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Zebrafish survival depends on escaping predators from a distance

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1

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Abstract

Predation is a fundamental interaction between animals, yet it is largely unclear how sensing and locomotion allow prey to survive encounters with predators. Using experiments and game modeling, we examined the effects of kinematic events on prey survival in interactions between zebrafish (*Danio rerio*) larvae and adult and juvenile predators of the same species. High-speed 3D kinematic measurements tracked the body position of prey and predator to determine the probability distributions of behavioral events by both fish. These measurements provided the basis for a game model that simulated the trajectories of predator and prey and well as capture events. The model was verified by replicating the measured number of strikes that prey survived before capture. We then conducted a sensitivity analysis to determine which kinematic events influence prey survival. Our results suggest that zebrafish prey may enhance survival only by expanding their response distance and that similar benefits cannot be achieved through changes in the speed or direction of an escape. Only by reducing speed below 50% of the observed mean did the model predict an effect on survivorship. Therefore, larval zebrafish escape with sufficient speed, but would benefit from responding from a greater distance. These findings suggest that the performance of predator detection is the decisive factor in the survival of fish prey. This identifies a suite of physiological characteristics that distinguish successful prey and may therefore play a major role in the evolution of prey strategy.

1. Introduction

Predator-prey interactions offer a behavioral context for understanding the biomechanics and neurophysiology of animals. It is commonly argued that the survival of evasive prey and the success of predators depend on fast or highly-maneuverable locomotion [1–5]. The rate and force of predatory strikes are similarly considered important factors [6,7], as is the ability of prey to sense an approaching predator at great distance [8,9]. However, it is not clear how these factors compare in strategic importance and it is consequently unknown what traits distinguish successful predators and prey. The aim of the present study was to determine the kinematic parameters that are most important in determining the survival of a fish when it becomes the prey of a larger fish.

Experimental approaches for understanding the dynamics of predators and prey are challenged by the interdependency of both animal's actions. Any motion by a prey may (or may not) be in response to the predator, which may (or may not) be a response to an earlier

motion by the prey. This behavioral coupling has the potential to make the actions of both animals appear as a stochastic chain of events. Multivariate statistics are generally insensitive to such dynamics, yet may succeed in resolving dominant features of successful prey [3] or predators [10]. Such analysis is enhanced by considering behavioral responses to an artificial predator or prey that is experimentally controlled [9,11,12]. An alternative approach attempts to formulate a behavioral algorithm of one animal by considering their responses to the measured kinematics of the other animal. For example, such an approach found that bat predators track evasive moths by maintaining their heading, rather than attempting to anticipate the prey's direction [13]. The present study introduces a variant on this technique by pairing an analysis of the kinematics of predator and prey with game modeling to examine the effects of kinematic parameters.

Pursuit-evasion game models consider the interactions between predator and prey by simulating how both animals move through space. This application of the classic 'homicidal chauffeur' model [14] has addressed the optimal direction of escaping fish prey [15] by assuming simplified kinematics. From this perspective, predator-prey strategy may be categorized by the speed of the prey relative to the predator [16]. The fast-predator domain is defined by a predator that moves 10-times the speed of the prey, where escape direction may matter little because the predator can overtake the prey, irrespective of its direction. When prey are faster than the predator, the slow-predator domain, a broad range of escape directions are equally effective. It is only in the intermediate domain, when the predator is slightly faster, that the prey benefits by conforming to an optimal strategy. Prey operating in all domains should benefit from initiating their escape from a greater distance. This theory offers a heuristic framework, but it is unclear how predictive it may be for biological systems. For example, these models generally assume that both predator and prey operate with perfect information and move with a constant velocity and heading in purely deterministic calculations that do not consider variation in behavior. In the interest of relaxing some of these assumptions while retaining an ability to explore strategy, we presently developed a pursuit-evasion model that incorporates measured variation in kinematic events into simulations for the motion of predator and prey.

We performed our experiments and modeling with a focus on zebrafish (*Danio rerio*). The larval stage of this species serves as a model for studying the neurophysiological [**?**,17–19] and biomechanical [20,21] basis of behavior. Predator-prey interactions may be experimentally replicated in the lab, where adults and juveniles strike at larvae with suction feeding and the larvae respond with a fast-start escape response [22], the two principle behaviors that are characteristic of a broad diversity of piscivorous interactions [3,15,23,24]. When approaching an

evasive prey, zebrafish predators will approach much more slowly than their maximum speed. This is a common phenomenon among suction-feeding fishes [23,24], perhaps because it allows greater control over the direction and timing of the strike, which is limited to a brief duration over a small range in front of the mouth [7,25]. In contrast, the high speed of the escape cause zebrafish larvae operate in the slow-predator domain and therefore could escape in a variety of directions with equal evasiveness [16]. We tested this prediction of differential game theory and additionally examined how prey survival depends on the response distance and speed of the escape. We performed experiments both with juvenile and adult predators to examine how the strategy of both predator and prey may vary with the relative size of both fish [26].

2. Material and methods

(a) Animal husbandry

All experiments were conducted on zebrafish (*Danio rerio*, Hamilton 1922), where larvae (5 – 7 days post fertilization, dpf) were preyed upon by older fish of the same species. To examine how these interactions vary with the size of the predator, we performed one set of experiments using adult (≥ 9 months old, 3.4 ± 0.5 cm, $N =$????) predators and another using juveniles ($3 - 4$ months old, 2.0 ± 0.4 cm, $N =$????), which were significantly smaller (One-way ANOVA, $N = 19$) All fish were bred from wild-type (AB line) colonies housed in a flow-through tank system (Aquatic Habitats, Apopka, FL, USA) that was maintained at $28.5\,^\circ$C on a 14:10 h light:dark cycle. To produce larvae, the fertilized eggs from randomized mating were cultured according to standard techniques [27]. Predators were motivated to feed by fasting for a period of 7 – 14 days prior to an experiment.

