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MANAGEMENT BRIEF

The Behavioral Responses of a Warmwater Teleost to Different Spectra of Light-Emitting Diodes

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Abstract
Freshwater ecosystems are threatened by a wide range of anthropogenic infrastructure related to hydropower, irrigation, municipal withdrawals, and industrial cooling. Technology can be used to mitigate the loss of fish associated with such infrastructure by exploiting the sensory physiology of a species through stimuli designed to manipulate their natural behaviors (e.g., to attract or repel). Technologies used for behavioral guidance often incorporate light; however, previous studies investigating light devices have focused on mercury vapor bulbs and thus have been limited in their exploration of the broader light spectra. Innovations in light-emitting diode (LED) technology provide opportunities for manipulating light spectra (i.e., color) as well as light-pulse frequency. We tested the behavioral response of Largemouth Bass *Micropterus salmoides* under 16 different LED color and light-pulse frequency combinations as well as in a control in which no light was emitted. Red, orange, yellow, and green were considered with four light-pulse frequencies (0, 120, 300, and 600 pulses/min). Using a large shallow arena, lateral fish movement in response to the light treatments was examined. Regardless of color or light-pulse frequency, fish were repelled by the light source. In contrast, when there was no light emitted, fish were evenly distributed throughout the arena. This work suggests that colored light accompanied with light-pulse frequencies produced by LEDs can induce an avoidance response in Largemouth Bass.

Freshwater ecosystems have been dramatically altered as a result of human activities and infrastructure, which has led to unprecedented declines in freshwater biodiversity, particularly in fluvial systems (Moog 1993; Dudgeon et al. 2006; Ziv et al. 2011). One of the ongoing threats to aquatic biota involves infrastructure related to hydropower development, including industrial cooling, irrigation, or municipal withdrawals, which have the potential to lead to fish entrainment (i.e., nonvolitional downstream movement, transfer into irrigation channels or ditches or structure machinery) or impingement (i.e., trapped against intake screens). Entrainment and impingement have often led to mortality or reductions in system productivity (e.g., Coutant and Whitney 2000; Schilt 2007) such that regulators require hydropower utilities to identify and implement mitigative strategies to minimize threats to fish (Noatch and Suski 2012).

Conservation physiology has brought an understanding of the ways in which tactile, auditory, and visual systems influence fish movement (Hasler et al. 2009). These sensory systems can be exploited with the use of stimuli in order to direct fish along desired paths (Coutant 1999, 2001). Researchers have expended great effort in developing and testing behavioral guidance strategies, including strobe lights (Brown 2000;

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Richards et al. 2007), bubble curtains (Sager et al. 2000; Stewart et al. 2014), and bioacoustics (Goetz et al. 2001; Flammang et al. 2014), both alone and in combination. A repulsion response can be used to deter fish from areas that may lead to injury or death through entrainment or impingement (Noatch and Suski 2012). An attraction response can be used to direct fish towards safe passageways that provide connectivity between waterways in the presence of man-made barriers (Brown 2000). Sensory capabilities (recognizing and processing environmental information) are species-specific (Popper and Carlson 1998) and will therefore influence the designs for systems used to protect fish (Richards et al. 2007; Noatch and Suski 2012).

Light in particular has been explored as a stimulus of interest for reducing entrainment and impingement of fish (Brown 2000) as sight is one of the primary sources of information used by many teleosts (Sager et al. 2000; Utne-Palm 2000). In previous studies on freshwater fish, light spectra has largely been neglected as a possible influence on fish behaviors. Light-emitting diodes (LEDs) have gained considerable attention because of this, as they are comprised of a wide range of spectra that can be applied at various light-pulse frequencies to track the spectral sensitivities of a given species (Gustafsson et al. 1992). Most of the early work on fish protection with light focused on the use of mercury vapor lights (Patrick et al. 1985; Rodgers and Patrick 1985; Nemeth and Anderson 1992) or white strobe lights (Mueller et al. 2001; Richards et al. 2007), which do not have the flexibility of a multispectral LED array for which color and light-pulse frequency can be adjusted.

