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The effect of intensified illuminance and artificial light at night on fitness and susceptibility to abiotic and biotic stressors★

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ABSTRACT:

Changing light conditions due to human activities represents an important emerging environmental concern. Although changes to natural light conditions can be independently detrimental, in nature, organisms commonly face multiple stressors. To understand the consequences of altered light conditions, we exposed a model amphibian (wood frog; *Lithobates sylvaticus*) to a control and two anthropogenic light conditions: intensified daytime illuminance and artificial light at night - ALAN (intensified daytime illuminance + extended photoperiod). We measured (1) metrics of fitness (hatching success as well as survival to, size at, and time to metamorphosis) (2) susceptibility (time to death) to a commonly co-occurring anthropogenic stressor, road salt (NaCl) and (3) susceptibility (infection load) to a common parasite (trematode). We also explored behavioral (swimming activity) and physiological (baseline corticosterone (CORT) release rates) changes induced by these light conditions, which may mediate changes in the other measured parameters. We found that both intensified daytime illuminance and ALAN reduced hatching success. In contrast, for amphibians that successfully hatched, neither treatment affected amphibian survival or time to metamorphosis but individuals exposed to ALAN were larger at metamorphosis. The light treatments also had marginal effects; individuals in ALAN treatments were more susceptible to NaCl and trematodes. Finally, tadpoles exposed to ALAN moved significantly less than tadpoles in the control and intensified daytime illuminance treatments, while light had no effect on CORT release rate. Overall, changes in light conditions, in particular ALAN, significantly impacted an amphibian model in laboratory conditions. This work underscores the importance of considering not only the direct effects of light on fitness metrics but also the indirect effects of light with other abiotic and biotic stressors. Anthropogenic-induced changes to light conditions are expected to continue increasing over time so understanding the diverse consequences of shifting light conditions will be paramount to protecting wildlife populations.

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1. Introduction

Anthropogenic activities have profound impacts on natural ecosystems (Goudie 2018). In particular, shifts in light conditions due to human activities have received increasing recent attention (Davies and Smyth, 2018; Gaston et al., 2013; Hölker et al., 2010; Macgregor et al., 2015; Sanders and Gaston, 2018). Light conditions are often quantified in terms of illuminance. Illuminance (measured in lux) describes the amount of light from a particular light source (either natural or synthetic) that is perceived by an individual (Cinzano and Falchi, 2014). Light conditions vary naturally based on a number of factors (e.g., transitions between different habitats, dominant foliage type, height, and color; Théry, 2001). However, due to human activities, natural light conditions are being rapidly altered in novel ways (Cinzano et al., 2001). For example, human activities such as deforestation, can result in unnaturally rapid changes in the magnitude of daytime illuminance (Endler and Thery, 1996). For conspecific interactions that are optimized at specific light conditions (e.g. reproductive displays in tropical birds; Endler and Thery, 1996), shifts in the magnitude of illuminance may have significant consequences. Yet, when considering the effects of light pollution, intensified daytime illuminance is often neglected.

Human activities can also change light conditions by modifying

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photoperiods. For instance, organisms living in or near human centers are commonly exposed to artificial light at night (ALAN) and face unnaturally long periods of light (Hölker et al., 2010). Exposure to ALAN has been shown to be detrimental to a number of taxa. For example, city lights near coastal areas interfere with sea turtle hatchlings' ability to orient themselves toward the ocean after hatching (Tuxbury and Salmon, 2005). Lights in urban areas have also been shown to interfere with songbird nighttime activity (Ouyang et al., 2017) and bird migration (Doren et al., 2017; La Sorte et al., 2017). Since ALAN is a frequent product of urbanization (Hopkins et al., 2018), it is likely to occur concurrently with intensified daytime illuminance due to the deforestation that permitted urbanization. Despite this, the majority of studies generally focus on extended photoperiod alone. Whether and how intensified daytime illuminance and extended photoperiods combine to influence natural populations is relatively less understood. In light of the rapidly increasing rates of human population growth, considering how anthropogenically-induced changes in light conditions modify natural ecosystems will become increasingly important.

Moreover, while changes to natural light conditions can be independently detrimental, organisms rarely contend with only one anthropogenic stressor (Sala et al., 2000). Habitats that are exposed to increased daytime illuminance or ALAN due to anthropogenic activities likely also face a variety of other man-made abiotic stressors including noise, chemical contamination, or other effects related to changes in land use practices (Lyytimäki, 2013). Chemical contaminants (e.g. road salts, heavy metals, pesticides) are particularly relevant because they not only occur in areas impacted by human-induced shifts in light conditions (e.g. urban areas, roadways) but can exacerbate the effects of shifting light conditions to more negatively affect wildlife. For example, past work demonstrated that the negative effect of ultraviolet (UV−B) light on amphibian mortality was strengthened when tadpoles were simultaneously exposed to the insecticide, carbaryl (Zaga et al., 1998). Evaluating how shifting light conditions influence susceptibility to other stressors can help shed light on the complex consequences of human activities on natural ecosystems.

