Effects of Invasive Jumping Worms (*Amyntas* spp.) On Microhabitat and Trophic Interactions of Native Herpetofauna

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Effects of invasive jumping worms (*Amyntas* spp.) on microhabitat and trophic interactions of native herpetofauna

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Abstract

Invasive species can affect native communities through multiple mechanisms, including ecosystem engineering and trophic interactions. In North America, invasive jumping worms (*Amyntas* spp.) may alter microhabitats used by native herpetofauna or serve as a novel food resource for herpetofauna predators. We investigated effects of *Amyntas* on leaf litter microhabitat and trophic interactions of native herpetofauna using an outdoor mesocosm experiment, laboratory feeding trials, and field surveys of predator stomach contents in Wisconsin, USA. In mesocosms, *Amyntas* reduced leaf litter biomass but did not have clear effects on soil conditions (pH, moisture, and temperature) or survival of American toad metamorphs (*Anaxyrus americanus*). In laboratory trials, American toads preyed on *Amyntas*, though toads were less successful at capturing *Amyntas* compared with alternative prey, namely European earthworms (*Lumbricus* spp.) and crickets (*Acheta domesticus*). The differences in prey attack success were likely due to unique defensive behaviors of *Amyntas*. *Amyntas* also spent less time moving than *Lumbricus*, which was associated with later prey detection times by toads. Based on diet surveys, *Amyntas* are consumed by common garter snakes (*Thamnophis sirtalis*) in southern Wisconsin. We did not find evidence of *Amyntas* consumption by American toads or red-bellied snakes (*Storeria occipitomaculata*), although additional surveys will be needed to definitively determine whether these taxa are consuming *Amyntas*. Our findings highlight the importance of studying multiple mechanisms by which invasive species affect native communities and suggest that unique anti-predator behaviors may influence how *Amyntas* are incorporated into food webs as a novel prey resource.

Keywords: invasive earthworm, *Amyntas*, herpetology, trophic interactions, ecosystem engineer

Introduction

Invasive species can affect recipient communities through a wide range of mechanisms, resulting in unforeseen consequences for native species. For instance, an invader may directly or indirectly influence trophic interactions, competition dynamics, disease transmission, or physical ecosystem structure (Crooks 2002; Carlsson et al. 2009; Vilcinskas 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. Determining overall effects of ecosystem engineers in invaded communities can be complex. For example, an invasive algal ecosystem engineer (*Halimeda incrassata*) causes multidirectional effects within a Mediterranean Sea community; depending on the species, native fish may be attracted to, unaffected by, or driven away from the altered ecosystem (Vivó-Pons et al. 2020). Another invasive engineer, the North American beaver (*Castor canadensis*), reduces macroinvertebrate diversity in Chilean streams but increases overall macroinvertebrate biomass and productivity (Anderson and Rosemond 2007). Assessing net effects of invasive ecosystem engineers is further complicated when the invader also directly interacts with native taxa.

While the predatory roles of some invaders have received longstanding research attention, the potential for invaders 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 utilize 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).

Jumping worms (*Amyntas* and *Metaphire* spp.) are widespread invasive species in the eastern United States and are recent invaders in several midwestern and northeastern states (Chang et al. 2018). As only species of the *Amyntas* genus are considered here, the group 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). Invasive *Amyntas* may induce changes in native communities through multiple mechanisms, though the overall effects of these changes on native species are largely unknown. *Amyntas* live on the surface and upper few centimeters of soil and consume detritus, which alters topsoil structure (Görres et al. 2019) and reduces leaf

litter layers (Qiu and Turner 2017). Invasive *Amyntas* indirectly alter composition of leaf litter invertebrate communities through mechanisms including resource competition (Snyder et al. 2011; Greiner et al. 2012; Gao et al. 2017) and have been known to outcompete previously established nonnative earthworms (e.g., *Apporectodea*, *Lumbricus*, and *Octolasion spp.*) (Chang et al. 2016b; Laushman et al. 2018). Additionally, invasive *Amyntas* potentially provide an abundant novel prey source, but anti-predator 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 earthworm species but 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). In the current study, we asked 1) how invasive *Amyntas* worms, established nonnative *Lumbricus* worms, and native American toads interact within leaf litter microhabitat, 2) how predator-prey interactions with American toad predators compare for *Amyntas* prey and alternative prey under laboratory conditions, and 3) whether native toads and snakes consume *Amyntas* in the field. We predicted that 1) *Amyntas* would reduce leaf litter mass, soil

moisture, and metamorphic toad survival, and increase soil temperature within mesocosms based on known effects of *Amyntas* on forest floor habitat, 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 (n=48; rim diameter = 45cm, height = 38cm). 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 approximately 460g of hand mixed leaf litter randomly collected 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 S1) 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 S1) 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 moisture (Extech Soil Moisture Meter MO750) by inserting meters 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 earthworms, we added 10

newly collected *Amyntas* to all *Amyntas*-treated mesocosms (for a total of 30 *Amyntas* worms added). 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 S2), collected from a jumping worm-free location (the UW-Madison Lakeshore 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 S2). 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 S2). 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

185 trials per toad per prey type (48 total trials). In both experiments, trials were performed two days
186 apart, and the order of prey for each toad was rotated between individual toads (Table S2).

