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Original Article

Water flow impacts group behavior in zebrafish (*Danio rerio*)

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INTRODUCTION

Phenotypes should be more flexible in variable or heterogeneous environments compared to more stable or homogenous environments, particularly when these environmental features impose a metabolic cost (Ellers and Van Alphen 1997; Weinig 2000; Van Kleunen and Fischer 2005; Snell-Rood et al. 2011). These plastic responses could involve changes to morphology during development, or more immediate changes to behavior. For example, stickleback (*Gasterosteus aculeatus*) and cichlid fish, *Astatotilapia burtoni*, have evolved morphological features that may help them to overcome the physiological costs associated with water currents (Kaeuffer et al. 2012; Hockley et al. 2014; Theis et al. 2014). Both vertebrates and invertebrates use complex behavioral responses to adjust locomotion to changing wind conditions and to navigate through turbulence created by different types of obstacles (Ravi et al. 2013; Sapir et al. 2014; Crall et al. 2015). Animals may also form groups to gain aerodynamic or hydrodynamic benefits under certain flow conditions (e.g., Hemelrijk et al. 2014; Becker et al. 2015; Croft et al. 2015). This sort of behavioral plasticity may be an important mechanism for coping with environmental fluctuations and increasing individual fitness (Van Buskirk 2012). Here, we use a controlled laboratory experiment to test the influence of water flow on the plasticity of social behavior of zebrafish (*Danio rerio*), considering also the impact of obstructions.

Flow affects behavior directly in several ways, sometimes imposing an important energetic constraint and sometimes reducing the costs of locomotion. Fluctuations in environmental factors such as the strength of tailwinds are especially important in predicting daily distance (Mellone et al. 2012) and flight speed (Safi et al. 2013; Horvitz et al. 2014) in migrating birds. Changes in wind can also have major impacts on the timing and routes chosen by insects and birds during migration (e.g., Grönroos et al. 2012; Chapman et al. 2016). In water, the fluid motion around fish bodies can influence their detection by potential predators or prey (Gemmell et al. 2013; Stewart et al. 2014), and group vigilance can be enhanced in flowing as opposed to still water (Chicoli et al. 2014). Parasites tend to thrive in low flow conditions, causing them to have small impact on social groups in high flow conditions (Lenihan et al. 1999; Barker and Cone 2000; Hallett and Bartholomew 2008).

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On the other hand, flow and turbulence may also obstruct sensory systems with background noise making it harder to communicate with social partners (Mogdans and Bleckmann 2012). Similarly, dominance hierarchies may be less stable when there is increased turbulence or a constantly changing environment (Sneddon et al. 2006). Nevertheless, guppies (*Poecilia reticulate*) and chub (*Leuciscus cephalus*) tend to form larger shoals in flowing than in still water (Allouche and Gaudin 2001; Hockley et al. 2014). Here, we test the effects of a weak flow, typical of some areas in which zebrafish are naturally found (Spence et al. 2011; Arunachalam et al. 2013; Parichy 2015; Suriyampola et al. 2015), and predict that shoals will be less cohesive and more active in the flow due to the alteration of fish motion by the water current. As fish work hard to maintain their positions in flowing water, we expect their energetic costs, stress, and aggression also to increase.

Physical obstructions can also influence social behavior by introducing structural complexity and possible turbulence. As air or water flows around an object, it creates forces that can push an animal in different directions, increasing the cost of locomotion, sometimes with fatal consequences (Ravi et al. 2013; Cryan et al. 2014; Crall et al. 2015). Vegetation and other forms of structural complexity can also impact behavior independently of flow, for example, leading to enhanced foraging (Gotceitas 1990), or potentially leading to complex interaction effects by creating regions of turbulence in flowing water. Structural complexity can also decrease aggression by decreasing visibility (Eason and Stamps 1992), the chance of encounter (Corkum and Cronin 2004), or perceived risk (Pettersson and Brönmark 1993; Hamilton and Dill 2002). The presence of landmarks may increase aggression by providing defensible resources (Barreto et al. 2011; Suriyampola and Eason 2015) or decrease aggression by allowing individuals to establish territories (Danley 2011). Structural complexity also limits the available space, increasing the density and thereby the rate of interaction between individuals (Kaspersson et al. 2010; Poot et al. 2012). Here, we predict that obstacles will accentuate the effects of flow, making groups to be even less cohesive and more aggressive than when in flowing water without obstructions.

