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Analysis of social swimming dynamics in the Mexican cavefish

Adam Patch[1], Alexandra Paz[3], Karla Holt[1], Erik Duboue[1,2], Johanna Kowalko[1], Alex Keene[2,3], and Yaouen Fily[1]

[1]Wilkes Honors College, Florida Atlantic University, Jupiter, FL 33458
[2]Jupiter Life Science Initiative, Florida Atlantic University, Jupiter, FL 33458
[3]Department of Biological Sciences, Florida Atlantic University, Jupiter, FL 33458

January 31, 2020

Abstract

Fish display a remarkable diversity in social behavior that ranges from highly social to largely solitary. While social behaviors are critical for survival and under stringent selection, surprisingly little is understood about how environmental pressures shape differences in collective behavior. The Mexican tetra, *Astyanax mexicanus*, is a model for studying the evolution of social behaviors. These fish exist as multiple blind cave populations in the Sierra de El Abra and extant ancestral surface fish populations that inhabit rivers and lakes throughout Mexico. Cavefish populations have converged upon many different morphological, physiological, and behavioral traits including reduced social behavior. Dynamically, groups of surface fish maintain close proximity, persistently shoaling or schooling, while their dark-cave-evolved counterparts swim independently, surveying walls and floors for food. To more carefully study evolved changes in individual and social behavior, we track free-swimming individuals in a large circular tank, varying group size and fish type. We find that group size and isolation impact swimming dynamics of cave and surface fish very differently, underscoring the mechanics of their distinct social dynamics. Highly-social surface fish respond to isolation by becoming more inactive while cavefish swimming becomes less persistent in the presence of others. In stark contrast, we find that cavefish actively evade each other upon encounter and respond to increased social density by slowing down. For abstract consideration of evasive interactions, we consider emergent effects of local evasive interactions by constructing and simulating a minimal active matter model and show how this behavior can generate group dynamics that may uniquely benefit exploration of confined environments in the absence of long-range sensory cues provided by vision. These findings reveal the convergent evolution of avoidance behavior in cavefish and support the use of *A. mexicanus* as a model for studying the evolution of social behavior.

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1 Introduction

Social behaviors differ dramatically between species as a result of their unique evolutionary history, diverse factors including predation, foraging strategy, sexual selection, and available sensory inputs such as vision, olfaction, and mechanoreception[24, 18, 13, 26]. Many fish species shoal, a collective behavior defined by a compact group from which individuals rarely stray away. Shoaling fish may also school, a more specific collective behavior defined by group-wide alignment and coordinated swimming[32, 14, 31, 16, 7]. The theoretical framework developed to study phase transitions and collective phenomena in condensed matter physics has been applied to and provided insight into the way collective animal motion emerges from the interactions between fish[44, 31]. Mathematical models based on this type of analogy, combined with motion tracking of fish groups, have been used to infer the nature of those interactions in a number of individual species [15, 14, 27, 20, 30, 5]. However, these models have rarely been applied to study the evolution of relevant interactions and group behaviors[19].

The Mexican tetra, Astyanax mexicanus, is a leading model to study behavioral evolution. These fish exist as a surface form that inhabits the rivers and lakes throughout Mexico and Southern Texas and at least 30 populations of a cavefish form in the Sierra del Abra region of Northeast Mexico[33, 17]. At least some of these cave populations have evolved from independent colonization events[17, 21], allowing for examination of the mechanisms underlying convergent evolution. All populations of A. mexicanus studied to date are interfertile, providing the opportunity to examine trait evolution in a single species with morphs that inhabit dramatically different environments [34]. Isolation in dark cave environments has resulted in the convergent evolution of numerous morphological and behavioral traits in cavefish populations. Some physical traits, including eye loss, albinism, and increased sensitivity of the mechanoreceptive lateral line, have repeatedly evolved in multiple cavefish populations and may represent adaptations to life in the absence of light [46, 43, 23, 38]. In addition, cavefish exhibit differences in behavior relative to surface fish, including sleep loss, hyperphagia, and reductions in aggression and schooling [9, 1, 29]. The ability to generate cave-surface crosses has been leveraged to identify the genetic architecture underlying many of these traits [34]. Thus, A. mexicanus offers an excellent opportunity to understand the fundamental behavioral and genetic mechanisms underlying evolution.

The loss of schooling in cavefish is thought to be linked to the reduced need for vision in the dark cave environment. Schooling is reduced in vision-deprived surface fish, suggesting visual inputs are necessary for social behavior, but it is also possible that vision-independent mechanisms contribute to the loss of schooling. For example, schooling is lost in some surface-cave hybrids with intact visual systems[29]. The relatively simple approaches previously used to distinguish between schooling and shoaling behavior provide limited ability to quantify many different evolved aspects of collective behavior. Recent developments in computer vision allow for precise and automated tracking of collective behaviors. Here, we use large scale analysis of video tracking data to finely quantify and compare the locomotor and social behaviors of three different cave populations and one surface population in groups of different sizes. This comparative approach in turn provides a basis for inferring evolved changes resulting from environmental perturbation.

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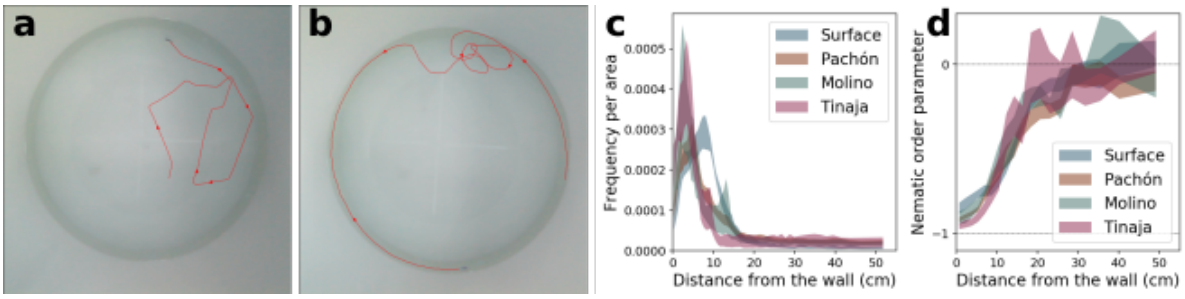

Figure 1: (a) Snapshot from a lone surface fish video. The red line with arrows shows the trajectory over the 25 seconds leading to the snapshot. (b) Similar snapshot and 25s-trajectory from a lone Pachónvideo. (c) Distribution of distance to the wall for a single fish for each population. The further the fish can be from the wall is 55.5cm corresponding to the center of the 111cm-diameter tank. (d) Average nematic alignment with the wall as a function of the distance to the wall. A nematic alignment value of -1 corresponds to perfect alignment parallel to the wall, 1 corresponds to perfect alignment perpendicular to the wall. In the center the nematic alignment goes to 0 suggesting a balanced mix of directions.

