***The response of common minnow (Phoxinus phoxinus) to visual cues under flowing and static water conditions***

**While fixed visual cues provide reliable reference points for navigation in static water, it remains unclear whether fish energetically benefit from their presence in flowing water. Furthermore, benefits of visual feedback from fixed sources may reduce for group members that can gain additional information from others. Using an open-channel flume, this study investigated how fish response to stationary visual stimuli is influenced by flow and group membership under two treatments: vertical black and white stripes placed on (1) both side-walls of the channel, (2) one side-wall only; compared to a control where both walls were uniform white. Fish response was compared under flowing and static water, and between individuals and groups of five. Fish exhibited a positive affiliation for the visual cues; travelling at a slower speed and spending more time closer to the striped walls. Fish were more edge oriented under flowing conditions, presumably utilising the lower velocities at the wall boundary to reduce energy expenditure. When only one wall presented visual cues, individual fish spent a median of 97% of time associated with it under flowing water, suggesting some energetic benefit in lotic conditions. This may result from a greater ability to maintain station or control position relative to a reference point, and/or the use of visual stimuli as a proxy indicator of physical structure that may provide drag-reducing refuge. A lesser association with the striped wall under static water suggests that visual cues provide other non-hydrodynamic benefits, such as physical refuge from predators or opportunities for crypsis. Conversely, less association with the striped walls exhibited by shoals may reflect a greater dependence on information provided by conspecifics, or increased security associated with being part of a group. This study indicates that fixed visual cues likely provide several benefits that vary depending on flow and group membership.**

*Keywords:* Collective behaviour, environmental stimulus, fish, group behaviour, optic flow Moving animals continuously monitor their surroundings while controlling their heading and speed to avoid collisions. Within dynamic environments such as air, wind can impede an animal’s ability to gain reliable information on its spatial position. Consequently, successful navigation of airborne animals, such as birds and insects, is highly dependent on visual cues (Hedenström & Åkesson, 2017; Schiffner & Srinivasan, 2015; Zeil et al., 2008). The apparent motion of these stimuli relative to the movements of the observing animal is known as optic flow. During flight, honeybees (*Apis mellifera*) and budgerigars (*Melopsittacus undulatus*) control their path, speed and altitude by maintaining a constant rate of optic flow on the retina (Bhagavatula et al., 2011; Linander et al., 2017). This enables them to move away from areas they perceive to be “cluttered”, reducing the risk of them colliding with physical objects (Lecoeur et al., 2019).

Although fish encounter similar challenges to insects and birds, they display distinctly different responses to optic flow. For example, when individual zebrafish (*Danio rerio*) are presented with one vertically and one horizontally striped wall on opposite sides of a tunnel under static water conditions, they react by swimming closer to the vertical stripes that provide stronger visual feedback because they are oriented perpendicular to the direction of swimming (Scholtyssek et al., 2014). This is in contrast to bees, which steer away from vertical stripes when presented with the same choice (Scholtyssek et al., 2014). It is suggested that the zebrafish may move towards stronger visual stimuli because they provide useful reference points in an environment that has the potential to be turbid (Scholtyssek et al., 2014).

The majority of studies focusing on fish response to visual cues have been conducted under static water conditions. However, water currents are a fundamental property of lotic aquatic environments that can be used to enhance navigation (Milner et al., 2012; Vowles et al., 2014), while fish suffer the risk of being displaced downstream if they cannot fix on a stationary point of reference (Arnold, 1974; Engelmann et al., 2002). How fish navigate or maintain position in flowing water has been a long-standing question (Arnold, 1974), and likely depends on the availability of visual, tactile and/or hydrodynamic information. Fish can compensate and perform rheotaxis with either the lateral line or vision blocked, but fail to orient to the flow if both senses are inhibited (Suli et al., 2012), indicating that rheotaxis is a multi-sensory process involving both vision and the lateral line system (Bak-Coleman et al., 2013; Kulpa et al., 2015; Olive et al., 2016). Although fish move closer to visual cues in static water (Scholtyssek et al., 2014); the role of vision in rheotaxis suggests energetic benefits may be gained from access to visual cues for navigation and station holding in flowing water.

