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ECOSYSTEM FUNCTIONAL CONSEQUENCES OF TOP PREDATOR MORTALITY DUE TO THE
INVASIVE SHRUB, *LONICERA MAACKII*

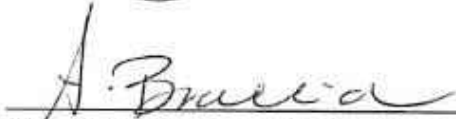
BY

JOSEY LEE-ANNE BERTA

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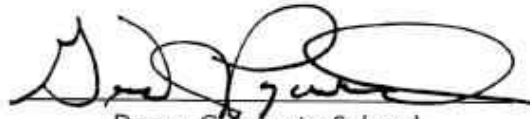
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ECOSYSTEM FUNCTIONAL CONSEQUENCES OF TOP PREDATOR MORTALITY DUE TO
THE INVASIVE SHRUB, *LONICERA MAACKII*

BY

JOSEY LEE-ANNE BERTA

Submitted to the Faculty of the Graduate School of
Eastern Kentucky University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

2019

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ABSTRACT

Lonicera maackii (Amur Honeysuckle) is an invasive woody plant species that is present across the United States. Previous studies have assessed the biotic effects of honeysuckle, as well as abiotic effects such as changes in soil chemistry, ground level light, and forest floor temperature. Although direct effects of *L. maackii* on native terrestrial plant communities are well studied, little is known about its indirect effects, especially in aquatic ecosystems. Based on limited prior studies, I predicted addition of *L. maackii* leaves to aquatic systems would increase mortality of a top amphibian predator due to the release of phenolic compounds that inhibit respiration. A mesocosm experiment was developed to characterize the cascading effects of increased top predator (*Ambystoma maculatum*) mortality on larval salamander growth, macroinvertebrate densities, zooplankton densities, leaf litter mass loss, chlorophyll abundance, biofilm growth, and availability of soluble nutrients. Of the 20 mesocosms that contained larval *A. maculatum*, only 11 produced metamorphs, and only one mesocosm with leaf litter from *L. maackii* produced metamorphs. All 10 mesocosms containing *A. maculatum* larvae and native leaf litter produced metamorphs. A total of 117 metamorphs were retrieved from all mesocosms, and only three of those 117 were retrieved from mesocosms with leaf litter from *L. maackii*. Salamander survival and growth rates (mm/day) were significantly lower in mesocosms with *L. maackii* than in mesocosms with native leaf litter alone. Mesocosms with *L. maackii* leaf litter also contained substantially more mosquito larvae, suggesting reduced water quality. There was no indication that apex predator mortality in *L. maackii* mesocosms altered aquatic

ecosystem functions. However, increased mass loss occurred in leaf packs containing *L. maackii* compared to leaf packs containing native leaf litter removed from the same mesocosm, which was not caused by greater invertebrate densities within *L. maackii* packs. I also found there to be an oily sheen on the water surface of mesocosms containing *L. maackii* leaf litter, which could hinder gill-breathing by amphibians. Relatively high invertebrate densities and diversity may have served as a buffer for lower trophic levels, such that they were not affected when *A. maculatum* individuals were eliminated from mesocosms due to exposure to *L. maackii* phenolic compounds. The results of this study will help complete the overall picture of the possible consequences of biological invasion.

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I. Introduction

Exotic species can become invasive, dominating habitats, and negatively impacting entire ecosystems (Didham et al. 2005, Hejda et al. 2009, Zedler & Kercher 2004, Civitello et al. 2008). Invasive plant species specifically have now become established across much of the Eastern United States, subsequently altering native ecosystems through direct and indirect pathways. Direct pathways through which invasive plants affect ecosystems include competition, (e.g., for sunlight or nutrients; Orrock et al. 2010, Watling et al. 2011c, Weidenhamer and Callaway 2010) and changes in habitat composition (Didham et al. 2005, Weidenhamer and Callaway 2010), such as shifts in canopy structure, shifts from herbaceous to woody plants (or vice versa), increased productivity and leaf litter deposition, changes in leaf litter mass loss, altered nutrient regimes, and increased or decreased flammability (Zedler & Kercher 2004). Some invasive plants, however, can affect ecosystems through indirect pathways, such as alteration of the soil chemical environment (Ehrenfeld 2003, Maerz et al. 2005, Watling 2011a,b; Wolfe & Klironomos 2005).

Several invasive plant families alter physical and chemical environments of the invaded habitat (Ehrenfeld 2003, Weidenhamer & Callaway 2010, Wolfe & Klironomos 2005), and of these, shrub species are the most common (McKinney & Goodell 2010). Amur Honeysuckle (*Lonicera maackii*) is an invasive shrub that creates a thick shrub layer that is absent in native, uninvaded forests (Collier et al. 2002), consequently reducing ground level light (McKinney & Goodell 2010), decreasing temperature (Herrera 1997),

and increasing humidity (Chen et al. 1999). Changes in these factors influence plant and animal species richness, evenness, and composition of invaded areas (Collier et al. 2002, McKinney & Goodell 2010, Watling et al. 2011c). *Lonicera maackii* also significantly alters soil chemistry by releasing water soluble phenolic compounds (tannins) that are toxic to many terrestrial and aquatic animals (Templer, Findlay & Wigand 1998, Rauha et al. 2001, Watling et al. 2011c). When certain phenolic compounds (i.e., apigenin and luteolin; Cipollini et al. 2018, Watling et al. 2011a) are dissolved in aquatic ecosystems, they can cause adverse behavioral changes and increase mortality of aquatic animals (Maerz 2005, Watling et al. 2011a,b,c). Native plants and animals are often adversely affected by these phenolic compounds because of their relatively short evolutionary history of co-occurrence, which prevents the former from evolving resistance to invasives' toxins (Zedler & Kersher 2004). Changes to aquatic taxa caused by phenolic compounds have the potential to cause subsequent negative impacts to entire populations and communities, yet such indirect effects are not well-understood, and further experimentation is needed to predict the ecosystem impacts of invasion by *L. maackii* (Weidenhamer and Callaway 2010, Watling et al. 2011c).

Watling et al. (2011b,c) monitored behavior and mortality of larval amphibians in response to *L. maackii* extracts, concluding that some amphibian species were more likely to die due to exposure, while other amphibian species displayed behaviors consistent with *L. maackii* reducing the quality of larval respiration. From these

studies, phenolic compounds in *L. maackii* appear water soluble and capable of significantly impacting amphibian survival and behavior, and evidence indicates that their results are specifically due to these compounds (Cippolini et al. 2008; Watling et al. b,c). Given these initial observations, it is possible that increased amphibian mortality could alter trophic cascades and associated aquatic ecosystem functions. Studies of the direct effects of *L. maackii* in aquatic systems are limited (Boyce et al. 2012; McNeish et al. 2012), and even less is known about indirect effects, such as the impacts of amphibian losses on lower trophic levels. Wilbur (1972) suggested that amphibian diversity is primarily regulated in the larval stage, even for subsequently terrestrial metamorphic populations, and density-dependent interactions among larval amphibians contribute to the stability of their associated aquatic communities. Based on the effects of *L. maackii* on amphibian larvae in lab and field experiments (Watling et al. 2011b,c), I hypothesize increased larval amphibian mortality induced by *L. maackii* could alter trophic interactions and associated ecosystem functions in ephemeral pond ecosystems.

Within forested ephemeral ponds, larval salamanders serve as apex predators, and staggered breeding phenology among adult salamanders produces considerable population and community size-structure and associated diversity of intra- and interspecific trophic interactions among salamander larvae (Boone 2005; Mott and Maret 2011, Figure 1). In a typical ephemeral pond in the Eastern United States, *Ambystoma maculatum* is one of the last salamander species to hatch, resulting in

smaller body sizes when co-occurring with other species, such as *Ambystoma opacum* (hatches earlier in the fall) and *Notophthalmus viridescens* (has an aquatic adult stage), which allows these larger species to prey on *A. maculatum* (Petranka 1998). However, in many cases *A. maculatum* is the sole salamander species in ephemeral ponds, in which case it would serve as the ecosystems' apex predator (Anderson et al. 2017). Macroinvertebrates and microcrustacea are the dominant food sources of larval salamander predators such as *A. maculatum* (Petranka 1998), but they exhibit dietary shifts to larger prey during ontogeny, up to and including cannibalism (Mott and Sparling 2016). Larval salamanders (Petranka 1998), along with macroinvertebrates (Webster and Benfield 1989) prey on tadpoles, microinvertebrates, and zooplankton, which often function as primary consumers (Altig, Whiles & Taylor 2007 & Petranka & Kennedy 1999) and detritivores (Webster & Benfield 1986), responsible for regulating detritus breakdown and algal productivity. Previous studies have established that loss of salamanders (top predators) in ephemeral ponds can result in trophic cascades (Anderson et al. 2013; Morin 1983).

Predator-prey interactions across trophic levels regulate ecosystem structure; however, interspecific competition (Anderson and Whiteman 2015, Morin 1986), intraspecific competition (Anderson et al. 2013, Anderson and Whiteman 2015), and cannibalism (Anderson et al. 2013) also regulate ecosystem structure. These ecological interactions in ephemeral ponds in turn influence the presence and intensity of various ecosystem functions, such as rates of leaf litter decomposition, primary productivity,

and availability of soluble nutrients (Hocking and Babbitt 2014; Mokany 2007; Petranka and Kennedy 1999). By measuring response variables associated with these ecosystem functions, we might infer how *L. maackii* might influence the ecosystem functional consequences of competitive and predator-prey interactions in aquatic systems. Decreases in salamander abundance result in a corresponding increase in their prey, which may subsequently alter ecosystem functions. Following reduction or elimination of salamander apex predators, increased predation pressure by tadpoles and herbivorous invertebrates may increase detritus breakdown and algae consumption.

In previous studies, when exposed to extracts of *L. maackii*, top predators (larval *Ambystoma spp.*) in ephemeral pond ecosystems are reduced or eliminated due to specific phenolic compounds released into the ponds either by plant roots or fallen leaves (Watling et al. 2011b). We predict the elimination or reduction of top predators by *L. maackii* will weaken trophic cascades (Figure 1) in ephemeral ponds. Lower order predators (i.e., macroinvertebrates) will exhibit increased densities due to reduced predation, in turn reducing densities of their largely herbivorous prey (i.e., microcrustacea and tadpoles), giving way to increased algal growth. Leaf litter is broken down by macroinvertebrates in the shredder functional group, so we expect to see an increase in leaf litter decomposition due to the generally increased number of macroinvertebrates (Figure 2).

