

Article submitted to journal

Subject Areas:

Animal behavior, biomechanics

Keywords:

pursuit-evasion model, locomotion, predation, sensing, strategy

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A faster escape does not enhance survival: experiments and modeling of prey strategy in zebrafish larvae

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Abstract

An escape response is a rapid maneuver that an animal executes to evade predators. It is commonly argued, but rarely tested, that this behavior may enhance an animal's survival if performed at greater speed or in a more strategically-favorable direction. We tested the relationship between locomotor performance and survival in zebrafish (*Danio rerio*) larvae during encounters with predators (adults and juveniles) of the same species. High-speed 3D kinematics were used to track the body position of prey and predator and to determine the probability of behavioral actions by both fish. These measurements provided the basis for a probabilistic pursuit-evasion model that simulated the trajectories of the animals, which we verified against measurements of the number of strikes survived by prey. Contrary to expectation, a parameter analysis of this model found that survival was not improved by increasing the speed or altering the direction of the escape. Rather, the locomotion of zebrafish larvae operates with sufficient performance due to the relatively slow approach and limited range of the suction feeding employed by fish predators. We did find that prey survive better when responding from a greater distance, which is an ability that depends on the capacity of the visual and lateral line systems to detect a looming threat. Therefore, performance in sensing, and not locomotion, is decisive for improving the survival of fish prey. These results may be applicable to the evolution of predator-prey strategy in a broad diversity of animals.

1. Introduction

An escape response allows prey to evade predators with fast locomotion [1]. Because of its potential to directly affect survivorship, natural selection may favor animals that can execute an escape response with high locomotor performance. Indeed, the physiology and mechanics of locomotion features many traits that are likely adaptations for rapid motion. Escape responses are controlled by large-diameter command neurons (e.g. the giant axon of squid [2]), which often recruit specialized muscles (e.g. the axial musculature of fish [3]), which sometimes animate an appendage that functions only during an escape (e.g. the uropods of crayfish [4]). Prey may direct this escape in an optimal direction [5], or may alternatively benefit from heading in an unpredictable [6] or variable [7] direction. However, it does not necessarily follow that any improvement in speed or variation in heading will have a positive effect on survivorship. Fish predators commonly approach their prey at a relatively slow speed [8,9] and this could permit an escape by prey exhibiting speed and directionality that is sufficiently

33 evasive, but well below physiologically-maximal performance. The aim of the present study
34 was to test whether improvements in locomotor performance affect prey survival by examining
35 predator-prey interactions in zebrafish.

36 We addressed this aim with a novel approach that combines experimentation with an
37 application of pursuit-evasion modelling. Our methodology was developed to meet the
38 challenges to understanding the coupled dynamics of predators and prey. This coupling emerges
39 because motion by the prey may (or may not) be in response to the predator, which may (or may
40 not) be a response to prior motion by the prey. Regression analyses are generally insensitive to
41 such interdependency, yet may succeed in resolving dominant features of successful prey [10] or
42 predators [11]. It is additionally helpful to study behavioral responses to an artificial predator or
43 prey that is experimentally controlled and therefore not coupled [11–15]. An alternative approach
44 attempts to formulate a behavioral algorithm of one animal by considering their responses to
45 the measured kinematics of the other. For example, this technique revealed that predatory bats
46 track evasive moths by maintaining their heading, rather than attempting to anticipate the prey's
47 direction [16].

48 The present study also included measurements of predator-prey kinematics, but these
49 provided a basis for modeling the behavior of both the predator and prey. Our model predicted
50 the trajectories of both animals such that the probability of behavioral actions matched our
51 observations when conducted over number simulations. This served as a probabilistic, agent-
52 based model with the payout being the number of strikes that the prey survived before
53 capture. The advantage of an experimentally-validated model is that it allows for an predictive
54 consideration of the effects of differences in behavior on prey survival.

55 We performed our study on zebrafish (*Danio rerio*). The larval stage of this species serves as a
56 model for studying the neurophysiological [17–19] and biomechanical [20,21] basis of behavior.
57 Predator-prey interactions may be experimentally replicated in the lab, where adults and juveniles
58 strike at larvae with suction feeding and the larvae respond with a fast-start escape response
59 [22]. These are the two principle behaviors that characterize a broad diversity of piscivorous
60 interactions [5,10]. When approaching an evasive prey, zebrafish predators approach much more
61 slowly than their maximum speed [22], which is common among suction-feeding fishes [8,9]. A
62 slow approach presumably allows greater control over the direction and timing of the suction
63 feeding, which is limited to a brief duration over a small region in front of the mouth [23,24].
64 The prey, by contrast, respond with an explosive escape response with speed that exceeds that of
65 the predator. As suggested by prior experiments [25] and pursuit-evasion models [5], the relative

66 speed of predator and prey greatly determines strategy. We, therefore, performed experiments
67 with juvenile and adult predators with a nearly two-fold difference in body length.

68 2. Material and methods

69 (a) Animal husbandry

70 All experiments were conducted on zebrafish (*Danio rerio*, Hamilton 1922) with larvae (5–7 days
71 post fertilization, dpf) that were preyed upon by older fish of the same species. To examine how
72 these interactions vary with the size of the predator, we performed one set of experiments using
73 adults (≥ 9 months old, Mean \pm 1 SD = 3.4 ± 0.5 cm, $N = 19$) and another using juvenile predators
74 (3–4 months old, 2.0 ± 0.4 cm, $N = 19$). All fish were bred from wild-type (AB line) colonies
75 housed in a flow-through tank system (Aquatic Habitats, Apopka, FL, USA) that was maintained
76 at 28.5°C on a 14:10 h light:dark cycle. To produce larvae, the fertilized eggs from randomized
77 mating were cultured according to standard techniques [26]. Predators were motivated to feed by
78 fasting for a period of 7–14 days prior to an experiment.

