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RESEARCH ARTICLE

The response of anguilliform fish to underwater sound under an experimental setting

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Abstract

Traditional physical screens designed to prevent fish entering dangerous areas (e.g., turbine intakes) can have negative impacts due to impingement or mechanical abrasion at high velocities. Behavioural deterrents may provide an alternative approach to screening. This study investigated the potential for a continuous broadband sound to modify the behaviour of two endangered species of anguilliform fish: European eel (*Anguilla anguilla*) and river lamprey (*Lampetra fluviatilis*). Experiments were conducted in an experimental channel. Eel and lamprey were, respectively, released upstream and downstream of an "acoustic maze". A single individual released per trial encountered two successive chambers that offered a choice of passage through either an ensonified or a control (ambient noise only) corridor with a speaker turned off. Two possible configurations were tested to control for any lateral bias with positions of the activated speakers reversed. The influence of treatment, chamber, and configuration on route selection, rejection, and time to pass were tested. No influence of any of the three factors on route selection was observed for eel. River lamprey tended to pass through the ensonified corridor more often under Configuration 2 but only in the first chamber. Both species were more likely to reject the ensonified corridors than the controls, and the time taken to pass these routes was greater for those that did so. For eel, the variation in time to pass was greater for the non-migratory (yellow phase) life stage. Although the acoustic signal used in this study influenced fish behaviour, the response observed would likely be insufficient to induce a strong deterrent effect in the field if used in isolation.

KEYWORDS
Anguilla, acoustic, deterrent, hydropower, *Lampetra*, screens, sound

1 | INTRODUCTION

Many populations of diadromous anguilliform fish have experienced substantial declines over recent decades. For example, since the 1980s, recruitment of the catadromous European eel (*Anguilla anguilla*) has reduced by nearly 90% throughout Europe (European Inland Fisheries Advisory Commission, 2006; Aalto et al., 2015; Bevacqua et al., 2015). Dekker (2003a) reported a reduction in the fisheries landings in the preceding two decades and suggested this to be consistent with a decline in the continental stock (Dekker, 2003b). Similarly, anadromous river lamprey (*Lampetra fluviatilis*) populations across Europe have suffered severe declines over the past 30 years (Kelly & King, 2001; Masters et al., 2006), with human activity identified as one of the main causes (Kelly & King, 2001). Multiple factors have been suggested to explain the collapse of populations of these species, including climate change (Bonhommeau et al., 2008), over fishing (eel—Feunteun, 2002; lamprey—Masters et al., 2006), pollution (eel—Maes et al., 2004; lamprey—Kelly & King, 2001), and fragmentation of habitat due to the placement of river infrastructure (eel—Kettle et al., 2011; lamprey—Igoe et al., 2004).

In response to concerns about declining populations, multiple pieces of legislation have been enacted to protect these species. These include the EU Eel Regulation (EC 1100/2007) that establishes a framework for the protection and sustainable use of stocks in the member states. It requires them to achieve escapement equivalent to 40% of the silver eel biomass expected to be produced under pristine conditions that would occur in the absence of human activity. Further, European eel are listed under Appendix II of the Convention on International Trade in Endangered Species that places strict restrictions on their trade. Lamprey are listed under Annex II and V of the EU Habitats Directive (EC 92/43/EEC) that requires member states to contribute towards ensuring biodiversity through conservation of natural habitats and of wild fauna and flora. Species listed benefit from the implementation of Special Areas of Conservation (Annex II) and measures to manage their exploitation (Annex V). River lamprey are also protected under Annex III of the Bern Convention. Despite this legislation, the major anthropogenic threats to population persistence remain.

River infrastructure, such as dams and weirs, negatively impact populations of diadromous fish, many of which are of high commercial interest, by delaying their migration or blocking access to critical habitats (Kemp, 2016). Furthermore, fish are likely to suffer physical injury and mortality if they enter dangerous areas such as turbine intakes (Calles et al., 2010) or water abstraction points (Piper et al., 2013). Physical screens are installed in an effort to reduce entrainment and guide fish towards alternative routes (e.g., bypass systems).

Although developed to mitigate environmental impacts, screens carry their own risks. If poorly designed, or incorrectly installed, and when water velocities are high relative to swimming capability, fish can become impinged on the screen face where they suffocate if unable to escape. Moreover, small-bodied fish can be entrained through the screens if mesh/gap size is too large. Because of their elongated morphology, low aspect ratio of their body cross section, and relatively weak burst swimming capabilities, anguilliform fish are at greater risk of impingement and entrainment than many other species (Russon et al., 2010). In the face of this challenge, one option to better protect anguilliform fish is to install very fine-spaced screens (e.g., 2-mm slot width for juvenile eel; Sheridan et al., 2014), which require larger surface areas to maintain equivalent through flows of water at greater costs to the owners and operators (e.g., water supply and electricity-generating companies) of the associated infrastructure. Devices that employ behavioural stimuli (e.g., acoustics, lights, bubbles, hydrodynamics, or multiple stimuli in combination; Ruebush 2011) to induce an avoidance response and deter fish have the potential to enhance the efficiency of traditional physical screens if used in combination (Deleau et al., 2019).

The development of behavioural deterrents for anguilliform fish, as for other fish species, is not new, and devices that are based on a range of stimuli, such as infrasound (Sand et al., 2000; Sand et al., 2001) and strobe lights (Patrick et al., 1982), are commercially available but tend to be used in isolation. Unfortunately, in many instances, they have been advanced through a process of trial and error, and their effectiveness seldom quantified by robust experimental studies; when evaluation has taken place, the results are often contradictory and inconclusive (e.g., see contradictions between Sand et al., 2000, MacNamara et al., 2012, and Piper et al., 2018 for eel response to infrasound under field settings). As a result, behavioural deterrents are generally considered less efficient than physical and mechanical screens. Nevertheless, behavioural screening devices remain appealing, should high efficiencies be attainable, as they represent a much sought-after non-contact solution to the challenge of developing sustainable water and electricity-generating infrastructure systems in a cost-effective manner.

For European eel, the use of strobe lights has recently returned promising results (Elvidge et al., 2018), whereas infrasound (<20 Hz) is suggested to influence their downstream swimming trajectories (Sand et al., 2000, Piper et al., 2018). For lamprey, however, there is little published information on their response to behavioural deterrents.

