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Danielle Crownover Date

Effects of LED lighting on amphibian movement at Cuscowilla, Virginia June-July 2019

By

Danielle Crownover

Master of Science

Environmental Science

John Wegner, PhD

Advisor

Gonzalo Vazquez-Prokopec, PhD

Committee Member

Lance Gunderson, PhD

Committee Member

Accepted:

Lisa A. Tedesco, Ph.D.

Dean of the James T. Laney School of Graduate Studies

Date

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An abstract of

A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of Master of Science
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2020

ABSTRACT

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Amphibian populations are decreasing as a result of anthropogenic factors such as habitat fragmentation and pollution. The urbanization of amphibian habitat often includes introducing artificial light at night. Light pollution is believed to affect the behavior of amphibians. Currently sodium-halogen lamps are frequently used for lighting at night, however, there is a shift towards using Light Emitting Diodes as a greener alternative. Although this study took place in Cuscowilla, Virginia along the edge of a beaver pond with no previous artificial light exposure, the intention was to emulate an urban artificial lighting confrontation experience for pond-dwelling amphibians. At the chosen study location, five sites were set up with nine pitfall traps on each side of an erosion control fence. A lantern capable of emitting both high intensity LEDs and low intensity LEDs was placed centrally at each site to attract amphibians coming from and to the pond. For twenty nights each site's lantern was randomly selected for high intensity, low intensity, or no light and left on for a period of two hours.

After the two-hour period, amphibians were identified and counted before traps were recovered. It was predicted that there would be a higher abundancy of amphibians found in pitfall traps located near high intensity LED sites in contrast to low intensity LEDs sites. Initial analysis determined that the data were non-normally distributed, therefor non-parametric Chi-squared tests using species counts (presence/absence trap success) were performed to determine if any species preferred one light intensity over the other. Only two species: *Acris crepitans* and *Lithobates sphenocephalus* were determined to respond significantly to the presence of LEDs over the absence. When conducting an additional non-parametric Kruskal-Wallis test using total amphibians collected, a significant difference between high intensity and no light treatment was found. A post-hoc Dunn test revealed that an additional species, *Gastrophryne carolinensis* significantly responded to LED presence over no light.

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1. INTRODUCTION

Anthropogenic forces such as urbanization are a known threat to amphibians (Daszak et al., 2003). Amphibians are unable to keep up with the increasing magnitude of stressors such as habitat loss and fragmentation (Wake and Verenburg 2008). Human population continues to rise and with it the associated effects of altering natural landscapes. Migratory amphibian species are experiencing substantial population declines as a result of habitat loss and landscape fragmentation (Horns and Sekercioglu 2018). Fragmentation can occur as a result of infrastructure development and its dividing impact on the surrounding environment. Fragmentation creates patches of undisturbed and disturbed environments that are defined by their difference in integrity. Migratory species are limited to the corridors created by the patches resulting from landscape development.

Humans have altered ecosystems by changing the composition, connectivity, and configuration of natural landscapes (Tischendorf and Fahrig 2000). When habitat fragmentation occurs, amphibian movement is prohibited. Many amphibian species migrate over longer distances from overwintering habitats to breeding ponds for summer, the mating season. Both during migration events and movements within the home range, novel ponds are affected by habitat fragmentation (Eigenbrod et al., 2008). In addition, the destruction and replacement of habitat can create barriers or filters to the movement of individuals between habitat patches (Eigenbrod et al., 2008). Roads are believed to be strong filters for many species, as not all are able to successfully cross (Eigenbrod et al., 2008). Despite the development of roads, amphibians continue to migrate via their historic routes notwithstanding the alteration of their historic wetlands. As a result of their inability to rapidly change their routes, amphibians are prone to contact with humans and human-made structures which may result in deadly encounters such as artificial light at night.

Artificial light at night results in light pollution which increases ambient illumination, disrupts photoperiod, and changes spectral properties of night light that may affect the physiology, behavior, and ecology and evolution of many animals, including amphibians (Buchanan 2006). Artificial light that alters the natural phenology of lighting in an ecosystem is referred to as ecological light pollution (Longcore and Rich 2004). Globally, light pollution is increasing rapidly, estimated at 6% annually (Hölker et al., 2010). There is both an expansion of electric lighting to previous unlit communities in the economically developing world, but also a greater density of lighting in many already heavily developed areas (Falchi et al., 2011). As human communities and lighting technologies develop, artificial light increasingly encroaches on the dark refuge of amphibians (Fuller et al., 2013).

As a result of artificial light being introduced, the natural lighting cues which mandate amphibian behavior may have less of an effect on their movement patterns. Moonlight or moon phases have been shown to affect the activity of several species of amphibians (Fitzgerald and Bider 1974). An amphibian that responds directly to solar cues for both movement and mating is *Acris crepitans* or the Northern Cricket Frog (Kenney and Stearns 2015). Additional species such as *Lithobates sphenocephalus*, *Lithobates palustris*, *Gastrophryne carolinensis*, *Hyla chrysoscelis*, *Lithobates catesbeianus*, and *Anaxyrus fowleri* are also expected to reside in similar environments of the Northern Cricket Frog and experience similar shifts in behavior as a result of artificial light at night (Jensen 2008).

Biologically, amphibians experience a physical change in their eyesight as a result of experiencing introduced artificial light at night. Rapid changes from the adaptational state of an amphibians' eye are likely to result in impaired vision (Muntz 1977). Buchanan (1993) writes, "It is unlikely the nocturnal behavior of amphibians would remain unaltered within the spectral range of a human observer, when the use of the introduced light causes a shift in the frogs' perceived illumination". In instances where amphibians are experiencing impaired vision, they may be more likely to experience predation.

Predation risk associated with light, natural or artificial, can result in both prey attraction and an increase in mortality of amphibians (Buchanan 2006). Natural light, in the absence of artificial light, is utilized by organisms as a resource and a source of information about their environment (Gaston et al., 2013). As a result of an increasingly artificially lit world, amphibians are experiencing a change in their phenology which historically depended on moon light or other natural light sources. Behavioral changes in amphibians in the presence of artificial light have been documented and represent a problem arising with migratory species. A study found that the reproductive behaviors of *Phsalameus pustulous* female frogs are less selective towards their male mates, preferring to mate quickly in order to avoid predation (Longcore and Rich 2004). An additional study found that aiming a spotlight on the pond at night reduced the calling frequency of male *Lithobates sphenocephalus* (Hall 2016). Research concerning male green frogs, *Rana clamitans melanota,* demonstrates changes in behavioral patterns with the presence of artificial light (Perry et al., 2008). Scientists predict that night lighting may prevent movement to and from breeding areas by stimulating lightresponsive behavior (Longcore and Rich 2004). If migratory species are encountering artificial light at night, their natural movement may be altered as a result. This draws into question which component of artificial light are amphibians responding to.

A potential influence on the movement of amphibians towards the direction of a light source is the initial attraction of prey species to the same source. Prey consumption has a supported direct relationship to the amount of prey available, the more prey present in a location will result in an increase of amphibians present and competing for available resources (Labanick 1976). Light pollution may have significant ramifications for the insect population, species or community level (Chittka and Raine 2006). Being attracted to the light, insects make themselves both visible and vulnerable to predators. It is currently unknown whether amphibians are drawn to light for feeding purposes, the light source itself or a combination of the two (Perry et al., 2008).

Experiments concerning light pollution found significance in light sources that emit short wavelength light versus long wavelength light (Van Grunsven et al., 2017). The amphibian's behavioral response to artificial light was found to be directly related to the spectral composition of the light. The light color which demonstrated the largest change in anuran behavior was determined to be blue light. Blue light is commonly emitted by light emitting diodes (Van Grunsven et al., 2017). Little to no research concerning LED intensity and amphibian congregation has been conducted in the field. LED lighting is a novel addition to outdoor artificial light at night as street lights are converted from sodium halogen lights.

In summary, amphibians may be vulnerable to artificial illumination for the following reasons: 1) amphibians use natural light as a cue for behaviors, 2) illumination may affect other community members that interact with amphibians (Brown et al., 2011). General relationships between amphibians and traditional out-door artificial lighting have been established, but little research has been published on the relationship between amphibian movement and light from light emitting diodes (LED). LED lighting is now used more frequently than ever to light offices, houses, and industrial/agricultural facilities as a cost-effective method over traditional lights (Magno et al., 2015).

With the use of LED lighting increasing, it is important to study the behavioral reactions of amphibians to the introduced lighting near their home ponds. Wetlands that have been transformed into new patches rich with LED artificial light may experience a decline in their amphibian population for a variety of reasons. This study captures the idea of a bright light collecting more prey at night, and thus likely more amphibians. To simulate urbanization occurring in a rural area two different LED intensities were tested: low and high intensity. This was conducted to determine if an impact in amphibian behavior could be correlated with one intensity over another.

2. METHODOLOGY

Field experiments were conducted in southern Virginia to evaluate the effects of different LED lighting on amphibian movement. Experiments included artificial drift-fences and pitfall traps to capture individual amphibians. Research and the capturing of amphibians took place during the summer near a beaver pond known for high abundance in amphibian populations. I predicted that there will be a higher abundancy of amphibians, such as Northern Cricket Frogs captured next to high intensity LEDs in contrast to low intensity LEDs and in greater abundance than in traps where there is no influence of light.

