'Selfish herds' of guppies follow complex movement rules, but not when information is limited

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Abstract

Under the threat of predation, animals can decrease their level of risk by moving towards other individuals to form compact groups. A significant body of theoretical work has proposed multiple movement rules, varying in complexity, which might underlie this process of aggregation. However, if and how animals use these rules to form compact groups is still not well understood, and how environmental factors affect the use of these rules even less so. Here, we evaluate the success of different movement rules, by comparing their predictions to the movement seen when shoals of guppies (*Poecilia reticulata*) form under the threat of predation. We repeated the experiment in a turbid environment to assess how the use of the movement rules changed when visual information is reduced. During a simulated predator attack, guppies in clear water used complex rules that took multiple neighbours into account, forming compact groups. In turbid water the difference between all rule predictions and fish movement paths increased, particularly for complex rules, and the resulting shoals were more fragmented than in clear water. We conclude that guppies are able to use complex rules to form dense aggregations, but that environmental factors can limit their ability to do so.

Keywords: selfish herds, aggregation, *Poecilia reticulata*, group living, turbidity, social behaviour

Introduction

Animal aggregations often arise in response to predation threat, and the anti-predator benefits of grouping have been extensively considered (e.g.[1-3]). These benefits include dilution [4], encounter-dilution [5, 6] and confusion effects [7-10], through which individuals benefit from reduced risk arising from the presence of con- or heterospecifics in close proximity. The selfish herd hypothesis [11] suggests a further benefit to individuals: risk for any particular individual in the group can be reduced, but at the expense of other group members, for whom risk is increased. Individual risk is defined by the ‘domain of danger’ (DOD), the area of space containing all points closer to the focal animal than to any other individual, and the selfish herd hypothesis suggests individuals should position themselves within groups to minimise the size of their own DOD [11]. A significant body of theoretical work has evaluated the success of various behavioural ‘movement rules’ in minimising DODs and creating compact groups of individuals either once stable aggregations have formed [11, 12, 15-17] or during the process of aggregation itself [14, 18]

In theoretical models, simple rules, by which animals move towards their nearest neighbour [11] tend to be outperformed by more complex rules, in which the position and distance of multiple neighbours are accounted for [16, 19, 20]. These complex rules generate more compact aggregations in which a greater proportion of the group are able to reduce the size of their DOD. Simple rules can, however, result in more rapid initial reduction in DOD area [18], which might be particularly important when animals have little time to respond following detection of a predatory threat [14]. Simple rules have been criticised for their inability to produce the dense groups seen in nature [12, 16], whereas more complex rules may be cognitively too complex for animals to follow [21, 22].

The empirical study of selfish herd movement rules lags behind theory, with limited examples providing opposing evidence. Fur seals (*Arctocephalus pusillus pusillus*) moving through areas of high risk of predation from white sharks (*Carcharodon carcharias*), appear to move towards their nearest neighbour rather than evaluating the position of multiple neighbours [22]. On the other hand, domestic sheep move towards the centre of the group when herded by a sheep dog [23]. Meanwhile, three-spined sticklebacks (*Gasterosteus aculeatus*) move towards an individual that can be reached more quickly rather than one which is spatially closer [24], although these latter two cases did not evaluate alternative rules.

To experimentally test the predictions of the selfish herd hypothesis, we investigate the selfish herd movement rules used by guppy shoals (*Poecilia reticulata*) in response to a simulated predator, comparing actual movement paths to the predictions of a simulation model. We assess the difference between the movement direction of each fish and the predicted direction if that fish were following a range of different rules, including simple and complex algorithms, and thus provide the first experimental comparison of multiple movement rules.

Theoretical models assume that individuals using a particular rule are able to gather all the information necessary to make an informed decision without error. In reality, errors in the evaluation of the position of neighbours may lead to movement patterns that are not consistent with optimal movement rules. As errors may be exacerbated by environmental conditions [20], we explore the impact of increasing environmental turbidity on the selfish herd responses of our guppy shoals. In aquatic systems, increasing turbidity degrades the visual environment, shortening response distances to conspecifics [25, 26], predators [27, 28] and prey [29-31] in many species including guppies [25, 26]. We predict that increasing turbidity will result in either a) a switch from more complex to simpler rules as fewer shoalmates can be detected or b) increased error in evaluation of the position of shoalmates, leading to increased error in following any rule.

Methods

Study species and husbandry

All fish were descendants of wild-caught guppies from Trinidad in 2005/6, from multiple populations that were subsequently mixed in 2011. Fish were maintained in groups of approximately 40 in stock aquaria (200x400x400mm) on a recirculating system at the University of Hull. Temperature was held at at ~26^{o}C on a 12:12hr light:dark cycle and fish were fed daily on ZM small granular feed (0.5-0.8mm ZM Systems, Hampshire, UK). Experimental shoals consisting of 10 guppies (N = 12 shoals) were created by taking female fish of similar size from stock tanks and placing them in separate holding tanks (20 x 20 x 20cm) for 24 hours before experiments began. All fish in a shoal measured within 5mm of every other, mean size of fish in shoals varied from 15 to 29mm. Shoals differed in mean body size (ANOVA: $F_{11,108} = 123.3$, $P < 0.001$), but there was no difference in shoal heterogeneity between shoals (Levene's test: $F_{11,108} = 1.31$, $P = 0.18$). Only females were used as they form the core of guppy shoals [32] and to reduce the confounding effect of sexual behaviour on association patterns. Shoals were kept in these tanks for 24 hours before experiments began.

Turbid water was created using a widely distributed unicellular, motile algae species *Chlamydomonas* (Phytotech lab, Kansas, USA), previously used to disrupt vision in fish [26, 33]. Algae was grown in a medium containing de-ionised water and Bold's Basal Medium Solution (Phytotech lab, Kansas, USA) at 20^{o}c, in cylindrical culture vessels (5cm in diameter, 50cm in height) with a constant light source and airflow. Cultures were left to reach high concentrations (~400NTU) and then diluted with water from the aquarium system for experiments to reach ~20 NTU, equating to a 10cm visual range measured using a Secchi disk. Using this species ensures algal turbidity remains relatively stable over a period of up to 75 minutes [26].

