Behavioural response of white sturgeon and walleye to a LED based light guidance device

Matthew Ian Ford
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Ottawa, Ontario

Dedication

I dedicate this thesis to everyone who has had an effect on my life. I would not be here if it were not for every little piece that everyone played. I especially want to thank my mother for always being the most supportive and helpful person anyone could ever ask for. To my entire family for always being there and giving me the best support system around to make it through any challenge, and to centre me when it is necessary. I want to thank all of my friends for helping me through the worst of times and giving me even better ones to remember forever. I want to thank the whole Cooke lab and everyone who played a role in my research and anyone who was there in the lab to talk to when I needed to lift my head from my computer, and maybe some times when I did not. I want to thank Steve and ATET-Tech for giving me this opportunity to experience and learn so much about the animals I love. Finally, I want to thank my girlfriend Kayla who has been a significant part of my life since we met and whom I want to continue being able to rely on and have the best conversations ever with for the rest of my life.

Abstract

With increasing water usages, water management is creating a growing need to protect fish. Behavioural guidance is used to reduce damage to fish from waterway infrastructure. A light guidance device designed by ATET-Tech, inc. was tested using both white sturgeon and walleye. White sturgeon were attracted to green light more than blue and red during the day. At night, white sturgeon were attracted to all lights. A louver-LGD system was tested using green light strobing at 20Hz to attract fish and red light strobing at 1Hz to deter fish. The louver was consistently effective at guiding white sturgeon toward the bypass with green light enhancing night bypass rates. Walleye were assed for their behavioural responses to green and orange light with either constant or 5Hz strobing light. Walleye avoided light significantly more compared the control. This thesis furthers understanding how fish react to light, potentially reducing entrainment and impingement.

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Co-authorship

Chapter 2: Preferences of age-0 white sturgeon Acipenser transmontanus for different colours and strobe rates of LED lights may be exploited to improve behavioural guidance strategies. Matthew I. Ford, Chris K. Elvidge, Dan Baker, Thomas C. Pratt, Karen E. Smokorowski, Paul H. Patrick, Michael Sills, and Steven J. Cooke.

This study is my own, but it could not have been done without the useful and informative guidance from Chris, Dan, Steve, Michael, and Paul. The idea was conceived by all of the co-authors and myself to create a synthesis for the background of light guidance. All writing and research was conducted by Ford. All of the co-authors played a significant role in the planning, editing, and feedback for this paper. This paper is currently still in review with a peer-reviewed journal.

Chapter 3: Evaluating a light-louver system for behavioural guidance of age-0 white sturgeon. Matthew I. Ford, Chris K. Elvidge, Dan Baker, Thomas C. Pratt, Karen E. Smokorowski, Paul H. Patrick, Michael Sills, and Steven J. Cooke.

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Chapter 4: Behaviour of age 0+ and 2+ walleye when exposed to strobing or constant light of different colours emitted from an LED behavioural guidance device. Matthew I. Ford, Chris K. Elvidge, Paul H. Patrick, Michael Sills, and Steven J. Cooke.

This study is my own, but it could not have been done without the useful and informative guidance from Chris, Dan, Steve, Michael, and Paul. The idea was conceived by all of the co-authors and myself to create a synthesis for the background of light guidance. All writing and research was conducted by Ford. All of the co-authors played a significant role in the planning, editing, and feedback for this paper. This paper currently being edited to make it ready for submission to a peer-reviewed journal.

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Chapter 1: Introduction

Hydropower and other water infrastructure have played a large role in human development with an increased need for water (Barnthouse 2013, Dudgeon et al. 2006). With increased demand for water and more manipulation to natural waterways there are greater risks for fish and other freshwater organisms subjected to the dangers of waterway management (Barnthouse 2013, EPRI 2012). Migratory species are especially susceptible to the dangers of waterway development as their movement exposes them to multiple waterway infrastructures (Baxter 1977, Scruton et al. 2008). The risk for entrainment (to be displaced by being sucked into water intakes or turbines) and impingement (to be pulled against a structure through flow) is dangerous for any fish coming into contact with hazardous infrastructure leading to a need for ways to help fish avoid danger (Schilt 2006, Poletto *et al.* 2014b). Barriers can be used to block or deter fish from moving towards hazardous structures (Hocutt 1981, EPRI 2001). Both physical barriers (EPRI 1998) and non-physical barriers (Noatch and Suski 2012) can be used to behaviourally guide fish away from the hazards of waterway development and reduce possible damage to populations at risk (Schilt 2006).

Behavioural guidance is a way to guide fish using stimuli to elicit a behavioural response (Noatch and Suski 2012). Behavioural guidance techniques usually fall into one of two categories, physical and non-physical barriers. For fish, physical barriers aim to physically block passage into an undesirable location. Objects such as screens (Gale et al. 2008), bar racks (Russon et al. 2010), and louvers (Amaral 2003) (louvers are a series of vertical slats angled to deflect oncoming matter and organisms), though louvers can be considered non-physical as well through modification of flow regimes] block fish from entering waterway infrastructure such as intake pipes and turbines. Physical barriers have some drawbacks since they require regular cleaning to remove debris (Poletto et al. 2014a) and can also allow smaller fish through depending on the size of the space between bars (Coutant and Whitney, 2000). Non-physical barriers target a specific stimuli that the fish may be sensitive to leading to a behavioural response (Noatch and Suski 2012). Drawbacks of non-physical barriers are that reactions to the stimuli are species-specific and not always uniform throughout the species (Schilt 2006), day (Amaral et al. 2001), season (Hocutt 1981), or age of the fish (Fore 1969). Light is one form of non-physical barriers that has been tested for decades and has new possibilities with advancements in current light technology.

Light has been used as a behavioural guidance technique for fish since the 1950's (Brett and MacKinnon 1953). Light aims to target the visual physiology of the fish species it is trying to guide (Noatch and Suski 2012). The light reacts with the photoreceptors (rods and cones) in the eye which are stimulated to send messages to the brain through the optic nerve (Stevens et al. 2011). The reaction of fish to light is based on the response of the rods and cones to the light. Rods and cones are stimulated differently depending on the intensity (rods) and sensitivity to specific wavelengths (cones) of light (Bond 1996). Rods are sensitive to light levels and are typically associated with greyscale of dark to light, though they can have colour sensitivities as well (Bond 1996). Cones absorb a specific wavelength in the visual spectrum and are stimulated (Stevens et al. 2011). There are different cones for specific colour sensitivities and even combinations of cones to combine sensitivities of two separate colours or increase the absorption of a single colour (Stevens et al. 2011). Within populations, there is variation in rod-cone ratios and small ranges of sensitivity to wavelength absorbances, which can be seen in white sturgeon (Sillman et al. 1990). The retinal composition of rods and cones plays a factor into how the fish will react to light (Bond 1996). There is very little, if any, research to determine how fish react based on the sensitivity of the rods and cones though there may be a link with other behavioural aspects (eg. foraging or predator avoidance). Strobing is another type of light guidance that typically deters fish using pulses of light (Baker 2008). Flicker fusion frequency (FFF) is an aspect of vision related to strobing where the light stimulates the photoreceptors for a certain period of time before they reset to take in more light (Ali and Klyne 1985). If light is coming in at a rate above the FFF, then it will appear as a constant light to the individual (Ali and Klyne 1985). The visual physiology of the fish plays a role in how effective behavioural guidance with light will be.

Light is used as a behavioural guidance tool that has been applied in many ways to either attract or repel fish. Light has been used to help guide fish away from places like hydropower turbines and water intake pipes (Schilt 2006). This research began in the 1950's (Fields et al. 1955) with lab work to determine how fish reacted to overhead lights, usually in combination with some type of physical barrier. Early research used incandescent bulbs to shine light in the water and determine the reaction of the fish (Brett and MacKinnon 1953, Field et al. 1954, Fields et al 1955). Many of the first studies done using light guidance were tested on salmonids (Noatch and Suski 2012). Early incandescent typically elicited an avoidance response in fish. Later mercury vapor bulbs were developed which could use colour. Mercury vapor bulbs were beneficial because, with the use of colour, they could attract fish. This attraction could help guide fish towards bypasses instead as an alternative to fish reacting to bright lights as deterrents. Mercury vapor bulbs had some drawbacks in high energy requirements and large heat outputs requiring large heat syncs. Mercury bulbs also are large enough to make creating waterproof housing difficult. Strobing lights were the next development, which began to be used heavily in the 1980's. Strobing lights typically elicited avoidance responses in fish in most tests but were still inconclusive in some studies (Noatch and Suski 2012). Incandescent, mercury vapor, and strobe lights all had flaws and benefits. Each of them had situations where they did not work and others where they did work. Species-specific reactions to each differed and there was no one light that worked in every situation. Light guidance has been difficult since every environment is different, making it very difficult to replicate results and study setups. LED lights have the possibility to solve this problem since they are extremely flexible and have the ability to be programmed. LED lights are a form of solid state lighting that have the ability to change colours based on expression of different wavelengths (Chang et al. 2014). LED's are much smaller (often less than 10mm x 10mm, and can be very thin) and have less harmful side effects (no mercury and lower energy requirements) than other lighting options making them more appealing (Steigerwald et al. 2002). The programmability of LED's allows the light to change colours through combinations of blue, green, and red output (Steigerwald et al. 2002) and strobing rates instantly, combining the benefits of past light technology while removing some of the negatives. There are still situations in which light will not work (eg. very high turbidity in water), yet the LED provides the flexibility to create a unique solution for almost any given situation.

This research utilizes a new LED based light guidance device (LGD). This LGD waterproof box that is about 12 inches wide by 4 inches tall and uses 162 LED modules. Since the LGD is waterproof and relatively small, is can be deployed in a vast amount of scenarios, even fixed to concrete walls. The LED lights used can change colour intensity (red: 560-1120 millicandels [mcd]; green: 1120-2240 mcd; blue: 280-560 mcd, each colour has a different base intensity) allowing for up to 16 million different colour combinations and can flash (maximum strobe rate of up to 40Hz). The LGD has the capacity to be programmed for scheduled computerized changes (scheduling can be defined by hour) or can be manually changed at any moment. The LGD does not include UV wavelengths in it spectrum. This device has been previously tested by Sullivan et al. (2016) and was seen to repel largemouth bass for red, green, orange, and yellow colours strobing at rates of 2Hz, 5Hz, 10Hz, and a constant light emission. The ability to change colour and strobing frequency increases the potential for finding effective colour-strobe combinations that can effectively behaviourally guide different species.

Research objectives

The objective of this research is to determine how white sturgeon and walleye behaviourally respond to different light stimuli. Based on previous research on both species, spectral sensitivities were identified in the photopigments. Using these specific sensitivities combined with different strobing rates, age 0+ (young of the year that have not lived a full year) white sturgeon along with age 0+ and 2+ (fish that have lived two years and are in their third year of life) walleye were tested for their reaction to the light. Since walleye and lake sturgeon (a relative of the white sturgeon that is closely related) can be found in the same water system, we will determine how they both behaviourally react to light stimuli. In chapter 2, I tested age 0+ white surgeon raised in a hatchery at Vancouver Island University to test their behavioural response to red, green, and blue light strobing at a rate of 20Hz, 1Hz, and constant light. This study gauged whether age 0+ white sturgeon were attracted, repelled, or indifferent to the different colour-strobing combinations. Chapter 3 furthered this research using the most attractive and least attractive settings and combined them with a louver. The most attractive setting attempted to guide fish towards a simulated bypass while a repulsion setting was used to scare fish away from the louver and simulated turbine entrance. Both of the settings were tested in combination with a reversed louver (a reversed louver angles the slats towards the flow instead of with the flow) to determine the effectiveness of an integrated louver-light guidance system. Chapter 4 assessed the behavioural response hatchery raised age 0+ and 2+ walleye to orange and green light with either a strobing rate of 5Hz or a constant light. Age 2+ walleye were tested for their behavioural response to the different settings while age 0+ and 2+ were tested for escapement rates from the light to determine if there was any ontogenetic differences between the age classes. This research could have implications for conservation since relatives of the white sturgeon and walleye can inhabit the same water system. Findings from this research could help protect multiple at risk species at once. Lastly, Chapter 5 combines all of the findings and the possible implications of this research and points out what I think are the next logical steps in the research.

Chapter 2: Preferences of age-0 white sturgeon Acipenser transmontanus for different colours and strobe rates of LED lights may be exploited to improve behavioural guidance strategies

Abstract

Many populations of migratory fish species, including white sturgeon (*Acipenser transmontanus* Richardson), are threatened due to modification of riverine systems and may experience downstream displacement or mortality at water intake structures. Efforts to reduce the impacts of these structures are beginning to incorporate behavioural guidance, where the sensory capabilities of fishes are exploited to repel them from high-risk areas or attract them towards desirable paths. Artificial lighting has been tested before, but consisted of single-spectrum lights. Using a new programmable LED-based light guidance device (LGD), I exposed age-0 white

sturgeon to light strobing at 1 Hz, 20 Hz, or constant illumination with colours (green, red, blue) matching the absorbance maxima of their retinal photopigments. The behavioural responses of the sturgeon were assessed using y-maze dichotomous choice tests under both day (light) and night (dark) conditions. Sturgeon demonstrated positive phototaxis under both day and night conditions, and approached the LGD more often when light was continuous or strobing at 20 Hz compared to strobing at 1 Hz. Green light elicited the greatest rates of attraction overall. The combination of strobing and colour may help to protect imperiled fish from waterway development and serve as an effective form of mitigation at hydropower facilities and other human infrastructure where fish may be entrained or impinged.

Introduction

Human modification of rivers through canalization, damming and water diversions has occurred for centuries and has had a number of negative consequences on aquatic life (Vörösmarty et al. 2010). Indeed, freshwater biodiversity is in decline (Dudgeon et al. 2006) and freshwater fish are among the most threatened group of organisms on the planet (Bruton 1995; Sala et al. 2000). The number of dams and water intake structures for irrigation, drinking water, and electricity production continues to rise in response to demand, not just in the developed world but increasingly in developing countries (Winemiller et al. 2016). For fishes (Rago 1984; Coutant 1999), dams and water intake structures create the risks of entrainment (downstream displacement through a water intake) and impingement (becoming trapped against barriers; Sager et al. 2000), both of which can have lethal outcomes.

The white sturgeon (*Acipenser transmontanus*) is a semi-anadromous fish species inhabiting rivers along the west coast of North America. Across their entire range, they are currently listed as Least Concern on the IUCN Red List; in Canada alone, there are four separate populations, three of which are considered endangered (upper Fraser River, upper Kootenay River, upper Columbia River) and the fourth considered threatened (lower Fraser River; COSEWIC 2015). Many anthropogenic factors, including fishing, have led to declines in white sturgeon populations (Birstein 1993; Birstein et al. 1997), but due to their migratory behaviour, river modifications have played a particularly significant role (Jager et al. 2001). White sturgeon may encounter multiple barriers and waterway modifications throughout their life history, potentially magnifying the risks of entrainment or impingement over time. As sturgeon do not reach maturity until they are 10+ years with females maturing later than males (Semakula & Larkin 1968), they are especially susceptible to population decline from even low levels of mortality above natural levels and any efforts to reduce mortality and guide fish away from danger (i.e., water intakes) would be of broad utility (Secor et al. 2002).

Extensive research has been done to reduce rates of entrainment and impingement at hydropower facilities and water intakes, including physical (e.g. bar racks and screens; Allen et al. 2012) and non-physical (e.g. electric current, lights, bioacoustics, bubble screens; Sager et al. 2000; Schilt 2007; Noatch & Suski 2012) barriers. Non-physical barriers target the sensory physiology of fishes to elicit a desired reaction and are used either alone or integrated with another guidance system (Coutant 2001). In practice, both methods have had equivocal success (Allen et al. 2012), but recently, the possibility of refining behavioural guidance techniques to achieve conservation and management targets for species of concern has received renewed attention. While artificial light has been used as a tool in behavioural guidance for many years (Haymes et al. 1984; Patrick et al. 1985; Noatch & Suski 2012), including with sturgeon (Kynard & Horgan 2001; Poletto et al. 2014b; Klimley et al. 2015), these attempts typically used mercury vapor bulbs that could only emit one spectral frequency and required substantial amounts of power. Advances in LED technology have created lights that can vary in spectra and strobing frequency, programmed to target different species and situations (see Sullivan et al. 2016 for example with largemouth bass *Micropterus salmoides* Lacepède).

