Wetlands (2024) 44:72
https://doi.org/10.1007/s13157-024-01808-5

ORIGINAL RESEARCH ARTICLE

Fish Invasion of Prairie Pothole Wetlands Reduces Amphipod Abundance, A Key Vertebrate Forage

Jake D. Carleen[1] · Danelle M. Larson[2] · Michael J. Anteau[3] · Megan J. Fitzpatrick[4] · Andrew W. Hafs[5] · Carl W. Isaacson[1] · Breanna R. Keith[1]

Received: 30 August 2023 / Accepted: 16 April 2024

Abstract
Fishes have spread into previously fishless wetlands, likely affecting other species. In the Prairie Pothole Region of North America, the invasion of fish into wetlands is facilitated by interactions of altered land use, climate, and hydrology. We aimed to understand the effects of fishes on amphipods, which are macroinvertebrates that vertebrates rely on as forage. We hypothesized the presence and abundance of fish, particularly benthivores, would have detrimental effects on amphipod abundance. Our study design targeted a large gradient of amphipod abundances among wetlands, including very high abundances of two amphipod species: *Gammarus lacustris* and *Hyalella azteca*. We found that fishless basins had twice as many amphipods as those with fish, on average. *Gammarus lacustris* were not detected in the presence of Black Bullhead *Ameiurus melas*. The abundance of both amphipod species had negative associations with the most common fishes, Fathead Minnow *Pimephales promelas* and Brook Stickleback *Culaea inconstans*. A multivariate community analysis showed the benthivore-fish functional feeding guild was negatively associated with the amphipod community, as hypothesized. However, our study design captured several wetlands with anomalies of high abundances of both fish and amphipods, obscuring their relationships. Our results aid resource managers by confirming several fish guilds and species are associated with lower abundances of amphipods. These findings can inform resource managers who make decisions about managing for fish and wildlife; for example, they may choose to manage existing fish populations or protect existing wetlands with high amphipod densities from new fish invasions.

Keywords Amphipoda · Benthivore fishes · Fish invasion effects · *Gammarus lacustris* · *Hyalella azteca* · Prairie Pothole Region wetlands

Introduction

Many wetland species provide services to humankind, and so the factors diminishing biodiversity need to be identified. Aquatic invertebrates are essential for many ecosystem functions but are vulnerable to habitat alterations, especially if the invertebrates have low dispersal abilities or are endemic to specific regions (Strayer 2006). Amphipods (crustaceans in the taxonomic order Amphipoda) are considered an "umbrella" species whose protection may extend to benefit many species. For example, amphipod abundance is positively associated with biodiversity and overall community structure of the wetland plant community (Larson et al 2022).

Amphipods are prominent and important organisms in the Prairie Pothole Region (PPR) of North America. The PPR

✉ Danelle M. Larson
dmlarson@usgs.gov

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

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

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

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

5 Bemidji State University, Aquatic Biology Program, Department of Biology, 1500 Birchmont Drive Northeast, Bemidji, MN 56601, USA

is an expansive mosaic of grasslands and depressional wetlands, covering approximately 800,000 square kilometers. The PPR is considered one of the most productive habitats for waterfowl in the world, and amphipods are key forage for species of conservation concern like lesser scaup *Aythya affinis* (Anteau and Afton 2008a; Anteau et al 2014). Lesser scaup primarily feed on amphipods and seek wetlands with high amphipod abundance (Lindeman & Clark 1999; Anteau and Afton 2009). Amphipod availability in this region is likely declining, as indicated by studies showing the diets from lesser scaup in 2000 contained 94% less amphipods when compared to diets from the same location in the 1980s (Anteau and Afton 2006, 2008a). Amphipod abundance is known to be affected by aquatic plants, insecticide concentrations, watershed land use, and fish (Anteau et al 2011; Hanson et al 2012; Janke et al 2019; Keith 2021; Larson et al 2022).

Observational and experimental studies have shown increased fish presence negatively affects amphipods, yet sometimes fish effects were strong and other times were moderate (Bouffard and Hanson 1997; Anteau et al 2011). However, the relationships between fish and amphipods across a large gradient of amphipod density have not been studied, particularly for wetlands with a very high abundance of amphipods. Prior studies surveyed a random sample of wetlands that yielded few wetlands with high amphipod densities, which limited knowledge of the magnitude to which various fish species and functional feeding guilds influence amphipod abundance (Anteau and Afton 2008b; Anteau et al 2011).

Over the past few decades, the lesser scaup population has declined concurrently with amphipods on scaup's important spring staging habitats in the PPR (Anteau et al 2014), and the scaup declines seemingly coincide with an increase in fish abundance in this region (Afton et al 1991; Anteau and Afton 2008b). Historically, prairie pothole wetlands underwent frequent drying and freezing that limited fish establishment. However, recent landscape-level hydrological changes like consolidation drainage and changes in precipitation events have synergistically resulted in greatly increased runoff into extant wetlands (Anteau et al 2016; McKenna et al 2019). Such changes have resulted in increased water levels and connectivity among wetland potholes (Anteau 2012; McCauley et al 2015), allowing for invasion and persistence of fish into previously fishless wetlands. Fish are now present in about 80% of semi-permanent and permanent wetlands and shallow lakes in the PPR (Anteau and Afton 2008b; Hanson et al 2012; McLean et al 2016), and one study documented a notable increase in the abundance of fishes in the PPR from 2004 to 2011 (Wiltermuth 2014).

Amphipod abundance likely declines in association with increases in fish abundance directly through predation and indirectly through fish-induced wetland alterations (Friederichs et al 2011; Maurer et al 2014). In the PPR, Fathead Minnow *Pimephales promelas* are very abundant and detrimental to macroinvertebrate densities by predation (Friederichs et al 2011), competition for detritus as food (Anteau et al 2011), and causing bioturbation that affects wetland quality (Maurer et al. 2014). In addition, other common PPR fishes like Brook Stickleback *Culaea inconstans* readily consume amphipods when available (Tompkins & Gee 1983). Benthivorous fish, like the invasive Common Carp *Cyprinus carpio* and native Black Bullhead *Ameriurus melas,* reduce submerged aquatic vegetation (SAV; Fischer et al. 2013; Mork et al. 2009) that is beneficial to amphipods (Hanson et al 2012; Larson et al 2022).

In this study, we evaluated how fish abundance, species composition, and functional feeding guilds affected amphipod abundance in PPR wetlands. We deliberately incorporated wetlands with high amphipod densities to better ascertain the effects of fish on amphipods compared to previous studies using a random selection of wetlands (Anteau & Afton 2008a, b; Janke et al. 2019). We hypothesized that amphipod abundance was negatively associated with the presence and abundance of fish because of the direct and indirect effects fish can have on invertebrates. We also hypothesized the benthivore-fish functional feeding guild would have the greatest effects on amphipods because both organisms would co-occur on the benthos (i.e., wetland bottom), where predation and bioturbidity would negatively affect amphipods.

Methods

Study Area & Design

The study area includes several large ecoregions (Lake Agassiz Plain, Northern Glaciated Plains, Northern Lakes and Forests, and Western Corn Belt Plains) from across Minnesota that represent the eastern portion of the PPR. We sampled 59 semi-permanent wetlands and shallow lakes (hereafter, wetlands) during the spring and summer of 2019 and 2020. Western Minnesota and specifically the PPR are known to have wetlands with conditions that help amphipods survive and sometimes thrive (Anteau et al 2011; Larson et al 2022).

We aimed to better understand the associations between fish and amphipods, so we designed an observational study that intentionally sampled two types of wetlands: wetlands with very high amphipod densities (> 500 individuals/m^3) and wetlands with a gradient of densities (randomly selected). Prior to this study, we used a rapid assessment protocol (Carleen 2022) to design and conduct rapid assessments on 111 wetlands in May 2018 to identify those with relatively high densities of amphipods. Roughly half of the 59 wetlands sampled for this study were selected based on the greatest amphipod densities detected during the rapid assessments (Figure S1, Table S1). The other half of sampled wetlands were randomly selected from the National Wetlands Inventory Database 2020 shapefiles to capture a gradient of amphipod densities. Candidates for selection included wetlands with semi-permanent or permanent hydroperiods, which helped ensure that selected wetlands were able to support amphipods without desiccation or winterkill and had similar wetland size distributions to the high amphipod density wetlands. We further constrained the selection of random wetlands, so they were within 24 km of a high amphipod abundance wetland to facilitate travel efficiency.