Experimental Design

Experiments were carried out in a white circular shoaling tank 50cm in diameter with graduated sides, such that the water depth decreased from 5cm in a central area (20cm in diameter) to 0.5cm at the edges. This discouraged guppies from swimming around the edge of the tank or using the tank sides as a potential refuge. Shallow water restricted shoals to closer to two dimensions, and facilitated tracking of individual fish in turbid water; such shallow water is also a realistic representation of much of the stream habitat of the source populations. Trials were recorded from above using a Microsoft Lifecam suspended 40cm above the surface of the water. A monofilament fishing line was attached to two points either side of the tank out of view of the fish, and ran over the centre of the tank, passing 5cm above the camera (45cm above the water surface) at a 45 degree angle. From this a model bird predator (an oval piece of black card 10cm long and 4cm at its widest point) was dropped such that it passed over the centre of the tank at a speed of approximately 3.8 m s^{-}

^{1}, without obscuring the view of the fish. The camera was sufficiently small (23mm diameter) that the predator was visible to the prey at all times as it passed over the tank.

Shoals were allowed to acclimatise in the shoaling tank for an hour. Then, at a point when the fish were dispersed across the tank (judged by eye), the model predator was released. Previous work has shown this is sufficient to elicit a clear and distinct anti-predator response in guppies [26]. Each shoal was tested twice, once in clear and once in turbid water in a randomised order. After the first trial guppies were placed back into the holding tank and tested 24 hours later in the alternate water treatment. Guppies show no acclimitisation to simulated aerial predation attempts on this timescale [26, 34]. The water in the tank was changed after every experiment to prevent the build up of any olfactory cues. At the end of the second trial fish were measured (standard body length) to the nearest 0.5mm using calipers, and returned to stock tanks. As the fish were not marked, it was not possible to identify individuals within shoals between the two treatments.

Movement rules: fish

To identify the movement pathways of individual fish, we used VirtualDub (http://www.virtualdub.org) to convert videos into a stack of images at 15fps for each shoal. These were then analysed in ImageJ (http://imagej.nih.gov/ij/) using the manual tracker plugin MtrackJ. Each fish was tracked by taking the XY coordinate (taken from the nose of each individual as we were interested in movement direction) starting from just before the simulated predator flew over the tank until they had stopped moving in response to the predator. As our interest lay in the aggregation rules used, we used only this part of the anti-predator response in our analysis. Fish typically respond to a threat using a range of responses including a C-start, darting and freezing motion: aggregation typically begins after this initial response (which was observed in all individuals in our experiments), and so we restricted our analysis to movement occuring after this. For each individual, we used only the movement in the first 6 frames (0.4 s) after it initiated aggregation, and calculated the movement speed of each individual (distance moved/time) for use in the modelling. Simultaneously, we recorded the position of every other fish in the shoal at the point at which the focal fish began aggregation, regardless of where in their own movement sequence they were. These positions were used as the start locations for the fish in modelling the predicted paths (see below). For individuals which did not initiate aggregation (remained frozen), we could not predict a path, and so these fish are excluded from our analysis as focal fish, but are included as group mates for other fish (N=3/120 individuals in clear water and 15/120 individuals in turbid water). Our results are robust to the choice of 6 frames (see Supplementary Material).

Movement rules: model predictions

Predicted paths were generated using the agent-based selfish herd modelling framework described in [14] and [18, 20, 35]. For each shoal 10 point-like agents representing the fish were placed into a circular arena at the positions defined by the locations of the fish in the experimental trials. We assume that all individuals follow the same movement rule, and track the predicted paths of each fish over 6 timesteps. We considered 5 different movements rules (see table 1), following previous work on the topic: nearest neighbour (NN), 2 nearest neighbours (2NN), local crowded horizon (LCH), group centre (GC) and movement away from the final position of the simulated predator (AP).

The start of the simulation represented the time at which the focal fish started moving, and all individuals began moving simultaneously [11, 12, 14, 16]. In each timestep t (t = 1/15th s to match the frame rate of the video), each prey identified its target location, and moved towards that location using the speed of that individual as measured from the video. All individuals moved simultaneously and updated their target location in each timestep.

At the end of the simulation, we calculated the difference in movement direction between the start and end points of the focal fish, and the start and end points of the predicted movement path of that fish for each of the rules, giving us a movement error measured in degrees (hereafter, 'error'; see Supplementary Material for example movement paths). The error measurement took values between 0° (representing an exact follow of the rule) and 180° (a fish moving in the opposite direction to the predictions of the movement rule). We also investigated how the predicted pathway of each rule for each fish differed, and if the best-performing rule acted in combination with movement away from the predator (See Supplementary Material). All modelling was carried out in MATLAB R2011a.

Shoal cohesion

To evaluate overall aggregation levels, we counted the number of neighbours within 3 body lengths [36] of each fish, one frame before the simulated predator threat, and once a stable aggregation had formed. As fish were variable in size, but it was not possible to individually identify fish from the video, we used the mean body length of each shoal as our measure of distance for that shoal.

Statistics

To assess the success of each rule in explaining the movement of the fish, we compared the error measurements (difference in movement angle between the fish and the prediction) for each rule using linear mixed effects models (LME), with rule and water type as fixed effects, and shoal identity a random factor to account for the repeated measures nature of the data. Error was square root transformed to meet the assumptions of normality. Non-significant interactions were removed and only main effects are presented here [37]. The model was then re-run on clear and turbid water separately, using rule as the fixed effect. Pairwise comparisons of rules were achieved by setting each movement rule as the main intercept (re-levelled the data) in clear and turbid water. To assess whether the error for each rule differed between clear and turbid water, we used paired Wilcoxon Signed Rank tests on each rule separately. If fish were moving randomly (i.e. not following any rule), we would predict a mean error of 90°, so we assessed whether movement was closer to each rule than to random movement (i.e. if error differed from 90°) using one-sample Wilcoxon Signed Rank tests. *P*-values were corrected for multiple testing using the Benjamini and Hochberg [38] False Discovery Rate control (FDR) method.

We assessed the effect of turbidity on the time (number of frames) taken to initiate aggregation and the effect of turbidity, predation threat and their interaction on number of near neighbours (within three body lengths) using generalised linear mixed effects models (GLMER) with Poisson error distributions (as appropriate for count data) and shoal identity as a random factor (to account for repeated measures). We added an observation level random effect [39] to account for any overdispersion in the data. Pairwise comparisons were made using the same model structure on subsets of the data. All analysis was carried out in R 3.1.2 (R Development Core Team 2011).

