## RESEARCH ARTICLE

# Low rate of population establishment of a freshwater invertebrate (*Gammarus lacustris*) in experimental conservation translocations

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Conservation translocations may be a useful tool for the restoration of declining freshwater invertebrates, but they are poorly represented in the literature. We conducted a before-after/control-impact (BACI) experiment to test the efficacy of conservation translocation for re-establishing abundant populations of the amphipod *Gammarus lacustris*, a declining species and wildlife food resource in depressional wetlands in the upper Midwest of the United States of America. Each study site (*n* = 19) contained at least one treatment wetland receiving translocated *G. lacustris* from a local donor and one control wetland. We selected study wetlands based on a suite of wetland characteristics and randomly assigned recipient versus control treatment. *Gammarus lacustris* was detected post-translocation at only 2 of 22 recipient wetlands (1 of 19 sites). Overall, there was a statistical increase in *G. lacustris* density in recipient wetlands compared to controls; however, the results were of minimal biological significance due to being driven by a single site with low *G. lacustris* densities. Accordingly, our results suggest that future conservation translocations of amphipods might be successful if limited to recently restored wetlands or informed by a more complex habitat suitability model to differentiate dispersal limitations from habitat limitations. To develop such a model would involve identifying the fewest, most influential physical and biological factors (e.g. wetland size/structure, fish, aquatic vegetation, and water chemistry) from the numerous inter-related factors that correlate with the abundance of naturally occurring *G. lacustris*; candidate wetlands to receive amphipods would be those for which the model predicts abundant *G. lacustris* but in which they do not presently occur.

Key words: Amphipoda, before-after/control-impact (BACI), freshwater biodiversity, Prairie Pothole Region, reintroduction, stocking

## **Implications for Practice**

- Experimental conservation translocations of a declining freshwater invertebrate yielded few new populations, despite high pre-release survival and large numbers of invertebrates released.
- Given *Gammarus lacustris* is declining in its native range and translocations have been unsuccessful, restoration practitioners may choose to avoid translocations to minimize disruption to healthy, native populations, until a more successful translocation process is developed.
- Abundance of naturally occurring wetland invertebrates often varies with multiple, correlated factors (e.g. fish abundance, water quality, and aquatic vegetation). Translocation efforts with wetland invertebrates may benefit from complex site-selection criteria guided by predictive models of abundance based on the fewest, most influential variables. Wetlands for which a model predicts high abundance but the species is not present would be promising recipient sites.

## Introduction

The biodiversity of freshwater systems is declining at an alarming rate worldwide, affecting ecosystem function, productivity, and ecosystem services (Reid et al. 2019; McLean et al. 2020; Albert et al. 2021). Aquatic invertebrates serve key roles in freshwater ecosystem functions and food webs, particularly as detritivores and secondary producers, and many species are vulnerable to habitat degradation and rapid environmental changes (e.g. global climate change, modification of river flow and wetland drainage,

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pollution, and invasive species dispersal) with implications for organic matter decomposition rates, nutrient cycling, and secondary production (Strayer 2006; Collier et al. 2016; Epele et al. 2022).

Many aquatic invertebrates have high levels of endemism and low dispersal ability, limiting their ability to recolonize aquatic ecosystems after extirpations (Strayer 2006; Cazzolla Gatti 2016). Thus, managing invertebrate populations following local extirpations or habitat restorations may be warranted for dispersal-limited species, especially for species that play important ecosystem roles such as the transfer of energy between trophic levels.

Conservation translocations are intentional movements of organisms to restore local populations or supplement populations to increase viability (Seddon et al. 2014). This practice, sometimes called stocking or conservation stocking, may be a useful conservation strategy for freshwater invertebrates. Translocations may restore a locally extinct species (e.g. McNaught et al. 1999), reduce the extinction probability of threatened or vulnerable species, restore gaps in the aquatic community, facilitate recolonization following habitat restoration, be a method for assisted migration in the era of global climate change, and enhance the prevalence of "flagship," "ambassador," or "umbrella" species that support biodiversity (Seddon et al. 2014; Jourdan et al. 2019; Larson et al. 2022). The protection and restoration of freshwater habitat is key to preserving aquatic invertebrate biodiversity, but species-specific actions like conservation translocations may complement habitat restoration and management (Strayer 2006; Collier et al. 2016).

Aquatic invertebrates typically receive less conservation support than vertebrates (Collier et al. 2016), and translocations are correspondingly rarer (Seddon et al. 2014). For example, Jourdan et al. (2019) documented only 40 freshwater macroinvertebrate reintroductions in a literature review, of which just 23 had monitoring data for at least one generation postrelease (Jourdan et al. 2019). Additionally, achieving success (self-sustaining populations) with translocations can be challenging, with potential difficulties stemming from factors both intrinsic and extrinsic to the animal, such as the quality of the release habitat (including abiotic conditions and the presence of competitors and predators); environmental conditions like harsh weather; a lack of genetic or phenotypic diversity, possibly combined with local adaptation; and handling effects during collection and release (Seddon et al. 2014; Jourdan et al. 2019; Berger-Tal et al. 2020). Consequently, post-translocation monitoring, research, and publication of results, including unsuccessful attempts, is crucial to assess unbiased success rates and improve translocation methods (Fischer & Lindenmayer 2000; Jourdan et al. 2019). Current priority research needs for freshwater conservation include rigorous evaluations like before-after/ control-impact (BACI) designs (Maasri et al. 2022), particularly in translocations.

In central North America's Prairie Pothole Region (PPR), wetland ecosystems may benefit from conservation translocations to establish and bolster populations of freshwater amphipods (primarily *Gammarus lacustris* and *Hyalella azteca*; Fig. 1). Amphipods are an important component of food webs and nutrient cycling in PPR wetlands and have declined since the mid-twentieth century, especially in Iowa and Minnesota (Anteau & Afton 2006, 2008*a*, 2008*b*). The PPR is a large, regionally flat area of the North American Great Plains, extending from Alberta, Canada, to Iowa, United States, containing thousands of shallow wetlands formed in depressions left by receding glaciers. These prairie wetlands typically lack permanent surface inlets and outlets, are primarily fed by precipitation (especially spring snowmelt), and tend to accumulate nutrients from the surrounding landscape (Hayashi et al. 2016). This leads to high productivity and makes the PPR an important staging and breeding area for North American water bird populations (Batt et al. 1989; Skagen et al. 2008; Hayashi et al. 2016).

In PPR wetlands, amphipods consume coarse particulate organic matter, algae, and bacteria from the benthos and aquatic macrophytes and are consumed by amphibians and fish (Olenick & Gee 1981), waterfowl (Brown & Fredrickson 1986; Benoy et al. 2002), and other invertebrates (Mathias & Papst 1981). Amphipods can reach densities of hundreds to thousands of individuals per square meter in some prairie wetlands (Wen 1992; Mushet et al. 2015; Larson et al. 2022), serving as an important prey resource for wildlife. Declines in amphipods have been linked to declines in the continental population of Lesser scaup (*Aythya affinis*), which specialize on amphipods as a food resource during migration and breeding (Lindeman & Clark 1999; Anteau & Afton 2004; Kahara & Chipps 2009). Although historical information is limited, regional amphipod population densities in 2004–2005 were



Figure 1. Photographs of (A) *Gammarus lacustris* specimen under magnification  $(53 \times)$ , (B) large number of *G. lacustris* collected in a sweep net during survey of a wetland with high *G. lacustris* density, (C) practitioners releasing *G. lacustris* into a recipient wetland as part of experimental conservation translocation, and (D) underwater view of translocated *G. lacustris* 1 day following release. In panel (C), *G. lacustris* have been collected into coolers in large numbers. Practitioners are gently scooping *G. lacustris* from coolers with a pitcher and pouring into auger hole in ice, which is framed by a bottomless plastic bucket (orange) for convenience. Black tubing provides water from another auger hole to flush amphipods away from the release hole. In panel (D), *G. lacustris* are mostly clinging to the underside of the ice near the top of the image, with some swimming. All photo credits are to the authors and not copyrighted. Photo (A) by B.R.K.; Photo (B and C) by D.M.L.; Photo (D) by M.J.F.; used with permission.

roughly an order of magnitude lower than reported historical values from wetlands and lakes in the PPR in the 1960s–1980s, with declines of up to 94% in specific waterbodies (Anteau & Afton 2008*a*). The aggregate percentage of amphipods in the scaup diet sampled at staging sites in northwestern Minnesota declined by 94% between the 1980s and early 2000s (Anteau & Afton 2006, 2008*b*), suggesting that developing methods to bolster amphipod populations on the PPR land-scape would benefit Lesser scaup and other predators.

