THE BEHAVIORAL RESPONSES OF TWO APPALACHIAN CRAYFISH TO COOL AND WARM SPECTRUM LED LIGHT AT NIGHT

A Thesis
by
JUSTIN RYAN FISCHER

December 2016
Department of Biology

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Abstract

THE BEHAVIORAL RESPONSES OF TWO APPALACHIAN CRAYFISH TO COOL
AND WARM SPECTRUM LED LIGHT AT NIGHT

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Ecological light pollution is increasing worldwide, and the use of artificial lighting is
expected to increase during the coming decades. The threats posed by light pollution to
freshwater ecosystems are not well-studied. Light-emitting diodes (LEDs) are currently the
preferred luminaire technology and have largely replaced incandescent, fluorescent and high-
intensity discharge lights across much of the developed world. Two different types of LEDs
are in widespread use. Cool-spectrum LEDs are characterized by shorter wavelength cool-
hued light whereas warm-spectrum LEDs are characterized by longer wavelength, warm-
hued lights. It is not clear how the different spectral emissions produced by these two LED
categories will affect freshwater animals. The New River crayfish (*Cambarus
chasmodactylus*) and the spiny stream crayfish (*Orconectes cristavarius*) are important
ecosystem engineers with influences on food webs, community structure and nutrient
processing. I used artificial stream microcosms to expose both species to cool (5000 K) and
warm (3000 K) spectrum lights at intensities of 15 lux. I recorded crayfish behaviors under
each light treatment and in a dark control during the first and third hours after artificial dusk.
In addition, I quantified total substrate displacement for *C. chasmodactylus* in these

treatments. I found that *O. cristavarius* sheltering behavior significantly increased and locomotor activity decreased while exposed to both 5000 K and 3000 K light. *Cambarus chasmodactylus* significantly increased sheltering behavior and exhibited decreased mobility in response to 5000 K but not 3000 K lights. Substrate displacement by *C. chasmodactylus* was not affected by light treatments. Reduced crayfish mobility may lead to fewer foraging opportunities and reduced bioturbation in light-polluted streams. Species-specific responses to artificial light complicate our ability to make generalizations about the effects of LEDs on freshwater ecosystems. Further investigations are needed to understand how the effects of LEDs differ among freshwater taxa as well as how long-term exposure to cool and warm LED spectral emissions may alter the behaviors of freshwater taxa and key ecosystem processes.

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Center and Refuge, and the University of North Texas. Thank you President Barack Obama for working to address climate change and biodiversity loss, future generations will be grateful for your efforts.

Mom, dad and Joseph, thank you for always supporting me and encouraging me to do what I love. Dad, I will always remember our walks on trails, and how you took me to any state park that I mentioned as a child. I hope you know that you stimulated my love for nature when it mattered most. Mom, to this day I receive the most encouraging words from you. Thank you for taking me to so many science activities. Joseph, you are a great brother and friend. Harness your creativity and excel at what you love. Never stop learning and don't forget about what the natural world can teach us.

Louann and Ramon Fagundo, without your constant love and support, I would not have met a single deadline. You were both crucial to the success of my research. Raquel, thank you for being my best friend, collaborator and wife. We have many great times ahead of us. Nica, if you weren't such a happy baby, I don't know if I would have completed this research. I can't wait to explore the world with you. Your mother and I will work hard to keep this planet healthy for you to enjoy.

Dedication

I would like to dedicate this work to my wife,

Raquel Fagundo,

and to our daughter,

Veronica "Nica" Fagundo Fischer.

May we never stop exploring nature as a family.

Table of Contents

List of Tables

List of Figures

Foreword

This research will be submitted to the peer-reviewed journal *Freshwater Science*. It has been formatted to fit the requirements for that journal.

INTRODUCTION

Anthropogenic light sources have illuminated the night on a large scale since the beginning

of the Industrial Revolution (Longcore and Rich 2004). Nighttime illumination is now

increasing at an annual rate of 6% worldwide (Hölker et al. 2010). Through increased

urbanization and technological advances, the quantity of lights is increasing and spectral

qualities of light installations are diversifying. Direct glare from individual lights and sky

glow from atmospheric refraction may influence the behavior of nocturnal and crepuscular

organisms (Gaston et al. 2014). This artificial illumination is referred to as *ecological light*

pollution (ELP). While there has been an awareness of the potentially negative impacts of

ELP (e.g., disorientation of turtle hatchlings) for a few decades, it is only recently that there

has been a concerted effort to understand its effects on organism behavior and ecosystem

processes (Longcore and Rich 2004).

Organisms perceive a range of electromagnetic wavelengths depending on optical

physiology. While human vision is in the 400-700 nm range, many species detect 300 nm

ultraviolet (UV) light, and various taxa use linearly polarized light for navigation (reviewed

in Horváth et al. 2009). Wavelength perception deserves attention due to the plethora of

lighting technologies that emit both broad and narrow-spectrum light. Further, changes in

lighting technology can drastically alter which organisms are affected (Davies et al. 2013,

Pawson and Bader 2014). Light-emitting diodes (LEDs) are becoming the preferred light

source in both urban and rural areas. Although LEDs reduce energy consumption and provide

more light per watt, their broad spectrum characteristics suggest that they could affect an

array of nocturnal and crepuscular behaviors. Efforts are being made to create more

biologically appealing LED models, with wavelengths emissions that are less detrimental to

various species in a variety of ecosystems (Longcore et al. 2015, Spoelstra et al. 2015, Žukauskas et al. 2014).

It is estimated that ~30% of vertebrates and >60% of invertebrates are nocturnal which means that ~1-5 million species may be impacted by altered light regimes globally (Hölker et al. 2010). To date, much of what we know about the effects of artificial light at night (ALAN) on nocturnal taxa has been elucidated from studies of just a few vertebrate taxa. Briefly, ALAN has been shown to affect orientation (Squires and Hanson 1918, Witherington and Bjorndal 1991), physiology (Navara and Nelson 2007, Fonken and Nelson 2014) and behavior (Miller 2006, Robertson et al. 2010, Kurvers and Hölker 2015) of vertebrate taxa. The effects of ALAN on invertebrates have not been extensively studied but entomologists have exploited the fixating capabilities of a range of artificial light sources for as long as they have existed (Williams 1939). Moore et al. (2001) found that ALAN alters *Daphnia* vertical migration. Nocturnal insects fixate upon, become disoriented by and are rapidly drawn to a range of ALAN sources (Frank 1988, Eisenbeis and Hassel 2000, Eisenbeis 2006). Research has shown that firefly (Coleoptera: Lampyridae) communication is hampered by ALAN (Lloyd 1994). Large lights in terrestrial habitats can change local invertebrate community composition over time (Davies et al. 2012) and Geffen et al. (2015) found that long-term exposure to ALAN may inhibit Geometrid moth reproductive hormone production. Effects of ALAN on aquatic invertebrate communities and freshwater ecosystems have not been well-documented (Gaston et al. 2015). It is crucial that researchers work to separate the effects of other stressors from ELP effects in order to understand the consequences of increased nocturnal illumination.

