Biological Response to the Dynamic Spectral-Polarized Underwater Light Field

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LONG-TERM GOALS

Camouflage in marine environments requires matching all of the background optical properties: spectral, intensity and polarization components— all of which can change dynamically in space and time. Current research suggests that polarization detection is more sensitive than other conventional detection methods in scattering media such as the ocean, hence underscoring the need to develop polarized camouflage technology. Our research investigates the biological challenge of camouflage in the near-shore littoral zone and near-surface marine environments in two distinct water types found in coastal environments around the globe (oligotrophic and eutrophic) with particular emphasis on the polarization properties. We aim to characterize the dynamic light field along with the behavioral and cellular response of camouflaging animals in these environments. Our long-term goal is to identify the biological pathways for concealment against the underwater spectral-polarized light field enabling us to identify design principles for future naval camouflage.

OBJECTIVES

(1) Measure and model the spectral-polarized light field in near-shore and near-surface environments
(2) Characterize the biological camouflage response of organisms to these dynamic optical fields
(3) Identify the internal controls and structural mechanisms that coordinate the camouflage response
**APPROACH**

Our first aim is to **measure and model the underwater spectral-polarized light field in distinct water types**. We characterize the light field by the simultaneous deployment of a comprehensive optical suite including underwater video-polarimetry (full Stokes vector video-imaging camera custom-built Cummings; and “SALSA” (Bossa Nova Technologies, CA) Gilerson), inherent optical properties (MASCOT, Sullivan), hyper-spectral multi-angular Stokes vector spectroradiometry (Gilerson,Ahmed), and hyperspectral benthic reflectance (Dierssen). These measurements will be used to refine development of, and make comparisons to, theoretical expectations from a fully 3-D radiative transfer model that solves for each of the polarization elements of the Mueller matrix transformation of the Stokes vector by (Kattawar). The first modeling objective of this proposal is to calculate the complete Mueller matrix/Stokes vector for any set of oceanic and atmospheric conditions for any region of the ocean. We will then use this modeling approach (i) to predict the 3-D light field; (ii) to calculate experimental conditions to measure biological responses; and (iii) to investigate the nature of the light field as it interacts with cells within the skin. Our approach is to develop a 3-D Monte Carlo model with full Mueller matrix treatment.

During field operations, we couple polarimetry measurements of live, free-swimming animals in their environments with a full suite of optical measurements (mentioned above) to completely characterize the **biological response to dynamic optical environments** (Cummings, Gilerson, Dierssen, Sullivan, Seibel, Ahmed). We also restrain live, awake animals to take polarimetry measurements (in the field and laboratory) under a complete set of viewing angles and incident polarization light fields. We use Mueller matrix modeling to determine the specific Mueller matrix elements of animals that differ from those of conventional structures currently deployed for open ocean camouflage (e.g. standard mirror). Furthermore, we examine in vivo “flickering” activity on the skin of several species of squid and its relationship to background matching (wave-induced fluctuations of the light field) (Gilly).

We identify the internal control of these field-studied organisms and structural mechanisms that coordinate the camouflage response by (a) characterizing different tissue layers and organelles associated with different species occupying a diverse array of marine habitats using a combination of light and SEM microscopy (Cummings, Gilly), (b) examining both iridophore and chromatophore control processes and the local vs peripheral control features using both pharmacological and electrical stimulation techniques (Gilly, Cummings), and  (c) developing a novel 3D Monte Carlo model to describe how the spectral-polarized light field interacts with cells within the skin (Kattawar).

**WORK COMPLETED**

a) Presented at Annual MURI review in Arlington, VA 2014 (Dierssen, Gilerson,Cummings, Kattawar).