To test these predictions, we use zebrafish in an experiment measuring the effects of water flow on behavior, and considering also the impact of obstacles. Zebrafish are small cyprinids native to India, Nepal, Pakistan, and Bangladesh (Arunachalam et al. 2013). Although zebrafish are typically described as occurring primarily in still water (lakes and ponds) in small, loose shoals (Spence et al. 2011; Parichy 2015), a recent field study also found zebrafish forming much larger and more coordinated schools in fast-flowing water (Suriyampola et al. 2015). Zebrafish thus live in a wide range of flow conditions and exhibit a very broad range of group cohesion in the wild, and make a suitable choice for a study of the impact of water flow, with and without obstructions, on social behavior.

METHODS

Study subjects

We collected wild zebrafish from West Bengal in northeastern India in June 2014, and exported them to our lab in the United States for this experiment. The collection site was geographically close to the FM site of Suriyampola et al. (2015), where fish occur in a variety of habitats including both still water and flows up to 14.1 cm/s, vegetated and non-vegetated areas. Individual fish are likely to experience a wide range of these habitats as conditions change seasonally with the monsoons. In the lab, we housed zebrafish in 37.8 L (10 gallon) tanks in standard conditions: mixed sex ratios, 28 ± 3°C, 14:10 h light: dark cycle, and fed ad libitum commercial flake food (Tetramin Tropical). Each housing aquarium was aerated with a small filter (Supreme® Ovation 210), which created a very weak flow. We began the experiment after the wild-caught fish had acclimated to our laboratory conditions for 2 months, thereby ensuring also that all of the zebrafish were adults and in good health. Note that this additional time in the lab likely also increased their overall aggression (Martins and Bhat 2014).

Treatment conditions and procedures

After the initial acclimation period, we formed 25 mixed-sex groups of 6 adult fish (3 males and 3 females in each) and allowed them to become familiar with each other for more than 30 days. In the morning following this period of group formation, we moved each group to a new 20.8 L (5.5 gallon or 43 × 23 cm) aquarium with shallow water (8 cm) for testing in a nearly 2-dimensional context. Each aquarium was fitted with a white plastic floor to enhance automated tracking of overhead video images by increasing contrast between the zebrafish and their backgrounds. Under that floor, we submerged a small aquarium filter (Supreme® Ovation 210), extending the tip above the surface through a PVC connector (4 cm tall, 2.5 cm diameter). Above the test arena, we installed a webcam (Logitech® c525 HD) for video-recording at 30 frames/s.

We placed each group of fish in one of the following treatment conditions (chosen at random for each group): 1) an empty arena, 2) obstructions, 3) flowing water, and 4) flowing water and obstructions. We constructed each test arena with the goal of enhancing tracking by maximizing contrast, for example, suspending a white plastic floor over the body of the filter and illuminating the arena with full-spectrum lights. For the flowing water treatments, we turned on the aquarium filter, generating a gentle flow of 4 cm/s. For the treatment with obstructions, we temporarily attached 4 small plastic plants, which were 8 cm in height (Green Foreground Plastic Aquarium Plants, painted white to increase contrast) to the white plastic floor. We placed these plants in a square formation (12 cm between plants), so that they could be used as landmarks identifying different parts of the available space. Together, however, the plants occupied a relatively small proportion of the available area (0.7% of the total volume and 3.5% surface area). Although we did not measure turbulence directly, it seems likely that the plants contributed to creating a number of turbulence peaks and gradients.

After about 20 h of acclimation to each treatment condition (about 1 h after lights came on the following morning), we video-recorded each group of fish engaged in undisturbed behavior for a total of 4 min. At the end of the trial, we sprinkled 500 mg of food on the surface of the water in the center of the test arena and recorded the time before all 6 fish approached the food. We then altered each test arena (adding or removing plants, turning on or off the flow), and left the groups to acclimate to the new testing conditions. We repeated this procedure on 4 consecutive days so that each group was exposed to each of the 4 treatment conditions in random order.

