

RESEARCH ARTICLE

Light and vision in the deep-sea benthos: I. Bioluminescence at 500–1000m depth in the Bahamian Islands

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SUMMARY

Bioluminescence is common and well studied in mesopelagic species. However, the extent of bioluminescence in benthic sites of similar depths is far less studied, although the relatively large eyes of benthic fish, crustaceans and cephalopods at bathyal depths suggest the presence of significant biogenic light. Using the *Johnson-Sea-Link* submersible, we collected numerous species of cnidarians, echinoderms, crustaceans, cephalopods and sponges, as well as one annelid from three sites in the northern Bahamas (500–1000m depth). Using mechanical and chemical stimulation, we tested the collected species for light emission, and photographed and measured the spectra of the emitted light. In addition, *in situ* intensified video and still photos were taken of different benthic habitats. Surprisingly, bioluminescence in benthic animals at these sites was far less common than in mesopelagic animals from similar depths, with less than 20% of the collected species emitting light. Bioluminescent taxa comprised two species of anemone (Actinaria), a new genus and species of flabellate Parazoanthidae (formerly *Gerardia* sp.) (Zoanthidea), three sea pens (Pennatulacea), three bamboo corals (Alcyonacea), the chrysogorgiid coral *Chrysogorgia desbonni* (Alcyonacea), the caridean shrimp *Parapandalus* sp. and *Heterocarpus ensifer* (Decapoda), two holothuroids (Elasipodida and Aspidochirota) and the ophiuroid *Ophiochiton ternispinus* (Ophiurida). Except for the ophiuroid and the two shrimp, which emitted blue light (peak wavelengths 470 and 455nm), all the species produced greener light than that measured in most mesopelagic taxa, with the emissions of the pennatulaceans being strongly shifted towards longer wavelengths. *In situ* observations suggested that bioluminescence associated with these sites was due primarily to light emitted by bioluminescent planktonic species as they struck filter feeders that extended into the water column.

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INTRODUCTION

Although bioluminescence is relatively rare in terrestrial habitats and nearly absent in freshwater, it is abundant in the ocean, particularly in the mesopelagic realm, where it is thought to serve a number of functions including luring, warning, crypsis, sexual display and defense against predation (reviewed by Widder, 2010; Haddock et al., 2010). In addition to more focused studies, four large surveys of oceanic bioluminescence have investigated its prevalence and taxonomic distribution and measured the spectra of the emitted light (Nicol, 1958; Herring, 1983; Widder et al., 1983; Haddock and Case, 1999). However, with the exception of a few taxa in Herring (Herring, 1983) and several remote-video studies (Gillibrand et al., 2007; Heger et al., 2007; Craig et al., 2011), the bioluminescence of bathyal benthic species at mesopelagic depths (200–1000m) is poorly understood. This is likely due to the fact that it is easier to catch pelagic animals in good condition with a suitable trawl net than it is to catch benthic species in similar condition *via* a dredge, especially one dragged over complex terrain.

There are several reasons to suspect that bioluminescence in the deep-sea benthos may differ from that found in the mesopelagic realm. First, the optical environment is different. Instead of a featureless world where light propagates unhindered in all directions,

the benthic zone can be complex, and emitted light is blocked by the sea floor and possibly by organic and inorganic structures. Because of this, certain functions of bioluminescence may be less efficient and others may be less relevant (e.g. counterillumination in epifaunal taxa and escape decoys in sessile taxa). In addition, because of sediments suspended by bottom currents, the water is likely less clear than that found in the water column above and possibly has a longer wavelength of maximal transmission. These bottom currents may also advect bioluminescent plankton onto benthic structures and sessile organisms (some of which may also be bioluminescent), leading to significant amounts of emitted light (see Craig et al., 2011). Also, in at least some benthic locations, the population density and species diversity may be much higher than in the mesopelagic realm. Finally, it appears that the eyes of certain benthopelagic predators are larger than those of related mesopelagic species at similar depths, suggesting that visual detection may be more important in the benthic realm (reviewed by Warrant and Locket, 2004).

In coastal benthic habitats, bioluminescent species comprise only a few percent of the total number, but the density of bioluminescent individuals can be high under certain conditions (reviewed by Morin, 1983). Bioluminescent taxa in this habitat include hydroids,

pennatulaceans, polychaetes, echinoderms and fish. Work on these and other species has suggested that foiling predation is the primary function of bioluminescence in coastal benthic species as compared with more complex and diverse functions in mesopelagic species (reviewed by Morin, 1983). It is also known that shallow benthic anthozoans, both luminous and non-luminous, often bear green-fluorescent proteins (GFPs), which, in the case of bioluminescent species, shifts the bioluminescence to a narrow emission spectrum centered at ~500 nm (reviewed by Haddock et al., 2010). In general, the emission spectra of both neritic and benthic coastal species are shifted to longer wavelengths relative to those of mesopelagic and epipelagic species, particularly among epifaunal benthic invertebrates (e.g. hydroids, pennatulaceans, ophiuroids and polychaetes), though there are exceptions to this rule (Herring, 1983; Morin, 1983).

This study describes a survey of bioluminescence at three benthic sites on the continental shelf in the Bahamas (500–1000 m depth). Using a manned submersible and taking advantage of recent advances in the sensitivities of both spectrometers and digital cameras, we collected numerous individuals of a diverse array of benthic invertebrate species and tested them for bioluminescence. Those that emitted light were then analyzed *via* spectroscopy and still and video imagery. We found that bioluminescence is both rare and prevalent at these sites because, although relatively few benthic species emitted light, bioluminescence was continually triggered by bioluminescent plankton impacting structures in the habitat. We also found that the characteristics of the light emitted by certain benthic species differed from that emitted by mesopelagic species, with the main difference being a shift to longer wavelengths in sessile taxa that reached up into the water column.

MATERIALS AND METHODS

Specimen collection

All animals were collected at three sites using the *Johnson-Sea-Link II* submersible operated from the RV *Seward Johnson* in the Bahamas (20–31 July 2009). The 'Memory Rock' site (eight dives) was located off the western shore of Grand Bahamas Island (~27°4'N, 79°19'W) at a depth of 600 m and consisted of a series of lithoherm – parallel, rocky ridges ranging in height from 30 to 50 m and up to 300 m long (Neumann et al., 1977; Messing et al., 1990). Ridge flanks and crests supported a diverse and often dense

assemblage of predominantly suspension-feeding invertebrates including hexactinellid sponges, demosponges, stylasterid and scleractinian corals, octocorals (Primnoidae, Plexauridae and Paragorgiidae), hydroids, actinians, antipatharians (black corals), stalked and comatulid crinoids, brisingid asteroids and euryalid ophiuroids. The dominant species, especially at the crests, was a new genus and species of Parazoanthidae (Hexacorallia: Zoantharia) (Fig. 1A) historically referred to as *Gerardia* sp. (e.g. Messing et al., 1990). The immediately surrounding unconsolidated substrates were relatively barren of epifaunal organisms, but complex species assemblages could be found on dead standing coral (Fig. 1B).

