

THESIS

EFFECTS OF INVASIVE SPECIES ON NATIVE HERPETOFAUNA AND POND
COMMUNITIES IN URBAN ENVIRONMENTS

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ABSTRACT

EFFECTS OF INVASIVE SPECIES ON NATIVE HERPETOFAUNA AND POND COMMUNITIES IN URBAN ENVIRONMENTS

Urban environments can provide high quality habitat for native species, yet these environments are also prone to species invasions via numerous introduction pathways. Invasive species may affect communities through multiple mechanisms including ecosystem engineering, predator-prey interactions, and resource competition, so isolating individual effects can be challenging. Further, urban environments commonly host multiple invasive species, which may interact with one another and amplify effects on native species. Despite their potential effects, however, the roles of invasive species in urban communities are often poorly understood. For example, the city of Madison, Wisconsin, USA is home to multiple invasive species, including jumping worms (*Amyntas* spp.), goldfish (*Carassius auratus*), and Chinese mystery snails (*Cipangopaludina* (= *Bellamya*) *chinensis*), but little is known about the community effects of these species. Jumping worms are non-native invasive earthworms that may act as ecosystem engineers in leaf litter habitat and potentially provide an abundant novel prey resource to native predators. Invasive goldfish and mystery snails have been widely introduced through aquarium trading and are known to co-occur in urban ponds. In the first study, we used a mesocosm experiment, laboratory feeding trials, and nonlethal stomach contents surveys to assess effects of invasive jumping worms on microhabitat and trophic interactions of native herpetofauna predators. We found that jumping worms reduced leaf litter biomass but did not influence soil conditions or survival of American toad (*Anaxyrus americanus*) metamorphs. Our results

revealed that jumping worms are viable prey for native herpetofauna, especially common garter snakes (*Thamnophis sirtalis*), but may be challenging to capture due to defensive behaviors. In the second study, we used a mesocosm experiment to test for facilitative or antagonistic interactions between goldfish and mystery snails, and to examine individual and combined effects of goldfish and mystery snails in urban pond communities. We found evidence that goldfish facilitate invasive mystery snails, and that goldfish drive shifts in community composition and food web structure through trophic cascades. Comparatively, mystery snails had limited effects in the experimental community. Overall, our studies emphasize the diversity of roles invasive species can play in communities as ecosystem engineers, prey, predators, or competitors, and that their effects on native species are dependent on these roles. Broadly, our findings highlight the value of understanding invasive species effects in urban environments to direct management and support native species conservation.

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TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
LIST OF TABLES	vii
LIST OF FIGURES	viii
CHAPTER 1 – EFFECTS OF INVASIVE JUMPING WORMS (<i>AMYNTHAS SPP.</i>) ON MICROHABITAT AND TROPHIC INTERACTIONS OF NATIVE HERPETOFAUNA	1
Introduction.....	1
Methods.....	5
Study site.....	5
Mesocosm experiment	5
Feeding experiments	7
Diet surveys	9
Analyses	10
Results.....	11
Mesocosm experiment	11
Feeding experiments	12
Diet surveys	13
Discussion	13
References	26
CHAPTER 2 – NONNATIVE FISH FACILITATE NONNATIVE SNAILS AND ALTER FOOD WEB STRUCTURE IN EXPERIMENTAL PONDS	32

Introduction.....	32
Methods.....	35
Experimental design.....	35
Mesocosm sampling.....	36
Analyses	37
Results.....	39
Goldfish effects	39
Mystery snail effects	40
Interactions between invaders.....	41
Discussion	42
References	53
APPENDICES	60
Appendix 1	60
Appendix 2.....	66

LIST OF TABLES

Table 1.1) Prey identified in stomach contents of herpetofauna during field diet surveys.....	25
Table A1.1) Initial and final numbers and masses of toads and earthworms in a mesocosm experiment.....	61
Table A1.2) Toad and prey masses, prey species, and capture success of trials during two laboratory feeding experiments.....	62
Table A1.3) Results of models assessing effects of earthworms on soil responses in a mesocosm experiment.....	63
Table A2.1) Numbers, masses, and length measurements of three snail species added to aquatic mesocosms	69
Table A2.2) Results of zero-inflated negative binomial models testing effects of goldfish and mystery snails on native snail densities in aquatic mesocosms	70
Table A2.3) Results of models testing effects of goldfish and mystery snails on responses measured at the end of an aquatic mesocosm experiment or averaged across all time points.....	71
Table A2.4) Results of models testing effects of goldfish and mystery snails on temporal response variables	72

LIST OF FIGURES

Figure 1.1) Mean dry masses of leaf litter in mesocosms across earthworm treatments.....	21
Figure 1.2) Mean toad metamorph survival in mesocosms across treatments and sample dates ..	22
Figure 1.3) Summary of attack data from toad feeding trial experiments	23
Figure 1.4) Prey movement time across prey types and the relationship between prey movement time and prey detection time in toad feeding trial experiments.....	24
Figure 2.1) Periphyton and filamentous algae biomass biomasses and amphibian survival and/or stage across treatments in aquatic mesocosms.....	48
Figure 2.2) Pond community measurements recorded over time across treatments in aquatic mesocosms	49
Figure 2.3) Photographs of a representative aquatic mesocosm from each treatment group	51
Figure 2.4) Invasive mystery snail and goldfish measurements in the presence and absence of the other invasive species	52
Figure A1.1) Soil measurements over time across earthworm treatments in mesocosms.....	60
Figure A1.2) Masses, lengths, and stomach contents of toads collected during field surveys.....	64
Figure A1.3) Masses, lengths, and stomach contents of snakes collected during field surveys....	65
Figure A2.1) Map of goldfish and mystery snail presence across pond locations in Madison, Wisconsin, USA, and photograph of a goldfish and mystery snail collected in Madison.....	66
Figure A2.2) Densities of zooplankton taxa collected over time across mesocosm treatments	67
Figure A2.3) Mean number of snail egg masses collected across mesocosm treatments.....	68

CHAPTER ONE:

EFFECTS OF INVASIVE JUMPING WORMS (*AMYNTHAS SPP.*) ON MICROHABITAT
AND TROPHIC INTERACTIONS OF NATIVE HERPETOFAUNA

Introduction

Invasive species can affect recipient communities through a wide range of mechanisms, sometimes resulting in unforeseen consequences for native species. For instance, an invasive species may directly or indirectly influence trophic interactions, competition dynamics, disease transmission, or habitat structure (Crooks 2002; Carlsson et al. 2009; Vilcinskis 2015; Dueñas et al. 2018). Effects on native community members are generally not isolated to a single type of interaction (Bennett et al. 2011; Kaemingk et al. 2017), complicating efforts to understand and predict consequences of species invasions. Untangling multiple mechanisms through which an invasive species affects native species may require a combination of research techniques.

Some invasive species serve as ecosystem engineers by altering the physical habitat structure to which native species have adapted (Crooks 2002; Ransom 2011). Native species may be directly affected by the structural changes or indirectly affected by resulting shifts in habitat conditions or community composition. Overall effects of ecosystem engineers in invaded communities can be complex and multidirectional. For example, in a Mediterranean Sea community invaded by an algal ecosystem engineer (*Halimeda incrassata*), native fish may be attracted to, unaffected by, or driven away from the altered ecosystem depending on the species (Vivó-Pons et al. 2020). Another invasive ecosystem engineer, the feral swine (*Sus scrofa*), dramatically reduced overall vegetation diversity in a Florida prairie, but promoted rapid growth of a single native forb (Boughton and Boughton 2014). Assessing net effects of invasive

ecosystem engineers is further complicated when the invasive species also directly interacts with native taxa.

While the predatory roles of some invasive species have received longstanding research attention, the potential for invasive species to serve as novel prey has been recognized more recently. In some cases, nonnative prey can have positive population-level effects on native predators (Carlsson et al. 2009). This can be particularly important if an invasive species outcompetes native prey because it can mitigate the effect of reduced native prey availability (Dijkstra et al. 2013; Cava et al. 2018). For example, invasive round gobies (*Apollonia melanostomus*) in the Great Lakes have been incorporated into diets of native predators including fish, birds, and snakes (King et al. 2006; Hensler et al. 2008; Coleman et al. 2012) and have been linked to population recovery of native snakes (King et al. 2006). Alternatively, if predators are unable to effectively consume an invasive species, due for instance to dietary specialization (Suárez et al. 2000) or prey defensive traits, this may lead to population declines in native predators. In a well-studied example, the toxicity of invasive cane toads (*Rhinella marina*) to many native Australian predators (Phillips et al. 2003) has resulted in population declines of native snakes, lizards, and other species (Shine 2018).

Widespread on every continent but Antarctica (Hendrix et al. 2008), invasive earthworms can alter habitat structure and trophic interactions within invaded ecosystems. Earthworms act as ecosystem engineers by restructuring soil and leaf litter microhabitats, which subsequently alters community structure and ecosystem functions, including decomposition rates, nutrient cycling, and primary production (Craven et al. 2017; Frelich et al. 2019). In addition, invasive earthworms may be important prey for a variety of predators (Maerz et al. 2005; Gao et al. 2017; Virgin and King 2019), though research on their roles in trophic interactions is limited. Because

earthworms reproduce with small cryptic cocoons, detection and widespread control are challenging, and nonnative earthworm range expansion may occur rapidly (Chang et al. 2021).

Jumping worms (*Amyntas* and *Metaphire* spp.) are widespread invasive species in the eastern United States and have recently invaded several midwestern and northeastern states (Chang et al. 2018). As only species of the *Amyntas* genus are considered in this study, jumping worms will hereafter be referred to as *Amyntas*. Originating in eastern Asia, including Japan and the Korean Peninsula (Blakemore 2003, 2014), these earthworms were likely spread to the United States through horticulture practices and use as fishing bait (Gates 1958; Görres and Melnichuk 2012). *Amyntas* can reproduce parthenogenically, have an annual life span, and produce cocoons throughout the summer months (Chang et al. 2016a). *Amyntas* have been known to outcompete previously established nonnative earthworms (e.g., *Apporectodea*, *Lumbricus*, and *Octolasion* spp.) (Chang et al. 2016b; Laushman et al. 2018). *Amyntas* behaviors differ from other nonnative earthworms and their establishment may elicit changes in habitat structure and food webs (Chang et al. 2021), especially since *Amyntas* may outcompete and cause population declines of other earthworm species (Chang et al. 2016b; Laushman et al. 2018). Unlike many nonnative earthworms in North America, *Amyntas* live only on the surface and upper few centimeters of soil and create an aggregated casting layer on the soil surface that is distinct from other earthworm castings (Görres et al. 2019; Chang et al. 2021). *Amyntas* reduce leaf litter layers (Qiu and Turner 2017), though the extent to which this differs from other earthworms is largely unknown (Chang et al. 2021). Invasive *Amyntas* indirectly alter composition of leaf litter and soil invertebrate communities through mechanisms including resource competition (Snyder et al. 2011; Greiner et al. 2012; Gao et al. 2017). Additionally, invasive *Amyntas* potentially provide an abundant novel prey source, but anti-predator

behavioral defenses such as their characteristic “jumping” behavior may make *Amyntas* challenging prey for native predators (Gorsuch and Owen 2014). Few studies have documented the role of invasive *Amyntas* in food webs (but see Gorsuch and Owen 2014 and Gao et al. 2017).

To examine how introduced *Amyntas* may affect native species through multiple mechanisms, we conducted a mesocosm experiment, laboratory feeding trials, and field diet surveys of native predators in Madison, Wisconsin, USA. In historically glaciated regions of the northern United States, including the Madison area, there are no native terrestrial earthworm species but there are several established nonnative earthworms (e.g., *Lumbricus* spp., *Aporrectodea* spp., *Octolasion* spp., *Eisenia* spp.) (Hendrix and Bohlen 2002; Bohlen et al. 2004). Southern Wisconsin is also home to native terrestrial herpetofauna that share habitat with and could potentially prey on *Amyntas*, including American toads (*Anaxyrus americanus*), red-bellied snakes (*Storeria occipitomaculata*), and common garter snakes (*Thamnophis sirtalis*) (Christoffel et al. 2008, 2009). American toads, particularly metamorphs, may be especially vulnerable to changes in cover and moisture content within leaf litter habitat and commonly co-occur with *Amyntas* in their invaded range (Heinen 1993; Rittenhouse et al. 2008). In the current study, we examined how *Amyntas* affect habitat and trophic interactions of terrestrial herpetofauna compared to established *Lumbricus* earthworms. We make comparisons with *Lumbricus* because they are a common local species who have experienced dramatic population declines associated with *Amyntas* invasion (Laushman et al. 2018). We expected differences in effects between the two species to reflect changes in local communities as *Amyntas* spread. We asked 1) how invasive *Amyntas* worms, *Lumbricus* worms, and native American toad metamorphs interact within leaf litter microhabitat, 2) how prey behavioral interactions with a

generalist predator (American toads) compare between *Amyntas* and alternative prey, including *Lumbricus*, under laboratory conditions, and 3) whether native toads and snakes consume *Amyntas* in the field. We predicted that 1) *Amyntas* would directly reduce leaf litter mass via consumption, indirectly reduce moisture, and increase temperature of soil through litter depletion and soil aggregation, and indirectly reduce metamorphic toad survival because of these habitat changes; 2) toads would have reduced success at capturing *Amyntas* compared with alternative prey due to defensive behaviors of *Amyntas*, and 3) native toads and snakes would consume *Amyntas*, despite their status as a novel prey source in this region.

Methods

Study site

We conducted the mesocosm experiment and diet survey at the University of Wisconsin-Madison Arboretum (43.041293, -89.428252; hereafter referred to as the UW Arboretum), a 1,200-acre restored public natural area and research center surrounded by urban development. *Amyntas* were discovered in the UW Arboretum in 2013, and their range has expanded rapidly (Laushman et al. 2018). Laboratory feeding trials were conducted at the University of Wisconsin-Madison.

Mesocosm experiment

To examine effects of *Amyntas* on toad microhabitat, we performed a 2 x 2 x 2 factorial mesocosm experiment with the following treatments: 1) presence or absence of 4 American toad metamorphs, 2) presence or absence of 20 *Amyntas* worms, and 3) presence or absence of 20 *Lumbricus* worms. We randomly assigned treatments to 50-liter plastic mesocosms (rim diameter = 45cm, height = 38cm). 46 total mesocosms were used, and each treatment was replicated in either 5 or 6 mesocosms (Table A1.1). We drilled holes in the bottom of the mesocosms for

drainage and raised 70% shade cloth over them to simulate canopy cover. The mesocosms were covered on the bottoms with garden fabric and on the top with fiberglass mesh lids to prevent worms and toads from escaping. We filled each mesocosm to an approximate height of 30cm with locally sourced screened topsoil from a nearby garden store, then topped the soil with an approximate wet mass of 460g of hand mixed leaf litter collected randomly from an *Amyntas*-free area of an oak-dominated forest in the UW Arboretum. Litter was composed predominantly of black oak (*Quercus velutina*, ~66% by volume), white oak (*Q. alba*, ~14%), shagbark hickory (*Carya ovata*, ~14%), and black walnut (*Juglans nigra*, ~3%).

We collected *Amyntas* for the mesocosms by hand from beneath leaf litter, and we collected *Lumbricus* by applying a mustard solution to the soil (a method which draws worms to the surface by irritating the skin), after which they were rinsed with clean tap water. We only collected juveniles (worms lacking a clitellum) of all taxa, as *Amyntas* were predominantly in the juvenile life stage at the time. Worms were weighed (Table A1.1) and added to mesocosms within 12 hours of collection. Based on known species compositions in the area (Laushman et al. 2018, Price-Christenson et al. 2020), *Amyntas* worms added to mesocosms were a combination of *Amyntas tokioensis* and *A. agrestis*, and *Lumbricus* worms in the experiment were *Lumbricus terrestris* and *L. rubellus*.

