Reflective Light Modulation by Cephalopods and Fishes in Shallow Nearshore Habitats

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LONG-TERM GOAL

The central question is: what optical principles are used by opaque organisms to achieve crypsis in shallow, nearshore marine habitats?

OBJECTIVES

Camouflage mechanisms are not well known despite the general misconception that they are. Moreover, quantification of camouflage (especially of opaque organisms) is particularly wanting. We have four objectives: (1) Acquire imagery of camouflaged animals and their backgrounds in coral reef and temperate rock reef environments; (2) collect corresponding *in situ* irradiance and reflectance data from the animal and background; (3) Develop a suite of image analysis methods to quantify the type and degree of crypsis; (4) Construct a comparative digital photographic and video library of shallow-water marine animals in the camouflage categories of Uniform, Mottle and Disruptive. The central focus is on cephalopods (octopus, cuttlefish and squid) because they have the most diverse and changeable camouflage patterns known in biology. Several fishes with changeable coloration (groupers and flounders in particular) are studied comparatively.

APPROACH

High-resolution digital still images are acquired under natural marine conditions. No flash is used to avoid making artificial shadows from the flash's light. A computer-controlled spectrometer (adapted for underwater use) takes downwelling and sidewelling irradiance data at the exact time of photography; then the animal reflectance data are recorded with the spectrometer (in both gross and fine detail on the animal's body) so that color- and contrast-matching can be quantified in the digital images. HDTV video (Panasonic HVX200) is used to follow foraging cephalopods and fish to document (a) speed of body patterning changes and (b) the range of microhabitats that they encounter

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and the pattern they choose to camouflage themselves in each microhabitat. Laboratory experiments on cephalopods are conducted occasionally to support or test field findings. Image analysis programs are being developed in a MATLAB toolbox to enable quantitative analysis of crypsis.

R. Hanlon conducts the field work, participates in image analysis and publication, and directs the grant project. Dr. Charlie Chubb is Co-Investigator and leads the efforts on image analysis. Dr. Chubb has extensive experience in this field and brings a wealth of quantitative skills to this set of tasks. He is adept in writing code for MATLAB and in applying psychophysics methods to our laboratory experiments. He is assisted in these tasks by Dr. Liese Siemann (postdoc who works part-time on this grant) and, very recently, by Ms. Derya Akkaynak who is a fourth year PhD candidate in the MIT/WHOI joint program in Mechanical Engineering and Oceanography. Ms. Akkaynak is co-mentored by Dr. Ruth Rosenholtz of MIT, R. Hanlon of MBL, and C. Chubb of UC Irvine. Ms. Justine Allen, now starting her third year in the Brown University/MBL Joint PhD Program (Neurobiology), will continue to participate in the field work on this grant.

WORK COMPLETED

Field work continued apace during late 2010 and again in April 2011. RTH completed 3 field trips (total of 35 SCUBA dives), acquired ca. 1,200 high-resolution digital still images of cephalopods and fishes, and recorded 12 hours of High Definition TV (HDTV) field video of crypsis and pattern change. Briefly, the breakdown is as follows: (1) Puerto Rico, December 2010, to film *Octopus vulgaris* using camouflage in various habitats, including seagrass, soft corals, patch reefs, and fully developed coral reefs with soft and hard corals; (2) Monterey, California, Hopkins Marine Station, to film *Octopus rubescens*, a species with exceptionally complex skin and patterning, in well developed kelp forests; (3) western Turkey (near Izmir) studying the cuttlefish *Sepia officinalis* in various sea grass and sandy environments. New irradiance and reflectance data (with spectrometers) were collected in Turkey for a variety of applications (see below).

