Preferences of age-0 white sturgeon for different colours and strobe rates of LED lights may inform behavioural guidance strategies

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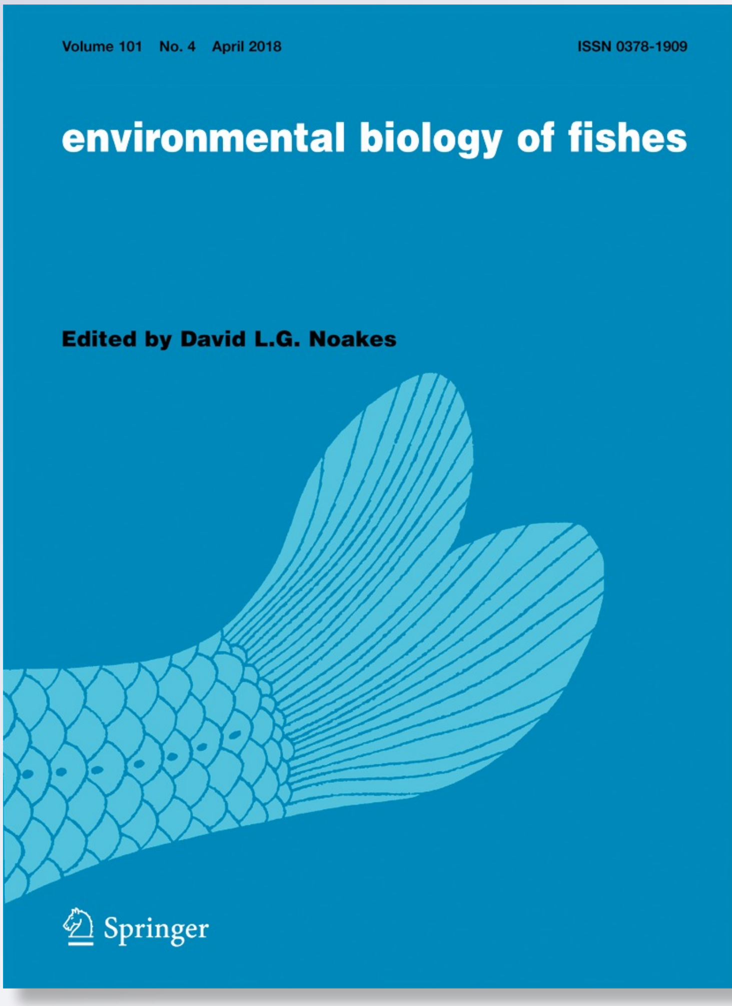

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Abstract Many populations of migratory fish species, including white sturgeon (*Acipenser transmontanus* Richardson), are threatened due to modification of riverine systems and may experience downstream displacement or mortality at water intake structures. 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), we 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.

Keywords Hydropower · Migration · Entrainment · Impingement · Phototaxis · Vision

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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 global decline (Dudgeon et al. 2006) and freshwater fishes are among the most threatened groups 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*, Richardson) 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. Following emergence from gravel substrates as larvae, juvenile sturgeon initially drift downstream (Auer 1996). Although information on juvenile stages is deficient, age 2+ white sturgeon have been observed undergoing diel and seasonal migrations (Parsley et al. 2008), ostensibly to optimize foraging and habitat conditions (Auer 1996). As sturgeon do not reach maturity until they are 10+ years old, with females maturing later than males (Semakula and 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 aimed 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 and 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 and Suski 2012), including with sturgeon (Kynard and Horgan 2001; Poletto et al. 2014; 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. 2014). Sturgeon are known to exhibit positive phototaxis to green light as larvae, while developing sensitivities to red and blue in later life stages (Loew and Sillman 1993). White sturgeon also have a specific rod cell sensitivity to green light (540 nm; Sillman et al. 1995). In this study, we tested the effectiveness of a new, LED-based light guidance device (LGD) at achieving behavioural guidance in age-0 white sturgeon. To do so, we 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, we 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

