ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

# Conspecific Identity Determines Interactive Space Area in Zebrafish Shoal

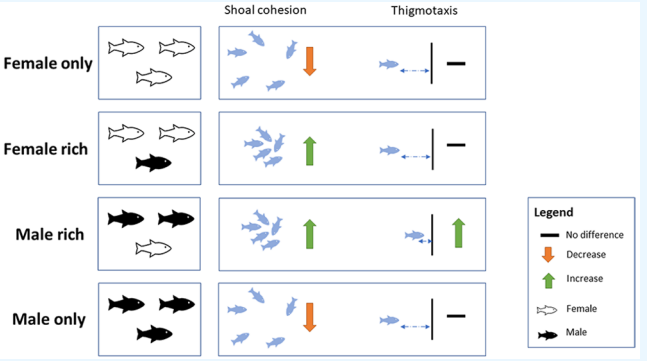
Aswathy Sivaraman, Rohit Nandakumar, and Binu Ramachandran*

Cite This: *ACS Omega* 2022, 7, 37351−37358

Read Online

ACCESS | Metrics & More | Article Recommendations

**ABSTRACT:** Sex ratio of shoals has been shown to influence shoaling behavior in many fishes. This study tests whether the conspecific identity influences shoal performance (shoal area, interactive distances, distance traveled, and thigmotaxis) of zebrafish (*Danio rerio*) via group tracking. We conducted a two-dimensional analysis of shoals with different sex ratios (male only, female only, male rich, and female rich) of a five-membered shoal. Parameters describing the shoal structure and individual behavior were derived using video tracking and a custom-made program. We found that mixed-sex shoals had significantly lesser shoal area and interactive distance compared to single-sex shoals (approximate difference of 80% for shoal area and 50% for interactive distance). Our findings shed light on complex interactive behaviors of zebrafish in a shoal that are affected by differences in sex ratios of interacting individuals. The outcomes from this study can be used to design better zebrafish shoaling experiments for clinically relevant research like human nerve disorders and social deficits.




## INTRODUCTION

Grouping behavior is exhibited by organisms across diverse taxa.[1] Animals gain a variety of benefits from grouping such as reduced risk from predators and parasites.[2−4] Among the diverse organisms that exhibit grouping behavior, fishes are convenient model organisms to study this behavior under laboratory conditions. This is because they are a good compromise between the convenience afforded by smaller invertebrate models (like short generation times, easy and cost-effective breeding, and maintenance) and behavioral and physiological similarities with "higher" vertebrates.[5]

In the wild, many fishes exhibit aggregatory behaviors known as shoaling and schooling.[6] Shoals are small and loose aggregations of fish with less polarized organization, whereas schools are dense, highly polarized, and have some synchronized swimming patterns.[7] In this paper, we have used the term shoaling to refer to all types of grouping behavior exhibited by fish.

Shoaling confers fish with similar advantages as other animals which show grouping behavior. A large shoal is believed to detect predators more easily than lone individuals and can transmit that information soon among the shoal mates (many-eyes hypothesis).[7] Odd preys in a group are more likely to be spotted by predators (oddity effect).[8] Hence, fish tend to form a homogeneous group to deceive predators (confusion effect).[9] Shoaling is also thought to increase a fish's foraging capability and reproductive success.

Fish in shoals make active decisions on which shoal to join based on various factors, and they can vary among different species. In general, fish tend to choose shoal members who are conspecifics,[10] phenotypically similar, have a similar body size[11] and coloration,[12] are familiar,[13] and have low parasitic loads.[14] There are passive sorting mechanisms as well, mechanisms that do not involve decisions taken by the fish—that restrict membership in shoals.[14] For example, the active swimming capacity of an individual is positively correlated with body length and negatively correlated with parasitic load and parasite-induced phenotypic changes.[15] This reduces the chances of fish joining shoals of dissimilar sizes and parasite loads.

Studies on shoal choices of female and male fish show that shoal choices differ based on the sexual and species identities of the individual and the shoal members. In guppies, it was shown that the females spent more time shoaling and had greater shoal cohesion than male guppies.[16] On the other hand, non-reproductive male three-spined sticklebacks were found to be more active in shoaling than females.[17]

**Received:** June 24, 2022
**Accepted:** October 4, 2022
**Published:** October 17, 2022





https://doi.org/10.1021/acsomega.2c03815
*ACS Omega* 2022, 7, 37351−37358

Previous shoaling studies on the effect of sex have largely dealt with a dichotomous shoal-choice experimental setup. In this type of study design, preference for an empty compartment of the tank and a compartment with stimulus fish shoals was inferred from the amount of time spent by the fish near each compartment.[18] But there are very few studies that look at how individuals of different sex freely interact in a shoal and affect the shoal structure as a whole. These types of studies are important because they create shoaling conditions that are closer to natural conditions, with the fish having access to social cues other than just visual.[19]

Zebrafish (*Danio rerio*, Hamilton, 1822) are model organisms widely used in various biological research fields. Their known genome, ease of genetic manipulation, and cost-effective breeding and maintenance make them attractive for laboratory experiments.[5,20] Zebrafish exhibit sexual dimorphism after reaching adulthood,[21] with male zebrafish being smaller and thinner than females and with yellowish color patterns on their bodies. Female zebrafish are more bluish and have a characteristic enlarged belly.[22] The ease of identifying the sexes along with the rising popularity of zebrafish in a variety of laboratory research across diverse biological fields makes them a suitable organism for investigating how fish of different sex affect the shoal structure and the general activity patterns in the shoal.

