**Abstract**

Spatial patterns in moving fish shoals originate from social interactions and are driven by factors that benefit individuals, such as predator avoidance or reduced energy expenditure. How shoaling is influenced by the presence and absence of a flow field is only partially understood. Adopting a reductionist approach, time spent shoaling, shoal structure, and information transfer between pairs (the smallest subsystem of a shoal) of Eurasian minnow (*Phoxinus phoxinus*) were investigated in a recirculating flume in the absence (control) and presence of flow (low and high). Minnow spent more time shoaling under high flowing conditions. The pairs tended to swim in a tandem and side-by-side configuration under static and high flowing water, respectively, while under low flow, the neighbour’s position was more uniformly distributed around the focal fish. Our approach involved analysis of fish accelerations and velocity correlations and indicated that, with the potential for hydrodynamic masking in flowing water, the relative positions adopted reflect the individual’s propensity to enhance information transfer with its neighbour by visual means so that an energy efficient configuration can be maintained. Conversely, when the energetic benefits of a side-by-side configuration are lost in the absence of flow, fish limit the costs of the partial visual impediment (on one side) imposed by the presence of a close neighbour by employing a “follow-the-leader” tactic.

**Keywords***: group behaviour, hydrodynamics, information transfer, schooling, shoaling, swimming efficiency*

Throughout the animal kingdom, social living has evolved as a means to outweigh the costs of being solitary through realising the potential benefits gained from the presence of conspecifics (Alexander, 1974). These include reduced risk as a result of increased probability of detecting and/ or confusing predators (Cresswell, 1994; Godin, Classon, & Abrahams, 1988; Jeschke & Tollrian, 2007), enhanced foraging success (Creel & Creel, 1995; Day, MacDonald, Brown, Laland, & Reader, 2001), and reduced energy expenditure of locomotion (Hemelrijk, Reid, Hildenbrandt, & Padding, 2014; Weimerskirch, Martin, Clerquin, Alexandre, & Jiraskova, 2001). However, costs of group membership may include a higher rate of detection and attack from predators (Pitcher & Parrish, 1993), elevated competition for resources (J. Krause & Ruxton, 2002), and an increased probability of spreading disease at higher densities (Lee, 1994).

The relative importance of different factors that influence the cost: benefit ratio associated with group living are often difficult to determine, as are the mechanisms by which they are realised. On one hand, the mechanisms may be purely passive (e.g. statistically lower probability of capture by being a member of a large herd, flock or swarm), while on the other hand, the ability to profit from the presence of others depends on group members actively interacting with others, and transferring information effectively. The emergence of group patterns following interactions between individuals is referred to as collective behaviour (Camazine et al., 2003; Couzin, 2009; Sumpter, 2006). Through these interactions groups of animals have shown to be able to maintain cohesion, synchronise movements, and respond to outside perturbations (e.g. a predator attack). Several approaches are employed to better understand information transfer between individuals and the fundamental mechanisms underlying emergent macroscopic properties such as collective movement. For example, modelling has been employed to reproduce the properties of groups by imposing simple interaction rules among individuals, such as the distance between them (e.g. Couzin, Krause, James, Ruxton, & Franks, 2002; Gautrais, Jost, & Theraulaz, 2008; Hemelrijk & Kunz, 2005; Huth & Wissel, 1992). However, similar characteristics of group behaviour can emerge in models that use distinctly different interactions rules, highlighting the value of empirical experimentation to aid the development and/ or validation of these (Katz, Tunstrom, Ioannou, Huepe, & Couzin, 2011; Lukeman, Li, & Edelstein-Keshet, 2010).

