Density and group size influence shoal cohesion, but not coordination in zebrafish (*Danio rerio*)

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Abstract

The formations made by gregarious animals can range from loose aggregates to highly synchronized and ordered structures. For very large, coordinated groups, both physical and social environments are important for determining the physical arrangement of individuals in the group. Here we tested whether physical and social factors are also important in determining the structure of small, loosely coordinated groups of zebrafish. We found that even though our fish were not crowded and did not use most of the available space, the distance between individual fish was explained primarily by the amount of available space (i.e., density). Zebrafish in a larger space spread out more and the total dimensions of the shoal were an additive function also of group size. We, however, did not find any impact of social or physical environment on the orientation of individual fish or shoal. Thus, both physical and social factors were important for shoal spatial arrangements, but not individual orientation and shoal alignment.

Keywords: density, group size, shoal cohesion, environmental structure, zebrafish

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When animals aggregate, their spacing patterns are the result of a complex tug-of-war between approach and avoidance in response to social and physical environmental factors (Bode, Faria, Franks, Krause, & Wood, 2010; Hemelrijk & Hildenbrandt, 2012). In large groups, the social environment can strongly influence spacing and alignment. For example, bird flocks (reviewed in Bajec & Heppner, 2009; Hemelrijk & Hildenbrandt, 2012) and lobster trails (reviewed in Wyatt, 2011) exhibit coordinated movements suggesting that individuals are responding to each other. The physical environment can also impact the spatial distribution and orientation of individuals in a large group. For example, crowding can transform loose groups of locusts into highly organized marches (Buhl et al., 2006). Whether social or physical environments are more important in shaping the spatial structure of the group may depend on whether the group is large and coordinated, or relatively small and loosely interacting.

Groups of animals often balance the competing demands of predator avoidance, information transfer and competition by varying group size dynamically (Focardi & Pecchioli, 2005; Ford & Swearer, 2013). These changes in group size may have important effects on the physical structure of the group (Hemelrijk & Hildenbrandt, 2012). For example, domesticated animals in small groups maintain longer distances between neighbors, whereas larger groups are more compact (reviewed in Estevez, Andersen, & Nævdal, 2007; sheep: Sibbald, Shellard, & Smart, 2000). In addition, individuals may prefer to aggregate in larger groups in the presence of a predator, but prefer smaller groups when food-deprived (Hoare, Couzin, Godin, & Krause, 2004). Very large groups may have improved vigilance and faster information flow (e.g., sandpipers: Beauchamp, 2012), but individuals within those groups are also more likely to compete with each other (marine fish: Stier, Geange, & Bolker, 2013, colobus: Teichroeb & Sicotte, 2012) or to collide during group locomotion (sandpipers: Beauchamp, 2012; locusts: Buhl et al., 2006). To reduce the likelihood of competition and collision, some animals in large groups space themselves in a well-defined pattern to avoid aggressive neighbors (Bazazi et al., 2008), stagger themselves behind a leader (Nagy, Ákos, Biro, & Vicsek, 2010; Yomosa, Mizuguchi, & Hayakawa, 2013), or oscillate among physical positions (Ballerini et al., 2008; Morrell, Ruxton, & James, 2011). Here, we ask whether group size has similar impacts on the physical properties of relatively small aggregations.

Enclosure size and configuration can also strongly influence the shape and behavioral repertoire of animal groups. For example, individuals in larger enclosures tend to disperse farther from their neighbors (chickens: Leone et al., 2010; cows: DeVries, von Keyserlingk, & Weary, 2004), have larger home ranges, and move along the edges of the area (Buijs et al., 2010; Horiuchi & Takasaki, 2012). Mice placed in a flat nest huddled together in a relatively flat, horizontal plane, whereas mice in a concave nest huddled in three dimensions, with pups piling on and crawling under littermates (Shelton & Alberts, 2013). Similarly, locusts crowded in a donut arena showed density-dependent transitions from muddled groups to highly aligned plagues, with evenly-spaced members marching in a single direction (Buhl et al., 2006).