(b) Kinematics

We arranged the lights and cameras for high-speed recordings of both fish with high-contrast images. A hemispherical aquarium ($\oslash = 8.5$ cm) was composed of white acrylic, which served as a translucent diffuser of the IR illumination (940 nm) provided by three lamps (CM-IR200-940, CMVision, Houston, TX, USA), positioned below (Fig. 1a). These lamps provided high-intensity illumination that was invisible to the fish [28], while visible illumination at low intensity was provided by overhead fluorescent lights. Each camera (FASTCAM Mini UX50, Precision Photron Inc., San Diego, CA, USA) was fitted with a with a 55mm lens (f/2.8 Micro Nikkor AIS, Nikon

Inc., Melville, NY, USA) and positioned at a distance to permit a view of the entire aquarium. The cameras were arranged above the aquarium to allow both fish to be viewed by at least two cameras when the fish were positioned close together. The cameras were synchronized to record at 1,000 fps (at 1024 x 1024 pixels) with a common TTL end-trigger and controlled with the manufacturer's software (PhotronFASTCAM Viewer).

Predation experiments were performed by recording the swimming of one predator and one prey fish in the aquarium (Fig. 1A). This began by placing the fish on opposite sides of a partition. Following a 15 min acclimation period, we lifted the partition and observed the fish until the predator successfully ingested the prey. Using a post-trigger to the high-speed cameras, we saved recordings from $\sim 0.5\,\text{s}$ before the first predatory strike and until $\sim 0.5\,\text{s}$ after the prey was captured.

Our video recordings were used to perform measurements of 3D kinematics. We calibrated the cameras by recording a static body that we constructed with 48 landmarks of known relative position, which was placed in the center of the aquarium. A direct-linear transform (DLT) was calculated using 'Digitizing Tools' software in MATLAB (2015a, MathWorks, Natick, MA, USA) [29] from manually-selected coordinates of these landmarks from the perspective of the three cameras. Using a custom script in MATLAB, we found the body positions of predator and prey fish by selecting landmarks from two camera views and using the DLT to determine the coordinates in 3D space. We used the position of the predator's two eyes to calculate a mean position that approximated the buccal cavity (Fig. 1a). The rostrum and posterior margin of the swim bladder were found on the prey's body. The posterior of the swim bladder approximates the center of mass [30]. We acquired the landmark positions at five key events in each interaction between predator and prey: (1) when the predator initiated an approach toward the prey, when the predator (2) began and (3) completed suction feeding and (4) at the initiation and (5) completion of the prey's escape response.

(c) Descriptive statistics

Descriptive statistics were used to characterize the probability of actions by the predator and prey during predation experiments. For all interactions recorded among our experiments, we found predator-specific parameters that consisted of the strike distance (s), the distance from the prey at which a strike was initiated, and the strike duration (τ), which is the period between the opening and closing of the mouth during suction feeding. For the prey, we found the reaction distance (l), which is the distance from the predator at which the escape response is initiated. The escape

was additionally characterized by the escape angle (θ), the angular change in heading from the resting orientation to the escape path. The escape duration (η) included the period for all stages of the C-start and subsequent undulatory swimming, until the larva ceased motion. The frequency distribution for each parameter was found to be well-approximated by the following log-normal probability density function:

$$f(x) = \frac{1}{x\sigma\sqrt{2\pi}}\exp\left[-\frac{(ln(x) - \mu)^2}{2\sigma^2}\right],\tag{2.1}$$

where x is a particular behavioral parameter (s, τ, l, θ, or η), μ is the log mean, and σ is the log standard deviation. We determined best-fit values for μ and σ for each behavioral parameter by maximum-likelihood (the 'fitdist' function in MATLAB).

The probability that the strike of a zebrafish predator is successful depends critically on the distance between the mouth and the prey [22]. Strikes were therefore measured as a function of distance. These measurements revealed that the probability of a successful capture (C) was well-characterized by the following sigmoidal function:

$$C(d) = \left[1 + e^{-r(d-d_0)}\right]^{-1},\tag{2.2}$$

where d is the distance between predator and prey, d_0 is the decay distance, and r is the decay rate. The best-fit values for d_0 and r were determined by least-squares ('sqcurvefit' function in MATLAB).

All parameters for the prey and predators were compared between experiments with adult predators and juvenile predators. These parameters failed to conform to normal distributions, so we performed comparisons with non-parametric statistics. In particular, probability-density functions (Eqn. 2.1) and capture probability (Eqn. 2.2) parameters were compared using a two-sample Kolmogorov-Smirnov test (i.e. KS-test) [31].

(d) Game model

A pursuit-evasion game model was developed to simulate the conditions of our experiments. This model predicted the 2D motion of a predator (i.e. pursuer) and prey (i.e. evader) according to algorithms that were specific to the behavioral state of each of these agents (Fig. 1b). The predator's states were Tracking (T) and Striking (S) and the prey's were Resting (R) and Escaping (E). The duration of states, probability of transitioning between states, and probability of capture were determined by random-number generators with probability distributions and a range of values that matched the results of our kinematic measurements. Therefore, the model treated the

predator and prey's actions as probabilistic, but each outcome of an interaction also depended on the determinism of the kinematics of the two agents. Simulations were scripted in MATLAB to calculate the motion of both agents and their behavioral states and consequently determined the number of unsuccessful strikes before prey capture.

Each simulation began with the predator in the Tracking state, where it moved at an approach speed U with a direction that was always headed toward the prey, with perfect information about the prey's position (Fig. 1b). If the prey was motionless, then the solver would advance in time to the strike or escape initiation, whichever was found to occur first. Otherwise, the predator tracked the motion of the prey with a delay, λ, calculated with a fixed time step of $5\,\text{ms}$. The predator's transition into the Striking state occurred when the prey was within a particular distance that was determined by the strike distance (s). This value for was determined *a-priori* by the generation of a random value (using the 'random' function in MATLAB) from the log-normal probability-density function (Eqn. 2.1) for measured values of s. The capture probability for a particular strike depended on the distance between the agents in the middle of a strike, according to our measured parameter values for this relationship (Eqn. 2.2). Our simulations used this relationship to generate a random value for the range within which the prey was determined to be captured. The simulation was terminated if a strike was successful, otherwise the predator reverted to the Tracking state after completion of the strike duration. Single values for the approach speed and predator delay were used for all simulations (Table 1) and were determined by trial-and-error to best replicate the distribution of the measured number of unsuccessful strikes before prey capture. These values approximated measurements reported in prior studies [22,32].

