Effects of Artificial Light at Night on Metamorphic Rate, Survival, and Size of Wood Frogs (*Lithobates sylvatica*)

Honors Thesis by Will Pfadenhauer ('20)
For the Academic Year (2019-2020)
Advisor: Dr. Megan Rothenberger
Committee: Drs. Kira Lawrence, Michael Butler
and Caleb Gallemore

Introduction

Increases in human population and development are closely linked with increases in the amount and extent of artificially generated light (Cauwels et al. 2014). Light pollution, known as artificial light at night (ALAN) is becoming more widely recognized as a threat to biodiversity (Hölker et al. 2010, Gaston and Holt 2018). In a recent study, ALAN dominated nighttime natural sky light at 81% of worldwide locations surveyed, which included rural areas (Kyba et al. 2015). ALAN is known to cause severe issues in animals that rely on regular and consistent light cycles (Longcore and Rich 2004, Davies et al. 2014, Gaston et al. 2017). Many animal processes (e.g. metabolic functions) are dependent on daily fluctuations in the functions of genes, which are initiated by the combination of internal circadian rhythms, light-dark cycles, and feeding patterns (Panda 2016). While animals' circadian rhythms cannot be altered with differences in light, the circadian rhythms can become unsynchronized with the release of certain hormones such as melatonin and many types of glucocorticoids (Bedrosian et al. 2016). Recent research indicates that exposure to ALAN is a major cause of this desynchronization in animals, leading to disruption of homeostatic processes and larger effects on physiology and behavior (Bedrosian et al. 2016).

An increasing number of scientific studies are revealing that light pollution negatively impacts amphibians. This is a serious conservation concern because one-third of all known amphibian species are threatened with extinction (U.S. Fish and Wildlife Service 2015). Although habitat loss is the single largest threat to amphibians, affecting nearly two-thirds of all known amphibian species worldwide (Halliday 2017), habitat loss is likely interacting with ALAN to alter amphibian habitat use, development, growth, and reproduction. For example, Feuka et al. (2017) suggested that blue-spotted salamanders may choose different substrates when exposed to ALAN that they would normally not prefer. Dananay and Benard (2018) observed shorter larval phases and less massive juveniles in American toads that were exposed to ALAN. Underhill and Hobel (2018) observed increased chorusing and breeding in Eastern Gray Treefrogs during nights that are characterized by intermediate levels of light, and female *Physalaemus pustulosus* frogs were found to be less selective of male mates when ALAN levels are elevated (Longcore and Rich 2004).

While light pollution has been established as a biological issue for amphibians, more research is needed to understand the precise ecological implications. Most ecological studies on the impacts of ALAN focused on stationary light sources, resulting in a gap in knowledge of the effects of light pollution from road traffic (Gaston and Holt 2018). Although road traffic worsens light pollution in numerous ways, vehicle headlights are the most intense, far-spreading, and inconsistent portions of light pollution from road traffic, making them potentially the most devastating to amphibian habitats (Lyytimäki et al. 2012). Furthermore, vehicular headlights remain largely underrecognized as a threat to biodiversity due to a number of social and psychological reasons (Lyytimäki et al. 2012). For example, the widespread usefulness of vehicle headlights and the difficulty of changing the current system of vehicular headlights likely leads some people into deliberate unawareness of the environmental issues of such a system, where facts are pushed aside and the burden of responsibility of addressing such issues are passed on to other people (Lyytimäki et al. 2012). Sky brightness (also referred to as skyglow), in comparison, is more widely recognized as a problematic form of light pollution and is therefore better understood. Light sources that significantly contribute to skyglow have been identified through previous research, but research into the factors that affect the spatial extent of skyglow is lacking (Kuechly et al. 2012).

I aim to address gaps in our knowledge regarding the impact of ALAN on amphibians with my honors thesis research by building on an existing amphibian conservation project started by faculty and former students at Lafayette College (Rothenberger et al. 2019). The ultimate goal of that project was to compare amphibian habitat quality among 15 natural, restored, and created vernal pools. Vernal pools are forested depressions that temporarily fill with surface runoff during the spring months each year. In northeastern North America, approximately 56% of frog, toad, and salamanders species frequent vernal pools for breeding, development, foraging, or hibernation (Colburn 2004). Rothenberger et al. (2019) used successful reproduction and metamorphosis of two vernal pool indicator species (the wood frog and spotted salamander) to measure and compare vernal pool quality. Results indicated that amphibian success is not necessarily related to pool type, but that quality of mitigation attempts is variable. and certain factors are more important for governing amphibian success than others (Rothenberger et al. 2019). The vernal pools used in this study vary in their proximity to roads, housing developments, and cities, and therefore also in their exposure to ALAN. Since previous studies that focused on amphibian success at vernal pools have overlooked light pollution as a potential variable, I propose a next step in this project that involves an experiment to quantity the impact of ALAN on wood frog growth and development by simulating both skyglow and vehicular light pollution in the laboratory at levels comparable to those at field sites.