79 (b) Kinematics

80 We arranged the lights and cameras for high-speed recordings of both fish with high-contrast
81 images. A hemispherical aquarium ($\varnothing = 8.5$ cm) was composed of white acrylic, which served as
82 a translucent diffuser of the IR illumination (940 nm) provided by three lamps (CM-IR200-940,
83 CMVision, Houston, TX, USA), positioned below (Fig. 1a). These lamps provided high-intensity
84 illumination that was invisible to the fish [27], while visible illumination at low intensity was
85 provided by overhead fluorescent lights. Each camera (FASTCAM Mini UX50, Precision Photron
86 Inc., San Diego, CA, USA) was fitted with a 55 mm lens (f/2.8 Micro Nikkor AIS, Nikon Inc.,
87 Melville, NY, USA) and positioned at a distance that permitted a view of the entire aquarium. The
88 cameras were angled above the aquarium to allow both fish to be viewed by at least two cameras
89 when the fish were positioned close together. The cameras were synchronized to record at a 1,000
90 fps (at 1024 \times 1024 pixels) with a common TTL trigger and controlled with the manufacturer's
91 software (PhotronFASTCAM Viewer).

92 Predation experiments were performed by recording the swimming of one predator and one
93 prey fish in the aquarium (Fig. 1A). This began by placing the fish on opposite sides of a partition.
94 Following a 15 min acclimation period, we lifted the partition and observed the fish until the

95 predator successfully ingested the prey. Using a end-trigger to the high-speed cameras, we saved
96 recordings from ~ 0.5 s before the first predatory strike and until ~ 0.5 s after the prey was
97 captured.

98 Our video recordings were used to perform measurements of 3D kinematics. We calibrated
99 the cameras by recording a static body that we constructed with 48 landmarks of known relative
100 position, which was placed in the center of the aquarium. A direct-linear transform (DLT) was
101 calculated using 'Digitizing Tools' software in MATLAB (2015a, MathWorks, Natick, MA, USA)
102 [28] from manually-selected coordinates of these landmarks from the perspective of the three
103 cameras. Using a custom script in MATLAB, we found the body positions of predator and
104 prey fish by selecting landmarks from two camera views and using the DLT to determine the
105 coordinates in 3D space. We used the position of the predator's two eyes to calculate a mean
106 position that approximated the buccal cavity (Fig. 1a). The posterior margin of the swim bladder
107 was found on the prey's body, which approximates the center of mass [29]. The initial heading
108 of the prey was approximated by matching an ellipsoid (using the 'regionprops' function in
109 MATLAB) to the body of the prey and measuring the angle of the major axis of the ellipsoid. All
110 subsequent heading measurements of the prey was defined as the average angular displacement
111 of prey during an escape and was relative to the prior heading of the prey. We acquired the
112 landmark positions at four key events in each interaction between predator and prey: at (1) the
113 initiation of a predator's approach toward the prey, (2) the middle time point of the duration
114 of suction feeding by the predator and the (3) initiation and (4) completion of the prey's escape
115 response.

116 (c) Descriptive statistics

117 Descriptive statistics were used to characterize the probability of actions by the predator and prey
118 during predation experiments. We recorded the predator-specific parameters of the strike distance
119 (s), the distance from the prey at which a strike (i.e. a suction feeding event) was initiated, and
120 the strike duration (τ), which was defined as the period between the opening and closing of the
121 mouth during suction feeding. For the prey, we found the reaction distance (l), the distance from
122 the predator at which the escape response was initiated. The prey's kinematics were additionally
123 characterized by the escape angle (θ), the angular change in heading from the resting orientation
124 to the escape path. The escape duration (η) included the period for all stages of the C-start and
125 subsequent undulatory swimming, until the larva ceased moving. The frequency distribution
126 for each of these parameters was found to be well-approximated by the following lognormal

¹²⁷ probability density function:

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

¹²⁸ where x is a particular behavioral parameter (s , τ , l , θ , or η), μ is the log mean, and σ is the log
¹²⁹ standard deviation. We determined best-fit values for μ and σ for each behavioral parameter by
¹³⁰ maximum-likelihood (the ‘fitdist’ function in MATLAB). Instances where the predator captured
¹³¹ the prey, parameters for the prey were not included in the dataset.

¹³² The probability that the strike of a zebrafish predator is successful depends critically on the
¹³³ distance between the mouth of the predator and the prey [22]. Strikes were therefore measured as
¹³⁴ a function of distance. We binned our distance data using an fixed number of bins and calculated
¹³⁵ the ratio of number of observations in the bin over the total sample size (Fig. 2f). These binned
¹³⁶ measurements revealed that the probability of a successful capture (C) was well-characterized by
¹³⁷ the following sigmoidal function:

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

¹³⁸ where d is the distance between predator and prey, d_0 is the decay distance, and r is the decay rate.
¹³⁹ The best-fit values for d_0 and r were determined by least-squares (using the ‘sqcurvefit’ function
¹⁴⁰ in MATLAB).