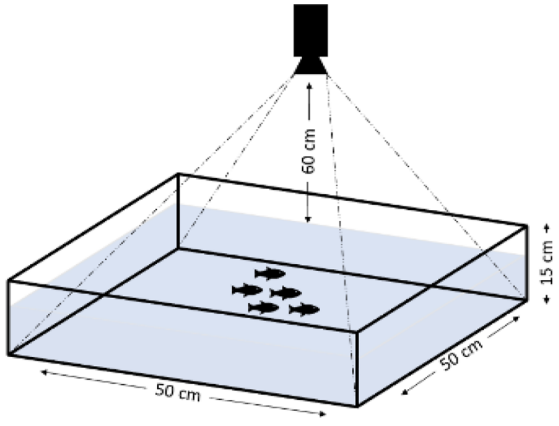
Our study looks at how shoal structure and the activity levels of individuals in them vary with differing sex ratios. We used zebrafish shoals with varying numbers of male and female fish and derived shoal-level and individual-level parameters. We used interfish distance (IFD), farthest neighbor distance (FND), nearest neighbor distance (NND), and shoal area as parameters that would describe the structure of the shoal as a whole. Total distance traveled (TDT) and thigmotaxis (wall-hugging) were used as parameters describing individual activity patterns within the shoal.

## MATERIALS AND METHODS

**Ethical Statement.** All research involving animals was in accordance with the Ethics committee of the University of Calicut. All efforts were made to minimize the number of animals used and their suffering.

**Experimental Setup.** A custom-made experimental tank with a measurement of 50 cm × 50 cm × 15 cm (length × width × height) was made to analyze the zebrafish shoaling behavior (Scheme 1). A high-resolution camera (30 fps) was placed 60 cm (focal length) perpendicular to the arena. The tank was designed to have two dimensions with a length that was ten-times the average body length of an individual fish. This allowed the fish to freely explore two dimensions. The experimental shoal size was kept at five fish.[23] Adult zebrafish having a body length of approximately 4−5 cm were taken for the behavioral experiments.

**Scheme 1. Experimental Setup**




**Experimental Protocol.** Before starting all behavioral experiments, the male and female fish were separated and put into separate preholding tanks. Preholding tanks had a capacity of 16 L, which could hold around 20−25 fish at a time. To reduce observer error, male and female zebrafish were identified based on a consensus arrived at by multiple researchers working on zebrafish. Five zebrafish were randomly selected at the time of the experiment from the preholding tanks and transferred to the pretreatment tank having a measurement of 30 cm × 20 cm × 20 cm and allowed to acclimatize for 5 min. The fish were then transferred to the experimental tank and allowed 3 min for acclimatization. The system water was used and maintained at a level of 6 cm from the bottom of the experimental tank. The water was changed after every experiment to avoid the subsequent subjects from being affected by the olfactory cues from the previous subjects.

The experiments were divided into four treatments: female-only (FO), female-rich (FR), male-only (MO), and male-rich (MR). FO shoals contained five female fish, FR shoals had three females and two males, MR had two females and three males, and MO had only males (Table 2). Each treatment group contained ten shoals.

Videos of each shoal were recorded at 30 frames per second for 6 min for each experiment using a high-resolution camera Canon EOS R (Mirrorless, Canon Inc., Ota, Tokyo, Japan). The camera was placed perpendicular to the arena and 60 cm above the arena. Four 10 W LED light plates with an antiglare diffuser were placed approximately 18 cm away from the four walls of the experimental tank to attain the uniform diffuse lighting necessary for accurate tracking. A total of 100 male and 100 female fish were used for all the behavioral experiments combined. None of the fish was used for more than one experiment. All fish were fed 2 h before the start of experiments and just after the completion of experiments (at 7.00 am and 4.00 pm). The behavioral experiments were conducted from 9.00 am to 4.00 pm IST.