2 Results

2.1 Experimental design and boundary effects

Although surface and cave populations of A. mexicanus have been studied extensively for evolved differences in locomotion, schooling and sleep, these assays typically involved derived environments that include the use of model fish or individually housed fish [48, 29]. Many aspects of behavior in these systems may not be reflective of fish motion under natural conditions. For example, accurate assessment of locomotion behaviors, including identifying the role of the walls, requires a tank large enough that the dynamics near its center (what physicists call the bulk region) is not affected much by the walls. The assays used to study behavior in A. mexicanus likely did not possess such a region. Further, most tanks are rectangular, which biases the statistics of the fish's orientation towards the orientation of the tank. Lastly, social behavior assays often rely on a contrived social setting including the use of a transparent partition[45, 28], or a school of model fish being moved near the actual fish[29] (Wark et al model school paper sticklebacks). Despite the successes of those assays at quantifying schooling and other social behaviors, it is not always clear how their results translate to social interactions in natural settings.

Table 1: Total Trials Collected

Group Size	Surface Fish	Pachón	Tinaja	Molino
1	20	20	3	3
2	10	9	3	3
5	12	10	3	3
10	8	4	1	1

To define collective behavior in semi-natural conditions, we recorded fish behavior in

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a large circular tank (111cm-diameter, 20 fish body lengths) in which the fish are freely interacting. We recorded behavior in groups ranging from 1 to 10 of the same population. Following a 10 minute acclimation period, behavior was recorded for 20 minutes (Figure 1A). Table 1 summarizes the amount of data collected for each population and each group size.

Previous single-fish experiments in a 30 cm-diameter circular tank [39] found surface fish trajectories were spread over the entire tank whereas cave fish trajectories closely followed the walls. Both surface and cave fish were found to align parallel to the wall. To quantify those behaviors in our experiments and critically assess the size of our tank, we measured the fish density (the probability per unit area of finding a fish at a certain location), shown in Figure 1c, and the nematic alignment with the wall as a function of the distance from the wall, shown in Figure 1d. The fish density is similar to the distribution of distance to the wall measured in [39] but corrects for the fact that there is more area available near the wall than in the center. The nematic alignment with the wall is a number between -1 and 1 where -1 corresponds to perfect alignment with the wall over the entire trial, 1 corresponds to perfect alignment perpendicular to the wall, and 0 means a balanced mix of orientations. In contrast with [39], we find that both the surface fish density and the cave fish density exhibit a sharp peak near the wall. Both densities then plateau in the central part of the tank ($>$ 20cm from the walls), consistent with a bulk region. Similarly, the nematic alignment parameter shows strong alignment parallel to the wall near the wall (values close to -1 less than 10cm from the wall) then drops to near zero in the central region ($>$ 25 cm from the wall).

Despite the overall similarity between surface and cave fish in isolation, we find that cave fish prefer to be closer to the wall than surface fish. The cave fish density peaks at 4.5 cm from the wall for Pachón, 2.3 cm for Molino, and 3.4 cm for Tinaja then falls quickly, whereas the surface fish density peaks about 7.4 cm from the wall, only falling to near its bulk value about 20cm from the wall. This slow fall of the surface fish density may explain why surface fish occupied the entire tank in [39]: the entire tank was within 15cm of the wall. Finally, neither the density nor the nematic alignment show any significant difference between the three cave populations. Together, these findings suggest under isolated conditions, the swimming dynamics are largely similar across A. mexicanus populations.

2.2 Shared differences in social locomotion across cave populations

Cave populations have evolved many differences in social behavior, including reduced aggression and schooling[11, 37, 29]. To determine how social locomotion differs in A. mexicanus under semi-natural conditions we measured the behaviors of group-housed fish in our large arena setting. Figure 2a shows the speed distribution (the fraction of the time spent moving at various speeds) of a surface fish in groups of 1 to 5. Each distribution is averaged over every trial for that population and group size and represented by shading the area between the mean minus one standard error and the mean plus one standard error. All three distributions are strikingly bimodal with a sharp peak at zero speed corresponding to inactivity and a broader peak around 15cm/s corresponding to actively moving fish. The base of the zero-speed peak, located around 1cm/s, provides a natural definition of active (moving faster

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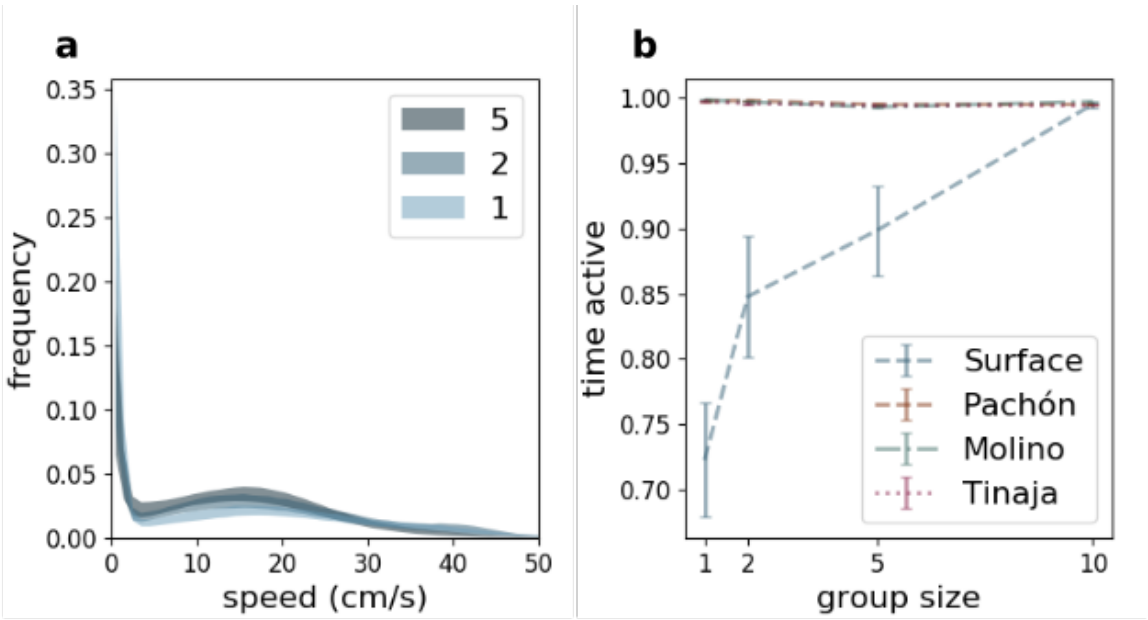

Figure 2: (a) Speed distribution of surface fish as a function of group size. A sharp peak near zero indicates fish not moving. The area under the peak is the fraction of the time the fish are not moving. (b) Fraction of time spent moving faster than 1cm/s ("active") as a function of group size. While cavefish are almost always moving, surface fish are less active with increased isolation.

than 1cm/s) vs inactive (moving slower than 1cm/s). The area under the zero-speed peak represents the fraction of time spent inactive.

