Exploring the Impacts of Urbanization and Aquatic Pollutants on Northern Leopard Frog (*Lithobates pipiens*) Populations in Southeastern Wisconsin

by

KristieRae Ellis

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Abstract

Amphibians are crucial pieces of the aquatic ecosystem, shuttling nutrients to higher trophic levels and providing energy to other species. However, in light of global amphibian decline and climate change, many amphibian species are disappearing. The northern leopard frog (*Lithobates pipiens*) is one of these species. The purpose of this study was to better understand the impacts that urbanization may be having on *L. pipiens*, especially where water quality and aquatic pollutants are concerned. Human population density per km^2 was used to represent the degree of urbanization while nutrient pollutants (phosphates and nitrates), specific conductivity, dissolved oxygen, and pH were used to analyze the impact that urbanization may be having on *L. pipiens* populations. Throughout the course of this study, *L. pipiens* was only identified at 1 of 5 sample locations. Thus, the results indicate that although *L. pipiens* is sensitive to degrading water quality, the species may be more sensitive to habitat loss, habitat fragmentation, and the resulting population growth of the green frog (*Lithobates clamitans*), a generalist species and common predator of *L. pipiens*.

Introduction

Many frogs and toads (known collectively as "anurans") fill an important ecological niche that is required to maintain a healthy and properly functioning ecosystem. As adults, anurans act as highly efficient energy "conveyor belts" within their habitats by ingesting macroinvertebrates - such as insect larvae - and directing approximately 50% of the resulting energy into physical growth. When the frogs are preyed upon by members of higher trophic levels, such as herons or large fish, the stored energy is free to move further up the trophic levels, providing the energy other species require for their life processes (Collins and Crump 2009; Molles 2013). Furthermore, many anurans also contribute significantly to the control of insect populations and have become increasingly useful in the medical field where amphibian skin secretions are being used to develop new pharmaceutical drugs (Collins and Crump 2009).

However, the last few decades have revealed an alarming decrease in amphibian populations around the globe (Collins and Crump 2009), threatening not only frogs and other amphibians, but also the health of aquatic ecosystems (Blaustein et al. 1994) and advances in medical technology. In the 1980s, the first documentations of global amphibian decline were recorded (Peacock and Rogeres 2012). Since then, local extinctions of amphibian species and declining populations have grabbed researchers' attention. Sadly, this has not always translated to public interest, resulting in a lack of research funding and resources (Boyer 1995). Thus, the major causes of global amphibian decline are somewhat of a mystery, with plausible causes ranging from habitat destruction to the introduction of invasive species and diseases (Hamer and McDonnel 2008). However, while researchers scramble to better understand this global disappearance, frog populations continue to deteriorate. As of 2008, 21% of all frog species were listed as "endangered" or "critically endangered" (Hamer and McDonnell 2008) and 88% of threatened amphibian species were being impacted by habitat loss, fragmentation, and degradation (Baillie et al 2004).

In light of current environmental issues, primarily global climate change and the resulting ecosystem disturbances, the conservation of amphibian species may be even more relevant today than in previous decades. Although amphibians are one of the least studied taxonomic groups (Hamer and McDonnell 2008), it has become widely accepted that their life history makes them an excellent indicator species for environmental health (Guzy et al. 2012). For instance, due to a large portion of their development taking place within the confines of an aquatic ecosystem, most amphibians tend to be incredibly sensitive to aquatic pollutants (Hamer and McDonnell 2008). Therefore, the presence or absence of mature amphibians in and around a water body has the potential to indicate the extent and impact of both overarching and local environmental problems (Storfer 2003, Guzy et al. 2012). However, some amphibians act as better indicator species than others as each species responds uniquely to urbanization based on their ecological requirements (Hamer and Parris 2011). If a species is a specialist – requiring a very narrow range of habitat characteristics - it will have a more difficult time adapting to changes in the ecosystem, making it a good indicator species.

In Wisconsin, there are a handful of frog species that could arguably be considered specialist species. Of these, the northern leopard frog (*Lithobates pipiens*) has been one of the most abundant species in the Great Lakes region (Harding 1997) and its natural habitat range spans much of North America, spreading throughout Canada and the northern United States. However, *L. pipiens* range has been shrinking since the 1960s and 1970s (Harding 1997).

Although *L. pipiens* is considered a "species of special concern" by the Wisconsin Department of Natural Resources and is considered a "sensitive species" by the United States Forest Service and Bureau of Land Management, the ICUN Red List of Threatened Species labels all Wisconsin frog and toad species as having stable populations and of "Least Concern."

While this may seem encouraging, the population stability of *L. pipiens* is still largely disputed. In 2011, the nineteen westernmost states were informed by the United States Fish and Wildlife Service that "the northern leopard frog does not warrant federal protection as a threatened or endangered species" under the Endangered Species Act and is considered globally stable (Rogers and Peacock 2012). This decision was made despite multiple studies suggesting that *L. pipiens* populations are declining and their extensive range is beginning to shrink, especially in the western United States where large populations of *L. pipiens* once existed (Rogers and Peacock 2012, Drost et al. 2011).

According to the Wisconsin Frog and Toad Survey, conducted by volunteers and the Wisconsin Department of Natural Resources, the *L. pipiens* population in Wisconsin has been steadily decreasing since the 1980s. However, there are currently no published studies exploring plausible causes for this overall decrease in population size in Wisconsin. With global amphibian decline becoming a prominent issue around the world, it is important to completely understand the various facets contributing to *L. pipiens* decline in western states and in Wisconsin. Only by understanding the problem in its entirety can we begin to restore *L. pipiens* populations to their native range and potentially reverse the population decline.

Literature Review

Global Threats to Amphibian Communities

1. Global Amphibian Decline

As the environment inevitably changes, species will either begin to adapt over time through the process of natural selection or will die off as a result of poor fitness. Extinction is a normal and, over long periods of time, a relatively common phenomenon in the natural world (Blaustein et al. 1994). However, in recent years, there has been an alarming increase in the rate and number of species extinctions, particularly vertebrates. In the 1980s, these extinctions became increasingly alarming with concern to amphibians (Blaustien et al. 1994, Collins and Crump 2009). Although amphibian populations are known to fluctuate widely in response to climatic factors - making it difficult to discern between natural variation and legitimate population decline (Guzy et al. 2012, Harding 1997) - James P. Collins and Martha L. Crump, coauthors of *Extinction in our Times: Global Amphibian Decline,* have estimated that of the 6,347 identified amphibian species, one-third are experiencing a decline in population size. And, by the 1990s, most researchers had come to the consensus that the fluctuations in many amphibian populations were abnormal (2009).

