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Artificial Lighting and Nocturnal Anuran Calling Behavior
in Northern Michigan Vernal Pools

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Abstract

Artificial lighting affects the physiology of nocturnal anuran amphibians; however, its effects on their behavior are still largely unknown. The goal of this study was to determine if artificial lighting significantly affects male nocturnal anuran breeding call behavior. Using the North American Amphibian Monitoring Program (NAAMP) protocol, seven vernal ponds in northern Wisconsin and Michigan were surveyed under unlit (0.1-0.00001 lux) and lit (800 lux) conditions using a high intensity floodlight. Although abiotic weather variables accounted for some of the calling variance, significantly fewer *Pseudacris c. crucifer* and *Hyla versicolor* called during lit surveys than unlit surveys. Future conservation efforts directed towards anurans must address artificial night lighting in order to account for this potentially harmful behavioral change.

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Concern over amphibian declines in the past twenty years (Wake, 1991) has led to increased use of monitoring programs (Weir et al., 2005) to assess amphibian populations. Amphibian call surveys such as the North American Amphibian Monitoring Program (NAAMP) have been proposed as an effective method for assessing anuran distribution patterns and population trends (Heyer et al., 1994; Weir and Mossman, 2005). This method is based on the species-specific advertisement calls made by males of many frogs and toads during the breeding season. Such calls can be easily detected and quantified and provide evidence of species presence and a rough index of adult population size.

Abiotic factors such as air temperature, rainfall, and moonlight have been of interest to scientists investigating intraspecific variability in anuran calls (Saenz et al., 2006; Oseen and Wassersug, 2002; Granda et al., 2008). Recent surveys conducted in Maryland (Weir et al., 2005) and central Texas (Pierce and Gutzwiller, 2007; Granda et al., 2008) have focused on the impact moonlight has on anuran calling behavior. For example, Pierce and Gutzwiller (2007) were able to correlate the presence of moonlight with decreased agreement between observers during anuran call surveys. Additionally, Granda et al. (2008) detected significantly more anuran species under low moonlight and Weir et al. (2005) found that moonlight negatively impacts probability of anuran species detection. These three studies suggested the relationship between moonlight and variability in species detection was due to frogs calling less during moonlight to avoid predation (Weir et al., 2005; Pierce and Gutzwiller, 2007; Granda et al., 2008).

Although no study has yet been published explicitly studying the effects of moonlight on anuran perception of increased predation, a few studies have addressed the effects of artificial lighting (e.g., urban glow or headlights) on anurans. Cornell and Hailman (1984) determined pupillary responses of *Rana pipiens* and *Rana forreri* to illumination when adapted to darkness. The researchers found that the two species required 10-20 min to fully constrict to light. They also found that at low illumination (5-50 lux), the anurans' pupils required 4 hr to fully redilate, longer than 4 hr with high intensity illumination (500-1500 lux), and longer than 24 hr with even higher illumination (5000 lux).

In addition to Cornell and Hailman's study of pupillary dilation, several studies have been conducted on phototactic behavior of anurans under different spectral ranges (Hailman and Jaeger, 1974; Jaeger and Hailman, 1976) and activity levels of anurans in response to ambient light (Hailman, 1982; Hailman, 1984; Jaeger and Hailman, 1981; Rand et al., 1997). For example, Buchanan (1993) tested the effects of different levels of artificial lighting on the efficiency of predatory behavior on male *Hyla chrysocelis*. Buchanan's goal was to determine if laboratory lighting or continuously shining a headlamp on an anuran affected their predatory behaviors. Buchanan found that high-intensity 'white' light (at an intensity equivalent to a standard headlamp) inhibited the eyesight of *H. chrysocelis* enough to negatively affect predatory behavior as measured by the amount of time taken to first orient to prey. Buchanan suggests that high-intensity 'white' light, commonly used during call surveys, can affect anuran predatory behavior due to impaired eyesight.