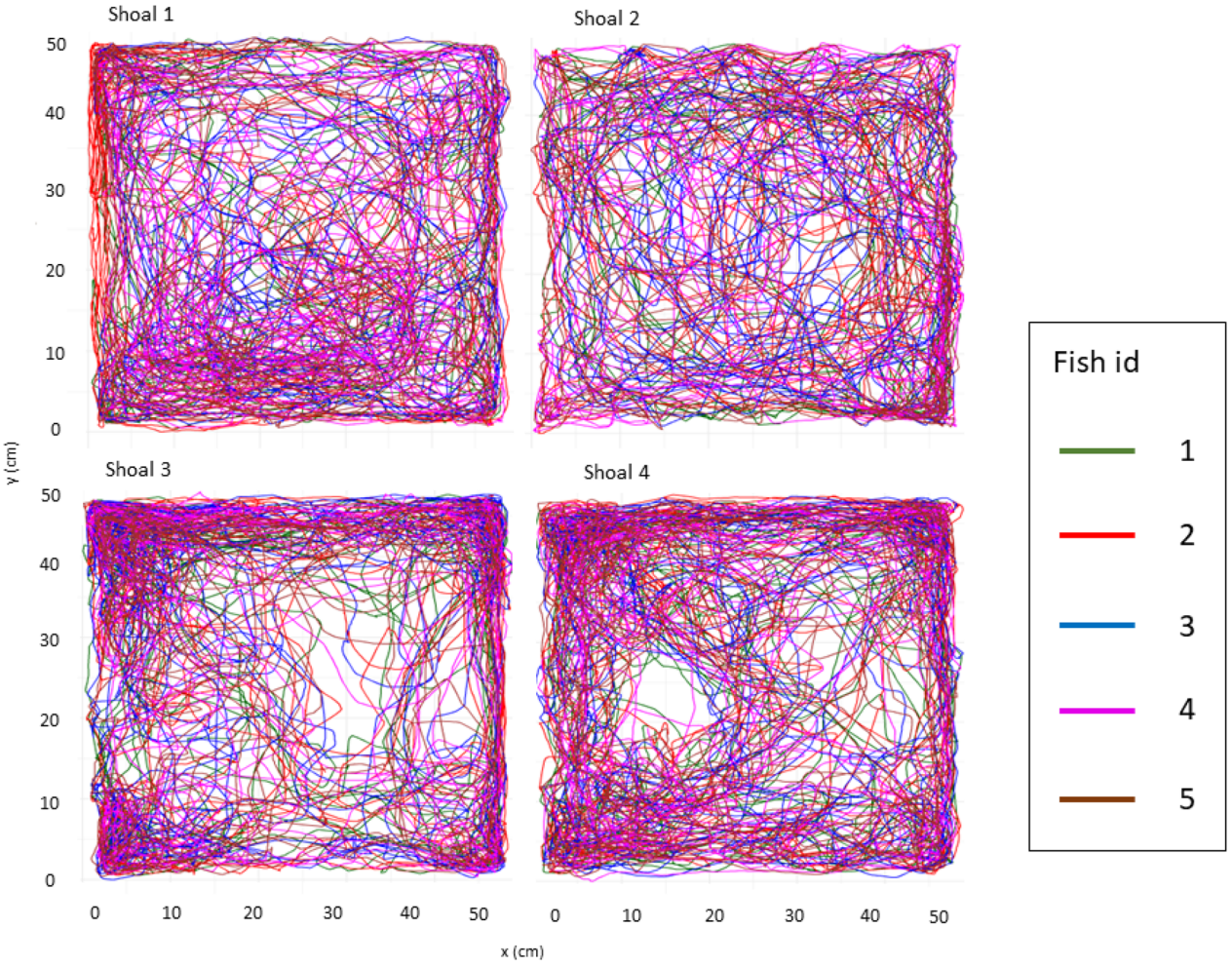
**Statistical Analysis and Derivation of Shoal Parameters.** The recorded videos were compressed using Adobe premiere pro-2020 (version 14.0, Adobe Inc., San Jose, California, US). The compressed videos were analyzed in idTracker 2.1.[24] idTracker is an automatic animal tracking software that is capable of identifying individuals and tracking the movements of multiple animals simultaneously using video recordings of animal groups. The output of the software is a spreadsheet with x and y coordinates of the fish in each frame of the video and a unique identifying number for each individual. Scheme 2 shows examples of 6 min tracks of four shoals. Shoal level parameters like shoal area (SA), interfish distance (IFD), farthest neighbor distance (FND), nearest neighbor distance (NND), total distance traveled (TDT), and thigmotaxis[23,25−27] (Table 1) for each shoal were derived from the track data using statistical programming package, R.[28]

We used Mann−Whitney U test to do a pairwise comparison of each of the parameters across the four treatments. A p-value less than 0.05 was considered statistically significant. All the statistical analyses were performed using Graph Pad Prism software (Graph Pad Software, Inc. San Diego, CA, USA).



https://doi.org/10.1021/acsomega.2c03815
ACS Omega 2022, 7, 37351−37358

**Scheme 2. Examples of Tracks of Fish in Shoals**[a]




[a]Each plot represents the tracks of positions of the fish in a shoal. Each color code represents an individual fish in the shoal. The shoal was allowed to explore for 6 min in the open tank (five fish per shoal).

**Table 1. Behavioral End Points and Explanations Used in the Current Study**

| parameters | definition |
|---|---|
| shoal area (SA) | area of the smallest polygon that contains all the fish at a given time |
| interfish distance (IFD) | distance between each shoal member's body center |
| farthest neighbor distance (FND) | distance between each fish's body center and the farthest neighboring fish |
| nearest neighbor distance (NND) | distance between each fish's body center and the nearest neighboring fish |
| total distance traveled (TDT) | total distance traveled by the zebrafish in the experimental tank |
| thigmotaxis (TS) | average distance between the individuals of the shoal and the tank's center |

## RESULTS

Our results show that MR and FR (mixed-sex shoals) were not significantly different from each other for any of the variables that were taken into consideration. Comparisons between MO and FO (same-sex shoals) also showed similar results. On the other hand, paired comparison between the mixed-sex shoal treatments and the same-sex shoal treatments (comparison between MR−MO, MR−FO, FR−MO, and FR−FO) revealed a significant difference between the treatments for many of the variables (Table 2, Table 3).

Among the variables that were considered, shoal area showed the highest percentage difference (between 77% and 79%) and the lowest p value between the different treatments. Figure 2 represents the shoal area as a radar plot for better comprehension of the variation of shoals in different treatments, while Figure 3 is a network representation of four example shoals representing associations between individual zebrafish within a shoal. IFD, NND, and FND exhibited similar percentage differences among each other (between 48% and 52%) but less than that of the shoal area (Figure 1).

Since IFD, NND, FND, and shoal area describe similar behavioral end points (i.e shoal cohesion), we refer to them collectively as "shoal cohesion" in this paper. The results also suggest that these parameters are correlated (but see ref 29 for an example where these parameters showed different patterns).

