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Short communication

A preliminary study into the effectiveness of stroboscopic light as an aversive stimulus for fish

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Abstract

To reduce fish mortality in nature it is often necessary to expel individuals from areas modified by humans (e.g., dams) where they could be injured or killed. We tested the effectiveness of stroboscopic light as an aversive stimulus for zebrafish (*Danio rerio*) in captivity. We used six groups of five zebrafish with the groups subjected to three sequential experimental treatments, which each lasted 7 days. Treatment 1 (Control): the fish were maintained in an aquarium, which was divided physically into left and right sides. Treatment 2 (Enrichment): the left side of the aquarium was enriched with a variety of objects. Treatment 3 (Stroboscopic light): a stroboscopic light was aimed at the enriched side (left) of the aquarium. In each treatment, fish were acclimatized for 3 days and then filmed for 90 min on four consecutive days. Using scan sampling with instantaneous recording of behaviour (30 s interval) we recorded the number of fish visits to each side of the aquarium. Fish did not show any side preference in Treatment 1 ($P > 0.05$), during Treatment 2 they preferred the enriched (left) side of the aquarium ($P < 0.05$) and in Treatment 3 they again showed no side preference ($P > 0.05$). A Friedman's test confirmed that avoidance of the stroboscopic light lasted until almost 22.5 min. These results suggest that stroboscopic light can be temporarily effective in removing fish from a preferred area.

Keywords: Avoidance behaviour; Stroboscopic light; Environmental enrichment; Aversive stimulus; Fish behaviour

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1. Introduction

Fish use a wide range of information in their external environment to guide themselves, such as light, sound, water flow and temperature. Although, bioengineering research in the early days of dam building developed successful combinations of physical structures and hydraulics to attract and pass adult fish upstream through fish ladders, similar success with guiding and passing juveniles in dam facilities before they enter turbine intakes or suction tubes (i.e., so-called ''areas of risk'') has not been achieved (Coutant, 2001a,b).

The unwanted injury (e.g., lacerations) and mortality of fish due to the activities of dam facilities are, unfortunately, a common problem (Goetz et al., 2001; Maiolie et al., 2001). It is possible to enhance physical barriers (e.g., screens) with behavioural barriers that guides a fish away from danger to safety (Mueller et al., 2001). Behavioural barriers have typically included lights, sound and electric fields (Patrick et al., 2001). Light is a stimulus to which fish frequently respond with avoidance (Marchesan et al., 2005). However, the nature of the response (avoidance or attraction) may be inconsistent and depends upon the state of acclimation of fish to ambient lighting and the intensity of the 'test' light (Patrick and Christie, 1985; Ploskey and Johnson, 2001; Taft et al., 2001; Marchesan et al., 2005).

A stroboscopic light is a device emitting extremely rapid and brilliant flashes of light. The problem with previous studies on the effectiveness of stroboscopic light as an aversive stimulus is that researchers simply measured whether fish would avoid this stimulus (Sager et al., 1987; Welton et al., 2002). A more appropriate test would be to show that this stimulus has the ability to remove fish, for a significant period of time, from an area for which they have previously shown a preference (see Dunlop et al., 2005).

In this study a model species was used to substitute for endangered species (e.g., *Paulicea jahu*) which are affected by river dams in Brazil. The use of a model species is common in the first stages of a conservation project where it is considered unethical to use an endangered species before a new methodology has been tested (Hardy, 1996). In this experiment we created a preferred area in an aquarium by using environmental enrichment (Young, 2003), and then evaluated if fish would stay in this area once a stroboscopic light was applied. Furthermore, our study investigated habituation to stroboscopic light, a factor overlooked in previous studies.

2. Material and methods

2.1. Animals, aquariums and handling

The study was conducted at the Pontifical Catholic University of Minas Gerais, Brazil. We used 30 zebrafish (*Danio rerio*) which were born in captivity, and acquired from an aquarium shop. They measured approximately 38 mm in total length during the experiment. Neither the specific age nor sex of the fish was known, nor were individuals tagged for identification. The 30 experimental fish were divided into six groups of five individuals (test groups 1 to 6) and placed into 17-L holding tanks (300 mm in diameter and 340 mm deep). The water in the tanks was initially treated with an anti-chlorine agent, then aerated and warmed. Water temperature was maintained at approximately 25 °C by a 10 J/s heater. The fishes were fed a balanced, commercial fish diet (Nutral Premium, Madrid, Spain) two times per day (a minimum of 1 h before and after experimentation).

The experimental aquarium measured 300 mm × 400 mm × 600 mm and had a capacity of 72 L. It was divided into two compartments using a plastic mesh with 11 mm hole size (diameter), which allowed fish and water to pass freely between both sides. The same water treatment and heating as per the holding tanks

were applied. Illumination was provided by a 38 lx fluorescent lamp (OSRAM Universal, Munich, Germany) installed in the cover of the aquarium, the photoperiod was adjusted to a 12:12 h light: dark cycle (lights on at 07:00 h). Both sides of the test aquarium were visually divided into equal sized quadrants using white tape on the outside of the aquarium to form nine quadrants on each side. We labelled the quadrants on the left as L1 to L9 (starting at top left then moving from left to right and from top to bottom), and the right quadrants were labelled R1 to R9 (starting at top left then moving from left to right and from top to bottom).

