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Coloured LED light as a potential behavioural guidance tool for age 0 and 2 year walleye *Sander vitreus*

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ABSTRACT

Based on existing laboratory research on the visual physiology of walleye *Sander vitreus*, we tested colours of known spectral sensitivity (*i.e.*, green and orange) using constant and strobing (5 Hz) illumination with an LED-based light guidance device (LGD). Hatchery-reared age 0 and 2 years *S. vitreus* were exposed to these four light combinations as well as an unilluminated control treatment during day and night trials. Age 2 years *S. vitreus* generally avoided the LGD when light was produced (negative phototaxis) compared with the control, with continuous illumination having a greater effect than strobing. The proportions of both age 0 and 2 year fish exiting illuminating zones of the trial arena did not differ with light colour or strobe rate, suggesting that phototactic behaviours in *S. vitreus* do not change with ontogeny in these age classes. Our findings confirm that typical behavioural responses of *S. vitreus* to light stimuli are characterised by avoidance and provide evidence that the use of light for behavioural guidance (deterrence) may be effective at reducing entrainment and impingement of this species on hydraulic barriers during migrations, independent of ontogenetic stage.

KEYWORDS
entrainment, impingement, migration, North America, ontogeny, phototaxis

1 | INTRODUCTION

Waterway modifications have contributed to declines in freshwater ecosystem biodiversity around the world and water demands for human consumption, agriculture, industrial processes and energy production continue to increase with population growth and development (Vörösmarty *et al*., 2010). The various infrastructure associated with water withdrawals or energy generation pose risks to fishes as they can become entrained downstream through water intakes (Allen *et al*., 2012; Schilt, 2007). Although physical barriers can be used to reduce likelihood of entrainment (Coutant, 1999; Noatch & Suski, 2012), fish may still become entrained and impinged with lethal outcomes.

Behavioural guidance strategies aim to mitigate risks of entrainment and impingement by exploiting sensory physiologies of target species to achieve desirable outcomes (Noatch & Suski, 2012). Typically, these guidance techniques are used to lead or repel fishes away from hazardous areas, such as hydropower turbines or unprotected water intake pipes (Coutant, 1999; Schilt, 2007). Alternatively, behavioural guidance can also be used to attract fish to desired pathways such as fishways or bypass channels. One of the inherent challenges in behavioural guidance is that many fishes undergo ontogenetic changes in their visual physiology and behaviour (Noakes & Godin, 1988), potentially altering the effects of a given stimulus at different life-history stages. For example, peak absorbances in the retinal photopigments of white sturgeon *Acipenser transmontanus* Richardson 1837 change as they grow from larvae to juveniles, with sensitivities to red and blue light spectra developing after *c*. 10 weeks (Loew & Sillman, 1993), while age 4 years lake sturgeon *Acipenser fulvescens*

Rafinesque 1817 demonstrated greater light avoidance than age 1 year fish in captive guidance trials (Elvidge *et al*., 2019).

Light has been evaluated in the past as a behavioural guidance tool (Hocutt, 1981; Noatch & Suski, 2012; Taft, 1986) but was largely abandoned based on a 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; Taft, 1986), but no one solution seemed to work as responses were highly variable between species. More recent advances in light technology include the development of light emitting diodes (LED), compact and powerful lights that can provide illumination in different colours and strobe rates (low or high frequency) and with greater energy efficiency. For these reasons, strobing LEDs show promise for use in the behavioural guidance of freshwater fishes (Elvidge *et al*., 2018; Ford *et al*., 2018; Jesus *et al*., 2019; Kim & Mandrak, 2017; Sullivan *et al*., 2016).

Walleye *Sander vitreus* (Mitchill 1818) is a perciform fish native to many rivers and lakes in the Midwest of North America (Bozek *et al*., 2011), where they are highly valued for recreation as well as for subsistence in many Canadian and US communities (Cooke *et al*., 2018; Lester *et al*., 2014). *Sander vitreus* plays a similar socio-ecological role to that of its congener, the pike-perch or zander *Sander lucioperca* (L 1758), in Europe. Owing to their popularity, *S. vitreus* populations are at risk from overfishing and waterway development and they are currently being stocked into many waterbodies for stock supplementation (Wilson *et al*., 2007). Walleye is a versatile predator, although competition with other top predators can limit population sizes in smaller waterbodies (Bozek *et al*., 2011). They can spawn in both rivers and lakes and sometimes undergo upstream migrations from lakes into tributaries to spawn (Bozek *et al*., 2011). In rivers, walleye use rapids as spawning grounds (Walburg, 1972) and in lakes, they use shallow areas like reefs and shoals (Eschmeyer, 1950). These upstream spawning migrations, subsequent downstream returns and seasonal movement patterns within lakes and rivers, can result in exposure to the hazards of waterway development. Walleye are generally crepuscular and nocturnal (Carlander & Cleary, 1949; SDRNF & Reed, 1962) and they spend the daylight hours at depth as they seek out low light conditions (Bozek *et al*., 2011; Kelso, 1978). This natural behaviour (negative phototaxis) suggests that walleye may react desirably to behavioural guidance through light, particularly as a repulsive stimulus.