The PPR has undergone extensive wetland drainage and habitat modification over the past century due to intensifying agriculture, and habitat alteration may be a cause of the decline of amphipods. Specific factors may include direct or indirect effects of increased fish abundance, contaminants, major hydrologic alterations, and changing aquatic plant communities (Keith 2021; Carleen 2022; Larson et al. 2022). However, some wetlands support high amphipod densities in this highly modified landscape while others do not (Mushet et al. 2015, 2022; Keith 2021). Amphipods' relatively passive means of dispersal (compared to wetland invertebrates like insects with a flighted adult stage) via animals or stream connections between wetlands (Swanson 1984; Keith 2021; Mushet et al. 2022), combined with conservation managers' concerns that dispersal rates are limited by patchily distributed extant populations, makes amphipods candidates for trialing human-assisted translocation as a means of restoration.

Historical amphipod translocations were conducted to support fisheries rather than amphibian or waterfowl forage. Translocations were conducted in Europe and Siberia in the nineteenth century, with results ranging from no population establishment to the establishment of invasive species, but documentation of methods and contemporary assessments of outcomes were minimal (Jażdżewski 1980; Berezina 2007). In the PPR, G. lacustris stocked into a constructed pond reproduced, but neonates experienced high mortality (Mathias & Papst 1981). However, amphipods were only surveyed through August of the release year (May-June release), and the conditions of an artificially created pond may differ substantially from natural prairie potholes. Well-documented studies with robust experimental designs are lacking but would be useful to assess the efficacy of translocation as a conservation tool for amphipods.

We focused on experimental conservation translocations of the largest common PPR amphipod species, *G. lacustris*. This species is widespread globally and inhabits lakes and wetlands at a variety of elevations in a temperate to subpolar range (Bousfield 1958; Sutcliffe 1993; Väinölä et al. 2017). In the PPR, *G. lacustris* exhibits precopulatory amplexus (mateguarding behavior) beginning in winter, while the surfaces of wetlands are ice-covered (Menon 1966; Sutcliffe 1993). Photoperiod or dim light acts as a stimulus for reproduction (DeMarch 1982). Females carry eggs in a marsupium, where they are fertilized and develop until released as live young (Sutcliffe 1992). Incubation duration is temperature-dependent (e.g. 2–4 weeks in prairie Canada; Menon 1966; Biette 1969). In low-elevation prairie lakes, *G. lacustris* has an approximately 1-year life cycle and reproduce once, although a small proportion of females may reproduce twice in the breeding season (Menon 1966; Biette 1969; Wilhelm & Schindler 2000). The average number of broods per season varies. Two to three broods per season were reported in streams in the Great Lakes region (Hynes & Harper 1972), and up to four broods have been reported from Europe (Hynes 1955; Golubev 1997). The number of eggs per brood varies from approximately 15 to 55, depending on female size and whether they are bearing their first or second brood (Menon 1966; Biette 1969; Wilhelm & Schindler 2000).

We conducted a replicated BACI experiment to test the efficacy of G. lacustris translocations into depressional wetlands lacking G. lacustris in the PPR and the adjacent forest transitional zone of Minnesota, United States. Minnesota has some of the lowest rates of amphipod occurrence and abundance in Lesser scaup's migratory path through the PPR (Anteau & Afton 2008a). Our objectives were to test (1) whether abundant new populations of G. lacustris were established in recipient wetlands after one generation (reproduction and mortality of translocated amphipods; about 9 months following translocation) and (2) whether G. lacustris densities in recipient wetlands increased over time (up to 3 years post-translocation), with the expectation that several years would be required for population growth as evidenced by increases in amphipod densities on revisits. Though we selected wetlands that we believed would generally support G. lacustris survival (refer to site-selection criteria in Methods), we anticipated that G. lacustris might not establish at all wetlands. Consequently, we conducted in-depth surveys of water quality and chemistry, fish, and aquatic vegetation communities post-translocation at as many study wetlands as possible within the time frame of our study, with the goal of assessing wetland characteristics associated with successful G. lacustris establishment.

## Methods

#### Study Design, Study Area, Site Selection

All data are original and publicly available (Fitzpatrick et al. 2024). We conducted our study in depressional wetlands (wetlands occurring within topographic depressions; Brinson 1993) with semi-permanent to permanent hydroperiods in the PPR and adjacent forest-transition zone of Minnesota (Fig. 2), where G. lacustris is native and sometimes occurs at high densities (Anteau & Afton 2008a; Larson et al. 2022). Wetlands were located on Waterfowl Production Areas managed by the U.S. Fish and Wildlife Service, Wildlife Management Areas managed by the Minnesota Department of Natural Resources, and private property. As is typical for semi-permanent and permanent wetlands in the region, study wetlands contained a central open water area (0.6–19.0 ha; Table 1) surrounded by a ring of emergent vegetation (typically narrowleaf and/or hybrid cattail, Typha angustifolia and/or Typha  $\times$  glauca). None of the wetlands had a permanent connection to other water bodies but may have connected intermittently via overland flow (e.g. Hayashi et al. 2016). None of our wetlands dried during the study period, as was common for wetlands classified as



Figure 2. Map showing 19 study sites selected for a before-after/control-impact study of *Gammarus lacustris* conservation translocation (stocking) in western Minnesota, United States. Large map shows extent of larger map (blue rectangle). Gray scale background shading denotes elevation (U.S. Geological Survey 2007). Black lines denote North American state and province boundaries (Natural Resources Canada and U.S. Geological Survey 2010). Each site includes one control wetland and one to three *G. lacustris*-recipient wetlands. Wetlands entered the study over the course of 3 years (three groups). *G. lacustris* were translocated into group 1 sites (n = 7 sites) in winter 2017–2018, group 2 sites (n = 5) winter 2018–2019, and group 3 sites (n = 7) in winter 2019–2020. Wetlands were surveyed for *G. lacustris* one time before translocation and up to 3 years following translocation.

semi-permanent during the multi-decadal wet cycle in which our study occurred (van der Kamp et al. 2016; McLean et al. 2022).

Our study had a paired BACI design (Stewart-Oaten et al. 1986; Smith 2002). We selected 19 study sites (areas containing multiple wetlands), whereby each site contained one or more recipient wetlands (wetlands into which *G. lacustris* would be released) and one control wetland (wetland on the same site that would not receive translocated *G. lacustris*). The maximum distance between control and recipient wetlands at a site was 15 km. There were 41 wetlands total in the study (22 recipient wetlands and 19 control wetlands), because two sites had more than one recipient wetland (Table S1). For sites with more than one recipient wetlands within each site for BACI analysis. The selection of which study wetland would be the control wetland at each site was random, with a few exceptions (Table S1).

We added new sites to the experiment over 3 years to incorporate varying annual conditions (air temperature and precipitation) (Table S1). We translocated *G. lacustris* into the recipient wetlands at seven sites in winter 2017–2018, five more sites in winter 2018–2019, and the final seven sites in winter 2019–2020. Each wetland was surveyed once for amphipods pre-translocation ( $\leq$ 7 months prior to the *G. lacustris* release date), and then once each autumn post-translocation through 2021, with few exceptions (Table S1).

Wetlands were selected using site-selection criteria and evaluated via a combination of landowner/land manager knowledge and a habitat suitability survey on a preliminary site visit. We developed an initial candidate pool of study wetlands by asking five public land managers to suggest water bodies that they thought would serve as quality amphipod habitat and could benefit migrating waterfowl, as if they were implementing a conservation translocation program. After public awareness of our plans to experimentally translocate amphipods developed (Larson 2018), several private landowners requested we release amphipods in their wetlands. In 2019, we scouted over 30 additional wetlands. The list of candidate sites and wetlands was screened annually to find study wetlands meeting our a priori wetland selection criteria. We obtained information about each wetland from the landowner/land manager and conducted at least one preliminary site visit, where we observed the wetland and searched for preexisting amphipods and excessively abundant fish using a sweep net at ≥4 locations reachable by technicians wading with chest waders.

