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The morphological effects of artificial light at night on amphibian predators and prey are masked at the community level☆

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ABSTRACT

Artificial light at night (ALAN) is a pervasive pollutant that influences wildlife at both the individual and community level. In this study, we tested the individual-level effects of ALAN on three species of tadpole prey and their newt predators by measuring prey pigmentation and predator and prey mass. Then we evaluated whether the individual-level effects of ALAN on pigmentation and mass had cascading community-level effects by assessing the outcome of predator-prey interactions. We found that spring peepers exposed to ALAN were significantly darker than those reared under control conditions. Additionally, wood frogs reared in ALAN conditions were significantly smaller than those reared in control conditions. In contrast, Eastern newts collected earlier in the spring that were exposed to ALAN were significantly larger than controls while those collected later in the spring were not affected by ALAN, suggesting phenological differences in the effect of ALAN. To understand how changes in pigmentation and size due to ALAN influence predation rates, we ran predation assays in both ALAN-polluted and ALAN-free outdoor environments. After the predation assay, the size disparity in wood frogs reared in ALAN was eliminated such that there was no longer a treatment difference in wood frog size, likely due to size-selective predation. This demonstrates the beneficial nature of predators' selective pressure on prey populations. Lastly, despite individual-level effects of ALAN on pigmentation and mass, we did not detect cascading community-level effects on predation rates. Overall, this study highlights important species-level distinctions in the effects of ALAN. It also emphasizes the need to incorporate ecological complexity to understand the net impact of ALAN.

1. Introduction

As human populations grow, the effect of environmental pollutants on natural ecosystems becomes more pervasive (Butchart et al., 2010; Geldmann et al., 2014; Halpern et al., 2015). In particular, artificial light at night (ALAN) is a growing pollutant of concern that is ubiquitous with human activity (Kyba et al., 2017). ALAN is defined as any anthropogenic light source, such as street lights, sky glow from large cities, flood lights, car headlights, etc. That is present at a time inconsistent with the natural day-night cycle (Longcore and Rich, 2004). Since the use of artificial lights is a relatively new phenomenon in the evolutionary history of most wildlife, ALAN has the potential to have a number of significant consequences from the individual-level to the ecosystem-level (Swaddle et al., 2015). Importantly, the impacts of ALAN at one organizational level can have cascading interactive effects at other organizational levels (Warne et al., 2019). For example, ALAN interferes with the physiological interpretation of day length, leading to behavioral changes that have significant impacts on ecological interactions, such as mating, foraging, and pollination (Gaston et al., 2017). Given the potential for cascading interactive effects, holistic approaches that integrate the effects of ALAN at multiple organization levels are important to assessing the impact of ALAN.

At the individual level, one of the most recognized consequences of ALAN is its suppressive effect on melatonin production (Aubé et al., 2013; Fleury et al., 2020; Grubisic et al., 2019; Jiang et al., 2020; Kernbach et al., 2020; Kumar et al., 2019; Lewy et al., 1980; Navara and Nelson, 2007; Russart and Nelson, 2018). Melatonin is a pineal hormone that fulfills a number of immunological and circadian functions in both

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vertebrates and some invertebrates (Filadelfi and Castrucci, 1996; Hardeland and Poeggeler, 2003; Pévet, 2003; Sugden et al., 2004; Vivien-Roels and Pévet, 1993). Melatonin is sometimes referred to as the "darkness" hormone, as its peak production occurs at night (Filadelfi and Castrucci, 1996; Hardeland and Poeggeler, 2003; Pévet, 2003; Sugden et al., 2004; Vivien-Roels and Pévet, 1993). In amphibians, melatonin is also responsible for the aggregation of melanosomes, organelles within melanophores (pigment cells) that contain the black-brown pigment melanin (Sugden et al., 2004). When melatonin reaches receptors in a melanophore, melanosomes aggregate to the center of the melanophore, leading to skin lightning (Sugden et al., 2004). Though the impact of ALAN on amphibian melatonin has not been tested, based on the highly repeatable effect of ALAN on melatonin across taxa, it is possible that ALAN suppresses melatonin in amphibians as well. While we expect that amphibians exposed to ALAN will be darker due to melatonin suppression (Dawson, 1975; Garcia and Sih, 2003; Norris and Lowe, 1964), these effects may differ by species since effects of ALAN can be species-dependent (Bailey et al., 2019; Barré et al., 2021; Feuka et al., 2017; McNaughton et al., 2021; Mena et al., 2021; Miller et al., 2017; Perry et al., 2008; Rotics et al., 2011; Senzaki et al., 2020). This in turn may lead to differential ecological consequences (i.e. changes in pigmentation may modify visibility of tadpoles to visual predators). Yet, to date, our understanding of the generalizability of individual- and community-level ALAN effects across species that vary in important life history traits (predator avoidance strategies, metamorphic rate, etc.) remains limited.

ALAN has also been found to impact the size of individuals (American toads, *Anaxyrus americanus* - Dananay and Benard, 2018; mice, *Mus musculus* - Kooijman et al., 2015; wood frogs, *Rana sylvatica* - Shidemantle et al., 2019; cane toads, *Rhinella marina* - Secondi et al., 2021). Specifically, disruptions to photoperiod can lead to a misalignment in circadian rhythms which cause mass gain and metabolic abnormalities (Fonken et al., 2010; Fonken and Nelson, 2014). Despite this, others have observed decreases in mass following ALAN exposure (Dananay and Benard, 2018), while others have found that ALAN has no impact on mass (Touzot et al., 2019, 2020). Understanding the impact of pollutants on size is important because size is often a proxy for fitness and can have significant effects on ecological interactions (Berven, 1990; Cabrera-Guzmán et al., 2013; Semlitsch et al., 1988). The equivocal impact of ALAN on size suggests that these effects may not be generalizable. As such, to better understand the effect of ALAN on size, there is a need to consider the effects of ALAN across taxa and guilds.

