Summary

Despite substantial interference from hurricanes Bonnie, Charley, and Katrina, ***Operations Deep-Scope 2004 and 2005*** were extremely successful. In addition to numerous discoveries (e.g. fluorescent sharks, new large deep-sea squid, UV vision in deep-sea crabs, the importance of polarized light and bioluminescent searchlights), these expeditions developed several new technologies. The Eye-in-the-Sea is now a robust stealth camera system; waveband, fluorescence, polarization, and UV imaging techniques are well-developed; and we can now collect deep-sea benthic species without damaging their eyes. Together these achievements place us in a unique position to explore the deep sea in innovative and exciting ways. In 2007 we propose to extend the envelope of this exciting frontier in ocean exploration in two ways: 1) developing further imaging and listening technologies, 2) using the currently developed methods to explore the cliffs in the Bahamas that range from the surface to 3000 feet in depth. Results from this proposal will characterize an important deep-sea benthic environment, and use new technologies to locate inorganic and organic ocean resources, fulfilling two of the main themes of Ocean Exploration. The proposed cliff sites range from shallow coral reefs to the abyssal plain and will allow us to explore a large number of benthic communities in a small location and learn how depth affects undersea life. Given the technological focus of our research however, if the ships' schedules should make it difficult to work in this area we would welcome the opportunity to test these new technologies at any biologically rich sites. Unlike many research cruises, which focus in depth on one problem and method, we propose a number of smaller projects that are linked by the methods and questions of visual ecology and optical oceanography. The ultimate goal of our highly interdisciplinary group of researchers is to explore and characterize the deep-sea world in these new ways.

PROJECT DESCRIPTION

Identification of problem

Most of what we know about life in the deep ocean is based on net sampling and brief excursions with bright, noisy submersibles. How many animals remain unknown to us because they evade our sampling and observation platforms? One of the mission objectives for OE is the "characterization of benthic and pelagic habitats and ecosystems". This has proven to be quite difficult because current technologies either frighten animals away, disturb normal behavior, harm and/or blind animals, or view the deep-sea world through an anthropomorphic lens that results in false or incomplete views of its ecology. The end result is that we know far less about deep-sea habitats and species than we do about any other environment on earth. In addition, because deep-sea exploration is costly and time-consuming, we have only examined a few habitats at discrete depths. While it is known that depth is a primary determinant of marine communities, little work has been carried out on examining the effect of depth in a single location.

Scientific Objectives and Methodology

Deep-Scope 2004 and ***2005*** brought together an international and multidisciplinary group of investigators. In the course of these two projects, a number of technologies were developed to the point where they could reliably explore the deep sea (as evidenced by the numerous discoveries in two truncated cruises). ***Operation Deep-Scope 2007*** seeks to use these technologies (in addition to newer ones) to study benthic habitats over a 3000 foot depth range.

1) The Eye-in-the-Sea: A Window into the Deep Sea (Widder)

During ***Deep-Scope 2004*** and ***2005***, the Eye-in-the-Sea camera system evolved into a robust, highly dependable tool for ocean exploration. This unique deep-sea observatory is an autonomous, battery-powered, video-capture and illumination system that uses far red light (680 nm light-emitting diodes with a high-pass filter for 700 nm and above) in combination with a highly sensitive camera that can record bioluminescence and compensate for the attenuation losses associated with using long wavelength illumination (Widder et al. 2005) (Figure 1). The system can be programmed to record in time-lapse and/or triggered mode. In triggered mode, a bioluminescent flash, detected by a PMT, activates the system. For Deep-Scope 2005 we succeeded in creating an illumination level that clearly recorded bioluminescence, while still providing enough red-light illumination to reveal the organisms. Deployments were made using baited traps and an electronic jellyfish lure capable of imitating four different luminescent displays, including highly conspicuous "burglar alarm" displays. All parameters are fully programmable by the user and EITS was operated in a variety of modes during each deployment.

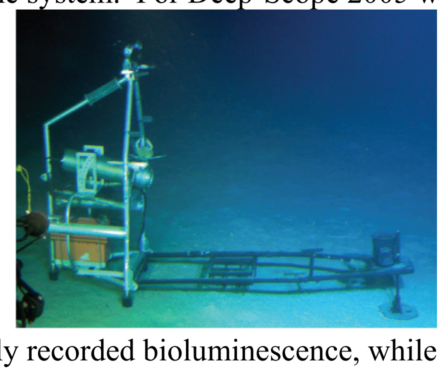

Figure 1: Eye-in-the-Sea *in situ* during Deep Scope 2005. The fold-down alignment mechanism in front of the camera holds the electronic jellyfish, bait box, and hydrophone.

In our 2004 OE proposal, we hypothesized that noise and/or light generated from the JSL submersible deter many deep-sea creatures. EITS video analysis from 2004 deployments in the Gulf of Mexico illustrated a decrease in the number of recorded species in the presence of a submersible. Additional experiments carried out with EITS in Monterey Bay confirmed that the EITS illuminators fitted with far-red filters did not elicit a behavioral response in deep-sea visual predators. We feel these experiments validate EITS's capabilities as an unobtrusive deep-sea viewing platform.

During Deep Scope 2004 in the Gulf of Mexico, when EITS was positioned near the edge of the Brine Pool, we recorded the first ever *in situ* footage of a large (> 2 m length), previously undescribed species of deep-sea squid. This squid is so new to science that it cannot be placed into any known family (M. Vecchione pers. comm.). The squid attacked just after the electronic jellyfish initiated a bioluminescent burglar alarm sequence. The fact that such a large, previously unknown predator was observed on the very first deployment of the EITS on this mission served as clear proof-of-concept for this novel method of exploration. During Deep Scope 2005 in the Gulf of Mexico, we once again recorded a large squid that attacked during a bioluminescent burglar alarm display and that appears to be the same unknown species as we saw in 2004. Clearly this innovative lure has fantastic potential for attracting large predators that are not scavengers.