Amphipod Field Sampling and Laboratory Processing

Crews began amphipod sampling in the southern portion of the study area and moved north following the timing of ice-out (March–April 2019 and 2020) to target adult amphipods, similar to (Anteau and Afton 2008b). We crafted novel amphipod sampling techniques most suitable for depressional, mucky wetlands as described by (Keith et al 2022). In summary, we sampled amphipods at eight random sampling locations along evenly spaced transects. Two sampling locations occurred along each transect, specifically at 2 m from the shoreline (shallow water) and 50 m from the shoreline (open, deeper water). Sampling was restricted to water depths between 0.3 m and 1.2 m to ensure accurate density estimates. We collected samples with two D-shaped sweep nets (1,200 µm mesh) from the benthos and water column, and all sweep net contents (including debris and invertebrates) were immediately preserved with 95% ethanol in containers.

We processed sweep net samples using a standard operating procedure in the laboratory (Keith et al 2022). In summary, we emptied the sample contents into a 500 µm sieve and rinsed to remove fine particles. We then placed a grid with four equal-sized openings over the sieve and processed the contents of one randomly selected grid at a time, removing all macroinvertebrates from the benthic debris under a 3 × lighted desk magnifier. We continued processing grids until at least 25% of the grids or 400 macroinvertebrates were picked, whichever came first. We identified amphipods under 110 × microscopy and identified individuals to either the genus-level (*Crangonyx spp.*) or to the species-level (*Gammarus lacustris* and *Hyalella azteca*). We reported speciated amphipod densities from the benthos and water column as individuals/m^3.

Fish Sampling

We sampled the fish community in April–June in 2019 and 2020, which was a few weeks after the amphipod sampling. The differences in sampling timing between organisms was based on sampling efficacy (e.g., adult amphipods were best detected in spring) and secondarily for logistics (e.g., we had larger field crews to sample fish in early summer). We also timed fish sampling to occur before potentially dense aquatic vegetation would hinder the setting, retrieval, and efficacy of fish gear in late summer. We surveyed fish count and biomass (both the total biomass of all fish and each fish species) in each wetland using one gill net and three mini fyke nets. We used a short gill net (21 m × 2 m composed of 3 m panels, ranging in mesh size from 1.9–7.6 cm) due to the small size of study wetlands (often < 1 hectare). In contrast to (Anteau et al 2011), we did not use minnow traps due to low effectiveness of capturing fish in wetlands with low fish abundances during our pilot studies (Carleen 2022). Instead, we used mini fyke nets (6.5 mm bar mesh with 4 hoops, 1 throat, 7.62 m lead, and a 0.69 × 0.99 m rectangular frame opening; Duluth Trading Company, Mt. Horeb, WI) to capture many fish species and sizes. Each net was placed along a randomly selected amphipod sampling transect. All fish gear were left for an overnight soak. Most gear were deployed for approximately 19 h. A few nets were deployed for shorter or longer periods (16–22 h) because of typical logistical constraints.

Crews processed catch on-site immediately after retrieving gear. All catch were identified, weighed, measured for length, and enumerated by species. Crews sub-sampled catch (minimum of 20% by weight) if the estimated time to process a net would be over 30 min. We calculated a variety of metrics, including overall total number of fish caught, total fish biomass, catch per unit effort (CPUE; defined as the number of fish caught per unit time and effort, which was 19-h overnight set using the three nets), and species-specific CPUE and biomass.

Wetland Characteristics as Key Covariates

Crews sampled SAV biomass because SAV biomass might represent habitat structure for both fish and amphipods. We sampled SAV using the methods described in Larson et al (2022). Briefly, we sampled SAV in July–August 2019 and 2020 from boats with the number of sampling locations scaled to wetland size. Crews dragged a plant rake alongside the boat for 1.5 m and identified aquatic plants species collected and their relative biomass. Crews used a Hach HQ30D multimeter to measure pH. We calculated wetland area using National Wetland Inventory Database 2020 shapefiles in ArcGIS.

Data Analyses

All data are publicly archived online (Carleen et al 2024). For aquatic vegetation and fish data entries into spreadsheets, we used double data entry and then compared entries with the R package 'arsenal' to minimize data entry errors. Any data entry errors were reconciled and corrected via referring to the original field data sheets. We periodically detected the amphipod *Crangonyx spp*. in study wetlands but omitted it from our analyses due to low relative abundance.

Two types of statistical analyses were used to test the hypotheses: (1) generalized linear models to test hypotheses that individual fish species had significant, negative associations with amphipods, and (2) redundancy analysis (RDA) to statistically test the fish functional feeding guild associations with amphipod abundance.

We modeled *G. lacustris* and *H. azteca* data separately using generalized linear models using R 4.1.3 (2022, R Development Core Team, Vienna, Austria) and the 'glmmTMB' package (Brooks et al 2017). Initially, a Poisson distribution was tested but the resulting models were over-dispersed, so we used a negative binomial distribution and log link due to right skew and improved model fits, similar to the methods of related studies (Anteau and Afton 2008b; Larson et al 2022). Conceptually, the response variable was the density of amphipod species per wetland (m^3). Model inputs were counts of amphipods per wetland with the natural log of the total water volume swept per wetland included as an offset variable. The offset variable controlled for the differing volumes of water swept according to wetland depths and allowed the model to compute density. We chose to report amphipod density rather than counts because it is more comparable to prior studies with different study designs and accounts for the water volume swept, which changed with the number of sample locations and water depths. Two wetlands were removed from the *G. lacustris* negative binomial analyses due to extreme data outliers (defined as 5 times greater than the interquartile range for Fathead Minnow counts).

We used CPUE as the predictor variables in the negative binomial models. We selected the primary predictor variables as CPUE of Fathead Minnow, Brook Stickleback, and Black Bullhead to match hypotheses and because these species were caught with high frequency and variable abundances. Black Bullhead and *G. lacustris* were nearly mutually exclusive, co-occurring in only one wetland, and therefore the relationship between them could not be modeled with regression. We also reported the results from the negative binomial analyses with the two outliers retained for full transparency (Supporting Information Table S2). For the *G. lacustris* model, the CPUE of Fathead Minnow and Brook Stickleback were square root transformed to improve the model's predictive power and meet model assumptions of linearity and equal variance.

We included amphipod habitat covariates to improve the model fit and obtain the best parameter estimates for the primary predictors of fish. Potential covariates initially included SAV biomass, average depth, wetland pH, total phosphorus, and wetland size. We graphically observed a threshold effect of wetland area in relation to the abundance of both amphipod species, so we created a binary variable that classified wetlands as either > 15 ha or < 15 ha. The primary predictors, which were the CPUE of select fish species and habitat covariates, were weakly correlated ($r < 0.35$).

We constructed a global model for each amphipod species that included the three primary fish predictors and potential covariates. Then, we manually used a backwards stepwise selection approach to remove environmental covariates with a p-value > 0.10 but did not remove our primary fish covariates regardless of statistical significance. We reported the relative magnitude of fish effects on amphipods, ± 90% confidence intervals from the model coefficients, and the p-value with degrees of freedom to assess ecological significance of fish associations with amphipods (Wasserstein et al 2019).

We examined the role of fish functional feeding guild on amphipod abundance using redundancy analysis (RDA). The RDA is an extension of multiple regression where the ecological community is constrained by linear combinations of the predictor variables (Legendre 2018). The response variables (*G. lacustris* and *H. azteca*) were amphipod count data that were log-transformed. The predictor variables included the CPUE of fish that we assigned into three functional feeding guilds (benthivore, piscivore, and planktivore; Table 1). The variance inflation factors found no redundancy in the model (VIF < 10), so all predictor variables were included. We conducted a permuted (perm = 9,999) multivariate analysis of variance (PERMANOVA) to assess the overall RDA models, RDA axes, and RDA terms.

