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INVASIVE ASIAN EARTHWORMS  
NEGATIVELY IMPACT WOODLAND  
SALAMANDERS: COMPETITIVE  
EXCLUSION, FORAGING INTERFERENCE,  
AND HABITAT DEGRADATION REDUCE  
SALAMANDER DENSITY

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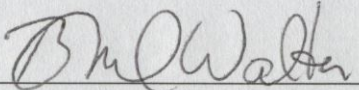
A Thesis Submitted to the  
Office of Graduate Studies  
College of Arts & Sciences of  
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in Partial Fulfillment of the Requirements  
for the Degree of  
Master of Science

By  
Julie L. Ziemba  
2015

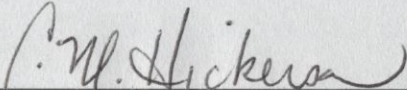
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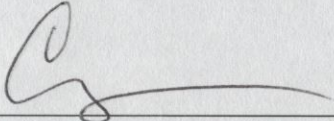
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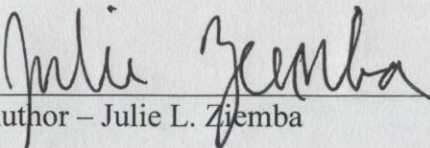
  
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**Invasive Asian earthworms negatively impact woodland salamanders: Competitive exclusion, foraging interference, and habitat degradation reduce salamander density**

**ABSTRACT**

Asian earthworms (*Amyntas* spp.) are invading North American forests and consuming the vital detrital layer that forest floor biota (including the Eastern Red-backed Salamander, *Plethodon cinereus*) rely on for protection, food, and habitat. Salamander population decline has been associated with European earthworm-mediated leaf litter loss, but there have been few studies on the interactions between *Amyntas* spp. and *P. cinereus*. Since the large, active *Amyntas* spp. earthworms spatially overlap with salamanders beneath natural cover objects and in detritus, they may compound the negative consequences of resource degradation by physically disturbing important salamander activities (foraging, mating, and egg brooding). I predicted that *Amyntas* spp. would exclude salamanders from high quality microhabitat, reduce foraging efficiency, and negatively affect salamander fitness. In laboratory trials, salamanders used lower quality microhabitat and consumed fewer flies in the presence of earthworms than when alone. In a natural field experiment conducted on salamander populations from “non-invaded” and “*Amyntas*-invaded” sites in Ohio, salamanders and *Amyntas* spp. shared cover objects ~60% less than expected. However, there was no effect of *Amyntas* spp. invasion on salamander body condition. *Amyntas* spp. density was negatively associated with juvenile and male salamander density, but had no relationship with female salamander density. Juvenile and non-resident male salamanders do not hold stable territories, which results in reduced access to prey and a higher risk of desiccation.

Degraded leaf litter layers in *Amyntas*-invaded forests may uniquely challenge juvenile and male salamanders, exposing them to increased risk of desiccation, predation, and starvation as they search for suitable, unoccupied surface microhabitat. If habitat degradation and physical exclusion of salamanders from cover objects reduce juvenile and male salamander performance, then recruitment and ultimately salamander abundance may decline following *Amyntas* spp. invasion.

## 1. Introduction

The invasion of non-native species is thought to be a major driver of worldwide biodiversity loss (Butchart et al. 2010). Exotic species can negatively influence native populations through diverse direct interactions, including predation, competition, and the introduction of disease (Crowl et al. 2008; Human and Gordon 1996; Wiles et al. 2003). Invasive species can also have important indirect, non-trophic effects by altering the physical environment and availability of resources as “ecosystem engineers” (Jones et al. 1994). Although some invasive ecosystem engineers [such as beavers (*Castor canadensis*) in Cape Horn, Chile; Anderson and Rosemond 2007], positively facilitate other biota by increasing habitat complexity (Jones et al. 1997), resource degradation is a common result of invasive ecosystem engineering (Crooks 2002). Interactions between indigenous and non-indigenous species are complex, and predicting the net effects of invasion requires identifying the presence, direction, and magnitude of direct and indirect pathways. Further, discerning the variable importance of these interactions with respect to seasonal, short-term, and long-term ecosystem processes is essential to understanding the consequences of invasion (Kéfi et al. 2012).

European and Asian earthworms are cosmopolitan ecosystem engineers that have invaded and colonized all continents, except Antarctica (Hendrix et al. 2008). During the Pleistocene epoch, glacial denudation of North America extirpated earthworms from forests covered by the Laurentide ice sheet, which includes the Great Lakes region (Tiunov et al. 2006). Since earthworms are limited by slow dispersal rates (0.5–1.0 km in 100 years; Hale 2006), these forests remained earthworm-free until ~1700 CE when European colonization introduced exotic species (James 1998). Subsequently,

anthropogenic activities, including composting, bait dumping, development, horticulture, and international commerce, have been the primary vectors for European and Asian earthworm dispersal (Bohlen et al. 2004; Callahan et al. 2006; Hale 2008).

Forest floor communities lacking native earthworms are particularly vulnerable to the transformative effects of invasive earthworms (Frelich et al. 2006; Gundale et al. 2005; Hale et al. 2005). Previously-glaciated forests evolved in the absence of ecological equivalents to earthworms. In these forests, shed organic matter gradually developed on the forest floor and formed a thick, multi-layered habitat (organic soil horizon, or O horizon) as a consequence of bacterial-dominated decomposition and nutrient cycling. This detrital duff layer traps moisture, creating a humid microhabitat that buffers inhabitants from thermal and hydric extremes, offers refugia from predation, and provides a nutrient base for the entire forest food web (Gessner et al. 2010). Rapid earthworm-driven consumption of the nutrient-dense, insulative O horizon that accumulated over thousands of years has exposed native biota to environmental conditions outside of their recent evolutionary experience.

The effects of earthworm species belonging to different ecological groups can vary based on particular trophic and microhabitat characteristics (Migge-Kleian et al. 2006). However, in general, invasive earthworms accelerate leaf litter decomposition and nutrient release, reduce the O horizon depth by consuming detritus, and alter edaphic properties via cast (excrement) production and bioturbation (Brown et al. 2000; Burtelow et al. 1998; Resner et al. 2015). Non-indigenous earthworms also reduce the diversity and abundance of native plant, microinvertebrate, amphibian, mammal, and avian species



(Bohlen et al. 2004; Migge-Kleian et al. 2006) while facilitating other exotics (i.e. invasional meltdown; exotic understory plants and deer; Dávalos et al. 2015).

Asian earthworms (*Amyntas* spp.) are rapidly expanding their range in previously-glaciated regions of the United States (Görres and Melnichuk 2014) and are causing a drastic reduction of leaf litter layers in both disturbed and undisturbed forests following colonization (Callaham et al. 2003; Greiner et al. 2012; Richardson et al. 2015; Schermaier 2013). *Amyntas* species are classified into the epi-endogeic functional group, meaning that they live at the soil surface and construct shallow, temporary burrows in upper topsoil layers. These earthworms voraciously consume leaf litter and associated microorganisms (Hendrix and Bohlen 2002), producing large quantities of casting material that changes the physical, chemical, and biotic properties of the topsoil (Burtelow et al. 1998; Redmond et al. 2014). Poorly-studied in comparison to longer-established European genera, *Amyntas* earthworms are highly active, with a thrashing, serpentine locomotion and large body size (65 mm average total length for *A. hilgendorfi*; Greiner et al. 2010) that makes them conspicuous surface occupants. *Amyntas* earthworms exhibit diet plasticity (Zhang et al. 2010) and are capable of both sexual and parthenogenetic reproduction (Görres and Melnichuk 2014), which contribute to their successful colonization and competitive domination of newly invaded environments (Snyder et al. 2009). Additionally, *Amyntas* earthworms are negatively associated with European earthworm species in previously-glaciated forests of Ohio (Schermaier 2013).

In contrast to many invasive European earthworm species, *Amyntas* spp. has an annual life cycle. *Amyntas* spp. juveniles begin to emerge in the spring, rapidly building biomass and reaching maximum abundances during the summer (>100 individuals per

m<sup>2</sup>; Görres and Melnichuk 2014). In the fall, large adults dominate a dense population and produce cocoons before a mass die-off from late November to early December (Callahan et al. 2003; Görres and Melnichuk 2014; Greiner et al. 2012). The desiccated cocoons are able to survive the cold North American winter, which corresponds to the monsoon season experienced by source and current populations in East Asian temperate forests (i.e., the native range; Richardson et al. 2009). Given their aggressive colonization and combination of unique morphological, locomotory, feeding, and life history characteristics, it is likely that the effects of an *Amyntas* spp. invasion on the soil layers and the organisms that inhabit the forest floor community differ from those of the well-studied European species.

