The Influence of Light Colour on the Behaviour of Atlantic Cod in an Experimental Setting

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Abstract

Fishing technologies often exploit the visual sensitivity of target species to alter their behaviours. Atlantic cod (*Gadus morhua* Linnaeus, 1758) are an economically important species, commonly targeted by fisheries in the North Atlantic, yet the behaviour of adult Atlantic cod in reaction to the simultaneous presentation of various light stimuli has not been assessed in an isolated setting to determine if there is a preference for certain light qualities. To assess the influence that artificial light may have on the behaviour of Atlantic cod, we investigated the movement of wild-caught cod in a laboratory setting with green, blue, and white light with a blank control presented in paired choice tests. We predicted that green light would have the greatest influence on cod behaviour and that they would preferentially spend more time in proximity to that light condition. The findings show that cod behaved consistently across trials and across experimental sessions, and that the right side of the arena was preferred regardless of the colours of light presented. These findings indicate that cod do not prefer to spend more time in proximity to certain colours of light in an artificial environment, which has direct implications for animal husbandry, fisheries research, and behavioural ecology.

Keywords: visual ecology, Atlantic cod, choice test, light preference, negative results, fisheries

Les techniques de pêches ciblant la sensibilité visuelle peuvent modifier les comportements. La morue franche (*Gadus morhua* Linnaeus, 1758) est une espèce d’importance économique couramment ciblée par les pêcheries de l'océan nord-Atlantique. Afin d’optimiser ses méthodes, il serait tout d’abord important de comprendre le comportement de la morue franche en contact avec divers stimuli lumineux présenté simultanément. Pour évaluer l'influence de la lumière artificielle sur son comportement, nous avons étudié le mouvement de la morue franche en laboratoire, avec des lumières vertes, bleues et blanches présentées avec un témoin à blanc lors

de tests à choix par paires. Dans cette étude, nous avions prédit que la lumière verte aurait la plus grande influence sur le comportement de la morue et que par conséquence, une plus grande proportion de temps passé dans la zone éclairée par la lumière verte serait observée. Les résultats montrent que les morues préfèrent systématiquement le côté droit de l'arène, sans distinction pour le type de couleur projeté. Ces résultats indiquent que la morue ne préfère pas passer plus de temps à proximité de certaines couleurs de lumière dans un environnement artificiel, ce qui a des implications directes pour l'élevage, la recherche halieutique, et l'écologie comportementale.

Introduction

Visually-oriented fishes, including many benthic and demersal species, depend on light to forage and interact with their environment (Woodhead 1966; Brynh et al. 2014; Winger et al. 2016; Humborstad et al. 2018). Predator avoidance, migration patterns (Woodhead 1966), and foraging (McMahon and Holanov 1995) are strongly influenced by fishes' visual systems and ambient light environment. As such, light may play an important role in the efficiency and effectiveness of fishing gear, as technologies can be used to intentionally manipulate and exploit the natural behaviours of animals. In fisheries, this often involves modifying or baiting fishing gears in ways that aim to attract (or repel, in some cases) specific taxa or species groups. Due to the natural role that vision plays in many harvested fishes, light modifications to gears are often used for such purposes. Bait and other lures are sensory stimuli that act to draw the target species toward or away from the gear and manipulate their behaviour to allow for their capture or avoidance. Though fishing gears often employ light stimuli in an attempt to improve catch, the visual ecology of the target species is sometimes overlooked in efforts to quickly apply enhancements directly to industry.

Atlantic cod (*Gadus morhua* Linnaeus, 1758) are a culturally and economically important species throughout the world and are currently listed by the International Union for Conservation of Nature and Natural Resources (IUCN) as Vulnerable as of 1996. Once a major fishery, a moratorium on commercial groundfishing in the Northwest Atlantic Ocean was imposed by the Government of Canada, owing to a collapse of Atlantic cod stocks through over-fishing, coupled with environmental changes and socioeconomic factors (Hutchings and Myers 1994; Myers et al. 1996; Milich 1999; DFO 2011; Sierra-Flores 2014). Since the moratorium was imposed, cod stocks have shown some recovery but have remained susceptible to depletion through over-fishing and mismanagement (Hutchings and Myers 1994; Shelton et al. 2006; DFO 2011; Rowe and Rose 2017). As well, climate-induced effects on demersal ecological conditions such as prey availability have also been ongoing (Buren et al. 2014). Cod populations in the Newfoundland and Labrador Designatable Unit are considered Endangered by the Committee on the Status of Endangered Wildlife in Canada as of 2010 (COSEWIC 2010). All that remains as a fishery for cod in Eastern Canada is a small-scale inshore fishery, with gillnets and handlines as the ubiquitous gear types used, where light enhancements could be easily implemented.

There have been varying results regarding the behaviour of Atlantic cod with respect to light. Some studies indicate that cod are attracted to light (Bryhn et al. 2014), while other studies have shown that light has no effect on the behaviour of cod (Grimaldo et al. 2017; Melli et al. 2018; Utne-Palm et al. 2018; Blackmore et al. 2022). To investigate the visual ecology of Atlantic cod and to inform the use of artificial lights as lures in fishing practices, the behaviour of Atlantic cod needs to be studied in response to various light stimuli in tandem.

Like all environmental constraints, an organism's light environment shapes its life history, physiology, and behaviour. The light environment at depth in the ocean is restricted to a range including only short-wavelength light, due to the absorption of light as it travels through the water column (Levine and MacNichol 1982). Adult Atlantic cod are well-adapted to their environment, living primarily in coastal and continental waters between tens to a few hundred metres depths (COSEWIC 2010), and have developed opsin pigments in their retina that allow maximum sensitivity and discrimination of wavelengths in the blue-green light range (Valen et al. 2014) associated with these coastal marine environments. Cones in the eyes of adult Atlantic Cod are maximally sensitive to 446 nm and 517 nm (Douglas and Djamgoz 1990, Figure 1), respectively. The broad absorption spectra of these photopigments and incorporation of rods for light detection allow cod to see in the low-light levels of the deep Atlantic Ocean. However, it is possible that colours of light that do not naturally occur within the range of natural light conditions could be perceived by cod, due to the broad absorption capabilities of their visual system and cause behavioural changes when presented via artificial stimuli.

Objectives and Hypotheses

The aim of this study is to examine the effect of three light conditions on the activity and movement of adult Atlantic cod in a controlled laboratory setting.

Atlantic Cod have spectral sensitivities adapted to pursue prey efficiently in blue-green limited light environments, and as such, can distinguish different light stimuli against a background that mimics natural light conditions. We predicted that all light conditions would show greater responses than the control, and that green light emitted at 510 nm would be the light quality to best stimulate a phototactic response by Atlantic Cod, as the wavelength of light closest to the peak sensitivity of the cone in the retina of cod. As such, we predicted that cod would spend more time in proximity to the green light stimulus than to other colours of light and the control.