At the community level, ALAN can increase foraging rates (Santos et al., 2010), increase disease-induced mortality (Kernbach et al., 2020), facilitate invasions (Komine et al., 2020; Thawley and Kolbe, 2020), and decrease biodiversity (Hölker et al., 2010; Secondi et al., 2019). Notably, ALAN has profound impacts on predator-prey dynamics (Gaston et al., 2021, 2013). Indeed, ALAN can alter the predator's ability to capture prey or reciprocally, the prey's ability to avoid predation (Bailey et al., 2019; Grenis et al., 2015; Manfrin et al., 2018, 2017; McMunn et al., 2019; Miller et al., 2017; Minnaar et al., 2015; Santos et al., 2010). For example, ALAN may provide a better visual environment for spotting prey (increasing predation) (McMunn et al., 2019). Similarly, prey may be more conspicuous (e.g. through changes in skin pigmentation) (Bolliger et al., 2020; Minnaar et al., 2015). The impacts of ALAN on size may also have cascading effects on predation rates. For example, changes in prey size may influence predation rates and predation success (e.g. large prey avoid predation by gape-limited predators; ratio of predator size to prey size is indicative of predation success; Barnes et al., 2010; Christensen, 1996; Nakazawa et al., 2011; Urban, 2007). Similarly, changes in predator size may also alter predation rates, as larger predators tend to eat more prey than smaller predators (Babbitt and Tanner, 1998). Overall, ALAN-induced changes in pigmentation and mass at the individual level likely have cascading consequences at the community level on predator-prey dynamics (McMunn et al., 2019). Therefore, it is important to understand how pollutants such as ALAN impact predators and prey both individually and when interacting with one another.

The goal of this study was to determine how ALAN influences prey and predators at the individual level and in turn their interactions with one another. We used amphibians in this study since amphibians are sensitive to environmental change and have already shown to elicit effects in response to ALAN (Baker and Richardson, 2006; Cope et al., 2020; Dananay and Benard, 2018; Feuka et al., 2017; Secondi et al., 2021; Touzot et al., 2021, 2020, 2019). We hypothesized that exposure to ALAN impacts tadpole pigmentation: specifically, tadpoles reared in ALAN would be significantly darker than controls. We hypothesized that ALAN would impact size, specifically leading to higher mass for both predators and prey. Finally, we hypothesized that the individual-level effects of previous ALAN exposure would impact predation rates differently in ALAN-free *vs.* ALAN-polluted environments because tadpoles may recover from prolonged ALAN exposure when placed in ALAN-free environments. We predicted that in both environment types, predation rates would be highest among treatments that had been previously exposed to ALAN due to individual effects on prey (pigmentation and size) and predators (size). We tested these hypotheses using three different species of larval amphibians (i.e., tadpoles) and one species of tadpole predator. We used multiple prey species in order to determine if the effects of ALAN were consistent across species.

2. Materials and methods

2.1. Animal collection

2.1.1. Larval collection

We used three species of larval amphibians for this study: wood frogs (*Rana sylvatica*), spring peepers (*Pseudacris crucifer*), and American toads (*Anaxyrus americanus*). Since each has a distinct breeding period, we collected eggs from each species separately. On 26 March 2021, we collected ten partial wood frog egg masses from Binghamton University's Nature Preserve (BUNP). Next, on 2 April 2021, we collected spring peeper eggs from four amplexing adult pairs from BUNP. On 6 May 2021, we collected ten partial American toad egg masses from Aqua-Terra Wilderness Area. At all collecting sites, nighttime illuminance was <0.01 lux. For each of the three collecting periods, we mixed eggs from the various clutches to maximize genetic diversity within our experiments and immediately sorted eggs into their experimental units and exposed them to experimental conditions.

2.1.2. Predator collection

We used adult Eastern newts (*Notophthalmus viridescens*) as tadpole predators in predation assays. Newts are common tadpole predators with an active, vision-based predation strategy (Hunsinger and Lannoo, 2005; Ramamonjisoa et al., 2018). It was important for us to choose a visual predator because ALAN can increase the conspicuousness of prey by providing a better visual environment for predators and a visual predator may be able to detect changes in prey pigmentation caused by ALAN. On 30 March and 6 April 2021, we collected newts from BUNP (Nuthatch Hollow pond). Newts collected on 30 March were used for predation assays with wood frogs, while those collected on 6 April were used for predation assays with spring peepers. On 10 May 2021, we collected newts from Aqua-Terra Wilderness Area for predation assays with American toads. We collected newts used for toad predation assays from this population because it is the same area in which the toads were collected. At all collecting sites, nighttime illuminance was <0.01 lux. Though collected at different time points and locations, the average size of newts used in the different predation assays (wood frog *vs.* American toad *vs.* spring peeper) did not statistically differ (Kruskal-Wallis chi-squared $= 3.7188$, p-value $= 0.1558$).

We transported all predators to the lab where they were immediately exposed to experimental conditions. All animals were collected and used according to the appropriate permits (New York State's Scientific

License to Collect and Possess: Scientific #2673).