Invasive earthworms co-occur with terrestrial salamanders, such as the Eastern Red-backed Salamander (*Plethodon cinereus*), in the leaf litter and beneath natural cover objects (rocks and logs; Görres and Melnichuk 2012). Although salamanders will eat juvenile *Amyntas* earthworms (Ivanov 2011) and smaller European species (Maerz et al. 2005), adults of larger species exceed red-backed salamander gape size, and in the case of *A. agrestis*, may be distasteful (Gorsuch and Owen 2014). Studies on the behavioral interactions between woodland salamanders and invasive earthworms have only investigated the effects of the deep-burrowing European earthworm, *Lumbricus terrestris*. Red-backed salamanders will use the permanent vertical burrows of *L. terrestris*, which can improve salamander overwintering success and provide refugia from predators (Cáceres-Charneco and Ransom 2010; Ransom 2011; 2012a; 2012b). *Lumbricus terrestris* burrows can provide an additional fossorial habitat for salamanders that may alleviate some of the negative impacts of leaf litter consumption that degrades

surface microhabitat (Ransom 2012b). Since *Amyntas* earthworms remove the detrital habitat of salamanders, but do not construct permanent soil burrows to serve as an alternate spatial resource, impacts of their invasion on *P. cinereus* populations likely differ from European species. The presence of the large, active *Amyntas* individuals in scant suitable surface microhabitat may physically disrupt normal salamander activities. Additionally, *Amyntas* earthworm consumption of the vital leaf litter buffer may expose salamanders to harsh surface conditions and increased predation pressure.

Despite their small body size [*P. cinereus* average ~40 mm in snout-vent length (SVL) and weigh ~1 g in Ohio; Anthony and Pfingsten 2013], red-backed salamanders contribute an impressive amount of biomass to northeastern temperate forest communities (> 2.8 individuals per m<sup>2</sup>; Burton and Likens 1975). As predators of microinvertebrates and prey for larger vertebrates, red-backed salamanders are integral trophic links in forest ecosystems and indirectly influence forest floor carbon dynamics (O'Donnell et al. 2014; Walton 2013). *Plethodon cinereus* are lungless, and require moist substrate to respire passively through the skin (Spotila and Berman 1976). Between periods of rainfall, when the dry forest floor becomes inhospitable for the desiccation-prone salamanders, natural cover objects are crucial refugia. Salamanders aggressively defend these valuable territories from intra- (Jaeger and Forester 1993; Mathis et al. 1995) and interspecific competitors (Hickerson et al. 2004; Hickerson et al. 2012; Jaeger 1971).

In addition to spatial overlap beneath cover objects, *Amyntas* spp. and *P. cinereus* share nocturnal activity patterns, as well as behavioral responses to changes in moisture conditions of the forest floor. Both taxa are more active during periods of

rainfall and at night, when there is reduced pressure from diurnal visual predators, decreased UV radiation, and cooler temperatures (Chuang and Chen 2008; Heatwole 1962). In the northeastern United States, populations of *P. cinereus* spend the winter and summer underground in natural soil crevices, avoiding suboptimal surface conditions, except for mild winter days and when rainy, cool nights allow for sporadic nightly foraging events in the summer. Since climatic factors constrain a salamander's use of its three main microhabitats (underground retreats, leaf litter, and the substrate beneath natural cover objects) at different times of year, it is possible that the habitat modification and physical effects of *Amyntas* earthworms may shift seasonally. *Amyntas* spp. ontogeny may also be an important factor to consider, as smaller juvenile earthworms could provide important food sources for gravid females in the spring (Maerz et al. 2005), but would be full-grown, and therefore too large for consumption by gravid females in the fall. The majority of salamander surface activity occurs in the spring and fall, during an extended breeding season (Anthony and Pfungsten 2013). In the fall, adult *Amyntas* earthworms may have the greatest potential to physically interfere with important salamander activities that occur both in the leaf litter and under cover objects. Dense populations of *Amyntas* adults could disturb salamander territorial defense, mating, egg brooding (in Ohio, 6-9 weeks following oviposition in late-July; Anthony and Pfungsten 2013), and foraging behavior.

In this study, I used manipulated laboratory trials and field surveys to investigate the potential interactions between *Amyntas* earthworms and salamanders. Laboratory experiments focused on isolating the direct effects of the physical interaction between *Amyntas* spp. and *P. cinereus* on salamander 1) microhabitat use and 2) foraging behavior.

I expected that the presence of large, active *Amyntas* individuals would physically disturb normal salamander activity. In cohabitation trials, I predicted *Amyntas* spp. would exclude salamanders from high quality microhabitat (beneath artificial cover objects), resulting in salamanders using high-quality microhabitat less frequently when paired with an earthworm than when alone. I reasoned that the disruptive physical activity of *Amyntas* earthworms would prompt salamanders to seek alternative, suboptimal microhabitat where they can avoid close proximity to the earthworms. For the foraging trials, I posited that the presence of *Amyntas* individuals would reduce salamander prey capture efficiency due to physical interference. I predicted that the presence of *Amyntas* spp. in the arena would lengthen a salamander's latency to the first attack and reduce the number of flies that a salamander is able to consume. Further, I used arenas with low and high habitat complexity to determine if the environmental structure (bare substrate vs. leaf litter matrix) influenced the magnitude of earthworm interference. I hypothesized that the magnitude of the effect of *Amyntas* spp. on salamander foraging would differ depending on the complexity of the environment through which salamanders try to locate and capture prey. The focus of the field study was to evaluate the differences between populations of *P. cinereus* in *Amyntas*-invaded and non-invaded forests of northeastern Ohio. I predicted that salamander populations in invaded forests would exhibit reduced body condition and fecundity compared to those in non-invaded forests. I reasoned that *Amyntas* earthworms would physically disturb salamanders and degrade leaf litter habitat. Specifically, I hypothesized that salamander density, body condition, and eggs per female would decrease with *Amyntas* spp. density. Since salamanders rely on leaf litter for camouflage from visual

predators, I also expected that rates of tail breakage (a proxy for predation pressure; Schoener 1979) would increase with *Amyntas* spp. density.

## 2. Materials and methods

### 2.1. Laboratory experiments

Adult (SVL > 35 mm; N = 120) red-backed salamanders (*Plethodon cinereus*) were collected from the Manatoc Boy Scout Camp property (MBSC; 41°13'37.2"N, 81°31'17.2 W) in Summit County, OH on 17 April 2014. European and Asian earthworms were observed throughout the field site. Wet mass and SVL were recorded in the laboratory for all salamanders using a digital scale and digital calipers. Snout shape and presence of eggs in the abdomen were used to determine the sex of the individuals (Anthony et al. 2008). *Plethodon cinereus* is polymorphic, and the “striped” and “unstriped” color morphs were collected for the laboratory trials. However, only “striped” individuals were used for the microhabitat trials, as there is evidence for slightly different thermal and moisture preferences between color morphs (Lotter and Scott 1977; Smith et al. 2015; Williams et al. 1968). Before the beginning of the experiment, salamanders were maintained individually in Pyrex© dishes (11 cm diameter; 470 ml volume) with leaf litter substrate (collected from MBSC), on a natural light: dark cycle at an average temperature of  $18.5 \pm 1^\circ\text{C}$ . Salamanders were fed wingless *Drosophila melanogaster ad libitum*.

*Amyntas gracilis* specimens were purchased online (The Worm Dude, California, USA). Only intact, clitellate individuals with total lengths > 65 mm were used

for trials. Earthworms were housed in plastic containers (5 earthworms per container; 20 cm long x 12 cm wide x 6 cm deep) with a layer of topsoil covered by moist detritus (collected from MBSC) until the experiment began.

### *2.1.1. Cohabitation trials*

For the cohabitation trials, a salamander and either 0 or 1 earthworm(s) were placed in a test arena (Fig. 1A) with zones of high and low microhabitat quality for 12 hours. I predicted that salamanders would use the high quality zone beneath the artificial cover object more frequently when alone in the arena than when paired with an *Amyntas* earthworm. I reasoned that the large, active earthworms would physically displace (exclude) salamanders from the high quality zone when both animals shared the same arena. Each salamander (N = 36) was tested twice, once alone and once paired with an earthworm for a total of 72 trials. The order of the trials for each salamander was assigned randomly. Each earthworm was used for only one trial.