2

We know comparatively little about the effects of ALAN on freshwater ecosystems and processes (Moore et al. 2001, Longcore and Rich 2004, Perkin et al. 2011). The seminal book *Ecological Consequence of Artificial Night Lighting* by Rich and Longcore (2006) contained a contribution by Moore et al. (2006) that was the first to predict that ALAN is likely to disrupt freshwater systems. Streams and their associated riparian zones are hotspots for biodiversity and sites of high productivity at the local and landscape scales. Streams and stream biota provide important ecosystem services including nutrient sequestration and processing, water purification and flood control. Although freshwaters cover a mere 0.8% of the planet, 9.5% of all animal species reside in these habitats (Balian et al. 2008). Perkin et al. (2011) outlined four research domains that emphasize the threats of ALAN to stream ecosystems: 1) effects on dispersal, 2) population genetics, 3) ecosystem functioning, and 4) interactions with other stressors. These domains have been the focus of subsequent studies that have not only provided some answers, but also introduced new questions.

Early studies investigating the role of light in aquatic invertebrate life histories assessed the effects of natural light regimes and predators on the foraging behaviors of mayflies (Wodsedalek 1911, Lyman 1945, Kohler and McPeek 1989, Scrimgeour and Culp 1994). During the daylight hours, many mayfly species remain on the underside of substrates, where they are safe from visually-feeding, vertebrate predators. As night approaches, these individuals typically move to the upper surface where periphyton growth is more abundant (Peckarsky 1996, Schloss 2002, but see Kohler 1983). Elliot (1968) and Waters (1972) investigated how mayfly adaptive compromises are influenced by natural photoperiod regimes. Relative light change (RLC) has been determined to be the key mechanism behind

the shift from diurnal foraging behavior to nocturnal foraging for the mayfly nymphs of *Stenonema modestum* (Schloss and Haney 2006).

Under naturally occurring photoperiods, aquatic insect richness, abundance and community structure remained unchanged (Guareschi et al. 2016). However, mounting evidence suggests that the introduction of artificial light sources disrupts activity periods, orientation and distributions of several stream taxa. For example, adult aquatic insects fixate on glare light sources (Nowinszky et al. 2012). Meyer and Sullivan (2013) studied aquatic insect emergence and riparian spider densities in illumination treatments of 0.1-12.0 lux, and found a decrease in spider density and emergent aquatic insect family richness with light. Henn et al. (2014) found that in high-clarity central Texas streams, aquatic insect drift abundance decreased by 37% under high light pollution. Interestingly, they did not find a difference in insect richness or diversity. However, Andersson (2015) did find a significant decrease in species richness in the drift proximate to ALAN. Emergent aquatic insects are drawn farther into the terrestrial environment in the presence of riparian light (Perkin et al. 2014a), possibly affecting aquatic-riparian nutrient transfer. Freshwater amphipods (*Gammarus*) have been studied due to their widespread distribution, with surprising results. Gammarid drift rates did not change once experimental enclosures were exposed to a range of illuminations, except when individuals were isolated in smaller enclosures (Perkin et al. 2014b). It has become apparent that the effects of ALAN on stream dispersal are variable and likely species-specific. Any change in drifting abundance and/or richness could have a detectable impact on community structure, population connectivity and resources for insectivorous fish.

Crayfish can influence the distributions and abundances of other macrovinvertebrates, macrophytes, detritus, algae and sediment (Creed 1994, Creed and Reed 2004, Lodge et al. 1994). Despite extensive studies of the ecological roles and life histories of several crayfish, there are still gaps in our knowledge of ecological interactions and conservation threats (Helms et al. 2013). ALAN studies may contribute to our understanding of how crayfish function in ELP-altered environments. Further, they may contribute to our understanding of crayfish invasions if invasive species are more tolerant of ELP. Crayfish activity periods can be measured as a proxy for understanding how ALAN might affect a number of things, such as inter- and intraspecific contact, ecosystem engineering, and foraging behaviors. Thomas et al. (2016) found that under high-pressure sodium light of 12 ± 5 lux, naturalized populations of the invasive signal crayfish (*Pascifastacus leniusculus*) showed reduced activity, increased sheltering, and reduced interactions with conspecifics. Theirs was the first study to investigate how light pollution might affect crayfish, as well as the first look at how an invasive species responds to ALAN.

Abeel et al. (2016) investigated the effects of cool (5500 K), neutral (3800 K) and warm (2600 K) LED light on the stress responses of the noble crayfish (*Astacus astacus*) in a closed system designed to simulate the conditions of crustacean aquaculture for the production of seafood. They applied what they considered to be bright (761 lux) and weak (38 lux) intensities. Both of these intensities are far higher than would be experienced by wild crayfish, yet they are common in aquaculture. They found indications of stress in response to brighter light, but not to different light colors. Crayfish are known to perceive polarized light, as do many other freshwater invertebrates (Muller 1973). It is probable that crayfish utilize polarized light as well as natural lighting for orientation during both diurnal

and nocturnal movements. The effects of ALAN on crayfish may be species- or even population-specific due to the range of water depths, turbidities, riparian covers, and land uses that comprise each habitat.

For this study, two regionally common crayfish species were selected for trials. The New River crayfish (*Cambarus chasmodactylus*) and spiny stream crayfish (*Orconectes cristavarius*) are both common in the headwaters of the New River in Watauga and Ashe counties, North Carolina, USA (Helms and Creed 2005, Fortino and Creed 2007). *Cambarus chasmodactylus* is endemic to the New-Kanawha River Drainage and occurs in low-to mid order streams with large rock slabs (Loughman et al. 2013). Although both *C. chasmodactylus* and *O. cristavarius* may occur syntopically, *O. cristavarius* is also abundant in gravel-bottomed pools and runs. *Orconectes cristavarius* is native to the New River, but also occurs in other Ohio River tributaries including the Big Sandy and Kentucky rivers (Taylor and Schuster 2005). Locally, *O. cristavarius* may be undergoing a range expansion as it has moved into and become abundant in tributaries of the South Fork of the New River (Fortino and Creed 2007, RP Creed pers. comm.).

The objectives of this study were to 1) determine if these two crayfish species show changes in nocturnal activity under ecologically relevant ALAN levels, and 2) discern whether these two species exhibit any differences in the responses to warm spectrum (3000 K) and cool spectrum (5000 K) LED lights. Comparing the responses of these two species will provide novel insights into how two co-occurring genera of crayfish are affected by artificial light. Comparing two types of LED light on crayfish behavior could also provide valuable information on which type of LED might have less of an impact on aquatic species.

The following hypotheses were made concerning the responses of both species to ALAN: 1) cool LED lights should cause a more pronounced change in crayfish behavior, and 2) the habitat specialist *C. chasmodactylus* would be more affected by nocturnal illumination, while the habitat generalist *O. cristavarius* would be less affected.

METHODS

LED Lights

All light technologies are marketed with a calculated CCT to indicate the color of the light, irrespective of lighting technology. The CCT correlates to the spectral characteristics of the light emissions. Lights with a CCT between 2000-3500 K are generally described as 'warm' colored, appearing more yellow-orange. Lights within 4500-6500 K are described as 'cool' colored, and appear bluer. Neutral white colors are between these two classifications. Warmer lights emit more light in the 600 – 700 nm range, and cooler lights are stronger in the 400-500 nm range. While these designations describe the light that humans perceive, different technologies emit different wavelengths to achieve these CCTs, which underscores the importance of considering the impacts of each type of LED light on biota. The concurrent prevalence of these two categories of LED lights in urban and suburban areas warrants the study of how they may impact stream species.