2.1 Determining locations for study sites

Virginia is home to a diverse population of amphibians with 111 recognized species (Beane et al., 2010). A possible explanation for the exceptional diversity of amphibians in this area is that southeastern Virginia is comprised of various ecotones: The Piedmont Plateau, Southern Atlantic Coastal Plain, and Northern Atlantic Coastal Plain (Figure 1A) (Micancin et al., 2012). The South East region is experiencing the largest net population growth in the U.S. as well as an expansion of large urban centers (O'Driscoll et al., 2010). The changes of the distribution of the amphibians in Virginia has been found to be a result of agriculture practices, dam building, mining, and costal development (Beane et al., 2010). Despite the importance of southeastern Virginia for amphibian diversity, suspected declines in this area have received modest scientific attention and research (Micancin et al., 2014).

Amphibians can be found throughout the Piedmont region in south central Virginia, including a community known as Cuscowilla (Figure 1B). The flood plains of Cuscowilla are composed of alluvial sand and silt and have a moderate growth of mixed hardwood trees (Micancin et al., 2012). Using the literature concerning the habitat of Cuscowilla, a singular pond was chosen to conduct this experiment (Figure 2). Cuscowilla, Virginia features a large freshwater reservoir surrounded by ponds that have existing amphibian populations (Figure 1B). Kerr reservoir is a federal park, but the surrounding wooded area containing the ponds lacks streetlights as well as other artificial lighting. This allows for novel behavioral observations to take place in the presence of high and low intensity LEDs. The particular pond used in this study was recommended via personal communication James Crownover and confirmed for pre-existing amphibian populations via Travis Land, a local Herpetology Curator (Figure 1B).

The selected location to build the 5 sites was confirmed to have calling amphibians, including Northern Cricket Frogs, Eastern Narrow-mouthed Toads, Coper's Gray Treefrogs and other amphibians (Pers. Comm. James Crownover) in May, prior to the study beginning in June. The songs of male amphibians were used as an indicator for the presence of breeding pairs. Adult males can generally be heard singing their advertisement calls around dusk but have also been recorded to call on warm humid days during the peak of their breeding season (Brown and Littlejohn 1972). Therefore, the hours of sampling began at around 9pm each night (dusk in Cuscowilla, VA). Sampling lasted 20 nights, beginning on June 15^{th} and ending on July 4^{th} 2019. Figure 2 displays a view of the chosen pond from site 1's location and shows the grassy area preceding the edge of the water. Figure 3 features the same pond with a view of a beaver's lodge and displays the biodiversity and habitat of the amphibians captured during the study.

2.2 Drift fence and pitfall traps

Drift fences and pitfall traps were established at each of five study sites (Figure 2). Drift fence and pitfall traps are commonly used for capturing amphibians (Van Grunsven et al., 2017). Sites were located horizontally within approximately 2.5 meters of one another to ensure capture of the same population of amphibians. Individuals captured during the experiment were not marked before being released.

At each site a drift fence (2.5 meters long and 0.9 meters tall) was erected. Fencing material was made of erosion control fabric and held up by 3 pre-installed wooden stakes along the length of drift fence. A lantern capable of emitting both high and low intensity light was located 30 centimeters above the fence, centrally positioned and supported by a metal garden hook. Nine pitfall traps were placed on either side of the drift fence at each study site, for a total of 18 pit traps at each site (Figure 5A). The total number of pitfall traps on each side of the fence was limited as a result of the width of each trap allowing for a total of 9 buckets along length of the drift fencing.

Plastic buckets were used as pitfall traps. Each one-gallon bucket had a top diameter of 18.7 centimeters and was 19.2 centimeters tall. The buckets were buried at equal intervals of approximately 25 centimeters (Figure 4B) such that the rim was flush with the ground. The inner edge of each trap was placed 5 centimeters from the drift fencing. The bottom of each bucket had 4 drainage holes that are approximately 2.5 centimeters in diameter. Funnels were installed for each gallon bucket and were attached to the pitfall trap via duct tape. Funnels allowed trapped amphibians access to fresh air and prohibited them from leaving (Figure 4A) (Perry et al., 2008). The distance between the buckets and the fencing was 5 centimeters to ensure the amphibians would fall into the traps upon encountering the fencing (Figure 4B).

Figure 4A shows a horizontal view of the installation. Figure 4A also displays the central location of the lantern in relation to the fencing. The lantern had two different lumen settings; the high intensity setting emitted 1000 lumens while the low intensity setting emitted 80 lumens. Each lantern was positioned above the 30.5 centimeters above the 1.21-meter tall drift fence. The penumbra extended along the 2.5 meters of fencing at each site. Study sites were built the 5th of June with testing beginning the second week in June on the 15th to allow the surrounding habitat to recover from disturbance (Figure 5).

2.3 Sampling

For each night of the study, each of the five study sites had three levels of light intensity; no light, high intensity and low intensity. For every site, the intensity level was determined using a random number generator. After the light intensity was established for a site, the lights were turned on. Treatments were turned on for a pre-condition 30-minute period before being left on with trap lids removed for two hours each night and then switched off after the allotted time period.

The illumination percentage of the moon was recorded each day for comparisons in relation to interference with light response behavior. Percentages range from 0% during a new moon to 100% illumination during a full moon. Exact illumination percentages of the moon for the study location were recorded at the beginning of the 30-minute adjustment period from TheWeatherChannel.com. After the 30-minute period, lid keeping amphibians out of traps were removed and placed 15 meters away in the hard-wooded area of the study location. Lanterns were for outdoor use and battery operated (Figure 6). Once the lights were turned off and amphibians counted, lids were replaced on all traps.

Each individual caught in a trap was identified to species. The number of amphibians found in each pitfall trap at each site was counted and categorized by site location and species. Amphibians were identified to species but age and sex were not determined per individual, however, it was noted that many amphibians captured during this duration were adolescents. After processing, organisms were released 10 meters away from the study site location. Post-counting, traps at each of the sites were covered with a fitted plastic lid to prevent additional organisms from becoming captured. As a result of individuals not being marked during the sampling process, there was a possibility of recapture from night-to-night treatments.

2.4 Statistical analysis

Initial analysis used counts of individuals rather than number of individuals caught. A count, otherwise known as trap success, was defined as a single individual of a species entering a trap. Trap success was defined by a site having at least count of an individual. The count data were not normally distributed therefore a non-parametric chi squared test was used to determine if the difference in light intensity affected the amphibian count. The P-Value was set at $P<0.05$. Proportions of each species collected were used for chi-squared results. Proportions were calculated by totaling species count per intensity and dividing it by the overall amphibian count.

Each site was randomized for light intensity treatment each night. By chance alone sample effort was uneven across treatments. Positive trap count was used for comparing the chi-squared values for each of the five sites to determine if one site received significantly more or less success than the other sites. A chi-squared test was then run to determine if intensity was significant for overall amphibian count before looking at individual species. To determine if lunar illumination had an effect on the trap success, a scatter plot was generated.

For each species' individuals, proportions to overall species count were monitored, as well as total amphibian numbers to derive additional relationships unclear by testing count alone. Chi-squared were first conducted using count data. To clarify if the Chi-squared test was failing for species fewer in count, Fisher exact tests were conducted. Bar graphs were generated to display visual relationships between individual sites and treatments for species with statistically significant results.

Amphibian abundance relationships for each site were calculated using number of amphibians captured to determine if there was a significant difference in behavior for species in relation to treatment. On account of the data for number of amphibians being non-normal, a non-parametric Kruskal-Wallis test of independence was used to test for significance. Following the Kruskal-Wallis test, a post-hoc Dunn test was conducted to determine if total number of individual species yielded a significant relationship for each light intensity treatment comparison (High versus Low, High versus None, and Low versus None). Dunn test results were compared to initial Chi-squared results to determine if count data varied from abundance data.

As a result of the abundance data being non-normal, medians were used in place of means for looking at individual species returns. Each species' medians were calculated using the total number of the species and displayed via histogram. The species which was highest in frequency was compared visually with the median number of species and species of lowest count. From the histogram, the species which had the highest, middle, and lowest count were revealed and then further graphed in proportion to overall trap success.

The Virginia Fish and Wildlife Management did not require a permit for the counting and returning of native amphibians. Institutional Animal Care and Use

Committee [IACUC] certification was achieved prior to the field study; protocol number 201800299, for handling amphibians in the field.

3. RESULTS

3.1 Illumination

Because sampling took place over 20 days, moonlight varied per day and was expected to affect the significance of the LED treatments. To test whether moonlight affected the capture of amphibians, a correlation was drawn using a scatter plot and linear regression. The brightness of the illumination of the moon was predicted to correlate with a higher chance of an amphibian being collected across all sites regardless of treatment. Illumination of the moon was found to be a non-confounding factor against the amphibian count for the artificial lighting treatments (Figure 8).

A full moon displays 1/4 lumens per square meter (Panayotova 2010). The difference in lumens of artificial light intensity treatment (1000s lumens versus 80 lumens) is expected to have less of an effect on the behavior of amphibians under the occurrence of a full moon. Beneath 100% illumination, it can also be predicted that the no-light traps would receive an increased number of individuals being collected as a result of the increase in light. To test for the possibility of this effect, a scatter plot was graphed to show correlation between amphibians collected and lunar illumination (Figure 8). Using a linear regression, no relationship between the amount of moon illumination and the total number of amphibians counted (P-value = 0.89). As a result, the slope is very close to zero, therefore, no direct relationship can be declared.

