Report for Friday Harbor Laboratories Fish Swimming Course 2019

Multiple visual stimuli in Pacific staghorn sculpin *Leptocottus armatus:* Can fish modulate their escape response while escaping?

Hibiki Kimura [1], Tilo Pfalzgraff [2] & Marie Levet [3]

[1] Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Nagasaki, Japan.
[2] Technical University of Denmark - DTU, North Sea Science Park Hirtshals, Denmark.
[3] Département de sciences biologiques, Université de Montréal, Montréal, Québec, Canada.

ABSTRACT

The effect of multiple visual stimuli replicating predator strikes from two opposite sides were investigated in the Pacific staghorn sculpin (*Leptocottus armatus*) at different time points during the escape response. To trigger the escape responses, we used a visual stimulation (e.g., a looming image of an approaching black circle) simulating a predator strike. For the control treatment, only a single visual stimulus was used while for the treatments we played a second stimulus, simultaneously or with a delay of 33 or 83 milliseconds. Our results revealed that a second stimulus with a delay of 33 ms affects the escape trajectory with a change of turning angle during the stage one of the escape response. Such findings tend to suggest that the sensory feedback mechanism modulates the escape response.

INTRODUCTION

In a situation of predation, the foremost defense mechanism employed by prey is the escape response. This predation avoidance behavior can be observed by a rapid displacement movement of the prey to maximize the chances of survival (Domenici et al., 2011a). The rapid displacement movement is the direct consequence of the stimulation of the prey's sensory system caused by the identification of an approaching object considered as a predation threat (Eaton and Hackett, 1984). This escape response consists of an initial turn and a propulsion phase (stage 1) followed by an angular refinement phase (stage 2) (Domenici and Blake, 1997; Eaton and Hackett, 1984; Eaton et al., 2001; Weihs, 1973).

Previous studies of escape responses have revealed kinematic mechanisms and neuronal networks as components contributing to the escape success (Domenici, 2010; Domenici and Blake, 1997; Eaton et al., 2001; Kimura and

Kawabata, 2018; Stewart et al., 2013). Most of these studies assumed a predator attack from one side despite the fact that some species are known to employ collaborative or cooperative hunting in a natural condition (Bshary et al., 2006; Herbert-Read et al., 2016; Steinegger et al., 2018). Therefore, the prey has to escape from multiple threads while fleeing away from a predation situation.

The escape trajectory is a crucial element of the escape response. By escaping away from its predators, the prey obtains higher escape success (Walker et al., 2005). As suggested by Domenici and Blake (1993), the escape trajectory has a multimodal distribution, which prevents predators from learning and predicting the escape path of their prey. This multiple distribution of escape trajectories was reported several times in species such as shrimps, fish and cockroaches (Arnott et al., 1999; Domenici and Blake, 1993; Domenici et al., 2008). Certainly, a pattern in the escape trajectory makes the prey more predictable and increases the risk of being preyed upon. As predators can adapt their trajectory to pursue a prey (McHenry et al., 2019), the prey itself should have variable escape patterns to increase the chances of avoiding a predator's attack and make the escape trajectory less predictable (Domenici et al., 2011a; Domenici and Blake, 1991; Eaton and Emberley, 1991). Interestingly, it has been suggested that the sensory feedback mechanisms can create a variation in escape trajectory modulating an initial turn even while the prey is escaping (Domenici and Blake, 1993; Eaton and Emberley, 1991). In fish, escape responses are initiated by the occurrence of a "C-start", where the fish bends its body into a C-shape during the stage 1 (Domenici and Blake, 1993; Eaton and Emberley, 1991). The onset of this escape response is typically mediated by the Mauthner cells and other neuronal pathways within the hindbrain of the fish (Brainstem escape network) (Eaton et al., 2001). Fish onset their escape response due to various stimulations such as visual and acoustic signals (Domenici and Batty, 1997; Paglianti and Domenici, 2006; Temizer et al., 2015). When fish receive a stimulus, a Mauthner cell on the side of the body closest to the stimulus fires and the opposite body trunk muscular contraction onsets after sensory and neural processing (Domenici and Hale, 2019). Fish need a particular time to onset their movements after they received a stimulus (escape latency). In that sense, multiple studies showed that the escape latency to a visual stimulus is longer than the latency to a mechano-acoustic stimulus (Burgess and Granato, 2007; Domenici and Hale, 2019; Liu and Fetcho, 1999). The brainstem escape network inhibits the opposite Mauthner

cell activation and another muscular body contraction to produce the appropriate escape response. This inhibition by the brainstem escape network intervenes in the duration before the end of stage 1 (Fetcho and Faber, 1988; Liao and Fetcho, 2008; Satou et al., 2009).

