Influence of land use change on amphibian species assemblages and larval development

by

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Abstract

Land use alterations can have significant impacts on wetland ecosystems. One of the most notable impacts is altered flood regimes which can influence organisms dependent upon those waters. Amphibians, with their recent dramatic declines, are among those influenced by land use alterations. This study combined field and laboratory techniques to elucidate some of the factors influencing amphibian occurrence and survival in altered wetlands. The field component consisted of active searches and automated recording devices on fifteen headwater slope wetlands in southern Alabama. More rare and sensitive species were detected in wetlands with more forest in the surrounding area. Several species were positively associated with agricultural land use and one species (*Eleutherodactylus planirostris*) was positively associated with impervious surface area. A mesocosm experiment was conducted to examine the effects of altered flooding regimes on tadpole development in four different species. A species adapted to habitats with minimal water level fluctuations (*Lithobates sphenocephalus*) had higher survival in the control treatment and the lowest survival in the flashy, urban treatment. *Hyla chrysoscelis* had larger body sizes in a gradual flooding treatment than in the control or urban treatment.

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Chapter 1: THE EFFECT OF LAND USE CHANGE ON AMPHIBIAN SPECIES ASSEMBLAGE AND LARVAL DEVELOPMENT

In recent decades it has become apparent that amphibian populations are declining on a global scale (Alford and Richards 1999). At the same time the human population is rapidly increasing. Estimates predict that the world population will increase by 2.3 billion within the next forty years, and since more than half the world population already lives in urban areas, it is expected that these areas will see most of this growth (U.N. 2011). As amphibians are known to be affected by various human activities, it is imperative to assess exactly how land use may change their populations and to determine ways to mitigate any negative effects. In 2011, this study began to investigate some of the potential ways that land use change may impact amphibians in headwater slope wetlands in southern Alabama.

Wetlands are extremely valuable ecosystems worldwide although their value has only recently been recognized. During early European settlement of North America, wetlands were frequently regarded as useless land and filled or drained for other purposes, most often agriculture (Mitsch and Gosselink 2007). Estimates have shown that approximately 118 million acres of original U.S. wetlands have been lost since this time (Dahl and Allord 2004). Technologies emerged for more efficient drainage and were advocated by the government until the 1970s when a shift in the understanding of the value of wetlands started to occur. Policy reversal has led to more incentives to protect and restore wetlands as well as understand their current status and ecological functions (Dahl and Allord 2004). Recent estimates have determined that there are approximately 110.1 million acres of wetlands in the United States. Ninety-five percent of these wetlands are freshwater and about half of those are forested wetlands (Dahl 2009). Wetlands have many well-recognized beneficial functions, including water quality improvement, flood control, recreation, and wildlife habitat (Mitsch and Gosselink 2007).

Issues still exist when protecting wetlands as focus tends to be on the flooded portion of the wetland, while disregarding transitional or isolated wetlands and critical adjacent upland habitat. Many animals have diverse life histories that require multiple habitat types (Gibbons 2003). Wetlands provide habitat and breeding locations for a diversity of plant and animal species. For example, many reptiles are semi- or fully aquatic and use wetland habitat for foraging, breeding, and cover (Gibbons et al. 2000), and many birds, especially waterbirds, use wetlands for reproduction, foraging, and as migration corridors (Haig et al. 1998). A number of amphibians use water for reproduction and have biphasic life cycles requiring an aquatic larval stage prior to an adult terrestrial stage (Wilbur 1980). All of these taxa are dependent on various wetland habitats.

Amphibians are an integral link between the ecosystems they occupy. They provide a remarkable source of biomass (Burton and Likens 1975, Gibbons et al. 2006) and are a means of moving energy between aquatic and terrestrial habitats. However, amphibian populations are declining worldwide (Houlahan et al. 2000; Alford and Richards 1999; Stuart et al. 2004). The causes of such declines are most likely from a combination of factors (Alford and Richards 1999; Davidson et al. 2002; Sodhi et al. 2008). Habitat destruction and modification leads to less area available for critical life functions and has been shown lower species richness and abundance (Lehtinen et al. 1999). Diseases, such as chytridiomycosis, occur globally and can devastate populations within short time periods even in relatively pristine, protected habitats (Berger et al. 1998, Rosenblum et al. 2010). Pollution decreases immune system functions and can cause death or deformities in amphibians (Carey and Bryant 1995). Climate change could affect survival and reproduction by altering local precipitation, temperatures, and pond hydroperiods, affecting microhabitats, ranges, and the timing and success of breeding (Donnelly and Crump 1998). Increased ultraviolet radiation also affects certain species and can magnify the effects of other stressors such as fungi (Blaustein et al. 1994, Kiesecker and Blaustein 1995). Understanding human effects on habitats such as wetlands could help address some of these issues related to habitat loss and modification.

Amphibian populations and health can often be considered an indicator of ecosystem health. They are closely connected to their habitats and often require specific conditions to thrive, such as a particular microclimate (Welsh and Droege 2001). Having semi-permeable skin and requiring aquatic larval stages as well increases the threats of pollution. Howe et al. (2009) showed that American toads and northern leopard frogs in larval stages were more affected by concentrations of common herbicides in agricultural fields than two fish species (channel catfish and rainbow trout). This is important because water quality regulations are often based on fish communities and suggests that amphibian larvae could serve as a better indicator of environmental changes. In comparison with other groups of animals too, concentrations of the common herbicide Roundup that were nontoxic to birds and mammals and had variable effects on fish and invertebrates were found to be moderately to highly toxic among six species of amphibian larvae (Relyea 2004).

The importance of assessing threats at the landscape context is a well-recognized issue in conservation planning (Guerry and Hunter 2002, Lehtinen et al. 1999, Simon et al. 2009). Animal communities respond to shifts in land use, which can often be seen through changes in species richness or abundance (Delis et al. 1996). Increases in the amount of habitat converted to urban or agricultural land use are often associated with declines in amphibian species (Davidson et al. 2002, Houlahan and Findlay 2003). Land manipulation, especially practices that clear land or drain shallow water bodies, has a drastic effect on amphibian reproduction and survival as both aquatic and terrestrial sites are necessary for a population's survival (Trauth et al. 2006, Semlitsch and Bodie 2003). Changes associated with urbanizing landscapes that may impact aquatic organisms include 1) an increase in the number of roads or paved surfaces, 2) an increase in artificial ponds such as stormwater retention ponds, 3) increased levels of sediments and pollutants in aquatic habitats, 4) increased contaminants associated with roads, such as heavy metals and salt, 5) increased nutrient levels, 6) destruction and fragmentation of uplands surrounding a wetland, and 7) altered hydrologic regimes within the wetland. Each of these factors is detailed below.

Roads

Roads can have numerous ecological effects on different taxa. For plant communities, road corridors are conducive to herbaceous species that thrive on the increased light and moisture from roads. Roadsides are frequently managed and mowed which favors disturbance-tolerant and invasive species and can result in higher species richness (Forman and Alexander 1998). Roads also fragment habitat and create movement barriers for many animal species. Noise and traffic densities affect animal behavior and avoidance (Forman and Alexander 1998). In addition, they increase the risk of mortality, especially for animals that migrate in mass during specific times of the year, such as many amphibian species (Glista et al. 2007). Glista et al. (2007) found that of 10,515 animals found as road-kill along 12 km of survey routes, 9,809 of them were amphibians. In compiling data from surveys of wetland complexes in Ontario, Canada, Findlay and Houlahan (1997) showed that species richness for reptile and amphibian species was negatively correlated with the density of paved roads within 2 km of the wetland. Overall, road edges have been shown to be an obstruction for moving amphibians though there could also be behavioral modifications in addition to the elevated mortality (Gibbs 1998a). Road networks also alter the hydrology of an area. The increase in impervious surfaces leads to less infiltration of precipitation and more surface flow of water after storm events (Walsh et al. 2005; Forman and Alexander 1998). Hydrographs of urban streams and wetlands show more frequent, flashier floods (Walsh et al. 2005). High flow events have been shown to wash salamander larvae from streams. In lab experiments, two-lined salamander larvae were flushed from streams at velocities between 0.2 and 0.6 m/s (Barrett et al. 2010). An increase in flashy flood events can lead to decreased larval survival in these streams. In addition, roads alter the way water flows, changing drainage patterns (Montgomery 1994) and concentrating it along the road, creating ditches (Forman and Alexander 1998). Ditching alters drainage patterns as well and lowers water tables which can exacerbate already dry conditions during droughts, leading to lower breeding success for amphibians (Babbitt and Tanner 2000).

Stormwater ponds

Stormwater retention ponds are made to slow the delivery of runoff and decrease the amount of pollutants such as oils, salts, sediment, and fertilizers that reach streams. Aquatic vegetation and standing water can attract breeding amphibians but potentially expose eggs and larvae to the pollutants in these ponds (Brand and Snodgrass 2010). Species' tolerances towards pollutants are variable. Survival of *Anaxyrus americanus* eggs and larvae was much greater than *Lithobates sylvatica* when exposed to sediment from stormwater ponds because of their relative sensitivities to contaminants. The metamorphosis rate of the toads was approximately 85%, while the wood frogs had 100% mortality within two weeks of exposure (Snodgrass et al. 2008). In a similar study, gray treefrog (*Hyla veriscolor)* larvae exhibited a 50% mortality rate when exposed to sediment from a stormwater pond known for high levels of metals and road salt (Brand et al. 2010). These experiments demonstrate the varying rates at which different species may be affected by contaminants. Although these ponds are not pristine habitats, they may be all that is available in suburban environments where land changes have replaced natural wetlands (Brand and Snodgrass 2010). Some frogs, such as *Lithobates sphenocephalus* and *L. grylio*, are known to utilize man-made ponds in addition to natural areas (Wilson and Porras 1983). In a study along a major highway in France, Scher and Thièry (2005) found only generalist, opportunistic amphibian species living in stormwater ponds, suggesting that only these species are capable of using these habitats.

Sedimentation/pollutants

Land conversion can lead to changes in the structure of streams and wetlands through erosion and sediment deposition. Construction and development expose soils and increase the rate at which sediment is carried away by runoff and deposited in stream channels (Paul and Meyer 2001). This can raise stream beds and fill wetlands, altering or destroying wildlife habitat (Ribaudo et al. 1999). These increased, high-intensity flows result in more flooding and more sediment deposition on the stream banks (Paul and Meyer 2001) which can impact aquatic habitats. Welsh and Ollivier (1998) found that three species of stream-dwelling amphibians (*Ascaphus truei, Dicamptodon tenebrosus, Rhyacotriton variegatus*) in Prairie Creek State Park in California were negatively affected by a combination of road development and a strong storm event that resulted in mass wasting and a large amount of soil erosion. They surmised the causes for declines were sensitivity to fine sediments, the filling of microhabitats, and reduced periphyton availability because of reduced growth or scouring by the sediment (Welsh and Ollivier 1998). When analyzing differences in sediment loads, agricultural and urban land use influenced streams in North Carolina had higher amounts of silt and sand (70%) while forested watersheds generally had larger substrate particle sizes and only 40% sand. Excess sediment fills in microhabitats such as spots under rocks or woody debris and can reduce the diversity of the habitat (Lenat and Crawford 1994). Some stream breeding species require flowing water or riffle habitats for reproduction and could be negatively impacted by the filling in of those places (Lind et al. 1996).

Since no single factor can be pinpointed as the cause of global amphibian declines, it has been suggested that multiple stressors are interacting to exacerbate any one cause (Boone et al. 2007). Pollutants and other anthropogenic changes may intensify the effects of various diseases and parasites that amphibians are normally resistant to. For example, Lefcort et al. (1997) found that salamanders exposed to silt were more susceptible than those in treatments without silt to the effects of a parasitic water mold (*Saprolegnia parasitica*) that was unintentionally introduced. Reylea (2004) found that in wood frogs (*Lithobates sylvatica*) the introduction of chemical cues from a predator greatly increased the mortality rate of tadpoles exposed to otherwise nonlethal concentrations of a common herbicide. In a test of the effects of different combinations of pesticides, Hayes et al. (2006) found that immune function was suppressed in tadpoles exposed to mixtures of low concentrations of common pesticides, which led to individuals being vulnerable to bacteria that caused deformities and death. Frogs also showed reduced growth and increased time to metamorphosis when exposed to the chemicals (Hayes et al. 2006).

Another negative effect of urban and agricultural land uses is an increase the amounts of pollutants present in a system. Taylor et al. (2005) found that tadpoles in wetlands closer to lawns and agricultural fields were more likely to have limb deformities due to chemical runoff than those in undisturbed sites, with those near agriculture having almost double the risk. In addition, exposure to pesticides has been shown to decrease the activity level of tadpoles, which results in less time spent foraging (Bridges and Semlitsch 2000). In using different combinations of sublethal levels of an insecticide, herbicide, and fertilizer to mesocosms of gray treefrogs (*Hyla versicolor*), Boone and Bridges-Britton (2009) found that the combinations resulted in higher survival of the tadpoles than any single chemical alone. The authors suggest that chemical interactions and changes in the food web complicate the analysis of the effects of contaminants. Exposure to chemicals can have different effects based on the life stage at which the animal is exposed. Embryos in water contaminated with atrazine, a common herbicide, took longer to hatch but larval salamanders metamorphosed earlier, possibly to escape the effects of the herbicide (Rohr et al. 2004). Water isn't the only medium that moves pollutants. Davidson et al. (2002) implicated wind-borne chemicals were negatively affecting the populations of four species of ranid frogs in California. The increased application of chemicals for pest control may have various effects on amphibians that are not fully predictable at this point.

Heavy metals

Toxins associated with roads are another concern with development. The amount of heavy metals found in systems increases with urbanization (Feng et al. 2004, Singh et al. 1997). Sources include industrial activities, vehicle use, and atmospheric deposition (Li et al. 2001). These metals tend to concentrate along roadsides and can be carried as runoff or assimilated by plants and animals (Forman and Alexander 1998). The presence of heavy metals in the water has been shown to affect amphibians, particularly in the larval state when they are in constant contact with water. Spotted frog (*Lithobates luteiventris*) tadpoles exposed to zinc, cadmium, lead, and soil from a contaminated site had reduced survival and growth rates when raised in tanks with these pollutants compared to those raised in clean water (Lefcort et al. 1998). Behavioral cues were altered as well. Tadpoles in contaminated water had decreased fright responses to the chemical cues of rainbow trout, spending more time in open water and altering their movements (Lefcort et al. 1998). In comparing the concentrations of heavy metals in sediment and in tadpoles collected from stormwater retention ponds, Casey et al. (2005) found that tadpoles showed signs of bioconcentration of the metals in their heads and intestines. Although concentrations in water and sediment tests may not be at levels to raise concern, evidence exists that these contaminants accumulate in the animals (Casey et al. 2005). Areas with more impervious surface and road traffic also have higher amounts of oils and greases in stormwater runoff. Fuels and motor oil from cars are significant sources and can be toxic to aquatic organisms (Khan et al. 2006). For instance, used motor oil in the water led to decreased growth rates and faster metamorphosis of mole salamander (*Ambystoma sp.*) larvae in a mesocosm study (Lefcort et al. 1997).

Salt

Salt is used to de-ice roads in many areas and can accumulate in sediment, groundwater, and surface pools. Although not regulated by U.S. law, high levels of salt in freshwater can make water unfit for human consumption and impact aquatic systems by altering chemical processes and changing community structure (Kaushal et al. 2005). The use of, and subsequently the concentration of, salt found in stream water has been shown to increase with the amount of impervious surface within a watershed (Kaushal et al. 2005). Concentrations spike seasonally and can be found at levels lethal to some aquatic biota and are beneficial to the intrusion of some saltwater plants and animals (Kaushal et al. 2005). Salinization of freshwater also has been shown to have a negative effect on amphibians. Spotted salamander (*Ambystoma maculatum*) and wood frog (*Lithobates sylvatica*) eggs and larvae have reduced survival rates at higher conductivities (Karraker et al. 2008) and are excluded from ponds with lethally high salt concentrations (Collins and Russell 2009). Behavioral modifications result as well. Tadpoles exposed to different concentrations of salt showed decreased speeds and amounts of movement with increasing amounts of exposure time to salt (Denoël et al. 2010).

Nutrients

Nutrient inputs and cycling patterns are affected by land use change. First of all, vegetation removal takes away a valuable nutrient sink. Then, increasing impervious surfaces along with increased drainage facilitate the faster transport of increased nutrient loads into streams and wetlands (Lee et al. 2006). Inputs can come from various sources. In urban and suburban areas, sewage and excess lawn fertilizers are sources of nutrients (Cooper 1993). Livestock waste adds ammonia that can be lethal to aquatic animals at high concentrations (Cooper 1993). Crop agriculture often requires inputs of nitrogen and phosphorus as fertilizers since these can limit plant growth (Cooper 1993). In addition, crops are typically fertilized in the spring and summer, during the same period that most North American amphibians are breeding and eggs and larvae are vulnerable to aquatic threats (Mann et al. 2009). Fertilizers, particularly from agricultural sources can alter the community structure by stimulating the growth of periphyton and altering food webs (Boone et al. 2007). Excess nutrients can change the plant community and increase algal growth resulting in large blooms. The algal blooms increase the turbidity of the water which decreases the amount of sunlight reaching aquatic macrophytes. As the algae dies, the oxygen demand increases within the system, reducing the amount available to aquatic fauna (Lee et al. 2006). Johnson and Chase (2004) implicated eutrophication for the increase in snails that serve as intermediate hosts for a trematode parasite responsible for amphibian malformations. In urban streams, nutrient levels are often higher not only because of increased inputs, but also because of reduced rates of uptake by stream biota (Meyer et al. 2005).

Fragmentation

Habitat loss is the primary threat to amphibian populations (Mann et al. 2009). As animals that typically rely on both wetlands and uplands for various portions of their life cycles, amphibians are vulnerable to losses in either component. Most amphibians are dependent upon upland habitat adjacent to wetlands for hibernation, foraging, and migration, and many species only return to water for breeding, spending the majority of their lives on land (Gibbons 2003). In Ontario, Findlay and Houlahan (1997) found that wetland reptile and amphibian species richness decreases when forest cover on adjacent land decreases and when the density of paved roads increases. The quantity and quality of upland habitat affects communities in a number of ways. When vegetation is removed, abiotic factors such as light intensity and air and soil temperature increase while soil moisture may decrease. The plant community adjusts itself to the change in structure. Plants on the edge receive more light and can put out more leaves, affecting the amount of light or shade that reaches the remaining forest (Murcia 1995). This subsequently can cause changes in the animal community. Differences in vegetation can attract or repel certain animals such as insects and birds (Murcia 1995). Frogs, too, have been found to be affected by the amount of light reaching breeding pools. Wood frogs (Lithobates *sylvatica*) had slower growth rates in shaded pools, and spring peepers (*Pseudacris crucifer*) were not found in heavily shaded pools in Connecticut (Halverson et al. 2003). Urbanization tends to disturb the soil and increase the number of weedy or invasive species found in a system (Ehrenfeld 2000). Vegetation influences important microhabitat components for amphibians such as the litter layer composition and soil moisture and temperature, affecting the surface activity of salamanders (Welsh and Droege 2001, Taub 1961). In a laboratory test of habitat selection, spotted salamanders (*Ambystoma maculatum*) consistently chose the substrate with a leaf litter layer (Rittenhouse et al. 2004). The availability of cover objects such as trees, rocks, and leaf litter is very important for maintaining temperature and moisture thresholds. If such refuge is not available during hot or dry conditions, amphibians are likely to desiccate (Seebacher and Alford 2002). In one study, frogs in clear-cut forests without refuge lost more water than frogs in the same conditions but provided with brush piles (Rittenhouse et al. 2008).

Upland habitat is important for a large component of many amphibians' life cycles. In a literature review of studies, Semlitsch and Bodie (2003) found that core habitat required by amphibians was between 159 m and 290 m from water. Often amphibian studies focus only on the breeding phase that occurs in aquatic environments. Maintaining habitat corridors for migration to occur is important for maintaining metapopulations in a changing landscape (Gibbons 2003). When wetlands dry or refill, animals have to move between them and require cover to do so (Gibbons 2003). Buffer zones are often established around wetlands for water quality purposes and in some cases are considered beneficial to animal populations (Houlahan and Findlay 2004). Regulated buffers however are often narrow, and amphibians frequently use these areas only during their breeding season and then disperse for the remainder of the year, so buffers do not provide protection to the majority of the adult population or the resources they require (Rittenhouse and Semlitsch 2007).

Soil moisture and structure influences amphibian survival, as well, and can be altered by changes along the forest edge. When frogs were placed in different microhabitats, forest ridgetop and forest drainage, Rittenhouse et al. (2008) found that frogs on the ridgetop had a higher proportion of water loss than those in the drainage even though both had a closed forest canopy. Lower soil moisture resulted in faster desiccation (Rittenhouse et al. 2008). Soil compaction is another issue facing terrestrial and fossorial amphibians. Terrestrial salamanders spend the majority of their time in burrows within the soil. Some species are capable of digging their own but many use existing burrows from small mammals. Rothermel and Luhring (2005) found that salamanders denied access to burrows lost more water and had lower survival than salamanders able to use burrows. Compaction can occur from heavy machinery used in logging, agriculture, and urban construction activities and may destroy burrows and inhibit amphibians and other animals from creating new ones (Rothermel and Luhring 2005).

Hydrology

The physical characteristics of streams and wetlands can change with the shift in land use, which in turn can affect the animal community. In forested watersheds, streams are typically shallow, cooler, and well-shaded with flood events characterized by gradual increases and decreases in water levels (Schoonover et al. 2006). They also generally have more water contributed from shallow groundwater. Urban streams tend to be deeper with warmer waters, more frequent, flashier floods, and decreased baseflow levels, while streams draining watersheds with pasture land may have higher baseflows than urban or forested watersheds (Schoonover et al. 2006). The baseflow trend in agricultural areas is likely because using land as pasture or for crops can increase infiltration capacities, vegetation removal decreases evapotranspiration, and the patchiness of remaining vegetation alters surface water flows (Schoonover et al. 2006, Gordon et al. 2008). As the amount of impervious surface within a watershed increases, less precipitation can infiltrate the ground, which results in greater surface water discharge. This leads to more frequent, flashier floods and increased contributions from surface flow within streams (Paul and Meyer 2001; Walsh et al. 2005). In addition, although the total annual discharge of water did not change, Gilliam and Skaggs (1986) found that peak runoff rates were higher in agricultural areas than undeveloped pine forests. Changes in flow regime can also change the width and depth of streams, usually resulting in straighter, deeper channels with reduced complexity (Walsh et al. 2005). These effects can eventually influence faunal communities. Wetlands with greater urban influence have demonstrated lower species richness (Lehtinen 1999) and shifts in the herpetofauna (Barrett and Guyer 2008). Urban watersheds have been shown to have fewer amphibian species overall than forested or pasture-dominated watersheds (Barrett and Guyer 2008).

In addition, water temperatures are higher in urban streams when riparian vegetation is removed and impervious surfaces heat runoff inputs (Paul and Meyer 2001). Helms et al. (2009) found increased water temperatures and decreased dissolved oxygen concentrations in streams with increasing impervious surface within their watersheds. Temperature can have a significant effect on amphibians at different stages of their life cycles. Air and water temperature are used as cues for breeding in many species. Climate change and the associated changes in temperature and precipitation can alter species' habitat use and breeding phenology (Alford and Richards

1999). Adults and larvae have different responses to environmental stressors (Price et al. 2010). The speed and size of larvae through development has been shown to be influenced by temperature when combined with other stressors (Newman 1998). Newman (1998) found that the metamorphosis of Couch's spadefoot toad (*Scaphiopus couchii*) tadpoles was affected by temperature when tadpoles were kept at high densities with decreasing amounts of food, with individuals metamorphosing earlier at low temperatures than at high temperatures. At high temperatures with high densities and low food, no tadpoles metamorphosed within the study period. Metabolic needs were most likely lower at the low temperatures (Newman 1998) allowing for energy to be spent on metamorphosis.

