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Quantification of cuttlefish (*Sepia officinalis*) camouflage: A study of color and luminance using *in situ* spectrometry

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Keywords: animal coloration, spectral angle, color match, body pattern, fish predator

Abstract

Cephalopods are renowned for their ability to adaptively camouflage on diverse backgrounds. *Sepia officinalis* camouflage body patterns have been characterized spectrally in the laboratory but not in the field due to the challenges of dynamic natural light fields and the difficulty of using spectrophotometric instruments underwater. To assess cuttlefish color match in their natural habitats, we studied the spectral properties of *S. officinalis* and their backgrounds on the Aegean coast of Turkey using point-by-point *in situ* spectrometry. Fifteen spectrometry datasets were collected from seven cuttlefish; radiance spectra from animal body components and surrounding substrates were measured at depths shallower than 5m. We quantified luminance and color contrast of cuttlefish components and background substrates in the eyes of hypothetical di- and trichromatic fish predators. Additionally, we converted radiance spectra to sRGB color space to simulate their *in situ* appearance to a human observer. Within the range of natural colors at our study site, cuttlefish closely matched the substrate spectra in a variety of body patterns. Theoretical calculations showed that this effect might be more pronounced at greater depths. We also showed that a non-biological method ("Spectral Angle Mapper"), commonly used for spectral shape similarity assessment in the field of remote sensing, shows moderate correlation to biological measures of color contrast. This performance is comparable to that of a traditional measure of spectral shape similarity, hue and chroma. This study is among the first to quantify color matching of camouflaged cuttlefish in the wild.

Introduction

Coleoid cephalopods are unrivaled in the animal kingdom in their ability to quickly and dynamically change their body patterns for signaling and camouflage (e.g., Hanlon and Messenger 1988; Hanlon and Messenger 1996). Pattern and color change are achieved through the physiological control of chromatophore organs (Florey 1969; Messenger 2001), and structural reflectors (iridophores and leucophores, Mäthger et al. 2009; Wardill et al. 2012). Many laboratory and field studies have shown that camouflage behavior in cuttlefish is visually driven (e.g. Chiao and Hanlon 2001a, b; Chiao et al. 2005; Barbosa et al. 2007; Chiao et al. 2007; Kelman et al. 2007; Barbosa et al. 2008a, b; Allen et al. 2009; Chiao et al. 2009; Zylinski et al. 2009a, b, c; Chiao et al. 2010; Barbosa et al. 2012). Counterintuitively, these animals adaptively tune their body patterns in response to their visual surrounds without the use of color vision (Marshall and Messenger 1996; Mäthger et al. 2006).

Until now, spectrophotometric quantification of cuttlefish camouflage had only been performed using data collected in the laboratory (Mäthger 2008; Chiao et al. 2011), but *in situ* measurements of cuttlefish skin radiance spectra under natural illumination and in natural habitats are crucial to provide empirical evidence to assess the degree of color matching. In this study, we collected radiance spectra from the common European cuttlefish, *Sepia officinalis*, and their visual surrounds (sand, gravel, pebbles, algae-covered rocks, peacock's tail alga, hydrozoans, *Pinna* mollusc shells, etc., hereafter, "substrates") underwater on the Aegean Coast of Turkey.

Studies such as this one are often constrained by technology and methodology (Johnsen 2007), thus we introduce an auxiliary methodology here because sometimes it may be necessary to assess the similarity of two spectra independently of the perception of a particular visual

system. For example, there may not be enough information about the photoreceptor types or ratios of a certain predator, or a result that can be generalized across visual systems may be desired. In such cases, purely mathematical measures of spectral similarity can provide a rough approximation. For this reason, we first used Spectral Angle Mapper (SAM), a technique commonly used to assess spectral shape similarity in the field of remote sensing, to quantify the similarity of cuttlefish and substrate spectra. Then, we developed a systematic analytical tool to compare the luminance and color contrast between animal and substrate spectra in the eyes of hypothetical di- and trichromatic fish predators and supplemented our spectral comparison by further analyzing cuttlefish color matching in human color space using the International Commission on Illumination (CIE) 1931 XYZ model. We found that SAM correlated moderately with our biological measures of color contrast, making it comparable to a traditional chroma and hue based measure of spectral shape similarity (Endler 1990).