Vision plays a central role in the collective behaviour of fish and is critical to the formation and maintenance of shoals and schools (Partridge & Pitcher, 1980). For example, shoaling is elicited when fish are exposed to a moving visual cue (Pitcher, 1986; Shaw & Tucker, 1965), and for members of a shoal that have been blinded in one eye, their ability to maintain position within the group is dependent on other fish being present on their intact, rather than sightless, side (Shaw, 1962). In flowing water, group members benefit from adopting formations that reduce the energetic cost of locomotion (Ashraf, Godoy-Diana, Halloy, Collignon, & Thiria, 2016; Hemelrijk, Reid, Hildenbrandt, & Padding, 2015), such as by swimming side-by-side (e.g. De Bie et al. 2020, for minnows, *Phoxinus phoxinus*) and synchronising frequency of tail beats at higher velocities (Ashraf et al., 2017 for red-nose tetra, *Hemigrammus rhodostomus*). The transfer of visual information between members of a group allows multiple individuals to benefit from a discovery made by a few. For example, goldfish (*Carassius auratus*) and minnows detect food patches more efficiently when in a larger group (Pitcher, Magurran, & Winfield, 1982), and forage at a faster rate and for longer compared to smaller groups that need to remain more vigilant of predators (Magurran & Pitcher, 1983; Morgan, 1988). Given the visual information transfer between conspecifics when navigating in groups, the necessity for members to maintain visual contact with the surrounding environment may be lower than for isolated individuals, and thus stationary visual cues may be of lesser importance.

This study used an experimental approach to determine fine-scale fish response to: (1) visual cues (vertical stripes) and how this is influenced by (2) flow and (3) group size. We predicted that: (i) fish will associate with visual cues; (ii) that this will be stronger in flowing than static water because they will gain energetic benefits (reduced risk of displacement and greater ability to control speed of movement) from the utilisation of fixed points of reference; and (iii) members of groups will have a lower affinity for visual cues than isolated individuals because information on relative position can be gained from others.

# Methods

To compare the response of individuals and groups of common minnow to visual cues in flowing and static water an experiment was conducted using an open channel flume under two treatments: [T1] vertical black and white stripes placed on one side-wall only, while the other was white (randomly alternated between trials to control for lateral bias of flume hydraulics and/or fish behaviour); [T2] vertical black and white stripes placed on both side-walls of the flume, and [C] a control with uniform white walls. The minnow was selected as the model because it is relatively common and of low conservation concern, easily captured and maintained, and tends to display natural behaviours, including the formation of cohesive shoals, under laboratory conditions.

## Experimental Setup

Experiments were conducted at the International Centre for Ecohydraulics Research (ICER; University of Southampton, UK), using a rectangular open-channel recirculating flume (16 m long x 0.6 m wide x 0.8 m deep), between 24 September and 25 October 2018. White laminate PVC sheeting was secured to the inner walls and base of the channel to reduce visual points of reference and to increase contrast between the fish and the background so that automated video processing could be enhanced. To reduce hydrodynamic complexity, a flow straightener was installed upstream of a 4.8 m long experimental section that was isolated from the remainder of the channel by mesh partitions. A blackout hide was erected over the experimental section to prevent disturbance to the fish, and LED strip lighting (Brillihood - LED-Batten - 4 FT - 36 W, 2950 lumen, frequency peaks: 450 nm & 550-600 nm) with reflectors and diffusers installed to enhance uniformity of illumination.