Proposed research for 2007

EITS will be an integral tool in the biodiversity assessment of proposed study sites. The unobtrusive nature of EITS will enable us to potentially film new species and behaviors, which were previously scared off by the lights and sounds of underwater vehicles. Building on the value demonstrated in 2004 and 2005 of using novel lures to attract predators (Figure 2), we now plan to develop a mechanical lure that mimics the vibrations associated with a struggling fish. This concept was first suggested by deep-sea fish biologist, Tracey Sutton. In addition to unobtrusive deployments, we also propose to test out new forms of lures and stimuli. During these experimental deployments, only the mechanical bait or the E-jelly will be used to lure in animals. Species' behavioral responses to each stimulus will be noted and compared with current knowledge about their sensory systems.

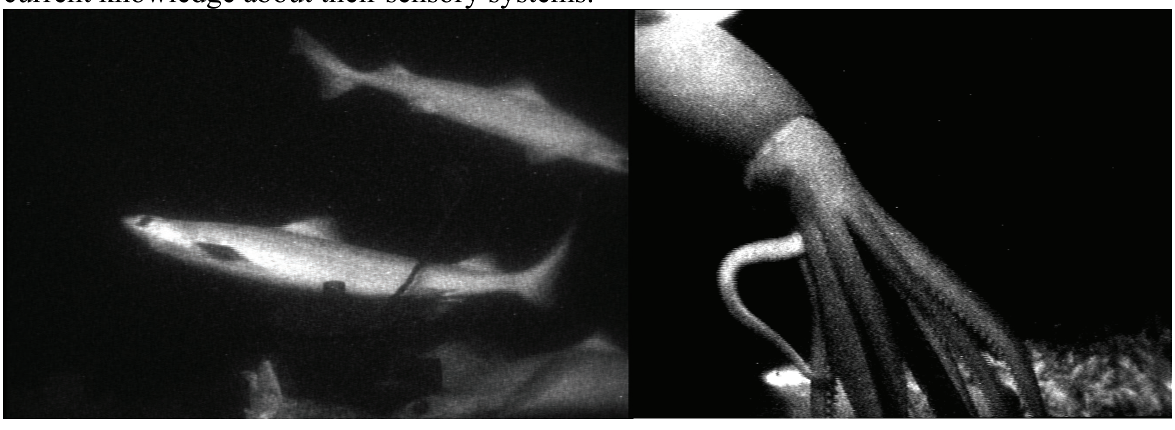

Figure 2: A. Eye-in-the-Sea recording of large (>6ft) unidentified squid, attacking electronic jellyfish lure during Deep Scope 2004. B. Never before seen schooling behavior of gulper sharks, *Centrophorus granulosus*, from Deep Scope 2005.

During two separate recoveries of EITS, the system was found tipped over or in a location away from its original deployment site. In both instances, the source of these relocations was not captured on video and therefore is unknown. In an effort to understand these periodic events, which are possibly caused by large predators, we propose to place a tilt sensor on EITS that will serve as a recording trigger when the camera is moved. Further proposed additions to the EITS include a wide-angle lens to increase the field of view and a higher density power source.

Our most exciting discoveries with the EITS have been made at locations that constitute biological oases in the deep ocean - places where high abundance and high diversity are likely to attract large predators. Ecologically-rich, tropical cliffs constitute exceptionally rich environments and the base of such cliffs, where carrion and detritus are likely to accumulate, are likely to be routinely patrolled by large predators. We therefore believe that locating the EITS at the base of such cliffs represents a very exciting venue for deep ocean exploration.

2) Ecological roles of fluorescence in marine organisms (Matz)

Fluorescence ecology in the open ocean

Animal fluorescence used to be overlooked by visual ecologists working in terrestrial or shallow-water environment since its contribution to the apparent color is usually negligible under full-spectrum illumination. In the monochromatic blue ocean environment, however, fluorescence becomes one of the best ways to generate a color signal, because it transforms the blue light into other colors. We initially hypothesized that this mechanism would work best at intermediate depths, where the downwelling light is already too close to monochromatic for absorption-based coloring to work but is still abundant enough to interfere with bioluminescence

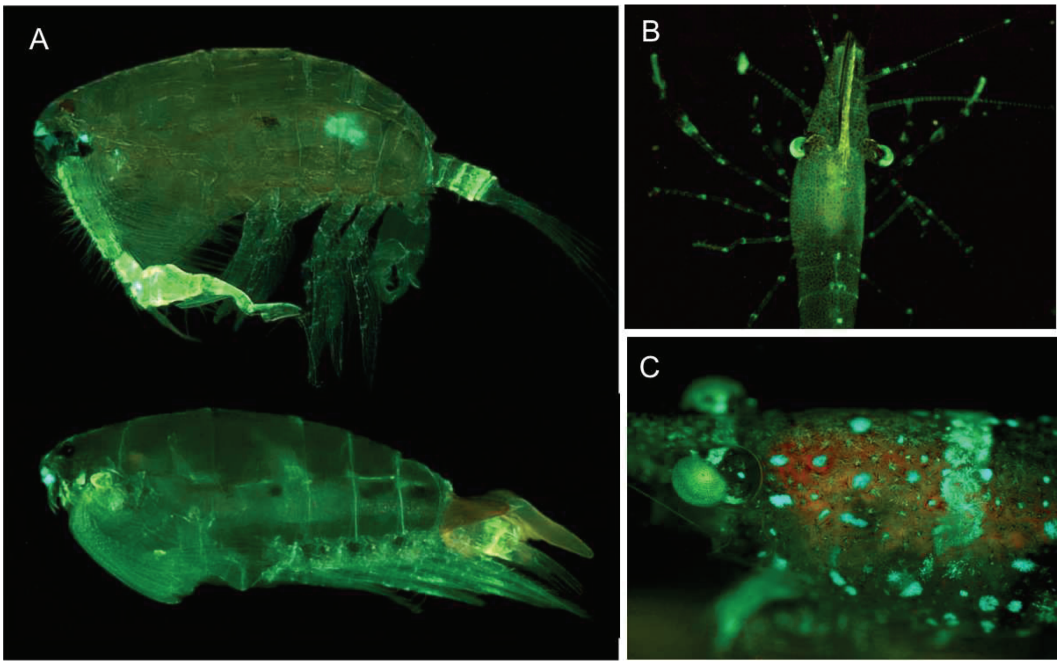

Figure 3: Some fluorescent crustaceans of the open ocean (from the DeepScope-2005 collection). **A**: Planktonic copepod *Pontella securifer*. Top – male, bottom – female. **B**: Sargassum shrimp. Note green fluorescent leg joints and yellow fluorescent rostrum. **C**: Close-up of another sargassum shrimp displaying three types of chromophore cells: fluorescent cyan, fluorescent green and non-fluorescent brown.

