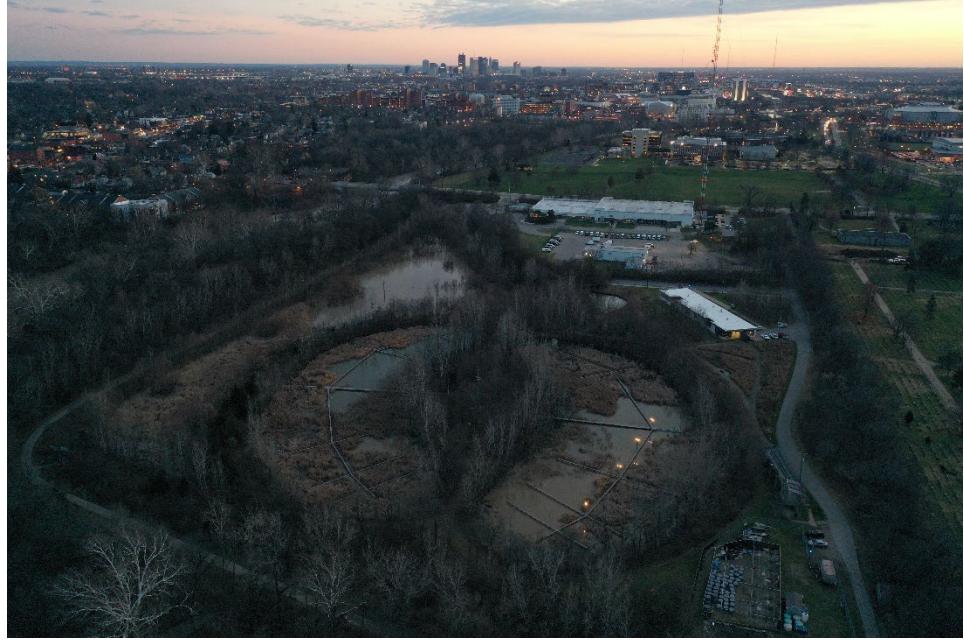# Ecological Design Rules for Roadway Lighting

Artificial lighting at night in Columbus, Ohio, including an experimental lighting set-up at the Olentangy River Wetland Research Park at The Ohio State University.

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16. Abstract
Artificial lighting at night alters natural patterns of light and dark in ecosystems and includes chronic or periodic illumination, unexpected changes in lighting, and direct glare that can have serious implications for ecological systems, such that it is increasingly referred to as "ecological light pollution". ALAN occurs at an extent and magnitude so great that it can be regarded as a significant perturbation to the Earth system and a threat to biodiversity and ecosystem function. However, current knowledge relative to the ecological impacts of ALAN is extremely limited, but critical in order to design appropriate best management practices (BMPs) for ecologically sensitive areas, such as riparian areas along roads and bridges over waterways. This is especially critical for Ohio, a highly populated state with expansive roadway infrastructure (e.g., 262,350 total lane miles). To address this challenge, we conducted a comprehensive literature search on the ecological impacts of ALAN and roadway lighting, coupled with field studies aimed at quantifying the effects of roadway lighting on wildlife and sensitive aquatic and riparian ecosystems. Our research was conducted in the Columbus Metropolitan Area (CMA), Ohio. From this information, we developed a series of recommendations to be used to guide roadway lighting designs that minimize ecological impacts from ALAN.

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We thank the valuable input from the members of the ODOT TAC including Kevin Duemmel, Jeremy Cessna, Jason Lutz, and Kathleen Dunlap.

1. Problem Statement

The footprint of human activity on Earth is distinctly visible from space in the form of artificial lighting at night (ALAN; 1, 2). ALAN (e.g., urban lighting, roadway lights, security lighting, and other light sources) has dramatically increased over recent decades (3, 4) and is expected to grow as the urban population of the planet increases from 50 to 70% by 2050 (5). ALAN alters natural patterns of light and dark in ecosystems and includes chronic or periodic illumination, unexpected changes in lighting, and direct glare that can have serious implications for ecological systems, such that it is increasingly referred to as "ecological light pollution" (6). In fact, ALAN occurs at an extent and magnitude so great that it can be regarded as a significant perturbation to the Earth system and a threat to biodiversity and ecosystem function on a global scale (4).

The impact of ALAN ranges across spatial scales and ecosystem types (4, 6). A classic example comes from marine systems, where lighting along beaches can lead to the disorientation of hatchling sea turtles (7). Recent research has explored the consequences of ALAN for higher levels of ecological organization. For example, Becker et al. (8) showed how ALAN alters fish communities within urban estuarine ecosystems, implicating unnatural top-down regulation of fish populations. Similarly, Davies et al. (9) found increased predators and scavengers in ground-dwelling invertebrate communities under street lights.

Here, we focus on riparian ecosystems (e.g., rivers and their adjacent riparian zones, wetlands) because they should be especially vulnerable to ALAN. In particular, riparian systems are disproportionately exposed to ALAN given that many cities and roadways developed along waterways. In addition to containing high biodiversity in general, riparian areas also support specialized species that may be more sensitive to ALAN. For example, aquatic invertebrates that form the food base of many aquatic-riparian ecosystems are known to use light as cues for reproduction and orientation (10, 11). Indeed, elevated levels of ALAN in urban streams were correlated with reduced diversity, body size, and biomass of larval aquatic insects emerging from the stream as adults (hereafter 'emergent aquatic insects') (12). Moreover, riparian zones tend to support high diversity and abundance of aerial insectivorous birds, which forage heavily on light-sensitive emergent aquatic insects (13, 14), yet are experiencing widespread population declines (reviewed in 15). Nocturnal organisms such as bats that forage on flying insects in illuminated riparian areas might also be expected to be adversely affected by light pollution (16).

Impacts to trophic and ecosystem-level processes remain poorly resolved, yet the potential for ALAN to cascade through food webs and alter ecosystem functions is significant. For instance, in terrestrial ecosystems, Lewanzik and Voigt (17) showed that ALAN reduces the seed-dispersing ability of fruit-eating tropical bats, with negative effects on forest biodiversity. ALAN can also alter nocturnal pollination networks critical to plant reproduction (18). Increased lighting also strongly mediates rates of primary productivity (i.e., of plants) in aquatic and riparian ecosystems and can shift total biomass and population size of insects with implications up the food chain (19). Thus, food-chain length (FCL) – a critical measure of food-web architecture that regulates energy flow through ecosystems, nutrient cycling, freshwater-atmospheric carbon exchange, and bioaccumulation of contaminants in humans via consumption of top predators (e.g., fish) (20 and references therein) – could be strongly affected by ALAN.

In addition to the presence of artificial lighting, the impacts of ALAN on organisms and ecosystems are expected to be dependent on the spectral characteristics of light (i.e., the relative abundance of different colors of light). Although many lighting types are common on the market, light-emitting diode (LED) lamps are quickly becoming the most common illumination on roadways across Ohio, the nation, and the world as they can provide high energy cost savings, reduced maintenance, and increased control (21). However, current knowledge relative to the ecological impacts of LEDs is extremely limited, but critical in order to design appropriate best management practices (BMPs) for ecologically sensitive areas, such as riparian areas along roads and bridges over waterways.

The potential impact of ALAN on sensitive species and ecosystems in is especially critical for Ohio, a highly populated state with expansive roadway infrastructure, covering 262,350 total lane miles. Thus, the need to further understand the potential ecological impacts of Ohio roadway lighting is critical in developing best-management practices for environmental conservation in Ohio.

2. Research Background

The overarching goals for this project were to (1) couple an in-depth, large-scale investigation of the potential impacts of ALAN on sensitive aquatic and riparian habitats of the Columbus Metropolitan Area, Ohio, with a comprehensive literature search, and (2) use this information to help establish BMPs that can be used by ODOT and other decision makers to address lighting concerns in ecologically sensitive areas near roadways. To meet the overarching goals, we addressed the following specific objectives:

1. *Conduct a thorough literature review on the ecological impacts of roadway lighting on wildlife and ecosystems.* Review existing literature on (1) the ecological effects of street/roadway ALAN on wildlife and ecosystem functions, and (2) existing lighting BMPs from other entities for use in ecologically sensitive urban and rural roadways.

2. *Field and mesocosm studies on impacts of LED luminaires on wildlife and ecosystem functions in aquatic and riparian areas in the CMA.* Conduct a series of field studies to determine the impacts of LED luminaires on a suite of organisms (insects, fish, and wildlife) in sensitive ecosystems including (a) stream-riparian ecosystems, (b) bridges crossing rivers/reservoirs, and (c) urban wetlands. Study sites in the CMA included a range of urban, suburban, and peri-urban settings, thus representing a broad range of watershed development. However, we were not able to include sensitive rural areas, as originally proposed, as we were unable to locate a sufficient number of suitable study sites that met the following criteria: rural, aquatic-riparian, adjacent to roadway lighting. As proposed, we investigated responses of a suite of organisms, including aquatic insects and fish. On the wildlife side, we decided to investigate responses of a suite of riparian mammal communities instead of riparian bird communities, as we anticipated that nocturnal mammals may be a better indicator of ALAN effects vs. diurnal birds. We also implemented a suite of controlled mesocosm studies to help identify potential mechanisms driving responses of aquatic insects and fish to ALAN.

3. *Synthesize research findings and compile data to support BMP recommendations.* Present recommended best-management practices as a table that highlights strategies to minimize ecological effects of ALAN.

3. Research Approach

Our approach consisted of a comprehensive literature review combined with a series of experimental and observational field and mesocosm studies in the CMA (Table 1). Any changes or deviations that were necessary – particularly relative to delays and changes due to the COVID-19 pandemic – were addressed through regular communications with the ODOT Technical Advisory Committee (TAC).

3.1 Literature Review. We performed a comprehensive literature review on the ecological impacts of ALAN, including LEDs, with a focus on roadway applications. We used a keyword search on Scopus, Web of Science, and Google Scholar that used the following inclusive key term search string: "artificial light* at night" OR "ecological light pollution" OR "light pollution" OR "urban light*" OR "night* light*." When possible, searches were restricted by field to exclude publications that focus solely on astronomical, engineering, and public health fields. We found 3,370 articles on Google Scholar, 1113 articles on Web of Science, and 1,331 articles on Scopus. Given that Google Scholar was more comprehensive, we continued only with the Google Scholar results. Each article was investigated individually to determine relevance in that it investigated an effect of artificial light at night on a biological response variable. In total, 293 papers articles were selected that ranged in biological topics from ALAN effects on animal behavior to ecosystem function (See Appendix: Literature Review Bibliography).

3.2 Study Site Selection. In selecting field sites, numerous factors were taken into consideration: characteristics of the roadway lighting environment, characteristics of the riparian area, physical access, likelihood of obtaining permission to access the site, and other potential constraints. Site selection began with a broad set of potential study sites, which was narrowed down based on the above factors, consultation with the ODOT TAC, knowledge of the study system from previous studies (22-24), and synoptic surveys of ALAN intrusion into focal ecosystems. The CMA covers 8,208 km^2 in central Ohio with a population of 2.4 million people. The CMA is in the Scioto River Watershed and is characterized by the Scioto River flowing through it. The Scioto River is a 6th-order tributary of the Ohio River that drains a 16,882-km^2 basin throughout Ohio (24). The upper section of the basin where the Scioto River flows through Columbus is mostly row-crop agriculture north of the city and suburban/urban land use moving toward the city (25). Field studies were complemented by a suite of mesocosm studies (i.e., controlled experiments in large outdoor tanks) designed to test mechanisms by which ALAN may affect insects and fish.