Aside from the deficiencies in knowledge regarding the effect of ALAN on amphibians, there is also little understanding about the spatial extent of light pollution from cities. Although remote sensing has been used for modeling spatial extents of sky brightness, satellites measure upward facing light as a proxy for sky brightness which reflects false differences in data due to varying policies and technologies (Kyba et al. 2013). Additionally, satellites are only capable of relatively coarse imagery, which does not work as well on regional scales (Elvidge et al. 2007). Citizen science has been proven to be effective in areas with high quantities of observations, but this presents accuracy issues for geographic areas with fewer observations (Kyba et al. 2013). Since there is currently no consistent way to predict how far meaningful light pollution from cities spreads into surrounding suburbs and wildlife habitats, I propose a complimentary analysis to the aforementioned laboratory experiment that evaluates the spatial variations in sky brightness between the vernal pools listed above, and attempts to provide a quick and consistent method for predicting these spatial variations in future scenarios.

I established that skyglow at the 15 vernal pool study sites ranges from 19.41 to 20.62 magnitudes per square arcsecond, indicating that they are being exposed to a moderate amount of light pollution. Vernal pools in Susquehannock State Forest, an area renown for its dark skies and lack of light pollution were exposed to skyglow in the range of 21.7 magnitudes per square arcsecond. Three of the vernal pools are also within 45 meters of paved roads and are exposed to varying levels of vehicular light pollution. Based on preliminary observations and results of previous studies of the effects of ALAN on amphibian development (Dananay and Benard 2018), I predict that skyglow and vehicular light pollution are additional environmental variables governing amphibian success at these pools. I hypothesize that, if I expose developing wood frogs to levels of ALAN comparable to those measured at field sites, then metamorphic duration and size of wood frogs at metamorphosis will both decrease when compared to wood frogs not exposed to intermediate levels of light pollution. Based on research by Mcdonald et al. (2009), I also hypothesize that biologically relevant levels of skyglow will persist within at least a 10-kilometer radius from the urban source of the skyglow, and that increasing levels of skyglow at the urban source will correspond with greater distances of persistence.

Proposed Methods

Quantifying ALAN at Vernal Pool Sites

Skyglow will be quantified at field locations through use of a Sky Quality Meter manufactured by Unihedron. This device is used frequently in the scientific community because of its ability to consistently quantify night sky brightness (Kyba et al. 2015, Jechow et al. 2016, Hänel et al. 2018). The frequency of vehicle headlights passing vernal pools in the field will be observed through use of a game camera. The intensity of vehicle headlights will be measured by a lux meter at the edge of vernal pools closest to the paths of vehicular traffic. Measurements of skyglow at vernal pools will be done once per month. Quantification of intensity and frequency of vehicular light pollution at vernal pools will be done on two separate nights in early March 2020.