Test arenas were Thermo Scientific™ Nunc™ Square BioAssay dishes with lids (24.5 cm x 24.5 cm x 2.5 cm; Anthony et al. 2007). Each arena included a piece of moistened filter paper (7.5 cm diameter) in a random corner of the dish with an artificial cover object (small ceramic tile; 7.5 cm x 7.5 cm x 0.9 cm), propped up by a 1 cm piece of plastic surgical tube (0.6 cm diameter), on the top of the filter paper (Fig. 1A). To control for potential differences in humidity, 1 ml of spring water was applied to the center of the dry filter paper immediately before an animal was added to an arena. Zone quality was defined by the level of moisture (protection from desiccation) provided. The high quality zone was the space beneath the artificial cover object and on top of moist

filter paper, and the low quality zone was the dry plastic bottom of the arena. Pilot data indicated that earthworms occupied the high quality zone both when alone in the arena and when paired with a salamander.

At the beginning of each trial individual(s) were placed in a random corner of the arena that did not contain the cover object (in paired trials, earthworms and salamanders were placed in opposite corners) and the movements of the animals were video-recorded for 12 hours. Three digital video cameras (Sony® HDR-CX240 Full HD Handycam camcorders) were used to record 18 trials per sampling date. Each video camera filmed a group of six arenas, with three replicates of each treatment (salamander only and paired) per group (Fig. 1B), arranged in a randomized block. Each salamander's set of trials occurred on consecutive days. I sampled 30 minute intervals of the 12-hour video to establish frequency of use for each zone. The salamander's location was determined based on which zone contained > 50% of the salamander's mid-body or 50% of the salamander's mid-body and its head. Trials were conducted on 9–15 August 2014 from 2100–0600 hours, under dim red illumination (Safelight B, 15-W bulb; Kodak, Rochester, NY, USA; Siddiqi et al. 2004) and from 0600–0900 hours under ambient illumination to approximate a natural night: day lighting regimen.

### *2.1.2. Foraging efficiency trials*

For the foraging efficiency trials, salamanders were given 20 minutes to consume 15 fruit flies and observed to determine if *Amyntas* spp. presence or the interaction between *Amyntas* spp. presence and environmental structure altered foraging behavior. I hypothesized salamanders in trials with an earthworm would have a longer latency to first



attack and consume fewer flies than those without an earthworm. Further, I predicted that there would be an interactive effect of earthworm presence and environmental complexity, with the magnitude of the effect of earthworm presence on salamander foraging efficiency differing between environments of low and high structure. I reasoned that *Amyntas* spp. would physically interfere with a salamander's movement or ability to locate prey and reduce foraging efficiency.

The foraging efficiency trials evaluated the effect of *Amyntas* spp. on salamander foraging behavior using a 2 x 2 factorial design, including environmental complexity and earthworm presence as factors. Salamanders were tested in arenas (Thermo Scientific™ Nunc™ Square BioAssay dishes with lids; 24.5 cm x 24.5 cm x 2.5 cm) of either low structure (moist paper towel substrate) or high structure (moist paper towel substrate and a layer of moistened leaves), with either 0 or 1 *Amyntas* earthworm(s). Sugar maple (*Acer saccharum*) and tulip poplar (*Liriodendron tulipifera*) leaves were dried in an oven for > 48 hrs to remove invertebrates and then soaked in distilled water for at least 24 hrs before being added to the arenas. The four treatment combinations (N=30 each) were: 1) low structure, 2) low structure + earthworm, 3) high structure, and 4) high structure + earthworm. Each animal was only used for one trial. Salamanders were ranked by mass and distributed evenly among treatments. Salamander morphs (striped and unstriped) and genders were also distributed evenly among treatments. Since light intensity has been shown to alter foraging efficacy in nocturnal amphibians (Buchanan 1993), all trials were conducted under dim red light (Safelight B, 15-W bulb; Kodak, Rochester, NY, USA; Siddiqi et al. 2004). Before the beginning of a trial, food was withheld from the salamander for 7 days to standardize the hunger level among individuals.

At the beginning of each trial, a salamander and either 0 or 1 *Amyntas* earthworm(s) were placed in opposite corners of the arena and allowed to acclimate for five minutes. During the acclimation period, the salamander was covered by an opaque petri dish (5 cm diameter). Following acclimation, 15 wingless *D. melanogaster* were added to the arena and the acclimation cover was removed. Salamander foraging behavior was observed and timed until the first fly was captured (latency to first attack). The trials lasted for 20 minutes and the number of flies consumed by the conclusion of the trial was recorded.

## 2.2. Field experiment

To evaluate the effect of *Amyntas* spp. invasion on the fitness and fecundity of *P. cinereus*, measures of salamander abundance, body morphometrics, predation pressure, and reproductive effort were compared between populations from “*Amyntas*-invaded” and “non-invaded” forests. I predicted that salamander populations at *Amyntas*-invaded sites would have lower densities, reduced body condition, greater rates of tail breakage, and fewer eggs per gravid female than those at non-invaded sites. I expected that the reduced leaf litter quality and physical disturbance from *Amyntas* earthworms would result in reduced salamander fitness and fecundity at invaded sites. I also hypothesized that *Amyntas* spp. would exclude salamanders from cover objects, resulting in less co-occurrence of earthworms and *P. cinereus* beneath the same cover object than expected by chance.

### 2.2.1. Site classification

All field sites were within previously-glaciated, beech-maple hardwood forests in northeastern Ohio (Table 1; Fig. 2). Vegetation, detrital fauna, and land use history were similar for all field sites. These sites were classified as *Amyntas*-invaded or non-invaded based on the presence of *Amyntas* individuals (or *Amyntas* casting material) and leaf litter quality (modified from a European earthworm invasion rapid assessment method; Loss et al. 2013). Leaf litter quality (LL score) of each site was evaluated on a scale of 1–3 within four random 1 m<sup>2</sup> quadrat samples. A score of 1 indicated a low quality forest floor, with a thin organic horizon composed of leaf-fall from only the previous year. A score of 3 indicated a high quality forest floor, with a thick, multi-layered organic horizon with 3+ years of leaf-fall. A score of 2 indicated intermediate forest floor quality. The maximum score that a site could receive was a 12 (an intact, healthy forest floor), while the minimum score a site could receive was a 4 (a highly disturbed, scant forest floor). Since there are likely very few forests in Ohio that completely lack earthworms (if any), sites were classified as “non-invaded” if there were intact, healthy leaf litter layers (LL score > 9) and low European earthworm burden (< 5 individuals found from raking through leaf litter during forest floor quality assessment and < 50 individuals found at the site in total).

### 2.2.2. Animal surveys

Salamander and earthworm data were collected during the day (0800–1700 hrs) from 18 September–25 October 2014, during the fall breeding season for *P. cinereus*. I visited the localities in semi-random order, alternating between *Amyntas*-invaded and

non-invaded forests to control for the effect of differences in precipitation on salamander detection (during and immediately following rainfall, salamanders will leave cover objects to forage in the leaf litter; Jaeger 1980a). Forests that contained both *Amyntas*-invaded and non-invaded areas were surveyed on consecutive days (The West Woods and Holden Arboretum; Table 1).

To obtain measures of earthworm (*Amyntas* spp. and surface-dwelling European spp.) and salamander densities, I flipped the first 100 natural cover objects encountered at the site and recorded how many individuals were found. I only flipped rocks or logs that were 25–50 cm<sup>2</sup> (containing no space for refuge from interaction) to ensure that I was measuring actual co-occurrence of *Amyntas* spp. and *P. cinereus*. I also recorded if salamanders were alone or sharing the cover object with an *Amyntas* earthworm. Salamander SVL, wet mass, color morph, and age class (juvenile: SVL < 35 mm, adult: SVL > 35 mm) were recorded. Tail breakage (or regrowth) was recorded as a proxy for predation pressure. The number of eggs per gravid female were counted by flattening the ventrum of the salamander against a petri dish with a sponge and palpating the lateral body walls to move the eggs closer to the surface of the skin. After the initial 100 cover objects were flipped to determine a standardized salamander density per site, I continued to flip any cover object encountered until I collected body morphometrics of at least 50 total salamanders per site.

