Opponent assessment and conflict resolution through mutual motor coordination

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Abstract:

Evolutionary game theory has been the theoretical tool of choice for analysis of social interactions in general and aggressive interaction in particular. Many important game theory models of aggression are formulated based on assumptions about short timescale behavior but are tested trough measuring long timescale behavior. The resulting methodology often yields ambiguous conclusions, because very different short timescale behaviors can generate similar effects on the long timescale. Here we circumvent problems with traditional methods by developing a machine learning pipeline that measures and classifies the behavior of individual unmarked animals on a sub-second timescale. The resultant dataset allows us to directly measure activity correlations between the attacker and the defender and to identify the cumulative assessment model as an appropriate description of zebrafish, *Danio rerio*, aggression without reference to covariation between resource holding potential and fight duration. Further analysis of phase specific movement rules unexpectedly revealed display-like characteristics in an attack behavior. A measurement of differences in dynamics between pre- and post resolution attacks suggested that highly energetically costly resolution phase attacks

occur because the winner is trying to increase its relative dominance over the loser. Overall, our data pipeline and associated framework can help researchers to develop improved descriptions of strategic behavior which span the full timescale from life history decisions down to the policies used for rapid motor coordination during individual behavioral acts.

1 Introduction

Animal behavior unfolds over multiple timescales. At the longest timescale, an animal faces a series of life history decisions such as whether it should adopt a bold or a shy temperament [1, 2, 3] or whether it is best to use a rapid breeding replication strategy over a slow-growth/low vulnerability replication strategy [4]. Typically, these decisions are mechanistically specified at the genetic or epigenetic level and are well approximated as being fixed throughout an individuals life cycle. On a shorter timescale spanning hours, minutes and seconds, an animal must analyze its surroundings and choose an appropriate ethological behavior to satisfy its current needs [5]. Elementary ethological behaviors themselves also have rich internal structure. The nervous system dynamically regulates the motor output of animals at a millisecond timescale in order to implement policies for achieving specific goals [6].

All the above statements are applicable to aggression as well. For example, the personality trait boldness typically influences aggressive tendencies over a long time-scale. On the intermediate time-scale, three game theory models of assessment- the war of attrition game (WOA) [7, 8], the cumulative assessment game (CAM) [9] and the sequential assessment game (SAM)[10]- have been used to analyze rules of decision making. On the second and sub-second time scale, we have many experimental documentations of short-lasting display and attack behaviors [11, 12].

The trigger for our current investigation was the observation that while the three game theory models of assessment are formulated based on certain assumptions about short timescale behavior, they are experimentally tested using intermediate timescale measurements concerning the overall duration of certain phases of conflict [13]. In order to determine the model used for conflict resolution in a certain species, it is recommended to analyze how the measured fight times covary with resource holding potential [13, 14], most likely because direct quantitative data about low level behavior is usually unavailable.

We circumvented the traditional analysis by testing the assumptions of the three models directly by measuring short timescale behavior. Each of the

three models is based on a different set of assumptions about how individual activity levels interact to determine costs. In WOA, individual signaling or incur a cost only to the individual generating the behavior. The same is true for the SAM, but additionally, the SAM require the two opponents to match display intensity. The most general model is the CAM, where any individual act may induce a cost both on the producer as well as the target of the behavioral act.

To test the assumptions of game theory models directly, we took inspiration from several pre-existing machine learning tools to create a system which allows for tracking and identifying unmarked animals as well as automatically annotating their behaviors [15, 16, 17]. The resulting system provides the user with trajectory data containing information about velocities, accelerations and relative positions of the fighting individuals as well as an automated ethogram which identifies the behavior performed by any animals at any given moment. Our set of tools provided us with the data necessary to examine which type of assessment game was occurring at the lowest possible level.

An orthogonal utility in measuring the fine structure of fights stems from the potential to expand domains in which evolutionary game theory can be tested. It has been speculated that predator and prey interactions during escape maneuvers are best characterized as a game where the prey makes unpredictable maneuvers in order to avoid easy capture [18, 19, 20]. Similar games might unfold within the multitude of elementary interactions occurring during a fight. Analyzing games which occur on a fast time-scale might be of interest not only because it provides opportunities to expand the domain where game theory is applied, but also because payoffs obtained in low-level games will determine the payoffs of various strategies for the long time-scale assessment games in which they are embedded. A similar methodology has already shown promise in the analysis of schooling behavior [21]. In the last section of our results, we take a first step towards game-theoretical analysis of movement rules.

Finally, our analysis increases the list of zebrafish behaviors which have been characterized at the lowest possible level. In addition to non-social and schooling rules, we document four additional rules of movements which will hopefully aid in the elaboration of in silico models of zebrafish brain and behavior [22].

