Behavioural and life-history responses of mosquitofish to biologically-inspired and interactive robotic predators

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SUPPLEMENTARY MATERIAL

MATERIALS AND METHODS

Robotic platform and bioinspired predator replicas

A robotic platform with three degrees of freedom was utilized to actuate the predator replica in the experimental arena. A total of three stepper motors were installed on the platform and two of them were used to translate the replica on the X-Y Cartesian plane, while the third motor was used to adjust the orientation of the fish. This mechanism allowed for mimicking in the swimming pattern of live basses in shallow waters. Starting with a commercially available Cartesian plotter (XY Plotter Robot Kit, Makeblock Co., Ltd, Shenzhen, China), we included a third stepper motor (NEMA 14, Pololu Corp., Las Vegas, NV, USA) on the end effector of the Cartesian plotter using a 3-D printed bracket. To control the motors and allow communication with the computer, we used a dedicated microcontroller for each motor (Kuman CNC Kit, Kuman Trade Co., Shenzhen, China). To interface each microcontroller with the computer, we utilized GRBL 0.9 tool (Grbl^{TM} v0.9, Copyright (c) 2012-2014 Sungeun K. Jeon) and we used Matlab R2018 (The MathWorks, Inc., Natick, MA, USA) to establish serial communication regarding position, speed, and turn rate data with the platform.

Experimental conditions and live tracking

To capture the position of the live fish and implement the closed-loop conditions (CL1 and CL2), a real-time tracking and control system was developed based on the computer vision toolbox in Matlab R2018 (The MathWorks, Inc., Natick, MA, USA). The tracking algorithm was a motion-based multiple object tracking. Specifically, focusing on a predetermined region of interest, an initial frame was obtained, cropped, and converted into a black and white image. Then, this initial frame was subtracted from the instantaneous frame to form a image mask. After filtering noise, the mask was utilized to identify the focal mosquitofish, whereby blob analyses were performed to track the centroids of the fish over time (trial).

If the system failed to identify the position of the focal fish, the position would be predicted by a Kalman filter based on the history of the trajectory, under constant velocity assumption. To help monitor the experimental procedure, two indicators were implemented to identify whether the robot was attacking or swimming and whether its distance from the fish was larger or smaller than 5 cm.

Data processing

Trajectories of mosquitofish and replicas were extracted from the videos to gather information on both behaviours and positions of fish and replicas. First, the obtained trajectories were smoothed using Gaussian smoothing with a moving window of 30 frames (1.5 s) to reduce measurement noise. Then, the smoothed data was processed to estimate distance moved (in cm), freezing (in s), speed variance during swimming (in cm^2/s^2), and mean distance from the replica (in cm) at each minute of a trial.

To calculate the total distance moved, the distance moved in each time frame (0.05 to 904 s) was calculated by taking the norm of the vector between positions in consecutive frames The same distance was divided by the time step (0.05 s) to obtain the instantaneous speed, which we used to isolate freezing instances. Freezing instances were excluded from the overall observation to compute the mean speed and speed variance. The distance of the fish from the replica was calculated by taking norm of the vector between the fish position and replica position. Then, the mean values of the distances were computed for each minute of the trial as well as for the overall trial.

RESULTS

Table S1 Phenotypic-correlation estimates between pairs of traits (body length, mass, and Fulton's *K*).

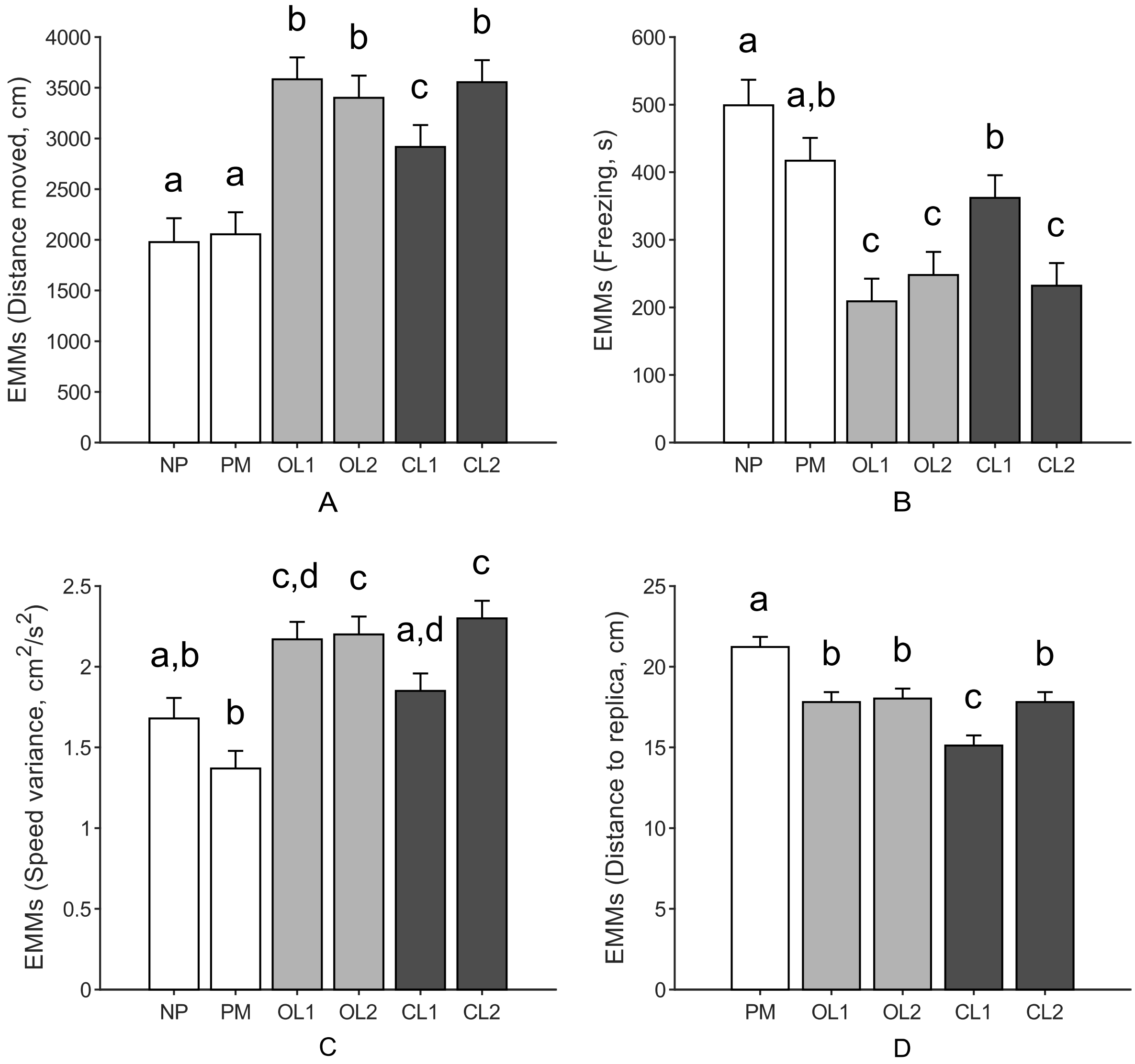
	Body length	Body mass	*K*
Body length	-	**0.624**	**-0.344**
Body mass	0.499; 0.745	-	-0.013
K	-0.492; -0.176	-0.184; 0.151	-

The best estimate of correlation coefficients (values above the diagonal) and their 95% credible intervals (values below the diagonal) are represented for each pair of traits. We used bivariate linear mixed-effects models using Markov Chain Monte Carlo techniques, including the individual as a random effect (that is, random intercepts) to account for repeated measures. Significant results correspond to correlation coefficients whose credible intervals do not overlap with zero (highlighted in bold).

Table S2 Results from LMMs with Fulton's *K* (body condition) as the dependent variable.

K				
Fixed factors	Mean Sq	*df*	*F*	*P*
Sex	0.009	1, 71	0.507	0.479
Week	0.927	1, 436	50.036	<0.001***
Condition	0.185	6, 436	9.990	<0.001***
Random effects	Estimate (SE)	ΔAIC	χ^2_1	*P*
V_{among}	0.018 ()	186.667	188.667	<0.001***
V_{within}	0.018 ()			
Repeatability	0.488			

Sex, week, and condition are included in the models as fixed factors, while random intercepts are included for each individual, which allowed variance decomposition. Within-individual variance (V_{within}), among-individual variance (V_{among}), and repeatability are shown. Test statistics (χ^2_1) and significant levels of the random effects (i.e., intercepts) were estimated using a LRT (*P*) and Akaike Information Criteria (AICs) between the full and the null model. Note that ΔAIC corresponds to the difference in AIC between the null models minus the AIC from the full model. The significance was set at α<0.05, and significant results are indicated with *** (<0.001).



76 **Figure S1** Estimated marginal mean (EMMs) differences represent adjusted mean
77 differences (+ SE) in distance moved, freezing, speed variance, and distance from the replica
78 across conditions once the contribution of fixed effects included in the model (that is,
79 Fulton's *K*, body mass, sex, week) is accounted for. White histograms correspond to control
80 conditions (NP and PM), light grey histograms to open-loop conditions (OL1 and OL2), and
81 dark grey histograms to closed-loop conditions (CL1 and CL2). Means not sharing a common
82 superscript are significantly different. The significance was set at $\alpha<0.05$.

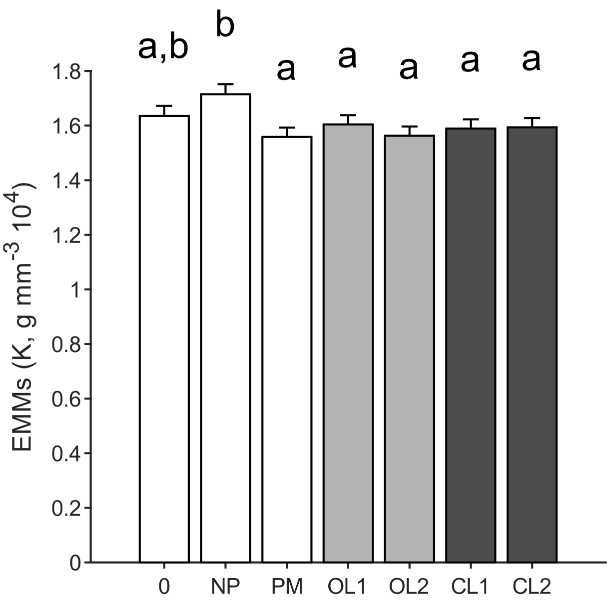


Figure S2 Estimated marginal mean (EMMs) differences represent adjusted mean differences (+ SE) in body condition (Fulton's *K*) across conditions once the contribution of fixed effects included in the model (that is, sex, week) is accounted for. Notably, the first histogram (0) refers to the baseline body condition measured before the beginning of the experiment. White histograms correspond to control conditions (0, NP, and PM), light grey histograms to open-loop conditions (OL1 and OL2), and dark grey histograms to closed-loop conditions (CL1 and CL2). Means not sharing a common superscript are significantly different. The significance was set at α<0.05.