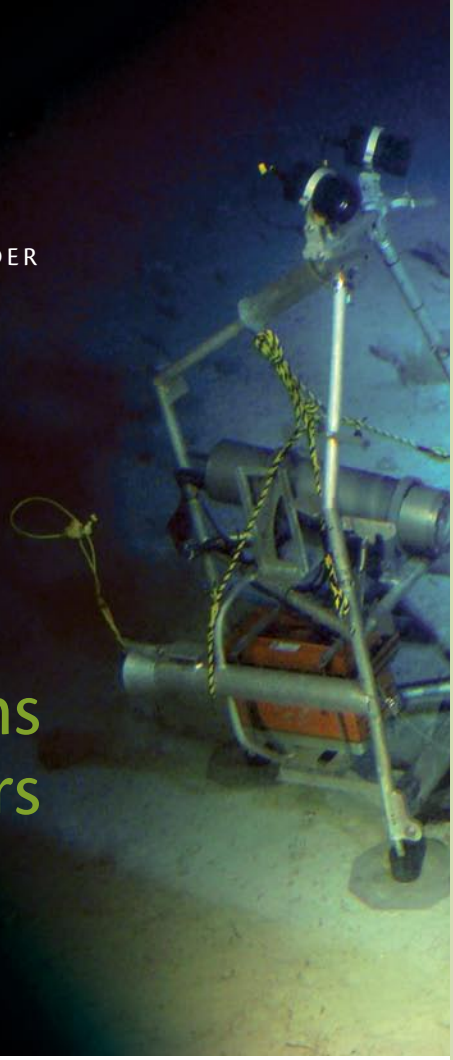
BY EDITH A. WIDDER

Sly Eye for the Shy Guy

Peering into the Depths with New Sensors



IT IS AN OFT-STATED statistic that 95% of the ocean is unexplored, but this number does not expose the full extent of our ignorance about the largest ecosystem on the planet—first because it refers to the ocean floor, not the almost unimaginably vast volume of water above it, and second because limited tools have been used to probe what little of the ocean has been explored. For centuries, nets were the primary means of exploration, but these move slowly compared to the swimming speeds of large, agile predators, and they destroy delicate fauna such as gelatinous zooplankton. Exploration with submersibles and remotely operated vehicles (ROVs) has opened new vistas, revealing the remarkable abundance and incredible adaptations of previously unknown fragile fauna, as well as equally diverse and fragile benthic communities associated with hydrothermal vents, hydrocarbon seeps (Fisher et al., this issue), and deep-water corals (Baco et al., this issue; Ross et al., this issue). However, such platforms use loud thrusters and bright white lights that are disruptive to organisms adapted to life in the dim, peaceful depths.

We must question how much our tools for exploration are biasing what we see. Recognizing that there may be animals we scare away, behaviors we don't see, and adaptations we don't understand because we lack a "fish-eye" perspective, the Deepscope missions funded by NOAA's Office of Ocean Exploration in 2004, 2005, and 2007 were designed to explore with new technological eyes and to focus those eyes on how animals perceive their environments.

Vision in the ocean is more challenging than it is on land because of the relative opacity of seawater. The scattering and absorptive properties of water greatly shorten the distances that light travels and biases the visible colors. In clear ocean water, blue light travels the furthest and is, therefore, the wavelength most favored for visual signaling. Blue is the dominant color of both environmental light and bioluminescent emissions; as a consequence, the ocean depths are often described as being essentially monochromatic. If this were true, it would eliminate an entire dimension of visual communication: color. It would also eliminate the opportunity to explore unobtrusively outside the visual waveband of the inhabitants. In actual fact,