The above impacts may depend critically on whether the groups are large and composed of unfamiliar animals moving in a synchronized fashion (e.g., a “school”) or smaller groups of familiar, loosely-interacting individuals (e.g., a “shoal”). In large groups, for example, social factors may be more important, as individuals copy the behavior of their neighbors (e.g., prairie dogs: Hare, Campbell, & Senkiw, 2014; kangaroos: Pays et al., 2009), creating waves of animals that are all simultaneously vulnerable to predators (Sirot & Touzalin, 2009). In smaller groups, animals may recognize each other as individuals and may thus be more likely to coordinate rather than synchronize behavior, taking turns at vigilance. Familiar shoals are more cohesive and display more predator evasion tactics than do groups of unfamiliar fish (reviewed in Ward & Hart, 2003). The parallel alignment and consistent spacing of large, synchronized groups may have a genetic basis, and thus be relatively fixed (e.g., Greenwood, Wark, Yoshida, & Peichel, 2013). In contrast, the physical properties of smaller, less-coordinated groups may be shaped more directly by physical factors that elicit taxes (light: Bode et al., 2010; Imada et al., 2010; water currents: Capello, Soria, Potin, Cotel, & Dagorn, 2013; Genin, Jaffe, Reef, Richter, & Franks, 2005), or constrain group motion (enclosure shape: Bazazi et al., 2008; Buhl et al., 2006; nest configuration: Shelton & Alberts, 2013).

In the present study, we test whether the physical shape of zebrafish shoals (relatively small groups of familiar, loosely-interacting individuals) are influenced more strongly by social or physical environments, by varying the number of individuals and the size of the arena in which they are tested. Zebrafish display complex social behavior (reviewed in Spence, Gerlach, Lawrence, & Smith, 2008) and are found in shoals of 2-10 fish in the wild (Pritchard, Lawrence, Butlin, & Krause, 2001). Zebrafish are susceptible to social factors, exhibiting strong preferences for shoaling partners with particular phenotypes (Engeszer, Ryan, & Parichy, 2004; Rosenthal & Ryan, 2005) and adopting distinct social roles (Vital & Martins, 2011, 2013), both of which could alter the spacing between members and the cohesion of the shoal. Zebrafish evolved in a wide diversity of natural habitats including lakes and streams of India, Nepal, and Pakistan (Bhat, 2004), and experience drastic seasonal changes in water velocity and other environmental properties with the Indian monsoons (Bhat, 2004; Sreekantha et al., 2007). Thus, they may also react directly to physical properties such as water flux and amount of available space. In this study, we test for differences in the spacing patterns of zebrafish, while varying group and arena size. If social factors are important in determining the physical properties of the shoal, then we expect to see differences between groups of four and eight fish. If the physical environment is more important, then we expect to see a difference between shoals according to the tank size or amount of available space.

Method

Subjects

We used adult zebrafish from the Scientific Hatcheries strain, an outbred, wildtype strain used recently in other behavioral studies (Moretz, Martins, & Robison, 2007; Oswald & Robison, 2008; Vital & Martins, 2011, 2013). We housed fish in 37.85 L (10-gallon) tanks, maintained at room temperature of 28°C ± 3°C with a 10:14 h light: dark cycle, and fed *ad libitum* commercial flake food (Tetramin Tropical).

Experimental Procedure and Scoring

We formed 20 groups of four fish and 20 groups of eight fish to compare the effects of group size, choosing adults of both sexes from larger groups that had been housed together for at least two weeks before testing. Our experimental arena was slightly smaller than a standard 37.85 L (10-gallon) aquarium and contained 14.5 cm of gently-flowing water (Fig. 1). We tested the effects of physical space by forming 9 additional groups of four fish and placing them in a shortened form of the arena, which reduced the volume of water per fish by half (Fig. 1). We used adjustable collimators to reduce turbulence and to vary the dimensions of the space available to the fish.

