An experimental method for evoking and characterizing dynamic color patterning of cuttlefish during prey capture

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Supplementary Information

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Supplementary Text

A. Movement phases of the robotic prey:

- Natural: Skewer arm rotates clockwise into the water until it is fully submerged and approximately perpendicular to the surface of the water ("90 deg down"), pauses, then "wiggles" 3 times. A single "wiggle" consists of moving 10 deg clockwise (CW), then 10 deg counter-clockwise (CCW), then 10 deg CW again; or 10 deg CCW, then 10 deg CW, then 10 deg CCW again. In between each wiggle, the skewer arm paused for 3 seconds. After 3 wiggles, the skewer arm is moved back out of the water to the "start position."

- Smooth: Skewer arm enters the water and travels smoothly through one full rotation back up to the start position. Each food offering alternates between CW and CCW rotations.

- Predictable: Skewer arm begins to enter the water along a smooth CW or CCW rotation. If the skewer reaches "90 deg down" and pauses, the skewer exits the water back in the direction from which it came. If the skewer enters the water and does not pause, the skewer continues in the same direction to exit the water. The "robotic prey" was designed to randomly choose whether or not to pause during each trial of this stage.

Supplementary Figures
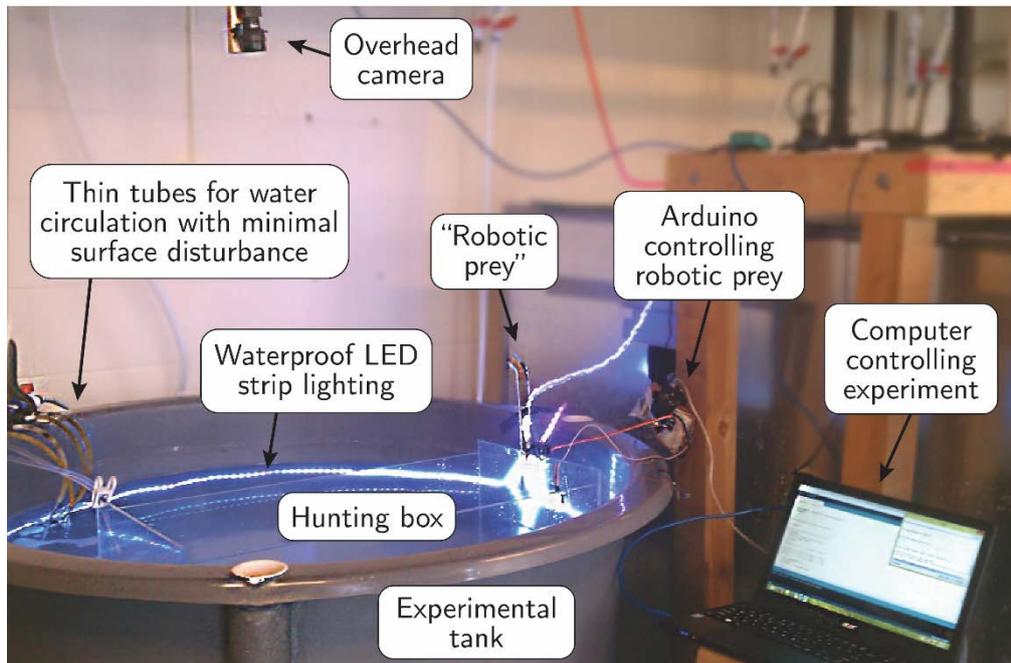

Figure S1: The experimental tank. The hunting box, with hardware setup controlling the robotic prey, inside the circular holding tank with LED lighting inside the holding tank and overhead Point Grey FlyCap2 camera.
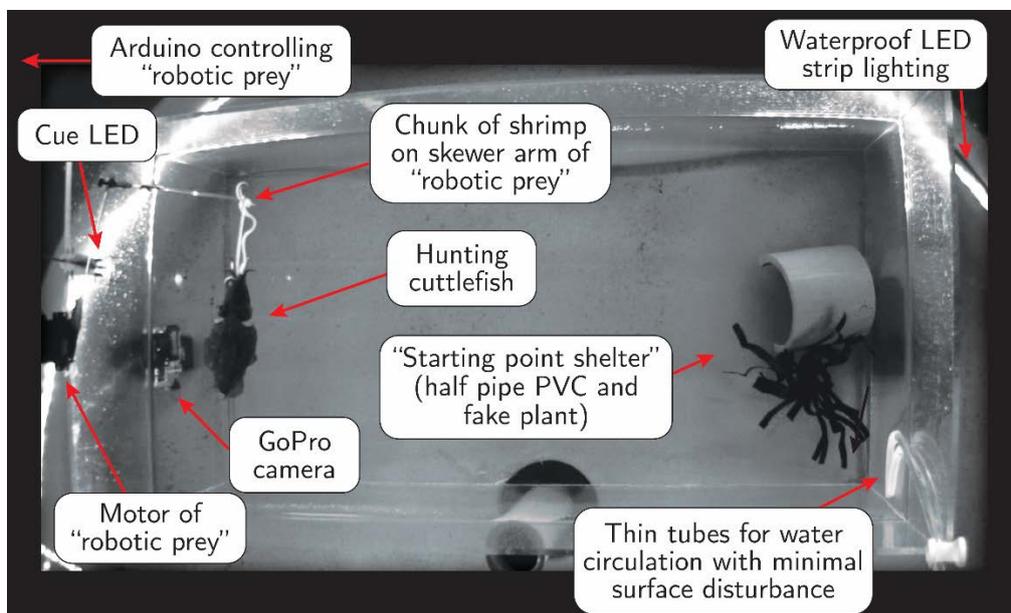
Figure S2: Overhead view of hunting box inside the holding tank. This image is a screenshot from a session video recorded by the overhead Point Grey FlyCap2 camera.

Figure S3: TGB Videos, spatial alignment. Example of the process of cropping and aligning a single frame of a Cuttle Shuttle hunting session video recording. Manual alignment of all "TGB videos" was done using Apple software Final Cut Pro and Adobe Premiere Pro by D. Kim.
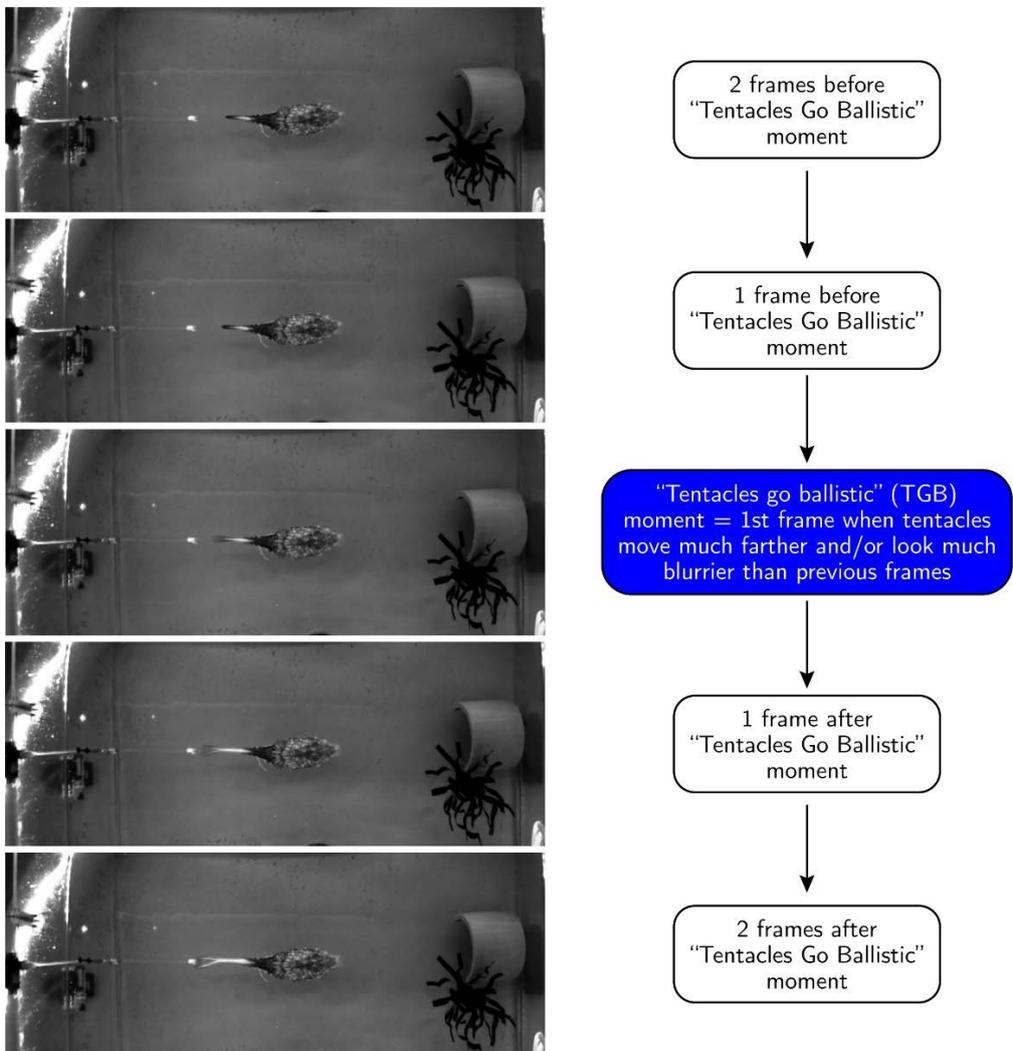
Figure S4: TGB Videos, temporal alignment. Example of how the TGB videos were aligned temporally on the frame in which "tentacles go ballistic" (TGB).