3.2 Count data

The species most frequently counted was the Northern Cricket Frog, followed by the Eastern Narrow-mouthed Toad. Because each individual was released within 10 meters of the study site, re-capturing of the same individual on different nights was possible. Although 7 total species were collected, only 2 species: Northern Cricket Frogs and Southern Leopard Frogs both displayed a statistically significance difference between species count number and treatment success rate. This significance was highest between the presence of LED lighting versus no light at all. Even though counts were high for the other 5 species, the similarity between the individual species counts and treatment type failed to produce any significant difference. Each of the 5 sites yielded different counts of amphibians regardless of treatment being tested. The variation between the sites' success rate can be seen in Figure 9.

Trap success rate varied per site (Figure 9). Site 5 received the lowest species counts compared to the other 4 sites; a possible result of being located in the hardwood area of the study site. Site 2 had the highest return compared to the other 4 sites, accounted for over 28% of total trap success. A chi-squared test including all site count data resulted in a statistically significant difference between each site's species count (Figure A-1).

The high intensity treatment had the highest species count as well as highest success rate among the other treatments (Figure 10). There was a significant difference between trap success among high light intensity and no light as well as a significant difference between low intensity light and no light treatment success rates. There was no significant difference between high and low light intensity trap success. Trap success was significantly different among the 3 light treatments. (See appendix B-2, B-3, B-4, B-1)

More Northern Cricket Frogs were found in the LED lit sites than in the sites with no light, thus the trap success for both high and low intensity lighting was high (Figure 11). The results displayed by Table 3 can be reflected by Figure 11. The results yielded a statistically significant difference between high and no-light treatment with high intensity receiving a higher proportion of individual species counts. There is also a significant difference between low intensity and no-light, where low intensity received the highest amount of counts. It is important to note that there is no statistically significant difference between high and low intensity. These results reflect the frogs' behavioral preference for the presence of light over the absence of light. There is a significant difference among all 3 possible treatments ($P= <0.05$) (See appendix C-2, C-3, C-4, C-1).

Southern Leopard Frogs display a trend that reflects higher success rate for high intensity LED sites over low intensity and no-light treated sites (Figure 12). No statistically significant difference was found between low light and no light treatment as a result of a low trap success rate for both treatments. There is no statistically significant difference between high and low intensity treatments. A statistical difference between high and no light treatment exists as well as a statistically significant difference among all 3 treatments (See appendix D-2, D-3, D-4, D-1).

3.2 Abundance data

Instead of using count data, actual numbers of each species collected were used in calculating abundancy per site per treatment. Figure 9 displays the same results as

Figure 13. Sites 4 and 5 experienced the least number of individuals. As a result of the data being determined non-normal, a Kruskal-Wallis test was used to determine if a significance between the median number of amphibians is significant per treatment. The Kruskal-Wallis statistical test determined that the sites have a significant difference in number of individuals visiting each treatment, (x^2= 12.74, DF= 2, P= 0.0017).

The treatment with the highest number of amphibians caught was the high intensity treatment with site 2 collecting 20 individuals (Figure 13). The intensity most frequently visited was the high intensity treatment with the proportion of traps being 69 individuals. The treatment with the lowest number of individuals caught was no light at 22 individuals. There is a significant difference between number of amphibians caught at each of the intensity treatments.

Running a Dunn test for all amphibian species at each of the possible treatment comparisons determined that a significant difference between the presence and absence of light is significant (Table 3). High intensity light returned significantly more individuals, a higher success rate, than the treatment no light. Comparing individual results between species for the Dunn Test and Chi-squared results, there was not one species which had a statistically significant P-value for both tests. Dunn test P-values reported in table 4 were adjusted. Only 3 species displayed statistical significance when considering both test types: The Northern Cricket Frog, the Eastern Narrow-mouth Frog, and the Southern Leopard Frog. The Eastern Narrow-mouthed Frog only displayed statistical significance for the Dunn test; this is a possible result of the Dunn test calculations using pair-wise rankings that are not used by the Chi-squared test.

Medians were used to compare species and treatment, as the data reported in Figure 14 were determined to be not normally distributed per site. The treatment with the highest median number of individuals being caught at high intensity LED traps. The next highest median number of species is greater than 1.5 for low intensity LED treatment and relatively 1 individual for no treatment. The median number of overall amphibian species is low due to the overall count of 138 individuals being low.

Comparing the trap success rate for the species with the highest, middle, and least number of individuals collected visually displays which species compromised the bulk of the statistics being conducted (Figure 15). This graph features the abundance in percentage of the three species captured for each treatment, 75% of each site's traps for each treatment were the Northern Cricket Frogs. Fowler's Toads individuals were collected 4 times over the duration of the study; similarly, only 11 Pickerel Frogs were captured thus they contributed significantly less to trap success than Northern Cricket Frogs (Table 1).

4. DISUCSSION

Amphibian behaviors are directly influenced by anthropogenic factors such as artificial lighting. The presence, location, and intensity of the light source may support a specific movement pattern from several species of amphibians. The results of the current study found that more amphibians were found at sites with LED lights than sites with no light source. No effect of intensity (high versus low) was found. This same effect was found in two species, Northern Cricket Frogs and Southern Leopard Frogs (Figures 10, 11). However, using a Dunn Test, Eastern Narrow-mouthed Frogs favored low intensity light over both high intensity and no-light (Table 4). Each night, light intensities were randomized to reduce site fidelity, as well as eliminate previously utilized dispersal paths by amphibians to and from the pond. A potential reason these species in particular were determined to have statistical significance lies partially in the fact that they were species which had higher yields. An un-even distribution of species with a single predominant such as the Northern Cricket Frog can lead to skewed variance in overall amphibian totals. The illumination of the moon progressing from new to full was expected to affect the collection of species. To avoid statistical problems arising when combining the multiple treatments with the change in moon illumination, an information theoretic approach was taken (Hall 2016). Illumination percentage was compared against the amphibian count via a scatter plot to determine if there was a statistical effect. Assuming a non-parametric response, a null distribution would correspond to the illumination of the moon having no effect on the treatments of light versus the number of amphibians captured (Hall 2016). The scatter plot failed to reject the null hypothesis in which full moon illumination was no different from the absence of illumination by the new moon. In a similar experiment, scientists determined an alternate effect in which different colored lights of the same wavelength actually deterred migrating toads in Philips, Amsterdam Netherlands (Van Grunsven et al., 2017). Pitfall traps have no way of measuring the number of individuals of a population that are deterred, however, there may be a connection between light intensity and light color with amphibian behavior.

Possible variables that drew the amphibians to the lighting at my study location include the increased visibility of the prey, as well as an increase in the presence of same species individuals. Several amphibians, which did not fall into the pit-fall traps, chose the erosion control fence as a mating location or were observed directly under the light source feeding on prey. The density of the prey varying across the light intensity treatments was not measured, however, this information could provide further detail into possible reasons amphibians chose certain sites over others. The limitations of this study concern aligning the study with the mating phenology of the amphibians.

Extending the duration of the study to early March allows not only for more individuals of mature age to be collected, but also provides an opportunity for adolescents to be sampled. The research design which inspired this study ran for a duration of 11 days (Van Grunsven et al., 2017). By increasing the collection period to 20 days a higher yield of individuals was expected; however, this was not the case. In the current study, several locations with additional sites were not created as I was single-handedly collecting amphibians each night. In an ideal experiment, the individuals captured by a pitfall trap would be marked pre-release by having a toe clipped. Doing so would prevent re-counting of previously captured individuals. In addition to capturing and marking amphibians, samples of insects and their estimated density at each light intensity would help in determining if a relationship exists between insect population and light intensity. As a result of these changes, a clear relationship between amphibian aggregation near higher intensity lighting and prey density could be measured.

Confounding factors such as the limited distance between each site may have led to over-lapping light exposure across sites. To better control the exposure of the LED's light, light-blocking tape should be placed on both sides of the lanterns. In doing so, the illumination of high or low intensity LED will be directed at either the pond side or wooded area of the study location. The height of the lanterns above the drift fencing was also a variable that possibly influenced the individual intensity exposures. Having a lantern directly flush the top of the fencing would allow for the experiment to be more robust. As a result of this design flaw, each of the traps across the 5 sites were not independent from one another. My original question and study were not strong enough to address a defining relationship between LED intensity and all amphibian behavior.

The amphibians collected and released in this study were located in rural Virginia where artificial lighting was not present. In order to explore the question further, a translocation between urban amphibians (living within areas that are artificially lit at night), and rural amphibians (having little to no light at night exposure) should take place. Placing the rural amphibians within a fenced study area would test whether their behavior towards the LED intensities would differ from other commonly used urban lighting such as sodium-halogen lights. Prey densities should be measured in the location of which the urban amphibians are sampled and then compared with densities present at the rural location.