The length of a wetland hydroperiod affects the species assemblage that can persist. In permanent bodies of water, predators such as fish are often present and only cryptic, less detectable, or unpalatable species can coexist (Kats et al. 1988). These species may also take longer to develop and can be outcompeted in temporary habitats (Snodgrass et al. 2000). In habitats with temporary hydroperiods, amphibian larvae must metamorphose quickly to escape before drying occurs or resources become limited, so the benefit is greater to forage actively and achieve a larger size quicker (Newman 1998). Invertebrate larvae also predate amphibian larvae in these habitats. Often a tadpole can only escape this threat by growing to a size where the predator can no longer easily attack and consume the tadpole. This situation offers advantages to animals that can forage and utilize refuge efficiently (Wellborn et al. 1996). Management recommendations for preserving amphibian diversity endorse conserving wetlands with a range of hydroperiod lengths to encompass the different needs of many species (Snodgrass et al. 2000).

Desiccation of developmental habitats will ultimately result in death for larvae that do not complete metamorphosis. The influences of fluctuating water levels within a wetland, however, are less clear. In Washington, Richter and Azous (1995) found that amphibian species richness was negatively related to increased water level fluctuations and urbanization within the watersheds of 19 wetlands. They suggest that fewer species could tolerate the stress of the increased flood frequency and intensity well enough to persist. Looking at the individual tadpole or certain species raises questions about the fate of larvae subjected to more variable flooding regimes. In laboratory experiments testing the influence of decreasing water levels, tadpoles have been shown to metamorphose sooner in treatments that lose water over time (Laurila and Kujasalo 1999, Gervasi and Foufopoulos 2008, Mogali et al. 2011). In some cases, smaller individuals resulted from faster metamorphosis (Laurila and Kujasalo 1999, Mogali et al. 2011) but not all (Gervasi and Foufopoulos 2008). Additionally, different species are known to have different responses to drying conditions (Leips et al. 2000). Some animals have adapted to the type of habitat that they typically breed in (i.e. permanent or temporary ponds and pools). Species utilizing transitory water sources can be more plastic in their response to water levels decreasing than those that use permanent waters (Leips et al. 2000). The response to changes in flooding regime is likely somewhat species specific.

Amphibians and headwater wetlands

Headwater slope wetlands are a class of forested wetland primarily driven by groundwater (Mitsch and Gosselink 2007). In the Coastal Plain physiographic region of Alabama, these are linear systems that occur at the headwaters of first order streams. These systems tend to have a dry period in the summer with little surface water. Typically, their canopy vegetation is dominated by sweet bays (*Magnolia virginiana*), red bays (*Persea borbonia*), swamp tupelo (*Nyssa biflora*), red maple (*Acer rubrum*), and slash pine (*Pinus elliottii*) (Noble et al. 2007, personal observation). Midstory shrubs include southern bayberry (*Morrella cerifera*) and titi (*Cyrilla racemaflora*). There is also often a thick fern understory consisting of royal fern (*Osmunda regalis*), netted chainfern (*Woodwardia areolata*), and cinnamon fern (*Onoclea cinnamonea*) (W. F. Barksdale personal communication).

Headwater wetlands serve as important habitat for many amphibians. In North America, 84 species of salamander and 28 species of frog are known to occur in headwater streams, seepages, and springs (Meyer et al. 2007). Studies examining the influence of urbanization on stream salamanders have been conducted (Price et al. 2010, Barrett et al. 2010, Willson and Dorcas 2003) but fewer have considered frog populations in similar habitats (Barrett and Guyer 2008). In the Pacific Northwest, headwater wetland amphibians have been studied as useful biotic indicators (Welsh and Hodgson 2008) and in respect to land management at different spatial scales (Stoddard and Hayes 2005); however similar studies are lacking for these systems in the Southeast U.S.

Baldwin County is one of Alabama's fastest growing counties. The 2000 U.S. Census recorded it as having a population of 140,415 while the 2010 Census recorded 182,265 (U.S. Census Bureau 2010), an increase of almost 42,000 people in ten years. It has 14 municipalities but most people live in unincorporated rural areas (Baldwin Co. 2002). As of the 2007 Census of Agriculture, Baldwin County had 189,815 acres in agriculture on 1,139 farms, with 80% of these farms less than 180 acres. Of that land, 54.28% is cropland with peanuts, forage, cotton, soybeans, and sod being the top crop items (USDA 2009). The population increase combined with the existing agricultural pressure makes this area a valuable study site to learn about the effects of human disturbance on wildlife communities.

Not all species respond to anthropogenic disturbances in the same manner. This project evaluated the question of this response on two levels. The first part is a survey to compare amphibian assemblages in headwater slope wetlands of Baldwin County, AL with different surrounding land uses at the watershed level and within buffers surrounding the wetlands. The second part is a mesocosm experiment testing the specific impact of the altered hydrology associated with urbanization on amphibians in their larval state, particularly anuran groups known for their different development strategies.

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Chapter 2: EVAULATING THE EFFECTS OF LAND USE ON HEADWATER WETLAND AMPHIBIAN ASSEMBLAGES IN COASTAL ALABAMA

Abstract: Anthropogenic land use (i.e. urban and agriculture) is known to impact aquatic ecosystems in several ways, including increased frequency and intensity of floods, stream channel incision, sedimentation and loss of microtopography, and shifts in vegetation present. Amphibians, are often tied to water, thus they are particularly susceptible to changes in wetland, riparian, and surrounding habitats. This study evaluated the amphibian assemblages of fifteen headwater slope wetlands in coastal Alabama across a gradient of land use change. Amphibians were surveyed on a seasonal basis using active searches and automated recording devices. Land use was delineated within wetland watersheds and within a 200-m buffer surrounding each wetland. Amphibian presence/absence and land use data were used to develop species occupancy probability models which were ranked using Akaike Information Criterion (AIC). Both urban and agricultural land use were shown to influence amphibian occurrence. Species richness ranged from five to ten species across sites; however, a number of species only occurred in wetlands surrounded by forested lands. Many species were detected more frequently on these wetlands compared to wetlands surrounded by urban or mixed land uses. Based on occupancy models, *Acris gryllus* was negatively associated with the amount of agriculture within a 200-m buffer of wetlands. *Hyla squirella, Lithobates clamitans,* and *L. sphenocephalus* were positively associated with the amount of agricultural land within the watershed. The non-native greenhouse frog (*Eleutherodactylus planirostris*) was found in several urbanized wetlands across the study area and was positively associated with the amount of impervious surface area (associated with urban land use) within a 200-m buffer of the wetlands.

INTRODUCTION

Biological assemblages, or the composition of species within a taxonomic subgroup (Wang et al. 2006), are variable and dependent upon certain biotic and abiotic conditions. Species can be included or excluded by factors such as vegetation, climate, predation, and interspecific competition (Jaeger 1971, Blaustein et al. 2001, Skelly et al. 2002, Riley et al. 2005), so it is logical that assemblages are influenced by changes in land use. Amphibians are a particularly responsive group with respect to their biological requirements. The presence or absence of certain species can depend upon environmental factors, many of which are influenced by anthropogenic activity (e.g. urban or agriculture) in surrounding lands. Land use often impacts amphibian assemblages present by changing the quantity and quality of wetland and terrestrial habitat available. Terrestrial habitat is important for many species of amphibian for migration, foraging, nesting, and hibernation (Gibbons 2003). Many species differ in their requirements for forest surrounding a wetland habitat at varying spatial scales (Porej et al. 2004; Guerry and Hunter 2002). For example, salamanders commonly use surrounding terrestrial forests extensively and are generally more sensitive to forest removal than frogs (Todd et al. 2009). In New Hampshire, Herrmann et al. (2005) found that most species were influenced by land conversion up to 1000 m from wetland habitats with forest cover >60% resulting in more diverse communities than wetlands surrounded by <40% forest cover. Findlay and Houlahan (1997) found reduced species richness for herpetofauna in areas of reduced forest cover around wetlands from 1000 to 2000 m. Species are affected differently and it has been suggested that more sedentary species may fare better in fragmented habitats where surrounding lands have been altered due to land use change. Since species like the redback salamander (*Plethodon cinereus*) are more likely to spend their lives in one location, they may be less vulnerable to the risks that come along with dispersing in a fragmented landscape (Gibbs 1998). Typically in primarily forested landscapes, dispersers, like the red-spotted newt (*Notophthalmus v. viridescens*), have the advantage of moving farther and colonizing new places, but after fragmentation, dispersers may be at a higher risk of ending up in unsuitable habitat (Gibbs 1998).

Many amphibians also exhibit sensitivity to wetland conditions, which may change as surrounding land is altered. Certain species are known to be more tolerant of degraded conditions and may be less impacted by land use changes. Increased impervious surface area commonly occurs with urbanization and can increase stream and wetland flood flashiness, decreasing habitat stability (Walsh et al. 2005). Barrett et al. (2010) found fewer (though larger) *Eurycea cirrigera* larvae in urban streams than forested streams in West Georgia and suggested that changes in flood regimes lead to lower survivorship in these streams. In Nova Scotia, wood frogs (*Lithobates sylvatica*) and spotted salamanders (*Ambystoma maculatum*) were excluded from ponds with high salt concentrations (as a result of road de-icing), while American toads (*Anaxyrus americanus*) showed high tolerance to increased salt concentrations and green frogs (*L. clamitans*) and spring peepers (*Pseudacris crucifer*) displayed intermediate tolerances (Collins and Russell 2009). This study showed that salt concentrations could act to structure amphibian communities in ponds based on roadway exposure. The treefrogs, *Hyla cinerea* and *H. squirella,* are known to use a wide variety of habitats including man-made gardens, buildings, and trash piles (Redmer and Brandon 2005, Mitchell and Lannoo 2005a), showing adaptability to anthropogenic landscapes. In Florida, Delis et al. (1996) found ranid frogs (*Lithobates sphenocephala*, *L. grylio*, and *L. catesbeianus*) in greater numbers in an urban housing development than natural areas nearby, suggesting that these frogs were not as heavily impacted by human settlement as other species detected in the study. They surmised that since ranids are more aquatic than many other frog species, they were less reliant on upland habitat. It was also suggested that man-made water bodies are beneficial to these frogs by providing more permanent sources of water (Delis et al. 1996). There are varying rates of sensitivity detected among species to pollutants as well. For example, American toad (*Anaxyrus americanus*) eggs and larvae were shown to be more tolerant and have higher survival rates in polluted sediment than gray treefrogs (*Hyla veriscolor*) which had 50% mortality or wood frogs (*Lithobates sylvatica*) which had 100% mortality (Snodgrass et al. 2008; Brand et al. 2010). Variation is also present among amphibian species within the same family. Bridges and Semlitsch (2000) showed that tadpoles of nine different species of the family Ranidae had different tolerances towards the same concentration of the pesticide (carbaryl), demonstrating varied sensitivities to pollution.

Urban and agricultural land use can also affect native species assemblages by facilitating the invasion of non-native predators or competitors (McKinney 2002, Blann et al. 2009). It is understood that increasing global travel by humans has increased the number of exotic plants and animals transported around the world. Introductions may be intentional or accidental. Although not many cases can be directly linked to native extinctions, when exotic species become established, they can negatively impact native populations (Gurevitch and Padilla 2004). The effects of existing stressors may be exacerbated through increased competition, predation, disease introductions, and habitat modification. Amphibians are by no means spared any of these ill effects. In a study of streams in southern California, Riley et al. (2005) found that when watershed area was >8% urban land use, the presence of invasive species (particularly predaceous crayfish and fish) increased, and the presence and abundance of native amphibian species decreased. Although not entirely the cause of the amphibians' absence, these exotics do prey upon native amphibians and are aided by the same changes in stream flow (increase in the intensity of floods and water velocity) and structure (reduction in the number of pools and increase in channelization) that are detrimental to amphibians (Riley et al. 2005).

Land use and land cover patterns are known to impact amphibian populations through many different mechanisms. The habitat required for reproduction, forage, and shelter is a logical focal point because without these three components, animal populations would not persist. This study began in 2011 as part of a larger effort to elucidate potential impacts of land use change on headwater slope wetlands in coastal Alabama. Land conversion in the area threatens the function of these wetlands by influencing hydrologic regimes and vegetation communities. Unaltered wetlands tend to have seasonal, shallow water levels that are groundwater driven, attributes suitable for amphibian breeding habitat. Amphibian assemblages were chosen as the study subject because of their close ties to aquatic environments and their potential response to anthropogenic influence on wetlands. The objective of this study was to determine the composition of amphibian assemblages of headwater slope wetlands in coastal Alabama across a gradient of land conversion. We expected to see a decrease in the frequency and number of sensitive species and an increase in the number of more tolerant species utilizing a wetland as more land within the watershed becomes converted from forest. Although there are exceptions within every group, presumed sensitive species included salamanders from the family Plethodontidae because of factors such as their fairly high densities, complete reliance on cutaneous respiration, and dependence on specific, easily disturbed microclimates such as leaf litter layer and soil moisture (Welsh and Droege 2000). Expected tolerant species included frogs from the family Ranidae because of their aquatic/semi-aquatic characteristics, ability to adapt to more variable hydroperiods, and somewhat reduced reliance on upland habitat (Delis et al. 1996).

METHODS

Study site selection

Prospective headwater slope wetlands for this study were identified from aerial photographs, USGS topographic surveys, and National Wetland Inventory maps of Baldwin County, Alabama. Headwater slope wetlands are freshwater, forested wetlands that are primarily groundwater driven in their natural state and occur at the headwaters of first order streams (Noble et al. 2007). Mature, canopy-sized sweet bays (*Magnolia virginiana*), characteristic of this type of wetland (Noble et al. 2007), were used as an indicator when identifying potential sites that had been altered. Over 30 sites were inspected in the field for conditions suitable with the study and 15 were selected across Baldwin County, Alabama (Figure 2.1) based on landowner permission, accessibility, and to represent a range of land use conditions surrounding wetlands and within their watersheds. Watersheds were delineated using ArcSwat version 2009.93.6 in ArcGIS 9.3 and adjusted based on aerial photographs and field verification of potential anthropogenic drainage features when appropriate. Impervious structures, roads, other paved surfaces (i.e. parking lots, driveways), unpaved roads, agricultural land, wetlands, forest, water, clear cut areas, and open spaces were delineated within the watershed and a 200-m buffer of the wetland boundary (Anderson et al. 1976). Agricultural land, impervious surface area, and forest land were chosen as relevant categories for evaluating land use effects. The amount of impervious surface area was used as a more precise measure of urban land use and better potential predictor of hydrologic alteration to wetlands. Watershed land use was used to assess potential changes in drainage while 200-m buffer land use was used to assess available adjacent terrestrial habitat. The surface cover within each boundary was digitized by hand based on 2009 aerial photographs in ESRI ArcGIS 9.3, verified in the field, and calculated as percentage of total land cover (Table 2.1).

Sites were characterized by surrounding land use to represent a range of conditions (Table 2.1). Four of the study sites had watersheds and buffers comprising of high forest cover (>80% within the watershed) and low agriculture and impervious surface area. These sites were considered reference conditions and were all within conservation areas, including a state park, wildlife management area, a national estuarine reserve, and local nature preserves. Seven sites were considered mixed-land use including urban, forest, and agricultural land. These sites for the most part had a fair amount of agricultural (watershed: 0-52.0%, 200-m: 0-31.0%) and/or residential land. The remaining four sites had higher amounts of urban land use (>10% ISA within the watershed, with the exception of No. 28 which had 6.6%). These sites all received extensive surface water drainage from roads, were located on unmanaged properties, and were dominated by non-native, invasive plant species (*Ligustrum sinense, Triadica sebifera, Lygodium japonicum)*.

Amphibian surveys

Area constrained searches were conducted in May 2011, December 2011, and May 2012. For each search, random, independent plots (100-400 m^2) were selected per wetland. The number and size of plots surveyed per wetland depended upon the wetland size and were conducted so that approximately 5% of each wetland was surveyed. Within a designated plot, two to three searchers raked all leaf litter and flipped any debris that could conceal animals. In areas with standing water, the water was swept with a dip net until the last ten sweeps of the net captured no new amphibians. The search time was limited to twenty minutes, which was deemed adequate to completely search any plot. From this, we obtained species presence and relative abundance (number of individuals ha^{-1}) of all amphibians detected at each site.

Automated recording devices (Wildlife Acoustics Song Meter SM1 and SM2) were used to record calling anurans. The devices (frog loggers) were deployed in May 2011, January 2012, March 2012, and May 2012 to record a range of anurans that are known to breed in different seasons. For each sampling period, the loggers were set to record for one minute every hour from18:00 to 3:00 and recorded for five consecutive nights to capture variation in weather conditions among nights. For each night recorded, tapes were listened to and all frog species were identified and designated a recorded number 1-3 to indicate the strength of the chorus (per protocol reported in Mossman and Hine 1984). The tapes provided species presence and call intensity per species on those nights from each wetland.

Data analysis

To initially assess species assemblages mean species richness was compared among wetlands in forested (reference), mixed, and urban surrounding land uses. Species cumulative data were summarized per land use group and an ANOVA was run in R on unadjusted species richness values from all wetlands per group. Because many species respond to stressors differently, these data were also analyzed on a species by species basis. Since animals often are not detected when present, detection probability was calculated and occupancy analysis was used to examine species presence across all sites in response to land use variables and habitat covariates within both the watershed and a 200-m buffer (MacKenzie et al. 2002). Land use variables were considered dependent of one another (if a piece of land is classified as agriculture it cannot also be forest, King et al. 2005), therefore no two were combined in any one model. The sampling method used and the month in which the data were collected were included as detection covariates to account for the effects that each may have had on species detection since some animals are more likely to be found by each method or during certain seasons. These analyses were done in the Program PRESENCE version 3.1 (Hines 2006) and models were ranked using Akaike Information Criterion (AIC). Of the 20 species found, only five were detected frequently enough across a range of conditions to build models. Land use data percentages were represented from 0 to 1 to make scales manageable for the models. Assumptions included that species were not falsely detected, that detection was independent at each site, and that heterogeneity in occupancy and detection can be explained by covariates (MacKenzie et al. 2006). We also assumed that sites were closed to changes in occupancy state between sampling occasions from May 2011 to May 2012.

Since rates of detection and occurrence vary by species, heterogeneity in species detectability was accounted for in estimating species richness among sites (Boulinier et al. 1998; Dorazio and Royle 2005). There are 42 species of amphibian that have ranges recorded within Baldwin County, Alabama (Mount 1975, USGS National Amphibian Atlas 2012). This model was used to estimate the total species richness across all wetlands using the detection probabilities and occupancies calculated from the species observed and estimated for the species that were not observed. The programs R and WinBugs were used for these analyses.

RESULTS

Surrounding land use and amphibian species trends

Watersheds and wetland buffers showed a range of conditions for the land use categories chosen (Table 2.1). Fifteen species of frog were detected using the frog loggers, and eight species of frog and four species of salamander were detected during the active searches (Table

2.2). Salamanders were not detectable using the loggers and four species were found during active searches. Seven species of frogs were detected by both techniques.

The species richness model accounting for occupancy and detectability estimated based on what was detected that there was a mean of 32.12 (σ=5.44) species present across all study sites. An ANOVA revealed no significant differences in unadjusted species richness values among land use types (forest, mixed, and urban) (df= 2, F= 0.64, p=0.544). Wetlands classified as forested land use had a range of 5 to 10 species present with 15 species cumulatively across sites and a mean of 6.75 (±1.02) (Table 2.3). Mixed land use wetlands ranged from 5 to 8 with a total of 12 species present and a mean of 6.29 (±0.44), and urban wetlands had 6 to 10 species with a total of 13 and a mean of 7.5 (±0.75). Although cumulative species richness was similar among wetland land use groups, several species (*Anaxyrus quercicus*, *Desmognathus conanti*, *Eurycea quadridigitata, Hyla femoralis*, and *Pseudacris ornata)* were only found on forested land use wetlands albeit on few occasions (Table 2.4). *H. gratiosa* was found on mixed and forested land use wetlands. *Siren intermedia* was found in urban and forested land use wetlands with standing water present. The non-native *Eleutherodactylus planirostris* was found in two urban wetlands and three mixed land use wetlands. *Gastrophryne carolinensis* was only found once during a survey on a mixed land use wetland. *Lithobates grylio* and *L. catesbeianus* were only heard at one urban wetland with permanent standing water. *Acris gryllus, A. terrestris, E. cirrigera, H. cinerea, H. squirella, L. clamitans, L. sphenocephalus,* and *P. crucifer* were found across the range of wetland land uses. Forested wetlands had the highest mean number of detections per wetland (180 ±86) compared to urban (141 ±55) and mixed (91 ±18) land use.

Amphibian occupancy models

Agricultural land use was included in top occupancy models for several species. The two of the top three models for *Acris gryllus* included the effect of agriculture both in the watershed and 200-m buffer (Table 2.5). *A. gryllus* also had high occupancy rate among sites (0.93-0.99). The top models for *Hyla squirella* showed a positive association with the amount of agricultural land and a negative association with forest cover (in both the watershed and buffer), with occupancy ranging from 0.18-0.93 (Table 2.5). The top model for *Lithobates clamitans* showed a positive association with the amount of agricultural land in the watershed with an occupancy rate of 0.48 (Table 2.5). The top model for *L. sphenocephalus* has a positive association with the amount of agricultural land as well, with an occupancy rate of 0.31. The models for *Eleutherodactylus planirostris* had impervious surface area as the top predictor covariate with an occupancy rate of 0.09 (Table 2.5).

Anaxyrus quercicus, Desmognathus conanti, Eurycea quadridigitata, Gastrophryne carolinensis, Hyla chrysoscelis, H. femoralis, H. gratiosa, Lithobates catesbeianus, L. grylio, Pseudacris ornata, and *Siren intermedia* were not detected frequently enough or at enough sites to run models. One species, *P. crucifer*, was detected at every site and therefore had an occupancy rate of 1.00 across sites. Models for *A. terrestris* were not computable probably because there were not enough detections or enough variation in the detections across the range of data. There were not enough detections across the range of sites for any covariate in the models for *E. cirrigera* to be supported. There was also not very strong support for any particular model for *H. cinerea*.

DISCUSSION

The objective of this study was to determine the amphibians present across a range of land uses. Over all 15 wetlands, 20 species of amphibian were detected either by area constrained searches or automated recording devices. The use of multiple techniques to detect amphibians was advantageous for this study. Though the automated recording devices could not detect salamanders, only seven species of frog were detected by both techniques. Eight frog species would have been missed if only the constrained searches had been used. The use of multiple techniques to determine herpetofaunal assemblages is generally preferred as no one technique has been proven to detect all species present (Ryan et al. 2002).

There was a wide range of land use composition represented across wetland watersheds and adjacent buffers (Table 2.1). Mean species richness among groups was 7.5 ±0.75 for urban sites, 6.75 ±1.02 for forested land use sites, and 6.29 ±0.44 for mixed land use sites (Table 2.3). Though there were similar species richness values across land use groups, several species in this study demonstrated sensitivity to land conversion and were only detected in relatively undisturbed wetlands (*Anaxyrus quercicus, Desmognathus conanti, Eurycea quadridigitata, Hyla femoralis,* and *Pseudacris ornata).* It is likely that these species require extensive terrestrial habitat and groundwater drainage that only these less disturbed wetlands provided. For instance, *A. quercicus* tend to occupy areas with well to poorly drained soils and shallow temporary pools such as wet prairies and oak and pine forests with open canopies (Punzo 2005). It is possible that many of the headwater streams used in this study did not provide the type of habitat necessary, though development and sedimentation (as seen in these urbanized wetlands) are cited as threats to *A. quercicus* (Punzo 2005). One forested land use wetland (No. 101) had particularly high species richness (n=10), and two species that only occurred there (*A. quercicus* and *D. conanti*) as well as some species that only occurred on one or two other sites (*E. cirrigera, E.*

quadridigitata, and *Siren intermedia*). This area was formerly the town of Blakeley, one of the oldest towns in Alabama but reached its peak in the 1820s. The town became a fort during the Civil War but was then abandoned afterward, and the land remained idle until it was designated as a state park in 1981 (Historic Blakeley State Park 2013). Since this land was left untouched for approximately 130 years, this could be what separates this site from other forested land use wetlands in richness. Though the others are all now conservation areas, most of them were logged within the past century.