This rapid decline of the world's amphibian populations has been attributed to many different issues, the majority of which are an indirect result of human activities (Blaustein, et al. 1994). As the human population continuously grows, natural spaces are being pushed out to make room for parking lots, supermarkets, and housing expansions. This not only reduces habitat availability, but it also has the potential to degrade habitat quality and decrease species dispersal (Hamer and McDonnel 2008). Furthermore, land use changes can even alter the hydrology (Meyer and Paul 2001), temperature, and resource availability of a natural system. This can compromise the availability of food and also has the potential to introduce diseases and chemical contaminants (Collins and Crump 2009).

2. Urbanization

The largest and most widely accepted factor driving global amphibian decline is urbanization (Baillie et al. 2004). It is defined by Hamer and McDonnell (2008) as "an increase in human density that generates significant changes in chemical, physical, and ecological conditions in areas of human development." Urbanization poses a threat to local ecosystems by creating conflicts in which the development and expansion of urban landscapes outweigh the preservation and conservation of natural areas.

With 3.3 billion people worldwide already living in urban landscapes, the global urban population expected to reach 5 billion people by 2030 (UNFPA 2007). Thus, urbanization is rapidly forging ahead to meet this anthropogenic demand for resources and infrastructure (Hamer and Parris 2011). Understanding of the impacts that urbanization can have on an ecosystem is crucial, but due to its overarching nature, the term "urbanization" encompasses a multitude of environmental issues and ecosystem disturbances. Species isolation, the introduction of new diseases, over-exploitation of anuran populations, aquatic pollutants, and invasive species are just a handful of the documented suspects contributing to global amphibian decline (Coltman 2008, Peacock and Rogers 2012, Collins and Crump 2009, Hamer and McDonnell 2008, Storfer 2003). Aquatic systems located in urban landscapes also tend to be surrounded by a higher road density, having fewer forested and wetland areas than aquatic systems in more rural locations (Rubbo et al. 2004). However, the most pervasive outcomes of urbanization are habitat loss, habitat fragmentation, and the deterioration of habitat quality. These three factors alone threaten approximately 37% of all amphibian species (Hamer and McDonnell 2008, Hamer and Parris 2011).

Habitat loss and fragmentation are direct results of urbanization. As natural areas are cleared to make room for urban landscapes, animal populations are forced to move elsewhere and large habitats are split into multiple smaller and disconnected pieces. This poses a threat to many anuran populations as many species not only require a variety of linked habitats to satisfy their complex life cycle needs, but also because habitat loss and fragmentation can prevent the distribution of amphibians and limit the success of their breeding (Rubbo and Kiesecker 2005). Ponds surrounded by green, open space have a higher connectivity and promote species dispersal, while simultaneously providing access to a diverse forging habitat and refuge from aquatic predators (Hamer and Parris 2011). Lack of amphibian distribution can reduce biodiversity in an ecosystem and if habitat loss or fragmentation is too extensive, local extinction of some species may occur (Hamer and McDonnell 2008, Hamer and Parris 2011).

Habitat loss and fragmentation can also degrade the quality of a habitat by altering the quantity and type of aquatic and terrestrial vegetation present. Decreased vegetation leaves adult amphibians and their larvae unprotected and more vulnerable to predation. Hamer and Parris (2011) and Rubbo and Kiesecker (2005) both documented a positive correlation between amphibian species richness (the number of different species present within a given area) and habitat complexity. Hamer and Parris (2011) showed that amphibian species richness tends to increase significantly in response to the amount open grassland surrounding a pond and moderately in response to an increasing quantity of aquatic vegetation. Rubbo and Kiesecker

(2005) documented a positive association between amphibian species richness and the presence of forest habitat.

It is also hypothesized that some anuran species are more sensitive to the impacts of urbanization than others (Rubbo and Kiesecker 2005). Species with more general habitat requirements may be able to survive in artificially created landscapes, such as retention ponds or dams (Hamer and McDonnell 2008). However, Hamer and Parris (2011) noted that species richness decreases in response to an increase in the surrounding human population density. In addition, a similar drop in species richness in response to human population density has also been documented in larval amphibian communities (Rubbo and Kiesecker 2005). Thus, while some generalist anuran species may be relatively robust in the face of habitat loss and fragmentation, this does not necessarily hold true for all anuran species.

Water Quality and Aquatic Pollutants

As cities expand and urbanization continues, it alters the hydrology and physical landscape of a region (Meyer and Paul 2001). The construction of roads, buildings, parking lots and other impervious, unnatural surfaces increases the quantity and rate at which water drains from the landscape during and after precipitation events. Unlike natural landscapes, the high density of impermeable landscapes in urban areas prevents the rainwater from soaking into the earth's soil and subsurface. Instead, the rainwater is rushed directly into artificially and naturally created ponds, rivers, and wetlands (Christopherson 2013). In addition to storm water runoff, aquatic ecosystems can also be exposed to municipal wastewater, sewer overflows, and agricultural runoff (Meyer and Paul 2001, Brown and Froemke 2012).

1. ***Toxins and Nutrients***

Many toxins entering the aquatic environment are nonpoint pollutants, meaning that they cannot be traced back to a single, specific origin or polluter. Common nonpoint aquatic pollutants include heavy metals, industrial chemicals, pharmaceuticals, and pesticides. Despite the hazardous impacts toxins can have on plant, animal and human health, the majority of these toxins still remain largely unregulated by the United States government (Brown and Froemke 2012). Brown and Froemke (2012) found that stressors contributing to the introduction of toxins are housing density, road density, agricultural cultivation, confined animal feeding, and atmospheric deposition.

Nutrient pollution is also proving to be problematic in urban environments. While nutrients may be naturally occurring, they can enter the environment in excess as a result of stressors such as housing density and atmospheric deposition (Brown and Froemke 2012). The most common nutrients that are hazardous in excess are nitrogen and phosphorus, with urbanization being a major contributor to higher phosphorus concentrations in waterways (Meyer and Paul 2001). While these nutrients are crucial for primary productivity in an ecosystem, too much of a good thing can also be dangerous. When nitrogen and phosphorus enter an aquatic ecosystem, the excess nutrients can cause algal blooms. The throughout its life cycle algae depletes the ecosystem of dissolved oxygen. In turn, less dissolved oxygen is available to other members of the aquatic community, such as fish and amphibians (Brown and Froemke 2012). When more dissolved oxygen is demanded than is actually available in a system, individuals and the population as a whole will become stressed and likely less healthy.