Although Weir et al. (2005) and Granda et al. (2008) observed that increased moonlight decreased anuran species detection probability and several studies have measured the effects of ambient or dim lighting on anuran activity levels (Hailman, 1982; Hailman, 1984; Jaeger and Hailman, 1981; Rand et al., 1997; Buchanan, 1993), no previous study has measured the effects of artificial lighting on breeding call behavior of nocturnal anurans in a natural setting. The purpose of this study was to determine if artificial light introduced to amphibian breeding habitats affects calling behavior. Two specific hypotheses were tested: 1) nocturnal anurans call less frequently under high-intensity artificial lighting 2) abiotic variables, especially moonlight, can predict nocturnal anuran calling frequency. Using several short call surveys and a high-intensity floodlight, seven vernal ponds in the northern peninsula of Michigan were used to compare breeding call intensities between lit and unlit ponds.

Methods

Amphibian call surveys were conducted from June 2 to June 17, 2009, using the North American Amphibian Monitoring Program (NAAMP) protocol (Weir and Mossman, 2005), except that surveys were carried out under all wind conditions. All surveys were conducted within the University of Notre Dame Environmental Research Center (UNDERC; [46° 13' N, 89° 32' W]). A map of vernal ponds within the UNDERC property was used to select sites. Vernal ponds are common breeding sites for many amphibian species in the northern U. S. (Colburn, 2004). Thirty-six sites were chosen and pre-tested once for 15 min to ensure anurans call from each site. Seven sites had detectable numbers of anurans calling during the pre-test and were used during experimental surveys.

Each auditory survey was conducted between 2100 and 0100 h by one or two observers. At each site, the observers first recorded survey time, air temperature (Celsius), water temperature (Celsius), cloud cover (percentage), visible moonlight (percentage), and any precipitation [0 = none, 1 = mist or light rain, or 2 = moderate or heavy rain]. During surveys, each observer independently recorded each species heard, the NAAMP calling intensity index (0, 1, 2, or 3), the estimated number of individuals calling (1, 2, 3-5, 6-10, or >10), and road noise (number of passing vehicles). When more than one observer surveyed the same site, one was randomly determined as the primary observer.

For call index, number of frogs calling, call latency and road noise, values recorded by the primary observer were used. Moonlight data for the date and hours of each survey were obtained from the U.S. Naval Observatory web site

(http://aa.usno.navy.mil/data/docs/RS_OneDay.html). To reduce interobserver variation, observers were trained to recognize ten anuran species that had the potential to occur within the survey area. Frog call quizzes (<http://www.pwrc.usgs.gov/frogquiz>) were conducted at least twice a week to ensure proficiency in recognizing anuran breeding calls.

Each lighting condition (lighting or no lighting) consisted of five 3-min surveys taken in succession. Shirose et al. (1997) found 3-min surveys have a high detection probability and that longer surveys do not appreciably increase call detection. Each site was surveyed under both lighting conditions with a 5-min gap between groups of five 3-min surveys. At each site, the order of survey type (lighting-no lighting and no lighting-lighting) was determined randomly. Each site was surveyed at least two different nights under both lighting orders between at least two different evenings. Two sites were tested per survey night. The order of the site tested during each night was randomly determined and, to avoid carryover effects from previous surveys, no site was tested two nights in a row.

To illuminate the sites, one high-intensity floodlight (810.4 lux; Brinkmann Model No. 800-2500-0) used in combination with a 12V rechargeable lead acid battery (EaglePicher Model No. CF-12V14L) was used to illuminate the largest proportion of the vernal pool possible. The pool was illuminated only during the lighted condition for the duration of the five 3-min surveys. Vehicles used to approach the sites were parked at least 25 m away with the headlights turned off. A headlamp covered with a red filter was sparingly used to aid in recording data. To measure the luminance of the floodlight, a LI-COR LI-1000 Data Logger was used in combination with a Quantum model light meter

positioned 1 m from the floodlight connected to a fully charged battery. The floodlight had a luminance of 810.4 lux at 1 m (lumen m⁻²; Meyer-Arendt, 1968).