Changes in light condition can also manipulate biological interactions. For example, in sea horses, increased daytime illuminance altered prey capture rates (James and Heck, 1994). Similarly, in an estuary ecosystem, ALAN resulted in higher predator abundance and predation risk compared to relatively dark nights (Becker et al., 2013). In amphibians, ALAN decreased the number of calls produced by male frogs and toads, compromising their interactions with females (Baker and Richardson, 2006; Hall, 2016; Steelman and Dorcas, 2010). While growing studies document the effects of light pollution on ecological interactions, how changes in light conditions impact host-parasite interactions has been relatively less explored and mostly limited to a handful of species (see Hevrøy et al., 2003; Ouyang et al., 2017; Kernbach et al., 2018). These interactions are particularly relevant as shifts in circadian rhythms associated with changing light conditions have important implications on overall health (Bonmati-Carrion et al., 2014; Chepesiuk, 2009). Further, parasites have a powerful influence on the structure of communities (Preston and Johnson, 2010) and have been shown to have negative cumulative effects on hosts when combined with other stressors (Marcogliese and Pietrock, 2011). Given the potential effect of light on health and the importance of parasites in shaping community structure, understanding how human-induced shifts in light influence host-parasite interactions has broad conservation and ecological implications.

Exposure to shifting light conditions (ALAN and intensified daytime illuminance) also has the potential to affect behavior and physiology (Baker and Richardson, 2006; Doren et al., 2017; Hall, 2016; Steelman and Dorcas, 2010). Evaluating behavioral or physiological responses to light may help elucidate mechanisms for how shifting light conditions influence fitness and susceptibility to abiotic and biotic stressors. For example, a reduction in behaviors, such as activity levels, may allow for more resources to be available for growth, metabolizing/detoxifying contaminants, or for mounting immune responses against parasites (i.e resource allocation theory; Brown et al., 2004). Shifts in behavior may also have indirect effects such as reducing feeding rates and ultimately growth and development or even altering the chances of encountering contaminants or parasites (Hanazato, 2001; Sheldon and Verhulst, 1996; Van Buskirk and Yurewicz, 1998). Similarly, changing light conditions can lead to physiological stress mediated by the production of glucocorticoid hormones such as corticosterone or cortisol (Romero, 2004). The overproduction of glucocorticoids (i.e. allostatic overload) can lead to negative consequences including reductions in muscle and bone mass and impairments in the ability to mount appropriate stress responses when exposed to novel stressors (Busch and Hayward, 2009; McCormick and Romero, 2017; McEwen and Wingfield, 2003). The mechanisms dictating how shifting light conditions influence fitness and susceptibility to contaminants and parasites are currently not understood, thus investigating shifts in behavior or stress hormones across light conditions may be promising initial mechanisms to explore.

To better understand the consequences of altered light conditions (intensified daytime illuminance and ALAN), we exposed a model amphibian (wood frog; *Lithobates sylvaticus*) to three different light conditions: control, intensified daytime illuminance, and ALAN (intensified daytime illuminance + artificial light at night). We measured how these light conditions affected (1) metrics of amphibian fitness (hatching success as well as survival to, size at, and time to metamorphosis), (2) susceptibility (time to death) to an anthropogenic stressor, road salt (NaCl), (3) susceptibility (infection load) to a common parasite (trematode), (4) tadpole behavior (activity levels), and (5) baseline stress via corticosterone (CORT) release rates. We hypothesized that intensified daytime illuminance would have negative consequences on amphibian fitness (decreased hatching success, size, and survival at metamorphosis) and would increase susceptibility to NaCl and trematodes relative to the control treatment (Zaga et al., 1998). Next, we predicted that the ALAN treatment would exacerbate the negative consequences on amphibian fitness and susceptibility to NaCl and trematodes compared to both the control and the intensified daytime illuminance treatment (Hevrøy et al., 2003; Ouyang et al., 2017; Kernbach et al., 2018). Finally, we predicted that amphibians in the intensified daytime illuminance treatment will differ in swimming activity and CORT release rates compared to the control treatment and that these differences would be intensified in the ALAN treatment (Bridges, 1997; Ouyang et al., 2018).

2. Materials and methods

2.1. Model species

Regarded as one of the most widespread anurans in North America, the wood frog's natural range spans from the southeastern United States to the northern reaches of Canada and the Arctic circle (Martof and Humphries, 1959). Wood frogs are prolific breeders that are ubiquitous in many woodland and wetland systems, and as a result are often a useful indicator of ecosystem health and function (Hilty and Merenlender, 2000). Adult wood frogs in the eastern United States have an innate preference for reproduction in closed-canopy (low light) ponds in mature forests rather than ponds under open canopies of early-succession forests, or

those in areas dominated by shrubs and other diminutive plant life (Demaynadier and Hunter, 1998). As such, wood frogs are vulnerable to the effects of deforestation and other human activities that alter the intensity of daytime illuminance or photoperiods (Alford and Richards, 1999; Becker et al., 2016; Ultsch et al., 1999), making them a useful model organism for studying the effects of shifting light conditions.