Results

Movement rule

There was no effect of turbidity on the time (number of frames) taken to initiate aggregation ($Z = -1.17$, $p = 0.241$). However, both water clarity ($F_{1,1121} = 32.1$, $P < 0.001$) and rule ($F_{1,1121} = 8.87$, $P < 0.001$) had an effect on error, but there was no significant interaction between them. In clear water, we found a significant effect of movement rule on error rate ($F_{4,571} = 7.74$, $P < 0.001$; figure 1a). More complex rules, accounting for more neighbours (GC and LCH), had a lower error relative to fish movement compared to the more simple rules (NN, 2NN), and movement away from the predator (AP). In terms of their ability to predict the path of the fish, there was no significant difference between GC and LCH or between the 3 simple rules, but GC and LCH were significantly better at predicting movement paths than NN or 2NN (table 2). In turbid water, we saw no effect of movement rule on error rate ($F_{4,509} = 2.61$, $P = 0.304$, figure 1b). Pairwise comparisons suggest AP is less good at predicting movement than 2NN, GC or LCH (table 2). We found the more complex rules, and movement away from a predator (AP) had lower errors in clear water compared to turbid (GC: $V = 3673$, $P = 0.002$, LCH: $V = 3477$, $P = 0.008$, AP: $V = 3411$, $P = 0.008$), whereas we found no difference in the use of more simple rules between clear and turbid water (NN: $V = 2895$, $P = 0.370$, 2NN: $V = 3164$, $P = 0.091$). In clear water, all rules were better (lower error) at predicting the movement path of fish than would be expected if movement were random (table 3, figure 1a). In turbid water, the more complex rules (2NN, GC, LCH) predicted movement more accurately than expected by chance while the simpler rules (AP, NN) were no better than chance at predicting movement (table 3, figure 1b).

Shoal cohesion

There was a significant interaction between treatment (clear and turbid) and time (before and after) on the number of near neighbours an individual had (table 4). There was no difference in cohesion between water types before the attack ($Z = -0.121$, $P = 0.904$), but number of neighbours increased after a simulated attack in both clear ($Z = -8.005$, $P < 0.001$)

and turbid ($Z = -3.160$, $P = 0.002$) water, but after the attack, shoals were more cohesive in clear water ($Z = -4.841$, $P < 0.001$).

Discussion

Our results demonstrate that shoaling guppies are more likely to use complex (LCH or GC) rather than simple (NN or AP) movement rules when aggregating under the threat of predation, resulting in the formation of more compact shoals, as predicted by the selfish herd hypothesis [11]. Our study provides the first evidence that grouping animals are able to use the position of multiple neighbours when making facultative aggregation decisions under the threat of an imminent predatory attack. We know from previous works that fish are able to consistently choose the numerically larger [40, 41] or denser of a pair of shoals [42] and are able to distinguish between shoal sizes of 40 and 60 individuals [43], yet pairwise interactions are sufficient to capture spatial patterns of shoaling in groups of 30 under non-threat conditions [44]. The ability of animals to use complex rules has been questioned [12, 16, 21], but our results suggest that guppies are cognitively capable of responding to the position of multiple group mates.

Under the degraded visual conditions associated with turbidity, we predicted that guppies would either switch from complex to simpler rules, or show a decreased ability to follow any particular rule. Our results support the second of these predictions: in turbid conditions, the difference between the predicted and actual paths of the fish increased, particularly for GC and LCH rules. This led to the formation of shoals that were more fragmented than those seen in clear water. Turbidity acts to reduce the visual information available to the individuals, and may explain why Cape fur seals move towards one or two nearest neighbours when under threat, rather than accounting for multiple group members [22]. An alternative explanation is that fish in turbid water have a reduced perception of risk (e.g. [45, 46], but see [26]) and so are less motivated to seek shelter with their group-mates than fish in clear water, reducing the need to use rules to aggregate. However, there was no effect of water clarity on the time (number of frames) it took fish to initiate aggregation, suggesting no difference in risk perception between clear and turbid water, although fish were more likely to remain frozen in turbid water (proportion test: $X_2 = 7.27$, $P = 0.007$; see[26]).

The inability to form cohesive groups in visually poor environments could ultimately alter predation risk and survival. Although in our study, the mean number of close neighbours did not differ between clear and turbid water before the simulated predation attack, previous work has shown that high levels of turbidity can lead to the formation of looser aggregations under non-threat conditions [25, 26]. This implies that already increased inter-individual distances could exacerbate the reduction in ability to respond to multiple neighbours we observed here, leading to further dispersal of prey shoals. If groups are less cohesive, then the anti-predator benefits associated with large, dense groups, such as confusion [8, 9] and dilution effects [5, 7] are likely to be weakened, increasing individual predation risk. Different types of turbidity may affect behaviour in different ways. In aquatic environments, suspended sediment reduces the transmission of light through water (light attenuation), increases scattering [47] and reduces visual range [48]. Algal turbidity (as used here) can additionally act to shift the spectral composition of light towards green wavelengths [49, 50], while dissolved organic matter shifts wavelengths into the longer orange/red [51]. A shift in spectral composition may impact on behaviour of animals, particularly those that rely on colour-based visual communication [51, 52]. The impact of different types of turbidity on selfish herd responses to predation is yet to be studied.

We found no evidence that fish were moving away from the likely location of a predatory threat (following an AP rule): error associated with movement towards conspecifics was lower than the error associated with moving away from the predator. One might expect that the direction of a predatory approach to have a significant effect on movement direction. Indeed, Viscido et al [15] predicted that movement paths should include movement both towards conspecifics and away from the predator, and this behaviour has been observed in fiddler crab (*Uca pugilator*) flocks [13] and mini herds separated from droves [53]. We found no evidence to support the suggestion that a combination of GC (one of the best predictors of movement) and AP resulted in a smaller error than GC alone (see Supplementary information). It is likely, therefore, that the directional information provided by the overhead stimulus was not sufficient to trigger this type of response, and our design more closely reflected the non-directional stimulus of Hamilton’s [11] ‘hiding lion’, in which prey perceive the threat, but receive no information as to the possible direction of attack.

Although we find support for complex movement rules, we considered only a single, relatively small group size of 10 individuals (although this falls well within the normal range of shoal sizes found in the wild for this species; [54]). Theoretical work predicts that group size and density may be important in determining the best movement rule to follow, with simpler rules favoured when shoals are larger and the individuals within them are more dispersed [14]. The cognitive complexity of using the position of multiple neighbours may also be dependent on group size, and in larger groups (for which LCH rules were developed; [16]) it may be more challenging for individuals to use these rules. Further work is needed to investigate whether patterns of rule following differ as a function of group size both within and between species, and whether there is commonality across species in the use of different rules. Different predation strategies, for example dispersing prey before attacking, or delaying the attack until further into the centre of the group, may favour the evolution of different avoidance strategies [55], either dynamically, as the same group faces different predators or threats, or as evolved responses across populations or species.

