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# Cuttlefish adjust body pattern intensity with respect to substrate intensity to aid camouflage, but do not camouflage in extremely low light

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ABSTRACT

Cuttlefish are able to camouflage to a wide variety of natural backgrounds that contain varying colors, intensities and patterns. Numerous studies have investigated the visual cues that influence cuttlefish body pattern expression, yet none have addressed experimentally how well overall intensity is matched between animal and substrate. Here, cuttlefish were tested on artificial and natural substrates that varied in intensity and were illuminated by different light levels; calibrated grayscale photographs were used to analyze the intensity of cuttlefish and their surrounding substrates. We found that cuttlefish scaled their body pattern intensity with respect to substrate intensity under bright and moderate lighting conditions, but not under low or extremely low lighting conditions. Surprisingly, in extremely low light ($<0.0001$ lux), cuttlefish did not camouflage to the substrate, but instead retracted most of their dermal chromatophores, assuming a pale appearance. This closed chromatophore body pattern may represent a low-energy choice when cuttlefish have extremely limited visual input. Overall, these results suggest that at light levels most often encountered in the wild, cuttlefish may achieve resemblance to the background by matching the intensity of the substrates on which they are settled, but they do not camouflage in low or extremely low lighting conditions. In addition, our results suggest the possibility that cuttlefish may be able to detect light at an order of magnitude darker than starlight ($<0.0001$ lux), as evidenced by the expansion of their chromatophores when exposed to this low light level; however, these cuttlefish did not appear to be able to distinguish patterns since they did not camouflage themselves with respect to the substrate.



## 1. Introduction

Cephalopods, like *Sepia officinalis*, are able to rapidly adapt their body pattern, color and intensity to camouflage on backgrounds that they encounter in the wild (Akkaynak et al., 2013; Hanlon, 2007; Hanlon and Messenger, 1988; Hanlon et al., 2011, 2013); this ability appears to be largely under visual control (Hanlon and Messenger, 1988; Marshall and Messenger, 1996). Cuttlefish are also capable of adjusting their body patterns to camouflage at night (Allen et al., 2010; Hanlon et al., 2007), and their night vision must be well developed to detect prey, as well as avoid predation. In fact, one experimental study has shown that *S. officinalis* are capable of dynamically camouflaging in very dim lighting conditions (the equivalent of starlight levels on land; Allen et al., 2010), suggesting that their vision is indeed especially sensitive in low light.

Although they are colorblind (Marshall and Messenger, 1996; Mäthger et al., 2006), cuttlefish are able to produce a wide variety of colors and intensities using their sophisticated skin (e.g., Mäthger et al., 2008, 2009; Wardill et al., 2012). A cuttlefish's appearance depends on which combinations of skin elements are expressed at any given time; light is reflected by pigmented chromatophores, structural reflectors (iridophores and leucophores), or both at the same time (Mäthger and Hanlon, 2007). The interaction between chromatophores and structural reflectors produces colors that encompass the whole visible spectrum (400–700 nm; Mäthger and Hanlon, 2007), allowing these animals to produce the abundance of colors and intensities that enable them to camouflage themselves to their natural surroundings.

Several recent studies have investigated the color- and luminance-matching capabilities of cuttlefish in the laboratory and in the wild, and have found that the spectral properties of cuttlefish and their surroundings (nearby objects and substrates) were closely related

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(e.g., Akkaynak et al., 2013; Chiao et al., 2011; Hanlon et al., 2013; Mäthger et al., 2008; Zylinski et al., 2011). Akkaynak et al. (2013) also showed that, in general, *S. officinalis* matched the luminance spectra of their surrounding substrates more closely than they matched color; the authors suggested that intensity-matching may be more important than color-matching for cuttlefish since most objects tend to appear blue–green underwater due to attenuation of longer wavelengths.

While the color- and luminance-matching capabilities of cuttlefish have been investigated, no study has addressed experimentally how well overall intensity is matched between animal and substrate. In addition, although cuttlefish have been shown to camouflage at night (Allen et al., 2010; Hanlon et al., 2007), their ability to intensity-match under low light conditions has not been examined previously. We tested whether cuttlefish scale their body pattern intensity with respect to the intensity of surrounding artificial or natural substrates under different lighting conditions that were close to what they may encounter in the shallow water they inhabit in the wild: daylight (2000 lux—typical overcast day), crepuscular conditions (56 lux—sunrise/sunset), and a moonless overcast night (<0.0001 lux; Johnsen, 2012). We used calibrated grayscale photographs to compare cuttlefish body intensity to the intensity of their surrounding benthic substrate (we use the term *intensity* to refer to the values recorded in the pixels of an image taken by a camera).

