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Deep-Sea Research I 52 (2005) 2077–2085

DEEP-SEA RESEARCH
PART I

www.elsevier.com/locate/dsr

Using red light for in situ observations of deep-sea fishes

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Received 13 December 2004; received in revised form 6 June 2005; accepted 9 June 2005
Available online 11 August 2005

Abstract

Observations of animals in the deep ocean typically require the use of bright lights that can damage eyes and disrupt normal behaviors. Although the use of infrared light is an effective means of unobtrusive observation on land, it is far less effective in the ocean where long wavelength light is rapidly attenuated by seawater. Here we describe in situ observations of the behavior of the sablefish, *Anoplopoma fimbria*, around a baited site under different lighting conditions. Fish were observed with low-light-level imaging that had adequate sensitivity to compensate for the attenuation losses associated with the use of long wavelength light in water. ROV-based experiments compared the number of sablefish seen around bait, illuminated alternately with red vs. white light. Significantly more fish were seen under red light than white light with the average number of sablefish observed per 10 min viewing interval under red light being 38.9 (±18.5 SD) compared to 7.5 (±7.1 SD) under white light. Under both red and white light sablefish spent only brief periods in the illumination field (10.5 s [±8.7 SD] under red light and 6.6 s [±8.7 SD] under white light). It appeared that sablefish were responding to competing drives of attraction to the bait and avoidance of the lights and that the avoidance was greater for white light than for red light. Observations were also made with the newly developed deep-sea observatory, Eye-in-the-Sea, using long wavelength LED illumination. The onset of LED illumination did not generally produce a startle response from fish around the bait, and in some cases invoked no response at all. However, in the majority of cases the fish moved out of the circle of red-light illumination during the 7.5 s recording period, indicating that the light was detectable and aversive to these fish. This was true with both 660 and 680 nm LED illuminators. We conclude that while a sharper short-wavelength cutoff of the illumination source is required to achieve truly unobtrusive observation, red light is nonetheless significantly less disruptive than white light for observing deep-sea fish behavior, and can provide adequate illumination when used in combination with image-intensified cameras.

Keywords: *Anoplopoma fimbria*; Behavior; Red light; Sablefish; Vision; USA; California; Monterey Bay

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doi:10.1016/j.dsr.2005.06.007

1. Introduction

In spite of recent developments in ocean exploration and monitoring, unobtrusive observation of biological activity remains a scientific frontier. Our primary means of viewing animals in the deep ocean or in surface waters at night requires the use of bright incandescent lamps that are disruptive to the life processes of the animals that live there. Animals capable of swimming often flee from the lamps or swarm around them (Verheijen, 1958; Forward, 1988). Sedentary animals often shrink back, temporarily ceasing their normal activities and animals with sensitive eyes may be permanently blinded (Herring et al., 1999). To really understand life in the oceans we must find ways to study oceanic communities and populations without modifying the habitat and frightening the inhabitants with intrusive, artificial lights. On land, this is accomplished using infrared illumination, which is invisible to the animals being observed but visible to infrared cameras that record their behavior. This methodology is generally impractical for in situ observations of marine animals, because infrared light is attenuated so rapidly in seawater that observations are restricted to distances of less than one attenuation length (1.5 m for 700 nm light) (e.g. Matsuoka et al., 1997). Therefore, most behavioral observations of marine organisms using far-red or infrared illumination have been conducted in a laboratory setting where the animals are confined to a very small viewing volume (e.g. Strickler, 1977; Batty, 1983; Widder, 1992; Fisher and Bellwood, 2003). In order to observe large animals in their natural state, a much larger viewing volume is needed. Here we describe in situ observations of fish behavior viewed with far-red illumination in combination with low-light-level (LLL) cameras that have adequate sensitivity to compensate for the long-wavelength attenuation losses.