Recent studies have begun to delineate what is considered 'ecologically relevant' ALAN (Thomas et al. 2016). Some have used unrealistically intense experimental treatments that are unlikely to be found in nature (Abeel et al. 2016), providing data that are irrelevant to ecologists. By designating ecologically relevant light pollution levels to be 1-20 lux, these studies made their findings more meaningful and applicable to the considerations of future urban planners.

Experimental design

       The laboratory setup consisted of five 38 l aquaria for acclimation, and three 38 l aquaria for experimental trials. Acclimation aquaria were on separate but adjacent shelves. All aquaria contained a uniform gravel substrate to a depth of 5 cm. Each aquarium contained a shelter created by placing two three-holed red clay bricks side-by-side to create three tunnels 10 cm long with a diameter of 4.5 cm (Figure 1). A 2000 l h^{-1} wave-maker pump (SunSun Model: JVP-110) was placed in the corner above each shelter to create a stream-like current and maintain dissolved oxygen saturation. Aquaria were covered by metal hardware cloth in order to prevent crayfish from escaping. All doors, and windows were blacked-out to prevent light penetration. The entire setup was kept in a 16˚C climate-controlled room. Each acclimation and experimental aquarium had a weekly 50% water replacement with water collected from the South Fork of the New River.

       Equiline LED 12V puck lights (Tresco, model L-POC-3EQFR-CNI-1; model L-POC-3EQFR-WNI-1) were placed 1.25 m above each tank. Two CCTs were used, 5000 K (cool white) and 3000 K (warm white). Lights were covered with a layered black plastic mesh in order to dim the intensity to the desired level without changing the spectral emissions. This setup produced ecologically relevant light pollution of 15 ± 1 lux at the water surface. This intensity was chosen as a result of field observations of riparian light pollution near the Appalachian State University (ASU) campus in Boone, NC. Hodges Creek at Boone Creek Drive had water-level light intensities of 22.5 lux. Boone Creek at Rankin Science Hall on the ASU campus had a stream-level light intensity of 6.6 lux. All light measurements were taken using a lux meter (Extech Instruments, model LT300).

Crayfish collection and acclimation

Light-naïve *Cambarus chasmodactylus* and *O. cristavarius* were collected from several stream reaches in the New River watershed in Watauga and Ashe counties, North Carolina, USA. No light pollution was present at the collection locations. Crayfish were caught by active collection with D-frame nets, seines and rock-turning by hand. Collected crayfish were immediately brought to the laboratory acclimation aquaria. Total carapace length (TCL) was measured, and sex was determined (Table 1, 2). Male gonopods were examined to determine whether the individual was in the form I or form II reproductive state. Two crayfish were acclimated in each aquarium, each with its own brick shelter. Individuals readily used these shelters, and minimal conflict was observed. Each animal was fed two pellets of TetraFin fish food every other day during acclimation. No food was given to individuals within 24 hours of their use in trials, nor during trials. Crayfish were returned to their respective collection sites after the completion of trials. Each animal was used in one trial. Measurements of each crayfish (*C. chasmodactylus* TCL 2.9-4.4 cm; *O. cristavarius* TCL 1.3-2.3 cm) did not indicate repeated use of any individuals.

For acclimation and trial aquaria, daytime illumination was created using full-spectrum fluorescent tube lights (Sylvania Model: F40DSGN50) at a water-surface intensity of 2100-3900 lux, depending on the measurement location in relation to the lights. This intensity is similar to natural illumination on a cloudy day, or under a partially shaded riparian canopy. Daytime lights were on from 0700-2000 hrs. Trials were conducted from April to June 2016.

Infrared night vision home security cameras (Netgear Arlo Q model: VMC3040) were used to record the nocturnal behaviors of crayfish during trials. Cameras were placed

facing into the front side of the aquarium with a view of the entire habitat. Supplemental infrared illumination was located above each aquarium in order to ensure adequate visibility during night vision mode.

LED trials

Each group of acclimated crayfish provided individuals for three consecutive nights of trials. Within the three trial aquaria, the location of the treatments (control darkness, 5000 K LED, 3000 K LED) was rotated each night to reduce the risk of a location effect due to any unforeseen difference in microcosm conditions.

On trial days at 1700 h, three crayfish were randomly chosen from the acclimation aquaria and each one was placed in a randomly selected experimental aquarium. Animals were allowed to acclimate to the experimental aquarium for 3 h. Crayfish acclimated to these aquaria rapidly due to their similarity with acclimation aquaria. At 1945 h, full spectrum lights were removed and cameras were placed in front of each tank. Blackout shelters constructed of matte black poster boards were created around, above and below each trial aquarium to isolate each treatment and to mimic the nocturnal environment. At approximately 2030 h, LED lights were turned on, and trials began. At the commencement of a trial period, the acclimation aquaria were completely enveloped in thick black plastic sheeting to avoid exposing them to experimental treatments.

Data collection and analysis

Video recordings of the first and third hours after the start of trials were analyzed. These hours were selected due to previously described crayfish activity periods (Page and Larimer

10

1972, Loughman et al. 2013). The total duration (seconds) and frequency per hour of sheltering behavior was recorded. Sheltering animals were either inside of the brick tunnels, between the bricks and the sides of the aquarium, or behind the bricks (e.g. Figure 2). These areas were considered shelter due to preliminary observations of crayfish retreating to these locations when threatened. Illumination was noticeably reduced in these locations.

Substrate disturbance was quantified for *C. chasmodactylus* only. A 1 cm^2 grid was placed on the front and back of each aquarium. At the start of each trial, substrates were leveled at 5 cm depth. Changes in substrate topography were recorded in cm^2 at 0700 h following each trial night. Photographs were taken of the substrate topography of each morning (example in Figure 3), and analyzed by counting the number of cells where substrate was displaced for each side of the aquarium. Preliminary observations of *O. cristavarius* did not find notable substrate disturbance. Sample size was not large enough for a comparison of sexes that would support drawing any conclusions, therefore these analyses were omitted.

Preliminary data analyses found that data violated the assumptions of the parametric one-way analysis of variance. Shapiro-Wilk tests found that normality was violated for both data sets. Bartlett and Levene tests found that data violated the assumption of equal variances. Transformations failed to correct for this. As a result, data were analyzed using a nonparametric Kruskal-Wallis one-way analysis of variance on ranks (Kruskal and Wallis 1952). A sequential Bonferroni correction (Holm 1979) was included to reduce the probability of a type I error ($\alpha = 0.025$). Post hoc Dunn's tests (Dunn 1964) were conducted for multiple comparisons when significant differences were found. Substrate disturbance data for *C. chasmodactylus* were analyzed using a Kruskal-Wallis test adjusted for ties (Conover

11

and Iman, 1981). All analyses were conducted using R software (R Development Core Team, 2016).

RESULTS

Cambarus chasmodactylus

Observation of *C. chasmodactylus* behavior at 2000 h (n = 13) found that under 5000 K light, the total time spent sheltering significantly increased (H = 7.63, d.f. = 2, *P* = 0.02; Figure 4a). At 2000 h, the frequency of sheltering decreased significantly under both light treatments (Table 3; Figure 4b). At 2200 hrs (n = 12), a non-significant increase in total sheltering time was observed under 3000 K light (Table 3; Figure 5a). At 2200 h, no significant changes in sheltering frequency were observed (Table 3; Figure 5b). Substrate disturbance for *C. chasmodactylus* (n = 13) was not significantly different among the treatments (Table 3).