Methods

Study site, animal & substrate measurements

Seven cuttlefish (mantle lengths between 15 and 22cm) were studied off the village of Çeşmealtı (İzmir) on the Aegean coast of Turkey in spring, 2011. Fifteen radiance datasets were collected; each data set consisted of one animal, one location and one body pattern. Radiance measurements were taken using a USB2000 spectrometer (sensitivity range: 200-850nm; Ocean Optics, Dunedin, FL) coupled with a Compaq iPaQ handheld computer in a custom underwater housing (Wills Camera Housings, Victoria, Australia). Optical fibers (50 and 100-micron diameter) were used to collect data from cuttlefish and surrounding substrates (Fig. 1a). We used a Spectralon (Labsphere, UK) target as a white reflectance standard. A CC3 cosine corrector

(Ocean Optics, Dunedin, FL) was attached to the optical fiber for collection of irradiance data and an LS-1-CAL tungsten light source (Ocean Optics) was used to obtain absolute intensity values. This calibration was done in a dark room to minimize stray light. Our equipment design is documented in (Roelfsema et al. 2006) and has been used for fieldwork by many others (Leiper et al. 2012, Hedley et al. 2012, Lim et al. 2009, Cheney et al. 2008, Matz et al. 2006, Cheney and Marshall 2009).

Photographs were taken with a 24-70 mm lens on a Canon EOS 1-Ds Mark II digital camera in a Subal housing equipped with a dome port. Videos recorded with a compact FlipCam (Irvine, CA) documented the underwater data collection. Measurements were taken under natural light, at depths shallower than 5 meters. Animals were carefully approached by two divers (DA & JJA) until they habituated to the divers' presence and did not show any signaling behavior (e.g., unilaterally expressed pattern components or Paired mantle spots as part of a Deimatic display; see Hanlon and Messenger 1988).

Animals were allowed to settle in a location of their choice and substrates were not altered, with one exception: before data acquisition, white rocks were placed near one cuttlefish to evoke a weak pattern (Fig. 2g). This pattern is known to be the camouflage response to white cues in a cuttlefish's visual surrounds (*S. pharaonis*, Chiao and Hanlon 2001; *S.officinalis*, e.g., Barbosa et al. 2007, Mäthger et al. 2007).

In each case, ten to fifteen radiance measurements were recorded for each body component (Fig. 1b), background element, or irradiance measurement and their average was used for the data analysis. If the cuttlefish moved or changed body patterns during data acquisition, the animal was allowed to re-habituate and measurements from the new location/body pattern were recorded as a new data set. Similarly, if lighting conditions changed

significantly (e.g., in response to passing clouds), a new set of irradiance and white standard measurements was recorded. In both cases, we only analyzed datasets that were taken under consistent lighting conditions. In all cases, the probe head of optical fiber was less than 3 cm away from the feature whose spectrum was being recorded. We ensured that the diver's hand did not shadow the area of interest.

Assessment of chromatic similarity between animal and background spectra using a non-biological measure (Spectral Angle Mapper)

In the field of remote sensing, automated spectral library search algorithms developed for hyper-spectral images (Chang 2003; Sweet 2003; Freek 2006; Nidamanuri and Zbell 2011) are used to compare reflectance spectra of known targets to those of novel spectra by computing a scalar similarity score between them. For these algorithms, stochastic methods are more frequently used than deterministic methods because imaging conditions can be imperfect and because the high spectral resolution of a hyper-spectral sensor often results in more than one material spectral signature in a given pixel. In our study, spectral data were point-by-point measurements of solid color patches where the area of the patch was much wider than the diameter of the spectrometer fiber. Hence, we use a deterministic method, Spectral Angle Mapper (SAM), from the field of hyper-spectral image classification in our assessment of spectral shape similarity. SAM is the most commonly used spectral angle-based similarity measure (Yuhas et al. 1992; Kruse et al. 1993) and it is the normalized inner product of two vectors. It is computed as follows:

(1)

where "$\mathbf{S_1}$" and "$\mathbf{S_2}$" are the two spectra vectors being compared. Each continuous radiance spectrum is vectorized (denoted) into 31 dimensions by taking its value every 10 nm in the visible range (400-700nm). "T" denotes the transpose of a matrix and the ||.|| symbol denotes Euclidian norm; the division by the vector norms indicates that SAM is indifferent to the magnitude of the vectors (brightness) and only calculates similarity of spectral shape (color). A small angle between two vectors indicates that the spectra are similar in shape.