2 Methods

2.1 Staging of contests

We used zebrafish of the AB strain approximately 1 year of age which were grown at a 10/14h light-dark cycle and fed with standard dry food flakes. We adapted a procedure from [12] where a pair of males were removed from their home tanks and kept in visual but not olfactory isolation for a period between 24 to 48 hours. In a slight departure from the previous procedure, the fight was staged in an arena which was different from and larger than the arena used for pre-fight isolation, because we wanted to avoid the confounding influence of walls on swimming behavior which occurs too frequently in smaller arenas. The fight was staged in a uniform rectangular arena with dimensions 32-by-24-by-12 cm, slightly rounded corners and water depth of approximately 7 cm. Care was taken to ensure a lack of sharp illumination gradients in the tank so as to facilitate later tracking. Recordings began when the two animals were simultaneously poured from the isolation tank to the fight arena. A typical recording lasted for 1 hour and was continued for another hour in the rare cases where the fight appeared unresolved after 1 hours time. Video data was acquired at 20 frames per second using MATLAB standard functions.

2.2 Tracking

Code and trajectory data are available on request:
https://goo.gl/eGCp3q
Aggressive contests in zebrafish pose three challenges. First, fighting is a 3D process and the maneuvers are facilitated by deep waters, which induces appearance changes as the depth of the fish varies. Second, fish change their appearance not only due to varying depth but also due to color changes during the fight. Third, collisions are more frequent during fighting than during schooling. This motivated the use of a hybrid system where a new version of idTracker [15] (idtracker.ai, Romero-Ferrero, Bergomi et. al, in preparation) utilizing deep convolutional networks was used for tracking when the animals were not colliding, because of the greater expressive capacity of learned templates compared to the hand-engineered template of classical idTracker. When the pair of fish collided, a Gaussian mixture model was used to separate the colliding animals (as in [17]) and identity information was propagated into the collisions by using a greedy acceleration minimization principle along the trajectory with the constraint that identities of both trajectories at the start and end of the collision had to be matched with the

predictions from idTracker (see [15] for an analogous algorithm for collision resolution).

The greedy acceleration minimization was implemented step by step. At each time step, two candidate coordinates (each representing the center of mass of a fish with unknown identity) originating from the GMM algorithm needed to be identified. We considered the identities of the coordinates at the previous two time steps to be fixed and then we calculated the absolute net linear acceleration along both trajectories for the two possible identity assignments. Whichever assignment resulted in the lower total acceleration was used for final identification and the cycle was repeated again and again until end of collision. During collisions, we had an identification accuracy of 98%.

2.3 Automated behavior classification and analysis

To improve data efficiency, we used a preprocessing method which was designed to reduce translational and rotational variance. For our four vectors at each time point, we transformed them into a new coordinate system where the zero was located at the joint center of mass of the pair of fish at time $t-K$. The x axis was aligned with a vector which pointed from fish 1 towards fish 2 at time $t-K$. All coordinates of the four vectors were converted into this coordinate system. After the preprocessing, the four processed vectors were then concatenated into a single vector and passed as input to the first layer of a standard multilayer perceptron with a ReLu hidden layer activation function, a cross entropy loss function. Using this preprocessing and a fairly small amount of annotated data (small as compared to the total corpus of data analyzed), we were able to train a perceptron with two hidden layers of size 250 neurons to have a test set accuracy of 95%.

All further analysis was done using custom-written MATLAB code. The forcemap technique was adapted from [23]. In order to avoid potential influences from walls, the symmetric phase forcemaps were analyzed only when both fish were further than 5 *cm* away from the nearest wall. During the asymmetric phase, the fish spent most of their time swimming very close to the wall, but we excluded the influence of corners on turning by removing all data when fish were closer than 5 *cm* to the nearest corner.

3 Results

We began our study by staging 34 contests between adult male zebrafish (see Methods for details of contest staging). In order to analyze the fine

details of aggressive behavior in fish, we augmented our software idTracker [15] (which allows tracking and identification of unmarked animals) with a custom-written collision resolution system that enabled us to resolve the identities of the animals while they were colliding as well (see methods for details on this and all other machine learning procedures). The output of the tracking system was a time series of trajectories for both contestants (sampling rate 50 ms, 20 Hz). We annotated a small fraction of our video data to indicate when attacks were taking place. These annotations were in turn used to train a neural network which could detect the presence of attacks from trajectory data with 95% reliability. Our behavior classification system differed from similar systems [16] through the use of end-to-end deep learning on trajectory data, which allowed us to eliminate an intermediate feature engineering step. By combining several augmented and improved machine learning tools into a common pipeline, we thus created a machine learning software that automatically provided information about the movement, behavior and identity of each animal on a subsecond timescale (Figure 1).

