Dynamic visual noise promotes social attraction, but does not affect group size preference, in a shoaling fish

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ARTICLE INFO

Article history:
Received 12 November 2020
Initial acceptance 7 December 2020
Final acceptance 9 March 2021
Available online 20 May 2021
MS. number: 20-00828R

Available online 20 May 2021

Keywords:
shoal attraction
shoal choice
social information
visual noise
water caustics

The survival of organisms is often determined by their ability to detect resources (Johnson & Andersson, 2002; Willson, 1971) or predators (Devereux, Whittingham, Fernández-Juricic, Vickery, & Krebs, 2006; Fleishman, 1986; How et al., 2015), while reproductive success is dependent upon an organism’s ability to seek, court and/or defend potential mates, a process that is underpinned by the detection of social cues (Endler & Thery, 1996; Marshall & Stevens, 2014; O’Donnell, Ford, Shine, & Mason, 2004). The efficacy of an organism to acquire information is determined not only by the salience of a given sensory cue but also by the relative levels of sensory noise in the environment (Merilaita, Scott-Samuel, & Cutilli, 2017). Indeed, the decision making of animals can be largely understood within the framework of signal detection theory (Green & Swets, 1966; Swets, 1964), whereby the ability of an animal to detect and respond to information is shaped by the level of sensory noise in the environment. Recent studies have documented how anthropogenic sources of noise (Jezek & Mathews, 2020; Kunc, McLaughlin, & Schmidt, 2016) can physically mask or disrupt the detection of signals or cues (Halfwerk et al., 2011; Halfwerk & Slabbekoorn, 2015; Kunc, Lyons, Sigwart, McLaughlin, & Houghton, 2014; Vasconcelos, Amorim, & Ladich, 2007), induce negative attentional shifts (Chan & Blumstein, 2011; Purser & Radford, 2011) and impact the cohesion of social groups (Herbert-Read, Kremer, Bruinjes, Radford, & Ioannou, 2017; Sarà et al., 2007). Sensory noise, however, is not limited to anthropogenic interference, with animals having been exposed to different forms of ecological noise over their evolutionary histories. Indeed, natural habitats contain a host of noisy features, which may serve to distract an organism’s attention or disrupt its ability to detect signals or cues of importance. One natural feature of both terrestrial and aquatic environments is the presence of visual noise, which arises as a by-product of irrelevant motion cues within an environment, ultimately affecting the ability of animals to extract biologically relevant cues from their surroundings. For example, the
presence of windblown vegetation can force animals to alter the visual signals exhibited during territorial displays (Bian et al., 2018, 2019; Ord, Peters, Clucas, & Stamps, 2007; Ord & Stamps, 2008; Peters et al., 2007, 2008; Ramos & Peters, 2016), as well as accentuate motion masquerade employed by mobile organisms (Bian, Elgar, & Peters, 2016; Fleshman, 1985, 1986; Gans, 1967; Ryerson, 2017). Moreover, recent investigations highlight that this effect is not limited to the motion of physical structures within a habitat, but extends to the false motion cues elicited by dynamic illumination, such as dappled light and water caustics (Cuthill, Matchette, Cheney, Marshall, & Negahdaripour, 2009).

Water caustics, or wave-induced light flicker, are an optical phenomenon created by the refraction and convergence of light rays through the curvature of a water surface (Berry & Upstill, 1980; Joets, 2012; Lock & Andrews, 1992; McFarland & Loew, 1983; Nye, 2018; Swirski, Schechner, Herzberg, & Negahdaripour, 2009). When projected upon a substrate parallel to the water surface, water caustics comprise a mosaic of low-intensity, polygonal patches that are irregularly enclosed by high-intensity light (Fig. 1a; Matchette, Cuthill, Cheney, Marshall, & Scott-Samuel, 2020; McFarland & Loew, 1983). These mosaics warp and stretch when projected over three-dimensional objects (Matchette et al., 2020; McFarland & Loew, 1983), and when the water surface is disturbed by wind and waves, the light intensity at any specific location flickers over time (Matchette et al., 2020; McFarland & Loew, 1983). Because their formation depends only on sunlight and water surface disruption (McFarland & Loew, 1983), caustics are a common feature of shallow aquatic habitats, including coral reefs, mangroves, rivers, streams, lakes and intertidal zones. Some studies highlight the possible influence of water caustics in fish patterning (Loew & McFarland, 1990) and the early evolution of colour vision (Maximov, 2000), while others highlight how caustics may aid the detection of objects in midwater (McFarland & Loew, 1983) but hinder the detection of objects near the substrate (Attwell, Ioannou, Reid, & Herbert-Read, 2020; Matchette et al., 2020).