Calculation of color difference using chroma and hue

A common way to assess the similarity of two spectra independent of a visual system is to compute their chroma (C), hue (H) and brightness (B) values, and calculate the Euclidean distance between them (D). A small Euclidean distance means that the two colors are similar. Here, we used the segment classification analysis of spectra from Endler (1990) to calculate hue and chroma. Our goal is to compare the performance of this method to that of SAM. SAM metric does not take brightness into account and for a fair comparison, before chroma and hue values are calculated each spectra should be multiplied with a constant to equalize their overall brightness (see Endler 1990 for further details). Following this, the distance between the two colors are found from:

(2)

where

Although the details of visual systems of cuttlefish predators are not known (*Serranus cabrilla* is the only fish species observed directly to prey on *S. officinalis* in the Mediterranean sea), we may speculate that cuttlefish are preyed upon by a variety of vertebrate and invertebrate predators with different visual systems (Hanlon and Messenger 1988; Hanlon and Messenger 1996). Thus, we chose one dichromatic fish and one trichromatic fish as their potential predators to simulate their views of these camouflaged cuttlefish. In dichromatic fish, the λ_{max} of S and M cones was 460 and 570 nm. In trichromatic fish, the λ_{max} of S, M, and L cones was 460, 540, and 570 nm. N.B., although the choice of these λ_{max} of dichromatic and trichromatic cones was arbitrary, shifting the λ_{max} of these cones up or down 10-20 nm did not visibly affect the results.

We carried out this analysis for photoreceptor ratios 1:1:1 and 1:2:2 for trichromats and 1:1 and 1:2 for dichromats; these are typical fish retina cone mosaic patterns (Shand et al. 1999, Cheney and Marshall 2009). The results of our analysis in both cases were similar, and we only present results for the ratios 1:1:1 for trichromats and 1:1 for dichromats.

Luminance contrast (

where

different perceptual distances. The XYZ tri-stimulus values cannot be visualized directly; to offer a visual comparison of the variety of cuttlefish and habitat colors at our field site, we converted the XYZ tri-stimulus values into the sRGB space and created color patch assemblies for both animal and substrate data. For details on converting XYZ tri-stimulus values to RGB color spaces, see Reinhard et al. (2008).

Chromaticity diagrams are also used in the studies of animal color vision (see Pike 2011 and Kelber et al. 2003) as graphical representations of perceived colors. We followed the methodology described in Kelber et al. (2003) to plot the loci of colors measured from cuttlefish and surrounding substrate on the Maxwell triangle for a hypothetical trichromatic observer with λ_{max} values for S, M, and L cones 460, 540, and 570 nm.

Simulation of color and luminance contrasts at depth

Underwater, the available light field changes in intensity and spectral composition, changing the appearance of objects in response to factors such as: depth, time of day, weather conditions and the amount of suspended particles in the water column. To illustrate the effect of this change on camouflage, data from a uniform dark animal collected at 1 m depth (an animal that did not appear well color matched to its surroundings) were used to simulate the appearance of its colors at a depth of 10 m using irradiance spectra collected at 10 m at our study site. The simulation was done as follows. First, the radiance spectra from the original dataset were converted into reflectance by dividing the difference of radiance of the feature of interest and dark noise by the difference of white standard radiance and dark noise. Second, these reflectance spectra were multiplied with irradiance spectra we recorded *in situ* at 10 meters depth. Third, simulated animal and substrate radiance spectra were assessed for similarity and discriminability

using methodology described above. Color patches were simulated in sRGB space using the tri-stimulus values obtained from the CIE 1931 XYZ model.

Results

In Fig. 2(i) radiance measurements taken in the field are summarized. Measurements taken from cuttlefish components are labeled on the outlines of photographs with black numbers, those taken from substrates are labeled with blue letters. Larger, high-resolution photographs are available in Online Resource 2. The same color convention is used in spectral curves and each curve is normalized by its maximum value to emphasize similarity of shape. The red curve in each plot is the (normalized) spectrum recorded from a Spectralon target and represents the shape of "white" under ambient light conditions.

Use of Spectral Angle Mapper for assessing spectral shape similarity between animal and background

Fig. 2(ii) shows the Spectral Angle Mapper scores computed between each cuttlefish component and background substrate measured. The limits of each plot were adjusted to the minimum and maximum values of SAM encountered across all datasets (0 and 0.4508, respectively). The score computed by SAM is a measure of how well two multi-dimensional vectors are aligned and does not carry a biological meaning. Therefore, while the SAM scores cannot give any information regarding the discriminability of two colors from the perspective of any visual system, they are informative about how similar the shapes of two spectra are, which can be compared to chromaticity. In general, the lower the SAM score, the more similar the colors. For example, in Fig. 2a, the cuttlefish has a uniform body pattern, which appears well matched to the surrounding substrates in color. The corresponding SAM plot has low scores

throughout. The animal in Fig. 2b also has a uniform pattern but, unlike Fig. 2a, it does not match the surrounding sand. This difference is captured in the magnitude of the values displayed in the SAM plots.