Table 1. Ecosystem types (including experimental mesocosms) represented in research approach, overview of experimental design, lighting characteristics, and taxonomic focus of investigation for field and mesocosm study components. Mesocosms are outdoor experimental tank systems (located at OSU's Olentangy River Wetlands Research Park (ORWRP)) used to test effects of ALAN under controlled conditions. LED = light-emitting diode. HPS = high-pressure sodium.

Ecosystems	Experimental Design	Lighting Intensity and Spectral Composition	Focus of Investigation
Stream-Riparian	10 paired lit-unlit 1st-3rd order stream-riparian sites + 2 additional sites (i.e., two paired unlit-unlit) - one within the CMA and one on TNC land ~70 km northwest of Columbus to avoid urban impacts – served as reference sites (Fig. 1). Unlit reaches were always located upstream from lit reaches to prevent downstream flow impacts from lit reaches being recorded at unlit reaches.	0.1-2.5 lux; mixture of HPS and LED.	Benthic Aquatic Insect, Fish, Riparian Mammal Communities
River-Reservoir	4 artificially lit bridge sites and 2 unlit control bridge sites on Scioto River between O'Shaughnessy Reservoir (north of Dublin, OH) and downtown Columbus (Fig. 2).	Urban: lit reach ~0.5-2.0 lux, unlit reach ~ 0.22 lux; Peri-urban: lit reach ~0.5-3.0 lux, unlit reach ~ 0.01 lux	Fish Communities
Wetland	2 experimental wetlands at OSU's ORWRP. At each, luminaires were introduced creating dark and lit sections (Fig. 3).	<0.1 – 20 lux from LED and HPS luminaires.	Emergent Aquatic Insect, Bat Communities
Mesocosm	Eight pairs of small mesocosms, each pair consisting of a lit	Lit treatment from LED luminaires: 13.46 ± 0.65 lux (mean ±	Mosquito Reproduction

	mesocosm and a dark control.	standard error); Control: 0.24 ± 0.01 lux.	
Mesocosm	20 tanks set up to test effects of light intensity and constancy on fish foraging efficiency.	Ambient, low light (1 lux), medium light (4 lux), high light (12 lux), intermittent high light (12 lux, 1 s on/off intervals); all lights were LED.	Fish (Bluegill) - Predation
Mesocosm	24 tanks set up to test effects of light intensity and turbidity levels on fish predation (Figure 4).	Ambient, low (0.5 lux), medium (3 lux), and high (10 lux). All lights were LED.	Fish (Bass) - Predation
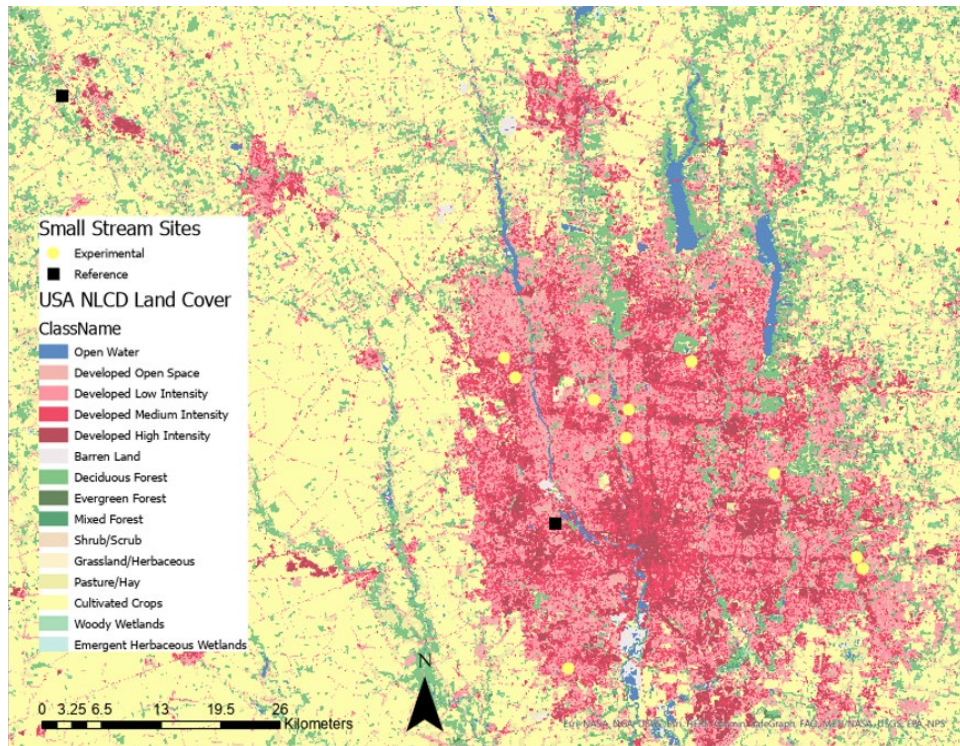
Figure 1. Map of steam-riparian sites with paired lit-unlit reaches (yellow dots) and reference sites with paired unlit-unlit reaches (black squares) over 2021 NLCD Land Cover data.
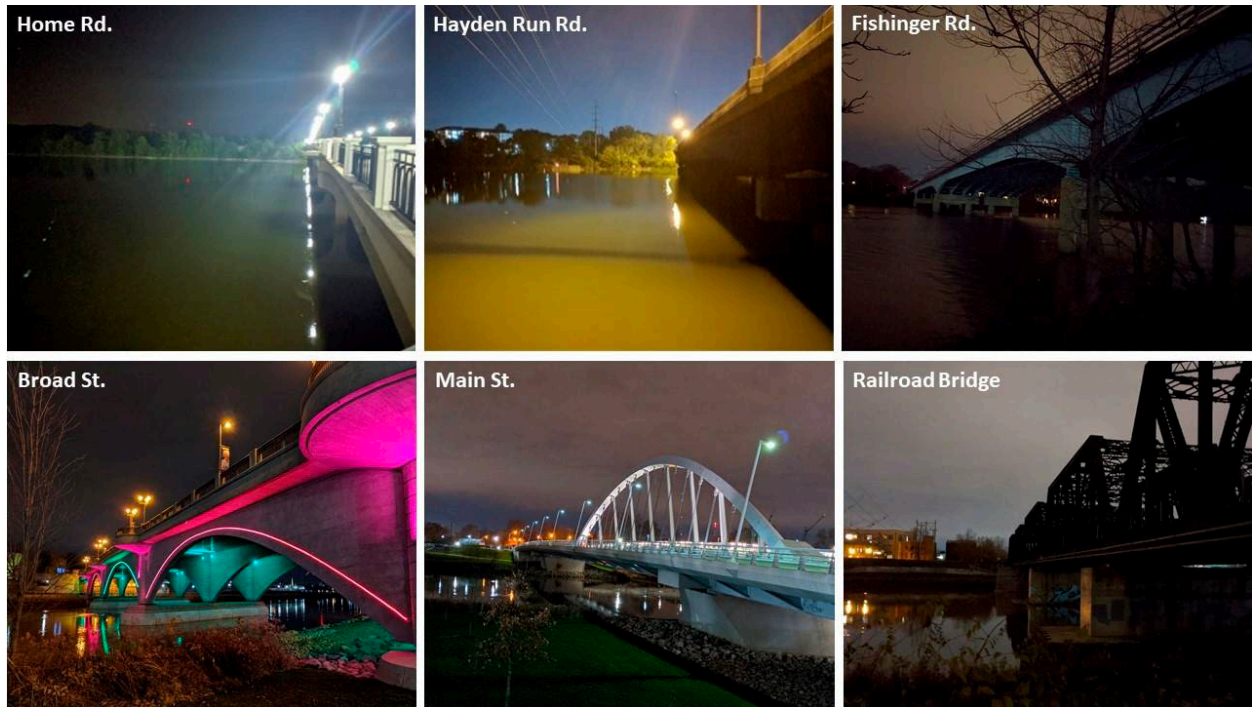
Figure 2. Home Rd. (top left), Hayden Run Rd. (top middle), and Fishinger Rd. (top right) cross broad, heavily impounded reservoirs in suburban areas and were considered peri-urban sites for this study. Broad St. (bottom left), Main St. (bottom middle), and the railroad bridge (bottom right) cross narrower portions of the river in downtown Columbus and were considered urban sites (photo credit: Susanna Harrison).

Figure 3. One of experimental wetlands at the ORWRP showing lit area/treatment in the foreground and unlit treatment in the background.

Figure 4. Mesocosm experiment at the ORWRP testing for potential effects of ALAN on fish predation (photo credit: Susanna Harrison).

3.3 Environmental Characteristics. To characterize both the aquatic and terrestrial light environments (day and night, above and below the water and in the riparian zone), simultaneous absolute irradiance (photons cm^{-2} nm^{-1} s^{-1}) and luminance (lux) measurements were made. For stream-riparian study sites (Fig. 5), light illuminance (lux) was measured on three transects (0m, 15m, 30m), two positions (left-middle, right-middle), and three heights (1cm below water surface, 1cm above surface, 1m above surface) using an ILR 1700 Radiometer with a waterproof illuminance (lx) sensor (SUD033/Y/W; International Light Technologies, Peabody, Massachusetts). Spectral composition of primary light sources was recorded using an Ocean Optics® Flame UV-VIS Spectrometer (Ocean Insight, Orlando, Florida). Light sources at each site were categorized as either high-pressure sodium (HPS) or light-emitting diode (LED) based on spectral distribution. Illumination and spectra were measured at each reach during Summer 2019, Autumn 2019, Winter 2020, Summer 2020, Autumn 2020, and Winter 2021 at least two hours after sunset on nights without cloud cover. For reservoir sites, lux levels were measured using an ILT® 1700 Research Radiometer along the same transects used for fish sampling, with three sub-transects (left bank, mid-channel, and right bank) sampled per transect. At each sub-transect, measurements were taken at the surface of the water, just below the surface, and 100 cm below the surface (or the nearest maximum depth in shallow areas). For wetlands, four pairs of area luminaires were positioned in each of two wetland cells such that each wetland cell was divided into a "lit" and "unlit" area. Luminaires were 1593 lumen 3000K LED (LEDALUX, Providence, RI, USA). Lux was measured at three positions (W, central, E) across eight transects in each wetland using the same methods as in the stream and reservoir studies above. Lit areas averaged 7.00 ± 1.02 lux (mean ± SE) while unlit areas averaged 0.03 ± 0.004 lux. Lit areas also had variance in light coverage created by distance from luminaire that replicates real field conditions (i.e., light coverage was not perfectly uniform).
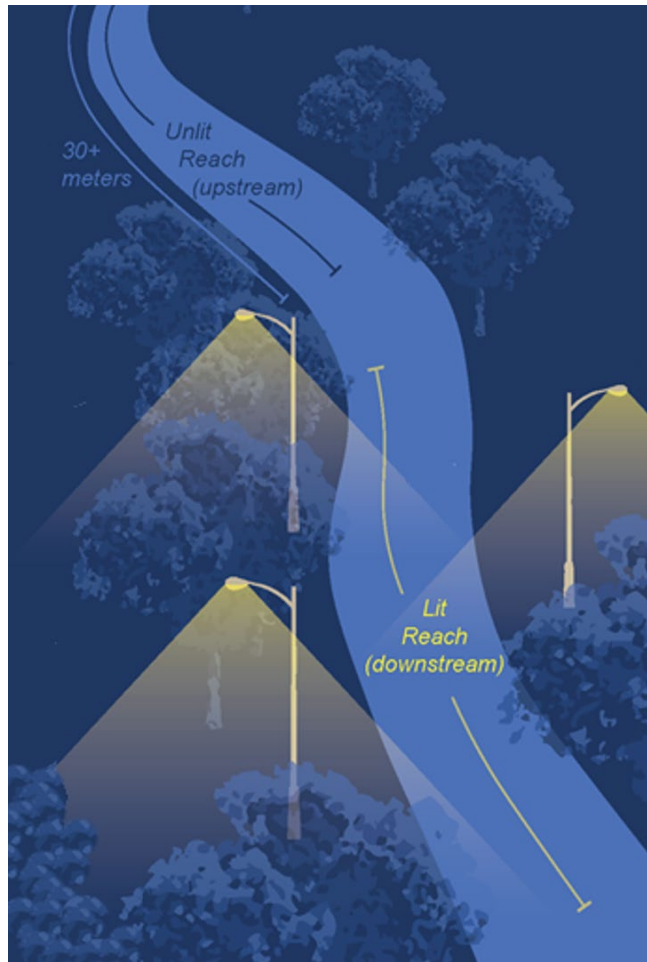
Figure 5. Schematic of study-design for paired unlit-lit stream-riparian research sites.