2.1.3. Experiment 1: tadpole pigmentation

Part 1: Rearing Conditions– We placed newly laid individual eggs (wood frogs, spring peepers, and American toads) into either a control or an ALAN treatment. For each species, we replicated each treatment 15 times for a total of 30 eggs per species. Eggs were individually housed in 250-mL plastic cups filled with 175 mL filtered well water on ten shelves. Half of the shelves were used for the control treatment and the other half were used for the ALAN treatment (for more detail on light treatment setup see Appendix Text 1). The control treatment consisted of light during the day (700–1100 lux) and darkness (<0.01 lux) at night while the ALAN treatment consisted of light during the day (700–1100 lux) and dim light at night (8–14 lux). We chose this range of ALAN because it is has been recorded in wetlands where these species live (Dananay and Benard, 2018) and it has been reported to elicit physiological and ecological effects on amphibians (Cope et al., 2020; Dananay and Benard, 2018; Feuka et al., 2017; Sanders et al., 2021). The laboratory was set to room temperature (20 °C) and we monitored temperature daily using digital thermometers (ThermoPro TP50, Atlanta, USA) on each shelf (Appendix Table 1). All species started the experiment as eggs (GS 2) and were exposed to the light treatments until one week after hatching at GS 25 (7 April – 12 days for wood frogs, 17 April – 15 days for spring peepers, and 19 May – 13 days for toads). Species were exposed for different durations in order to allow all species to reach GS 25, a safe handling stage. During the rearing period, once each species reached the tadpole stage, we fed them slurried Tetramin *ad libitum* every three days. We conducted a water change two days before photographing took place.

Part 2: Photographs and Analysis– Immediately after exposure to light treatments, we photographed the dorsum of each tadpole under standardized conditions (Appendix Fig. 1) using a Nikon D3500 camera with a DX Micro NIKKOR 40 mm lens (Nikon, Inc., Melville, NY, USA). Following photographing, we recorded each tadpole's stage using a dissecting scope (Olympus SZ61, Waltham, MA, USA) (Appendix Table 2). We did not measure pigmentation in newts because tadpole prey rely on olfactory, rather than visual, cues to detect aquatic predators (Babbitt and Tanner, 1998; Chivers and Smith, 1998; Christensen, 1996; Kats and Dill, 1998; Schoeppner and Relyea, 2009; Urban, 2007).

We analyzed photographs using ImageJ software version 1.53a (ImageJ, Bethesda, MD, USA) with the MicaToolbox plugin (Troscianko and Stevens, 2015). Linear color images were generated from each RAW image file. We used the polygon tool to trace the darkest part of the dorsum, taking care to exclude the eyes, tail, and any glare spots (Appendix – Fig. 2). We then measured percent reflectance under the three preset color filters and averaged their value to get the mean percent reflectance for each tadpole. The higher the percent reflectance, the lighter the pigmentation, while the lower the percent reflectance, the darker the pigmentation (Garcia and Sih, 2003). Included in our measurements was the standard deviation of the percent reflectance. This value was obtained during each of the three measurements and then averaged to yield an average standard deviation of percent reflectance. This value indicates the variation in a tadpole's pigmentation (i.e. higher standard deviation, more variation in pigmentation).

2.1.4. Experiment 2: predation assays

Part 1: Rearing Conditions– In Experiment 2, we reared newts and all three tadpole species in the same light treatments as those in Experiment 1 (Control *vs.* ALAN). Animals were housed on six shelves (three control, three ALAN) which followed the same setup as described for Experiment 1. Amphibians were group housed by species in 17-L bins filled with 8 L of filtered well water (tadpoles: 10 bins/species; newts: 14 bins). Twenty-five tadpoles of the same species were housed in each tadpole bin, while three newts were housed in each predator bin. Two 30-cm strands of *Elodea* sp. were added to each newt bin to provide a habitat for the newts to hide and perch. Prior to starting the rearing period, all newts were weighed to confirm that the mass of predators in control treatments did not differ from those in ALAN treatments (Appendix Table 4). Once tadpoles reached Gosner stage 25 (Gosner, 1960), we fed them slurried Tetramin *ad libitum*. Each newt was fed three non-experimental tadpoles every other day. We held tadpoles and newts in indoor experimental rearing conditions for two weeks and 10 days respectively.

Part 2: Predation Assays – Experiment 2a: Effect of ALAN on predator and prey mass: After exposure to control or ALAN rearing environments (Part 1), we measured the mass of each newt and a subset of wood frogs and American toads to determine if size differed between the two treatments. There were not enough spring peepers to sample a subset of those exposed to the rearing conditions, so we do not have data on spring peeper mass. The subset of tadpoles that we collected mass data from were not used in the predation assays. To measure mass, we used a digital scale (HRB103 scientific; 0.001 g/1 mg sensitivity). After recording mass, animals were moved to Binghamton University's Ecological Research Facility (ERF) to acclimate to outdoor conditions for 12 h for use in Experiment 2b and 2c. Tadpoles were allowed to acclimate in 100 L blue pools at a density of five tadpoles/pool, and newts were group-housed in separate 100 L pools. All animals were held separately by light condition experience until the start of the predation assay.

