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Acoustic barriers as an acoustic deterrent for native potamodromous migratory fish species

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This study focused on the use of sound playbacks as acoustic deterrents to direct native potamodromous migratory species away from all kind of traps. The effects of two acoustic treatments, a repeated sine sweep up to 2 kHz (sweep-up stimulus) and an intermittent 140 Hz tone, were tested in three fish species native to Iberia: *Salmo trutta*, *Pseudochondrostoma duriense* and *Luciobarbus bocagei*. In contrast with *S. trutta*, the endemic cyprinids *P. duriense* and *L. bocagei* exhibited a strong repulse reaction to the frequency sweep-up sound. The 140 Hz stimulus did not seem to alter significantly the behaviour of any of the studied species. These results highlight the potential of acoustic stimuli as fish behavioural barriers and their application to in situ conservation measures of native Iberian fish populations, to protect them from hydropower dams. In addition, this study shows that acoustic deterrents can be used selectively on target species.

KEYWORDS

acoustic deterrents, behavioural barrier, conservation measures, dam, endemic fishes

1 | INTRODUCTION

The effect of climate change, largely triggered by fossil fuel dependence, has driven strategic policies in Mediterranean countries aiming to implement energy plans based on renewable resources, such as river damming for hydroelectric production. However, dams disrupt

river connectivity and impose severe restrictions on fish migrations in diadromous and potamodromous fish communities (Noatch & Suski, 2012). In addition, the hydraulic structures of dams, mainly hydroelectric turbines, cause massive fish mortality due to abrupt changes in pressure, cavitation, shear forces, turbulence and mechanical shock (; Becker *et al.*, 2003; Cada *et al.*, 1997).

To reduce fish mortality, non-physical barriers based mostly on different aversive conditions have been tested, including electric and magnetic fields, water velocity barriers, hypoxia and hypercapnia, pheromones, strobe lights, bubble curtains and acoustic deterrents (Noatch & Suski, 2012). Such barriers triggering aversive behaviour have proved very effective in reducing the effects of dams on different species (McIninch & Hocutt, 1987; Patrick *et al.*, 1985; Sonny *et al.*, 2006) by allowing fish to avoid hazardous structures such as hydroelectric turbines, pumping systems and adducting pathways (Abernethy *et al.*, 2001). They can also be used to protect native fish populations by rerouting them to passages in hydroelectric power plants (Coutant & Whitney, 2000). Non-physical barriers can also be used to avoid or, at least, slow the spread of invasive alien species (IAS) (Taylor *et al.*, 2005; Vetter *et al.*, 2015).

The use of acoustic barriers depends on the hearing capabilities of target species. Fish present a continuum of hearing capabilities associated with the evolution of hearing structures (Popper & Fay, 2011). Fish that present morphological specializations connecting air-filled cavities, such as the swimbladder, to the inner ear have enhanced hearing capabilities and can detect sound pressure in addition to the kinetic component of sounds, i.e. particle motion. The Cyprinidae, are notably sensitive to sound and can detect a wide frequency range (up to thousands of Hz) (Popper & Fay, 2011, Popper & Schilt, 2008). Species with no hearing specializations, such as Salmonidae, are only able to detect particle motion and their hearing sensitivity is restricted to low frequency sounds of up to a few hundred Hertz; e.g., 400 Hz in the Atlantic salmon *Salmo salar* L. 1758 (see figure 2.1 in Popper & Schilt, 2008). Due to the large variation in hearing structures, different species may react differently to an acoustic barrier, emphasizing the need for species-dedicated behavioural evaluation tests.

Freshwater ecosystems of Iberia (Portugal and Spain) have suffered substantial modifications in recent years through construction of several new large dams, built mainly for hydropower purposes, but also for irrigation, water supply and flood prevention (Flores Montoya *et al.*, 2006; Horvath & Yagüe, 2000; INAG, 2012; Melo, 2012). This enhances the risk for native freshwater fishes, in particular migratory species, which are among the most threatened species in Portugal (Cabral *et al.*, 2005). For instance, severe habitat modifications are occurring in three main tributaries (Tâmega, Tua and Sabor) of River Douro in northern Portugal. Large dams are responsible for substantial changes of native fish fauna, in particular due to the breakdown of genetic continuity, reduced habitat availability (e.g., for reproduction, feeding and shelter) or the introduction of exotic species, particularly the expansion of predators (Dudgeon *et al.*, 2006), demanding in situ conservation measures, in particular for endemic fish species.

The use of behaviour-conditioning acoustic systems, specifically aimed at protecting endangered fish species, can have a positive effect on biodiversity conservation. Acoustic barriers can contribute to keep these species away from dangerous structures and reduce their mortality, or to direct fish to transposition systems, thus increasing their chances to access spawning grounds.

The objective of this study was to evaluate the potential of pure tones and broadband sound stimuli as behavioural barriers for native Iberian freshwater fish species. The deterrent effect of a repeated sine

sweep up to 2 kHz (sweep-up sound) and an intermittent 140 Hz tone, were tested in two endemic cyprinids, the northern straight-mouth nase *Pseudochondrostoma duriense* (Coelho 1985), which has a Vulnerable IUCN Red List conservation status (Crivelli, 2006), the Iberian barbel *Luciobarbus bocagei* (Steindachner 1864) and brown trout *Salmo trutta* L. 1758.