We created a priori wetland selection criteria based on a literature review of *G. lacustris* habitat characteristics and suggestions from peer scientists during grant proposal development (Larson 2018). Wetland criteria included the following: location within the prairie pothole or forest-transition zones of Minnesota, United States (because *G. lacustris* populations are low and landscape modifications were extensive); National Wetlands Inventory Circular 39 Type 4 or 5 classification ("inland deep freshwater marsh" or "inland open water"; Shaw & Fredine 1956, Minnesota Department of Natural Resources 2019), with semi-permanent or permanent

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logical characteristics of wetlands i one recipient (R) wetland that recei 'is were translocated into wetlands in information includes wetland locatic , averaged across years); total phosy recentage of sample points where SA and floating aquatic vegetation ta number of Tiger salamanders (TGS August through early October) from innow (Hybognathus hankinsoni), I elas), GSF = Green sunfish (Lepon cander vitreus), YEP = Yellow perc stry/quality using similar methods a and control wetlands were measurec tail (Ceratophyllum demersum). Fis	Location	46°6′8.2′N, 95°46′3.5′W 47°37′16.4′N, 95°19′37.9′W 47°37′10.1′N, 95°19′45.3′W 47°37′3.8″N, 95°19′48.3′W	45°49'53.2"N, 95°5'0.3"W 45°50'15.2"N, 95°4'57.4"W 45°23'58.2"N, 95°12'57"W	47°27'19.9"N, 94°44'8"W	46°7'13.1"N, 95°46'37.4"W 45°19'46.3"N, 94°3'46"W 47°18'48"N, 95°4'25.7"W	47°32′58.2″N, 95°40′51.4″W 47°39′38.4″N, 96°0′0.2″W	47°38′27″N, 96°1′43.8″W 47°33′12.2″N, 94°51′22.2″W	46°53'3.8"N, 95°56'19"W 47°25'59.2"N, 94°49'49.8"W	46°46′43.3″N, 96°5′33.4″W	47°37'4.8"N, 95°34'33.2"W 46°51'59.4"N, 95°38'38.7"W	47°30'19.1"N, 95°49'21.4"W	47°37'34″N, 95°36'2.5″W
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Fish species	Vo fish	DCE, FHM, GSF 3SB, CMM, FHN 3SB, CMM, DCE FHM	3SB, CMM 3LG, LMB 3SB, FHM Vo fish	3SB, FHM 3SB, CMM, DCE FHM	3SB, CMM 3SB, FHM, WAE 7HM <sup>3</sup>	
# Fish	0	221 I 18 H 405 H	72 H 202 H 610 H 0 N	1348 H 132 H	52 H 244 H F	$\begin{array}{c} 0\\1225\\13,848\\0\\296\end{array}$
Simpson's D		0.81 0.8	0.83 0.74 0.75	0.72 0.74	0.58 0.82	$\begin{array}{c} 0.62 \\ 0.76 \\ 0.87 \\ 0.58 \\ 0.75 \end{array}$
Avg. SBI		1.6 2.3	3.7 0.6 1.5	2.2 2.9	2 0.71	$\begin{array}{c} 0.9\\ 1.9\\ 0.6\\ 2.1\\ \end{array}$
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P SAV		96	100 100	100	+ 100	9 00 10 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9
Total (mg/I	0.017 0.03 0.053 0.0353 0.012 0.042	0 0.017 0.02	0.022 0.046 0.046 0.046 0.046 0.046 0.046 0.046	0.256 0.028 0.025	$0.01^{2}$ 0.025 $0.05^{2}$	$\begin{array}{c} 0.010 \\ 0.031 \\ 0.074 \\ 0 \\ 0 \\ 0.038 \end{array}$
Chl-a (µg/L)	0.99 0.99 0.82 0.25 0.25	0.69 1.22 1.75	1.25 0.24 4.08 0.61	16.71 1.78	$     \begin{array}{r}       1.73 \\       0.85 \\       3.91^2     \end{array} $	$\begin{array}{c} 0.61\\ 2.99\\ 16.02\\ 0.24\\ 2.56\end{array}$
Turbidity (NTU)	$\begin{array}{c} 1.65\\ 3.5\\ 3.54\\ 3.54\\ 3.02\\ 1.5\\ 0.98\\ 1.29\end{array}$	0.8 1.49 1.65	2.76 0.9 1.49 0.68	39.65 1.34 1.32	$\begin{array}{c} 0.98 \\ 1.11 \\ 9.41^2 \end{array}$	0.9 2.5 9.5 3.7
Hd	8.0 8.1 8.5 8.3 8.3 8.3 8.3	8.1 8.7 8.5	9 8.5 8.5 8.5	8.9 9.0 8.3	8.6 8.5 $9.0^{2}$	8.0 8.4 8.9 8.0 8.4 8.4
Temp. (°C)	10 17.8 17.8 17.3 11.5 10.4	7.8 7.8 18.9 10.9	17.1 19.5 10.4 13.3	10.6 9.5 10.7	14.5 8.9 27.2 <sup>2</sup>	7.8 14.4 20.2 7.8 13.6
Avg. dep. (m)		2.1	1 1.4 0.8	$0.7 \\ 1.1$	1.3 3.11	$\begin{array}{c} 0.8 \\ 1.5 \\ 2.7 \\ 0.7 \\ 1.3 \end{array}$
Max. lep. (m)		3.4 2.9	1.7 1.5 1.2	$0.9 \\ 1.2$	1.8 4.27	$\begin{array}{c} 1.1 \\ 2.5 \\ 7.3 \\ 0.9 \\ 1.8 \end{array}$
Area (ha) 6	$\begin{array}{c} 2.3\\ 0.6\\ 0.9\\ 0.7\\ 10.1\\ 1.0\\ 7.7\end{array}$	7.6 8.1 2.8	$   \begin{array}{c}     1.2 \\     1.2 \\     1.2.4 \\     1.8 \\   \end{array} $	18 2.6 1.2	5.7 4.3 1.9	0.6 5.9 0.6 5.5
Location	46°6/19.2"N, 95°45/49.8"W 47°37/14.1"N, 95°19'56.6"W 45°50/12.1"N, 95°19'56.6"W 45°23'33"N, 95°4'50.7"W 45°23'33"N, 95°12'39.5"W 47°26'1.6"N, 94°41'8.2"W 46°7'8.9'N, 95°46'43.9"W	47°21'41.11'N, 94°56'51.8''W 47°33'28.5'N, 95°41'1.1''W 47°38'53.5''N, 96°4'49''W	47°38'39.2"N, 96°3'25.9"W 47°32'33.5"N, 94°47'24.4"W 46°52'58.8"N, 95°55'538"W 47°24'20.9"N, 94°48'14"W	46°48°53.8″N, 96°610.8″W 47°35′17.5″N, 95°36'34.9″W 46°50'49.9″N, 95°38'32.6″W	47°30/13.3″N, 95°48/51.5″W 47°37/37.2″N, 95°36/56.2″W 46°20/20.0″N, 95°47/55.5″W 46°18/36.0″N, 95°51/53.3″W	
2 Translocation	2017–2018 2017–2018 2017–2018 2017–2018 2017–2018 2017–2018 2017–2018	2018–2019 2018–2019 2018–2019	2018–2019 2018–2019 2019–2020 2019–2020	2019–2020 2019–2020 2019–2020	2019–2020 2019–2020 N/A N/A	t min. t avg. max. vg.
$Typ\epsilon$	0000000		0000	0 0 0	d3d1 c c	cipient cipient cipient ntrol n ntrol a
Site	- 0 0 4 v v r	9 10	$112 \\ 122 \\ 123 $	15 16 17	18 N/# N/#	C C R K K

Restoration	Ecology
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Table 1. Continued

hydroperiod confirmed by landowner/land manager observation and average wetland water depth greater than 1 m (to reduce chances of G. lacustris desiccation or winterkill by ice); open water surface area less than approximately 20 ha (to allow statistical replication within the project budget); fish not abundant to the best knowledge of the land manager and per observation on preliminary site visit (fish not appearing in dip nets); existence of submerged and emergent aquatic vegetation communities; a comparable, nearby wetland (to support our BACI design); and reasonable accessibility (e.g. landowner permissions to repeatedly sample and the wetlands were reachable by snowmobile in winter). We avoided translocating amphipods into wetlands detected or suspected to have extremely abundant fish populations due to their known negative effects on G. lacustris (Anteau et al. 2011) and because translocation was intended to restore waterfowl and amphibian habitats rather than support fisheries. We did not avoid wetlands with low fish abundance because G. lacustris cooccurred with fish across the PPR (Anteau et al. 2011) and because fishless semi-permanent to permanent wetlands are extremely scarce in Minnesota's prairie pothole landscape.

Although we nonrandomly selected the study wetlands, we randomly selected which wetland would serve as a recipient versus control wetland at each site to make valid inferences from the BACI design. Per the study design, most (17 of 19) sites did not contain *G. lacustris* (as assessed via ice auger and sweep net surveys prior to translocation; refer to Amphipod Translocation below). We unintentionally included two sites containing *G. lacustris* prior to translocation (Sites 15 and 18). These sites were chosen for the study because *G. lacustris* was not visually detected in the sweep nets during preliminary site visits or pre-translocation amphipod surveys described below; however, we detected small numbers (<20 m<sup>-3</sup>) of *G. lacustris* in the sample material under closer examination in the laboratory after stocking had occurred (Fig. 3 & Table S1).

#### Study Wetland Characteristics

Following translocation, we surveyed wetlands for fish, aquatic vegetation, water depths, and water quality/chemistry (refer to Supplement S3 for detailed survey methods), with the goal of assessing habitat characteristics associated with the establishment of *G. lacustris*. In brief, we measured water chemistry at all wetlands during annual fall amphipod sweep net surveys (refer to Amphipod Monitoring below). We collected water samples and measurements from near-surface water at a single location near the center of the wetland at each visit (Supplement S3). With a few exceptions, we measured temperature, pH, and turbidity in all years (one to three times per wetland) and chlorophyll-*a* and total phosphorus one to two times per wetland.