Laboratory studies on walleye retinal physiology have revealed that their photoreceptors have two spectral sensitivities with peak absorbances at 533 nm (green) and 605 nm (orange) (Burkhardt *et al*., 1980), while the critical flicker-fusion frequency, the highest frequency at which the eye can differentiate flashes, of the walleye retina has a limit of *c*. 20 Hz (Ali & Anctil, 1977). With this knowledge and the flexibility of LED lights, we hypothesised an optimal combination of strobing and colour may be found to most effectively guide walleye using their sensory biases or sensitivities. The objective of this experiment was to determine the behavioural response of age 2 year walleye to two light spectra (green and orange), presented at constant illumination or strobing at 5 Hz. As the responses to light in other fishes (*e.g*., *A. transmontanus*: Loew & Sillman, 1993) shift as they grow, we then compared the behavioural responses of both age 0 and 2 year walleye to determine if there is an ontogenetic difference in reaction to light stimuli.

2 | METHODS AND MATERIALS

The care and use of experimental animals complied with national animal welfare laws, guidelines and policies as approved by the Canadian Council on Animal Care through Carleton University protocol no. 102925. Test fish were from captive-reared populations for stock augmentation programmes operated by the Ontario Ministry of Natural Resources and Forestry White Lake Fish Culture Station and all handling and transport was in accordance with hatchery protocols. No fish were lethally sampled and experimentation was limited to short-term behavioural observations. We observed no mortalities or lasting harm amongst the focal fish.

2.1 | Study site and species

This study took place at the White Lake Fish Culture Station, Sharbot Lake, Ontario, Canada, from 18 July to 8 August 2016. Fish tested were Lake Ontario provenance walleye age 0 year (n = 250; mean total length ± SE, L_T = 7.3 ± 0.69 cm) and age 2 years (n = 500; L_T = 21.4 ± 1.5 cm). The age 2 year fish were held in 2000 l cattle drums at densities of 300 fish per tank and the age 0 year fish were held in 2000 l cattle drums at densities of *c*. 10,000 per tank. Water temperature was maintained at 18°C using a combination of deep and surface-drawn water from White Lake. Test fish were taken off feed for the duration of the study (separate holding tanks were used to limit duration of no feed to <2 days). Age 2 year walleye were transported in 67 l black utility buckets with a garbage bag cover to prevent sun exposure and reduce stress while age 0 year walleye were transported in 20 l white utility buckets. After testing, the fish were placed in a super-trough recovery tank until the study was completed to prevent the resampling of individuals.

2.2 | Stimuli and trial arena

We used a light guidance device (LGD) developed by ATET-Tech, Inc. (www.atet-tech.com) for use in behavioural guidance of fish to test walleye reactions to coloured, strobing light. The LGD consists of 162 LED modules that can produce constant light or strobe up to 40 Hz and can produce any colour combination of light in the 400–670 nm spectrum by varying the saturation of red, green and blue (RGB) light. Following esrlier studies using strobing light (Baker, 2008; Johnson *et al*., 2005), we chose to use constant illumination and a strobe rate of 5 Hz, which is well below walleye critical flicker-fusion frequency (20 Hz: Ali & Anctil, 1977). Orange (605 nm) and green (535 nm) were chosen based on the spectral sensitivity of the walleye retina (Burkhardt *et al*., 1980), generating 5 treatment

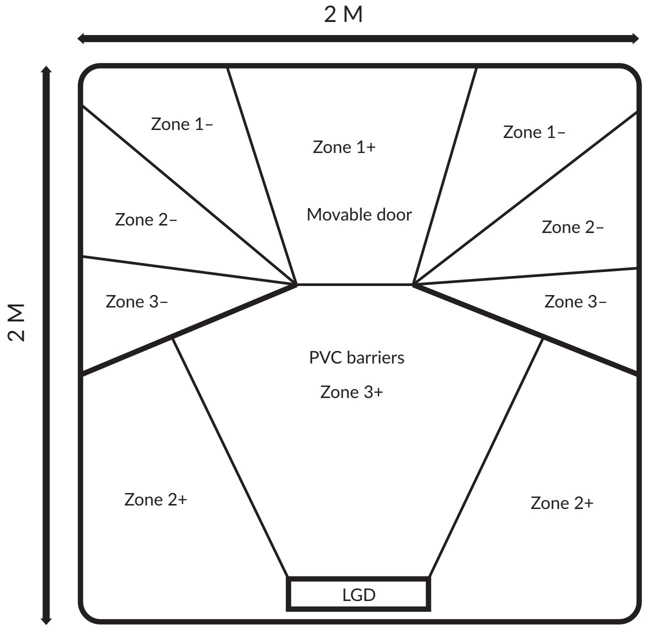

FIGURE 1 Trial arena layout (fibreglass tank measuring 2 × 2 m with rounded corners filled to 40 cm depth; *c*. 1600 l volume) showing light-zone overlay used for analysing *Sander vitreus* behaviour under different light conditions. Light zones correspond to dark and light areas ranging from darkest (3–, 1.0–1.7 lx) to brightest (3+, 681–9300 lx green, 1798–20,900 lx orange). Fish were held for acclimation directly behind the removable door in zone 1+

combinations including a control consisting of the LGD present but turned off. These 5 treatments were presented during the day as well as at night to identify any diel patterning in response.

