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Supplementary Materials for

Cuttlefish use stereopsis to strike at prey

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The PDF file includes:

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Legends for movies S1 to S4

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/2/eaay6036/DC1)

Movie S1 (.mp4 format). Method and animal behavior.
Movie S2 (.mp4 format). Example responses to control and disparate stimuli (relates to Fig. 1).
Movie S3 (.mp4 format). Example responses to quasi-monocular and binocular stimuli (relates to Fig. 2).
Movie S4 (.mp4 format). Example responses to correlated, anticorrelated, and uncorrelated random dot stimuli (relates to Fig. 3).

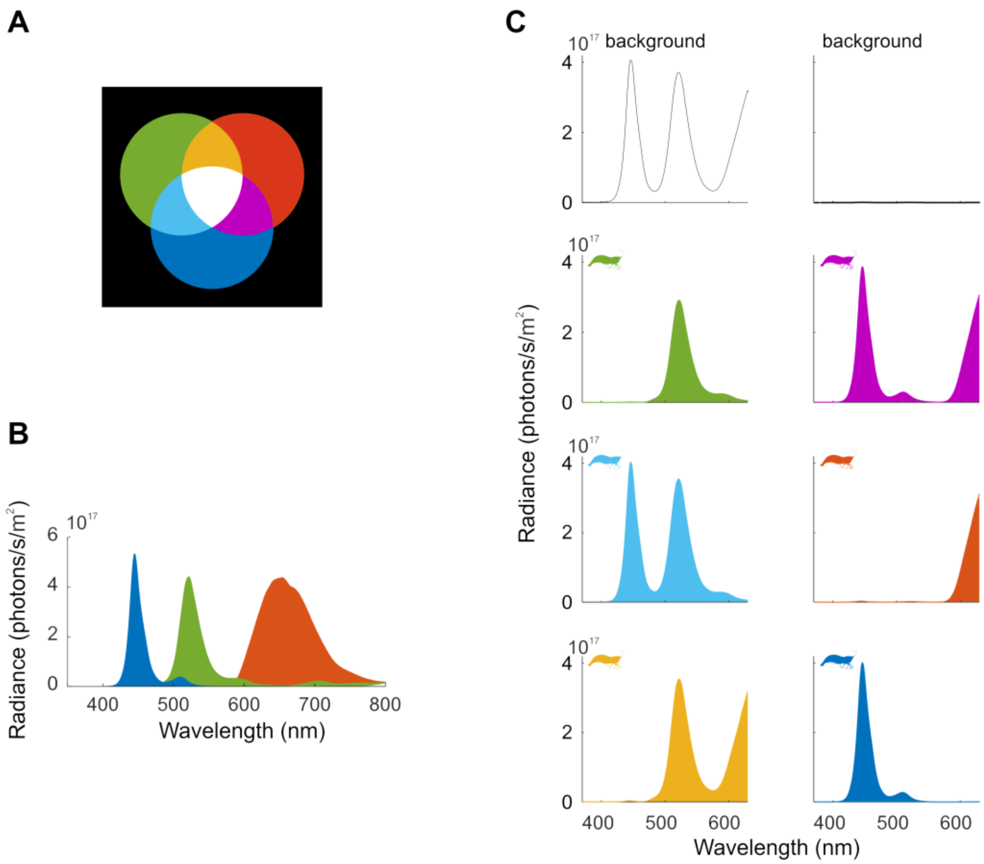

Fig. S1. Spectral content of screen and stimuli. (A) Additive color system of an LCD display. (B) Radiance spectra of the three primary LEDs of the screen display. (C) Spectral radiance of light emitted by each color of background and shrimp stimulus.

