



# Influence of acoustics on the collective behaviour of a shoaling freshwater fish

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## Abstract

1. Understanding how collective behaviour of animals is influenced by anthropogenic activity is important for their conservation in an increasingly urbanised world. River infrastructure, e.g. for transport and electricity generation, and associated construction and operation, produces sound that can disrupt ecological processes.
2. Adopting a reductionist manipulative experimental approach using Eurasian minnow (*Phoxinus phoxinus*) as a model shoaling species, we compared the response of individuals and groups of five fish to a broadband acoustic stimulus in a tank containing still water.
3. Four metrics were calculated 10 min immediately before (control–sound stimulus absent) and during the acoustic treatment: (1) swimming speed, (2) persistence of swim paths, (3) cohesion of the group, and (4) orientation of group members.
4. On presentation of the stimulus, groups exhibited a consistent escape response compared to individuals for which behaviour was more variable. Thereafter, individuals swam faster and their swim paths were less persistent than during the control; no difference was observed for groups. Conversely, group integrity became more cohesive and members were more likely to orient in a common direction during the treatment compared to the control.
5. This study provides insight into the importance of collective behaviour of fish in relation to antipredator-like response to anthropogenic noise. Short-term shifts in behaviour are context specific and depend on whether fish are members of a shoal or solitary. The results indicate the potential for negative impacts of unnatural sound on the ecology of shoaling species that inhabit engineered freshwater environments.

## KEYWORDS

anthropogenic disturbance, group behaviour, noise, schools, sound

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## 1 | INTRODUCTION

Collective behaviour, in which coordinated groups are formed as a result of local interactions among individuals, is widely observed in nature, e.g. in animals that form flocks, schools, and swarms. The benefits for individual fitness of group membership include enhanced antipredator defence (e.g. in spiders; Uetz, Boyle, Hieber, & Wilcox, 2002), foraging efficiency (e.g. in birds; Sullivan, 1984), thermoregulation (e.g. in huddling endotherms; Gilbert et al., 2010), and information transfer (e.g. in fish; Laland & Williams, 1997). Although understanding the mechanisms and significance of collective behaviour has been of great interest in the fields of ecology (Couzin, Krause, Franks, & Levin, 2005), ethology (Ballerini et al., 2008), and evolution (Couzin, Krause, James, Ruxton, & Franks, 2002) for decades, there has been little consideration of how it may be disrupted by anthropogenic disturbance, such as noise pollution.

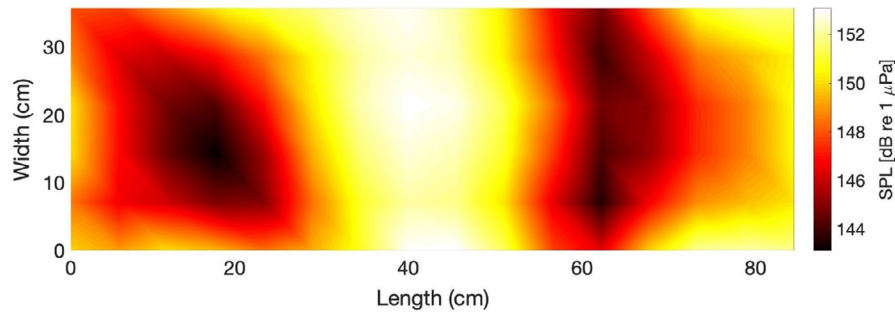
The impacts of acoustic disturbance on animal behaviour are relatively well studied for terrestrial systems (Barber, Crooks, & Fristrup, 2010) and the marine environment (Slabbekoorn et al., 2010). In particular, considerable attention has been directed towards understanding the response of marine mammals (usually cetaceans) (Shannon et al., 2016; Weilgart, 2007) and fishes (Hawkins & Popper, 2017; Herbert-Read, Kremer, Bruintjes, Radford, & Ioannou, 2017; Popper, Fewtrell, Smith, & McCauley, 2003), with the focus on the commercially important species for the latter. In comparison, how human-generated noise affects fish in the freshwater environment has received relatively limited attention (Holt & Johnston, 2015; Mickle & Higgs, 2017).

It is assumed fish perceive and respond to anthropogenic disturbances, such as those caused by underwater noise, in a way that is analogous to antipredator behaviours (Frid & Dill, 2002). These include increased vigilance, fleeing, and hiding, all of which divert time and energy from other fitness-enhancing activities such as feeding and reproduction. However, the results of previous studies are contradictory, and in many cases use playback recordings that may be inappropriate when viewed from the perspective of the typical habitat exploited by the subject species studied. For example, under laboratory conditions, three-spined stickleback (*Gasterosteus aculeatus*) and Eurasian minnow (*Phoxinus phoxinus*) are more frequently startled, and exhibit lower feeding rates, when field recordings of ships passing through harbours are played back (Voellmy, Purser, Flynn, et al., 2014). Furthermore, in a similar experiment, the sticklebacks respond more quickly to a visual predatory stimulus in the presence of noise than during control conditions, while minnows exhibit no difference in response (Voellmy, Purser, Simpson, & Radford, 2014b). Eurasian minnow has also recently been observed to exhibit complex behavioural group response to acoustic stimuli (Currie, White, Leighton, & Kemp, 2020). Conversely, juvenile European eel (*Anguilla anguilla*) is slower and less likely to exhibit a startle response to a simulated predator, increasing the probability of capture, again under treatments employing the playback of shipping noise (Simpson, Purser,