Assessment of luminance and color contrasts in the eyes of hypothetical di- and trichromatic fish predators

In Fig. 3(i), we present luminance contrast among cuttlefish components and luminance contrast between cuttlefish components and substrates. Contrast among cuttlefish components is an indicator of whether the cuttlefish pattern is uniform or non-uniform (i.e. mottle, zebra or weak), and contrast between animal and substrate is an indicator of how similar the cuttlefish components are from the substrate in brightness. Fig. 3(ii) shows color contrast between cuttlefish and substrates as seen by hypothetical di- and trichromats. In both (i) & (ii), a value of 1 is a "just noticeable difference" (JND) and is marked with a green line.

Pattern 1 **(Fig. 3a)**. Luminance contrast values below the JND = 1 line suggest this animal has a uniform pattern, and matches the substrates in intensity. The photograph in Fig. 2a confirms these observations. Low color contrast values suggest that this animal is difficult for both di- and trichromats to distinguish from its background.

Pattern 2 **(Fig. 3b).** Low luminance contrast among animal components indicates a uniform pattern. Almost all cases of luminance contrast between animal and substrates are above the visual threshold, indicating this animal did not match the substrate in luminance. Color contrast values suggest this animal can be distinguished from its background by both di- and trichromats, but note that color contrast values, overall, are lower for the dichromat (on average,

a trichromat. Indeed, there was a mismatch between this cuttlefish in a dark uniform body pattern (with weak zebra stripes) and the sand in its immediate surroundings as confirmed by Fig. 2b.

Pattern 3 **(Fig. 3c).** In this dataset, we observed that cuttlefish components had high and low luminance contrast values among themselves indicative of a non-uniform (mottle, zebra or weak pattern). The photograph in Fig. 2c shows a mottle/weak weak body pattern.

In both color and luminance contrast, when compared to the dark green *Posedonia* seagrass ("b"), all cuttlefish components scored above the visual threshold. In response to visual cues from a nearby three-dimensional structure with projections approximately the width of its arms, this animal raised its first pair of arms, a postural component of cuttlefish camouflage (Barbosa et al. 2012). Although some body components matched the gravel in luminance and color contrast, the arm posture and overall body pattern suggested this animal may have performed masquerade camouflage in response to visual cues from the three dimensional *Posedonia* seagrass (for discussions of masquerade camouflage, see Stevens and Merilaita 2009; Skelhorn et al. 2010; Buresch et al. 2011; Skelhorn and Ruxton 2011).

Pattern 4 **(Fig. 3d).** Luminance contrast plots suggest this animal has a uniform pattern and matches the substrate in intensity. The photograph in Fig. 2d shows that this animal had a uniform body pattern with some aspects of a mottle. While this animal is well matched to its surroundings in luminance, its overall pattern is detectable in terms of color contrast to both di- and trichromats (Fig. 3d,ii). It was difficult to distinguish this cuttlefish from nearby 3D objects, suggesting this animal might have performed masquerade camouflage instead of background matching (e.g., Buresch et al. 2011). Note that the radiance spectra measured from the components of this animal were similar to those measured from a dark brown hydrozoan roughly two mantle lengths away (Fig. 2d, inset).

***Pattern 5* (Fig. 3e).** The luminance contrast values suggest a mottle, zebra, or weak pattern and the mostly high color contrast values in (ii) suggest this animal may be easy to spot against the background. The photo shows that this animal had a mottle pattern with weak expression of the White square and Median mantle stripes, components often seen in weak body patterns. All animal body components closely matched the nearby *Pinna* mollusc shell ("a") in luminance contrast but had high contrast when compared to the gravel ("b"). Overall, this animal could be detected by a hypothetical di-or tri-chromatic predator.

