

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

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Abbreviations used: MBL, Marine Biological Laboratory which is a private, non-profit research organization affiliated with the University of Chicago and founded in Woods Hole, Massachusetts, United States in 1888; TGB, tentacles go ballistic. This is the moment when tentacles are ballistically ejected towards the prey or food item. The TGB moment happens during tentacle “shots,” also known as tentacle strikes; TGB videos, 6-second video clips taken from study video dataset. Each video was manually cropped and aligned to keep the cuttlefish in the same location and orientation in every frame, and temporally aligned such that the TGB moment occurred at 3.0 s; TSPs, tentacle shot patterns. The category of rapid, brief and high-contrast changes in body coloration that always occur with the TGB moment

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## ABSTRACT

Cuttlefish are active carnivores that possess a wide repertoire of body patterns that can be changed within milliseconds for many types of camouflage and communication. The forms and functions of many body patterns are well known from ethological studies in the field and laboratory. Yet one aspect has not been reported in detail: the category of rapid, brief and high-contrast changes in body coloration (“Tentacle Shot Patterns” or TSPs) that always occur with the ejection of two ballistic tentacles to strike live moving prey (“Tentacles Go Ballistic” or TGB moment). We designed and tested a mechanical device that presented prey in a controlled manner, taking advantage of a key stimulus for feeding: motion of the prey. High-speed video recordings show a rapid transition into TSPs starting 114 ms before TGB ( $N = 114$ ). TSPs are then suppressed as early as 470–500 ms after TGB ( $P < 0.05$ ) in unsuccessful hunts, while persisting for at least 3 s after TGB in successful hunts. A granularity analysis revealed significant differences in the large-scale high-contrast body patterning present in TSPs compared to the camouflage body pattern deployed beforehand. TSPs best fit the category of secondary defense called deimatic displaying, meant to briefly startle predators and interrupt their attack sequence while cuttlefish are distracted by striking prey. We characterize TSPs as a pattern category for which the main distinguishing feature is a high-contrast signaling pattern with aspects of Acute Conflict Mottle or Acute Disruptive Pattern. The data and methodology presented here open opportunities for quantifying the rapid neural responses in this visual sensorimotor set of behaviors.

**Keywords:** deimatic behavior, secondary defense, cephalopod, body patterning, *Sepia officinalis*

## INTRODUCTION

Cuttlefish, like other coleoid cephalopods, evolved their active camouflage abilities as a form of defense against predation after losing the hard shell common to other molluscs. Cuttlefish also use body patterning to sneak up, dazzle, or stun prey, as well as for communication, most clearly during agonistic bouts between rival males but also at other times [1–7]. Due to direct neural control, these changes in patterning can occur in less than one second [8–10]. Thanks to recent advances in high-speed video recording technology, cuttlefish researchers and recre-

ational divers have noticed a brief yet dramatic body patterning change in hunting cuttlefish that appears during prey capture events (Fig. 1).

The hunting behavior of *Sepia officinalis* when attacking prawns was first described in detail by Messenger in 1968 [11], although color changes in response to any attention-grabbing object were also described as early as Aristotle [12,13]. Messenger emphasized that cuttlefish are primarily visual; his descriptions focused on movements of the eyes and the whole body. Since then, there have been studies that describe differences in behavior of hunting cuttlefish, including differences in body pattern expression, based on prey type [1,14–16], the presence of

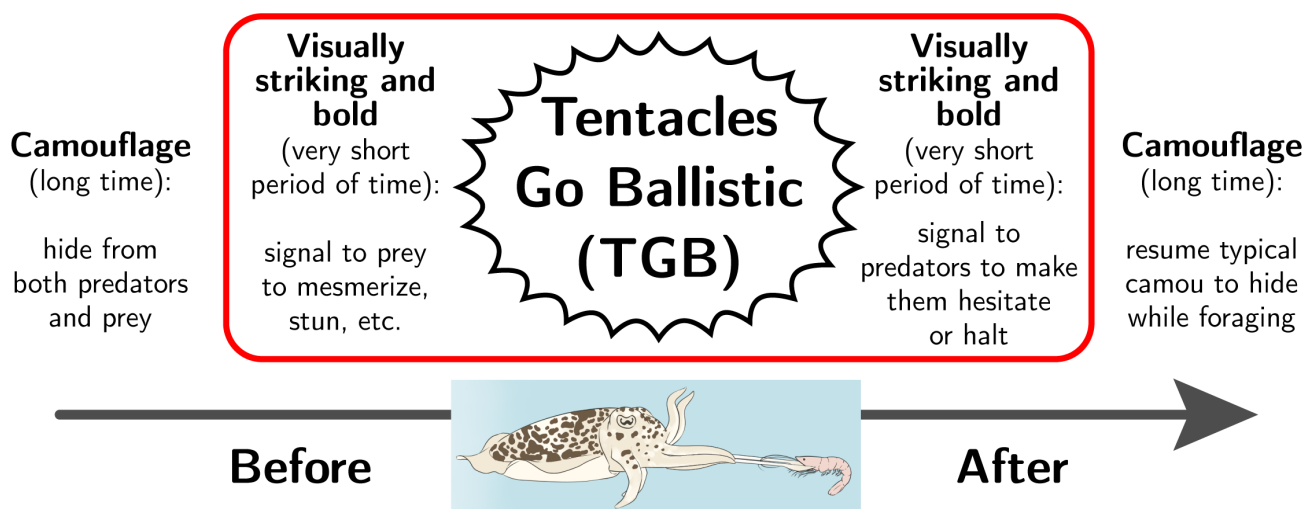
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potential predators [1], and whether the animal was moving or stationary while capturing prey [17].

These various body patterns deployed during hunting have been richly described qualitatively in previous literature, and while quantitative methods for distinguishing between still images of these body patterns exist, there are to date no published analysis methods that would allow researchers to automate and standardize the detection of various body patterns in videos of freely behaving animals. This study presents a low-cost, open-source method for reliably evoking and recording cuttlefish hunting behavior, then numerically characterizing the changes in body pattern during hunting. Specifically, the results described here focus on

an acute (meaning transient, as opposed to chronic [18]) body pattern that is specific to prey capture; this pattern has often been observed anecdotally in the field and was mentioned briefly in a previous laboratory study [1] (although see [19] for mention of a similar post-attack body pattern in the squid *Loligo vulgaris*). **Figure 1** represents a tentative framework for studying this phenomenon based upon published literature on camouflage sequences, yet here it is depicted in the context of approaching, seizing prey, and returning to camouflage. Our intent was to test this sequence in the lab to characterize its potential function with regard to defense against predators while the cuttlefish itself is vulnerable to predation as it concentrates on prey attack (**Fig. 1**).