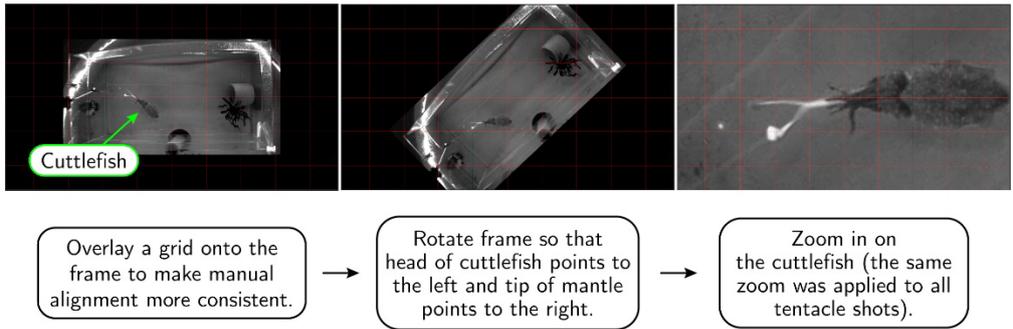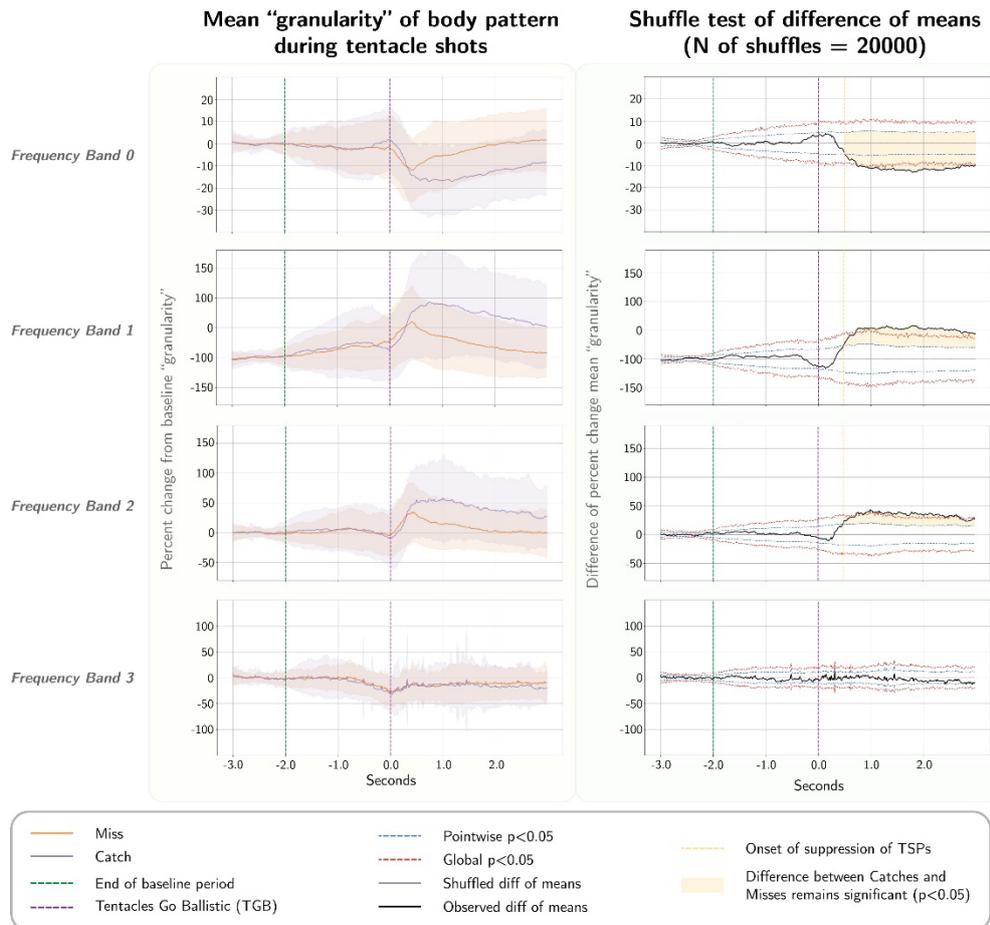**Figure S5: Quantifying dynamics of TSPs with "granularity" analysis, all frequency bands.** The left column shows the changes in mean "granularity" of body pattern during tentacle shots (measured by Process Cuttle Python, modified from [21], in 4 different spatial frequency bands), from 3 seconds before TGB to 3 seconds after TGB, normalized and pooled across all subjects. The right column shows shuffle tests (N=20,000 shuffles) of the difference of means (catch vs. miss) in each frequency band, and a vertical dashed yellow line indicates when the difference becomes significant (p<0.05).