Data Analysis. Paired *t*-tests were used to compare the absolute number of anurans calling between lit and unlit conditions. To correct for the increased risk of a Type I error associated with multiple *t*-tests, Bonferroni correction was applied. In order to determine if recorded abiotic factors significantly affected anuran calling behavior, a multiple squares least squares regression was used. An adjusted r^2 statistic was used for the multiple regression due the large number of independent variables simultaneously tested. When a range of anuran individuals (for example, 6-10 or 11-20) were heard during a survey, the mean number of the range (8 and 15.5, respectively) was used for data analysis. MYSTAT version 12.02.00 was used for all statistical procedures.

Results

Seven anuran species were detected across 14 survey nights from June 2 to June 17, 2009: *Bufo americanus*, *Hyla versicolor*, *Pseudacris c. crucifer*, *Rana catesbeiana*, *Rana clamitans*, *Rana pipiens*, and *Rana sylvatica*. Two of these species, *H. versicolor* and *P. c. crucifer*, were heard often enough for statistical analysis. Using Bonferroni correction, two paired *t*-tests were used to compare the number of frogs detected during lit and unlit surveys. Significantly fewer *P. c. crucifer* were detected during lit surveys ($\bar{x} = 2.667$, $SD = 2.786$) than unlit surveys ($\bar{x} = 3.176$, $SD = 2.982$, $t = 2.659$, $df = 104$, $p < 0.01$, see Fig. 1). Additionally, significantly fewer *H. versicolor* were detected during lit ($\bar{x} = 1.633$, $SD = 1.999$) surveys than unlit surveys ($\bar{x} = 2.200$, $SD = 2.434$, $t = 2.345$, $df = 59$, $p < 0.05$ see Fig. 2).

A multiple least-squares regression was used to assess variance in the number of calling anurans explained by abiotic factors. Both experimental conditions were included in this analysis. Abiotic factors included were: air temperature, water temperature, time of night, percentage of detected moonlight, percentage of cloud cover, and road noise. Precipitation was not used in the regression analysis due to the lack of precipitation during any survey. Moonlight was visible on four nights, and when not visible zero values were used for statistical analyses. This combination of abiotic factors had a small, statistically significant effect on the number of anurans calling, r^2 (adjusted) = 0.194, $F(6, 194) = 9.004$, $p < 0.001$. Further analysis of the multiple regression revealed minor statistically significant effects of water temperature, detected moonlight, and cloud cover on the number of anurans calling (Table 1). Neither air temperature, time of night, nor

road noise significantly affected the number of anurans calling (all p 's > 0.5). The following linear formula was generated based on the multiple regression:

$$N = 8.116157 - 0.290W - 2.146M + 1.185C$$

where N = the number of *P. c. crucifer* or *H. versicolor* calling, W = water temperature in °C, M = percentage of detected moonlight, and C = percentage of cloud cover. One outlier was omitted from this analysis.

Discussion

In this study, significantly fewer *H. versicolor* and *P. c. crucifer* called during lit than unlit call surveys. Additionally, water temperature, observed moonlight, and cloud cover explained a small portion of the variance between the numbers of frog species detected. Surprisingly, a decrease in water temperature led to increased calling behavior. Due to anuran amphibians being ectotherms, this seems counterintuitive. Indeed, temperature significantly affects behavioral performance in anurans (e.g., Putnam and Bennett, 1981). It is possible that the range of temperatures recorded during the present study's surveys were not of a wide enough range to make a meaningful assessment. A more elaborate study by Gayou (1984) examining the effects of temperature on male *H. versicolor* breeding calls criticized the lack of more broad temperature spectra used in past correlational temperature studies. In fact, Gayou (1984) demonstrated the opposite of the present study – that increased temperature is significantly associated with more energetic calling in *H. versicolor*. Additionally, the association of decreased moonlight and increased cloud cover with increased anuran calling frequency seen in this study agrees with previous studies that examined the effects of moonlight on anuran calling behavior (Weir et al., 2005; Pierce and Gutzwiller, 2007; Granda et al., 2008).