Prey fish usually escape away from predators attacking from the lateral side (Eaton and Emberley, 1991). If two predators attacked a prey fish from two opposite directions simultaneously, the prey should turn away from one and towards the other predator. In this case, the fish turning angle may be smaller due to the sensory feedback mechanism following the hypothesis of Domenici and Blake (1993). However, the brainstem escape network could be activated. Therefore, the brainstem escape network may prohibit the sensory feedback mechanism modulating the escape response in that situation. If the second predator attacked after the end of the activity of the brainstem escape network, the escape trajectory may be moderated via the stage 2 phase.

Therefore, the present study aimed to investigate whether a sensory feedback mechanism will modulate the escape trajectory when two predators attack a prey fish from left and right. To test this, we used Pacific staghorn sculpin (*Leptocottus armatus*) as the model species and subjected the fish to visual stimulation. The choice of the visual stimulation is explained by the fact that visual system has been recognized across a wide range of taxa as one of the most important sensory channels (Domenici, 2002; Nakagawa and Hongjian, 2010; Temizer et al., 2015; Yilmaz and Meister, 2013). Accordingly, the present study used a looming effect (a two-dimensional representation of an object simulating a predator strike) to elicit an escape response.

MATERIAL AND METHODS

(a) Model species and housing conditions

Staghorn sculpins were captured by beach seining at Jackson Beach, south of San Juan Island, Washington, U.S.A (48° 32’ N; 123° 05’ W) in July 2019. All individuals were transferred by boat - within two hours after fishing - to the Friday Harbor Laboratories facilities and left to acclimatize for at least 24 hours prior use in the experiment.

Fish were held under a natural photoperiod (14 h: 10 h light: dark cycle) in two tanks (57 cm L x 87 cm W x 14 cm D) alimented with flow-through seawater at a temperature of 12.5 ± 0.5 °C (mean ± SD). Fish were reared in two tanks according to size to avoid bigger fish to prey upon smaller conspecifics. During the time of the experiment, the fish were kept in accordance with IACUC regulations on animal welfare.

At the time of the experiment, the mean standard length of the fish was 14 ± 1.7 cm (mean ± SD). The animals were fed every other day with pieces of shrimps. Subsequently, at the end of the experiment, each individual was released, unharmed, at Jackson Beach.

(b) Experimental setup

Staghorn sculpin (*n* = 57) were tested individually in an experimental tank (125.5 cm L x 57 cm W x 35 cm D) with white panels on the walls to minimize visual disturbances; the apparatus was lightened by two 120-volt lamps placed above the tank to control for reflection in the water. After transfer to the experimental tank, fish were placed within an opaque PVC shelter (15.5 diameter) to acclimatize for 15 minutes. Two minutes after the removal of the shelter, the first stimulus was played. A square panel was placed under the bottom of the tank for placement reference. For each treatment, the fish were placed at the center of the tank (32.5 cm from the stimuli). Fish were placed at least at 1.5 body lengths of the walls to avoid any interference with the escape trajectory of the fish (Eaton and Emberley, 1991). The fish were placed at a 90° angle from the stimuli.

(c) *Visual stimulus and recording*

The stimulus used in our experiment was a simulation of an approaching object materialized by a black circle on a white background, similar to the one used by Paglianti and Domenici (2006). The stimulus simulated a predator of 24 cm diameter approaching from 200 cm distance from the center of the tank with a constant speed of 1 m / s. Four different looming animations (60 fps) were created by using loomeR (Carey, 2019) and FFmpeg (v. 4.2 “Ada”) on R (v.3.6.1). A control treatment, a simultaneous stimulation (0 ms treatment), a stimulation with one looming delayed by 33 ms (33 ms treatment) and one delayed by 83 ms (83 ms treatment) compared to the first stimulus. The delay times for the delayed stimulus were chosen, based on an

estimated escape latency of 60 ms (Paglianti and Domenici, 2006). The second stimulus with a delay time of 33 ms would reach a Mauthner cell during the act of the brainstem escape network with a stimulus not delayed (first stimulus). However, the second stimulus with a delay time of 83 ms reaches a Mauthner cell after the end of the act of brainstem escape network with a first stimulus.