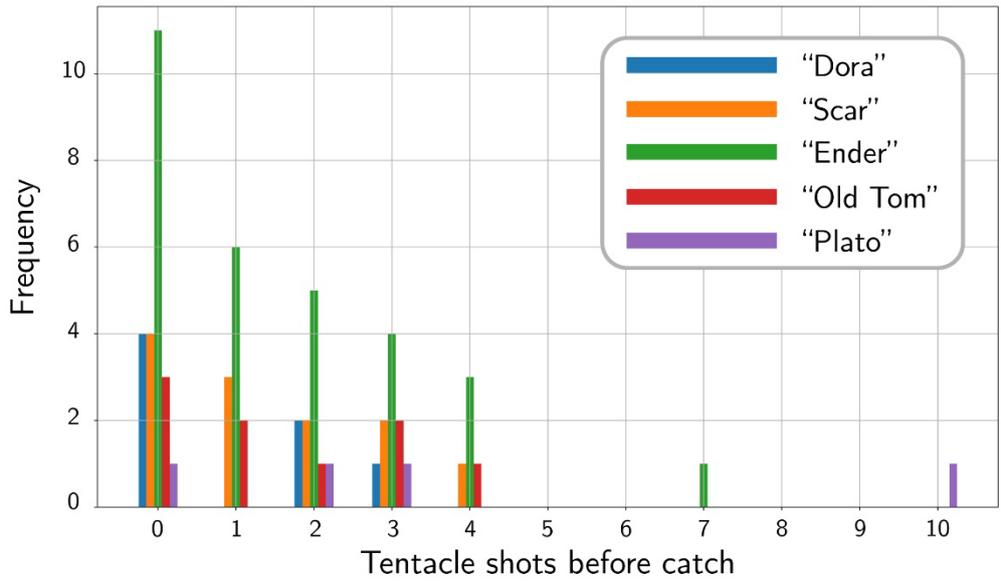
Figure S6: Number of tentacle shots before a catch for all animals. Summary plot showing the number of tentacle shots before a catch was successful; success frequency shown for all animals. Tentacle shots were annotated during data collection via Bonsai [15] and verified manually by re-watching overhead videos.
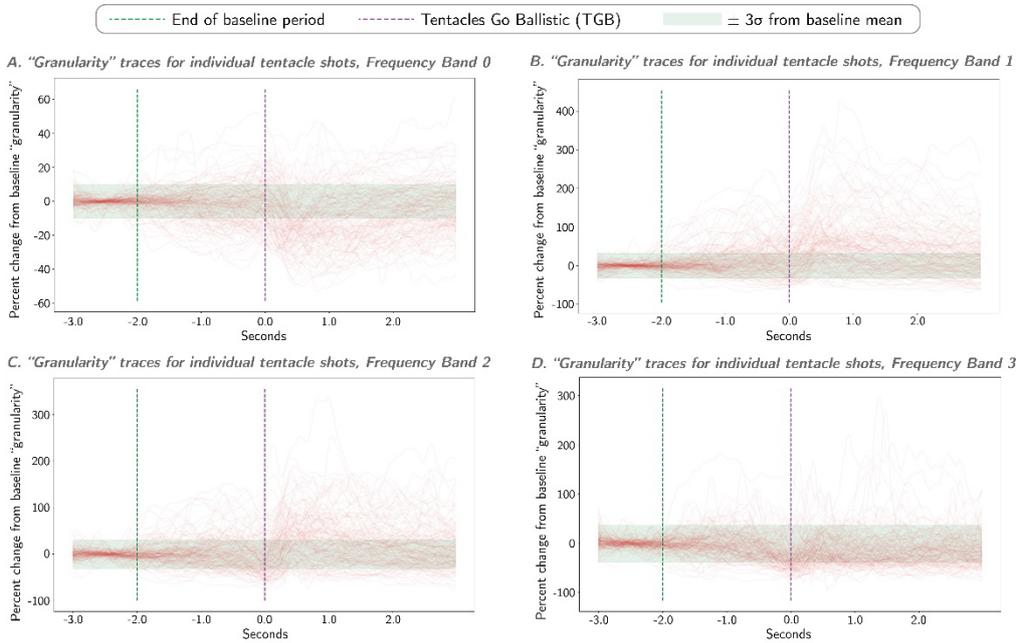
Figure S7: Descriptive plots showing "first exits" from baseline mean ± 3σ. These plots visualize when the "granularity" of the mantle pattern during individual tentacle shots deviated significantly (>3 standard deviations) from the mean of the baseline period (as measured by Process Cuttle Python, modified from [21]).