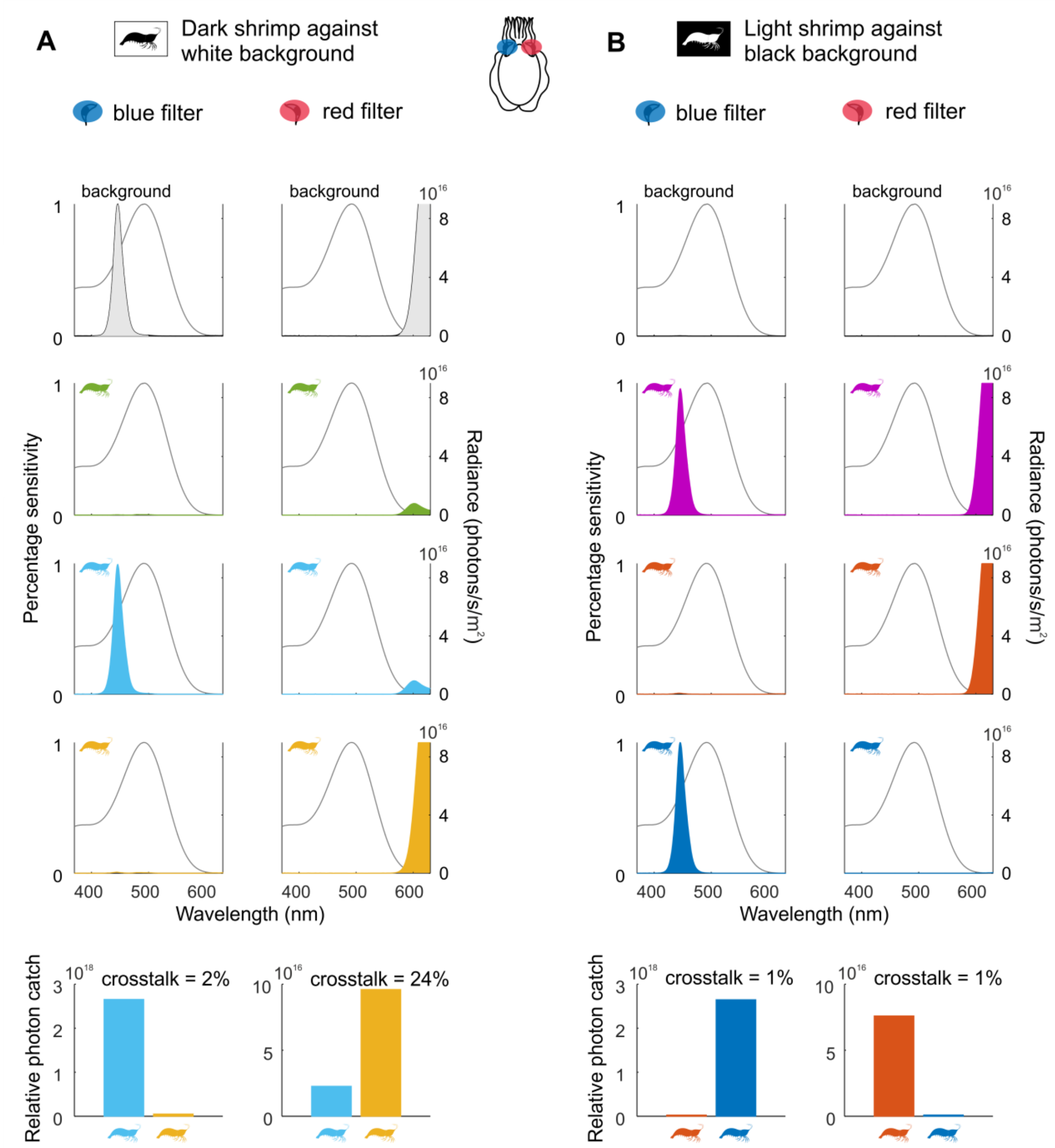

Fig. S2. Stimulus spectra, filter properties, and spectral content cross-talk measurements—red/blue glasses. (A) Stimulus radiance for each dark stimulus on white background as transmitted through the blue and red filters (y-axis on the left) overlaid on the cuttlefish spectral sensitivity curve. Sensitivity curve was calculated after Stavenga *et al*. (*31*). Bottom panels show the relative photon catch for each stimulus that was used to calculate crosstalk between the two channels. Crosstalk is the ratio of the relative photon catch of the stimulus intended for one eye by the other. (B) As in (A) but for light stimulus against dark background.

