

Effects of Artificial Light at Night on the Visual Acuity of Bluegill

Lepomis macrochirus

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Abstract

As worldwide human populations continue to increase and reside near aquatic environments, the impacts of such actions may become clearer. Artificial light at night is one way that humans have affected the natural environment. Bluegill (*Lepomis macrochirus*) are one of the most widespread species of fish throughout North America. This study tested the hypothesis that persistent artificial light at night affects the visual acuity of Bluegill. In order to test this, Bluegill were held under 4 different light treatments for approximately 3 months, which included high (15 lux), medium (8 lux), low (2 lux), and control (0 lux). Then, an experimental chamber was used to measure the reaction distance (i.e. maximum distance Bluegill could see the food) and latency (i.e. time to discover the food) of a Bluegill detecting freeze-dried shrimp under their respective light treatment. Two separate one-way ANOVA tests revealed no significant differences in reaction distance or latency when Bluegill were held under different light treatments. Ultimately, visual acuity of Bluegill was not significantly affected by artificial light at night under these intensities and spectral composition conditions.

Introduction

Artificial light at night (ALAN) has become a worldwide anthropogenic pollutant to natural environments, especially near human communities (Cinzano et al. 2001, Falchi et al. 2016). Given technological advancements and an increasing human population, the natural environment is becoming more affected by ALAN (Cinzano et al. 2001, Longcore and Rich 2004, Gaston et al. 2013). Humans tend to congregate near bodies of water, thus ALAN heavily affects nearby aquatic ecosystems (Kummu et al. 2011). Research has shown that ALAN affects both terrestrial and aquatic communities. For example, European blackbirds *Turdus merula* developed their reproductive system one month earlier and molted earlier when exposed to ALAN (Dominoni et al. 2013); hatchling loggerhead sea turtles *Caretta caretta* became disoriented when exposed to ALAN (Witherington and Karen 1991); circadian rhythms of European Perch *Perca fluviatilis* were altered due to ALAN (Brüning et al. 2015); and Smallmouth Bass *Micropterus dolomieu* exhibited more aggressive nest guarding under ALAN, potentially reducing their reproductive success due to elevated energetic demands (Foster et al. 2016).

Recent ALAN research has mostly focused on individual or population level effects, but there has been limited research expanded to higher community levels, such as species, communities, or ecosystems (Zapata et al. 2019). ALAN can alter community structure and ecosystem function in streams due to aquatic-terrestrial fluxes of invertebrates (Meyer and Sullivan 2013). The goal of my study is to provide more insight into the effect of ALAN on the visual acuity (i.e. sharpness of vision) of Bluegill and how this influences higher community levels.

Bluegill are one of the most widespread fishes in North America, particularly abundant in the central and eastern regions of the United States (Trautman 1981). They provide a crucial link in many aquatic and terrestrial food webs (Mittelbach and Osenberg 1993, Olson et al. 2003, Raikow 2004, Roth et al. 2007). Additionally, Bluegill are a valuable contributor to the economy through recreational fishing and as a food source (Hughes 2015). Bluegill are a preferred target for anglers compared to other sport fish, such as Largemouth Bass and Walleye, and fishermen show locational preference to waters with a strong Bluegill fishery (Tingley et al. 2019).

ALAN may alter the visual acuity of Bluegill, which we seek to better understand. Visual acuity is a measure of an organism's ability to discern an object from its surrounding, or the sharpness of vision (Cronin et al. 2014). Visual acuity can be experimentally measured by the reaction distance of an organism (Neave 1984). Reaction distance is the maximum distance that an organism can detect a visual stimulus (Nieman and Gray 2018). Reaction distance can decrease under reduced light intensity (Miner and Stein 1993, Fraser and Metcalfe 1997). Bluegill, a common centrarchid sunfish in Eastern North America, can locate invertebrate prey from a greater distance under greater light illumination (i.e. greater light intensity) than in less illuminated conditions (Vinyard and O'brien 1976). Also, it may take fish longer to locate prey and detect predators under reduced light intensity conditions (James and Heck 1994). Lux is a measure of light intensity as perceived by humans. Urban streams subjected to high ecological light pollution (ELP) recorded light intensities up to 2.1-4.0 lux and darker streams with low ELP were measured as 0.1-0.5 lux (Meyer and Sullivan 2013). However, in streams with open canopy near dense lighting of roads, stores, and stadiums, light intensities were 8-12 lux (Meyer and Sullivan 2013). Changes in visual acuity of Bluegill from the effects of ALAN may hinder their ability to forage for food (Vinyard and O'brien 1976, McMahon and Holanov 1995,

Nieman and Gray 2018), detect predators (James and Heck 1994), and find mates (van der Sluijs et al. 2011).