¹⁴¹ All parameters for the prey and predators were compared between experiments with adult
¹⁴² predators and juvenile predators. Because these measurements failed to conform to normal
¹⁴³ distributions, we performed statistical comparisons using non-parametric statistics. In particular,
¹⁴⁴ we used the two-sample Kolmogorov-Smirnov test (i.e. KS-test) [30], which does not assume any
¹⁴⁵ particular distribution for the data.

¹⁴⁶ (d) Probabilistic, agent-based model

¹⁴⁷ A probabilistic, agent-based model was developed to simulate the conditions of our experiments.
¹⁴⁸ This model predicted the 2D motion of a predator and prey [31] according to algorithms that
¹⁴⁹ were specific to the behavioral state of each of these agents (Fig. 1b). The predator’s states
¹⁵⁰ were Tracking and Striking and the prey’s were Resting and Escaping. The duration of states,
¹⁵¹ probability of transitioning between states, and probability of prey capture were determined by
¹⁵² random-number generation that conformed to the probability distributions and range of values
¹⁵³ that we measured. Therefore, the model treated the predator and prey’s actions as probabilistic,
¹⁵⁴ but each outcome of an interaction also depended on the determinism of the kinematics of the

155 two agents. Simulations were scripted in MATLAB to calculate the motion of both agents and
156 their behavioral states, which consequently determined the number of unsuccessful strikes before
157 prey capture.

158 Each simulation began with the predator in the Tracking state, where it moved at an approach
159 speed with a direction that was always headed toward the prey, with perfect information about
160 the prey's position (Fig. 1b). If the prey was motionless, then the solver would advance in time
161 to the strike or escape initiation, whichever was found to occur first. Otherwise, the solver would
162 resolve both predator and prey motion with a fixed time step of 5 ms. In this regime, the predator
163 adjusted its heading to track the prey with a temporal time delay, λ . The predator's transition into
164 the Striking state occurred when the prey was within a particular value for the strike distance. This
165 value was determined *a-priori* by the generation of a random value (using the 'random' function in
166 MATLAB) according to the lognormal probability density function (Eqn. 2.1) for measured values
167 of strike distance. The capture probability, C , for a particular strike depended on the distance
168 between the agents in the middle of a strike, according to our measured parameter values for
169 this relationship (Eqn. 2.2). The simulation was terminated if a strike was successful, otherwise
170 the predator reverted to the Tracking state after completion of the strike duration (Fig. 1b). The
171 value of strike duration was determined by the generation of a random value from the lognormal
172 probability density function from measured values. Single values for the predator speed and
173 delay were used for all simulations (Table 1) and were determined by trial-and-error to replicate
174 the distribution of the measured number of unsuccessful strikes before prey capture. These values
175 were found to approximate measurements reported in prior studies [22,32].

176 The model simultaneously determined the actions of prey (Fig. 1b). Prey behavior was
177 modeled with Resting and Escaping states because larval zebrafish generally remain still between
178 periods of rapid swimming initiated by an escape response [13,22]. The prey began each
179 simulation in the Resting state, where it was motionless and positioned at a random distance from
180 the predator that was within the aquarium diameter ($\Theta = 8.5$ cm). The prey transitioned into the
181 Escaping state when the predator moved within the reaction distance, after a latency [33]. During
182 an escape, the speed of prey varied as a single saw-toothed pulse, with the maximum value (the
183 peak of the sawtooth) attained at 0.2η , where η is the escape duration. We found that this function
184 well-characterized prey speed using a frame-by-frame kinematic analysis of escape swimming for
185 12 larvae. The amplitude of the saw-toothed pulse represented the maximum escape speed,
186 u , observed in our 12 recordings. During the escape, the prey was assumed to follow a straight
187 path in a direction determined by the escape angle and escape direction. The reaction distance,

188 escape angle, and escape duration were determined by random numbers with probability density
189 functions matching experimental measurements. The escape angle was defined with respect to
190 the prey's frame of reference, with $\theta = 0^\circ$ corresponding to and axis defined by forward motion.
191 This angle was directed with respect to the right or left side of the body by the escape direction.
192 The escape direction was defined as the probability that the escape angle was directed toward
193 the side of the body facing away from the predator, with a value (Table 1) that was previously
194 measured [13].

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

207 We designed a parameter analysis to evaluate the parameters that had the greatest effect on
208 prey survival. This was achieved by running monte carlo simulations of the model for a 1,000
209 simulations where one parameter was varied between -90% and 100% of their original mean
210 values at increments of 10%. For parameters described by a probability distribution, the log-mean
211 parameter, μ , was adjusted to create the desired percent-change in the mean of the distribution,
212 while σ was held constant. The range of possible random values for each distribution was also
213 adjusted to retain the the same cumulative probability range in the probability distribution. Each
214 parameter variation yielded 1000 trials, each where the prey has escaped a varying amount of
215 times. The effect of these manipulations were assessed by comparing the measured number
216 of escapes the prey made against the model's prediction without any parameter variation (%
217 change = 0) using a Kruskal-Wallis test. The results for these comparisons are displayed as
218 escape probabilities in the main text, though the number of escapes plots can be viewed in the
219 supplements (Fig. S2).