After a five-minute acclimation period, we recorded the fish with a Logitech® c525 HD video camera from above. The relatively low water level (14.5 cm) ensured that zebrafish movements were largely restricted to two-dimensions. We took 5 snapshots of each group at 15 s intervals, and used NIH Image J (Schneider, Rasband, & Eliceiri, 2012) to score nearest-neighbor distance (NND), the distance between each fish and its closest neighbor. We also scored shoal area and perimeter by creating a minimum convex polygon from the positions of the outermost fish, and measured shoal length as the distance between the two farthest fish. Finally, we scored individual orientation by marking the nose and midsection of each fish, drawing a line through the points, and measuring the angle of the fish in relation to the water current. For NND and individual orientation, we analyzed individual measures of four fish from each group (all fish from groups of four and four randomly-selected fish for groups of eight) to maintain equal sampling variances in the treatments. To measure shoal orientation, we found the long axis of the shoal and recorded its angle in relation to the water flow. Because motivation can affect orientation (sharks: Gardiner & Atema, 2014; mottled sculpin: Coombs & Grossman, 2006), we fed the fish prior to the beginning of each trial.

Analysis

We averaged measures of each parameter across the five images taken of each group, and then used an ANOVA with Tukey post-hoc tests to examine the effects of group and arena size (4 fish large tank 2.8 L/fish, 8 fish large tank 1.4 L/fish, or 4 fish small tank 1.4 L/fish) on average NND, shoal perimeter, shoal area, and shoal diameter. We used Type III sums of squares, which correct for unbalanced data (Shaw & Mitchell-Olds, 1993), and an alpha level of .05. We also log 10-transformed NND and shoal area, as required to obtain residuals that conformed to the assumptions of the ANOVA. To assess the effects of social and physical structure on orientation, we calculated the mean vector length, ρ of each individual and group. A vector length of 1 results from perfect concordance for all phase angles (a highly polarized collective of fish or a consistently responding shoal), whereas a vector length of 0 represents an asynchronous group of individuals and randomly oriented shoal. We conducted all statistical analyses in R (Team, 2012), using the 'base' package and 'circular' package as needed (Lund, Agostinelli, & Agostinelli, 2013).

Results

Zebrafish Aggregated, Using Much Less than the Total Available Space

Fish in this study clumped together in the relatively large testing arena (Fig. 1). The testing arena (771.75 cm^2) was at least 7 times larger than the average shoal area of the largest group size under the highest density condition (M = 104.8, 95% CI [91.00, 118.60]). Similarly, groups of four fish with similar available space had a mean shoal area (M = 23.2, 95% CI [15.91, 30.49]) that was over 15 times smaller than the area of the testing arena (385.79 cm^2). Groups of four fish in the same larger tank (but half the density) had even smaller shoal area to tank size ratio, 23 times smaller (M = 41.9, 95% CI [37.53, 46.27]).

Both Physical and Social Factors Influenced Shoal Cohesion

Groups of four fish in a large space (2.8 L / fish) maintained nearly twice the distances between neighbors (M = 3.7 cm, 95% CI [-.14, 7.54]) than did groups of four or eight fish in a smaller space (M = 2.4 cm and 2.4 cm, 95% CI [.58, 4.22] and 95% CI [-.19, 4.99], respectively). This resulted in a significant difference in NND across the three treatment categories ($F_{2, 46} = 8.32$, $p = .003$, $\eta^2 = .27$; Fig. 2). There was no significant difference between the groups of four and eight fish under the same available space conditions ($p = .90$, Tukey). There was a significant difference between groups of four fish tested in larger and smaller arenas (or at different densities; $p < .01$, Tukey).