### 2.3. *Statistical analysis*

Before all analyses, the Shapiro-Wilk and Levene's tests were performed to determine if the data met the test assumptions of normality and homoscedasticity. When necessary,

appropriate remedial transformations and weighting (by the inverse variance; Neter et al. 1990) were applied to non-normal and heteroscedastic data. For the microhabitat trials, two-tailed, paired t-tests were used to evaluate the effect of *Amyntas* spp. on salamander microhabitat use. For the foraging trials, the effects of earthworm presence and environmental complexity on a salamander's latency to attack and the number of flies consumed by the end of the trial were analyzed using a randomized complete block ANOVA. The main effects were earthworm presence (absent or present), environmental complexity (low or high structure), gender (male or female), and morph (striped or unstriped). All two-way interactions and main effects were included in the model.

Univariate linear regression was used to compare the relationship between *Amyntas* spp. density and variables of interest (LL score, European earthworm density, salamander density, number of eggs/female salamander, and tail breakage). For *Amyntas*-invaded sites, the probability of *P. cinereus* and *Amyntas* spp. co-occurrence beneath a cover object was evaluated using a chi-square test. Chi-square analyses were not completed for individual *Amyntas*-invaded sites because the data did not meet the test assumptions. Salamander body condition was calculated for each age class using the scaled mass index (Peig and Green 2009). Nested ANOVA analyses were used to compare salamander body condition between populations at invaded and non-invaded sites. For all analyses,  $\alpha = 0.05$ . Analyses were completed using SPSS v. 21.

### **3. Results**

#### *3.1. Laboratory experiments*

Including all salamanders (N=36) in the analysis, there was no effect of *Amyntas* spp. presence on salamander microhabitat use ( $t_{35} = -1.05$ ,  $P = 0.3$ ; Fig. 3A). However, there were seven salamanders that never used the high quality microhabitat during the 24-hour microhabitat trials when alone. Excluding those seven individuals from analyses, *Amyntas* spp. presence had a significant effect on the quality of microhabitat used by salamanders ( $t_{28} = -3.1$ ,  $P = 0.005$ ; Fig. 3B). Salamanders used low quality microhabitat ~34% more when paired with an earthworm than when alone.

Eleven salamanders did not attack a fly during the 20 minute foraging trials (N=120) and eight of those salamanders were in trials with earthworms. The interaction between *Amyntas* spp. presence and environmental structure was not significant for the number of flies consumed or latency to first attack. There were significant effects of earthworm presence ( $F_{1,102} = 6.288$ ,  $P = 0.014$ ; Fig. 4) and salamander gender ( $F_{1,102} = 10.463$ ,  $P = 0.002$ ) on the number of flies consumed. On average, salamanders in trials with *Amyntas* spp. ate ~19% fewer flies than those not paired with an earthworm, and males ate ~31% more flies than females, regardless of treatment. Environmental structure had no effect on the number of flies that salamanders consumed ( $F_{1,102} = 0.548$ ,  $P = 0.461$ ). There was no significant effect of *Amyntas* spp. presence ( $F_{1,108} = 0.065$ ,  $P = 0.8$ ) or environmental structure ( $F_{1,108} = 0.705$ ,  $P = 0.403$ ) on a salamander's latency to first attack. Yet, several salamanders were observed following the earthworm around the arena, displaying aggressive postures [all trunk raised (ATR); Jaeger 1984], and even biting at the earthworms throughout the trials.

### 3.2. Field experiment

No *Amyntas* earthworms were found at sites classified as “non-invaded” (N=7), while a total of 133 individuals were found at “*Amyntas*-invaded” sites (N=6). *Amyntas* spp. density at invaded sites ranged from 3–50 individuals (Table 1). Although only surface-dwelling European earthworms would have been detected with this sampling design, very few of these individuals were found at non-invaded (36) and *Amyntas*-invaded sites (3). There was a significant negative linear relationship between *Amyntas* earthworm density and LL score ( $R^2 = 0.886$ ,  $F_{1,11} = 85.634$ ,  $P < 0.0005$ ; Fig. 5A). Thinner, lower quality leaf litter layers were associated with higher densities of *Amyntas* earthworms. At sites where earthworms were detected, there was also a significant negative association between densities of *Amyntas* spp. and surface-dwelling European earthworms ( $R^2 = 0.544$ ,  $F_{1,8} = 9.562$ ,  $P = 0.015$ ; Fig. 5B).

Red-backed salamanders and *Amyntas* earthworms co-occurred beneath cover objects ~60% less often than expected ( $\chi^2 = 25.86$ ,  $df = 1$ ,  $P < 0.0001$ ; Table 2). Juvenile and male salamander density were negatively correlated with *Amyntas* spp. density (Table 3; Fig. 6). However, there was no association between *Amyntas* spp. density and overall or female salamander density (Table 2). *Amyntas* earthworm invasion had no effect on juvenile ( $F_{1,12.752} = 1.663$ ,  $P = 0.22$ ), adult male ( $F_{1,11.107} = 1.594$ ,  $P = 0.233$ ), or gravid female ( $F_{1,11.002} = 0.306$ ,  $P = 0.591$ ) salamander body condition (Fig. 7). There was no effect of *Amyntas* spp. invasion on the number of eggs/female salamander ( $R^2 = 0.064$ ,  $F_{1,11} = 0.748$ ,  $P = 0.406$ ) or salamander tail breakage ( $R^2 = 0.028$ ,  $F_{1,11} = 0.32$ ,  $P = 0.583$ ).

#### 4. Discussion

Invasive Asian earthworms (*Amyntas* spp.) are drastically altering the resource quality and indigenous biodiversity of detrital communities in the United States (Snyder et al. 2009; Schermaier 2013). Previously-glaciated forests that lack native earthworms are particularly vulnerable to habitat degradation following exotic earthworm invasion (Hale et al. 2005). *Amyntas* earthworms live at the soil surface and spatially overlap with *Plethodon cinereus*, an ecologically important forest floor amphibian (Görres and Melnichuk 2014). The purpose of this study was to investigate the interaction between an aggressive, understudied invasive earthworm and a native keystone salamander. I hypothesized that the physical presence and ecosystem engineering of *Amyntas* spp. would disturb normal salamander activities and reduce salamander fitness. Consistent with my predictions, *Amyntas* earthworms excluded salamanders from high quality microhabitat and reduced prey capture in laboratory trials. A comparison of *P. cinereus* populations from *Amyntas*-invaded and non-invaded forests of northeastern Ohio also supported my hypothesis because there was reduced juvenile and male salamander density at invaded sites, as well as fewer instances of *Amyntas*-salamander co-occurrence beneath cover objects than expected. In contrast to my predictions, there was no signal regarding the effect of *Amyntas* spp. invasion on salamander body condition, fecundity, or tail breakage.

Natural cover objects are vital for salamander success because they provide protection from desiccation when the forest floor is dry, as well as dependable access to prey and mates (Fraser 1976). Red-backed salamanders are territorial and aggressively defend the space beneath rocks and logs from con- and heterospecifics (Jaeger and



Forester 1993; Hickerson et al. 2004; Hickerson et al. 2012; Mathis et al. 1995). Data collected from laboratory trials and field observations indicate that *Amyntas* spp. competitively excludes *P. cinereus* from valuable cover object microhabitat. These findings are consistent with a previous laboratory microcosm experiment that found altered *P. cinereus* microhabitat use in the presence of *Amyntas* spp. and increased importance of cover object microhabitat following earthworm-mediated leaf litter consumption (Ziemba et al., unpublished data). In this study, salamanders used low quality microhabitat (dry areas outside of the cover object) significantly more often when paired with *Amyntas* earthworms than when alone in laboratory trials. In the field, *P. cinereus* and *Amyntas* spp. were found sharing cover objects significantly less than expected by chance. Although large, active *Amyntas* individuals may physically displace salamanders, there is evidence that *Amyntas agrestis* produces distasteful skin secretions when perturbed that may also deter salamanders (Gorsuch and Owen 2014). Yellow, mucous-like *Amyntas* spp. secretions were found in several arenas following microhabitat trials. Since red-backed salamanders have well-developed chemoreception via nasolabial grooves (Dawley and Bass 1988), potential chemical-based avoidance of *Amyntas*-occupied microhabitat should be investigated. As surface-dwelling animals that require moist substrate to respire through their skin, *Amyntas* spp. and *P. cinereus* are limited by natural cover object resource availability. Competitive domination of spatial resources by *Amyntas* earthworms may result in negative fitness consequences for salamanders, since they could be more prone to desiccation, have reduced access to microinvertebrate prey, and fewer mating opportunities without the maintenance of stable territories.