as an alternative mode of visual communication. As a result of the Deep Scope 2005 cruise, however, it became clear that organisms in the open ocean use fluorescence at the very surface, in plankton and sargassum communities (Fig. 3). Preliminary analysis of visual capabilities of two representatives of these surface communities - planktonic copepod *Pontella securifer* and sargassum shrimp (Figures 3 A and B) - indicated that their visual systems are tuned to their own fluorescent signals, because they are offset with respect to the blue background light. We currently assume that these animals use fluorescence to generate contrast against the blue water background – the mechanism that would work starting from the surface. To test this hypothesis, in addition to looking at more surface-living animals, we plan to analyze fluorescence and color in deeper organisms (at 50-200 m depth). We will collect plankton samples using the JSL's "Critter Gitter" attachment while stopping the submersible at different depths during ascent from the bottom. In addition, animals will be collected from various depths at night with a Tucker Trawl, fitted with a closing cod-end, for studies of their spectral sensitivity (see objective 4). The spectrum of downwelling light at these depths will be measured during plankton collections using the through-hull spectrometer probe mounted in the JSL and used for modeling the effect of fluorescence on visibility.

Benthic fluorescence

Our previous observations suggested that the bright green fluorescence of some benthic deep-sea predators, such as tube anemones (Figure 4), and fish (Figure 5) might be related to prey capture. This function is strongly suggested by the patterned fluorescence of these animals. Due to the extreme scarcity of light that may excite these fluorescent signals, such a function implies extraordinary visual adaptations and/or behavioral responses of the prey organisms. We plan to measure the abundance of organisms exhibiting hypothetically prey-attracting fluorescence over a wide depth range. We will also collect them and examine their gut contents using the technique of DNA barcoding, to determine the taxonomic affiliation of the prey and evaluate the degree of feeding specificity.

We will also continue surveys of deep-sea benthic organisms for unexpected cases of fluorescence, such as fluorescence of the chain cat shark – the only fluorescent shark documented thus far (Figure 6). We hope that repeated observations of animals such as this shark would provide an indication of the biological relevance of their fluorescent coloration.

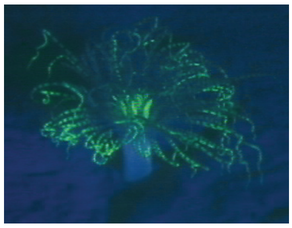

Figure 4: Fluorescence of the tube anemone (DeepScope-2004, *in situ* image).

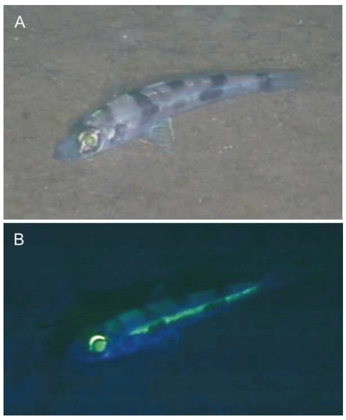

Figure 5: Shortnose greeneye fish, *Chlorophtalmus agassizi* **A**: White light. **B**: Fluorescence. (DeepScope-2004, *in situ* image).

Figure 6: Fluorescent chain catshark (DeepScope-2005, *in situ* image).

Bioprospecting for potential biotechnology markers

All the collected fluorescent organisms will be used for cloning the genes that determine fluorescence with the goal to develop novel *in vivo* imaging technologies for biomedical science. We will use approaches that we successfully applied previously for cloning proteins homologous to the green fluorescent protein (GFP) from cnidarians and arthropods (Shagin et al. 2004), including homology-based cloning using degenerate primers and screening of bacterial expression libraries. For potentially more exotic fluorescent cases (fish or echinoderms), we will apply proteomics to derive initial protein sequence fragment followed by amplification and expression of the corresponding full-length cDNA using established methods (Matz 2002; Matz et al. 2003). Towards this goal Matz has recently received a major 5-year R01 grant from the National Institutes of Health. This grant does not include funds for the actual ship time and submersible operations; however, it guarantees that the collections of fluorescent specimens made during such cruises would be immediately used to generate high-quality scientific results.

Non-biological fluorescent features

One of the brightest yellow fluorescent signals that we have observed at depth came from gas hydrates breaking through the sediment. Gas hydrates are of great interest as potential energy source, as well as due to their presumed role in global climate change and determination of sediment stability against landslides. Without fluorescent optics, gas hydrates are not nearly as conspicuous (Figure 7). Since the only data on gas hydrate fluorescence comes from our own observation during ***Deep Scope 2004***, it remains unclear what is the extent of variation of this fluorescence, whether it may be used to derive some information about the particular hydrate and even whether the fluorescence is always seen in the hydrates. In 2007, we hope to obtain more fluorescence images of gas hydrates, since the cruise is planned in the general area of the Blake-Bahamas Plateau that harbors multiple previously explored gas hydrate sites. Comparison of these images with the hydrates seen in the Gulf of Mexico in 2004 may become the first step in developing the new methodology of mapping and classifying gas hydrates *in situ*.