We collected American toad metamorphs around three ponds in Madison. Toads were weighed (Table A1.1) and added to mesocosms in similar size cohorts to minimize competition effects. Toads were presumed to feed on naturally occurring springtails, beetles, ants, and mites observed within leaf litter. We also added 10 to 20 flightless fruit flies evenly to each mesocosm every two days throughout the experiment to ensure adequate prey availability.

We began the experiment on July 26, 2019 and took soil measurements weekly. We measured soil pH and temperature (Hanna Instruments Direct Soil Measurement pH/Temperature Meter HI 99121) and percent soil moisture (Extech Soil Moisture Meter MO750, range = 0% to 50%) by inserting probes at the soil surface to a depth of ~10 cm. On day 36 (Aug 31), we counted and weighed all toads and disassembled one toad + *Amyntas* + *Lumbricus* mesocosm to assess earthworm survival. Due to concerns about low numbers of observed *Amyntas*, both in the broken-down mesocosm and on the surfaces of other mesocosms, we added 10 newly collected *Amyntas* to all *Amyntas*-treated mesocosms (for a total of 30 *Amyntas* worms added). While *Lumbricus* had also declined in the broken-down mesocosm, we were more uncertain about their survival across tanks because *Lumbricus* were not easily visible compared to the surface-dwelling *Amyntas*, so we chose not to add more *Lumbricus*. On day 61 (Sep 25), we collected, counted, and weighed remaining toads and earthworms from all mesocosms. At this time, we also collected all leaf litter from each mesocosm and dried it in an oven for 24 hours at 80° C to quantify litter dry mass.

Feeding experiments

To examine predator-prey behavioral interactions between toads and *Amyntas*, we conducted two no-choice feeding trial experiments in September 2019 (hereafter referred to as Exp. 1 and Exp. 2). For both experiments, we used the same 12 American toads (mean mass = 16.9 g; Table A1.2), collected from an *Amyntas*-free location (the UW-Madison Lakeshore Nature Preserve). Toads were housed individually in 5.7-liter plastic storage bins with moist peat moss substrate and access to distilled water, and they were fasted for at least 48 hours prior to each experiment. We spot-cleaned habitats and replaced water daily.

The two feeding trial experiments had slightly different designs. During each trial in Exp. 1, a toad was offered one live house cricket (*Acheta domesticus*), one *Amyntas* worm, or one *Lumbricus* worm. Each toad in Exp. 1 underwent one trial per prey type (36 total trials). Crickets were purchased from a local pet store and used as a positive control because they were expected to be readily consumed by toads. *Amyntas* were collected from the UW Arboretum, and *Lumbricus* (specifically, *L. terrestris*) were purchased from a local bait store. The average mass of the purchased *Lumbricus* (mean = 2.7 g) was approximately 3 times the mass of the *Amyntas* (0.9 g) in Exp. 1 (Table A1.2). Because we did not want prey size differences to confound possible differences in earthworm-toad interactions, we then conducted a follow-up experiment (Exp. 2) in which we used only comparably sized *Amyntas* (mean = 0.6 g) and *Lumbricus* (mean = 0.6 g) prey (Table A1.2). In Exp. 2, *Amyntas* and *Lumbricus* were hand-collected in the UW Arboretum. Toads were offered one *Amyntas* worm or one *Lumbricus* worm per trial, and we conducted two trials per toad per prey type (48 total trials). In both experiments, trials were performed two days apart, and the order of prey for each toad was rotated between individual toads (Table A1.2).

Feeding trials were conducted in a clear plastic arena with a rectangular base (29.7 x 19.3 x 20.3 cm.) and brown paper was attached to the walls to minimize external stimuli. During each trial, we placed a toad under a transparent cover in the arena for a five-minute acclimation period. A pre-weighed prey item was placed in front of the toad, and the cover was removed. The time of each attack or capture and total number of attacks were recorded. Attacks were defined as any attempt (successful or not) by the toad to capture prey with or without physical contact. A capture was an attack that resulted in the full consumption of a prey item. In Exp. 1, we removed the toad after prey consumption or after 15 minutes if the prey had not been consumed. Based on

a lack of captures observed in the last 5 minutes of Exp. 1 trials, we reduced the time limit to 10 minutes for Exp. 2. Due to observed interspecific variation in worm movement in Exp. 1 (see Discussion), we recorded each trial in Exp. 2 using an iPhone SE 2016 to quantify the proportion of total trial time a worm spent moving (hereafter referred to as “prey movement proportion”) and the time at which the toad first visibly detected the earthworm (hereafter referred to as “prey detection time”). The latter response variable was a consistently observed behavior in which a toad would visibly turn its head toward the prey item, often followed by other characteristic hunting behavior (e.g., crouching, stalking, attacking).

Diet Surveys

To determine whether American toads or colubrid snakes were consuming *Amyntas* worms in the field, we collected individual predators and nonlethally recovered their stomach contents. We opportunistically captured predators during visual encounter surveys and from beneath cover boards. Both survey methods were used in two disconnected areas of the UW Arboretum: an *Amyntas*-invaded site (“invaded”) and a site with no known *Amyntas* presence (“uninvaded”). The invaded site consisted of deciduous forest, oak savanna, prairie, and mowed/garden spaces. The uninvaded site contained pine forest, deciduous forest, oak savanna, and prairie. We surveyed the uninvaded site with the goal of comparing diets between the two areas; however, the number of predators captured in each location was too low to robustly analyze diet differences. To survey cover boards, we haphazardly placed a total of 50 corrugated tin and 20 plywood boards evenly in the two areas of the Arboretum. We checked boards about twice a week from May to September 2020. Upon capture of each toad and snake, we recorded mass and snout-vent length (SVL) (Fig. A1.2 and A1.3). We collected snake stomach contents by gently palpating the stomach to induce regurgitation (Seigel et al. 1987). We collected toad

stomach contents by using gastric lavage methods (Solé et al. 2005) with clean well water from an 8Fr (diameter = 2.3 mm) silicone veterinary feeding tube and 60 ml plastic syringe. Stomach contents were strained through a paper coffee filter. Retained prey items were preserved in 70% ethanol and later identified visually using a dissecting microscope.

Analyses

We analyzed treatment effects on leaf litter dry mass, soil parameters (pH, temperature, and log-transformed percent moisture), and toad survival within mesocosms. Our experimental design included toads as an independent variable to assess toad effects on earthworm survival, but we were unable to test this effect due to low earthworm survival in all treatments (see Table A1.1 and Discussion). Because toads did not affect leaf litter or soil responses and including toads as a predictor did not improve model performance, we grouped toad present and absent treatments for analyses of habitat variables. We tested effects on leaf litter dry mass using linear models (lm, ‘stats’ package) with fixed effects for presence/absence of *Amyntas*, presence/absence of *Lumbricus*, and their interaction. To test effects on soil responses, we used generalized additive mixed models (gamm, ‘mgcv’ package) with the same earthworm treatment predictors, a smoothing term for sample date, and a random intercept for mesocosm identity to account for repeated measurements (Wood 2017). We tested earthworm effects on toad percent survival using generalized linear mixed effects models (glmer, ‘lme4’ package) with fixed effects for *Amyntas*, *Lumbricus*, and sample date with full interactions, and a random intercept for mesocosm identity (Bates et al. 2015).

We analyzed feeding experiment data to test our hypothesis that toads would be less successful predators of *Amyntas* than alternative prey. Exp. 1 and 2 were analyzed independently. We analyzed effects of prey type on the total number of attacks per trial and on

the binary success or failure of each attack (“attack success”) using generalized linear mixed effects models (glmer, ‘lme4’ package) with a fixed effect for prey type and a random intercept for toad identity (Bates et al. 2015). We specified a Poisson distribution for the attack number model and a binomial distribution for attack success. For Exp. 1, each model was followed by Tukey-adjusted pairwise comparisons (pairs, ‘emmeans’ package) to directly compare between the three prey types (Lenth et al. 2021). We used a linear model (lm, ‘stats’ package) to test for effects of prey type on prey movement proportion (time moving/total trial time; logit-transformed). Lastly, we assessed the relationship between earthworm movement and toad hunting behavior in Exp. 2 using a linear mixed effects model (lmer, ‘lme4’ package) with prey detection time (log-transformed) as the response, prey movement proportion (logit-transformed) as a predictor, and toad identity as a random intercept (Bates et al. 2015). All analyses were performed in R version 4.03 (R Core Team 2020).

Results

Mesocosm experiment

Amyntas and *Lumbricus* earthworms altered leaf litter but did not substantially affect soil conditions or toad survival. *Amyntas* presence reduced dry leaf litter mass by 16% compared to controls ($\beta = -16.43\text{g}$, $\text{SE} = 4.20$, $t = -3.92$, $p = <0.001$) and *Lumbricus* reduced dry litter mass by 13% compared to controls ($\beta = -12.82\text{g}$, $\text{SE} = 4.104$, $t = -3.13$, $p = 0.003$) (Fig. 1.1). Mesocosms with both earthworms present had 26% less dry litter mass than controls, but we did not see evidence of an interaction between earthworms ($\beta = 3.10\text{g}$, $\text{SE} = 5.93$, $t = 0.52$, $p = 0.605$) (Fig. 1.1). Sampling date strongly affected all three soil responses (Fig. A1.1, Table A1.3). We did not detect significant effects of earthworms on soil, though *Amyntas* showed a nonsignificant positive association with soil temperature ($\beta = 0.11$, $\text{SE} = 0.06$, $t = 1.82$, $p =$

0.075) (Fig. A1.1, Table A1.3). Toad survival across all treatments averaged 52% on day 36 and 34% at the end of the experiment on day 61 (Fig. 1.2). The interaction between *Amyntas*, *Lumbricus*, and sample date showed a nonsignificant negative effect on percent toad survival ($\beta = -1.77$, $SE = 0.89$, $t = -1.98$, $p = -0.063$). Mesocosms with both earthworms had the lowest mean final toad survival at 21% compared with 30% for *Amyntas* mesocosms, 46% for *Lumbricus* mesocosms, and 38% for control mesocosms (Fig. 1.2).

Feeding experiments

In both experiments, toads attacked *Amyntas* more times per trial on average than other prey types, but attacks were less likely to result in a capture. We observed at least one attack in most trials with crickets (92%), and in comparably fewer trials with *Amyntas* (Exp. 1: 58%, Exp. 2: 42%) and with *Lumbricus* (Exp. 1: 33%, Exp. 2: 55%). In Exp. 1, the mean number of toad attacks per trial on *Amyntas* was over 2x the number on crickets (ratio = 2.21, $SE = 0.60$, $z = 2.94$, $p = 0.009$) and over 3x the number on *Lumbricus* (ratio = 3.82, $SE = 1.26$, $z = 4.05$, $p < 0.001$) (Fig. 1.3A). Similarly, the mean number of attacks per trial on *Amyntas* in Exp. 2 was 3x the number on comparably sized *Lumbricus* ($\beta = 1.11$, $SE = 0.25$, $z = 4.53$, $p < 0.001$) (Fig. 1.3B). Mean attack success on *Amyntas* in Exp. 1 was over 13x lower than on crickets (odds ratio = 0.06, $SE = 0.06$, $z = -3.06$, $p = 0.006$) and 9x lower than on *Lumbricus* (odds ratio = 0.10, $SE = 0.11$, $z = -2.15$, $p = 0.080$) (Fig. 1.3C). In Exp. 2, mean attack success on *Amyntas* was again 9x lower than on comparably sized *Lumbricus* ($\beta = -3.30$, $SE = 0.95$, $z = -3.48$, $p < 0.001$) (Fig. 1.3D). During Exp. 2, prey movement proportion was lower for *Amyntas* than for *Lumbricus* ($\beta = -2.18$, $SE = 0.54$, $t = -4.02$, $p < 0.001$) (Fig. 1.4A). Lastly, prey detection time in Exp. 2 occurred earlier during trials in which worms spent a larger proportion of time in motion ($\beta = -0.337 \pm 0.129$ SE, $t = -2.615$, $p = 0.012$) (Fig. 1.4B).

Diet surveys

Amyntas earthworms were detected in the stomach contents of common garter snakes, but not the other predators surveyed. We captured a total of 24 American toads, 35 red-bellied snakes, and 44 common garter snakes. We captured 11 of the toads at the *Amyntas*-invaded site and 14 at the uninvaded site. Only 1 toad was found under a cover board; all others were found incidentally. No *Amyntas* were identified in toad stomach contents, and only one earthworm (unidentifiable) was recovered from a toad in the *Amyntas*-uninvaded site. Other toad prey (reported as the % of total prey items) included 25% snails (2 toads), 19% ants (2 toads), 9% millipedes (4 toads), 7% dipterans (5 toads), 5% springtails (5 toads), and 7% unidentifiable prey (5 toads) (Table 1.1). “Unidentifiable prey” were prey items considered too degraded for any level of identification. Nearly all (96% of common garter and 91% of red-bellied) snakes were captured at the *Amyntas*-invaded site. Stomach contents were recovered from 37% of red-bellied snakes (13 individuals), and all identifiable prey consisted of nonnative slugs. Prey consisted of 65% *Deroceras reticulatum* (7 snakes), 12% *Arion subfuscus* (2 snakes), 18% unidentifiable slugs (3 snakes), and 6% unidentifiable prey (1 snake) (Table 1.1). Common garter snakes were the only species whose stomach contents contained *Amyntas*. We recovered stomach contents from 34% of common garter snakes (15 individuals). Prey items consisted of 26% *Amyntas* spp. (5 snakes), 26% *Lumbricus* spp. (3 snakes), 26% American toads (5 snakes), 11% unidentifiable earthworms, and 11% unidentifiable prey (2 snakes) (Table 1.1).

Discussion

Using mesocosm experiments, laboratory feeding trials, and field diet surveys, we were able to increase understanding of the mechanisms through which a recent *Amyntas* invasion affects native herpetofauna in the upper Midwest, USA. *Amyntas* reduced litter mass, but did

not cause strong changes to soil conditions or toad survival. Our laboratory feeding trials and diet surveys indicated that *Amyntas* are a viable prey source for native herpetofauna, particularly common garter snakes, though their defensive behaviors may make them difficult to capture for some species. Overall, we found that *Amyntas* alter microhabitat by reducing leaf cover and affect prey availability for native herpetofauna. The ability of herpetofauna to tolerate or benefit from these changes may depend on microclimatic requirements, hunting behaviors, and dietary flexibility. More broadly, our results suggest that combining experimental and field studies can be a useful approach to isolate distinct mechanisms mediated by invasive species and quantify their importance in communities.