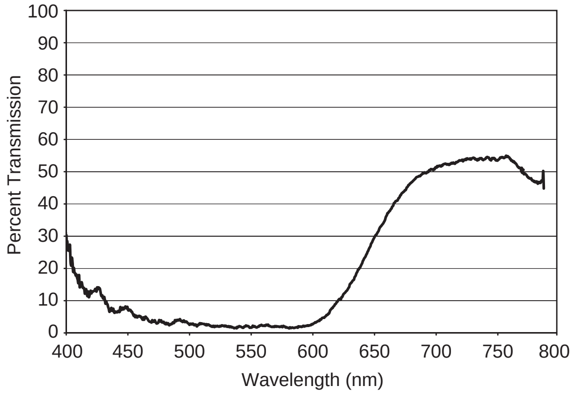

Fig. 1. Transmission through red plastic filters used on ROV *Ventana*'s camera lights for these experiments.

2. Materials and methods

2.1. ROV-based experiments

Experiments were conducted in October 2000 using the ROV *Ventana* fitted with an intensified video camera (Kongsberg Simrad ISIT) that has a photometric sensitivity of 5×10^{-6} LUX and a radiometric sensitivity of 25 mA/W at 700 nm. Illumination was provided by the ROV's four camera lights (DSPL HID 400 W daylight) of which two were fitted with red plastic filters with 10% transmission at 620 nm and 50% transmission at 680 nm (Fig. 1). Either the two red filtered lights or the two white unfiltered lights were illuminated for each period of observation. A bait cage, used to prevent scavengers from moving the bait outside the field of view of the camera, was placed approximately 2 m in front of the ROV at a depth of 520 m in Monterey Canyon (36°46.9′ N 121°55.0′ W). Recordings began at 20:00 and ended before 05:00 the following morning.

The ROV *Ventana* is trimmed to be positively buoyant in order to ensure recovery in the event of a power loss. This necessitates continuous use of down-thrust for the ROV to sit on the bottom. In order to achieve a truly unobtrusive observation capability, it is necessary to eliminate the acoustic, electrical, and mechanical disturbances associated with the ROV. This was a primary motivation for the development of the Eye-in-the-Sea (EITS) deep-sea observatory (Widder, et al. in prep).

2.2. Eye-in-the-sea experiments

The EITS is being developed as an unobtrusive sub-sea observatory (Fig. 2). Briefly, it consists of

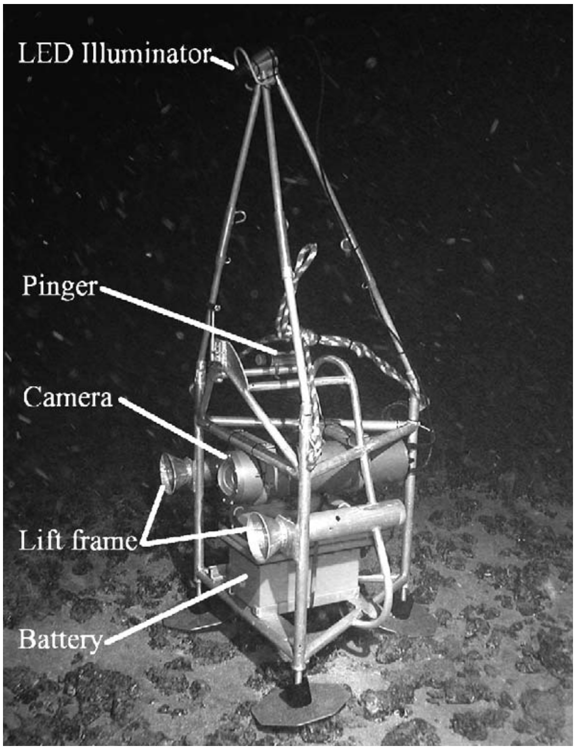

Fig. 2. Eye-in-the-Sea observatory in situ. The lift frame was designed for deployment by both the ROV *Ventana* and the *Johnson-Sea-Link* submersible. Tubes on the lift frame are designed to slide on and off stabs on the deployment platform.