Methods and Materials

Experiments were performed at the Joe Brown Aquatic Research Building (JBARB) of Memorial University of Newfoundland's Ocean Sciences Centre in Logy Bay, Newfoundland from August 26th to December 3rd, 2020. Thirty-four wild-caught Atlantic cod (acquired from Arnold's Cove, Newfoundland on November 28, 2019) were housed at low density in a 3 m diameter x 3 m tall 25,000 L surface-input flow-through aquarium (holding tank) beginning December 1, 2019, with water input flow rate set to 100 litres per minute, and regulated temperatures between 7-9 °C. Taxon identification was achieved using guidelines from Nozères et al. (2010). Two individuals were removed and euthanized on December 12, 2019, due to exophthalmia. Following their removal, the remaining fish (n = 32) were treated with anti-parasite formalin (Parasite-S) treatments for three days, starting December 17, 2019, as a preventative measure against further disease or parasitic infection. This treatment was advised by the JBARB staff as a well-studied chemical treatment but can lower the dissolved oxygen levels in the aquarium. Its application was not thought to affect the behaviour of cod as long as the controlled oxygenation levels of the holding tank remained high (see Leal et al. 2016 for a summary of its use in aquaculture of other fishes). Following the treatments, the behaviour of cod was monitored by JBARB staff to ensure no abnormalities such as loss of appetite or decreased activity were observed. Before trials were able to begin, COVID-19 safety protocol for research on campus at Memorial University was established, restricting access to the JBARB and temporarily postponing this research project. Access to the building was not given to the research team until late August 2020. Experimental trials began October 1, 2020, by which time seven more cod had perished, leaving 25 apparently healthy individuals for experimentation.

An identical aquarium next to the holding tank with the same water parameters and flow rate was used as the experimental arena. The light environment was altered in both tanks using a lighting filter (Lagoon Blue #172, LEE Filters Canada ™) that absorbs light above 550 nm (Figure 2a) to mimic the natural light environment of Atlantic cod at depth (see McMahon and Holanov 1995, Harant and Michiels 2017). Photoperiod was automated to follow a natural day-light cycle. For 7 fish (~20 % of the sample population) fork-length was measured to the nearest 0.1 cm using a metre stick (visible in Figure 3) and measured mass to the nearest gram using a Pesola spring scale (values in Supplementary Material). The Atlantic cod were fed a standard diet of a food mixture (40 % Atlantic mackerel, 40 % Atlantic herring, and 20 % squid) thrice weekly, rationed at 0.5 % of the average body mass per day (approximately 23 g per fish per week).

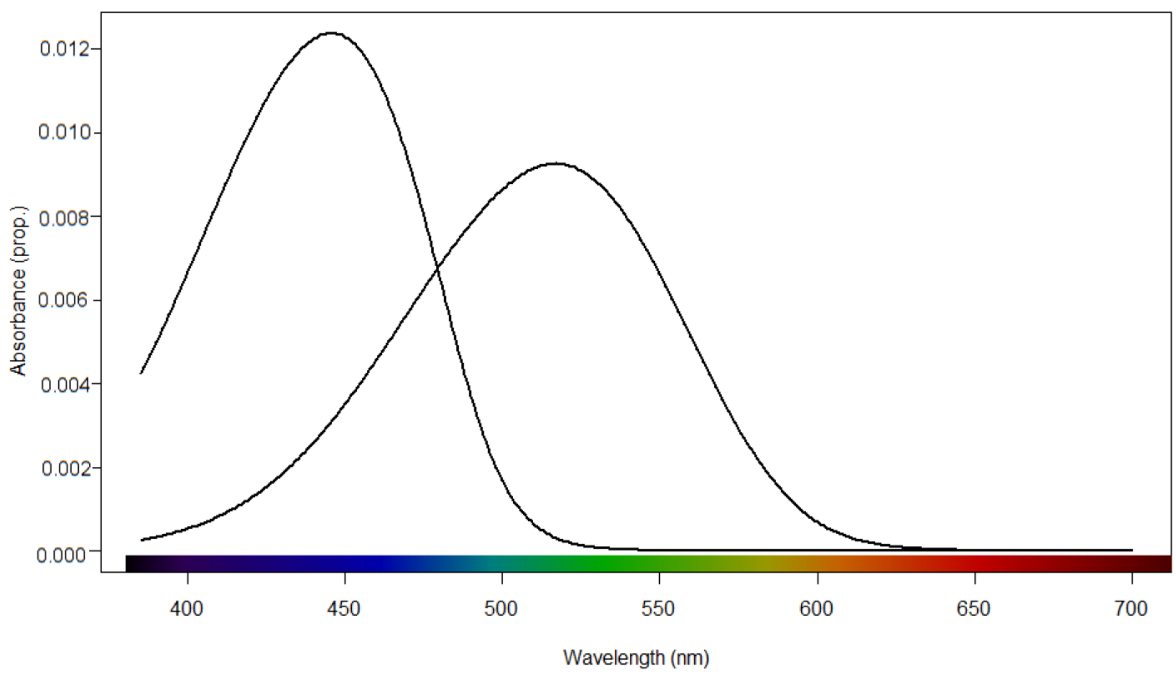

Figure 1. Spectral sensitivities of cones from *Gadus morhua* Linnaeus, 1758 as measured by microspectrophotometry; λmax SWS = 446 nm, λmax RH2A = 517 nm (Douglas and Djamgoz

1990). These probability curves were produced using a vertebrate template (Govardovskii et al. 2000) and may not accurately reflect true cod vision capabilities, because the range in the short wavelength may be cut off by ocular media in the eye (e.g., lens).