2.2. Model abiotic stressor - NaCl

To understand how different light conditions affect amphibian susceptibility to a common chemical contaminant, we used NaCl. NaCl, the primary and active ingredient in common road deicing salt, has been identified as a leading cause of secondary salinization in inland wetlands (Herbert et al., 2015). While peak NaCl contamination occurs during the winter months, high NaCl concentrations may be retained within wetlands into the spring and summer (Findlay and Kelly, 2011; Kaushal et al., 2005). Indeed, chloride concentrations of up to 2.7 g/L have been detected in wetlands (Benbow and Merritt, 2004). Due to wood frog reproduction in the late winter and early spring, developing individuals commonly overlap with periods of peak road deicing salt runoff (Findlay and Kelly, 2011). Finally, investigating susceptibility to NaCl is relevant since road salt and ALAN have both been identified as threats to wildlife near roadways (Kociolek et al., 2011).

2.3. Model biotic stressor – parasites (Echinostoma sp.)

We examined how different light conditions affected tadpole susceptibility to a common trematode, *Echinostoma* sp. Trematodes have a complex, multi-species, multi-host life cycle (Huffman and Fried, 2012; Kanev et al., 2000). In this study, we focus on the free-swimming stage of the parasite (cercariae). Trematodes rely on chemical cues to locate their amphibian host (Haas, 2003; Sears et al., 2012), therefore it is not expected that light conditions would influence their ability to locate and encyst in tadpole hosts. In contrast, the degree of cercariae encystment varies depending on host health, thus, it is expected that amphibian host susceptibility may be directly or indirectly affected by light conditions (Schotthoefer et al., 2003).

2.4. Animal collection

We collected 10 wood frog egg masses on 13 April 2018 from a densely canopied vernal pool from the Pennsylvania State Game Lands (40.9730° N, 76.3435° W). Following transport to Binghamton University, we randomly separated 42 eggs from each of the 10 masses for a total of 420 eggs.

2.5. Experimental conditions

On 16 April 2018, the 420 eggs (Gosner 10; Gosner, 1960) were individually placed into clear plastic 266 mL plastic cups filled with 150 mL of UV-filtered well water. Individuals were randomly assigned to one of three experimental light conditions: control (12 h 300 lux: 12 h darkness), intensified daytime illuminance (12 h 1200 lux: 12 h darkness; hereafter “intensified illuminance”), and ALAN (12 h 1200 lux: 12 h 300 lux). We chose values of illuminance based on ranges detected in nature with our control treatment mimicking the low light conditions of closed canopy areas preferred by wood frogs (Demaynadier and Hunter, 1998), the intensified illuminance treatment mimicking open canopy areas (Bennie et al., 2016), and the ALAN treatment mimicking open canopy areas contaminated with ambient light at night from artificial sources (Bennie et al., 2016; Dananay and Benard, 2018). In this study, “darkness” was functionally defined as negligible illuminance of 1 lux, the lower limit of the luxmeter's range. To create the 300 lux condition, we used a Sylvania 40 W equivalent LED Daylight bulb. For the 1200 lux condition, we used a 100-W LED Daylight bulb. Experimental illuminance levels were verified with the luxmeter (Tables SI–1).

All treatments were kept in the same temperature-controlled (20 °C) room for the entire experiment. We assessed hatching success by counting the number of individuals from each treatment ($n = 140$) that successfully reached Gosner stage 20 (Gosner, 1960). All embryos that successfully hatched were allowed to develop into the free-swimming tadpole stage (Gosner 25 - all embryos reached the tadpole stage within 6 h of each other). Once tadpoles reached Gosner 25, we fed all tadpoles slurried Tetramin fish flakes *ad libitum* and we randomly set aside 60, 30, and 20 individuals from each light treatment for Experiment 1 (amphibian survival, development, size), Experiment 2 (Time to Death Assay), and Experiment 3 (Parasite Susceptibility Assay), respectively. For Experiment 4 (Behavioral Assay) and Experiment 5 (CORT Assay), we used the 60 individuals set aside from Experiment 1, since both the behavioral and the CORT assays are non-invasive measures.

2.5.1. Experiment 1: survival to, size at, and time to metamorphosis

We tracked survival to, size at, and time to metamorphosis in 60 wood frogs/treatment. Survival was defined as individuals that reached metamorphosis (Gosner 45). To measure time to metamorphosis, we observed individuals every day and recorded the day each individual reached metamorphosis (Gosner 45). To assess metamorphic size, we measured mass and SVL (snout to vent length) of all animals when they reached metamorphosis (Gosner 45). For the duration of the experiment, we fed all tadpoles a slurried Tetramin fish flakes *ad libitum* and we conducted scheduled water changes every 5 d. Once individuals reached Gosner 45, all individuals were euthanized by submersion in 5 g/L MS-222 solution and preserved in 10% formalin solution. We measured mass, SVL, and tadpole stage using a grade digital scale (HRB103 scientific; 0.001 g/1 mg sensitivity), digital calipers (Mitutoyo Absolute IP67, Aurora, IL, USA), and a dissecting microscope (Olympus SZ61, Waltham, MA, USA), respectively.