Ethical statement

All work was approved by the School of Biological, Biomedical and Environmental Sciences and Faculty of Science and Engineering ethical review committees before work began, and followed the Association for the Study of Animal Behaviour/Animal Behavior Society Guidelines for the Use of Animals in Research (Animal Behaviour, 2006, 71, 245-253).

Competing interests

We have no competing interests

Data accessibility

Data and source code: Dryad doi:10.5061/dryad.gs390
(*http://dx.doi.org/10.5061/dryad.gs390*)

Author contributions

HSK carried out all experimental work, video and statistical analysis. LJM conceived the study and carried out the modelling work. Both authors participated in study design and manuscript preparation and gave final approval for publication.

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References

1. Krause, J. and G. Ruxton, Living in groups. Ecology and evolution, ed. P. Harvey and R. May. 2002, Oxford: Oxford University Press.
2. Davies, N.B., K.R. Krebs, and S.W. West, An introduction to behavioural ecology. 2012, Chichester: Wiley-Blackwell.
3. Beauchamp, G., Social predation. How group living benefits predators and prey. 2014, London: Academic Press.
4. Foster, W.A. and J.E. Treherne. 1981. Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. *Nature,* **293(5832)**, 466-467. doi: 10.1038/293466a0
5. Turner, G.F. and T.J. Pitcher. 1986. Attack abatement - a model for group protection by combined avoidance and dilution. *American Naturalist,* **128(2)**, 228-240. doi: 10.1086/284556
6. Wrona, F.J. and R.W.J. Dixon. 1991. Group-size and predation risk - a field analysis of encounter and dilution effects. *American Naturalist,* **137(2)**, 186-201. doi: 10.1086/285153
7. Landeau, L. and J. Terborgh. 1986. Oddity and the confusion effect in predation. *Animal Behaviour,* **34**, 1372-1380. doi: 10.1016/s0003-3472(86)80208-1
8. Milinski, M. 1977. Do all members of a swarm suffer same predation. *Zeitschrift Fur Tierpsychologie-Journal of Comparative Ethology,* **45(4)**, 373-388.
9. Krakauer, D.C. 1995. Groups confuse predators by exploiting perceptual bottlenecks - a connectionist model of the confusion effect. *Behavioral Ecology and Sociobiology,* **36(6)**, 421-429. doi: 10.1007/bf00177338
10. Ioannou, C.C., L.J. Morrell, G.D. Ruxton, and J. Krause. 2009. The effect of prey density on predators: Conspicuousness and attack success are sensitive to spatial scale. *American Naturalist,* **173(4)**, 499-506. doi: 10.1086/597219
11. Hamilton, W.D. 1971. Geometry for selfish herd. *Journal of Theoretical Biology,* **31(2)**, 295-&. doi: 10.1016/0022-5193(71)90189-5
12. Morton, T.L., J.W. Haefner, V. Nugala, R.D. Decino, and L. Mendes. 1994. The selfish herd revisited: Do simple movement rules reduce relative predation risk? *Journal of Theoretical Biology***(167)**, 73-79.

13. Viscido, S.V. and D.S. Wethey. 2002. Quantitative analysis of fiddler crab flock movement: Evidence for 'selfish herd' behaviour. *Animal Behaviour,* **63**, 735-741. doi: 10.1006/anbe.2001.1935

14. Morrell, L.J. and R. James. 2008. Mechanisms for aggregation in animals: Rule success depends on ecological variables. *Behavioral Ecology,* **19(1)**, 193-201. doi: 10.1093/beheco/arm122

15. Viscido, S.V., M. Miller, and D.S. Wethey. 2001. The response of a selfish herd to an attack from outside the group perimeter. *Journal of Theoretical Biology,* **208(3)**, 315-328. doi: 10.1006/jtbi.2000.2221

16. Viscido, S., M. Miller, and D.S. Wethey. 2002. The dilemma of the selfish herd. The search for a realistic movement rule. *Journal of Theoretical Biology***(217)**, 183-194. doi: 10.1006/yjtbi.3025

17. James, R., P.G. Bennett, and J. Krause. 2004. Geometry for mutualistic and selfish herds: The limited domain of danger. *J Theor Biol,* **228(1)**, 107-13. doi: 10.1016/j.jtbi.2003.12.005

18. Morrell, L.J., G.D. Ruxton, and R. James. 2011. The temporal selfish herd: Predation risk while aggregations form. *Proc Biol Sci,* **278(1705)**, 605-12. doi: 10.1098/rspb.2010.1605

19. Viscido, S.V., J.K. Parrish, and D. Grünbaum. 2005. The effect of population size and number of influential neighbors on the emergent properties of fish schools. *Ecological Modelling,* **183(2-3)**, 347-363. doi: 10.1016/j.ecolmodel.2004.08.019

20. Morrell, L.J., L. Greenwood, and G.D. Ruxton. 2015. Consequences of variation in predator attack for the evolution of the selfish herd. *Evolutionary Ecology,* **29(1)**, 107-121. doi: 10.1007/s10682-014-9743-6

21. Reluga, T.C. and S. Viscido. 2005. Simulated evolution of selfish herd behavior. *J Theor Biol,* **234(2)**, 213-25. doi: 10.1016/j.jtbi.2004.11.035

22. De Vos, A. and M.J. O'riain. 2012. Movement in a selfish seal herd: Do seals follow simple or complex movement rules? *Behavioral Ecology,* **24(1)**, 190-197. doi: 10.1093/beheco/ars153

23. King, A.J., A.M. Wilson, S.D. Wilshin, J. Lowe, H. Haddadi, S. Hailes, and A.J. Morton. 2012. Selfish-herd behaviour of sheep under threat. *Curr Biol,* **22(14)**, R561-2. doi: 10.1016/j.cub.2012.05.008

24. Krause, J. and R.W. Tegeder. 1994. The mechanism of aggregation behavior in fish shoals - individuals minimize approach time to neighbors. *Animal Behaviour,* **48(2)**, 353-359. doi: 10.1006/anbe.1994.1248

25. Borner, K.K., S. Krause, T. Mehner, S. Uusi-Heikkila, I.W. Ramnarine, and J. Krause. 2015. Turbidity affects social dynamics in trinidadian guppies. *Behavioral Ecology and Sociobiology,* **69(4)**, 645-651. doi: 10.1007/s00265-015-1875-3

26. Kimbell, H. and L.J. Morrell. 2015. Turbidity alters anti-predatory behaviour in guppy (poecilia reticulata) shoals. *Animal Behaviour,* **103**, 179-185.