a computer-controlled, intensified video camera (Philips LTC-0500 $\frac{1}{2}$" CCD with a Litton SPV1305 NiteMate GENIII MCP intensifier with photometric sensitivity of 10^{-5} LUX and radiometric sensitivity of 120 mA/W at 640 nm), an LED illumination source, a tripod, and an undersea battery (DSPL SB-24/42). Data are acquired by digitizing the video signal from the camera into the system RAM before transferring to a hard drive as BMP files. The amount of RAM available currently limits the duration of individual recorded events to 150 frames, which at the maximum frame rate of 20 fps represents an upper limit of 7.5 s per event. For these experiments, which took place in October 2002 and July 2003, the EITS was programmed to record events every 15 min for 22 h and to store the digitized files on the 40 GB hard disk. Experiments were begun in mid to late afternoon and ran through the night and into the next day. Illumination was provided by either 660 nm (60 mA) or 680 nm (20 mA) LEDs. Irradiance at 2 m in clear ocean water was $3.3 \times 10^{12}\,\mathrm{p\,cm^{-2}\,s^{-1}}$ from the 660 illuminator, and $1.2 \times 10^{12}\,\mathrm{p\,cm^{-2}\,s^{-1}}$ from the 680 illuminator. The ROV *Ventana* was used to place the EITS on Smooth Ridge (36°45.5′ N 121°59.5′ W) in Monterey Canyon at a depth of 600 m. A weighted bait bag was placed 2 m in front of the camera. The first frame of each recording was coincident with the onset of red light illumination.

2.3. Spectral measurements

Transmission of the red filters used in the ROV experiments was measured with a multichannel spectrometer (PS 1000, Ocean Optics, Inc). Spectral emissions of the LED illuminators used for the EITS experiments were measured in air with a spectroradiometer (OL 754 UV-Vis, Optronic Laboratories, Inc.) with an integrating sphere entrance port (OL IS-670, Optronic Laboratories, Inc.) and in water with an Ocean Optics USB2000 fiber optic spectrometer. The spectrometers were radiometrically calibrated with reference to a LLL, multi-filter calibration source (OL 310, Optronic Laboratories, Inc.).

3. Results

There was no difference in visibility or depth-of-field between red light and white light illumination when using the ISIT video camera mounted on the ROV. In fact, because the ISIT camera records in black and white and has automatic gain control, the only way to distinguish between red and white light illumination was with the time code (Fig. 3).

Sablefish, *Anoplopoma fimbria*, and Pacific hagfish, *Eptatretus stoutii* were the fish seen most frequently around the bait. There was no apparent difference in the number or behavior of hagfish (which have reduced eyes with no lenses) seen around the bait when viewed under red or white light. However, this was not the case for sablefish. Fig. 4 is a plot of the amount of time that sablefish were visible in the field of view of the camera under

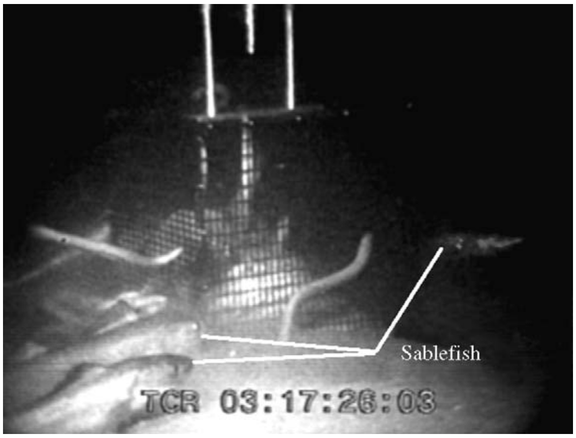

Fig. 3. A frame grab from an ISIT video recording made from the ROV *Ventana*, showing sablefish and hagfish clustered around the bait box under red light illumination.

red light compared to white light. The order of illumination was 1 h of red light, 15 min of darkness, 1 h of white light, and then 10 min of red, 10 min of white, 10 min of red and 10 min of white light. Recordings were analyzed in 10-min viewing intervals to determine the amount of time each fish was seen on camera. Bars in Fig. 4 represent the sum of these values for each 10 min viewing interval.