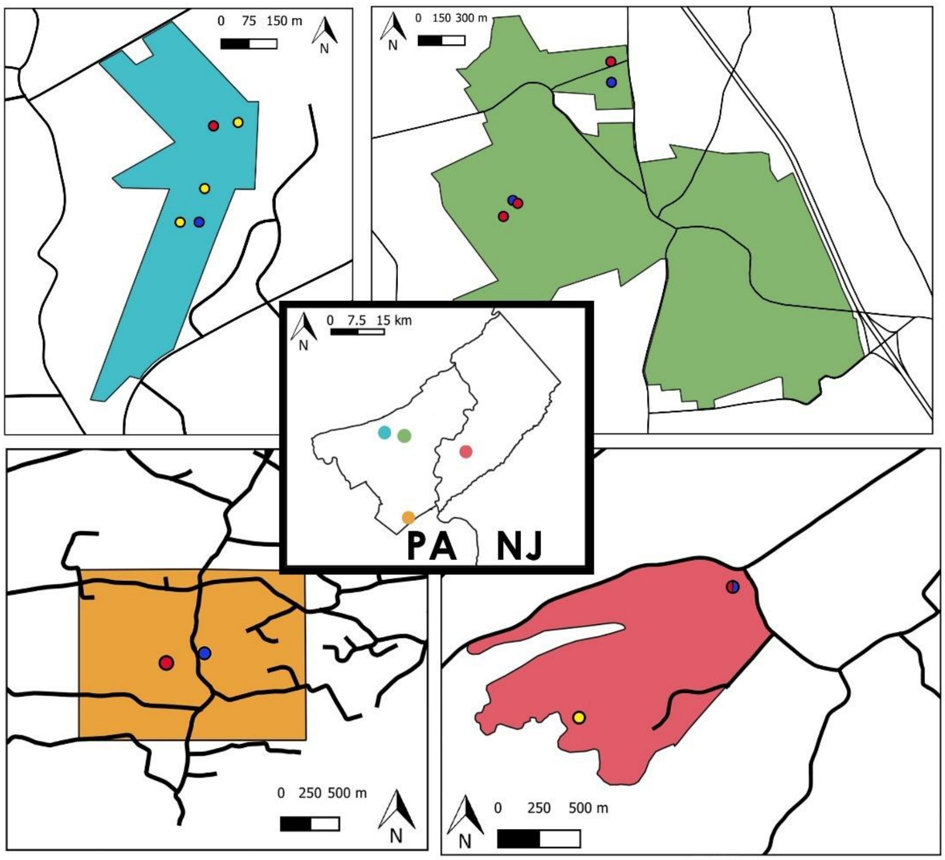

Figure 1. Vernal pools and surrounding properties from Rothenberger et al. (2019).

Recreating ALAN conditions in the laboratory

To simulate skyglow, blue, 13-watt, 120-volt fluorescent light bulbs will be used since they are relatively inexpensive and emit short wavelength light similar to that of light scattered by the atmosphere. The bulbs will be controlled and dimmed by a computerized light controller and mounted in such a way that the light emitted is reflected off solid black fabric above the aquaria containing developing wood frogs. Further dimming will be done by neutral density filters if necessary. The black fabric above the aquaria will be checked for accuracy with a Sky Quality Meter. To simulate vehicular light pollution, 60-watt, 120-volt halogen flood light bulbs will be used since they are also relatively inexpensive and halogen lights are still the most common form of vehicle headlights. These will be mounted such that they are directly facing the developing wood frogs, and these lights will be controlled and dimmed with the same light controller, and by neutral density filters if necessary. The light reaching each of the aquaria will be checked for accuracy with a luxmeter. The levels of simulated skyglow and vehicular light pollution in the lab will match field measurements from the vernal pools described above.

Impact of ALAN on wood frog growth and development

Experiments to assess the effect of ALAN on wood frog development will include four manipulation types: 1) no ALAN (control), 2) simulated vehicular light pollution, 3) simulated skyglow, and 4) combined simulated skyglow and vehicular light pollution. Each treatment will consist of three replicates. The levels of ALAN for the control will be equivalent to measurements of vehicular light pollution and skyglow taken at vernal pools in Susquehannock State Forest (mentioned above). Dependent variables will include wood frog survival rates, larval stage length, snout to vent length, and mass of wood frog individuals.

Retrieval of eggs

To obtain individuals for this experiment, we will collect two egg masses (each with about 500 eggs per mass) in March 2020 from one of our natural vernal pools. Collecting two egg masses will enable us to begin with about 80 eggs in each one of our twelve 38-L treatment tanks and a density of about 2 larvae per liter, which is comparable to our most successful vernal pools and to previous studies using wood frog larvae in experimental laboratory assays. Water quality in the aquariums will be maintained in accordance with parameters used in Barr et al. (2018) and the Association of Zoos and Aquariums (2012). These methods have already received approval from IACUC and the Pennsylvania DCNR.

The developing wood frogs will be raised in the laboratory until late April 2020 when they will be released at the vernal pool from which they were collected. During the experiment, developing wood frogs will be exposed to natural light-dark cycles with the added light simulations for the replicates in treatments 2-4 (outlined above).

Survival rates and larval stage length with be observed and recorded every third day of the experiment, while snout to vent length and mass measurements will be recorded once, immediately prior to release.

The vernal pools studied in Rothenberger et al. (2019) will mark the edges of the study area for spatial skyglow variations. Monthly skyglow measurements will be taken at each of 18 sites, spread out in a grid across the greater Easton, PA area (Fig. 2). These sites were selected in an attempt to create even spatial distribution of points between vernal pool sites, while selecting locations that were feasible for taking measurements. These measurements will be plotted in QGIS and analyzed to determine an equation of best fit that describes how skyglow changes as it extends farther from its source.