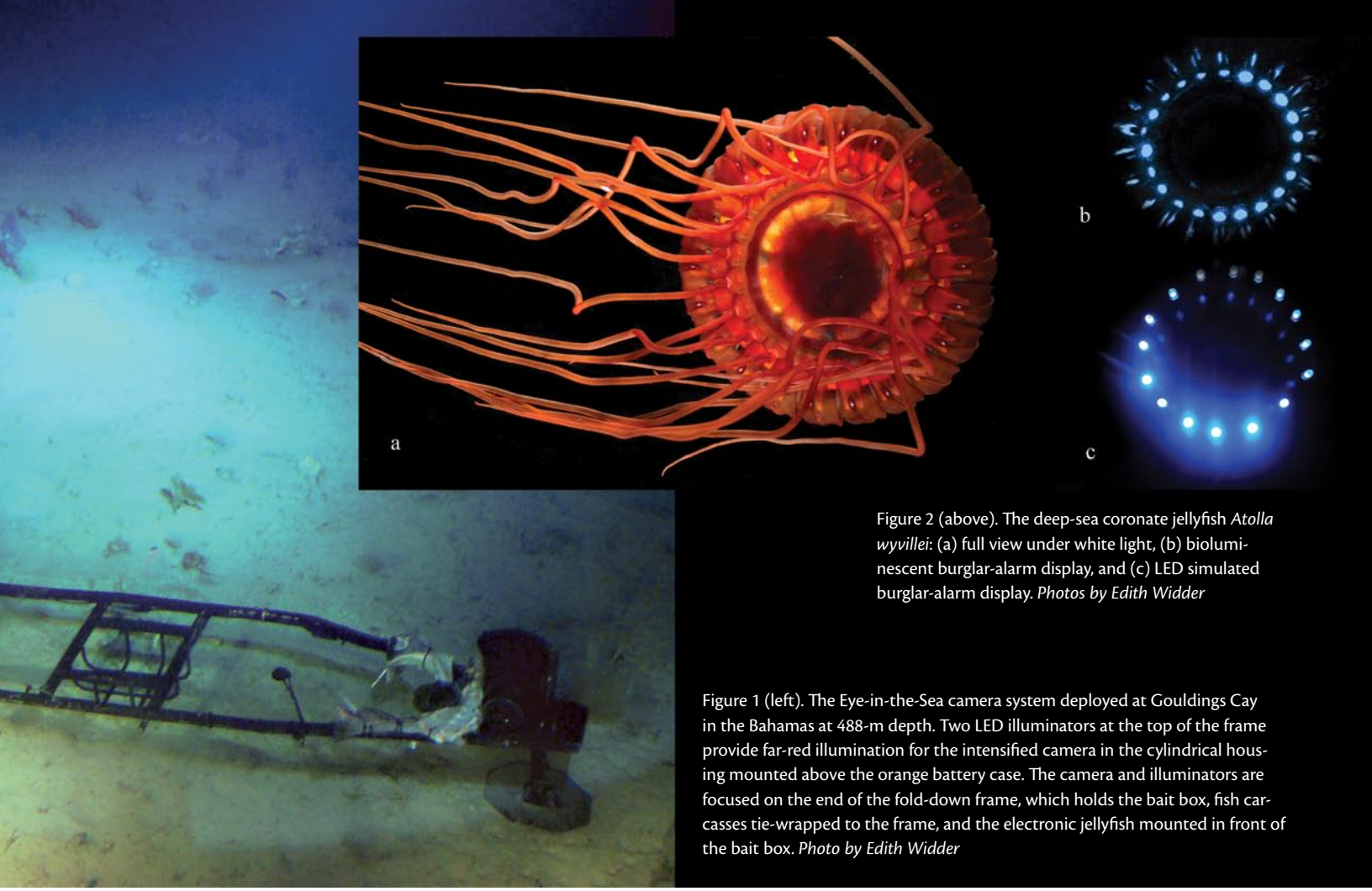


Figure 2 (above). The deep-sea coronate jellyfish *Atolla wyvillei*: (a) full view under white light, (b) bioluminescent burglar-alarm display, and (c) LED simulated burglar-alarm display. *Photos by Edith Widder*