2.5.2. Experiment 2: time to death assay

In order to measure the effect of the light treatments on tadpole susceptibility to NaCl, we conducted a time to death (TTD) assay. TTD assays are ideal tools for assessing relative differences in tolerance across treatments. While concentrations used in TTD assays are higher than concentrations typically found in nature, they are intended to serve as a proxy for tolerance at lower levels (Newman, 2009). On 30 April (11 d post-hatching), we placed 30 tadpoles from each treatment into 266 mL plastic cups filled with 150 mL of an 8 g/L NaCl solution. During the TTD assay, all tadpoles remained in their original light treatment conditions. We documented each individual's time to death by checking for mortality every 4 h for the first 12 h and then every 2 h until the 72-h mark was reached. At 72 h, all remaining animals were euthanized by submersion in 5 g/L MS-222 solution. All individuals were preserved in 10% formalin solution to later measure tadpole mass, stage, and SVL.

2.5.3. Experiment 3: parasite susceptibility assay

On 10 May (21 days post-hatching), we exposed 20 tadpoles from each experimental treatment to 50 trematodes (see Text SI-1 for details on obtaining parasites) and measured the number of trematodes that successfully encysted in the tadpole kidney. Prior to the addition of trematodes, tadpoles were individually transferred into 1 L plastic containers filled with 200 mL of UV-filtered

well water. During the parasite susceptibility assay, all tadpoles remained in their original light treatment conditions.

After 24 h, we euthanized all individuals by submersion in 5 g/L MS-222 and preserved them in a 10% formalin solution. We chose 24 h because previous studies have shown that this is sufficient time for cercariae to encyst in the tadpole kidney (Rohr et al., 2008). After preservation, individuals were assessed for size and developmental differences by recording mass, stage, and SVL. To quantify parasitic infection in each individual, the kidneys were removed and placed between microscope slides to be analyzed under a dissecting microscope to count the number of encysted trematodes.

2.5.4. Experiment 4: behavioral assay

To understand how light treatments affected amphibian behavior, on 20 May, using tadpoles set aside for Experiment 1, we measured swimming activity using a 24-h scan assay (Relyea and Mills 2001). Two hours prior to the start of the scan assay, we conducted a scheduled water change on all individuals. Tadpoles were placed in 1 L deli cups filled with 800 mL of UV-filtered well water with three drops of slurried Tetramin fish flakes. Starting at 8:30 p.m., we began the scan assay by recording whether each tadpole was moving or motionless every 3 h. We scanned each tadpole 10 times at each check and concluded the assay at 8:30 p.m. on 21 May. During dark hours, we monitored behavior using a red LED headlamp which we found did not elicit a behavioral response from tadpoles (Figure SI-1).

2.5.5. Experiment 5: corticosterone assay

In order to measure CORT release rates, we conducted a water borne immersion procedure (Gabor et al., 2013). All water samples were collected at 7:00 p.m. to control for the effects of time of day on CORT levels. CORT samples were collected from animals (Gosner stage 44.27 ± 0.032; average ± standard error) by immersing each individual in 20 mL of UV-filtered well water in a sterile 100 mm × 15 mm petri dish for 1 h with no disturbances. After the hour of immersion, the sample was transferred to a sterile plastic scintillation vial, which was immediately frozen at −20 °C for later sample processing and analysis using an enzyme immunoassay (See Text SI-2 for detailed hormone sample preparation and validation techniques).

2.6. Statistical analysis

2.6.1. Experiment 1: amphibian fitness metrics

To evaluate the effect of light treatment on hatching success, we conducted a generalized linear model using a binomial distribution with a probit-link function. Of the animals that successfully hatched, we found 100% survival until metamorphosis. Therefore, we did not conduct statistical analyses on wood frog survival to metamorphosis.

To understand the effect of light treatments on rate of metamorphosis and average time to metamorphosis, we conducted a Cox proportional hazard model (Relyea and Mills 2001) and an analysis of variance (ANOVA), respectively.

To understand the effect of light treatments on amphibian mass and SVL at metamorphosis, we conducted a multivariate analysis of variance (MANOVA). We also included amphibian stage in this model to confirm that all animals were at the same stage (Metamorphic stage- Gosner 45) when size data was collected.

2.6.2. Experiment 2: effects of light treatment on tadpole susceptibility to NaCl

To understand the effect of light treatments on tadpole rate of death when exposed to lethal concentrations of NaCl, we conducted a Cox proportional hazard model (Cox and Oakes, 1984). Since mass, SVL, and stage may affect tadpole rate of death, we added these variables as covariates in our model. However, because these metrics are related, we first conducted a dimension reduction to combine these variables into a single principle component (PC). We then used the PC value as the covariate in the Cox proportional hazard model.

To investigate the effect of light treatment on tadpole susceptibility to NaCl (average time to death), we conducted an ANOVA. A PC consisting of mass, SVL, and stage was included as a covariate.