220 3. Results

221 (a) Kinematics

222 The behavior of both predator and prey were similar whether the predators were juvenile or adult
223 zebrafish. Prey responded similarly, having indistinguishable differences in escape angle (KS-test:
224 $P = 0.86, N = 164$) and with modest, though significant, differences in reaction distance (KS-test:
225 $P < 0.001, N = 164$) and escape duration (KS-test: $P = 0.04, N = 153$) (Fig. 2b–c). For example,
226 prey reacted at a mean distance to juvenile predators ($\bar{l} = 0.84$ cm, $N = 91$), that was about two-
227 thirds the reaction distance to adults ($\bar{l} = 1.26$ cm, $N = 73$). Escape swimming lasted for about one-
228 third of a second, with the response to juveniles ($\bar{\eta} = 0.35$ s, $N = 91$) being only 50 ms longer than
229 to adults ($\bar{\eta} = 0.30$ s, $N = 62$). Prey escaped earlier to adult predators (KS-test: $P = 0.02, N = 89$)
230 by 41 ms, on average, relative to the mid-duration of suction feeding. Juvenile and adult predators
231 were not significantly different in either their strike distance (KS-test: $P = 0.08, \bar{s} = 7.6$ mm, $N =$
232 154), or strike duration (KS-test: $P = 0.87, \bar{\tau} = 44$ ms, $N = 107$) (Fig. 2d–e). Therefore, much of the
233 behavior of predator and prey were similar, despite the fact that the adults were nearly twice the
234 body length of the juveniles.

235 Despite having similar behavior, adult and juvenile predators differed in capture performance.
236 Juveniles did not succeed in capturing prey beyond a distance of 3.2 mm ($N = 91$), whereas
237 adults captured prey at a maximum distance that was about 3-times greater (10.4 mm, $N = 77$).
238 In the relationship between capture probability and distance (Eqn. 2.2), the decay distance was
239 used to indicate the spatial range of high capture probability. By this metric, the strike of adult
240 predators also exhibited a range that was slightly greater than 3-times the distance of juveniles
241 (Table 1, Fig. 2f). We tested whether this result was due to juveniles approaching the prey with
242 inferior accuracy by measuring the bearing angle of predators. This angle corresponds to the
243 radial position of the prey relative to the predator's heading, such that a predator is perfectly
244 accurate for a prey position of 0° . The bearing angle when they prey initiated an escape was not
245 significantly different (KS-test: $P = 0.15$) between juveniles ($N = 91$) or adults ($N = 77$). However,
246 bearing angles changed such that there was a significant difference (KS-test: $P = 0.008$) between
247 adults and juveniles when measured at the moment of mid-gape. In particular, adult predators
248 succeeded in achieving a median bearing of 13.1° (Mean = 35.3°), whereas the same metric was
249 30.0° (Mean = 39.2°) in juveniles. This suggests that adults were more accurate in their strikes
250 and more effectively adjusted their heading in the time between escape and strike.

251 (b) Probabilistic, agent-based model

252 The trajectories of predator and prey fish followed paths that were qualitatively similar to that
253 predicted by our pursuit-evasion model. For most of the duration of our experiments, predators
254 were observed to be swimming toward the prey (Fig. 3a). In contrast, the prey were generally
255 motionless, except when executing escape swimming. The predators and prey followed a more
256 circuitous path in the predation experiments than the motion prescribed by our model (Fig. 3b).
257 Nevertheless, the temporal sequence of events in the model offered a reasonable approximation
258 of the kinematics of live predator-prey interactions.

259 The model accurately predicted the broad quantitative patterns of our experimental results.
260 This was assessed by the probability of the prey surviving over a particular number of strikes.
261 In our experiments, prey exhibited the greatest probability of being captured on the first strike
262 with monotonically decreasing probabilities over subsequent strikes (Fig. 3c). Adults were more
263 successful on the first, second and third strikes than juveniles, which consequently exhibited
264 a more even probability distribution. The model was successful in replicating these trends,
265 which were found to be statistically indistinguishable for both adult (KS-test: $P = 0.93, N = 73$)
266 and juvenile (KS-test: $P = 0.86, N = 91$) predators. Furthermore, all trends from the parameter
267 analyses of the pursuit-evasion model were similar between the adult (Fig. 4) and juvenile (Fig.
268 S1) predators.

269 A parameter analysis of prey parameters revealed that escape speed and reaction distance
270 were the only parameters with a substantial effect on prey survival. This result was determined by
271 adjusting the probability distribution of each parameter to generate a percent change in the mean
272 value of that parameter among 1,000 simulations. Generating these changes in escape duration,
273 escape direction, and escape angle led to statistically insignificant or small changes in escape
274 probability (Fig. 4a). An increase in escape speed similarly had a negligible effect on survival, but
275 survival probability did decline when speed was reduced by 50% or more. However, survival
276 was most sensitive to reaction distance. Increases to the mean reaction distance caused escape
277 probability to increase up to 16% and decreases in the mean reaction distance by 30% or more had
278 a dramatic adverse effect on survival (Fig. 4a).

279 We examined how the effect of reaction distance varies with escape speed by conducting a two-
280 dimensional parameter analysis (Fig. 4b). It was not until speed was reduced by more than 50%
281 that the relationship between response distance and survivor was altered. For example, reducing
282 speed by 70% yielded a distinct curve, the shape of which was similar to the sigmoid generated at
283 higher speeds (Fig. 4c). Alterations in response distance did affect the relationship between escape

²⁸⁴ speed and survival, though the shape of this curve was largely unaltered (Fig. 4d). This suggests
²⁸⁵ little evidence for an interactive effect on survival between escape speed and response distance.