We 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

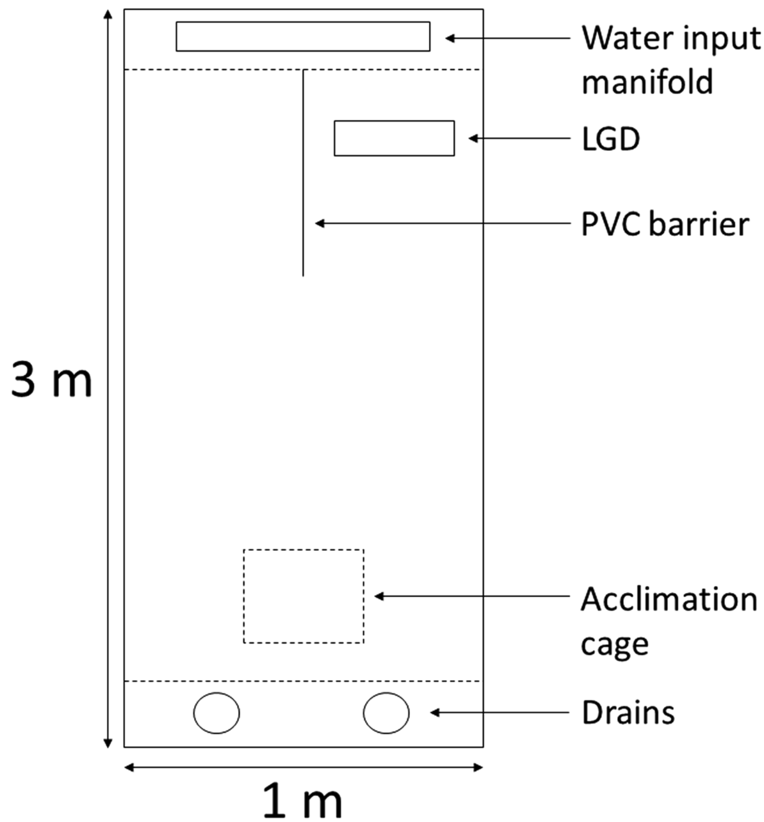
To simulate a stream setting, we equipped a green fibreglass raceway tank (3 m length × 1 m width) with a semi-closed, recirculating flow system. Water was added continuously to this system at a rate of 1 L·min^{-1} which allowed us to supply the trial arena with a constant flow rate of 0.24 m·s^{-1} (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).

We 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 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. We then replicated these tests under dark conditions (<5 lx background illumination) to simulate the availability of ambient light at night. Individual fish were exposed to one treatment combination each ($N = 20$ per treatment, $N = 400$ total).

Experimental protocol and analysis

Individual, naïve white sturgeon were placed into the arena at the downstream end under a wire cage (30 cm × 30 cm) for 3 min 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 time. If no choice was made (neither chamber was entered) after 1 min the trial was ended and scored as "no decision" (a neutral reaction). This allowed us to assign a binary score where a '1' indicated that the sturgeon approached the LGD and a '0' indicated that they did not (i.e., the fish entered the side of the y-maze without the LGD). The sturgeon that did not enter either side of 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 (latency to enter one of the chambers). Initially, we looked for evidence of laterality or side bias by isolating the control trials (no LGD output) and looking at the binary response in a generalized linear model with the side containing the LGD as a fixed-effects factor. Continuous behavioural responses (latencies to enter the y-maze) that did not meet the assumptions of normality (Shapiro-Wilk test, $p < 0.05$) were rank-transformed (Scheirer et al. 1976) and analyzed via three-way factorial ANCOVA 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 generalized linear models with binomial distributions. 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 models. All analyses were conducted using R version 3.2.4 (R Core Team 2016).

Fig. 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*)



Results

All conditions

Overall, 12.5% of age-0 white sturgeon made no choice (did not move upstream into the y-maze) under dark conditions ($N = 200$), while 20.5% sturgeon made no choice under light conditions ($N = 199$). Of the sturgeon that did make a clear behavioural choice in control trials, the proportion of white sturgeon approaching and entering the y-maze chamber containing the LGD was not influenced by side (Wald's $\chi^2_1 = 2.89$, $p > 0.05$), justifying our exclusion of laterality from the analyses.

The proportion of sturgeon approaching and entering the y-maze chamber containing the LGD was significantly influenced by colour ($\chi^2_8 = 42.33$, $p < 0.0001$), strobe frequency ($\chi^2_4 = 17.15$, $p < 0.01$) and light condition ($\chi^2_5 = 19.22$, $p < 0.01$), with a significant interaction between colour and background light condition ($\chi^2_3 = 8.35$, $p < 0.05$). There was no effect of fish length ($p = 0.58$). 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, independent 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. 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 the LGD when it was strobing at 1 Hz, while all three colours elicited faster approaches 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 ($\chi^2_3 = 17.93$, $p < 0.001$) and strobe frequency ($\chi^2_2 = 8.82$, $p < 0.05$). Green light consistently elicited the most approaches and red light the least, with the lowest proportion approaching in response to the red light when it was strobing at 1 Hz (Fig. 2a). Compared to the control treatment, red light at 1 Hz attracted a lower proportion of age-0 white sturgeon under 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 ($\chi^2_3 = 23.0$, $p < 0.0001$) and strobe frequency ($\chi^2_2 = 9.70$, $p < 0.01$) 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 resulted in fewer approaches than constant light or light strobing at 20 Hz (Fig. 3a), although any light treatment had more approaches 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

Our 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. Our 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 light has been used for many years as a 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 and 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.) from Lake Ontario, however, yielded equivocal findings, with strobe lights increasing capture success under laboratory conditions but not in field trials (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). The similarity in responses we observed to constant light or