5. CONCLUSION

Although this study took place in rural Virginia, the intention was to emulate an urban artificial lighting confrontation experience for pond-dwelling amphibians. This was conducted to reflect similar occurrences that other edge-dwelling amphibians may experience as a result of an increase in urbanization. Urban ecosystems are more complex than other types of ecosystems as a result of the socio-biophysical feedbacks that are driven by humans (Parris 2016). As previously mentioned, roads are a common result of urban infrastructure which can impede the movement of individual amphibians across natural paths, thus isolating populations. A change in natural dispersal of amphibians, as a result of introduced artificial lighting over time, subjugates the structural integrity of ecological communities (Parris 2016). The movement response patterns of amphibians in an environment that is experiencing an increase in artificial lighting such as LEDs, will determine whether those populations are directly affected by the presence of artificial light. Wise (2007) suggested that light pollution exacerbates amphibian decline although the mechanism is unknown.

On the global scale, an extensive proportion of terrestrial surfaces are considered light-polluted (Cinzano et al.,2001). Artificial light is relatively new on the evolutionary time scale and animals with pronounced nocturnal activity are more likely to be affected by the presence of artificial light (Van Grunsven et al., 2017). Currently, urban ecosystems are the fastest growing ecosystem, and are expanding at rates of 3% per year (Niemelä et al., 2011). With the current increase in urbanization, the dynamics of amphibian populations are at risk. This is an underlying result of an increase in patches created through urban development and an increase in the presence of artificial lighting. Monitoring amphibians' behavior response to novel artificial light will support whether the introduced light has an effect on the integrity of amphibian populations.

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6. TABLES

Table 1 Total trapping effort and number of sampling species captured per pitfall traps. *Invertebrate species captured in small numbers during study (e.g. wolf spider), ~ Excluded from analysis

Metric	Total Effort / Species # Collected
Total Trap-nights	1800
Total Amphibians	138
Acris crepitans (Northern Cricket Frog)	65
Gastrophryne carolinensis (Eastern Narrow-mouthed Toad)	25
Lithobates sphenocephalus (Southern Leopard Frog)	16
Lithobates palustris (Pickerel Frog)	11
Hyla chrysoscelis (Cope's Gray Treefrog)	9
Lithobates catesbeianus (American Bullfrog)	8
Anaxyrus fowleri (Fowler's Toad)	4
Grass Spider ~	9
Crayfish ~	5
Other* ~	12

Table 2 Chi-squared values for all species counts and treatment response (total treatment denominator used info from Table 2). Values (*) represent significant differences at P=0.05.

Species	Chi Squared Value	DF	P Value
Northern Cricket Frog	13.37	2	0.0012*
Eastern Narrow-mouthed Toad	1.096	2	0.5781
Southern Leopard Frog	6.246	2	0.0440*
Pickerel Frog	4.530	2	0.1038
Cope's Gray Tree Frog	2.29	2	0.3198
American Bullfrog	3.08	2	0.2143
Fowler's Toad	0.412	2	0.8136

Table 3 Non-parametric post-hoc Dunn Test complimenting Figure 15, ranked mean va lues for all amphibians at multiple treatment comparisons: High versus Low, High versus None, Low versus None. Values (*) represent significant differences at p=0.05.

Comparison	Z Value	P Unadjusted	P Adjusted
High vs. Low	1.807	0.0707	0.0707
High vs. None	3.559	0.0003*	0.0011*
Low vs. None	1.923	0.0543	0.0815

Table 4 Comparison of light intensity effects on frog species movement. The table displays the test statistic from both the Chi-squared and Dunn test for a non-parametric pairwise comparison between light levels of High versus low, High versus None, and Low versus None. NA represents too few numbers of that species were collected to conduct the test. Values (*) represent significant differences at P=0.05.

Species	High vs. Low		High vs. None		Low vs. None	
Test Type	Chi X^2	Dunn	Chi X^2	Dunn	Chi X^2	Dunn
Northern Cricket Frog	0.59	0.14	0.002*	0.46	0.0006*	0.78
Eastern Narrow-mouthed Toad	0.55	0.13	0.62	0.038*	0.27	0.76
Southern Leopard Frog	0.07	0.53	0.03*	0.59	0.58	1.00
Pickerel Frog	0.15	0.35	0.06	0.76	0.51	0.35
Cope's Gray Treefrog	0.50	0.39	0.50	1.0	0.38	0.23
American Bullfrog	0.26	N/A	0.11	N/A	0.60	N/A
Fowler's Toad	0.59	N/A	0.68	N/A	0.73	N/A

7. FIGURES

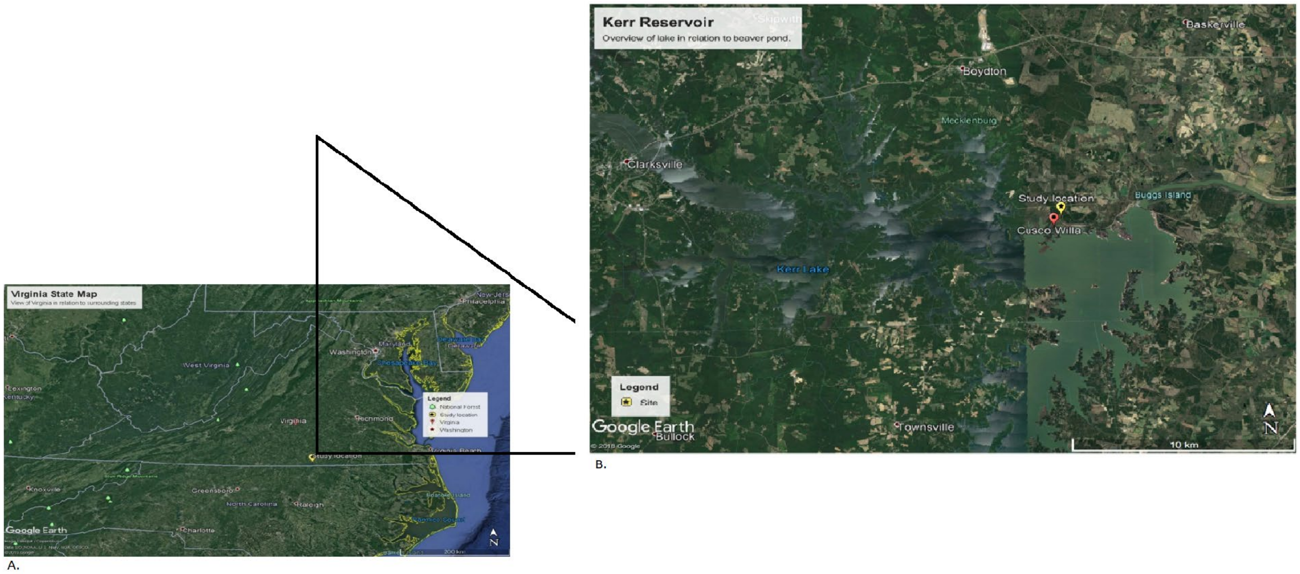

Figure 1 Location of study site in southern Virginia, USA. Map © Google Earth. (A) Virginia is bordered by North Carolina, Tennessee, Kentucky, West Virginia, and Maryland, (B) Kerr Reservoir (Latitude 36.601° N, Longitude -78.341 ° W). Map © Google Earth. Study sites were located in wetlands on the northern edge of the reservoir.

Figure 2 Eye level view of pond (background and left) and marshland (center and right) before the construction of Site 1. Beaver lodge is visible in the left upper background. Photograph by Danielle Crownover.

Figure 3 Eye level photograph of beaver pond adjacent to study site locations, taken at the edge of the water. Beaver's lodge is visible in the upper right-hand corner of the photograph. Photograph by Danielle Crownover.

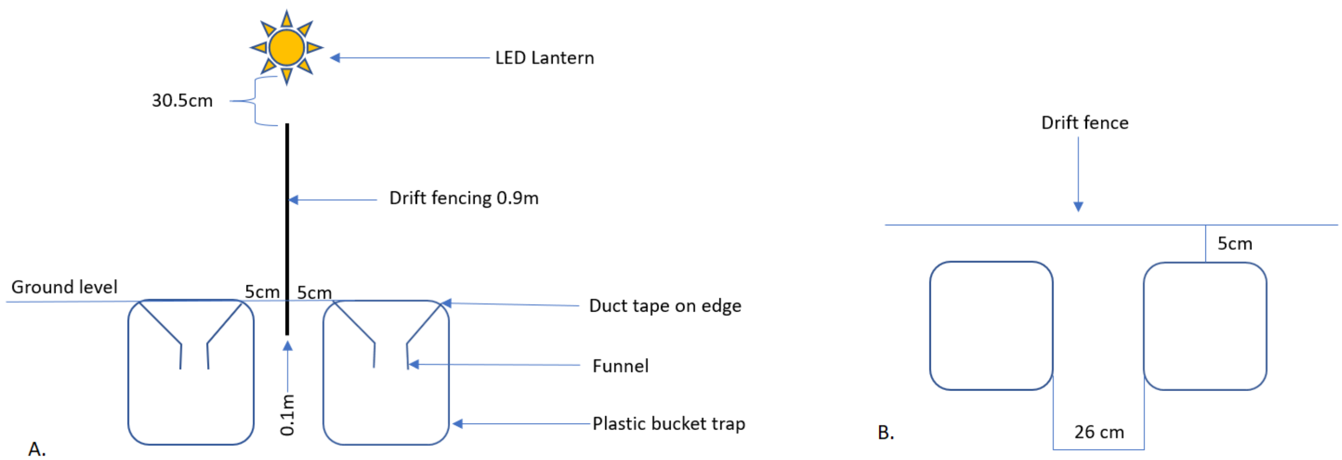

Figure 4 Schematic views of pit traps. A. Cross-section view, showing two traps separated by a drift fence. Light source was placed directly over the fence. B. Top-down view of traps in comparison to drift fencing. 9 Buckets were placed 26 centimeters from one another within 5 centimeters of the drift fence, this procedure applied to both sides of the fencing for each of the five sites. This example features 2 buckets buried out of 9 on each side of the fence.