The 'Burrow Cay' site (10 dives) was located west of Burrow Cay, a small island south-southeast of Grand Bahama Island, with nine dives at 26°25'N, 77°51'W, and one at 26°08'N, 77°35'W. The depths at this site ranged from 550 to 700 m; substrates consisted chiefly of extensive expanses of fine unconsolidated sediment interrupted by ledges, escarpments and hardgrounds. The dominant epifauna were echinoderms, particularly echinoids and ophiuroids (Fig. 1C), with smaller numbers of pennatulaceans, other octocorals, and galatheid and chirostylid crabs. Many of the ophiuroids and smaller crabs were found on various octocorals (Fig. 1D). Additional specimens were collected on a single training dive to 1000 m at a site south of Lucaya, Grand Bahama Island (26°24'N, 78°41'W). This site was deeper than the Burrow Cay site, but had similar characteristics.

Attempts were made during each dive to collect as many different epifaunal species as possible. On some dives, the submersible's lights were turned off. After the pilot and scientists had dark-adapted, the pilot used the submersible's manipulator to gently disturb various animals to stimulate them to emit light. Those that emitted light, and individuals of any untested species, were collected using the manipulator claw, grab or suction sampler. Those collected using the claw or grab were placed in a custom-built 'bio-box', a thermally insulated and light-tight container mounted on the front of the submersible. Specimens collected *via* suction were drawn into one of 12 clear acrylic buckets. During one dive, we positioned the submersible downstream of a parazoanthid fan, turned the lights off and filmed an area of ~0.5–1 m² for 10 min using an I²CCD intensified video camera (Intevacs NiteMate 1305/1306 CCTV Intensifier coupled to a Panasonic CCD; Santa Clara, CA, USA) operated from inside the submersible's passenger sphere.

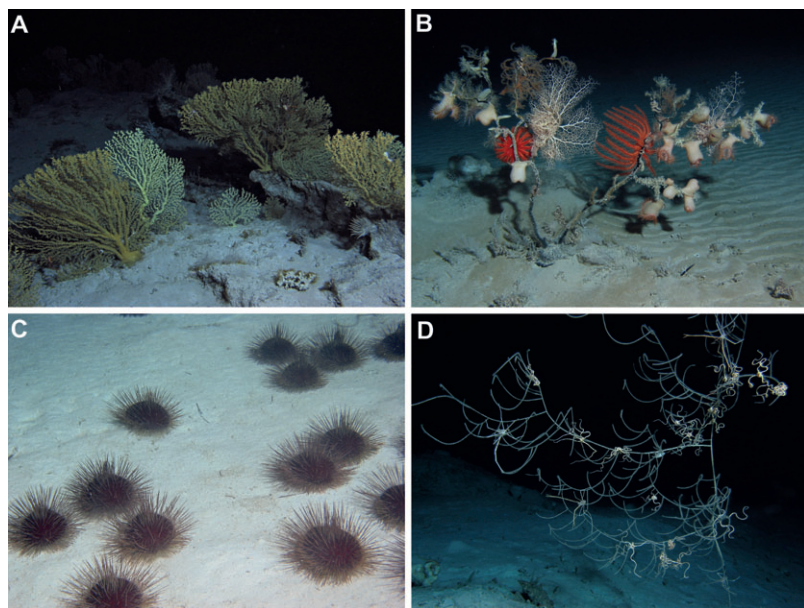


Fig. 1. Representative images of two of the three sites where animals were collected. (A) Crest of a ridge at the lithoherm site (Memory Rock site) showing fans of Parazoanthidae n. gen., n. sp. (B) Plain adjacent to a ridge at the lithoherm site with a community of organisms on a dead stand of coral. (C) Sediment plain in the Northwest Providence Channel (Burrow Cay site). (D) Ophiuroids and chirostylid crabs on isolated antipatharian (black coral) at the sediment plain site.

Following each dive, animals were placed in 4°C seawater and tested under darkness for bioluminescence, generally *via* gentle mechanical stimulation, in some cases augmented by addition of small amounts of KCl to the water (for details, see Herring, 1983; Widder et al., 1983). Those that did not emit light were allowed to recover for at least 12 h in a darkened environmental chamber kept at 4°C before being tested again. Those that did emit light were then used for spectroscopy and photographs.

Spectral measurements of bioluminescent emissions

Spectral measurements of bioluminescence were taken using a highly sensitive multichannel spectroradiometer designed to measure fluorescence (QE65000-FL, Ocean Optics, Dunedin, FL, USA). The spectrometer uses a back-illuminated Hamamatsu FFT-CCD detector with a 90% quantum efficiency and a two-dimensional construction that allows for a large signal-to-noise ratio (>1000:1 at peak signal compared with 250:1 for typical multichannel spectrometers). The spectrometer was equipped with a 200 µm entrance slit and was coupled to a 1000 µm diameter, 0.22 numerical aperture, fiber-optic cable.

Measurements of stimulated bioluminescence were taken shipboard in a dark, light-tight room. The bare end of the optical fiber was held within 2 mm of the source of the emitted light until a spectrum with an acceptable signal-to-noise ratio could be captured; the optimal integration time of the spectrometer was determined by trial and error. The spectra were measured from 360 to 750 nm at a resolution of 0.4 nm and then smoothed using a second-order Savitsky–Golay filter with a window of 30 nm. The smoothed spectra were stripped of their baselines and corrected for the spectral sensitivity of the system, resulting in spectra calibrated in relative photon units. The corrected spectra were then averaged in 5-nm-wide bins and normalized to a peak of one. Because we wished to compare the spectra with previously published spectra (Herring, 1983; Widder et al., 1983; Haddock and Case, 1999) that were calibrated in energy units (and often only reported in terms of peak wavelength and spectral width), we also calibrated the collected spectra in relative energy units (see Wampler, 1978).

Imaging of bioluminescent emissions

Because of the dimness of bioluminescence and the limitations of film and electronic sensors, few true-color images of bioluminescent emissions have been taken, particularly of marine species. Instead, most color images of bioluminescence have consisted of individual frames from intensified black-and-white video colorized to match the appearance as seen by humans. However, recent increases in the sensitivity of CCD arrays now permit direct color photography. In this study, stimulated animals were photographed using a Nikon D700 camera mounted to a copy stand bolted to a laboratory bench. This camera uses a full-frame (35 mm) CCD sensor that has a peak ISO sensitivity of 25,600 (64 times more sensitive than the most sensitive readily-available film). The noise levels at this ISO were higher than desired, so an ISO of 12,800 was used for most images. The camera was fitted with a Nikon 60 mm f2.8 Micro-Nikkor lens (generally used at full aperture). The exposure time ranged from 2 to 30 s and was determined by trial and error, in an attempt to balance image brightness against blurring due to motion of the specimen and rolling and vibration of the ship. During each exposure, the animal was briefly illuminated with a small, red light-emitting diode to record its position and form. The length of red light exposure was again determined by trial and error to achieve a balance that showed both the animal and its emitted light.