b) Published paper on bio-mimicry model for Polaro-cryptic mirror based on lab measurements and theoretical ocean contrasts for the lookdown, Selene vomer (Cummings; Brady et al. 2013; PNAS)

c) Completed analysis and submitted Group paper of 2011 (Florida Keys) and 2012 (Curacao) Field Campaigns for field test of polaro-cryptic model with new open ocean fish species including optical measurements: spectral stokes vector radiometery, polarization videoimagery, IOPs and VSF, partial size distributions, remote sensing reflectance, benthic reflectance spectrometry, and
sensor packages providing measurements of fish, camera and sun orientation (Cummings, Gilerson, Sullivan, Dierssen, Twardowski, Kattawar)

d) Published Group paper combining data and simulation using a vector radiative (RT) transfer code from two 2011 field campaigns that measured polarization characteristics of shallow water environments with diverse substrates. (Gilerson, Ahmed, Sullivan, Twardowski, Dierssen, Cummings; Gilerson et al. 2013, Applied Optics)

e) Revised group paper examining the biological structural mechanisms of polaro-crypsis and broadband reflectance in the Lookdown, Selene vomer. (Cummings, Kattawar)

f) Revised and submitted paper examining differences in polarization reflectance between species of different optical habitats, and peripheral vs central control of polarization reflectance. (Cummings)

g) Published paper on fish communication using polarized signalling including social modulation of polarized signals (Cummings; Calabrese et al. 2014 PNAS)

h) Continued field measurements (East Sound, WA and Lake Erie, MI) of the optical properties of different environments in an effort to better understand and model the effects of different particle fields on the resulting VSF and polarized light field synergistic to the goals of the MURI project and in conjunction with the Naval Research Laboratory, Stennis, MS (Sullivan, Twardowski, Kattawar).

i) Published paper examining the LIDAR extinction-to-backscatter ratio in the ocean (Sullivan, Twardowski).

j) Analyzed the White structural colorations from iridophores in the striped pyjama squid

k) Analyzed the angular distribution of diffuse reflectance from incoherent multiple scattering in turbid media

l) Extended a new Mueller matrix holographic method for small particle characterization

m) Studied the polarized extinction properties of plates with large aspect ratios

n) Studied the effects of incoherence on particle characterization using digital holography microscopy

o) Studied the scattering of partially coherent electromagnetic beams by water droplets and ice crystals

p) Did ab initio calculations on a chromatophore and produced the correct chromaticity coordinates in both reflection and transmission.

q) Simulated the reflectance and transmittance results for an iridophore and also showed how misleading results can be obtained by using sliced samples

r) Simulated the helical structure of the chromosome of K. brevis and showed that when the pitch of the helix matched the the wavelength of light we were able to get the highly enhanced backscattering that was observed in the S14 element of the Mueller matrix

s) Published paper on sensor design and calibration of hyperspectral imaging spectrometer (Dierssen; Mouroulis et al. 2014 Appl. Optics)

t) Published book chapter on underwater hyperspectral imaging for biogeochemical properties (Dierssen; Johnsen et al. 2013, Subsea Optics and Imaging, Ed. Watson and Zielinski)
Published a overview of hyperspectral remote sensing for mapping marine benthic habitats (Dierssen 2013, SPIE Imaging Spectrometry XVIII).

Completed entries on Bathymetry including optical and remote sensing methods for estimating bathymetry (Dierssen, in press, Encyclopaedia of Natural Resources, Taylor & Francis).

Published paper on hyperspectral remote sensing of seagrasses (Dierssen; Hill et al. 2013)


RESULTS

1) Microscope measurements of carangid fish (lookdown) reflectance layers reveals that the guanine platelet distribution along the vertical axis of the fish have a near uniform alignment but have a random distribution along the short axis of the fish, providing a selective reflectance and contributing to their polaro-crypsis. (Data not shown, currently in review; Cummings, Gilerson, Dierssen, Kattawar, Sullivan)

2) Swordtail fish exhibit social modulation of polarization signalling with males exhibiting higher polarization contrast (both within body and body to background) in social contexts than asocial contexts suggesting they may have facultative control of polarization signals (Cummings; Fig 1).

3) Model for polarimetric imaging of polarized targets in various water conditions was expanded by the analysis of the vector point spread function (VSPF) and reflective targets and further used for the comparison with the measurements by UT polarimeter during the Curacao campaign for the ocean background and the mirror demonstrating very good match for both intensity and degree of polarization (Gilerson, Cummings, Sullivan, Dierssen, Kattawar; Fig. 2).