Evaluations of sturgeon retinal sensitivities have demonstrated large degrees of similarity between species, with retinal cells consisting of ~40% cones having maximal absorbances in the red, green, and blue spectra (Sillman et al. 2007). Sturgeon cone cells are most sensitive during the daytime (light conditions), while rod cells are more sensitive at night (low light conditions; Tosini et al. 2014), concurrent with peaks in the activity levels of the organism (Poletto et al. 2014a). Sturgeon are known to exhibit positive phototaxis to green light as larvae, while developing sensitivities to red and blue in later life stages (Loew & Sillman 1993). White sturgeon also have a specific rod cell sensitivity to green light (540nm; Sillman et al. 1995). In this study, I tested the effectiveness of a new, LED-based light guidance device (LGD) at achieving behavioural guidance in age-0 white sturgeon. To do so, I used dichotomous choice tests in a y-maze and measured the preferences of fish for the unilluminated control arm of the y-maze versus the arm illuminated with the LGD, producing red, green, or blue light at constant output, or strobing at frequencies of 1 Hz or 20 Hz. Based on published data, I predicted that age-0 white sturgeon would demonstrate the greatest responses to green light under dark conditions, with those responses being more consistent with attraction (positive phototaxis) than repulsion (negative phototaxis).

Methods

Study site and species

I obtained age-0 (~4 months old, 153 ± 16 mm total length, mean ± SD) hatchery-reared white sturgeon of Fraser River stock from the International Centre for Sturgeon Studies (ICSS) at Vancouver Island University (VIU) in Nanaimo, B.C, Canada. The ICSS maintains their sturgeon indoors in dechlorinated, biofiltered and UV-treated municipal water at a temperature of 14 °C and a natural photoperiod determined by external light sensors. Subjects were held in 2000 L green cattle drum tanks with average densities of 500 fish per tank. Sturgeon were transported individually in 10 L buckets between their holding tanks and the trial arenas, and placed in net pens in the holding tanks following trials to prevent reuse.

Experimental apparatus

To simulate a stream setting, I equipped a green fibreglass raceway tank (3 m length × 1 m width) with a semi-closed, recirculating flow system. Water was added continuously to this system at a rate of 1 $L \cdot min^{-1}$ which allowed us to supply the trial arena with a constant flow rate of 0.24 $m \cdot s^{-1}$ (measured using a flow meter across multiple points in the tank and averaged) and maintain a depth of 20 cm. The temperature was constantly monitored by the LGD and water was added when necessary to make sure the temperature did not vary by more than 1 °C. Two panels of green wire mesh (1 cm mesh size) were placed 30 cm from the head and foot of the tank to confine the sturgeon to the trial arenas (2.5 m length × 1 m width). The upstream end of the tank was divided by grey opaque PVC sheeting (75 cm length × 20 cm height) to create a y-maze for dichotomous choice testing to determine if white sturgeon had a preference for, or an aversion to, a particular colour or strobe rate of light (Fig. 1).

I used a programmable underwater light guidance device (LGD) developed by ATET-Tech, Inc. (Thornhill, ON) as a behavioural guidance tool for migratory fishes. The LGD consists of 162 LED modules that can each produce red (605 nm), green (540 nm) and blue (460 nm) light at variable intensities and strobe at rates up to 40 Hz for all colour and intensity combinations. The light was placed on the bottom of the tank in one of the two y-maze chambers (and rotated between sides to account for positional biases) at the "upstream" end and set to produce red, green or blue light at one flash per second (1 Hz), twenty flashes per second (20 Hz) or constant illumination. Including a control treatment, where the device was present in the arena but turned off, there were 10 different light treatment combinations. I then replicated these tests under dark conditions (<5 lux background illumination) to simulate the availability of ambient light at night where fish were dark adapted in their 5 min acclimation before the test began. Individual fish were naive and exposed to only one treatment combination (N = 20 per treatment, N = 400 fish total).

Experimental protocol and analysis

Individual, naïve white sturgeon were placed into the arena at the downstream end under a wire cage (30 cm x 30 cm) for 3 mins with the light treatment active to allow the fish time to acclimate to the arena and detect the upstream stimulus. Following the acclimation period, the cage was removed and the sturgeon were observed and videotaped for 1 min to observe which side of the y-maze was chosen. Dark trials were visually monitored to determine choice and latency. To control for video to manual time, some daytime experiments were manually timed and compared to video latency to ensure accuracy of night manual timing. If no choice was made (i.e., neither chamber was entered) after 1 min the trial was ended and scored as "no decision" (a neutral reaction). This allowed me to assign a binary score where a '1' indicated that the sturgeon approached the light and a '0' indicated that they did not (i.e., the fish entered the side of the y-maze without the LGD). Sturgeon were scored as entering the y-maze when their entire body had crossed the plane of the barrier. The sturgeon that did not enter the y-maze were not included in the analysis. Subsequent video analysis of the trials enabled measurement of the time required for the initial decision to be made (i.e., the latency to enter one of the chambers). The behavioural responses were compared via three-way factorial ANCOVAs with light colour, strobe frequency and ambient light conditions as fixed effects and fish size (total length) as a linear covariate. Binary data were analyzed in general linear models with binomial distributions, while latencies to enter the y-maze were rank-transformed (Scheirer et al. 1976). Due to significant differences between ambient light conditions in the preliminary analyses, the data were separated by light condition, and light and dark periods were examined individually in two-way factorial ANCOVAs with length as a covariate to account for differences that may be derived from physical characteristics. . All analyses were conducted using R version 3.2.4 (R Core Team 2016).

Results

All conditions

Overall, 12.5% of age-0 white sturgeon made no choice (did not move upstream into the y-maze) in dark conditions (N = 200), while 20.5% sturgeon made no choice under light conditions (N = 200). Of the sturgeon that did make a clear behavioural choice, the proportion of white sturgeon approaching and entering the y-maze chamber containing the light device was significantly influenced by colour ($F_{3,329} = 10.25$, $p < 0.0001$), strobe frequency ($F_{2,327} = 7.78$, $p < 0.001$) and light condition ($F_{1,326} = 43.0$, $p < 0.0001$), with a significant interaction between colour and background light condition ($F_{3,318} = 6.18$, $p < 0.001$). There was no effect of fish length (p = 0.48). Sturgeon were most likely to approach the LGD when it was emitting green light, irrespective of strobe frequency. Constant light and light strobing at 20 Hz elicited more approaches than light strobing at 1 Hz, irrespective of colour. Both colour ($F_{3,378} = 5.52$, $p <$

0.01) and strobe frequency ($F_{2,378} = 10.32$, $p < 0.0001$), but not background light condition, had significant effects on the latency to approach the LGD, and fish size was a significant covariate ($F_{1,378} = 4.34$, $p < 0.05$), with smaller fish taking longer to approach the light source (Pearson's r = -0.11). In general, it took longer for sturgeon to approach when the LGD was strobing at 1 Hz, while all three colours were apparently more attractive than the control when the LGD was emitting constant light or strobing at 20 Hz.

Light conditions

In the lighted trials, the proportion approaching the light device was significantly influenced by colour ($F_{3,154} = 5.96$, $p < 0.001$) and strobe frequency ($F_{2,152} = 5.38$, $p < 0.01$). Green light was consistently the most attractive whereas red light was the least attractive, with the lowest proportion approaching in response to the red light at 1 Hz treatment (Fig. 2a). Compared to the control treatment, red light at 1 Hz appeared to have a deterrent or repellant effect on age-0 white sturgeon during light conditions. There were no significant differences in the times taken to approach the LGD between treatment combinations (Fig. 2b).

Dark conditions

Under dark conditions, both colour ($F_{3,171} = 12.42$, $p < 0.0001$) and strobe frequency ($F_{2,169} = 4.6$, $p < 0.05$) again influenced the proportion of sturgeon approaching the LGD as well as their latency to approach (colour: $F_{3,189} = 3.23$, $P < 0.05$; frequency: $F_{2,189} = 11.27$, $p < 0.0001$). Blue light consistently elicited the fewest approaches, and light strobing at 1 Hz appeared to be slightly less attractive than constant light or light strobing at 20 Hz (Fig. 3a), although any light treatment combination attracted more sturgeon than the no-light control under dark conditions.

Red light at 1 Hz resulted in the longest latency to approach times (Fig. 3b), with all light colours constant or flashing at 20 Hz eliciting faster approaches than the control.

Discussion

My results demonstrate that age-0 white sturgeon phototaxis varies depending on the colour and strobe rate of light stimuli, as well as ambient light conditions. Green light strobing at a high frequency (20 Hz) appears to be an attractant to the sturgeon, while red light strobing at a low frequency (1 Hz) elicited responses consistent with repulsion under light background conditions. Under dark background conditions, sturgeon did not demonstrate specific colour preferences and were instead positively phototactic, approaching any light stimuli. My findings suggest that variable light output (in terms of both colour and frequency) optimized to target fish species at varying ambient light conditions could potentially improve the success of behavioural guidance strategies.

Strobing lights have been used for many years as an additional tool in efforts to guide fish, although reactions have been both variable and species specific. Light strobing at irregular frequencies can induce avoidance responses in fish (Noatch & Suski 2012), and strobe lights have successfully reduced rates of entrainment of salmonids around a navigation lock (Johnson et al. 2005). Attempts to trap sea lamprey (*Petromyzon marinus* L.), however, found that this invasive species had varying reactions to strobe lights (Stamplecoskie et al. 2012). Taxon-specific differences in response may be the result of different physiological capacities to adjust to strobing lights (critical flicker frequency, CFF). The ability to adjust from low light to higher levels of light is dependent on time and the rod/cone ratio within the retina (Sager et al. 2000), with lower ratios corresponding to longer adjustment periods and lower limits on detectable strobe rates (CFF). My results suggest that lights strobing at 20 Hz may be above the CFF of age-0 white sturgeon and appear as constant light eliciting an attraction response, while strobing at 1 Hz acted as a deterrent by triggering negative phototaxis. Characterizing visual limits in other species may have important implications in determining strobing rates for desired responses as each species may respond differently to strobing lights.

The role of diel periods and associated biological rhythms is well understood in fishes (Zhdanova & Reebs 2006). Over the course of this study, white sturgeon were more active under dark-adapted conditions and approached the LGD at higher rates during low-light conditions compared to simulated daytime conditions. This may be a function of dark-adaptation and not diel rhythms. These findings may differ from a true diel rhythm test but this was logistically not possible in this study. This dark-adaptation activity pattern may be associated with the relatively high proportion of rod cells in their retina (~60%: Sillman et al. 1990). It is possible that under dark conditions, the stimulation of rod photoreceptors by any incumbent light was more likely to trigger an attraction than stimulation of cone receptors, an idea reinforced by both the dark-adapted switch of red from the least attractive colour during the day tests to the most attractive colour at night, and the general overall increase in attraction for all three colours tested at night. During simulated day conditions, constant ambient light may continuously stimulate the rod cells, resulting in larger colour-specific cone cell responses; during dark periods, stimulation of the rod cells may be the driving factor in fish attraction.

Several behavioural guidance techniques have used light as a deterrent stimulus. I observed an overall attraction to light (positive phototaxis), which could have implications for management in how light guidance is used to mitigate fish loss. In my study, different light colours elicited different responses and the high level of attraction to green light could be linked to their sensitivities to the green spectrum in both their rod and cone cells. Our results provide insight for conservation efforts focused on white sturgeon, and possibly other acipenserids more broadly. The knowledge that reactions of fishes may vary by colour, time of day, and strobe rate could facilitate the development of species-specific behavioural guidance strategies. That age-0 white sturgeon are generally attracted to light, particularly at night when they are also most active (Poletto et al. 2014a), has important implications for potentially reducing negative outcomes around water in-stream infrastructure. The potential use of light to guide age-0 white sturgeon away from potential entrainment and impingement mortality sources to areas of relative safety under low flow conditions provides an additional operation for protecting this species of conservation concern.

More research is needed into how ambient light, light stimulus intensity, water flow, age, colour, and strobing rates might affect white sturgeon for field applications and conservation management, including how these light parameters influence other fish species that would encounter these devices so that unintentional damage to other populations does not occur. The application of an LED-based LGD could significantly improve sturgeon survival and aid in management of populations at risk, particularly if used in combination with other physical (see Ford et al. in press for an evaluation of an integrated light-louver rack array system) or non-physical technologies. Additional benefits of using LED technology includes lower power requirements, allowing for additional flexibility in implementation, lower operational costs and even possibilities of remote installations using alternative energy sources (e.g. solar).

Figures

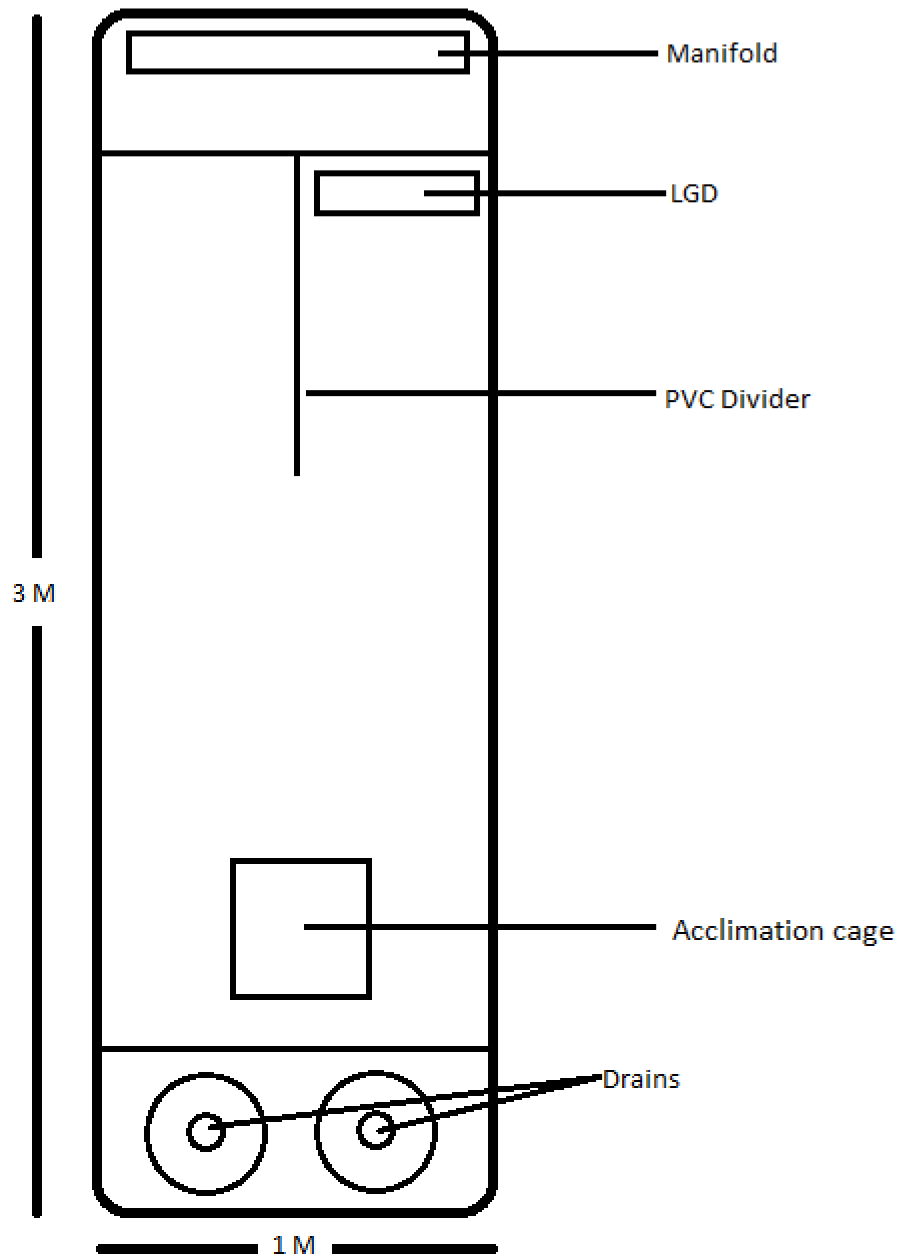

Figure 2-1. Schematic diagram of the y-maze used to detect preferences for specific colour and strobe rate combinations in age-0 white sturgeon (*Acipenser transmontanus*).