The objective of this research study was to determine if there are differences in visual acuity after being held under four different intensities of artificial light at night (ALAN). Bluegill held under high intensity (i.e. high lux) ALAN were predicted to exhibit greater visual acuity than those held under low intensity (i.e. low lux) ALAN.

Methods

Holding Tank Conditions

Bluegill (n =330) were acquired from Fender's Fish Hatchery in Baltic, Ohio to be the test subjects of this research study. The Bluegill were held at the Wilma H. Schiermeier Olentangy River Wetland Research Park located on the Ohio State University campus in Columbus, Ohio. They were cared for in holding tanks for approximately 3 months (August 6 – October 27, 2020) before they underwent experimental trials. Bluegill were held in 1,000-gallon cylindrical holding tanks at a density of 15 fish per tank. Each tank contained a filter, dissolved oxygen stone, and 4 plastic plants for cover. Four holding tanks per treatment were arranged in a 2 × 2 square cluster beneath a single light source (Figure 1). Additionally, two back-up tanks under control (i.e. 0 lux) conditions were set aside if needed. Bluegill from backup tanks were used to replace any Bluegill from treatment groups that dies in order to keep fish density in each tank constant.

To create treatment conditions, a Lepower 50-watt LED flood light was placed above each corresponding treatment group of 4 holding tanks. Spectral composition produced by an LED light is shown in Figure 2. Neutral density filters covered each light to create the 4 light

treatments including: high (15 lux), medium (8 lux), low (2 lux), and control (0 lux). Neutral density filters only change light intensity, they do not change the spectral composition of the light. The light intensity was the same for each of the 4 holding tanks within light treatments. The lights were turned on and off every day at sunset and sunrise, respectively. Black curtains enclosed each treatment group of holding tanks at night to keep stray light out and ensure accurate light intensity exposure to Bluegill. Water quality and environmental conditions were recorded to maintain the same conditions across treatments. Air temperature, water temperature, and dissolved oxygen measurements were taken twice daily. Conductivity, pH, ammonia, nitrite, salt concentration, and nocturnal light measurements were taken weekly. Bluegill were fed Optimal Bluegill floating pelleted fish food every night.

Experimental Trial Conditions

Reaction distance (i.e. maximum distance Bluegill could see the food) and latency time (i.e. time to discover the food) trials were performed on Bluegill from each of the 4 treatments: 15 lux ($n = 10$), 8 lux ($n = 10$), 2 lux ($n = 9$), and 0 lux ($n = 8$). The experimental chamber was a clear aquarium with dimensions of 86.0 cm long \times 21.5 cm wide \times 30.0 cm high (Figure 3). Black cloth curtains surrounded the tank on the back and sides to minimize external light and disturbance during the experiments. Neutral density filters were used to match the light intensity (i.e. lux) of the experimental chamber to the corresponding light intensity treatment of the holding tanks. A Bt-meter Digital Illuminance Light Meter measured the light intensity in lux for each experimental trial to ensure it matched holding tank conditions for that treatment. Clear well water was used to fill the experimental chamber, the same water source used in the holding tanks. A Marine Metal Aerator was used to ensure dissolved oxygen levels were at least 8 mg/L

or above before each trial began. A YSI Water Quality Meter measured temperature, dissolved oxygen, and conductivity before each trial to match conditions of the experimental chamber and holding tanks. A TDS Water Meter measured the approximate salt concentration to validate experimental chamber and holding tanks contained ~2,000 mg/L and added API Aquarium Salt if needed. An API Aquarium Test Kit measured ammonia and nitrite. A Vivosun pH probe measured pH levels. The experimental chamber conditions were similar to those of the holding tanks in temperature, dissolved oxygen, salt concentration, conductivity, PH, ammonia, nitrite, and light treatment intensity. Total length of each freeze-dried shrimp was measured and recorded before the trial began. A ruler was placed along the front of the tank for reference in later reaction distance analyses from the trial videos. An Amcrest camera recorder with night vision capabilities was placed on a tripod in front of the tank to record behaviors without interference from human presence. There was a black barrier in the middle of the chamber, which separated the individual Bluegill and prey item until the trial began.