To account for potential differences in water quality that might contribute to differences in biotic communities (i.e., beyond differences in lighting), water samples were measured for total nitrogen (TN; mg N/L), total phosphorus (TP; mg P/L), nitrate (mg NO_3-N/L), ammonia (mg NH_3-N/L), and orthophosphate (mg PO_4-P/L) at the Ohio Agricultural Research and Development Center Service Testing and Research Lab (STAR Lab), Wooster, Ohio. Additionally, continuous water-chemistry and flow measurements were recorded by deploying data probes in one representative urban, rural, and reference streams (n = 3 sensors) for the duration of the study.

At stream-riparian study sites, canopy cover was measured from canopy photographs photographed with a fish-eye lens camera during sampling periods in 2019, 2020, and 2021. Impervious surface distance (the shortest distance from the stream to an impervious surface) was measured four times at 10-meter increments along each reach using the "Measure" function in ArcGIS Pro (ESRI 2019) to quantify urbanization of each reach. Following Wolman (1954), sediment size was measured using a gravelometer at the top, middle, and bottom of each reach by completing pebble counts with a minimum of 75 pebbles to estimate the median sediment size (D_{50}) during Spring 2021.

3.4 Insects. Benthic macroinvertebrate (i.e., larval aquatic insects) samples were collected via subsamples at six regularly spaced intervals along the length of the stream reach using a 500-µm mesh Surber sampler and kick net following standard methods (Barbour et al. 1999). In order to collect a representative sample of all habitat types in the stream channel, the position of the kick net at each subsample location will be randomly assigned to mid-channel, towards the left bank or towards the

right bank. All samples were preserved in 70% ethanol. Emergent aquatic insect traps were our primary method for sampling adult insects. At each stream study reach, we deployed 3-5 floating emergence traps (1m^2, pyramid-shaped, covered with fine mesh screen). Traps were deployed for two, 2-week periods each sampling season (last week of May/1st week of June, 2nd/3rd week of July). In the wetlands, emergent traps were deployed and sampled weekly from June-September in 2021 and 2022. In the laboratory, aquatic insects were identified to the lowest practical taxonomic level.

Mesocosm Experiment. The layout of the experiment consisted of 8 blocks, each with two mesocosms and a light source. Blocks were arranged into two rows of four and each block was ≥ 3 m from its nearest neighboring block. Within each block, the three components were laid out in a 2 m equilateral triangle such that the luminaires were positioned interiorly and 2 m from each mesocosm, which, in turn, were positioned 2 m from each other. Mesocosms were either controls (i.e., dark/skyglow) or exposed to a 3000K LED luminaire (750 lumens, Energetic Lighting, Chino, California). At 2 m from the luminaire, each lit mesocosm received 13.46 ± 0.65 lux [mean ± standard error; equivalent to 1.25 ± 0.06 foot candles (fc)], while dark mesocosms received only skyglow (0.24 ± 0.01 lux; equivalent to 0.02 ± 0.0009 fc). These levels of lux have been found to be ecologically relevant in Columbus, Ohio (Meyer and Sullivan 2013, Sullivan et al. 2019). The luminaires were shielded with opaque black plastic material to direct light only to the treatment mesocosm in each block.

The mesocosms were 71×51×15 cm (length, width, depth) and held 37.9 L of water. Each of the pools received 100 g of leaf litter as a nutrient base and two filter papers in adjacent corners to serve as additional oviposition substrate for mosquitoes with skip oviposition strategies (e.g., *Aedes*, *Ochlerotatus*; distribute their eggs from the same batch among several different sites). Mesocosms were filled with water on June 1st, 2021. Egg rafts and filter papers were collected daily each morning for 7 days and transferred to a laboratory at ORWRP. Filter papers were replaced daily upon collection. Individual eggs (*Ochlerotatus*) were quantified under a microscope, left to dry for two days, and then added to individual hatching and rearing containers until they reached 4th instar for identification. Egg rafts (*Culex*) were immediately placed into hatching containers where they were likewise raised to 4th instar for identification. Species' identifications were based on Darsie and Ward (2005).

Analysis. Macroinvertebrate α-diversity (i.e., mean species diversity at a local site) was characterized using Hill numbers (i.e., effective number of species), which were calculated by exponentiating Shannon's entropy (Hill q = 1) (Jost 2006). Linear mixed effects models (LMMs) were used as our primary analytical method. Predictors included reach (lit, unlit), light technology (HPS, LED), season (summer, winter), mean stream depth, total nitrogen, total phosphorus, orthophosphate, and D$_{50}$. Response variables included species richness, α-diversity (Hill numbers), and abundance. Site was included as random effect in all models. Significance for linear mixed effects models was tested with Approximate F-Tests (Type III Satterthwaite). To evaluate how predictor variables affected fish assemblage composition, we performed permutational analysis of variance (PERMANOVA) on square-root transformed species abundances using *adonis2* in **vegan** 2.5-7 (Oksanen et al. 2018). PERMANOVA models contained the same predictor variables as linear models. Permutations were constrained by site, which is used as a blocking effect in adonis2. Permutational analysis of multivariate dispersion (PERMDISP) was used to check for homogeneity of group variances.

For the mesocosm study, the two main units of analyses were cumulative egg rafts oviposited on the water surface (*Culex*) per mesocosm and cumulative eggs oviposited on filter papers (*Ochlerotatus*) per mesocosm. We compared control and treatment mesocosms using a generalized linear mixed model (GLMM) with a Poisson distribution and log-link function where treatment (lit and control) was a fixed effect and block was a random effect. Significance was tested using a likelihood ratio test comparing the treatment model to a null model. We used **R** 4.0.2 (R Core Team 2017) for all analyses (α = 0.05), **lme4** 1.1-26 (Bates et al., 2015) for mixed effects models, **tidyverse** 1.3.1 (Wickham et al., 2019) for graphics and data manipulation, **ggsci** 2.9 (Xiao, 2018) for plot color schemes, **cowplot** 1.1.1 (Wilke, 2020) for plot themes, and **emmeans** 1.5.3 (Lenth, 2020) for marginal mean estimates.

3.5 Fish. In streams, each study reach was sampled four times over a two-year period for a total of 96 stream surveys. To capture maximum variation in ALAN, two surveys occurred during leaf-on (summer) and two surveys occurred during leaf-off (winter). Two-pass depletion electroshocking surveys were used to assess fish assemblages in each reach, which adequately captures fish community diversity in small streams of the region (26). All surveys were conducted at night >2 hours post sunset. Total length and mass were collected for the first 20 fish of each species and batch-weighed thereafter.

In reservoirs, field samples for this study were taken along the Scioto River in central Ohio. The Scioto River flows through central and southern Ohio and includes highly urbanized and modified segments, particularly in and around the city of Columbus. Four artificially lit bridge sites and two unlit control bridge sites were selected from a portion of the Scioto River between O'Shaughnessy Reservoir (north of Dublin, OH) and downtown Columbus. Three of these bridges cross heavily impounded reservoirs with wide, deep channels and minimal flow. These sites were classified as "peri-urban" and included two lit bridges (Home Rd. over O'Shaughnessy Reservoir and Hayden Run Rd. over Griggs reservoir) and one control dark bridge (Fishinger Rd. over Griggs Reservoir). The remaining three bridges cross the Scioto River in downtown Columbus, where the river is impounded by run-of-river low-head dams that create narrower channels and allow more variable flow. These sites, which included two lit bridges (Main St. and Broad St.) and one control dark bridge (an unnamed railroad span), were classified as "urban". The light sources on the lit bridges create clear lighting gradients on the waterway, which allowed us to easily differentiate between "lit" and "dark" areas. The control dark bridges were included in the study to account for the influence of the bridge structure and pilings, which might also influence fish communities along a similar (but more localized) gradient.

The fish community at each site was sampled during October and November 2020 using standard boat electrofishing protocols. We commenced each sampling session at least 2 hours after sunset. At each site, four transects were fished parallel to the bridge starting at 0 m and extending beyond the reach of the bridge light. Transects #1 and #2 (0 m and 20 m; Fig. 6) were considered the 'lit reach', while Transects 3 and 4 (100 m and 120 m; Fig. 6) were considered the 'unlit reach'. Captured fish were identified to species and the total length (TL; mm) and weight (g) of each fish was measured. Most fish were held in aerated coolers until sampling of all transects was completed, then released.
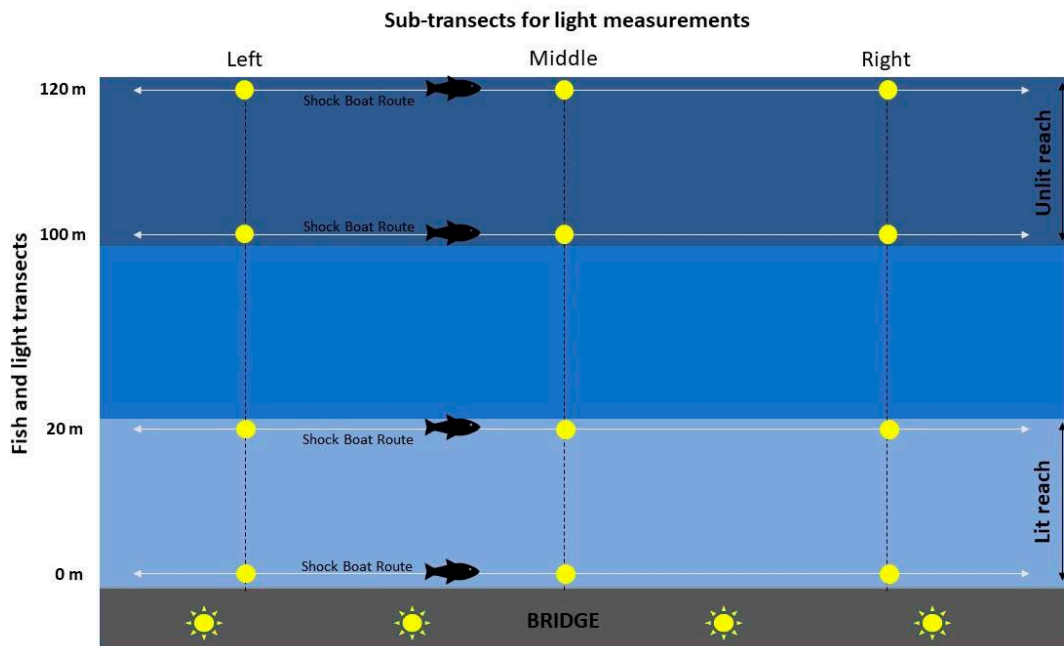
Figure 6. Diagram of transect and sub-transect divisions for fishing and light measurements. Yellow dots indicate light measurement locations. White arrows indicate approximate shock boat route.