2 | MATERIALS AND METHODS

2.1 | Study site

The experiments took place in raceway tanks, continuously supplied with water from an unpolluted headstream, the River Baceiro at the fish farm of Estação Aquícola de Castrelos, in north-eastern Portugal.

2.2 | Target species

The *S. trutta*, *P. duriense*, *L. bocagei*, were captured by electrofishing (Hans Grassl ELTII, DC 300/600 V; www.hans-grassl.com) in the River Sabor (Douro basin). Three hundred individuals of each species were selected with the following total length (L_T) and mass (M) (mean \pm s.d.): *S. trutta* $L_T = 26.3 \pm 1.3$ cm, $M = 232.7 \pm 37.7$ g; *P. duriense*: $L_T = 12.2 \pm 0.4$ cm, $M = 23.4 \pm 2.5$ g; *L. bocagei* $L_T = 13.1 \pm 0.5$ cm, $M = 31.2 \pm 3.4$ g. After the experiments all healthy fishes were released in the same river zone.

2.3 | Test stimuli

Three replicate trials and two different stimuli (140 Hz and a sweep-up sound) were used. The sound stimuli, generated with a laptop running Adobe Audition 3.0 (Adobe Systems; www.adobe.com), were delivered to an amplifier (Blaupunkt GTA 260; www.blaupunkt.com) via the digital-to-analogue converter of an USB audio capture device (Edirol UA-25, Roland 16 bit, 44.1 kHz; www.roland.com), The amplified stimuli were played back with an underwater loudspeaker (Electro-Voice UW30; www.electrovoice.com, 30 W, 8 Ohm, frequency response 0.1–10 kHz) placed in the channel1 area of the tank where the trials took place (see below).

The acoustic stimuli comprised 140 Hz tone pulses, 50 ms long, 3 ms ramps, delivered at a rate of 195 min and sine sweeps up to 2 kHz (sweep-up stimulus), 5 s long, delivered at a rate of 12 min (Figure 1(a),(b)). The playback stimuli were adjusted to an amplitude of 140 dB sound pressure level (SPL; re. 1 μ Pa) at 1 m in front of the speaker. These measurements were made with a Brüel & Kjær 8104 hydrophone (Brüel & Kjær; www.bksv.com; sensitivity -205 dB re. 1V/Pa; frequency response 0.1 Hz to 180 kHz) connected to a sound level meter (Mediator 2,238, Brüel & Kjær). The 140 Hz tone was well represented in the playback. The second harmonic (generated by any commercial speaker) was at least 20 dB below 140 Hz spectral peak. The sweep-up signal varied greatly in amplitude along the frequency range (Figure 1(d)) due to the non-linearity of the UW30 speaker (N.B. the frequency increases linearly with time in the sine-sweep stimulus). Both stimuli attenuated similarly along the tank (Figure 1(c)). As expected the attenuation was higher close to the speaker and smoothed out with distance.

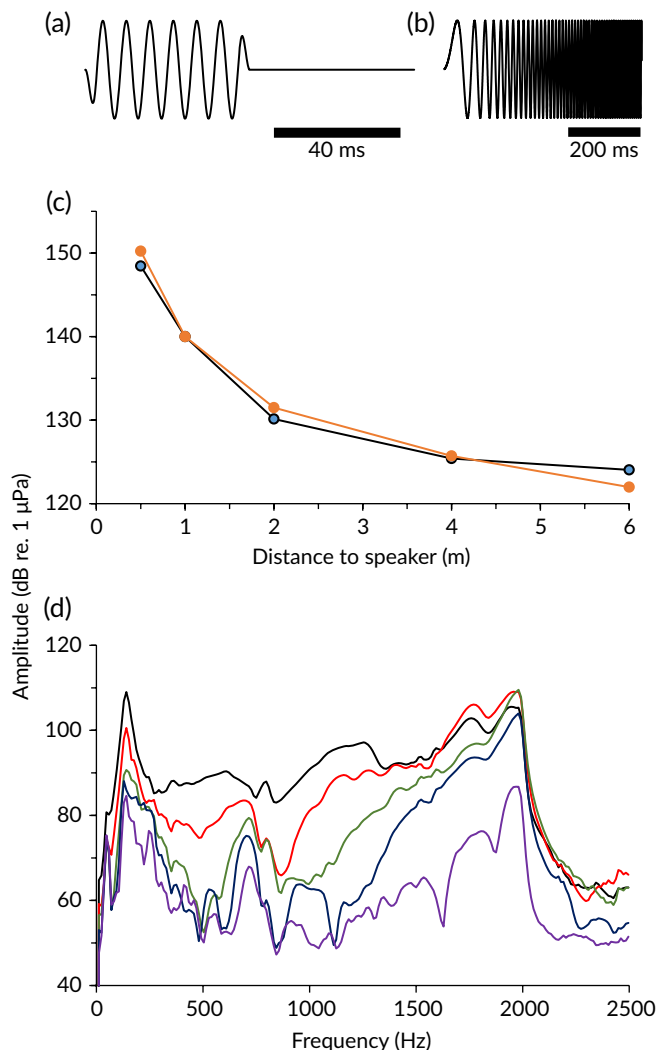


FIGURE 1 Test stimuli included (a) A 140 Hz tone pulse with 3 ms ramps and (b) A sweep-up stimulus, consisting of sine sweeps ranging up to 2 kHz (only 0.5 s are represented). (c) Attenuation of the sine sweep and 140 Hz stimuli along the experimental tank, calibrated to an amplitude of 140 dB re 1 μ Pa at 1 m from the loudspeaker (\bullet —) sweep-up, and (\bullet —) 140 Hz. (d) Power spectra of the sweep-up stimulus recorded at different distances from the speaker (as in (c)) (—) 0.5 m, (—) 1 m, (—) 2 m, (—) 4 m, and (—) 6 m. Note that the energy below 1,500 Hz, where fish are most sensitive, drops considerably after 4 m, i.e. in channel 3 area