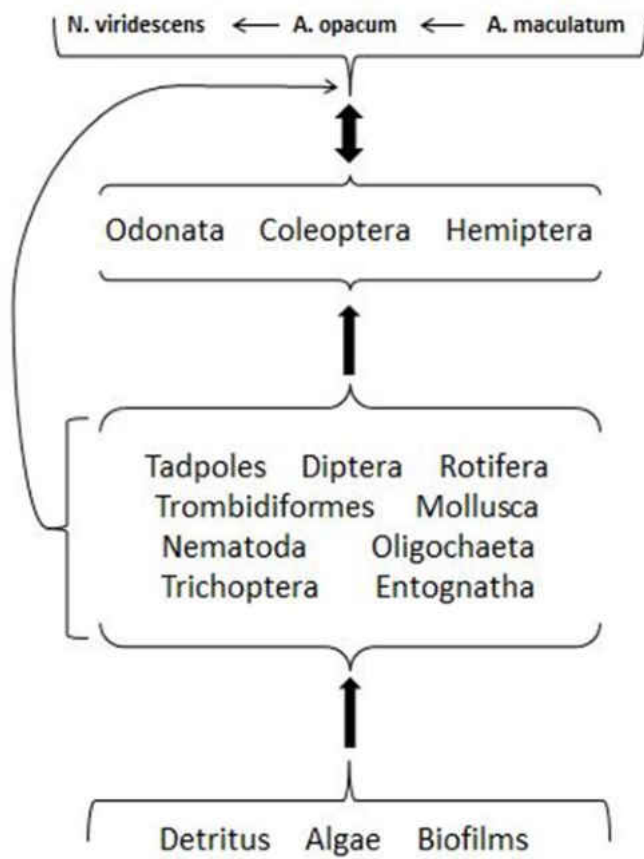


Figure 1. Simplified food web typical of forested ephemeral ponds in the Eastern United States, with predation occurring in each trophic level. Arrows point to the predator and double ended arrows indicate reciprocal predation based on similar size.

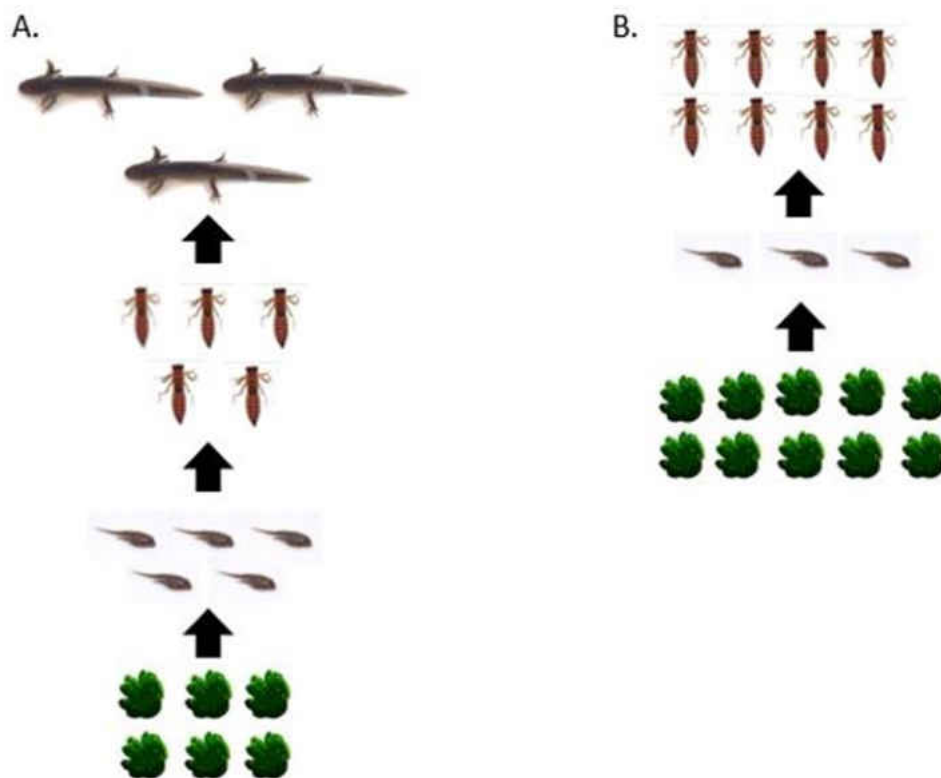


Figure 2. A) Example of trophic interactions in a natural ephemeral pond. Larval salamanders consume macroinvertebrates, macroinvertebrates consume tadpoles and tadpoles consume algae B) Example of trophic interactions in an ephemeral pond containing *L. maackii*. *Lonicera maackii* will severely reduce or eliminate salamanders as top predators, thus allowing macroinvertebrate populations densities to increase. More macroinvertebrate predation on tadpoles will reduce tadpole densities. Finally, with fewer tadpoles, algae will grow at a faster rate and/or there will be greater amounts of algae in mesocosms containing *L. maackii* leaves.

Because much of the Eastern United States has experienced invasion by non-native plants (Bradley et al. 2010; Pimentel et al. 2005), it is important to address the direct and indirect effects on native aquatic ecosystems. In this experiment, we determined the direct and indirect impacts of *L. maackii* on the trophic interactions of an aquatic

food web. We measured ecosystem functions (algal growth as surrogate for rates of primary productivity, and leaf litter mass loss as surrogate for rates of decomposition) in experimental mesocosms while maintaining the integrity and composition of a natural ephemeral pond, thereby striking a balance between realism and repeatability (Wilbur 1989). The results of this study will help complete the overall picture of the possible consequences of biological invasion and, provide more complete information for conservationists going forward.

II. Methods

Study Site

This research project was conducted at Taylor Fork Ecological Area (TFEA) (37.7166° N, 84.2958° W) at Eastern Kentucky University in Madison County, Kentucky.

Experimental Design

It is difficult to observe the complexity of trophic interactions in natural ephemeral ponds due to uncontrolled variables within a natural pond which aren't being measured. Artificial ponds, or mesocosms, have been employed to allow scientists to create and observe more controlled, but still realistic, aquatic environments (Boone 2005, Morin 1986, Walls and Williams 2001, Wilbur 1989). Our experiment was completed within 30 mesocosms (300-gal, Rubbermaid stock-tank, Model number: FG424700BLA) structured to replicate a natural ephemeral pond. In early February 2018, native (~208g/L dry weight) and *Lonicera maackii* (~7.5g/L wet weight) leaves were placed in 15 mesocosms; the remaining 15 mesocosms were only stocked with native (~208g/L) leaf litter. Native leaves consisted of *Acer rubrum*, *Platanus occidentalis*, *Carya* spp. and *Quercus* spp., all of which were recently senesced and collected from the surrounding area in November 2017. Leaves of *L. maackii* were collected *in vivo* at TFEA and surrounding areas in November 2017 and stored at -20°C for later use following Maerz et al. (2005). In early February 2018, *L. maackii* leaves were thawed and weighed. Leaves were placed in the center of each of the 15 mesocosms that were to contain treatments of *L. maackii* (Figure 3).

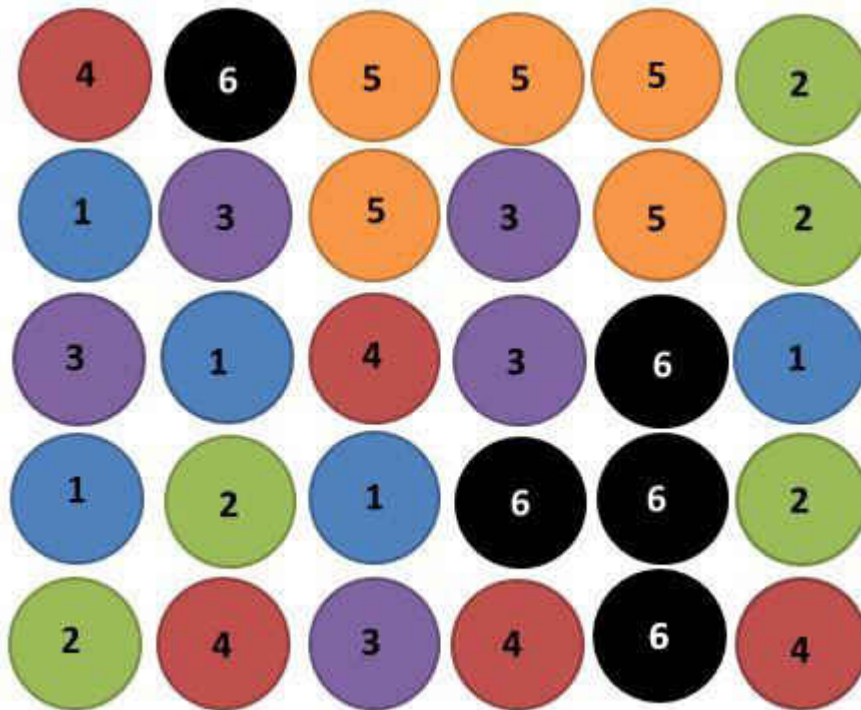


Figure 3. Experimental array of 30 mesocosms with five replicates of six treatments: 1) *Ambystoma maculatum* (apex predator) & *Anaxyrus americanus* (herbivore) with native leaf litter; 2) *A. maculatum* with native leaf litter; 3) *A. americanus* with native leaf litter; 4) *A. maculatum* and *A. americanus* with native & *L. maackii* leaf litter; 5) *A. maculatum* with native & *L. maackii* leaf litter; 6) *A. americanus* with native & *L. maackii* leaf litter.

Zooplankton, sediment, and aquatic macroinvertebrates were added to each of the 30 mesocosms from mid-February to mid-March following established procedures (Doyle and Whiteman 2008, Anderson and Whiteman 2015, Mott and Sparling 2016) to standardize mesocosm function prior to larval amphibian introduction. Zooplankton were collected from late February to early March from a cistern and natural pond at TFEA by skimming the surface of each with an 80- μ m conical Fieldmaster zooplankton

net (Wildlife Supply Company, Yulee, Florida). Approximately three liters of water containing concentrated zooplankton were added to each of the 30 mesocosms, and these concentrations reflect realistic *in situ* zooplankton densities (Doyle and Whiteman 2008, Anderson and Whiteman 2015, Mott and Sparling 2016). In March 2018, sediment was collected from a large pond at TFEA using shovels and buckets. All sediment collected was homogenized in a large plastic container, and approximately two liters of sediment was placed in the center of each of the 30 mesocosms, using a one-liter plastic scoop. On the same day, each mesocosm also received 950 mL of concentrated algae from an unused cattle tank. Mesocosms were left uncovered for the first two months of the experiment (early-March to early-May) to allow for deposition of volant aquatic invertebrates (Anderson and Whiteman 2015). Many invertebrate taxa colonized mesocosms independently; however, three Lymnaeid snails and three larval Zygoptera (damselflies) were collected and deposited into each mesocosm, since it was unlikely these groups would self-colonize quickly enough for this project. 1.85-m diameter lids constructed from 10 cm flexible chlorinated polyvinyl chloride (CPVC) pipe and 1-mm white mosquito netting (Memphis Net and Twine, Memphis, Tennessee) were used to cover each mesocosm. Lids were placed on mesocosms on May 9th, 2018 to prevent extra uncontrolled tadpoles in mesocosms following breeding of Cope's Gray Treefrog (*Hyla chrysoscelis*).

