publications Lists

FY2023 Publications – Farmland Group

Gallman, C. W., T. W. Arnold, **E. S. Michel**, and J. D. Stafford. *In Review*. Evaluation of fall-seeded cover crops for grassland nesting waterfowl in eastern South Dakota. *Wildlife Society Bulletin*.

Goebel, K. M., **N. M. Davros**, P. J. Rice, and D. E. Andersen. *In Revision*. Effects of insecticide spray drift on arthropod prey resources of birds in grasslands in Minnesota. *To be resubmitted to Journal of Wildlife Management*.

Jensen, W. F., K. L. Brackel, B. S. Kaskie, **E. S. Michel**, D. M. Grove, C. S. Bahnson, and J. A. Jenks. *In Press*. Pathogen exposure of adult female white-tailed deer and survival of associated fawns in the western Dakotas. *Journal of Wildlife Diseases*.

Jensen, W. F., **E. S. Michel**, and J. A. Jenks. *In Press*. What and why we hunt: a review of archaeological sites and hunting practices on the Northern Great Plains. *The Prairie Naturalist*.

Tucker, S. A., C. Bahnson, S. Courtney, J. A. Jenks, W. F. Jensen, J. G. Lundgren, and **E. S. Michel**. *In Review*. Presence of neonicotinoids insecticides in bobcats (*Lynx rufus*), fisher (*Pekania pennanti*), and river otter (*Lontra canadensis*) in North Dakota, USA. *Journal of Mammalogy*.

Woodley, S. E., C. F. Wahl, A. Tryforos, and R. Diaz. 2023. Biological control of invasive floating fern leads to rapid recovery of ecological functions in coastal freshwater wetlands in Louisiana. *Journal of Aquatic Plant Management* 61:42-54.