2.4 | Experimental design

Each experimental group was composed of 50 individuals of the same species. Each group was tested once. Before trials, wild fish spent a quarantine week in stock reservoirs (1.5 m diameter \times 0.85 m deep, 1,500 l). They were then transferred to raceway tanks similar to the test tank (9 m long, 0.9 m wide, 0.6 m deep, c. 5,000 l) and were allowed to acclimate for a week to local conditions. Fish health was visually checked every day and feeding was suspended on the day of the trial. These tanks had a continuous water supply at the upper end, promoting a constant flow (water speed c. 0.07 m s⁻¹) to stimulate the rheophilic response of fish. The underwater speaker, was attached to a concrete slab (0.3 m width \times 0.3 m length \times 0.4 m height) and placed at c. 2.5 m from the upper end of the trial tank, normally

preferred by the fish. A net installed across the tank in front of the speaker prevented the fish from swimming behind the speaker rear (Figure 2). Three different fish monitoring areas were defined (Figure 2). The area channel 1 was the closest to the speaker while channel 3 was the furthest. Fish position was recorded by three video cameras covering each monitoring area.

Trials were made during day light. Fish were allowed 2 h to adapt to the test tank. Each trial lasted 60:15 min of a pre-playback control period (control), 15 min of sound playback (PBK1), 15 min of post-playback period (post-PBK), followed by 15 min of a second sound playback period (PBK2). This second sound presentation was made to assess first stages of short-term habituation (hereafter just referred to as habituation; Thorpe 1963). Fish position was analysed for the three monitoring areas (channel 1, channel 2 and channel 3), in the four 15 min test periods (control, PBK1, post-PBK and PBK2). Trial efficiency (avoidance index, I_A) was determined by comparing the number of fish present in channel 1 during the control period and the test period as: $\%I_A = (1 - (\text{no. of fish channel 1 testing})/(\text{no. of fish channel 1 control}))^{-1} \times 100$.

Areas channel 2 and channel 3 were not considered for fish avoidance evaluation. However, they allowed assessment of fish distribution throughout the tank as well as validation of fish counts (channel 1 + channel 2 + channel 3 = 50). Fish were counted in each area (channel 1, channel 2 and channel 3) every 10 s by freezing the video images, resulting in six accumulated counts min⁻¹ and 180 counts per trial (Figure 2). Latency to stimulus response was also measured. We considered that fish were responding to the playback when more than half of the fish ($n > 25$) kept consistently away from channel 1 area.

2.5 | Statistical analysis

2.5.1 | Stimulus deterrence effect

To test for a sound playback effect the number of fish in channel 1 was compared between control and PBK1 and across time (three 5 min intervals within each experimental period) with repeated-measures ANOVA for each sound stimulus (140 Hz or sweep-up). Thus, repeated-measures ANOVA models included two repeated measures variables: playback treatment (sound) with 2 levels: control (silence) and sound (PBK1) and time with 3 levels, consisting of the first, second and third 5 min intervals of each experimental period. Three replicates were considered per experiment and species. Data transformations were necessary, namely for *P. duriense* and *L. bocagei* sweep-up data, where $\log(x + 1)$ and $\sqrt{(x + 3/8)}$ transformations were respectively used to achieve compound sphericity requirements.

2.5.2 | Carryover effect and habituation

To test for possible carryover effects of the sound playback and habituation to stimulus presentation the average number of fish in channel 1 was compared among experimental phases of the full experimental procedure with repeated-measures ANOVA. The analyses included one repeated-measures variable with four levels: control, PBK1, post-PBK and PBK2 periods. The repeated measures ANOVA assumptions were met. Post-hoc Tukey HSD tests were performed.