To determine the level of attraction, repulsion, or neutrality to the colours and strobe-rates being tested, a funnel choice test was designed in a 2 × 2 m fibreglass tank. The tank was divided by PVC sheets placed at angles of 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 aluminium bars, a guide was created for a PVC door that could be raised using a pulley system to minimise disturbances to 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 held in place using two 1 kg lead weights to illuminate the acclimation chamber once the door was lifted. The tank was filled to a depth of 20 cm using the hatchery water supply, with temperature maintained within 1°C of the holding tank temperature (18°C).

2.3 | Experimental protocol

Individual age 2 year walleye were placed in the centre of the acclimation chamber for 300 s before the LGD was activated on one of the 5 treatment settings under both day (*c*. midday) and night (*c*. midnight) conditions (n = 25 per treatment; n = 242 total observations due to video malfunctions during eight trials). Following the acclimation period, the door to the funnel entrance was removed and subsequent

TABLE 1 Proportions of trials where light-zone inspections, entries and complete passes through were observed in age 2 year *Sander vitreus* between light stimuli under day and night conditions (n = 21–27 per treatment combination)

Time	Light stimulus	Inspection	Entries	Passes
Day	Control	0.074	0.778	0.778
	Green constant	0.458	0.083	0.458
	Green 5 Hz	0.478	0.304	0.522
	Orange constant	0.619	0.091	0.545
	Orange 5 Hz	0.565	0.130	0.522
Night	Control	0.000	0.625	0.708
	Green constant	0.364	0.045	0.409
	Green 5 Hz	0.435	0.261	0.609
	Orange constant	0.364	0.300	0.667
	Orange 5 Hz	0.625	0.250	0.625

behaviour of walleye in the trial arena were recorded using a GoPro Hero 3+ (GoPro, Inc.; www.gopro.com) camera. At the end of each trial, the fish was removed, measured (L_T) and transported to the recovery tank.

To test ontogenetic differences the design was modified, with the LGD activated before each trial began with the door of the emergence chamber left open. Focal walleye (age 0 or 2 years) were released into the emergence chamber facing into the light beam on the acclimation side of the tank and subsequently monitored for 60 s. This was repeated during both day and night for both age classes (n = 25 per age–treatment combination).

2.4 | Statistical analyses

The trial arena was separated into six sections based on light levels recorded with a Dr. Meter LX1330B digital lux meter (Hisgadget; www.hisgadget.com). Light levels were scored as attraction (+) or avoidance (–) and divided into three zones based on light intensity. Dark zones were scored as –3 (1.0–1.7 lx), –2 (2.1–3.0 lx) and – 1 (3.0–3.8 lx), while light zones were scored as 1+ (125.8–405 lx green, 192–691 lx orange), 2+ (22–145 lx green, 30–356 lx orange) and 3+ (681–9300 lx green, 1798–20,900 lx orange; Figure 1). We scored the 2+ zone higher than the 1+ zone because fish had to pass through 3+ (brightest zone) to enter, which was not necessary to enter the 1+ zone. We recorded the number of zone changes and the amount of time spent in each zone and then calculated the variable light time–time in illuminated zone as a measure of total light exposure. We calculated light time as: (3 s spent in zone 3+) + (2 s spent in zone 2+) + (1 s spent in zone 1+) + (–1 s spent in zone –1) + (–2 s spent in zone –2) + (–3 s spent in zone –3). From the videos we recorded: (a) closest distance to the LGD (if the fish passed into the light beam); (b) number of light inspections (instances where the fish moved right to the edge of the light beam and either maintained position or turned away); (c) the number of passes through the light cone (times the whole body of the fish entered an illuminated zone). Number of entrances to