Image analysis during this year focused on developing methods *for predicting which cryptic body pattern will be effective in different visual habitats and quantifying the effectiveness of different body patterns*. We previously developed a MATLAB toolbox that incorporates many texture and feature detectors. To allow flexibility, the program can be easily altered to incorporate different animal templates or analyses as needed. The newest addition to this program focuses on comparing the pattern of a target animal to its adjacent substrate. It automatically extracts a circular region of the image surrounding an animal, with a radius that is proportional to the size of the animal and extracts a vector of statistics from the animal and the surrounding region. These statistics can be incorporated into a program designed to predict body patterns deployed by cuttlefish in different habitats.

We have begun devising a novel approach for assessing the effectiveness of body pattern. Although high-fidelity match between animal and background may seem to be the ultimate camouflage, it is rare for two reasons: there are too many backgrounds in nature; and a pattern need only resemble the background in those statistics to which the predator is spontaneously sensitive. We are deriving a measure that strives to reflect the degree to which a given camouflage pattern might be expected to elude detection by any visual system, including artificial systems augmented with many more dimensions of sensitivity than human vision.

We are also developing a method that allows accurate representation of color for underwater use because accurate color representation is critical for our image analysis efforts. There are two fundamental limitations to COTS digital cameras: (a) they are tuned exclusively to human vision with only 3 channels represented - Red, Green, and Blue (with green emphasized over red or blue) (b) manufacturers will not provide the detailed spectral sensitivity of their sensors. Every digital camera - even two cameras of the same make and model - will have a unique sensor array that records color information differently. Therefore for scientists publishing results derived from images on color-sensitive topics, it is of utmost importance to convert their "unique-to-camera" colors to a common, standardized color space so their work can be reproduced and compared with that taken by different cameras. Thus, we are extending the "terrestrial" work of Stevens *et al.* (2007) for use underwater.

RESULTS

<u>Crypsis Patterning.</u> We continued to make advances this year on nearly all of the major features and concepts of animal camouflage (see IMPACTS section below). We published four journal papers plus a book chapter and conference paper; most importantly perhaps a PNAS paper on HyperSpectral Imaging (HSI). Second, we continued to explore the link between *laboratory experiments* on visual control of camouflage patterning and *field work* of camouflaged cephalopods and have begun the ultimate challenge of predicting what camouflage pattern is appropriate on different natural backgrounds.

<u>Cryptic patterning in large and small fish.</u> Three efforts are in progress. First, our fieldwork to characterize camouflage in *large* fishes began with Nassau grouper, *Epinephelus striatus* (graduate student Anya Watson, University of Connecticut). With postdoc Liese Siemann we have refined the quantitative measurement of pattern types, and we now have a journal paper nearly completed for these groupers; we expect to submit it within a few months. Second, for the flatfish peacock flounder, *Bothus lunatus*, we conducted a field study to determine the pattern repertoire that this species exhibits in a coral reef habitat; the results are excellent and are nearly written up for submission to a journal. Third, to demonstrate capabilities of *very small* fish, we continue our study of the remarkable camouflage changing capability of the very small (1-2 inch) slender filefish *Monocanthus tuckeri*. Our images are yet to be fully analyzed, but we still plan to produce a publication in the upcoming year.

<u>*In situ* reflectance and irradiance data with Ocean Optics spectrometers.</u> The basic question is: when cuttlefish are camouflaged, do different parts of their bodies match the color and brightness of the adjacent background? Two major strides were made this past year. First, we finished analyzing our data from Australia and have a paper ready to submit to a journal. Second, we successfully acquired an extensive data set on the cuttlefish *Sepia officinalis* in Turkey in April 2011. These data will be analyzed in the coming year.

<u>Hyperspectral Imaging of cuttlefish camouflage</u>. After imaging cuttlefish in the summer of 2010 we spent this current year analyzing data. This paper was published in Proceedings of the National Academy of Sciences in February (Chiao *et al*., 2011) and was featured as a Research Highlight in Nature magazine as well. The basic question was: what does the camouflaged prey look like in the color space of the predator's visual system? This paper provides the first Proof of Principle that HyperSpectral Imaging can transform studies of visual perception. Camouflaged cuttlefish showed good color and pattern match when viewed by modeled dichromatic and trichromatic fish predators.