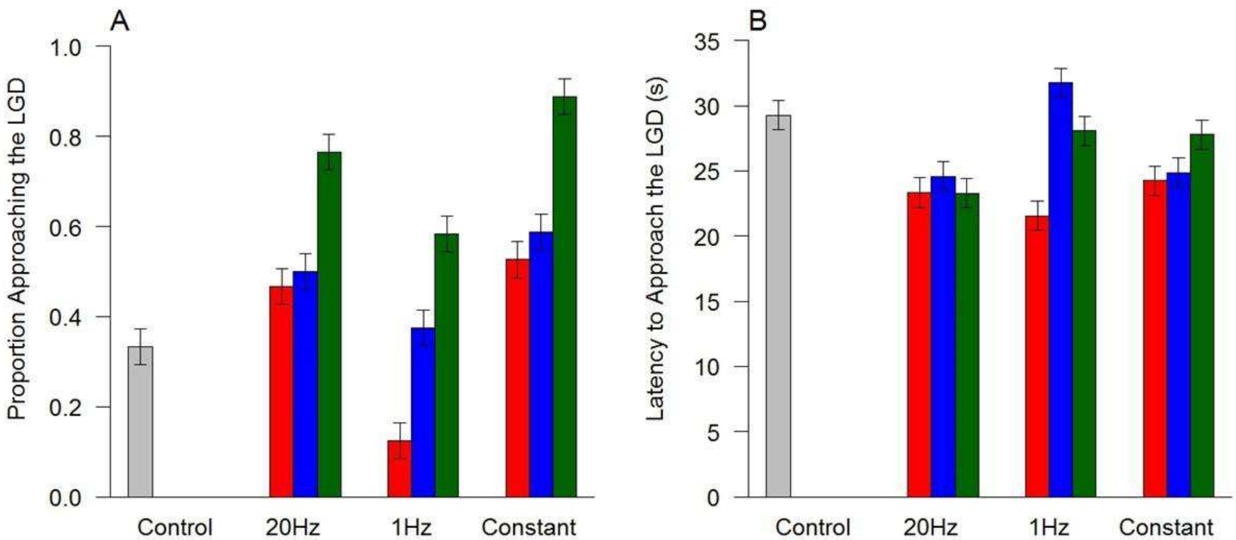

Figure 2-2. (A) Proportion of age-0 white sturgeon (*Acipenser transmontanus*) that approached the light device and (B) latency to approach under light conditions in a y-maze test. Bar colours indicate colour of light; control treatment had the LGD present but turned off.

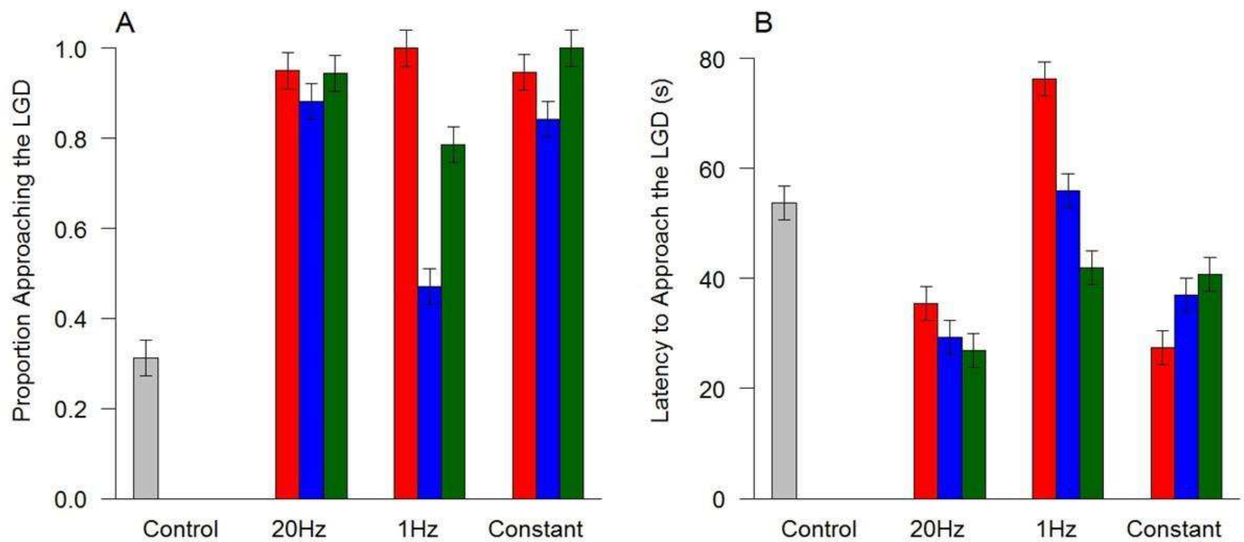

Figure 2-3. (A) Proportion of age-0 white sturgeon (*Acipenser transmontanus*) that approached the light device and (B) latency to approach under dark conditions in a y-maze test. Bar colours indicate colour of light; control treatment had the LGD present but turned off.

Chapter 3: Evaluating a light-louver system for behavioural guidance of age-0 white sturgeon

Abstract

Water diversions for hydropower and other applications are some of the most disruptive alterations affecting fish populations in lotic systems. Although many different strategies have been developed to reduce lethal encounters with such infrastructure, few studies have evaluated different forms of behavioural guidance concurrently. Here, I combine an LED-based light guidance device (LGD) equipped with adjustable wavelength and strobing output with a reverse-configured louver rack to assess the effectiveness of this two-part behavioural guidance system on downstream movement through a bypass by age-0 white sturgeon (*Acipenser transmontanus*). Several combinations of LGD and louver settings were tested under both simulated day and night (low light) conditions in a laboratory setting. In the absence of the LGD, louver slat spacings of 10 cm or 20 cm were most effective at achieving downstream bypasses with greater success rates (~ two-fold greater) under night conditions than under day conditions. Incorporating the LGD operating at the most attractive setting (green light strobing at 20 Hz) with the louver spacings of 10 cm or 20 cm achieved the highest rates of bypass usage (100% and 97%, respectively) under both day and night conditions while the control treatment (no LGD or louver) resulted in the lowest bypass rate (46%) among fish that moved downstream. Collectively, these results demonstrate that complementary cues can enhance the behavioural guidance of fishes and highlight the importance of continuing to explore the use of multiple strategies to mitigate entrainment for high priority fish species.

Introduction:

The growing demand for water in hydropower production and other diversions (e.g., irrigation, drinking water, industrial cooling) generates considerable problems for the conservation of aquatic systems (Vörösmarty *et al.*, 2010) and freshwater communities (Dudgeon *et al.*, 2006). Notably, the demand for water diversions increases the risk to fishes of entrainment through these structures and/or impingement on their debris racks (Barnthouse, 2013; Pracheil *et al.*, 2016), either of which can result in injury or mortality to affected organisms. Migratory species may be particularly susceptible as their movements may result in increased frequencies of encounters with these structures (Schilt, 2006; Poletto *et al.*, 2014a). Physical barriers like small spaced louver arrays (EPRI, 2001; Amaral, 2003) and bar racks (Russon *et al.*, 2010) or screens (Gale *et al.*, 2008) can potentially be used to prevent entry of aquatic organisms to intake pipes and turbines. However, smaller fishes may still be able to pass through many of these structures (Coutant and Whitney, 2000), and larger fishes may become impinged upon them (Swanson *et al.*, 1998). Non-physical barriers, by contrast, aim to exploit the sensory physiology of aquatic biota to repel them from potentially dangerous areas (negative taxis) or serve as an attractant (positive taxis) towards more desirable paths such as bypass channels (Noatch and Suski, 2012).

Behavioural guidance strategies have recently gained attention for their potential to decrease mortality rates associated with water diversion infrastructure (Coutant, 1999, 2001). Artificial lighting consisting of mercury vapor bulbs producing white light was one of the earliest behavioural guidance strategies (Rodgers and Patrick, 1985; Patrick *et al.*, 1985; Nemeth and Anderson, 1992), but with varying success. Strobing white lights have been used to deter Chinook (*Oncorhynchus tshawytscha*), coho (*O. kisutch*), and sockeye salmon (*O. nerka*), as well as steelhead (*O. mykiss*), from entering a navigation lock (Johnson *et al.*, 2005), and similar results were obtained for delta smelt (*Hypomesus transpacificus*; Hamel *et al.*, 2008). Conversely, the effects of strobe lights in behavioural guidance were equivocal in sea lamprey (*Petromyzon marinus*; Stamplecoskie *et al.*, 2012) and muskellunge (*Esox masquinongy*; Stewart *et al.*, 2014). One limitation of earlier light apparatus is that they were typically monochromatic (e.g., white mercury vapour), constraining their effects on fishes varying in diel activity patterns and sensitivities to different colours. White lights have been used, unsuccessfully, to guide white sturgeon in the past and had limited effectiveness at reducing rates of impingement on physical barriers (Poletto *et al.*, 2014a). Several different light devices (Nemeth and Anderson, 1992; Mueller *et al.*, 2001; Richards *et al.*, 2007) have also been tested, although light intensity, colours, and strobing rates have been evaluated independently (Mueller *et al.*, 2001; Richards *et al.*, 2007; Sullivan *et al.*, 2016). In the context of diel patterning, larger groups of kokanee (*O. nerka*) and rainbow trout (*O. mykiss*) have been observed around white lights at night compared to during the day (Simmons *et al.*, 2004), suggesting that single-colour lights may not be effective at achieving desirable behavioural outcomes throughout the full photoperiod.

Bubble screens (Sager *et al.*, 2000; Stewart *et al.*, 2014), electrical fields (Noatch and Suski, 2012; Clarkson, 2004), and acoustics (Goetz *et al.*, 2001; Flammang *et al.*, 2014) have all subsequently been incorporated into behavioural guidance strategies. Similarly, fish guidance efforts have tended to focus on the effectiveness of physical or non-physical barriers in isolation (EPRI, 2001; Noatch and Suski, 2012), while generally neglecting to explore any complementary effects arising from integrated multi-sensory approaches (*sensu* Ferrari *et al*., 2008; Elvidge *et al*., 2013). Louver arrays have been used as a behavioural guidance device since at least the 1950s (Bates and Vinsonhaler, 1957). Louvers have been evaluated for their potential to help guide many species, including American eels (*Anguilla rostrata*; Amaral, 2003), Atlantic salmon (*Salmo salar;* Scruton *et al*., 2008), rainbow trout (Shepherd *et al*., 2006), and shortnose (*Acipenser brevirostrum*) and pallid sturgeon (*Scaphirhynchus albus;* Kynard and Horgan, 2001). Louver systems function by altering the hydrodynamics of the water (Scruton *et al*., 2008b) in order to create turbulence that deters fish from passing through, which can also lead to reduced flow and power generation. A reversed louver array likely improves diversion of fish since the slat angle is reversed relative to the flow. This may allow flow to an intake to remain relatively unaltered while still creating turbulence and hydrodynamic conditions intended to deter fishes. Typical louver configurations place the slats at acute angles to the direction of flow (Kynard and Horgan, 2001; Shepherd *et al*., 2006), ranging from 7.2° (Shepherd *et al*., 2006) to 45° (Kynard and Horgan, 2001; Amaral, 2003).

White sturgeon (*Acipenser transmontanus*) are endemic to the Pacific coast in British Columbia, Washington, Oregon, and California. Some populations of this semi-anadromous species are listed as endangered under the Species at Risk Act in Canada (Fisheries and Oceans Canada, 2014), and overall the species is assessed as Least Concern on the IUCN Red List (Duke *et al*., 2004). Inhabiting rivers, bays, and estuaries along the coast, white sturgeon are the longest living freshwater fish in North America (Birstein, 1993) and have historically experienced pressures from fisheries harvesting that have been exacerbated by the development of hydropower facilities that impede their migrations (Boreman, 1997). Barriers to migration contribute to juvenile mortality when they encounter turbines (Beamesderfer and Farr, 1997), and alterations to river flow regimes reduce the amount and quality of habitat available to sturgeon populations (Fisheries & Oceans Canada, 2014). White sturgeon possess several characteristics suggesting that they may be an ideal candidate for behavioural guidance strategies: they are sensitive to light, especially the green and red spectra, during their juvenile stages (539 nm and 605 nm, respectively: Sillman *et al.*, 1990, 2007); they are subject to impingement on screens over water intake structures; they exhibit diel patterning of behaviour (Poletto *et al.*, 2014a); and other species of sturgeon have been experimentally guided by louver arrays (Kynard and Horgan, 2001).

Using age-0 white sturgeon in a simulated stream channel under both simulated day (light) and night (dark) conditions, we examined the effectiveness of a combination of a reversed louver array and an LED-based light guidance device (LGD) at eliciting bypass usage during downstream movements. The LGD, unlike other light sources that have been used in guidance strategies, can produce any wavelength of light in the 400 nm - 670 nm spectrum at adjustable intensity and constant output or strobing at frequencies up to 40 Hz. Based on earlier studies (Kynard and Horgan, 2001; Amaral, 2003), I predict that the presence of the louver will have a positive effect on bypass usage and that including the LGD as an attractant towards the bypass will increase bypass rates. Individually, I predict that the louver will provide more effective guidance under day conditions than under night conditions as both conditions will provide sturgeon with hydraulic cues while the louver will only be visible under day conditions. I predict the opposite pattern for the LGD, with light stimuli having greater effects on fish movement patterns under night conditions. These results may serve not only to inform conservation efforts for white sturgeon and other species of concern around areas where there is risk of entrainment, but also contribute to the design of integrated behavioural guidance strategies in the field that exploit the sensory perceptions of target species.

Methods:

Test fish

I obtained age-0 hatchery-reared white sturgeon of Fraser River, BC stock from the International Centre for Sturgeon Studies (ICSS) at Vancouver Island University in Nanaimo, BC Canada. The ICSS maintains their sturgeon indoors in dechlorinated, biofiltered and UV-treated municipal water at a temperature of 14 ^{o}C and a photoperiod determined by external light sensors. Age-0 sturgeon are held in 2000 L green cattle drum tanks with an average density of 500 fish per tank. Test fish were transported individually in 10 L buckets between their holding tanks and the trial arenas and placed in net pens in the holding tanks following trials to prevent reuse. All experimental work was conducted within the ICSS building between October 2015 and January 2016.

Experimental apparatus

A dark green, fibreglass raceway tank (3 m length × 1 m width × 0.75 m depth) was supplied with water diverted from the ICSS aquaculture system and filled to a depth of 0.2 m (total volume = 600 L). Using a semi-closed recirculating flow system described that added water to the system at a rate of 1 L/min, I produced a constant flow rate of 0.24 m/s and prevented temperature changes greater than 1° C. The raceway was outfitted with a reversed louver array (Figure 1) with the outer frame constructed out of 2.5 cm × 2.5 cm square aluminium bars in a rectangular shape measuring 122 cm × 36 cm (length × height). The louver frame was placed 1 m from the head of the raceway angled 70° to the side wall. Holes were drilled along the length of the frame at 5 cm intervals to allow slats to be inserted at different spacings. The louver slats consisted of grey PVC sheets measuring 25 cm × 30 cm × 0.6 cm (length × height × width) attached to the outer frame at top and bottom with galvanized screws. The slats were angled 45° to the louver frame in a "reversed" position such that they were 65° to the side of the tank. A guide bar was attached to the bottom of the slats on the headwater side which closed the gap below the slats and prevented sturgeon from passing underneath the louver. A manual adjustment bar was fastened to the tops of the slats so their positions could be adjusted simultaneously. At the downstream end of the tank I left a 20 cm gap between the louver frame and the tank wall to simulate a bypass. The effectiveness of different louver parameters was evaluated by manipulating the spacing of the slats and the presence or absence of the guide bar. Sturgeon were exposed to slat spacings of 5 cm, 10 cm, or 20 cm with the guide bar in place. Two additional configurations consisted of the louver frame and guide bar in place with no slats, and the louver frame without the guide bar or slats. Finally, I conducted movement trials with no louver infrastructure in place (control). This approach resulted in six different louver settings (5 treatments, 1 control).

In addition, I incorporated an LED-based LGD developed by ATET-Tech, Inc. (Thornhill, ON). This device can produce any colour in the 400-670 nm spectrum at constant intensity or strobing between 1 Hz – 40 Hz. The LGD was used with one of two output settings: green light (540 nm; cf. peak absorbance of 539: Sillman *et al.*, 1990) strobing at 20 Hz, which had an attractive effect on this population of age-0 white sturgeon, and red light (605 nm) strobing at 1 Hz, which had a repellant effect (Ford *et al.*, in review). The green setting involved placing the LGD downstream of the bypass to guide fish towards the passage, while the red setting was presented by placing the LGD behind the louver to deter the sturgeon from passing between the slats or through the louver frame. Movement trials were first conducted under day (light) conditions and later under night (dark) conditions. Over the course of the experiment, fish growth was sufficient to prevent them from being able to pass through the 5 cm slat spacing during night trials (day: 170.5 mm ± 1.16 mm; night: 196.7 mm ± 0.41 mm, mean total length ± SE), resulting in 35 different treatment combinations.