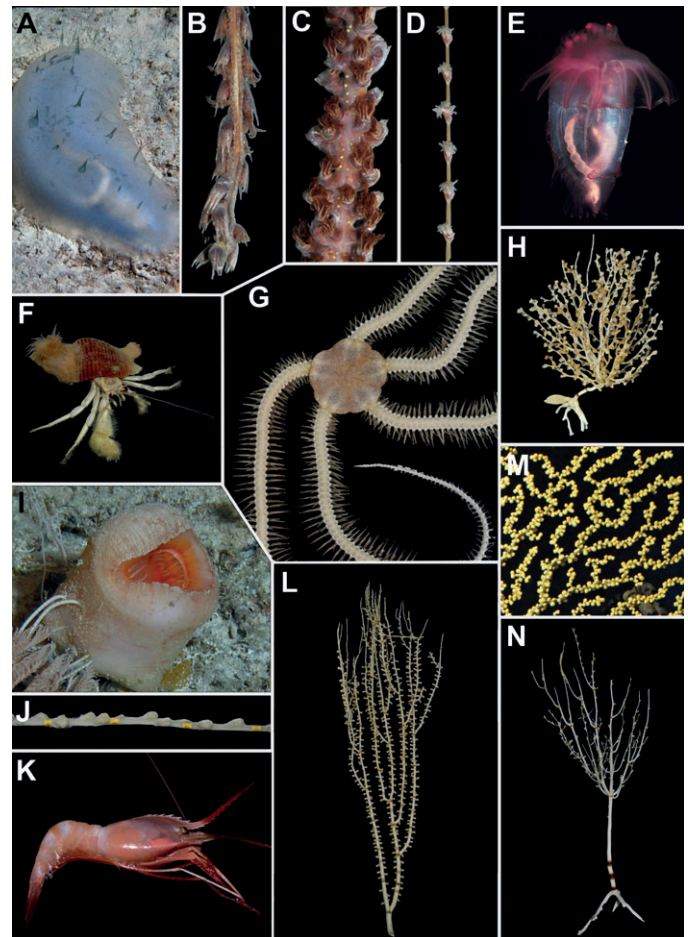


Fig. 2. Collected species that emitted light when stimulated.

(A) *Hansenothuria benti* (Holothuroidea: Aspidochirota: Synallactidae), (B) *Protoptilum* sp. (Anthozoa: Pennatulacea: Protoptilidae), (C) *Halipteris* sp. (Anthozoa: Pennatulacea: Halipteridae), (D) *Calibelemon* sp. or *Stylatula* sp. (Anthozoa: Pennatulacea: Scleroptilidae or Virgulariidae), (E) *Enypniastes eximia* (Holothuroidea: Elasipodida: Pelagothuridae), (F) undescribed hermit crab anemone (Anthozoa: Actinaria), (G) *Ophiochiton ternispinus* (Ophiuroidea: Ophiurida: Ophiochitonidae), (H) *Acanella* sp. (Anthozoa: Alcyonacea: Isididae), (I) *Actinoscyphia* sp. (Anthozoa: Actinaria: Actinoscyphiidae), (J) *Lepidisis* sp. (Anthozoa: Alcyonacea: Isididae), (K) *Parapandalus* sp. (Malacostraca: Decapoda: Pandalidae), (L) *Isidella* sp. (Anthozoa: Alcyonacea: Isididae), (M) Parazoanthidae n. gen., n. sp. (Anthozoa: Zoanthidea: Parazoanthidae), (N) *Acanella* sp. (Anthozoa: Alcyonacea: Isididae). Not shown are the chrysogorgiid octocoral, *Chrysogorgia desbonni* (Anthozoa: Alcyonacea: Chrysogorgiidae) and the pandalid shrimp *Heterocarpus ensifer* (Malacostraca: Decapoda: Pandalidae). In all but A and I, the backgrounds were darkened to clarify the presentation of the animal.

RESULTS

Species distribution of bioluminescence

Bioluminescent species were relatively uncommon among the animals from both primary sites, comprising less than 20% of the collected taxa (Figs 2, 3, Table 1, supplementary material Table S1). At these two sites, bioluminescence was most common among the bamboo corals (Alcyonacea: Isididae), sea anemones (Actinaria: Actinoscyphiidae) and sea pens (Pennatulacea: multiple families). It was also found in Parazoanthidae n. gen., n. sp., the dominant sessile organism at the lithoherm site (Zoanthidea: Parazoanthidae). *Chrysogorgia desbonni* Duchassaing and Michelotti 1864