Reaction Distance Experimental Process

All Bluegill were starved for 24 hours prior to any experimental trials to help standardize motivation to forage. The first Bluegill randomly netted from a holding tank was used for each trial per their respective treatment conditions. Experimental trials were conducted between the hours of 11:00 and 23:30 from October 18 to October 27, 2020. The Bluegill was placed on the right side of the barrier to acclimate to water conditions of the experimental chamber for 30 minutes. Then, a freeze-dried shrimp was placed on the left side of the barrier; the barrier was then removed. The fish was recorded for an additional 20 minutes after barrier removal to

observe when (latency) and from how far (reaction distance) they detected the prey item after the barrier was removed.

When the fish turned toward the prey and looked directly at it, the video was paused to measure reaction distance (Figure 4). Reaction distance was determined by measuring the distance from the eye of the Bluegill to the center of the floating freeze-dried shrimp. The software program, ImageJ, was used to compare a known distance on the ruler placed on the outside of the tank in the video to the distance between fish and prey. Latency was recorded as the time (s) between barrier removal and detection of prey. Total length of each Bluegill was measured and recorded at the end of the trial.

All Bluegill were humanely euthanized in clove oil at the conclusion of each trial in accordance with IACUC Protocol #2014A00000055-R2. Fish were preserved in 10% buffered formalin for future morphological assessment.

Statistical Analyses

Separate one-way ANOVAs were used to determine if there were significant differences in reaction distance and latency between light treatments. Normality and homogeneity of variance were tested using a Chi-square test and Levene's test, respectively, to ensure the data was within normal bounds. Significance tests were followed with post hoc Tukey's tests for all pairwise comparisons among treatments. All statistical analyses were conducted in the Excel Microsoft computer program.

Results

The dependent variables were the reaction distance and latency to detect the prey item, and the independent variable was the light treatments (15, 8, 2, 0 lux). Bluegill, used for both reaction distance and latency time trials, ranged in size from 65 to 117 cm in total length (mean total length \pm SE= 85.14 \pm 1.87). The freeze-dried shrimp ranged in size from 0.7 to 1.2 cm in total length (mean total length \pm SE= 0.86 \pm 0.03). There were no significant differences between light treatments for total length of Bluegill ($F_{3,34}=0.179$, $P = 0.910$) or total length of freeze-dried shrimp ($F_{3,34} = 1.659$, $P = 0.195$), so these were not significant factors on the targeted objectives. Experimental chamber conditions were similar between the holding tanks and experimental chamber in temperature, dissolved oxygen, salt concentration, conductivity, PH, ammonia, nitrite, and light treatment to reduce extraneous variables in this experiment (Figure 5). No significant differences were detected in the reaction distance between light treatments ($F_{3,34} = 0.482$, $P = 0.697$; Figure 6). In addition, there were no significant differences in latency to detect prey between light treatments ($F_{3,34} = 2.476$, $P = 0.864$; Figure 7). Ultimately, visual acuity of Bluegill was not significantly affected by the 3-month holding period under the respective light treatment.

Discussion

Although there was no significant difference in visual acuity or latency to detect prey across light treatments, these findings still demonstrate a biological interaction that aligns well with our predictions. These results can improve our understanding of the complicated interactions between ALAN and aquatic environments. One explanation for our results could

simply be that visual acuity in Bluegill is not impacted by ALAN or that these light conditions were too low to induce significant changes to be observed between treatments.

Visual acclimation to their light treatment conditions over the 3-month holding period could be one explanation for this lack of difference between treatments. If a Bluegill becomes accustomed or makes a rapid physiological change in vision to respond to a light intensity at night, then one may expect such an individual to react similarly during the experimental trial under the same light conditions (Landsberger et al. 2008). Their eyes may be adequately fit for seeing the greatest distance in those experimental conditions, which may provide one possible explanation for these results (Warrant 2004).