Fish have widely been used as models on which theories of collective behaviour have been developed and tested (e.g. Belyayev & Zuyev, 1969; Fish, 1999; Hemelrijk et al., 2014; Huth & Wissel, 1992). Over several decades, there has been much debate as to the primary driver for group formation in fish, with some proposing that shoals and schools occur primarily to reduce the energetic costs of swimming (e.g. Weihs, 1973), while others argue information transfer is the dominant determinant, e.g. in relation to predator avoidance (Domenici & Batty, 1997; Godin et al., 1988; Rieucau, Holmin, Castillo, Couzin, & Handegard, 2016) or enhanced foraging efficiency (e.g. Reebs, 2000). Recently, Ashraf, Godoy-Diana, Halloy, Collignon, and Thiria (2016) demonstrate that groups of red nose tetra (*Hemigrammus bleheri*) adopt a side-by-side configuration under flowing water conditions as a means to reduce energy expenditure. Using the most elementary subsystem of a school, pairs of fish tend to maintain a constant distance between one another independent of flow, but adopt an increasingly synchronised swimming mode (consisting of both “in-phase” and “out-phase” configurations) at higher velocities, an energy saving tactic. These results were repeated using larger groups (3-9 fish) of the same subject species, with all fish adopting a side-by-side (phalanx) configuration when forced to swim rapidly (Ashraf et al., 2017). In this case, nearest neighbour distance decreases at higher speed, while fish exhibit greater synchronicity and reduced tail beat frequency (a proxy for energy expenditure) when side-by-side. The authors argue that while the school geometry described will facilitate information transfer, and the distance between individuals is likely maintained by visual contact, the reason for adopting this pattern is enhanced energy efficiency (Ashraf et al., 2017). Of interest, however, is that despite investigating variation in school structure at different flow velocities, these studies do not explore the patterns adopted by groups in the absence of flow.

The side-by-side group pattern adopted under flowing conditions described for red nose tetra appears not to be unique to this species. Groups of eight giant danio (*Devario aequipinnatus*) also adopt a side-by-side configuration in the presence of flow, compared to static water conditions (Chicoli et al., 2014). However, the focus of their study is less on energy efficiency of swimming, and more on the detection of, and response (startle) to, an artificial predatory stimulus, and subsequent transmission of information. The authors observe that the probability of at least one individual detecting a looming visual predatory stimulus is higher in the presence of flow than in its absence. This is because the fish are better able to detect the visual looming predatory cue presented at the sides of the tank when they are rheotactically polarised, i.e. facing upstream. However, the internal transfer of information is lower in flow, as indicated by a lesser number of individuals responding. Chicoli et al. (2014) suggest that flow likely impairs the transmission of social information, in this case the propagation of a startle response, because hydrodynamic cues from neighbours may be masked under flowing conditions. Furthermore, the side-by-side positions adopted may prevent those within the interior of the group gaining direct visual contact with the stimulus, especially under shallow water two-dimensional conditions typical of many experimental settings. This indicates that the adoption the side-by-side group pattern may carry disadvantages, in terms of disrupted information transfer, as well as advantages through enhanced efficiency of swimming at high velocities (Ashraf et al., 2017; 2016). Although Chicoli et al. (2014) report that groups tend towards a circular shape under static water conditions (as opposed to an oblong one due to the side-by-side positioning in flow), information relating to the position adopted by individual fish relative to one another is limited.

In the current study we aim to further explore group structure and the transfer of information between members of fish pairs under flowing and static water conditions so that the effect of flow can be isolated. Building on the previous research described, we explore the potentially contradictory mechanisms that underpin the emergence of different group patterns. On the positive side, under flowing water conditions, and especially when fish are forced to swim rapidly, a side-by-side configuration can reduce the energetic costs of swimming (Ashraf et al., 2017; 2016) . However, by doing so information transfer between fish may be impaired as a result of the hydrodynamic noise created under flowing water conditions, and the visual isolation that results from close proximity to conspecifics (Chicoli et al., 2014). Using the reductionist approach adopted by (Ashraf et al., 2016) and others (e.g. Herbert-Read et al., 2011; Katz et al., 2011), and in an effort to expand understanding to a wider range of species, we use pairs (the smallest subsystem of a shoal) of Eurasian minnow (*Phoxinus phoxinus*) as our model due to their tendency to maintain cohesive shoals (Pitcher, 1973a) and ease of acquisition and maintenance in the laboratory. The minnow pairs were filmed in a section of a recirculating flume under either static or flowing water conditions. By using automated tracking software to obtain the position and orientation of both individuals through time, we were able to investigate the influence of flow on: (i) shoaling time, (ii) shoal structure, and (iii) information transfer between individuals, based on accelerations, and correlation analysis (Cavagna et al., 2010; Katz et al., 2011) of swimming speed, and turning as a function of distance and location between the fish. Due to the energetic benefits described, we predict that fish will be more likely to shoal in the presence of flow, and remain in close proximity to each other while adopting an energy efficient side-by-side configuration as previously described by others (Ashraf et al., 2017; 2016; Chicoli et al., 2014). In the absence of flow, and subsequent reduction of hydrodynamic noise, we predict that the value of shoaling, and in particular the adoption of a side-by-side configuration, will be lost as the maintenance of close proximity will no longer bestow energetic benefits, while continuing to impose the costs of visual obstruction by conspecifics. In this case, fish are likely to adopt an alternative configuration that has previously gained limited attention.