Experiment 2b: Does prior experience with ALAN impact predation rates in ALAN-free environments? We conducted three separate 2 (prey experience: Control *vs.* ALAN) X 2 (predator experience: Control *vs.* ALAN) fully factorial predation assays: [1] wood frog + newt, [2] spring peeper + newt, [3] American toad + newt. For each of these assays, we replicated the four treatments five times for a total of 20 experimental units. Experimental units were 100 L outdoor pools at Binghamton University's Ecological Research Facility (ERF). Each pool consisted of 40 L filtered well water and five oak leaves (*Quercus* sp.) that could be used as a refuge for tadpoles. Pools were covered by shade cloths to prevent unintentional entry of other predators and to control light conditions. Each pool contained one newt predator and started with five tadpoles.

Experiment 2c: Does prior experience with ALAN impact predation rates in ALAN-polluted environments? For this experiment, we replicated the design used for Experiment 2b. Experimental units were identical to Experiment 2b except pools were exposed to ALAN (n = 20) by placing three disc-shaped LED lights on top of the shade cloth (~20 lux; Appendix Text 1). Lights were on from 7:30 p.m.–7:30 a.m. throughout all assays, roughly aligning with sunset and sunrise respectively.

For both ALAN and ALAN-free conditions, all assays began shortly before sunset (7:30 p.m.) at which time the free-ranging predators were added to their respective tadpole pools. Throughout the assays, we periodically counted the number of tadpoles remaining in each pool. We used a red headlamp for nighttime observations since we have previously found that red light has no effect on amphibian behavior (Shidemantle et al., 2019). For wood frogs, checks occurred every 2 h until hour twelve, every 4 h until hour twenty-four, and finally every 12 h until hour thirty-six. For spring peepers, checks occurred every 2 h until hour six, then every 4 h until hour thirty-eight, and then every 12 h until hour ninety-six. Checks were less frequent and the assay was longer for the spring peepers because they are relatively inactive and therefore had longer predation rates (Lawler, 1989; Skelly, 1997). In the American toad assay, checks occurred every hour until hour four, then every 2 h until hour eight, then every 4 h until hour twenty.

At the end of each assay, newts and any remaining tadpoles were removed from the experimental pools. Remaining tadpoles were euthanized in an overdose of MS-222 and preserved in 10% formalin. The mass of these tadpoles was later measured as described above. Predators were returned to their respective collecting sites.

2.2. Statistical analysis

2.2.1. Experiment 1: tadpole pigmentation analysis

To determine if ALAN impacted pigmentation in three amphibian species, we performed a linear mixed effects model using the R package nlme (Pinheiro et al., 2013). The rank transformed average percent reflectance was used as the response variable (untransformed data was not normally distributed), while species, treatment, and species*treatment were used as the independent variables. The shelf that each tadpole was reared on was used as a random factor. We ran a Tukey test for any significant main effects. We verified that the transformed reflectance data met the homogeneity of variance assumption for ANOVAs by running a Levene's Test using the R package car (Fox and Weisberg, 2019).

To determine if ALAN impacted the variation in pigmentation, we performed a linear mixed effects model following the same protocol as above except that the average standard deviation of percent reflectance was used as the response variable. This data was normalized using a log transformation and met the assumption of homogeneity of variance. As with the average percent reflectance analysis, we ran a Tukey test to identify significant pairwise comparisons.

2.2.2. Experiment 2: predation assay analysis

Experiment 2a: Effect of ALAN on predator and prey mass: To determine how light conditions influenced the mass of newts, we ran separate linear mixed effects models for each group of newts using the R package nlme. We used the average mass of the three newts in a single bin as the response variable, treatment (ALAN or control) as the independent variable, and bin ID as the random factor to control for rearing location. For newts used in wood frog and American toad assays, the mass data met the normality and homogeneity of variance assumptions for parametric testing. For the newts used in spring peeper assays, the mass data was not normally distributed, so we performed a Tukey transformation (R package rcompanion; Mangiafico, 2016) which normalized the data. The Tukey-transformed mass data met the homogeneity of variance assumption for parametric testing.

To determine how light conditions impacted the mass of wood frogs and American toads before and after the predation assay, we performed linear models (lm) or generalized linear models (glm) for each assay depending on whether parametric assumptions had been met (R base package). For wood frogs, the mass data did not meet the homogeneity of variance assumption, so we ran a glm using rearing conditions (ALAN or control), assay (before assay *vs.* after assay), and rearing conditions*assay as fixed factors. For the toads used in the newt + toad assay, we removed an outlier (mass = 0.0113 g) that was more than three standard deviations from the mean mass of all toads (M = 0.0515 g, SD = 0.0110). We performed a lm with this data using the same fixed factors as the wood frog analysis. When necessary, we performed Tukey tests for pairwise comparisons.

Experiment 2b and 2c: Does prior experience with ALAN impact predation rates in (2b) ALAN-free and (2c) ALAN environments? To identify differences in predation rate among treatments, we conducted Cox mixed effects models using the R package coxme (Therneau, 2012). Each set of models was split by the pool condition so that predation rates were only compared among pools exposed to ALAN-free conditions (Experiment 2b) and then only compared among pools exposed to ALAN (Experiment 2c). We conducted separate models using a Bonferroni correction to adjust p-values for multiple comparisons. For all species, the data met the proportional hazards assumption of Cox mixed effects models. For all models, the pool replicate number was included as a random factor. Since each tadpole species differs in life history, the assays were performed at different times in outdoor pools, thus we were unable to control for factors such as temperature and natural light regime. Therefore, we did not make interspecific comparisons, and each assay was analyzed separately. All analyses were performed and all graphs (R package ggplot 2) were made using RStudio Version 1.4.1717.