Groups of eight fish maintained a shoal perimeter (M = 36.1, 95% CI [31.45, 40.75]) that was nearly twice the shoal perimeter of groups of four fish (M = 20.0, 95% CI [14.98, 25.02]) under similar density conditions and almost 25% larger than groups of four fish (M = 26.6, 95% CI [22.05, 31.15]) with double the available space. This led to a significant difference in shoal perimeter across all treatment conditions ($F_{2, 46} = 21.85$, $p < .0001$, $\eta^2 = .49$; Fig. 3). The difference between groups of four and eight fish tested under similar available space conditions was significant ($p < .0001$, Tukey). There was a significant difference between groups of four fish tested in larger and smaller arenas (or at different densities; $p = .04$, Tukey). Similarly, there was a significant difference between groups of eight fish and four fish tested in the same size arena (but consequently at different densities; $p < .0001$, Tukey). The pattern was consistent and nearly identical for shoal area and shoal diameter measures.

Social and Physical Environment Do Not Enhance Individual or Shoal Polarity

In all treatment conditions, individual fish oriented at random in response to the water flow, showing no signs of aligning with the direction of water flow (Fig. 4). We found no evidence of enhanced synchrony among individual fish (ρ ranged from 0.01 to 0.28; Fig. 4a). Groups also did not appear to orient with respect to the flow (ρ ranged from 0.98 to 0.99; Fig. 4b) in any of the social or physical conditions.

Discussion

We found that although the distance between individuals in a shoal depends primarily on the relative amount of available space, shoal dimensions depend also on group size. Although our experimental arena was much larger than the dimensions of the shoal, zebrafish spread themselves out more when there was more available space, indicating that the fish adjusted their proximity to neighbors according to the relative amount of space (i.e., density). Groups of four and eight fish tested at the same relative densities maintained the same distance between neighbors, with the groups of eight fish taking up roughly twice the amount of total space as a consequence. Because groups of four fish in a larger arena spread out more (larger NND), they occupied an intermediate amount of total space – not as much as groups of eight fish in the same sized arena, but more than groups of four fish in a smaller arena. We found no obvious differences in orientation or synchrony of individuals or shoals.

Even though our animals were not crowded, the relative amount of space was the most significant factor affecting spatial distributions in our study. Density is clearly important for shaping the spatial distribution of other species when in crowded conditions. For example, as the amount of allowable space per individual increases, domesticated rabbits transition from avoiding pen members to increasing proximity to conspecifics (Buijs et al., 2011). In chickens, groups under higher density conditions had smaller distances between neighbors than groups with more space per individual, irrespective of group size (Leone et al., 2010), and assumed spatial positions that were indicative of social attraction (Febrer, Jones, Donnelly, & Dawkins, 2006). Here, we show that density can also be highly relevant to space use when animals are not obviously crowded, clumping together in such a way that they occupy a small portion of the available space.

In large groups, spatial arrangement of individuals within the group can show substantial variation and complexity. For example, in large starling flocks the inner structure of the group is less dense than the border, as birds on the periphery push inwards to maintain cohesion (Ballerini et al., 2008; Cavagna, Queirós, Giardina, Stefanini, & Viale, 2013). Large schools and flocks also tend to have complex structures with pseudopodia, and pockets of high or low density (reviewed in Bajec & Heppner, 2009; Hemelrijk & Hildenbrandt, 2012). In contrast, we found that the shoal area, shoal perimeter, and shoal diameter of groups of eight zebrafish were nearly double that of groups of four fish under similar density conditions, suggesting that the arrangement of individuals in each of these relatively small groups were additive.

Animals that form very large groups also sometimes vary distance between individuals dynamically to balance the benefits of grouping with the need to minimize resource competition. Flocks of barnacle geese, for example, land as a tight, synchronized group, but then slowly expand in total dimension as individuals along the edges of the flock begin exploring (Carbone, Thompson, Zadorina, & Rowcliffe, 2003). Similarly, individuals along the periphery of large deer herds can venture so far away that the group fissions, separating the exploring individuals from others at the center of the herd that are tightly synchronized (Focardi & Pecchioli, 2005). As discussed by Hamilton (1971), perceived predation risk is also a major factor influencing whether groups are tightly cohesive and synchronized (e.g., starlings: Carere et al., 2009; cranes: Ge, Beauchamp, & Li, 2011). Although we found no evidence for social or physical factors impacting the alignment or synchrony of zebrafish shoals, additional studies are needed to determine whether individual behavior or social roles depend on group or enclosure size, or whether synchrony is simply less variable in smaller groups.