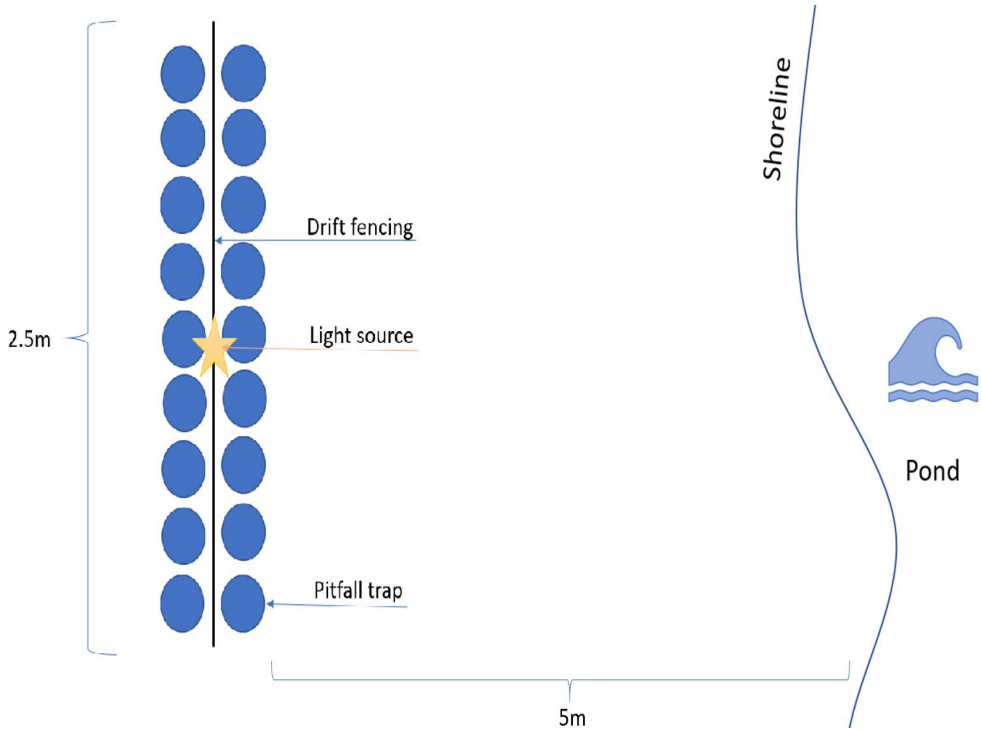

Figure 5 Layout of 18 traps at a single study. The trap array was located approximately 5 meters from the edge of the beaver pond.

Figure 6 Top-down view of site 5 at night featuring the traps post-collection period. Lids were placed on traps before high intensity treatment was turned off for the remainder of the night. Photograph by Danielle Crownover.

Figure 7 Photograph of a green morph Northern Cricket Frog inside a pitfall trap prior to being removed. A grass spider is visible to the right of frog. Photograph by Danielle Crownover.

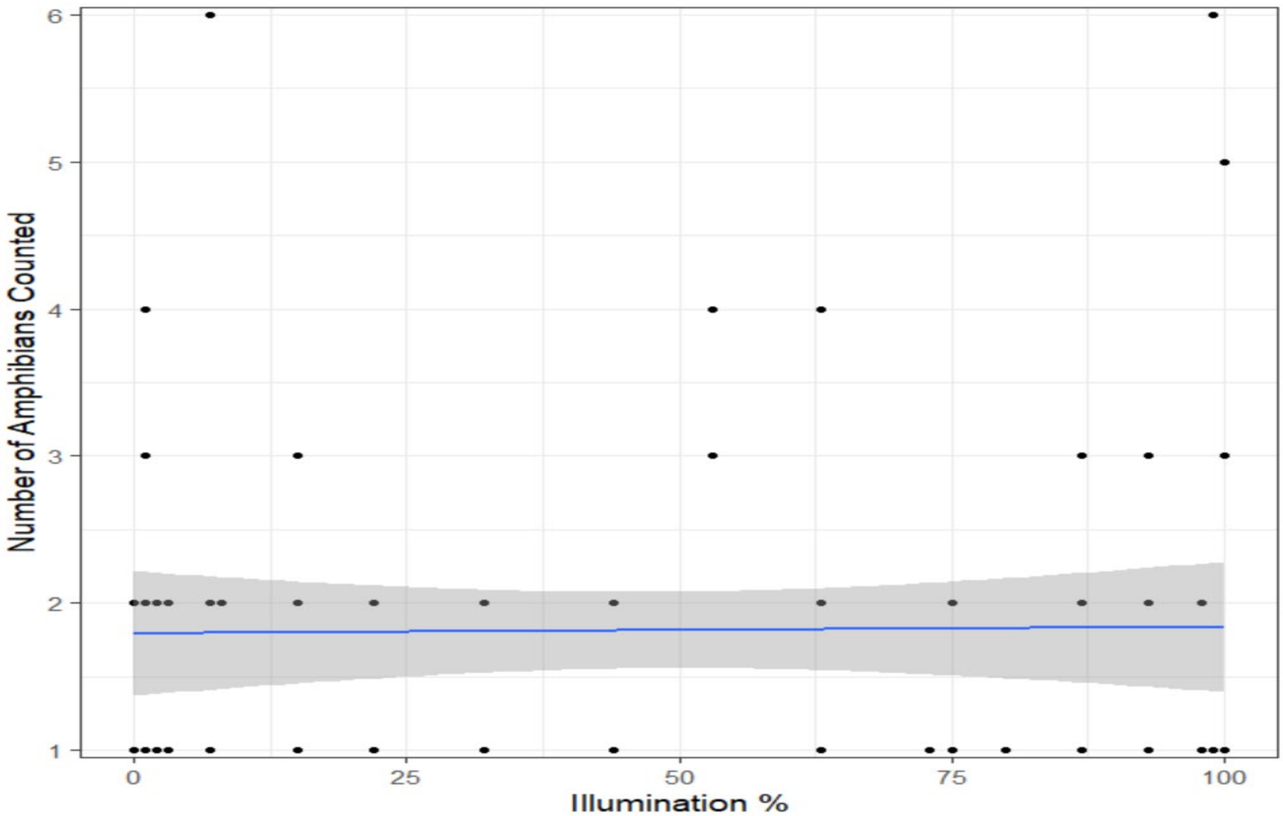

Figure 8 Scatter plot correlation of all amphibian species counted versus change in moon illumination percentage (0-100%) over 20-day duration. Each dot represents an amphibian that was caught and counted throughout the duration of the study. They gray area encompassing the slope displays a 95% confidence interval.

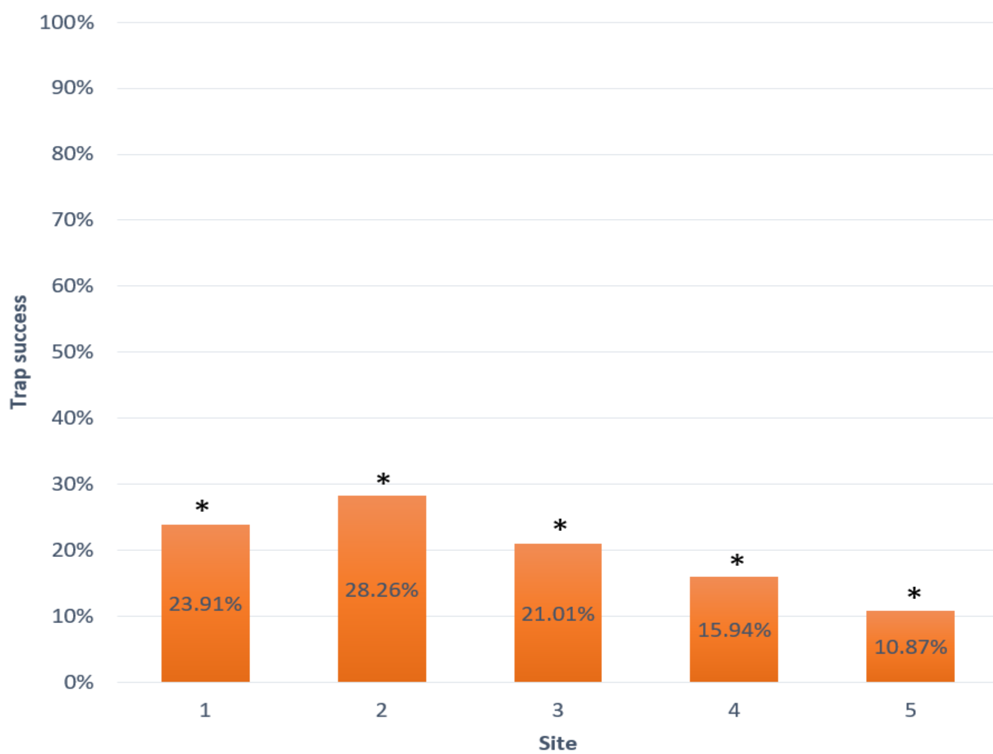

Figure 9 Success rate for each site that captured amphibians collected across all testing locations. Values (*) represent significant differences at P=0.05.