Using this definition, we computed the fraction of the time spent actively moving as a function of group size for each population. The results, shown in Figure 2b, show that whereas cave fish are almost always moving regardless of group size, surface fish only do so in larger groups (10 fish), with lone surface fish moving less than 75

Before looking at other aspects of fish motion, it is important to note that the interpretation of quantities like the orientation of the fish, the speed at which they turn, or the distance between them is bound to be different depending on whether the fish are moving or not. For example, the turning speed (or angular speed) while moving translates to a turning radius, with large turning speeds corresponding to sharp turns. This in turn may be discussed in terms of following a curved wall, or avoiding an obstacle. In contrast, turning while stopped has no impact on the trajectory. It may be driven by seemingly random water movements, or by the need to collect sensory input from a different direction. Thus, the analysis of the positions and orientations of the fish should be performed separately on those frames in which a fish is moving and those in which it is not. With this in mind, we focus on frames in which each fish is moving.

To determine how group size impacts swimming speed, we quantified the distribution of speed of every population as a function of group size (Figure 3). In this context, focusing on the active frames means removing the zero-speed peak. The swimming speed of active surface fish is fairly consistent across group sizes. In other words, group size primarily

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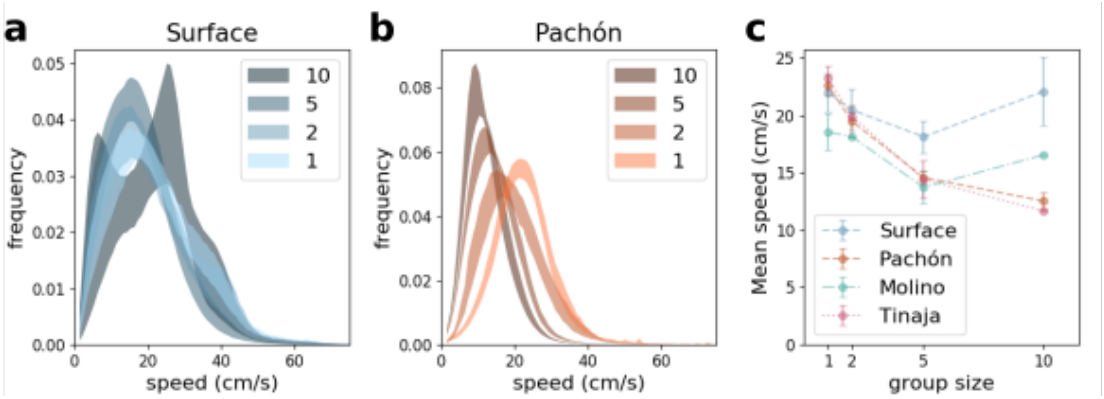

Figure 3: Speed distribution as a function of group size for (a) surface fish and (b) Pachón cave populations. Active surface fish swimming speed is multi-modal while cavefish are essentially unimodal. (c) Mean speed as a function of group size for all types shows a convergent social behavior. Cave fish swimming speed decreases with group size while surface fish maintain a constant speed.

affects how much they swim, not so much how fast they swim. Conversely, the swimming speed of cave populations decreases when the group size increases. What is more, the whole distribution shifts, suggesting the slow down is not limited to encounters with other fish but rather affects the overall behavior of the fish – both during and between encounters.

2.3 Group size affects turning dynamics in opposing ways

Let us now analyze each the fish's changes of direction. Do they swim mostly straight with occasional turns, or do they constantly change direction? Do they change direction suddenly or gradually? Much like the swimming speed we already discussed, the angular speed (the rate of turning) and its dependence on group size can tell us about the differences in collective behavior between surface and cave fish.

Figures 4a and 4c show the distribution of angular speed of groups of 1 to 10 surface (Figure 4a) or Pachón (Figure 4c) fish. The insets show the tail of the same distributions in logarithmic scale. All distributions are strongly non-Gaussian with peak at zero angular speed corresponding to straight motion and a heavy tail. Comparing the distributions of different group sizes suggests three key ranges of angular speed: mostly straight motion (< 1 rad/s), gradual turning (1-6 rad/s), and quick turning (> 6 rad/s). With those definitions, following the wall falls under straight motion (Figure 3 shows top speeds ~ 50cm/s, which in a 111cm-diameter tank corresponds to just under 1rad/s). In surface fish, increasing group size decreases both straight motion and quick turns in favor of gradual turning. Increasing group size in Pachón has the opposite effect: less gradual turning, more straight motion, more quick turning.

The balance of gradual vs quick turning is perhaps best seen in Figure 4b and 4d in which we weighted the distribution of angular speed by the angular speed itself in order to account for the fact that quicker turns can yield the same amount of turning (the same angle) in less time. The weighted distributions show that larger groups of surface fish do much of