Given the potential to use acoustics as a deterrent, this study aimed to investigate the possibility of developing a device that would deter multiple species of anguilliform fish (eel and lamprey) by testing their response using an acoustic maze that consisted of a series of two chambers, each with a choice of route through either an ensonified or control (ambient background sound only) corridor. The experiment was inspired by the classical Y-maze design used in studies that offer a choice (e.g., Olsén 1985). To achieve our aim, we attempted to answer three questions related to the influence of a continuous broadband sound (60 to 1000 Hz) on fish behaviour: (a) does sound affect selection of otherwise physically equivalent routes?; (b) does sound exposure influence probability of rejecting the selected route?; and (c) does sound impact the time taken to pass the selected route? The results obtained will be of value to those interested in designing behavioural deterrents for anguilliform fish in an effort to enhance their conservation.

2 | MATERIALS AND METHODS

2.1 | Fish collection and maintenance

Silver phase European eel (N = 81, Total Length: 300–782 mm, median = 547 mm; mass: 29–850 g, median = 261 g) were caught during their seaward migration using a fixed eel trap on the River Stour (50°46'31.6"N, 1°54'38.1"W), United Kingdom, in December 2014. Migrating adult river lamprey (N = 82, Total Length: 312–419 mm, mean = 368.2 mm, SD = 20.34; mass: 51–109 g, mean = 80.6 g, SD = 13.45) were trapped in the River Ouse (53°53'26.2"N, 1°5'36.8"W), United Kingdom, by a commercial fisherman in December 2014. Non-migratory (yellow phase) eel (N = 67, Total Length: 332–681 mm, mean = 447.5 mm, SD = 70.61; mass: 56–617 g, median = 133 g) were collected by electric fishing a small stream near Funtington (50°50'47.9"N, 0°50'45.5"W), United Kingdom, at the beginning of March 2015. Fish were transported to the International Centre for Ecohydraulics Research Facility, University of Southampton (50° 57'42.6"N, 1°25'26.9"W), in two large transportation tanks filled with aerated river water. The fish were maintained in five separate holding tanks (silver eel and river lamprey: 4 × 1,000 L outdoor tanks; yellow eel: 1 × 2,000 L indoor tank) equipped with individual filtration systems and separate air pumps. Water was monitored daily and maintained through regular water changes (50% weekly) using dechlorinated tap water (pH = 7.8, Nitrate: <40 ppm). Mean water temperature was 8.9°C (*SD* ± 0.8°C) for the lamprey and silver eel and 12.1°C (*SD* ± 0.6°C) for the yellow eel. As yellow eel were expected to continue feeding during this phase of their life cycle, they were daily fed live *Dendrobaena* worms.

2.2 | Experimental setup

The maze comprised a series of two chambers to enable the number of replicates to be increased by twice presenting each fish with a choice of route. This is in line with the principles of ethical research, which dictates that efforts should be made to reduce the numbers of individuals used, an ethos that is especially important when studying endangered species. This design, however, requires the importance of any influence of repeated exposure to be evaluated, for example, because processes such as learning or habituation may confound the results obtained. Furthermore, to control for any lateral bias (e.g., of flume characteristics or fish behaviour), tests were conducted under two configurations: 1) right and left hand channels ensonified in Chambers 1 and 2, respectively; and 2) the reverse. Each individual fish was tested under both configurations.

Both eel and lamprey can encounter a vast range of riverine conditions during their migration: from shallow and narrow natural streams to heavily engineered channels and large deep estuaries. The conditions created in this experiment, in terms of water depth and flow rate, are representative of a shallow and narrow stream environment that both species inhabit or an engineered channel typically encountered during migration (and which may coincide with water extraction points). Our experimental setting enabled the key factors of interest (acoustics and hydrodynamics) to be manipulated, whereas confounding factors are controlled.

A concrete block experimental channel (7.16 m long, 1.39 m wide, and 0.56 m deep) was constructed within an existing outdoor flume (60 m long) at the International Centre for Ecohydraulics Research (Figure 1). The channel was divided into two identical chambers (3.15 m long, 1.39 m wide), each comprised two corridors (2.25 m long, 0.37 m wide) separated by blocks. A 0.52-m wide entrance/exit was located centrally at the start/end of each chamber, and wire–mesh release/recapture enclosures (13 × 13-mm mesh, 1-mm gauge) were installed at the ends of the experimental area to facilitate safe introduction and retrieval of fish at the start and end of each trial.

Fish movements were recorded using eight closed-circuit television cameras (two cameras per corridor) with integrated infrared light units (AV-TECH 245 Sony Effio 580TVL charge-coupled device) mounted 1.5 m above the maximum water level (0.5 m). Six additional 15.0-W infrared lights provided additional illumination to enhance the contrast of the video recordings.