Orconectes cristavarius

For *O. cristavarius* at 2000 h (n = 11) total time sheltering increased significantly under both 5000 K and 3000 K light compared to the control (Table 3; Figure 6a). At 2000 h, there was no significant increase in sheltering frequency (Table 3; Figure 6b). At 2200 h (n = 9), a non-significant increase in sheltering occurred under 5000 K lights (Table 3; Figure 7a). At 2200 h, there was no significant increase in sheltering frequency (Table 3; Figure 7b).

A paired t-test compared the total time spent sheltering at 2000 h and 2200 h. Individuals did not change sheltering time between 2000 h and 2200 h in either species (*C. chasmodactylus*: t(35) = 0.581, *P* = 0.57; *O. cristavarius*: t(20) = 1.189, *P* = 0.25). A linear regression of total sheltering in relation to TCL was performed with combined data from both

hours and for both species (Figure 8), and while one relationship was significant the r^2 values suggested that body size was not a significant source of variation in the responses of either species (control: $R^2 = 0.008$, n = 45, $P = 0.55$; cool LED: $R^2 = 0.14$, n = 45, $P = 0.01$; warm LED: $R^2 = 0.065$, n = 45, $P = 0.09$).

DISCUSSION

My data suggest that ecologically relevant levels of LED light at night alter the behavior of two crayfish species. Both cool and warm-colored LEDs significantly increased the amount of time spent sheltering, and reduced nocturnal mobility of *Orconectes cristavarius* and *Cambarus chasmodactylus*. *Orconectes cristavarius* was slightly more responsive than *Cambarus chasmodactylus* to light intensities of 15 lux. The effects of LED light at night on the focal species were not related to body size. This suggests that the illuminated environment was the cause of changes in behavior, and not the size differences between the two species. Despite the effect of ALAN on activity, total nightly substrate disturbance by *C. chasmodactylus* was not different across treatments. The findings of this study highlight the responses to ALAN that are expected in freshwater, terrestrial and marine systems (Perkin et al. 2011) and suggest that the nocturnal behaviors of crayfish throughout the Appalachian region are likely to be altered, possibly with dramatic consequences for ecosystem processes.

This is the first study to investigate the impacts of ALAN on native crayfish. Our findings concur with those of Thomas et al. (2016). In summary, this study and Thomas et al. (2016) have demonstrated that both native and non-native crayfish of three genera (*Cambarus*, *Orconectes* and *Pacifastacus*) respond to commonly used urban lighting technologies at an illumination intensity of 15 lux. This study was also the first to investigate

the impacts of LED lights on native crayfish. Behavioral changes of *C. chasmodactylus* and *O. cristavarius* under LED light corroborate the conclusions of Thomas et al. (2016), yet the contrasting light emissions and underlying ecology are noteworthy. *Pacifastacus leniusculus* were exposed to narrow spectrum high-pressure sodium (HPS) lights in the aforementioned study, whereas I exposed *Cambarus* and *Orconectes* to broad spectrum LEDs. Thus, both HPS and LED lights inhibit nocturnal crayfish behavior.

Sheltering responses under ALAN are similar to responses documented when *Pacifastacus leniusculus* were exposed to chemical and visual predator stimuli (Blake and Hart, 1993). These observed behavioral changes also support previous works by Kennedy and Bruno (1961), and Fanjul-Moles et al. (1992) that show crayfish are especially sensitive to red light as adults, as are many freshwater and marine taxa (Lythgoe, 1988). Evolutionary pressures of the aquatic environment may have selected for enhanced perception of longer wavelength, low-energy red light since it is most quickly lost in deeper waters. It is plausible that *O. cristavarius* vision is more red-shifted than that of *C. chasmodactylus*, causing the greater response to 3000 K observed with *O. cristavarius*. The yabby crayfish (*Cherax destructor*) has keen eyesight and is capable of facial recognition during agonistic interactions with conspecifics (Van der Velden et al. 2008). Perception of long-wavelengths in freshwaters likely enhances vision in such intra- and interspecific communication across crayfish taxa. However, lower light levels were found to reduce communication efficiency among *Orconectes rusticus*, resulting in longer, less frequent agonistic behaviors (Bruski and Dunham 1987). The details of how crayfish species respond to unnatural illumination fluctuations likely depend on a combination of visual abilities, life history traits and resource requirements.

Other crustaceans are responsive to changes in light levels, although the group as a whole remains largely understudied. Only 11 of roughly 70,000 marine and freshwater crustaceans have been the focal species of ALAN research. Clearly, a lot of work is yet to be done. One of the first freshwater ALAN studies found that diel vertical movements of copepods (*Daphnia*) are suppressed by sky glow (Moore et al. 2001). While this study did not create sky glow conditions in the laboratory, determining the level of atmospheric light at night that may trigger a behavioral response in crayfish would advance our understanding of how extensive the impacts of ALAN may be. Perkin et al. (2014b) found that amphipods (Gammaridae) in artificial streams subjected to low fluorescent light did not alter drift rates, but in situ amphipods altered nocturnal activity in response to ALAN. Navarro-Barranco and Hughes (2015) tested the effects of both LED and halogen light on amphipod assemblages. They found that LED light attracted more amphipod species and individuals. Although amphipods and crayfish are only distantly related, the similarly strong effects of LEDs on both groups allude to the threat posed by broad spectrum ALAN.

Narrow spectrum emissions of HPS and Halogen lights are likely to affect species with peak sensitivities within the matching range, but broad spectrum lights are capable of altering the visual environment of all taxa with visual capabilities (Davies et al. 2013). Recently, Longcore et al. (2015) showed that broad spectrum LEDs can be fine-tuned to reduce emissions of wavelengths that are maximally attractive to moths. With the data from this study and published spectral sensitivity curves, it is possible that a light model could be designed that minimizes effects on freshwater species while continuing to illuminate public use areas.

Changes in the behavior of crayfish could have far-reaching implications in freshwater ecosystems. Increased nocturnal sheltering is likely to reduce foraging opportunities and lead to infrequent reproductive opportunities due to the highly reclusive nature of the focal species under ALAN. Crayfish are highly motile ecosystem engineers in southern Appalachian streams (Creed and Reed 2004, Helms and Creed 2005). Locomotion is an important component of crayfish feeding behaviors as well as the other ecologically relevant behaviors of crayfish (e.g., bioturbation). When crayfish are hesitant to move about the nocturnal benthos, individuals may settle for sub-prime food resources that are located closest to shelter, rather than hunting for high quality resources.

The impacts of ALAN on physiological processes are increasingly of interest to the scientific community. Bruening et al. (2015) investigated the impact of multiple illumination intensities on the melatonin and cortisol levels of European perch. Nocturnal release of melatonin was inhibited under illumination as low as 1 lux (a common level of background ALAN) suggesting that the circadian rhythms of freshwater fish may be already disrupted but they did not find any difference in cortisol levels between perch exposed to ALAN and control individuals. Crayfish are known to produce melatonin, serotonin and crustacean hyperglycemic hormone (CHH), all of which are likely influenced by circadian rhythms (Fanjul-Moles 2006). Changes in photoperiod or simply activity periods could impact hormone production. Even if individuals are not physically stressed, it appears that other aspects of physiology and behavior are changing in some taxa in response to ALAN.