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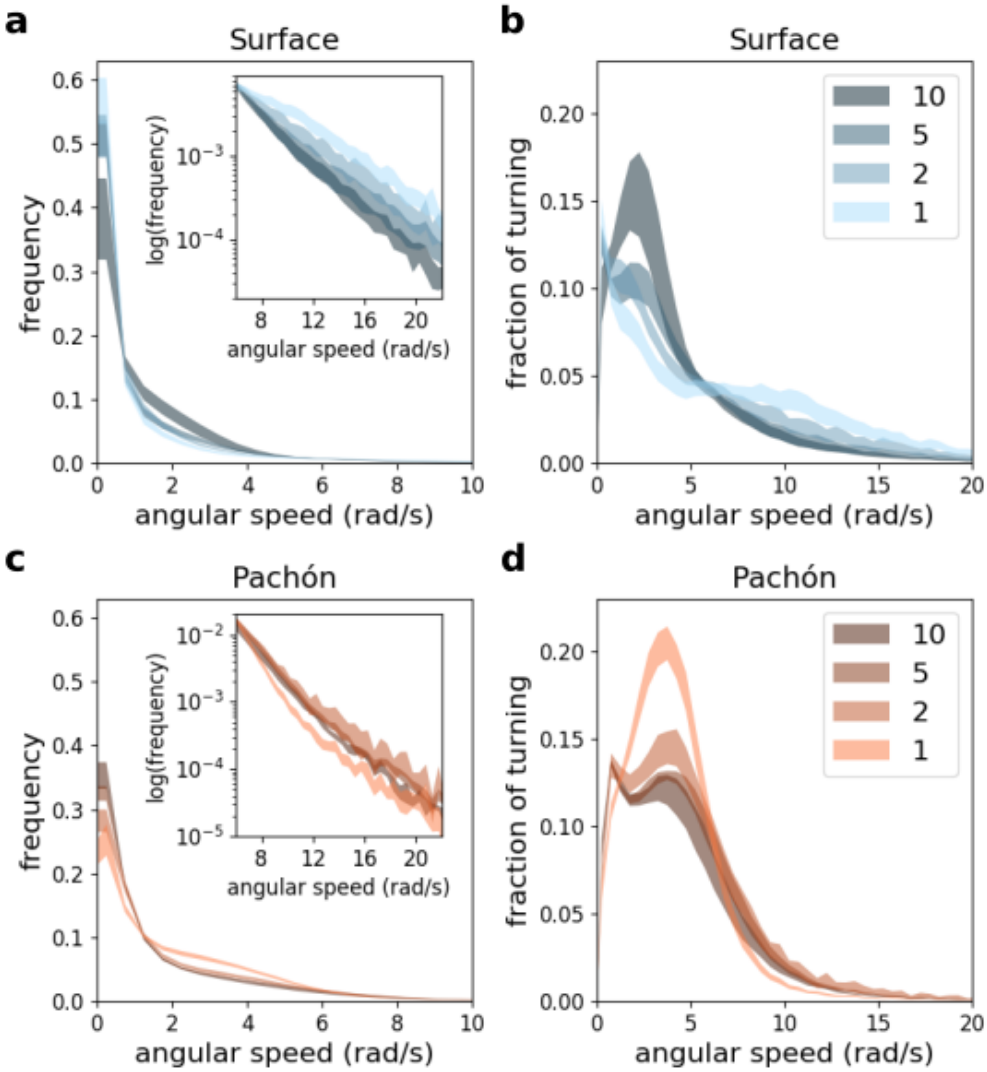

Figure 4: Turning distributions as a function of group size for (a,b) surface fish and (c,d) Pachon. Distributions of rotational speed for show non-Gaussian turning statistics in both types of fish. The reduction in peak (< 1 rad/s) for (a) surface fish signifies a reduction in persistent swimming with group size, while Pachon swim more persistently with increased group size. The tail of this distribution shows that (a-inset) surface fish turn less rapidly with increased group size while (b-inset) Pachon perform more rapid turns in groups. (b,d) The fraction of total turning by angular speed shows that (b) single surface fish make more rapid turns, but social swimming generates a new dominant mode of turning between 1-7 rad/s while (d) more rounded turning (1-7 rad/s) of single Pachon is reduced with increased group size.

their turning in the form of gradual turns. In contrast, in lone surface fish the gradual turning peak disappears in favor of an extended plateau in the quick-turning range. Direct inspection of the trajectories confirms those observations: lone surface fish tend to swim in straight bouts alternating with sharp turns whereas fish in larger groups make rounder turns.

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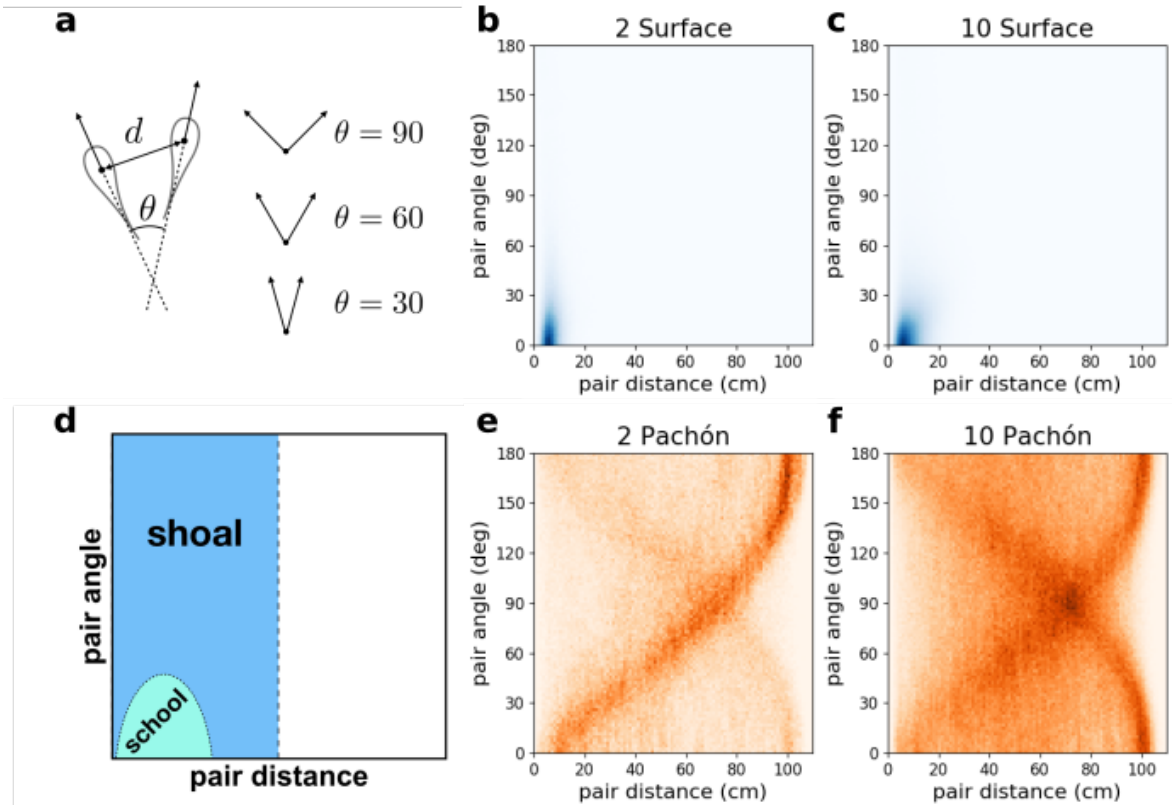

Figure 5: (a) Definition of relative distance and angle between two fish alongside reference angles. (d) Qualitative expectations for shoaling and schooling behavior as a probability density of finding two fish that are shoaling or schooling (a subset of shoaling) given a certain the pair distance (horizontal axis) and pair angle (vertical axis). (b, c) Surface fish school in tight groups of two and ten when active. The pair distance broadens as the group size increases. (e, f) Pachón do not school or shoal in groups of two or ten. Correlations in probability density arise from wall-following, where the larger group produces more swimming away from the walls.