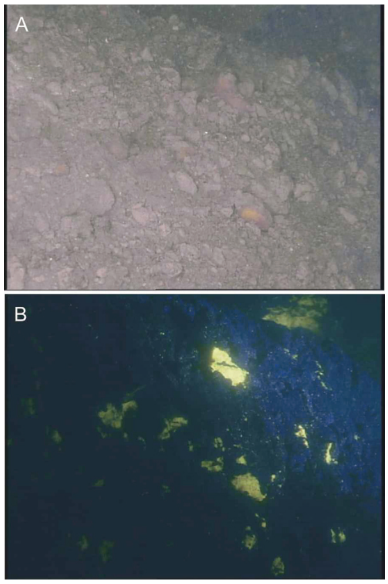

Figure 7: Gas hydrates observed at the Green Canyon site (DeepScope-2004, *in situ* images). **A**: White light. **B**: Fluorescence.

3) The deep-sea light environment as it is perceived by its inhabitants (Johnsen, Marshall, Widder)

Operation Deep Scope 2004 and ***2005*** gave us a much better view of how the deep-sea world is perceived by its inhabitants and what this means for camouflage and visual communication. ***Deep Scope 2004*** showed that the colors of deep-sea animals are far better adapted for camouflage against bioluminescent searchlights than against the ambient light, giving us the first solid evidence of the ecological importance of these ocular photophores. ***Deep***

Scope 2004 also showed that the birefringence of the tissues of pelagic animals may make them vulnerable to detection by animals that have the potential for polarization vision, such as crustaceans and cephalopods. Finally it showed that the color patterns on deep-sea galatheid crabs were an excellent form of disruptive coloration for their coral habitats. ***Deep Scope 2005***, though cut in half by hurricane Katrina, continued this work. The preliminary analyses from this

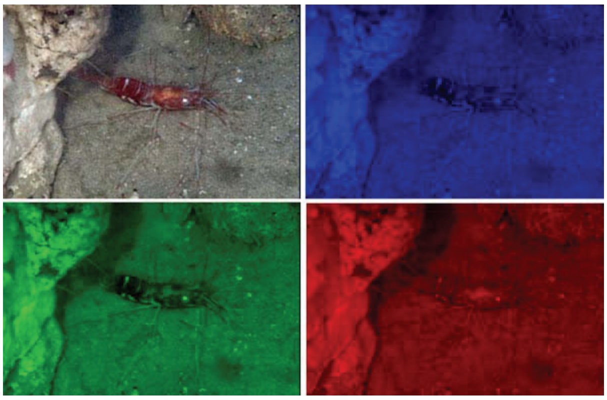

Figure 8: Yellow zooanthids on a glass sponge. Note that, though highly conspicuous under full light, they blend in with the sponge at blue wavelengths.

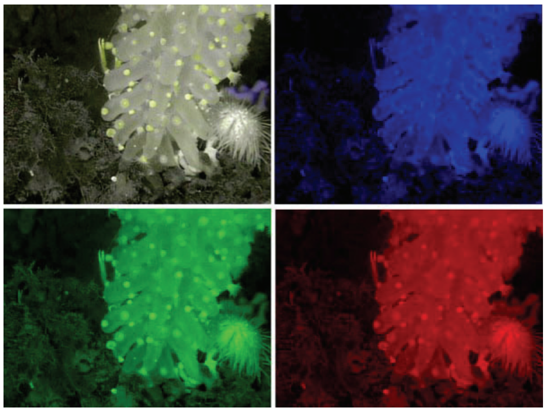

Figure 9: Deep-sea shrimp. Unlike what was seen at shallower depths, this animal is not most camouflaged at blue wavelengths (which is what occurs naturally). Instead it actually blends with the background best at red wavelengths.

cruise suggest that the camouflage of deep-sea benthic species is not as good at greater depths (e.g. Figures 8 and 9). This is exciting because it suggests that the selective pressure for camouflage decreases with decreasing light levels. It also raises the question of what the colors of animals at great depths are for. We propose to continue this work, focusing on two issues that arose during the previous cruises: 1) the effect of visual acuity on the appearance of objects at depth, and 2) the potential for novel colors of animals and bioluminescence at great depths.

Visual acuity and perception of the deep sea world

Humans are unusual in a number of ways, but one that is not often considered is our high visual acuity. Exceeded only by that of birds, our ability to discern small objects and fine patterns is vastly better than that of most species. This is particularly true of deep sea species, which must trade visual acuity for increased sensitivity by pooling the light from large numbers of photoreceptors to increase photon counts to detectable levels. Dark-adapted human vision uses the same process, which is one of the reasons why it is impossible to read at night. In deep-sea species, this process, known as temporal and spatial summation, leads to a visual acuity that would be considered legal blindness in a human. Therefore, our sharp photographs and movies portray the deep-sea environment in a way that none of its inhabitants ever experience. This gives us a false idea of what is easily seen and what is well-hidden, in addition to what features of the environment can be used for navigation and homing.

We propose to photograph and film various habitats at a range of depths from the *JSL* submersible. These images will have scaled objects placed within them, so that they can be calibrated for size and angular extent. The images will them be digitized and converted to their correct appearance using methods previously developed by Johnsen (Johnsen *et al.*, 2004; Blevins and Johnsen, 2004). The methods use the spatial and temporal resolutions of appropriate species (see objective 4 below) to create point spread functions. These point-spread functions, which are the images of single points, are convolved with the digitized images using Fourier methods (Figure 10) (see Johnsen et al., 2004). The resulting images show the appearance of the scene to an animal with a given spatial and temporal resolution.