Each movement trial consisted of an individual sturgeon being released into the centre of the arena at the upstream end. Using Hero 2 digital cameras (GoPro, Inc., San Mateo, CA) mounted above the arena, I recorded their movements over 1 min post-release for subsequent analysis based on whether or not: 1) each fish moved downstream; 2) each fish moved through the bypass channel; 3) each fish moved through the louver array; and 4) the time (in seconds) to move downstream through the bypass or through the louver array area, if applicable. Each sturgeon was exposed to one treatment and no fish were tested more than once. I analyzed the first three measures as general linear models with binomial distributions and time to passage as a linear model against louver spacing, LGD setting and light condition (day/night) as fixed-effects. Due to the size difference between fish tested under day and night conditions, I included individual body size (total length, mm) as a linear covariate in the analyses. The binary response variables were then converted to odds (where odds of 1 imply a 50% chance of either outcome) and odds ratios to highlight the effects of the LGD-louver settings on sturgeon behaviour in comparison to control trials. All analyses and figures were generated using R version 3.2.4 (R Core Team, 2016) and the 'gplots' (Warnes *et al.*, 2015) package.

Results:

Light intensity

Ambient light intensity and LGD output were measured using a Dr.Meter® LX1330B digital light meter (HISGADGET, Union City CA) with a range of 0 – 200 000 lux. Under dark and light conditions, ambient light intensity at the midpoint of the water column in the trial arena was 3 lux and 169 lux, respectively. Intensities of the different colours of light are illustrated in Figure 2; sturgeon were placed into the arena 200 cm away from the LGD.

Louver parameters

Of the fish used (*n* = 1349, ~4 months old, total length 182.7 mm ± 27 mm, mean ± SD), 60.6% (*n* = 818) of these moved downstream regardless of treatment (Table 1). LGD-control trials allowed us to test the effect of louver configuration on use of the downstream bypass and passage through the louver itself independent of the light stimulus. Overall, slat spacing (Wald's $\chi^2 = 19.5$, df = 5, P = 0.0015) and background light condition ($\chi^2 = 85.1$, df = 1, P < 0.0001) both significantly influenced whether or not a fish moved downstream in the trial arena (Figure 3a). Similarly, slat spacing (Wald's $\chi^2 = 37.4$, df = 5, P < 0.0001) and background light condition ($\chi^2 = 24.5$, df = 1, P < 0.0001) both had significant effects on bypass usage in the LGD-control trials, with a greater effect under night conditions (Figure 3b).

Within the subset of fish that did move downstream, only the interaction term between louver spacing and light condition was statistically significant in terms of latency (time) to use the bypass ($F_{4,457} = 3.75$, P = 0.0052; Figure 3c). Slat spacing ($\chi^2 = 42.6$, df = 7, P < 0.0001), light condition ($\chi^2 = 13.9$, df = 1, P < 0.0001) and their interaction term ($\chi^2 = 13.9$, df = 4, P = 0.0078) all significantly influenced the actual proportion of sturgeon using the bypass (Figure 2d;

Table 1, all rows where "LGD parameter" is "Control"). All fish that moved downstream but did not use the bypass instead passed through the louver itself or through its footprint area in the case of louver-control treatments. Fish size (total length) did not have a significant effect on any of these responses.

Under day conditions only, louver spacing did not have a significant effect on the overall proportion of sturgeon moving downstream through the bypass ($\chi^2 = 10.5$, df = 5, P = 0.061, Figure 3b). In the subset of fish that moved downstream, however, the presence of the louver significantly increased bypass usage ($\chi^2 = 12.6$, df = 5, P = 0.027) whenever slats were present (Figure 3d). Under night conditions, both the overall rate ($\chi^2 = 43.3$, df = 4, P < 0.0001) and the actual rate of bypass usage ($\chi^2 = 30.7$, df = 4, P < 0.0001) were significantly influenced by the presence of louver slats (Figure 3b & d). Overall, louver spacings of 10 cm or 20 cm were the most effective at eliciting bypass usage under both day and night conditions, with no significant difference found between them in post hoc testing.

Integrated LGD-louver system

Overall downstream movement was significantly influenced by LGD setting ($\chi^2 = 32.9$, df = 2, P < 0.0001), louver spacing ($\chi^2 = 16.3$, df = 5, P = 0.0061), light condition ($\chi^2 = 90.4$, df = 1, P < 0.0001), and the two-way interactions between LGD and louver spacing ($\chi^2 = 18.6$, df = 10, P = 0.045), LGD settings and light condition ($\chi^2 = 20.1$, df = 2, P < 0.0001) and louver spacing and light conditions ($\chi^2 = 9.9$, df = 8, P = 0.042; Table 1). LGD setting ($\chi^2 = 85.6$, df = 2, P < 0.0001), louver spacing ($\chi^2 = 18.9$, df = 5, P = 0.0019) and background light condition ($\chi^2 = 25.1$, df = 1, P < 0.0001) had significant effects on the overall proportion of age-0 white sturgeon using the bypass (Figure 4a). In addition, there were significant two-way interactions between

LGD and louver settings (χ^2 = 31.7, df = 10, P = 0.00045), louver settings and background light conditions (χ^2 = 10.3, df = 4, P = 0.036) and in the three-way interaction between LGD setting, louver spacing and background light condition (χ^2 = 25.8, df = 8, P = 0.0011) on the overall proportion of fish using the bypass. Sample sizes, proportions and mean latencies of bypass usage for each treatment combination are listed in Table 1.

Of the fish that moved downstream, bypass usage was influenced by LGD and louver settings, light condition, and body size, with significant interaction terms between all fixed-effects factors (all P < 0.05; Figure 4a & b). In general, smaller sturgeon were more likely to use the bypass while fish that passed through the louver tended to be larger (mean total length 184.9 mm vs 193.7 mm, respectively) although the mean difference was <10 mm. Latency to bypass was influenced by LGD setting ($F_{2,764}$ = 4.98, P = 0.0071), body size ($F_{1,764}$ = 21.62, P < 0.0001, Pearson's r = -0.19) and the interaction between louver spacing and light condition ($F_{4,764}$ = 3.42, P = 0.0088; Figure 4c & d).

Under day conditions, LGD setting (χ^2 = 9.1, df = 2, P = 0.01) and body size (χ^2 = 4.3, df = 1, P = 0.038) had significant effects on bypass usage (Figure 3a), while latency was influenced only by body size ($F_{1,288}$ = 17.75, P < 0.0001, r = -0.25; Figure 4c). While larger sturgeon took longer to use the bypass, fish passing through the louver itself were larger on average than fish using the bypass (183 mm vs 171 mm, respectively). Under night conditions, bypass usage was only influenced by LGD setting (χ^2 = 58.1, df = 2, P < 0.0001; Figure 4b) while latency was influenced by LGD setting ($F_{2,273}$ = 3.9, P = 0.021) and louver spacing ($F_{5,273}$ = 6.34, P < 0.0001; Figure 4d).

Independent of louver spacing, green light (540 nm) strobing at 20 Hz resulted in greater bypass usage overall under both day and night conditions, while red light (605 nm) strobing at 1

Hz tended to elicit fewer bypasses than either the green light or control treatments, particularly under night conditions (Figure 3a & b). In combination with the louver, green light strobing at 20 Hz and louver spacings of 10 cm or 20 cm resulted in the greatest odds of bypass usage (Table 1, last column).

Discussion:

My results demonstrate that an integrated light-louver system can be effective at behaviourally guiding age-0 white sturgeon towards a bypass while simultaneously decreasing the latencies of bypass approach and entry. These findings support the hypothesis that green light strobing at 20 Hz can serve as an attractant to age-0 white sturgeon. Conversely, when all trials are examined together, treatments involving red light strobing at 1 Hz had lower proportions of fish moving downstream, raising the possibility that red light was an effective repellent and inhibited downstream movement altogether as moving downstream in the trial arena required an individual to enter an area illuminated by red light. Background light conditions significantly influenced behavioural responses to the paired stimuli, with sturgeon demonstrating the greatest rate of bypass usage when the LGD was used to attract them to the bypass with green light under day (light) conditions, independent of louver parameters. My experiment demonstrated relatively high levels of diversion with a louver system, although not all louver parameters were equal, as the 5 cm and 10 cm spacings had 100% diversion rates compared to 90% diversion with the 20 cm spacing. By contrast, the use of either red or green light under simulated night (dark) conditions did not increase the effectiveness of the louver when slat spacings were 10 cm or 20 cm. Overall, rates of bypass usage were highest when the louver spacing was 10 cm, with significantly more fish using the bypass during the night trials compared to the day trials.

The use of strobing lights on age-0 white sturgeon affected the rate of bypass usage and latency to bypass. Bypass rates increased for the higher strobing frequency and latency to bypass increased with the lower strobe frequency setting. Since strobe rate and wavelength were linked by setting, it is possible that strobe rate influenced bypass rate even though it was not tested independently. Previously, I found significant differences in attraction to the LGD in dichotomous choice tests (Ford *et al.*, unpublished data), suggesting that both colour and strobe rate may affect the adoption of positive or negative taxis for behavioural guidance outcomes.

Colour vision is a known trait of fishes (Levine and MacNichol, 1982), and different species have demonstrated variable responses to different colours of light (Marchesan *et al.*, 2005). In my study, green light (540 nm) was a significant factor in increasing bypass usage, with a greater effect observed during the day than at night, while red light (605 nm) had a repellant effect. Spectral sensitivities (539 nm and 605 nm: Sillman *et al.*, 1990) interacting with colour preferences or aversions may be the cause of attraction to green light in age-0 white sturgeon, providing a putative explanation for why the “attraction” setting of the LGD was associated with higher rates of bypass. However, ontogenetic shifts in spectral sensitivity towards red and blue wavelengths (Sillman *et al.*, 1990) suggest that as white sturgeon mature, their reactions to green and red light may shift and therefore age-specific behavioural guidance strategies may be required.

The notable difference in the louver system I tested compared to earlier designs was that the slats were angled 65° to the flow direction, allowing more water passage. This did not change the success rate of diverting fish, as during both day and night conditions, there were no fish passing through the louver for the two smallest spacings (5 cm and 10 cm) and few (10%)

passing through the 20 cm spacing. The added benefit to this integrated light-louver system is that the combination of stimuli allows for guidance depending on varying responses to the individual stimuli.

Since white sturgeon do not react the same diurnally to different stimuli (Poletto *et al.*, 2014a), it is important to make sure that they can be guided at all times. During the day, the green light was a more effective guidance tool than the louver as it elicited greater rates of downstream movement. Together the two devices create the possibility for a 24 hour guidance strategy. It is conceivable that different individuals respond differently to behavioural guidance technologies and different times (e.g., day vs night) such that the use of combined approaches may lead to greater effectiveness. Most behavioural guidance systems use only one technique to guide fish, or if they do use multiple, they are typically multiple non-physical barriers (Noatch and Suski, 2012). The combination of using both a physical and non-physical barrier increased the effectiveness in guidance for age-0 white sturgeon, providing a better chance of being protected during different photoperiods. The louver was most effective in simulated night conditions. This effectiveness could have been because the fish avoided the complex currents created by the louver when they could not rely on vision. My findings suggest that age-0 white sturgeon downstream movement is greater at simulated night conditions, and similar results have been observed in other sturgeon species (shortnose and pallid [Kynard and Horgan, 2001]; green and white sturgeon [Poletto *et al.*, 2014a]); this may be a consequence of the higher activity levels and migratory movement of sturgeon at night (Poletto *et al.*, 2014a). These results may show benefits to the use of integrated behavioural guidance systems and how they may enhance desired outcomes through mutual reinforcement.

Behavioural guidance techniques in the past have primarily focused on avoidance by using different strategies to deter organisms from passing into a certain area, whereas my approach also examined the possibility of attracting fish towards safe passages. Based on my observations, using red light as a repellent from the louver was less effective than using green light to attract fish towards the bypass. Attraction to safe areas may be more beneficial to fish protection since fish may become habituated to the negative stimuli, attenuating the repulsive effect and increasing the risk of harm over repeated exposures. While my observed trends of slower approaches to the bypass and decreased likelihoods of downstream movement during the red light treatments may not be completely attributed to the repulsion setting, these could benefit from further exploration under field conditions. Repulsion strategies also can become a problem as fish do not respond consistently to the same stimuli depending on time of day (Poletto *et al.*, 2014b), as illustrated by reports of fish numbers increasing around an illuminated dam during night compared to day (Simmons *et al.*, 2004). If fish react differently depending on individuals and diel period then it may be possible that attraction would serve best to reinforce travel around hazards and decrease the number of encounters with harmful objects such as physical barriers.

The present research has shown promise for the use of integrated behavioural guidance systems. The advantage of the LGD during the day and the louver during the night has shown that age-0 white sturgeon can be guided towards safe passage in a laboratory setting through attraction. The use of an integrated guidance system to simultaneously repel (louver) fish from a danger area and to attract (LGD) fish towards safety could lead to applications around many waterway developments where single behavioural guidance techniques may not be sufficient to guide the majority of fish. It is important to note that this research was done in a laboratory, and if at all possible should be followed up with a study under fully natural conditions to confirm these results. Findings from this study may help lower risk of entrainment and impingement of white sturgeon, aiding populations that are threatened from many different stressors and thus improving population numbers. The use of integrated diversion systems could lead to better protection for many other imperiled fish and aquatic species.

Table

Table 3-1. Sample sizes, summary results and odds of downstream movement patterns demonstrated by age-0 white sturgeon (*Acipenser transmontanus*) exposed to different LGD and louver array parameters.

Parameters:				Proportions:		Latency	Odds of
Light condition	LGD	Louver	N	Moving downstream	Using the bypass[1]	to bypass (s)	bypass use[2]
Light	Control	Control	115	0.29	0.86	16.9	0.34
		Frame	25	0.59	0.68	21.3	0.67
		Guide bar	25	0.41	0.52	27.7	0.27
		20cm	25	0.43	0.91	32.9	0.63
		10cm	25	0.37	1	24.7	0.60
		5cm	20	0.3	1	25.4	0.43
	Red 1Hz	Control	115	0.2	0.6	30.6	0.14
		Frame	24	0.5	0.67	36.1	0.5
		Guide bar	25	0.6	0.8	25.2	0.92
		20cm	25	0.2	0.8	31.5	0.19
		10cm	25	0.28	0.86	46.9	0.32
		5cm	20	0.1	1	39.0	0.11
	Green 20Hz	Control	115	0.64	0.94	34.6	**1.5**
		Frame	25	0.68	0.88	23.1	**1.5**
		Guide bar	25	0.8	0.95	17.5	**3.17**
		20cm	25	0.84	0.95	39.1	**4.00**
		10cm	25	0.64	1	27.4	**1.78**
		5cm	20	0.8	1	21.4	**4.00**
Dark	Control	Control	115	0.83	0.32	25.7	0.36
		Frame	25	0.85	0.53	23.8	0.83
		Guide bar	25	0.81	0.61	21.2	0.97
		20cm	25	0.91	0.84	19.9	**3.17**
		10cm	25	0.69	1	20.7	**2.26**
	Red 1Hz	Control	115	0.8	0.8	27.7	**1.78**
		Frame	25	0.6	0.27	26.6	0.19
		Guide bar	25	0.76	0.42	18.6	0.47
		20cm	25	0.64	0.44	33.2	0.39
		10cm	25	0.68	0.71	25.9	0.92
	Green 20Hz	Control	115	0.8	1	30.9	**4.00**
		Frame	25	0.72	0.94	30	**2.13**
		Guide bar	25	0.88	0.82	20.7	**2.57**
		20cm	25	0.72	1	24.8	**2.57**
		10cm	25	0.76	1	25.2	**3.17**

[1]Proportion of individuals using the bypass are calculated as proportions of fish that moved downstream in each treatment combination.

[2]All odds of bypass usage >1 (i.e. greater than 50% chance) are listed in bold.