## METHODS

### *Experimental setup*

Experiments were conducted in a recirculating flume (12.0 m long, 0.3 m wide and 0.5 m deep) at the International Centre for Ecohydraulics Research (ICER), University of Southampton. Two 5 mm mesh panels were inserted in the channel to create a 1.0 m long experimental area (Fig. 1a) over which two 85 W PhotoSEL Fluorescent lights were placed on wooden platforms to provide uniform illumination. The glass walls were covered to prevent external disturbance and to prevent fish seeing reflections during the trials. The discharge was monitored using a flow-meter and controlled with a valve on a recirculating pipe. A sharp crested weir was located at the downstream end of the flume to control depth. An overhead camera was installed to record fish behaviour (Casio Exilim EX-F1 (12x optical zoom) with a spatial resolution of 640 x 480 pixels, frame rate of 30 fps, and resulting field of view that covered the experimental area, Fig. 1b) against a pale flume floor that enhanced contrast between the fish and the background during video tracking.

Experiments were conducted under: (1) static water (control) of 4.5 cm depth, and (2) low and (3) high velocity treatments under constant discharge of 0.001 m3/s and 6.0 cm and 3.0 cm depth, respectively (Table 1). Under these shallow water conditions, fish shoals were assumed to be two-dimensional (Katz et al., 2011). The magnitude of the high velocity treatment was determined through pilot experiments. At higher velocities, minnow struggled to maintain position against the flow and risked becoming impinged on the downstream screen. Across the two velocity treatments the Reynolds number , where *U* is flow velocity (m/s), *H* is water depth (m), and *ν* is kinematic viscosity (m2/s). was maintained constant by altering the water depth (Table 1) so that similar turbulence characteristics (turbulence intensity) were experienced, thus isolating the effect of flow velocity.

### *Experimental procedure*

Eurasian minnow were used as the model species in this study. They are a member of the Cyprinidae family and are known to form shoals throughout their life, making them a suitable candidate for experimental investigation of collective behaviour (Partridge, 1982; Pitcher, 1973a, 1973b). By using pairs of minnow, as the smallest subsystem of a shoal, we eliminated the confounding effects of multiple alternative sources of social information gained by an individual (Delcourt & Poncin, 2012). Such a reductionist approach has been commonly adopted previously. For example, under static water, Herbert-Read et al. (2011) analysed interactions in mosquitofish (*Gambusia holbrooki*) pairs and then increased shoal size to four and eight individuals; Katz et al. (2011) investigated golden shiner (*Notemigonus crysoleucas*) pairs followed by groups of, three, 10 and 30 fish; and Ashraf et al. (2016) studied red nose tetra in groups of two and three fish before expanding to larger schools in a later study (Ashraf et al., 2017).

A total of two-hundred minnow(mean body length [BL] ± SEM = 52.6 ± 0.5 mm; mean wet mass [M] ± SEM = 2.2 ± 0.1 g,) were captured from the river Itchen (51°3’5.7” N, -1°18’59.1” W) using a seine net on 8 April 2013. They were transported in aerated river water to the ICER facility and maintained in a 500 litre holding tank for two weeks prior to being placed into two 110 litre holding tanks (approx. 100 individuals per tank) close to the flume so that they could acclimatise for four days before the trials commencing. These tanks had a substratum consisting of gravel with small pebbles, and several objects (flower pots, pipes) that offered shelter for all individuals. The holding tanks contained dechlorinated and continuously oxygenated tap water. Water quality was maintained using submersible pond pumps and filter systems, and 50% of the volume was exchanged when levels of compounds toxic to fish exceeded thresholds (0.25 ppm for NH3 and NO2-, and 40 ppm for NO3-). All fish were exposed to a natural/ daily light:dark regime and were fed fish flakes twice daily. Disturbance to the fish in holding tanks was minimized by placing the tanks in locations with minimal human presence, except for the researchers.