Our objective was to identify the behavioral responses of Largemouth Bass *Micropterus salmoides* to various LED light spectra and light-pulse frequencies. Largemouth Bass are excellent candidates for studying behavioral responses to light as they are known to differentiate between colors and are particularly sensitive to red (Kawamura and Kishimoto 2002). Further, Largemouth Bass are susceptible to impingement and entrainment (Brown 2000; Spicer et al. 2000; Grimaldo et al. 2009). This study is the first to explore how colored LEDs influence freshwater fish movement in an enclosure.

METHODS

Field collections.—The study was conducted between July 14 and August 16, 2014, on Lake Opinicon, Ontario (44°33′56.0″N, 76°19′23.6″W). Adult Largemouth Bass (N = 107; total length = 20.9–48.2 cm [mean = 32.3 cm]) were angled from shallow weedy bays distributed throughout the lake using a 2-m-long rod and reel of medium strength, a 4.5-kg-break-strength braided fishing line, and passively fished soft-plastic lures.

Behavioral experiments.—All fish were caught and transported to the laboratory located on Lake Opinicon in large coolers supplied with fresh lake water. They were transferred to large acclimation tanks (2.6 m in diameter, water depth of 50 cm) and held for 24 h. A flow-through system was used to provide a constant supply of aerated, fresh lake water to the holding tanks. Fish were also not fed during the study to avoid any confounding variables that could be related to differences in metabolic rate.

After approximately 24 h, each fish was tested for their response to light stimuli in an in-lake experimental arena (Figure 1) consisting of a converted enclosed boathouse slip (2.6 m × 6.0 m). Sources of natural sunlight were reduced by covering the windows and slip entrance. Additionally, an observation blind made of black plastic was used to prevent disturbance during testing. This blind consisted of two windows (30 cm × 30 cm) through which the fish were observed. The experimental arena was enclosed by walls that were covered in mesh to allow for water flow. This mesh also provided a barrier to debris and possible fish entry or escape. The water temperature during the experimental period followed the natural temperature fluctuations of the lake (mean = 23°C; range = 20–26°C) and was consistent with the holding tanks due to the flow-through system design. The arena was separated into 1-m zones to measure fish location as a behavioral response during the experiment. An LED device (model 521-1045-ND and LED type TITAN RGB Light Engine) with capabilities of displaying various spectra and light-pulse frequencies was used. The light was put at the 0-m line and a 2.6-m × 1.0-m acclimation box was placed between the 2- and 3-m lines (Figure 1).

Testing was conducted between 0830 and 1830 hours. The acclimation period was approximately 30 min and consisted of two phases. The first phase was approximately 15 min of exploration in which the fish was able to freely move throughout the experimental arena. The second phase included the time the fish spent in the acclimation box to standardize the focal location of the fish at the start of each trial.

The primary colors of interest within this study were red and green as these colors can be emitted with strictly red or green LEDs (Figure 2). The secondary colors of interest were orange and yellow as these colors can be formed through a combination between red and green LEDs at different variations of relative power intensities. These colors were chosen because Largemouth Bass exhibit a chromatic-type response in which depolarization occurs to red light and hyperpolarization occurs to green light (Kawamura and Kishimoto 2002). Additionally, a high spectral sensitivity to red is noted through the luminosity response (Kawamura and Kishimoto 2002). Four variable light-pulse frequencies (0, 120, 300, and 600 pulses/min) were displayed with each color. A control was also conducted in which no color or light-pulse frequency was emitted. All treatments were replicated 20 times.