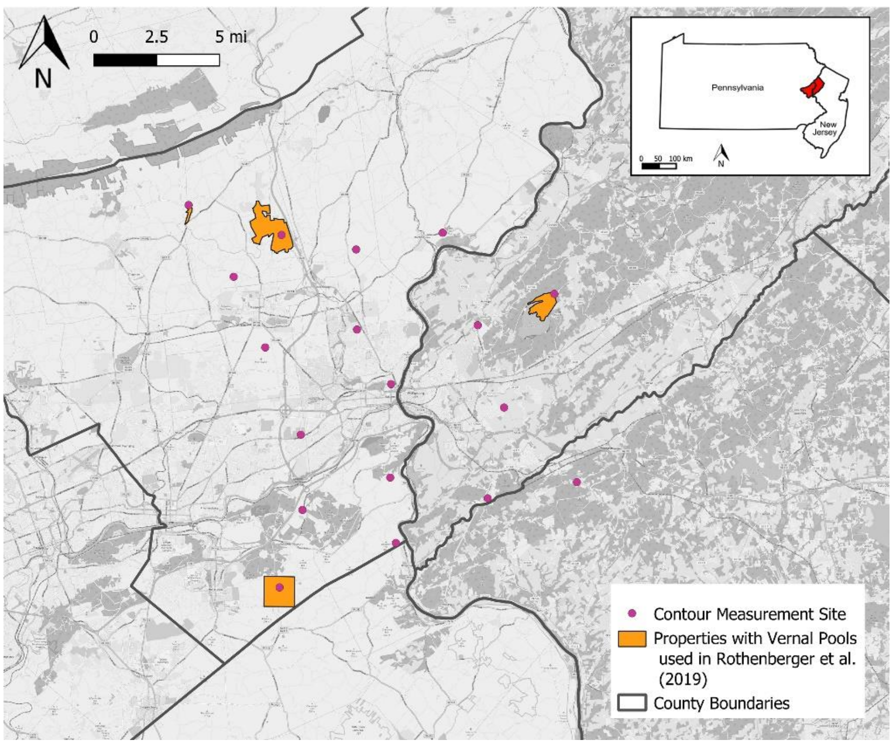

Figure 2. Night sky brightness measurement sites used for skyglow analysis.

Budget

This research will be funded in part through a $500 photosciences grant from the Zoological Lighting Institute. Sky quality meters, aquaria, a game camera, and a computerized light controller have already been purchased, meaning light bulbs, fixtures, fabric, and wood frog food are the main expenses that will be covered by the grant funds.

Literature Cited

Association of Zoos and Aquariums. 2012, April 4. Amphibian Husbandry Resource Guide. Assocation of Zoos and Aquariums.

Barr, J. M., J. R. Palmucci, O. J. Lambert, and P. P. Fong. 2018. Exposure to the antifouling chemical medetomidine slows development, reduces body mass, and delays metamorphosis in wood frog (Lithobates sylvaticus) tadpoles. *Environmental Science and Pollution Research* 25:10630–10635.

Bedrosian, T. A., L. K. Fonken, and R. J. Nelson. 2016. Endocrine Effects of Circadian Disruption. *Annual Review of Physiology* 78:109–131.

Cauwels, P., N. Pestalozzi, and D. Sornette. 2014. Dynamics and spatial distribution of global nighttime lights. *EJP Data Science* 3:2–27.

Colburn, E. A. 2004. *Vernal Pools: Natural History and Conservation*. McDonald and Woodward, Blacksburg, VA.

Dananay, K. L., and M. F. Benard. 2018. Artificial light at night decreases metamorphic duration and juvenile growth in a widespread amphibian. *Proceedings of the Royal Society B: Biological Sciences* 285:2–7.

Davies, T. W., J. P. Duffy, J. Bennie, and K. J. Gaston. 2014. The nature, extent, and ecological implications of marine light pollution. *Frontiers in Ecology and the Environment* 12:347–355.

Elvidge, C. D., J. Safran, B. Tuttle, P. Sutton, P. Cinzano, D. Pettit, J. Arvesen, and C. Small. 2007. Potential for global mapping of development via a nightsat mission. *GeoJournal* 69:45–53.