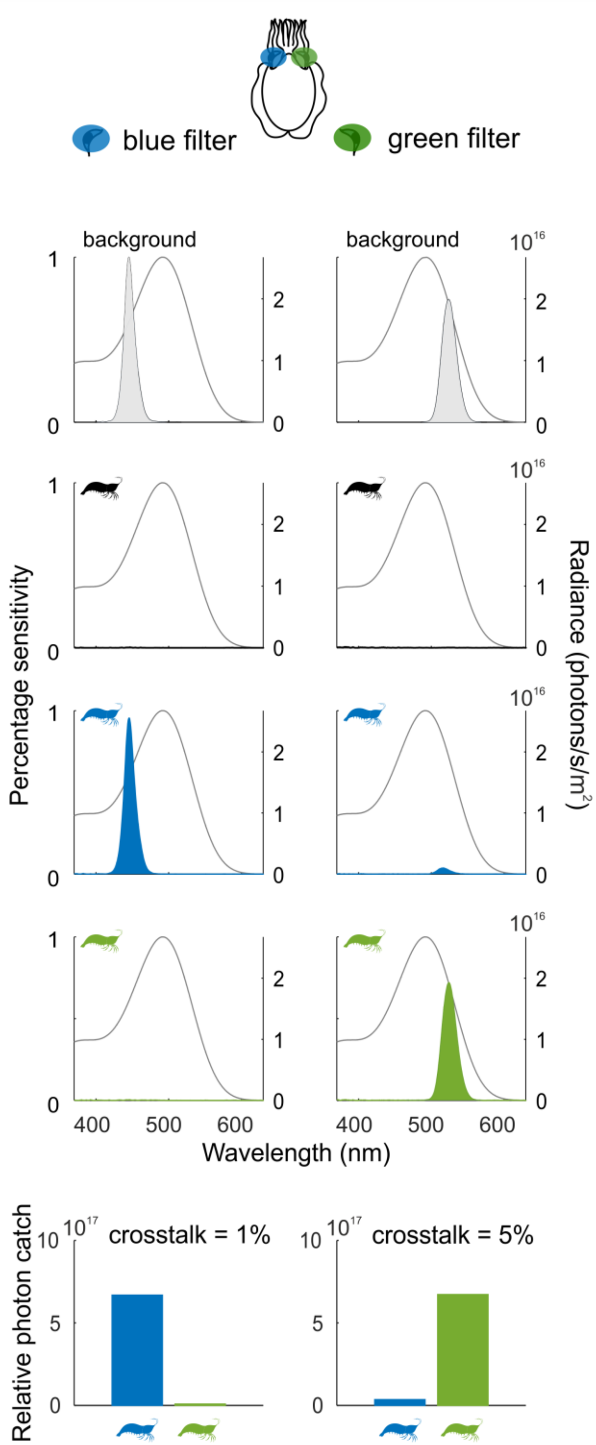

Fig. S3. Stimulus spectra, filter properties, and spectral content cross-talk measurements—blue/green glasses. As in fig. S2A, but for blue and green filters against a light background (See movies S1 to S4).