Figures

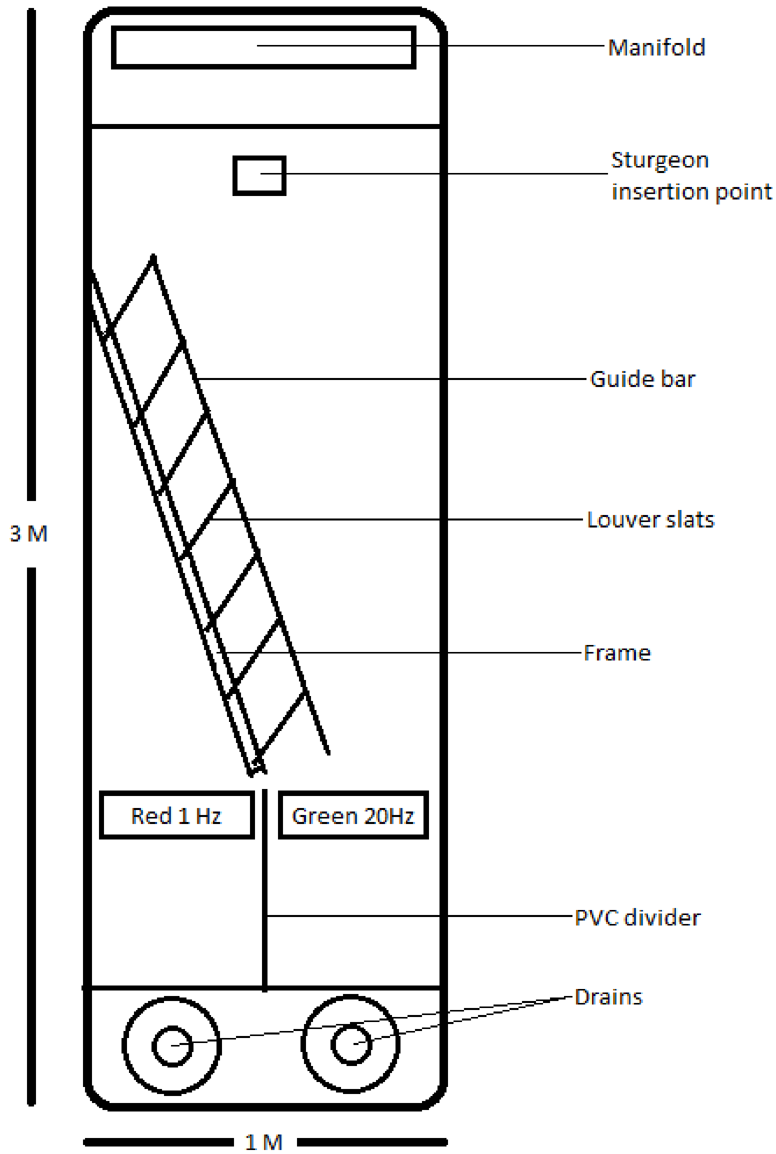

Figure 3-1. Schematic diagram of the raceway used to test the effectiveness of the integrated light-louver array on downstream passage of juvenile white sturgeon (*Acipenser transmontanus*). Louver slats could be removed entirely or spaced at 5 cm, 10 cm, or 20 cm. Distance between the LGD and the sturgeon insertion point is 200 cm.

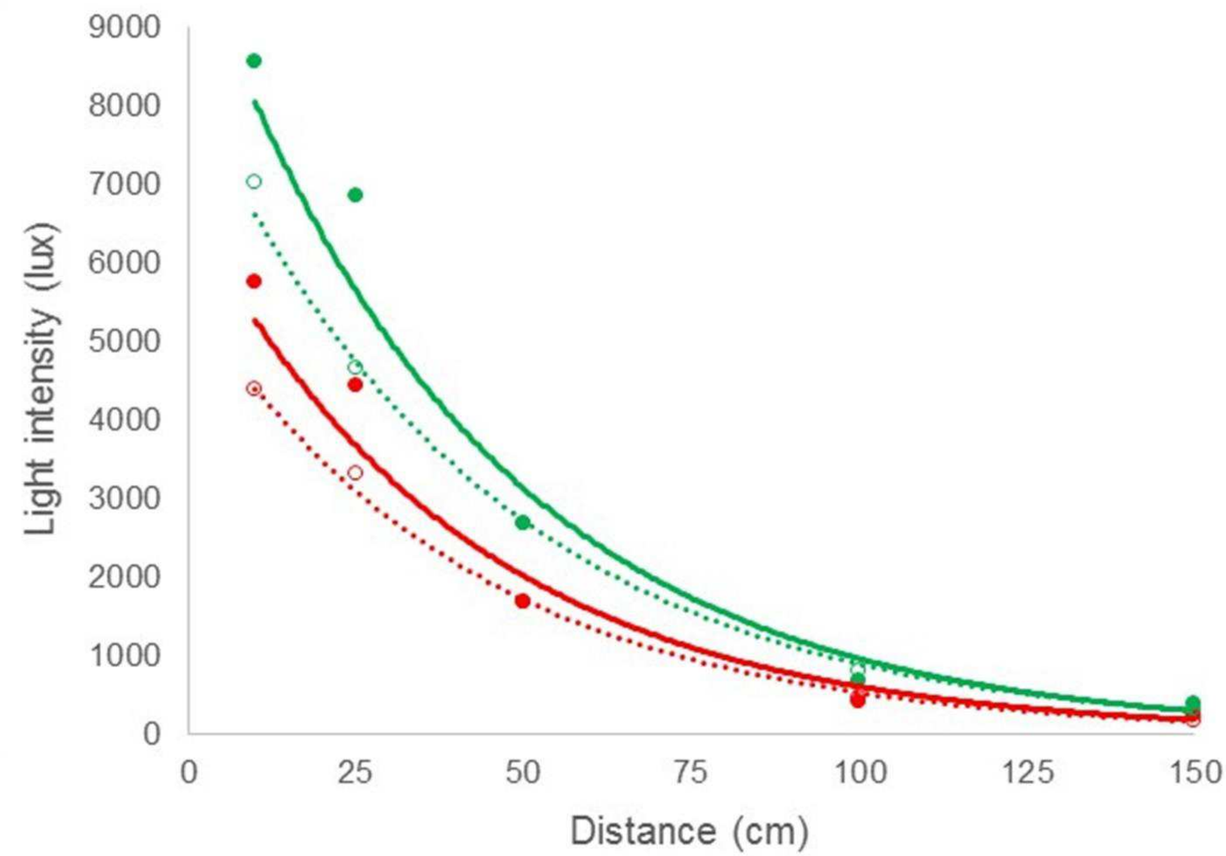

Figure 3-2. Light intensity (lux) of the colors used at distance intervals (10 cm, 25 cm, 50 cm, 100 cm, 150 cm) under dark (open circles, dotted lines) and light (closed circles, solid lines) conditions.

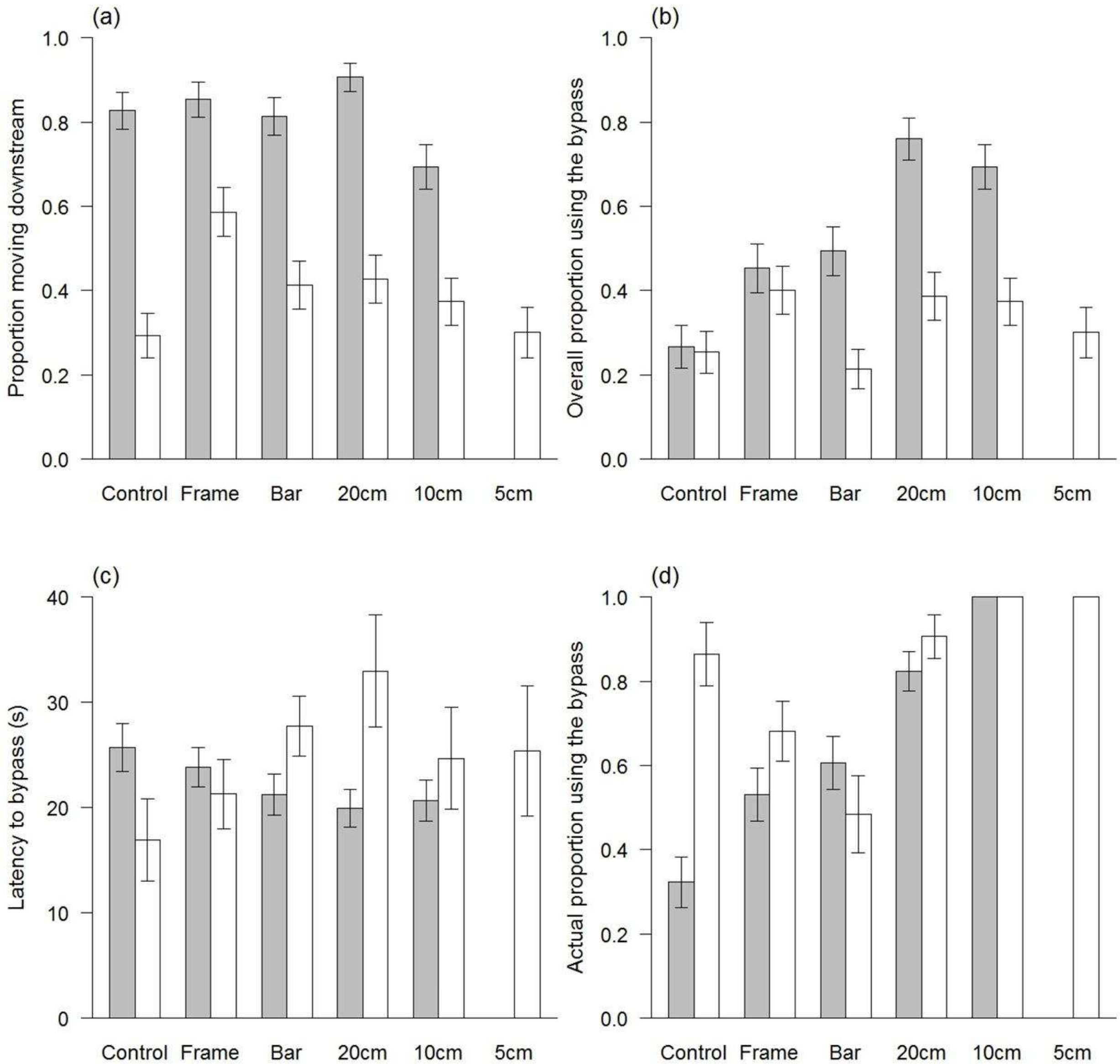

Figure 3-3. Overall proportions (± SE) of juvenile white sturgeon (*Acipenser transmontanus*) that (a) travelled downstream, and (b) used the bypass. For the sturgeon that moved downstream, (c) mean (± SE) latency to use the bypass and (d) the actual proportion that used the bypass with different louver configurations under both light (open bars) and dark (grey bars) conditions in the absence of illumination from the LGD.

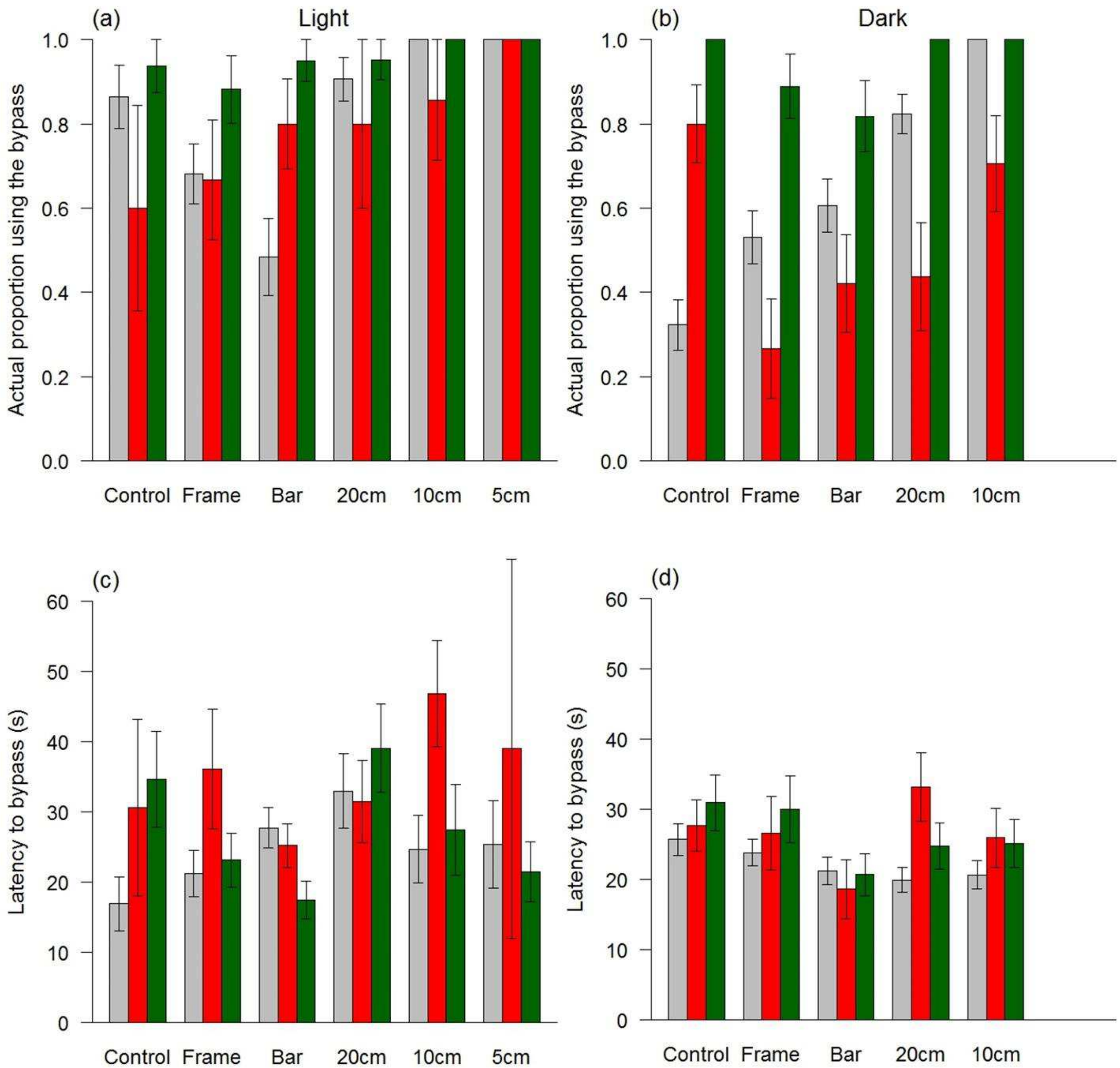

Figure 3-4. Proportion (± SE) of juvenile white sturgeon (*Acipenser transmontanus*) that moved downstream and that used the bypass (a, b) and their mean latency (± SE) to passage (c, d) with different louver configurations under light and dark conditions. Grey bars: LGD control; red bars: red light strobing at 1 Hz; green bars: green light strobing at 20 Hz.

Chapter 4: Behaviour of age 0+ and 2+ walleye when exposed to strobing or constant light of different colours emitted from an LED behavioural guidance device

Abstract

Much effort has been devoted to the development of behavioural guidance technologies to reduce fish entrainment and/or impingement yet the effectiveness of these mitigation tools vary widely and are known to be species- and context-specific. In the midwest of North America, Walleye are a culturally and socio-economically valuable species for which there is need to develop more effective behavioural guidance technologies. Therefore, I explored the possibility of using light emitting diode (LED) lights as a behavioural guidance tool for walleye. Based on existing laboratory research on the visual physiology of walleye, I tested colours of known spectral sensitivity (i.e., green and orange) using constant light and a strobing light with a frequency of 5Hz in short term tests. Age 2+ and 0+ walleye were tested using these settings against a control both during daytime and nighttime hours in a low light environment. Age 2+ walleye generally avoided the light guidance device when the light was on compared to the control, regardless of colour or strobing rate. To determine if there was any ontogenetic differences in walleye reaction to light, I tested both age 0+ and 2+ for their rate of escapement when exposed directly to the light. This test failed to identify significant differences in the amount of time either age class stayed in the light although it was apparent that fish of both age classes avoided the light, regardless of colour and strobing rate. This study determined that walleye behavioural responses to light is typically in the form of avoidance and that it appears to be consistent for both age 0+ and 2+ walleye.

Introduction

Waterway modifications have contributed to biodiversity declines in many of the world's freshwater ecosystems (Dudgeon et al. 2006; Vörösmarty et al. 2010). Water is increasingly necessary for agriculture, human consumption, industrial processes, and energy production (Vörösmarty et al. 2010). The various infrastructure associated with water withdrawl or energy generation poses a risk to fish as they can become entrained or impinged, which can be fatal (Schilt 2000; Allen et al. 2012). Although physical barriers can be used to reduce likelihood of entrainment (e.g., Coutant 1999; Noatch and Suski 2012), fish may still become impinged upon them.