4. Discussion

We found that the survival of larval fish does not increase by escaping at a faster speed or by varying direction, but only by responding from a greater distance. These results were attained through a parameter analysis of a model (Fig. 1*b*) that calculated the trajectories of predator and prey and the outcome of predatory strikes (Fig. 3*a,b*). This probabilistic, agent-based model successfully replicated the broad patterns of survival (Fig. 3*c*) by simulating behavioral actions that matched our measurements (Fig. 2). Our analysis of its predictions suggests that prey survival in fishes may be enhanced by increasing the performance of sensing and not locomotion.

(a) Locomotor performance and prey survival

The survival of prey depends largely on the actions of the predator. In contrast to the explosive speed of an escape response [20], zebrafish predators tend to approach their prey substantially slower than their capacity, often by braking [32]. The approach speed amounted to less than one-third the maximum speed of escaping larvae (Table 1), which is consistent with previous measurements [22]. The approach speed relates strategically to the mechanics of feeding. The suction feeding of fishes succeeds in capturing prey in only a small region around the mouth over a duration of merely tens of milliseconds [34–36]. A slow approach is common among suction-feeding fishes and is likely a means of enhancing strike accuracy [8,9]. This style of predation is seen over many species of fish [37]. Furthermore, our data suggest that zebrafish predators are more likely to capture prey when approaching larval zebrafish with slower approach speeds (Fig. S3). Therefore, the limited range of suction feeding may constrain some predators to a slow approach while offering prey an opportunity to escape [24]. Despite this strategic advantage for prey, adult zebrafish captured prey on the first strike more than one-quarter of the time and rarely needed more than three strikes to be successful (Fig. 3*c*).

The effectiveness of an escape has previously been considered by classic pursuit-evasion models of fish predation. Classic models resolve how the direction of an escape affects the distance between predator and prey with analytical mathematics [5,31]. They generally model a single encounter and assume that both animals move with a fixed heading and speed over time. A recently-developed version predicted that animals like zebrafish operate in a ‘slow-predator’ domain, where the predator moves more slowly than the prey [38]. In this domain, no optimal escape angle exists and prey may rather evade predators with a broad range of escape directions and this range is only modestly expanded by a faster escape. In contrast, the predator

gains a strategic advantage when the approach at a faster speed than the prey's escape [5]. Consistent with these ideas, we found a monotonic decrease in survival as we reduced escape speed below half of the observed value (Fig. 4a) in our simulations. In addition, we found only modest differences in survival between experiments using adult and juvenile predators (Figs. 2,3c), despite a nearly two-fold difference in body size. This would also be expected by classic theory because the interactions operate in the slow-predator domain in both cases. It follows from this theory that prey strategy benefits greatly by escaping faster than the predator, but further increases in speed offer diminishing returns.

Granted, in the 'fast-predator' domain, it could be postulated that there could be profound differences in the results from our model. Studies have shown that the kinematics of an escape response matter greatly when escaping predators [7,39]. For example, in classical pursuit-evasion models, the escape angle is a critical parameter for survival [5]. Furthermore in situations when the predator is faster than the prey, prey adapt other behaviors, such as shoaling, as a predator defense [40]. However, these studies do no consider the effect of sensation can have on prey survival. Currently, our model cannot address this domain of predator-prey interactions. However, future studies with the presented model could examine how important the kinematics of escape are relative to sensation.

The strategic conditions exemplified by zebrafish have the potential to be applicable to a diversity of fish species, but represent a special case among predator-prey interactions. Suction feeding is ubiquitous among fishes and a slow approach is common [8,9]. However, not all fish predators are slower than the prey. Ram-feeding fishes strike at prey while swimming at a relatively high speed and may thereby place prey at a strategic disadvantage. Success in ram feeding may, in-turn, require superior coordination in directing and timing a strike [11]. Ram feeding therefore shows greater similarity in strategy to flying predators such as birds [15], bats [16], and insects [41]. In these systems, the escape direction emerges as an important factor in prey strategy [42,43]. This direction may conform to an optimal value [5], or may benefit prey by being unpredictable [6]. Other factors, such as the turning radius of an escape trajectory [7], or the rate of changes in direction [6] additionally become important when the predator is faster than the prey.

346 (b) Prey survival depends on reaction distance

347 The reaction distance has broad strategic significance. The predictions of pursuit-evasion models
348 support the simple notion that prey are more evasive if they start from further away [5,31,38]. This

principle is consistent with evolutionary models that contrast the fitness benefit of responding from a distance against its potential costs [44,45]. For example, escape responses that are initiated at high frequency may be energetically expensive, prohibit foraging, or succeed in revealing cryptic prey [46]. Responding from a great distance may even be inferior on purely strategic grounds. A prey that is slower than a predator, but capable of executing a tight turn, may benefit from initiating this maneuver at the final moments of a predatory strike [7]. Therefore, a greater response distance offers a clear strategic benefit in zebrafish (Fig. 4), but may not be universally advantageous.

