

RESEARCH ARTICLE

Zebrafish larvae evade predators by sensing water flow

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SUMMARY

The ability of fish to evade predators is central to the ecology and evolution of a diversity of species. However, it is largely unclear how prey fish detect predators in order to initiate their escape. We tested whether larval zebrafish (*Danio rerio*) sense the flow created by adult predators of the same species. When placed together in a cylindrical arena, we found that larvae were able to escape 70% of predator strikes (mean escape probability $P_{\text{escape}}=0.7$, $N=13$). However, when we pharmacologically ablated the flow-sensitive lateral line system, larvae were rarely capable of escape (mean $P_{\text{escape}}=0.05$, $N=11$). In order to explore the rapid events that facilitate a successful escape, we recorded freely swimming predators and prey using a custom-built camera dolly. This device permitted two-dimensional camera motion to manually track prey and record their escape response with high temporal and spatial resolution. These recordings demonstrated that prey were more than 3 times more likely to evade a suction-feeding predator if they responded before ($P_{\text{escape}}=0.53$, $N=43$), rather than after ($P_{\text{escape}}=0.15$, $N=13$), a predator's mouth opened, which is a highly significant difference. Therefore, flow sensing plays an essential role in predator evasion by facilitating a response prior to a predator's strike.

Key words: lateral line system, bow wave, startle response, predator–prey interaction, suction feeding, zebrafish, larvae.

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INTRODUCTION

A fish's ability to survive an encounter with a predator depends on its ability to both detect and evade the predator's strike. Despite the importance of these abilities, it is generally unknown what features of the sensory and motor systems matter most to successful evasion in fish. The goals of the present study were to test whether prey fish use their flow-sensitive lateral line system to detect predator fish and to determine how the timing of the escape response affects evasion success. These goals were achieved with experiments that used zebrafish (*Danio rerio*) larvae as prey and zebrafish adults as predators.

After detecting a predator, prey fish may evade a strike with the 'fast start' startle response. This behavior is characterized by the body curling into a 'C' shape and then rapidly unfurling over a long excursion. This motion accelerates the body and initiates fast undulatory swimming (Weihs, 1972; Kimmel et al., 1974; Webb, 1976; Eaton et al., 1977). The fast start enables fish to evade a predatory strike (Webb, 1981; Webb, 1982; Webb, 1986; Blaxter and Fuiman, 1990; Fuiman, 1991; Fuiman, 1993; Walker et al., 2005; Van Wassenbergh and De Rechter, 2011; Yasugi and Hori, 2012), which may be triggered by the visual appearance of the predator (Dill, 1974a; Dill, 1974b; Webb, 1982; Fuiman et al., 2006). However, a reliance upon the visual system presents some strategic limitations for a prey fish. In addition to being hindered in dark or turbid environments (Cerri, 1983; Kamil, 1988), the central processing of visual stimuli can substantially delay an escape. For example, some prey fish exhibit a latency of 20 ms before initiating a startle response to a visual stimulus (Guthrie and Banks, 1990; Canfield, 2003), which is about twice the latency of mechanical stimuli (Liu and Fetcho, 1999; Liu et al., 2012) and slower than the strike of some fish predators (Wainwright et al., 2001).

The lateral line system may trigger a fast start rapidly enough to allow successful predator evasion (McHenry et al., 2009). In larval fish, this mechanosensory system is sensitive to the flow generated by the differential motion between the body and the surrounding water (McHenry et al., 2009; Stewart and McHenry, 2010). This stimulus is capable of triggering a startle response (Blaxter and Fuiman, 1989; Liu and Fetcho, 1999; McHenry et al., 2009). When encountering a predator, the lateral line of the prey may detect either the flow of a predator's approach (Muller and Osse, 1984; Visser, 2001) or the suction-feeding strike (Visser, 2001; Holzman and Wainwright, 2009). However, it is unclear whether the flow created by a predator can trigger a fast start or whether this behavior is effective at evading a predator's strike.

Zebrafish can serve as a model system for predator–prey interactions. Although many wild-type strains of this species have been bred in laboratory conditions for hundreds of generations, they retain the essential strike and escape characteristics of a piscivorous encounter. Furthermore, no other species offers a greater potential for understanding the functioning of the lateral line system (e.g. Kohashi and Oda, 2008; Nagiel et al., 2009; Nuñez et al., 2009; Mo and Nicolson, 2011; Trapani and Nicolson, 2011) and the startle response (e.g. Bhatt et al., 2007; Issa et al., 2011; Nikolaou and Meyer, 2012; Liu et al., 2012) across levels of organization. For these reasons, we have focused on zebrafish in the present study. This was achieved by comparing the ability of larvae with and without a functioning lateral line system to evade adult predators. The use of zebrafish adults as predators is appropriate because adults readily feed on zebrafish eggs and larvae (Spence et al., 2008). Furthermore, adult zebrafish are larger than prey, with body length proportions (10:1) that are comparable to other fish predators and prey, such as oscar cichlids and guppies (6:1) (Wainwright et al.,

2001), asp and goldfish (7:1) (Van Wassenbergh and De Rechter, 2011), largemouth bass and mosquitofish (9:1) (Sass and Motta, 2002), or clownfish and northern anchovy larvae (4–15:1) (Webb, 1981). To study the effects of the timing and distance of the response, we measured the kinematics of evasion by developing a novel translating camera dolly to track fish with high-speed video at high spatial resolution.

MATERIALS AND METHODS

We conducted two types of experiments to study predator evasion in zebrafish. Our lateral line ablation experiments compared the survival of prey with an ablated lateral line with that of untreated larvae in both light and dark conditions to test whether flow sensing plays a role in predator–prey interactions. High-speed kinematics experiments investigated how the success of an evasion varies with the timing of a startle response. This was achieved by recording the strikes of zebrafish adults on larvae under illumination with high-speed, high-magnification video. The results from both types of experiments yielded insight into the sensory signals that alert prey to attacking predators and the motor response that facilitates evasion.

Animal husbandry

Zebrafish larvae and adults were maintained according to standard protocols. All zebrafish, *Danio rerio* (Hamilton 1922), 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 14h:10h light–dark cycle. The fertilized eggs from randomized mating were cultured according to standard techniques (Westerfield, 1993) and larvae were raised in an incubator in E3 embryo media (Brand et al., 2002).

Lateral line ablation experiments

Experiments of predator–prey interactions were used to investigate the role of flow sensing in predator evasion. In each experiment, a single larva was placed in a cylindrical arena (20 cm diameter × 6 cm depth) with an adult. A camera ('Marlin', Allied Vision Technologies, Stadroda, Germany) was positioned above the arena to record the position of both fish (768 × 480 pixels; 20 × 20 cm field of view at 10 frames s⁻¹). Infrared panels were positioned below the arena to generate back-lit, high-contrast video images to visualize the animals in both illuminated and darkened conditions. Sheets of low-density polyethylene plastic (3.2 mm thick) were placed between the infrared panels and the arena to serve as a diffuser to provide uniform illumination. For illuminated recordings, a 45 W halogen bulb directed away from the arena provided diffuse ambient lighting. A partition separated predator and prey within the chamber for a 20 min acclimation period before recording of predator–prey interactions began. The partition was removed at the start of an experiment and the movements of predator and prey were video recorded until the prey was captured, or until 20 min had elapsed, whichever came first. This setup allowed for coarse measurements of the timing of strikes and a determination of whether they were successful. However, it was not possible in these experiments to resolve the details of the timing and relative position of predator and prey during an encounter between these fish. This limitation was addressed by our high-speed kinematics experiments, described in the following section.