Overall, the average number of fish appearances on camera was significantly greater under red light than white light (Student's *t*-test, $df = 14$, $P<0.001$) with an average of 38.9 (±18.5 SD, $N = 8$) appearances under red light per 10 min viewing period and 7.4 appearances (±7.1 SD, $N = 8$) under white light. The average amount of time that each fish was visible was also significantly longer under red light than under white light (Student's *t*-test, $df = 369$, $P = 0.001$). The

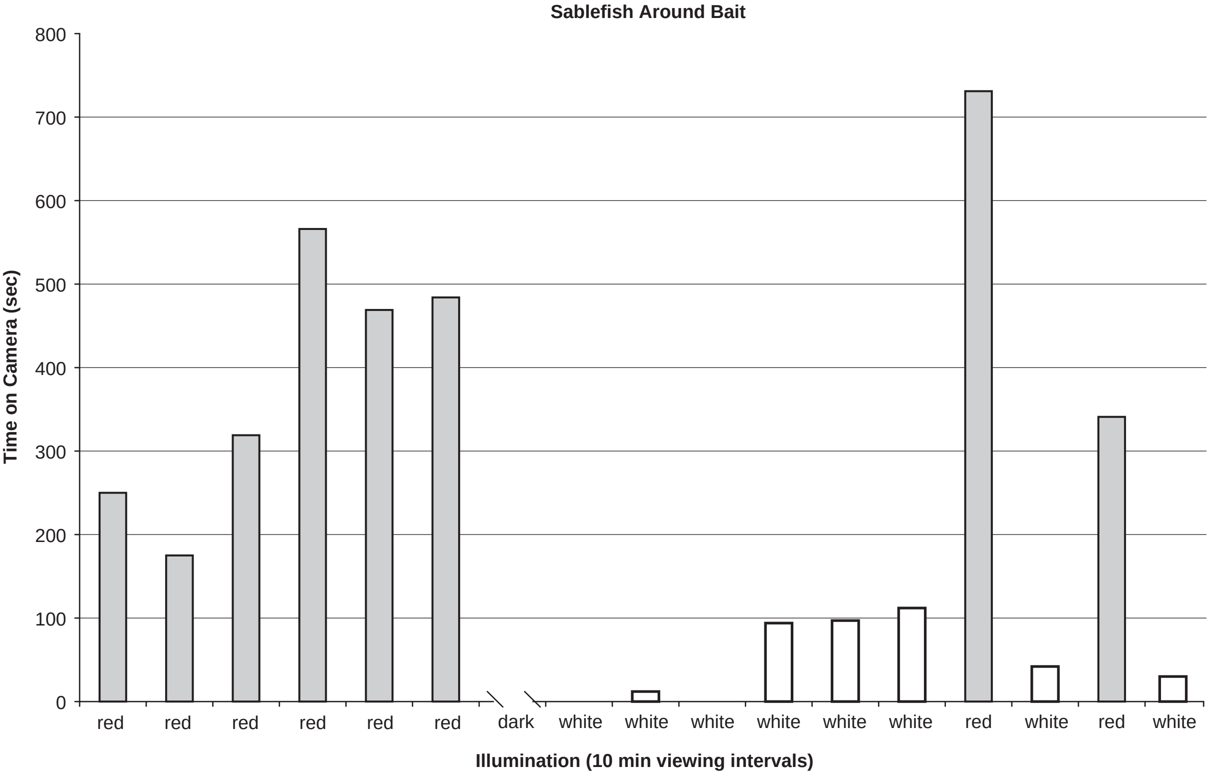

Fig. 4. The amount of time sablefish were visible on camera during ROV experiments under red light compared to white light, measured in 10 min intervals. The gap in the *x*-axis indicates a 15-min dark period.

average time on camera under red light was 10.7 s (±8.9 SD, $N = 312$), compared with 6.6 s (±8.7 SD, $N = 59$) under white light. Following the 15 min dark period, when the white light was first turned on, no sablefish were seen around the bait. However, because the automatic gain control blooms with sudden exposure to white light, there was a 2 s lag between the lights coming on and the scene being visible. Sablefish remained largely absent from the field of view during the first half hour of white light illumination and only began to appear in the field during the second half hour. When the white light was turned off and the red light turned on sablefish immediately approached the bait. When the white light was turned on and the red light turned off sablefish immediately departed the scene, but began to appear again in the latter part of the 10 min viewing period. Under white light it was possible to use the ROV's high-resolution camera set on high gain to look outside the field of view of the intensified camera and see that sablefish were swimming just outside the halo of illumination.