On the significant effect of artificial night lighting on calling behavior: this study agrees with previous studies that have found artificial lighting affects nocturnal anuran behavior (e.g., Buchanan, 1993). In addition to confirming behavioral effects of artificial night lighting, the present study raises concerns for its long-term ecological impact. Cinzano et al. (2001) calculated 18.7% of the Earth's terrestrial surface experiences night sky brightness considered polluted by astronomical standards (a brightness ratio 0.11

greater than unpolluted night skies). This impressive figure includes 61.8% of the United States and 85.3% of the European Union (Cinzano et al., 2001). The present study's findings suggest that anuran calling behavior, essential for anuran reproduction, will be adversely affected by the artificial lighting already impacting a large portion of the Earth's terrestrial surface. Furthermore, given the present study's findings, it is likely that artificial lighting adversely affects nocturnal anuran fitness and may impair reproduction, contributing further to amphibian declines.

Unfortunately, the current evidence cannot determine the long-term consequences of artificial night lighting. Although ascertaining these long term consequences was not a goal of this study, biologists and policy-makers alike could benefit from such data. In particular, a long term study measuring the fitness of anurans in an environment subjected to continuous artificial night lighting (i.e., urban glow) is necessary to determine if light pollution negatively affects anuran species survival.

In conclusion, the established concern for assessing and potentially reversing rapid amphibian declines can directly benefit from these findings. Future conservation efforts and policies directed towards anuran amphibians must account for the luminance of artificial night lighting, whether continuous or dynamic (e.g., passing automotive headlights) in order to account for this most-likely harmful behavioral change.

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Table 1. Results of multiple least-square regression analysis of abiotic factors on number of anurans calling. $df = 194$.

Abiotic factor	β_1	SE	t	p
Water Temperature (°C)	- 0.2904	± 0.1127	- 2.577	0.011
Detected Moonlight (%)	- 2.146	± 0.5590	- 3.839	< 0. 001
Cloud Cover (%)	1.185	± 0.5748	2.062	< 0.05