2.6.3. Experiment 3: effects of light treatment on tadpole susceptibility to trematodes

To investigate the effect of light treatments on tadpole susceptibility to trematodes, we used a generalized linear model. Prior to analyzing our data, we transformed our data using arcsin(SQRT) transformation (Conover and Iman, 1981; Warton et al., 2016). We incorporated tadpole mass, SVL, and stage as covariates as they have been shown to influence tadpole susceptibility to trematodes (Johnson et al., 2011). Again, because these variables are related, we conducted a dimension reduction to combine these variables into a single PC. We then included this PC as a covariate.

Pairwise comparisons: For all analyses with significant main effects in Experiments 1–3, we conducted planned contrasts (LSD; UPOV, 2007) to compare the effect of (1) intensified illuminance and ALAN vs. control light conditions and (2) intensified illuminance vs. ALAN.

2.6.4. Experiment 4: effects of light treatment on swimming behavior (activity)

To investigate the effect of light treatment on tadpole swimming behavior, we conducted a repeated measures ANOVA (rm-ANOVA). For significant main effects of light treatment, time, or interactions, we conducted Tukey's pairwise comparisons.

2.6.5. Experiment 5: effects of light treatment on CORT release rate

Hormone release rates were standardized by dividing by the SVL of the individual measured and by the time (1 h). Differences between light treatments did not meet assumptions of normality so we analyzed the data using a Kruskall-Wallis test.

All data in our experiment were analyzed using IBM SPSS software (Version 22, IBM, INC). For all analyses of variance, we tested all assumptions and if assumptions were not met, we ranked-transformed the data and ran a non-parametric analyses (Quinn and Keough, 2002).

3. Results

3.1. Effects of light treatments on hatching success

We found a significant main effect of light treatment on hatching success (Wilk's $\lambda = 7.2$, $p = 0.03$). Specifically, 99.3%, 92.1%, and 92.9% of embryos from the control, intensified illuminance, and ALAN treatments, successfully hatched, respectfully. Embryos reared in the control treatment had higher hatching success than embryos from both the intensified illuminance ($p = 0.005$) and ALAN treatments ($p = 0.003$). Embryos reared in the intensified illuminance and ALAN treatments did not differ in hatching success ($p = 0.82$).

3.2. Effects of light treatments on time to metamorphosis

We found no significant effect of light on tadpole metamorphic rate ($\chi^2 = 0.94$; $p = 0.62$; Fig. SI-2A). We found no significant main effect of the light treatment on average time to metamorphosis ($F_{2,194} = 1.8$; $p = 0.17$; Fig. SI–2B).

3.3. Effects of light treatments on mass, SVL, and stage

We found a significant overall multivariate effect of light treatments on tadpole mass, SVL, and stage (Wilk's $\lambda = 0.75$, $F_{6, 170} = 4.3$, $p < 0.001$; Fig. 1A and B; SI-3). We found a significant effect of light treatment on tadpole mass ($F_{2,87} = 9.3$, $p < 0.001$) and tadpole SVL ($F_{2,87} = 10.9$, $p < 0.001$) but not Gosner stage ($F_{2,87} = 1.6$, $p = 0.2$).

Tadpoles reared in the ALAN treatment were significantly heavier compared to tadpoles from the intensified illuminance treatment ($p < 0.001$; Fig. 1A) and tadpoles in the control treatment ($p = 0.001$). Tadpoles in the control treatment did not differ in mass compared to tadpoles from the intensified illuminance treatment ($p = 0.27$). Tadpoles reared in the ALAN treatment had significantly longer SVL compared to tadpoles from both the control treatment ($p < 0.001$; Fig. 1B) and the intensified illuminance treatment ($p < 0.001$). Tadpoles from the control treatment did not differ in SVL compared to tadpoles from the intensified illuminance treatment ($p = 0.95$).

3.4. Effects of light treatments on NaCl susceptibility

We found no significant effect of light on tadpole survival rate when exposed to NaCl ($\chi^2 = 2.3$; $p = 0.32$). We found a marginally significant main effect of the light treatment on average tadpole time to death ($F_{2,84} = 2.9$; $p = 0.06$; Fig. 2). Tadpoles raised in the ALAN treatment were more susceptible to NaCl compared to the control treatment ($p = 0.02$) but not the intensified illuminance treatment ($p = 0.16$). Tadpoles raised in the control treatment did not differ from tadpoles raised in the intensified illuminance treatment ($p = 0.34$).

3.5. Effects of light treatments on tadpole susceptibility to trematodes

We found a marginally significant main effect of the light treatments on tadpole susceptibility to trematodes ($\chi^2 = 5.7$; $p = 0.057$; Fig. 3). Tadpoles raised in the ALAN treatment were marginally more susceptible to trematodes compared to the control treatment ($p = 0.076$) and significantly more susceptible to trematodes compared to the intensified illuminance treatment ($p = 0.027$). Tadpoles raised in the control treatment did not differ from tadpoles raised in the intensified illuminance treatment ($p = 0.66$).