Our study also highlights the importance of using both individual and group measures to characterize social behavior. Although individual metrics (e.g., NND) are often used to approximate group properties (Miller & Gerlai, 2007; Parrish, Viscido, & Grünbaum, 2002; Buijs et al., 2011), our measures of NND suggested that the structure of zebrafish groups depended only on the available space, and not group size. It was only when we considered also measures of the group as a whole (e.g., shoal diameter) that we saw the effects of group size. We conclude that a better and more accurate characterization of zebrafish shoals involves both individual and group measures, as the individual and whole are dynamically linked (reviewed in (Parrish & Edelstein-Keshet, 1999). Future studies should explore under what context individual metrics sufficiently characterize the group and in which conditions group-level measures are needed to describe the group more accurately.

In summary, the goal of this study was to identify the impact of social (group size) and physical factors (tank size and density) on individual and group spatial distributions and orientation. Our results show that the spatial distribution of zebrafish in small groups is primarily determined by density, followed by group size, and that these effects can vary substantially depending on the combination of metrics. We found no evidence to suggest that group size had a fundamental impact on how individual fish spaced themselves in relation to neighbors or oriented in response to the water currents. In contrast, the dimensions of the shoal were clearly affected by enclosure size and number of fish in an additive manner. Studies such as this suggest that complex spacing patterns of small animal groups can be generated by simple mechanisms. Understanding these mechanisms is critical to understanding how complexity and order can arise.

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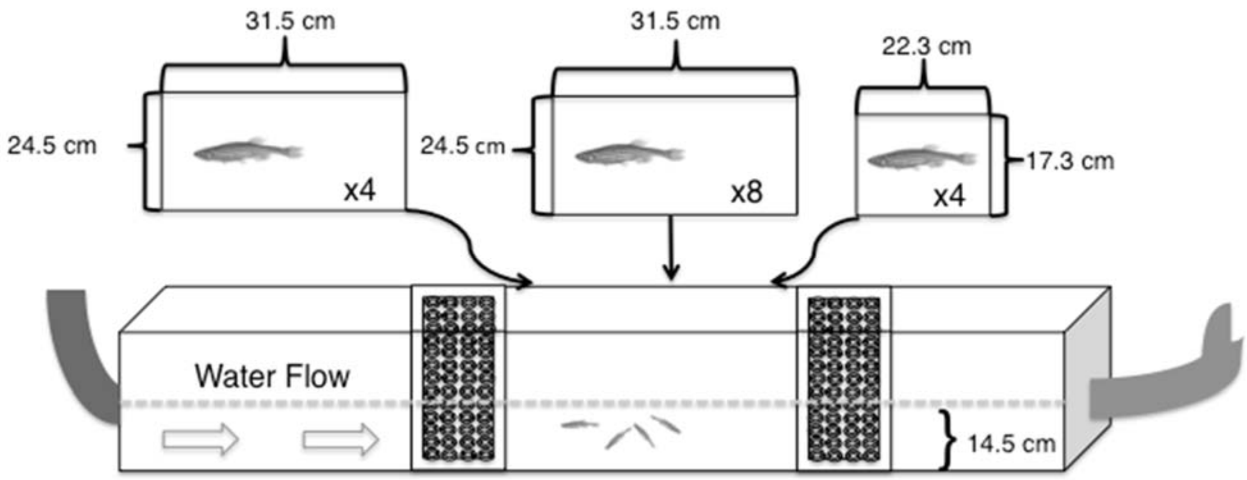

Figure 1. Dimensions of the observational areas for each of three treatment conditions: a group of four fish in a large tank (2.8L/fish), a group of eight fish in the same size tank but higher density (1.4L/fish), and a group of four fish in a smaller tank and consequently a higher density (1.4L/fish). The experimental arena was a fluvial tank with a unidirectional water flow system and two collimators designating an observation area and minimizing turbulence. We photographed the fish from above, keeping the water level low to restrict the movements of the fish to 2-dimensional space.