A 1 m long acclimatisation zone was created at the most downstream end of the experimental area using a retractable screen. Upstream of this was an approach zone (1.48 m long), devoid of visual cues, followed by the treatment zone of an equivalent length along which visual cues were displayed during trials (Figure 1). A further zone in which the test visual cues were absent extended 0.84 m upstream of the treatment zone. Two overhead Logitech webcams (HD Pro Webcam C920; 30 frames/second, Resolution = 1080p) were installed 1.3 m directly above the centre of the approach and treatment zones (Figure 1).

Vertical black and white stripes created strong optic flow on the walls of the treatment zone during the trials (Figure 1). A stripe width of 4 cm was selected to provide a spatial frequency (0.066 cycles/degree), as seen from the centre of the flume, that is within the optimum range for the visual acuity of zebrafish, the closest related species to minnow for which robust measurements of visual resolution exist (Tappeiner et al., 2012). The stripe width chosen was comparable to that presented during previous optic flow studies using zebrafish (Bak-Coleman et al., 2015; Scholtyssek et al., 2014).

## Flow Regimes

Unidirectional flow velocity was measured over a period of 10 seconds at 40 locations throughout the experimental area using a Valeport electromagnetic velocity meter (Model 801, Valeport Totnes, UK). Mean ± SD velocities were 0.34 ± 0.26 cm/s and 17.4 ± 2.56 cm/s for the ‘static’ and ‘flowing water’ regimes, respectively. Velocity during the ‘flowing water’ treatment was slightly lower than the published sustained swimming speed of common minnows of comparable size measured in an open channel (Holthe et al., 2009). Water depth was maintained at 17 cm for both regimes.

## *Fish Maintenance*

Common minnows were collected from the River Itchen Navigation, St. Catherine’s Hill, Winchester, UK, (lat: 51.049783 long: -1.311416), using a 5 m seine net, and transported in aerated river water to holding tanks at the ICER facility. No more than 350 minnows were collected on three separate occasions between 19 September and 18 October 2018. They were held for a maximum of 16 days before return to a location upstream of the fishing site.

The fish were maintained in two 1200 L holding tanks and fed daily. Water quality was monitored and regular water changes (20 – 50%) ensured a high standard was maintained (ammonia < 0.25 mg L−1, nitrite < 0.25 mg L−1, and nitrate < 50 mg L−1). Mean ± SD holding tank temperature was 16.4 ± 0.5 °C. Lighting was set to match the natural photoperiod throughout the study.

## *Experimental Protocol*

Fish were acclimatised in a perforated container located at the downstream end of the flume for at least 30 minutes prior to the start of trials. An individual or group of five fish were selected at random and moved to the acclimatisation zone at the downstream end of the channel for a further 10 minutes to encourage exploratory behaviour and, in the case of groups, the formation of a shoal. At the start of each trial the retractable screen was lifted, enabling fish access to the experimental area for 30 minutes, after which they were caught, weighed (g) and measured (fork length, mm). Fish length (mean ± SD = 47.6 ± 5.63 mm, N = 580; ANOVA: *F*11,196 = 0.51, *P* = 0.90) and mass (mean ± SD = 1.15 ± 0.40 g, N = 580; ANOVA: *F*11,196 = 0.58, *P* = 0.84) did not differ between treatments. Mean ± SD flume water temperature was 16.7 ± 0.6 °C and did not differ between treatments (ANOVA: *F*11,196 = 1.02, *P* = 0.43). To minimise the effect of confounding variables, treatments were randomised throughout the experimental period, and there was no bias in the start time of treatments (earliest: 07:59; latest: 18:00). Fish were used in one trial only and at least 14 repeats were conducted for each condition tested (Table 1).

## Behavioural and Statistical Analysis

Video data was post processed using custom-written tracking software (The MathWorks, 2018). The spatial coordinates of fish centroids were recorded every third of a second by identifying changes in contrast between fish silhouettes and the background. Where multiple fish were present within a single frame the coordinates of each fish were recorded, and the centroid of the group calculated by minimising the sum squared distances between each coordinate.