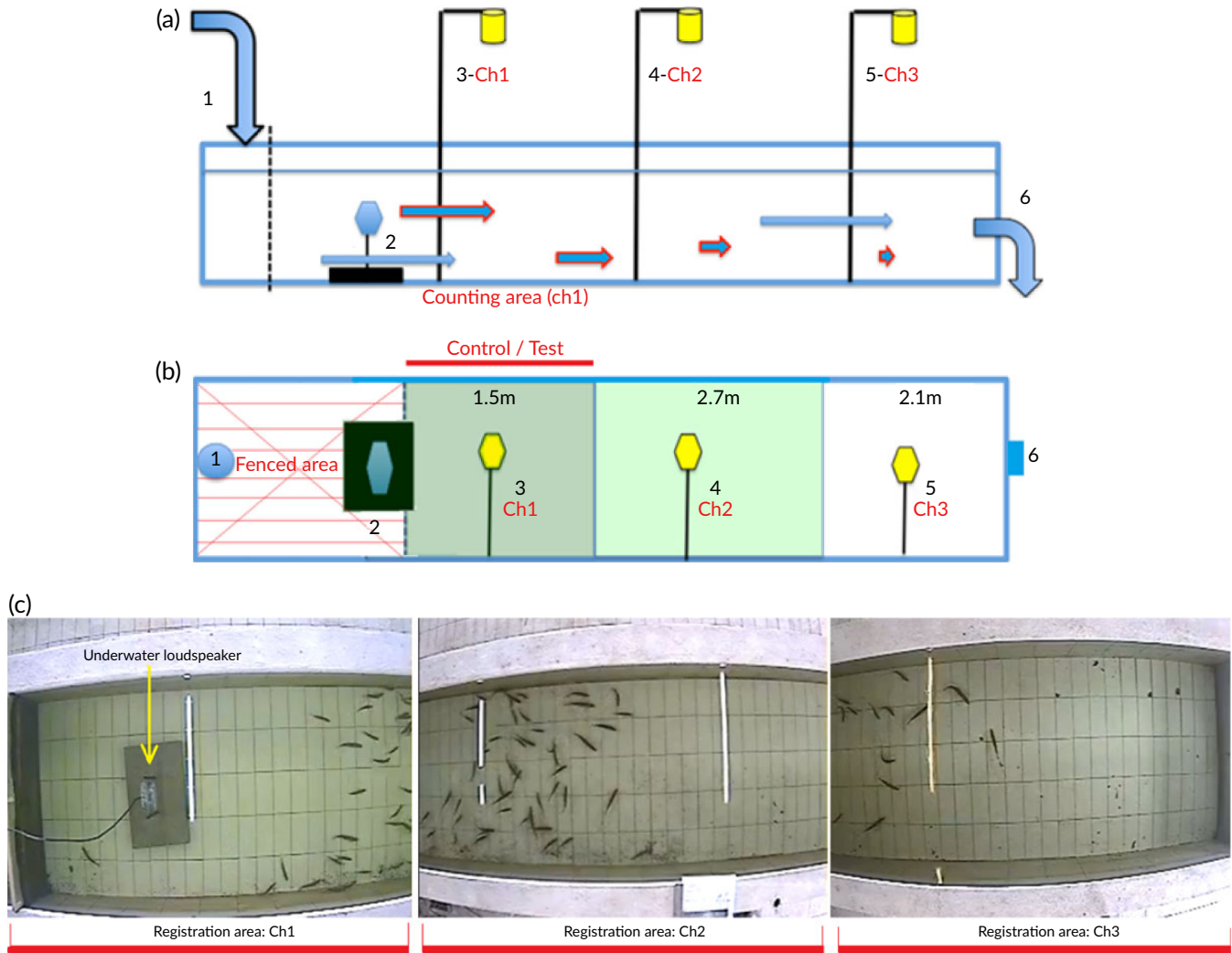


FIGURE 2 (a) Lateral and (b) top views of the trial tank, depicting different video registration areas: 1, water entrance; 2, speaker; 3, channel [Ch] 1 registration area; 4, channel 2 registration area; 5, channel 3 registration area; 6, water exit. (c) Examples of video (C) frames taken from recording made by the three video cameras (corresponding to Ch1, Ch2 and Ch3) strategically placed to record the entire acoustic trial tank

To test if fish distribution was affected by playback we tested if the number of fish 10^{-1} s in each tank area fitted a Poisson distribution (expected if fish presented a random distribution). The aim was to test if fish followed a random distribution within each tank area or stayed in groups and if the pattern was changed by the stimuli. These analyses were performed separately for the control and PBK1 periods and only in case of a significant sound playback effect (as above).

All tests were made using the software Statistica 13.0 (Dell Inc.; www.dell.com).

3 | RESULTS

The avoidance index (I_A) of the sweep-up treatment was high for the endemic cyprinid species, *L. bocagei* (95.9%) and *P. duriense* (87.9%) in contrast with what was observed for *S. trutta* (8.7%) (Table 1). Consistently, there was a significant deterrent effect of the sweep-up stimulus on *P. duriense* ($p < 0.05$) and on *L. bocagei* ($P < 0.01$), but *S. trutta* did not show a significant avoidance behaviour (Figure 3; Table 2).

The deterrent effect of the sweep-up treatment increased over time for both cyprinid species, as fewer fish were found in channel 1 in the second and third 5 min periods of the experimental protocol (Figure 3 and Table 2). Channel 1 was also the preferential area for the fish when there was no deterrent noise (Figure 3), since most individuals of all species were in channel 1 during control periods (*S. trutta* mean = 46; *P. duriense* mean = 38; *L. bocagei* mean = 44, all out of 50 fish). In contrast, only 5 and 2 individuals (mean values) for *P. duriense* and *L. bocagei*, respectively, remained in channel 1 area during the presentation of the sweep-up stimulus, while for *S. trutta* most individuals were not affected (42 *S. trutta* remained in the channel 1 area). On average, *L. bocagei* took 20.0 s (± 26.5 s s.d.) to consistently avoid the channel 1 area after the start of stimulus presentation while *P. duriense* showed a slower avoidance reaction: 146.7 s (± 32.1 s). Figure 4 depicts the distribution of fish across the experimental tank in the first 5 min of the experiment and shows that, during the sweep-up playback (PBK), most individuals of *L. bocagei* avoided channel 1 and stayed in channel 3 whereas in *P. duriense* most individuals stayed in channel 2.