187 Feeding trials were conducted in a clear plastic arena with a rectangular base (29.7 x 19.3
188 x 20.3 cm.) and brown paper was attached to the walls to minimize external stimuli. During each
189 trial, we placed a toad under a transparent cover in the arena for a five-minute acclimation
190 period. A pre-weighed prey item was placed in front of the toad, and the cover was removed. The
191 time of each attack or capture and total number of attacks were recorded. Attacks were defined
192 as any attempt (successful or not) by the toad to capture prey with or without physical contact. A
193 capture was an attack that resulted in the full consumption of a prey item. In Exp. 1, we removed
194 the toad after prey consumption or after 15 minutes if the prey had not been consumed. Based on
195 a lack of captures observed in the last 5 minutes of Exp. 1 trials, we reduced the time limit to 10
196 minutes for Exp. 2. Due to observed interspecific variation in worm movement in Exp. 1 (see
197 Discussion), we recorded each trial in Exp. 2 using an iPhone SE 2016 to quantify the proportion
198 of total trial time a worm spent moving (hereafter referred to as “prey movement proportion”)
199 and the time at which the toad first visibly detected the earthworm (hereafter referred to as “prey
200 detection time”). The latter response variable was a consistently observed behavior in which a
201 toad would visibly turn its head toward the prey item, often followed by other characteristic
202 hunting behavior (e.g., crouching, stalking, attacking).

203 *Diet Surveys* – To determine whether American toads or colubrid snakes were consuming
204 *Amyntas* worms in the field, we collected individual predators and nonlethally recovered their
205 stomach contents. We opportunistically captured predators during visual encounter surveys and
206 from beneath cover boards. Both survey methods were used in two disconnected areas of the UW
207 Arboretum: a jumping worm-invaded site (“invaded”) and a site with no known *Amyntas*

presence (“uninvaded”). The invaded site consisted of deciduous forest, oak savannah, prairie, and mowed/garden spaces. The uninvaded site contained pine forest, deciduous forest, oak savannah, 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 ~2 times a week from May to September 2020. Upon capture of each toad and snake, we recorded mass and snout-vent length (SVL) (Fig. S2 and S3). 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 – To test our hypothesis that *Amyntas* would affect metamorphic toad microhabitat and survival, we used linear models (lme4, Bates et al. 2015) to analyze treatment effects on leaf litter dry mass, soil metrics (pH, temperature, and log-transformed percent moisture, and toad survival proportion within mesocosms. We modeled effects on leaf litter dry mass using fixed effects for presence/absence of *Amyntas*, presence/absence of *Lumbricus*, presence/absence of toads, and all interaction terms. For each soil response model, we used the same treatment predictors and added a fixed effect for sample date (continuous) and a random intercept for mesocosm identity to account for temporal variation and repeated measurements. We modeled toad survival proportions using fixed effects for *Amyntas*, *Lumbricus*, and their

interaction, as well as a fixed effect for sample date and a random intercept for mesocosm identity.

We analyzed feeding experiment data to test our hypothesis that toads would be less successful predators of *Amyntas* than of alternative prey. Exp. 1 and 2 were analyzed independently. We analyzed the following response variables: attack number (total per trial), success or failure of each attack (“attack success”), and prey movement proportion (movement time/total trial time; Exp. 2 only). We tested for effects on each response using linear mixed effects models (lme4, Bates et al. 2015) with a fixed effect for prey type. We included a random intercept for toad identity for all responses except prey movement proportion because individual prey items were only used once. We specified a Poisson distribution for the attack number model, a binomial distribution for attack success, and Gaussian distributions for prey movement proportion. We logit-transformed prey movement proportion to approximate a normal distribution. For Exp. 1 only, every model was followed by Tukey-adjusted pairwise comparisons (emmeans; Lenth et al. 2021) to directly compare between the three prey types. To test the correlation between earthworm movement and toad hunting behavior in Exp. 2, we also used a regression model with prey detection time (log-transformed) as the response, prey movement proportion (logit-transformed) as a predictor, and toad identity as a random intercept. All analyses were performed in R version 4.03 (R Core Team 2020).

