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Survival depends critically on predator detection in zebrafish larvae

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Abstract

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1. Introduction

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The present study addressed the strategic importance of sensing and locomotion with a combination of experiments and mathematical modeling. High-speed kinematics were recorded of swimming by a single zebrafish larva as it was pursued by an adult predator of the same species, as in previous studies [1,2]. Descriptive statistics of these interactions were used to characterize the probability of behavioral actions by both the predator and prey. These findings provided the basis of a game model that was used to simulate the conditions of our experiments. Once verified, an analysis of this model was performed to evaluate the sensitivity of prey survival on the behavioral parameters of the predator and prey.

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, $3.4 \text{ cm} \pm 0.5 \text{ cm}$) predators and another using juveniles (3 – 4 months old, $2.0 \text{ cm} \pm 0.4 \text{ cm}$). 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°C on a 14:10 h light:dark cycle. To culture larvae, the fertilized eggs from randomized mating were cultured according to standard techniques [3]. Predators were fasted for a period of 7 – 14 days prior to an experiment to motivate feeding.

(b) Kinematic measurements

We arranged the lights and cameras for high-speed recordings of both fish with high-contrast images. The hemispherical aquarium ($\varnothing = 8.5 \text{ cm}$) 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 (CITE), 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 that viewed the entire aquarium. The cameras were distributed 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 1000 fps (at 1280 x 1024 pix) 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 a hemispherical aquarium (Fig. 1A). Experiments were set up by placing the fish on opposite sides of a partition. Following a 15 min acclimation period, each experiment began by lifting the partition and ended when the predator successfully ingested the prey. We recorded ~ 0.5 s before the first predatory strike and ended at ~ 0.5 s after the prey was captured.

The 3D kinematics of both fish were measured from the video recordings. The cameras were calibrated by recording a body with 48 landmarks of known relative position that was placed in the center of the aquarium. Manually selecting the position coordinates from the view of the three cameras provided the basis for a direct-linear transform (DLT), which was calculated using 'Digitizing Tools' software in Matlab (2015a, MathWorks, Natick, MA, USA) [4]. Using a custom script in Matlab, we found the body positions of predator and prey fish by manually selecting landmarks from two camera views and using the DLT to find the coordinates in 3D space. For the predator, we found the position of the two eyes from which we calculated a mean position that approximated the buccal cavity (Fig. 1b). The rostrum and posterior margin of the swim bladder were found on the prey's body, which is a position that approximates the center of mass [5]. We acquired the landmark positions at four key events in the interactions between predator and prey: (1) when the predator fish initiated an approach, when the predator (2) began and (3) completed suction feeding and (4) at the initiation 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. The predator-specific parameters consisted of the strike distance (s), the proximity 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 proximity from the predator at which the escape response is initiated. The escape was additionally characterized by the the escape angle (θ), the angular change in heading from the resting, and the escape duration. 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 calculated for the interactions of all experiments and found to be well-approximated by the following log-normal probability density function:

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

where x is a particular behavioral parameter, 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 [6]. The capture probability (i.e. the ratio of captures to strikes) was measured as a function of distance, divided into intervals of ???, for all experiments. This probability (C) was well-characterized by the following sigmoidal function:

$$C(d) = \frac{1}{1 + e^{-r(d-d_0)}}, \quad (2.2)$$

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 (the 'sqcurvefit' function in Matlab).

(d) Game theory

A pursuit-evasion game model was developed to simulate the conditions of our experiments. This game predicts the 2D motion of a predator (i.e. pursuer) and prey (i.e. evader) according to algorithms that are specific to the behavioral state of each of these agents (Fig. 1c). The predator's states are Tracking (T) and Striking (S) and the prey's are Resting (R) and Escaping (E). The duration of states, probability of transitioning between states, and probability of capture are determined by random-number generators with probability distributions and range of values that match the results of our kinematic measurements. Therefore, the game treats the predator and prey's actions as probabilistic, but each outcome of the interaction also depends on the determinism of the kinematics of the two agents. We developed a solver for this game, scripted

in Matlab, that calculates the motion of both agents and their behavioral states and consequently finds the payoff, the ultimate score for a game model [7], as the number of strikes before capture.