As fish volitionally explored the experimental area during each 30-minute trial, multiple paths through the approach and treatment zones were recorded in both the upstream and downstream directions. Consequently, data from the approach and treatment zone were analysed independently. The positions of fish as they passed through the approach and treatment zones were used to determine their response to visual cues through the following three metrics: (1) *Visual cue association* - quantified as the time spent in the treatment zone expressed as a proportion of total time spent in the approach and treatment zones, (2) *Ground speed* - calculated as quotient of distance and time between consecutive frames, and (3) *Distance to closest wall* - quantified as the distance to the closest wall from the individual fish or group centroid. The closest member of the group to a wall in each frame was also analysed but as the results did not differ, the group centroid was chosen for the final analysis. Only passes in the upstream direction and in the mid 90 cm of each zone were used to calculate *Ground speed* and *Distance to closest wall* because the behaviour in the approach zone may have been influenced by the presence of stripes in the upstream treatment zone, and flow influenced the ground speed of fish swimming downstream. Due to the group splitting between zones and times where fish did not form cohesive shoals, frames containing fewer than three fish were excluded to ensure that data was representative of group behaviour. This resulted in 22% of group data being removed from the analysis.

A factorial experimental design was used to quantify the influence of flow regime (static versus flowing water) and group membership (individuals versus group of five) on fish response to visual cues (T1, T2, and C), resulting in a total of 12 conditions. Statistical analysis was performed using R software (R Core Team, 2020). Each metric was analysed separately with visual cue, flow regime and group membership as explanatory variables. Interactions between explanatory variables were also tested. Mean *Ground speed* and *Distance to closest wall* were calculated for each pass and then averaged by trial to avoid pseudoreplication due to disproportionately more data obtained from longer passes or trials that contained a greater number of passes. Where the error distribution of the response variable deviated from normality, an appropriate transformation was used to normalise the data or error distribution assumed within the model. Model fit was assessed using the DHARMa package (Hartig, 2020). Chi-square and F statistics were calculated using the car package (Fox, John & Weisberg, 2011). Post-hoc tests were conducted using the phia package (De Rosario-Martinez, 2015). As mean water temperature and fish size did not differ between treatments they were not included as covariates.

Proportion data was arcsine square-root transformed prior to use in the analysis of *Visual cue association* using a two-way Anova. A linear mixed model (LMM) (Lme4 package; Bates et al., 2015) was used to compare the *Ground speed* between the approach and treatment zones with a random effect variable of *Trial ID* to account for data from the same fish in both zones during a trial. The response viable was log10 transformed to normalize the model residuals. *Ground speed* and *Distance to closest wall* were log10 and square-root transformed, respectively, prior to analysing the effect of explanatory variables using Anova.

## Ethical Note

Ethics was reviewed by the Animal Welfare and Ethics Review Board and approval granted by the University of Southampton Ethics and Research Governance committee (ID: 45048). Permission was obtained from the UK Environment Agency to extract common minnows from their natural habitat and to return them to the source river shortly after completion of the trials. Experiments were carried out in compliance with Home Office regulations. Individuals were handled with care, and handling time was kept to a minimum. There was no evidence of stress or fatigue from exposure to the visual cues or flowing water treatments during the 30-minute trial time.

# Results

## Response to Visual Cues

Fish spent a greater proportion of time in the treatment zone when either one (mean ± SE = 0.67 ± 0.05; ANOVA: *F*1,203 = 49.6, *P* < 0.001) or two walls (mean ± SE = 0.69 ± 0.07;ANOVA: *F*1,203 = 64.4, *P* < 0.001) displayed visual cues compared to the control (mean ± SE = 0.48 ± 0.05; Figure 2), but no difference was observed between the one and two striped wall visual treatments (ANOVA: *F*1,203 = 1.36, *P* = 0.25).