Half of these experiments used larval prey with an ablated lateral line system. We will refer to the groups of larvae with functioning and ablated lateral line systems, respectively, as 'untreated' and 'treated' groups. Lateral line ablation was achieved by inducing cell death in the lateral line hair cells by exposure to a 250 μmol l⁻¹

solution of neomycin sulfate for 1 h prior to experiments. This technique was developed in previous studies (Harris et al., 2003; McHenry et al., 2009), where it was shown through visualization and behavioral analysis to leave inner ear hair cells intact. Although this treatment is highly effective at diminishing lateral line function, zebrafish are capable of rapidly regenerating lateral line hair cells. It therefore cannot be assumed that the lateral line system is completely disabled (Harris et al., 2003; McHenry et al., 2009). To ensure that treated larvae were healthy, we only used larvae for experiments that exhibited normal behavior such as routine burst and coast swimming and a motivation to feed.

Predator–prey experiments were conducted under four different conditions. The first experiment was performed in an illuminated chamber with untreated larvae. After 12 h, each predator was used in a second experiment with a new untreated larva in the dark. This was followed by a third experiment, conducted 12 h later under lighted conditions with a treated prey larva. The fourth experiment occurred 12 h after the third, using a treated larva under darkened conditions. The order of light *versus* dark conditions among experiments was randomized for each predator. All four experiments were successfully completed with 10 predators; predators were tested with a single prey during each of the four experiments (40 total prey used, 5–8 days post fertilization, d.p.f.). There was no significant difference in age between untreated and treated larvae in the illuminated experiments (*t*-test, d.f.=21, *P*=0.09). Data were collected on three additional predators feeding on untreated prey in illuminated and dark conditions, but unsuccessful lateral line ablation treatments prevented experiments with these predators and treated prey.

All predators were trained in the 2 days prior to experiments. This consisted of conducting four predator–prey interactions with untreated larvae under illumination at 12 h intervals. The purpose of this training period was to verify that predators were capable of feeding and to ensure that any increase in predator feeding ability due to experience would occur before experiments began. Of 16 predators, 13 were capable of preying on larvae and were consequently included in the study.

We measured the position of predator and prey from the video recordings from these experiments. This analysis and all other analytical approaches in the present study were achieved with software developed within Matlab (v.2010a, with the image processing toolbox, MathWorks, Natick, MA, USA). The centroid body position of predator and prey were identified in each video frame of a recording. This semi-automated tracking procedure required manual position selection for larvae when they came into contact with a predator or the walls of the arena. Position data were used to estimate the speed and location of prey and predators throughout the experiments, which were used to identify encounters, prey startle responses and predator strikes. Startle responses by larvae were identified as swimming that exceeded 15 body lengths s⁻¹ (Kimmel et al., 1974). Predator strikes were defined as instances where a predator changed its heading and accelerated directly toward the prey. An encounter between the predator and prey was identified as an instance when the margins of the predator and prey bodies were within 2 prey body lengths.

Predator–prey encounters were classified from video recordings to characterize the behavioral differences between experimental conditions. We used four categories for encounters: passive encounters, unprovoked responses, evasions or captures. A passive encounter occurred when neither the predator nor prey exhibited a change in heading or speed during an encounter. An unprovoked response occurred when the prey initiated a startle response and the predator did not strike. An evasion occurred when a prey successfully

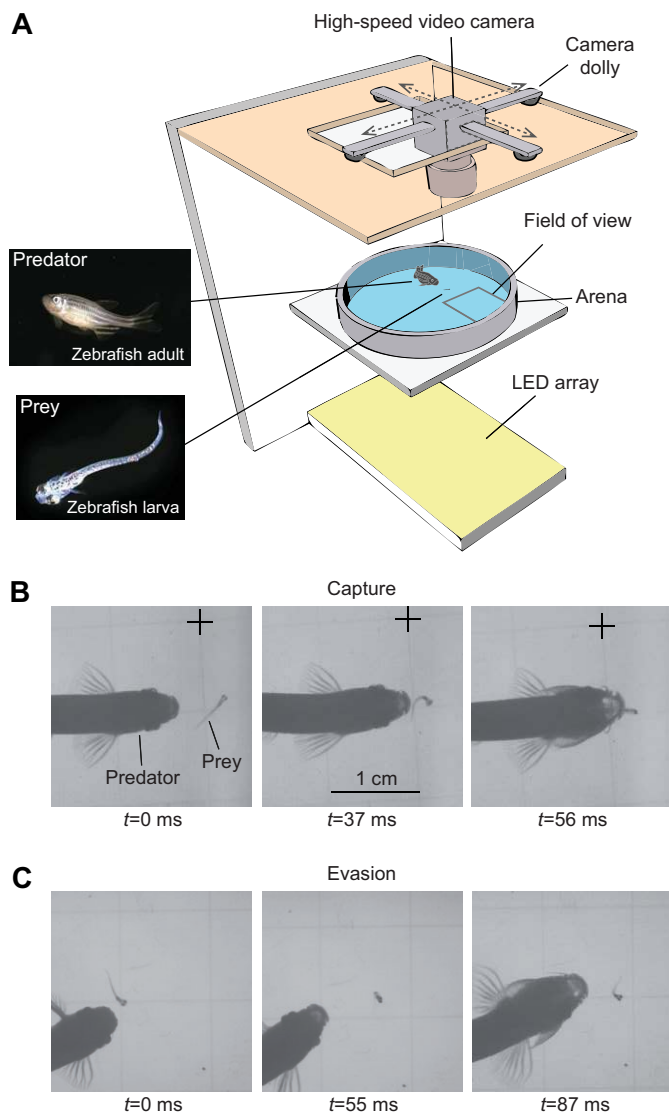


Fig. 1. High-speed video recordings of predator–prey interactions using a translating camera dolly. (A) A schematic illustration of the experimental setup used to record zebrafish (*Danio rerio*) adults feeding on larvae of the same species. Ball bearings within the dolly system permitted the planar motion of a high-speed video camera at a fixed distance from the arena (16 cm diameter) in which a predator pursued its prey. The camera was manually operated to follow these fish through the arena with a relatively small field of view (4.5×4.5 cm). (B, C) Video frames from two representative predator–prey interactions illustrate a capture (B) and a successful evasion (C). (B) The position of a background grid was digitally tracked (crosses) to correct for the movement of the camera during recordings.

evaded a predator's strike with a startle response. Finally, captures occurred when the predator ingested the prey during a strike. The frequencies of each interaction type were compared among the four experimental conditions to evaluate the effects of flow sensing and vision on prey survival.

We measured the effectiveness of a prey's startle response as the escape probability (P_{escape}) for an experiment. This was calculated as the ratio of the number of successful evasions (n_{evasion}) to the total number of strikes (n_{strike}) for each prey during a single recording:

$$P_{\text{escape}} = \frac{n_{\text{evasion}}}{n_{\text{strike}}} \quad (1)$$

We also measured the response probability of the prey (P_{response}) during an experiment. This was calculated as the ratio of the number of startle responses (n_{response}) to the total number of encounters ($n_{\text{encounter}}$) for each prey during a recording:

$$P_{\text{response}} = \frac{n_{\text{response}}}{n_{\text{encounter}}} \quad (2)$$

We recorded the duration before prey capture, which was the survival time of the prey (t_{survival}) for each recording. To test the effect of the lateral line system on predator evasion, P_{escape} , t_{survival} and n_{evasion} were compared between recordings involving predators and untreated *versus* treated prey with paired *t*-tests (Sokal and Rohlf, 1995).