Fig. 5 is a frame grab from a video sequence collected with the EITS showing sablefish around the bait bag, illuminated with 660 nm light from the LED illuminator. In order to assess the effects of the onset of red light illumination, sablefish behavior observed within each 7.5 s viewing period (the duration maximum set by the RAM) was broken down into four categories and then enumerated as shown in Fig. 6A.

- "Unresponsive" describes sablefish that remained in the field of view of the camera during the 7.5 s recording period and exhibited no apparent reaction to the light. In most cases this consisted of fish resting on the bottom next to the bait bag with no change in position.
- "Responsive" describes sablefish that remained in the field of view during the entire recording period but appeared to respond to the light. This consisted of a change in swimming direction, usually away from the illumination source or the initiation of swimming in fish that were holding position around the bait bag.
- "Enters" refers to sablefish that swam into the field of view of the camera during the recording period.
- "Exits" refers to sablefish that swam out of the field of view during the recording period. In cases where a fish swam into and out of the field of view during the same recording period both behaviors were logged.

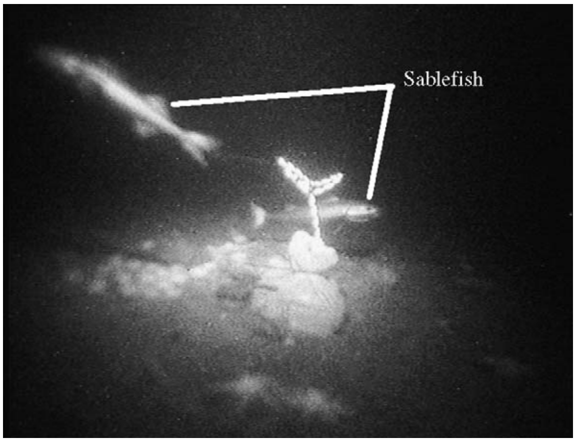

Fig. 5. A single frame from the Eye-in-the-sea showing two sablefish and the bait bag illuminated with the 660 nm LED illuminator. A polypropylene rope is floating above the bag, a cluster of sea urchins is visible to the left of the bag and two starfish are in the foreground.

There were a few instances where fish appeared to exhibit a strong aversive response to the red light, as evidenced by rapid swimming out of the illumination field, but the most common response to light onset was slow swimming away from the 660 nm field of illumination (Fig. 6A). In three instances fish swam up to the edge of the brightest circle of illumination, paused and then swam away. Although, this clearly appeared to be a reaction to the red light, since they did not remain within the field of view, each of these was logged as an entrance and exit in order to be consistent with our behavior categories.

An identical experiment was carried out using 680 nm LEDs. Although, in this case the illumination was noticeably dimmer in the camera recordings, the results were the same, with the number of exits significantly exceeding entrances (Fig. 6B).

