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Shoaling Preferences in Zebrafish: Exploring the Interplay of Phenotypic and Genotypic Factors with Group Size

Shabnam Rahman

Department of Zoology, Mrinalini Datta Mahavidyapith, Kolkata 700051, India

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Abstract: Zebrafish have long been a key model in developmental biology and genetics, but interest in their behavior has only recently surged. Their small size and high reproductive rate make them ideal for high-throughput screening. A notable characteristic of zebrafish is their tendency to form groups, a behavior called shoaling. This has led to the suggestion that zebrafish could serve as a model organism for studying the genetics of vertebrate social behavior. However, shoaling behavior in zebrafish is not yet well understood. In fish like zebrafish (*Danio rerio*), shoaling is crucial for survival, balancing the advantages of group living with its risks. This study investigates how zebrafish prioritize their shoal choices based on genetic and phenotypic factors compared to group size. Through binary choice experiments, zebrafish consistently favored genetically related sibling groups over larger, genetically different groups, despite the potential predator avoidance benefits of larger groups. The study underscores the complex decision-making involved in shoaling behavior, influenced by genetic relatedness, phenotypic similarities, and group size, offering insights into the evolutionary forces shaping social behaviors in aquatic environments. It highlights the intricate dynamics of zebrafish shoaling behavior, showing how genetic relatedness and group size interplay in their decision-making processes. Even though larger shoals provide safety benefits, zebrafish preferred smaller groups of genetically related siblings over larger, genetically and phenotypically diverse groups. This preference persisted until the size difference between the conspecific shoal and the sibling group became substantial, illustrating a complex trade-off between genetic relatedness and predator avoidance strategies.

Keywords: Zebra fish, Shoaling, Group size, Genotypic similarity, Relatedness

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Introduction

Shoaling behavior in fish is widespread and crucial for their survival, yet many aspects of how and why fish form shoals remain poorly understood (Hensor *et al.*, 2005). Fish often join shoals to reduce competition and avoid predators, benefiting from safety in numbers. This safety is enhanced by effects like confusion, where predators struggle to target one fish in a group of similar-looking individuals, and oddity, where distinct individuals are more likely to be attacked (Ward *et al.*, 2002). Fish tend to shoal with others that look like them, a behavior known as

assortative shoaling, which maximizes the benefits of confusion and minimizes the risks of oddity (Rosenthal and Ryan, 2005). Several adaptive benefits are provided by enhancing feeding efficiency and reducing vulnerability to predators as group improves vigilance, aiding in predator detectionas well. Studies show that shoal size is crucial, as larger groups decrease the chances of individual predation (Krause and Ruxton, 2002; Frommen *et al.*, 2009). Other factors like familiarity and kinship also influence shoal preferences, as kin are more likely to cooperate in risky situations like predator encounters (Hamilton, 1964). Shoal formation is influenced by a variety of environmental factors such as vegetation, water flow rate, temperature, fish age, location, existing shoal size, predation risk, early life experiences, and food availability (Ruhl and McRobert, 2005; Miller and Gerlai, 2007, 2011; Arganda *et al.*, 2012; Suriyampola *et al.*, 2016; Orger and de Polavieja, 2017; Groneberg *et al.*, 2020).

Shoaling also entails several drawbacks. Close proximity among many individuals facilitates the rapid spread of pathogens and intensifies competition for food and mates (Pitcher and Parrish, 1993; Krause and Ruxton, 2002; Miller and Gerlai, 2011). Shoaling with kin carries risks such as inbreeding, which can lead to genetic problems in offspring (Charlesworth and Charlesworth, 1987). Furthermore, large shoals increase visibility to predators compared to solitary fish, which can hide more effectively in vegetation (Suriyampola *et al.*, 2016). The position of an individual within a shoal is crucial in weighing these advantages and disadvantages. Peripherally located individuals may access food more quickly but face higher predation risk, whereas those in the center are safer from predators but may struggle to reach food (Bumann *et al.*, 1997; Miller and Gerlai, 2011). This balance between safety from predators and access to resources likely explains the short-term fluctuations in shoal cohesion observed previously (Miller and Gerlai, 2007). However, research on various fish species has shown mixed results regarding the influence of kinship on shoal formation, with some species showing strong kin associations while others do not (Naish *et al.*, 1993; Hauser *et al.*, 1998; Pouyaud *et al.*, 1999). Shoaling is a complex and intriguing behavior among zebrafish, reflecting a delicate interplay of cognitive processes. This study aims to explore how conflicting preferences for joining larger shoals versus shoaling with genetically and phenotypically similar individuals affect decision-making in zebrafish (*Danio rerio*) siblings. By testing scenarios with equal numbers of size-matched and size-unmatched shoals and varying the proportions, we seek to understand which preference-- group size or similarity-- is more influential in their shoaling decisions. Understanding these dynamics can shed light on the complex factors influencing social behavior in fish and how they balance conflicting preferences when choosing shoal mates.