4) Newly developed target with various orientantions of polarizers in the open environment and on the mirror was successfully deployed in the open ocean and moderate coastal waters on CCNY polarimetric system (polarimeter + camera) during NASA Ship-Airborne Bio-Optical Research (SABOR) cruise, summer 2014 showing availability of the polarized light and its dependence on the azimuth orientation of the target (Gilerson, Ahmed, Fig. 3)

5) Quantum catch color discrimination modeling on hyperspectral imagery of Sargassum crabs reveal a high degree of crypsis for fish predators, but not for seabirds which have visual sensitivities into the red portion of the visible spectrum. Similar approaches can be applied to determine efficacy of spectral camouflage in a variety of marine and terrestrial habitats (Dierssen)

6) Hyperspectral benthic reflectances measured in a variety of shallow water habitats reveal subtle spectral differences that alter the light environment for marine organisms and can be incorporated into remote sensing models to characterize coastal habitats (Dierssen)

7) Humboldt squid chromatophores are used in two basic modes – strong, synchronous “flashing” of most chromatophores over the entire body at a frequency of 2-4Hz and weak, asynchronous “flickering” with poor spatial synchronization and variable temporal components in the 2-6 Hz range. Frequency and phase of flashing (with respect to another flashing squid) can be altered, providing a large behavioral repertoire. Flashing is presumably controlled by motor output from the brain and...
appears to function in intra-specific communication. Flickering may operate in the absence of excitatory input from the brain, but it can be quickly and globally halted, presumably due to inhibitory neural control. Spectral components of flickering resemble those of fluctuations of downwelled light in the water column, suggesting that flickering plays a role in dynamic crypsis in the open-ocean environment inhabited by Dosidicus gigas (Gilly; Fig. 4, 5).

8) Patch voltage-clamp experiments to be carried out on squid chromatophore muscle fibers revealed voltage-gated Ca and K currents but no Na currents, indicating that electrical excitability in these muscle fibers is due to graded Ca-action potentials and not all-or-nothing Na action potentials. These new observations are consistent with the lack of effect of tetrodotoxin, a specific blocker of Na channels, on spontaneous chromatohore activity in intact skin preparations in Dosidicus gigas or denervated Loligo opalescens. In turn, these observations are consistent with our hypothesis of a peripheral network that permits communication between chromatophores in the absence of ordinary descending motor-control from the brain (Gilly).

9) Use of specific fluorescent probes for muscle and nerve cells in squid skin revealed that chromatophore muscle fibers branch extensively and that branches from neighboring chromatophores sometimes appear to fuse. Fusion is more common in Dosidicus gigas, a pelagic open-ocean species, than in Loligo opalescens, a neritic coastal species, but the frequency of fusion in Loligo appears to increase after chronic denervation. Dosidicus and denervated Loligo both show tetrodotoxin-resistant spontaneous chromatohore activity, but normal (innervated) Loligo skin does not. These observations suggest that direct communication between muscle fibers of adjacent chromatophores may be an important element of peripheral control of squid chromatophores (Gilly).

10) Microscopic measurements (TEM) and two-stream radiative transfer theory simulations of squid skin show that reflected light increases at oblique viewing angles, and polarization increases near Brewster angles (Kattawar; Fig 6).

11) Measurements and simulations of leucophore reflectance properties characterize their lambertian qualities (Kattawar, Fig 7-9).

12) Measurements of 3 surfaces (mirror, diffuse metal reflector and live carangid fish) in natural, near surface open ocean environments reveal that carangid fish reflectance provides greater overall polarization and intensity camouflage than the other two surfaces. (Full Dataset not shown, currently in review; Cummings, Gilerson, Dierssen, Kattawar, Sullivan; portion shown in Fig 10,11)

13) Crypsis in the open ocean requires different types of surface reflectance at different viewing angles. The ideal camouflage reflector may represent a composite of carangid-fish like reflectance properties; mirror-like and diffuse-mirror-like reflectance with each surface optimized for a specific viewing angle (Full Dataset not shown, currently in review; Cummings, Gilerson, Dierssen, Kattawar, Sullivan; portion shown in Fig 10,11).

IMPACT/APPLICATIONS

New technology (in-situ holographic microscope) that was leveraged/developed to explore innovative methods to better understand the factors (i.e. particle dynamics) that control the spatial-temporal dynamics of the underwater polarized light field (MURI goal) has resulted in finding ubiquitous particle orientation in the coastal oceans and has lead to a new ONR funded activity: “Effects of
natural, undisturbed particle fields on light transmission and dispersion in coastal waters” (Sullivan, Twardowski, Kattawar).