While some studies have demonstrated that visual noise created through the motion of physical structures (e.g. windblown vegetation) can influence social behaviours (Bian et al., 2019; Ord et al., 2007; Ord & Stamps, 2008; Peters, Hemmi, & Zeil, 2007), little is known about the effects of dynamic illumination on social behaviour. One context where this could be important is in the formation and maintenance of social groups, where in many aquatic species such as fishes, the formation of shoals is often reliant upon the detection of conspecífics, underpinned in part by individuals’ visual systems (Grünebaum, 1998; Herbert-Read, 2016; Herbert-Read et al., 2017; Partridge & Pitcher, 1980; Pitcher, 1979). Indeed, the changes to a light environment induced by an increase in turbidity have been shown to alter not only the colour pattern expression of fish, but also their shoaling behaviour (Ginnaw et al., 2020; Kelley, Phillips, Cummins, & Shand, 2012). Because caustics reduce the likelihood of an individual detecting and extracting information from its environment (Attwell et al., 2020; Matchette et al., 2018, 2020), social behaviour could be affected by this form of visual noise in two ways. First, caustics may increase individuals’ reliance upon social information, which could be reflected in individuals choosing to associate with larger social groups, either to access more social information, or to dilute the risks associated with having limited perception (e.g. a reduced ability to detect predators in noisy environments). Alternatively, caustics may hinder an animal’s ability to detect social cues, therefore disrupting its ability to respond to conspecífics. This may be reflected in a reduction in the ability to distinguish between group sizes or a change in social attraction towards conspecífics.

Here we asked whether the presence of varying levels of water caustic flicker affects the social decisions of three-spined sticklebacks, Gasterosteus aculeatus. When given a choice between two shoals, a variety of fish species, including sticklebacks, prefer to associate with the larger of two shoals (Dougherty, Ledesma, Bauer, & McRobert, 2010; Krause & Godin, 1994; Krause & Ruxton, 2002; Lachlan, Crooks, & Laland, 1998), underpinned by the associated benefits to grouping (Hamilton, 1971; Krause & Ruxton, 2002; Pulliam & Caraco, 1984), including reduced individual predation risk (Godin, Classon, & Abrahams, 1988; Godin & Morgan, 1985; Landeau & Terborgh, 1986; Lima, 1995; Lima & Zollner, 1996), increased foraging success (Krause, Hartmann, & Pritchard, 1999; MacGregor, Herbert-Read, & Ioannou, 2020; Pitcher, Magurran, & Winfield, 1982) and information sharing (Couzin, 2009; Ioannou, 2017; Mathis, Chivers, & Smith, 1996; Sumpter, Krause, James, Couzin, & Ward, 2008; Ward, Herbert-Read, Sumpter, & Krause, 2011). We used this experimental paradigm to assess the influence of caustic flicker on sticklebacks’ social behaviour. We predicted that increased visual noise could affect the social behaviour of sticklebacks in two ways. First, visual noise could either increase or decrease the likelihood that fish would associate with the larger

![Figure 1](image-url)

**Figure 1.** (a) Frame from an experimental trial showing the experimental arena with an individual (focal) fish within the central compartment and two differently sized (‘stimulus’) shoals in the end compartments, separated by transparent Perspex. The simulated caustics, a moving mosaic of low-intensity, polygonal patches that are irregularly enclosed by high-intensity regions, are shown. (b) Histogram of the focal fish’s distance from the centre of the tank to the end compartments across the longest tank axis. Blue bars indicate data where the fish was defined as interacting with the stimulus shoals.
conspecific shoals. Second, increased visual noise could affect the social attraction towards conspecifics, measured by either an increase or decrease in the distance individuals maintain from shoal members. A difference in a fish’s shoal choice or shoal attraction in different levels of noise could be affected by individuals showing more risk-averse behaviour (freezing, reduced exploration) in increased levels of visual noise. To account for this possibility, we measured (1) how often the fish visited each side of the arena, and (2) the activity of the fish measured as the fish’s median speed, as a function of visual noise.

**METHODS**

**Animals**

Three-spined sticklebacks (*N = 285*) were caught with hand nets from the network of streams that connect to the River Cam around Cambridge, U.K., where water caustics are often observed (S.R. Matchette & J.E. Herbert-Read, personal observation). Fish were released at the same locations once the study was complete. All procedures were approved by the Institutional Animal Care and Use Committee (IAUC Protocol Number: Z0080/20). Fish were housed in three large glass aquaria (tank 1: 1200 x 450 mm and 500 mm deep; tank 2: 1200 x 600 mm and 600 mm deep; tank 3: 1150 x 450 mm and 450 mm deep) that were exposed to a 12 h light cycle (0700–1900 hours) in a temperature-controlled room (12 °C). Aquaria were filled with aged and conditioned water, which underwent a 20% water change on a weekly basis. The average water temperature across aquaria was 10.5 °C. Housing aquaria contained several aerators, as well as a host of real and artificial plants for shelter and environmental enrichment. Each aquarium received three frozen cubes (12.5 g) of blood worm (Superfish®) daily.

**Experimental Equipment**

Two experimental arenas were positioned next to each other in a visually isolated cubicle, surrounded by black cloth to minimize external disturbances. Each experimental arena (500 x 250 mm and 205 mm deep) was made of opaque white Perspex and contained three independent compartments: one central compartment (300 x 250 mm and 205 mm deep) bookended by two end compartments (100 x 250 mm and 205 mm deep; Fig. 1a). The compartments were divided by transparent Perspex, which was sealed to stop water mixing between the end compartments and the central compartment (i.e. removing the potential for chemical cues from the stimulus shoals to influence the focal fish’s behaviour). Each section of the arena contained a 2 cm layer of Roman sand (Pettex, www.pettex.co.uk). Each experimental arena was filled with aged and conditioned water to 125 mm depth, with 1 litre of this total volume coming from a housing aquarium to saturate the arena with chemical cues. Between every fourth trial, 1 litre of water was removed from the experimental aquaria and replaced with 1 litre of aged and conditioned tap water.