Agricultural land use was the top predictor of species presence for several amphibians in this study and positively associated with several of them. Pasture and cropland can potentially increase infiltration, decrease evapotranspiration, and alter surface flow through changes in the vegetative cover (Blann et al. 2009). The effects of agriculture on drainage vary considerably depending on specific use and is often not quite the same effect as urban land use might have on aquatic systems (i.e. decreased infiltration, decreased evapotranspiration, and increased flood intensity and frequency; Schoonover et al. 2006). *Acris gryllus* was negatively associated with agricultural land within a 200-m buffer and at the watershed scale. Though their exact dependence on upland habitat is unknown, *A. gryllus* have been observed at fairly large distances from aquatic habitats, and lower numbers of *A. gryllus* have been reported for silvicultural lands in comparison to native habitats, suggesting that these frogs use uplands substantially, most likely for foraging (Jensen 2005). Barrett and Guyer (2008) did not find *A. gryllus,* or any hylid frogs, in urban streams sampled for herpetofauna and suggest that disturbances within the stream and riparian zone may be responsible for the absence of these frogs and Plethodontid salamanders in those watersheds. Though *A. gryllus* were found at many sites in this study (n=13), they were detected most frequently at two forested and two mixed land use sites (Table

2.4) and these model results suggest a similar dependence upon undisturbed land as cricket frogs were negatively associated with the amount of agricultural land surrounding the wetland. *Hyla squirella* was positively associated with the amount of agricultural land within the watershed in its top model and negatively associated with the amount of forest in the watershed and within a 200-m buffer. *H. squirella* are generalists and found in a wide variety of habitats including around human establishments so long as they have sufficient food, moisture, and cover (Mitchell and Lannoo 2005a). Models in this study support this as *H. squirella* were tolerant of watersheds with agricultural land use.

Several ranid frog species were detected across wetlands. *Lithobates clamitans* was another species that was widespread across study sites and was positively associated with agricultural land use in this study. *L. clamitans* are aquatic frogs and typically are found close to open water sources (Pauley and Lannoo 2005), which was consistent with where they were typically located during active searches in this study. Contrary to these results, Houlahan and Findlay (2003) found *L. clamitans* to be positively correlated with forested land over a larger area in Ontario, Canada, and suggested that forest cover may also function as a measure of reduced agricultural activity and reduced fertilizer and pesticide runoff. *L. sphenocephalus* was positively associated with agricultural land within the watershed as well too, and other top models for this species were negatively associated with impervious surface area and forest cover. *Lithobates catesbeianus* and *L. grylio* were only detected on tape at one wetland which was permanently flooded and near another permanent golf course pond (No. 28). Not all studies have linked increased ranid species to land use change. Johnson et al. (2011) found that the presence of northern leopard frogs (*L. pipiens)* was negatively correlated with urban and suburban land use and positively correlated with grassland. Delis et al. (1996) however found greater numbers of ranid frogs (*L. sphenocephalus, L. grylio, and L. catesbeianus*) in an urban area than in a nearby, natural environment, suggesting that they were resilient to the changes occurring in the surrounding landscape or potentially benefiting because of increasing open water habitat. Many of the agricultural areas in this part of Alabama have small, built ponds that may enhance ranid populations.

It is very likely that not all species present in these wetlands were detected by the sampling methods used. The species richness model accounting for detection probability and occupancy estimated a mean of 32.12 (σ=5.44) species present across all study sites. The southeastern U.S. was under drought conditions for the first year of this study (NOAA 2013), which likely influenced amphibian activity (Walls et al. 2013). Frog calling activity increased in from May 2011 to May 2012 when the drought lessened (Table 2.1, Appendix A) however it was suspected that drought conditions may have influenced the detectability of some salamander species as well. Only one *Desmognathus* salamander was found throughout the entire study. No salamanders of the genus *Ambystoma* were found in this study. Ambystomatid salamanders typically breed in the winter when temperature and precipitation conditions are appropriate but otherwise spend the majority of their time underground. It is likely that sampling periods missed the breeding season for these salamanders or that these habitats were not appropriate for their breeding.

Permanently aquatic salamanders were also not detected frequently enough for analyses, though their presence in this region is known. *Siren intermedia* was only found at two sites, an urban and a forested land use wetland) both with standing water. *S. intermedia* are permanently aquatic salamanders that live in swamps and weedy ponds and can survive dry periods by burrowing in mud or hiding in crayfish burrows (Mount 1975). Another permanently aquatic salamander, *Amphiuma means,* was found dead at a forested state park site on one occasion after a flood and not during a sampling event, suggesting that they may have been present in the area. Amphibian species characteristic of larger streams were also not detected but this was probably due to the nature of the headwaters being studied.

Three species of plethodontid salamander were found during this study, *Desmognathus conanti, Eurycea cirrigera* and *E. quadridigitata,* and they were only found at four sites. *E. cirrigera* are typically abundant in their range and can be found in fairly polluted waters (Pauley and Watson 2005). In this study, *E. cirrigera* were only found as larvae, and therefore, only found in areas with water present which limits the range of sites where they could be detected (sites T2, 28, and 101). However, it would have been possible to find adults during searches. *D. conanti* was found at one site (101) and E. *quadridigitata* were detected at two sites, both with high forest cover (101 and T3). These salamanders breed and live around ponds, swamps, seeps, and springs and can be found in pine flatwoods and sandhills (Means 2000, Bonett and Chippindale 2005). These habitats have been altered in Baldwin County by human development, so thought *E. quadridigitata* was likely more common, they have declined due to habitat loss. Unfortunately, there were not enough detections to make predictions from their presence as dry conditions likely reduced salamander activity and detection (Walls et al. 2013).

The small number of detections across sites is likely the cause of the poor model results for *Anaxyrus terrestris* and *Eurycea cirrigera*. Models for *A. terrestris* were not computable, possibly because there were not enough detections in upper end of spectrum for 200-m buffer impervious surface area and agricultural land and not enough variation in the detections. None of the models for *H. cinerea* and *E. cirrigera* had strong support and AIC weights were distributed among eight models for each of them. Though *H. cinerea* was detected at 12 wetlands, the detections were not distributed equally which may have interfered with the model (Table 2.5). One mixed land use wetland with permanent standing water (No. 28) had 56 detections while the others all had 17 or less, with three sites only having one detection and two sites having two.

E. planirostris was most strongly associated with impervious surface area in these models. This species is known to thrive in natural, agricultural, and urban areas and can be frequently found under trash piles (Wilson and Porras 1983). These frogs were first recorded in Alabama in Fairhope (west Baldwin County) in 1982 (Carey 1982) with no published records since. The presence of calling males in five sites spread across Baldwin County shows that these frogs have spread since their initial introduction. Greenhouse frogs have direct development (hatch from an egg into a miniature adult) and do not rely upon standing water for completion of a larval stage (Kraus et al. 1999). *Eleutherodactylus* frogs are known to be transported in greenhouse materials and can be associated with the horticultural trade (Kraus et al. 1999). The wetlands where these frogs were found in this study were observed to be washed out sites with little microtopography and standing water. These conditions are common in urban headwater wetlands and may be the result of excessive sedimentation (Barksdale and Anderson, in review). *E. planirostris* were also found in sites that had other commonly occurring frog species present (*A. gryllus, A. terrestris, H. cinerea, H. chrysoscelis, L. clamitans,* and *P. crucifer*). In another amphibian example, Wilson and Porras (1983) suggested that *Bufo marinus* moved into areas made unsuitable to *Anaxyrus terrestris* in south Florida after substantial changes in vegetation and water supply. It is possible that a similar situation is occurring with *E. planirostris* being able to utilize wetlands that other species are not.

Agriculture was the most frequent determinant of amphibian presence in this study though this effect is not consistent among other land use studies. Knutson et al. (1999) found a positive relationship between anurans and agriculture in Wisconsin but negative associations for a few species in Iowa. They suggested that remnant forests provide refuge for amphibians within agricultural lands in Wisconsin where agricultural practices are not as intense as in Iowa (Knutson et al. 1999). Beebee (1983) also found species specific responses to the conversion of heathland to agricultural land in southeast England. The author cited the change in the availability of suitable breeding pools as a cause for the absence of the newt, *Triturus cristatus*, but suggested that *T. helveticus* was more able to adjust to low pHs and conductivities than other newt species and therefore was found in more ponds (Beebee 1983). Relyea (2004) found that tadpoles of six species were killed by relatively low doses of a common herbicide (glyphosate) and that the chemical was twice as lethal when the stress of chemical cues from a predatory newt was added, demonstrating the added danger of increased pesticide usage within a watershed. Forest cover has also been suggested to be an important variable for amphibian species assemblages. Houlahan and Findlay (2003) found a positive relationship between forest cover and amphibian species richness and a positive relationship between species richness and proportion of adjacent wetlands, showing that more forest and wetland habitat were beneficial. Gibbs (1998) found different responses among five amphibian species to the amount of forest fragmentation along transects, with a species known for its dispersal abilities (*Notophthalmus v. viridescens*) being one of the most heavily affected. Habitat modification (such as forest clearing and wetland drainage) have been implicated as one of the leading causes of global amphibian declines (Alford and Richards 1999, Collins and Storfer 2003) and has demonstrable effects on amphibians and other wildlife, though this study supports that effects can be variable and may not be easily predicted.

One improvement to this study would have been to use more sites. Fifteen was a relatively low number of sites but was limited by landowner permission, time constraints, and the number of automated recording devices available. More sampling occasions for the active searches could have improved the models for salamander species; however, this was also limited by time and amount of disturbance allowable for accurate assessment. (We adequate wanted time to pass in between constrained searches so that the habitat would recover from the disturbance caused by raking leaf litter and flipping debris). Either of these increases in effort could have allowed for more detections and possibly better models describing some of these species' habits.

CONCLUSION

This study examined the relationship between amphibian species presence and land use surrounding headwater slope wetlands in coastal Alabama. Two methods were used to detect amphibians: automated recording devices and active searches. Aerial photographs were used to delineate the land use/land cover of watersheds and a 200-m buffer surrounding wetlands. Based on surrounding land use, wetlands often supported similar species richness however wetlands surrounded by forest often had species deemed to be more sensitive and were less frequently encountered. Occupancy models were run in Program PRESENCE and ranked by AIC. Of the species that had sufficient detections to run models, AIC indicated agricultural land as highest for several species. From model results, *A. gryllus* was negatively associated with agriculture within a 200-m buffer, while *H. squirella, L. clamitans,* and *L. sphenocephalus* were positively associated with the amount of agriculture within the watershed. The non-native frog, *E. planirostris,* was found to have increased its distribution across Baldwin County, AL since the last published record of it in 1982. These frogs were positively associated with the amount of impervious surface area within 200-m of the wetland, which fits with their lack of dependence on water for reproduction and tendency to be spread through the plant nursery industry. This work supports a growing body of literature documenting the influences of anthropogenic land use change on animals such as amphibians and demonstrates that these species frequently respond to changes in their surroundings.

Table 2.1. Watershed areas and 200-m buffers and percentages of forest, impervious surface area (ISA), and agriculture.

		Watershed Percentages				Buffer Percentages			
Wetland ID	Watershed Area (ha)	Forest (%)	ISA (%)	Agriculture (%)	Buffer Area (ha)	Forest (%)	ISA (%)	Agriculture (%)	Surrounding LULC characteristic
28	62.9	47.1	6.6	0.0	22.2	57.3	4.3	0.0	Urban
68	151.1	16.0	15.1	20.2	57.3	45.1	7.1	34.1	Urban
71	39.5	20.0	24.0	7.7	44.5	31.9	16.5	4.2	Urban
100	111.7	17.9	34.0	0.0	27.2	57.6	16.5	0.0	Urban
T3	81.3	91.8	0.1	0.5	52.2	98.5	0.0	0.0	Forest
41N	16.7	80.8	0.0	0.0	42.6	57.7	0.3	8.7	Forest
85	59.6	95.8	1.7	0.0	87.4	93.7	1.4	0.0	Forest
101	36.5	93.8	0.6	0.0	31.6	98.2	0.0	0.0	Forest
T2	16.4	57.6	0.0	42.2	33.2	86.0	0.0	13.3	Mixed
9	83.2	19.3	4.4	52.0	36.8	22.4	1.3	7.8	Mixed
26	87.9	72.7	0.8	18.0	26.0	89.5	1.8	0.0	Mixed
40	97.9	37.6	1.2	37.4	25.7	64.4	2.4	31.0	Mixed
41S	194.9	78.1	0.7	8.2	37.9	66.0	0.0	0.0	Mixed
67	46.3	40.3	2.9	7.5	24.7	34.8	3.3	14.7	Mixed
102	26.9	84.5	4.8	0.0	39.4	80.3	4.8	0.0	Mixed

Table 2.2. Species detected across all wetland sites and by which method they were detected.

Scientific Name	Common Name	Logger	Survey
Acris gryllus (LeConte, 1825)	Southern Cricket Frog	X	X
Anaxyrus quercicus (Holbrook, 1840)	Oak Toad	X	
Anaxyrus terrestris (Bonnaterre, 1789)	Southern Toad	X	X
Desmognathus conanti (Rossman, 1958)	Spotted Dusky Salamander		X
Eleutherodactylus planirostris (Cope, 1862)	Greenhouse Frog	X	
Eurycea cirrigera (Green, 1831)	Southern Two-lined Salamander		X
Eurycea quadridigitata (Holbrook, 1842)	Dwarf Salamander		X
Gastrophryne carolinensis (Holbrook, 1835)	Eastern Narrowmouth Toad		X
Hyla chrysoscelis (Cope, 1880)	Cope's Gray Treefrog	X	
Hyla cinerea (Schneider, 1799)	Green treefrog	X	X
Hyla femoralis (Bosc, 1800)	Pine Woods Treefrog	X	
Hyla gratiosa (LeConte, 1856)	Barking Treefrog	X	
Hyla squirella (Bosc, 1800)	Squirrel Treefrog	X	X
Lithobates catesbeianus (Shaw, 1802)	Bullfrog	X	
Lithobates clamitans (Latreille, 1801)	Bronze Frog	X	X
Lithobates grylio (Stejneger, 1901)	Pig Frog	X	
Lithobates sphenocephalus (Cope, 1886)	Southern Leopard Frog	X	X
Pseudacris crucifer (Wied-Neuwied, 1838)	Spring Peeper	X	X
Pseudacris ornata (Holbrook, 1836)	Ornate Chorus Frog	X	
Siren intermedia (Barnes, 1826)	Lesser Siren		X

Table 2.3. Amphibian species detected at each wetland and per surrounding land use type.

	Urban				Forest				Mixed						
Species	28	68	71	100	T3	41N	85	101	T2	9	26	40	41S	67	102
Acris gryllus	X	X	X	X	X	X	X	X	X	X			X	X	X
Anaxyrus quercicus								X							
Anaxyrus terrestris	X	X	X	X		X	X	X	X	X	X	X		X	X
Desmognathus conanti								X							
Eleutherodactylus planirostris			X	X								X		X	X
Eurycea cirrigera	X							X	X						
Eurycea quadridigitata					X			X							
Gastrophryne carolinensis										X					
Hyla chrysoscelis			X					X	X						X
Hyla cinerea	X	X	X	X	X	X	X			X	X	X	X		X
Hyla femoralis					X										
Hyla gratiosa							X					X			
Hyla squirella		X	X						X	X	X			X	
Lithobates catesbeianus	X														
Lithobates clamitans	X	X		X		X		X	X	X	X	X	X		
Lithobates grylio	X														
Lithobates sphenocephalus	X	X				X			X	X	X		X		
Pseudacris crucifer	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pseudacris ornata						X									
Siren intermedia	X							X							
Total	10	7	7	6	5	7	5	10	8	8	6	5	5	6	6

Table 2.4. The number of detections of each species at each wetland. Numbers are combined for loggers and searches.

	Urban				Forest				Mixed						
Species	28	68	71	100	T3	41N	85	101	T2	9	40	41S	67	102	26
A. gryllus	7	1	2	4	4	136	15	7	8	37	0	14	1	3	1
A. quercicus	0	0	0	0	0	0	0	23	0	0	0	0	0	0	0
A. terrestris	2	20	39	4	0	18	6	27	9	3	6	0	14	7	1
D. conanti	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
E. planirostris	0	0	26	15	0	0	0	0	0	0	8	0	3	30	0
E. cirrigera	26	0	0	0	0	0	0	1	4	0	0	0	0	0	0
E. quadridigitata	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0
G. carolinensis	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
H. chrysoscelis	0	0	1	0	0	0	0	7	1	0	0	0	0	1	0
H. cinerea	56	10	1	2	1	17	2	0	0	11	1	6	0	0	3
H. femoralis	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
H. gratiosa	0	0	0	0	0	0	4	0	0	0	1	0	0	0	0
H. squirella	0	14	3	0	0	0	0	0	2	5	0	0	16	0	3
L. catesbeianus	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L. clamitans	150	60	0	2	0	108	0	34	7	9	1	1	0	0	55
L. grylio	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L. sphenocephalus	11	1	0	0	0	64	0	0	11	4	0	2	0	0	12
P. crucifer	2	10	28	20	83	80	19	53	79	61	22	13	72	16	73
P. ornata	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
S. intermedia	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Total	301	116	100	47	91	428	46	155	121	131	39	36	106	57	148

Table 2.5. AIC results of models for each species run in Presence. Models with delta AIC within 2 units were included along with AIC weight, psi/Ψ (probability that a site is occupied), and if the association with that land use variable was negative or positive. Psi is occupancy, p is detection probability, and terms in parentheses are the covariates used to influence each measure. (.) indicates that no covariate was used in that model and psi or p did not change. 200=variable was measured within a 200-m buffer of the wetland, WS=variable was measured at the watershed scale.

Species	Model	Delta AIC	AIC weight	Association	Ψ
Acris gryllus	psi(200 Ag),p(method, month)	0.00	0.399	-	0.998
	psi(.),p(method, month)	1.77	0.165		0.933
	psi(WS Ag),p(method, month)	1.94	0.151	-	0.989
Eleutherodactylus planirostris	psi(200ISA),p(method)	0.00	0.344	+	0.097
	psi(200ISA),p(.)	0.47	0.272	+	0.097
Hyla squirella	psi(WS Ag),p(method, month)	0.00	0.333	+	0.187
	psi(WS Forest),p(method, month)	0.30	0.287	-	0.894
	psi(200 Forest),p(method, month)	1.57	0.152	-	0.927
Lithobates clamitans	psi(WS Ag),p(method, month)	0.00	0.331	+	0.477
	psi(.),p(method, month)	1.42	0.163		0.667
Lithobates sphenocephalus	psi(WS Ag),p(method, month)	0.00	0.233	+	0.311
	psi(.),p(method, month)	0.51	0.181		0.467
	psi(200 ISA),p(method, month)	0.66	0.168	-	0.606
	psi(WS ISA),p(method, month)	1.53	0.109	-	0.554
	psi(200 Forest),p(method, month)	1.94	0.088	-	0.719

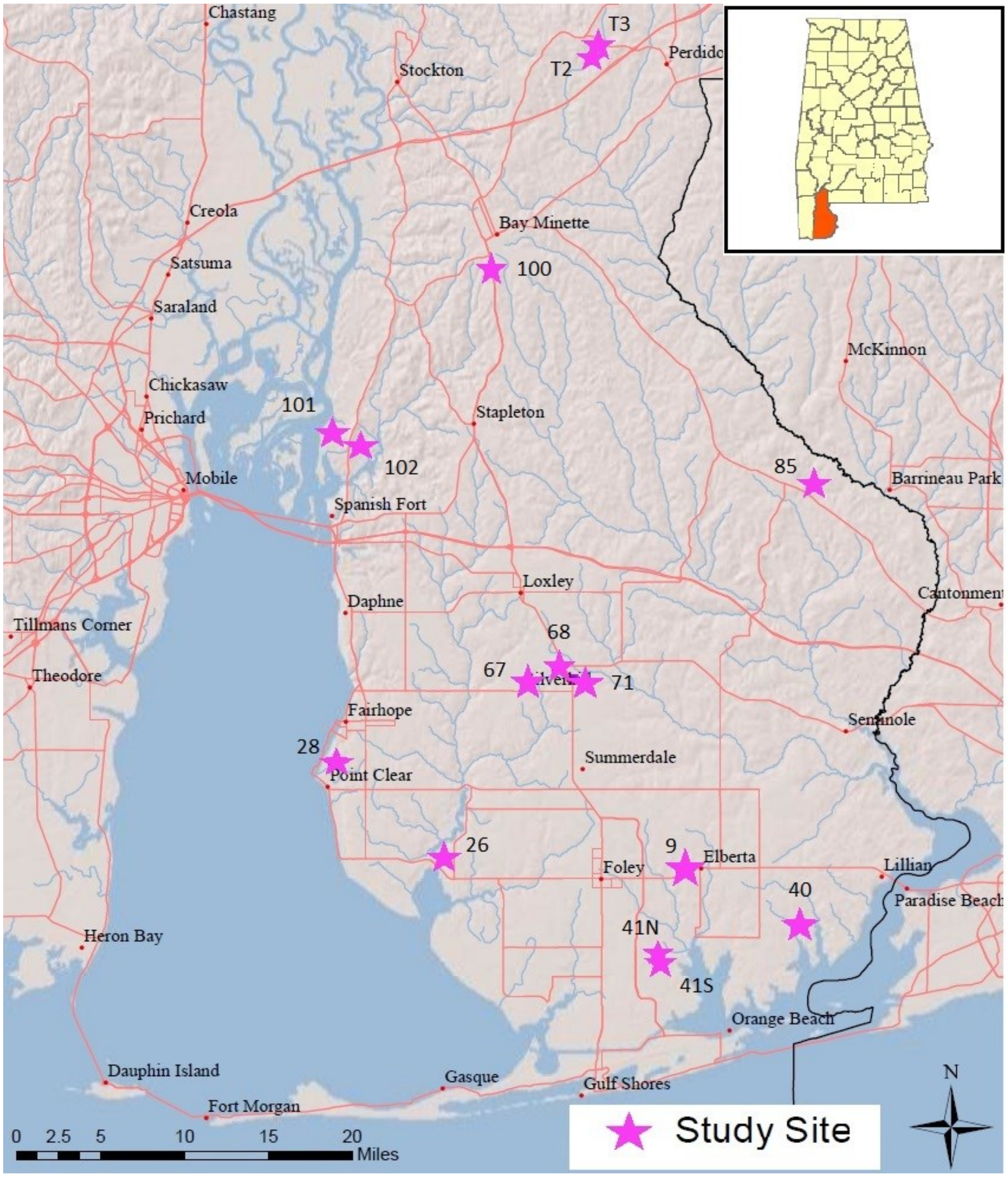

Figure 2.1. Map of fifteen wetland study sites surveyed for amphibian assemblages.

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Chapter 3: THE INFLUENCE OF URBAN AND FOREST FLOODING ON THE DEVELOPMENT AND SURVIVAL OF LARVAL ANURANS IN AQUATIC HABITATS

Abstract: Land use alterations can impact riparian wetland flooding regimes, often resulting in flashier, high-energy floods with lower groundwater levels between flooding events. Frogs with aquatic larval stages, like most North American species, may be impacted by these hydrologic changes through accelerated larval development rates, reduced survivorship, and overall reduced fitness. To examine these potential effects, we subjected tadpoles of four species with different breeding preferences (*Lithobates sphenocephalus, Hyla chrysoscelis, Hyla squirella,* and *Gastrophryne carolinensis*) to experimental flooding regimes intended to imitate flashy urban flooding, gradual flooding within a forested watershed, and a control of stable water levels. Periodic development rates, daily mortality, and final mass and length were measured for each species. Variable responses between were found species. *L. sphenocephalus* had the least mortality in the stable water level, followed by gradual floods, fitting with this species' preferred breeding habitat of lentic waters. *H. chrysoscelis* had high survival in all treatments; however, tadpoles in the gradual flooding regime had larger body sizes at the completion of the trials than the other two treatments. These findings suggest that altered flooding regimes caused by land use change may affect frog species differently depending on their breeding preferences through altered mortality rates and fitness at metamorphosis.