A predator begins the game in the Tracking state, where it moves at an approach speed U with a direction that is always headed toward the prey, with perfect information about the prey's position. In this state, the predator is capable of tracking the motion of the prey after a delay λ . The predator transitions into the Striking state when the prey is within a value for strike distance (s), which is 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 depends 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 simulation uses this relationship to generate a random value from this distance-specific probability to find the range of a strike, with the prey considered captured if it is within that range. The game ends if the strike is successful, otherwise the predator reverts to the Tracking state after completion of the strike duration (τ). Single values for the approach speed and predator delay were used for all simulations (Table 1), determined by trial and error and were found to approximate the mean values observed in prior studies [6,8].

The game model simultaneously determines the actions of the prey fish. The behavior of prey was modeled with Resting and Escaping states because we presently found that larval zebrafish generally remain still between periods of rapid swimming initiated by an escape response, as reported previously [?]. The prey begins each game in the Resting state, where it is motionless at a distance equal to the aquarium diameter ($\phi = 8.5$ cm) at a random radial position with respect to the predator's heading. After a latency (χ) [9], the prey transitions into the Escaping state when the predator moves within the reaction distance (l). The prey follows a straight path in the direction of the escape angle (θ) during the Escaping state, and varies in speed according to a saw-toothed function of time, where the escape speed (u) is achieved at 20% of the escape duration (η). The reaction distance, escape angle, and escape duration are determined by random-numbers with probability density functions matching experimental measurements, similar to the actions of the predator. The escape angle is defined with respect to the prey's frame of reference, with $\theta = 0^\circ$ corresponding to straight-ahead motion and $\theta = 90^\circ$ corresponding to a perpendicular turn. The escape direction (v) is the probability that this angle is directed away from the predator and was previously measured [1].

This model abstracts many aspects of the complexity of predator-prey interactions in the interest of establishing a predictive first-order approximation. It assumes that the dynamics may

122 be reasonably approximated with two-dimensional motion and does not bound that motion by
123 the dimensions of an aquarium. Simulations were stopped if prey successfully escaped on 20
124 occasions, which reflected the observed maximum and guarded against an errant simulations
125 from executing for an infinite duration. The model's probabilistic approach considers the effects
126 of biomechanics and neurophysiology without articulating those elements. For example, capture
127 success is treated as a distance-specific probability (Eqn. 2.2) that does not parse the effects of a
128 predator's suction-feeding hydrodynamics, nor the propulsive forces generated by an escaping
129 prey. The model was tested by comparing the results of simulations against our experimental
130 findings using a Kolmogorov-Smirnov test (CITE). This test was chosen over a Kruskal-Wallis
131 test because of its emphasis on the shape of the distribution, rather than the median value. The
132 number of successful prey escapes before capture for all experiments were compared to the same
133 metric for 1,000 simulations.

134 A sensitivity analysis determined which characteristics of the predator and prey behavior
135 have the greatest effect on prey survival. This was achieved by running batches of 1,000
136 simulations with parameters varied individually between -90% and 100% of their original values
137 at increments of 10%. For parameters described by a probability distribution, the log-mean μ
138 parameter and the range of values were adjusted by these factors to create a change in the
139 distribution's values. The effect of these manipulations were assessed by comparing escape
140 probability against the experimentally-validated prediction using a Kruskal-Wallis test.