Each fish was independently presented with three randomly selected color or color and light-pulse frequency combinations, consecutively displayed through a series of

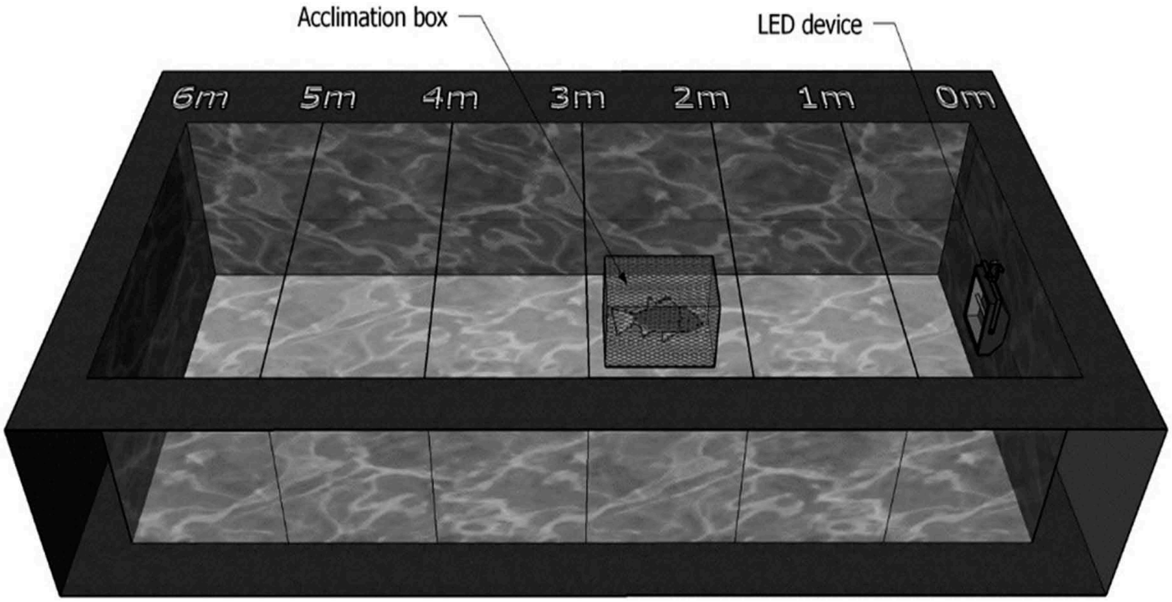

FIGURE 1. Schematic representation of the experimental arena used within this study. All zones are labeled relative to the distance from the LED device, which was placed within the foremost right zone of the diagram (at 0 meters), while the acclimation box is located within the middle (between 2 to 3 meters).