Visual acuity varies broadly within and among aquatic invertebrate taxa as well as developmental stages of crustaceans (Fanjul-Moles et al. 1992). Long-wavelength red light is absorbed 100 times more rapidly than blue light in water. Water also scatters short-

16

wavelength blue light to a greater extent, meaning that if intensities were equal at the surface, red and blue light penetration is not equal at the level of the substrate. Light penetration to the benthos is greater in low-order streams. *Orconectes cristavarius* typically inhabits fourth order systems with more open canopies whereas *C. chasmodactylus* is more abundant in third order streams with more forested riparian zones (Fortino and Creed 2007). Perhaps large fourth order streams have exposed *O. cristavarius* to less riparian cover over millennia due to open canopies over wide bodies of water. This would expose communities to the full fluctuations of lunar cycles, providing a greater range of light intensities than would be found under a dense canopy in a headwater stream.

Regional characteristics including landscape slope and aspect may influence the severity of ALAN impacts on stream systems. The southern Appalachian region is characterized by high elevation gradients and a high concentration of headwaters. Due to the rugged terrain, highways, communities and agricultural activities are typically most abundant in the valleys adjacent to the streams. Urban and street lighting as well as automobile headlights are common in some Appalachian stream valleys. Considering the enhanced presence of ALAN in southern Appalachian streams, it is possible that behaviors of stream macroinvertebrates are more likely to be influenced than macroinvertebrates in other rural regions of the southeastern US.

It is not clear if individual responses may change due to repeated exposure to artificial light. It is unknown what the latency to respond is once ALAN is applied both suddenly and with a phase transition. Illumination thresholds are understudied in most freshwater invertebrates. While this study had small samples of males, females and reproductive forms, possible life-history specific differences in response should be explored further.

It is conceivable that disrupted nocturnal activity patterns may translate to altered diurnal activities in crayfish. Reduced nocturnal feeding could cause compensatory daytime activities, making individuals more vulnerable to visual-predators. When crayfish do not voluntarily expose themselves in search of habitat, feeding opportunities, burrowing or intraspecific interaction, a substantial food resource is eliminated for predators of crayfish such as rock bass (Fortino and Creed 2007). In contrast, illuminated environments may reduce searches for ideal habitat, leading to crayfish settling in sub-prime habitats where they face more competition and are more vulnerable to predators. Community-level field research is needed that incorporates ALAN and crayfish, along the lines of Meyer and Sullivan (2013). By reducing mobility, ALAN may reduce bioturbation and alter diet. Conducting field experiments similar to Creed and Reed (2004) and Helms and Creed (2005) with the addition of ALAN would provide an excellent starting point.

Efforts have been made to determine if customized spectral emissions possess the ability to minimize effects of ALAN on biota (Spoelstra et al. 2015). With each species likely responding uniquely to short- and long-wavelength light, challenges lie ahead in determining which technologies have the least impact. It is clear that the most effective means of reducing ALAN effects on stream ecosystems is to reduce light intensity altogether. The ecological functions of vegetative riparian buffers are crucial to the health of all freshwaters (Xiang et al. 2016). Riparian buffers are going to be of even greater importance in the future as ALAN spreads in developing countries.

Seasonal differences in riparian canopy and vegetation are likely to alter the ALAN intensities in freshwaters (Longcore and Rich 2004). Crayfish activity is decreased during the cooler water temperatures of winter (Fortino 2006), complicating our understanding of how

18

seasonality may affect crayfish in temperate zones. It is also possible that seasonal changes in nocturnal light emissions occur in mid- to high latitudes (Gaston et al. 2012). During winter, benthic light intensities may increase dramatically in light-polluted areas due to a loss of canopy and changes in ALAN emissions. Further field data collection is needed to understand the extent of seasonal changes in light exposure on stream communities.

Light pollution is becoming a ubiquitous feature of nocturnal landscapes (Cinzano et al. 2001, Gaston et al. 2014). Considering the multitude of species that are crepuscular or nocturnal (Hölker et al. 2010), my study acknowledges the importance of addressing species-specific ecology, life history and visual aspects of physiology that translate to behavioral activity patterns in both dark and artificially illuminated environments. Experimental light treatments used field-calibrated ecologically relevant light levels. This translates to more relevant research results that are useful for urban planners considering the ecosystem impacts of artificial illumination. If nocturnal behaviors are altered, the effects of ALAN could be more far-reaching than currently known. Reduced mobility may have adverse effects on feeding, mating, habitat selection and predator-prey interactions. In a controlled microcosm, my data indicate that light-naïve Appalachian crayfish display reduced locomotor activities and increased sheltering under cool and warm color temperature LED light at night. The implications of these conclusions warrant greater efforts to protect freshwaters from ecological light pollution.

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Table 1. *Cambarus chasmodactylus* total carapace length (TCL = cm), sex, male form and collection location.

Trial MM/DD/YYYY	Treatment	Tank	TCL (cm)	Sex	Form (males)	Source Location
4/22/2016	control	left	3.2	female		Howards Creek, Watauga Co, NC
4/22/2016	5000 K	center	4.3	male	I	Howards Creek, Watauga Co, NC
4/22/2016	3000 K	right	3.5	female		Howards Creek, Watauga Co, NC
4/23/2016	control	center	3.4	male	I	Howards Creek, Watauga Co, NC
4/23/2016	5000 K	right	3.7	female		Howards Creek, Watauga Co, NC
4/23/2016	3000 K	left	3.0	female		Howards Creek, Watauga Co, NC
4/24/2016	control	right	3.4	female		Howards Creek, Watauga Co, NC
4/24/2016	5000 K	left	2.9	male	II	Howards Creek, Watauga Co, NC
4/24/2016	3000 K	center	3.2	female		Howards Creek, Watauga Co, NC
4/29/2016	control	left	3.7	male	I	Howards Creek, Watauga Co, NC
4/29/2016	5000 K	center	3.4	female		Howards Creek, Watauga Co, NC
4/29/2016	3000 K	right	4.4	male	I	Howards Creek, Watauga Co, NC
4/30/2016	control	center	4.0	male	I	Howards Creek, Watauga Co, NC
4/30/2016	5000 K	right	3.8	female		Howards Creek, Watauga Co, NC
4/30/2016	3000 K	left	3.5	female		Howards Creek, Watauga Co, NC
5/1/2016	control	right	4.2	male	I	Howards Creek, Watauga Co, NC
5/1/2016	5000 K	left	3.5	female		Howards Creek, Watauga Co, NC
5/1/2016	3000 K	center	3.7	female		Howards Creek, Watauga Co, NC
5/21/2016	control	left	3.6	female		Howards Creek, Watauga Co, NC
5/21/2016	5000 K	center	4.2	male	II	Howards Creek, Watauga Co, NC
5/21/2016	3000 K	right	3.2	male	II	Howards Creek, Watauga Co, NC
5/22/2016	control	center	3.5	female		Howards Creek, Watauga Co, NC
5/22/2016	5000 K	right	3.7	male	II	Howards Creek, Watauga Co, NC
5/22/2016	3000 K	left	3.7	female		Howards Creek, Watauga Co, NC

Table 1 continued.