The primacy of reaction distance underscores the strategic importance of predator detection. Fish sense water flow with the mechanosensory lateral line system and this sensory modality is necessary for zebrafish larvae to survive predation [22]. The bow wave of flow generated ahead of a gliding predator succeeds in triggering a fast start in zebrafish larvae within a distance of 1.3 cm [13], which is a range that includes many of the responses that we recorded (Fig. 2c). Although flow-mediated responses permit a close approach by the predator, the response is rapid and may trigger an escape in less than 10 ms [47]. Escape responses may also be elicited by a looming visual stimulus [48], but the demands for visual processing necessitate a latency of at least 200 ms [49]. Nonetheless, the greatest reaction distances that we observed were likely generated by the visual appearance of the predator. Therefore, the visual system offers prey fish the means to enhance survival by responding to a predatory threat from a distance (Fig. 4). Vision and flow-sensing may be augmented by olfactory cues [50], though zebrafish do not acquire a sensitivity to the alarm pheromone Schreckstoff until a later stage of growth (>48 dpf) [50].

Summary

We found that zebrafish larvae operate in a slow-predator strategic domain when preyed upon by adults and juveniles of the same species. As a consequence, increasing the speed or varying the direction of an escape response shows a negligible effect on survival (Fig. 4). Survival may instead be enhanced by initiating the escape from greater distance by rapidly identifying the predator as a threatening visual stimulus. These findings offer valuable insight into the key strategic factors that govern predator-prey interactions in a diversity of fishes and other animals that operate with a similar strategy.

378 Authors' contributions

379 The study was designed in collaboration between AN and MJM. AN and CN performed all
380 experiments and kinematic analysis. The probabilistic, agent-based model was created by AN,
381 with guidance from MJM. The manuscript was written collaboratively by AN and MJM.

382 Competing interests

383 We declare we have no competing interests.

384 Funding

385 This research was supported by grants to MJM from the National Science Foundation (IOS-
386 1354842) and the Office of Naval Research (N00014-15-1-2249).

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⁵³³ Figures & Tables

Table 1. Behavioral parameters and probability distributions

Variable	State	Adult predator	Juvenile predator
<i>Predator</i>			
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 → S	$\mu_d = -4.980, \sigma_d = 0.448 (N = 51)$	$\mu_d = -5.100, \sigma_d = 0.648 (N = 103)$
Strike duration, τ (s)	S	$\mu_\tau = -3.166, \sigma_\tau = 0.331 (N = 53)$	$\mu_\tau = -3.208, \sigma_\tau = 0.399 (N = 54)$
Capture probability, C	S	$r = -0.573, d_0 = 5.20 (N = 77)$	$r = 1.99, d_0 = 1.60 (N = 91)$
<i>Prey</i>			
Reaction distance, l (m)	R → E	$\mu_l = -4.546, \sigma_l = 0.587 (N = 73)$	$\mu_l = -4.941, \sigma_l = 0.582 (N = 91)$
Escape angle, θ (rad)	E	$\mu_\theta = 0.144, \sigma_\theta = 0.449 (N = 206)$	$\mu_\theta = 0.144, \sigma_\theta = 0.449 (N = 206)$
Escape duration, η (s)	E	$\mu_\eta = -1.369, \sigma_\eta = 0.552 (N = 62)$	$\mu_\eta = -1.167, \sigma_\eta = 0.5234 (N = 91)$
Escape direction, v	E	$v = 0.696 (N = 206)$	$v = 0.696 (N = 206)$
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).

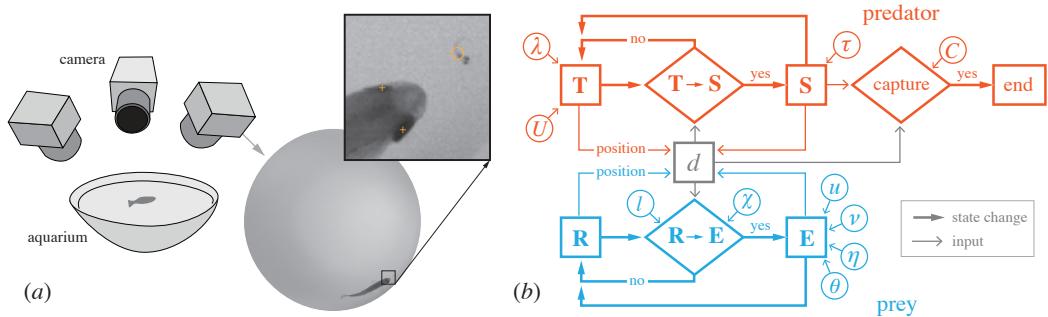


Figure 1. Kinematic measurements and probabilistic, agent-based modeling for studying predator-prey interactions in zebrafish. (a) Three high-speed video cameras recorded video of one larval prey and one predator fish (adult or juvenile) that were placed in a hemispherical aquarium. A representative video frame (cropped to the margin of the aquarium) shows an adult in close proximity to the prey. In the inset, orange markers denote the locations of morphological landmarks used to describe the position of the two fish. This consisted of the position of the two eyes for the predator ("+" and the posterior margin of the swim bladder in the prey (open circle). (b) A flow chart illustrates the major components of the model used to simulate the interactions between predators and prey (see Table 1 for symbol definitions and parameter values). Each fish behaves according to an algorithm specific to a particular behavioral state and the probability of transitioning between states is determined by random-number generators with probability distributions matching kinematic measurements (Fig 2). Predators (in red) operate between Tracking (T) and Striking (S) states and prey are either Resting (R) or Escaping (E). The outcome of a strike is determined by the capture probability (C , Eqn. 2.2). See Materials and methods for details.