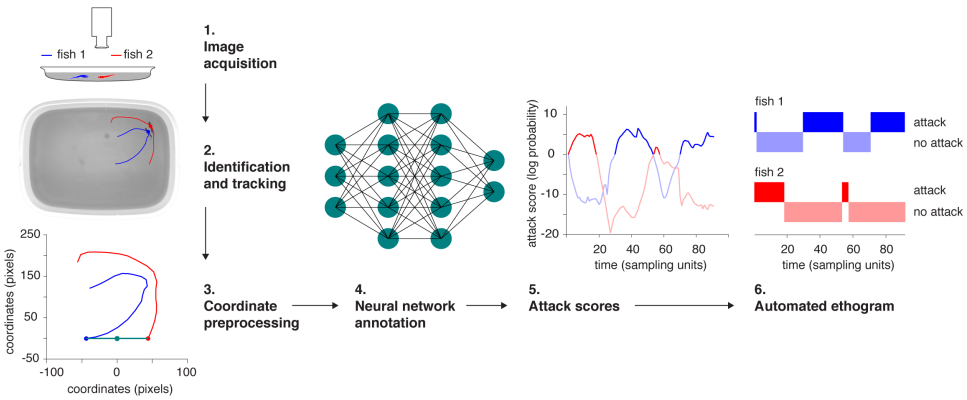

Figure 1: **The computer vision pipeline**. 1: the raw video. 2: unmarked animals after identification with idTracker [15] and a short span of the trajectory of each animal overlaid. 3: preprocessing of a local chunk of trajectory for neural network analysis. 4: schematic of the neural network classifier which was trained to mimic human annotations. 5: a time series of attack scores for two animals as produced by the neural network classifier. High attack score values indicate a high internal confidence of the network that an attack is taking place. 6: an automatic ethogram calculated by thresholding the attack score.

3.1 Analysis of activity correlations and assessment models

As a preliminary to studying activity correlations, we first sought to characterize the large-scale patterns of aggressive behavior in our dataset. We found clear signs of aggression in 27 of the 34 staged contests. When we examined the attack rates of our contestants during individual contests, there was evidence that fights consisted of two distinct types of phases (Figure 2A, see also supplementary video S1). During what we called the symmetric phase (Figure 2A, 24-28 minutes), both individuals engaged in mutual attack behavior. During the asymmetric phase, mainly one individual performed attack behaviors (Figure 2a, 34-60 minutes). When a symmetric phase was present (N=15 fights), the most common pattern (N=8 of 15 fights) was for there to be a pre-fight phase with very few attacks (Figure 2A 0-22 minutes), followed by a symmetric phase, which was in turn followed by an asymmetric phase where only one individual engaged in attacks. It is thus likely that the symmetric phase is similar to the contest phase described in many other model systems of aggression, whereas our asymmetric phase resembles the resolution phase [24, 25].

However, not all fights followed the aforementioned progression. In some cases, the symmetric phase was not followed by an asymmetric phase and in other cases, an asymmetric phase both proceeded and followed the symmetric phase. Interestingly, the individual who was dominant before the symmetric phase was not necessarily the same one who engaged in attacks after the symmetric phase (see Figure S1 for example plots of fight progression in the more rare cases). In 12 fights, no symmetric phase was present and the only phase present was the asymmetric one. In the next sections, our analysis will focus on 14 of the 15 fights were the symmetric phase was present unless we state otherwise (with 1 fight excluded from analysis since its long duration posed a threat to animal welfare and had to be prematurely stopped).

We used the outputs of our machine learning pipeline to analyze coarse kinematic parameters of attacks. Attacks in both the symmetric and asymmetric phase were associated with high velocities compared to the pre-fight phase. Pre-fight, the fish had an average speed of 5.3 ± 0.84 cm/s (N=13, mean $\pm$ standard deviation), which during the symmetric phase rose to 10.3 ± 1.9 cm/s (N=14) for the attacker and 10.9 ± 1.4 cm/s (N=14) for the defender. We note that here and elsewhere, the roles of attacker and defender were not fixed during the analysis of a fight but were calculated dynamically for each individual at each moment in time based on the outputs of our classifier. Fish swimming speed rose further during the asymmetric phase where the attacker attacked with speed 13.5 ± 1.6 cm/s (N=11)

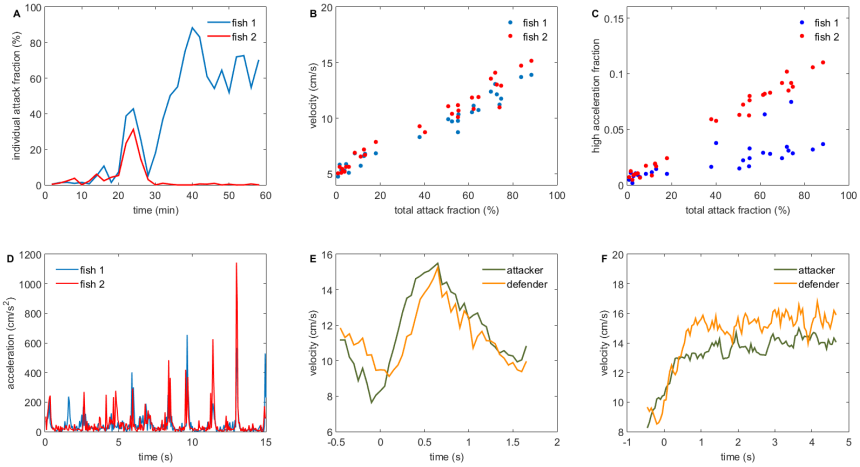

Figure 2: **A kinematic characterization of a fight**. A: The fraction of an animal's time budget used in attacks (the individual attack fraction) over the course of the fight (blue and red curves mark the two different individuals here and elsewhere). Analysis is conducted in non-overlapping 2 minute time windows. Minutes 24-28 correspond to the symmetric phase. The asymmetric phase approximately spans the time from 34-60 minutes. B: correlation between total attack fraction (sum of individual attack fractions) and velocity. C: very high intensity acceleration bouts ($a > 128\ cm/s^2$) occur mostly during attacks. D: a time series of acceleration during the symmetric phase of the fight depict the occurrence of sudden acceleration bouts. E: the velocity waveforms of the attacker (green) and defender (orange) during an average attack (N=114) in the symmetric phase. Attacks begin at time 0. F: same as before but for the asymmetric phase (N=116).