& Radford, 2015). However, European eel has been shown to exhibit avoidance behaviours towards underwater sound (Deleau, White, Peirson, Leighton, & Kemp, 2020a, 2020b) and specifically infrasound (Piper, White, Wright, Leighton, & Kemp, 2019). While appreciating that there is likely to be interspecific variability in response to noise that may partially explain the conflicting results obtained, there is a need to enhance understanding of fish behaviour by adopting a more reductionist approach in which the in-tank acoustic fields are accurately measured, and frequencies and intensities better defined than in previous experiments, reflecting those likely to be encountered in nature.

Returning to considerations of collective behaviour in fish, a common tendency in previous studies of response to sound is to focus on the individual, rather than the group in species that commonly aggregate. Until recently, observations of group response to sound was usually anecdotal, and not specifically focused on the impacts on collective behaviour per se (e.g. evasive diving by schools in response to approaching motorised vessels, Gerlotto & Fréon, 1992). However, in a recent study Herbert-Read et al. (2017) explored the response of groups of sea bass (*Dicentrarchus labrax*) to playback of either ambient background natural sound or pile-driving. In contradiction to expected observations of antipredator-like behaviour, the schools became less cohesive and directionally oriented under the pile-driving treatment. Further, these shoals became less correlated in speed and directional changes. In contrast, a recent study looking at pulsed puretones on the group behaviour of Eurasian minnow noted opposite impacts regarding cohesion, speed and directionality (Currie et al., 2020). Although such studies provide useful insights to enhance understanding of the collective behaviour of fish in response to human-generated sound, more work is needed to compare the response between groups and individuals to isolate the influence of collective behaviour.

The current investigation adopted a reductionist experimental approach to quantify the influence of underwater sound, a well-defined broadband random noise field (60–2,000 Hz), on the behaviour of solitary individuals and groups of five Eurasian minnow. To meet this aim, four key objectives and associated working hypotheses based on expectations of response to sound being analogous to an antipredator-like response, were developed. These focused on quantifying: (1) swimming speed for individuals and shoals (Hypothesis 1: swimming will be faster during exposure to a sound stimulus, but less so for groups compared to individuals as a result of perceived safety in numbers); (2) persistence of the swim path for individuals and shoals (Hypothesis 2: paths will be less predictable and more erratic under the sound treatment, but less so for groups than individuals); (3) group cohesion (Hypothesis 3: shoals will be more closely grouped under the sound treatment); and (4) orientation of group members (Hypothesis 4: shoals will be more polarised in the presence of sound). The results of this study are valuable because they provide important information relating to variation in response between individuals and groups of fish under controlled experimental settings and that differ from those obtained previously for marine species.



**FIGURE 1** Sound pressure level (SPL) using a 60–2,000 Hz broadband stimulus across an experimental water tank, measured over a  $5 \times 5$  cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of an acoustic field on shoaling behaviour of the Eurasian minnow (*Phoxinus phoxinus*). The colour scale represents the SPL at a point, expressed in dB re  $1 \mu\text{Pa}$ , with dark shades corresponding to lower intensities than light shades

## 2 | METHODS

### 2.1 | The use of tank experiments and selection of the model species

There has been recent debate over the validity of tank versus field studies in the investigation of fish response to acoustics, with recent recognition that tank experiments are a valid approach provided care is taken to accurately measure in-tank acoustic fields (Leighton et al., 2019). Indeed, some important field scenarios (e.g. when acoustic deterrents are placed within freshwater infrastructure to deter fish from water extraction points) resemble tank conditions more than they do *natural* conditions (Leighton et al., 2019). We employed the classical manipulative experiment, which allowed quantification using videography of fine-scale behaviours exhibited by the fish in response to the manipulation of the factor of interest, in this case exposure to a well-defined acoustic field (rather than the commonly used playback recordings of anthropogenic activities, such as shipping noise, obtained in the field), while confounding variables are controlled. This allowed quantification of fish response to predefined acoustic fields to be achieved.

The model species was selected due to their propensity to shoal, and ease of capture and maintenance in captivity, so enabling their history (e.g. health) to be ascertained and monitored prior to testing, and their conservation status (least concern). We exposed the fish to a broadband random noise field (60–2,000 Hz), which was selected to cover the hearing range expected for the Eurasian minnow based on current understanding for other similar species (e.g. fathead minnow, *Pimephales promelas*, Scholik & Yan, 2001). This frequency range is also representative of anthropogenic derived sound (such as boat traffic) in shallow waters (Kozaczka & Grazyna, 2011) that freshwater fish would be likely to experience.