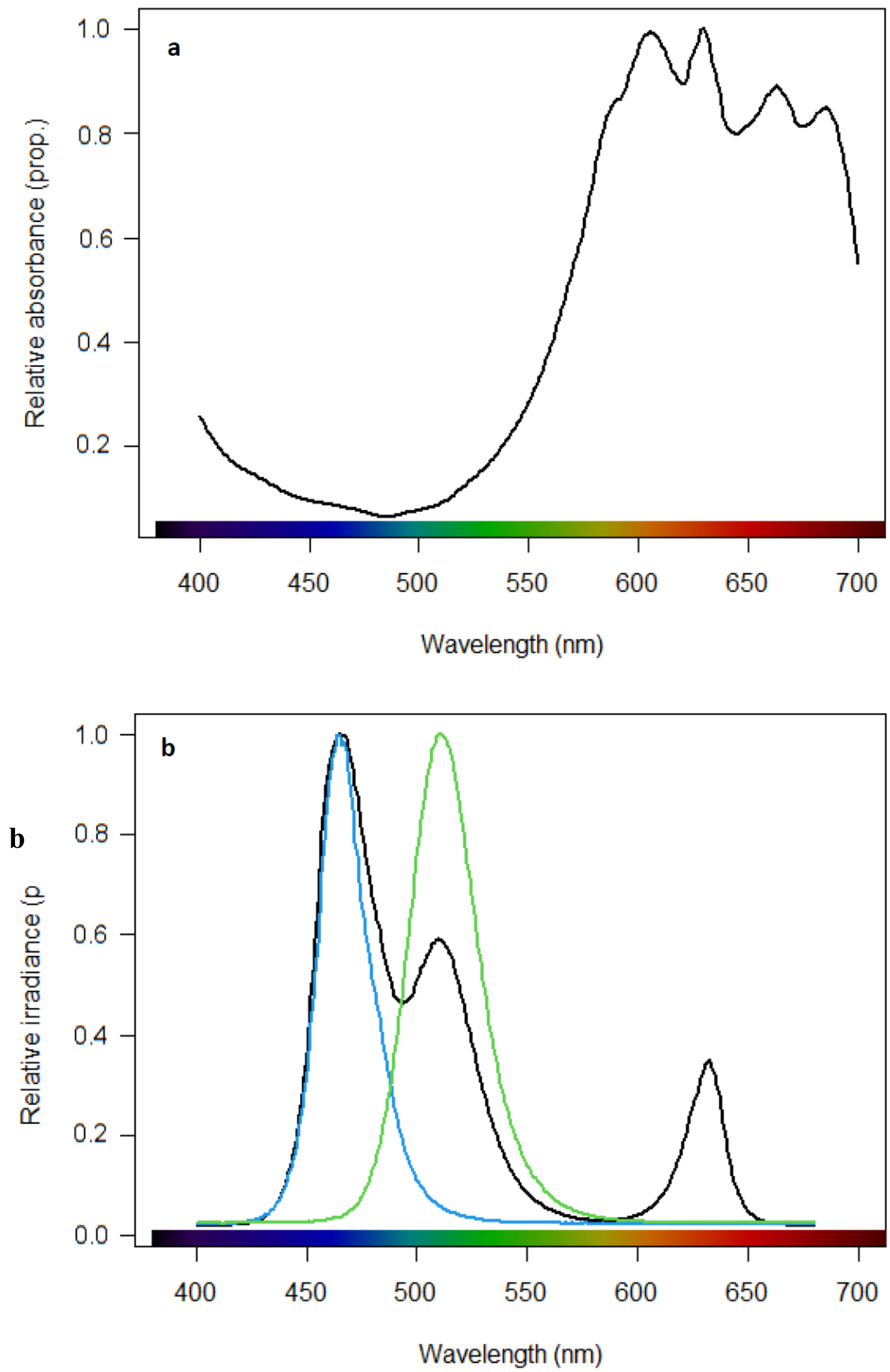

Figure 2. Spectrophotometric measurements of a) the relative absorbance of light transmitted through LEE Filter #172 "Lagoon Blue" via a calibrated light source and b) the relative intensity

of light output from UNPAD LED devices on the “White” (black line), “Green” (green line) and “Blue” (blue line) settings.

The behaviour of cod was assessed when presented with four conditions: green, blue, and white light, as well as a control with no light. Wireless waterproof multicolour LED devices (UNPAD, Figure 2b) were used, which have settings for blue, green, and white light emittance. White light was chosen as it contains peaks at both blue and green, as well as a slight peak in the red (Figure 2b), to account for any influence that light outside of the expected perception of the visual system of cod might have on behaviour. The devices were measured using a spectrophotometer (Jaz® spectrophotometer, OceanInsight, Orlando, U.S.A.) fitted with a cosine-corrected optic fibre (CC-3-UV, Ocean Insight) in scope mode. The filter was measured using the same spectrophotometer in absorbance mode, using a bare optic fibre from the output (the internal light source), and a cosine corrector on the receiving fibre. The ends of each fibre were placed facing each other with a spacing of 1 cm, with the full light cone contained within the cosine corrector. The reference measurement was taken using the light only, the dark reference was measured while covering the cosine corrector completely, and the measurement of the filter was taken by applying it directly to the cosine corrector. Integration time was set during the reference measurement to automatic with dark noise correction.

The green LED was measured to emit light at a peak intensity of 510 nm (Figure 2b). The blue LED was measured to emit light at a peak intensity of 464 nm (Figure 2b). The “white light” is produced by a combination of blue, green, and red LEDs with an overall peak emission at 633 nm. The relative intensity of light emitted from each LED used on the “white” setting was lower at longer wavelengths, such that compared to the blue (465 nm) emittance, the green (509 nm) light was emitted at 59.12 % intensity and the red (633 nm) light was emitted at 34.77 % intensity (Figure 2b), optimized by the device itself.

Study Design and Procedure

Our chosen four light conditions were tested in pairwise combination, such that all six possible combinations of green, blue, white, and no light were presented. These six combinations were tested in random order and random sides of the tank, forming six 10-minute experimental trials. For clarity throughout, we refer to an experimental "session" as consisting of these six 10-minute trials, and a flow-chart diagram below (Figure 4) shows the order of operations. All Atlantic cod were subjected to two experimental sessions. For their first session, twenty-five cod were individually tested by haphazard selection, transferred from the holding tank to the arena using a dip-net and allowed to acclimatize to the arena for 5 minutes. All cod were tagged with a subdermal ID tag (Floy T-bar Anchor Fish Tag, Figure 3) near the first spine of the dorsal fin for individual recognition immediately prior to their first experimental session. Each trial was initiated by the lowering of LEDs into the tank, where LED devices were attached to weights and simultaneously lowered into the tank to allow the presentation of each light condition from opposite sides of the arena (Figure 4, 5). Trials involving the control condition still began with the lowering of both LEDs, but the LED being used as the control was turned off. Fish were given approximately 30-180 seconds between trials, while the light condition was set for the upcoming trial. A colour CCD infrared camera (National Electronics Inc. DN-IR36) was placed above the centre of the experimental tank to record all trials. Once each fish finished their first experimental session, it was returned to the holding tank with the others. Once all 25 fish had completed one session, they were haphazardly selected for a second experimental session, in which the order and side that light conditions were presented were randomized to control for order effects. The first experimental sessions occurred in September and October 2020, and the second sessions occurred in November and December 2020. Cod were not fed on the days that testing took place to account for satiation as a factor affecting behaviour. All cod were euthanized by blunt-force trauma to the head following the completion of their second session.

Figure 3. Example of Floy T-bar Anchor Fish Tag used to identify Atlantic cod (*Gadus morhua* Linnaeus, 1758) and its placement on an individual.