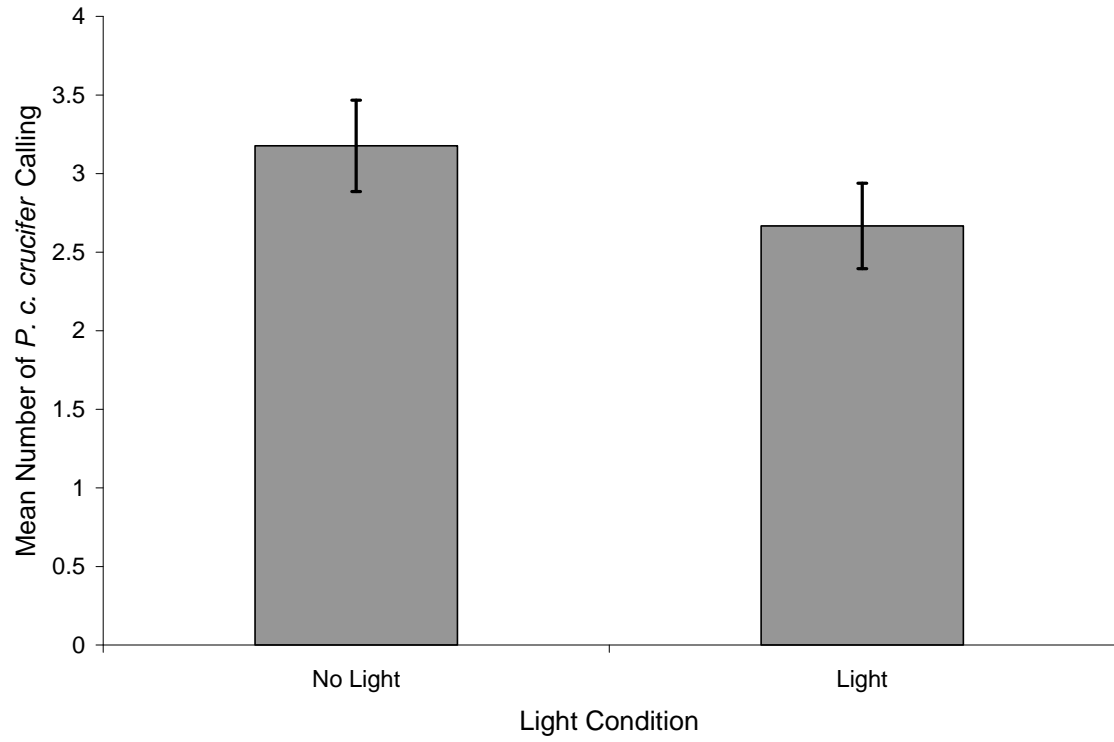


Figure 1. Results of a paired *t*-test comparison between lit and unlit anuran call surveys. Significantly fewer *P. c. crucifer* called during lit surveys than unlit surveys, $p < 0.01$.

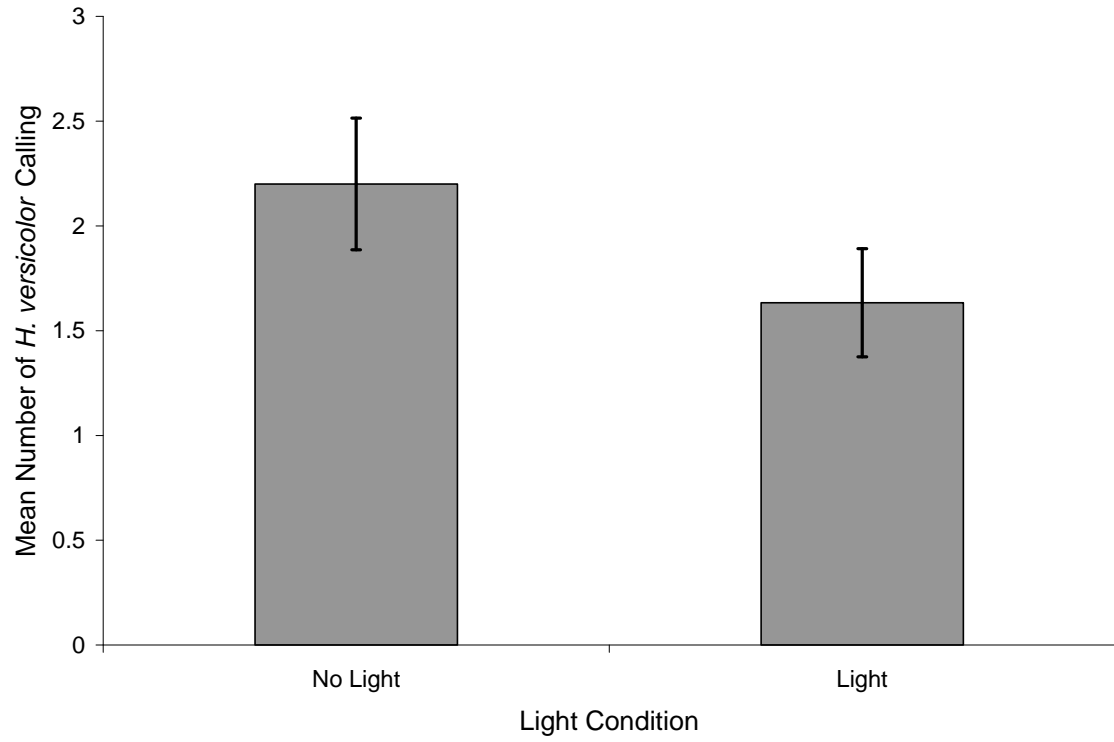


Figure 2. Results of a paired *t*-test comparison between lit and unlit anuran call surveys. Significantly fewer *H. versicolor* called during lit surveys than unlit surveys, $p < 0.05$.