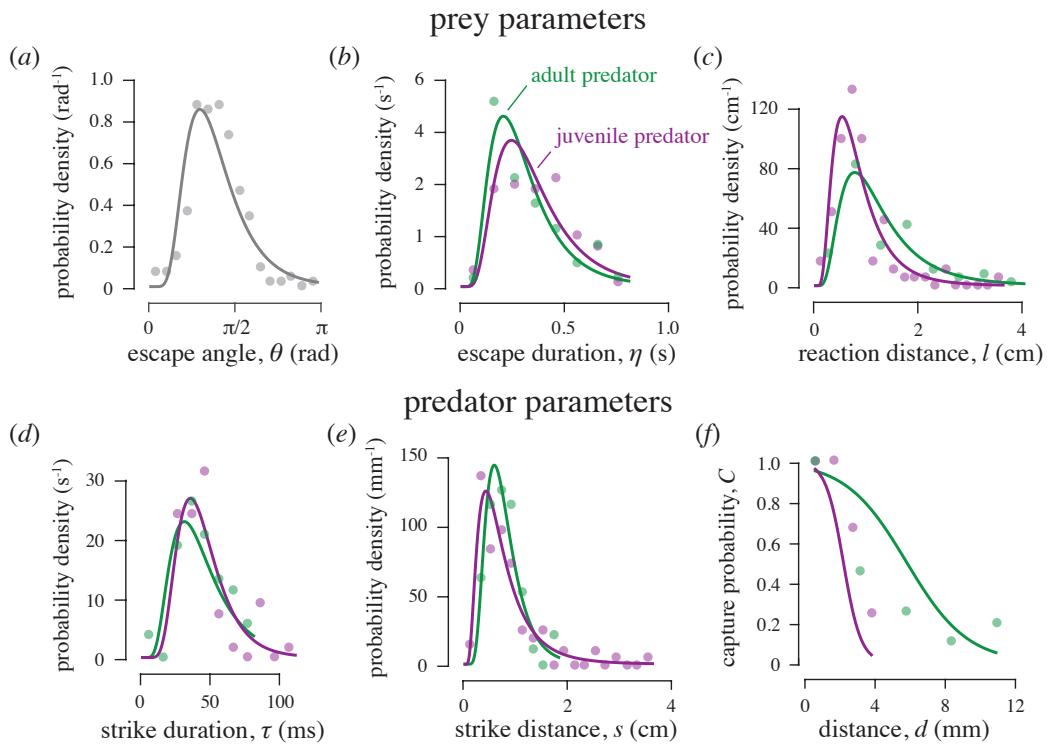


Figure 2. Descriptive statistics of swimming kinematics. (a-e) The probability measurements (circles) and probability density function (Eqn. 2.1) fits for experiments where the predator was a juvenile (purple) or adult (green) predator. Parameters were measured from the kinematics of prey (a-c) and predators (d-e) (See table 1 for sample sizes). Points on graph denotes measured probability density. Each point represents a bin of the respective data and the size of the bins were determined using the Freedman-Diaconis rule. The value of each bin is the probability density and is calculated as the ratio of the number of observed samples of data in the bin over the product of the total sample size and the bin width. The curves represent the continuous, least squares fit to the discrete data. (f) The capture probability was examined as it varies with distance between the predator and prey (Eqn. 2.2).