Also, water conditions and structure may play a role in limiting the effect of ALAN on a Bluegill's visual acuity. For example, if turbid water or submerged structures block ALAN or change the light intensity, fish may not experience changes in visual acuity after being subjected to various light treatment conditions (Oldham 2018, Nieman et al. 2018). The experimental trials were performed in Columbus, Ohio in late October, when temperatures start to decrease as winter approaches. Their slowed metabolism due to colder temperatures may reduce their need to forage during the experiments and could influence the effectiveness of measures based on these assumptions (Shoup and Wahl 2011). In addition, trials were recorded during the day and evening, which could explain some differences in their rhythmic need to forage if the Bluegill have been accustomed to being fed at night (Davis and Bardach 1965). In natural settings, Bluegill often feed on live prey in the water column with movement, so experimental trial conditions that can replicate this may elicit a better feeding response than floating stationary prey (Werner and Hall 1974).

Future research could explore how visual acuity of Bluegill changes when subjected to ALAN other than the light intensity they were held under. In addition, research could identify how long it takes for physiological changes, such as visual acuity, to occur after being subjected to ALAN. Research projects could test more extreme light intensities, which may show a more significant interaction than the light intensities we tested. Other shifts induced by ALAN could be explored through field sampling of macroinvertebrates and fish to determine relative abundance of species, dietary differences, or gonadal differences between various intensities of ALAN that may influence an individual's fitness. The visual acuity of various fish species may be affected differently by ALAN. For example, fish species relying primarily on vision to find prey (e.g. Saugeye) may be impacted more than those that rely more on chemoreception (e.g. Channel catfish). Research exploring the effects of ALAN on the connection between terrestrial and aquatic environments may be beneficial as well. For example, consumption of terrestrial flying insects by fish near ALAN may become more pronounced and lead to community shifts (Meyer and Sullivan 2013).

Fixed pupils in a fish's eyes effect the amount of light that enters the eye (Cronin et al. 2014). Larger eyes allow more light to enter, thus individuals with larger eye size have an increased proportional effect on their visual capabilities (Fernald 1993). Greater visual acuity was positively correlated with larger eye in 159 species of ray-finned fishes (Caves et al. 2017). Morphological adaptations, such as larger eye size and larger pupil diameter, increased visual abilities in aquatic systems of low light levels (Pankhurst 1989). Pupil diameter can be used as a measure of visual acuity (Oldham 2018). In future research studies, pupil diameter could be used as a proxy to understand the effects of ALAN on visual acuity between various light intensities.

One would expect greater pupil diameter length to demonstrate greater visual acuity in fishes, such as Bluegill.

As the results indicate, understanding the effects of ALAN on the visual acuity of individual Bluegill may provide more insight into widespread effects of ALAN as human populations continue to increase. These results may provide further understanding into the effects of ALAN on the vision of other *Lepomis* fishes, such as Green Sunfish or Longear Sunfish. This may provide links to observed changes in higher communal levels, such as population or community shifts. For example, invertebrate community composition was changed by moderate to high levels of ALAN, particularly favoring predators and detritivores (Sullivan et al. 2019). Understanding the effects of ALAN may help to inform legislation and government agencies, such as the Ohio Department of Transportation, on optimal lighting practices at night, specifically concerning fish and aquatic environments. Continued communication between various organizations may provide adequate solutions to minimize human impacts on the natural world.

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Figures



Figure 1. Holding tanks grouped into clusters of 4 tanks per light treatment.