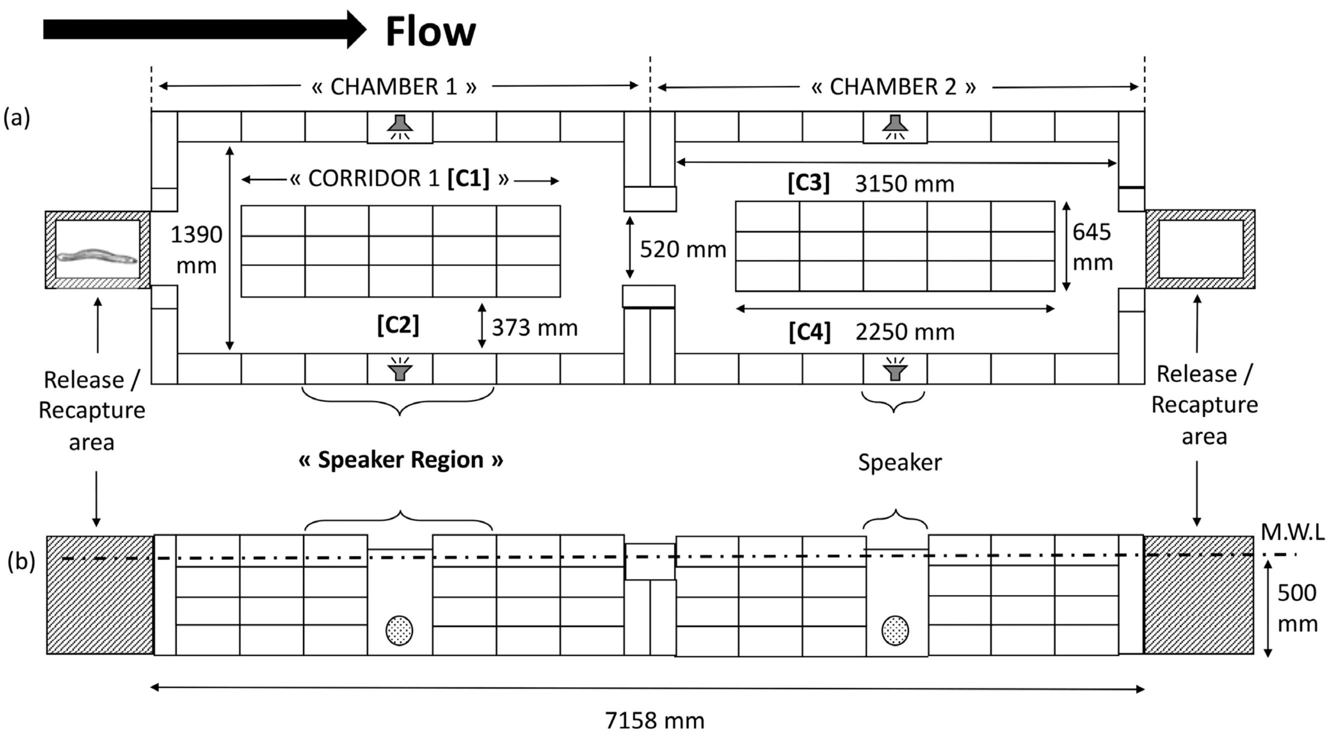

FIGURE 1 An "acoustic maze" installed in an outdoor recirculating flume at the International Centre for Ecohydraulics Research, University of Southampton. During experimental trials, the acoustic maze twice presented European eel and river lamprey with a choice of route through either an ensonified or control corridor. Plan (a) and elevation (b) views are depicted with the maximum water level (M.W.L.) represented by a dashed line. The grey boxes at both ends represent the release/recapture enclosures that restrained the fish within the experimental area of the maze

A constant flow (depth: 48–50 cm; mean velocity: 0.1 m/s, *SD* ± 0.01 m/s) was maintained using three centrifugal pumps. Depth and velocity was measured using a rule and an electromagnetic flow meter (Valeport, 801—flat) that recorded over 10 s.

2.3 | Acoustic stimuli

The stimulus selection was based on an audiogram constructed for European eel (Jerkø et al., 1989) that indicated sensitivity between 60 and 400 Hz, with a maximum sensitivity at approximately 80 Hz. There is currently no available audiogram in the literature for river lamprey, but Teague and Clough (2013) speculate that neither low frequency nor ultrasound deterrents would be effective for lamprey as they are hearing non-specialists. Nevertheless, Popper (2005) encourages further investigation into the hearing capabilities of lamprey and their behavioural response to sound.

During a pilot study, a series of experiments were conducted to define an appropriate acoustic stimulus relevant for the test species. Fish response to a series of specific test tones was assessed, and behaviours were compared with those observed under a control in which there was no externally generated sound.

Preliminary results indicated that sounds with frequencies that ranged from 60 to 2,000 Hz may have the potential to deter fish. In the current study, a continuous broadband (60—1,000 Hz) sound was generated by four underwater speakers (ElectroVoice UW30), one installed in each corridor. During trials, the set-up was divided into one of two test configurations in which one of two speakers in each chamber was turned on. The position of the ensonified corridor alternated between chambers under each configuration (Figure 2). The sound production system consisted of a laptop (Dell© Latitude E6430) linked to a National Instruments data acquisition box (National Instruments© USB-6251) driving a power amplifier (SkyTronic© Mini AV Digital Surround Amplifier 103.100) to which the speakers were connected.

To understand the sound field within the corridors containing the speakers, one can consider each corridor as a waveguide because their length is very much greater than their width. The cross section through a corridor was bounded on the sides by concrete blocks, underneath by a concrete floor (both assumed to be acoustically rigid), and on the upper surface by air (assumed to be a pressure release boundary). The sound field in a waveguide can be represented as the sum of modes. The mode of order (n_x, n_y) has a pressure field that takes the form of the real part of

$$p_{n_x,n_y} = A_{n_x,n_y} \cos\frac{n_x \pi_x}{L_x} \cos\left(n_y + \frac{1}{2}\right)\frac{\pi_y}{L_y} e^{i(2\pi f t - k_z z)}, \tag{1}$$

where L_x and L_y are the width of the corridor (0.373 m) and the water depth (0.5 m), respectively; z defines the horizontal direction parallel to the walls; f is the frequency; and A_{n_x,n_y} is the modal amplitude. The order of the modes are the non-negative integers n_x and n_y. If c is the sound speed, then the component of the wavenumber in the z direction, k_z, for a particular mode, (n_x, n_y), is given by

$$k_z = \sqrt{\left(\frac{2\pi f}{c}\right)^2 - \left(\frac{n_x \pi}{L_x}\right)^2 - \left(\left(n_y + \frac{1}{2}\right)\frac{\pi}{L_y}\right)^2}. \tag{2}$$

This component of the wavenumber only takes on a real value for frequencies greater than the cut-on frequency of that mode, f_{n_x,n_y}.

$$f > f_{n_x,n_y} = \frac{c}{2}\sqrt{\left(\frac{n_x}{L_x}\right)^2 + \left(\left(n_y + \frac{1}{2}\right)\frac{1}{L_y}\right)^2}. \tag{3}$$

Below this frequency, k_z is imaginary, physically corresponding to a mode which is nonpropagating, that is, evanescent. Table 1 shows the frequencies of the lowest few modes for this experimental configuration and demonstrates that in the frequency band of 60–1,000 Hz, only the (0,0) mode contains any propagating energy. All the higher order modes do not propagate; they only cause pressure fluctuations that decay in evanescent manner, so their contribution to the distant acoustic field can be neglected. The (0,0) mode is characterized by a pressure field, which is uniform in the cross-corridor, x, direction.

The sound field within the experimental arena was measured using a calibrated hydrophone (Brüel & Kjær © 8105, sensitivity—205 dB re 1 V/μPa) and recorded at two depths (1 and 25 cm) between the tip of the hydrophone and the channel floor. The sound pressure levels generated within the maze ranged from 100 to 150 dB re 1 μPa (Figure 2). These levels were selected to maximize the loudspeakers output without distorting the acoustic signal. Moreover, a difference of 50 dB between ensonified and control corridors were deemed sufficient for the purpose of the experiment because they created two distinct "quiet/control" and "loud/ensonified" areas.