Amyntas reduced leaf litter biomass in our mesocosms, which supports prior research (Greiner et al. 2012; Ziemba et al. 2015; Qiu and Turner 2017). *Lumbricus* also reduced litter mass, but the extent to which the functional roles of *Amyntas* and *Lumbricus* in leaf litter breakdown differ is unclear from our experiment, and we found no evidence of interactive effects. Our results show weak evidence that *Amyntas* may raise soil temperatures, but this effect was inconclusive, and we saw no other effects of *Amyntas* or *Lumbricus* on soil responses. Prior studies of *Amyntas* effects on soil conditions show mixed results, but studies have found *Amyntas* to be associated with increased pH (Laushman et al. 2018; Bethke and Midgley 2020), altered thermal properties (Görres et al. 2019), and increased moisture loss (Görres et al. 2019). Litter decomposition processes and soil aggregation through casting are proposed indirect mechanisms for these soil effects (Chang et al. 2021). In our study, remaining unconsumed leaf litter and ambient environmental conditions (e.g., light levels, relative humidity, and rainfall) may have buffered soil from meaningful temperature and moisture changes. Both *Amyntas* and *Lumbricus* survival was very low at the end of the experiment

(Table A1.1). While we cannot be certain of the cause, heavy flooding that prevented proper mesocosm drainage during the week before the experiment ended likely caused at least a portion of the mortality. Nevertheless, the substantial differences in leaf litter masses between controls and earthworm treatments provide strong evidence that enough earthworms of both species survived long enough to cause leaf litter reduction, which was the main mechanism we predicted to have cascading effects on soil conditions and toad survival. That said, the magnitude of earthworm effects on leaf litter, soil properties, and amphibians in our experiment was likely affected by changes in earthworm densities over time. Future experiments in which *Amyntas* densities are carefully manipulated across the range of naturally observed densities would be useful to further clarify their direct and indirect effects.

The combination of *Amyntas* and *Lumbricus* had an increasingly negative association with toad survival over time, but this effect was relatively weak. Toad survival was low (<50% on average) across all treatments but tended to be slightly lower in tanks with *Amyntas* (Fig. 1.2). Earthworms reduced leaf litter, which in turn might have reduced cover, humidity, and prey habitat (Rittenhouse et al. 2008; Kazemi et al. 2009) which may have affected toad mortality. Earthworm invasions have been linked to effects on other terrestrial amphibians, namely eastern red-backed salamanders (*Plethodon cinereus*) (Maerz et al. 2009; Ransom 2011, 2012; Ziemba et al. 2015). For example, nonnative *Lumbricus* earthworms are associated with declines in salamander abundance in the northeastern United States (Maerz et al. 2009), and consumption of leaf litter by *Amyntas* can have sublethal effects on salamanders through increasing movement and thus energy expenditures (Ziemba et al. 2015). American toads are somewhat less susceptible to desiccation than other amphibians (Rittenhouse et al. 2008), so negative effects of earthworms may have been stronger on other amphibian species. Research comparing lethal and

sublethal effects of *Amyntas* among multiple litter-dependent herpetofauna taxa would help assess habitat modification effects across a broader native community.

In the feeding experiments, American toads hunted and attacked *Amyntas*, *Lumbricus* and crickets, but toads were often unsuccessful when attacking *Amyntas* compared with alternative prey. We observed a higher number of attacks on *Amyntas* per trial because of the frequent failed attacks. The repeated attacks on *Amyntas* suggest that low attack success was likely a result of worm defensive behaviors rather than unpalatability (e.g., toxins or distasteful compounds). Gorsuch and Owen (2014) found that, compared with other nonnative earthworms (*Aporrectodea longa* and *L. rubellus*), *Amyntas* are more successful at evading capture by nonnative wandering broadhead planarians (*Bipalium adventitium*), native seal salamanders (*Desmognathus monticola*), and native ribbon leeches (*Nephelopsis obscura*). We observed three distinct defensive behaviors by *Amyntas*: jumping, freezing, and autotomy (tail separation). The jumping defense is a behavior characterized by rapid undulations by an *Amyntas* worm to propel itself away from a physical disturbance and potentially startle a predator (Gorsuch and Owen 2014). Freezing was characterized by an immediate cease in movement for seconds to minutes at a time following a physical disturbance. This behavior has not been previously reported in the literature to our knowledge. As sight-based hunters, the toads commonly stopped displaying hunting behaviors when worm movement ceased, making this an effective strategy. Autotomy was the complete detachment of the tail region; this response occurred only once during our trials and has also been reported in prior research (Gorsuch and Owen 2014). In our study the toad consumed the separated tail and afterward captured and consumed the full worm, further evidencing the palatability of *Amyntas* to toads.

Overall movement patterns of *Amyntas* differed substantially from those of *Lumbricus*. *Amyntas* movement was sporadic and varied, due in part to the freezing behavior, while *Lumbricus* typically moved throughout most of the trial regardless of attacks. Additionally, *Amyntas* movement sometimes only involved the head or mouthpart while the rest of the body was stationary, which may have influenced toad hunting behavior. Worm movement patterns were associated with toad hunting behavior; prey detection time and thus hunting activity occurred earlier during trials with more active worms. Divergent life histories of *Amyntas* and *Lumbricus* may help explain movement differences. The species of *Amyntas* in this study dwell on or near the soil surface (epigeic to epi-endogeic) and are not known to create vertical burrows (Richardson et al. 2009; Qiu and Turner 2017), while *Lumbricus* are shallow-dwelling to deep-burrowing (epi-endogeic to anecic), depending on species (Shipitalo et al. 1988; Hale et al. 2006). For *Lumbricus*, especially anecic species like *L. terrestris*, constant movement may itself be a defense mechanism if its purpose is to seek a burrow to escape. *Amyntas* may have evolved more diverse behavioral defenses because living on the soil surface results in more frequent exposure to a wide range of terrestrial predators in their native range. *Amyntas* are highly abundant on the soil surface in recently invaded areas (Laushman et al. 2018), so although they are likely harder to capture than *Lumbricus*, they also may be more readily available to certain predators. While laboratory trials can help us predict the outcome of novel trophic interactions between invasive and native species, field studies are needed to reveal more clearly how invasive species integrate into natural food webs.

In our herpetofauna diet surveys, we found *Amyntas* in the stomach contents of garter snakes but not of American toads or red-bellied snakes. American toad stomach contents were primarily composed of arthropods and gastropods, though one toad consumed a non-*Amyntas*

earthworm (Fig. A1.3). This aligns with prior, though limited, findings that toads predominantly consume arthropod prey (Smith and Bragg 1949; Bellocq et al. 2000; Bolek and Coggins 2000), but may opportunistically prey on earthworms (Bush 1959). Earthworms have also been recorded in diets of other amphibians (Careddu et al. 2020), including the related genus *Bufo* (Evans and Lampo 1996; Vallvé and Sánchez-Iglesias 2018; Kolenda et al. 2019) and may be especially important prey after rain events (Maerz et al. 2005). Our sample size of American toads was relatively small, particularly at the *Amyntas*-invaded site, and a larger dataset would be useful to definitively conclude whether or not American toads are consuming *Amyntas* in the field. Additionally, soft-bodied prey like earthworms are digested more quickly than arthropod prey and therefore may be missed more often in visual stomach contents surveys (Pompanon et al. 2012). Surveying at night when toads are more likely to be feeding can help reduce this bias (Dodd 2010). Based on our laboratory trials, American toads may opportunistically consume *Amyntas* if they can capture them, but due to the inefficient hunting behavior we observed, hunting *Amyntas* may result in a higher energy expense and lower prey yield compared to alternative prey. This is important because *Amyntas*-induced extirpations of earthworms including *Lumbricus* (Chang et al. 2016b; Laushman et al. 2018) and shifts in broader invertebrate communities (Snyder et al. 2011; Gao et al. 2017) may change the prey assemblages available to American toads and other species. More field-based research, including quantification of energy flows into herpetofauna predators, would be needed to test this idea. Additionally, while we captured similar numbers of toads in invaded and uninvaded areas, research over a longer time period and larger sample size could more clearly assess whether *Amyntas* have population-level effects on toads.

Our study provides the first evidence that we are aware of for common garter snake consumption of *Amyntas*. Common garter snakes are highly opportunistic predators and are known to consume nonnative prey including *Lumbricus* and *Apporectodea* earthworms (Virgin and King 2019). We expect that as *Amyntas* compete with and reduce populations of other earthworm taxa (Chang et al. 2016b; Laushman et al. 2018), *Amyntas* will become an increasingly important component of common garter snake diets. This trend may have significance for other snakes of local conservation concern, such as the Butler's garter snake (*Thamnophis butleri*), which is an earthworm specialist and is considered a species of conservation concern in areas including Wisconsin (Wisconsin Department of Natural Resources 2017). Possibly, garter snakes and other predators that hunt using chemoreception may be able to better overcome some of the defensive behaviors of *Amyntas*, such as jumping and freezing, compared with predators that depend largely on sight-based hunting strategies (e.g., toads). Two other taxa, native centipedes (families Cryptopidae and Scolopocryptopidae) and native rusty crayfish (*Oronectes rusticus*), have displayed relative success at capturing invasive *Amyntas* (Gorsuch and Owen 2014; Gao et al. 2017) and are also known to use chemoreception (Müller et al. 2011; Kraus-Epley et al. 2015). Laboratory feeding trials testing chemoreception of snakes or other species (see Burger 1991) with *Amyntas* prey could test this idea. Additionally, it is unclear how the nutritional and energetic value of *Amyntas* as prey compare with other prey species, which could be another informative research direction.

Interestingly, red-bellied snakes in our study consumed only nonnative slugs, which corroborates previous research (Virgin and King 2019). Common garter and red-bellied snakes are both widespread and relatively common in urban areas (Kjoss and Litvaitis 2001). The dietary flexibility that allows predators to opportunistically switch to nonnative prey may also

help them succeed in urban areas because anthropogenic disturbances strongly affect prey availability (Seress et al. 2018; Siqueira and Marques 2018). Accordingly, we suggest future research focus on species-specific traits that make native predators successful at utilizing nonnative prey. Further, we captured far more common garter and red-bellied snakes where *Amyntas* were present, which could be associated with differing prey availabilities due to nonnative species, though we have little evidence for this relationship. Research investigating direct and indirect effects of nonnative prey (e.g., *Amyntas* and slugs) on snake populations while controlling for environmental and habitat variables could assess this possible mechanism.

Due to the potential for multiple, multidirectional effects of invasive species on native communities, an integrative approach to invasive species research is essential. While teasing apart the individual mechanisms that affect native species can be complex, from a conservation standpoint it is important to understand the net effects of invasive species interactions within native communities. Our research helps to advance understanding of the effects of *Amyntas* on native herpetofauna microhabitat and trophic interactions. More generally, our findings emphasize the potential importance of nonnative species as novel food resources to native consumers (Carlsson et al. 2009). This often-overlooked ecological role should be evaluated alongside the more commonly studied effects of invasive species as consumers, competitors, and disease vectors to obtain a more holistic understanding of invasion biology.

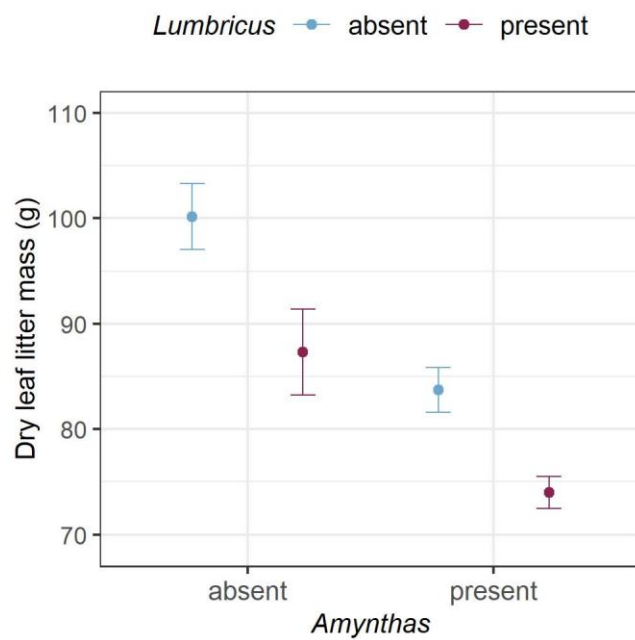


Figure 1.1) Mean dry mass of leaf litter in the mesocosm experiment for each earthworm treatment group (*Amynthus* presence/absence and *Lumbricus* presence/absence). 460g wet mass of leaf litter was originally added to each tank, and the experiment was run from July to September 2019. Error bars represent the standard error of each treatment mean.

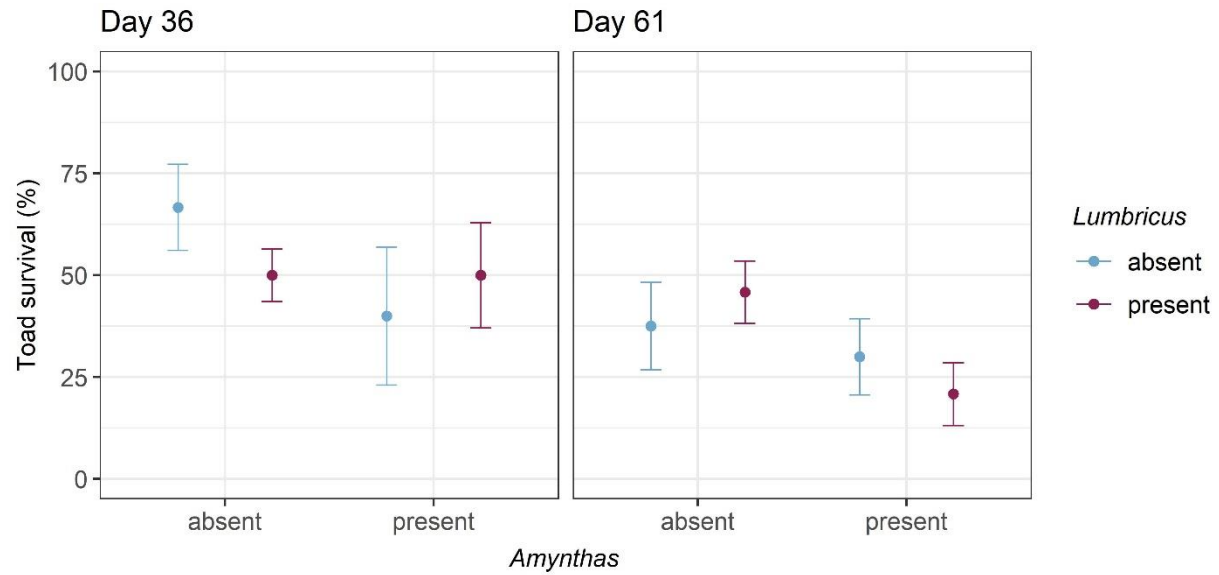


Figure 1.2) Mean percentage of American toad metamorphs (*Anaxyrus americanus*) surviving in the mesocosm experiment at each sample date (Aug 31 and Sep 25, 2019) for each earthworm treatment group. Error bars represent the standard error of each treatment mean.