and the defender swam with velocity $14.0 \pm 1.6\ cm/s$ (N=11). As a further check of our analysis, we binned data from each fight into consecutive non-overlapping 2 minute long segments and calculated the average speed and the total percentage of time that attacks were occurring (the total attack fraction) during each time bin. There is a strong linear correlation between average movement speed and attack percentage (Figure 2B, $r = 0.90 \pm 0.06$, N=28 individuals).

Fighting is associated not only with an increase in velocity but also with the occurrence of bursts of high acceleration (Figure 2D). As with speed, there was a strong correlation between the total attack fraction and the total fraction of time each animal spent performing acceleration bursts (Figure 2C, $r = 0.88 \pm 0.15$, N=28).

From our analysis, it becomes clear that an attack induces a strong energetic cost not only for the attacker but also for the defender. This point is further reinforced if we time-align individual attacks and calculate the average velocity waveform for both the attacker and the defender during both the symmetric (Figure 2E) and the asymmetric (Figure 2F) phase. From Figure 2E,F it is apparent how attacks begin with an increase in the velocity of both the attacker and the defender. In fact, the locomotor costs for the defender are on average even higher than those for the attacker as defenders swim with a higher average speed in 20 out of the 25 conflict phases analyzed (p=0.004, two-tailed binomial test). These findings are most compatible with the assumptions of the cumulative assessment model and seem to violate the assumptions of WOA and SAM.

Next, we compared the inferences derived from our new methodology with more established methods of analysis. The first step involved finding an indicator of resource holding potential. In our dataset, size was a statistically significant indicator of resource holding potential (RHP) as the larger animal ended up as the dominant individual in 20 out of 25 fights where we could identify a clear winner (p=0.002, one-tailed binomial test, analysis includes fights both with and without a symmetric phase). In fights where a symmetric phase was present, there was a statistically significant trend for large size differences to be associated with longer fights (r=-0.47, p=0.045, one-tailed t-test for Pearson correlation coefficient). A linear regression analysis of the effects of the sizes of both contestants yielded a model where the larger individual's size had a negative effect on fight duration and the smaller individual size had a weaker but positive effect of fight duration, although the latter value was not statistically significantly different from zero ($c_{large} = -0.14$, $c_{small} = 0.04$, $p_{large} = 0.02, p_{small} = 0.52$).

A negative relationship between body mass difference and fight time is expected in all three models (WOA, CAM, SAM) [13]. A negative effect of larger individual body size on fight duration is incompatible with a WOA model of contest behavior. Our result, where the size of the larger individual has a stronger effect on fight times than the body size of the smaller individual is atypical of SAM and CAM, but as we show in a mathematical addendum, it is in principle consistent with the CAM model (see supplement, Mathematical analysis of the cumulative assessment model). Overall, the analysis of resource holding potential versus body size is consistent with our claims of a cumulative assessment model for zebrafish fights, which we posited above without reference to fight time and RHP covariance analysis.

In addition to size, another weak predictor of fight outcome was color. We found that zebrafish exhibited a transient darkening which occurred specifically during the symmetrical contest phase (see Figure S2 and supplementary

methods). On average, the symmetric fight phase was accompanied by an $8\% \pm 4\%$ (N=28) darkening of appearance in both fighters and this transient largely disappeared irrespective of weather the fight ended with asymmetric chasing or not. The eventual looser tended to darken more than the winner. In 9 out of 10 fights, the eventual looser had a higher intensity change relative to pre-fight intensity than the eventual winner (p=0.02, 2-tailed binomial test). However, color change was a weak predictor of how the fight ended, since unequal changes in color were also associated with fights that ended without a clear way to determine the winner because chasing behavior was absent.

3.2 Analysis of movement rules

Zebrafish fight maneuvers have a complicated spatio-temporal structure, which may potentially contain useful information about strategic incentives. In order to analyze this structure, we utilized the tool of forcemaps which was originally developed to study cooperative movement coordination during schooling (see Methods for details). Briefly, during each moment, we transformed our data into a coordinate system where the focal fish is located at the center and the y axis is aligned to the instantaneous velocity of the focal fish. We then measured the distribution of locations for the partner fish in this new focal fish coordinate frame. We also calculated how the location of the partner fish influenced the tendency of the focal fish to turn and to speed up. As with the analysis of speed, we separated our data into four categories depending on whether the focal fish was attacking or defending and whether the current phase of the fight was symmetric or asymmetric (in Figures 3 and 4 which follow, the colored fish on the inset images A,E always illustrates which fish is the focal fish for a given row of the figure).