We suspended an Epson EB-915W projector (Seiko Epson; Suwa, Japan, www.epson.co.uk/) 1540 mm above the experimental arenas so that it projected into both experimental arenas below. A HD Logitech C920 Pro webcam (Logitech; Lausanne, Switzerland; www.logitech.com/en-gb) was fixed in place at the same level and filmed both experimental arenas at the same time. The camera recorded each trial at 24 fps (1280 x 720 aspect ratio). The projector and the webcam were connected to a laptop (Macbook Pro) whereby the video playbacks of water caustics and the recording software could be managed. The projector made it possible to bathe the experimental aquaria with differing levels of visual noise of simulated water caustics. To create the playbacks of different levels of visual noise, 600 images of sequentially moving caustics were created using the Caustics Generator Pro software (Dual Heights, www.dualheights.se/caustics/; see software settings in Table A1), cropped to an aspect ratio of 3840 x 2159 pixels, mirroring those used by Attwell et al. (2020). These images were stitched together using MATLAB (The Mathworks Inc; Natick, MA, U.S.A.; www.uk.mathworks.com/products/matlab) so that, as the images were looped through, they created an animation of simulated caustics. To create animations with different levels of visual noise, different spatial and temporal properties of the caustics, such as the spatial complexity or contrast, could be manipulated. However, such manipulations would also change the average light intensity in the arenas. Therefore, we only manipulated the speed at which the animations looped, effectively changing how quickly the caustics flickered. We constructed four animations of different flicker speeds (noise levels; see Video S1); zero flicker (no looping of animation), slow flicker (animation looped every 80 s), medium flicker (animation looped every 10 s) and fast flicker (animation looped every 2.5 s). Each animation lasted 5 min and 20 s. From these animations, we constructed four different playbacks, where each playback contained all four different noise levels (Table A2). The order of noise levels within the playbacks was devised using a Latin square design, ensuring that each noise level occurred at each of the four possible time segments across the playbacks. Between each different noise level within the playbacks, transitional periods (e.g. from fast to slow, slow to medium; ranging from 80 to 90 s in length) were added to minimize abrupt changes to the visual scene. Overall, each playback lasted for a total of ca. 30 min. For each trial, focal fish were exposed to one of these four playbacks. We ensured that we presented an equal number of the different playbacks across all trials, and that the order of playbacks between trials was presented in a randomized block design (see Supplementary material for a data sheet).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.anbehav.2021.04.017

**Experimental Protocol**

The following protocol describes the experimental process for one trial of the two experimental arenas; however, this protocol occurred simultaneously for the pairs of experimental arenas trialled together. Two groups of fish (hereafter, ‘stimuli shoals’) were removed from one of the housing aquaria and placed in the two end compartments of the experimental arena. The average body length (hereafter, ‘size’) of the fish in each compartment was visually inspected to ensure an approximate match between the two stimulus shoals. The number of fish in each compartment was one of 12 different combinations, termed ‘shoal choice treatments’ (Table 1). For every treatment, the number of fish in one of the stimulus shoals was larger than the other. In fishes, discriminating between shoal sizes appears to rely upon their relative rather than their absolute differences in size, a feature that can be attributed to Weber’s Law (of Just Noticeable Difference; Gallistel & Gelman, 2000; Gómez-Laplaza & Gerlai, 2011; Moyer & Landauer, 1967; Thünken, Eigster, & Frommen, 2014). This perceptual difference between shoal sizes can be captured using the Michelson contrast (Michelson, 1927), a useful comparative metric with the following formula: \[ \text{Michelson contrast} = \frac{N \text{shoal1} - N \text{shoal2}}{N \text{shoal1} + N \text{shoal2}} \] where larger values indicate greater contrast difference and thereby ease of distinguishing between the shoal sizes. Indeed, this metric has been applied to understand the rules that underpin animal social decision making (Arganda, Pérez-Escudero, & De Polavieja, 2012; Gómez-Laplaza & Gerlai, 2011; Pern, Granovskiy, Garnier, Nicolis, & Labédan, 2012). The threshold Michelson contrast for
discriminating between shoal sizes varies between species and context. Zebrafish, *Danio rerio* (Arganda et al., 2012; Pritchard, Lawrence, Butlin, & Krause, 2001) and angelfish, *Pterophyllum scalare* (Gómez-Laplaza & Gerlai, 2011) show a preference for larger shoals at a Michelson contrast of ca. 0.33, while three-spined stickleback shoals (Fischer & Frommen, 2013; Frommen, Hiermes, & Bakker, 2009; Thinken et al., 2014) and creek chub, *Semotilus atromaculatus* (Krause, Godin, & Rubenstein, 1998) can distinguish between shoals more similar in size, at contrasts of 0.17 and 0.09, respectively. The contrast scale used in this study captured a range of contrasts that sticklebacks had, and had not, previously been shown to distinguish between (Table 1). The individual shoal sizes used in each comparison all fall within the natural range of stickleback shoals observed in the wild (Ward et al., 2017).