Feuka, A. B., K. E. Hoffmann, M. L. Hunter, and A. J. K. Calhoun. 2017. Effects of light pollution on habitat selection in post-metamorphic wood frogs (Rana sylvatica) and unisexual blue-spotted salamanders (Ambystoma laterale × jeffersonianum). *Herpetological Conservation and Biology* 12:470–476.

Gaston, K. J., T. W. Davies, S. L. Nedelec, and L. A. Holt. 2017. Impacts of Artificial Light at Night on Biological Timings. *Annual Review of Ecology, Evolution, and Systematics* 48:49–68.

Gaston, K. J., and L. A. Holt. 2018. Nature, extent and ecological implications of night-time light from road vehicles. *Journal of Applied Ecology* 55:2296–2307.

Halliday, T. 2017, March 3. Worldwide Amphibian Declines.

Hänel, A., T. Posch, S. J. Ribas, M. Aubé, D. Duriscoe, A. Jechow, Z. Kollath, D. E. Lolkema, C. Moore, N. Schmidt, H. Spoelstra, G. Wuchterl, and C. C. M. Kyba. 2018. Measuring night sky brightness: methods and challenges. *Journal of Quantitative Spectroscopy and Radiative Transfer* 205:278–290.

Hölker, F., C. Wolter, E. K. Perkin, and K. Tockner. 2010. Light pollution as a biodiversity threat. *Trends in Ecology & Evolution* 25:681–682.

Jechow, A., F. Hölker, Z. Kolláth, M. O. Gessner, and C. C. M. Kyba. 2016. Evaluating the summer night sky brightness at a research field site on Lake Stechlin in northeastern Germany. *Journal of Quantitative Spectroscopy and Radiative Transfer* 181:24–32.

Kuechly, H. U., C. C. M. Kyba, T. Ruhtz, C. Lindemann, C. Wolter, J. Fischer, and F. Hölker. 2012. Aerial survey and spatial analysis of sources of light pollution in Berlin, Germany. *Remote Sensing of Environment* 126:39–50.

Kyba, C. C. M., K. P. Tong, J. Bennie, I. Birriel, J. J. Birriel, A. Cool, A. Danielsen, T. W. Davies, P. N. den Outer, W. Edwards, R. Ehlert, F. Falchi, J. Fischer, A. Giacomelli, F. Giubbilini, M. Haaima, C. Hesse, G. Heygster, F. Hölker, R. Inger, L. J. Jensen, H. U. Kuechly, J. Kuehn, P. Langill, D. E. Lolkema, M. Nagy, M. Nievas, N. Ochi, E. Popow, T. Posch, J. Puschnig, T. Ruhtz, W. Schmidt, R. Schwarz, A. Schwope, H. Spoelstra, A. Tekatch, M. Trueblood, C. E. Walker, M. Weber, D. L. Welch, J. Zamorano, and K. J. Gaston. 2015. Worldwide variations in artificial skyglow. *Scientific Reports* 5.

Kyba, C. C. M., J. M. Wagner, H. U. Kuechly, C. E. Walker, C. D. Elvidge, F. Falchi, T. Ruhtz, J. Fischer, and F. Hölker. 2013. Citizen Science Provides Valuable Data for Monitoring Global Night Sky Luminance. *Scientific Reports* 3:1835.

Longcore, T., and C. Rich. 2004. Ecological light pollution. *Frontiers in Ecology and the Environment* 2:191–198.

Lyytimäki, J., P. Tapio, and T. Assmuth. 2012. Unawareness in environmental protection: The case of light pollution from traffic. *Land Use Policy* 29:598–604.

Mcdonald, R. I., R. T. T. Forman, P. Kareiva, R. Neugarten, D. Salzer, and J. Fisher. 2009. Urban effects, distance, and protected areas in an urbanizing world. *Landscape and Urban Planning* 93:63–75.

Panda, S. 2016. Circadian physiology of metabolism. *Science* 354:1008–1015.

Rothenberger, M. B., M. K. Vera, D. Germanoski, and E. Ramirez. 2019. Comparing amphibian habitat quality and functional success among natural, restored, and created vernal pools. *Restoration Ecology* 27:881–891.

Underhill, V. A., and G. Höbel. 2018. Moonlighting? - Consequences of lunar cues on anuran reproductive activity. *Acta Oecologica* 87:20–28.

U.S. Fish and Wildlife Service. 2015, May 28. Conserving Amphibians: What the Amphibians are Telling Us and Why We Should Listen.