The stimuli were then played via two screens placed centrally on either side of the experimental tank (DELL 2000FP, 1600 x 1200 pixel, 60 Hz). A mirror was placed on the top of the left monitor and pointed towards the camera in order to record the exact frame at which the fish reacted to the first stimulus.

Each fish was exposed to the first stimulus in randomized direction (whether it comes from left or right) and in randomized order to all stimuli. Between two treatments, the fish were placed back into the PVC shelter to allow changes in the stimulus and avoid stimulation prior to the treatment; a minimum of two minutes to replace the fish and allow it to recover from the previous stimulation was used. If the individual showed signs of an increased stress level, such as a high ventilation rate, extra time was given before playing the next stimulus.

A high-speed camera (Olympus Stylus TG-870, 640 × 360 pixels, 240 fps) positioned at 110 cm above the tank was used to record the escape response.

(d) *Data and statistical analysis*

Each video sequence was analyzed frame by frame using Logger pro software (v.3.15). Thus, the *x* and *y* coordinates of the tip of the head and the center of mass of the fish [CM calculated to be 35 percent of the total length frame when the fish was straight (Paglianti and Domenici, 2006)] were digitalized frame by frame. The start and end of the digitalization were set at 10 frames prior to the start of the movement of the fish and at 10 frames after the end of stage 2. Based on these *x* and *y* coordinates, we were able to look at the following locomotor and behavioral variables: escape trajectory, apparent looming threshold (ALT), initial orientation, maximum speed, maximum acceleration, stage 1 turning angle, stage 1 duration, stage 1 turning rate, maximum stage 1 turning rate, stage 2 turning angle, stage 2 duration, stage 2 turning rate, cumulative distance and type of c-start.

As the escape trajectory and stage 1 turning angle have a bimodal distribution, we used the information-theoretic (I-T) approach to test differences between treatments. This approach is analogous to ANOVA and Tukey-Kramer test for the

unimodal normal distribution (Burnham et al., 2011; Dayton, 1998; Sugiura, 1978). We recategorized our four treatments into the possible groups and made the possible combinations, which include four initial treatments (Table S1). Then, we calculated the log-likelihood of each group as the homoscedastic bimodal Gaussian distribution with the Mclust (v.5.4.5) on R (v.3.6.1). Finally, we calculated Akaike's information criterion (AIC) (Akaike 1974) for each combination using the log-likelihood of the groups and we calculated the difference of AIC between best model and each other model (ΔAIC). Models that have small ΔAIC (less than 2) were considered possible models (Burnham et al., 2011). Because the escape trajectory did not distribute around 0/360 degrees, we assumed the escape trajectory as a linear variable. The difference of the responsiveness, the directionality to the first stimulus and the type of C-start were tested by χ^2 test of goodness-of-fit. Other variables were tested for normal distribution by Shapiro–Wilk test. If variables were normally distributed, they were tested for homogeneity of variances by Levene's test. Multiple comparisons for treatments were carried out by one-way ANOVA and Tukey-Kramer test or Kruskal-Wallis test and Steel-Dwass test. Significant differences were considered when $p < 0.05$ and results are presented as mean ± SD. All calculations for variables and all statistical analyses were performed using R (v.3.6.1).

RESULTS

Altogether, 57 fish were tested during the experiment, resulting in a total of 228 played visual stimulation trials. One fish moved during the visual control stimulation and therefore, the result of that trial was excluded from the analyses. Of the 227 trials, 64 stimuli resulted in an escape response of the tested fish. Of the 56 trials of the control treatment, 12 times fish reacted to the stimulus. Of the 57 trials with 0 ms, 33 ms or 83 ms delay, 19 fish reacted to the 0 ms treatment, 16 fish reacted to the 33 ms treatment, and 17 fish responded to the 83 ms treatment. The responsiveness was consequently the highest in the 0 ms treatment (33 %) and lowest in the control treatment (21 %), however not significantly different ($\chi^2 = 2.09$, d.f. = 3, $p = 0.55$). Of the 18 trials of simultaneous stimulation, 16 fish (89 %) escaped with double bend response. Of the 16 trials with 33 ms treatment, 13 fish (81 %) escaped with double bend response. Of the 17 trials of the 83 ms treatment, 11 fish (65 %) escaped with

double bend response. Of the 12 control trials, 11 fish (92 %) escaped with double bend response. There was no significant difference between treatments in the type of C-start (χ^2 = 4.54, d.f. = 3, p = 0.21). The number of stimuli used for the statistical analysis of the data for each treatment is stated in table S2 together with further results.