The game model simultaneously determined the actions of prey (Fig. 1b). Prey behavior was modeled with Resting and Escaping states because larval zebrafish generally remain still between periods of rapid swimming initiated by an escape response [11,22]. The prey began each simulation in the Resting state, where it was motionless at a random distance equal to, or less than, the aquarium diameter ($\oslash = 8.5\,\text{cm}$) and at a random orientation with respect to the predator. The prey transitioned into the Escaping state when the predator moved within the reaction distance (l) after a latency (χ) [33]. During an escape, the prey was assumed to follow a straight path in the direction of the escape angle (θ). Using a frame-by-frame kinematic analysis of escape swimming for 12 larvae, we found that prey varied speed as a saw-toothed function of time that approximately attained its maximum value at 20% of the duration duration. We consequently modeled variation in swimming speed for prey to attain the maximum speed (u) was achieved at 0.2η, where η is the escape duration. The reaction distance, escape angle, and escape duration

were determined by random numbers with probability density functions matching experimental measurements. The escape angle was defined with respect to the prey's frame of reference, with $\theta = 0°$ corresponding to forward motion. The escape direction (υ) was defined as the probability that this angle was directed away from the predator, with a value (Table 1) that was previously measured [11].

This model simplified many aspects of the complexity of predator-prey interactions. It assumed that the kinematics of the two fish may be reasonably approximated with two-dimensional motion that is not bounded by an aquarium. Simulations were stopped if prey successfully escaped on 20 occasions, which reflected the observed maximum and guarded against an errant simulation of infinite duration. The model's probabilistic approach considered the effects of biomechanics and neurophysiology without articulating those elements. For example, capture success was treated as a distance-specific probability (Eqn. 2.2) that parsed neither the effects of a predator's suction-feeding hydrodynamics, nor the propulsive forces generated by an escaping prey. The number of successful escapes before capture for all experiments were compared to the same metric for 1,000 simulations. This comparison was executed by a two-sample Kolmogorov-Smirnov test, chosen over a Kruskal-Wallis test because of its emphasis on the shape of the distribution.

A sensitivity analysis evaluated the characteristics of the predator and prey behavior which had the greatest effect on prey survival. This was achieved by running batches of a 1,000 simulations with parameters varied individually between -90% and 100% of their original mean values at increments of 10%. For parameters described by a probability distribution, the log-mean μ parameter and the range of values were adjusted to create the desired percent-change in the mean of the distribution. The effect of these manipulations were assessed by comparing the measured escape probability against the model's prediction using a Kruskal-Wallis test.

3. Results

(a) Kinematics

The behavior of both predator and prey were similar whether the predators were juvenile or adult zebrafish. Prey responded with similar behavior, having indistinguishable differences in escape angle (KS-test: $P = 0.86, N = 164$) and with modest, though significant, differences in reaction distance (KS-test: $P < 0.001, N = 164$) and escape duration (KS-test: $P = 0.04, N = 153$) (Fig. 2b–c). For example, prey responded at a mean distance to juvenile predators ($\bar{l} = 8.4\,\text{mm}, N = 91$), at

about two-thirds the reaction distance to adults ($\bar{l} = 12.6$ mm, $N = 73$). Escape swimming lasted for about one-third of a second, with the response to juveniles ($\bar{\bar{\eta}} = 0.35$ s, $N = 91$) being only 50 ms longer than to adults ($\bar{\bar{\eta}} = 0.30$ s, $N = 62$). Juvenile and adult predators were significantly different in neither their strike distance (KS-test: $P = 0.08$, $\bar{s} = 7.6$ mm, $N = 154$), nor strike duration (KS-test: $P = 0.87$, $\bar{\tau} = 44$ ms, $N = 107$) (Fig. 2*d–e*). Therefore, much of the behavior of predator and prey were similar, despite the fact that the adults were nearly twice the body length of the juveniles.

The major exception in this comparison was in the effectiveness of strikes by suction feeding. Juveniles did not succeed in capturing prey beyond a distance of 3.2 mm ($N = 91$), whereas adults captured prey at a maximum distance that was 3-times greater (10.4 mm, $N = 77$). In the relationship between capture probability and distance (Eqn. 2.2), the decay distance indicates the spatial range of high capture probability. By this metric, the strike of adult predators also exhibited a range that was slightly greater than 3-times the distance of juveniles (Table 1, Fig. 2*f*). Therefore, the spatial proximity of suction feeding is the key feature that distinguishes the performance of juvenile and adult predators. We tested whether this result was due to juveniles approaching the prey with inferior accuracy. This was achieved by measuring the radial position of the prey, relative to the predator's heading, at the time when the strike was initiated. By this metric, prey positioned ahead of accurate predators would be clustered around angular values close to zero. However, we found no significant difference (KS-test: $P = 0.15$) in the probability distribution for the angular position of prey when approached by juveniles ($N = 91$) or adults ($N = 77$). Therefore, the observed differences in capture probability (Fig. 2*f*) may likely be attributed to hydrodynamic differences in the suction feeding of adults and juvenile predators.

(b) Game model

The trajectories of predator and prey fish followed paths that were qualitatively similar to that predicted by our game model. For most of the duration of our experiments, predators were observed to be swimming toward the prey (Fig. 3a). In contrast, the prey were generally motionless, unless interrupted by undulatory swimming, initiated with a fast-start. The predators and prey followed a more circuitous path toward to prey than the motion prescribed by our model (Fig. 3b). Nevertheless, the temporal sequence of events in the model offered a reasonable approximation of the kinematics of live predator-prey interactions.

The model accurately predicted the broad quantitative patterns of our experimental results. This was assessed by the probability of the prey surviving over a particular number of strikes. In

our experiments, prey exhibited the greatest probability of being captured on the first strike with monotonically lesser probabilities over subsequent strikes (Fig. 3c). Adults were more successful on the first, second and third strikes than the juveniles, which consequently exhibited a more even probability distribution. The model was successful in replicating these trends, which were found to be statistically indistinguishable for both adult (KS-test: $P = 0.93$, $N = 73$) and juvenile (KS-test: $P = 0.86$, $N = 91$) predators. Furthermore, all trends from the sensitivity analyses of the game model were similar between the juvenile and adult predators. Therefore, adult game model results are presented in this results section and juvenile predator results from the game model are presented in the supplemental material (Fig. S1).