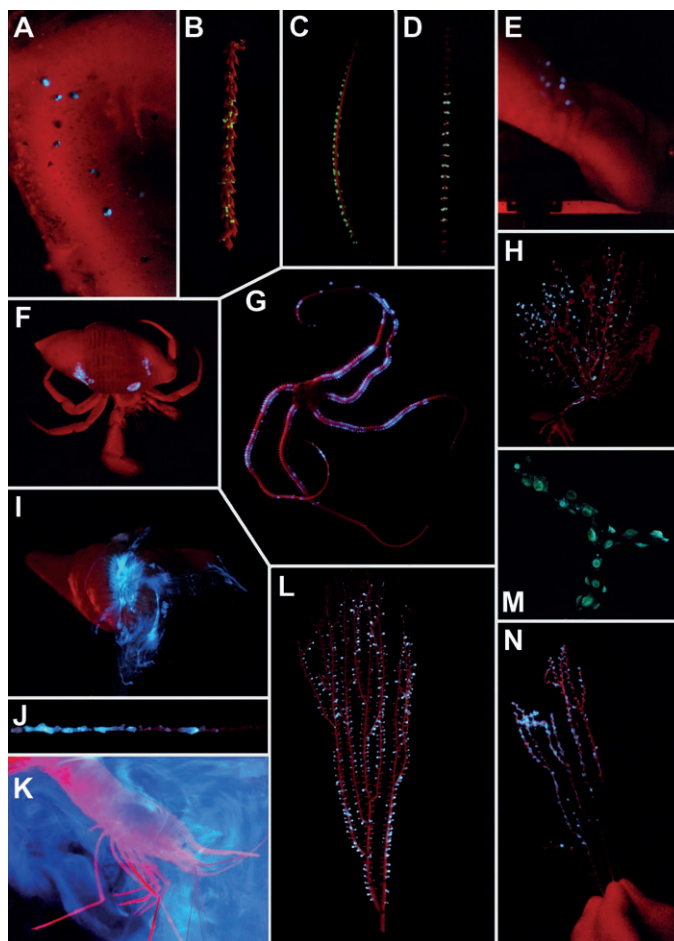


Fig. 3. Natural color images of bioluminescence. (A) *Hansenothuria benti*, (B) *Protoptilum* sp., (C) *Halipteris* sp., (D) *Calibelemonn* sp. or *Stylatula* sp., (E) *Enypniastes eximia*, (F) undescribed hermit crab anemone, (G) *Ophiuchiton ternispinus*, (H) *Acanella* sp., (I) *Actinoscyphia* sp., (J) *Lepidisis* sp., (K) *Parapandalus* sp., (L) *Isidella* sp., (M) Parazoanthidae n. gen., n. sp., (N) *Acanella* sp. Not shown are the chrysogorgiid octocoral *Chrysogorgia desbonni* and the pandalid shrimp *Heterocarpus ensifer*. The red coloration of the animals is not natural, but is instead due to brief illumination by a red light-emitting diode. See Fig. 2 legend for higher-level taxonomy.

(Alcyonacea: Chrysogorgiidae) was the only non-isidid bioluminescent coral, and the caridean shrimp *Parapandalus* sp. Borradaile 1900 and *Heterocarpus ensifer* (Milne-Edwards 1881) (Decapoda: Pandalidae) were the only bioluminescent benthic crustaceans. The holothuroid *Hansenothuria benti* Miller and Pawson 1989 (Aspidochirota: Synallactidae) was found to have bioluminescent dorsal papillae, but bioluminescence was not found in any other echinoderm species from the two primary sites, despite the diversity of species collected and the prevalence of bioluminescence in this group, particularly among the ophiuroids. Interestingly, the sea lily *Neocrinus blakei* (Carpenter 1884) (Isocrinida: Isocrinidae) did not appear to be bioluminescent, but a congener, *Neocrinus decorus* Thomson 1864, found locally in shallower water (275–522 m) but not seen or collected during this expedition, produces flashes of light along its stalk (C.G.M., unpublished observations).

Of the few specimens collected from the dive to the 1000 m Lucaya site, two were bioluminescent: the benthopelagic elasipodid holothuroid *Enypniastes eximia* Théel 1882 (Elasipodida:

Pelagothuridae) and the ophiuroid *Ophiuchiton ternispinus* Lyman 1883 (Ophiurida: Ophiuchitonidae). Given that the one dive here was a certification dive with limited collecting opportunities, it is unknown whether bioluminescent species were more common at this site than at the other two.

In situ video of bioluminescence

During a 10 min *in situ* video of a fan of the zoanthidean Parazoanthidae n. gen., n. sp., 24 bioluminescent events were observed that appeared to be a consequence of organisms contacting the fan. In the majority of cases, the luminescence appeared to adhere to the fan (Fig. 4A,B). In only two cases did the luminescence appear to pass through the fan. As the fan itself is bioluminescent, it is possible that non-bioluminescent organisms or objects made contact with the fan and stimulated bioluminescence. To test this, we lightly struck the fan with the robotic arm and observed the kinetics of the response. We also stimulated other fans *in situ* and collected multiple samples of this species and tested their light emission kinetics onboard the ship. In all cases, both gentle and heavy stimulation resulted in a glow that persisted for almost a full minute (Fig. 4C) and was visibly greener than planktonic bioluminescence. In contrast, the kinetics of the other bioluminescent events we observed *in situ* generally produced a flash or multiple flashes with durations varying from 0.25 to 10 s. Multiple other observations from the darkened submersible confirmed the continual presence of bioluminescence that appeared to be caused by plankton striking filter feeders that reached up into the water column.

Spectral properties of bioluminescent emissions

Newly measured bioluminescent spectra generally fell into two categories (Fig. 5, in black and gray). Most ($N=13$) approximated the shape of a skewed Gaussian curve with peak wavelength ($\lambda_{\max}$; calibrated in photons) that ranged from 455 to 495 nm (mean $\pm$ s.d.=480 $\pm$ 12 nm), with the emission spectra from Parazoanthidae n. gen., n. sp. having the longest wavelength peak in this category (495 nm). The full-width half-max (FWHM; the wavelength range over which the value is at least half the peak value) of these spectra ranged from 65 to 90 nm (mean=78 $\pm$ 8 nm). In contrast, the spectra from the three newly measured pennatulaceans (*Halipteris* sp., *Protoptilum* sp. and *Stylatula/Calibelemonn* sp.) fell into a second category and were long-wavelength shifted (peaking at 505, 510 and 535 nm, respectively) and narrower (FWHMs of 25, 25 and 40 nm, respectively). These spectra had the classic characteristics of emission spectra in which bioluminescence co-occurs with GFP-based fluorescence, with a narrow peak and a long-wavelength shoulder (see Wampler et al., 1971; Haddock et al., 2010).

The previously published spectra also fell into these two categories with similar values for $\lambda_{\max}$ and FWHM [Fig. 5, in red and pink, see legend for higher taxonomy; data from Widder et al. and Herring (Widder et al., 1983; Herring, 1983)]. The $\lambda_{\max}$ of the spectra of the non-pennatulaceans ($N=8$) ranged from 455 to 525 nm (mean=486 $\pm$ 24 nm) and the FWHM ranged from 60 to 100 nm (mean=77 $\pm$ 14 nm). The asteroid *Plutonaster bifrons* (Wyville Thomson 1873) was a significant outlier with a $\lambda_{\max}$ of 525 nm. The six spectra of the three previously measured pennatulaceans were more complex. In two [*Umbellula magniflora* K  llicker 1880 and *Distichoptilum verrilli* (Pallas 1766)], the spectra of light emitted from the stalk were similar to those measured in this study ($\lambda_{\max}$ =505, 505 nm; FWHM=55, 40 nm). However, spectra from light emitted from the plumes of the animals were significantly broader ($\lambda_{\max}$ =495, 505 nm; FWHM=100, 105 nm). In the remaining pennatulacean, *Stachyptilum superbum* Studer 1894, the majority