The escape trajectories of 0 ms and 33 ms treatments were smaller than the ones of 83 ms and control treatments [0 ms: 86.09 ± 34.26 (n = 18), 33 ms: 89.24 ± 44.71 (n = 16), 83 ms: 104.27 ± 57.97 (n = 17), Control: 100.85 ± 37.41 (n = 12); Table 1]. According to I-T approach, the model in which escape trajectories of 33 ms treatment was separated from others (Top row of table 1; i.e 33 ms treatment was different from other treatments) was the best model (AIC = 597.46, ⊿AIC = 0.00; 33 ms: $Mean_1$ = 87.58 degree, $Mean_2$ = 257.60 degree; 0 ms + 83 ms + Control: $Mean_1$ = 95.07 degree, $Mean_2$ = 269.91 degree; Table 1; Figure 1). Escape trajectory of 33 ms treatment was smaller than of the other treatments. The model in which all treatments were considered as the same (Second row of table 1; i.e. There was no difference between treatments) was the second best model (AIC = 597.58, ⊿AIC = 0.12). The model in which treatments were divided into two groups (Third row of table 1; i.e. 0 ms treatment and 33 ms treatment were different from 83 ms treatment and control) was the third best model (AIC = 598.59, ⊿AIC = 1.13). Stage 1 turning angles of 0 ms and 33ms treatments were smaller than the ones of 83 ms and control treatments [0ms: 37.71 ± 29.39 (n = 18), 33ms: 45.17 ± 45.11 (n = 16), 83ms: 61.02 ± 51.01 (n = 17), Control: 55.03 ±37.78 (n = 12); Table S2]. According to I-T approach, the model in which treatments were divided into two groups (Forth row of table 1; i.e 0 ms treatment and 33 ms treatment were different from 83 ms treatment and control) was the best model (AIC = 524.29, ⊿AIC = 0.00; 0 ms + 33 ms: $Mean_1$ = 30.19 degree, $Mean_2$ = 155.17 degree; 83 ms + Control: $Mean_1$ = 41.15 degree, $Mean_2$ = 167.24 degree; Table 1; Figure 2). There were no other models with ⊿AIC smaller than 2. Stage 2 turning angles had no trends [0ms: 33.71 ± 9.01 (n = 16), 33ms: 30.27 ± 9.06 (n = 13), 83ms: 29.87 ± 10.28 (n = 11), Control: 31.00 ± 10.63 (n = 11); Table S2]. Stage 2 turning angle had no significant difference between treatments (Kruskal-Wallis rank sum test: $\chi2$ = 1.70, df = 3, p = 0.64; Figure 3). Results of statistical analyses of other variables are show in table 2. All other variables have no significant difference.

Table 1: The top 3 (AIC) results of the information-theoretic (I-T) approach. The top row of each variable shows the best model. The boldface shows that the model of ⊿AIC, which is the difference of AIC between each model and the best model compared, was less than 2. All results are shown in table S1.

Variable	Model	AIC	⊿AIC
Escape Trajectory	**0 ms + 83 ms + Control; 33 ms**	**597.46**	**0**
	0 ms + 33 ms + 83 ms + Control	**597.58**	**0.12**
	0 ms + 33 ms; 83 ms + Control	**598.59**	**1.13**
Stage 1 Turning Angle	**0 ms + 33 ms; 83 ms + Control**	**524.29**	**0**
	0 ms + 33 ms + 83 ms; Control	530.6	6.31
	0 ms + 33 ms; 83 ms; Control	531.05	6.76

Table 2: The result of multiple comparisons for treatments.

Variable	Test	Statistical value	d.f.	p-value
Apparent Looming Threshold	Kruskal-Wallis test	$\chi^2 = 2.52$	3	0.47
Initial Orientation	Kruskal-Wallis test	$\chi^2 = 1.99$	3	0.57
Maximum Speed	One-way ANOVA	$F = 0.31$	3, 59	0.82
Maximum Acceleration	Kruskal-Wallis test	$\chi^2 = 0.31$	3	0.96
Stage 1 Duration	Kruskal-Wallis test	$\chi^2 = 3.62$	3	0.31
Stage 1 Turning Rate	One-way ANOVA	$F = 1.95$	3, 59	0.13
Maximum Stage 1 Turning Rate	One-way ANOVA	$F = 2.01$	3, 59	0.12
Stage 2 Duration	Kruskal-Wallis test	$\chi^2 = 1.84$	3	0.61
Stage 2 Turning Rate	Kruskal-Wallis test	$\chi^2 = 1.68$	3	0.64
Cumulative Distance	One-way ANOVA	$F = 1.27$	3	0.29