Results

Amphipod Densities Were Greater in Fishless Wetlands

Among our surveyed wetlands, 19% were fishless, while the other 81% had fish (Fig. 1). The average densities of amphipod species were nearly double in fishless wetlands compared to those with fish (Welch's t-test: $p > 0.10$ for both amphipod species). Wetlands with fish had uniformly low

Table 1 A summary of 23 species of fish captured in 59 wetlands in western Minnesota, USA during springs and summers of 2019–2020. The names (American Fisheries Society abbreviation, common name, and scientific name), functional feeding guild ("FFG"; PLK = planktivore, BEN = benthivore, PIS = piscivore), prevalence (percent of the 59 sampled wetlands where species was detected), total counts captured from 59 wetlands (the capture per unit effort was a 19-h overnight soak for one gill net and two fyke nets), and mean counts in a wetland (including wetlands with no detections)

Abbreviation	Common Name	Scientific Name	FFG	Prevalence (%)	Count (total)	Count (mean)
FHM	Fathead Minnow	*Pimephales promelas*	PLK	68	21,418	363.00
BSB	Brook Stickleback	*Culaea inconstans*	PLK	51	961	16.29
BLB	Black Bullhead	*Ameiurus melas*	BEN	29	1,318	22.34
GSF	Green Sunfish	*Lepomis cyanellus*	PLK	12	132	2.24
DCE	Dace spp.	*Chrosomus spp.*	PLK	10	2,596	44.00
WAE	Walleye	*Sander vitreus*	PIS	10	159	2.70
YEP	Yellow Perch	*Perca flavescens*	PLK	8	138	2.34
CMM	Central Mudminnow	*Umbra limi*	PLK	8	140	2.37
CAP	Common Carp	*Cyprinus carpio*	BEN	8	22	0.37
NOP	Northern Pike	*Esox lucius*	PIS	8	19	0.32
OSS	Orange-Spotted Sunfish	*Lepomis humilis*	PLK	7	98	1.66
PMK	Pumpkinseed	*Lepomis gibbosus*	PLK	7	16	0.27
WTS	White Sucker	*Catostomus commersonii*	BEN	5	206	3.49
GSH	Golden Shiner	*Notemigonus crysoleucas*	PLK	5	13	0.22
LMB	Largemouth Bass	*Micropterus salmoides*	PIS	5	6	0.10
BLC	Black Crappie	*Pomoxis nigromaculatus*	PLK	5	11	0.18
BLG	Bluegill	*Lepomis macrochirus*	PLK	5	165	2.80
BMB	Bigmouth Buffalo	*Ictiobus cyprinellus*	BEN	3	10	0.17
BOW	Bowfin	*Amia calva*	PIS	3	6	0.10
MSK	Muskellunge	*Esox masquinongy*	PIS	2	1	0.02
TPM	Tadpole Madtom	*Noturus gyrinus*	BEN	2	10	0.17
YLB	Yellow Bullhead	*Ameiurus natalis*	BEN	2	7	0.12

amphipod density while amphipod density varied widely among fishless wetlands, indicating that wetlands with fish were likely to lack amphipods and that fishless wetlands were less predictable for amphipod density. The greater variability in fishless wetlands could also arise from minor differences in fish or amphipod sampling or the differences of habitats between fish- and fishless wetlands. Several wetlands had anomalies of very high abundances of either amphipod species in the presence of fish.

Amphipod Densities were Lower with Greater Fish Abundances

Catch of fish varied considerably, with total fish catch ranging from 0–2,631 fish/19 h, with the median value being 185 fish/19 h. Total fish biomass also varied widely, ranging from 0–41 kg/19 h. The three most encountered fish species were Fathead Minnow, Brook Stickleback, and Black Bullhead (Table 1). Black Bullhead and Fathead Minnows were the primary contributors to total fish biomass, accounting for 26% and 24% of biomass, respectively. Twenty-two species of fish were sampled and varied widely with respect to prevalence and CPUE (Table 1). The most prevalent species was Fathead Minnow (68% of wetlands), with CPUE up to 2,621 fish/19 h in a single wetland. Brook Stickleback occurred in 51% of wetlands with CPUE up to 265 fish/19 h. Black Bullhead were present in 29% of wetlands with CPUE towards 400 fish/19 h. Dace *Chrosomus spp.* had only 10% prevalence but were often in high abundance, if present.

Amphipods Were Negatively Associated with Several Common Fish Species

Gammarus lacustris were negatively associated with the three dominant fish species: Black Bullhead, Fathead Minnow, and Brook Stickleback (after accounting for other environmental covariates). *Gammarus lacustris* were not detected in the presence of the Black Bullhead, a benthivore, except for one basin with co-occurrence of very low density of *Gammarus* (Fig. 2). The perfect separation of *G. lacustris* and Black Bullhead detections prohibited modeling their relationship further. As hypothesized, *G. lacustris* counts were also negatively correlated with Fathead Minnow and Brook Stickleback CPUE (Table 2, Fig. 2, Fig. 3).