Pachón, although their turning becomes comparatively quicker in larger groups, always turn fairly gradually when compared to lone surface fish.

2.4 From schooling to avoiding

Although we have shown A. mexicanus adapt the way they move to their social settings, we have yet to examine the way the fish move in relation to each other, specifically whether they swim close to each other and whether they swim in the same direction. To do so, we compute the joint probability of observing two fish at a distance d from each other with their headings making an angle θ with each other (see explicative sketch and probability distribution in Figure 5). For surface fish, we find a strong preference for both proximity (in two-fish groups fish are less than 10 cm apart 87% of the time and in ten-fish groups all other fish are within 20 cm of each other 92% of the time) and alignment (quantify in some

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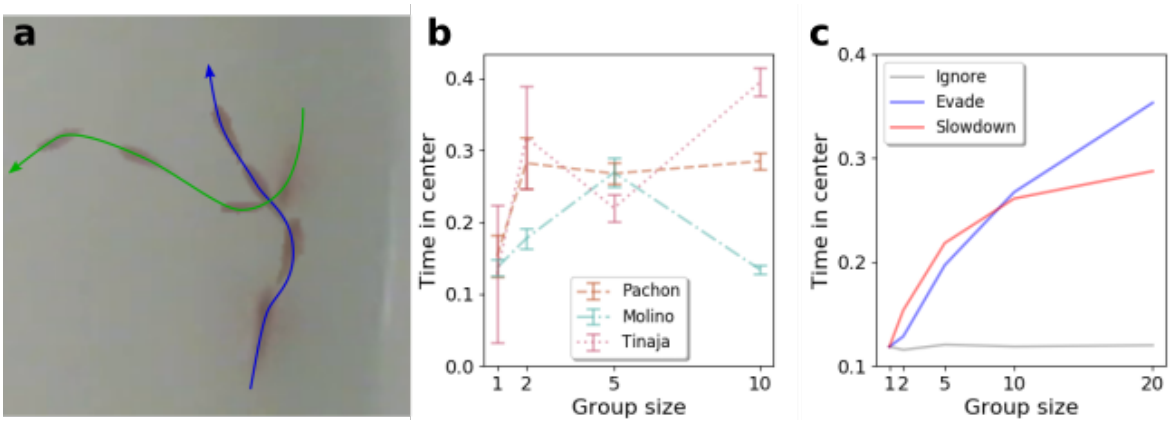

Figure 6: (a) Evasive encounter between two Pachón leading to a complete change of direction. (b) Fraction of the time spent in the center of the tank as a function of group size in experiment, and (c) in simulations. If fish ignore each other, they are unlikely to spend more time in the center of the tank, but evasive interactions and slowing down with social density result in more time away from the walls.

way, e.g., the relative angle is less than 60 degrees 91% of the time), indicative of schooling rather than mere shoaling.

Unlike that of surface fish, the joint probability distribution of relative distance and relative angle in cave fish (Figure 5) reveals neither a preference for proximity nor one for alignment. The most striking pattern it exhibits is a pair of arches due to the fish's tendency to follow the walls, as evidenced by the red dashed lines showing the relationship between the distance between two points on the circular tank's wall and the angle between their tangents. Interestingly, this pattern subsides in larger groups, suggesting the fish don't swim along the walls as much. We explore this behavior in more detail in the next section.

2.5 Cave fish avoidance promotes exploration away from the walls

The schooling metric used in the previous section suggests cave fish spend more time away from the wall when part of a larger group. To further quantify this behavior, we computed the fraction of the time spent in the central part of the tank defined as a circle with the same center as the tank and half its area. With this definition, a fish equally likely to be anywhere in the tank would spend half of its time in the central region. Figure 6b shows the fraction of the time spent in the central region as a function of group size for our three cave populations. As expected from the previous section, all three populations spend more time in the wall region (the outer half) than in the central region, and increasing group size increases the time spent in the central region.

Preference for the walls is a very generic property of persistent motion, from *E. coli* to sperm cells and abstract self-propelled particle models[8, 40, 10]. It is often associated with a large persistence length, i.e., a tendency to travel long distances without changing direction. Thus, decreased speed and increased turning both have the potential to decrease the time spent near the walls. From Figure 3b it is clear that cave fish do slow down when in larger groups. Direct observation of cave fish motion shows larger groups may also increase

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turning through avoidance events wherein two cave fish passing near each other perform a sudden wide turn such as the one shown in Figure 6a. Fish following the wall are quite likely to encounter other fish following the wall, and those avoidance events could contribute to sending them away from the walls and into the central region.

To explore those possible mechanisms, we simulated several variants of a simplified model of cave fish. All models include a short range repulsion that prevents the fish from passing through each other and a torque that aligns them with the walls. The *evade* model additionally has fish turning away from each other upon close encounter, whereas the slowdown model adjusts the swimming speed as a function of group size according to the mean speed observed in Pachón fish (Figure 3c). The details of the models are presented in Section 4.9. The mean fraction of the time each variant of the simulated cave fish spends in the central part of the tank is shown in Figure 5 along with the experimental data. We find that the *slowdown* model and the evade model both result in an increase of the time spent in the central region as the group size increases that is not present in the *ignore* model (the control model), suggesting both an overall slowdown and evasive turns may contribute to real cave fish's increased presence in the center in larger groups. On the other hand, the increases seen in the slowdown model and the evade model are too similar to allow us to conclude which mechanism contributes most to the phenomenon.

2.6 Surface fish in the dark: wall-following without shoaling or evasion

Previous work has shown that surface fish do not school or shoal in the absence of light, suggesting that vision is necessary for these collective behaviors[29]3. To determine the role of vision on the subcomponents of collective behaviors, we have run a small set of trials tracking single surface fish and groups of 10 surface fish in the dark. Our results support previous observations that surface fish do not school in the dark and that they spend more time following the wall[39]. Acclimation to the tank seems to take longer than in the light, but we find that between 10-20 minutes after being placed in the tank the fish settle into a wall following behavior.