TABLE 1 Relative efficiency (%) of acoustic treatments in *Salmo trutta*, *Pseudochondrostoma duriense* and *Luciobarbus bocagei*

Species	Trial	Date	Channel 1 (n)		Relative efficiency (%)
			Control	Testing	
<i>S. trutta</i>	140 Hz	December 5, 2011	4,350	4,060	6.67
		December 6, 2011	3,691	2,708	26.63
		December 7, 2011	3,719	3,264	12.23
		Total	11,760	10,032	14.70
	Sweep-up	December 27, 2011	4,119	3,905	5.20
		December 28, 2011	4,141	3,910	5.58
		December 29, 2011	4,014	3,390	15.55
Total	12,274	11,205	8.70		
<i>P. duriense</i>	140 Hz	December 5, 2011	3,445	1,563	54.63
		December 6, 2011	3,024	3,087	-2.08
		December 10, 2012	659	289	56.15
		Total	7,128	4,939	30.71
	Sweep-up	January 2, 2012	1,898	413	78.24
		January 3, 2012	4,021	453	88.73
		January 4, 2012	4,320	374	91.34
Total	10,239	1,240	87.89		
<i>L. bocagei</i>	140 Hz	January 24, 2012	3,909	3,085	21.08
		January 25, 2011	4,500	4,500	0
		January 26, 2012	4,500	3,267	27.40
		Total	12,909	10,852	15.93
	Sweep-up	January 18, 2012	2,792	2	99.93
		January 19, 2012	4,500	253	94.38
		January 23, 2012	4,500	228	94.93
Total	11,792	483	95.90		

Note. Number of fish counted in registration area channel 1 in each trial is denoted. n = Number of fish.

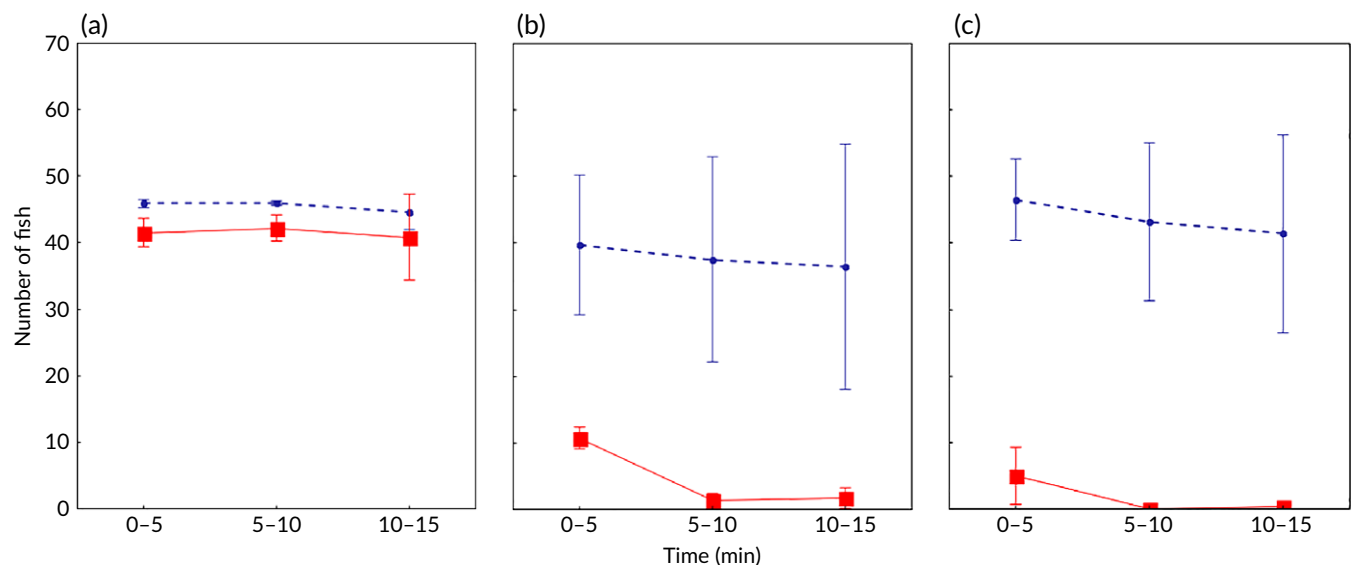


FIGURE 3 The number (mean \pm s.d.) of individual (a) *Salmo trutta*, (b) *Pseudochondrostoma duriense* and (c) *Luciobarbus bocagei*, observed in channel (Ch)1 over the three 5 min experimental periods for the control and the sweep-up treatments (●) Control, and (■) Sweep-up

The I_A of the 140 Hz tone treatment was low in all species (*S. trutta* I_A = 14.7%; *P. duriense* I_A = 30.7%; *L. bocagei* I_A = 15.9%; Table 1). The 140 Hz tone did not cause any significant avoidance effect in any of the studied species ($P > 0.05$, Table 2).