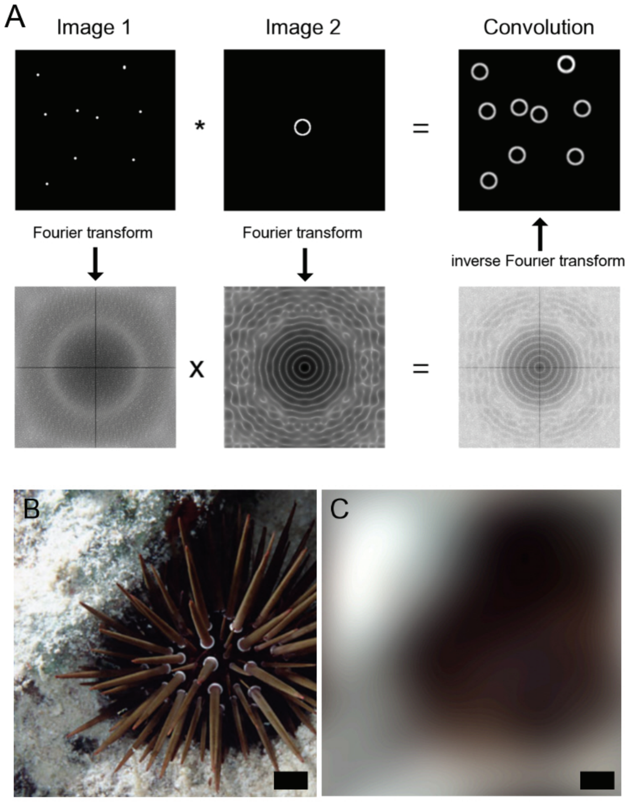

Figure 10: a) The convolution of image 1 and image 2 (denoted by the "*" operator) can be calculated by multiplying the Fourier transforms of the two images and then inverse Fourier transforming the product. This process, which replaces each point in image one by the circle in image 2, can be used to show how images look under reduced visual acuity. **b)** Image of *Echinometra lucunter* in burrow. **c)** Same image convolved to show how it would appear to another urchin placed a few centimeters away. Scale bars are 20°.

These techniques will be combined with the color techniques developed in the previous cruises to create the most species appropriate images of the deep-sea world to date. Since the proposed study sites involve a great range of depths, which correlates with spatial resolution (due to decreasing light levels), we will see how the perception of these environments depends on both the environments and the visual abilities of their inhabitants.

The spatial resolutions of some of these species are already known. In species where this information is not available it will be calculated using morphological and histological techniques. In crustaceans, it will be determined from ommatidial diameters and angular spacing. In vertebrates it will be determined from retinal ganglion cell spacing (reviewed by Land and Nilsson, 2002). Animals will be collected using methods developed in the two previous cruises. Temporal resolution will be determined as described in objective 4

The potential for novel colors of animals and bioluminescence at great depths.

Most deep-sea exploration has occurred at depths where at least some solar light is still detectable. This light is overwhelmingly blue, due to the filtering effects of the water. For this reason, animals at these depths have eyes that are most sensitive to this color. In addition, most bioluminescence is also of this color, since it needs to be detected by animals which primarily have blue sensitivity. In other words, the downwelling light has locked both vision and bioluminescence into a limited color range.

However, at greater depths, below approximately 900 m in the clearest waters, and at shallower depths in murkier waters, the solar light is too dim to be detected by any animal. Animals at these depths, provided they do not migrate into shallower waters, are freed from this selective pressure. Therefore, the color range of vision and bioluminescence may be much larger. Recent research on animals at sunless depths has suggested that this is indeed the case (Haddock et al. 2005), with animals producing red lights using novel combinations of fluorescence and bioluminescence. We propose to examine the bioluminescence and colors of animals at these great depths to see if this pattern holds. In addition to opening up a fascinating new world of color, this work has the potential to find unusual fluorescent and bioluminescent compounds. The fact that sensitivity to near-UV light in a deep-sea crab was recently discovered (Deep-Scope 2005) lends support to the premise that unusual bioluminescent compounds may be present at these depths.

Animals will be collected during the deepest dives using the *JSL* submersible. The spectrum of their bioluminescence will be measured using a newly developed and highly sensitive multichannel spectrometer designed by Ocean Optics Inc. Their colors will be measured using methods developed by Johnsen during ***Deep Scope 2004*** (Johnsen 2005). A UV-visible xenon light source will be used to illuminate the surface of the animal. Its reflectance (calibrated using a diffuse white standard), will be measured using a UV-visible spectrometer (USB2000). Animals will be collected over a wide range of depths in the same location to look for depth-related patterns.

4) Examination of photosensitivity, polarization sensitivity, and spectral sensitivity of pelagic and deep-sea benthic organisms (Frank)

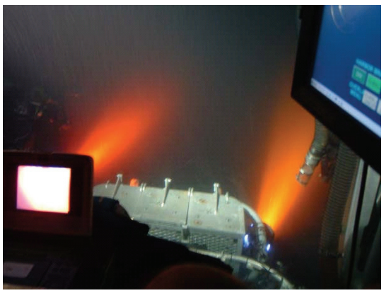

Figure 11: Orange and red filtered HMI lights on JSL

Figure 12: Benthic trap

Figure 13: Chaceon crabs protecting traps

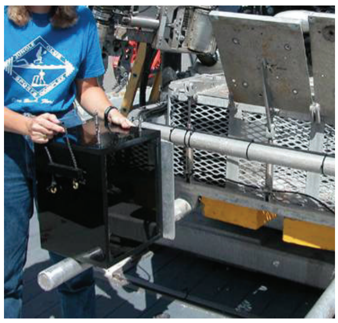

Figure 14: One of the BioBoxes being loaded onto the submersible

Previous Observations

During Operation Deep-Scope 2005, new techniques were used which proved to be very successful at allowing us to obtain deep-sea benthic crustaceans with intact photoreceptors. The JSL's DeepSea Power and Light HMI lights were fitted with orange and red cut-off filters (minimum cut-off wavelength 580 nm – Figure 11). Previous work has shown that deep-sea species are very insensitive to wavelengths above 570 nm (Frank and Case, 1988, Frank and Widder, 1999), so collections could be made without blinding the animals. Two collection techniques proved to be very successful. A new, open trap design was used (Fig. 12), which allowed the scent of the bait inside to spread much further. The traps were recovered under orange/red light and deposited into a black BioBox (Fig 14), which was thermally insulated and light tight when closed at depth. Using the suction sampler to pick up crabs and deposit them into the BioBox also proved to be a very effective way to collect live animals with intact eyes. Even though over half the cruise was lost due to Hurricane Katrina, we managed to collect 7 crustaceans – 1 *Gastroptychus sp* (Figure 15), 3 *Eumunida picta*, 1 *Munidopsis tridentate*, 1 *Bathynectes longispina*, and 1 large, as yet unidentified caridean shrimp. A surprising discovery from electrophysiological recordings revealed that *Gastroptychus* has an ultraviolet photoreceptor, in addition to the usual blue receptor. What such a deep-living crab, found at 550 m at the South Florida lithoherm site, is doing with an ultraviolet receptor remains a mystery, but preliminary data from the caridean shrimp suggests that it too possesses UV sensitivity, while the other three crab species, found at the same depths, do not.