Behavioural guidance is a common mitigation technique for entrainment and impingement and relies on exploiting an organisms sensory physiology to guide them (Noatch and Suski 2012). Typically these guidance techniques are used to lead fish away from hazardous situations, such as hydropower turbines or unprotected water intake pipes (Coutant 1999, Schilt 2007). However, it can also be used to attract fish to desired pathways (e.g., a fishway or bypass channel). One of the inherent challenges for behavioural guidance is that fish undergo ontogenetic changes in visual physiology and behaviour. This can affect how a desired stimulus will affect the guidance of a fish depending on life stage. For example, the photo-response of white sturgeon changes as they advance from larvae to juveniles and become less attracted to green light (Loew and Sillman 1993). Light has been used in the past as a behavioural guidance tool (Hocutt 1981, Noatch and Suski 2012, ERPI 1986) but was largely abandoned based on lack of flexibility and technical limitations. Early on, mercury vapour bulbs (Haymes et al. 1984) were used but they were not as bright as desired and required large energy inputs. Later developments in the field included the ability of lights to strobe or produce different colours

(Hocutt 1981, ERPI 1986), but no one solution seemed to work as species specific reactions vary greatly for light guidance. Advances in light technology in recent years have led to the creation of light emitting diodes (LEDs). LEDs are very powerful lights that can be very small and have the ability to change colours and strobe (at low or high frequency), something lights of the past could not easily do. Moreover, LEDs are much more efficient, requiring less energy. For these reasons, LEDs show promise for use in the behavioural guidance of fish (e.g., Sullivan et al. 2016; Ford et al. In Press).

Walleye (*Sander vitreus*) are a perciform fish that is native to many rivers and lakes in Canada and the northern Unites States (Bozek et al. 2011). Walleye are highly valued, as both recreational fish and for nourishment in many communities in the midwest of North America (e.g., Lester et al. 2014). Due to their value, they are at risk from overfishing and waterway development, leading to walleye currently being stocked into many waterbodies to maintain populations (Wilson et al. 2007). Walleye are a versatile top predator, though competition with other top predators can influence population sizes in smaller waterbodies (Bozek et al. 2011). They can spawn in both rivers and lakes, and sometimes even undergo migration from lakes into tributaries to spawn (Bozek et al. 2011). In rivers they use rapids (Walburg 1972) as spawning grounds and in lakes they use shallow areas such as reefs (Eschmeyer 1950). The increased movement for spawning along with natural movement patterns within lakes and rivers are exposing these fish to the hazards of waterway development. Walleye are known to be a nocturnal fish (Reed 1962, Carlander and Cleary 1949) and they generally spend the day at depth as they seek out low light conditions (Bozak et al. 2011, Kelso 1978). This natural behaviour indicates that walleye may react well to behavioural guidance through light as they typically prefer low light environments. Laboratory studies on the retinal physiology of the walleye has revealed that they have two spectral sensitivities with peak absorbances at 533nm (green) and 605nm (orange) (Burkhardt et al. 1980). The critical flicker-fusion frequency (CFF) (highest frequency at which the eye can differentiate flashes) of the walleye retina was examined to have a limit around 20Hz with some variation (Ali and Anctil 1977). With this knowledge and flexibility of LED lights, an optimal combination of strobing and colour may be found to best guide walleye using their behavioural response to light stimuli.

The objective of this experiment was to determine the behavioural response of age 2+ walleye to known spectral sensitivities (green and orange) using a constant light and a strobing light (5Hz). Walleye were monitored for 300 seconds to determine their reaction to the light as avoidance, attraction, or a lack of reaction. As the responses to light in other fishes [like the white sturgeon (Loew and Sillman 1993) shift as they grow, I tested the behavioural responses of both age 0+ and 2+ walleye to determine if there is an ontogenetic difference in reaction to the light stimuli. The majority of the data were collected on age 2+ walleye so those data are presented first with the comparison between 0+ and 2+ walleye being presented thereafter.

Methods

Study site and species

This study took place at the White Lake Fish Culture Station, Ontario Canada, from July 18 to August 8 2016. Fish tested were 0+ (Average total length = 7.3 ± 0.69) and 2+ (Average length = 21.4 ± 1.5) year walleye hatched from Lake Ontario eggs. 2+ fish at the hatchery were held in 2000L cattle drums at densities of 300 fish per tank. 0+ walleye were being grown in 2000L cattle drums with a density of about 10,000 per tank. Water temperature was maintained at 18°C using lake water from White Lake (a combination of deep and surface water). Walleye were taken off feed for the duration of the study (separate tanks were sampled at a time to reduce duration of no feed). 2+ walleye were transported in 67L black utility buckets with a garbage bag cover to prevent exposure to sun during transportation and reduce stress while 0+ walleye were transported in 20L white utility buckets. After use, walleye were placed in a super-trough until study was completed to prevent reuse.

Trial arena and stimuli

To determine level of attraction, repulsion, or neutrality to the colours being tested along with their combination of strobing rates, a funnel choice test was designed in 2m×2m fiberglass tanks. The tank was divided by PVC sheets that were placed at angles of around 60° (relative to the dark side of the tank) on opposite sides and extended into the middle, forming the walls of a funnel entrance to the light chamber (Figure 1). Using u-shaped aluminum, a guide was created for a PVC door that could be lifted up from a distance using a pulley system to minimize disturbances for the test fish. Walls were taped along the two sides in contact with the tank to prevent light passage through the seams. The LGD was placed in the light chamber against the wall facing the door and weighted down using two kg weights to prevent floating. The tank was filled to a depth of 20cm using the hatchery water supply, with temperature maintained within 1°C of the holding tank temperature (18°C).

To test walleye reactions to strobing light, I used a light guidance device (LGD) developed by ATET-Tech, Inc. (Thornhill, ON), for use in behavioural guidance of fish species. The LGD consists of 162 LEDs that can produce constant light or strobe up to 40Hz and can produce any colour combination of light in the 400-670nm spectrum by varying the saturation of red, green, and blue light. The LGD was placed in the light chamber facing the removable door so that light could shine into the acclimation chamber once the door was lifted. I used a strobe rate of 5Hz as well as constant light were chosen to be tested to determine the effect of strobing lights on the walleyes reaction to colour. 5Hz was chosen based on previous studies using strobing light (Johnson et al. 2011 and Baker 2008) along with knowledge that walleye CFF limit is close to 20Hz (Ali and Anctil 1977). Two colours were chosen to be tested based on the spectral sensitivity of the walleye retina (Burkhardt et al. 1980). From this study the colour of green (535nm) and orange (605nm) were chosen based on the sensitivity of the single cone and double cone formations respectively. Both colours were tested with both constant light and strobing at 5Hz, along with a control of no light emissions from the LGD. These 5 treatments were replicated during the night to assess level of activity differences as walleye are known to be nocturnal (N=25 per treatment, N=242 total observations).

Experimental protocol and analysis

Walleye were placed in the centre of the acclimation chamber and allowed to acclimate for 5 minutes before the LGD was activated on one of the 5 settings and the door to the funnel entrance was removed. The subsequent behaviours of the walleye in the trial arena were recorded using GoPro Hero 3+ cameras for 5 minutes. At the end of each trial, the fish was removed, measured (total or standard length?), and transported to the recovery tank (super-trough).

To test ontogenetic differences the design was modified. The light was turned on from the beginning and the door was left open. Fish were then released in the light beam on the acclimation side of the tank (attempts were made to make fish face light as they were released) and monitored for 1 minute. This study was done during day and night with 0+ and 2+ year class walleye (N=25 per treatment, N=500 total).

Analysis

We transcribed the following behavioural responses from the videos: i) closest distance to light (if walleye passed into the light beam); ii) number of inspections (instances where the walleye moved right to the edge of the light beam and either maintained position or turned away); and iii) the number of passes (times the whole body of walleye entered the light). Entrances, passes, and inspects were changed in R to either a 0 (for no action of this nature) or a 1 (action taken, e.g. passed though the light) for better analysis using a GLM. The arena was separated into six section according to light levels. Light levels were taken using the Dr. Meter digital lux meter (Model LX1330B). Light levels were scored as attraction (+) or avoidance (-) and broken up into 3 zones based upon light intensity. Dark zones were scored as 1- (1.0-1.7 Lux), 2- (2.1-3.0 Lux), and 3- (3.0-3.8 Lux). Light zones were scored as 1+ (125.8-405 Lux green, 192-691 Lux orange), 2+ (22-145 Lux green, 30-356 Lux orange), and 3+ (681-9300 Lux green, 1798-20900 Lux orange) (reference Figure 1). 2+ was scored higher than 1+ because fish had to pass through 3+ (brightest zone) to enter which was not necessary for 1+. Light time used as a measure of these zones to create one continuous variable displaying proportion of time spent in light and dark areas. Calculation for light time used is: (3*seconds spent in zone 3+) +(2*seconds spent in zone 2+) +(1*seconds spent in zone 1+) +(-1*seconds spent in zone 1-) +(-2*seconds spent in zone 2-) +(-3*seconds spent in zone 3-). Distance was measured as a percentage of the closest a fish came to the LGD with “0” being the walleye touched the LGD and “100” being the furthest away from the light possible. Distance was only measured for walleye that entered the light beam (zone 1+) (N=151 for experiment 1, experiment 2 had fish inserted directly into light so a measure was always taken). Linear models were used to determine significance for light time and distance. Number of zone changes and amount of time spent in each zone was recorded and analyzed. Analysis of these metrics was done using R version 3.2.4 (R Core Team 2016).

Results

Experiment 1 – Age 2+ reaction to light

Using a GLM test, walleye were observed to be significantly less likely to enter the LGD side of the tank for treatments of both the green ($p<0.001$) and orange ($p<0.001$) light settings. Fish responses were independent of body size ($p=0.451$), strobing vs constant light ($p=0.087$), and diel period ($p=0.702$). Both passes (Green: $p<0.01$, Orange: $p<0.01$) and inspections (Green: $p<0.05$, Orange: $p<0.05$) were also significant for the colours green and orange but were not significant for any other factors. Walleye were more likely to have passed through the 1+ zone, inspected the edge of the light (or where it would be for controls), and entered the LGD side of the tank when the LGD was not emitting light. There were no significant interactions. The Hosmer-Lemeshow goodness-of-fit-test was used for all glm models and determined to be of good fit for entrances ($p=0.582$), inspects ($p=0.581$), and passes ($p=0.801$).

Through the use of a linear regression model, light time was significantly affected by colour, both green (t value= -4.067, $p<0.001$) and orange (t value= -4.153, $p<0.001$), when compared to the control. There was no significant effect from length of the fish (t value= -1.187, $p=0.236$), strobing vs constant light (t value= -0.822, $p=0.412$), and diel period (t value= =1.089, $p=0.277$). Fish were more likely to have a lower score (on average more time spent in the dark zones) when the light was on compared to when the light was turned off. A linear model of distance fish came within the LGD showed a significant difference in the colour setting for both green (t value= 4.338, $p<0.001$) and orange (t value= 6.137, $p<0.001$) as well as a significant difference for constant light vs strobing light (t value= -2.435, $p<0.05$). There was no effect on walleye distance to the LGD based upon fish length (t value= 0.823, p=0.412) or diel period (t value= -0.437, p=0.663). There were no significant interactions. On average, fish kept a larger distance between them and the LGD when the light was on both green and orange lights and were more likely to be closer if the light was strobing.

Experiment 2 – Ontogenetic difference in reaction to light for age 0+ and 2+ walleye

From the analysis of a linear model of closest distance between the fish and the LGD I observed significant difference for both the green (t value= 3.107, p<0.01) and orange (t value= 3.348 p<0.001) colours as well as a difference in reaction based on age (t value= -3.019, p<0.001). There was no significant effect from the length of the fish (t value= 0.526, p=0.599), diel period (t value= -1.431, p=0.153), or strobing (t value= 0.418, p=0.676). There were no significant interactions between variable but interaction between orange and age, diel period and age were both close to being significant (p=0.052 and p=0.0874 respectively). The three-way interaction between orange, diel period, and age was also close with a p-value = 0.088. This model shows that fish stayed further away from the LGD when the light was on regardless of colour when compared to no light and that age 2+ walleye were more likely to get closer to the LGD than 0+ walleye.

A linear model for light time revealed that only colour played a significant role for the study. Green light (t value= -3.458, p<0.001) and orange light (t value= -4.182, p<0.001) both has significantly lower levels of light time when compared to the control. Length (t value= -0.558, p=0.577), diel period (t value= -0.349, p=0.727), age (t value= 0.786, p=0.432), and strobing (t value= 0.218, p=0.828) had no significant effect on the model. There were no significant interaction between variable.

The rate at which fish left the light after being exposed to it quicker if the light was on, regardless of colour. Both green light (t value= -2.393, $p<0.05$) and orange light (t value= -3.364, $p<0.001$) saw fish escape the light significantly quicker than the control when no light was emitted. There was no effect from fish length (t value= 0.094, p=0.925), diel period (t value= 0.585, p=0.559), age (t value= -0.709, p=0.479), or strobing (t value= 1.100, p=0.272) and there were no significant interactions. Walleye of age 0+ had more fish that did not leave the light, where fish froze and sat on the bottom the entire test (N=21 for 2+ and N=43 for 0+). On average, there was no significant difference between the two age groups in distance to the LGD, light time, and time till escape. Both age 0+ and 2+ walleye avoided the light when the light was on regardless of strobing or colour.

Discussion

The results of this study show the difference in individual walleye's reaction to light emitted from an LED light guidance device. Both orange and green lights lowered the amount of time that walleye spent in the light when measured against the control. The activity level of the walleye was higher in control settings, which can be seen by larger numbers of passes and inspects. Walleye were more likely to approach the LGD and come closer on average to the device when the light was off. From the results shown, the overall reaction of walleye to the LGD appears to be repulsion.

Behaviour of the walleye was significantly affected by the LGD emitting light, with their use of the arena switching to utilize the darker portions of the tank more readily when the light was on, regardless of colour emitted or strobing rate. This kind of avoidance behaviour has been observed in many fish species when interacting with light (Johnson et al. 2005, Sager et al. 2000, and Johnson et al. 2005). Though light has been known to attract fish (Marchesan et al. 2005) at times it has also been used in the past as a deterrent for many species, especially salmonids (Johnson et al. 2005, Nemeth and Anderson 2011, Puckett and Anderson 1988). Typically, strobing has been used to increase efficiency of repulsion since fish tend to avoid constant flashing (Johnson et al. 2005). The strobing light in the study did not alter the reaction of the walleye to the LGD although only a low flash rate was used (5 Hz). For walleye, the larger factor for avoidance behaviour was the light itself, regardless of if the light was strobing or not. One possible explanation for this is that walleye are photosensitive to both green and orange light (Burkhardt et al. 1980). This in itself may not explain my observations but in a similar study white sturgeon were observed to be attracted to one of their spectral sensitivities (Ford et al., 2017). Walleye are known to be nocturnal (Reed 1962, Carlander and Cleary 1949) so the presence of a bright light may be the overriding difference in their behavioural differences noted in this study. This knowledge benefits guidance techniques since the avoidance to light is uniform. With the ability to deter walleye regardless of colour, the colour can be altered to best adapt for different environmental conditions (e.g. turbidity, colour of water). This allows flexibility of colours to allow for targeting of other species (to also deter another species based on its colour preference) or to account for environmental conditions such as water clarity (e.g. if orange does not penetrate then green may be more useful). This flexibility can also favour strobing rates in the same manner as colour.

Walleye activity was affected by the presence of light. Walleye were more active and explored the arena more during control studies. The number of walleye to inspect the light beam and pass through it (rather where it would be if the light were on) was significantly higher in control studies showing that walleye were less bold when the light was on, regardless of colour or strobe settings. Activity levels are normally higher for walleye at low light levels (Kelso 1978). This behavioural difference, however, does not explain the difference in passes and inspections because the study was conducted both at night and during daytime hours and still no significant difference was observed in behaviour. The only significant factor affecting activity levels was the presence of light emitted from the LGD. This knowledge could be beneficial for behavioural guidance of walleye since it appears that walleye are repelled by light in both day and night conditions. This allows for a more universal protection system that could deter entrainment and impingement levels in walleye, even when they are most active.