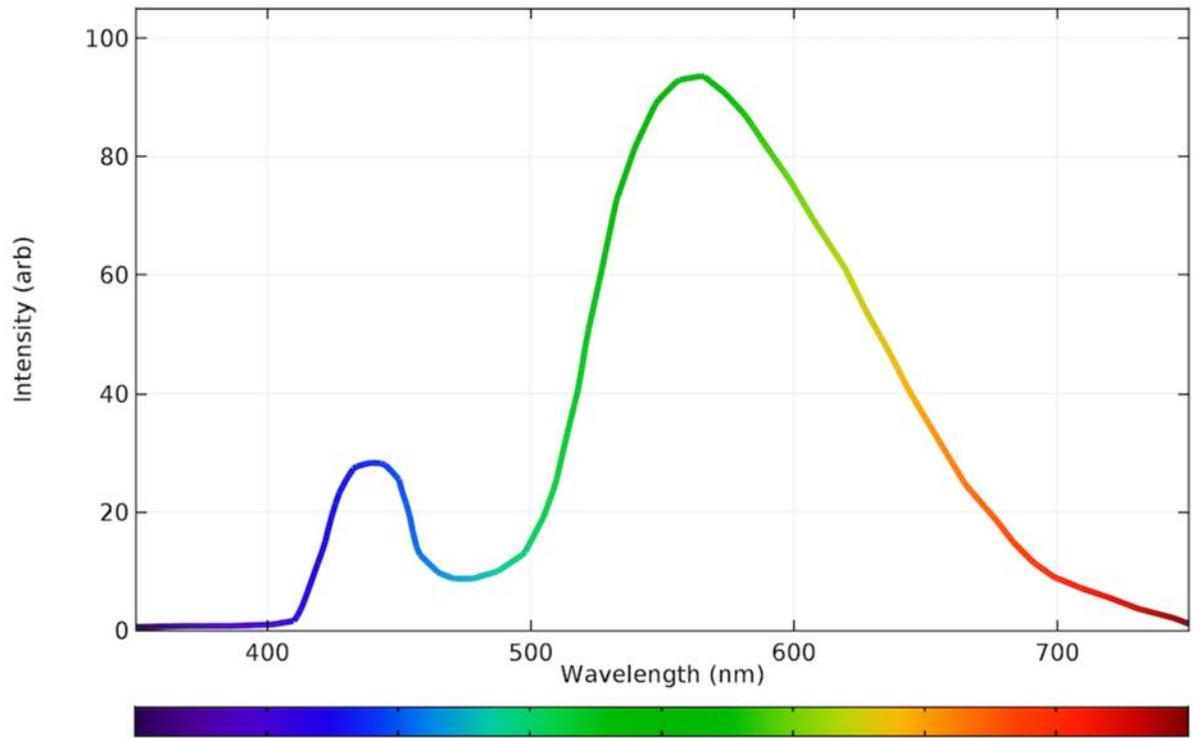


Figure 2. Spectral composition of LED light Bluegill were exposed to in both the experimental chamber and holding tanks.

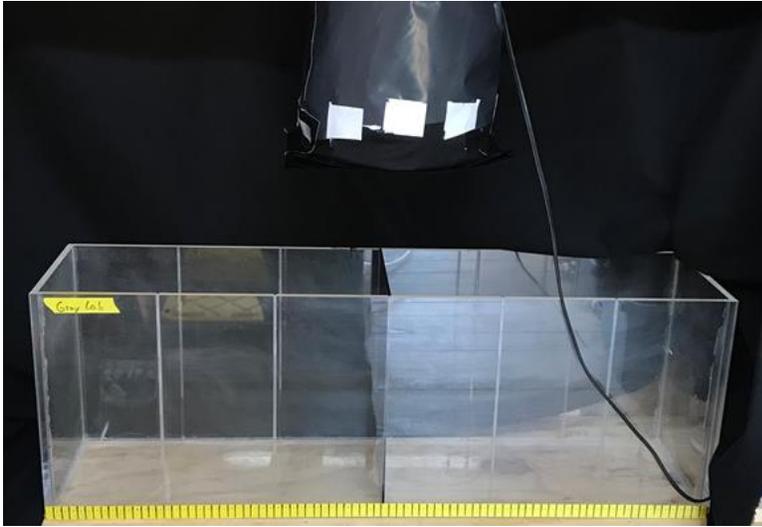


Figure 3. Experimental chamber design consisted of an artificial light overhead, reference measurements, and black barrier present in the middle.



Figure 4. Reaction distance measurement of a Bluegill as it turns upward to look at the floating freeze-dried shrimp.

	Experimental Chamber	Holding Tanks
High Light Treatment (lux)	15.0	15.0
Medium Light Treatment (lux)	8.3	8.0
Low Light Treatment (lux)	1.6	2.0
Control Light Treatment (lux)	0	0
Dissolved Oxygen (mg/L)	9.76	7.64
Temperature (°C)	12.85	10.93
Conductivity (S/m)	3184.00	3283.25
PH	7.30	8.02
Salt concentration (mg/L)	2200	1900
Ammonia (mg/L)	0	0
Nitrite (mg/L)	0	0

Figure 5. Average conditions were similar between experimental chamber and holding tanks.