Trial MM/DD/YYYY	Treatment	Tank	TCL (cm)	Sex	Form (males)	Source Location
5/23/2016	control	right	4.1	male	II	Howards Creek, Watauga Co, NC
5/23/2016	5000 K	left	3.6	female		Howards Creek, Watauga Co, NC
6/4/2016	3000 K	right	3.3	male	II	Three Top Creek, Ashe Co, NC
6/5/2016	control	center	3.2	female		Three Top Creek, Ashe Co, NC
6/5/2016	5000 K	right	3.7	male	II	Three Top Creek, Ashe Co, NC
6/5/2016	3000 K	left	4.1	female		Three Top Creek, Ashe Co, NC
6/19/2016	control	left	3.7	female		Three Top Creek, Ashe Co, NC
6/19/2016	5000 K	center	3.3	female		Three Top Creek, Ashe Co, NC
6/19/2016	3000 K	right	3.9	female		Three Top Creek, Ashe Co, NC
6/20/2016	control	center	4.0	female		Three Top Creek, Ashe Co, NC
6/20/2016	5000 K	right	3.5	female		Three Top Creek, Ashe Co, NC
6/20/2016	3000 K	left	4.2	female		Three Top Creek, Ashe Co, NC

Table 2. *Orconectes cristavarius* total carapace length (TCL = cm), sex, male form and collection location.

Trial MM/DD/YYYY	Treatment	Tank	TCL (cm)	Sex	Form (males)	Source Location
5/7/2016	control	left	1.9	male	II	S Fork New River, Watauga Co, NC
5/7/2016	5000 K	center	1.9	male	II	S Fork New River, Watauga Co, NC
5/7/2016	3000 K	right	2.3	male	I	S Fork New River, Watauga Co, NC
5/9/2016	control	center	2.1	male	I	S Fork New River, Watauga Co, NC
5/9/2016	5000 K	right	1.9	male	I	S Fork New River, Watauga Co, NC
5/9/2016	3000 K	left	1.7	male	I	S Fork New River, Watauga Co, NC
5/10/2016	control	right	1.9	male	II	S Fork New River, Watauga Co, NC
5/10/2016	5000 K	left	1.3	male	II	S Fork New River, Watauga Co, NC
5/10/2016	3000 K	center	1.5	male	I	S Fork New River, Watauga Co, NC
5/16/2016	control	right	1.9	male	II	S Fork New River, Watauga Co, NC
5/16/2016	5000 K	left	1.3	male	II	S Fork New River, Watauga Co, NC
5/16/2016	3000 K	center	1.5	male	I	S Fork New River, Watauga Co, NC
5/28/2016	control	left	1.7	female		S Fork New River, Watauga Co, NC
5/28/2016	5000 K	center	1.8	male	II	S Fork New River, Watauga Co, NC
5/28/2016	3000 K	right	1.6	male	II	S Fork New River, Watauga Co, NC
5/29/2016	control	center	1.7	male	II	S Fork New River, Watauga Co, NC
5/29/2016	5000 K	right	2.0	male	II	S Fork New River, Watauga Co, NC
5/29/2016	3000 K	left	1.9	male	II	S Fork New River, Watauga Co, NC
5/30/2016	control	right	1.9	male	II	S Fork New River, Watauga Co, NC
5/30/2016	5000 K	center	1.7	female		S Fork New River, Watauga Co, NC
5/30/2016	3000 K	left	1.5	male	II	S Fork New River, Watauga Co, NC
6/12/2016	control	left	1.9	male	II	S Fork New River, Watauga Co, NC
6/12/2016	5000 K	center	1.4	female		S Fork New River, Watauga Co, NC
6/12/2016	3000 K	right	1.7	female		S Fork New River, Watauga Co, NC

Table 2 continued

Trial MM/DD/YYYY	Treatment	Tank	TCL (cm)	Sex	Form (males)	Source Location
6/13/2016	3000 K	left	1.8	male	II	S Fork New River, Watauga Co, NC
6/13/2016	control	center	1.5	male	II	S Fork New River, Watauga Co, NC
6/13/2016	5000 K	right	1.6	female		S Fork New River, Watauga Co, NC
6/14/2016	3000 K	left	2.1	female		S Fork New River, Watauga Co, NC
6/26/2016	control	left	1.9	female		S Fork New River, Watauga Co, NC
6/26/2016	5000 K	center	2.1	female		S Fork New River, Watauga Co, NC
6/26/2016	3000 K	right	1.4	male	II	S Fork New River, Watauga Co, NC
6/27/2016	control	center	1.9	female		S Fork New River, Watauga Co, NC
6/27/2016	5000 K	right	1.7	female		S Fork New River, Watauga Co, NC
6/27/2016	3000 K	left	2.0	male	II	S Fork New River, Watauga Co, NC
6/28/2016	control	right	1.7	female		S Fork New River, Watauga Co, NC
6/28/2016	5000 K	left	1.5	female		S Fork New River, Watauga Co, NC
6/28/2016	3000 K	center	2.0	female		S Fork New River, Watauga Co, NC

Table 3. Kruskal-Wallis ANOVA. Results of Kruskal-Wallis one-way analysis of variance on ranks to compare total seconds and frequencies of sheltering (SH) activities under the dark control, 5000 K cool LED light and 3000 K warm LED light. P values followed by an asterisk were determined to be significant using a sequential Bonferroni analysis (initial α = 0.025).

Species	Hour	Behavior	H	d.f.	P
C. chasmodactylus	2000	Total SH	7.63	2	0.02*
C. chasmodactylus	2200	Total SH	4.70	2	0.10
O. cristavarius	2000	Total SH	14.96	2	<0.001*
O. cristavarius	2200	Total SH	6.67	2	0.04*
C. chasmodactylus	2000	Frequency SH	10.38	2	0.01*
C. chasmodactylus	2200	Frequency SH	5.01	2	0.08
O. cristavarius	2000	Frequency SH	6.31	2	0.04
O. cristavarius	2200	Frequency SH	2.41	2	0.30