Fish tended to swim in close proximity to the flume walls, independent of visual cue treatment, resulting in a bimodal lateral distribution for the control and two striped wall conditions (Figure 3). However, when only one wall displayed visual cues the lateral distribution was skewed as fish tended to associate with the striped wall (Figure 3; bold text in Table 1).

When visual cues were present fish swam roughly twice as close to the channel walls (One wall: median [IQR] = 6.3 [6.8] cm, ANOVA: *F*1,188 = 16.1, *P* < 0.001; Two walls: median [IQR] = 6.2 [8.5] cm, *F*1,188 = 14.0, *P* < 0.001) compared to the control (median [IQR] = 12.2 [10.8] cm; Figure 4). There was no difference in the *Distance to closest wall* between the one and two wall visual cue treatments (ANOVA: *F*1,188 = 0.02, *P* = 0.88). In the absence of visual cues (control) there was no difference in the median *Ground speed* between approach and treatment zones (LMM: *F*1,201 = 0.30, *P* = 0.59). However, considering the treatment zone in isolation, all three explanatory variables influenced *Ground speed* (ANOVA: *F*11,188 = 5.51, *P* < 0.001), including an interaction between group membership and visual cue treatment (ANOVA: *F*2,188 = 5.9, *P* = 0.003, Figure 5).

## Influence of Flow on Response to Visual Cues

Association with visual cues was greatest in flowing water (ANOVA: *F*1,203 = 14.8, *P* = 0.01), with fish spending on average 16.8% more time in the treatment zone (mean ± SE = 0.74 ± 0.07) compared to when they were in static water (mean ± SE = 0.63 ± 0.06, Figure 2). Fish also associated more closely with the striped wall (Figure 3; Table 1) and swam closer to the flume walls in flowing water (median [IQR] = 5.5 [5.7] cm) compared with static water (median [IQR] = 12.4 [11.2] cm) for all visual cue treatments including the control (ANOVA: *F*1,188 = 76.4, *P* < 0.001).

The *Ground speed* of minnows was slightly slower in flow (median [IQR] = 14.7 [10.9] cm/s) than static water (median [IQR] = 18.9 [11.1] cm/s; ANOVA: *F*1,188 = 4.98, *P* = 0.026).

## Influence of Group Membership on Response to Visual Cues.

Group size did not influence the proportion of time spent in the treatment zone (ANOVA: *F*1,203 = 1.6, *P* = 0.21), but individual fish associated with the striped wall to a greater extent (Figure 2; Table 1) and swam closer to the flume walls (median [IQR] = 5.5 [7.0] cm) compared to groups (median [IQR] = 12.2 [8.6] cm, ANOVA: *F*1,188 = 69.1, *P* < 0.001; Figure 4).

Groups of fish did not vary their *Ground speed* across any of the visual cue treatments (ANOVA: *F*2,188 = 2.04, *P* = 0.13). However, individual fish swam slower when more visual cues were displayed. Specifically, individuals swam slower when one (ANOVA: *F*1,188 = 15.9, *P* < 0.001) or two walls (ANOVA: *F*1,188 = 45.3, *P* = 0.001) displayed visual cues compared to the control and when two walls displayed visual cues compared to just one (ANOVA: *F*1,188 = 8.17, *P* = 0.02; Figure 5).

# Discussion

This study quantified the response of minnows to visual cues and investigated how that was influenced by flowing water and group membership. In-line with previous work with zebrafish (Scholtyssek et al., 2014), minnow swam in proximity to visual cues in static water, but as predicted their association became stronger in flowing water. In addition, individual fish showed a stronger affinity to the stripes compared to groups of five. The stronger association in flowing water indicates that fish may accrue energetic benefits by using visual reference points to control their swimming. In groups, information transfer between conspecifics may reduce the necessity for fixed reference points, and/or individual fish may be more inclined to seek refuge alongside the visual cues because the advantages gained from being a member of a group are absent.