We obtained more complicated results for TDT and thigmotaxis. In general, mixed-sex shoals tended to travel less distance compared to same-sex shoals, but the differences in TDT were low (6−10%). TDT was significantly different only for MO−MR and FO−MR. FR had a lower but nonsignificant TDT compared to MR, and this might be the reason why pairs involving FR and same-sex shoals did not show any significant differences. On the other hand, thigmotaxis was generally higher for mixed-sex shoals with MR having the highest levels of


https://doi.org/10.1021/acsomega.2c03815
ACS Omega 2022, 7, 37351−37358

**Table 2. Percentage Differences between the Pairs of Treatments Compared[a]**

| | | | TREATMENT COMBINATIONS | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | MO - MR | MO - FR | MR - FO | FO - FR | MR - FR | MO - FO |
| PARAMETERS | Shoal level | Shoal area | 79.07 | 77.77 | -79.04 | 77.73 | -5.87 | 0.16 |
| | | IFD | 51.25 | 50.48 | -51.65 | 50.88 | -1.56 | -0.81 |
| | | NND | 49.8 | 48.22 | -50.88 | 49.33 | -3.05 | -2.15 |
| | | FND | 50.64 | 50.32 | -51.83 | 51.53 | -0.63 | -2.42 |
| | Individual activity | Distance | -6.27 | -8.93 | 6.74 | -9.39 | -2.84 | 0.5 |
| | | Thigmotaxis | -11.81 | -5.12 | 6.57 | 0.51 | 7.05 | -5.61 |

[a]Green colored cells have values that were significantly different between the treatments compared.

**Table 3. Showing p Values of Each Shoal Comparison[a]**

| Treatment | Statistical parameter | MO - FO | MO - MR | MO - FR | FO - MR | FO - FR | MR - FR |
|---|---|---|---|---|---|---|---|
| SA | p-value | 0.9118 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.7394 |
| | Exact/approximate | Exact | Exact | Exact | Exact | Exact | Exact |
| | Sum of ranks | 103, 107 | 155, 55 | 155, 55 | 155, 55 | 155, 55 | 100, 110 |
| | Mann-Whitney U | 48 | 0 | 0 | 0 | 0 | 45 |
| IFD | p-value | 0.9705 | <0.0001 | <0.0001 | 0.0002 | 0.0002 | 0.9118 |
| | Exact/approximate | Exact | Exact | Exact | Gaussian | Gaussian | Exact |
| | Sum of ranks | 106, 104 | 155, 55 | 155, 55 | 155, 55 | 155, 55 | 103, 107 |
| | Mann-Whitney U | 49 | 0 | 0 | 0 | 0 | 48 |
| FND | p-value | 0.8534 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.8798 |
| | Exact/approximate | Exact | Exact | Exact | Exact | Exact | Gaussian |
| | Sum of ranks | 102, 108 | 155, 55 | 153, 57 | 155, 55 | 155, 55 | 107.5, 102.5 |
| | Mann-Whitney U | 47 | 0 | 2 | 0 | 0 | 47.5 |
| NND | p-value | 0.7623 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.7394 |
| | Exact/approximate | Gaussian | Exact | Exact | Exact | Exact | Exact |
| | Sum of ranks | 100.5, 109.5 | 155, 55 | 152, 58 | 155, 55 | 155, 55 | 100, 110 |
| | Mann-Whitney U | 45.5 | 0 | 3 | 0 | 0 | 45 |
| TDT | p-value | 0.5787 | 0.2475 | 0.0433 | 0.4813 | 0.0147 | 0.315 |
| | Exact/approximate | Exact | Exact | Exact | Exact | Exact | Exact |
| | Sum of ranks | 97, 113 | 89, 121 | 78, 132 | 95, 115 | 73, 137 | 91, 119 |
| | Mann-Whitney U | 42 | 34 | 23 | 40 | 18 | 36 |
| TM | p-value | 0.0962 | 0.0052 | 0.1431 | 0.1655 | 0.9705 | 0.0962 |
| | Exact/approximate | Gaussian | Exact | Exact | Exact | Exact | Gaussian |
| | Sum of ranks | 82.5, 127.5 | 69, 141 | 85, 125 | 86, 124 | 106, 104 | 127.5, 82.5 |
| | Mann-Whitney U | 27.5 | 14 | 30 | 31 | 49 | 27.5 |

[a]The p values were calculated using a two-tailed Mann-Whitney U test, graph pad prism (n = 40). Statistically significant values are highlighted (green). Gaussian refers to p values estimated using Gaussian approximation.

thigmotaxis; except for MO−MR, none of the pairwise differences was significant (Figure 4). (Statistical comparison of shoals is given in Table 3).

## DISCUSSION

Previous studies that looked at shoaling behavior of zebrafish in a similar free-shoaling setup have found that zebrafish prefer to shoal with conspecifics compared to heterospecifics.[10] Our experiments suggest that in zebrafish, the sex ratio of the conspecific shoals influences shoal cohesion. Mixed-sex shoals tend to have higher shoal cohesion parameters than same-sex shoals, but the relative number of males and females in the mixed shoals did not make a difference. Similarly, the sexual identity of same-sex shoals also did not significantly affect shoal cohesion parameters. Regardless of the sexual identity or the relative proportion of the sexes, whether a shoal is mixed-sex or not is what determines shoal cohesion.

A similar study using only mixed-sex shoals found that females in shoals preferred to swim close to other females, but males did not show any such preference.[30] Hence, increased shoal cohesion in mixed-sex shoals in our study might be because of the female preference to be with individuals of the same sex. But