## Camouflage and Signaling sequences associated with Prey Capture by Cuttlefish



**Figure 1. Camouflage and signaling sequences associated with prey capture by cuttlefish.** Tentative framework for the sequence of body patterning during prey capture (see also [7]). This study focuses on the 3 s immediately before and after prey capture, circled in red in this diagram. Illustration by Jennifer Deutscher.

### MATERIALS AND METHODS

Experiments were conducted in the Marine Resources Center at the Marine Biological Laboratory (MBL) in Woods Hole, USA. The behavior paradigm used for these experiments was based on a “shuttling” paradigm used by the Intelligent Systems Lab with rodents, modified to accommodate an aquatic environment and the behavioral repertoire of cuttlefish. Captive *Sepia officinalis* ( $N = 6$ , age = 15 months, gender unknown) were trained to hunt “robotic prey” (a  $5 \times 2$  mm piece of defrosted king prawn, *Penaeid sp.* [20], presented on a motorized skewer). Cuttlefish are active carnivores that use vision for predation, and motion is a necessary stimulus for feeding.

#### Robotic prey

Plastic skewers commonly used to feed cuttlefish were attached to a continuous rotation hobby servo motor (HS-232, Hitachi, Japan), which was controlled by an Arduino Uno (Arduino, Italy) microcontroller

board. The skewer arm of the “robotic prey” always started out of the water and was originally designed to progress through three stages of increasingly complex movement. See **Supplementary Text**, section A for details of each movement phase.

#### Experimental setup

The experimental tank consisted of a  $43 \times 43 \times 81$  cm rectangular box with an open top and transparent walls (**Fig. 2**) placed within a larger “holding tank” (**Fig. S1**). The “robotic prey” was installed at one end of the long axis of the experimental tank. A “starting point shelter” was set up at the other end where the cuttlefish could hide until they were acclimated to the experimental context. The holding tank was lined with waterproof LED strip lighting to provide uniform, multidirectional luminance to the experimental arena. Small plastic tubes moved water into and out of the experimental tank, which was filled with water to a height of approx. 27 cm; during experiments, the water flow was kept minimal to reduce surface perturbations that would interfere with

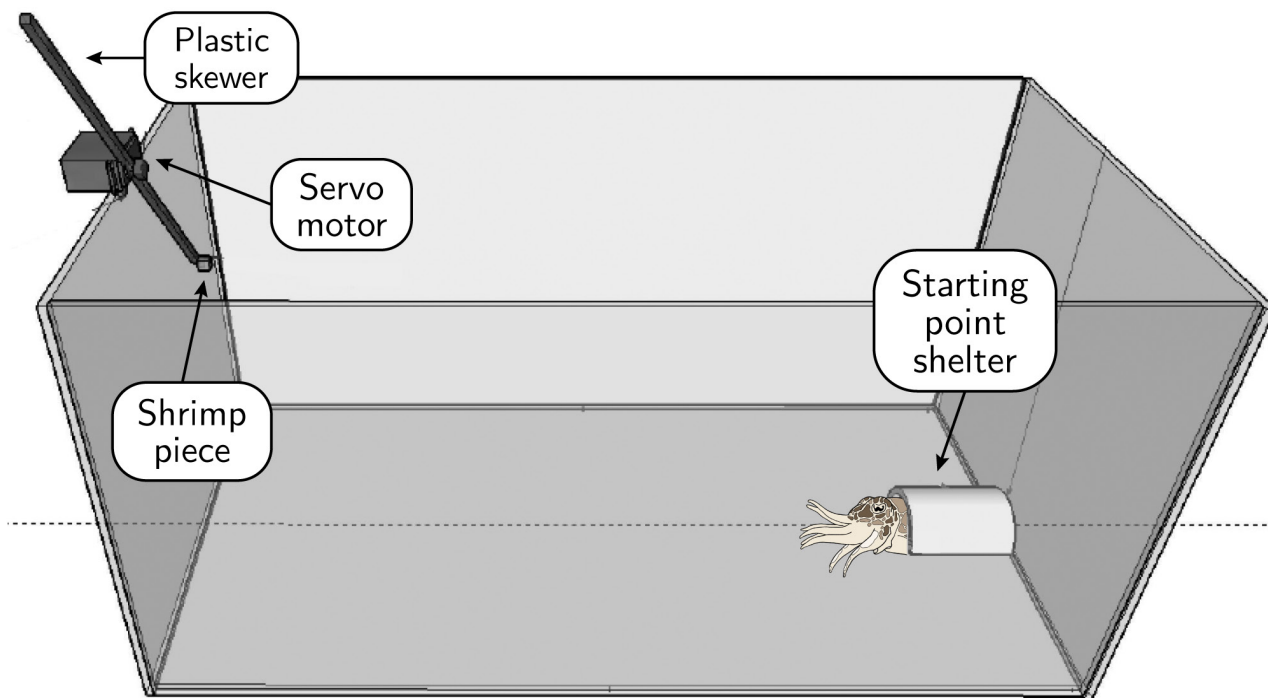
overhead filming (Fig. S2).

A laptop computer coordinated the various hardware components involved in the experimental setup *via* the Bonsai Visual Reactive programming language [21] and collected video footage from the overhead camera (Flea3 monochrome USB camera, resolution: 1280 × 960; frame rate: 70 fps. PointGrey, USA). This overhead camera was used to remotely monitor each hunting session, during which certain letters on the laptop keyboard could be pressed to log timestamps of “moments of interest” in a CSV file. These “moments of interest” included:

- ✓ returns to “home base;”

- ✓ “orienting” events: “attention” or “positioning” as defined by [11] (any rapid changes to color, posture, or whole-body alignment by the cuttlefish in response to the robotic prey);
- ✓ tentacle shots: equivalent to the “seizure” phase as defined by [11];
- ✓ catches (tentacle shots that resulted in catching the shrimp on the robotic prey).