Figure 15 *Gastroptychus* sp, a benthic species found at 550 m, possesses UV sensitivity

Figure 16 *Eumunida picta,* a benthic species found at 550 m, does not have UV sensitivity

Data from flicker fusion frequency studies indicate that these are extraordinarily slow eyes, with critical flicker fusions ranging from 6 to 12 Hz, suggesting that these photoreceptors are designed for extremely long integration times in order to enhance their sensitivity. Eyes from all crabs collected have also been fixed for histology, to determine their spatial resolution as well as whether any novel optical adaptations are present to further enhance sensitivity.

Further exploration

How prevalent is UV sensitivity in the deep-sea benthos? Having now demonstrated that it is possible to retrieve benthic species with intact photoreceptors, we are excited by the possibility of what further explorations may bring. The discovery of novel UV photosensitivity in the small sample size available raises the possibility that there may be other benthic species with this unusual adaptation, and brings forth the question of what characteristics are shared by the UV sensitive species. In several species of deep-sea pelagic carideans that possess UV and blue visual pigments, the answer appears to lie with their bioluminescence (Frank and Case 1988; Cronin and Frank, 1996). The benthic species discovered thus far with UV photoreceptors are not bioluminescent, so the answer may lie in bioluminescent prey that emit UV light, currently thought to be a very rare phenomenon. The possibility that many deep-sea benthic animals can see ultraviolet light opens up a whole new chapter on behavioral interactions in the deep-sea benthos

Are low spatial and temporal resolution characteristic of photoreceptors of deep-sea benthic species? Further studies on temporal resolution will be conducted utilizing flicker fusion frequency and response latency. Photoreceptor tissue will also be fixed for light and transmission electron microscopy, and spatial resolution will be calculated from inter-ommatidial distances.

Are fluorescent planktonic crustaceans adapted for enhanced sensitivity to fluorescent wavelengths? After submersible dives have been completed for the day, a small (2 m x 3 m mouth opening) Tucker Trawl will be launched over the side of the ship. A light-tight cod-end on the end of the Tucker Trawl can be closed at depth via a net timer, allowing us to collect planktonic organisms from a variety of depths without exposing their eyes to light. Spectral sensitivity measurements will be made from fluorescent species (as determined in objective 2), and compared to the spectral sensitivities of non-fluorescent species.

Polarization sensitivity: while most crustaceans possess tiered rhabdoms that gives them the potential to detect polarized light, physiological evidence of this ability has never been determined for any deep-sea planktonic or benthic species. Utilizing a 90^{o} twisted nematic liquid crystal display (Doug Bryant, Liquid Crystal Institute, Kent State University), we will be able to test the responses of the eye to two polarization planes, differing by 90^{o}. These liquid crystal molecules are sandwiched between 2 pieces of glass, with a polarizing filter on one surface. When no charge is applied to the crystals, the light coming in through the polarized surface is rotated 90^{o}. When a charge is applied to the crystals, all the molecules will line up parallel to the surface of the glass, and the light is no longer rotated, effectively giving light whose primary E-vector orientation is 90^{o} to that in the uncharged state. Utilizing this system on the end of the light guide transmitting light to the eyes of the animal ensures that the position and angle of the light guide does not move when switching polarization (as it might when reaching into the Faraday cage to attempt to manually manipulate a polarizing filter), an absolutely critical requirement for an experiment of this type.

Are deep-sea benthic species being blinded by science? The extraordinary sensitivity of the benthic eyes studied to date (based on flicker fusion – intensity vs. response data and latency response data are still be analyzed) suggests the real possibility that normal submersible illumination is blinding. Our earlier study demonstrated that crabs collected under white light and brought to the surface in unprotected containers are blind. However, these sub light exposures were of quite long duration, were directly focused on the animals, and include exposure to blinding surface light levels as well. It is important to assess the effect of more normal submersible illumination, such as sweeping passes with submersible lights as transects are conducted, or indirect illumination as other organisms are collected, without the confounding factor of surface illumination. Now that we can reliably collect benthic species with intact photoreceptors, studies will be conducted using calibrated dosages of white light, identical to those used on the submersible, to determine the light tolerance of these delicate photoreceptors. This will be assessed both electrophysiologically and histologically, as many studies have demonstrated structural damage resulting from excessive light exposure (Gaten et al, 1998; Herring et al, 1999). Some crabs will be exposed to white submersible lights in situ, collected under orange and red light, and then tested electrophysiologically. Other dark-collected crabs will be set-up in the experimental chamber, and a dark-adapted intensity vs. response curve will be generated to get a baseline for each animal. Exposure to a set duration and irradiance of white light will occur with a light hanging from the top of the Faraday cage. Further intensity vs. response curves will be generated 12, 24 and 36 hours after exposure, to see what light dosage the animals can recover from.

5) Enhancing vision in pelagic environments with UV and Polarization vision. (Marshall, Johnsen)

Both ***Deep Scope 2004*** and ***Deep Scope 2005*** taught us much about strategies for camouflage and camouflage breaking in the ocean. Much of this work became focused upon ultraviolet (UV) vision and polarization vision, both adaptations found in animals inhabiting pelagic environments and both with the potential to help break transparency camouflage. Unlike us, many near-surface animals can see ultraviolet and polarized light (Losey et al. 1999, Leech and Johnsen 2003). Animals that are invisible to us can be quite visible to animals with different visual abilities. During ***Deep Scope 2004*** and ***2005*****,** polarization and UV video and photographs taken on several open ocean SCUBA dives and during submersible dives showed that UV and polarization vision have the potential to greatly increases the ability to see otherwise invisible animals and objects (Figure17).