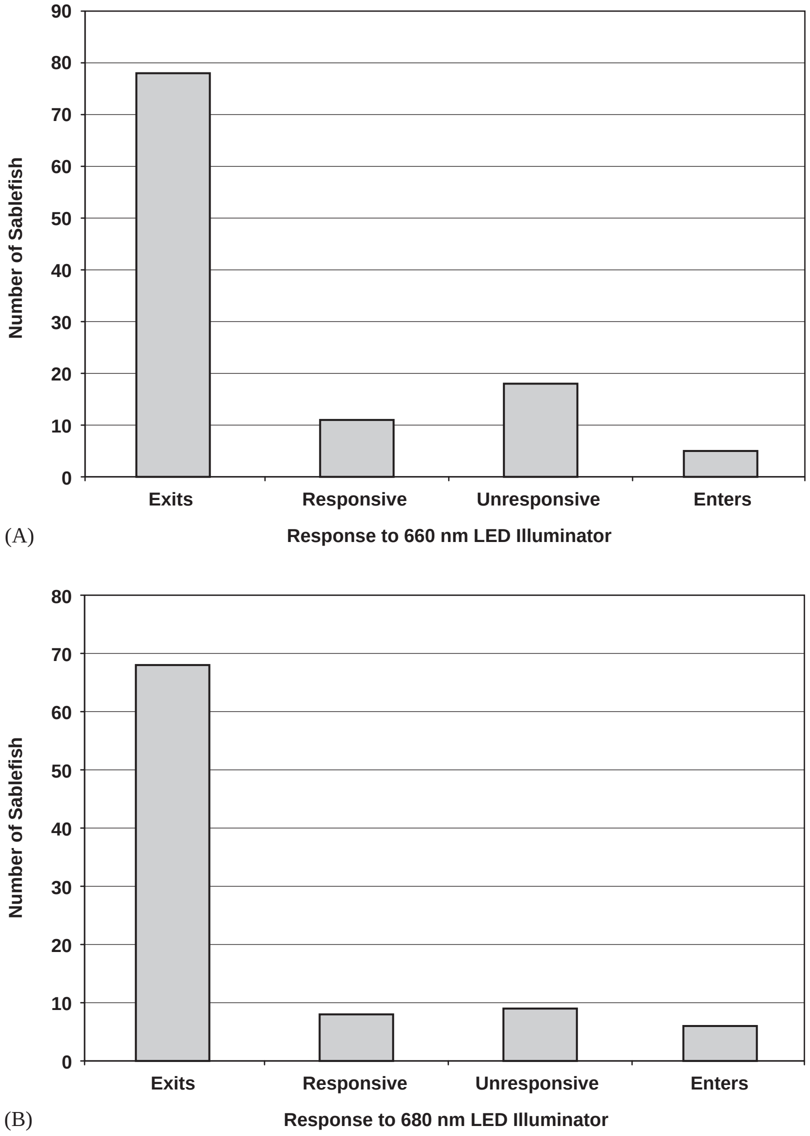

Fig. 6. Enumeration from Eye-in-the-Sea recordings of sablefish behaviors observed during 7.5 s recording periods under (A) 660 nm LED illumination and (B) 680 nm LED illumination.

4. Discussion

In the ROV-based experiment sablefish were seen more often around the bait site when it was illuminated with red light than with white light. This difference was highly significant and apparently not unduly influenced by the rate of arrival of fish to the bait given the extreme differences seen at the end of the experiment, when red light was alternated with white light at 10 min intervals (Fig. 4). With either illumination method the fish would generally enter the light field to inspect the bait and then leave a few seconds later. It was primarily the number of such events that varied between the two illumination methods, although the length of time in the light field was also several seconds longer under red light than under white light. This behavior suggests that the red light was not unobtrusive, but merely less aversive than white light. The fish appeared to be responding to competing drives of attraction to the food and aversion to the light, which drove them to swim into the light field to approach the bait, but then leave to avoid the light. The fact that these visits to the bait were significantly more frequent and longer under red light, indicates that the red light was less aversive than white light.

The degree of aversion appeared to be influenced by the state of visual adaptation. Fish did not approach the bait under white light illumination immediately following a 15-min dark period, but did begin to approach the bait during the second half of the viewing period. When red light was alternated with white light at 10-min intervals, the fish rushed in with the onset of red light and then dispersed with the onset of white light. However, after the red light, fish began to approach the bait much sooner under the white light than they did following the 15-min dark period. This could be explained if the red light caused some light adaptation.