A second underwater camera (Hero2 GoPro camera inside a waterproof case, color, frame rate: 60 fps.) recorded another view from just outside the experimental tank on the robotic prey side (Fig. 2).



**Figure 2. Experimental tank setup (not to scale).** An acrylic box (43 × 43 × 81 cm, open top) with “robotic prey” (shrimp piece on a plastic skewer moved by an Arduino-controlled servo motor) on the left, perched on top of the experimental tank wall, and “starting point shelter” on the right, where cuttlefish can hide during acclimation. An overhead camera (not shown) provided a top-down, monochrome recording of the setup, while an underwater camera (also not shown) recorded from another angle in color.

## Experimental protocol

The cuttlefish were housed individually in partitioned home tubs, in which the animals were separated by panels of acrylic but shared water intake and outflow. Water in both home tubs and experimental tank was filtered, recirculated natural sea water. During each week of the experiment, the animals were fed freely for one day, deprived of food for 2 d, then spent 4 d in the experimental tank.

When an animal entered the experimental tank, it was given 30 min to eat as much food as it could catch while hunting the robotic prey. The animal triggered the start of “food offerings” by settling in or near the “starting point shelter” while deploying a body pattern that was not primarily white (“total paling” of the whole body is considered to be a sign of distress in cuttlefish [2]). A “food offering” was made when the Arduino-controlled skewer brought the shrimp into the water and moved it around. Once triggered, “food offerings” were made at random intervals ranging between 30 to 60 s. A green LED, located next to the

motor, turned on 2 s before each food offering. If a cuttlefish caught the prey and removed the shrimp piece from the skewer end, the skewer was rotated out of the water and a new piece of shrimp was placed on the skewer. Afterwards, the cuttlefish had to return to the “starting point shelter” to trigger more food offerings.

## Video pre-processing

We manually created 6-second clips of every tentacle shot using the video editing softwares Final Cut Pro and Adobe Premier Pro (Fig. S3 and S4). These 6-second clips were temporally aligned on the “Tentacles Go Ballistic” (TGB) moment during each tentacle shot, which was defined as the first frame when the tentacles suddenly accelerated towards the prey, fast enough to be seen as a blur even with a 70 fps recording rate; this is in contrast to when the tentacles first appear from within the cuttlefish arms, at which point they are moving much more slowly (Fig. S4). In each of these 6-second video clips (referred to as

“TGB videos”), the TGB moment occurs at 3.0 s. Spatially, each frame was cropped and aligned such that (in descending priority):

- ✓ the cuttlefish was oriented with its head and arms pointing to the left side of the frame and the posterior tip of the mantle pointing to the right side of the frame;
- ✓ the backs of the eyes created an axis parallel to the vertical axis of the frame;
- ✓ the long axis of the cuttlefish’s body was vertically centered and as parallel to the horizontal axis of the frame as possible; and
- ✓ the cuttlefish body filled the frame as much as possible without any part of its body getting cut out of the frame (a single zoom value was chosen and applied across all tentacle shots).

The TGB videos were then downsampled to 60 fps. Note that the TGB videos were all 6 s long regardless of the full timespan of each prey capture attempt.

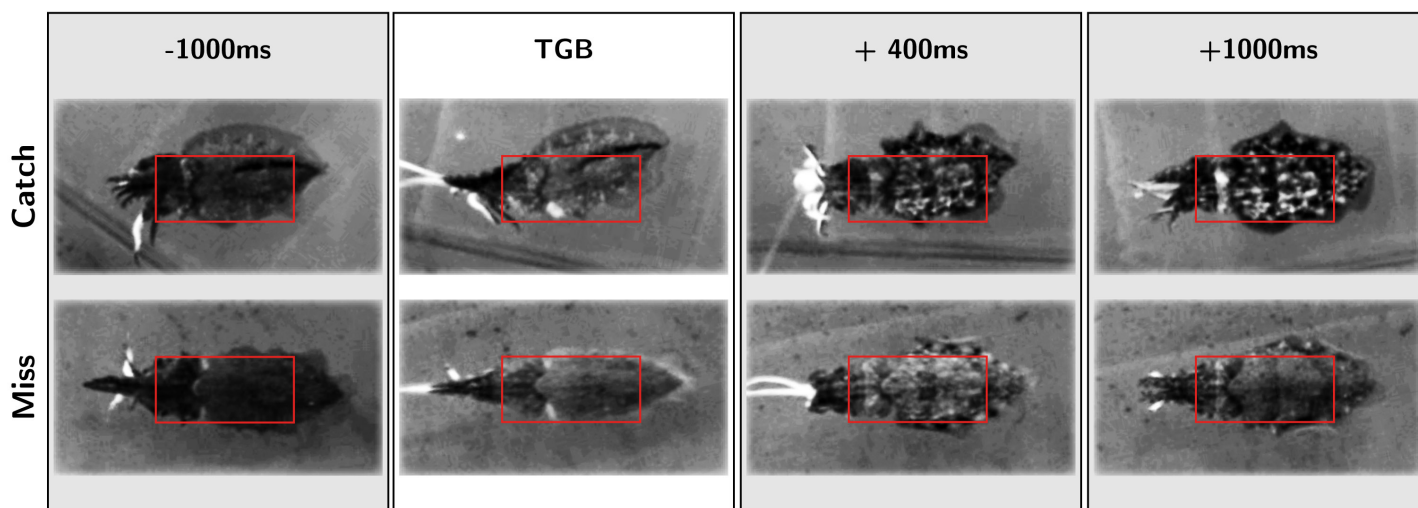
### Video analyses—characterizing TSPs

The visual characteristics of the body pattern for each frame of the TGB videos were quantified surrounding a prey capture event. We focused our characterization on a rectangular region of interest (ROI) that contained as much of the cuttlefish mantle as possible without including any background (see Fig. 3), and then measured the change in “granularity” using a modified version of a granularity analysis method

originally developed to discriminate between uniform/stipple, mottle, and disruptive camouflage body patterns in still images of cuttlefish (for details, see [22]). Each frame of the TGB videos was quantified as follows:

- ✓ the two-dimensional Fast Fourier Transform (FFT) of the frame was computed;
- ✓ four spatial frequency filters were applied by masking the FFT using the following spatial frequency bands (Fig. 4):
  - “Frequency band 0”: full ROI window (rectangular) to 125.44 mm/cycle (x-axis),
  - “Frequency band 1”: 125.44 to 62.72 mm/cycle,
  - “Frequency band 2”: 62.72 to 31.36 mm/cycle, and
  - “Frequency band 3”: 31.36 to 15.68 mm/cycle;
- ✓ the sum of the squared pixel values in the resulting filtered images gave the total energy of the original video frame in that particular frequency band.