High-speed kinematics

Video recordings with high spatial and temporal resolution were used to measure the kinematics of predator–prey interactions (Fig. 1). An adult predator and larval prey were placed in a water-filled circular arena (16 cm diameter×2.5 cm depth) and separated by a partition for a 20 min period so the animals could adjust to this environment before experiments began. The partition was then removed and the predator's attack was recorded with a high-speed video camera (Photron Fastcam 1024, Photron USA, San Diego, CA, USA) mounted above the arena. The high-speed camera collected images (1024×1024 pixels) at 1000 frames s⁻¹ through a macro lens (Nikkor 35–70 mm, Nikon Corp., Tokyo, Japan) that was connected to an extension tube (Nikon Pk-3, Nikon Corp.) for enhanced magnification. Only attacks occurring away from the arena walls (>1 cm) were considered for analysis. Infrared panels positioned underneath the arena produced high-contrast video images while overhead fluorescent lights (25 W) provided ambient visible illumination. Recordings of attacks in dark conditions were not possible as predators did not feed in the dark. After the prey was captured, this procedure was repeated with the same predator and a new larval prey. Experimentation on an individual predator ceased once a predator had ingested 8 prey. In total, 67 strikes were used in our analysis, which involved 1–8 strikes from 18 predators (mean ± s.d. body length, 4.0±0.25 cm) and 67 prey (5–7 d.p.f.; 0.42±0.44 cm body length). Predators used in these high-speed recordings were unique from the predators used previously in the lateral line ablation experiments. All predators were trained before experiments, whereby predators were fed 2–3 live larvae each day for 1 week. Only a single strike from each predator–prey interaction could be recorded before the animals rapidly swam out of the camera's field of view. For this reason, the response and escape probability values were not anticipated to be equivalent to those measured during ablation experiments.

A translating camera dolly was developed to record predatory strikes with high spatial resolution by focusing on freely swimming larvae (Fig. 1A). This consisted of a stage supported by ball bearings that allowed manual planar translation of the attached camera. The camera was focused on a field of view (4.5×4.5 cm) that covered a small portion of the 16 cm diameter arena. We were able to follow the prey in the arena during an experiment by moving the dolly and attached camera. An infrared panel (3×4 cm) and diffuser plate were positioned underneath the arena to provide high-contrast, back-lit video images. Because the infrared panel was also connected to the camera dolly, the camera and light source translated in unison to provide consistent lighting during experiments. A grid (1 cm squares) was drawn on the floor of the arena to track the camera's position during recordings. Our coordinate measurements were calculated by subtracting changes in grid position.

We acquired kinematic measurements that tested how the relative position and orientation of predator and prey affect escape probability. We manually selected landmarks from video images of the two fish with custom-written software in Matlab. The distance between the animals (d , Fig. 2) was calculated as the span between the anterior tip of the predator's rostrum and the closest margin of the prey body. This margin was measured with a spline curve fit (the 'spline' function in Matlab with default settings) to six manually selected coordinates. We determined the orientation of a predator from the centroid position of its two eyes, which defined the lateral axis of the cranium. The heading was defined as the axis perpendicular to this lateral axis. The heading of the larva was defined from three coordinates: the tip of the rostrum, the posterior margin of the swim bladder and a point that was equidistant between the lateral margins. The heading axis was determined from a least-squares linear fit to these points. The body's center of mass was estimated as the midpoint between the prey's rostrum and the posterior margin of the swim bladder, which is consistent with prior work (Stewart and McHenry, 2010). The angular position of the predator (θ) was calculated as the angle between the prey's heading and the line connecting the anterior tip of the predator's rostrum to the prey's center of mass (Fig. 2). The angular position of the prey (ϕ) was defined as the angle between the closest margin of the prey and the heading of the predator (Fig. 2).

Kinematic measurements focused on the times at which a larva initiated its startle response and at the beginning of a predator's strike. The time of the strike (t_{strike}), was determined from the video frame when the predator first began to open its jaws for suction feeding. The time of the response (t_{response}) was defined relative to t_{strike} (with negative values prior to the strike) and was identified by the video frame showing the first lateral movement of the prey's rostrum when initiating a startle response. The response distance (d_{response}) and strike distance (d_{strike}) were measures of d at t_{response} and t_{strike} , respectively. The time to mouth opening (t_{MO}) was recorded when maximum jaw protrusion occurred after the time of strike. Linear regression was used to investigate the relationship between d_{strike} and t_{MO} . The predator's approach angle (θ_{approach}) was recorded at the time of the prey's response. The speed of this approach (u_{approach}) was measured as the average speed over 20 ms prior to the prey response by tracking the anterior tip of the predator's rostrum. Time-resolved analyses of approach speed in four randomly selected recordings showed minimal acceleration during this 20 ms period. For those few prey that did not respond, approach speed was calculated in the 20 ms before the predator's strike. Prey that were spontaneously swimming when approached were identified by low-amplitude undulatory body movements during the 20 ms preceding the time strike.

A variety of statistical analyses were employed to evaluate how the kinematics of predator and prey affected escape probability. We first tested whether individual differences among our 18 predators could be neglected by pooling the results of all recordings. This was achieved with a series of Model II ANOVA tests that categorized individual predators as a random effect. Each test evaluated one of the following kinematic parameters as a dependent variable: t_{response} , d_{response} , θ_{approach} , u_{approach} and d_{strike} . As reported in Results, the non-significant results ($P > 0.05$) of these tests justified pooling the results from different predators in subsequent analyses.

We tested whether escape probability depended on the timing of the startle response and the relative position of predator and prey. The effects of five predictor variables (u_{approach} , θ_{approach} , d_{strike} ,

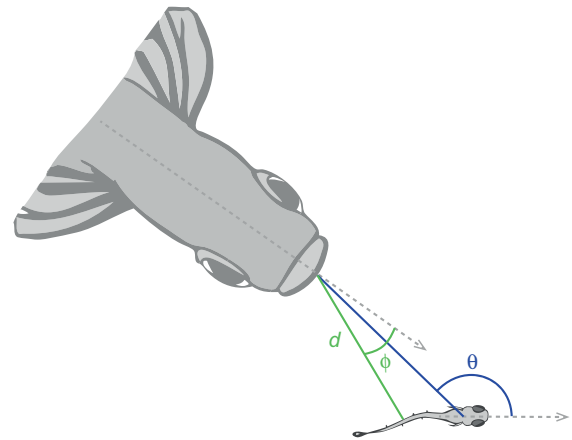


Fig. 2. Kinematic variables of predator-prey interactions. The direction of the approaching predator (θ) was measured as the angle between the prey's heading (dashed arrow extending from prey) and the line connecting the tip of the predator rostrum to the prey's center of mass (blue line). The distance separating the predator and prey (d , green line) was measured as the span between the predator's rostrum and the closest margin of the prey body. The angular position of the prey (ϕ) was found as the angle between this closest margin and the predator's heading (dashed arrow extending from predator).

d_{response} and t_{response}) on escape probability were evaluated by logistic regression (Quinn and Keough, 2002) for a binomial distribution ('glmfit' function in Matlab). We evaluated the form of relationships between predictor variables and escape probability by binning recordings by the values of the predictor variables. For each variable, the boundaries of these bins were set to contain at least 10 individuals, with the remainder placed in the final interval. For example, the effect of approach speed on escape probability was examined by grouping encounters by six uneven intervals of approach speed, which produced five bins of equal sample size ($N=11$) and one additional individual in the final bin ($N=12$). Significant differences between groups were determined by comparing the escape probability and its 95% confidence intervals (calculated for a binomial distribution) (Johnson et al., 2005) for each bin. A group was considered significantly different if its 95% confidence intervals did not span the escape probability of another group.

We also tested how that predator's speed and direction of approach affected the prey's ability to respond. The effect of approach angle (θ_{approach}) on the response distance (d_{response}) was tested using a 1-way ANOVA after prey were grouped based on θ_{approach} . Linear regression analysis (Sokal and Rohlf, 1995) characterized the relationship between approach speed (u_{approach}) and the response distance (d_{response}).

The spatial distribution of interactions was examined by mapping our measurements of escape probability from the predator's frame of reference. The prey's position was defined in polar coordinates using the distance separating predator and prey (d) and ϕ (Fig. 2). These probability maps were constructed at the times of prey response and predator strike. Finally, we tested whether prey are less responsive when swimming by grouping encounters by whether larvae were swimming at the time of a predator's approach. The probability of a response and the probability of escape were compared for significant differences between these groups by a comparison of 95% confidence intervals and mean values.