We surveyed fish, vegetation, and water depths of wetlands in 2019 and 2020, with a focus on recipient wetlands. We did not survey all wetlands due to logistical constraints (travel constraints and staff shortages during the COVID-19 pandemic), but we surveyed as many wetlands as possible. Vegetation (aquatic submerged, floating, and emergent) and depth surveys were conducted in July–August 2020. We surveyed aquatic plants in 12 of the 19 study sites (including recipient wetlands

at 12 sites and reference wetlands at 8 of the 12 sites) using the methods described in Larson et al. (2022) (Supplement S3). We surveyed submerged aquatic vegetation using a plant rake from a canoe (10-44 gridded points per wetland, scaled to wetland area; Supplement S3; Larson et al. 2022). We also surveyed floating vegetation in a 1-m<sup>2</sup> area alongside the canoe at each sample point and recorded the dominant emergent vegetation type at each sample point adjacent to shore (Supplement S3). We recorded relative biomass (5-point categorical scale ranging from 0 to 4) and taxon presence/absence for submerged and floating aquatic vegetation, with most plants identified to species (Supplement S3; Larson et al. 2022). We surveyed submerged and floating vegetation at depths up to 5.1 m (the maximum reach of our plant rake) but did not find aquatic vegetation at depths greater than 3.7 m due to light attenuation (Supplement S3). We measured water depth at all sample points using a weighted, marked rope (Supplement S3).

We conducted fish surveys in July–August of 2019 and 2020 using methods described in Carleen (2022) at 14 of the 19 study sites (14 recipient wetlands and 11 controls) (Supplement S3). In brief, we surveyed relative fish abundance and biomass in each wetland using one 21-m (70-ft) gill net and three small fyke nets ("Mini Fyke Net," Duluth Nets, Duluth, Minnesota) set overnight (Supplement S3). Following net retrieval, fish were sorted to species, weighed (total species mass), and counted. Dace species (*Chrosomus*) were grouped due to difficulty of differentiating hybrids. If the time required to process a net was estimated to be greater than 30 minutes, a subsample of each small (<30 cm) species with numerous individuals was weighed and counted to estimate the total count (Supplement S3).

#### **Amphipod Translocation**

We collected *G. lacustris* from donor wetlands within our study region of Minnesota and released them into recipient wetlands during late winter. We collected amphipods in late winter because *G. lacustris* migrates vertically from the benthic zone to more oxygenated water near the underside of the ice at this time (Kolar & Rahel 1993), allowing large quantities to be collected with minimal extraneous debris. Collection and release were also conducted in the winter to avoid heat stress. Annual timing (mid-January to mid-March) varied based on ice cover condition for safety during collection and *G. lacustris* phenology.

We collected amphipods from two donor wetlands (Table 1). One wetland (donor 1) was used primarily, and a second wetland (donor 2) was used in the final year of the study to avoid overharvesting at donor 1. Donor 1 contained only *G. lacustris* amphipods, as determined in a concurrent study (Larson et al. 2022). In winter 2019–2020, volumetric subsamples (described below) from donor 2 were identified under a microscope, verifying that the harvested amphipods were *G. lacustris*. Donor 1 was a large wetland (47.7 ha, average depth 3.1 m) surveyed for fish, vegetation, and water quality/chemistry as part of a concurrent study (Keith 2021; Carleen 2022; Larson et al. 2022). Turbidity, pH, total phosphorus, and chlorophyll-*a* (collected in summer) were within the range of values in recipient wetlands (Table 1). The fish



Figure 3. (A) *Gammarus lacustris* density at 19 study sites in a 3-year before-after/control-impact study of *G. lacustris* conservation translocation. Each study site had at least one recipient wetland (receiving translocated *G. lacustris*) and one control wetland. Most sites had one recipient wetland, except Site 3 (two recipients) and Site 2 (three recipients). Densities are shown for surveys before ("B") and 1–3 years after ("A1," "A2," and "A3") translocation. Sites entered the study over the course of 3 years, such that the earliest sites had 3 years of after-translocation *G. lacustris* surveys and the latest sites had 1 year of after-translocation surveys. Table S1 shows the data underlying this graph. (B) Box-and-whisker plots of site-level *G. lacustris* before and after translocation in most wetlands, such that boxes and whiskers are clustered at 0, and non-zero values are outliers (defined as values more than 1.5 times the interquartile range below the 25th percentile or above the 75th percentile; shown as dots). Number of zero values represented by the collapsed box-and-whiskers are labeled (n = 17 before translocation; n = 33 after translocation). Recipient wetland values were averaged for sites with more than one recipient wetland. A Mann–Whitney *U* test of these data found that amphipod densities were statistically higher after translocation (U = 297.5, p = 0.08), but the result was driven by change at a single site (Site 3). The data underlying this figure are shown in Table S8.

community was similar to that of most recipient wetlands, with a relative abundance of 241 Fathead minnows and Brook stickleback, although also including a few (relative abundance 3) small (<5 cm) Walleye (*Sander vitreus*) due to this wetland being used for Walleye rearing by the Minnesota Department of Natural Resources (Tables 1 & S4; Fig. S3). The aquatic vegetation community included species found commonly in recipient wetlands (Table S2), although submerged aquatic vegetation coverage was lower than most recipient wetlands (Table 1), likely due to the deeper average depth in this wetland. Donor wetland 2 was smaller (1.9 ha). It was not formally surveyed, but anecdotal observations indicate the presence of common fish (fathead minnows) in recipient wetlands and submerged aquatic vegetation (coontail), sago pondweed (*Stuckenia pectinata*), and star duckweed (*Lemna triscula*), similar to at least some recipient wetlands (e.g., Sites 13, 18; Table S2; Fig. S2).

Our capture and holding methods of *G. lacustris* were based on prior private (not formally studied) translocation attempts using the same holding facilities, in which high survival from capture to release was observed. We collected *G. lacustris* using an underwater, remotely operated vehicle (ROV) towing a plankton net. We deployed the ROV through a  $0.7\text{-m} \times 1.5\text{-m}$ hole cut through the ice. We erected a shelter over the hole to avoid exposing amphipods to sub-freezing air temperatures, which could damage appendages and increase mortality risk. The *G. lacustris* were collected into coolers of water from the donor wetland and transported to a holding facility, where they were held in captivity for 1–5 days. Hold time varied according to logistical needs, including time to collect enough *G. lacustris* and ambient air temperature above freezing to reduce mortality risk during transit.

At the holding facility, we held *G. lacustris* in 4542-L (1200 gal) metal tanks (approximately  $10^6$  individuals per tank) with a constant stream of fresh water (well water;  $8.3^\circ$ C) and supplemental aeration with bubble air stones. Tank water temperature was not measured but was cooler than incoming water temperature, as the building was unheated in winter. During the short-term captivity, we fed *G. lacustris* chopped organic potatoes and dead White sucker (*Catostomus commersonii*). The *G. lacustris* consumed all food except potato skins and fish bones. However, the amount of food provided varied and was not recorded. Food was suspended in 12.7-mm mesh pens to keep the water clean.

On the day of release, we transferred G. lacustris from the tanks into water-filled coolers using fine mesh dip nets and transported them to a recipient wetland. We released G. lacustris at a density of 18.7 L/ha of wetland surface area (2 gal/acre). This volumetric measurement refers to the contents of the coolers containing amphipods in water and equated to an average of 10 individuals/m<sup>2</sup> wetland surface area (range:  $2.1-15.7 \times m^{-2}$ ; Table S5), based on counts of amphipods in known-volume samples of cooler contents (n = 25 samples from 11 wetlands). We released amphipods at multiple locations based on wetland size: at minimum, two release locations for wetlands less than or equal to 4 ha, with an additional release hole for every additional 4 ha in size (i.e., three holes for wetlands 4.1-8 ha, four holes for wetlands 8.1-12 ha, etc.). However, amphipods were sometimes released at more locations (irregularly), or fewer locations if necessary due to unsafe ice conditions (Table S6).

To gradually transition amphipods to recipient wetland water temperature prior to release at each auger hole, we transferred approximately half of the amphipods from each cooler into an empty cooler and filled both coolers with recipient wetland water to obtain an approximately 1:1 mix of tank and wetland water for 1–2 minutes prior to release. In winter 2017–2018, we collected 100-mL samples of *G. lacustris* from coolers following acclimation to assess mortality and injury rates immediately prior to release. Amphipods were visually examined for mortality and visible injury (missing or broken limbs) in trays on-site.

Amphipods were then scooped from coolers with a pitcher and gently poured into release holes (Video S1). We used a hose with an electric pump to pipe water from a second auger hole into the release hole during release, aiding amphipod dispersal away from the hole.