27. Meager, J.J., P. Domenici, A. Shingles, and A.C. Utne-Palm. 2006. Escape responses in juvenile atlantic cod gadus morhua l.: The effects of turbidity and predator speed. *J Exp Biol,* **209(Pt 20)**, 4174-84. doi: 10.1242/jeb.02489

28. Miner, J.G. and R.A. Stein. 1996. Detection of predators and habitat choice by small bluegills: Effects of turbidity and alternative prey. *Transactions of the American Fisheries Society,* **125(1)**, 97-103. doi: 10.1577/1548-8659(1996)125<0097:dopahc>2.3.co;2

29. Abrahams, M. and M. Kattenfeld. 1997. The role of turbidity as a constraint on predator-prey interactions in aquatic environments. *Behavioral Ecology and Sociobiology,* **40(3)**, 169-174. doi: 10.1007/s002650050330

30. De Robertis, A., C.H. Ryer, A. Veloza, and R.D. Brodeur. 2003. Differential effects of turbidity on prey consumption of piscivorous and planktivorous fish. *Canadian Journal of Fisheries and Aquatic Sciences,* **60(12)**, 1517-1526. doi: 10.1139/f03-123

31. Gregory, R.S. and C.D. Levings. 1998. Turbidity reduces predation on migrating juvenile pacific salmon. *Transactions of the American Fisheries Society,* **127(2)**, 275-285. doi: 10.1577/1548-8659(1998)127<0275:trpomj>2.0.co;2

32. Croft, D.P., J. Krause, and R. James. 2004. Social networks in the guppy (poecilia reticulata). *Proceedings of the Royal Society B-Biological Sciences,* **271**, S516-S519. doi: 10.1098/rsbl.2004.0206

33. Johannesen, A., A.M. Dunn, and L.J. Morrell. 2012. Olfactory cue use by three-spined sticklebacks foraging in turbid water: Prey detection or prey location? *Animal Behaviour,* **84(1)**, 151-158. doi: 10.1016/j.anbehav.2012.04.024

34. Seghers, B.H. 1974. Geographic variation in responses of guppies (poecilia-reticulata) to aerial predators. *Oecologia,* **14(1-2)**, 93-98. doi: 10.1007/bf00344900

35. Morrell, L.J., G.D. Ruxton, and R. James. 2011. Spatial positioning in the selfish herd. *Behavioral Ecology,* **22(1)**, 16-22. doi: 10.1093/beheco/arq157

36. Pitcher, T.J. and J.K. Parrish, Functions of shoaling behaviour in teleosts. Fish and fisheries series; behaviour of teleost fishes, second edition, ed. T.J. Pitcher. Vol. 7. 1993: Chapman and Hall Ltd., 2-6 Boundary Row, London SE1 8HN, England; Chapman and Hall, Inc., 29 West 35th Street, New York, New York, USA. 363-439.

37. Crawley, M., The r book. 2007, Chichester: John Wiley & Sons, Ltd.

38. Benjamini, Y. and Y. Hochberg. 1995. Controlling the false discovery rate - a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Methodological,* **57(1)**, 289-300.

39. Harrison, X.A. 2014. Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ,* **2**, e616. doi: 10.7717/peerj.616

40. Keenleyside, M.H.A. 1955. Some aspects of the schooling behaviour of fish. *Behaviour* **8**, 183 -248.

41. Krause, J., J. Godin, and D. Rubenstein. 1998. Group choice as a function of group size differences and assessment time in fish: The influence of species vulnerability to predation. *Ethology,* **104(1)**, 68-74.

42. Frommen, J.G., M. Hiermes, and T.C.M. Bakker. 2009. Disentangling the effects of group size and density on shoaling decisions of three-spined sticklebacks (gasterosteus aculeatus). *Behavioral Ecology and Sociobiology,* **63(8)**, 1141-1148. doi: 10.1007/s00265-009-0767-9

43. Thünken, T., M. Eigster, and J.G. Frommen. 2014. Context-dependent group size preferences in large shoals of three-spined sticklebacks. *Animal Behaviour,* **90**, 205-210. doi: 10.1016/j.anbehav.2014.01.024

44. Katz, Y., K. Tunstrom, C.C. Ioannou, C. Huepe, and I.D. Couzin. 2011. Inferring the structure and dynamics of interactions in schooling fish. *Proceedings of the National Academy of Sciences of the United States of America,* **108(46)**, 18720-18725. doi: 10.1073/pnas.1107583108

45. Gregory, R.S. 1993. Effect of turbidity on the predator avoidance-behavior of juvenile chinook salmon (*oncorhynchus tshwytshca*). *Canadian Journal of Fisheries and Aquatic Sciences,* **50(2)**, 241-246. doi: 10.1139/f93-027

46. Engström-Öst, J. and J. Mattila. 2008. Foraging, growth and habitat choice in turbid water: An experimental study with fish larvae in the baltic sea. *Marine Ecology Progress Series,* **359**, 275-281. doi: 10.3354/meps07345

47. Utne-Palm, A.C. 2002. Visual feeding of fish in a turbid environment: Physical and behavioural aspects. *Marine and Freshwater Behaviour and Physiology,* **35(1-2)**, 111-128. doi: 10.1080/10236240290025644

48. Davies-Colley, R.J. and D.G. Smith. 2001. Turbidity, suspended sediment, and water clarity: A review. *Journal of the American Water Resources Association,* **37(5)**, 1085-1101. doi: 10.1111/j.1752-1688.2001.tb03624.x

49. Ranaker, L., P.A. Nilsson, and C. Bronmark. 2012. Effects of degraded optical conditions on behavioural responses to alarm cues in a freshwater fish. *PLoS One,* **7(6)**, e38411. doi: 10.1371/journal.pone.0038411

50. Kirk, J.T.O., Light and photosynthesis in aquatic ecosystems. Vol. Third Edition. 2011: Cambridge University Press.

51. Kelley, J.L., B. Phillips, G.H. Cummins, and J. Shand. 2012. Changes in the visual environment affect colour signal brightness and shoaling behaviour in a freshwater fish. *Animal Behaviour,* **83(3)**, 783-791. doi: 10.1016/j.anbehav.2011.12.028

52. Engstrom-Ost, J. and U. Candolin. 2007. Human-induced water turbidity alters selection on sexual displays in sticklebacks. *Behavioral Ecology,* **18(2)**, 393-398. doi: 10.1093/beheco/arl097

53. Mclain, D.K., A.E. Pratt, and K. Kirschstein. 2005. Predator-driven fragmentation of fiddler crab droves into selfish miniherds of biased composition. *Journal of Experimental Marine Biology and Ecology,* **315(1)**, 1-15. doi: 10.1016/j.jembe.2004.08.024

54. Croft, D.P., B.J. Arrowsmith, J. Bielby, K. Skinner, E. White, I.D. Couzin, A.E. Magurran, I. Ramnarine, and J. Krause. 2003. Mechanisms underlying shoal composition in the trinidadian guppy, poecilia reticulata. *Oikos,* **100(3)**, 429-438. doi: 10.1034/j.1600-0706.2003.12023.x

55. Demsar, J., C. Hemelrijk, K., H. Hildrenbrandt, and I. Bajec, L. 2015. Simulating predator attacks on schools. Evolving composite tactics. *Ecological Modelling,* **304**, 22-33.