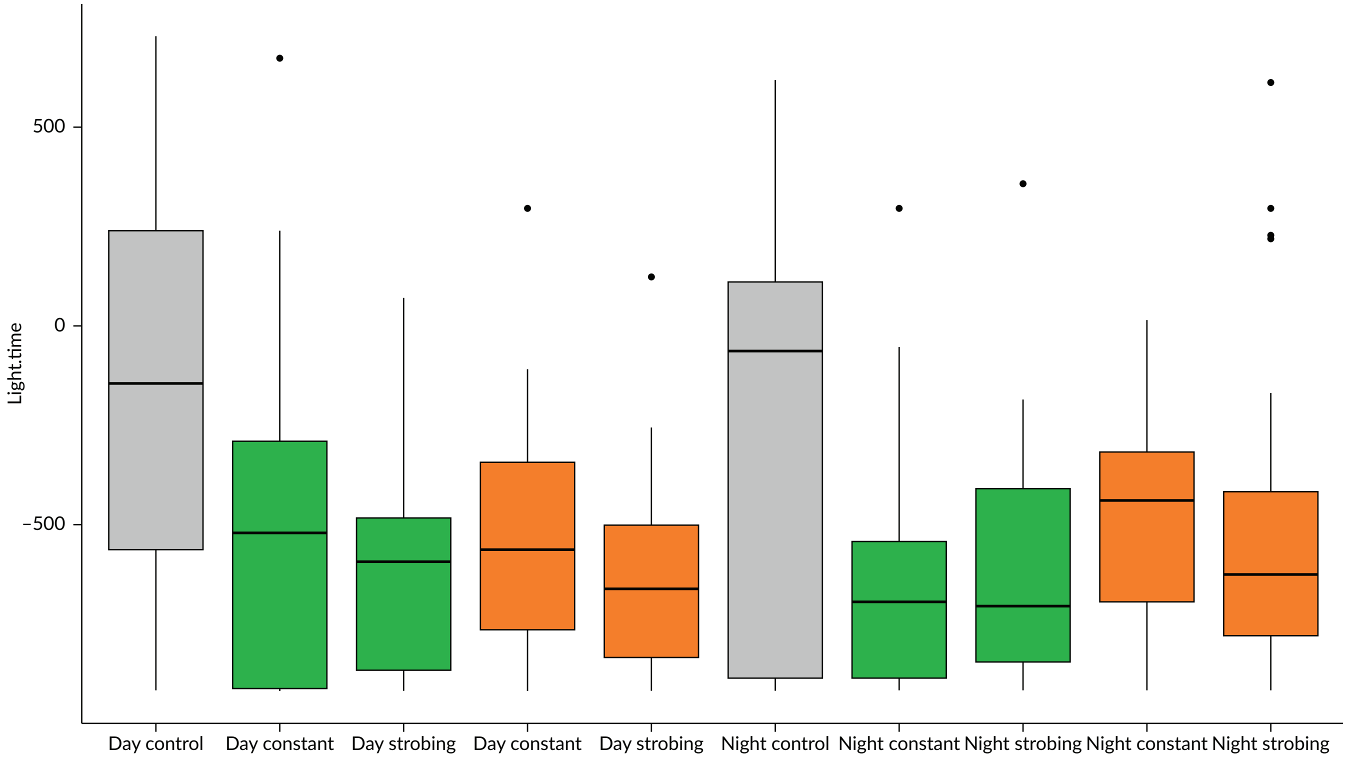

FIGURE 2 Boxplots (——, mean; □, 25_75th interquartile range; |, ± 95% CI; •, outliers) of light time of naïve age 2 year *Sander vitreus* (*n* = 242) during 300 s trials for each light treatment during the day and at night. Light time represents cumulative amounts of time spent in all light zones. Strobe rate 5 Hz; □, Unilluminated control; □, green light; □, orange light