2. *Specific Conductivity*

Combined, many toxins and nutrient pollutants can contribute to a high specific conductivity in an aquatic ecosystem. Specific conductivity can be defined as the concentration of ions dissolved in water. The most common freshwater ions are magnesium and calcium, followed by lesser concentrations of other ions, including sodium, potassium, sulfate, nitrate, and phosphate (Dodson 2005). Hamer and Parris (2011) documented a high specific conductivity in ponds located in urban areas and noted that amphibian species richness seemed to decrease as specific conductivity increased. They speculated that the increased specific conductivity was a result of local disturbances and land use changes that had caused storm water runoff to drain into the ponds, carrying chemical and nutrient ions with it from the urban landscape (Dow and Zampella 2000, Paul and Meyer 2001, Hamer and Parris 2011).

Water Quality and the Northern Leopard Frog

Lithobates pipiens (previously classified as *Rana pipiens*) is commonly known as the northern leopard frog. The species is native to the Great Lakes region and is also prevalent throughout much of Canada and the mid-west and north-western United States. In 1965, the species' range was considered "more extensive than that of any other North American anuran", reaching as far south as Panama (Dole 1965). As fully mature adults, they are approximately 5.0 to 11.1 cm in length (Harding 1997). They range in color from green to brown, with black spots covering their back and sides. The black spots are commonly surrounded by a golden halo while the dorsal folds along the animal's back are white.

Figure 2. Lithobates pipiens. Sand Pond, Illinois Beach State Park, Zion Illinois. Photo credit Trinette Ellis.

L. pipiens is generally most active in the warmer, summer months when the air temperature is between 22 - 25°C, with water temperatures ranging from 18 - 24°C (Nace et al. 1996). The species prefers no wind or fairly calm winds. Due to the fact that amphibians have sensitive and highly permeable skin, strong winds have the potential to cause physiological water loss and drying out. For this reason, *L. pipiens* is also most active during light or periodic precipitation events, sometimes even migrating a significant distance away from their home waters during such events (Dole 1965).

In the spring, *L. pipiens* congregates in marshes, wetlands, lakes, and ponds to breed. After breeding, many adult frogs will radiate out into the surrounding environment, particularly into grassier areas such as wet meadows and sedge meadows (Dole 1965, Harding 1997, Kendell 2002). These areas have the benefit of not only being moist, but also offering better protection from predators. As summer eases into autumn, adult *L. pipiens* return to their home waters to hibernate, usually during the months of September and October. A suitable over-wintering habitat is characterized by relatively high dissolved oxygen levels (7 – 10ppm), cooler water temperatures (<4°C), and moderate water circulation, as this would likely prevent the water body from freezing through completely (Kendell 2002).

Prior to the 1960s and 1970s, *L. pipiens* was one of the most common frog species in Great Lakes Basin and the surrounding region (Harding 1997). However, in recent years there has been an obvious decline in their population and range within the United States and Canada, especially in the western United States where *L. pipiens* populations are disappearing from locations that have historically boasted of their presence (Rogers and Peacock 2012, Drost et al, 2011). The decrease in *L. pipiens'* range and population size has been largely attributed to their collection as biological supply specimens and as fish bait (Harding 1997), but other factors such as habitat fragmentation and over-withdrawal of water have also been shown to impact the species (Drost 2011).

Purpose of Study

The purpose of this study was to analyze the potential impacts of urbanization on *Lithobates pipiens* populations and to explore the role that this may play in the decline of *L. pipiens* populations in Wisconsin. With the understanding in mind that *L. pipiens* is more sensitive to aquatic pollutants than other native amphibian species (Harding 1997), it was anticipated that water quality would be a determining factoring in distinguishing ideal *L. pipiens* habitats from inhospitable habitats. In addition, it was also expected that *L. pipiens* would be more abundant in ponds with complex surrounding habitats. The species is known to disperse into grasslands and prairies during the summer months after breeding (Kendell 2002, Harding 1997; Dole 1965). Therefore having breeding and wintering ponds located near grasslands would be advantageous for frog reproduction and foraging.

Based on this information and the knowledge that water pollution tends to be more pronounced in urbanized areas (Brown et al. 2012), I hypothesized that *L. pipiens* would have a greater relative abundance in more rural ponds with a smaller human population density (per km^2) than in urban ponds with a larger human population density (per km^2).

Methods

Sample Sites

Sampling sites are located in southeastern Wisconsin and northeastern Illinois near the state line. Five sites were chosen based on human population density and location, creating a gradient of urbanization, with human population density representing the degree of urbanization (**Table 1**). Sample sites with a higher human population density were considered urban, while sample sites with a lower human population density were considered to be rural. Population density was determined per km^2 by using the 2014 United States Census data from ESRI, a geographic information systems data provider, and served as the primary factor in choosing sample locations. For example, Lincoln Park in Kenosha, Wisconsin had the highest human population density (2,777people/km^2) and was considered the most urban sample site, while Bristol Woods in Bristol, Wisconsin had the lowest human population density (69people/km^2) and was considered the more rural sample site.

The surface area of the ponds ranged from approximately 800m^2 to 77,000m^2 (**Table 1**). Although it would have been ideal for all the sampled water bodies to be relatively homogonous in terms of surface area, resource and time constraints prevented this from being a possibility. For example, Bristol Woods pond was selected as a substitute for a pond with a larger pond that was selected initially, but could not be accessed safely due to the opening of duck hunting season. Thus, there was a large variation in pond surface area and the mean surface area of the sampled ponds was 25,607$m^{2.}$

Table 1: Description of sample locations**.** Population density was determined per km^2 using 2014 United States Census data from ESRI. Degree of urbanization was determined based on human population per km^2. Thus, Lincoln Park was considered the most urban sample site, while Bristol Woods was considered most rural. Surface area was calculated using ESRI ArcGIS Online™ software.

	Location	**Population (per km^2)**	**Surface Area (m^2)**
Lincoln Park	Kenosha, WI	2,777	15,966
Anderson Park	Kenosha, WI	1,372	26,457
32nd Avenue	Pleasant Prairie, WI	497	7,948
IL Beach State Park	Zion, IL	287	76,866
Bristol Woods	Bristol, WI	69	800

Sampling Procedure: Visual Encounter Survey

All five sites were sampled at least three times. Samples were collected between October 5th and October 23rd 2014. This is a convenient time of year to sample *Lithobates pipiens*, because the larvae will have developed into juvenile frogs, making them easier to distinguish from other larval anuran species, and adult frogs will be returning from grasslands and prairies to the ponds for winter hibernation (Harding 1997, Kendell 2002). In addition, sampling during the fall also eliminates the need to compensate for the dispersal of juvenile, post-metamorphic *L. pipiens*, which radiate into the surrounding wetlands and ponds regardless of habitat quality. Thus, if this study had included samples from the period of juvenile dispersal, such as late summer months, it is likely that some *L. pipiens* would have been encountered in areas not necessarily beneficial to their health or development (Germaine et al. 2009).