Figure 1 (left). The Eye-in-the-Sea camera system deployed at Gouldings Cay in the Bahamas at 488-m depth. Two LED illuminators at the top of the frame provide far-red illumination for the intensified camera in the cylindrical housing mounted above the orange battery case. The camera and illuminators are focused on the end of the fold-down frame, which holds the bait box, fish carcasses tie-wrapped to the frame, and the electronic jellyfish mounted in front of the bait box. *Photo by Edith Widder*

other colors do travel through seawater, albeit less efficiently than blue, and therefore the opportunity exists for both increased complexity of visual communication and unobtrusive exploration.

STEALTH CAMERA

Deep-sea animals have evolved extremely sensitive eyes, tuned to detect the faintest glimmer of bioluminescence or the subtlest hint of downwelling sunlight. To explore and observe unobtrusively requires using illumination outside the waveband of their sensitivity. On land, this investigation is accomplished with infrared light, but underwater these wavelengths travel such short distances that they are totally inadequate for anything beyond centimeters, rather than the meters required for observing large fauna and exploring significant volumes. The Eye-in-the-Sea (EITS) camera system (Figure 1) was developed to address this challenge by replacing infrared with far-red illumination that is still invisible to most deep-sea inhabitants. It also uses an intensified video camera that helps to compensate for the reduced illumination and permits recordings of bioluminescence.

Preliminary experiments conducted during a series of deployments in Monterey Canyon concentrated on finding a compromise between unobtrusive, but adequate, illumination and settled on illuminators that combine 680-nm LEDs with short-wavelength cut-off filters, which eliminate visible wavelengths below 625 nm (Widder et al., 2005; Raymond and Widder, 2007). A new kind of optical lure was also produced during this development phase and dubbed the electronic jellyfish or e-jelly. Given the brevity of missions that are generally available for exploration, we sought to maximize our opportunities for viewing fauna by luring animals into the camera's field of view. Traditionally, this enticement is accomplished with bait, but carrion attracts primarily scavengers. Because we also sought to attract active predators, we devised a visual lure that imitated burglar-alarm displays, including one exhibiting characteristics of the deep-sea coronate jellyfish *Atolla wyvillei* (Figure 2) (Herring and Widder, 2004). According to the burglar-alarm hypothesis, bright showy displays such as the pinwheel frenzy of an *Atolla* have evolved to attract secondary predators

that may attack the primary predator, thereby affording the jellyfish prey an opportunity for escape. These displays are sometimes described as being akin to the fear screams of some birds and monkeys, which may serve the same purpose of attracting secondary predators. In the ocean, many animals "scream" with light.

To further maximize our chances of seeing something new, we chose oases on the bottom of the ocean—regions of high abundance and diversity in the midst of barren plains. We reasoned that such locations are likely to be frequented by large predators in search of prey. In the Gulf of Mexico in 2004 and 2005, these oases included patches of deep-water coral and cold seeps where high concentrations of methane and hydrogen sulfide nourish communities of symbiont-containing fauna, such as tubeworms and mussels. In the Bahamas in 2007, the Eye-in-the-Sea was placed near cliff-face communities, which concentrate biomass in narrow vertical corridors. These locations require site-specific deployments with an ROV or submersible so that the camera can be placed in proximity to specific targets. This scheme is in contrast to lander systems that are simply dropped over the side of a ship. For recovery, landers float to the surface following activation of an acoustic release, making launch and recovery of such systems simpler and less weather dependent than the EITS, but ill-suited for exploration of complex topography.