The sound mapping measured the sound pressure levels at each location in the maze. As the preceding discussion predicts, most of the acoustic energy from the speaker does not propagate very far in within the maze. In particular, the sound field in a corridor is close to the background noise level in the vicinity of the entrance.

The sensitivity of fish to the particle motion component of the sound field is well established (Slabbekoorn, 2016), and this component provides important directional information for fish. In our experiment, no significant standing wave fields are set-up (Figure 2), and in such travelling-wave conditions, a weak pressure field implies that the particle motion will be small. Thus, the acoustic cues available to fish when selecting a corridor by which to pass through the maze are not strong. Therefore, for the frequency range tested, energy at frequencies above 724 Hz will propagate in the (0,0) mode; below this frequency, the (0,0) mode will be evanescent (with evanescent waves decaying slowly with distance close to the cut-on frequency; Table 1).

2.4 | Experimental trials

Trials took place between February 26 and March 18, 2015 for river lamprey and silver eel (mean flume temperature: 9.8°C; *SD* ± 0.8°C)

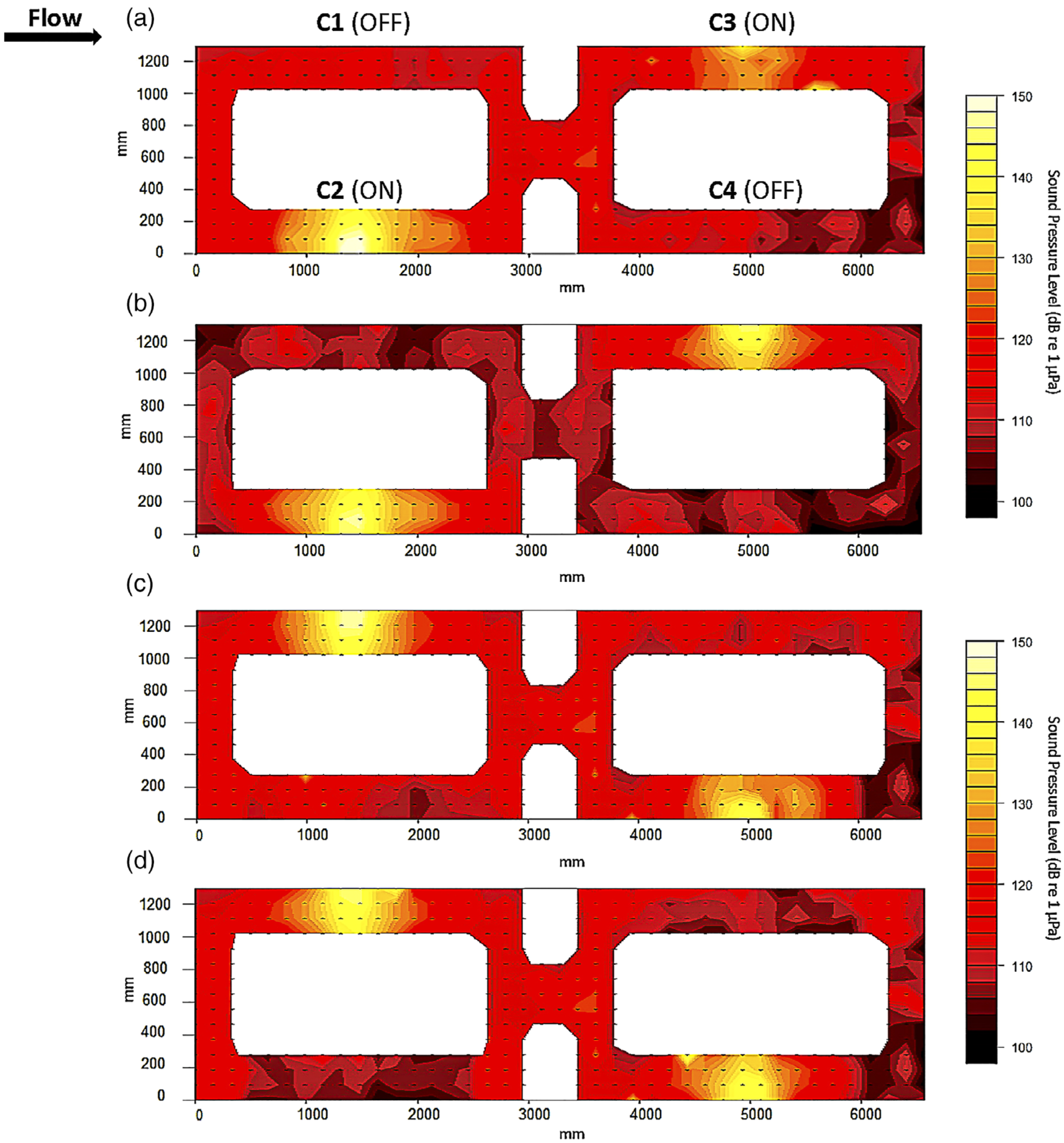

FIGURE 2 Acoustic field generated within the maze at two different depths (a and c = 1 cm; b and d = 25 cm from the channel floor) under Configuration 1 (a,b), in which Corridors (C) 2 and 3 were ensonified, and the reverse Configuration 2 (c,d) in which speakers C1 and C4 were turned on. The sound field was measured at regular spatial intervals along a grid using a hydrophone (dots indicate measurement points). White areas correspond to the concrete blocks structures delimiting the chambers and corridors [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 The mode numbers and cut-off frequencies for the lowest few modes of this experimental arrangement for a sound speed of 1447 m/s (L_x = 0.373 m, L_y = 0.5 m, water temperature 10°C)

Mode number in x (n_x)	Mode number in y (n_y)	Cut-on frequency (Hz)
0	0	724
1	0	2,070
0	1	2,171
1	1	2,911

and between March 19 and 21, 2015 for yellow eel (mean flume temperature: 10.6°; *SD* ± 0.5°).

A total of 462 trials (162 silver and 134 yellow eel; 164 river lamprey), each using a single fish, were conducted during hours of darkness (between 18:00 p.m. and 06:00 a.m.): half under Configuration 1 (stimulus activated in C2 and C3; see Figure 2) and half under Configuration 2 (stimulus activated in C1 and C4). Thus, fish were presented with the option of moving through each chamber via either an ensonified or control corridor.