INTRODUCTION

Many amphibians in North America have an aquatic larval stage before metamorphosing into a terrestrial adult. At this pivotal life stage, amphibians are susceptible to changes in hydrology and water chemistry in addition to pressures from competition, predation, and climate

(Newman 1992). There has been a growing body of knowledge focusing on human impacts to aquatic environments, and it is reasonable to expect that amphibian larvae are influenced as well. Urbanization and land use change have numerous effects on aquatic systems including increased inputs of nutrients and pollutants (Hatt et al. 2004). The removal of streamside vegetation and drainage of pavement and other impervious surfaces has been shown to artificially increase water temperatures (Paul and Meyer 2001). Land use alterations can influence stream and wetland hydroperiods and flooding regimes as well (Paul and Meyer 2001). Increased impervious surface area within the watershed can increase stormwater runoff and its velocity into streams and wetlands, causing floods with flashier hydrographs (Walsh et al. 2005). Further, the increase in runoff and decrease in upland soil infiltration may reduce groundwater recharge and reduce baseflow levels (Schoonover et al. 2006). All of these changes have the potential to influence aquatic animals dependent on streams and riparian wetlands, and larval amphibians provide excellent study organisms to reveal the potential effects of these changes. Tadpoles are closely connected to changes in water quality and quantity and have demonstrated plasticity to environmental changes in other studies (Laurila and Kujasalo 1999, Leips et al. 2000, Reylea 2001). They are generally available in large quantities and fairly easy to maintain in captivity.

It has been shown that land use changes to wetland and riparian flooding regimes can influence amphibian species. Field studies have demonstrated various negative impacts on amphibian species richness and the presence of eggs or larvae. Richter and Azous (1995) found that wetlands with water level fluctuations of greater than 20 cm were more likely to have a lower species richness (three or fewer) than wetlands with lower fluctuations. Lind et al. (1996) studied the presence and abundance of *Lithobates boylii* egg masses below a dammed section of river in northern California and found that the number of egg masses present declined severely after extreme high flow events. Barrett et al. (2010) found lower survival of two-lined salamander larvae in urban streams in west Georgia, though surviving larvae appeared to grow faster than in forested streams, possibly due to smaller larvae being washed out by intense floods. These studies all indicate that altered flooding regimes may influence species assemblage, reproductive output, and larval development of amphibians.

Many species breed in temporary waters, and tadpoles have demonstrated abilities to respond to changes in water levels, though the mechanisms for these responses are not fully understood. Accelerated water loss has been shown to trigger responses to induce faster metamorphosis (Laurila and Kujasalo 1999, Gervasi and Foufopoulos 2008). Laurila and Kujasalo (1999) and Mogali et al. (2011) found that decreasing water levels in laboratory experiments led to tadpoles metamorphosing earlier and at smaller sizes than tadpoles kept at constant water levels. Denver et al. (1998) found that *Scaphiopus hammondii* tadpoles not only accelerated their development rate and had smaller body sizes in response to desiccating pools but could reverse this response if pools refilled and gain mass to compensate. Brady and Griffiths (2000) found that tadpoles of three different species in desiccating ponds all metamorphosed at smaller sizes, though not any faster, than in constant water levels. Some species may have evolved different strategies based on the habitats that they use for breeding (Leips et al. 2000). In comparing a species that typically uses temporary ponds (*Hyla gratiosa*) to one that uses permanent ponds (*H. cinerea*), Leips et al. (2000) found *H. gratiosa* to be more plastic in the timing of and size at metamorphosis in response to different rates of declining water levels.

The purpose of this study was to investigate the effects of a flashier hydroperiod, such as in an urbanizing wetland or riparian area, on amphibians in the larval stage. This study was conducted using mesocosms which have been used often to study many aspects of larval amphibian ecology including the effects of density (Wilbur and Collins 1973), predation (Relyea 2001), chemical contamination (Boone and James 2003, Rohr et al. 2004, Lefcort et al. 1998), food or resource limitation (Alford and Harris 1988), and desiccation (Brady and Griffiths 2000, Denver et al. 1998). Although mesocosms offer less realism, they do provide the advantage of increased replication in a controlled environment (Ahn and Mitsch 2002). In this experiment, flashier, more variable hydroperiods (typical of urban riparia) were expected induce more rapid development of amphibian larvae with less fitness (smaller body sizes) and overall reduced survival. Comparing species, we also expected species that typically breed in ephemeral pools (e.g. *Gastrophryne carolinensis*) to have higher survival and a better final body condition than species better adapted for permanent breeding pools with constant water levels (e.g. *Lithobates sphenocephalus*).

METHODS

Mesocosm set-up

To evaluate the potential development response by amphibian larvae, two different hydroperiod treatments and a stable control were prescribed for a mesocosm experiment. Experimental hydroperiods were designed to mimic different flooding conditions expected in urban and non-urban wetlands (see Figure 1). The ‘urban’ hydroperiod had post-storm water fluctuations that simulated a flashier, urban flood while including a reduced base flow (i.e., lower water levels in between storm events). The ‘forest’ hydroperiod was characterized by post-storm floods with a more gradual rising/falling limb, lower flood depths, and longer flood durations. These conditions were intended to mimic riparian wetlands in more forested watersheds. A third set of mesocosms was kept as a control and maintained at a constant water level. The experimental hydroperiods were generalized from patterns typical of streams in urban and forested watersheds (Paul and Meyer 2001) but also based on hydrologic data collected directly from wetlands and riparian areas in other studies throughout the SE United States (Rose and Peters 2001, Schoonover et al. 2006, Barksdale 2012, unpublished data). The frequency of storm events (pulses) were based on long term precipitation data of the frequency of spring-frontal systems moving through coastal Alabama (NOAA 2011, <http://www.ncdc.noaa.gov/>) and was set at 10 day intervals.

Mesocosms were established in a greenhouse on the Auburn University campus in Auburn, Alabama. Each mesocosm consisted of an 18.9-L painter bucket with nearly vertical sides. A series of trials were established for each of four species selected for the study starting on 24 March 2012. Each trial consisted of 12 mesocosms with a total of 4 mesocosms per species and hydroperiod treatment (Table 1). Light and temperature conditions were consistent for mesocosms during a trial with the exception of changing water levels. The average day time temperature throughout the study was 27.3°C (81.1°F) and the average night temperature was 23.7°C (74.7°F), with a range of 17.8°C-37.8°C (64°F-100°F). Conditioned or aged tap water was found to kill nearly all young tadpoles in early trials. Therefore, approximately 190 L of water were collected from a nearby pond every 8-10 days, kept in the greenhouse for a day to reach ambient temperature, and filtered before used in mesocosms (with the exception of *G. carolinensis*, see below). Each mesocosm had a plexi-glass ramp to provide structure and edge for tadpoles and reduce the influence of the straight-sided containers.

Tadpole monitoring and hydrologic manipulation

Amphibian egg masses and newly emerged tadpoles were collected as they became available spring 2012 from wild populations in eastern Alabama. For each species, tadpoles/eggs were collected from a common source which included woodland depressions, old field pools, forest dirt road swales, and an abandoned detention pond. Our intent was to examine species that selected for different breeding habitats (Table 1), therefore, four different species were used, *Lithobates sphenocephalus* (southern leopard frog)*, Hyla chrysoscelis* (Cope's gray treefrog)*, Hyla squirella* (squirrel treefrog)*,* and *Gastrophryne carolinensis* (eastern narrowmouth toad). *L. sphenocephalus* and *G. carolinensis* were collected as eggs, and *H. chrysoscelis* and *H. squirella* were collected as newly-hatched tadpoles. Egg masses were kept in 18.9-L buckets until hatched.

Once hatched, tadpoles were maintained similarly aside from hydrologic treatment. The same numbers of tadpoles per mesocosm were kept to avoid any initial density effects on their development. *L. sphenocephalus* were started at five individuals per mesocosm due to their larger size while the other species were started at ten per mesocosm. Initially, three extra buckets of each species/treatment were maintained to provide replacements in order to maintain similar densities in treatment mesocosms for as long as possible. These buckets received the same water level changes, cleaning, disturbance, and food regimes as the mesocosms. Once a tadpole was removed from an extra bucket, the tadpoles in that bucket were excluded from the study.

Food quantity was not meant to be limited in this study. *L. sphenocephalus, H. chrysoscelis,* and *H. squirella* were fed fish meal-based floating food sticks (Tetrafauna ReptoMin), approximately 24 mg during the first ten days and then 43 mg for the remainder of the experiment. Because of its carnivorous habit, a plankton net was used to collect food for *G. carolinensis* from the same pond where water was collected. To increase the likelihood of food availability, *G. carolinensis* mesocosm water was not filtered to provide any additional zooplankton present. For all mesocosms, excess food and tadpole waste was carefully removed on a daily basis using a fine fish net prior to changing water levels.

Water level fluctuations began after one day of acclimation in each mesocosm. Experimental hydroperiods ran through three flooding periods or 30 days for *H. chrysoscelis, H. squirella,* and *G. carolinensis*. *L. sphenocephalus* was run for another ten days due to their longer development period, ending at 40 days (Table 1). Water was carefully added or removed over the experiment to change daily water levels per designated hydroperiods (Figure 1). The control mesocosms received clean water on five consecutive days, similar to the reference treatment water changes. Throughout the experiment, any time that one mesocosm was disturbed all other mesocosms were disturbed as well. An error was made on July 20 when the urban flood treatment of *H. squirella* was only flooded to 10 L instead of the full 16 L.

Tadpole mortality was recorded daily and daily survival probabilities were calculated for each species treatment. Tadpole development rates for each mesocosm were assessed by determining the larval development stage (Gosner 1960) of each animal per pulse (every ten days). At the conclusion of the experiment, the length, mass, and stage of each surviving animal was measured. Mass was measured on to the nearest milligram. Total animal length (including tail) and snout-vent length were measured to the nearest millimeter.

Data Analysis

The Kaplan-Meier procedure (Pollock et al. 1989a) was used to estimate survival functions for each species per treatment. This method estimates the probability of an animal's survival over time using the equation:

$$\hat{S}(t) = \Pi\left(1 - \frac{d_j}{r_j}\right), \qquad j|a_j < t$$

where d_j is the number of deaths at time j, r_j is the number at risk at j, t is time, and a is the time at which a death occurs. Presumed assumptions met for this procedure included random samples, survival times that were independent of each other, capture and handling that did not influence survival, and that the censoring mechanism was random and unrelated to each animal's fate. Staggered entry was not required as all animals were started at the same time (Pollock et al. 1989a). Our censoring mechanism was not entirely random. When the buckets of extras were used, individuals remaining in that bucket were censored, though the individuals initially assigned to those buckets were random. Also some *H. chrysoscelis* individuals (approximately 12) escaped from their mesocosms shortly after metamorphosis and were also censored in this analysis since their fate was unknown. Formulas were calculated and survival curves generated and compared for each treatment x species combination.

Cox-proportional hazard models were run in R to compare the survival of treatments among species. This provided for comparisons of survival functions for each treatment that allow the function to change with increasing time (Hosmer et al. 2008). Using the model results, potential pairwise differences between treatments ($p<0.05$) were examined. Odds ratios calculated from the estimated coefficients (β^e) were used for comparisons and to describe treatments that were more or less likely to elicit animal death compared to another. Assumptions for this model are similar to Kaplan-Meier: random sample, survival not influenced by capture, and independent survival times (Pollock et al. 1989b).

A linear mixed-effects model was used to compare between treatments for the larval development data with mesocosm as a blocking variable to account for individuals within the same mesocosm and avoid pseudoreplication. Autocorrelation between times was not considered an issue because the previous measurement did not necessarily affect how much each tadpole would develop by the next measurement. Assumptions included that samples were random and independent, the relationship was linear, error was homoscedastic and normally distributed, and that the dependent variable was continuous.

Another linear mixed-effects model was also used to compare the final masses and snout-vent lengths between treatments with mesocosm as a blocking variable to avoid pseudoreplication. Pairwise comparisons were performed on significant interactions. R was used to run all linear effects models with significance set at p=0.05.

Because of the high and consistent survivorship of *H. chrysoscelis* across treatments, a post-hoc comparison was conducted to examine body size relative to development stage. The relationships between mass and stage were plotted for the animals remaining at the end of the experiment, compared between treatments, and fitted for polynomial trend lines.

RESULTS

Tadpole Survival

Overall, *H. chrysoscelis* tadpoles had the highest survival rate throughout the experiment (Figure 3.2b, Table 3.2). Urban *H. chrysoscelis* had a higher survival probability (0.81; 95% CI =0.80-0.82) than the other two treatments (forest=0.72; 95% CI=0.71-0.73 and control=0.80; 95% CI=0.79-0.81) although all were high. Based on Cox proportional hazard models, *H. chrysoscelis* in the forest and urban treatments were 1.54 (p=0.25; 95 % CI=0.74-3.17) and 1.23 (p=0.61; 95% CI=0.56-2.70) times more likely to die as the control, though neither difference was statistically significant (Table 3.3). No significant difference was detected for comparison between forest or urban (p=0.56). For the other three species, mortality increased at points of major physiological changes (mostly when front or hind limbs were emerging). *L. sphenocephalus* in the control mesocosms had higher survival probabilities (0.52; 95% CI=0.50-0.54) than the other treatments (forest=0.19; 95% CI=0.18-0.21 and urban=0.16; 95% CI=0.15-0.17, Figure 3.2a, Table 3.2). According to the Cox proportional hazards model, *L. sphenocephalus* tadpoles in the forest and urban treatment were 1.73 (p=0.13, 95% CI= 0.86-3.50) and 2.49 (p=0.01; 95% CI= 1.30-4.74) times as likely to die as the control, respectively and urban *L. sphenocephalus* were 1.44 (95% CI=0.76-2.76) times as likely to die as forest tadpoles (Table 3.3). Urban *H. squirella* had a much higher survival probability (0.60; 95% CI=0.59-0.61) than the other treatments (forest=0.09; 95%=0.09-0.10 and control=0.18; 95% CI=0.17-0.19, Figure 3.2c, Table 3.2) and based on the hazard model, *H. squirella* tadpoles in the forest treatment were 1.22 (p=0.38; 95% CI=0.78-1.92) times as likely to die as the control, and tadpoles in the urban treatment were 0.33 (p<0.001; 95% CI=0.18-0.59) times as likely to die (Table 3.3). *G. carolinensis* had low survival overall (Figure 3.2d, Table 3.2). Urban *G. carolinensis* had the highest survival probability (0.22; 95% CI=0.21-0.23) while the control had a probability of 0.11 (95% CI=0.11-0.12) and the forest treatment had the lowest (0.05; 95% CI=0.04-0.05) (Table 3.2 and Figure 3.2d). In this hazard model, *G. carolinensis* in the forest treatment were 2.50 times as likely to die (p<0.001; 95% CI=1.63-3.86) as the control and tadpoles in the urban treatment were 0.99 times as likely to die (p=0.97; 95% CI=0.62-1.58) (Table 3.3).

Tadpole Development

Within each mesocosm, tadpoles grew at different rates and often there were one or two tadpoles noticeably larger or smaller than the others. *L. sphenocephalus* in the forest treatment developed faster than the other treatments until the last staging interval when the urban tadpoles were farther along (Figure 3.3a) however no significant difference was detected among stages. *H. chrysoscelis* were consistent with the forest treatment being slightly more developed than the other two (Figure 3.3b) but not significantly at any stage. The forest treatment tadpoles also developed faster in *H. squirella* with the urban treatment being the slowest, although the urban treatment also had the highest survival compared to the other two treatments (Figure 3.3c, Table 2). *G. carolinensis* only had 15 individuals remaining at the completion of the experiment. The one tadpole in the forest treatment was more developed than any of the others. The control tadpoles also appeared to have grown faster than the urban treatment towards the end, though with fewer individuals remaining (Figure 3.3d, Table 3.2). Average stages for all species within each treatment at each interval are provided in, Appendix B.

L. sphenocephalus, H. chrysoscelis, and *H. squirella* had no significant differences in stages between treatments at each time (Figure 3.3). *G. carolinensis* had significant differences in stage between treatments at day 10, 20, and 30, however by the second staging, only 18 tadpoles total remained in the experiment and only two tadpoles remained in the forest treatment (Figure 3.3d) so comparisons are influenced by small sample sizes.

Tadpole Final Mass

L. sphenocephalus showed no significant differences between treatments in final mass or length (Table 3.4). Among the 30 *H. squirella* tadpoles remaining at the conclusion of the trial, no significant differences were detected between treatments for mass or length (Table 3.4); however, sample sizes were unbalanced between treatments with 23 in the urban treatment and only three and four remaining in the other treatments (Table 3.2). Likewise, 15 *G. carolinensis* survived to the end of the experiment however only one tadpole remained in the forest flood regime, five in the control, and nine in the urban treatment, making comparisons questionable (Table 3.2). There were no significant differences in size detected between treatments of *G. carolinensis* (Table 3.4).

Among the 89 *H. chrysoscelis* measured after 30 days, the mean masses of tadpoles were 0.34 g ±0.03 g (p=0.021) in the forest treatment, 0.26 g ±0.02 g in the control, and 0.27 g ±0.02 g (p=0.64) in the urban treatment (Table 3.4). Forest tadpoles also had significantly longer snout-vent lengths (0.11 cm ±0.04 cm; p=0.021) than control tadpoles, as did urban tadpoles 0.03 cm (±0.04 cm; p=0.402). Figure 3.4 shows the relationship between final mass and stage of tadpoles. A peak in mass was detected around stage 40 for most tadpoles preparing to grow front legs and to resorb their tails. Trend lines showed that tadpoles in the forest treatment generally had larger masses midway through development than the urban treatment or the control.

DISCUSSION

The goal of this experiment was to determine if changes in wetland and riparian flooding regimes that result from urban development have an impact on anuran larvae. Examining four different amphibian species with different breeding preferences, we found that some species did seem to respond to water level fluctuations while others did not. The mortality results of *L. sphenocephalus* seemed to fit with its breeding habitat preferences which typically are shallow, lentic pools where the threat of desiccation is minimal (Butterfield et al. 2005). Control mesocosms with stable water levels had the highest survival, followed by the gradual water level changes, while the flashy, urban treatment had the lowest survival. These results also fit with the findings of Wilbur (1987) who found that *L. sphenocephalus* only survived in low-density, no predator treatments that had slow drying or no drying at all. Leopard frogs breed early in the spring and tadpoles have a relatively long larval period, approximately 50-70 days from hatching to metamorphosis (Butterfield et al. 2005), so breeding habitats need to be stable enough to be flooded for that length of time.

H. chrysoscelis lay their eggs in natural or disturbed, man-made shallow pools that can be temporary or semi-permanent (Cline 2005). Their high survival rate in this experiment may be a reflection of how *H. chrysoscelis* utilize a wide range of oviposition sites and could be tolerant of a wide range of conditions, including variable flooding regimes. *H. chrysoscelis* was the only species that showed an effect of treatment on their final size (Table 3.4). The mean mass of tadpoles from the forest treatments (0.34 g, p=0.021) was notably larger than both urban and control (0.27 g and 0.26 g) respectively although there was only a statistical difference with control. Tadpoles during their mid-stages (36-40) in the forest treatment were larger than the control and urban treatment (Figure 3.4). Having a larger size at this stage of development could be beneficial in that these tadpoles will have more energy stores to complete metamorphosis. A gradual flood could provide the advantages of more water and space available per tadpole, decreasing the number of interactions and diffusing waste in the water. Generally, a larger larval size is advantageous to amphibians. Berven (1990) found that wood frogs (*L. sylvatica*) that metamorphosed at larger sizes had greater survival to return to breeding pools in following years. In addition, Smith (1987) found that though a larger size did not affect survival, western chorus frogs (*Pseudacris triseriata*) that metamorphosed earlier and at a larger size were more likely to reproduce sooner, only one year later rather than two.

H. squirella are also generally not particular in their habitat selection and are known to breed in various shallow water bodies, including wetlands, roadside ditches, and ponds (Mitchell and Lannoo 2005a). This species had a similar trend as *H. chrysoscelis* though lower overall survival, with significantly fewer deaths in the urban treatments than the control. Though insignificant, the urban treatment had the largest body mass and the shortest length compared to other treatments. The urban treatment also had the most individuals remaining (Table 3.2). The tadpoles left in the control and forest treatment were all past stage 40 by the completion of the trial. It is possible that the larger individuals in the other treatments died off sooner while going through metamorphosis.

Feeding was an issue that likely influenced the survival and other measures of *G. carolinensis*. *G. carolinensis* is one of the few species in North America that are carnivorous filter feeders as tadpoles, primarily feeding on zooplankton in the water. Whereas with the other species tested, it is known that they received enough food daily to have surplus, this was not clear with *G. carolinensis* and the quantity of zooplankton they received daily was uncertain. *G. carolinensis* tends to breed in fairly ephemeral pools that may include flooded fields, temporary ponds, flooded ditches, and edges of permanent ponds (Mitchell and Lannoo 2005b). If survival had been higher, perhaps they may have done well in the flashy hydroperiod since they are adapted to use habitats subject to rapid desiccation.

There were no significant relationships found between treatments in the development rates for any species, though we expected tadpoles in urban treatments to develop faster to escape the potential desiccation. In this experiment, tadpoles were subjected to floods periodically and experienced them three or four times so this disturbance impacted them at early, intermediate, and late stages. Tadpoles have shown plasticity in their responses to threats within their aquatic environment (Denver et al. 1998, Relyea 2001); however, in this case neither of the experimental treatments resulted in significantly faster development than the control. This treatment may not have been substantial enough to prompt that type of response. It is possible that water levels in this experiment did not drop low enough to trigger a response to potential desiccation. Other studies have shown that tadpoles can respond to water levels decreasing by accelerating development (Laurila and Kujasalo 1999, Loman 1999, Bridges 2002, Gervasi and Foufopoulos 2008), or a decrease in size of tadpoles in desiccating pools (Brady and Griffiths 2000) or both (Marquez-Garcia et al. 2009). Denver et al. (1998) found that *Scaphiopus* tadpoles could reverse their accelerated development and gain mass to compensate if pools refilled.

Studies suggest that the rate of tadpole development is not necessarily always set at the same time during the larval period. It can be dependent upon multiple factors available resources and conditions of their environment. Alford and Harris (1988) found that *Anaxyrus woodhousei fowleri* tadpoles subjected to different food levels responded to the most recent condition of food availability in terms of growth and timing of metamorphosis. After experiments altering food levels for *H. gratiosa* and *H. cinerea* tadpoles, Travis (1984) and Leips and Travis (1994) found that only changes in the earliest time period affected the timing of metamorphosis, suggesting that the rate of metamorphosis is set early in their development. The quantity of food was held constant between all mesocosms regardless of the number of tadpoles so mesocosms with fewer tadpoles had access to more food. In this case, our tadpoles received more food later in their development as their density was reduced. According to Alford and Harris (1988), this should have made tadpoles in those treatments larger by the end, but according to Travis (1984) and Leips and Travis (1994), this rate would have been set earlier when densities were consistent among mesocosms and later mortality of neighbors would not have allowed any advantage later on. In this case, we can consider differences in tadpole size and development regardless of unbalanced numbers.