#### **Amphipod Monitoring**

**Ice Auger Surveys.** In the first year of the study (January 2018), we surveyed wetlands pre-translocation using an ice auger and catch net. We sampled amphipods along transects that radiated out from the center of the wetland. We aimed to place one transect on wetlands less than or equal to 2 ha, and two transects on wetlands 2.6–4 ha, with an additional transect added for every 4-ha increase in size above 4 ha (three transects for

wetlands 4.1–8 ha, four transects for wetlands 8.1–12 ha, etc.). However, wetland sizes were estimated due to time constraints, and we sometimes sampled one to two more or fewer transects than prescribed based on wetland size measured in aerial imagery after the fact (Table S6). We placed the first transect at a randomly selected bearing (0–359), and distributed the remaining transects evenly around the wetland (e.g. two transects 180° apart, three transects 120° apart, etc.). On each transect, amphipods were sampled at a site 5 m from shore and a site 50 m from shore. Total number of sample points ranged from 2 to 10 (one to five transects) per wetland.

We drilled two side-by-side holes (diameter 20 cm) using a propane-powered ice auger (Video S2). We moved snow away from the holes to form a depression containing the two holes. We then fitted a sieve into one of the holes, inserted the auger into the other hole, and ran the auger for 15 seconds. The auger drew water onto the ice surface, and water flowed through the sieve. Invertebrates drawn up with the water either came to rest on the ice or were captured in the sieve. We stored invertebrates in 95% ethanol and transported them back to the laboratory for identification.

Flow rate tests in February 2018 (Supplement S1) indicated substantial variation in water volume sampled via this method (2.9  $\pm$  0.9 L/s). Consequently, we switched to sampling known water quantities with sweep nets during the ice-free season to obtain more precise quantitative amphipod densities for the remainder of the study.

Sweep Net Surveys. We sampled amphipods at all wetlands using sweep nets after the first season of the study. We primarily sampled in late August to late October (with two sites sampled in late July 2018). At this time, we expected G. lacustris reproduction to have slowed or stopped and juveniles to be large enough to be captured in sweep nets (Menon 1966; Biette 1969). We sampled from canoes using D-frame sweep nets (1200-µm mesh), similarly to prior studies (Anteau & Afton 2008a, 2011; Janke et al. 2019). We created amphipod sample point locations in advance using ArcGIS (versions 10.6-10.7, ESRI, Redlands, CA, U.S.A.) and navigated to sample points in the field using handheld GPS units (Montana 650 or Montana 680 t, Garmin Ltd., Olahe, KS, U.S.A.). We placed sample points along transects radiating from the center of the wetland to the shore. Wetlands with open water area ≤4 ha had two transects, and we added an additional transect for each 4 ha increase in wetland size (i.e. wetlands 4.1-8 ha had three transects, 8.1-12 acres had four transects, etc.; Table S6). We placed the first transect at a randomly selected bearing and distributed the remaining transects evenly around the wetland. On each transect, we placed one amphipod sample point at a randomly selected distance within 5 m of shore (i.e. 1, 2, 3, 4, or 5 m from shore) and another sample point at a randomly selected distance greater than 5 m from shore. This resulted in 4-12 sampling points (two to six transects) per wetland  $(1.4-7.5 \text{ m}^2 \text{ or } 0.002-0.03\%)$ wetland area; Table S6).

At sample points, we measured the water depth to the nearest 5 cm (Table S6). We moved sample locations closer to shore

along the transect where water was too deep for sampling (>140 cm), and away from shore if water was less than the height of the sweep net (30 cm). We conducted a horizontal sweep, skimming the top 1-2 cm of sediment for a distance equal to the water depth, and then raised the net to the surface at a 45° angle from horizontal. We stored the sweep net contents in 95% ethanol for laboratory analysis.

In the laboratory, we counted and identified amphipods from sweep net material as described in Keith et al. (2022). Individual sample points were processed separately. In short, we randomly subsampled material volumetrically using a grid frame placed over a flat mesh sieve. Sample grid material was transferred to a white plastic tray, suspended in water, and inspected with a lighted magnifier ( $3 \times$  magnification; Luxo KFM 17115 Magnifier). We picked all amphipods from a minimum of 20% of the sample volume. Picked amphipods were stored in 70% ethanol and identified (*Hyalella azteca*, *G. lacustris*, or *Crangonyx* spp.) using a trinocular stereomicroscope ( $6.5-53 \times$  magnification, Laxco MZS4-Z33A). Taxon-specific total numbers per sample were estimated based on the amphipod count in the subsample and the proportion of the sample picked.

We calculated *G. lacustris* density at each sample point as the number of individuals per water volume swept (benthic sweep plus  $45^{\circ}$  sweep through water column; Fig. S4) for statistical analysis:

$$D_{\rm vol} = \frac{C}{aw\left(1 + \left(1/\cos(45^\circ)\right)\right)}$$

where  $D_{\text{vol}}$  is *G. lacustris* density (m<sup>-3</sup>), *C* is the *G. lacustris* count (adjusted for subsampling), *a* is the area of the sweep net opening (m<sup>2</sup>), and *w* is water depth (m). We averaged  $D_{\text{vol}}$  across sample points to obtain an average *G. lacustris* density for each field sampling date for each wetland.

We also converted *G. lacustris* density to number of individuals per horizontal area swept to facilitate comparison with release densities, which were conducted on a per-area basis:

$$D_{\rm area} = D_{\rm vol} \times W$$

where  $D_{\text{area}}$  is the *G. lacustris* density (m<sup>-2</sup>),  $D_{\text{vol}}$  is the volumetric density (m<sup>-3</sup>), and *w* is water depth (m).

**Video Monitoring.** We opportunistically monitored translocated *G. lacustris* at a small local wetland (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 the potential for mass mortality immediately and days following release. We observed *G. lacustris* immediately following release, one time per day over the next 3 days, and then one time per week until 5 weeks post-release, when underwater monitoring ceased 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 (Fig. 1D; Video S3). We also checked new locations within 20 m of the release hole beginning 1 week after release to look for dispersal. In addition, we cut a larger hole (approximately  $0.6 \times 0.6$  m) in the ice near (6 m from) 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.

## Data Analyses

We used a Mann–Whitney U test as the BACI analysis to test whether translocation increased *G. lacustris* densities compared to densities at the associated control wetlands. The predictor variable was period (before or after translocation). The response variable was the difference in amphipod densities between the recipient and control wetlands at each site at each timepoint  $(D_{pik})$ . That is,

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

(after Smith 2002, eqn 3, but using a nonparametric Mann– Whitney U test rather than a t test due to the non-normal distribution of our response variables) where R is G. lacustris density (m<sup>-3</sup>) in recipient wetland p (p = 1, 2,..., 19 sites) at time ik, where i represents period (before or after translocation) and k represents time within period (k = 1 within i = before and k = 1, 2, or 3 within i = after). C is G. lacustris density in control wetland p at time ik. We chose a Mann–Whitney U test after finding that transformations did not sufficiently improve the normality of  $D_{pik}$  for a t test (Fig. S5).

To test whether G. lacustris densities increased through time (up to 3 years) following translocation, we conducted a nonparametric Kruskal-Wallis test with G. lacustris densities from post-translocation samples as the dependent variable and time since translocation as the independent variable (A1 = first fall,approximately 9 months, after translocation; A2 = second fall post-translocation; A3 = third fall post-translocation). As in the Mann-Whitney test, our response variable was the difference in amphipod densities between the recipient and control wetlands at each site (but only from post-translocation samples). We used  $\alpha = 0.10$  as evidence of the statistical significance of translocation effectiveness in all tests. Additionally, to place results in the context of our translocation methods, we estimated the potential post-stocking density for G. lacustris given amphipod release densities  $(m^{-2})$  and winter sex ratios, reproductive rates, and juvenile mortality rates of naturally occurring prairie populations from the literature (Supplement S2).

## Results

## **Study Wetland Characteristics**

Recipient wetlands averaged a pH of 8.4 (range 8.0–8.9), turbidity of 2.47 nephelometric turbidity units (NTU) (range 0.89–9.5 NTU), chlorophyll-*a* concentration of 2.99  $\mu$ g/L (range 0.61–16.02  $\mu$ g/L), and total phosphorus of 0.03 mg/L (range 0.01–0.07 mg/L) (Table 1). Control wetland water characteristics were similar

(Table 1). Maximum depth ranged from 1.1 to 7.3 m in recipient wetlands (Table 1). Submerged aquatic vegetation prevalence (percentage of sample points with submerged vegetation) in recipient wetlands was high (94-100%), with average biomass values ranging from 0.9 to 3.5 (average 1.9; Table 1). Floating species prevalence was variable (range 0-100%: Table 1). Simpson's diversity index (Supplement S3) averaged 0.76 (range 0.63–0.87; Table 1). Aquatic plant species composition varied (Table S2; Fig. S2), but most recipient wetlands had a high prevalence of macroalgae (Chara spp.), coontail (Ceratophyllum demersum), or both, and all wetlands contained pondweeds (Potamogeton and/or Stuckenia spp.). Bladderwort (Utricularia spp.) and water bulrush (Schoenoplectus subterminalis) were also common (Table S2; Fig. S2). Control wetland plant communities were similar (Table S3; Fig. S2).