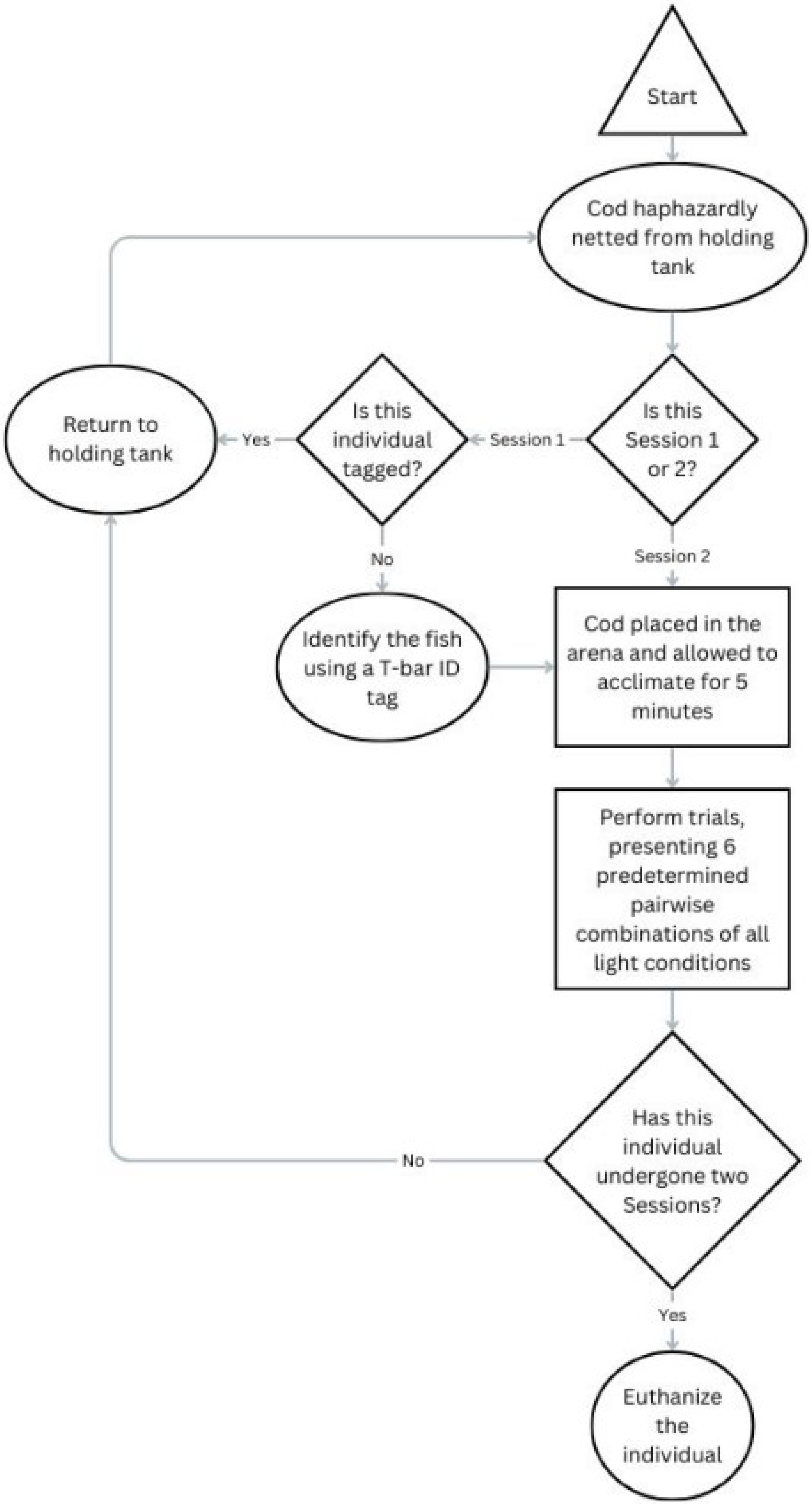
Start
Cod haphazardly netted from holding tank
Is this Session 1 or 2?
Session 1
Is this individual tagged?
Yes
Return to holding tank
No
Identify the fish using a T-bar ID tag
Session 2
Cod placed in the arena and allowed to acclimate for 5 minutes
Perform trials, presenting 6 predetermined pairwise combinations of all light conditions
Has this individual undergone two Sessions?
No
Yes
Euthanize the individual

Figure 4. Flow-chart showing the procedural order of events throughout which all Atlantic cod (*Gadus morhua* Linnaeus, 1758) were subjected to experimental conditions. Session 1 was completed by all 25 individuals before Session 2 began.

Analysis of videos

Behavioural data were extracted from videos of the trials by trained assistants who were blind to the treatments and to the research question. A custom-built program allowed assistants to extract positional data from videos (available at https://github.com/rfh473/fish). Assistants were tasked with placing their cursor above the centre of mass of the fish and following the fish's movements for the duration of each trial. The user's cursor position was normalized to 0-1, where the origin was the upper left corner of the video frame. The relative position of the cursor to the bounds of the recording was therefore equivalent to the relative position of the fish to the bounds of the arena. For each frame of the video (1080 x 720p, 48 fps), the normalized x and y coordinates were written to a comma-separated-value (.csv) file for each test.

Positional data were categorized for proximity to the light sources presented on the right side of the tank (right zone) or the left (left zone, Figure 5a). These zones were created as equal tangent circles, where the positions of each light source were the origins and the radii were half the distance between the light sources. The proportion of total time that fish spent in the left and right zones was used as a measure of time spent in proximity to each light source.

Proportion of time values were calculated for each 10-minute trial, as well as for the first 60 seconds of each trial. The initial minute of behaviour was assessed separately from the entire trial to capture the immediate reactive behaviours of cod upon being presented with lights.

In cases where data were missing for one of the two sessions, or the behaviour of the fish changed such that it was determined to not meet the participation requirements for the experiment (i.e., an individual moved considerably during its first session, but floated at the top of the tank during the entire second session, which may have been caused by some sort of trauma), that session was considered to be an outlier and was excluded, with the remaining session chosen for analysis. While all fish were subjected to two experimental sessions, many had missing or excluded data. Such that all 25 cod could be assessed, including those with an excluded session, fish with two valid sessions (n = 7) had one session picked by random assortment, and that same session was then used for all subsequent modelling exercises.