Survival to metamorphosis is typically low in natural populations. Calef (1973) found that tadpole survival to metamorphosis of *Lithobates aurora* in British Columbia was around 5%, with 60-80% dying within the first three weeks. Herreid and Kinney (1966) also found a mortality rate of 96% for *L. sylvatica* in several ponds in Alaska. Various environmental factors such as climate, temperature, desiccation, predation, and the prevalence of fungal infections can greatly influence survival but other biological processes play a part in mortality as well (Herreid and Kinney 1966, Anderson et al. 1971, Calef 1973). *L. sphenocephalus* had the highest survival in the control with no water level fluctuation, while *H. squirella* and *G. carolinensis* had the highest survival in the urban treatments. This reflects the natural tendencies of *L. sphenocephalus* to choose pools filled early in the spring that should not be in danger of desiccation or dramatic fluctuations. *H. squirella* is capable of taking advantage of many different bodies of water, including those only temporarily available after storms, such as pastures and ditches. *G. carolinensis* lays eggs in highly ephemeral pools that flood after storm events and can be subject to water level changes faster than some other types of habitat. *H. chrysoscelis* had fairly high survivorship among all treatments, possibly because they are also adaptable to a range of conditions in their breeding habitat.

In spite of best attempts, mesocosms cannot fully mimic natural conditions for larval anurans. Even though ramps were provided for structure, these did not perform the way a natural pond shoreline would and likely did not allow for temperature stratification that typically occurs (Jacobs et al. 1997). The food supply was relatively constant and did not vary with floods. If a natural pond is filled with water, tadpoles may have access to more plant material recently submerged along the edge. Intense floods also have the potential to flush eggs or larvae from their developmental pools but may allow for competitive release and benefit those individuals remaining. Petranka and Sih (1986) found that during years of intense floods, fewer larval smallmouth salamanders (*Ambystoma texanum*) survived the early larval period but had greater survival later and metamorphosed sooner and at larger sizes with overall survival rates were similar to years without floods. In a comparison of streams surrounded by different land uses, Barrett et al. (2010) found larger two-lined salamander larvae in urban watersheds, though those populations typically had lower survivorship, and suggested that the cause may be the flushing of larvae in streams with increased spate frequency and magnitude. This effect was not evaluated in this study as tadpoles were contained in their mesocosms though the displacement or additional mortality likely gives the survivors an advantage. In addition, floods may not have fluctuated enough to elicit a response to the threat of desiccation from any of these species. If this experiment were repeated, more replicates could be used to buffer for the expected mortality and the minimum water levels lowered.

CONCLUSION

A mesocosm study was conducted in 2012 to examine the role of flooding regime on amphibian larval development. Four species (*L. sphenocephalus, H. chrysoscelis, H. squirella,* and *G. carolinensis*) were subjected to three different riparian flooding regimes: 1) flashier floods and lower base-level, typical of urban conditions, 2) a more gradual flood typical of forested watersheds, and 3) a control kept at a stable water level. Examining the influence of hydroperiod on larval amphibian survival, development rate, and size, this study suggests that effects differ depending on the species. A species that typically selects for stable, lentic waters, *L. sphenocephalus,* had greater survival in the control and forest hydroperiod than in the urban treatment and may suffer from increased disturbance in habitats with severe water level fluctuations. *H. chrysoscelis* tadpoles in the forest treatment had larger body sizes than in stable and urban treatments with high survival across all treatments, indicating it was fairly adaptable to all hydroperiods but having a slight preference for the forested treatment which potentially confers the benefits of flooding but without the rapid fluctuation. Flood water levels likely influence amphibian larvae in conjunction with other flooding effects (e.g., increased flow and sediment loads) to alter environments and act to subtly structure species assemblages.

Table 3.1. Mesocosm species, trial dates (2012), number of mesocosms, and breeding preferences for each species used in mesocosm study.

Species	Trial Date	Number of Mesocosms per Hydroperiod Treatment (n=)			Reported Breeding Habitat Preference
		Forest	Urban	Control	
Lithobates sphenocephalus	3/24-5/2	4	4	4	Permanent/semi-permanent woodland (Mount 1975, Butterfield, Lannoo, and Nanjappa 2005)
Hyla chrysoscelis	5/17-6/14	4	4	4	Semi-permanent or temporary (Mount 1975, Cline 2005)
Hyla squirella	7/18-8/16	4	4	4	Semi-permanent (Mitchell and Lannoo 2005a)
Gastrophryne carolinensis	7/21-8/19	4	4	4	Highly ephemeral (Mitchell and Lannoo 2005b)

Table 3.2. Final survival probabilities (95% confidence interval) and tadpole count for each species per hydroperiod treatments and control.

	Forest		Urban		Control	
Species	Surv. Prob.	Final No.	Surv. Prob.	Final No.	Surv. Prob.	Final No.
L. sphenocephalus (40 days)	0.19 (0.18-0.21)	5	0.16 (0.15-0.17)	4	0.52 (0.50-0.54)	12
L. sphenocephalus (30 days)	0.66 (0.64-0.68)	20	0.35 (0.34-0.36)	13	0.78 (0.76-0.79)	18
H. chrysoscelis	0.72 (0.71-0.73)	25	0.81 (0.80-0.82)	26	0.80 (0.79-0.81)	38
H. squirella	0.09 (0.09-0.10)	3	0.60 (0.59-0.61)	23	0.18 (0.17-0.19)	4
G. carolinensis	0.05 (0.04-0.05)	1	0.22 (0.21-0.23)	9	0.11 (0.11-0.12)	5

Table 3.3. Results of Cox proportional hazards model comparing treatments for each species, including coefficient values, exp(coefficient), standard errors, and p-values. Coefficient values are raised to *e* for interpretation. Treatment listed first was used as reference in the model.

	Control x Forest				Control x Urban				Forest x Urban			
	Coeff.	Exp(coef)	SE	p	Coeff.	Exp(coef)	SE	p	Coeff.	Exp(coef)	SE	p
L. sphenocephalus	0.55	1.73	0.36	0.13	0.91	2.49	0.33	0.01	0.37	1.44	0.33	0.26
H. chrysoscelis	0.43	1.54	0.37	0.25	0.21	1.23	0.40	0.61	-0.22	0.80	0.38	0.56
H. squirella	0.20	1.22	0.23	0.38	-1.12	0.33	0.30	<0.001	-1.31	0.27	0.28	<0.001
G. carolinensis	0.92	2.50	0.22	<0.001	-0.01	0.99	0.24	0.97	-1.06	0.35	1.09	0.33

Table 3.4. Mean (± SE) of final body mass (g) and snout vent lengths (SVL) (cm) for species subjected to different hydroperiod treatments. Letters denote significant differences ($p<0.05$) between hydrologic treatments per species based on linear effects model.

Species	Forest	Urban	Control
		Mass	
L. sphenocephalus	0.74 (0.19)a	0.81 (0.12)a	0.73 (0.06)a
H. chrysoscelis	0.34 (0.03)a	0.27 (0.02)ab	0.26 (0.02)b
H. squirella	0.18 (0.03)a	0.25 (0.02)a	0.23 (0.01)a
G. carolinensis	0.19a	0.09 (0.02)a	0.16 (0.03)a
		SVL	
L. sphenocephalus	1.72 (0.14)a	1.78 (0.11)a	1.73 (0.08)a
H. chrysoscelis	1.28 (0.02)a	1.20 (0.03)ab	1.17 (0.02)b
H. squirella	1.30 (0.10)a	1.13 (0.04)a	1.23 (0.11)a
G. carolinensis	1.10a	0.76 (0.05)a	0.94 (0.05)a

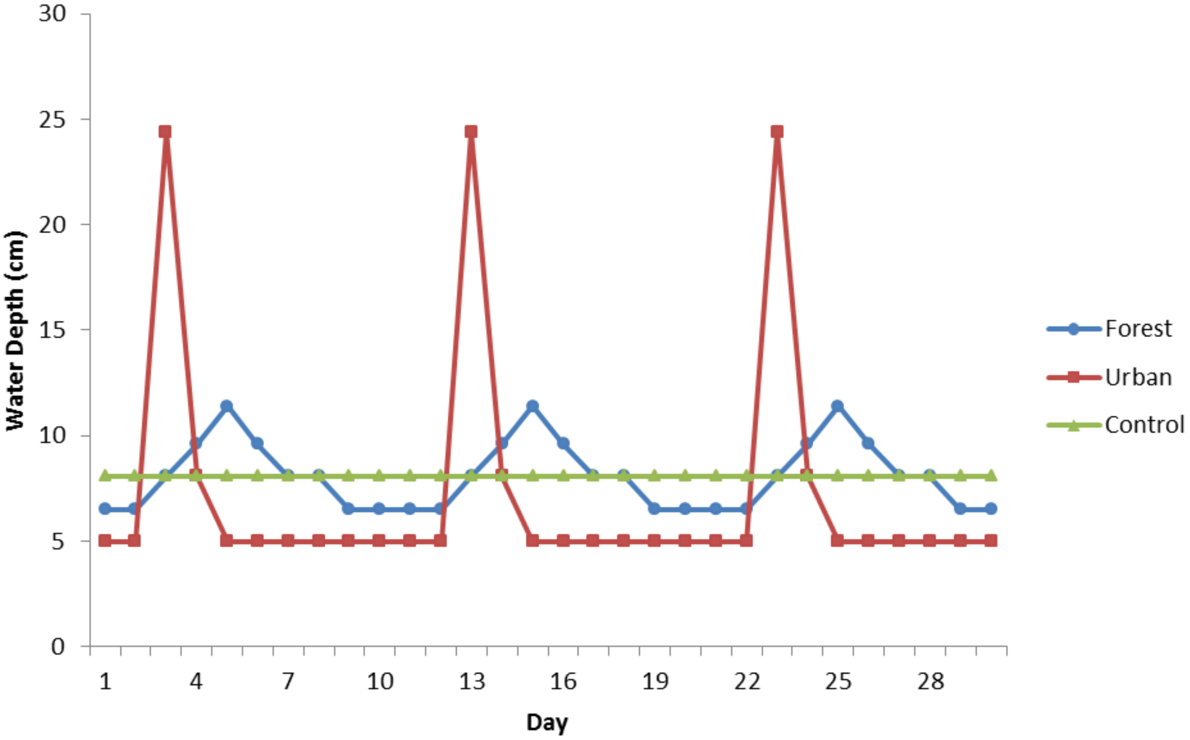


Figure 3.1. Hydrograph of experimental and control hydroperiods used in wetland mesocosms.

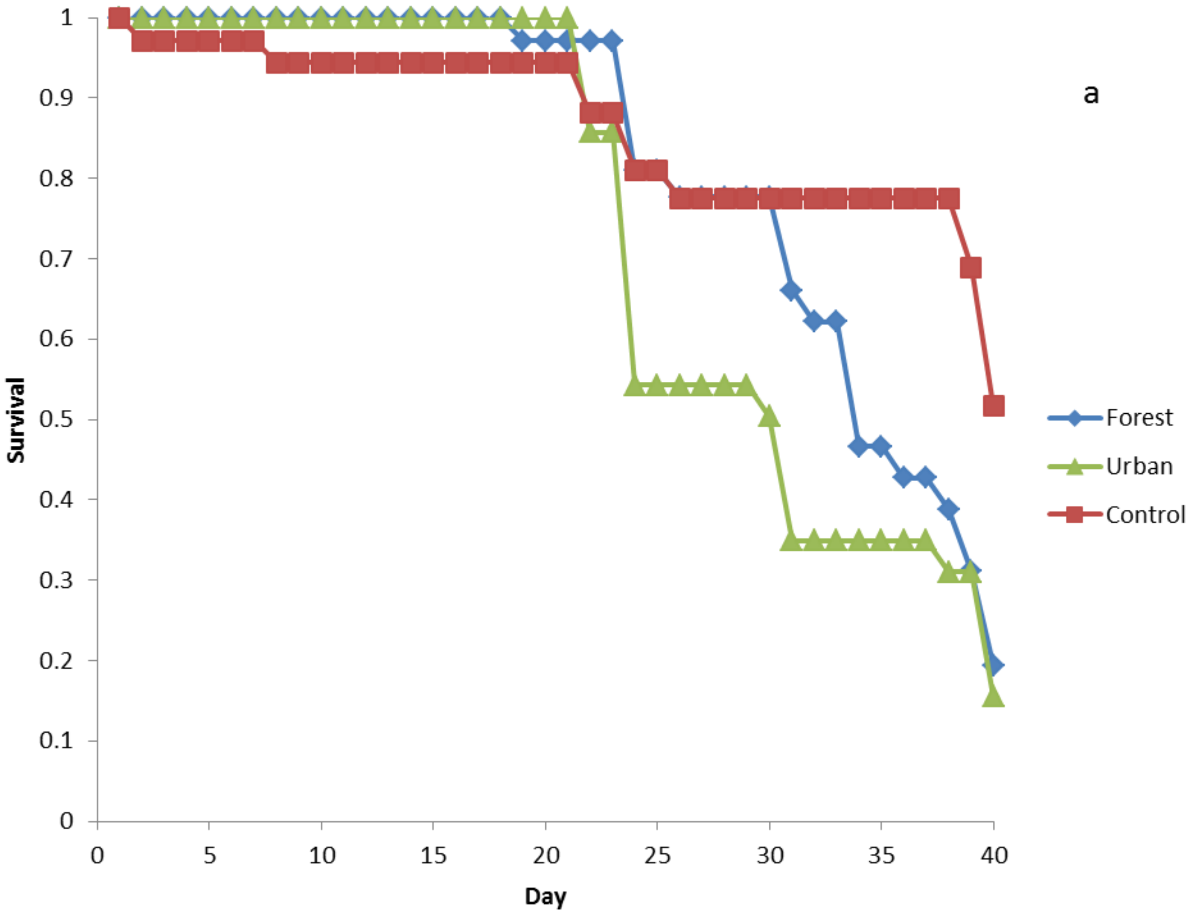
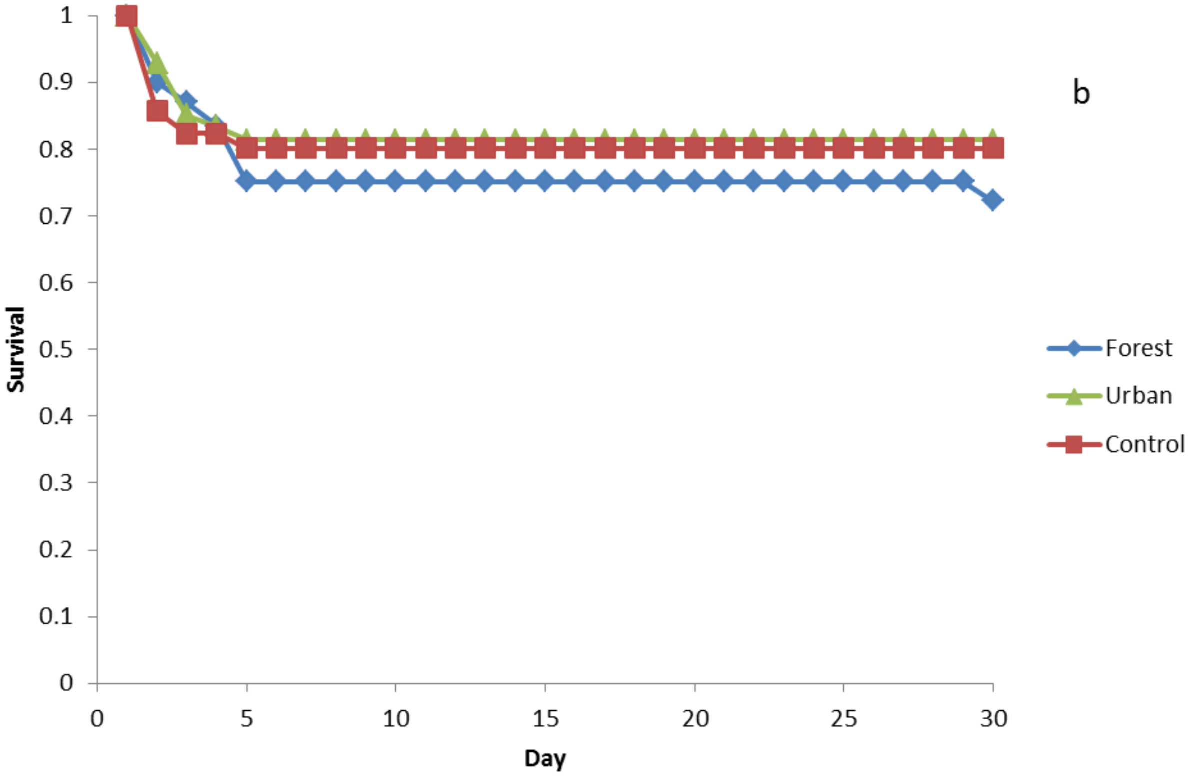


Figure 3.2. Kaplan-Meier survival functions of **a)** *L. sphenocephalus,* **b)** *H. chrysoscelis,* **c)** *H. squirella,* and **d)** *G. carolinensis* for hydrologic treatments and control.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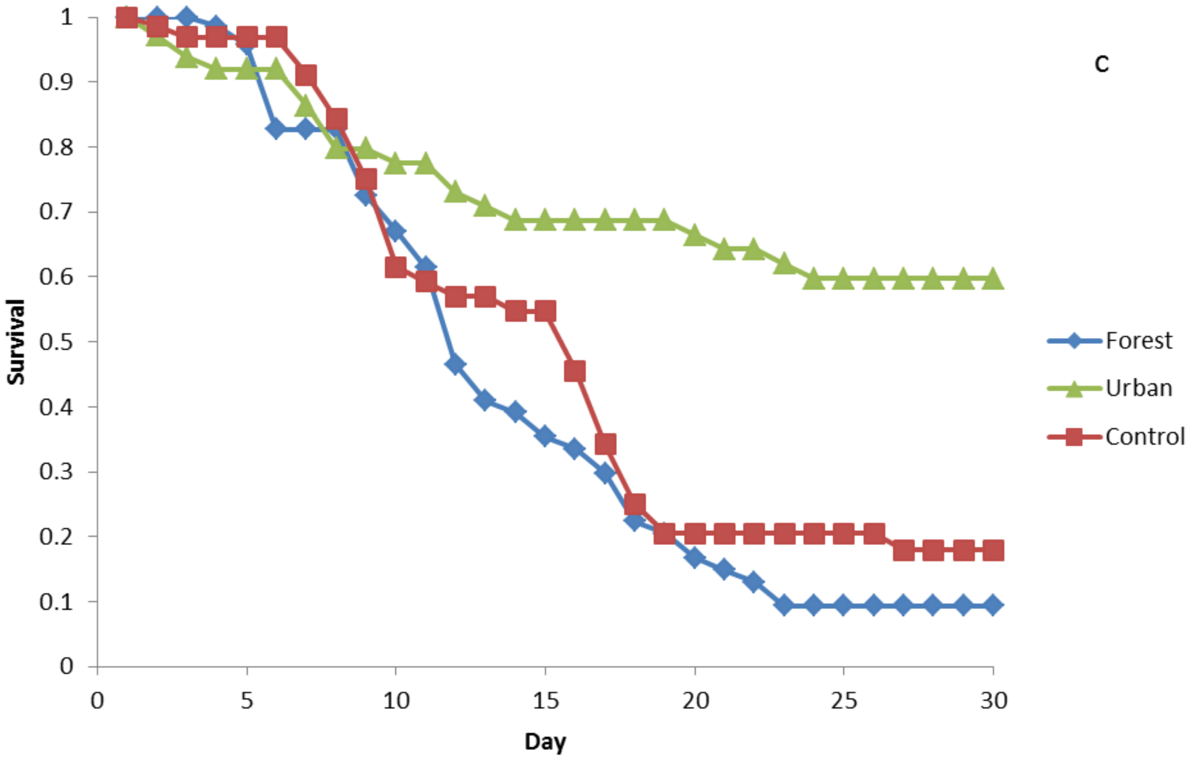

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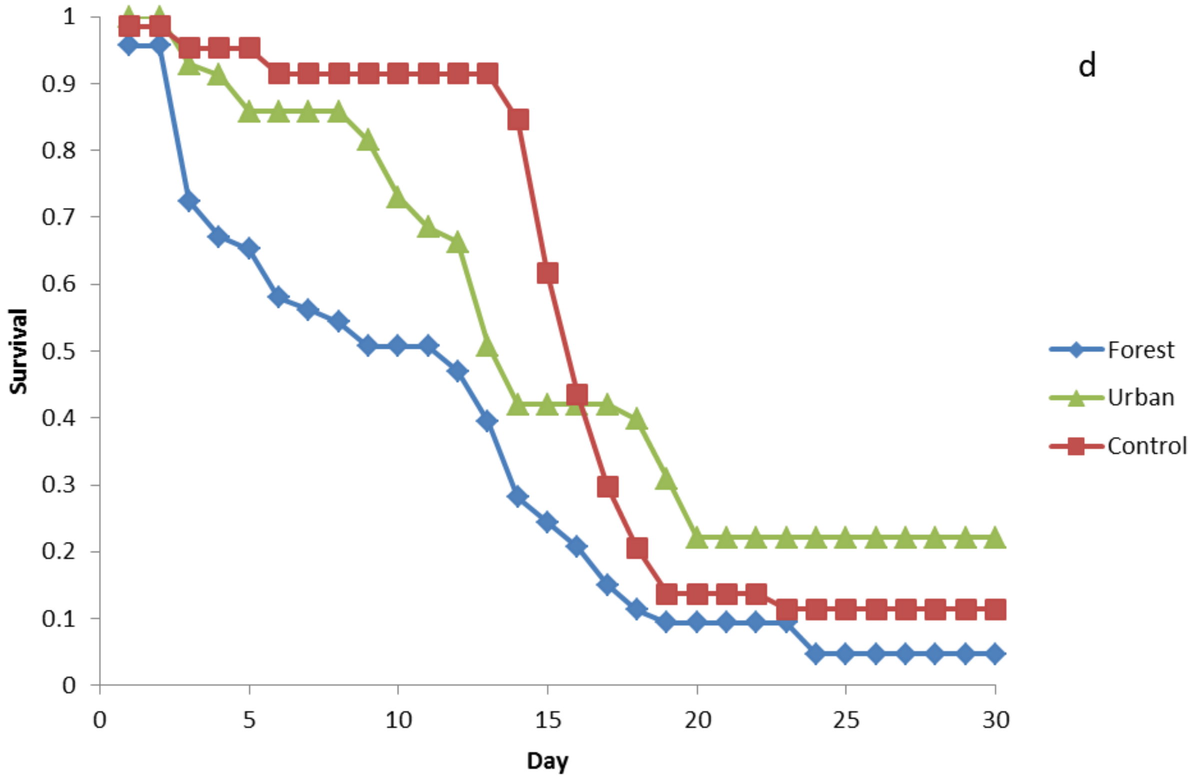
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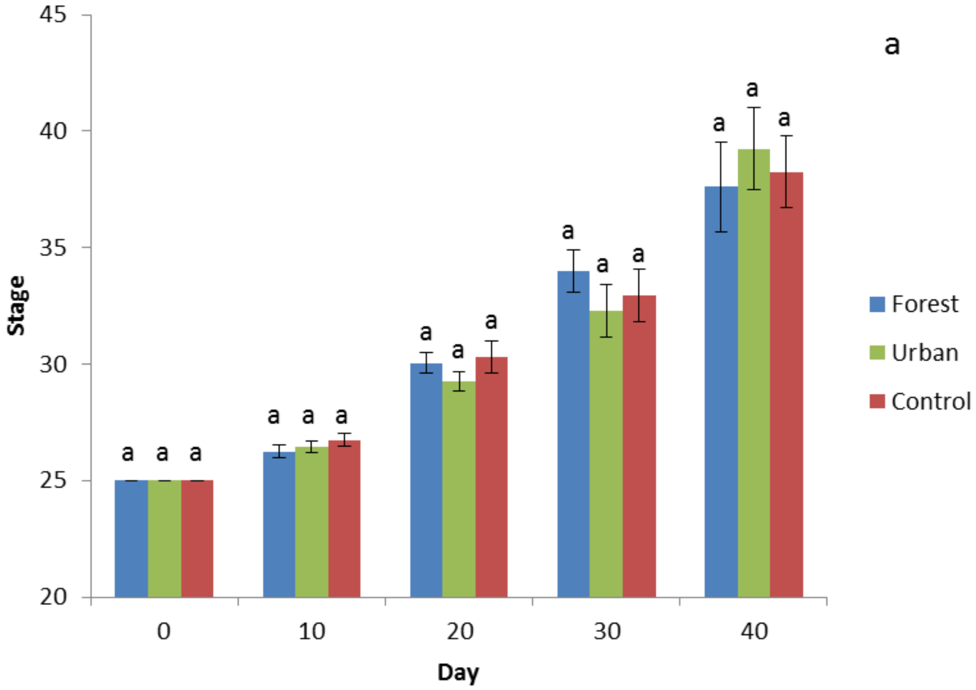
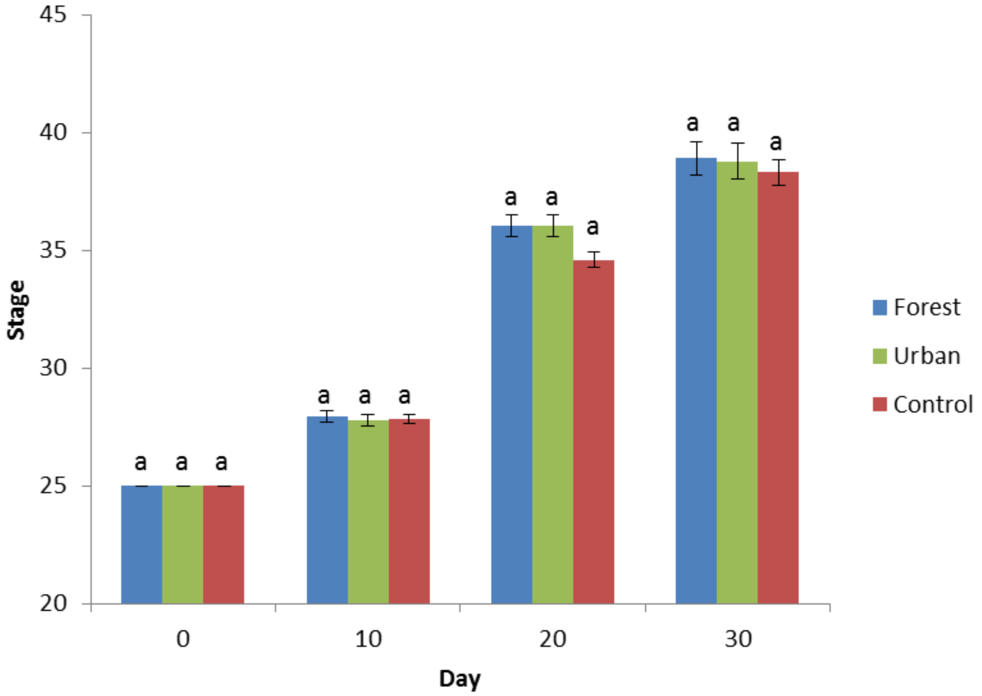