Fish occurred in 11 of the 14 surveyed recipient wetlands (Table 1). In most recipient wetlands with fish, counts varied by an order of magnitude (52-690 fish; 390-3424 g biomass). One outlier wetland (Site 11) contained an exceptionally large number of fish (13,848 fish; 25,948 g), primarily small Black bullheads (Ameiurus melas). Most recipient wetlands with fish contained primarily a mix of dace and Fathead Minnows (Pimephales promelas), sometimes with smaller numbers of Central mudminnows (Umbra limi) and/or Brook stickleback (Culaea inconstans) (Tables 1 & S4; Fig. S3). Six wetlands contained Black bullheads (including the outlier Site 11) (Tables 1 & S4; Fig. S3). The three recipient wetlands with no fish were at Site 3 (two recipient wetlands) and Site 13 (one recipient wetland). Control wetlands had similar variation in fish communities (Tables 1 & S4; Fig. S3), although without Black bullheads, and two control wetlands were fishless (Sites 3 and 14). Tiger salamanders (Ambystoma tigrinum), an amphipod predator, occurred as an incidental catch in all fishless wetlands.

We found two additional amphipod taxa (*Hyalella azteca* and *Crangonyx* spp.) in the study wetlands during pre- and post-translocation periods (Fig. S1). *Hyalella azteca* was present in 95% of wetlands (0–1500 individuals/m<sup>3</sup>; Fig. S1). Fourteen (35%) wetlands contained *Crangonyx* spp. (0–605 individuals/m<sup>3</sup>; Fig. S1).

#### Condition of Amphipods Prior to Release and Projected Densities

The majority (mean 98%) of *Gammarus lacustris* were alive and uninjured while in coolers immediately prior to release. In pre-release spot checks (n = 22 samples from coolers at seven recipient wetlands), we observed a mean mortality rate of 1.8% of amphipods ( $\pm 2.7\%$  standard deviation) (Table S7). An additional small proportion (mean <1%) were visibly injured but alive (Table S7). Approximately 25% were paired in the precopulatory amplexus (Table S7). Given our release densities, and literature values of winter sex ratios, reproductive rates, and juvenile mortality rates for prairie amphipods, we projected second-generation densities of roughly 2.2–25.1 *G. lacustris* × m<sup>-2</sup> in the first fall following translocation (Supplement S2).

## Amphipod Densities

We conducted a total of 56 amphipod surveys at the site-level (19 pre-translocation surveys and 37 post-translocation surveys; Table S1; Fig. 3). As described in Methods, most sites did not contain *G. lacustris* prior to stocking by design, but we unintentionally included two sites containing small numbers (<20 m<sup>-3</sup>) of *G. lacustris* in the pre-stocking sweep net surveys. Post-translocation, we detected *G. lacustris* at only 1 of the 19 sites. At this site (Site 3, which contained two recipient wetlands and one control wetland), no *G. lacustris* were detected prior to translocation in any wetland. After translocation, the two recipient wetlands reached maximum *G. lacustris* densities of  $35.2 \text{ m}^{-3}$  ( $31.7 \text{ m}^{-2}$ ) and  $2.5 \text{ m}^{-3}$  ( $2.1 \text{ m}^{-2}$ ), while *G. lacustris* remained absent in the control.

Amphipod density was statistically higher after translocation (Mann–Whitney test, U = 297.5, p = 0.08); however, this result was driven by the single site where post-translocation *G. lacustris* were detected (Site 3; Table S8; Fig. 3). The average density of *G. lacustris* in recipient wetlands was only 0.52 *G. lacustris* × m<sup>-3</sup> higher after translocation than before translocation, and the average recipient-control difference was only 1.7 *G. lacustris* × m<sup>-3</sup> higher after translocation than before translocation. The Kruskal–Wallis test of post-translocation samples indicated no significant difference in *G. lacustris* density among the first, second, and third years following translocation ( $\chi^2 = 1.39$ , df = 2, p = 0.49), reflecting our zero-heavy dataset (Table S9).

## Video Monitoring

Underwater videos at the recipient wetland of Site 14 showed living *G. lacustris* clustered on the underside of the ice in high density around the release hole throughout the first week following translocation (Video S3). Lower numbers of *G. lacustris* were observed swimming in the water column and near the wetland bottom. Some *G. lacustris* were observed in the precopulatory amplexus. We found small numbers of *G. lacustris* at holes drilled up to 20 m away from the release hole 1 week after release (<10 individuals per hole). However, from the perspective of our auger hole viewpoints, *G. lacustris* appeared to gradually decline in density in weeks 2–5. There was no evidence of mass mortality in sweep nets or videos, although our methods could not rule out this possibility.

## Discussion

We found *Gammarus lacustris* in recipient wetlands at only 1 of 19 study sites following translocation (2 of 22 recipient wetlands, with the 2 wetlands located on the same site). Correspondingly, translocation increased *G. lacustris* population densities by a very small amount that is not likely to be pertinent to wildlife (<1 *G. lacustris* × m<sup>-3</sup> on average across study sites). In the same vein, *G. lacustris* densities did not increase significantly through time in the first 3 years following translocation, although *G. lacustris* did increase through time in the single wetland where they were detected twice post-translocation (Site 3). The low establishment rate of new

populations was likely due to biological and physical conditions of the wetlands (often anthropogenically influenced) and possibly because of some aspects of our translocation methods that prevented *G. lacustris* from establishing and becoming abundant. Use of a habitat suitability model capable of disentangling environmental limitations from limitations due to other aspects of the translocation process may increase the probability of successful translocations of *G. lacustris* in the future.

#### **Densities of Translocated Amphipods**

We projected potential second-generation densities of 2.2–25.1 *G. lacustris* × m<sup>-2</sup> in the first fall following translocation. At Site 3 where *G. lacustris* were detected posttranslocation, density in the first fall following translocation (4.8 m<sup>-2</sup>) fell within the projected range and subsequently increased, as expected. The maximum density (31.7 m<sup>-2</sup>; 35.2 m<sup>-3</sup>) achieved at Site 3 may provide wildlife forage, given that amphipod densities greater than approximately 26 m<sup>-3</sup> promote efficient feeding by Lesser scaup (Anteau & Afton 2009). Given that *G. lacustris* did not establish at any other site, we cannot rule out the possibility that the response at Site 3 was from a chance natural colonization event not related to our translocation effort. However, given the sample size of sites in this BACI study (n = 19), it is likely that the *G. lacustris* at Site 3 was the result of translocation.

#### **Environmental Limitations**

Native aquatic vegetation communities, wetland connectivity, fish, and insecticide concentrations exert a strong pressure, limiting amphipod abundance in the PPR (Anteau et al. 2011; Keith 2021; Larson et al. 2022). The PPR has undergone extensive consolidation of drainage and increased connectivity among wetlands, which negatively affects amphipod abundance (Wiltermuth 2014; McCauley et al. 2015; Keith 2021). We suspect that such factors contributed to the unsuccessful translocations, although this is challenging to assess given our result of establishment at only one site. By selecting 19 sites for our BACI experiment using reasonable criteria consistent with available literature about G. lacustris habitat, we had expected to translocate G. lacustris into a number of different wetlands and find G. lacustris establishment in at least several wetlands, allowing us to compare habitat characteristics between wetlands where G. lacustris did and did not establish to refine our understanding of G. lacustris habitat needs for successful reintroduction. Our results were further complicated by unexpected limitations to our ability to survey all wetlands for all characteristics within the study time frame due to COVID-19 pandemic travel restrictions. Still, comparison of the characteristics of our surveyed study wetlands to G. lacustris habitat characteristics documented in the literature suggests that wetland characteristics may have prevented G. lacustris establishment in at least some study wetlands.

For example, fish likely had a negative effect on the chances of *G. lacustris* establishment in some recipient wetlands. Carleen (2022) found that *G. lacustris* were particularly sensitive to Black bullheads, with Black bullhead presence nearly perfectly explaining the absence of *G. lacustris* in wetlands in western Minnesota, potentially due to direct predation and the effects of bottom-feeding on water quality. In our posttranslocation surveys, we found Black bullheads at 46% of the sites that we surveyed for fish (6 of 13 sites; Sites 5, 8, 10, 15, 11, and 17). Black bullheads may thus have occurred and prevented *G. lacustris* establishment in about 10 (46%) of our study wetlands.