2.2. Experimental protocol

A test group was transferred from its home tank to the experimental aquarium 3 days before we started behavioural observations, to allow them to acclimatise. We undertook 24 observation periods (six groups × 4 days) for each of the three treatments. Each observation period lasted for 90 min during which the behaviour of the fish was recorded using a video camera (SONY DCR-HC1000, Tokyo, Japan), which was programmed to film 2 s of behaviour at 30 s intervals (i.e., time-lapse). All filming was conducted in the morning between 09:00 h and 10:30 h.

Three sequential experimental treatments were applied. Treatment 1 (control): the test aquarium was identical on both sides, with no environmental enrichment. Food was distributed, in equal amounts, on both sides of the aquarium. This treatment was used as a control to test whether the fish had an inherent left–right side bias.

Treatment 2 (enrichment): identical to Treatment 1, but the left side of the experimental aquarium was enriched with some artificial plants, gravel and two PVC tubes (approximately 100 mm in length and 30 mm in diameter). Food was distributed only on the left side of the aquarium. The objective of this treatment was to determine if it would be possible to create a side preference in the fish.

Treatment 3 (stroboscopic light): identical to Treatment 2, but a stroboscopic light (Coghlan's emergency light strobe, Winnipeg, Canada, 60 flashes/min) was aimed at the enriched (left side) of the aquarium about 50 mm from the front (in front of quadrant L8, which was chosen because of the high frequency of fish visiting this quadrant in the enrichment treatment; see Fig. 1). The purpose of this light was to act as an aversive stimulus (Ploskey and Johnson, 2001). The light was left on continuously during the 90 min of observations.

Visits to quadrants were transcribed onto checksheets from the video tapes. To analyse quadrant visits we used scan sampling with instantaneous recording of behaviour with an interval of 30 s to score the number of fish in each quadrant (Martin and Bateson, 1993).

2.3. Data analysis

For each group per treatment we calculated mean frequency values for quadrant visits. An Anderson–Darling test was used to test whether our data had a normal distribution, which they did not, and, therefore, all statistical tests applied were non-parametric. Wilcoxon matched-paired tests were applied to test for side preferences within each treatment. Spearman's Rank Correlations were made for each treatment, between mean frequency of visits to quadrant L8 (per test) and cumulative time (in 7.5 min intervals) of the treatment. For each treatment, cumulative time was divided-up into 7.5 min intervals and the number of visits to quadrant L8 during these time intervals was compared between the three treatments using a Friedman's test and *post-hoc* Tukey tests.

3. Results

In Treatment 1, fish expressed no side preference ($W = 5.0$; $N = 6$; $P = 0.834$), in Treatment 2, fish showed a preference for the enriched (left) side (67.91%) ($W = 0.0$; $N = 6$; $P < 0.05$) and in Treatment 3 fish, again, showed no side preference ($W = 4.0$; $N = 4$; $P = 0.208$).