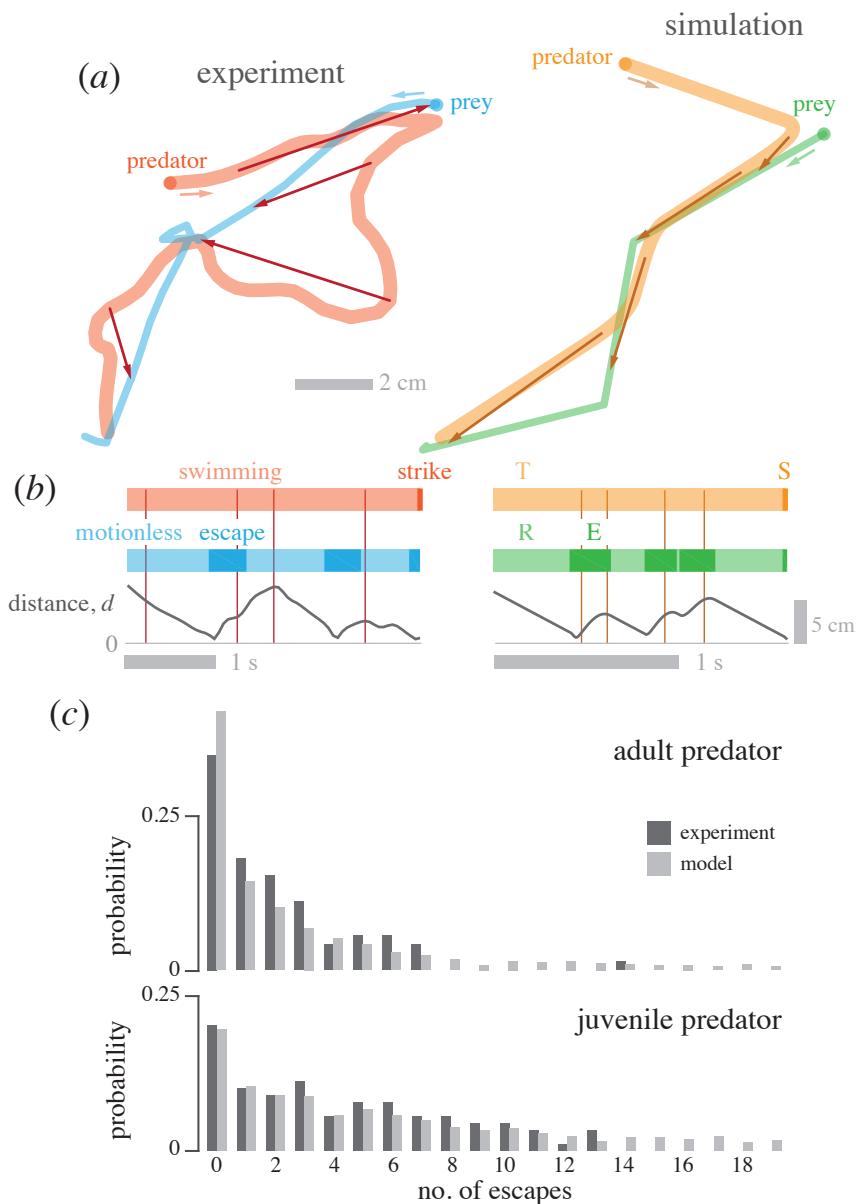


Figure 3. Comparison between experimental measurements and modeling. (a) Trajectories of predator and prey from a representative experiment (left) and simulation (right). The position of predator and prey that correspond to particular time points are shown with connecting arrows. (b) Ethograms for these trajectories illustrate the temporal changes in the predator's swimming and strike (left), which are respectively modeled by the Tracking (T) and Striking (S) (Fig. 1b) states (right). The prey's behavior while motionless and during escape (left) were respectively modeled as Resting (E) and Escape (E) modes (right). For both ethograms, the distance (d) between predator and prey are shown. Particular moments in the trajectories are highlighted with vertical lines that correspond with the same-colored arrows in (a). (c) The probability that a prey survives over a particular number of strikes is shown adult (above) and juvenile (below) predators for experiments (dark gray) and simulations (light gray).

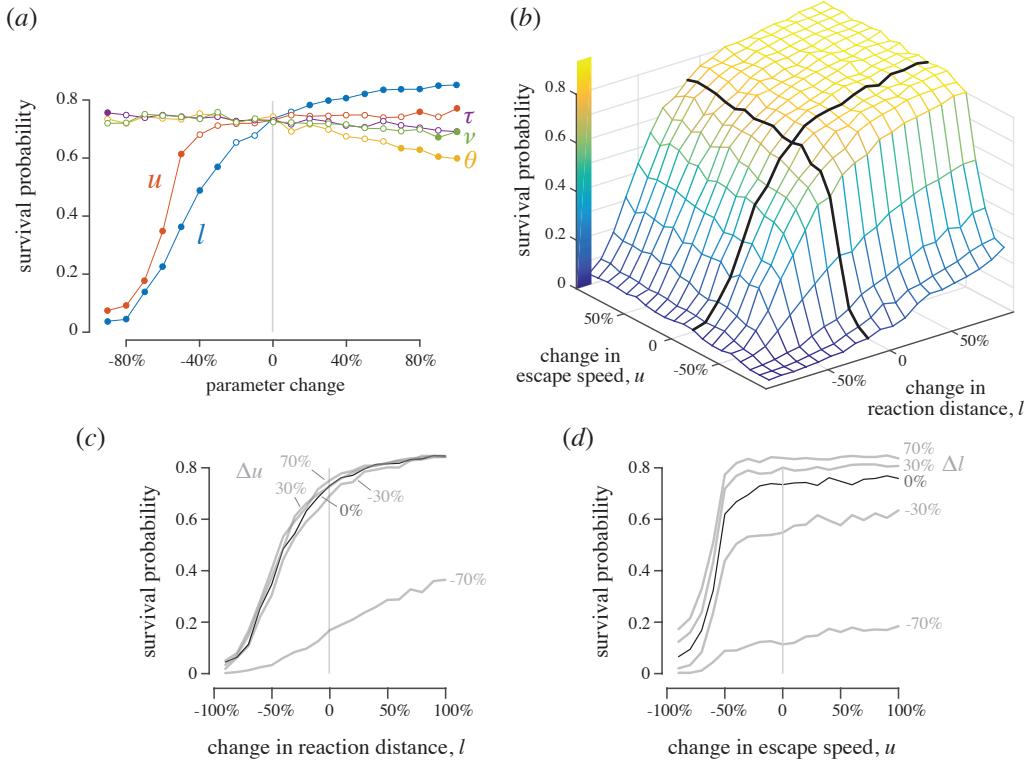


Figure 4. Parameter analysis of the probabilistic, agent-based model to examine the effects of parameters on escape probability. (a) We individually varied the mean parameter value among simulations by manipulating the distribution (Fig. 2) of our measurements (see Table 1 for parameter definitions and values). Each point represents the survival probability of prey among 1000 simulations and filled circles denote a significant difference (KS-test: $P < 0.05$) from the observed probability. Simulations that varied in escape angle (θ) differed by an interval of 0.127 rad. (b) Variation in escape probability was examined with respect to both escape speed and reaction distance. The same simulation results are shown with respect to changes in escape speed (c) and reaction distance (d). All simulations used an adult predator, although similar results were obtained with a juvenile predator (Fig. S1).