Tables

Table 1: Description of the proposed movement rules for individuals aggregating under the threat of predation (adapted from [14]).

Rule	Description
Movement away from predator (**AP**) [15]	Individuals move in the opposite direction (180° angle) away from movement of predator (i.e. a potential strike location)
Nearest neighbour (**NN**) [11]	Individuals moves towards closest neigbour in space
2 nearest neighours (**2NN**) [12]	Individuals moves towards the average location of 2 nearest neighbours
Group centre (**GC**) [15, 22]	Individuals move towards the area in the centre of all individuals within the group
Local crowded horizon (**LCH**) [16]	Individuals moves towards the area with the densest concentration of conspecifics. Closer individuals have a stronger influence on direction, whereas distant individuals exert a weaker force. The perception function used is f(x) = 1/1+kx, where x is the distance from the focal individual, and k = 0.375 [16].

Table 2: Pairwise comparisons of the ability of the 5 different movement rules to predict the movement path of the fish in clear (white) and turbid (shaded) water. Significant p-values are highlighted in bold. In all cases, N = 12 shoals of 10 fish each.

Rule	AP	NN	2NN	GC	LCH
AP		t = 0.71 *P* = 0.48	t = -1.17 *P* = 0.24	t = -3.72 ***P* < 0.001**	t = -3.3 ***P* = 0.001**
NN	t = -0.81 *P* = 0.42		t = -1.88 *P* = 0.061	t = 4.440 ***P* < 0.001**	t = 4.02 ***P* < 0.001**
2NN	**t = -2.1** ***P* = 0.04**	t = -1.28 *P* = 0.20		t = 2.55 ***P* = 0.011**	t = 2.14 ***P* = 0.033**
GC	**t = -2.5** ***P* = 0.01**	t = 1.68 *P* = 0.09	t = 0.4 *P* = 0.69		t= 0.41 *P* = 0.68
LCH	**t = -2.59** ***P* = 0.01**	t= 1.78 *P* = 0.08	t = 0.5 *P* = 0.62	t = -0.096 *P* = 0.92	

Table 3: Results (*P* values correct for multiple testing using FDR) from one-sample Wilcoxon signed rank tests, testing if the movement used by the fish is significantly different from random (90^{o}) for each of the movement rules. N = 12 shoals each containing 10 individuals.

	AP	NN	2NN	GC	LCH
Clear water	V = 2352 ***P* = 0.004**	V = 2220 ***P* <0.001**	V = 1357 ***P* <0.001**	V = 620 ***P* <0.001**	V = 643 ***P* <0.001**
Turbid water	V = 2795.5 P = 0.97	V = 2181 P = 0.082	V = 1875 **P = 0.009**	V = 1719 **P = 0.002**	V = 1702 **P = 0.002**

Table 4: Results from generalised linear mixed effects model evaluating the effect of treatment (clear or turbid) and time (before or after the simulated attack) on the number of near neighbours an individual had within 3 body lengths.

	Estimate	Std. Error	Z value	P
(Intercept)	1.483	0.074		
Time	-0.567	0.075	-7.566	**<0.001**
Treatment	-0.332	0.070	-4.721	**<0.001**
Time*Treatment	0.324	0.109	2.959	**0.003**

Figures

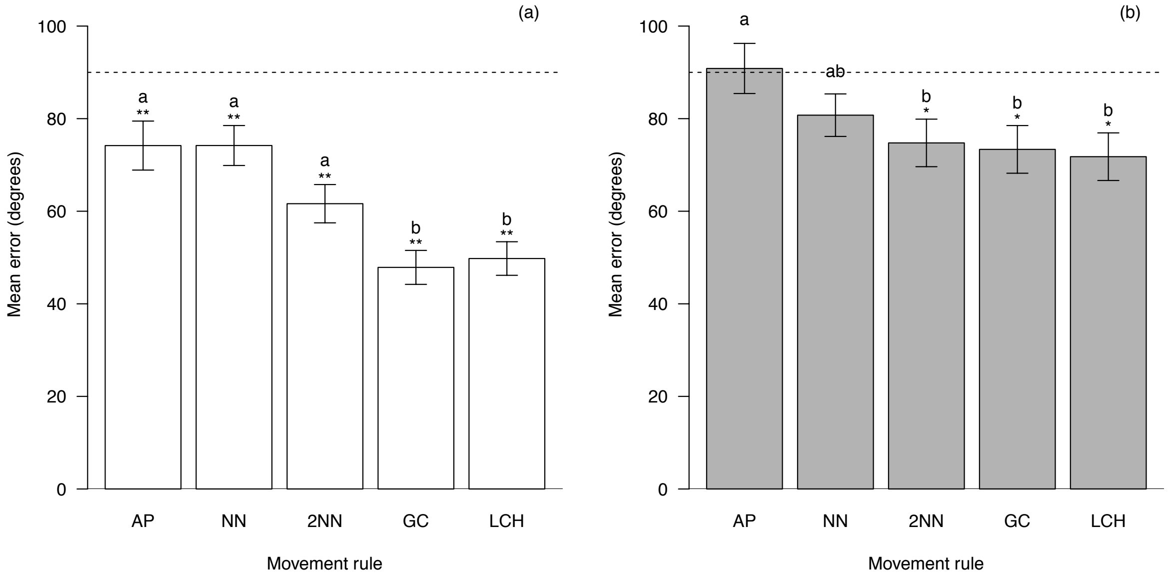

Figure 1. Mean error (degrees) ± S.E. between the movement path used by the fish in response to a predator attack and the 5 different movement rules (AP: away from predator, NN: nearest neighbour, 2NN: two nearest neighbours, GC: group centre, LCH: local crowded horizon) in (a) clear water and (b) turbid water. Dashed line at 90° is the prediction of random movement, asterisks indicate significant differences from this (* $p<0.01$, ** $p < 0.001$, table 3). Letters indicate homogenous subsets (table 2).