Five half-hour trials were conducted per night (after 8 pm to minimise the amount of scattered light present) during May 2013, until a total of 30 (10 replicates per treatment) had been completed. Before each trial, two minnow were selected at random from the holding tanks (mean ± SEM temperature = 20.2 ± 0.03 °C) and transferred to the experimental area, with one of two overhead lights switched on (alternated between trials) to prevent sudden exposure to full lighting. After five minutes the second light was switched on, and after a further five minutes of acclimation the trial commenced. At the end of each trial, fish were collected, measured and weighed. The sex of each individual was not determined as it was not a factor of interest for this study. Eurasian minnow form mixed-gender shoals in the wild, thus pre-selection based on sex was not deemed essential. Each fish was used once only, and treatments were alternated daily. After trials the test fish measured and weighed before being transferred to two other 110 litre holding tanks (specifically defined for used fish), similar in size and substrate/ shelter; and maintained and fed until completion of the experiment. Mean ± SEM flume water temperature at the start of trials was 20.7 ± 0.16 °C. Pilot experiments indicated that acclimation minimised startle response and erratic movements during trials.

### *Tracking and behavioural analysis*

Video records of fish movements were analysed. Fish were deemed to shoal whenever they were within 4 BL of each other (Hensor, Couzin, James, & Krause, 2005; Katz et al., 2011). After excluding non-shoaling time, a five minute sample was randomly selected from the remaining available video data for each trial and analysed. Fish movements were tracked (Ctrax v. 0.3.9), enabling determination of the location and orientation of individuals through time (Fig. 1b). Track data was loaded into Matlab (R2012a) and the selected fragments checked for errors on a frame-by-frame basis before final export. This allowed determination of the x, y coordinates of each fish (i.e. their centre of mass) based on an arbitrary origin, as well as the orientation (radians) through consecutive frames. Tracks were smoothed using a moving average of ten frames.

Shoaling time was expressed as a percentage of total time (excluding periods in which fish exhibited attempted escape behaviour by interacting with the screens) spent shoaling during each trial. Mean shoaling time was calculated per treatment.

Shoal structure was quantified by tracking the fish pair. In consecutive frames of each sampled video fragment, the focal fish was placed at the centre of a Cartesian coordinate system (Fig. 2) and the relative position of the neighbour added to the corresponding bin. Total counts in all bins were combined for all video samples of the same treatment and expressed as a percentage of the most visited following the approach of Katz et al. (2011) and Chicoli et al. (2014). Resulting density plots of shoal structure (sample size approx. 180k per treatment) were produced in Matlab. Fish orientation in each sample were combined for all videos for each treatment for which rose diagrams were produced.

Information transfer between individuals was determined based on information relating to fish velocity (m/s) and acceleration (m/s2). These were calculated by numerical differentiation of the smoothed fish coordinates and velocities, respectively, in consecutive frames. Both speed and acceleration were decomposed in a ‘speed’ (along the x- axis) and ‘turning’ (along the y- axis) component (Fig. 2). First, the speed of the focal fish compared to the neighbour, in terms of speed and turning acceleration, was calculated as a function of the distance between fish. Second, interactions between individuals, and resulting information transfer, was quantitatively expressed by means of a correlation analysis (Cavagna et al., 2010; Katz et al., 2011). Velocity (speeding and turning) correlation functions were calculated as a function of time lag (dt, up to 1 s or 30 frames of video material) and of the relative position of the neighbour with respect to the focal fish (Eqn. 1).

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|  |  | (1) |

where s1 is the velocity of the focal fish and s2 the velocity of its neighbour. For each time lag under consideration, velocity data from both fish were pooled, considering each individual as the focal fish, and the ensemble average was used, denoted by the angle brackets in (Eqn. 1). The location of the neighbour was categorised as 4 equal sectors (Fig. 2): (i) in front of, (ii) to either side of, and (iii) behind the focal fish. Correlation analysis was carried out for each of these sectors. For flow treatments, correlation analysis was restricted to frames where both fish were oriented against the flow. Drifting while oriented downstream was thus excluded, but holding station included.

The magnitude of Cs provides information on the level of correlation between the focal fish and its neighbour. At a given time lag, a low and high value for Cs indicates that the velocity of the focal fish is weakly or strongly related to that of the neighbour, respectively. Furthermore, the presence of a peak, or local maximum, in the correlation curve indicates that at the associated time lag, the degree of similarity between both fish’s velocity has spiked; this is indicative of the response time of the focal fish. When such a peak is absent, information transfer to the focal fish can be considered weak.

### *Statistical analysis*

Shoaling time percentage data were arcsine square root transformed prior to conducting tests of normality and homogeneity of variance using a Shapiro-Wilk and Levene’s test, respectively. A one-way ANOVA was used to assess the influence of treatment (fixed factor) on shoaling percentage (dependent variable), followed by a Tukey HSD post-hoc test to identify which treatments were different. Speed and turning accelerations were tested for normality and homogeneity of variance using Shapiro-Wilk and Levene’s tests. As the data failed to meet these requirements, non-parametric Kruskal-Wallis tests were used to explore differences between treatments.