The potential carryover effect and habituation to the sweep-up treatment was investigated for *L. bocagei* and *P. duriense*. In both species avoidance of channel 1 area continued after the sound playback until the second test period (*L. bocagei* $F_{3,6} = 49.53$, $P < 0.001$;

TABLE 2 Effect of sound playback (sound) and time on fish avoidance from the tank speaker area (registration area channel1) in three *Salmo trutta*, *Pseudochondrostoma duriense*, *Luciobarbus bocagei*

Species	Sound	Variables	ANOVA	P-value
<i>S. trutta</i>	140 Hz	Sound	$F_{1,2} = 7.60$	> 0.05
		Time	$F_{2,4} = 6.14$	> 0.05
		Time × sound	$F_{2,4} = 3.59$	> 0.05
	Sweep-up	Sound	$F_{1,2} = 7.08$	> 0.05
		Time	$F_{2,4} = 0.29$	> 0.05
		Time × sound	$F_{2,4} = 0.12$	> 0.05
<i>P. duriense</i>	140 Hz	Sound	$F_{1,2} = 1.53$	> 0.05
		Time	$F_{2,4} = 2.28$	> 0.05
		Time × sound	$F_{2,4} = 1.20$	> 0.05
	Sweep-up	Sound	$F_{1,2} = 26.13$	< 0.05
		Time	$F_{2,4} = 15.95$	< 0.01
		Time × sound	$F_{2,4} = 7.46$	< 0.05
<i>L. bocagei</i>	140 Hz	Sound	$F_{1,2} = 3.58$	> 0.05
		Time	$F_{2,4} = 0.72$	> 0.05
		Time × sound	$F_{2,4} = 0.75$	> 0.05
	Sweep-up	Sound	$F_{1,2} = 517.12$	< 0.01
		Time	$F_{2,4} = 15.21$	< 0.01
		Time × sound	$F_{2,4} = 0.98$	> 0.05

P. duriense $F_{3,6} = 7.59$, $P < 0.05$; Figure 5). However, in *P. duriense* some fish returned to the channel 1 after the sound presentation as the difference in the number of fish between the control and the post-PBK period became marginally non-significant (Tukey HSD test, $P > 0.05$; Figure 5). Once *P. duriense* were re-exposed to the sweep-up stimulus the number of fish in channel 1 decreased (difference between control and PBK2, $P < 0.05$). These results indicate that the deterrence action of the sweep-up was stronger for *L. bocagei* having a prolonged carryover effect. In *P. duriense* the carryover effect was not so pronounced but the reaction to a second sweep-up

presentation suggests no habituation, at least with the considered experimental period. Congruently, I_A for *P. duriense* was similar in both PBK periods (Table 1) and in *L. bocagei* the avoidance effect could not even be calculated in 2 out of 3 trials as fish remained in channel 3 after PBK1 (Figures 4 and 5).

The sweep-up stimulus presentation did not affect fish distribution for *P. duriense* and *L. bocagei*. In both the control and PBK1 periods the number of fish per tank area counted per 10 s did not fit a Poisson distribution (*P. duriense* $\chi^2 = 8,263.7-9,223.7$, d.f. = 3, $P < 0.001$; *L. bocagei* $\chi^2 = 9,315.7-9,438.0$ d.f. = 3, $P < 0.001$). In addition, for both species, the observed variance was c. 23–30 fold higher than the average number of fish counted per 10^{-1} s per area suggesting a clumped distribution.

In summary, the sweep-up treatment had a significant deterrent effect on the endemic cyprinids, namely for *P. duriense* and especially for *L. bocagei* where the 140 Hz stimulus did not seem to alter significantly the behaviour of any of the studied species.

4 | DISCUSSION

Fish behavioural barriers have been commonly used for fish guidance, altering fish migration routines, in order to protect native species or to avoid and limit the spread of invasive alien species (Noatch & Suski, 2012; Perry *et al.*, 2012; Schilt, 2007; Vetter *et al.*, 2015). Several of such deterrence techniques have been used as management tools in freshwater systems including electrical, chemical, visual and acoustic stimuli. In the present work, we found significant differences in the reaction to potential acoustic deterrents between *S. trutta* and two cyprinids, *P. duriense* and *L. bocagei*. We showed that the sweep-up (up to 2 kHz) sound presented a significant and fast repulsive response for both cyprinid fishes ($I_A > 80\%$) and a non-significant effect for the salmonid species. This differential behaviour towards