***Pattern 6* (Fig. 3f).** The low values of luminance contrast among animal components suggest a uniform pattern. The photograph shows (Fig. 2f) that the animal actually had a weak zebra pattern, showing low overall contrast. This animal allowed us to collect data from individual light and dark stripes within two body pattern components: the Posterior mantle bar ("20") and the White mantle bar ("3"). As in the case of pattern 3, the cuttlefish components better matched the green *Posedonia* seagrass in luminance than in color contrast (substrates "a" and "b"). This result is intuitive as the cuttlefish is not capable of producing a green color with its pigmented chromatophores, but can alter the intensity of skin to appear light or dark. Overall, this animal could be detected by a hypothetical di-or tri-chromatic predator.

***Pattern 7* (Fig. 3g)**. Luminance contrast among cuttlefish components suggest that this animal has a uniform body pattern; however from Fig. 2g, we see that it had a weak pattern. Its weak pattern was turned on after white rocks were placed nearby. When compared among cuttlefish components and substrates, luminance contrast values were high; indicating the animal's components are darker than the surrounding white rocks, with the exception of its White square. The luminance of the White Square ("2") was similar to the luminance of the other components. The cuttlefish components were most similar to substrates ("c" and "d") in luminance contrast,

and nearly indistinguishable in the eyes of both di- and trichromats in terms of color contrast. Note that the White square was not pure white, rather, closer to a light brown color. White square luminance and color are modulated by pigmented brown, orange and yellow chromatophore organs overlying the structural reflectors (lecuophores) responsible for whiteness in this animal (M äthger et al. 2009)

Comparison across all patterns

Fig. 4a summarizes the mean JND values across all datasets. In almost all cases, the dichromat JND values were equal to or smaller than the trichromat JND values, suggesting that it was more difficult for the dichromats to distinguish the cuttlefish components from the substrates. Similarly, the mean luminance contrast of the cuttlefish components, when compared to the surrounding substrate, was less than or slightly higher than 1 JND; the cuttlefish generally did a good job matching the luminance of the substrates. For datasets “b” and “b10m” (the latter is the animal whose appearance is simulated at 10 meters, described in the “Simulation of spectra at depth” subsection), the luminance contrast was nearly unchanged, while the mean color contrast fell to the JND = 1 limit. The mean values in dataset “e” are likely to be affected by the measurement of the dark eyespots; these spots do not usually appear as a part of a camouflage pattern and were displayed as a warning to the divers.

Fig. 4b shows the percentage of pairs of components that had JND values less than 1. Animals in datasets “a” and “c” appear to have the best overall color and luminance match in the eyes of both di- and trichromatic predators, and those in “d”, “e” and “f” seem to have done a better job matching luminance than color. Overall, the animal in dataset “b” was the worst color matched animal. Its simulated appearance at 10 meters depth was more conspicuous than the animal at 1-meter depth.

The relationship between SAM, chroma and hue based color difference (D), and color contrast

One cuttlefish (see Fig. 2b; data taken at 1m depth) poorly matched its surrounding sandy substrate. We used the irradiance profile we recorded at our study site at a depth of 10 meters to obtain a theoretical ambient light field and simulate this animal's radiance spectra had they been collected at that location (see Methods for details). Simulated spectra, SAM, luminance and color contrast results are presented in Fig. 6a. At this depth, the shapes of all spectra become similar in shape due to the ambient light conditions as shown by the spectral curves. Overall, the SAM scores decreased by about 50%; indicating that the spectra became more similar in shape at depth. Luminance contrast remained nearly the same. This is expected, as luminance contrast is a ratio of the quantum catches, and remains unchanged when the same process attenuates both spectra. The color contrast plots show that in the eyes of both di- and trichromat, the color match at 10 m depth has gotten better, and it is now harder to distinguish this animal from its background.

In Fig. 6b Maxwell triangles show the change in the appearance of colors from 1 m to 10m. At 10 meters, cuttlefish and substrate colors form tighter clusters than they did at 1-meter depth. The sRGB appearance of colors shows that at 10 meters, the cuttlefish and substrate colors are indistinguishable.