### *Ethical note*

At the time of the study, licences were not required to extract Eurasian minnow from UK waters. Permission to do so was obtained from the Environment Agency, Natural England, and the landowner. Experiments were carried out in compliance with Home Office regulations. No potential harmful manipulations were identified, and no tags or data loggers were used on the fish. In accordance with Home Office regulations, all minnows were killed after the experiment was completed. It was done by following the Home Office Schedule 1 appropriate methods of humane killing for which the lead author held a licence. This follows a two-step procedure consisting of administering a lethal overdose of anaesthetic followed by confirmation of brain death by severing the spinal cord.

## RESULTS

Shoaling time:Minnow spent the majority of time under all treatments shoaling, and tended to shoal more under flowing than static water (Fig. 3). Shoaling time differed between treatments (*F*2,27 = 4.523, *P* = 0.020) and was greater under high flow than static water (*P* = 0.017).

Shoal structure: In static water, the distribution of the neighbour relative to the focal fish formed an ellipse with major and minor axis up to 1.0 and 0.5 BL, respectively. The neighbour tended to be present more often in regions immediately in front of or behind the focal fish, thus the pair could be considered swimming in a line behind each other, or in a ‘tandem’ configuration (Fig. 4a). Fish predominantly oriented themselves along the 0° and 180° direction relative to the *x*-axis (Fig. 4b). Under low flow, the neighbour showed a more uniform distribution around the focal fish at distances of up to 1 BL, comparable on the *x*-axis to those observed for the static water condition (Fig. 4c). Conversely, under high flow the neighbour tended to occupy positions to the side of the focal fish, to a maximum of approximately 0.5 BL away (Fig. 4e). Under both flow treatments, fish tended to adopt more polarised orientations (between -20° and 20°) compared to the control, facing the direction of flow, while active downstream movement was rare (Fig. 4d, f).

Information transfer: Under all treatments, the focal fish reacted to the position of its neighbour by modulating its speed. When the neighbour was behind, the focal fish decelerated, and when the neighbour was in front the focal fish sped up. This is indicated by negative and positive values for speed acceleration, respectively (Fig. 5a, c, e), and shows that information transfer between the two fish takes place when they adopt a tandem configuration. Speed accelerations differed between treatments (Kruskal-Wallis: *H2* = 86.68, *P* < 0.001, with differences between standing water (median 0.0002 m/s2)and each flow treatment *P* < 0.001 and the difference between low and high flow *P* < 0.05 (medians -0.009 m/s2 and -0.006 m/s2, respectively). However, the small difference between the medians across treatments (< 0.01 m/s2) indicates that the distribution of the data is highly similar as shown in Fig. 5 a, c, e. Similarly, the focal fish exhibited turning movements that matched those of the neighbour, i.e. the turning component of the focal fish’s acceleration was negative (indicating a turn to the left) as the neighbour moved farther to the left, and it became positive when the neighbour moved farther to the right (Fig. 5b, d, f). It can thus also be inferred that there was exchange of information between the two fish with regards to directional changes. The exact nature of these interactions can be further illustrated using the velocity correlation functions (Fig. 6). Peaks in the velocity correlation functions (where present) were typically between 0.3- 0.5 s under all treatments, reflecting the timescale over which a response of the focal fish was exhibited. However, the occurrence of these peaks in all velocity correlation curves, except for those when the neighbour was located behind the focal fish, indicated that information transfer did not flow from back to front.

In static water, temporal correlations of both components of each fish’s velocity were highest when the neighbour swam in front, i.e. the focal fish responded best when swimming behind its neighbour (Fig. 6a, b). This suggests that the front fish is leading, and not responding to velocity changes of the trailing fish. In turn, the trailing fish detects (presumably using vision and the lateral line) movements of the front fish and responds accordingly. This is in agreement with a “follow-the-leader” or tandem configuration predominantly observed under this treatment. Under flowing water treatments, both the speeding and turning velocity correlation functions showed distinctly different features compared to those in static water. First, generally higher values for correlation of the speeding velocity component (Fig. 6c, e) indicated that, in addition to shoaling more (Fig. 3), fish interacted in a more coordinated way in moving water. Second, highest correlations for the turning component of the velocity were observed for the side-by-side configuration (Fig. 6d, f). Finally, regardless of which individual was chosen as the focal fish or neighbour, the correlation functions always displayed a maximum, indicating that the exchange of information between the two was mutual and no longer in one direction, as observed for the tandem configuration. The combined observations suggest that under flowing water conditions, remaining behind the front fish is not as advantageous as adopting a side-by-side configuration, where coordinated movements are enhanced, particularly when changing direction. However, this observation is less frequent under static water when the tandem configuration appears to be beneficial.