The new results we have obtained will help us understand the fundamental mechanisms of camouflage in cephalopods and teleosts. The results from the study comparing three different reflective materials (mirror, diffuse metal reflector and live carangid fish) give material scientist a template from which to potentially create a super material that would optimally camouflage under all viewing angles. It will also aid in understanding the complete polarization properties of the oceans using only the inherent optical properties.

Peripheral vs. central control mechanisms for squid chromatophores remains an important open question and is central to a deeper understanding of how his complex system operates for various functions, including communication and camouflage. Our work is designed to identify the structural and physiological mechanisms of the hypothesized peripheral control pathway. Central vs. peripheral control is a basic property of all complex systems and is thus of fundamental importance to many aspects of both the natural and man-made world.

RELATED PROJECTS

Our 3D Monte Carlo has been modified to compute the spectral extinction curves for Type 1a supernovae far better than any calculation done so far. We have also used it to calculate the spatial offset reflectance from compacted quartz which will be the new gold standard for reflecting materials.

Synergistic 3-D spectral modeling in seagrass canopies has been undertaken as part of a follow-on research funded by NASA Ocean Biology and Biogeochemistry in collaboration with John Hedley (Environmental Computer Science, Ltd). Modeling to be presented at Ocean Optics XXII (Dierssen).

Hyperspectral airborne PRISM imagery has been obtained over the Florida Bay shallow water stations in collaboration with NASA Jet Propulsion Laboratory and Ocean Biology and Biogeochemistry. The benthic reflectance spectra obtained as part of the MURI are being incorporated into modeling algorithms for retrieving bottom depth and composition.

Supported projects to continue with squid chromatophore work:

“EAGER: Natural Chromogenic Behaviors of Squid in Oceanic Waters” – Proposal awarded by NSF ($250,000, 2 years, Gilly and Rosen)

“Natural behaviors of free-swimming Dosidicus gigas studied with an animal-born-video package and data loggers” – Proposal awarded by National Geographic CRE ($5,000, 1 year, Rosen and Gilly)

“Natural behaviors of Humboldt squid in relation to oxygen-limited habitats” – Proposal awarded by National Geographic CRE ($25,000, 1 year, Gilly and Rosen)
REFERENCES


PUBLICATIONS


Gilerson, Alexander A; Stepinski, Jan; Ibrahim, Amir I; You, Yu; Sullivan, James M; Twardowski, Michael S; Dierssen, Heidi M; Russell, Brandon; Cummings, Molly E; Brady, Parrish; Ahmed, Samir A; Kattawar, George (2013). Benthic effects on the polarization of light in shallow waters. Applied Optics. 52 8685-8705


Meng Gao, Xin Huang, Ping Yang, and George W. Kattawar (2013). Angular Distribution of Diffuse Reflectance from Incoherent Multiple Scattering in Turbid Media. Applied Optics. 52 5869-5879, [published, refereed]