The significance threshold was set at $p < 0.05$ for all statistical analyses.

3. Results

3.1. Experiment 1: tadpole pigmentation

We found no significant effect of treatment ($F_{1,79} = 0.858$; $P = 0.357$) on average percent reflectance. However, there was a significant effect of species (Fig. 1; $F_{2,79} = 47.5$; $P < 0.001$) and the interaction between species and treatment ($F_{2,79} = 6.49$; $P = 0.003$). Post hoc comparisons reveal that wood frogs raised in both ALAN and control conditions had significantly lighter pigmentation than spring peepers and American toads reared in both ALAN and control conditions (Table 1). We also found that spring peepers reared in control conditions were more lightly pigmented than those reared in ALAN ($P = 0.014$), while wood frogs and American toads did not differ by treatment ($P = 1.00$; $P = 0.724$ respectively).

For variation in tadpole pigmentation, we found neither significant effect of treatment ($F_{1,79} = 1.86$; $P = 0.175$) nor the interaction between treatment and species ($F_{2,79} = 2.19$; $P = 0.119$). However, we did find a significant effect of species (Fig. 2; $F_{2,79} = 148$; $P < 0.001$). Pairwise comparisons reveal that all species differed significantly from one another ($P < 0.001$ for all comparisons) with spring peepers having the highest variation in pigmentation and American toads having the lowest variation in pigmentation.

3.2. Experiment 2: predation assays

Experiment 2a: Effect of ALAN on predator and prey mass*:* The mass of newts reared in control or ALAN treatments did not differ for newts used in the spring peeper assay ($F_{1,12} = 0.274$; $P = 0.610$) or the American toad assay ($F_{1,12} = 0.395$; $P = 0.541$). However, for newts used in the wood frog assay, newts reared in ALAN were significantly larger than newts reared in control conditions ($F_{1,12} = 5.80$; $P = 0.033$).

For wood frogs, there was a significant effect of treatment ($t = 3.22$;

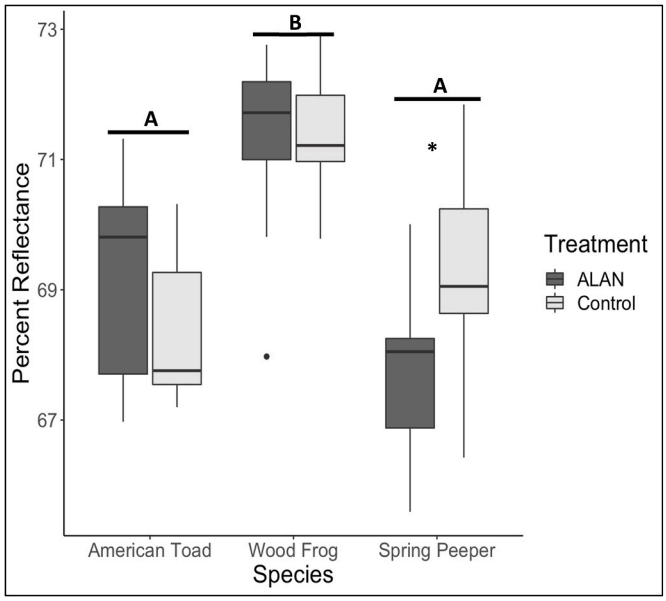

Fig. 1. Percent reflectance of tadpoles reared in ALAN and control conditions according to species (n = 15 tadpoles/treatment/species). Higher percent reflectance values indicate lighter pigmentation, while lower percent reflectance values indicate darker pigmentation. Species with different letters are significantly different from one another. Asterisks demonstrate a difference between ALAN and control treatments. For each box plot, the box represents the interquartile range (IQR), the thick black line represents the median value, and the whiskers extend to 1.5 times the IQR.

Table 1
Pairwise comparisons for the significant interaction between Treatment and Species in percent reflectance. Asterisks represent significant pairwise comparisons.

Treatment:Species Comparisons	Difference	P Value
Control:Toad-ALAN:Toad	−9.067	0.724
ALAN:Wood Frog-ALAN:Toad	34.5	<0.001*
Control:Wood Frog-ALAN:Toad	33.01	<0.001*
ALAN:Peeper-ALAN:Toad	−15.1	0.206
Control:Peeper-ALAN:Toad	7.00	0.886
ALAN:Wood Frog-Control:Toad	43.6	<0.001*
Control:Wood Frog-Control:Toad	42.1	<0.001*
ALAN:Peeper-Control:Toad	−6.05	0.940
Control:Peeper-Control:Toad	16.1	0.140
Control:Wood Frog-ALAN:Wood Frog	−1.47	1
ALAN:Peeper-ALAN:Wood Frog	−49.7	<0.001
Control:Peeper-ALAN:Wood Frog	−27.5	<0.001*
ALAN:Peeper-Control:Wood Frog	−48.2	<0.001*
Control:Peeper-Control:Wood Frog	−26.1	0.002*
Control:Peeper-ALAN:Peeper	22.1	0.014*