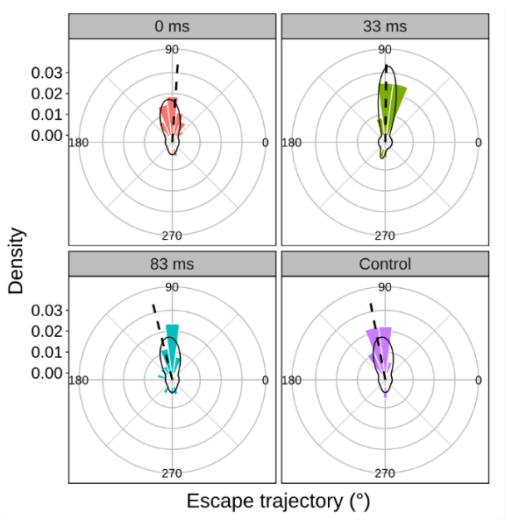


Figure 1: Escape trajectories

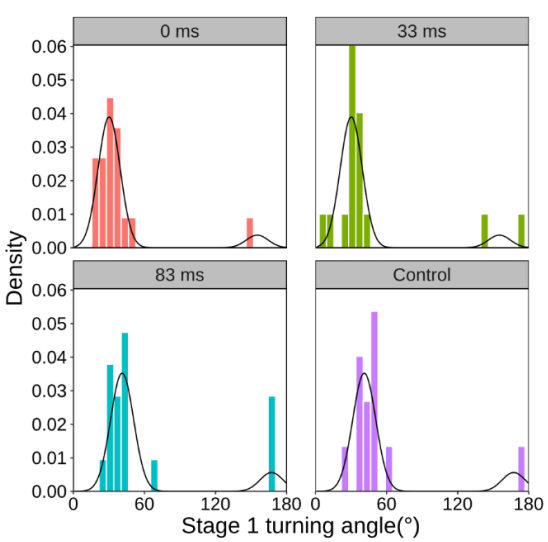
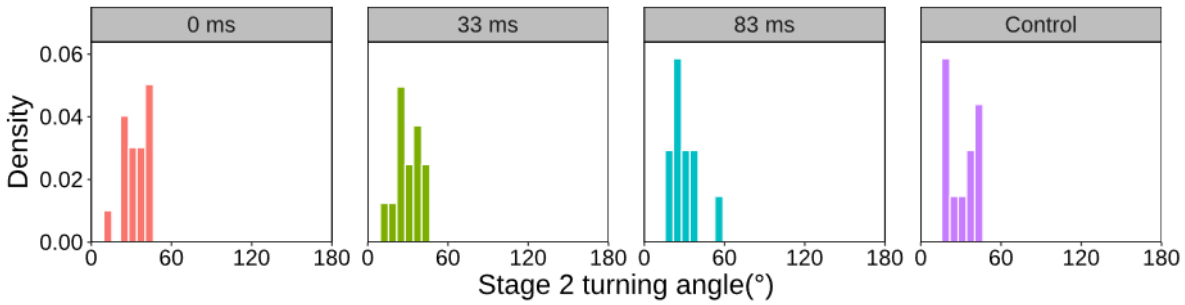


Figure 2: Stage 1 turning angle (degree)

Figure 3: Stage 2 turning angle (degree)



DISCUSSION

Escape trajectory in the 33 ms treatment was closest to a 90-degree angle. Stage 1 turning angle was smaller when the second stimulus came simultaneously or after a 33 ms delay. However, stage 2 turning angle and other kinematic variables had no significant difference between treatments. In the 33 ms treatment, the second stimulus came within the escape latency of the first stimulus. Our results showed that the escape trajectory was modulated through the change of stage 1 turning angle when the second stimulus started during the escape latency, which is within the duration of the act of the brainstem neural network. Escape trajectory is a consequence of the stage 1 turning angle and the stage 2 turning angle (Domenici and Blake, 1997; Domenici and Blake, 1993; Eaton et al., 2001). Domenici and Blake (1993) suggest that the sensory feedback mechanism could modulate the stage 1 turning angle. Our result was consistent with this hypothesis. Therefore, the sensory feedback may have a superiority over the brainstem escape network in 33 ms treatment.