Looking at the maps of the defender (Figure 3B,F), it is clear that attacks of the symmetric and the asymmetric phases induce very different histograms of relative inter-individual positioning. During the symmetric phase, the attacker is located about half a body length away from the defender and is positioned to the left or to the right of the defender rather than staying directly behind it (Figure 3B). In contrast, during asymmetric phase attacks the attacker is typically located a whole body length behind the defender (Figure 3F). In order to understand what caused these differences, we compared how individual acceleration responses to each other differed during the two phases.

We first focused our attention on the speeding maps. The speeding response of the defender to the attacker was rather similar in both phases. For both phases, we see a repulsive interaction between the attacker and the

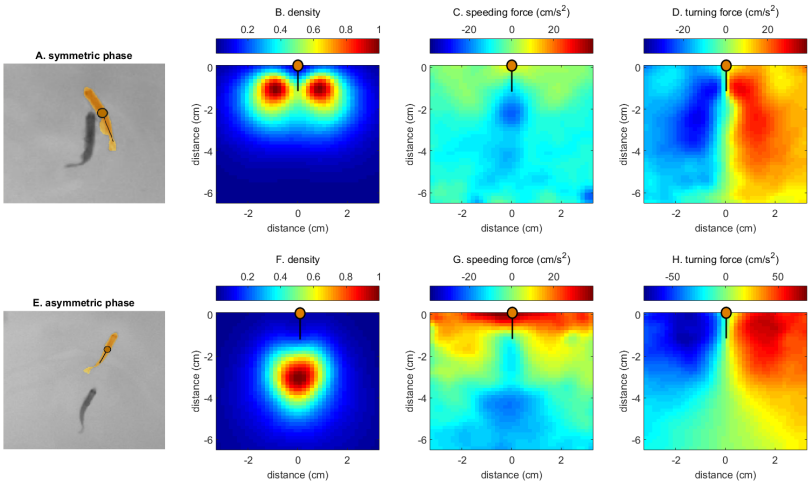

Figure 3: **Forcemaps of the defender in the symmetric (top row) and asymmetric (bottom row) phase**. Average forcemaps (N=14 fights, 230 000 time-points). The top inset image shows a typical configuration of the two fish during the symmetric phase (same for asymmetric phase at the bottom). In this figure, the focal fish is always located at the top middle of the map (orange dot, point 0,0) and its velocity vector is oriented up. A: a typical configuration of the two fish during the symmetric phase. B: a probability density map of the position of the attacker relative to the defender during the symmetric phase. C: the speeding force as a function of the relative location the attacker during the symmetric phase. D: the turning behavior of the defender as a function of the relative location of the attacker during the symmetric phase. E: a typical configuration of the two fish during the asymmetric phase. F-H: same as B-D for the asymmetric phase.

defender at close distances (Figure 3C,G; Figure 4C,G), which is apparent because at such close distances the defender attempts to speed up (Figure 3C,G yellowish-red areas at the top of the maps) while the attacker tries to slow down (Figure 4C,G, blue areas at the bottom of the maps). This response by the defender is expected as it is motivated to keep a distance between itself and the attacker in order to avoid bites. If the attacker is far away, then the defenders tendency to slow down (Figure 3C,G blue areas at the bottom) might be explained as a strategy to save some energy while the attacker is too far away to be dangerous. The response of the attacker is perhaps more puzzling, since it is naively expected to try to close the distance between itself and the target of its attacks, which should lead to a permanent pattern of acceleration (see more below). However, such a pattern is clearly

not observed. Overall, the speeding maps of both parties appear set up so as to maintain a small fixed distance between them in both phases.

Compared to the speeding maps, the turning maps of both the attacker and the defender had more qualitative phase-specific differences. If we look at the asymmetric phase turning map of the defender (Figure 3H) in the region where the attacker is most likely to be found (compare with Figure 3F), we see a zone of neutrality (a triangle-shaped zone near the bottom of the panel mostly green in color), where the tendency to turn is weakly developed. If the attacker ventures too close to the side of the defender, there is a tendency for the defender to perform strong turning maneuvers, but this tendency is absent where the attacker is most likely to be found. Overall, the speeding and turning map together explain why asymmetric phase attacks tend to look like extended periods of chasing where the fish maintain a convoy-like configuration (Figure 3E).

The aforementioned convoy-like configuration is mostly absent during symmetric phase attacks. As mentioned before, during a symmetric phase attack, the attacker is located predominantly near the sides of the defender (Figure 3A,B). Our initial impression was that this behavior occurs because the attacker makes a great effort to swim to this position so he could deliver biting attacks to the vulnerable sides of his opponent. We were surprised to see that rather than turning away from its attacker, the defender instead responded to this by exhibiting a statistical tendency to turn towards the attacker and exposing the sides of its body even further (Figure 3D). Unlike the extended chasing maneuvers of the asymmetric phase where the two fish tended to swim in a straight line, the symmetric phase attacks showed a tendency for the two fish to move in a semicircular trajectory while maintaining a T-like configuration (Figure 3A).

A further surprise comes from examining the symmetric phase turning maps for the attacker. In the symmetric phase, the attackers body is pointed directly towards the center of the defender as if poised to attack (Figure 4A,B). Yet the speeding map and the turning map both show how at close distances, the supposed target of the attack actually exerts a repulsive effect on the attacker (Figure 4C blue zone at the bottom, Figure 4D triangular zone at the bottom). This effect is not the result of physical contact forces because the maps have the same qualitative features even if we exclude from analysis the periods where the bodies of the two fish are in physical contact (see Figure S3).