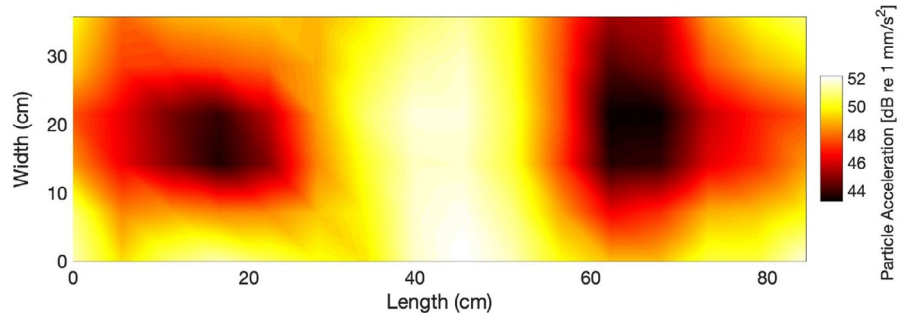
### 2.2 | Experimental set-up

An experiment to investigate the behavioural response of individuals and groups of minnow to sound was conducted in a transparent acrylic tank with 1 cm thick walls (30 cm wide, 30 cm deep, 300 cm long)

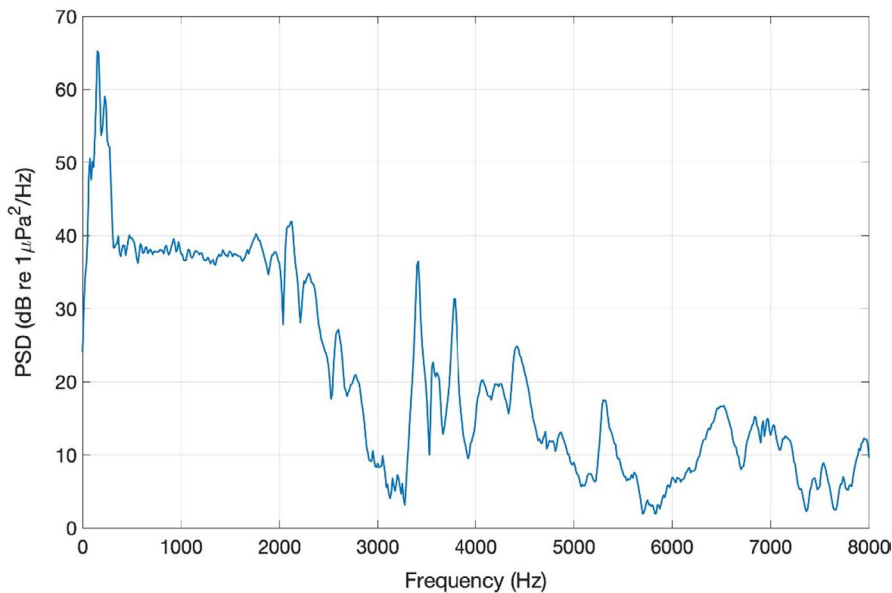
holding standing water at the International Centre for Ecohydraulics Research (ICER), University of Southampton, U.K. An experimental arena (85 cm long) in which the subject fish were introduced during trials was created in the centre of the tank by installing two white partitions at either end. A speaker (Electro-Voice UW-30) was placed in mid-water 10 cm behind each partition. White sheeting was placed around the tank to increase contrast of the video recordings obtained from an overhead camera (Logitech c920 Webcam) mounted 1.5 m above the floor. Illumination was provided from lighting units placed below the tank. An even distribution of lighting was achieved by using photographic diffusers and by projecting light onto the white tank walls. The camera was connected to a laptop running QuickTime to capture the video data at 30 Hz frame rate. Water temperature was measured throughout the experimental period at  $16.2 \pm 1.1^\circ\text{C}$  (mean  $\pm$  SD).

A data acquisition (DAQ) system (National Instruments USB-6341), controlled via a laptop computer, was used to generate the stimulus and measure the acoustic field. The stimulus was created by constructing a signal in MATLAB by filtering pseudo-random Gaussian noise using a sixth-order band-pass Butterworth filter, with the pass band limits being 60 and 2 kHz. This signal was played, via the DAQ, to a Skytronic 103.100 Mini AV digital amplifier connected to the underwater speaker. The signal was pulsed on for 2 s and off for 1 s, from both speakers simultaneously, and repeated for 10 min. The loudspeakers were switched on during the control period, without playing the stimulus, to control for electric field effects. The experimental area was subsequently mapped using a hydrophone (Bruel and Kjaer 8103) connected to a charge amplifier (Bruel and Kjaer 2635) and acquired on to the computer via the DAQ. The sound pressure level was measured over a  $5 \times 5$  cm grid pattern at three depths (2, 13, and 24 cm above tank floor) and seven frequencies (80, 100, 200, 400, 800, and 1,000 Hz and the broadband stimulus) to quantify the acoustic field within the tank (e.g. Figure 1). The particle acceleration of the sound field was computed using the same dataset and exploiting a gradient based approximation (Figure 2). Equation 1 was used to calculate the particle acceleration ( $a$ ), where  $\rho$  represents the ambient density and  $P$  the complex pressure amplitude (Kinsler, Frey, Coppens, & Sanders, 1982).