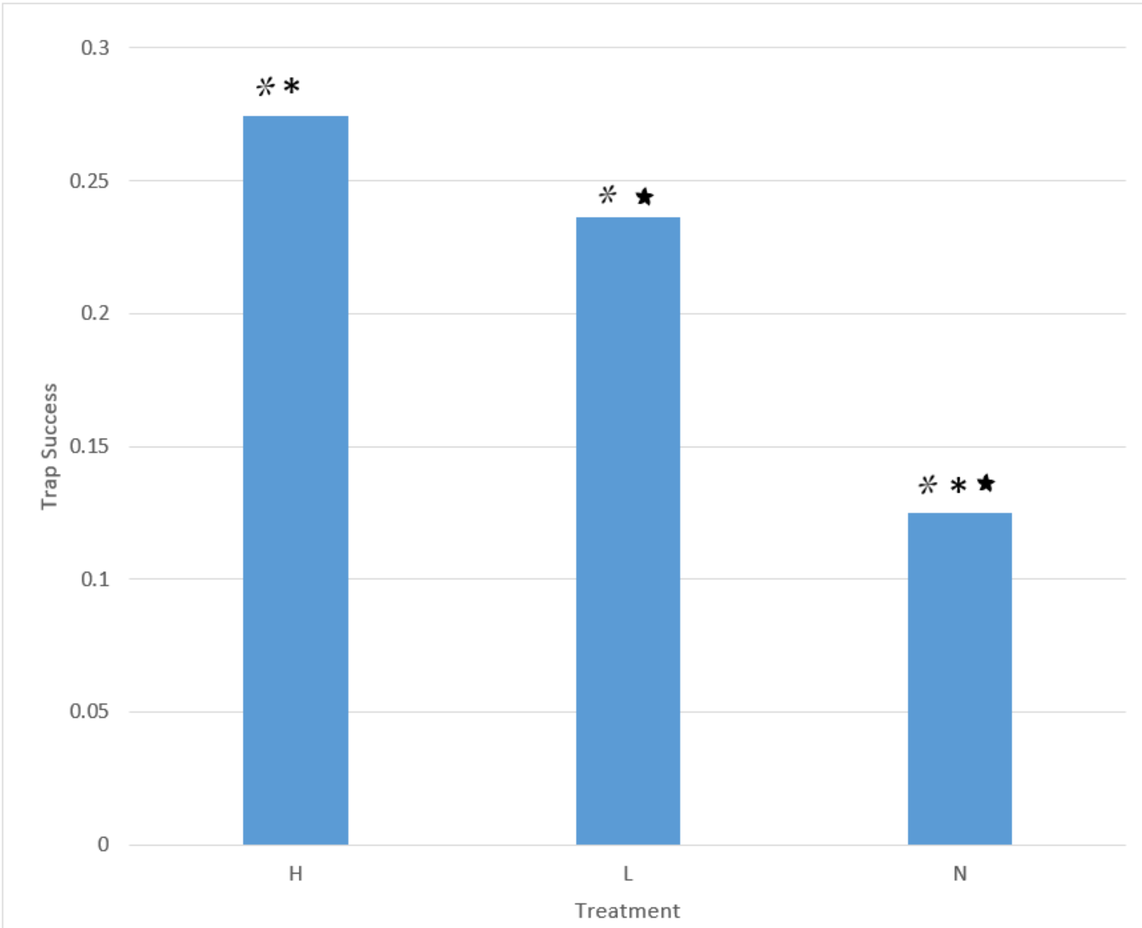

Figure 10 Trap success for all amphibians for each treatment: High, Low, and None. Values of various (*) represent significant differences at P=0.05.

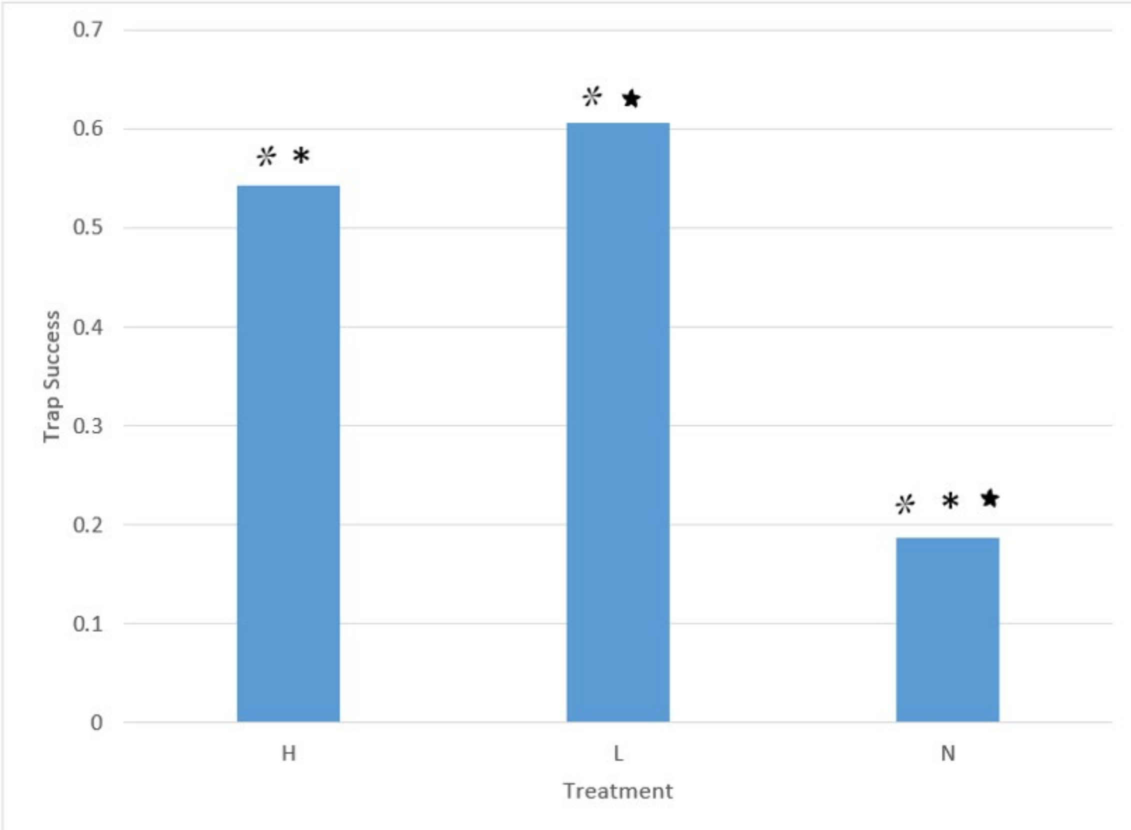

Figure 11 Success rate of Northern Cricket Frog counts across treatments: High, Low, and None. Values of various (*) represent significant differences at P=0.05.