Note that each frequency band has a very different size scale of the light and dark bits of the pattern, with band 1 the largest scale and band 3 smallest scale; this is a key feature of “granularity” in this pattern descriptor. Applying the modified granularity method to the TGB videos resulted in four time series of numeric values, one for each frequency band, which describe the body pattern during each tentacle shot.



**Figure 3. Ethogram of body pattern changes during hunting behavior in captive *Sepia officinalis* (exemplary screenshots from overhead video).** The top row shows body pattern changes (TSP) during a successful attack, while the bottom row shows body pattern changes during an unsuccessful attack. All images are frames from manually cropped and aligned video clips of tentacle shots made by the same animal. Red boxes indicate ROI described in Methods section “Video analyses—characterizing TSPs”.

### Statistical analyses

Given the small sample sizes obtained in this experiment (5 animals, number of tentacle shots per animal, mean = 29.0, std = 13.957), we pooled the tentacle shots from all animals (total number of tentacle shots = 140) using a baseline normalized measure (percent change from baseline) computed by dividing the power in each band by the power present in the first second of that clip, then subtracting 1 (to center around 0) and multiplying by 100 (to convert to percentage).

To calculate the timing of the appearance and disappearance of tentacle shot patterns (TSPs), we set an upper and lower bound that

was 3 standard deviations above and below the mean baseline. We defined the appearance and disappearance of TSPs according to the following criteria:

- ✓ the percent change in granularity exits “3 sigma bounds” in the direction of the general trend in that frequency band (*i.e.*, below on frequency band 0 and above on frequency bands 1 and 2, see Fig. 5);
- ✓ percent change in granularity re-enters “3 sigma bounds” after TGB; and
- ✓ if percent change exits and re-enters multiple times after

TGB, TSPs begin at the exit from “3 sigma bounds” closest in time to TGB.

To determine significant differences in TSPs following successful versus unsuccessful tentacle shots, we used a shuffle test for significance (number of shuffles = 20000) to calculate when the mean values describing successful tentacle shots became significantly different from the mean values describing unsuccessful tentacle shots. For each frame and for each frequency band, we did the following:

- ✓ pooled all values for this frame from all tentacle shots, regardless of whether they were successful or not;
- ✓ randomly assigned 59 of these values as “catch” and 81 of these values as “miss” (corresponding to the observed data of 59 successful versus 81 unsuccessful tentacle shots in our experimental dataset);
- ✓ calculated the means for the randomly assigned “catch” values and the randomly assigned “miss” values;
- ✓ subtracted the shuffled means from each other;
- ✓ repeated this shuffling (steps 2–4) 20000 times to create a bootstrapped gaussian distribution of differences of means at each frame.

This procedure generated a frame-by-frame (or “pointwise”) threshold for  $P < 0.05$ . To correct for our bootstrapping, we then generated random traces for each frequency band by doing the following:

- ✓ pooled all values for each frame from all tentacle shots, regardless of whether they were successful or not;

- ✓ randomly chose a value from the combined pool of values at each frame;
- ✓ combined the randomly chosen value from each frame into a 6-second long time series;
- ✓ repeated steps 1–3 above 1000 times to generate 1000 random traces;
- ✓ calculated the max and min of these 1000 traces at each frame.

These max and min values at each frame became our global thresholds for  $P < 0.05$  (for each frequency band). If the observed difference in mean percent change of “catch” versus “miss” trials crossed outside the global  $P < 0.05$  threshold, then the difference in the means became significant when the difference of the means crossed the pointwise threshold.

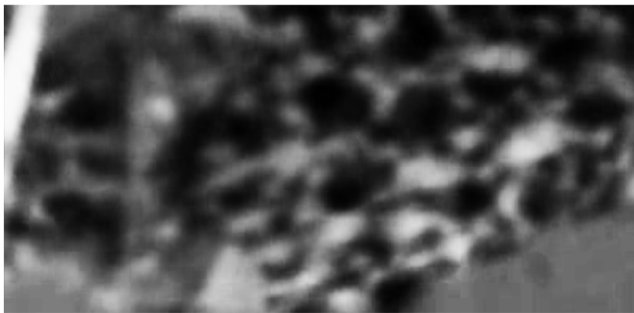
### Experimental dataset

The full dataset for this experiment, including all videos from the overhead view monochrome camera and annotations, is shared online via the Harvard Dataverse and can be found at <https://dataverse.harvard.edu/dataverse/CuttleShuttle>. See Movies S1 for a low-resolution version of the full video dataset.

### Analysis code repository

All code used to analyze this dataset can be found online at [https://github.com/everymind/CuttleShuttle\\_Paper](https://github.com/everymind/CuttleShuttle_Paper).

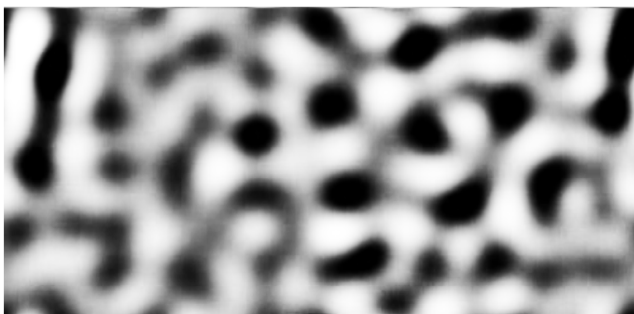
#### A. Frequency Band 0 (unfiltered)