$$a = -\frac{1}{\rho} \nabla P \quad (1)$$



**FIGURE 2** Particle acceleration as measured from a 60–2,000 Hz broadband stimulus across an experimental water tank, measured over a  $5 \times 5$  cm grid at a depth of 13 cm above the tank floor. The tank was used in an experiment to quantify the effects of an acoustic field on shoaling behaviour of the Eurasian minnow (*Phoxinus phoxinus*). The colour scale represents the particle acceleration at a point, expressed in dB re  $1 \text{ mm/s}^2$ , with dark shades corresponding to lower accelerations than light shades



**FIGURE 3** Power spectral density (PSD) frequency response as measured from the centre of the tank with the stimulus playing

Using the same acoustic equipment as used for the mapping, the power spectral density of the stimulus was measured at the central location in the experimental arena. For this measurement, a stimulus of 60 s duration was employed (Figure 3).

### 2.3 | Fish collection and maintenance

Eurasian minnow ( $n = 120$ , mean  $\pm$  SD total length and mass =  $56.90 \pm 4.55$  mm,  $1.66 \pm 0.47$  g) were collected from the River Itchen (St. Catherine's Hill, Winchester, U.K., 51.049783–1.311416) using a 10 m seine net on the 13 May 2016. The site of capture (mean  $\pm$  SD width =  $18.07 \pm 2.03$  m; depth =  $0.46 \pm 0.24$  m) is typical of southern English rivers and those commonly found across Europe. The bed is formed of chalk with loose clay and silt with occasional flint pebbles. There is minimal submerged vegetation but a large quantity of riparian vegetation during the spring and summer months. The banks are engineered with the most common materials being solid concrete reinforced with metal sheeting, concrete sandbags supporting wooden planking and compressed chalk.

This is typical of a riverine freshwater environment in the U.K. in that it is not a *pristine freshwater ecosystem* but it is extensively anthropogenically modified and influenced (Maltby et al., 2011).

After capture, the fish were transported in a 100-L aerated container to the holding facility at ICER where they were maintained in a tank (150 cm wide, 150 cm long, 100 cm deep) with a water depth of 70 cm prior to conducting the trials. The holding tank water was filtered and aerated and quality tested daily. Regular water changes (approximately 25%) ensured high quality was maintained (nitrite  $< 1$  mg/L and nitrate  $< 50$  mg/L). The mean  $\pm$  SD water temperature in the holding tank remained stable throughout the study at  $17.0 \pm 1.6^\circ\text{C}$ . Fish were held for a mean of 180.4 hr (range = 189.8 hr) and fed a diet of dried animal protein based pellet food until satiation at the end of each working day.

### 2.4 | Experimental trials

Forty 50-min trials were conducted between 29 and 31 May 2016, and treatments alternated between using a solitary individual and a

group of five fish. Fish were placed in the experimental area at the start of a 30-min acclimation period. A further 10-min pre-treatment (control) period followed during which the acoustic stimulus was absent, before the fish were exposed to the sound (treatment) for 10 min. At the end of each trial, the fish were weighed and measured. No fish was used more than once.

## 2.5 | Fish behaviour

Analysis of video recordings allowed both qualitative and quantitative measures of behaviour to be determined. Behaviours observed during the acoustic treatment were compared with those recorded 10 min prior (pre-treatment control). The influence of sound on behaviour was quantified using the: (1) speed fish moved at; (2) persistence of the swimming paths; (3) cohesion of the shoal; and (4) orientation of members of the group. Qualitative descriptions of initial behaviour in response to the first presentation of the stimulus were recorded, either as: (1) startle, in which the test fish exhibited a sudden rapid burst and brief acceleration of velocity (Andraso, 1997); (2) station holding, in which a fish stopped moving and momentarily maintained position in the same location; and (3) no discernible response, where no change in behaviour was apparent. For shoals, the response of the largest group was noted; however, in practice there was no variation from this majority.