Although 0 ms treatment and 33 ms treatments were different from 83 ms treatment and control treatment in the stage 1 turning angle, only 33 ms treatment was different from the others in the escape trajectory. The second stimulus came within the escape latency both in the 0 ms treatment and 33 ms treatment. Fish usually escape away from the stimulus (Domenici and Blake, 1997; Eaton et al., 2001; Kimura and Kawabata, 2018). In this study, all fish also escaped away from the control stimulus. In the 0 ms treatments, the away response rate to the stimulus that was played on the monitor, which always played the first stimulus in the other treatments was the lowest (61 %). Thus, 39 % of fish in the 0 ms treatment escaped to the stimulus that was simultaneously played on the second monitor. The escape trajectory was defined as the angle of the fish to the stimulus played on the monitor that played the first stimulus in the other treatments. Therefore, the escape trajectory should be considered from both stimuli, since they were played at the same time. Thus, the results of the escape trajectory in 0 ms treatment may not be consistent. Further analyses are needed to correctly compare turning angles and escape trajectory in the 0 ms treatment.

When the treatments are divided into two groups, one group is 0 ms and 33 ms treatments, another group is 83 ms and control treatments, this model of escape

trajectory has a small ⊿AIC (1.13). Furthermore, the mean escape trajectory of 0 ms treatment is close to the one of 33 ms treatment. Therefore, it is possible that the escape trajectory in both 0 ms and 33 ms treatments were different from the one in 83 ms treatment and control treatment. It is consistent with our result of the stage 1 turn angle. The escape trajectory may be the result of the control of the sensory feedback in stage 1. However, another model of escape trajectory that contained all four treatments also has a small ⊿AIC (0.12). Hence, escape trajectories could not be different between treatments. This result of the escape trajectory may be attributed to our experimental tank shape.

A previous study of the same species using a single looming stimulus (Paglianti and Domenici, 2006) resulted in mean escape trajectories of 132 degrees, while our study's escape trajectory in the control treatment with a single stimulus was 100.85 ± 37.41 (n = 12). This difference could be explained by the wall effect that modulates the escape trajectory to avoid collision into a wall of the tank, even though the distance from our fish to the walls was a minimum of 1.5 times the total length of the fish. This distance should have been enough to prevent the wall effect (Eaton and Emberley, 1991). However, our tank had a rectangular shape (125.5 cm L x 57 cm W), so fish may prefer lateral direction (90 or 180 degrees) to escape over a longer distance. However, optimal escape trajectory should be different from each treatment because the optimal escape trajectory to a single stimulus is 135 degrees (Domenici et al., 2011b). In future studies, a square or circular tank should be used to prevent preferred escape trajectories parallel to the long side of the tank.

In conclusion, our results show that in Pacific staghorn sculpin, a sensory feedback mechanism of the escape response exists during the initial phase of the escape response. The escape trajectory was modulated to be close to 90 degrees by the sensory feedback mechanism, possibly because a 90-degree angle should be the optimal escape trajectory to maximize the distance between the prey and two stimuli from opposite sides. In future research, it would be interesting to assess, if a modulation of the escape response via sensory feedback can be verified in more species.

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SUPPLEMENTAL INFORMATION

Table S1: The results of the information-theoretic approach. Top row of each variable shows the best model. The boldface shows that the model of ⊿AIC, which is the difference of AIC between each model and the best model compared, was less than 2.