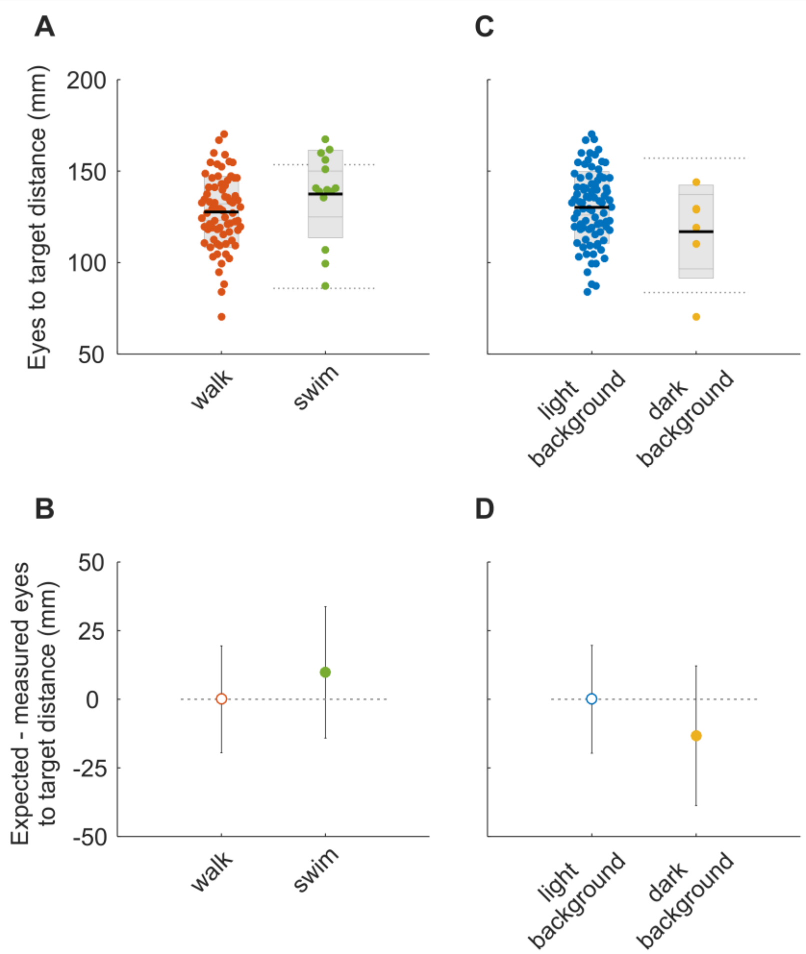

Fig. S4. Speed of stimuli and polarity or background contrast do not alter the perceived location of the 3D prey. (A) and (C) Distance from the animal's eyes to the screen, for different shrimp movement (walking, n = 5, vs swimming, n = 2) and shrimp-background contrast polarity, both for the zero-disparity condition (light background, n = 5, and dark background, n = 1). Black line = mean, inner grey box = SEM, outer grey box = SD, dotted line is 95% CI from bootstrapping. (B) and (D) The expected cuttlefish position when stereopsis is employed was calculated for each stimulus disparity and trial, as shown in A and C, and the swimming shrimp stimulus and dark background experiments were not found to be different from their counterpart stimulus profile (Bootstrap: p = 0.461 and p = 0.333 respectively).

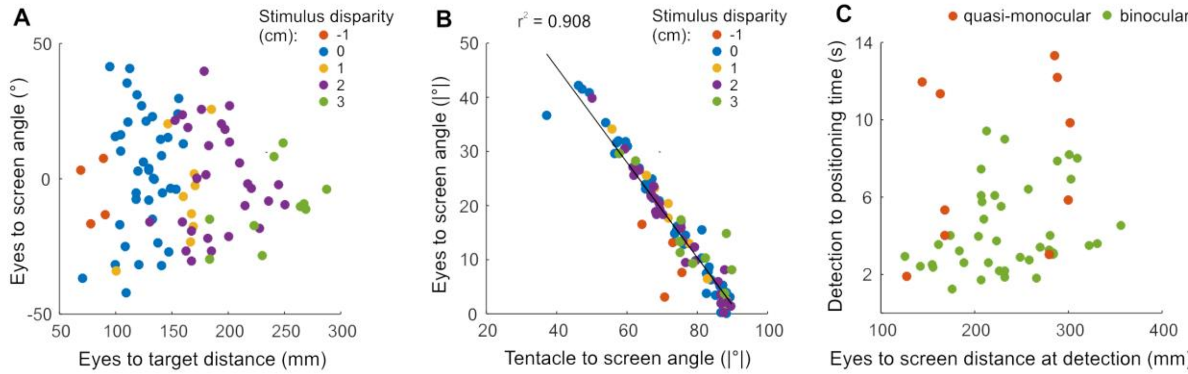

Fig. S5. Control analyses for different hunting behavior parameters. (A) Relation between i. the distance between the animal's eye to the shrimp on screen distance and ii. the angle of the eye axis (an imaginary line perpendicular to the line joining both eyes) to the shrimp on the screen for all stimulus disparities, $r^2 = 0.0030$. (B) Relationship between the absolute angle of the tentacles to the screen and the absolute angle of the eyes to the screen for all disparity conditions. For A-B, n = 2, 5, 2, 5, 2 for -1, 0, 1, 2, 3 cm disparities. (C) Relationship between i. the animal's distance to shrimp on screen when the stimulus was detected and ii. Time elapsed since the stimulus was detected until the tentacles were visible (detection to positioning), combined quasi-monocular and binocular data: $r^2 = 0.0436$, quasi-monocular alone: $r^2 = 0.0679$, n = 3, binocular alone: $r^2 = 0.0860$, n = 5.