Red-backed salamanders use both ambush (vision-based, under bright or dim lighting) and active (chemosensory-based, in the dark) predation methods to opportunistically consume leaf litter microinvertebrates (Playck and Graves 2001). In laboratory foraging trials, *Amyntas* spp. presence significantly reduced the amount of prey captured by salamanders, but did not affect the latency to a salamander's first attack. A salamander's latency to first attack seemed to be heavily influenced by the initial proximity of a prey item to the salamander, and therefore may not be the best measure of predation efficiency. The presence of an earthworm reduced prey capture by salamanders by 16% which could result in a substantial energy loss for salamanders in natural conditions. The reduced prey capture in *Amyntas*-present trials may be due to visual impediment (large earthworms block salamanders from locating or attacking prey) or distraction (salamander aggression toward earthworms). Salamander behavior was not quantified for this study, but several individuals were observed following the earthworm around the arena and displaying aggressive behaviors (ATR, look toward, and biting; Jaeger 1984) instead of foraging. Aggression toward earthworms could accrue an energy cost for salamanders (expensive aggressive activity combined with lost foraging time; Jaeger 1984). Behavioral trials to evaluate salamander aggression toward earthworm invaders may provide insight into *P. cinereus*-*Amyntas* spp. competition dynamics and associated energetic costs. Surprisingly, there was a significant effect of gender, with male salamanders consuming more prey than females, but no effect of environmental structure on salamander foraging efficiency. There is evidence that male red-backed salamanders are more territorial than females (Wrobel et al. 1980). We expected for males to exhibit territorial responses toward *Amyntas* spp. more often or for a longer

duration than females, resulting in reduced foraging efficiency (Jaeger et al. 1983). It is unclear as to why females consumed fewer flies in this study. However, it is likely that there was insufficient difference between the low and high structure treatments to create a realistic simulation of bare substrate vs. leaf litter foraging conditions. The flies were not able to move beneath the leaves, so foraging on the flat leaf substrate was identical to foraging on the paper towel substrate. Future laboratory studies that attempt to quantify differences in *P. cinereus* foraging under different environmental conditions should more realistically replicate forest floor habitat (at the expense of the ability to visually observe a salamander's latency to the first attack) and focus on quantifying total prey consumed.

Consistent with previous studies, *Amyntas*-invaded forests had thinner, lower quality leaf litter layers and an abundance of casting material covering the soil surface (Burtelow et al. 1998; Greiner et al. 2012; Richardson et al. 2015). Red-backed salamanders rely on a thick detrital matrix to buffer extreme thermal and hydric surface conditions, provide refugia from predators, and gain access to microinvertebrate prey (Petranka 1998). Although there was no signal concerning the body condition (Milanovich and Maerz 2013) of salamanders from *Amyntas*-invaded vs. non-invaded forests, juvenile and male salamander densities were negatively associated with *Amyntas* spp. density. These findings are consistent with past studies that reported declines in red-backed salamander populations following exotic earthworm-mediated leaf litter loss (Maerz et al. 2009). However, this study is the first to find different responses among age/sex classes of salamanders, since unlike that of juveniles and males, female salamander density was not affected by *Amyntas* spp. invasion.

Overall, red-backed salamanders have low vagility (dispersal less than 80 m/year; Cabe et al. 2007), small home ranges (5–25 m; Kleeberger and Werner 1982) and exhibit site tenacity to cover object territories from year-to-year (Gillette 2003). Dispersal in *P. cinereus* is thought to be male-biased, occurring both during the juvenile stage (allowing smaller salamanders to avoid resource competition with larger adults; Marsh et al. 2004), and after maturation (allowing non-resident males to avoid mate competition with territorial residents; Liebgold et al. 2011). The absence of a leaf litter buffer could be a major dispersal barrier to desiccation-prone terrestrial salamanders. It is possible that juveniles and males in *Amyntas*-invaded forests must disperse farther, through habitat lacking leaf litter cover to successfully find territories unoccupied by conspecifics or earthworms. Thus, juvenile and male salamander success could be reduced in *Amyntas*-invaded forests because of increased risk of desiccation or predation during dispersal. Compared to adult salamanders, juveniles have a higher surface area: volume and therefore experience greater physiological constraints on surface activity because they desiccate more easily (Peterman and Semlitsch 2014). Therefore, juvenile salamanders may not be able to reach unoccupied spatial resources, and subsequent competition with superior adults could reduce recruitment rates (Jaeger 1980b). Since there was no difference found in the number of eggs per female salamander at *Amyntas*-invaded vs. non-invaded sites, it is likely that the decline in juvenile density is related to the success of individuals following oviposition. There has been no research into the effects of exotic earthworm invasion on salamander brooding or hatchling success. Earthworm casting material that covers the soil surface has different chemical, physical, and biotic characteristics than topsoil (Burtelow et al. 1998; Mclean and Parkinson 1997). Pellet-

like, granular *Amyntas* spp. casts have higher pH, retain less moisture, and contain different microflora than soil (Dedeke et al. 2010; Schermaier 2013), so it is possible that salamander eggs may develop differently in *Amyntas*-invaded forests. *Amyntas* spp. does not construct permanent burrows, and it is unknown if there are animal burrows or natural crevices present in earthworm casting. Since red-backed salamanders do not excavate their own burrows for oviposition, but instead rely on burrows made by other animals and natural crevices in the soil (Anthony and Pfungsten 2013; Test and Heatwole 1962), a lack of appropriate oviposition sites could reduce reproductive success. Additionally, *Amyntas* spp. could physically disturb brooding female salamanders, compromising their efforts to protect the eggs from desiccation, predation, and lethal microbial infection (Anthony and Pfungsten 2013; Highton and Savage 1961). More research concerning salamander egg development, egg brooding, and recruitment in *Amyntas*-invaded forests is needed.

The differing effects of *Amyntas* earthworm invasion on the densities of adult male and female salamanders may be due to variable surface activity between genders. Field data indicated that male salamander density decreased with increasing *Amyntas* spp. density, but there was no relationship between *Amyntas* spp. invasion and female salamander density. In Ohio, the greatest surface activity for red-backed salamanders occurs during the breeding season (late fall–early spring; Anthony and Pfungsten 2013), when males aggressively vie for access to mating partners at the soil surface (Gergits and Jaeger 1990). Male *P. cinereus* are reproductive annually, whereas females only reproduce once every two years (due to the high cost of egg production; Petranka 1998). Since males are actively searching for mates at the soil surface every year (and

particularly in the fall when adult *Amyntas* spp. density is greatest), there is a greater overlap with *Amyntas* spp., which could explain the unique negative consequences for males found in this study.

Reduced salamander prey availability following earthworm-mediated leaf litter consumption would negatively impact red-backed salamander populations. However, there are conflicting findings concerning the effect of *Amyntas* spp. on microinvertebrate diversity and abundance (Maerz et al. 2009; Migge-Kleian et al. 2006; Schermaier 2013). It is possible that short-term vs. long-term impacts of earthworm invasion may differently influence leaf litter arthropods. For instance, initial earthworm invasion rapidly releases nutrients from bound up in detrital layers, potential bolstering microinvertebrates, but unused nutrients will be leached leading to reduced resources over time (Migge-Kleian et al. 2006; Schermaier et al. 2013). It is unlikely that *Amyntas* spp. can adequately replace lost microinvertebrate prey in salamander diets because they may be distasteful and are too large for consumption by the fall. Over time, if microinvertebrate abundances are reduced by *Amyntas*-mediated leaf litter consumption, then salamanders may have to expend more energy to locate prey. Further, although there was no relationship between *Amyntas* spp. invasion and salamander tail breakage found in this study, extended foraging time in thin/absent leaf litter layers could increase salamander exposure to predators. More direct measures of salamander prey availability and predation pressure from larger vertebrates could clarify how *Amyntas* spp. invasion influences trophic relationships of *P. cinereus*.

The lack of data concerning *Amyntas* spp. invasion history in northeastern Ohio forests was a potential drawback of this study. Although *Amyntas* spp. density and LL

score may be used as crude measures of how long *Amyntas* earthworm populations have been established, there were no data available confirming a site's invasion history (Ricciardi 2003). It is possible that some signals of *Amyntas* spp. invasion on salamander fitness and fecundity could have been obscured by differences in the amount of time that *Amyntas* spp. have been established at the invaded sites. Also, despite low detection of surface-dwelling European earthworms at the sites used for this study, between-site differences in the presence of other invasive species (flora and fauna) might have confounded the ability to obtain a clear measure of *Amyntas* spp. invasion on salamander populations. Surveying efforts to track *Amyntas* spp. population establishment and subsequent ecological impacts on an accurate temporal scale are essential for the ability to quantify short term vs. long-term effects of *Amyntas* earthworm invasion (Migge-Kleian et al. 2006; Schermaier 2013).