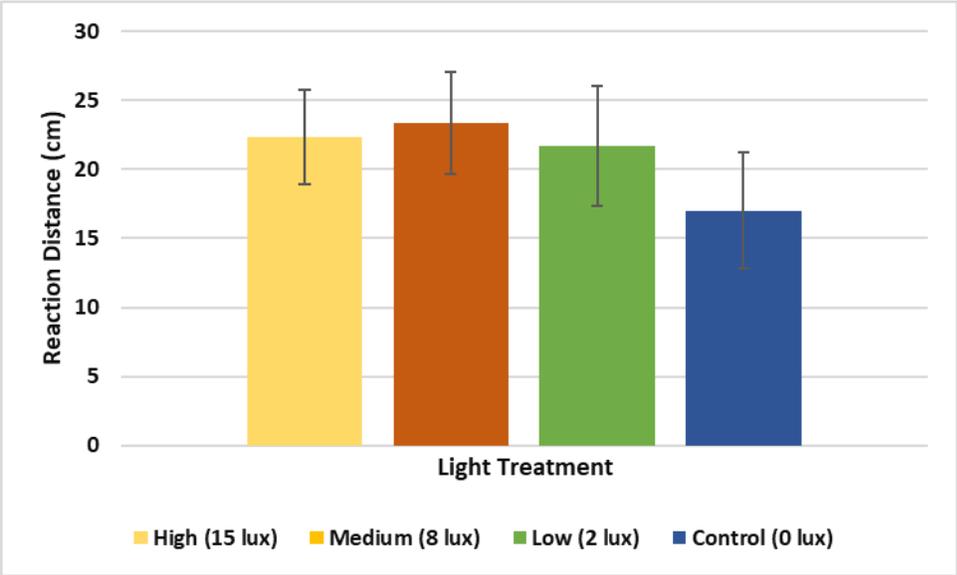


Figure 6. Mean reaction distance (\pm SE) between light treatments. One-way ANOVA ($F_{3,34} = 0.482, P = 0.697$) indicates that they are not significantly different from each other.

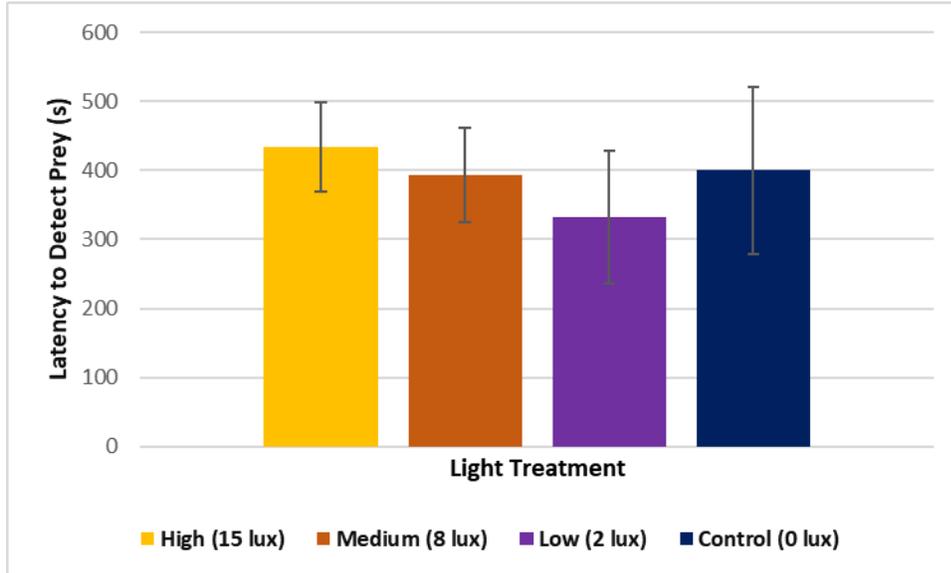


Figure 7. Mean latency time (\pm SE) between light treatments. One-way ANOVA ($F_{3,34} = 2.476$, $P = 0.864$) indicates that they are not significantly different from each other.