Several other recipient wetland characteristics were consistent with naturally occurring (not translocated) G. lacustris habitat but may have presented challenges to the establishment of new populations via translocation. Five of our surveyed wetlands lacking Black bullheads contained other fish species. Gammarus lacustris co-occur with these fish species in other prairie pothole wetlands at similar abundances (Anteau et al. 2011; Carleen 2022). Fish counts in recipient wetlands lacking Black bullheads (up to 690 fish) fell well within the range of fish counts in wetlands with naturally occurring G. lacustris in a concurrent study with the same survey methods (up to 2631 fish; Carleen 2022). However, previous work has found a negative relationship between G. lacustris density and fish (Anteau et al. 2011; Carleen 2022), such that fish presence reduced the probability of G. lacustris establishment. Translocated G. lacustris may have been especially vulnerable to fish predation due to their large numbers and tendency to cluster on the underside of the ice surface near the release location immediately following release. If any recipient wetlands were anoxic at depth, fish and amphipods may have been particularly likely to encounter each other near the surface. The two wetlands at Site 3 where G. lacustris established were fishless; however, G. lacustris did not establish in our other fishless recipient wetland (Site 13), indicating that a lack of fish alone is not sufficient for G. lacustris (This requires further testing given our extremely small sample size of three fishless wetlands.).

We note that Site 13's recipient wetland contained Tiger salamanders, a *G. lacustris* predator in prairie pothole wetlands (Olenick & Gee 1981), but so did Site 3 where translocated *G. lacustris* was established. Tiger salamanders have been found to have less influence on prairie pothole invertebrate communities than fish (Zimmer et al. 2000; Wiltermuth 2014), and this may be reflected by the establishment of *G. lacustris* at Site 3 only. Further research could assess the effects of Tiger salamanders and other predators on *G. lacustris* abundance and translocation success or failure.

Aquatic vegetation positively influences *G. lacustris* (Anteau et al. 2011; Larson et al. 2022). Anteau et al. (2011) found a positive relationship between the density of naturally occurring *G. lacustris* and the prevalence of submerged aquatic vegetation. Thus, the uniformly high (>94%) submerged aquatic vegetation prevalence in our recipient wetlands was likely supportive of *G. lacustris*, possibly by providing foraging substrate or shelter from predation. Similarly, Larson et al. (2022) found a positive relationship between *G. lacustris* density and submerged aquatic vegetation diversity in a concurrent study, where Simpson's diversity index values (computation methods comparable to those herein) of 0.80 or higher were associated with high

*G. lacustris* densities (Larson et al. 2022). Although all our surveyed recipient wetlands had Simpson's diversity values within the range in which *G. lacustris* occurred in Larson et al. (2022) (0.53-0.85 in Larson et al. 2022; 0.62-0.87 herein), four (33%) of our surveyed recipient wetlands (including fishless Sites 13 and 18 lacking Black bullheads) had values at the lower end of this range. Lower aquatic plant diversity or associated factors, such as wetland productivity (amphipod food resources), may have reduced the probability of *G. lacustris* establishment in those four wetlands.

Water chemistry, including total phosphorus and turbidity, may also have reduced the probability of G. lacustris establishment. Naturally occurring G. lacustris abundance is correlated with moderate-to-high productivity and turbidity in prairie pothole wetlands (Anteau et al. 2011; Larson et al. 2022). Total phosphorus and turbidity in recipient wetlands in this study were on the low end of the range where G. lacustris occurred in Larson et al. (2022) (total phosphorus 0.01-0.07 mg/L in this study versus 0.01-0.26 in Larson et al. 2022; turbidity 0.9-9.5 NTU in this study versus 0.73-61.5 in Larson et al. 2022). G. lacustris may benefit from at least moderately turbid water for several reasons, including reduced visibility to predators and increased food availability associated with higher nutrient concentrations (Anteau et al. 2011). On the other hand, we note that pH values for recipient wetlands in this study were likely appropriate for G. lacustris, given similarities to wetlands with G. lacustris in Larson et al. (2022) (8.0-8.9 in this study versus 8.0-9.6 in Larson et al. 2022). Wetland pH was likely not a factor in the lack of establishment.

Competition with other amphipod species was likely not a factor limiting establishment in our study, as Hyalella azteca and Crangonyx were found in high densities (>2000 and  $>200 \text{ m}^{-3}$ , respectively) in the Site 3 wetlands where G. lacustris were detected post-translocation, and G. lacustris failed to establish in wetlands with lower densities of H. azteca and Crangonyx species. However, we acknowledge that competition may have been masked by other factors limiting translocation success in most wetlands (other environmental factors or translocation methods). Gammarus lacustris has a narrower ecological niche compared to H. azteca, being more sensitive to benthivorous fish abundance (Anteau et al. 2011; Carleen 2022), salinity (Mushet et al. 2015; McLean et al. 2016), hydroperiod (Mushet et al. 2022), water nutrient concentrations (Keith 2021; Larson et al. 2022), and aquatic vegetation diversity (Larson et al. 2022), and is less common on the landscape (Anteau et al. 2011; Larson et al. 2022).

We did not assess pesticide or other contaminant concentrations in this study. However, Keith (2021) found a negative relationship between naturally occurring *G. lacustris* abundance and sediment pyrethroid concentrations in a concurrent study. *Gammarus lacustris* may experience negative physiological effects of these and other common pesticides in the PPR, including neonicotinoids and glyphosate (Demirci 2022), even when they occur at sublethal levels.

Our results suggest that more complex criteria for selecting recipient wetlands are likely necessary to achieve higher establishment rates. As a next step, a model relating naturally

occurring G. lacustris density to fish and plant communities, water quality parameters, connectivity, and surrounding land use, based on a meta-analysis of prior research with naturally occurring G. lacustris in the region, could be used to select recipient wetlands in which G. lacustris would be more likely to persist. Wetlands for which a model predicts high G. lacustris abundance (no habitat limitation), but G. lacustris are not present (dispersal limitations), ought to be good candidates for translocation. Combining information from prior research into a single model based on the fewest, most influential variables from the pool of correlated ecological and biological factors in wetlands (e.g. fish, aquatic vegetation, and water chemistry) would help target the most important types of preliminary surveys needed by a management organization contemplating translocating amphipods. For purposes of both model creation and model use, geographic information system derived predictor data (e.g. land cover and hydrology) would be logistically preferable to site-specific field data because it is available from many wetlands without the cost and effort of site visits. However, given that amphipods are sensitive to factors that are generally not derivable from aerial imagery at a broad scale (e.g. aquatic plants, insecticides, and fishes; Keith 2021; Carleen 2022; Larson et al. 2022), an accurate predictive model may need to be based on site-measured habitat data from many sites.

Wetlands that have recently been restored or enhanced may be particularly good candidates for G. lacustris translocations. Restoration actions may include drawdowns to improve water quality and aquatic vegetation communities and remove fish, fish barriers, piscicide treatment, or hydrological restoration to reduce wetland connectivity. Our work was partially inspired by an earlier translocation attempt (not part of this study) conducted by the Minnesota Department of Natural Resources in a shallow lake in southern Minnesota (Smith Lake, Cokato, MN, U.S.A.), which appeared to be successful because G. lacustris was detected post-translocation in multiple years (F. Bengston 2021, Minnesota Department of Natural Resources, personal written communication). A key difference from our experimental wetlands is that G. lacustris was translocated into Smith Lake after it was restored (i.e. the drawdown and installation of fish barriers). Although this example is only a single case, translocation into recently restored wetlands may be promising.

#### **Translocation Process**

Although recipient wetland conditions likely influenced our low establishment rate, we cannot discount the possibility that other aspects of our translocation process limited *G. lacustris* survival and reproduction. We found that most (>98%) *G. lacustris* were alive with intact limbs immediately prior to release, approximately 25% commenced or maintained reproductive behavior (precopulatory amplexus) throughout the collection and transport process, and video monitoring and sweep net sampling under ice at Site 14 did not reveal evidence of mass mortality immediately following release. However, our methods did not assess non-lethal stress that could affect reproductive rates or the long-term survival of translocated individuals. Experimental

translocations into recipient wetlands selected by a habitat model based on the occurrence of non-translocated *G. lacustris*, as described above, would help disentangle translocation-based limitations from environmental limitations.

Future research could also examine the optimization of the translocation methodology. For example, experiments with ad libitum feeding and tracking of tank water conditions in relation to donor and recipient wetland water conditions could be undertaken. Additionally, we translocated *G. lacustris* in winter (to avoid heat stress and for practical reasons of collecting large numbers of animals from under the ice surface); however, natural wetland colonization by *G. lacustris* takes place during the ice-free season (e.g. Mushet et al. 2022). Translocation in warmer seasons may correspond better with seasonal physiology (stress response and behavior). Collection of sufficient numbers of *G. lacustris* for translocation in the ice-free season would be challenging but has been done in the past (Mathias et al. 1982) and could be tried experimentally.