Materials and Methods

The study involved 2 years old *Danio rerio* larvae bred from gravid pairs and adult conspecifics over one year old obtained from collection from local sites acclimatized in the laboratory fish tank. Each fish's standard length and body weight were measured (length: 4.3 ±2 cm, weight: 0.5 ± 0.2 g) for consistency. For conducting this binary choice experiment, a custom-made experimental three-compartment tank was taken with a measurement of 50 cm × 50 cm × 75 cm (length × width × height) was made to analyze the zebrafish shoaling behavior. On one side the old known siblings were kept, the central compartment was for test fish and on the other side new unknown conspecifics were kept (Fig. 1). The partition was clear for the test fish to interact on both the sides. The test fish was released in the central compartment of the aquarium and there was partition between the different shoals, the test fish was allowed to interact with both the old known sibling shoals and new, unknown conspecific shoal. Shoaling preferences were assessed using standard binary choice experiments conducted over a total of 15 min. During the experiment, test

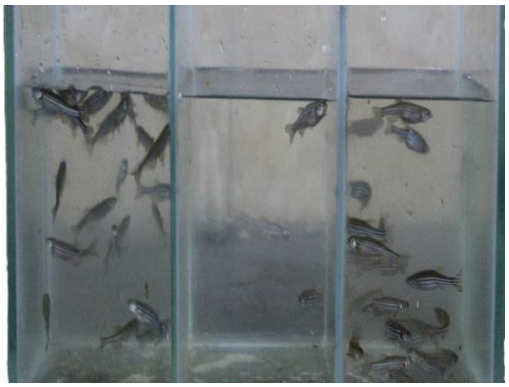

Fig. 1: Experimental tank. Left compartment(L) has unknown new conspecifics, central compartment (C) has the test fish, right compartment(R) has old known siblings.

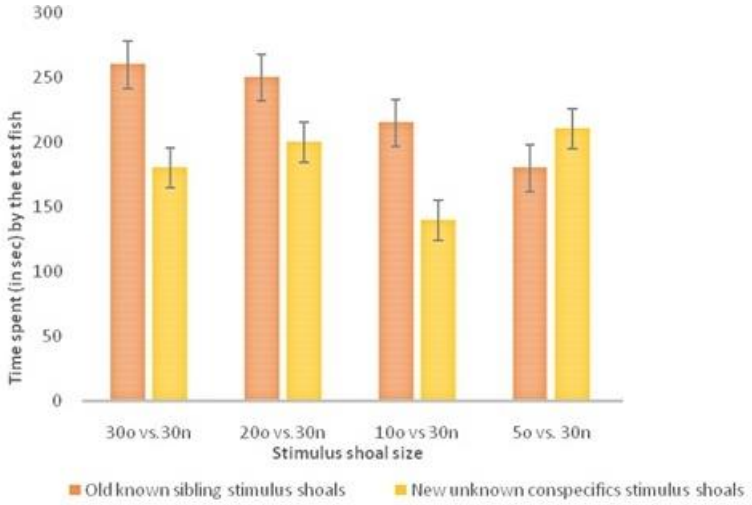

Fig. 2: Shoaling preferences of individual *Danio rerio* (Zebra fish) for similar size old known sibling vs new unknown conspecifics. Mean time spent in seconds ±SE. n=20.

fish were given 10 min to observe and evaluate two stimulus shoals. Subsequently, their behavior was observed for the remaining 5 min behind a screen to minimize disturbance. Data analysis was performed using a parametric dependent sample two-tailed t-test.

Results

The study revealed that the test fish consistently preferred smaller shoals of genetically related siblings over larger groups of genetically and phenotypically different conspecifics, despite the potential safety benefits of larger groups against predators. This preference was evident until the conspecific shoal was visually higher in number of individuals. Notably, when tested against equal-sized conspecific shoals (30 old siblings vs. 30 new conspecifics), the fish did not show a preference, likely due to the perceived differences in the new conspecifics group. As the number of new conspecifics increased to thirty-five individuals while maintaining a total of fifty individuals (20 old siblings vs. 30 new conspecifics), the preference for known sibling shoals persisted. However, as the number of new conspecifics further increased to forty-five individuals with corresponding decreases in known siblings to five, the preference for known sibling shoals diminished. In these scenarios (10 old siblings vs. 30 new conspecifics, 5 old siblings vs. 30 new conspecifics), the test fish exhibited random behavior between compartments. As per t test, at P(T<=t) two-tail value is 0.003, t critical value is 3.18; the results were significant (Fig. 2).