https://doi.org/10.1021/acsomega.2c03815
ACS Omega 2022, 7, 37351−37358

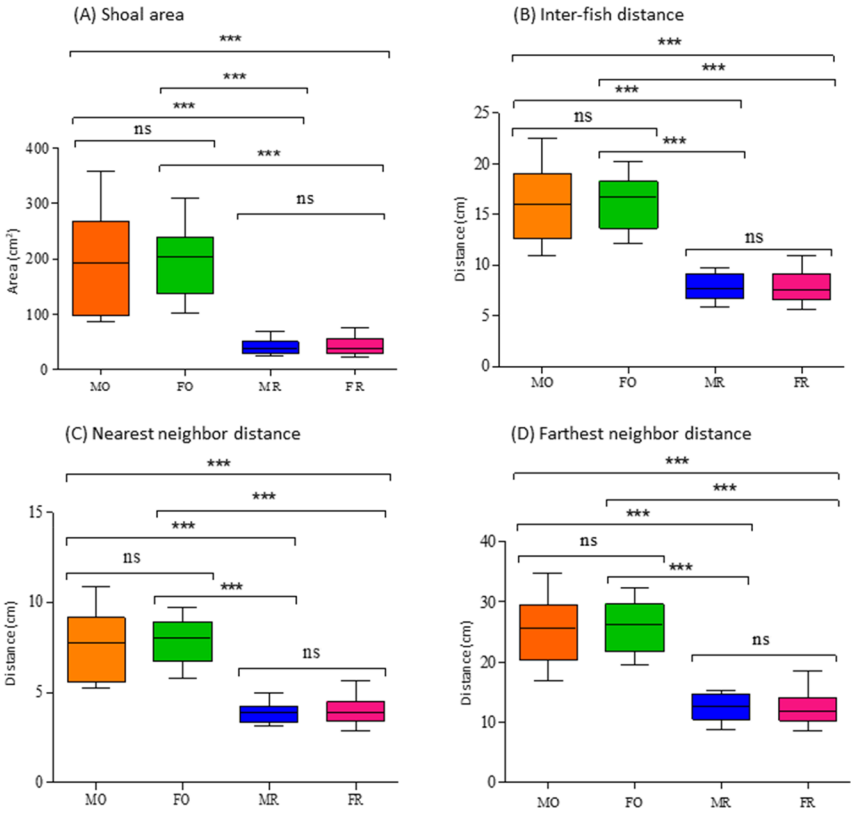



**Figure 1.** Boxplots showing the distribution of (A) shoal area, (B) IFD, (C) NND, and (D) FND values across the four treatments: MO, FO, MR, and FR. The asterisks (***) mean high statistical significance ($p \leq 0.0001$), and "ns" indicates the statistical nonsignificance between groups, respectively. The lower and upper ends of the boxes represent the first and third quartiles, and the horizontal line within each box is the median. The whiskers are (1.5× interquartile range) added to the quartiles (comparison done using Mann−Whitney U test; $n = 40$, 5 fish per shoal and 10 shoals per treatment).

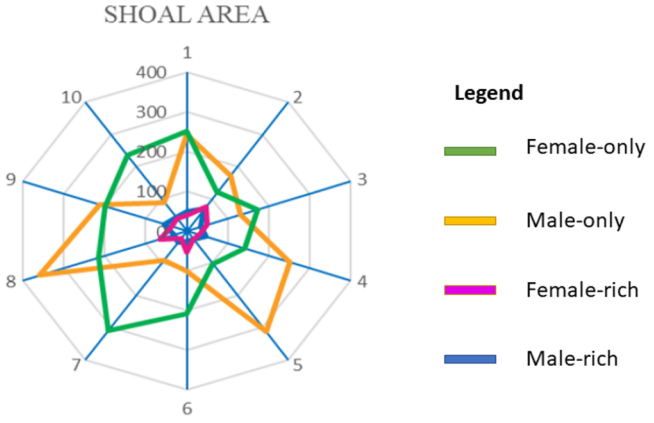



**Figure 2.** Radar plot of shoal area: all 10 axes in the radar graph represent the 10 shoal observations of the four different shoals. A total of 40 observations were done for the analysis. The center point of the radar starts from a value of zero and all the values fall within a maximum value of 400. The highest shoal area was reported in the male-only (MO) and female-only (FO) shoal and the lowest shoal area in the male-rich (MR) and female-rich (FR) shoal.

it is interesting to note that the female−female cohesion decreases in an all-female shoal.

Zebrafish interfish distances vary with environmental conditions and physiology.[1] Interfish distance tends to be positively correlated with food availability and negatively correlated with predator threat.[19] In general, tightening the shoal by reducing shoal area, and reduced IFD, NND, and FFD are related to anxiety and stress.[31] This has also been validated by studies that show that shoal cohesion parameters and thigmotaxis increase when treated with anxiolytic drugs and reduce when treated with anxiogenic drugs.[32]

The increased shoal cohesion in mixed-sex shoals could also be a result of increased stress levels, though the reason for an increase in stress is open to speculation. It is suggested that a lot of group-living animals exhibit sexual segregation because of differences in predation risk, required resources, and in general a lack of synchrony in activities.[33] In many taxa, sexual segregation happens also due to sexual harassment of females by males.[34] Hence, the mixed-sex shoals might be under higher stress because a male-female ratio almost equal to one might be unusual.

Contrary to the findings of this study, Way et al. found no effects of sex on shoaling behavior in zebrafish in a dichotomous shoal preference test.[35] The contradiction between the two studies might be due to the difference in study designs. The dichotomous preference test tells us the preference of an individual fish to interact with a shoal, whereas interactions in a free-swimming shoal test tell us about the behavior of the shoal as a whole. This underlines the importance of behavioral assays conducted with the least amount of restrictions and as close to natural conditions as possible.