## 2. Materials and methods

### 2.1. Animals and experimental setup

Ten adult *S. officinalis* were used for each set of experiments. Animals were reared from wild-collected eggs and ranged in size from 6.8 cm–8.7 cm in mantle length. To minimize stress to the animals, no more than three trials per day were performed with a single cuttlefish. Experiments were performed in a black tent inside of a light-occluding galvanized steel box (2.0 mm thick). A circular arena (15 cm diameter) was placed inside of a tank with running seawater to confine the animals to the substrates. The arena was illuminated with a ring of broad-spectrum white LED lights (high brightness daylight white, Environmental Lights, San Diego, CA; spectral distribution shown in Supplementary Fig. S1). Cuttlefish were tested at four light levels: 1) bright (LED ring illuminated without any filters), 2) moderate (LED ring with two neutral density filters (LEE Filters #211, Burbank, CA, USA)), 3) low (inside of closed black tent inside of a galvanized steel box with steel door slid open 10 cm) and 4) extremely low (inside of closed black tent inside of a galvanized steel box with steel door closed completely). Light levels were measured inside the arena using a hand-held Field Max II radiometer with an OP-2VIS sensor (Coherent, Inc, Santa Clara, CA) to produce a measurement in watts/cm$^2$ and with an International Light Technologies Research radiometer with a SED033/Y sensor (International Light Technologies, Peabody, MA) to produce a measurement in lux. Cuttlefish behavior was monitored via a television screen outside of the steel box and photographs were taken remotely with a Canon Rebel XS digital camera with a Canon Speedlite 580EX flash. A white and black standard was placed on the outside of the arena—inside the field of view of the camera, but outside the view of the cuttlefish—to standardize the intensity in each photograph.

### 2.2. Experiment 1: artificial substrates

We tested cuttlefish on four computer-generated substrates that were placed on the floor and the walls of the arena (Fig. 1A): dark gray (RGB 61), medium gray (RGB 122), light gray (RGB183), and black and white checkerboard (known to evoke a Disruptive body pattern) at four light levels (bright: 408 μW/cm$^2$, 2100 lux; moderate: 40.1 μW/cm$^2$, 56 lux; low: 2.04 nW/cm$^2$, <0.0001 lux; extremely low: 1.02 nW/cm$^2$, <0.0001 lux) to determine whether light intensity affected cuttlefish body intensity and/or body pattern. The substrate and light intensity combinations were presented to cuttlefish in a randomized order. At the beginning of each trial, cuttlefish were placed inside of the experimental arena and allowed to acclimate to extremely low light for 20 min. After acclimation, a flash photograph was taken of the cuttlefish using a remote trigger (modified optical interrupter, Mumford Microsystems, Santa Barbara, CA). Then, a second photograph was taken using the following procedure: The lights inside of the experimental arena were turned on to the setting to be tested in that trial and the cuttlefish were allowed to acclimate to the experimental light setting for 20 min. After 20 min, the lights inside of the experimental arena were turned off, which immediately (i.e., 5 μsec) triggered the camera to take a flash photograph; this speed is faster than cephalopods are known to respond with a change in body pattern (e.g., in *Hapalochlaena lunulata*, the blue-ring flashes can be shown in as little as 0.3 s (Mäthger et al., 2012)). All photographs were taken under identical conditions (using an external camera flash in the dark), regardless of the experimental light setting. This procedure allowed the photographs to record any differences in cuttlefish body pattern intensity according to the experimental light levels without affecting the overall intensity of the images.

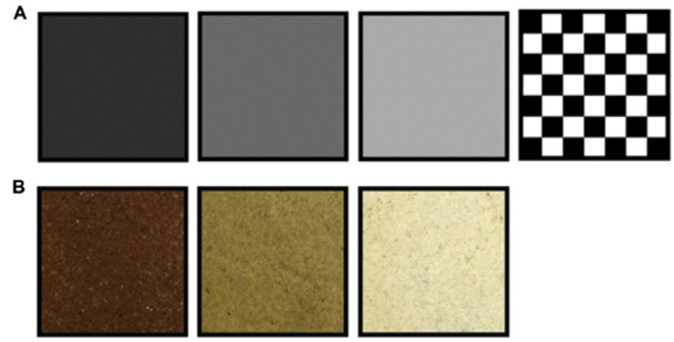


**Fig. 1.** Experimental substrates used to test for scaling of body pattern intensity with respect to substrate intensity in *Sepia officinalis*. A) Artificial gray substrates: dark gray (RGB = 61), medium gray (RGB = 122), light gray (RGB = 183) and black (RGB = 0) and white (RGB = 255) checkerboard. B) Natural sand substrates: dark brown sand, light brown sand and white sand.

### 2.3. Experiment 2: natural substrates

We tested whether cuttlefish scale their body intensity on natural substrates that were glued to the floor and the walls of the experimental arena: dark brown sand, light brown sand and white sand (Fig. 1B) at four light levels (bright: 408 μW/cm$^2$, 2100 lux; moderate: 40.1 μW/cm$^2$, 56 lux; low: 2.04 nW/cm$^2$, <0.0001 lux; extremely low: 1.02 nW/cm$^2$, <0.0001 lux). Photographs were taken using the same method and time intervals as in Experiment 1. Since substrates were made from natural sand that was not homogeneous, there was some natural variation in the intensity of each substrate.

### 2.4. Image analysis

Images were captured in camera raw format and were manually processed in MATLAB (Mathworks, Inc. Natick MA) as described in Akkaynak et al. (2014). White and black photographic calibration targets were placed outside of the arena—inside the field of view of the camera, but outside the view of the cuttlefish—to standardize the intensity in each photograph. Demosaicing was done with the default algorithm used by Adobe DNG converter (Adobe, Inc., version