Eight unglazed ceramic tiles (4.7 x 4.7 x 0.5 cm) were used to monitor algal productivity in each experimental mesocosm. Algal tiles were placed on the south side

of each of the 30 mesocosms, suspended above leaf litter and sediment by nylon string. Eight nylon mesh bags (27cm x 17cm, 0.5 cm mesh) containing 5g of dried native leaf litter (*Acer rubrum*, *Platanus occidentalis*, *Carya spp.* and *Quercus spp.*, Boulton and Boon 1991) were also placed in each of the 30 mesocosms. In addition to the mesh bags containing native leaf litter, five nylon mesh bags (27cm x 17cm, 0.5 cm mesh) containing 5 g of dried *L. maackii* leaf litter were also added to each of the 15 mesocosms previously stocked with leaf litter from *L. maackii*. All leaf litter bags were weighed down with small pieces of gravel to keep them in place. In mesocosms containing native and *L. maackii* leaf litter, leaf packs were alternated on the mesocosm floor starting on the south side of the mesocosm, with a native leaf litter pack, and moved along the wall to the east side.

Egg masses (~20) of *A. maculatum* were collected on March 23rd at Miller-Welch Central Kentucky Wildlife Management Area (CKWMA). Egg masses were brought back to the vivarium facilities at Eastern Kentucky University and maintained in environmental chambers at 11.4° C and 12L:12D photoperiod until hatching. Egg mass hatching occurred from March 30th - April 20th. On April 20th all salamander larvae were placed in a single container (Anderson and Whiteman 2015), and 30 larvae were removed and temporarily placed in each of 20 containers (15cm x 15cm, ~ 5 cm deep), filled half way with deionized water, and labeled corresponding to the 20 mesocosms to receive *A. maculatum* larvae (Figure 2). Larvae were photographed for subsequent measurement and then transported to the mesocosms. Each container was placed in

its corresponding mesocosm for 40 minutes to allow larvae to acclimate to water temperatures, after which larvae were released into mesocosms.

Approximately 900 tadpoles of *Anaxyrus americanus* were collected in a puddle at CKWMA on April 26th, brought back to the vivarium facilities at Eastern Kentucky University, and placed in the environmental chamber under the aforementioned temperature and photoperiod. The next day tadpoles were homogenized, divided into groups of 30 tadpoles each, photographed, and released into corresponding mesocosms using the same methods used with larval *A. maculatum*.

Experimental mesocosms were divided into five replications of six separate treatments. Treatments consisted of different larval amphibian combinations in either the presence or absence of leaves of *L. maackii*: 1) *Ambystoma maculatum* (Spotted Salamander) and *Anaxyrus americanus* (American Toad) with native leaf litter; 2) *A. maculatum* with native leaf litter 3) *A. americanus* with native leaf litter; 4) *A. maculatum* and *A. americanus* with native and *L. maackii* leaf litter; 4) *A. maculatum* with native and *L. maackii* leaf litter; 5) *A. americanus* with native and *L. maackii* leaf litter (Figure 2). Because *A. americanus* is an herbivore, it served as a relatively large vertebrate herbivore that is also consumed by larval *A. maculatum*. If numbers of *A. americanus* were altered, it was assumed that algal growth within mesocosms would increase or decrease, depending on the changes in tadpole abundance. By having these different structural combinations, we could determine how *L. maackii* affects

each amphibian species directly, as well as the possible direct or indirect community effects of *L. maackii*.

Sampling

Mesocosms were sampled from May-September 2018, which coincided with the short larval life stage of *A. maculatum* (Petranka 1998). Mesocosms were sampled about every 35 days for a total of four sampling events. In each mesocosm at each sampling event, we recorded larval amphibian survival rates/densities and body size variation, as well as invertebrate species composition and density (Robinson et al. 1998), leaf litter mass loss (Boulton & Boon 1991), biofilm mass (Rosemond et al. 1993), zooplankton densities (Mott and Sparling 2010), and nitrate and phosphate concentrations. Hand-held meters were also used to measure chlorophyll a, pH, dissolved oxygen, and temperature of each mesocosm.

At each sampling event, one leaf litter bag containing native leaves was removed from each of the 30 mesocosms, and one leaf litter bag containing leaves of *L. maackii* was also removed from each of the 15 mesocosms also containing leaf litter from *L. maackii*. Bags were sealed in individual Whirl-Paks with 70% ethanol and Rose Bengal stain. An algal tile was removed from each mesocosm and scraped with a razor blade, with contents preserved in individual plastic specimen cups in 2% glutaraldehyde. Zooplankton samples were taken at each sampling event by a single vertical dip of an 80- μ m Conical Fieldmaster Student Zooplankton Net (Wildlife Supply Company, Yulee,

Florida). Samples were immediately preserved in 70% ethanol stained with Rose Bengal (Mott and Sparling 2010). Aquatic macroinvertebrates were collected using quantitative enclosure sampling (Shaffer et al. 1994) from a single benthic leaf litter sample per sampling event using a Fieldmaster Mighty Grab (Wildlife Supply Company, Yulee, Florida). Samples were preserved individually in Whirl-Paks with 70% ethanol and Rose Bengal stain. Three minnow traps (Cabella's Promar collapsible, 40cm x 25cm x 25cm) were placed in each mesocosm monthly to estimate densities of larval salamanders and tadpoles. Traps were placed in mesocosms one night prior to sampling to allow them to soak overnight, before sampling the next morning. Larval salamanders and tadpoles captured in minnow traps were photographed in a tray with a ruler for later measurement with Image J (Abramhoff et al. 2004; Mott and Steffen 2013), and individuals were then returned to their respective mesocosms. Water samples that would be used to determine nitrate and phosphate concentrations were removed from each mesocosm and immediately placed on ice in a cooler. After field sampling was complete, water samples were stored in the freezer at -20° C.

In the lab, macroinvertebrates were collected from leaf litter bags, identified to the lowest useful taxonomic level and functional group, enumerated (Robinson et al. 1998), and the remaining leaf litter was dried for 120 hours at 65° C and weighed. From these weights, rates of leaf litter mass loss were determined (Boulton and Boon 1991) by subtracting the final leaf mass from the original leaf mass (5 g). Periphyton samples collected from algal tiles were dried for 48 hours at 80° C and weighed to

estimate biovolume (Rosemond et al. 1993) and provide an indicator of biofilm growth. Zooplankton density estimates were determined by pipetting 1-mL subsamples into Sedgewick-Rafter counting chambers (Wildlife Supply Company, Yulee, Florida). Under 32x dissection microscopy, zooplankters were enumerated, identified to order (Smith 2001), and used with sample volumes to estimate zooplankton densities (Mott and Sparling 2010). Macroinvertebrate densities were estimated using quantitative enclosure sampling (Shaffer et al. 1994) obtained from the Mighty Grab during field sampling. Under 32x dissection microscopy, stained macroinvertebrates were picked from the samples, enumerated, and identified to the lowest useful taxonomic level and functional group. Nitrate and phosphate water samples were removed from the freezer and allowed to thaw to room temperature. A Trilogy Laboratory Fluorometer with nitrate and phosphate models was used to determine nitrate and phosphate readings.

Total body lengths of hatchling *A. maculatum* were determined using ImageJ before releasing hatchlings into mesocosms (Abramoff et al. 2004; Mott et al. 2010 and; Schneider, Rasband, & Eliceiri 2012). Image J was also used to record total body length and snout-vent lengths from images of *A. maculatum* larvae and metamorphs captured in minnow traps during field sampling. Using a software program to take measurements of *A. maculatum*, as opposed to physically measuring them with calipers, reduced handling stress for the animals and field time for the investigators (Mott et al. 2010). Average growth rates of larval salamanders in each mesocosm and

treatment were determined by taking measurements of the initial size and size of *A. maculatum* individuals at the last sampling event.

After salamanders began to show signs of metamorphosis (i.e., loss of gills and tail fins, development of eyelids and juvenile coloration (Petranka 1998)), they were removed from mesocosms, and measurements of total length were recorded using photographs and ImageJ (Schneider et al. 2012) and a final patted dry mass was also taken.

Metamorphs were euthanized by immersion in a 250 mg L⁻¹ aqueous solution of benzocaine to eliminate the spread of disease associated with releasing metamorphs to the wild. After euthanization, specimens were immediately preserved in a 70% ethanol for possible future study.

Statistical Analyses

Using a series of three multivariate analyses (Chalcraft & Resetarits Jr. 2003) of variances (MANOVA) in RStudio (R Core Team 2013), we assessed the effects leaves of *L. maackii* had on different trophic levels among the mesocosms. The first MANOVA assessed treatment effects on survival and growth rates of metamorphosed *A. maculatum*. The second MANOVA included response variables of zooplankton and invertebrate densities, as well as densities of invertebrates separated into their appropriate functional groups (i.e., Shredders, Grazers, Collectors, Filterers and Predators) to determine how changes to salamander (mortality) impacted other organisms in mesocosms. The third MANOVA tested the six treatments against

chlorophyll a (as indicator of primary production), biofilm mass (as indicator of biofilm growth), rates of leaf litter mass loss, and soluble phosphate and nitrate measurements. In a separate analysis solely among *L. maackii* mesocosms, MANOVA was used to compare mass loss (g) and invertebrate densities between native leaf litter bags and *L. maackii* leaf litter bags. Both leaf litter bag types were taken from the same mesocosms that contained both *L. maackii* leaves and native leaves. Finally, an analysis of similarity (ANOSIM) of Bray-Curtis similarity measures was used to compare the relative structures of macroinvertebrate communities by functional groups among treatments (Marchant et al. 2000; Clarke et al. 2006)

III. Results

Larval *A. maculatum* were placed in mesocosms on 20 April 2018, and the experiment was terminated on 20 September 2018 at the last sampling event when no additional metamorphic *A. maculatum* could be recovered from mesocosms, and no remaining larvae were detected. Metamorphosis of larval *A. maculatum* began on 31 May 2018 and continued through 18 September 2018. Of the 20 mesocosms that contained larval *A. maculatum*, only 11 (55%) produced metamorphs, and only one mesocosm with leaf litter from *L. maackii* produced metamorphs. All 10 mesocosms containing *A. maculatum* larvae and native leaf litter produced metamorphs. A total of 117 metamorphs were retrieved from all mesocosms, and only three of those 117 (2.5%) were retrieved from mesocosms with leaf litter from *L. maackii*. *Anaxyrus americanus* tadpoles were placed in 20 assigned mesocosms on 27 April 2018. A single metamorphic *A. americanus* was found on a minnow trap during the first sampling event (5 June 2018), but there were never any tadpoles captured in minnow traps or other metamorphs found throughout the duration of the experiment. Tadpoles of *Anaxyrus americanus* may not have survived past the first week after introduction into mesocosms due to a large temperature shift of their surroundings, potentially leading to cold shock and subsequent mass mortality. Because only one surviving metamorph was retrieved, tadpole survival was not incorporated into the statistical analyses.