Behavioral scoring and analysis

We used EthoVision XT10 (Noldus Information Technology 2013) software to track the zebrafish automatically from the video recordings. The software determined the *x* and *y* coordinates of each fish (from above in 2-dimensional space) every 0.03 s (1790 moments/min). We then used those coordinates to calculate several measures of social behavior. Ethovision tracked all 6 fish relatively well in our test arenas, although we had to drop about 7.8% of the 7160 moments in each trial because the software was unable to locate one or more of the fish. We did not see any differences between experimental treatments in this proportion.

We estimated shoal *Cohesion*, first using the rgeos package (Bivand and Colin 2014) of R (R Development Core Team 2014) to estimate the smallest convex polygon that included all 6 of the fish, and then calculating both the median distance of each fish from the centroid of that polygon and the maximum distance between 2 vertices of that polygon (i.e., "group diameter").

Next, we scored *Chase Rate*, operationally defined as the frequency of episodes of fast acceleration in which a fish with a velocity greater than 20 cm/s exhibited also an acceleration towards a second fish (the "Weighted Movement To" measure from Ethovision) for more than 0.3 cm. We chose these parameters based on preliminary analyses which showed that with these, we could accurately identify chases scored by a human observer from the same video. Our measure of aggression is thus also a subset (those that continue for more than 0.3 cm) of the "charges" described by Way et al. (2015). Our measure of Chase Rate, however, includes both temporal and group dimensions because we determined whether any fish in the group had engaged in a chase during each of the 7160 moments, summed across the trial as a whole, and finally divided by 4 min to get a number of chase moments/min.

Finally, we estimated locomotor activity as total *Distance Moved* (m/4 min), summing across the 6 fish in the group at each moment, and then taking the median across the 7160 moments in the trial. This measure of activity is similar to that used in Abril de Abreu et al. (2015), however the distances moved by the 6 fish at each moment of the trial were not normally distributed, making the median a better description of their central tendency. As the medians were normally distributed, we analyzed these using standard parametric statistics as described below (e.g., calculating a mean of medians). Please note that Distance Moved is also not an ideal measure of activity in flowing water because a fish that is working hard to maintain its location in flowing water may not move any distance at all. We also used the measure of *Group Latency to Feed* (time for all 6 fish to approach food) as a second general measure of activity, although latency to feed may also serve as a measure of boldness (Moretz et al. 2007) or hunger (Oswald and Robison 2008).

We used 2-way repeated-measures analysis of variance (ANOVAs) to test the effects of Flow and Obstructions on each of the 4 behavioral measures (Cohesion, Chase Rate, Distance Moved, and Group Latency to Feed), although also taking into account that each group was measured in all 4 contexts. We did all calculations using the "aov" function in R (R Development Core Team 2014), checking residuals for each model to confirm that the usual ANOVA assumptions were not violated. Using Pearson's correlations, we also estimated the magnitude of the relationship between different measures of behavior, and also the repeatability of measures taken from the same group in different contexts.

RESULTS

Zebrafish formed less cohesive groups when tested in flowing water

Flow was associated with less cohesive zebrafish shoals in our experiment (Figure 1). Zebrafish formed more cohesive groups when tested in still water (mean group diameter = 18 ± 0.7 cm; median distance from centroid = 6 ± 0.3) but formed significantly looser groups (mean group diameter: 21 ± 0.8 cm; median distance from centroid = 7 ± 0.3) in flowing water (diameter: $F_{1,70} = 4.9$, $P = 0.03$; Median distance from centroid: $F_{1,70} = 4.8$, $P = 0.03$). Although zebrafish formed the tightest shoals in the empty testing arena (no flow and no obstructions), obstructions had little impact on group diameter in flowing water (Figure 1). Neither the main effect of Obstructions (Diameter: $F_{1,70} = 0.4$, $P = 0.51$; Median distance from centroid: $F_{1,70} = 0.8$, $P = 0.38$) or the interaction between flow and obstructions was statistically significant (Diameter: $F_{1,70} = 1.0$, $P = 0.31$; Median distance from centroid: $F_{1,70} = 1.8$, $P = 0.18$). Across groups, we also found a marginally significant flow × obstruction interaction effect (diameter: $F_{1,22} = 4.2$, $P = 0.05$) or Obstructions (Median distance from centroid: $F_{1,22} = 4.1$, $P = 0.06$) in our repeated-measures ANOVAs, suggesting that some groups responded consistently differently to the 4 treatment conditions than did others.