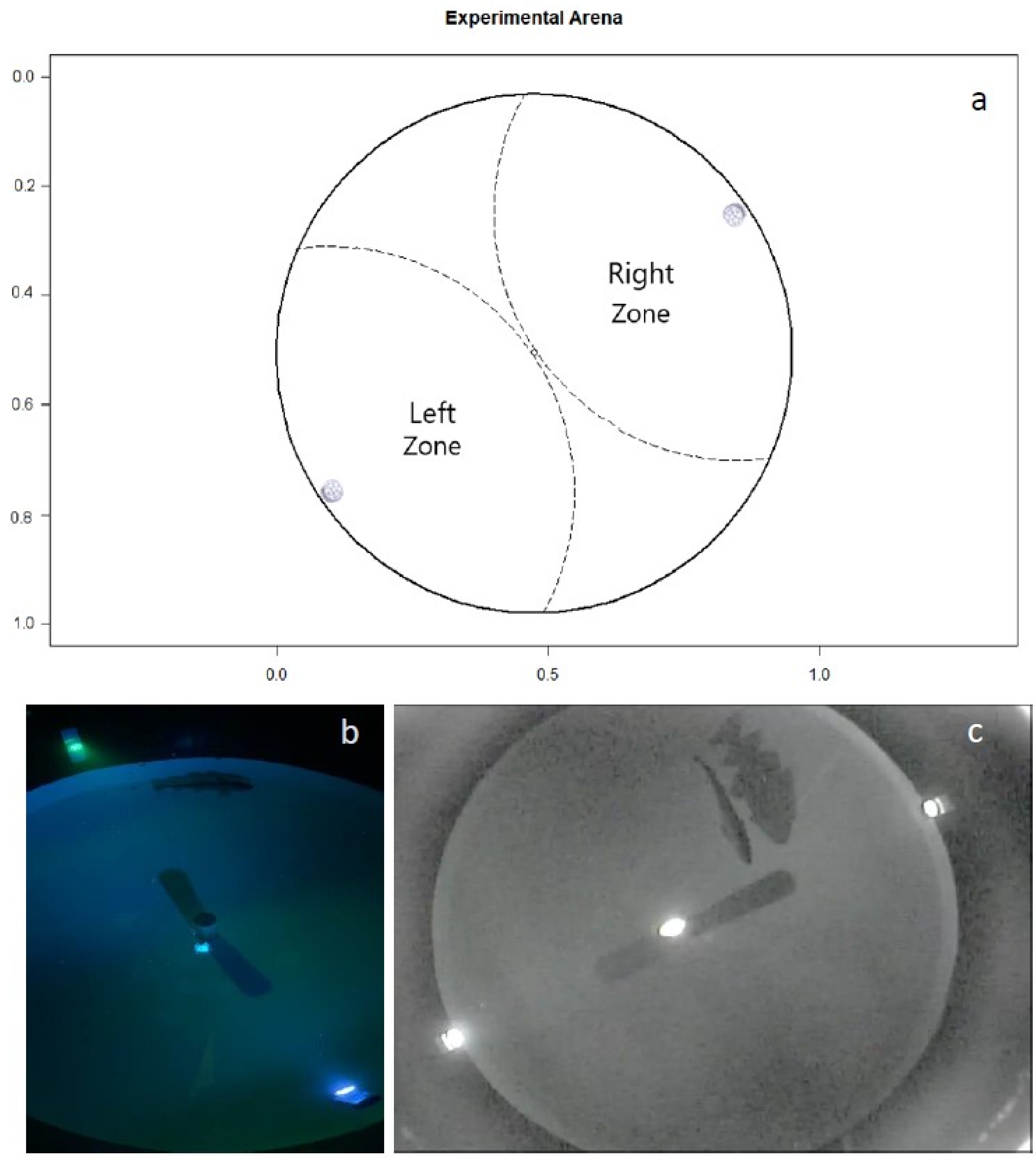

Figure 5. a) The design of the arena, showing the right and left zones associated with each light source. b) The view of the arena from the perspective of the researcher. c) The view of the arena from the overhead infrared camera.

Statistical Analysis

A measure of individual activity was calculated using the *spDists* function in R (sp v. 1.6-0 package, Pebesma and Bivand 2005) as the mean absolute deviation of total distance travelled throughout the arena during a single session by each fish. With the exception of those excluded from analyses due to a lack of movement, all fish spent the ten minutes of each trial moving in some capacity, so time spent moving could not be used as a way to correct other metrics, so we used distance moved as an approximation for activity. Every individual had a total distance travelled for their one session, and this value was subtracted from the grand mean of all individuals to get a mean absolute deviation for each subject. To assess behavioural repeatability during trials, the activity measure for each fish was analyzed using the *rpt* function in R (rptR v. 0.9.22 package, Stoffel et al. 2017). The repeatability measure was calculated to determine if the behaviour of activity differed among fish (n = 25) and then differed for individual fish between sessions (n = 7). The sample size available for the between-session analysis was low, and did not involve all 25 cod, due to the aforementioned exclusions due to erroneous behaviour or faults in the video capture accounting for the loss or removal of one of the two sessions, leaving only seven individuals with two entirely valid sessions. Each of these analyses were generated with 1,000 bootstrap simulations to create 95 % confidence intervals around the estimated repeatability measures.

Two dependent measures were modelled; the proportion of time that fish spent in each zone during the first minute of each trial, and the proportion of time that fish spent in each zone during the 10-minute trial, to investigate differences in the initial response against a prolonged response to the light conditions. Twenty-five fish were included in both models, where fish identity was treated as a random factor, and the colour of light, side of the tank the light was presented on, and individual activity measure were treated as fixed factors.

Data were fitted to generalized linear mixed models using the glmmTMB package (v. 1.15, 2022, Brooks et al. 2017) in R (R Core Team 2022). Models were first fit using a Gaussian distribution of residuals and were assessed for fit using the *simulateResiduals* function of the DHARMa package (v. 0.4.6, Hartig 2022), which assesses homogeneity and fits the residuals to Q-Q plots to check normality. Both models were zero-inflated and otherwise normally distributed, bound at 0 and 1, so a model was built using a Tweedie distribution, as well as a hurdle modelling approach with a zero-inflation parameter paired with a student's t-distribution for the error structure. Akaike information criteria (AIC, Akaike 1974) were used to compare the two models through the *Anova* function in the car package (Fox and Weisberg 2019). P-values associated with each explanatory variable in the model were assessed through the *anova* and determined to be significant if less than $\alpha = 0.05$. *Post hoc* investigations of a variable were performed if the p-value was less than $\alpha = 0.10$.

For the boxplot produced using the *ggplot* function in ggplot2 package, default settings for the interquartile range and whiskers were used, such that the box represents the 25^{th}, 50^{th} (median), and 75^{th} percentiles, whiskers represent 1.5 times the interquartile range, and dots represent outlying values outside of 1.5 times the range. For the violin plot, data are scattered in an overlay on the violin for visibility.

Ethics and Permits

Cod were held under General Broodstock Holding IAC Protocols DB 18-01. Experiments were performed with approval from Memorial University of Newfoundland Animal Care Committee under Animal Use Protocol 20-01-WM.

Results

Repeatability tests

The repeatability of activity, as measured by the total distance travelled by each individual, was calculated between trials within a session (n = 25 individuals, 6 replicates each), as well as between sessions (n = 7 individuals, 2 replicates each). Repeatability estimates showed that, within a single session, behaviour was highly repeatable ($R^2 = 0.794$, log-Likelihood = -601.1, $p \ll 0.001$). Between sessions, behaviour was also repeatable but the sample size was too low for the p-value to be considered significant ($R^2 = 0.472$, log-Likelihood = -78.87, $p = 0.128$). Bootstrapping models corroborated the results of the repeatability estimates, with $\mu_{1000} = 0.783$ between trials and $\mu_{1000} = 0.436$ between sessions.

The effect of light on space use

A model evaluation using AICs showed that the hurdle model factors for the colour of light presented, side of the tank, individual activity measure, and fish ID was the best fit for the first minute of trials. We found that the right zone was preferred overall ($\beta = 0.121 \pm 0.012$, df = 1, $p < 0.001$), but that the three light treatments were not preferred to the control condition (blue: $\beta = -0.002 \pm 0.018$, df = 3, $p = 0.931$, green: $\beta = -0.001 \pm 0.018$, df = 3, $p = 0.972$, white: $\beta = -0.005 \pm 0.017$, df = 3, $p = 0.791$). Activity was not a significant factor for 1-minute trials ($p = 0.745$, df = 1).