The three principal characteristics of the Eye-in-the-Sea observatory that set it apart as a tool for exploration are its unobtrusiveness, its use of an optical lure to attract visual predators, and its capacity for site-specific deployments at biological oases. The first time all of these elements came together was on the NOAA Ocean Exploration mission to the Gulf of Mexico in 2004. Proof of concept came with the first deployment at the *NR-1* brine pool in the northern Gulf of Mexico. The camera was placed on the edge of the pool along with the electronic jellyfish and a bait bag. After four hours of standard recordings, the electronic jellyfish was activated for the first time. Just 86 seconds after the pinwheel burglar-alarm display began, a squid was recorded (Figure 3) with characteristics so unique that it cannot be assigned to any known family (video may be viewed at www.oceanrecon.org/research). Remarkably, during the 2005 Ocean Exploration mission to the Gulf of Mexico, the same unknown species of squid was recorded at a site over 660 km from the first, once again in apparent response to an e-jelly burglar-alarm display. The fact that such a large (~ 1–2 m) unknown squid was so readily recorded by the EITS on two separate occasions was a testament to this new approach to exploration—one that takes into account how the deep-ocean environment is perceived by its inhabitants.

"I SPY WITH MY LITTLE EYE"

Color

An object or organism is visible—that is, distinguishable from its background—because of the difference in the photons reflected or emitted from it and those reflected or scattered from its background. This perception is defined as contrast and is a critical matter for predators trying to locate prey. Many prey that cannot out-swim their predators avoid attack by being cryptic, which means blending into the background. Still others avoid attack by being toxic or distasteful and advertising that fact with some striking visual characteristic, like the brightly colored wings of the Monarch butterfly. In the ocean depths where the light field is predominantly blue and photons are highly scattered over very short distances, both of these strategies are a challenge.

To better understand how animals meet this challenge, the Deepscope team

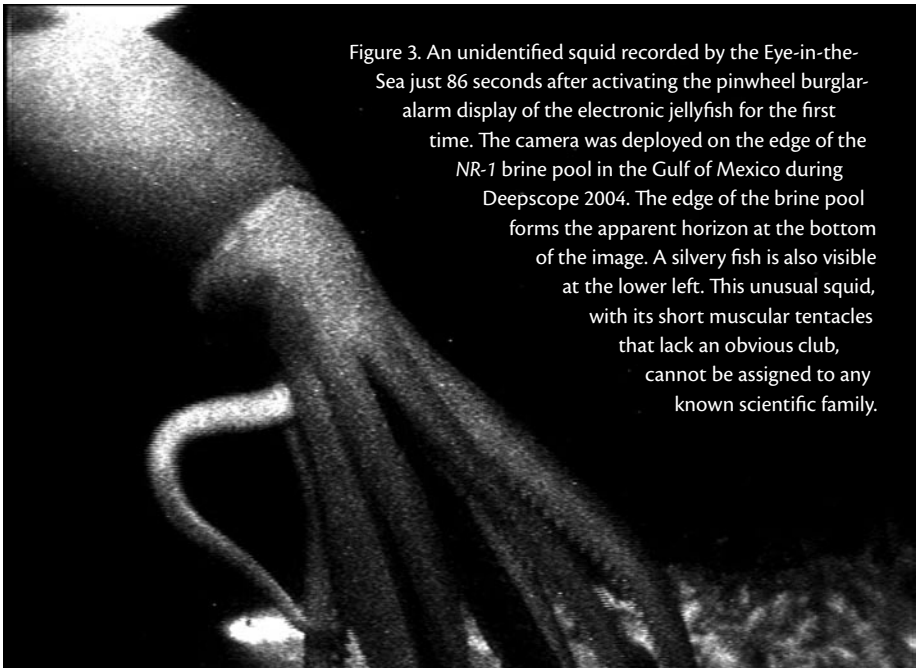


Figure 3. An unidentified squid recorded by the Eye-in-the-Sea just 86 seconds after activating the pinwheel burglar-alarm display of the electronic jellyfish for the first time. The camera was deployed on the edge of the *NR-1* brine pool in the Gulf of Mexico during Deepscope 2004. The edge of the brine pool forms the apparent horizon at the bottom of the image. A silvery fish is also visible at the lower left. This unusual squid, with its short muscular tentacles that lack an obvious club, cannot be assigned to any known scientific family.