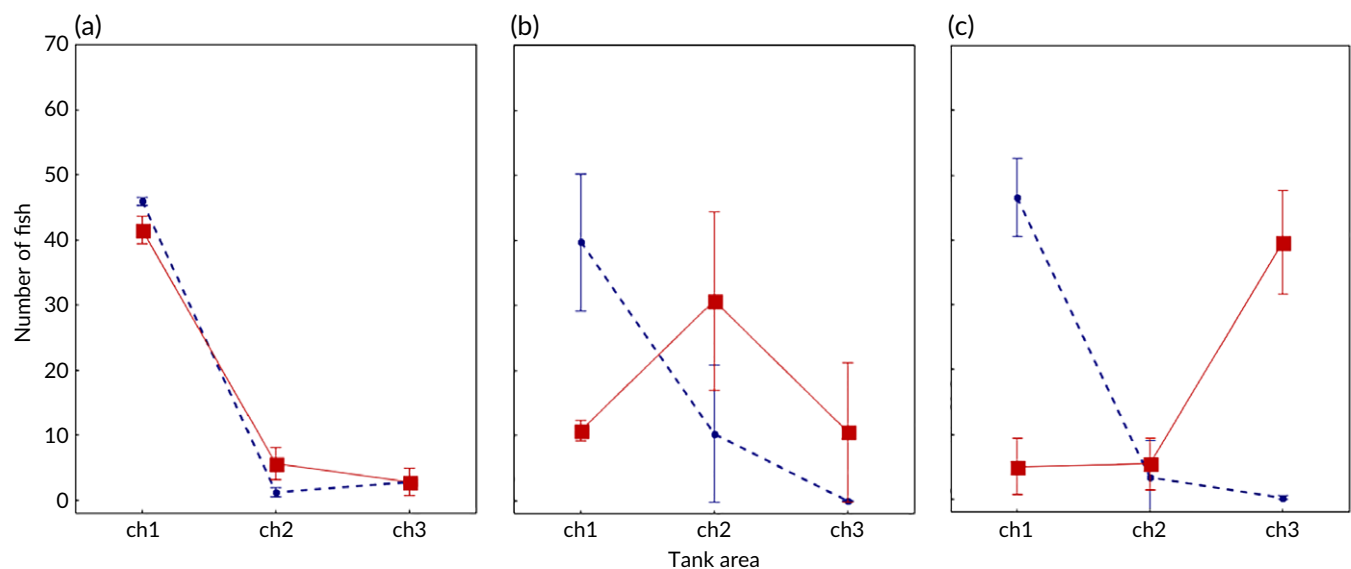


FIGURE 4 The number (mean \pm s.d.) of (a) *Salmo trutta*, (b) *Pseudochondrostoma duriense* and (c) *Luciobarbus bocagei* observed in the three registration areas (channel (Ch)1, channel 2 and channel 3) of the experimental tank in the first 5 min experimental period for the control and the sweep-up treatments (● Control, and ■ Sweep-up)

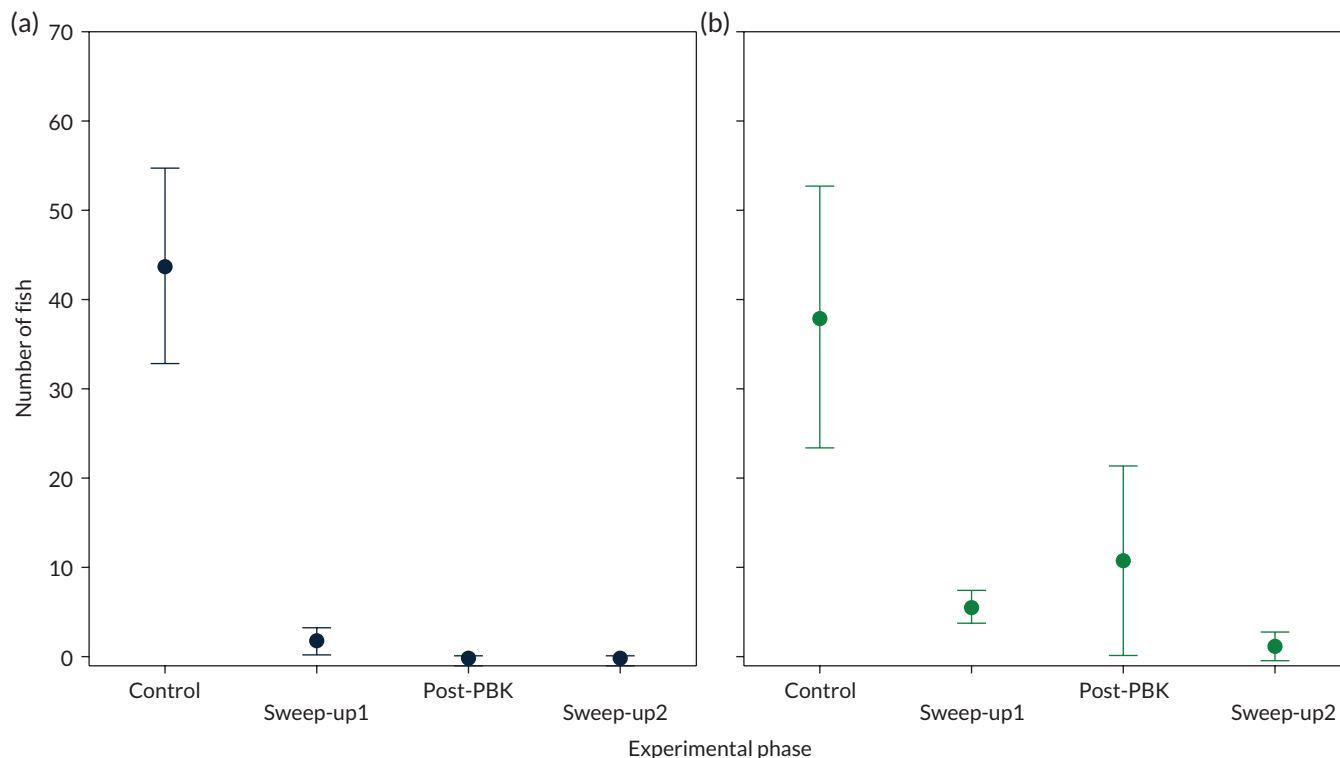


FIGURE 5 The number (mean \pm s.d.) of individual (a) *Luciobarbus bocagei* and (b) *Pseudochondrostoma duriense* observed in channel 1 over the four experimental phases of the full experimental procedure. PBK, Sound playback

the sweep-up stimulus is probably related to the higher acoustic sensitivity of the two native cyprinid species (Popper & Schilt, 2008) with cyprinids not only detecting a higher range of frequencies but also perceiving the sweep-ups as louder, as stimuli are further above the hearing threshold. Similarly, Lambert *et al.* (1997) identified that sounds ranging in frequency from 10 Hz to 3 kHz approximately 20 dB above background level generally induce behavioural avoidance. Importantly, the present study indicates that in the studied cyprinids distribution is not affected by the sweep-up stimulus, but there is a relatively prolonged deterrence effect and no short-term habituation either within the 15 min sound exposure or between sound exposures (PBK1 and PBK2). However, the habituation results need to be treated with caution and further tests should involve more steps and longer periods, when using the same groups of fish. Vetter *et al.* (2017) also found that the bighead carp *Hypophthalmichthys nobilis* (Richardson 1845) (Cyprinidae) did not show short-term habituation to the exposure of broadband sounds, but an increase in tolerance over longer exposure periods should not be excluded (Nedelec *et al.* 2016).