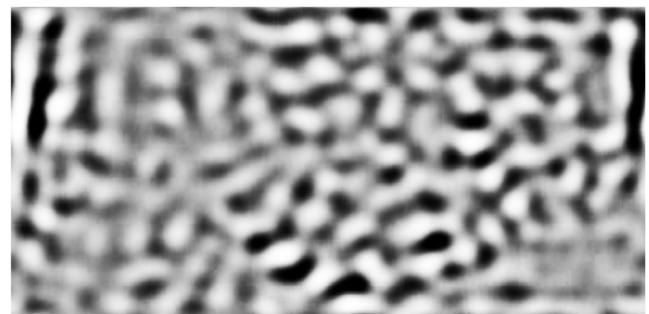
#### B. Frequency Band 1



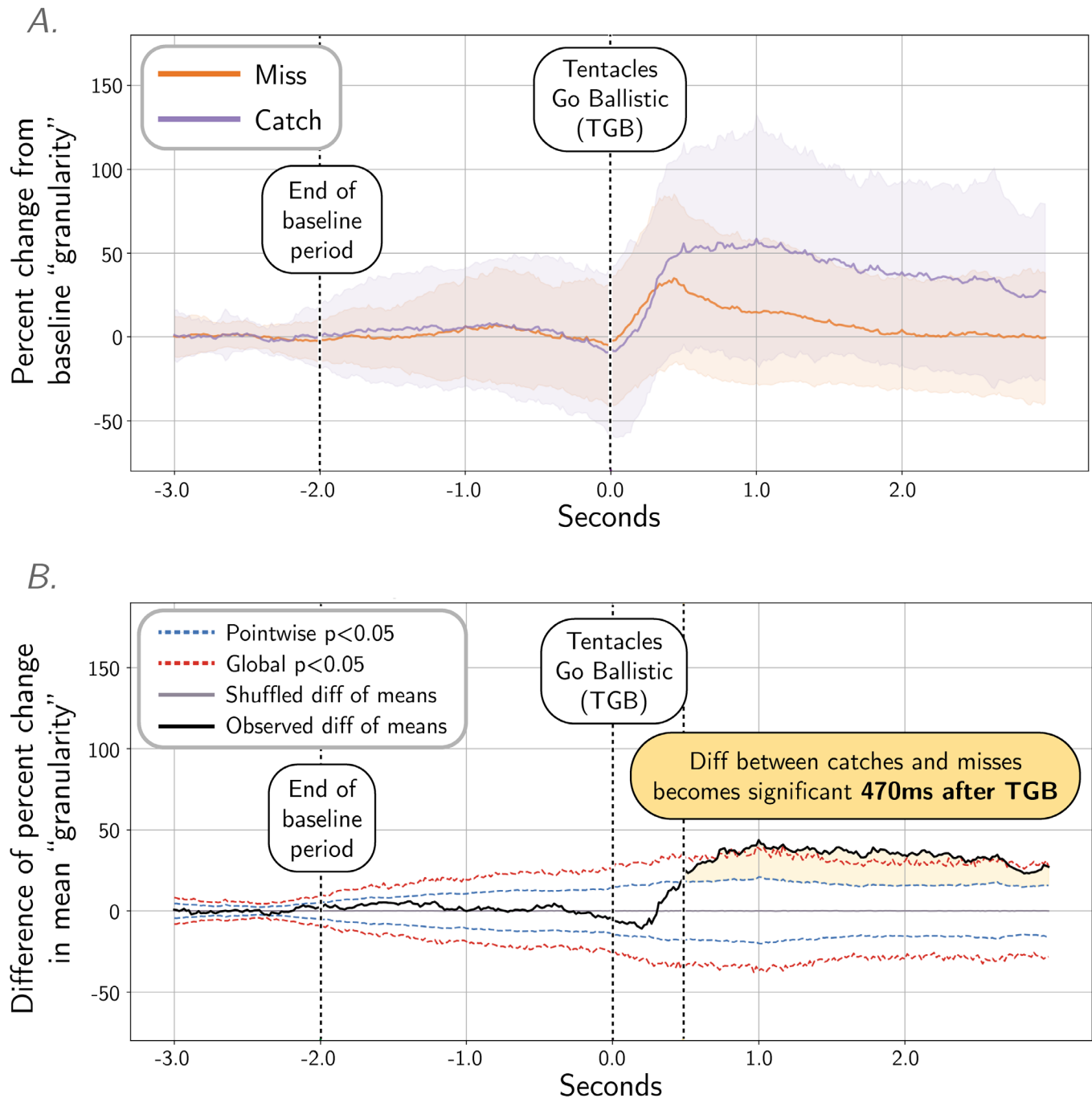
#### C. Frequency Band 2



#### D. Frequency Band 3



**Figure 4. Example screenshot of mantle body pattern after filtering for analysis.** A screenshot of the mantle body pattern (+1000 ms after TGB) is shown after filtering through the 4 frequency bands used by Process Cuttle Python (modified from [21]) to analyze the TSP.



**Figure 5. Quantifying the dynamics of TSPs with “granularity” analysis, frequency band 2.** **A.** Mean “granularity” of body pattern during tentacle shots in frequency band 2 (measured by Process Cuttle Python, modified from [21]), from 3 s before TGB to 3 s after TGB, normalized and pooled across all subjects. **B.** A shuffle test ( $N = 20000$  shuffles) of the difference of means (catch vs. miss) show significance at 470 ms after TGB. See **Figure S5** for granularity analysis and shuffle test plots at all spatial frequencies.