Consistent with the first prediction, minnows tended to show an innate association with visual cues irrespective of flow and group membership. Fish reduced their *Ground speed* and chose to spend more time alongside the visual cues. In addition, fish associated with the striped side of the channel when offered a choice. This supports the findings of previous studies that found fish to favour the side of the tank displaying the stronger visual feedback (Scholtyssek et al., 2014). This contrasts insects and birds that balance bilateral optic flow. If fish were balancing optic flow across both eyes they would move away from the striped wall if offered a choice or move directly down the centre of the flume when both walls displayed the same cues. Although fish exhibited a general attraction to visual cues, abiotic (flow) and biotic (presence of conspecifics) cues influenced the magnitude of affiliation.

In agreement with the second prediction, minnows clearly associated with visual cues to a greater extent in flowing compared to static water. It was expected that fish would utilise the lower velocities at the channel boundaries to reduce their energetic costs in flowing water (Watson et al., 2018), but the clear preference for the single striped wall when the opposite was uniform indicates that there are likely benefits associated with visual cues. In nature, holding position in a current may provide multiple ecological advantages, including the lessening of energetic costs of locomotion, improved access to food, and reduced probability of detection by a predator through motion camouflage (Johansen et al., 2019). Fish living in a unidirectional flowing environment have adapted behaviours such as rheotaxis and position holding, to avoid being displaced downstream. Vision has been demonstrated to play an important role in rheotaxis (Arnold, 1974), and therefore the stripes may have provided fixed reference points to help fish hold position. However, fish associate with visual cues even in static water, suggesting other benefits in addition to reduced energetic expenditure. The visual complexity associated with a striped, rather than uniform landscape, may indicate structural complexity and thus attract fish to areas where they might seek shelter from unfavourable hydrodynamic conditions or predators (Ioannou & Krause, 2009; Orpwood et al., 2008). Alternatively, a complex striped background may itself bestow benefits in terms of reduced predator detection if the outline of the fish is disrupted, independent of whether physical structure is present (Kjernsmo & Merilaita, 2012; Scharf et al., 2006).

Groups of minnows were less inclined to spend time close to the stripes and tended to show more exploratory behaviour compared to individuals, supporting the third prediction. Shoaling plays an important role in lowering predation risk, and so individuals may seek alternative strategies by using cover (Pitcher, 1986). For example, Orpwood et al. (2008) found that minnows tended to school in areas of low habitat complexity but sought refuge and remained still in more complex habitats. Attraction of individuals to visual cues may, therefore, reflect a sheltering or refuge seeking response with the stripes offering a form of visual camouflage. Considering minnow are a social species, when conspecifics were absent individuals may have experienced higher stress levels and this could provide an alternative explanation for the increased association. The importance of vision on the collective behaviour of fish has been demonstrated (Lemasson et al., 2018; Partridge & Pitcher, 1980; Pita et al., 2015), with subtle forms of information transfer between schooling fish, usually for resource or predator detection (Brabazon, Cui, & O’Neill, 2015; Magurran & Higham, 1988). Additionally, groups of fish adopt side-by-side formations to enhance visual information transfer to benefit from energy efficient formations in flow (de Bie et al., 2020). Given that fish gain information on their relative position from conspecifics, a reduced onus on group members to maintain visual contact with environmental cues is logical, and would explain the looser association of groups with the visual cues in this study. Alternatively, the energetic benefits groups gain by adopting energy efficient formations (Ashraf et al., 2016) may outweigh those gained by controlling position using fixed visual cues.

## Conclusions

Based on our observations, the benefits of association with the visual cues provided by striped walls are likely to be greatest when isolated minnow inhabit flowing water. This is likely because fish in flowing water use visual cues as reference points to control position for energetic benefit. Furthermore, the vertical stripes may resemble macrophytes in the minnow’s natural habitat and the observed association may reflect the seeking of physical refuge from flow or predators. For groups, information transfer between conspecifics may explain their reduced need to use visual cues, either to save energy by fixing on a known location, or as an indicator of refuge, e.g. from a predator or adverse flow.