MANOVA indicated a significant overall effect of treatment on survival and growth rate (mm/day) of *A. maculatum* ($F_{3,16} = 4.3945$, $P < 0.01$). Subsequent one-way ANOVAs for

individual response variables showed survival ($F_{3,16} = 9.851$, $P < 0.001$) and growth rate ($F_{3,16} = 7.604$, $P < 0.01$) were both significantly influenced by treatment. A Tukey post-hoc test of survival showed significant differences between mesocosms containing *A. maculatum* with native leaf litter and mesocosms containing *A. maculatum* & *A. americanus* with native & *L. maackii* leaf litter, as well as between mesocosms containing *A. maculatum* with native leaf litter and mesocosms containing *A. maculatum* with native & *L. maackii* leaf litter (Figure 4). With all native mesocosm treatments combined and all *L. maackii* mesocosm treatments combined, survival of *A. maculatum* larvae in mesocosms containing *L. maackii* leaves (1%) was significantly reduced relative to mesocosms containing only native leaf litter (38%). Mesocosms containing *A. maculatum* & *A. americanus* with native leaf litter (Treatment 1) exhibited 28.7 % survival of *A. maculatum* and mesocosms containing *A. maculatum* with native leaf litter (Treatment 2) exhibited 47.3% survival of *A. maculatum* while mesocosms containing *A. maculatum* & *A. americanus* with native & *L. maackii* leaf litter (Treatment 4) exhibited 2% survival of *A. maculatum* and mesocosms containing *A. maculatum* with native & *L. maackii* leaf litter (Treatment 5) exhibited 0% survival of *A. maculatum* (Figure 4). The three sole metamorphic *A. maculatum* recovered from the *A. maculatum* & *A. americanus* with native & *L. maackii* leaf litter treatment (Treatment 4) was from the same single mesocosm.

A one-way ANOVA of growth rate ($F_{3,16} = 7.604$, $F < 0.01$) showed significant effects of treatment. A Tukey post-hoc test of salamander growth rate (mm/day) showed

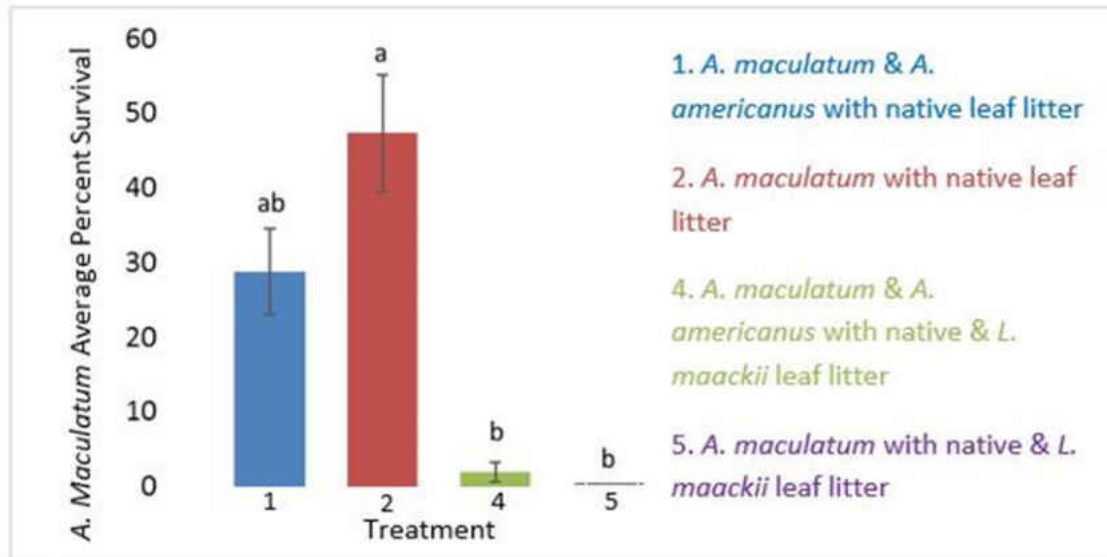


Figure 4. Survival (% ± S.D.) of *A. maculatum* metamorphs by mesocosm treatment

significant differences between mesocosms containing *A. maculatum* with native & *L. maackii* leaf litter (Treatment 5) and mesocosms containing *A. maculatum* & *A. americanus* with native leaf litter (Treatment 1), as well as between mesocosms containing *A. maculatum* with native leaf litter (Treatment 2) and mesocosms containing *A. maculatum* with native & *L. maackii* leaf litter (Treatment 5) litter (Figure 5), which appears to be driven by the fact that there were no larvae in mesocosms containing *A. maculatum* with native & *L. maackii* leaf litter. It should be mentioned that there were never any surviving metamorphic *A. maculatum* removed from mesocosms treated with native and *L. maackii* leaf litter, so there were never any final metamorph measurements from which to subtract the original hatchling measurements. This resulted in growth rates of 0 mm/day, leading to the significant differences between the first two mesocosms mentioned. Despite overall low sample

size in mesocosms with *L. maackii*, a trend towards reduced growth in *L. maackii* mesocosms was evident from the last two mesocosm treatments (mesocosms containing *A. maculatum* with native leaf litter and mesocosms containing *A. maculatum* & *A. americanus* with native & *L. maackii* leaf litter) mentioned, of which the *L. maackii* mesocosms had a total of three metamorphs removed.

MANOVA indicated no significant influence of treatment on zooplankton densities (Figure 6), macroinvertebrate densities (Figure 7) or invertebrate densities broken down into their separate functional groups (collectors (Figure 8), filterers (Figure 9), grazers (Figure 10), predators (Figure 11) and, shredders (Figure 12)) ($F_{1,28} = 1.34$, $P =$

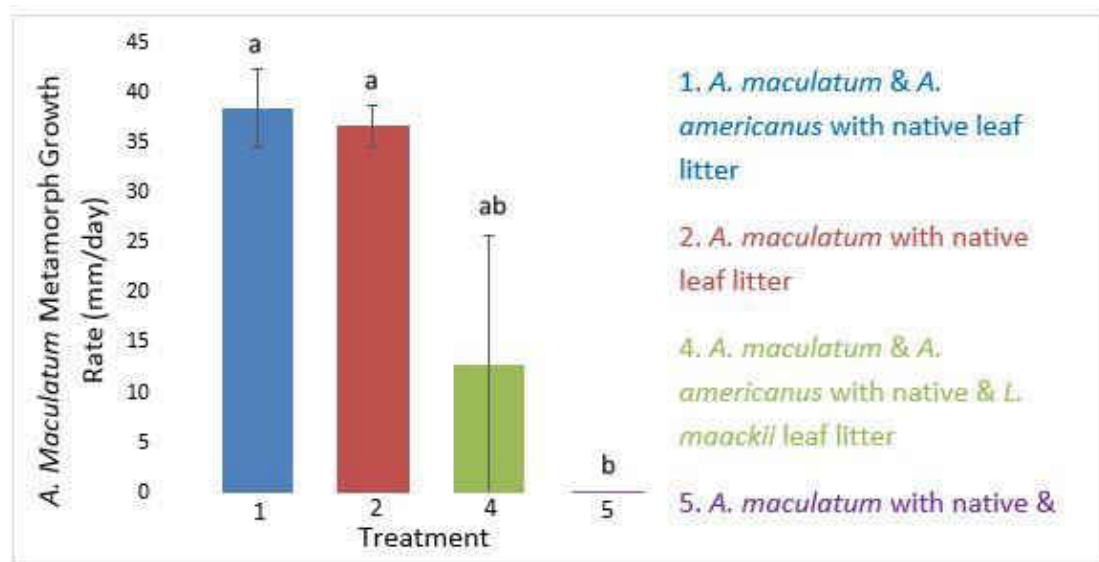


Figure 5. Average *A. maculatum* growth rate (mm/day \pm S.E.) among mesocosms in which treatments included *A. maculatum*.

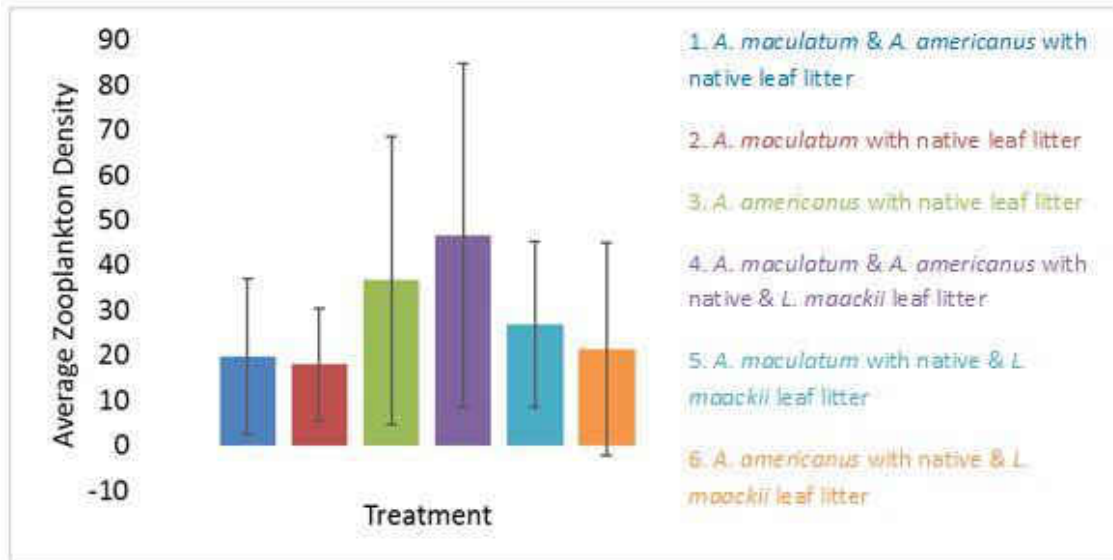


Figure 6. Average zooplankton density (\pm S.D.) taken from mesocosms on the 4th sampling event, 20 September 2018.

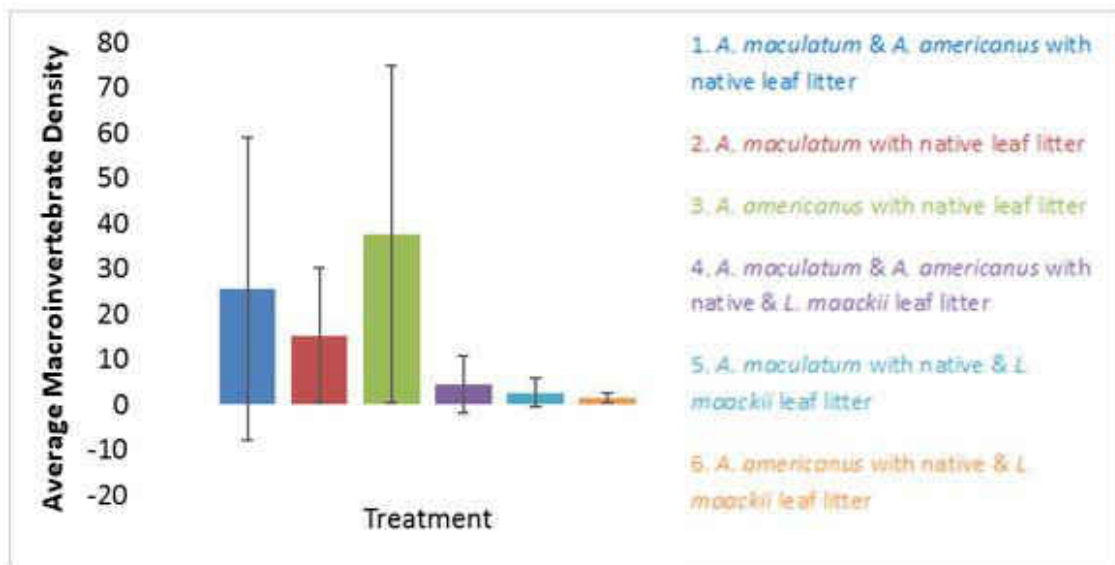


Figure 7. Average macroinvertebrate density (\pm S.D.) taken from mesocosms on the 4th sampling event, 20 September 2018.