Results

Mesocosm experiment – Both earthworm taxa altered leaf litter but did not affect soil conditions or toad survival. *Amyntas* presence reduced dry leaf litter mass by 18.4g (SE = 5.61) on average relative to controls (lm: $t = -3.28$, $p = 0.002$) and *Lumbricus* reduced dry litter mass

by 14.7g (SE = 5.82) on average (lm: $t = -2.53$, $p = 0.016$) (Fig. 1). Toads did not affect leaf litter mass (lm: $\beta = -7.9\text{g}$, SE = 5.82, $t = -1.36$, $p = 0.183$), and there were no significant interaction terms (Fig. 1). Sampling date affected the soil responses, but we did not detect significant effects of earthworms or toads (Fig. S1, Table S3). Toad survival across all treatments averaged 52% on day 36 and 34% at the end of the experiment on day 61. Neither *Amyntas* nor *Lumbricus* presence had significant effects on toad survival (glmer: *Amyntas*: $\beta = -17.08$, SE = 12.9, $t = -1.32$, $p = 0.202$; *Lumbricus*: $\beta = -4.17$, SE = 12.31, $t = -0.34$, $p = 0.739$). The *Amyntas*-present mesocosms had lower mean survival than *Amyntas*-absent mesocosms on both day 36 (13.3% lower) and day 61 (16.3% lower), but this effect was not statistically significant (Fig. 2).

Feeding experiments – In both experiments, toads attacked *Amyntas* more than other prey types, but attacks were less successful. Toads attacked crickets in most trials (91.7%) and did so in comparably fewer trials for *Amyntas* (Exp. 1: 58.3%, Exp 2: 41.7%) and *Lumbricus* (Exp. 1: 33.3%, Exp. 2: 54.7%). The mean number of toad attacks on *Amyntas* in Exp. 1 was over 2x the number for crickets (pairs: ratio = 2.21, SE = 0.60, $z = 2.94$, $p = 0.009$) and over 3x the number for *Lumbricus* (pairs: ratio = 3.82, SE = 1.26, $z = 4.05$, $p < 0.001$) (Fig. 3A). Similarly, the mean number of attacks on *Amyntas* in Exp. 2 was 3x the number for comparably sized *Lumbricus* (glmer: $\beta = 1.11$, SE = 0.25, $z = 4.53$, $p < 0.001$) (Fig. 3B). Mean attack success for *Amyntas* in Exp. 1 was over 13x lower than for crickets (pairs: odds ratio = 0.06, SE = 0.06, $z = -3.06$, $p = 0.006$) and 9x lower than for *Lumbricus* (pairs: odds ratio = 0.10, SE = 0.11, $z = -2.15$, $p = 0.080$) (Fig. 3C). In Exp. 2, mean attack success for *Amyntas* was again 9x lower than for comparably sized *Lumbricus* (glmer: $\beta = -3.30$, SE = 0.95, $z = -3.48$, $p < 0.001$) (Fig. 3D). During Exp. 2, prey movement proportion was lower for *Amyntas* than for *Lumbricus* (lm: $\beta = -2.18$, SE = 0.54, $t = -4.02$, $p < 0.001$) (Fig. 4A). Lastly, prey detection time in Exp. 2 occurred

earlier during trials in which worms spent a larger proportion of time in motion (lmer: $\beta = -0.337 \pm 0.129$ SE, $t = -2.615$, $p = 0.012$) (Fig. 4B).

Diet surveys – We captured a total of 25 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 the total prey items) included 25.3% snails (2 toads), 18.9% ants (2 toads), 9.5% millipedes (4 toads), 6.3% dipterans (4 toads), 5.2% springtails (5 toads), and 7.4% unidentifiable prey (5 toads) (Table 1). “Unidentifiable prey” were considered too degraded for any level of identification. Most snakes were captured at the *Amyntas*-invaded site (Table 1). Stomach contents were recovered from 37% of red-bellied snakes (13 individuals), and all identifiable prey consisted of nonnative slugs. Prey consisted of 62.5% *Deroceras reticulatum* (6 snakes), 12.5% *Arion subfuscus* (2 snakes), 18.8% unidentifiable slugs (3 snakes), and 11.4% unidentifiable prey (4 snakes) (Table 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 26.3% *Amyntas sp.* (5 snakes), 26.3% *Lumbricus sp.* (3 snakes), and 21.1% unidentifiable prey (4 snakes) (Table 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. We found evidence that *Amyntas* act as ecosystem engineers in leaf litter microhabitat and are a viable prey source for native

herpetofauna, although their defensive behaviors may make them difficult to capture for some species. Our findings do not provide strong support for *Amyntas* altering survival of litter-dwelling herpetofauna via habitat modifications in the mesocosm experiment. Broadly, our results suggest that combining experimental and field studies can be a useful approach to isolate distinct mechanisms and quantify their importance in invaded 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), but we did not detect significant effects of *Amyntas* on soil pH, moisture, or temperature, or on metamorphic toad survival. Previous results on how *Amyntas* affect soil conditions are mixed, but some 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). In our study, remaining unconsumed leaf litter, the *Amyntas* cast layer within the mesocosms, and ambient environmental conditions (e.g., light levels, relative humidity, and rainfall) may have buffered soil from temperature and moisture changes. Mean toad survival was marginally lower in *Amyntas*-present treatments, but we could not definitively link this result to *Amyntas*. American toads are somewhat less susceptible to desiccation than other amphibians (Rittenhouse et al. 2008), so results may have differed using other amphibian species. 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). Other nonnative 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). Research comparing lethal and sublethal effects of *Amyntas*

among multiple litter-dependent herpetofauna taxa would help assess ecosystem engineering impacts across a broader native community.