The sampling method used was modeled after Crump and Scott's Visual Encounter Survey (1994) found in *Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians* (Heyer et al. 1994). This method is fairly common and has been recommended and used in several other amphibian studies (Germaine 2009, Peacock and Rogers 2012, Klaver et al. 2013, Kendell 2002). It is especially useful for monitoring specific species; however, it is somewhat limited because it is based on the following assumptions: (1) each individual of each species has an equal chance of being observed during the survey period, (2) each species has the same chance of being observed as other species, regardless of season, weather, or other ecological events (such as predation or competition), and (3) each individual is recorded only once throughout the course of the survey (Crump and Scott 1994).

For the purpose of this study, the survey method was time constrained; limited to 15 minutes at each site. This time limit was chosen in part because the perimeter of some sample ponds was made inaccessible by dense, emergent vegetation. Walking through such vegetation would likely disturb basking frogs and yield poor survey results. Kendell (2002) suggests that time-constrained sampling be carried out for at least 20 minutes. However, 15 minutes is approximately the length of time required to walk the perimeter of the smallest site, the Bristol Woods pond (perimeter of ~0.13 kilometers) at steady, but slow pace.

At each site, we walked around the perimeter of the water body, as near to the water as possible without causing a disturbance among the vegetation. To assure that an approximately equal area was sampled at each location, care was taken to walk at an even pace and to avoid lingering at a single location along the perimeter. Each time a basking frog was spotted, it was identified by species and the information was recorded. The total number of all frogs observed and the number of individuals of each identified species were recorded. For example, if 13 frogs were observed in total at one location, ten of these frogs may have been northern leopard frogs and while the remaining three may have been western chorus frogs.

In the case of green frogs (*Lithobates clamitans),* species identification was either visual or vocal. When disturbed, *L. clamitans* will often produce a short, high pitched vocalization that can be used as a distinguishing characteristic for the species. This vocalization consists primarily of a single, sharp “peep” and allows for easy identification if the frog disappears before a visual observation can be made. This technique was used frequently in identifying *L. clamitans*, as their standard response to disturbances is to dive beneath the water surface.

Although *L. pipiens* does not produce a vocalization when agitated, their response to disturbances is much less abrupt and consists largely of decreasing physical movement and crouching nearer to the substrate (although usually not becoming submerged completely) rather than diving below the surface of the water. This makes visual observations very easy and in many instances, it was actually possible to study the same northern leopard frog for several minutes. On occasions when a frog was seen, but the species could not be identified (primarily because it had dipped under the surface of the water before a visual observation could be made and did not produce an identifying vocalization), it was simply tallied as part of the total number of frogs seen at the location.

Sampling Procedure: Weather Conditions

Because many frog species are sensitive to weather conditions, their activity is highly dependent upon time of day, temperature, precipitation, and wind (Kendall 2002). Therefore, these factors were all recorded throughout the duration of the sampling period. During each sampling session, air temperature was measured at three different locations around the pond perimeter. Night air temperature was established through forecast history provided by the National Oceanic and Atmospheric Association (NOAA).

However, precipitation and wind were recorded as discrete data. For example, precipitation was recorded as “light” or “none”. This data collection decision was justified based on the assumption that when it comes to precipitation and wind, anurans are most influence by broad, observable differences, rather than by small, minute changes. This assumption is reflected in the *Survey Protocol for the Northern Leopard Frog* (Kendell 2002), which also records precipitation and wind as discrete data.

Sampling Procedure: Aquatic Pollutants

To establish water quality, three different methods were used. The first method required the use of an electronic monitoring instrument known as a water quality sonde, made by HydroLab®. The sonde has multiple sensors, located at the tip of the instrument, and each sensor is responsible for measuring a single water quality parameter. When the sensors are completely submerged in a water sample, the water moving over the sensors allows the sonde to calculate the desired information. This information is then displayed on a handheld electronic screen that is attached to the sonde (known as the surveyor). For the purposes of this study, the HydroLab® sonde was used only to measure pH, specific conductivity (µS/cm), water temperature (°C), and dissolved oxygen concentration (mg/L).

Three measurements were recorded at each pond, with the individual measurements being collected at different locations around the perimeter of the pond. Water quality was measured within one meter of the shore, as this is where frogs are most easily observed, hiding amongst emergent vegetation and floating algal mats or leaves. These measurements were calculated <0.5 meters below the water surface. However, the sonde sensors must be completely immersed in water to calculate measurements and the majority of the sampled shoreline areas were shallow enough that it was necessary to lay the sonde horizontally, rather than vertically, in the water for the sensors to be completely submerged. Even if it was possible to place the sonde vertically into the water, completely immersing the sensors, the depth was still <0.5 meters.

Water samples were collected in small containers and phosphorus levels were measured the same day the samples were collected. Following the LaMotte® water pollution kit (Model AM-21) procedure for a standard field test, a chemical titration method was used to analyze the phosphorus content of the samples. Inorganic phosphate levels are determined by the reaction of phosphate with a molybdate solution. This forms a phosphomolybdate, which appears blue in color when reduced. The intensity of the color indicates the level of phosphorus present in the water sample.

Nitrate, nitrite, hardness, alkalinity, and chlorine were measured using Teta EasyStrips™, scientific quality aquarium water test strips. However, the collected water samples were not able to be tested with EasyStrips™ until approximately 1 month after the samples had been collected. Regardless, the strips were dipped into the water samples for 1 second and allowed to dry for 60 seconds, following the instructions provided by the manufacturer. Each strip was then compared to the colors on a chart, also provided by the manufacturer, to establish the concentration of nitrate (mg/L), nitrite (mg/L), and chlorine (mg/L) while also indicating the hardness (GH – general hardness)(ppm) and alkalinity (KH – carbonate hardness)(ppm) of each water sample.

After the data was collected, it was entered into IBM SPSS Statistics software for analysis. To determine the statistical differences amongst the five sample sites and the significance of these differences, one-way analysis of variance (ANOVA) was run on the dependent variables pH, dissolved oxygen (mg/L), water temperature (°C), and specific conductivity (µS/cm), using sample location as the independent variable ($\alpha = 0.05$). A Tukey HSD (honest significant difference) post-hoc test was conducted to confirm suspected differences and similarities between the sample means at each location. This post-hoc test also combines locations into groups, referred to as subsets, based on statistical similarities. These subsets were used to help understand the levels of variation between urban sample locations and rural sample locations.