Swimming speed, persistence of swim paths, group cohesion, and orientation of fish within the group relative to other members were quantified. Using data obtained from the video recordings, 2D coordinates of fish position relative to the walls of the tank were obtained using an automated image processing routine implemented in MATLAB 2016a which provided location and orientation of the fish in each video frame. The position of the  $i^{\text{th}}$  fish in the  $n^{\text{th}}$  video frame was represented as the vector  $\underline{X}_i(n)$  which is defined as:

$$\underline{X}_i(n) = (x_i(n), y_i(n))^t \quad (2)$$

$x_i(n)$  representing distance along the length of the tank of the  $i^{\text{th}}$  fish in frame  $n$  and  $y_i(n)$  corresponding distance across the width of the tank. During video processing, the position of an individual fish was defined as the centre of mass of those pixels associated with it.

When analysing the movement of the groups of fish, location was based on the shoal's centroid,  $\underline{X}_c(n)$ . The shoal's centroid position was calculated using:

$$\underline{X}_c(n) = (x_c(n), y_c(n))^t = (\underline{X}_1(n) + \underline{X}_2(n) + \underline{X}_3(n) + \underline{X}_4(n) + \underline{X}_5(n))/5 \quad (3)$$

(i) *Swimming speed* was calculated for both shoals and individuals,  $v_c(n)$ , based on the motion of the centroid of the group or individual. It was evaluated by first computing the change in position between two frames. For shoals this was  $d\underline{X}(n) = \underline{X}_c(n) - \underline{X}_c(n-1)$ , whereas for individuals it was  $d\underline{X}(n) = \underline{X}(n) - \underline{X}(n-1)$ . The speed was the length of

this vector divided by the time interval between two frames (in this case  $\delta = 0.033$ , corresponding to 30 frames/s):

$$v_c(n) = d\underline{X}_c(n)/\delta \quad (4)$$

(ii) *Persistence* of the swim path provided a measure of its predictability, low persistence corresponding to more erratic movements. Persistence described the difference between expected, based on the trajectory of prior positions, and observed location recorded for individuals or groups at each time step. Specifically, using the locations of the fish/group in two preceding frames,  $n-2$  and  $n-1$ , then assuming the fish was swimming at constant speed along a straight line, the location in the  $n^{\text{th}}$  frame was predicted. The prediction denoted as  $\underline{P}_c(n)$  was defined as:

$$\underline{P}_c(n) = \underline{X}_c(n-1) + \underline{X}_c(n-1) - \underline{X}_c(n-2) = 2\underline{X}_c(n-1) - \underline{X}_c(n-2) \quad (5)$$

Persistence was based on the distance between the predicted (4) and observed location, so that:

$$\underline{E}(n) = \underline{X}_c(n) - \underline{P}_c(n) \quad (6)$$

The persistence is quantified by the norm (length) of the vector  $\underline{E}(n)$ .

(iii) *Cohesion* of the group was quantified by measuring the standard deviations of the locations on the x and y axis and combining them. This was defined as:

$$\sqrt{\frac{1}{5} \sum_{i=1}^5 (x_i(n) - x_c(n))^2} + \sqrt{\frac{1}{5} \sum_{i=1}^5 (y_i(n) - y_c(n))^2} \quad (7)$$