Ontogenetic differences have been observed in walleye for many different behaviours (Bozek et al. 2011). Behaviour is a factor that can change as fish mature. I observed little difference in the escapement time and light time between age 2+ and age 0+ walleye. This may mean that regardless of age, walleye avoid strong light sources. This knowledge is interesting since I would expect there to be a difference between the older and younger walleye since walleye undergo multiple ontogenetic behavioural differences (Bozek et al. 2011). Differences in foraging and niche utilization have been seen to be affected by age (Forney 1966, Colby et al. 1979). These differences have been linked to visual behaviour as well (Ali and Anctil 1977). Changes in the retinal structure of the walleye allow them to function better at dim light conditions as they develop (Ali and Anctil 1977). All of these ontogenetic behavioural differences did not make any changes in reaction to the light between 0+ and 2+ walleye. This could reaffirm that light is the main deterrent for walleye regardless of age. The knowledge that walleye avoid light regardless of age could be beneficial for behavioural guidance as it would allow for uniform targeting of walleye to light guidance. This could help protect walleye of all age classes easier than having to target specific sensitive life stages.

The results of this study may enhance the behavioural guidance of walleye. The knowledge that walleye will mostly avoid light can be used to help limit entrainment and impingement circumstances for age 0+ and 2+ walleye and possibly walleye of other ages. Further testing of the LGD as a behavioural guidance tool for walleye is needed to determine behaviour both in longer term lab testing and in field testing as this study mainly focused on the walleye's behavioural reaction to lights at known photosensitive wavelengths. This study shows promise of the LGD as a useful tool to guide walleye along with the flexibility to possibly guide other at risk organisms.

Figures

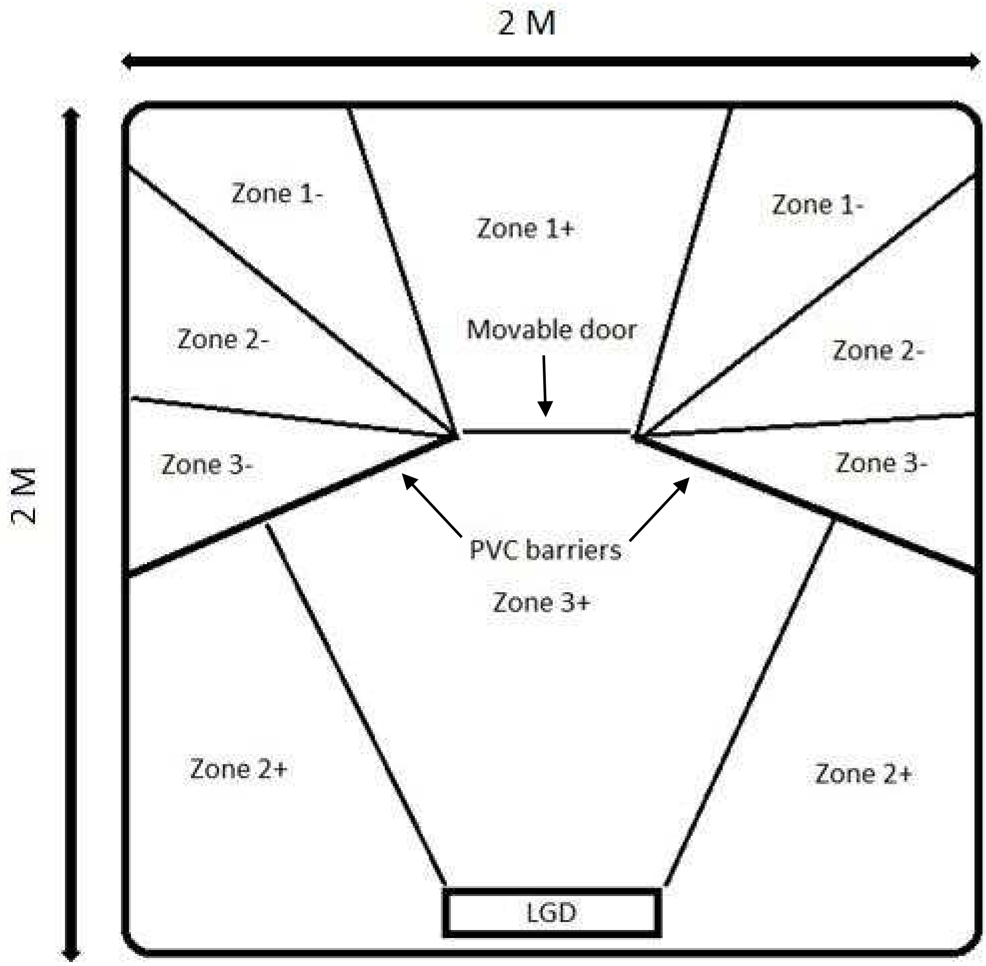

Figure 4-1. Tank setup with physical design and showing zone overlay used for analysis. Fiberglass tank measuring 2Mx2M with rounded corners and water 40 cm deep (volume about 1600L). Light zones correspond to dark and light areas ranging from darkest (3-) to brightest (3+). Dark zones were scored as 1- (1.0-1.7 Lux), 2- (2.1-3.0 Lux), and 3- (3.0-3.8 Lux). Light zones were scored as 1+ (125.8-405 Lux green, 192-691 Lux orange), 2+ (22-145 Lux green, 30-356 Lux orange), and 3+ (681-9300 Lux green, 1798-20900 Lux orange).

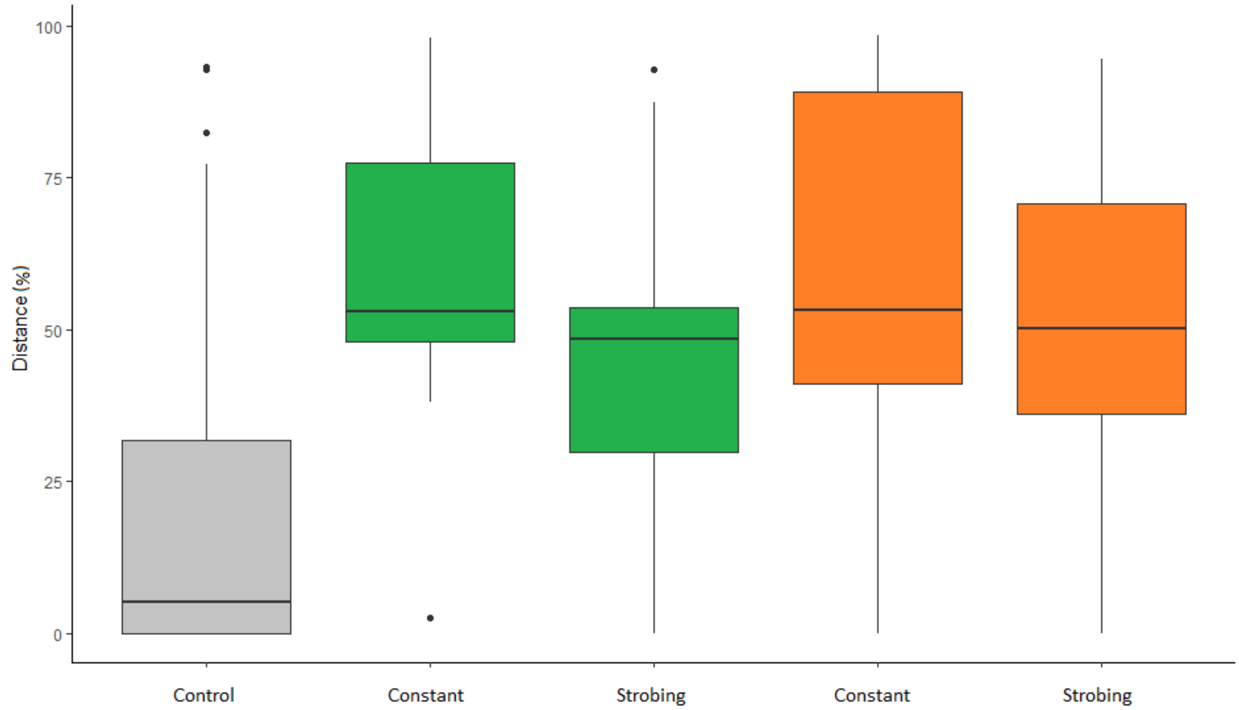

Figure 4-2. Boxplot showing the average distance (percentage) that naive age 2+ walleye (N=242) were away from the LGD over a 300 second trial for each treatment. Only fish that entered the light beam were measured for distance (N=91 did not enter light). Distance is measured as a percentage of distance from the LGD with “0” being touching the light box and “100” being the furthest possible distance from LGD. Legend: Strobing = 5Hz.

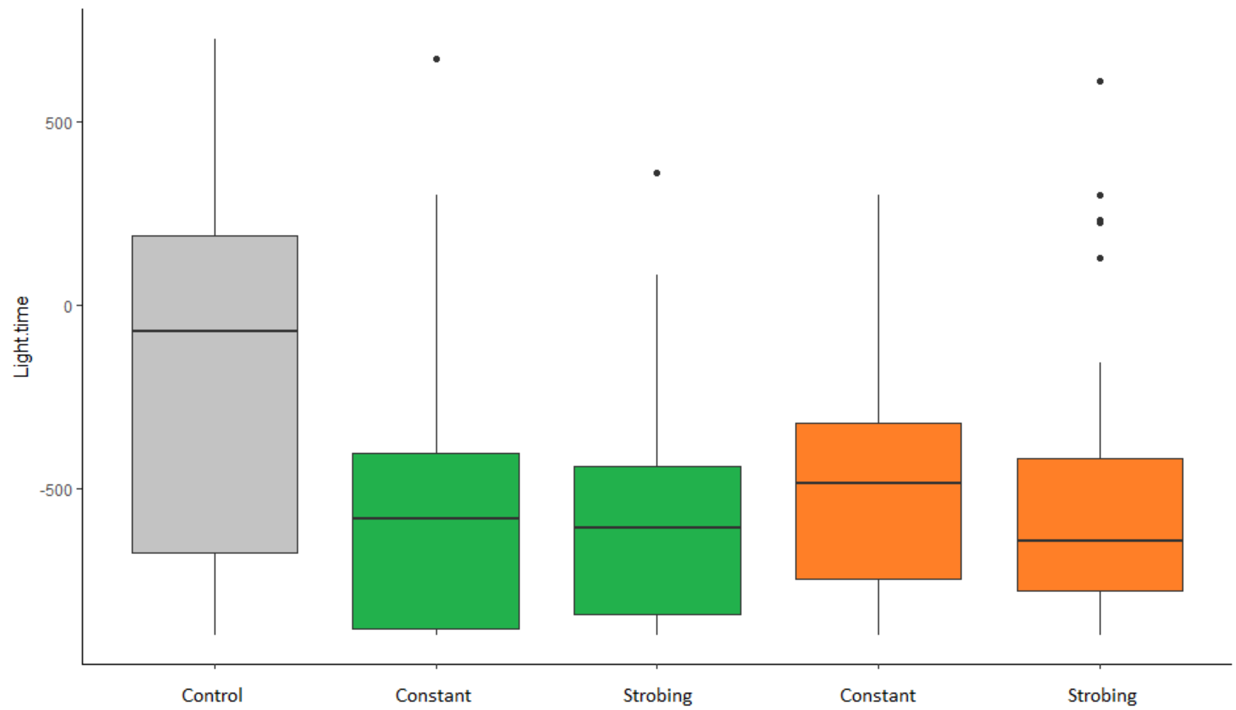

Figure 4-3. Boxplot showing the average light time of each naive age 2+ walleye (N=242) over a 300 second trial for each treatment and diel period. Light time was measured using the amount of time spent in each light zone combined to create a scale of how the arena was used. Equation was set to (3*seconds spent in zone 3+) +(2*seconds spent in zone 2+) +(1*seconds spent in zone 1+) +(-1*seconds spent in zone 1-) +(-2*seconds spent in zone 2-) +(-3*seconds spent in zone 3-) creating an overall score of proportion for time spent in light zones. Legend: Legend: Strobing = 5Hz.

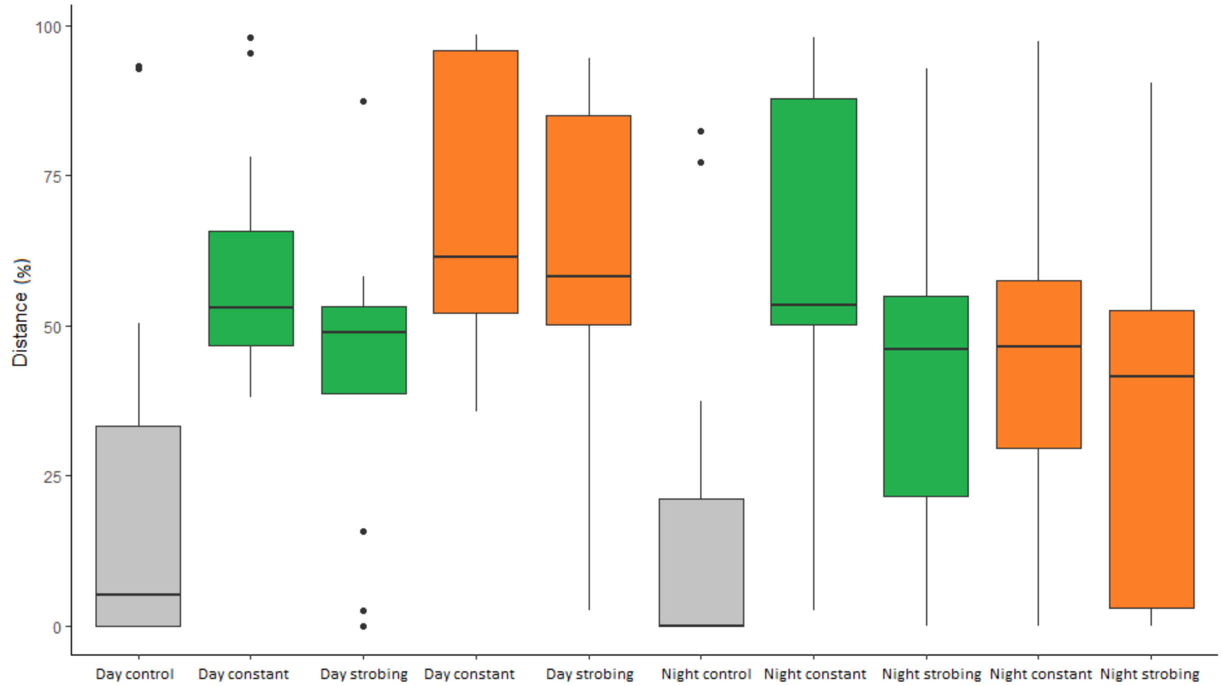

Figure 4-4. Boxplot showing the average distance (percentage) to LGD that naive age 2+ walleye (N=151) came within over a 300 second trial for each treatment and diel period. Only fish that entered the light beam were measured for distance (N=91 did not enter light). Distance is measured as a percentage of distance from the LGD with "0" being touching the light box and "100" being the furthest possible distance from LGD. Legend: Strobing = 5Hz.