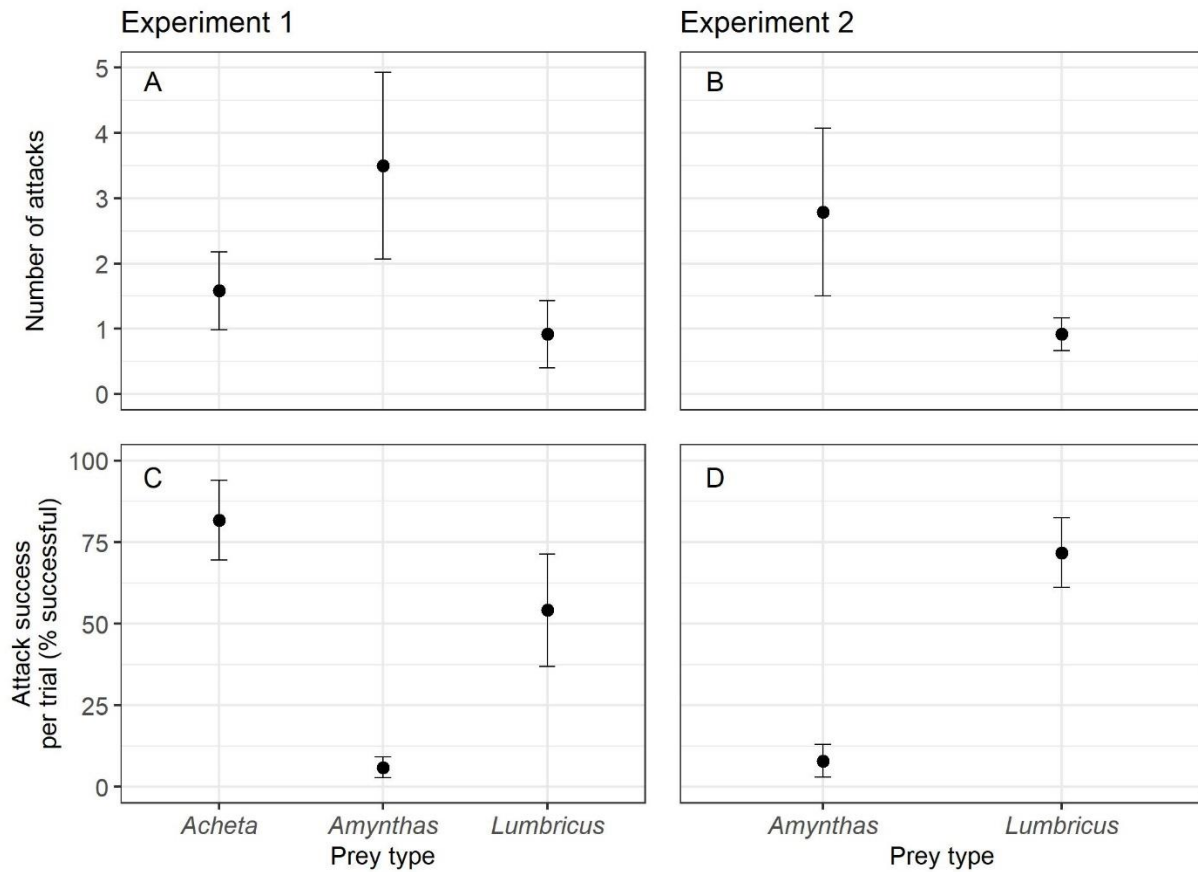


Figure 1.3) Summary of attack data from feeding trial experiments showing A) mean total number of attacks by toads per trial on crickets (*Acheta*), *Amyntas*, and *Lumbricus* in Exp. 1., B) mean total number of attacks by toads per trial on *Amyntas* and *Lumbricus* in Exp. 2., C) mean attack success (number of successful attacks/number of total attacks) by toads per trial for each prey type in Exp. 1, and D) mean attack success by toads per trial for each prey type in Exp. 2. Error bars represent the standard error of each treatment mean.

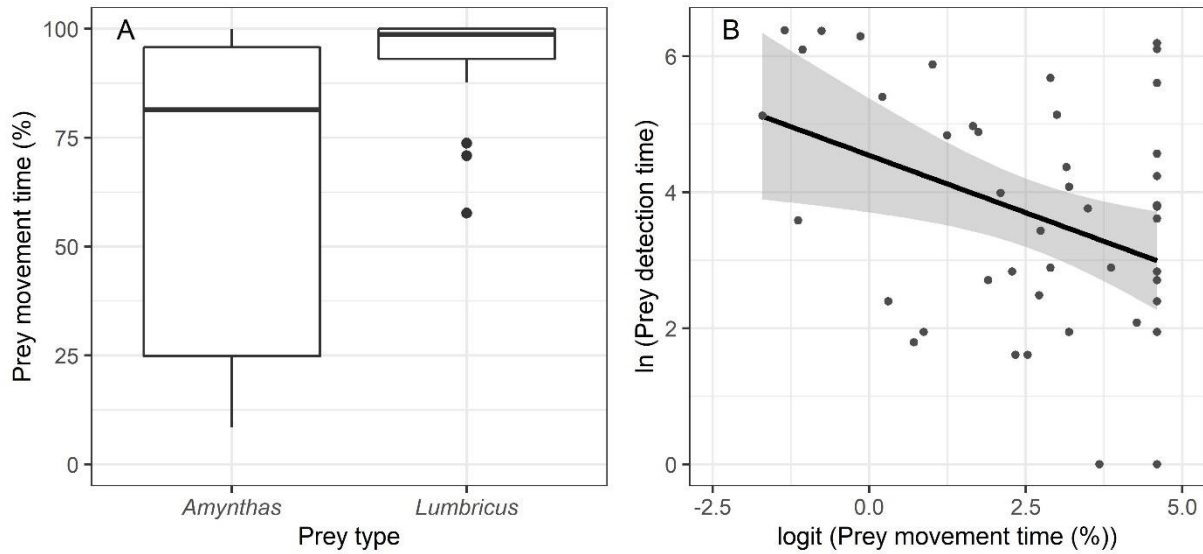


Figure 1.4) A) Boxplot showing prey movement time (time moving/total trial time) for *Amynthus* and *Lumbricus* prey in Exp. 2. The center lines represent the median percentages. B) Linear model of log-transformed prey detection time (time at which toad was observed to first detect prey) predicted by logit-transformed prey movement time in Exp. 2. The grey area represents a 95% confidence interval.

Table 1.1) Prey identified in stomach contents of herpetofauna predators from field diet surveys at the UW Arboretum (Madison, WI, USA). Prey count is the total number of individuals of a certain prey type across all predator individuals of a certain species. Predator count is the total number of predators that consumed one or more of each prey type.

Species	Prey Type	Prey Count	Predator Count
<i>Anaxyrus americanus</i> (n = 24)	Araneae	4	3
	Blattodea	9	2
	Chilopoda	1	1
	Coleoptera	5	4
	Collembola	5	5
	Diplopoda	9	4
	Diptera (adult)	1	1
	Diptera (larva)	6	4
	Gastropoda (snail)	24	2
	Gastropoda (slug - <i>Deroceras reticulatum</i>)	1	1
	Hymenoptera (Formicidae)	18	4
	Hymenoptera (unknown)	1	1
	Isopoda	1	1
	Nematoda	1	1
	Oligochaeta	1	1
	Trombidiformes	1	1
	unidentified	7	5
	no prey recovered	-	7
<i>Storeria occipitomaculata</i> (n = 35)	<i>Arion subfuscus</i>	2	2
	<i>Deroceras reticulatum</i>	11	7
	unidentified slug	3	3
	unidentified	1	1
	no prey recovered	-	22
<i>Thamnophis sirtalis</i> (n = 44)	<i>Amyntas</i> spp.	5	5
	<i>Anaxyrus americanus</i>	5	5
	<i>Lumbricus</i> spp.	5	3
	unidentified earthworm	2	2
	unidentified	2	2
	no prey recovered	-	29

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CHAPTER TWO:

NONNATIVE FISH FACILITATE NONNATIVE SNAILS AND ALTER FOOD WEB STRUCTURE IN EXPERIMENTAL POND COMMUNITIES

Introduction

Invasive species form novel interactions with both native and nonnative species, potentially altering community structure and dynamics. Interactions include consumptive (Doherty et al., 2016) and competitive (Davis, 2003) effects, which may cause declines of native community members if they cannot alter defensive strategies or resource use in response (Dueñas et al., 2021). However, the direction and magnitude of invasive species effects depends on the trophic position of the native and nonnative species (David et al., 2017). For example, native community members may benefit from consuming nonnative species or from competitive or consumer release that results from invasions (Rodriguez, 2006). Thus, species invasions often drive complex changes to community structure, making it important to understand invader interactions in a multi-species community context (David et al., 2017).

In an increasingly globalized world, invasions rarely occur in isolation from one another, highlighting the need to understand interactions between nonnative species (Dawson et al., 2017). Nevertheless, interactions between invaders have received comparatively less research attention than the traditional focus of invasion ecology on understanding and predicting effects of a single invader on native community members (Jackson, 2015). The “invasional meltdown hypothesis” posits that the presence of one invasive species may facilitate the invasion of another, potentially amplifying undesirable effects on native species (Simberloff, 2006; Simberloff & Von Holle, 1999). For example, invasive crabs (*Carcinus maenas*) facilitate invasive whelks (*Urosalpinx cinerea*) in California estuaries by outcompeting a major predator

of the whelks, which subsequently caused a sharp decline in native oysters (*Ostreola conchaphila*) (Kimbrow et al., 2009). Despite examples of facilitation between invasive species, a 2015 meta-analysis found that negative or neutral interactions between invasive species are more commonly recorded in the literature than facilitation (Jackson, 2015). Thus, the relative ecological importance of facilitation between invaders has been debated, and experimental evidence of invasional meltdown remains rare.

In aquatic ecosystems, co-occurring invasions are prevalent due to numerous introduction pathways (Havel et al., 2015), habitat connectivity (Kao et al., 2021), and co-occurring anthropogenic change (Johnson et al., 2008). Aquatic introductions are often cryptic, sometimes allowing species to spread undetected until they are well established (Morais & Reichard, 2018). Urban freshwaters may be particularly prone to species introductions due to their proximity to humans who may intentionally or unintentionally introduce nonnative species into waterbodies (Copp et al., 2010; Hassall, 2014). Recreational boating and fishing, fish stocking, and ballast water are commonly cited invasion pathways in urban freshwaters (Bobeldyk et al., 2015; Tricarico, 2012). Increasingly, the trade in aquarium species is also recognized as a major driver of aquatic species introductions (Florescu et al., 2018; Padilla & Williams, 2004; Preston et al., 2022).

Goldfish (*Carassius auratus*) and Chinese mystery snails (*Cipangopaludina* (= *Bellamya*) *chinensis*; hereafter referred to as “mystery snails”) are widespread invasive species that are common in the aquarium trade and are known to co-occur in the Midwest and northeastern United States (Brown, 2019; Marsden & Hauser, 2009; Mills et al., 1996). For example, field surveys of 102 ponds conducted in 2019 and 2020 around Madison, Wisconsin, USA found goldfish in 10 ponds, mystery snails in 4 ponds, and both invaders together in 3 ponds (Fig.

A2.1) (Trovillion et al., in prep.). Goldfish are native to eastern Asia and have been bred for over 1000 years, making them the oldest known ornamental fish (Roos, 2019). Goldfish are also used as fishing bait, which is an additional introduction pathway (Courtenay Jr. & Stauffer Jr., 1990). Spread of goldfish is aided by their high salinity, temperature, and pollutant tolerance, allowing them to thrive in multiple lotic and lentic habitats types, including highly disturbed areas and even estuaries (Jia et al., 2019; Schofield et al., 2006; Spotila et al., 1979; Tweedley et al., 2017). Goldfish are generalist omnivores and can facilitate a shift away from clear, macrophyte dominated water via consumptive effects, bioturbation, and nutrient excretion (Razlutskiy et al., 2021; Richardson et al., 1995). Despite widespread presence of goldfish across six continents, however, community effects of invasive goldfish have received limited research attention.

Mystery snails are a large (up to 6.5 cm shell length) species of viviparous, gonochoric snail capable of rapid reproduction (Solomon et al., 2010; Stephen et al., 2013). Native to eastern Asia, mystery snails have been introduced to freshwater throughout North America, western Europe, and Hawai'i (Bury et al., 2007; Collas et al., 2017; Cowie, 1998; McAlpine et al., 2016). Along with the aquarium trade, mystery snails have been spread by trade for human consumption (Cowie, 1998) and accidental boat transportations (Solomon et al., 2010), aided by their ability to survive prolonged desiccation periods (Havel, 2011). While densities vary, invaded habitats have supported up to ~ 40 snails m^{-2} and mystery snails may reach a biomass density that is orders of magnitude above native community members (Chaine et al., 2012; Johnson et al., 2009; Solomon et al., 2010). Thus, nonnative mystery snails may be an important food source if predators are able to overcome their large size and thick shells (Olden et al., 2009; Twardochleb & Olden, 2016). Mystery snails feed both by grazing and filtering (Olden et al., 2013), which may shift primary production patterns and cause competition with native herbivores (Johnson et

al., 2009). Little is known about how goldfish and mystery snails influence overall food web structure in ponds or how they interact within co-invaded systems.

We conducted a mesocosm experiment to broaden understanding of the effects of goldfish and mystery snails in pond communities and to assess how these invasive species interact with one another. We asked 1) how goldfish and mystery snails independently and combined affect a native pond community, and 2) whether there is evidence of facilitation or antagonistic interactions between goldfish and mystery snails. We predicted that goldfish would 1) reduce survival of native invertebrates and amphibians via predation, and 2) increase turbidity and primary production through bioturbation and indirect effects mediated by native grazer reduction. We predicted that mystery snails would 3) reduce algae and turbidity through grazing and filtration, and 4) indirectly reduce growth of native herbivores via resource competition. Finally, we predicted that 5) mystery snails would be less susceptible to predation by goldfish than native species due to their large size, potentially leading to facilitation of mystery snails via competitive release from other grazers.

Methods

Experimental design

We conducted a mesocosm experiment at the University of Wisconsin-Madison Arboretum (Madison, Wisconsin, USA) using a 2 x 2 full factorial design with presence or absence of mystery snails crossed with presence or absence of goldfish (4 treatments). We replicated each treatment 5 times for a total of 20 mesocosms. Mesocosms were made from 50-gal (189 L) black Rubbermaid cattle tanks covered with fiberglass screen lids. We added 8 L washed sand and 100g dried leaf litter to provide substrate and cover. We filled each mesocosm with well water sourced on site. Throughout the experiment, we topped off mesocosms with well

water as needed to maintain consistent water levels. We added chemical nutrients (1.12 mg/L KH_2PO_4 and 6.07 mg/L NaNO_3) to achieve nitrogen and phosphorus concentrations that reflected mean values from local stormwater ponds in the Madison area (Dodson 2008). We also added 60ml of rabbit chow per mesocosm as a supplementary nutrient and food source for herbivores at the start of the study. A 20.3 x 25.4 cm plexiglass sheet was leaned against a wall in each mesocosm to provide a surface from which to count snail eggs and juveniles, and to sample periphyton. In mystery snail-positive treatments, we added 5 adult mystery snails (Table A2.1), and in goldfish-positive treatments we added 3 goldfish (~3.5cm mean length), all of which were collected from local ponds. Goldfish were naïve to mystery snails, but mystery snails came from a site that also contained goldfish.

To simulate a native Wisconsin pond community, we added phytoplankton, zooplankton, native snails, and native anurans to each mesocosm. Community members were sourced from several goldfish- and mystery snail-free ponds in Madison, WI. We added 330ml of a concentrated and homogenized phytoplankton-pond water mixture to each mesocosm. We collected zooplankton (mostly cladocerans, copepods, and ostracods) using a zooplankton tow net and added 400ml of a concentrated zooplankton-pond water mixture to each mesocosm. We added 20 pond snails (*Physa acuta*), 10 ramshorn snails (*Planorbella trivolvis*), 15 green frog (*Lithobates clamitans*) and 10 gray tree frog (*Hyla versicolor*) tadpoles to each mesocosm (see Table A2.1 for mean snail measurements). Green frogs hatched on ~June 20, and gray tree frogs hatched on ~July 6. The experiment was run for approximately 8 weeks (July 15 to Sept. 14).

Mesocosm sampling

We measured relative phytoplankton fluorescence and turbidity of each mesocosm weekly using a Turner Designs AquaFluor handheld fluorometer/turbidimeter. We sampled

zooplankton every 2 weeks by submerging a PVC tube 5 times in each mesocosm (once in each corner and the center), filtering the collected volume (~1.3L per mesocosm) through a 250-micron sieve, and preserving zooplankton in 70% ethanol. Zooplankton were classified as cladocerans, copepods, or ostracods and counted under a dissecting microscope. We quantified snail reproduction weekly by identifying and counting the number of egg masses and juvenile snails attached to the plexiglass sheet in each mesocosm. We checked mesocosms daily for frog metamorphs; if metamorphs (Gosner stage ≥ 44) were present, we removed them, recorded the date of metamorphosis, and measured their weight and snout-vent length (SVL) (Gosner, 1960).