## RESULTS

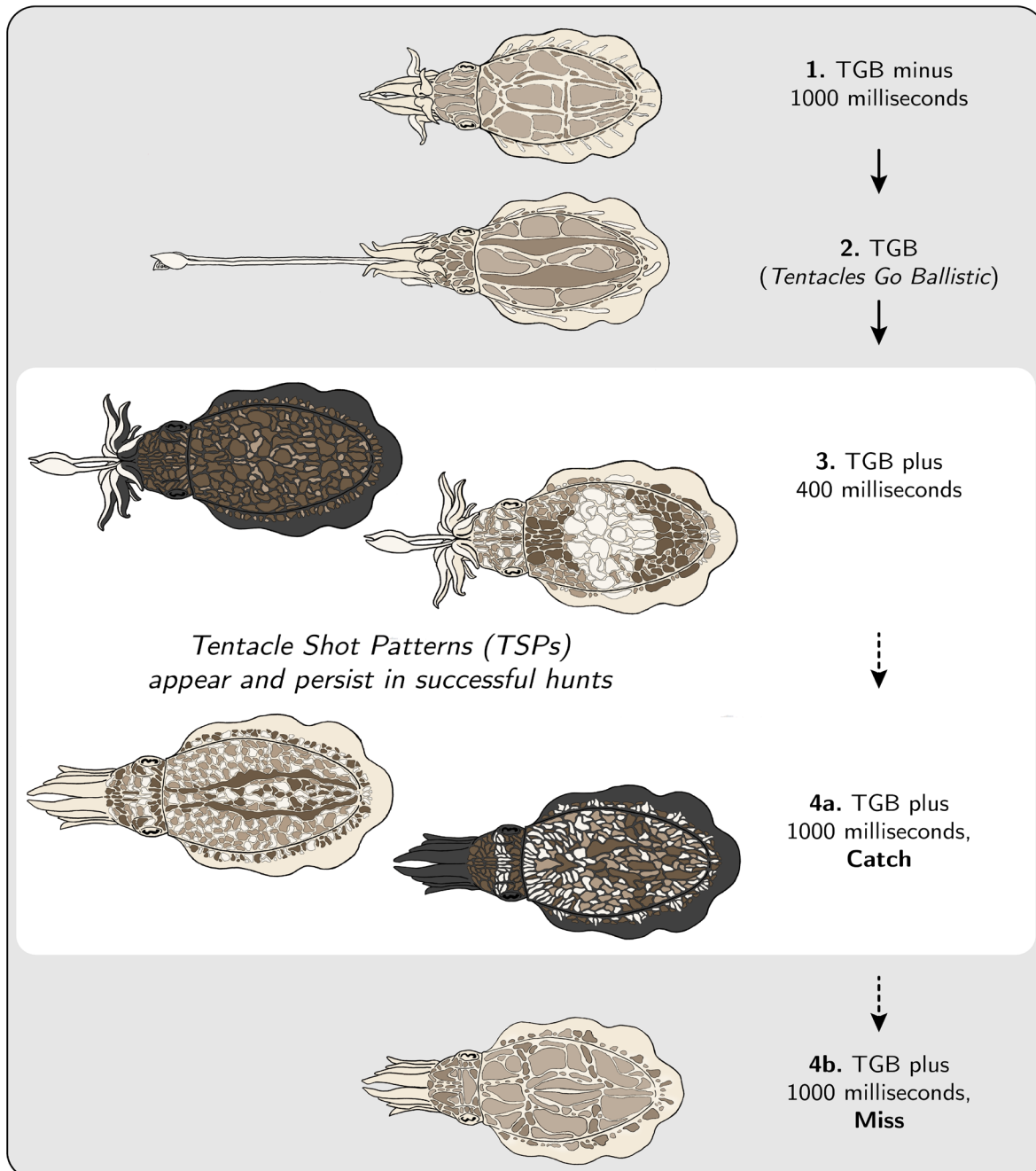
The high-speed video recordings confirmed the occurrence of a very brief and highly conspicuous category of body patterns that has been observed both in the lab [1] and in the wild (Fig. 1) during prey

capture events. We refer to this category as “Tentacle Shot Patterns” (TSPs) because they always appeared when the cuttlefish made a tentacle strike (equivalent to the “ejection” subphase of phase “seizure,” as defined by [11]).