The model with the best fit for the 10-minute trials was also a hurdle model. The proportion of time that fish spent in proximity to green ($\beta = -0.003 \pm 0.019$, df = 3, $p = 0.889$), blue ($\beta = 0.005 \pm 0.019$, df = 3, $p = 0.710$), and white ($\beta = 0.0001 \pm 0.019$, df = 3, $p = 0.90$) light did not differ significantly from the control throughout all conditions (Figure 6), nor did any experimental light condition differ from another (Figure 6). The proportion of time that a fish spent near a given light condition was not-significantly influenced by their activity ($p = 0.079$, df = 1), but fish spent significantly more time in the right zone, regardless of the light condition that was presented (Figure 7; $\beta = 0.122 \pm 0.0127$, df = 3, $p < 0.001$).

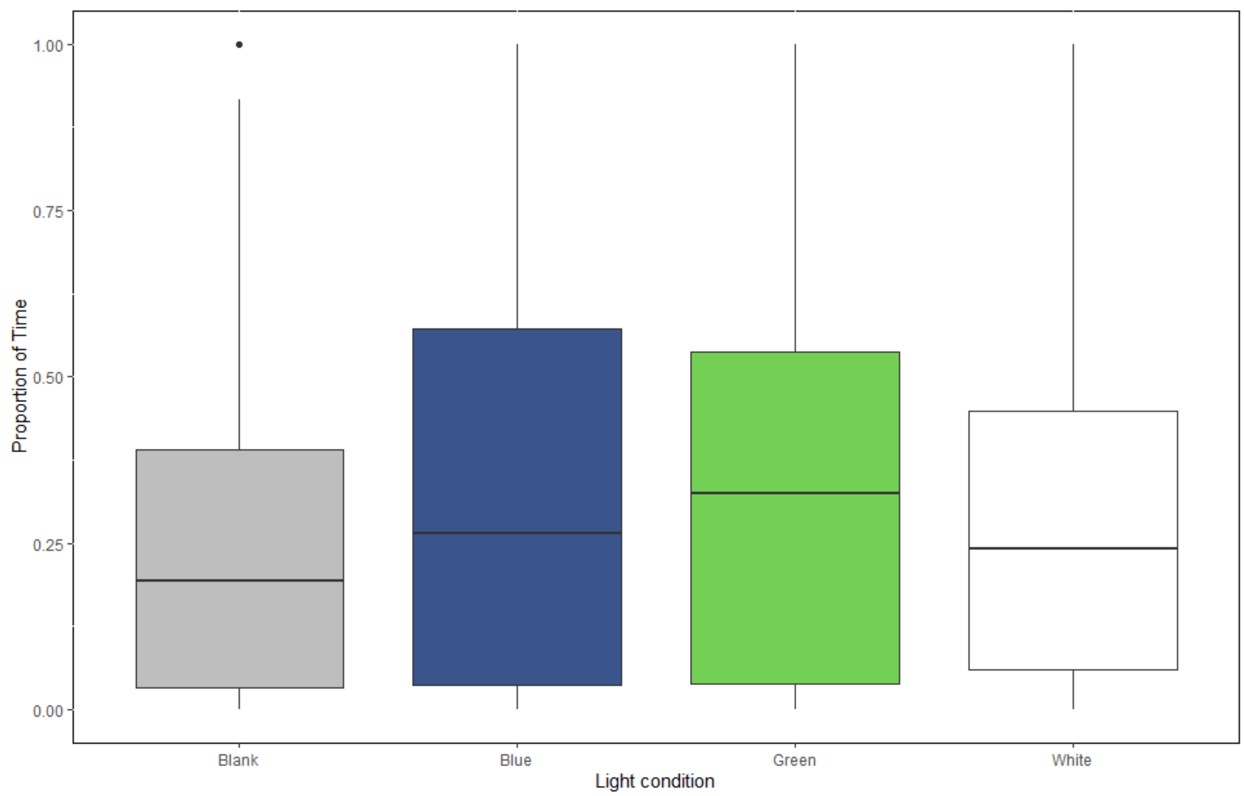

Figure 6. Total proportion of time that Atlantic cod (*Gadus morhua* Linnaeus, 1758) spent in proximity to each of the four experimental light conditions, binned by the side of tank on which it was presented.

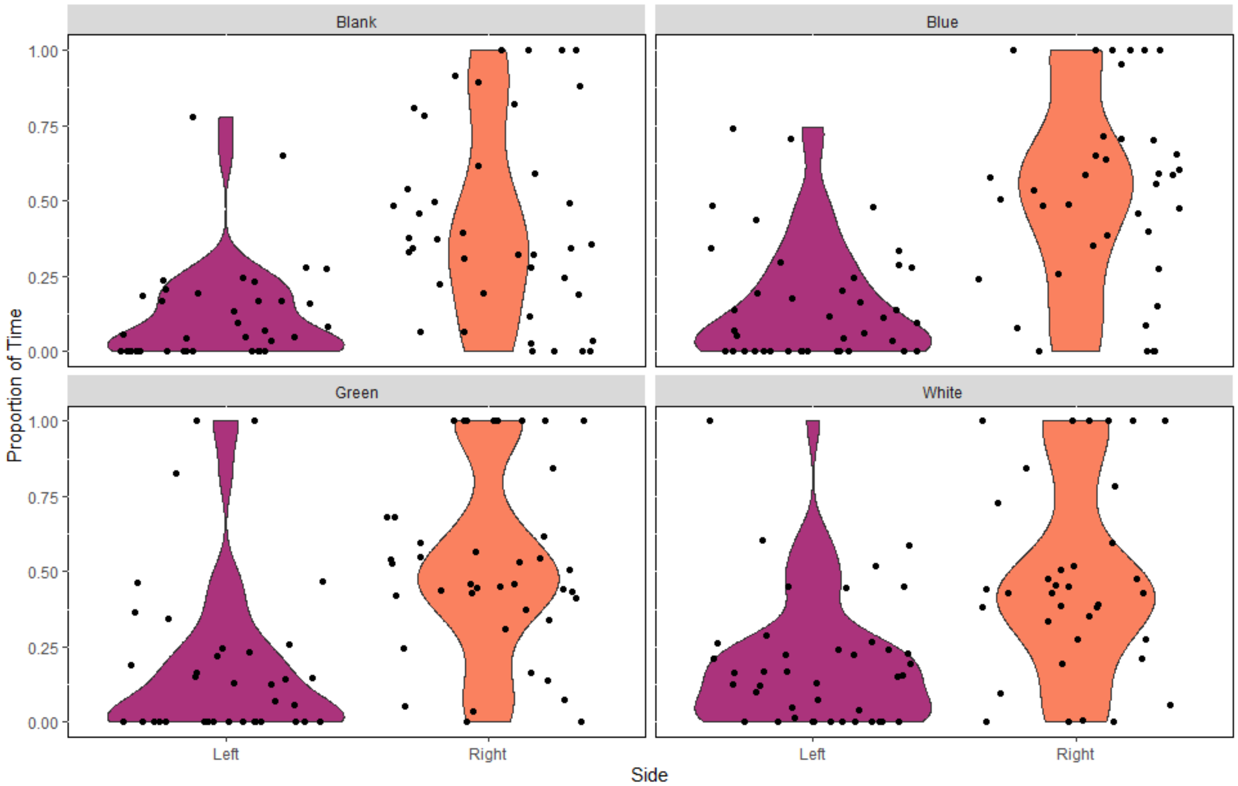

Figure 7. Total proportion of time that Atlantic cod (*Gadus morhua* Linnaeus, 1758) spent in either the right or left zone, split by light condition.