141 3. Results

142 Data collected from live predator prey experiments alone could not discern performance
143 differences between locomotion and sensing. Live predator-prey interactions between an adult
144 (9+ months old) and larval (5 days post fertilization) zebrafish and between a juvenile (2-
145 3 months old) and larval zebrafish were recorded and analyzed. For the live experiments, a
146 suite of variables pertaining to locomotion and sensing for the prey (larval zebrafish) and the
147 predator (adult or juvenile zebrafish) were collected from the predator-prey recordings (Table
148 2.1). Differences between successful escapes and failed escapes in each prey or predator parameter
149 were insignificant (Mann-Whitney U test: $P > 0.05$, $N =$ see Table 1). This suggested that neither
150 locomotion or sensing had an significant impact on whether a prey escape attempt was successful
151 or not. This seemed unreasonable since it well known both sensory and motor systems are
152 important for prey survival (CITE). We suspect this discrepancy emerged from the fact that the
153 behavior of predator zebrafish and larval zebrafish were dependent on each other. This led to

154 a coupled system of behavior where there were not strict measurable controls. Therefore, we
 155 decided to computationally model the observed predator-prey interactions, allowing the ability
 156 to control for various aspects of the predatory encounter.

157 The probabilistic model confidently mimicked the live predator-prey experiments. The
 158 probabilistic model was able to simulate predator-prey interactions between adult and larval
 159 zebrafish and between juvenile and larval zebrafish. For the model to replicate these two different
 160 predator-prey interactions, two different parameters sets were derived from the recordings of
 161 adult-larval zebrafish interactions and juvenile-larval zebrafish interactions (Table 2.1). Juvenile
 162 and adult zebrafish were morphologically similar, but were different in scale. Adult zebrafish
 163 were significantly longer in body length and had a larger gape width than juvenile zebrafish ($P \ll$
 164 0.001, One-way ANOVA, $N = 19$, Supp. Fig). Reaction distance and escape duration of the larval
 165 zebrafish were significantly different between the adult-larval zebrafish interactions and juvenile-
 166 larval zebrafish interactions ($P < 0.05$, Two-sampled Kolmogorov-Smirnov test). The capture
 167 probability of predator fish between juvenile and adult zebrafish also varied between parameter
 168 sets as well (Supp. Fig). In the model, both the predator and prey agent behaved similarly to
 169 their biological counterparts (Fig. 2A). This was true for both the adult-larval parameter set and
 170 juvenile-larval parameter set. To quantitatively compare the model and the live predator-prey
 171 experiments, we collected the number of successful escapes larval zebrafish and the virtual prey
 172 agent made before being captured and compared for significant differences. The comparison
 173 revealed that the data from the live experiments and the model were quantitatively similar for
 174 adult-larval interactions and juvenile-larval interactions (Kolmogorov-Smirnov test: $P \gg 0.05$, $N =$
 175 73 for adult-larval experiments and 91 for juvenile-larval interactions, $N = 1000$ for each). Given
 176 the probabilistic model can replicate our predator-prey experiments, we conducted the rest of
 177 our analysis using the probabilistic model. Furthermore, many of our results observed with the
 178 adult-larval zebrafish parameter set were mirrored by the juvenile-larval zebrafish parameter set.
 179 Therefore, the results in this paper will focus on the results of the adult-larval zebrafish parameter
 180 set.

181 A sensitivity analysis of the prey parameters revealed that escape speed and reaction distance
 182 were the most impactful parameters for prey survival. We conducted a sensitivity analysis where
 183 we modulated each prey parameter. For each parameter, the mean value of the parameter was
 184 changed by increments of 10%. For each incremental change in the parameter, we observed a
 185 change in the escape probability, the chance a prey agent will escape for any given predatory strike
 186 (Fig. 3). Changes in escape duration, escape direction, and escape angle led to minimal changes

in escape probability. However, reaction distance and escape speed had much more substantial effect on escape probability compared to the rest of the prey parameters. By increasing the mean value of the reaction distance, escape probability increased significantly. However, decreasing the mean value of the reaction distance created greater, significant decreases to the escape probability. Modulating escape speed had similar effects. However, 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.