Increased investment in foraging might be necessary for females because of the higher energy costs associated with tissue generation.[36] Hence, one might expect females to have a higher activity level in general, with TDT being a proxy for fish activity. Studies on the cichlid fish *Pelvicachromis taeniatus* found that shoals with a higher proportion of females engaged in shoaling had higher shoal activity in terms of swimming speed and


https://doi.org/10.1021/acsomega.2c03815
ACS Omega 2022, 7, 37351−37358

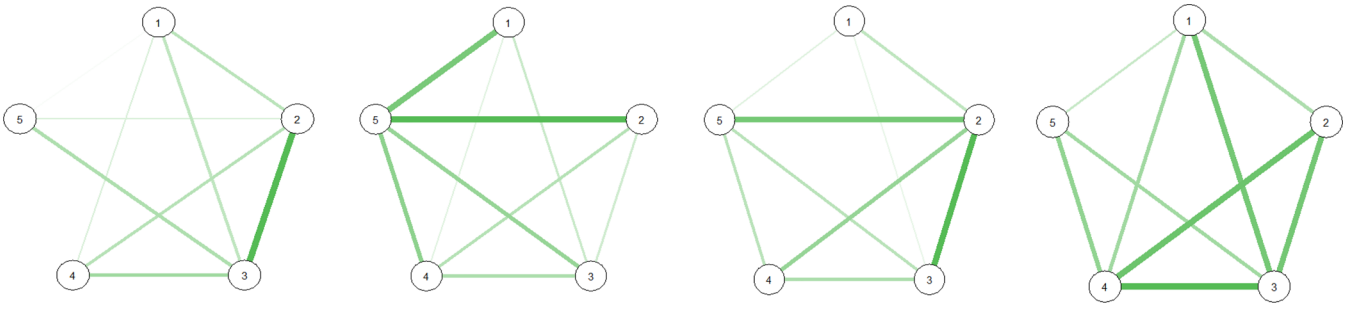



**Figure 3.** Network diagrams representing interfish distances between individual members of a shoal for four different shoals. Thicker lines indicate a close association or less interfish distance, and the thinner line indicates a high interfish distance. Although we did not track individual fish for our study, the software we used "idTracker" is capable of tracking specific individuals throughout a video.

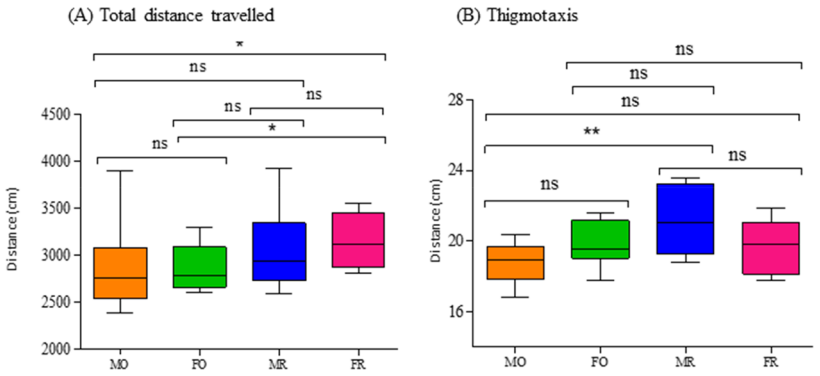



**Figure 4.** Boxplots showing the distribution of (A) TDT and (B) thigmotaxis values across the four treatments: MO, FO, MR, and FR. The asterisks (**) mean high statistical significance ($p \leq 0.0001$), and "ns" indicates the statistical nonsignificance between groups, respectively. The lower and upper ends of the boxes represent the first and third quartiles, and the horizontal line within each box is the median. The whiskers are (1.5× interquartile range) added to the quartiles (comparison done using Mann−Whitney U test; n = 40, 5 fish per shoal and 10 shoals per treatment)

distance traveled.[37] Though we did not track the sexual identity of individual fish in our experiments, we would have expected FO and FR shoals to have higher TDT than other treatments. But in our results, FO and MO showed similar levels of activity. Only fish in FR showed significantly higher activity levels than same-sex shoals. MR showed higher activity than same-sex shoals, but the difference was not significant.

A possible explanation for the TDT results is the mating-related behaviors that fish exhibit once in a mixed-sex shoal. Spence and Smith showed that courtship behaviors increase in female-biased shoals.[38] Although this corresponds to the activity patterns of the zebrafish in our study, we believe that courtship behavior probably does not influence our results as the experiments in our study were conducted after the peak spawning period.[39] Some of our experiments could have picked up intrasexual territorial behaviors exhibited right after the spawning peak, but whether this behavior is modified with changes in sex ratio is yet to be known. One can also argue that given the small effect sizes and low p values, the differences in activity level are not big enough to be biologically meaningful.

For thigmotaxis, MR showed a significantly higher value compared to MO. All the other pairwise comparisons yielded nonsignificant results. Aggressive behavior among males in a female poor environment might be an explanation for this. Female−female aggression is also reported in zebrafish as a part of dominant−subordinate hierarchies.[40]

Changes in shoal cohesion parameters and activity levels have behavioral consequences on zebrafish. Increased IFD may limit the intrashoal transfer of cues, which in turn will affect the stability of the shoal.[33] Shoal cohesion represents a trade-off: high shoal cohesion increases safety from predators but increases intrashoal competition for resources.[11] Higher activity, as shown in the Chinese sturgeon, *Acipenser sinensis*, allows for more foraging effort by covering bigger areas, resulting in higher food intake.[41]

Future studies could use free shoaling experiments to explore the interaction of various factors with the sexual identity of zebrafish. For instance, Blonder and Tarvin used a dichotomous preference test to see whether male zebrafish preferred to shoal with familiar males over unfamiliar males.[42] Exploring the effect of the sexual identity of zebrafish on their interaction with familiar and unfamiliar fish in a free-swimming shoal might yield useful insights.

Though this paper has used zebrafish as a model organism to study behavioral, we believe that the results of this study could have wider implications for biomedical, genetic, molecular, and neurological research. Human neurological disorders associated with social communication deficits including autism spectrum disorders, schizophrenia, etc. are widely investigated based on the shoaling behavior of zebrafish.[29] Increased shoal area and interindividual distance can be correlated with shoal disruption and social communication in the case of neurological disorders.