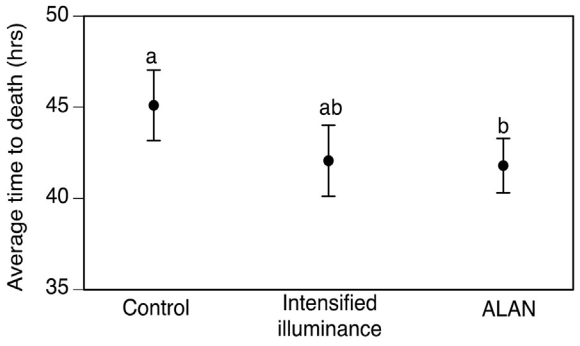

Fig. 2. The effect of light treatments on average tadpole time to death. Treatments with different letters are significantly different. Error bars represent standard error.

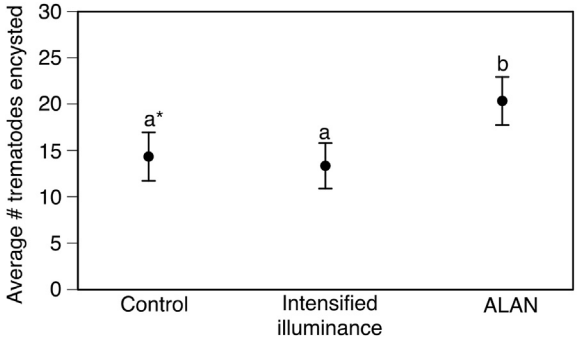

Fig. 3. The effect of light treatments on tadpole susceptibility to trematodes. Treatments with different letters are significantly different from each other. * Indicates that tadpoles exposed to the control treatment were marginally different than those in the ALAN treatment ($p = 0.076$). Error bars represent standard error.

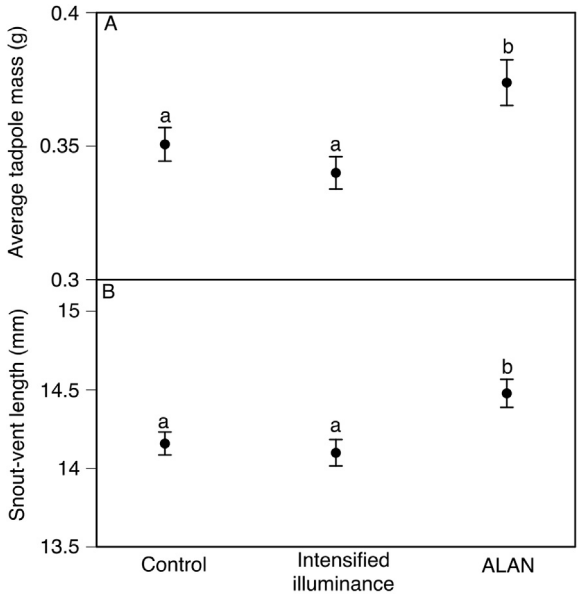

Fig. 1. The effect of light treatments on tadpole (A) mass and (B) SVL at metamorphosis. Treatments with different letters are significantly different from each other. Error bars represent standard error.

3.6. Effects of light treatments on wood frog swimming behavior (activity)

We found a significant effect of light ($F_{2,177} = 30.7$; $p < 0.001$), time ($F_{8,170} = 22.5$; $p < 0.001$; Fig. 4), and light*time ($F_{16, 170} = 12.5$; $p < 0.001$) on tadpole swimming activity. To better understand the

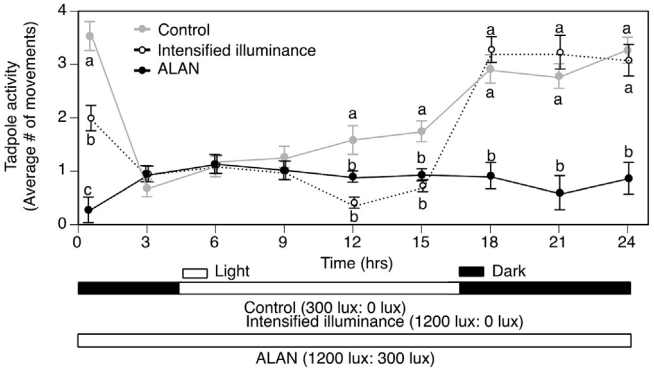

Fig. 4. The effect of light treatments on tadpole swimming activity. Treatments with different letters are significantly different from each other. Error bars represent standard error.

interaction we compared tadpole movement in each of the three treatments at each of the nine time points (Fig. 4). We found no significant differences in tadpole movement between the light treatments at hours 3, 6, and 9 ($p > 0.05$). However, we did find a significant difference in tadpole movements across all three treatments at hour 1 of the assay ($p < 0.001$). At hour 12, tadpoles reared in the control treatment were more active than tadpoles reared in both the intensified illuminance and the ALAN treatment ($p < 0.001$, $p = 0.051$, respectively). Similarly, at hour 15, tadpoles reared in the control treatment were more active than tadpoles reared in both the intensified illuminance and the ALAN treatment ($p < 0.001$, $p = 0.001$, respectively). In contrast, at hour 18, 21, and 24, tadpoles reared in the ALAN treatment were significantly less active than tadpoles reared in both the control and intensified illuminance treatment ($p < 0.001$ for all comparisons).