Top view of
Acclimation and trial tank setup

Powerhead

Rear

Two-brick
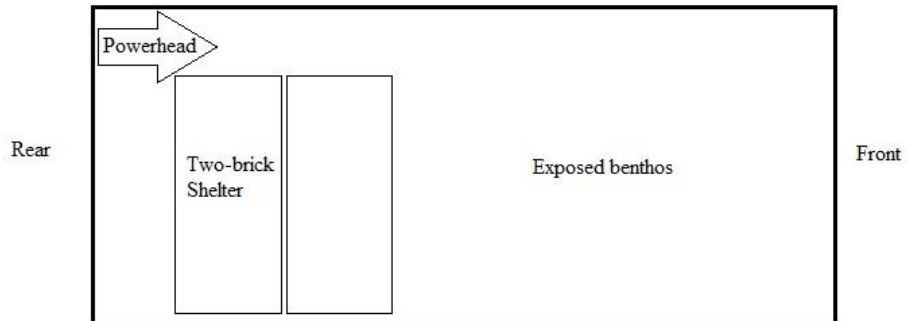Shelter

Exposed benthos

Front

Figure 1. Laboratory aquarium setup. A powerhead is used to maintain flow, two bricks are laid side-by-side for shelter, and an exposed open area allows for movement. Tanks were covered with mesh wire to prevent escape. Benthos consisted of uniform gravel at a depth of 5 cm. 1 cm^2 grids were placed on the front and rear.
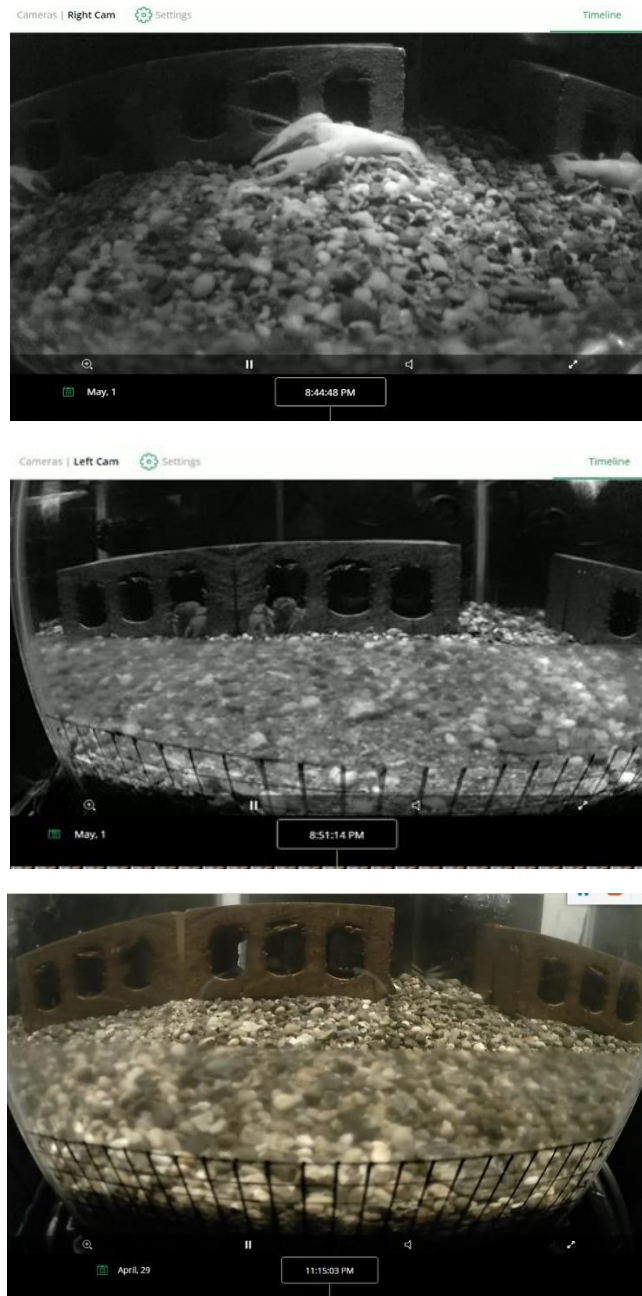
Figure 2. Examples of video recordings of crayfish behavior. Top left is a dark control illuminated by infrared, with a crayfish exposed mobile; top right is 15 lux cool LED light, with a crayfish sheltering; bottom is 15 lux warm LED with a crayfish sheltering behind the bricks.

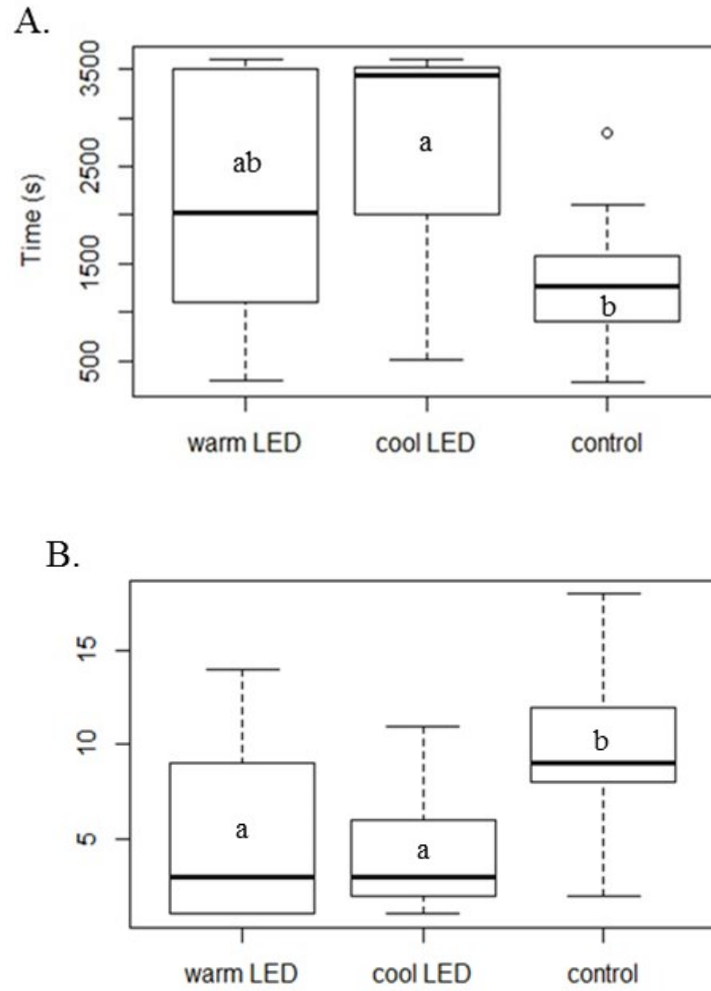Figure 3. An example of a 1 cm^2 grid at dawn following a trial night.

A.

B.

Figure 4. Boxplots of *Cambarus chasmodactylus* behaviors from 2000-2100 hrs.
Total sheltering (A), and frequency of sheltering events (B). Medians with the
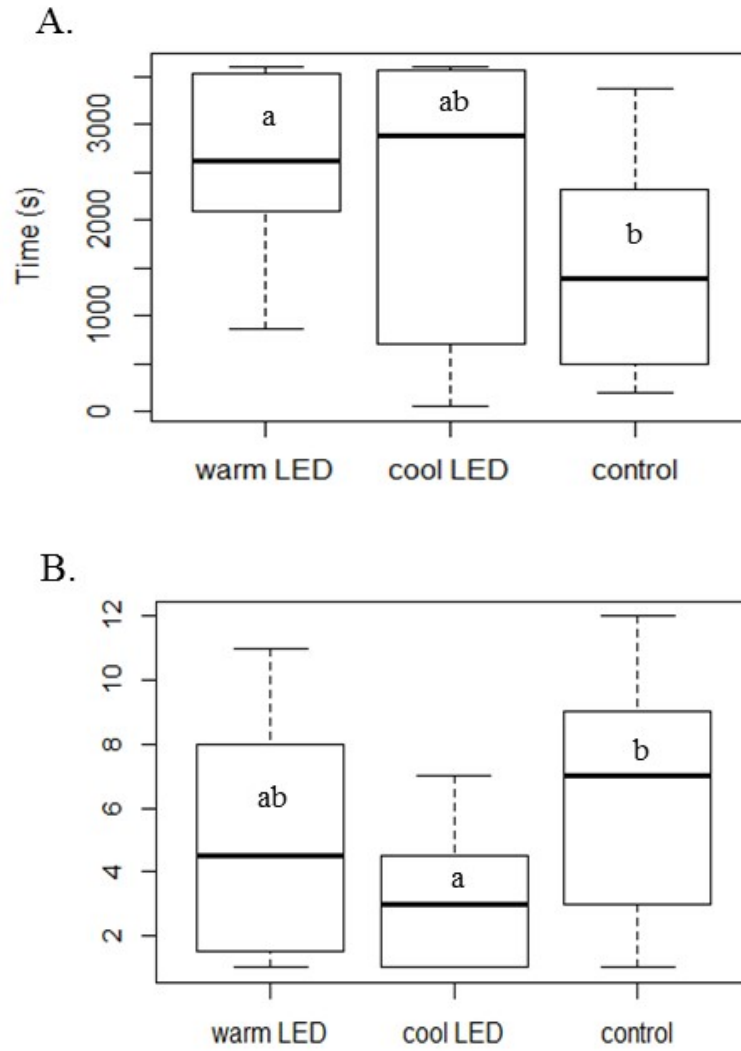same letter are not significantly different.