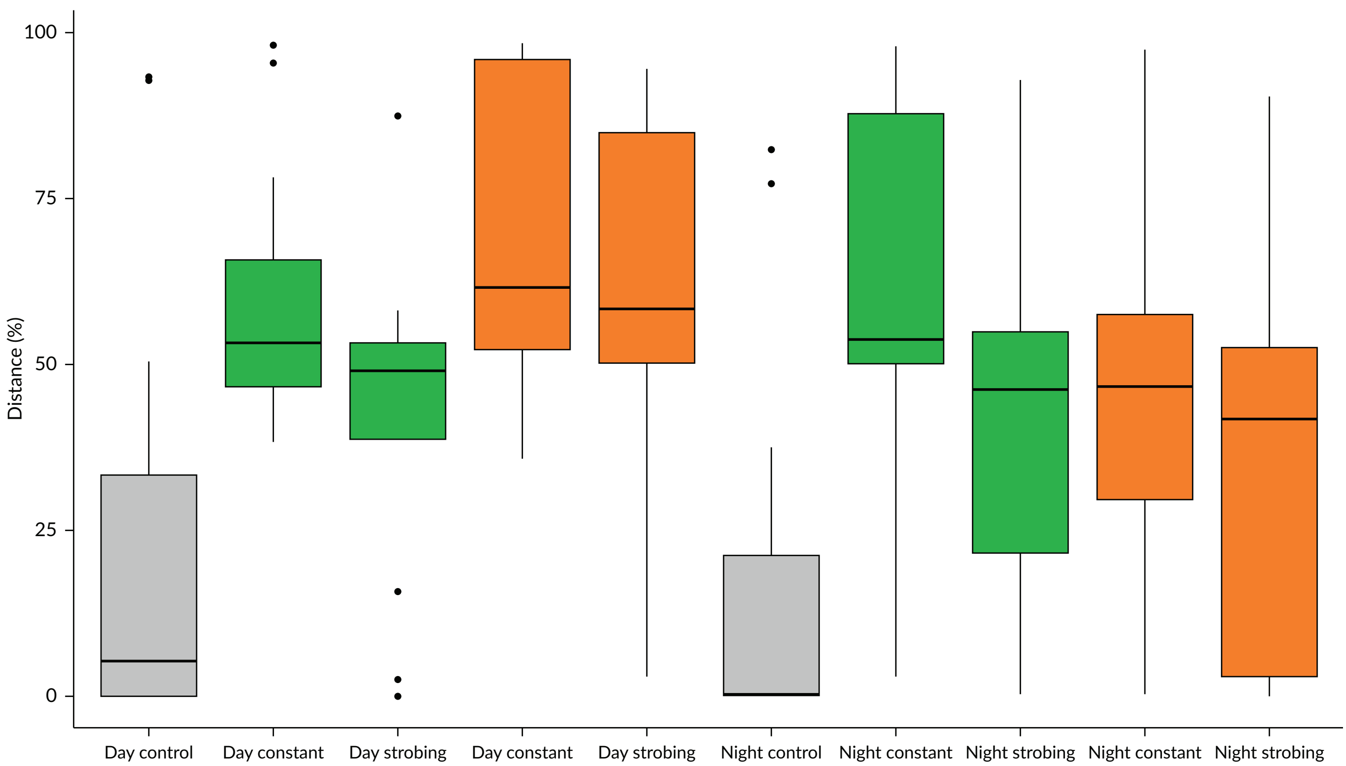

FIGURE 3 Boxplots (——, mean; □, 25_75th interquartile range; |, ± 95% CI; •, outliers) shortest distance that naïve age 2 year *Sander vitreus* (*n* = 242) approached the light guidance device (LGD) during 300 s trials during the day and at night. Distance was only measured for fish that entered the light beam (*n* = 91 were excluded). Distance was measured as 0% being representing physical contact and 100% the furthest possible distance from the LGD. Strobe rate 5 Hz; □, Unilluminated control; □, green light; □, orange light

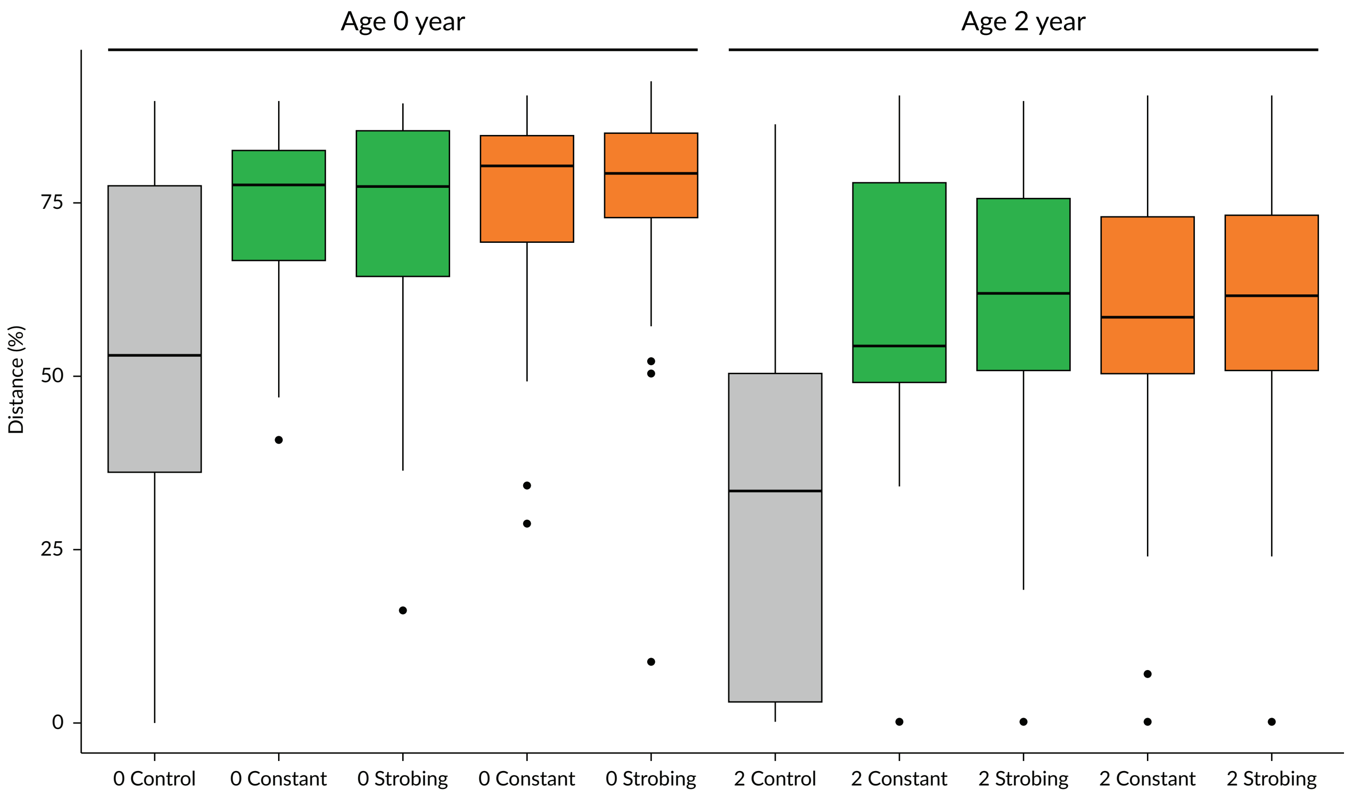

FIGURE 4 Boxplots (——, mean; □, 25_75th interquartile range; |, ± 95% CI; •, outliers) of distance from the light guidance device (LGD) of naive age 0 (*n* = 250) and 2 year (*n* = 250) *Sander vitreus* during 60 s trials for each treatment during both day and night. Distance was measured as 0% being representing physical contact and 100% the furthest possible distance from the LGD. Strobe rate 5 Hz; ■, Unilluminated control; ■, green light; ■, orange light