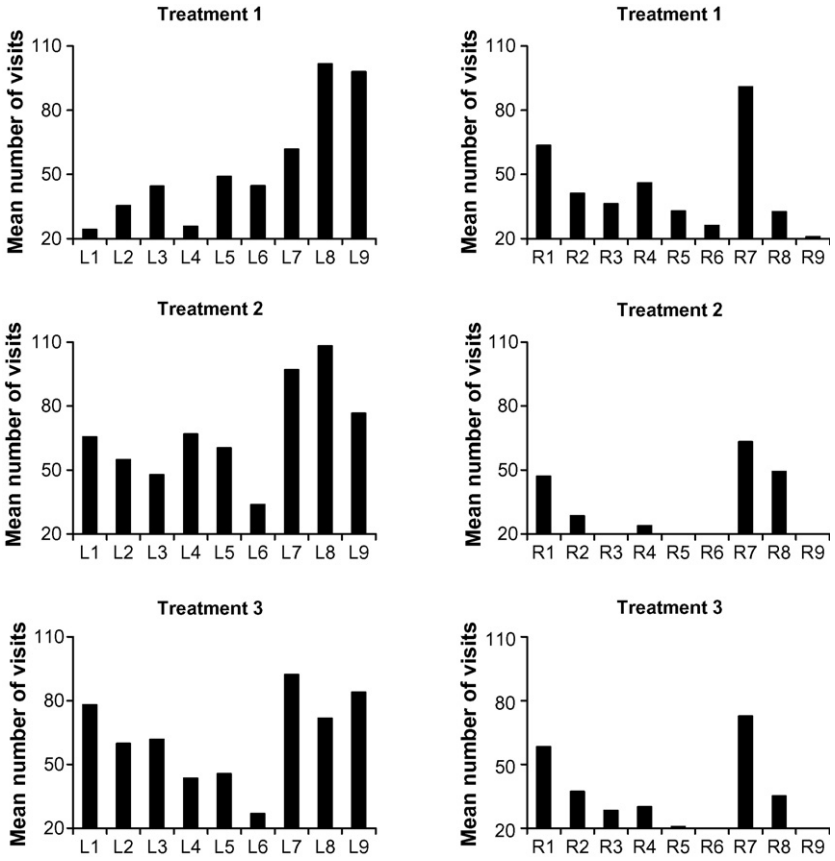

Fig. 1. Mean visitation rates (per 90 min observation) by six groups of five zebrafish to each aquarium quadrant (on the *x*-axis L = left side and R = right side) during three consecutive treatments (Treatment 1 = control; Treatment 2 = enrichment; Treatment 3 = stroboscopic light). The aquarium was divided into left and right side, and each side was subsequently divided into nine quadrants (numbered 1–9 from starting at top left (1) and finishing at bottom right (9).

Table 1
Friedman's and *post-hoc* Tukey tests results comparing visits (mean total for each 7.5 min interval) to the quadrant where the stroboscopic light was focussed (L8) in relation to cumulative time of each treatment (until there was no significant difference)

Time (min)	Friedman			Comparisons (Tukey test)			Mean			Median		
	Fr	*P*	d.f.	T1 × T2	T1 × T3	T2 × T3	T1	T2	T3	T1	T2	T3
7.5	11.44	0.0033	2	$P > 0.05$	$P < 0.05$	$P < 0.05$	8.0	8.2	4.17	6.0	9.0	2.5
15	7.52	0.0233	2	$P > 0.05$	$P > 0.05$	$P < 0.05$	7.6	12.0	4.92	6.0	10.5	3.5
22.5	5.77	0.055	2	$P > 0.05$	$P > 0.05$	$P > 0.05$	7.46	9.72	5.83	4.5	9.5	4.5

Fr = Friedman test value, d.f. = degree of freedom, T1 × T2 = *P*-value for comparison between Treatment 1 (Control) and 2 (Enrichment), T1 × T3 = *P*-value for comparison between Treatment 1 (Control) and 3 (Stroboscopic light), T2 × T3 = *P*-value for comparison between Treatment 2 (Enrichment) and 3 (Stroboscopic light); T1 = Treatment 1; T2 = Treatment 2 and T3 = Treatment 3.

Fig. 1 shows that there was a high frequency of visits to quadrants L7, L8 and L9, especially in Treatment 1. On the right side of the aquarium the preference appeared to be for quadrant R7. Spearman's Rank Correlations between mean frequency of visits to quadrant L8 and cumulative time showed no significant result for Treatment 1 ($r_s = 0.406$; $N = 12$; $P = 0.191$) or Treatment 2 ($r_s = -0.329$; $N = 12$; $P = 0.297$), but a significant and positive correlation for Treatment 3 ($r_s = 0.890$; $N = 12$; $P < 0.001$), showing that only in the stroboscopic light treatment did fish initially avoid the light at quadrant L8. The Friedman's test showed that in Treatment 3 fish were deterred from entering quadrant L8 until almost 22.5 min into the test (Table 1).

4. Discussion

In this study we showed that an induced area preference in zebrafish can be temporarily reduced through the application of stroboscopic light. Therefore, stroboscopic light can be considered to be an aversive stimulus for zebrafish. However, we also showed that fish habituated to this stimulus in approximately 22.5 min of continuous application. During the first 22.5 min of exposure to the stroboscopic light we did not observe 100% avoidance of the area to which the light was applied. It is possible that varying light intensity, wavelength or flash-rate may produce a stronger behavioural response in fish, which may also reduce the problem of habituation (Patrick and Christie, 1985; Sager et al., 1987).

Our tests provide conservative estimates of stroboscopic light effectiveness because they were conducted under artificial lighting (see Ploskey and Johnson, 2001). The insides of dam structures are normally completely dark and this may enhance the effectiveness of stroboscopic light as an aversive stimulus. However, high water turbidity may reduce the effectiveness of such a stimulus (Amaral et al., 2001). The other important consideration is the natural behaviour of fish; the species we tested is considered to be diurnal but with a preference for dark hiding places (Serra et al., 1999). It may be that stroboscopic light would be more effective with strictly nocturnal species of fish (Welton et al., 2002). Amaral et al. (2001) conducted tests in an experimental aquarium and reported that Chinook salmon (*Oncorhynchus tshawytscha*) exhibited strong avoidance of stroboscopic lights only during night time. Interestingly, it is nocturnal species of fish that suffer the highest rates of injury inside dam structures (Godinho, unpublished data).

5. Conclusion

The efficacy of our stroboscopic lights in moving fish away from a 'risky area' remains to be tested in a real life situation. Here we have shown that, for a limited period of time, stroboscopic light can deter fish from entering a previously preferred area. However, stroboscopic light in our experiment was not 100% effective in deterring fish from entering a preferred area. This suggests that we need to investigate characteristics of stroboscopic light such as flash frequency, and that to remove all fish from areas of risk a combination of behavioural and other barriers may be necessary.

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