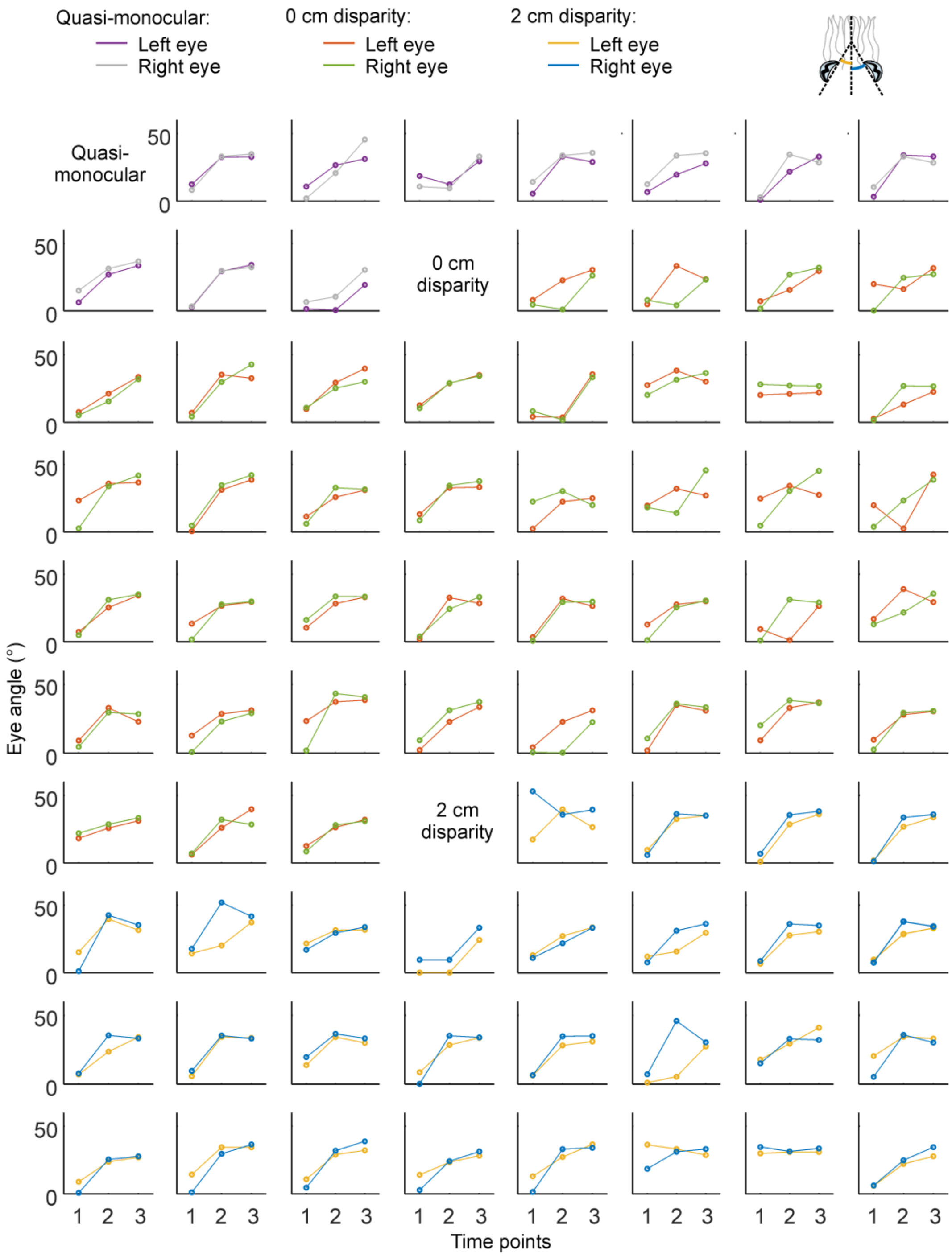

Fig. S6. Cuttlefish eye angles vary greatly during the hunt. Eye angle at three time points during predatory behavior, for all trials from quasi-monocular as well as binocular (0 cm and 2 cm disparity) stimuli. T1 = Immediately prior to shrimp presentation, T2 = After animal has rotated its body to view the screen, and is moving forwards, T3 = During ballistic tentacle strike where 0° is the eye looking laterally.