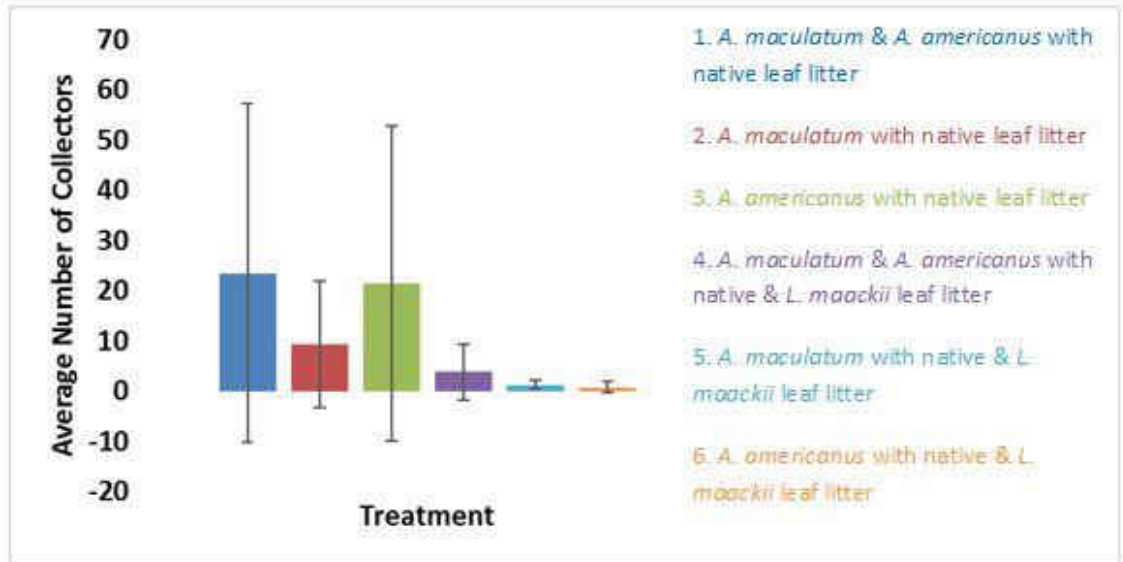


Figure 8. Average density of macroinvertebrate collectors (\pm S.D.) taken from mesocosms on the 4th sampling event, 20 September 2018.

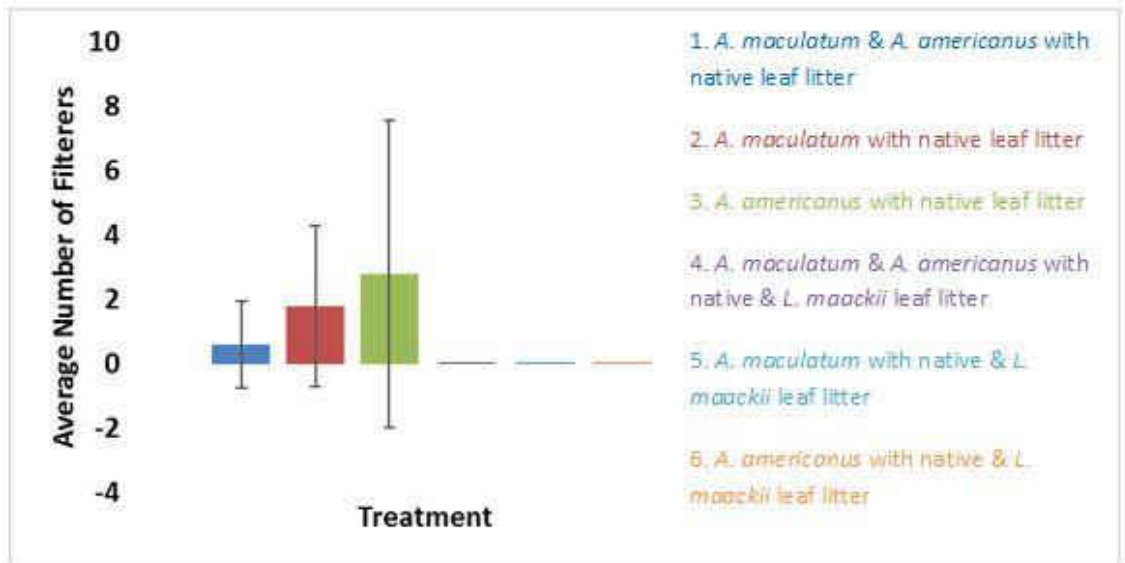


Figure 9. Average density of macroinvertebrate filterers (\pm S.D.) taken from mesocosms on the 4th sampling event, 20 September 2018.

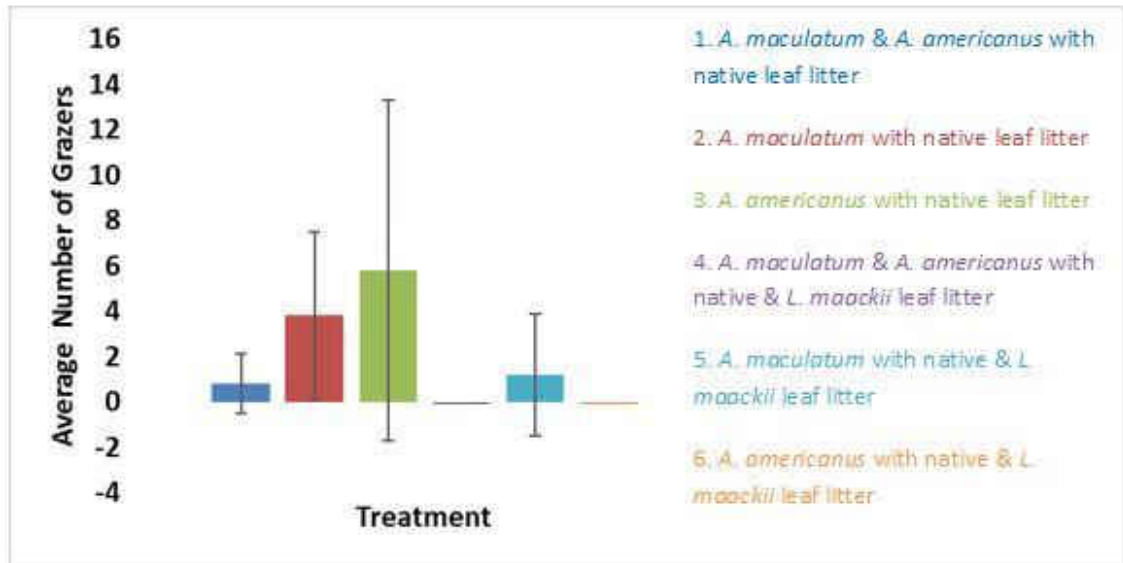


Figure 10. Average density of macroinvertebrate grazers (\pm S.D.) taken from mesocosms on the 4th sampling event, 20 September 2018.

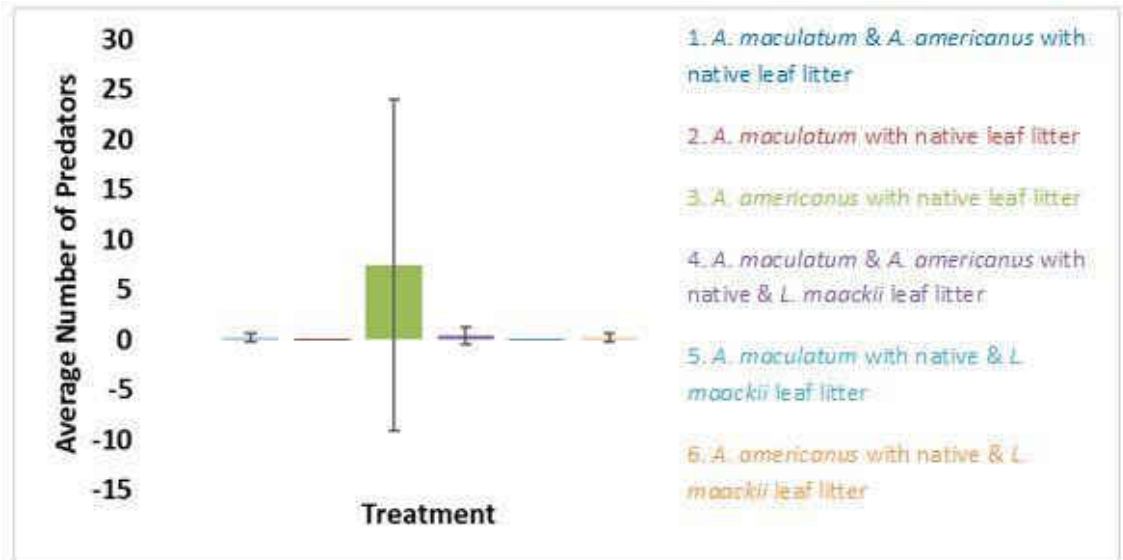


Figure 11. Average density of macroinvertebrate predators (\pm S.D.) taken from mesocosms on the 4th sampling event, 20 September 2018.

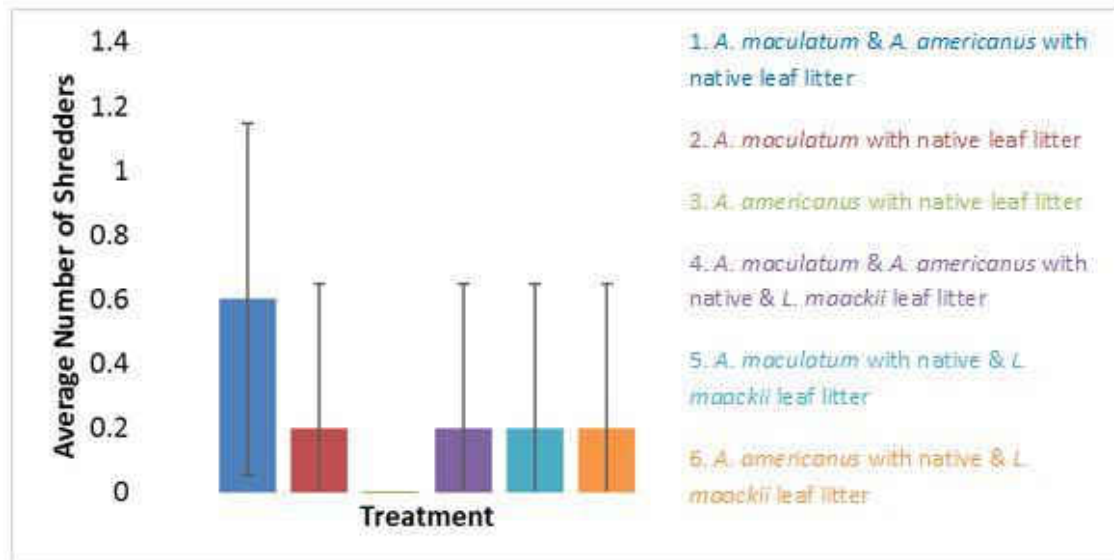


Figure 12. Average density of macroinvertebrate shredders (\pm S.D.) taken from mesocosms on the 4th sampling event, 20 September 2018.