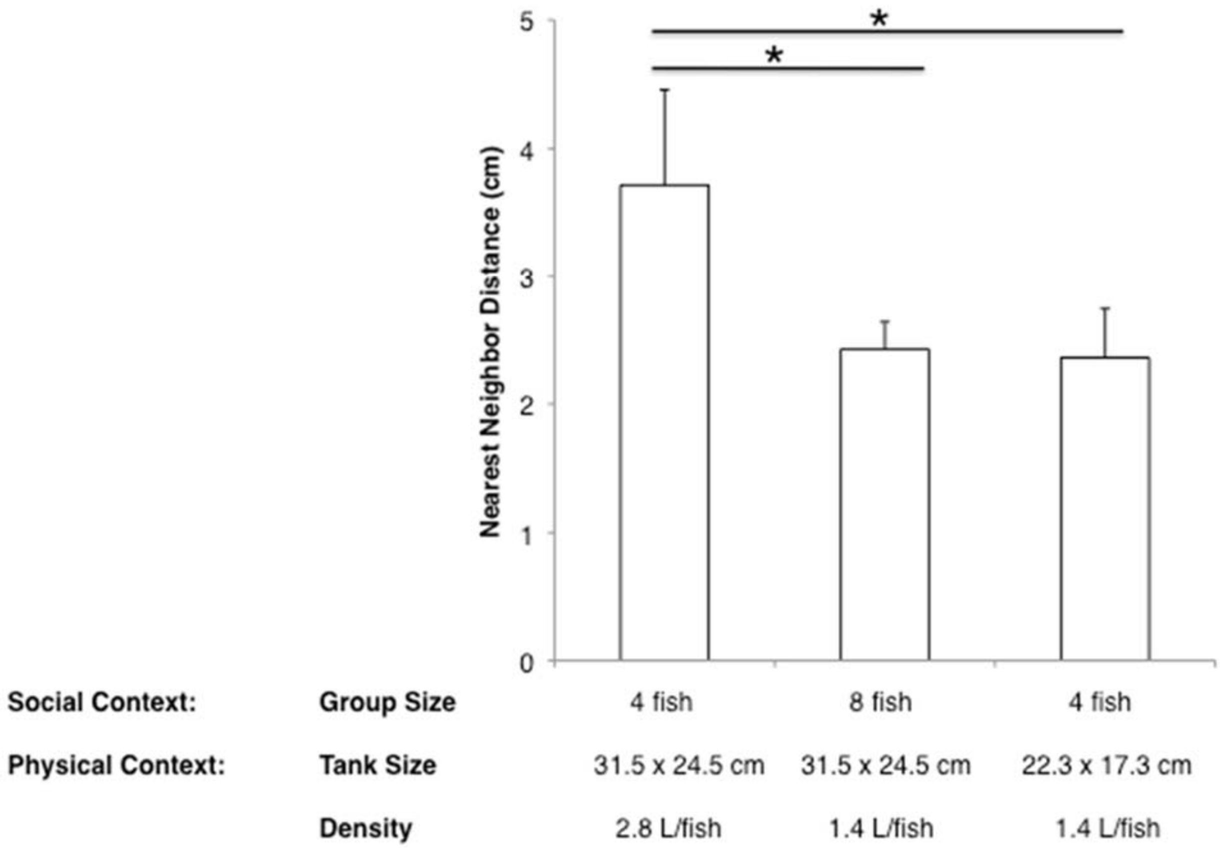

Figure 2. Nearest Neighbor Distance is more influenced by the physical environment than the social structure. Groups of four fish in a relatively large arena (2.8 L / fish) were more dispersed than groups of eight fish in the same size space but at a higher density (1.4 L / fish) and groups of four fish in a smaller arena (1.4 L / fish). Groups of four and eight fish were similarly dispersed when density was equal. Error bars are one standard error. *Corresponds to significant Tukey post-hoc comparisons at $p < .05$.