<u>Prediction of body pattern types from backgrounds</u> can be pursued several ways. One approach depends on the idea that cuttlefish body patterns can be quantified and differentiated based on the scale and size of the pattern elements. We have analyzed a series of laboratory and field images. A vector of

statistics was extracted from the cuttlefish and from a circular disk of substrate surrounding the animal (the annulus). These statistics included the animal and annulus granularity band energies and ratios between these band energies, Canny and Laplacian of Gaussian edges and Harris corners in the annulus, the presence of very light/white and very dark/black regions in the annulus, and contrast between pixel pairs in the annulus (GLCM contrast). When the contrast in the annulus remains low across a range of distances up to and beyond the width of the white square, cuttlefish reliably deploy a Uniform pattern. For higher contrast backgrounds, we have discovered that the ratio of band 2+3 to band 4+5 energies (23/45 ratio) in the annulus is highly correlated to the same ratio in the animal in a set of 90 laboratory and field images ($r = 0.84$) (Figure 1). This ratio, combined with other band energy ratios, can be used to categorize cuttlefish patterns as Mottle or Disruptive. Our preliminary prediction model, based on granularity band ratios and GLCM contrast, correctly predicts the body pattern a cuttlefish will choose on natural laboratory substrates over 80% of the time. In a smaller sample of field images, the same model correctly predicts the pattern a cuttlefish will choose close to 70% of the time. We have used linear regression, coupled with residual analysis, to identify images with cuttlefish that are more or less disruptive than predicted, and these images will be analyzed further to investigate features in the substrate that might cause the animal's pattern to deviate from what is predicted by the space-averaged granularity spectrum and overall contrast of the annulus.

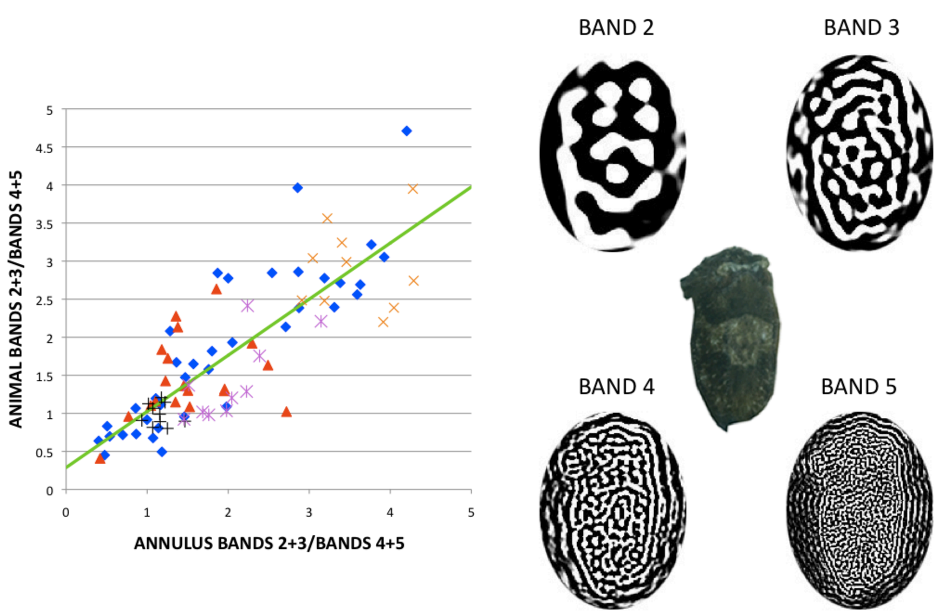

Figure 1. Linear regression analysis of the band 23/45 ratios of cuttlefish and their surrounding backgrounds. Blue diamonds are from laboratory images with different substrates; red squares are from field images; and orange crosses, purple stars, and black pluses are from sets of lab images with different animals on the same substrates. The images on the right show an example of a cuttlefish from a field image, along with the corresponding band filters used for granularity analysis, indicating the sizes of pattern components for each granularity band.