A sensitivity analysis of prey parameters revealed that escape speed and reaction distance were the only parameters with a substantial effect on prey survival. Changes in escape duration, escape direction, and escape angle led to statistically insignificant or small changes in escape probability (Fig. 4a). In contrast, modulating reaction distance and escape speed changed the escape probability substantially. On average, changes in reaction distance and escape speed created a 23% and 15% greater change in escape probability, respectively, than changes in the other three parameters. By increasing the mean value of the reaction distance, escape probability increased significantly by upwards of 16%. However, decreasing the mean value of the reaction distance created greater, significant decreases to the escape probability, as great as 70% with a 90% decrease in the reaction distance. Increasing escape speed did not lead to a significant increase in escape probability and significant decreases to escape probability only occurred when escape speed was reduced by 50% or more of its original value.

We examined whether escape speed and reaction distance interactively affect escape probability by conducting a two-dimensional sensitivity analysis (Fig. 4b). This yielded results which were consistent with our initial analysis (Fig. 4a). For a particular escape speed, increasing reaction distance above the measured value slightly, though significantly, increased the overall escape probability. However, a decrease in reaction distance generated large decreases in escape probability (Fig. 4c). For example, at the measured escape speed value, increasing the reaction distance by 30% of its mean value increased the escape probability by 6.6%. Conversely, decreasing the reaction distance by 30% at the same escape speed generated a 18.7% decrease in escape probability. Increasing escape speed while the reaction distance was held constant did not substantially increase escape probability (< 2% increases in escape probability, on average). However, decreasing the escape speed by 70% led to a 43% change, on average, throughout all tested values of reaction distance.

4. Discussion

The present study succeeded in testing how the locomotion of prey affects their ability to survival an encounter with a predator. This was achieved through the development of a game model that successfully replicated the broad patterns of survival (Fig. 3*c*) by incorporating the measured probabilities of behavioral events in zebrafish (Fig. 2). A sensitivity analysis of the model revealed that prey survival may be improved only by expanding the distance at which an escape is initiated (Fig. 4). The observed escape speed is sufficiently rapid and the escape may be successfully executed in a variety of directions, with little effect on survival. These results were largely similar whether the predator was a juvenile or adult zebrafish. These findings offer valuable insight into the key strategic factors that govern predator-prey interactions in a diversity of fishes.

(a) Prey survival depends on response distance

Our finding on the importance of reaction distance is consistent with prior theoretical and experimental studies. The reaction distance commonly serves as a metric of the sensitivity of prey to an approaching predator in behavioral ecology studies [34]. The strategic advantage to responding at a greater distance is supported by the predictions of differential game models, particularly if both animals maintain a fixed heading [14–16]. Quite simply, a prey's ability to maintain some distance from a predator is aided by initiating an escape from further away.

Nonetheless, there are a variety of conditions where one might expect a response from a great distance to have adverse consequences. Ydenberg and Dill [35] illustrated a myriad number of ways in which escaping in response to a predator may incur costs that exceed the benefits of predator evasion. Escape responses may be energetically costly or prohibit foraging and an escape may succeed in revealing a cryptic prey that may have otherwise remained hidden from the predator [34,36]. These costs are largely removed in our experiments, where prey are provided with little alternative to predator evasion, but could have been factors in the evolution of zebrafish behavior. Responding from a great distance may even be inferior on purely strategic grounds. For example, escaping along a straight path, as we observed in zebrafish larvae (Fig. 3*a*), may indicate a destination where the prey may be intercepted by an accelerating predator. Such a possibility is neglected by differential game models that assume a fixed speed and heading [14,15]. We did not find evidence for an adverse effect of responding from a distance on the survival of prey in zebrafish (Fig. 2*f*), but it remains a possibility in other species.

The primacy of escape distance in our findings underscores the strategic importance of predator sensing. Our model is not explicit about any particular sensory modality, but our measurements of reaction distance (Fig. 2c, Table 1) are within the range possible for vision [37], olfaction [38], or flow sensing by the lateral line system. Olfactory cues probably did not play a major role in our experiments. Zebrafish are sensitive to the smell of the alarm pheromone Schreckstoff, which is released by conspecifics when the skin is damaged. However, this sensitivity is not acquired until a later stage in zebrafish growth (>48 dpf) [38] and the water in our experimental aquarium was changed between trials. It remains possible that the scent of adult zebrafish could directly trigger an escape response, or perhaps enhance the sensitivity of another sensory modality.

Escape responses are clearly elicited in larval zebrafish by a looming visual stimulus [39] and by the bow wave of flow ahead of an approaching predator [11]. Vision offers superior range, but its demands for neuronal processing require a latency of at least $200\,\text{ms}$ [40], in contrast to the responses to flow, which may be triggered in less than $10\,\text{ms}$ [41]. The lateral line system is a modality necessary for zebrafish larvae to survive encounters with adult predators [22], but its limited range fails to stimulate an escape response beyond a distance of $1.3\,\text{cm}$ [11]. Therefore, flow sensing may play a role in stimulating the responses that we measured that were in close proximity to the predator (Fig. 2c, Table 1). Beyond this range, responses were likely stimulated by the visual appearance of the predator as a looming stimulus. Therefore, our findings suggest a strategic advantage for the prey that are highly sensitive to a threatening visual stimulus.

(b) Zebrafish larvae are sufficiently fast

Consistent with previous work [22], we found that larvae escape at a speed that is many times faster than approaching predators. Although larger zebrafish certainly have the capacity to move faster than larvae, they approach prey at a relatively slow speed, often by breaking [32]. This is likely executed in the interest of controlling the timing and direction of suction-feeding with greater precision, as observed in other fishes [23,24]. In contrast, the prey execute a c-start escape response that offers the most explosively rapid behavior for which they are capable [20]. Our findings suggests that this behavior would be no more effective if executed at greater speed. The probability of surviving an encounter with a predator showed little improvement by increasing speed in our sensitivity analysis and we began to find an adverse effect when the speed was reduced by half the observed value (Fig. 4a,c). Therefore, zebrafish larvae escape with a speed that is more than sufficient to survive encounters with predators.