Figure S8: Boxplots showing distribution and mean onset of TSPs. Visualization of data from Table 2.

Supplementary Tables

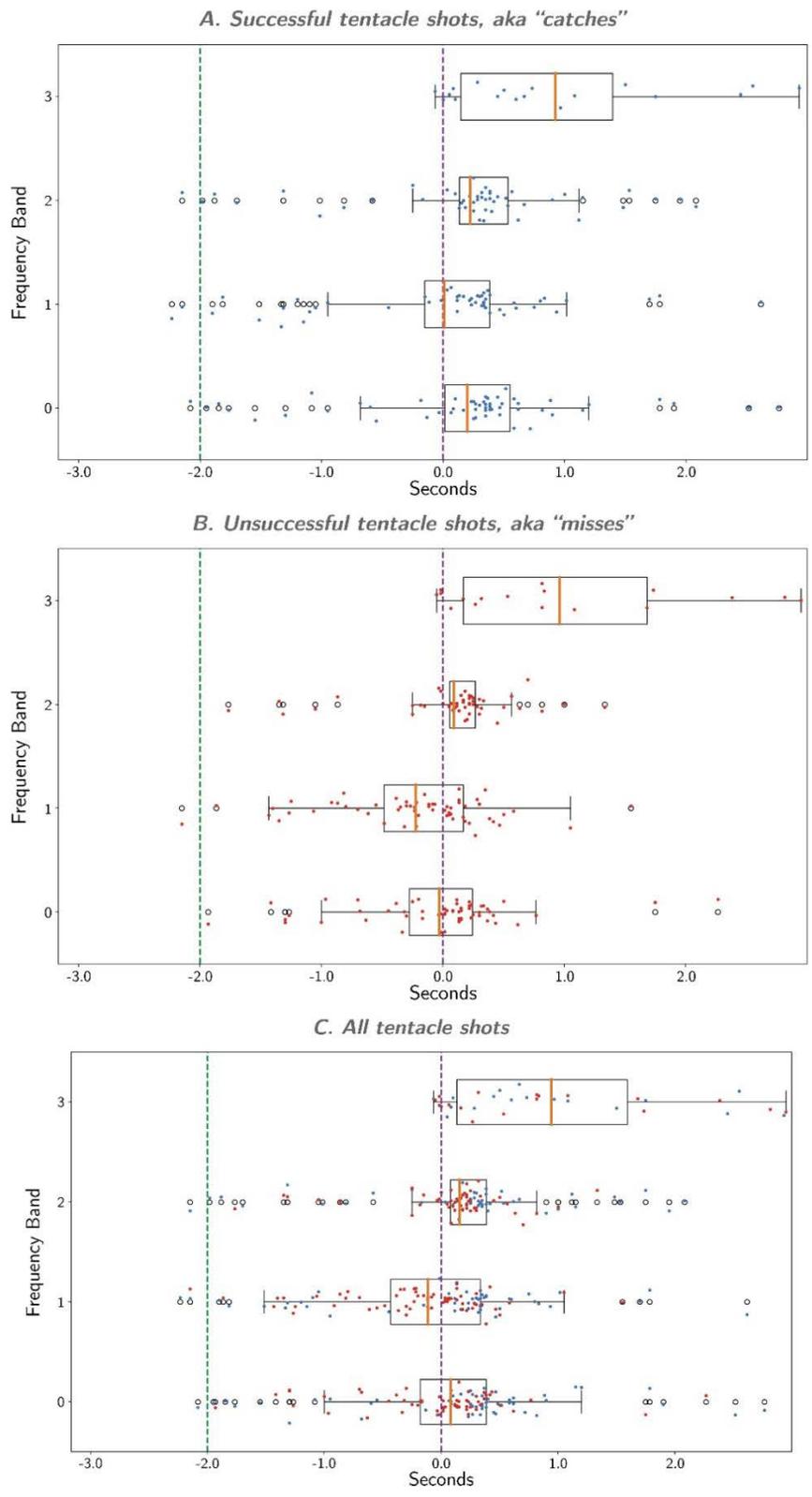| Name of Animal	'L1-H2013-01', aka 'Dora'	'L1-H2013-02', aka 'Scar'	'L1-H2013-03', aka 'Ender'	'L7-H2013-01', aka 'Old Tom'	'L7-H2013-02', aka 'Plato'
% of shots successful on first try	57.14	20.00	37.93	33.33	25.00
% of shots successful on first or second try	57.14	50.00	62.07	55.56	25.00
% of shots successful on first, second, or third try	85.71	70.00	75.86	66.67	50.00

Table S1: Accuracy of seizure via tentacle shot while hunting robotic prey, for all animals throughout the entire experimental protocol.

Frequency Band	0	1	2
Appearance of TSPs during **Catches** (milliseconds after TGB)	199 ± 960 (N = 55)	10 ± 955 (N = 53)	225 ± 909 (N = 49)
Appearance of TSPs during **Misses** (milliseconds after TGB)	-27 ± 663 (N = 59)	-222 ± 652 (N = 61)	91 ± 537 (N = 51)
Appearance of TSPs during **All Tentacle Shots** (milliseconds after TGB)	82 ± 828 (N = 114)	-114 ± 815 (N = 114)	157 ± 746 (N = 100)
Difference between Catches and Misses becomes significant at (milliseconds after TGB)	500	480	470

Table S2: Summary of the dynamics of TSPs, showing mean timing of appearance of TSPs, and timing of significant ($p<0.05$) divergence between successful and unsuccessful hunts.

Supplementary Movies

Movie S1: Videos of TSPs, low resolution. YouTube playlists of overhead-view videos (low-res) of each tentacle shot by all animals. Visit the full dataset on Harvard Dataverse for full-session, high-resolution videos (https://doi.org/10.7910/DVN/J6XLGK).