<u>The effectiveness of body pattern</u> has been assessed using a "goodness-of-general-resemblance metric" based on the signal detection theory. In brief, for any possible value v that might be assigned to a pixel

of a texture *J*, the histogram $h_J(v)$ gives the expectation of the proportion of pixels in a patch of *J* that will take the value *v*. If two textures *I* and *J* are identically distributed (i.e., the joint distributions characterizing their pixel ensembles are identical), then it is obvious that the following condition must hold: for any image transformation *T*, the output images *T*(*I*) and T(*J*) have identical histograms. What may not be so obvious is that the reverse implication holds as well. That is, if *T*(*I*) and *T*(*J*) have identical histograms for any image transformation *T*, then *I* and *J* are identically distributed. The metric we are deriving is founded on this observation. Let *Q* be the patch of texture filling a target situated against a background texture *R*. Suppose the histograms of *Q* and *R* differ, and consider the real valued function *f*. It can be shown that the function maximizes the signal-to-noise ratio. By applying the function *f* pixel-by-pixel to the image *J*, we derive what we might call an optimal histogram-based "spotlight" for making the target *Q* stand out against the background *R*, whereby "spotlight" we mean a real-valued output image whose mean value inside *Q* differs as strongly as is mathematically possible from the mean value across the background *R* relative to the standard deviation of differences between the two regions.

Equation $$f(v) = h_Q(v) - h_R(v) / h_Q(v) + h_R(v)$$

An example of the application of our goodness-of-general-resemblance metric is given below using the transformation functions *fTgrayscale* derived only using intensity histograms of the target cuttlefish (*Q*) and its background (*R*) pixels, *fTr/g/b* derived using histograms of red, green and blue channels separately, or *fTrgb* derived using histograms of all channels combined. It is apparent that when using transformation functions *fTgrayscale* and *fTr/g/b*, the target detection is relatively poor (Figures 2a, 2b), but a dramatic improvement can been seen when the transformation function *fTrgb* was applied (Figure 2c). This example highlights the importance of how much information colors carry in an image. We expect that this result will improve further when HSI images are used, since an RGB approach is a limitation of the cameras we commonly use, which is optimized for the human visual system.