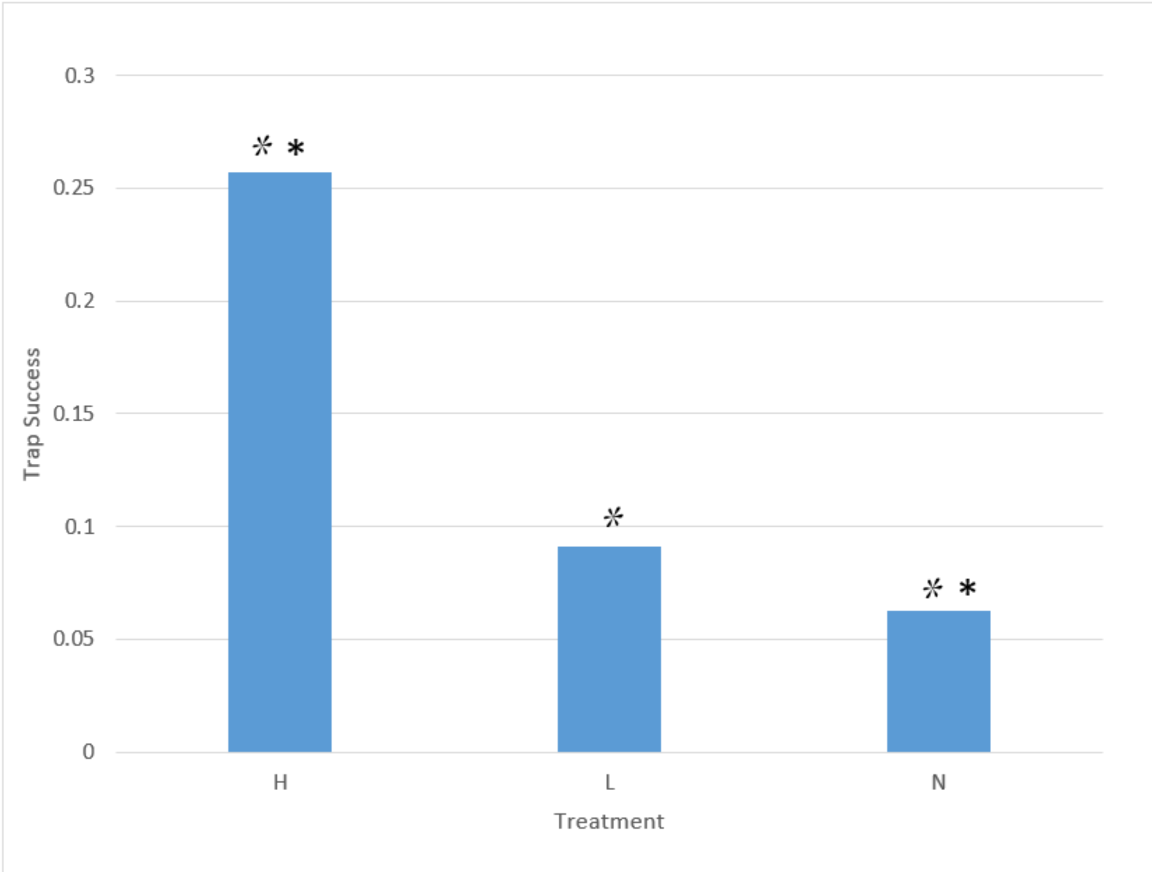

Figure 12 Trap success for Southern Leopard Frogs counts across treatments: High, Low, and None. Values of various (*) represent significant differences at P=0.05.

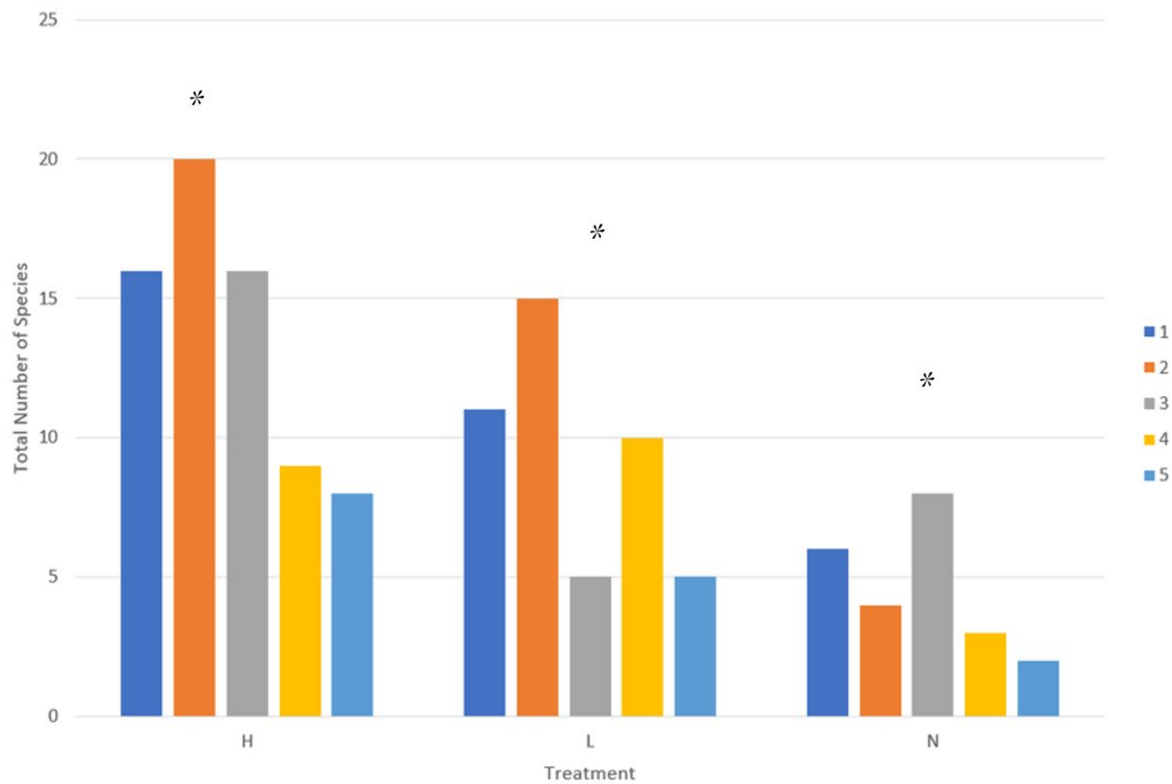

Figure 13 Total number of amphibians across each testing location per treatment: High, Low, and None. Value (*) represent significant differences at P=0.05.

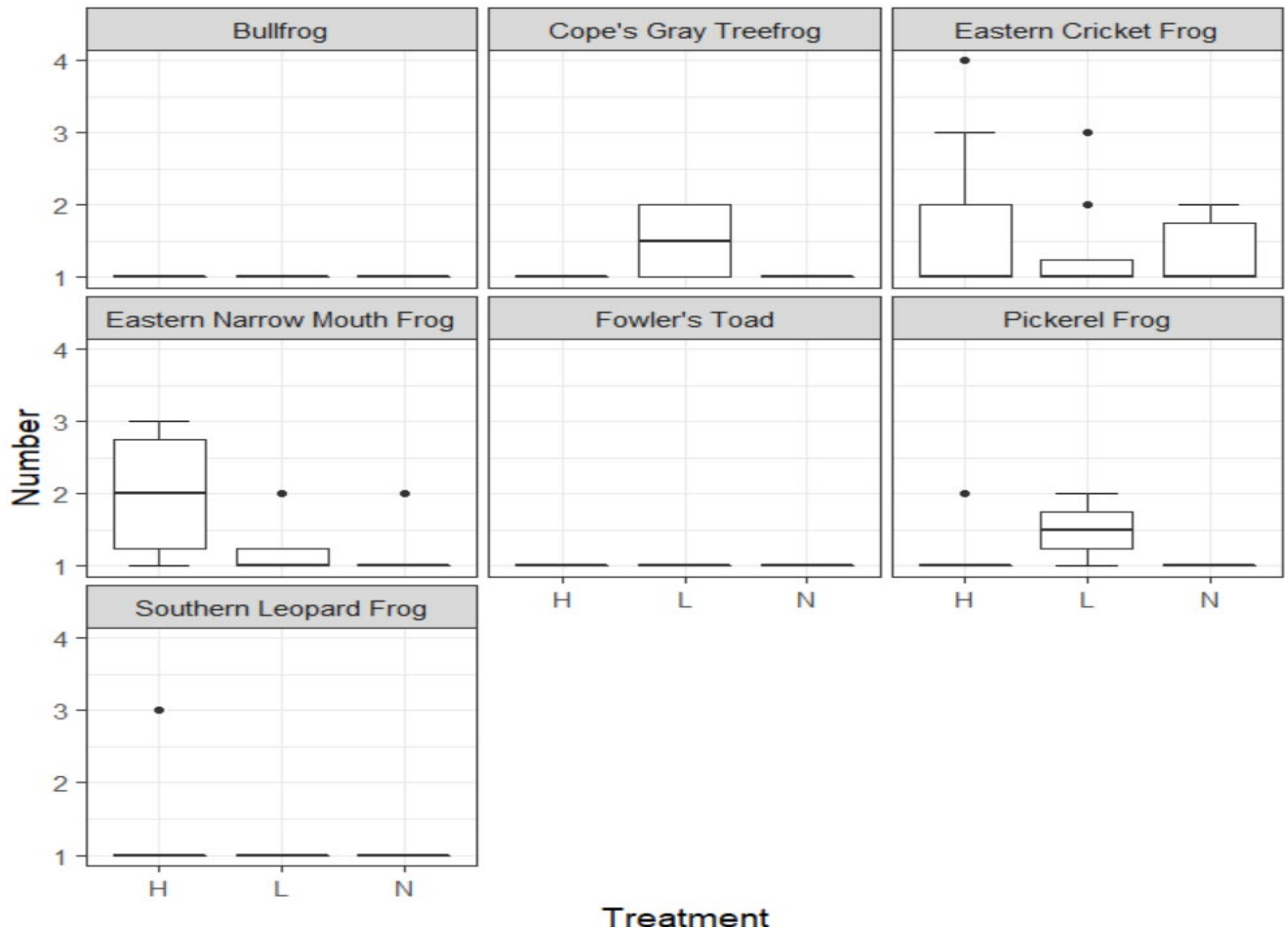

Figure 14 Plot of medians for all amphibian species per treatment. High intensity LED display the highest mean at greater than 2.5 individuals, low intensity at greater than 1.5 individuals, and no light at relative 1 individual.