Mesocosm Experiments – Fish (Bluegill). We tested for the effect of ALAN on foraging abilities in Bluegill (*Lepomis macrochirus*) using five treatments (n = 4 tanks/treatment): control (ambient night light, <1 lux); low light (1 lux), medium light (4 lux), high light (12 lux), intermittent high light (12 lux, 1 s on/off intervals). Overhead LED lights were turned on at sunset and off at sunrise. Fish were held under these treatment conditions for 5 weeks (n = 15 fish/tank). Feeding trials were conducted on each tank weekly for five weeks. Food was withheld for 24 hours prior to each trial to standardize foraging motivation of the Bluegill. Trials commenced at least 2 hours after sunset and lasted for 30 minutes each. Prior to each trial, turbidity (NTU) and light intensity (lux) were measured in the trial tanks using a Hach 2100Q Portable Turbidimeter and a BTMeter BT-881D Digital Luminance Light Meter, respectively. Security cameras (Amcrest Video Security System AMDV960H8-8B) with infrared night vision were installed above each tank to record the trials. Once the cameras were in place and recording began, the Bluegill in the trial tank were fed a set number of floating freeze-dried river shrimp based on the number of fish in the tank (i.e., 2 shrimp per fish). At the end of each trial, the number of shrimp remaining were counted to determine the total number of prey consumed. The percentage of prey consumed was calculated as (*n* prey consumed ÷ *n* prey available)*100. Video recordings were used to determine the average number of prey strikes (defined by Oldham et al. as a distinct and rapid forward motion; 2018) per tank per night.

Mesocosm Experiments – Fish (Largemouth Bass). To test the hypothesis that ALAN can disrupt predator-prey dynamics by increasing predation rates, we utilized a full factorial field mesocosm experiment where we exposed predators and prey to four levels of ALAN (a = 4; 0, 0.5, 3, and 10 lux) crossed with two levels of turbidity (t = 2; clear and 40 NTU). The mesocosm array consisted of three blocks (b = 3), each of eight mesocosms (a × t = 8), replicated across two trials (r = 2) for a total of 48 mesocosm experimental units (b × r × a × t= 48 = n). Each mesocosm received one largemouth bass (*Micropterus salmoides*) as the predator, 30 fathead minnows (*Pimephales promelas*) as prey, and three hollow cinder blocks to function as habitat structure. Luminaires were suspended above each mesocosm within a fixture that prevented light trespass to neighboring mesocosms within each block. Mesocosms experienced natural lighting during the daytime and luminaires were left on during the entire experiment to produce ALAN. Fathead minnow abundance was monitored in each mesocosm every day for twenty days (or until all prey were consumed) to determine predation rates. We expected an interaction between ALAN and turbidity such that ALAN will increase predation rates, but this effect will be greater in low turbidity treatments.

Analysis. For stream fish, fish assemblage α-diversity was characterized using Hill numbers (i.e., a diversity index of the effective number of species), which were calculated by exponentiating Shannon's entropy (Hill *q* = 1) (Jost 2006). Linear mixed effects models (LMMs), which consists of regression and ANOVA techniques with data grouping structures, were used as our primary analytical method. Predictors included reach (lit, unlit), light technology (HPS, LED), season (summer, winter), mean stream depth, total nitrogen, total phosphorus, orthophosphate, and D_{50}. Response variables included illuminance, canopy cover, species richness, diversity (Hill numbers), abundance, relative abundance, and $\log_{10}$-transformed mean fish mass. Nitrate and ammonia were significantly positively correlated with TN (*r* = 0.87 and *r* =0.81, respectively), thus only TN was used in analyses to prevent multicollinearity. Total phosphorus and orthophosphate were not correlated (*r* = -0.11). A reach * season interaction was included in the model when analyzing illuminance and canopy cover, but not for other response variables. Mean stream depth was modeled as a 2nd-order polynomial within all LMMs, while orthophosphate was modeled as a 2nd-order polynomial in the fish mass analysis. Site was included as random effect in all models and species was included as a random effect in relative abundance and mean fish mass models. Significance for linear mixed effects models was tested with Approximate *F*-Tests (Type III Satterthwaite). To evaluate how predictor variables affected fish assemblage composition, we used permutational analysis of variance (PERMANOVA), which is a multivariate statistical technique that simultaneously analyses the abundances of multiple species at each location. The PERMANOVA was performed on square-root transformed species abundances and modeled using *adonis2* in **vegan** 2.5-7 (Oksanen et al. 2018). PERMANOVA models contained the same predictor variables as linear models. Permutations to do significance tests were constrained by site,

which is used as a blocking effect in *adonis2*. Permutational analysis of multivariate dispersion (PERMDISP) was used to check for homogeneity of group variances, which is a critical assumption of PERMANOVA.

For all analyses α = 0.05. We used **R** 4.0.2 (R Core Team 2017) and the following R packages for formal statistical analyses: **lme4** 1.1-26 (Bates et al. 2015) for mixed effects models, **lmerTest** 3.1-3 (Kuznetsova et al. 2015) for approximate *F* tests, **MuMIn** 1.43.17 for conditional (full model) and marginal (fixed effect) R^2 values (Barton 2020), **tidyverse** 3.3.3 (Wickham et al. 2019) for graphics and data manipulation, and **vegan** 2.5-7 (Oksanen et al. 2018) for PERMANOVA, PERMDISP, and NMDS scores. We used **ggsci** 2.9 (Xiao 2018) for plot color schemes, **cowplot** 1.1.1 (Wilke 2020) for plot themes, **ggConvexHull** 0.1.0 (Martin 2017) for NMDS convex hulls, **kableExtra** 1.3.1 (Zhu 2020) for output formatting, **psych** 2.0.12 (Revelle 2020) for data summaries, and **car** 3.0-10 (Fox and Weisberg 2019) for model diagnostics.

To evaluate potential differences in river-reservoir fish assemblages, we calculated species evenness (Pielou's *J'*), richness (S; the count of the number of species), abundance (the number of individuals per species), and diversity (an index that takes into account richness and abundance). We characterized fish assemblage diversity with Hill numbers (calculated by exponentiating Shannon's entropy; Hill *q* = 1), which describe the effective number of common species in the assemblage. Linear mixed effects models (LMMs) with a Gaussian distribution were used to test whether these fish assemblage characteristics varied with light intensity. Akaike Information Criterion (AIC) was used to select which independent variables to include in each LMM. Only models with ΔAICc less than 2 were considered for analyses (Symonds and Moussalli 2011). Independent variables considered for model selection included lux (i.e., light intensity), transect width, bridge type (i.e., lit sites vs. control dark sites), and the interaction between lux and transect width. Transect width (m) was included as an independent variable to account for the differences in flow, depth, channel width, and ambient light levels between the urban and peri-urban sites. Transect width also acts as a proxy for electrofishing pass duration (s); in other words, the wider the transect, the longer it took to complete an electrofishing pass. We tested for significance (α = 0.05) with a type III analysis of variance (ANOVA) using Satterthwaite approximation for degrees of freedom. Permutational analysis of variance (PERMANOVA) was used to evaluate the influence of the same independent variables considered in the LMMs on fish assemblage structure and non-metric multidimensional scaling (NMDS) was used to visualize PERMANOVA results.

For the bluegill mesocosm study, we assessed three response variables: foraging efficiency (n prey captured:total n of prey strikes); success rate (n prey captured ÷ n prey available); strike rate (n prey strikes ÷ n prey available). We used separate LMM (linear mixed models) analyses for each of the three behavioral variables with lighting treatment as a fixed effect, turbidity (the degree to which water loses its transparency due to suspended particulates; which increased in all tanks over the course of the experiment) and the interaction between turbidity and treatment as fixed variables, and tank ID number and trial number (i.e., the week of the experiment during which the trial took place) as random effects.

For the bass mesocosm study, we analyzed the number of surviving minnows over time in each mesocosm. To do this, we used LMMs to model surviving minnows as our response variable and light treatment × turbidity treatment as our main predictor. We used mesocosm and day into experiment (day) as random variables to control for repeated-measures of each mesocosm. We tested for significance (α = 0.05) with a type III analysis of variance (ANOVA) using Satterthwaite approximation for degrees of freedom.

3.6 Ground-dwelling Mammals. At stream-riparian sites, small mammals were trapped using Sherman live traps (7.62 x 8.89 x 22.86 cm, galvanized steel doors and treadle; H.B. Sherman Traps, Tallahassee, Florida, USA) in a paired transect design (Pearson and Ruggiero 2003). Each transect contained seven pairs of traps (14 total) to accommodate multiple captures at the same location, with each pair of traps spaced 5 m from other pairs. Transects ran parallel to the stream, 5 m from the

stream bank. Trapping transects were opened for three consecutive nights at each reach, with the lit and unlit reach of each paired study site being trapped concurrently. Traps were baited with a mixture of oats and peanut butter with cotton ball bedding included. Trapping occurred from August 2020 to November 2021, with each site being sampled three times, once in the late summer/early autumn of 2020, once in the summer of 2021, and once in the autumn of 2021. All trapping was carried out in accordance with the standards set by the OSU Institutional Animal Care and Use Committee (IACUC, Protocol 2010A0003-R3). All captured small mammals were placed in handling bags to reduce stress (Sikes et al. 2011). Standard measurements were taken on all captures as well as recording age class, sex, and species identification. Identifications were based on guidelines from (27). Ear tags (1005-1 Ear Tags, National Tag and Band Company International Identification Inc., Newport, KY, USA) were attached to a single ear on all captured individuals to record mark-recapture data. For shrews that cannot have ear tags, we used permanent marker color patterns on their ventral side to create individualized marks. All captured small mammals were released at the same place they were captured.

The presence of larger mammals was assessed with RECONYX Hyperfire 2 High-Output Trail Cameras (RECONYX, Holmen, Wisconsin, USA) that were deployed at paired reaches simultaneously with live-trapping periods. Two cameras were deployed at each reach, one each at lit/unlit reaches in four separate sampling events: May - June 2021, July 2021, October – November 2021, and January – February 2022. Cameras were set for 10 consecutive days during each sampling period and all pictures were digitally stamped with the date and time. All cameras were attached to trees ~1 m above the ground with a field of view that encompassed the entire 30 m reach. Cameras were set facing away from trails, roads, or any high human usage area and followed the recommendations of Herrera et al. (2021). Cameras were set to a three-shot trigger, a 5 s delay between triggers, and activated IR flash for nighttime photographs. Due to difficulty in identifying different individuals, mammals of the same species observed by the camera within 20 minutes of the most recent sighting were considered a single independent encounter, unless multiple individuals were observed at a single time. Observations of the same species outside of 20 minutes of the most recent encounter, even if they are potentially the same individual, were considered separate independent encounters. Sites that were compromised by damaged or destroyed camera traps were resampled during the following 10 days. Encounters during both daytime and nighttime were included in the analysis due to ALAN potentially impacting the timing of activity of nocturnal animals, creating changes to daytime encounters.

Analysis. For ground-dwelling mammals, linear mixed-effect models (LMMs) were used to assess differences in total mammals encountered and species richness (*S*; number of species) between reaches. Season, time of day, light intensity (lux), light technology, impervious surface, sediment size (D_{50}), and reach were included as fixed effects in the models. Site was included as the random effect. The amount of variation explained by site (the random effect) in the LMMs is the difference between the total variation of the model (Conditional R^2) and the variation of the model explained by fixed effects (Marginal R^2).