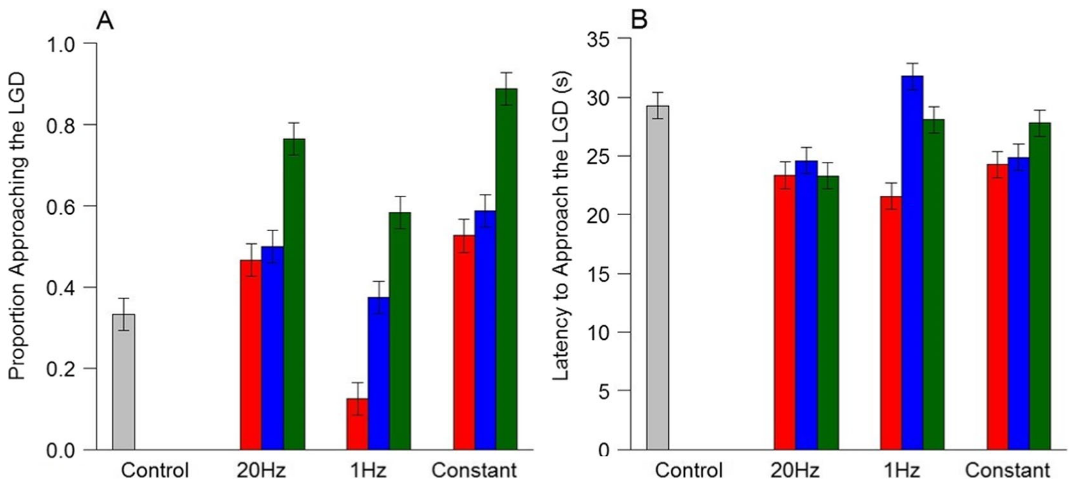


Fig. 2 **a** Proportion of age-0 white sturgeon (*Acipenser transmontanus*) that approached the light device and (**b**) latency to approach under light conditions in a y-maze test. Bar colours indicate colour of light; control treatment had the LGD present but turned off

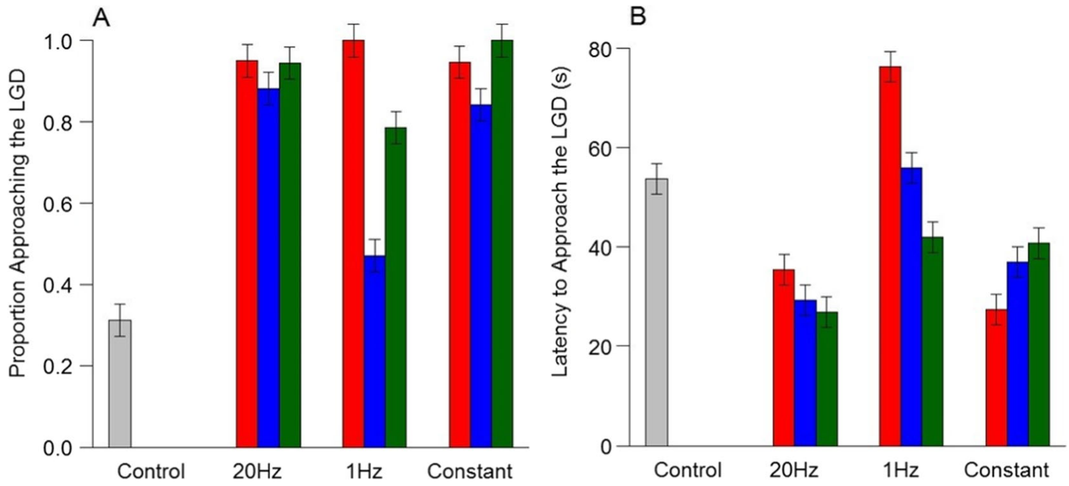


Fig. 3 **a** Proportion of age-0 white sturgeon (*Acipenser transmontanus*) that approached the light device and (**b**) latency to approach under dark conditions in a y-maze test. Bar colours indicate colour of light; control treatment had the LGD present but turned off

light strobing at 20 Hz suggest that 20 Hz may be above the CFF of age-0 white sturgeon and appear as constant light, eliciting an attraction response, while light strobing at 1 Hz instead 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 and Reebs 2006). Over the course of this study, white sturgeon were more active under night conditions and approached the LGD at higher rates during low-light conditions compared to simulated daytime conditions. This diel 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 diel switch in red from being 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 under night conditions. Under day conditions, constant ambient light may continuously stimulate the rod cells, resulting in larger colour-specific cone cell responses, while stimulation of the rod cells may be the driving factor in fish attraction under dark conditions.

Several behavioural guidance studies have used light as a deterrent stimulus (e.g., Haymes et al. 1984; Sager et al. 2000; Johnson et al. 2005; Stamplecoskie et al. 2012). We 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 our 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. 2014), has important implications for 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 to limit any negative impacts. 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. 2017 for an evaluation of an integrated light-louver rack array system) or non-physical technologies. Additional benefits of using LED technology include stable output spectra (Pulli et al. 2015), lower power requirements, lower operational costs, and possibilities of remote installations using alternative energy sources (e.g., solar; Pimputkar et al. 2009).

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