All fish are exposed to both configurations, so that the number of fish used is reduced, consistent with ethical principles, while potentially increasing statistical power. Fish from each of the three groups (silver, yellow eel, and lamprey) were randomly divided into two separate batches (B1 and B2). B1 were first tested under Configuration 1, followed by B2 under Configuration 2. After a period of recovery that ranged from 2 to 10 days, B1 was tested under Configuration 2, followed by B2 under Configuration 1.

Eel were released from the upstream end of the maze, whereas lamprey were released downstream, reflecting the migratory life history (catadromy and anadromy, respectively) of the species. Trials were completed once the fish had passed through the maze or 60 min had elapsed.

Prior to the start of each trial, water temperature in the flume was compared with that of the holding tanks to ensure that temperatures never differed by more than 2°C to limit stress to the fish. During both experimental periods, the temperature difference between tanks (silver eel and lamprey: mean = 9°C, *SD* ± 0.8°C; yellow eel: mean = 12.3°C, *SD* ± 0.6°C) and the flume (silver eel and lamprey: mean = 9.8°C, *SD* ± 0.8°, and yellow eel: mean = 10.6°C, *SD* ± 0.5°C) never exceeded the 2°C limit. In addition, fish were acclimated to flume conditions in a porous container in the channel for at least 1 hr before transfer to the "release area" (Figure 1).

2.5 | Fish behaviour and data analysis

2.5.1 | Route selection

The movement of fish through the maze was recorded and video data analysed. Only trials in which fish passed the entire maze (i.e., both chambers) were included in the analysis (98.8% and 100% of the silver and yellow eel, respectively, and 85.9% of river lamprey). Passage was deemed to have occurred when the test fish passed through the entire length of a corridor. In cases when a fish passed through a single chamber multiple times (after returning in the opposite direction), only the first pass was recorded and included in further analysis. The number of passes that occurred through the ensonified corridors was calculated as a proportion of the total number of passes through both control and treatment routes. Pearson's $\chi 2$ tests were used to evaluate whether the number of passes differed with treatment, chamber, and configuration.

2.5.2 | Rejection

The number of fish that expressed a rejection, when their direction of travel was reversed, within the region immediately adjacent to the speakers ("speaker region" extending 450 mm upstream and downstream from the speaker edges—total length of 1,350 mm, Figure 1) was quantified. When rejecting, only eel were observed to swim backwards, whereas both eel and lamprey turned around and swim away from the speaker. The number of trials in which a rejection was displayed was expressed as a proportion of the total conducted. Chi-squared tests were used to assess whether the observed number of fish displaying a rejection differed between treatment, chamber, and configuration.

2.5.3 | Time to pass

The time taken to pass the speaker region was calculated. For the fish that first rejected the speaker region but eventually passed within the same chamber, the "time to pass" was calculated as the duration from first entering the speaker region to eventually leaving it, that is, the "time to pass" includes the period when the fish temporarily left the speaker region. In cases when the fish enters the speaker region in one chamber, rejects that speaker, and completes its passage in the alternative speaker chamber, the "time to pass" was calculated as the time between entering the speaker region in the first chamber and exiting the speaker region in the second chamber, that is, the time to pass between chambers is included in the time to pass.

A mixed effect model was used to interpret these data by including the following factors: (a) "wall" (fixed factor with two levels) = "speaker" or "opposite," indicating whether or not the fish passed along the wall in which the speaker was installed; (b) "sound" (fixed factor with two levels) = "on" or "off"; (c) "configuration" (fixed factor with 2 levels) = "Configuration 1" or "Configuration 2," indicating the acoustic configuration fish were tested under; (d) "chamber" (fixed factor with two levels) = "first" or "second"; (e) "batch" (fixed factor with two levels) = "B1" or "B2," indicating the batch each individual had been assigned to; (f) body "length"; (g) body "mass" (continuous factors); (h) "rejection" (fixed factor with two levels) = "yes" or "no," indicating if the fish performed a rejection during the trial; (i) "ID" (discreet random factor) identifying each individual; and (j) "day" discrete random factor defining the day on which the trial was conducted. "ID" and "day" were included in the model as random uncontrolled factors to test whether response differed among fish and day.

3 | RESULTS

3.1 | Route selection—does sound affect route selection?

More lamprey passed through the ensonified corridor of the first chamber in Configuration 2 compared with Configuration 1 (df = 1, χ^2 = 6.82, p = .009, Figure 3). Lamprey were more likely to pass through the ensonified corridor than the control in the first chamber (df = 1, χ^2 = 19.18, p = .001) but not in the second (df = 1, χ^2 = 3.18, p = .0743). Route selection did not differ between chambers. For both yellow and silver eel, route selection did not differ between treatments (Figure 3), configuration, or chamber.

3.2 | Rejection—does sound exposure influence probability of rejecting the selected route?

For silver eel and lamprey, rejection was greater when fish were exposed to sound (silver eel—Configuration 1: χ^2 = 4.86, *df* = 1, *p* = .03/Configuration 2: χ^2 = 8.62, *df* = 1, *p* = .003; river lamprey—Configuration 1: χ^2 = 4.74, *df* = 1, *p* = .03/Configuration 2: χ^2 = 5.97, *df* = 1, *p* = .01, Figure 4). For yellow eel, there was no effect of treatment under Configuration 2. Only one rejection of the control corridor was observed, that being by a silver eel. For all species/life stages, neither chamber nor configuration influenced the number of fish that exhibited a rejection.

3.3 | Time to pass—does sound impact the time taken to pass the selected route?