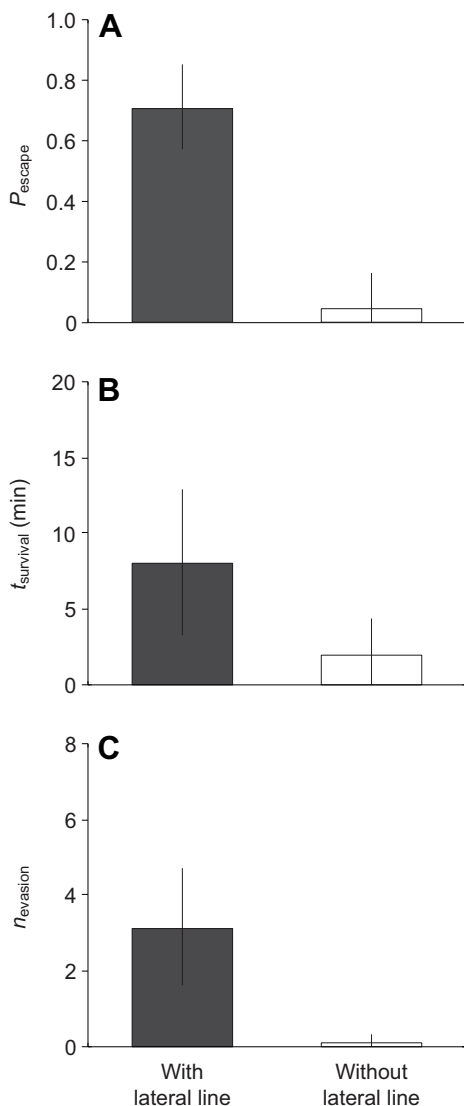


Fig. 3. The effect of the lateral line on evasion success. The lateral line system was ablated in a group of larval zebrafish ('Without lateral line', white bars) and the results of their encounters with predators were compared with those of untreated larvae ('With lateral line', gray bars). Results are shown ($\pm 95\%$ confidence intervals) for experiments conducted under illuminated conditions. (A) Untreated prey exhibited a higher probability of escape (P_{escape}) than treated prey (paired t -test, d.f.=19, $P < 0.01$). (B) The mean survival time (t_{survival}) showed that prey without a lateral line survived for a shorter duration (paired t -test, d.f.=19, $P = 0.046$) and (C) evaded significantly fewer strikes (n_{evasion}) than untreated prey (paired t -test, d.f.=19, $P = 0.003$).

RESULTS

The effect of flow sensing on predator evasion

We found that prey were substantially more successful at evading predators when they possessed a functioning lateral line system (Fig. 3). Untreated prey successfully evaded 70% of predator strikes ($P_{\text{escape}} = 0.70$, $L_1 = 0.56$, $L_2 = 0.85$, where L_1 and L_2 are, respectively, the lower and upper 95% confidence intervals for the mean, $N = 13$), whereas prey whose lateral line systems had been ablated only survived about 5% of attacks ($P_{\text{escape}} = 0.05$, $L_1 < 0.00$, $L_2 = 0.16$, $N = 10$), which is a highly significant difference (paired t -test, d.f.=19, $P < 0.01$). Untreated prey survived 4 times longer

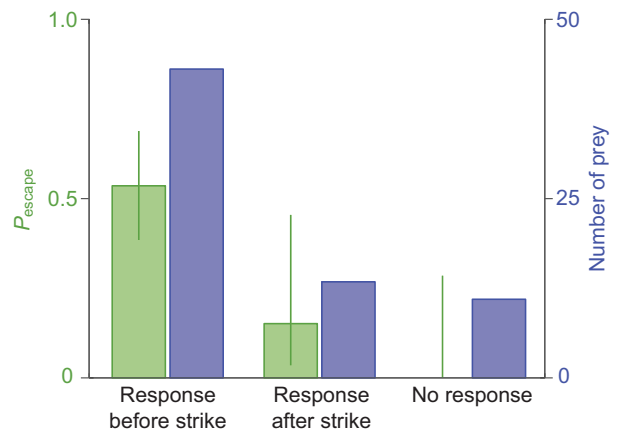


Fig. 4. Escape probability at different times in a predator–prey encounter. The number of prey (purple bars) and associated escape probability ($\pm 95\%$ confidence intervals, green bars) of prey that responded before and after the predator initiated the strike and for prey that did not respond is shown.

($t_{\text{survival}} = 8.1$ min, $L_1 = 3.3$, $L_2 = 12.9$, $N = 13$) than treated prey ($t_{\text{survival}} = 1.9$ min, $L_1 = 0$, $L_2 = 4.4$, $N = 10$), which was also a significant difference (paired t -test, d.f.=19, $P = 0.046$). Untreated prey also evaded significantly more predator strikes before being captured ($n_{\text{evasion}} = 3.1$, $L_1 = 1.6$, $L_2 = 4.7$, $N = 13$) than treated prey ($n_{\text{evasion}} = 0.1$, $L_1 = 0$, $L_2 = 0.3$, $N = 10$; paired t -test, d.f.=19, $P = 0.003$) (Sokal and Rohlf, 1995).

Under darkened conditions, adult zebrafish did not feed on larvae, but larvae did respond to predator encounters. Untreated larvae that were within two body lengths of a predator responded with an escape response almost two-thirds of the time ($P_{\text{response}} = 0.61$, $L_1 = 0.45$, $L_2 = 0.77$, $N = 13$), which was significantly greater than that in treated prey ($P_{\text{response}} = 0.01$, $L_1 \leq 0.01$, $L_2 = 0.01$, $N = 10$; paired t -test, d.f.=19, $P < 0.01$). This shows that prey with an intact lateral line system were much more responsive to nearby predators in the dark. However, in these experiments, treated larvae (7.0 ± 0.9 d.p.f.) were about a day older than untreated larvae (5.9 ± 0.9 d.p.f.), which was a significant difference (t -test, d.f.=20, $P = 0.01$). It is therefore possible the difference in responsiveness of larvae in the dark may be attributed either to lateral line ablation or to this difference in age.

The kinematics of predator–prey interactions

Our high-speed video recordings revealed how the outcome of a strike depends on the behavior of both predator and prey. Predators generally initiated a strike by directing their swimming toward the prey and then striking in close proximity. Individual differences between predators were found to be insignificant (Model II ANOVA with individual as a random effect) for t_{response} , d_{response} , θ_{approach} , u_{approach} and d_{strike} (Table 1). In addition, escape probability was not significantly different between predators, as determined by bounds of the 95% confidence intervals. Upon pooling our results, we found that most prey responded to an approach with a startle response before the predator opened its mouth (Figs 1, 4). Specifically, 43 prey responded before the predator's mouth opened, 13 prey responded after mouth opening and 11 prey did not respond. Prey responding before the strike were more than 3 times as likely to evade a predator ($P_{\text{escape}} = 0.53$, $L_1 = 0.38$, $L_2 = 0.69$, $N = 43$, where L_1 and L_2 are the respective lower and upper 95% confidence intervals, $N = 13$) than if they responded afterwards ($P_{\text{escape}} = 0.15$, $L_1 = 0.019$, $L_2 = 0.45$, $N = 13$) and no larva survived that failed to respond ($P_{\text{escape}} = 0$, $L_1 = 0$, $L_2 = 0.28$, $N = 11$). These values are lower than those

Table 1. Model II ANOVA results for five kinematic variables using predator individual as the random effect

Kinematic variable		
t_{response}	$F_{17,38}=0.91$	$P=0.57$
d_{response}	$F_{17,37}=1.23$	$P=0.29$
d_{strike}	$F_{17,49}=0.86$	$P=0.62$
u_{approach}	$F_{17,49}=0.98$	$P=0.49$
θ_{approach}	$F_{17,48}=0.86$	$P=0.62$

t_{response} , prey response time; d_{response} , prey response distance; d_{strike} , predator strike distance; u_{approach} , predator approach speed; θ_{approach} , predator approach angle.

measured in the ablation experiments (Fig. 3A) because the camera imaged a small portion of the feeding arena and therefore captured only one feeding strike before the animals swam out of the field of view. Therefore, the high-speed recordings provided a high-precision measurement of an escape whereas the long-duration recordings provided a comprehensive measure of a prey's ability to escape all encounters with a predator.