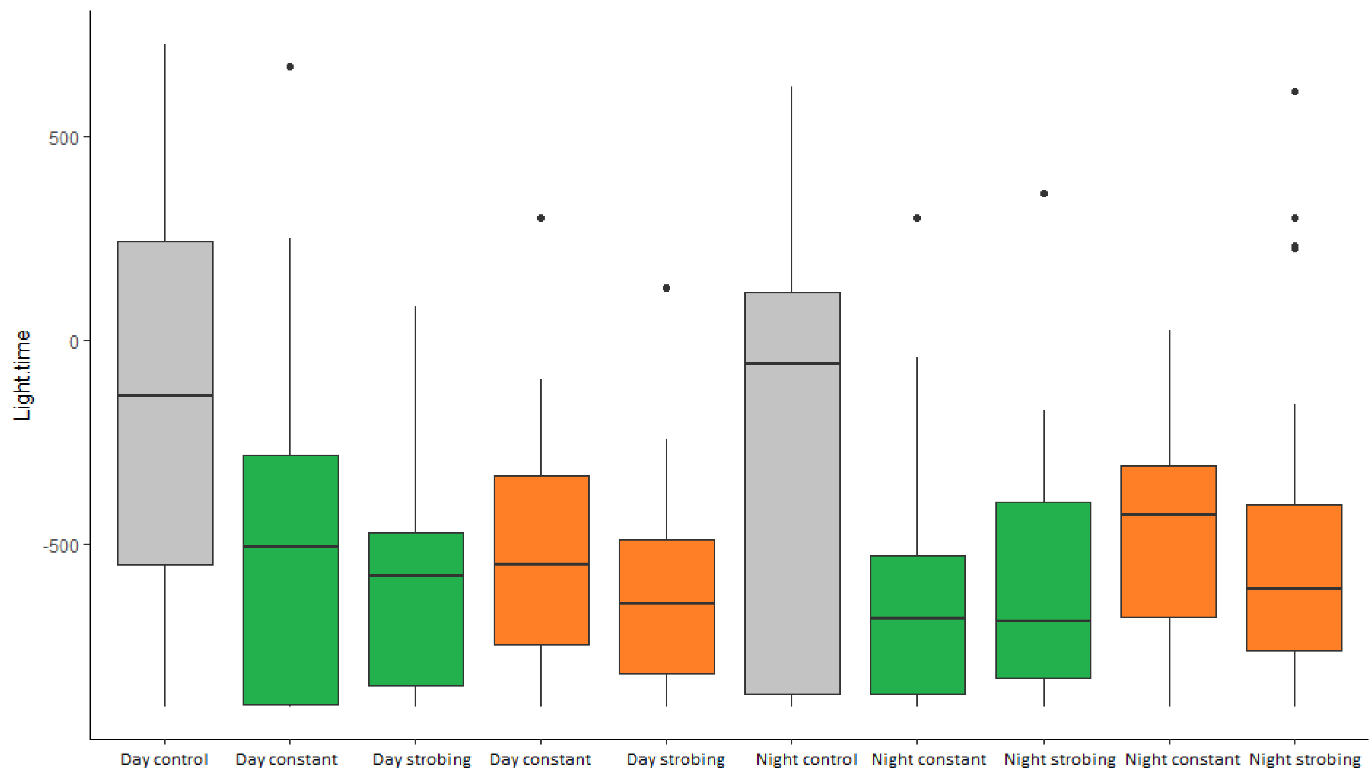

Figure 4-5. Boxplot showing the average light time of each naive age 2+ walleye (N=242) over a 300 second trial for each treatment and diel period. Light time was measured using the amount of time spent in each light zone combined to create a scale of how the arena was used. Equation was set to (3*seconds spent in zone 3+) +(2*seconds spent in zone 2+) +(1*seconds spent in zone 1+) +(-1*seconds spent in zone 1-) +(-2*seconds spent in zone 2-) +(-3*seconds spent in zone 3-) creating an overall score of proportion for time spent in light zones. Legend: Legend: Strobing = 5Hz.

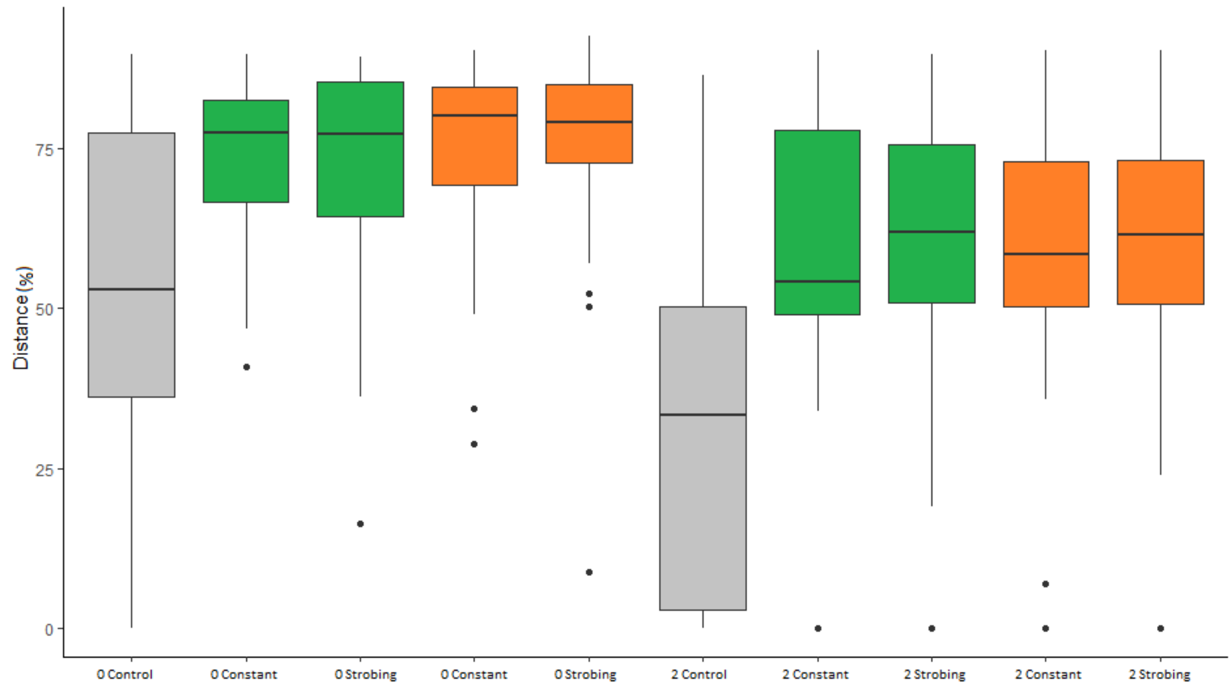

Figure 4-6. Boxplot showing the average distance (percentage) away from the LGD of naive age 2+ (N=250) and 0+ walleye (N=250) over a 60 second trial for each treatment and diel period. Averaging the total light time for each fish with both age 0+ and 2+ walleye combined (N=500). Distance is measured as a percentage of distance from the LGD with "0" being touching the light box and "100" being the furthest possible distance from LGD. Legend: Strobing = 5Hz.

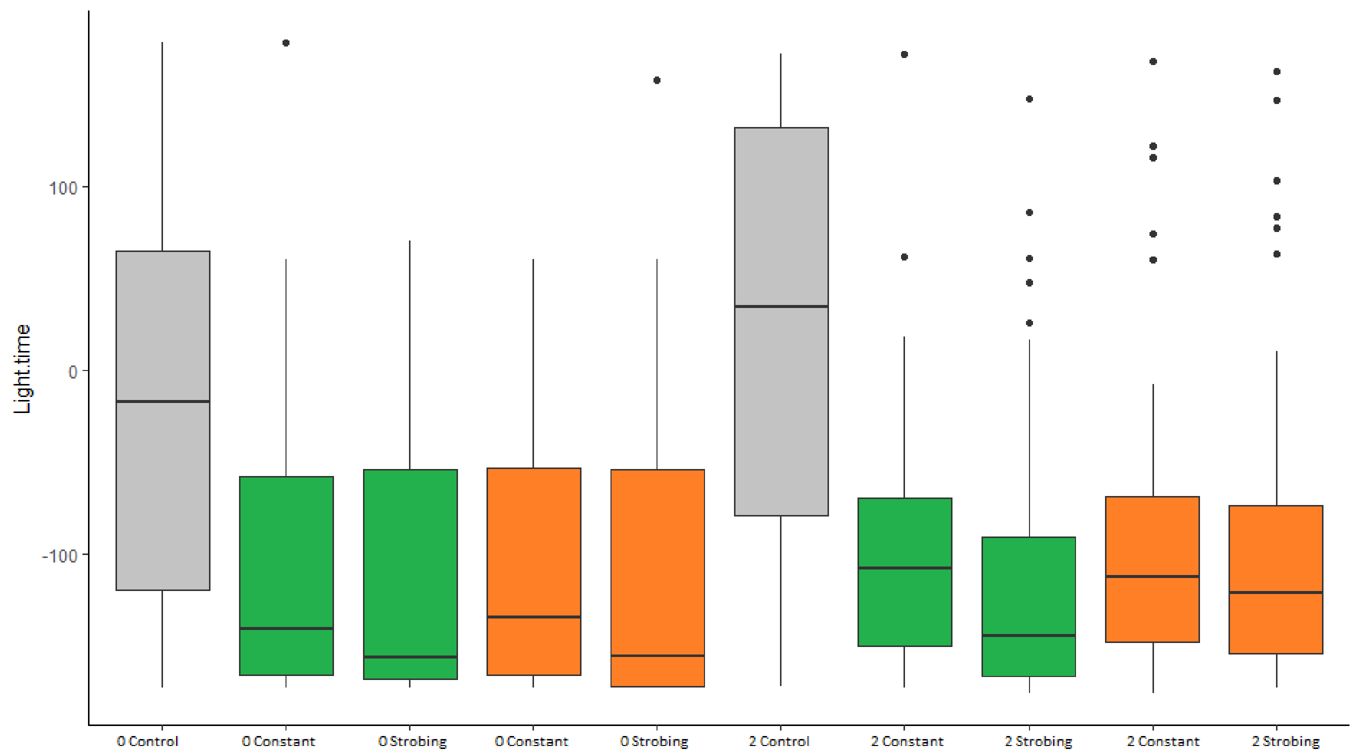

Figure 4-7. Boxplot showing the average light time of naive age 2+ (N=250) and 0+ walleye (N=250) over a 60 second trial for each treatment and diel period. Averaging the total light time for each fish with both age 0+ and 2+ walleye combined (N=500). Light time was measured using the amount of time spent in each light zone combined to create a scale of how the arena was used. Equation was set to (3*seconds spent in zone 3+) +(2*seconds spent in zone 2+) +(1*seconds spent in zone 1+) +(-1*seconds spent in zone 1-) +(-2*seconds spent in zone 2-) +(-3*seconds spent in zone 3-) creating an overall score of proportion for time spent in light zones. Legend: 0 = 0+ walleye, 2 = 2+ walleye, D = Day, N = Night, C = Control, G = Green, O = Orange, N = Constant light, S = Strobing (5Hz).

Chapter 5: General Discussion

Findings and implications

In the introduction, I found that there has been research in the field of light behavioural guidance since the 1950's. With this, studies have been performed on many different species in multiple countries and in varying conditions. From all of this research there is no clear trend as to how any given fish will react to a given light stimuli. Behavioural reactions have been seen to be species-specific, light-specific, and environment specific. There have been multiple success stories and multiple studies where is was concluded that light would not be a suitable guidance tool. The implications of this research is that it is impossible to know exactly how a fish may react to the light. This makes research into the field hard because what may work in the lab might not work in the field. It also means that almost every guidance attempt has to be individually tested to determine the reaction in that specific environment. This makes for a very complex solution to guidance using lights and there has not been a one size fits all answer yet, if there ever will be.

In chapter 2 I found that during the day, age-0 white sturgeon were attracted to green light more so than either blue or red when measured against the control. The attraction to green light was significant regardless of strobing rates. Blue and red had some variation in their reaction based on strobing but there was not one solution that held true throughout. At night, age-0 white sturgeon were attracted to all colours and strobing rates when compared to the control. The results from this experiment could be confounded by the tank used as it was green so a future test using another coloured tank that is different from their raising tanks could be beneficial to test biases. The results that sturgeon are attracted to green light throughout the day and that the other colours tested vary can be used for possible behavioural guidance applications. It may be possible to attract age-0 white sturgeon away from waterway hazards using a coloured light. The use of a strobing light could prove to be useful in other ways as it has been seen that many fish are deterred by strobing lights. If other fish are deterred but white sturgeon are attracted, there is possibilities for specialized and multi-species targeting using light as a behavioural guidance tool where white sturgeon are found. The possibilities with colour also allow for modifications of a guidance system depending on the environment that requires the guidance. Certain colours may penetrate further in coloured/turbid waters, making the reaction to different colours important as to how the light systems are deployed. Deployment of the lights is another factor to consider since Polleto et al. (2014b) found that white sturgeon were not deterred away from a water intake by strobing white lights. If colour were used to attract white sturgeon away from the water intake, it may be more effective. Comparing my results to Polleto et al.'s (2014b) research shows how the implications of this thesis may be important for the protection of white sturgeon.

In chapter 3 I examined the effects of an integrated louver-light guidance system. The analyses was changed from chapter 3 to incorporate the influence of the louver and two light settings. Louvers and lights combined proved more effective at guiding age-0 white sturgeon than either guidance stimuli on their own. The most effective setting was to use green light strobing at 20Hz as an attractant to a bypass while covering the hazardous infrastructure with a reversed-louver system. Appling this strategy around hydropower and other high risk locations for white sturgeon could help mitigate loses. Furthermore, results demonstrate that the louver was always effective at guiding age-0 white sturgeon while light increased effectiveness at night. Given that sturgeon are more active at night (Poletto et al. 2014a), scheduled or managed light systems could help conserve energy, through reduced use of light, while still effectively protecting white sturgeon. Hence, using both stimuli when they are most active should increase chances of guiding white sturgeon successfully.

In chapter 4, I found that light as a behavioural guidance tool can reduce walleye entrainment and impingement. Chapter 4 used simple ANOVA's and linear models best fitted to the data recorded for walleye trials. Regardless of colour, time of day, or strobing rate, walleye of both age classes avoided the light. Yet, past research shows that walleye are more active during low light hours (Reed 1962, Carlander and Cleary 1949). Thus, walleye could be effectively guided away from water intake pipes and hydropower with lights, especially during their peak active hours but also during low-risk hours. Since the colour of light, in this study, did not play a role in the deterrence rate, walleye can easily be included in specialized and multi-species targeting systems whereby colour variability may affect other species of fish. In other words, if one colour is adversely affecting a specific species it may be possible to change the colour used and still successfully deter walleye while accounting for the other species. In addition, the findings of the ontogenetic portion of this research are important to help protect walleye. The implications of the similar reactions despite age is that lights could help protect walleye through behavioural guidance at any life stage. Guidance efforts may be more important for certain life stages depending on the environment. With the knowledge that light can guide any stage, we may be able to mitigate losses due to entrainment and impingement.

In conclusion, my thesis demonstrates that management can use light effectively in certain situations. Despite there being some situations where light guidance may be ineffective, there is a wider array of possibilities when combining colour and strobe to target and guide specific species of fish using their natural reactions to light. The LGD does not have the capacity to produce UV wavelengths which fish can respond to, this was a limitation of our testing though all physiology background done on the species tested was using the visual spectrum. The research put forward on age-0 white sturgeon demonstrates light has the potential to help guide white sturgeon away from hazardous waterway infrastructure. The research put forward on walleye shows that there is the possibility any light can deter them, allowing management to better protect walleye where they may be at risk. It is possible the visual differences of white sturgeon and walleye could account for the behavioural differences in light reaction. Yet, these results require further testing, possibly in the field, to establish effectiveness in different environments. Even though light reactions are species-specific, light has the potential to improve behavioural guidance efforts with specific tailoring to the situation.

Future research directions

Even though this research has taken steps forward in both the behavioural guidance field and for the specific species studied, there is still a need for future research into the use of behavioural guidance using light to mitigate fish loses. Colour that can be modified is one future that LEDs pose, both in the form of diurnal changes in reaction to light and ontogenetic changes. Since fishes reactions to light are not uniform, even for a given species, research should further pursue how a given fishes reacts in a particular environment. Knowing that species have different spectral sensitivities and FFF's can guide research towards testing specific colours and strobing rates.

Research for white sturgeon must continue to be tested. The next logical step is to develop a field test determining if the laboratory findings hold true. To test this one could use strobing green and red lights to perform net pen research on young white sturgeon in the field.

Sturgeon should be tested for ontogenetic differences using the same tests as chapter 2 with multiple life stages. This will inform possible differences in behaviour and could affect implantation of the LGD. After the previously mentioned studies, a full scale study should be tested at a hydropower facility or water intake pipe known to endanger white sturgeon.

Walleye should move forward into field testing as well. Field tests should be done using this device and settings listed in chapter 4 to determine how walleye react to the locations specific environmental conditions. Field tests would help remove the possibility of biases from tank colour and hatchery conditions that may play a role in the walleye's behavioural response. Using the results from this study, the LGD should be tested on walleye around water intakes and hydropower facilities to determine the effectiveness of light as a deterrent.

Acclimatization to light needs to be examined in future research. Studies done in this thesis did not look at timespans over 5 minutes and used naïve fish. Long term experiments should be done for both species tested to determine if they develop habituation towards the light.

Finally, light should be further explored for its potential impacts to the ecosystem. There are ecosystems that both sturgeon (lake sturgeon) and walleye inhabit. Systems containing both species should be tested using the LGD incorporated with other behavioural guidance tools to determine if we can effectively protect both species at once using light and other guidance systems. Lights could have adverse effects on other organisms within the ecosystem in which the lights are employed. Many examples in the past describe how application of a tool for aid negatively affect another species. As such, light should not be used without fully examining the potential adverse effects.

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