Figure 3.3. Mean (± 1 SE) stage of tadpoles per treatment after each flooding cycle for **a)** *L. sphenocephalus,* **b)** *H. chrysoscelis,* **c)** *H. squirella,* and **d)** *G. carolinensis.* Letters denote significant differences ($p<0.05$) between treatments among staging days per mixed-effects model.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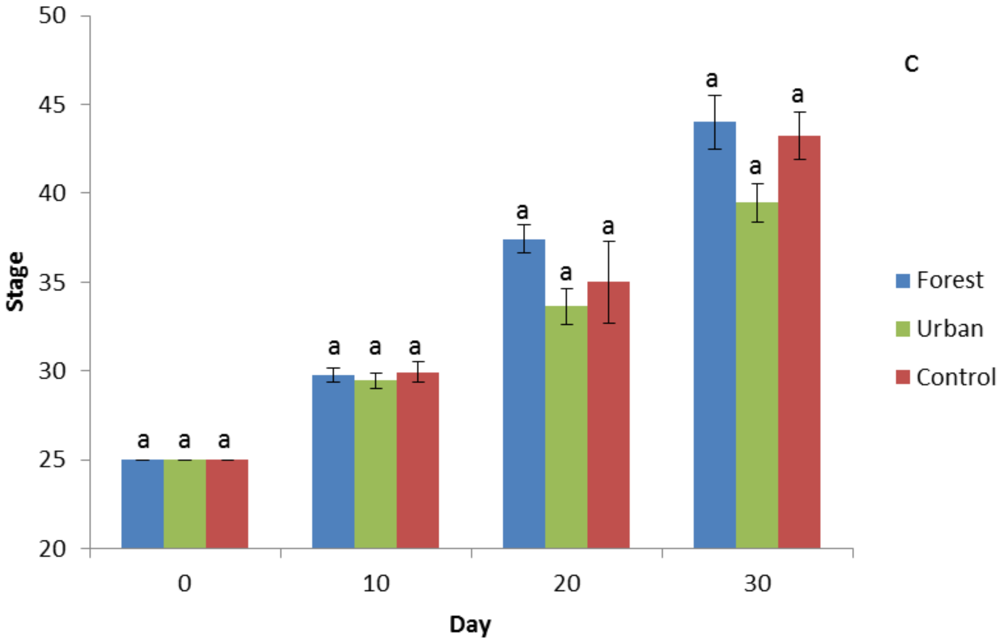
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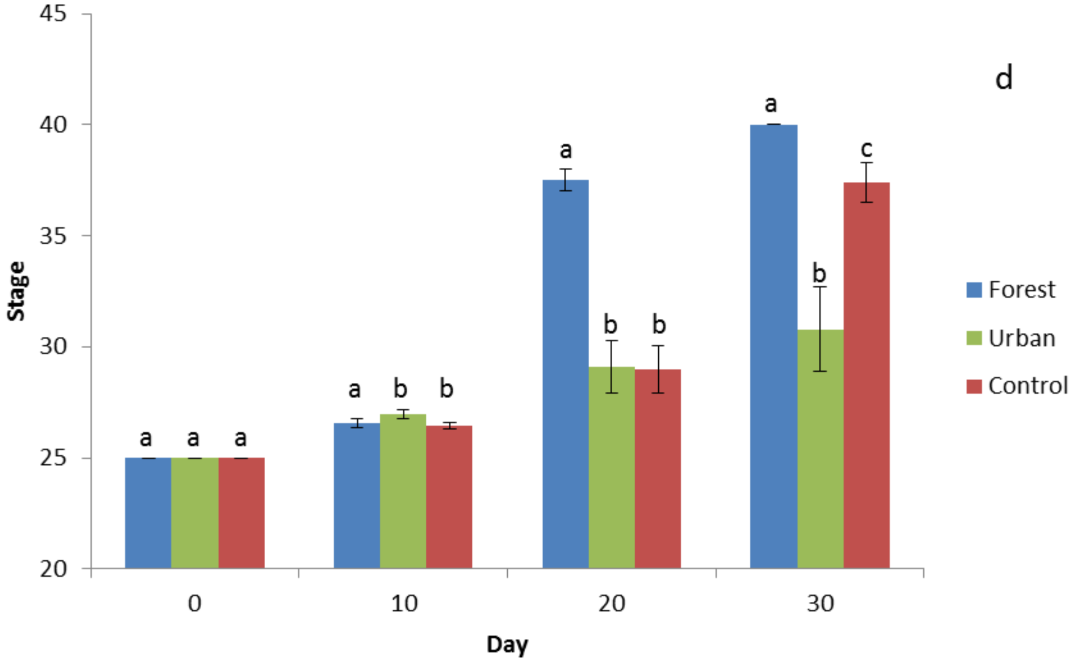
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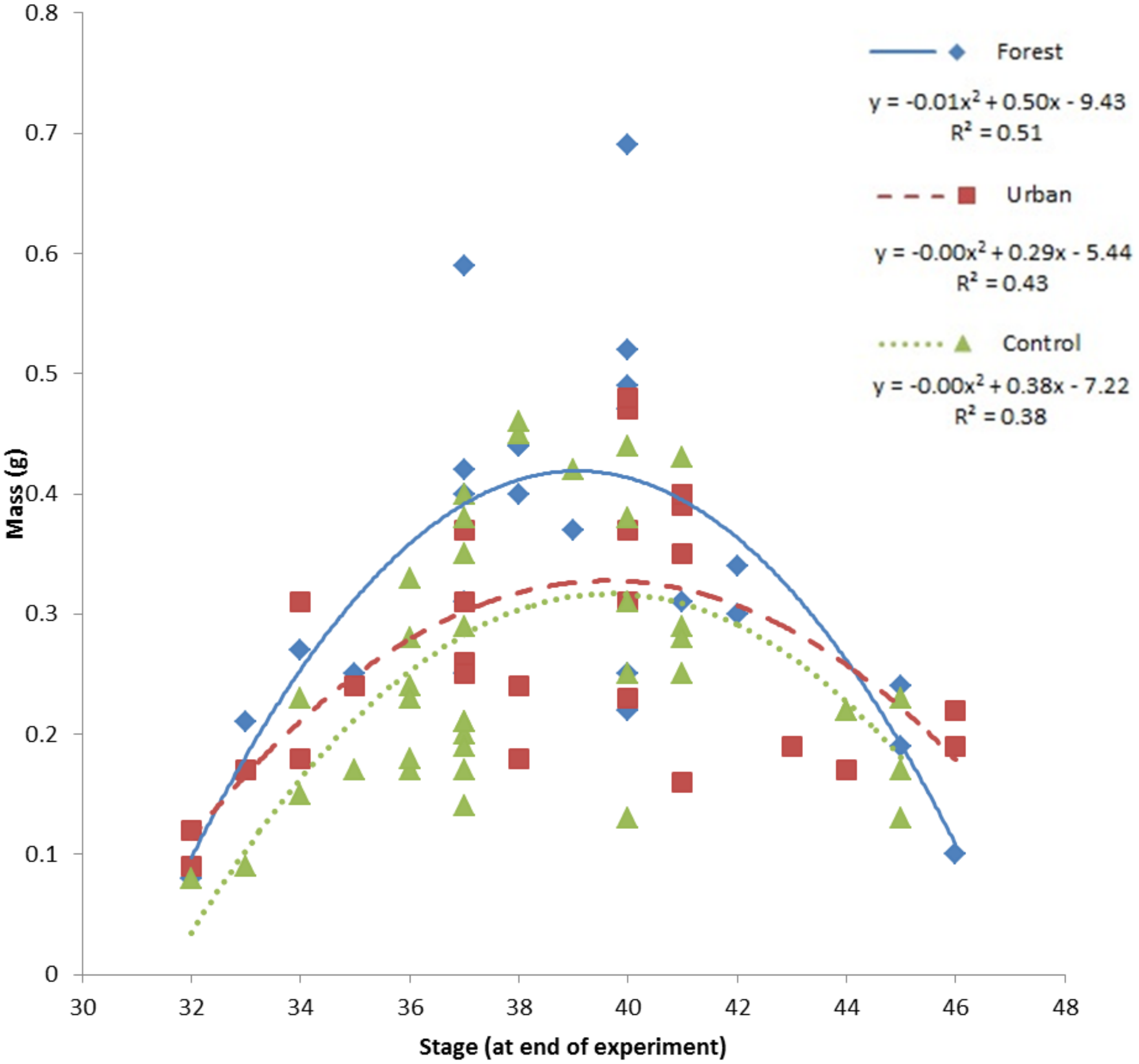

Figure 3.4. Relationship between final mass and stage for *H. chrysoscelis* for hydroperiod treatments and control.

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Chapter 4: SUMMARY

Land use alterations are known to impact wildlife populations and habitat in numerous ways. Some of the most influential ways are through the direct loss and modification of land necessary for life functions. Amphibians are particularly vulnerable as a group considering their dependence upon multiple habitat types, semi-permeable skin, and sometimes aquatic larval stages. Global amphibian declines have been recognized since the 1970s and more attention has been directed at the numerous causes that tend to interact. Urbanization and land clearing influence stream and wetland hydrologic regimes. Increases in impervious surface area within watersheds cause storm water to runoff faster and create flashier floods that are more disruptive to aquatic habitats. This was the basis for a study started in 2011 in Baldwin County, Alabama to examine the impacts of land use change on amphibians within this region.

This study tied together a field project and a mesocosm experiment. The field portion found that though species richness values were similar between wetlands with different surrounding land uses, wetlands within a forested landscape had more incidents of more rarely detected, sensitive species. We also found that species responded to different land uses differently. *Acris gryllus* was negatively influenced by agricultural land use surrounding wetlands, while *Hyla squirella, Lithobates clamitans,* and *L. sphenocephalus* were found to have positive associations with agricultural land use. Only one species was shown to respond to urban land use around wetlands. The non-native *Eleutherodactylus planirostris* was positively associated with impervious surface area within a 200-m buffer of wetlands.

A mesocosm experiment tested the response of tadpole development and survival to different flooding regimes expected based on urban and forest land cover in wetland watersheds. Results varied once again dependent upon the species examined. *L. sphenocephalus,* a species typically associated with deeper, stable water sources for breeding, fared better in stable water level treatments than in treatments with water level fluctuations. *H. chrysoscelis* and *H. squirella,* two generalist species in their breeding preferences, represented this tendency in different ways. *H. chrysoscelis* had fairly high survival in all treatments but individuals remaining in the forested treatment at the end of the trials were longer and had larger body masses than tadpoles in urban or control water level treatments. Though not statistically significant, *H. squirella* tadpoles had higher survival in urban water level treatments. *Gastrophryne carolinensis* was tested as well but had high mortality likely due to issues with feeding so it was difficult to make any inferences from this trial.

The results of these two projects demonstrate that the influences of land use change upon amphibians are not necessarily consistent and depend on the species examined. Some animals can benefit from human modifications, such as *L. sphenocephalus* that may be taking advantage of farm ponds present in agricultural landscapes, but are negatively influenced within an urban landscape where hydrologic fluctuations can cause increased tadpole mortality. Some amphibians are less likely to be found among altered habitats and may only persist in unaltered wetlands, such *A. quercicus, Eurycea quadridigitata* and *Desmognathus conanti* that were only found in wetlands surrounded by forested land.

We also demonstrated that wetlands within a mixed landscape composition can serve as suitable habitat for a number of species, especially in this region for those adapted to agricultural land use. Two ranid frog species, *Lithobates clamitans* and *L. sphenocephalus* were positively associated with agricultural land use. Agriculture can impact the landscape in various ways depending on what crops or livestock are being kept, how land is cleared, and the irrigation systems in place. Approximately 190,000 acres are in agricultural production in Baldwin County, AL with about 80% of operating farms on relatively small tracts of land (<180 acres) (USDA 2009). The top crops in this area are peanuts, forage, cotton, soybeans, and sod, comprising 54.28% of cropland in use (USDA 2009). Although not investigated in this study, agricultural ponds can serve as breeding habitat for amphibians within mixed landscapes, particularly ponds surrounded by crops and lacking fish (Knutson et al. 2004). Even ponds maintained for cattle access may provide valuable breeding habitat for some amphibian species (e.g. *Anaxyrus americanus*) while reproductive output may be reduced for others (e.g. *L. clamitans*) (Burton et al. 2009). Agricultural pesticides can also negatively impact amphibians exposed to them. Pesticides can directly kill larvae in the aquatic environment or combine with other stressors to increase lethal effects (Relyea 2004) or may become wind-borne and impact amphibians farther from the source of the direct disturbance (Davidson et al. 2002).

The response to impervious surface area within watersheds was not as strong as anticipated. Only one species, *Eleutherodactylus planirostris*, was determined by models to be influenced by impervious surface area. This frog is a non-native species introduced through the plant nursery trade and reproduces through direct development, which does not require an aquatic larval stage (Kraus et al. 1999). These animals were found in wetlands that typically contained little surface water and appeared impacted by altered flood regimes and sedimentation. Interestingly, the species impacted by flashy floods in the mesocosm experiment, *L. sphenocephalus*, did not co-occur with *E. planirostris* in any of these wetlands. *L. sphenocephalus* appears to be somewhat of a generalist; these frogs did occupy some of the degraded wetlands and models indicated a positive response to agricultural development. The hydrologic alterations that accompany urbanization, however, could potentially be the factor excluding *L. sphenocephalus* and giving *E. planirostris* an advantage.

Terrestrial habitat is well recognized as an important component of amphibian population survival. Many species use wetland habitat during the breeding season and return to terrestrial uplands for the remainder of the year for cover, forage, and hibernation/aestivation (Gibbons 2003). It has been shown that wetlands with more forest cover tend to support more diverse communities and higher species richness than disturbed wetlands (Findlay and Houlahan 1997). In addition, corridors are necessary for exchange of individuals and metapopulation persistence (Gibbons 2003). This study supports the idea that undisturbed wetlands with surrounding forest cover can harbor higher amphibian diversity and activity (Table 2.3, Table 2.4) than altered ecosystems.

Habitats with variable hydroperiods are necessary for maintaining a diversity of species with different hydroperiod requirements for larval development. Babbitt et al. (2003) found that different species of amphibian utilized wetlands with short, intermediate, and long hydroperiods, indicating that maintaining a matrix of wetlands with variable hydroperiods is important for amphibian conservation. Snodgrass et al. (1999) also concluded that wetlands with short hydroperiods supported unique species assemblages. This field study examined sites with a range of hydroperiods that were largely influenced by storm events and stormwater runoff. One wetland (No. 28) that was permanently flooded tied for the highest species richness (n=10) with a relatively undisturbed site (No. 101) at a state park. This wetland had four species of ranid frog present including *Lithobates catesbeianus* and *L. grylio*, which were not detected at any other wetland, as well as a fully aquatic salamander and salamander larvae (Table 2.3). In this case, the presence of permanent water boosted the number of species detected.

Our results of species specific responses to stressors are not surprising. Amphibians are a diverse group and variation should be anticipated. Leips et al. (2000) also found developmental differences between two closely related species with different preferences for breeding habitat hydroperiods. *Hyla gratiosa* was more plastic in the timing of development in response to water level manipulation than *H. cinerea*, which breeds in permanent ponds (Leips et al. 2000). Davidson et al. (2002) also found varied responses to land use change and wind-borne agricultural pesticides among declining populations of amphibians in California with some species persevering in modified habitats.

Incorporating uncertainty in sampling wildlife populations was another important component of this project. Unless a species has a detection probability of 1 (such as *Pseudacris crucifer* in our study), it is unlikely that it will be found at each site it is present at on every survey occasion (MacKenzie et al. 2002). For this study, we were able to use estimates of detection probability and occupancy across all sites to determine the number of species that were potentially present (Dozario and Royle 2005) as 32.12 (σ=5.44) which is considerably more than the 20 species actually observed. This estimate seems realistic though given that 42 species could have potentially occurred in wetlands in this region (Mount 1975, National Amphibian Atlas 2012) and seasonal sampling may have missed some species breeding periods. This demonstrates that headwater slope wetlands are important habitats for local biodiversity.

Watershed level protection of habitats is essential for amphibian conservation. As most species do not have the same breeding and microclimate requirements, a variety of habitats should be available for preserving biodiversity (Babbitt et al. 2003). Examining the influence of altered flood regimes on wetland habitat, species persistence, and developing amphibian larvae, the importance of stormwater management in urban watersheds becomes apparent if one hopes to maintain amphibian assemblages. Various low impact development practices such as pervious pavements, bioretention ponds, and grassed swales can be used to minimize surface water runoff and pollutant transport (Dietz 2007). Forested wetlands represent approximately half of the freshwater wetlands present in the United States currently (Dahl 2011) and provide numerous benefits such as water quality improvement, recreation, and flood control in addition to their value as wildlife habitat (Mitsch and Gosselink 2007). Maintaining unimpacted forested wetland habitat within changing landscapes may be able to provide refuge for amphibian populations and will be pivotal for future conservation.

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Appendix A

Table 2.1. Average call intensity and number of times heard calling for each species at each wetland by species.

Wetland ID		T2		T3		9		26		28		40		41N		41S	
		Ave.	# times	Ave.	# times	Ave.	# times	Ave.	# times	Ave.	# times	Ave.	# times	Ave.	# times	Ave.	# times
A. gryllus	May	0.0	0.0	0.0	0.0	1.0	5.0	0.0	0.0	2.0	3.0	0.0	0.0	2.3	39.0	0.0	0.0
	January	0.0	0.0	2.5	2.0	1.0	1.0	0.0	0.0	1.0	2.0	0.0	0.0	1.0	2.0	1.0	1.0
	March	0.0	0.0	0.0	0.0	1.2	10.0	2.0	1.0	0.0	0.0	0.0	0.0	2.5	43.0	1.0	2.0
	May	1.9	8.0	0.0	0.0	2.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	43.0	1.4	9.0
A. quercicus	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A. terrestris	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	1.2	9.0	0.0	0.0	1.3	3.0	0.0	0.0	1.5	2.0	2.2	6.0	2.2	18.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E. planirostris	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	8.0	0.0	0.0	0.0	0.0
H. chrysoscelis	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

	May	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
H. cinerea	May	0.0	0.0	1.0	1.0	0.0	0.0	1.0	2.0	2.3	18.0	0.0	0.0	0.0	0.0	1.0	3.0
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	18.0	0.0	0.0	2.0	1.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	2.3	11.0	0.0	0.0	2.7	20.0	0.0	0.0	2.2	15.0	3.0	3.0
H. femoralis	May	0.0	0.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
H. gratiosa	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	1.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
H. squirella	May	1.0	2.0	0.0	0.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	1.0	4.0	1.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L. catesbeianus	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	22.0	0.0	0.0	0.0	0.0	0.0	0.0
L. clamitans	May	0.0	0.0	0.0	0.0	0.0	0.0	1.0	11.0	1.8	46.0	0.0	0.0	1.0	10.0	0.0	0.0
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	1.3	4.0	0.0	0.0	1.0	2.0	1.2	5.0	1.6	40.0	0.0	0.0	1.5	47.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	1.4	5.0	1.3	38.0	2.0	49.0	0.0	0.0	1.6	49.0	0.0	0.0

L. grylio	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	21.0	0.0	0.0	0.0	0.0	0.0	0.0
L. sphenocephalus	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0
	January	0.0	0.0	0.0	0.0	2.0	1.0	0.0	0.0	2.5	11.0	0.0	0.0	1.21	14.0	0.0	0.0
	March	1.0	4.0	0.0	0.0	0.0	0.0	1.0	12.0	0.0	0.0	0.0	0.0	1.5	36.0	0.0	0.0
	May	1.0	7.0	0.0	0.0	1.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	14.0	1.0	1.0
P. crucifer	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	January	1.3	29.0	2.8	35.0	2.3	31.0	2.4	36.0	1.0	2.0	1.5	2.0	2.3	48.0	3.0	13.0
	March	2.5	45.0	2.6	48.0	1.3	30.0	2.4	37.0	0.0	0.0	2.2	20.0	2.5	32.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P. ornata	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	5.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2.1. Cont. Average call intensity and number of times heard calling for each species at each wetland by species.