In contrast with the sweep-up, the 140 Hz tone induced a reduced repulse response, with low values for the I_A , varying between 14.7% for *S. trutta* and 30.7% for *P. duriense*. This result is not surprising since pure tones have been found to be effective only when they present very low frequencies or very high sound pressure levels (Knudsen *et al.*, 1994, 1997, Lambert *et al.* 1997, Sonny *et al.*, 2006), which are extremely difficult and costly to produce. In addition, infrasound, can have a harmful effect on man, fauna and structures, as described by Gužas and Klimas (2009).

Consistent with the present study, Vetter *et al.* (2015) showed that in the silver carp (Cyprinidae) aversive behaviour was much

stronger in relation to complex tones (0–10 kHz) than to pure tones (500 and 2000 Hz). In addition, these authors found that silver carp *Hypophthalmichthys molitrix* (Valenciennes 1844) may adapt easily to pure but not to complex tones (Vetter *et al.*, 2015). The greater efficiency of complex sounds such as the sweep-up stimulus used in our study, which goes through a larger array of frequencies, has been suggested as the most efficient solution for the majority of species as they allow its application to species with broader sensory capabilities and avoids potential habituation to a single frequency (Lambert *et al.*, 1997; Vetter *et al.*, 2015).

It is also important to consider total duration and pattern of sound exposure when assessing behavioural responses to sound (Neo *et al.* 2015). In the present study the 140 Hz tone was shorter and had a shorter on-time despite being presented at a higher rate than the sweep-up. A shorter total exposure combined with a higher number of presentations (which could cause habituation) could have lessened the reaction to 140 Hz tones in relation to the sweep-ups (Nedelec *et al.* 2016, Neo *et al.* 2015). Future work should investigate the effect of total exposure time and patterning of acoustic barriers.

The present study was performed in tanks with a capacity of 5,000 l, with concomitant altered sound fields and spatial restrictions (Akamatsu *et al.*, 2002). Therefore, extrapolation of the results to real conditions should be made with caution and supported by complementary studies performed under natural conditions (Hawkins *et al.* 2014; Neo *et al.*, 2016; Slabbekoorn, 2015). Nevertheless, laboratory trials allow high control conditions and the analysis of robust behavioural characteristics that can support experiments in real scenarios (Slabbekoorn, 2015). In addition, tank-based and out-door experiments can produce comparable results (Neo *et al.* 2016).

The development of fish behavioural barriers with the use of acoustic stimuli presents a wide array of possibilities as sound propagates much faster in the underwater environment than in air, imposing an intense effect on aquatic animals (Mann, 2006). One of the potentials of this acoustic tool is its selective use on target species. Indeed, as supported by our work, it is possible to develop selective behavioural systems (repulsive or routing systems) specifically aimed towards particular species, since stimuli that are audible to some species are inaudible to others (Amundsen & Landro, 2011). Indeed, audiograms obtained under quiet laboratory conditions for salmonid species show that these species only detect sound louder than 90–100 dB SPL re 1 μ Pa whereas cyprinids are able to detect sound pressure above c. 45 dB SPL re 1 μ Pa and therefore are more sensitive to a much wider acoustic frequency range (Popper and Schilt, 2008). In addition, aversive responses to noise may vary between species and populations irrespective of hearing abilities, possibly due to differences in stress response or other factors such as genetic background or environmental context (Kastelein *et al.*, 2008; Pottinger, 2010).

Mediterranean freshwater ecosystems are known as hotspots of biodiversity threatened by human activities leading to the habitat fragmentation and disruption (Clavero *et al.*, 2004; Collares-Pereira & Cowx, 2004). In Iberia, river regulation is responsible for dramatic habitat modifications affecting the potamodromous reproductive migration of several endemic species. Non-physical barriers to guide fish movement can contribute to the conservation of these native species. With respect to *S. trutta*, several studies have described their behaviour, including their reaction to anthropogenic noise (Nedwel *et al.*, 2006), but none has focussed on southern European populations, including the Iberian Peninsula. For these reasons, the success of these techniques implies the knowledge of specific behavioural responses namely of the endemic species, like *P. duriense* and *L. bocagei*, since the reaction and response effects to other stimuli remain unknown.

In conclusion, our results suggest that sweep-up sounds have the potential to be an important management tool for the conservation of endemic cyprinids of Iberia. This deterrent technology should however be tested in natural conditions, on its own, or integrated with other behavioural barriers (e.g., bubble curtains, stroboscopic light), to safeguard threatened fish species. For example, the sound field generated by speakers in concrete tanks (namely in terms of particle motion, not considered in this study) will differ from sounds generated in the field. The development of behavioural barriers specifically adapted to these endemic species of northern Iberia represent an important mitigation measure to overcome fragmentation in regulated rivers, when coupled with environmental flows or fish passages.

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