## AUTHOR INFORMATION

### Corresponding Author

**Binu Ramachandran** − *Neuronal Plasticity Group, Department of Zoology, University of Calicut, Malappuram, Kerala 673635, India;* orcid.org/0000-0002-0443-4741; Email: binuramachandran@uoc.ac.in



https://doi.org/10.1021/acsomega.2c03815
*ACS Omega* 2022, 7, 37351−37358

### Authors

**Aswathy Sivaraman** − *Neuronal Plasticity Group, Department of Zoology, University of Calicut, Malappuram, Kerala 673635, India*

**Rohit Nandakumar** − *Nature Conservation Foundation, Mysore, Karnataka 570017, India*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c03815


### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This work was funded by DST-SERB Early Career Research Grant (ECR/2018/002479).

## ■ REFERENCES


(1) Krause, J.; Ruxton, G. D. *Living in Groups*; Oxford, 2002.

(2) Hamilton, W. D. Geometry for the Selfish Herd. *J. Theor. Biol.* **1971**, *31* (2), 295−311.

(3) Mooring, M. S.; Hart, B. L. Animal Grouping for Protection From Parasites: Selfish Herd and Encounter-Dilution Effects. *Behaviour* **1992**, *123* (3−4), 173−193.

(4) Beauchamp, G. Group-Size Effects on Vigilance: A Search for Mechanisms. *Behav. Processes* **2003**, *63* (3), 141−145.

(5) Spagnoli, S.; Lawrence, C.; Kent, M. L. Stress in Fish as Model Organisms. In *Fish Physiol.*; Schreck, C. B., Tort, L., Farrell, A. P., Brauner, C. J., Eds.; Academic Press, 2016; Vol. *35*, pp 541−564. DOI: 10.1016/B978-0-12-802728-8.00013-8.

(6) Miller, N.; Gerlai, R. From Schooling to Shoaling: Patterns of Collective Motion in Zebrafish (Danio Rerio). *PLoS One* **2012**, *7* (11), No. e48865.

(7) Pitcher, T. J. Functions of Shoaling Behavior in Teleosts. *Behavior of Teleost Fishes*; Springer, 1993. DOI: 10.1007/978-94-011-1578-0_12

(8) Penry-Williams, I. L.; Ioannou, C. C.; Taylor, M. I. The Oddity Effect Drives Prey Choice but Not Necessarily Attack Time. *Ethology* **2018**, *124* (7), 496−503.

(9) Murali, G.; Kumari, K.; Kodandaramaiah, U. Dynamic Colour Change and the Confusion Effect against Predation. *Sci. Rep.* **2019**, *9* (1), 1−6.

(10) Saverino, C.; Gerlai, R. The Social Zebrafish: Behavioral Responses to Conspecific, Heterospecific, and Computer Animated Fish. *Behav. Brain Res.* **2008**, *191* (1), 77−87.

(11) Miller, N. Y.; Gerlai, R. Shoaling in Zebrafish: What We Don't Know. *Rev. Neurosci. (Berlin, Ger.)* **2011**, *22* (1), 17−25.

(12) McROBERT, S. P.; Bradner, J. The Influence of Body Coloration on Shoaling Preferences in Fish. *Anim. Behav.* **1998**, *56* (3), 611−615.

(13) Griffiths, S. W.; Magurran, A. E. Familiarity in Schooling Fish: How Long Does It Take to Acquire? *Anim. Behav.* **1997**, *53* (5), 945−949.

(14) Krause, J.; Hoare, D. J.; Croft, D.; Lawrence, J.; Ward, A.; Ruxton, G. D.; Godin, J. J.; James, R. Fish Shoal Composition: Mechanisms and Constraints. *Proc. R. Soc. London. Ser. B* **2000**, *267* (1456), 2011−2017.

(15) Hoare, D. J.; Ruxton, G. D.; Godin, J. J.; Krause, J. The Social Organization of Free-ranging Fish Shoals. *Oikos* **2000**, *89* (3), 546−554.

(16) Richards, E. L.; van Oosterhout, C.; Cable, J. Sex-Specific Differences in Shoaling Affect Parasite Transmission in Guppies. *PLoS One* **2010**, *5* (10), No. e13285.

(17) Rystrom, T. L.; Clement, V. F.; Rick, I. P.; Bakker, T. C. M.; Mehlis, M. Shoal Sex Composition and Predation Risk Influence Sub-Adult Threespine Stickleback Shoaling Decisions. *Behav.Processes* **2018**, *157*, 495−501.

(18) Engeszer, R. E.; da Barbiano, L. A.; Ryan, M. J.; Parichy, D. M. Timing and Plasticity of Shoaling Behaviour in the Zebrafish, Danio Rerio. *Anim. Behav.* **2007**, *74* (5), 1269−1275.

(19) Miller, N.; Gerlai, R. Quantification of Shoaling Behaviour in Zebrafish (Danio Rerio). *Behav. Brain Res.* **2007**, *184* (2), 157−166.

(20) Tavares, B.; Santos Lopes, S. The Importance of Zebrafish in Biomedical Research. *Acta Med. Port.* **2013**, *26* (5), 583−592.

(21) Conradsen, C.; McGuigan, K. Sexually Dimorphic Morphology and Swimming Performance Relationships in Wild-Type Zebrafish Danio Rerio. *J. Fish Biol.* **2015**, *87* (5), 1219−1233.

(22) Avdesh, A.; Chen, M.; Martin-Iverson, M. T.; Mondal, A.; Ong, D.; Rainey-Smith, S.; Taddei, K.; Lardelli, M.; Groth, D. M.; Verdile, G. Regular Care and Maintenance of a Zebrafish (Danio Rerio) Laboratory: An Introduction. *J. Visualized Exp.* **2012**, *18* (69), No. e4196.