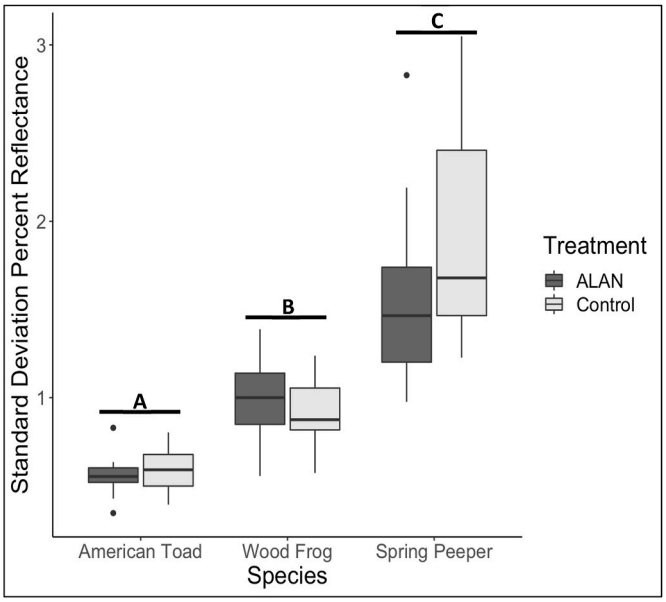

Fig. 2. Standard deviation of the percent reflectance of tadpoles reared in ALAN and control conditions according to species (n = 15 tadpoles/treatment/species). Higher standard deviation indicates higher variation in pigmentation, while lower standard deviation indicates lower variation in pigmentation. Species with different letters are significantly different from one another. For each box plot, the box represents the interquartile range (IQR), the thick black line represents the median value, and the whiskers extend to 1.5 times the IQR.

P = 0.002), assay (t = 7.78; P < 0.001), and an interaction between treatment and assay (Fig. 3; t = −3.15; P = 0.002). Pairwise comparisons show that prior to the predation assay, wood frog tadpoles reared under ALAN were significantly smaller than those reared in control conditions (P = 0.007). After the predation assay, however, the surviving tadpoles reared in control and ALAN conditions did not differ in size (P = 0.809). In the ALAN and control treatments, the average mass of the surviving tadpoles was significantly larger compared to the average mass of tadpoles prior to the start of the assay (P < 0.001; P = 0.033 respectively). Finally, tadpoles reared in ALAN were significantly smaller before the predation assay than control tadpoles after the predation assay (P < 0.001), and tadpoles reared in control conditions were significantly smaller than those reared in ALAN after the predation assay (P < 0.001).

For American toads, there was no significant effect of rearing conditions (t = −1.41; P = 0.162) or rearing conditions*assay (t = 0.815; P = 0.418). However, there was an effect of assay (Fig. 4; t = 1.98; P = 0.052) such that the average mass of toads before the predation assay was smaller than the average mass of toads after the predation assay.

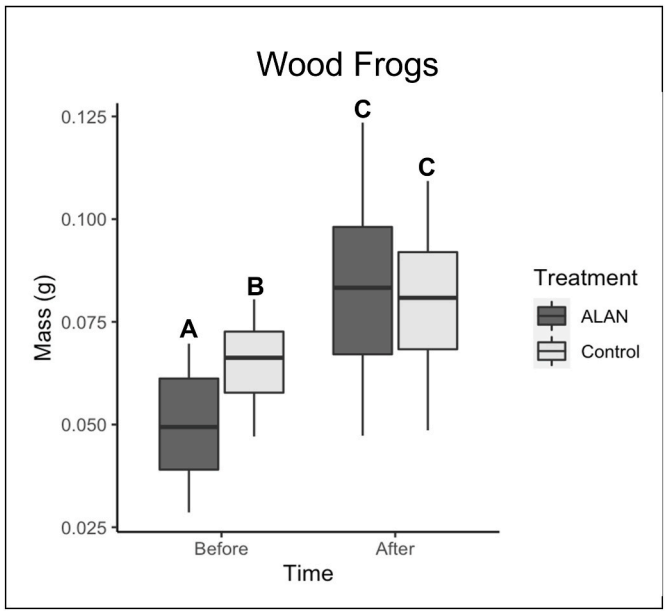

Fig. 3. Mass of wood frogs reared in ALAN and control conditions before and after the predation assay. Box plots with different letters are significantly different from one another. For each box plot, the box represents the interquartile range (IQR), the thick black line represents the median value, and the whiskers extend to 1.5 times the IQR. Sample sizes can be found in Appendix Table 3.

Experiment 2b: ALAN-free environmental conditions: For all three assays, (wood frog assay, spring peeper assay, and American toad assay) there were no significant differences between the predation rates of any of the treatments (Appendix Tables 6–8 and Appendix Fig. 3).

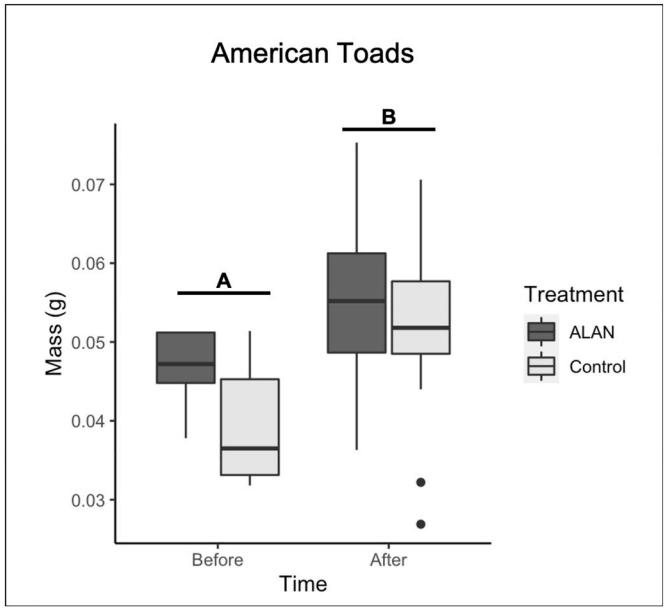

Fig. 4. Mass of American toads reared in ALAN and control conditions before and after the predation assay. Box plots with different letters are significantly different from one another. For each box plot, the box represents the interquartile range (IQR), the thick black line represents the median value, and the whiskers extend to 1.5 times the IQR. Sample sizes can be found in Appendix Table 3.