The EITS experiments provided further evidence of sablefish sensitivity to red light. Although sablefish rarely exhibited startle responses with the onset of red light illumination, evaluation of fish behavior indicated that both the 660 and 680 nm LEDs were aversive. If normal sablefish behavior in total darkness were to swim toward and then away from the bait, with random viewing intervals one would expect to see an equal number of entrances and exits of the light field. Instead we found that sablefish were significantly more likely to leave the field of illumination than to enter it, indicating that the light was not unobtrusive.

Most deep-sea fish have only one visual pigment with a sensitivity maximum centered in the blue wavelengths between 470 and 495 nm (Partridge et al., 1988, 1989). Studies on visual pigment extracts of *A. fimbria*, have revealed a sensitivity maximum of 491 nm (Beatty, 1973). Although a comparison of this visual pigment sensitivity with the emission spectrum of the 680 nm LED illuminator (Fig. 7A) might suggest that this illumination should be invisible to these fish, it is important to remember that visual systems have an impressive dynamic range covering many orders of magnitude. When the same data are plotted on a logarithmic scale (Fig. 7B), it is apparent that there may be considerable overlap. Just how much overlap will depend on the absolute sensitivity of sablefish vision.

The best means of determining an animal's visual sensitivity is with behavioral evidence. In one investigation, juvenile sablefish were used in a laboratory experiment that examined how different levels of illumination influenced their ability to detect and respond to a towed net (Olla et al., 1997). It was found that the threshold light intensity required to induce swimming was five times lower for juvenile sablefish than for juvenile Alaska pollock, *Theragra chalcogramma*. The irradiance at which the sablefish stopped swimming, which was 2.4×10^{10} photons/cm^2/s (PAR 400–700 nm), was presumed to be the threshold at which they could no longer see the net. By comparison, the irradiance from the 680 nm LED illuminator that produced a response from sablefish in our study was 1.2×10^{12} photons/cm^2/s. If one figures in the relative sensitivity of the visual pigment over the spectral range of the LED, then this irradiance is comparable to 3.8×10^9 photons/cm^2/s, approximately an order of magnitude less than the threshold for juvenile sablefish.

Juvenile sablefish are pelagic and found in surface waters (Armstrong, 1996), while adults are bathydemersal and occur between 305 and 2740 m (Eschmeyer et al., 1983). Therefore, one might expect an increase in visual sensitivity as these fish mature and move into deeper water. In fact, an irradiance of 10^9 photons/cm^2/s is still a high value when compared to the absolute sensitivity of goldfish, which is approximately 5×10^4 photons cm^2/s (Powers et al., 1988). Given that deep-sea fishes are believed to be at least 10–100 times more sensitive than this (Denton, 1990), it is not surprising that sablefish were able to detect the 680 nm LEDs used on the EITS observatory.

These results clearly demonstrate just how challenging it will be to make truly unobtrusive observations of deep-sea fish. The width of the visual pigment absorption spectrum on the one