Additionally, we find that surface fish swim about half as slow in the dark. We find mean speed for single fish and groups of ten to be 11 ± 2 cm/s and 12 ± 7 cm/s, compared to 22 ± 2 cm/s and 22 ± 3 cm/s in the light. In contrast with the straight bursts and sporadic turns we described earlier, fish swimming in dark trials swim with a more Gaussian distribution of turning speeds with standard deviation of 5 ± 1 cm/s and 12 ± 2 cm/s for single fish and groups of ten respectively whereas in the light we find a devation 3.1 ± 0.2 and 2.5 ± 0.1. Slower swimming in combination with a wider distribution of turning speeds means that surface fish swim much less persistently in the dark compared to the light.

In contrast to our finding that cavefish slow down with increased group size, we do not find a significant change in swim speed across group size in dark trials of surface fish, as reported above. The variance of turning speed is greater in dark trials of surface fish as compared with cavefish as the standard deviation of single and ten-fish Pachón are 2.97 ± 0.07rad/s and 2.7 ± 0.1 rad/s). Moreover, the variance of dark surface fish turning speed increases with group size, something we do not find to be the case for any cavefish type we tested.

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Without light, surface fish have less persistent swimming than cavefish and this swimming becomes even less persistent in groups, suggesting surface fish explore dark environments with a slower, more localized swimming style than their cave counterparts.

Qualitatively, we also saw differences in social interactions between surface fish in the dark and cavefish. We do not observe evasive maneuvers. Instead, in some encounters we observe transient bouts of following. Further analysis will allow us to quantify the nature of these interactions.

3 Discussion

In this paper, we systematically investigated collective behavior in A. mexicanus surface fish and three populations of independently evolved cavefish. To generate semi-realistic conditions that allow for modeling and statistical analysis of interactions, we used a circular tank that is comparatively much larger than those used in previous studies of locomotion and social behavior. We then applied automated tracking [41, 4] to quantify locomotion and discuss interactions between individuals. These studies identified a significant loss of collective behavior across all three populations of cavefish. In addition, the changes in multiple aspects of collective behavior are conserved across all three populations of cavefish, revealing evolutionary convergence on shared mechanisms of collective behavior.

The assay and the data analysis pipeline we developed for this project automatically screen motion data to account for temporarily inactive fish and motion tracking limitations (mainly overlapping fish) to extract meaningful information about specific modes of swimming. The data analysis pipeline can be readily adapted to suit the needs of different swimming experiments, and updates to the analysis methodology can be run on the entire data set (over 100 hours of footage) in approximately one day. The ability to quantify diverse aspects of locomotion behavior including velocity, wall proximity, angular velocity, and spatial relationships between fish represent an advance over previous analyses that quantified simple proximity to neighbors or exclusively examined locomotor activity[29, 48]. Similar forms of tracking behavior have been used in fruit flies and mice, and in conjunction with unsupervised learning approaches to identify specific subcomponents of behavior [47, 25].

Using this data-oriented approach we find new evidence that A. mexicanus cave fish have evolved behavior that enhances their exploration of confined environments while avoiding interactions with each other, in stark contrast with surface populations whose highly social behaviors, including shoaling and schooling, continue to be studied extensively. Rather than maintaining proximity with others, we find that cavefish display two forms of avoidance behavior. First, their slower swimming at higher social density decreases the frequency of their encounters with other fish. Moreover, evasive maneuvers prod wall-following cavefish to explore away from the walls. To find out whether some of those behaviors may be merely due to the lack of visual cues, we also study the motion of surface Astyanax in the absence of light. Similar to cavefish, we find that they no longer school or shoal. However, we also find that they exhibit neither of the two cavefish avoidance mechanisms we just discussed, perhaps as a way to avoid straying too far from the group in less confined environments than caves. Alongside their biological relevance, these aspects of cavefish behavior make them interesting objects of study for quantitative modeling of collective behavior, including trying

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to infer the nature of local interactions as in [27].

At an even finer level of detail, this approach to measuring fish movement has further improved our ability to compare different, independently-evolved cave populations. Previous research suggested that the three cave populations examined here all displayed reduced schooling and shoaling behavior relative to surface fish [29]. Here, we confirm that finding. While previous studies were unable to determine if cavefish social behavior is reduced or completely lost, we demonstrate that cavefish do modify their behavior in response to the presence of other fish, however they do so in a way that keeps them away from each other rather than near other. Further, previous results did not identify differences between the social behavior of cavefish from different populations[29]. Here we find important similarities and differences between cavefish from different populations. Cavefish from all three cave populations slow down as the group size increases (with the exception of groups of 10 Molino fish) and exhibit similar turning statistics, suggesting convergence on these aspects of social behavior relative to surface fish. Behavioral similarities like these may reflect something universal underlying the response to the selective pressures of cave environments.

Additionally, fish from the Molino population displayed distinct behavioral differences compared to Tinaja and Pachón cavefish. For example, the effect of group size on mean speed and time away from walls were intermediate between surface fish and Pachón and Tinaja fish. These differences could reveal differences in evolutionary history between these populations. Pachón and Tinaja fish are more closely related to one another than to Molino fish, were are derived from a different surface stock and a separate colonization event[17, 21]. Indeed, a number of traits have been identified that are intermediate in value between surface fish and Pachón cavefish in Molino cavefish[12, 35]. Thus, together, the existence of these intermediate phenotypes could be due to differences in evolutionary history between different cave populations. Alternatively, differences in habitat between the different caves could result in these behavioral differences. Further ecological work, and the connection between any ecological differences and these differences in social behavior social behavior, would answer this question.

Taken together, these findings provide a detailed analysis of collective behavior in an evolutionarily tractable system. These approaches could be extended to examine many different behaviors in this model including aggression, sleep, stress and chemosensory behaviors[11, 2, 6, 22]. Fine quantification of those behaviors in surface-cave hybrids could be used to uncover the genetic basis of those behaviors. The application of behavioral modeling and behavioral assessment under conditions that more realistic mimic the natural environment can potentially improve our understanding of trait evolution in this model.