Experiment 2c: ALAN environmental conditions: For the wood frog assay and spring peeper assay, there were no significant differences between the predation rates of any of the treatments (Appendix Tables 6–8 and Appendix Fig. 4). However, in the American toad + newt assay, tadpoles and predators reared in control conditions had marginally lower predation rates than the predation rate for predator and prey reared in ALAN conditions ($z = -2.57$; adj P value = 0.06; P = 0.01).

4. Discussion

The first goal of this study was to determine if ALAN affects pigmentation in amphibians using three tadpole species as a model. Consistent with our predictions, we found that spring peepers were significantly darker when reared under ALAN conditions compared to control conditions, but ALAN did not impact the pigmentation of the other two species. This is the first evidence that exposure to ALAN impacts skin pigmentation in amphibians. Although we did not directly measure or manipulate melatonin levels in our experiments, inhibition of melatonin is a potential mechanism behind this effect based on the relationship between melatonin and pigmentation (Sugden et al., 2004). However, it is important to note that a recent study in common toads (*Bufo bufo*) (Touzot et al., 2021) found that ALAN did not affect the expression of melatonin-related genes. Future studies are necessary to determine if melatonin suppression is responsible for pigmentation differences in our study. While we found that ALAN conditions induced pigmentation changes in spring peeper tadpoles, all tadpoles were reared in black background conditions (Appendix Fig. 5a) and all predation assays occurred in pools with blue backgrounds (Appendix Fig. 5b). Thus, whether the ALAN conditions induced pigmentation changes in spring peeper tadpoles is adaptive or maladaptive is beyond the scope of our study, as we did not manipulate background color. Towards this end, future studies should consider manipulating background color to evaluate whether ALAN-induced shifts in pigmentation allow spring peeper tadpoles to blend into different background conditions or whether it makes the tadpoles more conspicuous.

Of the three anuran species tested, we only detected ALAN-induced pigmentation changes in spring peepers. Many prey species perceive a higher predation threat when exposed to ALAN (Baker and Richardson, 2006; Hall, 2016). Spring peeper tadpoles may be more sensitive to the predation threat under ALAN and in response change pigmentation under ALAN conditions. Spring peepers are relatively inactive tadpoles that spend most of their time in the benthos (Lawler, 1989; Skelly, 1997). For this reason, they may have to rely more heavily on crypsis than other species like wood frogs and American toads, which are more active (Marino, 2016). These natural history differences may explain why wood frog and American toad tadpoles did not show differences in skin pigmentation when reared under ALAN. Future studies should consider whether variation in life history at adult stages (arboreal *vs.* terrestrial) might contribute to these effects as well. Overall, we see that ALAN can impact skin brightness, but those effects are species-dependent. This reflects the importance of investigating the response of multiple species to pollutants such as ALAN before conclusions are drawn regarding the impact of that pollutant on a taxonomic group as a whole.

Along with average skin reflectance, we also considered skin pigmentation variation (i.e. how variable the pigmentation is). We found that ALAN had no impact on variation in pigmentation in any of the three species. However, we found a significant effect of species on skin pigmentation variation such that all three species differed significantly from one another. Spring peepers had the most variable pigmentation, followed by wood frogs, and then American toads. A higher level of pigmentation variation suggests that spring peepers may have more phenotypically plastic pigmentation compared to the other species. Like average reflectance, species-level differences in pigmentation variation may be due to differences in life history. Spring peepers may show a higher level of variation to blend in to a wider diversity of background conditions since they are less active and therefore may invest less energy into selecting matching backgrounds (Lawler, 1989; Skelly, 1997). This variation may not be necessary in species such as wood frogs and American toads which are more active (can move to different areas to blend in) and employ other predator avoidance strategies (i.e. bufo toxins, developmental plasticity) (Benard and Fordyce, 2003; Marino, 2016; Petranka and Hayes, 1998). Although ALAN did not contribute to differences in variation, species-level differences in pigmentation variation may have relevant consequences on crypsis, underscoring the importance of considering species life history when evaluating the ecological effects of ALAN.

We also considered the effects of ALAN on newt and tadpole size. In previous studies, ALAN increased mass in wood frog tadpoles (Shidemantle et al., 2019) and increased gut mass in adult cane toads (Komine et al., 2020). Similarly, we found that newts used in wood frog assays were significantly larger after exposure to ALAN compared to newts in the control treatment. In contrast, we did not observe size differences in newts from the spring peeper or American toad assays. This may reflect phenological differences in the effect of ALAN. Namely, newts from the wood frog assays were collected earliest to match the early wood frog breeding season. It is possible that newts are most affected by ALAN earlier in the spring when they are recovering from overwintering (Jiang and Claussen, 1992). Future studies evaluating the interaction between phenology and ALAN are necessary to more fully understand the consequences of prolonged ALAN exposure. For tadpoles, contrary to predictions, we found that wood frogs reared in ALAN were significantly smaller than those in control conditions, and we did not observe ALAN-induced differences in mass among toad tadpoles. It is likely that the direction and magnitude of impact is species- and experimental condition- (i.e. duration of exposure, light intensity, etc.) dependent (Dananay and Benard, 2018; Shidemantle et al., 2019). Future studies that standardize experimental conditions would be helpful in identifying generalizable effects of ALAN on wildlife.