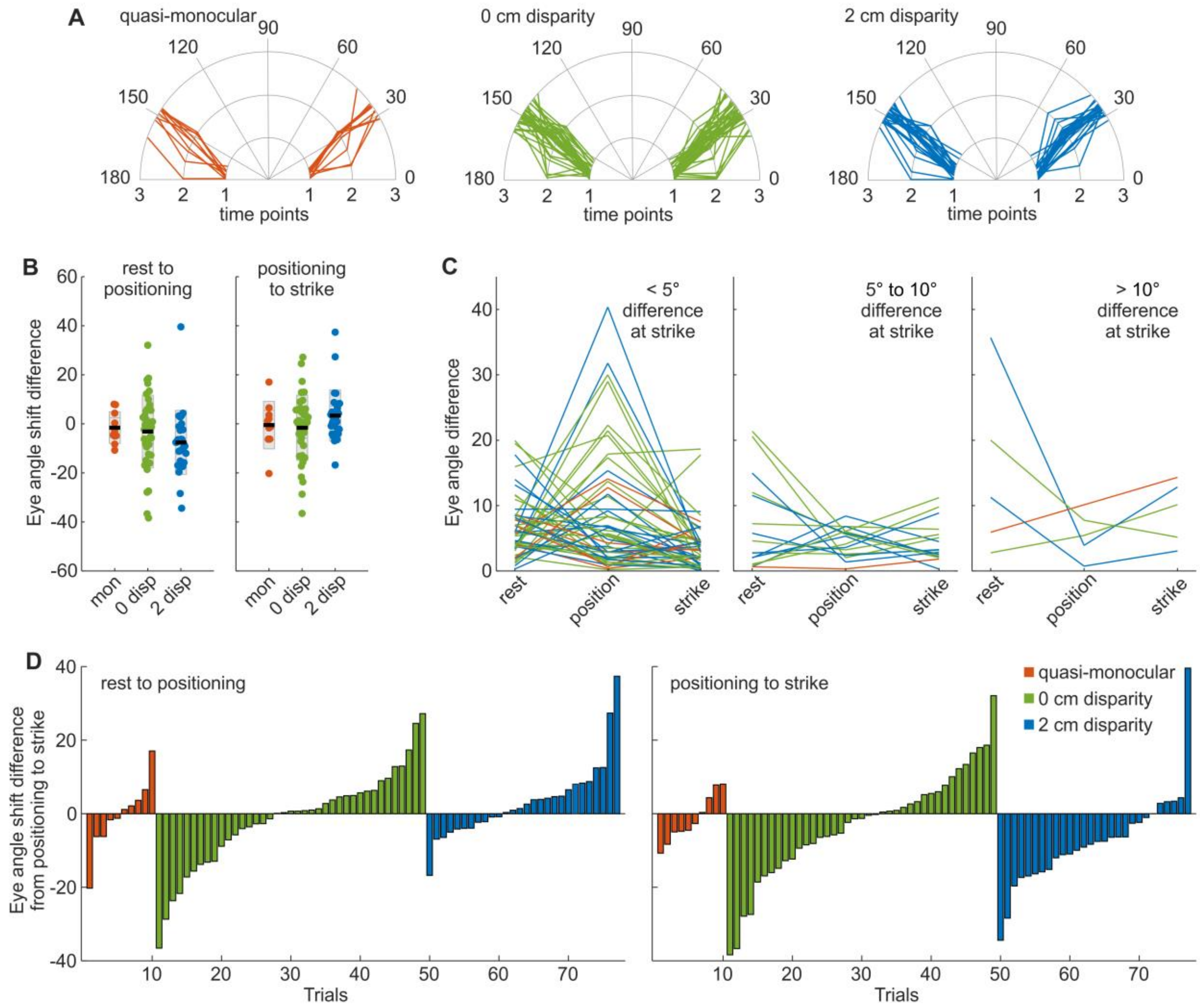

Fig. S7. Diversity in cuttlefish eye vergence during the hunt. These panels provide greater information than the mean and standard deviations shown in Fig. 4C. (A) Eye vergence changes over time between the phases of the hunt (0° = right, 90° = anterior, 180° = left). T1 = Immediately prior to shrimp presentation, T2 = After animal has rotated its body to view the screen, and is moving forwards, T3 = During ballistic tentacle strike. (B) Quasi-monocularly and binocularly stimulated animals have similar eye vergence shifts (for quasi-monocular vs. 0 cm disparity p= 0.404; for 0 vs. 2 cm disparity p = 0.369; for quasi-monocular vs. binocular p = 0.245). (C) The difference in angular position (eye angle) between left and right eye for the phases of the hunt (rest, positioning and striking), categorized into groups which had <5°, 5 to 10°, and >10° angle differences. (D) Distribution of eye angle shifts, from rest to positioning and positioning to striking, among groups (same data as shown in B). n = 3, 5 & 5 for quasi-monocular and binocular 0 & 2 cm disparities respectively.