The speed and direction of a predator's approach had a minor influence on the prey's ability to escape (Table 2). The 95% confidence intervals of the escape probability showed no significant differences among strikes that were grouped by approach speed (Fig. 5A) and the logistic regression analysis showed no significant relationship between escape probability and approach speed (Wald $t=0.13$, $P=0.9$). About one-fifth of the variation in the prey response distance correlated with approach speed (Fig. 5B, regression: $R^2=0.21$, $P=0.0023$). Though a weak relationship, this result does favor the hypothesis that larvae respond from further away when a predator approaches at higher speed. In contrast, the direction of the predator's approach (θ_{approach}) did not significantly affect escape probability (determined from confidence intervals, Fig. 6A) or response distance (1-way ANOVA, d.f.=54, $P=0.21$, Fig. 6B).

The success of a startle response varied with the distance between a predator and prey at the time of a prey's response (Fig. 7). A significant relationship between response distance and escape probability was reported (Fig. 7A, logistic regression, Wald $t=2.1$, $P=0.03$). Prey responding at distances between 3.5 and 6.0 mm showed the highest chance of escape ($P_{\text{escape}}=0.82$, $L_1=0.48$, $L_2=0.98$, $N=11$), while prey responding at greater distances were less likely to survive ($P_{\text{escape}}=0.5$, $L_1=0.19$, $L_2=0.81$, $N=10$). We found that predators were capable of changing their heading after prey responded. For interactions when the prey responded before the strike, predators altered their heading with a great degree of variation (6.43 ± 9.58 deg) between the time of the prey response and the time of the strike. Prey that responded in close proximity to the predator's mouth exhibited a substantially lower chance of survival. For example, less than a third of startle responses were successful

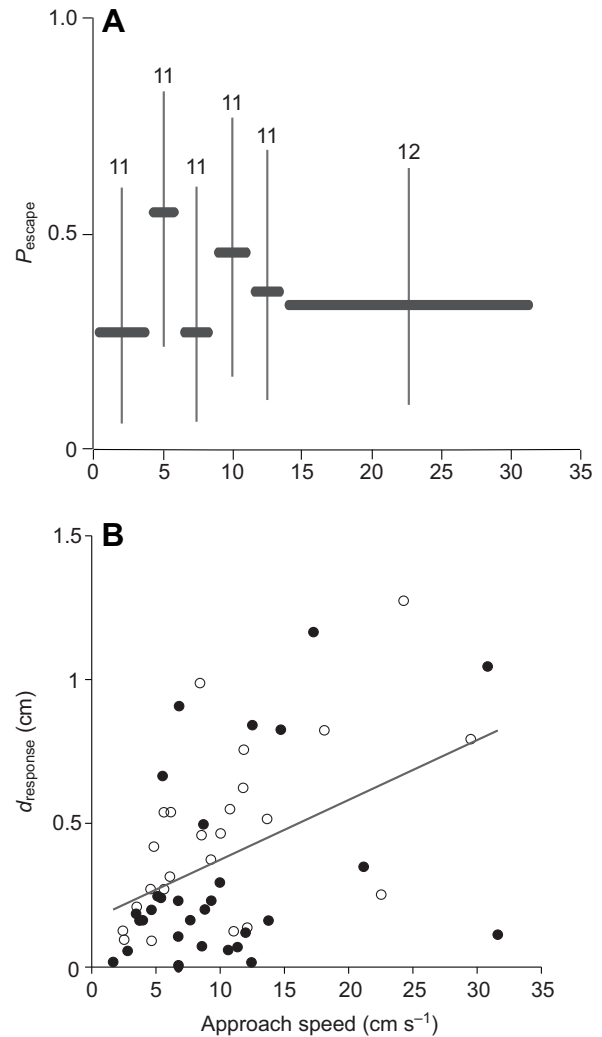


Fig. 5. Effects of a predator's approach speed on the ability of the prey to respond and escape. (A) The escape probability of the prey as a function of the approach speed of the predator. Prey were binned into groups based on the approach speed of the predator, and the probability measurements ($\pm 95\%$ confidence intervals) were calculated for each group. The range of each bin is indicated by the width of the thick bars, with sample sizes denoted above each bin. (B) The response distance of the prey (d_{response}) is positively correlated with the predator's approach speed ($R^2=0.21$, $P<0.01$). Prey that successfully evaded a predator's strike (open circles, $N=24$) and prey that were captured (filled circles, $N=30$) are shown.

when larvae responded within 3 mm ($P_{\text{escape}}=0.33$, $L_1=0.16$, $L_2=0.49$, $N=33$, Fig. 7A). Therefore, prey were most successful when they responded to an approaching predator at an intermediate distance.

Table 2. Summary of kinematic variables for the predator strike

Variable	μ	μ_e	μ_c
u_{approach} (cm s ⁻¹)	10.2 \pm 2.8 (67)	10.3 \pm 6.9 (25)	10.1 \pm 6.9 (42)
d_{strike} (cm)	0.28 \pm 0.25 (67)	0.50 \pm 0.24 (25)	0.15 \pm 0.12 (42)*
t_{MO} (ms)	14.9 \pm 6.2 (59)	18.5 \pm 6.8 (23)	12.6 \pm 4.5 (36)*
θ_{approach} (deg)	84.5 \pm 46.1 (66)	78.2 \pm 39.6 (25)	88.4 \pm 49.6 (41)

u_{approach} , predator approach speed; t_{MO} , predator time to mouth opening; d_{strike} , predator strike distance; θ_{approach} , predator approach angle.

μ , mean for all strikes; μ_e , mean for evasions; μ_c , mean for captures (expressed as mean \pm 1 s.d., sample size in parentheses).

*Significant difference (t -test, $P<0.001$) between μ_e and μ_c .

Escape probability increased with distance from the predator at the time of a strike (Fig. 8). For example, larvae showed only a 13% chance of escape ($P_{\text{escape}}=0.13$, $L_1=0.05$, $L_2=0.27$, $N=45$) when strikes occurred within 3 mm of the prey. However, this increased to 86% ($P_{\text{escape}}=0.86$, $L_1=0.65$, $L_2=0.97$, $N=22$) at a distance between 3 and 16 mm. Logistic regression confirmed that escape probability increased significantly with strike distance (Wald $t=4.0$, $P<0.001$). Variation in the predator's time to mouth opening predicts only about one-third of the variation in strike distance (regression, $R^2=0.35$), with a significant ($P<0.001$) positive regression. In contrast, the angular position of the prey with respect to the predator had little effect on the prey's ability to escape. We found that differences in ϕ (Fig. 2) measured at the time of prey response (Fig. 7F) and at the time of predator strike (Fig. 8B) did not significantly affect the escape probability (determined from confidence intervals).

Prey that were spontaneously swimming when approached by a predator showed a decreased ability to respond (Fig. 9). About three-quarters (76%) of larvae were stationary at the time of a predator's approach and these larvae were significantly more likely to respond ($P_{\text{response}}=0.89$, $L_1=0.77$, $L_2=0.96$, $N=54$) than prey that were swimming ($P_{\text{response}}=0.62$, $L_1=0.32$, $L_2=0.86$, $N=13$). Stationary prey exhibited a greater probability of escaping a strike ($P_{\text{escape}}=0.43$, $L_1=0.29$, $L_2=0.57$, $N=54$) than swimming prey ($P_{\text{escape}}=0.15$, $L_1=0.02$, $L_2=0.45$, $N=13$), but this difference was not statistically significant.