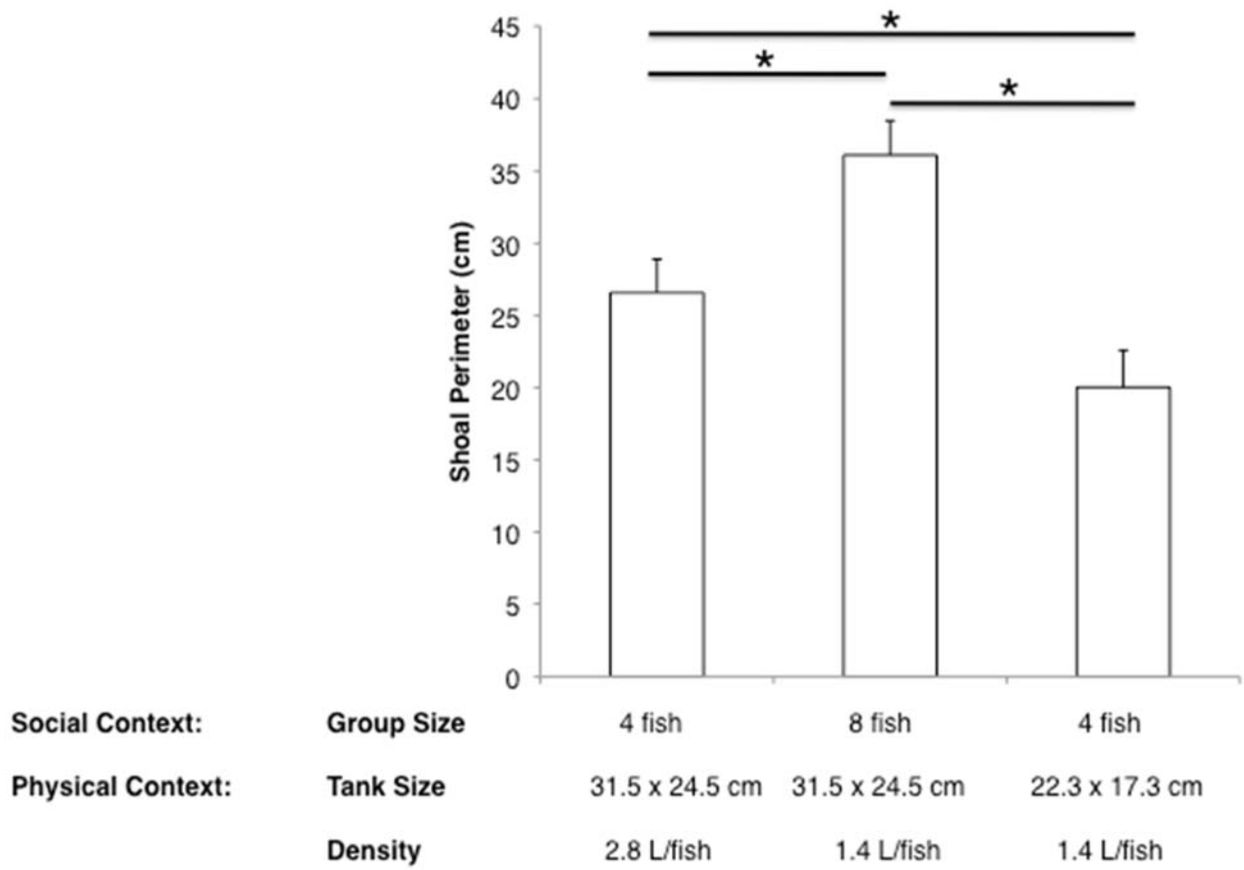

Figure 3. Social and physical environments affect shoal perimeter. Groups of eight fish in a relatively large arena (1.4. L / fish) were more dispersed than groups of four fish under the same density conditions, but smaller space (1.4 L / fish) and groups of four fish in a larger arena and at half the density (2.8 L / fish). Groups of four fish at higher densities had smaller shoal perimeters than groups of four fish at lower densities. Error bars are one standard error. *Corresponds to significant Tukey post-hoc comparisons at $p < .05$.