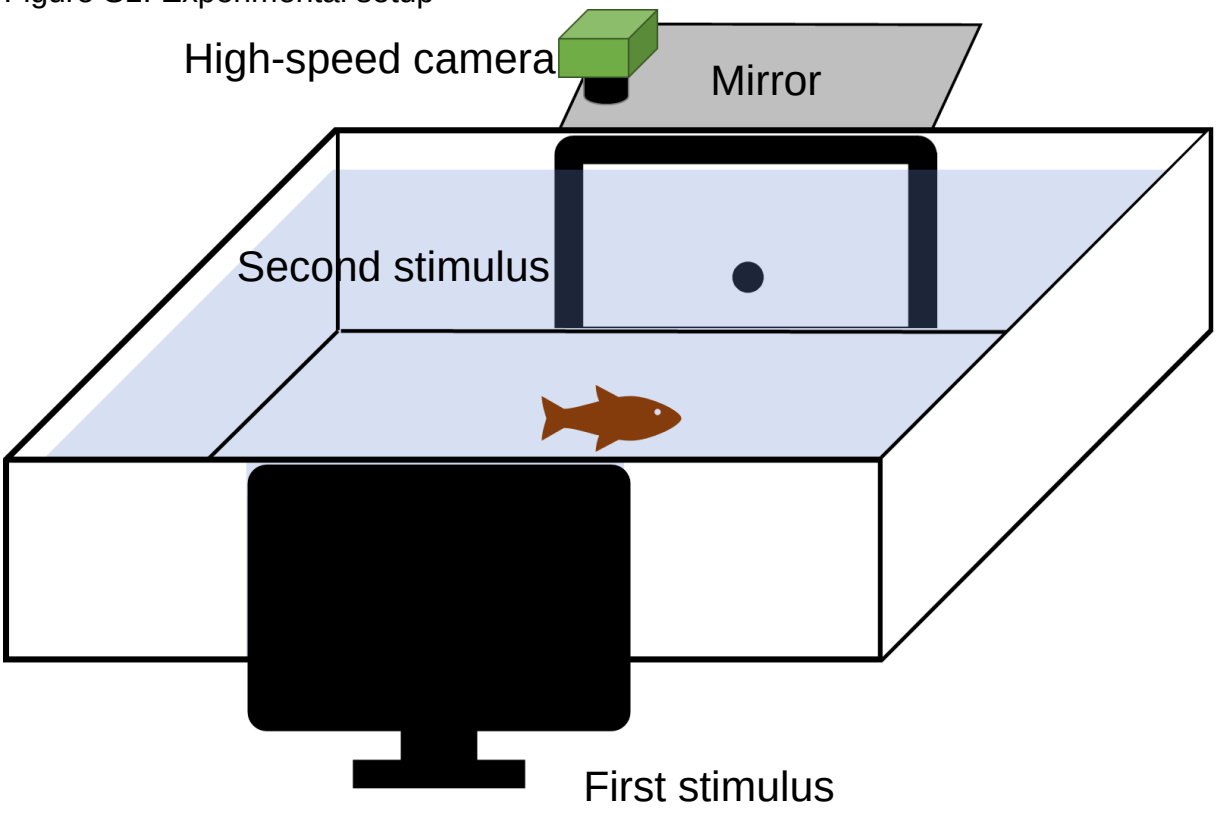
Variable	Model	AIC	⊿AIC
Escape Trajectory	**0 ms + 83 ms + Control; 33 ms**	**597.46**	**0**
	0 ms + 33 ms + 83 ms + Control	**597.58**	**0.12**
	0 ms + 33 ms; 83 ms + Control	**598.59**	**1.13**
	0 ms + 83 ms; 33 ms + Control	600.6	3.14
	0 ms + 33 ms + Control; 83 ms	600.92	3.46
	0 ms; 33 ms; 83 ms + Control	601.03	3.57
	0 ms; 33 ms + 83 ms + Control	602.65	5.19
	0 ms + 83ms; 33 ms; Control	602.78	5.32
	0 ms + Control; 33 ms; 83 ms	603.06	5.6
	0 ms + 33 ms + 83 ms; Control	603.13	5.67
	0 ms + 33 ms; 83 ms; Control	604.78	7.32
	0 ms; 33 ms + 83 ms; Control	605.03	7.57
	0 ms + Control; 33 ms + 83 ms	605.25	7.79
	0 ms; 33 ms; 83 ms; Control	607.21	9.75
	0 ms; 33 ms + 83 ms; Control	609.4	11.94
Stage 1 Turning Angle	**0 ms + 33 ms; 83 ms + Control**	**524.29**	**0**
	0 ms + 33 ms + 83 ms; Control	530.6	6.31
	0 ms + 33 ms; 83 ms; Control	531.05	6.76
	0 ms + 33 ms + 83 ms + Control	532.9	8.61
	0 ms + 83 ms + Control; 33 ms	536.45	12.16
	0 ms + 83ms; 33 ms; Control	537.63	13.35
	0 ms + 33 ms + Control; 83 ms	538.91	14.63
	0 ms + Control; 33 ms + 83 ms	540.9	16.62
	0 ms + 83 ms; 33 ms + Control	541.57	17.29
	0 ms + Control; 33 ms; 83 ms	544.02	19.74
	0 ms; 33 ms; 83 ms + Control	568.2	43.91
	0 ms; 33 ms + 83 ms + Control	571.04	46.76
	0 ms; 33 ms + 83 ms; Control	571.84	47.55
	0 ms; 33 ms; 83 ms; Control	574.96	50.67