Pearson product-moment correlation coefficients between measures of the same group in different treatment conditions were relatively weak and statistically insignificant ($r < 0.23$, df = 24 or 25, $P > 0.05$ in all cases), confirming that this pattern of group-level consistency was not strong. In contrast, measures of group diameter and median distance from centroid were very strongly correlated with each other ($r = 0.98$, df = 96, $P << 0.01$), suggesting that both measures reflect the same aspect of group Cohesion.

Zebrafish were more aggressive in the presence of flow and obstructions

Both water flow and the plastic plants were associated with increased aggression in our experiment (Figure 2). Zebrafish in our trials were not very aggressive in absolute terms, with an individual fish chasing others during fewer than 3.8% of the scored moments in each trial. Zebrafish chased each other about twice as often in flowing (median = 218 chases/min/group) than in still water (109

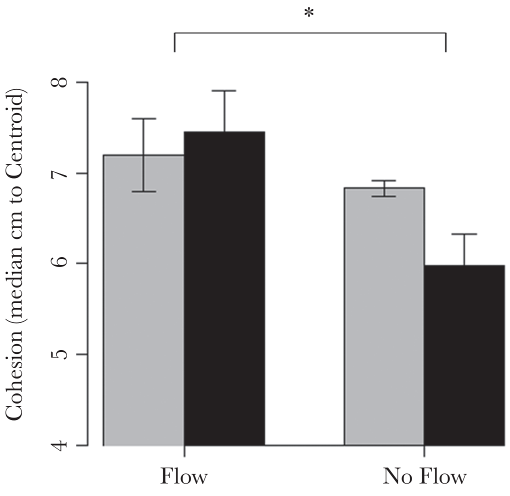

Figure 1
Zebrafish shoals were less cohesive in flowing water compared with still water treatments, exhibiting a longer median distance to the group centroid. Grey bars reflect zebrafish behavior in arenas with obstructions (4 small plastic plants), whereas black bars reflect behavior in empty arenas. The effect of plant obstructions and the plant × flow interaction effects were not statistically significant in our repeated-measures ANOVA ($P > 0.05$, see Results for additional details).

chases/min/group), and this difference was statistically significant ($F_{1,70} = 6.9$, $P = 0.01$).

Zebrafish tested in arenas with plants also chased each other about twice as often (median = 249 chases/min/group) as did zebrafish in arenas without obstructions (102 chases/min/group), a difference which was again statistically significant ($F_{1,70} = 12.5$, $P << 0.01$). We found no evidence for an interaction between flow and obstructions ($F_{1,70} < 0.1$, $P = 0.96$) or for significant differences among groups ($P > 0.05$) in our 2-way, repeated-measures, ANOVA.

Using Pearson correlations, we found some evidence of consistency between groups measures of Chase Rate in the 2 flowing water treatments ($r = 0.57$, df = 22, $P < 0.01$), obstructions with and without flow: $r = 0.48$, df = 22, $P = 0.02$), and also between group measures in the 2 no-flow treatments ($r = 0.55$, df = 22, $P < 0.01$). There was also a strong relationship between measures of the same groups in arenas with obstructions but no flow and in arenas with flow but no obstructions ($r = 0.67$, df = 22, $P < 0.01$). These consistent group differences were not detected in the ANOVA because there was no relationship between measures of the same group across flow conditions (no obstructions with and without flow: $r = 0.01$, df = 22, $P = 0.95$; or between measures in arenas without obstructions or flow and those in arenas with obstructions and flow ($r = 0.04$, df = 22, $P = 0.85$).