Table 1. Bioluminescent species collected during this study

Species	Collection site	Depth (m)	$\lambda_{\max}$ (nm)	FWHM (nm)
Cnidaria				
Anthozoa				
Hexacorallia				
Actinaria				
<i>Actinoscyphia</i> sp.	L,P	600–650	483	70
<i>Actinoscyphia</i> sp. (acontial filaments)	L,P	600–650	455	75
Undescribed hermit crab actinian	P	500	485	70
Undescribed hermit crab actinian	P	500	490	70
Zoanthidea				
Parazoanthidae n. gen., n. sp.	L	600–650	495	85
Octocorallia				
Pennatulacea				
<i>Calibelemnon</i> sp. or <i>Stylatula</i> sp.	P	675	505	30
<i>Halipterus</i> sp.	P	675	510	25
<i>Protoptilum</i> sp.		675	535	35
Alcyonacea	P			
<i>Acanella</i> sp.	P	400	480	85
<i>Acanella</i> sp.	P	700	480	80
<i>Chrysogorgia desbonni</i>	P	700	Unk.	Unk.
<i>Isidella</i> sp.	P	650	480	80
<i>Lepidisis</i> sp.	P	700	475	70
Crustacea				
Malacostraca				
Decapoda				
Caridea				
<i>Heterocarpus ensifer</i>	P	690	462*	63*
<i>Parapandalus</i> sp.	P	690	455	60
Echinodermata				
Ophiuroidea				
Ophiurida				
<i>Ophiochiton ternispinus</i>	DP	1000	470	80
Holothuroidea				
Elasipodida				
<i>Enypniastes eximia</i> (benthopelagic)	P	1000	470	78
Aspidochirota				
<i>Hansenothuria benti</i>	P	700	475	75

$\lambda_{\max}$, peak wavelength; FWHM, full-width half-max.

Collection sites include: L, lithoherm site (Memory Rock); P, sediment plain in NW Providence channel (Burrow Cay); and DP, deeper plain off Lucaya, Grand Bahama Island. See Fig. 5 for emission spectra.

*Not measured in this study; values are from Herring (Herring, 1976) and may be unreliable because they were not corrected for the spectral sensitivity of the detector.

of the specimens emitted light with a $\lambda_{\max}$ of 535 nm and a FWHM of 55 nm, but a small fraction of specimens emitted light with a shorter $\lambda_{\max}$ of 505 nm and a FWHM of 30 nm. This is similar to what has been found in the shallow-water pennatulacean *Renilla reniformis* (see Wampler et al., 1971).

Fig. 6 shows $\lambda_{\max}$ plotted against FWHM for the spectra from this study combined with previously published spectra of deep-sea benthic species. These spectra were compared with emission spectra from 115 mesopelagic species (48 cnidarians, 24 ctenophores, two cephalopods, 32 crustaceans and nine teleosts; see Fig. 6 legend for data sources). The $\lambda_{\max}$ and FWHM for all species, both benthic and mesopelagic, were calculated from spectra calibrated in energy units to allow comparison with previous data that reported only $\lambda_{\max}$ and FWHM calibrated in energy units. In general, the $\lambda_{\max}$ and FWHM of the deep-sea benthic and mesopelagic spectra were similar. In particular, the emission spectrum of the caridean shrimp *Parapandalus* sp. closely approximated that reported for mesopelagic decapod crustaceans (Herring, 1983) (the spectrum of the shrimp *Heterocarpus ensifer* could not be measured). However, several groups had markedly different spectra from mesopelagic species. The spectra of ophiuroids (with the exception of *O. ternispinus*) were shifted to longer peak wavelengths and larger FWHMs and, as mentioned above, the spectra of the pennatulaceans

(with the exception of the plumes of *Umbellula magniflora* and *Distichoptilum verrilli*) were shifted to longer wavelengths with substantially smaller FWHMs. Although only four emission spectra from deep-sea asteroids have been measured, they are quite disparate, ranging from 450 to 525 nm in $\lambda_{\max}$ and 64 to 115 nm in FWHM. In contrast, the 10 deep-sea holothuroid spectra had a narrower range in both parameters ($\lambda_{\max}$ =462–495 nm; FWHM=70–96 nm). The $\lambda_{\max}$ of Parazoanthidae n. gen., n. sp. was longer than all but one of the mesopelagic spectra [that of an undescribed bathyctenid ctenophore (see Haddock and Case, 1999)].

Dynamic properties of bioluminescent emissions

Most of the emissions observed in the present study followed dynamics typical for mechanically stimulated bioluminescence, with a sharp rise in intensity after stimulation followed by an exponential decay with a half-life on the order of 1 s. The exceptions were Parazoanthidae n. gen., n. sp., which had a substantially longer half-life, the pennatulacean *Halipterus* sp., which pulsed with a period of ~0.5 s, and the isidid octocoral *Acanella* sp., in which different polyps turned on and off asynchronously in a 'twinkling' display. The bioluminescence of the anemone *Actinoscyphia* sp. and the holothuroid *Enypniastes eximia* both consisted of adhesive luminescent secretions (or possibly detached luminescent epidermis)

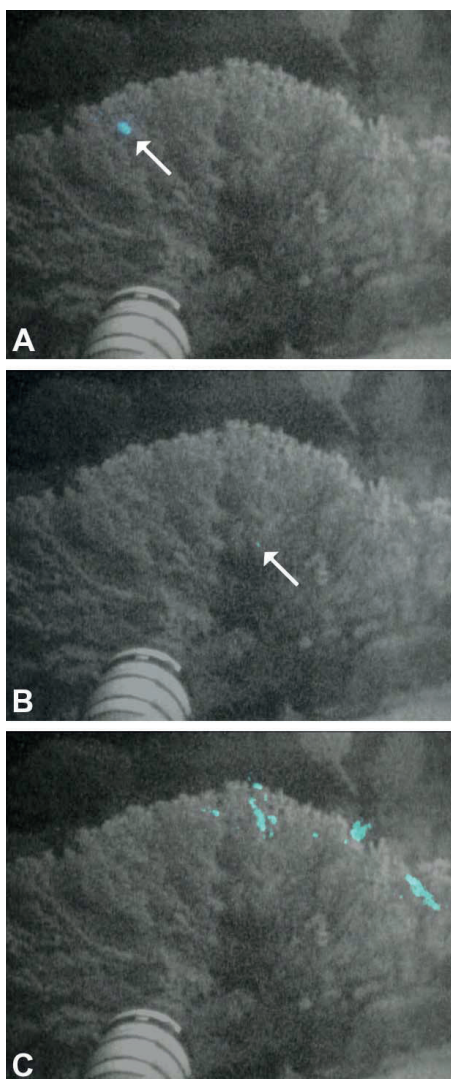


Fig. 4. Intensified *in situ* video of bioluminescence at the Memory Rock site. Each panel is a composite of an image of a stand of *Parazoanthidae* n. gen., n. sp. (taken under dim submersible lights) and an image of bioluminescence taken with the submersible's lights turned off. The bioluminescence is (falsely) colored blue to distinguish it from the background. (A,B) Light emitted when a planktonic animal struck a colony of *Parazoanthidae* n. gen., n. sp. The bioluminescence image in B is the sum of 13 video frames to increase the signal-to-noise ratio. (C) Light emitted from the colony itself after being mechanically stimulated by the claw on the submersible.