Stimuli shoals were given 10 min to acclimatize to the experimental aquaria. For each trial, an individual fish (hereafter, ‘focal fish’) was transferred from a housing aquarium to the central compartment of the experimental arena and allowed 5 min to acclimatize. Focal fish and stimulus fish were always taken from separate housing aquaria, thereby removing any potential effects of familiarity. All acclimatization was completed with the static noise level projected into the arena and in the full view of both stimulus shoals. Following acclimation, recording started, and the focal fish was exposed to one of the four playbacks. Upon completion of the trial, the focal fish was removed and placed in a housing aquarium separate to the original housing aquaria to prevent retrial; no focal fish were reused between trials. To minimize spatial bias, the two stimulus shoals were swapped between end compartments after two trials. After four consecutive trials, the stimulus shoals were replaced entirely for two new stimulus shoals. Fish that had been used as stimulus fish were housed in separate 40-litre housing aquaria and labelled according to the number of times the stimulus fish had been used. Stimulus fish were never reused on the same day and were used a maximum of three times. We ensured that the shoal choice treatments and video playbacks were presented evenly across each of the stimulus fish’s use. We housed stimulus fish in groups of ca. 30–40 individuals, making it unlikely that the same individuals were used in the same stimulus groups more than once. A total of 144 focal fish were tested. Each of the 12 shoal choice treatments had 12 replicates.

### Tracking, Trajectory Analysis, and Variables Measured

To track the fish in the trials, videos were uploaded to the software, Loopy (Loopbio, Vienna, Austria; [www.loopbio.com](http://www.loopbio.com)). Each video was subsequently cropped to include only the central compartment (containing the focal fish). Loopy implements a supervised machine learning method (termed ‘key point detection’) to learn and then identify specified objects in videos. We trained a model to identify the focal fish’s centroid in each video by manually annotating the focal fish’s position within ca. 200 frames of the trials. The trained model was then used to predict the location (x,y coordinates) of each focal fish across all frames of the videos. Tracks were then imported into MATLAB. We assessed the accuracy of the tracking data, first, by identifying the proportion of frames where the fish were tracked throughout the trials, and second, by comparing the automated tracking data to a manually tracked subset of randomly selected frames (for more details, see Appendix 1). Fish were tracked on average 98±2% (mean±1 SD) across all frames, and >99% of the automated coordinates were within half a body length of the manually tracked coordinates (Fig. A1). The size of each focal fish was measured using a bespoke MATLAB script, whereby the length of the fish was manually identified. The average total body length of the focal fish was 31±8 mm (mean±1 SD).

We used the fish’s trajectories to calculate the proportion of time the focal fish spent interacting with each shoal in each level of noise (excluding transitional periods). We classified a fish as interacting with a stimulus shoal when it was within 100 pixels (ca. 2.5 body lengths, ca. 80 mm) of the shoal. We chose this value as the distribution of observations beyond this distance was relatively uniform, suggesting less influence of the stimulus shoal on the focal fish’s behaviour (Fig. 1b). We then calculated a focal fish’s preference for the larger shoal as the proportion of time the fish spent interacting with the larger shoal minus the proportion of time the fish spent interacting with the smaller shoal at each level of noise. Preference scores greater or less than zero indicate the fish spent more time interacting with the larger or smaller shoal, respectively.

We also measured whether the strength of the focal fish’s attraction towards either shoal changed as a function of the level of visual noise level. To do this, on each frame we calculated the distance between the focal fish and the closest point along each of the two Perspex dividers. We then took the smaller of these two distances as the distance to the closest shoal and took a median of these two Perspex dividers. We used the *Mahalanobis distance* to learn and then identify specific objects in videos. We trained a model to identify the focal fish’s centroid in each video by manually annotating the focal fish’s position within ca. 200 frames of the trials. The trained model was then used to predict the location (x,y coordinates) of each focal fish across all frames of the videos. Tracks were then imported into MATLAB. We assessed the accuracy of the tracking data, first, by identifying the proportion of frames where the fish were tracked throughout the trials, and second, by comparing the automated tracking data to a manually tracked subset of randomly selected frames (for more details, see Appendix 1). Fish were tracked on average 98±2% (mean±1 SD) across all frames, and >99% of the automated coordinates were within half a body length of the manually tracked coordinates (Fig. A1). The size of each focal fish was measured using a bespoke MATLAB script, whereby the length of the fish was manually identified. The average total body length of the focal fish was 31±8 mm (mean±1 SD).

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Finally, we assessed how the level of noise affected the fish’s activity in the arena. To do this, we calculated the fish’s instantaneous

### Table 1

| Shoal choice treatment | No. of fish in large shoal | No. of fish in small shoal | Absolute difference | Michelson contrast | Shoal choice | Mean preference score | Lower/upper confidence interval | Post Holm–Bonferroni correction |
|------------------------|---------------------------|---------------------------|---------------------|-------------------|--------------|-----------------------|-------------------------------|---------------------------------|
| 1                      | 8                         | 3                         | 5                   | 0.45              | *            | 0.24                  | 0.03 / 0.45                   |                                 |
| 2                      | 7                         | 3                         | 4                   | 0.40              | *            | 0.40                  | 0.19 / 0.61                   | *                               |
| 3                      | 8                         | 4                         | 3                   | 0.33              | -0.07        | 0.07                  | -0.29 / 0.15                  |                                 |
| 4                      | 6                         | 3                         | 3                   | 0.33              | 0.09         | 0.09                  | 0.19 / 0.37                   |                                 |
| 5                      | 7                         | 4                         | 3                   | 0.27              | 0.04         | 0.04                  | 0.12 / 0.20                   |                                 |
| 6                      | 8                         | 5                         | 3                   | 0.23              | 0.03         | 0.03                  | -0.27 / 0.33                  |                                 |
| 7                      | 6                         | 4                         | 2                   | 0.20              | 0.21         | 0.21                  | 0.01 / 0.41                   |                                 |
| 8                      | 7                         | 5                         | 2                   | 0.17              | 0.14         | 0.14                  | 0.11 / 0.39                   |                                 |
| 9                      | 8                         | 6                         | 2                   | 0.14              | 0.16         | 0.16                  | 0.06 / 0.38                   |                                 |
| 10                     | 6                         | 5                         | 1                   | 0.09              | 0.17         | 0.17                  | 0.07 / 0.41                   |                                 |
| 11                     | 7                         | 6                         | 1                   | 0.08              | 0.14         | 0.14                  | -0.09 / 0.37                  |                                 |
| 12                     | 8                         | 7                         | 1                   | 0.07              | 0.04         | 0.04                  | -0.22 / 0.14                  |                                 |