Discussion

Though within the visual sensitivity range for this species, this study found no evidence that the presentation of blue (465 nm), green (510 nm) or broad-spectrum white LEDs influence the movement behaviour of Atlantic cod. No significant differences were observed during the first minute of each trial, nor throughout the entire 10-minute duration of trials, and the colour of light presented did not affect the time that Atlantic cod spent in proximity to the stimuli. Light is used in many contexts in fisheries worldwide but is primarily and historically used as an attractive feature of fishing gear meant to lure target fishes into capture (Nguyen and Winger 2018). Some examples of lights being used in fisheries targeting Atlantic cod have shown that they are attracted to light (Bryhn et al. 2014), while other studies have shown that lights do not affect cod behaviour (Grimaldo et al. 2017; Melli et al. 2018; Blackmore et al. 2022). Light can also be used in artificial settings, to modify the physiology (e.g., Brandsen et al. 2005; Taranger et al. 2006), as well as the behaviour or distribution (Stien et al. 2014; Hansen et al. 2018; Xu et al. 2022) of fish in aquaria, which can have direct husbandry implications. In a similar study, Utne-Palm et al. (2018) found no evidence that wild-caught cod were attracted to light between 448-560 nm in an artificial setting. The findings presented here expand on the results of Utne-Palm et al. (2018) by providing further information on the effect that light has on the movement behaviour of Atlantic cod in a laboratory setting. However, the potential range of spectral sensitivity in Atlantic cod ranges from <400 to >650 nm, so other qualities of light could influence the behaviour and movements of cod. Our study could not replicate the true depth at which cod would naturally forage and was restricted to a 3 m aquarium. As such, there may not have been sufficient differentiation in the perception of light stimuli across such a short distance when presented in pairwise combination. Within these restrictions, however, the implications of the results are still relevant to fishing efforts, husbandry, and the basic visual ecology of Atlantic cod, as their tendency to approximate to one colour of light over another would have applications across these fields at that distance. Our study aimed to understand the component of behaviour associated with phototaxis, therefore, cod were not fed on days that experimentation took place, and we did not present food alongside the lighting conditions, so as not to introduce satiation levels and food-association as factors into the experimental design.

In these experiments, the right side of the tank was preferred ($p < 0.001$) and the time spent on either side of the tank was unequal. This could be due to the position of extraneous components of the tank setup. The tanks used as the holding tank and experimental arena were identical, with a seawater input flow from the left side (right side in the holding tank) at the water's surface towards the centre of the tank, and a drain in the centre of the tank floor (Figure 5). The movement of surface water from this inflow could have caused an avoidance of this area, meaning that fish spent more time in the right side of the tank. Temperature gradients can be stronger stimuli for inducing behavioural changes in fish than lighting (Pavlov et al. 2005, Stien et al. 2018), therefore although likely minimal, it is possible that minor differences in water temperature near the inflow on the left side compared to the right side of the tank may have affected the potential distribution effects of light stimuli in this study. Ideally, the water input would have been positioned in the centre of the camera's point-of-view, such that any disturbance that it caused would be evenly distributed across the two "zones" of the tank. Similarly, the soundscape throughout the laboratory was not controlled for in our experiment, lending variation in noise from areas of the space that may have altered behaviour in our focal individuals. Cod are able to hear sounds in their environment and use noise to forage (Hawkins and Picciulin 2019; Hawkins and Popper 2020), as well as to communicate with conspecifics during courtship and spawning behaviour (Rowe and Hutchings 2006; Hawkins and Picciulin 2019), and anthropogenic noise is known to elicit a startle response in larval Atlantic cod (Nedelec et al. 2015), therefore their sensitivity to background anthropogenic noise should have been considered for its contribution to differences in observed behaviour throughout this experiment.

The activity of fish, modelled as mean absolute deviation of distance travelled, also significantly affected the time that fish spent in the left or right zones. However, since the distance measure was calculated using the distance travelled by a fish throughout the entire 60-minute session, this differs from the fish's behaviour within the first minute of each trial.

The repeatability of behaviour of Atlantic cod in a laboratory setting has been studied greatly (Meager et al. 2018), in both juveniles (Beukeboom et al. 2022) and adults (Zimmermann et al. 2012; Reynisson and Ólafsdóttir 2018; Villegas-Ríos et al. 2018) as measures of animal personality. Prior studies have found consistent individual differences in behavioural traits of Atlantic cod for both boldness and exploration (Zimmermann et al. 2012; Reynisson and Ólafsdóttir 2018; Villegas-Ríos et al. 2018; Beukeboom et al. 2022). Within-individual repeatable behavioural differences in animals affect spatio-temporal dynamics (Spiegel et al. 2016), and thus management and conservation strategies for the species (Collins et al. 2022). With Atlantic cod there are direct implications of movement behaviours for interactions with fishing gears (Olsen et al. 2012; Bøe 2013) and space use, i.e., distribution and migration (Thorsteinsson et al. 2012; Reynisson and Ólafsdóttir 2018; Villegas-Ríos et al. 2018; Beukeboom et al. 2022). The findings of this study show variation among individuals, and that individual cod behaved consistently across trials ($R^2 = 0.794$), as well as across the two experimental sessions ($R^2 = 0.472$), showing aspects of animal personality. The repeatability of movement behaviour within individuals in this study validates the experimental design and is compatible with the study by Villegas-Ríos et al. (2018). Consistent individual behaviours have implications for the outcomes of the study, in that light preference may have been influenced by an individual's tendency to roam. Highly active individuals, particularly if they had been considered to be exploring the arena as a novel environment, may not have spent more time in one zone near a certain colour of light over another and spent the entirety of the trials moving throughout the arena. The reverse is also true, in that inactive individuals may have preferred one light quality over another, but this preference was masked by their inactivity. Due to the high repeatability of behaviour across trials and across sessions within individuals, light had no effect on the measure of activity, and that activity was consistent across all combinations of light colours presented. If this experimental arena is considered a novel environment, it would be of interest to perform a similar test for proximity to light in a familiar environment or *in situ* within the natural home range of a wild population (e.g. Thorsteinsson et al. 2012).