At the end of the experiment (Sep 14), we collected all remaining snails, fish, and tadpoles, and we measured biomass of periphyton and of floating filamentous algae. We euthanized snails by freezing and euthanized fish and tadpoles using an MS222 solution. For tadpoles we measured SVL and Gosner stage (Gosner, 1960), and for fish we measured the total length of each individual. We counted all pond, ramshorn, and mystery snails, measured shell lengths of all individual snails, and dried juveniles to measure total juvenile dry mass per mesocosm for each species. We sampled periphyton biomass by scraping both sides of each plexiglass sheet (total of 1031cm²) with a razor blade, and then we dried and weighed the collected periphyton biomass. For filamentous algae, we collected, dried, and weighed all floating or suspended algae mats in each mesocosm. Juvenile snails, periphyton, and phytoplankton were each dried for 24h at 80°C.

Analyses

We analyzed the effects of goldfish and mystery snails on pond communities with R version 4.1.1 (R Core Team, 2020) using a combination of linear models (LMs), linear mixed effects models (LMMs), generalized linear models (GLMs), and generalized linear mixed effects

models (GLMMs). Models included categorical predictors for goldfish presence and mystery snail presence unless otherwise specified. For all responses measured over time, we also included a continuous predictor for time in weeks. Each model contained all possible interactions between predictors unless otherwise specified. For responses that were measured multiple times or on multiple individuals within a single mesocosm, we included a categorical random intercept for mesocosm identity. We used LMMs ('lme4' package) to test for invader effects on turbidity and relative phytoplankton fluorescence (square-root transformed) (Bates et al., 2015). To test effects on total zooplankton density (cladocerans + copepods + ostracods), we used GLMMs ('lme4' package) specifying a Poisson distribution. We tested for effects on native snail egg masses using a GLM ('lme4' package) specifying a negative binomial distribution. Due to low numbers of egg masses at most time points, we pooled values across time and summed pond and ramshorn snail egg masses. We tested treatment effects on juvenile pond snail and ramshorn snail densities using zero-inflated negative binomial GLMMs ('glmmTMB' package) (Brooks et al., 2017). In the zero-inflated portion of each native snail model, we included only a fixed effect for goldfish presence and random intercept for mesocosm identity. In the negative binomial portion of each native snail model, we included all predictors and the random intercept with an interaction term between mystery snail presence and time. We omitted the interaction between goldfish presence and week because native snails in goldfish treatments showed no variation over time. We used LMs ('stats' package) to test effects of treatments on final periphyton biomass (square-root transformed), filamentous algae biomass (square-root transformed), and mean green frog stage per mesocosm. For green frog and gray tree frog survival, we used GLMMs with binomial distributions. Due to zero tree frog survival across every goldfish-present mesocosm, we excluded these treatments (and the corresponding goldfish predictor) from

analyses of tree frog responses. We used a Cox proportional hazard model ('survival' package) to assess effects of mystery snails on emergence rates of surviving tree frogs throughout the experiment (Therneau & Lumley, 2015). We did not analyze green frog emergence because few green frogs metamorphosed during the experiment. To analyze mystery snail effects on goldfish, we used LMs with mystery snail presence as a predictor and final mean goldfish length as the response. For goldfish effects on mystery snails, we analyzed total dry biomass of juvenile snails (square-root transformed) and individual juvenile length (shell aperture to apex; log-transformed). The DHARMA package was used for model diagnostics and to ensure relevant assumptions were met (Hartig & Hartig, 2017).

Results

Goldfish effects

Goldfish affected nearly all primary producers and native herbivores. Periphyton biomass was 23x higher on average in goldfish-only mesocosms (hereafter, "goldfish mesocosms") compared to control mesocosms ($\beta = 0.25$, $SE = 0.06$, $t = 4.21$, $p < 0.001$) (Fig. 2.1a). Relative phytoplankton fluorescence was about 3x higher on average in goldfish mesocosms than controls ($\beta = 0.25$, $SE = 0.06$, $t = 4.22$, $p < 0.001$) (Fig. 2.2a). Conversely, the average mass of filamentous algae was 85% lower in goldfish mesocosms than controls ($\beta = -2.81$, $SE = 0.76$, $t = -3.69$, $p = 0.002$) (Fig. 2.1b). Goldfish increased turbidity towards the end of the experiment (goldfish*time, $\beta = 5.62$, $SE = 1.76$, $t = 3.20$, $p = 0.002$) (Fig 2.2b). These differences in primary production and turbidity were visibly apparent, with more filamentous algae cover and higher water clarity observed in controls than goldfish mesocosms (Fig. 2.3). Goldfish dramatically reduced zooplankton ($\beta = -4.00$, $SE = 0.43$, $z = -9.18$, $p < 0.001$), with an average of 98% reduction in zooplankton density per week in goldfish mesocosms (mean = 25.7/liter) compared

to controls (mean = 1387/liter) (Fig. 2.2c). Each individual zooplankton taxon (cladocerans, copepods, and ostracods) showed similar patterns (Fig. A2.2). Native snail egg mass production was 6x higher in goldfish mesocosms relative to controls ($\beta = 1.79$, $SE = 0.46$, $z = 3.9$, $p < 0.001$) (Fig. A2.3). However, goldfish affected juvenile native snails in the opposite direction. In goldfish mesocosms, we found an increased likelihood of observing a snail density value of zero for both juvenile pond snails ($\beta = 6.41$, $SE = 1.34$, $z = 4.77$, $p < 0.001$) (Fig. 2.2d) and juvenile ramshorn snails ($\beta = 5.22$, $SE = 1.41$, $z = 3.70$, $p < 0.001$) (Fig. 2e). On average, goldfish mesocosms had a 99% lower density of juvenile pond snails and 97% lower density of juvenile ramshorn snails than controls (see Table A2.2 for full analyses).

Goldfish reduced both green frog and gray tree frog survival, but the magnitudes of effects differed between frog species. Tree frog survival was reduced to zero across every mesocosm containing goldfish compared with a mean survival rate of 76% when goldfish were absent (Fig. 2.1c). Green frog survival was 29% lower in goldfish mesocosms than control mesocosms ($\beta = -2.26$, $SE = 0.96$, $z = -2.36$, $p = 0.018$) (Fig. 2.1d). Goldfish presence had a strong positive effect on green frog development, as measured by Gosner stage ($\beta = 3.97$, $SE = 1.26$, $t = 3.16$, $p = 0.006$) (Fig. 2.1e). Excluding frogs that had metamorphosed, the average stage of surviving green frogs in goldfish mesocosms was >35 compared to an average stage of >31 in controls. Additionally, of the 7 green frogs that metamorphosed during the experiment, all were in mesocosms with goldfish.

Mystery snail effects

Mystery snails affected anuran development but did not show strong effects on other members of the pond community. Mystery snails slowed the rate of gray tree frog development, as measured by date of emergence ($\beta = -1.54$, $SE = 0.32$, $z = -4.83$, $p < 0.001$) (Fig. 2.2f). On

average, tree frogs that emerged did so 4 days earlier in control mesocosms than in mystery snail mesocosms. Furthermore, 92% of surviving tree frogs (35 out of 38) in control mesocosms emerged compared with 42% of surviving tree frogs (15 out of 36) in mystery snail mesocosms. Mystery snails were marginally associated with reduced development rates in green frogs, as measured by Gosner stage ($\beta = -2.63$, $SE = 1.26$, $t = -2.10$, $p = 0.053$) (Fig. 2.1e). Green frogs in mystery snail mesocosms had a final average stage of 29 compared with an average of over 31 in control mesocosms. We did not see evidence for mystery snail effects on any other response variables (Table A2.3 and A2.4).

Interactions between invaders

We found evidence for facilitation of mystery snails by goldfish. Final total biomass of juvenile mystery snails was about 7x higher on average when goldfish were present ($\beta = 1.97$, $SE = 0.72$, $t = 2.73$, $p = 0.026$) (Fig. 2.4a). Juvenile lengths were 15% higher with goldfish present, but this effect was not statistically significant ($\beta = 0.14$, $SE = 0.10$, $t = 1.46$, $p = 0.21$) (Fig. 2.4b). In contrast, mystery snails were associated with a 6% lower total goldfish length at the end of the study ($\beta = -0.35\text{cm}$, $SE = 0.15$, $t = -2.27$, $p = 0.031$) (Fig. 2.4c). We did not see strong evidence of interactive effects between goldfish and mystery snails on the greater pond community, suggesting that their effects were largely additive, rather than synergistic. Green frog survival was lowest in goldfish + mystery snail mesocosms at 32.0%, compared with 66.7% survival in goldfish mesocosms and 86.7% in mystery snail mesocosms, however the goldfish-by-mystery snail interaction was not statistically significant ($\beta = -1.06$, $SE = 1.31$, $z = -0.81$, $p = 0.42$) (Fig. 2.1d). Mean Gosner stage of surviving green frogs in goldfish + mystery snail mesocosms was higher (>34) than in mystery snail mesocosms (29), but the interaction was not significant ($\beta = 3.24$, $SE = 1.83$, $t = 1.77$, $p = 0.097$) (Fig. 2.1e).

Discussion

In experimental pond communities, goldfish strongly altered species composition and food web dynamics, while mystery snails had more limited effects. These differences largely stemmed from the distinct trophic levels of the two invaders and the relative strength of consumptive effects of a predator (goldfish) compared to an herbivore (mystery snails). Our results also suggest that trophic interactions of goldfish indirectly facilitated mystery snail reproduction and/or juvenile survival, providing experimental evidence for the invasional meltdown hypothesis. However, facilitation appears to have been only in one direction, as we found slightly reduced growth of goldfish in mesocosms with mystery snails. Taken together, our results emphasize the importance of studying invasive species effects within a food web context that incorporates direct and indirect effects, while also assessing interactions between multiple invaders that increasingly co-occur in nature.

Our study highlights the potential for introduced generalist omnivores to alter community composition, as goldfish affected every measured component of the experimental mesocosms. Goldfish substantially reduced survival and/or biomass of native snails, native frogs, zooplankton, and filamentous algae through direct consumption. The potential for goldfish to shift community structure is corroborated by a study by Trovillion et al. (in prep.), which found that invasive cyprinids (goldfish and common carp (*Cyprinus carpio*)) were associated with reduced invertebrate richness in ponds. Mean invertebrate richness in urban and urban greenspace ponds with goldfish ($n = 6$) was 10.2 taxa compared to an average of 16.6 taxa in ponds without goldfish ($n = 40$) (Trovillion et al., in prep.). Prior stomach contents surveys have found insects, zooplankton, eastern mosquitofish (*Gambusia affinis*), and large proportions of vegetation, cyanobacteria, and detritus in goldfish diets (Morgan & Beatty, 2007; Richardson et

al., 1995). By reducing herbivores, goldfish indirectly reduced herbivory on periphyton and phytoplankton, leading to strong increases in both via trophic cascades. This supports previous research, which linked zooplankton and macrophyte consumption by Prussian carp (*Carassius gibelio*) to phytoplankton production (Razlutskiy et al., 2021). Periphyton and phytoplankton increases in the mesocosms may have been amplified by goldfish consumption of filamentous algae, which likely reduced light levels and utilized nutrients in mesocosm without goldfish. Although studies of cyprinid effects on nutrients have shown mixed results (Razlutskiy et al., 2021; Zhang et al., 2016), a study of crucian carp (*Carassius carassius*) provides evidence for cyprinid excretion as a direct mechanism increasing growth of phytoplankton and cyanobacteria (Kolmakov & Gladyshev, 2003). Changes in primary production appeared to benefit some herbivores that were not susceptible to predation. For instance, despite the strong negative effect of goldfish on abundance and biomass of juvenile native snail, egg masses of native snails were more abundant in tanks with goldfish. Adult snails were not consumed by goldfish and were therefore released from competition and capable of higher egg production as other herbivores declined. Our research also supports previous findings that goldfish increase turbidity via bioturbation (Crivelli, 1995; Richardson et al., 1995; Richardson & Whoriskey, 1992), which may exacerbate increases in nutrients and phytoplankton.

The traits of native species can mediate the effects of nonnative species, as demonstrated by the inconsistent effects of goldfish on amphibians in our experiment. Goldfish eliminated gray tree frogs across all mesocosm. In previous research, goldfish have also been associated with reductions in other amphibians including European common frogs (*Rana temporaria*) and eastern long-toed salamanders (*Ambystoma macrodactylum columbianum*) (Meyer et al., 1998; Monello & Wright, 2001). Conversely, while goldfish were associated with green frog mortality

in our experiment, many green frogs survived and coexisted with goldfish. In fact, surviving green frogs grew faster when goldfish were present, likely due to release from inter- and intraspecific competition. While faster amphibian development may be associated with predator presence in an effort to avoid unfavorable conditions (Melotto et al., 2020), green frogs were also larger on average when goldfish were present (1.7cm mean SVL in goldfish mesocosms and 1.3cm SVL in controls), supporting the facilitation mechanism. Whereas gray tree frogs are palatable to and rarely co-occur with native or nonnative predatory fish (Hecnar & M'Closkey, 1997; Shulse et al., 2013; G. Smith & Smith, 2015), green frogs are more common in permanent wetlands, are unpalatable to certain predators, and have co-evolved with predatory fish (Adams et al., 2011; Babbitt, 2005; G. R. Smith et al., 2019). Similar results have been found in a separate study system in which nonnative mosquitofish reduced survival of native Pacific tree frogs, but increased growth of unpalatable western toad larvae through competitive release (Preston et al., 2012), suggesting this may be a general phenomenon when amphibian larvae differ in palatability. In urban areas including our study site of Madison, Wisconsin, stormwater retention and land use changes have caused shifts from natural wetlands with shorter hydroperiods to human-modified permanent ponds (Rubbo & Kiesecker, 2005; Sauer et al., in review). This habitat change increases suitability of ponds for fish, and likely increases subsequent declines of palatable amphibians and other species susceptible to fish predation (Semlitsch et al., 2015; Shulse et al., 2010). Importantly, our research suggests that goldfish effects on amphibians are species-specific, and that goldfish will induce community shifts in favor of amphibian species that co-evolved with fish.

Compared to goldfish, mystery snails had relatively few effects in the community. Mystery snails showed sublethal competitive effects on gray tree frogs by delaying development

rates. While we expected mystery snails to affect primary production through grazing and filtering, we did not observe these effects in mesocosms. Unlike native snails, mystery snails were rarely observed on the plexiglass sheets used to sample periphyton, so mystery snails may have preferentially grazed periphyton from the benthos or walls, which we did not measure. Mystery snail effects on benthic algae have been observed in previous mesocosm studies (Johnson et al., 2009). Previous research has shown that mystery snails feed both by grazing and filter feeding (Olden et al., 2013), but we did not find strong evidence of reduced phytoplankton due to filter feeding by mystery snails either. We note, however, that relative phytoplankton fluorescence was lower in mystery snail treatments, but this difference was not significant. Per capita filter feeding rates of mystery snails increases with snail density, which suggests that mystery snails filter feed facultatively depending on the availability of benthic resources (Olden et al., 2013). Unexpectedly, we also saw no effects of mystery snails on native snails. This contrasts results of a prior mesocosm study in which mystery snails negatively affected native *Physa gyrina* and *Limnaea stagnalis* snails in Wisconsin (Johnson et al., 2009). According to a review by David et al. (2017), competitive effects of invasive species tend to be less extreme than predatory effects and are unlikely to cause extirpations. Our research supports this idea, as competitive effects of mystery snails were sublethal or undetectable compared to multiple highly lethal predatory effects of goldfish.