Discussion

The utility of in situ *spectrometry in studying animal camouflage*

Here we presented spectrophotometric field data collected from camouflaged cuttlefish (*Sepia officinalis*) and some of their surrounding substrates. We have advanced previous studies by collecting data in the cuttlefish's natural benthic, near-shore environment where the daylight spectrum is affected by atmospheric conditions, water quality and depth (Jerlov 1968, 1976; Tyler and Smith 1970). Studying cuttlefish camouflage under natural conditions is essential for the study of animal and background luminance and color because it is under these conditions that

cuttlefish camouflage body patterns have evolved to successfully deceive the eyes of predators. Recently, field spectrometry data collected by Hanlon et al. (submitted) were analyzed for a different species, the giant Australian cuttlefish (*Sepia apama*), using a similar approach to quantify camouflage in the eyes of potential fish predators. Studies assessing the color signals of animals through the visual systems of their known predators have been done for chameleons (Stuart-Fox and Moussalli 2008), spiders (Heiling et al. 2003), (Théry and Casas 2002), fish (Marshall and Vorobyev 2003) and birds (Endler and Mielke Jr 2005). Spectrometers, however, are not ideal instruments for the assessment of overall animal patterns because they only record point-by-point samples. This makes it difficult, if not impossible, to collect spatial information from high-frequency textures, i.e. textures that are not solid color patches. In addition, animal coloration studies using spectrometers require getting the optical fiber very close to the skin of the animal; this is challenging while studying freely behaving animals in their natural habitats. The use of such equipment underwater further complicates the data collection process due to the rapidly changing light field from the undulations of the sea surface, limited light penetration at depth, particles suspended in water that affect visibility. Furthermore, wave surge, currents, and practicality issues such as bulky watertight housings affect the speed and maneuverability of divers, and the corrosion of equipment from seawater and reduced performance from most batteries due to low operating temperatures also distinguish such marine studies from terrestrial endeavors.

Imagers that record continuous spectra for every pixel in an image, namely multi- and hyper-spectral cameras, are becoming common in many fields of science, and their use has been pioneered by Chiao et al. (2011) in the field of animal coloration. While the costs of such imagers are still prohibitive, rapid developments in technology are making their deployment in

the field as fast as commercial off-the-shelf digital cameras, and they will likely replace spectrometers to become the standard in studies of coloration in the next decade.

A non-biological measure, SAM, is a rough estimate of color contrast,

advantage of the opponency of color channels while SAM is only mathematical measure of shape. These results should be interpreted with caution since they do not relate to any biological system and most terms such as luminance, color, chromaticity etc. are meaningful with respect to visual systems. More specifically, they don't refer to the visibility of colors. We expect a more solid understanding of the relationship between mathematical and biological measures of color contrast to emerge as future studies adopt the calculation of a SAM score along with their biological analysis. From our study, it can be concluded that there is more to the discrimination of colors than simply a spectral shape mismatch. For example CIE

human visual system. This visual representation provided a way to assess the similarity of spectral properties between animal and background. Cuttlefish body components and substrates, were constrained to a general area around the point x ≈0.4 & y≈0.35 near the locus of the "white point" at x = 0.33, y = 0.33 (Fig. 5a). Colors close to this point would appear gray under most lighting conditions. This narrow distribution implies that while cuttlefish chromaticity values are remarkably close to those of substrates, our study site was not very colorful, at least in comparison with terrestrial colors that humans are used to (see black squares that show colors from a Macbeth ColorChecker, representative of colors that a human might observe terrestrially on a daily basis).

In our analysis of luminance contrast, we only presented results for trichromats. Since long- wavelength receptors are thought to be responsible for luminance contrast in fish (Marshall et al. 2003) and we used

Broad-spectrum sunlight can be available under clear water at shallow depths on a cloudless day, especially in kelp or coral reef habitats. Under those conditions, colored objects (e.g., sand, rocks, algae, coral, tunicates, sponges, etc.) will appear colorful (Jerlov 1976; Chiao et al. 2000; Marshall et al. 2003a,b; Hochberg et al. 2004). However, many underwater light fields are not made up of broad-spectrum sunlight because light is scattered by particles suspended in water (e.g., plankton, sediment, algae, etc.) and attenuates non-uniformly with depth and wavelength (Tyler and Smith 1970). Therefore, many marine habitats do no appear particularly colorful, even if the substrate contains colorful objects. In shallow coastal areas, such as our dive site, water turbidity is key in limiting the spectral composition of daylight. At great depths, it is thought that camouflage by intensity matching is more effective than color matching, since the appearance of most objects become blue-green (see references in Mäthger et al. 2006).At a depth of 10 m, color contrast between the simulated animal spectra and substrate spectra was less substantial than differences between actual animal and substrate spectra collected at a depth of 1 m. Luminance contrast, however, remained practically unchanged, as the ratios of quantum catches using attenuated spectra did not change significantly. This result suggests that the animal would have been less distinguishable from its background in terms of color had the spectra been measured at this depth (Fig. 6), but would have appeared to have the same luminance contrast relative to the surrounding substrate despite attenuated ambient light. Mäthger et al. (2008) performed a similar simulation using laboratory data and showed that color match differences adjusted for a depth of 10 m were half as substantial as differences measured in a few centimeters of water.