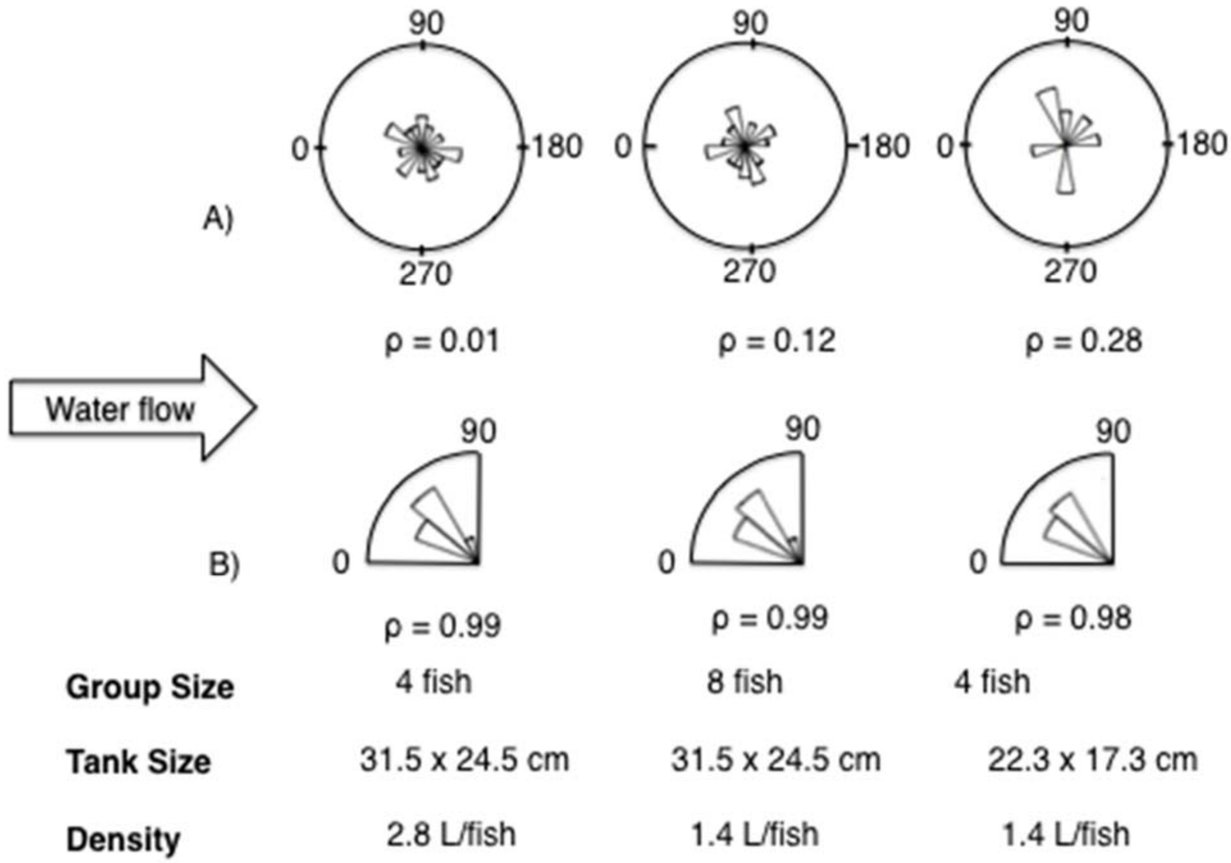

Figure 4. Individuals randomly orient and shoals maintain consistent polarity independent of social and physical contexts. The direction of the water flow in relation to the orientation of the fish and shoals is depicted by the arrow. The synchrony of individual fish and the polarity of the shoal are indicated by ρ. In all tank and group sizes, individual fish orient randomly (A). The shoal's polarity varies independently of tank size and number of fish within the shoal (B). We set each bin to encompass an angular range of 18 degrees (so 0–18 degrees, 18–36 degrees, etc.).