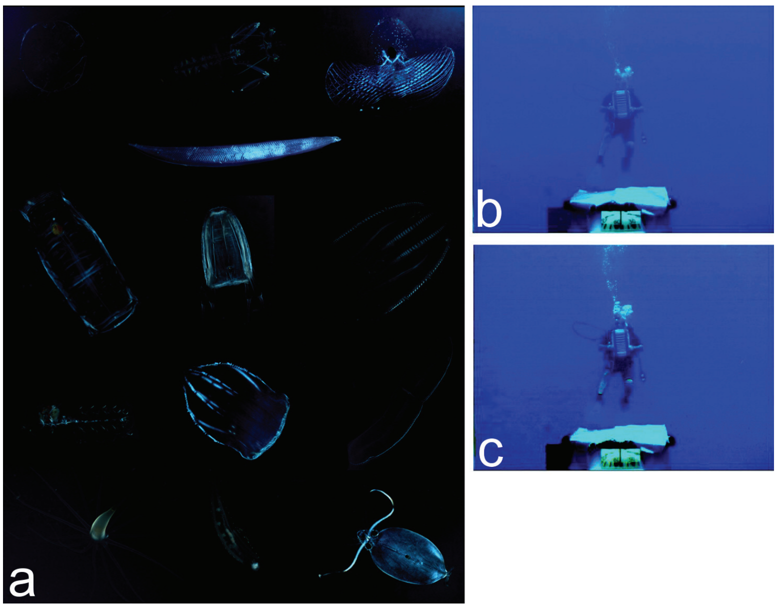

Figure 17: a) Images of common species viewed through crossed polarizers showing birefringent tissues that may make these animals more visible to polarization-visual species. **b)** Underwater image from ***Deep Scope 2004***. **c)** Same image dehazed using polarization information. Note the increased visibility of the tank hose and other features.

Our aim in ***Deep Scope 2007*** is to complete this story and finally be able to quantify just how effective this camouflage mechanism is. Also, using new discoveries from ***Deep Scope 2005*** and extensions of this work in 2007, we will be able to measure the efficiency of the visual systems of animals breaking this camouflage. Spectral sensitivities (including UV) of pelagic animals were recorded in 2005 and this work will continue in 2007 with the addition of polarization sensitivities. While there is much anecdotal and some photographic evidence that both UV vision and polarization vision can help increase the contrast of animals trying to use transparency as a camouflage strategy, nobody has managed to gather all the bits of information required to quantify this. Storm interruption and the subsequent change in ocean optical properties prevented us completing the environmental measurements started in 2004 and collecting enough animals for lab work was also impossible. Deep Scope 2007 promises to finally answer exactly how much contrast is gained by extending vision into the UV and polarized light domains and tell us more about under what conditions this is possible. What depths can such strategies extend to and over what distances (horizontally) can these mechanisms work are also questions to be tackled.

We therefore propose to examine the sensory world of UV and polarization-visual species by filming transparent animals underwater and in the lab using specialized UV and polarization cameras and by continuing to measure the underwater UV and polarization levels using spectroradiometers and adapted cameras. Lab photography and measurements will be set up in order to mimic open ocean environments. This data will be combined with spectral and polarization sensitivity measurements to complete the picture. Any study that tries to see the world through the eyes of animals needs 4 bits of information: what are the theoretical capabilities of the visual system, what is the nature of light in the environment where the animals are found, what are the colours (or lack of them) of the objects of interest - this includes the animals and their background - and finally how are these aspects combined to allow effective behaviors. The more precise measurements to be made in 2007 will enable us to calculate, rather than guess, how much more visible animals become to other animals rather than to us.

Proposed Study Sites

In order to be able to investigate the impact of depth and light gradients on camouflage and visual adaptations, we propose to work along the steep, biologically diverse walls found in the Bahamas (Figure 18). The Bahamas are famous for these escarpments that draw SCUBA divers from all over the world because of their diverse and colorful fauna, including sponges, seafans, seawhips, bryozoans and black coral. Many of these walls extend far below the range of SCUBA and have consequently been little explored at these greater depths. Excellent potential sites include Chub Cay, Gouldings Cay (south of Nassau), San Salvador Island, and the SW tip of Eleuthera Island, NE of Exuma Sound. In addition to these island walls, there is an atoll along Tartar Bank off the South tip of Cat Island that rises to 90 ft depth at the top and is surrounded by deep water, dropping steeply on all sides to ~1500 ft. Although we will not be able to explore all of these sites in the time available, we hope that the wide range of lees that they afford may mitigate the number of dive days lost to bad weather, which was a frustration during Deep Scope 2004 and 2005.

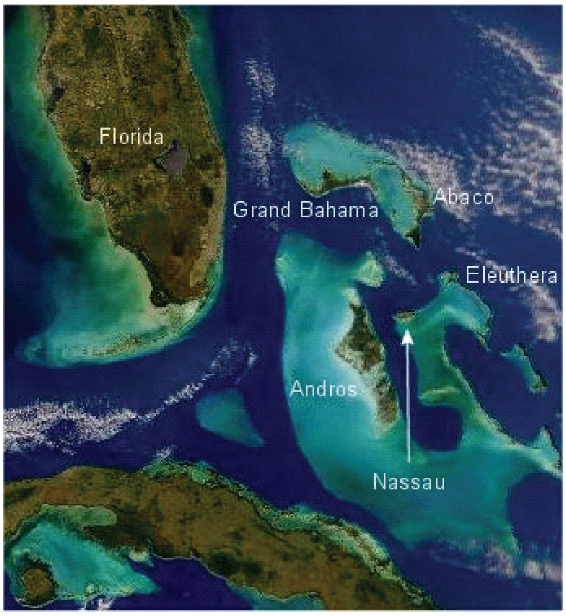

Figure 18: Satellite view of the Bahamas showing deep water in close proximity to islands.