Wetland ID		67		68		71		85		100		101		102	
		Ave.	# times	Ave.	# times	Ave.	# times	Ave.	# times	Ave.	# times	Ave.	# times	Ave.	# times
A. gryllus	May	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-
	January	1.0	1.0	0.0	0.0	1.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	2.0	1.0	0.0	0.0	1.0	1.0	1.0	4.0	1.0	1.0	1.0	1.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	1.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0
A. quercicus	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	23.0	0.0	0.0
A. terrestris	May	0.0	0.0	1.1	14.0	1.0	12.0	-	-	-	-	-	-	-	-
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	1.9	14.0	2.3	6.0	2.2	26.0	2.3	6.0	2.0	3.0	3.0	4.0	3.0	6.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	23.0	0.0	0.0
E. planirostris	May	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	3.0
	May	1.0	3.0	0.0	0.0	1.4	26.0	0.0	0.0	1.0	15.0	0.0	0.0	1.1	27.0
H. chrysoscelis	May	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0	0.0	0.0	2.0	7.0	1.0	1.0

H. cinerea	May	0.0	0.0	1.2	5.0	0.0	0.0	-	-	-	-	-	-	-	-
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	2.8	5.0	0.0	0.0	3.0	1.0	2.0	2.0	0.0	0.0	0.0	0.0
H. femoralis	May	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
H. gratiosa	May	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	3.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0
H. squirella	May	1.0	9.0	1.1	9.0	1.0	3.0	-	-	-	-	-	-	-	-
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	1.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	1.0	7.0	1.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L. catesbeianus	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L. clamitans	May	0.0	0.0	1.0	2.0	0.0	0.0	-	-	-	-	-	-	-	-
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	1.5	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	1.5	47.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	34.0	0.0	0.0

L. grylio	May	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L. sphenocephalus	May	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P. crucifer	May	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-
	January	2.4	30.0	3.0	5.0	1.9	10.0	1.0	1.0	0.0	0.0	1.3	12.0	0.0	0.0
	March	2.4	42.0	1.4	5.0	2.3	18.0	1.9	18.0	2.3	20.0	2.2	40.0	1.6	16.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P. ornata	May	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-	-	-	-	-
	January	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	March	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	May	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix B

Table 3.1. Staging data and tadpole count per ten-day intervals for four species.

		L. sphenocephalus			*H. chrysoscelis*			*H. squirella*			*G. carolinensis*		
Treatment	Day	Mean	SE	Count	Mean	SE	Count	Mean	SE	Count	Mean	SE	Count
Forest	10	26.3	0.3	20	27.9	0.3	34	29.8	0.4	31	26.6	0.2	27
	20	30.1	0.4	20	36.0	0.5	34	37.4	0.8	7	37.5	0.5	2
	30	34.0	0.9	20	38.9	0.7	25	44.0	1.5	3	40.0	-	1
	40	37.6	1.9	5	-	-	-	-	-	-	-	-	-
Control	10	26.8	0.3	20	27.9	0.2	40	29.9	0.6	27	26.4	0.1	40
	20	30.3	0.7	20	34.6	0.3	40	35.0	2.3	5	29.0	1.1	6
	30	33.0	1.1	20	38.3	0.5	38	43.3	1.3	4	37.4	0.9	5
	40	38.3	1.6	12	-	-	-	-	-	-	-	-	-
Urban	10	26.5	0.3	20	27.8	0.2	34	29.5	0.4	33	27.0	0.2	33
	20	29.3	0.4	20	36.1	0.5	34	33.6	1.0	27	29.1	1.2	10
	30	32.3	1.2	14	38.8	0.8	26	39.5	1.1	23	30.8	1.9	9
	40	39.3	1.8	4	-	-	-	-	-	-	-	-	-

Table 3.2. Results of linear effects models for *H. chrysoscelis* comparing final mass (g) and snout-vent length (cm) among treatments and control. Treatment listed first was used as the reference in the model.

	Mass			Snout-vent length		
	Value	SE	p	Value	SE	p
Control x Forest	0.0854	0.0305	0.021	0.108	0.0397	0.021
Control x Urban	0.01457	0.0305	0.641	0.03375	0.0383	0.402
Forest x Urban	-0.0708	0.0332	0.062	-0.075	0.0413	0.104

Table 3.3. Staging data for *L. sphenocephalus.*

Treatment	Bucket	Stage	Date	Day
Forest	R3	26	2-Apr	10
Forest	R3	26	2-Apr	10
Forest	R3	27	2-Apr	10
Forest	R3	25	2-Apr	10
Forest	R3	25	2-Apr	10
Forest	R2	25	2-Apr	10
Forest	R2	27	2-Apr	10
Forest	R2	26	2-Apr	10
Forest	R2	25	2-Apr	10
Forest	R2	25	2-Apr	10
Forest	R1	25	2-Apr	10
Forest	R1	27	2-Apr	10
Forest	R1	25	2-Apr	10
Forest	R1	27	2-Apr	10
Forest	R1	25	2-Apr	10
Forest	R4	27	2-Apr	10
Forest	R4	27	2-Apr	10
Forest	R4	29	2-Apr	10
Forest	R4	28	2-Apr	10
Forest	R4	28	2-Apr	10
Control	S1	25	2-Apr	10
Control	S1	27	2-Apr	10
Control	S1	27	2-Apr	10
Control	S1	27	2-Apr	10
Control	S1	26	2-Apr	10
Control	S4	27	2-Apr	10
Control	S4	28	2-Apr	10
Control	S4	26	2-Apr	10
Control	S4	28	2-Apr	10
Control	S4	28	2-Apr	10
Control	S3	28	2-Apr	10
Control	S3	25	2-Apr	10
Control	S3	25	2-Apr	10
Control	S3	25	2-Apr	10
Control	S3	25	2-Apr	10
Control	S2	28	2-Apr	10
Control	S2	28	2-Apr	10
Control	S2	28	2-Apr	10
Control	S2	27	2-Apr	10
Control	S2	27	2-Apr	10
Urban	U3	25	2-Apr	10

Urban	U3	25	2-Apr	10
Urban	U3	25	2-Apr	10
Urban	U3	27	2-Apr	10
Urban	U3	27	2-Apr	10
Urban	U1	27	2-Apr	10
Urban	U1	25	2-Apr	10
Urban	U1	28	2-Apr	10
Urban	U1	27	2-Apr	10
Urban	U1	27	2-Apr	10
Urban	U2	26	2-Apr	10
Urban	U2	27	2-Apr	10
Urban	U2	28	2-Apr	10
Urban	U2	28	2-Apr	10
Urban	U2	28	2-Apr	10
Urban	U4	25	2-Apr	10
Urban	U4	27	2-Apr	10
Urban	U4	25	2-Apr	10
Urban	U4	25	2-Apr	10
Urban	U4	27	2-Apr	10
Forest	R3	28	12-Apr	20
Forest	R3	28	12-Apr	20
Forest	R3	29	12-Apr	20
Forest	R3	31	12-Apr	20
Forest	R3	29	12-Apr	20
Forest	R2	30	12-Apr	20
Forest	R2	31	12-Apr	20
Forest	R2	30	12-Apr	20
Forest	R2	28	12-Apr	20
Forest	R2	30	12-Apr	20
Forest	R1	33	12-Apr	20
Forest	R1	28	12-Apr	20
Forest	R1	29	12-Apr	20
Forest	R1	28	12-Apr	20
Forest	R1	31	12-Apr	20
Forest	R4	31	12-Apr	20
Forest	R4	33	12-Apr	20
Forest	R4	35	12-Apr	20
Forest	R4	28	12-Apr	20
Forest	R4	31	12-Apr	20
Control	S3	36	12-Apr	20
Control	S3	32	12-Apr	20
Control	S3	31	12-Apr	20
Control	S3	33	12-Apr	20

Control	S3	31	12-Apr	20
Control	S1	28	12-Apr	20
Control	S1	34	12-Apr	20
Control	S1	31	12-Apr	20
Control	S1	25	12-Apr	20
Control	S1	28	12-Apr	20
Control	S4	28	12-Apr	20
Control	S4	27	12-Apr	20
Control	S4	28	12-Apr	20
Control	S4	29	12-Apr	20
Control	S4	29	12-Apr	20
Control	S2	29	12-Apr	20
Control	S2	29	12-Apr	20
Control	S2	34	12-Apr	20
Control	S2	36	12-Apr	20
Control	S2	28	12-Apr	20
Urban	U3	28	12-Apr	20
Urban	U3	31	12-Apr	20
Urban	U3	31	12-Apr	20
Urban	U3	31	12-Apr	20
Urban	U3	29	12-Apr	20
Urban	U4	32	12-Apr	20
Urban	U4	25	12-Apr	20
Urban	U4	27	12-Apr	20
Urban	U4	31	12-Apr	20
Urban	U4	29	12-Apr	20
Urban	U1	27	12-Apr	20
Urban	U1	30	12-Apr	20
Urban	U1	31	12-Apr	20
Urban	U1	31	12-Apr	20
Urban	U1	29	12-Apr	20
Urban	U2	28	12-Apr	20
Urban	U2	27	12-Apr	20
Urban	U2	29	12-Apr	20
Urban	U2	31	12-Apr	20
Urban	U2	28	12-Apr	20
Forest	R3	31	22-Apr	30
Forest	R3	40	22-Apr	30
Forest	R3	37	22-Apr	30
Forest	R3	37	22-Apr	30
Forest	R3	35	22-Apr	30
Forest	R2	29	22-Apr	30
Forest	R2	32	22-Apr	30

Forest	R2	32	22-Apr	30
Forest	R2	39	22-Apr	30
Forest	R2	33	22-Apr	30
Forest	R1	37	22-Apr	30
Forest	R1	36	22-Apr	30
Forest	R1	31	22-Apr	30
Forest	R1	27	22-Apr	30
Forest	R1	28	22-Apr	30
Forest	R4	40	22-Apr	30
Forest	R4	36	22-Apr	30
Forest	R4	37	22-Apr	30
Forest	R4	35	22-Apr	30
Forest	R4	28	22-Apr	30
Control	S3	37	22-Apr	30
Control	S3	29	22-Apr	30
Control	S3	37	22-Apr	30
Control	S3	29	22-Apr	30
Control	S3	36	22-Apr	30
Control	S1	39	22-Apr	30
Control	S1	36	22-Apr	30
Control	S1	29	22-Apr	30
Control	S1	27	22-Apr	30
Control	S1	25	22-Apr	30
Control	S4	31	22-Apr	30
Control	S4	35	22-Apr	30
Control	S4	30	22-Apr	30
Control	S4	28	22-Apr	30
Control	S4	35	22-Apr	30
Control	S2	41	22-Apr	30
Control	S2	34	22-Apr	30
Control	S2	36	22-Apr	30
Control	S2	25	22-Apr	30
Control	S2	40	22-Apr	30
Urban	U1	37	22-Apr	30
Urban	U1	31	22-Apr	30
Urban	U1	28	22-Apr	30
Urban	U1	35	22-Apr	30
Urban	U3	36	22-Apr	30
Urban	U3	37	22-Apr	30
Urban	U3	35	22-Apr	30
Urban	U3	33	22-Apr	30
Urban	U3	28	22-Apr	30
Urban	U4	35	22-Apr	30

Urban	U4	36	22-Apr	30
Urban	U4	25	22-Apr	30
Urban	U4	31	22-Apr	30
Urban	U4	25	22-Apr	30
Forest	R3	40	2-May	40
Forest	R3	40	2-May	40
Forest	R2	33	2-May	40
Forest	R2	33	2-May	40
Forest	R4	42	2-May	40
Control	S3	41	2-May	40
Control	S3	36	2-May	40
Control	S2	45	2-May	40
Control	S2	37	2-May	40
Control	S2	44	2-May	40
Control	S2	38	2-May	40
Control	S1	44	2-May	40
Control	S1	40	2-May	40
Control	S4	25	2-May	40
Control	S4	35	2-May	40
Control	S4	37	2-May	40
Control	S4	37	2-May	40
Urban	U3	41	2-May	40
Urban	U4	41	2-May	40
Urban	U4	34	2-May	40
Urban	U1	41	2-May	40

Table 3.4. Staging data for *H. chrysoscelis*.

Treatment	Bucket	Stage	Date	Day
Forest	R1	30	26-May	10
Forest	R1	32	26-May	10
Forest	R1	26	26-May	10
Forest	R1	29	26-May	10
Forest	R1	28	26-May	10
Forest	R1	29	26-May	10
Forest	R1	28	26-May	10
Forest	R2	28	26-May	10
Forest	R2	28	26-May	10
Forest	R2	28	26-May	10
Forest	R2	27	26-May	10
Forest	R2	29	26-May	10
Forest	R2	25	26-May	10
Forest	R2	29	26-May	10
Forest	R2	29	26-May	10
Forest	R3	29	26-May	10
Forest	R3	29	26-May	10
Forest	R3	28	26-May	10
Forest	R3	27	26-May	10
Forest	R3	26	26-May	10
Forest	R3	28	26-May	10
Forest	R3	28	26-May	10
Forest	R3	29	26-May	10
Forest	R3	26	26-May	10
Forest	R3	28	26-May	10
Forest	R4	27	26-May	10
Forest	R4	27	26-May	10
Forest	R4	25	26-May	10
Forest	R4	27	26-May	10
Forest	R4	29	26-May	10
Forest	R4	31	26-May	10
Forest	R4	27	26-May	10
Forest	R4	26	26-May	10
Forest	R4	28	26-May	10
Control	S1	26	26-May	10
Control	S1	28	26-May	10
Control	S1	27	26-May	10
Control	S1	26	26-May	10
Control	S1	27	26-May	10
Control	S1	26	26-May	10
Control	S1	28	26-May	10

Control	S1	27	26-May	10
Control	S1	30	26-May	10
Control	S1	28	26-May	10
Control	S2	27	26-May	10
Control	S2	27	26-May	10
Control	S2	26	26-May	10
Control	S2	28	26-May	10
Control	S2	27	26-May	10
Control	S2	28	26-May	10
Control	S2	28	26-May	10
Control	S2	29	26-May	10
Control	S2	29	26-May	10
Control	S2	30	26-May	10
Control	S3	29	26-May	10
Control	S3	29	26-May	10
Control	S3	27	26-May	10
Control	S3	28	26-May	10
Control	S3	25	26-May	10
Control	S3	27	26-May	10
Control	S3	27	26-May	10
Control	S3	29	26-May	10
Control	S3	29	26-May	10
Control	S3	29	26-May	10
Control	S4	29	26-May	10
Control	S4	29	26-May	10
Control	S4	27	26-May	10
Control	S4	30	26-May	10
Control	S4	27	26-May	10
Control	S4	28	26-May	10
Control	S4	28	26-May	10
Control	S4	28	26-May	10
Control	S4	30	26-May	10
Control	S4	27	26-May	10
Urban	U1	28	26-May	10
Urban	U1	28	26-May	10
Urban	U1	27	26-May	10
Urban	U1	25	26-May	10
Urban	U1	28	26-May	10
Urban	U1	30	26-May	10
Urban	U1	27	26-May	10
Urban	U1	28	26-May	10
Urban	U2	30	26-May	10
Urban	U2	28	26-May	10

Urban	U2	28	26-May	10
Urban	U2	26	26-May	10
Urban	U2	27	26-May	10
Urban	U2	29	26-May	10
Urban	U2	27	26-May	10
Urban	U2	28	26-May	10
Urban	U3	29	26-May	10
Urban	U3	28	26-May	10
Urban	U3	25	26-May	10
Urban	U3	25	26-May	10
Urban	U3	29	26-May	10
Urban	U3	28	26-May	10
Urban	U3	27	26-May	10
Urban	U3	27	26-May	10
Urban	U3	31	26-May	10
Urban	U4	26	26-May	10
Urban	U4	29	26-May	10
Urban	U4	28	26-May	10
Urban	U4	29	26-May	10
Urban	U4	27	26-May	10
Urban	U4	28	26-May	10
Urban	U4	29	26-May	10
Urban	U4	28	26-May	10
Urban	U4	27	26-May	10
Forest	R1	37	5-Jun	20
Forest	R1	35	5-Jun	20
Forest	R1	41	5-Jun	20
Forest	R1	38	5-Jun	20
Forest	R1	36	5-Jun	20
Forest	R1	35	5-Jun	20
Forest	R1	37	5-Jun	20
Forest	R2	41	5-Jun	20
Forest	R2	40	5-Jun	20
Forest	R2	38	5-Jun	20
Forest	R2	37	5-Jun	20
Forest	R2	37	5-Jun	20
Forest	R2	36	5-Jun	20
Forest	R2	34	5-Jun	20
Forest	R2	40	5-Jun	20
Forest	R3	30	5-Jun	20
Forest	R3	34	5-Jun	20
Forest	R3	31	5-Jun	20
Forest	R3	37	5-Jun	20

Forest	R3	36	5-Jun	20
Forest	R3	33	5-Jun	20
Forest	R3	36	5-Jun	20
Forest	R3	36	5-Jun	20
Forest	R3	34	5-Jun	20
Forest	R3	33	5-Jun	20
Forest	R4	32	5-Jun	20
Forest	R4	33	5-Jun	20
Forest	R4	37	5-Jun	20
Forest	R4	34	5-Jun	20
Forest	R4	40	5-Jun	20
Forest	R4	40	5-Jun	20
Forest	R4	35	5-Jun	20
Forest	R4	36	5-Jun	20
Forest	R4	36	5-Jun	20
Control	S1	34	5-Jun	20
Control	S1	35	5-Jun	20
Control	S1	37	5-Jun	20
Control	S1	34	5-Jun	20
Control	S1	34	5-Jun	20
Control	S1	33	5-Jun	20
Control	S1	34	5-Jun	20
Control	S1	40	5-Jun	20
Control	S1	35	5-Jun	20
Control	S1	35	5-Jun	20
Control	S2	34	5-Jun	20
Control	S2	33	5-Jun	20
Control	S2	36	5-Jun	20
Control	S2	37	5-Jun	20
Control	S2	36	5-Jun	20
Control	S2	34	5-Jun	20
Control	S2	34	5-Jun	20
Control	S2	37	5-Jun	20
Control	S2	33	5-Jun	20
Control	S2	35	5-Jun	20
Control	S3	38	5-Jun	20
Control	S3	35	5-Jun	20
Control	S3	34	5-Jun	20
Control	S3	37	5-Jun	20
Control	S3	33	5-Jun	20
Control	S3	36	5-Jun	20
Control	S3	28	5-Jun	20
Control	S3	34	5-Jun	20

Control	S3	36	5-Jun	20
Control	S3	37	5-Jun	20
Control	S4	37	5-Jun	20
Control	S4	31	5-Jun	20
Control	S4	31	5-Jun	20
Control	S4	34	5-Jun	20
Control	S4	33	5-Jun	20
Control	S4	32	5-Jun	20
Control	S4	36	5-Jun	20
Control	S4	33	5-Jun	20
Control	S4	36	5-Jun	20
Control	S4	33	5-Jun	20
Urban	U1	40	5-Jun	20
Urban	U1	29	5-Jun	20
Urban	U1	38	5-Jun	20
Urban	U1	38	5-Jun	20
Urban	U1	36	5-Jun	20
Urban	U1	38	5-Jun	20
Urban	U1	32	5-Jun	20
Urban	U1	37	5-Jun	20
Urban	U2	37	5-Jun	20
Urban	U2	32	5-Jun	20
Urban	U2	34	5-Jun	20
Urban	U2	35	5-Jun	20
Urban	U2	34	5-Jun	20
Urban	U2	38	5-Jun	20
Urban	U2	38	5-Jun	20
Urban	U2	37	5-Jun	20
Urban	U3	33	5-Jun	20
Urban	U3	41	5-Jun	20
Urban	U3	36	5-Jun	20
Urban	U3	37	5-Jun	20
Urban	U3	40	5-Jun	20
Urban	U3	38	5-Jun	20
Urban	U3	37	5-Jun	20
Urban	U3	36	5-Jun	20
Urban	U3	36	5-Jun	20
Urban	U4	34	5-Jun	20
Urban	U4	35	5-Jun	20
Urban	U4	30	5-Jun	20
Urban	U4	37	5-Jun	20
Urban	U4	36	5-Jun	20
Urban	U4	36	5-Jun	20

Urban	U4	37	5-Jun	20
Urban	U4	38	5-Jun	20
Urban	U4	36	5-Jun	20
Forest	R1	38	15-Jun	30
Forest	R1	42	15-Jun	30
Forest	R1	37	15-Jun	30
Forest	R1	40	15-Jun	30
Forest	R1	37	15-Jun	30
Forest	R2	46	15-Jun	30
Forest	R2	45	15-Jun	30
Forest	R2	45	15-Jun	30
Forest	R2	37	15-Jun	30
Forest	R2	40	15-Jun	30
Forest	R2	33	15-Jun	30
Forest	R2	42	15-Jun	30
Forest	R3	38	15-Jun	30
Forest	R3	37	15-Jun	30
Forest	R3	39	15-Jun	30
Forest	R3	40	15-Jun	30
Forest	R3	40	15-Jun	30
Forest	R3	32	15-Jun	30
Forest	R3	41	15-Jun	30
Forest	R3	40	15-Jun	30
Forest	R4	35	15-Jun	30
Forest	R4	40	15-Jun	30
Forest	R4	34	15-Jun	30
Forest	R4	38	15-Jun	30
Forest	R4	37	15-Jun	30
Control	S1	37	15-Jun	30
Control	S1	32	15-Jun	30
Control	S1	34	15-Jun	30
Control	S1	34	15-Jun	30
Control	S1	38	15-Jun	30
Control	S1	37	15-Jun	30
Control	S1	37	15-Jun	30
Control	S1	40	15-Jun	30
Control	S1	40	15-Jun	30
Control	S2	45	15-Jun	30
Control	S2	45	15-Jun	30
Control	S2	38	15-Jun	30
Control	S2	36	15-Jun	30
Control	S2	36	15-Jun	30
Control	S2	40	15-Jun	30

Control	S2	36	15-Jun	30
Control	S2	37	15-Jun	30
Control	S2	40	15-Jun	30
Control	S2	35	15-Jun	30
Control	S3	44	15-Jun	30
Control	S3	44	15-Jun	30
Control	S3	45	15-Jun	30
Control	S3	41	15-Jun	30
Control	S3	33	15-Jun	30
Control	S3	37	15-Jun	30
Control	S3	41	15-Jun	30
Control	S3	40	15-Jun	30
Control	S3	39	15-Jun	30
Control	S4	36	15-Jun	30
Control	S4	36	15-Jun	30
Control	S4	37	15-Jun	30
Control	S4	36	15-Jun	30
Control	S4	37	15-Jun	30
Control	S4	37	15-Jun	30
Control	S4	37	15-Jun	30
Control	S4	37	15-Jun	30
Control	S4	41	15-Jun	30
Control	S4	41	15-Jun	30
Urban	U1	37	15-Jun	30
Urban	U1	46	15-Jun	30
Urban	U1	44	15-Jun	30
Urban	U1	46	15-Jun	30
Urban	U1	41	15-Jun	30
Urban	U1	33	15-Jun	30
Urban	U1	41	15-Jun	30
Urban	U2	35	15-Jun	30
Urban	U2	40	15-Jun	30
Urban	U2	34	15-Jun	30
Urban	U2	37	15-Jun	30
Urban	U3	40	15-Jun	30
Urban	U3	34	15-Jun	30
Urban	U3	40	15-Jun	30
Urban	U3	32	15-Jun	30
Urban	U3	37	15-Jun	30
Urban	U3	40	15-Jun	30
Urban	U3	41	15-Jun	30
Urban	U4	37	15-Jun	30
Urban	U4	38	15-Jun	30

Urban	U4	38	15-Jun	30
Urban	U4	41	15-Jun	30
Urban	U4	32	15-Jun	30
Urban	U4	43	15-Jun	30
Urban	U4	41	15-Jun	30
Urban	U4	40	15-Jun	30