illuminated zones, passes and inspections towards the light zones or the LGD itself were scored in binary as 0 (for no action) or 1 (action taken; *e.g.*, passed through the light) for analysis using a generalised linear model (GLM) with binomial error distribution, while numbers of inspections and passes were analysed with Poisson distributions.

Distance was measured as a percentage of the closest a fish came to the LGD with 0% representing physical contact with the LGD and 100% indicating that it remained as far away from the light as possible within the trial arena. Distance was only measured for walleye that entered the light beam (*i.e.*, zone 1+; *n* = 151 for the experiment 1; experiment 2 had fish inserted directly into the illuminated zone so a measure was always taken: Figure 1). Light time and distance were examined as linear models in base R, GLMs with either binomial or Poisson distributions with the car package (Fox & Weisberg, 2011) and all analyses used fully factorial models in R 3.5.2 (www.r-project.org).

3 | RESULTS

3.1 | Experiment 1: age 2 year walleye reaction to light

Whether or not a focal fish entered the illuminated zones during a trial was significantly influenced by light colour (binomial GLM, likelihood ratio χ^2 = 40.09, *df* = 2, $P < 0.001$), with the control trials having greater rates of entry than the trials with either green or orange light. Both number of passes through the light zone (Poisson GLM, χ^2 = 8.605, *df* = 2, $P < 0.05$) and number of inspections of the light zone (Poisson GLM, χ^2 = 29.657, *df* = 2, $P < 0.001$) differed significantly between colours (Table 1), but not between any other factors or interactions (all $P > 0.05$). Walleye were more likely to have passed through the 1+ light zone, inspected the edge of the light (or approached its position in control trials) and entered the LGD side of the arena when the LGD was not producing light in control trials (Table 1).

Time spent in the illuminated zones was found to be significantly influenced by colour (linear model LM, $F_{2,231}$ = 18.26, $P < 0.001$), with fish spending significantly greater time during control trials than in trials with orange or green light (Figure 2). This time was not influenced by strobe rate, fish size, diel period, or any of the interaction terms (all $P > 0.05$). Conversely, test fish spent more time on average in the dark zones when the LGD was on compared to when it was turned off. The closest distance a fish came to the LGD differed significantly between trials with different light colours (LM, $F_{2,143}$ = 19.62, $P < 0.001$), strobe rates (LM, $F_{1,143}$ = 5.18, $P < 0.05$) and during different diel periods (LM, $F_{1,143}$ = 6.02, $P < 0.05$), but not with fish size or any interaction terms (all $P > 0.05$). During daytime trials, fish demonstrated graded responses to the light stimuli, with closest approaches to the LGD occurring in control trials, greatest distances during orange light trials and green light trials eliciting intermediate distances (Figure 3). At night, fish again demonstrated closer approaches during control trials and greater nearest distances during both green and

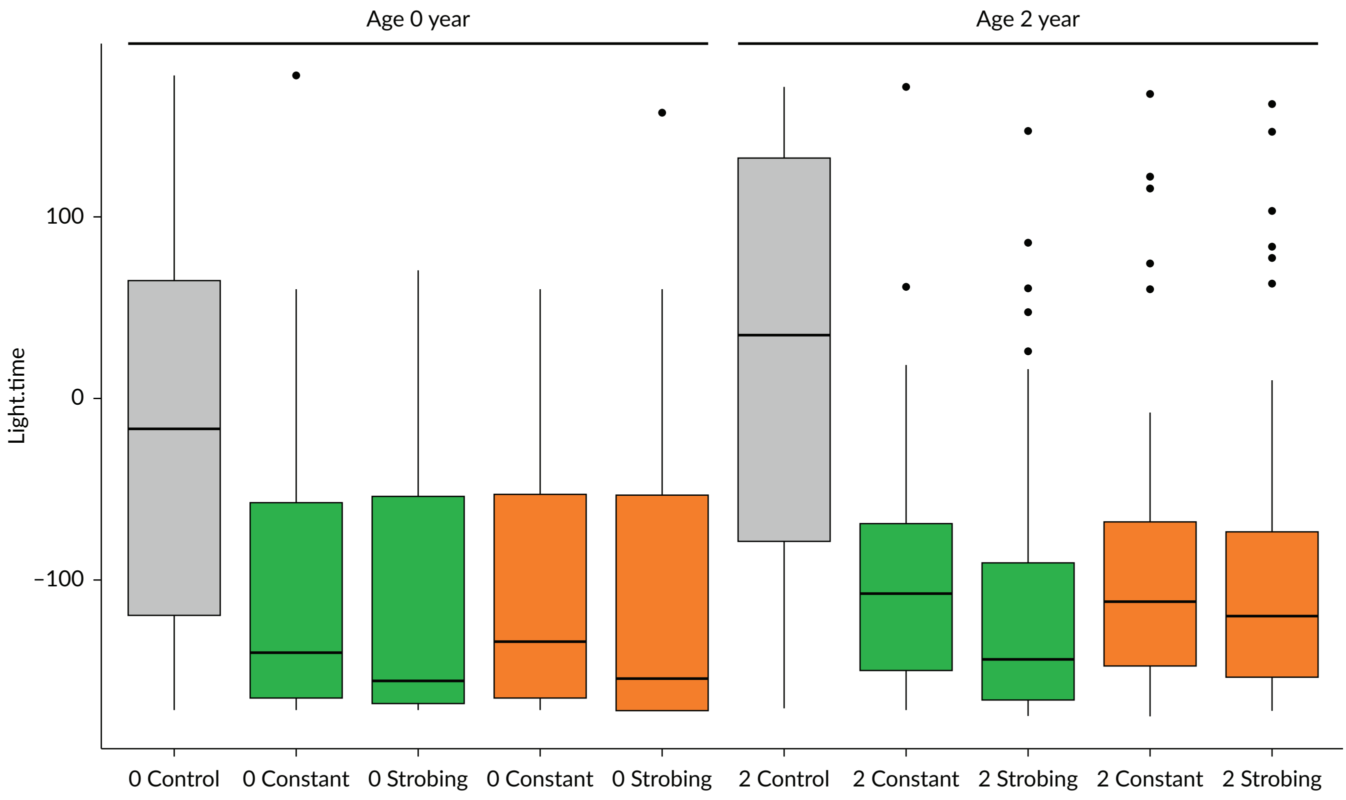

FIGURE 5 Boxplots (——, mean; □, 25_75[th] interquartile range; |, ±95% CI; •, outliers) of light time of naïve 0 (n = 250) and age 2 year (n = 250) *Sander vitreus* during 60 s trials for each treatment during both day and night. Light time is the cumulative amounts of time spent in all light zones. Strobe rate 5 Hz; □, Control; □, green light; □, orange light

orange light trials, with constant green light eliciting greater distances than green light strobing at 5 Hz (Figure 3). Overall, light (both green and orange) resulted in greater approach distances than control trials and constant light had greater distances than strobing light (Figure 3).

3.2 | Experiment 2: ontogenetic differences in reaction to light in age 0 and 2 year walleye

The closest distance between each fish (as a percentage of total arena size) and the LGD was significantly greater for the illuminated trials than the control trials (LM, $F_{2,478}$ = 61.88, $P < 0.001$) and in age 0 *v.* 2 year fish (LM, $F_{1,478}$ = 4.65, $P < 0.05$). There were no significant effects of diel period, strobe rate, or any interaction term. Overall, test walleye stayed further away from the LGD when light was being produced, independent of colour and strobe rate and age 2 year fish approached the LGD more closely than age 0 year fish (Figure 4).

LGD colour setting significantly influenced time spent in illuminated zones (LM, $F_{2,479}$ = 24.83, $P < 0.001$), with green and orange light eliciting lower times on average than control trials (Figure 5). Strobe rate, fish age, diel period and the interaction terms had no significant effects on time spent in light zones. Similarly, age 0 and 2 year walleye left the illuminated zones in significantly less time during green or orange light trials than during controls (LM, $F_{2,479}$ = 24.83, $P < 0.001$). However, fewer age 0 year fish left the light zones, instead demonstrating freezing behaviour and remaining motionless (n = 21 for age 2 years and n = 43 for age 0 years). In general, there was no significant difference between the two age groups in distance to the LGD, light time and time to leave the light zones. Both age 0 and 2 year walleye avoided LGD-influenced areas when the light was on independent of strobing or colour.

4 | DISCUSSION

The relatively young (age 0 and 2 years) walleye used in this study demonstrated strong negative phototaxis in response to both colours (orange and green) of light they were exposed to. They also avoided light in general, but their aversion to constant light appeared greater than their aversion to light strobing at 5 Hz. Walleye locomotor activity levels were higher in control treatments, as indicated by greater numbers of passes and inspections through the zones in the trial arena. Walleye were also more likely to approach the LGD and come closer on average to the device during unilluminated control trials. Based on these observations, LED light stimuli appear to have an overall repulsive effect towards age 0 and 2 year walleye.