4 Materials and Methods

This experimental assay exploits video collected from many trials of fish swimming in an unobstructed circular tank after a period of acclimation. Through analyzing the video with custom-built software, we first determine positions of fish and utilize video frame rate to calculate translational and angular velocities and accelerations of each fish. With these quantities and we then make reasonable cuts to our data based on periods of inactivity (when fish are essentially stopped) and tracking errors due to occlusions and misidentification of

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fish. In this section, we provide more detail about the stages of this analysis. Further details are referenced in Supplementary Information.

4.1 Experimental Procedure

This experimental assay exploits video collected from many trials of fish swimming in an unobstructed circular tank after a period of acclimation. Through analyzing the video with custom-built software, we first determine positions of fish and utilize video frame rate to calculate translational and angular velocities and accelerations of each fish. With these quantities and we then make reasonable cuts to our data based on periods of inactivity (when fish are essentially stopped) and tracking errors due to occlusions and misidentification of fish. In this section, we provide more detail about the stages of this analysis. Further details are referenced in Supplementary Information. 4.1 Experimental Procedure

4.1.1 Husbandry

Animal husbandry was carried out as previously described [42, 3] and all protocols were approved by the IACUC of Florida Atlantic University (protocol). Fish were housed in Florida Atlantic University core facilities at a water temperature of 23 ± 1° C in 10 or 20 gallon tanks on a 14:10 hr light-dark cycle. Light intensity was maintained between 24 and 40 lux. Experimental fish were bred in the lab from descendants of either cave fish originally collected from the
Pachon, Molino, and Tinaja caves or from two different surface populations originating from Texas or Mexico.

4.1.2 Behavioral experiments

All experimental fish were adults housed in groups of 5-10 fish for 10 gallon tanks and 10-25 fish for 20 gallon tanks. Tanks and groups were carefully chosen to avoid repeatedly assaying the same groups of fish and a minimum of two weeks was allowed before assaying fish from a previously used tank. Fish to be assayed were carried to a designated behavior room in a 2.5 L carrier tank and were then gently netted into the experimental arena and allowed to acclimate for 10 minutes.

Experiments were conducted in a round tank (111 cm diameter x 66 cm height) filled to a depth of 10 cm with water taken directly from the tank system. A video camera (Genius WideCam F100, Dongguan Gaoying Computer Products Co., Guangdong, China) was affixed to a custom-built PVC stand that allowed recording from above the center of the tank. Lighting was provided via four white 75-watt equivalent halogen light bulbs (Philips A19 Long Life Light Bulb, Amsterdam, Netherlands) mounted in clamp lights with 5.5 in shades (HDX, The Home Depot, Georgia, United States) to diffuse light. For experiments conducted in the dark, videos were collected by removing the infrared filter from within the camera, and whitelight bulbs were replaced with 940 nm Infrared bulbs (Spy Camera Specialists, Inc., New York, United States) and supplemented with four additional infrared lamps (IR30 WideAngle IR Illuminator, CMVision, Texas, United States). Videos were collected at 30 fps using OBS Studio (Open Broadcaster Software).

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4.2 Data collection

Table 2: Total Tracked Time of Trials (min)

Group Size	Surface Fish	Pachón	Tinaja	Molino
1	385	385	60	60
2	184	164	61	60
5	231	192	61	60
10	199	82	20	20

Data is collected in video files that are run from the time fish are released in the tank and run continuously for longer than 30 minutes, where a few runs have been run for shorter times. We disregard the first 10 minutes of the video to allow the fish to acclimate to the tank environment and then collect until the 30-minute mark so that each run is weighted equally. Table 1 show the total number of trials that we collected and Table 2 shows the total time collected for use in our analysis.

4.3 Tracking

In order to more directly control the intricacies of our analysis, we have developed our own Python-based software package that implements computer vision to track fish from raw video and allows us to process all stages of analysis to the final results presented in this paper. For those interested in the finer details of this code or in using our software, it is available for download on github. Trials conducted in the dark were tracked with Ethovision XT v. 13.0.1220 (Noldus Information Technology, Wageningen, Netherlands).

4.3.1 Tracking with computer vision

Fish tracking is achieved using a modified version of the tracktor library[41], built on basic functionality of the OpenCV library. For each frame in a video, this involves:

- tracing outlines of fish using local light intensity contrast
- determine position and director of fish via geometric moments of pixels from valid outlines
- identify fish by estimating proximity to location at previous frame

While this is not optimized for specifically identifying fish, we have the ability to add this functionality following Ref. [36] for future studies, and this already yields interesting results for the sake of this paper.

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4.4 Post-processing

Once we have estimated the location and direction of each fish in terms of pixels in the video, it becomes necessary to transform this into an estimate of physical space. This involves first transforming pixels to account for the camera's wide-angle lens, then locating the edges and center of the tank and using the known diameter of the tank to convert pixel distance to physical distance. After all of these steps, we can then calculate kinematic values from position and direction as a function of time.

4.5 Fish-eye transformation

Transformation of data to account for the wide-angle projection involves calibrating our camera according to the OpenCV fisheye library[4]. Using a printable pattern, we estimate values associated with the lens that will be consistent across runs since we do not change the camera. Using these values, we apply a fish-eye transformation to our data in post-processing.

4.6 Assessing tank location and wall

To ensure accuracy of tank edge location, we employ a GUI to draw a circle along the edge of the tank by allowing the user to select three points along the edge of the tank from a single random frame in a run, and then verify that a proper trace has been drawn. All data is then transformed into the reference frame of the circle center and the tank diameter is used to convert from pixels to tank frame distance.

4.7 Calculating kinematics

Kinematic quantities like velocity and acceleration can then be calculated for each fish using positions from frame to frame. For each fish at this stage, the software has collected a discrete set of positions as a function of time $\mathbf{p}_t = (x_t, y_t)$ with a time step Δt. Using these positions, we calculate two-dimensional velocity $\mathbf{v}_t = (v_{x,t}, v_{y,t})$ by

$$v_{x,t} = \frac{x_{t+1} - x_{t-1}}{2\Delta t} \tag{1}$$

$$v_{y,t} = \frac{y_{t+1} - y_{t-1}}{2\Delta t} \tag{2}$$

and similarly, we calculate two-dimensional acceleration $\mathbf{a}_t = (a_{x,t}, a_{y,t})$ by

$$a_{x,t} = \frac{v_{x,t+1} - v_{x,t-1}}{2\Delta t} \tag{3}$$

$$a_{y,t} = \frac{v_{y,t+1} - v_{y,t-1}}{2\Delta t} \tag{4}$$

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Angular kinematics are determined in this way as well, where instead of position, we consider the director angle θ to calculate the angular velocity ω and angular acceleration α.

$$\omega_{x,t} = \frac{\Delta_{\min}\big(\theta_{t+1}, \theta_{t-1}\big)}{2\Delta t} \tag{5}$$

$$\alpha_{x,t} = \frac{\omega_{t+1} - \omega_{t-1}}{2\Delta t} \tag{6}$$

where $\Delta_{\min}(\theta_2, \theta_1)$ is a function determines the minimum difference between angles for $(\theta_2 - \theta_1) \in [0, 2\pi)$, assuming that at this time-resolution, the minimum angular distance is correct one.