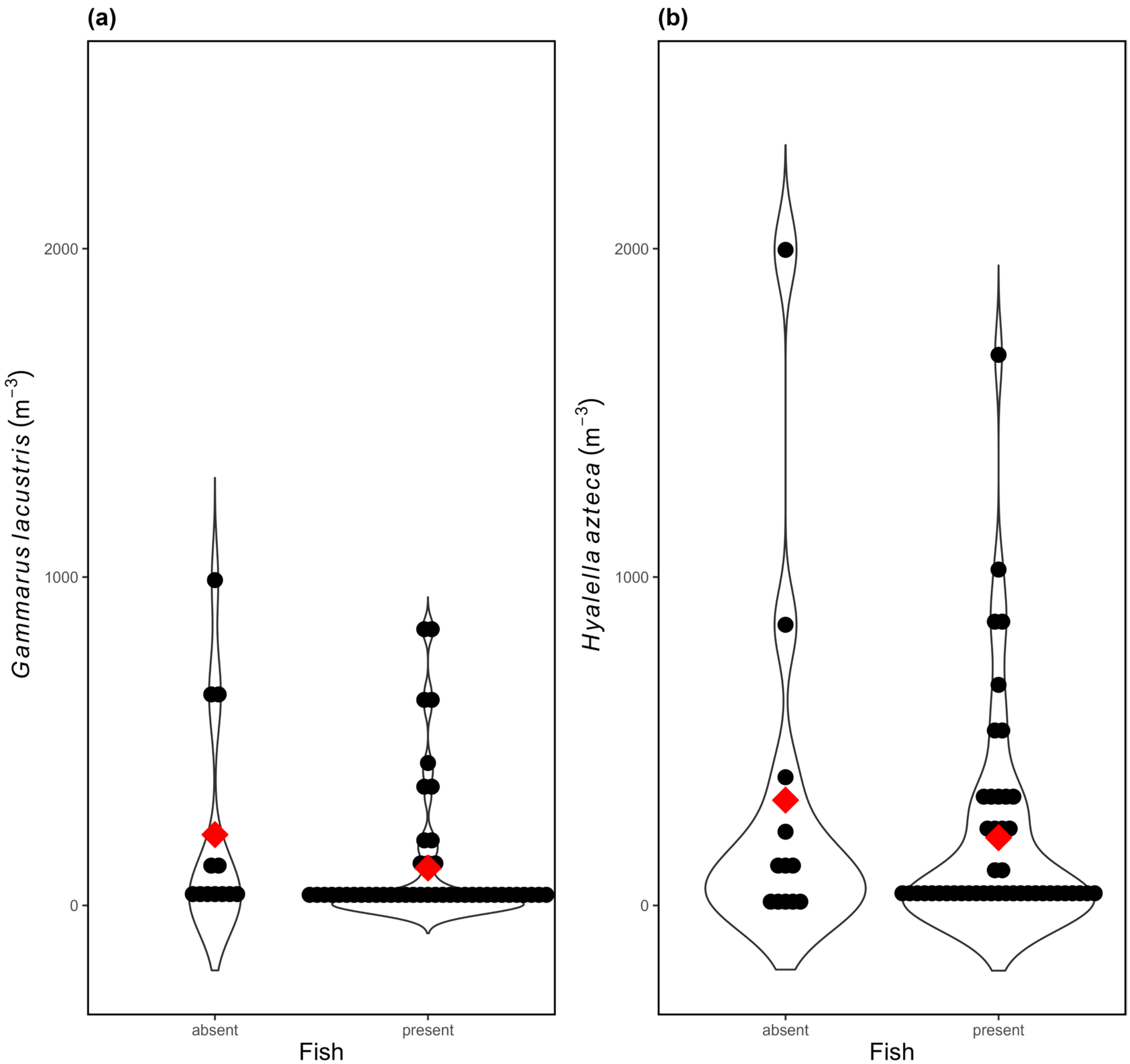

Fig. 1 The densities of *Gammarus lacustris* (panel a) and *Hyalella azteca* (panel b) in 59 prairie pothole wetlands of Minnesota, USA in years 2019–2020. The violin plots contrast the wetlands that are fishless (fish not detected) to those with any number and species of fish (fish present), and the impacts to amphipod densities. The catch per unit effort (CPUE) for this study was a 19-h overnight soak for one gill net and two fyke nets. The black dots are amphipod densities from 59 wetlands, the red diamond indicates the mean amphipod density, and the violin plots show the data distributions

Gammarus had a non-linear response to Brook Stickleback and were predicted to be absent when Brook Stickleback counts exceeded 100 CPUE. The covariates indicated a positive correlation of *G. lacustris* with wetland pH and negative correlations to SAV biomass and wetland size over 15 hectares, analogous to previous studies guiding the use of these covariates (Anteau et al 2011; Larson et al 2022). The negative binomial regression results were similar when removing or retaining the two outliers (Table 2, Table S2).

Hyalella azteca varied in its responses to various fish species (Table 2, Fig. 2, Fig. 4). *Hyalella azteca* was relatively abundant in wetlands with low to moderate CPUE of Fathead Minnow; however, *Hyalella*'s response to high CPUE of Fathead Minnow was either positive or negative and had large confidence intervals encompassing 0 m^{-3}. In contrast, *H. azteca* were negatively associated with CPUE of Brook Stickleback and Black Bullhead. The covariate of SAV biomass indicated positive correlation with *H. azteca*, like Larson et al. (2022). The negative binomial

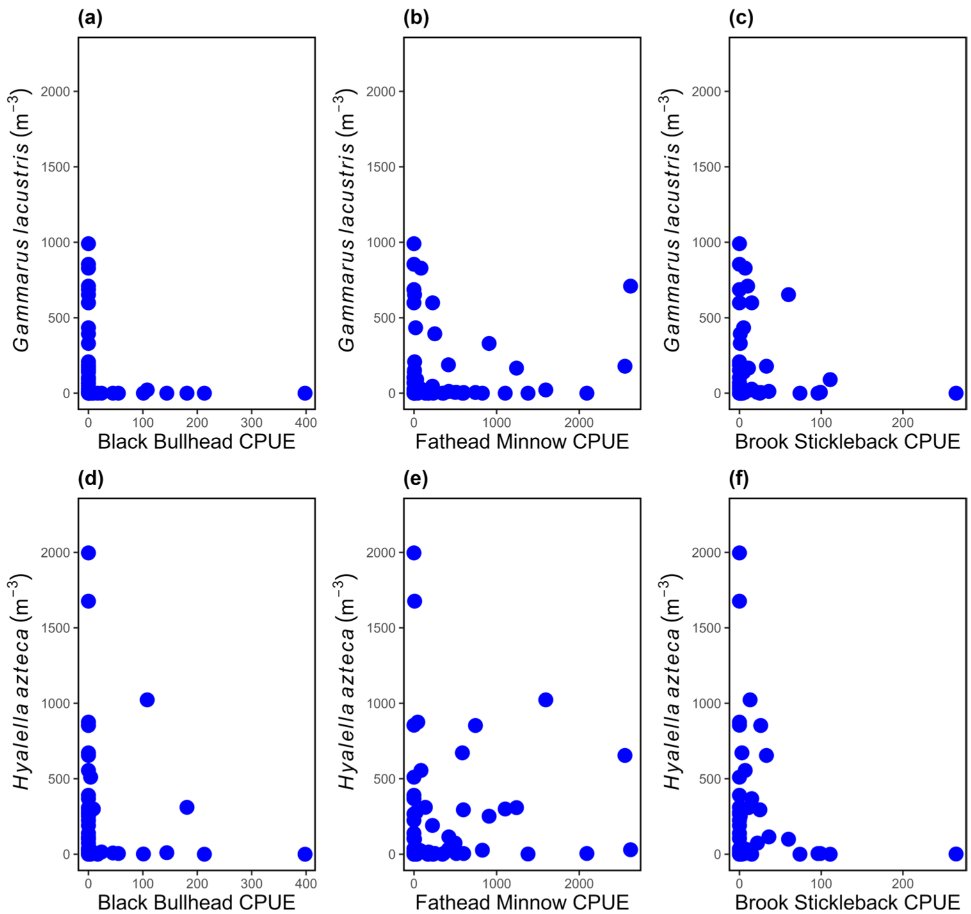

Fig. 2 The raw (unmodeled) associations between three common fish species and two amphipod species (*Gammarus lacustris* and *Hyalella azteca*) in 59 wetlands sampled in 2019–2020 from prairie pothole wetlands of Minnesota, USA. The three fish species include Black Bullhead *Ameiurus melas*, Fathead Minnow *Pimephales promelas*, and Brook Stickleback *Culaea inconstans*. The catch per unit effort (CPUE) was a 19-h overnight soak for one gill net and two fyke nets. The two extreme outliers (defined as greater than 5 times the interquartile range) of Fathead Minnow CPUE (> 2,500 Fathead Minnow) were retained in panel (b) and panel (e)

Table 2 Results of final models using negative binomial regression analysis for *Gammarus lacustris* and *Hyalella azteca* (*n* = 57 wetlands; two extreme statistical outliers were removed) in prairie pothole wetlands of Minnesota, USA in years 2019– 2020. The fish terms are primary hypothesized variables, and the other variables (plants, pH, and wetland size) are covariates included to improve parameter estimates for fish effects. The counts of Fathead Minnow and Brook Stickleback are square root transformed (^{2}) in the *Gammarus lacustris* model to improve the model's predictive power and meet model assumptions. Wetland size threshold is a binary variable used to classify wetlands either as > 15 ha and < 15 ha

Effect on *Gammarus lacustris*	*E*	SE	*Z*-score	*P*
Fathead Minnow count ^{2}	-1.10e-06	6.29e-07	-1.75	0.08
Brook Stickleback count^2	-2.61e-04	1.66e-04	-1.58	0.11
Submerged aquatic plant (SAV) biomass	-1.89	0.60	-3.15	< 0.01
pH	2.63	1.03	2.55	0.01
Wetland size threshold	-2.55	0.97	-2.63	< 0.01
Effect on *Hyalella azteca*	***E***	**SE**	***Z*-score**	***P***
Fathead Minnow count	1.19e-04	6.25e-04	1.91	0.05
Brook Stickleback count	-2.32e-02	7.05e-03	-3.29	< 0.01
Black Bullhead count	-1.12e-02	-5.89e-03	-2.06	0.04
Submerged aquatic plant (SAV) biomass	0.62	0.33	1.89	0.06

regression results were similar regardless of removing or retaining the two outliers with high Fathead Minnow biomass (Table 2, Table S2).