We conclude that during symmetric phase attacks, at close range there is a paradoxical tendency for the defender to turn towards the attacker and for the attacker to avoid turning towards the body of the defender. Although we do occasionally observe contact and biting attacks between the contestants,

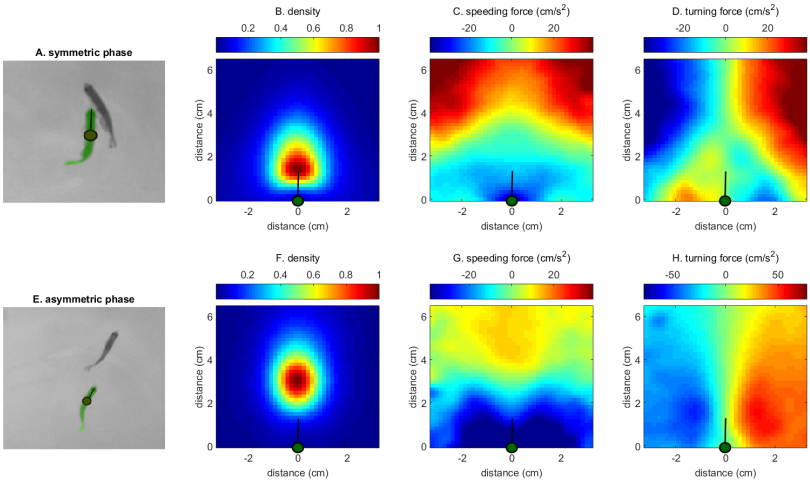

Figure 4: **Forcemaps of the attacker in the symmetric (top) and asymmetric (bottom) phase**. Average forcemaps (N=14 fights, 140 000 time-points). The focal fish for each row is marked in color on an inset image (A,E). On these forcemaps, the focal fish is always located at the bottom middle of the map (marked as a circle with a tail on the maps) and its velocity vector is oriented up. A: a typical configuration of the two fish during the symmetric phase. B: a probability density map of the position of the defender relative to the attacker during the symmetric phase. C: the speeding force as a function of the relative location the defender during the symmetric phase. D: the turning behavior of the attacker as a function of the relative location of the defender during the symmetric phase. E: a typical configuration of the two fish during the asymmetric phase. F-H: same as B-D for the asymmetric phase.

the statistical trend is towards mutual short-range avoidance. One possible interpretation is that symmetric phase attacks function not only as ways to damage the opponent but also as opportunities to engage in size and risk tolerance displays. If the predominant function of symmetric phase attacks is to enable displays, then it is more understandable why opportunities for attacks are not more efficiently exploited.

One final feature of the maps is worth noting. The maps can often be reliably calculated from data gathered only during individual fights. These individual maps have a structure that is qualitatively very similar to the plots we show in the main paper, where we pooled data across all 14 conflicts which exhibited a symmetric phase (see Figure S4,S5). These movement maps thus appear to be a very reliable feature of zebrafish aggression.

In addition to detailed analysis of attack bouts, we also found evidence for a heretofore undocumented type of aggressive maneuver which we termed the splash (Figure 5 top panels). We named the behavior splash after the characteristic wave-like ripple pattern which occurred on the water surface after the maneuver was performed. The splash behavior typically occurred as one zebrafish approached another. As the two zebrafish made contact, one or both of them responded with a sudden acceleration maneuver (Figure 5A) which resulted in the orientations of the two fish being completely reversed and the two fish being propelled apart by a distance of a few body lengths or more (Figure 5C). Typically, a 180 degree change in orientation (Figure 5D) was completed in less than 50 ms.

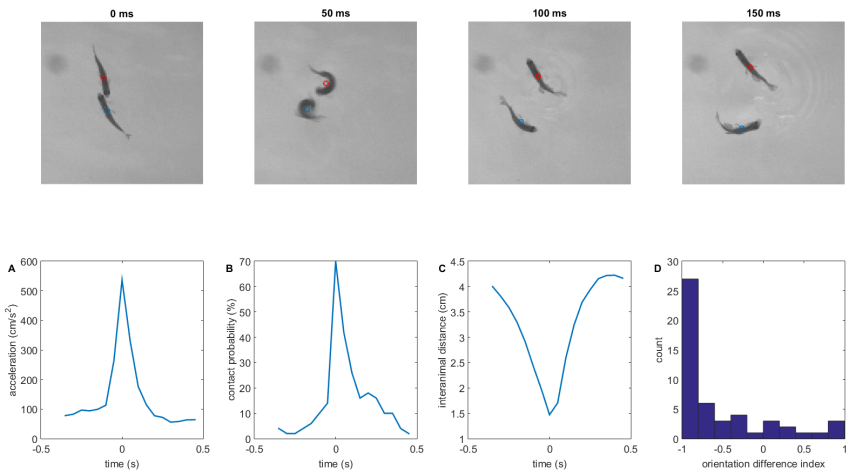

Figure 5: **The splash behavior**. Top panels: a time-lapse series of 4 consecutive frames (sampled 50 ms apart in time) during an example splash behavior. Bottom panels: A: the average acceleration of an individual during a splash. The splash takes place at time 0. B: The probability of contact during a splash. C: The average time evolution of inter-animal distance during a splash. D: A histogram of the orientation change index during 50 acts of splash. An index of -1 corresponds to a 180 degree change in orientation, an index of 1 corresponds to orientation being unchanged (see methods for details). For all plots, N=50 splash behaviors. Due to their short duration and comparatively rare occurrence, the splash behaviors were found by manual annotation.