(23) Séguret, A.; Collignon, B.; Halloy, J. Strain Differences in the Collective Behaviour of Zebrafish (Danio Rerio) in Heterogeneous Environment. *R. Soc. Open Sci.* **2016**, *3* (10), 160451.

(24) Pérez-Escudero, A.; Vicente-Page, J.; Hinz, R. C.; Arganda, S.; de Polavieja, G. G. IdTracker: Tracking Individuals in a Group by Automatic Identification of Unmarked Animals. *Nat. Methods* **2014**, *11* (7), 743−748.

(25) Miller, N.; Gerlai, R. Automated Tracking of Zebrafish Shoals and the Analysis of Shoaling Behavior. In *Zebrafish protocols for neurobehavioral research*; Springer, 2012; pp 217−230. DOI: 10.1007/978-1-61779-597-8_16.

(26) Pham, M.; Raymond, J.; Hester, J.; Kyzar, E.; Gaikwad, S.; Bruce, I.; Fryar, C.; Chanin, S.; Enriquez, J.; Bagawandoss, S.; Zapolsky, I.; Green, J.; Stewart, A. M.; Robison, B. D.; Kalueff, A. v. Assessing Social Behavior Phenotypes in Adult Zebrafish: Shoaling, Social Preference, and Mirror Biting Tests. In *Zebrafish Protocols for Neurobehavioral Research*; Kalueff, A. v, Stewart, A. M., Eds.; Humana Press: Totowa, NJ, 2012; pp 231−246. DOI: 10.1007/978-1-61779-597-8_17.

(27) Audira, G.; Sampurna, B. P.; Juniardi, S.; Liang, S. T.; Lai, Y. H.; Hsiao, C. A Versatile Setup for Measuring Multiple Behavior Endpoints in Zebrafish. *Inventions* **2018**, *3* (4), 75.

(28) R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing, 2013.

(29) Franscescon, F.; Souza, T. P.; Müller, T. E.; Michelotti, P.; Canzian, J.; Stefanello, F.; Rosemberg, D. B. Taurine Prevents MK-801-Induced Shoal Dispersion and Altered Cortisol Responses in Zebrafish. *Prog. Neuro-psychopharmacol. Biol. Psychiatry* **2021**, *111*, 110399.

(30) Etinger, A.; Lebron, J.; Palestis, B. G. Sex-Assortative Shoaling in Zebrafish (Danio Rerio). *Bios* **2009**, *80* (4), 153−158.

(31) Speedie, N.; Gerlai, R. Alarm Substance Induced Behavioral Responses in Zebrafish (Danio Rerio). *Behav. Brain Res.* **2008**, *188* (1), 168−177.

(32) Bencan, Z.; Sledge, D.; Levin, E. D. Buspirone, Chlordiazepoxide and Diazepam Effects in a Zebrafish Model of Anxiety. *Pharmacol., Biochem. Behav.* **2009**, *94* (1), 75−80.

(33) Conradt, L.; Roper, T. J. Activity Synchrony and Social Cohesion: A Fission-Fusion Model. *Proc. R. Soc. London. Ser. B* **2000**, *267* (1458), 2213−2218.

(34) Croft, D. P.; Morrell, L. J.; Wade, A. S.; Piyapong, C.; Ioannou, C. C.; Dyer, J. R. G.; Chapman, B. B.; Wong, Y.; Krause, J. Predation Risk as a Driving Force for Sexual Segregation: A Cross-Population Comparison. *Am. Nat.* **2006**, *167* (6), 867−878.

(35) Way, G. P.; Kiesel, A. L.; Ruhl, N.; Snekser, J. L.; McRobert, S. P. Sex Differences in a Shoaling-Boldness Behavioral Syndrome, but No Link with Aggression. *Behav. processes* **2015**, *113*, 7−12.

(36) Blumer, L. S. Parental Care Sex Differences in the Brown Bullhead, Ictalurus Nebulosus (Pisces, Ictaluridae). *Behav. Ecol. Sociobiol.* **1986**, *19* (2), 97−104.

(37) Schons, R. F.; Vitt, S.; Thünken, T. Environmental Habituation and Sexual Composition Affect Juveniles' Shoaling Activity in a Cichlid Fish (Pelvicachromis Taeniatus). *J. Fish Biol.* **2021**, *99* (4), 1307−1317.

(38) Spence, R.; Smith, C. Male Territoriality Mediates Density and Sex Ratio Effects on Oviposition in the Zebrafish, Danio Rerio. *Anim. Behav.* **2005**, *69* (6), 1317−1323.

(39) Hutter, S.; Magee, S.; Zala, S. M.; Penn, D. J. Reproductive Behaviour of Wild Zebrafish (Danio Rerio) in Large Tanks. *Behaviour* **2010**, *147* (5−6), 641−660.

(40) Pandolfi, M.; Scaia, M. F.; Fernandez, M. P. Sexual Dimorphism in Aggression: Sex-Specific Fighting Strategies across Species. *Front. Behav. Neurosci.* **2021**, *136*, 1 DOI: 10.3389/fnbeh.2021.659615.
(41) Qian, X.; Cui, Y.; Xie, S.; Lei, W.; Zhu, X.; Xiong, B.; Yang, Y. Individual Variations in Growth, Food Intake and Activity in Juvenile Chinese Sturgeon Acipenser Sinensis Gray. *J. Appl. Ichthyol.* **2002**, *18* (4−6), 695−698.
(42) Blonder, A. F.; Tarvin, K. A. Male Zebrafish (Danio Rerio) Do Not Preferentially Associate with Familiar over Unfamiliar Conspecifics. *J. Fish Biol.* **2022**, *100* (4), 1025−1032.



https://doi.org/10.1021/acsomega.2c03815
*ACS Omega* 2022, 7, 37351−37358