The time to pass the selected route was not influenced by sound for either life stage of eel or lamprey (Table 2). Body length had an effect on time to pass for silver eel (*df* = 312, *F* value = 4.05, *p* = .04) and

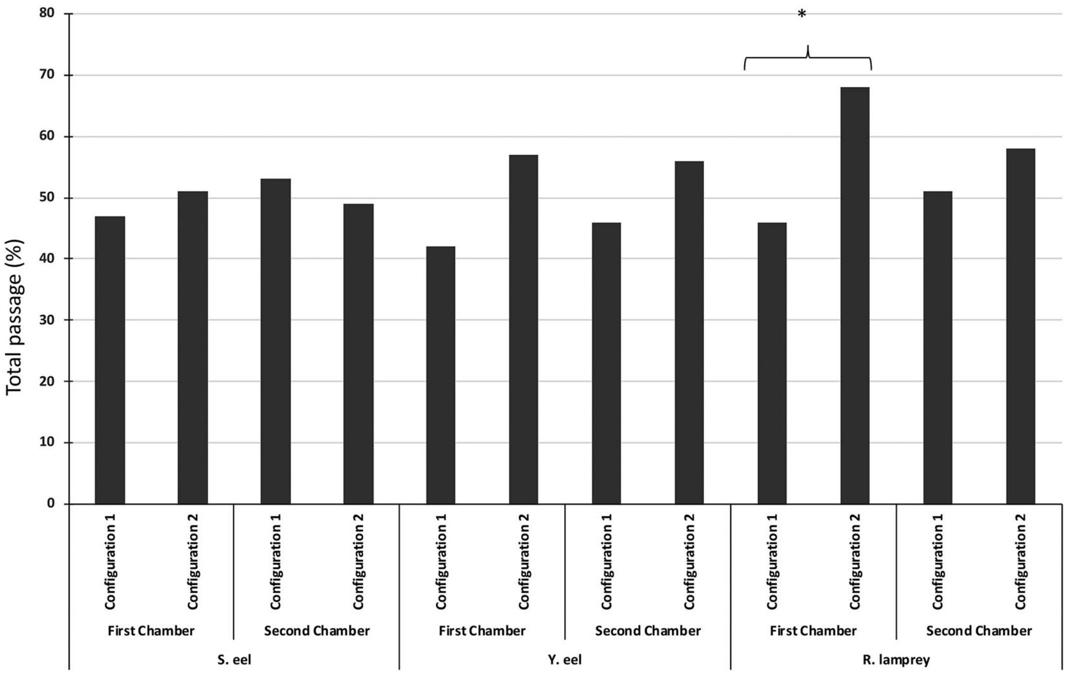

FIGURE 3 The percentage of total passes by silver and yellow eel and river lamprey through the ensonifed treatment corridor presented in two chambers of an acoustic maze under two configurations tested in which treatment route was alternated. Asterisk indicates a significant difference

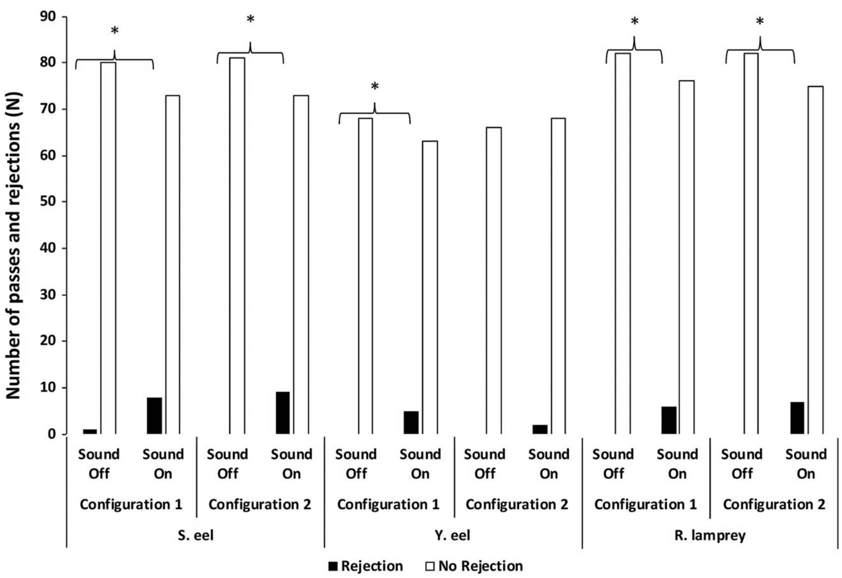

FIGURE 4 Number of passes (clear bars; all species) and rejections (solid bars) of the speaker in the treatment and control corridors under both configurations. Asterisks indicate a significant difference

TABLE 2 Results from the fixed factors model for fish passage time and percentages of variability due to the random factors (ID and Day)

Variable	Passage time		
Species	Silver eel	Yellow eel	River lamprey
Factors			
Sound	p = .7254	p = .1829	p = .5503
Chambers	p = .0628	p = .4406	p = .0492*
Configuration	p = .2778	p = .1003	p = .2490
Weight/Length	Weight (0.0564) Length (0.0452*)	Weight (0.7229) Length (0.9511)	Weight (0.5920) Length (0.0339*)
Wall	p = .0020*	p = .0334*	p = .8728
Rejection	p ≤ .001	p ≤ .001	p ≤ .001
Interindividual variability (ID)	ID = 40.4%	ID = 50%	ID = 13.4%
Variability between Days (Day)	Day = 51.9%	Day < 1%	Day = 19.9%

*Significant at the 5% level.

lamprey (df = 276, F value = 4.56, p = .03) but not for yellow eel, with longer individuals taking a greater time to pass. The time to pass for eel was the same for the two chambers. In contrast, lamprey passed more rapidly through the first chamber than the second (df = 276, F value = 3.91, p = .05). The time to pass was not influenced by configuration for either eel or lamprey.

Both life stages of eel tended to pass more rapidly along the wall with the speaker (silver eel, df = 312, F value = 9.72, p = .002; yellow stage eel, df = 259, F value = 4.58, p = .03), and time to pass was greater when both eel and lamprey exhibited a rejection (silver eel, df = 312, F value = 135.55, p < .0001; yellow stage eel, df = 259, F value = 80.70, p < .0001; lamprey, df = 276, F value = 109.3, p < .0001; Figure 5).

The inter-subject (ID) variability was high for eel, especially the yellow phase, and greater than that for day (ID = 50% and Day < 1%) in yellow eel. This was not the case, however, for silver eel for which a greater day effect was evident (ID = 40.4% and Day = 51.9%). For lamprey, the percentage of variation in the data explained by the difference among individuals and day was not as pronounced as that for eel (ID = 13.4% and Day = 19.9%). There was no influence of batch.