The design of these experiments was created without insight into whether wild-caught cod from the Northwest Atlantic would behave consistently across trials nor what degree of activity differences would be observed between individuals. Therefore, the behavioural tests were designed in such a way that reproducible preferences in light conditions could be captured given any possible consistent among-individual variation in movement behaviours. The experimental tank is not unique enough from the holding tank in which the sample population is housed to constitute a novel environment, therefore the measured trait is solely referred to as "activity", since "exploration" requires a novel environment (Réale et al. 2007). Though these two traits are often referred to in conjunction with one another as a behavioural syndrome, movement behaviours differ in Atlantic cod when in a known vs. unknown environment (Beukeboom et al. 2022). The experimental design also included randomizing the sampling of subjects, randomizing the order and side of the presentation of light variables, and testing all individuals in two sessions at different times. This approach allows for variation in behavioral attributes (heterogeneity) in the sample population while being tested in a single laboratory setting and still accounts for the influence of time and experience of the individuals tested (von Kortzfleisch et al. 2020).

No covariates were assessed to explain the repeatability measurements but given that all individuals were caught simultaneously and housed in the holding tank for nearly a year prior to experimentation, randomization justifies the covariation. Cod show individual differences in behaviour linked to sex (Hutchings et al. 1999) and by association, size (Villegas-Rios et al. 2018), which can influence personality traits including movement behaviours linked to activity and exploration (Chapman et al. 2013). Cod were tested alone, in an experiment where sex and other physiological characteristics were not considered, so we acknowledge this as a potential oversight and encourage its investigation in future research. The length of time that cod were kept in housing may also have affected their behaviour, as they had nearly a year to acclimate and adjust to their holding tank. However, since all fish were kept for the same length of time, and the parameters (temperature, lighting, feeding regime, etc.) remained consistent both during their time spent in the holding tank and while being tested, we do not see this as an issue with regards to the experimental design.

We hypothesized that Atlantic cod would show distinct behavioural differences when presented with different light qualities in an artificial setting and predicted that cod would spend more time in proximity to light conditions than the control, and to green light over other colours. Instead, these results are consistent with similar studies, indicating that cod do not modify their behaviour or show a preference when presented with various qualities of light. This result has direct application to the Newfoundland and Labrador inshore stewardship ground-fishery and elsewhere, in that overall, light has very little effect on the movement and foraging behaviours of Atlantic cod, so modifying the light environment near fishing gears through gear modifications or attachments may not be an effective strategy of exploiting their behaviour.

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Competing Interests

The authors declare there are no competing interests for this research.

Author Contributions

R. J. B.: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Visualization, Project Administration, Funding Acquisition.

W. A. M.: Conceptualization, Methodology, Resources, Writing – Review and Editing, Funding Acquisition.

R. F. B. H.: Software, Data Curation, Writing – Review and Editing.

P.-P. B: Conceptualization, Methodology, Resources, Writing – Review and Editing, Funding Acquisition.

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Data Availability

The script written for tracking fish is available here: https://github.com/rfh473/fish.

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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Supplementary Material

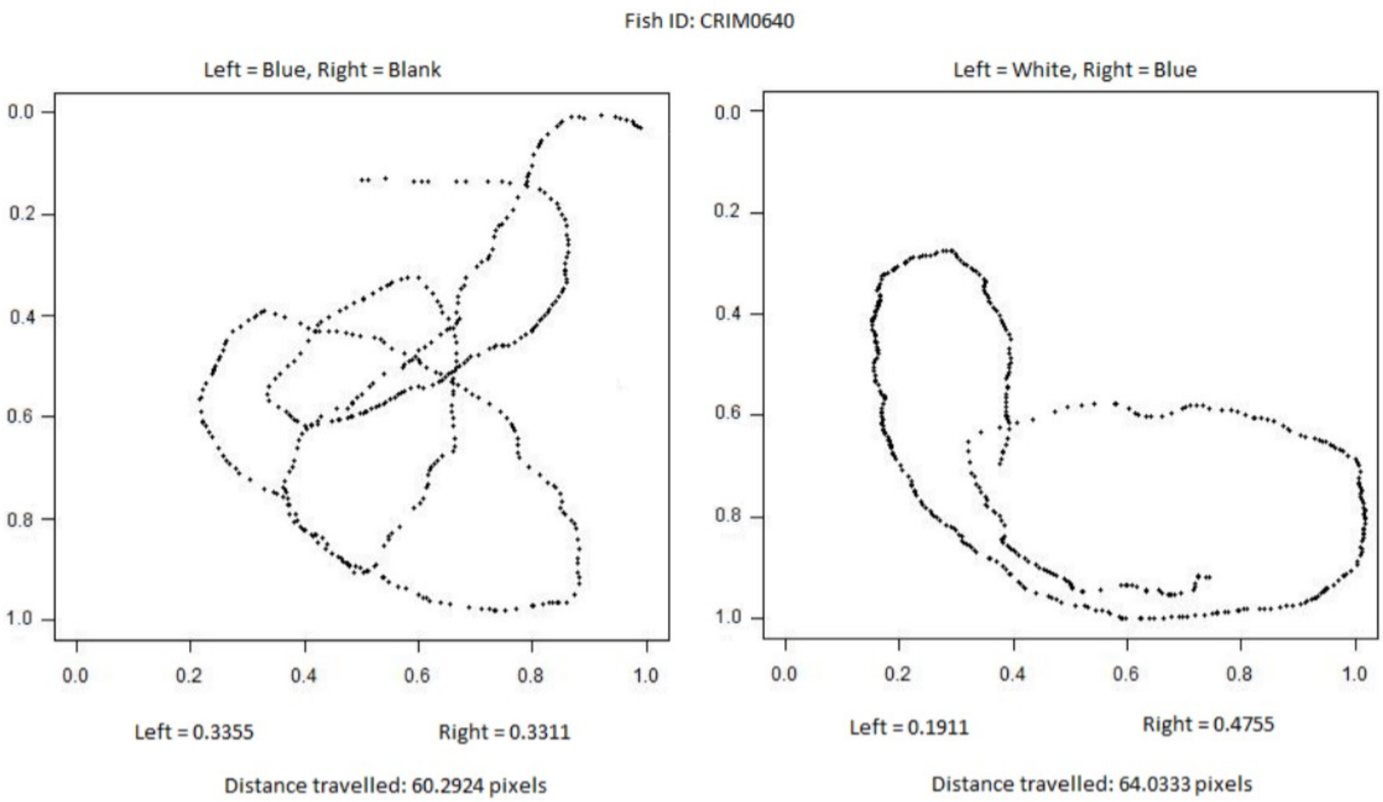

Figure S1. Two example movement paths of Atlantic cod "CRIM0640" around the arena during experimental trials within one session. X- and Y-axes represent the coordinates of the video frame from 0 to 1, points represent the centre of mass of the cod for each frame of video. The proportion of time spent in the left and right zone are labelled below each graph.

A subsample of seven wild-caught Atlantic cod were measured for body length and mass by staff members of the Joe Brown Aquatic Research Building prior to experimentation and the values were provided as follows: the mean fork-length to the nearest 0.1 cm = 53.5 cm FL, and mean body mass to the nearest gram = 1,527 g.