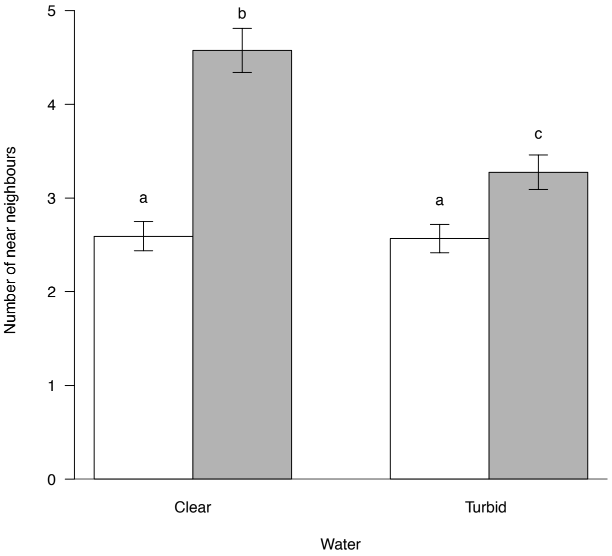

Figure 2: The mean (± S.E) number of neighbours within three body lengths, before (open bars) and after (shaded bars) a simulated predator attack in both clear and turbid water. Letters indicate homogenous subsets.

Supplementary Material for:

‘Selfish Herds’ of guppies follow complex movement rules, but not when information is limited

Helen S. Kimbell & Lesley J. Morrell

a) Choice of 6 frames as a reference point

For each individual, we report the error (difference between the movement direction of the fish and the predicted movement direction from the modelling) calculated 6 frames after movement began. To assess the robustness of this choice, we evaluated the error at 4, 8 and 12 frames, and found that the mean errors for each rule are very similar to those at 6 frames (table S1). Thus, our findings are robust to the timeframe we chose.

Table S1. Mean (1 SE) error (in degrees) evaluated at 4 different time points for all 5 rules, in clear (white) and turbid (shaded) water. In all cases, N = 12 shoals of 10 fish.

Time point	AP	NN	2NN	GC	LCH
4 frames	72.7 (5.2)	77.3 (4.5)	60.1 (4.4)	49.9 (3.6)	50.9 (3.7)
6 frames	73.7 (5.3)	74.6 (4.3)	61.8 (4.1)	47.8 (3.7)	50.0 (3.6)
8 frames	72.4 (5.2)	71.6 (4.5)	60.3 (4.5)	47.3 (3.8)	49.8 (3.8)
12 frames	71.2 (4.9)	72.2 (4.5)	59.4 (4.3)	48.5 (3.8)	52.8 (3.8)
4 frames	89.0 (5.4)	78.5 (4.7)	73.0 (5.2)	71.8 (5.2)	70.2 (5.2)
6 frames	92.5 (5.4)	81.8 (4.6)	75.8 (5.1)	73.7 (5.2)	72.2 (5.1)
8 frames	90.1 (5.3)	82.6 (4.7)	75.0 (4.9)	73.9 (5.1)	73.7 (4.9)
12 frames	90.6 (5.3)	86.7 (4.8)	76.9 (5.1)	71.3 (5.1)	74.4 (5.3)