We predicted that the impacts of ALAN on spring peeper tadpole pigmentation would carry over to the community level (predator-prey interactions). However, despite ALAN-induced pigmentation changes in spring peepers, predation rates were not affected in either the ALAN-free or ALAN-polluted environments. This may be for a few reasons. First, the changes in pigmentation that we observed in the spring peepers might not be enough for the newt predators to distinguish. In our pigmentation analysis we could not take into account the visual system of newt predators, so it is possible that newts may not be able to perceive differences in tadpole pigmentation. Future studies should incorporate the spectral sensitivity of newts to determine if the pigmentation change is ecologically meaningful (Wuthrich et al., 2022). Next, spring peepers are relatively inactive tadpoles that experience low predation rates compared to wood frogs and American toads (Lawler, 1989; Skelly, 1997). In general, less active tadpoles are less susceptible to predation (Skelly, 1994). Indeed, previous work in our lab demonstrates that tadpoles decrease overall activity when reared under ALAN (Shidemantle et al., 2019). It is possible that in this system, activity is more important in terms of conspicuousness than color is. Future studies should consider the relative contributions of activity level and cryptic coloration to predation rates under ALAN-polluted environments.

Overall, despite the effects of ALAN detected in the lab setting, for all three outdoor predation assays, we found limited evidence that ALAN experience influences predation rates. Throughout existing literature, the effect of ALAN on predator-prey interactions is equivocal. While many studies have found that ALAN increases predation rates (Bailey et al., 2019; Manfrin et al., 2018, 2017; McMunn et al., 2019; Miller et al., 2017; Minnaar et al., 2015; Owens et al., 2020; Sanders et al., 2021; Santos et al., 2010), others have found no effect (Cope et al., 2020; Grenis et al., 2015). One potential explanation for these discrepancies may be that different studies utilize predators that differ in predation strategies (e.g. active *vs.* sit and wait). A pilot study in our lab using a sit-and-wait tadpole predator (dragonfly nymph – *Anax* sp.) found no

effect of ALAN on predation (Appendix Table 10), but additional studies investigating predators with different predation strategies and prey with different predator avoidance strategies are warranted. It is also possible that ALAN may affect net predation rates in the newt-tadpole system, but we were unable to detect the effects using our experimental design. Notably, other studies show that predation rates are often higher under ALAN due to increased prey and predator abundances (Manfrin et al., 2017; McMunn et al., 2019; Owens et al., 2020; Sanders et al., 2021). In our predation assays, we controlled for both prey ($n = 5$) and predator ($n = 1$) abundance. In more realistic pond settings, ALAN could alter the abundance of both prey and predators, resulting in changes to predation rates. Future studies should consider differences in abundance in ponds exposed to ALAN and those under controlled lighting conditions to get a more holistic understanding of how predator-prey dynamics are impacted in this system.

Interestingly, while we found an effect of ALAN on average wood frog tadpole mass prior to the predation assay, this effect was eliminated after the predation assay, likely due to selective foraging by predators on smaller prey. Indeed, consistent with past studies (Barnes et al., 2010; Christensen, 1996; Nakazawa et al., 2011), we found that tadpoles (both wood frogs and American toads) that survived the predation assay were on average larger than the average size of tadpoles that entered into the predation assay. In this way, by consuming the smaller tadpoles, newt predators dampened the effect of ALAN on wood frog size. In amphibians, larger individuals are considered to be more fit (Berven, 1990; Cabrera-Guzmán et al., 2013; Semlitsch et al., 1988). The results observed in our study reflect how predators can be beneficial to their prey population as a whole by serving as a powerful selective pressure for higher fitness (e.g., larger size). Without experiments that examine effects of ALAN at multiple levels of organization, this effect would not be detected. Collectively, these results also underscore the importance of integrating more realistic ecological conditions (i.e. size-selective predation) in effort to understand the net impact of ALAN on wildlife fitness.

5. Conclusions

By using three species of larval amphibians, each with distinct behaviors and life histories, we were able to identify morphological and ecological effects of ALAN. Our study is the first to show evidence of ALAN impacting the pigmentation of larval amphibians. Although this result was not consistent across all three species, it emphasizes the need to consider a variety of species in ALAN research. This further underscores the need to have a foundational understanding of species life history which will be critical to shaping the way we design studies regarding the impacts of ALAN and other global changes. Next, we found that despite impacts of ALAN on both prey and predator size, previous exposure to ALAN had no impact on predation rates in both ALAN-free and ALAN-polluted environments for all three tadpole species. However, size-selective predation left only the largest tadpoles remaining in the population. This effect eliminated prey size differences due to ALAN, suggesting that predators can mask the effects of ALAN on prey. This finding emphasizes the need for studies that examine both individual-level effects and community-level effects of pollutants since individual-level effects of pollutants like ALAN may only be detected when considering community-level interactions.

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Credit author statement

Grascen Shidemantle: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Project administration, Funding acquisition, Jurnee Blackwood: Investigation, Methodology, Kelsey Horn: Investigation, Methodology, Isabela Velasquez: Investigation, Emily Ronan: Investigation, Beth Reinke: Methodology, Writing – review & editing, Jessica Hua: Resources, Supervision, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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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.2022.119604.

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