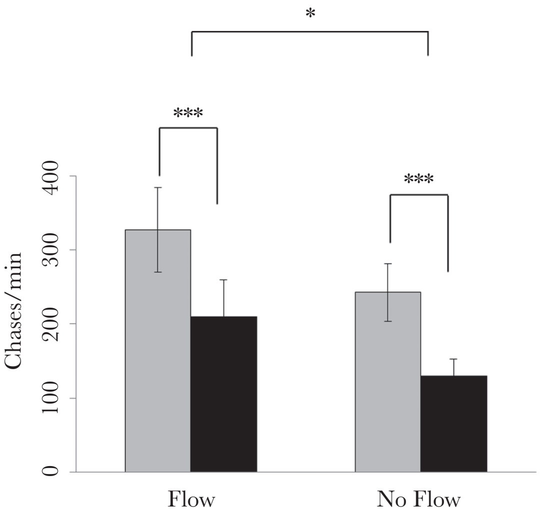

Figure 2
Zebrafish chased more often when tested in arenas with flowing water or with obstructions. Grey bars reflect zebrafish behavior in arenas with obstructions (4 small plastic plants), whereas black bars reflect behavior in empty arenas. We found significant main effects of flow and plants in our repeated-measures ANOVA, but no significant interaction effects.

Distance moved increased in flowing water and with obstructions

Zebrafish in our experiment moved continuously, with a group of 6 fish together moving up to 171.2 m during the 4-min trial. Most groups moved more slowly, such that the median distance moved during a 4-min trial (summing across all 6 fish) was 16.9 m (Figure 3a). Zebrafish moved greater distances in flowing (median = 0.16 m) than in still water (median = 0.15 m), leading to a significant main effect of Flow in the within-groups component of our ANOVA model ($F_{1,70} = 4.3$, $P = 0.04$). Zebrafish also moved more in the presence of the plastic plants (median = 0.17 m) than in an empty arena (median = 0.15 m; main effect of obstructions: $F_{1,70} = 7.4$, $P = 0.01$). We found no evidence for an interaction effect between flow and obstructions ($F_{1,70} = 0.01$, $P = 0.94$), or for significant differences between zebrafish groups ($P > 0.05$). We found some evidence of group-level consistency between measures in arenas with obstructions (but no flow) and those in arenas with flow (but no obstructions: $r = 0.54$, df = 22, $P = 0.01$), but there were no significant relationships between groups measured in any of the other contexts ($P > 0.05$ in all cases).

Our 2 measures of activity (Distance Moved and Latency to Feed) were not closely related to each other ($r = 0.12$, df = 96, $P = 0.22$). However, both responded similarly to flow (Figure 3). Zebrafish groups took less time to approach food in flowing than in still water (Figure 3b). Although most zebrafish in our study approached the flake food within a few seconds, a few groups took longer than 1 min to begin feeding. Groups in the flow treatments approached the food more quickly (median = 3 s), whether or not

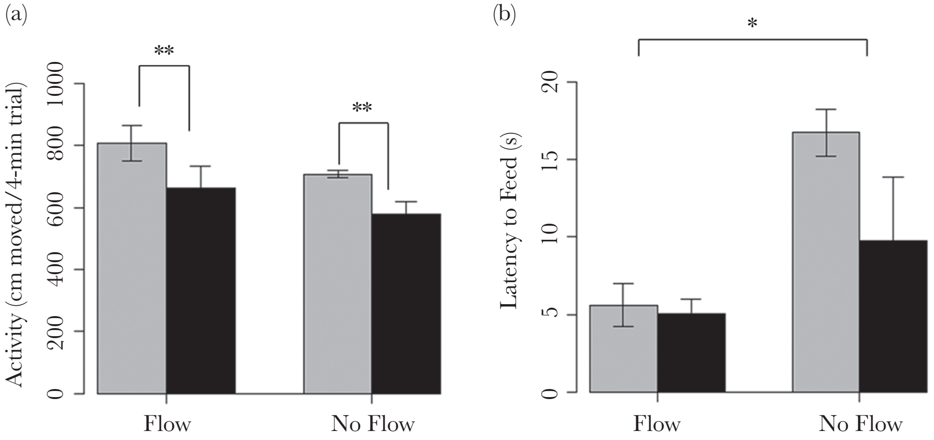

Figure 3
Zebrafish were more active in flowing water than they were in still arenas. (a) Average distance moved was greater in flowing arenas and in the presence of plastic plants than they were in still or empty arenas. We found no evidence for a significant interaction in our repeated-measures ANOVA. (b) Zebrafish moved more quickly towards food in flowing than in still water. However both the effects of plants and the interaction were not statistically significant in our repeated-measures ANOVA (see Results for additional details). Grey bars reflect zebrafish behavior in arenas with obstructions (4 small plastic plants), whereas black bars reflect behavior in empty arenas.