The speed of an escape affects the significance of escape direction [14,15]. We found that larvae moved with a maximum speed that was at least three-fold greater than the predators (Table 1), which places them in the slow-predator strategic domain that is modeled by differential game theory [16]. In this domain, a broad range of escape directions are equally effective. For example, larvae may escape at angles from zero to $70.5°$ (for adults) or $82.8°$ (for juveniles) with optimal effectiveness [16] and suffer only a modest penalty in performance for deviating slightly outside of this range. In this context, it is unsurprising that we found differences in escape angle to have little effect on prey survival (Fig. 4a, S1). If follows that escape direction should become important for predators that approach at a speed that is equal to, or faster than, the prey [16].

(c) Juvenile and adult predators are similar

Despite a nearly two-fold difference in size, the differences in strategy between experiments featuring juvenile and adult predators were subtly distinct. Larvae responded from a slightly greater distance from adults than juveniles, likely because the adults presented a threshold visual at greater distance (Fig. 2c). However, predators were similar in the kinematics of suction feeding (Fig. 2d–e) and in the direction and duration of the escape that they stimulated (Fig. 2a–b). Therefore, adults and juveniles appeared to strike with equivalent accuracy.

The greatest distinction between predators was in the spatial range at which prey were successfully captured (Fig. 2c). Adults showed an ability to capture prey at a distance that was about three-fold greater than juveniles. This difference is somewhat surprising. The flow speed generated at the mouth is proportional to the rate of buccal expansion and inversely proportional to the area of the mouth opening [25,42]. The duration of a strike is similar between juveniles and adults (Fig. 2d) and negative allometry in gape diameter and hyoid depression [43] suggest that the feeding apparatus is similar in dimensions and therefore likely to generate similar flow fields. It is therefore unresolved how adults manage to operate as more effective suction feeders than juveniles (Fig. 2f).

Summary

We found that that prey survival in zebrafish may be improved only by expanding the response distance (Fig. 4), which is an ability that depends on the sensitivity of a prey to visual cues. Fishes that swim substantially faster than their predators do not benefit from swimming even faster and survival does not depend strongly on the direction of an escape. Predators such as zebrafish may

adopt a similar strategy, yet may differ significantly in the effectiveness of suction feeding. These findings offer valuable insight into the key strategic factors that govern predator-prey interactions in a diversity of fishes.

Authors' contributions

The study was designed in collaboration between AN and MJM. AN and CN performed all experiments and kinematic analysis. The game model was created by AN, with guidance from MJM. The manuscript was written collaboratively by AN and MJM.

Competing interests

We declare we have no competing interests.

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References

1. Alexander, R. M., 2003 *Principles of Animal Locomotion*.
   Princeton, NJ.
2. Wilson, A. M., Lowe, J. C., Roskilly, K., Hudson, P. E., Golabek, K. A. & McNutt, J. W., 2013 Locomotion dynamics of hunting in wild cheetahs.
   Nature **498**, 185–189.
3. Walker, J. A., Ghalambor, C. K., Griset, O. L., McKenney, D. & Reznick, D. N., 2005 Do faster starts increase the probability of evading predators?
   Func. Ecol. **19**, 808–815.
4. Domenici, P., Blagburn, J. & Bacon, J. P., 2011 Animal escapology I: theoretical issues and emerging trends in escape trajectories.
   J. Exp. Biol. **214**, 2463–2473.
5. Howland, H. C., 1974 Optimal strategies for predator avoidance: The relative importance of speed and manoeuvrability.
   J. Theor. Biol. .
6. deVries, M. S., Murphy, E. A. K. & Patek, S., 2012 Strike mechanics of an ambush predator: the spearing mantis shrimp.
   J. Exp. Biol. **215**, 4374–4384.
7. Holzman, R. & Wainwright, P. C., 2009 How to surprise a copepod: Strike kinematics reduce hydrodynamic disturbance and increase stealth of suction-feeding fish.
   Limnol. Oceanogr. **54**, 2201–2212.
8. Dill, L., 1972 Visual mechanism determining flight distance in zebra danios (*Brachydanio-Rerio* Pisces).
   Nature **236**, 30–32.

9. Gabbiani, F., Krapp, H. G. & Laurent, G., 1999 Computation of object approach by a wide-field, motion-sensitive neuron.
J. Neurosci. **19**, 1122–1141.

10. Wainwright, P. C., Ferry-Graham, L., Waltzek, T., Carroll, A., Hulsey, C. & Grubich, J., 2001 Evaluating the use of ram and suction during prey capture by cichlid fishes.
J. Exp. Biol. **204**, 3039–3051.

11. Stewart, W. J., Nair, A., Jiang, H. & McHenry, M. J., 2014 Prey fish escape by sensing the bow wave of a predator.
J. Exp. Biol. **217**, 4328–4336.

12. Heuch, P. A., Doall, M. H. & Yen, J., 2007 Water flow around a fish mimic attracts a parasitic and deters a planktonic copepod.
J.Plank. Res. **29**, i3–i16.

13. Ghose, K., Horiuchi, T. K., Krishnaprasad, P. S. & Moss, C. F., 2006 Echolocating bats use a nearly time-optimal strategy to intercept prey.
PLoS biology **4**, e108.

14. Isaacs, R., 1965 Differential Games. A Mathematical Theory with Applications to Warfare and Pursuit, Control and Optimization.
John Wiley & Sons, Inc., New York.

15. Weihs, D. & Webb, P., 1984 Optimal avoidance and evasion tactics in predator-prey interactions.
J. Theor. Biol. **106**, 189–206.

16. Soto, A., Stewart, W. J. & McHenry, M. J., 2015 When optimal strategy matters to prey fish.
Int. Comp. Biol. **55**, 110–120.
(doi:doi:10.1093/icb/icv027).

17. Bianco, I. H. & Engert, F., 2015 Visuomotor transformations underlying hunting behavior in zebrafish.
Curr. Biol. **25**, 831–846.

18. Bagnall, M. W. & McLean, D. L., 2014 Modular organization of axial microcircuits in Zzebrafish.
Science **343**, 197–200.