Results

In terms of the number of frogs observed at each location, there was positive correlation between decreasing human population density and total frog population, with the total number of observed frogs increasing as human population decreased. The highest number of frogs observed on one day at a single location was 32. This number was recorded both at the Bristol Woods sample site, the least urban of the five locations (69 people/km^2) and Sand Pond (287 people/km^2). However, the Bristol Woods pond had the highest mean frog count (24 individuals). The lowest mean number of frogs observed was 0.3 individuals. This was at the Lincoln Park sample site, the most urban of the five locations (2777 people/km^2). Only one frog was observed here throughout the course of sampling. The mean number of frogs observed had a positive correlation with decreasing human population density ($r^2 = 0.330$) (**Figure 1**), with the total number of frogs steadily increasing as urbanization decreases, and also a positive correlation with increasing specific conductivity ($r^2 = 0.731$) (**Figure 2**).

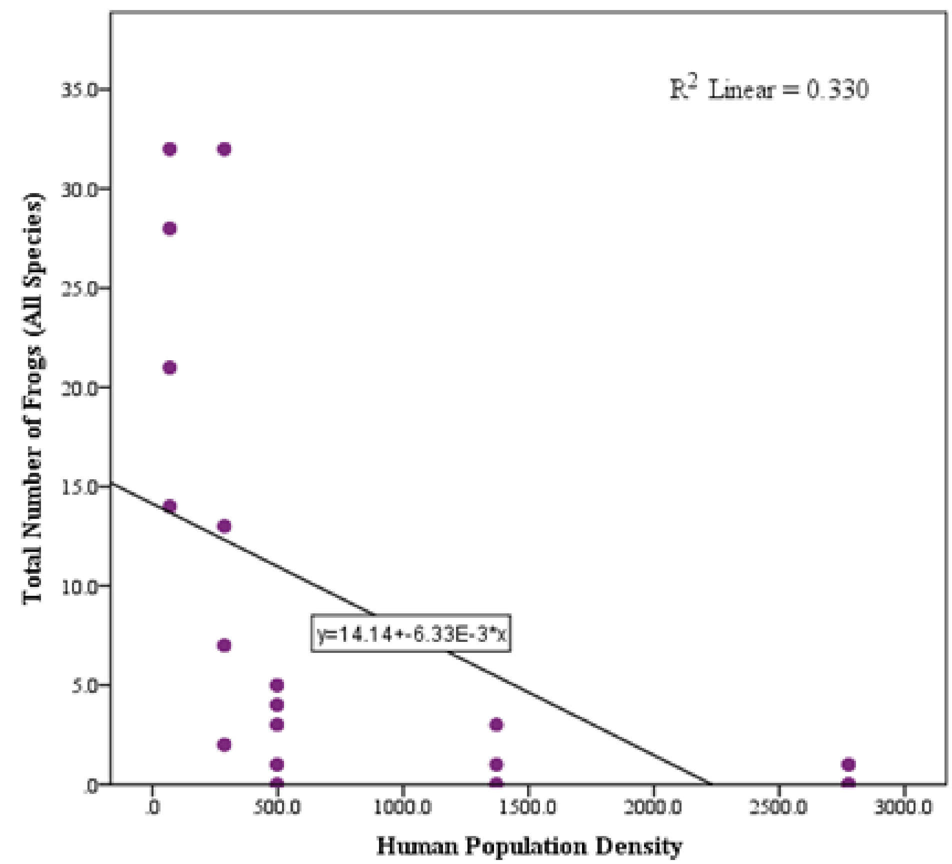

Figure 1: Correlation between human population density (number of people/ 1km^2) and the total number of frogs observed of all species. There is a negative correlation between the total number of frogs and increasing human population density.

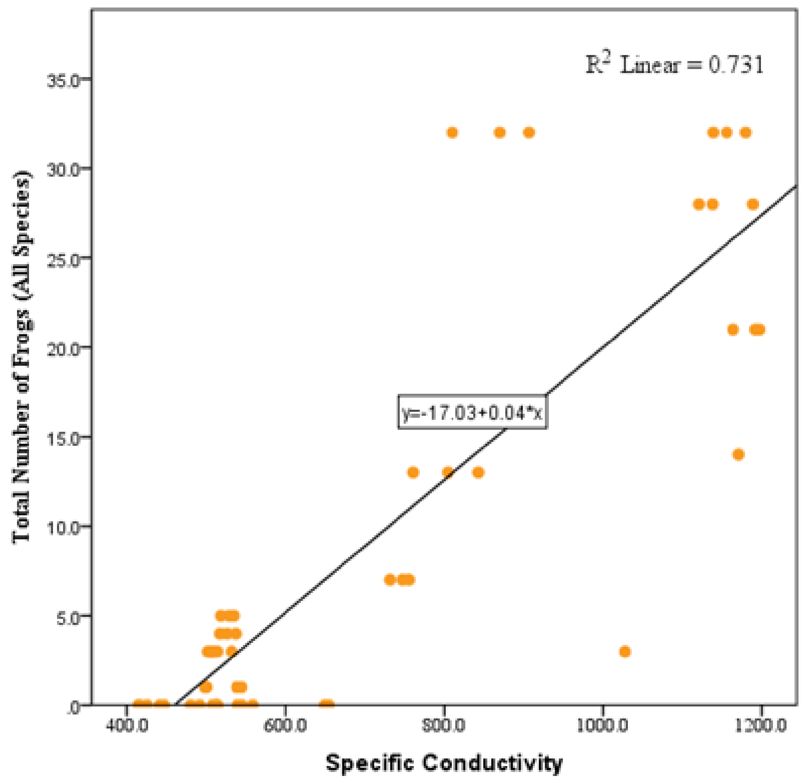

Figure 2: Correlation between specific conductivity (µS/cm) of water and the total number of frogs observed of all species. There is a positive correlation between increasing specific conductivity and the total number of frogs.

However, regardless of the total number of frogs observed at each location, *Lithobates pipiens* was only observed and identified at Sand Pond in Illinois Beach State Park in Zion, Illinois. In contrast, *Lithobates clamitans* (green frog), was identified at the Anderson Park pond, the 32nd Avenue pond, and the Bristol Woods pond. The only other frog species identified at these sites was one *Lithobates catesbeianus* (American bullfrog) and it was identified at the 32nd Avenue Pond. *L. pipiens* was the only frog species identified at Sand Pond within the 15 minute time-constrained survey. However, while walking the site later to collect water samples, three *Pseudacris triseriata* (western chorus frog) were also observed and able to be identified, but were not included in the data as they were only observed after surveying was complete.