plants were present. Groups in still water took longer to reach the food (median = 4 s), leading to a significant main effect of Flow (within-groups) in our repeated-measures ANOVA ($F_{1,70}$ = 4.8, $P = 0.03$). Although zebrafish groups also took longer to reach the food when in treatments with obstructions (Figure 3b), this effect was mostly detected in the still water treatment and so was not statistically significant as a main effect of Obstructions ($F_{1,70}$ = 1.9, $P = 0.18$) or an interaction effect ($F_{1,70} = 2.0$, $P = 0.16$).

Again, there was no evidence of significant differences between zebrafish social groups in our repeated-measures ANOVAs ($P >$ 0.25). Nevertheless, we found some evidence of consistent relationships between measures of the same groups in different treatment conditions in our Pearson correlations. There was a significant positive relationship between measures in arenas with plastic plants without flow and arenas with flow: $r = 0.54$, df = 23, $P < 0.01$. Other measures were did not show significant correlations ($r < 0.38$, $P > 0.06$ for all pairwise comparisons). Note that as a consequence of the variability, the above ANOVA results are from models using log-transformed measures of latency to feed.

In general, our behavior measures were not closely associated with each other. We found a significant positive relationship between measures of aggression and activity (combining data across all 4 contexts: $r = 0.84$, df = 96, $P < 0.001$), despite the differences in how each measure responded to treatment conditions above. Group Cohesion was not closely associated with chase rate ($r = -0.06$, df = 96, $P = 0.57$), distance moved ($r = 0.10$, df = 96, $P = 0.34$) or latency to feed ($r = 0.02$, df = 96, $P = 0.82$). Chase rate was closely associated with distance moved ($r = 0.84$, df = 96, $P <<$ 0.01), but not latency to feed ($r = 0.16$, df = 96, $P = 0.82$).

DISCUSSION

Our results show that water flow can have an important impact on shoaling behavior, as zebrafish rapidly adjusted group dynamics in response to differences in water flow. Even in the weak flow used in this study, zebrafish were more aggressive, maintained less cohesive shoals, and approached food more quickly than they did in still water. We did not find any evidence that physical obstructions or turbulence further influenced the impact of flow, but zebrafish were more active and more aggressive when in a structurally complex context. These results suggest that zebrafish adjust their behavior to respond to small changes in their environment, and that both seasonal or anthropogenically caused habitat shifts may have important impacts on group behavior.

Flow can affect motion by increasing energetic constraint (i.e., of swimming against the flow) or by speeding locomotion (when swimming with the flow). Flow may also obstruct sensory systems making it harder to communicate with group members (Mogdans and Bleckmann 2012), or reduce predation risk (Allouche and Gaudin 2001). The increased activity level displayed by zebrafish in flowing water in our study suggests that the main impact of flow was to impose a cost as animals attempted to maintain group cohesion although moving against the current. Others have reported fish forming larger, more cohesive and oriented groups in fast-flowing water (Allouche and Gaudin 2001; Suriyampola et al. 2016) or no effect of flow on group cohesion (Bhat et al. 2015). The stark contrast between these and our own results suggests that other factors such as predation risk (Allouche and Gaudin 2001) or population-level effects (Martins and Bhat 2014; Bhat et al. 2015) may play important roles in the plasticity of social behavior in response to flow. Genetic differences clearly underlie many aspects of zebrafish behavior, including shoaling and boldness (Robison and Rowland 2005; Wright et al. 2006). There may also be a non-linear or threshold effect underlying the relationship between flow and group cohesion, for example, with only much faster-flowing water triggering the formation of well cohesive groups.