We found experimental evidence for facilitation of one invasive species by another, supporting the invasional meltdown hypothesis. The biomass of juvenile mystery snails was higher when goldfish were present, suggesting either higher survival of juveniles or increased reproductive success of adults. Despite higher abundance, juvenile mystery snails were larger in tanks with goldfish, though this pattern was not significant. Despite their generalist feeding

behavior, goldfish did not consume juvenile mystery snails. Relative to juvenile pond and ramshorn snails, juvenile mystery snails are much larger with a minimum shell length of 6.5mm recorded at the end of our study, compared to a minimum of 2.3mm for pond snails and 1.8mm for ramshorn snails. Mystery snails also have thick shells, which likely reduces their susceptibility to predation by goldfish and other predators such as crayfish (Johnson et al., 2009). As with green frogs, the ability of mystery snails to coexist with goldfish led to benefits from reduced competition and higher resource availability. In Madison, Wisconsin urban ponds where goldfish and mystery snails coexisted, mystery snails were found in high densities (Trovillion et al. in prep.), suggesting a similar ability to coexist with goldfish in natural settings. Research assessing stage-structured interactions with mystery snails and goldfish would help to clarify effects across populations, and additional field research could determine whether facilitation is occurring in natural waterbodies. The similar facilitative effects on green frogs, native snail egg production, and biomass of juvenile mystery snails suggest that competitive release driven by goldfish may be generalizable across multiple herbivore groups that can resist predation. Mystery snails had a small negative effect on goldfish by reducing goldfish length. This interaction may be due to exploitative competition involving shared resources or to a reduction in goldfish prey biomass via competition with mystery snails, though we do not have strong support for either mechanism. Longer term experiments across multiple goldfish generations may provide a clearer understanding of the effects of mystery snails on goldfish.

Our results have several implications for pond management and the trade in aquarium species. To protect amphibian and invertebrate biodiversity, support ecological function, and maintain water clarity in urban waterbodies, prevention of goldfish introductions and removal of established populations via pond draining could be beneficial. Preventing introductions is

complicated by the fact that goldfish are widely available through the aquarium trade. The aquarium trade is a multi-billion dollar industry that is often poorly regulated (Maceda-Veiga et al., 2019; Patoka et al., 2018; Penning et al., 2009). Increasing regulations may help to prevent releases, though enforcement of these regulations can be a challenge (Maceda-Veiga et al., 2019), especially because of the volume of sellers and diversity of venues. For instance, invasive or potentially invasive snails, fish, and other aquatic species are readily available for purchase both in stores and from abundant online sellers (Olden et al., 2021; Preston et al., 2022). Partnering directly with organizations and individuals in the aquarium trade may also be an effective strategy for implementing conservation and invasive species education initiatives (Maceda-Veiga et al., 2016; Seekamp et al., 2016).

The effects of goldfish and mystery snails in our experiment highlight the wide variation in community interactions of invasive species depending on their trophic positions. Our findings provide novel evidence of facilitation of invasive mystery snails by invasive goldfish and reveal substantial shifts in producer and herbivore populations associated with goldfish introductions. We suggest future research analyze goldfish-mystery snail interactions in field settings, and further investigate implications of invasive goldfish to conservation and ecosystem services across urban waterbodies. Broadly, our results emphasize the need to study invasive species in a food web context to capture direct and indirect effects across multiple trophic levels.

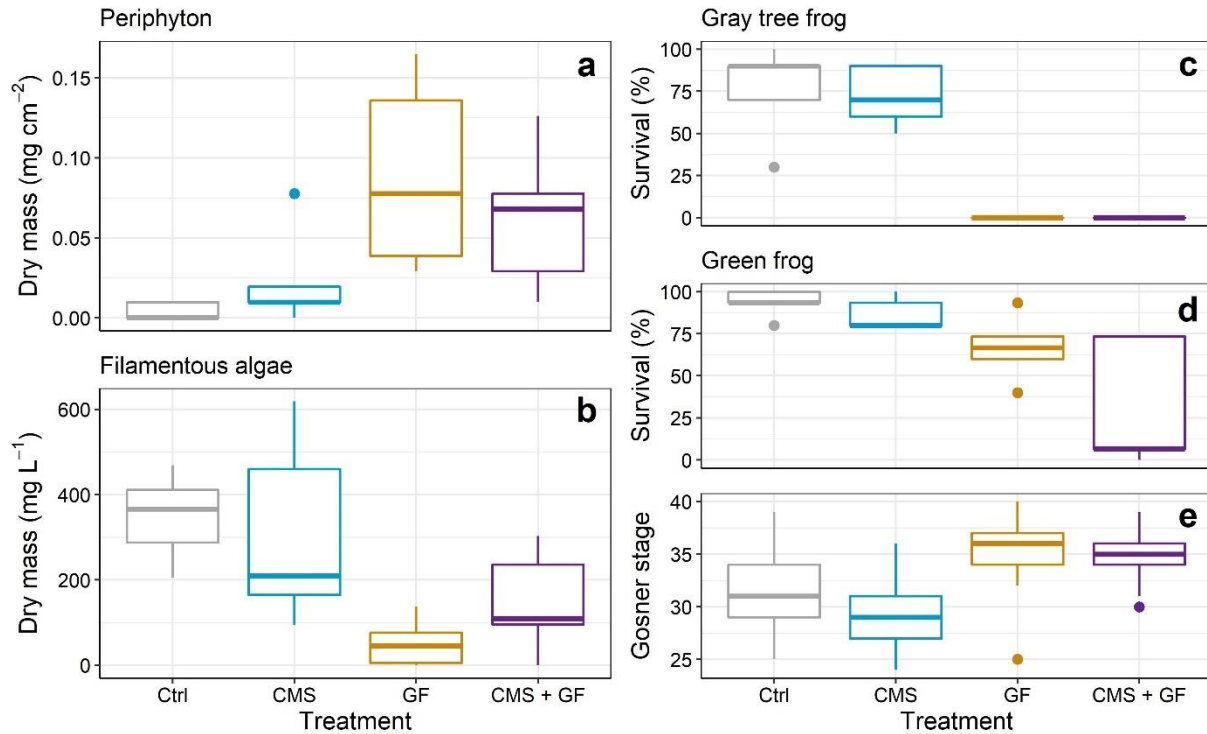


Figure 2.1) Boxplots showing final values for a) periphyton dry mass (collected from 20.3 x 25.4 cm plexiglass sheets), b) filamentous algae dry mass, c) final percent survival of gray tree frogs (out of 10 added), and final d) percent survival (out of 15 added), and e) Gosner stage of green frog tadpoles. Thick lines represent median values, and lower and upper box boundaries represent the first and third quartiles, respectively. Treatment groups are abbreviated as “Ctrl” (control), “CMS” (Chinese mystery snails present), “GF” (goldfish present), and “CMS + GF” (Chinese mystery snails and goldfish present).

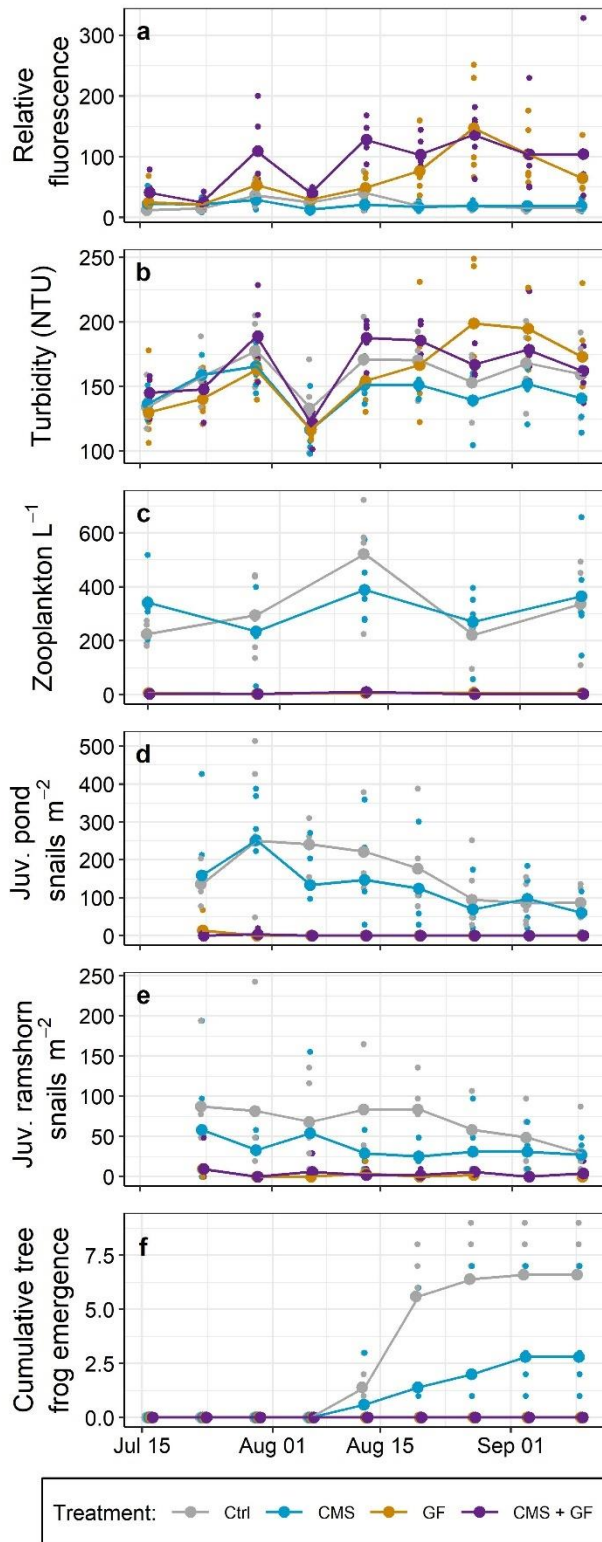


Figure 2.2) Measurements of a) relative phytoplankton fluorescence, b) turbidity, c) total zooplankton density, d) juvenile pond snail (*Physa acuta*) density, e) juvenile ramshorn snail

(*Planorbella trivolvis*) density, and f) cumulative emergence (i.e., complete metamorphosis) of gray tree frogs (*Hyla versicolor*) recorded weekly or bi-weekly during a nine-week mesocosm experiment. Large points connected by lines represent mean values per week for each treatment group, and small points represent raw data. Treatment groups are abbreviated as “Ctrl” (control), “CMS” (Chinese mystery snails present), “GF” (goldfish present), and “CMS + GF” (Chinese mystery snails and goldfish present).

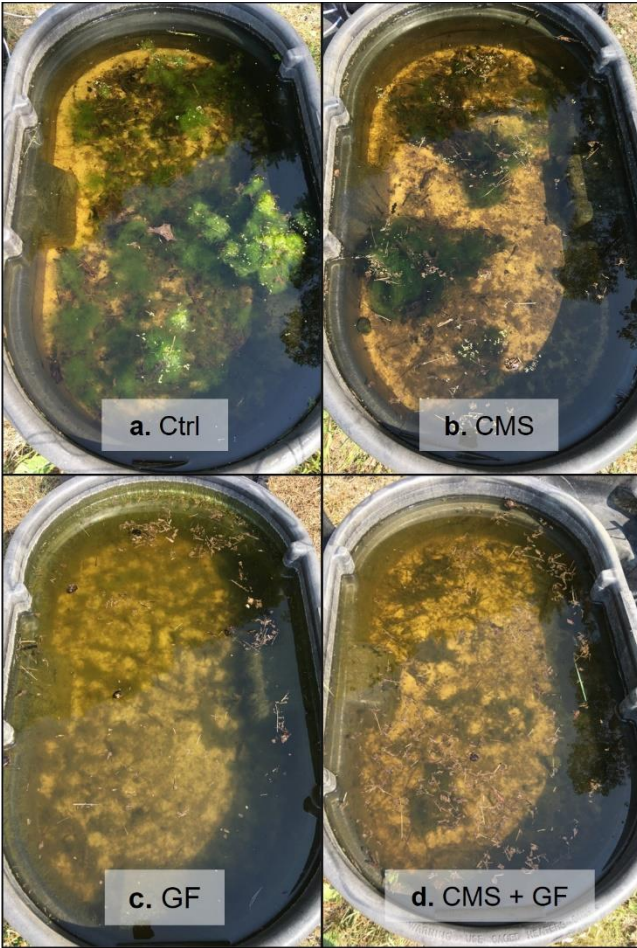


Figure 2.3) Photographs of a representative experimental mesocosm from each treatment group on August 25, 2020 (day 40 of 60) showing differences in water clarity and primary production for each treatment. Treatment groups are a) control (“Ctrl”), b) Chinese mystery snails present (“CMS”), c) goldfish present (“GF”), and d) Chinese mystery snails and goldfish present (“CMS + GF”).

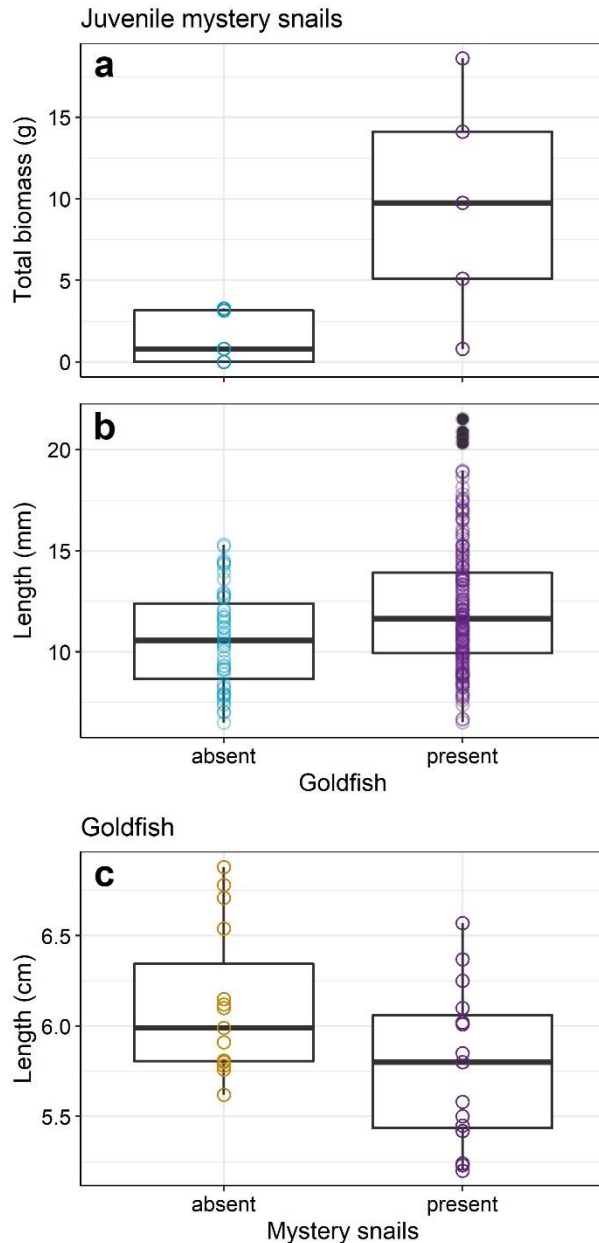


Figure 2.4) Boxplots showing a) total biomass and b) shell length of juvenile mystery snails in tanks with and without goldfish, and c) final length of goldfish in tanks with and without mystery snails. Thick lines represent median values, and lower and upper box boundaries represent the first and third quartiles, respectively. Open circles represent raw values.