(Fig. 3E,I), whereas that of the shrimp *Parapandalus* sp. and *Heterocarpus ensifer* were of the 'spew' variety, in which the reagents are released into the water where they mix to generate light (Fig. 3K).

DISCUSSION

The simultaneous prevalence and rarity of bioluminescence at the two primary sites

Bioluminescent benthic species were relatively uncommon at both primary collection sites. Bioluminescence is generally considered to be common in mesopelagic species (reviewed by Haddock et al., 2010). Although a reliable estimate is of course difficult to obtain (or even define), it appears that ~80% of mesopelagic fish and crustaceans are capable of emitting light (Herring, 1976; Herring and Morin, 1978;

Hastings and Morin, 1991). Bioluminescence is also nearly ubiquitous in mesopelagic cephalopods and gelatinous zooplankton, although it is less common in certain mesopelagic taxa (e.g. copepods and amphipods) and rare or absent in others (e.g. heteropods, pteropods, chaetognaths, salps and doliolids) (Herring, 1987; Hastings and Morin, 1991; Haddock and Case, 1999). In contrast, less than 20% of the benthic species collected at the three sites emitted light. However, before we entertain any adaptive reasons for this, we must consider potential confounding factors. First, the relative dearth of bioluminescent taxa may be location or depth-specific and not a universal condition. It is intriguing that the one short dive to a greater depth in this study uncovered a higher percentage of bioluminescent taxa, though the sample size was far too small to draw any conclusions. Unfortunately, no other systematic surveys of the species distribution of deep-sea benthic bioluminescence have been performed. Interestingly, the few surveys of bioluminescence in coastal benthic species found that it was also uncommon (1–2% of species), primarily being confined to certain ophiuroids, hydroids, pennatulaceans, polynoid and other polychaetes, and fish (reviewed by Morin, 1983). Second, it is possible that the threshold for triggering bioluminescence is higher in benthic species than in mesopelagic ones, and that we thus mislabeled some taxa as non-bioluminescent. One might expect that benthic taxa are physically disturbed more often than mesopelagic taxa, as many are sessile and exposed to current-borne particles as well as occurring in higher population densities, and thus have a higher disturbance threshold. However, the benthic species that did emit light always did so with stimulation comparable to that used to stimulate emission in mesopelagic species. Finally, it is possible that the collection process caused apparently non-light-producing taxa to exhaust their bioluminescence within the bio-box or suction sampler during the remainder of the dive, leaving them incapable of producing further light for a time. However, the 12 h wait before retesting (see Materials and methods) should have allowed for at least some recovery of bioluminescent potential. In summary, given the authors' extensive experience with stimulating bioluminescence in marine species and the multiple attempts made with each specimen, we are confident that a high percentage of the animals that did not emit light did not have the potential to do so. Therefore, we believe that, for the primary two sites at least, bioluminescence is relatively rare in epifaunal benthic species (infaunal species were not tested).

It is at first difficult to understand why this might be the case. Although the water near the deep-sea floor has some suspended sediment and thus is not as clear as mesopelagic water, it is by no means murky and is likely as clear as near-surface coastal waters that have abundant bioluminescence at night. One possibility is that benthic bioluminescence can potentially be blocked by the more complex habitat and is therefore a less efficient signal than mesopelagic bioluminescence, which usually can be broadcast equally in all directions. This is consistent with the fact that bioluminescence was more common in the less complex Burrow Cay site and also more common in species that reached higher into the water column (e.g. *Parazoanthidae* n. gen., n. sp. and the pennatulaceans). Another possibility is that certain functions of bioluminescence, such as counterillumination, are less relevant to benthic species, especially to epifaunal invertebrates that cannot enter the water column. These hypotheses are all necessarily speculative.

However, it is important to realize that, although not common among benthic species, bioluminescence was nevertheless significant at both primary study sites. First, the zoantharian *Parazoanthidae* n. gen., n. sp. – the dominant species on the crests of the ridges at the lithoherm site – was not only bioluminescent, but its light was bright, easily triggered and exceptionally long-lasting. Second, and more