0 ms; 33 ms + Control; 83 ms	578.9	54.61	

Table S2: Result summary table, values mean ± SD

Variable	0 ms	33 ms	83 ms	Control
Escape Trajectory (°)	86.09 ± 34.26 (n = 18)	89.24 ± 44.71 (n = 16)	104.27 ± 57.97 (n = 17)	100.85 ± 37.41 (n = 12)
Apparent Looming Threshold (rad/s)	3.18 ± 1.82 (n = 18)	3.34 ± 3.08 (n = 14)	3.51 ± 2.03 (n = 16)	2.55 ± 1.99 (n = 12)
Initial Orientation (°)	83.59 ± 11.68 (n = 18)	88.62 ± 11.29 (n = 16)	86.76 ± 8.79 (n = 17)	87.78 ± 9.98 (n = 12)
Maximum Speed (cm/s)	92.77 ± 28.54 (n = 18)	95.29 ± 33.16 (n = 16)	95.26 ± 29.47 (n = 17)	103.29 ± 29.94 (n = 12)
Maximum Acceleration (cm/s/s)	6109.23 ± 2156.49 (n = 18)	6166.71 ± 2458.32 (n = 16)	5997.9 ± 2026.82 (n = 17)	5743.34 ± 1759.27 (n = 12)
Stage 1 Turning Angle (°)	37.71 ± 29.39 (n = 18)	45.17 ± 45.11 (n = 16)	61.02 ± 51.01 (n = 17)	55.03 ± 37.78 (n = 12)
Stage 1 Duration (s)	0.04 ± 0.03 (n = 18)	0.04 ± 0.03 (n = 16)	0.05 ± 0.03 (n = 17)	0.04 ± 0.01 (n = 12)
Stage 1 Turning Rate (°/s)	1025.55 ± 354.46 (n = 18)	1024.79 ± 458.09 (n = 16)	1188.98 ± 422.41 (n = 17)	1351.62 ± 444.27 (n = 12)
Maximum Stage 1 Turning Rate (°/s)	1451.81 ± 452.24 (n = 18)	1489.76 ± 718.62 (n = 16)	1765.51 ± 713.2 (n = 17)	1957.3 ± 660.32 (n = 12)
Stage 2 Turning Angle (°)	33.71 ± 9.01 (n = 16)	30.27 ± 9.06 (n = 13)	29.87 ± 10.28 (n = 11)	31 ± 10.63 (n = 11)
Stage 2 Duration (s)	0.04 ± 0.01 (n = 16)	0.04 ± 0.01 (n = 13)	0.04 ± 0.01 (n = 11)	0.04 ± 0.01 (n = 11)
Stage 2 Turning Rate (°/s)	866.18 ± 268.33 (n = 16)	784.52 ± 275.1 (n = 13)	753.74 ± 345.85 (n = 11)	770.14 ± 398.02 (n = 11)
Cumulative Distance (cm)	4.19 ± 1.32 (n = 18)	4.18 ± 1.57 (n = 16)	4.45 ± 1.52 (n = 17)	5.1 ± 1 (n = 12)
Type of C-start	Double Bend Response = 16; Single Bend Response = 2	Double Bend Response = 13; Single Bend Response = 3	Double Bend Response = 11; Single Bend Response = 6	Double Bend Response = 11; Single Bend Response = 1

Figure S1: Experimental setup



LoomeR script

```
library(loomeR)

# 1. Create a model

# Simple constant speed model
# (speed in cm/s, frame rate in Hz, all other inputs in cm)

x <- constant_speed_model(
        screen_distance = (57+5)/2,    # How far from the screen is your observing specimen?
        frame_rate = 60,               # Frame rate you want the final animation to be
        speed = 100,                   # Speed of the simulated oncoming object
        attacker_diameter = 24,        # Diameter of the simulated oncoming object
        start_distance = 200           # Starting distance of the simulated oncoming object
)

looming_animation_calib(
        correction_range = c(0.02, 0.03),
        width = 1600,
        height = 1200,
        ruler = 10
)

# 2. Use the model to create the animation

looming_animation(
        x,
        correction=0.02222,
        width=1600,
        height=1200,
        background = "white",
        fill="black",
        frame_number = T,
        filename = "Looming_for_2stimuli"
)
```