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APPENDICES

Appendix 1.

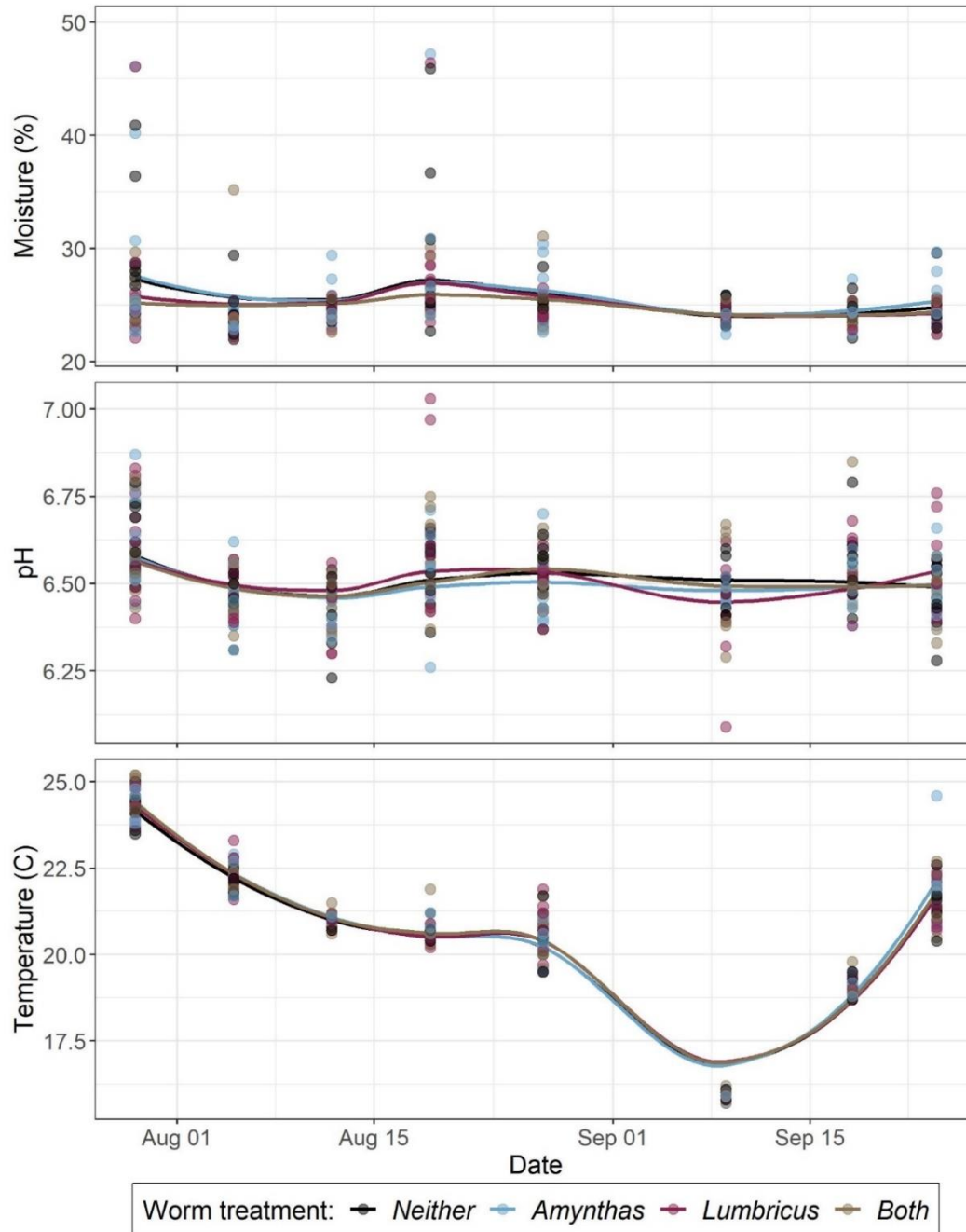


Figure A1.1) Soil measurements in each earthworm treatment over time during the July-September 2019 mesocosm experiment. Points are raw mesocosm values and smoothed lines represent change in means. pH means are calculated from H⁺ concentration means.

Table A1.1) Initial and final number (n or mean n), mean mass, and standard error of the mean mass (SE) of individual American toads (*A. amer.* = *Anaxyrus americanus*), *Amyntas* earthworms (*Amy.*), and *Lumbricus* earthworms (*Lum.*) for tanks in each treatment group (“Reps.” = number of treatment replicates) in a July-September 2019 mesocosm experiment. Only tanks with survivors are considered in final mean mass/SE values.

Initial										
Treatment	Reps.	<i>A. americanus</i>			<i>Amyntas</i>			<i>Lumbricus</i>		
		n	mean mass (g)	SE	n	mean mass (g)	SE	n	mean mass (g)	SE
<i>A. amer.</i> + <i>Amy.</i> + <i>Lum.</i>	6	4	0.35	0.05	30	0.31	0.004	20	0.26	0.001
<i>A. amer.</i> + <i>Amy.</i>	5	4	0.38	0.05	30	0.31	0.005	0	-	-
<i>A. amer.</i> + <i>Lum.</i>	5	4	0.45	0.04	0	-	-	20	0.26	0.009
<i>A. amer.</i>	6	4	0.42	0.04	0	-	-	0	-	-
<i>Amy.</i> + <i>Lum.</i>	6	0	-	-	30	0.31	0.008	20	0.25	0.004
<i>Amy.</i>	6	0	-	-	30	0.30	0.006	0	-	-
<i>Lum.</i>	6	0	-	-	0	-	-	20	0.28	0.008
control	6	0	-	-	0	-	-	0	-	-
Final										
Treatment	Reps.	<i>A. americanus</i>			<i>Amyntas</i>			<i>Lumbricus</i>		
		mean n	mean mass (g)	SE	mean n	mean mass (g)	SE	mean n	mean mass (g)	SE
<i>A. amer.</i> + <i>Amy.</i> + <i>Lum.</i>	6	0.83	0.36	0.08	1.8	0.79	0.06	1.5	1.27	0.22
<i>A. amer.</i> + <i>Amy.</i>	5	1.2	0.25	0.03	1.2	0.70	0.11	0	-	-
<i>A. amer.</i> + <i>Lum.</i>	5	1.83	0.36	0.03	0	-	-	3.8	1.25	0.22
<i>A. amer.</i>	6	1.5	0.31	0.05	0	-	-	0	-	-
<i>Amy.</i> + <i>Lum.</i>	6	0	-	-	0.4	0.89	NA	1.2	1.74	0.30
<i>Amy.</i>	6	0	-	-	1.2	0.69	0.01	0	-	-
<i>Lum.</i>	6	0	-	-	0	-	-	1.7	1.38	0.21
control	6	0	-	-	0	-	-	0	-	-

*Includes mass of *Amyntas* added initially (n=20), not those supplemented in August (n=10)

Table A1.2) American toad (*Anaxyrus americanus*) and prey masses in September 2019 feeding trial experiments in Madison, WI, USA. Whether or not each trial resulted in successful capture of the prey is provided in the column labelled “cap.?”. The following abbreviations are used for prey types: *Ach.* = *Acheta domesticus*, *Amy.* = *Amyntas* spp., *Lum.* = *Lumbricus* spp.

Experiment 1													
toad ID	toad mass (g)	trial 1			trial 2			trial 3			trial 4		
		prey type	prey mass (g)	cap.?	prey type	prey mass (g)	cap.?	prey type	prey mass (g)	cap.?	prey type	prey mass (g)	cap.?
1	13.2	<i>Ach.</i>	0.1	yes	<i>Amy.</i>	0.67	no	<i>Lum.</i>	2.4	no	-	-	-
2	32.5	<i>Amy.</i>	1.7	no	<i>Lum.</i>	3.39	no	<i>Ach.</i>	0.2	yes	-	-	-
3	25.9	<i>Lum.</i>	4.1	yes	<i>Ach.</i>	0.18	no	<i>Amy.</i>	1.0	no	-	-	-
4	22.5	<i>Ach.</i>	0.2	yes	<i>Amy.</i>	1.15	no	<i>Lum.</i>	2.7	no	-	-	-
5	23.3	<i>Amy.</i>	0.7	yes	<i>Lum.</i>	2.16	yes	<i>Ach.</i>	0.1	yes	-	-	-
6	13.1	<i>Lum.</i>	3.1	no	<i>Ach.</i>	0.12	yes	<i>Amy.</i>	1.0	no	-	-	-
7	25.2	<i>Ach.</i>	0.1	no	<i>Amy.</i>	0.61	yes	<i>Lum.</i>	2.9	yes	-	-	-
8	27.7	<i>Amy.</i>	1.1	no	<i>Lum.</i>	3.1	no	<i>Ach.</i>	0.2	yes	-	-	-
9	5.7	<i>Lum.</i>	2.1	yes	<i>Ach.</i>	0.18	yes	<i>Amy.</i>	0.5	no	-	-	-
10	4.7	<i>Ach.</i>	0.1	no	<i>Amy.</i>	0.63	no	<i>Lum.</i>	2.4	no	-	-	-
11	3.5	<i>Amy.</i>	1.1	no	<i>Lum.</i>	2.32	no	<i>Ach.</i>	0.1	yes	-	-	-
12	4.6	<i>Lum.</i>	2.2	no	<i>Ach.</i>	0.11	yes	<i>Amy.</i>	0.5	yes	-	-	-
Experiment 2													
1	12.1	<i>Amy.</i>	0.4	no	<i>Lum.</i>	0.54	no	<i>Amy.</i>	0.6	no	<i>Lum.</i>	0.6	no
2	30.9	<i>Lum.</i>	0.7	no	<i>Amy.</i>	0.54	no	<i>Lum.</i>	0.3	no	<i>Amy.</i>	1.0	no
3	22.4	<i>Amy.</i>	0.4	yes	<i>Lum.</i>	0.6	yes	<i>Amy.</i>	0.6	no	<i>Lum.</i>	0.8	yes
4	20.5	<i>Lum.</i>	0.4	yes	<i>Amy.</i>	0.85	no	<i>Lum.</i>	0.9	yes	<i>Amy.</i>	0.7	no
5	21.8	<i>Amy.</i>	0.7	yes	<i>Lum.</i>	0.36	yes	<i>Amy.</i>	0.7	no	<i>Lum.</i>	0.4	yes
6	11.5	<i>Lum.</i>	0.6	no	<i>Amy.</i>	0.76	no	<i>Lum.</i>	0.8	no	<i>Amy.</i>	0.7	no
7	26.6	<i>Amy.</i>	0.5	yes	<i>Lum.</i>	0.69	yes	<i>Amy.</i>	0.6	no	<i>Lum.</i>	0.8	yes
8	23.4	<i>Lum.</i>	0.6	no	<i>Amy.</i>	0.5	no	<i>Lum.</i>	0.8	no	<i>Amy.</i>	0.7	no
9	6.1	<i>Amy.</i>	0.5	no	<i>Lum.</i>	0.36	yes	<i>Amy.</i>	0.5	no	<i>Lum.</i>	0.4	yes
10	4.4	<i>Lum.</i>	0.5	no	<i>Amy.</i>	0.69	no	<i>Lum.</i>	0.7	no	<i>Amy.</i>	0.4	no
11	3.3	<i>Amy.</i>	0.5	no	<i>Lum.</i>	0.78	no	<i>Amy.</i>	0.5	no	<i>Lum.</i>	0.4	no
12	4.6	<i>Lum.</i>	0.4	yes	<i>Amy.</i>	0.4	no	<i>Lum.</i>	0.8	yes	<i>Amy.</i>	0.5	No

Table A1.3) Effects of sample date and treatment effects on soil responses in a 2019 mesocosm experiment in Madison, WI, USA. The following generalized additive models were used: soil response ~ *Amyntas* * *Lumbricus* + s(sample date) + (1 | mesocosm ID). *Amyntas* spp., and *Lumbricus* spp. are categorical predictors and sample date is a continuous predictor.

Soil Response	Coefficient	Value	SE	t	p
% moisture	<i>Amyntas</i>	0.005	0.025	0.20	0.84
	<i>Lumbricus</i>	-0.016	0.024	-0.66	0.51
	<i>Amyntas</i> * <i>Lumbricus</i>	-0.008	0.035	-0.24	0.81
	s(sample date)	-0.168	0.050	-3.37	>0.001
pH	<i>Amyntas</i>	-0.009	0.019	-0.49	0.63
	<i>Lumbricus</i>	0.005	0.018	0.29	0.77
	<i>Amyntas</i> * <i>Lumbricus</i>	0.002	0.027	0.07	0.95
	s(sample date)	-0.309	0.054	-5.69	>0.001
temperature (°C)	<i>Amyntas</i>	0.112	0.061	1.83	0.08
	<i>Lumbricus</i>	0.072	0.060	1.20	0.24
	<i>Amyntas</i> * <i>Lumbricus</i>	-0.072	0.087	-0.83	0.41
	s(sample date)	1.003	0.256	3.92	>0.001

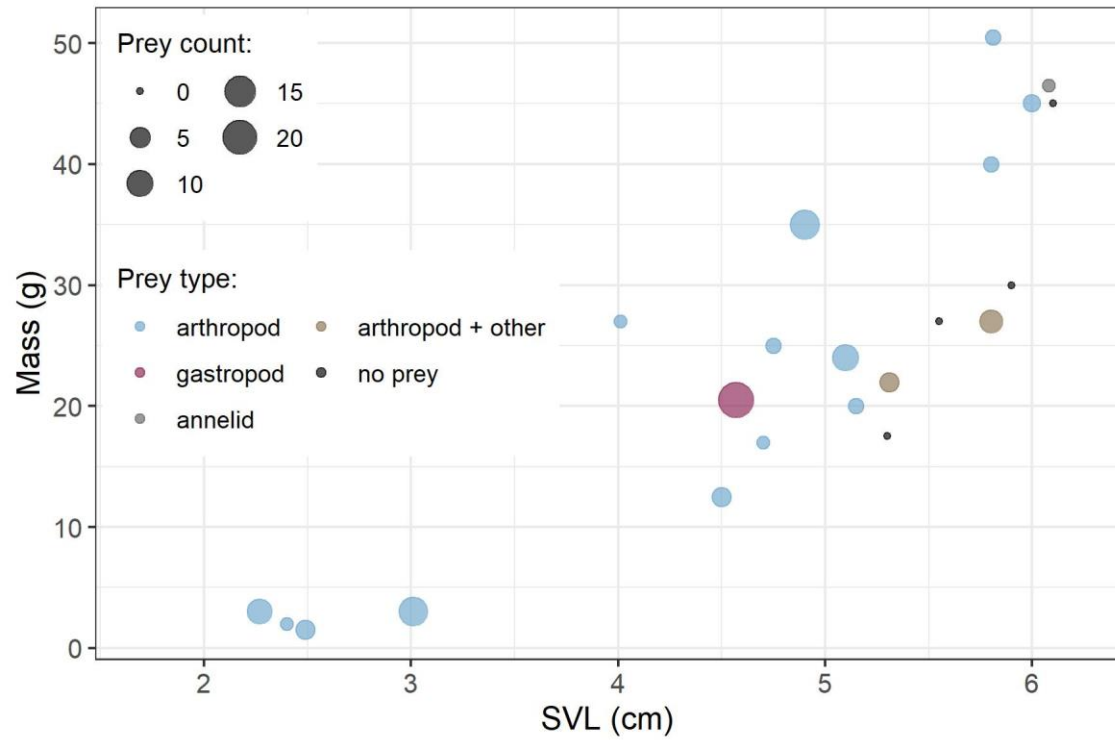


Figure A1.2) Mass by snout-vent length (SVL) of American toads (*Anaxyrus americanus*) collected during 2020 herpetofauna diet surveys at the UW Arboretum (Madison, WI, USA). Number and category of prey items are represented by point size and color, respectively.