Analysis of mammal community composition was conducted in three ways: small mammal captures, camera-trap species encounters, and camera-trap guild (i.e., group of species with similar requirements that play a similar role within a community) encounters. For small mammal captures, abundance was estimated at each reach using two capture-recapture models: the Cormack-Jolly-Seber Model (CJS model) and Pollock's Robust Design. The CJS model used capture-recapture data with survival intervals between sampling events, meaning estimates of abundance and survival/emigration can be estimated from at least three sampling events (Cormack 1964, Jolly 1965, Seber 1965). Pollock's Robust Design integrates the CJS model with multiple sampling events, known as "trapping intervals," occurring between survival intervals. This allows for estimation of abundance, survival/emigration, and birth/immigration (Pollock 1982). While attempting to use the white-footed mouse data, the small sample sizes resulted in the Cormack-Jolly-Seber and Pollock's Robust Design mark-recapture models not providing reliable estimates of population parameters. All further analysis was done on raw capture data rather than abundance estimates, with the number of captures summarized by site, reach, and season available. At each level (small mammal captures, camera-trap species encounters, camera-trap

guild encounters), the most encountered species/groups were compared at lit versus unlit reaches using paired *t*-tests.

To measure the difference between reaches based on their species/guild compositions, Bray – Curtis dissimilarity was calculated for all reaches. Bray – Curtis dissimilarity is calculated using a matrix where each row is a different reach and each column is a different species/guild, with the values of the matrix being the number captured/encountered at that reach. The Bray – Curtis dissimilarity value is a non-Euclidian distance measure that quantifies the differences between samples of ecological abundance data at different locations (Bray and Curtis 1957).

Species and guild compositions were also qualitatively assessed using non-metric multidimensional scaling (NMDS) plots with convex hulls representing fixed effects. NMDS plots allow for the projection of reaches into a 2-dimensional plot based on multi-dimensional factors (in this case, species/guilds). Following Anderson (2001), Permutational Multivariate Analysis of Variance (PERMANOVA) was used to analyze the effects of year, sediment size, impervious surface, and season on small mammal capture community, the effects of time of day, reach, sediment size, impervious surface, and season on total mammal camera-trap capture community, and the effects of time of day, reach, sediment size, impervious surface, and season on mammal guild camera-trap encounter community. PERMANOVAs were performed using the Bray-Curtis distance matrix with blocking by site (n_{perms} = 9999). Additional ALAN variables (light intensity, spectral composition) were removed from the analysis due to violations of dispersion. All data analysis was completed in **R** (R Core Team 2018) using base packages and the **vegan** package (Dixon and Palmer 2003). In all statistical tests, an α = 0.05 (p < 0.05) was used to indicate statistical significance; an α = 0.10 (p < 0.10) was used to indicate a trend of possible differences under a greater sample size or different lighting regime.

3.7 Bats. At our wetland site in the Olentangy River Wetland Research Park, we monitored for the presence of bat activity within the experimental lit and unlit areas across two summer seasons (June-Sept 2021 & 2022). Eight bat monitors (Song Meter SM4BAT FS, Wildlife Acoustics, Maynard, MA, USA) were evenly distributed across lit and unlit areas (two monitors per area per wetland cell). Monitors had a 16k high filter with a sample rate of 256 kHz and a minimum and maximum duration of 2.0 and 200 ms, respectively. The minimum trigger frequency was 18 kHz, the trigger level was 24 dB, and the trigger window was 2 s. Data were collected weekly from each monitor for the duration of each summer sampling period. See Appendix Figures 1 and 2 for additional details on experimental design.

Analysis. In initial analyses, we used the number of hits per recorder as a measure of bat activity and as our main response variable. We used linear mixed models (LMM) to compare the number of hits between treatments: lit and unlit areas in the wetlands. Year and treatment were fixed factors in the analysis, while sample week was a random variable to control for repeated-measures. We set α = 0.05 (p < 0.05) to indicate statistical significance.

3.8 Food Webs. In addition to community surveys (described above), we collected tissue samples of wildlife to be processed and submitted for analysis of naturally abundant isotopes (^{13}C and ^{15}N) in order to measure food-web and trophic relationships, such as food-chain length using Bayesian mixing models (28). Given delays in processing of stable-isotope samples (COVID-19 pandemic led to isotope labs shutting down and/or severely restricting services), we only present the results for riparian mammals in this report.

Analysis. Studying food-web interactions is important for understanding how communities and ecosystems function (Post et al. 2000). One way to understand food web linkages is by using naturally abundant stable isotopes to estimate relative diet proportions and trophic feeding position of organisms (Kling et al. 1992). To understand complex trophic interactions, two stable isotopes are commonly used: ^{13}C and ^{15}N. Because δ^{13}C varies little with trophic position but greatly between primary producers (Post 2002), δ^{13}C in consumer tissue can be used to determine basal resource pathways from which an organism derives its energy (DeNiro and Epstein 1978, Post 2002). The δ^{15}N in

consumer tissue can be used to estimate relative trophic position because $\delta^{15}N$ shows consistent enrichment as trophic level increases (DeNiro and Epstein 1981).

For stable isotope analysis, mammal hair, macroinvertebrate (terrestrial and emergent), terrestrial vegetation, detritus, and periphyton samples were oven dried (60°C, 48 h) and homogenized using a ball mill grinder. Homogenized samples were packed into tin capsules. Tissue from multiple macroinvertebrates and vegetation classes within the same group were combined into a composite sample to reduce variation (Lancaster and Waldron 2001).

Stable isotope samples were analyzed for carbon (C) and nitrogen (N) using elemental analysis isotope ratio mass spectrometry (EA-IRMS) at the Washington State University Stable Isotope Core (Pullman, Washington, USA) and the Southern Methodist University Stable Isotope Laboratory (Dallas, Texas, USA). Stable isotope results are reported in δ notation, where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. C values are expressed relative to Vienna Pee Dee Belemnite (a standard isotope reference) and N values are expressed relative to atmospheric air (N_2).

4. Research Findings and Conclusions

4.1 Lighting Environment. Characteristics of ALAN varied across our study locations. Lighting across the CMA was primarily a mixture of LED and HPS (Fig. 7), and intensity (lux) in riparian and aquatic ecosystems varied broadly based on light source, distance, and other mitigating factors such as natural shielding (e.g., vegetation; e.g. Fig. 8). Across our stream-riparian study sites, we observed lighting levels from 0.1-11.2 lux (equivalent to 0.009-1.04 fc). At our river-reservoir sites, lighting intensity ranged from 0.01 at unlit reaches to 6.9 lux (0.64 fc) at the water surface near bridges. Our experimental work in wetlands and mesocosm tested ecological responses of LED up to 12-20 lux (1.11-1.86 fc).
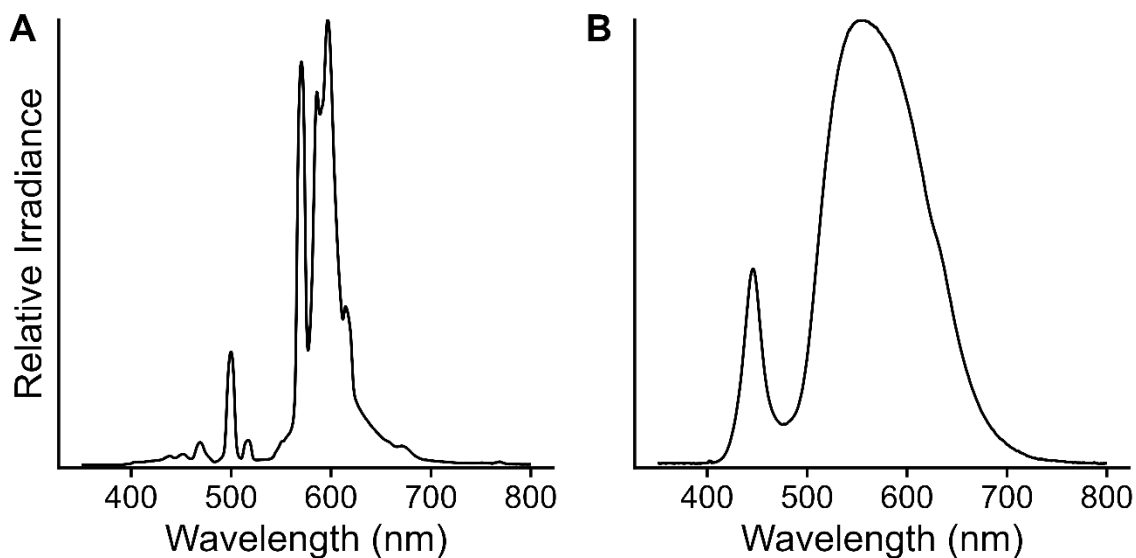
Figure 7. Spectral graphs of wavelength (nm) and frequency for LED (A) and HPS (B) lights at stream-riparian study sites. There was variation between lights at different sites (A) Grove City High School and (B) Dublin Rec Center.
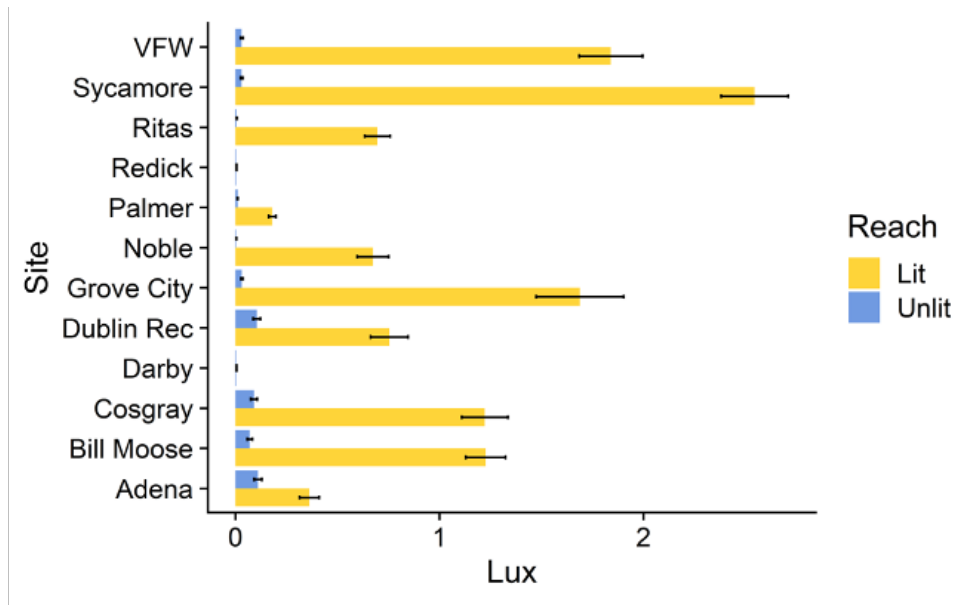
Figure 8. Mean lux values at night comparing lit and unlit reaches at our stream-riparian study sites. Error bars= +/- 1 SE. None of the reaches at reference sites (Darby, Redick) exhibited sufficiently high lux values to register on the ILT1700.

Our results also show that mean light trespass into urban streams is approximately doubled during leaf-off in winter months (1.97 lux ± 0.28; equivalent to 0.18 ± 0.03 fc) compared to leaf-on in summer months (1.00 lux ± 0.19; equivalent to 0.09 ± 0.02 fc), highlighting the important interceptive effect of canopy coverage in small systems.