The splash behavior may have two related potential uses. First, it may enable one fish to halt an approach as it is becoming too dangerous. In support of this, notice how the splash is usually deployed right as the attacker

is making first contact with the defender (Figure 5B). Second, the potential option to engage in the splash behavior may also mitigate the risk associated with engaging in the display-like attacks which leads the defender into a vulnerable configuration. The vulnerable configuration which occurs during the displays may be stabilized because the attacker knows that any attempt to exploit the vulnerability can be countered with a splash maneuver by the defender.

3.3 Modeling of the asymmetric phase

The literature on game theory and dyadic aggression is rich but appears to be primarily focused on the symmetrical/assessment phase of the conflict (see for example Chapter 2 of [25]). In addition to an assessment phase, our fights also have a structured post-resolution phase, where both the winner and the looser engage in stereotypical behaviors. We were motivated to seek a theoretical treatment of the post-conflict phase by the observation that both the winner and the looser engage in a contact-free but high-velocity chases, which are costly for both the winner and the looser. Why does the winner feel compelled to waste energy on attacks even after it has established its dominance in the symmetric phase?

In more natural conditions, animals including fish often fight to defend territories and establish dominance [26, 27, 24]. Post-fight behaviors either serve the function of chasing the looser out of the territory or maintaining dominance rank. In order to achieve either of the aforementioned aims without risking the possible loss of dominance, it is necessary for the winner to perform behaviors which damage the looser equally or more than the winner. We can therefore think of the post-resolution fight as a zero-sum game, where the reward of the winner is $r_w = C_l - C_w$ and the reward of the looser is $r_l = C_w - C_l$ (C_w and C_l designate the costs incurred by the looser and the winner respectively).

Zebrafish incur costs either through rapid swimming or by receiving bites from the opponent. During the post-resolution phase, the dominant engages in rapid swimming with an approximately constant velocity (Figure 2F) while staying a constant distance away from the subordinate (Figure 3F). Our analysis must explain why the dominant never accelerates enough to touch and deliver bites to the opponent, which would certainly help to selectively reduce subordinate fitness. In order to maintain a stable velocity v, the winner fish must generate a force $F(v)$ which carries a cost $C(F)$. If the looser also swims with velocity v, it will also incur a cost $C(F)$. If the winner engages in biting, it will deliver to the looser an additional cost C_b. However, it is reasonable to expect that the opportunity to deliver bites at velocity

v will not come without a cost to the attacker as well. It must produce extra force in order to generate some pressure between his own mouth and the body of the looser. In addition, extra energy may be needed for moving the jaws and potentially suffering a less streamlined posture because of the bending needed to deliver the bites. The extra force needed δF will induce a greater cost of $C(F + \delta F)$, while the looser incurs a cost of only $C(F)$.

For real fish, the functions $F(v)$ and $C(F)$ are obviously not completely generic. $F(v)$ is monotonically increasing, because higher velocities require higher forces in order to overcome increased drag. The function $C(F)$ is likely to be not only monotonically increasing, but also convex, since maintaining higher forces requires recruitment of more energetically inefficient muscle groups [28]. For convex functions, $\Delta C = C(F + \delta F) - C(F)$ is an increasing function of F as well. At equilibrium, it must be the case that the winner cannot increase his reward by switching from steady chasing at velocity v_e and force $F(v_e)$ to a biting attack at velocity v_e and force $F + \delta F$. Therefore, at equilibrium, $\Delta C(F) = C_b$. Since $\Delta C(F)$ is an increasing function of F, it is a mathematical inevitability that there exists a value of F high enough for this to condition to be true. Another solution to the model involves limits on the range of possible values of F. If the value F has a biological maximum (F_{max}) which is smaller than the value of F where biting costs become equal to attack costs, then the equilibrium value v_{eq} is given by $F(v_{eq}) = F_{max} - \delta F$. In both cases, the equilibrium involves a stable value of v_{eq}.

From our theory, we conclude that it is necessary for the looser fish to maintain the high velocity v_{eq} as well because otherwise biting attacks become profitable for the winner and the looser will further descend in relative capacity. The winner in turn must maintain a high velocity and a close distance from its opponent or else the looser may respond by slowing down since the dominant is too far away to attack. The caudal deceleration zones apparent in the speeding map of the defender in the asymmetric phase (Figure 3G) may well be a mark of such strategic responses. In summary, we believe to have shown a plausible link between game theory equilibria, well-known features of fish biomechanics/physiology and the observed long duration chasing which often concludes zebrafish fights.