19. Huang, K. H., Ahrens, M. B., Dunn, T. W. & Engert, F., 2013 Spinal projection neurons control turning behaviors in zebrafish.
Curr. Biol. **23**.

20. Müller, U. K. & van Leeuwen, J. L., 2004 Swimming of larval zebrafish: ontogeny of body waves and implications for locomotory development.
J. Exp. Biol. **207**, 853–868.

21. Li, G., Müller, U. K., van Leeuwen, J. L. & Liu, H., 2016 Fish larvae exploit edge vortices along their dorsal and ventral fin folds to propel themselves.
J. R. Soc. Interface **13**, 20160068.

22. Stewart, W. J., Cardenas, G. S. & McHenry, M. J., 2013 Zebrafish larvae evade predators by sensing water flow.
J. Exp. Biol. **216**, 388–398.

23. Higham, T. E., 2007 Feeding, fins and braking maneuvers: locomotion during prey capture in centrarchid fishes.
J. Exp. Biol **210**, 107–117.

24. Higham, T. E., Malas, B., Jayne, B. C. & Lauder, G. V., 2005 Constraints on starting and stopping: behavior compensates for reduced pectoral fin area during braking of the bluegill sunfish *Lepomis macrochirus*.
J. Exp. Biol **208**, 4735–4746.

25. Holzman, R., Collar, D. C., Day, S. W., Bishop, K. L. & Wainwright, P. C., 2008 Scaling of suction-induced flows in bluegill: morphological and kinematic predictors for the ontogeny of feeding performance.
J. Exp. Biol. **211**, 2658–2668.

26. Fuiman, L. A., 1994 The interplay of ontogeny and scaling in the interactions of fish larvae and their predators.
J. Fish. Biol. **45**, 55–79.

27. Westerfield, M., 1995 *The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish, (Brachydanio rerio).*
Eugene, OR: University of Oregon Press.

28. Robinson, J., Schmitt, E. A., Hárosi, F. I., Reece, R. J. & Dowling, J. E., 1993 Zebrafish ultraviolet visual pigment: absorption spectrum, sequence, and localization.
Proc. Natl. Acad. Sci. U.S.A. **90**, 6009–6012.

29. Hedrick, T. L., 2008 Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems.
Bioinspir. Biomim. **3**, 034001.

30. Stewart, W. J. & McHenry, M. J., 2010 Sensing the strike of a predator fish depends on the specific gravity of a prey fish.
J. Exp. Biol. **213**, 3769–3777.

31. Massey, F. J., Jr., 1951 The Kolmogorov-Smirnov Test for Goodness of Fit.
J. Am. Stat. Assoc. **46**, 68–78.

32. McHenry, M. J. & Lauder, G. V., 2005 The mechanical scaling of coasting in zebrafish (*Danio rerio*).
J. Exp. Biol. **208**, 2289–2301.

33. Nair, A., Azatian, G. & McHenry, M. J., 2015 The kinematics of directional control in the fast start of zebrafish larvae.
J. Exp. Biol. **218**, 3996–4004.

34. Cooper, W. E. & T, B. D., 2015 *Escaping From Predators*.
Cambridge: Cambridge Univ. Press.

35. Ydenberg, R. C. & Dill, L. M., 1986 The economics of fleeing from predators.
Adv. Stud. Behav. **16**, 229–249.

36. Broom, M. & Ruxton, G. D., 2005 You can run—or you can hide: optimal strategies for cryptic prey against pursuit predators.
Behav. Ecol. **16**, 534–540.

37. Dunn, T. W., Gebhardt, C., Naumann, E. A., Riegler, C., Ahrens, M. B., Engert, F. & Del Bene, F., 2016 Neural Circuits Underlying Visually Evoked Escapes in Larval Zebrafish.
Neuron **89**, 613–628.

38. Waldman, B., 1982 Quantitative and developmental analyses of the alarm reaction in the zebra danio, *Brachydanio rerio*.
Copeia **1982**, 1–9.

39. Bianco, I. H., Kampff, A. R. & Engert, F., 2011 Prey capture behavior evoked by simple visual stimuli in larval zebrafish.
Front. Syst. Neurosci. **5**, 101.

40. Burgess, H. A. & Granato, M., 2007 Modulation of locomotor activity in larval zebrafish during light adaptation.
J. Exp. Biol. **210**, 2526–2539.

41. Liu, K. & Fetcho, J. R., 1999 Laser ablations reveal functional relationships of segmental hindbrain neurons in zebrafish.
Neuron **23**, 325–335.

42. Muller, M., Osse, J. & Verhagen, J., 1982 A Quantitative Hydrodynamical Model of Suction Feeding in Fish.
J. Theor. Biol. **95**, 49–79.

43. Hernandez, L. P., 2000 Intraspecific scaling of feeding mechanics in an ontogenetic series of zebrafish, Danio rerio.
J. Exp. Biol **203**, 3033–3043.

**Table 1.** Behavioral parameters and probability distributions

| Variable	State	Adult predator	Juvenile predator
Predator			
Approach speed, U (m s^{-1})	T	$U = 0.13$	$U = 0.05$
Predator delay, λ (ms)	T	$\lambda = 10$	$\lambda = 10$
Strike distance, s (m)	T $\rightarrow$ S	μ_d = -4.980, σ_d = 0.448 (N = 51)	μ_d = -5.100, σ_d = 0.648 (N = 103)
Strike duration, τ (s)	S	μ_τ = -3.166, σ_τ = 0.331 (N = 53)	μ_τ = -3.208, σ_τ = 0.399 (N = 54)
Capture probability, C	S	$r = 0.573$, $d_0 = 5.20$ (N = 77)	$r = 1.99$, $d_0 = 1.60$ (N = 91)
Prey			
Reaction distance, l (m)	R $\rightarrow$ E	μ_l = -4.546, σ_l = 0.587 (N = 73)	μ_l = -4.941, σ_l = 0.582 (N = 91)
Escape angle, θ (rad)	E	μ_θ = 0.144, σ_θ = 0.449 (N = 206)	μ_θ = 0.144, σ_θ = 0.449 (N = 206)
Escape duration, η (s)	E	μ_η = -1.369, σ_η = 0.552 (N = 62)	μ_η = -1.167, σ_η = 0.5234 (N = 91)
Escape direction, υ	E	$\upsilon = 0.696$ (N = 206)	$\upsilon = 0.696$ (N = 206)
Escape latency, χ (ms)	E	$\chi = 8$ (N = 15)	$\chi = 8$ (N = 15)
Escape speed, u (m s^{-1})	E	$u = 0.4$ (N = 12)	$u = 0.4$ (N = 12)