0.281). An analysis of similarities (anosim) was used in the program R (Package: vegan) to compare the relative structures of macroinvertebrate communities by functional groups among treatments, (Figure 13), which also showed no significant treatment effect ($R = 0.1178$, $P = 0.079$).

Basic water chemistry data are presented in Table 1. I found no significant differences in chlorophyll a (Figure 14), periphyton mass (Figure 15), leaf litter mass loss, phosphate concentrations, or nitrate concentrations based on treatment (MANOVA $F_{1,26} = 1.69$, $P = 0.179$). All nitrate readings were almost zero, which led us to believe that mesocosms had not been established long enough prior to or during the experiment to allow release of such compounds to occur via invertebrate processing.

While leaves begin to break down about two weeks after introduction to an aquatic ecosystem (Benfield et al. 2017), most aquatic systems have different stages of leaf decomposition within them from leaf litter decomposition from previous years. My mesocosms only contained freshly fallen leaves which had not had time to be decomposed previously. Based on this hypothesis, the MANOVA model was run again excluding nitrate, though no significant effect of treatment on the remaining indicators of ecosystem function was observed ($F_{1,26} = 2.18$, $P = 0.12$).

Within mesocosms containing *L. maackii* (Treatments 3, 4 and 5), MANOVA indicated a significant overall treatment effect on leaf mass loss and

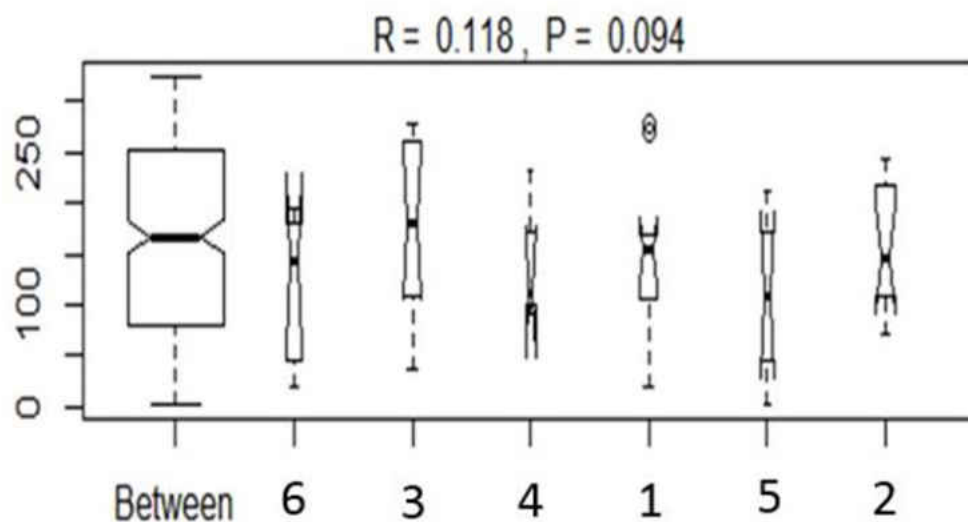


Figure 13. Comparison of similarity among invertebrate functional groups by treatment: 1) *A. maculatum* & *A. americanus* with native leaf litter, *A. maculatum* with native leaf litter, 3) *A. americanus* with native leaf litter, 4) *A. maculatum* & *A. americanus* with native & *L. maackii* leaf litter, 5) *A. maculatum* with native & *L. maackii* leaf litter, 6) *A. americanus* with native & *L. maackii* leaf litter.

Table 1. Average Dissolved Oxygen, Temperature, and pH obtained during the 4th and final sampling event (19-20 September 2018) for each treatment.

Treatment	Replicates	Dissolved Oxygen %	Temperature (C°)	pH
1. <i>A. maculatum</i> & <i>A. americanus</i> with Native leaf litter	5	46.48 ± 19.08	26.92 ± 0.18	7.368 ± 0.19
2. <i>A. maculatum</i> with Native leaf litter	5	59.06 ± 17.38	27.04 ± 0.48	7.706 ± 0.47
3. <i>A. americanus</i> with Native leaf litter	5	47.96 ± 8.53	26.64 ± 0.31	7.66 ± 0.76
4. <i>A. maculatum</i> & <i>A. americanus</i> with Native & <i>L. maackii</i> leaf litter	5	33.02 ± 28.53	26.44 ± 0.30	7.456 ± 0.52
5. <i>A. maculatum</i> with Native & <i>L. maackii</i> leaf litter	5	19.36 ± 4.01	27.02 ± 0.25	7.298 ± 0.10
6. <i>A. americanus</i> with Native and <i>L. maackii</i> leaf litter	5	21.72 ± 11.77	26.58 ± 0.35	7.34 ± 0.28

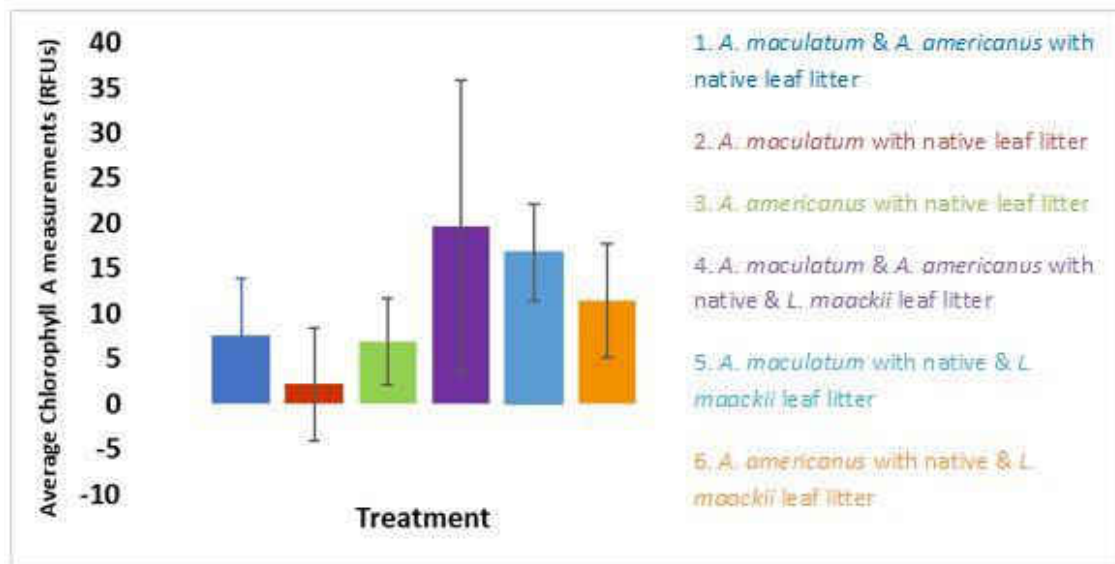


Figure 14. Average chlorophyll measurements (RFUs ± S.D.) taken from mesocosms during the 4th sampling event, 20 September 2018. RFU=Relative Fluorescence Units

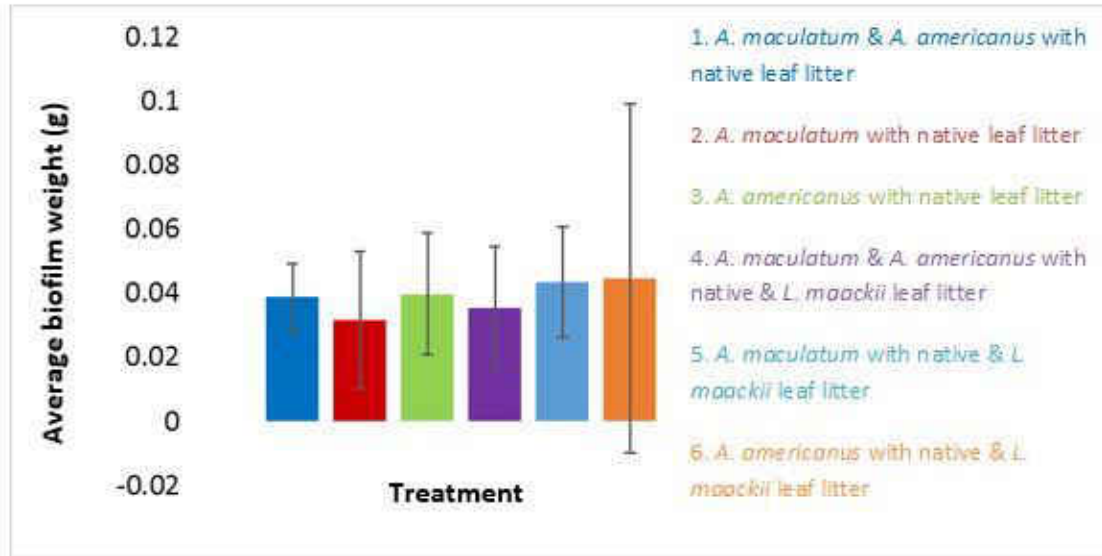


Figure 15. Average mass of biofilm on algal tiles (g; \pm S.D.) that were placed in mesocosms at the initiation of the experiment and removed on the 4th sampling event, 20 September 2018.

macroinvertebrate density between native leaf litter and *L. maackii* leaf litter ($F_{5,22}=4.5547$, $P<0.001$). Subsequent one-way ANOVAs for individual response variables showed mass loss was significantly greater in bags containing leaves of *L. maackii* relative to bags containing native leaves (Figure 16; $F_{5,22}=27.274$, $P<0.001$).

Invertebrate density was not significantly different between treatments ($F_{5,22} = 0.896$, $P = 0.501$). Although macroinvertebrate abundance was not significantly different among treatments, there was a greater density of invertebrates in the *L. maackii* leaf litter packs than in the native leaf litter packs (Figure 17).

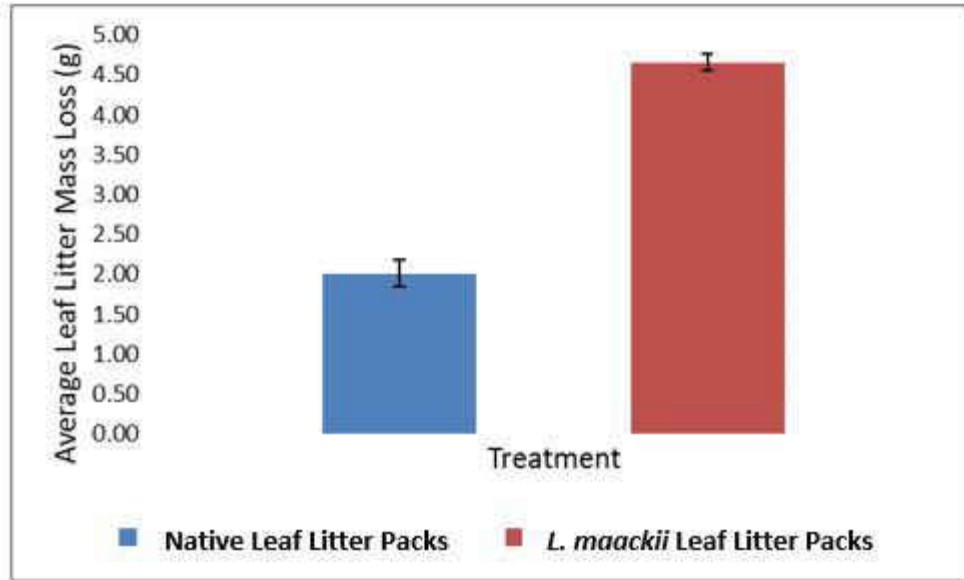


Figure 16. Average leaf litter mass loss (g; \pm S.D.) from native and *L. maackii* leaf litter packs placed in *L. maackii* treated mesocosms at the initiation of the experiment and removed on the 4th sampling event, 20 September 2018.