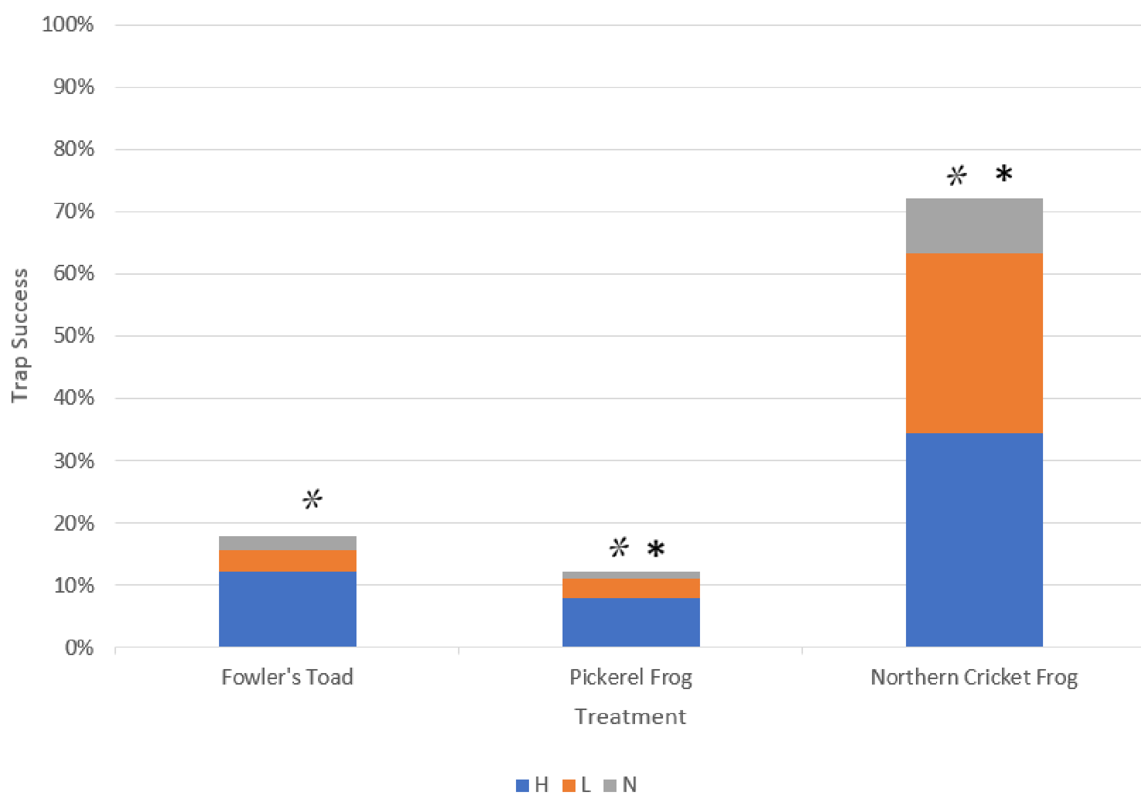

Figure 15 Trap success for all species count, visualizing 3 species: Northern Cricket Frogs, Pickerel Frogs, and Fowler's Toads. Values of various (*) represent significant differences at P=0.05.

8. APPENDIX

Table A.1 Chi-Squared values for Figure 9 displaying chi squared values for each of the 5 testing locations mentioned in Figure 3. P-value is non-significant.

OBSERVED:

	+	-	
Site 1	33	57	90
Site 2	39	51	90
Site 3	29	61	90
Site 4	22	68	90
Site 5	15	75	90
	138	312	450

EXPECTED:

	+	-	
Site 1	27.60	62.40	90.00
Site 2	27.60	62.40	90.00
Site 3	27.60	62.40	90.00
Site 4	27.60	62.40	90.00
Site 5	27.60	62.40	90.00
	138.00	312.00	450.00

CHI-SQUARE:	**18.3528**
DF:	**4**
P-VALUE:	**0.0011**

Table B.1 Chi-squared values displayed reflection Figure 10 comparing all treatments for all amphibians captured. P-value is significant.

OBSERVED:

	+	-	
H	48	127	175
L	39	126	165
N	20	140	160
	107	393	500

EXPECTED:

	+	-	
H	37.45	137.55	175.00
L	35.31	129.69	165.00
N	34.24	125.76	160.00
	107.00	393.00	500.00

CHI-SQUARE:	**11.8065**
DF:	**2**
P-VALUE:	**0.0027**

Table B.2 Chi-squared values for Figure 10 displaying difference between no light treatment high intensity treatment for all amphibian species. P-value is significant.

OBSERVED:

	+	-	
H	48	127	175
N	20	140	160
	68	267	335

EXPECTED:

	+	-	
H	35.52	139.48	175.00
N	32.48	127.52	160.00
	68.00	267.00	335.00

CHI-SQUARE:	**11.5138**
DF:	**1**
P-VALUE:	**0.0007**

Table B.3 Chi-squared values for Figure 10 displaying difference between no light treatment and low intensity treatment for all amphibian species. P-value is significant.

OBSERVED:

	+	-	
L	39	126	175
N	20	140	160
	59	266	325

EXPECTED:

L	31.77	143.23	175.00
N	29.05	130.95	160.00
	60.82	274.18	335.00

CHI-SQUARE:	**7.1609**
DF:	**1**
P-VALUE:	**0.0075**

Table B.4 Chi-squared values for Figure 10 displaying difference between low intensity treatment and high intensity for all amphibian species. P-value is non- significant.

OBSERVED:

	+	-	
L	39	126	175
H	48	127	175
	87	253	340

EXPECTED:

	+	-	
L	44.78	130.22	175.00
H	44.78	130.22	175.00
	89.56	260.44	350.00

CHI-SQUARE:	**1.1940**
DF:	**1**
P-VALUE:	**0.2745**

Figure B-4 Chi-squared values for Figure 10 displaying difference between low intensity treatment and high intensity for all amphibian species. P-value is non- significant.

Table C.1 Chi-squared values represented in Figure 11, Chi-squared depicts difference between each of the three treatment types for Northern Cricket Frog count. P-value is significant.

OBSERVED:

	+	-	
H	19	16	35
L	20	13	33
N	6	26	32
	45	55	100

EXPECTED:

	+	-	
H	15.75	19.25	35.00
L	14.85	18.15	33.00
N	14.40	17.60	32.00
	45.00	55.00	100.00

CHI-SQUARE:	**13.3757**
DF:	**2**
P-VALUE:	**0.0012**

Table C.2 Chi-squared values represented in Figure 11, Chi-squared depicts difference between high intensity and no light treatment types for Northern Cricket Frog count. P-value is significant.

OBSERVED:

	+	-	
H	19	16	35
N	6	26	32
	25	42	67

EXPECTED:

	+	-	
H	13.06	21.94	35.00
N	11.94	20.06	32.00
	25.00	42.00	67.00

CHI-SQUARE:	**9.0247**
DF:	**1**
P-VALUE:	**0.0027**

Table C.3 Chi-squared values represented in Figure 11, Chi-squared depicts difference between low intensity and no light treatment types for Northern Cricket Frog count. P-value is significant.

OBSERVED:

	+	-	
L	20	13	35
N	6	26	32
	26	39	65

EXPECTED:

	+	-	
L	14.00	21.00	35.00
N	12.80	19.20	32.00
	26.80	40.20	67.00

CHI-SQUARE:	**11.6399**
DF:	**1**
P-VALUE:	**0.0006**

Table C.4 Chi-squared values represented in Figure 11, Chi-squared depicts difference between high intensity LED and low intensity treatment for Northern Cricket Frog count. P-value is non-significant.

OBSERVED:

	+	-	
H	19	16	35
L	20	13	33
	39	29	68

EXPECTED:

	+	-	
H	20.07	14.93	35.00
L	18.93	14.07	33.00
	39.00	29.00	68.00

CHI-SQUARE:	**0.2774**
DF:	**1**
P-VALUE:	**0.5984**

Table D.1 Chi-squared values represented in Figure 12, Chi-squared depicts difference between each of the three treatment types for Southern Leopard Frog count. P-value is significant.

OBSERVED:

	+	-	
H	9	26	35
L	3	30	33
N	2	30	32
	14	86	100

EXPECTED:

	+	-	
H	4.90	30.10	35.00
L	4.62	28.38	33.00
N	4.48	27.52	32.00
	14.00	86.00	100.00

CHI-SQUARE:	**6.2460**
DF:	**2**
P-VALUE:	**0.0440**

Table D.2 Chi-squared values represented in Figure 12, Chi-squared depicts difference between high intensity and no light treatment types for Southern Leopard Frog count. P-value is significant.

OBSERVED:

	+	-	
H	9	26	35
N	2	30	32
	11	56	67

EXPECTED:

	+	-	
H	5.75	29.25	35.00
N	5.25	26.75	32.00
	11.00	56.00	67.00

CHI-SQUARE:	**4.6152**
DF:	**1**
P-VALUE:	**0.0317**

Table D.3 Chi-squared values represented in Figure 12, Chi-squared depicts difference between low intensity and no light treatment types for Southern Leopard Frog count. P-value is non-significant.

OBSERVED:

	+	-	
L	3	30	35
N	2	30	32
	5	60	65

EXPECTED:

	+	-	
L	2.69	32.31	35.00
N	2.46	29.54	32.00
	5.15	61.85	67.00

CHI-SQUARE:	**0.2938**
DF:	**1**
P-VALUE:	**0.5878**

Table D.4 Chi-squared values represented in Figure 12, chi-squared depicts difference between high intensity LED and low intensity treatment for Southern Leopard Frog count. P-value is non-significant.

OBSERVED:

	+	-	
H	9	26	35
L	3	30	33
	12	56	68

EXPECTED:

	+	-	
H	6.18	28.82	35.00
L	5.82	27.18	33.00
	12.00	56.00	68.00

CHI-SQUARE:	**3.2297**
DF:	**1**
P-VALUE:	**0.0723**