3.7. Effects of light treatments on wood frog tadpole CORT release rates

We found no significant effect of light treatment on corticosterone release rates ($F = 0.32$; $p = 0.8515$; Fig. SI-4). One individual from the control treatment and one individual from the intensified illuminance treatment had CORT release rates that fell below the standard curve and where therefore excluded from the analysis.

4. Discussion

Embryos exposed to the intensified illuminance treatment and the ALAN treatment both had lower hatching success than embryos exposed to the control treatment. Similarly, Cook and Rust (2002) found that fish embryos raised in a low illuminance treatment (1 lux) had higher hatching success than those in a high illuminance treatment (563 lux). While the mechanisms driving hatching success across light treatments are beyond the scope of this study, previous work in chickens suggest that decreased hatching success in response to intensified illuminance may be associated with thyroid hormone (thyroxine AKA T4; Yu et al., 2018). In contrast, we found no difference in hatching success between the intensified illuminance and ALAN treatments, suggesting that the addition of an extended photoperiod to higher illuminance did not exacerbate the negative effects on hatching success. Overall, while we found a statistically significant effect of light treatments on hatching success, it is important to note that hatching success was relatively high across all treatments (92.1%, 92.9%, 99.3% for intensified illuminance, ALAN, and control, respectively). Future studies should consider the relative influence of this reduction in hatching success on natural populations dynamics.

For amphibians that successfully hatched, we did not find an effect of intensified illuminance or ALAN on wood frog survival to metamorphosis. Similarly, Dananay and Benard (2018) found no effect of ALAN on the survival of *Anaxyrus americanus* (American toad) larvae. Our results suggest that following successful hatching, exposure to the intensified illuminance and extended photoperiods used in this study are not directly lethal to amphibians from the larval through metamorphic stages. We also did not find an effect of intensified illuminance or ALAN on time to metamorphosis. The effects of light on amphibian development in the literature and in this study are equivocal. Edwards and Pivorun (1991) found that *Xenopus laevis* (African clawed frogs) larvae exposed to an extended photoperiod (23L:1D) took more time to complete metamorphosis than tadpoles exposed to a shortened photoperiod (1L:23D). In contrast, Dananay and Benard (2018) found that *A. americanus* tadpoles exposed to ALAN (15 lux) accelerated time to metamorphosis. Lux levels were not reported in Edwards and Pivorun (1991), but the photoperiod in our study differed from the Edwards and Pivorun (1991) study (24 h of light: 12 h at 1200 lux and 12 h at 300 lux). Notably, while environmentally relevant (Bennie et al., 2016), the nighttime illuminance levels (300 lux for 12 h) used in our study were almost an order of magnitude more intense compared to those used in Dananay and Benard (2018; 15 lux for 11 h). Thus, the disparities in the effect of light on time to metamorphosis may reflect the variation in experimental methodologies across studies (i.e. different photoperiods, illuminance values, experimental venues - lab vs. field, and model species). While we did not find an effect of intensified illuminance and extended photoperiod on survival to or time to metamorphosis, given the disparity in results across the literature, it is important for future studies to continue assessing the consequences of light conditions across different light scenarios and species.

Contrary to our hypothesis, amphibians from the ALAN treatment were larger at metamorphosis compared to tadpoles in both the intensified illuminance and control treatments. Previous work suggests that disruptions to light conditions can lead to a misalignment in circadian rhythms that lead to weight gain and metabolic abnormalities (Fonken et al., 2010; Fonken and Nelson, 2014). Kooijman et al. (2015) demonstrated that extended photoperiods led to increased weight in rats, but this was due to decreased energy expenditure rather than increasing food intake or locomotor activity. The results of our behavioral assay are consistent with Kooijman et al. (2015). Overall, tadpoles in the ALAN treatment reduced swimming activity by 59% and 50% compared to the control and intensified illuminance treatment, respectively. This suggests that decreased energy expenditure may be one potential mechanism for why tadpoles in the ALAN treatment are larger. Alternatively, Fonken et al. (2010) exposed rats to ALAN and found that these rats consumed more during daylight hours when their metabolic cycles are less efficient causing them to retain more weight than rats not exposed to ALAN. In our study, because tadpoles were fed *ad libitum* and we did not specifically assess tadpole feeding behavior, we cannot rule out increased feeding in the ALAN treatment as an alternative mechanism for increased amphibian size. This mechanism should be considered in future studies.

When exposed to lethal concentrations of NaCl, none of the three light treatments affected mortality rates. However, we detected an increase in tadpole susceptibility to NaCl (average time to death) in the ALAN treatment compared to tadpoles in the control treatment. Few studies have assessed how exposure to light pollution influences susceptibility to chemical contaminants. Of the studies that do, most focus on the effects of intensifying ultraviolet (UV) radiation rather than ALAN or intensified daytime light (Bancroft et al., 2008; Kiesecker and Blaustein, 1995; Long et al., 1995; Zaga et al., 1998) A meta-analysis performed by Bancroft et al. (2008) demonstrated that UV light in combination with additional stressors such as acidity, pesticides, fertilizers, and disease resulted in increased amphibian mortality across a wide variety of species. Collectively, the increase in tadpole susceptibility to NaCl in the ALAN treatment makes a case for future studies to consider the potential for interactive effects of ALAN and chemical contaminants. This is especially relevant since many habitats experiencing ALAN are already in close proximity to humans, increasing their chances of being exposed to additional anthropogenic stressors (Lyytimäki, 2013).