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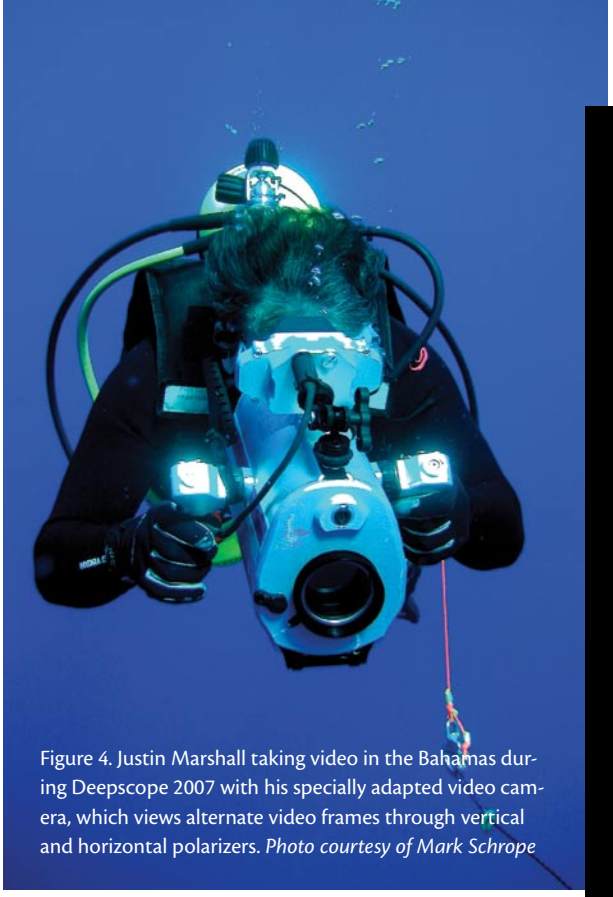

Figure 4. Justin Marshall taking video in the Bahamas during Deepscope 2007 with his specially adapted video camera, which views alternate video frames through vertical and horizontal polarizers. *Photo courtesy of Mark Schrope*