4.2 Aquatic Insects. The abundance and diversity of benthic macroinvertebrates are key indicators of healthy stream ecosystems. Surveys of benthic macroinvertebrates did not detect effects of ALAN on benthic macroinvertebrate diversity (Fig. 9A, 2B).
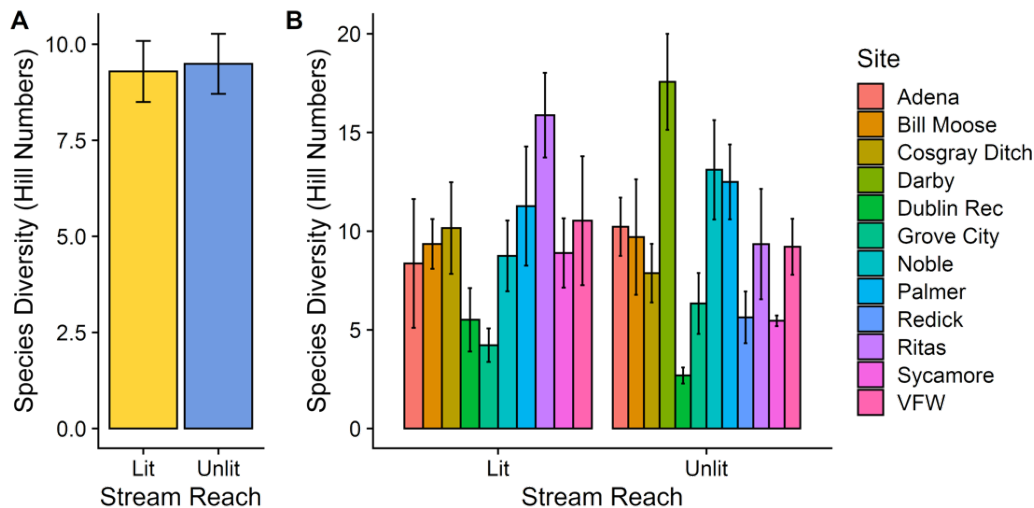
Figure 9. (A) Mean macroinvertebrate species diversity measured in Hill numbers across lit and unlit stream reaches sampled in CMA. (B) Mean macroinvertebrate species diversity measured in Hill numbers at each site sampled in CMA.

Many larval aquatic insect species transition to terrestrial, winged adults when they emerge from the aquatic environment. These emergent aquatic insects serve as critical prey sources for a suite of

riparian consumers including birds, bats, small ground mammals, and spiders. As part of our ALAN-wetland research, we found that emergent aquatic insect density was significantly higher at lux >1-20 compared to control/skyglow conditions (<0.1 lux; Fig. 10A), but we observed no difference in density between LED and HPS (Fig. 10B). However, we did observe a difference in the community composition of emergent aquatic insects between LED and HPS lighting types (Fig. 10C).
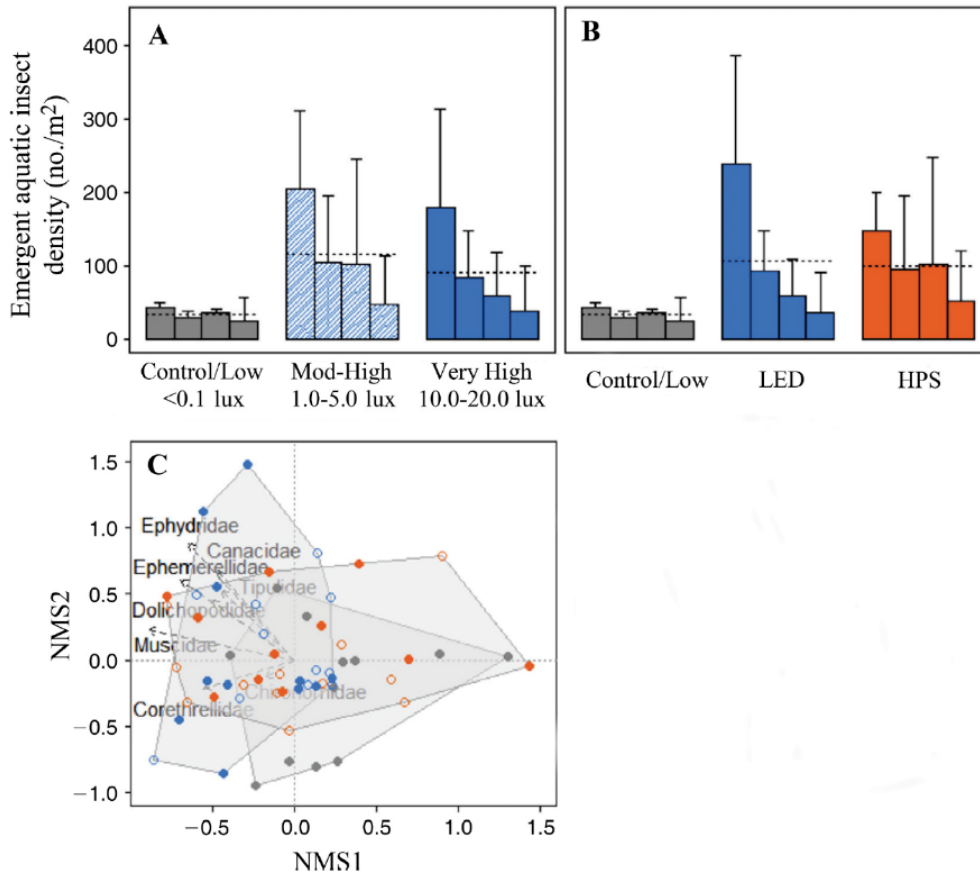
Figure 10. Emergent aquatic insect density in response to (A) light intensity and (B) lighting spectra across four sample dates. Both effects were significant: light intensity, $F_{2,22}$ = 7.53, P = 0.003, and lighting spectra, $F_{2,22}$ = 3.96, P = 0.034. At α = 0.05, post-hoc contrasts were significant between the moderate-to-high light intensity and control/skyglow only in panel A; at α = 0.1, post hoc contrasts were significant between the very high light intensity and both moderate-to-high light intensity and control/skyglow intensity in panel A and between the control/skyglow and both LED and HPS lighting spectra in panel B (all post hoc contrasts were adjusted by FDR). In panel C, the emergent aquatic insect community composition data across four sample dates were ordinated by nonmetric multidimensional scaling (NMS; stress = 0.23). Samples (circles) are coded by treatment type (i.e., lighting spectra and intensity): solid, gray, control/skyglow illuminance; open, blue, light-emitting diode (LED), moderate-to-high illuminance; solid, blue, LED, very high illuminance; open, orange, high-pressure sodium (HPS), moderate-to-high illuminance; and solid, orange, HPS, very high illuminance. Formal testing by permutational multivariate analysis of variance (PERMANOVA; with blocking by habitat and sample date) suggested a difference with respect to lighting spectra ($F_{2,55}$ = 1.28, P = 0.010; the three convex hulls are grouping the control/skyglow, LED and HPS samples, respectively); mainly in response to LED lighting (pairwise PERMANOVAs significant at α = 0.05 for control/skyglow vs. LED and LED vs. HPS, with FDR adjustment). Data are from the wetland field experiment; light-intensity treatments were control/low, <0.1 lux; moderate-to-high, 1–5 lux; and very high, 10–20 lux. In panels A and B, the dashed horizontal bars are overall means (across all dates and sites) per treatment and values are means and SD (error bars) (from Sullivan et al. 2019).

In experimental mesocosms, we used mosquitoes as an insect model to compare oviposition (egg-laying) rates in habitats exposed to light-emitting diode (LED) luminaires (3000K, ~13 lux) and dark controls. Mosquitoes had species-specific responses to ALAN (Fig. 11). Mean cumulative *Culex* egg raft oviposition was greater in control pools (20.97 ± 2.85) than in ALAN pools (10.13 ± 1.58). We observed no response of *Ochlerotatus*, potentially reflecting the risk associated with the alternative oviposition strategies of the two species (eggs rafts vs. skip oviposition).
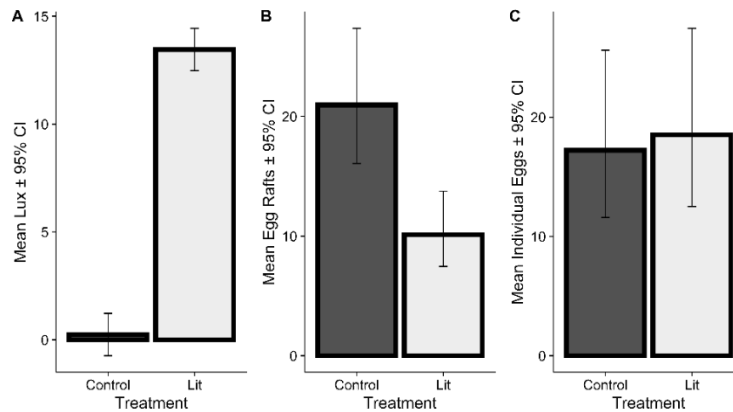
Figure 11. (A) Mean lux values of the two treatments groups. (B) Mean total egg rafts oviposited by *Culex restuans* on surface water of the two treatments groups. C) Mean total individual eggs oviposited by *Ochlerotatus japonicus* on filter paper of the two treatment groups. Error bars represent 95% confidence intervals.

4.3 Fish. At our stream study sites, we observed no differences in fish assemblages (i.e., abundance, relative abundance, biomass, diversity, guild composition; Figs. 12, 13) between lit and unlit reaches (> 100 m apart). Species richness and α-diversity (Hill numbers) scaled positively with mean stream depth. Nutrient concentrations were negatively related to mean fish mass and fish assemblage diversity. Species richness scaled negatively with total phosphorus and α-diversity scaled negatively with orthophosphate.
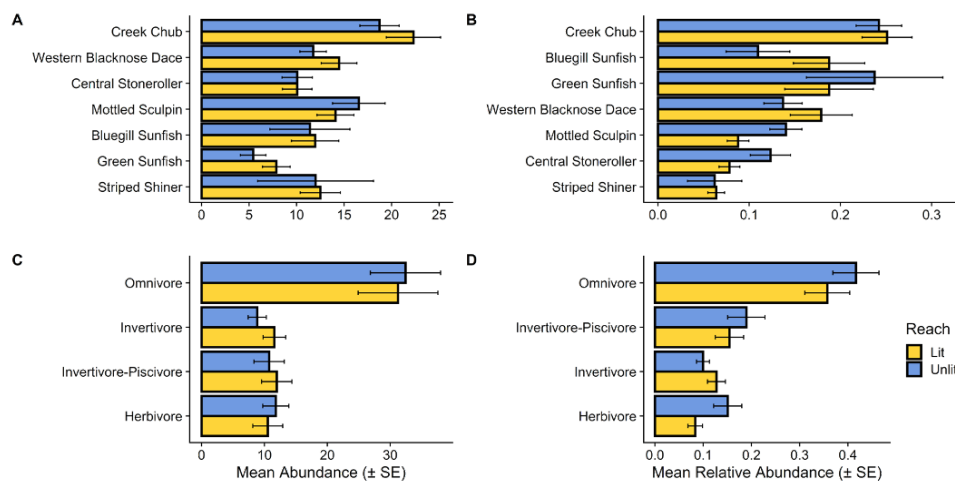
Figure 12. (A) Mean abundance (± SE) of the seven most common fish species. (B) Mean relative abundance (± SE) of the seven most common fish species. (C) Mean abundance (± SE) of each fish guild. (B) Mean relative abundance (± SE) of each fish guild. Lit reaches are represented in yellow, while unlit reaches are in blue.
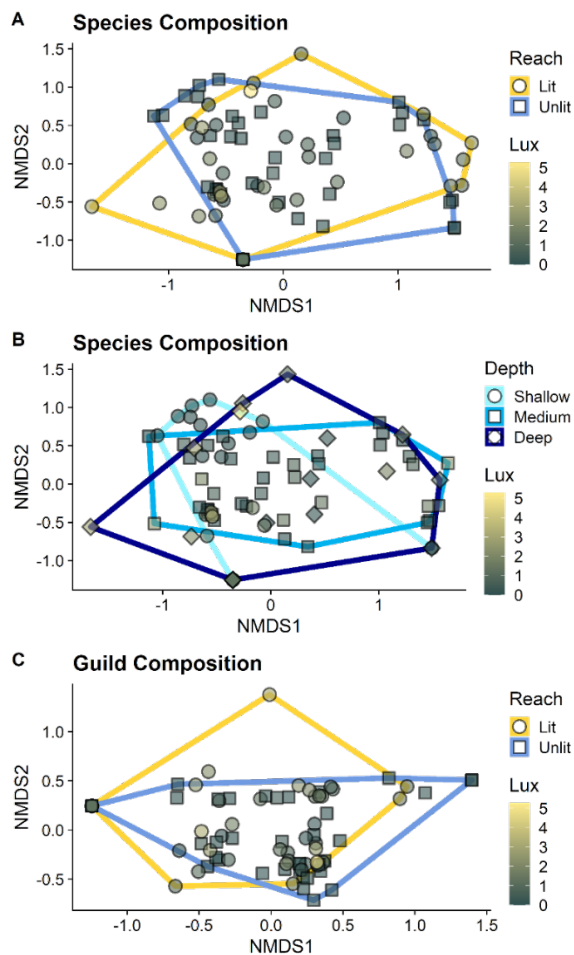
Figure 13. Non-metric multidimensional scaling plots for fish assemblages showing species composition (stress = 0.144) across (A) reaches (P = 0.154) and (B) depths (P = 0.0278). (C) Feeding guild composition (stress = 0.080) across reaches.