- Catches: https://youtube.com/playlist?list=PLM8kdf7qFeq_aefMtjuaXoTNRXyrNvkWo
- Misses: https://www.youtube.com/playlist?list=PLM8kdf7qFeq_zURYyS0C-CaGl4lEypVkm
- Cropped and aligned clips used for analysis: https://youtube.com/playlist?list=PLM8kdf7qFeq-ewYerw0KcYMe-xdBvc5CT

Movie S2: Video ethograms of body pattern changes during hunting behavior in captive *Sepia officinalis*. In these videos, individual tentacle shots are combined into a "matrix" video, categorized by whether the tentacle shot resulted in a catch or a miss:
- Short introductory video contextualizing the video ethograms, starring L1-H2013-03, aka "Ender": https://youtu.be/CgWJwalk8c8
- Matrix video of all catches (cropped and aligned) by L1-H2013-01, aka "Dora": https://youtu.be/RNf6kitSMNA
- Matrix video of all misses (cropped and aligned) by L1-H2013-01, aka "Dora": https://youtu.be/ul4HmHMjlmw
- Matrix video of all catches (cropped and aligned) by L1-H2013-02, aka "Scar": https://youtu.be/30bQeTUZXJg
- Matrix video of all misses (cropped and aligned) by L1-H2013-02, aka "Scar": https://youtu.be/aXr4AM1dFnE
- Matrix video of all catches (cropped and aligned) by L1-H2013-03, aka "Ender": https://youtu.be/6gbaBxuSVLM
- Matrix video of all misses (cropped and aligned) by L1-H2013-03, aka "Ender": https://youtu.be/xUKc1EQ0-SY
- Matrix video of all catches (cropped and aligned) by L7-H2013-01, aka "Old Tom": https://youtu.be/oN3YhthIfq8
- Matrix video of all misses (cropped and aligned) by L7-H2013-01, aka "Old Tom": https://youtu.be/SM0_UCbvH4Y
- Matrix video of all catches (cropped and aligned) by L7-H2013-02, aka "Plato": https://youtu.be/ipJFmIQV3R0
- Matrix video of all misses (cropped and aligned) by L7-H2013-02, aka "Plato": https://youtu.be/iCZwtaFnkAg

Movie S3: Mean Luminance of body pattern mapped to frequency. Using Bonsai [15], we summed the pixel values within a region of interest in each video frame and called this the "luminance score" of each frame. We then took that frame's "luminance score" and mapped it

to a frequency within human hearing range by normalizing the dynamic range of luminance scores to the range of frequencies audible to human ears: https://youtu.be/1COkRuZ3GqU

Movie S4: Video summary of this experiment.
https://youtu.be/G3Xx_AnrMyw

References

1. Adamo SA, Ehgoetz K, Sangster C, Whitehorne I (2006) Signaling to the enemy? Body pattern expression and its response to external cues during hunting in the cuttlefish Sepia officinalis (Cephalopoda). The Biol Bull 210(3):192-200.

2. Holmes W (1940) The colour changes and colour patterns of Sepia officinalis L. Proceedings of the Zoological Society of London 110 (1-2): 17-35.

3. How MJ, Norman MD, Finn J, Chung WS, Marshall NJ (2017) Dynamic skin patterns in cephalopods. Front Physiol 19(8):393.

4. Langridge KV (2006) Symmetrical crypsis and asymmetrical signalling in the cuttlefish Sepia officinalis. Proceedings of the Royal Society B: Biological Sciences 273(1589):959-67.

5. Langridge KV, Broom M, Osorio D (2007) Selective signalling by cuttlefish to predators. Current Biology 17(24):R1044-5.

6. Shashar N, Rutledge P, Cronin T (1996) Polarization vision in cuttlefish in a concealed communication channel?. Journal of Experimental Biology 199(9):2077-84.

7. Hanlon RT, Messenger JB (2018) Cephalopod Behaviour. 2nd edition. Cambridge University Press.

8. Hill AV, Solandt DY (1935) Myograms from the chromatophores of Sepia. J Physiol 83:13-4.

9. Hanlon, R (2007) Cephalopod dynamic camouflage. Current Biology 17(11):R400-R404.

10. Hadjisolomou, SP, El-Haddad, R, Kloskowski, K, Chavarga, A, & Abramov, I (2021) Quantifying the speed of chromatophore activity at the single-organ level in response to a visual startle stimulus in living, intact squid. Front Physiol 12:921.