There were compensatory effects for escape probability between escape speed and reaction distance. Since escape speed and reaction distance were the two parameters that had the greatest impact on escape probability of the prey agent, we conducted a two-dimensional sensitivity analysis where reaction distance and escape speed were both incrementally changed (Fig. 5A). By increasing and decreasing both parameters simultaneously, escape probability increased and decreased respectively. However, escape speed and reaction distance affected each other in different manners (Fig. 5B,C). Reflecting the patterns seen in the one-dimensional sensitivity analysis, reaction distance modulated the patterns of escape speed has on escape probability (Fig. 5B). By keeping escape speed constant, increasing reaction distance slightly increased the overall escape probability. However, decreasing reaction distance while escape speed was held constant created large, significant decreases to escape probability. Decreasing escape speed was the only way to observe significant changes in escape probability when reaction distance was held constant (Fig. 5C). Increasing escape speed while the reaction distance was held constant did not substantially increase escape probability.

4. Discussion

Sample equations

Sample equations.

$$\begin{aligned}\frac{\partial u(t, x)}{\partial t} &= Au(t, x) \left(1 - \frac{u(t, x)}{K}\right) - B \frac{u(t - \tau, x)w(t, x)}{1 + Eu(t - \tau, x)}, \\ \frac{\partial w(t, x)}{\partial t} &= \delta \frac{\partial^2 w(t, x)}{\partial x^2} - Cw(t, x) + D \frac{u(t - \tau, x)w(t, x)}{1 + Eu(t - \tau, x)},\end{aligned}\tag{4.1}$$

$$\begin{aligned}\frac{dU}{dt} &= \alpha U(t)(\gamma - U(t)) - \frac{U(t - \tau)W(t)}{1 + U(t - \tau)}, \\ \frac{dW}{dt} &= -W(t) + \beta \frac{U(t - \tau)W(t)}{1 + U(t - \tau)}.\end{aligned}\tag{4.2}$$

$$\begin{aligned} \frac{\partial(F_1, F_2)}{\partial(c, \omega)} \bigg|_{(c_0, \omega_0)} &= \begin{vmatrix} \frac{\partial F_1}{\partial c} & \frac{\partial F_1}{\partial \omega} \\ \frac{\partial F_2}{\partial c} & \frac{\partial F_2}{\partial \omega} \end{vmatrix} \bigg|_{(c_0, \omega_0)} \\ &= -4c_0q\omega_0 - 4c_0\omega_0p^2 = -4c_0\omega_0(q + p^2) > 0. \end{aligned} \quad (4.3)$$

Data accessibility

Authors' contributions

Competing interests

We declare we have no competing interests.

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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 \rightarrow 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	$r = 573.8, d_0 = 0.0052$ ($N = 77$)	$r = 1990, d_0 = 0.0016$ ($N = 91$)
<i>Prey</i>			
Reaction distance, l (m)	R \rightarrow 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; d_0 , decay distance.

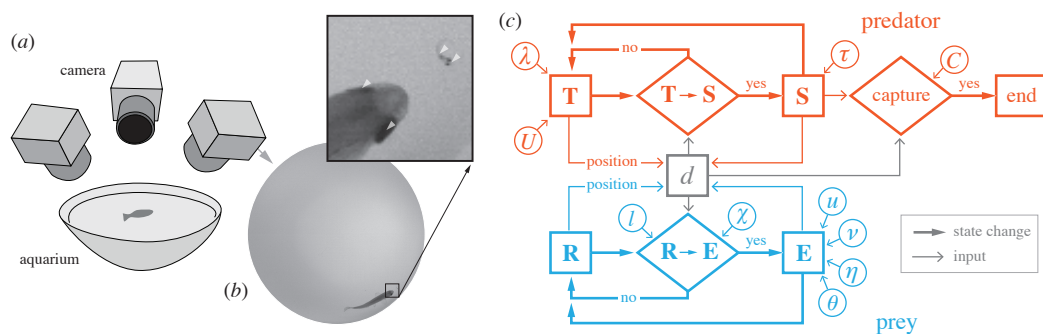


Figure 1. Experimental and mathematical techniques for studying predator-prey interactions. (a) Three high-speed video cameras recorded video of one larval prey and one adult predator fish that were placed in a hemispherical aquarium. (b) A representative video frame (cropped to the margin of the aquarium) 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. (c) 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).