T, Tracking; S, Striking; R, Resting; E, Escaping; μ, log mean; σ, log standard deviation; r, decay rate (mm^{-1}); d_0, decay distance (mm).
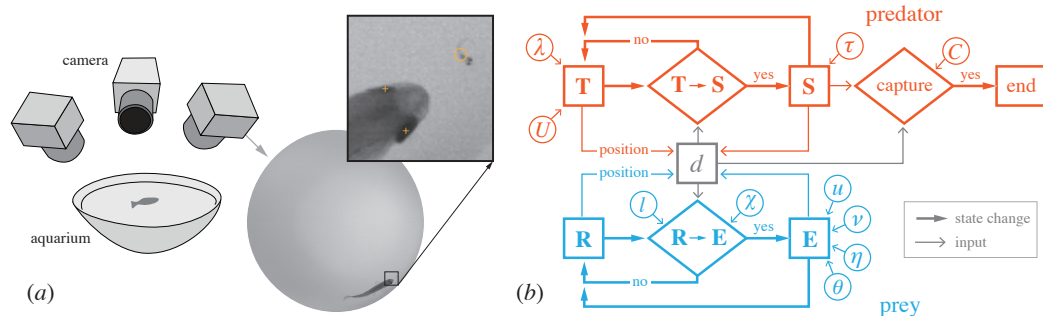
Figure 1. Experimental and mathematical techniques for studying predator-prey interactions. (*a*) Three high-speed video cameras recorded video of one larval prey and one adult predator fish that were placed in a hemispherical aquarium. A representative video frame (cropped to the margin of the aquarium) shows an adult in close proximity to the prey. In the inset, white triangles denote the locations of morphological landmarks used to describe the position of the two fish. (*b*) A flow chart illustrates the major components of the game model used to simulate the interactions between predators and prey (see Table 1 for symbol definitions and parameter values).
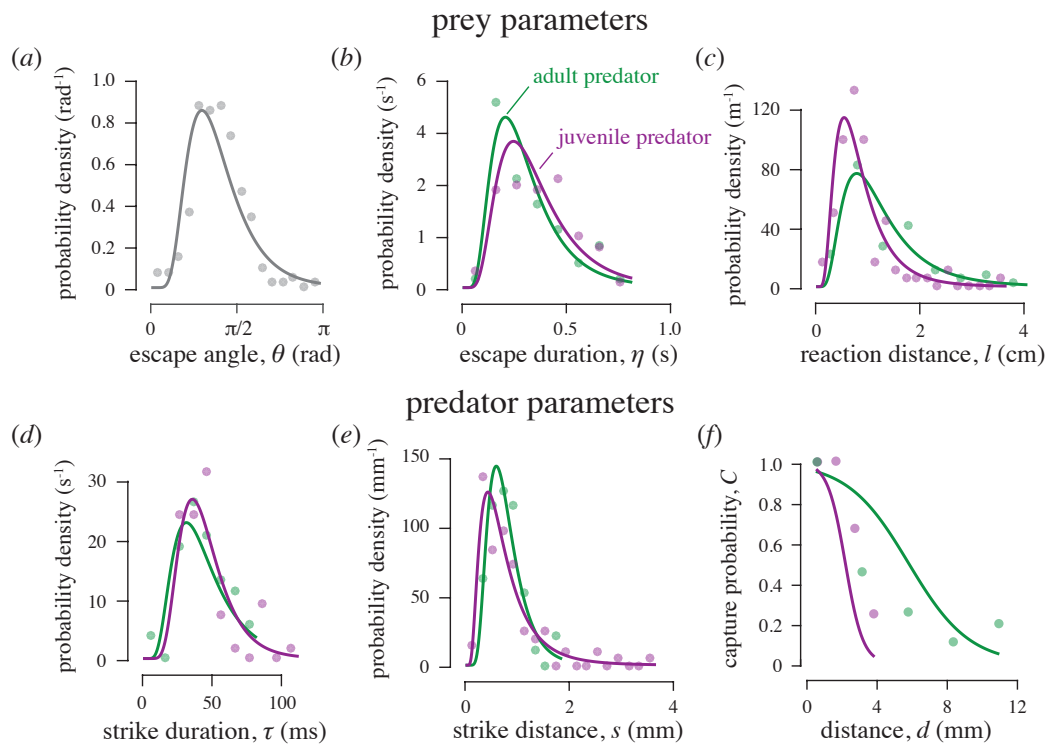
Figure 2. Descriptive statistics of swimming kinematics. (*a-e*) The probability density measurements (circles) and function (Eqn. 2.1) fit for experiments where the predator was a juvenile (purple) or adult (green) predator, which were significantly different, except for escape angle (*a*), shown in gray. Parameters were measured from the kinematics of prey (*a-c*) and predators (*d-e*). (*f*) The capture probability was examined as it varies with distance between the predator and prey (Eqn. 2.2).
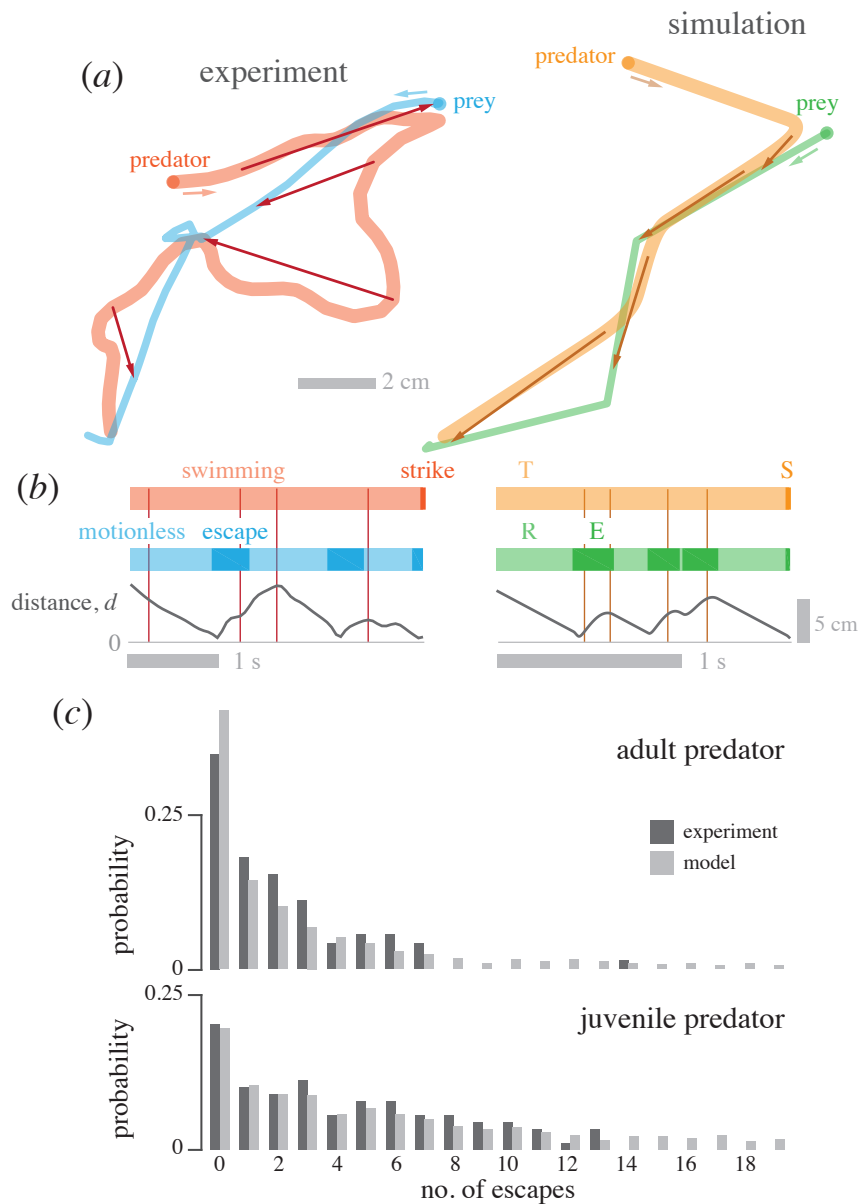