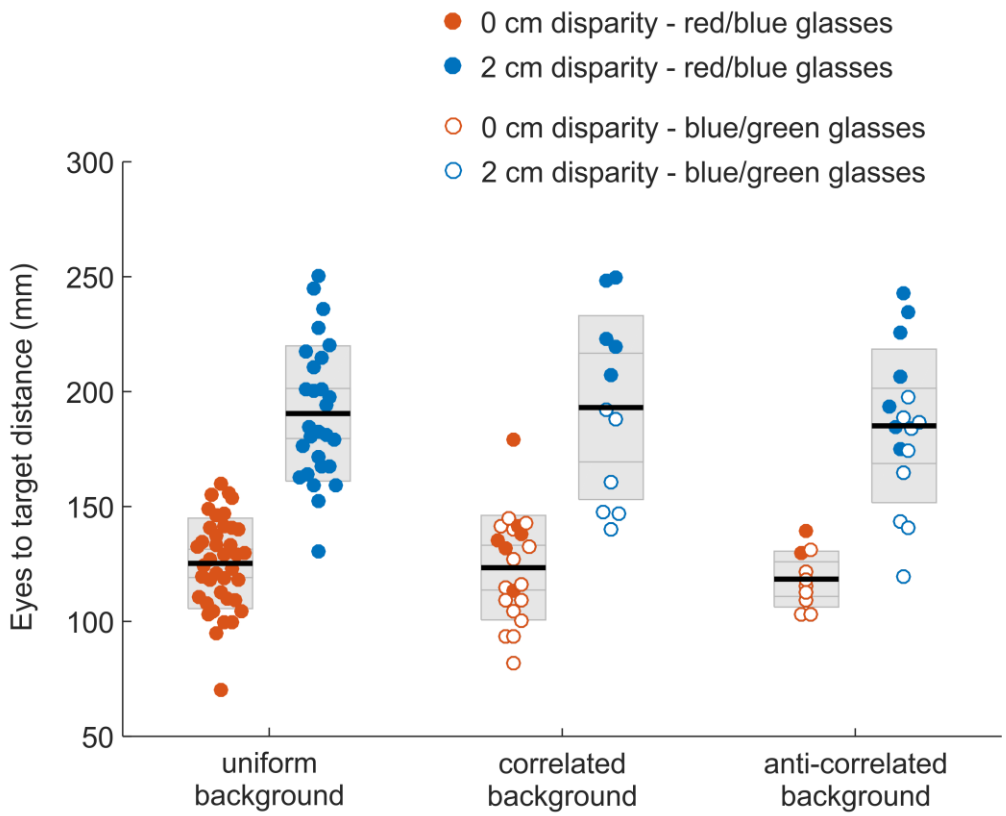

Fig. S8. Positioning does not differ between stimuli and glasses types. Distance to target chosen by the animals is consistent across background types, both for the 0 and 2 cm disparity stimuli. There was no difference found for the 0 cm disparity stimuli across the three background conditions (red dots) nor across the 2 cm disparity stimuli (blue dots) (Uniform: n = 5 for 0 & 2 cm disparities; Correlated: n = 6 & 3 for 0 & 2 cm disparities respectively; Anti-correlated n = 5 & 4 for 0 & 2 cm disparities respectively). Statistical results: 0 cm disparity, uniform vs correlated: p = 0.998, uniform vs anti-correlated: p = 0.977, correlated vs anti-correlated: p = 0.996; 2 cm disparity, uniform vs correlated: p = 1.000, uniform vs anti-correlated: p = 0.987, correlated vs anti-correlated: p = 0.972. Filled and empty markers reflect animals with red/blue glasses and green/blue glasses, respectively.

Movie S1. Method and animal behavior.

Movie S2. Example responses to control and disparate stimuli (relates to Fig. 1).

Movie S3. Example responses to quasi-monocular and binocular stimuli (relates to Fig. 2).

Movie S4. Example responses to correlated, anticorrelated, and uncorrelated random dot stimuli (relates to Fig. 3).