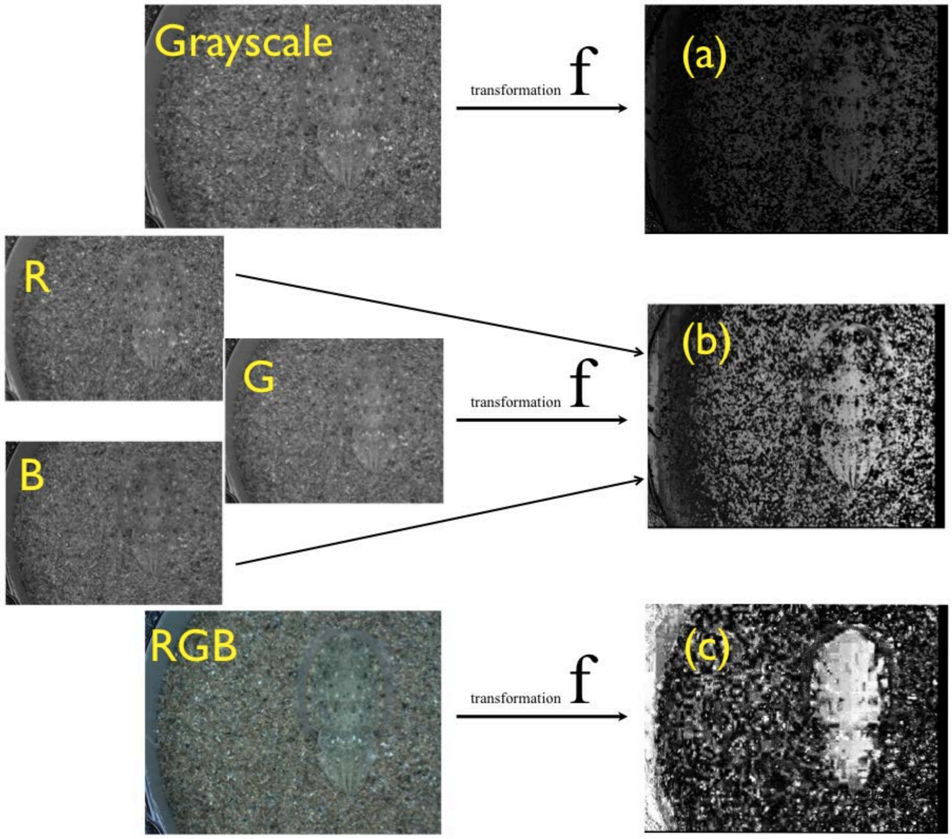

Figure 2. Examples of histogram-based transformation functions for revealing a target cuttlefish. (a) Transformation function applied to the grayscale image does not strongly reveal the target cuttlefish. (b) Transformation function applied to the Red, Green, or Blue channels also does not strongly reveal the target. (c) Transformation function applied to the three color channels combined clearly reveals the target cuttlefish as a bright region.

Habitat specific color chart. Professional photographers commonly use a calibrated color chart (e.g. Gretag Macbeth chart) to derive a transformation from the camera-raw RGB space (a camera color space) to the XYZ color space (a device-independent space for human vision). A Macbeth chart contains 24 color patches selected to represent realistic colors that are likely to be found in many daily scenes (i.e. dark skin, light skin, foliage, etc.) and each patch retains a constant reflectance value when viewed under varying terrestrial illumination conditions. An equivalent chart for underwater photography does not exist. Furthermore, the fast and uneven attenuation of long-wavelength light underwater will not yield reliable values for color patches viewed underwater. Thus, for underwater applications, the solution is *in situ* reflectance spectrometry and **derivation of habitat-specific color charts**. We developed the methodology and a prototype chart (Figure 3) in western Turkey in May 2011 (Akkaynak *et al.* 2011). We took various waterproof color charts underwater, to different depths at different times of day, and their reflectance values were recorded with our Ocean Optics spectrometer (SubSpec) as well as our Canon SLR to build an "underwater color appearance" database. This information was used to convert camera raw RGB values to the standardized XYZ space, representing the actual color of that underwater habitat. Such a habitat-specific color chart optimizes the camera raw RGB to XYZ transformation for that dive site. A huge advantage of this method is that our previously taken photos (thousands) can be retroactively color-corrected once the required "color appearance data" are collected for that specific dive site. When combined with new HSI imagery, we will have a spectrally rich as well as spatially rich image library. We expect that other research groups may adopt this methodology since COTS digital cameras are so widespread.