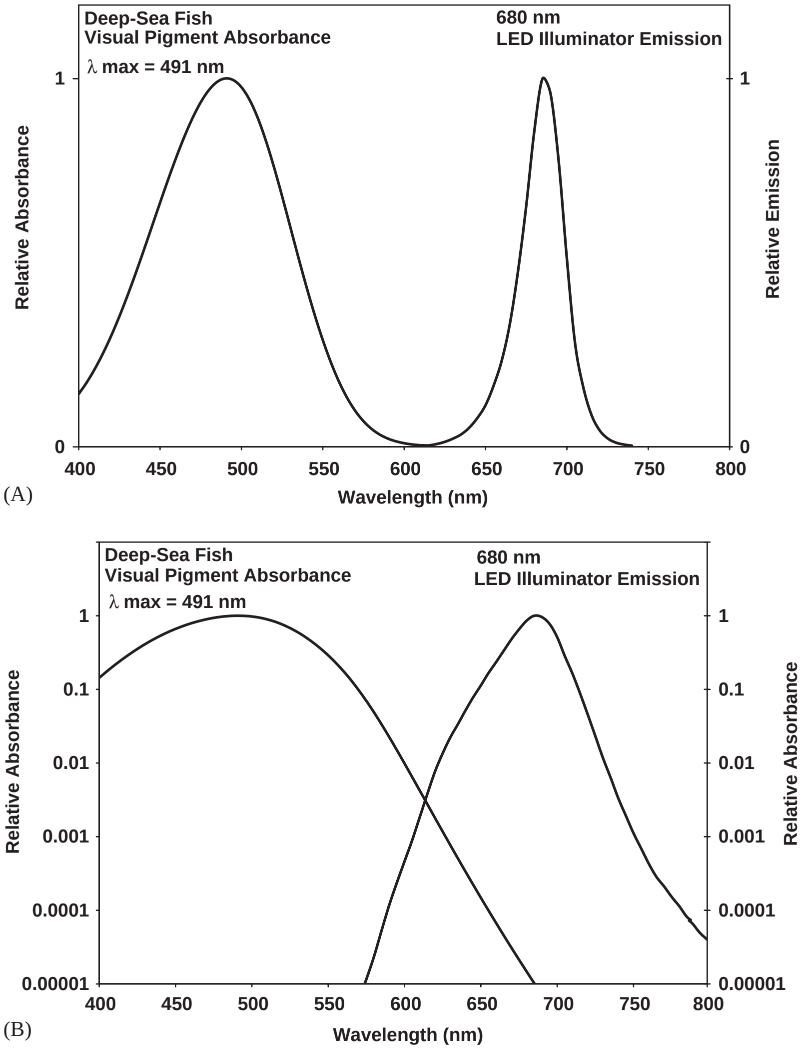

Fig. 7. Normalized visual pigment absorbance of a visual pigment with a 491 nm absorbance maximum (Govardovskii et al., 2000) compared to the normalized spectral emission of the 680 nm LED illuminator used on the Eye-in-the-Sea plotted on (A) linear and (B) logarithmic scales.

hand, and the extreme attenuation by seawater at wavelengths outside of that range on the other hand, puts very tight constraints on illumination systems. One attenuation length (the distance over which irradiance due to unscattered photons is diminished by 1/e or ~37%) is 52 m for blue light at 470 nm. By contrast, one attenuation length for red light at 700 nm is only 1.5 m (Smith and Baker, 1981). It was apparent from our investigation that the 680 nm LED illuminator we used was near the limit of providing adequate illumination for the camera but, to be unobtrusive, it will require a longer wavelength maximum, or at least a shorter wavelength cutoff, which will further decrease illumination.

We gain some encouragement that this is still an achievable goal from the bioluminescent emission spectra of the deep-sea fish *Malacosteus niger*, which peaks at 705 nm and has a steep, short-wavelength cutoff at just below 700 nm. The red filter covering the suborbital photophore of *M. niger* confines the broad spectral emission of the bioluminescence (which would otherwise peak at 660 nm) to the far-red portion of the spectrum, an adaptation that is believed to make these emissions unobtrusive (Widder et al., 1984; Douglas et al., 1998). Therefore, with this same goal in mind, we are currently redesigning the EITS with a new, more sensitive camera system and a refined illumination system with a steeper short-wavelength cutoff. Given the highly significant difference that we recorded between red light and white light observations (Fig. 4) there can be no doubt that red light is less disruptive than white light and should be used whenever possible for behavioral observations of deep-sea fishes.

Acknowledgments

We thank MBARI's Operations group, in particular, ROV *Ventana*'s pilots and the captain and crew of the Point Lobos, for their support in conducting this work. We thank Lee Frey, Erika Raymond, Farley Shane, and Dave Smith, for shore-based support, Jeff Drazen and Rob Sherlock for assistance at sea and Kimberly Ackerman for assistance with the video data analysis. We thank Tamara Frank, Peter Herring, Sonke Johnsen, and Tracey Sutton for critical reading of the manuscript. This work was supported in part by the Monterey Bay Aquarium Research Institute and Harbor Branch Oceanographic Institution, and grants from NOAA's Office of Ocean Exploration and the Office of Naval Research. HBOI Contribution No. 1599.

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