## **5. Conclusions**

This is the first study to identify disparate effects of Asian earthworm invasion on age classes and genders of red-backed salamanders. I found that *Amyntas* spp. invasion negatively impacts red-backed salamander populations in northeastern Ohio, resulting in declines in juvenile and male salamander densities. The physical disturbance and resource degradation caused by *Amyntas* spp. synergistically excludes salamanders from high quality microhabitat, hinders salamander foraging effort, and depletes detrital resources. *Amyntas* spp. domination (natural cover objects) and removal (leaf litter) of spatial resources in forest floor communities may challenge the social, trophic, and physiological success of red-backed salamanders. Although *P. cinereus* populations are

quite robust throughout the species' range, decreases in red-backed salamander populations could have important trophic ramifications for detrital communities. Also, there is cause for concern for populations of less-abundant, ecologically similar forest floor plethodontids that may be negatively affected by *Amyntas* earthworm invasion. As *Amyntas* spp. rapidly expands its range (Reynolds et al. 2015), understanding how native forest floor biota are impacted will be vital for monitoring detrital community stability and informing conservation priorities.

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**Table 1**

Field sites in northeastern Ohio. Sites were classified as “non-invaded” or “*Amyntas*-invaded” based on presence of *Amyntas* spp. (or *Amyntas* spp. casting material) and the quality of the leaf litter (LL; based on Loss et al. 2013). LL was evaluated at four random locations within the site, each of which was scored from 1–3, with a lower score indicating lower quality. The maximum score a site could receive was a 12 (indicating an intact, healthy forest floor composed of LL from 2+ years), while the minimum score a site could receive was a 4 (indicating a highly disturbed, scant forest floor containing only LL from the previous year).

	Coordinates	Collection date	# <i>Amyntas</i>	# European earthworms	LL score
<b>Non-invaded sites</b>					
Chapin	41°35'29.3"N 81°21'55.7"W	9/18 & 9/25/14	0	2	11
The West Woods	41°27'36.4"N 81°17'46.0"W	9/26/14	0	5	11
Hinckley	41°13'13.9"N 81°42'34.4"W	10/3/14	0	16	11
Holden Arboretum	41°36'49.6"N 81°16'59.5"W	10/4/14	0	0	12
Swine Creek	41°25'58.4"N 81°01'26.2"W	10/10/14	0	0	12
Rocky River	41°24'45.3"N 81°52'46.8"W	10/17/14	0	13	10
Mill's Stream Run	41°18'18.3"N 81°46'42.0"W	10/22/14	0	0	12

**Table 1 (Continued)**

	Coordinates	Collection date	# <i>Amyntas</i>	# European earthworms	LL Score
<b><i>Amyntas</i>-invaded sites</b>					
Doan Brook	41°29'37.0"N 81°35'37.9"W	9/25 & 9/30/14	50	0	4
The West Woods	41°27'27.9"N 81°18'01.8"W	9/27/14	21	0	6
Holden Arboretum	41°36'39.6"N 81°16'48.4"W	10/5/14	36	1	4
Chagrin River	41°26'47.9"N 81°24'38.9"W	10/9/14	14	0	4
Bedford	41°23'15.8"N 81°32'33.9"W	10/24/14	3	0	9
Squire	41°29'38.6"N 81°24'50.5"W	10/25/14	9	2	7



**Table 2**

Natural cover object (N=100 per site) occupancy data for *Amyntas*-invaded sites.

Site	Unoccupied	Salamander only	<i>Amyntas</i> spp. only	Co-occurrence
All	378	113	94	15
Doan Brook	41	12	37	9
The West Woods	72	12	13	3
Holden Arboretum	58	12	29	1
Chagrin River	70	20	10	0
Bedford	65	33	1	1
Squire	71	24	4	1

**Table 3**

Linear regression results for the relationship between *Amyntas* spp. density and salamander density.

<i>Salamander density</i>	$R^2$	<i>dfn, dfd</i>	$F$	$P$
All	0.179	1, 11	2.405	0.149
Female	0.018	1, 11	0.206	0.658
Juvenile	0.501	1, 11	11.064	<b>0.007</b>
Male	0.301	1, 11	4.738	<b>0.05</b>

**Fig. 1.** Cohabitation arena and trial set-up. Cohabitation arenas (A) had zones of high quality (greatest protection from desiccation) and low quality (least protection from desiccation). The high quality zone (Area = 39 cm<sup>2</sup>; 6.5% of arena) was the space between the artificial cover object and the moist filter paper (upper right corner). The low quality zone (Area = 561 cm<sup>2</sup>; 93.5% of arena) is the space on the dry plastic bottom of the arena. In A, an *Amyntas* spp. earthworm is visible beneath the cover object (high quality zone), while a salamander is outside the cover object (low quality zone). Groups of six arenas, with three replicates of each treatment, were arranged in a randomized block (B) with visual barriers between them for video-recording.

**Fig. 2.** Study site localities in northeastern Ohio. Areas of Ohio denuded by the Laurentide ice sheet during the Wisconsinan glaciation (containing no native earthworms) are shaded gray. Non-invaded sites were: Rocky River (RR), Mill's Stream Run (MR), Hinckley (HI), Chapin (CH), The West Woods (WW), Holden Arboretum (HA), and Swine Creek (SC). *Amyntas*-invaded sites were: Doan Brook (DB), Bedford (BE), Squire (SQ), Chagrin River (CR), The West Woods (WW), and Holden Arboretum (HA).

**Fig. 3.** Equal probability plots for high quality (HQ) microhabitat use when salamanders were alone or paired with an *Amyntas* spp. earthworm. The diagonal line indicates no difference in a salamander's HQ zone use when they were alone vs. paired with an earthworm. A point above the line represents a salamander that used the HQ zone more often when alone, while points below the line represent salamanders that used the HQ

zone more often when paired with an earthworm. Part A shows the spread of data when all salamanders (N=36) are included in the analysis. Part B shows the spread of data when the seven salamanders that did not use HQ microhabitat when alone in the arena were removed.

**Fig. 4.** The effect of *Amyntas* spp. presence on the number of flies consumed in salamander foraging efficiency trials. Salamanders consumed fewer flies in the presence of earthworms than when alone ( $F_{1,102} = 6.288$ ,  $P = 0.014$ ). The bars represent standard error.

**Fig. 5.** Significant negative relationships between *Amyntas* spp. density and leaf litter score (A;  $R^2 = 0.886$ ,  $F_{1,11} = 85.634$ ,  $P < 0.0005$ ) and surface-dwelling European earthworm density (B;  $R^2 = 0.544$ ,  $F_{1,8} = 9.562$ ,  $P = 0.015$ ).

**Fig. 6.** The effect of *Amyntas* earthworm invasion on juvenile and male salamander density from forested sites in northeastern Ohio. Juvenile salamander density was negatively associated with *Amyntas* spp. density ( $R^2 = 0.501$ ,  $F_{1,11} = 11.064$ ,  $P = 0.007$ ). There was a strong trend of decreasing male salamander density with increasing *Amyntas* spp. density ( $R^2 = 0.301$ ,  $F_{1,11} = 4.738$ ,  $P = 0.05$ ).

**Fig. 7.** A comparison of *Amyntas* spp. density and salamander body condition from forested sites in northeastern Ohio. There was no significant effect of *Amyntas* spp. invasion on salamander body condition ( $P > 0.05$  for all salamander categories).