b) Example movement paths of fish compared with rule predictions

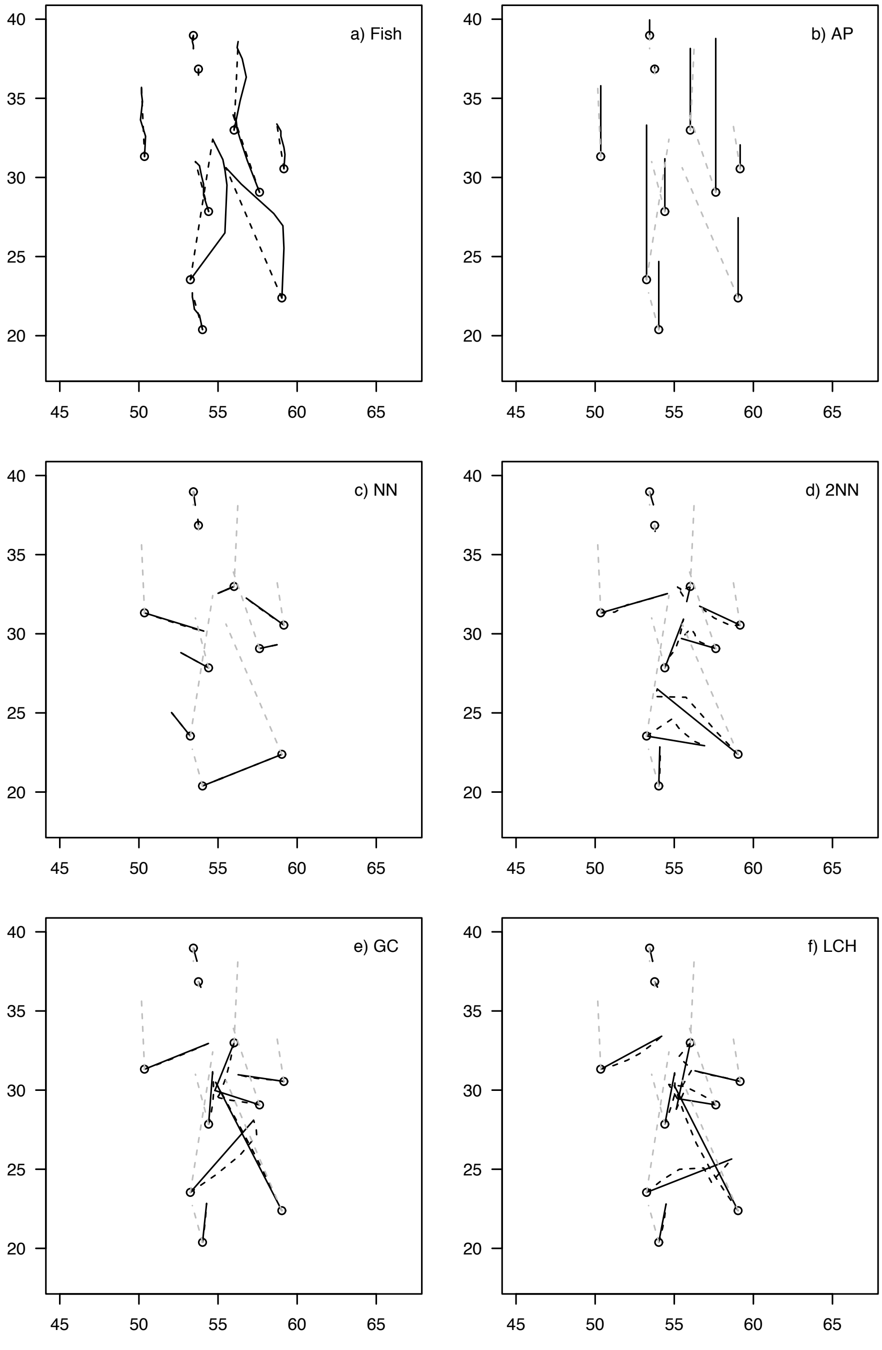

Figure S1: **a**) Movement paths of fish (solid lines) and movement direction (dashed line) in an example shoal. Panels **b-f** show the predicted movement paths (black dashed lines) and direction (solid line) for each movement rule. Grey dashed lines in panels **b-f** show the movement direction of the fish. The error (difference between the movement direction of the fish and the predicted movement direction from the modelling) is the minimum difference in angle between the solid and grey dashed line.

c) Combined AP and GC rules

Movement away from a predator (AP) may act in combination with other movement rules (NN, 2NN, GC or LCH) to affect the direction of movement. Viscido et al (2001) predicted that movement pathways of aggregating animals would be influenced by a combination of predator direction and the location of conspecifics.

Methods

To assess this for this, we generated a rule that combined AP and GC rules at different ratios, so that the strength of the effect of the direction of the predator decreased in 10% increments from a AP:GC ratio of 100:0 (pure AP) to 0:100 (pure GC). We then compared each of these combinations to the movement pathways of individual fish using identical methology to that of the main paper.

Results

Rules including a higher level of influence from the direction of the predator (AP rule) increased the error observed, the rule was more accurate (i.e. lower error) when just GC rules (AP:GC ratio of 0:10) were compared with the movement pathway of fish, in both clear and turbid water (figure S1)

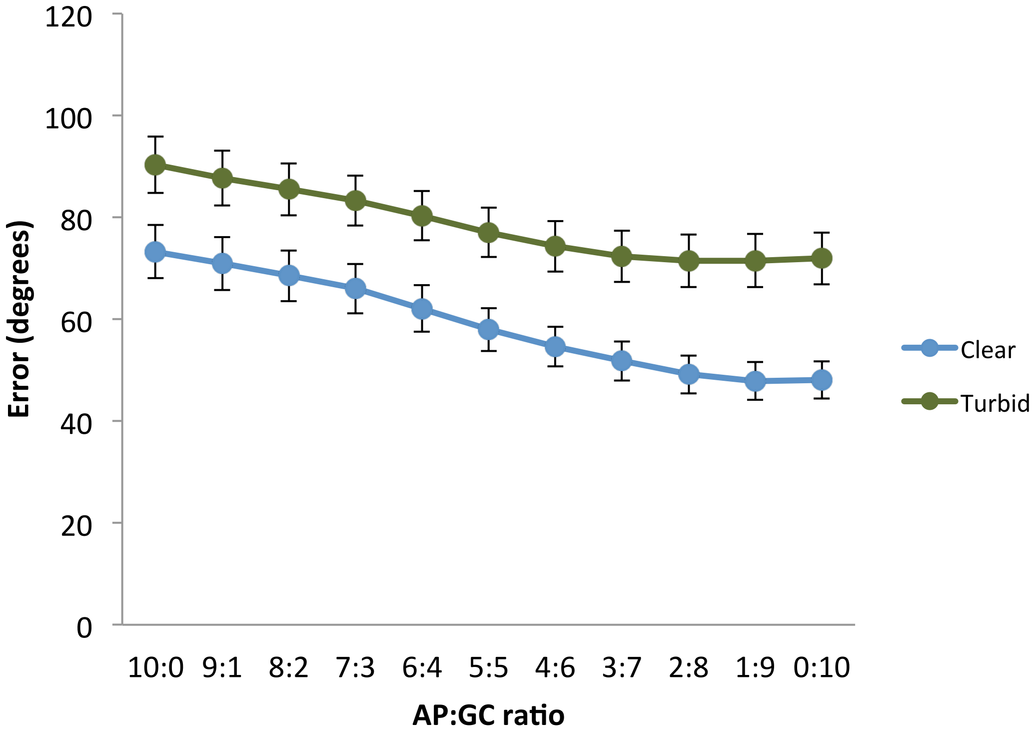

Figure S2: Mean error (± S.E.) when comparing the movement pathways of individual fish against a combined rule containing AP and GC rules at different ratios, represented here as a decreasing influence of the AP rule.

d) Rule comparisons

Some pairs of movement rules may predict similar movement paths, for example, movement following a GC rule may be similar to movement following a LCH rule as both account for multiple individuals within the small groups we evaluated. To assess the similarity of movement rule predictions, we explored the difference in predicted angle between each possible pair of rules for each individual fish. We tested whether the error was significantly different from 0° (what we would expect if the rules predicted the fish moved in the same direction) by using a one sample Wilcoxon Rank Sign test, correcting for multiple testing using the FDR method (Benjamini and Hochberg 1995)

This analysis revealed that GC and LCH made the most similar predictions (mean difference of 27.8 ± 2.5; table 1a), and that 2NN was similar to LCH (mean difference of 40.1 ± 2.9 degrees), but all pairs of rules were significantly different from one another (table S2).

Table S2: Comparison in the error (angle) ± S.E. between the different rules. A lower angle represents a similar direction. In all cases, d.f. = 2200. P-values are after correction using Benjamini & Hochberg's (1995) False Discovery Rate control method.

Rule 1	Rule 2	Mean difference	SE	V	P
AP	NN	92.2	3.4	24753	< 0.001
	2NN	90.5	3.5	24753	< 0.001
	GC	87.3	3.5	24753	< 0.001
	LCH	89.7	3.4	24753	< 0.001
NN	2NN	45.6	3.0	24753	< 0.001
	GC	66.8	3.2	24753	< 0.001
	LCH	55.1	3.1	24976	< 0.001
2NN	GC	59.3	3.5	24753	< 0.001
	LCH	40.1	2.9	24753	< 0.001
GC	LCH	27.8	2.5	24753	< 0.001

References

Benjamini, Y. & Hochberg, Y. 1995. Controlling the false discovery rate - a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Methodological,* 57**,** 289-300.

Viscido, S. V., Miller, M. & Wethey, D. S. 2001. The response of a selfish herd to an attack from outside the group perimeter. *Journal of Theoretical Biology,* 208**,** 315-328.