Table 3.5. All staging data for *H. squirella.*

Treatment	Bucket	Stage	Date	Day
Forest	R1	28	27-Jul	10
Forest	R1	31	27-Jul	10
Forest	R1	29	27-Jul	10
Forest	R1	27	27-Jul	10
Forest	R1	30	27-Jul	10
Forest	R1	30	27-Jul	10
Forest	R1	29	27-Jul	10
Forest	R1	29	27-Jul	10
Forest	R2	30	27-Jul	10
Forest	R2	29	27-Jul	10
Forest	R2	27	27-Jul	10
Forest	R2	31	27-Jul	10
Forest	R2	31	27-Jul	10
Forest	R2	32	27-Jul	10
Forest	R2	28	27-Jul	10
Forest	R2	33	27-Jul	10
Forest	R2	28	27-Jul	10
Forest	R2	30	27-Jul	10
Forest	R3	27	27-Jul	10
Forest	R3	34	27-Jul	10
Forest	R3	31	27-Jul	10
Forest	R3	28	27-Jul	10
Forest	R4	37	27-Jul	10
Forest	R4	30	27-Jul	10
Forest	R4	27	27-Jul	10
Forest	R4	31	27-Jul	10
Forest	R4	29	27-Jul	10
Forest	R4	31	27-Jul	10
Forest	R4	30	27-Jul	10
Forest	R4	28	27-Jul	10
Forest	R4	28	27-Jul	10
Control	S1	27	27-Jul	10
Control	S1	33	27-Jul	10
Control	S1	33	27-Jul	10
Control	S1	30	27-Jul	10
Control	S1	28	27-Jul	10
Control	S1	34	27-Jul	10
Control	S1	27	27-Jul	10
Control	S2	25	27-Jul	10
Control	S2	25	27-Jul	10
Control	S2	35	27-Jul	10

Control	S2	33	27-Jul	10
Control	S2	29	27-Jul	10
Control	S2	29	27-Jul	10
Control	S3	29	27-Jul	10
Control	S3	31	27-Jul	10
Control	S3	28	27-Jul	10
Control	S3	30	27-Jul	10
Control	S3	31	27-Jul	10
Control	S3	30	27-Jul	10
Control	S3	29	27-Jul	10
Control	S3	36	27-Jul	10
Control	S4	30	27-Jul	10
Control	S4	34	27-Jul	10
Control	S4	27	27-Jul	10
Control	S4	29	27-Jul	10
Control	S4	29	27-Jul	10
Control	S4	27	27-Jul	10
Urban	U1	28	27-Jul	10
Urban	U1	31	27-Jul	10
Urban	U1	30	27-Jul	10
Urban	U1	29	27-Jul	10
Urban	U1	31	27-Jul	10
Urban	U1	29	27-Jul	10
Urban	U1	26	27-Jul	10
Urban	U1	27	27-Jul	10
Urban	U2	27	27-Jul	10
Urban	U2	28	27-Jul	10
Urban	U2	26	27-Jul	10
Urban	U2	28	27-Jul	10
Urban	U2	31	27-Jul	10
Urban	U2	29	27-Jul	10
Urban	U2	30	27-Jul	10
Urban	U2	34	27-Jul	10
Urban	U2	30	27-Jul	10
Urban	U3	26	27-Jul	10
Urban	U3	32	27-Jul	10
Urban	U3	32	27-Jul	10
Urban	U3	34	27-Jul	10
Urban	U3	29	27-Jul	10
Urban	U3	30	27-Jul	10
Urban	U3	27	27-Jul	10
Urban	U3	28	27-Jul	10
Urban	U3	29	27-Jul	10

Urban	U3	30	27-Jul	10
Urban	U4	28	27-Jul	10
Urban	U4	27	27-Jul	10
Urban	U4	31	27-Jul	10
Urban	U4	36	27-Jul	10
Urban	U4	30	27-Jul	10
Urban	U4	29	27-Jul	10
Forest	R1	39	6-Aug	20
Forest	R1	37	6-Aug	20
Forest	R1	36	6-Aug	20
Forest	R2	37	6-Aug	20
Forest	R3	34	6-Aug	20
Forest	R3	40	6-Aug	20
Forest	R3	39	6-Aug	20
Control	S2	37	6-Aug	20
Control	S2	31	6-Aug	20
Control	S2	43	6-Aug	20
Control	S2	33	6-Aug	20
Control	S2	31	6-Aug	20
Urban	U1	36	6-Aug	20
Urban	U1	36	6-Aug	20
Urban	U1	40	6-Aug	20
Urban	U1	27	6-Aug	20
Urban	U1	27	6-Aug	20
Urban	U1	40	6-Aug	20
Urban	U1	26	6-Aug	20
Urban	U1	27	6-Aug	20
Urban	U2	37	6-Aug	20
Urban	U2	31	6-Aug	20
Urban	U2	40	6-Aug	20
Urban	U2	38	6-Aug	20
Urban	U3	37	6-Aug	20
Urban	U3	40	6-Aug	20
Urban	U3	28	6-Aug	20
Urban	U3	30	6-Aug	20
Urban	U3	31	6-Aug	20
Urban	U3	37	6-Aug	20
Urban	U3	38	6-Aug	20
Urban	U3	26	6-Aug	20
Urban	U3	37	6-Aug	20
Urban	U3	31	6-Aug	20
Urban	U4	29	6-Aug	20
Urban	U4	28	6-Aug	20

Urban	U4	41	6-Aug	20
Urban	U4	33	6-Aug	20
Urban	U4	37	6-Aug	20
Forest	R3	46	11-Aug	25
Forest	R3	45	11-Aug	25
Forest	R3	41	16-Aug	30
Control	S2	46	9-Aug	23
Control	S2	45	15-Aug	29
Control	S2	41	16-Aug	30
Control	S2	41	16-Aug	30
Urban	U1	45	15-Aug	29
Urban	U1	45	16-Aug	30
Urban	U1	29	16-Aug	30
Urban	U1	31	16-Aug	30
Urban	U1	43	16-Aug	30
Urban	U1	37	16-Aug	30
Urban	U1	41	16-Aug	30
Urban	U1	37	16-Aug	30
Urban	U2	46	9-Aug	23
Urban	U2	42	16-Aug	30
Urban	U2	39	16-Aug	30
Urban	U3	45	12-Aug	26
Urban	U3	45	15-Aug	29
Urban	U3	44	16-Aug	30
Urban	U3	42	16-Aug	30
Urban	U3	30	16-Aug	30
Urban	U3	36	16-Aug	30
Urban	U3	39	16-Aug	30
Urban	U3	41	16-Aug	30
Urban	U3	31	16-Aug	30
Urban	U3	37	16-Aug	30
Urban	U4	40	16-Aug	30
Urban	U4	43	16-Aug	30

Table 3.6. Staging data for all *G. carolinensis.*

Treatment	Bucket	Stage	Date	
Forest	R1	26	30-Jul	10
Forest	R1	27	30-Jul	10
Forest	R1	29	30-Jul	10
Forest	R1	26	30-Jul	10
Forest	R1	25	30-Jul	10
Forest	R1	26	30-Jul	10
Forest	R1	25	30-Jul	10
Forest	R1	26	30-Jul	10
Forest	R1	26	30-Jul	10
Forest	R2	27	30-Jul	10
Forest	R2	29	30-Jul	10
Forest	R3	26	30-Jul	10
Forest	R3	27	30-Jul	10
Forest	R3	27	30-Jul	10
Forest	R3	26	30-Jul	10
Forest	R3	26	30-Jul	10
Forest	R3	27	30-Jul	10
Forest	R3	27	30-Jul	10
Forest	R3	28	30-Jul	10
Forest	R3	26	30-Jul	10
Forest	R4	28	30-Jul	10
Forest	R4	27	30-Jul	10
Forest	R4	25	30-Jul	10
Forest	R4	26	30-Jul	10
Forest	R4	27	30-Jul	10
Forest	R4	26	30-Jul	10
Forest	R4	26	30-Jul	10
Control	S1	26	30-Jul	10
Control	S1	25	30-Jul	10
Control	S1	25	30-Jul	10
Control	S1	28	30-Jul	10
Control	S1	26	30-Jul	10
Control	S1	27	30-Jul	10
Control	S1	28	30-Jul	10
Control	S1	26	30-Jul	10
Control	S1	26	30-Jul	10
Control	S1	27	30-Jul	10
Control	S2	27	30-Jul	10
Control	S2	25	30-Jul	10
Control	S2	27	30-Jul	10

Control	S2	26	30-Jul	10
Control	S2	26	30-Jul	10
Control	S2	26	30-Jul	10
Control	S2	26	30-Jul	10
Control	S2	26	30-Jul	10
Control	S2	27	30-Jul	10
Control	S2	27	30-Jul	10
Control	S3	26	30-Jul	10
Control	S3	26	30-Jul	10
Control	S3	28	30-Jul	10
Control	S3	28	30-Jul	10
Control	S3	26	30-Jul	10
Control	S3	26	30-Jul	10
Control	S3	27	30-Jul	10
Control	S3	25	30-Jul	10
Control	S3	26	30-Jul	10
Control	S3	26	30-Jul	10
Control	S4	28	30-Jul	10
Control	S4	26	30-Jul	10
Control	S4	26	30-Jul	10
Control	S4	27	30-Jul	10
Control	S4	25	30-Jul	10
Control	S4	28	30-Jul	10
Control	S4	26	30-Jul	10
Control	S4	27	30-Jul	10
Control	S4	26	30-Jul	10
Control	S4	27	30-Jul	10
Urban	U1	29	30-Jul	10
Urban	U1	25	30-Jul	10
Urban	U1	28	30-Jul	10
Urban	U1	27	30-Jul	10
Urban	U1	29	30-Jul	10
Urban	U1	28	30-Jul	10
Urban	U1	28	30-Jul	10
Urban	U1	27	30-Jul	10
Urban	U1	28	30-Jul	10
Urban	U1	27	30-Jul	10
Urban	U2	25	30-Jul	10
Urban	U2	26	30-Jul	10
Urban	U2	26	30-Jul	10
Urban	U2	27	30-Jul	10
Urban	U2	26	30-Jul	10
Urban	U2	29	30-Jul	10

Urban	U2	26	30-Jul	10
Urban	U2	27	30-Jul	10
Urban	U2	27	30-Jul	10
Urban	U2	27	30-Jul	10
Urban	U3	28	30-Jul	10
Urban	U3	26	30-Jul	10
Urban	U3	27	30-Jul	10
Urban	U3	26	30-Jul	10
Urban	U3	28	30-Jul	10
Urban	U3	28	30-Jul	10
Urban	U3	25	30-Jul	10
Urban	U3	28	30-Jul	10
Urban	U3	27	30-Jul	10
Urban	U3	26	30-Jul	10
Urban	U4	25	30-Jul	10
Urban	U4	28	30-Jul	10
Urban	U4	26	30-Jul	10
Forest	R2	37	9-Aug	20
Forest	R2	38	9-Aug	20
Control	S1	28	9-Aug	20
Control	S3	29	9-Aug	20
Control	S3	34	9-Aug	20
Control	S4	29	9-Aug	20
Control	S4	27	9-Aug	20
Control	S4	27	9-Aug	20
Urban	U3	26	9-Aug	20
Urban	U3	27	9-Aug	20
Urban	U3	31	9-Aug	20
Urban	U3	27	9-Aug	20
Urban	U3	29	9-Aug	20
Urban	U3	27	9-Aug	20
Urban	U3	33	9-Aug	20
Urban	U3	29	9-Aug	20
Urban	U3	25	9-Aug	20
Urban	U3	37	9-Aug	20
Forest	R2	40	19-Aug	30
Control	S3	36	19-Aug	30
Control	S3	40	19-Aug	30
Control	S4	39	19-Aug	30
Control	S4	36	19-Aug	30
Control	S4	36	19-Aug	30
Urban	U3	26	19-Aug	30
Urban	U3	26	19-Aug	30

Urban	U3	40	19-Aug	30
Urban	U3	28	19-Aug	30
Urban	U3	33	19-Aug	30
Urban	U3	26	19-Aug	30
Urban	U3	30	19-Aug	30
Urban	U3	28	19-Aug	30
Urban	U3	40	19-Aug	30

Table 3.7. Final measurements for *L. sphenocephalus.*

Treatment	Bucket	Stage	Mass (g)	Snout-vent length (cm)	Total Body Length (cm)
Forest	R2	33	0.48	1.4	3.6
Forest	R2	33	0.41	1.4	3.7
Forest	R3	40	1.21	2.1	5.2
Forest	R3	40	0.92	1.9	4.6
Forest	R4	42	0.7	1.8	4.2
Control	S1	40	1.03	1.8	5.2
Control	S1	44	0.94	2.1	0
Control	S2	37	0.47	1.5	4
Control	S2	38	0.83	1.8	4.6
Control	S2	44	0.57	1.8	2.6
Control	S2	45	0.76	2.2	0
Control	S3	36	0.54	1.5	4
Control	S3	41	1.06	1.6	5
Control	S4	25	0.45	1.3	2.5
Control	S4	37	0.66	1.7	4.6
Control	S4	37	0.86	1.9	4.6
Control	S4	35	0.55	1.5	3.9
Urban	U1	41	0.73	1.9	3.9
Urban	U3	41	0.95	1.7	4.9
Urban	U4	41	1.03	2	5
Urban	U4	34	0.51	1.5	3.9

Table 3.8. Final measurements for *H. chrysoscelis*

Treatment	Bucket	Stage	Mass (g)	Snout-vent length (cm)	Total Body Length (cm)
Forest	R1	38	0.4	1.4	3.8
Forest	R1	42	0.34	1.3	4.1
Forest	R1	37	0.4	1.3	3.6
Forest	R1	40	0.49	1.4	4
Forest	R1	37	0.42	1.3	3.8
Forest	R2	46	0.1	1.2	-
Forest	R2	45	0.24	1.3	-
Forest	R2	45	0.19	1.3	1.4
Forest	R2	37	0.59	1.4	3.5
Forest	R2	40	0.52	1.5	3.9
Forest	R2	33	0.21	1.2	2.9
Forest	R2	42	0.3	1.4	3.7
Forest	R3	38	0.44	1.3	3.8
Forest	R3	37	0.31	1.3	3.5
Forest	R3	39	0.37	1.2	3.5
Forest	R3	40	0.22	1.2	3.6
Forest	R3	40	0.25	1.2	3.5
Forest	R3	32	0.08	1	2.4
Forest	R3	41	0.31	1.2	3.7
Forest	R3	40	0.47	1.2	3.9
Forest	R4	35	0.25	1.1	3.2
Forest	R4	40	0.69	1.5	4.2
Forest	R4	34	0.27	1.3	3
Forest	R4	38	0.44	1.2	3.6
Forest	R4	37	0.25	1.2	3.3
Control	S1	37	0.29	1.2	3.2
Control	S1	32	0.08	1	2.6
Control	S1	34	0.15	1.1	3
Control	S1	34	0.23	1.1	3.1
Control	S1	38	0.46	1.4	3.5
Control	S1	37	0.38	1.2	3.5
Control	S1	37	0.35	1.2	3.4
Control	S1	40	0.44	1.2	3.6
Control	S1	40	0.38	1.2	3.7
Control	S2	45	0.13	1.3	1.3
Control	S2	45	0.17	1.2	1.2
Control	S2	38	0.45	1.4	3.7
Control	S2	36	0.23	1.1	2.8

Control	S2	36	0.33	1.2	3.4
Control	S2	40	0.31	1.1	3.3
Control	S2	36	0.28	1.1	3.1
Control	S2	37	0.19	1	3.1
Control	S2	40	0.13	1.1	3.5
Control	S2	35	0.17	1	2.7
Control	S3	44	0.22	1.4	1.5
Control	S3	44	0.22	1.5	1.6
Control	S3	45	0.23	1.3	1.4
Control	S3	41	0.25	1.2	3.5
Control	S3	33	0.09	1	2.9
Control	S3	37	0.4	1.3	3.6
Control	S3	41	0.28	1.1	3.5
Control	S3	40	0.25	1.1	3.1
Control	S3	39	0.42	1.3	3.6
Control	S4	36	0.24	1.1	2.9
Control	S4	36	0.17	1.1	3.1
Control	S4	37	0.2	1.2	3
Control	S4	36	0.18	1	2.5
Control	S4	37	0.14	1	2.6
Control	S4	37	0.21	1	3.1
Control	S4	37	0.17	1.1	3.2
Control	S4	37	0.21	1.1	3
Control	S4	41	0.43	1.3	4
Control	S4	41	0.29	1.2	3.7
Urban	U1	37	0.37	1.2	3.3
Urban	U1	46	0.22	1.3	-
Urban	U1	44	0.17	1.3	1.6
Urban	U1	46	0.19	1.2	-
Urban	U1	41	0.4	1.3	3.7
Urban	U1	33	0.17	1	3.1
Urban	U1	41	0.4	1.3	3.8
Urban	U2	35	0.24	1	2.9
Urban	U2	40	0.37	1.3	4
Urban	U2	34	0.31	1.1	3
Urban	U2	37	0.26	1.2	3.3
Urban	U3	40	0.47	1.2	3.7
Urban	U3	34	0.18	1	2.7
Urban	U3	40	0.31	1.3	3.1
Urban	U3	32	0.09	1	2.5
Urban	U3	37	0.25	1.1	2.8
Urban	U3	40	0.48	1.4	4.3
Urban	U3	41	0.39	1.5	4.1

Urban	U4	37	0.31	1.1	2.8
Urban	U4	38	0.24	1.2	3.7
Urban	U4	38	0.18	1.1	2.9
Urban	U4	41	0.16	1.2	3.3
Urban	U4	32	0.12	1.1	2.8
Urban	U4	43	0.19	1.3	3.1
Urban	U4	41	0.35	1.5	4.4
Urban	U4	40	0.23	1.1	3.5

Table 3.9. Final measurements for *H. squirella.*

Treatment	Bucket	Stage	Mass (g)	Snout-vent length (cm)	Total Body Length (cm)
Forest	R3	46	0.16	14	-
Forest	R3	45	0.15	14	15
Forest	R3	41	0.23	11	29
Control	S2	46	0.22	15	-
Control	S2	45	0.26	13	16
Control	S2	41	0.2	10	30
Control	S2	41	0.25	11	30
Urban	U1	45	0.25	12	13
Urban	U1	45	0.2	13	14
Urban	U1	29	0.1	8	21
Urban	U1	31	0.16	9	23
Urban	U1	43	0.25	13	27
Urban	U1	37	0.27	11	29
Urban	U1	41	0.31	11	32
Urban	U1	37	0.26	10	33
Urban	U2	46	0.16	13	-
Urban	U2	42	0.47	15	38
Urban	U2	39	0.52	14	37
Urban	U3	45	0.14	10	11
Urban	U3	45	0.16	11	14
Urban	U3	44	0.2	12	24
Urban	U3	42	0.22	13	27
Urban	U3	30	0.13	8	20
Urban	U3	36	0.19	10	26
Urban	U3	39	0.27	11	33
Urban	U3	41	0.36	12	31
Urban	U3	31	0.15	8	24
Urban	U3	37	0.23	9	28
Urban	U4	40	0.38	11	34
Urban	U4	43	0.4	15	36

Table 3.10. Final measurements for *G. carolinensis*.

Treatment	Bucket	Stage	Mass (g)	Snout-vent Length (cm)	Total Body Length (cm)
Forest	R2	40	0.19	11	26
Control	S3	36	0.13	9	24
Control	S3	40	0.19	11	30
Control	S4	39	0.24	10	28
Control	S4	36	0.09	8	22
Control	S4	36	0.13	9	24
Urban	U3	26	0.04	6	15
Urban	U3	26	0.07	6	18
Urban	U3	40	0.16	10	25
Urban	U3	28	0.04	7	17
Urban	U3	33	0.12	8	19
Urban	U3	26	0.03	7	16
Urban	U3	30	0.07	7	18
Urban	U3	28	0.05	7	18
Urban	U3	40	0.2	10	28

Table 3.11. Kaplan-Meier survival probabilities calculated for all four species.

	Lithobates sphenocephalus			Hyla chrysoscelis			Hyla squirella			Gastrophryne carolinensis		
Day (t)	Forest	Stable	Urban	Forest	Stable	Urban	Forest	Stable	Urban	Forest	Stable	Urban
1	1	1	1	1	1	1	1	1	1	0.9571	0.9857	1
2	1	1	1	0.9	0.8571	0.9286	1	0.9857	0.9714	0.9571	0.9857	1
3	1	1	1	0.87	0.8229	0.8512	1	0.9693	0.9390	0.7247	0.9529	0.9286
4	1	1	1	0.8352	0.8229	0.8342	0.9857	0.9693	0.9203	0.6703	0.9529	0.9131
5	1	1	1	0.7517	0.8012	0.8133	0.9571	0.9693	0.9203	0.6522	0.9529	0.8583
6	1	1	1	0.7517	0.8012	0.8133	0.8274	0.9693	0.9203	0.5798	0.9147	0.8583
7	1	1	1	0.7517	0.8012	0.8133	0.8274	0.9111	0.8639	0.5616	0.9147	0.8583
8	1	0.9714	1	0.7517	0.8012	0.8133	0.8274	0.8428	0.7975	0.5435	0.9147	0.8583
9	1	0.9714	1	0.7517	0.8012	0.8133	0.7261	0.7517	0.7975	0.5073	0.9147	0.8154
10	1	0.9714	1	0.7517	0.8012	0.8133	0.6702	0.6150	0.7753	0.5073	0.9147	0.7296
11	1	0.9714	1	0.7517	0.8012	0.8133	0.6144	0.5922	0.7753	0.5073	0.9147	0.6853
12	1	0.9714	1	0.7517	0.8012	0.8133	0.4654	0.5695	0.7310	0.4697	0.9147	0.6632
13	1	0.9714	1	0.7517	0.8012	0.8133	0.4096	0.5695	0.7089	0.3946	0.9147	0.5085
14	1	0.9714	1	0.7517	0.8012	0.8133	0.3910	0.5467	0.6867	0.2818	0.8461	0.4201
15	1	0.9714	1	0.7517	0.8012	0.8133	0.3537	0.5467	0.6867	0.2442	0.6175	0.4201
16	1	0.9714	1	0.7517	0.8012	0.8133	0.3351	0.4556	0.6867	0.2067	0.4345	0.4201
17	1	0.9714	1	0.7517	0.8012	0.8133	0.2979	0.3417	0.6867	0.1503	0.2973	0.4201
18	1	0.9714	1	0.7517	0.8012	0.8133	0.2234	0.2506	0.6867	0.1127	0.2058	0.3979
19	0.9714	0.9714	1	0.7517	0.8012	0.8133	0.2048	0.2050	0.6867	0.0939	0.1372	0.3095
20	0.9714	0.9714	1	0.7517	0.8012	0.8133	0.1676	0.2050	0.6646	0.0939	0.1372	0.2211
21	0.9714	0.9714	1	0.7517	0.8012	0.8133	0.1489	0.2050	0.6424	0.0939	0.1372	0.2211
22	0.9714	0.9067	0.8571	0.7517	0.8012	0.8133	0.1303	0.2050	0.6424	0.0939	0.1372	0.2211
23	0.9714	0.9067	0.8571	0.7517	0.8012	0.8133	0.0931	0.2050	0.6203	0.0939	0.1143	0.2211
24	0.8095	0.8341	0.5429	0.7517	0.8012	0.8133	0.0931	0.2050	0.5973	0.0470	0.1143	0.2211
25	0.8095	0.8341	0.5429	0.7517	0.8012	0.8133	0.0931	0.2050	0.5973	0.0470	0.1143	0.2211
26	0.7771	0.7979	0.5429	0.7517	0.8012	0.8133	0.0931	0.2050	0.5973	0.0470	0.1143	0.2211
27	0.7771	0.7979	0.5429	0.7517	0.8012	0.8133	0.0931	0.1794	0.5973	0.0470	0.1143	0.2211

28	0.7771	0.7979	0.5429	0.7517	0.8012	0.8133	0.0931	0.1794	0.5973	0.0470	0.1143	0.2211
29	0.7771	0.7979	0.5429	0.7517	0.8012	0.8133	0.0931	0.1794	0.5973	0.0470	0.1143	0.2211
30	0.7771	0.7979	0.5041	0.7228	0.8012	0.8133	0.0931	0.1794	0.5973	0.0470	0.1143	0.2211
31	0.6606	0.7979	0.3490									
32	0.6217	0.7979	0.3490									
33	0.6217	0.7979	0.3490									
34	0.4663	0.7979	0.3490									
35	0.4663	0.7979	0.3490									
36	0.4274	0.7979	0.3490									
37	0.4274	0.7979	0.3490									
38	0.3886	0.7979	0.3102									
39	0.3109	0.7092	0.3102									
40	0.1943	0.5319	0.1551									