The shoal choice column shows the output of the linear models, testing whether the preference score at each Michelson contrast in turn differed from zero (following line 4 nomenclature in R): preference score ~ 1 + (1|fish,identity). Positive t values denote an overall preference towards the larger shoal and negative t values denote an overall preference towards the smaller shoal. Asterisks denote significance with and without post Holm–Bonferroni correction (final column).
speed between frames, and then took the median of these speeds within each level of noise. We also manually scored the number of times the fish crossed the arena’s midline as an additional proxy for its activity in each level of noise.

**Statistical Analysis**

All statistical analyses were performed in R v. 3.3.2 (R Foundation for Statistical Computing, www.R-project.org), where we used linear mixed models (function lmer in the lme4 package; Bates et al., 2017). Preference scores, the median speed of fish, the distance to the closest shoal (inverse transformation) and the number of midline crossings (square-root transformation) were modelled with Gaussian error structures. Initially, each response model contained the full complement of fixed effects (termed, ‘maximal model’): Michelson contrast (continuous fixed effect), noise level (ordinal fixed effect) and their two-way interaction, fish size (continuous fixed effect), time segment (continuous fixed effect), the reuse of stimuli fish up to three times in the study (hereafter ‘stimuli reuse between days’; continuous fixed effect), and the reuse of stimuli fish for four consecutive trials (hereafter ‘stimuli reuse within days’; continuous fixed effect). Each maximal model was fitted with the full complement of random effects, including focal fish ID as a random intercept, time segment as a random slope (to control for any differences in the change in behaviour of individuals over the course of the trial), playback as a random intercept and the stimulus group identity as a random intercept. Using model simplification, we sequentially removed each fixed effect from the maximal model, and compared the deviance between models with and without the component in question (Crawley, 2011). Nonsignificant components were removed in turn (see Table A3 for a full model breakdown). The change in deviance between the models was tested against a chi-square distribution with degrees of freedom equal to the difference in degrees of freedom between the models. The model addressing the distance to the nearest shoal as the dependent variable included the inverse transformation of fish size as a fixed effect, given the latter shares the same units scale as the former (i.e. mm). Normality Q-Q plots and residuals versus fitted values were visually inspected to interpret the dispersion and distribution of the residuals. The effect sizes for each model component are also provided, represented by Cohen’s D, with larger absolute values of D denoting larger statistical effect size. Effect sizes were calculated using the lme4 dscore function from the EMAtools package (Kleiman, 2017).

To determine whether focal fish showed a preference for larger or smaller shools regardless of the Michelson contrast presented (shoal choice treatments), we tested whether the intercept of the preference score model was statistically different from zero. This is functionally equivalent to performing a paired t test (time with larger shoal versus time with smaller shoal), albeit with the inclusion of random effects. Here, the model contained the full complement of random effects outlined above (minus the random slope of time) but with a single intercept term replacing the fixed effects. Using lme4 nomenclature in R, the form of the linear mixed model was Preference score = 1 + (time | fish identity) + (1 | playback) + (1 | stimulus shoal identity), with ‘1’ representing the intercept term.

As a further a posteriori analysis, we assessed whether the focal fish showed a preference for the larger shoal at each individual Michelson contrast in turn. Like the overall shoal preference model above, we constructed linear mixed models for each Michelson contrast separately and tested whether the intercept term of each model statistically differed from zero. Random intercept terms included fish ID, to account for individual variation in shoal choice, and playback, to account for the variation in presentation order. In this analysis, a Holm–Bonferroni correction was applied to the alpha levels of significance to account for the effects of multiple testing (Holm, 1979). For completeness, we also tested whether noise level had an effect on preference score for the individual contrast levels in which fish showed a shoal size preference (as above, prior to applying the correction).

**RESULTS**

Focal fish showed a weak overall preference to associate with the larger of the two shools (mean proportion of time spent with larger shoal – mean proportion of time spent with smaller shoal = 0.13; t_{142} = 3.75, P < 0.001; Fig. 2a). However, the preference scores of focal fish were not affected by either Michelson contrast, noise level (Fig. 2a) or their interaction (Table A3). When individually assessing whether the fish showed a preference for the larger shoal at each Michelson contrast in turn, we could only detect that focal fish preferred the larger over the smaller shoal when the Michelson contrast was 0.45 (eight versus three fish), 0.40 (seven versus three fish) and 0.20 (six versus four fish; Table 1). When following the Holm–Bonferroni correction method, the only contrast level at which these preferences were statistically maintained was 0.40 (Table 1). At this contrast level, there remained no effect of visual noise level on the fish’s preference scores (contrast = 0.40, X^2 = 2.44, P = 0.486), nor was there an effect of visual noise at the contrast levels of 0.45 (X^2 = 1.40, P = 0.707) or 0.2 (X^2 = 1.42, P = 0.701). Fish size, time segment and the reuse of stimuli fish between days had no effect upon the fish’s preference score (Table A3). The reuse of stimuli fish within days, however, appeared to influence the fish’s preference score (Table A3, Fig. A2a), with fish exhibiting less preference for the larger shoal as the number of trials that stimulus shools were used for increased.