Note that at this stage of post-processing, director angle is verified using a combination of the velocity and the director. Since this work focuses on fish that are actively swimming, align the measured director with the velocity to account for the fact that the director calculation in the tracking software has no preferred direction and may be reversed.

4.8 Filtering

The analysis presented in this paper primarily focuses on presenting statistically meaningful histograms that should clearly show how dynamic quantities vary for different populations and group sizes as they swim in our assay. In general, we calculate standard errors by calculating variance across mean values from each trial. For each distribution we present, the thickness of the line shows the width of the standard error, as calculated for each histogram bin, where we average density-normalized histograms collected for each trial.

In order to focus on the most relevant portions of our data set, we make strategic cuts to our data to account for inactivity and tracking issues. Since our data set is large, we make these cuts to avoid fixing tracking issues at across an unreasonably large set of times. Here is a brief rationalization of these cuts.

4.8.1 Activity cuts

We consider fish to be inactive if they are swimming slower than 1 cm/s, so we remove any data corresponding to a fish swimming slower than this speed, and to account for frame-to-frame correlations in translational and angular speed, we also remove data in the two time-adjacent frames before and after the inactive frame. Furthermore, if more than half of the frames in a trial are inactive, we remove the entire trial, in accordance with Ref. []. We validate this method by comparing velocity distributions with and without cuts and find this is a smooth and optimal way to identify instances of inactivity.

4.8.2 Tracking cuts

Considerable work has been done in recent years to effectively track individuals as they move within groups. While there are many potential improvements for this experiment, we find that our simple setup allows us to gain a cursory but meaningful comparison of behavior across morphs. Our custom software allows us to track the majority of frames, but several issues remain, and we account for these by making additional straightforward cuts.

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In the case that two fish are swimming over and underneath each other or simply too close to one another, our adaptive threshold may not distinguish them as two separate individuals. This well-known issue is called *occlusion*. While our software does not attempt to separate the fish, it is capable of accurately identifying occlusion events. Once an occlusion is identified, we ignore that data from those two fish in the frames of occlusion. To further account for issues in kinematic calculations before and after these events, we exclude three time-adjacent frames for each occlusion event.

Occasionally, we also encounter issues when tracking larger groups, where errors from occlusion or misidentification propagate to otherwise easily-tracked fish. In these cases, an identified individual is traced as swimming across unrealistic distances or turning at unrealistic speeds. In order to eliminate these erroneous frames, we cut unusually high swimming and turning speeds. We cut all data for fish swimming faster than 100 cm/s or turning faster than 25 cm/s, and we additionally exclude three time-adjacent frames for each unreasonably rapid event.

4.9 Simulations

4.10 Models

Here we present a minimal model evasive active Brownian particles (ABPs) as preliminary consideration of collective effects that may result from this kind of orientational interaction. This two-dimensional, agent-based model considers particles that move according to dynamical equations,

$$\dot{\boldsymbol{r}}_i = v_0\, \hat{\boldsymbol{e}}_i + \mu\big[\boldsymbol{F}_i^{\mathrm{w}} + \sum_j \boldsymbol{F}_{ij}\big] \tag{7}$$

$$\dot{\theta}_i = \eta_i(t) + \boldsymbol{\tau}_i^{\mathrm{w}} + \boldsymbol{\tau}_{ij} \tag{8}$$

$$\tag{9}$$

where position $\boldsymbol{r}_i$ of the ith particle with constant speed v_0 along unit director $\hat{\boldsymbol{e}}$. Particles experience repulsive-only harmonic interactions with a circular wall

$$\boldsymbol{F}_i^{\mathrm{w}} = -k_{\mathrm{w}}(R_{\mathrm{t}} - |r_i|)\hat{\boldsymbol{r}}_i \tag{10}$$

whenever $R_{\mathrm{t}} - |r_i| < 0$ and is elsewhere zero. Particles additionally experience repulsive-only interaction forces with other particles according to

$$\boldsymbol{F}_{ij} = -k_{\mathrm{p}}(d_0 - |d_{ij}|)\hat{\boldsymbol{d}}_{ij} \tag{11}$$

whenever $d_0 - |d_{ij}| < 0$ and is elsewhere zero.

Unit director $\hat{\boldsymbol{e}} = (\cos\theta_i, \sin\theta_i)$ changes according to dynamical equation of its planar angle θ_i primarily according to uncorrelated Gaussian noise $\eta_i(t)$ defined with a mean of zero, $\langle\eta_i(t)\rangle = 0$ and variance D_r, $\langle\eta_i(t)\eta_j(t')\rangle = D_{\mathrm{r}}\delta(t-t')$. We model wall avoidance using a torque term

$$\tau_i^{\mathrm{w}} = -c_{\mathrm{w}}(\hat{\boldsymbol{e}}_i \cdot \hat{\boldsymbol{n}}_i)\frac{(\hat{\boldsymbol{e}}_i \times \hat{\boldsymbol{n}}_i)}{|r_i| - R_{\mathrm{t}}} \tag{12}$$

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and is zero in the cases where $\hat{\boldsymbol{e}}_i \cdot \hat{\boldsymbol{n}}_i \leq 0$ or $(|r_i| - R_\mathrm{t}) \leq 0$. In essentially the same way, we define avoidance interaction torques according to

$$\tau_i^\mathrm{w} = -c_\mathrm{w}(\hat{\boldsymbol{e}}_i \cdot \hat{\boldsymbol{d}}_{ij})\frac{(\hat{\boldsymbol{e}}_i \times \hat{\boldsymbol{d}}_{ij})}{|d_{ij}| - d_0} \tag{13}$$

and is zero in the cases where $\hat{\boldsymbol{e}}_i \cdot \hat{\boldsymbol{d}}_{ij} \leq 0$ or $(|d_{ij}| - d_0) \leq 0$.

Using this model, we simulate avoidance interactions at short range are able to turn them off by simply dropping the term τ_{ij}.

4.10.1 Simulations

We simulate our model using a standard Brownian dynamics algorithm.

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