These findings highlight the complex decision-making processes involved in the shoaling behavior of *Danio rerio*, where genetic relatedness often takes precedence over the advantages of joining larger groups that are genetically and phenotypically different, particularly in terms of reducing predation risk.

Discussion

The collective behavior of large animal aggregations is a remarkable natural phenomenon, often self-organizing into complex patterns without external stimuli. Simple rules of interaction among individuals suffice to produce such collective behavior, yet empirical knowledge about these rules remains limited (Alex and Thomas, 2011). In contrast to previous findings by Alex and Thomas (2011) where *Puntius sarana* showed a preference for larger shoals over size-matched smaller sibling shoals, the present study revealed that fish prioritize their preferences contextually, considering both group size and genetic relatedness in their shoaling decisions. While studies suggest that fish use relative magnitude of continuous variables like total area to judge group size (Agrillo *et al.*, 2009), the present findings indicated that an increase in total area alone does not always attract fish to larger shoals. This was evident as test fish did not preferentially associate with size-unmatched shoals of conspecifics. However, when the number of individuals in the known sibling shoal was significantly lower compared to unknown conspecific shoals of larger size, the test fish actively discriminated and associated with the unknown larger conspecifics. Here, fish traded off their preference for phenotypic similarity and genotypic relatedness with sibling shoals in favor of joining larger groups as a crucial strategy for predator avoidance.

Maintaining aggregation cohesion is a critical biological requirement shaped by evolutionary pressures for survival (Turesson and Bronmark, 2007). Flocks and shoals adapt flexibly to enormous density changes in open spaces while maintaining cohesion, benefiting from earlier predator detection and diluting individual risk of predation (Rodgers *et al.*, 2011). However, maintaining homogeneity within shoals is crucial to avoid the 'oddity effect,' where visually distinct individuals are selectively preyed upon. The influence of body size on shoaling decisions was pronounced in the present study, suggesting that anti-predator benefits such as dilution, oddity, and confusion effects depend on individual characteristics. Preferences for kin association imply mechanisms for kin recognition, involving direct and indirect familiarity (phenotype matching). Phenotype matching relies on correlating phenotypic traits with genotype, facilitating the identification of kin and optimizing social behaviors such as nepotism and mate choice (Tang-Martinez, 2001). When the number of individuals in the sibling shoal was larger, fish prioritized homogeneity; whereas with fewer individuals, they favored size-mismatched conspecifics with similar coloration. Nevertheless, genetic relatedness may still influence shoaling preferences when conspecifics are involved.

Furthermore, this study indicates that *Danio rerio* does not merely associate with perceived larger shoals but actively evaluates group characteristics before exhibiting associational preferences. This capacity for judgment likely relies on social cognition and information processing abilities. Understanding how social environments influence fish shoaling choices is biologically relevant, shedding light on factors favoring social learning abilities in fishes.The present findings underscore the complexity of shoaling decisions in *Danio rerio*, where considerations of group size, phenotypic traits, and genetic relatedness interplay dynamically in shaping collective behavior and optimizing survival strategies.

Conclusion

The present study suggests that zebrafish prioritize homogeneity and genetic relatedness when shoal sizes are similar. However, if the sibling shoal becomes significantly smaller, they prefer larger conspecific groups to reduce

predation risks. This behavior highlights the importance of genetic recognition and social cognition in zebrafish, showing that they do not just follow size cues but actively evaluate group characteristics. This research offers valuable insights into the social behaviors of zebrafish, revealing that their shoaling decisions are context-dependent and influenced by a nuanced balance of genetic, phenotypic, and environmental factors. These findings enhance our understanding of the adaptive advantages of shoaling and the cognitive processes underlying collective behavior in fish. Although there may be biases due to the inability to control all variables, the shoaling preference test remains a simple and versatile tool that can provide new insights into neuropsychiatric and neurodevelopmental disorders characterized by social impairments. Applying this test in neurobehavioral research has two main benefits: it allows for the examination of a single individual rather than a group, unlike traditional shoaling and schooling assays.

Ethical Statement

This study was in accordance with the Ethics Committee of the Mrinalini Datta Mahavidyapith College. All efforts were made to minimize the number of animals used and handled with all precautions.

Conflict of Interest

The author declares no conflicts of interest.

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