While the level of visual noise did not affect the focal fish’s preference scores, it did affect their social attraction towards the shools (Table A3, Fig. 2b). In particular, as visual noise level increased, fish moved significantly closer to the nearest shoal (regardless of whether this was the larger or smaller of the two shools), indicative of stronger social attraction. The variability in shoal proximity within each noise level was highest for the lowest noise levels (interquartile range: static = 21.5, slow = 15.6, medium = 11.6, fast = 13.4 mm). There was also an effect of fish size on the distance to the stimulus shools (Table A3), with smaller fish being closer to the nearest shoal. This is to be expected considering smaller fish can maintain physically closer distances to the shools. Social attraction was not affected by Michelson contrast, time or stimuli reuse (Table A3). There was also no interaction between noise level and Michelson contrast (Table A3) on the distance focal fish maintained from the stimulus shools.

There was no evidence that visual noise reduced the activity of fish, which would have suggested an increase in risk-averse behaviour. Instead, fish swam faster in higher levels of visual noise (Table A3, Fig. 2c), while the number of midline crossings did not increase or decrease in increased levels of visual noise (Table A3). Fish were not more likely to make crossings in the arena as a function of Michelson contrast (Table A3). The fish did, however, change their behaviour over the course of the trial. Both the median speed of fish and the number of midline crossings increased over time within a trial (Table A3), consistent with the fish habituating to the arena. As expected, the median speed of fish increased with fish size, but fish size did not affect the number of midline crossings (Table A3).

The reuse of stimuli fish between days affected the speed of focal fish (Table A3, Fig. A2), with the median speed decreasing as the number of times stimuli fish were used increased. Furthermore,
the reuse of stimuli fish within days influenced both measures of activity, with both the median speed of fish (Table A3, Fig. A2) and the number of midline crosses (Table A3, Fig. A2) decreasing as the number of trials that stimuli fish were used for increased.

**DISCUSSION**

While we predicted that the presence of visual noise could either increase or decrease the likelihood that sticklebacks associate with larger shoals, there was no evidence that visual noise or Michelson shoal contrast influenced the preference of fish to associate with different shoal sizes. Despite focal fish exhibiting a weak overall preference to associate with the larger of the two shoals, when assessing the preferences for each shoal contrast in turn, this preference could only be detected at the extreme shoal contrast levels. In particular, sticklebacks only showed significant preferences for the larger of the two shoals at a Michelson contrast of 0.40. At this contrast (seven versus three fish), the preference to associate with the larger shoal was also not affected by visual noise. In line with our second prediction, however, higher levels of visual noise increased social attraction towards conspecifics, with focal fish moving closer to the stimulus shoals, irrespective of the shoal’s size, in increased levels of visual noise.

In our study, while there was a weak overall attraction towards the larger versus smaller shoals, there was not enough power to detect a shoal size preference for Michelson contrasts below 0.40 (seven versus three). Other studies, however, have demonstrated discrimination by sticklebacks with similar sample sizes at contrasts of 0.33 (10 versus five, six versus three fish), 0.29 (nine versus five fish), 0.23 (eight versus five fish), 0.20 (12 versus eight, 60 versus 40 fish) and even as low as 0.17 (seven versus five fish; Fischer & Frommen, 2013; Krause et al., 1998; Thünken et al., 2014). These contrast values highlight the variation between studies in the size of shoals for which sticklebacks show preferences. Shoal size preference can be shaped by factors such as body size disparity (Hoare, Krause, Peuhkuri, & Godin, 2000; Lachlan et al., 1998; Mikheyev, 1995; Rodgers, Ward, Askwith, & Morrell, 2011) and prior experience (DeWissel, et al., 2010). Indeed, there are cases where fish may instead actively favour small shoals over larger ones (see Krause et al., 1998), or may simply exhibit a preference to associate with any shoal, irrespective of its size, rather than remain alone (Hoare et al., 2000; Krause & Ruxton, 2002; Pitcher & Parrish, 1993). This could occur, for example, when the costs and benefits of belonging to different shoal sizes are balanced (Agrillo & Dadda, 2007; Lachlan et al., 1998). The sensitivity of these shoal size preferences is further highlighted by our finding that preference scores changed with the reuse of stimulus fish within a day, unlike the protocols used in other studies (Fischer & Frommen, 2013; Krause et al., 1998; Thünken et al., 2014). If the shoal members’ behaviour changed over the course of these trials, which is likely considering the focal fish’s activity changed even within a trial, this could influence visual features of the shoals, such as their activity level (Gómez-Laplaza & Gerlai, 2012; Pritchard et al., 2001), density (Frommen et al., 2009) and visual saliency (Botham, Kerfoot, Louca, & Krause, 2005). Such changes to the visual features of shoals could have resulted in changes to the shoal size preferences. There is scope for these relatively simple assays to highlight how distinguishing and selecting between different shoal sizes are impacted by the sensory cues of shoals and individual behavioural states.