4 Discussion

We have introduced a machine vision pipeline for the study of aggression in zebrafish which allows both automated identification and tracking (see methods) of unmarked animals as well as individual-level automated classification of ethologically relevant behaviors on a sub-second timescale (compare

with the lack of identification and end-to-end deep learning in [16, 17]). The tracking of unmarked animals has the experimental benefit of eliminating the marking step in the experimental procedure. If marking is no longer necessary then this also eliminates the need for control experiments to establish a reasonable similarity between the behaviors of marked and unmarked animals. Automated annotation also reduces the workload of the investigator.

Our methods also have the additional advantage of allowing for some parallelization. Though we focused here on experiments in large arenas to avoid confounding influence of walls on our analysis, it is possible to fit up to 4 smaller fight arenas into the field of view of our camera. The tracking can also be done in parallel without modifications to the code. Hence, it is feasible for certain experiments to increase the setup throughput by a factor of 4 if needed.

In our work, we have also demonstrated how the ability to gather high resolution trajectory data can be of aid in the process of deciding which of the many assessment models provides the best description of the fight. For example, we observed a strong correlation between the velocity of the attacker and the defender during individual attacks. The mutual correlation in activity levels rules out WOA models of contests as good descriptions of zebrafish aggression. We reach the conclusion because these models posit individual elementary signals to have an effect on the energy budget of only the one who is producing the signal and not on the target of the signal- a hypothesis clearly violated in our data.

The assumptions underlying the sequential assessment game also appear to be violated by our dataset. In its standard form, the sequential assessment game requires the two opponents to match their rates of signaling and individuals accrue costs only due to their own displays. In our dataset, contestants do not necessarily match signaling rates (see supplementary Figure S1 for examples). Also, individual behavioral acts induce a cost not just for the initiator of the act, but also for the other animal who needs to increase its locomotor activity levels in response. Thus, both assumptions of the sequential assessment game are violated in our data.

Having ruled out both the self-assessment and the sequential assessment models, we are left by elimination with the cumulative assessment game as the only suitable description of zebrafish fighting. We note that we have reached this conclusion without reference to resource holding potential, but rather through the process of analyzing directly the activity levels occurring during individual behavioral acts. As modern machine learning tools increase in sophistication and become more easily accessible to the casual user, our approach may become viable in other species as well. Our ability to measure instantaneous velocities and accelerations may also provide a good proxy

measure of the costs associated with fighting and further facilitate a closer connection between theory and experiment.

Analysis of trajectory level data also proved useful in clarifying the nature of certain behaviors. In the beginning we believed that the primary function of attacks in the symmetric phase was to maneuver the attacker into a position where he might be able to elicit further damage trough direct contact and biting. We were surprised to find in our forcemaps that rather than avoiding such attacks, the defender had a statistical tendency to turn its flank towards such attacks. Even more surprisingly, the attacker had a tendency to incompletely exploit the resulting vulnerable configuration as evidenced by the presence of weak repulsion zones in the turning rule of the attacker. The willingness of the defender to expose its flank may thus be at least partly a display behavior intended to signal its ability to maneuver and/or withstand damage. The potential risk of the display may be mitigated by the opportunity to engage in the splash behavior if the vulnerability were excessively exploited, since the violent maneuver of the splash is capable of rapidly breaking any dangerous configuration.

We have used the tool of movement rules, originally developed for schooling behaviors, to analyze aggression. Movement rules have been conceptualized as policies which regulate the movement decisions of agents in social contexts on a fast time-scale [23, 21]. While it has been known that the movement rules themselves can be regulated on a slower timescale by environmental or developmental processes [29, 30], we have provided evidence that the movement rules can also change very rapidly on a subsecond timescale as animals switch between non-aggressive swimming, attacks and retreats. Therefore, even though the movement rules regulate processes on a subsecond timescale, they themselves may also be regulated on a fast timescale as well. In general, we speculate that he ability to rapidly switch between different movement policies may be of key importance in contexts of strategic conflict, where evolutionarily stable strategies are often not monolithic, but consist of random mixtures of various deterministic policies.

Finally, we hope that the study of trajectory level data will open up a new frontier in he study of strategic conflict. With the ability to record high resolution data, we may be able to get a better handle on the biomechanical determinants of movement during contests. This may finally allow us to study the long-ago stated goal of examining not just how displays are used, but what factors determine the form and the fine dynamics of the displays as well [31]. Or in other words, we may eventually be able to study the movement subgames taking place within the larger assessment games. We took a small step in that direction by explaining the qualitative patterns of locomotion during the chasing phase through a game theory analysis, but there is also

a clear need for better theoretical methods to analyze the extended games which occur when acceleration decisions influence inter-individual distances over time.

Author Contributions

Designed project: MJ AL GP. Performed the experiments: MJ AL. Wrote code: AL. Analyzed the data: MJ AL GP. Obtained models: MJ AL. Contributed to the writing of the manuscript: MJ AL GP.

Conflict of interest statement

The authors declare no conflict of interest exists

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Supplementary video link

https://drive.google.com/open?id=0Bz9JP7PG5u41S1phejlyWU5Wb3M

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