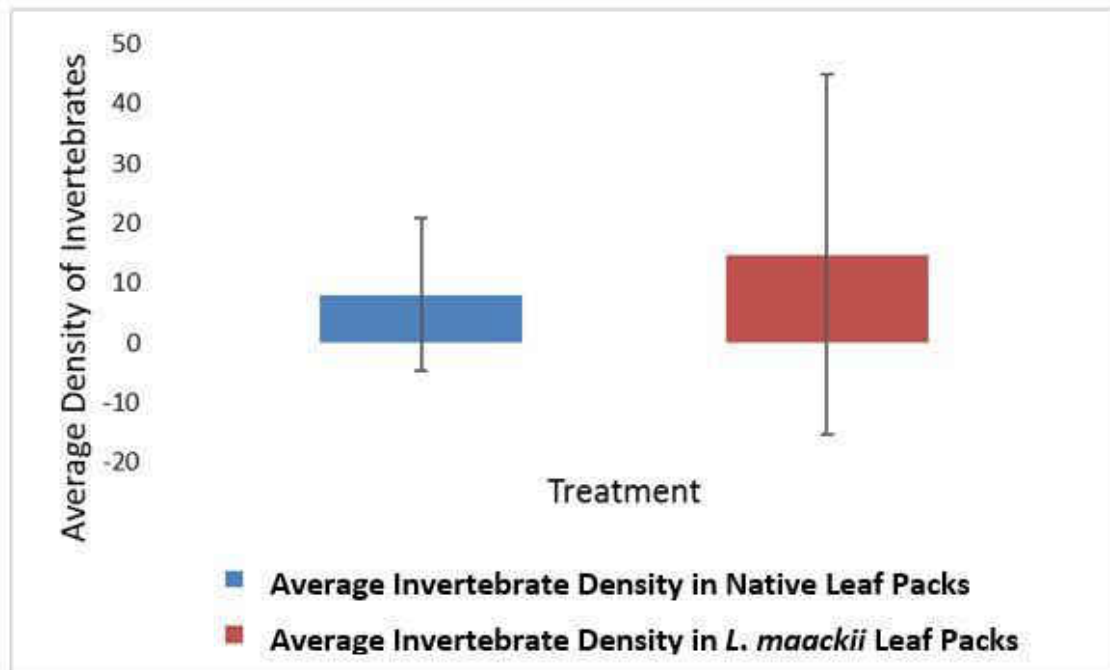


Figure 17. Average density of invertebrates (\pm S.D.) found in Native leaf packs vs. *L. maackii* leaf packs found in mesocosms containing Native and *L. maackii* leaf litter.

IV. Discussion

The results of my study demonstrate decreased survival and growth rates of larval *A. maculatum* when exposed to leaves of *L. maackii*. There was no indication that increased mortality of larval *A. maculatum* due to exposure to *L. maackii* affected lower trophic levels or our surrogate indicators of ecosystem functions. When comparing native and *L. maackii* leaf packs collected from the same mesocosm, significantly more mass loss occurred in packs containing *L. maackii* than those containing native leaf litter, though there were no significant differences in invertebrate densities between packs.

The effects of *L. maackii* on terrestrial ecosystems have been well-studied (Chen et al. 1999; Collier et al. 2002; Herrera 1997; McKinney and Goodell 2010); however, whether similar impacts occur in aquatic ecosystems is generally less clear (Weidenhamer and Callaway 2010; Watling et al. 2011c). Phenolic compounds in *L. maackii* are toxic to many species of flora and fauna in invaded habitats because the recency of co-occurrence has prevented adequate time for native species to evolve resistance to its phenolic compounds (Zedler and Kersher 2004). Negative behavioral effects on, and decreased survival of, some larval amphibians in response to *L. maackii* phenolic compounds has been observed in both controlled laboratory settings (Maerz et al. 2005, Watling et al. 2011b) and in field observations (Watling et al. 2011a,c). These studies clearly indicate negative direct effects of *L. maackii* on aquatic species, which supports a wider array of research demonstrating that phenolic compounds

produced by a variety of invasive plants directly reduce larval amphibian survival (Brown et al 2006; Cohen et al. 2012; Maerz et al. 2005; Martin and Blossey 2013). Leaves of native plant species, however, also release phenolic compounds (Cipollini et al. 2008, Watling et al. 2011a,c) and native species are the dominant contributor to leaf litter in ephemeral ponds, including ponds invaded by *L. maackii* (Watling et al. 2011a). Because both native and invasive plants contribute to the pool of phenolic compounds in aquatic ecosystems, holistic approaches to measuring their impacts must include both leaf types in experimental mesocosms to characterize their combined effects on amphibians. Moreover, leaves of native plant species often release phenolic compounds at even higher concentrations than invasive plants (Cipollini et al. 2008; Watling et al. 2011a), suggesting negative impacts on aquatic species are not mediated by the total concentration of all phenolic compounds, but rather by the identity of specific phenolic compounds found in invasive plants like *L. maackii* (Watling et al. 2011a,b). For example, apigenin and luteolin are phenolic compounds considered toxic to native flora and fauna, and such compounds occur in high densities in *L. maackii* but not at such densities in native plant species (Cipollini et al. 2008). In comparison to other aquatic stressors, we know little about the complex interactions and impacts phenolic compounds have on aquatic ecosystems and specifically on amphibians (Kerby et al. 2010); consequently, further study of novel phenolic compounds (Callaway and Ridenour 2004) and the roles they play in native ecosystems is needed.

A laboratory study (Watling et al. 2011b) found no differences in survival between larval *A. maculatum* in treatments with extracts from native plants versus extracts from *Lonicera spp.*, but tadpoles of *A. americanus* were over four times more likely to die upon exposure to extracts from *Lonicera maackii*. Similar amphibian laboratory and/or mesocosm studies have observed decreases in survival of *Anaxyrus* sp. (Cohen et al. 2012; Maerz et al. 2005; 2006) and *A. maculatum* (Martin and Blossey 2013) in response to exposure of phenolic compounds of other invasive plant species. My mesocosm experiment showed larval *A. maculatum* were almost 40 times more likely to die upon exposure to *L. maackii*, and this result demonstrated a more realistic assessment of the potential impacts of *L. maackii* on ephemeral pond ecosystems by incorporating: a) exposure to a combination of phenolic compounds from native leaves and leaves of *L. maackii*; and b) complex ecological interactions occurring within aquatic communities that could exacerbate the effects of invasive phenolic compounds (Abhilasha et al. 2008; Watling et al. 2011b). Stressors causing high mortality rates and changes in developmental rates already exist in ephemeral ponds and may have impacted my experimental mesocosms. For example, high larval densities (Petranka 1989) and invertebrate densities resulting in more intense competition and cannibalism place pressure on larval *A. maculatum*. In previous studies, other natural and anthropogenic stressors such as anthropogenic chemicals (Boone et al. 2005) and disease (Parris and Cornelius 2004) reduced amphibian survival rates, and the additive or greater-than-additive effects of these stressors when

combined with phenolic compounds associated with *L. maackii*, may heighten already high rates of larval mortality (Boone et al. 2007).

In addition to its effects on larval survival, *Lonicera maackii* exerted a direct negative impact on growth rates of larval *A. maculatum*. To my knowledge, previous studies have not attempted to calculate growth rate changes in salamanders caused by *L. maackii*, but laboratory studies have concluded that exposure to *L. maackii* may accelerate development (time to metamorphosis) in other amphibians (Watling et al. 2011a). Reduced growth rates in larval *A. maculatum* exposed to *L. maackii* in my study would cause larvae to remain in mesocosms longer than larvae only exposed to native leaf litter. Larvae remaining in ephemeral ponds longer at smaller body sizes experience increased associated risk of desiccation due to pond drying prior to metamorphosing (Rowe and Dunson 1995) and macroinvertebrate predation (Formanowicz, Jr. and Brodie, Jr. 1982), the latter of which may not be affected directly by exposure to *L. maackii*. Previous studies have found amphibians are forced to metamorphose more quickly due to environmental pressures like competition (Barnet and Richardson 2002; Resetarits et al. 2004), predation risk (Skelly and Warner 1990; Wilbur and Fauth 1990), food availability (Niecieza 2000), shorter hydroperiod (Crump 1989; Hom 1987; Newman 1992; Wilbur 1987), chemical exposure (Boone et al. 2001; Cauble and Wagner 2005), and in the presence of *L. maackii* (Watling et al. 2011a). Larvae who metamorphose early due to these stressors do so at smaller body sizes, and smaller sizes at metamorphosis may decrease fitness by decreasing the

chances of survival and reproduction in the terrestrial environment (Berven 1990; Semlitsch et al. 1988). These patterns of salamander size at metamorphosis and timing of metamorphosis in my mesocosms may be due to complex ecological interactions or random effects, as only three larval *A. maculatum* metamorphosed from one of ten mesocosms in which they were exposed to *L. maackii*. This small sample size may not provide accurate estimates of salamander growth and timing of metamorphosis in mesocosms containing *L. maackii*, and additional studies using sublethal concentration of extracts from *L. maackii* would be useful in determining its effect on larval salamander development.

Reproductively active female amphibians can assess the suitability of habitats for oviposition and/or larval survival based on factors such as hydroperiod (Egan and Paton 2004), pesticide exposure (Gertzog et al. 2010), predation risk (Blaustein et al. 2004) and, optimum temperature (Seale 1982). Following previous observations of altered amphibian community composition following non-native plant invasion (Watling et al. 2011a,b,c; Marez et al. 2005), female amphibians may reduce or avoid oviposition in ephemeral ponds invaded by *L. maackii* due to chemosensory or other cues regarding habitat quality. Qualitatively, I observed an oily sheen on the water surface and a foul smell that was only associated with mesocosms containing *L. maackii*. These sensory cues might be detected by ovipositing female salamanders. If female salamanders are unable to assess aquatic habitat quality in ephemeral ponds containing invasive plants, exposure to metabolites produced by invasive plants can

significantly decrease embryo mortality and cause embryonic malformation (Sacerdote and King 2014) even prior to hatching.

Although my results indicate that metamorphosis of *A. maculatum* is unlikely in ponds containing leaf litter from *L. maackii*, previous studies have identified subsequent effects of invasive plants on emerging metamorphs in adjacent terrestrial habitats. Metamorphic amphibians emerging into terrestrial habitats containing invasive plant species may display decreased developmental rates (Brown et al. 2006; Martin and Blossey 2013), reduced foraging performance (Brown et al. 2006), deformities (Sacerdote and King 2014) and decreased reproductive fitness (Berven 1990; Semlitsch et al. 1988). At community scales, terrestrial field studies have documented reduced adult amphibian species evenness, richness and composition due to changes in temperature and humidity caused by dense *L. maackii* shrubs (Watling et al. 2011c). When these changes occur, entire populations of amphibians can be altered, and it is hypothesized to inevitably lead to the loss of amphibian species (Martin and Blossey 2013).