11. Messenger JB (1968) The visual attack of the cuttlefish, Sepia officinalis. Animal Behaviour 16(2-3):342-57.

12. Sereni E (1930) The chromatophores of the cephalopods. The Biol Bull 59(3):247-68.

13. Thompson DW (1910) Aristotle: Historia animalium. The works of Aristotle translated into English, book IV. The Classical Review.

14. Cole PD, Adamo SA. Cuttlefish (Sepia officinalis: Cephalopoda) hunting behavior and associative learning (2005) Anim cogn 8(1):27-30.

15. Duval P, Chichery MP, Chichery R (1984) Prey capture by the cuttlefish (Sepia officinalis L): an experimental study of two strategies. Behavioural processes 9(1):13-21.

16. Feord RC, Sumner ME, Pusdekar S, Kalra L, Gonzalez-Bellido PT, Wardill TJ (2020) Cuttlefish use stereopsis to strike at prey. Sci Adv 6(2):eaay6036.

17. Zylinski S, Osorio D, Shohet AJ (2009) Cuttlefish camouflage: context-dependent body pattern use during motion. Proceedings of the Royal Society B: Biological Sciences 276(1675):3963-9.

18. Hanlon RT, Messenger JB (1988) Adaptive coloration in young cuttlefish (Sepia officinalis L.): the morphology and development of body patterns and their relation to behaviour. Philosophical Transactions of the Royal Society of London. B, Biological Sciences 320(1200):437-87.

19. Carreno Castilla A, Hernandez-Urcera J, Gouraguine A, Guerra Á, Cabanellas-Reboredo M (2020) Predation behaviour of the European squid Loligo vulgaris. J Ethol 38:311-22.

20. Panetta D, Solomon M, Buresch K, Hanlon RT (2017) Small-scale rearing of cuttlefish (Sepia officinalis) for research purposes. Mar Freshw Behav Physiol 50(2):115-24.

21. Lopes G, Bonacchi N, Frazão J, Neto JP, Atallah BV, Soares S, Moreira L, Matias S, Itskov PM, Correia PA, Medina RE (2015) Bonsai: an event-based framework for processing and controlling data streams. Front Neuroinform 9:7.

22. Barbosa A, Mäthger LM, Buresch KC, Kelly J, Chubb C, Chiao CC, Hanlon RT (2008) Cuttlefish camouflage: the effects of substrate contrast and size in evoking uniform, mottle or disruptive body patterns. Vision Res 48(10):1242-53.

23. Packard A, Hochberg FG (1977) Skin patterning in Octopus and other genera. In Symp. Zool. Soc. Lond 38: 191-231.

24. Kier, W. M., & VanLeeuwen, J. L. (1997). A kinematic analysis of tentacle extension in the squid Loligo pealei. Journal of Experimental Biology, 200(1), 41-53.

25. Graving JM, Chae D, Naik H, Li L, Koger B, Costelloe BR, Couzin ID (2019) DeepPoseKit, a software toolkit for fast and robust animal pose estimation using deep learning. Elife 8:e47994.

26. Mathis A, Mamidanna P, Cury KM, Abe T, Murthy VN, Mathis MW, Bethge M (2018) DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. Nat Neurosci 21(9):1281-9.

27. Nath T, Mathis A, Chen AC, Patel A, Bethge M, Mathis MW (2019) Using DeepLabCut for 3D markerless pose estimation across species and behaviors. Nat Protoc 14(7):2152-76.

28. Messenger, J. B. (2001). Cephalopod chromatophores: neurobiology and natural history. Biological Reviews, 76, 473-528.

29. Laan A, Gutnick T, Kuba MJ, Laurent G (2014) Behavioral analysis of cuttlefish traveling waves and its implications for neural control. Current Biology 24(15):1737-42.

30. Reiter S, Hülsdunk P, Woo T, Lauterbach MA, Eberle JS, Akay LA, Longo A, Meier-Credo J, Kretschmer F, Langer JD, Kaschube M, Laurent G (2018) Elucidating the control and development of skin patterning in cuttlefish. Nature 562(7727):361-6.

31. Chiao, C. C., Chubb, C., & Hanlon, R. T. (2015). A review of visual perception mechanisms that regulate rapid adaptive camouflage in cuttlefish. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 201, 933-945.