The results of the multivariate ANOVA show that there is a significant statistical similarity ($p = 0.988$) in mean pH between the 32nd Avenue pond (mean pH = 9.50) and Anderson Park pond (mean pH = 9.42), with 32nd Avenue having the highest mean pH of all sample sites. Lincoln Park pond, however, located in the most densely populated area (2,777 people/km^2) and considered the most urban sample location, had a pH more similar to that of the two more rural ponds, Sand Pond (287 people/km^2) and the Bristol Woods pond (69 people/km^2). Tukey's post-hoc test revealed that while Sand Pond and Bristol Woods pond can be divided into one subset (A), the Lincoln Park pond still overlaps with the more urban locations (C) (**Table 2**). The lowest mean pH (8.81) was recorded at Sand Pond in Illinois, following the Bristol Woods pond (8.84) and the Lincoln Park Pond (8.94). In addition, there was a slight negative correlation between increasing pH (water becoming more basic) and the total number of frogs observed ($r^2 = 0.305$) (**Figure 3**).

Table 2: Results of Tukey's HSD (honest significant difference) Post-hoc Test, expressing the means of pH, specific conductivity (µS/cm), dissolved oxygen (mg/L), and water temperature (°C). A, B, and C represent different subsets. Sites labeled as AB or BC were somewhat similar to other subsets and could not be placed in a unique subset. The blue box shows the strong differences between specific conductivity in urban locations compared to more rural locations, those in the red and green boxes.

	Lincoln Park Pond	Anderson Park Pond	32nd Ave Pond	Sand Pond	Bristol Woods Pond
pH	8.94 (AB)	9.42 (BC)	9.50 (C)	8.81 (A)	8.84 (A)
Specific Conductivity	491.00 (A)	507.02 (A)	568.72 (A)	803.29 (B)	1164.70 (C)
Dissolved Oxygen	9.61 (AB)	10.30 (AB)	12.70 (B)	8.90 (A)	12.59 (B)
Water Temperature	12.23 (A)	12.18 (A)	13.35 (AB)	14.67 (B)	12.60 (AB)

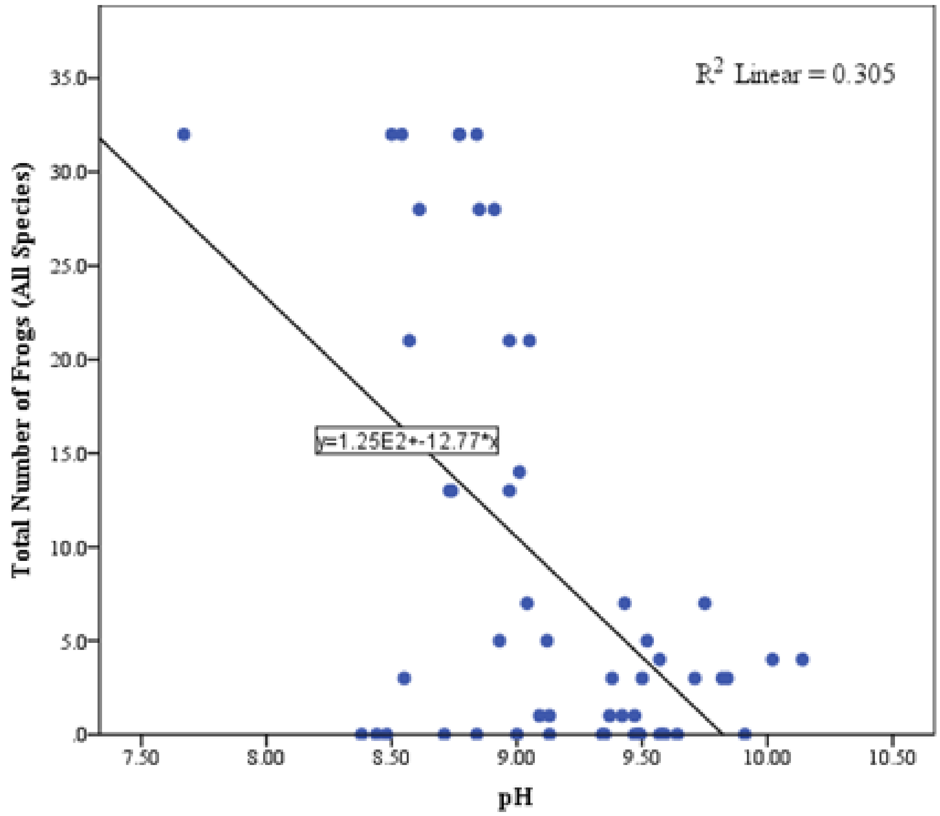

Figure 3: Correlation between water pH the total number of individual frogs observed of all species. There is a negative correlation between increasing pH and total number of frogs observed.

Specific conductivity varied greatly between the five locations. The pond with the lowest mean specific conductivity was the Lincoln Park pond (491.00 µS/cm), the most urban site, while the pond with the highest mean specific conductivity was the Bristol Woods pond (1164.70 µS/cm), the most rural sample site. Tukey's post-hoc tests shows the three most urban sites (Lincoln Park, Anderson Park, and 32nd Avenue) can be grouped into one subset (A), while Sand Pond (287 people/km^2) and the Bristol Woods pond (69 people/km^2) are in their own subsets (B and C, respectively) (**Table 2**). There is a very large statistical difference between the Bristol Woods pond and all other sample sites ($p = 0.000$ for all sites). This is also true for Sand Pond ($p = 0.000$ for all sites). The most statistically similar ponds were the Anderson Park pond and the Lincoln Park pond ($p = 0.663$). **Figure 4** shows how specific conductivity increases in response to decreasing human population density ($r^2 = 0.400$).