Effects of *L. maackii* on the survival of *A. americanus* cannot be determined from this experiment due to collecting only one metamorph throughout the experiment, paired with no observations of tadpoles a week after mesocosm introduction. When exposed to extracts from *Lonicera*, *Anaxyrus americanus* are exponentially more likely to die than individuals exposed to native extracts (Maerz et al. 2005; Watling et al. 2011b)

however, it is unclear why all *A. americanus*, regardless of treatment, did not survive in my experiment. Mortality of *A. americanus*, regardless of treatment, could have been due to a temperature shock upon introduction to mesocosms. Tadpoles were collected from a shallow puddle on a warm spring day and placed relatively soon after into cooler and deeper water in experimental mesocosms. The loss of tadpoles posed a problem for the project's experimental design, as tadpoles were anticipated to function as the dominant herbivores, and their loss represented the loss of a trophic level (i.e., vertebrate primary consumers) in relevant experimental mesocosms. Since tadpoles were primary consumers in the mesocosms, their loss would have released predation pressure on primary productivity, thereby increasing algal densities, rates of primary production, or both. Based on other studies, *L. maackii* may have exerted direct negative impacts on survival of tadpoles of *A. americanus* (Watling et al. 2011a,b) similar to effects on survival of larval *A. maculatum*, resulting in complete loss of amphibians exposed to leaves of *L. maackii*.

Changes in salamander mortality did not impact lower trophic levels within mesocosms, indicating there were no trophic cascading effects due to substantial losses of larval *A. maculatum* in mesocosms containing *L. maackii*. Previous field studies report ambystomatid salamander predation may not influence overall prey densities when prey are abundant (Van Buskirk and Smith 1991), and thus the loss of larval *A. maculatum* in mesocosms may have little indirect effect on zooplankton or even macroinvertebrate prey. My results support a broad array of research indicating

a lack of connectivity between predator and prey densities in some systems (e.g. Brodie and Giordano 2013; Mehner 2010; Mikola and Setälä 1998), wherein the loss of predators has no consequences for lower trophic groups. Trophic cascades may be dampened or eliminated due to several ecological factors, such as the presence of mid-level omnivores that can switch prey items when preferred prey items are eliminated (Pace et al. 1999; Stein et al. 1995), as well as effects of species diversity and species replacement (Pace et al. 1999). Experimental mesocosms may also have been mediated by “bottom-up” processes, and thus intermediate trophic levels in this system (i.e., invertebrates) would not be regulated by predation by larval salamanders but rather by primary producers (Crutsinger et al. 2006) which are regulated by nutrient availability. Support for bottom-up regulation in my mesocosms comes from high invertebrate abundances, which buffer overall predatory effects of salamanders (Holomuzki et al. 1994; Strong 1992), and high invertebrate diversity within mesocosms, in which no single species has a larger trophic influence than others (Strong 1992). Meta-analytical approaches have indicated that predator-prey ratios range from 0.24 in species-poor communities to 0.46 in the most species-rich communities (Warren and Gaston 1992). In contrast, mesocosms with native leaves in my experiment (i.e., where apex predators were present) exhibited predator-zooplankton prey ratios of <0.0001 , indicating that salamander predators were likely not limited by prey availability and that prey abundance buffered the predatory effects of larval salamanders on trophic cascades. Greater invertebrate diversity and density may be indicative of bottom up regulation due to their dependence on primary

producers as a main food source. While this study assumed indirect effects of *L. maackii* on macroinvertebrates and zooplankters via effects on larval salamanders, general similarities in invertebrate densities between all mesocosms indicate that *L. maackii* did not directly impact survival of macroinvertebrates or zooplankters because there was no indication of increased invertebrate mortality among treatments.

Previous studies have found mixed results on the effects of invasive plants on invertebrates (Palmer et al. 2004; Stiers et al. 2011; Tallamy 2004), and a laboratory microcosm study of only leaf litter and invertebrates could confirm these findings

Increased leaf mass loss occurred in leaf packs containing *L. maackii* compared to leaf packs containing native leaf litter that were both removed from the same mesocosm.

Leaf mass loss primarily occurs due to processing by macroinvertebrates shredders, but we found no difference shredder densities within the two types of leaf packs.

Thus, greater mass loss in leaf litter packs containing *L. maackii* was not due to macroinvertebrate processing, but more likely due to inherent differences in leaf textures and decomposition rates. Previous studies have documented faster rates of aquatic decomposition of *L. maackii* compared to native leaves (Lewis and Brown 2010; McNeish et al. 2012; Fargen et al. 2015), as well as higher nitrogen and lower lignin content in *L. maackii* than some native leaves, conditions which increases rates of decay (Fargen et al. 2015; Trammell et al. 2012) even if macro- and micro-invertebrate densities were not affected.

While overall benthic macroinvertebrate and zooplankton densities were not impacted by *L. maackii*, there were substantially more mosquito larvae and adults recorded in all mesocosms containing *L. maackii* than mesocosms containing only native leaf litter (Figure 18). Previous field studies have assessed mosquito oviposition in forests invaded with *L. maackii* and found that increased densities of *L. maackii* negatively influence mosquito oviposition (Conley et al. 2011), which contrasts with my observations of *L. maackii* seemingly attracting mosquito oviposition. My results did not align well with Conley et al. (2011) likely due to our differing environmental conditions (Open field vs. dense forest). Mosquitoes are known vectors of many diseases that affect humans and wildlife (Apperson et al. 2004; Farajollahi et al. 2011), indicating *L. maackii* may have an indirect effect on human and wildlife health. Additional studies on the relationship between honeysuckle invasion, mosquito colonization, and prevalence of mosquito-borne disease are needed to better predict such unanticipated consequences of plant invasions.

There were no significant changes in our surrogate variables associated with ecosystem functions among treatments. Mortality of larval *A. maculatum* did not influence invertebrate densities, and therefore it is expected that chlorophyll a, biofilm mass, leaf litter mass loss, and soluble nutrient availability would also not be altered. Trophic cascades are only likely to occur when there is a keystone predator present and low species diversity (Strong 1992) throughout the community because the loss of keystone predators is often what drives top-down trophic effects (Paine 1980, Strong

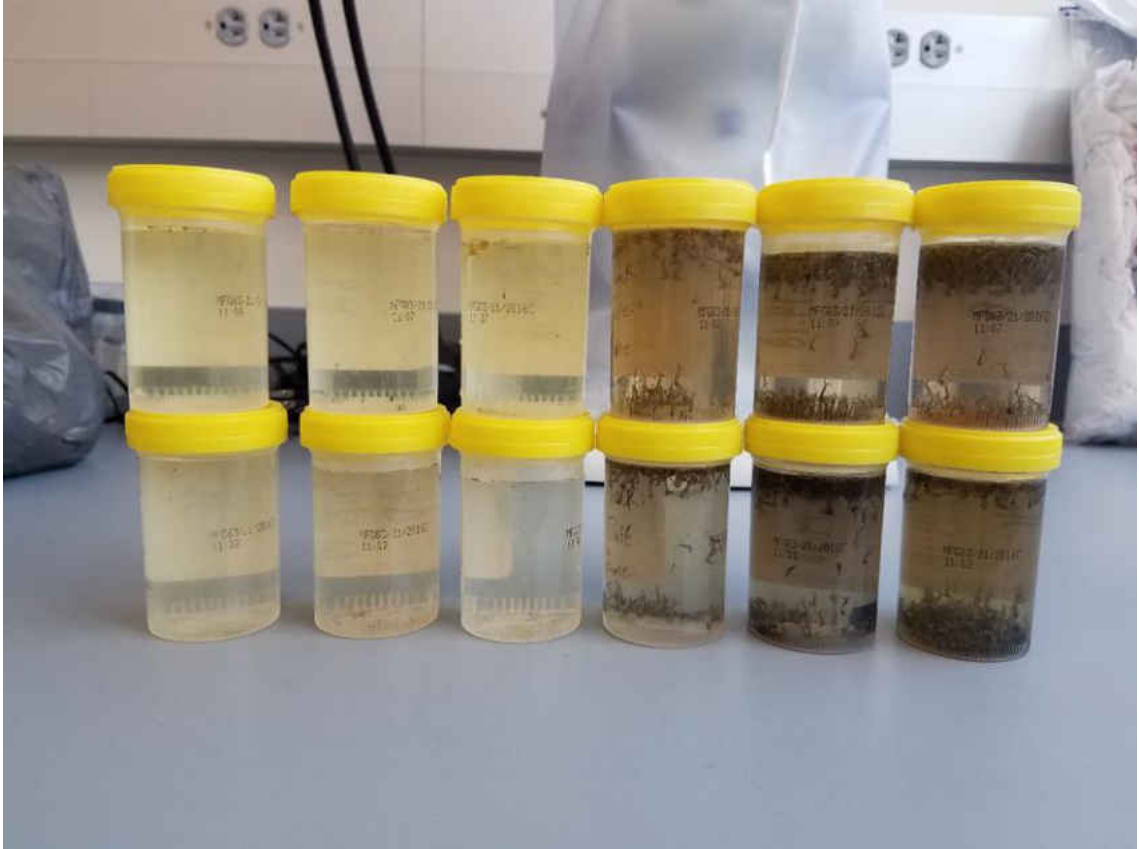


Figure 18. Qualitative differences in mosquito densities between mesocosms with native leaf litter (six specimen cups on left) and mesocosms containing native and *L. maackii* leaf litter (six specimen cups on right). The dark coloration in specimen cups to the right are masses of individual mosquito larvae.

1992). Without such conditions, trophic cascades do not always occur as a result of changes in predator densities within many ecosystems (e.g. Brodie and Giordano 2013; Mehner 2010; Mikola and Setälä 1998). High invertebrate densities most likely served as a buffer in my mesocosms, preventing changes in predator densities from trickling down the trophic web to the lowest level of ecosystem function. Whether it be because of high invertebrate densities or because of bottom-up regulation, the lack of a trophic cascading event showed a strong stability of the ecosystem within the

mesocosms (McCann and Hastings 1997; McCann et al. 1998). It is also possible that this experiment did not allow adequate time for *L. maackii* to fully affect the mesocosms. Effects of invasive species may take years to develop, and restricting experimentation to a single season may not adequately mimic natural invasion processes (Brown et al. 2006).

Lonicera maackii is not only a threat to terrestrial flora and fauna in the Eastern United States, but also to aquatic species, communities, and ecosystems. Although the presence of *L. maackii* did not produce the predicted trophic cascading effects, it still exerted a major direct impact on the apex predator in this system, larval *A. maculatum*. This research has laid the ground work for subsequent studies that will contribute to a more comprehensive understanding of the effects of invasive plants on aquatic landscapes. Currently, 40% of all known amphibian species are threatened with extinction (IUCN 2019), with large numbers of additional data-deficient and/or declining species. Therefore, understanding the impacts invasive plants play in amphibian larval development will be critical in developing a holistic approach to understanding and managing aquatic ecosystem functional consequences of species invasions.

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