## DISCUSSION

This study investigates the collective behaviour and shoaling dynamics of pairs of minnow in the absence and presence of flow by quantifying time spent shoaling, shoal structure, and information transfer between the individuals. A greater proportion of time is spent shoaling under the highest flow, compared to the static water condition (Fig. 3), during which fish tend to adopt a side-by-side configuration as previously reported by others (Ashraf et al., 2017; 2016; Chicoli et al., 2014) (Fig. 4e). While our observations support the findings of (Ashraf et al., 2017; 2016), they appear to contradict those of Chicoli et al. (2014), which indicate information transfer is reduced in flow, even though the probability of detecting a looming predatory stimulus is enhanced. Conversely, our results suggest bidirectional information transfer between two individuals is improved under the flowing conditions (Fig. 6c- f). Furthermore, we describe an alternative “follow-the-leader” or tandem configuration in the absence of flow (Fig. 4a), which appears to limit transfer of information from the front to back fish (Fig. 6a, b), but which may nevertheless have ecological benefits when considering the detection and response to other important stimuli.

As predicted based on the evidence of energetic benefits bestowed by forming cohesive groups (Ashraf et al., 2017; 2016), minnow spend more time shoaling under higher flow compared to the static water treatment. In their studies, Ashraf et al. (2017; 2016) observe that energy efficiency of swimming is enhanced when schooling at higher velocities, as tetra move closer to their neighbours as they synchronise slower tail beats. Previous studies to investigate the influence of flow rate on shoaling or schooling measure a variety of metrics and report contradictory results. In our study we use percentage of total time as an indicator of the propensity to shoal, based on the assumption that an energetic or other advantage is gained when swimming together against higher velocities. In another experimental study that uses the same model species, shoaling appears to decline during a high flow event, as indicated by a reduction in shoal size, which remains smaller for an hour thereafter (Garner, 1997). However, the author cautions that the level of variability between replicates is high. In another case, the ratio of the number of fish in a shoal to the shoal length (linear distance from nose of the most upstream to tail of the most downstream fish) is used to define an aggregation index (Allouche & Gaudin, 2001). Using an experimental stream to explore the response of juvenile chub (*Leuciscus cephalus*) to a simulated avian predator, no influence of flow velocity on the level of aggregation is observed when the stimulus is absent. In the presence of the predatory cue, greater aggregation occurs at high flow, but only in pools. Hockley, Wilson, Graham, and Cable (2014) find that shoal size of guppies (*Poecilia reticulata*), held in an experimental re-circulatory flume, is smaller under flowing than static water conditions. Their study investigates the interacting effects of flow and parasite infection and suggests that infection reduces shoal cohesion (increases nearest neighbour distance), although the magnitude of difference varies with treatment, being greater under the static water condition at the group level. Neither parasite infection nor flow treatment appears to influence the time spent shoaling. The explanation for such contradictory results is not clear, although it is apparent that the aims of previous studies tend to be multifaceted, considering the interaction between flow and other factors (e.g. predation, parasitism) resulting in more complex experimental designs in comparison to the current study that adopts a high resolution and much more reductionist approach to ensure confounding variables are controlled. Nevertheless, it is clear that relatively few studies are conducted to explore the response of shoaling fish to variation in flow velocity, particularly by directly comparing results from static and flowing water conditions, and as such understanding of the ecological drivers and consequences remain limited.