Although we expected obstacles to exaggerate the effect of flow, shoal cohesion does not appear to be influenced by obstacles, at least not when the obstacles are small. Small obstacles may not impose a significant constraint on zebrafish that are derived from a population that typically experiences a ranges of microhabitats such as turbulence, pools, and riffles as water run through various obstructions like vegetation. In our study, plants also increased aggression in ways that were consistent with Bhat et al. (2015), who found that zebrafish in vegetated context chased one another more frequently. Although the amount of space available can impact near-neighbor distances (Shelton et al. 2015), the limitation of space or visibility is unlikely to be the cause for the increase in aggression as plastic plants that we used to create structural complexity occupied only 0.7% of the volume and 3.5% of the surface area of the test arena. However, presence and/or the spatial arrangement of vegetation may have increased the perceived safety of the habitat (Gotceitas 1990; Pettersson and Brönmark 1993), provided defendable resources (Barreto et al. 2011), spatial cues (Roy and Bhat 2016) or landmarks that delineate boundaries (Suriyampola et al. 2015) making them more attractive and thus leading to an increase in agonistic interactions. Future studies teasing apart the impact of visual obstructions and turbulence may be particularly useful.

Animals can cope with environmental fluctuations and can increase individual fitness by varying behavior to match their specific context (Van Buskirk 2012). Wild zebrafish have evolved in variable habitats that undergo major habitat shifts annually with the Indian monsoons, which produce enough rain to flood large rivers and fundamentally change the landscape and microhabitat of zebrafish (Bhat 2004; Sreekantha et al. 2007). In addition to seasonal variation, zebrafish natural habitats are under the influence of recent human-induced habitat alterations. In recent years, the Ganges River has experienced decreased water levels and increased vegetation due to urban pollution (Vass et al. 2010) and global climate change is also expected to have profound impact on water flow and vegetation of these habitats (McDonald et al. 2011). Although the impact of climate change on freshwater fish may be primarily through its effects on water quality (e.g., turbidity, pH, pollution), our results suggest that even small changes in water flow can have important consequences. Thus, examining behavioral plasticity of social groups will provide important insight to understand how these animals will respond to future climate-induced changes to the hydrology, which is likely to occur more frequently in the future. Behavioral plasticity can slow adaptive evolution by buffering animals from the action of natural selection (Ghalambor et al. 2007). Integrating the mechanisms forging these plastic responses will allow us to determine which populations and phenotypes are more susceptible than others and which habitat measures are best candidates for intervention.

Understanding the influence of immediate context and genetic factors on behavior is vital as zebrafish are becoming an increasingly significant model system in biomedical research including studies of addiction (Klee et al. 2011; Stewart et al. 2011), neurodegenerative disease (Sager et al. 2010; Lee and Freeman 2014), and autism (Elsen et al. 2009; Stewart et al. 2014). Although much of the available information on zebrafish behavior is derived from laboratory studies, the habitat differences between field and laboratory studies make the generalization of results from one context to another challenging (Parichy 2015). Despite abundance evidence of personality types or behavioral syndromes in a wide diversity of animals (Sih et al. 2004) including fishes (Conrad et al. 2011), our results agree with some earlier studies in finding virtually no evidence of consistent individual differences in zebrafish behavior (Moretz et al. 2007), although others have found evidence of moderate repeatability (Way et al. 2015). Within populations, ecological factors such as water flow seem to be the main forces underlying phenotypic plasticity. Genetic factors appear to be considerably more important in determining population- or strain-level differences (Robison and Rowland 2005; Martins and Bhat 2014). Studies like ours provide insight to better understand how fundamental properties of the physical environmental may team up with genetic factors to trigger specific behavioral responses and their underlying physiological processes.

In summary, results of this study reveal the effect of water flow on the plasticity of zebrafish shoaling behavior. Even a weak flow, as tested here, has an important, immediate, effect on shoaling behavior as they rapidly adjusted their activity level, aggression, and shoal cohesion in response to the presence of water flow. The effect of flow does not appear to be exaggerated by obstacles or turbulence generated from vegetation, at least not when the vegetation is minimal. Given zebrafish have evolved in habitats that undergo seasonal variations in flow, their ability to adjust behavioral responses in order to suit the conditions of its immediate context is an important mechanism for coping with these fluctuations. Further investigating the underlying mechanisms of these plastic responses may allow us to understand the influence of environmental fluctuation on the shoaling behavior and possibly the evolution of phenotypic traits in response to future environmental changes.

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