Walleye behaviour and use of space were strongly influenced by light output from the LGD, with their use of the arena favouring the darker areas of the tank the light was on, regardless of colour or strobe rate. This kind of avoidance behaviour has been observed in several other fish species when exposed to artificial light; *e.g.*, perciforms and clupeiforms (Sager *et al.*, 2000) and salmonids (Johnson *et al.*, 2005), although light has also been demonstrated to have an attractive effect on some species; *e.g.*, perciforms and mugiliforms (Marchesan *et al.*, 2005). In walleye, other laboratory experiments have demonstrated that light is less effective than sound at preventing escapement past a non-physical barrier, although observed increases in escapement when light was present in addition to sound do not preclude avoidance of light stimulus. In practice, light has typically been employed as a deterrent stimulus, particularly for salmonids (Johnson *et al.*, 2005; Nemeth & Anderson, 1992; Puckett & Anderson, 1988). Strobing lights have been used to increase the efficiency of repulsion as many fishes tend to avoid strobing over constant illumination (Johnson *et al.*, 2005), possibly as a result of habituation to constant stimuli. The strobing light at 5 Hz used in this study appeared to diminish the reactions of the walleye to the LGD relative to constant illumination, although we cannot claim that similar results would be obtained with higher strobe rates. For walleye, the main factor governing avoidance behaviour in our study was the production of light, independent of constant or strobing output.

One possible explanation for our results is that walleye are photosensitive to both colours of light that were tested; *i.e.*, green and orange (Burkhardt *et al.*, 1980). In a similar study, *A. transmontanus* demonstrated strong levels of attraction to light matching one of their documented spectral sensitivities, green (Ford *et al.*, 2018), leaving the possibility that walleye may not demonstrate the same degree of light avoidance if presented with colour spectra that do not match their retinal sensitivities. However, if walleye avoidance of light is uniform across the visible spectrum (as our results suggest), the ability to deter them independent of colour enables guidance strategies to be adapted to different environmental conditions (*e.g.*, turbidity, water colour) by selecting spectra that achieve the greatest attenuation distances. This could also confer great flexibility in colour selection to concurrently target other species for guidance, or to select colours that do not elicit a response in non-target species.

Walleye generally prefer low-light conditions and demonstrate greater levels of activity at night (SDRNF & Reed, 1962), so the presence of bright artificial lighting is probably the driving factor behind the behavioural differences found in this study. Walleye were more active and explored the arena more during control trials and greater numbers inspected the light beam and passed through it during control trials, independent of the time (day *v.* night) they were tested. Although activity levels are normally higher for walleye at low light levels (Kelso, 1978), our results indicate that illumination from the LGD was the only significant factor influencing activity levels between night and day periods. In terms of behavioural guidance, artificial lighting could serve to deter walleye from entering high-risk areas under both day and night conditions, independent of their diel activity patterning.

Ontogenetic differences in many different behaviours have been described in walleye (Bozek *et al.*, 2011) and behaviour is a phenotypic trait that typically demonstrates some degree of plasticity as fish mature (Noakes & Godin, 1988). We observed little difference in area use and light time between age 0 and 2 year walleye, suggesting that negative phototaxis is a behavioural pattern that does not change with ontogeny. Differences in foraging and niche utilisation reflect differences in age and size (Colby *et al.*, 1979; Forney, 1966; Xia *et al.*, 2018), as do shifts in visual behaviour (Elvidge *et al.*, 2019). Ontogenetic changes in the retinal structure of the eye of walleye allow them to function better under dim light conditions as they develop (Ali & Anctil, 1977), but our results demonstrate that developmental changes between age 0 and 2 year fish did not result in significant overall changes in behavioural responses to light stimuli. These findings reaffirm that light is a strong deterrent for walleye, independent of age. The knowledge that all age classes of walleye avoid light could be beneficial for behavioural guidance as it would allow for uniform targeting light guidance to walleye. Similarly, the use of light as a guidance stimulus for other potential sympatric target species such as *A. fulvescens* (Elvidge *et al.*, 2019) may reliably be viewed as having consistently repulsive effects on non-target species, including walleye, within the same basin (Kim *et al.*, 2019; Kim & Mandrak, 2017).

The results of this study have two broad implications for the guiding the behaviour of walleye. First, if walleye generally and consistently avoid light, light stimuli can be used to help limit occurrences of entrainment and impingement for migratory life-history phases in this species and possibly in congenerics (*e.g.*, *S. lucioperca*) by using light as a repellent stimulus in areas of elevated hazard. Second, the use of light as an attractant towards desirable areas such as passageways for other species, particularly migratory species (Elvidge *et al.*, 2018; Ford *et al.*, 2017), is unlikely to have effects beyond repulsion on walleye. Further testing of the LGD as a behavioural guidance tool for walleye is needed to determine behaviour both over longer terms under laboratory conditions and under field conditions. However, this study demonstrates the potential of strobing LED light as a useful tool to behaviourally guide walleye concurrently with other negatively or positively phototactic co-occurring species.

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CONTRIBUTIONS

M.I.F., C.K.E. and S.J.C. designed the experiment; M.I.F. conducted the experiment; M.I.F. and C.K.E. analysed the data; P.H.P. and M.S. developed the L.G.D.; M.I.F. and C.K.E. wrote the manuscript; all authors contributed to editing the manuscript; P.H.P., M.S. and S.J.C. obtained funding as senior investigators.

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