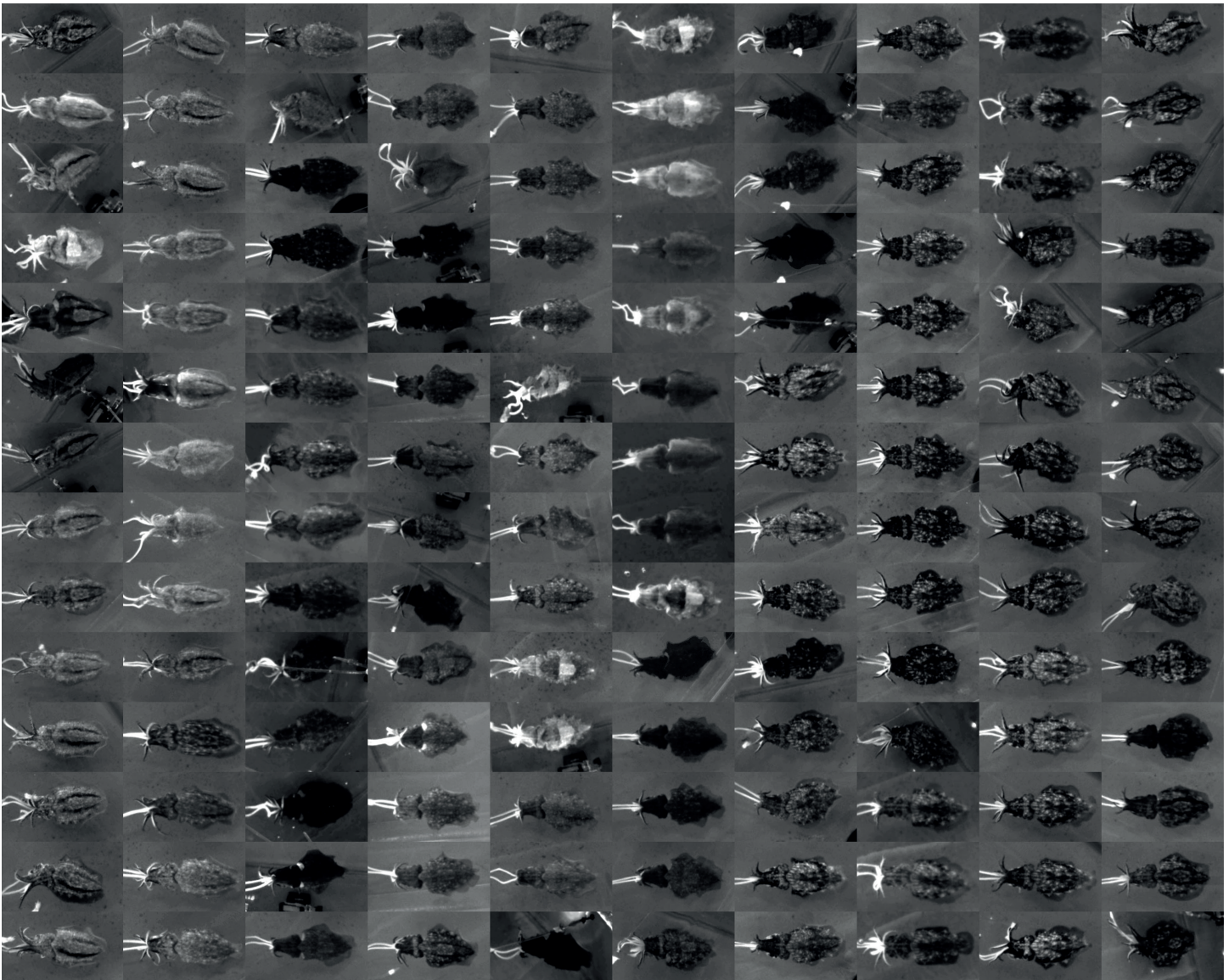
## Hunting behavior ethograms

Figure 3 shows a visual still-image ethogram constructed from TGB videos of two tentacle shots, one successful and one unsuccessful, by animal L1-H2013-03, aka “Ender.” See Movies S2 for links to video ethograms of these body pattern changes, during all tentacle shots for all experimental animals, from 3 s before TGB until 3 s after TGB.

Visual inspection confirmed that a new category of large-scale, high-contrast signaling patterns, characterized by aspects of high-contrast Acute Conflict Mottle or Acute Disruptive Pattern (TSPs) appears surrounding TGB for all tentacle shots of all animals. Figure 6 and Figure 7 illustrate sample variations of these two body patterns.



**Figure 6. Schematic of body pattern changes during prey capture, shown in 4 stages.** 1. TGB minus 1000 ms: Animal is typically transitioning from the positioning phase to the seizure phase of the hunt (see [8]), and tentacles are just beginning to show from within the arms. 2. TGB: This is the moment when tentacles are ballistically released towards the prey or food item. 3. TGB plus 400 ms (Tentacle Shot Patterns appear): By 400 ms after TGB, the “granularity” of the deployed body pattern has increased significantly as compared to the “granularity” of the body pattern deployed during baseline (TGB minus 3000 ms to TGB minus 2000 ms). 4a. TGB plus 1000 ms, Catch (TSPs persist): When the hunt is successful, the “granularity” of the deployed body pattern remains high. See Figure 7 for all examples of TSPs in the dataset. 4b. TGB plus 1000 ms, Miss (TSPs disappear): When the hunt is unsuccessful, the “granularity” of the deployed body pattern returns to baseline. Illustration by Danbee Kim.



**Figure 7. Examples of tentacle shot patterns.** This composite image shows screenshots of all tentacle shots by all animals at 233.5 ms after TGB, the timepoint when TSPs are most strongly displayed, regardless of whether the animal caught or missed the prey.

Behaviorally there was a noteworthy difference in the duration of the TSPs. At 1.0 second after TGB, TSPs persist during tentacle shots that result in a successful catch; however, for most ( $N = 64$  out of 81, or 79.01%, as measured by frequency band 2) tentacle shots that result in a miss, TSPs have disappeared by this time, or never fully appeared in the first place (see criteria defined in Methods section “Statistical analyses”). During the few “miss” tentacle shots that retain TSPs, the animal’s tentacles got stuck on either the plastic skewer of the robotic prey or the sides of the tank.

### Accuracy of prey capture

We measured the accuracy of the cuttlefish when catching the robotic prey with their tentacles by calculating the percentage of catches made after one, two, or three tentacle shots. Among the animals that made catches ( $N = 5$ ), the mean percentage of catches after the first tentacle shot was 34.68% (variance across animals = 1.65%); the mean percentage of catches after the first or second shot was 49.95% (variance

across animals = 1.71%); and the mean percentage of catches after the first, second, or third shot was 69.65% (variance across animals = 1.38%). See **Table S1** and **Figure S6** for more detailed summaries of the accuracy of our animals while hunting the robotic prey.

### Quantitative characterization of the dynamics of TSPs

To quantify the temporal dynamics of the onset and offset of TSPs, we performed a modified form of the “granularity spectrum” analysis, as described in [22], at 3 s before and after the TGB. The granularity analysis revealed dynamic changes in cuttlefish skin patterning at different spatial frequencies during a prey capture event (**Fig. S5**). Out of the four frequency bands used in our analysis, only the three lowest frequency bands (0, 1, 2) proved useful for quantifying TSPs (see Materials and Methods, section “Video analyses—characterizing TSPs” for quantifications of these frequency bands), as these frequency bands showed both a deviation from baseline around the TGB moment (**Fig. S7** and **S8**) and a significant difference between the mean granularity of successful



versus unsuccessful tentacle shots after the TGB moment (**Fig. S5**).

Band 0, the lowest spatial frequency band, exhibited a strong decrease in contrast energy starting immediately after TGB, while higher frequency bands 1 and 2 showed large increases (**Fig. S5**), consistent with our classification of TSPs as high-contrast signaling patterns of high-contrast Acute Conflict Mottle or larger-scale pattern components of Acute Disruptive Pattern (see [18,23] for definitions of these terms). Bands 0, 1, and 2 showed significant ( $> 3$  sigma) changes from baseline (**Fig. S7**), allowing us to measure their onset and offset timing (**Table S2**). The onset of TSPs was detected earlier during unsuccessful versus successful tentacle shots for all frequency bands. During both successful and unsuccessful tentacle shots, mean onset of TSPs was detected as early as 114 ms before TGB in frequency band 1, and as late as 157 ms after TGB in frequency band 2. The onset is linked tightly to the TGB moment, but the offset is linked to the success of the prey capture event.

TSPs are quickly suppressed following an unsuccessful prey capture attempt, and so we characterized the disappearance of TSPs by identifying when a significant deviation between successful (“catches”) and unsuccessful (“misses”) body patterns occurred following TGB. This analysis was performed non-parametrically (see Methods) *via* a shuffle test at each frame of the 360-frame long TGB videos. We found that the means become significantly ( $P < 0.05$ , pointwise; corrected for global  $P < 0.05$  at upper bound = 99.993 and lower bound = 0.007) different from each other as early as 500, 480, and 470 ms after TGB in frequency band 0, 1, and 2, respectively ( $N = 140$ , see **Fig. 5** and **Table S2** for more details).

## DISCUSSION

We developed an experimental paradigm that can reliably evoke a category of visually conspicuous acute chromatophore expressions in lab-cultured *Sepia officinalis*. These TSPs evoked by our assay have been observed in a variety of cuttlefish species, by several researchers, both in the lab and the wild, but have not been studied in detail or characterized numerically.