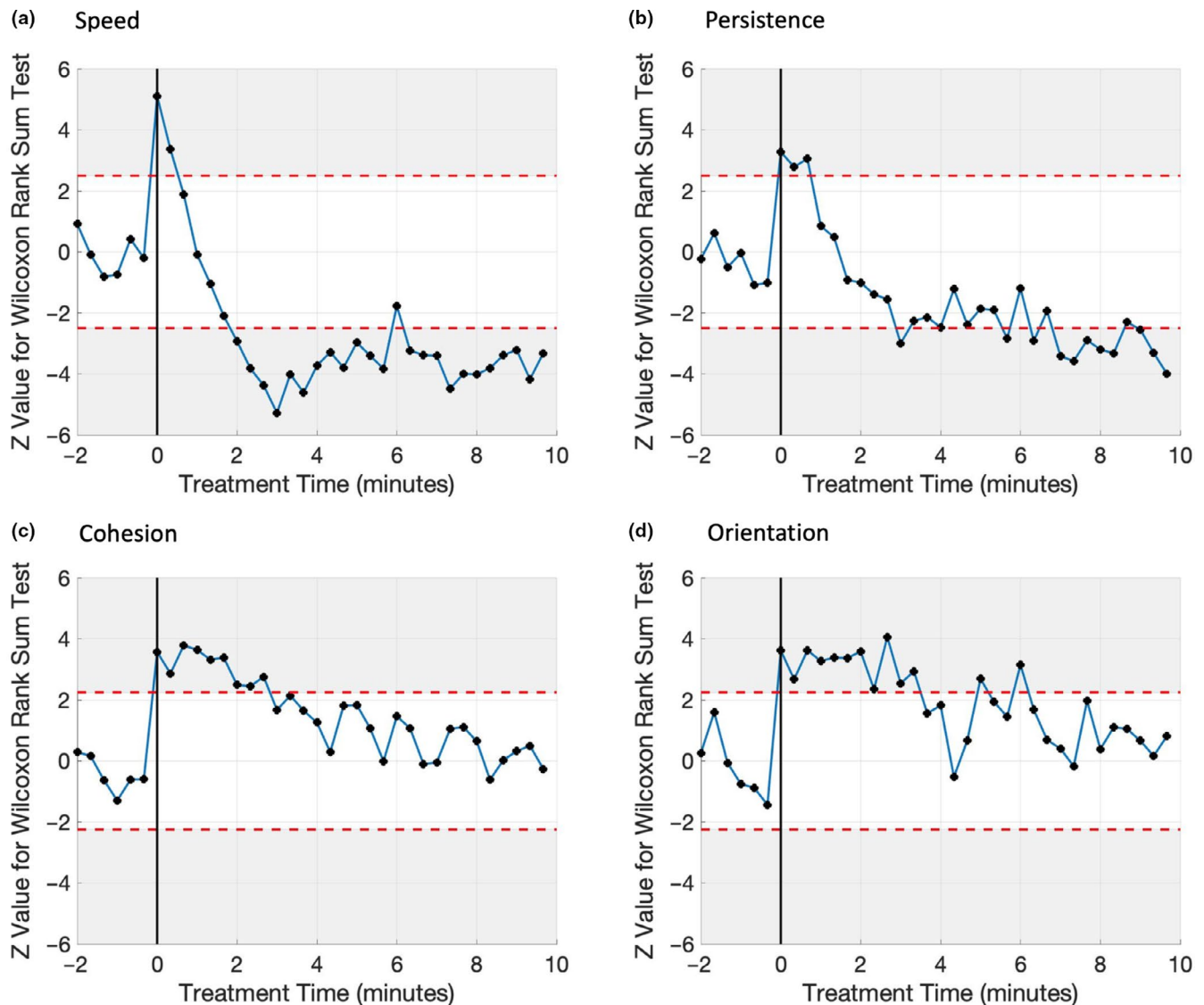
(iv) *Orientation* of each individual fish,  $(\theta_i(n))$  was represented by an angle in the range  $-90^\circ$  to  $90^\circ$  and represented the direction in which the body of the fish was aligned. This orientation does not account for the heading of the fish due to an inability of the analysis software to determine heading. For example, the software does not recognise the difference between a fish swimming horizontally to the right or left. Mean orientation for the group was defined as:

$$\theta_c(n) = (\theta_1(n) + \theta_2(n) + \theta_3(n) + \theta_4(n) + \theta_5(n))/5 \quad (8)$$

How dissimilarly oriented individual fish were in relation to each other was defined as the standard deviation of the orientations:

$$\theta(n)_{SD} = \sqrt{\frac{1}{5} \sum (\theta_i(n) - \theta_c(n))^2} \quad (9)$$

The standard deviation of the orientation is a measure of how aligned the fish are relative to each other (a low value of  $\theta(n)_{SD}$  corresponding to a high degree of alignment). Note that the absence of flow and use of optical screens worked to reduce anisotropic external stimulus.



**FIGURE 4** Z-values calculated using the Wilcoxon rank sum test (WRST) of four behaviours exhibited by fish under experimental conditions. The figure above displays the swimming speed of individual fish (a), the persistence of individual fish swim paths (b), the cohesion of 20 groups of five fish (c) and the similarity of orientation of 20 groups of five fish (d). The figure displays 2 min of pre-treatment followed by 10 min of treatment time. The black line at 0 indicates the start of the treatment. The circles indicate the data points with lines connecting them to show behavioural trends over time. The white area in the middle is an area where values display no significant difference from previously measured behaviour. The grey areas, marked by the dashed red line, at the top and bottom of the plots indicate where significant differences were observed. Sections a and b have this line set at 2.5 and -2.5, which reflects a significance threshold of  $p = 0.0125$ . Sections c and d have this line set at 2.25 and -2.25, which reflects a significance threshold of  $p = 0.025$ . The Y axis contains the Z-values (test statistic) from the WRST where a stronger behaviour results in a more positive number, except for b where a more positive reading refers to less persistent behaviour

The four metrics were calculated for every frame in the image. The data were averaged for over 1 s (30 frames). To reduce noise and mitigate against tracking artefacts, a median value was calculated for every 20 s (block) for each trial. For each time block, the distributions of the values arising from the 20 trials (10 control and 10 treatment) were compared using a Wilcoxon rank sum test. This allowed quantification of the significance of an effect as a function of time, providing some information about the temporal evolution of the effects. We sought an overall probability of a type I error, which is 5%. Since multiple tests were applied to the data a Bonferroni correction was

applied, reducing the threshold for significance for each individual test. Such a correction assumes that tests are statistically independent; in this instance, the metrics tested were likely to be dependent, consequently the application of Bonferroni was expected to result in an overall likelihood of a type I error lower than 5%. Therefore, the corrected significance threshold for individual behaviours (speed and persistence) to a significance threshold of 0.025 and the group behaviours tested (speed, persistence, cohesion, and orientation) were corrected to a significance threshold of 0.0125. Effect size was also considered by computing Cohen's  $d$  where 0.8 is a large effect

size and 0.5 is a medium effect size. We were not able to measure small effect sizes with our sample size of  $n = 20$ . The tests compared data from control and treatment blocks that were separated by 10 min, where the control block represented a 20-s period 10 min prior to the corresponding 20-s treatment block. This process resulted in a sequence of 30 Z-values covering each of the 20-s blocks in the 10-min treatment period. This time-series of Z-values allowed the temporal persistence of changes in behaviour to be quantified (Figure 4).