A fundamental understanding of how fish react to environmental stimuli may help inform environmental engineers and fisheries managers, particularly in relation to fish guidance. In this respect, further research to investigate whether fish habituate to the presence of visual stimuli, or whether such signals can be used to elicit a consistent response over a range of environmental conditions and for multiple species, remains an avenue of interest.

# Acknowledgements

This work was supported by the Natural Environment Research Council (NERC) as part of a NERC studentship [grant number 1786331]. We thank Toru Tsuzaki and members of the ICER team for their assistance and technical advice. Data published in this article are available from the University of Southampton repository at DOI: <https://doi.org/10.5258/SOTON/D1833>.

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# Tables and figure captions

Table . Proportion of time associated with striped walls

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Visual cue treatment | Flow regime | Group size | Proportion by stripe / right | IQR | Sample size (N) |
| C | N  **N**  N | 1 | 0.456 | 0.252 | 19 |
| **T1** | **1** | **0.738** | **0.274** | **20** |
| T2 | 1 | 0.558 | 0.218 | 20 |
| C | Y | 1 | 0.325 | 0.660 | 20 |
| **T1** | **Y** | **1** | **0.970** | **0.298** | **21** |
| T2 | Y | 1 | 0.475 | 0.599 | 21 |
| C | N | 5 | 0.408 | 0.255 | 14 |
| **T1** | **N** | **5** | **0.668** | **0.160** | **16** |
| T2 | N | 5 | 0.547 | 0.333 | 14 |
| C | Y | 5 | 0.612 | 0.734 | 14 |
| **T1** | **Y** | **5** | **0.906** | **0.267** | **15** |
| T2 | Y | 5 | 0.636 | 0.450 | 15 |

Median proportion of time associated with the one striped wall (T1, rows in bold) and the right side of the flume in the two striped wall treatment (T2) and control (C) in the treatment zone. IQR = interquartile range.

Figure 1. Plan of the experimental channel used to assess the response of individual and groups (5 fish) of common minnow to visual cues under static and flowing water. Fish were released from the acclimatisation area and flow was from right to left (arrow). Fish behaviour was recorded by overhead cameras positioned above the centre of the approach and treatment zones. Visual cues were displayed on the flume walls within the treatment zone during treatments (T1 and T2) only.

Figure 2. Proportion of time minnows spent within the treatment zone compared to the approach zone under the three visual cue treatments (C – Control, T1 – One-striped wall, T2 – Two-striped walls) in (a) - static and (b) - flowing water. Black bar represents the median and the box represents the interquartile ranges (IQR) for each treatment. Dashed line at 0.5 represents equal time between the two zones.

Figure 3. Probability density plot for lateral distribution of fish in the treatment zone for all 12 treatment groups. (a - d) control (C), (e – h) one-striped wall (T1) and (i – l) two-striped walls (T2). Line type varies for the different visual cue treatments. The solid line in (e – h) represents trials when the visual cues were on the left side of the flume and the dashed line, the right. Plots on the left (a, b, e, f, I, j) individuals and on the right (c, d, g, h, k, l) groups. The first and third columns (a, e, I, c, g, k) were trials in static water and the second and forth columns (b, f, j, d, h, l) flowing water. Y-axis is log2 transformed. Shaded grey plots show the underlying density distribution for each trial.

Figure 4. The median distance to closest wall for each treatment (C – Control, T1 – One-striped wall, T2 – Two-striped walls) for (a, b) individuals, (c, d) groups and (a, c) static verses (b, d) flowing water. Error bars represent the interquartile ranges.

Figure 5. Median ground speed stratified by visual cue treatment (C – Control, T1 – One-striped wall, T2 – Two-striped walls) for (a, b) individuals, (c, d) groups and (a, c) static verses (b, d) flowing water. Error bars display interquartile ranges.