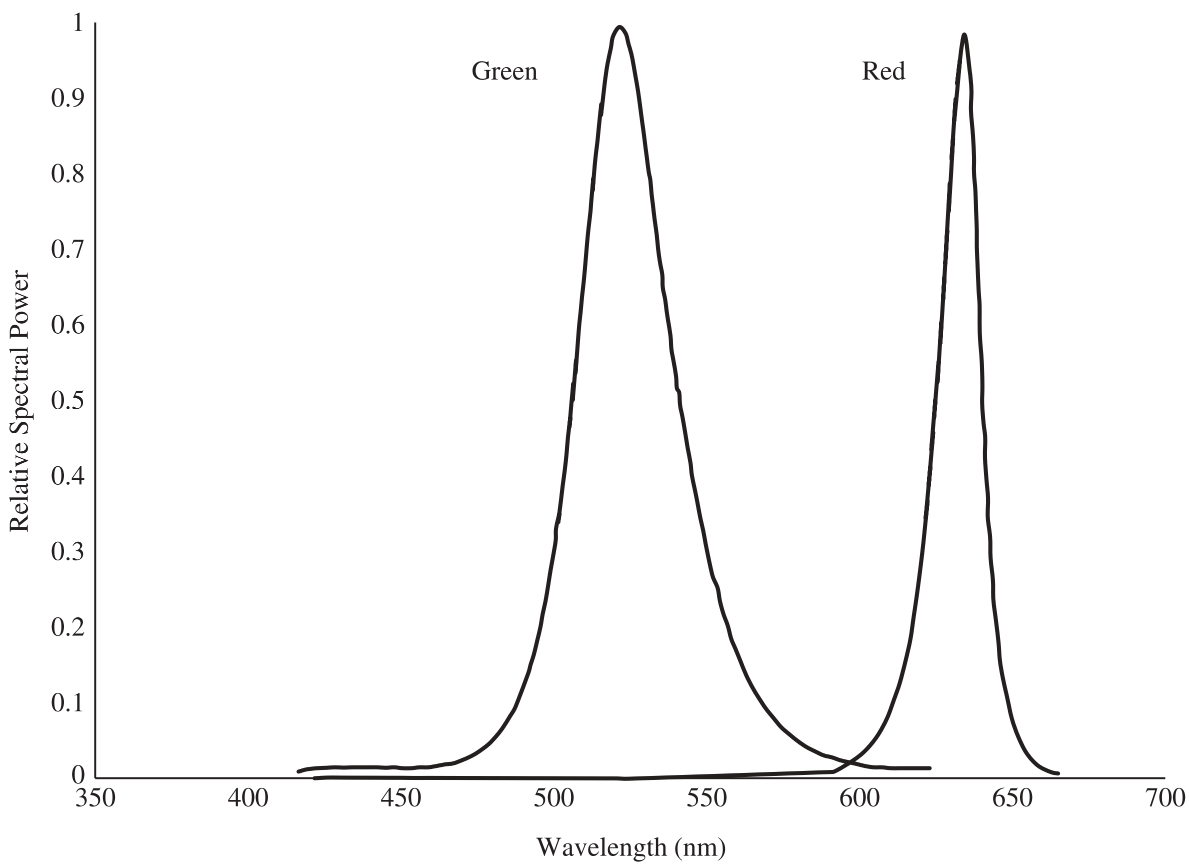

FIGURE 2. Relative spectral power of red and green light emitting diodes for a given range of wavelengths (nm) in which yellow and orange are a combination of these light emitting diodes based on relative power intensities.

observational periods. Each observational period began with the removal of the acclimation box from the experimental arena as soon as the light device displayed one of the selected colors and light-pulse frequencies. The fish's location relative to the LED device was recorded every 20 s for 5 min for a total of 15 observations per individual per trial. We considered the fish to be attracted to the light when it was within 2 m of the light, neutral when it was from 3 to 4 m from the light, and repulsed if it was from 5 to 6 m from the light. At the end of the observation period, the fish was dipnetted from the experimental arena with a rubberized fishing net (50 cm in diameter) and returned to the acclimation box (<10 s) for 10 min in preparation for the next selected color and light-pulse frequency. Following the three consecutive behavioral tests, each fish was fin-clipped (tip of the caudal fin) to ensure it was not retested and was then directly released into Lake Opinicon.

Model type, model selection, and model validation.—To estimate how fish responded to the light source, a generalized linear mixed-effects model was used that treated the response variable as a count (Poisson distribution), calculated as the number of observations at a given distance from the light source within the experimental arena. Distance from the light source would be an appropriate response variable if it was measured consistently over the experimental period; however, only six possible distances were measured, and therefore we analyzed the number of observations at a given distance, where distance (meters from the light source) was treated as a fixed effect. Light-pulse frequency and color were also treated as fixed effects, and fish identification was treated as a random intercept. To account for overdispersion of the residuals, an observation-level random intercept was included in the model (Harrison 2014). We considered two potentially important interactions: location in the experimental arena × color and location × light-pulse frequency. We modeled light to determine the following: (1) whether Largemouth Bass behavioral response varies in the presence and absence of the light, (2) whether Largemouth Bass behavioral response varies between colors, and (3) whether Largemouth Bass behavioral response varies between light-pulse frequencies for a given color. The model was graphically validated following Zuur et al. (2009). Analyses were performed in R (version 2.15.1; R Foundation for Statistical Computing, Vienna) using the package lme4 (Bates et al. 2015). Confidence limit overlap was used to determine relationships between experimental treatments with 95% confidence intervals.