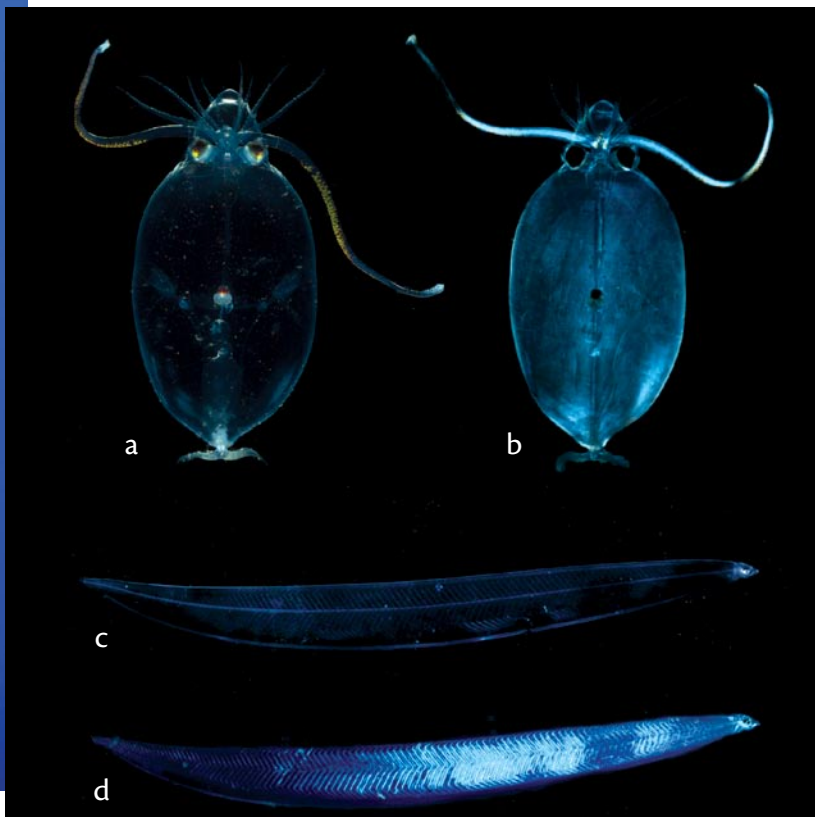

Figure 5. Images of a cranchid squid viewed (a) without polarizers and (b) between crossed polarizers, and of an eel leptocephalus larva viewed (c) without polarizers and (d) between crossed polarizers, demonstrating the dramatically increased contrast of birefringent tissues. This design is comparable to how predators with polarization vision might detect such transparent animals when viewed against highly polarized background light. *Photos by Edith Widder*

focused in on characterizing the deep-sea light environment. To accomplish this analysis, measurements of downwelling spectral irradiance were made to depths of 500 m using an ultra-high-sensitivity scanning spectrophotometer in combination with a through-hull fiber-optic penetrator installed in the rear dive chamber of the *Johnson-Sea-Link* submersible. Measurements were also taken of the reflectivity of various pelagic and benthic inhabitants found at depths where sunlight still penetrates, but where bioluminescence is prevalent. Comparison of these measurements with bioluminescent emission spectra previously taken from ocular photophores (Widder et al., 1983) revealed that the colors of deep-sea animals are far better adapted for camouflage against bioluminescent search lights than against the ambient light, providing the first solid evidence of the ecological significance of these searchlights (Johnsen, 2005).

Transparency

Another common form of crypsis (the ability of an organism to avoid observation) in the epipelagic and mesopelagic zones is transparency. Being transparent would appear to be the ideal adaptation for hiding from predators as it allows an animal to blend in with any background. However, a possible counter-adaptation may be polarization sensitivity in the eyes of some crustaceans and cephalopods, which can increase the contrast of transparent but birefringent tissues when they are viewed against a polarized background. This mechanism is akin to a contrast-enhancing trick used by photographers, where a linearly polarized filter is used on the camera lens and is cross polarized with respect to the illumination source. Members of the Deepscope team have been exploring the use of polarization technologies in the open ocean with cameras fitted with Polaroid filters (Figure 4). Animals collected during blue-water dives have also been imaged in the laboratory with crossed polarizers, demonstrating how effective this method of contrast enhancement could be for helping predators to track down invisible prey (Figure 5). Besides providing insight into open-ocean visual ecology,

Figure 6. The short nose green-eye fish, *Chlorophtalmus agassizi,* as seen with white light (below) and fluorescence (above). The green fluorescence seen in the eyes is a pigment in the lenses, which absorbs the predominantly blue ambient light and lets through longer wavelengths, potentially of the fish's own fluorescence, thereby enhancing contrast. *Photos by Edith Widder*

this line of research also has exciting applications for extending the range of underwater imaging through the development of polarization-based, haze-removing algorithms (Sönke Johnsen of Duke University and Justin Marshall of University Queensland, *pers. comm.,* August 2005; Schechner et al., 2003).