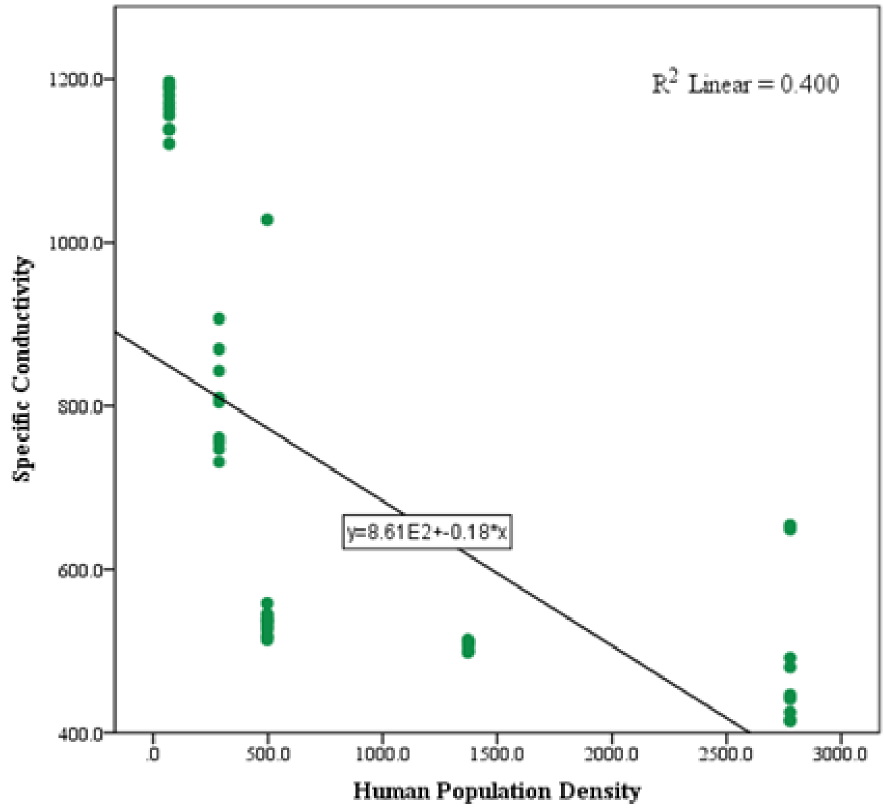

Figure 4: Correlation between human population density (number of people/1km^2) and specific conductivity. There is a negative correlation between increasing human population density and specific conductivity.

Moving into dissolved oxygen (DO), the multivariate ANOVA indicated that the most significantly different locations were the 32nd Avenue pond and Sand Pond ($p = 0.016$), in addition to the Bristol Woods pond and Sand Pond ($p = 0.036$). This was also reflected in Tukey's post-hoc test, as Sand Pond was represented as one subset (A) while 32nd Avenue and the Bristol Woods pond were sorted into a different subset (B) (**Table 2**). The two remaining sample locations, the Anderson Park pond and the Lincoln Park pond, were The pond with the lowest mean dissolved oxygen level was Sand Pond (mean DO = 8.90 mg/L), while the pond with the highest dissolved oxygen level was the 32nd Avenue pond (mean DO = 12.70 mg/L). However, there was little to no correlation between the total number of frogs observed and change in dissolved oxygen concentration ($r^2 = 2.456 \times 10^{-4}$).

The water temperature also varied, with there being the greatest significant difference when comparing Sand Pond to the Anderson Park pond ($p = 0.15$) and the Lincoln Park pond (0.031). There was only a moderate statistical difference when comparing Sand Pond to the Bristol Woods pond ($p = 0.080$). Sand Pond was also recorded as having the warmest mean water temperature (14.67 °C), while the Anderson Park pond had the coldest mean water temperature (12.18 °C), followed by the Lincoln Park pond (12.23 °C). In addition, Sand Pond also had the highest mean air temperature (19.55 °C) while the Anderson Park pond had the coldest mean air temperature (14.39 °C), followed once again by the Lincoln Park pond (16.55 °C). As expected, there was a slight positive correlation between water temperature and air temperatures ($r^2 = 0.277$) and a slight positive correlation between increasing air temperature and the total number of frogs observed ($r^2 = 0.232$). However, there was little correlation between the total number of frogs observed and the temperature of the water ($r^2 = 7.419 \times 10^{-5}$).

As far as nutrient pollution is concerned, there has been little conclusive data. The LaMotte® water pollution kit results showed that all sample sites had a < 1ppm concentration of phosphorus and it was not possible to determine a relative difference in concentration among the five samples. Nitrate, nitrite, hardness, chlorine, and alkalinity, measured with the EasyStrips™ showed similar results, with there being little obvious difference from one location to the next. The Lincoln Park pond and the Anderson Park pond showed slightly elevated levels of nitrate, but nitrite and chlorine were not present in measurable amounts at any of the five locations. The most obvious trend was the positive correlation between increasing alkalinity and decreasing urbanization. The Lincoln Park pond had the lowest alkalinity (80 – 120ppm) while Sand Pond and the Bristol Woods pond had the highest alkalinity (>300ppm), meaning that the less urban sites have a higher capacity to neutralize acid than the less urban sites (Dodson 2005).

Discussion

Lithobates pipiens was identified at only one site, Sand Pond in Illinois Beach State Park in Zion, Illinois, throughout the course of sampling. This location had the most neutral mean pH (8.81) of all the sites. According to Kendell (2002), *L. pipiens* requires breeding ponds with a non-acidic pH ranging from 6.5 – 8.5. Although Sand Pond is slightly more basic than this, it also has a high alkalinity (>300ppm), meaning that the pond has the potential to neutralize acids without dramatically changing pH (Dodson 2005). However, the difference in pH between Sand

Pond and the Bristol Woods pond is not significantly different statistically ($p = 1.00$) and both exhibit the same alkalinity reading (>300ppm).

Interestingly, Sand Pond had the lowest mean dissolved oxygen concentration (8.90mg/L), but this measurement still falls within the 7mg/L – 10mg/L parameter that Kendell (2002) suggests, as do the two most urban locations, Lincoln Park (9.61 mg/L) and Anderson Park (10.30 mg/L). The relatively low dissolved oxygen concentration could potentially be explained in part by the high mean water temperature (14.67°C), as dissolved oxygen concentration tends to decrease as water temperature increases. However, due to the fact that the dissolved oxygen data was collected in the autumn, when ponds and lakes are beginning to experience seasonal turnover, this data could hold little significance overall. During turnover, the normal stratification layers of water bodies are disturbed the sinking of water at the surface. This causes the oxygen and temperature of the ponds to become more evenly distributed throughout (Dodson 2005). If dissolved oxygen data were collected during a different time of year, when the ponds were not experiencing a seasonal turnover, it is quite possible that this dissolved oxygen concentration measurements would tell a different story.

One of the most unexpected results of this study is the trend in specific conductivity. Specific conductivity is used as a parameter in many water quality studies to act as a rough indication of the concentration of the total dissolved ions present in the water. These dissolved ions can often be classified as aquatic pollutants, such as heavy metals, magnesium, nitrates, phosphates, etc. (Dow and Zampella 2000). Hamer and Parris (2011) documented that higher specific conductivity was associated with ponds in more urban areas and that amphibian species richness decreased as specific conductivity increased. Thus, it was expected that specific conductivity would decrease as the surrounding human population decreased.

However, while specific conductivity can seem be used as a rough estimate for the concentration of aquatic pollutants, Dodson (2005) notes that "there is a general correlation between total conductivity and the concentration of plant nutrients." This means that specific conductivity can also act as a proxy for the amount of nutrients available for primary production (i.e. the growth of emergent macrophytes and algae as a result of photosynthesis and nutrient availability) (Prepas 1983).