Figure 3. Comparison between experimental measurements and mathematical modeling. (*a*) Trajectories of predator and prey from a representative experiment (left) and simulation (right). (*b*) Ethograms for these trajectories illustrate the temporal changes in the predator's swimming and strike (left), which are respectively modeled by the T and S (Fig. 1*b*) modes (right). The prey's behavior while motionless and during escape (left), where modeled with the R and E modes of the model (right). For both ethograms, the distance between predator and prey are shown and particular moments in the trajectories are highlighted with vertical gray lines. (*c*) The probability that a prey survives over a particular number of strikes is shown for when the predator was and adult (above) and juvenile (below) for experiments and model simulations.
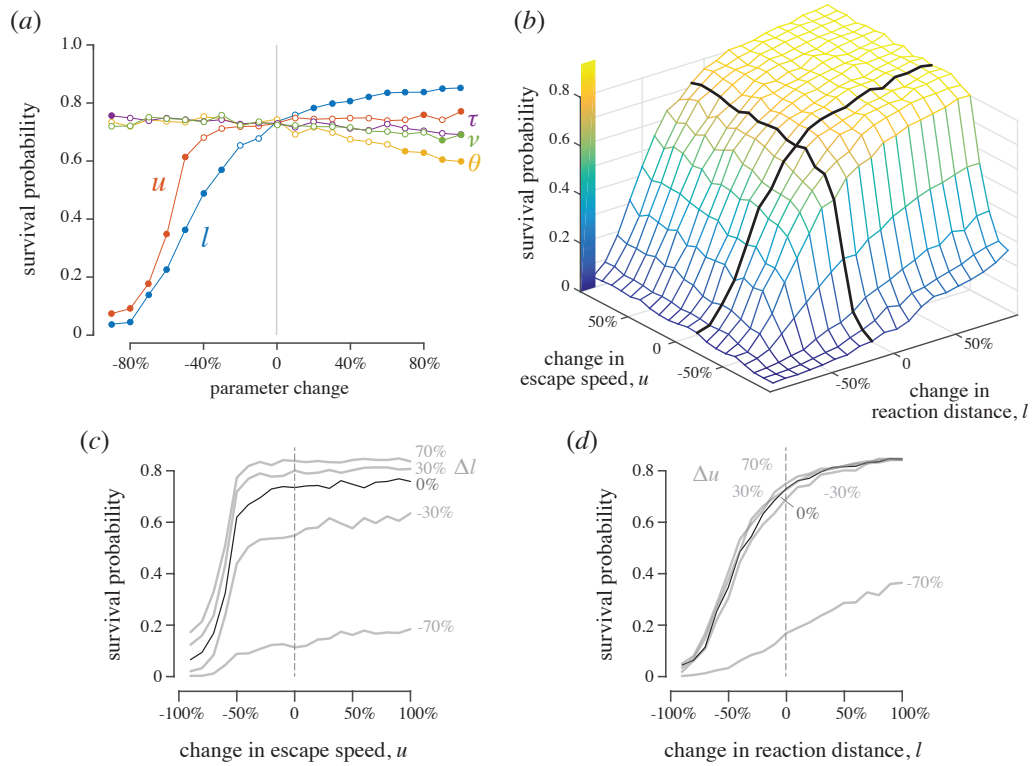
Figure 4. Sensitivity analysis of game model to examine the effects of parameter variation on escape probability. (*a*) Varying the the mean of the distribution by manipulating the log-mean value (see Table 1 for parameter definitions and values), with each point representing the result of 1000 simulations. (*b*) Variation in escape probability was examined with respect to both escape speed and response distance the same simulation results are shown with respect to changes in escape speed (*c*) and response distance (*d*).