Fluorescence

Not all animals benefit from blending in with the background. Some need to stand out in order to advertise their distastefulness to predators or their attractiveness to potential mates. One way to stand out is with color. However, because ambient light in the deep ocean is predominantly blue, color signaling might seem unlikely. One of the hypotheses being tested during the Deepscope missions was that fluorescence could provide a way to generate a color signal by means of the Stokes shift, which causes light absorbed at one wavelength to be re-emitted at a longer wavelength, thereby providing a color that contrasts with the background light field. To test this hypothesis, two of the white lights on the submersible were fitted with blue filters, while the camera was fitted with a yellow filter and the scientist observer wore yellow glasses of the same filter material to block out the intense excitation wavelengths. This approach proved highly effective and led to some surprising discoveries, including a brightly banded anemone, a brilliantly fluorescent chain cat shark (http://www.oceanexplorer.noaa.gov/explorations/05deepscope/logs/aug22/media/movies/fluorescent_shark_video.html), and a beautiful fluorescence pattern on the short nose green-eye fish, *Chlorophtalmus agassizi* (Figure 6). Although the green eyes, which give this fish its common name, are readily apparent under white light, it is under blue light that they truly stand out. Interestingly, the fluorescent pigment in the eyes is actually in the lenses and appears to provide the same functionality as the yellow filters used by the Deepscope team—absorbing the predominantly blue ambient light and letting through the longer wavelengths of the fish's own fluorescence. This design may serve a dual function as it enhances contrast and perhaps the attractiveness of potential mates, as well as the visibility of potential bioluminescent prey. The upward-pointed eyes of these fish indicate that they search the downwelling light field for the silhouette of any tasty mouthful swimming overhead. Many open-ocean inhabitants thwart this common predatory strategy with counterillumination—production of bioluminescence from their ventral surfaces to mask their silhouettes. It is believed that yellow filters have evolved to break this camouflage by making the more turquoise-colored light of bioluminescence stand out against the deep blue background (Muntz, 1976).

These fluorescence studies also provide an opportunity for bioprospecting for potential biotechnology markers. Fluorescent proteins have had an enormous impact on in vivo imaging technologies, and there is great interest in locating new proteins and new colors. Therefore, the fluorescent organisms collected during the Deepscope missions are screened as potential sources of genes that can be cloned for new fluorescent proteins. For the short nose green-eye fish, proteomics were applied to derive an initial protein sequence fragment followed by amplification and

expression of the corresponding full-length cDNA using established methods (Matz et al., 2003).

SEEING THE LIGHT

Color, transparency, and fluorescence are in the eye of the beholder. Therefore, to truly comprehend the nature of life in the deep ocean, it is essential to define how this realm is viewed from the standpoint of its inhabitants, rather than depending on the distorted view provided by our high-resolution, light-insensitive cameras and bright white floodlights. The problem is how to study the eyes of deep-ocean dwellers without damaging them in the process of collection. Most of these ultra-sensitive eyes lack eyelids or contractible irises for protection against bright lights, so the intrusion of submersibles or ROVs with their bright spotlights are not only disruptive, they can be permanently blinding. In the midwater, this challenge has been met by using nets fitted with thermally insulated and light-tight collection reservoirs that can be closed at depth and then opened in a shipboard laboratory where the animals are sorted under dim red light. For benthic animals, the early Deepscope missions attempted something similar with the development of thermally insulted, light-tight, baited traps for retrieval of living benthic organisms with intact visual systems for electrophysiological characterization. However, these traps proved ineffective because of unwanted scavengers such as hagfish and large crabs that were too large to enter the traps but guarded their entrances and warded off smaller crustaceans. Therefore, an alternative approach was developed that involved putting red filters on the submersible lights and then collecting individual crustaceans that were placed into a thermally insulated, light-tight box for transport to the surface.

Studies on the eyes of animals retrieved in this manner revealed very slow flicker fusion frequencies, which is not surprising, as use of long integration intervals is a well-known sensitivity-enhancing trick. What was surprising was the discovery of a deep-sea crab, *Gastroptychus spinifer*, with an ultraviolet photoreceptor in addition to the more typical blue receptor (Frank, 2006). This discovery was a testament to how much we still have to learn about life in the deep sea. What purpose does UV sensitivity serve in such a deep-living (550-m depth) crab? Is there ultraviolet bioluminescence in the depths yet to be discovered or does UV sensitivity play some role for this crab in locating food or mates? Understanding how the deep ocean is viewed by its inhabitants is providing new tools for exploration, new insights into deep-ocean ecology, and new evidence of how much we still have to learn about Earth's final frontier.

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