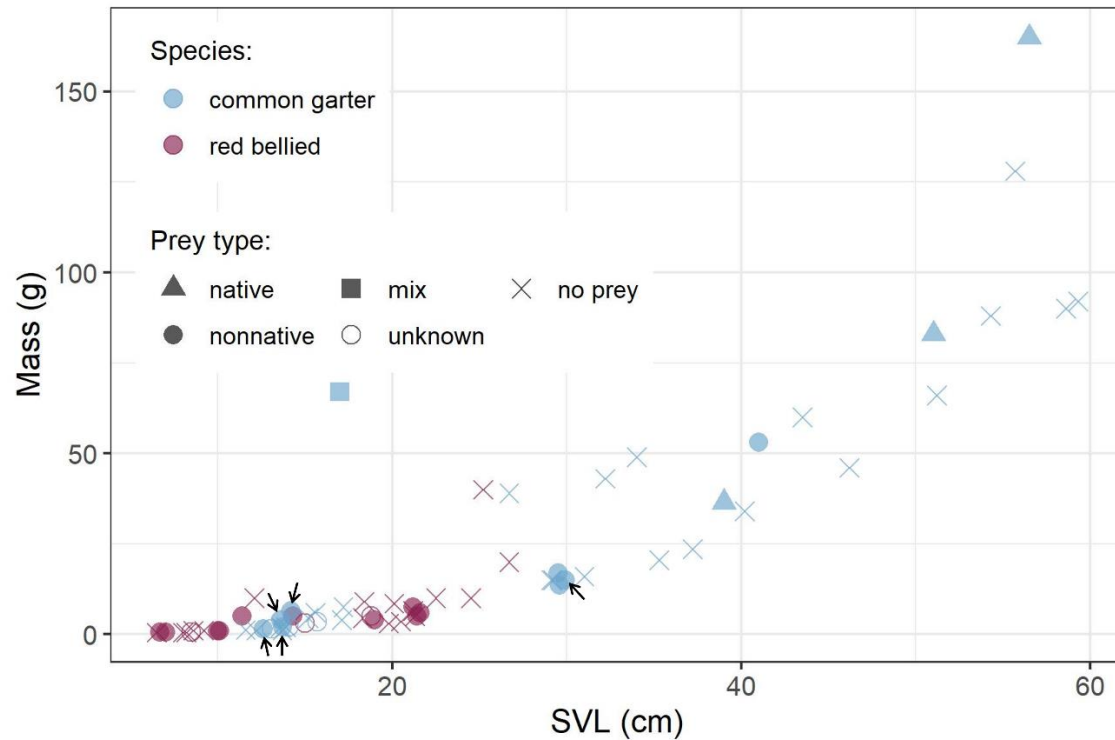


Figure A1.3) Mass by snout-vent length (SVL) of common garter snakes (*Thamnophis sirtalis*) and red bellied snakes (*Storeria occipitomaculata*) collected during 2020 herpetofauna diet surveys at the UW Arboretum (Madison, WI, USA). Prey native or nonnative status is represented by point shapes, and black arrows point toward the five individual common garter snakes from which *Amyntas* spp. prey were recovered.

Appendix 2.

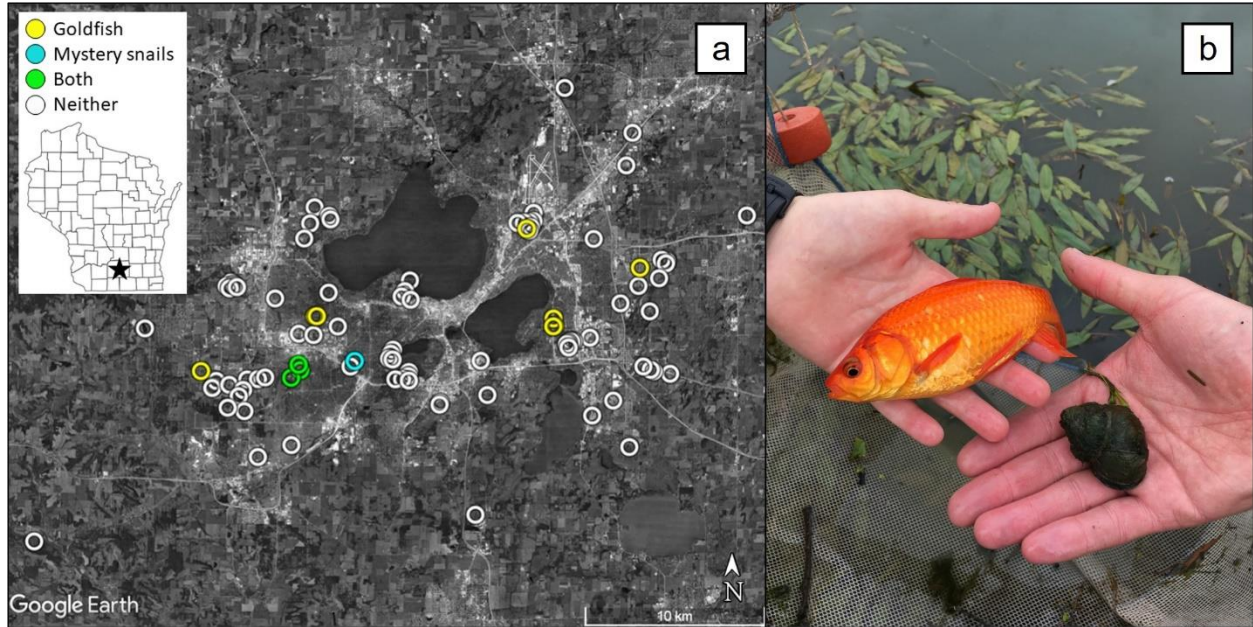


Figure A2.1) a) Map of Madison, Wisconsin, USA showing sites of ponds surveyed in summer 2019 and 2020. Goldfish (*Carassius auratus*) were detected in 10 ponds and Chinese mystery snails (*Cipangopaludina* (= *Bellamya*) *chinensis*) in 4 ponds out of 102 surveyed. Goldfish and mystery snails overlapped in 3 ponds. b) Photo of a goldfish and mystery snail collected in one seine net sweep at Elver Park pond in Madison.

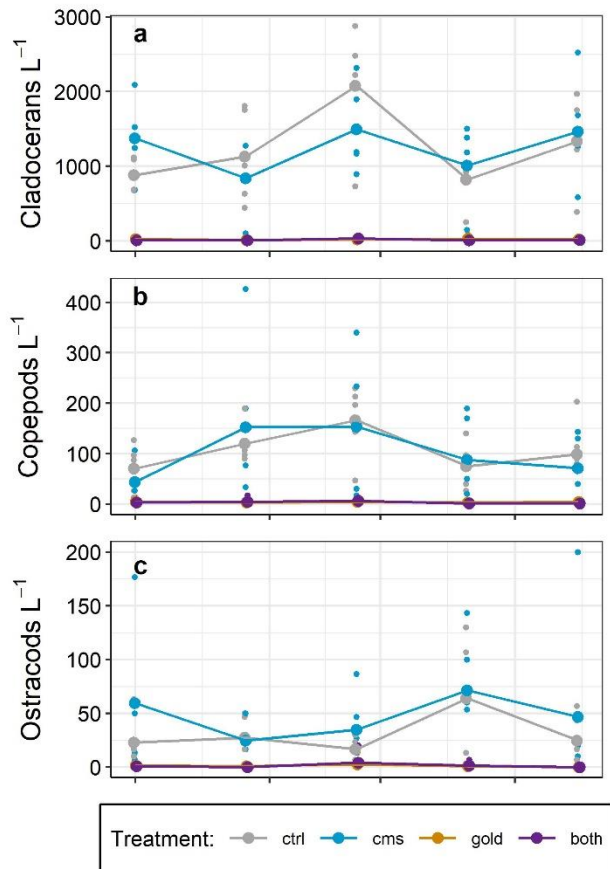


Figure A2.2) Density measurements over time of the following zooplankton taxa: a) cladocerans, b) copepods, and c) ostracods. Zooplankton were collected bi-weekly in 300ml samples. Large points connected by lines represent mean values per week for each treatment group, and small points represent raw data. Treatment groups are abbreviated as “Ctrl” (control), “CMS” (Chinese mystery snails present), “GF” (goldfish present), and “CMS + GF” (Chinese mystery snails and goldfish present).

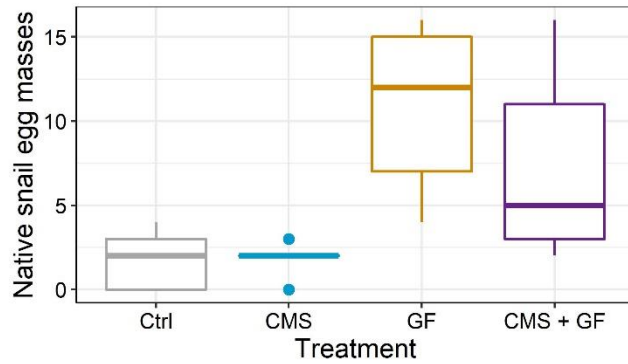


Figure A2.3) Native snail egg masses across mesocosm treatments, including both ramshorn (*Helisoma trivolvis*) and pond snails (*Physa acuta*). Egg mass values for each mesocosm were summed across 8 sampling dates (one per week) from July 23 to September 10, 2020. Egg masses were counted from a plexiglass sheet (one side = 516cm²) placed in each mesocosm at the start of the experiment. Thick lines represent median values, and lower and upper box boundaries represent the first and third quartiles, respectively. Treatment groups are abbreviated as “Ctrl” (control), “CMS” (Chinese mystery snails present), “GF” (goldfish present), and “CMS + GF” (Chinese mystery snails and goldfish present).

Table A2.1) Number, mean mass, mean shell length, and mean aperture width (widest distance) of snails added to mesocosms (n = 5) of each treatment group. Snail species were Chinese mystery snails (“CMS”, *Cipangopaludina* (= *Bellamya*) *chinensis*), pond snails (*Physa acuta*), and ramshorn snails (*Helisoma trivolvis*).

Treatment	Species	Number	Mean mass (g)	Mean shell length (mm)	Mean aperture width (mm)
Ctrl	CMS	0	-	-	-
	pond snail	20	0.04	7.1	2.1
	ramshorn snail	10	0.72	14.7	7.6
CMS	CMS	5	20.63	45.4	25.3
	pond snail	20	0.04	6.9	2.2
	ramshorn snail	10	0.74	14.8	7.6
GF	CMS	0	-	-	-
	pond snail	20	0.03	6.7	2.0
	ramshorn snail	10	0.75	14.9	7.6
CMS + GF	CMS	5	20.15	45.3	24.6
	pond snail	20	0.04	7	2.1
	ramshorn snail	10	0.72	14.6	7.6

Table A2.2) Zero-inflated negative binomial models for juvenile pond and ramshorn snail densities in mesocosms over time. Models include a random intercept for mesocosm identity.

Response	Predictor	Coefficient	SE	z	p
Juvenile pond snail density	Negative binomial coefficients:				
	CMS	0.12	0.38	0.31	0.754
	GF	-1.54	0.79	-1.93	0.053
	week	-0.16	0.04	-3.76	0.000
	CMS * GF	-1.42	1.32	-1.08	0.281
	CMS * week	-0.07	0.06	-1.21	0.226
	Zero-part coefficients:				
	GF	6.41	1.34	4.77	< 0.001
Juvenile ramshorn snail density	Negative binomial coefficients:				
	CMS	-0.13	0.55	-0.23	0.818
	GF	-1.52	0.66	-2.32	0.020
	week	-0.13	0.05	-2.85	0.004
	CMS * GF	0.45	0.92	0.49	0.624
	CMS * week	-0.01	0.08	-0.15	0.883
	Zero-part coefficients:				
	GF	5.22	1.41	3.70	< 0.001

Table A2.3) Models for measurements taken at the end of the mesocosm experiment or averaged across time points (native snail egg masses only). Linear models were used for filamentous algae and periphyton responses, a generalized linear model was used for the native snail egg masses response, a linear mixed effect model was used for green frog stage, and generalized linear mixed effect models were used for green frog survival and tree frog survival. All mixed effects models include a random intercept for mesocosm identity. Test statistic represents a t-value for all models except green frog survival and tree frog survival, in which test statistic represents a z-value. Model distribution of non-Gaussian distributed models are listed in parentheses below the model response.

Response	Predictor	Coefficient	SE	Test statistic	p
Filamentous algae	CMS	-0.40	0.76	-0.53	0.604
	GF	-2.81	0.76	-3.69	0.002
	CMS * GF	1.45	1.08	1.35	0.196
Periphyton	CMS	0.08	0.06	1.44	0.169
	GF	0.25	0.06	4.22	0.001
	CMS * GF	-0.14	0.08	-1.65	0.119
Native snail egg masses (neg. binomial)	CMS	0.00	0.55	0.00	1.000
	GF	1.79	0.46	3.86	0.000
	CMS * GF	-0.38	0.66	-0.57	0.568
Green frog stage	CMS	-2.63	1.26	-2.10	0.053
	GF	3.97	1.26	3.16	0.006
	CMS * GF	3.24	1.83	1.77	0.097
Green frog survival (binomial)	CMS	-0.94	0.99	-0.95	0.343
	GF	-2.26	0.96	-2.36	0.018
	CMS * GF	-1.06	1.31	-0.81	0.419
Tree frog survival (binomial)	CMS	-0.33	0.79	-0.42	0.674

Table A2.4) Models for temporally measured responses. Linear mixed effect models were used for Turbidity and relative fluorescence (log-transformed) responses, and a generalized linear mixed effect models was used for total zooplankton (cladocerans + copepods + ostracods) density. Models include a random intercept for mesocosm identity. Model distribution of non-Gaussian distributed models are listed in parentheses below the model response.

Response	Predictor	Coefficient	SE	z	p
Turbidity	CMS	-2.90	13.82	-0.21	0.836
	GF	-21.07	13.82	-1.53	0.138
	week	2.02	1.24	1.62	0.107
	CMS * GF	26.64	19.54	1.36	0.183
	CMS * week	-2.38	1.76	-1.36	0.177
	GF * week	5.62	1.76	3.20	0.002
	CMS * GF * week	-2.24	2.49	-0.90	0.370
log(Relative fluorescence)	CMS	-0.02	0.25	-0.07	0.946
	GF	0.13	0.25	0.53	0.597
	week	0.00	0.03	-0.07	0.943
	CMS * GF	0.57	0.35	1.64	0.106
	CMS * week	0.01	0.04	-0.19	0.847
	GF * week	0.19	0.04	4.39	< 0.001
	CMS * GF * week	0.05	0.06	-0.74	0.458
Total zooplankton density (zoop./100ml) (Poisson)	CMS	0.08	0.20	0.38	0.707
	GF	-3.90	0.32	-12.03	< 0.001
	week	0.02	0.01	3.88	< 0.001
	CMS * GF	-0.40	0.49	-0.82	0.415
	CMS * week	-0.01	0.01	-1.22	0.224
	GF * week	-0.01	0.04	-0.27	0.789
	CMS * GF * week	-0.01	0.07	-0.18	0.855