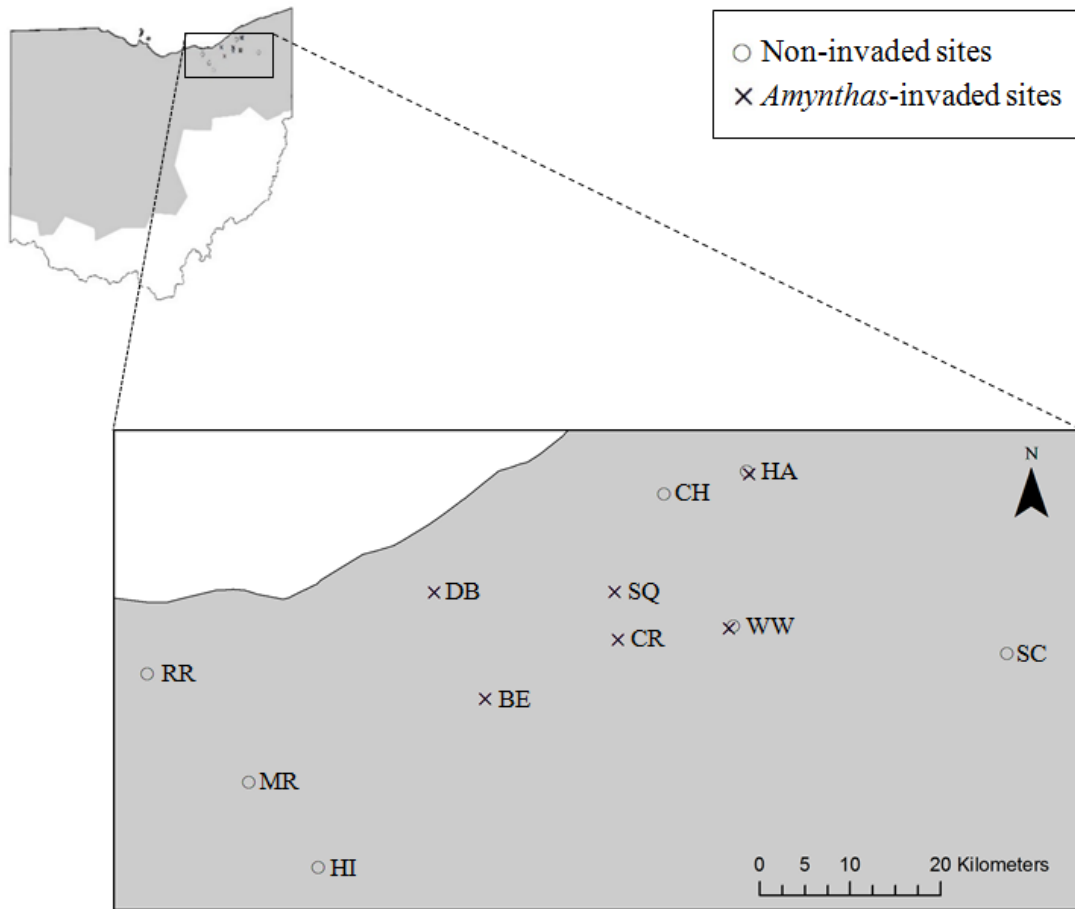
(A)



(B)

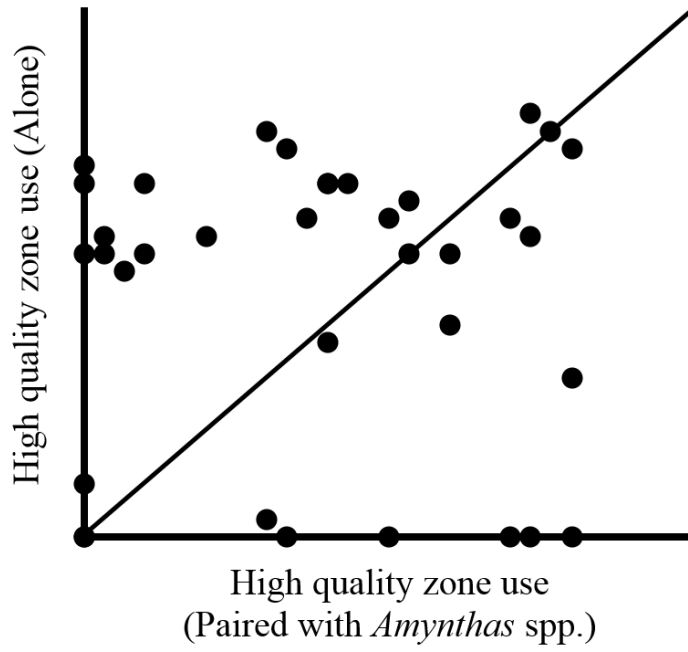


**Fig. 1**

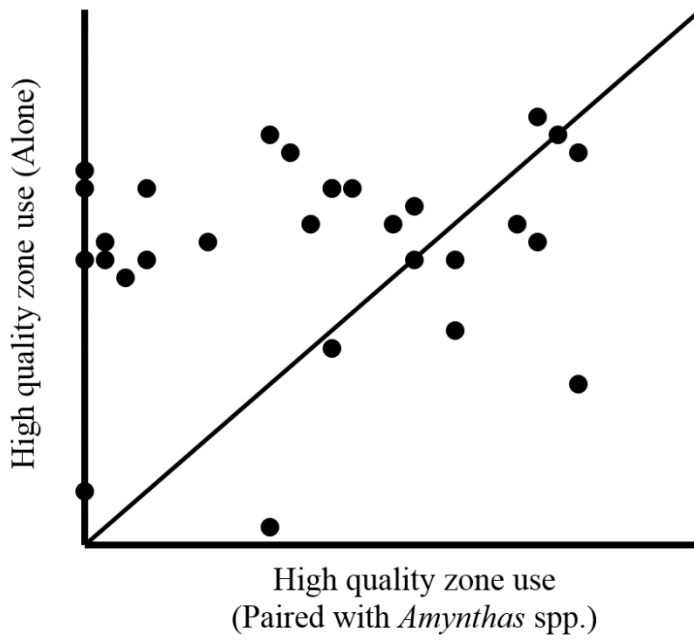


**Fig. 2.**

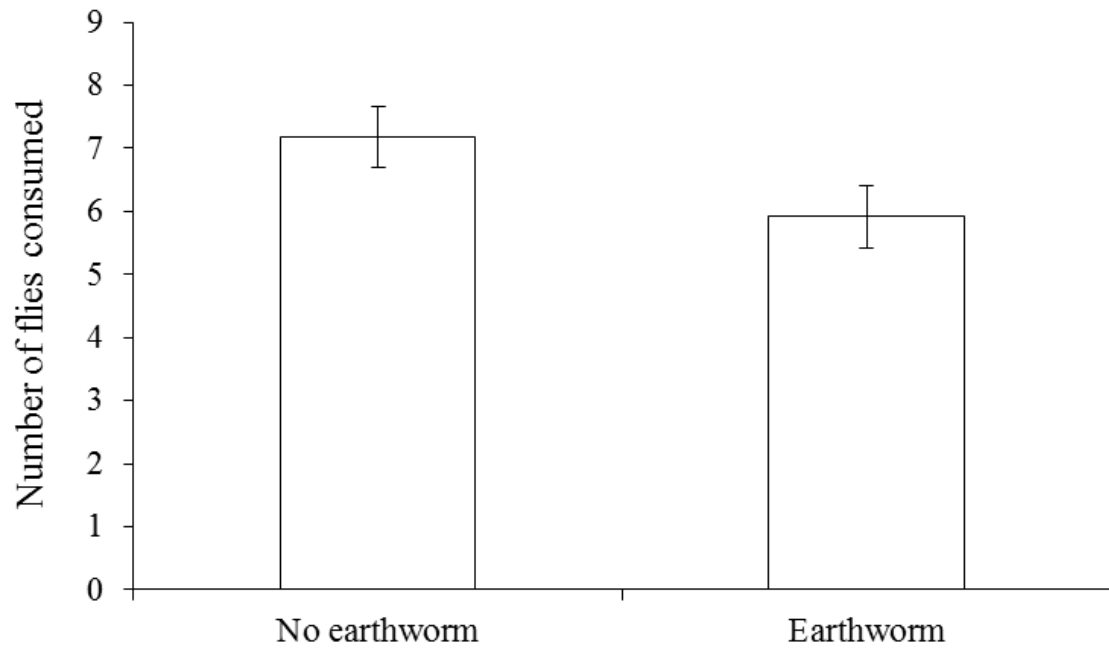
(A)



(B)