### 3 | RESULTS

All groups exhibited a startle response on activation of the acoustic stimulus (Table 1), whereas only 45% of individuals did so. Thirty percent of individuals held station when the treatment was initiated, whereas the remainder (25%) displayed no obvious reaction.

#### 3.1 | Swimming speed

Individual fish initially swam more rapidly during the treatment period than under the control. For 20 s immediately after the stimulus was turned on, there was a large effect ( $Z = 5.11, p < 10^{-7}, d = 1.14$ ; Figure 4a), which remained for the first 40 s. After the initial period of rapid swimming had subsided, the swimming speed slowed, so that after 5 min there was a medium sized effect observed resulting in the swimming speed significantly lower than during the pre-treatment control period for all but a single data point. For groups, there was only one time block (2 min after treatment onset) when swimming speed reduced significantly below observed levels in the control period ( $Z = -2.54, p < 0.01, d = 0.56$ ).

#### 3.2 | Persistence

For individuals, the swim paths were less persistent, i.e. more erratic, during the treatment compared to the control. Immediately after the

**TABLE 1** Behaviours displayed by Eurasian minnow on initiation of an acoustic stimulus

	Startle	Holding station	No discernible reaction
Group	20	0	0
Individual	9	6	5

*Note:* One reaction type is noted per trial. The behaviours were defined as: (1) startle, in which the test fish exhibited a sudden rapid burst and brief acceleration of velocity (Andraso, 1997); (2) station holding, in which a fish stopped moving and momentarily maintained position in the same location; and (3) no discernible response—where no change in behaviour was apparent. For shoals, the response of the largest group was noted. The behaviour of individual fish, maintained in the experimental tanks in isolation, was compared to groups to identify any important changes in the three reaction types.

onset of the stimulus, for the differences between control and treatment there was a medium effect ( $Z = 3.27, p < 0.0011, d = 0.73$ ) that remained for a further 20 s (Figure 4b). From 3 min onwards a medium sized effect was once again apparent, but this time because the paths were more persistent (less erratic) than during the pre-treatment control, and remained so for the majority of the remainder of the trial. For groups, there was generally no difference in persistence between treatment and control, with only one instance where the group was less persistent during the first 20 s measurement ( $Z = 2.20, p = 0.03, d = 0.49$ ), but reverted to a non-significant level after this period. Note that this value did not drop below the significance threshold of  $p = 0.0125$ .

#### 3.3 | Cohesion

For groups, initially there was a large effect on cohesion, with fish being more closely gathered together immediately following activation of the acoustic stimulus; this effect persisted for 2 min 40 s ( $Z = 3.57, p < 10^{-4}, d = 0.82$ ; Figure 4c).

#### 3.4 | Orientation

There was a large effect on the orientation of groups, with fish being more commonly oriented for 3 min 20 s after the acoustic stimulus was turned on, and the difference between treatment and control was greatest during the first 20 s ( $Z = 3.61, p < 0.0003, d = 0.802$ ; Figure 4d).

### 4 | DISCUSSION

Previous research to investigate the response of fish to anthropogenic sound have typically been biased towards solitary individuals and marine species, and as a consequence the influence of collective behaviour in species that frequently form aggregations, and the potential impacts in freshwater environments, has largely been ignored. Our study addressed this by comparing the response of solitary individuals and groups of a common shoaling freshwater species, the Eurasian minnow, to an accurately measured and well-defined appropriate acoustic field, rather than using play back of human-generated sounds collected in unrepresentative environments. The results support the hypothesis that the response to sound is akin to that exhibited in the presence of a predatory threat (Frid & Dill, 2002), and that this differs between groups and individuals. As predicted, solitary fish tended to swim more rapidly and follow less predictable trajectories immediately after exposure to an acoustic signal, during which a startle response was exhibited by 45% of individuals. For groups, the startle response at the onset of the treatment was universal and fish became more closely grouped and aligned, although this response declined with time. This study offers interesting insight into similarities and differences between responses to

acoustic disturbance and predatory threat for individuals and groups of freshwater fish.