RESULTS

Our model indicated that Largemouth Bass tended to be repulsed by the presence of light (Figure 3; Supplementary Table S.1 available in the online version of this article). For example, when the light source was green and constant, fish were observed 8.3 times (95% CI = 6.4–10.4) at the furthest distance from the light source compared with only 2.7 times (95% CI = 1.8–4.0) at the closest distance to the light source. Fish were similarly repulsed by each of the other colors under constant light. We observed fish at the furthest distance

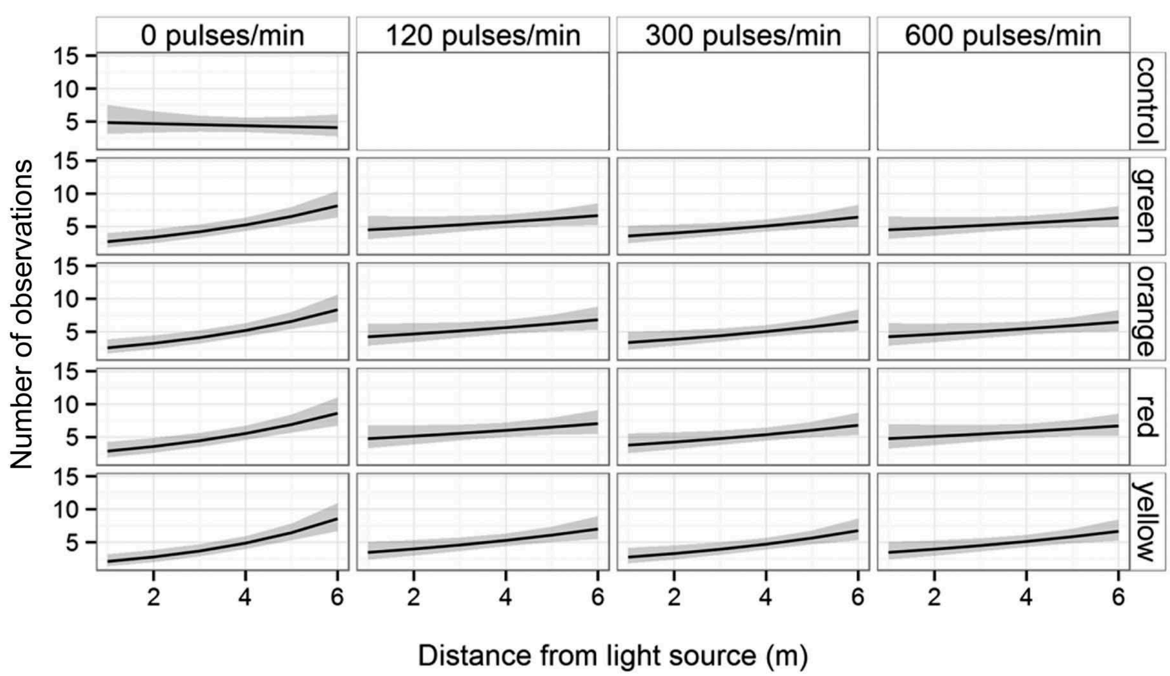

FIGURE 3. Fitted values (GLMM) for the number of observations (± 95% confidence intervals) made of a Largemouth Bass at a given distance from the light source (m), for each light-pulse frequency (0 min^{-1}, 120 min^{-1}, 300 $min^{-}1$, 600 min^{-1}), and light color (red, green, yellow, orange).