Even in the shoal size treatments where we could detect preferential association with the larger shoal sizes, noise level did not affect these preferences. Given a major component of shoal size discrimination is visual (Botham et al., 2005; Frommen et al., 2009; Pritchard et al., 2001), we predicted that increased visual noise might constrain a fish’s ability to extract information related to shoal size, much in the same way that visual noise disrupts a predator’s ability to extract information relating to a prey organism (Matchette et al., 2018, 2019, 2020). Indeed, the presence of dynamic visual noise can accentuate the concealment of motion cues (Matchette et al., 2018, 2019, 2020; Bian et al., 2016; Fleishman, 1985, 1986; Gans, 1967; Ryerson, 2007), impacting the detection of dynamic visual signals (Bian et al., 2018, 2019; Ord & Stamps, 2008; Peters et al, 2007, 2008; Ramos & Peters, 2016). We did not find this effect, however, with the overall shoal preference remaining unchanged as visual noise increased. This could be for two reasons. First, it may be that the strength of the visual cues used by fish to assess shoal size are so salient that visual noise did not mask them, therefore unaffecting shoal size discrimination. Alternatively, the detection of visual social cues may have been affected by visual noise, but moving closer to the stimulus shoals may have compensated for a reduction in ability to detect such cues. Because many species use visual cues to maintain cohesion (Grünbaum, 1998; Herbert-Read, 2016; Herbert-Read et al., 2017; Partridge & Pitcher, 1980; Pitcher, 1979), increasing social attraction in times of increased visual noise may allow them to improve their access to social cues, mitigating the effects that noise has on their ability to detect social information. Indeed, little
is known about how the structure and behaviour of shoals may change as a function of visual noise. Here, the stimulus shoals were also bathed in caustics; hence it is possible that their behavioural response to visual noise (e.g. in terms of activity level or density) may in turn promote increased social attraction by the focal fish. Further experiments that assess changes to shoal structure as a function of visual noise in finer detail are warranted.

Another reason social attraction may increase in times of increased visual noise is to compensate for a reduction in the private information that is available from the environment in noisy conditions. Because visual noise reduces the likelihood of an individual detecting information, such as the location of prey in the environment, animals may adapt their behaviour to mitigate the effects noise has on their perceptual abilities. Animals can do this by avoiding visually noisy areas (Attwell et al., 2020), changing their activities in times of increased visual noise (Evans, Dall, & Righart, 2018) or by changing information sampling strategies in noisy conditions (Corcoran & Moss, 2017). Here we further suggest that increasing reliance on social rather than private information in times of compromised perception may allow individuals to supplement limited private information in noisy conditions. Crucially, one of the primary benefits of grouping is access to social information (Couzin, 2009; Ioannou, 2017; Mathis et al., 1996; Sumpter et al., 2008; Ward et al., 2011), enhancing the detection of resources (Krause et al., 1999; MacGregor et al., 2020; Pitcher et al., 1982) and predators (Godin et al., 1988; Godin & Morgan, 1985; Landeau & Terborgh, 1986; Lima, 1995; Lima & Zollner, 1996). By increasing their responsiveness to conspecifics during times of increased visual noise, individuals may be able to mitigate the effects of compromised private information detection.

Increased social attraction could also result from individuals mitigating risk in environments where information is difficult to detect. Indeed, fish may perceive the visually noisier environments as inherently riskier owing to a reduced likelihood of detecting threats. Animal groups often form more cohesive structures in times of increased risk (Carere et al., 2009; Nettstead & Axelsen, 1999; Romensky et al., 2020), which can be attributed to individuals reducing their limited domains of danger (Hamilton, 1971). On the other hand, if environments with increased visual noise were perceived as riskier, we may have expected the fish to reduce their activity and speed to reduce their own likelihood of being detected by predators. Indeed, reduced activity is an indicator of a stress response in fish (Blaser, Chadwick, & McGinnis, 2010; Blaser & Gerlai, 2006), including in three-spined sticklebacks (Norton & Gutiérrez, 2019), and animals often reduce activity to avoid being detected (Broom & Ruxton, 2005; Scarratt & Godin, 1992; Smith, 1991). In fact, we found that fish increased their speed in noisier environments, as well as finding that there was no significant difference in the number of midline crossings across noise levels. Attwell et al. (2020) similarly found no evidence that visually noisy environments increased or decreased refuge use (a behaviour associated with minimizing risk) in sticklebacks, suggesting that fish were not adapting their behaviour to mitigate risk. We suggest that instead of changes in social attraction being driven by the perception of increased risk, it is more likely that increased social attraction is driven by a reduction in access to private and social information in times of compromised perception.

We highlight that in our experiments, we used simulated, rather than natural, water caustics. This decision allowed maximal control of the level of sensory noise presented, as well as ensuring that all fish were subject to identical noise treatments. However, it remains important to assess the natural range of caustics present in different environments. The formation of water caustics is governed by a host of abiotic features, including the intensity and angle of sunlight, the water clarity and the degree of water surface disruption (McFarland & Loew, 1983). Therefore, the variability in the patterns, movements and intensities of caustics will vary considerably depending on the conditions. It will be important to measure this variability, as well as to consider that some populations of animals may experience different levels of visual noise in their habitats. Indeed, some habitats may vary considerably in the abiotic factors that govern the creation of caustics (e.g. streams, lakes, mangroves, reef flats), and therefore will exhibit large variation in the caustics observed. Assessing the natural diversity of caustics in the environments where animals live, in combination with controlled behavioural studies, remains a key consideration for future research.