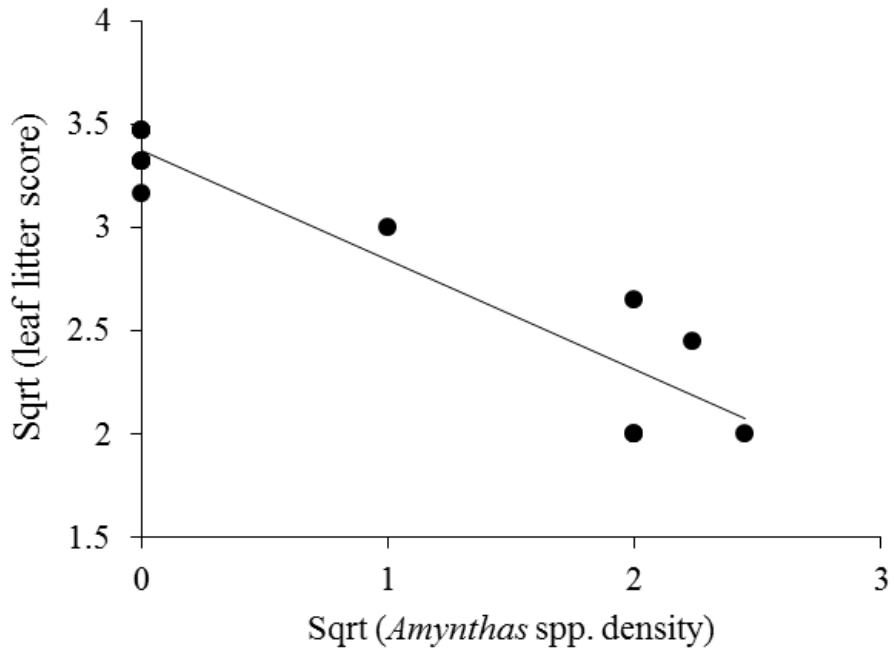
**Fig. 3.**



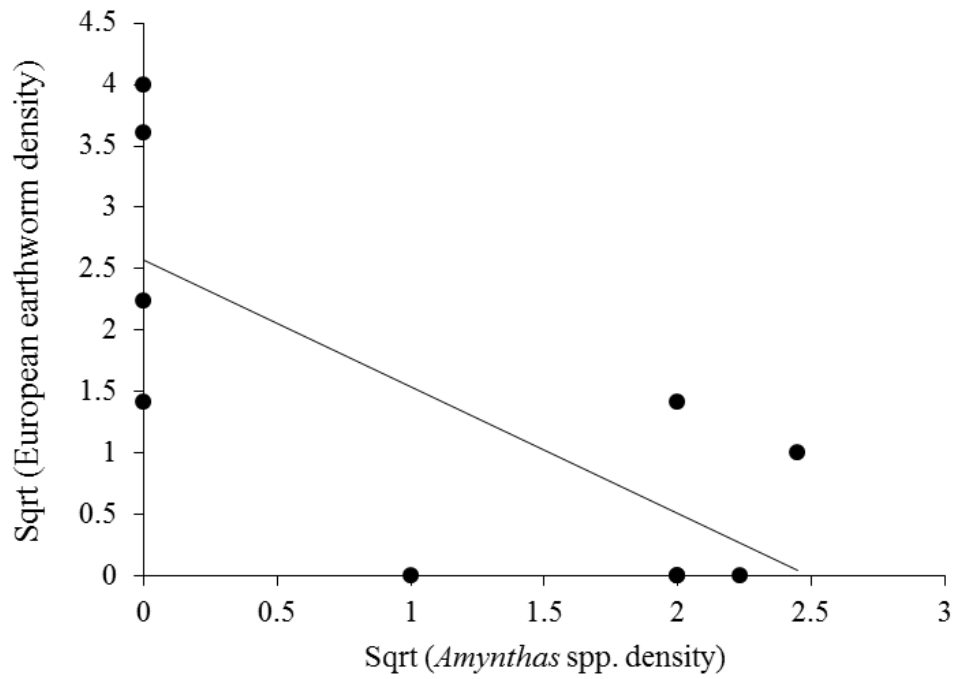
**Fig. 4.**



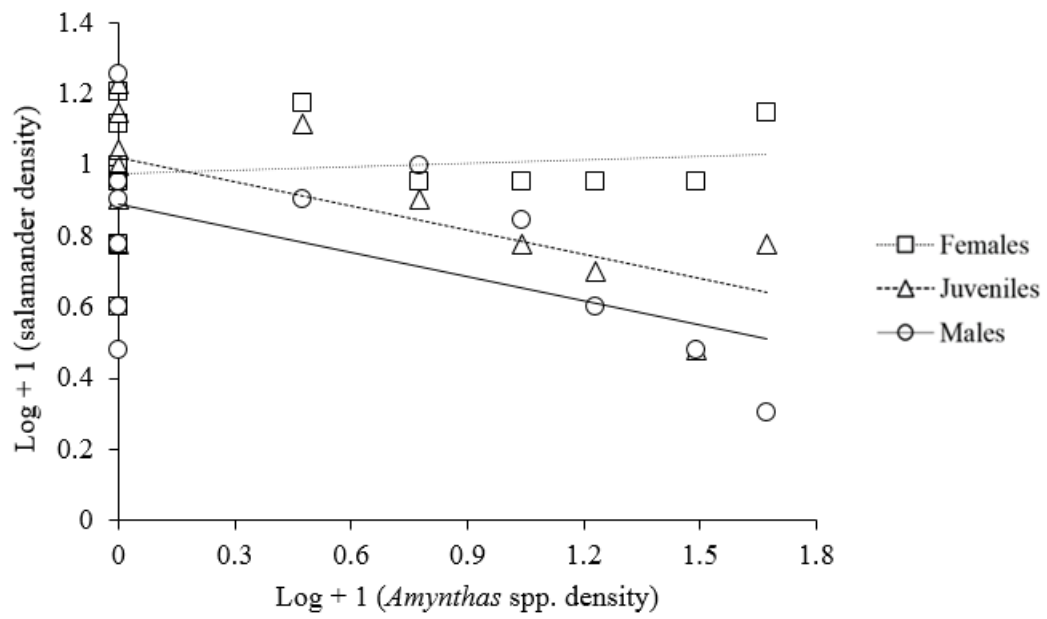
(A)



(B)



**Fig. 5.**



**Fig. 6.**

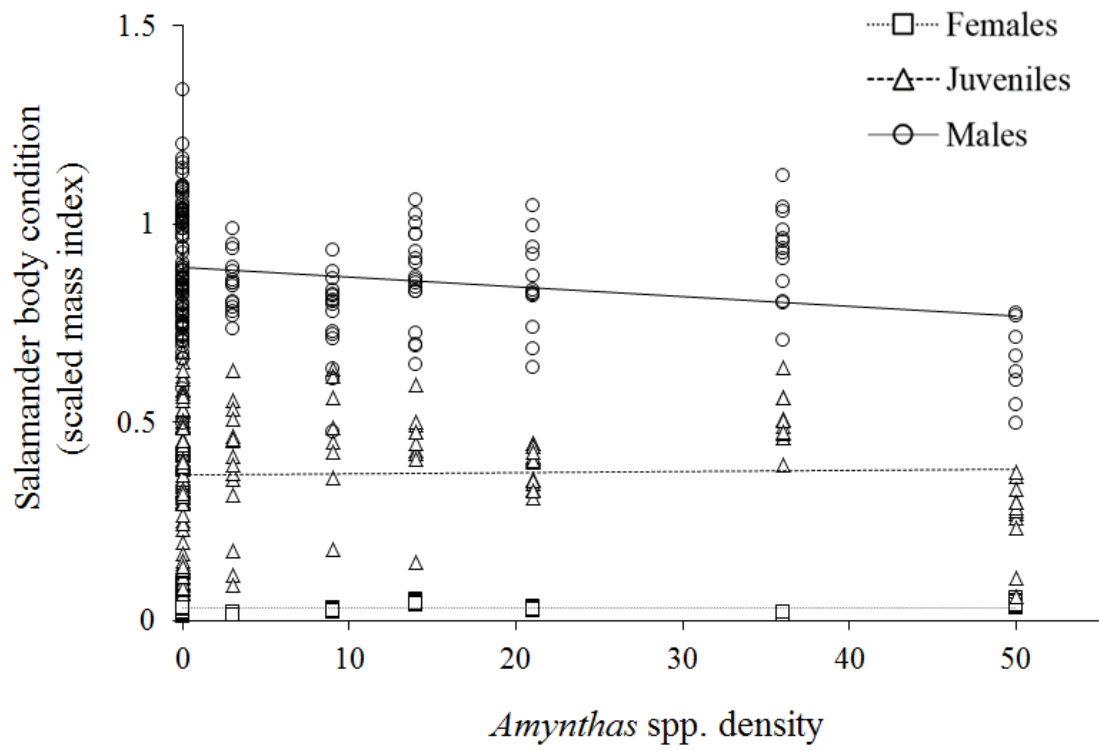


Fig. 7.