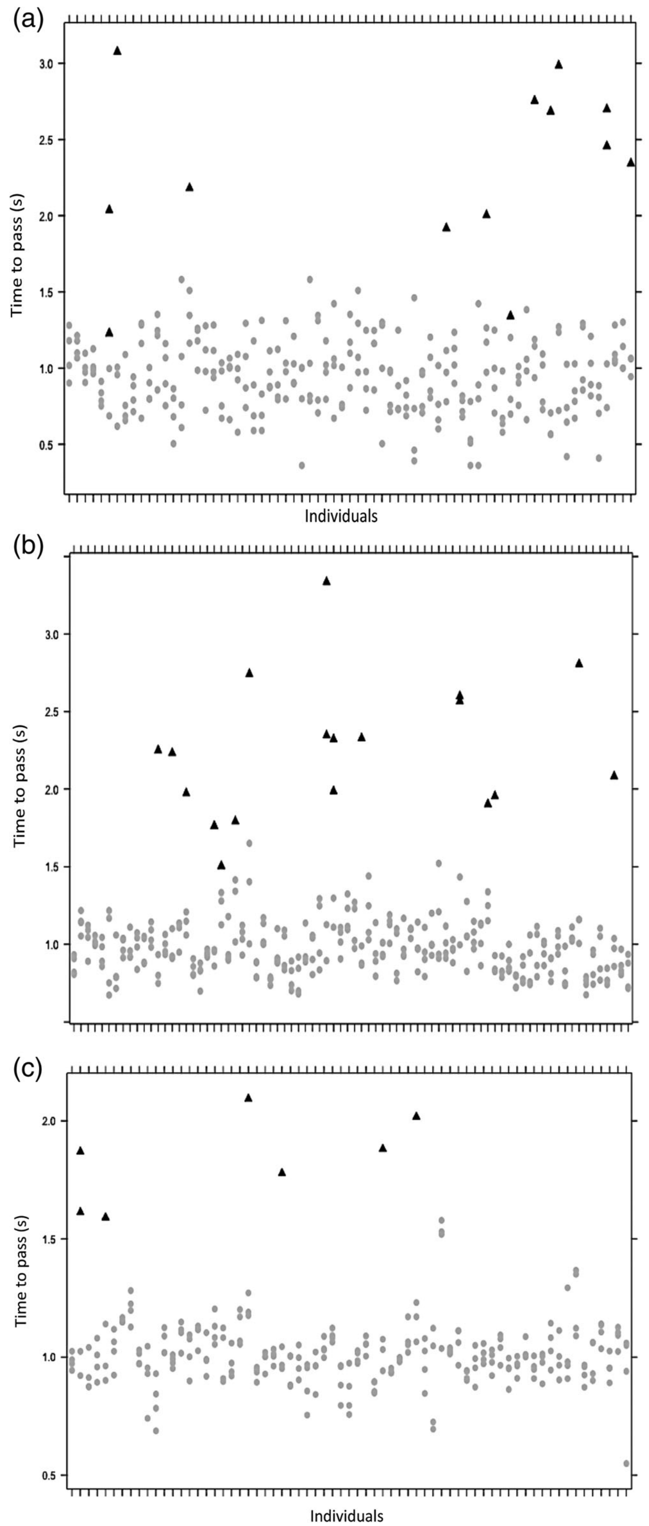

FIGURE 5 Time to pass for (a) river lamprey, (b) silver eel, and (c) yellow eel. A Log10 has been applied to the data to optimize graphs resolution (y-axis). Black triangles and grey circles indicate time to pass during attempts with and without a rejection, respectively. Data shown have been transformed via a Box–Cox transformation used in the mixed effect model

4 | DISCUSSION

In this experimental study, we investigated the behavioural response of European eel (silver and yellow stage) and adult river lamprey to a broadband stimulus (60 to 1,000 Hz) using a test that twice offered a choice of route through either an ensonified corridor or a control route. Returning to the questions posed, first, sound did not affect route selection, with the exception of lamprey that tended to pass through the ensonified route in the first chamber encountered only under Configuration 2. Second, although rejection was relatively uncommon, when it did occur, it was overwhelmingly after a fish had entered the ensonified corridor (only one rejection was observed for the control route). Those fish that did reject a corridor subsequently took longer to pass than those that did not. Despite this, overall, there was no influence of sound on the time taken to pass the maze for any of the species/life stage tested.

At the point of making a decision, the lack of a clear preference for selecting either corridor may reflect an absence of a sufficiently strong acoustic cue needed to induce avoidance. Specifically, at the junction between the corridors, the sound pressure levels were close to those of the background ambient noise, suggesting that the signal-to-noise ratio was not substantial enough to encourage a choice to be made at this point.

Our results indicate that, despite inducing an avoidance response (rejection) in a few individuals of both species, the acoustic stimulus encountered did not alter the overall probability of passing a particular route or time taken to do so. A lack of spatial avoidance in response to acoustic cues (100–1,000 Hz broadband sound) in cichlids and zebrafish (Sabet et al., 2016) was suggested to be a result of a restricted behavioural response due to the confined environment in which the experiment was conducted. This might also be an explanation for the limited rejections observed in our experiment.

To our knowledge, this is the first study to test river lamprey response to sound. The apparent preference for the ensonified channel in the first chamber encountered under one configuration was unexpected. This could have reflected a weaker acoustic signal close to the channel floor where lamprey may have travelled (see Russon et al., 2010 for description of river lamprey utilizing boundary zones at the channel walls), a suggestion supported by more rapid passage observed under that treatment. For eel, previous studies investigating response to sound, however, have provided inconclusive and sometimes contradictory results. Whereas Sand et al. (2000) describe the potential of low frequency (11.8 Hz) sound to divert downstream migrating eel in the field, MacNamara (2012) and Piper et al. (2018) were unable to replicate their findings at other sites. With regard to river lamprey, Maes et al. (2004) did not observe a significant reduction in the percentage of deflected fish (and other Pleuronectiformes) compared with other Clupeoid species, when deploying a 20–600 Hz sound projector at a power station intake. The author suggested that the response to sound by species lacking a swimbladder (lamprey), or a swimbladder connection to the inner ear structures (e.g., eel), might have been weakened by the absence of such anatomical features. While deploying an infrasound projector (16 Hz) at a power station water intake, Sonny et al. (2006) observed similar results, with more than 90% of fish (which included European eel) deflected being cyprinids (hearing specialists). As a result, the justification for employing infrasound deterrents on anguilliform species remain uncertain, especially considering the cost and difficulty of installing what are typically large units that generate limited sound fields due to the weak propagation under shallow water conditions associated with fluvial environments (Noatch & Suski, 2012). Our experimental design used narrow corridors, one of which was ensonified, to assess whether sound could be employed as a barrier to fish movements. Despite observing rejection and other avoidance behaviour (e.g., increased swimming speed and movements away from the ensonified wall), the sound used here did not create a barrier. However, this study suggests that sound might have some potential to guide the subject species to more preferred alternative routes (see Deleau et al., 2019). More research is needed to identify the appropriate frequencies, intensities, and patterns needed to achieve this more effectively.