Our study quantified the contrast strength and size scale, or “granularity,” of this body pattern category during the 3 s before and after the TGB moment (**Fig. 6**). The TGB moment of each tentacle shot was defined as the moment during a hunt when the tentacles suddenly accelerate towards the prey, as if they were ballistically flung towards the target [24]. When compared to the baseline body pattern at TGB-minus-3 s, TSPs are characterized by a rapid increase in granularity starting as early as 114 ms before TGB. In unsuccessful hunts, TSPs are suppressed as early as 470–500 ms after TGB; whereas in successful hunts, TSPs persist for at least 3 s after TGB.

### Function of tentacle shot patterns

Our results support a role for TSPs as a visual signaling defense against predation when a cuttlefish is exposed and cannot effectively camouflage. The duration of the TSPs was contingent upon whether the tentacle shot successfully caught prey. Since cuttlefish are moving and visually distracted during attacks on prey, they are vulnerable to detection and ambush by nearby visual predators, especially when their tentacles are extended or when consuming prey. We observed cuttlefish using body patterns not seen at any other time during their behavioral repertoire to distract predators during this moment of vulnerability. Spe-

cifically, the cuttlefish in our study deployed highly conspicuous body patterns, which are characterized as high-contrast signaling (as opposed to camouflage) patterns containing strong elements of high-contrast Acute Conflict Mottle or Acute Disruptive Pattern (**Fig. 7**), perhaps as an attempt to cause predators to halt or hesitate for a few seconds. The rapid suppression of TSPs following an unsuccessful prey capture attempt suggests that TSPs are not the preferred pattern when camouflage patterns are possible, and when the cuttlefish is more attentive to its surroundings rather than prey submission. The slightly longer duration of TSPs after successful prey capture supports this explanation, as the cuttlefish is still distracted with manipulating a struggling prey within its arms and must get the prey to its mouth to bite and disable the prey before it can visually assess its surroundings and resume a camouflage body pattern.

### Methodological improvements upon the current study

It would be helpful to replicate this study in younger *Sepia officinalis*, preferably fully developed but young adults (older than 17 weeks, as defined by [11]). Given the typical life expectancy of 18 months for captive *Sepia officinalis*, our animals were at the end of their life expectancy. When compared to previous research documenting the accuracy of cuttlefish hunting live prawns [11,15], our cuttlefish were noticeably less accurate with tentacle strikes, which could be a result of hunting robotic prey, the age of the animals in the study, or both.

Replication datasets with more hunting trials would also help to better characterize TSPs, as the data shown here are highly variable, as well as if/how cuttlefish learn to hunt robotic prey. One interesting observation from this study was that although there were occasions when animals did not settle in the “starting point shelter” and thus did not ever trigger any “food offerings,” the overhead videos showed that the animals were in “hunting mode,” identified *via* behavior such as raised 1<sup>st</sup> pair of arms, burrowing movements (although no sand was present), and slow sneaking punctuated by dramatic and rapid body pattern changes (as if the animal were trying to spook or flush out prey). Future studies could benefit from characterizing and quantifying these displays of predatory behavior.

### Machine learning tools for studying cuttlefish behavior

Computer vision methods at the time of data collection and analysis for this paper were incapable of automatically detecting and tracking the cuttlefish in our videos. Any replications of this study would be aided by recently published tools for estimating the pose of individuals from laboratory and field video recordings [25, 26]. For example, Deep Lab Cut 2.0 [27] has produced promising preliminary results for tracking the cuttlefish in our videos.

Further studies can also investigate the dynamics of TSPs in greater detail by increasing the analysis window beyond the 6 s surrounding the TGB. In the analysis presented here, we used the first second of our 6-second tentacle shot video clips as our baseline period; however, a closer look at the behavior during this period shows that in many of the tentacle shot trials, animals were already in the “positioning” phase of the hunt. A more behaviorally relevant baseline would be based on the body pattern of cuttlefish before they enter the “attention” phase of the hunt, when the animal is not engaged in any hunting behavior at all. Additionally, while this study kept the hunting arena bare to better isolate TSPs, using natural substrates in the hunting tank would also provide more natural camouflage body patterns before and after the

attack. This could lead to additional insight into the dynamics of body pattern changes not only during all phases of prey capture but also during other behaviors in the cuttlefish behavioral repertoire.

### Implications for neuroscience

The methods we present here enable a precise examination of TSPs and other body pattern changes during prey capture. Because these body pattern changes are controlled directly from the central nervous system *via* neuro-muscular action [28], these methods present a promising opportunity to learn how cuttlefish nervous systems use sensory information during a hunt and orchestrate many thousands of chromatophore organs in the skin to rapidly generate intricate and complex body patterns.

More generally, cuttlefish and other cephalopods offer the opportunity to non-invasively study whole organism behavior and single-unit neural activity simultaneously during many sensorimotor behaviors. The methods presented here were inspired by a rodent behavior paradigm established to use high-speed and high-definition video recordings to untangle a subtle difference in motor response based on the predictability of the environment [29]; however, the rodent study methodology relied heavily on invasive procedures to access neural activity and anatomy related to the behavior. In the face of the possibility that their actively camouflaging skin could be treated as an objective measure of their perception and a natural “read-out” of activity in their nervous systems, cephalopods, and in particular cuttlefish, are re-emerging as a promising class of model species for neuroscience research [30–32], and in particular for non-invasive neuroscience research.

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## Supplementary information

**Text S1.** Movement phases of the robotic prey.

**Figure S1.** The experimental tank.

**Figure S2.** Overhead view of hunting box.

**Figure S3.** TGB Videos, spatial alignment.

**Figure S4.** TGB Videos, temporal alignment.

**Figure S5.** Quantifying dynamics of TSPs with “granularity” analysis, all frequency bands.

**Figure S6.** Attempts until catch.

**Figure S7.** First exits from baseline.

**Figure S8.** Boxplots, onset of TSPs.

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

**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.

**Movie S1.** Videos of TSPs, low resolution.

**Movie S2.** Video ethograms.

**Movie S3.** Mean luminance of body pattern mapped to frequency.

**Movie S4.** Video summary of this experiment.

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