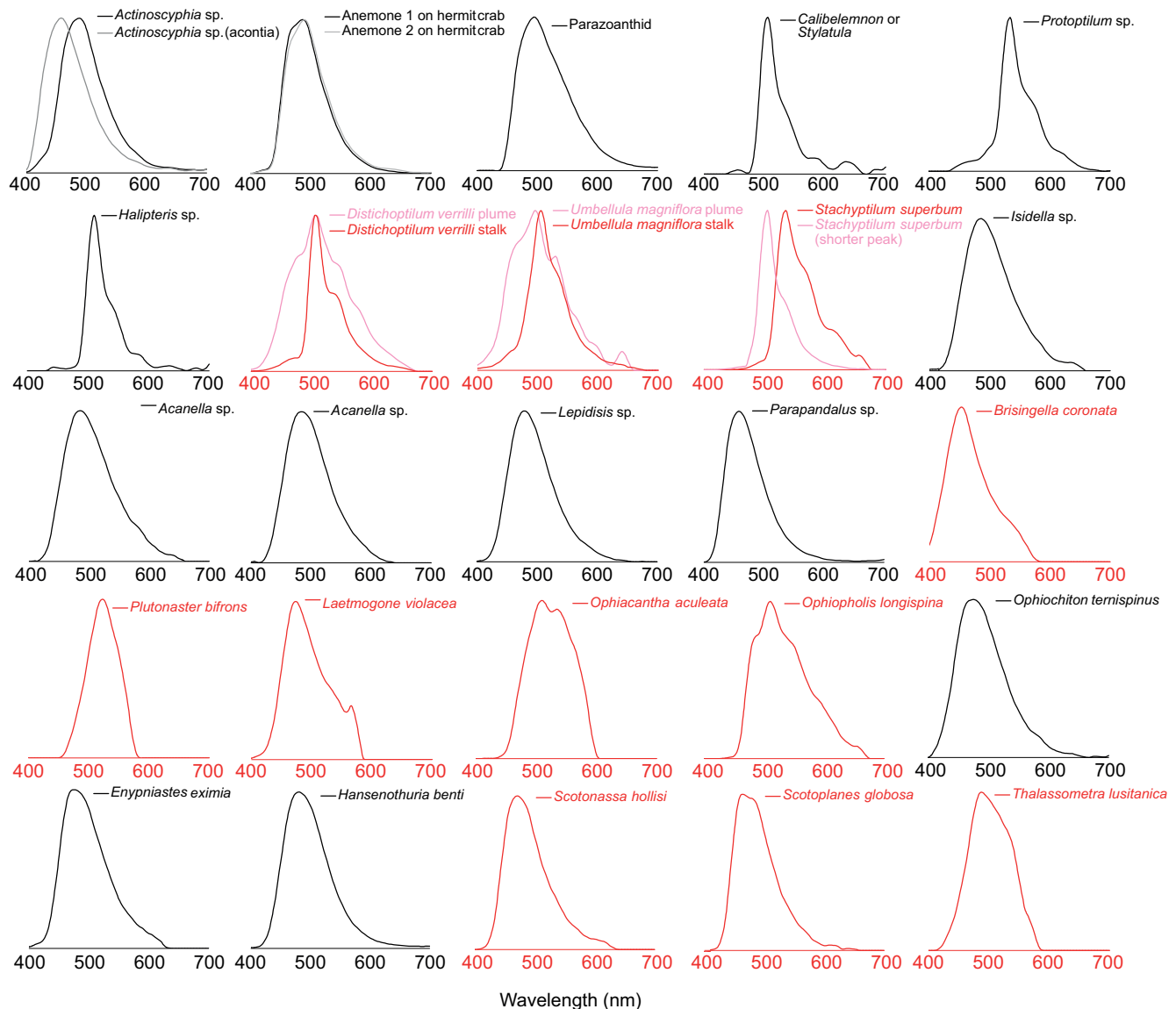


Fig. 5. Emission spectra of the collected species (in black or grey) combined with previously published spectra (in red or pink) of other deep-sea benthic species. Of the previously published spectra, those from *Distichoptilum verrilli* (Anthozoa: Pennatulacea: Protophilidae), *Stachyptilum superbum* (Anthozoa: Pennatulacea: Stachyptilidae), *Umbellula magniflora* (Anthozoa: Pennatulacea: Umbellulidae), *Scotoplanes globosa* (Holotheuroidea: Elapodidae: Elpididae), *Scotonassa hollisi* (Holotheuroidea: Elapodidae: Elpididae) and *Ophiopholis longispina* (Ophiuroidea: Ophiurida: Ophiactidae) are from Widder et al. (Widder et al., 1983). Those from *Laetmogone violacea* (Holotheuroidea: Elapodidae: Laetmogonidae), *Ophiacantha aculeata* (Ophiuroidea: Ophiurida: Ophiacanthidae), *Brisingella coronata* (Asteroidea: Brisingida: Brisingidae), *Plutonaster bifrons* (Asteroidea: Paxillosida: Astropectinidae) and *Thalassometra lusitanica* (Crinoidea: Comatulida: Thalassometridae) are from Herring (Herring, 1983). All spectra are calibrated in photons and normalized to have the same maximum. See Table 1 for further details on the taxonomy and collection depths of the animals collected in this study.

importantly, observations from a darkened submersible at both primary sites showed that bioluminescent plankton transported by the bottom current frequently emitted light as they impacted the stands of Parazoanthidae n. gen., n. sp., the taller octocorals and other structures that stood high in the water column [see Craig et al. (Craig et al., 2011) for similar *in situ* observations at depths of 2000–3000 m]. In the mesopelagic zone, bioluminescence is primarily triggered by the interactions of larger nekton with bioluminescent plankton (Widder and Johnsen, 2000), and is thus more rare. Long-term observations from submersibles trimmed to neutral buoyancy (to avoid motion relative to the plankton) have shown that spontaneous bioluminescence in the mesopelagic realm is nearly non-existent (see Widder and Johnsen, 2000). Counterillumination is of course widespread in fish, crustaceans and cephalopods, but is by definition

nearly invisible. In contrast, bioluminescence is relatively common in structurally complex benthic habitats due to the motion of plankton-laden water relative to the substrate and sessile organisms. In fact, the presence of bioluminescence triggered by the incidental impacts of plankton with the benthos may help explain why it is relatively rare among the benthic species examined. Certain functions of bioluminescence, such as attracting higher-order predators, startling predators and luring prey, may be less successful in the presence of significant amounts of accidentally generated light.

The species composition of benthic bioluminescence

Aside from the general paucity of bioluminescence in local benthic taxa, two unusual taxon-specific results emerged. First, to our knowledge, we found the first bioluminescent anemones:

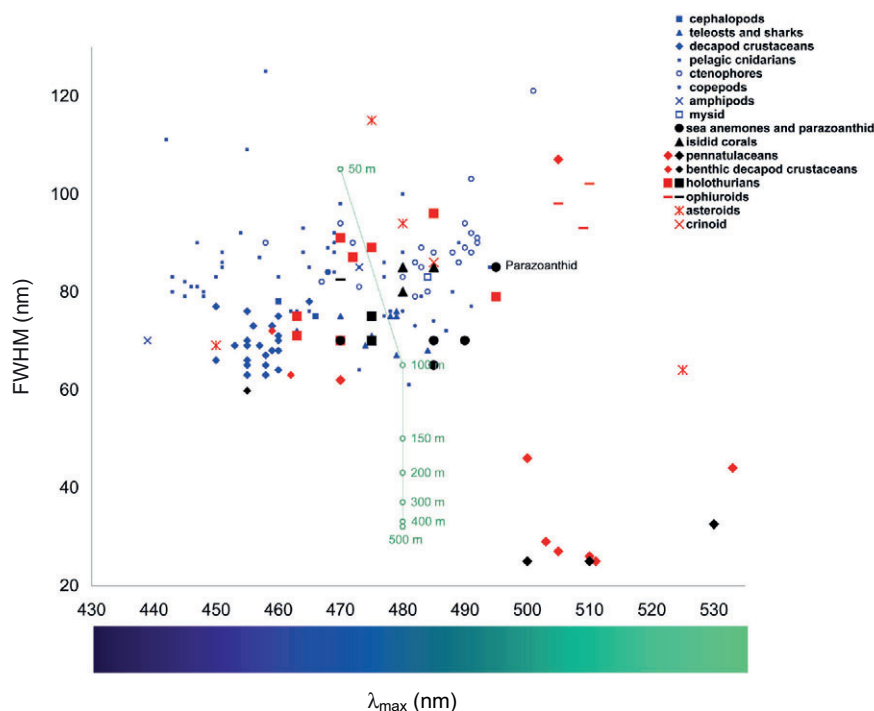


Fig. 6. Peak wavelength ($\lambda_{\max}$) versus emission width [full-width half-max (FWHM)] for the light emissions from deep-sea benthic and mesopelagic species. Black symbols represent deep-sea benthic species from this study. Red symbols represent deep-sea benthic species from Herring (Herring, 1983) and Widder et al. (Widder et al., 1983). Blue symbols represent mesopelagic species (excluding emissions used for counterillumination). The green line gives the peak wavelength and spectral width of the downwelling irradiance in clear, oceanic waters as a function of depth [data from Johnsen et al. (Johnsen et al., 2004)]. The parazoanthid spectrum is labeled because it is a dominant organism in the lithoherm site. The mesopelagic spectral data are from Nicol (Nicol, 1960), Swift et al. (Swift et al., 1973; Swift et al., 1977), Biggley et al. (Biggley et al., 1981), Herring (Herring, 1983), Denton et al. (Denton et al., 1985), Widder et al. (Widder et al., 1983), Herring et al. (Herring et al., 1992; Herring et al., 1993) and Haddock and Case (Haddock and Case, 1999). All spectra are calibrated in energy units (instead of photons) to facilitate comparison with previously published work.