To visualize this attenuation further, we used Maxwell triangles to represent colors from the visual system of a hypothetical trichromat predator (data only shown for one case, see Fig.

6b). In the case of one animal that appeared to have a bad color match to its surroundings (Fig. 2 & 3b) at 1 m, the cuttlefish and substrate colors were relatively widely dispersed on the Maxwell triangles (Fig. 6b). When the appearance of this animal was simulated at a depth of 10 meters (Fig. 6, ii), the loci of colors on the Maxwell triangle almost completely overlapped, indicating that this animal's body pattern would be better camouflaged in the eyes of trichomatic predators at a depth of 10 meters.

Color matching in colorblind cuttlefish

Researchers have been puzzled by the color-matching aspect of cuttlefish camouflage because cuttlefish are known to be colorblind (Brown and Brown 1958; Marshall and Messenger 1996; Mäthger et al. 2006). Mäthger et al. 2008 suggested that the spectral properties of *S. officinalis* body patterns and many natural objects are generally similar, thereby rendering color match less difficult. Our field data confirm their speculation for *S. officinalis*, at least for this particular study site. *S. officinalis* encounters a wide range of habitats including temperate rock reef environments throughout the Mediterranean and coral habitats off the west-central African coast. Certainly, some colors are not in the color repertoire of cuttlefish skin as Mäthger et al. (2008) showed in laboratory studies. Although cuttlefish body pattern spectra were remarkably similar to many of the natural substrates at the Aegean study site, the color spectra of those particular background substrates and objects were limited (Fig. 5) and natural substrates that cuttlefish cannot match undoubtedly exist within its geographical range. For example, none of the parts of the animals in Fig. 2, c & f closely matched the dark green *Posedonia* in color contrast while many of the measured body pattern components closely matched tan and brown sand and gravel substrates.

As cuttlefish approach sexual maturity, their skin undergoes a physiological and morphological change where iridophores and leucophores develop to form White zebra bands (Hanlon and Messenger 1988), an important component of body patterns used for sexual signaling. Although the White zebra bands can be masked by the overlying chromatophores, they are permanent and are often partially visible while a mature cuttlefish is camouflaged. One cuttlefish (Fig. 2f) showed a weak zebra pattern and spectral measurements from individual light and dark bands had low luminance and color contrast relative to some of the surrounding substrates. This result supports the speculation of Hanlon and Messenger (1988) that, in addition to their role in signaling, the White zebra bands can contribute to camouflage when their bright contrast is modulated by actively masking them to varying degrees by overlying chromatophores.

Overall, cuttlefish skin pattern components for camouflage closely resembled the luminance and color of surrounding substrates in the eyes of hypothetical di- and trichromatic fish predators we modeled, but the range of colors found in this particular habitat on both cuttlefish and substrates was narrow. Nevertheless, our light-field and animal/substrate measurements corroborate that the spectral properties of chromatophores and natural objects are similar, thus facilitating color matching by cuttlefish. Moreover, cuttlefish can neurally control the expression of chromatophores thus selectively reveal underlying reflector cell types such as leucophores, which also have some capability to tone match and perhaps reflect ambient wavelengths (Messenger 1974). Despite apparent colorblindness in cuttlefish, the tone and color matches between animal and background make cuttlefish camouflage superb in the animal kingdom (see selected images in Hanlon et al. 2009). It remains a future challenge to discover how cephalopods achieve color resemblance to multiple backgrounds, and to what degree this is an active vs. passive process. This can be approached in the near future by testing the color

matching abilities of cuttlefish in more chromatically diverse habitats (e.g., a coral reef) with hyper-spectral imagers and fuller characterization of the light field; currently, little is known about the color matching abilities of cuttlefish that live different habitats. Another approach, albeit logistically difficult, would be to transport cuttlefish native to a chromatically poor habitat to a chromatically rich habitat. Many such challenges remain in the field of sensory ecology, not just of cephalopods in marine habitats but many taxa involved in visual predator/prey camouflage interactions.

Acknowledgements

We thank Elron Yellin, Sinan Akkaynak and Çeşmealtı Güzelleştirme Derneği for their help with data collection. We are grateful to Dr. Felice Frankel for helping with data presentation and to four anonymous reviewers for their constructive comments. JJA is grateful for support from a National Defense Science and Engineering Graduate Fellowship. This study was funded by ONR grant N000140610202 to RTH.