DISCUSSION

The present study contributes to our understanding of predator–prey interactions because of its novel experimental approach. We have shown that flow sensing is crucial for fish larvae to survive encounters from live predator fish and revealed how prey escape (Fig. 1A). Studies that have investigated the mechanics of prey capture in fish have appropriately used tethered prey (e.g. Higham et al., 2006) or introduced prey to a specific region of a feeding chamber (e.g. Sass and Motta, 2002) in order to control the position of a strike before a fixed camera. By recording freely swimming fish with high resolution, we have been able to examine how the kinematics of a prey influence the effectiveness of the startle response.

Predator–prey interactions between zebrafish adults and larvae exhibit characteristics that are general to predator–prey interactions among fish species. The size ratio of zebrafish adults and larvae (10:1) is comparable to that of other pairs of predator and prey fish (for a review, see Godin, 1997). Adult zebrafish exhibit rapid suction-feeding strikes ($t_{\text{MO}}=14.9\pm 6.2$ ms), which are customary of other piscivores like bluegill sunfish or largemouth bass (Higham et al., 2006). In addition, the escape probability of untreated zebrafish larvae reported here ($P_{\text{escape}}=0.70$) is similar to that of other fish predator–prey interactions, such as smallmouth bass or tiger muskellunge feeding on fathead minnow ($P_{\text{escape}}=0.88$ and 0.54 , respectively) (Webb, 1982).

The role of the lateral line system in predator evasion

Our results demonstrate, for the first time, that the lateral line system is necessary for predator evasion. Larvae with a disabled lateral line system evaded only 5% of predator attacks, whereas larvae capable of sensing flow were 14 times more likely to escape (Fig. 3A). This finding builds upon previous work that established the capacity of the lateral line system to trigger a startle response in larval fish. For example, several marine teleosts require a functioning lateral line system to respond to an approaching probe (Blaxter and Fuiman, 1989). Zebrafish larvae trigger a fast start in response to a fluid jet

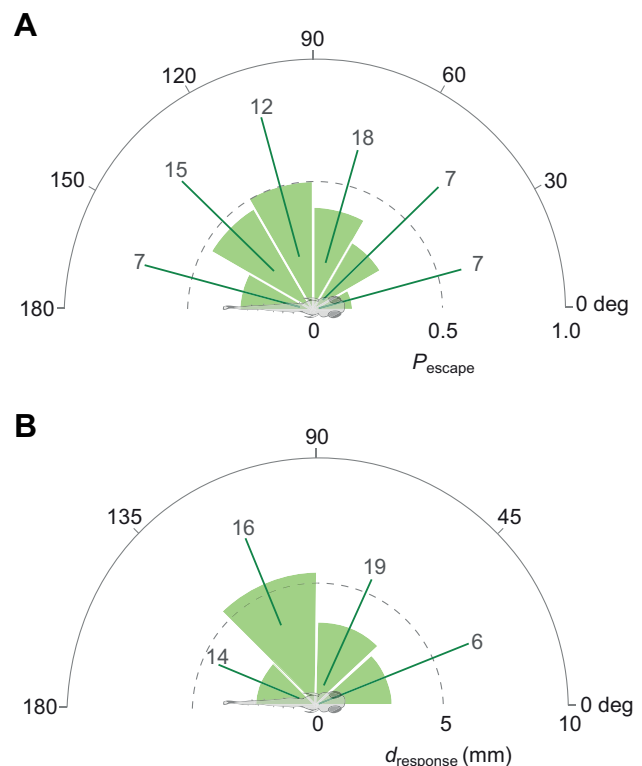


Fig. 6. The relationship between the direction of an approaching predator and the probability of prey response and escape ($\pm 95\%$ confidence intervals). Numbers above each bin denote the sample size of each group. (A) Approach angle (θ_{approach}) did not affect escape probability as indicated by the lack of significant differences among bins. (B) The approach angle also did not significantly affect the response distance of the prey (ANOVA, d.f.=54, $P=0.21$).

(Liu and Fetcho, 1999) and require the lateral line to respond to a rapid pressure field (McHenry et al., 2009). The present results build upon these findings and validate the hypothesis that the sensitivity of larval fish to water flow provides a crucial defense against predation (Blaxter and Fuiman, 1989).

Larval fish are not the only aquatic prey that employ flow sensing to detect a predator. Crustaceans use mechanosensation as both prey (Fields and Yen, 1997; Viitasalo et al., 1998; Visser, 2001) and predators (Doall et al., 2002; Browman et al., 2011). For example, copepods sense flow with setae located along the first antennae to detect attacking predator fish (Yen et al., 1992; Kiørboe and Visser, 1999; Heuch et al., 2007). The mechanosensory system of crustaceans appears to be sensitive to different cues from those used by the lateral line system as a result of morphological differences. The setae of copepods are located along antennae that extend from the body into the surrounding fluid environment (Yen et al., 1992). This allows copepods to detect predators or prey located within a three-dimensional volume surrounding the copepod body (Doall et al., 2002; Browman et al., 2011). While the present study shows that zebrafish larvae can respond to predators approaching from all directions within the horizontal plane (Fig. 6B), the ability of zebrafish larvae to detect a vertical attack has yet to be evaluated. Nonetheless, flow sensing appears to be crucial for predator evasion among a broad diversity of zooplankton.

Vision has been classically viewed as the dominant sensory modality used by prey fish for predator detection (Dill, 1974a; Dill,