In this study, a startle response was common for solitary fish on exposure to the sound stimulus, followed by an increase in swimming speed immediately thereafter, and then a decrease over time to levels lower than the pre-treatment control. At the same time, solitary minnows initially moved along less predictable paths, as evidenced by a lower persistence, but then settled to exhibit more predictable movement than during the pre-treatment control. In contrast, although all groups startled, presumably because of a higher probability that one or more members would elicit a response that would propagate through the shoal, the response was not as dramatic as for individuals, with no change in swimming speed or persistence. Herbert-Read et al. (2017) observed a decrease in swimming speed of juveniles of a marine species, the sea bass, during acoustic treatments, which is contrary to previous observations of faster swimming reported by others (e.g. Fewtrell & McCauley, 2012, for several marine species). Although a lack of a change in speed appears to contradict the concept of fleeing, slow speeds may bestow benefits related to the identification and greater monitoring of risk, such as a chasing predator (Domenici, 2010). In the case of the current study, although a lack of an effect was unforeseen, a lesser influence of an acoustic signal on group swimming speed and persistence compared to individuals was predicted, indicative of greater security associated with being a member of a group due to antipredator benefits. Alternatively, a lack of a change in these behaviours after the initial startle may have indicated the maximisation of information transfer and accuracy, rather than speed of response, a logical strategy in the absence of visual evidence of an imminent directional threat.

After startling, all shoals grouped closer together under the acoustic treatment in line with our hypothesis. This differs from the observations of Herbert-Read et al. (2017) who found that the shoals of juvenile sea bass became less cohesive during the acoustic treatment. However, our results are similar to those previously described by others in which greater group cohesion is linked to an acoustic signal (e.g. Fewtrell & McCauley, 2012 for several species of caged marine fish; Neo et al., 2015 for zebrafish [*Danio rerio*] in a small tank; Neo et al., 2014 for European sea bass enclosed in an outdoor basin). However, in our study the increased shoal cohesion observed on first exposure to the stimulus was relatively short-lived, with a return to the pre-treatment levels after only approximately three minutes. Although fish appeared to exhibit an antipredator-like response on initial exposure to the stimulus, this waned relatively quickly, presumably due a lack of reinforcing stimuli that may operate through alternative modalities (e.g. mechanosensory of visual systems).

In the current study the aligned polarity of the shoals initially increased on exposure to sound as predicted, but like group cohesion the effect was relatively short-lived, potentially reflecting the lack of reinforcement associated with alternative signals (such as a visual threat). Maintaining a common direction of orientation is likely to enhance the coordination of escape, as indicated in others studies of fish response to sound (e.g. Domenici & Batty, 1997 for schools

of herring [*Clupea harengus*]). Interestingly, a recent modelling study to simulate schooling predicted that a slower but more accurate response would be exhibited by groups that become more commonly oriented after detecting a threat, than for those that are already strongly aligned (Chicoli & Paley, 2016).

The impact of anthropogenic noise on the marine environment has been a subject of conservation concern for a number of years as evidenced by its consideration in international legislation, such as the EU Marine Strategy Framework Directive (2008/56/EC). Despite recent attention directed to the use of passive acoustics (Desjonquères, Gifford, & Linke, 2020; Rountree & Juanes, 2020), ecoacoustics (Linke & Deretic, 2020; Linke, Gifford, & Desjonquères, 2020) and freshwater acoustic monitoring (Desjonquères, Rybak, et al., 2020; Gottesman et al., 2020), the particular issue of the *impact* of anthropogenic noise in freshwater environments remains much less often considered than in the marine environment (Hanache et al., 2020; Higgs & Humphrey, 2020). This is of particular importance when considering the potential magnitude of influence probably being higher due to the extent of human activities (e.g. urbanisation, industry, transportation, agriculture) and engineering that occur along the world's rivers and lakes (Leighton et al., 2019). Whilst the causes and impacts of disturbance over larger temporal periods are being increasingly understood, for example flooding disturbance and trophic structure (Jellyman & McIntosh, 2020), this study provides experimental evidence of the impact of acoustic disturbance on the short-term behaviours of shoals and individuals of a model species. Furthermore, it indicates that the response within a species is context dependent, and varies depending on whether fish are members of a group or solitary. This is especially important when considering the changes in behaviour displayed over the time scales described in this study, and the ubiquitous presence of anthropogenic sound in many of the worlds developed rivers. The influence of anthropogenic sound on ecology should continue to be considered in addition to more commonly understood impacts, such as the fragmentation and degradation of physical habitat, disruption to hydrological regimes (Jellyman & McIntosh, 2020), and reductions in water quality, associated with human activity.

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#### ETHICAL APPROVAL

Ethical approval for this study was provided by the University of Southampton Animal Welfare and Ethical Review Board (ERGO ethics submission no. 19783). Permission to remove Eurasian minnow from the River Itchen was provided by the Environment Agency (permit reference EP/EW083-L-263/5065/02).



## DATA AVAILABILITY STATEMENT

The datasets generated and analysed during the current study are openly available from the University of Southampton repository at <https://doi.org/10.5258/SOTON/D1572>.

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