At our river-reservoir sites fish assemblage diversity and evenness were significantly lower in more brightly lit areas, indicating that these illuminated zones were dominated by a few abundant species. Bluegill were the second-most abundant species captured and likely represented one of these species dominating the fish assemblage in lit areas.

In our experimental mesocosm facility, we tested for influences of ALAN on Bluegill and Smallmouth Bass. In the Bluegill foraging experiment, we found no effect of treatment on foraging efficiency ($F_{5,84}$ = 2.25, P = 0.673). However, the intermittent lighting treatment significantly impacted success rate and strike rate (Kruskal-Wallis Chi-square = 38.78, df = 4, P < 0.0001), with both success and strike lower on average in the fish exposed to flashing light (Fig. 14). Results of the Bluegill mesocosm experiment suggest that high-intensity light pollution allowed fish to locate and strike at prey more quickly. Additionally, flashing high intensity lights (intended to mimic the effect of passing car headlights on a busy highway) appeared to inhibit the ability of fish to successfully capture prey. Our results demonstrate the potential for light pollution (especially flashing lights) to interfere with the behavior of this ecologically and economically important sportfish.
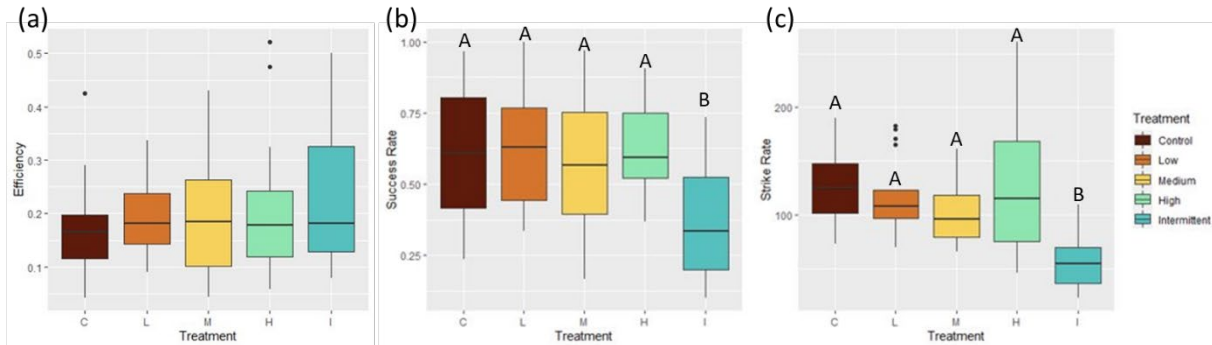
Figure 14. Foraging ability by lighting treatment. Box plots of (a) foraging efficiency (n prey captured:total n of prey strikes); (b) success rate (n prey captured ÷ n prey available); c) strike rate (n prey strikes ÷ n prey available). Box extends from 25th to 75th percentile; band represents the mean; whiskers range from lowest to highest datum within the 1.5x inter-quartile range interval. Different letters indicate significant differences between groups.

In our bass mesocosm experiment, the effects of ALAN to modulate predation rates in a predator-prey system were tested. The 0.5 lux treatment exhibited the lowest survival rate compared to other treatments, but a linear mixed model revealed no statistically significant difference among treatments over the course of the experiment (Fig. 15), however, a formal survival analysis that models time to event will provide the clearest answer.
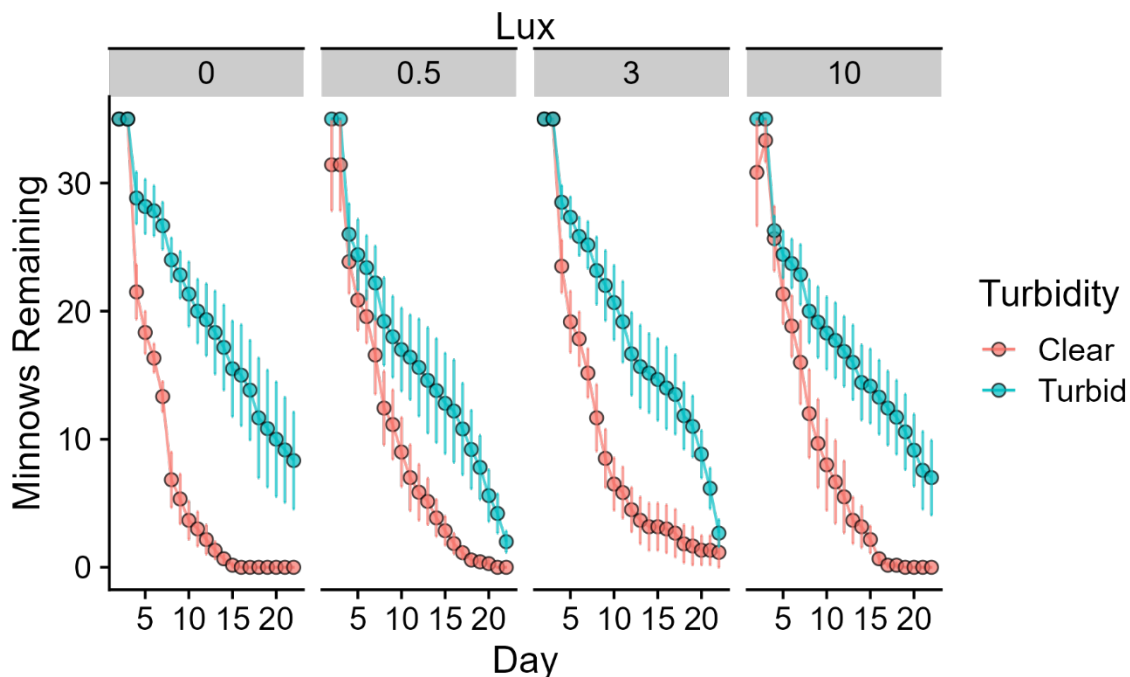
Figure 15. Largemouth bass (*Micropterus salmoides*) predation rate across four light levels (0, 0.5, 3, and 10 lux) measured by the daily number of minnows remaining in a field mesocosm experiment.

4.4. Mammals. In the riparian areas of small streams in the Columbus Metropolitan Area, we did not find evidence that ALAN influenced mammal diversity (Tables 2, 3) or community composition (Fig. 16). There were no differences between individual species or guild encounters at lit reaches compared to unlit reaches. Seasonality influenced the mammal community response across levels of our study, regardless of ALAN presence. Year also may have had an influence on the small mammal community,

which is supported by other studies of small mammals that show precipitation and resource differences between years have strong influences on small mammal communities (Wolff 1996). More recently, studies have shown that even when faced with massive habitat transformations, community compositions still varied widely from year to year (Chapman et al. 2018). While mammals often maintain their daily activity patterns even when overlapping other species (Mori et al. 2019), some mammals exposed to stressors can adjust their daily activity patterns (Diao et al. 2021).

Table 2. Total captures for small mammal species within riparian stream reaches of the Columbus Metropolitan Area for lit and unlit reaches at paired study sites, the number of reaches at which each species was captured (# of reaches), and the number of captures at lit and unlit reaches. Species with >5 captures have results of paired *t*-test comparing the number of captures of that species at lit vs unlit reaches. Paired *t*-tests were not run for the unidentified deer mouse species since those individuals could have been two different species.

Common Name	Species Name	Captures	# of Reaches	Captures - Lit Reaches	Captures - Unlit Reaches	*t*	*df*	*p*
White-footed Mouse	*Peromyscus leucopus*	168	18	72	96	-1.439	9	0.184
Eastern Chipmunk	*Tamias striatus*	13	10	9	4	1.342	9	0.213
Deer Mouse Species	*Peromyscus spp.*	9	5	4	5	-	-	-
Short-tailed Shrew	*Blarina brevicauda*	9	6	6	3	1.152	9	0.279
Deer Mouse	*Peromyscus maniculatus*	8	5	3	5	-1.000	9	0.343
Virginia Opossum	*Didelphis virginiana*	2	1	2	0	-	-	-
Meadow Vole	*Microtus pennsylvanicus*	1	1	0	1	-	-	-

Table 3. Relative Abundance Index (RAI – independent encounters/100 trap nights) for mammal species within riparian stream reaches of the Columbus Metropolitan Area for lit and unlit reaches, the total number of encounters for each species, and the number of reaches each species was observed at (# of reaches). Species with >20 encounters have results of paired t-tests comparing the number of encounters of that species at lit vs unlit reaches.