8.6 times (95% CI = 6.7–10.9) with yellow light, 8.6 times (95% CI = 6.7–11.0) with red light, and 8.3 times (95% CI = 6.5–10.6) with orange light. In contrast, when no light was emitted (i.e., the control treatment) Largemouth Bass were found equally throughout the arena (Figure 3).

Under variable light conditions, fish were also repulsed and responded similarly for each given color and light-pulse frequency combination (Figure 3). For instance, under orange light conditions fish were observed at the furthest distance from the light source 6.8 times (95% CI = 5.3–8.8) at a low light-pulse frequency, 6.6 times (95% CI = 5.1–8.4) at an intermediate light-pulse frequency, and 6.5 times (95% CI = 5.1–8.6) at a high light-pulse frequency.

DISCUSSION

This study is the first to consider colored LEDs for manipulating freshwater fish movement and has shown that Largemouth Bass tend to be repulsed from constant and pulsed red, orange, yellow, and green LEDs (Figure 3). Largemouth Bass ambush their prey in low-lit dense vegetation or other complex cover (McMahon and Holanov 1995). This species possesses a high spectral sensitivity to red, which may indicate better color analysis at longer wavelengths (Kawamura and Kishimoto 2002). It would thus be expected for Largemouth Bass to respond differently to red light than to the other colors of light. However, given that this species hunts in dimly lit areas, exposure to bright light of any kind would be unnatural and could explain the consistency in the avoidance response across color treatments at 0 pulses/min.

The addition of an unnatural light-pulse frequency combined with a light source has the potential to elicit an avoidance response (Noatch and Suski 2012). Such avoidance has been observed in Channel Catfish *Ictalurus punctatus*, Bluegill *Lepomis macrochirus*, and to a lesser extent Northern Pike *Esox lucius* (Brown 2000). The critical fusion frequency is the light-pulse frequency at which the visual system is unable to differentiate between constant and pulsed light stimuli. This may be determined by the rod-to-cone ratio of the fish's eye (Sager et al. 2000). Cones are understood to mediate photopic vision under bright-light conditions, while rods are understood to mediate scotopic vision under low-light conditions (Stenkamp 2011). A previous study that considered multiple size-classes, including young-of-the-year Largemouth Bass (total size range between 50 and 250 mm) found that a white light-pulse frequency of 400 pulses/min only slightly repelled this species, with no avoidance seen at 500 and 600 pulses/min (Michaud and Taft 2000). In comparison, our study found that adult Largemouth Bass (size range from 209 to 482 mm) elicited an avoidance response for all colors (red, yellow, orange, green) with each light-pulse frequency (120, 300, and 600 pulses/min). It is understood that teleost fish increase their rod-to-cone ratio through life stage development (Li and Maaswinkel 2007:184–185). As such, it is likely that large adult Largemouth Bass are better equipped to detect changes in light conditions (i.e., light-pulse frequencies) than their younger counterparts (Stenkamp 2011). This suggests that the difference in responses may have been influenced by fish size. An alternative explanation for the difference in these responses could be that Largemouth Bass visual sensitivity is increased with wavelengths that are within the range of their color-sensitive cones. This may help Largemouth Bass detect photopic conditions better than white light alone. As such, the use of color may allow fish to detect a wider range of light-pulse frequencies.

These findings provide the foundation for testing LED light as a useful technology to reduce the entrainment and impingement of Largemouth Bass and other fish species, thus expanding the range of behavioral guidance strategies available to practitioners. Noatch and Suski (2012) reported that lights and other nonphysical barriers may be more effective when used in combination with other fish diversion systems, such as angled screens and louvers. This could be achieved by installing light devices in areas that pose potential harm to fish to promote an avoidance response. Further studies will be needed to assess species that are at risk to impingement and entrainment and that possess color vision, such as Bluegill (Hawrynshyn et al. 1988) and Walleye *Sander vitreus* (Burkhardt et al. 1980; Michaud and Taft 2000).

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