In the feeding experiments, American toads hunted and attacked *Amyntas*, *Lumbricus* and crickets, but toads were often unsuccessful 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 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. Tail separation 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. *Amyntas* 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). So, 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. This aligns with prior 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 toad species in the related genus *Bufo* (Evans and Lampo 1996; Vallvé and Sánchez-Iglesias 2018; Kolenda et al. 2019). Our sample size of American toads was relatively small, particularly in the *Amyntas*-invaded area, so we cannot say with much certainty whether or not American toads are consuming *Amyntas* in the field. Additionally, soft-bodied prey like earthworms are digested more quickly and are therefore may be missed more often in visual stomach contents surveys than arthropod prey (Pompanon et al. 2012). 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.

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). While we have no data on common garter snake diets within the study area prior to *Amyntas* invasion, 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.

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 of nonnative species should be evaluated alongside the more commonly studied effects of invaders as consumers, competitors, and disease vectors to obtain a more holistic understanding of invasion biology.

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Declarations:

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Conflicts of interest

The authors declare no conflicts of interest.

Availability of data and material

All data sets and statistical code used in this study will be made freely available prior to publication and is available upon request.

Authors' contributions

EC, DP, BH, and DD conceived the ideas and designed the studies. EC, ES, and DP helped with set up mesocosm setup and statistical analyses. EC collected data and wrote the manuscript draft. All authors contributed to revisions.

Ethics approval

All use of research animals was approved by UW-Madison College of Agriculture and Life Sciences IACUC (Protocols A006183-A02 and A006186-A03). Permits were obtained from the Wisconsin Department of Natural Resources for sampling and collection (#SRLN-19-32 and #SRLN-20-12).

Consent to participate

Not applicable.

Consent for publication

All authors provide their consent for publication.

638 **Table and Figure Captions**

639 Figure 1. Mean dry mass of leaf litter in the 2019 mesocosm experiment at for each treatment
640 group: American toad (*Anaxyrus americanus*) presence, *Amyntas* presence, and *Lumbricus*
641 presence. Error bars represent the standard error of each treatment mean

642 Figure 2. Mean percentage of American toad metamorphs (*Anaxyrus americanus*) surviving in a
643 mesocosm experiment at each sampling date (Aug 31 and Sep 25, 2019) for each earthworm
644 treatment group. Error bars represent the standard error of each treatment mean

645 Figure 3. Summary of attack data from September, 2019 feeding trial experiments. A) Mean total
646 number of attacks by toads per trial on crickets (*Acheta*), *Amyntas*, and *Lumbricus* in Exp. 1. B)
647 mean total number of attacks by toads per trial on *Amyntas* and *Lumbricus* in Exp. 2. C) mean
648 success rate of attacks by toads per trial (successful attacks/unsuccessful attacks) for each prey
649 type in Exp. 1, and D) mean success rate of attacks by toads per trial (successful
650 attacks/unsuccessful attacks) for each prey type in Exp. 2. Error bars represent the standard error
651 of each treatment mean

652 Figure 4. A) Boxplot showing prey movement proportion (time moving/total trial time) for
653 *Amyntas* and *Lumbricus* prey in feeding experiment 2, conducted in September 2019. The
654 center lines represent the median proportions. B) Linear model of log-transformed prey detection
655 time (time at which toad was observed to first detect prey) predicted by logit-transformed prey
656 movement proportion in feeding experiment 2, which reflects a statistically significant negative
657 relationship ($p = 0.012$). The grey area represents a 95% confidence interval

658 Table 1. Prey identified in stomach contents of herpetofauna predators from 2020 field diet
659 surveys at the UW Arboretum (Madison, WI, USA). Prey count is the total number of a prey
660 type across all predator individuals of one species. Predator count is the total number of
661 predators that consumed one or more of each prey type.

662

663

Species	Prey Type	Prey Count	Predator Count
<i>Anaxyrus americanus</i> (n = 25)	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 spp.</i>	5	5
	<i>Anaxyrus americanus</i>	5	5
	<i>Lumbricus spp.</i>	5	3
	unidentified earthworm	2	2
	unidentified	2	2
	no prey recovered	-	29

Supplementary Files

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