Common Name	Species Name	Encounters	# of Reaches	RAI - Lit Reaches	RAI - Unlit Reaches	*t*	*df*	*p*
Whitetail Deer	*Odocoileus virginianus*	613	20	88.5	64.75	1.0262	9	0.3316
Eastern Gray Squirrel	*Sciurus carolinensis*	503	20	69.5	56.25	0.35835	9	0.7283
Raccoon	*Procyon lotor*	424	16	60.25	45.75	0.58388	9	0.5736
Red Fox	*Vulpes vulpes*	72	6	8.5	9.5	-0.1745	9	0.8653
Eastern Chipmunk	*Tamias striatus*	50	5	3	9.5	-1.0419	9	0.3246
House Cat	*Felis catus*	47	10	3.25	8.25	-0.9632	9	0.3606
Eastern Cottontail	*Sylvilagus floridanus*	32	6	3	5	-0.8300	9	0.4280
Virginia Opossum	*Didelphis virginiana*	25	5	2.75	3.5	-0.3448	9	0.7381
Groundhog	*Marmota monax*	18	7	0.25	4.25	-	-	-
Coyote	*Canis latrans*	13	6	2	1.25	-	-	-
Long-tailed Weasel	*Mustela frenata*	12	4	0.5	2.5	-	-	-
Red Squirrel	*Tamiasciurus hudsonicus*	10	2	2.25	0.25	-	-	-
Striped Skunk	*Mephitis mephitis*	6	3	0.75	0.75	-	-	-
Fox Squirrel	*Sciurus niger*	5	2	0.5	0.75	-	-	-
Mink	*Neogale vison*	3	1	0.75	0	-	-	-
White-footed Mouse	*Peromyscus leucopus*	2	2	0.25	0.25	-	-	-
Domestic Dog	*Canis lupus familiaris*	1	1	0	0.25	-	-	-

Our results comparing the influence of time of day and season on mammal guild camera-trap encounter compositions (Figs. 16, 17) suggest potential daily and seasonal differences in mammal guild encounter compositions, but no difference by ALAN.
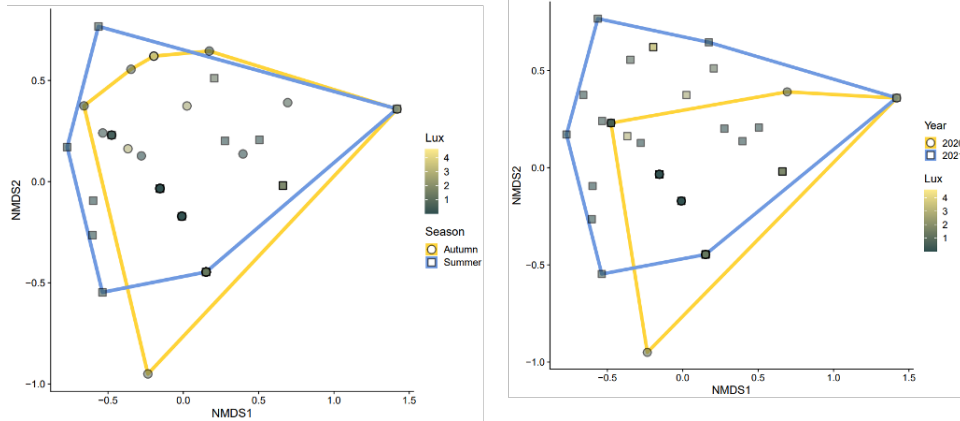
Figure 16. 2-D nonmetric multidimensional scaling (NMDS) (NMDS axes 1 and 2) ordination graphs of small mammal species capture composition (stress = 0.14), showing the convex hull polygon for season (left) and year (right). Point fill color is average lux. The results from the NMDS comparing the influence of season and year on small mammal species capture compositions suggest potential seasonal and annual differences in small mammal species capture composition, but no difference by ALAN. PERMANOVA supported these observations, suggesting a significant influence of season (Pseudo F = 1.465, P = 0.011) and a trend for year (Pseudo F = 1.414, P = 0.056).
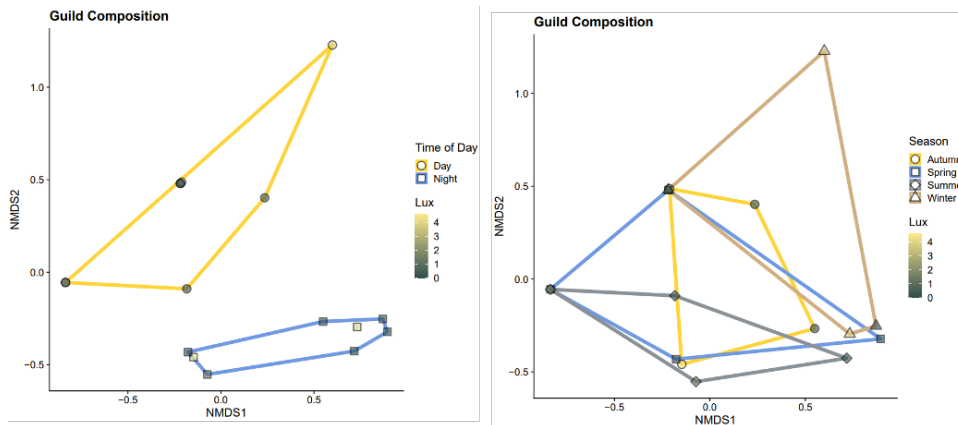
Figure 17. 2-D nonmetric multidimensional scaling (NMDS) (NMDS axes 1 and 2) ordination graphs of guild camera encounter composition (stress = 0.09), showing the convex hull polygon for time of day (left) and season (right). Points are sites with fill color of average lux.

We also hypothesized that any trends in mammal community composition, encounters, or species richness would have greater correlation at lit reaches with LED lights compared to HPS lights. None of the data supported this hypothesis. Deer exhibit different electroretinography (ERG) responses to multiple light wavelengths (Crivelaro et al. 2018), indicating different light wavelengths from ALAN should change their responses. Mice can observe and respond to some wavelengths of UV light (Peirson et al. 2018) while some bats exhibit positive phototaxis to green light (Voigt et al. 2017) and red light but not to white light (Voigt et al. 2018). Mammals being influenced by different wavelengths of light indicates that light technologies with different spectral ranges should show a difference in mammal communities, but the data does not support that. No differences based on light technology could be due to the increasing prevalence of LED lighting in urban areas where they are often becoming the standard lighting choice because of their brightness and energy saving qualities (Longcore et al. 2015). It is possible that urban mammals are not affected by LED night lighting since they are exposed to it more often. Since white LED lights have such a broad emittance spectrum that encompasses the entire

spectrum that HPS lights emit (29), mammals that are not being impacted by LED lights would most likely not be impacted by HPS lights either.

From our wetland study, we observed that bat responses to ALAN were relatively even among treatment areas (Fig. 18). We observed greater variability in 2022 compared to 2021, but no significant differences were noted ($P > 0.05$). However, data parsed by species can potentially reveal more nuanced patterns as some species are light-shy (Spoelstra et al. 2017, Azam et al. 2018), while others congregate around light to forage for insects (30).
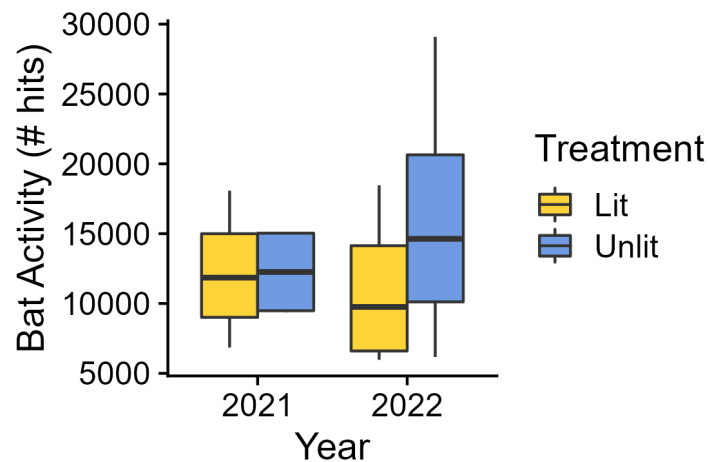
Figure 18. Bat activity measured by the number of hits (detections) on monitors positioned in Lit and Unlit areas of the wetlands across two years.

4.5 Food webs. ALAN presence was related to the proportion of energy derived from aquatic vs. terrestrial primary producer pathways in the genus *Peromyscus* (deer mice), the only small mammals with enough samples to estimate diet proportions. At lit reaches, *Peromyscus* nutritional subsidies derived from aquatic primary producer pathways that originate from stream periphyton were 1.2% lower than at unlit reaches. Canopy cover was also associated with the proportion of energy derived from a terrestrial primary producer pathway that is indirectly consumed by *Peromyscus* (i.e., originating from aquatic detritus). Site as a random effect in linear-mixed models explained the greatest amount of variation in the proportion of energy derived from different primary producer pathways. Overall, we found evidence that ALAN was associated with changes in small mammal trophic interactions, but only by small amounts (>2% change in diet).

5. Recommendations for Implementation

We carried out multiple field and mesocosm studies to directly quantify the impacts of ALAN on sensitive aquatic ecosystems and biodiversity. Aquatic insects exhibited some of the strongest relationships with ALAN, with more nuanced associations with fish and riparian mammals. From our field studies, our results imply that the relatively low-levels of ALAN observed in canopied streams may exert only subtle effects on small stream insect, fish, and mammal assemblages, which are difficult to detect amongst the multiple, confounded stressors impacting these relatively species-poor systems with urban-adapted, tolerant species.

The lower lighting intensity found at our field sites elicited weaker responses than the higher lux levels of our experimental mesocosm studies, where we found that elevated ALAN intensity was associated with responses in insects, predatory fish, and bats. We only found limited evidence pointing to differences in ecological effects of HPS vs. LED lighting, yet the literature cautions that large-scale

adoption of energy-efficient white LED lighting may exacerbate ecological impacts due to increased blue-light radiation (31, 32).

Vulnerability to ALAN is a function of exposure to artificial lighting combined with the sensitivity of taxa and ecosystem processes to altered light regimes. Thus, there are multiple complex processes interacting that ultimately determine the effects of light trespass into ecologically sensitive areas, such as streams, riparian zones, wetlands, and rivers.

Coupled with an extensive literature search, our findings support the following recommendations (Fig. 19).

ARTIFICIAL LIGHTING AT NIGHT (ALAN)

Recommendations for Roadway Lighting

Artificial lighting at night alters natural patterns of light and dark and can have serious implications for wildlife and ecosystems.

Best management practices for Ohio roadway lighting should consider:

Maintaining intensity of light trespass to less than 1 lux.

Moonlight ≈ 0-1 Lux

Reducing lighting trespass into sensitive aquatic and riparian ecosystems through light shielding and by maintaining healthy riparian buffer zones.

Minimizing lighting duration by using automatic timers and motion sensors.

Avoiding strobing lights from passing traffic by minimizing the use of slatted railings on bridges.

Unless managing for specific species, focusing mitigation efforts on light intensity may be most beneficial. If LED are used, limiting blue-light will be an important conservation consideration.

HPS vs. LED

Figure 19. Recommended ALAN best-management practices for Ohio roadways.

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Appendix A

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Appendix B

Additional Figures
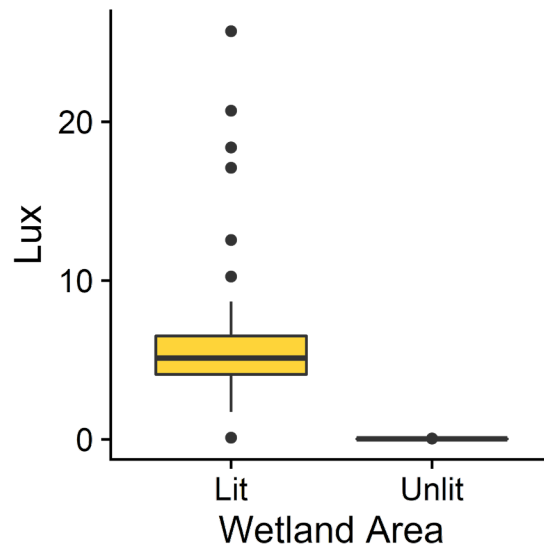
Appendix B Figure 1. Boxplot showing first quartile, median, and third quartiles of lux data in lit and unlit areas of the wetland experiment. Whiskers represent minimum and maximum lux (range) while individual points mark outliers.

Appendix B Figure 2. Experimental wetland light design at OSU's ORWRP.