Our observations support the findings of others that describe the adoption of a side-by-side shoaling configuration under flowing water conditions (Ashraf et al., 2017; 2016; Chicoli et al., 2014). The proposition by Ashraf et al. (2017; 2016) is that the side-by-side structure enhances the efficiency of swimming kinematics, as evidenced by a lower tail beat frequency. Such a configuration may optimize thrust by facilitating the exhibition of in-phase synchronicity and enabling fish to use the channelling effects between (Ashraf et al., 2017), especially at small clearances of around 0.5 BL, which is similar to those observed in this study. However, it is the switch of configuration to a “follow-the-leader” tandem structure under static water conditions that is perhaps the most interesting observation in our experiment. Rather than forming an elongated linear school shape in the absence of flow, as is the expectation based on our findings, (Chicoli et al., 2014) describe one that was more circular. Interestingly, and despite the lack of a static water control treatment and the use of large groups, (Ashraf et al., 2017; 2016) also describe an increased probability of individuals adopting linear downstream positions relative to their neighbours at lower flow velocities, as the energetic benefits are lost. This presents an interesting question; what is the reason for no longer maintaining a side-to-side shoal structure under static water conditions? That is, in the absence of energetic benefits gained by more efficient swimming as a result of adopting this configuration at high flows. The answer likely relates to information transfer, particularly under two-dimensional shallow water scenarios.

From the perspective of information transfer, the advantage to an individual of positioning itself laterally to other members of the shoal is that it can enhance acquisition of visual cues about its conspecifics (Chicoli et al., 2014). By using velocity correlation functions, and especially those associated with turning motions, to quantify information transfer between two fish that comprise a pair, we illustrate bidirectional information transmission to be highest when they are positioned next to each other under high flow. A greater dependence on visual cues may be especially important if unidirectional flow compromises the ability of the lateral line to detect oscillatory flows generated by swimming neighbours (Chicoli et al., 2014). Indeed, in early studies in which the lateral line is ablated, even under static water conditions fish tend to associate more with neighbours positioned alongside, compared to control and blinded individuals (Partridge & Pitcher, 1980). Furthermore, visual contact between members of the shoal that adopt a side-by-side configuration under energetically challenging high flows enable fish to synchronise swimming to enhance efficiency (Ashraf et al., 2016).

The downside of a side-by-side configuration is that an individual’s wider visual field is obscured, especially under two-dimensional shallow depths commonly presented under simplified experimental conditions where fish are unable to alter their position in the water column to any great extent. Such visual isolation occurs on one side only in the case of a pair, such as in this study, or an individual positioned at the edge of a larger school, but on both-sides for those that are more centrally located (Chicoli et al., 2014). Thus, in the absence of energetic benefits of side-by-side swimming, or the hydrodynamic noise created under unidirectional flow, the cost: benefit ratio shifts under static water conditions so that an alternative pattern, such as a “follow-the-leader” tandem configuration, becomes more advantageous. This pattern likely enhances the wider visual field, presumably aiding the detection of stimuli that emanate from outside the shoal (e.g. drifting particles of food or an approaching predator).

In our study, velocity correlation functions indicate that information transfer in the forward direction is weak (low correlation values and an absence of peaks), which is unsurprising considering that one fish is positioned in the blind spot of the other. Nevertheless, while information transfer between individuals is lower than when adopting a side-by-side pattern, it is not entirely lost, as cues continue to be passed in the backward direction to the trailing fish that responds to changes in the movement of the leader, enabling both to benefit if they periodically change position. However, such mechanisms are complex and likely to vary dependent on context and perhaps also with species. Indeed, Chicoli et al. (2014) describe improved stimulus detection of a visual cue under the flowing condition, probably because their fish are rheotactically polarised and thus align in a common upstream direction, making it easier to detect external cues presented from the side of the tanks. Conversely, our study focuses only on internal transfer of information between members of a pair.

In nature, minnow shoals usually consist of many more than two members (Kottelat & Freyhof, 2007). In such large groups, individuals are likely in proximity of multiple conspecifics that may take up positions both to the side and in the front/ back at the same time (Ashraf et al., 2017; Chicoli et al., 2014). With so many possible sources of social information, identifying the detailed interactions that occur between individuals are very difficult to detect and quantify. It is therefore essential, as a first step, to investigate pairwise interactions. Studies that have adopted a similar strategy showed that under static water, members in groups up to 30 individuals integrate information from multiple social sources by speed regulation rather than orientation matching and are most likely to respond to a single or two nearest neighbours instead of averaging the response of all within a certain area around it (Herbert-Read et al., 2011; Katz et al., 2011). Under flowing conditions such information is limited, but under flows of appr. 15 cm s-1, Ashraf et al. (2017) showed that groups of nine individuals consistently organized themselves in a side-by-side configuration, synchronized their swimming movements (which can be seen as a form of speed regulation) and individuals also reacted to the nearest neighbour on both sides. However, shoals of e.g. hundreds of individuals are unlikely to extend only in the lateral direction, as the domain can be limited and taking up peripheral positions is not without risk (Jens Krause, 1993). Having described the pairwise interactions in this study, a valuable continuation of the current research will be to identify whether the results presented here hold for larger minnow shoals groups, similar in size and structure as to those observed in nature.