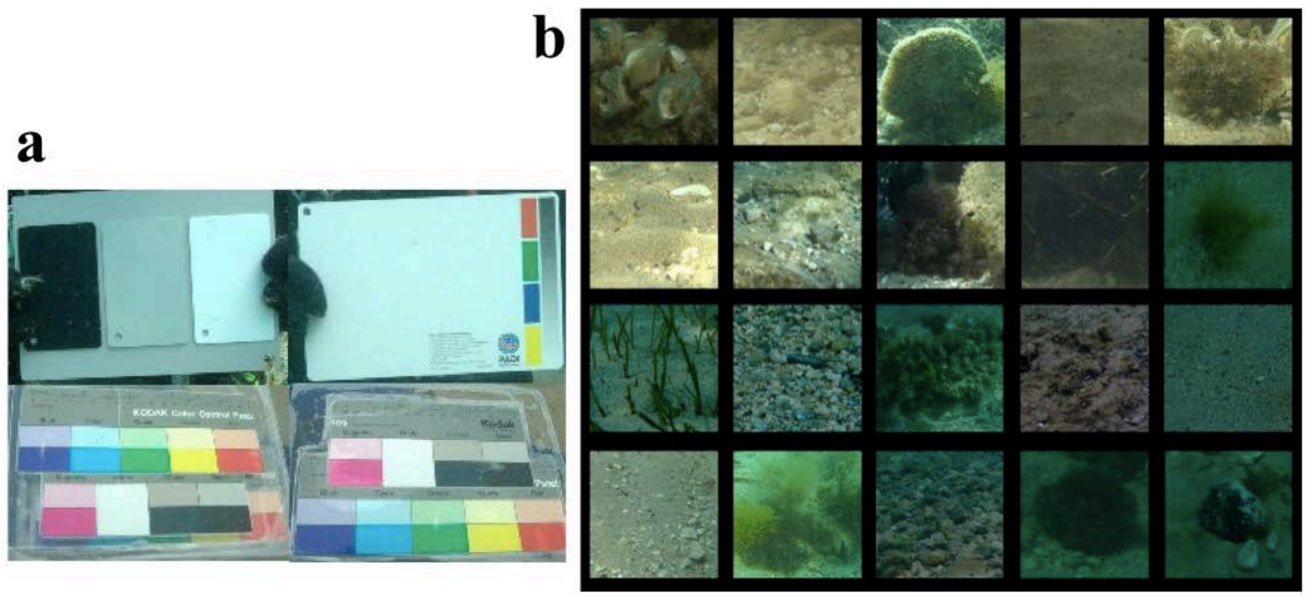

Figure 3. A habitat-specific color chart. (a) Array of waterproof color charts photographed at different depths at different times of day. (b) Prototype habitat-specific color chart developed for a dive site in Turkey.

IMPACT/APPLICATIONS

Concept development of camouflage visual mechanisms continued to mature this year. Following the book we published a year ago entitled Perspectives on Camouflage: Art and Science (Hanlon, 2010), we published a book chapter summarizing some key concepts learned from rapid adaptive camouflage in cephalopods (Hanlon, et al., 2011). These developments have spurred interest by several DoD agencies and discussions are continuing.

Our 2011 published paper on Hyperspectral Imaging (HSI) was well received by the scientific communities that focus on visual perception, coloration and patterning. Fortunately we have just been funded to build a small fast HSI unit adapted for camouflage and signaling studies underwater. This development, combined with our description above for a new method to derive habitat-specific color charts, will substantially expand our capabilities in quantitatively assessing camouflage under natural conditions, and will provide a large advantage over point-source spectrometry readings that have been used by us and others in recent years. This is a case in which a technology breakthrough in a different field opens up new and more sophisticated avenues of inquiry.

Our quantitative methods for analyzing animal body patterns have advanced well this year and we have taken steps towards the ultimate goal of predicting what body pattern will provide the best crypsis in any given habitat. In addition, we have produced a histogram-based transformation function for revealing a target cuttlefish. The potential applications of such developments will be obvious to most readers.

TRANSITIONS

A few of our findings on mechanisms of camouflage are being considered by ARL, SOCOM and DARPA.

RELATED PROJECTS

The PI has a 4-yr AFOSR grant that focuses on skin papillae and white leucophores in cephalopod camouflage. We also have a 1-yr contract from ARL to perform lab experiments on visual background stimuli that evoke camouflage in cuttlefish. Furthermore, we are one of the teams funded on a 4-yr ONR Basic Research Challenge (Rice University is lead organization); this is a materials-related grant, but we will address the biological question of color-blind camouflage in cephalopods by studying distributed light sensors in the skin (Mäthger *et al*., 2010). Importantly, we were funded by NSF in summer 2011 to build an underwater HyperSpectral Imager.

C. Chubb is PI on a grant from NSF (Perception, Action & Cognition) to analyze the dimensions of human visual sensitivity to various classes of textures. This research promises to have important implications for understanding how camouflage patterns operate to elude detection by human observers.

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