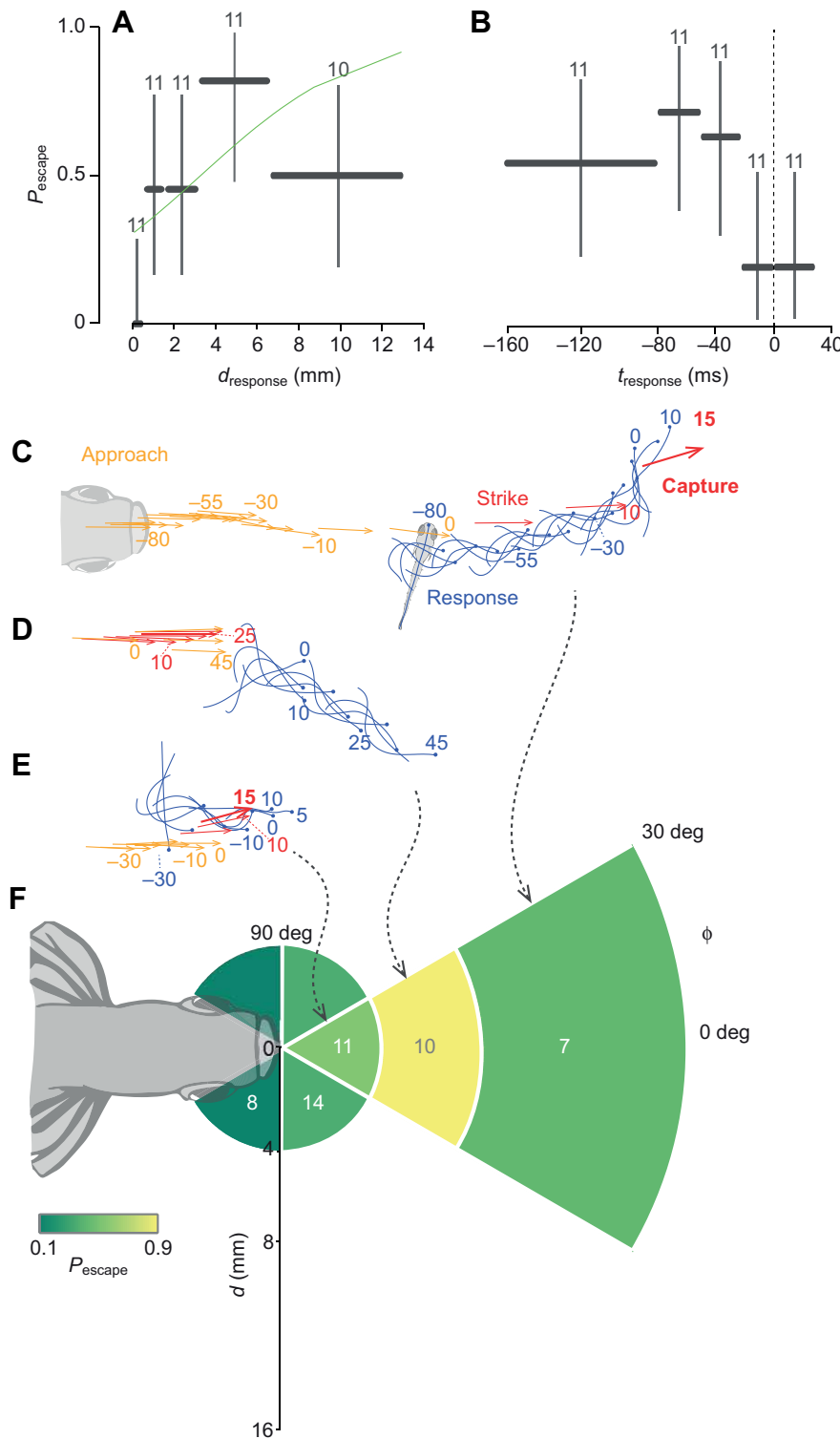


Fig. 7. How the timing of the startle response relates to its probability of success. (A,B) The escape probability of the prey as a function of the response distance and response time. Prey were grouped into bins of equal sample size and the width of the thick bars denotes the range of each bin, with sample sizes shown above. A shows the significant relationship between d_{response} and P_{escape} (logistic regression, Wald $t=2.1$, $P=0.03$) (green line). (C–E) Midline traces of zebrafish adults (orange and red) and larvae (blue) during predator–prey interactions at 5 ms intervals. The midline of the prey body (with the rostrum indicated by the filled circle) is shown during a fast start in response to the approach (orange) and strike (red) of the predator, with capture indicated in bold. In C, the prey was captured (thick red arrow) after responding far from the predator. In D, the prey exhibited an intermediate response distance and evades the predator. In E, the prey is captured after responding close to the predator. (F) A probability map illustrates the likelihood of escape based on the location of the prey at the time of prey response. P_{escape} is coded by color (see key) for different regions relative to the mouth of the predator (numbers indicate sample size), which is plotted with mirror symmetry about the horizontal axis. Prey location was defined by predator–prey distance (d) and ϕ (see Fig. 2) at the time of prey response.

1974b; Webb, 1982). This is consistent with the view that a wide diversity of terrestrial and aquatic prey use vision to detect an approaching predator from a great distance (Cronin, 2005). The broad visual field provided by the eyes of fish (Fernald, 1988) allows prey to monitor their surroundings for the appearance of predators from a wide range of directions (Cronin, 2005). In spite of this sophisticated sensory system, vision alone may not be sufficient for predator detection among fish in all situations. Other sensory modalities are favored at night (Cerri, 1983), in cloudy water or when a predator is cryptic (Saidel, 1988). Vision is also a relatively

slow sensory system. Visual cues require extensive nervous processing in comparison to other sensory systems. This processing delays a prey's startle response. For example, the response latency to a visual stimulus is over 10 times greater than that to an acoustic stimulus in red drum larvae (Fuiman et al., 1999). Cichlids possess a relatively fast visual system with latencies of a little more than 15 ms. However, this is over 3 times the latency to acoustic stimuli (Canfield, 2003). No comparable latency measurements have been conducted for the lateral line system, but the lateral line likely exhibits latency values that are comparable to those of the acoustic

system. As for hearing, a fish's ability to sense flow is mediated by hair cells with afferent neurons that project to the lateral dendrite of the Mauthner neuron that stimulates the fast start escape response (Korn and Faber, 1975). In support of this idea, zebrafish larvae respond to the initiation of a flow stimulus in less than 13 ms (McHenry et al., 2009). This appears fast relative to the feeding strikes of many predators (several cichlids can capture prey in less than 15 ms) (Wainwright et al., 2001). In contrast, visually initiated startle responses may not afford prey adequate time to evade an attack.

The kinematics of predator evasion

The timing of a startle response affects the escape probability. Escape probability increased with distance from the predator at the time of the strike (Fig. 9B). This result is unsurprising in light of the pressure gradient produced by suction feeding, which decreases exponentially with distance from the predator's mouth (Wainwright et al., 2001; Higham et al., 2006). Consequently, the forces generated by a strike draw prey from a proximity of only about one gape diameter of the mouth (Day et al., 2007; Wainwright and Day, 2007). Prey located further away at the time of a strike are therefore dramatically less threatened by a predator. This may be partially offset by the behavior of the predator, which we found prolonged their mouth opening when striking at distant or at successfully evasive prey (Table 2). A similar result was found when asp feed on goldfish, which is thought to aid in capturing more responsive or evasive prey (Van Wassenbergh and De Rechter, 2011).

Prey were most successful when responding at an intermediate distance from a predator (Fig. 7A). As argued for other species (Webb, 1976; Fuiman, 1993; Walker et al., 2005; Yasugi and Hori, 2012), prey responding very close to a predator have little time and space to evade an attack and risk being captured. In contrast, prey that trigger their startle response from a great distance allow predators to track the maneuver and direct their strike to intercept the prey after the maneuver (Howland, 1974). In the present study, successful prey may be responding at a strategically optimal distance that affords space and time to initiate a startle response without allowing the predator to adjust its attack. This principle is consistent with predator-prey interactions between marine fish adults and larvae (Fuiman, 1993; Scharf et al., 2003) and in terrestrial mammals (Curio, 1976).

Predators were able to capture nearby prey with a poorly aimed strike. An inaccurate strike may be identified by a large angular position of the prey (e.g. $\phi > 30$ deg, Fig. 8B). The ability of predators to change their heading likely improved their aim over the course of a strike. In addition, predators may have been successful because of the large mouth of zebrafish adults, which is proportionately larger than that of other suction-feeders, such as *Lepomis* (Higham et al., 2006). This causes suction forces to influence a proportionately larger region (Higham et al., 2006).

The direction of a predator's approach did not affect the prey's ability to escape (Fig. 6A), a result that could be related to the plasticity of the fish startle response. While fish most often direct the initial 'C-bend' of the startle response away from a threatening stimulus (Domenici and Blake, 1997), evasive swimming during later stages of the response can be directed over a large range of trajectories (Webb, 1976). The prey of the present study were able to evade attacks irrespective of the prey's body orientation, suggesting that escape responses can be successful when initiated from a wide range of positions relative to the predator.

Swimming prey were less responsive to predators than were stationary prey (Fig. 9). This result agrees with the finding that

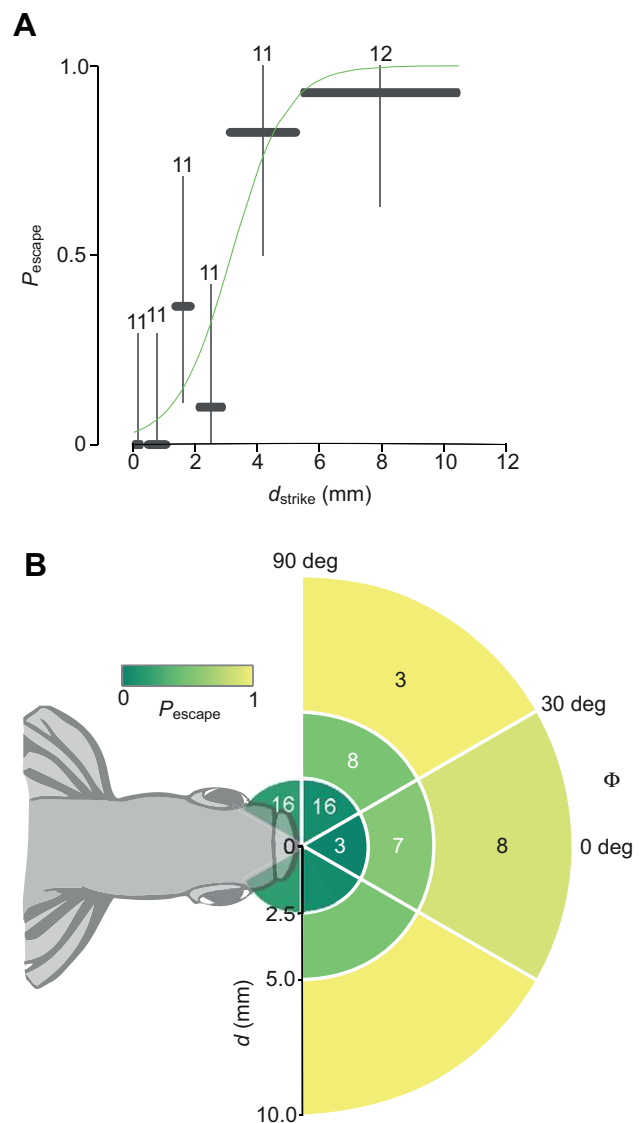


Fig. 8. How the timing of a strike relates to escape probability. (A) The escape probability ($\pm 95\%$ confidence intervals) of the prey as a function of the strike distance (d_{strike}) of the predator. Prey were binned into groups of equal sample size (values above; see Materials and methods) and the width of the thick bars indicates the bin range. The significant relationship between d_{strike} and P_{escape} (logistic regression, Wald $t=4.0$, $P<0.001$) is plotted (green line). (B) A probability map illustrating the spatial distribution of escape probability. Values of P_{escape} are indicated by color (see key) for different regions relative to the mouth of the predator (values denote sample size), plotted with mirror symmetry. Prey location was defined by predator-prey distance (d) and ϕ (see Fig. 2) at the time of predator mouth opening.

spontaneously swimming zebrafish larvae are less likely to respond to a flow stimulus (Feitl et al., 2010). The observed reduction in responsiveness may be the consequence of mechanical interference from self-generated flow or from the activity of efferent nerves that decrease flow sensitivity while swimming (Russell and Roberts, 1974; Roberts and Meredith, 1989).

The flow stimulus

The initiation of a startle response prior to suction feeding suggests that a prey detects the flow generated by an approaching predator.

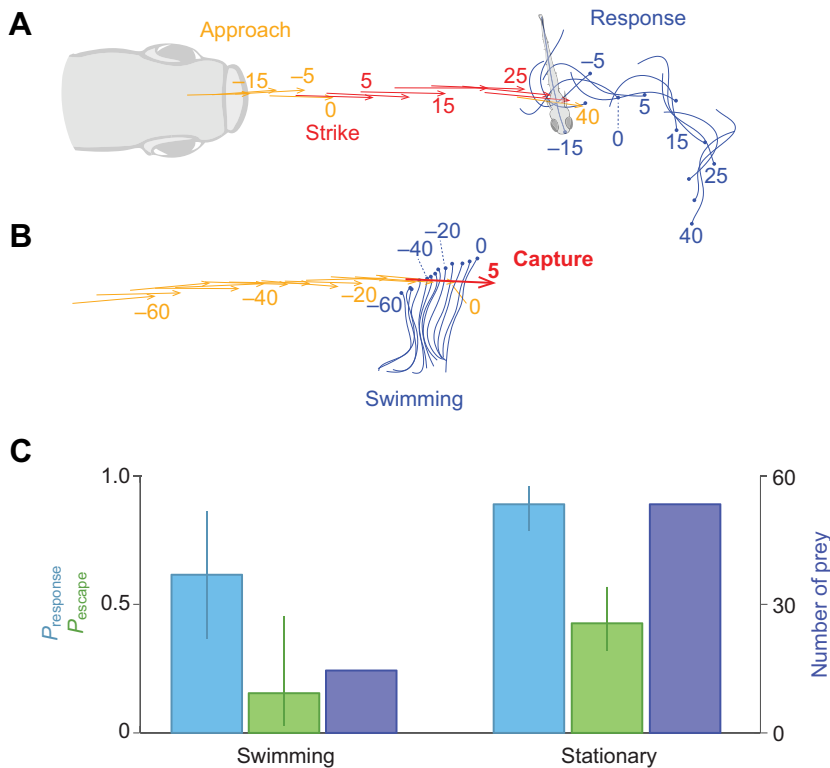


Fig. 9. The influence of spontaneous swimming on the prey's ability to evade capture when attacked. (A,B) Midline traces of a zebrafish adult (orange and red) and larva (blue, with rostrum indicated by the circle) during two representative predator–prey interactions at 5 ms intervals (numbers indicate time in ms). (A) A stationary prey responds to the predator's approach and escapes with a startle response. (B) A swimming prey does not respond to the approaching predator and is captured. (C) Bars denote number of prey (purple) and the associated probability ($\pm 95\%$ confidence intervals) of response (P_{response} , blue) and escape (P_{escape} , green) for prey that were spontaneously swimming and for prey that were stationary at the time of predator mouth opening.

A swimming fish disturbs a volume of water ahead of the body, sometimes called a bow wave (Ferry-Graham et al., 2003; Holzman and Wainwright, 2009), with a flow velocity that increases with proximity to the body surface (Muller and Osse, 1984; Windsor et al., 2010). No simple analytical model exists to predict the flow field within the bow wave. Furthermore, the stimulus to the lateral line is generated by a fluid–structure interaction between a flow field and the body of the prey (Stewart and McHenry, 2010). Therefore, it remains unclear what flow stimulus generated by a gliding fish triggers an escape response. However, it is clear that the bow wave may alert prey to an approaching predator before suction feeding begins (Visser, 2001). This opportunity to respond to the approach provides a prey with more time to complete a startle response and thereby evade a predator (Holzman and Wainwright, 2009). Copepods are similarly sensitive to the bow wave of a predator fish (Viitasalo et al., 1998; Heuch et al., 2007). For flow sensing in both fish and crustacean prey, the flow velocity and pressure gradient produced by a bow wave are substantially smaller stimuli than during suction feeding (Holzman and Wainwright, 2009). Although suction feeding may offer a stronger stimulus (Fields and Yen, 1997; Holzman and Wainwright, 2009; Stewart and McHenry, 2010), our results (Fig. 4) offer compelling evidence that the bow wave may provide a sufficient stimulus and an advantage of an early warning to a predator's strike.

The positive relationship between the speed of a predator's approach and the response distance of a prey may be attributable to the hydrodynamics of the bow wave. We found that prey fish responded to fast-swimming predators from a greater distance (Fig. 5B). A similar relationship was found when flow-sensitive copepods were approached by suction-feeding fish (Viitasalo et al., 1998). Considering the importance of flow sensing revealed by the present study, the effect of predator approach speed on the response distance of prey might be explained by the flow of the predator's bow wave. The magnitude of pressure and shear stress at the head

of a gliding fish increases with speed (Windsor et al., 2010). Therefore, a faster predator may exhibit a larger gradient in flow in front of the animal (Viitasalo et al., 1998; Visser, 2001; Kiørboe and Visser, 1999), which may alert flow-sensitive prey from a greater distance.

In summary, we have demonstrated for the first time that flow sensing is necessary for a larval prey fish to evade a predator fish with a high probability of success. High-speed kinematics of predator–prey interactions have shown that prey fish most often respond before the predator opens its mouth, which suggests that prey detect the flow produced during the predator's approach. We found that successful prey respond to predators from an intermediate distance with a startle response that displaces them away from the predator before suction feeding begins. Together, these results demonstrate how flow sensing and a well-timed escape maneuver can be crucial to the survival of larval fish.

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