HONORS/AWARDS/PRIZES

George W. Kattawar of TAMU won the Jerlov Award of The Oceanography Society for 2014
Fig. 1. Social modulation of Polarization Contrast in swordtail fish (Xiphophorus nigrensis). Mean DoLP (Degree of Linear Polarization) contrast and standard error bars of male (n=12) and female (n=17) measured with a videopolarimeter while swimming alone or with conspecifics. Asterisks indicate significant differences (P<0.05). Figure from Calabrese et al. 2014.
Fig. 2. Measured by UT polarimeter and simulated DOLP, I for the light from the ocean water (top) and from the mirror (bottom), Curacao campaign. The mirror surface was perpendicular to the camera’s viewing axis.
Fig. 3. Polarized target (top left), image of the target by CCNY camera (S1 component) at 90 deg azimuth angle near Chesapeake Bay Tower (top right), measured S0 (bottom left) and DoLP (bottom right) by UT polarimeter and simulated DOLP for different polarizers as a function of the azimuth angle.
Fig 4. Flashing behavior. A. Sequential frames from video show out-of-phase flashing between the primary squid and a conspecific. The white box on the primary squid indicates the approximate area over which skin color was quantified. The projected straight-line distance between the base of the outer-most arms is ~ 15 cm. B. Time course of change in skin color averaged over the entire boxed area before and during an episode of flashing. C. Flashing is highly synchronized over the dorsal surface of the primary squid’s head. The light red traces represent skin color measured in 18 10x10 pixel boxes in an array of 6 rows by 3 columns. The heavy black trace is the mean. D. Flickering was quantified in an analogous way in the same squid at a different time in the video clip.
Fig. 5. Spectral analysis of fluctuations of downwelled sunlight versus chromogenic flickering viewed on the head of the primary squid. Ai-iii. Examples of spectra for fluctuations of reflected sunlight with the squid in the upper 1-2 m of the water column. Data were collected for 7.1 s (i), 16.5 s (ii) and 9.1 s (iii). Bi-iii. Analogous data for periods of flickering at depth where sunlight-related fluctuations are of very low amplitude. Data were collected for 5.8 s in each case. Average depths for squid were 43.5 m (i), 47.2 m (ii) and 50.1 m (iii).
Fig. 6. Measured and simulated optical properties of pyjama squid (S. lineolata) white stripe. (A) Binarized version of TEM panorama of pyjama squid iridophore cells used in FDTD simulation; iridosome (black) refractive index = 1.59; inter-plate (white) refractive index = 1.33. Outlined area indicates computational domain; large arrow indicates incident light direction, smaller arrows indicate reflected light. Sub-regions, boxes 1–10, were selected to model reflectance spectra from individual Bragg stacks. (B) Predicted reflectance spectra for non-polarized light at normal incidence from FDTD simulation and two-stream radiative transfer theory. (C) Predicted reflectance spectra for 10 sub-regions found in (A) using FDTD modeling. Low reflectance values are likely due to incoherent orientation of plates. (D) The skin reflects light diffusely for a range of viewing/incident angles (<15–35°), but at increasingly oblique viewing/incident angles, reflected light increases. Plot shows averages and s.e. (n =10). Inset shows set-up angles for measured data obtained using goniometer. Measurement direction was vertical, given by microscope position. Two-stream radiative transfer theory predicts that reflectance increases with incident angle (dashed line). (E) Polarization percentage. At angles up to <25°, polarization is low (<1–2%). At angles around Brewster's angle (50°), polarization increases to its maximum value but remains partial (<13–20%). Plot shows averages and s.e. (n =3). Two-stream radiative transfer theory corroborates increase in polarization near Brewster's angle.
Fig. 7. a) A microscopic view of a 3D iridophore volume, which consists of multiple-layered iridosomes. b) 2D FDTD simulation shows that the system approximates a lambertian surface with reflection about 25%. This value is comparable with the measured reflection (average 45%) when considering iridophore thickness difference. c) A microscopic view of a 3D leucophore volume, which consists of spherical leucosomes (radius 200–400nm). d) ADDA results show that the packed spheres can simulate the leucophore very well. Note the big phase function backward peak is due to the specular reflection of these cutting sphere surfaces at the volume boundaries.
Fig. 8. This plot shows the reflection and transmission using the inferred index of refraction through the single scattering Mie code.

Fig. 9. This chromaticity plot shows the data taken from Sutherland et al. and the simulation using the inferred index of refraction run through the Mie code.
Fig. 10. Full Stokes—FS (a,e), Intensity—I (b,f), Q (c,g) and U (d,h) contrasts data for the mirror (M), DMR, and Fish (bigeye scad) across measurements of one specimen on July 9th 2012 (N=147 of bigeye scad) collected (A-E). Data for chase angles only (e-h) (N=38 of bigeye scad) is defined as $|\Phi|\geq45^\circ$ which represents both predator chase angles and prey chasing angles. All data points shown, averages denoted by black bars statistical significant differences denoted by * (p<0.05) and ** (p<<0.01).
Fig. 11. The proportion that each surface reflector (a, bigeye scad (green), DMR (white), and M, mirror (blue)) which exhibited the lowest Stokes contrast relative to the other surfaces across all measurements (Top, N=147) and chase angles (Bottom, N=38) of one specimen on July 9th 2012. The best performing surface (F, M or DMR) for the full Stokes contrast is graphed based on relative fish yaw verses observer inclination (b) and on relative fish yaw verses observer azimuth angles (c).