In addition, Klaver et al. (2013) showed that *Anaxyrus boreas* (boreal toad) responded positively to specific conductivity, with their occupancy increasing in water bodies with higher specific conductivities. Klaver et al. hypothesized that higher specific conductivity aids in the osmoregulatory processes of these toads, and making them less susceptible to diseases that interfere with osmoregulation, such as chytridiomycosis, also known as Chytrid fungus. In addition, Klaver et al. (2013) also noted that *Lithobates luteiventris* (Columbia spotted frog), a relative of *L. pipiens*, expressed little to no relationship between specific conductivity and occupancy.

In contrast, Klaver et al (2013) did show that *Pseudacris maculata* (the boreal chorus frog), a relative *Pseudacris triseriata* (the western chorus frogs seen only at Sand Pond), had a higher occupancy at sample sites with lower specific conductivity. Further study would be needed to confirm the similarities in response to specific conductivity between *P. maculata* and *P. triseriata*. However, because these species are often classified as subspecies (Minnesota Department of Natural Resources 2014), it seems reasonable to conclude that *P. triseriata* may also be sensitive to high specific conductivity and the population would have been less evident at locations with high specific conductivity, such as Sand Pond, and more evident at sample sites with lower mean specific conductivity readings.

In this study, there was a positive correlation between decreasing human population density and increasing specific conductivity ($r^2 = 0.400$) and the positive correlation between increasing specific conductivity and increasing total frog population ($r^2 = 0.731$), suggests that specific conductivity is measuring beneficial ions, such as nitrates and phosphates, present in the sampled ponds. Visual observation supports the hypothesis that specific conductivity could potentially act as a proxy for nutrient availability, as the more rural ponds (Sand Pond and the Bristol Woods pond) not only had a greater flora and fauna diversity, but also a much higher density of riparian vegetation. In some places, especially at Sand Pond, one could not easily reach the water's edge due to the dense vegetative growth. However, in the two more urban locations (Lincoln Park and Anderson Park), it was very easy to access to the water, with the exception of small areas around the pond perimeter where cattails or willows were growing. In addition, the urban locations also had a large quantity of mowed lawn, as did the 32nd Avenue Pond and the Bristol Woods pond. Sand Pond was the only sample site with relatively little mowed grasses and where these areas were present, it was seemed only to maintain walking paths and parking lots for public use.

L. pipiens also requires a variety of habitat types to meet life cycle requirements. *L. pipiens* tends to winter and breed in larger ponds, but radiates into prairie and grasslands during the summer, after breeding (Kendell 2002). Locations such as Sand Pond, offer a vast prairie and a variety of smaller ponds scattered within close vicinity. The Bristol Woods pond offered a similar landscape, but was surrounded by a greater amount of forest and agricultural land than Sand Pond.

In addition, it is important to note that *L. pipiens* and *L. clamitans* were never identified in the same ponds throughout the course of this study. The anuran occupancy at the sample sites were overwhelmingly one species or another, with *L. catesbeianus* being identified at only one location, indicating that the anuran species richness at all sites was low. However, it is possible that limiting survey time to only 15 minutes prevented the identification of other, less prominent anuran species within the ecosystem. This is certainly true to some extent, because three *Pseudacris triseriata* were identified at Sand Pond while water samples were being collected.

Harding (1997) mentions in his text that *L. clamitans* and *L. catesbeianus* can act as natural predators of *L. pipiens.* This seems to be the best explanation for why *L. pipiens* was not identified at locations with a high *L. clamitans* occupancy. In addition, *L. clamitans* is also a more generalist species, meaning it is capable of thriving in a wider variety of habitat conditions. Hamer and McDonnell (2008) noted in their review that amphibians with broad habitat requirements, such as *L. clamitans*, would be more likely to persist in urban areas because they can take advantage of man-made habitats such as retention ponds. Thus, *L. clamitans*' ability to cope with ecological stressors, such as poor water quality, habitat fragmentation, and habitat loss, may provide it with the opportunity to outcompete and also to prey upon the more sensitive *L. pipiens* population.

Future Implications

While this study shows that *L. pipiens* is clearly influenced to some extent by urbanization and is likely quite sensitive to habitat fragmentation and habitat loss, it is still undoubtedly limited in scope. For example, although many facets of water quality were tested, the information gained just skimmed the surface of many potential water quality issues. If more sensitive water pollution testing equipment had been utilized and a broader range of pollutants had been tested, it is likely that there would be a significant difference in aquatic pollutants and their concentrations. These differences could help clarify the specific conductivity findings and also shine a light on how *L. pipiens* and *L. clamitans* respond differently to these varying water quality parameters.

Future versions of this study or expansions upon it would be well advised to delve deeper into the water quality aspects of this study and attempt to understand the full spectrum of influences that aquatic pollutants can have. It would also be interesting to further investigate the relationship between *L. pipiens* and *L. clamitans* to determine with certainty if *L. clamitans* is outcompeting and preying upon *L. pipiens* in a manner significant enough to influence the *L. pipiens* population decline.

Lastly, it would also be recommended to sample ponds of more uniform surface areas or to sample a greater number of ponds, having multiple ponds representing each portion of the urbanization continuum. Many studies take this route, sampling a variety of water bodies of a variety of sizes. Peacock and Rogers (2012), sampled upwards of 97 sites, including the reaches of multiple streams. Gibbs et al (2005) sampled 323 sites and Guzy et al. (2012) collected data from 42 wetland locations. Due to time and resource constraints, this large of a study area was not possible, but certainly would have helped clarify the impact that pond surface area had on data.

Conclusion

While *L. pipiens* does require certain water quality parameters to survive and thrive within a given habitat, the data collected throughout the duration of the study suggests that *L. pipiens* is more susceptible to habitat loss and fragmentation than to urban runoff and aquatic pollutants. Because *L. clamitans* (green frog) does not require such a specific set of habitat requirements to meet life cycle needs, this generalist species has the ability to thrive in areas where *L. pipiens* cannot, such as in retention ponds in urban landscapes. *L. clamitans* could potentially be exploiting *L. pipiens* populations, the more sensitive of the two species, through resource competition and predation. Thus, *L. pipiens* is not only directly impacted by urbanization through habitat loss, degradation, and fragmentation, but *L. pipiens* is also indirectly impacted by urbanization through the opening it has provided for *L. clamitans* to occupy ecological niches that are unsuitable to *L. pipiens.* In many ways, urbanization has provided *L. clamitans* with a 'leg up' ecologically while it has hindered the growth and dispersal of *L. pipiens* populations.

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