Fish Functional Feeding Guilds Affected Amphipods

Amphipods were negatively associated with the fish community and fish functional feeding guilds (PERMANOVA: $F_{3,55} = 2.63$, $p = 0.013$). Amphipods were strongly and

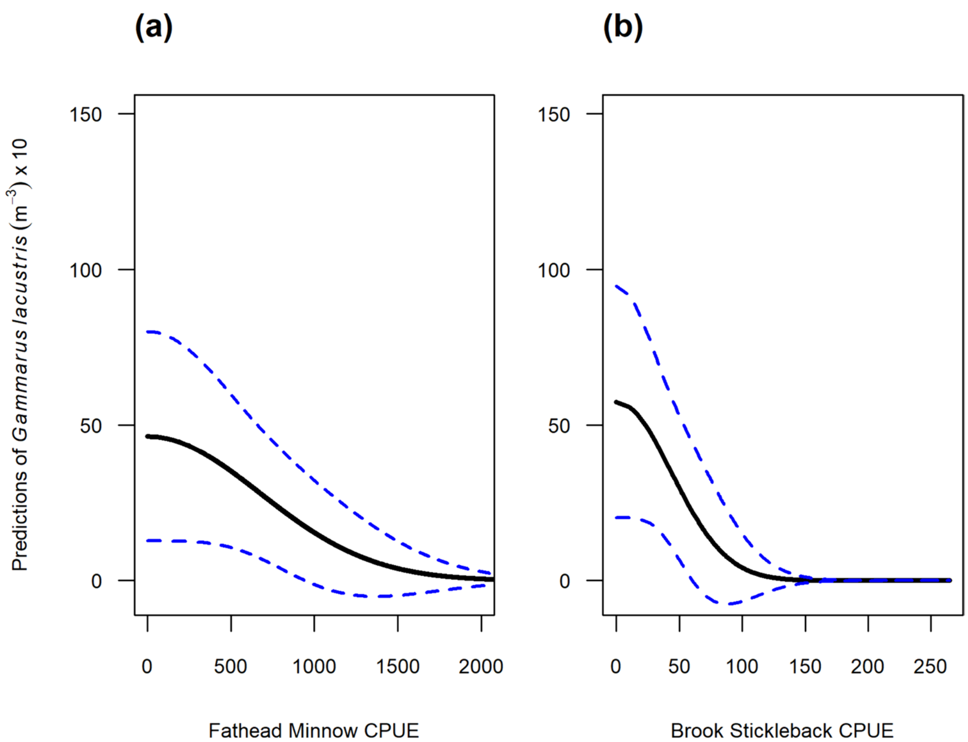

Fig. 3 Model-predicted densities of *Gammarus lacustris* in response to counts of Fathead Minnow *Pimephales promelas* (panel a) and Brook Stickleback *Culaea inconstans* (panel b) in 59 wetlands sampled in 2019–2020 from prairie pothole wetlands of Minnesota, USA. The catch per unit effort (CPUE) for this study was a 19-h overnight soak for one gill net and two fyke nets. The other model covariates in Table 2 were held constant at their mean values. Blue dashed lines represent 90% confidence intervals

negatively associated with benthivore fishes along the RDA1 axis ($F_{1,56}=7.53$, $p=0.010$). The Black Bullhead were the most prevalent benthivore (29% prevalence), followed by Common Carp (8% prevalence). The RDA2 axis and the associated functional feeding guilds of planktivore and piscivore were not significantly associated with amphipod community abundance ($F_{1,56}=0.51$, $p=0.922$).

Graphically, the RDA revealed further relationships between the fish and amphipod communities (Fig. 5). Most wetlands were clustered in ordination space, indicating similar fish assemblages among wetlands. Although fish assemblages were often similar, the fish guild abundances were along a strong gradient of few fish to very high abundances. The planktivores and piscivores were positively correlated, but not correlated to benthivores. Both amphipod species had slight positive correlations with each other, indicating that wetlands can house both amphipod species.

Discussion

High amphipod densities are essential forage for certain wildlife like scaup and tiger salamander, *Ambystoma tigrinum* (Olenick and Gee 1981; Strand et al 2008). Our results and others (Hanson and Butler 1994; Anteau et al 2011) show that fishless wetlands often support greater amphipod densities than wetlands with fish. Amphipod densities decline non-linearly with greater CPUE of the three most common fishes. The PPR has been extensively modified in ways that support greater fish abundances and movements among wetlands. Resource managers are faced with the decisions and challenges of fish management for bolstering amphipod densities, and we discuss tools that managers may consider implementing in the altered PPR landscape that wildlife continually depend on.

Fish Presence and Abundance Negatively Affected Amphipod Densities

Our results, along with other observational and experimental studies (Hanson and Butler 1994; Anteau et al 2011), show that fish presence and abundance are not generally observed with high densities of amphipods. The PPR was extensively modified in favor of fish movement and high abundances, yet our study and others show that ~ 20% of wetlands remain fishless (Anteau and Afton 2008b; Anteau et al 2011; Hanson et al 2012). Considering the marked effects that fish likely have on amphipods and other invertebrate communities, a landscape survey and predictive model that can identify semi-permanent and permanent wetlands that remain fishless would be a valuable tool for conservation because it could be used to prioritize conservation towards preventing the spread of fish. In wetlands with existing fish populations, resource managers need to weigh multiple tradeoffs to make decisions on whether to manage either fish or amphipods (Hanson and Butler 1994; Potthoff et al 2008).

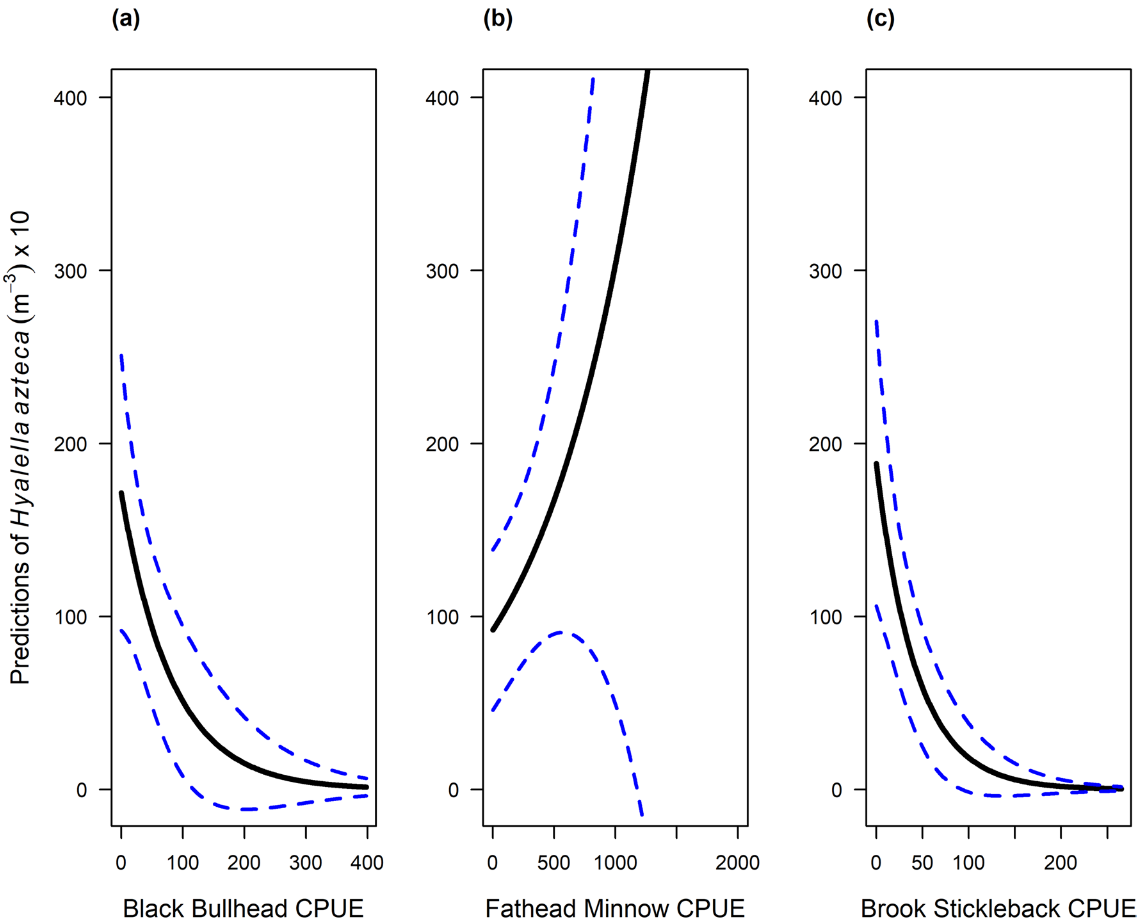

Fig. 4 Model-predicted densities of *Hyalella azteca* in response to counts of Black Bullhead *Ameiurus melas* (panel a), Fathead Minnow *Pimephales promelas* (panel b), and Brook Stickleback *Culaea inconstans* (panel c) in 59 prairie pothole wetlands of Minnesota, USA in years 2019– 2020.The catch per unit effort (CPUE) for this study was a 19-h overnight soak for one gill net and two fyke nets. The other model covariates in Table 2 were held constant at their mean values. Blue dashed lines represent 90% confidence intervals

In our study, amphipods were not detected in the presence of Black Bullheads. Black Bullhead are one of the most common native fish species found in Minnesota wetlands and are known to be tolerant to wider environmental conditions than many fish (Mork et al 2009). This species can reach high densities and may have increased due to anthropogenic influences like increased wetland connectivity and consolidation. Diet studies from other regions indicated Black Bullhead are highly opportunistic feeders and will readily consume amphipods (Repsys et al 1976; Strand et al 2008). Black Bullhead and other benthivores like Common Carp may also indirectly affect the quality of wetland habitats that amphipods rely on by increasing turbidity and decreasing aquatic vegetation (Zimmer et al 2002; Fischer et al 2013; Kowal et al 2022). Therefore, we hypothesize that Black Bullheads are excluding amphipods, but it is also possible that the two organisms rely on different wetland types.

Amphipods had a strong, negative association with Brook Stickleback. The Brook Stickleback are efficient at colonizing wetlands (Wieker et al 2016) and tolerant to a broad range of environmental conditions (McLean et al 2016). Although Brook Stickleback has a small gape (mouth), they may be consuming amphipods by picking them apart or eating *Gammarus* in their early life stage when small. In Manitoba prairie wetlands, *G. lacustris* was the most consumed food item by weight for Brook Stickleback (Moodie 1986). Further, Brook Stickleback preferentially consumed ovigerous female amphipods in a controlled experiment (Lewis and Loch-Mally 2010). We hypothesize that the mechanism for amphipod reduction is direct predation because we found no support in the literature for the notion that Brook Stickleback are responsible for major habitat degradation.

The effects of Fathead Minnow on amphipod densities remain unclear. Our study shows Fathead Minnow had a negative effect on *G. lacustris* densities, similar to previous work (Zimmer et al 2002; Anteau et al 2011). The effects of the planktivorous Fathead Minnow on *G. lacustris* are

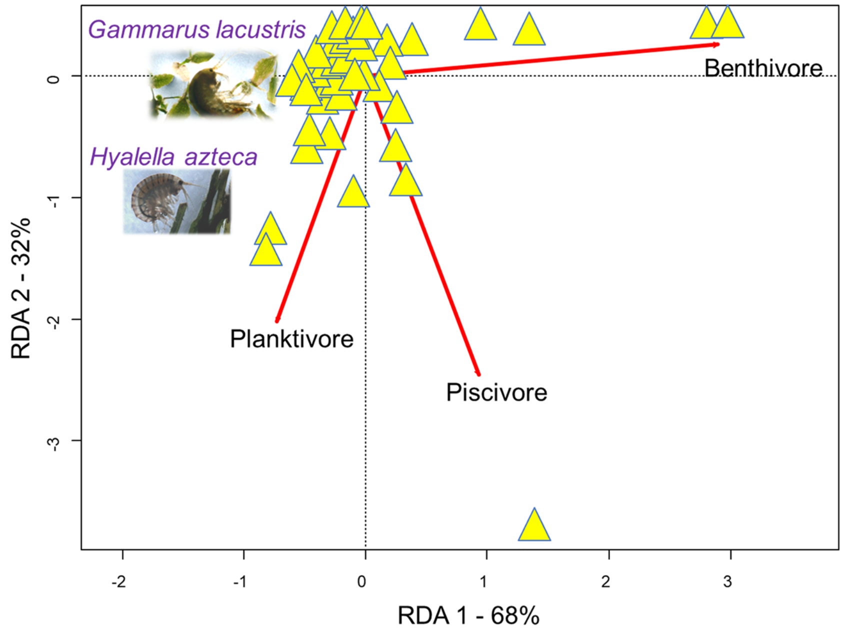

Fig. 5 Redundancy analysis (RDA) biplot of the amphipod community (*Gammarus lacustris* and *Hyalella azteca* counts) in relation to the catches of fish within three fish functional feeding guilds (benthivore, planktivore, and piscivore) from 59 wetlands in western Minnesota, USA. The counts of amphipods and fish were standardized for sampling effort. The fishes catch per unit effort (CPUE) for this study was a 19-h overnight soak for one gill net and two fyke nets. The yellow triangles represent individual wetlands, and the red lines are vectors showing the abundance gradients of fishes within each functional feeding guild. Vectors at < 90° angles are positively correlated, vectors at 90° angles are uncorrelated, and vectors at 180° angle are negatively correlated

likely to be both direct and indirect, though this study did not elucidate these effects due to insufficient sample sizes for such a model. Fathead Minnow could readily consume amphipods and likely compete with amphipods for detritus as food, and cause bioturbation (Zimmer et al 2002). Interestingly, the tiger salamander can affect trophic structure of prairie potholes similarly to planktivorous fish like the Fathead Minnow; yet, tiger salamander gut contents showed high amphipod consumption but not a strong effect on overall wetland amphipod densities (Benoy 2008; Wiltermuth 2014).

Contrary to our hypothesis, we did not detect a negative association between Fathead Minnows and *H. azteca;* however, this result had a weak, positive coefficient and a marginal *p*-value. Our finding contrasts with results found by Anteau et al. (2011), in which *H. azteca* showed greater decline in response to low Fathead Minnow abundances compared to *G. lacustris*. Based on Anteau et al. (2011) and our observations, Fathead Minnows and amphipods thrive in similar ecological niches. For example, a wetland must be deep enough to support overwinter survival of each species, but shallow enough not to support large abundances of large, predatory fish. This habitat-driven co-occurrence makes discerning cause and effect mechanisms of species interactions impossible in this observational study. However, it is also possible that some wetlands have conditions that can support productivity of both species. Two wetlands were excluded from our models due to having high populations of both *G. lacustris* and Fathead Minnows, and future research that targets wetlands with high co-occurrence may reveal what wetland characteristics support this anomaly. Further, we are aware of two wetlands in North Dakota with long-term data that also supported sustained populations of Fathead Minnows and amphipods (National Ecological Observatory Network 2023a; National Ecological Observatory Network 2023b). We believe further study would provide valuable insights into the mechanisms driving amphipod productivity.

The Interactions Among Aquatic Vegetation, Fish, Waterfowl, and Amphipods Remain Unknown

In both *G. lacustris* and *H. azteca* models, SAV biomass was an informative covariate. Interestingly, *G. lacustris* responded negatively to SAV biomass while *H. azteca* responded positively in this study and associated work (Larson et al 2022). We postulated reasons that amphipods respond differently to SAV, including use as a food resource, structural habitat, water purification, water-depth associations (e.g., lacustrine versus palustrine habitats), and mediating fish predation. Future research using more data than we currently have could explore interactions to learn whether plant biomass and plant diversity negates fish predation on amphipods.

The effects of predation on amphipods from either waterfowl or fish remain unclear from this study and the literature. Following ice-out when we sampled amphipods (April–May), millions of waterfowl would be using the PPR under high energetic demands for migration and reproduction. For example, scaup feed extensively on amphipods during spring migration (Anteau et al 2014) and will target wetlands with high densities of amphipods (Lindeman & Clark 1999) . Therefore, waterfowl may or may not negatively affect amphipod densities in this study and could be a confounding factor not controlled for in our study design. Future work could sample waterfowl use days, fish, and amphipods concurrently to better investigate their relationships or model out the effects of waterfowl to better understand the fish effects.

Managing Fish to Increase Amphipods for Wildlife Forage

Throughout the PPR, high amphipod densities are sometimes a management goal to support wildlife (Bouffard and Hanson 1997; Larson et al 2018). High amphipod densities can support migrating and breeding scaup (Lindeman and Clark 1999; Anteau and Afton 2006; Kahara and Chipps 2009), tiger salamanders (Benoy et al 2002; Benoy 2008), and likely other species that eat macroinvertebrates. Our results indicated that complete fish removal or reduced abundances may allow for greater densities of amphipods to serve as vertebrate food. A case study of Lake Christina, Minnesota, USA, suggested fish removal markedly increased amphipod densities until fish reinvaded (Hanson & Butler 1994). Exclusion of Common Carp from Delta Marsh, Manitoba, Canada, resulted in improved wetland conditions, SAV, and increased use of the marsh by migrating waterfowl (Kowal et al 2022; Bortolotti et al 2023).

Chemicals and water level drawdowns are two tools for reducing fish abundance in many wetland types. Rotenone is a non-selective piscicide that inhibits cellular respiration and leads to fish death. Non-target organisms, including amphipods, can be affected by Rotenone treatments but macroinvertebrate communities had quick recovery (Skaar et al 2017). Different species of fish require different concentrations of Rotenone; therefore, the target fish species should be identified before treatments for maximum effectiveness. Wetland drawdowns can also be an effective method of removing nuisance fish while increasing SAV that benefit amphipods (Larson et al 2020), but severe dewatering would likely harm amphipods as they cannot survive desiccation and are poor colonizers. Admittedly, removing fish from wetlands can be challenging, expensive, and often unsuccessful.

Stocking piscivorous fish to induce a trophic cascade may help reduce small nuisance fish (like Fathead Minnow). Biomanipulation via stocking Walleye *Sanders vitreus* and Northern Pike *Esox lucius* successfully suppresses Fathead Minnow (Potthoff et al 2008). In Minnesota's wetlands stocked with Walleye fry, the amphipods responded positively although the effect was short-lived; therefore, repeated fry stocking might be needed for lasting impacts (Potthoff et al 2008). In our study, the wetland named 'Cuba' has very high densities of both amphipod species (> 5,000 individuals per m^3) yet is commonly used as a Walleye rearing pond by Minnesota Department of Natural Resources. However, there is uncertainty whether piscivorous fish would preferentially consume amphipods if smaller fish were not available or if the stocked fry began to age and had greater energy demands.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13157-024-01808-5.

Acknowledgements We thank three anonymous peer reviewers who provided suggestions that improved our research proposal prior to funding. We thank Fred Bengtson and Ricky Lien for study conceptualization and funding acquisition, Brian Herwig for sharing fish gear and advice, students from Bemidji State University and Augsburg University for help sampling, and to Dr. Jeff Ueland and Dr. William Sea for helpful feedback on early versions of the manuscript. This study was performed under the authority of the Minnesota Department of Natural Resources fisheries permit (special permit # 24,192 to Danelle Larson and designees) and prohibited invasive species, aquatic plants, and water permit (permit #418 to Danelle Larson and designees). Fish were handled in accordance with American Fisheries Society guidelines for the use of fishes in research: https://fisheries.org/policy-media/science-guidelines/guidelines-for-the-use-of-fishes-in-research/. Research on U.S. Fish and Wildlife Service wetlands was supported by a research and monitoring special use permit (permit #SUP-18-O1R to Danelle Larson and designees). Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Funding Funding for this project was provided by the Minnesota Environment and Natural Resources Trust Fund as recommended by the Legislative-Citizen Commission on Minnesota Resources (LCCMR) as cited in Larson et al 2018. Additional funding and equipment were provided by the Minnesota Department of Natural Resources. The U.S. Geological Survey Northern Prairie Wildlife Research Center, U.S. Geological Survey Upper Midwest Environmental Science Center, and Minnesota Space Grant Consortium provided in-kind support for the project.

References

Afton AD, Hier RH, Paulus SL (1991) Lesser Scaup diets during migration and winter in the Mississippi flyway. Canadian Journal of Zoology 69:328–333. https://doi.org/10.1139/z91-053

Anteau MJ (2012) Do interactions of land use and climate affect productivity of waterbirds and prairie-pothole wetlands? Wetlands 32:1–9. https://doi.org/10.1007/s13157-011-0206-3

Anteau M, Afton A (2006) Diet shifts of lesser scaup are consistent with the Spring Condition Hypothesis. Canadian Journal of Zoology 84:779–186

Anteau MJ, Afton AD (2008) Diets of Lesser Scaup during Spring Migration throughout the Upper-Midwest are Consistent with the Spring Condition Hypothesis. Waterbirds 31:97–106. https://doi.org/10.1675/1524-4695(2008)31[97:dolsds]2.0.co;2

Anteau MJ, Afton AD (2008) Amphipod densities and indices of wetland quality across the upper-Midwest, USA. Wetlands 28:184–196. https://doi.org/10.1672/07-53.1

Anteau M, Afton A (2009) Wetland use and feeding by lesser scaup during spring migration across the upper Midwest, USA. Wetlands 29:704–712

Anteau MJ, Afton AD, Anteau ACE, Moser EB (2011) Fish and land use influence Gammarus lacustris and Hyalella azteca (Amphipoda) densities in large wetlands across the upper Midwest. Hydrobiologia 664:69–80. https://doi.org/10.1007/s10750-010-0583-2

Anteau MJ, DeVink J-M, Koons DN et al (2014) Lesser Scaup (Aythya affinis). The Birds of North America Online. https://doi.org/10.2173/bna.338

Anteau MJ, Wiltermuth MT, van der Burg MP, Pearse AT (2016) Prerequisites for Understanding Climate-Change Impacts on Northern Prairie Wetlands. Wetlands. https://doi.org/10.1007/s13157-016-0811-2

Bajer PG, Beck MW, Hundt PJ (2018) Effect of non-native versus native invaders on macrophyte richness: are carp and bullheads ecological proxies? Hydrobiologia 817:379–391. https://doi.org/10.1007/s10750-018-3592-1

Becker GC (1984) Fishes of Wisconsin. Copeia. https://doi.org/10.2307/1445079

Benoy GA (2008) Tiger Salamanders in prairie potholes: a “fish in amphibian’s garments?” Wetlands 28:464–472. https://doi.org/10.1672/07-07.1

Benoy GA, Nudds TD, Dunlop E (2002) Patterns of habitat and invertebrate diet overlap between tiger salamanders and ducks in prairie potholes. Hydrobiologia 481:47–59. https://doi.org/10.1023/A:1021260920095

Bortolotti LE, Emery RB, Kowal PD et al (2023) Migrating Ducks and Submersed Aquatic Vegetation Respond Positively After Invasive Common Carp (Cyprinus carpio) Exclusion from a Freshwater Coastal Marsh. Wetlands 43:25. https://doi.org/10.1007/s13157-023-01670-x

Bouffard SH, Hanson MA (1997) Fish in Waterfowl Marshes : Waterfowl Managers’ Perspective. Wildlife Society Bulletin 25:146–157

Brooks ME, Kristensen K, van Benthem KJ, et al (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R Journal. https://doi.org/10.32614/rj-2017-066

Carleen JD (2022) The effects of fish on amphipods in wetlands of Western Minnesota. Bemidji State University, Thesis

Carleen JD, Larson DM, Anteau MJ, et al (2024) Abundance, biomass, and density data for fishes and amphipods in prairie pothole region wetlands in 2019 and 2020: U.S. Geological Survey data release at https://doi.org/10.5066/P1Y9GBJK