This study adopts an analytical approach to help improve understanding of social interactions and information transfer in shoaling fish under both static water and flowing conditions. Many exciting avenues for future work remain, which include developing understanding of group response for a greater range of species and under more realistic conditions that accommodate properties of the flow which may induce simultaneous responses that occur independently from each other (e.g. turbulence intensity and integral length scales, Halsey, Wright, Racz, Metcalfe, and Killen, 2018) and depth (enabling a three-dimensional group structure to be attained). Ideally, the understanding gained will need to be validated in the field, provided biological and physical parameters are measured at a sufficiently high spatial and temporal resolution. This information will help to develop and improve interaction rules, such as nearest neighbour distance, attraction, repulsion, e.g. Couzin et al. (2002), that form the basis of commonly used Agent Based Models to test predictions of collective behaviour.

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**Data accessibility**

Data supporting this study will be made openly available from the University of Southampton repository at <https://doi.org/10.5258/SOTON/D1335>.

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**Table 1.** Hydraulic conditions encountered by Eurasian minnow pairs swimming in a recirculating flume under static water and two flow treatments.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment** | **Water depth**  **(cm)** | **Mean velocity (cm/s)** | **Reynolds number (Re)** | **Froude number (Fr)** |
| Static water | 4.5 | 0 | 0 | 0 |
| Low flow | 6.0 | 5.74 ± 0.03 | 3021 ± 17 | 0.07 ± 0.0004 |
| High flow | 3.0 | 11.57 ± 0.08 | 3045 ± 21 | 0.001 |

**Figure 1.** (a) Plan of the experimental setup in a recirculating flume used at the International Centre for Ecohydraulics Research, University of Southampton, to investigate the group response of pairs of Eurasian minnow (*Phoxinus phoxinus*) under static and flowing water conditions. Thick black lines represent the sides of the flume, with two mesh panels that bounded the domain (grey lines); the circle represents the position of the overhead camera; rectangles represent locations of overhead light sources. (b) Snapshot overhead image of a minnow pair taken under the ‘High flow’ treatment. It shows the experimental area within the mesh panels on the left and right, and the two fish identified by the Ctrax tracking software.

**Figure 2**. Focal fish coordinate system and division of its surrounding area into three regions. In consecutive frames, the focal fish (black) is placed at the origin of a fixed Cartesian coordinate system and the neighbouring fish (grey) at distance r away. Each fish has an orientation angle with the horizontal x-axis. Velocity can be decomposed into a speeding (along the x-axis) and turning (along the y-axis) component. The neighbouring fish can be placed into a specific region depending on its location with regards to the focal fish, defined as either in front of (I), to either side (II) or behind (III).

**Figure 3.** Shoaling time of Eurasian minnow pairs expressed as the percentage of total time spent shoaling, after this was adjusted for escape behaviour at the mesh panels. Shoaling time was calculated per treatment, each consisting of 10 trials. Error bars denote ± SEM.

**Figure 4.** Contour density plots and rose diagrams of shoaling Eurasian minnow pairs. The position of the neighbouring fish with respect to the focal fish (top) and the frequency of orientations of both fish (bottom) are shown for static water (a-b), low flow (c-d) and high flow (e-f). For the density plots, the domain plotted is [-3 BL, 3 BL], where BL is the average body length of fish used in the associated treatment. The black fish shape denotes the location of the focal fish in the origin. Length of each rose histogram is a measure of the frequency (percentage) with which Eurasian minnow are oriented in a particular direction. In graphs (c-f), flow is from right to left.

**Figure 5.** Mean accelerations of the focal fish vs the distance of the neighbour. The speeding and turning component of the acceleration is shown in the top and bottom panel, respectively, for static water (a-b), low flow (c-d) and high flow (e-f). For the speeding components, positive (negative) distances indicate a neighbouring fish in front of (behind) the focal fish. For the turning components, positive (negative) distances indicate a neighbouring fish to the left (right) of the focal fish.

**Figure 6.** Temporal velocity correlations depending on relative position of neighbouring fish. Mean correlations (with 95% CI) of the focal fish at time *t+dt* with respect to the neighbour at time *t*, when it is either in front (blue), behind (red) or to the side (black). The speeding and turning component of the velocity is shown in the top and bottom graph, respectively, for static water (a-b), low flow (c-d) and high flow (e-f).