Relevance to the goals of NOAA's Ocean Exploration Program

The proposed research directly addresses four of the six main themes of the Ocean Exploration Program (from pages 2 and 3 of the funding announcement)

3) and 5) Discover new communities with novel relationships and using* in situ *tehcniques: In order to characterize oceanic ecosystems, it is critical that observations be conducted as unobtrusively as possible in order to avoid scaring animals away as well as to obtain data about genuine animal interactions. Animals with well-developed sensory and locomotory systems can easily detect and evade our standard sampling tools. Therefore, the tools that we are currently using to explore our oceans are biasing population assessments as well as providing examples of disrupted, startle behavior, rather than data on environmentally relevant behavioral interactions, data that are critically needed to properly characterize ecosystems. The Eye-in-the-Sea observatory provided views of a new species of large predator as well as behaviors never observed before in deep-sea species, demonstrating its ability to provide fundamental and novel data on normal undisturbed biological activity in the benthic habitat. Eye-in-the-Sea also provides an excellent test bed for new technology and defined stimuli, such as novel lures.

In addition to observing *in situ* behavior, the varied areas of expertise brought together on this mission will enable the science team to provide a comprehensive characterization of how the benthic and pelagic worlds are viewed from the standpoint of the animals, rather than the distorted view we get from cameras and white floodlights. Not only is the level of detail and range of coloration seen under artificial illumination not perceived by animals in deep-sea ecosystems, but our view of the near-surface zone is also distorted, because of our lack of UV and polarization sensitivity. As demonstrated on ***Deep-Scope 2004***, transparency is in the eye of the beholder, and further studies will be undertaken on this mission to quantify how effective UV and polarization sensitivity are as a means of breaking transparent camouflage. The research proposed here will allow us to show the oceanic world as it is actually seen from the animals' viewpoint. In addition, new collection techniques will allow for the study of the visual physiology of deep-sea benthic species, data that are needed in order to model the benthic environment as it actually appears to its inhabitants as well.

Fluorescence was shown to be remarkably prevalent in the marine world on ***Deep-Scope 2004*** and ***2005*,** and the studies in the current proposal will build on these results, and perhaps lend support to the remarkable idea that fluorescence is a major mechanism of visual communication between organisms at intermediate depths.

2) and 6) New ocean resources: New methods of imaging (polarization, UV, fluorescence) have the potential to lead to new and better methods of locating organic and inorganic materials. This was demonstrated on ***Deep-Scope 2004*** and ***2005***, in which fluorescence imaging demonstrated that weak orange fluorescence in the sediments is due to the presence of oil, and that methane hydrates produce a very bright yellow fluorescent signal, which can be seen long before the hydrate becomes visible under white light illumination. In addition, polarization imaging showed great promise for allowing us to locate otherwise invisible objects and to see farther into the water than we have before. Further observations utilizing the imaging techniques described in this proposal may lead to new ways of mapping and locating other inorganic materials.

Benefits of proposed research to general public

The public appeal of this research has already been evidenced by the news coverage and public response to the ***Deep-Scope 2004 and 2005*** **OE** website. The discoveries made on both cruises went out as AP stories that were picked up by the national news (e.g. *CNN, ABC, National Geographic*) as well as by a large number of smaller media sources (*Scuba Diver*, *Tampa Tribune* etc.). Discovery Channel Canada broadcast an interview with T. Frank for their "Daily Planet" series about the discovery of UV photoreceptor, fluorescent shark and Eye-in-the-Sea footage. In addition, the Discovery Science Channel produced a program on the Eye-in-the-Sea development saga as part of their "Mid-Water Mysteries" series, which first aired on June 9, 2003 and has been rerun many times since. As a follow-on, independent producer/cinematographer James Lipscomb, who has won 6 EMMYs for sea oriented television specials, is very enthusiastic about producing a special about the continued development and deployments of EITS as well as its possible installation on the MARS observatory.

This is a very exciting time in ocean science that promises to revolutionize the field, but the high cost of ocean exploration demands that we muster as much public support as possible. The titillating possibility of discovering a new species, or finding new methods of communication between marine organisms, is a wonderful hook on which to hang the bigger picture of the important scientific and societal issues related to Ocean Exploration. The inevitable discoveries that will result from the explorations proposed here will undoubtedly excite great public interest and hopefully heighten public awareness of the amazing discoveries still to be made.

In addition to commercial media coverage, The Media Lab at Harbor Branch Oceanographic Institution has been producing a website specifically designed for near real-time coverage of oceanographic missions for the past 6 years. This website can be found at www.at-sea.org. The HBOI Media Lab will provide the same type of coverage as the oceanexplorer.noaa.gov Webmaster, including Pre-Mission coverage, illustrating the exciting nature and importance of the upcoming mission, as they did for our two previous missions. Because this content will be posted prior to the mission, it will include a variety of high-bandwidth media, discussing discoveries from previous missions, and explanations of work to be conducted on the upcoming mission. Coverage during the mission will be provided by Media Lab personnel on board ship, who will transmit daily logs and digital pictures of discoveries made during the mission. An "Ask an Explorer" forum will be incorporated into the mission coverage, so that readers can submit questions to the participating scientists, and receive answers during the mission. Images and essays on discoveries made during the mission will always be accessible to the general public after the mission as well. Funding to cover the costs of one Media Lab personnel on the expedition, as well as costs for satellite transmission of mission highlights to the @sea and Ocean Explorer websites are included in the budget. Finally, a very popular post-expedition professional development workshop was held in Ft. Pierce, Florida, after Deep-Scope 2005, during which educators participated in lesson plan activities directly related to the mission and talked with the expedition's scientists. We hope to have a similar professional development workshop at the HBOI campus based on the next Deep-Scope cruise.

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