Fischer JR, Krogman RM, Quist MC (2013) Influences of native and non-native benthivorous fishes on aquatic ecosystem degradation. Hydrobiologia 711:187–199. https://doi.org/10.1007/s10750-013-1483-z

Friederichs SJ, Zimmer KD, Herwig BR et al (2011) Total phosphorus and piscivore mass as drivers of food web characteristics in shallow lakes. Oikos 120:756–765. https://doi.org/10.1111/j.1600-0706.2010.19244.x

Hanson MA, Butler MG (1994) Responses to food web manipulation in a shallow waterfowl lake. Hydrobiologia 279–280:457–466. https://doi.org/10.1007/BF00027877

Hanson MA, Herwig BR, Zimmer KD et al (2012) Comparing Effects of Lake- and Watershed-Scale Influences on Communities of Aquatic Invertebrates in Shallow Lakes. PLoS ONE. https://doi.org/10.1371/journal.pone.0044644

Janke AK, Anteau MJ, Stafford JD (2019) Prairie wetlands confer consistent migrant refueling conditions across a gradient of agricultural land use intensities. Biological Conservation 229:99–112. https://doi.org/10.1016/j.biocon.2018.11.021

Kahara SN, Chipps SR (2009) Predicting lesser scaup wetland use during spring migration in eastern South Dakota. Great Plains Research 19:157–167

Keith B (2021) Interactions between wetland condition and amphipod abundance across Western Minnesota’s diverse landscape. Bemidji State University, thesis

Keith BR, Carleen JD, Larson DM, et al (2022) Protocols for Collecting and Processing Macroinvertebrates from the Benthos and Water Column in Depressional Wetlands. Open File Report 2022–1029, U.S. Geological Survey 1–32. https://doi.org/10.3133/ofr20221029

Kowal PD, Badiou P, Emery RB, et al (2022) Improvements in water clarity and submersed aquatic vegetation cover after exclusion of invasive common carp from a large freshwater coastal wetland, Delta Marsh, Manitoba. Frontiers in Environmental Science. https://doi.org/10.3389/fenvs.2022.992690

Larson DM, Cordts SD, Hansel-Welch N (2020) Shallow lake management enhanced habitat and attracted waterbirds during fall migration. Hydrobiologia 846:3364–3379. https://doi.org/10.1007/s10750-020-04342-7

Larson DM, DeJong D, Anteau MJ et al (2022) High abundance of a single taxon (amphipods) predicts aquatic macrophyte biodiversity in prairie wetlands. Biodiversity and Conservation 31:1073–1093. https://doi.org/10.1007/s10531-022-02379-9

Larson DM, Anteau M, Isaacson C, et al (2018) Restoring Wetland Invertebrates to Revive Wetland Habitats. Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 08g

Legendre P (2018) Numerical ecology. Encyclopedia of Ecology. https://doi.org/10.1016/B978-0-12-409548-9.10595-0

Lewis SE, Loch-Mally AM (2010) Ovigerous female amphipods (gammarus pseudolimnaeus) face increased risks from vertebrate and invertebrate predators. Journal of Freshwater Ecology 25:395–402. https://doi.org/10.1080/02705060.2010.9664382

Lindeman DH, Clark RG (1999) Amphipods, Land-Use Impacts, and Lesser Scaup (Aythya affinis) Distribution in Saskatchewan Wetlands. 19:627–638

Maurer KM, Stewart TW, Lorenz FO (2014) Direct and indirect effects of fish on invertebrates and tiger salamanders in prairie pothole wetlands. Wetlands 34:735–745. https://doi.org/10.1007/s13157-014-0538-x

McCauley LA, Anteau MJ, Van Der Burg MP, Wiltermuth MT (2015) Land use and wetland drainage affect water levels and dynamics of remaining wetlands. Ecosphere 6:1–22. https://doi.org/10.1890/ES14-00494.1

McKenna OP, Kucia SR, Mushet DM et al (2019) Synergistic interaction of climate and land-use drivers alter the function of North American, prairie-pothole wetlands. Sustainability. https://doi.org/10.3390/su11236581

McLean KI, Mushet DM, Stockwell CA (2016) From “Duck Factory” to “Fish Factory”: Climate Induced Changes in Vertebrate Communities of Prairie Pothole Wetlands and Small Lakes. Wetlands 36:407–421. https://doi.org/10.1007/s13157-016-0766-3

Moodie GEE (1986) The population biology of Culaea inconstans, the brook stickleback, in a small prairie lake. Canadian Journal of Zoology 64:1709–1717. https://doi.org/10.1139/z86-258

Mork MD, Bisping SM, Fischer JR, Quist MC (2009) Population characteristics of black bullhead (ameiurus melas) in iowa natural

lakes. Journal of Freshwater Ecology 24:635–644. https://doi.org/10.1080/02705060.2009.9664341
National Ecological Observatory Network (2023a) Macroinvertebrate collection (DP1.20120.001). In: https://data.neonscience.org .
National Ecological Observatory Network (2023b) Fish electrofishing, gill netting, and fyke netting counts (DP1.20107.001). . In: https://data.neonscience.org .
National Wetlands Inventory Database (2020) U.S. Fish and Wildlife Service.https://www.fws.gov/program/national-wetlands-inventory/data-download
Olenick RJ, Gee JH (1981) Tiger salamanders (Ambystoma tigrinum) and stocked rainbow trout (Salmo gairdneri): potential competitors for food in Manitoa prairie pothole lakes. The Canadian Field-Naturalist 95:129–132
Potthoff AJ, Herwig BR, Hanson MA et al (2008) Cascading food-web effects of piscivore introductions in shallow lakes. Journal of Applied Ecology 45:1170–1179. https://doi.org/10.1111/j.1365-2664.2008.01493.x
Repsys AJ, Applegate RL, Hales DC (1976) Food and Food Selectivity of the Black Bullhead, Ictalurus melas, in Lake Poinsett, South Dakota. Journal of the Fisheries Research Board of Canada 33:768–775. https://doi.org/10.1139/f76-094
Skaar DR, Arnold JL, Koel TM et al (2017) Effects of Rotenone on Amphibians and Macroinvertebrates in Yellowstone. Yellowstone Science 25:28–34
Strand KA, Chipps SR, Kahara SN et al (2008) Patterns of prey use by lesser scaup Aythya affinis (Aves) and diet overlap with fishes during spring migration. Hydrobiologia 598:389–398. https://doi.org/10.1007/s10750-007-9178-y
Strayer DL (2006) Challenges for freshwater invertebrate conservation. Journal of the North American Benthological Society 25:271–287
Tompkins AM, Gee JH (1983) Foraging behavior of brook stickleback, Culaea inconstans (Kirtland): optimization of time, space, and diet. Canadian Journal of Zoology. https://doi.org/10.1139/z83-329
Wasserstein RL, Schirm AL, Lazar NA (2019) Moving to a World Beyond "p <0.05." American Statistician 73:1–19. https://doi.org/10.1080/00031305.2019.1583913
Wieker JE, Schoonover CM, Gaines RK et al (2016) Effects of Introduced Brook Stickleback (Culaea inconstans) on Benthic Macroinvertebrate Communities in the Nearshore Area of Lentic Systems in Turnbull National Wildlife Refuge, Washington. Northwest Science 90:278–289. https://doi.org/10.3955/046.090.0306
Wiltermuth M (2014) Influences of climate variability and landscape modifications on water dynamics, community structure, and amphipod populations in large prairie wetlands. North Dakota State University, thesis
Zimmer KD, Hanson MA, Butler MG (2000) Factors influencing invertebrate communities in prairie wetlands: a multivariate approach. Canadian Journal of Fisheries and Aquatic Sciences 57:76–85. https://doi.org/10.1139/f99-180
Zimmer KD, Hanson MA, Butler MG (2002) Effects of fathead minnows and restoration on prairie wetland ecosystems. Freshwater Biology 47:2071–2086. https://doi.org/10.1046/j.1365-2427.2002.00952.x