34

A.

B.

Figure 5. Boxplots of *Cambarus chasmodactylus* behaviors from 2200-2300 hrs. Total sheltering (A), and frequency of sheltering events (B). Medians with the same letter are not significantly different.
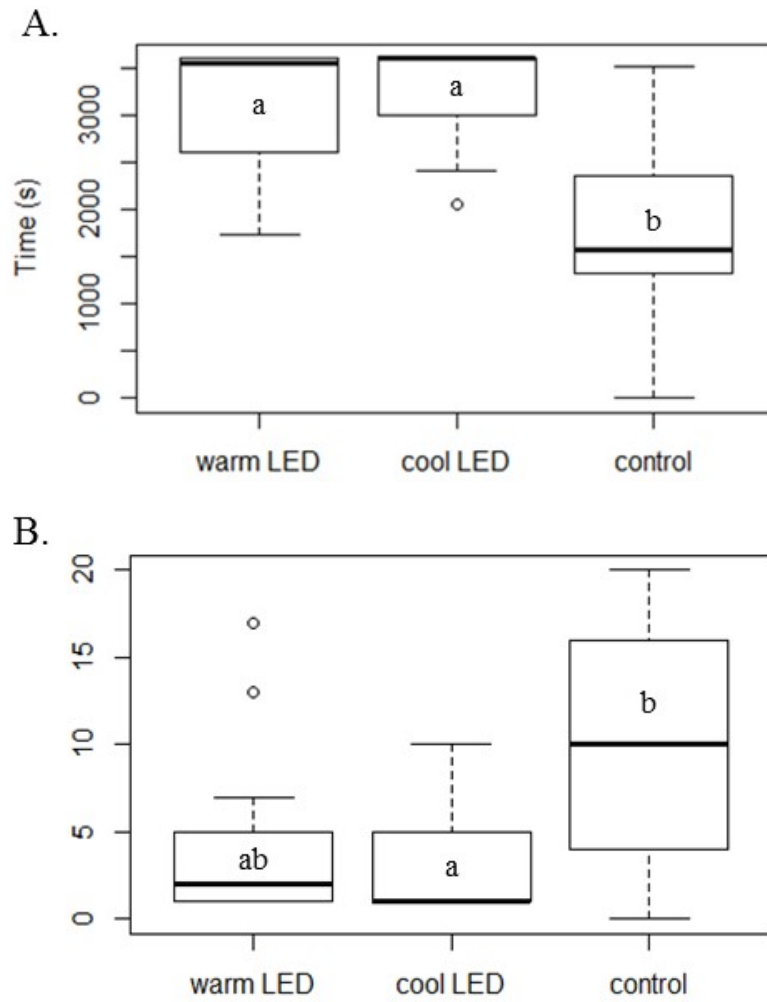
Figure 6. Boxplots of *Orconectes cristavarius* behaviors from 2000-2100 hrs. Total sheltering (A), and frequency of sheltering events (B). Medians with the same letter are not significantly different.
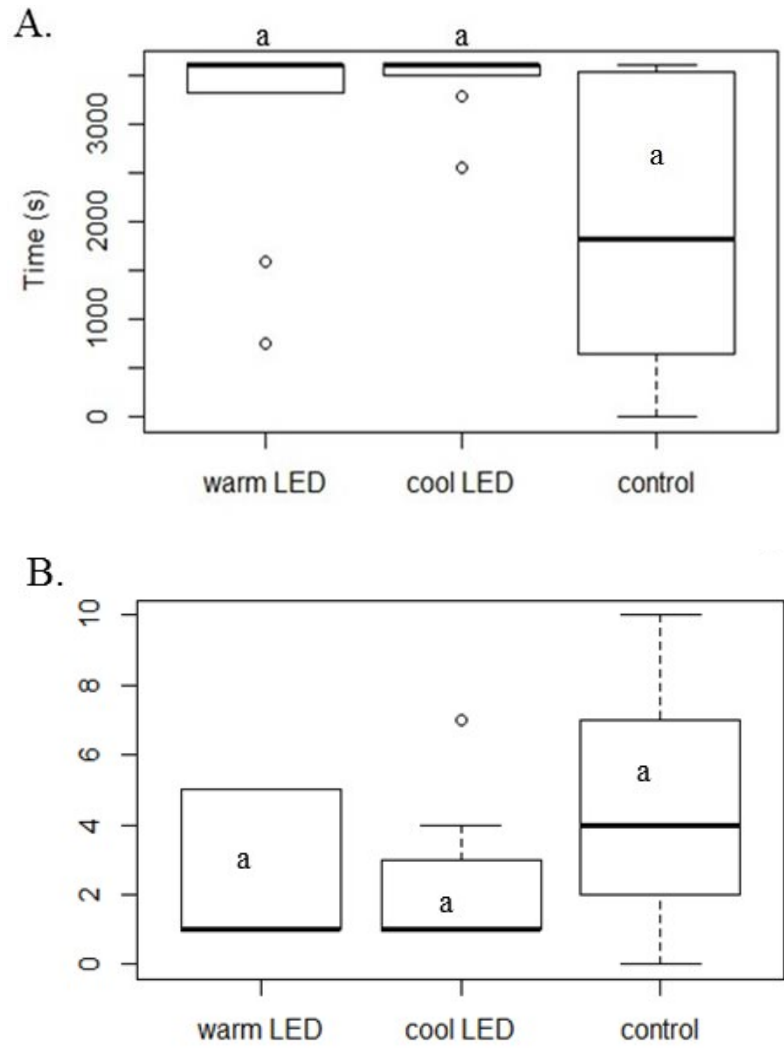
Figure 7. Boxplots of *Orconectes cristavarius* behaviors from 2200-2300
hrs. Total sheltering (A), and frequency of sheltering events (B). Medians
with the same letter are not significantly different.

Vita

Justin Ryan Fischer was born in Bedford, Texas to Veronica Gonzalez and Paul Fischer in 1991. He considers his home ecoregion to be the 'cross timbers' oaks and prairies of North Texas. He received his Bachelor of Science degree from the University of North Texas in 2014. Justin is most passionate about restoring degraded landscapes for the benefit of all creatures. In addition to being a stream ecologist, he is an avid birdwatcher and an advocate for the use of native plants in urban landscaping. He currently resides near Raleigh, NC with his wife Raquel, and his daughter Nica.