Abbreviations

B Brightness

C Chroma

D Euclidean distance between hue, chroma and brightness of two spectra

H Hue

SAM Spectral Angle Mapper

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Figure Legends

Fig. 1 (a) Spectral data were collected by two divers (DA and JJA). One diver operated the spectrometer while the other took still photographs and video to document the sequence of measurements. All measurements were taken under natural daylight after the cuttlefish had habituated to the presence of divers. (b) Cuttlefish body pattern components measured in this study, numbered and capitalized according to their description in Hanlon and Messenger (1988). 1 = white posterior triangle; 2 = white square; 3 = white mantle bar; 13 = white head bar; 14 = white arm triangle; 17 = anterior transverse mantle line; 18 = posterior transverse mantle line; 19 = anterior mantle bar; 20 = posterior mantle bar; 21 = paired mantle spots; 22 = median mantle stripe; 29 = anterior head bar; 39 = white square papillae.

Fig. 2 (a-g) Presentation of seven datasets; in (i) we present a photograph of the scene, an outline of the scene showing the spots radiance spectra were recorded from and normalized radiance spectra; in (ii) the Spectral Angle Mapper score computed between the spectra of each animal component and background substrate are shown. For example, for the animal in (a), the bars represent the SAM score between pairs of features: (“a”) & (“1”), (“a”) & (“2”), (“a”) & (“3”), (“a”) & (“19”), (“a”) & (“20”), (“b”) & (“1”), (“b”) & (“2”), (“b”) & (“3”), (“b”) & (“19”), (“b”) & (“20”) and so on. They are sorted in descending order. See text for details. We repeat here the pattern names we have used in text for each photo: (a) uniform/stipple, (b) dark uniform with weak zebra stripes, (c) mottle/weak weak, (d) mottle, (e) mottle, (f) weak zebra, and (g) weak. See supplementary online resource 2 for larger, high resolution images.

Fig. 3 (a-g) In (i), luminance contrast is shown for two cases: amongst the spectra of the cuttlefish components and between the spectra cuttlefish components and the background substrates. In (ii), color contrast calculated between spectra of cuttlefish components and substrates are presented for hypothetical di and tri-chromats we modelled. Luminance contrast values shown are for trichromats only and are identical for dichromats. See text for details. In all plots, the green line indicates a "just noticable difference" of 1; pairs of features that fall below this value cannot be distinguished by the visual system under consideration.

Fig. 4 (a) Mean just noticable difference (JND) values shown for each dataset and (b) the percent of cuttlefish components that are below 1 JND for each dataset. **(b)** We found that SAM has moderate correlation ($0.5<|r|<0.7$) to

measured. Each color patch in an assembly is accompanied by a corresponding white patch, representing the way "white" looked under the ambient conditions. Depending on depth, time of day, visibility etc., white may appear as shades of green and blue.

Fig. 6 Simulation of the spectra of the animal and nearby substrate from Fig. 2&3b, at a depth of 10m: (a) Normalized radiance spectra of cuttlefish components and substrates; red line indicates normalized spectra from of a Spectralon white target; SAM scores are almost halved when compared to Fig. 3b; luminance contrast remains unchanged and color contrast has decreased significantly. (b) Visualization of the colors of cuttlefish and substrate patches on a Maxwell triangle. In the 1 m case the loci of colors are relatively widespread, but they become almost coincident at 10 m. sRGB representation of color appearance suggests colors are indistinguishable to the human visual system at 10 meters depth.

Online Resource 1: Traditionally, hue and chroma values have been used as rough estimates of spectral shape. In this example we demonstrate how SAM scores compare to hue and chroma by computing color differences between every pair of color patches in the Macbeth ColorChecker, shown in (a). (b) The reflectance spectra of each color patch. (c) Normalized intensity of the CIE D65 illuminant. D65 is commonly used as an approximation to noon daylight. Here it is used to compute the radiance spectra of each color patch through multiplication with their reflectance spectra. (d) The similarity scores between color patches computed by SAM. Chroma and hue differences between each color patch, (e) and (f), respectively. Chroma and hue values are calculated according to (Endler 1990) and the similarity scores were found by

and therefore it is more meaningful to compare their combination with SAM, rather than individually. Euclidean distance between the chroma and hue of two colors is found as follows: