Biology Letters — Supplementary Material

Condition-dependent responses of fish to motorboats

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Methods

Acoustic stimuli

For the motorboat treatment, motorboats were driven continuously 10–200 m from the experimental setup with various steering patterns. Representative recordings were taken of the ambient conditions, of each motorboat used in the experimental trials, and of the holding-tank conditions in the Lizard Island Research Station (LIRS) aquaria (where fish were held overnight prior to trials), in both acoustic-pressure and monoaxial particle-acceleration domains.

Acoustic pressure was measured with a calibrated omnidirectional hydrophone (HiTech HTI-96-MIN with inbuilt preamplifier, manufacturer-calibrated sensitivity -164.3 dB re 1V/µPa; frequency range 0.02–30 kHz; calibrated by manufacturers; High Tech Inc., Gulfport MS). Monoaxial particle acceleration was measured using a calibrated triaxial accelerometer (M20-040; sensitivity 0–3 kHz; Geospectrum Technologies, Dartmouth, Canada). Both measurement devices were connected to a digital eight-track field recorder (Zoom F8 field recorder, sampling rate 48 kHz, Zoom Corporation, Tokyo, Japan). All recording levels were calibrated using a 1 kHz pure sine wave signal of known voltage recorded in-line with an oscilloscope. For analysis, 20 s of motorboat passes from each boat used in the experiments (n=5) were appended together into a single recording; 20 s from each of the eight different ambient recordings were similarly appended into a single recording; and a single 1-min holding-tank recording was used. All recordings were analysed with the paPAM acoustics analysis package [1] using MATLAB v2014a. The power spectral density of each recording was determined (Figure S1). Power spectral densities are presented over a frequency range of 0–3000 Hz as that is likely to fully include the hearing range of the study species. *C. viridis* communicate using a series of clicks between 500 and 1000 Hz during both agonistic and courtship interactions [2], with their hearing range likely covering the same frequency bandwidth.

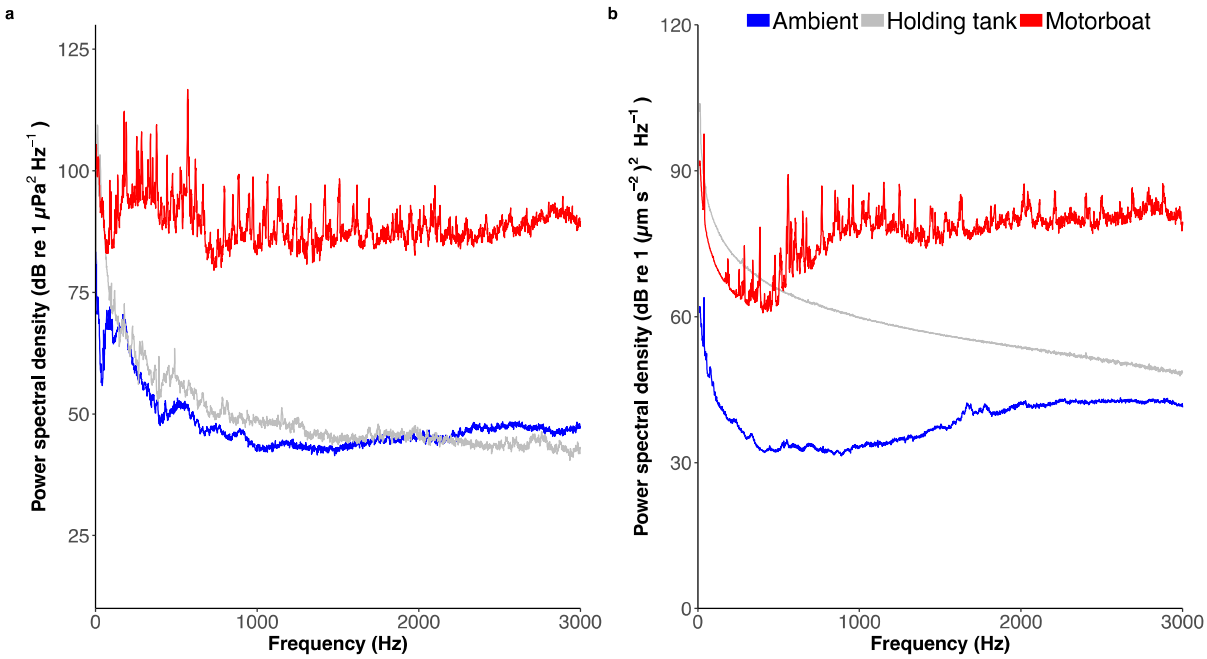

Figure S1. Analysis of acoustic conditions. Spectral content of ambient and motorboat field recordings, as well as the holding-tank conditions, measured in both (a) acoustic pressure and (b) particle acceleration. Mean power spectral densities of all conditions are shown. Sounds analysed with the paPAM acoustics analysis package [1] using MATLAB v2014a; FFT length = sampling frequency (48 kHz for ambient/motorboat noise, 44.1 kHz for holding-tank conditions), Hamming evaluation window, 50% window overlap.

Experimental overview

C. viridis were collected by SCUBA divers using a monofilament barrier net from a single site and transported to holding facilities at LIRS (fish were not fed during the holding period). Following measurement of individuals to assess relative body condition, the 'poor' and 'good' condition fish were kept in two separate aquaria; pooling individuals at this stage avoided the stress of social isolation, but meant individual body conditions could not be matched to their respective results from either experiment. The day after capture, fish were transported in temporary holding containers to the testing sites approximately 0.4–1 km away. Transport was either on foot (approx. 5 min) or by kayak (approx. 20 min), to avoid unwanted exposure to vehicle or motorboat noise prior to testing. At the testing site, fish were held in 250 L temporary aquaria under shade cloth on the beach; the water was refreshed regularly to maintain temperature and oxygen levels. At the end of each day, all fish were released back onto the reef from which they had been collected, but not the same location as capture to avoid the potential for retesting the same fish. Translocation

experiments of *Chromis viridis* indicate that it is unlikely the tested adults will have returned to their original colony [3].

For each physiology trial, individual fish were transferred into separate 200 ml plastic containers; four containers were used simultaneously. Acoustic transparency was predicted to be high for the plastic containers, given typical impedance values of plastic polymers (PET and PP) compared to sea water. To control for the influence of activity on OBR, fish activity level was characterised using a three-point ordinal scale: 1 (no swimming), 2 (occasional swimming; periods of rest interspersed with bouts of swimming) or 3 (constant swimming in the tube) [4]. Data were extracted from individual videos using QuickTime Player, with the play speed reduced to allow accurate counting of opercular beats. Individual trial videos of fish OBR were excluded from further analysis if the camera malfunctioned and part of the trial was lost or if the fish was out of sight and OBR could not be determined (n=9).

For each behavioural trial, an individual fish was transferred into a 500 ml transparent plastic pot that was held in place with a bungee cord on a concrete block positioned on the seabed at 1–2 m depth (see Figure S2). Acoustic transparency of the plastic pot was predicted to be high (see above). ImageJ (https://imagej.nih.gov/ij/) was used to measure the distance between the stimulus and the position of the fish upon startling (measurement taken from tip of the looming stimulus to the closest point on the fish). The distance scale was calibrated with a known distance in the experimental setup. Videos were removed from analysis if fish were facing away from the stimulus upon release or were out of sight of the camera in the top of the chamber, if there was camera failure or if the trial was disrupted by green sea turtles *Chelonia mydas* (n=9).

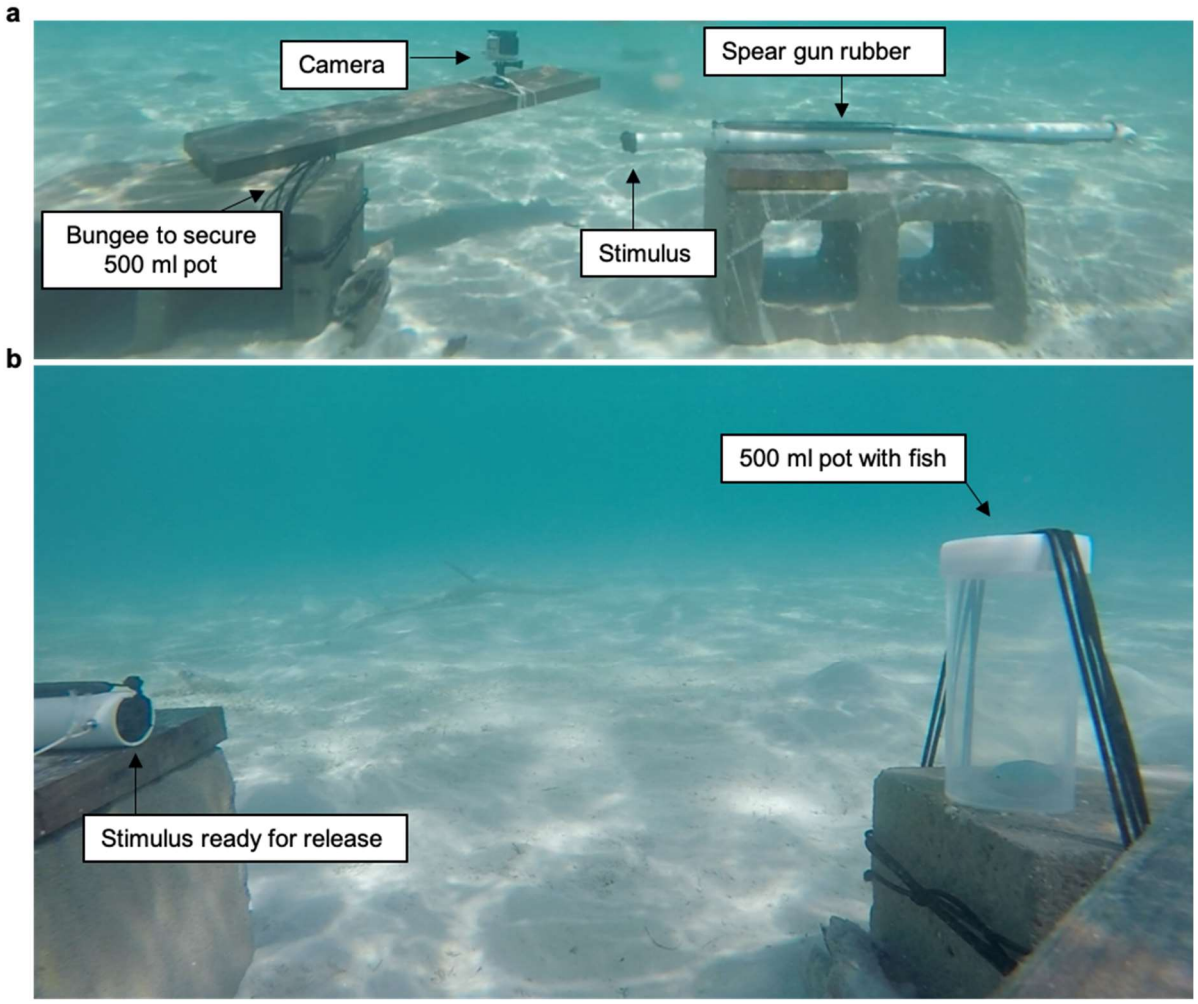

Figure S2. Images showing the standardised setup for the looming-stimulus experiment. **(a)** Experimental setup: the 500 ml pot containing the fish (absent from this photo) was held in place by the black bungee cord seen on the left-hand side of the image. The stimulus consisted of a 73-cm section of PVC pipe with a black end cap (seen here, partly protruding from the larger PVC pipe within which it was normally housed). The GoPro camera in the background was used to record each trial. All equipment was anchored to the seabed by attachment to the two breeze blocks. **(b)** Experimental setup as viewed from the camera used to record the trials.

Statistical analysis

All statistical analyses were carried out in R (Version 3.2.2) [5]. Mixed-effects models were used to take account of random as well as fixed terms. In all analyses, results were determined by comparisons excluding the term of interest; all post-hoc tests were performed using the *emmeans* package [6].

Pre-testing OBR in the two conditions was compared with a Welch two-sample t-test, following visual assessment for normality and the presence of outliers (1.5 times the interquartile range). Treatment OBR data were analysed with a linear mixed model, following visual assessment of the residual frequency distribution, quantile–quantile plot and residual vs fitted plot to confirm these data met the assumptions of normality of residuals and homogeneity of variance; Cook's distance was determined to check for outliers. Removal of data points (based on a cut-off value of $4/n$) did not qualitatively alter any statistical output so all results presented are based on the complete dataset. OBR was analysed with treatment (motorboat vs ambient), body condition (good vs poor) and the interaction between treatment and body condition as fixed factors. Fish activity level and cohort (representing the population of fish caught each day) were included as random terms; activity was scored per minute and subsequently combined to produce an overall score for the 5-min exposure period.

For the looming-stimulus experiment, the likelihood of fish exhibiting a startle response and the distance from the stimulus at the point of startle were analysed with generalised linear mixed models with a binomial and gamma error distribution, respectively. These error distributions were selected because they improved model fit, as assessed using visual inspection of quantile–quantile plots. Both models included treatment (motorboat vs ambient), body condition (good vs poor) and their interaction as fixed factors, and cohort (representing the population of fish caught each day) as a random term. For the continuous data, the normality of standardised residuals and homogeneity of variance were assessed using quantile–quantile plots and by plotting the residuals against the fitted values. Cook's distance was used to assess for the presence of outliers. Removal of detected data points (based on a cut-off value of $4/n$) did not qualitatively alter post-hoc comparisons; as such, all analysis was conducted on the complete dataset. For the binomial data, scaled residual diagnostic plots (quantile–quantile plots and residual vs predicted) were checked using the *DHARMa* package [7].

Results

The change in OBR from pre-testing period to exposure period was not significantly affected by treatment, body condition or their interaction (ambient: mean difference = 1.33 [95% CI = -7.68–10.1]; motorboat: mean difference = 0.32 [95% CI = -8.81–8.86]); see Table S1 for the full model output.

Table S1. Linear mixed model output for the response in opercular beat rate.

Parameter (reference)	Estimate	Standard Error	Confidence interval (95%)	*t*-value	Variance	SD
model<-lmer(OBR change ~ Noise*Condition+(1|Activity Exposure Period)+(1|Cohort), data = Harding et al data)						
Treatment (Motorboat)	1.50	4.55	-7.39–10.35	0.33		
Body condition (Poor)	2.23	4.49	-6.64–10.88	0.50		
Treatment (Motorboat): Body condition (Poor)	-3.98	6.31	-16.02–8.73	-0.63		
Random term (Activity level)					56.87	7.54
Random term (Cohort)					22.64	4.76

The presence or absence of a startle response to a looming stimulus was assessed in relatively 'good' or 'poor' condition fish exposed to either the ambient or motorboat treatment. See Table S2 for full model output.

Table S2. Generalised linear mixed model output for the startle response.

Parameter (reference)	Estimate	Standard Error	Confidence interval (95%)	*z*-value	Variance	Standard Deviation
model_bin<-glmer(Startle (presence/absence) ~ Noise*Condition + 1(1|Cohort), data = Harding et al data, family = binomial(link = "logit"))						
Treatment (Motorboat)	-1.36	0.88	-3.37–0.25	-1.54		
Body condition (Poor)	-1.23	0.90	-3.27–0.43	-1.37		
Treatment (Motorboat): Body condition (Poor)	1.29	1.12	-0.84–3.67	1.15		
Random (Cohort)					0.01	0.07

In fish that did startle, the distance from the stimulus to the fish at the moment of startle was assessed. See Table S3 for the full model output.

Table S3. Generalised linear mixed model output for the distance to startle.

Parameter (reference)	Estimate	Standard Error	Confidence interval (95%)	*t*-value	Variance	Standard Deviation
model<-glmer(Distance.to.startle..cm. ~ Noise*Condition + (1|Cohort), data = Harding et al data, family = Gamma(link = "sqrt"))						
Treatment (Motorboat)	0.32	0.21	-0.09–0.72	1.55		
Body condition (Poor)	0.01	0.20	-0.38–0.40	0.04		
Treatment (Motorboat): Body condition (Poor)	-0.61	0.29	-1.17– -0.05	-2.15		
Random (Cohort)					0.02	0.12

References

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