#### Local Adaptation

Local adaptation of source populations for translocation, which is especially likely in taxa with limited dispersal like amphipods, can pose an additional challenge to choosing appropriate recipient sites (Jourdan et al. 2019). Studies of amphipod taxa such as H. azteca and G. fossarum have revealed the existence of local genetic adaptation and even species complexes (cryptic species), often with varying responses to environmental factors such as predators and contaminants (Åbjörnsson et al. 2004; Weston et al. 2013; Weigand et al. 2020). Given that our donor wetlands contained fish predators and were not pristine (i.e. adjacent to roads and farmlands with associated runoff, and not occurring on remnant or restored prairie), we suspect that this was not a major cause of low population establishment in this study. However, to our knowledge, the genetic diversity of G. lacustris in North America has not been explored, and future translocation attempts would benefit from the study of genetic diversity within the PPR. In addition to informing the choice of recipient wetland conditions, such information would help inform the choice of source populations to conserve intraspecific diversity.

#### Sampling Intensity

Apparent *G. lacustris* absences post-translocation could reflect limitations in our ability to detect very low densities of amphipods using our sampling methods. We sampled 4–12 points per wetland, based on wetland size, resulting in sampling areas of 1.4–7.5 m<sup>2</sup> per wetland (0.002–0.03% wetland area). These sampling areas, comparable to prior studies of naturally occurring amphipods (e.g. Lindeman & Clark 1999; Anteau et al. 2011; Janke et al. 2019), would have allowed us to detect *G. lacustris* at densities of  $0.7 \times m^{-2}$  if they were evenly distributed around the wetland. Our sampling intensity was likely sufficient to detect the establishment of *G. lacustris* abundances that would provide substantial wildlife forage (e.g. ≥26 m<sup>-3</sup>; Anteau & Afton 2009), such that future translocations could consider selecting recipient wetlands using a habitat model. However, given our result of detectable *G. lacustris* at only one site post-translocation, future studies of this species could sample more locations per wetland to detect extremely low densities, in the interest of troubleshooting translocation methods and site selection.

#### **Emergent Vegetation**

Most of our study wetlands had a fringe of emergent vegetation (usually Typha spp.) that we could not sample with our sweep net method. Consequently, similarly to previous work that identified regional amphipod population declines (e.g. Anteau & Afton 2008a), we sampled the open water zone of wetlands. It is possible that we failed to detect G. lacustris within the emergent fringe, a potential limitation noted in Larson et al. (2022). However, it is unlikely that our inability to sweep within the emergent fringe caused us to miss any populations of G. lacustris large enough to provide substantial wildlife forage because G. lacustris associates with patches of submerged aquatic vegetation in addition to the emergent fringe (Menon 1969; Hanson 1990; Larson et al. 2022), and our sampling points usually fell within submerged aquatic vegetation beds. Still, if future researchers desired to search for low G. lacustris densities in emergent fringe, activity traps or polyvinyl chloride pipe samplers (Christensen & Crumpton 2010) could be used.

## Sampling Phenology

In the future, more frequent sampling to disentangle translocated amphipod mortality, reproduction, and juvenile mortality could improve researcher ability to assess amphipod translocations. Our first post-translocation sampling occurred in September-October approximately 9 months after stocking, when prairie populations of G. lacustris consist primarily of new recruits. with the parental generation having ended their annual lifespan (Menon 1966; Biette 1969). This timing was intended to assess comparative amphipod densities after a complete reproductive cycle, but this sampling regime left us unable to disentangle mortality and reproductive rates. Consequently, we could not differentiate whether low G. lacustris occurrence was caused by adult mortality, poor recruitment and subsequent senescence of translocated adults, juvenile mortality, or some combination of these factors. In winter, ice auger sampling, although not reliably quantitative, could be used to detect presence/absence and complement more labor- and equipment-intensive methods (e.g. underwater videos). Assessment of sampled females for the occurrence of eggs/young in brood pouches (Biette 1969; Menon 1969; Wilhelm & Schindler 2000) could also provide useful diagnostic information. For example, 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 the widespread occurrence of unfertilized or malformed eggs or dead broods can be indicators of sublethal physiological stress in amphipods, potentially due to environmental conditions (Wiklund & Sundelin 2001) or translocation. High juvenile

mortality could indicate predators that select smaller prey, including predation by adult *Gammarus* (Macneil et al. 1999).

#### **Potential Paths Forward**

Our results highlight the challenging nature of conservation translocation for freshwater invertebrates, similar to other experiments (Jourdan et al. 2019). Our BACI study design allowed for rigorous testing of *G. lacustris* translocation efficacy in wetlands, with the conclusion that translocation was not effective at increasing densities within a few years post-translocation using our translocation methods. However, with population establishment at only two wetlands on a single site (and thus fairly similar to each other), our ability to assess wetland characteristics facilitating or inhibiting population establishment was limited.

However, documentation of our translocation methods and BACI experiment is instructive for future work with prairie pothole amphipods. In the absence of sufficient establishment rates to rigorously evaluate wetland characteristics correlated with reintroduction success, a predictive model for selecting recipient wetlands based on habitat characteristics associated with naturally occurring G. lacustris, may provide a path toward restoring G. lacustris via translocation. Wetland habitat restorations to achieve model-informed ideal conditions for recipient wetlands may be useful. Given the low establishment rate in this study, we recommend that such future work start with a small number of wetlands that can be rigorously sampled (more sample points) throughout the year following translocation to detect even very low densities of G. lacustris, disentangle adult mortality from recruitment and juvenile mortality, and (if possible) provide proof-of-concept for translocation methods.

More broadly, freshwater invertebrate conservation translocation projects with new (not previously translocated) species that encounter similar challenges to ours may benefit from experimentation with a very small (less than our n = 19) sample size of well-surveyed pilot sites with habitat characteristics selected based on a model of natural-occurring abundance of the species, in restored or enhanced sites if necessary. For invertebrate species, which are often understudied, this may entail one or more extensive preliminary studies of habitat characteristics associated with species abundance or occurrence. Study of the natural history of local populations (e.g. number of broods per year) would also help inform expectations and the sampling effort necessary to monitor translocated populations. Investment in this additional pilot work to confirm that translocation methods can be successful in ideal habitat conditions (or to refine methods of translocation or refine understanding of ideal habitat conditions) and to choose appropriate monitoring methods may help facilitate effective follow-up BACI-style experiments with larger sample sizes for rigorous quantitative assessment of success rates and cost-benefits.

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## **Supporting Information**

The following information may be found in the online version of this article:

**Supplement S1.** Flow rate tests for ice auger sampling of amphipods.

**Supplement S2.** Estimates of numbers of *Gammarus lacustris* that might survive to fall sampling, given release densities and mortality rates from the literature.

Supplement S3. Wetland characteristics survey methods.

 Table S1. Average Gammarus lacustris densities (individuals/m³) from ice auger (January 2018) and sweep net (all other timepoints) surveys.

Table S2. Prevalence (percentage of sample points where taxon was present) of submerged and floating aquatic plant macroalgae taxa in a subset of recipient wetlands. Table S3. Prevalence (percent of sample points where taxon was present) of submerged and floating aquatic plant and macroalgae taxa in a subset of control wetlands. Table S4. Relative abundance of fish taxa in subset of wetlands from a before-after/

control-impact study of analysis of *Gammarus lacustris*) conservation translocation. **Table S5.** Estimated release density of *Gammarus lacustris* (individuals/m<sup>2</sup> of wetland surface area).

**Table S6.** Number of *G. lacustris* release locations and release density; number of points sampled for *G. lacustris*; and area and depths sampled with sweep nets.

**Table S7.** Percentages of dead, visibly injured, and paired (in precopulatory amplexus) *Gammarus lacustris* in samples collected from transport coolers immediately prior to release in winter 2017–2018.

 
 Table S8. Recipient wetland-control wetland G. lacustris density differences at varying timepoints before and after G. lacustris translocation.

**Table S9.** Recipient wetland-control wetland G. lacustris density differences at varying timepoints after G. lacustris translocation for 19 sites.

**Figure S1.** Amphipod densities (individuals/m<sup>3</sup>) measured via dip net and ice auger surveys in a before-after/control-impact study of *Gammarus lacustris* translocation. **Figure S2.** Prevalence (percent of sample points where taxon was present) of sub-

merged and floating-leaved aquatic plant taxa in a subset of study wetlands. **Figure S3** Palative abundance of fish taxa in a subset of (A) recipient wetlands the

**Figure S3.** Relative abundance of fish taxa in a subset of (A) recipient wetlands that received translocated *Gammarus lacustris* amphipods and (B) associated control wetlands.

Figure S4. Diagram illustrating volume of water and horizontal distance swept by sweep net in amphipod surveys.

**Figure S5.** Histograms showing distribution of the differences in *G. lacustris* density (recipient minus control wetland) for each site at each timepoint.

**Video S1.** Demonstration of *Gammarus lacustris* release into a recipient wetland as part of a 3-year before-after/control-impact study of *G. lacustris* conservation translocation.

Video S2. Demonstration of survey method for *Gammarus lacustris* in winter using an ice auger.

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Video S3. Monitoring translocated *Gammarus lacustris* using an underwater camera as part of a 3-year before-after/control-impact study of *G. lacustris* conservation translocation.

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