Tadpoles exposed to the ALAN treatment were more susceptible to trematodes than tadpoles from the intensified illuminance treatment and marginally more susceptible to trematodes from the control treatment. A similar phenomenon of increased parasite susceptibility when exposed to ALAN has been observed in birds and salmon (Hevrøy et al., 2003; Ouyang et al., 2017). Additionally, exposure to other anthropogenic stressors (e.g. chemical contaminants) has also been shown to increase parasite susceptibility in

wood frog tadpoles (Buss and Hua, 2018) and other larval amphibian species (Rohr et al., 2008). Past work demonstrates that larger tadpoles (Wersebe et al., 2019) as well as tadpoles that have lower activity levels are more susceptible to infection by trematodes (Johnson and Hoverman, 2014; Koprivnikar et al., 2006). While metamorphic individuals (Experiment 1) from the ALAN treatment were larger than those in both the control or the intensified illuminance treatments, tadpoles in the parasite assay (Experiment 3) from the ALAN treatment did not differ in size compared to the other treatments. However, tadpoles from the ALAN treatment did exhibit consistently lower activity levels suggesting that decreased movement (i.e. anti-parasitic behaviors) may be one mechanism for why wood frog larvae exposed to ALAN are marginally more susceptible to trematodes. ALAN has been shown to increase predator abundance and therefore predation risk (Becker et al., 2013). Thus, while a decrease in tadpole activity level may reduce tadpole vulnerability to visual predators, reductions in activities may increase vulnerability to parasites like trematodes. In this study, behavioral assays were conducted in the absence of trematodes. It is possible that the presence of trematodes may modify these observed behavioral patterns. Therefore, future studies should consider evaluating behavior in the presence of trematodes to further elucidate the potential mechanisms driving the effects of ALAN on trematode susceptibility. Collectively, this study underscores the value of considering potential consequences of ALAN by evaluating other measures of amphibian fitness (i.e. disease susceptibility) in addition to traditional measures (i.e. survival, mass, and development).

Interestingly, the intensified illuminance treatment differed from the control treatment for only one response variable, hatching success. In contrast, the ALAN treatment resulted in changes relative to the control treatment for several metrics (hatching success, metamorphic size, susceptibility to NaCl and trematodes). Collectively, this suggests that the higher daytime illuminance of the intensified illuminance treatment alone is not enough to initiate an effect for most parameters. Rather, the combination of both higher illuminance and extended photoperiod is required. While open canopy ponds are not the preferred habitat of larval wood frogs in eastern North America, wood frogs are still detected in these habitats and are likely exposed to light conditions similar to the intensified illuminance treatment (Bennie et al., 2016; Blomquist and Hunter, 2010; Demaynadier and Hunter, 1998). In our study, we collected wood frogs from a heavily-canopied pond that was unlikely to encounter light pollution via either intensified illuminance or ALAN. This suggests that perhaps the lack of response when exposed to intensified illuminance treatment may be because these conditions are within the natural range that wood frogs can tolerate despite their natal pond conditions. In contrast, exposure to ALAN may represent a more novel environmental condition.

Contrary to our predictions, exposure to changing light conditions did not alter CORT release rates. Past studies indicate that many contaminants elicit a nearly ubiquitous stress response mediated by the release of glucocorticoids (GCs), such as cortisol and corticosterone (Romero, 2004). Thus far, no other studies have investigated amphibian CORT levels in response to light pollution, although it has been examined in other vertebrates with equivocal results (Alaasam et al., 2018; Ouyang et al., 2018; Russart and Nelson, 2018). Our study found that light pollution did not result in differences in CORT release rate and thus may not be a likely mechanism for the effects of shifting light conditions that we detected in amphibian fitness, susceptibility to NaCl, and susceptibility to trematodes. However, because CORT release rates differ across development and time of day, future studies should consider measuring CORT release rates across development as well as across time. Further, our study only considers baseline CORT. While valuable, the ability to mount an acute stress response when faced with future stressors is also critical for survival (Romero, 2004). Future studies should consider how light pollution influences amphibian ability to mount an appropriate acute response to future stressors.

5. Conclusions

We found that changes in light conditions, in particular ALAN, had significant consequences on an amphibian model in laboratory conditions. Particularly, this work highlights the importance of considering both direct effects of light on fitness metrics and its effects on susceptibility to other abiotic and biotic stressors. For the majority of metrics, we found that higher daytime illuminance alone did not have significant consequences; the addition of the extended photoperiod of the ALAN treatment was often necessary to elicit a response. Finally, we investigated tadpole swimming activity and CORT release rates, and found that swimming activity may be important in influencing the effect of shifting light conditions on amphibian mass and susceptibility to trematodes. As light conditions across the globe continue to change, understanding the potential environmental consequences will be critical to protecting natural ecosystems.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.05.016.

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