In summary, our results demonstrate how the presence of increased visual noise within a habitat can promote shoal attraction by individual fish but does not affect shoal size preferences. This is likely to be a consequence of the disruption of private and/or social information acquisition in noisy environments, leading instead towards increased reliance on social information. Our study highlights how social behaviour can be directly influenced by the presence of caustic flicker, a widespread and prevalent form of visual noise in aquatic environments.

Data Availability

Data can be found on the data depository, Dryad, at https://datadryad.org/stash/share/pzlA8j-aX3h4aK3VmaFQ_J81fDuyta-I331PCksEw.

Acknowledgments

We thank the Department of Zoology’s Teaching Laboratory Technicians (Daniel White and Jacek Zalewski) for capturing the fish and providing projection equipment, as well as the Aquatics Culture Technician (Nigel Hall) for the maintenance of fish husbandry throughout the study. Thanks also to Innes Cuthill for useful discussions. This work was supported by the Whitten Program in Marine Biology and a Swedish Research Council (grant number 2018–04076) and an Isaac Newton Trust grant.

Supplementary Material

Supplementary material associated with this article can be found online at https://doi.org/10.1016/j.anbehav.2021.04.017.

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Appendix 1

In addition to quantifying the percentage of frames where fish were tracked, we also calculated how close these point estimates were (in mm) from manual (human) point estimates. Using a bespoke MATLAB script, we first extracted five random video frames from each noise level for each fish in each trial. Within each of these frames, we manually determined the centroid coordinates of the fish. This amounted to a total of 2880 manual fish tracks (i.e. five frames, per noise level, per focal fish; 5 x 4 x 14). These manual coordinates were then compared to the original tracking data obtained by the Loopy model, calculating the distance (mm) between the automated and manual pairs of coordinates. Overall, the median distance between manual and automated tracks was 3.7 ± 7.5 mm (mean ± 1 SD). Furthermore, only 0.3% frames (10 of 2880) pertained to distances that exceeded the average body length recorded in the experiment (31 mm; Fig. A1). Therefore, the tracking software was able to accurately track the fish even with a moving background (visual noise projection), highlighting the effectiveness of these new machine learning tracking algorithms for automatic quantification of behaviour.

Appendix 2

Table A1

| File | Noise level 1 | Noise level 2 | Noise level 3 | Noise level 4 |
|------|---------------|---------------|---------------|---------------|
| A    | SL            | M             | F             | ST            |
| B    | F             | ST            | SL            | M             |
| C    | ST            | F             | M             | SL            |
| D    | M             | SL            | ST            | F             |

ST = static, SL = slow, M = medium and F = fast.

Table A2

| Parameter settings used to generate the images of caustics in Caustics Generator Pro |
|-----------------------------------------------|
| Depth                                         |
| 5.0                                           |
| Intensity                                     |
| 0.05                                          |
| Atritude filter                               |
| 1.36                                          |
| Frequency filter                              |
| 1.5                                           |
| Time filter                                   |
| 40.06                                         |
Table A3
A statistical breakdown of the fixed effects included within the maximal model for each response measure

|                                | Preference score | Shoal proximity | Speed of fish | Midline crosses |
|--------------------------------|------------------|-----------------|---------------|----------------|
| **Noise level**                | \( X^2 = 1.80, P = 0.615 \) | \( X^2 = 42.42, P < 0.001, D = 0.65 \) | \( X^2 = 629.96, P < 0.001, D = 3.78 \) | \( X^2 = 5.99, P = 0.424 \) |
| **Michelson contrast**         | \( X^2 = 2.08, P = 0.721 \) | \( X^2 = 6.50, P = 0.343 \) | \( X^2 = 8.41, P = 0.078 \) | \( X^2 = 2.49, P = 0.646 \) |
| **Michelson contrast * Noise level interaction** | \( X^2 = 1.31, P = 0.723 \) | \( X^2 = 2.00, P = 0.572 \) | \( X^2 = 6.93, P = 0.074 \) | \( X^2 = 0.48, P = 0.923 \) |
| **Time segment**               | \( X^2 = 1.62, P = 0.204 \) | \( X^2 = 3.27, P = 0.071 \) | \( X^2 = 53.60, P < 0.001, D = 1.35 \) | \( X^2 = 7.19, P < 0.007, D = 0.26 \) |
| **Fish size**                  | \( X^2 = 1.15, P = 0.284 \) | \( X^2 = 12.10, P < 0.001, D = 0.62 \) | \( X^2 = 36.78, P < 0.001, D = 1.23 \) | \( X^2 = 0.59, P = 0.441 \) |
| **Reuse of stimuli fish (for four consecutive trials)** | \( X^2 = 8.43, P < 0.004, D = -0.62 \) | \( X^2 = 0.21, P = 0.646 \) | \( X^2 = 12.69, P < 0.001, D = -0.65 \) | \( X^2 = 8.76, P < 0.003, D = -0.58 \) |
| **Reuse of stimuli fish (up to three times in study)** | \( X^2 = 0.05, P = 0.831 \) | \( X^2 = 0.01, P = 0.926 \) | \( X^2 = 3.93, P < 0.048, D = -0.74 \) | \( X^2 = 0.05, P = 0.831 \) |

The fixed effects that, when included in the maximal model, did not significantly alter the model (not in bold) were dropped from the final model. Asterisks denote an interaction term.