Actinoscyphia sp. and two specimens of what may be one species of an unidentified hermit crab anemone. This contrasts with the widespread occurrence of bioluminescence among a variety of octocorals, particularly pennatulaceans. Second, although bioluminescence is relatively common among echinoderms (particularly among the holothuroids, asteroids and ophiuroids), it was rare in the species we examined. Despite initial expectations to the contrary and the testing of numerous specimens of multiple species from all five classes, only three of the 27 echinoderms tested were bioluminescent: the ophiuroid *Ophiochiton ternispinus*, and the holothuroids *Hansenothuria benti* and *Enypniastes eximia*. Although this low percentage appears puzzling, as previous studies of deep-sea benthic bioluminescence (Herring, 1983; Widder et al., 1983) found a number of bioluminescent holothuroids, asteroids and ophiuroids, as well as a crinoid, our taxon sampling may offer at least a partial explanation. Fully half of our tested taxa were crinoids, relatively few of which are known to be bioluminescent, and three of the five ophiuroid species tested were Euryalida (snakestars and basketstars), none of which are known to be bioluminescent (Herring, 1987).

The spectra of benthic bioluminescence compared with mesopelagic bioluminescence

Although some of the currently and previously measured benthic taxa (e.g. holothuroids, corals and shrimp) had emission spectra that fell within the general range of those found in mesopelagic species, others, including ophiuroids, Parazoanthidae n. gen., n. sp. and especially the pennatulaceans, had spectra that were significantly shifted to longer wavelengths. In the case of the sea pens, this is likely due to the presence of GFP as an accessory pigment (see Wampler et al., 1971; Haddock et al., 2010). Asteroids [as measured by Herring (Herring, 1983)] were a special case, displaying an impressive diversity in both the peak and width of the emission spectrum. The functional reason for the long-shifted spectra in these taxa is not known and any hypotheses are speculative.

One possibility is that the greener light is transmitted further than bluer light in the deep benthic environment. It is generally accepted that mesopelagic bioluminescent spectra peak in the blue-green

portion of the spectrum because these are the wavelengths of light that transmit best in the mesopelagic realm (Nicol, 1958; Herring, 1983; Widder et al., 1983; Haddock and Case, 1999). However, because of suspended sediment, some of which may contain chlorophyll remnants, it is possible that the wavelength of maximal light transmission in near-bottom waters is longer than that measured for the mesopelagic zone. Unfortunately, measuring this involves deploying a multi-wavelength transmissometer (e.g. WETLabs ac-9) within a meter of the deep-sea floor. Doing this *via* the usual ship-based deployment would be extraordinarily risky and, to our knowledge, has not been attempted. A safer approach would be to use a transmissometer mounted to a submersible or remotely operated vehicle. Previous studies used a scuba-deployed transmissometer within 10 cm of a coral reef and found that the inherent optical properties were highly variable and strongly affected by both dissolved organic matter released by organisms and particle uptake by filter feeders (Zaneveld et al., 2001; Boss and Zaneveld, 2003). Until a similar study is performed in deeper water, there is no way to determine whether the inherent optical properties of the deep benthic environment are significantly different from the water above it, though a slight shift to longer wavelengths in the visual pigments of deep-sea demersal fish (Douglas et al., 1995) suggests that they may be. However, even if they did differ, it is difficult to understand why certain deep-sea bioluminescent species would take advantage of this and others would not.

Another possibility is that the long-wavelength shifted taxa are signaling their toxicity or unpalatability, in much the same way as many red-colored taxa do in terrestrial habitats (e.g. Coppinger, 1970; Roper, 1990). Although it is certainly possible, and even likely, that some of the ophiuroids, pennatulaceans and Parazoanthidae n. gen., n. sp. are at least unpalatable, the success of such signals requires color vision in the viewers, which appears to be rare in the deep sea. Because multiple visual channels decrease the sensitivity of each channel, and because deep-sea animals generally operate near the absolute limits of vision in their light-limited environment, color vision comes at a significant cost. Indeed, extensive microspectrophotometry-based surveys of the visual pigments of mesopelagic fish (Douglas

et al., 1998) and electroretinography-based surveys of mesopelagic crustaceans (Frank and Case, 1988; Frank and Widder, 1999) have found that only a fraction of these species have multiple spectral channels and thus the potential for color vision (but see Frank et al. 2012). A final, related possibility is that the bioluminescence is greener to minimize the distance over which it can be seen. If the transmission properties of the deep-sea benthic environment are similar to those found in the mesopelagic environment, 525 nm light will not transmit as far as 480 nm light. This, combined with the fact that deep-sea visual systems are generally not optimized for detecting longer-wavelength light, would create a situation where pennatulacean bioluminescence acts as a short-range signal, a common characteristic of aposematism (reviewed by Ruxton et al., 2004).

Because, with the exception of asteroids, the emission spectra tend to group by taxa, phylogenetic effects are also possible. Indeed, the long-shifted emission spectra of the deep-sea pennatulaceans are quite similar to those found in shallow-water representatives of the same group (Wampler et al., 1973). A rigorous test of this using mesopelagic, epipelagic, shallow-benthic and deep-sea benthic emission spectra mapped onto a well-supported phylogeny would help address this possibility and is currently in progress. Indeed, because shallow and deep benthic species are often more closely related to each other than they are to pelagic species, properly controlling for phylogenetic effects is crucial for determining whether the long-shifted spectra of deep-sea benthic species have an adaptive function or are due to a shared history with related shallow-water taxa. Interestingly, previous surveys that included coastal bioluminescence (Herring, 1983; Morin, 1983) also showed that the emission spectra of benthic species are long-shifted relative to those of neritic species, though the difference is less pronounced than what we observed at depth.

Conclusions

This preliminary study raises more questions than it answers. Bioluminescence was uncommon in epifaunal benthic species collected from the two primary sites, though significant levels of bioluminescence were observed *in situ* as a result of bioluminescent plankton impacting the habitat. In certain benthic groups, the emitted light was significantly long-shifted. Further work needs to continue to sample bioluminescence in other deep-sea benthic habitats (including the abyssal plain), determine the inherent optical properties of the water in this realm, and investigate the spectral sensitivity and potential for color vision in benthic and benthopelagic species. Only when this is done can we more truly understand the visual environment of the largest benthic habitat on earth.

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