Identification of appropriate acoustic stimuli is the first challenge facing those that intend to develop acoustic deterrents for fish. High frequency sound (e.g., >20 kHz) can elicit responses within some families, such as the salmonids and clupeids (Popper & Carlson, 1998), although this has not been reported for anguilliform species. The sound source used in this study was designed to provide a small and relatively easy to deploy unit capable of generating broadband acoustic signals in the range of 60–1,000 Hz. This ensured that the frequencies employed corresponded with those identified to be within the hearing sensitivity of eel based on the audiogram produced by Jerko (1989), in which greatest sensitivity was determined to be between 80 and 100 Hz. We are unaware of any audiogram published for river lamprey and recommend that clarification of hearing sensitivity in this species is an area worthy of future investigation.

Earlier work suggests that frequencies above the infrasound range can influence eel response, as observed for juveniles and adult American eel (*A. rostrata*; <1,000 Hz; Patrick et al., 2001). However, better understanding is needed on how intensities and temporal patterns of acoustic signal likely influence response, with suggestions that intermittent sounds may inhibit rates of recovery (time taken to return to a baseline behaviour) in some species (e.g., European seabass, *Dicentrarchus labrax*, Neo et al., 2014). Indeed, in a follow-up experiment that explored the use of sound to improve the effectiveness of physical screens, Deleau et al. (2019) compared the response of eel with the continuous broadband signal (60–1,000 Hz) used in this study and an intermittent pulsed stimulus (100 Hz). Overall, they observed similar responses, although passage times were shorter for eel exposed to the continuous broadband sound. Similarly, Vetter et al. (2017) also investigated the effectiveness of broadband sound (playback of recordings of outboard motors) and pure tones on bighead carp (*Hypophthalmichthys nobilis*), which resulted in a significant negative phonotaxis response in presence of the broadband stimulus. The pure tones did not show any significant deterrent effect in comparison with broadband sound. With regards to eel and the work of Deleau et al. (2019) previously mentioned, a lesser (but significant) deterrent effect can be achieved with both broadband sound and pure tones (100 Hz), but this is also potentially dependant of the temporal pattern of the signal (continuous vs. pulsed). This has also been investigated by Neo et al. (2015), who tested different pulse repetition interval of 600 Hz pure tone (sound pressure level = 157 dB re 1 μPa) on captive European sea bass (*Dicentrarchus labrax*). They concluded that changes in pulse repetition interval had a significant effect on the immediate change of behaviour (e.g., group cohesion and swimming depth). Contrary to these studies, which achieved a deterring effect, Febrina et al. (2015) observed that ayu (*Plecoglossus altivelis)* were attracted to both pure tones (200 Hz) and a broadband stimulus (playback of recordings of sound associated with a fish ladder). Further research is required to test a wider variety of frequencies, intensities, and temporal patterns of sound to help select those most appropriate for advancing effective fish guidance systems.

When developing environmental impact mitigation technology, such as behavioural deterrents designed to protect populations of migratory fish, intraspecific variability in response is an important determinant of overall operating efficiency. Unlike for lamprey, eel in our study, and particularly the yellow life stage, exhibited high levels of inter-subject variability in passage time. Furthermore, in silver eel and lamprey, body length influenced passage time, with large silver eels taking longer. Previous research has also demonstrated intraspecific variability in European eel response to sound. In one study, eel that were in poor condition were more likely to exhibit negative physiological (increased ventilation rates) and behavioural (reduced startle in the presence of a looming predatory stimulus) responses to playbacks of shipping noise (Purser et al., 2016). In a similar manner to the present study, but with regard to a different species (ayu), Febrina et al. (2015) observed a difference in response to sound, with adults exhibiting higher preference to the stimuli than the juvenile. This suggests, as in European eel, that the developmental stage can greatly influence the behavioural response to sound. Furthermore, our results indicate that the passage time of river lamprey and silver eel (not yellow stage) was dependent on the day of the experiment. As reviewed by Rochard and Elie (1994), several environmental parameters (e.g., temperature, rainfall, and photoperiod) influence the migratory process of eel and other species (Jonsson, 1991). As yellow eel are the "sedentary" stage of the life cycle, they may be expected to be less affected by daily variations of environmental factors. Further research is needed to quantify intraspecific variability and daily variation of environmental factors in response to stimuli tested and to incorporate this into design and operation of future deterrent devices developed to protect fish populations.

Our study demonstrated that acoustic stimuli induced behavioural avoidance, all be it in a limited manner when viewed from a holistic perspective, in some European eel and river lamprey under the experimental conditions described. This is important because it indicates that sound may have potential in the development of behavioural deterrents that might be used either in isolation or in combination with other stimuli or traditional screening devices (Noatch & Suski, 2012). Earlier work has already investigated the response of fish to combined mitigations measures. For example, Pegg & Chick (2004) successfully combined electric, acoustic, and bubble barriers in an attempt to deter Asian carp, achieving 87% rejection by the fish. In another study, Perry et al. (2014) investigated the effectiveness of a bio-acoustic fish fence (BAFF) to guide juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in the Sacramento River, United States. The BAFF uses a combination of sound (96 sound projectors mean sound: 152 dB re 1 μPa, frequency range: 5–600 Hz), strobe lights, and bubbles. They found that under low river flows, entrainment into the Georgiana Slough, a route known to result in lower survival, was reduced by 14.6% when the BAFF was switched on. Ruebush (2011) also reviewed the deterrent effect of a sound–bubble–strobe light barrier, primarily targeting silver and big head carp. The author concluded that the sound–bubble–strobe light barrier was efficient at deterring carp but also other nontargeted species, and therefore the unforeseen negative impacts of the deployment of such devices should also be considered.

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DATA AVAILABILITY STATEMENT

All data supporting this study are openly available from the University of Southampton repository at: https://doi.org/10.5258/SOTON/D1035

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